Optimal activation methods for maximizing the concentrations of platelet-derived growth factor-BB and transforming growth factor-β1 in equine platelet-rich plasma

Kentaro FUKUDA1), Taisuke KURODA1), Norihisa TAMURA1), Hiroshi MITA1) and Yoshinori KASASHIMA2)*

1)Clinical Veterinary Medicine Division, Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke-shi, Tochigi 329-0412, Japan
2)The Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke-shi, Tochigi 329-0412, Japan

ABSTRACT. Platelet-rich plasma (PRP) therapy has been widely applied in various medical fields including humans and horses. This study aimed to establish an optimal activation method to stably and reproducibly maximize the concentrations of platelet-derived growth factor-BB (PDGF-BB) and transforming growth factor-β1 (TGF-β1) contained in equine PRP. Autologous PRP was prepared from 11 Thoroughbreds. For the activation test, PRP was activated by either a single freeze-thaw cycle (Fr) or adding calcium and autologous serum containing thrombin (Ca). PDGF-BB and TGF-β1 concentrations in Fr, Ca, nonactivated (No), and platelet-poor plasma (PPP) samples were determined using ELISA and compared. For repetitive freeze-thaw test, PRP was subjected to single (Fr1), double (Fr2), triple (Fr3), or quadruple (Fr4) freeze-thaw cycles and the concentrations of both growth factors in samples were compared similarly. The PDGF-BB concentration in Ca was significantly higher than that in other preparations. The TGF-β1 concentrations in Fr and Ca were significantly higher than those in PPP and No, with no significant differences between Fr and Ca. The concentrations of both factors were significantly increased in PRP treated with multiple cycles of freeze-thaw compared with that in PRP treated with a single cycle. No significant differences were noted among Fr2, Fr3, and Fr4. Our findings suggest that activation by adding calcium and autologous serum is optimal for instant use of PRP and that double freeze-thawing is an easier and optimal activation method for cryopreserved PRP.

KEY WORDS: equine platelet-rich plasma, freeze-thawing, platelet activation, platelet-derived growth factor-BB (PDGF-BB), transforming growth factor-β1 (TGF-β1)
and TGF-β1 in these samples were also determined and compared to the concentrations in the pre-treated supernatant samples. To determine the effect of the freeze-thaw treatment itself on growth factor concentration, the concentrations of PDGF-BB and TGF-β1 in the supernatant, dissolved precipitate, and washed nonactivated (No) samples were centrifuged under the same conditions before collection of the supernatant. Ca, for 5 min at 4°C before collection of the supernatant. Similarly, Fr and nonactivated (No) samples were centrifuged under the same conditions before collection of the supernatant. Ca, Fr, No, and PPP supernatants were preserved at 4°C for the analysis of growth factors.

The concentrations of PDGF-BB and TGF-β1 in samples were determined in duplicate using ELISA kits (Quantikine Human PDGF-BB ELISA DBB00 and Quantikine Human TGF-β1 ELISA DB100B, R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions. These kits were designed for testing human samples but have also been validated for use in horses [3, 11, 28, 30, 31]. All procedures were performed within 6 hr of preparation to ensure the stability of growth factors [11].

To observe morphological changes in platelets and leukocytes, smears of PPP, No, Fr, and Ca supernatants were stained using a commercial kit (Diff-Quick staining kit, Sysmex Corp.) and examined microscopically.

**PRP repetitive freeze-thaw test**

PRP samples were frozen for 1 month and were subjected to single (Fr1), double (Fr2), triple (Fr3), or quadruple (Fr4) freeze-thaw cycles, with repeated overnight freezing at −30°C and thawing at room temperature. All samples were divided into two aliquots after each freeze-thaw cycle (Fig. 1). One aliquot was centrifuged at 10,000 × g for 5 min at 4°C before collection of the supernatant. The remaining precipitate was dissolved and incubated for 30 min at room temperature with 0.5% TritonX-100 (SIGMA-ALDRICH, Co., St. Louis, MO, USA) PBS solution containing a protease inhibitor (Complete™ ULTRA Tablets, Roche Ltd., Basel, Switzerland). To dissolve platelet membranes and determine the total growth factor content in PRP samples, another aliquot was incubated for 30 min at room temperature using the Triton buffer described above (washed Fr). The concentrations of PDGF-BB and TGF-β1 in the supernatant, dissolved precipitate, and washed Fr (total concentration) were determined in the same manner as described previously and compared.

