RT-PCRによるウシ胎子におけるIgf2ならびにIgf2r遺伝子の発現解析

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—Research Note—

Analysis of Igf2 and Igf2r Genes Expression by RT-PCR in Bovine Fetuses

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Abstract: *In mammals, insulin-like growth factor 2 (Igf2) plays a key role in mammalian growth and its receptor, Igf2r, which are imprinted and expressed exclusively from paternal and maternal alleles, respectively. Here we examined that expression of Igf2 and Igf2r genes in blastocysts and fetuses ranging from 30 to 180 days of gestation by RT-PCR. The expression of both genes was significantly low at the blastocyst stage, but high expression of both genes was detected throughout the fetal period. These results suggest that Igf2 and Igf2r are important genes for growth regulation in bovine fetuses as well as mice.*

Key words: *Igf2, Igf2r, Bovine embryos, Bovine fetuses, Gene expression*

Fetal growth and development are regulated by complicated mechanisms in fetal-maternal interactions through the placenta. One of the main factors for regulating fetal growth is insulin-like growth factor 2 (*Igf2*) and its receptor (*Igf2r*), which are known as imprinted genes in mice and are expressed solely from paternal and maternal alleles, respectively [1–4]. *Igf2* is also imprinted in human and sheep [5, 6]. It has been reported that fetuses derived from cultured embryos often result in the fetal overgrowth, which is called large calf syndrome [7–9]. The reason why this occurs is still not known, but *Igf2* and *Igf2r* genes may be involved. Data on the expression of these genes in cattle is quite limited. Here we analyze the expression of *Igf2* and *Igf2r* genes in bovine blastocysts and fetuses by RT-PCR with specific promoters for bovine.

Blastocysts were produced by fertilization with *in vitro* matured oocytes. Oocytes were aspirated from ovarian follicles [10]. Oocytes with tightly packed cumulus cells were matured *in vitro* by culturing for 20 h in TCM-199 medium (Gibco BRL) supplemented with 10% (v/v) calf serum (Gibco BRL), penicillin-G (100 U/ml), and streptomycin (50 μg/ml) in an atmosphere of 5% CO_2 and 95% air at 39°C. The matured oocytes were transferred to a sperm suspension in BO medium supplemented with 5 mM caffeine (Sigma), 5 IU/ml heparin (Wako), and 10 mg/ml bovine serum albumin (Sigma). Six hours after insemination, the oocytes were co-cultured with the cumulus cells in TCM-199 medium supplemented with 5% (v/v) calf serum, penicillin-G (100 U/ml), and streptomycin (50 μg/ml) for 7 to 9 days.

Bovine fetuses from 30 to 180 days of gestation, which was estimated from their body size, were obtained from a slaughterhouse. Whole fetuses or parts of their organs were quickly frozen in liquid N_2 and stored until used for assay. Total RNA was extracted from about 1 μg of fetal tissue with ISOGEN (NipponGene) according to the protocol. Genomic DNA in total RNA fraction was digested by treatment with RQ1 DNase 2.0 μl (2U) (Promega) at 37°C for 30 minutes to prevent contamination of genomic DNA. Messenger RNA was extracted from day 8 blastocysts with a QuickPrep Micro mRNA Purification Kit (Amersham Pharmacia Biotech). First-strand cDNA was synthesized from 1 μg of total RNA by Superscript reverse transcriptase II (Gibco BRL) according to the manufacturer's instructions. The cDNA was subjected to PCR, which was carried out with 1.25 U of ExTaq DNA polymerase (Takara), 0.2 mM of dNTPs, 1.0 mM of $MgCl_2$ for β-actin, 1.5 mM for *Igf2* gene and 2.0 mM for the *Igf2r* gene. The synthesis of cDNA from mRNA, which was extracted with a QuickPrep Micro mRNA Purification Kit, was continuously subjected to Ready/To/Go RT-PCR Beads (Amersham Pharmacia Biotech).

