



Preparation and validation of platelet rich plasma from goat blood using a double centrifugation protocol

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Abstract

The development of species-specific protocols for platelet-rich plasma (PRP) preparation is critical for ensuring quality and reproducibility in veterinary regenerative medicine. This study aimed to standardise the preparation of homologous PRP from goat blood using a double centrifugation method and to validate its efficacy based on platelet enrichment and recovery. Venous blood was collected from bucks and baseline haematological parameters were assessed. A two-step centrifugation protocol was employed: a soft spin at 300×g for 5 min. followed by a hard spin at 700×g for 17 min. The resulting PRP was analysed for erythrocyte, leukocyte and platelet concentrations, and quantitative indices including platelet enrichment factor (PEF) and platelet recovery rate (PRR) were calculated. Compared with whole blood, PRP exhibited a marked higher concentration of platelet ($1465.50 \pm 124.42 \times 10^3/\mu\text{L}$ vs. $413.50 \pm 24.95 \times 10^3/\mu\text{L}$), with an average PEF of 3.74 ± 0.21 and PRR of 78.49 ± 3.94 per cent. Red and white blood cells were substantially reduced in PRP, confirming successful separation. The protocol provided effective platelet concentration while minimising contamination, although separation challenges due to the small size of goat platelets and erythrocytes were noted. These findings support the feasibility of standardised homologous PRP preparation in goats and highlight the need for further refinement to improve purity.

Keywords: Platelet-rich plasma, goat, double centrifugation, platelet enrichment

Platelet-rich plasma (PRP) is an autologous blood-derived concentrate containing platelets at levels above baseline. Owing to their rich reservoir of growth factors, cytokines and antioxidants, platelets play a pivotal role in tissue regeneration, angiogenesis and protection from oxidative damages (Bharti et al., 2024). PRP has therefore gained widespread use in both human and veterinary medicine for applications in orthopaedics, dermatology and assisted reproduction. In livestock species, homologous PRP is of particular interest because it offers a safe, cost-effective and biologically compatible therapeutic option for enhancing regenerative and reproductive outcomes.

The quality and efficacy of PRP depend on the protocol employed for its preparation. Various methods such as single-spin (Hernández-Corredor et al., 2020), double-spin (Salama et al., 2024), filtration-based (Jakfar et al., 2024)

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and commercial kits (Okumo et al., 2023) have been described, with double centrifugation generally reported to provide superior platelet yield and concentration. Double-spin method allows more efficient separation of platelets from other blood components by first removing red and white blood cells at a lower centrifugal force, followed by concentrating platelets in a second high-speed spin (Dhurat & Suresh, 2014). This two-step process minimises red cell contamination and ensures a higher platelet concentration compared to single-spin or commercial kit-based methods (Harrison et al., 2020), which are often optimised for human blood and may not account for the smaller cell size and density differences in goats. However, the optimal protocol must be tailored to species-specific haematological characteristics. In goats, PRP preparation presents inherent challenges due to the unique morphology of their blood cells. Goat erythrocytes are the smallest among domestic animals, their close similarity in size and density complicates the separation process during centrifugation, often resulting in partial red cell contamination of the platelet fraction. Conversely, leukocytes, being considerably larger, can be more effectively removed. These biological constraints highlight the importance of refining PRP preparation protocols in goats to achieve high platelet yield with minimal contamination.

Despite the growing application of PRP in veterinary medicine, only limited studies have attempted to standardise protocols for small ruminants. Previous reports suggest that adjusting centrifugation speeds and durations can enhance platelet concentration and platelet volume in goat and sheep. Nevertheless, systematic validation of PRP preparation in goats remains scarce. The present study was therefore undertaken to standardise the preparation of homologous PRP from goat blood using a double centrifugation method, and to validate the protocol through quantitative assessment of platelet indices in comparison to whole blood.

Materials and methods

Selection of donor animals

Three adult, healthy Malabari bucks aged two to three years, with body weight of 42 to 46 kg, maintained under uniform feeding, housing and managemental conditions at Artificial Insemination centre, Mannuthy were selected as blood donors for the preparation of PRP. The blood samples from donors were analysed in an automated haematology analyser (Mythic 5Vet PRO, ORPHEE SA, Geneva, Switzerland) before the preparation of homologous PRP to assess the baseline values of platelets count ($247-912 \times 10^3 \mu\text{L}$), erythrocyte count ($8-18 \times 10^6 \mu\text{L}$) and total leukocytes ($4-13 \times 10^3 \mu\text{L}$) in each animal. The analyser uses electrical impedance and optical flow cytometry principles for counting. Red blood cells, white blood cells, and platelets were counted by the

Coulter method, while the five-part WBC differential was determined by laser light scattering.

