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# Effects of hyperprolactinemia and ovariectomy on the tibial epiphyseal growth plate and bone formation in mice

## *Efeitos da hiperprolactinemia e ooforectomia sobre o disco epifisário da tíbia e formação óssea em camundongos fêmeas*

Original Article

**Keywords**

Growth plate/metabolism
Tibia/metabolism
Ovariectomy
Hyperprolactinemia
Hyaline cartilage

**Palavras-chave**

Lâmina de crescimento/metabolismo
Tíbia/metabolismo
Ovariectomia
Hiperprolactinemia
Cartilagem hialina

## Abstract

**PURPOSE:** To evaluate the effects of ovariectomy and the hyperprolactinemia procedure in the tibial epiphyseal growth plate of female mice. **METHODS:** In this study, the epiphyseal growth plate of ovariectomized (OVX) and/or rendered hyperprolactinemic female mice by 50 days of treatment with 200 μg metoclopramide (M) was evaluated morphologically, morphometrically and immuno-histochemically. Forty female and adult mice were divided into four groups according to treatment: V group — animals treated with saline solution; H group — hyperprolactinemic animals; Ovx/V group — ovariectomized animals and treated with saline solution; Ovx/H group — hyperprolactinemic and ovariectomized animals. After the treatment period, the animals were sacrificed, tibia was removed and fixed in 10% buffered formalin and decalcified in 10% formic acid. The material was immersed in paraffin and subjected to histological processing in paraffin. The sections were stained with Masson's trichrome and immunohistochemistry was carried out for the pro-apoptotic protein BCL-2. The images for the morphological and morphometric study were analyzed with the imaging program AxioVision 4.8 (Carl-Zeiss®, Germany). **RESULTS:** The combination of hyperprolactinemia and the ovariectomy procedure decreased the number of resting chondrocytes 1.5-fold, the number of proliferative chondrocytes 1.8-fold; the percentage of resting cartilage 2.4-fold and the percentage of trabecular bone 2.1-fold, compared with respective control animals. **CONCLUSION:** The procedure of ovariectomy combined with the metoclopramide-induced hyperprolactinemia in female mice has showed marked bone degeneration due to significant decrease of cell proliferation in the epiphyseal growth plate and bone formation.

## Resumo

**OBJETIVO:** Avaliar os efeitos do procedimento de ooforectomia e da hiperprolactinemia no disco epifisário da tíbia de camundongos fêmeas. **MÉTODOS:** Neste estudo, o disco epifisário de camundongos fêmeas ovariectomizadas (OVX) e/ou com hiperprolactinemia induzida por tratamento com 200 μg de metoclopramida por 50 dias (M) foi avaliado morfologicamente, morfometricamente e imunohistoquimicamente. Quarenta camundongos fêmeas e adultas foram divididas em quatro grupos, segundo o tratamento: Grupo V — animais tratados com solução salina; Grupo H — animais hiperprolactinêmicos; Grupo Ovx/V — animais ooforectomizados e tratados com o solução salina; Grupo Ovx/H — animais ooforectomizados e hiperprolactinêmicos. Após o período de tratamento, os animais foram sacrificados, as tíbias removidas e fixadas em formalina tamponada a 10% e descalcificadas em ácido fórmico a 10%. O material foi emblocado em parafina e submetido a processamento histológico em parafina. Os cortes foram corados pelo tricrômico de Masson e foi feita a imunohistoquímica para a proteína pró-apoptótica BCL-2. As imagens para o estudo morfológico e morfométrico foram analisadas com o programa de imagem AxioVision 4.8 (Carl-Zeiss®, Alemanha). **RESULTADOS:** A combinação da hiperprolactinemia e do procedimento de ovariectomia levou à redução do número de condrócitos de repouso em 1,5 vezes; o número de condrócitos proliferativos em 1,8; a percentagem de cartilagem de repouso em 2,4, e a percentagem de osso trabecular em 2,1 vezes, em comparação com os respectivos animais controles. **CONCLUSÃO:** O procedimento de ooforectomia combinado com a condição de hiperprolactinemia induzida pela metoclopramida em camundongos fêmeas evidenciou degeneração óssea acentuada, devido à diminuição significativa da proliferação celular no disco epifisário e da formação óssea.

