

Animal model of osteochondral injury in rabbits



<https://doi.org/10.56238/uniknowindevolp-109>

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ABSTRACT

Object: To study the alterations in an osteochondral surface, submitted to a standardized mechanical lesion. **Methods:** 30 young male rabbits were used for this experiment, being the experimental procedure performed on 60 knees. After anesthesia and antisepsis, the animals were submitted to surgical procedure for osteochondral lesion production with 4,2 X 5,0 millimeters (mm) in their condilus femoralis medialis with standardized instrumental. Three weeks after these animals were evaluated, concerning the lesion. **Results:** The tissue regeneration was incompatible with spontaneous resolution, developing cicatricial tissue different from the normal cartilage. **Conclusions:** The developed model animal is useful for study regeneration of the cartilage after mechanical lesion. The model is simple and easy to control and execute, as well as preserves the animals, which may used in other experiments, or for later observations of the previously described and analyzed phenomena in this first report.

Keywords: Animal Model Swine, Cortical cancellous bone, Tibia Proximal.

1 INTRODUCTION

Methods of restoration and replacement of joint defects represent a great contribution of Medicine for the twentieth century¹, and it is clear that the restoration of the surface is fundamental for the use of the joint in a physiologically adequate way ^{2,3}.

The greatest difficulty is not to diagnose the cartilaginous lesion, because both clinically and by imaging tests can identify and classify the destruction of the joint covering. The cure or the best handling of the lesion is what brings apprehension to the therapist⁴.

Despite the frequency and impact caused by degenerative joint disease, it has been considered inevitable in advancing age and an unpleasant fact, detrimental to a better quality of life.

As it is not possible to carry out research in "anima nobili", it is used for the study of joint biological responses, animals that, obeying the bioethical principles (Federal Law 6.638/79),* provide data to be verified the biological responses related to tissue healing.



The literature on corrections in joint lesions of laboratory animals shows that the researches carried out to correct them started from a surface that had hitherto been intact and that it was attacked only when it was replaced by detailed biological, synthetic or semi-synthetic products⁵.

In view of the above, some questions can be considered:

- Wouldn't it be necessary for the joint to be previously injured to receive treatment?
- Would the joint response, in biological terms of healing, be identical whether it was normal or injured?
- Can an experimentally produced injury lead to a degeneration similar to that acquired naturally?

Starting from these questions, some suggestions can be formulated:

- Only after having an injury could one perform its treatment.
- It is necessary to have an animal model in which, when an injury is caused, a biological response comparable to that of the injured joint by natural means is developed.

Based on these criteria, we began the planning of an experimental model that would be carried out in animals of easy obtainment and management, in an appropriate environment and with standardized and reproducible procedures, aiming to obtain an osteochondral lesion as close as possible to the naturally produced.

This work aims not only to report an experiment, but mainly to describe possible future therapeutic approaches.

*Law that established norms for didactic-scientific practice of the vivisection of animals, which allows only third degree establishments to carry out activities of this nature as long as they do not cause suffering to animals.

2 MATERIAL AND METHODS

2.1 SAMPLE

We used 30 white male rabbits (*Oryctolagus cuniculus*), aged less than six months, of the New Zealand lineage, weighing from 1670 grams (g) to 3,550g, from and housed in the Rabbit of the Federal Agricultural College Senador Gomes de Oliveira (CAFSGO) of Araquari -Santa Catarina in partnership with the University of the Region of Joinville-UNIVILLE, kept in metal cages 0.81 meter (m) X 0.60 m X 0.45 m, with a maximum of two specimens per cage, in a protected environment, isolated from noise, fed with feed suitable for the species and water at will.

The choice of breed was made for greater homogeneity of the sample, as well as the option for males avoiding hormonal variability and in the case of mixed housing the crosses.

The animals were submitted to veterinary follow-up and operated in a specially constructed surgical environment under specific disinfection, anti-infection and asepsis standards.



This was followed by the Animal Ethics Protocol governed by Federal Law 6.638/79

2.2 PROCEDURE

The preoperative procedures, anesthetic induction, anesthesia, antisepsis, surgery, immediate and late postoperative care as well as euthanasia were performed at the Laboratory of Experimental Surgery of CAFSGO.

The rabbits were kept with water and appropriate feed, at will, until the moment of the operation.

After weighing, the animals were submitted to pre-anesthetic medication with acepromazine 1%, intramuscular (IM) in *regal gluteal* (gluteal region), at a dose of 0.2 milligrams per kilogram of body weight (0.2mg.kg⁻¹).

After fifteen minutes, they received xylazine 10 mg.kg⁻¹ and ketanin 25mg.kg⁻¹ as anesthetic medication by the same route and site as the pre-anesthetic medication (Table 1).

