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OPTIMIZATION OF ISOLATION, PURIFICATION, AND FREEZE-DRYING FOR BOVINE PLATELET-RICH PLASMA (PRP) TO DEVELOP BIOLOGICAL PRODUCT

Optimasi Isolasi, Pemurnian, dan *Freeze-Drying Platelet-Rich Plasma* (PRP) Sapi untuk Pengembangan Produk Biologis

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Abstract

Platelet-rich plasma (PRP) is an autologous plasma fraction enriched with platelets and growth factors that support tissue repair. In veterinary applications, bovine PRP shows therapeutic potential, but its clinical use is limited by the short stability of fresh preparations. Freeze-drying offers a promising method to improve PRP shelf-life and usability. This study aimed to develop a standardized protocol for bovine PRP isolation, characterization, and freeze-drying. Blood samples (400 mL) were collected from six healthy cows using CPDA-1 anticoagulant. Hematological screening was performed using an automated analyzer. PRP was isolated via a double-spin centrifugation protocol and subsequently lyophilized using a controlled freezing and freeze-drying system. Data were analyzed using descriptive statistics, Shapiro-Wilk normality tests, and Spearman correlations. Hematology profiles showed wide biological variation in WBC, HGB, and HCT, while RBC, MCV, and PLT remained within stable ranges. Double-spin centrifugation effectively increased platelet concentration up to threefold from baseline. Freeze-drying produced stable PRP powder with low residual moisture, suitable for storage at room temperature. Significant positive correlations were found among RBC-HGB-HCT, reflecting normal erythropoietic physiology. The developed protocol successfully standardized bovine PRP isolation and freeze-drying procedures, producing stable and concentrated PRP suitable for extended storage. Freeze-dried bovine PRP has strong potential

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as a practical biological product for veterinary regenerative applications and cattle health management.Further studies are recommended to evaluate the biological stability and clinical efficacy of freeze-dried bovine PRP in various veterinary therapeutic applications.

Keywords: bovine PRP, freeze-drying, platelet-rich plasma

Abstrak

Platelet-rich plasma (PRP) merupakan fraksi plasma autolog yang diperkaya dengan trombosit serta berbagai faktor pertumbuhan yang berperan penting dalam proses perbaikan dan regenerasi jaringan. Dalam bidang kedokteran hewan, PRP sapi menunjukkan potensi terapeutik yang menjanjikan, namun penerapan klinisnya masih terbatas akibat stabilitas sediaan segar yang relatif singkat. Proses *freeze-drying* (liofilisasi) menjadi salah satu pendekatan yang menjanjikan untuk meningkatkan daya simpan serta kemudahan penggunaan PRP. Penelitian ini bertujuan untuk mengembangkan protokol standar dalam proses isolasi, karakterisasi, dan *freeze*-drying PRP sapi. Sampel darah sebanyak 400 mL dikoleksi dari enam ekor sapi sehat menggunakan antikoagulan CPDA-1. Pemeriksaan hematologi dilakukan menggunakan *automated hematology analyzer*. Isolasi PRP dilakukan dengan metode sentrifugasi dua tahap (*double-spin centrifugation*), kemudian dilanjutkan dengan proses liofilisasi menggunakan sistem pembekuan terkontrol dan *freeze-dryer*. Analisis data dilakukan secara deskriptif, dilanjutkan dengan uji normalitas *Shapiro-Wilk* serta analisis korelasi *Spearman*. Profil hematologi menunjukkan variasi biologis yang cukup luas pada parameter leukosit (WBC), hemoglobin (HGB), dan hematokrit (HCT), sementara jumlah eritrosit (RBC), volume eritrosit rata-rata (MCV), dan trombosit (PLT) berada dalam rentang yang relatif stabil. Protokol sentrifugasi dua tahap secara efektif meningkatkan konsentrasi trombosit hingga tiga kali lipat dibandingkan nilai awal. Proses *freeze-drying* menghasilkan serbuk PRP yang stabil dengan kadar air residu rendah, sehingga memungkinkan penyimpanan pada suhu ruang. Analisis korelasi menunjukkan hubungan positif yang signifikan antara parameter RBC, HGB, dan HCT, yang mencerminkan fisiologi eritropoiesis yang normal. Protokol yang dikembangkan dalam penelitian ini berhasil menstandarkan tahapan isolasi dan *freeze-drying* PRP sapi, serta menghasilkan PRP dengan konsentrasi trombosit tinggi dan stabilitas yang baik untuk penyimpanan jangka panjang. PRP sapi kering beku memiliki potensi besar untuk dikembangkan sebagai produk biologis praktis dalam aplikasi regeneratif veteriner dan manajemen kesehatan sapi.

