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124 IDENTIFICATION OF LUTEINIZING HORMONE RECEPTOR ISOFORMS DURING FOLLICLE DEVELOPMENT IN CATTLE

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Abstract

The expression of the LH receptor (LHR) is required for the transition from FSH to LH-dependence during the establishment of follicular dominance in cattle. The aim of this study was to identify LHR isoforms expressed before, during, and after follicle deviation, using as models dairy breeds with different dominant follicle sizes at deviation. Mural granulosa cells (GC) were collected using an adapted ultrasound-guided follicular aspiration system (Arashiro *et al.* 2012 Reprod. Fertil. Dev. **24**, 175) from follicles of 6, 8, 10, or 12 mm in diameter of Holstein (*Bos taurus*), and of 4, 6, 8, or 10 mm of Gir (*Bos indicus*) 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 from GC using a commercial RNeasy Micro Kit (Qiagen), quantified in a spectrophotometer (Nanodrop), and reverse transcribed using the commercial Superscript III kit (Invitrogen). The generated cDNA were PCR amplified using a specific primer for the LHR and designed to detect a region of known occurrence of isoforms. The samples were previously tested for theca cell contamination using a primer to detect the CYP17A1 gene, and those showing contamination were excluded. Results of PCR were analysed by electrophoresis in 5% native acrylamide gel. The frequency of occurrence of the different isoforms was compared by the chi-squared test. In Holstein, the full-length form of the LHR mRNA (459 bp) was detected in all samples. The isoform with total deletion of exon 10 and partial of exon 11 (isoform II; 113 bp) was observed in 4 of 6 follicles of 6 mm and in 4 of 5 follicles of 8 mm. The isoform with total deletion of exon 10 (isoform III; 378 bp) was observed in 4 of 6 follicles of 6 mm, and in all follicles of 8 mm (5/5). The isoform with partial deletion of exon 11 (isoform IV; 194 bp) was observed in 4 of 6 follicles of 6 mm and in 4 of 5 follicles of 8 mm. These 3 alternative isoforms were present in all follicles of 10 mm (4/4) and 12 mm (11/11). There was no difference ($P > 0.05$) in the frequency of occurrence of the different isoforms. In Gir, the expression of LHR was less regular, no isoform was present in all samples, and no follicle size class showed all isoforms. The full-length LHR mRNA was detected in 2 of 7 follicles of 4 mm, 6 of 9 follicles of 6 mm, 2 of 6 follicles of 8 mm and in all follicles of 10 mm (6/6). The isoform II was observed in 3 of 7 follicles of 4 mm, 3 of 9 follicles of 6 mm, 2 of 6 follicles of 8 mm, and in all (6/6) follicles of 10 mm. The isoform III was observed in 6 of 7 follicles of 4 mm, 7 of 9 follicles of 6 mm, and in all follicles of 8 mm (6/6) and 10 mm (6/6). The isoform IV was observed in 5 of 7 follicles of 4 mm, 6 of 9 follicles of 6 mm, 5 of 6 follicles of 8 mm, and in 5 of 6 follicles of 10 mm. In the Gir breed, the isoform with deletion of exon 10 was the most frequent one ($P < 0.01$). More than one isoform was observed in most samples. In conclusion, 1) LHR is expressed in GC before follicle deviation, and 2) the expression of LHR isoforms is affected by follicle diameter and breed.

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