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Effect of cGMP synthesis stimulation on the lipid content of bovine oocytes and cumulus cells during in vitro maturation

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Resumo

Lipids are important energy source for cells, but excessive accumulation renders them more likely to undergo oxidative stress. Studies have indicated that cGMP pathway may be involved in the lipid metabolism of bovine COCs during IVM. The aim of this study was to compare the effect of different stimulators of cGMP synthesis (NPPB=natriuretic peptide type B and PTPF=protoporphyrin IX, activators of membrane and soluble guanylyl cyclase, respectively) on the lipid content in bovine oocytes (OO) and cumulus cells (CC) after 9 and 24h IVM. COCs (20-25/group/replicate) were matured in TCM199 (0.2 mM sodium pyruvate, 10 µg/ml gentamicin, 0.5 µg/ml FSH, 10% FCS) with NPPB (10-7 M, Schefer et al, Anim Reprod, v.15, p.442, 2018) or PTPF (10-5 M, Schwarz et al, Theriogenology, v.81, p.556-564, 2014) or without stimulators (control). In Experiment 1, lipids were assessed in denuded OO (9 and 24h IVM), stained with Nile Red (1 µg/ml for 15 min), imaged by epifluorescence microscopy and fluorescence intensity (FI) was measured by ImageJ. In Experiment 2, lipids were assessed in CC (9 and 24h IVM), by staining COCs with BODIPY 493/503 (20 µg/ml for 1 h), which were imaged by confocal microscopy. Four random 1µm2 CC areas were selected from each image and lipid area determined in images using ImageJ nucleus counter (lipid area/ total cells area). Data (five replicates/group) were tested for normality of results and homogeneity of variance, then subjected to statistical analysis by ANOVA followed by Tukey test (GraphPad Prism software) at 5% significance level. In Experiment 1 analyzing OO at 9h IVM, lipid FI for NPPB (3.09±0.04) was lower than control (3.28±0.05, $P<0.05$) and PTPF (3.80±0.05, $P<0.05$), which was in turn higher than control ($P<0.05$). At 24h, NPPB (3.01±0.07) remained lower ($P<0.05$) than control (3.31±0.06) and PTPF (3.34±0.07), which did not differ from the control ($P>0.05$). In Experiment 2 analyzing CC at 9h IVM, no difference in lipid area/µm2 was observed (0.018± 0.03 to 0.039±0.018, $P>0.05$), but at 24h, NPPB (0.008±0.001) was lower ($P<0.05$) than control (0.019±0.002) and PTPF (0.021±0.006), which was similar to control ($P>0.05$). In conclusion, stimulators of different cGMP synthesis enzymes show distinct effects on lipid amounts in bovine COCs and dependent on IVM time; NPPB was more effective to reduce lipids in both OO and CC.

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