Histology of the *rectus abdominis* muscle in rats subjected to cranial and caudal devascularization[1]

Histologia do músculo reto do abdome de ratos submetidos à desvascularização cranial e caudal

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ABSTRACT

PURPOSE: To investigate the microscopic changes in the *rectus abdominis* muscle in rats subjected to five delay procedure.

METHODS: 30 male *holtzmann* rats, weighting between 250 and 350 grams, were used. The animals were divided into five groups (n=6): **A** – cranial section of the right muscle; **B** – caudal section of the right muscle; **C** – craniocaudal section of the right muscle; **D** – cranial section reflecting the right muscle in the craniocaudal direction; **E** – caudal section reflecting the right muscle in the caudocranial direction. On the seventh day after surgery, a resection of the cranial and caudal fragments of the right and left muscles, respectively, was performed for microscopic analysis. Histological alterations were quantified and the right and left (control) muscle fragments compared. Fisher's exact test was used for statistical purposes with a significance level of 5%.

RESULTS: The comparison between right and left muscles showed statistically significant differences in group A – inflammatory infiltrate in the cranial fragment (p=0.015); in group C – inflammatory infiltrate (p=0.000) and necrosis (p=0.015) in the caudal fragment; and in group E – edema in the caudal fragment (p=0.000). No significant alterations were noted in groups B and D. **CONCLUSIONS**: Irrigation exclusively through the perforating muscle vessels is inappropriate; irrigation exclusively through the cranial pedicle causes milder, insignificant histological alterations, when compared with irrigation exclusively through the caudal pedicle; irrigation exclusively through the caudal pedicle causes more pronounced, but still insignificant, histological alterations when compared with other forms of devascularization.

Key words: Surgical Flaps. Rectus Abdominis. Mammaplasty. Rats, Sprague-Dawley.

RESUMO

OBJETIVO: Investigar as alterações microscópicas no músculo reto do abdome de ratos submetidos a cinco técnicas de autonomização.

MÉTODOS: Utilizaram-se 30 ratos machos *Holtzmann*, pesando entre 250 e 350 gramas. Os animais foram distribuídos em cinco grupos (n=6): **A** - secção cranial do músculo direito; **B** - secção caudal do músculo direito; **C** - secção cranial e caudal do músculo direito; **D** - secção cranial rebatendo o músculo direito no sentido cranial-caudal; **E** - secção caudal rebatendo o músculo direito no sentido caudal-cranial. No sétimo dia pós-operatório, ressecou-se um fragmento cranial e caudal dos músculos direito e esquerdo, respectivamente, para estudo microscópico. As alterações histológicas foram quantificadas e comparados os fragmentos dos músculos direito aos do esquerdo (controle). Utilizou-se, para fins estatísticos, o teste exato de Fisher, com nível de significância de 5%.

RESULTADOS: A comparação entre os músculos direito e esquerdo demonstrou significância estatística no grupo A - infiltrado inflamatório no fragmento cranial (p=0,015); no grupo C - infiltrado inflamatório (p=0,000) e necrose (p=0,015) no fragmento caudal; e no grupo E - edema no fragmento caudal (p=0,000). Não foram observadas alterações significativas nos grupos B e D. **CONCLUSÕES**: A irrigação exclusiva pelos vasos musculares perfurantes é inadequada; a irrigação exclusiva pelo pedículo cranial causa alterações histológicas menos acentuadas, não-significantes, em comparação à irrigação exclusiva pelo pedículo caudal; a irrigação exclusiva pelo pedículo caudal causa alterações histológicas mais acentuadas, não-significantes, em comparação com as demais formas de desvascularização.

Descritores: Retalhos Cirúrgicos. Reto do Abdome. Mamoplastia. Ratos Sprague-Dawley.

