Retinol binding protein 4 is expressed in chondrocytes of developing mouse long bones: implications for a local role in formation of the secondary ossification center

Histochemistry and Cell Biology

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Online Resource 1 RT-PCR analysis of Rbp4 expression in hindlimbs from E14.5

Real-time RT-PCR analysis of Rbp4 expression in hindlimbs shows detectable Rbp4 expression from E14.5. Hindlimbs were dissected from mice at the indicated ages (n=3/age) and RNA extracted and real-time RT-PCR analyses conducted, essentially as described by Yang et al (2012). Rbp4 primers, designed to detect mouse Rbp4 (NCBI Reference Sequence: NM_001159487.1), were as follows: forward primer, 5’ – cagacatggtggcactttc – 3’ and reverse primer, 5’ – tccagtgtcagttcttc – 3’. The data were normalized to Ubiquitin C and Cytochrome c-1, which were identified as the two most stable genes across the sample set using the geNorm™ Housekeeping Gene Selection kit (PrimerDesign Ltd, Southampton, UK) and qBasePLUS software (Biogazelle, Ghent, Belgium) as outlined by Yang et al (2012). Rbp4 expression at E14.5 has been set at 1 for comparative purposes. Data between groups were compared by ANOVA and are presented as means with 95% confidence intervals.