

Expression levels of *FSHR*, *IGF-1R*, *AMH*, *AMHR2*, *EGFR*, *Bax* and *Caspase-3* in cumulus cells are correlated developmental competence of bovine oocytes.

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The generation of animal embryos by in vitro production (IVP) methods and of human embryos by artificial reproductive techniques (ART), is hampered by the failure of many of the embryos to develop to the blastocyst stage. This problem occurs even when cumulus oocyte-complexes (COCs) with good morphology are visually selected and used for culture. Thus, although variation in quality of COCs cannot be identified morphologically, it can have a significant effect on the success or failure of embryo development. Cumulus cells (CCs) are important for oocyte maturation and subsequent embryo development. The relationship between CCs status and the developmental competence of the oocyte is unknown; here, we hypothesized that the condition of CCs must reflect oocyte competence. Therefore, we investigated the relationship between the expression of genes in bovine CCs that are related to follicle development and to competence for blastocyst development.

COCs were aspirated from bovine ovaries obtained in a slaughterhouse. The CCs located closest to the oocyte were collected with a glass pipette (about 100 cells). The collected CCs were washed in PBS and stored at -80°C until used for real-time PCR analysis. The COCs from which the CCs were collected were used for in vitro maturation, in vitro fertilization, and in vitro embryo development. Oocyte developmental competence was evaluated by screening for cleavage and development to the blastocyst stage under a stereomicroscope at 7 days after insemination. CCs collected before in vitro maturation were used for real-time PCR after RNA extraction to quantify the mRNA levels of *FSHR*, *IGF-1R*, *AMH*, *AMHR2*, *EGFR*, *ERβ*, *Bax*, and *Caspase-3*; the relative levels of expression were measured using the $\Delta\Delta C_t$ method. The CCs of COCs that developed into blastocysts were assigned to the blastocysts (BL) group, and those that failed to reach the blastocyst stage were assigned to the 2cell-Morula (2c-M) group. Gene expression levels were compared between the two groups of CCs.

We found that *FSHR*, *IGF-1R*, *AMH*, and *EGFR* expression levels were higher and *Bax* and *Caspase-3* levels were lower in the BL group than in the 2c-M group ($P < 0.05$). Positive correlations were found between *FSHR* and *IGF-1R* expression levels ($r = 0.59$) and between *ERβ* and *EGFR*

expression levels ($r = 0.43$) in the BL group ($P < 0.05$).

These results suggest that the state of the cumulus cells of bovine COCs that more developed in vitro. The expression levels of FSHR, IGF-1R, AMH, EGFR, Bax, and Caspase-3 in CCs offer a non-invasive means of predicting oocyte developmental competence.