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# Imprinted Gene mRNA Expression during Porcine Peri-implantation Development*

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**ABSTRACT :** Imprinted genes are essential for fetal development, growth regulation, and postnatal behavior. However, little is known about imprinted genes in livestock. We hypothesized that certain putatively imprinted genes affected normal peri-implantation development such as embryo elongation, initial placental development, and preparation of implantation. The objective of the present study was to investigate the mRNA expression patterns of several putatively imprinted genes during the porcine peri-implantation stages from day 6 to day 21 of gestation. Imprinted genes were selected both maternally (*Dlk1*, *IGF2*, *Ndn*, and *Sgce*) and paternally (*IGF2r*, *H19*, *Gnas* and *Xist*). Here, we report that the maternally imprinted gene *IGF2* was expressed from day 6 (Blastocyst stage), but *Dlk1*, *Ndn*, and *Sgce* were not expressed in this stage. These genes were first expressed between days 12 and day 14. All the maternally imprinted genes studied showed significantly high expression patterns from day 18 of embryo development. In contrast, paternally imprinted genes *IGF2r*, *H19*, *Gnas*, and *Xist* were first expressed from day 6 of embryo development (BL). Our data demonstrated that the expression of *H19* and *Gnas* genes was significantly increased from day 14 of the embryo developmental stage, while *IGF2r* and *Xist* only showed high expression after day 21. This study is the first to show that the putatively imprinted genes were stage-specific during porcine embryonic development. These results demonstrate that the genes studied may exert important effects on embryo implantation and fetal development. (**Key Words** : Imprinted Genes, Peri-implantation, mRNA Expression, Early Development, Pig)

## INTRODUCTION

In mammals including humans and mice, paternal and the maternal genomes are both required for normal embryonic and brain development (Tilghman et al., 1999). A previous study reported that their functional non-equivalence was mediated to genomic imprinting, an epigenetic mechanism that gives rise to differential expression of paternally and maternally inherited alleles of certain genes (Young et al., 2003). An utmost consequence of imprinting is that parthenogenetic mouse embryos that have two maternal genomes and no paternal genomes are small and die shortly after implantation. This is because they lack imprinted genes that are expressed from the paternal allele (Surani et al., 1984, 1990). While the expression and epigenetic differences of imprinted genes have been extensively characterized in the mouse and human system, little is known about imprinted genes in livestock.

At this point, only mammals have renounced parthenogenesis, a means of producing posterity solely from maternal germ cells or paternal germ cells. Among the mammals studied, there are 49 human genes and 65 mouse genes that show some level of imprinting displaying monoallelic gene expression patterns. Of these genes, only 10 genes have been reported to show imprinting in sheep (*GTL2*, *DLK1*, *DAT*, *PEG11*, *antiPEG11*, *MEG8*, *MEST*, *IGF2*, *H19* and *IGF2r*; Killian et al., 2001), 8 in cattle (*IGF2r*, *XIST*, *IGF2*, *GTL2*, *NESP55*, *H19*, *PEG3* and *NNAT*) and 3 in pigs (*IGF2*, *IGF2r*, and *IGF2-AS*, *DLK1*; University of Otago, 2006; Li et al., 2008). During development, these DNA imprints are erased in the primordial germ cells (PGCs) and reestablished during gametogenesis (Ruddock et al., 2004), and at the pre- and post embryo implantation stages (Ross et al., 2003).

Porcine blastocysts hatch on days 6-7 and exhibit a prolonged peri-implantation period of development until

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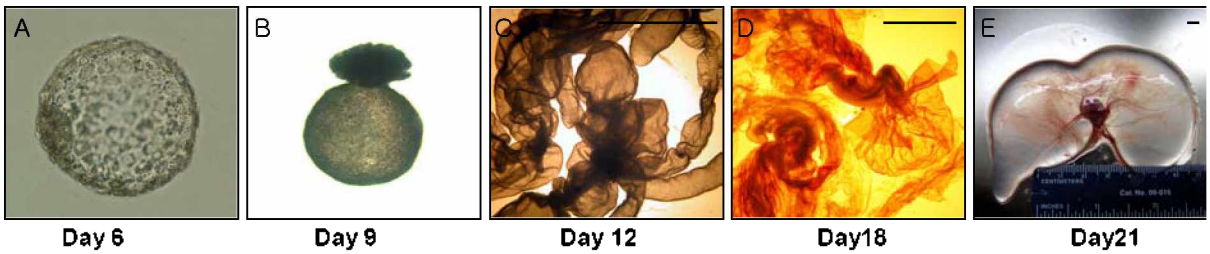


