Comparison of the methods for platelet rich plasma preparation in horses

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Abstract
Platelet rich plasma (PRP) is popularly used in the horse industry to enhance regeneration of tissue injury that has limitation of blood supply. This study aimed to compare the methods for platelet rich plasma preparation since they have not been established yet. Blood was collected from six horses and platelets were concentrated by three different methods (2-step centrifugation, separated centrifugation and separated centrifugation using histopaque). Concentrated blood was analyzed using Advia hematology systems. In the result, separated centrifugation using histopaque showed the significantly lower number of red blood cells than other groups. The 2-step centrifugation showed the significantly higher number of white blood cells than other groups, while it contained the highest concentration of red blood cells among three groups. In the 2-step centrifugation, separated centrifugation and separated centrifugation with histopaque, platelets were concentrated 4.5, 5.3 and 5.6 times, respectively. And no significant difference of the platelet concentration between the three groups was found. This study demonstrated that separated centrifugation using histopaque was the best method for platelet rich plasma preparation because of the proper amount of platelets and the separation of red blood cells from platelet rich plasma.

Keywords: Platelet rich plasma, Red blood cells, White blood cells, Horse

Introduction
Platelet rich plasma (PRP) is the blood plasma which contains a high concentration of platelets. Recently, PRP is largely used in the treatment of equine musculoskeletal, soft tissue, skin injuries [1, 2]. It includes α-granules that secrete the growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) [3–5]. The growth factors help the migration of the cells and the regeneration of blood vessels, ligaments, tendons, bones and skin [1, 6]. In horses, there are 100,000–350,000 platelets/ul in the blood and platelet rich plasma should contain three times to five times of platelets [7]. However, there is no establishment of the methods for platelet rich plasma preparation [8]. Therefore, the purpose of this study is a comparison of the three different methods for platelet rich plasma preparation.
was centrifuged at 200 g for 15 min and plasma was collected and then centrifuged again at 900 g for 15 min. The supernatant was discarded and collected the plasma. Residual blood cells were collected into a different tube and mixed with PBS (1:1). Histopaque was added into the tube and the suspension was centrifuged at 400 g for 30 min. White blood cells were separated and washed with PBS, then centrifuged again at 200 g for 10 min. PBS was removed and white blood cells were added into the plasma tube. Complete blood count was performed (Fig. 1). All the

![Diagram of platelet rich plasma preparation methods]

**Fig. 1** Summary of the methods for platelet rich plasma preparation. **a** 2-step centrifugation. **b** Separated centrifugation. **c** Separated centrifugation with histopaque
data obtained was analyzed by a statistical software (SPSS Inc., IBM, USA) using non-parametric Mann-Whitney (statistical significance was considered at \( P < 0.05 \)) and Kruskal-Wallis tests (statistical significance was considered at \( P < 0.017 \)).

**Results**

Red blood cells were significantly lower in the method of separated centrifugation using histopaque (mean 0.09 ± 0.05 \( \times 10^6/ul \)) and significantly higher in the 2-step centrifugation method (mean 8.50 ± 2.31 \( \times 10^6/ul \)) than in other groups. The significantly higher number of white blood cells was shown in the 2-step centrifugation (mean 35.20 ± 3.65 \( \times 10^3/ul \)) and the lowest number was shown with separated centrifugation with histopaque method (mean 12.53 ± 2.59 \( \times 10^3/ul \)). However, a significant difference of the number of white blood cells between the separated centrifugation and separated centrifugation with histopaque was not found. The platelets were the most concentrated with separated centrifugation with histopaque method. In the 2-step centrifugation, separated centrifugation and separated centrifugation with histopaque, platelets were concentrated 4.5, 5.3 and 5.6 times, respectively. However, no significant difference of the platelet concentration between the three groups was found (Table 1).

**Discussion**

In a recent study, platelet rich plasma has been thought to take a therapeutic effect in the aspect of not only platelets but also red blood cells and white blood cells [9]. Red blood cells and white blood cells have been identified to have important roles in immune-mediated response [9]. Therefore, regulating the amount of blood cells was important in this study. Since red blood cells increased immune-mediated factors such as interleukin-1 and TGF-\( \alpha \), it is important to reduce the amount of red blood cells during preparation of platelet rich plasma [6]. Also, it was demonstrated that immune-mediated factors were increased when there were high concentration of red blood cells in platelet rich plasma which have the low number of white blood cells [6]. The efforts to reduce red blood cells were conducted by using single or double centrifugation [10] and difference of time and the gravitational force of centrifugation [11]. In this study, separated centrifugation with histopaque showed the significantly lower number of red blood cells than the 2-step centrifugation and separated centrifugation. This is because histopaque separated the layer of white blood cells from the layer of red blood cells.

