Effect of Melatonin on Ovarian Function by Over-expression and Down -Regulation of Genes Related to Steroidogenesis in Pinealectomized Rats

Maganhin CC, Simões RS, Girão JHRC, Fuchs LFP, Sasso GRS, Carbonel AAF, Reis LA, Simões MJ, Baracat EC, Soares Jr JM

1. Gynecology Department, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil
2. Department of Obstetrics and Gynecology, University of São Paulo, USP-Brazil
3. Department of Morphology and Genetic, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil
4. Department of Molecular Biology, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil
5. Department of Nephrology, Federal University of São Paulo, Paulista School of Medicine UNIFESP, Sao Paulo, Brazil

Objective: To analyze the expression of genes related to steroidogenesis in ovary of pinealectomized rats. Methods: 32 adult female virgin rats, with regular estrous cycle, were pinealectomized (PNE) and equally divided into two groups, as follows: GI – control group (PNE that received vehicle solution) and GII (PNE treated with melatonin, 10µg/night, per animal), during 60 consecutive days. After treatment, the animals were euthanized by overdose of ketamine and xilazine, some ovaries were collected, kept in liquid nitrogen and stored at -80°C for posterior expression analyses by cDNA microarray (Kit GeneChip® Rat Genome 230 2.0 Array, Affymetrix) of genes related to ovarian functions. The microarray assay was carried out in triplicate for each group. Data were normalized and subjected to the GeneChip® Operating Software and later confirmed by the DNA-Chip Analyzer (dChip) software of secundar analyses. Gene expressions were considered significantly different when they were 1.5x over or low expressed, when compared to the control group. Some samples were appropriately kept for posterior RT-PCR analyses and others were processed for paraffin embedding.

Results: The GII showed one hundred one overexpressed genes and seventy-two low expressed genes compared to GI. The genes related to ovarian steroidogenesis were statistically significant overexpressed (Inibin beta-A, Folistatin and Abl-Interactor1) and low expressed (Prostaglandin D2 synthase, LIM Homeobox 9 and Glutathione S-transferase Mu 1), in the GII. Among the overexpressed genes, the Inibin beta-A showed the higher expression. Based on these data, we later confirmed the overexpression of Inibin beta-A by RT-PCR (GII > GI, p<0.01) and by immunohistochemistry, which showed higher immunoreactivity in GII (74.43±2.89) in the follicular and interstitial cells, as well as in the inner teca cells, compared to GI (54.32±4.32).

Conclusion: Our data show that melatonin may influence ovarian function by the overexpression and down-regulation of steroidogenesis related genes in ovary of PNE rats.

References:

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Microarray e RT-PCR

Fig. 1A – List of up-regulated and down-regulated genes in GII in relation to GI. B – Mean and standard deviation of $2^{-\Delta\Delta C_t}$ from the RT-PCR of the INHBA gene between GII and GI, using the same samples used in microarray experiments (INHBA- $p=0.007$). C – Mean and standard deviation of $2^{-\Delta\Delta C_t}$ from the RT-PCR of the FST gene in GII and GI, using the same microarray samples ($FST\,p=0.04$).

Immunohistochemical- Inhibin

Fig. 2 – Photomicrographs of sections of ovaries belonging to groups (GI and GII). Observe the expression of inhibin was higher in GII (pineallectomized treated with melatonin) (GII = 74.43 ± 2.89 *) in granulosa cells (GC) and theca interna (arrow) compared with GI (pineallectomized treated with vehicle) . (GI = 54.32 ± 4.32 *) (* $p <0.05$). 400x Increase Counter-Staining: Hematoxylin; negative control (CN).