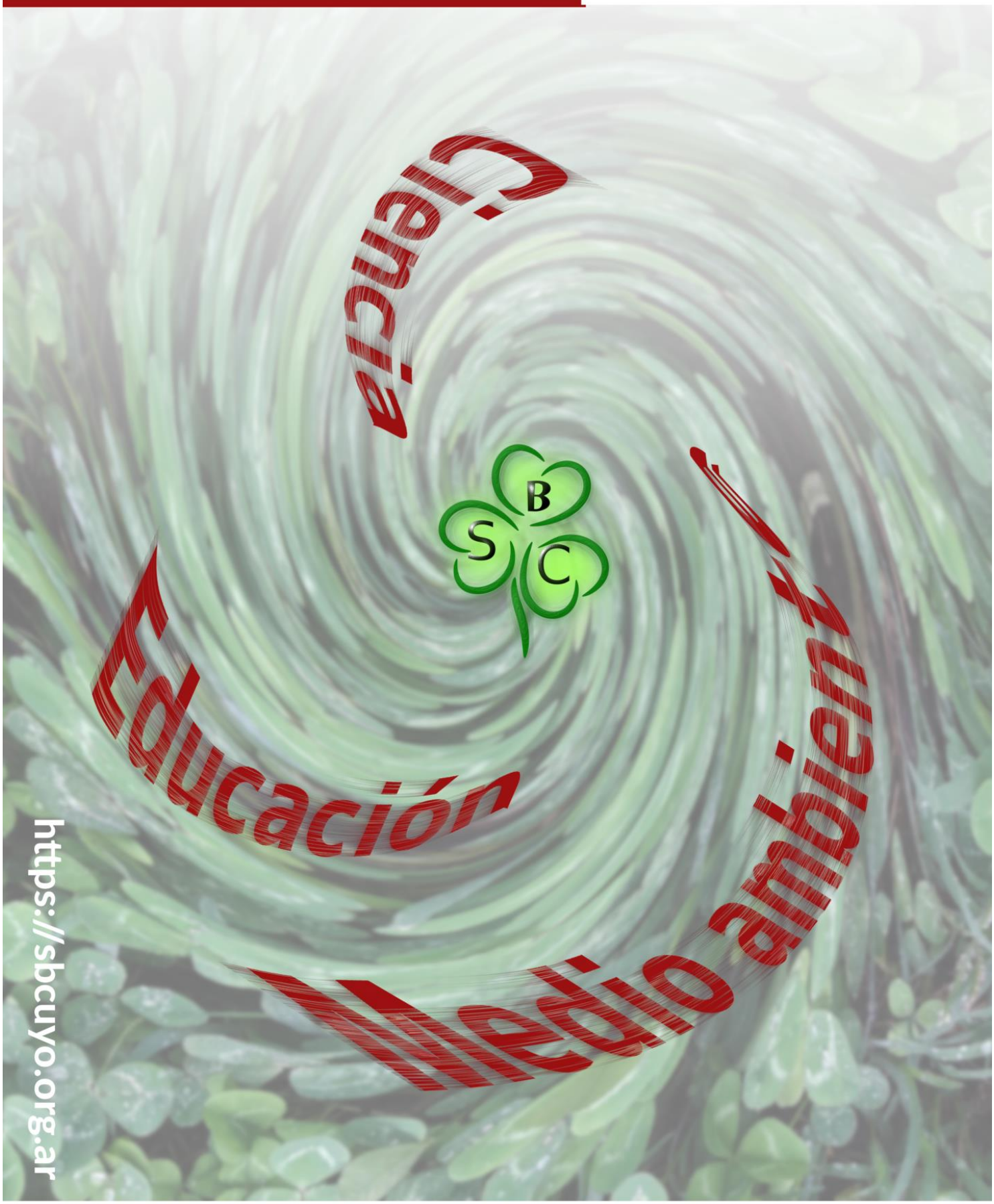
Sociedad de Biología de Cuyo

XXXVII Reunión Científica Anual

5 y 6 dic 2019 - San Luis



Libro de Resúmenes

XXXVII Reunión Científica Anual

Sociedad de Biología de Cuyo

5 y 6 de Diciembre de 2019

Centro Cultural José La Vía

Avenida Lafinur esquina Avenida Illia
San Luis
Argentina

Paoletti Geuna G, Delsouc MB, Serán C, Conforti RA, Vallcaneras SS, Casais M
Laboratorio de Biología de la Reproducción (LABIR), Universidad Nacional de San Luis, IMIBIO-SL CONICET. Ejército de los Andes 950, CP D5700HHW, San Luis, Argentina. ssvallca@unsl.edu.ar