Introduction

The Transversal *Rectus Abdominis* Myocutaneous flap (TRAM) has been considered the standard autogenous tissue for breast reconstruction ever since its description by Hartrampf *et. al.*[1], despite reported complications among at-risk patients such as smokers, the obese and diabetics[1-3]. Among 300 patients in good health who were subjected to breast reconstruction employing this flap, however, complications were rare, with the most frequent one being partial or total loss of the flap, which occurred in 6.2% of the patients. This complication occurs due to tension in the vascular pedicle, hematomas and technical errors. Among the patients that presented complications, 32% had previously been subjected to radiotherapy and 26% were smokers[3-5].

Considering the importance of the *rectus abdominis* muscle in breast reconstruction, this study was conducted on rats with the goal of assessing microscopic alterations in this muscle after the use of five devascularization techniques.

Methods

This was a prospective study of 30 males, Holtzmann, whose weight varied between 250g and 350g. The project was approved by the Animal Experimentation Ethics Committee, Protocol No. 106/2006 (CETEA/UFMG). In all stages of the experiment, the ethical precepts that guide animal experimentation were observed[6-8].

The 30 animals were randomly distributed into five groups of six. In each group of animals, a different type of devascularization of the right *rectus abdominis* muscle was performed as described below:

- Group A – section of the cranial epigastric vessels
- Group B – section of the caudal epigastric vessels
- Group C – section of the cranial and caudal epigastric vessels
- Group D – section of the cranial and perforating epigastric vessels, maintaining irrigation through the caudal epigastric vessels
- Group E – section of the caudal and perforating epigastric vessels, maintaining irrigation through the cranial epigastric vessels

Anesthesia and preparation for the surgical procedure

All the animals were subjected to intraperitoneal anesthesia using ketamine chloridrate (Ketalar®), at a dosage of 50mg/kg, together with 2-(2.6xilidino)-5.6dihidro-4H-1.3-tiazine (Rompun®), at a dosage of 1.0 ml/kg, administered through a plastic insulin syringe (U-100) connected to 13x8 needles. Next, trichotomy of the abdomen, antisepsis with povidone-iodine detergent and the placement of sterile drapes were performed.

Surgical procedure

The surgical procedure was performed in accordance with the specific group of animals:

- Group A – Transversal incision of the skin and superficial fascia, one centimeter wide, in the cranial threshold of the right *rectus abdominis* muscle. Section of the anterior aponeurosis and of the muscle at this point, and cauterization of the cranial epigastric vessels with a bipolar scalpel. Suture of the anterior aponeurosis with two simple stitches and continuous intradermic suture of the skin, both with mononylon 6-0 thread.
- Group B – Incision and suture identical to Group A in the caudal threshold of the right *rectus abdominis* muscle and cauterization of the caudal epigastric vessels.
- Group C – Incision and suture identical to groups A and B, in the cranial and caudal thresholds of the right *rectus abdominis* muscle and cauterization of the cranial and caudal epigastric vessels (Figure 1).
- Group D – Median xifopubic incision exposing the anterior aponeurosis of the right *rectus abdominis* muscle. Transversal section of the aponeurosis and muscle at the cranial threshold, cauterization of the cranial epigastric vessels. Medial and lateral sections of the anterior aponeurosis, 3.5 cm wide, and the release of the right muscle from its posterior aponeurosis, in the cranio-caudal direction. Suture of the aponeurosis identical to that of the skin (Figures 2 and 3).
- Group E – incision and exposition identical to group D. Transversal section of the aponeurosis and muscle at the caudal threshold, cauterization of the caudal epigastric vessels. Median and lateral section of the anterior aponeurosis, 3.5 cm wide, and release of the right muscle from its posterior aponeurosis in the caudocranial direction (Figures 4 and 5).