Collection of blood

Ten mL venous blood was aseptically collected from the jugular vein in 10 mL sterile syringes containing one mL of 3.8 per cent sodium citrate solution. Syringes were capped and immediately transported to the laboratory for the preparation of homologous PRP.

Platelet-rich plasma production protocol

Double centrifugation (Salama et al., 2024) with the syringe method (Philip, 2022) was selected and used for the production of homologous PRP. The double centrifugation procedure was carried out in pre-cooled high speed, fixed angle refrigerated centrifuge (Remi Elektrotechnik Ltd., Vasai, India) at 20°C, balanced by volume and the syringes were kept inside the centrifuge with the capped side up. The first centrifugation (soft spin) with 300xg for five minutes caused the separation of the blood into three components: red blood cells at the lowest level, buffy coat in the middle layer, and platelet suspended plasma in the upper layer.

These syringes were removed from the centrifuge with capped side up and syringes were attached to the 3-way cock (VEIN-O-LINE, Romsons, India), the platelet-suspended plasma above the buffy coat was cautiously aspirated and, depending on the aspirated volume, transferred to a 15 mL sterile centrifuge tube (SPINWIN centrifuge Tube, Tarsons, Kolkota).

The platelet suspended plasma in centrifuge tubes were again subjected to high-speed centrifugation (hard spin) at 700xg for 17 min. to settle the platelet rich pellet at the bottom and two-thirds of the platelet poor plasma (PPP) on the upper layer was discarded. The platelet rich pellet was mixed by agitation to form PRP. The PRP from these tubes were transferred into a centrifuge tube and were subjected to quality analysis. The above mentioned optimised centrifugation parameters were selected based on the results of preliminary trials.

Quantitative assessment of platelet-rich plasma

Platelet count in the prepared PRP was assessed using the automated haematology analyser. The platelet counts were verified manually to cross-check automated analyser accuracy. Blood collection, PRP preparation and qualitative assessment were performed six times using blood samples collected from these bucks. A quantitative assessment of PRP was performed by comparing platelet counts in whole blood and PRP. Dashore et al. (2021) have provided the basic formulas concerning PRP such as platelet enrichment factor and platelet recovery rate. The platelet enrichment factor was evaluated by comparing the platelet count in PRP to the platelet count in whole blood.

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

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

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

Scanning electron microscopy of PRP

The PRP were subjected to scanning electron microscopy (SEM) on the day of preparation to assess platelet morphology. The PRP (0.5 mL) were fixed in 2.5 per cent glutaraldehyde (Merck, Germany) at 4°C for 30 min. followed by centrifugation at 160×g for 5 min. The supernatant was aspirated and removed to mix the pellet with 1 mL phosphate buffer saline (PBS) (HiMedia Laboratories Ltd., Mumbai) after which another centrifugation at 160×g for 5 min. was done. The supernatant was removed and the pellet was gently washed five times with sufficient amount of PBS. After removal of PBS, a thin smear of the sample was made on a clean glass cover slip. Air dried sample was subjected to sputter coating (SC7620, Sputter Coater, Quorum Technologies, UK) for 60 sec. at 10 mA current. Then final imaging of the platelets was done at a magnification ranging from 3000 – 4000× and 15000 – 17000× using SEM (TESCAN VEGA-3-LMU, Czech Republic).

Results and discussion

Platelet-rich plasma production protocol

In the present study homologous platelet-rich plasma was successfully prepared using double centrifugation (Salama et al., 2024) with the syringe method (Philip, 2022). The procedure involved a soft spin at 300×g for five min., followed by followed by a hard spin at 700×g for 17 min. to concentrate the platelets. The platelet-rich pellet obtained was re-suspended in one-third of platelet-poor plasma to obtain the final PRP. The

process, conducted at 20°C in a refrigerated centrifuge, ensured effective platelet concentration with minimal contamination, yielding a sample suitable for quality evaluation.

