

## Melatonin Can Affect the CYP19 Immunoexpression of Female Rat Ovary

Maganhin CC<sup>1</sup>, Simões RS<sup>2</sup>, Fuchs LFP<sup>2</sup>, Sasso GRS<sup>3</sup>, Carbonel AAF<sup>3</sup>, Reis LA<sup>5</sup>, Calió ML<sup>4</sup>, Simões MJ<sup>3</sup>, Baracat EC<sup>2</sup>, Soares Jr JM<sup>2</sup>.

<sup>1</sup> Gynecology Department, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil

<sup>2</sup> Department of Obstetrics and Gynecology, University of São Paulo, USP-Brazil

<sup>3</sup> Department of Morphology and Genetic, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil

<sup>4</sup> Department of Molecular Biology, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil

<sup>5</sup> Department of Nephrology, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil

**Objective:** to determine the immunoexpression of cytochrome P-450 (CYP19), an enzyme associated with the conversion of androstenedione to estradiol in the ovaries of pinealectomized female rats after melatonin treatment.

**Methods:** Thirty female rats (*Rattus norvegicus albinus*), adult, virgin from Universidade de São Paulo – Escola Paulista de Medicina (UNIFESP/EPM) biotery were separated in three groups of 10 animals each: GI – Sham (falsely pinealectomized) received vehicle; GII – pinealectomized who received vehicle; GIII – pinealectomized with melatonin reposition (10µg/night, each animal) for 60 consecutive days (Fig. 1 and 2). After this period, animals were anesthetized and ovaries were collected, fixed in 10% buffered formaldehyde and processed for paraffin embedding. From the paraffin blocks, 5µm thick sections were collected to silanized slides and submitted to immunohistochemistry for CYP19 detection. Images were obtained using a light microscope (AxioLab Standard 2.0 - Carl Zeiss) attached to a high definition camera (AxioCam MRC - Carl Zeiss) and by the image analyzing image (AxioVision Rel. 4.8.2 - Carl Zeiss). Reaction expression was analyzed and quantified according with the color intensity with the aid of the Image J Pro Plus, having photographed 5 fields each slide, with the 40× objective. Obtained data was submitted to statistical analysis using ANOVA test complemented by the Tukey-Kramer test ( $p < 0,05$ ).

**Results:** Histological sections underwent immunostaining CPY19 showed that the expression of CPY19 was higher in pinealectomized (GII =  $84.43 \pm 5.90$  \*) compared to Sham groups (GI =  $64.71 \pm 4.06$ ) and pinealectomized treated with melatonin (GIII =  $58.35 \pm 8.90$ ) (\*  $p < 0.05$ )( Fig. 3)

**Conclusions:** Our results showed that melatonin affects ovarian steroidogenesis in female rats. [2]

### References:

- [1] Maganhin CC, Fuchs LF, Simões RS, Oliveira-Filho RM, de Jesus Simões M, Baracat EC, Soares JM Jr Eur J Obstet Gynecol Reprod Biol. 166 (2013), p. 178.
- [2] Financial support by FAPESP - 2006/60412-7; 2007/54398-4; 2011/51581-8

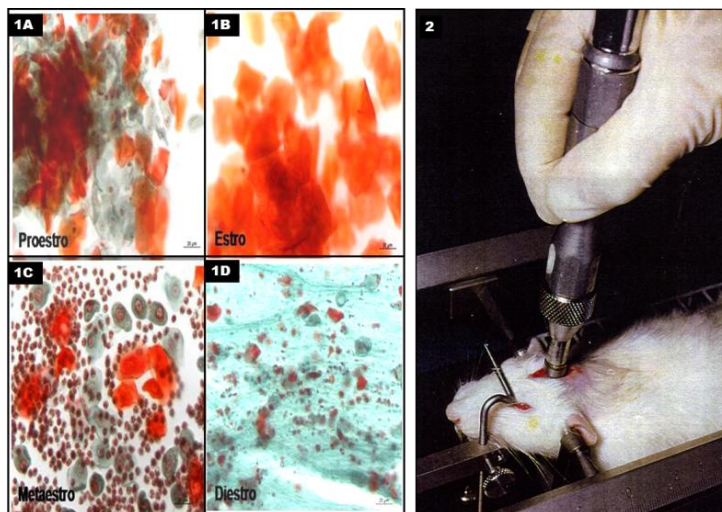


Figure 1 – Photomicrographs of vaginal smears in rats: proestrus (A), estrus (B), metestrus (C) and diestrus (D) - Colour: Shorr-HARRIS. Figure 2 - Surgical procedure of pinealectomy.

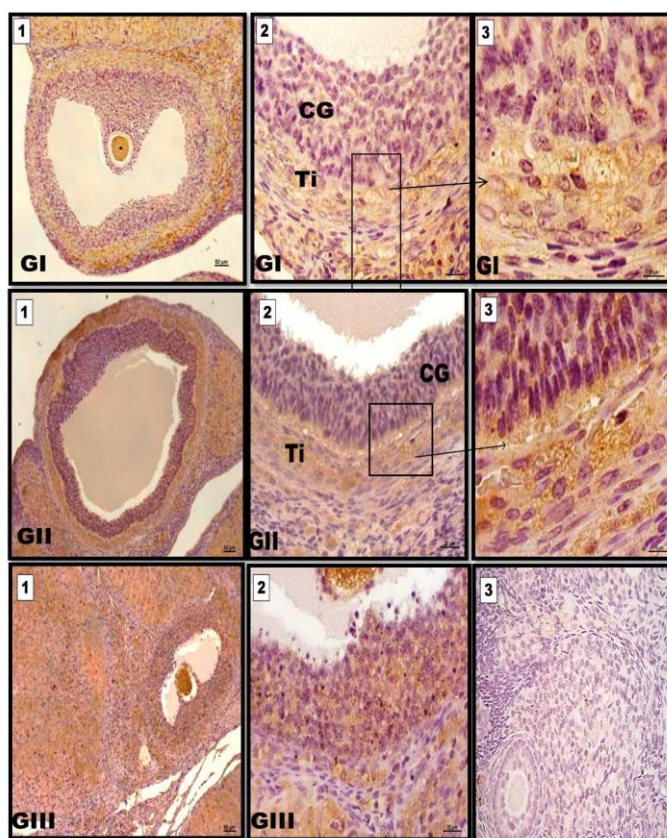


Figure 3 - Photomicrographs of ovarian sections after immunostaining the expression of Cyp19a1. GI - Sham group; (GII) pinealectomized or (GIII) pinealectomized with melatonin replacement. Note the positive reaction of granulosa cells it's more intense than of Cyp19a1 in the GII. The number three represent the respective negative control (non-specific goat antibodies) of immunohistochemical reaction of Cyp19a1. Bars = 20  $\mu$ m; 400X.