Kata kunci: *freeze-drying, platelet-rich plasma*, PRP sapi

INTRODUCTION

Platelet-Rich Plasma (PRP) is a component of blood in the form of plasma obtained through a simple centrifugation method. This component contains higher platelet levels than other blood components (Rossi *et al.*, 2023). PRP therapy is popular due to its ability to aid in the body's regeneration process. PRP is derived from the patient's own blood and contains more than a thousand types of proteins and bioactive factors that play a role in accelerating wound healing and tissue repair. These factors include immune system molecules, growth factors, enzymes, and various other components that support tissue recovery (Boswell *et al.*, 2012, Marques *et al.*, 2015). PRP contains various important growth factors such as PDGF, TGF-β1, TGF-β2, VEGF, bFGF, IGF, HGF, and EGF. These growth factors play a role in biological processes such as chemotaxis, cell migration, cell proliferation and differentiation, new blood vessel formation (angiogenesis), and extracellular matrix synthesis (Pavlovic *et al.*, 2016). In veterinary medicine, PRP is used to accelerate wound healing, treat joint problems, muscle injuries, tendon inflammation, and in various animals, including cattle (Iacopetti *et al.*, 2020).

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PRP from cattle has great potential as a therapy for various conditions, such as reproductive disorders, inflammation of the uterus, mastitis, and wounds to soft tissue and bone.

However, the use of fresh PRP has limitations because it must be administered immediately after production and is prone to quality deterioration due to rapid protein and growth factor degradation (Caterino *et al.*, 2023). A promising method to overcome these limitations is through preservation techniques such as freeze-drying. This method removes water content from PRP, thereby increasing product stability, extending shelf life, and allowing for more flexible storage and distribution processes without the need for ultra-low temperature storage facilities (Andia *et al.*, 2020).

The literature discussing the isolation, purification, and freeze-drying of bovine PRP is still very limited. Most studies use human or non-ruminant animal PRP, or only assess the clinical effects after application. Therefore, this study aims to develop a simple, efficient, and standardized protocol for the isolation, purification, and freeze-drying of bovine PRP. The resulting protocol is expected to produce stable PRP that is easy to store and rehydrate, thereby potentially becoming a practical veterinary biological product that can be used in various livestock conditions in Indonesia and supporting the utilization of cattle blood waste from slaughterhouses as a source of valuable biological material.

RESEARCH METHODS

Ethical suitability of laboratory animals

Blood samples were collected as part of routine veterinary health examination procedures, without any additional invasive procedures. This study only involved minimal-risk procedures in the form of blood collection from healthy cattle, which was carried out in accordance with veterinary practice standards and applicable animal welfare guidelines. Therefore, at the time the study was conducted, formal ethical approval based on institutional policy was not required.

Research object

Blood samples were obtained from six healthy female cows raised at the Teaching Farm of Faculty of Health Sciences, Medicine, and Natural Sciences, Airlangga University, Banyuwangi. The animals were aged 2-4 years, weighed 300-400 kg, were of mixed breed, and were confirmed not to be pregnant. Clinical examinations and basic hematological evaluations were conducted to ensure that the animals did not have systemic disease symptoms, metabolic disorders, infections, or abnormal findings that would exclude them from the study.

Research design

This study used a laboratory experimental observational design, conducted without clinical treatment on animals. The study was descriptive and exploratory in nature, focusing on observing the hematological parameters of healthy cattle blood and evaluating the process of isolating, purifying, and freeze-drying bovine PRP using a double-spin protocol. No therapeutic trials or clinical interventions were performed on animals.