Endometriosis (EDT) is a chronic gynecological disease that affects 10-15% of all women of reproductive age. The most common clinical signs of this pathology are menstrual irregularities, chronic pelvic pain, dysmenorrhea, dyspareunia, dysuria, fatigue, and depression. Progestin use can effectively treat the symptoms of endometriosis, although the mechanisms of action are not fully known. Therefore, the aim of this work was to elucidate whether Progesterone modifies the expression of sympathetic and sensory innervation markers, as well as neurotrophins, in endometriotic lesions induced in mice. EDT was induced experimentally by autotransplanting three pieces of the right uterine horn to the intestinal mesentery in C57BL/6 mice (Protocol CICUA-UNSL #B-262/17). From day 1 after the operation, the animals of the control group (n= 7) received sterile corn oil, while the animals of the experimental group (n= 7) received 2.5 mg Progesterone/kg bw/day (P0130 Sigma-Aldrich) subcutaneously for 1 month. The mice were then sacrificed by cervical dislocation and the lesions were removed and stored at -80°C for subsequent analysis of gene expression, by reverse transcription-polymerase chain reaction (RT-PCR), of tyrosine hydroxylase (TH), vesicular monoamine transporter 2 (VMAT2), calcitonin gene-related peptide (CGRP), Substance P (SP), brain-derived neurotrophic factor (BDNF) and neural growth factor (NGF). The protein expressions of both neurotrophins BDNF and NGF were analyzed by an indirect enzyme-linked immunosorbent assay (ELISA). Data were evaluated using T-test ($p<0.05$). In reference to the results obtained, the treatment with Progesterone did not alter the gene expression of the sympathetic innervation markers (TH and VMAT2). However, the gene expression of the sensory innervation markers (CGRP and SP) decreased in lesions, in comparison with the control group ($p<0.05$). In addition, the hormonal treatment showed an effect on BDNF, decreasing its gene expression ($p<0.01$) and its protein expression ($p<0.05$) in ectopic tissue, without altering the expression of NGF compared to the control. In conclusion, our findings suggest that Progesterone is a modulator of the expression of sensitive markers and BDNF in EDT. These results postulate a possible mechanism by which Progesterone would produce pain relief in patients.

30. INTRACELLULAR IONS AND WATER FLOWS ARE REQUIRED FOR ACROSOMAL SWELLING AND EXOCYTOSIS IN HUMAN SPERM

Poblete S[1], Mata-Martínez E[1], Arias R[1], De Blas GA[1,2]
[1]LaTIT – IHEM, UNCuyo - CONICET. [2]Área Farmacología, FCM - UNCuyo. Mendoza, Argentina. lspoblet92@gmail.com

The acrosome reaction (AR) is the exocytosis of the acrosome, asecretory vesicle located in the apical area of the sperm head. During exocytosis, it has been observed that, in order to achieve secretion, the acrosome must undergo morphological changes, including increased volume (acrosomal swelling). In other cell types, the increase in volume of secretory vesicles is crucial to carry out exocytosis, with ions and water flows being required. We assume that molecular machinery similar to the one responsible for swelling of other secretory vesicles is involved in AR. Consequently, we proposed that chloride, potassium and water channels are present in human sperm acrosome and are required for its swelling and AR. To test this we used capacitated and permeabilized human sperm as a strategy to study if intracellular ion channels were involved. First, we incubated the sperm with inhibitors of chloride, potassium or water channels and, then, the AR was induced with 10µM of free calcium. The results obtained support our hypothesis and also suggest the participation of intracellular aquaporins (7 and 8), the chloride transporters (ClC-2 and ClC-3), and the auxillary subunit of voltage-dependent channels Kvβ1 during AR. Furthermore, we identified the presence of all these proteins in the human sperm by Western Blot assays, as well as the subcellular localization in the acrosomal region of AQP-7 and Kvβ1 by immunofluorescence assays. In summary, our results indicate that intracellular water, chloride and potassium flows are required to complete acrosomal swelling and thus perform exocytosis in human sperm.

