Reparação do nervo laríngeo recorrente – Estudo experimental

Repair of the recurrent laryngeal nerve: Experimental study

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ABSTRACT

Intraoperative lesions of the recurrent laryngeal nerve (RLN) are rare, but have serious consequences. The authors present a new technique for repairing the RLN based on an experimental study using a vein-muscle graft between the tops of the RLN. The goal is to restore normal movements of the vocal cord. Nine animals were operated on, six in the nerve reconstruction group (NRG) and three in the control group (CG). Eleven months later, the vocal cord movements were analysed, the voice was recovered and the histological analysis of the graft was carried out. The results showed that all the goats (100 %) of the NRG regained movement of the vocal cords. In addition, in five animals, the reinnervation occurred with movements symmetrical to those of the other vocal cord and in another animal, they were almost symmetrical. The results for analysis of voice evolution of the NRG goats, obtained with objective and subjective tests, showed a good recovery of the "normal voice". As for the histological study, only 50 % of the NRG cases demonstrated complete regeneration of the RLN. The results also showed that there was a selective / discriminative nervous tropism. Abductor and adductor fibres were attracted to similar ones on the other top, effectively reinnervating the laryngeal muscles. The method tested in this study, which was applied for the first time to repair RLN, is effective, inexpensive, simple and leaves no anatomical or functional sequelae. It is possible to extrapolate the results to humans.

Keywords: *recurrent laryngeal nerve; thyroid surgery; nervous per operatory lesions; nervous repair.*

RESUMO

As lesões intraoperatórias do nervo laríngeo recorrente (NLR) são raras, mas com consequências graves. Os autores apresentam uma nova técnica para reparação do NLR, baseada num estudo experimental utilizando um enxerto de veia-músculo entre os topos do NLR. O objetivo é o restabelecimento dos movimentos normais da corda vocal. Foram operados nove animais, seis no grupo reconstrução do nervo (NRG) e três no grupo controle (CG). Onze meses depois foram analisados, filmes dos movimentos das cordas vocais, a recuperação da voz e efetuada análise histológica do enxerto. Os resultados mostraram que todas as cabras (100 %) do NRG recuperaram o movimento das cordas vocais. Além disso, em cinco animais, a reinervação ocorreu com movimentos simétricos aos da outra corda vocal e no outro animal foram quase simétricos. Os resultados para análise da evolução voz das cabras NRG, obtidos com testes objetivos e subjetivos, mostraram boa recuperação da "voz normal". Quanto ao estudo histológico, apenas 50 % dos casos NRG demonstraram regeneração completa NLR. Os resultados também mostraram que houve um tropismo nervoso seletivo/discriminativo. As fibras abdutoras e adutoras foram atraídas para as similares do outro topo, reinervando de forma eficiente os músculos da laringe. O método ensaiado neste estudo, que foi pela primeira vez aplicado para reparação do NLR, é eficaz, barato, simples e não deixa sequelas anatómicas ou funcionais. É possível a extrapolação dos resultados para o homem.

Palavras-chave: *Nervo recorrente laríngeo, cirurgia tiroideia, reparação nervosa.*

INTRODUCTION

Recurrent laryngeal nerve (RLN) is a concern for all surgeons performing thyroid lobectomies. The peroperative lesions are rare, but have very unpleasant or even serious consequences for the patient, depending on whether the damage is unilateral or bilateral. No method, so far tried, for nerve repair or remobilization of the vocal cord, has managed to achieve full recovery of the mobility of the vocal cord.

The restoration of the correct movement of the vocal cord, has had two lines of investigation: 1) that of repairing the RLN itself; 2) that comes from the research for peripheral nerve repair. Both are based on animal experimentation. The two lines have hardly crossed, except for the last few decades. Direct neurorrhaphy is the most successful and widely used repair method for peripheral nerves. However, Crumley[1] has shown that this type of repair is not effective in RLN. This happens because this nerve has, in the same trunk, fibres that command the adduction and others the abduction and when the tops are united, the regeneration is done not for the homologous fibres, but some for the ones of opposite nervous function. Brain orders for adduction may continue through an abductor fibre, and the vocal cord will become synkinetic[2], but without mobility.