Amplification of each gene was carried out with cDNA

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transcribed from 0.1 to 100 ng total RNA for semi-quantitative analysis. The amplification was done as follows: β-actin gene, a total of 30 cycles at 94°C for 1 minute, 57°C for 1 minute and 72°C for 1 minute; *Igf2* gene, a total of 30 cycles at 94°C for 30 seconds, 65°C for 30 seconds and 72°C for 1minute; *Igf2r* gene, a total of 35 cycles at 94°C for 1 minute, 57°C for 2 minutes and 72°C for 2 minutes. When blastocysts were used, ten cycles were added for PCR amplification.

When primers designed for human *Igf2* and *Igf2r* genes [11] were used, fine amplification of the genes was not detected (data not shown). Several sets of primers for bovine *Igf2* and *Igf2r* genes were therefore designed based on the sequence that was obtained from the database (accession no. Z6815: *Igf2*, no. J03527: *Igf2r*). The most efficient primer sets shown below were used to analyze expression of *Igf2* and *Igf2r* genes.

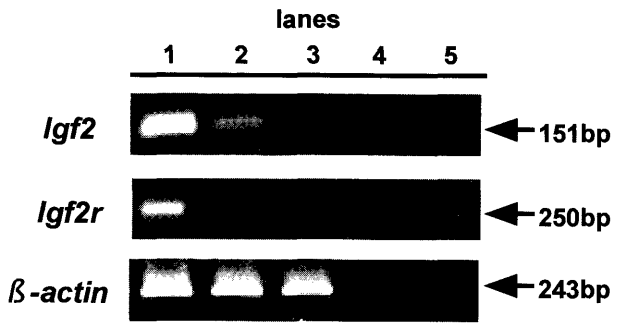

Fig. 1. RT-PCR analysis of *Igf2* and *Igf2r* expression in bovine blastocysts. RT-PCR products amplified from cDNA which was synthesized with mRNA extracted from 10 (lane 1), 5 (lane 2), 2 (lane 3), 1 (lane 4), and 1/2 blastocyst (lane 5).

The primer pair sets were:

Igf2: (5') AGCTGGTGGACACCCTCCAGT / (3') GTGGCACAGTAAGTCTCCAGCAG;

Igf2r: (5') ACCTACGACCTCTCGTCTCT / (3') CACAAGTCACCGTCAACGTAC.

β-actin [12]: (5') CGTGGGCCGCCCTAGGCACCA / (3') TTGGCCTTAGGGTTCAGGGGGG;

The amplified product was 243 bp for β-actin, 151 bp for *Igf2*, and 250bp for the *Igf2r* gene. After RT-PCR, cDNA products were detected by a 3% agarose gel electrophoresis in 0.5 × TBE and dyed with 0.5 μl/ml Ethidium Bromide.

Expression of *Igf2* and *Igf2r* genes in the blastocyst was first detected with total RNA extracted from 5 and 10 embryos, respectively (Fig. 1), whereas the expression of β-actin was detected from half of the embryo (Table. 1). The low expression of the genes at the blastocyst stage was coincident with the report by Watson et al. [11], who tested the gene expression with primers designed for human. On the other hand, in mice it is known that *Igf2* and *Igf2r* genes are expressed throughout embryogenesis commencing at the 2-cell and 4 to 8-cell stages, respectively [13–15].

Semi-quantitative analysis of gene expression by RT-PCR showed that both *Igf2* and *Igf2r* genes were strongly expressed through the fetal growth period from 30 to 180 days of gestation. Expression of the *Igf2* gene seemed to be stronger than that of the *Igf2r* gene (Fig. 2). The expression of *Igf2* and *Igf2r* genes in the brain of 150-day fetus was weaked than that in liver, heart and muscle (Table 1). When compared with the expression level during fetal growth, both genes were expressed strongly throughout the ontogenesis from 30

