Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 633-640, 2010.

DOI: 10.2298/AVB1006633L UDK 619:617.089

# THE NEW EXPERIMENTAL DESIGN OF ARTERIALIZED VENOUS FLAP ON THE RABBIT EAR MODEL

LALKOVIĆ M*, KOZARSKI J*, PANAJOTOVIĆ LJ*, ŠIJAN G*, ĐURĐEVIĆ D*, MIKOVIĆ N**, APOSTOLOVIĆ M***, PAVLOVIĆ M**** and GVOZDIĆ D****

**Military Medical Academy, Clinic for plastic surgery and burns, Institute for medical research, Belgrade, Serbia; **University of Belgrade, Faculty of Dentistry, Hospital of maxillofacial surgery, Belgrade, Serbia; ***University of Belgrade, Faculty of Medicine, Institute of orthopedic surgery "Banjica", Belgrade, Serbia; ****University of Belgrade, Faculty of Veterinary Medicine, Serbia*

(Received 13th May 2010)

*The aim of this study was to test a new experimental design of venous system arterialization on the rabbit ear arterialized venous flap (AVF) model. Total number of 10 "Big Chinchila" rabbits were divided in two experimental groups. On both ears of the five rabbits (Group 1) we have performed our original method of venous system arterialization with microsurgical arterialization of the central artery and vein with the preservation of central and peripheral vascular perfusion; at both ears of five rabbits (Group 2) we have performed AVF according to Byan et al., (1995). Vital AVF surface and necrosis percentage were determined in both experimental groups at day 1 and day 14 and results were compared using Student t-test. The results of our experiment indicate that our new experimental design of the AVF on rabbit ear model has better hemodynamic conditions, improves AVF survival and gives significantly bigger vital flap surface at 14 days after venous system arterialization.*

*Key words: arterialized venous flap, new experimental design, rabbit ear*

## INTRODUCTION

All surgeons, especially plastic and reconstructive surgeons aim for anatomic and functional reconstruction of loosened body parts, performing surgery with the minimal donor area morbidity (Panajotovć, 1998). Understanding of the changes in the circulation and biological characteristics of microvascular flaps after free flap transplantation is essential for reconstructive surgeons. It improves selection for better reconstruction according to the biological values and functional demands of the recipient region (Kozarski, 2000).

The arterialized venous flap (AVF) in the hierarchy of the reconstructive methods, represents a complex surgical procedure and demands good equipment and educated staff. It gives an opportunity for planning and transfer of thin tissue blocks with the minimal donor area morbidity (Nichter *et al*., 1995).

Those flaps have two independent vein zones. One zone is anastomosing with the recipient artery and another is anastomosing with the recipient vein for blood drainage. This surgical procedure requires an absence of vein valves or 180º flap rotation according to the donor area, bringing up the incompetence of the valve apparatus (Lee, 1993). Despite previous good results in the small simple arterialized flap transfer, complex tissue transfers did not achive expected clinical results. Those results showed a greate percentage of complications, including partial or total flap necrosis. Concepts of perfusion and gas exchange on the capillary level with arterial inflow and vein outflow do not give a full explanation of success in the survival of unconventional perfusion in the vein flap and in arterialized venous flap (Lee, 1993; Inoue *et al*., 1991; Cheng *et al*., 2004; Kelly *et al*., 2004; Kushima *et al*., 2002; Nakazawa *et al*., 2004).

Many experimental models were developed in reconstructive surgery aiming for bigger surface of arterialized venous flaps, using the method of flap delay. Byun *et al*. (1995) reported the method of AVF delay in rabbit ear model. Other authors reported similar results of AVF survival surface in their experimental models (Woo *et al*., 1998; Alexander, 2001). Analysing previous experimental models of the delayed AVF in rabbits, we have noticed that these models were potentially hemodynamicly compromised. There were indications that some veins in the rabbits ear circulation system were excluded, aiming for better flap survival conditions in the delay method. Consequently we have developed a new experimental design of AVF on the rabbits ear model. The aim of this study was to test our original experimental design of venous system arterialization of the rabbits ear AVF model.