The result will be the same if we use a sensory nerve graft[3]. Other methods have been described as: neurotization, reinnervation by nerve top implantation in injured muscle experienced by Su[4] and developed by Millesi[5]; anastomoses of the RLN to the phrenic nerve in termino-terminal (T-T)[7,8]; or termino-lateral (T-L)[9,10]; neuromuscular flaps such as from the omohyoid for posterior cricoarytenoid (PCA)[11,12,13], combined or not with other techniques, such as the coupling of an electrode with a pacemaker to stimulate the nerve pedicle of the flap[14]; anastomosis of the RLN to the cervical loop of the hypoglossus, as advocated by Crumley[15,16]; or directly to this nerve (XII)[17,18]; or, finally, the neuromuscular flap with a branch of the ansa cervicalis and fragment of the sternohyoid muscle implanted in the thyroaritenoidal muscle[19]. The authors had very irregular, and sometimes bad results with the application of these techniques.

Politis[20] described nervous chemotaxis, later confirmed by Lundborg[21] and Frey[22], and opened the door to the research of new methods of nerve repair, namely, for the development of techniques based on conducting channels of the regenerating nerve. The use of a vein as a conduit for nerve regeneration between two stumps was tested by Chiu[23] in 1982, but with frequent collapses of the vein. In 1984, Keynes[24] tested

and demonstrated that nerve regeneration through skeletal muscle was possible. Brunelli[25], in 1993, instituted the latter two methods and tested a vein graft filled with muscle, which avoids vein collapse, to reconstruct peripheral nerves with lesions between 1 and 2 cm (small and medium distances) in rats. In 2000, Battiston[26] advanced a little further and tested the application of this technique to nerve defects greater than 3 cm (great distance) in rats and in humans[27,28], also obtaining good results. This muscle vein graft has almost all the characteristics required of an ideal canal[29] and also maintains the distal stump protected and in good condition, a fundamental phenomenon, to promote the known chemotaxis between the stumps[30].

The authors proposed to test the reconstruction of the recurrent laryngeal nerve by interposing a peripheral vein graft filled with surrounding skeletal muscle between the stumps. This is the first time that this technique is applied for the reconstruction of the recurrent laryngeal nerve and described in the medical literature for this purpose. The hypotheses are: 1) to demonstrate that nerve regeneration is done through the protected muscle fed through the vein wall; and 2) that there is selective / discriminative tropism between the fibres of both stumps, in order to restore an effective movement of the vocal cord.

ANIMALS AND METHODS

Animal handling followed the guidelines of the EU Directive 86/609 / EEC on animal care, and experienced scientists performed all procedures with accreditation by the Federation of European Laboratory Animal Science Associations (FELASA) category C.

Nine goats of the Serrana (Capra hircus) breed were used. They were non-pregnant and non-lactating, aged between three and six years (4.2 ± 1.2 years) and weighing between 32 and 43 kg (37, 1 ± 3.84 kg). The selection of goats was made taking into account the ease of vocalization and age.

The nine animals included in the study were divided into two groups: Nerve Repair Group (NRG), with six goats (numbers 1, 2, 3, 4, 5 and 6) submitted to the caudal surgical section of the recurrent laryngeal nerve, followed by nerve repair; and a Control Group (CG), with three animals (numbers 7, 8 and 9) in which only a caudal surgical section of the RLN was performed.

Surgical Procedures

Approximately 30 minutes before the intervention the animals were medicated with Cephalexin (Ceporex Vet Inj®), 7 mg / kg administered intramuscularly in a single dose. After non-invasive hemodynamic monitoring a sufficient amount of *bolus* of 2 % Propofol (Lipuro, B. Braun Melsungen AG, Germany), 5-10 mg / kg, was administered to allow direct visualization of vocal cords (preoperative laryngoscopy). After tracheal intubation, anaesthesia was maintained with Isoflurane (Abbott, Amadora, Portugal) throughout the surgical procedure. After removing the intubation, the vocal cords were visualized (postoperative laryngoscopy).

In the NRG animals, the intervention began by harvesting a vein fragment from the saphenous vein in the right hind limb, which was washed and stored in saline solution. In both groups, after a median longitudinal cervicotomy of about 12 cm below the hyoid bone and withdrawal of the thyroid lobe, we visualized the left laryngeal caudal (corresponding to the laryngeal nerve in the human) nerve until its entry into the lateral cricoarytenoid, in an extension of about 6 cm. The nerve resection was performed 1 cm before its passage behind the cricopharyngeal muscle, and a 5-6 mm fragment was excised so as to obtain a clearance of 8-10 mm from the tops. At this point the operation on the goats of the CG ended.

In the NRG, a muscle fragment from the region was isolated, moulded to fit within the vein already harvested, where it was introduced (Figure 1).

