

EFFECTS OF PROGESTERONE AND ITS CORRELATION WITH INSULIN CASCADE ENZYMES ON BOVINE OOCYTE *IN VITRO* MATURATION

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Progesterone (P4) is a steroid hormone synthesized by the ovary and it is known to influence follicular growth, ovulation and luteinization. One of the biological actions of P4 is its ability to induce *Xenopus* oocytes maturation by the activation of phosphatidylinositol 3, an enzyme of insulin pathway. This work aims the study of bovine oocyte *in vitro* maturation in the presence of progesterone. Cumulus-oocyte complexes obtained from ovaries collected at local slaughterhouses were *in vitro* matured in 199 medium, at 38,5°C, 5% CO₂, during 16h. Oocytes were also cultured in maturation medium added with progesterone (5ug/mL, 10ug/mL) in order to verify the influence on chromosome migration during oocyte nuclear maturation. The evaluation of metaphasic plate (by Hoescht 33342 staining) showed that on both cases, the chromosome migration occurred in an altered pattern, assuming configurations similar to phases later to second metaphase. Similar results were obtained with addition of SB216763, a GSK3 inhibitor, which suggests that progesterone could be influencing GSK3 activity. GSK3 is an important enzyme, with its activity regulated by insulin pathway. To first verify this hypothesis, we decided to measure glucose concentration on oocytes after 16h of *in vitro* maturation with progesterone and SB. Treatments with progesterone (5ug/mL) and SB had the same glucose concentration (0,08ug/oocyte), while control had 0,25ug/oocyte and progesterone (10ug/mL) 0,05ug/oocyte. Moreover, phosphorylation on GSK3 Ser⁹ residue (as determined by Western-blot) indicates that progesterone treatment inhibits GSK3 in a dose dependent way and correlates with glucose concentration. Further studies on the role of progesterone and GSK3 on oocyte maturation are on the way and may contribute for the optimization of *in vitro* bovine embryos production.

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