## MATERIAL AND METHODS

*Experimental animals*. Total number of 10 "Big Chinchilla" rabbits, weighting between 3-3.5 kg, aged 8-12 months, from the Military Medical Academy Experimental Veterinary Station were divided in two groups. All animals were kept for 10 days before the start of the experiment in the Vivarium of the Institute for Medical Research at the Military Medical Academy, for adaptation. Feeding and water supply of animals were "*ad libidum*". Possible infections were prevented by administration of 100 mg oxytetracycline i/m in a single daily dose. All procedures were performed according to ethical principles of scientific research on experimental animal models at the Military Medical Academy (No. 282-12, 20th November 2002).

*Rabbit ear AVF experimental model*. The first experimental group (Group 1) is represented by 5 experimental animals with AVF on both ears (10 samples) carried out by our new venous flap arterialization method (Figure 1). Total anesthesia was performed using ketamine chloride (35 mg/kg body weight, BW) and acepromazin maleate (1 mg/kg BW). Ears were cut throught all levels with a surgical knife, preserving the anterior marginal vein. Microsurgical technique of central artery and vein anastomosis was performed by single sutures, using 20x magnification. The second experimental group (Group 2) were 5 experimental animals, with AVF (Byun *et al.*, 1995) on both ears (10 samples) (Figure 2).

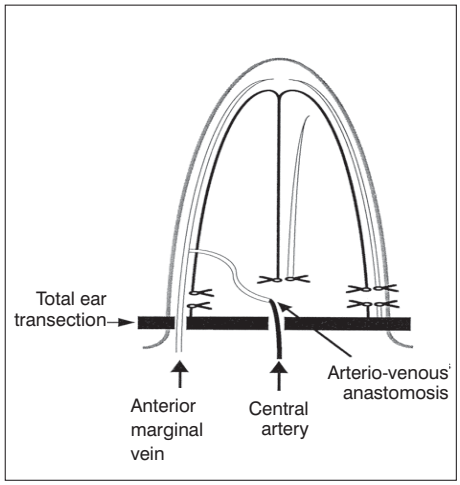


Figure 1. Scheme of the arterialized flap by Byan *et al*. (1995) model

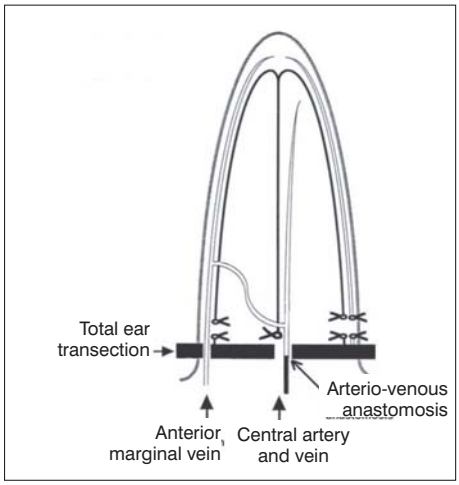


Figure 2. Scheme of our original arterialized flap model

*AVF vitality determination*. The arterialized venous flap surface in both experimental groups was determined using the following measurements: 1) rabbits ear length in cm, measured by the longest axis, 2) rabbits ear wideness in cm, measured by the widest axis. The flap surface in $cm^2$ was calculated: rabbits ear length x rabbits ear wideness.

The AVF necrosis surface was defined as a region of the rabbits ear full thickness without the phenomena of bleeding (sterile needle test), as well as not showing signs of superficial epithelisation and being covered with crusts. The AVF necrosis surface measurements were performed at day 1 and day 14 after the vein flap arterialization in both groups. Flap surface is measured after previously marking flap borders on transparent foil with regions of flap necrosis. Marked AVF necrosis surfaces were calculated using computer program with integrals and trapezoid rules (Kozarski, 2000).

*Statistical analysis*. The results are expressed as mean (M), standard deviation (SD) and coefficient of variation (CV) for each group of experimental animals. Probability and statistical significance of differences between mean values were calculated using Student's t-test.

