

## Background and Introduction

- Few studies have been published to standardize in-house protocols for the obtention of platelet-rich plasma (PRP) and platelet-concentrate (PC).
- PRP and PC are blood derivatives with high platelet concentrations (PC>PRP).
- They have 2 main uses: intraoperative musculoskeletal clinical use and as an alternative culture media.
- Platelet activation provides a high supply of growth factors that trigger cellular responses like proliferation and differentiation.

## Objectives

- The main objectives set to achieve are to:
- Validate a protocol for the obtention of canine hematological derivatives: platelet-rich concentration, poor in leukocyte concentration.
  - Obtain a final PC with a platelet fold  $\geq 3x$  whole blood concentration.
  - Determine possible wastages during the process and calculate the % of recovery of platelets in plasma from basal values.

## Materials and Methods

A double-centrifugation method was used to obtain PRP from 10 canine Beagle blood donors. An additional centrifugation resulted in PC.

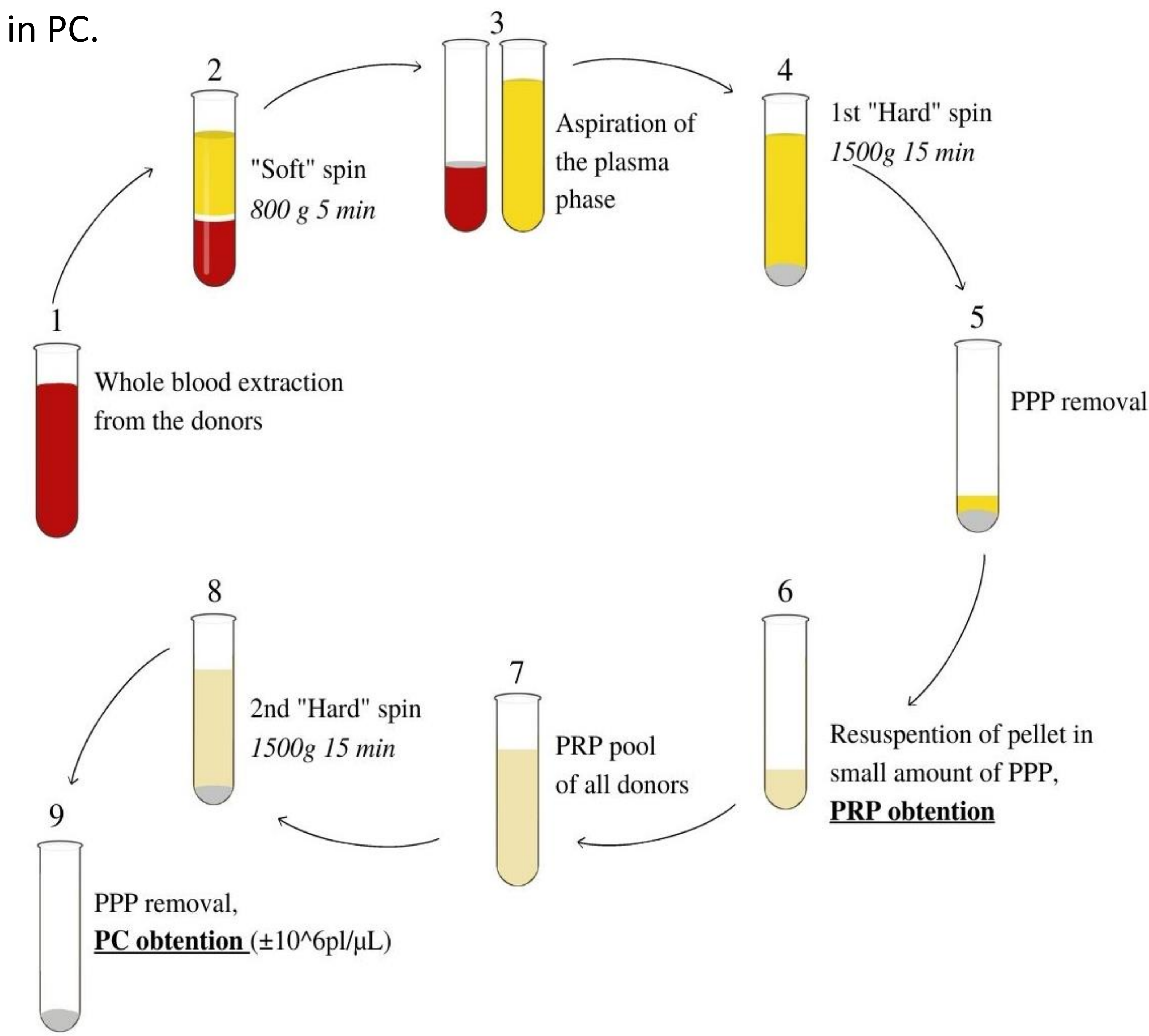


Figure 1. Diagram of the process followed to obtain platelet-rich plasma and/or platelet concentrate.

