

INHIBITION OF THE EFFECT OF GDF-9 AND BMP-15 ON MEIOTIC MATURATION IN VITRO OF CANINE OOCYTES

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Transforming growth factor (TGF- β) super family members are important products of growing follicles and oocytes, and among them growth differentiation factor 9 (GDF-9) and bone morphogenetic protein 15 (BMP-15) have especial roles in follicle growth and oocyte development. Therefore, both proteins are considered as essential regulators of fertility in many species. Although we were able to detect for the first time in canines the presence of GDF-9 and BMP-15 in granulosa/theca cells as well as in the oocytes during in vitro maturation (IVM), to date, the relative contribution of each of these factors in oocyte development has not been reported neither defined in canines. The aim of this study was to investigate the effects of antibodies (Ab) against GDF-9 and BMP-15 upon development during IVM of canine oocytes.

Ovaries at random stages of the estrous cycle were collected from adult bitches following ovariohysterectomy. In each replicate, cumulus oocyte complexes (COC) from antral follicles (~0.25- 9.5 mm in diameter) were arranged in three different experimental groups of 10 COCs which were cultured in: 1) TCM199-Hepes, supplemented with 10% fetal calf serum (FCS), 0.25 mM pyruvate, 10 IU/mL of hCG, 100 IU/ mL of penicillin, 20 μ g/mL of streptomycin and 2 μ g/mL of estradiol, as basal medium for Control group; 2) basal medium plus 1/100 anti mouse GDF-9 antibody (Ab) (C-18, Santa Cruz); and 3) basal medium plus 1/50 anti-human BMP-15 Ab (R&D Systems) for 72h at 38°C, 5% CO₂ in air and maximum humidity. The nuclear status was determined using 1 μ g/mL of DAPI staining, mounted on microscope slides with a epifluorescence microscopy. Data were evaluated by ANOVA.

The exposure to Ab against GDF-9 or BMP-15 during IVM had a negative impact on meiotic development. Higher number of oocytes ($P<0.05$) were arrested in GVBD stage when they were incubated with either GDF-9 Ab (64.4%) or BMP-15 Ab (67%) in comparison to those in Control group (32.4%). In contrast, more ($P<0.05$) oocytes in control groups reached MI-MII stages (47.6%) comparing to those groups with GDF-9 Ab (23.1%) or BMP-15 Ab (22.3%) ($P<0.05$). These results suggest that these proteins may play a role in regulating the oocyte maturation, being potential targets for fertility control in canines.

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