Quantitative assessment of platelet-rich plasma

Haematological analysis revealed a marked reduction in red and white blood cell concentrations in PRP compared to whole blood (Table 1). The average RBC concentration decreased from $12.25 \pm 1.30 \times 10^6/\mu\text{L}$ in whole blood to $0.26 \pm 0.09 \times 10^6/\mu\text{L}$ in PRP, while the WBC concentration dropped from $7.45 \pm 1.30 \times 10^3/\mu\text{L}$ to $2.10 \pm 0.67 \times 10^3/\mu\text{L}$. This reduction demonstrates effective removal of erythrocytes and leukocytes during centrifugation. In goats, white blood cells are considerably larger (10–15 μm) than platelets, facilitating their separation easy (Menaka & Singh, 2006). However, erythrocytes (2.5–3.9 μm) and platelets (~2.2 μm) are almost similar in size and density (Byers et al., 2010; Stayt, 2022), making it more difficult to prevent red cell contamination. The low RBC values obtained in the present study confirm that the adopted protocol minimised this limitation.

Platelet concentration increased significantly, from $413.50 \pm 24.95 \times 10^3/\mu\text{L}$ in whole blood to $1465.50 \pm 124.42 \times 10^3/\mu\text{L}$ in PRP, representing nearly a fourfold rise. The calculated platelet enrichment factor (PEF) was 3.74 ± 0.21 , and the platelet recovery rate (PRR) was 78.49 ± 3.94 per cent (Table 2). In a study on preparation of PRP from rat blood, a PEF of 4.5 and PRR of 45.30 per cent was reported by Reji et al. (2025). In this study, these indices validate the efficiency of the method and fall within the range reported in other domestic animals, where PRP typically contained three to seven times more platelets than whole blood (Samadi et al., 2019).

Studies have shown that using lower centrifugation speeds for a longer duration can enhance platelet concentration and increase the average platelet volume in PRP derived from goats and sheep (Clemmons et al., 1983). The concentration of thrombocytes in platelet-rich plasma (PRP) is three to seven times higher than that of normal whole blood (Samadi et al., 2019). In this study, double centrifugation was performed by first

Table 1. Analysis of whole blood of goat and homologous PRP for its cellular components (Mean ± SE) (n=6)

Blood cells (Unit)	Initial concentration in whole blood	Final concentration in PRP
RBC ($10^6/\mu\text{L}$)	12.25 ± 1.30	0.26 ± 0.09
WBC ($10^3/\mu\text{L}$)	7.45 ± 1.30	2.10 ± 0.67
Platelets ($10^3/\mu\text{L}$)	413.50 ± 24.95	1465.50 ± 124.42

Table 2. Evaluation of prepared goat PRP (Mean ± SE) (n=6)

Platelet concentration in		PEF	PRR
Whole blood ($10^3/\mu\text{L}$)	PRP ($10^3/\mu\text{L}$)		
413.50 ± 24.95	1465.50 ± 124.42	3.74 ± 0.21	78.49 ± 3.94

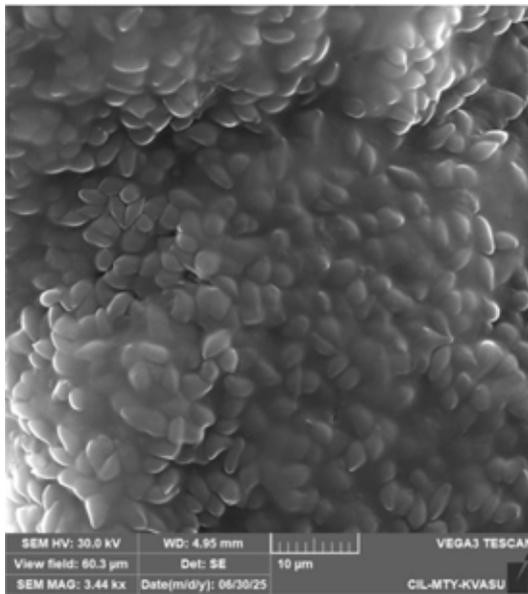


Fig. 1. SEM image of PRP (×3440)

applying a lower gravitational force, followed by a higher one, as recommended in earlier researches (Foster et al., 2009; Hoareau et al., 2014). These findings support the approach adopted in the present work. Sodium citrate solution (3.8%) was chosen as the anticoagulant for PRP preparation, based on its beneficial effects in preserving platelet integrity, as highlighted by do Amaral et al. (2016) in their comparative study evaluating anticoagulant efficiency.

