

## Effects of the Corticosteroids in the Lesions by Radiofrequency on Rats' Thigh in Different Age Groups

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### Abstract

**Background:** The corticosteroids limit the late growth of the lesions by radiofrequency (RF) on the rats' infants' thighs, but the effects on the pubescent and adult rats are unknown.

**Objective:** Evaluate the effects of the corticosteroids in the healing of the lesions by RF on the rats' thighs muscles in different age groups.

**Methods:** Ablation was performed on the thigh muscle of 30 rats (1 lesion per animal): infants (30 days old, weight 73 g, n = 10), pubescents (60 days old, weight 230 g, n = 10) and 10 adults (90 days old, 310 g, n = 10), subdivided in control and treated groups, that received Hydrocortisone (10 mg/kg IM post-RF) and Betametason (3.5 mg/kg IM, twice a week, for 29 days). The rats were sacrificed 60 days after the ablation for hystopathological and planimetric analysis with specified software (ImageJ™).

**Results:** In the infant, pubescent and adult groups, the weight gain in the follow up did not differ between the control and the treated ones. In the control group, the lesions of the infants and pubescents were superior ( $p = 0.01$ ) to the adults'. The treatment reduced the size of the lesion in the infants ( $5.58 \pm 0.61 \text{ mm}^2$  vs  $4.02 \pm 0.23 \text{ mm}^2$ ;  $p < 0.01$ ) and pubescents ( $5.20 \pm 0.47 \text{ mm}^2$  vs  $4.16 \pm 0.48 \text{ mm}^2$ ;  $p < 0.01$ ), but not in the adults ( $4.44 \pm 0.50 \text{ mm}^2$  vs  $4.79 \pm 0.53 \text{ mm}^2$ ,  $p = \text{NS}$ ). Infant and pubescent treated groups presented lower collagen deposition and less fibrotic bands invading the healthy tissue from the central fibrosis area, and forming lesions with remarkably more reduced dimensions than their controls. There were no differences in the adult groups.

**Conclusion:** The corticosteroids seem to reduce the late growth of lesion, in addition to attenuate the fibrotic proliferation in the infant and pubescent rats. (Arq Bras Cardiol 2010; 95(2): 207-214)

**Key words:** Corticosteroids; rats; myocardium/injuries.

### Introduction

Currently, the catheter ablation with radiofrequency (RF) energy is the chosen procedure for the treatment of patients with tachyarrhythmias, including pediatric ones<sup>1</sup>, due to its efficacy and safety. However, the RF ablation effects are not restricted only to the energy application moment. Late effects can also appear up to six months after the procedure, such as, for example, the late atrioventricular block after tachycardia ablation by atrioventricular nodal reentry<sup>2</sup>. The mechanisms of these late effects still remain obscure, but seem to be related to the lesion growth during its healing, probably by inflammatory process progression and/or damage to microcirculation or ultra structural damage to the surrounding tissue.

Saul et al<sup>3</sup> demonstrated that the lesions by RF in the ovine myocardium present remarked late growth and invasion

of the normal myocardic tissue by fibroelastic tissue; these findings indicate possible pro-arrhythmic effects in pediatric populations who underwent RF ablation, eventually leading to sudden death. As the inflammatory process seems to participate in the late extension of the lesion, we have as hypothesis that the corticosteroids could attenuate this process<sup>4</sup>. In this aspect, we demonstrated in a previous publication that the corticosteroids limit the late growth of the lesions by RF on the rats' infants' thigh muscle<sup>5</sup>. However, this investigation presented some relevant limitations: the effects of the treatment in the lesion dimensions were considered only qualitatively, without any quantification; as the animals were sacrificed on the last treatment day (30 days after the ablation), the final result of healing was not established; finally, it was not determined the group age in which the late growth stop occurring, as the intermediary age group (pubescents) was not investigated. The explanation of these issues motivated the present paper.

Therefore, we evaluated qualitative and quantitative aspects of the consolidate healing of the lesions by RF under effect of corticosteroids on the rats' thigh muscle in different age groups (infants, pubescents and adults).

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## Methods

This study was approved by the Ethics Committee on Research of the *Universidade Federal de São Paulo/Hospital São Paulo*.