31. THYROXINE EXCESS INCREASES FETAL GROWTH, PLACENTAL AND MILK IMMUNE CELL INFILTRATION IN LATE PREGNANCY AND EARLY LACTATION

Sánchez MB[1], Moreno Sosa MT[1], Neira FJ[1], Pietrobon EO[1], Soaje M[1], Valdez SR[1], Jahn GA,[1], Mackern-Oberti, JP[1].
1.IMBECU, UNCuyo, CONICET. sanchezbelen564@gmail.com.

Thyroid dysfunctions cause reproductive disorders that lead to preterm birth and profound endocrine alterations during lactation with deficient milk production and quality. Currently, whether thyroid hormones (THs) modulate immune cells in maternal milk and placenta is unknown. Therefore, the aim of our work was to assess the influence of hyperthyroidism (hyper) on placental immune cells as well as its impact in pregnancy and lactation. To this end, 10-12 weeks old *Wistar* rats were injected with a daily dose of T_4 (0.1 or 0.25 mg/kg s.c) to induce hyper, or vehicle in control animals. Rats were mated 8 days after starting T_4 treatment and euthanized on day 19 (G19), 20 (G20) of gestation and 2 of lactation (L2). Placenta samples and milk were minced to reach single cell suspension and dyed. Then, resident placental and milk immune cells ($CD45^+$, $CD3^+$, $CD11b/c^+$) were analyzed by flow cytometry and mRNA content of hormone receptors by qPCR. Also, placental (G19-20), fetus (G19-20) and offspring weights were measured. The fetuses of hyper 0.25 mg/kg mothers weighed more in G19 and G20 ($p<0,001$). The placentas of the hyper 0.25 mg/kg mothers were heavier than controls only in G19 ($p<0,001$). Furthermore, we showed a decrease in the expression of progesterone, estrogen and β_2 thyroid receptors in hyper 0.1 mg/kg ($p<0.05$). On G19, the percentage of placental leukocytes was significantly higher in both hyper groups ($p<0.05$). On G20 we showed an increase in leukocyte infiltrate compared with G19 in the control ($p<0.001$) but not in the hyper group. We observed that hyper mothers had a higher mortality rate than the control group (14,14% and 3,18% respectively). Furthermore, the hyper group offspring presented lower weight on days 1 and 2 ($p<0,001$). Rat milk had an increase in the percentage of $CD45^+$ cells in the Hyper ($p<0,05$). In addition, the absolute quantity of $CD3^+$ cells/ µl increased in respect to the control group while

the number of CD11 b/c$^+$ diminished ($p<0,05$). These results suggest that T_4 administration accelerates fetal development, changes placental sensitivity to ovarian steroids and impairs early lactation. Additionally, placental and milk leukocytes would be modulated by T_4. To our knowledge, this is the first report that shows the modulation of resident immune cells by thyroid hormones.

32. HYPOTHYROIDISM INDUCES A DECREASE IN UTERINE NK CELLS DURING THE IMPLANTATION PROCESS

Troncoso M[1,2], Rinaldini E[1],Gamarra-Luques C[1,3], Hapon MB[1,2]
[1]Instituto de Medicina y Biología Experimental de Cuyo (IMBECU) – Universidad Nacional de Cuyo (UNCuyo), CONICET,
[2]Facultad de Ciencias Exactas y Naturales – UNCuyo, [3]Facultad de Ciencias Médicas – UNCuyo. troncoso.mariana@gmail.com

