THE DESIGN OF THE ARTERIOVENOUS VASCULAR LOOP DOES NOT AFFECT ITS PATENCY: EXPERIMENTAL STUDY

O FORMATO DA ALÇA VASCULAR NÃO AFETA A PATÊNCIA ARTERIOVENOSA: ESTUDO EXPERIMENTAL

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ABSTRACT

Objective: To evaluate the effect of the design of a femoral vascular loop with anastomosis in the femoral artery of rabbits on the presence of flow (patency) after seven days. Methods: A total of 39 rabbits underwent arteriovenous microanastomosis using the microsurgical technique. Two loop designs were used: one circular and the other angled. The parameters evaluated were presence or absence of flow, signs of hemolysis and hemodynamic changes. Results: After seven days, flow was present in 68% of the angled loops and 75% of the circular loops (p > 0.05). There was a significant intragroup decrease in pCO2 and a significant increase in pH. For the other parameters evaluated, no significant differences between the two loop models were found. Conclusions: A reproducible vascular loop model was shown. There was no significant difference between the two vascular loop models about the presence of flow after seven days. Level of Evidence V, Animal experimental study.

Keywords: Arteriovenous Anastomosis. Vascular Grafting. Femoral Vein.

INTRODUCTION

Vascular loops enable the insertion of microsurgical flaps in areas where local recipient vessels are damaged. These loops have been used in head and neck, as well as trunk and limb, reconstruction. While they can be done during anesthesia for flap transposition, loops can also be prepared days earlier, thereby dividing a long and exhausting procedure into two shorter, more manageable surgeries.

Despite speculation regarding the factors that can lead to its failure, the literature lacks controlled models that demonstrate which of them actually affect the patency of a loop. The primary objective of this study was to standardize and maintain a vascular loop model in rabbits, thereby determining whether there is a difference in blood flow patency between two different loop designs, “circular” and “angled” (Figure 1).

All authors declare no potential conflict of interest related to this article.
After completion of the anastomosis (when the loop was positioned), an external collaborator, without direct contact with the procedure, told the surgeon what type of loop to make. This information was generated by a randomization table created on the website www.randomization.com and grouped into permutation blocks ranging from 2, 4 or 6 positions (Figure 4).

**MATERIALS AND METHODS**

The secondary objective was to determine the effects of the different loop designs on heart rate, respiratory rate, blood gas analysis and presence of hemolysis.

**Surgical and Anesthetic Protocol**

Our research involved New Zealand rabbits that were provided by the Central Animal Laboratory of the Medical School, Universidade de São Paulo. Male and female adult rabbits were used based on availability.

The weight of the animals ranged from 2,350 g to 6,081 g. The animals were anesthetized with ketamine (40 mg.kg\(^{-1}\)) and midazolam (2 mg.kg\(^{-1}\)), propofol at a 5 mg/kg dosage and isoflurane diluted in 100% oxygen by means of the Mapleson D anesthetic circuit.

The animals were prepared for surgery under aseptic conditions; a surgical microscope was used.

Solutions were prepared with 1 ml of 2% lidocaine to allow vasodilation; a 5000 IU heparin in 10 ml of normal saline was used to prevent thrombus formation in the vessel lumen.

Blood gas, electrolyte, bilirubin and liver enzyme tests were collected from a portion of the femoral artery distal where the anastomosis was performed. An insulin needle was used (0.45 mm x 13 mm).

A medial incision was made in the right thigh, from the inguinal ligament to the knee. The femoral artery and vein were identified from the inguinal ligament to the emergence of the genicular branches (Figure 2). The branches of the femoral vein were ligated, and the vein was detached from its bed. The vein was attached just above the origin of the genicular branches. The distance between the ligature and the point where the vessel crossed the inguinal ligament was measured and noted as the length of the vessel (Figure 3).

An end-to-end anastomosis was performed in the femoral artery immediately after the emergence of the deep femoral artery. A 10-0 mononylon yarn was used with a 75-micrometer needle.
The formed loop was then introduced into one of two possible positions, drawn by lot at the end of the anastomosis, to avoid influencing the surgeon’s preference:

a) “ANGLED,” folded over itself at the midpoint of its length. The vein was inserted so there would be contact between the descending and ascending sections of the loop, but without constriction of its walls. This shape reaches a more distal region of the foot (Figure 5).

b) “CIRCULAR,” introduced to be closer to a circular shape. With a balance between length and width, this loop design reaches a more proximal region of the foot when compared with the “ANGLED” loop positioning (Figure 6).