One half of the supernatant was frozen at −30°C overnight and thawed at room temperature for 30 min (treated supernatant) to determine the effect of the freeze-thaw treatment itself on growth factor concentration. The concentrations of PDGF-BB and TGF-β1 in these samples were also determined and compared to the concentrations in the pre-treated supernatant samples. Additionally, to clarify the amount of growth factors accumulated during freeze-thawing, the concentrations of growth factors
in supernatants collected after freeze-thawing PRP were compared with those in freeze-thawing PRP supernatant (treated supernatant). Smear examination was also performed for each Fr sample as mentioned above.

**Statistical analysis**

Growth factor concentrations were compared using one-way ANOVA, and post-hoc analyses were performed using Tukey’s test. Concentration ratios were compared using the Kruskal-Wallis test, and the subsequent post-hoc analysis was performed using the Steel-Dwass test. Concentrations of the freeze-thawing supernatants were compared using paired t-tests. Analyses were performed using Microsoft Excel 2013 Macro applications (Excel TOKEI ver. 7.0, ESUMI Co., Ltd., Tokyo, Japan). Statistical significance was set at \( P < 0.05 \).

**RESULTS**

Platelet and leukocyte counts in whole blood and PRP are shown in Table 1. Microscopic examination of smear samples revealed that leukocytes in PRP predominantly comprised lymphocytes (approximately >90%). Neither leukocytes nor platelets were observed in PPP or the supernatants of Ca gel. Platelets appeared to maintain their morphology, whereas leukocytes were not observed in Fr1, Fr2, and Fr3 (Fig. 2).

**PRP activation test**

The concentration of PDGF-BB in PPP and No was below the linear range of measurement (<31.2 pg/ml). The concentration of PDGF-BB in Ca (5,222 ± 3,957 pg/ml, mean ± SD) was significantly higher than that in other preparations (Fr, 1,369 ± 1,138 pg/ml, Fig. 3A). The concentrations of TGF-β1 in Fr (7,235 ± 2,842 pg/ml) and Ca (8,084 ± 2,257 pg/ml) were significantly higher than those in PPP (1,779 ± 461 pg/ml) and No (1,994 ± 442 pg/ml), with no significant differences between Fr and Ca (Fig. 3B).

**PRP repetitive freeze-thaw test**

The total PDGF-BB concentrations in Fr1, Fr2, Fr3, and Fr4 samples (9,486 ± 553 pg/ml, 11,545 ± 1,873 pg/ml, 12,295 ± 1,709 pg/ml, and 11,288 ± 1,591 pg/ml, respectively) were significantly higher than those in the respective supernatants (2,698 ± 1,510 pg/ml, 5,224 ± 913 pg/ml, 6,294 ± 1,254 pg/ml, and 6,351 ± 1,254 pg/ml, respectively) and precipitates (3,512 ± 2,045 pg/ml, 2,906 ± 654 pg/ml, 2,236 ± 344 pg/ml, and 2,069 ± 572 pg/ml, respectively) (Fig. 4A). No significant difference was observed between the Fr1 supernatant and precipitate. However, in the Fr2, Fr3, and Fr4 samples, the concentration of PDGF-BB in supernatants was significantly higher than that in precipitates. The concentrations of PDGF-BB in the Fr1 and Fr2 supernatants were significantly decreased by freeze-thawing with concentrations in the Fr1 and Fr2 treated supernatants of 2,154 ± 1,188 pg/ml and 4,218 ± 852 pg/ml, respectively, which were significantly lower than those in freeze-thawing PRP supernatant (Fig. 4B). The concentrations of PDGF-BB in the Fr2, Fr3, and Fr4 supernatants were significantly higher than that in the Fr1 supernatant, whereas no significant differences in PDGF-BB concentration were noted among the Fr2, Fr3, and Fr4 supernatants (Fig. 4C). There were no significant differences in precipitate or total PDGF-BB concentrations among the four groups.