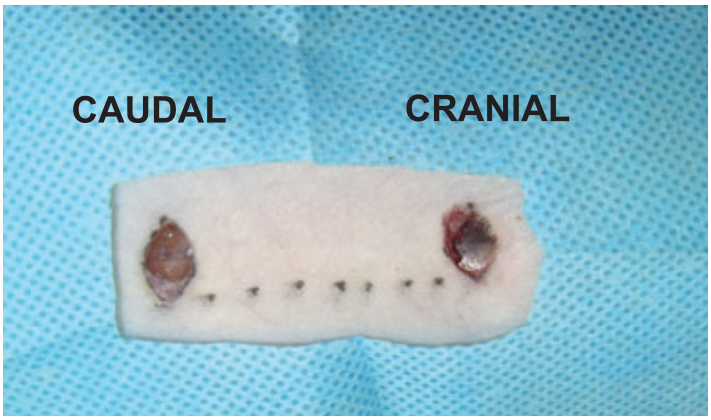

FIGURE 1 – Group C - anterior abdominal wall of rat displaying both cranial and caudal devascularization of the right *rectus abdominis* muscle.

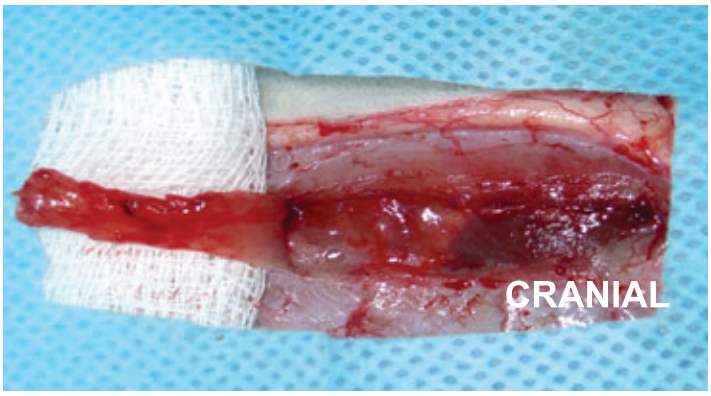

FIGURE 2 – Group D – anterior abdominal wall of rat displaying cranial devascularization of the right *rectus abdominis* muscle, reflected in the craniocaudal direction.

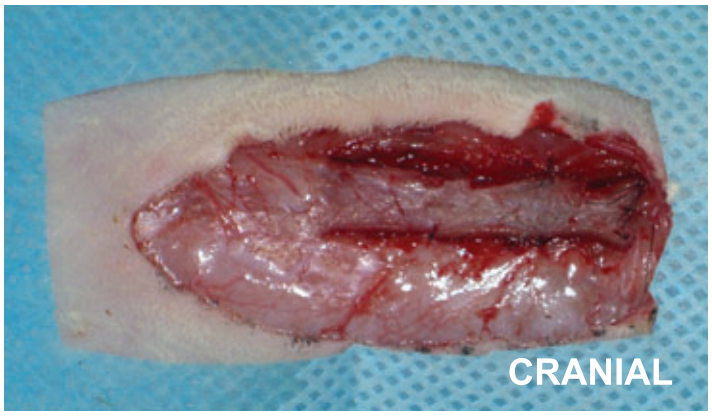

FIGURE 3 – Group D - anterior abdominal wall of rat displaying cranial devascularization of the right *rectus abdominis* muscle, sutured in its bed after the muscle was reflected in the craniocaudal direction.

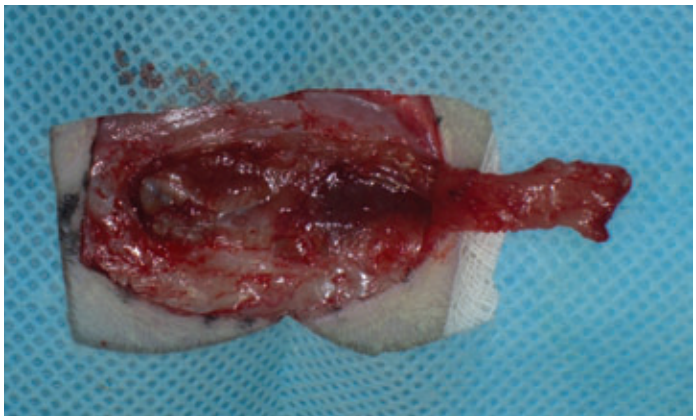

FIGURE 4 – Group E- anterior abdominal wall of rat displaying caudal devascularization of the right *rectus abdominis* muscle, reflected in the caudocranial direction.