## RESULTS

The results of average AVF surface area values, necrosis area ($cm^2$), necrosis percentage and AVF's vital surface percentage on days 1 and 14 after venous system arterializations of the experimental Group 1 are presented in Table 1.

Table 1. Mean AVF's surface area (M) at day 1 and day 14 after venous system arterializations, necrosis area, necrosis percentage and AVF's vital surface percentage in experimental Group 1

| Parameters | n | M | SD | CV |
|---|---|---|---|---|
| AVF vital surface area ($cm^2$), Day 1. | 10 | 106.92 | 9.52 | 8.90 |
| AVF vital surface area ($cm^2$), Day 14. | 10 | 36.22 | 10.72 | 29.59 |
| Necrosis area ($cm^2$), Day 14. | 10 | 70.70 | 2.09 | 5.77 |
| Necrosis %, Day 14. | 10 | 66.83 | 3.31 | 4.95 |
| AVF vital surface %, Day 14. | 10 | 33.17 | 3.12 | 9.41 |

Legend: n – number of AVFs

The results presented in Table 1 show that the AVF vital surface area at day 1 was 106.92 ± 9.52 $cm^2$, decreasing to 36.22 ± 10.72 $cm^2$ at day 14 after venous system arterialization. This represents 33.17 ± 3.12% of the initial AVF vital surface area with 66.83% of necrotic AVF surface area.

The results of average AVF surface area values, necrosis area ($cm^2$), necrosis percentage and AVF's vital surface percentage at day 1 and 14 after venous system arterializations of the experimental Group 2 are presented in Table 2.

Table 2. Mean AVF's surface area (M) at day 1 and day 14 after venous system arterializations, necrosis area, necrosis percentage and AVF's vital surface percentage in experimental Group 2

| Parameters | n | M | SD | CV |
|---|---|---|---|---|
| AVF vital surface area, Day 1. | 10 | 104.16 | 4.32 | 4.14 |
| AVF vital surface area, Day 14. | 10 | 1.74 | 0.55 | 31.61 |
| Necrosis area ($cm^2$), Day 14. | 10 | 102.42 | 4.11 | 4.01 |
| Necrosis %, Day 14. | 10 | 98.33 | 0.44 | 0.44 |
| AVF vital surface %, Day 14. | 10 | 1.67 | 0.77 | 46.11 |

Legend: n – number of AVFs

The results presented in Table 2 show that the AVF vital surface area at day 1 was 104.16 ± 4.32 $cm^2$, decreasing to 1.74 ± 0.55 $cm^2$ at day 14 after venous system arterialization. This represents only 1.67 ± 0.77% of the initial AVF vital surface area with the extreemly high necrotic surface area (98.33 ± 0.44%).

The results of statistical significance of differences between means using Students t-test are presented in Table 3.

Table 3. Analysis of the differences between means of AVFs vital surface area, necrosis area, necrosis percentage and AVFs vital surface percentage in both experimental groups

| Parameters | n | Group 1 | Group 2 | t | P |
|---|---|---|---|---|---|
| AVF vital surface area, Day 1. | 10 | 106.92 | 104.16 | 0.835 | >0.05 |
| AVF vital surface area, Day 14. | 10 | 36.22 | 1.74 | 10.158 | <0.0001 |
| Necrosis area ($cm^2$), Day 14. | 10 | 70.70 | 102.42 | 21.755 | <0.0001 |
| Necrosis %, Day 14. | 10 | 66.83 | 98.33 | 29.832 | <0.0001 |
| AVF vital surface %, Day 14. | 10 | 33.17 | 1.67 | 30.997 | <0.0001 |

The results presented in the Table 3 show that the initial mean values of the AVF vital surface area were almost equal in both experimental groups (106.92 $cm^2$ and 104.16 $cm^2$, Group 1 and 2, respectively). However, 14 days later there were statistically significant differences between mean values of the AVF vital surface area and percentage, necrosis area and percentage between the two AVF experimental designes. These data indicate that our new experimental design of the arterialized venous flap on the rabbit ear model is haemodinamicly superior compared to the Byun's model.

