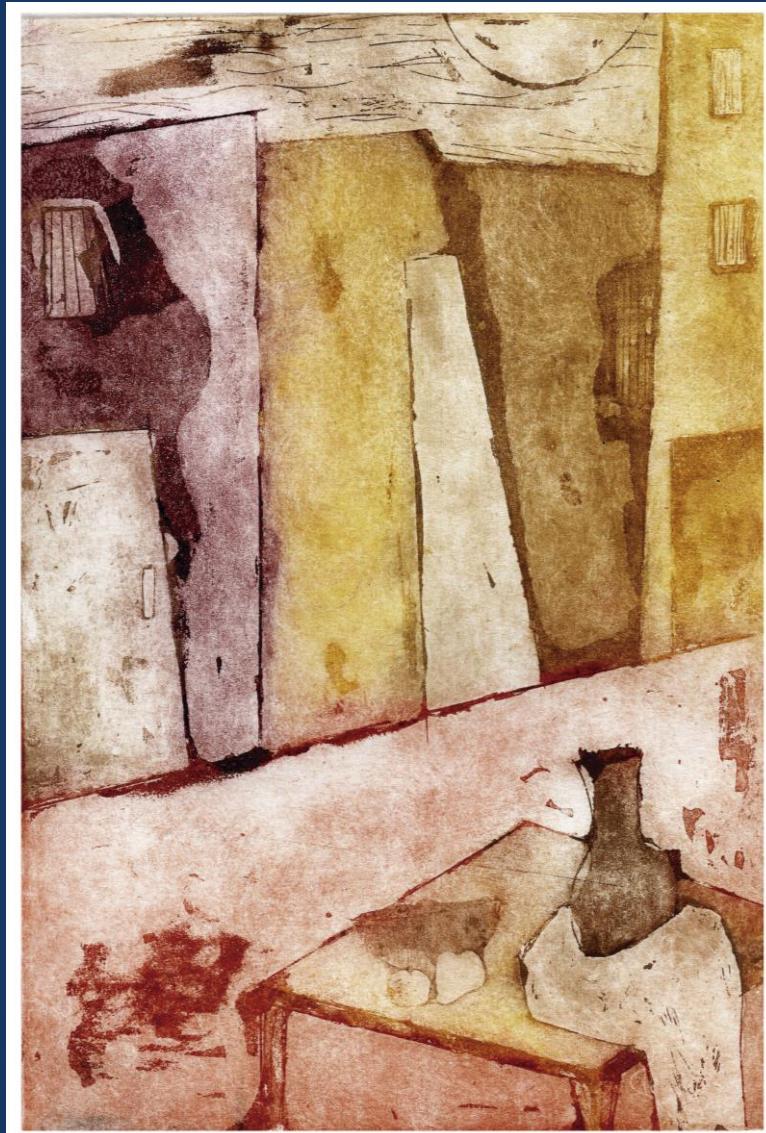


# medicina

BUENOS AIRES VOL. 79 Supl. IV - 2019

*80º Aniversario*



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BUENOS AIRES, VOL. 79 Supl. IV - 2019

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La Tapa (Ver pág. 4)

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MEDICINA (Buenos Aires) – Revista bimestral – ISSN 0025-7680 (Impresa) – ISSN 1669-9106 (En línea)

## REVISTA BIMESTRAL

Registro de la Propiedad Intelectual N° 02683675  
Personería Jurídica N° C-7497

Publicación de la Fundación Revista Medicina (Buenos Aires)

Propietario de la publicación: Fundación Revista Medicina

Queda hecho el depósito que establece la Ley 11723

Publicada con el apoyo del Ministerio de Ciencia, Tecnología e Innovación Productiva.

MEDICINA no tiene propósitos comerciales. El objeto de su creación ha sido propender al adelanto de la medicina argentina.

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Aparece en MEDLINE (PubMed), ISI-THOMSON REUTERS (Journal Citation Report, Current Contents, Biological Abstracts, Biosis, Life Sciences), CABI (Global Health), ELSEVIER (Scopus, Embase, Excerpta Medica), SciELO, LATINDEX, BVS (Biblioteca Virtual en Salud), DOAJ, Google Scholar y Google Books.

