Open Access

Check for updates

Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.1.13-19

Modified syringe technique to prepare platelet rich plasma from rat blood, its quantitative assessment, and evaluation of platelets stored at 4°C to determine its suitability for homologous application#

Reji Varghese[1*], S. Anoop[1], Syam K. Venugopal[1], S. Sooryadas[2], V.N. Vasudevan[3], Varuna P. Panicker[4], Sudheesh S. Nair[5], Deny Jennes[1] and K.D. John Martin[6]

[1]Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, India, [2] Department of Veterinary Surgery and Radiology, College of Veterinary and Animal Sciences, Pookode, Wayanad – 673 576, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, [3] Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, [4] Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, [5] Ross University School of Veterinary Medicine, Saint Kitts, West Indies, [6] University Veterinary Hospital, Kokkalai, Thrissur-680 021, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

Citation: Reji, V., Anoop, S., Syam, K.V., Sooryadas, S., Vasudevan, V.N., Varuna, P.P., Sudheesh, S.N., Deny, J. and John Martin, K.D. 2025. Modified syringe technique to prepare platelet rich plasma from rat blood, its quantitative assessment, and evaluation of platelets stored at 4 °C to determine its suitability for homologous application.
J. Vet. Anim. Sci. **56** (1):13-19

Received: 21.08.2024 Accepted: 20.09.2024 Published: 31.03.2025

Abstract

The study was designed to evaluate a modified syringe technique to prepare platelet rich plasma (PRP) from rat blood. Blood was collected by terminal cardiac puncture from adult male Wistar rats. It was subjected to double centrifugation for the separation of platelets. The PRP thus prepared was stored at 4 °C. The platelet recovery parameters such as platelet enrichment factor and platelet recovery rate were calculated for the quantitative assessment of PRP. Platelet count, mean platelet volume and platelet distribution width were obtained for assessment of platelets during storage. Microbiological, cytological and ultrastructural evaluations were done. The platelet recovery parameters suggested that the PRP obtained, satisfied the criteria to be used clinically and was of reasonable quality. Indicators of platelet activation suggested that there was no inadvertent activation of platelets during storage. The cytological and ultrastructural evaluation also suggested the same. It was concluded that PRP prepared from rat blood using modified syringe technique and stored at 4 °C could safely and effectively be used for daily homologous application for up to one week that depended on in situ activation of platelets.

Keywords: *Platelet rich plasma, rat, modified syringe technique, assessment, storage*

Platelet rich plasma therapy is gaining popularity due to its potential ability in regenerative medicine. Platelet rich plasma has been defined as an autologous concentration of platelets that is 3 to 5 times greater than the physiologic concentration of thrombocytes in whole blood. Platelets are formed in the bone marrow from megakaryocytes having

#Part of Ph. D thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode
**Corresponding author: reji@kvasu.ac.in, Ph. 9745055033*

a life span of about 7 to 10 days (Pavlovic *et al*., 2016). The recommended therapeutic concentration of PRP for clinical use is 1-1.5 million/µL (Dashore *et al*., 2021). Various methods have been described for the preparation and storage of PRP. But a balance needs to be maintained to avoid inadvertent activation of platelets, prevention of bacterial contamination and maintaining a therapeutic concentration during storage, especially when it is intended for daily application, with *in situ* activation for soft tissue applications. Even though many commercial systems are available to prepare PRP, they are expensive and researchers have been trying to develop more economic methods for its preparation. The modified syringe method is one such alternative and this study aims to evaluate the PRP prepared using this technique and its suitability for laboratory animal research, on storage at 4 °C.

Materials and methods

The study was conducted in eight adult male Wistar rats (*Rattus norvegicus*) weighing 320-390g. They were used to produce PRP. Six samples of PRP were subjected to storage studies. The Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, approved the study as per order number CVAS/MTY/IAEC/23/41 dated13.09.23.