## DISCUSSION

At the beginning of the ischemic state, tissue flap is nourished by plasmatic imbibition mechanisms, using diffusion of oxygen from the recipient bed, which has higher oxygen partial pressure, as in the case of free tissue transfer. These mechanism provides gas diffusion and oxygen usage on the pre-capillary level, until conventional neo-vascularization is established (Woo *et al.*, 1998).

Many theories about survival mechanisms of the non conventional flaps nowdays consider that gas transportat in the capillary bed is essential for flap vitality until neo-vascularisation occurs. In the explanation of blood flow through the capillary system the "rolling" mechanism of blood flow is considered. In the total vein and arterialized vein flaps, blood is entering the capillary bed with the help from already existing arterial-vein anastomosis. Harris suggested that because of the parallel arrangement of bigger arterioles and venules in skeletal muscles, where arterial-vein anastomosis are less developed, mutual oxygen exchange appeared. The diffusion between arterioles and venules can decrease oxygen partial pressure in the tissue and increase it in the venules. This mutual exchange is considered harmful for oxygen delivery. According to this theory, part of the oxygen in arterial blood diffuses in the venous blood, making a diffusion gradient in the shunt. In the presence of Borh's effect, mutual exchange increases oxygen partial pressure in tissues, according to mathematical models. This effect is especially emphasized in hypoxia, which is intensified by lactic acidosis. During hyperoksemia, Borh's effect does not show a significant influence on oxygen

partial pressure, because oxyhemoglobin is completely saturated. Because of that, mutual oxygen exchange behaved as an oxygen diffusion shunt, decreasing oxygen partial pressure and preventing toxic influence of hyperoksemie. Mutual gas exchange, according to the previously explained diffusion gas transport, can be another mechanism which helps the survival of the non-conventional miocutaneous flaps in the initial ischemic state without relying on the oxygen and carbon oxide exchange at the capillary level (O'Tool *et al.,* 2004).

The AVF's clinical appearance at the beginning is characterized with edema and congestion which withdraws slowly, between 5-10 days postoperatively. Congestion is an understandable result of the neo-vascularisation and vascular network adaptation in the flap. The neo-vascularisation is induced by hypoxia, and adaptation by AV shunts opening and pressure increase in the venous vascular network. An important role in these processes have the oscillated veins (Krishnan *et al*., 2005). This phenomena is also seen in our experimental model. The venous system is not anatomically adapted to high blood pressure. In the newly developing conditions, filtration pressure at the end of venous network is increased. Albumin and other blood plasma proteins exudate through capillary fenesters in the interstitial space. The blood plasma proteins are followed by electrolytes and water, thus initiating edema. The lymphatic system under normal conditions drains albumins, electrolytes and water, and takes them again into the vein drainage system. In the AVF's conditions lymphatic vessels are not capable to take over their transportation role, because the muscle pump is missing. The edema brings a cascade reaction of hypoxia and tissue adaptation to new conditions. In both our experimental groups during arterialization of the vein system, nerves were cut. Denervation causes widening of the venous vascular network and a decrease of the vascular wall tonus. It is assumed that opening of A-V shunts and pre-capillary sphincter is more expressed when denervation is done (Adanali *et al.*, 2002).

Byun *et al*. (1995) have reported the rabbit model with demonstration AVF carry out "shorted" circle of perfusion in rabbit ear with anastomosis of afferent central artery and anterior branch of central vein without delay method. The arterial blood perfusion in the vein vascular space has no possibilities for perfusion in the distal parts of the ear. The total flap necrosis occurs in all AVF's, despite previous T-T micro-anastomosis. A high percentage of AVF's necrosis is similar to the results reported by other authors (Cho *et al*., 1998; Moshammer *et al.*, 2003) and on pedicle vein flap on rats (Cutting *et al*., 1980; Daniel *et al*., 1990; Sano *et al*., 2003; Baser *et al*., 2005).

