

# The Effect of Platelet Rich Plasma on The Survival of Follicles in Hair Transplantation: An Experimental Study on Rats

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**Abstract:** *Background:* Platelet Rich Plasma (PRP) is currently used for a wide variety of medical applications and involves IGF, VEGF, EGF which are important in angiogenesis and endothelial differentiations. EGF, IGF, TGF-  $\beta$ , are other substance of PRP and simultaneously effect to hair growth.

*Objectives:* The authors decided to observe the influence of PRP to the success rate and vascularization rate of hair transplantation.

*Methods:* Control and PRP application sides are determined on the dorsal skin of rats. 2x1 cm skin islands were excised and punched to get out hair follicle grafts. Then hair follicles were transplanted into subcutaneous dorsal pockets. Injection of 0.2 ml PRP was made in to the pouch. 1 (20 rats) and 2 (20 rats) months after transplantation, skin sample including the hair follicle were removed and analyzed by immunochemical CD31 and direct microscope.

*Results:* After 1 month, control and PRP groups exhibited similar density of follicle shaft. But degree of capillary formation in PRP group was higher than that of the control groups. After 2 months, degree of capillary formation and follicle shaft numbers were significantly higher than that of control groups.

*Conclusions:* Increase of capillaries were correlated PRP involving IGF, VEGF, EGF which are important in angiogenesis and endothelial differentiations. Increase of hair shaft number was correlated with PDGF's mitogen and chemoattractant effect to mesenchymal cells and stem cells. Also PRP involves EGF, IGF, TGF-  $\beta$ , these affect hair growth.

In conclusion, PRP and hair transplantation collocation to may enhance the success of hair transplantations.

**Keywords:** Platelet Rich Plasma (PRP), hair loss, hair loss treatment, experimental study.

## INTRODUCTION

First hair transplantation has been designed at Newyork city by Norman Orentreich in 1952 [1]. At present hair transplantation is the gold standard of hair loss treatment [2]. According to surgical evolution of hair transplantation, it was started with macro punch technique, after than the technique was developed from micro punch to follicular unit transplantation (FUT) technique or follicular unit extraction (FUE) technique. Both of these technique aimed to transfer 30 unit/cm<sup>2</sup> follicular units for per area for satisfactory result [2-5]. Despite several advantages of FUE technique to the punch techniques, but requirements of adequate donor area and viable graft ratio are still challenging on advanced case. Successful results are required enough experiences of the team and good tissue quality of hair follicles [6].

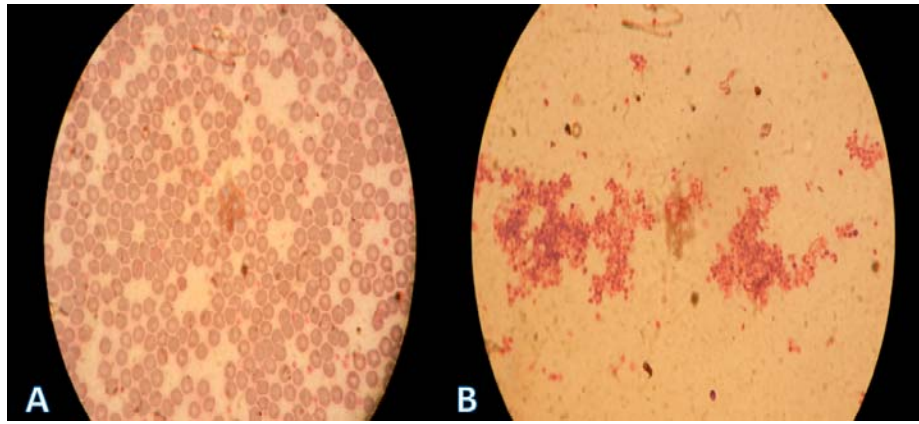
Platelet Rich Plasma (PRP) is obtained from the plasma *via* simple centrifugation. It contains bioactive proteins, cytokines, chemokine's, coagulation and

adhesion proteins and various growth factors [7]. These rich components promote regeneration potential on problematic chronic wound healing, maxillofacial surgery and spinal surgery [8], in addition to that is used in aesthetic surgery and as an antiaging agent for facial regeneration/rejuvenation [7]. According to our literature review, despite some of PRP ingredients, i.e. PDGF, TGF-B, VEGF, IGF-1, FGF are likely to improve the growth and regeneration of the hair follicle [9], but here is a few experimental animal study to test the effects of PRP on the survival of follicles in hair transplantation. We studied the effect of PRP on vascularization and follicle graft survival.

