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Free omental graft without vascular microanastomosis in the cutaneous wound healing of rabbits

[*Enxerto omental livre sem microanastomose vascular na cicatrização de feridas cutâneas em coelhos*]

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ABSTRACT

This study aims to evaluate the influence of free omental graft without vascular microanastomosis (FOGWVA) on experimental skin healing in rabbits. Through celiotomy, a 9cm^2 free omental fragment was collected in 36 rabbits, with subsequent production of a deep linear cutaneous wound in the dorsal midline measuring 3cm. In 18 animals from the omental group (OG), the omental fragment collected was fixed subcutaneously with six simple interrupted stitches using a 4-0 polyamide thread. In both treated and control (CG) groups, intradermal dermorrhaphy was performed with 4-0 polyamide thread. Experimental wounds were clinically evaluated every day. Each of the groups was divided into three subgroups of 6 animals each for histopathological evaluation on the 7th, 14th, and 28th days of postoperative. In the OG wounds, the increase in volume (omentum activation) stood out after the second postoperative day. Macroscopy showed an organic reaction to the graft on day 7, with a progressive reduction in addition to neovascularization towards the omental graft. The intense presence of mononuclear cells and collagen deposition on day 7 demonstrated an accelerated process of tissue remodeling and repair. The FOGWVA omental graft remained viable and positively influenced the cutaneous healing of the experimental wounds in rabbits.

Keywords: collagen, neovascularization, omentum, repair, skin

RESUMO

Neste estudo, objetiva-se avaliar a influência do enxerto omental livre sem microanastomose vascular (FOGWVA) na cicatrização cutânea experimental em coelhos. Por meio de celiotomia, foi coletado fragmento omental livre de 9cm^2 em 36 coelhos, com posterior produção de ferida cutânea profunda linear na linha média dorsal medindo 3cm. Apenas em 18 animais, do grupo omento (GO), o fragmento omental coletado foi fixado no subcutâneo com seis pontos simples interrompidos utilizando fio poliamida 4-0. Em ambos os grupos, tratado e controle (GC), efetuou-se dermorrafia intradérmica com fio poliamida 4-0. As feridas experimentais foram avaliadas clinicamente todos os dias. Cada um dos grupos foi dividido em três subgrupos, com seis animais cada, para avaliação anatomopatológica no sétimo, 14º e 28º dias de pós-operatório. Nas feridas do GO, destacou-se aumento de volume (ativação do omento) a partir do segundo dia pós-operatório. A macroscopia evidenciou reação orgânica ao enxerto no dia sete, com redução progressiva, além de neovascularização em direção ao enxerto omental. Intensa presença de células mononucleares e deposição de colágeno no dia sete demonstraram acelerado processo de remodelamento e reparo tecidual. O FOGWVA manteve-se viável e influenciou positivamente na cicatrização cutânea de feridas experimentais em coelhos.

Palavras-chave: colágeno, omento, neovascularização, pele, reparo

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INTRODUCTION

Challenges in wound repair are frequent in medical practice and, due to this fact, easy and accessible therapies have been pursued, which can stimulate and accelerate the repair process on these occasions (Silva, 2012). The properties of the omentum in stimulating neovascularization (Zhang *et al*., 1997, Litbarg *et al*., 2007), lymphatic drainage (Platell *et al*., 2000), tissue reconstitution, filling defects, and increased healing, even in the presence of infection (Platell *et al*., 2000; Litbarg *et al*., 2007; Fernandes *et al*., 2020), are widely discussed in the literature, in clinical and experimental reports (Karl and Dupré, 2012). In addition to being classically used as an adjuvant in the drainage and repair of intra-abdominal viscera (Morison, 1906), other studies have reported its use in the repair of extraperitoneal lesions, through free pedicled omental flaps (Wang *et al*., 2012; Falcão *et al*., 2016) with vascular anastomosis (Roa *et al*., 1999; Pap-szekeres, 2003) and free without vascular anastomosis (Fernandes *et al*., 2020; Teixeira *et al*., 2020).

The form traditionally used is the pedicled extraperitoneal omental flap (Wang *et al*., 2012; Falcão *et al*., 2016; Bruzoni *et al*., 2015), which maintains its blood supply through a vascular pedicle, however, the mobilization of the omental flap through subcutaneous tunnels to distant regions of the abdomen makes its use difficult. In human surgery, free omental grafts with vascular microanastomosis, in which the larger omental vessels are surgically anastomosed to those of the recipient bed has also been widely used. The use of free omental grafts with vascular microanastomosis becomes even more challenging in veterinary medicine (Roa *et al*., 1999; Pap-szekeres, 2003), particularly in small animals, due to the small size of the omental vessels, and because it demands specific knowledge and costly instruments. In this scenario, FOGWVA begin to gain prominence, being considered an easy, fast, and low-cost technique (Teixeira *et al*., 2020). The efficiency of the FOGWVA has been reported in organs such as the esophagus (Azari *et al*., 2012) and heart (Vineberg, 1967), in bone healing (Ree *et al*., 2018), and more recently in skin healing (Fernandes *et al*., 2020; Teixeira *et al*., 2020).

Knowing the capacity of the omentum to stimulate tissue repair, as well as its different therapeutic applicability, and due to the few reports of its use in free form without vascular anastomosis, the present study aimed to evaluate experimentally, through clinical and anatomopathological analysis (macroscopic and microscopic), the influence of FOGWVA at first intention healing of skin wounds experimentally produced in rabbits.

MATERIALS AND METHODS

Thirty-six male New Zealand rabbits, aged between three and four months, weighing on average 2.5 kg, from the Laboratory of Chemotherapy and Veterinary Experimental Parasitology of the Federal Rural University of Rio de Janeiro were used. The work was approved by the Ethics Committee on Animal Use of the Veterinary Institute of the Federal Rural University of Rio de Janeiro (n^{o} 8056140219).

The animals were divided into two groups of equal number (eighteen animals for each group), divided into Control Group (CG) and Omentum Group (OG). Each of the groups was divided into three subgroups, containing 6 animals each, from which material for histopathological evaluation was obtained after euthanasia at 7, 14, and 28 days.

The animals were sedated using ketamine (15mg/Kg), midazolam (0.5mg/Kg), and morphine (0.5mg/Kg), intramuscularly (IM), thus allowing for a wide trichotomy of the ventral abdominal, dorsal thoracic, and the left pelvic limb regions for venous access into the saphenous vein. Thereafter, the induction and maintenance of the anesthetic plan were performed via a face mask using isoflurane to the effect, with an inhaled oxygen fraction equal to 100%.