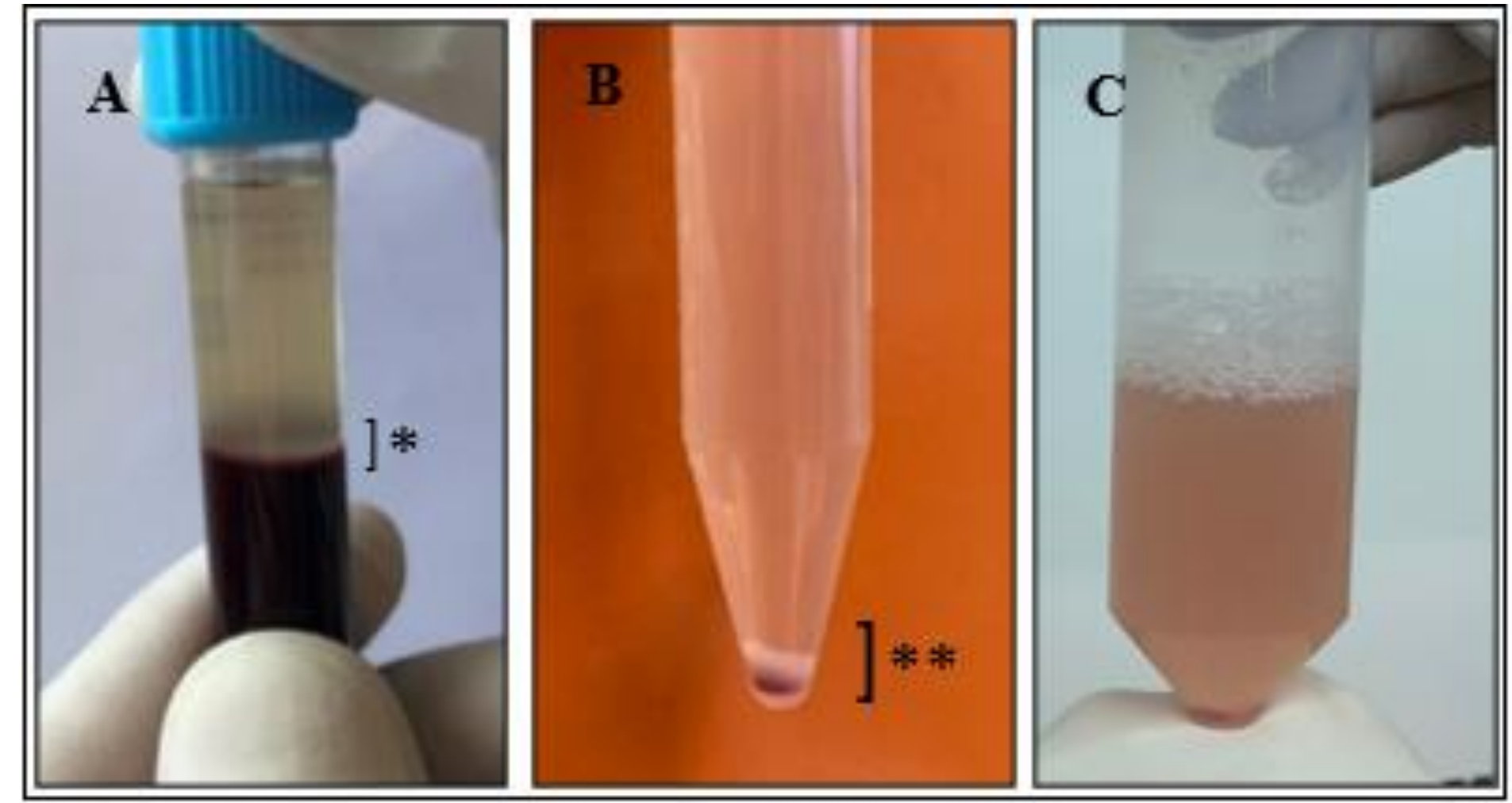


Figure 2. A Three-phase separation after the first soft spin. The asterisk (\*) marks the buffy coat. B Double asterisk (\*\*) marks the platelet-rich pellet after the second hard spin. C Pooled PRP.