Table 1 – Calculation for pre-anesthetic, anesthetic and analgesic medication.

WEIGHT (grams)	ACEPROMAZINE (ml)	QUETANINA 1/2 XILAZINA 1/2 (ml)	FLUNIXIN - MEGLUMINA (ml)
2000	0,4	0,8	0,2
2500	0,5	1,0	0,2
3000	0,6	1,2	0,3
3500	0,7	1,4	0,3

Once the anesthetic plane was obtained, identification was performed by tattooing on the inner face of the left ear and the manual removal of hair from the anterior, lateral and medial regions of the knees.

Installed on the operating table in dorsal decubitus, antisepsis was performed with 2% iodized alcohol in the region of the knee joints and delimited operative areas with disposable fields.

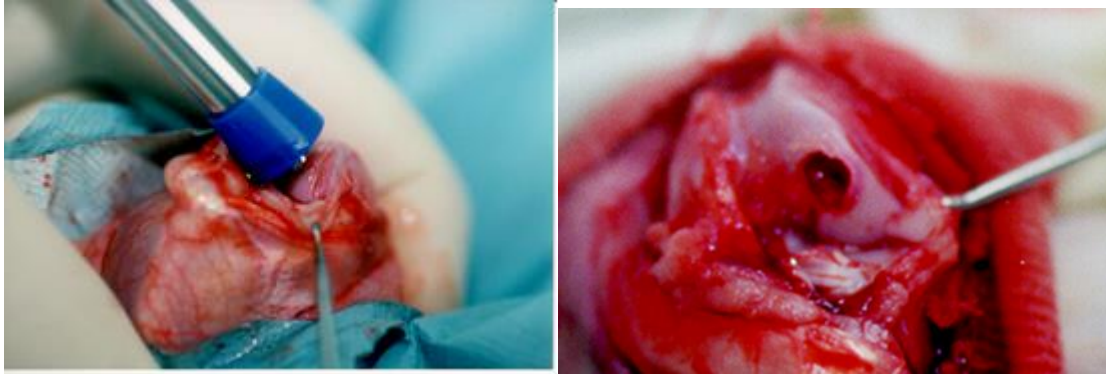
Delimited operative fields, the cutis (skin) dieresis of three cm was performed *on the medial surface of the* knee joint, with opening of the medial facies (*medial* retinaculum) and the articular capsule (*joint capsule*) and then *dislocated laterally the patella*.

With instruments designed by Biológica-GMReis Campinas – Brazil, the lesion was made in the *condilus femoralis medialis* (medial femoral condyle) in the standardized measurement of 4.2 mm. in diameter and 5 mm. in depth (Figs.1).



The synthesis of the *medialis facies* (medial retinaculum) and the *cutis* (skin) was performed with separate stitches of monofilament nylon 4.0 and the same procedure was performed contralaterally.

Figure 1 – Femoral condyle (*condilus medialis*) being injured through the standardized instrument and respective lesion produced.



Surgery was completed that ranged from 15 to 37 minutes (min.) (average of 22.5min.)

After the surgery, the dressings were made and the animals were taken to the recovery room where they received flunixin-meglumine 10 mg.kg⁻¹ as analgesic and anti-inflammatory IM and when they sketched reaction they were released to their respective cages.

For three weeks the rabbits had specific care for feeding, water at will and daily dressings. There were no deaths in the period.

The reoperation was performed three weeks after the primary surgery, and the same procedure of induction, anesthesia, antisepsis, placement of fields, incisions by anatomical planes and after inspection of the area submitted to primary aggression (Fig. 2). Then, it was removed, using standardized material²⁰ cylinder of 5 mm. X 6 mm. corresponding to the area of the lesion caused.

The closure by planes was repeated and the same procedure was performed contralaterally, removing the scar block for analysis. According to protocol, the animal proceeded to recovery, in its respective cage.