There were no significant differences in TGF-β1 concentrations within samples of the same freeze-thaw cycle number, except in total concentration of the Fr4 (55,506 ± 22,461 pg/ml), which was significantly higher than that of the precipitate of the Fr4 (15,166 ± 3,210 pg/ml) (Fig. 4D). The concentration of TGF-β1 in the Fr3 supernatant (40,386 ± 14,338 pg/ml) was significantly higher than that of PPP (1,779 ± 461 pg/ml) and No (1,994 ± 442 pg/ml), with no significant differences between Fr and Ca (Fig. 3B).

**Fig. 1.** Schematic representation of the platelet-rich plasma (PRP) repetitive freeze-thaw test. Fr PRP: freeze-thawing PRP.
Table 1. The summary of obtained platelet-rich plasma (PRP) (n=11)

|                 | Leukocyte (×10^6/ml) | Platelet (×10^8/ml) |
|-----------------|-----------------------|---------------------|
| Whole blood     | 6.7 ± 1.2             | 1.2 ± 0.2           |
| PRP             | 3.7 ± 1.6             | 9.2 ± 1.6           |
| Concentration ratio | 0.6 ± 0.3             | 7.4 ± 0.9           |

Mean ± SD. Concentration ratio is the value of PRP/whole blood.

Fig. 2. Micrographs of Diff-Quick-stained whole blood smears (A), fresh platelet-rich plasma (PRP) (B), single freeze-thawing PRP (C), and triple freeze-thawing PRP (D). Platelets maintaining native morphology are indicated with black arrows. Bars=10 μm.

Fig. 3. The concentrations of platelet-derived growth factor-BB (PDGF-BB) (A) and transforming growth factor-β1 (TGF-β1) (B) in platelet-poor plasma (PPP), nonactivated platelet-rich plasma (PRP) (No), freeze-thawing PRP (Fr), and PRP to which calcium and autologous serum were added (Ca) (mean ± SD, n=6). Different letters indicate significant differences among groups (P<0.05).
Fig. 4. Platelet-derived growth factor-BB (PDGF-BB) (A) and transforming growth factor-β1 (TGF-β1) (D) concentrations in single (Fr1), double (Fr2), triple (Fr3), and quadruple (Fr4) freeze-thawing platelet-rich plasma (PRP) (mean ± SD, n=5). Comparisons among total concentration, concentration in the supernatant, and concentration in the precipitate obtained after centrifugation (*P<0.05, **P<0.01). PDGF-BB (B) and TGF-β1 (E) concentration in freeze-thawing PRP supernatant (mean ± SD). Freeze-thawing PRP in (B) and (E)=supernatant in (A) and (D), respectively. Dashed lines indicate comparisons of the supernatant concentrations before and after freeze-thawing (*, †P<0.05; **, ††P<0.01). PDGF-BB (C) or TGF-β1 (F) concentration ratios compared to Fr1 (mean ± SE). (C) The concentrations in the Fr2, Fr3, and Fr4 supernatants were significantly higher than that in the Fr1 supernatant. (F) The concentrations in the Fr2 and Fr4 supernatants were significantly higher than that in the Fr1 supernatant. No significant differences were observed among the Fr2, Fr3, and Fr4 supernatants (***P<0.05).
decreased by freeze-thawing with concentrations in the Fr3 treated supernatants of 27,074 ± 5,336 pg/ml (Fig. 4E). The Fr3 supernatant contained significantly higher levels of TGF-β1 than the freeze-thawing Fr2 supernatant (23,044 ± 3,887 pg/ml). The concentrations of TGF-β1 in the Fr2 and Fr4 supernatants were significantly higher than that in the Fr1 supernatant, whereas no significant differences in TGF-β1 concentration were noted among the Fr2, Fr3, and Fr4 supernatants (Fig. 4F). There were no significant differences in precipitate or total TGF-β1 concentrations among the four groups.

