# The Role of Platelet Plasma Growth Factors in Male Pattern Baldness Surgery

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**Background:** Follicular units are commonly used in baldness surgery, and they have become a global procedure for both male and female patients. The yield from micrografts varies between 70 and 85 percent. Yield is determined by factors such as quality of the harvested donor area, preparation of the units, care taken during the implantation procedure, and follicular apoptosis. To improve hair density and stimulate follicular unit growth, an experimental study was designed using platelet plasma growth factors obtained from the patient's autologous plasma.

**Methods:** The author established a protocol within a group of 20 patients with male pattern baldness. The data showed a gaussian distribution; to compare the two procedures involved in this clinical trial, the paired *t* test was used.

**Results:** The author observed a significant difference in the yield of follicular units when comparing the experimental with the control areas of the scalp ($p < 0.001$). The areas treated with platelet plasma growth factors demonstrated a yield of 18.7 follicular units per $cm^2$, whereas the control areas yielded 16.4 follicular units per $cm^2$, an increase in follicular density of 15.1 percent. Among patients who used the experimental protocol, some experienced only 3 percent and others experienced a 52 percent increase in density.

**Conclusions:** This study provides a new perspective and contribution to baldness surgery with follicular unit megasessions, and demonstrates an improvement that can be introduced into baldness surgery clinics with less morbidity and a low cost-to-benefit ratio. Further studies may improve the efficiency of the technique and allow digital programs to better evaluate the increase in hair density. (*Plast. Reconstr. Surg.* 118: 1458, 2006.)

Baldness surgery with micrografts and minigrafts performed in megasessions was described in 1989 and 1991,[1,2] and today it is a widely used technique for treating both male and female hair pattern baldness. The procedure transplants great quantities of follicular units harvested from the posterior occipital area of the scalp and places them in bald regions. These transplanted units carry a good genetic histology, providing the future hair with the same quality of growth, durability, and characteristics of the donor area.

Implanted hair growth has an individual cycle. During the first 2 weeks of implantation, the catagen phase occurs, marked by an inflammatory process in which redness in the scalp and shedding of the hair shaft is common. The patient then enters the telogen phase, which lasts between 3 and 4 months. This is followed by the latency period, which precedes the third phase, anagen. During this phase, the future hair begins to grow. During the resting period, substantial follicular unit loss can occur because of apoptosis. Between 15 and 30 percent of the implanted grafts will be either eliminated or absorbed by the scalp. Therefore, only 70 to 85 percent of the implanted hair will sprout. Considering this important hair loss fact, a clinical trial using platelet plasma growth factors obtained from the patient's own plasma was developed. This

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experimental research was submitted to and approved by the Ethics Committee of the Pontificia Universidade Catolica do Rio Grande Do Sul, Division of Plastic Surgery.

## PLATELET GROWTH FACTORS AND HAIR STEM CELLS

The first articles on growth factors derived from plasma appeared during the 1970s and 1980s[3–8] as an application for tissue repair and hemostasis during the healing process of ulcers and undermined wound surfaces. More recent works in orthopedics and odontology[9–11] demonstrated the role of such factors in bone graft recomposition and in teeth osteosynthesis. Man et al. in 2001[12] and Bhanot and Alex in 2002[13] reported new applications of platelet-rich plasma in wound areas of cosmetic procedures.

The growth factors contained in platelets of blood plasma are basically three: platelet-derived growth factor (PDGF), transforming growth factor (TGF)-$\beta$, and vascular endothelial growth factor (VEGF). They are protein molecules which, in contact with their respective receptors, act in tissue angiogenesis, stimulating the healing and growth of new organic structures.[14–17] The action of growth factors on the germinative hair cycle has already been studied in both its embryologic and its adult phases[18–26]; however, it has not yet been studied in hair micrograft implantation surgery. No clinical trial or experimental protocol has previously been performed to verify the efficiency of those factors in the growth and density of implanted follicular units. Growth factors act in the bulge area, where stem cells are found, and they interact with cells of the matrix, thus activating the proliferative phase of the hair (Fig. 1). Stem cells are more primitive and of ectodermal origin. They give origin to epidermal cells and sebaceous glands. Germinative cells of the matrix, which are found at the dermal papilla, are of mesenchymal origin (Fig. 2). Both cells needs each other, and when they get together through the action of various growth factors (PDGF, TGF-$\beta$, and VDGF), they give rise to the future follicular unit, which consists of the hair shaft, sebaceous glands, erectus pilus muscle, and the perifolliculum. Headington described this histologic unit in 1984.[27] It is the complete and developed follicular structure, being in the anagen hair cycle phase, which lasts from 3 to 6 years in our scalp.

