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Changes in glucose concentration during refrigerated storage of caprine platelet-rich plasma: A reliable biochemical marker of platelet storage lesions

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Abstract

Platelet-rich plasma (PRP) is increasingly used in regenerative medicine because of its high concentration of platelets and growth factors. However, platelets remain metabolically active during storage and undergo progressive biochemical deterioration known as platelet storage lesions (PSL). The present study evaluated changes in glucose concentration in caprine PRP stored at 4°C for seven days. Blood samples collected from eight (n = 8) clinically healthy goats were processed using a double centrifugation technique to obtain PRP. Platelet additive solution was incorporated before storage, and glucose concentration was estimated at predetermined intervals using the glucose oxidase-peroxidase (GOD-POD) method. A progressive decline in glucose concentration was observed throughout the storage period, decreasing from 3.23 ± 0.33 mmol/L at 0 h to 0.54 ± 0.05 mmol/L on Day 7. The findings indicate continuous glycolytic activity despite refrigerated storage and demonstrate that glucose depletion is an early biochemical marker of platelet storage lesions. Monitoring glucose concentration may therefore serve as a useful quality indicator for stored caprine PRP intended for regenerative applications.

Keywords: Platelet-rich plasma, caprine, GOD - POD, platelet storage lesion, glycolysis, biochemical marker, veterinary regenerative medicine, veterinary transfusion medicine

1. Introduction

Platelets are small, anucleate cytoplasmic fragments derived from bone marrow megakaryocytes that play an essential role in haemostasis, thrombosis, inflammation and tissue repair. Beyond their traditional role in blood coagulation, platelets have gained considerable attention in regenerative medicine because they contain numerous biologically active growth factors capable of stimulating cellular proliferation, angiogenesis and extracellular matrix synthesis (Aibibula *et al.*, 2018) ^[1]. These properties have led to the widespread use of platelet-rich plasma (PRP) in both human and veterinary medicine for the treatment of musculoskeletal injuries, chronic wounds, tendon disorders, osteoarthritis and various orthopedic conditions.

Platelet-rich plasma is prepared by concentrating platelets from autologous whole blood through differential centrifugation. Compared with whole blood, PRP contains several-fold higher platelet concentrations along with platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) (Aibibula *et al.*, 2018) ^[1]. These bioactive molecules accelerate tissue repair by promoting fibroblast proliferation, collagen synthesis, angiogenesis and modulation of inflammatory responses. Consequently, PRP has emerged as a valuable biological therapeutic agent in regenerative medicine.

Although PRP is preferably used immediately after preparation, storage becomes inevitable in clinical practice, research laboratories and blood banking. During storage, platelets continue to remain metabolically active despite being outside the physiological environment. Ongoing cellular metabolism gradually induces structural, biochemical and functional alterations collectively known as platelet storage lesions (PSL) (Bruns & Knowler, 2009) ^[2].

These alterations include glucose depletion, lactate accumulation, reduction in pH, oxidative stress, membrane damage, potassium leakage, enzyme alterations and diminished platelet functionality.

Among these biochemical changes, glucose depletion is one of the earliest and most reliable indicators of platelet metabolic activity during storage. Platelets utilize glucose as their principal energy substrate through glycolysis and oxidative phosphorylation to generate adenosine triphosphate (ATP), which is essential for maintaining membrane integrity, cytoskeletal organization, ion transport and platelet activation (Aibibula *et al.*, 2018) [1]. As storage duration increases, platelets increasingly depend on anaerobic glycolysis, resulting in progressive glucose consumption and lactate accumulation. These metabolic alterations eventually compromise platelet viability and therapeutic efficacy.