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**Received**
06/20/2014

**Accepted with modifications**
07/15/2014

**DOI:** 10.1590/S0100-720320140005065

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Conflict of interests: none.

## Introduction

The bone formation through endochondral ossification is a process initiated by mesenchymal cell condensation, and it leads to the formation of a cartilaginous scaffold that will later be replaced by bone[1]. The outcome of this process is chondrocyte differentiation that leads to proliferation, hypertrophy, and concomitant expression of specific genes of cartilaginous tissue[2]. The terminal cartilage chondrocyte differentiation induces mineralization of cartilaginous extracellular matrix (ECM) and chondrocyte death by means of programmed cell death (PCD)[3]. However, in diarthroid joints, the cartilage scaffold is not fully replaced by bone. Two regions in these joints remain cartilaginous: the one that will be replaced (the growth plate) and the other that will never be replaced (articular cartilage)[4]. The growth plate is an epiphyseal hyaline cartilage located between the epiphysis and diaphysis of long bones, and it is responsible for the growth of these bones through an endochondral ossification process. This cartilage is divided into five distinct zones: resting, proliferative, hypertrophic, calcified, and ossified[1,2].

In the resting cartilage, the mesenchymal cells are flattened, isolated or paired with another cell, arranged irregularly in the matrix, and differentiated into proliferative chondrocytes[3]. In the proliferative cartilage, the chondrocytes that are already differentiated become flattened and are arranged within gaps in a longitudinal direction and have a typical columnar orientation. The cells in this cartilage zone are able to proliferate; this is one of the factors leading to longitudinal bone growth[4]. In the hypertrophic cartilage, the chondrocytes are still arranged within the gaps in longitudinal columns, but these columns are less organized than those in the proliferative cartilage zone. These chondrocytes are large in size as a result of the PCD process. The process of cartilage matrix mineralization begins by the release of intracellular calcium in vesicles[2,4]. In the calcified cartilage, the cavities created by chondrocytes after apoptosis are invaded by blood capillaries and undifferentiated mesenchymal cells migrating from the adjacent connective tissue (perichondrium). These cells differentiate into osteoblasts to initiate bone matrix synthesis. Subsequently, the chondrocytes trapped in the mineralized matrix undergo apoptosis, and then cavities separated by thin strands of cartilage matrix begin to appear. Following this event, the formation of the bone marrow channel occurs via bone resorption activity of osteoclasts, which are responsible for resorption and remodeling of the bone[1,5]. In the ossification zone, myeloid tissue and blood vessels invade the spaces created by the chondrocytes, and osteoblasts originating from the undifferentiated cells produce bone matrix on the residual cartilage matrix, giving rise to gaps and channels. Thereafter, the osteoblasts become osteocytes, because they are entrapped in the bone matrix, and primary bone formation with complete calcification of the cartilaginous matrix is achieved[6].

The processes of cellular proliferation and bone formation are influenced by several growth factors — epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), sex steroids (estrogen, progesterone, and testosterone), and hormones (parathyroid hormone, growth hormone, and prolactin)[7,8].

Prolactin and its receptor are related to cell differentiation and proliferation as well as bone mineralization. Besides breast enlargement and stimulation of milk production by the mammary glands, prolactin also influences bone formation by calcium demand. Its action on cartilage and bone has been suggested because of the presence of prolactin receptors (PRL-R) in these tissues[9]. Metoclopramide has been used in treatments for 50 days to elicit experimental hyperprolactinemia (high levels of prolactin). There is evidence that hyperprolactinemia leads to hypogonadism with a consequent decrease in the levels of sex steroids, leading to the degeneration of cartilage and bone tissue in mice and humans[10,11].

Bilateral ovariectomy (OVX) is also used to induce hypogonadism and causes the degeneration of cartilage and bone in mice. However, the effects of hyperprolactinemia and OVX alone or in combination on cartilage and bone tissue are not well understood[10]. Therefore, the present study extends previous research in our laboratory[12] by investigating the effects of hyperprolactinemia and OVX on the epiphyseal cartilage of the growth plate and bone formation in female mice.

