RESEARCH ARTICLE

Atlas of tissue- and developmental stage specific gene expression for the bovine insulin-like growth factor (IGF) system

Mani Ghanipoor-Samami1,2*, Ali Javadmanesh1,2*,a, Brian M. Burns3, Dana A. Thomsen1,2, Greg S. Nattrass4, Consuelo Amor S. Estrella1,2b, Karen L. Kind2, Stefan Hiendleder1,2*

1 Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia, 2 JS Davies Epigenetics and Genetics Group, Davies Research Centre, School of Animal and Veterinary Sciences, Roseworthy Campus, The University of Adelaide, Roseworthy, South Australia, Australia, 3 Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Rockhampton, Queensland, Australia, 4 Livestock Systems, South Australian Research and Development Institute (SARDI), Roseworthy, South Australia, Australia

☯ These authors contributed equally to this work.
a Current address: Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
b Current address: University of the Philippines, Laguna, Philippines
* stefan.hiendleder@adelaide.edu.au

Abstract

The insulin-like growth factor (IGF) axis is fundamental for mammalian growth and development. However, no comprehensive reference data on gene expression across tissues and pre- and postnatal developmental stages are available for any given species. Here we provide systematic promoter- and splice variant specific information on expression of IGF system components in embryonic (Day 48), fetal (Day 153), term (Day 277, placenta) and juvenile (Day 365–396) tissues of domestic cow, a major agricultural species and biomedical model. Analysis of spatiotemporal changes in expression of IGF1, IGF2, IGF1R, IGF2R, IGFBP1-8 and IR genes, as well as lncRNAs H19 and AIRN, by qPCR, indicated an overall increase in expression from embryo to fetal stage, and decrease in expression from fetal to juvenile stage. The stronger decrease in expression of lncRNAs (average —16-fold) and ligands (average —12.1-fold) compared to receptors (average —5.7-fold) and binding proteins (average —4.3-fold) is consistent with known functions of IGF peptides and supports important roles of lncRNAs in prenatal development. Pronounced overall reduction in postnatal expression of IGF system components in lung (—12.9-fold) and kidney (—13.2-fold) are signatures of major changes in organ function while more similar hepatic expression levels (—2.2-fold) are evidence of the endocrine rather than autocrine/paracrine role of IGFs in postnatal growth regulation. Despite its rapid growth, placenta displayed a more stable expression pattern than other organs during prenatal development. Quantitative analyses of contributions of promoters P0-P4 to global IGF2 transcript in fetal tissues revealed that P4 accounted for the bulk of transcript in all tissues but skeletal muscle. Demonstration of IGF2 expression in fetal muscle and postnatal liver from a promoter orthologous to mouse and human promoter P0 provides further evidence for an evolutionary and developmental shift...
from placenta-specific P0-expression in rodents and suggests that some aspects of bovine IGF expression may be closer to human than mouse.

**Introduction**

The insulin-like growth factor (IGF) system is essential for pre- and postnatal growth and development [1–4] and consists of two growth factors (IGF1, IGF2), type 1 and 2 receptors (IGF1R, IGF2R), the insulin receptor (IR) with short and long isoforms (IR-A, IR-B), six major IGF binding proteins (IGFBP1–6) and several lower-affinity binding proteins (IGFBP7 to IGFBP10) [1, 5, 6]. The IGF1 and IGF2 peptides have strong growth promoting endocrine and paracrine/autocrine actions in a wide range of pre- and postnatal tissues and undergo pronounced changes in expression during prenatal development and after birth [7–13]. Consistently, the IGF1 and IGF2 genes have been identified as quantitative trait loci for growth and development in several mammalian species, including mouse, pig, bovine and human [14–25].

Expression of *IGF1* starts early with transcripts detected in preimplantation stage bovine and human embryos and in midgestation rat embryos [26–28]. Transcription of *IGF1* can be initiated from exon 1 or 2, producing *IGF1* class 1 and 2 mRNAs that yield identical mature IGF1 proteins [29–32]. The IGF2 gene is subject to genomic imprinting and paternally expressed in prenatal mammalian tissues, but switches to biallelic expression in a promoter- and tissue specific manner postnatally [33–38]. Interestingly, in mouse, a sequence in *Igf2* intron 2 encodes for an imprinted miRNA that targets non-imprinted *Igf1* transcripts [39–41]. In sheep, pig, bovine and human, *IGF2* transcripts are expressed from four promoters (*IGF2*-P1-4) in a tissue- and developmental stage specific manner [16, 42–47]. The structure of mouse *Igf2* differs significantly from other mammals and transcripts originate from *Igf2*-P1-P3, which are orthologous to *IGF2*-P2-P4 in species discussed above [48], and an additional placenta-specific promoter (P0). Transcripts equivalent to mouse P0 transcripts have also been identified in human fetal skeletal muscle and several postnatal tissues, including heart, lung, liver, muscle and kidney [49, 50]. Furthermore, in mouse, a previously unknown promoter (Pm) is activated preferentially in mesoderm derived tissues by the expression of antisense *H19* long non-coding RNA (91H). This 91H-mediated *Igf2* activation is counteracted by a large excess of *H19* transcripts [51].

The reciprocally imprinted and maternally expressed long non-coding RNA *H19* is located immediately downstream of *IGF2* and expression of both genes is intrinsically linked through shared control elements such as CTCF binding sites [45, 52–56]. More recent analyses in mouse have shown that *H19* harbors miRNAs, one of which regulates cell proliferation and placental size, most likely by targeting *IGF1R* transcript [57]. Furthermore, correlations between *H19* transcript abundance and bovine fetal skeletal muscle and bone mass suggest that development of other organs may be regulated by *H19* [58, 59].

Both IGF ligands signal through combinations of IGF1R and IR homo- and heterodimers, albeit with different affinities. In bovine and human, alternative splicing of the *IR* transcript produces the two receptor isoforms, IR-A and IR-B, that exclude or include exon 11 [60]. Both form heterodimers with each other and IGF1R [61, 62]. IR-A isoform displays higher affinity for IGF2 than IGF1, while IR-B has a high specificity for insulin [63]. The IGF1 peptide signals through homodimers of IGF1R and heterodimers of IGF1R and IR-A or IR-B, while IGF2 peptide signals through homodimers of IGF1R and IR-A and heterodimers of IGF1R and IR-A or IRA and IR-B [61, 63−65]. The IGF1R gene is expressed ubiquitously and has a major role in maintenance of tissue growth and development [66, 67].
to growth retardation and/or growth failure more severe than IGF1 deletion [68]. In human, but not mouse, lack of functional IR leads to severe intrauterine growth retardation [69, 70]. In contrast to IGF1R and IR receptors, multifunctional IGF2R is primarily a regulator of IGF2 bioavailability and acts as a scavenger receptor that internalizes IGF2 and targets it for lysosomal degradation [71]. However, studies on stimulation of human trophoblast cell invasion by IGF2 suggest intrinsic signaling functions for IGF2R in placenta via the MAPK pathway [72]. The IGF2R gene is imprinted and maternally expressed in all investigated species, including bovine, with the exception of human, where imprinting appears to be polymorphic [73–77]. Ablation of IGF2R in mouse results in severe fetal overgrowth [78] and association of IGF2R alleles with postnatal growth parameters in cow suggests a general role for IGF2R in growth regulation [79]. Imprinted expression of mouse Igf2r is controlled by a reciprocally imprinted antisense of Igf2r non-protein coding RNA Airn; orthologues of Airn are also expressed in bovine and human [80–82]. However, data on tissue specific developmentally regulated expression of this RNA is lacking.

The IGFBPs modulate bioavailability of IGFs with affinities up to 50-fold higher than IGF1R [83]. Deletion and overexpression models demonstrated organ-specific and general effects of IGFBPs on growth and development [84–87]. The discovery of low affinity IGFBP-related proteins, including IGFBP7 and IGFBP8, has led to the proposal of an IGFBP superfamily [5, 83, 88]. Mice deficient in IGFBP8 die in the perinatal period due to respiratory failure and displayed generalized chondrodysplasia [89].

Expression patterns of genes in the IGF system are highly developmentally regulated [13, 90–100], but changes in tissue specific expression across pre- and postnatal stages by quantitative PCR have not been systematically examined in any species. Here we comprehensively characterize changes in transcript abundances of IGF system genes and associated regulatory long non-coding RNAs in a range of tissues at key developmental time points, i.e., (i) transition from embryo to fetal stage, (ii) fetal stage entering accelerated growth phase and (iii) juvenile stage around puberty. The resulting atlas of tissue- and developmental stage specific expression of the insulin-like growth factor system in bovine is a valuable resource that provides important reference data for future studies of the mammalian IGF system and yields novel insights into similarities and differences between animal model and human.

**Materials and methods**

**Animals and tissues**

All animal experiments and procedures described in this study were approved by the University of Adelaide, Adelaide, Australia, Animal Ethics Committee (No. S-094-2005 and S-094-2005A) and the Department of Agriculture, Fisheries and Forestry (DAFF) Animal Ethics Committee, Queensland, Australia (No. SA 2008/01/227 and SA 2010/12/339). We used dams and sires of the two subspecies of domestic cow, Bos taurus taurus (Angus, A) and Bos taurus indicus (Brahman, B), to generate a large number of purebred and reciprocal cross Day 48 embryos (n = 60) and Day 153 fetuses (n = 73) for samples of prenatal tissues. A set of Day 278 calves (term, Day 277–291, n = 17) was delivered by cesarean section for near term placental samples and later used to obtain samples from juveniles at 12–14 months of age. Further information on samples for RNA extraction and cDNA synthesis (see below) is provided in [S1 Table](https://doi.org/10.1371/journal.pone.0200466.s001).

To establish pregnancies for recovery of embryos, fetuses and calves, including placenta, dams were subjected to standard commercial estrous cycle synchronization protocols using Cidirol—Heat Detection and Timed Insemination (HTI) and Cidirol—Timed Insemination (TI) procedures as described previously [101]. All pregnancies were confirmed by ultrasound scanning and embryo, fetal and juvenile tissues obtained after sacrificing animals in an
RNA isolation and reverse transcription

All fetal and juvenile tissue samples and cesarean section placental samples were homogenized with ceramic beads (MoBio Laboratories, Carlsbad, CA, USA) using the Precellys™ 24 tissue homogenizer (Bertin Technologies, Saint Quentinen Yvelines Cedex, France). Total RNA was extracted using TRI Reagent™ (Ambion, Life Technologies™, Inc., Carlsbad, CA, USA) according to the manufacturer’s instructions. Embryonic tissues were homogenized with ceramic beads (MoBio Laboratories, Carlsbad, CA, USA) and the PowerLyzer™ 24 homogenizer (MoBio Laboratories, Carlsbad, CA, USA). Total RNA from embryonic liver and placenta was isolated with TRI Reagent™. Due to small sample size for embryonic brain and heart, AllPrep™ DNA/RNA Micro Kits (Qiagen GmbH, Inc., Hilden, Germany) were used for extraction of nucleic acids. Quantity of RNA was determined by repeated measurements with NanoDrop (ThermoFisher Scientific, Waltham, MA, USA). Quality of RNA was assessed using the Agilent RNA 6000 Nano Kit with a Bioanalyzer 2100 (Agilent Technology Inc., Santa Clara, CA, USA). The mean RNA integrity numbers (RIN) of extracted RNAs from different tissues measured by Bioanalyzer System (Agilent Technologies Inc., Santa Clara, CA, USA) are presented in S2 Table.

Complementary DNA (cDNA) was synthesized from 500 ng DNase I (RQ1-DNase, Promega, Madison, WI, USA) treated RNA of each individual tissue sample using SuperScript™ III First-Strand Synthesis System (Invitrogen, Life Technologies™, Inc., Carlsbad, CA, USA) and random hexamer oligonucleotides following the manufacturer’s instructions.

Target transcript amplification strategy and quantitative real time PCR

Transcripts quantified included IGF1 global transcript and the splice variants IGF1 class 1 and IGF1 class 2; IGF2 global transcript and promoter specific transcripts originating from P0, P1 (two transcripts, P1e2 and P1e3), P2 (two splice variants, P2e4 and P2e5), P3 and P4; IGF1R- and IGF2R transcript, IR global transcript and splice variants IR-A and IR-B; IGFBP1–8, as well as H19 and AIRN long noncoding RNA transcripts. Primers were designed to be isoform-specific and span two exons or an exon/intron junction to avoid amplification of genomic DNA sequences. Primer information for all amplicons of target genes is detailed in S3 Table. Primer design for promoter and splice variant specific IGF2 transcripts required extensive in silico analyses in order to be able to assess the complex transcript structure of this gene. Sequences and exon/intron structures for these analyses were retrieved from the literature [16, 43], National Center for Biotechnology Information (NCBI) GenBank database (NCBI reference sequence: AC_000186.1; Gene ID: 281240) and Ensembl project database (ENSBTAG00000013066.5). Since transcripts from P0 promoter were not previously identified in bovine, we performed a sequence similarity search using Basic Local Alignment Search Tool [102] of NCBI, and identified a highly conserved region upstream of bovine IGF2 exon 2 that corresponded to the human P0 promoter showing (69% homology) [50]. Therefore we hypothesized the existence of a putative orthologous promoter in bovine. An overview of our identification and quantification strategy for IGF2 transcripts in the context of the genomic structure of INS/IGF2 (GenBank accession no. EU518675.1) is presented in Fig 1.