## PATIENTS AND METHODS

Twenty male patients aged 25 to 55 years with male hair pattern baldness in the frontal, parietal, or occipital area were selected for this experiment. Two symmetric 2.5 × 2.5-cm bald areas were delineated (Fig. 3). On the right side, follicular units embedded with platelet plasma growth factors were implanted; on the left side, untreated follicular units were implanted as controls. Both areas

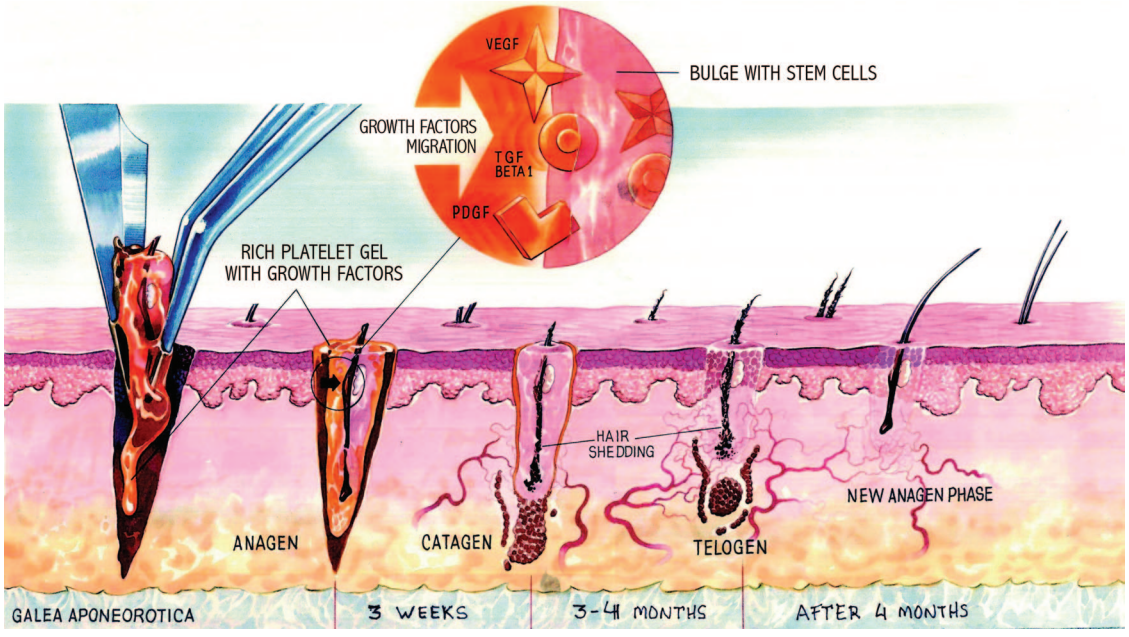


**Fig. 1.** Schematic view of the follicular units being implanted with platelet plasma growth factors, showing the dystrophic shading phase and the new proliferative phase with an intense vascular endoneogenesis supporting the new hair development to the anagen phase. There is an intense growth factor migration into the stem cells in the bulge area.

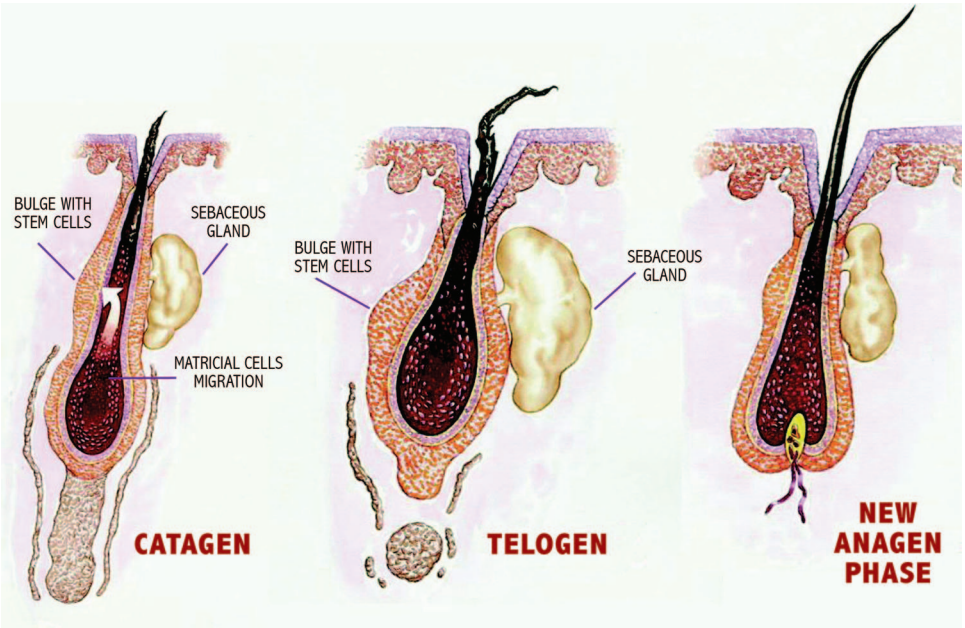


**Fig. 2.** Hair follicle cycle. The meeting between matricial cells from the papilla and the stem cells in the bulge area starts the growing phase of the new hair follicle.