Several studies have reported that glucose concentration decreases progressively during storage of blood and platelet products. Sacks *et al.* demonstrated that glucose declines continuously because of ongoing glycolysis, particularly when processing is delayed. Chan *et al.* similarly observed progressive glucose depletion in stored blood samples and emphasized rapid sample processing to preserve glucose stability. Bruns and Knowler further highlighted that glucose reduction during storage represents an important pre-analytical variable affecting biochemical interpretation. More recently, Grzych *et al.* and Pleus *et al.* confirmed that storage conditions significantly influence glucose stability, emphasizing the importance of proper preservation techniques (Sacks *et al.*, 1990; Chan *et al.*, 1995; Bruns & Knowler, 2009; Grzych *et al.*, 2019; Pleus *et al.*, 2022) [6, 3, 2, 4, 5].

Despite increasing applications of PRP in veterinary regenerative medicine, limited information is available regarding glucose metabolism in stored caprine PRP. Understanding glucose utilization during storage is essential for determining the biochemical stability and quality of platelet concentrates intended for therapeutic use. Therefore, the present study was undertaken to evaluate changes in glucose concentration during refrigerated storage of caprine platelet-rich plasma and to determine its usefulness as an indicator of platelet storage lesions.

2. Materials and Methods

2.1 Ethical approval

The present investigation was carried out after obtaining approval from the Institutional Animal Ethics Committee of the Veterinary College and Research Institute, Tirunelveli. Experimental procedures were performed in accordance with CPCSEA guidelines governing the use of animals for research.

2.2 Experimental animals

Eight apparently healthy adult goats of either sex maintained at the District Livestock Farm, Abishekapatti, Tirunelveli and Livestock Farm Complex, Veterinary College and Research Institute, Tirunelveli, were selected for the study. Animals were clinically examined before blood collection to ensure normal physiological status.

2.3 Collection of blood samples

Approximately 8 mL of blood was collected aseptically from the jugular vein of each animal using sterile disposable syringes fitted with 20-gauge needles. Blood samples were immediately transferred into sterile centrifuge tubes containing Acid Citrate Dextrose (ACD) anticoagulant to

prevent platelet activation and coagulation during processing.

2.4 Preparation of PRP

PRP was prepared by the double centrifugation protocol optimized in our lab. Initially, whole blood samples were centrifuged at 2500 rpm for 10 minutes to separate plasma and buffy coat from erythrocytes. The plasma layer containing buffy coat was carefully aspirated and subjected to a second centrifugation at 3500 rpm for 15 minutes. Following centrifugation, the upper platelet-poor plasma was discarded while the lower platelet-rich plasma fraction was retained. To improve platelet preservation during storage, Platelet Additive Solution (PAS) was incorporated into PRP at a ratio of 65% PAS and 35% PRP. Samples were gently mixed to avoid platelet activation before storage.

2.5 Storage and analysis of PRP

Prepared PRP samples were stored under refrigerated conditions at 4 °C for seven days. Glucose concentration was estimated immediately after PRP preparation (0 h), followed by analysis at 12 h, 24 h, 36 h, 48 h, Day 3, Day 5 and Day 7.

2.6 Glucose estimation

Glucose concentration was determined using the Glucose Oxidase–Peroxidase (GOD–POD) enzymatic colorimetric method employing a commercially available diagnostic kit (Delta Diagnostics, India). The principle of the assay is based on enzymatic oxidation of glucose by glucose oxidase to produce gluconic acid and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with phenol and 4-aminoantipyrine to produce a pink-colored quinoneimine complex (Trinder, 1969) [7]. The intensity of the colour formed is directly proportional to glucose concentration and was measured at 505 nm using double beam uv – vis spectrophotometer (UH 500 Hitachi, Japan) The glucose concentration was expressed in mmol/L.

2.7 Statistical analysis

Data obtained at different storage intervals were expressed as Mean $\pm$ Standard Error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences among storage intervals were considered statistically significant at $P < 0.05$.