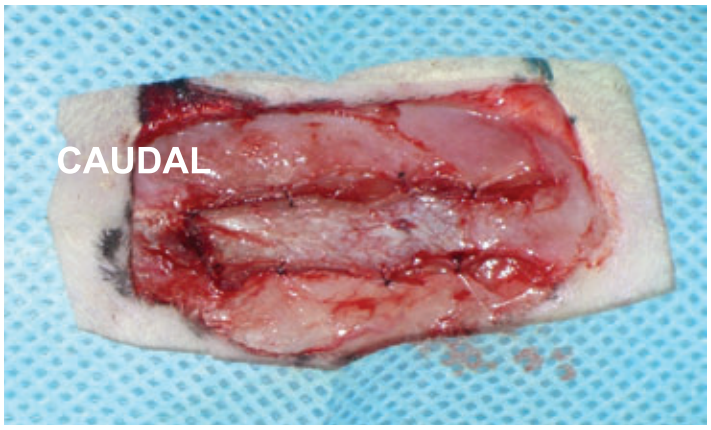

FIGURE 5 – Group E- anterior abdominal wall of rat displaying caudal devascularization of the right *rectus abdominis* muscle, sutured in its bed after the muscle was reflected in the caudocranial direction.

Specimen collection for histopathology and euthanasia of the animals

On the seventh day following surgery, the animals were anesthetized as in the surgical procedure and subjected to a block resection of the right *rectus abdominis* muscle, associated with the extirpation of the left *rectus abdominis muscl*e, which served as a control. Two fragments, of one centimeter each, of the left and right muscles of the cranial and caudal localization, respectively, were extracted, identified and fixed in formaldehyde.

The animals were euthanized while still under anesthesia through the sectioning of the abdominal aorta.

Anatomopathological exam

The muscle fragments were processed in blocks of paraffin and the histological cuts were colored with hematoxylin and eosin (HE), and analyzed through optic microscopy. The

following alterations were investigated in the five groups of animals by the same pathologist: inflammatory infiltrate, muscle fiber necrosis, vascular alterations, foreign body type reactions and edema (Figure 6), quantitatively classified as:

- **N** (Normal) - no alterations present;
- + - alterations of up to 33%;
- ++ - alterations of between 34 and 66%;
- +++ - alterations of between 67 and 100%.

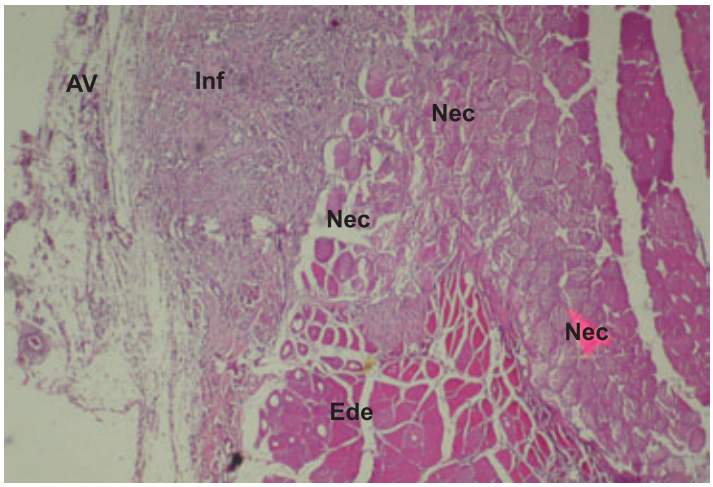

FIGURE 6 – Group C – colored with HE (50x). Histological cut of the caudal fragment of the right *rectus abdominis* muscle.
Inf=inflammatory infiltrate (++), **Nec=**muscle fiber necrosis (++), **AV=**vascular congestion (+), **Ede=**edema (++).

