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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 derivates: 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.

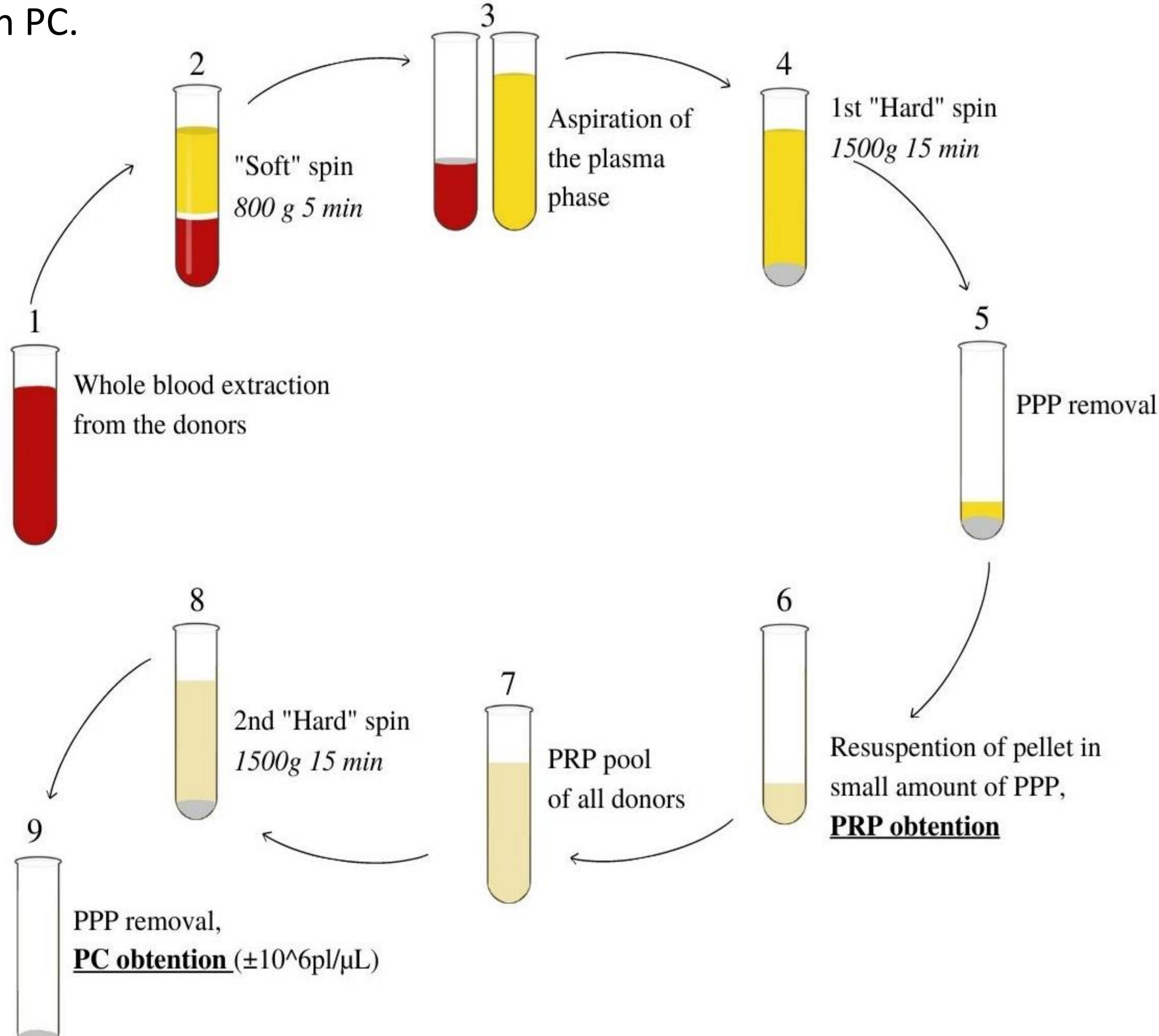


Figure 1. Diagram of the process followed to obtain platelet-rich plasma and/or platelet concentrate.