Research variables

In this study, the independent variable was the PRP preparation process, which included double-spin centrifugation as well as freezing and freeze-drying stages. The dependent variables comprised blood hematology parameters (WBC, RBC, HGB, HCT, MCV, and PLT), platelet concentration obtained from PRP isolation, and the physical characteristics of freeze-dried PRP preparations. The control variables included donor animal conditions (healthy, non-pregnant cows aged 2–4 years), blood volume collected (400 mL), anticoagulant type (CPDA-

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1), sample processing time, centrifugation speed and duration, and standardized freezing and freeze-drying conditions.

Blood Sampling

Sampling procedures were performed by veterinarians using venipuncture on the jugular vein. A total of 400 mL of blood was collected using sterile blood bags containing CPDA-1 (Citrate-Phosphate-Dextrose-Adenine-1) anticoagulant (HL Haemopack). This volume is within safe limits (<10% of the total blood volume of an adult cow), in accordance with AVMA guidelines and animal welfare standards. After sterilizing the skin area for several centimeters, blood is drawn using an 18-gauge needle (Terumo) through the external jugular vein. Blood is collected in a 450 mL blood bag containing CPDA-1 anticoagulant (composition per 100 mL: monohydrate citric acid 0.327 g; sodium citrate dihydrate 2.63 g; sodium phosphate monobasic dihydrate 0.251 g; anhydrous dextrose 2.90 g; adenine 0.0275 g). The blood bag is stored at 4°C and used no later than 24 hours after collection (Lange-Consiglio *et al.*, 2021).

Hematology Examination

Blood samples were taken from the CPDA-1 blood bag immediately after collection, then placed in a tube containing ±2 mL for hematology analysis. Complete blood analysis was performed using an impedance-based automatic hematology analyzer and flow cytometry (Mindray BC-2800Vet). The parameters measured included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), and platelet count (PLT). The platelet count varied from a minimum value of 154 × 10^3/µL to a maximum of 602 × 10^3/µL (Lange-Consiglio *et al.*, 2021).

PRP isolation

The entire procedure was performed aseptically using a class II biosafety cabinet and a centrifuge (Kubota 2800). A total of 400 mL of blood collected in a CPDA-1 bag was slowly transferred to a 50 mL conical tube and processed immediately within two hours after collection. The first centrifugation step (soft spin) aimed to separate plasma from erythrocytes without excessively sedimenting platelets. The tubes were centrifuged at 1,200 rpm for 8-10 minutes at room temperature, resulting in three layers: plasma (PPP + PRP), buffy coat, and erythrocytes. The top layer of plasma is then carefully removed using a sterile pipette without disturbing the buffy coat, and transferred to a new tube for further processing.

The plasma resulting from the soft spin then undergoes a second centrifugation (hard spin) at 1750 rpm for 10-15 minutes to precipitate the platelets. At this stage, a platelet pellet forms at the bottom of the tube and a supernatant in the form of platelet-poor plasma (PPP). Most of the supernatant (about 70-80%) is transferred and discarded aseptically, leaving 20-30% of the PPP volume to resuspend the platelet pellet, resulting in PRP with good homogeneity without premature activation. The PRP is then gently mixed using a soft pipetting technique and stored at 4 °C if it will be used within 24 hours, or stored at −80 °C for further processing such as freeze-drying.

Freeze-dried PRP

PRP samples are first pipetted into 50 mL conical tubes, each containing 10 mL of volume. All tubes are then frozen at a gradual temperature: 4°C for 2 hours, then transferred to −20 °C overnight, then transferred to −80 °C overnight to ensure complete freezing and stable ice matrix formation. After that, the freeze-dryer machine is turned on about 30 minutes in advance to lower the chamber temperature to the initial setting and ensure that conditions reach a stable point before the lyophilization process begins.

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The frozen PRP tubes are placed upright in the chamber on a pre-cooled rack. The vacuum system is activated to begin the primary drying phase, during which the pressure is lowered until sublimation conditions are reached. This process continues for approximately 72 hours, or until all the ice in the sample has evaporated, leaving only a dry matrix.

In the secondary drying phase, the machine maintains vacuum conditions while slightly increasing the temperature to remove residual bound moisture to a stable low moisture content. After the process is complete, the freeze-dried PRP samples are removed from the vacuum chamber and collected in airtight containers to prevent rehydration. The product is stored at room temperature or 2-8 °C.