**DISCUSSION**

There are many methods for preparing PRP with various cellular and molecular components. Furthermore, the methods of PRP activation are diverse [29]. Consequently, although PRP therapy is widely applied in human and equine orthopedic surgery, the clinical outcomes vary [4, 24, 29]. Because instantly effective ingredients are present in the supernatant of activated PRP, we examined an activation method to stably maximize the growth factor concentration in PRP supernatant. In addition, we posited that the variation in outcomes can be minimized by using such PRP for treatment.

Upon injury, PDGF stimulates mitogenicity and chemotaxis of leukocytes, fibroblasts, and smooth muscle cells to the wound site, and also enhances angiogenesis via upregulation of VEGF gene expression [2]. TGF-β1 also plays a crucial role in angiogenesis and connective tissue regeneration in wound healing. Additionally, TGF-β1 is a potent inhibitor of metalloproteinase preventing collagen breakdown [2]. In humans, because the levels of these growth factors are decreased in chronic wounds, recombinant human variants of PDGF-BB have been successfully administered for such lesions [2]. In equine practice, because autologous growth factor therapy may be considered a safer application than xenogeneic therapy [30], it seems clinically important to use PRP with high concentrations of these growth factors for treatment.

PRP activation tests revealed that activated PRP contains a high concentration of growth factors regardless of the activation method. Activation of PRP by the Ca method resulted in the highest PDGF-BB concentrations. In contrast, no significant differences in TGF-β1 concentrations between the Fr and Ca methods were observed. Similar conclusions have been reported by Textor et al [30].

Our results suggest that single freeze-thawing PRP contains an abundance of PDGF-BB in the precipitate, similar to that in the supernatant. Therefore, it is likely that the single freeze-thaw method is insufficient to entirely release PDGF-BB from platelets in PRP. Compared with the addition of calcium and serum, this method results in lower levels of PDGF-BB. However, there was no difference in the TGF-β1 concentration between the Fr and Ca methods. There was a large variation in the TGF-β1 concentrations between individual PRP supernatants activated by the single freeze-thaw method. It was previously reported that TGF-β1 from disrupted leukocytes contributes to its concentration in PRP [35]. In this study, although we attempted to prepare PRP in such a manner that the presence of leukocytes was kept as low as possible, the contamination of low-dose leukocytes was inevitable. We considered the possibility that the variation in TGF-β1 concentration among individual samples was due to a non-negligible amount of TGF-β1 released from leukocytes, as has been previously reported in humans [35]. Consequently, we did not observe differences in TGF-β1 concentrations corresponding to the activation method. Our results show that a method involving the addition of calcium and autologous serum may be superior for activation of PRP for instant use. However, the concentrations of growth factors in Ca supernatants showed high variability. Therefore, it was considered important to establish an activation method capable of more stably attaining high growth factor concentrations.

Our results also suggested that repeated freeze-thaw cycles result in higher growth factor concentrations in the supernatant of PRP than that obtained by a single freeze-thaw cycle. In humans, it was reported that double freeze-thawing results in higher PDGF concentrations in PRP supernatant than that obtained by single freeze-thawing [35]. It was also reported that the concentrations of PDGF-BB, TGF-β1, EGF, and FGF increased by repetitive freeze-thawing and plateaued at 3 and 5 cycles [26]. We found that the supernatants of PRP that were freeze-thawed multiple times contained significantly higher concentrations of growth factors than those subjected to single freeze-thawing. On the other hand, no significant differences were noted among PRP that was freeze-thawed two or more times. We hypothesized that concentrations of both PDGF-BB and TGF-β1 would increase in PRP supernatants because the number of freeze-thaw cycles should cause increased disruption of platelets and growth factor release. However, we found that the concentration of neither growth factor was increased with increasing cycles of freeze-thaw. We speculate that PDGF-BB is considerably vulnerable to degradation upon freeze-thawing. Conversely, the increase in growth factor concentrations in freeze-thawed PRP is caused by de-novo release of growth factors from disrupted platelets remaining in the precipitate. Therefore, it is reasonable to hypothesize that additional cycles do not significantly affect growth factor concentration in PRP. Consequently, we did not observe differences in the TGF-β1 concentration between the Fr1, Fr2, Fr3, and Fr4 supernatants. Based on our findings, the optimal growth factor concentration in PRP supernatant may be obtained by freezing and thawing at least twice.

