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*Original article*

# Temporomandibular joint arthrocentesis in a rabbit model: technique and recommendations in the study of temporomandibular disorders

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## Abstract

**Introduction:** Arthrocentesis has been used in the temporomandibular joint (TMJ) to analyze components of the synovial fluid or as a therapeutic procedure associated or not with the administration of a drug. The rabbit is one of the most commonly used animal species as a model for pathologies that affect the TMJ. The aim of this study was to propose a specific technique to perform arthrocentesis on the rabbit TMJ, emphasizing descriptions of reference points and measurements for a successful puncture without complications. **Materials and methods:** Fourteen adult rabbits (*Oryctolagus cuniculus*) were used. The project was approved by the Scientific Ethics Committee of the Universidad de La Frontera (File N°083/2016). **Results:** The description of the technique was divided into three steps: 1) Location of the rabbit TMJ, 2) Positioning of the needles in the TMJ, and 3) Passage of fluid through the TMJ. **Conclusions:** This arthrocentesis technique could help to simplify the procedure and give the investigator a guide for joint washing and extraction of synovial fluid in the rabbit TMJ.

**Key words:** temporomandibular joint, temporomandibular joint disorders, arthrocentesis, rabbit

## Introduction

Arthrocentesis is defined as the puncture of a joint with a sterile needle to remove synovial fluid (SF) for the diagnosis or treatment of a joint disease and sometimes to then inject a drug into the joint cavity (Frost and Kendell 1999, Alpaslan and Alpaslan 2001).

Arthrocentesis enables the elimination of inflammatory mediators associated with pathological processes such as osteoarthritis (OA) (Goiato et al. 2016). It has been used in the temporomandibular joint (TMJ) to analyze the SF components or as a therapeutic procedure associated or not with the injection of an anti-inflammatory or lubricant (Goiato et al. 2016). Arthrocentesis in the TMJ releases the joint disc from the fibrous tissue that forms between the disc and the joint capsule by washing out the upper joint space and/or applying hydraulic pressure, thus improving the free movement of the disc (Nitzan 2003).

Animal studies are frequently used to investigate the pathological mechanisms that affect the human TMJ and to develop new therapies. The rabbit is one of the most commonly used species as a model for pathologies that affect the TMJ, being used mainly for the study of disc or inflammatory disorders such as OA. One of the advantages of using this species as a model is the morphofunctional similarity of the TMJ between a rabbit and a human (Savalle et al. 1990, Güler et al. 2011). With respect to its bone and joint characteristics, the TMJ stands out as presenting a high rate of replacement, with osteonal remodeling similar to that of a human, which means that physiopathological and inflammatory processes can be quickly noted. Additionally, the rabbit is easily cared for, reproduces rapidly, is easy to feed and has suitable dimensions.

Despite the evidence contributed by the scientific literature regarding arthrocentesis in the human TMJ and in animal models, this procedure has not yet been described in detail. Therefore, the aim of this study was to propose a specific technique to perform arthrocentesis on the rabbit TMJ, emphasizing descriptions of reference points and measurements for a successful puncture without complications.

## Materials and Methods

For the implementation of the arthrocentesis protocol, fourteen male rabbits (*Oryctolagus cuniculus*, New Zealand race) were used, weighing approximately 3 kg, obtained in the Experimental Surgery Unit of the Center for Morphological and Surgical Studies of the Universidad de La Frontera, Temuco, Chile. They were kept in controlled environmental conditions

in terms of temperature, environmental noise and a cycle of 12h light/12h darkness, food and water *ad-libitum*. The procedures were carried out according to the instructions in the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 2011) and the recommendations of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) humane endpoints in animal experimentation. The project was approved by the Scientific Ethics Committee of the Universidad de La Frontera (File N°083/2016).

The animals were anesthetized with ketamine (40 mg/kg), xylazine (5 mg/kg) and acepromazine (1 mg/kg) intramuscular (Green et al. 1981) in order to avoid suffering and to enable the correct execution of the arthrocentesis. Once anesthetized, the auricular region of the TMJ was shaved and disinfected with 70% ethyl alcohol.

## Statistical methods

The statistical analysis was performed using the SPSS /MAC 20.0 (SPSS, Chicago, IL software). The normality analysis of the data was done with a Kolmogorov-Smirnov test. Means and their standard deviations were calculated using the t-test for related samples for the inferential analysis. The value  $p \leq 0.05$  was considered significant.

## Results

### Description of the arthrocentesis technique

#### *Location of the rabbit TMJ:*

In each animal the distance was measured between the lateral part of the rima palpebrarum and the TMJ to establish a reference point to locate the joint. The measurement was taken by palpating the lateral pole of the mandibular condylar process during opening and closing movements performed by the operator. In both TMJs the measurements were taken with a metal millimeter ruler (Fig. 1). The average distance in the animals studied was  $18.4 \pm 1.167$  mm on the right side and  $18.36 \pm 2.061$  mm on the left side, with no statistically significant differences between the two sides ( $p=0.724$ ). This procedure was used as a reference to locate the TMJ when beginning the arthrocentesis technique.

#### *Positioning of the needles in the rabbit TMJ:*

Using the previously indicated point of reference, the first 15 mm needle (25G x 5/8") was introduced into the TMJ at a 45° angle to a depth of 10 mm (Fig. 2).



Fig 1. Measurement of the distance between the lateral part of the rima palpebrarum and the temporomandibular joint using a metal millimeter ruler.