Data analysis

This study used a cross-sectional study design by observing research subjects without any intervention or treatment. Data analysis used descriptive statistical methods (mean, median, standard deviation (SD), min, max for each hematology parameter) due to the limited number of samples (n=6). Blood samples were tested using a hematology analyzer to obtain WBC, RBC, HGB, HCT, MCV, and PLT parameters. Hematology data were analyzed using the Shapiro-Wilk normality test to determine the distribution and then using the Spearman nonparametric correlation test to assess the relationship between hematology parameters.

RESULTS AND DISCUSSION

Results

Hematology

The hematology evaluation results from six individuals showed considerable biological variation in several parameters, particularly WBC, HGB, and HCT (Table 1). Descriptively, WBC values showed a wide range (1.5-10.3 × 10^9/L) with high standard deviation. Similar variability was also seen in HGB and HCT, which showed a fairly wide range and high SD. Meanwhile, the RBC, MCV, and PLT parameters showed a more consistent and homogeneous distribution, characterized by a relatively low standard deviation. The number of platelets in the blood circulation was still within normal limits (325.17±60.42 10^9/L), making it a candidate for PRP products.

The Shapiro-Wilk normality test (Table 2) confirmed that most parameters did not follow a normal distribution. WBC, HGB, and HCT consistently had $p<0.05$ values, indicating a non-normal distribution due to extreme variation and possible biological outliers. Conversely, RBC, MCV, and PLT tended to be normally distributed ($p>0.05$), but there were limitations in the form of a small sample size (n=6).

The results of Spearman's correlation analysis (Table 3) showed that several hematological parameters had a strong relationship with each other. Strong positive correlations were found between RBC-HGB ($r = 0.899$; $p = 0.0149$) and HGB-HCT ($r = 0.956$; $p = 0.0029$), reflecting the normal physiological relationship that an increase in erythrocyte count is directly proportional to hemoglobin and hematocrit levels. In addition, WBC-PLT ($r = 0.928$; $p = 0.0077$) also showed a positive correlation. Conversely, MCV was strongly negatively correlated with almost all other parameters, especially WBC ($r = -1.00$; $p = 0.000$) and PLT ($r = -0.928$; $p = 0.0077$).

PRP Isolation and Freeze-drying

The second centrifugation process (hard spin) successfully separated the plasma fraction into Platelet-Rich Plasma (PRP) and Platelet-Poor Plasma (PPP) effectively. The PRP layer was concentrated at the bottom of the plasma fraction, while PPP was at the top and could be easily

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separated without disturbing the platelet sediment (Figure 1). The obtained PRP was then placed in a freeze-dryer for lyophilization (Figure 1.d). During this process, the water in the PRP is successfully removed through the stages of freezing and vacuum sublimation, resulting in stable freeze-dried PRP that is ready for storage or use in further analysis (Figure 1.e).

Discussion

The wide biological variation in several parameters, particularly WBC, HGB, and HCT, indicates differences in immune responses between individuals, both in terms of increased leukocytes (leukocytosis) and decreased leukocytes (leukopenia). Leukocytosis in cattle, which is an increase in white blood cell count, is generally caused by bacterial infections that trigger neutrophilia (Ferrer, 2020). Conversely, leukopenia or a decrease in white blood cell count can occur due to disruption of the myeloid cell differentiation process. This can originate from the environment or husbandry management. Bovine leukosis caused by the BLV virus is often associated with reproductive and management factors, such as abortion, artificial insemination, and the use of shared syringes. These conditions can increase the risk of infection and stress in animals, thereby suppressing immune function and reducing white blood cell production (Conde-Muñoz *et al.*, 2023).

High variability in HGB and HCT indicates heterogeneity in erythropoiesis status or relative anemia in some samples. Hemoglobin (HGB) levels show fluctuations related to transport stress, mainly due to changes in hydration status and body fluid balance dynamics. The hematocrit (HCT) parameter is influenced by the degree of hemoconcentration and the intensity of stress experienced by the animals (Perayadhista *et al.*, 2022).

