

Biochemical Characterization of Bovine Platelet-Rich Plasma: a Review

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Abstract

Platelet rich-plasma (PRP) is a valuable biological fraction obtained from animal blood, with significant potential in veterinary medicine due to its complex composition, rich in proteins, growth factors, immunoglobulins and bioactive molecules. This review analyzes the chemical composition of PRP and modern characterization methods and techniques such as spectrophotometry, electrophoresis, high-performance liquid chromatography (HPLC) and mass spectrometry. Emerging therapeutic applications of PRP are also presented, especially in the context of bovine gynecological health, where it is used for the treatment of metritis, endometritis and stimulation of postpartum uterine regeneration. Through its immunomodulatory and healing actions, PRP offers a promising alternative to classical therapies, contributing to reducing the use of antibiotics and improving fertility in dairy farms.

Keywords: analytical characterization, biochemical composition, bovine, platelet rich-plasma

1. Introduction

Platelet-rich plasma (PRP) represents a concentrated fraction of blood plasma enriched with platelets, proteins, growth factors, and other bioactive substances. While initially developed for human health, PRP has gained popularity in veterinary science due to its regenerative, immunomodulatory, and healing properties [1]. It is a valuable therapeutic tool in veterinary medicine because of its complex biochemistry, which includes structural proteins such as fibrin and fibronectin, growth factors including vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF- β), and platelet-derived growth factor (PDGF), as well as cytokines and immunoglobulins [2].

In dairy cattle, postpartum uterine infections such as metritis and endometritis represent major challenges, as they significantly reduce fertility and productivity [3]. The use of PRP in these cases offers a promising alternative to conventional therapies, reducing antibiotic use and improving reproductive outcomes.

Notably, the therapeutic efficacy of PRP is closely linked to its biochemical composition. Platelet-derived growth factors and adhesive proteins stimulate angiogenesis, cell proliferation, and extracellular matrix remodeling. These biological processes account for most of the regenerative effects observed in reproductive medicine and other veterinary applications [4, 5].

Consequently, identifying the specific molecular components of bovine PRP and their relative concentrations is critical for connecting laboratory findings to therapeutic outcomes. Moreover, variability in PRP formulations resulting from preparation methods highlights the necessity of standardized protocols and rigorous biochemical characterization [6].

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Thus, analyzing PRP's chemical composition and applying modern characterization techniques, together with exploring its therapeutic applications in bovine veterinary medicine, are essential steps for validating and implementing this innovative therapy in clinical practice.

This review aims to synthesize current knowledge on the biochemical composition of bovine PRP, summarize the methods employed for its analysis, and highlight opportunities for future research that will support the reproducible and effective use of PRP in veterinary medicine.

2. Results and discussion

Preparation PRP

PRP is the fraction of autologous blood plasma with a platelet level higher than the baseline [7], enriched in platelet-rich growth factors (GFs), chemokines, cytokines, and clotting factors, which usually remain at their normal physiological levels [8]. Despite its substantial use for therapy in various fields, no standardized procedure has yet been set up for the preparation of PRP.

A multitude of protocols is available, implying single or double centrifugation, and all of them have shown an increase in the platelet concentration, allowing a therapeutic effect [9]. However, significant differences in components are observed in different separation methods and may have specific results on treated tissue [10]. Each preparation method is intended to create an end product with a particular bioaction, and consequently, with a specific clinical application and not just one single final blood derivative containing plasma and high concentrations of platelets [11]. Elevated concentrations of platelet-released GFs might have inhibitory effects on healing [12]. Briefly, PRP is a prepared product from blood collected on an anticoagulant via a single or double stage centrifugation protocol. First, the collected blood is submitted to centrifugation to separate the red blood cells (lower layer, approx. 45%) from the platelet-rich plasma and platelet-poor plasma (upper layer, approx. 55%). Then the upper separated fraction can be used as it is, or submitted to a second, centrifugation to obtain a platelet concentrated plasma or pure PRP, which is represented by the lower layer (Figure 1).