## Methods

Forty adult female mice (*Mus musculus*), with ca. 100 days and 35 g birth weight, from facilities of Cedeme – Federal University of São Paulo (UNIFESP) Center for Development of Experimental Models, were employed. The animals were housed in plastic cages with metal bars and artificial lighting, with a photoperiod of 12 hours light/12 hours dark. They were fed with standard chow (Labina-Purina®, Paulínia, Brazil) and water *ad libitum*; the average environment temperature was 22°C.

All experimental protocols were approved by the Research Ethics Committee on Animal Experimentation of UNIFESP-EPM/project 1148/09 and the animals were maintained in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals[13].

The vaginal smear of the animals was collected daily for a week for monitoring the regularity of the estrous cycle and the characterization of the estrous cycle phases by

Harris-Shorr staining cytological analysis[14]. Once observation of regular cycling as an indication of normal gonad activity was provided, the mice were randomly divided into 4 groups (n=10 animals for each group) and treated daily during 50 consecutive days, unless otherwise state, as follows: Vehicle (V): naïve controls, injected subcutaneously with 0.2 mL of the vehicle (saline solution – NaCl 0.9%); Metoclopramide rendered hyperprolactinemic (H): mice injected subcutaneously with 200 µg of metoclopramide (M) (Sigma®, St. Louis, United States) dissolved in 0.2 mL of the vehicle; Ovariectomized Vehicle (Ovx/V): Ovx mice treated subcutaneously with 0.2 mL of the vehicle; ovariectomized metoclopramide-hyperprolactinemic (Ovx/H): Ovx mice treated subcutaneously with 200 µg of M dissolved in 0.2 mL of the vehicle.

After being anesthetized with 16 mg/kg of xylazine (Anasedan® – Vetbrands Int, Florida, United States) and 16 mg/kg of ketamine (Dopalen® – Vetbrands Int, Florida, United States) subcutaneously, the animals were positioned ventrally, and an about 1.5cm length skin incision was made bilaterally. The ovaries, uterine horn tip and the ovarian adjacent fat were exteriorized and then ovaries and surrounding fat were sectioned. Stitches were made with a 3.0 silk thread (Ethicon® – Johnson & Johnson, New Jersey, United States). After the procedure, the animals were placed in individual cages and 10 mg/kg of an association of dipyrone, adifenine hydrochloride and promethazine hydrocloride (Lisador® – Farmasa, United States) were administered in drinking water. After a 7-day recovery period, vaginal smears were taken daily during 21 days to verify vaginal atrophy as indicative of female sex hormones depletion.

After 50 days of treatment, as already described, the mice were sacrificed by deep anesthesia (xylazine 20 mg/kg plus ketamine 100 mg/kg, Vetbrands Int, Florida, United States), subcutaneously. The animals were positioned dorsally and tibiae were taken. The bones were fixed in formaldehyde (10%) in phosphate buffer during 24 hours and decalcified in 10% formic acid. After that, they were dehydrated in increasing concentrations of alcohol, diaphanized in xylene and embedded in paraffin. Inclusion of longitudinal sections was performed and 5 µm thick slides were prepared for further analyses[15].

The slides with the sections were immersed in xylene, then in absolute ethanol, and then in Harris haematoxylin for 5 minutes, in solution A (fuchsin acid, acetic acid and distilled water) for 5 minutes, in solution B (fosfomolibdic acid and distilled water) for 5 minutes, and finally in solution C (aniline blue, acetic acid and distilled water). After that, they were immersed 3 times in acetic acid 1%, and finally in ethanol, in xylene and mounted with coverslips and Ettelan® (Merck Chemicals, Germany)[16].

Photomicrographs were taken from regions of the tibia in the proximal region of each slide using a field representative for each area and analyzed using a computerized system consisting of a light microscope (Carl Zeiss®, Germany), fitted to a high resolution camera – Axio Cam MRC (Carl Zeiss®, Germany) and a color video monitor (Samsung®, Korea). The images were obtained using the image analysis program AxioVision 4.8 (Carl Zeiss®, Germany). The morphological and morphometrical evaluation of the slides included: five regions of the epiphyseal growth plate at 400X objective, to obtain measures (µ) of total thickness and of each zone of cartilage (resting, proliferative and hypertrophic); five regions of the epiphyseal growth plate at 400X objective, to obtain the counting of chondrocytes in each zone of cartilage (resting, proliferative and hypertrophic) and four regions of the epiphyseal growth plate at 100X objective, using a Weibel's reticle of 36 points, which was superimposed on each image, to obtain the percentage of remaining cartilage, trabecular bone and bone marrow, determining the percentage of bone formation[17].