### Experimental Design

#### The sample

Thirty rats were used (*Rattus norvegicus*) EPM-1 Wistar, males, divided into six groups after the ablation with RF, namely:

- 1) 05 (five) infant rats 30±03 days old - Controls (1m-C).
- 2) 05 (five) infant rats 30±03 days old - Treated (1m-T).
- 3) 05 (five) pubescent rats 60±03 days old - Controls (2m-C).
- 4) 05 (five) pubescent rats 60±03 days old - Treated (2m-T).
- 5) 05 (five) adult rats 90±03 days old - Controls (3m-C).
- 6) 05 (five) adult rats 90±03 days old - Treated (3m-T).

The group of infant, pubescent and adult rats would correspond approximately in humans, respectively, to infants and toddlers, to the beginning of puberty and to adolescents and young adults.

### Ablation protocol

In the anesthetic induction, it was used: Acepromazine at 0.2% (0,1 mg/kg IM), and association of tiletamine hydrochlorides and Zolazepam (30 mg/kg IM); with maintenance of the surgical anesthetic plan through the inhalation of isoflurane, under spontaneous ventilation.

Trichotomy and anti-sepsia of the rat's right posterior limb were performed, the incision was performed on the thigh muscle region of approximately 1.0 cm. Skin and fascia were dissected and removed, exposing the region of the medial muscles of the pelvic member (Semitendinosus and Gastrocnemius)<sup>5</sup>.

A conventional ablation catheter (Marinr, Medtronic™, 7Fr - tip of 4 mm), connected to the RF generator (Atakr™, Medtronic™ CardioRhythm) with maximum power of 50 Watts, was positioned in parallel, under constant manual pressure, over the exposed muscular tissue<sup>5</sup>. Unipolar application of RF (one per animal) was performed, under temperature control (70° C, 60 seconds), between the ablation catheter distal electrode and the indifferent plate-electrode, underpositioned on the medial face of the pelvic member. During each application, power, impedance, temperature of the catheter tip and time were monitored and their means recorded for posterior analysis. After the ablation, the wound was sutured.

The procedures were performed by the same operator, under sterile conditions, to prevent infections. Prophylactic antibiotics were not used. All over the protocol, the left thigh (the ablation was performed on the right thigh) was used for the inoculation of the anesthetics and corticosteroids.

### Post-ablation protocol

Half of the population received corticosteroids according to the respective weight range, while the other half received nothing, serving as control group. Before this procedure,

the initial weight of each rat was recorded for dosage adjust of the corticosteroids; hydrocortisone (10 mg/kg IM) inoculated immediately after the ablation and, later, Betametasone (3.5 mg/kg IM) twice a week - Mondays and Thursdays - for 29 days after the procedure. For adjusting the dosages, the rats were weighed weekly, immediately before each inoculation.

The animals were kept in the lab, under medical-veterinary supervision, being sacrificed 60±05 days after the ablation, that is, 30 days after the end of the therapy, in order to evaluate the consolidated aspect of the lesion healing.

### Macroscopic analysis

At the end of the follow-up, the rats were weighed again to evaluate the ponderal gain (Table 1) and submitted to inhalatory euthanasia by isoflurane for extracting the thigh muscular block. The piece was examined with the naked eye regarding its macroscopic aspect and their qualitative impressions recorded for later comparison.

As in our previous study<sup>5</sup>, the measurement of the macroscopic dimensions of the chronic lesions was not performed for the infant, pubescent or adult rats. The main reason for this is that such lesions are badly delimited, mainly in the two first groups, making the measurement very imprecise.

### Histological analysis

The removed pieces were individually preserved in formol at 10%. The lesion was sliced in several histological cuts to encompass it totally. The slides (three slides/piece) were stained by H.E. and Masson's trichrome. During the qualitative analysis, the lesions were delimited on the slide itself (Figure 1) by the pathologist. Blinded for the study groups, for performing the quantitative analysis.

### Qualitative analysis

In addition to the histopathological aspects of each lesion, special attention was taken to the reading of the fibrotic proliferation and of the inflammatory cellular infiltrate. As previously demonstrated in ovines<sup>3</sup> and in the rats' thigh muscle<sup>5</sup>, the acute lesions by RF in adults and infants are similar. However, while in the adults the chronic lesion dimensions are comparable to the acute lesions, in the infants, they remarkably increase. Therefore, in the present study, by definition<sup>5</sup>, the later growth of the lesion was characterized by the increase in the lesion by RF dimension in relation to the adults, while its late extension was defined as the occurrence of multiple fibroblast tissue extensions that, from the central fibrotic area, invade the healthy muscle tissue surrounding the chronic lesions by RF.