Collection of blood

All the animals were anaesthetised using a cocktail of Inj. Xylazine hydrochloride (5 mg/kg) and Inj. Ketamine hydrochloride (50 mg/kg) given intraperitoneally. The ventral side of the animals was shaved starting from mid-sternum to anterior abdomen and was prepared aseptically by scrubbing with a detergent and painted with povidone iodine (5% solution). The skin was incised caudal to xiphoid and the incision was extended cranially, followed by sternotomy to expose the heart. A 20-gauge needle attached to a 10 mL disposable syringe was used for blood collection. One millilitre of 10 per cent sodium citrate solution was drawn into the syringe. The plunger was then drawn backwards and the syringe was rotated multiple times to allow uniform coating of the anticoagulant inside the barrel of the syringe. The needle was inserted into the left ventricle and 10 mL of blood was withdrawn. (Fig.1a). The syringe was detached from the needle and gently rotated for uniform mixing of the blood. The syringe was plugged with the cap of a three-way stop cock; the finger handles and the distal portion of the plunger leaving two centimetres, were removed using scissors to accommodate the syringes in the centrifuge tube. Following collection of blood from the heart, an overdose of inj. thiopentone sodium (10 %) was injected intracardiac through the needle in place, to euthanise the animals (Fig. 1b).

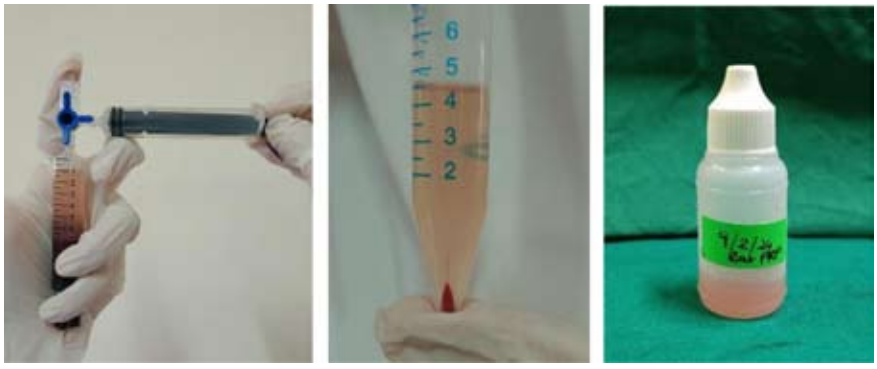
Syringe method of PRP preparation using differential centrifugation

The modified syringe method suggested by Laiju (2022) was used for the preparation of PRP. The differential centrifugation protocol used by Messora *et al*. (2011) was employed for the separation of platelets. From the collected blood, 0.25 mL was transferred to an Eppendorf tube and a baseline complete blood count was obtained using an automated haematology analyser (Mythic 18 vet, Orphee, Switzerland).

Thermo Scientific Heraeus Biofuge® Stratos refrigerated centrifuge was used for the differential centrifugation. Two syringes prepared identically, were placed in opposite slots of the centrifuge for balanced centrifugation. The first spin was carried out at 160 x *g* for 20 min at 22 °C. The red blood cells were settled at the bottom and the straw-coloured plasma was seen as the top layer. Separating these two layers, there was a fine layer of white blood cells – the buffy coat.

The cap was removed and the syringe was attached to the female Luer lock port of a three-way stopcock. A second 10 mL disposable syringe was attached to the second female Luer lock port and slight pressure was applied to the plunger to push the plasma into the fresh syringe. Care was taken to avoid red blood cells getting into the fresh syringe (Fig. 2a). The plasma thus separated was transferred into a sterile 15 mL sterile falcon tube with a conical bottom and screw cap.