The wound was cleaned and coated with antiseptic and repellent spray to discourage licking. A collar was attached to block neck movement but did not disturb the animal’s feeding.

The animals were kept in individual cages with food and water available ad libitum; their environment was clean and air-conditioned with a light and dark cycle.

Postoperative

Following the same protocol, after seven days the animals underwent an additional round of anesthesia for evaluation and subsequent euthanasia.

The loop was identified, the plastic film cut with microsurgery instruments (Figures 8 and 9), then measured, with blood samples taken from a portion of the distal loop for analysis (Table 1). The loop was then sectioned in its distal third, and the flow was evaluated as “present” when a continuous flow filled the cavity, or “absent” if it was a simple drip. Finally, a dose of 1.0 to 2.0 mEq/kg potassium chloride was administered.

The skin was closed with 4-0 nylon with separate stitching and a continuous running suture to ensure wound closure.
RESULTS

Forty-three surgeries were performed in a controlled and randomized manner. Four specimens died: three during induction of anesthesia and the fourth on the first postoperative day. Information for these four specimens was removed from the data analysis. Data were recorded and analyzed for 39 surgeries. Twenty-nine rabbits were female, and ten male. The “circular” loop group comprised 20 specimens, and the “angled” loop group 19. The parameters HR, RR and temperature, measured pre- and post-anesthesia, were similar in both groups. During sectioning and vessel ligation, the relative percentages of vessel shortening were 26.6% in the “circular” group and 33.5% in the “angled” group. Presence or absence of flow after seven days regarding the vascular loop design (Figures 11 and 12):

| ANGLED loops – Patency after 7 days |
|-------------------------------------|
| ABSENTE 32%                         |
| PRESENT 68%                         |

| CIRCULAR loops – Patency after 7 days |
|--------------------------------------|
| ABSENTE 25%                          |
| PRESENT 75%                          |

When subjected to statistical analysis using the chi-square test, the comparison between the “angled” and “circular” loop groups showed no significant difference (Table 1). All blood gas and electrolyte parameters measured showed normal distributions when subjected to the Kolmogorov–Smirnov test (Table 2).
When comparing the initial blood gas and electrolyte values, both the “circular” and “angled” loop groups showed similar baseline values. The laboratory hemolysis markers LDH, total bilirubin (TB), DB and IB are measured in Table 3. The concentrations of alanine aminotransferase transaminase (ALT) and aspartate aminotransferase (AST) were measured to rule out hepatopathy concomitant with bilirubin increase, as described in Tables 3 and 4.

A normal distribution of ALT, AST, and LDH was found. The test rejected the hypothesis of normality for TB, DB and IB.

No significant difference was found in any sample in either the intra-group comparison (“circular” or “angled”) or the intergroup comparison ("circular" versus "angled") regarding hemolysis tests (LDH, TB, DB and IB) and transaminases (ALT and AST) was found. Flow, in mL·min⁻¹, was measured in the first 12 specimens (Figure 13). The distribution was unusual, with no significant intra- or intergroup differences between initial surgery and euthanasia (Figure 13).

![Figure 13. Distribution of initial and final flow in mL min⁻¹ in the two loop designs.](image)

**Table 2. Electrolytes and blood gases at the start of surgery.**

| “CIRCULAR” LOOP | Minimum | Maximum | Mean | Standard deviation |
|------------------|---------|---------|------|--------------------|
| Initial pH       | 7.20    | 7.42    | 7.2616 | 0.07141            |
| Initial pCO₂ (mmHg) | 38.70  | 91.90   | 68.8273 | 17.87032           |
| Initial pO₂ (mmHg) | 99.60  | 399.00  | 213.6909 | 94.88694           |
| Initial sat O₂ (%) | 0.00   | 100.60  | 36.3364 | 50.41730           |
| Initial base excess (mmol/L) | -0.40  | 9.90    | 3.4655  | 3.33866            |
| Initial sodium (mEq/L) | 137.00 | 148.00  | 140.5455 | 3.17376            |
| Initial potassium (mEq/L) | 3.10   | 4.60    | 3.7636  | 0.48015            |
| Initial calcium (mg/dL) | 5.80   | 6.83    | 6.3818  | 0.29144            |
| Initial chloride (mEq/L) | 96.00  | 116.00  | 104.2727 | 6.03475            |