### Quantitative analysis

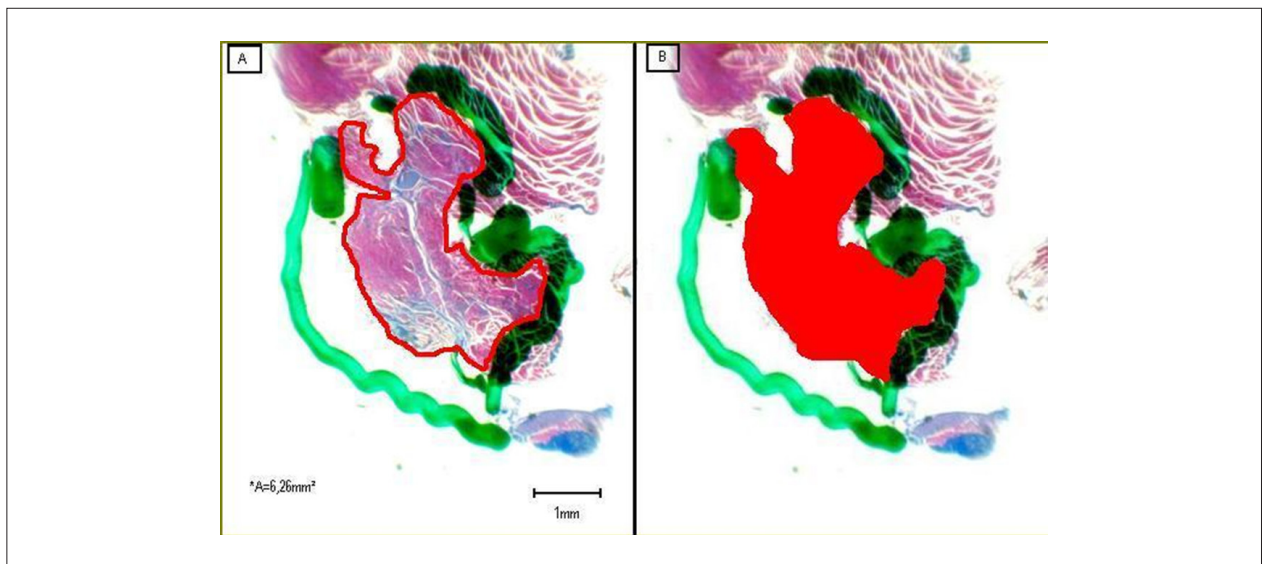
For the planimetric evaluation of the lesions, ImageJ™<sup>6,7</sup> software was used - broadly disseminated in cytometry<sup>8</sup> and other planimetric studies<sup>9-12</sup> - which automatically calculates the lesion area (Figure 1) with good precision and measurement converter to any desired unit by the operator. The measurement is manual, using the computer mouse that,

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**Table 1 - Biometric data of rats, biophysical parameters of applications and dimension of the lesions by radiofrequency of the infant, pubescent and adult groups**

