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EXPRESSION OF L-TYPE VOLTAGE CALCIUM CHANNELS IN EQUINE GRANUOSA CELLS: A MOLECOLAR AND FUNCTIONAL STUDY

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The ovary is dependent on pituitary gonadotropins to ensure folliculogenesis which in turn relies on intracellular calcium signaling, induced by follicular stimulating hormone to promote oocyte activation and maturation [1]. The intracellular calcium concentration $[Ca^{2+}]_I$ rise is due in part to L-voltage-gated calcium channels (L-VOCCs). The role of L- VOCCs in the ovarian follicular dynamic has been assessed in the granulosa cells (GCs) of several animals [2] but not in the mare, whose follicular growth is primarily controlled by the changing daylength that drives the reproductive seasonality. This study aims to evaluate the presence, quantification, and localization of L-VOCCs in equine GCs recovered from follicles of different diameters and their involvement in modulating $[Ca^{2+}]_I$ during follicles development. GCs were recovered from follicles of ovaries obtained during the year from a local slaughterhouse. An average of 5±1 mares were analyzed each month. Based on the cyclic periodicity, the number of follicles varied. Follicles were categorized as small, medium, and large [3]. GCs from each type of size-follicle were pooled and processed by a) western blot, to assess the expression and quantify L-VOCCs employing a rabbit affinity-purified primary antibody against the α_1 subunit of L-VOCCs and a biotinylated universal secondary antibody (Vectastain Elite ABC Universal kit, Vector- Laboratories, Burlingame, CA, USA). The positive hybridization was revealed by DAB substrate kit (Vector-Laboratories, Burlingame, USA); b) immunocytochemistry, to localize L-VOCCs by the same primary antibody used in the western blot procedure and a FITC- conjugated secondary antibody. c) fluorescent measurements of L-VOCCs activity and cytosolic Ca^{2+} dynamics employing Fura-2-AM fluorescent dye and Bay K-8644 and Nifedipine, which are agonist and antagonist, respectively, of the L-VOCCs using a QuantiCell 900 integrated image system (VisiTech International, Sunderland, UK). Data were analyzed by parametric tests. Results showed that all categories of GCs expressed L-VOCCs, which, as expected, appeared as a doublet of bands at different molecular weights. Fluorescence immunocytochemistry evidenced positive signals at the surface of GCs. The quantification of the L-VOCC levels showed that small follicles increased their expression in February (January *vs* February, $P<0.05$), just before the beginning of the equine breeding season. Moreover, it has been found that the used agonist and antagonist of the L-VOCCs induced a significant ($P<0.001$) change in the $[Ca^{2+}]_i$ in follicles of all sizes. Large follicles resulted in a greater change in $[Ca^{2+}]_I$ after addition of Bay K-8644 or Nifedipine when compared to the basal condition. The results obtained indicate the existence of a regulatory system of the function of the L-VOCCs associated with the functional state of the follicle during its growth and maturation.

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[2] Bahena-Alvarez et al. Calcium signaling and expression of voltage-gated calcium channels in the mouse ovary throughout the estrus cycle. Biol. Reprod., 100: 1018-1034, 2019.
[3] Dell'Aquila et al. Meiotic competence of equine oocytes and pronucleus formation after intracytoplasmic sperm injection (ICSI) as related to granulosa cell apoptosis. Biol. Reprod., 68:2065-2072, 2003.