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25.

FERTILIZATION INDUCES A TRANSIENT EXPOSURE OF PHOSPHATIDYLSERINE IN MOUSE EGGS

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Phosphatidylserine (PS) is a phospholipid localized in the inner leaflet of the plasma membrane, and its exposure is a marker for apoptosis. However, recent evidence suggests that this exposure could also be associated with non-apoptotic events as well as to viral fusion. Considering the similarities between this fusion event and sperm-egg fusion, our aim was to evaluate the involvement of PS in gamete interaction. First, we observed that the addition of fluoresceinated annexin 5 (ANX5, a protein that specifically binds PS) during gamete co-incubation, did not affect the percentage of penetrated eggs at any of the concentrations tested. Surprisingly, fertilized eggs presented a positive labelling for ANX5 on their surface that was observed in intact and zona pellucida-free eggs. Non-fertilized oocytes, eggs not exposed to sperm, and eggs activated with Ca^{2+} ionophore did not present labelling, suggesting that the exposure of PS would be mediated by sperm. The follow-up of the labelling showed that it disappeared from the sperm-entry site in the decondensed head stage, it is faint in the 2-pronuclei stage and, finally, non-detectable in 2-cell embryos. Altogether, results show for the first time the existence of a transient exposure of PS in fertilized eggs not associated with apoptosis and that would be induced by sperm.

26.

PARTICIPATION OF NEURAL CADHERIN IN HUMAN GAMETE INTERACTION

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Introduction: Previous studies from our laboratory have described the localization of the adhesion molecule neural cadherin (N-cad) in the acrosomal region of intact human spermatozoa and in the equatorial segment of reacted cells. *Aims:* To evaluate the participation of N-cad in sperm interaction with the *zona pellucida* (ZP) and the oolemma. *Methods:* The hemizona and ZP-free hamster oocyte sperm penetration assays were carried out, in which gametes were preincubated with anti N-cad antibodies directed towards different extracellular domains of the protein (clone GC-4, Sigma, domain 1; H-63, Santa Cruz Biotech., domain 3-4). *Results and Discussion:* Sperm preincubation with either H-63 (10 and 100 µg/ml) or GC-4 (20 and 200 µg/ml) did not affect their ability to bind to the homologous ZP in the hemizona assay (data not shown). Contrasting, preincubation of both gametes with anti N-cad antibodies led to a significant decrease ($P<0.01$) in the number of sperm penetrations per oocyte in comparison with controls (% inhibition for H-63 20 µg/ml: 57±12%, mean±SEM, n=3 assays; GC-4 200 µg/ml: 51±11%, n=8 assays). A similar effect was found when only oocytes were preincubated with the antibodies. N-cad was immunodetected in human and hamster oocytes. The results suggest that N-cad has a role in gamete adhesion/fusion but would not participate in sperm-ZP interaction.

27.

GnRH EXPRESSION AT HYPOTHALAMUS OF *LAGOSTOMUS MAXIMUS* (PLAINS VIZCACHA)

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Gonadotropin-releasing hormone (GnRH) is synthesized in a pulsatil manner from puberty to menopause, except during pregnancy, to regulate folliculogenesis. Plains Vizcachas show natural polyovulation with corpus luteum abundance and ovulation during pregnancy. To understand the modulation of hypothalamus-pituitary-gonadal axis (HPG), the aim of this work was to characterize hypothalamic GnRH expression in the vizcacha. Vizcachas of both sexes (n=5 each), captured at Estación de Cría de Animales Silvestres (ECAS), were anesthetized with ketamin-xilacin and sacrificed with Eutanyl®. Coronal brain slices were dyed with Hematoxylin and the hypothalamic regions Preoptic Area (POA), Ventromedial Nucleus (VMN), Medial Eminence (ME) and Arcuato Nucleus (AN) were localized by comparison with histological brain atlas of rat and guinea pig. Specific immunolocalization of GnRH was observed in the cytoplasm of neurons at POA and AN (at both, somas y ramifications), and at varicosities of POA, VMN and ME. Similar GnRH distribution was detected in animals of both sexes. GnRH expression level was studied in male and female plains vizcachas and no significant differences were determined between sexes. Tissue GnRH localization and its description at hypothalamic regions involved on HPG axis in this animal would allow the comprehension of HPG axis modulation with extrapolation into human fertility pathologies.

28.

LOCALIZATION OF PITUITARY AND EXTRAPITUITARY GONADOTROPINS BY *IN SITU* HIBRIDIZATION IN PEJERREY *Odontesthes bonariensis*

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In a previous study by RT-PCR it was demonstrated the presence of α and β gonadotropins (GtHs) subunits in pejerrey brain and gonads. In this work, using *in situ* hybridization, it was possible to identify these transcripts in the brain, pituitary, and gonads of adult pejerrey of both sexes. As it was expected, the three GtHs subunits RNAm were detected in cells of pituitary *pars distalis proximalis* and *pars intermedia* and conspicuously in neurons of *nucleous lateralis lemniscus*. The three GtHs subunits were identified in testicular spermatogonia and spermatocytes, whereas in the ovary they were observed in oocytes at different developmental stages. In spite of, extrapituitary GtHs function are not known yet, these results suggest that they can play novel roles acting as brain neuromodulator and as regulators of the gonads.