The results (mean ± standard deviation) were analyzed by ANOVA followed by Tukey's *post hoc* test with the computer program Prizma® (California, United States)[18]. Differences at $p \leq 0.05$ were taken as significant.

For immunohistochemistry analysis of the pro-apoptotic protein – BCL-2 antibody, sections were initially submitted to deparaffinization in xylene and alcohol series for 5 minutes, with subsequent recovery of antigenic sites on steam fluent (pot value) for 30 minutes. The slides were washed twice for 5 minutes in a shaker with 0.1 M phosphate buffered saline (PBS), pH 7.5 and then blocking endogenous peroxidase was carried out by immersion in hydrogen peroxide at 1% for 30 minutes, at room temperature. Then, the slides were washed again in PBS (2x 5 min) and blocking of nonspecific sites was performed with normal goat serum diluted in 0.2 M PBS, pH 7.4.

After incubation overnight at 4°C in a dark chamber with primary antibodies monoclonal anti-BCL-2 antibody (IMUNY™, Rheabiotech Ltda., Brazil) diluted 1:400 in PBS, the samples were subjected to repeated washses in PBS, and then incubated for 30 minutes at room temperature with secondary antibody anti-mouse IgG conjugated with biotin (LSAB 2 Kit, Dako) at a dilution of 1:400.

The samples were subsequently incubated with AB reagent (streptavidin-biotin complex, LSAB 2, DAKO) for 30 minutes at room temperature, and developed with DAB (Sigma ChemicalCo, St. Louis, MO, United States) for 1 minute at room temperature. The sections were then counterstained with Harris hematoxylin solution for 20 seconds, fixed in alcohol series – xylene and mounted with cover slip and mounting medium Permount (Fisher, United States).

Immunohistochemistry was performed at the Laboratory Rheabiotech/Imuny (Campinas, São Paulo, Brazil).

We adopted as negative control the absence of primary antibody and the appearance of brown cells and extracellular matrix of the growth plate for positive pattern. The results of the evaluation of the expression of BCL-2 antibody were qualitatively guaranteed by the presence of cell marking.

## Results

Figure 1A shows the morphology of the mouse epiphyseal growth plate of the naïve control group (V). The chondrocytes of the epiphyseal growth plate cartilage are organized into morphologically distinct zones: resting, proliferative, and hypertrophic. The area of the resting cartilage is characterized by a layer of flattened chondrocytes with small spaces between the cells. The proliferative cartilage is characterized by a large number of chondrocytes with small spaces between rows of flattened and arranged chondrocytes. Finally, the hypertrophic cartilage is characterized by small number of rounded and arranged chondrocytes.

In the group treated with metoclopramide (H; Figure 1B), the resting and proliferative cartilages have fewer and less organized chondrocytes with more spaces between them, and the hypertrophic cartilage has a larger number of chondrocytes, which are much more rounded and less arranged, in comparison with the control group.