Scanning electron microscopy of PRP

Scanning electron microscopic (SEM) evaluation of platelets in PRP was carried out. Platelets exhibited a smooth surface and were predominantly round to ovoid in shape, with an average diameter of 2.57 µm, indicating their quiescent nature (Fig. 1 and 2).

Goat erythrocytes are comparatively smaller among domestic animals, ranging from 2.5–3.9 µm in diameter, while platelets average only 2.2 µm (Stayt, 2022). Their close similarity in size and density complicates the separation process during centrifugation, often resulting in partial red cell contamination of the platelet fraction. In this study, the preparation was carried out at a temperature of at 20°C in a pre-cooled fixed angle refrigeration centrifuge to prevent platelet activation during the procedure. SEM images revealed round to ovoid platelets without any pseudopodia which ensure that platelets are not activated during the process of PRP preparation.

Conclusion

The present study successfully standardised a double centrifugation protocol for the preparation of homologous platelet-rich plasma (PRP) from goat blood.

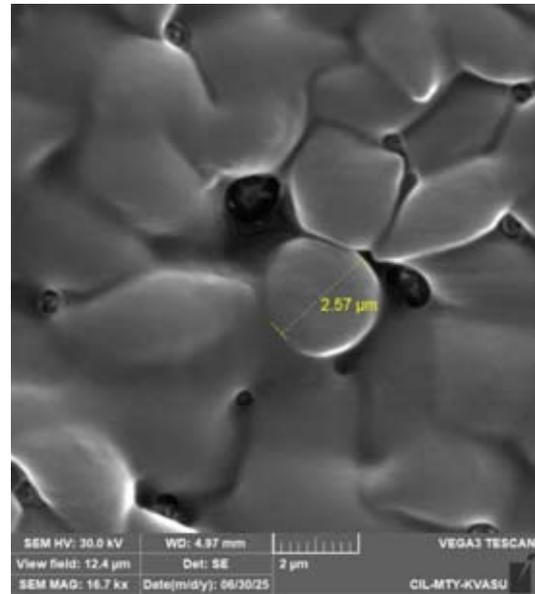


Fig. 2. SEM image of platelets in PRP, which are round to ovoid in shape (×16700)

The method yielded a nearly fourfold increase in platelet concentration compared to whole blood, with a recovery rate close to 80 per cent, while effectively reducing erythrocyte and leukocyte contamination. These findings confirm the suitability of the protocol for obtaining PRP of acceptable quality in goats. Nevertheless, the inherent similarity in size and density between goat erythrocytes and platelets remains a limiting factor that can compromise purity. Further refinement of centrifugation parameters or the development of species-specific separation strategies may help to overcome this challenge. Standardised goat-derived PRP has promising potential for application in veterinary medicine and reproductive biotechnologies because of its known role in tissue regeneration, angiogenesis and protection from oxidative damages, which warrants future research into its functional efficacy.

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Conflict of Interest

The authors declare no conflicts of interest.

References

- Bharti, D., Ajith, Y., Sharun, K., Banu, S. A., Kumar, A., Bhardwaj, A., Sidar, S. K., & Dhaleshwari. (2024). Therapeutic applications of canine platelets and their derivatives: A narrative review. *Topics in Companion Animal Medicine*, 58, 100840.

