Expression of Estrogen-Related Receptor GPR30 in Mouse Metaphase II Oocytes and Its Effect on Fertilization-Induced Ca^{2+} Oscillations

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Estrogen receptors such as classical receptors alpha (ERα) and beta (ERβ), and estrogen-related receptor GPR30 play a key role in the hormonal regulation of female reproduction. They may be also targeted by numerous exogenous agents, such as endocrine disrupting compounds (e.g., plasticizers such as bisphenol A) or substances used in cancer treatment (e.g., tamoxifen), which may modify their activity and, in consequence, disrupt female fertility. To address this issue, we wish to investigate whether these receptors participate in the regulation of calcium homeostasis in oocytes, a process crucial for early embryo development. Fertilization-induced oscillations of the cytoplasmic concentration of free calcium ions ($[Ca^{2+}]_i$) serve as a mechanism responsible for activation of the embryo development. Moreover, the pattern of $[Ca^{2+}]_i$ oscillations influences implantation and postimplantation development of the embryo. In our study, we used mouse oocytes as a model. Our results indicate that mRNAs for all three receptors are present in mouse metaphase II (MII) oocytes with mRNA encoding ERβ being the most highly expressed. Subsequently, using immunofluorescence staining, we found that all three receptors were present in the oocyte cytoplasm, but ERβ and GPR30 were also localized in the cell membrane and thus may affect oocyte physiology in a more direct way, which do not involve their migration into the nucleus and regulation of gene expression. Finally, we focused on GPR30, a potential target for xenoestrogens such as bisphenol A, and assessed if its activity influenced the fertilization-induced $[Ca^{2+}]_I$ oscillations. We matured prophase I (GV) oocytes *in vitro* in the presence of either GPR30 agonist (G1) or antagonist (G15). Subsequently, we followed $[Ca^{2+}]_i$ in *in vitro* fertilized MII oocytes. Our preliminary data suggest that the number and total duration of $[Ca^{2+}]_i$ transients were decreased in MII oocytes if GPR30 was activated during *in vitro* maturation. Interestingly, inhibition of GPR30 also shortened the duration of $[Ca^{2+}]_i$ oscillations but did not affect the number of $[Ca^{2+}]_i$ transients. Taken together, our initial data indicate that estrogen-related receptor GPR30 might influence fertilization-induced $[Ca^{2+}]_i$ oscillations. Although we still need to determine whether ERβ can affect fertilization-induced Ca^{2+} oscillations, our results strongly suggest that deregulation of estrogen receptors may affect oocyte ability to respond to fertilization.

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