**Figure 1.** Morphology of conceptuses during the peri-implantation development. Conceptuses were obtained from bred gilts on day 6, 9, 12, 18, and 21 of gestation. (A), (B) Blastocyst stage conceptus at day 6 and 9 after insemination. (C) Conceptus of filamentous form at day 12. (D). (E) Conceptus attached by interlocking mirovilli to form the epitheliochorial placenta. Bar 5 mm.

day 12, when the conceptus initiates attachment to the uterine luminal surface following a dramatic morphological change of the trophoblast (Geisert et al., 1997). On the other hand, murine blastocysts hatch from the zona pellucida on day 4 and implant into the endometrium soon after on day 4.5 of development (Perry et al., 1981). During this stage, swine exhibit high rates (~18%) of loss of pre-implantation conceptus following artificial insemination or natural mating (Bennett et al., 1989). This morphological alteration occurs via hyperplasia in the sheep and cow, whereas in the pig the initial stages of trophectoderm elongation take place through cellular reorganization and differentiation, and proliferation is a later event (Geisert et al., 1982; Pusateri et al., 1990). Asynchrony during the timing of elongation among D11 and D12 porcine conceptuses *in utero* may play an important role in conceptus survival; blastocysts differentiating earlier may have a competitive edge over their lagging cohorts in obtaining the uterine surface necessary for further development (Bazer et al., 1993). In the most critical period coinciding with elongation, D12 porcine conceptuses also synthesize and secrete estrogens that serve as an important molecular signal in establishing the maternal recognition of pregnancy (Pusateri et al., 1990; Geisert et al., 1997).

Since imprinted genes play crucial roles in fetal and placental development, it is important to establish the expression patterns of several imprinted genes. In the present study, we examined the expression levels of maternally (*Dlk1*, *IGF2*, *Sgce* and *Ndn*) and paternally (*IGF2r*, *H19*, *Gnas* and *Xist*) imprinted genes in conceptus during peri-implantation development (Day 6, 9, 14, 18 and 21 after mating).

## MATERIALS AND METHODS

### Collection of conceptus

Yorkshire gilts were monitored for estrus once a day by exposure to healthy mature Yorkshire boars and mated 12 and 24 h after estrus detection (one boar was used for all natural mating). Pregnant gilts (3 to 5 animals/status per day) were hysterectomized through midventral laparotomy as previously described (Gries et al., 1989). The day of the first mating was designated as Day 0. As shown in Figure 1, each day's conceptus (Day 6, 9, 12, 18 and 21 of gestation) was flushed from the uterine horn with 30 ml of PBS (phosphate buffered saline) supplemented with 1% FBS (fetal bovine serum). Each conceptus collected *in vivo* was washed three times in PBS. For RNA preparation, only the conceptuses having identical morphology were pooled and stored in 5 μl of diethyl pyrocarbonate-treated phosphate-buffered saline at -80°C until further use.

### Total RNA extraction and first strand cDNA synthesis

Total RNA was extracted from the conceptus using TRIzol reagent (Invitrogen, USA). The RNA was treated with RNas-free DNase I to remove possible contaminating DNA and stored at -80°C. Total RNA extracted was employed for synthesis of first-strand cDNA by reverse transcriptase using a cDNA synthesis kit (Invitrogen, USA). Briefly, RNA/primer mixtures were prepared in sterile 200 μl tubes (RNA/primer mixtures 10 μl; RNA 2 μl, 50 μM oligo (dT) 1 μl, 10 mM dNTP 1 μl, and DEPC-treated water 6 μl; the mixture was briefly centrifuged before use. Each sample was incubated at 65°C for 5 min and then incubated on ice for at least 1 min. The reaction mixtures were prepared by adding each component in the sample (9 μl mixture component; 10×RT buffer 2 μl, 25 mM Mgcl 4 μl, 0.1 M DTT 2 μl, and RNaseOUT™ Recombinant RNase Inhibitor 1 μl). The reaction mixture was added to each RNA/primer mixture, mixed gently, and collected by brief centrifugation. The sample was then incubated at 42°C for 2 min, after which 1 μl of SuperScript II™ reverse transcriptase (50 U/μl; Invitrogen, CA) was added to each tube except the no RT control. After mixing, the reaction was incubated at 42°C for 50 min (total reaction mixture 20 μl). The reaction was terminated at 70°C for 15 min and chilled on ice. The reactions were collected by brief centrifugation. All cDNA samples were stored at -20°C until amplification.