Variables investigated and statistical analysis

The dependent variable was represented by the devascularized right *rectus abdominis* muscle and the independent variable by the surgical devascularization techniques. For each group of animals, the microscopic alterations in the cranial and caudal fragments of the right muscle (devascularized) were compared with the left muscle (control) through Fisher's exact test with a significance level of 5%.

Results

The comparison of the histological alterations between the left and right *rectus abdominis* muscles showed significant results in the following groups (Table 1):

- **Inflammatory infiltrate** in the cranial segment of group A (p = 0.015) and in the caudal segment of group C (p = 0.000);
- **Necrosis** in the caudal segment of group C (p = 0.015);
- **Edema** in the caudal segment of group E (p = 0.000). In groups B and D, there were no significant differences between the left and right muscles in terms of the investigated histological alterations.

TABLE 1 - p-values for the comparison between the left and right muscles for groups A, B, C, D and E in terms of the percentage with some alteration in the variables investigated.

Alteration	Fragment	Groups				
		A	B	C	D	E
Inflammatory Infiltrate	Cranial	**0.015***	1.000	0.455	0.455	0.545
	Caudal	1.000	0.242	**0.000***	1.000	0.061
Muscle fiber necrosis	Cranial	1.000	0.182	1.000	0.455	0.080
	Caudal	0.182	0.182	**0.015***	0.567	0.061
Vascular alteration	Cranial	1.000	1.000	1.000	1.000	1.000
	Caudal	1.000	1.000	0.061	1.000	0.455
Foreign body type reaction	Cranial	1.000	1.000	1.000	1.000	0.545
	Caudal	1.000	1.000	1.000	1.000	0.061
Edema	Cranial	0.242	0.545	0.182	0.061	0.061
	Caudal	0.567	0.242	0.545	0.242	**0.000***

*p value with statistical significance

An increase in necrosis and inflammatory infiltrate found in the animals in groups D and E when compared with the other groups was also noted, however it was not statistically significant (Figure 7).

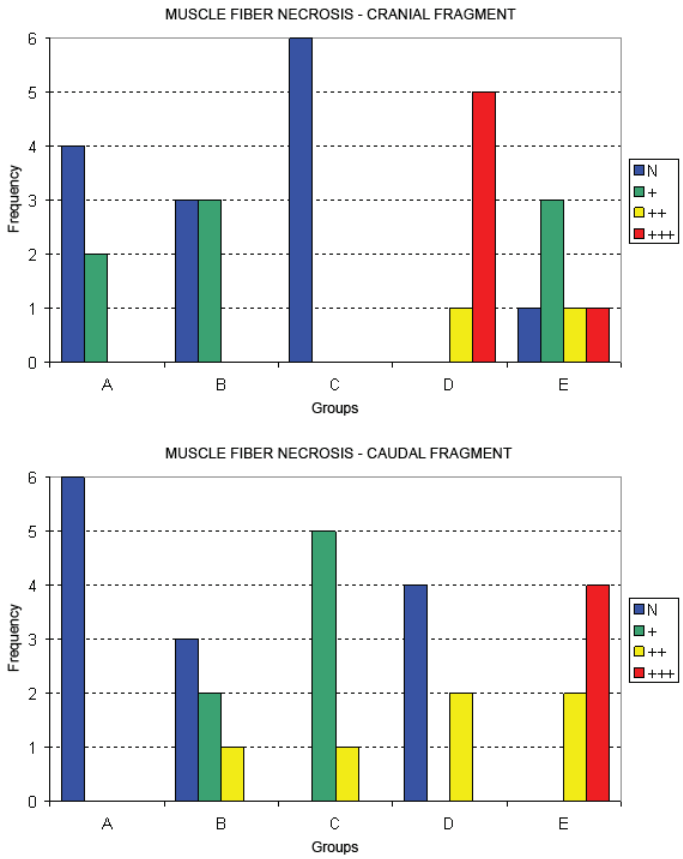

FIGURE 7 - Frequency and intensity of muscle fiber necrosis in groups A, B, C, D and E.