Once the adequate anesthetic plan was obtained, the rabbits were placed in the supine position to perform a median pre-umbilical longitudinal celiotomy. After accessing the abdominal cavity, inspection and removal of a distal fragment of the greater omentum were performed with the aid of a caliper, measuring approximately 3 cm on the omental edge and 3 cm on each side, by ligating the omental vessels with 3-0 polyglactin 910 thread (Figs. 1A, 1B and 1C), which was

then stored in a sterile saline solution. It should be noted that the omental fragment was collected from all 36 animals from both CG and OG to maintain the same surgical trauma and approximately the same surgical time in all animals, and also to later evaluate possible intra-abdominal adhesions caused by the omental fragment removal in all rabbits. However, the omental fragment collected was implanted only in 18 animals from the OG.

After celiorrhaphy and cleaning of the abdominal surgical wound, the animals were repositioned in the prone position to produce a 3 cm linear skin incision in the dorsal line (Fig. 1D), starting at a location corresponding to the imaginary line connecting the caudal edges of the scapulae and extending caudally. In the CG, the incision was deepened to include the entire thickness of the subcutaneous cell tissue, with a blade 15 scalpel, and the skin suture was performed in an intradermal pattern with 4-0 polyamide thread. In the OG animals, the same surgical incision was made, however, the omental fragment of 9 cm^2 was implanted using six simple stitches separated with 4-0 polyamide thread (one on each end and one on each side, at the midpoint of the fragment) (Fig. 1E), followed by dermorrhaphy (Fig. 1F) as previously described.

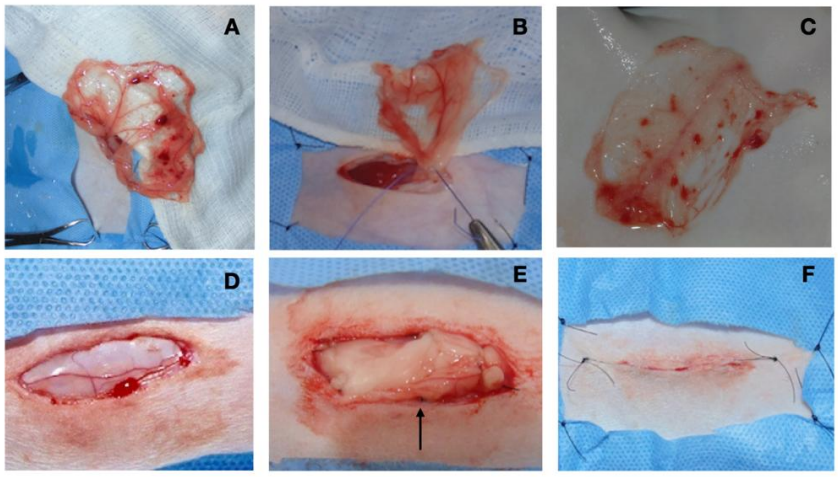

Figure 1. Photos of the execution of the surgical technique to collect the free omental graft through a median longitudinal celiotomy procedure with the subsequent creation of an experimental wound in the dorsal thoracic region (from the caudal scapular edges, 3 cm experimental wound) and implantation of free autologous omental fragment without vascular anastomosis in rabbits. **A**. Exposure of the omental edge to collect the fragment. **B**. Ligation of the omental vessels. **C**. Collected omental fragment (approximately 3 cm x 3 cm fragment for later implantation). **D**. Experimental linear surgical wound (3 cm) in the skin and subcutaneous cellular tissue in the dorsal thoracic region. **E**. Experimental surgical wound with an omental fragment fixed with six separate simple stitches, one on each end and one on each side at the midpoint of the fragment (arrow) using 4-0 polyamide thread. **F**. Experimental surgical wound after dermorrhaphy with intradermal pattern and 4-0 polyamide thread.

After recovery from anesthesia, protective collars and adhesive plaster gloves were placed on the extremities of the rabbits' limbs to avoid mutilation. As a postoperative medication, enrofloxacin (5 mg/kg, SID, for 3 days, SC) for bacterial wound prophylaxis, tramadol hydrochloride (3mg/kg, BID, for 3 days, SC) and dipyrone (25 mg/kg, BID, for 3 days, SC) were administered for pain control. Only the surroundings of the wounds were cleaned daily with cotton soaked in sterile saline, without applying excessive force and pressure, and always after clinical evaluations of the experimental wounds.

The wounds were observed daily and evaluated on days 1, 3, 5, 7, 14, and 28 after surgery for color, presence of crusts, devitalized tissue, and secretion, according to the adapted score from Tatarunas *et al*. (1998), where: 0 (absent), 1 (discrete), 2 (moderate) and 3 (accentuated). Also, based on the observations of Teixeira *et al*. (2020), increases in volume palpated in the wound region were measured daily with a pachymeter, in its length, width, and height.

Segments of skin and underlying tissues to a depth of the thoracic fascia were collected, using a conventional scalpel, after euthanasia for

microscopic examination on the 7th, 14th, and 28th day after the procedure, in both groups (Control and Omentum). If there were any changes, such as in color or volume increase in the region of the experimental wound, the full extent of this change was collected. The macroscopic characteristics evaluated at the time of collection of the skin fragments were documented for further descriptive analysis.

After fixation, the samples were cut and stained with Hematoxylin-Eosin (H&E) and Masson's trichrome. Segments of the superficial dermis containing the wound and of the deep dermis were microscopically evaluated, always by the same histopathologist, according to the adapted score from Garros *et al*. (2006), regarding the presence and intensity (absent=0; discrete=1; moderate=2; accentuated=3) of angiogenesis, mononuclear cells, polymorphonuclear cells, fibroblastic proliferation, collagenization, keratinization, hemorrhage, edema, and necrosis.

The abdominal cavity of each animal was inspected postmortem to assess for adhesions and postoperative findings.

Statistical analysis was performed using SigmaPlot software version 11.0 (Systat Software, San Jose, CA).

The results of the clinical evaluation of experimental surgical wounds were compared between treatments (Omentum Group and Control Group) using the Mann-Whitney U Test, and between the evaluation moments (days 1, 3, 5, 7, 14, and 28) using the Kruskal-Wallis test and Dunn's post test. Until the seventh day of clinical evaluation, each group (OG and CG) consisted of 18 animals, but due to the euthanasia for collection of histopathological material on days 7 and 14, from the seventh to the fourteenth day each group had 12 animals, and from the fourteenth to the twenty-eighth day, each group had 6 animals.

