

The Morphology of Paca (*Cuniculus paca*) Testis with High Dose of Letrozole an Aromatase Inhibitor

La Morfología del Testículo de Paca (*Cuniculus paca*) con Altas Dosis de Letrozol, un Inhibidor de la Aromatasa

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SUMMARY: The study reported the influence of the high and acute dose of Letrozole on the testis morphology in paca (*Cuniculus paca*), an aromatase inhibitor that reduces the endogenous estrogen, the essential hormone for spermatogenesis. Morphological changes were observed in seminiferous epithelium with germ cells with apoptotic characteristics and presence of vacuoles and nuclei in pycnose.

KEY WORDS: Gonadal; Toxic; Spermatogenesis.

INTRODUCCIÓN

The paca belongs to the Rodentia order, it is important as source of meat, and also has a scientific impact because this animal becomes an alternative experimental model.

Letrozole is a non-steroidal aromatase inhibitor that reduces endogenous estrogen when administered orally. The dosage of 0.15mg/kg, applied in swine during the pre pubertal phase, increased the number of sustentacular cells (Sertoli cells), the size of the testis and also sperm production (Berger & Conley, 2014). Several studies have shown the positive effects of small and chronic dosage for animal reproduction (Berger *et al.*, 2008). Thus, this study, at this point, showed how the high and acute dosage of letrozole influences the morphology of the paca (*Cuniculus paca*) testis.

MATERIAL AND METHOD

Animals: Pubertal paca (*Cuniculus paca*) testis samples were taken from the group of lowland pacas at the Sector of Wild Animals of FCAV/UNESP, registered by the Brazilian

Institute of Environment and Renewable Natural Resources (IBAMA) as a breeding facility of specimens of the Brazilian fauna for scientific purposes (Registration Record 482508). The experiment was approved by the Animal Use Ethics Committee of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA/FMVZ) under number: 8735070714.

Orchiectomy and Letrozole: The unilateral orchiectomy was done firstly. For this purpose, meperidine (3 mg/kg) associated to midazolam (1 mg/kg), IM, were used followed by anaesthesia with ketamine (25mg/Kg) and xylazine (0.5 mg/kg), IM. After the complete animal anaesthesia recovery, 1.0 mg/kg Letrozole (single dose) was orally administered. After seven days, the orchiectomy was carried out for the remaining testis.

Histology, transmission electron microscopy and immunohistochemistry (IHC). The protocols for the histology and the transmission electron microscopy were in accordance with Simões *et al.* (2016). The PCNA IHC was based on usual protocols.

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RESULTS

On light microscopy, the germinal epithelium of the control testis was intact with sustentacular cells, spermatocytes and rounded spermatids (Fig. 1A). The letrozol- treated testis showed apoptotic cells with intraepithelial vacuolization and pyknotic nuclei (Fig. 1B).

The PCNA immunohistochemistry showed germ cell proliferation in letrozol-treated testis as well as in control testis (Figs. 1C, D). This cellular proliferation was also evident at the ultra-structural level (Fig. 1E). In the control, spermatocyte in diplotene and zygotene, spermatid in differentiation was observed (Fig. 1F).