Discussion

Flaps are broadly employed in the reconstruction of defects caused by surgery, trauma, burns, etc. This type of reconstruction may, however, result in complications, the most common of which is partial or total necrosis of the flaps. This has stimulated research aimed at reducing the risks of said complication.

Miller *et al.*[9] investigated the supply of blood to the TRAM through angiographies of the internal mammary artery and the observation of the vascular anatomy of patients that had been subjected to breast reconstruction, demonstrating the applicability and safety of this flap.

Ishii *et al.*[10]and collaborators proposed the bipediculated transversal flap of the *rectus abdominis* muscle for high risk patients, aiming at increasing local blood supply. Results were satisfactory, showing a reduced number of complications, but were not confirmed by other authors[11-13].

Wagner *et al.*[14] reported their experiences with 341 unilateral breast reconstructions. They recognized increased tissue necessity, larger flap extension and microvascular dysfunction as conditions which justify the use of the bivascular flap. The univascular flap, they stated, should be reserved for patients with no risk factors.

Boyd *et al.*[15] identified, in humans, a dominance of the role played by the caudal epigastric vascular pedicle in the *rectus abdominis* muscle, along with the presence of numerous perforating muscular vessels acting in the local collateral circulation.

The rat has proven an excellent study model for TRAM research[16], having been used by several authors. Özgentas *et al.*[17,18] demonstrated, through microangiography, the physiological dominance of the cranial epigastric vessels in relation to the caudal epigastric vessels.

The study of the *rectus abdominis* muscle for the purpose of reconstructing defects in the corporal surface are part of a line of research initiated by Lopes Filho and Lázaro da Silva[19]. These authors adopted the term Retramcur (Transversal *Rectus Abdominis* Myocutaneous flap) and performed various types of blood flow dissections aimed at bilateral cranial autonomization of the skin, superficial fascia and *rectus abdominis* muscle. The group of animals in which only the deep superior epigastric vessels were sectioned presented a smaller area of necrosis in the flap[19].

Based on knowledge of the vascular anatomy of the anterior abdominal wall of the rat, various devascularization models for the *rectus abdominis* muscle were produced in an attempt to clarify, from a histological perspective, the possible repercussions resulting from the modification of its normal vascular physiology.

In the present study, the simultaneous interruption of blood flow in the cranial and caudal epigastric vessels caused necrosis in the distal muscle fragment among the animals in Group C. This proved the inadequacy of irrigation exclusively through the perforatory vessels. Despite the dominance of the cranial epigastric vascular pedicle in rats having been defined in the literature[17,18], no significant histological alterations were found between the animals in Group D and their respective controls. This probably was due to the smaller size of the group sampled. An increase in necrosis and inflammatory infiltrate was found among the animals in groups D and E, but this was not statistically significant (Figure 7).

Considering that TRAM is one of the most widely employed techniques in breast reconstruction, studies show that complications persist. It is therefore necessary to proceed with further studies, especially concerning blood irrigation of the muscles, in order to improve the technique.

Conclusions

The irrigation through perforating muscular vessels alone and through the caudal pedicle of the *rectus abdominis* muscle in rats cause more severe histological alterations. These vascular pedicles, however, are not employed in the TRAM technique. On the other hand, irrigation exclusively through the cranial pedicle causes less severe histological alterations, provided that this is the vascular pedicle utilized in the TRAM procedure.

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