**Table 1.** Primer sequences used for Real-time RT-PCR

| Genes | Primer sequence | Annealing temp. (°C) | GenBank accession number |
|---|---|---|---|
| p-H19 | Foward 5'-AAA GAG CAT CTC AAG CGA GT-3'<br>Reverse 5'-GCT CCT GTA CCT GCT ACT AA-3' | 55 | AY044827 |
| p-IGF2r | Foward 5'-ATA AAC ACC AAT ATA ACA CT-3'<br>Reverse 5'-GCA CAC GTT AAT ATA AAA CT-3' | 55 | AF342812 |
| h-Gnas | Foward 5'-ATT GAA ACC ATT GTG GCC GC-3'<br>Reverse 5'-GTG GAA GTT GAC TTT GTC CA-3' | 62 | NM019132 |
| p-Xist | Foward 5'-ACT AGT GAT GGT TAT GAA AA-3'<br>Reverse 5'-GTA AGA GGA AAG AAA TGA AG-3' | 61 | AJ429140 |
| p-IGF2 | Foward 5'-CTC GTG CTG CTA TGC TGC TT-3'<br>Reverse 5'-CAG GTG TCA TAG CGG AAG AA-3'. | 65 | NM213883 |
| p-DLK1 | Foward 5'-AGG TGA GGT TCG AGT GTC TG-3'<br>Reverse 5'-AGT GCT CTT GGT GAG CTC CT-3' | 60 | AY172651 |
| h-Sgce | Foward 5'-GAG CGC CTG AAC GCC ATA AA-3'<br>Reverse 5'-CAC-TTC CTG ATA GGT GGA CA-3' | 58 | NM003919 |
| b-Ndn | Foward 5'-AACGTGCTGCGCATCTTG-3'<br>Foward 5'-TCAGGTAGTTCTGCTGGACGAA-3' | 58 | AY360449 |
| p-βactin | Foward 5'-CAC TGG CAT TGT CAT GGA CT-3'<br>Reverse 5'-GGC AGC TCG TAG CTC TTC TC-3' | 65 | U07786 |

### Quantitative real-time PCR

Real-time PCR reactions were performed using a LightCycler (Roche, Germany) and conducted according to the manufacturer's instructions (Roche, Germany). The cDNA of target genes and β-actin was detected by real-time PCR using specific primer pairs shown in Table 1. The β-actin mRNA was used as an internal standard. The FastStart DNA SYBR green 1 (Roche, Germany) contained a Taq DNA polymerase enzyme and a reaction mixture (SYBR Green, optimized PCR buffer, 5 mM $MgCl_2$, and a dNTP mix that included dUTP). The PCR reaction was performed under following conditions: 95°C (10 min), 45 cycles of 95°C (10 sec), 56-64°C (30 sec) and 72°C (30 sec), and then a melting curve program (65-95°C) by using each specific forward and reverse primers as shown Table 1. The fluorescence data were acquired after the extension step during PCR reactions containing SYBR Green 1 thereafter; PCR products were analyzed by generating a melting curve. For the mathematical model, it was necessary to determine the crossing points (CP) for each transcript; CP was defined as the point at which the fluorescence increased significantly above the background fluorescence. The relative quantification of gene expression was analyzed using the 2-ddCt method (Livak and Schmittgen, 2001). The sizes of PCR products were confirmed by gel electrophoresis on a standard 2% agarose gel stained with ethidium bromide and visualized by exposure to ultraviolet light.