Growth factor concentrations in Fr1 samples were higher than those in Fr samples in the activation test, although these were similar supernatants activated by the single freeze-thaw method. McClain and McCarril indicated that the growth factor content in equine PRP varies with long-term cryopreservation [22]. In our activation test, it was necessary to determine the concentrations of growth factors in PRP preparations within 6 hr in order to measure stable concentrations consistently between all sample types.
REFERENCES

1. Aiko, J. L., Rodriguez, A. E., Abdelghany, A. A. and Oliveira, R. F. 2017. Autologous platelet-rich plasma eye drops for the treatment of post-LASIK chronic ocular surface syndrome. J. Ophthalmol. 2017: 2457620. [Medline] [CrossRef]

2. Barrientos, S., Stojadinovic, O., Golinko, M. S., Brem, H. and Tomic-Canic, M. 2008. Growth factors and cytokines in wound healing. Wound Repair Regen. 16: 585–601. [Medline] [CrossRef]

3. Bosch, G., van Schie, H. T., de Groot, M. W., Cadby, J. A., van de Lest, C. H., Barneveld, A. and van Weeren, P. R. 2010. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: A placebo-controlled experimental study. J. Orthop. Res. 28: 211–217. [Medline] [CrossRef]

4. Brossi, P. M., Moreira, J. J., Machado, T. S. and Baccarin, R. Y. 2015. Platelet-rich plasma in orthopedic therapy: a comparative systematic review of clinical and experimental data in equine and human musculoskeletal lesions. BMC Vet. Res. 11: 98. [Medline] [CrossRef]

5. De Rossi, R., Coelho, A. C., Mello, G. S., Frazílio, F. O., Leal, C. R., Facco, G. G. and Brum, K. B. 2009. Effects of platelet-rich plasma gel on skin healing in surgical wound in horses. Acta Cir. Bras. 24: 276–281. [Medline] [CrossRef]

6. Dhurat, R. and Sukesh, M. 2014. Principles and methods of preparation of platelet-rich plasma: A review and author’s perspective. J. Cutan. Aesthet. Surg. 7: 189–197. [Medline] [CrossRef]

7. Everts, P. A., Knape, J. T., Weibrich, G., Schönberger, J. P., Hoffmann, J., Overdevest, E. P., Box, H. A. and van Zundert, A. 2006. Platelet-rich plasma and platelet gel: a review. J. Extra Corpor. Technol. 38: 174–187. [Medline] [CrossRef]

8. Fukuda, K., Miyata, H., Kuwano, A., Kuroda, T., Tamura, N., Kotoyori, Y. and Kasashima, Y. 2017. Does the injection of platelet-rich plasma and activated for clinical use 

9. Giles, B. M., Underwood, T. T., Benhadji, K. A., Nelson, D. K., Grobeck, L. M., Lin, B., Wang, S., Fill, J. A., Man, M., Pitts, K. R. and Bamberg, A. 2018. Analytical characterization of an enzyme-linked immunosorbent assay for the measurement of transforming growth factor β1 in human plasma. J. Appl. Lab. Med. 3: 200–212. [Medline] [CrossRef]

10. Hammond, J. W., Hinton, R. Y., Curl, L. A., Muriel, J. M. and Lovering, R. M. 2009. Use of autologous platelet-rich plasma to treat muscle strain injuries. Am. J. Sports Med. 37: 1135–1142. [Medline] [CrossRef]

11. Hauschild, G., Geburek, F., Goshgeher, G., Eveslage, M., Serrano, D., Streitbürger, A., Johannülken, S., Menzel, D. and Mischke, R. 2017. Short- and long-term storage stability at room temperature of two different platelet-rich plasma preparations from equine donors and potential impact on growth factor concentrations. BMC Vet. Res. 13: 7. [Medline] [CrossRef]