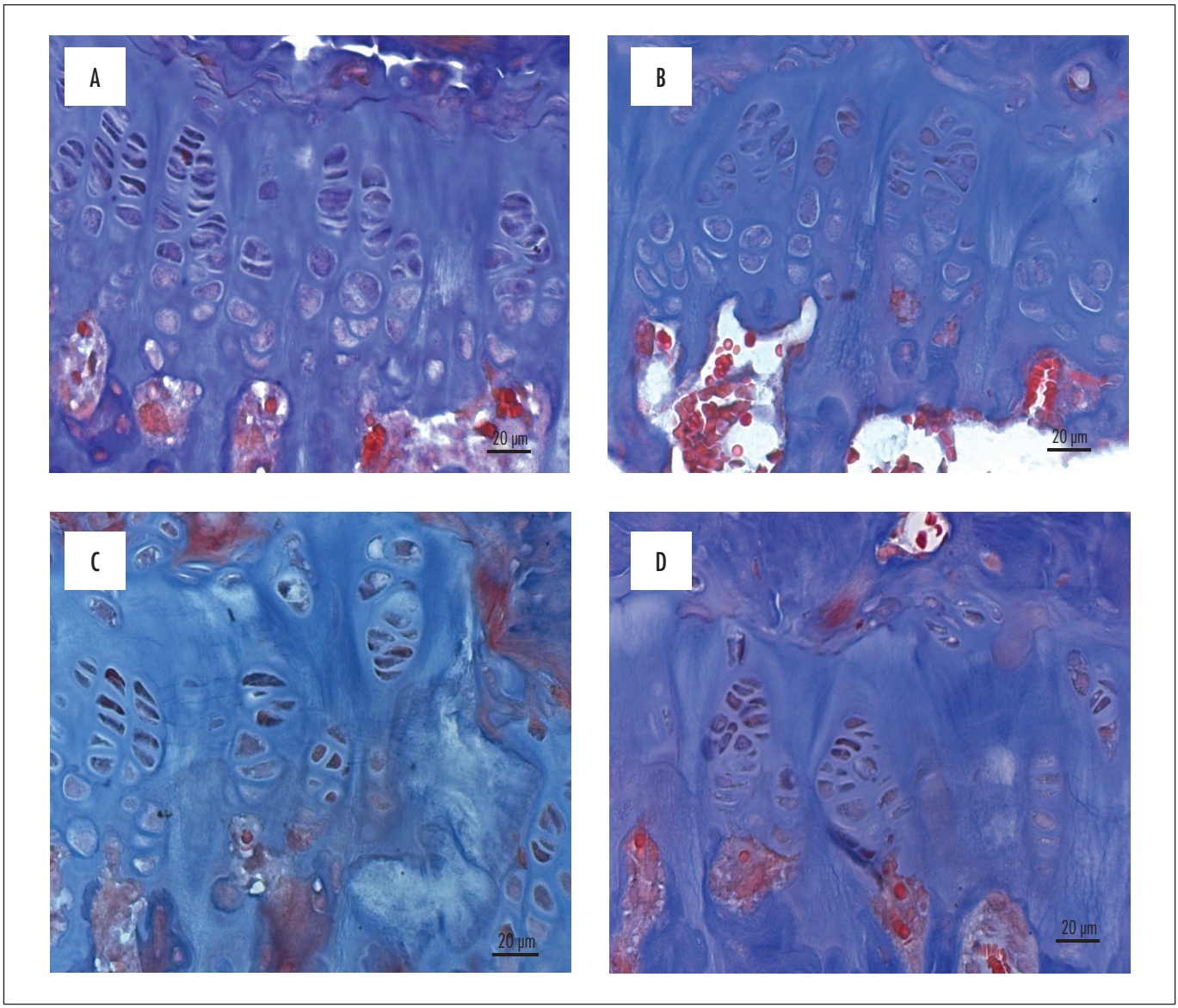


**Figure 1.** Photomicrographs of the tibial epiphyseal growth plate of the groups (n=10). (A) vehicle (V); (B) hyperprolactinemic (H); (C) ovariectomized vehicle (Ovx/V); (D) ovariectomized hyperprolactinemic (Ovx/H), with a 40X objective. Stained with Masson's trichrome.

In the group subjected to bilateral OVX and treated with vehicle (Ovx/V; Figure 1C), the resting and proliferative cartilages showed fewer disorganized flattened chondrocytes with larger spaces between them, and the hypertrophic cartilage showed a larger number of rounded and disorganized chondrocytes, in comparison with the control group.

In the group subjected to OVX and metoclopramide-hyperprolactinemia (Ovx/H; Figure 1D), the resting and proliferative cartilages showed few flattened, widely spaced, and disorganized chondrocytes in a small number of rows with large spaces between them, and the hypertrophic cartilage showed a great amount of rounded and disorganized chondrocytes, in comparison with the group treated only with metoclopramide-hyperprolactinemia (H).

The morphometrical results obtained from the evaluation of the resting cartilage percentage (Table 1; p≤0.05) showed that this percentage in the control (V) group (a) was not significantly different from that in the H and Ovx/V groups, but it was different from that in the Ovx/H group. In the evaluation of the trabecular bone percentage (Table 1; p≤0.05), in the V group (a), this percentage was significantly different from that in the H, Ovx/V, and Ovx/H groups; in the H group (b), this percentage was significantly different from that in the Ovx/V and Ovx/H groups. In the evaluation of the bone marrow percentage (Table 1; p≤0.05), in the V group (a), this percentage was significantly different from that in the H, Ovx/V, and Ovx/H groups; in the Ovx/V group (b), this percentage was significantly different from that in the Ovx/H group.