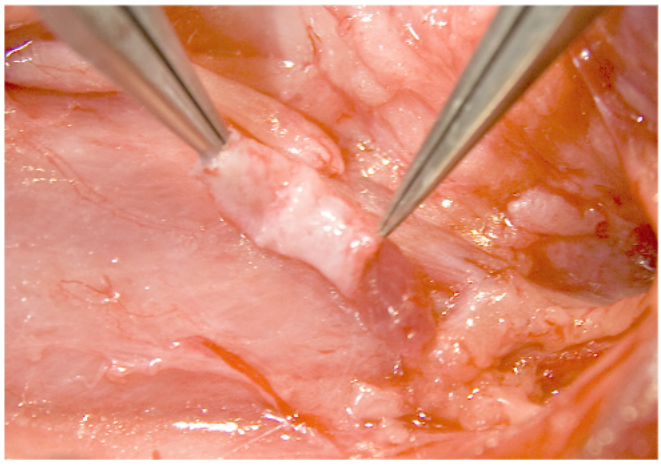

Figure 1 – **Introduction of a muscle fragment inside the harvested vein.**

The stumps of the RLN can be seen.

This muscle vein graft (vm) was placed between the stumps of the RLN and telescopically "anastomoses" were performed on the nerve ie the nerve stumps were introduced into the vein and a microsurgical suture was performed to the epineurium with Ethilon® 10 / 0 (Johnson & Johnson, Portugal) (Figure 2), for fixation. Wound closure.

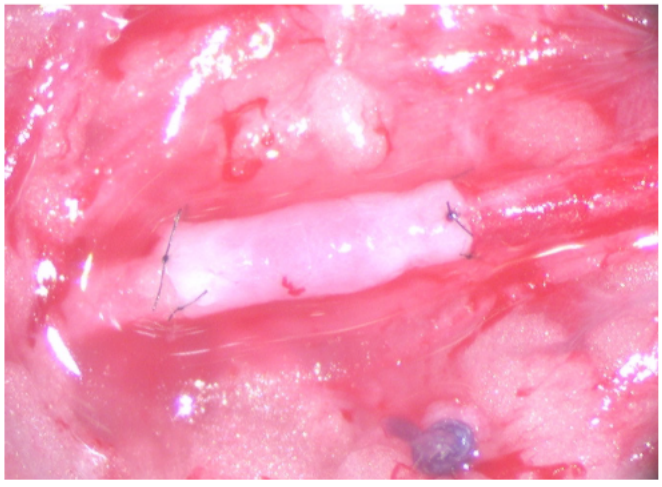

Figure 2 – **Telescopic suture of the nervous stumps to the vein-muscle graft.**

The same anaesthetic procedures were performed on the animals submitted to the second surgical procedure, on average 367 days later, to evaluate the results and harvest the treated nerve tissue.

Direct visualisation of the vocal cords

The direct observation of the vocal cords (vc) was performed under general anaesthesia with a Fujinon UGI – FP7 fibroscope (Fuji Photo Optical, Japan). For each animal, four recordings of at least one abduction / adduction movement was performed: 1) before the first surgical intervention (V1 ic1), to document the normal movement of vc; 2) at the end of the first intervention (V2 ic1), to demonstrate paralysis of the homolateral vocal cord; 3) before the second operation (V1 ic2), to know the state of the movement of the vc; and 4) at the end of this (V2 ic2), to show that the vc had paralyzed again.

The videos were analysed and the observations classified according to the grid shown in Table 1.

Table 1 – **Grid for classification of vocal cord mobility in second surgery pre-operative videos.**

Classification	Justification of the criteria
4	The vocal cords move symmetrically or almost
3	The vocal cord is not paralyzed in middle position, it moves 2/3 of the amplitude of the other
2	The vc is not paralyzed in middle position, but hardly moves
1	The vc is almost paralyzed in middle position (diminutive movements)
0	The vc only flies or is stuck in the middle position

Study of the voice

The induction of bleating was done through sympathy or social interaction, suggested by Carbonaro[31] and the recorded voice (96 kHz / 24 bit) in a lossless format,

was done in four different periods: preoperative, as voice control (VC) or "normal voice "; during the first week after surgery (1stV); between the 30th and 40th postoperative day (2ndV); and between 140 and 160 days after surgery (3rd V).

For the objective evaluation of the voice, as in other studies[32,33,34], two acoustic parameters were obtained using Praat software (P. Boersma & D.Weenink, University of Amsterdam, The Netherlands) to analyse the bleating: Jitter and Harmonics to Noise Ratio (HNR). Table 2 shows the grid for the classification of the records.

