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Short Communication

Variation in sequences and mRNA expression levels of growth hormone (GH), insulin-like growth factor I (IGF-I) and II (IGF-II) genes between prolific Lezhi black goat and non-prolific Tibetan goat (*Capra hircus*) ☆

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ABSTRACT

Growth hormone (GH), insulin-like growth factor-I (IGF-I), and II (IGF-II) play a key role in the development of preantral to preovulatory follicles in some species. To better understand the role of these genes in controlling follicular development and fecundity in goats, in the present study, we first cloned the cDNA encoding GH, IGF-I and IGF-II from prolific Lezhi black goat and non-prolific Tibetan goat (*Capra hircus*), and their mRNA expression between the two breeds were compared. By reverse transcriptase-polymerase chain reaction (RT-PCR) strategy, we obtained full-length 688-bp GH, 493-bp IGF-I, and 566-bp IGF-II cDNAs, encoding for 217 amino acid (aa) GH, 154 aa IGF-I, and 179 aa IGF-II putative proteins. Analysis of their nucleotide and amino acid sequences revealed a high degree of identity between the two breeds, although one base change in GH resulted in one amino acid substitution in the translated proteins. However, two base changes in IGF-I and IGF-II did not lead to any amino acid changes. Real-time PCR analyses revealed that in the middle of estrus, GH, IGF-I and IGF-II genes were expressed, albeit at different levels, in all three tissues (anterior pituitary, endometrium and ovary) examined. GH was most highly expressed in ovary ($P < 0.01$) and its expression was greater in all three tissues examined in Lezhi black goat than in Tibetan goat ($P < 0.05$). IGF-I and IGF-II genes were expressed at a higher ($P < 0.05$) level in anterior pituitary of Lezhi black goat than that in Tibetan goat, but they had a similar expression pattern in endometrium and ovary. These results provide the foundation of information required for future studies of these gene effects on goat fecundity.

1. Introduction

Reproductive rate, especially fecundity, is one of the most economically important traits in animal production and is regulated by genetic and environmental factors. Ovulation rate can be a major determinant of reproductive efficiency. In cattle, single ovulations occur most frequently, and in sheep and goats the number of ova released can range from one to many depending upon the breed, whilst the pig is polyovular. The processes of recruitment and selection determine the number of ovulatory follicles in all these species with FSH and subsequently LH playing major roles (Hunter et al., 2004; Silva et al., 2009). However, primordial to early antral follicle development has generally been considered to be largely gonadotrophin-independent and mechanisms governing the initiation of growth of the primordial follicles are not completely known. There is a growing body of evidence that growth hormone (GH) and members of the insulin-like growth factors (IGF-I and IGF-II) family system (Baker et al., 1996; Echternkamp et al., 2004; Hastie and Haresign, 2006; Hunter et al., 2004; Li et al., 2011; Lucy, 2011; Reinecke, 2010; Shimizu et al., 2008; Silva et al., 2009; Zhao et al., 2001; Zhao et al., 2002; Zhou et al., 1996) play a key role both in the development of preantral to preovulatory follicles and in the process of follicular atresia. Growth hormone exerts direct and/or indirect effects on virtually every organ in the body, with IGF-I mediating its indirect actions (Butler and Le Roith, 2001; Duan et al., 2010), whereas placental-specific IGF-II is a major modulator of placental and fetal growth (Constância et al., 2002; DeChiara et al., 1990; Velazquez et al., 2008). Growth hormone, a single-chain protein belongs to the structurally and functionally related prolactin, somatolactin, and placental

☆ The goat GH, IGF-I, and IGF-II cDNA sequences reported in this paper have been deposited in the GenBank database as the following accession numbers: JF813118, JF896275, and JF896277 for Lezhi black goat, and JF896274, JF896276, and JF896278 for Tibetan goat respectively

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lactogen family and is synthesized predominantly by the somatotrophs of the anterior pituitary (Ayuk and Sheppard, 2006; Filby and Tyler, 2007), but also by a number of extrapituitary sites (Abir et al., 2008; Carlsson et al., 1993; Le Roith et al., 2001). IGF-I and IGF-II are single-chain polypeptides with structural homology to proinsulin. Mature IGF-I and IGF-II consist of B, C, A, and D domains (Filby and Tyler, 2007; Funes et al., 2006; Jones and Clemmons, 1995). IGF-I is produced in organs of reproductive significance such as hypothalamus, anterior pituitary, ovaries, oviducts, and uterus (Adam et al., 2000; Bach and Bondy, 1992; Constância et al., 2002; Eppler et al., 2007; Filby and Tyler, 2007; Funes et al., 2006; Gonzalez-Parra et al., 2001; Hastie and Haresign, 2006; Hunter et al., 2004; Iida, 2005; Irwin and Van Der Kraak, 2011; Jevdjovic et al., 2007; Mikawa et al., 1995b; Olchovsky et al., 1993; Silva et al., 2008; Velazquez et al., 2008). However, most of the IGF-I measured in blood is produced by the liver (Funes et al., 2006; Yakar et al., 1999). IGF-II mRNAs were also detected in liver and in variety of extrahepatic tissues, as was IGF-I mRNA (Funes et al., 2006; Silva et al., 2009). Recently, the IGF system has garnered more attentions for its potential role in ovarian development with the discovery of the gonad specific IGF-III, a third form of IGF (Berishvilia et al., 2010; Irwin and Van Der Kraak, 2011; Reinecke, 2010).