Uterine natural killer (uNK) cells are the major leukocyte present in the endometrium during implantation. The role of these cells is important in maintaining a healthy pregnancy. The main function of uNK cells is to regulate maternal uterine vasculature remodeling. It has been demonstrated that uNK cells produce proangiogenic factors such as VEGF, growth factors and provide local IFN-γ for initiation of spiral artery formation during pregnancy. It is known that angiogenesis is a critical process in the uterine endometrium for embryo implantation, maintenance of early pregnancy, and development of the placenta. Our lab is focused on understanding the effect of hypothyroidism in the embryo implantation process. Hypothyroidism is one of the most common endocrine abnormalities implicated in the recurrent loss of pregnancy. We had shown that hypothyroidism in the rat is associated with a lower number of pups per litter due to a lower number of implantation sites. We had also observed a decrease in the proliferation (PCNA) of the endothelial and decidua cells and reduced vascularization during the process of implantation of the embryo. In this work, we hypothesized that hypothyroidism induces a decrease in uNK cells during the embryo implantation process. Therefore, the aim of this work was to study the effect of hypothyroidism in the uNK cells during the embryo implantation process, in two early stages of pregnancy. Hypothyroidism was induced in female Wistar rats bred in our laboratory by daily administration of 6-propyl-2-thiouracil (PTU) 0.1 g/l in drinking water. On day five (G5) and seven (G7) of gestation, uterine vascularization and the presence of uNK cells were evaluated by indirect immunofluorescence (IFI). Our results show that hypothyroidism significantly decreases the number of uNK cells before the implantation process (G5). However, when implantation occurs (G7), there is no significant difference between the animals treated with PTU and the controls. In conclusion, hypothyroidism affects the number of uNK cells present in the uterine decidua, before implantation occurs (G5). This could be related to the decrease in vascularization, a process necessary for embryo implantation to occur, reported before by our group. However, further studies are necessary to corroborate these hypothesis.

BIOQUÍMICA, FISIOLOGIA Y NEUROQUÍMICA (33-46)

33. MEMORY AND LEARNING DECLINE ARE RELATED WITH TEMPORAL CHANGES OF COGNITIVE FACTORS IN AGED RATS

Altamirano FG[1], Ponce IT*[2], Coria Lucero CD[1], Castro Pascual IC[1], Ferramola M[2], Delgado SM[2], Anzulovich AC[1,2], Lacoste MG[1,2]*
**equal contribution*
[1]Laboratorio de Cronobiología, IMIBIO, Universidad Nacional de San Luis, CONICET; [2] Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis. San Luis, Argentina. fergabalt@gmail.com

Aging brain undergoes several changes leading to a decline in cognitive functions. *Bdnf* and its receptor *TrkB*, are expressed in different brain regions, including hippocampus, and regulate a wide range of functions such as synaptic plasticity, long-term potentiation and neurogenesis, which are fundamental for memory and learning. Interestingly, previous results from our laboratory showed a loss of the circadian variation in the expression of *Bdnf* and *TrkB* mRNA in the hippocampus of older rats.The objective of this work was to evaluate the effect of aging on cognitive processes and compare it with the temporal expression of *Bdnf* and *TrkB* in the rat hippocampus. Holtzman male rats were separated into two experimental groups: young adult (3- months old) and old (22- months old) rats. Cognitive performance was assessed by the Barnes maze (BM) test for spatial learning and memory and by the Novel Object Recognition (NOR) test for contextual learning. In the BM test, older rats showed a significant lower exploratory frequency of the target region ($p<0,05$), longer total exploratory activity ($p<0,01$), greater numbers of errors in reaching around the target hole ($p<0,05$), longer escape box latencies ($p<0,05$), higher distance traveled on the platform ($p<0,05$) and lower percentage (%) of exploration of the meta holes ($p<0,05$), compared to young adult rats. In the case of the NOR test, older rats showed a significant shorter time for novel objects exploration ($p<0,01$), compared with the young adult rats. Taken together, the exposed evidence suggests that the loss of Bdnf and TrkB rhythmicity in the hippocampus could play a central role in the deterioration of memory and learning in aging. Thus, the alteration in the cognitive factors would be reflected in the low performance of the aged animals in both behavioral tests. Understanding the basis of cognitive impairment during normal aging is essential to develop strategies to prevent aging-related diseases and improve the quality of life of our older adults.

34. FORCED SWIMMING REVERT THE METABOLIC ALTERATION PRODUCED BY CHRONIC PRENATAL STRESS

Becerra K[1], Sosa E[1], Scoppa GI[2], Echegaray NA[2], Huck G[3], Liaudat AC[1], Rodriguez N[1].