Microscopic evaluations were compared between treatments (Omentum Group and Control Group) using the Mann-Whitney U Test, and between evaluation moments (days 7, 14, and 28) using the Kruskal-Wallis test and Student-Newman-Keuls post test.

In all assessments, a significance level of 5% was considered.

RESULTS

Both in the CG and OG, there was an increase in volume with a soft consistency located around the experimental wound in the first postoperative days. This soft consistency volume in the OG remained from the first to the fifth postoperative day, being significantly higher than in the CG ($p<0.001$), in which the increase in soft volume was maintained only until the third postoperative day.

In the OG, it was also possible to observe an increase in the volume of firm consistency, in the most central part of the experimental wound, from the second day after surgery. This volume of firm consistency increased significantly from the third day of evaluation ($p<0.05$), remaining until the twenty-eighth day.

The intensity of the staining in the CG wounds was discrete (rosy) over the evaluation days, evolving to pale (normal) on all animals from the seventh day of evaluation onwards. Yet in the OG, the color intensity was more intense (rosy and purplish) over the days of assessment when compared to the CG, especially on days 3 ($p<0.01$), 5 ($p<0.001$), and 7 ($p< 0.001$) of postoperative. From the seventh day onwards, there was a softening in the intensity of the coloration in the OG, being possible to observe at 14 days a rabbit with rosy color and one with a purplish color and at 28 days still one animal with rosy color.

It was possible to visualize cutaneous vessels in the groups from the first to the seventh day of evaluation, however, from that day onwards this visualization was more frequent in the animals of the OG, with a statistical difference between groups on the fourteenth day of evaluation ($p<0.05$).

Both groups showed similar patterns with regards to the crust formation on the wound, and it was possible to observe a slight amount of crust from the first to the seventh day of evaluation. No secretion, devitalized tissue, or necrosis were observed in the clinical evaluation of any of the experimental animals.

On all the OG animals, the region of the increased volume of the experimental wound, observed in the clinical evaluation, corresponded to an area of firm adherence of the omental graft to the dermis (Fig. 2). There was notable neovascularization originating from the thoracodorsal vessel towards the region of the omental graft, surrounded by connective tissue (Fig. 3). The measurement of the length, width, and height of the swelling in the omental region of the OG animals was similar to the measurements performed on the same days in the clinical evaluation.

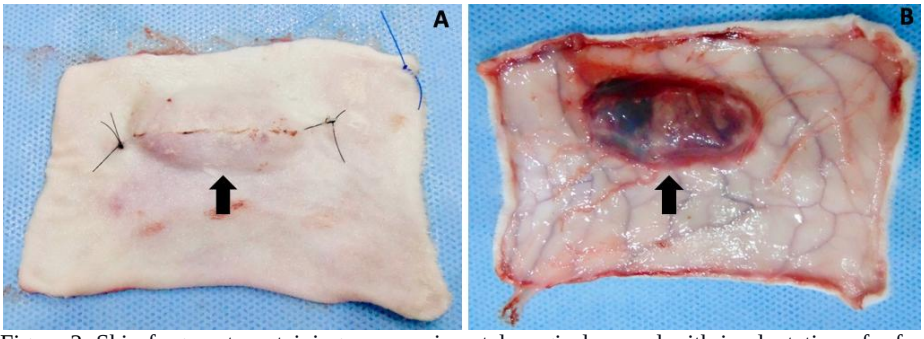

Figure 2. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of a euthanized rabbit after 7 days, collected for histopathological analysis. **A.** External surface of the skin fragment containing the experimental wound, demonstrating a notable increase in volume in the wound region (arrow). **B.** Internal surface of the same skin fragment containing the experimental wound, showing adherence of the omental graft (arrow) to the dermis area corresponding to the increase in volume in the clinical evaluation.

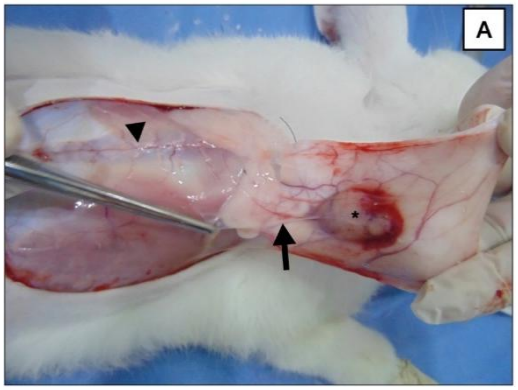

Figure 3. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of a euthanized rabbit after 7 days. **A.** The skin was retracted demonstrating neovascularization (arrow) originating in the thoracodorsal vessel (arrowhead), surrounded by connective tissue, and directing to the omental graft (asterisk).

In the OG animals euthanized after 7 days, the omental graft region was purplish with the presence of periomental hemorrhagic foci, with engorged subdermal vessels and also purplish in color (Fig. 4). Adhesions of the omental graft to the thoracic musculature were not observed.

In the OG animals euthanized at 14 days, the omental graft region was yellowish with purplish spots and few or no periomental hemorrhagic foci, in addition to containing engorged and purplish colored subdermal vessels (Fig. 5). Furthermore, in these animals, a greater density of connective tissue around the omental region was observed (Fig. 5), with adherence to the thoracic muscles in 2 animals (Fig. 6).

In four animals from the OG euthanized at 28 days, it was observed that the region of the omental graft was pale-yellow with slightly purplish spots around it and without periomental hemorrhagic foci, with slightly engorged and purplish-colored subdermal vessels (Fig. 7). In two animals, in which it was no longer possible to measure length, width, and height, the region of the omental graft presented a small elevation with a firm texture and pale-yellow color, and it was possible to observe the 3-0 nylon threads used for fixation of the graft, without hemorrhagic foci and subdermal vessels with normal color and appearance (Fig. 8). It was also possible to observe, in all animals in this group, the formation of dense connective tissue, with a veiled aspect, around the omental region (Figs. 7 and 8).

