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Expression of LH receptor isoforms during follicle deviation in cattle

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The aim of this study was to evaluate the changes in the expression of LHR isoforms associated with follicle divergence in cattle, using dairy breeds with different dominant follicle sizes at deviation as models. Granulosa cells (GC) were collected using an adapted ultrasound-guided follicular aspiration system (Arashiro et al., Reprod Biol Endocrinol, 11:73, 2013) from follicles of 6, 8, 10 or 12 mm in diameter of Holstein, and of 4, 6, 8 or 10 mm of Gir heifers. The recovered follicular fluid was centrifuged and the cells were washed with NaCl 0.9% saline and kept in RNA Later (Ambion). Total RNA extraction was performed using a commercial RNeasy Micro Kit (Qiagen), quantified in spectrophotometer (Nanodrop), and reverse transcribed using the commercial Superscript III kit (Invitrogen). The samples were tested for theca cell contamination using a primer to detect the CYP17A1 gene, and those showing contamination were excluded from analysis. The cDNAs were PCR amplified to screen for the presence of isoforms. Our previous results showed that follicles from all diameters evaluated and from both breeds presented the full-length form and/or the isoforms screened (Wohlres-Viana et al., Reprod Fertil Dev 26:176, 2014). In the present study, the relative expression of these isoforms during follicular development was evaluated. The cDNAs were submitted to Real Time PCR using three primers specific for each isoform (S10, S11, and S10+11 primers). The beta-actin gene was used as an endogenous control, and the results were analyzed using the REST software. Expression values are shown as mean±SEM. The expression results in samples from 10 mm in Holstein and from 8 mm follicles in Gir heifers were standardized as 1.00 and this value was used for further comparisons within breed. For Holstein, the S11 isoform was under-expressed ($P<0.05$) in 10 mm follicles, when compared to 8 mm (11.69±5.11) or 12 mm follicles (21.59±8.76). The same pattern was observed for Gir samples, in which the S11 isoform was under-expressed ($P<0.01$) in 8 mm follicles, when compared to 6 mm (230.05±213.16) or 10 mm follicles (5,474.99±5,560.00). Although not significant, the same trend was observed for S10 and S10+11 isoforms. In both breeds, this reduction in isoform expression occurred in follicles sampled after deviation, when the transition from FSH to LH dependence is expected to take place. Our previous results demonstrated that the expression of the LHR was maximum at 10 mm and 8 mm follicles in Holstein and Gir, respectively (Wohlres-Viana et al., Anim Reprod 10:425, 2013), suggesting a predominance of the full-length form of the LHR at this moment. Altogether, these results suggest a possible role of the modulation of LHR isoforms expression in the fine regulation of LHR function during follicle deviation in cattle.

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