## MATERIALS AND METHODS

Male (Inbred) Spraque-Dawley rats (n=70, XX months old, weighing around 250 g), were obtained from Bezmi Alem Vakif University Research Center (Istanbul, Turkey). The study has the permission of the ethics review committee for ethics in animal experiments of the Bezmi Alem Vakif University (2014/278), and guidelines for the care and use of laboratory animals were strictly followed. The animals were anesthetized with 35 mg/kg ketamine hydrochloride, 5 mg/kg xylazine hydrochloride. 10

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**Figure 1:** (A-B) Direct microscopic view of platelets' concentration on PRP specimen.

animals were sacrificed to harvest blood (8 cc per animal) by intracardiac puncture. PRP was separated by ready disposable sterile PRP tube (8 cc liquid capacity, 16x125 mm, involve 0.1 molar/ml sodiumcitrate, medium layer include 3 gr polyester gel, the lower layer 2ml polysaccharide sodium diatrizoate) by 3200 speed, 8 minute centrifugation (Figure 2A). PRP' s platelets concentration were counted and confirmed under microscopy and automatic hemogram blood test (average 2.5-3 fold) (Figure 1).

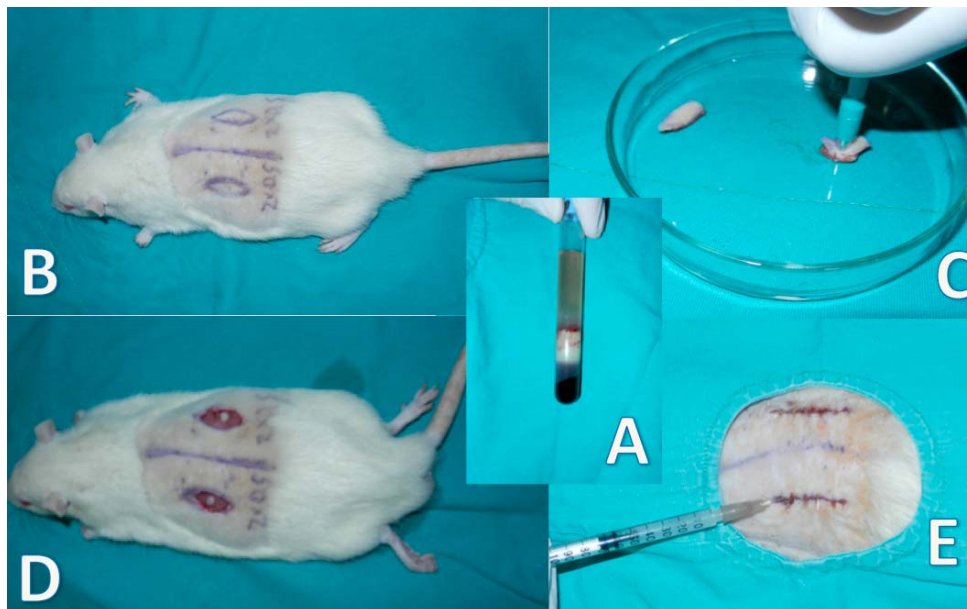
### Surgical Procedure

Dorsal skin island (2x1 cm) on left and right side were excised and harvested hair follicles on these skin grafts by 4 mm punch (Figure 2B-C). Hair follicle grafts were transplanted into subcutaneous dorsal pockets

