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Progesterone Receptor Membrane Component-1 in cell division: its role in bovine granulosa cells mitosis.

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The present studies were aimed to assess Progesterone Receptor Membrane Component-1 (PGRMC1) role in regulating bovine granulosa cells (bGC) mitosis. First, we performed immunofluorescence studies on *in vitro* cultured bGC collected from antral follicles, which showed that PGRMC1 localizes to the spindle apparatus in mitotic cells. Then, to evaluate PGRMC1 effect on cell proliferation we silenced its expression with RNA interference technique (RNAi). Quantitative RT-PCR and immunoblotting confirmed down-regulation of PGRMC1 expression, when compared to CTRL-RNAi treated bGC ($p < 0.05$). After 72h of culture, PGRMC1 silencing determined a lower growth rate ($p < 0.05$) and a higher percentage of cells arrested at G₂/M phase as assessed by flowcytometry ($p < 0.05$). Accordingly, live imaging studies revealed more aberrant mitosis and a delayed M-phase in PGRMC1-RNAi treated cells compared to CTRL-RNAi group ($p < 0.05$). These data confirmed that PGRMC1 is directly involved in bGC mitosis and ongoing preliminary studies are aimed to elucidate its putative mechanisms of action. Since PGRMC1 is a membrane protein, we hypothesize its possible involvement in vesicular trafficking and endocytosis, which is in turn an important process to assure proper cell division. To assess this hypothesis, we have preliminarily conducted immunofluorescence and *in situ* proximity ligation assay experiments that showed PGRMC1 co-localization and direct interaction with clathrin. This is important since clathrin is an essential protein for both endosomes formation, and cell division acting directly on the spindle apparatus. Thus our studies set the stage for analysis aimed to further characterize PGRMC1's mechanism of action in mitotic cells.

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