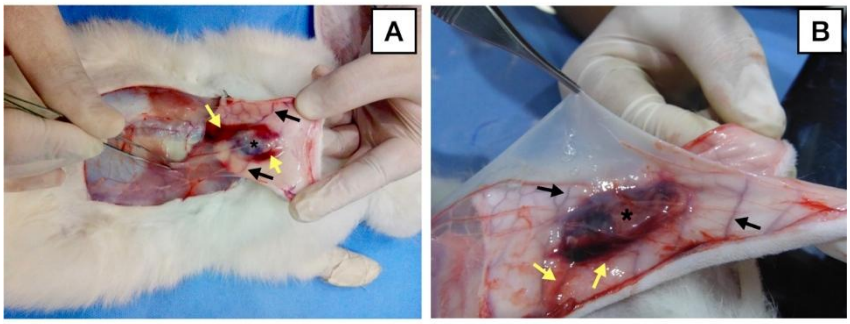

Figure 4. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 7 days. **A.** The skin was retracted, demonstrating the purplish appearance of the omental graft region (asterisk) with intense periomental hemorrhage (yellow arrow) and engorged subdermal vessels with a purplish color (black arrow). **B.** The skin was refolded, demonstrating the purplish appearance of the omental graft region (asterisk) with periomental hemorrhage foci (yellow arrow) and purplish engorged subdermal vessels (black arrow). Note that none of the photos show adhesions of the omental graft to the musculature.

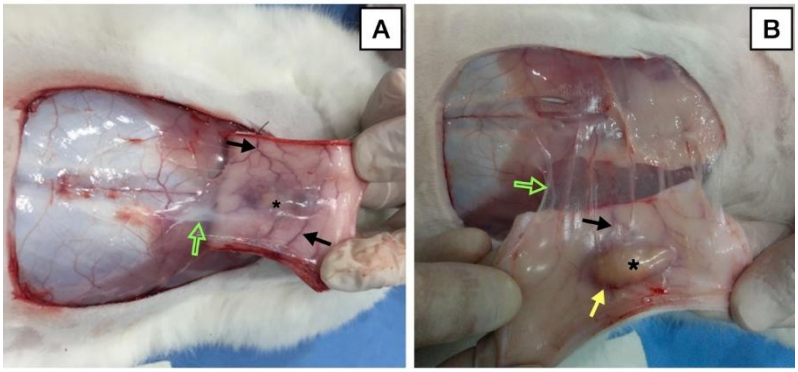

Figure 5. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 14 days. **A.** The skin was refolded, demonstrating the yellowish appearance with purplish foci in the region of the omental graft (asterisk) with engorged subdermal vessels of purplish color (black arrow), and the presence of greater density of connective tissue (arrow with green outline) around the region of the omental graft. **B.** The skin was refolded, demonstrating the yellowish appearance of the omental graft region (asterisk) with engorged subdermal vessels of purplish color (black arrow), small periomental hemorrhagic focus (yellow arrow), and the presence of greater density of connective tissue (arrow with green outline) around the omental graft region.

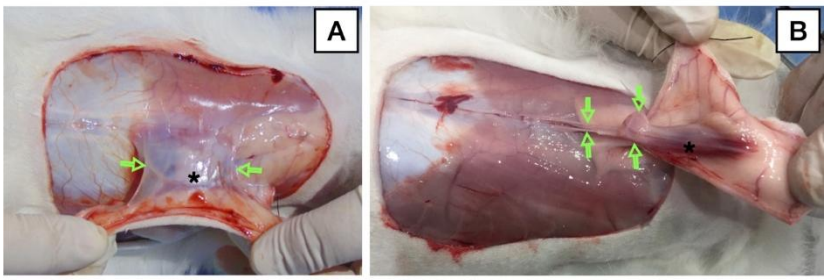

Figure 6. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 14 days. **A and B.** The skin was folded back, demonstrating the presence of adhesion (arrows with green outline) of the omental graft region (asterisk) to the thoracic musculature.

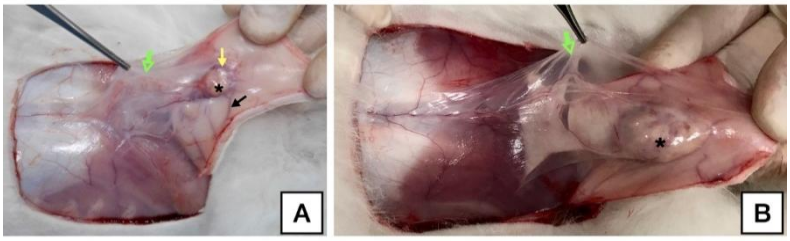

Figure 7. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 28 days. **A.** The skin was refolded, demonstrating a pale-yellow appearance in the region of the omental graft (asterisk) with slightly purplish spots around it (yellow arrow) and no foci of periomental hemorrhage, slightly engorged and purplish-colored subdermal vessels (black arrow), in addition to the presence of dense connective tissue (arrow with green outline) with veiled aspect around the omental graft region. **B.** the skin was showing the folded pale-yellow appearance in the omental graft region (asterisk) and the presence of dense connective tissue (arrow with green outline) around the omental graft region.

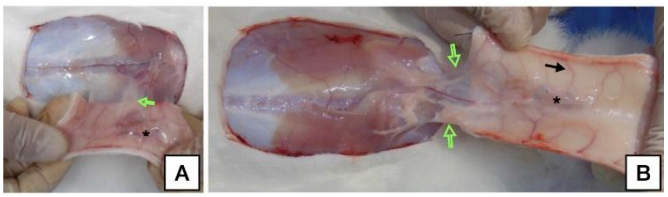

Figure 8. Skin fragment containing an experimental surgical wound with implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 28 days, in which it was no longer possible to measure the length, width, and height in the omental graft region. **A.** The skin was refolded, demonstrating a slight elevation of firm texture and pale-yellow color in the omental graft region (asterisk) without foci of periomental hemorrhage and presence of dense connective tissue (arrow with green outline), with a veiled aspect, around the omental graft region. **B.** The skin was refolded, demonstrating a slight elevation of firm texture and pale-yellow color in the omental graft region (asterisk) without periomental hemorrhage foci and subdermal vessels with normal color and appearance (black arrow), in addition to the presence of dense connective tissue (arrow with green outline), with a veiled aspect around the omental graft region.

In the CG, the experimental wound area presented a slight elevation of firm texture at all evaluation moments (more evident in animals euthanized at 7 days), similar in color to the surroundings and with subdermal vessels of normal color and appearance (Fig. 9). In 4 animals euthanized at 7 days, there were small hemorrhagic foci around the experimental wound. There was no neovascularization originating from the thoracodorsal vessel towards the region of the experimental wound in any of the animals.
In the macroscopic evaluation of the abdominal cavity of the 36 animals, no adhesions were observed at the region where the omental fragment was collected, to any viscera or the abdominal wall.

The median values and interquartile ranges referring to the objective analysis of the microscopic findings of the superficial dermis are shown in Table 1.