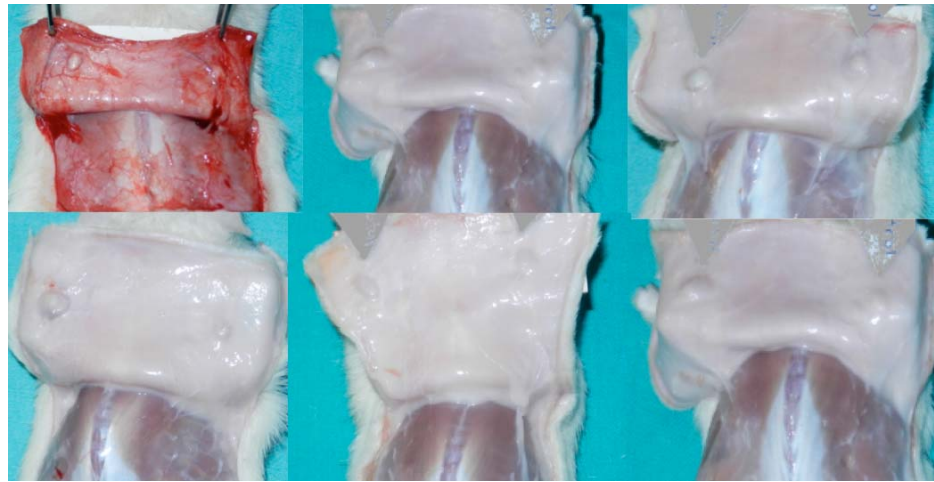
which was formed by the excision skin graft (Figure 2D). After the closure of the wound single 0.2 cc PRP injection was done in to the pouch only in the experiment group (Figure 2E).

After transplantation on first (20 rats) and second (20 rats) months, skin sample involving the hair grafts were removed and fixed in a 10% formaldehyde solution, embedded in paraffin, and sectioned in 4 nanometer increments (Figure 3).

These were stained with haematoxylin-eosin reagent and immunochemical CD31. Perifollicular capillary and follicle shaft were photographed and evaluated on each field. Histological assessment was performed on same section, was calculated average to the 3 highest number of vessels and follicle shafts number.



**Figure 2:** Experiment stages: centrifugation of blood by sterile disposable tube (A), dorsal skin is signed (B), excised skin graft is punched by 4 mm punch for harvesting hair follicular unit (C). Hair follicle graft were transplanted into subcutaneous dorsal pockets on right and left sides (D), 0,2 ml PRP is injected left side (E).



**Figure 3:** Skin sample involving the hair graft were removed.

Paired T test was used to evaluate of significance differences. P values of <0.05 were considered.

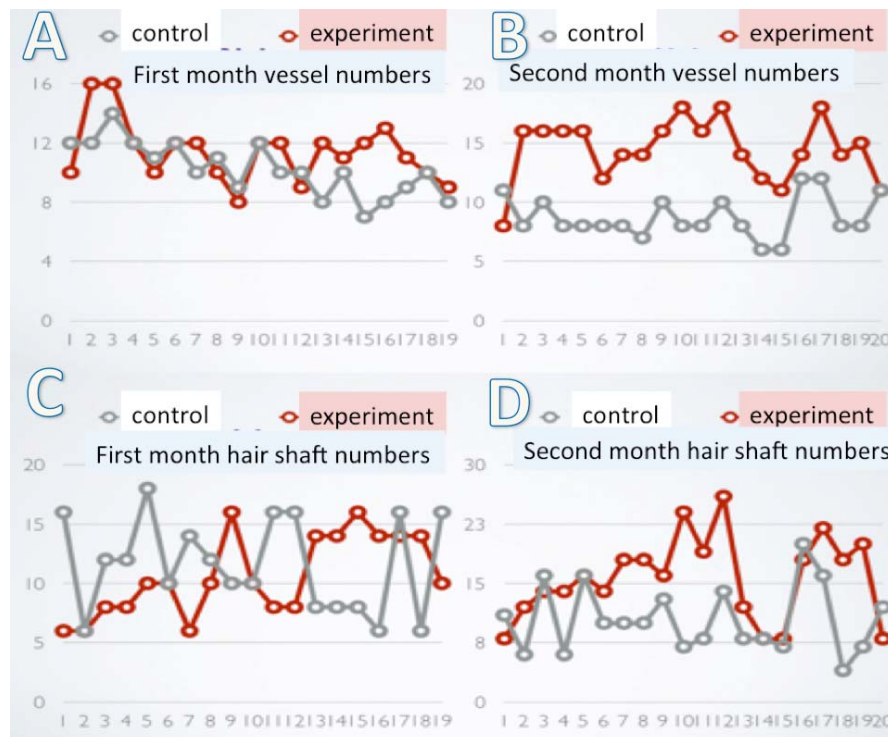
**RESULTS**

Only one animal discarded from the study because of the general state of disorder. There were numerical quantity differences between control and PRP groups about vessel and follicle shaft numbers (Figure 4). At first month, PRP and control groups’ follicular shafts were observed similar position (average 11.5, 10.6

n=19) (Figure 5), but, at second month, follicle shaft in PRP group was higher than control groups (average 10.4, 15.6 n=20) (Figure 6). At first and second month, PRP groups’ vessel numbers were significantly higher than control groups (average 11.4,10.2 n=19, average 14.4, 8.6 n=20) (Figure 7).