The platelet count is still within normal limits (325.17±60.42 10^9/L), making the blood sample a candidate for PRP production. This is supported by several other observers who stated that the range of platelet counts in the initial blood was between 154 × 10^3/µL and 602 × 10^3/µL, with an average of 348.46 × 10^3/µL and a standard deviation of 93.47 × 10^3/µL. After the preparation process, all PRP obtained showed an increase in platelet concentration of approximately three times the baseline value, namely 462 × 10^3/ µL to 1,806 × 10^3/µL, with an average of 1,045.07 × 10^3/µL and a standard deviation of 280.42 × 10^3/µL (Lange-Consiglio *et al.*, 2021).

The Shapiro-Wilk normality test confirmed that most parameters did not follow a normal distribution. This heterogeneity of distribution between parameters indicates that the data groups are heterogeneous, both physiologically and statistically. A strong positive correlation was found between RBC-HGB and HGB-HCT, reflecting the normal physiological relationship that an increase in red blood cell count is directly proportional to hemoglobin and hematocrit levels. Red blood cell count is directly proportional to hemoglobin and hematocrit levels. RBC, HGB, and HCT are three interrelated blood parameters because they all describe the condition of red blood cells. The number of erythrocytes (RBC) determines how much hemoglobin (HGB) is carried in the blood, because hemoglobin is found in every red blood cell. When the number of erythrocytes increases, hemoglobin levels generally increase as well. The same applies to hematocrit (HCT), which is the percentage of blood volume filled by erythrocytes; the more red blood cells, the higher the hematocrit value. Therefore, these three parameters usually change in the same direction, whether the animal is experiencing anemia, dehydration, or other stressful conditions (Bunga *et al.*, 2019; Mordak *et al.*, 2024).

A positive WBC-PLT correlation reflects a general hematopoietic response or certain inflammatory/subclinical conditions. Conversely, MCV correlates negatively with almost all other parameters, especially WBC and PLT, indicating that animals with smaller red blood cell volumes tend to have higher WBC and PLT levels, although this finding requires careful

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interpretation due to the very small sample size (n=6). These results are consistent with the findings (Machado *et al.*, 2022), which stated that no correlation was found between neutrophil count, protein secretion from isolated neutrophils, and platelet concentration in bovine blood. The number of platelets in circulation was also not associated with neutrophil viability or protein production capacity. Overall, significant correlations mainly occurred in parameters that are physiologically interrelated (RBC-HGB-HCT), while the negative MCV pattern reflects quite extreme inter-individual variation. These findings can be used to support the interpretation of the hematological condition of donor cows in PRP research, but must still be viewed with the limitation of the sample size.

The separation of plasma fractions during the second centrifugation stage (hard spin) produces a clear layer between the platelet-poor plasma (PPP) at the top and the platelet concentrate (PRP) that settles as a pellet or platelet-rich zone at the bottom of the tube; the PPP can be aspirated without disturbing the platelet deposit. This double-spin technique uses an initial soft spin to separate erythrocytes and a subsequent hard spin to concentrate platelets. It is a simple yet effective method for concentrating platelets and controlling the leukocyte composition in the final product (Machado *et al.*, 2022). These results have also been reported in other studies, namely that in the centrifugation process, each spin stage in the double-spin technique produces the separation of different blood components. The first spin (soft spin) uses low centrifugal force to separate whole blood into three layers, namely red blood cells, buffy coat, and plasma. Then, in the second spin (hard spin), which uses higher centrifugal force, the platelets are concentrated and settle at the bottom of the plasma fraction (Nazaroff *et al.*, 2021). We did not use commercial kits or special tools to obtain PRP but only used standard laboratory equipment and did not use any additional materials in each process.

Currently, there are many different protocols and methods for obtaining PRP, resulting in differences in comparisons between studies. Reporting of PRP preparation protocols in various clinical studies is still inconsistent and does not provide sufficient information to enable replication of the procedure. In addition, the current reporting of procedures and PRP composition does not allow researchers to compare the PRP products administered to patients. Therefore, a detailed, accurate, and step-by-step description of the PRP protocol is needed to enable accurate comparisons between studies and ensure the reproducibility of the method (Chahla *et al.*, 2017; Dhurat and Sukesh, 2014).