The necessity of white blood cells in platelet rich plasma is still controversial. McCarrel T et al. observed that white blood cells and corticosteroids were effective in the treatment of chronic lateral epicondylitis in horse [12]. Also, platelet rich plasma, including white blood cells except neutrophils had an effect on anterior cruciate ligament fibroblast [13]. Therefore, we had effort to retain white blood cells in platelet rich plasma. However, there are some adverse effects when white blood cells

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**Table 1** Results of platelet rich plasma analysis

| Horse | RBC Wbc | Abc | Babc | Cw,bc | Wbc | A,b,c | Bw,a | Cw,a | Platelet Ww,b,c | A,w | Bw | Cw |
|-------|--------|-----|------|-------|------|------|------|------|---------------|-----|-----|-----|
| 1 Halla Female 13 yrs | 6.96 | 9.03 | 2.51 | 0.14 | 7.04 | 36.56 | 14.85 | 17.06 | 79 | 368 | 556 | 605 |
| 2 Halla Male 7 yrs | 7.07 | 10.82 | 4.15 | 0.11 | 5.94 | 33.25 | 18.55 | 10.69 | 71 | 274 | 439 | 380 |
| 3 TB Female 4 yrs | 11.26 | 11.56 | 4.65 | 0.12 | 8.88 | 34.77 | 19.2 | 13.5 | 104 | 503 | 680 | 650 |
| 4 TB Female 4 yrs | 7.76 | 6.45 | 2.46 | 0.03 | 7.8 | 34.1 | 21.3 | 13 | 106 | 602 | 427 | 629 |
| 5 Halla Male 7 yrs | 6.92 | 6.57 | 2 | 0.02 | 7.9 | 41.6 | 12 | 10.7 | 133 | 494 | 543 | 499 |
| 6 Halla Female 13 yrs | 6.61 | 6.57 | 4.56 | 0.13 | 7.97 | 30.89 | 22.72 | 10.25 | 118 | 497 | 610 | 652 |
| Mean | 7.76 | 8.50 | 3.39 | 0.09 | 7.59 | 35.20 | 18.10 | 12.53 | 101.83 | 456.33 | 542.50 | 569.17 |
| SD | 1.75 | 2.31 | 1.19 | 0.05 | 1.00 | 3.65 | 4.02 | 2.59 | 23.35 | 116.20 | 97.67 | 108.60 |

\(A\) 2-step centrifugation, \(B\) Separated centrifugation, \(C\) Separated centrifugation with histopaque, \(TB\) Thoroughbred, \(W\) Whole blood, \(RBC\) Red blood cell, \(WBC\) White blood cell, \(\text{w,a,b,c}\) Different letters represent significant differences between groups (\( P \)-value: \(W\) vs. \(A, B, C\), Mann-Whitney test, \( P < 0.05\); \(A\) vs. \(B\) vs. \(C\), Kruskal-Wallis test, \( P < 0.017\))
are concentrated [12]. Immune-mediated factors such as interleukin-1 and TGF-α could be increased because of concentrated neutrophils [11] and platelet rich plasma, including a high concentration of white blood cells decreased synthesis of extracellular matrix [9]. In this study, white blood cells were significantly higher in the 2-step centrifugation method than other groups. The lowest number of white blood cells was observed with the method of separated centrifugation using histopaque but not significantly different with the separated centrifugation. This may be due to loss of white blood cells during washing procedure.

Different methods for preparation of platelet rich plasma has been developed [6, 7, 9, 13]. The proper number of platelets of platelet rich plasma was three to five times higher than whole blood [6]. We prepared platelet rich plasma with the proper number of platelets, since the concentration of platelets in 2-step centrifugation, separated centrifugation and separated centrifugation with histopaque method was 4.5, 5.3 and 5.6 times respectively, with no significant difference.

Conclusion
The purpose of this study was comparing the methods for platelet rich plasma preparation to ascertain the most appropriate method. We intended to reduce red blood cells and to preserve white blood cells during the process of platelet rich plasma. The method of using histopaque was considered the most appropriate since platelets were concentrated while red blood cells were removed the most and white blood cells were included.

Acknowledgments
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Republic of Korea. (NRF-2017R1C1B1006038).

Funding
This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Republic of Korea.

Availability of data and materials
The data generated or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
JWK performed the experiments, analyzed the results. EBL participated in analysis of results, and was a major contributor in writing the manuscript. JPS designed the experiments and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval
The experimental procedure and methods were approved by the Experimental Animal Committee of Veterinary Medicine of Jeju National University, Jeju, Republic of Korea.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 26 April 2018 Accepted: 6 August 2018
Published online: 18 August 2018

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