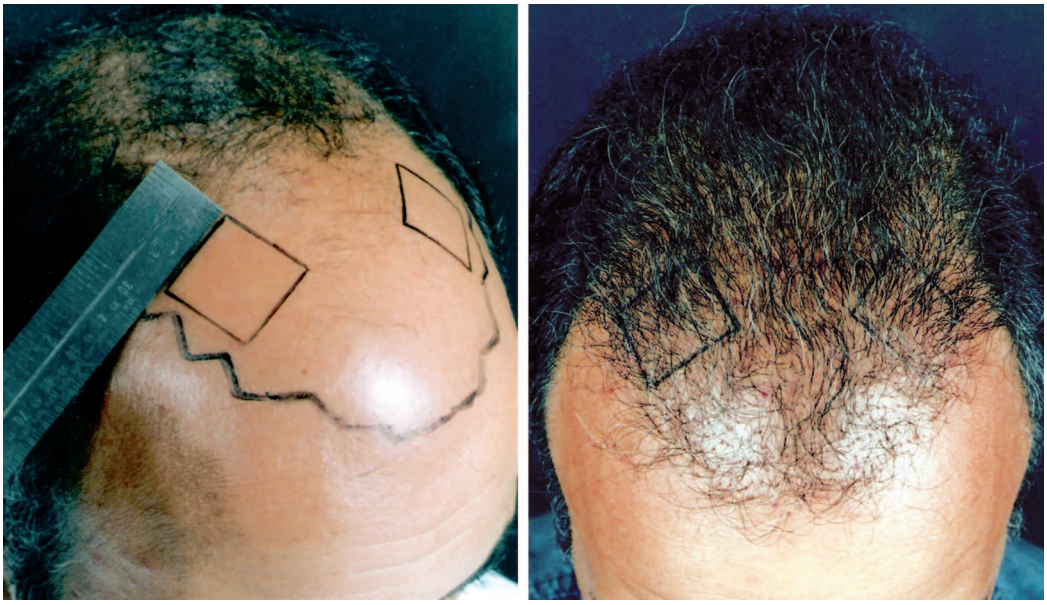

**Fig. 3.** Two identical areas of 2.5 × 2.5 cm are delineated in the bald area. On the right side of the patient's head, follicular units imbibed with platelet plasma growth factors were implanted; on the left side, standard follicular units were implanted as a control.

were implanted with an equal number of micrografts. All patients were duly informed about the clinical trial and signed informed consent documents. The research was submitted and approved by the Ethics Committee of Pontificia Universidad Catolica do Rio Grande do Sul. The following surgical routine was observed.

## Technique

### Harvesting of Follicular Units

In all cases, a hair-bearing ellipse flap was taken from the occipital area of the scalp above the neck. The flap size varied according to the amount of follicular units needed. For medium-type baldness,

an ellipse is obtained that is usually 15 cm long by 2 cm wide from which 1200 units can be obtained (Fig. 4). The donor area is closed, without tension, using an intradermal or continuous suture, to enable, if necessary, a secondary harvesting of grafts 3 to 4 years later if the patient desires. Two groups of 180 follicular units were harvest and prepared—one group was imbibed with platelet plasma growth factors and the other was kept wet with saline solution on an acrylic surface.