Fig 2. Location of the needle in the temporomandibular joint at a 45° angle and a depth of 10 mm.

The mandible was manipulated by opening and closing, producing a pendulum movement of the needle which accompanied the mandibular movement. Then the second needle of equal dimensions was then introduced, one millimeter behind the first to the same depth. In order to ensure the location of the needles inside the TMJ, the manipulation maneuver was repeated, creating a synchronous pendulum movement between the two needles. In the cases where this did not occur, the needles had to be redirected to enable a successful technique.

#### *Passage of fluid through the rabbit TMJ:*

A hypodermic syringe with 3 mL of physiological saline solution in the posterior needle was connected and approximately 1 mL was carefully injected until the fluid appeared through the anterior needle and was collected in an Eppendorf tube, creating a circuit. The remainder of the physiological saline solution was then injected, recovering all the fluid (Fig. 3). There were two error markers in the technique: 1) not producing the circuit once the first mL was injected, and 2) increased volume in relation to the TMJ.



Fig 3. Positioning of the needles and passage of the fluid through the rabbit's temporomandibular joint.

## Discussion

The TMJ is a complex structure composed of bones, ligaments and joint capsule that form a synovial joint. Different animal models have allowed the study of pathologies that affect the TMJ and its therapeutic tests. Arthrocentesis is a minimally invasive procedure used mainly in inflammatory and/or degenerative pathologies of the TMJ, where a puncture in the upper joint compartment is made, accompanied by the washing out and removal of the inflammatory mediators present. This technique was empirically developed by different working groups taking the arthroscopic technique as an example (Marti et al. 2016). Currently, there are different arthrocentesis techniques, the most frequent being done through a circuit produced by two needles, where physiological saline solution or Ringer's lactate solution is introduced into one of the needles, and the fluid exits through the second needle. The arthrocentesis technique has been used, associated or not, for the infiltration of a drug such as corticosteroid or hyaluronic acid (HA) in order to reduce inflammation, alleviate pain and improve joint lubrication (Marti et al. 2016).

Another use that has been attributed to arthrocente-

sis is the harvesting of SF from the TMJ for analysis. SF is ultrafiltered plasma that presents high concentrations of HA, which is secreted by the  $\beta$  cells present in the synovial lining (Brandt et al. 2000, Xinmin 2004). The presence of inflammatory mediators in SF has been assessed in pathologies such as TMJ OA. Among the most recognized inflammatory mediators are arachidonic acid derivatives, related enzymes and some cytokines (Uchôa and Constantino 2012). With respect to the cytokines, it has been observed that interleukin-1 $\beta$  and the tumor necrosis factor alpha (TNF- $\alpha$ ) can produce bone resorption and synovial proliferation, causing the destruction of the cartilage (Hirota 1998). These cytokines are produced by monocytes, synovial fibroblasts and epithelial cells which, in addition, can stimulate the production of metabolites of arachidonic acid, such as some prostaglandins (Hirota 1998), increasing joint damage and HA degradation. Iturriaga et al. (2017) conducted a systematic review of studies that evaluated the effect of HA as a treatment for OA of the human TMJ, demonstrating that its application has a positive effect on the regulation of inflammatory mediators.

It is worth noting that the rabbit is one of the most commonly used animal models to investigate patholo-

gies of the human TMJ, and different techniques have been described for the replication of disc dislocations, disc perforations and OA. However, there are few descriptions of the arthrocentesis technique used in these models. The rabbit has a mandibular fossa formed by the root of the zygomatic process of the temporal bone with a small joint surface, slightly concave in the front and convex in its sagittal section. The condylar process is four times longer than the fossa. Rostrally it has a spherical shape, narrowing at a caudal point. The joint disc is positioned on the rostral spherical portion of the condyle, where the marginal area is thicker in the central and caudal region of the disc (Savalle et al. 1990). Consequently, the joint space for performing the arthrocentesis technique is small, making it necessary to be very detailed in locating the TMJ, positioning the needles and infiltrating the fluid. The mandibular manipulation with opening and closing movements was a useful tool throughout the procedure, helping to locate the TMJ and position the needles, ensuring that these were within the joint and generating the pendulum movement associated with greater success when creating the wash-out circuit. The average distance from the lateral part of the rima palpebrarum to the TMJ is a value that will provide a reference point in future studies to improve the location of the joint and to facilitate the technique. It is important to consider that this distance was similar between the right and left sides, which is why the proposed protocol is indiscriminately applicable to the TMJ discussed. This study demonstrated that in cases where the fluid injected was not collected within a few seconds or where the area presented an increase of volume, it was necessary to reposition the needles, mainly changing their depth, thereby avoiding damage to the structures or rupture of the joint capsule. In addition, the amount of infiltrated fluid will depend on the correct generation of the circuit and not on the distension capacity of the joint capsule. Therefore, this arthrocentesis technique could help to simplify the procedure and give the investigator a guide for joint washing and SF extraction and infiltration of substances in the rabbit TMJ.

## Conclusions

The rabbit is one of the most commonly used animal species as a model for pathologies that affect the TMJ. The arthrocentesis technique in the TMJ of rabbits should include the three suggested steps to facilitate the procedure and achieve clinical success, so this arthrocentesis technique could help to simplify the procedure and give the investigator a guide for joint washing, SF extraction and infiltration of substances

in the rabbit TMJ, emphasizing descriptions of reference points and measurements for a successful puncture without complications.

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