In the evaluation of the number of resting chondrocytes (Table 2; p≤0.05), the V group (a) had a larger number than the Ovx/V and Ovx/H groups; the H group (b) had a larger number than the Ovx/H group. Moreover, the Ovx/V group (c) also had a larger number than the Ovx/H group. In the evaluation of the number of proliferative chondrocytes (Table 2; p≤0.05), the V group (a) had a larger number than the H, Ovx/V, and Ovx/H groups; the H group (b) had a larger number than the Ovx/V and Ovx/H groups; and the Ovx/V group (c) had a larger number than the Ovx/H group. In the evaluation of the number of hypertrophic chondrocytes (Table 2; p≤0.05), the numbers were not different between the V (a) and the H groups, but the V group had a smaller number than the Ovx/V and Ovx/H groups. In addition, the H group (b) had a smaller number than the Ovx/H group.

In the evaluation of the resting cartilage zone thickness (Table 3; p≤0.05), the V group (a) had a thicker zone than the H, Ovx/V, and Ovx/H groups; the Ovx/V group (b) had a thicker zone than the Ovx/H group. In the evaluation of the proliferative cartilage zone thickness (Table 3; p≤0.05), the V group (a) had a thicker zone than the H, Ovx/V, and Ovx/H groups; the H group (b) had a thicker zone than the Ovx/V and Ovx/H groups; and the Ovx/V group (c) had a thicker zone than the Ovx/H group. In the evaluation of the hypertrophic cartilage zone thickness (Table 3; p≤0.05), the V group (a) had a thicker zone than the H, Ovx/V, and Ovx/H groups; the H group (b) had a thicker zone than the Ovx/H group. In the evaluation of total cartilage thickness (Table 3; p≤0.05), the V group (a) had a thicker cartilage than the H, Ovx/V, and Ovx/H groups, and the Ovx/V group (b) had a thicker cartilage than the Ovx/H group.

The immunohistochemistry results (Figures 2A to 2D) showed only positive staining for early hypertrophic

**Table 1.** Analysis of the bone formation percentage (p≤0.05) of the groups

| | Bone formation percentage | | | |
|---|---|---|---|---|
| | V | H | Ovx/V | Ovx/H |
| Resting cartilage | 3.7 a | 3.1 | 2.8 | 1.6 |
| Trabecular bone | 9.3 a | 7.2 b | 5.4 | 4.3 |
| Bone marrow | 21.1 a | 27.7 b | 25.6 | 30.0 |

V: vehicle; H: hyperprolactinemic; Ovx/V: ovariectomized vehicle; Ovx/H: ovariectomized hyperprolactinemic.

Groups: (A) resting cartilage (a - comparison with Ovx/H); (B) trabecular bone (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/V and Ovx/H); (C) bone marrow (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/H).

**Table 2.** Analysis of the chondrocytes number (p≤0.05) of the growth plate cartilage zones of the groups

| | Analysis of the chondrocytes number | | | |
|---|---|---|---|---|
| | V | H | Ovx/V | Ovx/H |
| Resting chondrocytes | 11.2 a | 10.2 b | 9.1 c | 7.1 |
| Proliferative chondrocytes | 46.2 a | 38.8 b | 30.1 c | 26.2 |
| Hypertrophic chondrocytes | 28.0 a | 29.6 b | 31.6 | 33.6 |

V: vehicle; H: hyperprolactinemic; Ovx/V: ovariectomized vehicle; Ovx/H: ovariectomized hyperprolactinemic.

Groups: (A) resting (a - comparison with Ovx/V and Ovx/H; b - comparison with Ovx/H); (B) proliferative (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/V and Ovx/H; c - comparison with Ovx/H); (C) hypertrophic (a - comparison with Ovx/V and Ovx/H; b - comparison with Ovx/H).