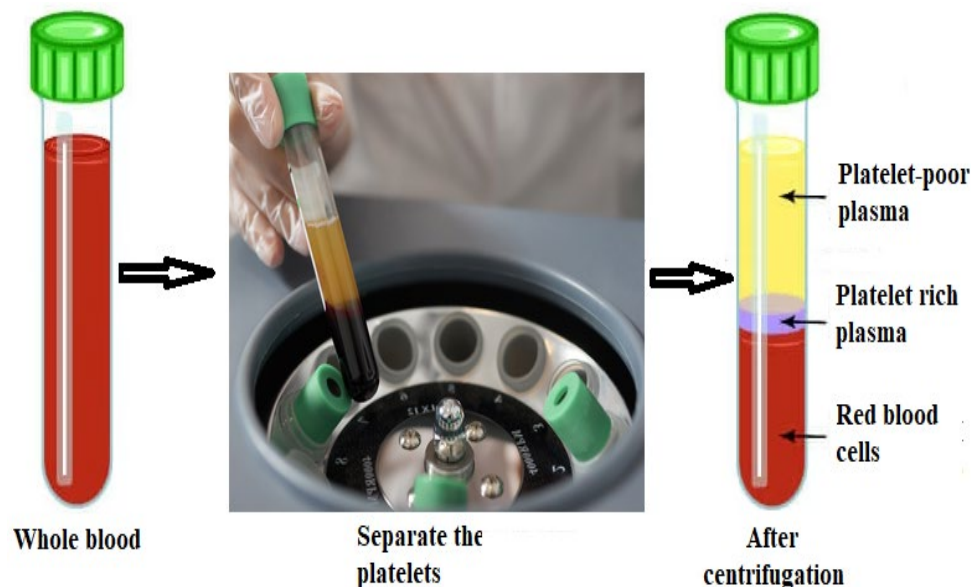


Figure 1. Obtaining Platelet-Rich Plasma from blood via centrifugation

Biochemical characterization of bovine platelet-rich plasma

Biochemical characterization includes determining the concentration and profile of proteins and peptides (growth factors and cytokines), assessing

platelet activation status, identifying residual plasma components, and, ideally, establishing a proteomic fingerprint of PRP.

Commonly applied techniques include spectrophotometry for total protein quantification,

electrophoresis (SDS-PAGE, 2D gels) for protein separation, high-performance liquid chromatography (HPLC) for quantification of peptides and proteins, and mass spectrometry (MS) for detailed proteomics, post-translational modifications, and peptide mapping (Table 1).

Table 1. Comparative analysis of techniques used for the characterization of bovine platelet-rich plasma (PRP)