Neovascularization of the superficial dermis was discrete in both groups at the three evaluation moments. A slight presence of edema was also observed in the first two days of evaluation, with a subsequent significant reduction ($p<0.05$) on the twenty-eighth day in both experimental groups.

The little presence of polymorphonuclear cells remained in the first two moments of evaluation, in both groups, decreasing on day 28, but with a statistical difference only in the CG ($p<0.05$). There was a slight presence of mononuclear cells in both groups, however, unlike OG, in which the presence of mononuclear cells was maintained, in the CG there was a significant reduction on the twenty-eighth day ($p<0.05$).

There was a gradual evolution in the degree of fibroblastic proliferation in the OG over the days of assessment, while in the CG this parameter

remained discrete at the three evaluation moments. The presence of collagen remained higher in the OG, with a significant difference between groups on the seventh day of evaluation ($p<0.01$). In the CG, the presence of collagen was discrete in the first days of evaluation, evolving significantly on day 28 ($p<0.05$).

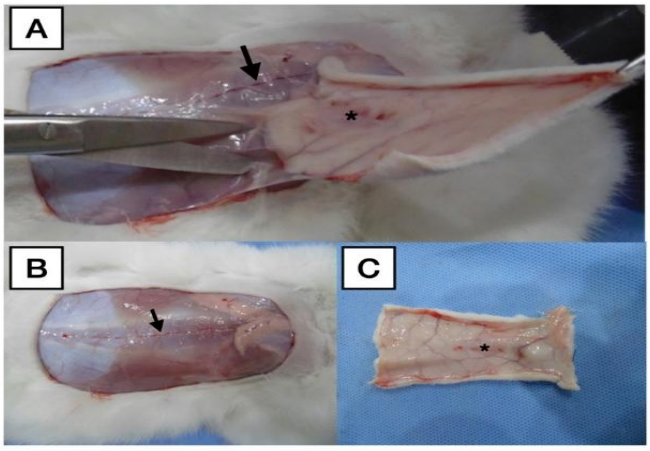

Figure 9. Procedure for collecting a skin fragment containing an experimental surgical wound without implantation of a free omental graft in the dorsal thoracic region of two euthanized rabbits after 7 days for histopathological analysis (**A, B, and C**). **A**. The skin was retracted demonstrating the region of the experimental wound (asterisk) and the thoracodorsal vessel (black arrow) in the fascia of the thoracic musculature. **B.** Bed after collection of the skin fragment containing the experimental wound for histopathological analysis demonstrating the thoracodorsal vessel in the thoracic musculature fascia (black arrow). **C.** Internal surface of the skin fragment containing the experimental wound in its central portion (asterisk).

Table 1. Results of the objective analysis of the histopathological findings in the superficial dermis of the region of surgical wounds experimentally produced on the back of rabbits, with or without implantation of a free omental graft (Omentum and Control Groups, respectively), expressed as median values and interquartile intervals (25% and 75%) relative to the scores obtained on days 7, 14, and 28 postoperatively. The intensity of the findings was classified as absent (0), discrete (1), moderate (2), and accentuated (3). OG – Omentum; CG – Control. Equal letters on the same line show a statistical difference in the same group between the days of evaluation (7, 14, and 28), with $p<0.05$. Equal symbols in the same column show statistical difference between groups (OG and CG), being: † $p<0.05$; § $p<0.01$.

Histopathological Findings	Group	7 days	14 days	28 days
Neovascularization	**OG**	1 (1-1)	1 (1-1)	1 (1-1)
	CG	1 (1-1)	1 (1-1)	1 (1-1)
Edema	**OG**	1 (1-2)[a]	1 (1-2)	0 (0-1)[a]
	CG	1 (1-1)[a]	1 (1-1)	0 (0-0)[a]
Polymorphonuclear Cells	**OG**	1 (1-1)	1 (0-1)	0 (0-1)
	CG	1 (0-1)	1 (1-1)[a]	0 (0-0)[a]
Mononuclear Cells	**OG**	1 (1-1)	1 (1-2)	1 (1-1)†
	CG	1 (1-1)	1 (1-1)	0 (0-1)†
Fibroblastic Proliferation	**OG**	1 (1-1)	1.5 (1-2)	2 (2-2)
	CG	1 (1-2)	1 (1-1)	1 (1-2)
Collagen (Masson)	**OG**	2 (1-2) §	2 (1-2)	2 (2-2)
	CG	1 (0-1)[a] §	1 (1-1)[b]	2 (2-2)[a,b]
Keratinization	**OG**	2 (2-3)	3 (3-3)	3 (3-3)
	CG	2.5 (2-3)	3 (3-3)	3 (3-3)

Keratinization levels remained high in both groups in all assessments. No animal presented superficial dermis necrosis in the three evaluation moments.

The median values and interquartile ranges referring to the objective analysis of the microscopic findings of the deep dermis are shown in Table 2.

Table 2. Results of the objective analysis of the histopathological findings in the deep dermis of the region of surgical wounds experimentally produced on the back of rabbits, with or without implantation of a free omental graft (Omentum and Control Groups, respectively), expressed as median values and interquartile intervals (25% and 75%) relative to the scores obtained on days 7, 14, and 28 postoperatively. The intensity of the findings was classified as absent (0), discrete (1), moderate (2), and accentuated (3). OG – Omentum; CG – Control. Equal letters on the same line show a statistical difference in the same group between the days of evaluation (7, 14, and 28), with $p<0.05$. Equal symbols in the same column show statistical difference between groups (OG and CG), being: † $p<0.05$; § $p<0.01$; ¥ $p<0.001$

Histopathological Findings	Group	7 days	14 days	28 days
Neovascularization	**OG**	2 (2-2) §	1.5 (1-2)	2 (1-2) §
	CG	1 (1-1)^{a} §	1 (1-1)^{b}	0 (0-1)^{a,b} §
Edema	**OG**	2.5 (2-3)^{a,b} †	1.5 (1-2)^{a}	1 (0-1)^{b}
	CG	1.5 (1-2)^{a} †	1 (0-1)	0 (0-0)^{a}
Polymorphonuclear Cells	**OG**	2 (1-2)^{a}	1.5 (1-2)^{b} †	0 (0-0)^{a,b}
	CG	1 (0-2)	0.5 (0-1) †	0 (0-0)
Mononuclear Cells	**OG**	2.5 (2-3) †	2 (2-3) §	2 (2-2) §
	CG	1.5 (1-2)^{a} †	1 (1-1)^{b} §	0 (0-0)^{a,b} §
Fibroblastic Proliferation	**OG**	3 (2-3)	2 (2-2)	2 (2-2)
	CG	2 (1-3)	1 (1-2)	2 (1-2)
Collagen (Masson)	**OG**	2 (1-2)^{a}	2 (1-2)	3 (3-3)^{a} §
	CG	1 (1-1)	1 (1-1)	2 (2-2) §
Hemorrhage	**OG**	2 (1-2)^{a,b} †	0 (0-0)^{a}	0 (0-0)^{b}
	CG	1 (0-1) †	0 (0-0)	0 (0-0)