Fig. 1a. Intracardiac blood collection; **1b.** Euthanasia with thiopentone



2a **2b** **2c**

Fig. 2a. Syringe method, **2b.** Cell pellet after second spin, **2c.** Final PRP in dropper bottle

Two tubes prepared in this manner were subjected to the second spin at 400 x *g* for 15 min at 22 °C. The platelet rich cell pellet settled at the conical bottom with a top layer of platelet poor plasma (PPP) (Fig. 2b). Two-third of the PPP was transferred to a sterile Eppendorf tube using a micropipette for further use. The cell pellet at the bottom was resuspended in the residual PPP using a micropipette to prepare platelet rich plasma. The volume was measured and transferred to another sterile dropper bottle (Fig. 2c). It was labelled and a complete blood count was obtained using an automated haematology analyser. One millilitre of PRP was obtained from 10 mL whole blood.

Storage of PRP

The dropper bottles containing PRP were kept in an insulated container protecting it from direct light and drastic changes in ambient temperature and stored at 4 °C in a refrigerator. Six samples were used for the storage study.

Quantitative assessment of PRP

Quantitative assessment of PRP was performed by comparing the platelet counts in whole blood and PRP. Dashore *et al*. (2021) have provided the basic formulas in relation to PRP:

$$\text{Platelet Enrichment Factor (PEF)} = \frac{\text{PRP platelet concentration}}{\text{Whole blood platelet concentration}}$$

Platelet recovery rate is the percentage of platelets that could be recovered in PRP from the collected volume of whole blood.

$$\text{Platelet Recovery Rate (PRR)} = \frac{(\text{PRP platelet concentration} \times \text{PRP volume}) \times 100}{(\text{Venous blood platelet concentration} \times \text{venous volume})}$$

Assessment of platelets during storage at 4 °C

The platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) were evaluated during the storage period. These parameters were obtained using an automated haematology analyser. The day on which the platelet count fell below 1 x 10^6/µL, was considered as the end point.

Bacteriological evaluation

The PRP sample after the end point of study was streaked on brain heart infusion agar and incubated at 37 °C for 72 h for detecting any bacterial contamination during storage.

Microscopic evaluation

Smears were prepared daily on clean glass slides from samples one and two, from day zero till the end point. They were stained with Field's stain and observed under a binocular light microscope (Labomed), to evaluate the microscopic appearance of platelets.

Scanning electron microscopy (SEM)

The platelets were subjected to SEM on day 0 and day 6 to assess their ultrastructure. The platelets were fixed in 2.5% glutaraldehyde for 24 h and centrifuged at a speed of 1500 rpm for five minutes. The cell pellet was washed with freshly prepared phosphate buffered saline and smeared on glass coverslips for sputter coating. SEM images were captured using Tescan Vega -3LMU Scanning Electron Microscope, at Central Instruments Laboratory of the College of Veterinary and Animal Sciences, Mannuthy.

Results and discussion

Collection of blood

In the present study, we could consistently draw 10 mL of whole blood by intracardiac puncture from Wistar rats weighing around 350g. Parasuraman *et al*. (2010) and Kumar *et al*. (2017) had described the various blood collection methods in rats. The different collection sites were: 1. lateral saphenous vein, 2. lateral tail vein, 3. dorsal pedal vein, 4. jugular vein, 5. retro orbital sinus

and 5. cardiac puncture. The method depended on the quantity of blood needed for the investigation. All the venipuncture sites yielded only a small quantity of blood enough for routine haematology. For the preparation of PRP, we needed large volumes of blood and hence cardiac puncture, a terminal procedure, was more suited for this study. Messora *et al*. (2011) used a jugular cannulation technique to collect 3.5 mL of whole blood from rats for the preparation of autologous PRP. But the volume of PRP was also limited and hence not suitable for such a study.