| “ANGLED” LOOP | Minimum | Maximum | Mean | Standard deviation |
|---------------|---------|---------|------|--------------------|
| Initial pH    | 7.14    | 7.40    | 7.2812 | 0.07608            |
| Initial pCO₂ (mmHg) | 42.40  | 76.10   | 59.8182 | 11.72446           |
| Initial pO₂ (mmHg) | 40.40  | 400.00  | 236.0727 | 131.00080          |
| Initial sat O₂ (%) | 0.00   | 99.90   | 28.9455 | 41.62591           |
| Initial base excess (mmol/L) | -0.10  | 6.60    | 2.3609  | 2.22143            |
| Initial sodium (mEq/L) | 134.00 | 145.00  | 137.7273 | 3.00303            |
| Initial potassium (mEq/L) | 3.10   | 4.10    | 3.6818  | 0.36423            |
| Initial calcium (mg/dL) | 6.10   | 6.76    | 6.4255  | 0.19831            |
| Initial chloride (mEq/L) | 96.00  | 107.00  | 101.5455 | 3.47458            |

**Table 4. Distribution of biochemical hemolysis markers.**

| Circular design | Minimum | Maximum | Mean | Standard Deviation |
|-----------------|---------|---------|------|--------------------|
| Initial ALT (IU/L) | 20      | 67      | 36.75 | 14.710             |
| Initial AST (IU/L) | 11      | 37      | 25.25 | 6.811              |
| Initial TB (mg/dL) | 0.0     | 0.1     | 0.017  | 0.0389             |
| Initial DB (mg/dL) | 0.0     | 0.0     | 0.000  | 0.0000             |
| Initial IB (mg/dL) | 0.0     | 0.1     | 0.017  | 0.0389             |
| Initial LDH (IU/L) | 160.7   | 617.8   | 333.825 | 126.8985           |
| Final ALT (IU/L) | 12      | 48      | 30.36  | 11.102             |
| Final AST (IU/L) | 13      | 39      | 22.00  | 8.450              |
| Final TB (mg/dL) | 0.0     | 0.1     | 0.018  | 0.0405             |
| Final DB (mg/dL) | 0.0     | 0.0     | 0.000  | 0.0000             |
| Final IB | 0.0     | 0.1     | 0.018  | 0.0405             |
| Final LDH (IU/L) | 124.2   | 345.2   | 232.973 | 66.7961           |

| Angled design | Minimum | Maximum | Mean | Standard Deviation |
|---------------|---------|---------|------|--------------------|
| Initial ALT (IU/L) | 17      | 73      | 38.42 | 21.043             |
| Initial AST (IU/L) | 18      | 62      | 30.17  | 12.988             |
| Initial TB (mg/dL) | 0.0     | 0.1     | 0.025  | 0.0452             |
| Initial DB (mg/dL) | 0.0     | 0.0     | 0.000  | 0.0000             |
| Initial IB (mg/dL) | 0.0     | 0.1     | 0.025  | 0.0452             |
| Initial LDH (IU/L) | 89.8    | 542.7   | 274.758 | 124.3742           |
| Final ALT (IU/L) | 11      | 173     | 42.09  | 45.034             |
| Final AST (IU/L) | 11      | 62      | 27.36  | 14.603             |
| Final TB (mg/dL) | 0.0     | 0.0     | 0.000  | 0.0000             |
| Final DB (mg/dL) | 0.0     | 0.0     | 0.000  | 0.0000             |
| Final IB | 0.0     | 0.0     | 0.000  | 0.0000             |
| Final LDH (IU/L) | 117.9   | 480.0   | 237.218 | 121.2778           |

