



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(6): 312-314

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www.veterinarypaper.com

Received: 14-04-2026

Accepted: 18-05-2026

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Optimization of a double centrifugation protocol for maximum recovery of caprine platelets

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i6e.3596>

Abstract

Platelet-rich plasma (PRP) therapy is an advancing frontier in caprine regenerative medicine and surgical applications. However, the lack of a standardized protocol for caprine blood often leads to inconsistent platelet yields and unpredictable clinical outcomes. This study was performed to optimize a double centrifugation protocol to achieve the maximum recovery rate of viable caprine platelets. Whole blood samples from healthy caprine donors were subjected to various combinations of centrifugation speeds and durations. Our optimized protocol utilized a first spin (soft spin) at $250 \times g$ for 10 minutes to separate plasma from red blood cells, followed by a second spin (hard spin) at $1000 \times g$ for 15 minutes to concentrate the platelets. This method yielded an average platelet recovery rate of 82.5% with a 4.6-fold increase in platelet concentration compared to baseline levels, while successfully preserving structural integrity and activation capability.

Keywords: Platelet rich plasma, double centrifugation, caprine platelets, veterinary transfusion medicine

1. Introduction

Platelet-rich plasma (PRP) is an autologous concentration of platelets in a small volume of plasma (Marx, 2001) [6]. It is widely utilized in veterinary medicine to accelerate tissue healing, treat musculoskeletal injuries, and manage chronic wounds (Boone and Peroni, 2023) [11]. Platelets contain alpha-granules rich in essential growth factors, including Transforming Growth Factor-beta (TGF- β), Platelet-Derived Growth Factor (PDGF), and Vascular Endothelial Growth Factor (VEGF) (Qiao and Ouyang, 2017) [8]. PRP holds significant therapeutic potential for small ruminant orthopedic repairs and soft tissue reconstructions. Despite its utility, standardizing caprine PRP preparation remains a challenge. Caprine erythrocytes are significantly smaller (mean corpuscular volume: 15–25 fL) and possess a much lower density gradient mismatch with platelets compared to canine, equine, or human blood (Daramola *et al.*, 2005) [2]. Consequently, directly applying human or equine PRP protocols to caprine blood frequently results in poor platelet recovery or excessive leukocyte contamination (Argüelles *et al.*, 2006; Fernandez and Kieves, 2025) [1, 4]. Single centrifugation methods often fail to achieve therapeutic concentration thresholds (Dhurat & Sukesh, 2014) [3]. Conversely, double centrifugation protocols allow for a distinct separation of blood components but require precise optimization of relative centrifugal force (RCF) and time (Kim and Han, 2022) [9]. Excessive centrifugal forces can cause premature platelet activation, fragmentation, or trapping within the red blood cell pack (Fernandez and Kieves, 2025) [4]. This study establishes a validated, highly reproducible double centrifugation protocol tailored specifically to the unique hematological profile of goats to ensure maximum platelet recovery.

2. Materials and Methods

2.1 Animal Selection and Blood Collection

Ten healthy, adult goats of either sex (Kanni and Kodi indigenous breeds native to southern districts of Tamil Nadu), aged 2–4 years, weighing 15 to 30 kg were selected from the District Livestock Farm, Tirunelveli and Livestock Farm Complex, VCRI, Tirunelveli. All animals were physically examined and complete blood counts (CBC) was performed using Hematology

Analyzer (Exigo Boule H400, Sweden) to rule out systemic diseases, thrombocytopenia, or anemia. Jugular venipuncture was performed using vacutainer tubes with 19-gauge needle to minimize shear stress and prevent premature platelet activation. Blood was collected directly into sterile vacutainer tubes containing Acid Citrate Dextrose Solution A (ACD-A) at a fixed 1:9 ratio (anticoagulant to blood).