- Byers, S. R., Kramer, J. W., Weiss, D. J., & Wardrop, K. J. (2010). Normal hematology of sheep and goats. In D. J. Weiss & K. J. Wardrop (Eds.), *Schalm's veterinary hematology* (6th ed., pp. 836–842). Wiley-Blackwell.
- Clemmons, R. M., Bliss, E. L., Dorsey-Lee, M. R., Seachord, C. L., & Meyers, K. M. (1983). Platelet function, size and yield in whole blood and in platelet-rich plasma prepared using differing centrifugation force and time in domestic and food-producing animals. *Thrombosis and Haemostasis*, *50*, 838–843.
- Dashore, S., Chouhan, K., Nanda, S., & Sharma, A. (2021). Preparation of platelet-rich plasma: National IADVL PRP taskforce recommendations. *Indian Dermatology Online Journal*, *12*(Suppl. 1), S12–S23.
- Dhurat, R., & Sukesh, M. (2014). Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. *Journal of Cutaneous and Aesthetic Surgery*, *7*, 189–197.
- do Amaral, R. J. F. C., da Silva, N. P., Haddad, N. F., Lopes, L. S., Ferreira, F. D., Filho, R. B., Cappelletti, P. A., de Mello, W., Cordeiro-Spinetti, E., & Balduino, A. (2016). Platelet-rich plasma obtained with different anticoagulants and their effect on platelet numbers and mesenchymal stromal cell behavior in vitro. *Stem Cells International*, *2016*, 7414036.
- Foster, T. E., Puskas, B. L., Mandelbaum, B. R., Gerhardt, M. B., & Rodeo, S. A. (2009). Platelet-rich plasma: From basic science to clinical applications. *The American Journal of Sports Medicine*, *37*, 2259–2272.
- Harrison, T. E., Bowler, J., Levins, T. N., Cheng, A. L., & Reeves, K. D. (2020). Platelet yield and yield consistency for six single-spin methods of platelet-rich plasma preparation. *Platelets*, *31*, 661–666.
- Hernandez-Corredor, L., Leon-Restrepo, S., Bustamante-Cano, J., Baez-Sandoval, G., & Jaramillo, X. (2020). Effect of the incorporation of platelet-rich plasma on the spermatozoa physiology of ram semen. *Journal of Dairy, Veterinary & Animal Research*, *9*, 34–38.
- Hoareau, G. L., Jandrey, K. E., Burges, J., Bremer, D., & Tablin, F. (2014). Comparison of the platelet-rich plasma and buffy coat protocols for preparation of canine platelet concentrates. *Veterinary Clinical Pathology*, *43*, 513–518.
- Jakfar, S., Ningsih, D. S., Lin, T. C., Chen, Z. Y., Lin, F. H., Gani, B. A., Syafriza, D., & Kusuma, H. (2024). Evaluation of a membrane filter and platelet-rich plasma product obtained from a prototype PRP kit based on a new cell-dimension separation method. *AIP Advances*, *14*.
- Menaka, R., & Singh, I. S. (2006). Cytomorphological studies on the blood cells of goat. *Indian Veterinary Journal*, *83*(1): 68–71. <http://www.indvetjournal.com>
- Okumo, T., Sato, A., Izukashi, K., Ohta, M., Oike, J., Yagura, S., Okuma, N., Koya, T., Sunagawa, M., & Kanzaki, K. (2023). Multifactorial comparative analysis of platelet-rich plasma and serum prepared using a commercially available centrifugation kit. *Cureus*, *15*, e48918.
- Philip, L. M. (2022). *Autologous platelet-rich plasma for treatment of sole lesions and expression profile of associated biomarkers in dairy cattle* (Ph.D. thesis, Kerala Veterinary and Animal Sciences University, Pookode).
- Reji, V., Anoop, S., Syam, K. V., Sooryadas, S., Vasudevan, V. N., Varuna, P. P., Sudheesh, S. N., Deny, J., & Martin, J. K. D. (2025). Modified syringe technique to prepare platelet-rich plasma from rat blood: Quantitative assessment and evaluation of platelets stored at 4 °C for homologous application. *Journal of Veterinary and Animal Sciences*, *56*(1), 13–19.
- Salama, M. S., Shehabeldin, A. M., Ashour, M. A., Al-Ghadi, M. Q., Marghani, B. H., El-Kon, I., & Shukry, M. (2024). Effect of the addition of platelet-rich plasma to Boer buck semen on sperm quality and antioxidant activity before and after cryopreservation and in vivo fertility. *Small Ruminant Research*, *230*, 107167.
- Samadi, P., Sheykhhasan, M., & Khoshinani, H. M. (2019). The use of platelet-rich plasma in aesthetic and regenerative medicine: A comprehensive review. *Aesthetic Plastic Surgery*, *43*, 803–814.
- Stayt, J. (2022). Hematology of sheep and goats. In D. J. Weiss & K. J. Wardrop (Eds.), *Schalm's veterinary hematology* (6th ed., pp. 1012–1018). Wiley-Blackwell. ■