Although the use of PRP has been widely accepted, a number of limitations remain and provide opportunities for research exploration, technological development, innovation, and commercial potential, particularly in the field of veterinary medicine. The biggest obstacle is that PRP is perishable and used autologously (Anitua *et al.*, 2021). Stability and long shelf life are very important for bioproducts because they make them easier to use and produce in large quantities without compromising quality. PRP itself is prone to rapid deterioration, so it should be used within 4-6 hours after blood collection (Weibrich *et al.*, 2004). Therefore, freeze-drying is an appropriate method to extend the shelf life of PRP, as it can maintain the stability of bioactive molecular components and preserve its therapeutic activity for a longer period of time. In this study, we produced freeze-dried PRP preparations in an effort to obtain PRP preparations that are more stable, have a longer shelf life, and still maintain their biological quality. Research by Gatin *et al.* (2008) states that one stable preservation method is freeze-drying, where the first step is to freeze the substance and then reduce the amount of solvent (water). There are two drying processes: primary and secondary. This reduction in water content prevents biological activity and chemical reactions that could potentially damage the bioproduct.

In their study, Freitas *et al.* (2022) compared the VEGF and PDGF growth factor content in fresh PRP and freeze-dried PRP using ELISA and stated that the freeze-drying process did not

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have a negative effect on the VEGF and PDGF content, so that the stability of the growth factors was maintained after freeze-drying. In addition, Freitas *et al.* (2022) also tested the duration of freeze-dried PRP storage on the VEGF and PDGF growth factor content. The data showed that the quantification of VEGF and PDGF growth factors did not change significantly up to 90 days of storage. The stability of freeze-dried preparations that we found is also in line with the findings of other researchers, da Silva *et al.* (2018), who also reported that the freeze-drying process is able to maintain the quality and content of growth factors optimally.

CONCLUSION AND SUGGESTIONS

Conclusion

Hematologic profiles show wide variations in WBC, HGB, and HCT parameters, while RBC, MCV, and PLT show more stable distributions. Normality and correlation analyses confirm that significant relationships occur mainly in RBC-HGB-HCT parameters, while extreme variations in MCV and WBC reflect individual heterogeneity with a limited sample size. The PRP preparation process using the double-spin method proved effective in separating PRP and PPP fractions without using commercial kits. The freeze-drying stage was able to produce stable dry PRP preparations. Overall, this study proves that freeze-dried PRP can be a more durable, stable, and storable alternative bioproduct.

Suggestions

Further research is needed to evaluate the biological stability and clinical efficacy of freeze-dried bovine PRP in various veterinary therapeutic applications.

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Tables

Table 1. Hematology results

Parameter	1	2	3	4	5	6	Ref**
WBC (10^9/L = 10^3/mcL)	5.5	3.5	10.3	10	1.5	7.1	4.0-12.0
RBC (10^{12}/L = 10^6/mcL)	6.13	4.44	6.12	6.2	2.52	4.42	5.0-10.0
HGB (g/L)	80	54	60	65	29	40	80-150
HCT (%)	36.4	26.6	26.6	26.9	15.2	19.6	-
MCV fL	59.5	60.1	43.5	43	60.4	44.4	40-60
PLT (10^9/L=10^3/mcL=10^3/µL)	350	305	379	389	219	318	100-800

*WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet. **Fielder (2024).

Table 2. Shapiro-Wilk W test

Parameter	n	Mean	SD	Min	Max	Shapiro-Wilk W	p-value	Distribution
WBC (×10^9/L)	6	6.37	3.58	1.50	10.30	0.872	0.207	Non-normal
RBC (×10^{12}/L)	6	4.97	1.47	2.90	6.50	0.911	0.413	Normal (marginal)
HGB (g/L)	6	53.83	17.71	33.00	77.00	0.844	0.132	Non-normal
HCT (%)	6	25.22	7.26	14.80	33.70	0.892	0.303	Non-normal
MCV (fL)	6	51.90	8.88	41.70	66.60	0.954	0.788	Normal
PLT (×10^9/L)	6	325.17	60.42	259	403	0.928	0.540	Normal

*WBC: White blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, PLT: Platelet.