Rat nr	Group	Initial weight (g)	Weight at sacrifice (g)	Ponderal gain (%)	Lesion area (mm <sup>2</sup> )	Impedance (Ohms)	Power (Watt)	Temperature (°C)	Time (sec)
1	IC	75	375	400	5.30	136	2	70	60
2	IC	75	350	366	5.27	115	1	70	60
3	IC	75	452	466	4.89	83	1	71	60
4	IC	65	350	438	6.18	123	1	70	60
5	IC	75	350	366	6.26	134	2	70	60
	TOTAL	73±4	†370±32	407±44	*5.58±0.61	118±21	1.4±0.5	70.2±0.4	60.0±0.0
6	IT	70	275	292	3.97	134	2	69	60
7	IT	70	260	271	4.23	182	2	69	60
8	IT	75	350	366	3.76	136	2	70	60
9	IT	75	300	300	3.86	111	2	70	60
10	IT	75	360	380	4.28	100	2	70	60
	TOTAL	73±3	†309±44	322±48	4.02±0.23	132±31	2.0±0.0	69.6±0.5	60.0±0.0
11	PC	225	350	55	5.23	154	2	70	60
12	PC	230	350	52	4.87	150	2	69	60
13	PC	230	350	52	5.97	127	2	70	60
14	PC	240	400	66	4.78	147	2	69	60
15	PC	240	400	66	5.16	119	2	70	60
	TOTAL	233±7	†370±27	58±7	§ 5.20±0.47	139±15	2.0±0.0	69.6±0.5	60.0±0.0
16	PT	250	470	88	3.73	127	2	69	60
17	PT	240	400	66	3.92	96	2	70	60
18	PT	245	450	83	4.79	145	2	70	60
19	PT	240	400	66	4.57	105	2	70	60
20	PT	245	450	83	3.81	98	2	70	60
	TOTAL	244±4	†434±32	77±10	4.16±0.48	114±21	2.0±0.0	69.8±0.4	60.0±0.0
21	AC	325	475	46	5.01	136	2	70	60
22	AC	320	450	40	3.95	115	1	70	60
23	AC	320	450	40	3.89	83	1	71	60
24	AC	325	475	46	4.58	123	1	70	60
25	AC	330	500	51	4.78	134	2	70	60
	TOTAL	324±4	†470±20	45±4	‡4.44±0.50	118±21	1.4±0.5	70.2±0.4	60.0±0.0
26	AT	300	475	58	4.77	99	2	69	60
27	AT	290	425	46	4.15	145	2	69	60
28	AT	290	400	37	5.53	144	2	69	60
29	AT	300	500	66	5.04	152	3	69	60
30	AT	295	450	52	4.48	149	2	70	60
	TOTAL	295±5	†450±39	52±10	4.79±0.53	137±21	2.2±0.5	69.2±0.4	60.0±0.0

IC - infant control; IT - infant treated; PC - pubescent control; PT - pubescent treated; AC - adult control; AT - adult treated. (\*)  $p < 0.001$  in relation to IT; (†)  $p < 0.001$  in relation to the initial weight; (‡)  $p = 0.01$  in relation to IC and PC. (§)  $p < 0.01$  in relation to PT.



**Figure 1** - Measurement of the lesion area with ImageJ software. Slide of rat nr. Five of Infant Control group, stained by Masson's Trichrome in a 10X increase. (A) The thick external line surrounding the lesion was marked on pen by the pathologist, while the thin, internal line was marked by the ImageJ operator's mouse. (B) The area marked by the fine line (shaded) was calculated by the software as estimate of the lesion size.

contouring the edges of the lesion, generates automatically the measurement result.

In each animal, the slide presenting the biggest lesion area (width and length in mm<sup>2</sup>) was used as size estimate of the lesion. Due to the complex geometry of the lesions, very irregular and spread, mainly in infants, despite the relatively large number of histological cuts performed, it was not possible to quantify the volume of the lesions.

### Statistical analysis

The variables are expressed as mean  $\pm$  standard deviation. Variance analysis (ANOVA) was used for comparing the sample groups. The  $p < 0.05$  values were considered as significant.

## Results

### Biophysical parameters of the RF applications

A lesion by RF was created in the thigh of each animal. As shown in Table 1, there was no difference between the groups in relation to the evaluated parameters.

### Animal development and evolution after the ablation

During the follow-up, no animal presented another occurrence, or local or systemic infections. From the procedure to the euthanasia, all the groups presented significant ponderal gain (Table 1), with no significant differences between the control and treated groups. As expected, the mean ponderal gain of the infants (control 407% and treated 322%) was superior to the pubescent's (control 58%, treated 77%) and adults' (control 45%, treated 52%).

### Lesions macroscopic analysis

In the infants and pubescents, the lesions were extensive

(approximately 2 cm wide), pale colored and with irregular edges, making difficult the visualization of their limits and making unfeasible the macroscopic evaluation of their dimensions. In adults, in turn, the lesions were much more delimited, with regular edges and characterized by rounded, pale colored areas, around 1 cm wide. In all the groups, the lesions were shallow, around 2 mm deep.