12. Hsu, W. K., Mishra, A., Rodeo, S. R., Fu, F., Terry, M. A., Randelli, P., Canale, S. T. and Kelly, F. B. 2013. Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. J. Am. Acad. Orthop. Surg. 21: 739–748. [Medline] [CrossRef]

13. Iacopetti, I., Perazzi, A., Ferrari, V. and Busetto, R. 2012. Application of platelet-rich gel to enhance wound healing in the horse: A case report. J. Equine Vet. Sci. 32: 123–128. [CrossRef]

14. Ito, Y., Nakachi, K., Imai, K., Hashimoto, S., Watanebe, Y., Inaba, Y., Tamakoshi, A., Yoshimura T., JACC Study Group 2005. Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta, soluble Fas, and superoxide dismutase activity for the JACC study. J. Epidemiol. 15 Suppl 1: S67–S73. [Medline] [CrossRef]

15. Jee, C. H., Eom, N. Y., Jung, H. M., Jung, H. W., Choi, E. S., Won, J. H., Hong, I. H., Kang, B. T., Jeong, D. W. and Jung, D. I. 2016. Effect of autologous platelet-rich plasma application on cutaneous wound healing in dogs. J. Vet. Sci. 17: 79–87. [Medline] [CrossRef]

16. Kim, J. H., Park, C. and Park, H. M. 2009. Curative effect of autologous platelet-rich plasma on a large cutaneous lesion in a dog. Vet. Dermatol. 20: 123–126. [Medline] [CrossRef]

17. Kushida, S., Kakudo, N., Morimoto, N., Hara, T., Ogawa, T., Mitsuji, T. and Kasumoto, K. 2014. Platelet and growth factor concentrations in platelet concentrates. J. Oral Maxillofac. Surg. 62: 489–496. [Medline] [CrossRef]

18. Lanca, J. F. S. D., Purita, J., Paulus, C., Huber, S. C., Rodrigues, B. L., Rodrigues, A. A., Madureira, J. L. Jr., Malheiros Luzo, Â. C., Belangero, W. D. and Annichino-Bizzacchi, J. M. 2017. Contributions for classification of platelet rich plasma—proposal of a new classification: MARSPILL. Regen. Med. 12: 565–574. [Medline] [CrossRef]

19. Magalon, J., Chateau, A. L., Bertrand, B., Louis, M. L., Silvestre, A., Giraudou, L., Veran, J. and Sabatier, F. 2016. DEPA classification: a proposal for standardising PRP use and a retrospective application of available devices. BMJ Open Sport Exerc. Med. 2: e000060. [Medline] [CrossRef]

20. Marx, R. E. 2004. Platelet-rich plasma to support its use. J. Oral Maxillofac. Surg. 62: 489–496. [Medline] [CrossRef]

21. Marx, R. E., Carlson, E. R., Eichstaedt, R. M., Schimmele, S. R., Strauss, J. E. and Georgeff, K. R. 1998. Platelet-rich plasma: Growth factor enhancement for bone grafts. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 85: 638–646. [Medline] [CrossRef]

22. McClain, A. K. and McCarrel, T. M. 2019. The effect of four different freezing conditions and time in frozen storage on the concentration of commonly measured growth factors and enzymes in equine platelet-rich plasma over six months. BMC Vet. Res. 15: 292. [Medline] [CrossRef]

23. Mei-Dan, O., Lippi, G., Sánchez, M., Andia, I. and Maffulli, N. 2010. Autologous platelet-rich plasma: a revolution in soft tissue sports injury

[11]. In contrast, frozen PRP stored for at least 1 month was thawed and measured in the repetitive freeze-thaw test, which may be the cause of the difference in values obtained. We found that high concentrations of growth factors can be maintained even after 1 month of PRP cryopreservation. Furthermore, 2.4-fold higher PDGF-BB and 1.5-fold higher TGF-β1 concentrations were obtained using double freeze-thawing for activation than by using single freeze-thawing. This indicates that double freeze-thawing can result in growth factor concentrations and stability that are comparable to calcium and serum addition. Consequently, PRP activated by the Ca method can be applied quickly and appears to be suitable for instant use. However, growth factors once extracted into the supernatant are prone to inactivation. Hence, if activated PRP is intended for repeated long-term use, it is easier to prepare PRP in large cryopreserved batches by dispensing it in small quantities and double freeze-thawing when used.