Technique	What it can measure / detect	Advantages and Limitations
Spectroscopic methods (UV-Vis, fluorescence, absorbance)	This method determines total protein concentration using absorbance at 280 nm or colorimetric assays. It is applied to quantify protein levels in plasma and in platelet releasates after activation, and is sometimes used for the detection of small molecules or contaminants [16].	The method is simple, inexpensive, and rapid; however, it cannot identify individual proteins, is subject to interference from hemolysis or lipids, and cannot distinguish between proteins and growth factors [16].
Electrophoresis (SDS-PAGE, 2D gels)	This technique separates proteins according to their size or charge and is used to profile bovine PRP proteins as well as to detect degradation products or isoforms. Western blotting, enables the identification of growth factors such as PDGF, VEGF, and TGF- β . Similarly, protein separation methods allow visualization of multiple proteins, facilitate quantification through spot intensity, and permit excision of bands or spots [17].	This method visualizes protein patterns and of sample purity. However, it has lower sensitivity for minor proteins and is antibody-dependent. While it provides good resolution for many proteins, the high dynamic range of plasma—where a few proteins are highly abundant and many are of low abundance—can minor proteins. In addition, gel-based methods are laborious [17].
High-Performance Liquid Chromatography (HPLC)	Separates proteins, peptides, and growth factors; in PRP, it specifically distinguishes fibrinogen, albumin, and cytokine fractions [18]. Enables resolution of complex mixtures, supports fractionation before MS analysis, allows quantification, and facilitates the measurement of small molecules as well [18].	Provides high resolution, reproducibility, and quantitative capability; however, it requires advanced instrumentation and meticulous sample preparation. The method is highly precise and reproducible, yet low-abundance proteins may be lost, and the process can be relatively slow when analyzing multiple fractions [18].
Mass Spectrometry (MS)	This technique provides proteomic profiling of bovine PRP, enabling the identification of growth factors, cytokines, and plasma proteins. It also detects post-translational modifications such as glycosylation and phosphorylation [19].	This method is highly sensitive, capable of identifying hundreds of proteins and detecting post-translational modifications. However, it is costly, requires significant expertise, and involves complex sample preparation [19].
Immunoassays (ELISA, Western blot, etc.)	This method allows specific quantification of known growth factors or cytokines (e.g., PDGF, TGF- β , VEGF) as well as other proteins [20].	This method offers high specificity and sensitivity for targeted analytes, but it requires high-quality antibodies. It is not suitable for the detection of unknown proteins, may be only semi-quantitative in certain cases, and has limited multiplexing capacity [20].

Although limited, some studies have investigated bovine PRP composition. Gutiérrez et al. [13] developed a two-step centrifugation protocol for bovine PRP and quantified platelets, leukocytes, and transforming growth factor beta-1 (TGF- β 1),

highlighting variability across preparations. Other research has focused on bovine plasma proteomics under stress or inflammatory conditions.

For instance, Reolon et al. [14] identified significant alterations in bovine plasma proteins in

response to heat stress using LC-MS/MS proteomics, while Grantz et al. [15] compared the platelet and plasma proteomes of postpartum dairy cows with high systemic inflammation versus healthy controls, detecting proteins involved in platelet degranulation and plasma immune responses.

These works demonstrate the utility of MS-based proteomics and electrophoretic separation for studying bovine plasma and platelet proteins, although few studies have applied them specifically to PRP.

PRP applications

In recent years, regenerative therapies have attracted increasing attention in both human and veterinary medicine, due to their potential to stimulate natural healing processes and reduce reliance on conventional treatments such as antibiotics. Among these therapies, platelet-rich plasma (PRP) is a blood-derived fraction enriched in proteins, growth factors, immunoglobulins, and bioactive molecules. These components play an important role in tissue repair and regeneration [21]. Platelet-rich plasma (PRP) has been increasingly investigated for the treatment of bovine uterine diseases, particularly endometritis and metritis, due to its regenerative and immunomodulatory properties. Several studies have evaluated its intrauterine application as an alternative or adjunct to conventional antibiotic therapies [22-24].

Marini et al. [22] reported that intrauterine administration of PRP in cows enhanced progesterone receptor expression in the endometrium and, in vitro, stimulated endometrial cell proliferation while simultaneously downregulating pro-inflammatory genes such as IL-1 β , IL-8, and COX-2 in response to lipopolysaccharide challenge. These findings underscore PRP's capacity to modulate inflammation and promote tissue repair in the bovine uterus.

More recently, Amer et al. [23] conducted a comparative clinical trial in Holstein dairy cows diagnosed with postpartum endometritis. Cows treated with autologous PRP exhibited significant reductions in uterine wall thickness and circulating pro-inflammatory cytokines (TNF- α , IL-6), demonstrating efficacy superior to that of

conventional oxytetracycline and second only to nano-oxytetracycline.