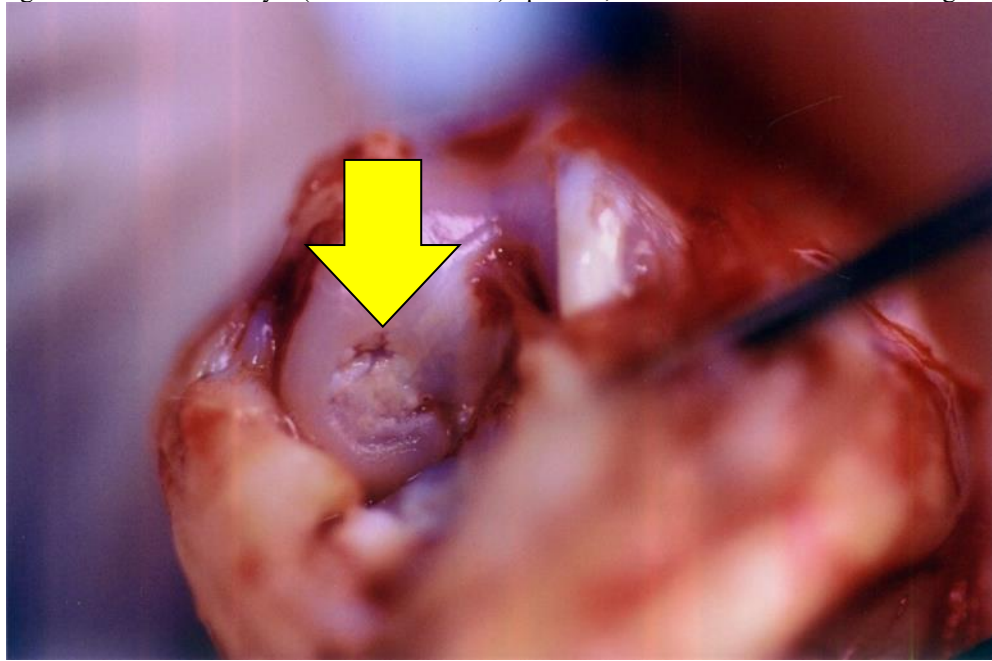
After harvesting, the blocks were deposited together in a container suitable for the containment of anatomical pieces and fixed with 10% buffered formaldehyde.

Two osteochondral blocks were removed from a rabbit without previous surgery with the same characteristics of age, sex, race and weight in order to have an undamaged condylar surface (control). After removal, they were fixed with 10% buffered formaldehyde and packed in individualized containers.

After this procedure, the animals were euthanized with intravenous injection of 3.0 milliliters (ml) of 10% potassium chloride.



Figure 2 – Femoral condyle (condilus medialis) operated, arrow shows the area in healing.



The histological evaluation was performed at the Laboratory of Pathological Anatomy of the Hospital Dona Helena - Joinville, Santa Catarina in a randomized manner (randomly chosen) of this group, 10 blocks and the two of the rabbits without previous surgery.

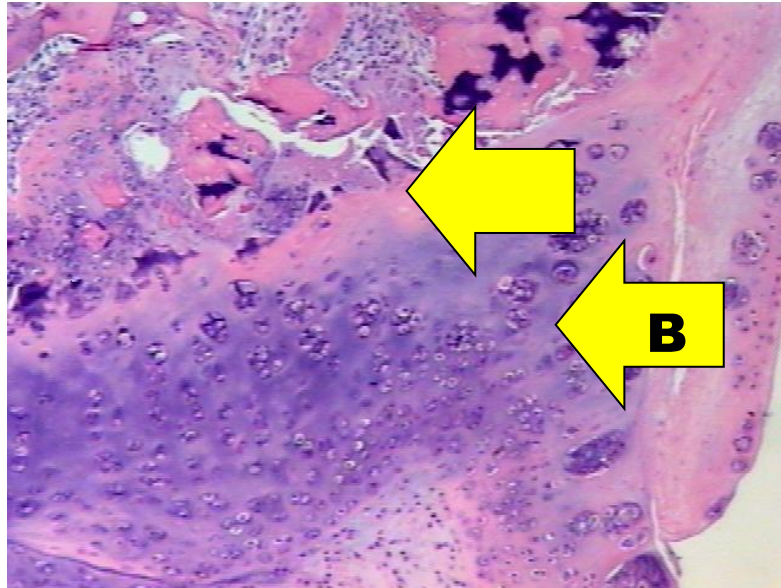
After decalcification of the cylinders with 5% nitric acid, sagittal sections were made, being dehydrated in alcohol in progressive concentrations, diaphanized with xylol and included in paraffin. The blocks were submitted to five-micrometer sections and stained with hematoxylin and eosin (H-E), Gomori's Trichrome staining and analyzed under an optical microscope (OM) (Fig. 3).

In the cartilage analysis, the conventional morphological description was used, which considers according to CALANDRUCCIO AND GILMER ⁷, being analyzed through criteria of presence or absence of lesion in the cartilage and presence or absence of hyperplasia of chondrocyte regeneration, in the randomized group and in the control blocks.

For statistical analysis, Student's t-test was applied to independent groups, assuming equivalent variation proven by the "F" test.



Figure 3 - Photomicrograph of randomized cylinder (HE 200X) showing presence of lesion (A) and regenerative hyperplasia of chondrocytes (B).



3 FINDINGS

The weight of the animals showed an increase between the first and the second surgery, with no significant difference between the initial and final weight means by the Student's "t" test for independent groups assuming equivalent variation proven by the "F" Test.