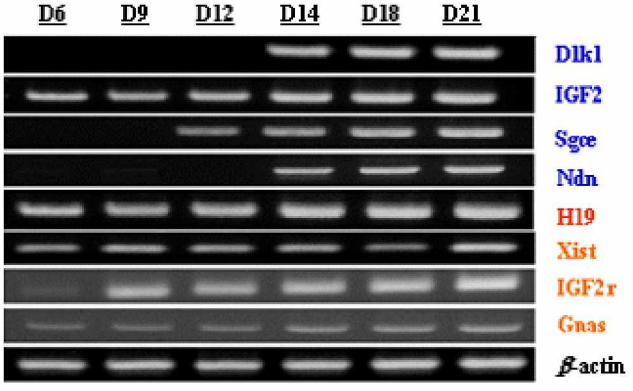


**Figure 2.** Representative gels from real-time RT-PCR analysis. Blue: Maternally imprinted genes; Red: Paternally imprinted genes.

### Statistical analysis

Data on mRNA expression were analyzed using the SigmaSat (Jandel Scientific, San Rafael, CA) software package. One-way repeated-measures ANOVA (followed by multiple pair-wise comparisons using Student-New-man-Kleus method) were used for the analysis of differences in mRNA expression assayed by quantitative RT-PCR. Differences of $p<0.05$ were considered significant.

### Results

The relative abundance of the gene transcripts studied is shown in Figure 3 and 4. Transcripts for maternally (*Dlk1*, *IGF2*, *Sgce* and *Ndn*) and paternally (*IGF2r*, *H19*, *Gnas* and *Xist*) imprinted genes were detected throughout the peri-implantation development.

### Messenger RNA expression of paternally imprinted genes

According to the photographs of representative gels from real-time RT-PCR analysis (Figure 2), our selected paternally imprinted genes (*IGF2r*, *H19*, *Gnas* and *Xist*

transcripts) were detected at all time points analyzed. *H19* transcripts were always detected on days 6, 9 and 12 of gestation. However, following completion of conceptus elongation on day 14, *H19* mRNA expression was significantly increased ($p<0.05$). Conceptus mRNA expression was more than 1100-fold higher on day 14, compared to day 6 of pregnancy. At Day 21, the expression levels of the gene were remarkably higher than before day 18. The pattern of expression of *Gnas* mRNA increased gradually from day 14 up to day 21, just before implantation. *Gnas* mRNA expression was 4.1-, 12.4- and 16.9-fold greater on days 14, 18 and 21, respectively, compared to that observed at day 6 in the blastocyst stage. Additionally the transcription levels of *IGF2r* and *Xist* remained fertilized thereafter, but significantly increased on Day 21, which was over 7.4- and 23.1-fold greater compared to the conceptus collected on day 6 of pregnancy, respectively ($p<0.05$) (Figure 3).

**Messenger RNA expression of maternally imprinted genes**

Figure 2 shows photographs of representative gels from RT-PCR analysis. Conceptus *IGF2* mRNA expression was detected at all time points analyzed from days 6 to 21, and was similar on days 6, 9 and 14. However, on days 18 and 21, *IGF2* mRNA expression was significantly increased ($p<0.05$). Conceptus mRNA expression was over 2112-7434-fold higher on day 18 and 21, compared to day 6 of pregnancy (Figure 4).

However, following completion of conceptus elongation on day 14, *Dlk1* and *Ndn* were first detected on day 14, and the *Sgce* gene was first detected on day 12. However, the patterns of expression of *Dlk1*, *Ndn* and *Sgce* as well as *IGF2* mRNA were similar during days 6-21 (Figure 4).

## DISCUSSION

During the pre-implantation period, mammalian embryogenesis is remarkable in terms of many critical events, both pre- and post-implantation of the embryo, that affect the genome (Latham and Schult, 2001). These events include changes related to genomic imprinting and changes in DNA methylation, chromatin structure, and the array of transcription factors. All these lead to the establishment of a nuclear structure that supports embryogenesis and allows for the successful performance of the developmental program (Latham and Schult, 2001).

