

Materials and Methods

RNA isolation and real-time PCR

RNA was purified from follicles using the Qiagen RNeasy Micro Kit according to the manufacturer's protocol (Qiagen, Valencia, CA, USA). RNA quality and quantity were assessed by NanoDrop (Thermo Scientific, Wilmington, DE, USA). From each condition, 214 ng of RNA was used for synthesis of full-length cDNA using the AccuScript High Fidelity 1st Strand cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA, USA). Real-time PCR was per-

formed on the ABI PRISM[®] 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using both the Taqman[®] Universal PCR Master Mix (Applied Biosystems) and Taqman[®] Gene Expression Assays (Applied Biosystems) according to the manufacturer's specifications. Gene-specific probes were labeled with the FAM reporter dye at the 5' end whereas a non-fluorescent quencher was linked to the 3' end of the probe. Each sample was performed in technical and biological duplicate and all results were normalized to the endogenous control ribosomal protein L18 (*Rpl18*). Analysis of relative gene expression was done using the comparative C_t ($2^{-\Delta\Delta C_T}$) method.