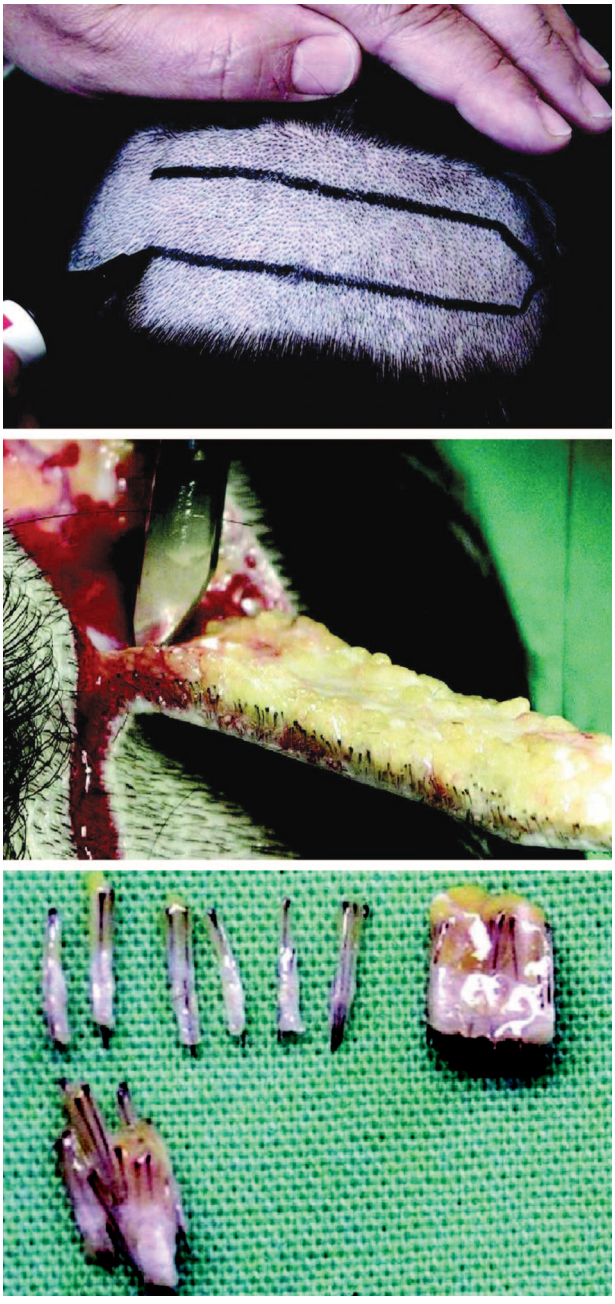

**Fig. 4.** Harvesting of the follicular units in the posterior occipital area, where the best histologic and genetic quality of hair is present. The units are implanted by the "stick-and-place" punctiform technique.

**Obtaining the Platelet Plasma Growth Factors**

Before surgery, 80 cc of blood was withdrawn from the patient in eight vacuum flasks, with each

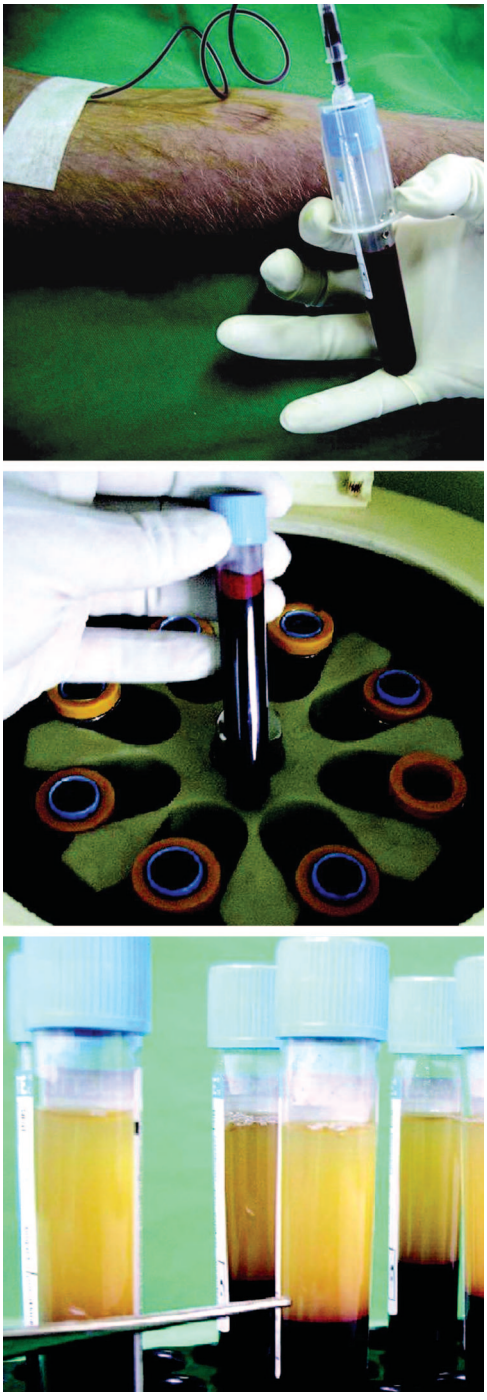

**Fig. 5.** Eighty cubic centimeters of autologous blood is withdrawn and centrifuged at 1000 rpm to avoid discharging the platelets to the flask bottom. The total plasma is then recentrifuged into four new flasks at 5000 rpm for 10 minutes and 2 cc of platelet-rich plasma is concentrated in the bottom, containing a high density of growth factors. The floating platelet-poor plasma is discharged.

one containing 1 ml of anticoagulant, 3.2% trisodium citrate (Vacuette; Greiner Bio-One, Kremsmuenster, Austria) (Fig. 5). The eight flasks were centrifuged at 1000 rpm for 10 minutes. The slow speed is important so that platelets are not displaced to the bottom of the flasks. The plasma is then dispensed into four other flasks for a second centrifugation of 5000 rpm for 10 minutes. The floating plasma is then removed, leaving only 2 cc of concentrate, which is the platelet-rich plasma with four to six times more platelets than normal plasma and therefore containing a high concentration of growth factors. This concentrate is then added to the follicular units before implantation. The follicular units are kept in the platelet growth factor solution for 15 minutes to allow the growth factors to attach to the stem cells located in the bulge area. Next, 10 drops of 10% calcium chloride is added to the mixture for the purpose of converting fibrinogen into fibrin, thereby producing the plasmatic gel that will seal the growth factors around the micrografts (Fig. 6).