## CONCLUSION

Non-conventional circulation in the flap is a relatively new and cheaper method in plastic and reconstructive surgery, that has been underestimated by many surgeons. We have created a new experimental model which gives 33.17% survival of the AVF's surface. Our original experimental AVF's model reported in this work is hemodynamicly favorable comparing to the Byun's *et al*. (1995) experimental AVF model. However, the experimental AVF models used in this

research have some limitations. The rabbit ear is the ideal experimental base for AVF research, because it has a specific vascularisation and no vein valves. In clinical research this comparison does not exist. We assume that the limiting factor for AVF's survival is basely high pressure in the vein system. Further research is needed concerning histological changes on the venous blood vessels, blood vessels size and T-T micro-anastomosis during the state of increased blood pressure in the small veins for AVF's perfusion.

Address for correspondence:
Lalković Mikica
Military Medical Academy
Clinic for plastic surgery and burns
Institute for medical research
Crnotravska 17
11000 Belgrade
Serbia
E-mail: drlalko@infosky.net

## REFERENCES

1. *Adanali G, Seyhan T, Turegun M, Senen D, Sensoz O*, 2002, Effects of different vascular patterns and the delay phenomenon on rat ventral island flap viability, *Ann Plast Surg*, 48, 6, 660-4.
2. *Alexander G*, 2001, Multistage type III venous flap or 'pre-arterialisation of an arterialized venous flap', *Br J Plast Surg,* 54, 8, 734.
3. *Baser NT, Silistreli OK, Sisman N, Oztan Y*, 2005, Effects of surgical or chemical delaying procedures on the survival of proximal predicled venous island flaps: an experimental study in rats, *Scand J Plast Reconstr Surg Hand Surg*, 39, 4, 197-203.
4. *Byun JS, Constantinescu MA, Lee A, May JW*, 1995, Effects of delay procedures on vasculature and survival of arterialized venous flap: an experimental study in rabbits, *Plast.Reconstr Surg,* 96, 7, 1650-8.
5. *Cheng TJ, Chen YS, Tang YB*, 2004, Use of a sequential two-in-one free arterialized venous flap for the simultaneous reconstruction of two separate defects on the foot, *Br J Plast Surg*, 57, 7, 685-8.
6. *Cho BC, Lee MS, Lee JH, Byun JS, Baik BS*, 1998, The effects of surgical and chemical delay procedures on the survival of arterialized venous flaps in rabbits, *Plast Reconstr Surg,* 102, 4, 1134-43.
7. *Cutting C, Bardach J, Rosewall D*, 1980, Skin flap delay procedures: proximal delay versus distal delay, *Ann Plast Surg,* 4, 4, 293-6.
8. *Daniel RK, Kerrigan CL*, 1990, Principles and Physiology of SkinFlap Surgery, In: McCarthy JG (ed), Plastic Surgery, Philadelphia, WB Saunders Co, Ch 9.
9. *Inoue G, Maeda N, Suzuki K*, 1991, Closure of big toe defects after wrap-around flap transfer using the arterialized venous flap, *J Reconstr Microsurg,* 7, 1, 1-8.
10. *Kelly JL, Eadie PA, Orr D, Al-Rawi M, O'Donnell M, Lawlor D*, 2004, Prospective evaluation of outcome measures in free-flap surgery, *J Reconstr Microsurg,* 20, 6, 435-8, discussion 439.
11. *Kozarski J,* 1998, Procena biloške vrednosti transplantiranih mikrovaskularnih režnjeva, Beograd: Doktorska disertacija, VMA.
12. *Kozarski J,* 2000, Biloška vrednost transplantiranih mikrovaskularnih režnjeva, Beograd: Zavod za udžbenike i nastavna sredstva , 1st edition, Ch.III, 65.
13. *Krishnan KG, Schackert G, Steinmeier R*, 2005, The role of near-infrared angiography in the assessment of post-operative venous congestion in random pattern, pedicled island and free flaps, *Br J Plast Surg*, 58, 3, 330-8.