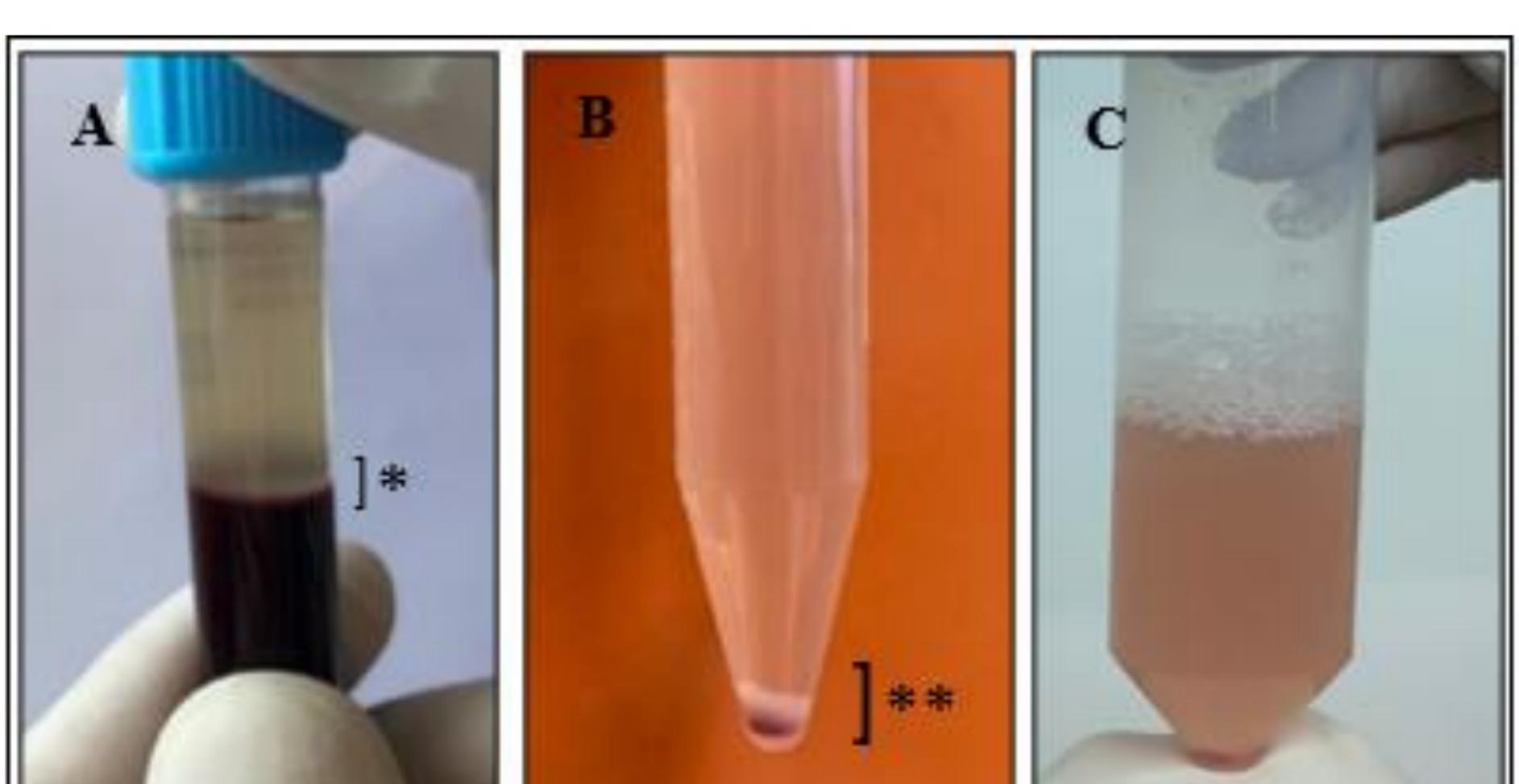


Figure 2. A Three-phase separation after the first soft spin. The asterisk (*) marks the buffy coat. B Double asterisk (**) marks the platelet-rich pellet after the second hard spin. C Pooled PRP.