### Lesions qualitative analysis

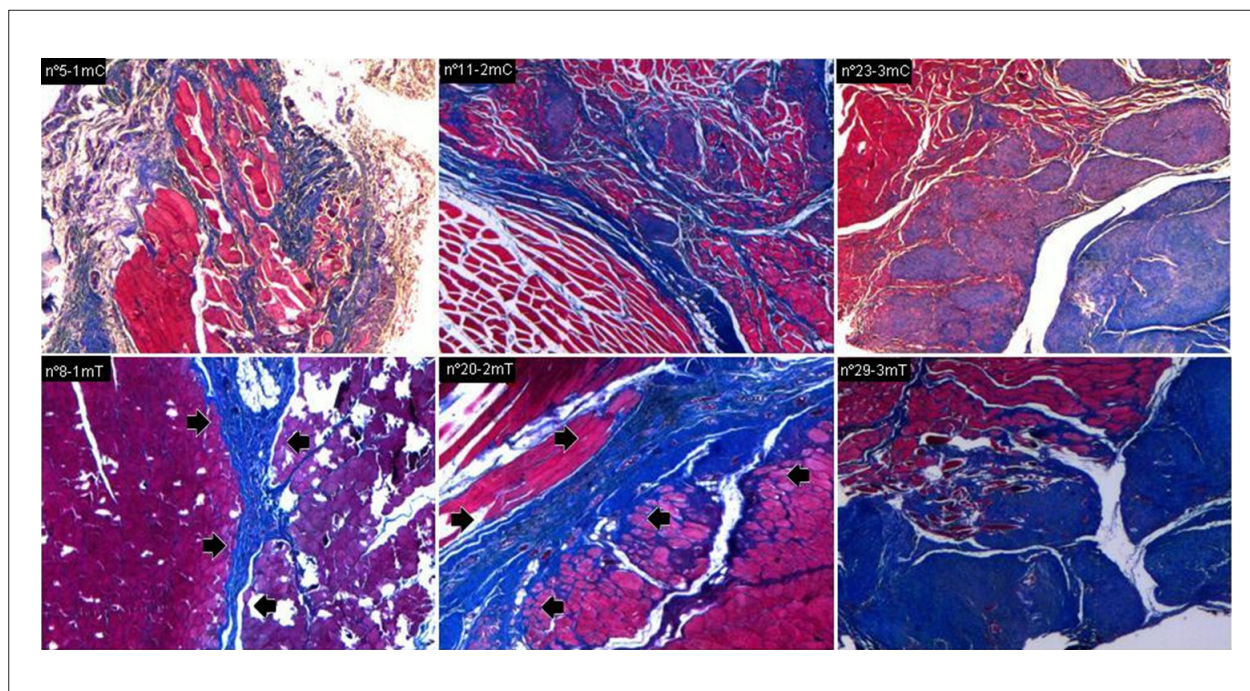
The qualitative findings were very consistent in all groups, as observed on Figure 2. The biggest lesions were seen in the infant controls (Figure 3), presenting large badly delimited fibrosis areas, with remarkable late extension, that is, invasion of the adjacent muscular tissue by multiple bands of myofibroblastic tissue, sometimes involving healthy muscle fibers. The pubescent controls showed lesions with a little smaller fibrosis areas than the infants', but better delimited. In this group, it was also observed a late extension, though the fibrosis bands presented a smaller number and extension when compared to the infants'. In the adults control, the lesions were smaller than in the other groups, well delimited and without late extension. The scars were well organized, with replacement of the myocytes by fibrous tissue.

In the treated infants and pubescents, the lesions presented notably reduced dimensions in relation to the controls. The scars were immature, with delayed healing, characterized by smaller collagen deposition and presence of granulation tissue. In addition, there were less fibrotic bands invading the healthy tissue from the central fibrosis area. On adults treated, noticeable differences were not seen in relation to the controls.

### Lesions quantitative analysis

As illustrated in Table 1 and Figure 4, in the control group, the infants' ( $5.58 \pm 0.61$  mm<sup>2</sup>) and pubescents' ( $5.20 \pm 0.47$





**Figure 2** - Qualitative comparison between the chronic lesions in the different age groups (the animals are identified on the slide). Slides stained by Masson's Trichrome in 40X increases. In the 1mC group (Infant Control) and 2mC (Pubescent Control), the lesions are large, irregular, badly delimited and with stress late extension, that is, large fibroblastic bands invade and spread on the healthy muscles increasing the lesion dimension, more remarked phenomenon in the infant group. It is noted that only segments of the lesions are showed, because their dimensions surpass broadly the limits of the visual field of the slide. In the 1mT group (Infant Treated) and 2mT (Pubescent Treated), the lesions present remarkably reduced dimensions (arrows), with more concentrated collagen deposition and lower late extension. In the 3mC groups (Adult Control) and 3mT (Adult Treated), the lesions were well delimited and without late extension, there not being an observable difference between them. It is also noted that, in this cases, almost the whole lesion is contained in the slide visual field.