The first part of this study was designed to systematically measure expression of IGF system components across a broad range of developmental stages and tissues. In light of the fundamental problem to identify stable reference genes across tissues of such rapidly changing developmental stages we opted for a cDNA pooling strategy to assess spatial and temporal differences.
in expression level. An equal proportion of cDNA from each individual was combined and pooled cDNA used as template in real-time qPCR reactions. The number and sex of individual

---

**Fig 1.** Bovine IGF2 gene and transcript structure with primer locations for amplification of promoter specific transcripts and splice variants. The exon-intron structure of bovine insulin/insulin-like growth factor 2 (INS/IGF2, GenBank accession no. EU518675.1) with locations of five promoters (P0, P1, P2, P3 and P4) is shown at the top with promoters (IGF2-P0 –P4) and splice variant specific transcripts indicated below. Red and green boxes depict untranslated and protein coding exons, respectively. Forward (F) and reverse (R) primers are indicated with region spanned, including intron where applicable, symbolized by a black bar between primers above the transcript. According to the transcription initiation site of human IGF2-P0 transcript, the putative orthologous bovine transcript is predicted to originate from a highly conserved region located upstream of the splice donor site of transcript P1 exon 2. We could specifically amplify bovine IGF2-P0 using a strategically designed forward primer within this unique 5'-UTR sequence and the reverse primer located within exon 2. The two splice variants of P1 promoter transcripts include leading exon 1 which is alternatively spliced onto exons 2 and 3 (IGF2-P1e2) and exon 3 (IGF2-P1e3) plus the coding exons. In order to amplify the P1 promoter transcripts, two pairs of primers located within exon 2 (for IGF2-P1e2) and exons 3 and 8 (for IGF2-P1e3) were used. This approach was necessary because specific amplification of transcripts derived from P1 promoter failed due to lack of suitable PCR primer sequence in exon 1. Since IGF2-transcript P1 exon 2 is part of the first exonic region of transcript IGF2-P0, and exon 3 is present in both IGF2-P0 and IGF2-P1 transcripts, the IGF2-P1e2 and -P1e3 amplicons could potentially derive from P0 and/or P1 promoters, depending on tissue and developmental stage. We quantified transcript abundances for two splice variants derived from IGF2-P2 promoter which comprise leading exon 4 (IGF2-P2e4) or leading exons 4 and 5 (IGF2-P2e5) as well as the protein coding exons. The forward primer for IGF2-P2e4 was designed to span the junction of exons 4 and 8, and for IGF2-P2e5 was in exon 5 with the reverse primer for both splice variants in exon 8. To amplify IGF2-P3 and IGF2-P4 transcripts, forward primers were designed within exons 6 and 7 with the reverse primer located within exon 8. All primers are detailed in S3 Table.

https://doi.org/10.1371/journal.pone.0200466.g001
tissue cDNA samples from different developmental stages used in cDNA pools is summarized in **S1 Table**. An equal proportion of cDNA from all tissue- and developmental stage specific cDNA pools was again pooled to generate a cDNA template for standard curve analysis. The standard curve included 3-fold serial dilutions of initial pooled cDNA template over eight data points. Three replicates were used for each dilution of the cDNA template. Real-time qPCR reactions were performed using Fast Start Universal SYBR Green Master (Roche Diagnostics GmbH, Mannheim, Germany) in an Eppendorf Mastercycler® ep realplex Real-time PCR System (Eppendorf Inc., Hamburg, Germany) following minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines [103]. The CT (threshold cycle) values of the standards were used to derive a standard curve which shows the CT values as a linear function of natural logarithm of the specified amounts of cDNA. All qPCR reactions were performed in a total volume of 12 μl, containing 6 μl of SYBR master mix, 4 μl of cDNA (equivalent to 12.5 ng of starting RNA), 0.8 μl of primers (5 pmol/μl) and 1.2 μl of double distilled nuclease-free water. PCR was carried out with a 10 minute initial denaturation/activation step at 95°C, followed by 40 cycles of 95°C for 20 seconds, 57–62°C (annealing temperatures, **S2 Table**) for 30 seconds and 72°C for 20 seconds. Tissue- and developmental stage specific qPCR reactions were performed in triplicate and all investigated tissues and developmental stages were covered in one 96 well plate for each target transcript. Target specificity and integrity was confirmed via sequencing of selected amplicons on a 3730xl DNA Analyzer (Applied Biosystems, Inc., Foster City, CA, USA), plots of the melting curve derived by Mastercycler® ep Realplex software (Eppendorf, Inc., Hamburg, Germany), and by electrophoresis of PCR products on a 2% agarose gel (Agarose low EEO, AppliChem GmbH, Darmstadt, Germany) and visual inspection under UV with Gel Doc™ 1000 Single Wavelength Mini-Transilluminator, using Quantity One image analyzing software (Bio-Rad Laboratories, Inc., Hercules, CA, USA) after staining with GelRed™ Nucleic Acid Stain (Biotium, Inc., Hayward, CA, USA). Melt-curve dissociation analyses were performed to ensure that amplifications were free of primer dimers; amplification efficiencies were ≥ 0.9.

The relative abundance of each target transcript was calculated by the relative standard curve method, with determination of PCR amplification efficiency, and expressed in relative units. Transcript abundances are presented in logarithmic scale due to the magnitude of differences between tissues and developmental stages. Since pooled cDNA was used in the quantitative real-time RT-PCR reactions for this part of the study, expression data was not normalized using reference genes. We deemed it not appropriate to perform statistical significance tests on technical replicates to compare the average transcript abundances between developmental stages. Rather, we present means and their respective standard deviations from triplicate analyses. Indeed, magnitudes of the vast majority of differences in transcript abundances are such that any statistical testing would have added no meaningful additional information.

**Contribution of IGF2 promoter specific transcripts to global IGF2 transcript**

In the second part of this study we quantified transcript abundances of IGF2 global and promoter specific transcripts in individual RNA samples of Day 153 fetuses for each of the studied tissues and using Johnson’s Relative Weight procedure determined the contribution of the promoter specific transcripts to global expression [104, 105]. This procedure is based on using individual sample values. It requires normalization against reference genes and provides robust estimates for relative promoter-specific contributions to global transcript, including confidence intervals. Each qPCR experiment for this analysis was performed in duplicate and the mean of both CTs used to calculate the amount of target transcript. An equal proportion of cDNA from
all fetuses in each tissue was pooled to generate a cDNA template for standard curve analysis. The standard curve included 2-fold serial dilutions of pooled cDNA template over eight data points, analyzed in triplicate. The relative abundance of each target transcript was automatically calculated by Mastercycler ep Realplex software (Eppendorf, Inc., Hamburg, Germany) using the relative standard curve method.

We determined expression levels of seven putative reference genes identified by a literature search, including actin beta (ACTB), ribosomal protein S9 (RPS9), ubiquitin B (UBB), H3 histone family 3A (H3F3A), TATA box binding protein (TBP) and vacuolar protein sorting 4 homolog A (VPS4A), in each fetal tissue. In placenta, expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined instead of H3F3A. Details of primers for amplification of reference genes are given in S4 Table. As the variation in expression of reference genes frequently differs between tissues, we used NormFinder [106] to identify the most stably expressed genes in each tissue for normalization following recommended procedures. NormFinder uses a model-based approach which enables ranking of reference genes based on stability values, suggesting the best combination of most stably expressed genes. Tissue-specific stability values for all putative reference genes are summarized in S5 Table. Expression levels of IGF2 global and promoter specific transcripts in each tissue were normalized to the geometric mean of the expression levels of identified reference genes [107].

The relative contribution of each promoter specific transcript to global IGF2 transcript abundance was then calculated by Johnson’s Relative Weight procedure [104, 105] using an SPSS program developed previously [108]. The following linear regression models were used to analyze tissue-specific relative contributions of promoter specific transcripts (P0, P2, P3 and P4) to global IGF2 expression:

- $IGF2_{skeletal\ muscle} = IGF2-P0 + IGF2-P2e4 + IGF2-P2e5 + IGF2-P3 + IGF2-P4$
- $IGF2_{liver} = IGF2-P2e4 + IGF2-P2e5 + IGF2-P3 + IGF2-P4$
- $IGF2_{placenta, heart, lung, kidney} = IGF2-P2e5 + IGF2-P3 + IGF2-P4$

where $IGF2$ is the relative expression normalized to the reference genes of global IGF2, and $IGF2-P0$, $IGF2-P3$ and $IGF2-P4$ are relative expression of the transcripts derived from P0, P3 and P4 promoters, respectively. $IGF2-P2e4$ (transcript with untranslated leader exon 4) and $IGF2-P2e5$ (transcript with untranslated leader exons 4 and 5) are relative expression of the splice variants derived from P2 promoter. Not every equation includes all promoters due to tissue-specific expression.

Results

Tissue- and developmental stage specific expression of IGF system components

Insulin-like growth factors 1 and 2. Across tissues and developmental stages juvenile liver and fetal skeletal muscle displayed the highest levels of global IGF1 transcript. Expression in brain and heart peaked at the fetal stage and was lower in most juvenile tissues, with a notable 60.7-fold reduction in lung (Fig 2 and S6 and S7 Tables). An exception was juvenile liver, where IGF1 expression was 36.5-fold higher than in fetal liver (Fig 2 and S7 Table). Generally, across tissues and developmental stages, the pattern of IGF1 class 1 expression was similar to global IGF1 expression, while IGF1 class 2 showed a very different pattern. In addition, postnatal increase in liver IGF1 class 1 and class 2 transcript differed significantly at 13-fold and 165-fold, respectively (Fig 2). The highest level of IGF1 class 2 transcript amongst all prenatal tissues was measured in embryonic placenta, from where it declined towards term (Fig 2).

Global IGF2 transcript levels were highest in embryonic and fetal liver, and fetal lung and skeletal muscle, while liver was the major tissue expressing IGF2 in juveniles. Brain displayed
Fig 2. Tissue-specific expression profiles of IGF system ligands in bovine pre- and postnatal developmental stages. Abundances of global IGF1 transcript and splice variants IGF1 class 1 and 2, global IGF2 transcript and promoter and splice variant-specific IGF2-P0, IGF2-P1e2, IGF2-P1e3, IGF2-P2e4, IGF2-P2e5, IGF2-P3 and IGF2-P4 transcript were measured in tissues of Day 48 embryos, Day 153 fetuses and 12–14 month-old juveniles. Placental samples were obtained from Day 48 embryos, Day 153 fetuses and term calves born by Caesarean section (C-section) at Day 277/278 of gestation. Means and standard deviations of means for each transcript and tissue were calculated based on triplicate measures of pooled cDNA comprising up to 60 embryonic cDNA samples, 73 fetal cDNA samples, 5 placental cDNA samples of C-section calves and 17 juvenile cDNA samples. Transcript abundances were calculated by the standard curve method and expressed in relative units, and are presented in logarithmic scale. ‘m’ denotes missing tissue such as kidney that is not yet present in embryos, where transcript abundances could not be determined.

https://doi.org/10.1371/journal.pone.0200466.g002
the lowest expression levels of global IGF2 transcript across all tissues and developmental stages but expression was still subject to substantial developmental change and 6-fold lower in juveniles as compared to prenatal stages (Fig 2 and S6 and S7 Tables). A drastic decline in global IGF2 expression was evident in juvenile lung where transcript abundance was 355-fold lower as compared to the fetal stage. In contrast, IGF2 expression in juvenile liver was only 4-fold lower than in fetal liver (Fig 2 and S7 Table).

Prenatal expression of transcripts from IGF2-P0 promoter was confined to fetal skeletal muscle and testis, with a 30-fold higher abundance in skeletal muscle. Postnatally, the IGF2-P0 transcript was only present in liver and at a level comparable to fetal skeletal muscle (Fig 2). The IGF2-P1e2 amplicon was not detected in embryo tissues, but present in fetal skeletal muscle, lung and liver. In juveniles, it was restricted to liver with 294-fold higher abundance than in the fetus. In the embryo, IGF2-P1e3 amplicon was only detected in liver, but by the fetal stage it was also present in heart, kidney and, at the highest level of all prenatal tissues, skeletal muscle. In juveniles this amplicon was again restricted to liver at a level 218-fold higher than at the fetal stage (Fig 2).

Comparison of expression patterns of IGF2-P2 splice variants revealed that IGF2-P2e4 expression was highest in embryonic and fetal liver and 68-fold lower in the juvenile organ. Transcript abundances for IGF2-P2e5 followed a similar pattern as IGF2-P2e4 except for lung, where it was also detected in juveniles, and for placenta, where it declined from embryo to term (Fig 2).

Expression of IGF2-P3 promoter transcript was higher in embryonic heart and liver than at the fetal stage. Apart from brain, where expression across developmental stages was very low, this transcript was subject to striking developmental changes. In juveniles, compared to the fetal stage, expression was reduced by 4639-fold in lung, 775-fold in kidney, 179-fold in skeletal muscle, 113-fold in heart and 74-fold in liver. In placenta, expression of IGF2-P3 declined throughout gestation with a 20-fold reduction from fetal stage to term (Fig 2). The developmental and tissue specific expression pattern of IGF2-P3 was similar to IGF2-P4 except for fetal lung, where IGF2-P2-P4 transcript was expressed at the same level as fetal kidney; both IGF2-P3 and IGF2-P4 expression patterns were more similar to that of global IGF2 transcript than any other IGF2 promoter specific transcript (Fig 2).