PRP preparation

Fukaya and Ito (2014) suggested an economical method for the preparation of PRP using disposable syringes, 3-way cock and extension tubes, employing a double centrifugation method. The finger-holders were cut to accommodate the syringes in the centrifuge tube. This was modified by Laiju (2022) for the preparation of bovine PRP and was adopted in this study. Sonnleitner *et al*. (2000) used a double centrifugation protocol with the first spin at 160 x *g* for 20 min and the second centrifugation was done at 400 x *g* for 15 min for the preparation of PRP. Messora *et al*. (2011) used these parameters with a refrigerated centrifuge for standardising a protocol for preparing PRP from rat blood. They achieved a four-fold increase in the number of platelets. The same technique was used in this study. Macey *et al*. (2002) stated that cooling might retard platelet activation and this might be essential in obtaining PRP with viable platelets. We used a refrigerated centrifuge with the temperature setting of 22 °C and the same was used by Messora *et al*. (2011). Acid citrate dextrose (ACD-A) is the preferred anticoagulant for the preparation of PRP (Dashore *et al*., 2021). In this study, 10% sodium citrate was used, as suggested by Messora *et al*. (2011). There are various commercially available systems to produce autologous PRP utilising the principle of differential centrifugation (Pavlovic *et al*., 2016). Most of these systems were expensive and the platelet recovery rate was low to medium as per the DEPA (Dose of injected platelets, Efficiency of production, Purity of the PRP, Activation of the PRP) classification (Magalon *et al*., 2016). A similar platelet recovery rate was achieved in the present study.

Storage of PRP

Platelets remained active for 5–7 days at room temperature or up to 10 days at temperatures of 0–6 ⍰ (Kim *et al*., 2020). It was opined by Jennes *et al*. (2023) that the reduction in platelet count and the chance of microbial contamination were less when autologous canine PRP was stored at -20 °C compared to 4 °C for seven days. In the present study, the PRP was stored at 4 °C till the platelet concentration fell below one million/µL (therapeutic concentration), which was considered as the end point. The end point for the six samples was 13th, 10th, 12th, 6th, 6th and 6th day respectively.

Quantitative assessment of PRP

The complete blood count parameters were obtained from whole blood samples and PRP. They were used to calculate the PEF and PRR (Table 1). The mean PEF was 4.5 and PRR was 45.3 per cent. Pavlovic *et al*. (2016) opined that the platelet concentration in PRP should be three to five times the normal physiological concentration of whole blood. Segabinazzi *et al*. (2021) suggested that the value obtained in whole blood was considered as the absolute value, and an increase or decrease in platelets or blood cells was used to attain the enrichment factor. Hence, the mean PEF of 4.5 obtained in this study was in concurrence with the definition of PRP.

Magalon *et al*. (2016) proposed the DEPA (Dose of injected platelets, Efficiency of production, Purity of the PRP, Activation of the PRP) classification to extend the characterisation of the injected PRP preparation. The platelet capture efficiency corresponded to the percentage

Table 1. Quantitative assessment of PRP

Sl. No.	Initial			Final			PEF*	PRR
	RBC	WBC	Platelet	RBC	WBC	Platelet		
	(10^6/µL)	(10^3/µL)	(10^3/µL)	(10^6/µL)	(10^3/µL)	(10^3/µL)		%
1	6.98	4.8	499	0.23	0.7	2354	4.7	47.2
2	6.62	6.2	308	0.06	1.3	935	3.0	30.4
3	6.44	4.3	594	0.17	1.5	3699	6.2	62.3
4	6.04	4.9	472	0.08	0.8	2025	4.3	42.9
5	5.78	3.8	601	3.96	6.2	2806	4.7	46.7
6	6.43	5.2	586	0.14	1.9	3058	5.2	52.2
7	6.62	3.4	656	0.04	0.8	3094	4.7	47.2
8	6.18	4.8	664	0.02	0.3	2220	3.3	33.4
Mean	**6.39**	**4.7**	**548**	**0.59**	**1.7**	**2524**	**4.5**	**45.3**

*PEF: Platelet Enrichment Factor; PRR: Platelet Recovery Rate

Table 2. Comparison of platelet count, MPV and PDW.