14. *Kushima H, Iwasawa M, Maruyama Y,* 2002, Recovery of sensitivity in the hand after reconstruction with arterialized venous flaps, *Scand J Plast Reconstr Surg Hand Surg,* 36, 6, 362-7.
15. *Lee WPA*, 1993, Arterialized venous flap for treating multiple skin defect of the hand (discussion), *Plast Reconstr Surg,* 91, 303.
16. *Moshammer HE, Schwarzl FX, Haas FM, Maechler H, Pierer G, Wiltgen M et al*., 2003, Retrograde arterialized venous flap: an experimental study, *Microsurgery*, 23, 2, 130-4.
17. *Nakazawa H, Kikuchi Y, Honda T, Isago T, Morioka K, Itoh H*, 2004, Use of an arterialized venous skin flap in the replantation of an amputated thumb, *Scand J Plast Reconstr Surg Hand Surg,* 38, 3, 187-91.
18. *Nichter LS, Jazayeri MA,* 1995*,* The physiologic basis for nonconventional vascular perfusion, *Plast Reconstr Surg,* 95, 2, 406-12.
19. *O'Toole G, Mac Kenzie D, Poole M*, 2004, Therapeutic angiogenesis, *Br J Plast Surg*, 57, 2, 179.
20. *Panajotović Lj,* 1998, Ratne rane sa defektom tkiva, In: Dragović M, Todorić M, editors, Urgentna i ratna hirurgija, Beograd: Velarta, 86-108.
21. *Pitet B, Phyllis Chang Ph, Cederna P, Cohen M, Blair W, Cram A*, 1996, The role of neovascularization in the survival of an arterialized venous flap, *Plast Reconstr Surg*, 97, 3, 621-9.
22. *Sano K, Hallock GG, Rice DC*, 2003, Venous "supercharging" augments survival of the delayed rat TRAM flap, *Ann Plast Surg*, 51, 4, 398-402.
23. *Woo SH, Kim SE, Lee TH, Jeong JH, Seul JH*, 1998, Effects of blood flow and venous network on the survival of the arterialized venous flap, *Plast Reconstr Surg,* 101, 5, 1280-9.

## NOVI EKSPERIMENTALNI DIZAJN ARTERIJALIZOVANOG VENSKOG REŽNJA NA MODELU UHA KUNIĆA

LALKOVIĆ M, KOZARSKI J, PANAJOTOVIĆ LJ, ŠIJAN G, ĐURĐEVIĆ D, MIKOVIĆ N, APOSTOLOVIĆ M, PAVLOVIĆ M i GVOZDIĆ D

SADRŽAJ

U radu je prikazan novi eksperimentalni dizajn arterijalizovanog venskog režnja (AVR) na modelu uha kunića. Ispitivanje je izvršeno na ukupno 10 kunića rase "velika činčila", koji su podeljeni u dve ogledne grupe. Kod 5 kunića je na oba uha sproveden originalni postupak mikrohirurške arterijalizacije centralne arterije i vene uz očuvanje centralne i ivične vaskularne perfuzije. Na 5 oglednih životinja druge grupe je na oba uha izvršena arterijalizacija venskog sistema uha kunića prema modelu Byan-a i sar. (1995). Vitalna površina AVR određivana je prvog i 14-og dana nakon arterijalizacije venskog režnja. Izračunate su srednje vrednosti vitalne površine AVR-a i procenat nekrotične površine. Poređenje rezultata sprovedenih eksperimentalnih procedura je izvršeno Studentovim t-testom.

Analizom različitih eksperimentalnih modela AVR na uhu kunića, kako neodloženih tako i onih kod kojih je radi povećanja vitalne površine primenjena metoda odlaganja režnja, utvrđeno je da su dosadašnji modeli neodloženih AVR, potencijalno hemodinamski nepovoljni. Rezultati našeg istraživanja su ukazali da se novim ekperimentalnim dizajnom postiže statistički veoma značajno smanjenje procenta nekrotične površine AVR i povećanje vitalne površine režnja.