mm<sup>2</sup>) lesions presented late growth, being significantly ( $p = 0.01$ ) higher than the adults' ( $4.44 \pm 0.50$  mm<sup>2</sup>). The infants' lesions were bigger than the pubescents', but this difference was not statistically significant ( $p > 0.10$ ). Remarkable: the treatment reduced the lesion area in the infants ( $5.58 \pm 0.61$  mm<sup>2</sup> vs  $4.02 \pm 0.23$  mm<sup>2</sup>;  $p < 0.001$ ) and in the pubescents ( $5.20 \pm 0.47$  mm<sup>2</sup> vs  $4.16 \pm 0.48$  mm<sup>2</sup>;  $p < 0.01$ ), but not in the adults ( $4.44 \pm 0.50$  mm<sup>2</sup> vs  $4.79 \pm 0.53$  mm<sup>2</sup>,  $p = \text{NS}$ ). The magnitude of the lesion area reduction was similar in the infants' (28%) and pubescents' (20%) groups.

## Discussion

### Major findings

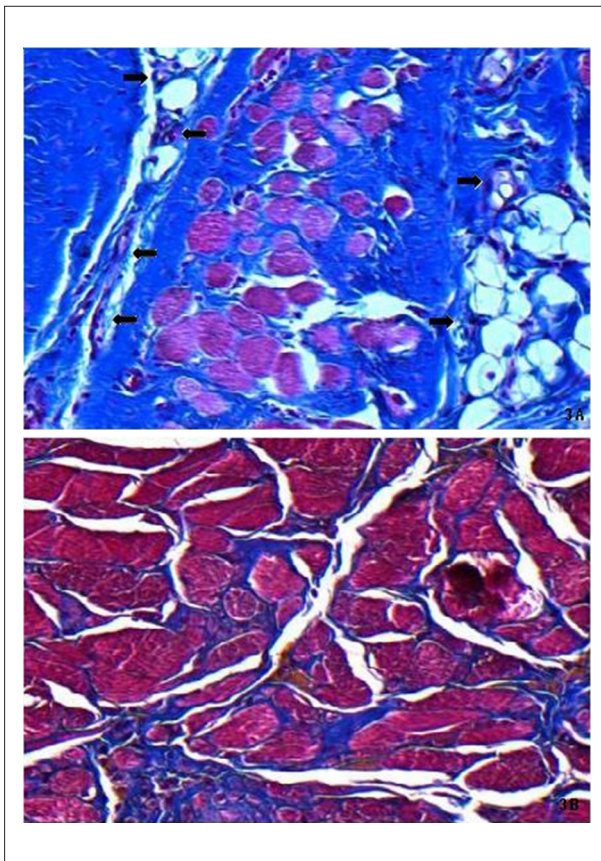
In this ablation model by RF on the rats' thigh muscle, we can demonstrate, qualitatively and quantitatively, that: 1) the lesions in the infants and pubescents presented late growth and extension, more remarkably in the infants, which did not occur in the adults's group; 2) it seems that the corticosteroids attenuate such phenomena in these age groups. These findings corroborate and expand our previous observations in this experimental model, when we demonstrate that the corticosteroids reduced the late lesion growth in infants<sup>5</sup>.

In the present study, we observed the final the corticosteroid effects, because the euthanasia was

performed 30 days after the end of the treatment, totalling a 60-day follow-up after the ablation. Thus, we studied already consolidated lesions. In our initial study, the sacrifice was immediately after the end of the treatment (30 days after the ablation). Therefore, it was not known if the corticosteroids effect would be kept in the long term, which now we can confirm.

Additionally, in our pioneer paper<sup>5</sup>, the treatment effects on the lesions dimensions could not be quantified, based exclusively on qualitative histological criteria. In the present paper, on the other hand, for the first time in the literature, the lesions were measured through specific software<sup>6-12</sup>, confirming that the corticosteroids reduce the lesion area in the infants and pubescents. These results can not be attributed to individual variations or to discrepancies in the ablation procedure. The biophysical parameters of the RF applications and the weight gain of the animals during the follow-up did not differ between the control and treated groups. Additionally, the qualitative and quantitative histological findings were very consistent in all the groups, indicating that the casuistry was adequate.