Incluida en el Núcleo Básico de Revistas Científicas Argentinas del CONICET.

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1427 Buenos Aires, Argentina  
Tel. 5287-3827 Int. 73919 y 4523-6619  
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Vol. 79, Supl. IV, Noviembre 2019

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p.R84H) were found in a child with septo optic dysplasia, a child with CPHD and a third patient with GH and TSH deficiency, respectively. Transient transfection of HEK293T cells with human wild-type or mutant hLHX3/ hLHX4 showed an impairment in transcriptional reporter activity by the mutant variants, except for variant LHX4 p.R84H. Collectively, using the first screening panel for known genes and candidate genes for CH, we identified a significant number of variants in a large cohort of patients associated with the complex phenotype. Our studies will facilitate early diagnosis and prognosis, assessing the risk of future affected individuals. Furthermore, understanding the mechanisms behind new genes involved in CH would lead us to develop new tailor-made therapies that could benefit the patients.

This work was supported by the Agencia Nacional de Promoción Científica y Técnica, Buenos Aires, Argentina (grant PICT 2016-2913 y PICT 2017-0002).

## **0591 - EXPRESSION OF RECOMBINANT FATTY ACID DESATURASE IN A BOVINE MAMMARY GLAND CELL LINE INDUCES CHANGES IN LIPID PROFILES**

**Alejandro Ernesto FILI** (1) | Bianca Ana OPIZZO BALZA(1) | Romina Marisa HEREDIA(1) | Diego Oscar FORCATO(1) | Gloria Inés LUCCHESI(1) | Wilfried KUES(2) | Pablo BOSCH(1)

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**Abstract/Resumen:** Vertebrates are unable to synthesize a subset of polyunsaturated fatty acids (PUFA) known as omega 3 and omega 6. The nematode *C. elegans* is able to synthesize them thanks to a family of lipid desaturases (delta desaturases, i.e., FAT2). Our hypothesis proposes that heterologous expression of a FAT2 in a bovine mammary gland cell line (MAC-T) will induce synthesis of PUFA. The aim of the present work was to transpose the *C. elegans* fat2 gene into the genome MAC-T and to study the resulting PUFA profile. Cotransfections of MAC-T with the Sleeping Beauty (SB) transposon system were performed. Two transposons, one carrying a cassette for the expression of a GFP, and a second one for expression of the FAT2 enzyme and neomycin resistance were used. For transfection essays, 2:0.5:0.5 molar ratios of FAT2, GFP transposons and SB-helper plasmid were used. After cotransfection, MAC-T cells were subjected to antibiotic selection (G418). After 15 days, fluorescent and resistant colonies were isolated and expanded. PCR confirmed presence of the FAT2 sequence in five clonal cell lines. Two transgenic cell lines and one unmodified cell clone were grown to confluence in order to analyze the profile of the cellular phospholipid. Gas chromatography analysis of phospholipids confirmed the presence of linoleic acid (C18:2) in both transgenic cell lines, and the absence of linoleic acid in the unmodified cell line. The results indicate that recombinant FAT2 is functional, since it catalyzed the synthesis of linoleic acid, an omega-3 FA. Experiments are ongoing in order to confirm FAT2-mediated production of longer omega-3 and omega-6 lipids (C20 and C22), which are derived from C18:2. In conclusion, we successfully use the SB transposon system to generate stable transgenic bovine cell lines that express functional recombinant FAT2 enzyme. These results pave the way for the production of genetically engineered animals with improved PUFA profiles in tissues or milk for human consumption.