Table 2 – **Grids for the classification of recovery of "normal voice" on objective (HNR in decibels and *Jitter* in %) and subjective tests (bs-best sample; gve-good voice evolution).**

Classification	Objective Tests		Subjective Test
	HNR dB	*Jitter* %	
4	< 1.2	< 0.5	>80% 3rd is the bs + >80% gve
3	1.21 – 2.4	0.51 – 1.0	>55% 3rd is the bs + >55% gve
2	2.41 – 3.6	1.01 – 1.5	>30% 3rd is the bs + >30% gve
1	3.61 – 4.8	1.51 – 2.0	>20% 3rd is the bs + >20% gve
0	4.81 – 6.0	2.01 – 2.5	3 rd is not bs or <20% gve

In terms of the subjective techniques, an adaptation of the method for human voice *Comparison Category Rating* (CCR)[35] was implemented to evaluate the evolution of the voice or time recovery, compared to the "normal voice", that is, the bleating preoperative period. The voice samples, recorded after the surgical procedures in all goats (NRG and GC), were heard sequentially, by the subjects, for each animal, comparing with the respective VC[36] recordings. Each individual classified the post-operative voice recordings according to the subjective test. The set of samples from each goat was heard twice, and after the second, the subjects identified the voice sample that was most similar to VC (best sample – bs), to evaluate if the bleating progressed to the preoperative state (good voice evolution – gve).

Histologic Study

In the second operation, after exposure of the v-m graft, the macroscopic characteristics were recorded and the grafts were resected, together with about 5 mm of each nerve top, for a histological study (n = 9).

The tissues were fixed in a solution containing 10% of phosphate buffered formaldehyde for eight hours at room temperature and then processed for routine embedding in paraffin. Paraffin-embedded tissue sections (3 µm) were deparaffinized with xylene, rehydrated through an ethanol water series, and stained with hematoxylin and eosin. Additionally, the Masson trichrome staining was also performed. The slides were sequentially submitted to Solution A (plasma stain), which contains acid fuchsin, xylidine Ponceau, glacial acetic acid, and distilled water, Solution B, that contains 1% of phosphomolybdic acid in distilled water, and finally Solution C fast green.

Table 3 – **Grid for classification of histological studies of nerve anastomoses of operated animals.**

Histological study classification	Justification of the criteria
4	Continuity of the fibers of the proximal and distal tops; regeneration with maintenance of normal histological pattern of peripheral nerve (perfect) or with some distortion
3	Nevroma, continuity of the fibers between the tops; regeneration equivalent at both ends; perineurium with increased production of mucopolysaccharides
2	Nevromas; without continuity of the fibers between the tops; regeneration more intense at the distal end; perineurium with increased production of mucopolysaccharides
1	Lack of fibers continuity between the tops; at the proximal end is not observed nerve; distal end with few images of regeneration
0	Lack of fibers continuity between the tops; absence of regeneration; nerve atrophy at both tops

Based on the microscopic observations of the muscle-vein graft pieces collected in the second intervention, a scoring system was established, presented in Table 3, with a five-point scale, similar to that used to evaluate other parameters.

Statistics

The comparisons within and between groups were performed using the Wilcoxon Signed test and the Mann-Whitney U test, respectively. As both samples were small (NRG-6 and CG-3) the results, according to the classification grids explained above, are given in absolute frequencies instead of percentages, and in the average score for each group of goats. Where relevant, Confidence Intervals were also calculated for the 95 % average.

RESULTS

There were no postoperative mortality nor early postoperative complications, such as vomiting or suture dehiscence, or delayed, such as cervical trauma that could condition anastomoses to the nerve or superficial or deep infection of the operative wound.

The operations and recoveries were uneventful and all of goats remained without intercurrent diseases during the observation period.

Movement of the vocal cords

Video analysis performed before the first intervention, V1 ic1, showed that in all of animals the vocal cords had coordinated and symmetrical movements, synchronized with breathing.

As expected, V2 ic1, performed after the first operation, showed all of vocal cords submitted to the work protocol paralyzed in the median region (0 points in Table 2), but it was not possible to obtain video recording in an NRG animal, goat 2, due to agitation and hypersalivation.

The same result was obtained in V2 ic2, post-operative videos of the second intervention.