It was observed that neovascularization in the deep dermis of the OG remained intense at all evaluation moments, differently from what was observed in the CG, with a significant difference between the groups on days 7 ($p<0.01$) and 28 ($p<0.01$). Edema in the deep dermis was also more intense in the OG when compared to the CG, especially on the first day ($p<0.05$) of the evaluation. In the CG, edema was moderate on the first day of the evaluation, gradually reducing until day 28 ($p<0.05$), while in the OG it was intense on day 7, significantly reducing on day 14 ($p<0.05$) and 28 ($p<0.05$). It should be noted, however, that on the first two days of the evaluation, the edema in the OG was mainly located around the omental graft, while on the last day of the evaluation it was predominantly located inside the graft. The presence of hemorrhage was observed only on the seventh day, in both groups, being more intense in the OG ($p<0.05$).

It was possible to visualize a greater number of polymorphonuclear cells in the deep dermis of the OG when compared to the CG in the first two days of evaluation, especially on day 14 ($p<0.05$). In both groups, there was a gradual reduction in the presence of these cells until day 28, with this reduction being significant in the OG ($p<0.05$). There was a greater number of mononuclear cells at all evaluation moments in the OG when compared to the CG (day 7: $p<0.05$; days 14 and 28: $p<0.01$). In the OG, the presence of mononuclear cells remained throughout the days of evaluation, while in the CG it showed a gradual and significant reduction until day 28 ($p<0.05$).

The degree of fibroblastic proliferation in the deep dermis remained high in both groups and at all evaluation moments. The presence of collagen in the OG evolved from moderate on day 7 to intense on the twenty-eighth day ($p<0.05$), while in the CG it remained similar at all evaluation moments, with a significant difference between the groups on days 14 and 28 ($p<0.01$).

It was possible to observe discrete foci of necrosis in the deep dermis of a few animals only in the first two days of evaluation in both the OG and the CG.

The main findings observed in the microscopic evaluation of the deep dermis on postoperative days 7, 14, and 28 are shown in Fig. 10.