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Table 3. Results of Spearman's correlation analysis

Variable	ρ (Spearman)	Strength & Direction	Interpretation
WBC - RBC	–0.03	Very weak, negative	There is almost no connection between leukocytes and erythrocytes.
WBC - HGB	0.03	Very weak, positive	It does not show a meaningful biological.
WBC - HCT	–0.03	Very weak, negative	There was no association between inflammation/leukocytosis and hematocrit.
WBC - MCV	–0.54	Moderate, negative	The tendency for high WBCs to appear in lower MCVs, but not statistically significant.
WBC - PLT	0.09	Very weak, positive	No indication of an immune-thrombopoiesis relationship.
RBC - HGB	0.60	Moderate-strong, positive	Physiologically appropriate: an increase in erythrocytes followed by an increase in hemoglobin.
RBC - HCT	0.83	Strong, positive	Very physiologically consistent: HCT increases with the number of erythrocytes.
RBC - MCV	0.03	Very weak	There is no relationship between cell size and erythrocyte count.
RBC - PLT	–0.26	Weak, negative	No thrombopoiesis-erythropoiesis relationship.
HGB - HCT	0.77	Strong, positive	Normal physiological relationship: high HGB → high HCT.
HGB - MCV	0.20	Weak, positive	Meaningless.
HGB - PLT	–0.09	Very weak	No apparent biological relationship.
HCT - MCV	0.20	Weak, positive	Meaningless.
HCT - PLT	–0.09	Very weak	No meaningful correlation.
MCV - PLT	–0.03	Very weak	There is no tendency for erythrocyte size to be related to platelet count.
HGB - MCV	0.20	Weak, positive	Meaningless.

*WBC: white blood cell, RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscural volume, PLT: Platelet.

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Figure

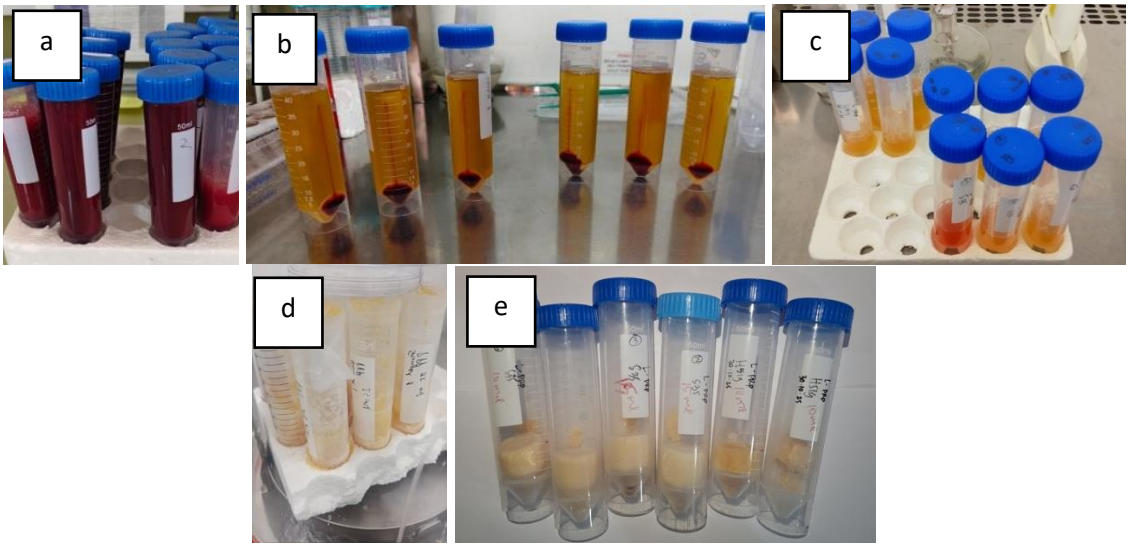

Figure 1. The process of freeze-dried PRP manufacturing flow; (a): Cow's blood in a conicle 50 mL, (b): Results of the second centrifugation (hard spin), (c): Results of PRP (1/3-1/4 of the bottom) that were successfully isolated, (d): PRP freeze-drying process (e): Final result of freeze-dried PRP.