Insulin-like growth factor receptors 1 and 2 and insulin receptor. In comparison to other receptors, IR displayed less variation in expression across tissues and developmental stages (Fig 3 and S6–S8 Tables). The highest global IR transcript levels of all embryonic and postnatal tissues were measured in liver while expression at the fetal stage was highest in skeletal muscle and at similar high levels in heart, kidney, liver, lung and testis. Reduction in postnatal IR expression was modest with 3.7-, 2.8- and 1.5-fold lower global transcript levels in juvenile kidney, lung and skeletal muscle than in respective fetal tissues. In placenta, global IR transcript abundance remained constant from embryo until term (Fig 3 and S6–S8 Tables). The relative temporal-spatial expression pattern for IR-A transcript was strikingly similar to global IR (Fig 3). As compared to the fetal stage, expression of IR-A was 2-fold lower in juvenile brain, heart and liver, and 4- and 3-fold lower in postnatal kidney and lung, respectively (Fig 3). The IR-B transcript displayed a somewhat stronger postnatal decline with 2-, 4-, 6- and 9-fold lower transcript levels in juvenile heart, brain, lung, and kidney, respectively than in fetal tissues. In placenta, IR-A and IR-B transcript levels were remarkably stable throughout gestation (Fig 3).

In brain and liver, IGF1R was expressed at similar levels and declined from embryo- to fetal- and juvenile stage. Expression in heart peaked at the fetal stage. In juvenile lung and kidney, transcript levels were 17- and 20-fold lower, respectively, as compared to fetal organs. The highest transcript abundance of IGF1R for juveniles was measured in heart and skeletal muscle
Compared to other prenatal tissues, IGF1R transcript was less abundant in placenta, where it was lowest at the fetal stage and highest at term (Fig 3).

Expression of IGF2R transcript was highest in fetuses and lowest in juveniles in all investigated tissues. The increase in expression from embryo to fetal stage ranged from 2.5-fold in liver to 4.9-fold in heart with highest transcript levels observed in fetal heart, liver and lung and lowest levels in brain (Fig 3 and S6 Table). In juveniles, IGF2R expression was highest in liver followed by skeletal muscle. Lung revealed the most remarkable postnatal change with a 58.6-fold lower transcript level in juvenile compared to the fetal stage. In contrast, postnatal expression of IGF2R in skeletal muscle was only 2.4-fold lower than at the fetal stage (Fig 3 and S7 Table).

Insulin-like growth factor binding proteins. We analyzed expression patterns of high affinity IGFBP1 to 6 as well as low affinity IGFBP7 and IGFBP8 (Fig 4). The IGFBP1 gene displayed a unique expression pattern among IGFBPs with almost exclusive expression in liver at all studied developmental stages where transcript abundance in juvenile was 2-fold lower compared to prenatal stages (Fig 4, S6 and S7 Tables). At the embryo stage, IGFBP2 expression was highest in brain with a 14-fold lower transcript abundance by the fetal stage and only a slight further decline in juvenile (Fig 4, S6 and S7 Tables). By the fetal stage, and amongst all sampled tissues, the highest amount of IGFBP2 transcript was measured in liver. High expression was also detected in kidney, while the lowest amounts of IGFBP2 transcript were observed.
in fetal lung and placenta. In juvenile, IGFBP2 expression was still highest in liver at a level similar to the embryo stage, while transcript abundance in kidney, heart and skeletal muscle were 3–16-, 67- and 106-fold lower than at the fetal stage (Fig 4 and S7 Table). Expression of IGFBP2 was comparatively low and constant in embryonic and fetal placenta, and further declined by 10-fold at term (Fig 4 and S7 Table). Expression of IGFBP3 was highest in liver, where it remained stable across developmental time points, and in placenta and testis. Expression was lower in heart and brain, with a peak at the fetal stage. The 15-fold decline in postnatal expression of IGFBP3 in lung was higher than in any other tissue (Fig 4 and S7 Table). The high level of embryonic placental IGFBP3 expression was also observed at late gestation (Fig 4). The
IGFBP4 gene was the only gene from the IGFBP family whose expression showed a postnatal organ-specific increase in expression level. Here, transcript abundance in juvenile liver was 4.5-fold higher than at the fetal stage. In other juvenile tissues, transcript abundances were lower than at the fetal stage and at a similar level with a more pronounced 18-fold reduction in skeletal muscle. Brain and placenta exhibited the lowest IGFBP4 transcript levels at all developmental stages (Fig 4 and S7 Table). Expression of IGFBP5 peaked at the fetal stage in brain, heart and liver and declined in juvenile tissues. The highest transcript levels of IGFBP5 were measured in fetal heart and kidney. Brain and lung showed the strongest postnatal decline by 16.4- and 13-fold, respectively. In juveniles, IGFBP5 transcript abundance was highest in heart and skeletal muscle and lowest in brain and liver (Fig 4 and S7 Table). Placenta displayed comparatively low and stable expression levels of IGFBP5 from embryo to term (Fig 4). Abundance of IGFBP6 transcript increased from embryo to fetal stage and was highest in fetal testis and skeletal muscle. Expression remained high in juvenile skeletal muscle and only kidney and lung displayed a 6-fold decline in transcript abundance compared to the fetal stage. Similar to IGFBP5, expression of IGFBP6 was lowest in liver and placenta, but unlike IGFBP5, expression did not decline markedly in postnatal brain and liver (Fig 4 and S7 Table).

We measured transcript abundances for two low affinity IGFBPs, IGFBP7 and 8. Expression of IGFBP7 was highest in skeletal muscle and heart and lowest in placenta at all developmental stages. At the fetal stage, transcript abundance in skeletal muscle was substantially higher than in other tissues followed by a 12.7-fold decline in juvenile skeletal muscle. Postnatal expression of IGFBP7 in kidney also declined by 10-fold (Fig 4 and S7 Table). Abundance of IGFBP8 transcripts was highest in heart and lowest in placenta at all developmental stages. Fetal and juvenile skeletal muscle and fetal testis also showed high levels of IGFBP8 expression. Expression of IGFBP8 in juvenile tissues was lower with an approximately 6-fold decline in liver and kidney from the fetal stage (Fig 4 and S7 Table).

**Tissue- and developmental stage specific expression of IncRNAs**

We investigated expression of the two imprinted long non-coding RNA genes associated with the IGF system, H19 and AIRN. Across all tissues, expression of H19 was highest in the embryo, declined slightly by the fetal stage and was substantially lower in juvenile (Fig 5, S6 and S7 Tables). Embryonic and fetal liver displayed the highest, and brain the lowest, H19 transcript levels of all prenatal tissues. Similar and comparatively high levels of H19 transcript were found in fetal kidney, lung and skeletal muscle with somewhat lower levels in heart and testis. Kidney displayed the strongest decline in postnatal H19 expression with more than 100-fold lower transcript abundance as compared to the fetal stage. Amongst postnatal tissues, H19 transcript level was highest in skeletal muscle which displayed a 9-fold reduction from fetal to juvenile stage. Expression of H19 in placenta declined 2-fold from embryo to fetal stage and was unchanged at term (Fig 5, S7 and S8 Tables).

Expression of AIRN transcript increased from embryo to fetal stage and declined in juvenile tissues (Fig 5, S6 and S7 Tables). Across developmental stages AIRN transcript was most abundant in liver with 10-fold lower expression in juvenile. Expression was also high in fetal kidney and lung, which showed the strongest decline in transcript abundance with 58- and 29-fold lower transcript levels in juvenile. Brain and heart displayed similar AIRN expression levels at the embryo and juvenile stages and brain, heart and skeletal muscle had a similar, milder reduction in expression level in juvenile as compared to fetal tissues (Fig 5, S7 Table). Placental expression of AIRN was similar at the embryo and fetal stage, but 6-fold lower at term (Fig 5 and S8 Table).
Contribution of promoter specific IGF2 transcripts to global IGF2 transcript abundance in fetal tissues

We determined for the first time the contribution of promoter-specific IGF2 transcripts (Figs 1 and 2) to global IGF2 transcript in fetal tissues including placenta by multiple regression analysis (Fig 6). Estimated means with 95% confidence intervals are presented in S9 Table. Contributions of 53%, 61%, 72% and 90% to global IGF2 transcript identified IGF2-P4 as the predominant promoter in bovine fetal liver, lung, heart and kidney, respectively. This promoter was also responsible for 64% of all IGF2 transcripts measured in placenta. Amongst all tissues studied, IGF2-P4 was least active in skeletal muscle but it still contributed 28% of global transcript. The second most abundant transcript in fetal lung, heart and placenta was IGF2-P2 derived IGF2-P2e5, accounting for 31%, 24%, and 24% of global transcript, respectively. However in fetal liver and skeletal muscle, the alternative splice variant derived from IGF2-P2 promoter, IGF2-P2e4 transcript, dominated and accounted for 44% and 35% of global IGF2 expression, respectively. Although IGF2-P0 accounted for 30% of global IGF2 and was therefore one of the most common IGF2 transcripts in muscle, it did not contribute to IGF2 expression in any other fetal tissue or placenta. Promotor IGF2-P3 was active at low levels with contributions of 1–8% in fetal tissues and 12% in placenta.

Discussion

We provide here an atlas of tissue- and developmental stage specific gene expression for the bovine insulin-like growth factor (IGF) system, including imprinted long non-coding (Inc) RNAs H19 and AIRN. This mammalian IGF expression catalogue informs basic and comparative IGF research and provides reference data for an important agricultural species and biomedical model.

Our comprehensive profile of expression patterns and comparisons of pre- and postnatal changes in expression of IGF ligands support established roles of IGF1 in growth and development [109]. Across developmental stages and tissues, global IGF1 expression was highest in juvenile liver, consistent with data from sheep [31, 110], mouse [111] and rat [112–114]. However, IGF1 expression in all other tissues peaked at the fetal stage, a clear indication of the
significant role of IGF1 in mammalian prenatal growth [2, 97, 115, 116]. As reported previously [117], expression was highest in fetal muscle where IGF1 has protein anabolic effects [118]. The expression pattern for IGF1 class 1 transcript was more similar to IGF1 global.

Fig 6. Relative contribution of promoter and splice variant-specific IGFB2 transcripts to global IGFB2 transcript abundance in fetal tissues and placenta. IGFB2-P0, IGFB2-P3 and IGFB2-P4 are percent transcript abundance derived from P0, P3 and P4 promoters, respectively. Splice variants of promoter P2 transcript are IGFB2-P2e4 with untranslated leader exon 4 and IGFB2-P2e5 with untranslated leader exons 4 and 5. Estimated means are from 73 fetal cDNA samples per tissue and 95% confidence intervals are detailed in S9 Table. Transcript abundances were calculated by the standard curve method, normalized with reference genes and expressed in relative units. The relative contribution of each promoter-specific transcript to global IGFB2 transcript abundance was calculated by Johnson’s Relative Weight procedure.

https://doi.org/10.1371/journal.pone.0200466.g006
transcript, suggesting that class 1 is the predominant transcript across tissues and developmental stages. In skeletal muscle and liver of the third trimester sheep fetus, IGF1 class 1 transcript was also much more abundant than class 2 transcript [97]. Expression of IGF1 class 2 transcript displayed considerable developmental stage and tissue specificity, with strongest expression in postnatal liver. Stronger postnatal hepatic expression of IGF1 class 2 compared to class 1 transcript could be due to greater dependency of this transcript on growth hormone as shown in sheep [110]. Our data thus extends previous limited information on IGF1 class 1 and 2 transcript expression in pre- and postnatal tissues of cattle [119, 120], sheep [97, 110, 121], pig [122, 123], mouse [124] and rat [125] to earlier embryo-fetal stages.

We found that expression of IGF2 was broadly similar in embryonic and fetal bovine tissues, but much lower in juvenile tissues. Postnatal decline in expression of global IGF2 transcript was considerably stronger than for IGF1, highlighting the special role of IGF2 in prenatal growth and development described previously [126–128]. Significant downregulation of IGF2 after birth has been reported for cattle, sheep and human [90–92, 98, 129]. In rat, IGF2 transcripts were undetectable by Northern blot in all adult tissues except brain, spinal cord and striated muscle [13, 130, 131]. Postnatal decline in IGF2 expression was least in liver, the major source of circulating IGF2 in adults [8] and consistent with considerable IGF2 levels in adult cattle [132]. The higher expression of IGF2 in postnatal liver compared to other tissues may be caused by tissue-specific relaxation of IGF2 imprinting or a change from imprinted to non-imprinted promoter use as previously demonstrated in human [37, 133]. The exclusive expression of IGF2-P0 transcript in juvenile liver and high levels of IGF2-P1e2 and -P1e3 transcripts in this tissue may reflect a combined imprinted/non-imprinted promoter scenario. In human, P0 transcript is expressed from the paternal allele while P1 transcripts show biallelic expression at all developmental stages [37, 50].