Before the second intervention, on average 367 days after the first, in all animals of Group I (100 %), the remobilization of the vocal cord was reinnerved, while the CG animals this was not present ($p = 0.015$). In the NRG group, five goats (5/6 = 83.3 %) had completely symmetrical vocal cord movement (Figure 3), but in one animal (1/6 = 16.7 %) the movement was considered almost symmetrical ($P = 0.02$ when compared to post-lesion vocal cord function. We considered a 3 points result in goat number 2, because the vocal cord only moved 2/3 of the movement it should, but the contralateral vocal cord moved 1/2 to 2/3 of the normal movement.

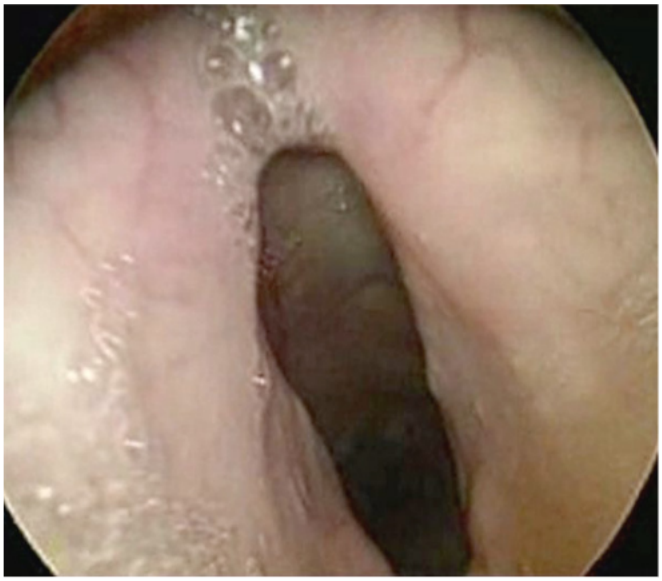

Figure 3 – **Open vocal cords with completely symmetrical movement.**

In all the goats, the vocal cords moved symmetrically and synchronously with the breathing.

In the CG, two goats had vc paralyzed in the median region (2/3 = 66.7 %), as expected, but in one (1/3 = 33.3 %), goat 7, vc moved almost symmetrically (Table 4).

In the NRG, 6/6 goats (100 %) recovered after surgery. In CG, 1/3 goats (33 %) recovered without surgery.

The evolution between the moments after the first operation and before the second, were different from zero with statistical significance, in the NRG (pNRG = 0.020 – Wilcoxon test), while the null hypothesis had to be maintained (pCG = 0.317 – Wilcoxon test). In addition, the results obtained before the second operation in NRG goats were significantly different from those obtained in CG (p = 0.015 – Mann-Whitney test).

Table 4 – Results of vocal cords movements
(V1 s1 – first surgery pre-operative video; V2 s1 – first surgery post--operative video;
V1 s2 – second surgery pre-operative video; V2 s2 – second surgery post-operative video).

Goat	*Group*	1st s pre-op v	1st s post-op v	2nd s pre-op v	2nd s post-op v
1	*NRG*	4	0	4	0
2		4	-	3	0
3		4	0	4	0
4		4	0	4	0
5		4	0	4	0
6		4	0	4	0
7	*CG*	4	0	3	0
8		4	0	0	0
9		4	0	0	0

Therefore, we can consider that the surgery was clinically significant in the recovery of goats from the NRG and that the recovery of movement in goat 7 from CG can be considered spontaneous non-significant.

Voice recordings

The voice induction method we used, by Carbonaro, failed, because the goats stopped vocalizing and we only managed to recordings that could be analyzed in four goats. Considering the objective and subjective evaluations of the grid application shown in Table 2, the results are shown in Table 5.

It was observed that in the NRG, except for the HNR in one case (goat 6), all were quoted with 3 and 4 points, i.e., there was a gradual approximation of the postoperative samples to the "normal voice" of the animals, for HNR, particularly in the third register.

Regarding the subjective tests, we would like to point out that for all NRG goats, most the subjects surveyed considered that the 3rd postoperative was the one that most resembled the preoperative and, because of this, the best sample. There was a concordance between objective and subjective tests, except for goat 6.