Imprinted genes play an important role to regulate fetal growth, feto-placental development, and postnatal behavior in several animals (Surani et al., 1984, 1990; Constancia et al., 2002). Imprinted genes in mammals are expressed from only one of maternal or paternal chromosomes. As some

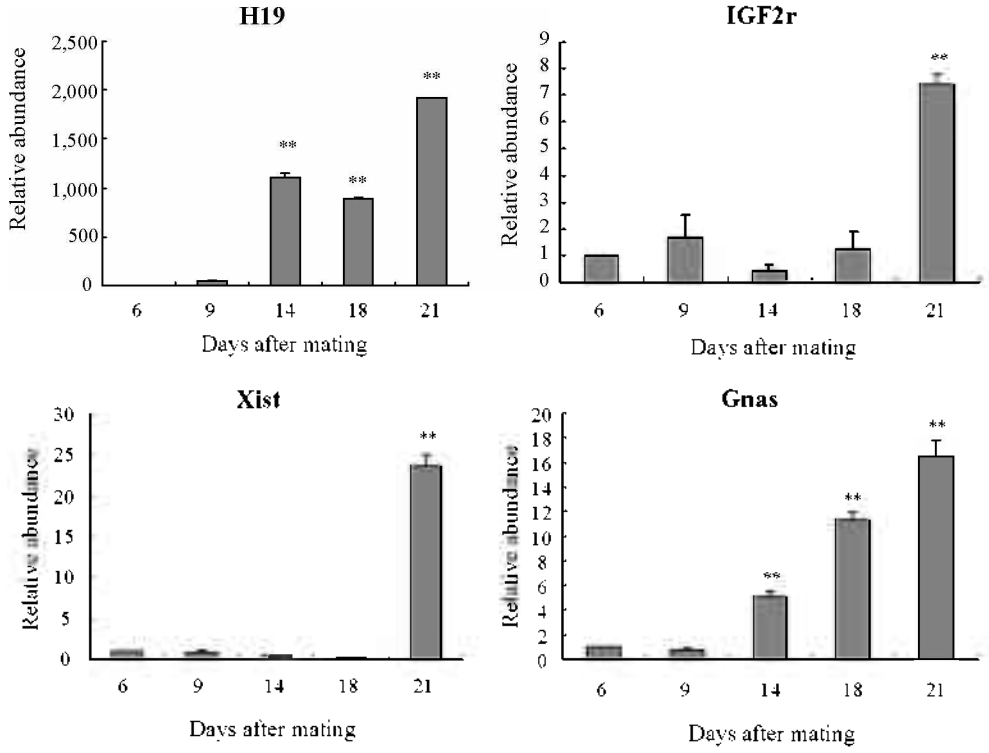


**Figure 3.** Relative expression levels of putative imprinted genes (paternally imprinted genes) from day 6 to day 21 fetus by real-time PCR. β-Actin gene expression was used as an internal standard (for real time PCR). The experiment was repeated five times. Bars indicate the standard errors of means. Asterisks denote significant differences from day 6 ($p<0.05$).