Exclusive expression of IGF2-P0 transcript in bovine fetal skeletal muscle and testis, is in agreement with reported IGF2-P0 expression in human fetal skeletal muscle [50]. To our knowledge fetal testis has not yet been tested for expression of IGF2-P0 transcript in human or any other species. Human and bovine thus indicate an evolutionary shift in IGF2 expression from mouse, where Igf2-P0 transcript is confined to placenta [127]. We also demonstrate for the first time a developmental shift in tissue-specificity of IGF2-P0, which is no longer active in juvenile bovine skeletal muscle but active in juvenile liver. This contrasts with ubiquitous IGF2-P0 expression in adult human tissues [50] although some of these differences may be explained by the different developmental age of bovine tissues studied.

Splice variants of IGF2-P1 transcripts including exon 1, which is alternatively spliced onto exons 2 and/or 3 as well as the coding exons, were previously observed in bovine [16], pig [44], human [134, 135] and sheep [42]. Considering sequence based restrictions of our IGF2 promoter 1-specific transcript amplification strategy described in Fig 1, and our finding that IGF2-P0 transcript is the predominant transcript in bovine fetal skeletal muscle, IGF2-P1e2 and IGF2-P1e3 transcripts detected in this tissue could potentially originate from the IGF2-P0 promoter. However, we conclude that IGF2-P1e2 and IGF2-P1e3 transcripts in liver, whose abundance increased in postnatal tissue, predominantly derive from IGF2-P1 promoter activity. This is based on an IGF2-P0 expression level in juvenile liver similar to that in fetal skeletal muscle and the fact that relative transcript abundance of IGF2-P1e2 and IGF2-P1e3 in juvenile liver was 130-fold and 15-fold higher than in fetal muscle. Our demonstration of increased activity of IGF2-P1 promoter in postnatal liver where IGF2-P2, IGF2-P3 and IGF2-P4 activity decreases, is consistent with the developmental shift in promoter activity reported for human [46, 50], pig [44], sheep [42, 91] and bovine [92].

Temporal-spatial expression patterns of the two splice variants that originate from IGF2-P2 promoter, IGF2-P2e4 and P2e5, showed some similarities (Fig 2), but quantitative analyses of
promoter-specific transcripts in fetal tissues revealed that only IGF2-P2e4 variant is a major contributor to IGF2 transcript abundance in fetal liver and skeletal muscle (Fig 6). This suggests that IGF2-P2e4 could be actively involved in the production of endocrine IGF2 in the bovine fetus.

Analysis of contributions of promoter specific IGF2 transcripts to global IGF2 transcript expression revealed that IGF2-P3 and IGF2-P4 are the least and most active promoters, respectively, in all bovine fetal tissues except kidney. This is in agreement with data from fetal mouse and rat, where IGF2-P3, which is orthologous to bovine IGF2-P4 promoter, was most active [48], but differs somewhat from human where IGF2-P3 and IGF2-P4, both orthologues of the respective bovine promoters, were active at high and moderate levels [136].

Amongst receptors studied, relative spatio-temporal expression patterns for global IR and splice variant IR-A were more stable than those for IGF1R and IGF2R. This may be explained by predominant involvement of insulin receptors in metabolic pathways, rather than in engendering growth responses [137]. Mice without Insr display virtually unimpaired prenatal development with only slight reduction in birth weight [70] and catastrophic loss of metabolic control only after birth [7, 138]. The similarity in expression patterns of IR global transcript and IR-A suggests that IR-A is the predominant isoform of insulin receptor expressed in tissues. The most notable change, and consistent with other relative changes in expression patterns in the IGF system, was the decline in postnatal IR-B transcript in kidney and lung.

The similarity in expression patterns of IGF2R and IGF2 is consistent with the crucial role of IGF2R for normal development [139]. Observed concomitant downregulation of IGF2R and IGF2 could be expected from the essential regulatory function of IGF2R for IGF2 bioavailability [7, 61, 96, 140]. Evidence for active signaling of IGF2 through IGF2R has been reported and several lines of evidence suggest that IGF2 stimulates trophoblast migration through IGF2R [72, 141, 142]. This is consistent with the stable expression of IGF2 and IGF2R in placenta from embryo to fetal stage and the decline in both transcripts at term in the present study.

Transcript abundances for IGFBPs revealed exclusive strong IGFBP1 expression in liver across all developmental stages. A similar expression pattern has been reported for fetal liver in mouse and human [143, 144] and fetal and adult liver in rat [145, 146] and reflects the endocrine role of IGFBP1 [147–150]. The postnatal decrease in expression of IGFBP2 in skeletal muscle, heart and kidney suggests a significant role before birth. This is supported by higher circulating IGFBP2 levels in the fetus as compared to adults in a number of species including rat, human, pig and rhesus monkey [99, 151–154]. The relative expression pattern of IGFBP3 in postnatal tissues showed similarity to the pattern observed for postnatal IGFI expression. In the sheep fetus, circulating IGFBP3 levels are positively correlated with IGFI [117, 155].

Expression of IGFBP3 in placenta remained high throughout gestation. In human and rhesus monkey placenta IGFBP3 is co-expressed with IGF2 and has been proposed to modulate IGF2 effects in an autocrine/paracrine fashion [156, 157]. The IGFBP4 gene stands out as the only binding protein gene that displayed an increase in transcript abundance in a juvenile tissue, i.e., liver. This is consistent with increased IGFBP4 expression in neonatal pig liver [100] and increased circulating IGFBP4 in sheep after birth [155]. It is possible that the postnatal increase in hepatic IGFBP4 expression is associated with increased expression of IGFI. It has been demonstrated that IGFBP4 enhances growth stimulatory effects of endocrine IGFI by increasing bioavailability of IGFI via an IGFBP4 protease-dependent mechanism [158]. High levels of IGFBP6 expression in fetal and juvenile bovine skeletal muscle may be explained by the involvement of this gene in inhibiting IGF2-induced proliferation and differentiation of myoblasts [159]. Similarly, high level of IGFBP7 expression in bovine fetal skeletal muscle may also be associated with a potential role in inhibiting myoblast differentiation [160, 161].
The most pronounced changes in transcript levels from pre- to postnatal stages were evident in lncRNAs, consistent with the fundamental roles of this RNA class in growth and development [162–165]. Substantially greater spatio-temporal changes in expression of H19 as compared to AIRN highlights the pivotal functions of H19 RNA as source of regulatory miRNA that impact IGF1R and Smad transcription factors [57, 166] and thus contribute to its role as a major regulator of an imprinted gene network [167] that controls growth [168]. Across tissues and for each developmental stage, relative expression patterns for H19 were highly similar to those for IGF2, which is consistent with current models for coordinated regulation of both genes [169–171]. Cell lineage dependent H19 expression has been described in sheep [172] and the human fetus [173] where, as in the present study, transcript abundance was lowest in brain. We found that expression of H19 in bovine fetal kidney, lung and liver was at similar high levels or higher than in fetal skeletal muscle, where it was originally described as a major regulator of prenatal growth and differentiation [168]; whether H19 has a similar role in these tissues remains to be elucidated. Expression of H19 in juvenile tissues was much lower than at prenatal stages, except for skeletal muscle, where significant transcription persists. This suggests that growth regulatory functions of H19 in bovine prenatal muscle [168] may continue well into postnatal development. High levels of H19 RNA have also been found in postnatal skeletal muscle of mouse, where H19 encoded miRNAs promote differentiation and regeneration [166].

In light of observed similarities in H19/IGF2 expression patterns, the different spatio-temporal patterns for AIRN and IGF2R transcript abundances are somewhat unexpected. Considering the antisense nature of AIRN/IGF2R transcripts and their mutually exclusive, interdependent mode of expression in mouse, where AIRN RNA silences IGF2R [81, 174, 175] an inverse relationship could have been expected. The seemingly unrelated expression patterns may indicate species differences and/or further, possibly organ-specific, roles of AIRN in silencing additional imprinted genes [175] or involvement in trans-regulatory processes similar to those identified for H19 [165].

Lung and kidney showed the highest and liver the lowest relative postnatal reduction in expression of IGF system components. This may be explained by additional prenatal functions of lung and kidney as the flow of fluid from the fetal lung and bladder are major contributors to amniotic fluid [176, 177]. Interestingly, amniotic fluid is a significant source of IGFs [178] as large amounts of fluid are swallowed by the fetus [179] and growth factors cross the gut to enter systemic circulation [180]. The lesser changes in postnatal liver likely reflect the continuing role of this organ as a major source of circulating IGF system components [8, 181–183].

To our knowledge, this is the first comprehensive study in any species to investigate changes in expression of IGF system components and their major regulatory lncRNAs across tissues and developmental stages using real time qPCR. Our expression atlas for the bovine insulin-like growth factor system provides important reference data for future studies of the mammalian IGF system. This includes dissection of prenatal effects on postnatal phenotype where IGF system components and epigenetic mechanisms regulating them, including imprinting and miRNAs, appear to be major programming components [181, 184–187].

Supporting information

S1 Table. Number and sex of individuals used for developmental stage and tissue-specific cDNA pools.

(DOCX)

S2 Table. RNA integrity number (RIN) of RNA extracted from different tissues.

(DOCX)
S3 Table. Details of forward (F) and reverse (R) primers used for amplification of transcripts of target genes.

(S3 Table)

S4 Table. Details of forward (F) and reverse (R) primers used for amplification of transcripts of reference genes.

(S4 Table)

S5 Table. Stability values for studied reference genes and best combination of genes for different tissues as calculated by NormFinder.

(S5 Table)

S6 Table. Comparison of changes in gene expression in the bovine IGF system between Day 48 embryo and Day 153 fetal stages.

(S6 Table)

S7 Table. Comparison of changes in gene expression in the bovine IGF system between Day 153 fetal and 12–14 month juvenile stages.

(S7 Table)

S8 Table. Comparison of changes in gene expression in the bovine IGF system of placenta from embryo stage to term.

(S8 Table)

S9 Table. Estimated relative contributions of IGF2 promoter-specific transcripts to global IGF2 transcript abundance with 95% confidence intervals for each fetal tissue.

(S9 Table)

Acknowledgments

We are grateful for access to South Australian Research Development Institute research facilities and to Struan Research Centre staff for animal management. We would also like to thank former members of the JS Davies Epigenetics and Genetics Group for their help in sample collection.

Author Contributions

Conceptualization: Karen L. Kind, Stefan Hiendleder.

Data curation: Mani Ghanipoor-Samami, Ali Javadmanesh.

Formal analysis: Mani Ghanipoor-Samami, Ali Javadmanesh, Consuelo Amor S. Estrella, Karen L. Kind.

Funding acquisition: Stefan Hiendleder.

Investigation: Mani Ghanipoor-Samami, Ali Javadmanesh, Brian M. Burns, Dana A. Thomsen, Consuelo Amor S. Estrella, Stefan Hiendleder.

Methodology: Mani Ghanipoor-Samami, Ali Javadmanesh, Greg S. Nattrass, Karen L. Kind, Stefan Hiendleder.

Project administration: Dana A. Thomsen, Stefan Hiendleder.

Resources: Brian M. Burns, Greg S. Nattrass, Stefan Hiendleder.

Supervision: Karen L. Kind, Stefan Hiendleder.
Validation: Mani Ghanipoor-Samami, Ali Javadmanesh, Karen L. Kind, Stefan Hiendleder.

Visualization: Mani Ghanipoor-Samami, Ali Javadmanesh.

Writing – original draft: Mani Ghanipoor-Samami.

Writing – review & editing: Karen L. Kind, Stefan Hiendleder.