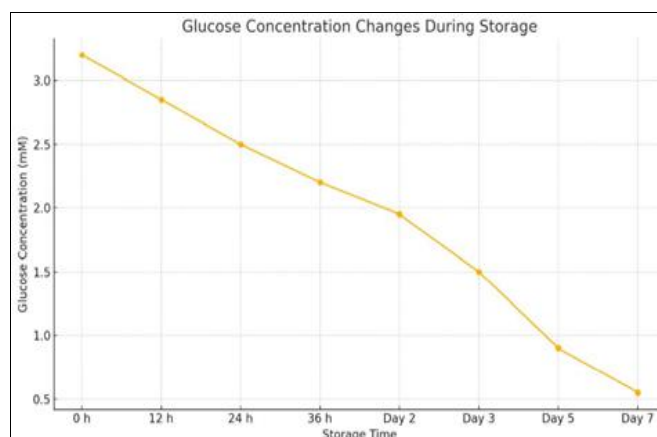
3. Results

3.1 Changes in glucose concentration during refrigerated storage of caprine PRP

Glucose concentration in caprine platelet-rich plasma declined progressively throughout the seven-day storage period under refrigerated conditions (4 °C). The mean glucose concentration at the time of preparation (0 h) was 3.23 ± 0.33 mmol/L, which gradually decreased to 2.84 ± 0.29 mmol/L after 12 h of storage. Further reductions were observed at 24 h (2.50 ± 0.25 mmol/L), 36 h (2.20 ± 0.20 mmol/L) and 48 h (1.94 ± 0.20 mmol/L). The decline became more pronounced during prolonged storage, with glucose concentration falling to 1.50 ± 0.15 mmol/L on Day 3, 0.90 ± 0.09 mmol/L on Day 5 and reaching the lowest value of 0.54 ± 0.05 mmol/L by Day 7. Statistical analysis revealed a significant ($P < 0.05$) decrease in glucose concentration with increasing storage duration. The overall reduction in glucose concentration from Day 0 to Day 7 was approximately 83.3%, indicating continuous metabolic activity of platelets despite refrigerated storage.

Table 1: Mean ($\pm$ SE) glucose concentration in caprine PRP during storage

Storage interval	Glucose (mmol/L)
0 h	3.23 $\pm$ 0.33
12 h	2.84 $\pm$ 0.29
24 h	2.50 $\pm$ 0.25
36 h	2.20 $\pm$ 0.20
48 h	1.94 $\pm$ 0.20
Day 3	1.50 $\pm$ 0.15
Day 5	0.90 $\pm$ 0.09
Day 7	0.54 $\pm$ 0.05

**Fig:** Progressive decline in glucose concentration in caprine platelet-rich plasma during seven days of refrigerated storage.

4. Discussion

Platelets remain metabolically active after collection and continue to consume glucose throughout storage to maintain intracellular ATP production (Aibibula *et al.*, 2018) ^[1]. The present investigation demonstrated a continuous decline in glucose concentration in caprine platelet-rich plasma stored at 4°C, indicating sustained glycolytic metabolism even under refrigerated conditions. These findings suggest that refrigeration slows but does not completely inhibit platelet metabolism.

Glucose serves as the principal energy substrate for platelets and is essential for maintaining membrane integrity, ion transport, cytoskeletal rearrangement and activation responses. Platelets generate ATP through both oxidative phosphorylation and glycolysis (Aibibula *et al.*, 2018) ^[1]. During storage, mitochondrial efficiency gradually decreases, causing platelets to increasingly rely on anaerobic glycolysis. Consequently, glucose is continuously consumed and converted into pyruvate, which is subsequently reduced to lactate. This metabolic shift explains the progressive decline in glucose concentration observed in the present study.

The gradual reduction in glucose concentration during storage observed in this investigation agrees with earlier findings reported by Sacks *et al.*, who demonstrated that blood glucose decreases continuously because of ongoing glycolysis by blood cells. They reported that glucose concentration may decline by approximately 5–7% per hour at room temperature if plasma separation is delayed. Similarly, Chan *et al.* reported progressive glucose depletion during storage and concluded that refrigeration considerably slows glycolysis compared with room-temperature storage. These observations support the present findings, where refrigerated storage reduced but did not completely prevent glucose utilization.