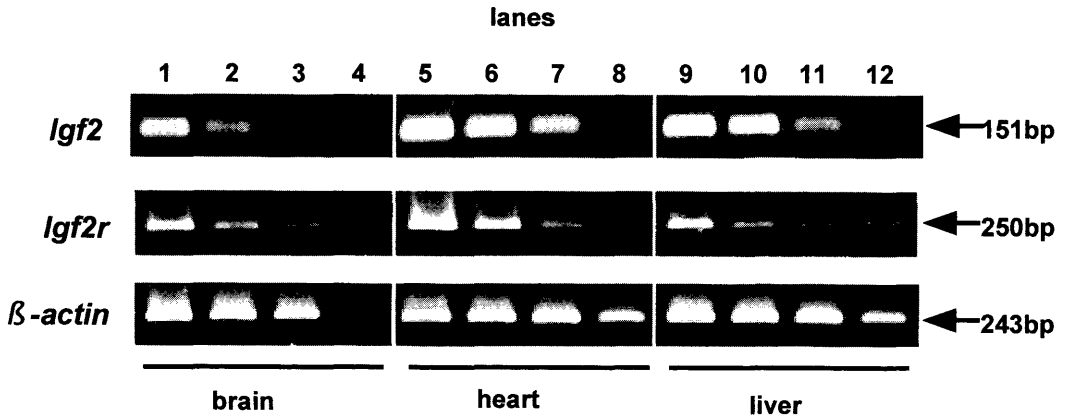

Fig. 2. RT-PCR analysis of *Igf2* and *Igf2r* expression in brain, heart and liver in a day 150 bovine fetuse. PCR products amplyfied from 10^{-1} cDNA (lane 1, 5, 9), 10^{-2} (lane 2, 6, 10), 10^{-3} (lane 3, 7, 11), and 10^{-4} (lane 4. 8, 12).

Table 1. The expression in *Igf2* and *Igf2r* genes

embryos	part	gene	10 blastocsyts	5 blastocsyts	2 blastocysts	1 blastocyst	1/2 blastocyst
8 days	blastocyst	*Igf2*	+	+	−	−	−
		Igf2r	+	−	−	−	−
		β-actin	+	+	+	+	+
fetuses	part	*gene*	10^{-1}	10^{-2}	10^{-3}	10^{-4}	(μg cDNA)
30 days	whole	*Igf2*	+	+	+	±	
		Igf2r	+	+	+	−	
		β-actin	+	+	+	+	
50 days	whole	*Igf2*	+	+	+	+	
		Igf2r	+	+	+	−	
		β-actin	+	+	+	+	
90 days	brain	*Igf2*	+	+	+	+	
		Igf2r	+	+	+	±	
		β-actin	+	+	+	+	
	liver	*Igf2*	+	+	+	±	
		Igf2r	+	+	+	−	
		β-actin	+	+	+	+	
	muscle	*Igf2*	+	+	+	+	
		Igf2r	+	+	+	−	
		β-actin	+	+	+	+	
150 days	brain	*Igf2*	+	+	−	−	
		Igf2r	+	+	+	±	
		β-actin	+	+	+	+	
	heart	*Igf2*	+	+	+	±	
		Igf2r	+	+	+	+	
		β-actin	+	+	+	+	
	liver	*Igf2*	+	+	+	±	
		Igf2r	+	+	+	±	
		β-actin	+	+	+	+	
180 days	heart	*Igf2*	+	+	+	±	
		Igf2r	+	+	+	+	
		β-actin	+	+	+	+	
	liver	*Igf2*	+	+	+	+	
		Igf2r	+	+	+	±	
		β-actin	+	+	+	+	

to 180 days of gestation (Table 1).

Paternally expressed *Igf2* is known as the major growth factor for mammalian fetuses, whereas maternally expressed *Igf2r* binds with *Igf2* and acts to degrade its activity [16]. During the preimplantation stage, these genes are biallelically expressed in mice, and after implantation they become expressed solely from paternal and maternal alleles, respectively [15, 17]. So far, there is no direct evidence that *Igf2* and *Igf2r* are imprinted and are active in fetal growth in cattle, but together with studies in human and mice [4, 18], the present results suggest that *Igf2* and *Igf2r* are important genes for growth regulation in bovine fetuses as well. It is reported that a range of developmental abnormalities in sheep [19], cattle [20–22] and mice [23, 24] are associated with somatic cell nuclear transfer. Although there is no clear evidence yet, inappropriate expression of imprinted genes may be involved in the abnormalities [25]. To obtain further insight into the function of *Igf2* and *Igf2r* genes in bovine fetuses including cloned embryos, more

sensitive quantitative analysis, such as real-time quantitative PCR, should be carried out.

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