References
1. Allan GJ, Flint DJ, Patel K. Insulin-like growth factor axis during embryonic development. Reproduction. 2001; 122(1):31–9. PubMed PMID: WOS:000169731400004. PMID: 11425327
2. Woods KA, Camacho-Hübner C, Savage MO, Clark AJL. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med. 1996; 335(18):1363–7. https://doi.org/10.1056/NEJM199610313351805 PMID: 8857020.
3. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth Factor I in mouse postnatal growth. Dev Biol. 2001; 229(1):141–62. https://doi.org/10.1006/dbio.2000.9975. PMID: 11133160
4. Baker J, Liu J-P, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. Cell. 1993; 75(1):73–82. https://doi.org/10.1016/s0092-8674(05)80085-6 PMID: 8402902
5. Kim H-S, Nagalla SR, Oh Y, Wilson E, Roberts CT, Rosenfeld RG. Identification of a family of low-affinity insulin-like growth factor binding proteins (IGFBPs): Characterization of connective tissue growth factor as a member of the IGFBP superfamily. Proc Natl Acad Sci U S A. 1997; 94(24):12981–6. PMID: 9371786
6. Denley A, Cosgrove LJ, Booker GW, Wallace JC, Forbes BE. Molecular interactions of the IGF system. Cytokine Growth Factor Rev. 2005; 16(4):421–39. https://doi.org/10.1016/j.cytogfr.2005.04.004.
7. Nakae J, Kido Y, Accili D. Distinct and overlapping functions of insulin and IGF-I receptors. Endocr Rev. 2001; 22(6):818–35. https://doi.org/10.1210/edrv.22.6.0452 PMID: 11739335.
8. O’Dell SD, Day INM. Molecules in focus Insulin-like growth factor II (IGF-II). Int J Biochem Cell Biol. 1998; 30(7):767–71. https://doi.org/10.1016/S1357-2725(98)00048-X. PMID: 9722981
9. Roth DL, Scavo L, Butler A. What is the role of circulating IGF-I? Trends Endocrinol Metab. 2001; 12(2):48–52. https://doi.org/10.1016/S1043-2760(00)00349-0. PMID: 11167121
10. Holly JMP, Wass JAH. Insulin-like growth factors; autocrine, paracrine or endocrine? New perspectives of the somatomedin hypothesis in the light of recent developments. J Endocrinol. 1989; 122(3):611–8. https://doi.org/10.1677/joe.0.1220611 PMID: 2478648
11. Klover P, Hennighausen L. Postnatal body growth is dependent on the transcription factors signal transducers and activators of transcription 5a/b in muscle: A role for autocrine/paracrine insulin-Like growth factor I. Endocrinology. 2007; 148(4):1489–97. https://doi.org/10.1210/en.2006-1431 PMID: 17158201.
12. Shoba L, An MR, Frank SJ, Lowe Jr WL. Developmental regulation of insulin-like growth factor-I and growth hormone receptor gene expression. Mol Cell Endocrinol. 1999; 152(1–2):125–36. https://doi.org/10.1016/S0303-7207(99)00045-3. PMID: 10432320
13. Brown AL, Graham DE, Nisley SP, Hill DJ, Strain AJ, Rechler MM. Developmental regulation of insulin-like growth factor II mRNA in different rat tissues. J Biol Chem. 1986; 261(28):13144–50. PMID: 3759952
14. Jeon JT, Carlborg O, Tornsten A, Giaufra E, Amarger V, Chardon P, et al. A paternally expressed QTL affecting skeletal and cardiac muscle mass in pigs maps to the IGF2 locus. Nat Genet. 1999; 21(2):157–8. PubMed PMID: ISI:000076399500013. https://doi.org/10.1038/3938 PMID: 99886263
15. Vykoukalová Z, Knoll A, Dvořák J, Čepička S. New SNPs in the IGF2 gene and association between this gene and backfat thickness and lean meat content in Large White pigs. J Anim Breed Genet. 2006; 123(3):204–7. https://doi.org/10.1111/j.1439-0388.2006.00580.x PMID: 16706926
16. Goodall JJ, Schmutz SM. IGF2 gene characterization and association with rib eye area in beef cattle. Anim Genet. 2007; 38(2):154–61. https://doi.org/10.1111/j.1365-2052.2007.01576.x PubMed PMID: ISI:000245311300010. PMID: 17403010
17. Heude B, Ong KK, Luben R, Wareham NJ, Sandhu MS. Study of association between common variation in the insulin-like growth factor 2 gene and indices of obesity and body size in middle-aged men and women. J Clin Endocrinol Metab. 2007; 92(7):2734–8. https://doi.org/10.1210/jc.2006-1948 PMID: 17488802.
18. Fan B, Onteru SK, Du Z-Q, Garrick DJ, Stalder KJ, Rothschild MF. Genome-wide association study identifies loci for body composition and structural soundness traits in pigs. PLoS One. 2011; 6(2). https://doi.org/10.1371/journal.pone.0014726 PubMed PMID: WOS:000287761700004. PMID: 21383979

19. Van Laere AS, Nguyen M, Braunenschweig M, Nezer C, Collette C, Moreau L, et al. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature. 2003; 425(6960):832–6. https://doi.org/10.1038/nature02064 PubMed PMID: ISI:000186118500044. PMID: 14574411

20. Sutter NB, Bustamante CD, Chase K, Gray MM, Zhao K, Zhu L, et al. A single IGF1 allele is a major determinant of small size in dogs. Science. 2007; 316(5821):1102–5. https://doi.org/10.1126/science.1137045 PMID: 17412960

21. Rosen CJ, Churchill GA, Donahue LR, Shultz KL, Burgess JK, Powell DR, et al. Mapping quantitative trait loci for serum insulin-like growth factor-1 levels in mice. Bone. 2000; 27(4):521–8. https://doi.org/10.1016/1098-2795(00)00354-9. PMID: 11033447

22. Zhao Q, Davis ME, Hines HC. Associations of an AciI polymorphism in the IGF-II gene with growth traits in beef cattle. 7th World Congress on Genetics Applied to Livestock Production; Montpellier, France 2002.

23. Zwierzchowski L, Siatkowska E, Oprzadek J, Flisikowski K, Dymnicki E. An association of C/T polymorphism in exon 2 of the bovine insulin-like growth factor 2 gene with meat production traits in Polish Holstein-Friesian cattle. Czech J Anim Sci. 2010; 55(6):227–33. PubMed PMID: WOS:000279340300002.

24. Sherman EL, Nikrumah JD, Murdoch BM, Li C, Wang Z, Fu A, et al. Polymorphisms and haplotypes in the bovine neuropeptide Y, growth hormone receptor, ghrelin, insulin-like growth factor 2, and uncoupling proteins 2 and 3 genes and their associations with measures of growth, performance, feed efficiency, and carcass merit in beef cattle. J Anim Sci. 2006; 86(1):1–16. https://doi.org/10.2527/jas.2006-799 PubMed PMID: 17785604

25. Ouni M, Gunes Y, Belot M-P, Castell A-L, Fradin D, Bougnères P. The IGF1 P2 promoter is an epigenetic QTL for circulating IGF1 and human growth. Clin Epigenetics. 2015; 7(1):1–12. https://doi.org/10.1186/s13148-015-0062-8 PMID: 25789079

26. Lonergan P, Gutierrez-Adan A, Pintado B, Fair T, Ward F, De La Fuente J, et al. Relationship between time of first cleavage and the expression of IGF-I growth factor, its receptor, and two housekeeping genes in bovine two-cell embryos and blastocysts produced in vitro. Mol Reprod Dev. 2000; 57(2):146–52. https://doi.org/10.1002/1098-2795(200010)57:2<146::AID-MRD5>3.0.CO;2-E PubMed PMID: WOS:000089086100005. PMID: 10984414

27. Lighten AD, Hardy K, Winston RML, Moore GE. Expression of mRNA for the insulin-like growth factors and their receptors in human preimplantation embryos. Mol Reprod Dev. 1997; 47(2):134–9. https://doi.org/10.1002/1098-2795(199706)47:2<134::AID-MRD2>3.0.CO;2-N PubMed PMID: WOS:A1997WW60700002. PMID: 91361113

28. Rotwein P, Pollock KM, Watson M, Milbrandt JD. Insulin-like growth factor factor expression during rat embryonic development. Endocrinology. 1987; 121(6):2141–4. https://doi.org/10.1210/endo-121-6-2141 PMID: 3678142

29. Fu Q, Yu X, Callaway CW, Lane RH, McKnight RA. Epigenetics: intrauterine growth retardation (IUGR) modifies the histone code along the rat hepatic IGF-1 gene. FASEB J. 2009; 23(8):2438–49. https://doi.org/10.1096/fj.08-124768 PMID: 19364764

30. Jansen E, Steenbergh PH, Vanschaik FMA, Susenbach JS. The human IGF-I gene contains two cell type-specifically regulated promoters. Biochem Biophys Res Commun. 1992; 187(3):1219–26. https://doi.org/10.1016/0006-291x(92)90433-I PubMed PMID: WOS:A1992JQ81200003. PMID: 14177977

31. Dickson MC, Saunders JC, Gilmour RS. The ovine insulin-like growth factor-I gene: characterization, expression and identification of a putative promoter. J Mol Endocrinol. 1991; 6(1):17–31. https://doi.org/10.1677/jme.0.0060017 PMID: 2015053

32. LeRoith D, Roberts CT. Insulin-like growth factor I (IGF-I): A molecular basis for endocrine versus local action? Mol Cell Endocrinol. 1991; 77(1):C57–C61. https://doi.org/10.1016/0303-7207(91)90054-V

33. DeChiara TM, Robertson EJ, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. Cell. 1991; 64(4):849–59. PMID: 1997210

34. Giannoukakis N, Deal C, Paquette J, Goodyer CG, Polychronakos C. Parental genomic imprinting of the human IGF2 gene. Nat Genet. 1993; 4(1):98–101. https://doi.org/10.1038/ng0593-98 PubMed PMID: WOS:A1993LA33700022. PMID: 8099843

35. McLaren RJ, Montgomery GW. Genomic imprinting of the insulin-like growth factor 2 gene in sheep. Mamm Genome. 1999; 10(6):588–91. PubMed PMID: ISI:000080383300012. PMID: 10341091
36. Dindot SV, Kent KC, Evers B, Loskutoff N, Womack J, Piedrahita JA. Conservation of genomic imprinting at the XIST, IGF2, and GTL2 loci in the bovine. Mamm Genome. 2004; 15(12):966–74. https://doi.org/10.1007/s00335-004-2407-z PubMed PMID: ISI:000225371100004. PMID: 15599555

37. Vu TH, Hoffman AR. Promoter-specific imprinting of the human insulin-like growth factor-II gene. Nature. 1994; 371(6499):714–7. https://doi.org/10.1038/371714a0 PMID: 7935819

38. Ekstrom TJ, Cui H, Li X, Ohlsson R. Promoter-specific IGF2 imprinting status and its plasticity during human liver development. Developmental. 1995; 121(2):309–16. PMID: 7768174

39. Qi Y, Ma N, Yan F, Yu Z, Wu G, Qiao Y, et al. The expression of intronic miRNAs, miR-483 and miR-483*, and their host gene, Igf2, in murine osteoarthritic cartilage. Int J Biol Macromol. 2013; 61:43–9. https://doi.org/10.1016/j.ijbiomac.2013.06.006. PMID: 23791756

40. Ma N, Wang X, Qiao Y, Li F, Hui Y, Zou C, et al. Coexpression of an intronic microRNA and its host gene reveals a potential role for miR-483-5p as an IGF2 partner. Mol Cell Endocrinol. 2011; 333(1):96–101. https://doi.org/10.1016/j.mce.2011.11.027. PMID: 21146586

41. Qiao Y, Zhao Y, Liu Y, Ma N, Wang C, Zou J, et al. miR-483-3p regulates hyperglycemia-induced cardiomyocyte apoptosis in transgenic mice. Biochem Biophys Res Commun. 2016; 477(4):541–7. https://doi.org/10.1016/j.bbrc.2016.06.051. PMID: 27346130

42. Ohlsen SM, Lugenebel KA, Wong EA. Characterization of the linked ovine insulin-like growth factor-II genes. DNA Cell Biol. 1994; 13(4):377–88. PubMed PMID: WOS:A1994NL87100006. https://doi.org/10.1089/dna.1994.13.377 PMID: 8011164

43. Curchoe C, Zhang SQ, Bin YF, Zhang XQ, Yang L, Feng DY, et al. Promoter-specific expression of the imprinted IGF2 gene in cattle (Bos taurus). Biol Reprod. 2005; 73(6):1275–81. https://doi.org/10.1095/biolreprod.105.044727 PubMed PMID: WOS:000233580700024. PMID: 16120826

44. Amarger V, Nguyen M, Van Laere AS, Braunschweig M, Nezer C, Georges M, et al. Comparative sequence analysis of the INS-IGF2-H19 gene cluster in pigs. Mamm Genome. 2002; 13(7):388–98. https://doi.org/10.1007/s00335-001-3059-x PubMed PMID: ISI:000177236700009. PMID: 12140686

45. Ohlsson R, Hedborg F, Holmgren L, Walsh C, Ekstrom TJ. Overlapping patterns of IGF2 and H19 expression during human development: biallelic IGF2 expression correlates with a lack of H19 expression. Development. 1994; 120(2):361–8. PubMed PMID: WOS:A1994MW03600012. PMID: 8149914

46. Depagterholthuizen P, Jansen M, Vanderkammen R, Oosterwijk C, Vandenbrande JL, et al. The human insulin-like growth factor-II gene contains 2 development-specific promoters. FEBS Lett. 1987; 214(2):259–64. PubMed PMID: WOS:A1987H130300007. PMID: 3569524

47. van Dijk MA, van Schaik FMA, Bootsma HJ, Holthuizen P, Sussenbach JS. Initial characterization of the four promoters of the human insulin-like growth factor II gene. Mol Cell Endocrinol. 1991; 81(1-2):81–94. https://doi.org/10.1016/0303-7207(91)90207-9. PMID: 20634819

48. Holthuizen PE, Cleijnsen CBJM, Veenstra GJC, van der Lee FM, Koonen-Reemst AMCB, Sussenbach JS. Differential expression of the human, mouse and rat IGF-II genes. Regul Pept. 1993; 48(1–2):77–89. PMID: 8265819

49. Moore T, Constancia M, Zubair M, Bailleul B, Feil R, Sasahki H, et al. Multiple imprinted sense and antisense transcripts, differential methylation and tandem repeats in a putative imprinting control region upstream of mouse IGF2. Proc Natl Acad Sci U S A. 1997; 94(23):12509–14. PubMed PMID: WOS:00019974F39300049. PMID: 9356480