These findings suggest that PRP provides clinically relevant benefits for restoring uterine health while also minimizing reliance on antibiotic medication. Preliminary investigations have also been carried out in beef heifers. Puttman in 2019 found that intrauterine infusion of PRP in heifers with asymptomatic endometritis reduced endometrial inflammation, indicating that it has therapeutic promise even in subclinical cases. Despite the small sample size, our findings contribute to a growing body of evidence demonstrating that PRP can improve reproductive performance and increase uterine healing in cattle [24]. Overall, these findings indicate that PRP is a promising therapeutic method for the treatment of endometritis and metritis in dairy and beef cattle. PRP has the potential to expedite uterine repair, reduce infection-related inflammation, and act as a long-term alternative to standard antibiotic therapy in reproductive management programs.

3. Conclusions

Bovine PRP is a complex biological fraction, enriched with proteins, growth factors, immunoglobulins, and bioactive molecules that underpin its regenerative and immunomodulatory potential in veterinary medicine. The biochemical composition of PRP plays a decisive role in its therapeutic efficacy, particularly in bovine reproduction, where it supports uterine regeneration and reduces inflammation associated with metritis and endometritis.

The characterization of bovine PRP cannot rely on a single analytical technique, as each method provides complementary information. Spectroscopic approaches are useful for rapid protein quantification, electrophoresis, and immunoassays enable visualization and targeted detection of growth factors, while HPLC and mass spectrometry provide high-resolution proteomic profiles and insights into post-translational modifications. Integrating these analytical strategies allows for a more accurate and standardized assessment of PRP composition, which is essential for linking its biochemical properties to therapeutic outcomes and ensuring reproducibility in veterinary applications.

