



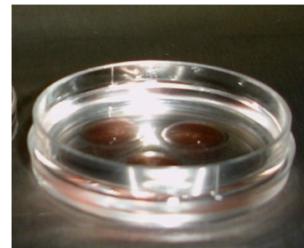
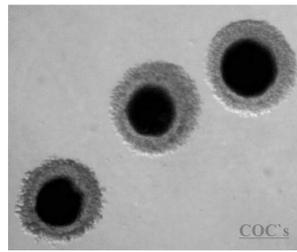
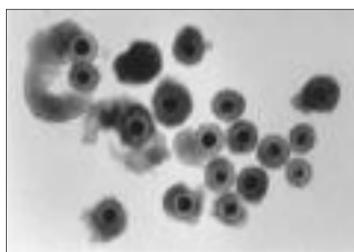
INHIBITION OF THE EFFECT OF GDF-9 AND BMP-15 ON MEIOTIC MATURATION IN VITRO OF CANINE OOCYTES

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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 in canines



The ovaries were collected from bitches of different breeds after neutering. The stage of estrous cycle was assessed according the ovarian structures and by measurements of progesterone.



- 1) Control
- 2) GDF-9 antibody (Ab)
- 3) BMP-15 antibody (Ab)

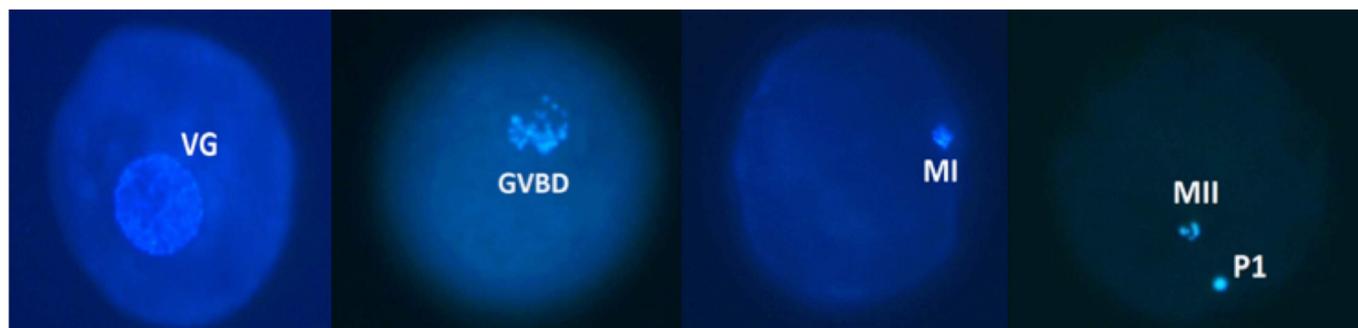
AIM

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.

MATERIAL AND METHODS

Ovaries at specific stages of the estrous cycle were collected from adult bitches following ovariectomy. Groups of cumulus-oocytes complexes (COCs) from antral follicles were arranged in three different groups and then cultured for 72 h in a supplemented TCM199-Hepes medium as:

RESULTS



Meiotic development in canine oocytes stained with DNA-specific binding dye, DAPI (4'-6-diamidino-2-phenylindole) and visualized with epifluorescence microscope. (A) Germinal vesicle (GV); (b) Germinal vesicle breakdown (GVBD) with condensed chromatin; (C) First metaphase (MI); (D) Second metaphase (MII) with polar body (P1). Magnification is x200

Table 1
Effects of temporal exposure to antibody (Ab) against growth differentiation factor-9 or bone morphogenetic protein-15 during IVM on the canine meiotic development

Treatment	Meiotic Stage (%)	\pm SD	Total Oocytes (N)
Control	GV: 19.9 \pm 8.6 ^a GVBD: 32.4 \pm 7.8 ^a	MI+MII: 47.6 \pm 1.3 ^{bc}	86
+ Ab GDF-9	GV: 12.5 \pm 6.7 ^{ab} GVBD: 64.4 \pm 2.1 ^{cd}	MI+MII: 23.1 \pm 4.7 ^a	70
+ Ab BMP-15	GV: 10.5 \pm 2.7 ^{ab} GVBD: 67.2 \pm 4.9 ^d	MI+MII: 22.3 \pm 4.0 ^a	67

GV, germinal vesicle; GVBD, germinal vesicle breakdown; MI, first metaphase; MII, second metaphase. Values with different superscript letters within the same column differ significantly (P<0.05). Ab GDF9, antibody against growth differentiating factor 9; Ab BMP15, antibody against bone morphogenetic protein15.

CONCLUSION

These results suggest that these proteins may play a role in the regulation of oocyte maturation, which likely contribute to the meiotic resumption being potential targets for fertility control in canines.