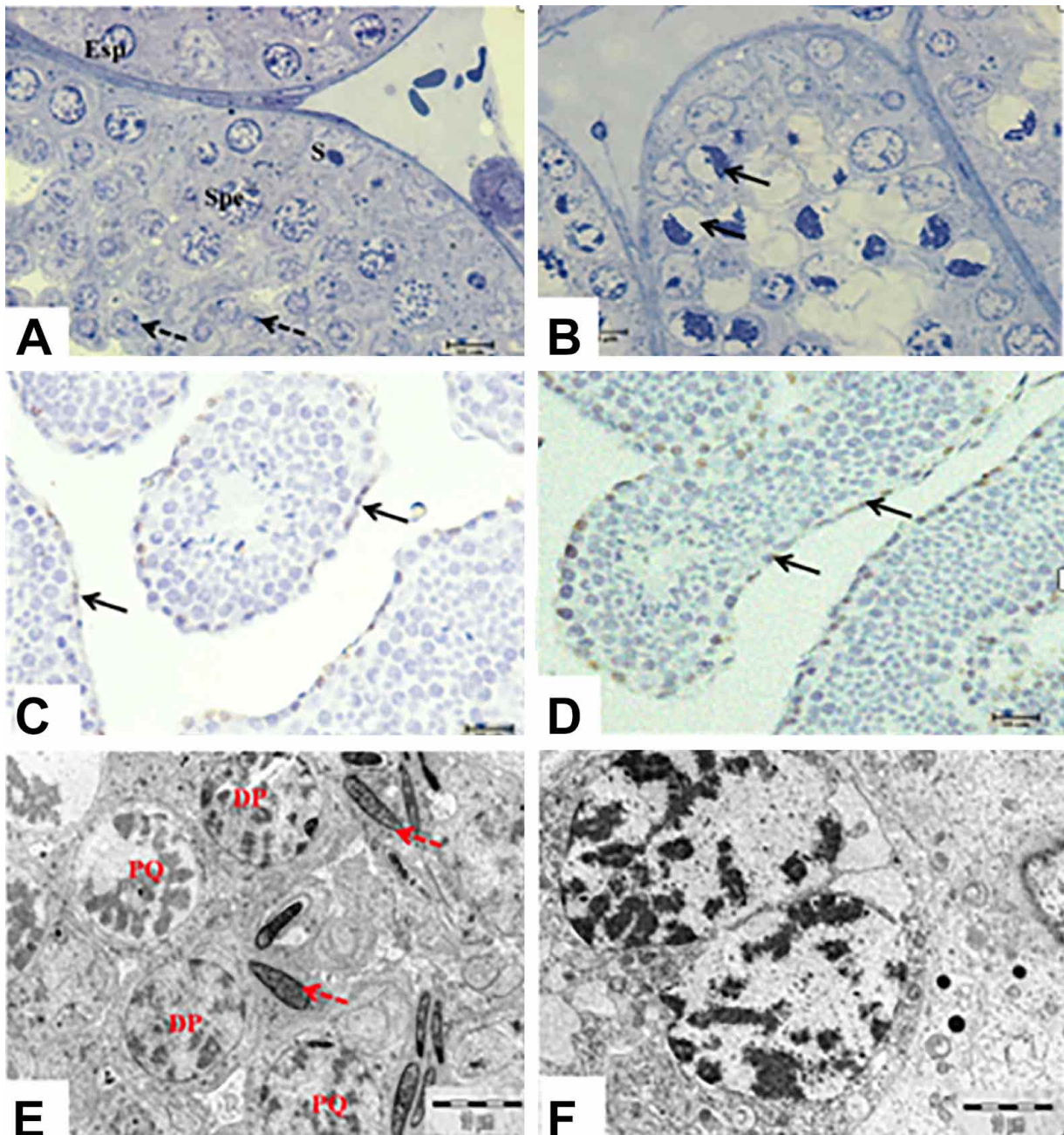


Fig. 1. Semithin sections of the pubertal testis. Control paca (A). Germinal epithelium with a presence of Sertoli (S) cells, spermatocytes (SPE) and spermatids (dashed arrows). Testis treated with Letrozole (B) germ cells with nuclei in picnose, with apoptotic characteristics (arrows). Stain: Blue Toluidine. Immunohistochemical for PCNA (C, D). Germ cells (arrows) is observed. Transmission electron microscopy (E, D). Control testis (E) evidences the differentiation of the spermatocytes in the diplotid (Dp) and pachytene (Pq) and spermatids (E); F observes a cell division of germ cells.

DISCUSSION

The high and acute dose of Letrozole in paca resulted in germinal epithelium degeneration suggestive of toxic effects, probably with impairment in spermatozoa and hormone production. The effects of toxic agents, such as chemotherapy and pesticides, have recently been investigated in reproduction (Fallahzadeh *et al.*, 2017; Saito *et al.*, 2017).

The apoptosis and loss of spermatogenic cells was reported in rats after the treatment with Di-(2 ethylhexyl)phthalate (dehp), found in the manufacture of plastics (Park *et al.*, 2002). In our case, apoptotic cells were also observed as well as the cellular proliferation, showed by PCNA marker.

The aromatase inhibition, by using 0.1 mg/kg weekly for 16 weeks letrozole treatment, has been reported to increase the number of sustentacular cells and the testicular size in pubertal boars, contributing to sperm production (Berger *et al.*). As shown by these authors, the small dose for long periods has positive effect for improving the porcine reproduction.

Thus, the dose and the frequency of letrozole treatment should be adjusted for each species. At this point of our study, for large rodents, the 1 mg/kg dose, after seven days, has demonstrated to be toxic for testis. We do not know whether or not the morphology and function of testicular epithelium could recover after a period longer than seven days or, if the degenerative process is permanent.

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RESUMEN: El objetivo de este estudio fue analizar la influencia de una dosis alta de Letrozol en la morfología de los testículos de la paca (*Cuniculus paca*), un inhibidor de la aromatasa que reduce el estrógeno endógeno, la hormona esencial para la espermatogénesis. Se observaron cambios morfológicos en el epitelio seminífero con células germinales con características apoptóticas y la presencia de vacuolas y núcleos en picnosis.

PALABRAS CLAVE: Gónada; Tóxico; Espermatogénesis.

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