**Implanting the Follicular Units**

The entire bald area on the scalp is massively infiltrated with saline solution containing epinephrine in a concentration of 1:200,000. This tumescent technique, which we call "scalp ballooning,"[2] and the vasoconstriction obtained with the epinephrine injection avoids bleeding and enables implanting the micrografts more easily. In the outlined area to the right, the units imbibed in platelet-rich plasma growth factors were implanted; in the left, the units considered controls were placed. On both sides, the same number of grafts was implanted, thus allowing greater control for the clinical trial (Fig. 7). For this procedure, microblades (BD Beaver, Becton, Dickinson and Co., Franklin Lakes, N.J.; and lance tip 15 DEG, Ellis Instruments, Madison, N.J.) and jeweler-type microforceps were used. The technique used is the "stick-and-place" method published by the au-

**Fig. 6.** After 15 minutes of imbibition, 10 drops of 10% calcium chloride is added to transform fibrinogen into fibrin. The plasma gel with the growth factors seals the follicular units and they are ready to be implanted.

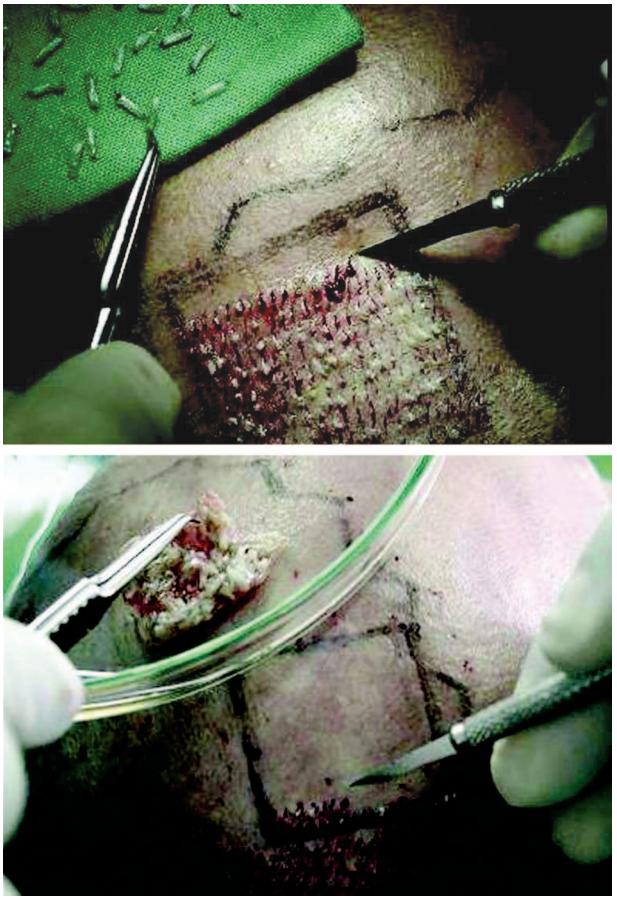

**Fig. 7.** (*Above*) The standard follicular units are implanted. (*Below*) The same number of grafts with platelet plasma growth factors are placed.