Table 5 – Results of voice control observations in four goats.
(CV – control voice; 1stV – 1st week after surgery; 2ndV – 30th to 40th days after surgery;
3rdV – 140th to 160th days after surgery; bs-best sample; gve-good voice evolution)

	Goat	Group	CV	1stV	2ndV	3rdV	Score
Jitter	**3**	*NRG*	1.454	2.731	2.607	1.695	4
	5		0.517	2.595	2.646	0.402	4
	6		1.950	2.379	1.167	0.653	2
	7	*CG*	0.641	2.012	0.647	1.148	3
HNR	**3**	*NRG*	6.732	2.731	2.900	5.205	3
	5		7.409	1.027	2.785	5.515	3
	6		6.720	2.551	5.180	5.745	4
	7	*CG*	9.426	5.483	7.753	7.490	3
Subjec.	**3**	*NRG*	93.5% bs is 3rd – 87.1% gve				4
	5		58.1% bs is 3rd – 83.9% gve				3
	6		55.0% bs is 3rd – 65.0% gve				3
	7	*CG*	45.0% bs is 2nd – 65.0% gve				2

Due to difficulties in inducing the goats' voices, it was not possible to define the moment, the day, when the animals recovered the voice quality they had before the intervention, the "normal voice". Still, we can say that the three NRG goats recovered up to the 19th week and goat 5, in five weeks.

Comparing the 95%CI for the mean scores of the three NRG goats with the score obtained by the CG goat, the following results were obtained:

Jitter: the CG goat score = 3.00 stands within the limits of the NRG 95%CI = [2.027; 4.640], which means that the CG Jitter score must be considered equal to the NRG Jitter score (p>0.05).

HNR: CG goat score = 3.00 stands within the limits of the NRG 95%CI = [1.868; 4.132], which, likewise, means that the CG HNR score must be considered equal to the NRG HNR score (p>0.05).

Subjective: the CG goat score = 2.00 stands below the NRG 95%CI = [2.680; 3.986], thus allowing to conclude that the CG subjective score is poorer (p=0.05) than the NRG Subjective score.

Only in subjective tests, the NRG goats achieved better results than the CG goat; in Jitter, the results were negatively impaired by the score of goat 6, while in HNR the results are rather inconclusive.

Histological study

Applying the methods of Table 4 the results obtained are shown in Table 6:

In the NRG there was recovery of the normal nerve pattern in only goats 1 and 6 (2/6 = 33 %); despite the good results in terms of vocal cord mobility and normal voice recovery, in the other goats the results were: goat 4 – 3 points (16.7%); goats 3 and 5 – 1 point (33 %); and goat 2 – 0 points (16.7 %).

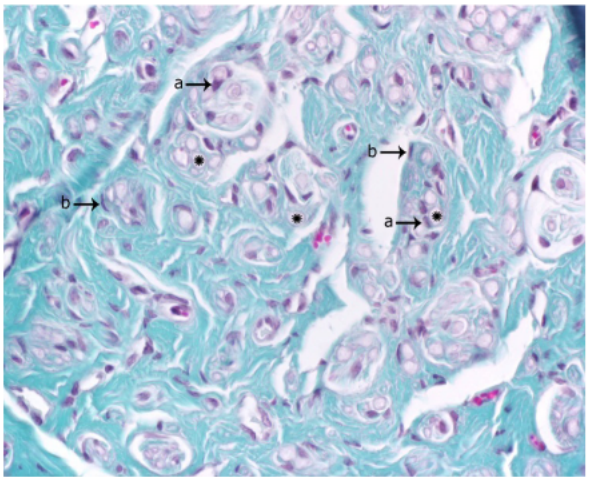

Figure 4 – *Axonal cytoplasm. Goat 1. Cross section of the middle portion of the graft. a) Schwann cells; b) fibroblasts. TM 60x.

In the CG, all animals had 1 point (100 %).

Figure 5 (A and B) shows the histological slide of goat 1 (4 points) its graft has become a normal nerve.

Table 6 – Summary of the results of: 2nd surgery pre-operative video (2nd s pre-op v); voice with Jitter, HNR and Subjective tests (Subj); and histology (Histol)

	Goat	2nd s	Voice			Histol
		pre-op v	***Jitter***	**HNR**	**Subj**	
NRG	**1**	4				4
	2	3				0
	3	4	4	3	4	1
	4	4				3
	5	4	4	3	3	1
	6	4	2	4	3	4
CG	**7**	3	3	3	2	1
	8	0				1
	9	0				1
mean	**NRG**	3,83	3,33	3,33	3,33	2,17
	CG	1,33	3,00	3,00	2,00	1,00
p-value		**0,015**	>0,05	>0,05	**<0,05**	0,394

The histological evaluation was inconclusive (p = 0.394).

DISCUSSION

This work demonstrates that the repair of the recurrent laryngeal nerve in goats with a muscle filled vein graft has promising results. It also shows that there was a selective / discriminative nervous tropism. The adductor and abductor fibres were attracted to the like on the opposite stump, efficiently reinnervating the laryngeal muscles.