It is important to emphasize that it was not possible to measure the volume of the lesions, as their geometry is very irregular (mainly in its surroundings), and therefore we opted by the measurement of the lesion central area in mm<sup>2</sup>. Thus, among the several histological cuts of a same piece, we selected the one that presented bigger dimensions in width



**Figure 3** - Qualitative aspect of the Infant Control (1mC) and Treated (1mT) groups illustrating the treatment effects on the lesions. In the controls (panel A), there are several rounded nuclei of fibroblasts (arrows) indicating intense proliferation; large collagen bands dominate the visual field and invade the interstice, leaving only few healthy muscle fibers. In the treated ones (panel B), the lesion is less invasive, because it is not observed proliferative activity and the fibrotic bands that enter the normal muscle tissue are thin. Colored by Masson's Trichrome, 100X.

and length as representative of the lesion. Although it does not make possible to determine the total volume of the lesion, the method used was adequate and reproducible for estimating the lesion size and comparison among the groups - our major objective. Reinforcing the adequacy of our methodology, it was observed a reduction in the lesion area secondary to the treatment for the infants (28%) and pubescents (20%), but not for the adults, for which it was already demonstrated that the corticosteroids do not reduce the lesion by RF size<sup>4</sup>. Finally, the quantitative findings were corroborated by the qualitative analysis performed by the pathologist.

Another new contribution of the present study was to demonstrate that the lesions by RF on the pubescents still presented late growth and extension, although less remarkable than in the infants. We have already reported that the evolution of these lesions in infant rats (corresponding to infants and toddlers) and adults (corresponding to adolescents and young adults) was similar to the one observed in the ovine<sup>3</sup> and canine<sup>4</sup> myocardium. While in adults, histologically, the chronic lesion is characterized by a

well-defined scar, with delimited edges, in the infants there is a remarkable lesion growth, with badly delimited and extensive scars, with evidence of invasion of the adjacent muscle by multiple fibroblastic tissue bands. The lesions in the pubescents (corresponding to the beginning of puberty) presented intermediary characteristics, between the infants' and adults' characteristics. Although the lesions revealed increase of their dimensions in relation to the adults (late growth), the scar is presented better delimited than the infants' and with smaller invasion of the adjacent tissue by fibrous bands (late extension).

The increased lesion by RF size in immature muscular tissues has been attributed to two factors: passive distention secondary to the muscle growth, as happens to the atriotomy scars created in dog infants<sup>13</sup>, and cellular proliferation of the constituent elements of the lesion, responsible for the fibrosis bands invading the surrounding tissue. This happens, in this phase, because, in contrast with the adult muscle, the muscle and interstitial cells divide actively<sup>14,15</sup>. Our findings suggest that, in the pubescents, the passive growth predominates in relation to the cellular proliferation, while in the infants the two components prevail.