References

1. Dohan Ehrenfest, D. M., Andia, I., Zumstein, M. A., Zhang, C. Q., Pinto, N. R., Bielecki, T., Classification of platelet concentrates (Platelet-Rich Plasma-PRP, Platelet-Rich Fibrin-PRF) for topical and infiltrative use in orthopedic and sports medicine: Current consensus, clinical implications and perspectives, *Muscles, Ligaments and Tendons Journal*, 2014, 4(1), 3–9.
2. Everts, P. A., Napolitano, M., Perelli, S., Cenacchi, T., Platelet-Rich Plasma: New Insights for the Future of Regenerative Medicine, *International Journal of Molecular Sciences*, 2024, 25(14), 7914.
3. Sheldon, I. M., Lewis, G. S., LeBlanc, S., Gilbert, R. O., Defining postpartum uterine disease in cattle, *Theriogenology*, 2009, 71(9), 1021–1030.
4. Marx, R.E., Platelet-rich plasma: evidence to support its use, *J Oral Maxillofac Surg.*, 2004, 62(4), 489–496.
5. Anitua, E., Sánchez, M., Orive, G., Potential of endogenous regenerative technology for in situ regenerative medicine, *Adv Drug Deliv Rev.*, 2015, 84, 167–185.
6. Mishra, A., Harmon, K., Woodall, J., Vieira, A., Sports medicine applications of platelet-rich plasma, *Curr Pharm Biotechnol.*, 2012, 13(7), 1185–1195.
7. Alves, R. Grimalt, R., Randomized Placebo-Controlled, Double-blind, half-head study to assess the efficacy of platelet-rich plasma on the treatment of androgenetic alopecia, *Dermatologic Surgery*, 2016, 42(4), 491–497.
8. Wroblewski, A.P., Melia, H.J., Wright, V.J., Application of Platelet-Rich Plasma to Enhance Tissue Repair, *Operative Techniques in Orthopaedics*, 2010, 20, 98–105.
9. Croisé B., Paré, A., Joly A., Louisy A., Laure, B., Goga, D., Optimized centrifugation preparation of the platelet rich plasma: Literature review, *Journal of Stomatology, Oral and Maxillofacial Surgery*, 121, 2, 2020, 150–154.
10. Mazzocca, A. D., McCarthy, M. B. R., Chowaniec, D. M., Cote, M. P., Romeo, A. A., Bradley, J. P., Arciero, R. A., Knut Beitzel, K., Platelet-Rich Plasma Differs According to Preparation Method and Human Variability, *J Bone Joint Surg Am.*, 2012, 94, 308–16.
11. Bos-Mikich, A., de Oliveira, R., Frantz, N., Platelet-rich plasma therapy and reproductive medicine, *Journal of Assisted Reproduction and Genetics*, 2018, 35(5), 753–756.
12. Collins, T., Dinesh, A., Bilal, B., Platelet-rich plasma: a narrative review, *EFORT Open Rev.*, 2021, 6(4), 225–235.
13. Gutiérrez, L., Carmona, J.U., López, C., Standardization of a protocol for obtaining platelet-rich plasma from bovine blood, *Veterinary Medicine International*, 2017, 1950401.
14. Reolon, H. G., Abduch, N. G., Freitas, A. C., Silva, R. M. O., Fragomeni, Bd. O., Lourenco, D., Baldi, F., Paz, CCPd., Stafuzza, N. B., Proteomic changes of the bovine blood plasma in response to heat stress in a tropically adapted cattle breed, *Front. Genet.*, 2024, 15, 1392670.
15. Grantz, J. M., Thirumalaikumar, V. P., Jannasch, A. H., Andolino, C., Taechachokevivat N., Avila-Granados, L. M., Neves, R. C., The platelet and plasma proteome and targeted lipidome in postpartum dairy cows with elevated systemic inflammation, *Sci Rep.*, 2024, 28, 14(1), 31240.
16. Kitamura, Y., Suzuki, M., Tsukioka, T., Isobe, K., Tsujino, T., Watanabe T., Watanabe T., Okudera, H., Nakata K., Tanaka T., Kawase T., Spectrophotometric determination of platelet counts in platelet-rich plasma, *International Journal of Implant Dentistry*, 2018, 4, 29.
17. Deng, L., Han, Y., Tang, C., Liao, Q., Li, Z., Label-Free, Mass Spectrometry-Based Quantitative Proteomics Analysis of Serum Proteins During Early Pregnancy in Jennies (*Equus asinus*), *Front. Vet. Sci.*, 2020, 7, 569587.
18. Henning, A. H., Groschup, M. H., Mettenleiter, T. C., Karger, A., Analysis of the bovine plasma proteome by matrix-assisted laser desorption/ionisation time-of-flight tandem mass spectrometry, *The Veterinary Journal*, 2014, 199, 1, 175–180.
19. Sun, D., Zhang, H., Guo, D., Sun, A., Wang, H., Shotgun Proteomic Analysis of Plasma from Dairy Cattle Suffering from Footrot: Characterization of Potential Disease-Associated Factors, *PLoS ONE*, 2013, 8(2), e55973.
20. López, C., Duque-Madrid, P. C., Ceballos-Márquez, A., Carmona, J. U., Effect of allogeneic pure platelet-rich plasma, sodium cloxacillin, and their combination for the treatment of subclinical mastitis in crossbred cows, *Front. Vet. Sci.*, 2024, 11, 1432354.
21. Everts, P. A., Onishi, K., Jayaram, P., Mautner, K., Platelet-rich plasma: New performance understandings and therapeutic considerations in 2020, *International Journal of Molecular Sciences*, 2020, 21(20), 7794.
22. Marini, M. G., Perrini, C., Esposti, P., Corradetti, B., Bizzaro, D., Riccaboni, P., Cremonesi, F., Effects of platelet-rich plasma in a model of bovine endometrial inflammation in vitro and in vivo, *Reproductive Biology and Endocrinology*, 2016, 14(58), 1–10.
23. Amer S. S., Rezk S. G., Dakroury, M. F., El Amrawi, G. A., Advanced Clinical Trials in Treatment of Postpartum Endometritis in Holstein Dairy Cows, 2024, *MJVM*, 4, 1.
24. Puttman, E., Use of Platelet Rich Plasma for the Treatment of Subclinical Endometritis in Beef Heifers, Undergraduate Thesis Submitted to Oregon State University, 2019.