

## A Histomorphological Study of Intervertebral Nucleus Pulposus of the Rabbit

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### Original Research Article

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**Abstract:** The nucleus pulposus is a vestigial gelatinous structure derived from notochord that is located within intervertebral discs. Nucleus pulposus from rabbit were obtained and processed to carry out a histological study using simple staining methods. Results show that nucleus pulposus from rabbit was composed of vacuolated cells aggregated in groups or cords immersed in an extracellular matrix composed of collagen type II and acid and sulfated mucopolysaccharides. Further studies will be necessary to characterize cells and extracellular matrix and to compare with nucleus pulposus from several origins.

**Keywords:** Nucleus pulposus, histology, physaliphorous cells, collagen type II, mucopolysaccharides.

### INTRODUCTION

The nucleus pulposus, a gelatinous structure located in the centre of intervertebral discs and surrounded by fibrous cartilage, absorbs axial compression forces and protects vertebral plates [1]. It derives from notochord, an embryonic structure that appears at third week after fecundation and that evolves notochordal plate, channel and finally to notochordal cord, which induces the apparition of neural plate at fourth week after fecundation with the essential participation of the Sonic hedgehog factor from the notochordal cells. After this fact in the nervous system conformation [2, 3], the cordal tissue remain as a vestigial structure in humans and superior animals but not in some animals such as tunicates and amphioxus [4]. However, nucleus pulposus is a poor studied structure. The objective of this short article is to provide a histological study of the rabbit nucleus pulposus.

### METHODS

Six male rabbits (*Oryctolagus cuniculus*) (male New Zealand white rabbits), weighing 2.5 to 3.0 kg, were purchased from the Centre for Scientific Instrumentation (Granada University). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Ethical Committee of Granada. Rabbits were sacrificed by intravenous injection of over dose of 3% pentobarbital sodium. Then the rabbit spines were obtained in sterile condition and intervertebral discs (4 for each rabbit) were fixed in formaldehyde 10% for 48 hours. Afterwards, they were included in paraffin blocks and paraffin sheets (5 µm) were obtained using a rotary microtome, Microm HM325 (Microm, Walldorf, Germany). Paraffin sheets were placed over microscopy slides and were left drying for 24 hours at room

temperature. Samples were deparaffinized and dehydrated. Afterwards, they were stained with common protocols of haematoxylin-eosin, Masson's trichrome for collagen type I and III, alcian blue pH 1 for acid mucopolysaccharides, toluidin blue for sulfated mucopolysaccharides, periodic acid-Schiff (PAS) for neutral mucopolysaccharides, picosirius for collagen type I, II and III and Gomori's reticulin for reticulin, collagen type III [5, 6, 7]. Moreover, picosirius samples were analyzed using polarized light in order to detect collagen type I [8]. Samples were mounted with a cover slip and resin and were left drying. Afterwards, they were observed and photographed with a photographic microscope.

### RESULTS AND DISCUSSION

Haematoxylin-eosin staining of nucleus pulposus showed a basophilic matrix with vacuolated cells which showed a very acidophilic cytoplasm and a pyknotic nucleus seeming “signet ring cells”. These cells were aggregated in groups or cords (Figure 1A and B). These vacuolated cells (physaliphorous cells) with a string arrangement probably contain various storage substances such as glycogen and proteoglycans (acidophilic), proteins (basophilic) or lipids (vacuolated cytoplasmic structures). Masson's trichrome staining revealed a negative staining for collagen I in contrast to surrounding fibrous cartilage which was highly positive (Figure 1C). On the other hand, nucleus pulposus was positive to both Alcian and toluidin staining indicating the presence of acid (hyaluronic acid) and sulfated (chondroitin sulfate, heparan sulfate, keratan sulfate) mucopolysaccharides. In fact, the presence of hyaluronic acid explains the nucleus pulposus function as an axial forces absorber [9]. By contrast, PAS staining was negative (Figure 1D and E) indicating that does not contain neutral ones. The presence of a great amount of mucopolysaccharides (basophilic) in relation to collagen type II (acidophilic) (see below) could explain the hematoxylin-eosin staining (basophilic matrix). In addition, samples were positives for picosirius red which detects the presence of collagen I, II, or III (Figure 2A and B). When these stained samples were visualized with polarized light, a negative birefringence (characteristic of collagen II and III) in nucleus pulposus matrix was observed. However, surrounding fibrous cartilage showed a positive birefringence (Figure 2C and D). Finally, Gomori's reticulin staining was negative in nucleus pulposus matrix (brown color) and weakly positive (black staining) in surrounding nucleus pulposus cells (Figure 2E and F). It could be due to the existence of a basement membrane - like structure although more histochemical studies will be necessary in order to demonstrate this fact. Previous studies about physaliphorous cells expose they contain many reserve substances such as glycogen and secretion proteoglycans, lipids or proteins. These results support our histological studies in which physaliphorous cells reveals acid cytoplasmic substances stained with toluidine blue (probably carbohydrates), basic cytoplasmic compounds stained with picric acid (probably proteins) and vacuoles (probably lipids lost in histological processing of the samples). Moreover, in the extracellular matrix, Risbud *et al.* [10] described the presence of collagen type II, but McCann *et al.* [11] demonstrated that nucleus pulposus contains collagen type III as a main extracellular matrix collagen.