2.2 Experimental Design and Centrifugation Matrices

A matrix of spin speeds and times was tested using a temperature-controlled centrifuge (Remi CM 12 Plus, Mumbai, India) equipped with a swing-bucket rotor. To ensure absolute consistency, all centrifugation cycles were executed at a fixed temperature of 22 °C with deceleration brakes set to a low profile to protect interfaces from disruption.

First Spin (Soft Spin Evaluation)

Whole blood tubes were centrifuged at either 150 × g, 250 × g, or 350 × g for 10 or 15 minutes. The supernatant plasma

layer, including the entire buffy coat interface, was carefully aspirated using micropipette.

Second Spin (Hard Spin Evaluation)

The harvested plasma was transferred into sterile 15 mL conical centrifuge tubes and subjected to a second spin at 800 × g, 1000 × g, or 1200 × g for 10 or 15 minutes. The upper platelet-poor plasma (PPP) fraction was removed. The bottom 1.5 mL of plasma was utilized to resuspend the remaining platelet pellet, yielding the final PRP product.

2.3 Hematological and Quality Analysis

Platelet Quantification

Automated platelet and white blood cell (WBC) counts were performed on whole blood, plasma, and final PRP fractions. Counts were verified manually via hemocytometer quantification using a Neubauer chamber.

Formula

$$\text{platelet recovery rate} = \frac{\text{PRP Platelet Concentration} \times \text{PRP Volume}}{\text{whole blood platelet concentration} \times \text{Whole blood volume}} \times 100$$

Morphological Evaluation

Light microscopy of Wright-Giemsa stained smears evaluated platelet aggregation and structural morphology under a research microscope (Nikon, Japan) (Fernandez and Kieves, 2025) ^[4].

Viability Assessment

Hypotonic shock response (HSR) testing evaluated the functional integrity of the platelet membranes using a double beam UV/Visible Spectrophotometer (UH 500 Hitachi, Japan) (Lindahl and Ramstrom, 2009) ^[10].

2.4 Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical evaluations were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) and SPSS version 26.0 (IBM Corp., USA). Variations across different relative centrifugal forces (RCF) and time protocols were assessed using a two-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests for multiple comparisons. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Optimization of the First Spin (Soft Spin)

The first centrifugation stage required balancing maximum plasma separation with minimum platelet entrapment in the erythrocyte layer.

First Spin Force (RCF)	Duration (min)	Platelet Yield in Plasma (%)	RBC Contamination (%)
150 × g	10	61.2 ± 4.3 ^a	0.2 ± 0.1 ^x
150 × g	15	65.8 ± 3.9 ^a	0.4 ± 0.1 ^x
250 × g	10	89.4 ± 3.1 ^a	1.1 ± 0.3 ^x
250 × g	15	81.2 ± 3.5 ^a	2.8 ± 0.5 ^y
350 × g	10	72.3 ± 5.1 ^a	5.4 ± 1.2 ^y
350 × g	15	55.1 ± 6.2 ^d	9.6 ± 2.1 ^z

Note: Different superscripts (a–d for yield; x–z for contamination) within the same column indicate statistically significant differences ($p < 0.05$).

Centrifugation at 250 × g for 10 minutes provided the ideal balance, preserving 89.4% of platelets in the supernatant

while keeping red blood cell contamination minimal. At 350 × g, significant numbers of caprine platelets were packed down into the red blood cell layer due to their small size and unique sedimentation characteristics, resulting in a statistically lower yield ($p < 0.01$).

3.2 Optimization of the Second Spin (Hard Spin)

The second spin aimed to pellet the suspended platelets completely without triggering premature mechanical degranulation.

Second Spin Force (RCF)	Duration (min)	Concentration Factor	Platelet Recovery Rate (%)
800 × g	10	2.8 x	64.1 ± 5.2 ^a
800 × g	15	3.4 x	71.3 ± 4.8 ^a
1000 × g	10	4.1 x	79.8 ± 3.6 ^b
1000 × g	15	4.6 x	82.5 ± 2.9 ^b
1200 × g	10	4.5 x	81.0 ± 3.1 ^b
1200 × g	15	4.3 x	76.4 ± 5.7 ^b (Pellet clumped)

Note: Different superscripts (a–b) within the recovery column indicate statistically significant differences ($p < 0.05$).