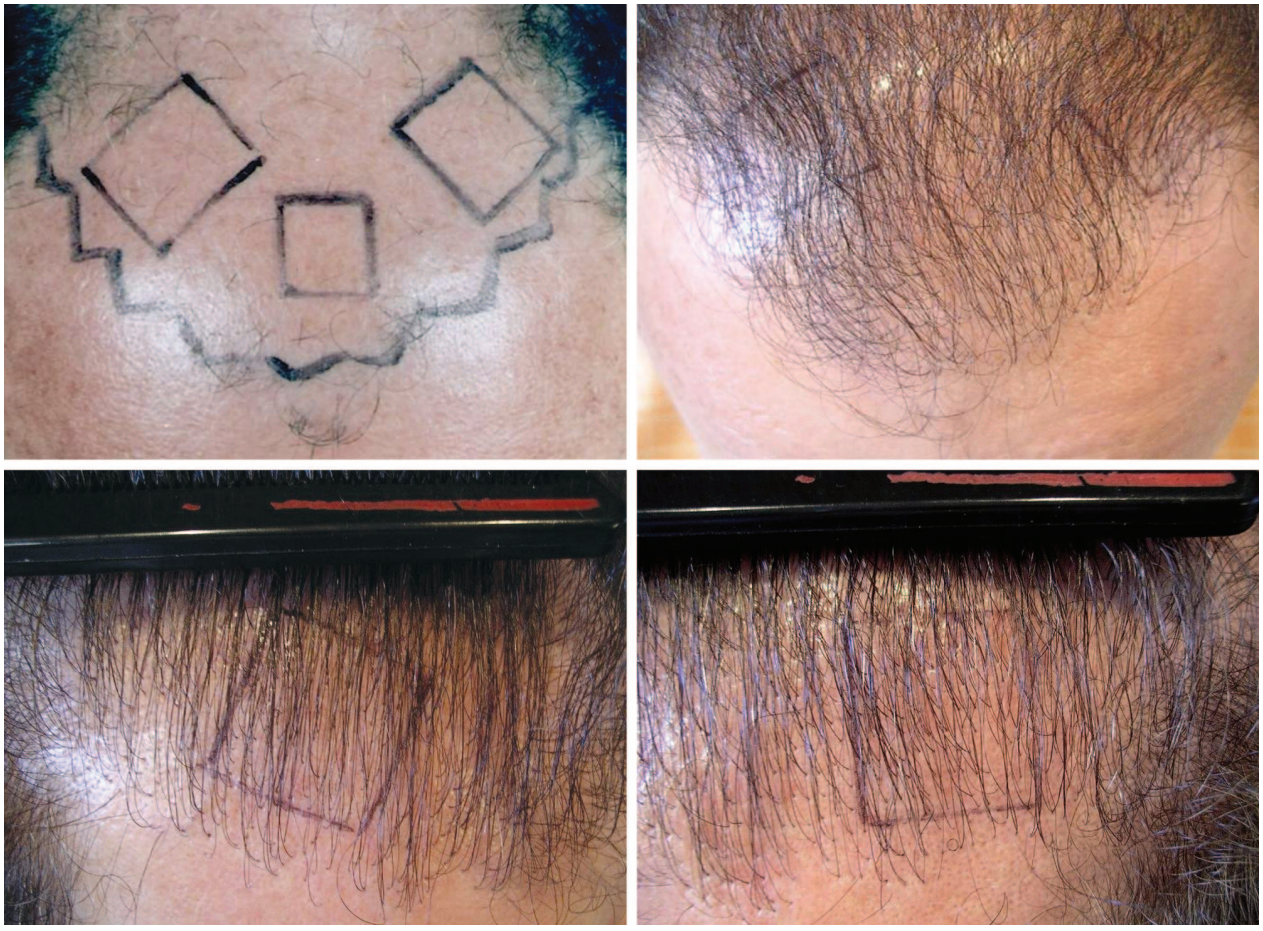

**Fig. 8.** A 38-year-old patient with 150 implanted follicular units. After 7 months, 114 are counted on the right and 95 on the left. This is an improvement of 20 percent.

**Table 1. Patient Characteristics, Implanted and Yield Follicular Units per 2.5 × 2.5-cm² Areas, and Normal and Rich Plasma Platelets (*n* = 20)***

| | | | Yield Follicular Units | | Platelets | |
|---|---|---|---|---|---|---|
| Age (yr) | Baldness Region | Implanted Follicular Units | Control | Experimental | Normal Plasma | Rich Plasma |
| 35 | F | 138 | 117 | 135 | 224,000 | 460,000 |
| 45 | F | 170 | 129 | 134 | 185,000 | 417,000 |
| 49 | F | 155 | 92 | 107 | 234,000 | 614,000 |
| 39 | F | 164 | 92 | 119 | 268,000 | 658,000 |
| 31 | F | 128 | 72 | 94 | 227,000 | 640,000 |
| 50 | F | 170 | 121 | 139 | 187,000 | 390,000 |
| 29 | F | 160 | 102 | 117 | 183,000 | 380,000 |
| 36 | F | 150 | 95 | 114 | 180,000 | 420,000 |
| 32 | F | 145 | 99 | 108 | 217,000 | 390,000 |
| 48 | F | 153 | 94 | 143 | 144,000 | 536,000 |
| 36 | O | 110 | 86 | 99 | 224,000 | 460,000 |
| 32 | F | 137 | 92 | 95 | 184,000 | 391,000 |
| 48 | F, P, O | 150 | 108 | 121 | 195,000 | 600,000 |
| 54 | F, P, O | 137 | 123 | 135 | 150,000 | 420,000 |
| 23 | F | 121 | 97 | 101 | 311,000 | 343,000 |
| 22 | F | 125 | 101 | 116 | 270,000 | 1202,000 |
| 42 | O | 135 | 103 | 107 | 165,000 | 656,000 |
| 29 | F | 137 | 108 | 127 | 248,000 | 1076,000 |
| 38 | F | 130 | 108 | 119 | 140,000 | 321,000 |
| 39 | F, P | 125 | 105 | 110 | 200,000 | 191,000 |
| 38 ± 9 | — | 142 ± 17 | 102 ± 14 | 117 ± 15 | 206,800 ± 44,942 | 528,250 ± 243,586 |

F, frontal; P, parietal; O, occipital.
*Data are presented as means ± SD.