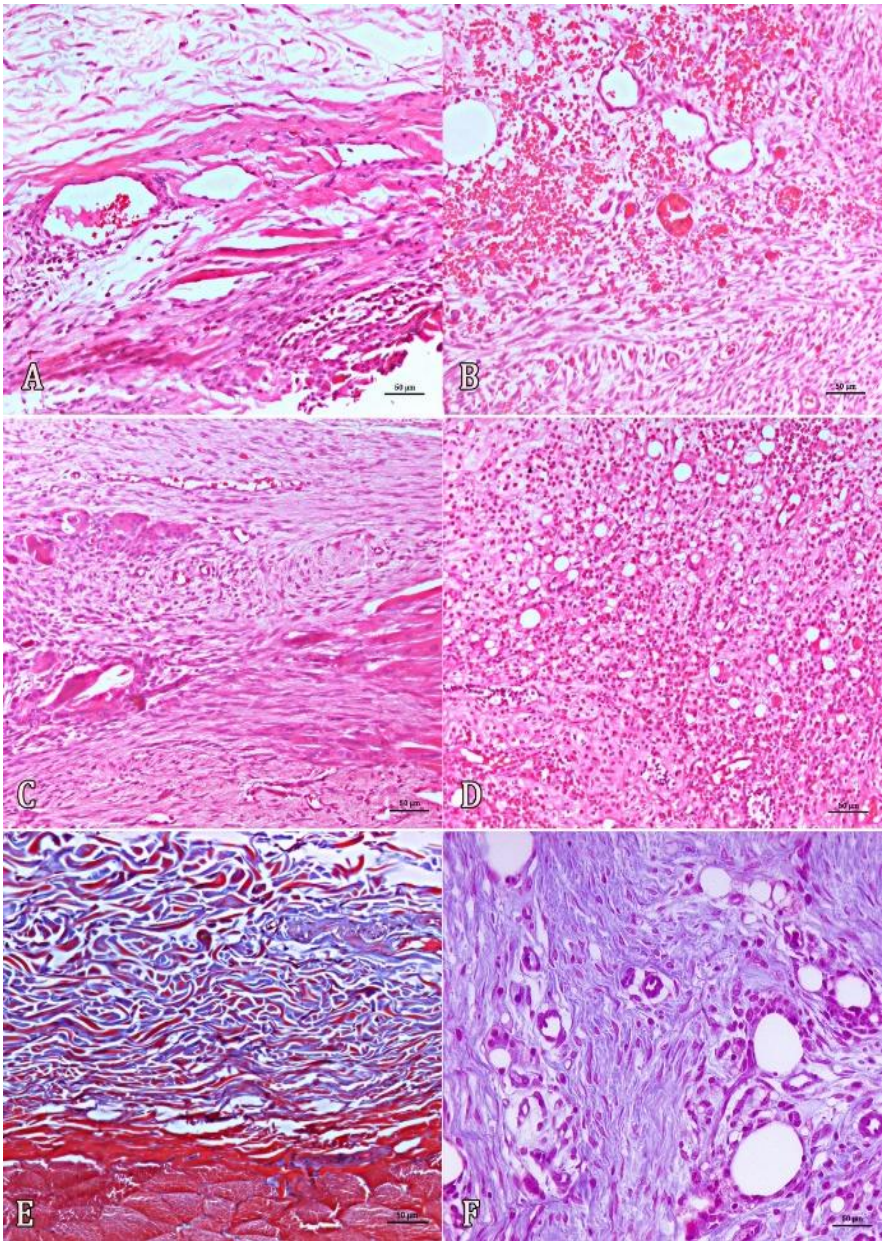

Figure 10. Photomicrographs of the deep dermis of skin fragments containing an experimental surgical wound with and without implantation of a free omental graft (Omentum and Control Group, respectively) in the dorsal thoracic region of euthanized rabbits with 7 (**A and B**), 14 (**C and D**) and 28 (**E and F**) days after surgery. **A.** Moderate macrophage infiltration and discrete focal blood vessels in deep rabbit dermis without euthanized free omental graft implantation 7 days postoperatively. H&E staining, obj. 20x. **B.** Moderate infiltrate with macrophages, neovascularization, multifocal hemorrhage, and diffuse edema in the deep dermis of rabbit with implantation of a free omental graft euthanized 7 days after surgery. H&E staining, obj. 20x. **C.** Discrete infiltration of focal macrophages and lymphocytes in the deep dermis of rabbits without implantation of a free omental graft euthanized 14 days after surgery. H&E staining, obj. 20x. **D.** Marked infiltration of neutrophils and eosinophils, moderate presence of macrophages and discrete giant cells in the deep dermis of rabbits with implantation of a free omental graft euthanized 14 days after the operation. H&E staining, obj. 20x. **E.** Presence of collagen fibers in the deep dermis of rabbits without euthanized free omental graft implantation 28 days after surgery. Masson's trichrome, obj. 20x. **F.** Diffuse presence of collagen fibers, moderate lymphocyte infiltration, and neovascularization in the deep dermis of rabbits with implantation of a free omental graft euthanized 28 days after surgery. Masson's trichrome, obj. 20x.

DISCUSSION

No studies were found that describe the influence of FOGWVA omental graft, its behavior, evolution, and organic reactions on skin healing. In the present study, the results of clinical and anatomopathological evaluations (macroscopic and microscopic) suggested that the graft had a positive influence on the healing of experimental wounds, with favorable behavior and evolution, and organic reactions indicative of stimulation of tissue repair.

The execution of surgical procedures involved in the collection and implantation of the free omental graft did not cause trans and postoperative complications and, according to Saifzadeh *et al*. (2009), a favorable point when compared to the use of the pedicled omental flap, which is associated with higher rates of complications such as hemorrhages, peritonitis, and herniations. Other authors used this modality of grafting in the esophagus (Azari *et al*., 2012), in orthopedic procedures (Baltzer *et al*., 2015; Ree *et al*., 2016, 2018), and only one in skin healing (Teixeira *et al*., 2020), and did not obtain complications resulting from the surgery. The fact that no changes were observed in the postmortem inspection of the rabbits' abdominal cavity in the present study reinforces these considerations.

The only limitation encountered by the surgeons in the experiment, with regards to the performance of the surgical technique, referred to the execution of intradermal suture in the experimental wounds with implantation of the omental graft due to the interposition of the omentum between the margins of the skin wound at the time of its approach. Teixeira *et al*. (2020) did not report any difficulty in performing the skin suture, possibly because they removed the subcutaneous cellular tissue before fixing the omentum fragment, which was not performed in the present study. This difficulty, however, did not prevent the favorable performance of the technique, nor did it lead to further trans or postoperative complications, as reported by Baltzer *et al*. (2015) and Ree *et al*. (2016), who used free omental graft over metallic implants in the treatment of fractures of the radius and ulna and arthrodesis, respectively.

The increase in volume observed in the region of the experimental surgical wound was an outstanding feature in the clinical evaluation of rabbits, suggesting important organic reactions regarding the omental graft. It is believed that the increase in soft volume palpated in the first 24 hours after surgery is related to the beginning of the inflammatory response with edema production, in both groups (OG and CG), and because it is a procedure of grafting, in which the omental fragment does not have vascular and lymphatic connections, the physiological and metabolic reactions in the OG were more intense and prolonged, as well as the cardinal signs of inflammation. Other studies using free omental graft in orthopedic surgeries (Baltzer *et al*., 2015; Ree *et al*., 2016, 2018) also observed edema and swelling in the region of the surgical wound with implantation of an omental graft, however, as, in these studies, this change probably did not act negatively on the repair, as there were no complications such as suture dehiscence, devitalized tissue or necrosis.

The firm volume increase palpated from the second postoperative day onwards in the most central region of the experimental surgical wound with omental graft suggests, together with the findings of Thompson and Pollock (1945), that this is the moment when the vascular ramifications and release of growth factors begin after graft adherence to the implantation site. This change in volume with firm consistency is possibly related to the "activation" of the omentum against tissue injury, which, according to Litbarg *et al*. (2007), triggers a 15- to 20-fold increase in its mass, accompanied by an intense release of cytokines and stimulus to repair processes.

The presence of rosier color in the clinical evaluations of both groups suggests hyperemia, commonly associated with the inflammatory process, and the fact that the OG has a more intense color in the initial moments of evaluation is expected due to the presence of the graft and, possibly, it is also related to “activation” of the omentum that caused a more accentuated metabolic process. Clinical studies using free omental graft in orthopedic surgeries (Baltzer *et al*., 2015; Ree *et al*., 2016, 2018) also demonstrate the presence of erythema in the surgical wound region and, as in this study, this change did not generate major complications and

did not progress to suture dehiscence or tissue necrosis. It is also worth noting that the "activation" of the omentum triggers the process of angiogenesis through the release of 2 to 3 times more Endothelial Growth Factor (VEGF), as observed by Zhang *et al*. (1997) and Litbarg *et al*. (2007), which may explain higher scores of neovascularization in deep dermis in the OG, reflecting the presence of rosier color for a longer period. The purplish color of the skin of some of the OG animals in the first days of evaluation may be due to the greater density of blood vessels in the deep dermis associated with the possible vascular congestion caused by the inflammatory process.

Both in the clinical and microscopic evaluation of the animals in the OG, there was a reduction in edema between 7, 14, and 28 days after the surgery, which suggests the occurrence of lymphangiogenesis, as well as a slowdown in the inflammatory response against the omental graft, being also observed in other studies that used free omental graft in orthopedic surgeries (Baltzer *et al*., 2015; Ree *et al*., 2016, 2018). It was observed in the microscopic evaluation that in some animals there was still slight edema in the deep dermis, more precisely inside the omental graft, between the 14th and 28th postoperative days, which may be associated with a slower lymphatic drainage process in this period, which can be explained by the growing increase in the degree of fibroplasia observed in the region of the omental graft at 28 days, which possibly made the residual edema more organized and located inside the graft.

Claro Junior *et al*. (2014) microscopically evaluated samples of pedicled omental flap sutured to the abdominal wall of rats eight weeks after the operation, reporting a high degree of fibrosis and contraction when compared to the intra-abdominal omentum. Despite being another way of using the greater omentum, the results by Claro Junior *et al*. (2014) are similar to what was found in this study regarding the observation of contraction and intense fibroplasia in the omental graft over the days of evaluation, demonstrated by the gradual decrease in firm volume in the region of the graft, which evolved to a small increase in firm texture in two rabbits evaluated 28 days after surgery. It should also be noted that these processes of contraction and fibroplasia do not seem to have generated any negative consequences for tissue repair in the present study.