The findings are also consistent with the observations of Bruns and Knowler, who emphasized that continued glycolysis is responsible for declining glucose concentrations

in stored blood samples and that sodium fluoride alone is insufficient to completely inhibit glucose consumption immediately after collection. Their study highlighted the importance of rapid sample processing and appropriate storage conditions for preserving glucose stability.

Although previous studies largely investigated whole blood or plasma, similar metabolic principles apply to platelet-rich plasma because platelets retain active glycolytic enzymes throughout storage (Aibibula *et al.*, 2018) ^[1]. The progressive decline in glucose observed in the present investigation indicates that platelet metabolism remains functional despite refrigerated conditions.

An interesting observation in the present study was that glucose depletion accelerated after approximately 48 hours of storage. During the initial phase of storage, platelets appeared capable of maintaining metabolic homeostasis with relatively moderate glucose utilization. However, as storage progressed, metabolic stress increased, resulting in more rapid glucose consumption. This phenomenon likely reflects increased dependence on anaerobic glycolysis resulting from progressive mitochondrial dysfunction during storage.

The reduction in glucose concentration corresponded closely with increasing lactate concentration and decreasing pH observed during the present investigation. As glycolysis proceeds, pyruvate is converted into lactate by lactate dehydrogenase, generating hydrogen ions that contribute to acidification of the storage medium. The simultaneous decline in glucose and pH therefore represents characteristic biochemical features of platelet storage lesions.

Reduced glucose availability has several important biological consequences. ATP synthesis declines progressively (Aibibula *et al.*, 2018) ^[1], limiting energy-dependent membrane transport mechanisms, calcium homeostasis and maintenance of phospholipid asymmetry. Energy depletion also affects platelet aggregation, granule secretion and responsiveness to physiological agonists. Consequently, prolonged storage eventually compromises the therapeutic potential of platelet-rich plasma.

Another important consequence of glucose depletion is increased oxidative stress. Reduced ATP availability impairs antioxidant defense mechanisms, facilitating reactive oxygen species generation and lipid peroxidation. Previous investigations have demonstrated that oxidative stress increases progressively during platelet storage and contributes to membrane damage, potassium leakage and deterioration of platelet quality (Aibibula *et al.*, 2018) ^[1]. Therefore, glucose depletion should not be considered an isolated metabolic event but rather an early indicator of a cascade of biochemical alterations associated with platelet storage lesions.

Although refrigeration effectively slows cellular metabolism, the present findings demonstrate that significant glucose utilization continues even at 4 °C. This observation has practical implications for veterinary regenerative medicine. Platelet-rich plasma intended for therapeutic application should preferably be used as soon as possible after preparation to maximize platelet viability and preserve growth factor release. Extended refrigerated storage may reduce biological activity owing to progressive metabolic deterioration.

The findings of the present investigation therefore establish glucose estimation as a reliable biochemical marker for evaluating the metabolic status of stored caprine platelet-rich plasma. When interpreted together with lactate production, pH changes, oxidative stress markers and membrane integrity

parameters, glucose concentration provides valuable information regarding platelet quality during storage.

5. Conclusion

The present study demonstrated a significant progressive decline in glucose concentration in caprine platelet-rich plasma during seven days of refrigerated storage. Continuous glucose depletion reflects persistent platelet metabolism despite storage at 4 °C and represents one of the earliest biochemical manifestations of platelet storage lesions. The marked reduction in glucose concentration after prolonged storage suggests increasing dependence on anaerobic glycolysis and declining platelet viability. Routine monitoring of glucose concentration may therefore serve as a simple, reliable and sensitive quality control parameter for evaluating stored platelet-rich plasma intended for regenerative medicine and transfusion applications. Further studies correlating glucose metabolism with platelet function and growth factor release will improve understanding of platelet storage biology and assist in optimizing storage protocols for veterinary use.

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Conflict of Interest

Not available

Financial Support

Not available

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