## Results

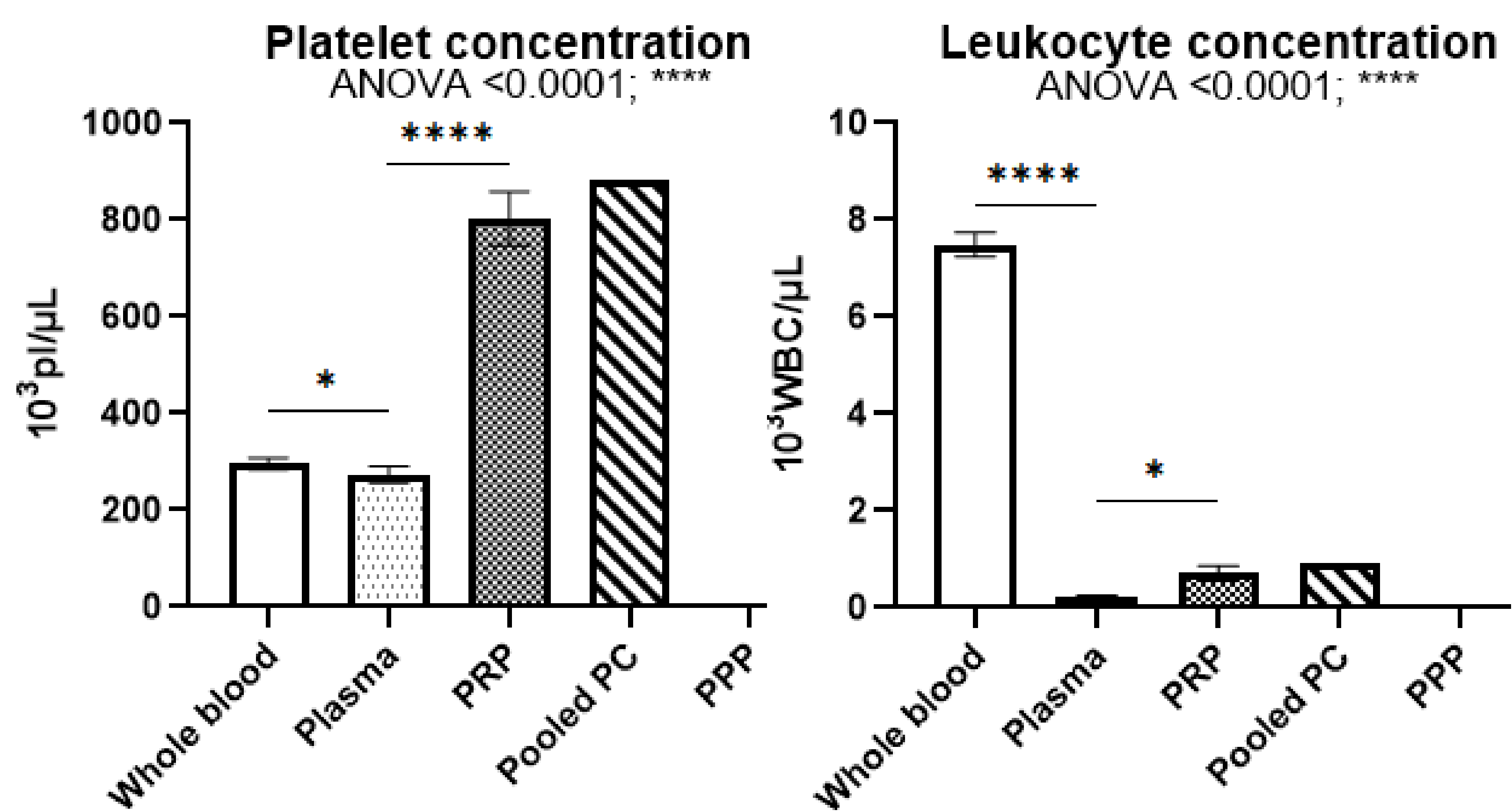


Figure 3. Variation of the dependent variables of the study, A platelet concentration and B leukocyte concentration.

Table 1. Quantification data of platelet and leukocyte concentration throughout the protocol process.

	Platelet concentration ( $\times 10^3/\mu\text{L}$ )	Leukocyte concentration ( $\times 10^3/\mu\text{L}$ )
Whole blood (Mean $\pm$ SD)	296.90 $\pm$ 37.97	7.5 $\pm$ 0.78
Plasma (Mean $\pm$ SD)	273.90 $\pm$ 52.40	0.2 $\pm$ 0.11
PRP (Mean $\pm$ SD)	802.8 $\pm$ 176.62	0.7 $\pm$ 0.48
Pooled PC	882	0.93
PPP	5	0
% of recovery in plasma from whole blood (Mean $\pm$ SD)	91.7 $\pm$ 8.26	-
PC fold from whole blood	2.97x	-

SD=standard deviation; PPP= platelet-poor plasma.

## Discussion

Platelet concentrates should be 3-7x fold the basal values to be effective. Centrifugation velocities of 500-1000g on the 1<sup>st</sup> spin combined with a 2<sup>nd</sup> spin at 1500 g 15 minutes are the most profitable. The 3<sup>rd</sup> spin contributes to a higher concentration (9.37%) from PRP to PC.

## Conclusions

- The protocol proposed obtains a 2.97x ( $\pm 3x$ ) fold the basal values and a complete leukoreduction during the process.
- No important preventable platelet losses were considered directly through this protocol.
- Critical points as well as limitations were established during the process.
- Further investigation is required on a higher number of samples and in search for factors that may alter the process and product.

## Main references

1. Strandberg, G., Sellberg, F., Sommar, P., Ronaghi, M., Lubenow, N., Knutson, F., & Berglund, D. (2017). Standardizing the freeze-thaw preparation of growth factors from platelet lysate. *Transfusion*, 57(4), 1058–1065.  
2. Shin, H., Woo, H., & Kang, B. (2017). Optimisation of a double-centrifugation method for preparation of canine platelet-rich plasma. *BMC Vet Res*, 13(1), 1–8.  
3. Chahla, J., Cinque, M. E., Piuze, N. S., Mannava, S., Geeslin, A. G., Murray, I. R., Dornan, G. J., Muschler, G. F., & LaPrade, R. F. (2017). A Call for Standardization in Platelet-Rich Plasma Preparation Protocols and Composition Reporting: A Systematic Review of the Clinical Orthopaedic Literature. *J Bone Joint Surg (Am)*, 99(20), 1769–1779.