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Preparation of Autologous Platelet Rich Plasma in Kathiawari and Thoroughbred Horses

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A B S T R A C T

Platelet rich plasma (PRP) is a newly emerging autologous product in regenerative medicine. There are various methods of preparation available worldwide. But still there is no unique method for preparation of platelet rich plasma. Autologous platelet rich plasma is safe from disease transmission, immune reaction and cross contamination. Platelet rich plasma is a blood derivative which is prepared by different centrifugation method generally greater than two to four times higher when compared with baseline value. In this study, platelet rich plasma was prepared by double centrifugation method. There was a significant increase of platelets in PRP when compared to whole blood.

Introduction

PRP has a pool of growth factors like platelet derived growth factor (PDGF), transforming growth factor (TGF-ß), vascular endothelial growth factor (VEGF) as well as cytokines such as platelet factor-4 (PF4) and CD40L.

They are widely used in dental implant surgery, orthopaedic surgery, muscle/tendon repair, osteoarthritis and skin ulcer (Anitua*et al.*, 2004). Platelet degranulation releases the growth factors and other substances. These growth factors promote tissue repair and influence the reactivity of vascular and other blood cells in inflammation and angiogenesis (Fortier and Smith, 2008).

Materials and Methods

Total of six horses were included in this study. They were free from any systemic diseases and had normal body condition. Fifteen milliliters of whole blood was collected in EDTA vials by using 16 gauge needled syringe (Amaral *et al.*, 2016) from jugular vein of each horse.

The blood was transferred into 15 mL graduated centrifuge tube and centrifuged at 120g for 5 minutes by using refrigerated Eppendorf centrifuge 5430 R at 4°C (Bi *et al.*, 2010). The first 50% of the top supernatant plasma fraction adjacent to the buffy coat was collected and centrifuged at 240g for 5 minutes. The bottom fourth was considered as

pure platelet rich plasma (P-PRP) (Rios *et al.*, 2015). The process was carried out within half an hour after blood collection.

The platelet concentration was analyzed using Auto Hematology Analyzer (mindray BC-2800Vet). The whole blood and PRP ratio was calculated as followed by Bosch *et al.*, (2010).

$$\frac{\text{Platelet count of PRP}}{\text{Platelet count of whole blood}}$$

Results and Discussion

The mean platelets and leukocytes concentration of whole blood, first centrifuge and second centrifuge is given in table 1. The platelet concentration was increased ranging from 2.1 to 3.4 fold from whole blood concentration. Statistical analysis revealed a highly significant increase ($P<0.01$) in platelet concentration in second centrifuge from whole blood. PRP of first and second centrifuge were stained with Giemsa stain (Figure 1).

Table.1 Platelet and leukocyte concentration in PRP

	Platelets				Leukocytes		
Horse No	**Base value**	**First centrifuge**	**Second centrifuge**	**P:L ratio**	**Base value**	**First centrifuge**	**Second centrifuge**
1	131000	529000	450000	3.4	8500	22100	18600
2	140000	412000	345000	2.5	8400	9000	7000
3	124000	374000	355000	2.9	4200	10500	8500
4	111000	381000	252000	2.3	9300	11600	5500
5	175000	521000	360000	2.1	8500	3400	3300
6	201000	471000	412000	2.1	11300	30000	24000

Table.2 Mean ± SE Platelets and WBC concentration

	Whole blood	First centrifuge	Second centrifuge	P value
Platelets	147000±13940.34[a]	448000±28095.08[c]	362333.33±27498.69[b]	**0.001****
WBC	8366.67±946.46	14433.33±3982.68	11150±3358.65	**0.459**[NS]

* Significant difference $P < 0.05$, ** Highly significant $P < 0.01$ and
[NS] Non-significant $P > 0.05$, each group was significant from each other

Fig.1 Photomicrograph showing platelets (arrows) and leukocytes after first centrifuge (1a) and second centrifuge (1b) x 1000- Giemsa staining

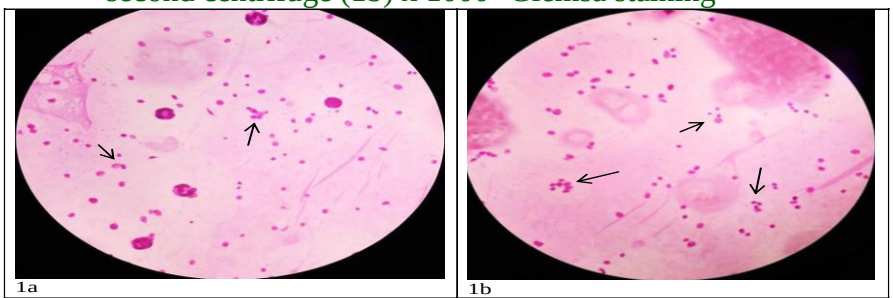

Figure 1: Photomicrograph showing platelets (arrows) and leukocytesafter first centrifuge (1a) and second centrifuge (1b) x 1000- Giemsa staining.

This concentration was used intra-articularly in the same horse for osteoarthritis. No

adverse effects were notice with this leukocyte concentration. In conclusion, this type of double centrifugation method provide adequate amount of platelet concentration with reduced leukocyte concentration. This PRP preparation is simple, cost effective and non-invasive procedure and this can be prepared at the time of use.

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