**Table 3.** Analysis of the thickness (μm; p≤0.05) of the growth plate cartilage zones of the groups

| | Analysis of the cartilage zones thickness | | | |
|---|---|---|---|---|
| | V | H | Ovx/V | Ovx/H |
| Resting thickness | 32.5 a | 26.7 | 28.1 b | 25.4 |
| Proliferative thickness | 52.0a | 41.2 b | 33.9 c | 30.8 |
| Hypertrophic thickness | 26.3 a | 29.1 b | 30.9 | 32.7 |
| Total thickness | 111.0 a | 97.1 | 93.0 b | 89.0 |

V: vehicle; H: hyperprolactinemic; Ovx/V: ovariectomized vehicle; Ovx/H: ovariectomized hyperprolactinemic.

Groups: (A) resting (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/H); (B) proliferative (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/V and Ovx/H; c - comparison with Ovx/H); (C) hipertrophic (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/H); (D) total (a - comparison with H, Ovx/V and Ovx/H; b - comparison with Ovx/H).

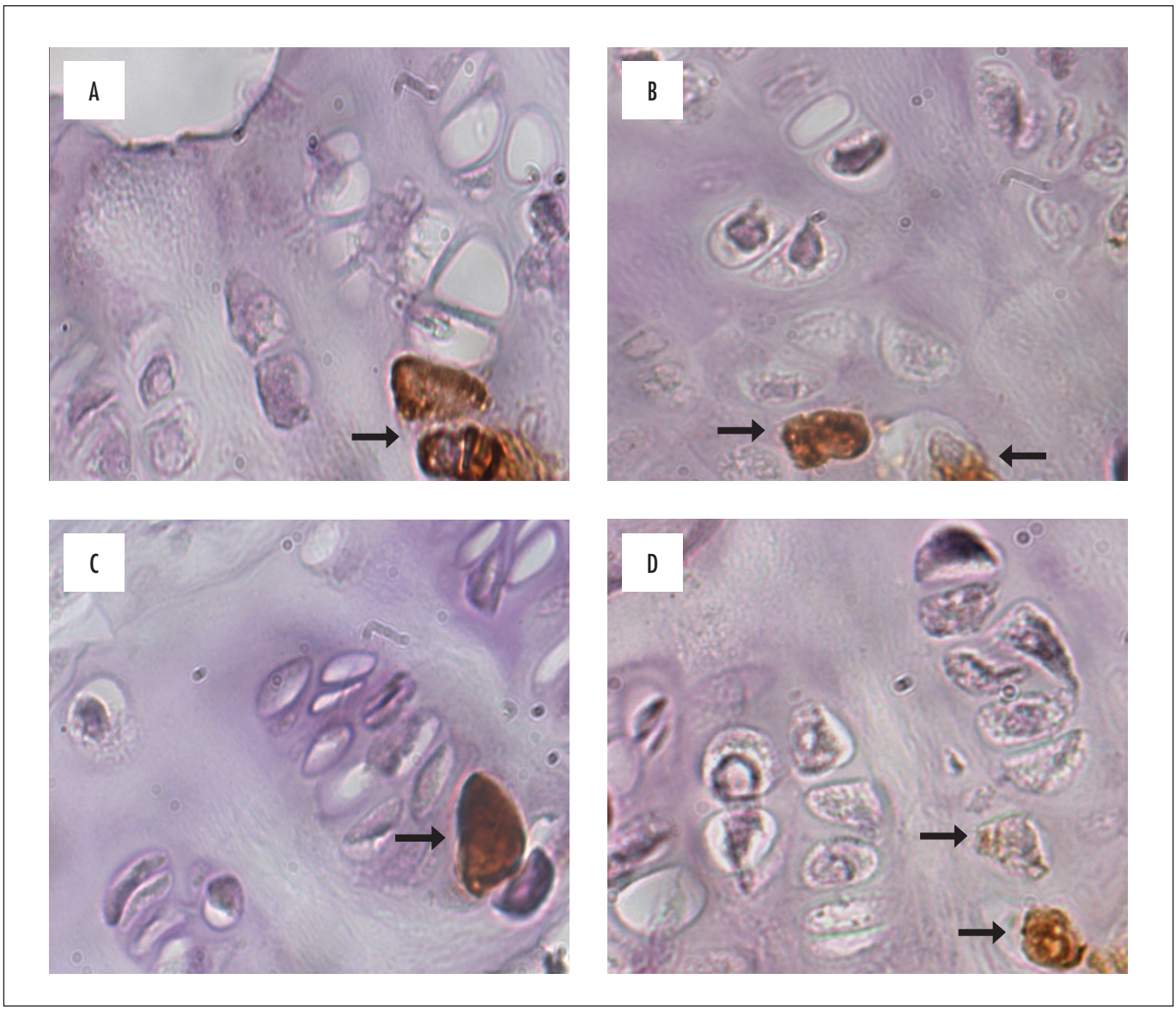


**Figure 2.** Photomicrographs of the tibial epiphyseal growth plate evidencing positive marking (arrows) for immunohistochemistry analysis of the pro-apoptotic protein - BCL-2 antibody. (A) vehicle (V); (B) hyperprolactinemic (H); (C) ovariectomized vehicle (Ovx/V); (D) ovariectomized hyperprolactinemic (Ovx/H), with a 100X objective.

chondrocytes and mature chondrocytes, and no proliferative chondrocytes were observed. This is because of the aging of the animals (Figure 2A), bilateral OVX (Figure 2B), and hyperprolactinemia (Figures 2C and 2D).

## Discussion

The growth plate is responsible for the growth of long bones via the process of endochondral ossification, and it goes through a series of events called "bone turnover"[6]. These events lead to the formation of a cartilaginous scaffold that will be replaced by bone[1]. The outcome of this process is chondrocyte differentiation leading to proliferation and hypertrophy that induces the mineralization of cartilaginous ECM and chondrocyte death by means of PCD[2,3].

Complex relationships exist between systemic factors and the paracrine, autocrine, and intrinsic influences that act together with the sex steroids to maintain cartilage homeostasis[7,19]. Thus, disturbances in this network can lead to damage in the cartilage and result in inadequate bone formation[3]. Our data show that disturbances in this network occurred in mice following ovariectomy and hyperprolactinemia. The animals treated with the vehicle developed the best quality of the growth plate cartilage and bone. The ovariectomized animals developed poor quality cartilage and bone compared with the V group; after treatment with metoclopramide, the hyperprolactinemic animals developed poorer quality cartilage and bone than the other two groups. In fact, after treatment with metoclopramide, the ovariectomized