Parameters	Day 0	Day 6	t value	p value
Platelet	1360.17 ± 131.91	1059 ± 93.69	1.79	0.134
MPV	7.11 ± 0.85	8.00 ± 1.50	0.78	0.472
PDW	9.55 ± 0.58	12.21 ± 1.79	1.79	0.134

of platelets recovered in the PRP from the blood. According to their classification, the platelet recovery rate (%) was classified as A (>90, high), B (70-90, medium), C (30-70, low) and D (<30, poor). As per this classification, the PRR of the current study came under 'C' category, which was comparable to the other methods studied by Magalon *et al*. (2016).

Assessment of platelets during storage at 4 °C

The platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) were evaluated during the storage period. The day on which the platelet count fell below 1 x 10^6/µL, was considered as the end point. The platelet counts in three samples maintained a therapeutic concentration even after 10 days. Since three of the six samples, reached the end point on day 6, the above parameters for the six samples were compared on day 0 and day 6. Paired t-test was done to compare the results (Table 2). A decline in platelet count was noticed on 6th day. The MPV and PDW values increased on 6th day. As the p value was > 0.05, these differences were not statistically significant. Boos *et al*. (2007) and Vagdatli *et al*. (2010) used MPV and PDW as surrogate markers of platelet activation. They opined that an increase in these values corresponded to activation of platelets. Though the results of the present study indicated an increase in these values, more research is warranted in this regard, using a larger sample size to establish its statistical significance.

Bacteriological evaluation

The PRP sample after the end point of study was streaked on brain heart infusion agar and incubated at 37 °C for 72 h. No growth was obtained in this study indicating that the storage conditions were ideal for the short-term preservation of rat PRP. Jennes *et al*. (2023) got contradicting results in their study conducted on canine PRP, where they obtained bacterial contamination in samples stored at 4 °C after seven days. They also opined that the storage at -20 °C was better to maintain the quality of platelets. A controlled prospective study with a larger sample size is indicated to establish a correlation between storage temperature and quality of PRP.

Microscopical evaluation

Platelet smears were prepared daily from samples one and two, from day zero, till the end point. The endpoint for sample one was day 13 and for sample two was day 10. The smears revealed purplish-blue coloured platelets that were evenly distributed. Changes in platelet morphology were not appreciated. Even though clumping of platelets was noted on 6th day in sample two, no such clumping was noticed on the days that followed till the end point (Fig. 5). Jennes *et al*. (2023) observed changes in platelet morphology during the storage of canine PRP. They also reported clumping of platelets during storage which was similar to the present study. Boos *et al*. (2007) reported that platelet activation led to platelet shape change (increasingly spherical), swelling (increase in platelet mass and volume), degranulation with consequent adhesion and aggregation (with increasing platelet clumping). No such changes, except for the aggregation on 6th day in one sample, were appreciated in the present study. From the above findings, it could be inferred that the storage of rat PRP at 4 °C did not lead to inadvertent platelet activation.

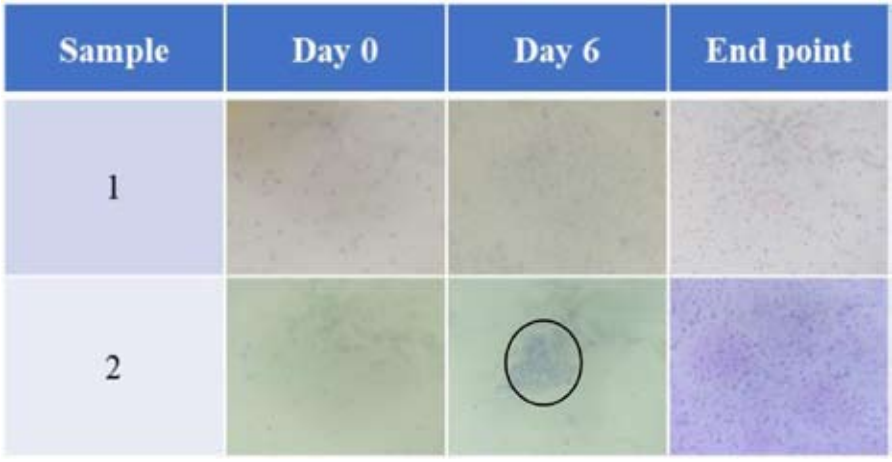


Fig. 5. Appearance of platelets at different time points (Field's stain, 100x).
Note: In sample 2, there was clumping of platelets on day 6 (marked by black circle).