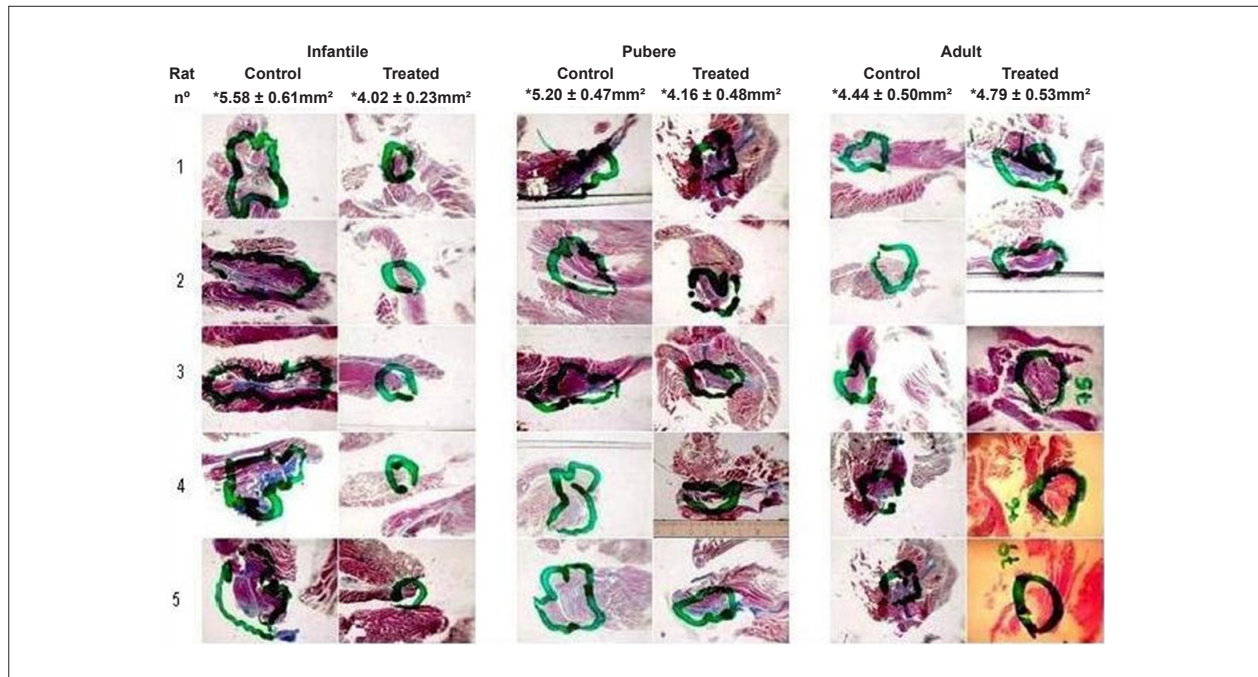
During the follow-up, the ponderal gain of the infants was of 350% and of the pubescents 70%, expected values for these age groups. The qualitative histological analysis was consistent when observing that the infants' lesions were bigger than those of the pubescents. However, although the Infants' lesion area ( $5.58 \pm 0.61 \text{ mm}^2$ ) has been bigger than the one of the pubescents ( $5.20 \pm 0.47 \text{ mm}^2$ ), this difference was not statistically significant ( $p > 0.10$ ). This can be related to the sample size or the measuring methodology used, which measures only the central area of the lesion and tends to subestimate extensive lesions with irregular geometry, as the infant rats' characteristics are.

Our study was not able to identify the pathways through which the corticosteroids reduce the late growth of the lesions by RF on infant and pubescents rats' thighs. These actions can be correlated to the complex effects of these drugs in the healing process<sup>16-18</sup>, such as progressive decrease of the fibroblast number, thus, the collagen synthesis with consequent delay in healing. And it is also possible that, in addition to the leukocytes and fibroblasts inhibition, the corticosteroids exercise these actions through the cytokines reduction and cellular growth factors<sup>17</sup>.

### Clinical implications

Our findings suggest that the corticosteroids reduce the late growth of the lesion by RF not only in infant rats but also in the pubescent rats. If confirmed by posterior studies, our results indicate the possibility of limiting the late extension of the lesions by RF through pharmacological interventions, what could be clinically useful specially in the infants and toddler children's ablation, age group in which the lesion growth has been associated to pro-arrhythmic effects<sup>19</sup>. The fact that the lesions in the pubescents is smaller, more homogeneous and with lower intensity of fibrous bands in comparison to the infants', suggest a lower propension to the formation of arrhythmogenic substrate in this age group. However,





**Figure 4** - Slides used for measuring the chronic lesions in several groups; coloring by Masson's Trichrome, 10X, ruler in mm. The thick line surrounding the lesion was marked on pen by the pathologist. Late, as illustrate in Figure 1, the lesion area in mm<sup>2</sup> was calculated by the Imaj software and used as estimate of the lesion size. It is noted that the lesion areas are consistent in each group. In the infant and pubescent treated groups, they are visibly lower than their treated peers, while in adults, there are no remarkable changes. It is also observed that in the controls, the lesion in infants and pubescents are higher than those in adults, characterizing the late growth. \* - mean area ± standard deviation of each group.

new investigations are necessary in order to determine the functional meaning of these lesions.

### Limitations

The study was performed on the normal skeletal muscle of rodents. Therefore, the results are not for direct inference to the human heart of pediatric patients with arrhythmias<sup>5</sup>. The formulation and the corticosteroids dosage were arbitrary, not being determined the most effective dose<sup>20,21</sup>. The acute lesions were not studied. However, our previous study<sup>5</sup> had already demonstrated that the acute lesions in infants and adults are similar. The age group of the rodents may not correspond exactly to the one of the humans.

### Conclusions

In this model, the lesions by RF on the infant and pubescent rat's thigh present late growth and extension, more remarkably in the infants - which does not occur in adults. The corticosteroids seem to reduce these phenomena in infants

and pubescent subjects. These findings can have implications to the ablation by RF in pediatric populations.

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### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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### Study Association

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