Table 7 shows the comparison of the results obtained in this series with those of other publications that used animals for research[37,38,39,40,10,41]. As you can see, the method we used is the only one that achieves 100 %, both in voice recovery and in vocal cord mobility.

Table 7 – **Comparison of results obtained in this series with those of other publications that used animals for research**

Best series	Nº of animals	Employed technique	Lesion's localization	Time lesion-reconstruction	cv mobility recuperation	Voice recuperation
Lith-Bijl 1991	12 cats	RLN – phrenic anastomose	2,5 cm	9 months	33%	No reference
Green 1998	6 dogs	NLR – sternothyroid branch of ansa cervicalis anast	2 cm	Minutes	0%	No stimulation – 0% Stimulation – 100%
Nonomura 1993	18 dogs	Suture of cricothyroid to lateral crico-arytenoid	Before entry laryngeal muscles	Minutes	0%	Improvement
Debnath 2008	6 dogs	neurotization of the NLR to posterior crico-arytenoid	3 cm	Minutes	100% (?)	No reference
Liu 2005	15 rats	End to side RLN – phrenic anastomose	7º anel da traqueia	Minutes	0%	No reference
Silveira 2012	**6 goats**	**Vein-muscle graft**	**1 cm**	**Minutes**	**100%**	**100%**

We reaffirm what we refer to in the Introduction. The top-to-top anastomosis of the RLN is not effective, for the reasons presented[1] and confirmed by several authors, both in experimental animals and in humans. For this reason *it was not justified to use this method in the Control Group*.

From the various techniques described in the literature, we discussed nine different methods for RLN repair or vocal cord remobilization. The first one used neurotization, reinnervation of the thyroarytenoid muscle by the ansa cervicalis applied by SU[4] in 2007: 30 % of patients recovered their normal voice; in 30 % it improved a lot; 20 % only improved; and 20 % did not improve, but no movement of the vocal chords or arytenoid was detected. Frazier[6] described the second method, in 1924, testing the RLN anastomosis for the descending branch of the hypoglossal nerve, with good functional results in two months, but resulting in important sequelae in the tongue. The termino-terminal (T-T) RLN anastomosis to the phrenic nerve was described by Colledge[7] in 1927 or Brøndbo[8] in 1986. These techniques were abandoned because the vocal cord had very strong contractions and caused paralysis of the diaphragm. Another method was proposed to avoid this inconvenience by Balance[42] in 1924. It suggested a triple anastomosis: RLN at the proximal top of the phrenic in TT, from the distal top of the phrenic to the proximal of the *descendens noni* in TT, and from the distal top of this to the hypoglossus results, even, ercetto secundum-lateral (TL), to overcome the paralysis of the diaphragm. However, the problem remained. Colledge[9] in 1928 and Liu[10] in 2005 proposed T-L RLN-phrenic anastomosis. This method allowed only a quarter of the abduction movement of the vocal cord, which was sufficient to avoid a tracheostomy.

A sixth method, described by Tucker[11] in 1976, was based on neuromuscular flaps, from omohyoid to CAP. This technique only achieved 40 % of good results in vocal cord mobilization, but the patient breathed well; combined with surgical medialization, it was possible to restore loudness and tone of voice in 71 % of patients[13]. Broniatowski[14] later refined this technique in collaboration with Tucker by applying an electrode to a pacemaker to stimulate the pedicle of the neuromuscular flap in cases of total paralysis of the larynx. They reported good mobilization of vocal cords and recovery of voice. However, this technique

has become very complicated and expensive, and has not yielded comparable results in the hands of other authors. Crumley in 1986[15] and 1991[16] performed the anastomosis of the RLN on the sternothyroid branch of the ansa cervicalis of the hypoglossal nerve. This method obtained 60 % of good results in voice recovery, but none of the vocal cords regained movement.

The eighth method, the direct anastomosis of the RLN to the XII, proposed by Paniello in 2000[17] and 2001[18] achieved some vocal cord mobilization, between five and ten months in five of the nine treated patients (55.6 %). However, it has the drawback of leaving a sequelae on the tongue, which is not negligible. The ninth and last method used a branch of the cervical loop and a fragment of the sternohyoid muscle. It was described by Yumoto[19] in 2010 and achieved good recovery of all parameters of the voice, but poor mobility of the vocal cords.

These studies did not show satisfactory results. Many authors have obtained good and reasonable voice recovery, the most important parameter. But almost no technique was able to properly mobilize the vocal cords.