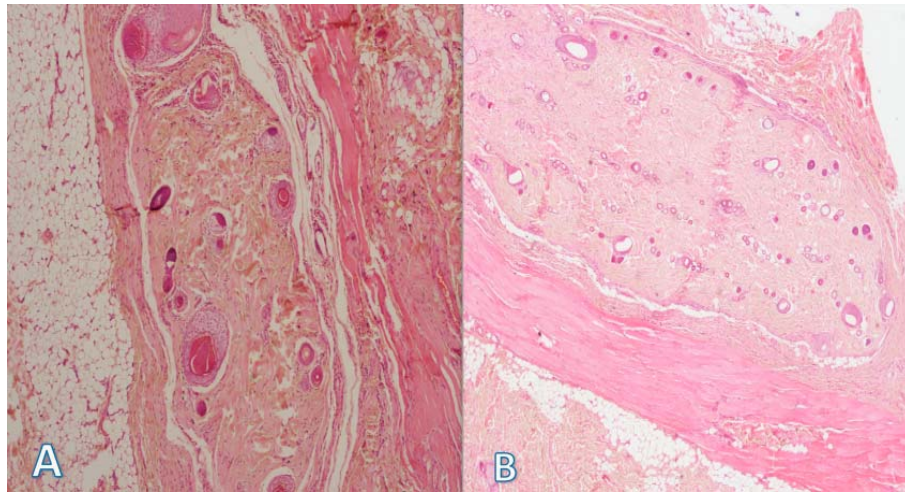
**DISCUSSION**

Hair lost is general aesthetic problem of not only manliness but also some sort of women. At last 3

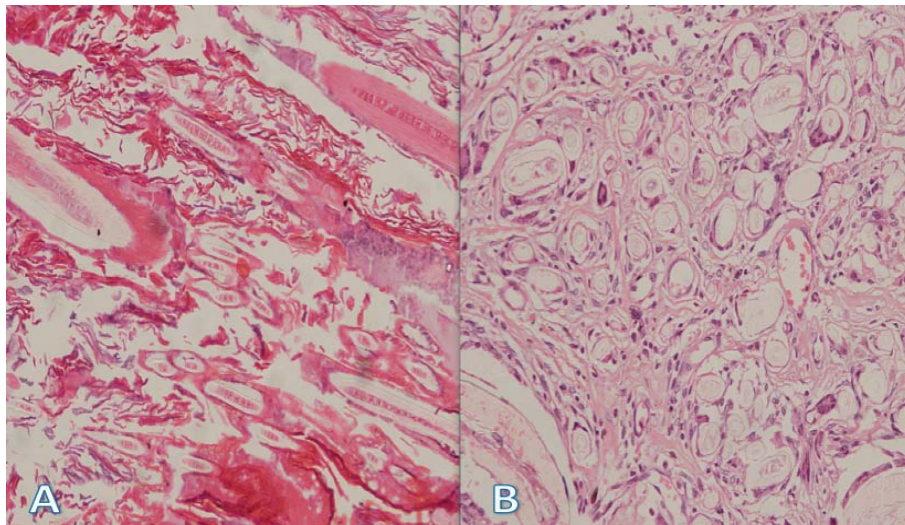


**Figure 4:** Hair shaft and vessel numbers. At first (A) and second (B) month, PRP groups’ vessel numbers were significantly higher than control groups (average 11.4,10.2 n=19, average 14.4, 8.6 n=20). At first month, PRP and control groups’ follicular shaft were observed similar position (average 11.5, 10.6 n=19) (C), but, at second month, follicle shaft in PRP group was higher than control groups (average 10.4, 15.6 n=20) (D).

