A057 Folliculogenesis, Oogenesis and Superovulation

Luteinizing hormone receptor gene expression during follicular divergence in *B. taurus* vs *B. indicus* dairy breeds

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The establishment of follicular dominance is a key phenomenon to determine the specie-specific ovulation rate, and involves the transition from FSH to LH-dependence by the dominant follicle. This physiological event occurs at different moments in *B. taurus* and *B. indicus* cattle. Previous studies of our group analyzed the relationship between the diameter at deviation and the progression of the intrafollicular steroidogenesis in dairy breeds (Arashiro et al., Reprod Fertil Dev 25:235, 2013). The objective of the current study was to evaluate the expression of the LH receptor (LHR) gene during follicular deviation in two dairy breeds. Mural granulosa cells (GC) were recovered from Holstein (*B. taurus*; n=10) and Gir (*B. indicus*; n=10) heifers, as described before (Arashiro et al., Reprod Fertil Dev 24:175, 2012). GC were collected by ultrasound-guided follicular aspiration of follicles at 6, 8, 10, and 12 mm in diameter from Holstein heifers, and 4, 6, 8 and 10 mm from Gir heifers. The recovered follicular fluid was centrifuged and the cells were washed with NaCl 0.9% saline and kept in RNA Later (Ambion, Austin, TX, USA). Total RNA extraction was performed using the RNeasy Micro Kit (Qiagen, Hilden, Germany), quantified in spectrophotometer (Nanodrop), and cDNA was synthesized using the Superscript III kit (Invitrogen, Carlsbad, CA, USA). The obtained cDNA underwent real-time PCR, using LHR specific primers in a region without occurrence of isoforms and thus producing a single fragment, a primer pair for the CYP17A1 gene as a marker of thecal cell contamination, and a primer pair for the GAPDH gene as an endogenous control. Samples with thecal cell contamination were discarded. Results were analyzed by the software REST© and are presented as means±SEM. In both breeds, LHR expression was identified in follicles of all size categories. The expression of LRH in 4 and 6 mm follicles (for Gir and Holstein, respectively) was used as a reference value (=1). A peak in LHR expression (11.0±5.8 and 10.7±8.0 -fold the reference value) was observed in 10 mm (Holstein) and 8 mm (Gir) follicles, i.e., diameters only reached after deviation in these breeds (8.6±0.4 and 6.3±0.2; Holstein and Gir, respectively). The increase in LHR expression occurred in parallel to the previously described increase in intrafollicular estradiol concentrations at this same interval of follicular growth (Arashiro et al., 2013). Results of the present study demonstrate that LHR gene is expressed even in follicles smaller than the expected diameter at deviation in both *Bos taurus* and *Bos indicus* females. Also, relative LHR expression increases during the establishment of dominance, consistently with the progression of steroidogenesis. Further studies will evaluate whether and in which proportion LHR isoforms are present in these follicles.

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