The location where the nerve is most frequently injured is between 0.5 and 1.5 cm before its passage below the cricopharyngeal [43,44]. In addition, the lesion should not be larger than 8-10 mm, except in cancer surgery. Therefore, in the present study, it was decided to perform an injury of about 8-10 mm long, located about 1 cm before the entry of the recurrent laryngeal nerve in the laryngeal muscles. The lesion was repaired with a muscle filled vein (v-m) graft.

The good results obtained with this method may in the future be identical to some research materials in bioengineering[45]. These may replace the muscle-vein graft described above but will be much more expensive and difficult to acquire. *The v-m graft*, because it uses components of the patient, *does not suffer rejection and the vein wall is an ideal channel for protection of nerve regeneration through the muscle*.

It was observed that regeneration was done through the muscle fibres inserted into a vein fragment and not by any other means, because when the nerve stumps and muscle vein graft were excised for histological study, the vocal cords paralyzed again.

We used the histological study of the v-m graft, but most authors now suggest that biopsies of the muscles are more reliable to know whether or not they have been reinnervated.

Immediate repair may have contributed to the favourable results obtained. Also in human surgery, it is important that the lesion is identified immediately, so we believe that the intraoperative monitoring proposed by Randolph[46] may be useful. If the nerve does not respond to electrostimulation, injury should be investigated and, if confirmed, repair should be performed immediately.

Although this work has been done on goats, the authors consider this animal to be a good experimental model. The goat was chosen for this research, because the vocal tract is very similar to that of human[47,48]. In addition, it usually vocalizes easily and has less cervical fat than sheep.

Regarding goat 2 it is difficult to explain the results, since both vocal cords move less than expected, not reaching the median region. One possible explanation – probable bilateral lesion by the endotracheal tube, in the first intervention. Unfortunately, this was the only animal in which it was not possible to obtain the postoperative video of the first intervention, in order to confirm this hypothesis.

Although the method used to induce bleating was not the best, as we said ago, it was only possible to analyze the records of four goats. Obviously if we had obtained good records of all animals, we are convinced that the results would confirm, with more vigor, those we have presented. Even so, the results obtained with the objective evaluation in all the NRG goats, where the voice was analysed, showed very good to excellent recovery of the "normal voice", except for the HNR in goat 6. These tests should be applied to a larger number of animals in order to be validated. In the subjective tests, the results were also classified with 4 and 3 points.

Nerve repair through a muscle filled vein graft[25] has been known for more than 20 years, but has only been applied for the reconstruction of peripheral

nerves[26,27,28,29,49] which have only one type of sensitive fibres (for a region) or motor (to perform a certain movement). *This is the first time that this method has been applied for the repair of the recurrent laryngeal nerve*, which has two types of fibres with conflicting effects, and *this is also the first time a selective and discriminative tropism has been demonstrated the adductor fibres were attracted to the other stump's adductor and the same happened to the abductor fibres in the nerve regeneration, through the vein-muscle graft, restoring the movements of the vocal cords with perfect symmetry and synchronously with the breathing.*

CONCLUSIONS

The technique of vein-muscle grafting to repair the recurrent laryngeal nerve is simple, cheap, easy to reproduce and without functional sequelae, and nerve regeneration results occurs in 100 %. These results are unique, considering the methods hitherto known. It is not known whether the application of this method in humans will provide the same results. However, if these results are confirmed when applied in humans, it will be a major evolution in the treatment of recurrent laryngeal nerve lesions.

Final comment

The authors are hopeful that in humans, it is also possible to obtain 100 % good results, solving a problem that led Frank Lahey[50] in 1938 to mention "One of the difficulties of suture of the recurrent laryngeal nerves is that of approximating abductor fibres with abductor fibres and adductor fibres with adductor fibres, as well as nerve carrying impulses to opposing muscles… It may be possible that it will never be effected in humans". And Roger Crumley, in 1986[15], to regret that "A voice could be regarded as normal only if the apposed vocal folds approximate without deficits on phonation. Significant improvement could occur if the paralyzed lateral cricoarytenoid (LCA) muscle, thyroarytenoid (TA) and PCA muscles could be reinnervated by re-anastomosing the *correct specific neural bundles* of the recurrent laryngeal nerve. This is of course not possible." (p. 615).

With the muscle vein graft, it was possible to specifically connect the adductive nerve fibres to the adductors and the abductors to the abductors, the only way to obtain an integral reconstruction of the vocal cord function.

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