**Table 5. Flow measured in mL min⁻¹.**

| Design | Minimum | Maximum | Mean | Standard Deviation |
|--------|---------|---------|------|--------------------|
| “ANGLED” Flow at start of surgery | 34      | 156     | 85.33 | 40.215             |
| Flow at euthanasia (mL min⁻¹) | 0       | 101     | 42.71 | 39.037             |
| “CIRCULAR” Flow at start of surgery | 23      | 170     | 72.46 | 39.928             |
| Flow at euthanasia (mL min⁻¹) | 0       | 160     | 44.33 | 50.060             |
The anesthesia protocol was sufficient to allow the animals to survive procedures of up to four hours. There were four deaths in this series, with undefined cause for the three deaths that occurred during induction. The death on the first postoperative day resulted from heavy intraoperative bleeding.

The saphenous vessels were not used, as the arrangement in humans is different, with two veins and one artery composing the same bundle, since this pattern would make the measurement of flow in a single vessel nonviable. We chose to use the femoral vein, from the confluence of the genicular veins to the confluence of the deep femoral vein. It was necessary to ligate the muscular branches along the entire path of this vessel. The absence of necrosis in the foot shows that the deep femoral artery is sufficient to supply the limb with blood in the absence of the femoral artery. Concerning the mold that keeps the loop in position, three questions were answered: the biocompatibility of the material; the minimum vessel compression; and the stability of the material when used in the rabbit’s thigh (a site of substantial movement).

Hard molds were tested and discarded for causing vessel compression. Instead, we chose to use flaps of Biocclusive® sterile plastic film (Johnson & Johnson, USA). The period of seven days between loop construction and the evaluation of patency was chosen in accordance with several clinical trials that waited the same period to evaluate loop maturation and the patient’s clinical stabilization.13-15

A sample power statistical study was conducted and showed significantly similar numbers of patent vessels in the “circular” and “angular” loops. The proportions of patent vessels in the “angled” and “circular” loop populations were 55% and 64%, respectively, thus the sample size calculation, allowing for an alpha error of 5% and a statistical power of 80%, indicated that 466 specimens would be required for each group, for a total of 932. This sample size would make the project nonviable from ethical, logistical and economic perspectives.

Upon the study completion, the patency rates for the “angled” and “circular” loops were 68% and 75%, respectively, which are similar to the success rate of a large clinical series, where flaps made after the loop showed a success rate of 66%.16 However, the values are slightly below the success rate for vascular loops in rats, estimated at 77%.17

The flow measurement results did not reflect differences between the two groups. Perhaps a greater number of studies, such as performed by Asano et al.,18 can provide different results. Regarding blood gas analysis, there was a decrease in the pCO2 rate from 68.8 mmHg to 46.5 mmHg in the specimens receiving the “circular” loop. This difference was significant and could be explained by an increase in cardiac output, resulting from decreased peripheral vascular resistance, generating an increased respiratory rate and a decreased CO2 level. However, no significant increases in HR and RR were found. This may be explained by an increase in alveolar permeability, which was not measured using this method.

In specimens subjected to the “angled” loop construction, a minimal alveolar permeability, which was not measured using this method, was performed by Asano et al.,18 can provide different results. The presented thrombosis rate does not preclude comparison between the loop shapes. The same anastomosis procedure was performed on all animals, and the surgeon was advised of the loop only after the anastomosis was completed, requiring only the positioning of the same in its bed, in the “angled” or “circular” shape. The anesthesia and surgery protocols, as well as the surgical technique for creation of the loop, were simplified to facilitate their reproducibility and use in exploring other gaps in scientific knowledge (such as providing blood flow to flaps or preparation of vascularized composite grafts in reconstructive microsurgery).

A major contribution of this study is to start breaking the paradigm that an angled loop has a greater chance of thrombosis than a circular one. In reconstructive microsurgery this may be the difference between a loop reaching a distal point or less in the limb that needs a microsurgical flap, covering the poorer areas in covering tissue, such as the distal third of the leg and ankle.

CONCLUSION

In this study, whether the design of the vascular loop was “angled” or “circular” did not affect the presence of flow in the vessel after seven days. The blood gas analysis was minimally affected. This is a suitable and reproducible model of vascular loops.

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