50. Monk D, Sanches R, Arnaud P, Apostolidou S, Hills FA, Abu-Amero S, et al. Imprinting of IGF2P0 transcript and novel alternatively spliced INS-IGF2 isoforms show differences between mouse and human. Hum Mol Genet. 2006; 15(8):1259–69. https://doi.org/10.1093/hmg/ddi041 PubMed PMID: WOS:000236613300002. PMID: 16531418

51. Tran VG, Court F, Duputie A, Antoine E, Apetl N, Milligan L, et al. H19 antisense RNA can up-regulate IGF2 transcription by activation of a novel promoter in mouse myoblasts. PLoS One. 2012; 7(5):e37923. https://doi.org/10.1371/journal.pone.0037923 PMID: 22662250

52. Engel N, West AG, Felsenfeld G, Bartolomei MS. Antagonism between DNA hypermethylation and enhancer-blocking activity at the H19-DMD is uncovered by CpG mutations. Nat Genet. 2004; 36(8):883–8. https://doi.org/10.1038/ng1399 PMID: 15273688

53. Ainscough JFX, Dandolo L, Azim Surani M. Appropriate expression of the mouse H19 gene utilises three or more distinct enhancer regions spread over more than 130 kb. Mech Dev. 2000; 91(1–2):365–8. https://doi.org/10.1016/S0925-4773(99)00289-0. PMID: 10704866

54. Bartolomei MS, Zemel S, Tilghman SM. Parental imprinting of the mouse H19 gene. Nature. 1991; 351(6322):153–5. https://doi.org/10.1038/351153a0 PMID: 1709450

55. Zhang SQ, Kubota C, Yang L, Zhang YQ, Page R, O'Neill M, et al. Genomic imprinting of H19 in naturally reproduced and cloned cattle. Biol Reprod. 2004; 71(5):1540–4. https://doi.org/10.1095/bi orep.104.031807 PubMed PMID: ISI:000224713000018. PMID: 15240429
56. Li C, Bin YF, Curchoe C, Yang L, Feng DY, Jiang QY, et al. Genetic imprinting of H19 and IGF2 in domestic pigs (Sus scrofa). Anim Biotechnol. 2008; 19(1):22–7. https://doi.org/10.1080/10495390701758563 PubMed PMID: WOS:000258687500003. PMID: 18228173

57. Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, et al. The H19 IncRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. Nat Cell Biol. 2012; 14(7):659–65. http://www.nature.com/ncb/journal/v14/n7/abs/ncb2521.html#supplementary-information. PMID: 22684254

58. Xiang R, Lee AMC, Eindorf T, Javadmanesh A, Ghanipoor-Samami M, Gugger M, et al. Widespread differential maternal and paternal genome effects on fetal bone phenotype at mid-gestation. J Bone Miner Res. 2014; 29(11):2392–404. https://doi.org/10.1002/jbmr.2263 PMID: 24753181

59. Xiang R, Ghanipoor-Samami M, Johns WH, Eindorf T, Rutley DL, Kruk ZA, et al. Maternal and paternal genomes differentially affect myofibre characteristics and muscle weights of bovine fetuses at midgestation. PLoS One. 2013; 8(1):e33402. https://doi.org/10.1371/journal.pone.0033402 PMID: 23341941

60. Moller DE, Yokota A, Beitner-Johnson D, Roberts CT. Molecular and cellular aspects of the insulin-like growth factor-I receptor. Endocr Rev. 1995; 16(2):143–63. PubMed PMID: WOS:A1990DP95000008. PMID: 2369896

61. Mosthal L, Grako K, Dull TJ, Coussens L, Ulrich A, McClain DA. Functionally distinct insulin receptors generated by tissue-specific alternative splicing. EMBO J. 1990; 9(8):2409–13. PubMed PMID: WOS: A1990DP95000008. PMID: 2369896

62. Pandini G, Frasca F, Mineo F, Sciacca P, Vigneri R, Belfiore A. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem. 2002; 277 39684–95. https://doi.org/10.1074/jbc.M202766200 PMID: 12138094

63. Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr Rev. 2009; 30(6):586–623. https://doi.org/10.1210/er.2008-0047 PMID: 19752219.

64. Frasca F, Pandini G, Scalici P, Sciacca L, Mineo R, Costantino A, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol. 1999; 19(5):3278–88. PMID: 10207053

65. Leroth D, Werner H, Betnnerjohnson D, Roberts CT. Molecular and cellular aspects of the insulin-like growth-factor-I receptor. Endocr Rev. 1995; 16(2):143–63. PubMed PMID: WOS:A1995QT90200002. https://doi.org/10.1210/edrv-1995-2-143 PMID: 7540132

66. Belfiore A, Malaguarnera R, Vella V, Lawrence MC, Sciacca L, Frasca F, et al. Insulin receptor isoforms in physiology and disease: An updated view. Endocr Rev. 2017; 38(5):379–431. https://doi.org/10.1210/er.2017-00073 PMID: 28973479

67. Hwa V, Fan P, Derr MA, Fiegerlova E, Rosenfeld RG. IGF-I in human growth: lessons from defects in the GH-IGF-I axis. In: Gillman MW, Gluckman PD, Rosenfeld RG, editors. Recent Advances in Growth Research: Nutritional, Molecular and Endocrine Perspectives. Nestle Nutrition Institute Workshop Series. 712013. p. 43–55.

68. Jospe N, Kaplowitz PB, Furlanello RW. Homozygous nonsense mutation in the insulin receptor gene of a patient with severe congenital insulin resistance: Leprechaunism and the role of the insulin-like growth factor receptor. Clin Endocrinol (Oxf). 1996; 45(2):229–35. PubMed PMID: WOS: A1996VQ87300016.

69. Louvi A, Accili D, Efstratiadis A. Growth-promoting interaction of IGF-II with the insulin receptor during newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol. 1999; 19(5):3278–88. PMID: 10207053

70. Ghosh P, Dahms NM, Kornfeld S. Mannose 6-phosphate receptors: new twists in the tale. Nat Rev Mol Cell Biol. 2003; 4(3):202–13. https://doi.org/10.1038/nrm1050 PMID: 12612639

71. McKinnon T, Chakraborty C, Gleeson LM, Chidiac P, Lala PK. Stimulation of human extravillous trophoblast migration by IGF-II is mediated by IGF Type 2 receptor involving inhibitory G protein(s) and phosphorylation of MAPK. J Clin Endocrinol Metab. 2001; 86(8):3665–74. https://doi.org/10.1210/jcem.86.8.7711 PMID: 11502794

72. Barlow DP, Stoger R, Herrmann BG, Saito K, Schweifer N. The mouse insulin-like growth factor type-2 receptor is implanted and closely linked to the Tme locus. Nature. 1991; 349(6304):364–7. https://doi.org/10.1038/349084a0 PMID: 1845916

73. Killian JK, Nolan CM, Wylie AA, Li T, Vu TH, Hoffman AR, et al. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. Hum Mol Genet. 2001; 10(17):1721–8. PubMed PMID: IS1:0001711256000001. PMID: 11532981
75. Young LE, Schnieke AE, McCreath KJ, Wieckowski S, Konfortova G, Fernandes K, et al. Conservation of {\textit{IGF2-H19}} and {\textit{IGF2R}} imprinting in sheep: effects of somatic cell nuclear transfer. Mech Dev. 2003; 120(12):1433–42. PMID: 14654216

76. Xu YQ, Goodyer CG, Deal C, Polychronakos C. Functional polymorphism in the parental imprinting of the human {\textit{IGF2R}} gene. Biochem Biophys Res Commun. 1993; 197(2):747–54. PubMed PMID: WOS:000147380000010. doi:10.1016/0006-291X(93)90661-7.

77. Bebber D, Bauersachs S, Fürst RW, Reichenbach H-D, Reichenbach M, Medugorac I, et al. Tissue-specific and minor inter-individual variation in imprinting of {\textit{IGF2R}} is a common feature of {\textit{Bos taurus}} concept and not correlated with fetal weight. PLoS One. 2013; 8(4):e59564. doi:10.1371/journal.pone.0059564.

78. Wang ZQ, Fung MR, Barlow DP, Wagner EF. Regulation of embryonic growth and lysosomal targeting by the imprinted {\textit{Igf2r}} gene. Mol Cell Endocrinol. 1991; 78(1–2):87–96. PMID: 20131936.

79. Berkowicz EW, Magee DA, Berry DP, Sikora KM, Howard DJ, Mullen MP, et al. Single nucleotide polymorphisms in the imprinted bovine insulin-like growth factor 2 receptor gene (IGF2R) are associated with body size traits in Irish Holstein-Friesian cattle. Anim Genet. 2012; 43(1):81–7. doi:10.1111/j.1365-2052.2011.02211.x.

80. Farmer WT, Farin PW, Piedrahita JA, Bischoff SR, Farin CE. Expression of antisense of insulin-like growth factor II (IGF-II) mRNA expression. Mol Cell Endocrinol. 1991; 78(1–2):87–96. PMID: 11111936.

81. Latos PA, Pauler FM, Warczok KE, Ambros PF, Oshimura M, et al. Identification of the human insulin-like growth factor-II (IGF-II) gene transcript with a targeted disruption of the insulin-like growth factor-II promoter region by calcineurin activation of NFAT in skeletal muscle. Am J Physiol Cell Physiol. 2007; 292(5):C1887–C94. doi:10.1152/ajpcell.00506.2006.

82. Schofield PN, Tate VE. Regulation of human IGF-II transcription in fetal and adult tissues. Development. 1987; 101(4):793–803. PMID: 3503629.

83. O’Mahoney JV, Brandon MR, Adams TE. Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression. Mol Cell Endocrinol. 1991; 78(1–2):87–96. PMID: 1936528.

84. Bouille N, Schneid H, Listrat A, Holthuizen P, Binoux M, Groyer A. Developmental regulation of bovine insulin-like growth factor-II (IGF-II) gene-expression—Homology between bovine transcripts and human IGF-II exons. J Mol Endocrinol. 1993; 11(2):117–28. PubMed PMID: WOS:000066341500020.

85. Yotova IY, Vlatkovic IM, Pauler FM, Warczok KE, Ambros PF, Oshimura M, et al. Identification of the human homolog of the imprinted mouse Air non-coding RNA. Genomics. 2008; 92(6):464–73. doi:10.1016/j.ygeno.2008.08.004.

86. Airn MN, Brinkman AS, Solverso P, Pun W, Pintar JE, Ney DM. Exogenous GLP-2 and IGF-I induce a differential intestinal response in IGF binding protein-3 and-5 double knockout mice. Am J Physiol Gastrointest Liver Physiol. 2012; 302(8):G794–G804. doi:10.1152/ajpgi.00372.2011.

87. Kostecza Z, Blahovec J. Animal insulin-like growth factor binding proteins and their biological functions. Vet Med (Praha). 2002; 47(2–3):75–84. PubMed PMID: WOS:000178212800001.

88. Silha JV, Murphy LJ. Minireview: Insights from insulin-like growth factor binding protein transgenic mice. Endocrinology. 2002; 143(10):3711–4. doi:10.1210/en.2002-220116.

89. Costantini F. Induction of chondrogenesis and angiogenesis during skeletal development. Development. 2003; 130(12):2779–81. doi:10.1242/dev.00505.

90. Schofield PN, Tate VE. Regulation of human IGF-II transcription in fetal and adult tissues. Development. 1987; 101(4):793–803. PMID: 3503629.

91. O’Mahoney JV, Brandon MR, Adams TE. Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression. Mol Cell Endocrinol. 1991; 78(1–2):87–96. PMID: 1936528.

92. Bouille N, Schneid H, Listrat A, Holthuizen P, Binoux M, Groyer A. Developmental regulation of bovine insulin-like growth factor-II (IGF-II) gene-expression—Homology between bovine transcripts and human IGF-II exons. J Mol Endocrinol. 1993; 11(2):117–28. PubMed PMID: WOS:000066341500020.

93. Allerri CM, Evans-Anderson HJ, Yutzey KE. Developmental regulation of the mouse IGF-I exon 1 promoter region by calcineurin activation of NFAT in skeletal muscle. Am J Physiol Cell Physiol. 2007; 292(5):C1887–C94. doi:10.1152/ajpcell.00506.2006.

94. Yotova IY, Vlatkovic IM, Pauler FM, Warczok KE, Ambros PF, Oshimura M, et al. Identification of the human homolog of the imprinted mouse Air non-coding RNA. Genomics. 2008; 92(6):464–73. doi:10.1016/j.ygeno.2008.08.004.

95. Silha JV, Murphy LJ. Minireview: Insights from insulin-like growth factor binding protein transgenic mice. Endocrinology. 2002; 143(10):3711–4. doi:10.1210/en.2002-220116.

96. Costantini F. Induction of chondrogenesis and angiogenesis during skeletal development. Development. 2003; 130(12):2779–81. doi:10.1242/dev.00505.

97. Schofield PN, Tate VE. Regulation of human IGF-II transcription in fetal and adult tissues. Development. 1987; 101(4):793–803. PMID: 3503629.

98. O’Mahoney JV, Brandon MR, Adams TE. Developmental and tissue-specific regulation of ovine insulin-like growth factor II (IGF-II) mRNA expression. Mol Cell Endocrinol. 1991; 78(1–2):87–96. PMID: 1936528.