In the evaluation of the presence (+) or absence (-) of Cartilage Lesion **in the Randomized and Control Groups**, as in the evaluation of the presence (+) or absence (-) of Chondrocyte Regeneration Hyperplasia **in the Randomized and Control Groups**, with respective percentages, **the randomized group had statistical significance when compared to the control group (Tables 2 and 3).**

TABLE 2 - Evaluation of the presence (+) or absence (-) of **Cartilage Lesion in the Randomized and Control Groups** with respective percentage of presence.

<i>RABBIT</i>	<i>PRESENCE OF INJURY (+)</i>	<i>ABSENCE OF INJURY (-)</i>
RANDOMIZED		
01	X	
02	X	
03	X	
04	X	
05	X	
06	X	
07	X	
08	X	
09	X	
10	X	
TOTAL %	100	0
CONTROL		
01		X
02		X
TOTAL %	0	100



TABLE 3 - Evaluation of the presence (+) or absence (-) of Chondrocyte Regeneration Hyperplasia in the Randomized and Control Groups with respective percentage of presence.

RABBIT	WITH HYPERPLASIA (+)	WITHOUT HYPERPLASIA(-)
RANDOMIZED		
01	X	
02	X	
03	X	
04	X	
05	X	
06	X	
07	X	
08	X	
09	X	
10	X	
TOTAL %	100	0
CONTROL		
01		X
02		X
TOTAL %	0	100

4 DISCUSSION

The use of rabbits in this study as experimental animals confirmed what different authors were aware of regarding the ease with which they can be handled, housed and acclimatized well^{2,5,8,9}.

The choice of a single breed, made for greater homogeneity in the sample, as well as the option for males avoiding hormonal variability and crosses, in the case of mixed housing proved to be effective.

The weight of the animals showed an increase between the first and second surgeries demonstrating that it did not interfere in their metabolism, with no significant difference between the initial and final weight means by the Student's "t" test for independent groups (assuming equivalent variation proven by the "F" Test).

The use of pre-anesthetic decreased the instability of the animal, reassuring it for the anesthetic act. 1% acepromazine, a phenothiazine tranquilizer, potent neuroleptic¹⁰ was used in all animals with expected results obtained.

For anesthesia, ketamine and xylazine¹¹ were used, which provided a safe anesthesia in the animals as soon as it was administered, ending its effect in a timely manner for the procedures. It enabled a good surgical time as reported by LIPMAN, MARINE E ERDMAN¹² and a low level of discomfort to the animal.

All animals survived the anesthesia and surgery procedures.

An animal model, according to BURR^{13, 10} produce a lesion that does not regenerate spontaneously, has to in addition to compromising the basal layer, reach the subchondral region. Its diameter should exceed 3 to 4 mm^{14,15,16,17}.



The operated rabbits were less than 06 months old, because WEI AND MESSNER (1999)¹⁸ described that the durability of the reparative scar in these animals decreases with their maturation. If it is necessary to continue the research of lesional therapies, the local response would be in full operation.

We preferred that our animals remain without immobilization in order to, according to MORAN ET AL.¹⁸, effect the regenerating potential of active joint mobility, as well as surgery on both knees prevent the predominant use of a "healthy" knee, distorting the response of the injured knee.

Histological examinations proved to be a recent regenerative lesion, demonstrating moderate structural disorganization. The chondrocytes were arranged in sparse groups, irregularly distributed in the middle of the hyaline matrix, in agreement with observations by CALLANDRUCCIO AND GILMER (1962)⁷.

The analysis of the data Cartilage lesion and Regenerative hyperplasia of chondrocytes became an obvious result when compared to that of the control group, since there is joint integrity in the latter group.

We agreed with the findings of authors^{20,21,22,23} and the presence of cells with mesenchymal characteristics and local fibrocartilage was verified at 3 weeks; evolution compatible with the degree of injury voluntarily caused in this study.

The present proposal of osteochondral lesion is unprecedented in three aspects:

- In the dimensions, because there is no bibliographic reference in this specific size of 4.2 mm X 5.0 mm, contemplating requirements for a spontaneous non-healing.
- It provided a regenerative lesion, according to the data collected at microscopy, leaving a joint with osteochondral lesion prepared for any subsequent therapeutic tests, whether surgical or not.
- It has macroscopic and microscopic aspects incompatible with spontaneous remission, and scar tissue has developed different from intact cartilage.

5 CONCLUSIONS

- The model is simple, easy to control and execute.
- Indicated in the study of cartilaginous changes of regeneration after mechanical injury.
- It preserves the animals, which may serve in other experiments or for late observations of the phenomena described, evaluated in this first report that considers only the early lesions.



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