However, there is little information about GH and IGF system in goats (Mikawa et al., 1995a,b; Silva et al., 2008; Yamano et al., 1988; Yato et al., 1988). The present study, for the first time, investigated the nucleotide sequences and mRNA expression levels of GH, IGF-I and IGF-II genes in the Lezhi black goats, a local Chinese breed famous for its high fecundity (producing 3–5 kids per kidding), and Tibetan goats (*Capra hircus*), a single-birth breed characterized by adapting to cold, hypoxic ecological conditions in the Qinghai-Tibet Plateau. This intends to examine whether base mutations or expression levels of mRNA of these genes could be associated with the reproductive difference in goats.

2. Materials and methods

2.1. Animals and sample collection

All experimental procedures were performed according to the guide for animal care and use of laboratory animals of the Institutional Animal Care and Use Committee of Southwest University for Nationalities. Lezhi black goats were supplied by the Lezhi Black Goat Breeding Farm in Lezhi County (30°30′N, 105°02′E and 596.3 m altitude), China, and Tibetan goats were purchased from Li County (31°42′N, 103°16′E and 2800 m altitude) of Qinghai-Tibet Plateau in China during the breeding season (October). Lezhi black goats were kept in shelters, and Tibetan goats were grazed on the pasture. Animals aged 3–5 year, with a history of multiple births (twin or triplet births) for Lezhi black goats ($n = 6$) and single birth for Tibetan goats ($n = 6$) were selected to investigate the nucleotide sequences and mRNA expression levels of GH, IGF-I, and IGF-II genes. Estrus was detected twice a day, and a doe was considered in estrus only when she allowed the male to mount. Goats were slaughtered at 12–24 h after onset of estrus (in the middle of estrus) for collection of anterior pituitaries, ovaries, and endometria. Part of the removed tissue samples was snap-frozen in liquid nitrogen and then stored at −80 °C to be used for RNA extraction.

2.2. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from the tissue samples (pituitaries, ovaries, and endometria) using RNAprep pure Tissue Kit (Tiangen Biotech, Beijing), following the manufacture's instructions. The RNA samples were spectrophotometrically quantified at $A_{260\ nm}$ and $A_{280\ nm}$, and then analyzed for their integrities on agarose gel (1.2%). Reverse transcription was performed using RNA PCR kit (AMV) (TaKaRa, Dalian, China) in a volume of 10 μL reaction mixture (Zi et al., 2012). The generated cDNA was then amplified using gene specific primers (GH: 5′-CAATGGGAAAAATCAGCA GTCT-3′/5′-GAGGGGTAGTAACAACAGATGG-3′; IGF-I: 5′-CAATGGGA AAAATC AGCAGTCT-3′/5′-ATT CTTCGCTCTTTAGGAAGGGC-3′; GF-II: 5′-GCA-GAGACATCAATGGG GATC-3′/5′-ACTTTGGCTCACT TCTAATCGC-3′, sense and antisense, respectively) designed from regions of GH, IGF-I, and IGF-II cDNAs conserved between sheep, goat and yak available in the NCBI GenBank database. PCR amplification was carried out in a volume of 25 μL of reaction mixture (Zi et al., 2012). The purified PCR products were ligated into a pMD19-T vector and transformed into DH5α (*Escherichia coli*) using standard techniques (Sambrook et al., 1989). The DNA sequence was determined with an ABI PRISM 3700 automated DNA sequencer by Shanghai Invitrogen Biotechnology (Shanghai, China).

2.3. Sequence analysis

The sequences of goat GH, IGFI and IGFII cDNA were subjected to BLAST analysis to verify that the sequence was of GH, IGFI and IGFII. The nucleotide and deduce amino acid sequence identity was performed using the Clustal option in MegAlign (Lasergene Software, DNASTAR).

2.4. Quantitative real-time PCR (qPCR)

GAPDH gene was chosen as reference gene for normalizing expression levels of target genes. Primers specific for target goat genes were designed with Beacon Designer 7.0 software (Premier Biosoft International, Palo Alto, CA USA) according to manufacturers guidelines (GH: 5′-CTGAAGGACCTGGAGGAA-3′/5′-GGA GAGCA GACCGTAGTT-3′; IGF-I: 5′-TGCTCTCCAGTTCGTGTG-3/5-CATCTCCA GCCTCCTCAG-3′; IGF- II: 5′-CACCCTCCAGTTTGTCTG-3′/5′-GGCA-CAG TAAGTCTCCAG-3′; GAPDH: 5′-AGTTCCACGGCACA GTCAAG-3′/5′-ACTCAGCACCAGCATCACC-3′). Total RNA of different tissues was extracted, and reverse transcribed as above. Real-time PCR was performed using on an iCycler iQ5 Real-Time Detection System (Bio-Rad, CA, USA) with the SsoFast™ EvaGreen Supermix (Bio-Rad, CA, USA) in a volume of 10 μL. The cycle parameters were 3 min at 95 °C, followed by 45 cycles of 30 s at 95 °C and 5 s at T_m, followed by a melting curve analysis. The efficiency of each primer pair and mean C_t (threshold cycles) values were calculated and used for determination of target gene RNA transcript levels (Pfaffl, 2001). Each sample was tested in triplicate.

2.5. Statistical analysis

All data were expressed as mean values ± SEM. Statistical differences were assessed by Student's *t*-test or one-way ANOVA followed by Dunn's multiple pairwise comparison test.

3. Results

3.1. Cloning and molecular characterization of cDNAs for GH, IGF-I, and IGF-II genes

As expected, the goat GH, IGF-I, and IGF-II cDNA sequences were found to consist of 688, 493, and 566 bp, respectively. They were respectively submitted to NCBI GenBank Accession Nos. JF813118, JF896275, and JF896277 for Lezhi black goat, and JF896274, JF896276, and JF896278 for Tibetan goat respectively. The coding region (nucleotides 6–656) of GH cDNA encoded a