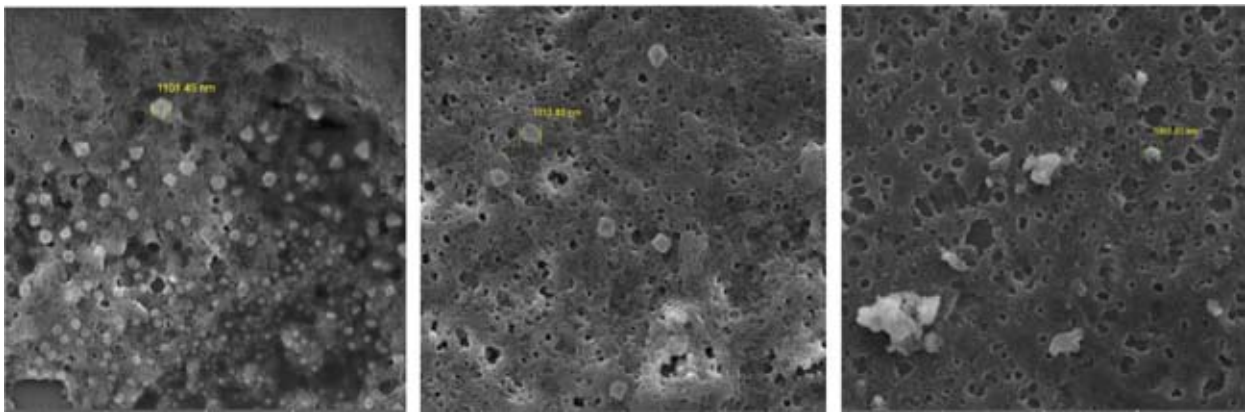


Fig. 6a. Day 0 (8.95 kx) **6b.** Day 0 (9.95 kx) **6c.** Day 6 (9.64 kx)

Note: No major change in the dimensions. Clumping of platelets noticed on day 6.

Scanning electron microscopy (SEM)

The scanning electron micrographs did not indicate any major change in the platelet dimensions between day 0 and day 6 (Fig. 6). Platelet clumps were appreciated on day 6 (Fig. 6c). Apakupakul *et al*. (2020) described the presence of pseudopods emerging from the margins of discoid platelets as an indicator for platelet activation. No such observations were made in this study.

Conclusion

The modified syringe technique is an economic and a reasonably efficient method of PRP preparation from rat blood as indicated by the platelet recovery parameters. A high degree of variability was observed in the parameters studied to assess storage of platelets at 4 °C. The non-significant changes in MPV and PDW, inconsistent appearance of platelet clumps in the smears and ultrastructural studies indicated that storage of rat PRP at 4 °C did not cause inadvertent activation of platelets. Thus, it could be concluded that rat PRP stored at 4 °C, could safely and effectively be utilised for homologous application for up to one week. This is indicated in conditions that depended on *in situ* rather than extrinsic activation of platelets. It is suggested that a controlled prospective study using a larger sample size is indicated to validate the results of this study.

Acknowledgements

The authors would like to acknowledge the Dean, College of Veterinary and Animal Sciences, Mannuthy, Professor and Head, UVH, Kokkalai and the staff of Central Instruments Laboratory for the facilities provided and help rendered.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Apakupakul, J., Sattasathuchana, P., Chanloinapha, P. and Thengchaisri, N. 2020. Optimisation of a rapid one-step platelet-rich plasma preparation method using syringe centrifugation with and without carprofen. *BMC Vet. Res.* **16**: 1-8.

