

Effect of Supplementation with GDF9 and BMP15 at IVM media of Prepubertal Goat Oocytes on Intracytoplasmic GSH and Parthenogenic Blastocyst Production.

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The oocyte-secreted factors (OSFs) bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) are well recognized to play a pivotal role in the normal proliferation and differentiation of granulosa cumulus cells (CC) and in the acquisition of oocyte developmental competence (reviewed by Belli and Shimasaki, *Vitam Horm*, 107;317-348, 2018). Oocytes are dependent of CC to perform fundamental metabolic processes on its behalf. The OSFs regulate CC glycolysis and affect antioxidant glutathione (GSH) and ROS of oocytes (reviewed by Richani et al., *Hum Reprod Update*, 27(1):27-47, 2021). BMP15 and GDF9 present species-specific roles. In goats, we have shown the positive effect of IVM supplementation with BMP15 on blastocyst development after parthenogenetic activation (PA), cumulus-oocyte communication through transzonal projections and EGFR expression both in the oocyte and their cumulus cells (Ferrer-Roda et al., *Reprod Fert Devel* 35(2):241-241, 2022; Ferrer-Roda et al., *Anim Reprod*, 20(2):0, 2023). Our aim is to study the effect of adding GDF9, alone or in combination with BMP15, to IVM media of prepubertal goat oocytes on PA-blastocyst production and ROS and GSH levels of IVM-oocytes. Cumulus-oocyte complexes (COCs) from 1-2 months old goats were collected by ovary slicing and matured in TCM-199 with FSH, LH, estradiol, EGF and cysteamine during 24h at 38.5°C with 5% CO₂. IVM media of the experimental group was supplemented with 100 ng/ml of GDF9 (GDF9 group) or in combination with 100 ng/ml of BMP15 (GDF9+BMP15 group). The control group was IVM medium without GDF9 or BMP15. A total of 173-192 matured oocytes per group (4 replicates) were parthenogenetically activated. Oocytes were denuded, put on PBS with 5µM ionomycin 4 min and then on TCM-199 with 2mM 6-dimethylamino-purine 4h. Afterwards, activated oocytes were embryo cultured for 8 days at 38.5°C with 5% CO₂ and 5% O₂ in BO-IVC medium (Bioscience, UK). Blastocysts were stained and fixed with 25 µg/ml Hoechst 33258 in pure ethanol overnight. Intra-oocyte ROS and GSH levels of 26-31 oocytes (3 replicates) were quantified after IVM by fluorescence imaging by staining with 10 µM 2',7'-dichlorodihydrofluorescein diacetate (Molecular Probes Inc., USA) and 10 µM Cell Tracker Blue (Molecular Probes Inc.) in PBS for 15 min. Data were statistically analyzed by two-way ANOVA followed by Tukey's correction. There were no significant differences in blastocyst percentages and cell number among groups. Control, GDF9 and GDF9+BMP15 groups showed a blastocyst development of 17.9 % ± 3.6, 16.1 % ± 2.37 and 12.5 % ± 3.7 respectively, and a total blastocyst cell number of 187.1 ± 20.8, 176.6 ± 17.5 and 195.5 ± 29.0 respectively. Neither ROS nor GSH levels were statistically significant. Results of ROS levels were 7.6 ± 2.1, 8.05 ± 1.2 and 5.2 ± 1.0, and GSH levels were 59.3 ± 5.4, 60.9 ± 3.9 and 64.4 ± 6.2 arbitrary units respectively, for control, GDF9 and GDF9+BMP15 groups. In conclusion, GDF9 alone or in combination with BMP15 did not improve PA-blastocyst development or intracytoplasmic antioxidant GSH in prepubertal goats. In cattle, BMP15 alone, GDF9 alone or the two combined increased the proportion of oocytes that reached the blastocyst stage post-insemination from 41% (controls) to 58%, 50% and 55%, respectively (Hussein et al., *Dev Biol*, 296(2):514-521, 2006). Study funded by the Spanish Ministry of Science and Innovation (PID2020-113266RB-100).