Results

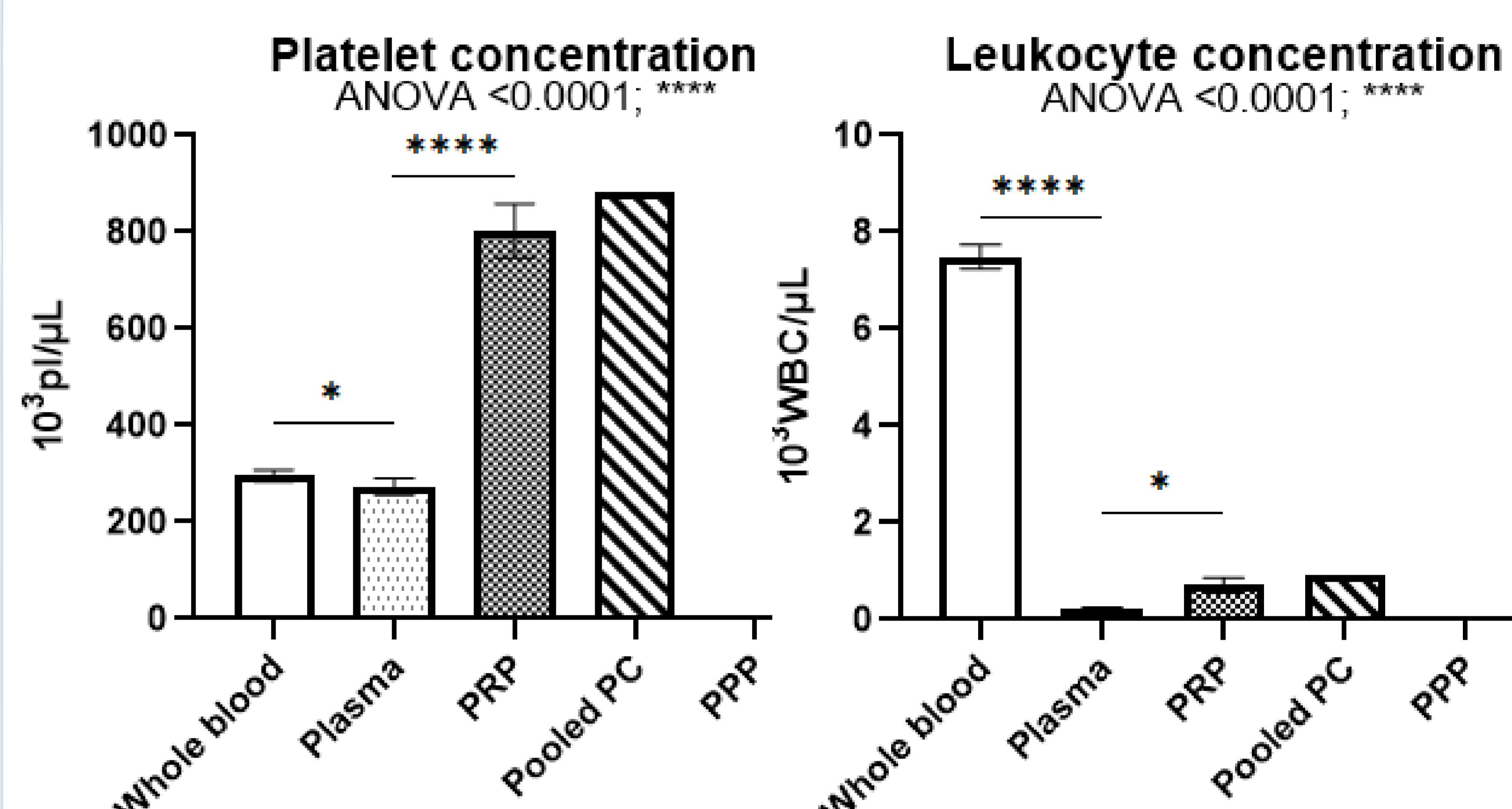


Figure 3. Variation of the dependent variables of the study. A platelet concentration and B leukocyte concentration.

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 1st spin combined with a 2nd spin at 1500 g 15 minutes are the most profitable. The 3rd 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

- 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.
- 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.
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