Boos, C.J., Beevers, G.D. and Lip, G.Y.H. 2007. Assessment of platelet activation indices using the ADVIA™ amongst 'high-risk' patients with hypertension. *Ann. Med*. **39**: 72-78.

Dashore, S., Chouhan, K., Nanda, S. and Sharma, A. 2021. Preparation of platelet-rich plasma: National IADVL PRP taskforce recommendations. *Indian Dermatol. Online J.* 12: S12-23.

Fukaya, M. and Ito, A. 2014. A new economic method for preparing platelet-rich plasma. *Plast. Reconstr. Surg. Glob. Open.* **2**: 1-7.

Jennes, D., Ramankutty, S., Anoop, S., Madhavan Unny, N., Sudheesh, S.N. and Martin, J.K. D. 2023. Evaluation of keeping quality of canine platelet rich plasma under different storage conditions. *J. Vet. Anim. Sci.* **54**: 91-97.

Kim, J.I., Bae, H.C., Park, H.J., Lee, M.C. and Han, H.S. 2020. Effect of storage conditions and activation on growth factor concentration in platelet-rich plasma. *Inc. J. Orthop. Res*. **38**: 777–784.

Kumar, M., Dandapat, S., Sinha, M.P., Kumar, A. and Raipat, B.S. 2017. Different blood collection methods from rats: A review. *Balneo Res. J.* **8**: 46-50.

Laiju, M.P. 2022. Autologous platelet rich plasma for treatment of sole lesions and expression profile of associated biomarkers in dairy cattle. *PhD thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 248p.

Macey, M., Azam, U., McCarthy, D., Webb, L., Chapman, E. S., Okrongly, D., Zelmanovic, D. and Newland, A. 2002. Evaluation of the anticoagulants EDTA and citrate, theophylline, adenosine and dipyridamole (CTAD) for assessing platelet activation on the ADVIA 120 haematology system. *Clin. Chem*. **48**: 891-899.

Magalon, J., Chateau, A.L., Bertrand, B., Louis, M.L., Silvestre, A., Giraudo, L., Veran, J. and Sabatier, F. 2016. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. *BMJ Open Sport Exerc. Med.* **2**: 1-5.

Messora, M.R., Nagata, M.J.H., Furlaneto, F.A.C., Dornelles, R.C.M., Bomfim, S.R.M., Deliberador, T.M., Garcia, V.G. and Bosco, A.F. 2011. A standardised research protocol for platelet rich plasma (PRP) preparation in rats. *Revista Sul-Brasileira de Odontologia (RSBO).* **8**: 299-304.

Parasuraman, S., Raveendran, R. and Kesavan, R. 2010. Blood sample collection in small laboratory animals. *J. Pharmacol. Pharmacother*. **1**: 87-93.

Pavlovic, V., Ciric, M., Jovanovic, V. and Stojanovic, P. 2016. Platelet Rich Plasma: a short overview of certain bioactive components. *Open Med.* **11**: 242-247.

Segabinazzi, L.G.T.M., Podico, G., Rosser, M.F., Nanjappa, S.G., Alvarenga, M.A. and Canisso, I.F. 2021. Three manual noncommercial methods to prepare equine platelet-rich plasma. *Animals*. **11**: 1-16.

Sonnleitner, D., Huemer, P. and Sullivan, D.Y. 2000. A simplified technique for producing platelet-rich plasma and platelet concentrate for intraoral bone grafting techniques: A technical note. *Int. J. Oral Maxillofac. Implants*. **15**: 879–882.

Vagdatli, E., Gounari, E., Lazaridou, E., Katsibourlia, E., Tsikopoulou, F. and Labrianou, I. 2010. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia*. **14**: 28-32. ■