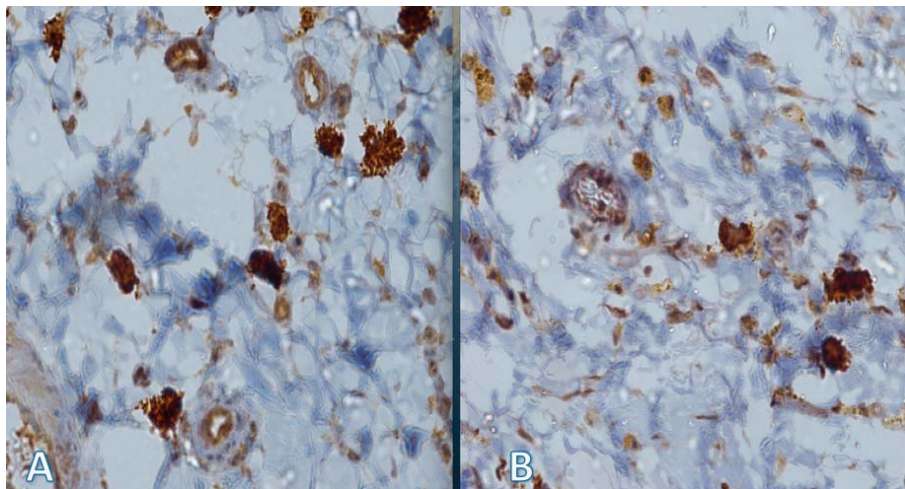




**Figure 5:** First month, hair shaft histologic view of control (A) and experiment (B) sides.



**Figure 6:** Second month, hair shaft histologic view of control (A) and experiment (B) sides.



**Figure 7:** Perifollicular vessel histologic view (CD31), control (A) and experiment (B) sides.

decades, there are many medications like minoxidile, finasteride, ketoconazole, dutasterid, latanoprost,

namidile, lemuteporfin, but they may develop many complications like undesirable hipertrikozis,

gynecomastia, empotans, immune suppression, healing problems, sexual dysfunction, ocular problems [10]. Low laser therapy increase ATP synthesis on skin. But still nowadays hair transplantation is gold standard on hair lost treatments [2]. Success of hair transplantation needs to transplant 35x45 follicle/cm<sup>2</sup> for enough density [6]. Other important condition is receiving environment's blood supply. Hair transplantations success rate is very low on scar tissue [11].

Hair transplantation was done with macro-punch technique initially, scarring at donor area and lack of homogeneity of implanted follicles are main disadvantages. Following micro-punch technique was developed, but it also could not completely solve those problems. Finally FUT or FUE technique was popularized to improve above-mentioned disadvantages. Remaining challenges were the need of adequate donor area, viable graft ratio and skills of the team, since it is absolutely a team work, the experiences of the team is important determinants of the successful results [1]. Adequate donor area is an important subject, which can be solved with tissue engineering studies. Such as to improve the viable ratio of follicles as like as our study.

Platelet Rich Plasma (PRP) is generally ten times concentrated form of platelets, is obtained from the plasma via simple centrifugation. PRP involves more than 20 growth factors like PDGF, TGF-beta, EGF, FGF, VEGF, and IGF. FGF and VEGF have angiogenesis potentially and EGF, IGF, TGF-beta [12].

There are many publicans about PRP's vascularization and regeneration effects on wound healings. An experimental study exposed to increase of PRP hair transplantation's success [13]. Rastegar *et al.* published to the PRP and some herbal products have a regulatory effect on dermal papilla [14]. Another study demonstrated that PRP reduced on alopecia areata effect on hair [15].

De Sousa *et al.* illustrated PRP action on androgenic alopecia at Wnt pathway [16]. Valente *et al.* found out that PRP's positive impression on dermal papilla [17]. PRP was evaluated positive exchange of stem cell and hair follicle by Li *et al.* [18]. PRP accelerate to grow of hair follicle [12]. Our study also supported that PRP has a positive impact on survival of hair follicles, it is proved in our study and demonstrated with the statistically significant improved difference of the groups. According to result, this statistically

significant difference is very important, since systemic effects due to the resorption of PRP can be judged but if this is a reality it can be a factor of both sides in the animal, whereas we obtained the significant difference on vascularization on the experiment side.

Our study is a simple research i.e., there was only one variable, or only the presence of PRP was the variable. All other terms and conditions were constant.

Rat's hair cycle (anagen, katagen, telogen) is 1 month and study aimed to obtain effect of PRP on first and second hair cycles on rats [19]. The main contribution of our study in this area of researches that to demonstrate PRP effect of vascularization and viability of hair graft on hair transplantation.

## CONCLUSION

PRP's effect on hair follicle entitled studies continue as just. Author found out increments of vessels and follicular shaft on PRP groups on hair transplantation experimental model. It may be make sense of PRP has a positive effect on hair follicle viability after transplantation.

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