and hyperprolactinemic animals developed the poorest quality cartilage and bone compared with all the groups. This outcome can potentially have a negative impact on locomotor system functioning[20].

Upon bilateral OVX, the production of sex steroids, such as estrogen and testosterone, ceases[12]. These steroids as well as progesterone are very important in the maintenance of cellular proliferation and bone formation[21]. If they are produced in lesser quantities in the body, the cartilage is greatly damaged and bone formation reduces, leading to very poor quality cartilage and bone[21-24].

The combination of OVX and hyperprolactinemia resulted in the lowest number of mesenchymal cells present in the resting cartilage zone, which is vital to counteract cartilage damage[20] This explains why this combined treatment results in the greatest cartilage degeneration and decreased bone formation, as observed in our experiments[12].

Prolactin acts in cartilage and bone through its local PRL-R. The transcription of these receptors is observed only in osteoblasts and not in osteoclasts. This fact suggests that prolactin is involved in bone formation and the maintenance of bone density by regulating calcium levels in conditions of increased demand, e.g., during gestation and lactation, and in an event of bone mass loss (osteoporosis)[10,19,22,23,25].

Prolactin secretion is maintained under hypothalamic inhibitory control mediated by the release of dopamine and the much more effective gamma-aminobutyric acid[26]. Plasma concentrations of prolactin can vary and sometimes increase because of physiological, pathological, and pharmacological issues[27,28]. Hyperprolactinemic states are related to decreased secretions of estrogen, progesterone, and testosterone (i.e., a hypogonadism-like state) followed by a decreased bone formation[10,29]. As shown by the results reported in this study, metoclopramide-induced hyperprolactinemia[12] negatively affects the quality of cartilage.

A drastic reduction in sex steroid production resulting from the combination of OVX and hyperprolactinemia and the resulting hypogonadism diminished cellular proliferation of cells in the growth plate epiphyseal cartilage as well as diminished bone formation in the tibia, resulting in the strongest negative effect on bone quality.

## Acknowledgement

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (Author's Scholarship).

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