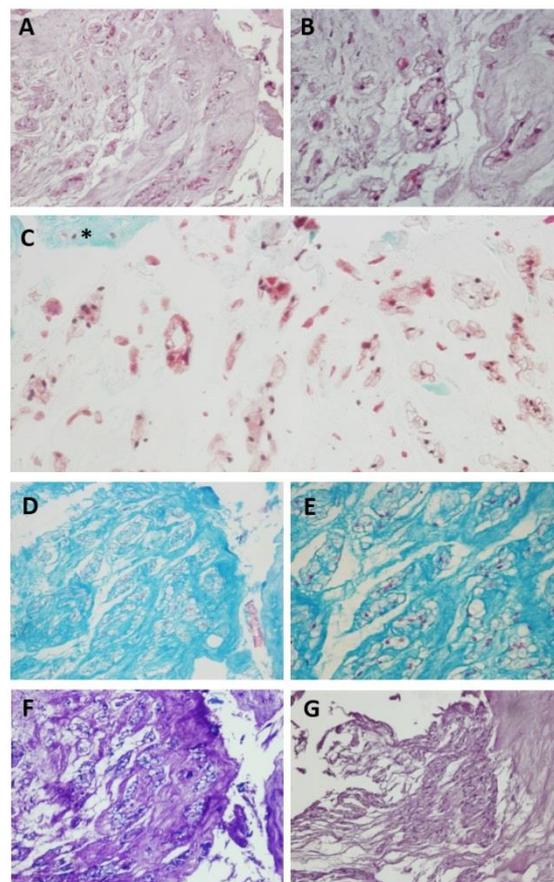


Fig-1

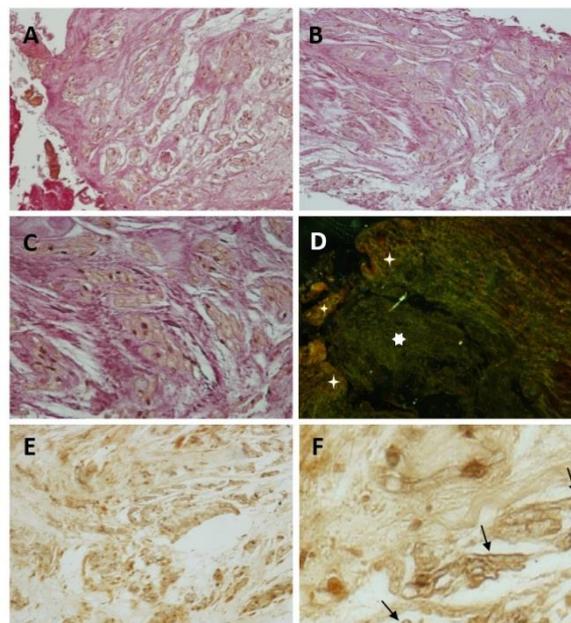


Fig-2

## CONCLUSIONS

Hystologic analysis of the rabbit nucleus pulposus showed that contains vacuolated cells immersed in an extracellular matrix composed of collagen type II, acid and sulfated mucopolysaccharides. A more extensive

immunohistochemically analysis will be necessary in order to characterize nucleus pulposus cells and to study extracellular matrix deeply and the possible existence of a basement membrane - like structure. It would be interesting to characterize cytoplasmic and surface markers of nucleus pulposus cells in order to demonstrate their possible potential as stem cells in cell therapy and tissue engineering.

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