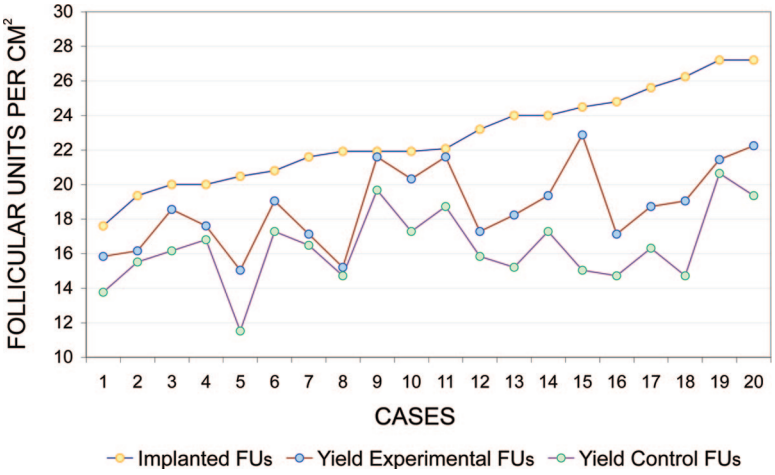


**Fig. 9.** Total implanted follicular units (*FUs*) and yield of follicular units for both experimental and control groups ($p < 0.001$).

thor in 1989 and 1991.[1,2] After the two demarcated areas were implanted, implantation of the entire remaining bald area was completed using standard follicular units. Moist gauze was applied to the implanted area and this was secured by an elastic bandage, which was kept in place for 24 hours. After that time, the patient removed the bandage and washed the entire implanted area with an antiseptic neutral shampoo.

### Endpoint Evaluation

All patients were evaluated monthly for 7 months, and the yield of follicular units was counted in the outlined areas. An accurate inspection, counting the number of follicular units within the two areas, was performed by staining four nankin tint spots (Fig. 8). The counting was performed at, the end of 7 months, with a magnifying glass by the surgeon, and recounted by two assistants for confirmation.

### Statistical Analysis

The data were summarized using mean ± SD (Table 1). To compare the two procedures involved—platelet plasma growth factor protocol and control group—the paired $t$ test was used, because data showed a gaussian distribution. Analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, Ill.). The significance level was set to $\alpha = 0.05$.

## RESULTS

There was a statistically significant difference observed in the yield of follicular units when comparing the two groups ($p < 0.001$). The experimental group with the platelet plasma growth factor showed a density of 18.7 follicular units per cm$^2$, whereas the control group showed 16.7 follicular units per cm$^2$. The difference of 2.4 follicular units per cm$^2$ (95 percent confidence interval, 1.6 to 3.2 follicular units per cm$^2$) represented a 15.1 percent increase in the yield of follicular unit density between the two groups (Figs. 9 and 10). This means that if there is a 100-cm$^2$ (10 × 10-cm) bald area to be implanted, one can obtain 240 follicular units more, or approximately 480 hair shafts, assuming two shafts per follicular unit. It is also important to point out that some patients have experienced only a 3 percent increase using the platelet-rich plasma growth factor protocol, whereas others have shown a 52 percent increase in follicular unit density. Figure 9 demonstrates a line plotting the total implanted follicular units and the yields for both experimental and control groups. There was a 15.1 percent increase of growth and density between these groups.