The marked neovascularization in the deep dermis of the OG, especially on the seventh and twenty-eighth days of evaluation, as observed by Litbarg *et al*. (2007), probably occurred due to the angiogenic capacity of the omental graft when "activated". In the macroscopic evaluation, it was possible to notice vascular congestion, especially on the seventh day, as well as the formation of new blood vessels towards the omental graft, suggesting not only the graft viability but also its angiogenic capacity. It was also observed on macroscopy, especially on the seventh day of evaluation, around the omentum, an intensely reddish area that was associated with periomental hemorrhage, also seen on microscopy, but it can be proposed that this change corresponds to marked neovascularization, increased density of blood vessels and blood content in the omentum area. Although the neovascularization of the superficial dermis on microscopy has not shown to be different between the groups, the visualization of cutaneous vessels in the clinical evaluation of the OG remained until the fourteenth day of evaluation, which may suggest a greater blood supply to the region of the experimental wound, possibly due to metabolic stimuli generated by the "activation" of the omentum in the deep dermis.

The FOGWVA implanted under the skin wounds in rabbits, showed similar evolution, in some respects, to the implantation of this type of graft in other places such as lung (Thompson and Pollock, 1945) and bone (Oloumi *et al*., 2006). On the seventh postoperative day, the adhesion of the omental graft to the experimental wound, similar to what routinely occurs in the abdominal cavity in situations of injury (traumatic or surgical), demonstrated that even freely, the omentum maintained its largely known adhesion characteristic (Koppe *et al*., 2014). This adhesion, associated with the neovascularization observed towards the graft, corroborates the hypotheses of Thompson and Pollock (1945) regarding the vascular connections of the omentum after one week of implantation. It is suggested that the purplish color of the omental graft region on the seventh postoperative day can be explained by the intense organic reaction and vascular congestion of the omentum at that time

of evaluation, evolving to its natural yellowish color on the fourteenth postoperative day. The appearance of the omental graft on day 14 was similar to the observations by Oloumi *et al*. (2006), who also visualized firm adherence of a yellowish color to the bone, yet the microscopic evaluation in the present work showed that adipocytes were normal and viable at this time of evaluation. The gradual reduction of reddish areas around the omentum, maintenance of neovascularization, reduction in volume, and pale-yellow color observed in animals euthanized at 28 days may suggest a possible slowdown in the organic responses to the graft, and evolution to the repair of the experimental wound.

The fact that the experimental wound in this work was sutured with an intradermal pattern, which has marked advantages, including adequate apposition with minimal tension and better epithelialization with reduced scar tissue (Sylvestre *et al*., 2002), associated with the accelerated expression of cellular and biochemicals in rabbits (Salgado *et al*., 2007) may explain why keratinization was complete in both groups at the first time of evaluation (7 days).

The implantation of the omental graft seems to have caused a more intense inflammatory process, which may be associated with the grafting procedure, albeit an autogenous material, and the fact that the omentum is a source of cytokines, chemotactic factors, and inflammatory mediators (Litbarg *et al*., 2007; Azari *et al*., 2012; Uchibori *et al*., 2017), reflecting the higher and longer presence of polymorphonuclear and mononuclear cells in the deep dermis. Also, according to Litbarg *et al*. (2007), the activation of the omentum is characterized by a 15- to a 20-fold expansion of its non-fat mass, composed of clusters of immune system cells called "milky spots", which would also explain the highest number of inflammatory cells, especially mononuclear cells, in the deep dermis of the OG animals. The slow decrease in the number of polymorphonuclear cells and maintenance of a high number of mononuclear cells, as early as the seventh day after surgery, seems to indicate chronicity in the inflammatory response, but observed characteristics in the clinical and anatomopathological evaluation suggest a reduction in inflammation and evolution to repair tissue. Clinically, it was possible to evaluate a marked reduction in edema in the OG, evidenced by the reduction in soft volume over the days, with a reduction in staining scores, indicating a slowdown in the cardinal signs of inflammation. In the macroscopic evaluation of the skin fragment with implantation of the omentum, a decrease in vascular congestion and hemorrhage was observed between the moments of evaluation, as well as a gradual decrease in the volume of the omentum, indicating less intense organic responses. Furthermore, the reduction in edema and hemorrhage associated with angiogenesis and the accelerated synthesis of collagen in the OG support the hypothesis that there was possibly a reduction in the pro-inflammatory activity and an acceleration in the process of repairing the injured tissue. Studies using immunohistochemistry and PCR demonstrate that implantation of the omentum can facilitate the expression of signals that mobilize inflammatory cells (especially mononuclear cells such as macrophages and lymphocytes) that produce anti-inflammatory cytokines and are mainly associated with remodeling and repair (Uchibori *et al*., 2017), which may suggest that the presence of mononuclear cells in greater quantities in the OG, as early as the seventh day, may be related to the faster healing process.

The marked presence of collagen fibers, especially in the superficial dermis of animals in the OG compared to the CG on the seventh day of evaluation, suggests an acceleration in the process of cutaneous repair and healing. The accelerated collagen synthesis was also described by Azari *et al*. (2012), who used free omental graft for esophageal healing, which is associated with the angiogenic capacity and release of growth factors by the omentum.

The results of the present study open the possibility for the use of the FOGWVA, simple, low-cost, and easy to perform technique, which have advantages over other forms of omental use, reducing complications related to the use of the pedicled omental flap, enabling the implantation of the graft in places distant from its origin with greater ease of manipulation, without the need for specific and expensive surgical materials, as observed in the use of FOGWVA (Brockman *et al*., 1996). Still, the use of the

FOGWVA reduces the transoperative time when compared to the pedicled omental flap, since it is not necessary to perform subcutaneous tunnels (Brockman *et al*., 1996). Once studies using the FOGWVA have shown no effective success in veterinary medicine (Roa *et al*., 1999; Pap-szekeres *et al*., 2003), the present work suggests promising results in the use of the FOGWVA as a stimulus to the skin healing process.

CONCLUSION

It is concluded that the free omental graft without vascular microanastomosis exerts a positive influence on the healing of skin wounds experimentally produced in rabbits, accelerating collagen synthesis and repair, without causing deleterious effects and remaining viable at the implantation site.

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