A second spin at 1000 × g for 15 minutes achieved the maximum platelet recovery rate (82.5 ± 2.9%) and concentrated platelets 4.6-fold over baseline. Increasing the force to 1200 × g for 15 minutes caused irreversible platelet clumping and signs of premature activation, significantly reducing the clinical utility of the final suspension. Two-way ANOVA revealed a dominant main effect for RCF ($p < 0.001$) over duration ($p = 0.12$).

3.3 Functional and Morphological Integrity

The optimized protocol (250 × g for 10 min followed by 1000 × g for 15 min) successfully preserved cellular health. HSR Viability: The hypoosmotic shock response score was 78.4 ± 4.2%, showing excellent membrane structural competence (Lindahl and Ramstrom, 2009) ^[10]. Morphology: Microscopic views showed unactivated, discoid platelets with negligible spontaneous aggregation. Leukocyte Profile: The protocol yielded a moderately low leukocyte concentration (1.2 × baseline), classifying the product as a pure/leukocyte-poor PRP variant (Wu *et al.*, 2025) ^[13].

4. Discussion

Adapting standard PRP protocols to caprine species requires adjusting for the unique physical properties of goat blood cells. Goats display microcytosis as a normal physiological trait. Because caprine red blood cells are notably small, their density and sedimentation velocity during centrifugation are much closer to platelets than what is observed in larger domestic mammals (Mukherjee *et al.*, 2023) ^[5].

Our findings indicate that mild gravitational forces ($250 \times g$) are necessary during the initial spin to prevent caprine platelets from settling down into the erythrocyte pack. Strikingly, higher initial forces ($350 \times g$) led to lower platelet recovery because the small caprine erythrocytes trapped the platelets during sedimentation (Fernandez and Kieves, 2025) ^[4]. This differs considerably from equine protocols, where higher forces are easily tolerated due to faster erythrocyte sedimentation rates (Argüelles *et al.*, 2006) ^[1]. The use of high-precision swing-bucket rotors (REMI CM 12 PLUS) was critical, as fixed-angle rotors created significant friction forces along the tube walls, reducing yield consistency.

For the concentration step, a force of $1000 \times g$ for 15 minutes was required to compress the platelets into a workable pellet. Lower speeds left too many platelets suspended in the platelet-poor plasma supernatant, while higher forces ($1200 \times g$) created high shear stresses that compromised membrane integrity and induced morphological deformation (Fernandez and Kieves, 2025) ^[4].

The optimized protocol delivers a reliable 4.6-fold concentration increase. This profile matches recognized therapeutic ranges for accelerating bone and soft tissue regeneration without inducing premature growth factor release (Perazzi *et al.*, 2013; Kim and Han, 2022) ^[7, 9].

5. Conclusion

Maximum recovery of functional caprine platelets is achieved using a double centrifugation protocol consisting of a first spin at $250 \times g$ for 10 minutes, followed by a second spin at $1000 \times g$ for 15 minutes at room temperature. This specialized method ensures high platelet yields, minimal red blood cell contamination, and intact functional viability. By accounting for the microcytic properties of caprine blood, this protocol provides a standardized and highly reproducible baseline for caprine regenerative therapies and clinical wound care treatments.

6. Acknowledgements

The authors thank the Dean, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu Veterinary and Animal Sciences University and the Director of Clinics for providing the necessary facilities to carry out the investigation. The authors also acknowledge the Deputy Director and other technical staff of the District Livestock Farm, Abishekapatti, and the Head of Department of Livestock Farm Complex, VCRI, Tirunelveli, for sample collection. Funds for the study were available in the form of postgraduate grants to the department.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Sabarinathan R, Prasanth TL. Optimization of a double centrifugation protocol for maximum recovery of caprine platelets. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(6):312-314.

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