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

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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:
Maganhin CC, Fuchs LF, Simões RS, Oliveira-Filho RM, de Jesus Simões M, Baracat EC, Soares JM Jr. Effects of melatonin on ovarian follicles. Eur J Obstet Gynecol Reprod Biol. 2013;166(2):178-84.

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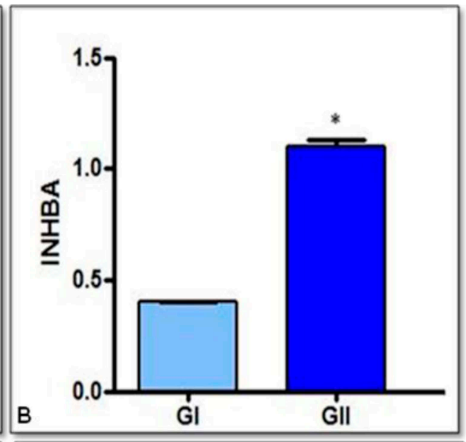
Microarray e RT-PCR

Up-regulated genes	Symbols	R
Inhibin Beta-A	INHBA	↑ 2,400
Follistatin	FST	↑ 2,030
Abl interactor 1	ABI1	↑ 2,010
Ectoderm-neural cortex protein 1	ENC1	↑ 1,990
T-box transcription factor18	TBx18	↑ 1,890
Glutamine-rich protein 1	QRICH1	↑ 1,860
Gremlin-2 homolog, cysteine knot superfamily, (Rattus norvegicus)	GREM2	↑ 1,760
Hepatitis A virus cellular receptor 1 homolog	Havcr1	↑ 1,730
Small nuclear ribonucleoprotein D1 polypeptide	Snrpd1	↑ 1,20
Centromere-associated protein E	Cenpe	↑ 1.710

A R = Ratio between GII and GI



Down-regulated genes	Symbols	R
Prostaglandin D2 synthase (brain)	Ptgds	↓ 3.062
LIM homeobox protein Lhx9	Lhx9	↓ 2.857
Glutathione S-transferase Mu 3 (Mus musculus)	Gstm3	↓ 2.132
Rab40b, member RAS oncogene family	Rab40b	↓ 1.863
Solute carrier family 34 (Sodium phosphate), member 2	slc34a2	↓ 1.815
Major histocompatibility complex, class II, Alpha DM	HLA-DMA	↓ 1.757
Aldehyde dehydrogenase family 1, member A3	Aldh1a3	↓ 1.744
Major histocompatibility complex, class II, Beta DM	HLA-DMB	↓ 1.739
Chemokine (C-X-C motif) ligand 10	Cxcl10	↓ 1.735
Basic helix-loop-helix family, member e41	Bhlhe41	↓ 1.728

R =Ratio between GII and GI

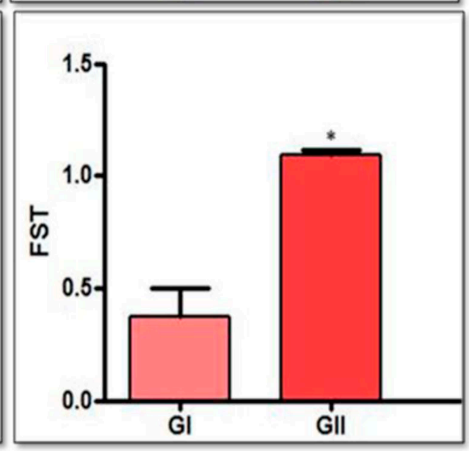


Fig. 1A – List of up-regulated and down-regulated genes in GII in relation to GI. B – Mean and standard deviation of 2-ΔΔCt from the RT- PCR of the INHBA gene between GII and GI, using the same samples used in microarray experiments ilNHBA- p=0.007). C – Mean and standard deviation of $2^{-\Delta\Delta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

Immunohistochemical- Inhibin

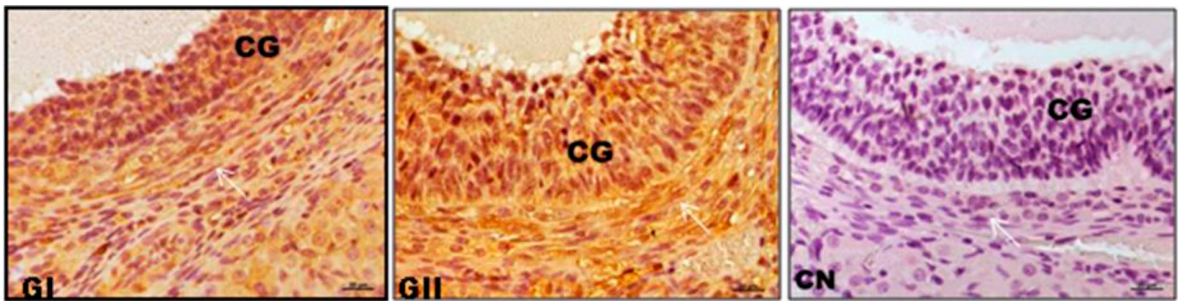


Fig. 2 – Photomicrographs of sections of ovaries belonging to groups (GI and GII). Observe the expression of inhibin was higher in GII (pinealectomized treated with melatonin) (GII = 74.43 ± 2.89 *) in granulosa cells (GC) and theca interna (arrow) compared with GI (pinealectomized treated with vehicle)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

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