In this study, we investigated only two growth factors and did not investigate other components in PRP. On the other hand, as mentioned above, it is known that these two growth factors, which are abundant in platelets, are essential contributors to the wound healing process. Therefore, when considering the clinical effects of PRP therapy, these growth factors must be focused on. However, further studies are needed to determine the appropriate application method of equine PRP optimally prepared and activated for clinical use [29]. In conclusion, our findings suggest that activation by adding calcium and autologous serum is optimal for instant use of PRP and that double freeze-thawing is an easier and optimal activation method for cryopreserved PRP.
management? Phys. Sportsmed. 38: 127–135. [Medline] [CrossRef]
24. Navani, A., Li, G. and Chrystal, J. 2017. Platelet rich plasma in musculoskeletal pathology: A necessary rescue or a lost cause? Pain Physician 20: E345–E356. [Medline] [CrossRef]
25. Sano, K., Takai, Y., Yamanishi, J. and Nishizuka, Y. 1983. A role of calcium-activated phospholipid-dependent protein kinase in human platelet activation. Comparison of thrombin and collagen actions. J. Biol. Chem. 258: 2010–2013. [Medline]
26. Strandberg, G., Sellberg, F., Sommar, P., Ronaghi, M., Lubenow, N., Knutson, F. and Berglund, D. 2017. Standardizing the freeze-thaw preparation of growth factors from platelet lysate. Transfusion 57: 1058–1065. [Medline] [CrossRef]
27. Sundman, E. A., Cole, B. J. and Fortier, L. A. 2011. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. Am. J. Sports Med. 39: 2135–2140. [Medline] [CrossRef]
28. Sutter, W. W., Kaneps, A. J. and Bertone, A. L. 2004. Comparison of hematologic values and transforming growth factor-β and insulin-like growth factor concentrations in platelet concentrates obtained by use of buffy coat and apheresis methods from equine blood. Am. J. Vet. Res. 65: 924–930. [Medline] [CrossRef]
29. Tambella, A. M., Martin, S., Cantalamessa, A., Serri, E. and Attili, A. R. 2018. Platelet-rich plasma and other hemocomponents in veterinary regenerative medicine. Wounds 30: 329–336. [Medline]
30. Textor, J. A. and Tablin, F. 2012. Activation of equine platelet-rich plasma: comparison of methods and characterization of equine autologous thrombin. Vet. Surg. 41: 784–794. [Medline] [CrossRef]
31. Textor, J. A., Norris, J. W. and Tablin, F. 2011. Effects of preparation method, shear force, and exposure to collagen on release of growth factors from equine platelet-rich plasma. Am. J. Vet. Res. 72: 271–278. [Medline] [CrossRef]
32. Waselau, M., Sutter, W. W., Genovese, R. L. and Bertone, A. L. 2008. Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in Standardbred racehorses. J. Am. Vet. Med. Assoc. 232: 1515–1520. [Medline] [CrossRef]
33. Xie, X., Zhao, S., Wu, H., Xie, G., Huangfu, X., He, Y. and Zhao, J. 2013. Platelet-rich plasma enhances autograft revascularization and reinnervation in a dog model of anterior cruciate ligament reconstruction. J. Surg. Res. 183: 214–222. [Medline] [CrossRef]
34. Yin, W., Xu, H., Sheng, J., Zhu, Z., Jin, D., Hsu, P., Xie, X. and Zhang, C. 2017. Optimization of pure platelet-rich plasma preparation: A comparative study of pure platelet-rich plasma obtained using different centrifugal conditions in a single-donor model. Exp. Ther. Med. 14: 2060–2070. [Medline] [CrossRef]
35. Zimmermann, R., Arnold, D., Strasser, E., Ringwald, J., Schlegel, A., Wiltfang, J. and Eckstein, R. 2003. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. Vox Sang. 85: 283–289. [Medline] [CrossRef]