99. Bouille N, Schneid H, Listrat A, Holthuizen P, Binoux M, Groyer A. Developmental regulation of bovine insulin-like growth factor-II (IGF-II) gene-expression—Homology between bovine transcripts and human IGF-II exons. J Mol Endocrinol. 1993; 11(2):117–28. PubMed PMID: WOS:000066341500020.

100. Allerri CM, Evans-Anderson HJ, Yutzey KE. Developmental regulation of the mouse IGF-I exon 1 promoter region by calcineurin activation of NFAT in skeletal muscle. Am J Physiol Cell Physiol. 2007; 292(5):C1887–C94. doi:10.1152/ajpcell.00506.2006.
94. Li ZC, Wu ZF, Ren GC, Zhao YX, Liu DW. Expression patterns of insulin-like growth factor system members and their correlations with growth and carcass traits in Landrace and Lantang pigs during postnatal development. Mol Biol Rep. 2013; 40(5):3569–76. https://doi.org/10.1007/s11033-012-2430-1 PubMed PMID: WOS:000317075300012. PMID: 23269622

95. Werner H, Woloschak M, Adamo M, Shen-Orr Z, Roberts CT, LeRoith D. Developmental regulation of the rat insulin-like growth factor I receptor gene. Proc Natl Acad Sci U S A. 1989; 86(19):7451–5. PMID: 2477843

96. Nissley P, Kiess W, Sklar M. Developmental expression of the IGF-II/mannose-6-phosphate receptor. Mol Reprod Dev. 1993; 35(4):408–13. https://doi.org/10.1002/mrd.1080350415 PMID: 8398120

97. Fowden AL. The insulin-like growth factors and feto-placental growth. Placenta. 2003; 24(8):803–12. https://doi.org/10.1016/S0143-4004(03)00080-8.

98. Delhanty PJ, Han VK. The expression of insulin-like growth factor (IGF)-binding protein-2 and IGF-II genes in the tissues of the developing ovine fetus. Endocrinology. 1993; 132(1):41–52. https://doi.org/10.1210/endo.132.1.7678219 PMID: 7678219

99. Lee CY, Chung CS, Simmen FA. Ontogeny of the porcine insulin-like growth factor system. Mol Cell Endocrinol. 1993; 93(1):71–80. https://doi.org/10.1016/0303-7207(93)90141-6. PMID: 7686518

100. Gerrard DE, Okamura CS, Grant AL. Expression and location of IGF binding proteins-2, -4, and -5 in developing fetal tissues. J Anim Sci. 1999; 77(6):1431–41. PMID: 10375221

101. Anand-Ivell R, Hiendleder S, Vilegas C, Martin GB, Fitzsimmons C, Eurich A, et al. INSL3 in the ruminant: A powerful indicator of gender- and genetic-specific fetomaternial dialogue. PLoS One. 2011; 6(5):e19821. https://doi.org/10.1371/journal.pone.019821 PMID: 21603619

102. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3):403–10. https://doi.org/10.1016/S0022-2836(05)80360-2 PubMed PMID: WOS:A1990ED16700008. PMID: 2231712

103. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009; 55(4):611–22. https://doi.org/10.1373/clinchem.2008.112797 PMID: 19246619

104. Johnson JW. A heuristic method for estimating the relative weight of predictor variables in multiple regression. Multivariate Behav Res. 2000; 35(1):1–19. https://doi.org/10.1207/S15327906MBR3501_1 PMID: 26777229

105. Johnson JW. Factors affecting relative weights: the influence of sampling and measurement Error. Organ Res Methods. 2004; 7(3):283–99. https://doi.org/10.1177/1094428104266018

106. Andersen CL, Jensen JL, Ørntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Cancer Res. 2004; 64(15):5245–50. https://doi.org/10.1158/0008-5472.CAN-04-0496 PMID: 15289330

107. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology. 2002; 3(7):research0034.1—research.11. PubMed PMID: https://doi.org/10.1186/gb-2002-3-7-research0034

108. Lorenzo-Seva U, Ferrando P, Chico E. Two SPSS programs for interpreting multiple regression results. Behav Res Methods. 2010; 42(1):29–35. https://doi.org/10.3758/BRM.42.1.29 PMID: 21602833

109. Ohlsson C, Mohan S, Stjören K, Tivesten Å, Isgaard J, Isaksson O, et al. The role of liver-derived insulin-like growth factor-I. Endocr Rev. 2009; 30(5):495–535. https://doi.org/10.1210/endo.2009-0010 PMID: 19589948.

110. Pell JM, Saunders JC, Gilmour RS. Differential regulation of transcription initiation from insulin-like growth factor-I (IGF-I) leader exons and of tissue IGF-I expression in response to changed growth hormone and nutritional status in sheep. Endocrinology. 1993; 132(4):1797–807. https://doi.org/10.1210/endo.132.4.8462477 PMID: 8462477

111. Ohhtsuki T, Otsuki M, Murakami Y, Maekawa T, Yamamoto T, Akasaka K, et al. Organ-specific and age-dependent expression of insulin-like growth factor-I (IGF-I) mRNA variants: IGF-IA and IB mRNAs in the mouse. Zoolog Sci. 2005; 22(9):1011–21. https://doi.org/10.2108/zsj.22.1011 PMID: 16219982

112. Bornfeldt KE, Amqvist HJ, Enberg B, Mathews LS, Norstedt G. Regulation of insulin-like growth factor-I and growth hormone receptor gene expression by diabetes and nutritional state in rat tissues. J Endocrinol. 1989; 122(3):651–6. https://doi.org/10.1677/joe.0.1220651 PMID: 2809476

113. Lund PK, Moatststaa BS, Hynes MA, Simmons JG, Hansen M, Dercole AJ, et al. Somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II mRNAs in rat fetal and adult tissues. J Biol Chem. 1986; 261(31):4539–44. PubMed PMID: WOS:A1986E668100028.
Liu J-P, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell. 1993; 75(1):59–72. https://doi.org/10.1016/S0092-8674(05)80084-4. PMID: 8402901

Hiendleder S, Wirtz M, Mund C, Klempt M, Reichenbach H-D, Stojkovic M, et al. Tissue-specific effects of in vitro fertilization procedures on genomic cytosine methylation levels in overgrown and normal sized bovine fetuses. Biol Reprod. 2006; 75(1):17–23. https://doi.org/10.1095/biolreprod.105.043919. PMID: 16554415

Kind KL, Owens JA, Robinson JS, Grant PA, Walton PE, et al. Effect of restriction of placental growth on expression of IGFs in fetal sheep: relationship to fetal growth, circulating IGFs and binding proteins. J Endocrinol. 1995; 146(1):23–34. https://doi.org/10.1677/joe.0.1460023. PMID: 7561617

Shen W, Wisniewski P, Ahmed L, Boyle DW, Denne SC, Liechty EA. Protein anabolic effects of insulin and IGF-I in the ovine fetus. Am J Physiol Endocrinol Metab. 2003; 284(4):E748–E56. https://doi.org/10.1152/ajpendo.00399.2002. PMID: 12488244

Wang Y, Price SE, Jiang H. Cloning and characterization of the bovine class 1 and class 2 insulin-like growth factor-I mRNAs. Domest Anim Endocrinol. 2003; 25(4):315–28. https://doi.org/10.1016/j. domaniend.2003.06.001. PMID: 14652133

Zhang J, Zhang G, Yang R, Niu S, Bai W, Liu D, et al. Cloning and characterization of four new splice variants of insulin-like growth factor-I gene in Chinese red steppes. Journal of Animal and Veterinary Advances. 2011; 10(18):2459–64. https://doi.org/10.3923/javaa.2011.2459.2464

O’Sullivan DC, Szestak TAM, Pell JM. Regulation of hepatic insulin-like growth factor I leader exon usage in lambs: effect of immunization against growth hormone-releasing factor and subsequent growth hormone treatment. J Anim Sci. 2002; 80(4):1074–82. PubMed PMID: WOS:000174808700027. PMID: 12002514

Xiao S, Li S, Zhang J, Zhang S, Dai L, Bao Y, et al. Cloning and characterization of class 1 and class 2 insulin-like growth factor-I mRNA in Songliao black pig. Mol Biol Rep. 2009; 36(2):415–21. https://doi.org/10.1007/s11033-007-9196-y. PMID: 18157703

Weller PA, Dickson MC, Huskisson NS, Dauncey MJ, Buttery PJ, Gilmour RS. The porcine insulin-like growth factor-I mRNA variants of insulin-like growth factor-I gene expression and differential regulation in the developing kidney. Endocrinology. 1992; 131(6):2793–9. https://doi.org/10.1210/en.131.6.1446616. PMID: 1446616.

Randhawa R, Cohen P. The role of the insulin-like growth factor system in prenatal growth. Mol Genet Metab. 2005; 86:84–90. https://doi.org/10.1016/j.mgen.2005.07.026. PMID: 16165387

Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, et al. Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature. 2002; 417(6892):945–8. PubMed PMID: WOS:000176441200038. https://doi.org/10.1038/nature00819. PMID: 12087403

Powden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res Paediatr. 2006; 65(suppl 3)(Suppl. 3):50–8.

Randhawa R, Cohen P. The role of the insulin-like growth factor system in prenatal growth. Mol Genet Metab. 2005; 86:84–90. https://doi.org/10.1016/j.mgen.2005.07.026. PMID: 16165387

Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res Paediatr. 2006; 65(suppl 3)(Suppl. 3):50–8.

Huang Y-Z, Zhan Z-Y, Sun Y-J, Cao X-K, Li M-X, Wang J, et al. Intragenic DNA methylation status and type 1 IGF2 gene expression in different developmental stages. Gene. 2014; 534(2):356–61. https://doi.org/10.1016/j.gene.2013.09.111. PMID: 24140490

Soares MB, Turkten A, Ishi D, Mills L, Episkopou V, Cotter S, et al. Rat insulin-like growth factor II gene: A single gene with two promoters expressing a multitranscript family. J Mol Biol. 1986; 192(4):737–52. PMID: 2438416

Gray A, Tam AW, Dull TJ, Hayflick J, Pintar J, Cavenee WK, et al. Tissue-specific and developmentally regulated transcription of the insulin-like growth factor-II gene. DNA-A Journal of Molecular and Cellular Biology. 1987; 6(4):283–95. https://doi.org/10.1089/dna.1987.6.283. PubMed PMID: WOS: A1987J730900001. PMID: 3652904

Sullivan TM, Micke GC, Perkins N, Martin GB, Wallace CR, Gatford KL, et al. Dietary protein during gestation affects maternal insulin-like growth factor, insulin-like growth factor binding protein, leptin...
concentrations, and fetal growth in heifers. J Anim Sci. 2009; 87(10):3304–16. https://doi.org/10.2527/jas.2008-1753 PMID: 19617516

133. Davies SM. Developmental regulation of genomic imprinting of the IGF2 gene in human liver. Cancer Res. 1994; 54(10):2560–2. PMID: 8168079

134. Holthuizen P, van der Lee FM, Ikejiri K, Yamamoto M, Sussenbach JS. Identification and initial characterization of a fourth leader exon and promoter of the human IGF-II gene. Biochim Biophys Acta. 1990; 1087(3):341–3. PMID: 2249882

135. Li X, Cui H, Sandstedt B, Nordlinger H, Larsson E, Ekstrom TJ. Expression levels of the insulin-like growth factor II gene (IGF2) in the human liver: Developmental relationships of the four promoters. J Endocrinol. 1996; 149(1):117–24. PubMed PMID: WOS:A1996UD87000014. PMID: 8676043

136. Sussenbach JS, Steenbergh PH, Holthuizen P. Structure and expression of the human insulin-like growth factor genes. Growth Regul. 1992; 2(1):1–9. PMID: 1463311

137. Frasca F, Pandini G, Sciaccia L, Pezzino V, Squatrito S, Belfiore A, et al. The role of insulin receptors and IGF-I receptors in cancer and other diseases. Archives of Physiology and Biochemistry. 2008; 114(1):23–37. https://doi.org/10.1038/sj.jn.3102872 PMID: 18465356

138. Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, et al. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet. 1996; 12(1):106–9. https://doi.org/10.1038/ng0196-106 PMID: 8528241

139. Ludwig T, Eggenschwiler J, Fisher P, Dercole AJ, Davenport ML, Efstratiadis A. Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igif1 null backgrounds. Dev Biol. 1996; 177(2):517–35. PubMed PMID: WOS:A1996VA82900012. https://doi.org/10.1006/dbio.1996.0182 PMID: 8806828

140. Pellegrini M, Pilia G, Pantano S, Luchini F, Uda M, Fumi M, et al. Gpc3 expression correlates with the phenotype of the Simpson-Golabi-Behmel syndrome. Dev Dyn. 1998; 213(4):431–9. https://doi.org/10.1002/(SICI)1097-0177(199812)213:4<431::AID-AJA8>3.0.CO;2-7 PubMed PMID: WOS:000077534500008. PMID: 9853964

141. El-Shewy HM, Lee M-H, Obeid LM, Jaffa AA, Luttrell LM. The insulin-like growth factor type 1 and insulin-like growth factor type 2/mannose-6-phosphate receptors independently regulate ERK1/2 activity in HEK293 cells. J Biol Chem. 2007; 282(36):26150–7. https://doi.org/10.1074/jbc.M703276200 PMID: 17620396

142. Chu C-H, Tzang B-S, Chen L-M, Kuo C-H, Cheng Y-C, Chen L-Y, et al. IGF-II/mannose-6-phosphate receptor signaling induced cell hypertrophy and atrial natriuretic peptide/BNP expression via Gαq inter-action and protein kinase C-α/CaMKII activation in H9c2 cardiomyoblast cells. J Endocrinol. 2008; 197(2):381–90. https://doi.org/10.1677/JOE-07-0619 PMID: 18434368

143. Ludwig T, Eggenschwiler J, Fisher P, Dercole AJ, Davenport ML, Efstratiadis A. Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igif1 null backgrounds. Dev Biol. 1996; 177(2):517–35. PubMed PMID: WOS:A1996VA82900012. https://doi.org/10.1006/dbio.1996.0182 PMID: 8806828

144. Schuller AGP, Vannecck JW, Lindenbergkortleve DJ, Groffen C, Dejong I, Zwarthoff EC, et al. Gene expression of the IGF binding proteins during post-implantation embryogenesis of the mouse; comparison with the expression of IGF-I and -II and their receptors in rodent and human. In: LeRoith D, Rainey J, editors. Current Directions in Insulin-Like Growth Factor Research. Advances in Experimental Medicine and Biology. 3431993. p. 267–77.