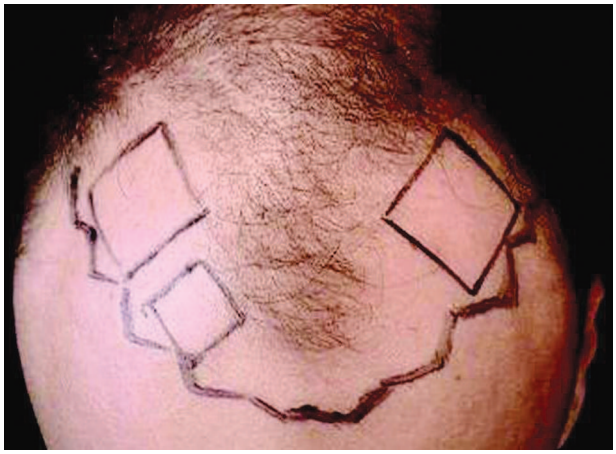

**Fig. 10.** A 32-year-old patient had 125 follicular units implanted on each side.

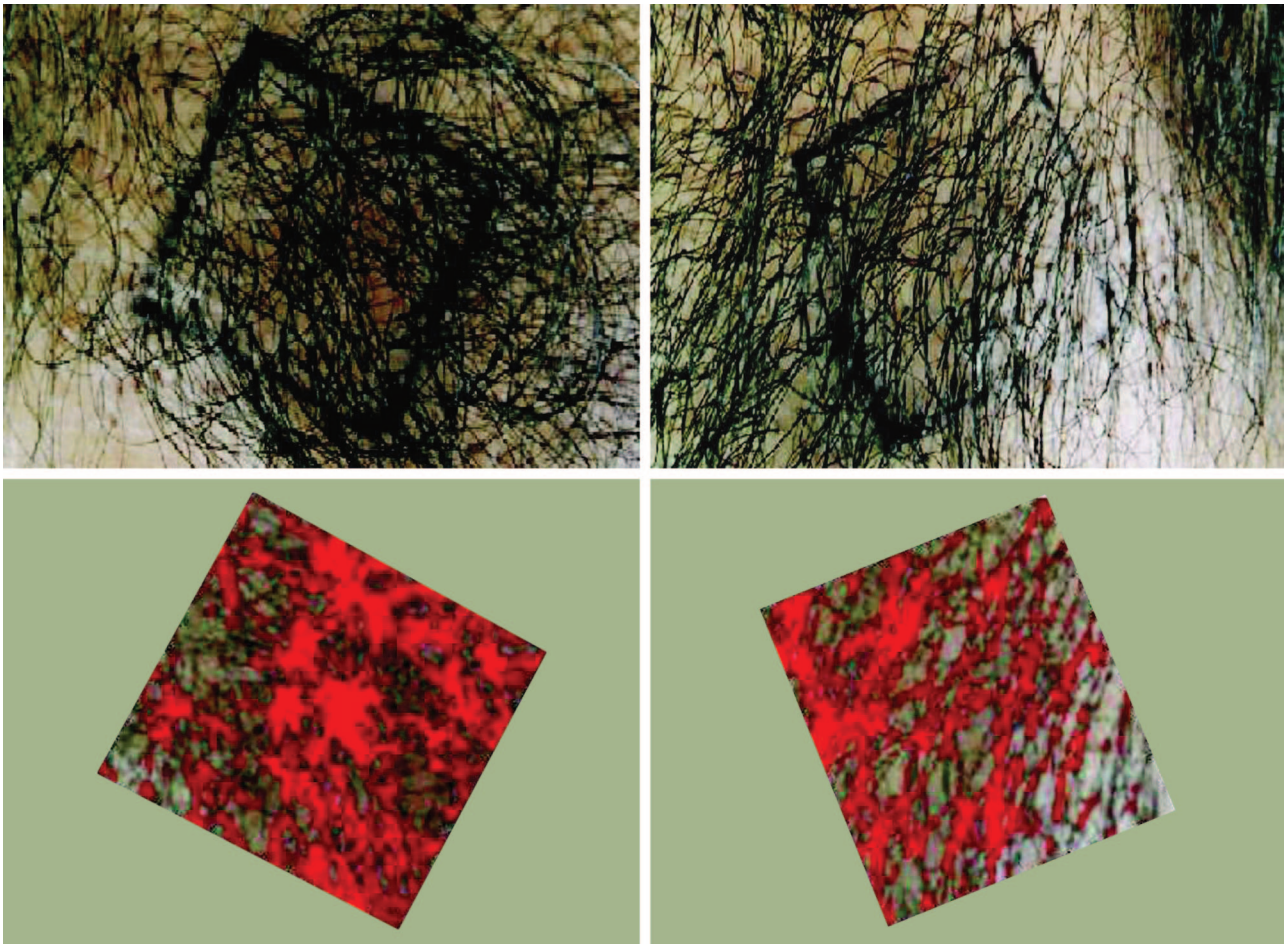

**Fig. 11.** Same patient as shown in Figure 10. (*Above*) One hundred seventeen follicular units were counted on the right and 93 were counted on the left; 26 percent more hair is noted in the area implanted with platelet plasma growth factors. (*Below*) Software image analysis demonstrated a similar density of 28 percent on the right side.

## DISCUSSION

According to the results obtained in this clinical trial, there was 15.1 percent more hair yield in follicular units and density in the area treated by platelet plasma growth factors. This new development for hair transplant surgery demonstrates the use of autologous platelet growth factors to improve capillary density with low cost and low morbidity. Especially for those patients with less density and very thin hair in the donor area, this technique should be a great contribution. Although these results are significant, further research, such as a double-blind test, should be performed to evaluate the final results with outside assistance, in asymmetric areas. The use of digital imaging will be an important comparative tool for density. For one patient, a digital camera (5.0-megapixel, 24-bit color) was used at a fixed distance from the scalp. Pictures were recorded in raw format. Image-Pro Plus 4.5 image analysis software (Media Cybernetics, Silver Spring, Md.) was used to perform morphometric analysis and to derive objective measures of the hair color density from the marked areas on the right and left sides. A color threshold level was selected interactively by an experienced observer[28] and applied on both images from each patient. The scale used was 40 pixels/cm. The area covered by hair was divided by the total area measured on every image. The patient shown in Figures 10 and 11 demonstrates an image of 28 percent more density on the right side. By counting the follicular units with a magnifying glass, an increase of 26 percent was observed in that area. This indicates that both systems are practically the same and that the digital program could perhaps be improved in future studies.

## CONCLUSIONS

With 20 male hair pattern baldness patients in this study, a considerable effect of platelet growth factors on the yield of follicular units was observed

and a significant improvement over conventional techniques. The method is simple and efficient and has a low cost and minimal morbidity. Further studies may perhaps improve the efficiency of the technique and allow digital programs to better evaluate the increase in hair density obtained. This opens a new perspective and is an important contribution to baldness surgery with follicular unit megasessions.

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## DISCLOSURE

*The author has no financial interest in the products, devices, or drugs mentioned in this article.*

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