## **0622 - SCANNING ELECTRON MICROSCOPY ANALYSIS OF HAIR TREATED WITH PYLORIC CECAE EXTRACT FROM PACU (PIARACTUS MESOPOTAMICUS)**

**Juan Marcelo LOPEZ** | Gabriela GOMEZ | Laura Cristina LEIVA | Andrea Carolina VAN DE VELDE

**LABORATORIO DE INVESTIGACIÓN EN PROTEÍNAS/NEA IQUIBA-UNNE**

**Abstract/Resumen:** Hair is composed mainly of keratin (90 %) is a fibrous and insoluble protein with high content of amino acid cysteine, responsible of disulfide bonds presence, which gives it high resistance to degradation. Hair structure consists of a medulla, cortex, and cuticle. This last cover the cortex, made of long filaments packed together (microfibrils). Currently, the industrial processing of animal sources generates significant volumes of highly polluting waste. The meat packaging and tannery industry, hairdressing salon, generate waste of keratinous nature. On the other hand, aquaculture discards considerable amounts of viscera, but these are considered an alternative source of enzymes with potentials industrial applications. Treatment of these protein-rich wastes is an attractive option that results in products with high added value. In the present study we evaluated the degradative capacity on hairs of a pyloric cecae extract from pacu (*Piaractus mesopotamicus*) under reducing and non-reducing conditions. The extract was prepared by mechanical digestion of pacu viscera in buffer pH 7.8, 1:5 g tissue/ml and proteolytic activity were assayed over Na-Benzoyl-dl-arginine-p-nitroanilide as substrate. Hairs were pre-treated with buffer 7.8, 1 % 2-mercaptoethanol (2-ME), for 20 min at 100°C, then 5.0 U/ml pacu extract was added (1:5) and incubated for 1h, 3h, 24h and 7 days at 37°C. Hairs were observed at scanning electron microscopy (SEM). Other samples of the same hair were exposed in parallel under different treatments, such as the absence of reducing agent, heat or fish extract. SEM analysis showed that viscera extract, only in presence of 2-ME and heat, was able of degrading the hair cuticle, exposing the cortex microfibrils just at the first day. Results demonstrate that pyloric cecae pacu extract is able to degrade hair pre-treated with heat and reducing agent, so this treatment could be used in the recovery of hair keratins.

## **0663 - PROTEOMIC STUDY OF BREAST CANCER CELL LINE AFTER HEMEOXYGENASE-1 MODULATION BY HEMIN TREATMENT**

**Karen SCHWEITZER** (1) | Lucia FERNÁNDEZ CHÁVEZ(1) | Exequiel Gonzalo ALONSO(1) | Marilina MASCARÓ(1) | Gerardo Martín ORESTI(2) | Abril BORISOV(1) | María Marta FACCHINETTI(1) | Alejandro Carlos CURINO(1) | Reinhard FÄSSLER(3) | Georgina Pamela COLÓ(1) | Norberto Ariel GANDINI(1)

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**Abstract/Resumen:** Hemoxygenase-1 (HO-1) is a microsomal enzyme that catalyzes the degradation of the heme group and it can be translocated to multiple subcellular compartments. Our laboratory, among others, has shown that HO-1 regulates several processes related to cancer progression such as: proliferation, invasion migration, metastasis and the epithelial-mesenchymal transition. The aim of this work is to investigate the role of HO-1 in the proteome modulation in a breast cancer cell line. Protein extracts of LM3 cell line treated with hemin, a pharmacological HO-1 inducer (80 µM, 24 h), were obtained and studied by Western blot and Mass Spectrometry (MS). MS-data analysis showed 1,033 from 7,292 proteins were modulated after hemin treatment (ANOVA,  $p < 0.05$ ). We observed that 595 proteins were increased, including HO-1 and 353 proteins were decreased in the group treated with hemin respect to their controls. Hemin treatment in LM3 cells induce lipid metabolism, heme and iron related protein expression. By thin layer chromatography, we observed an increase fraction of phosphatidyl serine, phosphatidylinositol, phosphatidylethanolamine and triglycerides after hemin treatment in LM3 cells, confirming the role of HO-1 in lipids metabolism. In addition, hemin treatment decreases the ribosomal RNA biogenesis and cytoskeleton and microtubules related proteins. These results show the multiple physiological effects of HO-1 in a breast cancer cell line.