145. Han VKM, Matsell DG, Delhanty PJD, Hill DJ, Shimaski S, Nygard K. IGF-binding protein mRNAs in the human fetus: tissue and cellular distribution of developmental expression. Horm Res Paediatr. 1996; 45(3–5):160–6.

146. Ooi GT, Grötzsch CC, Brown AL, Becker RE, Unterman TG, Rechle MM. Different tissue distribution and hormonal regulation of messenger RNAs encoding rat insulin-like growth factor-binding proteins-1 and -2. Mol Endocrinol. 1990; 4(2):321–8. PubMed PMID: BIOSIS:PREV1990081903275. https://doi.org/10.1210/mend-4-2-321 PMID: 1691819

147. Murphy LJ, Seneviratne C, Ballejo G, Croze F, Kennedy TG. Identification and characterization of a rat decidual insulin-like growth factor-binding protein complementary DNA. Mol Endocrinol. 1990; 4(2):329–36. PubMed PMID: WOS:A1990CR02700019. https://doi.org/10.1210/mend-4-2-329 PMID: 1691820

148. Lewitt MS, Denyer GS, Cooney JG, Baxter RC. Insulin-like growth factor-binding protein-1 modulates blood glucose levels. Endocrinology. 1991; 129(4):2254–6. https://doi.org/10.1210/endo-129-4-2254 PMID: 1717244

149. Brismar K, Fernqvist-Forbes E, Wahren J, Hall K. Effect of insulin on the hepatic production of insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, and IGF-I in insulin-dependent diabetes. J Clin Endocrinol Metab. 1994; 79(3):872–8. https://doi.org/10.1210/jcem.79.3.7521354 PMID: 7521354

150. Lee PDK, Conover CA, Powell DR. Regulation and function of insulin-like growth factor-binding protein-1. Proc Soc Exp Biol Med. 1993; 204(1):4–29. https://doi.org/10.3181/00379727-204-43630 PMID: 7690486
Hall K, Brismar K, Grissom F, Lindgren B, Povoa G. IGFBP-1. Production and control mechanisms. Acta Endocrinol (Copenh). 1991; 124:48–54. PubMed PMID: WOS:A1991FV94700009.

Donovan SM, Oh Y, Pham H, Rosenfeld RG. Ontogeny of serum insulin-like growth factor binding proteins in the rat. Endocrinology. 1989; 125(5):2621–7. https://doi.org/10.1210/endo-125-5-2621 PMID: 1708333.

Lassarre C, Hardouin S, Daffos F, Forestier F, Frankeenne F, Binoux M. Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res. 1991; 29(3):219–25. https://doi.org/10.1203/00006450-199103000-00001 PMID: 1709729.

Lee CY, Bazer FW, Etherton TD, Simmen FA. Ontogeny of insulin-like growth factors (IGF-I and IGF-II) and IGF-binding proteins in porcine serum during fetal and postnatal development. Endocrinology. 1991; 128(5):2336–44. https://doi.org/10.1210/endo-128-5-2336 PMID: 1708333.

Liu F, Powell DR, Styne DM, Hintz RL. Insulin-like growth factors (IGFs) and IGF-binding proteins in the developing rhesus monkey. J Clin Endocrinol Metab. 1991; 72(4):905–11. https://doi.org/10.1210/jcem-72-4-905 PMID: 1706351.

Carr JM, Owens JA, Grant PA, Walton PE, Owens PC, Wallace JC. Circulating insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs) and tissue mRNA levels of IGFBP-2 and IGFBP-4 in the ovine fetus. J Endocrinol. 1995; 145(3):545–57. https://doi.org/10.1677/joe.0.1450545 PubMed PMID: WOS:A1995RB15700018. PMID: 7543554.

Han VKM, Carter AM. Spatial and temporal patterns of expression of messenger RNA for insulin-like growth factors and their binding proteins in the placenta of man and laboratory animals. Placenta. 2000; 21(4):289–305. https://doi.org/10.1053/plac.1999.0498. PMID: 10833363.

Coulter CL, Han VKM. The pattern of expression of insulin-like growth factor (IGF), IGF-I receptor and IGF binding protein (IGFBP) mRNAs in the rhesus monkey placenta suggests a paracrine mode of IGF-IGFBP interaction in placental development. Placenta. 1996; 17(7):451–60. PubMed PMID: WOS:A1996VL49500009. PMID: 8899874.

Miyakoshi N, Qin X, Kasukawa Y, Richman C, Srivastava AK, Baylink DJ, et al. Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism. Endocrinology. 2001; 142(6):2641–8. https://doi.org/10.1210/endo.142.6.8192 PMID: 11356715.

Bach LA, Salemi R, Leeding KS. Roles of insulin-like growth factor (IGF) receptors and IGF-binding proteins in IGF-II-induced proliferation and differentiation of L6A1 rat myoblasts. Endocrinology. 1995; 136(11):5061–9. https://doi.org/10.1210/endo.136.11.7588242 PMID: 7588242.

Haugk KL, Wilson H-MP, Swisshelm K, Quinn LS. Insulin-like growth factor (IGF)-binding protein-related protein-1: an autocrine/paracrine factor that inhibits skeletal myoblast differentiation but permits proliferation in response to IGF. Endocrinology. 2000; 141(1):100–10. https://doi.org/10.1210/endo.141.1.7235 PMID: 10614628.

Damon SE, Haugk KL, Swisshelm K, Quinn LS. Developmental regulation of Mac25/insulin-like growth factor-binding protein-7 expression in skeletal myogenesis. Exp Cell Res. 1997; 237(1):192–5. https://doi.org/10.1006/excr.1997.3787. PMID: 9417882.

Fatica A, Bozzi I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet. 2014; 15(1):7–21. https://doi.org/10.1038/nrg3606 PubMed PMID: WOS:000358299200009. PMID: 24296535.

Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013; 152(6):1298–307. https://doi.org/10.1016/j.cell.2013.02.012 PubMed PMID: WOS:000316192500011. PMID: 23489338.

Santoro F, Barlow DP. Developmental control of imprinted expression by macro non-coding RNAs. Semin Cell Dev Biol. 2011; 22(4):328–35. https://doi.org/10.1016/j.semcdb.2011.02.018 PubMed PMID: WOS:000295212900002. PMID: 21333747.

Galowy A, Jamnes H, Dandolo L. The H19 locus: Role of an imprinted non-coding RNA in growth and development. Bioessays. 2010; 32(6):473–80. https://doi.org/10.1002/bies.200900170 PubMed PMID: WOS:000278709500005. PMID: 20486133.

Dey BK, Pfeifer K, Dutta A. The H19 long noncoding RNA gives rise to microRNAs miR-675-3p and miR-675-5p to promote skeletal muscle differentiation and regeneration. Genes Dev. 2014; 28(5):491–501. https://doi.org/10.1101/gad.234419.113 PMID: 24532688.

Varrault A, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, et al. Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. Dev Cell. 2006; 11(5):711–22. https://doi.org/10.1016/j.devcel.2006.09.003. PMID: 17084362.
168. Gabory A, Ripoche M-A, Le Digarcher A, Watrin Fo, Ziyat A, Forné T, et al. H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. Development. 2009; 136(20):3413–21. https://doi.org/10.1242/dev.036061 PMID: 19762426

169. Merkenschlager M, Odom Duncan T. CTCF and cohesin: linking gene regulatory elements with their targets. Cell. 2013; 152(6):1285–97. https://doi.org/10.1016/j.cell.2013.02.029. PMID: 23498937

170. Ma N, Zhou L, Zhang Y, Jiang Y, Gao X. Intragenic microRNA and long non-coding RNA: novel potential regulator of IGFl2-H19 imprinting region. Evol Dev. 2014; 16(1):1–2. https://doi.org/10.1111/ede.12057 PMID: 24399462

171. Nordin M, Bergman D, Halje M, Engström W, Ward A. Epigenetic regulation of the Igfl2/H19 gene cluster. Cell Prolif. 2014; 47(3):189–99. https://doi.org/10.1111/cpr.12106 PMID: 24738971

172. Lee RSF, Depree KM, Davey HW. The sheep (Ovis aries) H19 gene: genomic structure and expression patterns, from the preimplantation embryo to adulthood. Gene. 2002; 301:67–77. PMID: 12490325

173. Lustig O, Ariel I, Ilan J, Lev-Lehman E, De-Groot N, Hochberg A. Expression of the imprinted gene H19 in the human fetus. Mol Reprod Dev. 1994; 38(3):239–46. https://doi.org/10.1002/mrd.1080380302 PMID: 7917273

174. Braidotti G, Baubec T, Pauler F, Seidl C, Smrzka O, Stricker S, et al. The Air non-coding RNA: An imprinted cis-silencing transcript. Cold Spring Harb Symp Quant Biol. 2004; 69:55–66. https://doi.org/10.1101/sqb.2004.69.55 PubMed PMID: WOS:000232272000008. PMID: 16117633

175. Sleutels F, Zwart R, Barlow DP. The non-coding Air RNA is required for silencing autosomal imprinted genes. Nature. 2002; 415(6873):810–3. PubMed PMID: ISI:000173833900051. https://doi.org/10.1038/15810A PMID: 11845212

176. Beall MH, van den Wijngaard JPHM, van Gemert MJC, Ross MG. Amniotic fluid water dynamics. Placenta. 2007; 28(8–9):816–23. https://doi.org/10.1016/j.placenta.2006.11.009. PMID: 17254633

177. van Otterlo LC, Wladimiroff JW, Wallenburg HCS. Relationship between fetal urine production and amniotic fluid volume in normal pregnancy and pregnancy complicated by diabetes. BJOG. 1977; 84(3):205–9. https://doi.org/10.1111/j.1471-0528.1977.tb12556.x

178. Merimee TJ, Grant M, Tyson JE. Insulin-like growth factors in amniotic fluid. J Clin Endocrinol Metab. 1984; 59(4):752–5. https://doi.org/10.1210/jcem-59-4-752 PMID: 6384254

179. Tomoda S, Brace RA, Longo LD. Amniotic fluid volume and fetal swallowing rate in sheep. Am J Physiol Regul Integr Comp Physiol. 1985; 249(1):R133–R8.

180. Bloomfield FH, Breier BH, Harding JE. Fate of 125I-IGF-I administered into the amniotic fluid of late-gestation fetal sheep. Pediatr Res. 2002; 51(3):361–9. https://doi.org/10.1203/00006450-200203000-00016 PMID: 11861943

181. Holt RIG. Fetal programming of the growth hormone–insulin-like growth factor axis. Trends Endocrinol Metab. 2002; 13(9):392–7. https://doi.org/10.1016/S1043-2760(02)00697-5. PMID: 12367821

182. Le Roith D, Bondy C, Yakar S, Liu J-L, Butler A. The somatomedin hypothesis: 2001. Endocr Rev. 2001; 22(1):53–74. https://doi.org/10.1210/edrv.22.1.0419 PMID: 11159816

183. LeRoith D, McGuinness M, Shemer J, Stannard B, Lanau F, Faria TN, et al. Insulin-like growth factors. Neurosignals. 1992; 1(4):173–81.

184. Young LE. imprinting of genes and the Barker hypothesis. Twin Res. 2012; 4(5):307–17. Epub 02/01. https://doi.org/10.1375/twin.4.5.307

185. Vickers MH. Early life nutrition, epigenetics and programming of later life disease. Nutrients. 2014; 6(6):2165–78. https://doi.org/10.3390/nu6062165 PubMed PMID: WOS:000338192000003. PMID: 24892374

186. Borel C, Antonarakis SE. Functional genetic variation of human miRNAs and phenotypic consequences. Mamm Genome. 2008; 19(7–8):503–9. https://doi.org/10.1007/s00335-008-9137-6 PubMed PMID: WOS:000261180800004. PMID: 18787897

187. Chang A, Pogribny I. Considering maternal dietary modulators for epigenetic regulation and programming of the fetal genome. Nutrients. 2015; 7(4):2748. PubMed PMID: https://doi.org/10.3390/nu7042748 PMID: 25875118