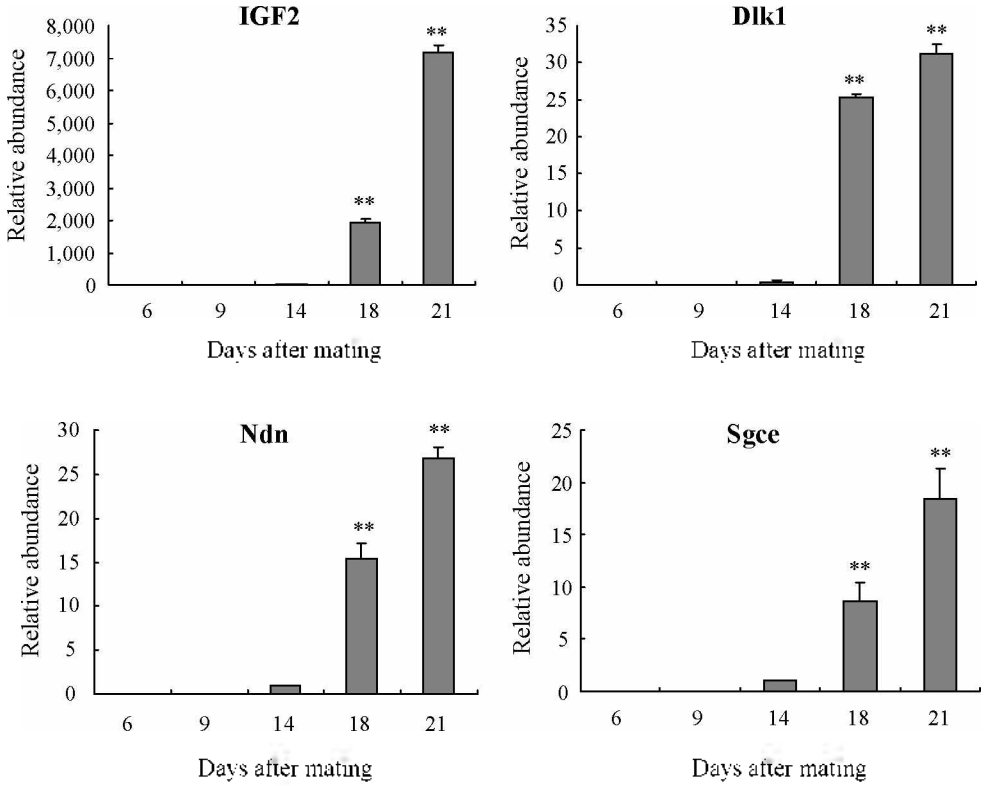


**Figure 4.** Relative expression levels of putative imprinted genes (maternally imprinted genes) from D6 to D21 fetus by real-time PCR. β-Actin expression was used as an internal standard (for real time PCR). The experiment was repeated five times. Bars indicate the standard errors of means. Asterisks denote significant difference from day 6 ($p<0.05$).

gene exhibits species-specific imprinting, the *ASCL2* gene was maternally expressed in the mouse, but detected by biallelic transcriptional activation in pig and human (Chung et al., 2007). Many studies have focused on genomic imprinting to investigate gene expression pattern, and quantitative trait loci (QTL), which underlies the genetic variance in animals, to map many chromosome regions (Lee et al., 2003; Lee et al., 2007).

In the present study, we first examined the expression of eight putative imprinted genes: *H19*, *IGF2r*, *Xist*, *Gnas*, *IGF2*, *Dlk1*, *Ndn* and *Sgce* in porcine conceptuses at days 6, 9, 12, 14, 18 and 21 after mating. *IGF2* and *H19* were the most widely studied imprinting genes, and they exhibited reciprocal expression by the parental allele. These genes were clustered within imprinting domain chromosomes 7, 11 and 2, in the mouse, human, and pig genomes, respectively (Reik et al., 2001; Amarger et al., 2002). Of the maternally imprinted genes, *IGF-2* encodes a growth factor that plays a crucial role in tissue differentiation, fetal growth (DeChiara et al., 1990; Efstratiadis et al., 1998) and placental development (Constancia et al., 2002). *H19* encodes for an untranslated RNA molecule (Brannan et al., 1990) and is expressed abundantly in the human placenta and in several embryonic tissues (Goshen et al., 1993). During the early development of the peri- and post-implanted embryo, the expression of *IGF2* was sharply increased on day 18 and 21 conceptus, compared to day 9 of pregnancy. Also, the level of the paternally imprinted gene, *H19* was highly increased after completion of conceptus elongation on day 14. Furthermore, we found that the other maternally imprinted genes, *Dlk1*, *Sgce* and *Ndn* started expression on day 14 of the embryo stage, and increased gradually up to day 21 of development. *Dlk1* mRNA is expressed in mouse embryos during the post-implantation development, beginning between embryonic day 7 and 11 (Schmidt et al., 2000). *Sgce* mRNA is dominantly expressed from a paternal allele in mouse adult tissue including the brain, and is also expressed in mouse embryos (Piras et al., 2000). *Ndn* is paternally expressed in the newborn mouse brain, and is highly expressed in human brain and placental tissues (McDonald et al., 1997).

In the case of the other paternally imprinted genes, expression of *IGF2r*, *Xist* and *Gnas* gradually increased from the blastocyst stage to various embryonic development stages. At day 21 of the embryonic stage, which is the stage post-implantation, transcription levels were highly expressed and almost completed the implantation. Expression of these imprinting genes appeared to coincide with the period shortly after porcine trophoblast attachment to the uterine surface in the pig. In general, mesodermal

outgrowths from the ICM (Inner cell mass) occurred when the porcine conceptuses were approximately 5 mm in spherical diameter on day 11 of gestation (Geisert et al., 1982). Differentiation of the mesoderm corresponded to an increase in conceptus estrogen synthesis and preceded rapid trophoblast elongation in the pig (Geisert and Yelich, 1997). The major conceptus loss occurs prior to gestational day 20, with the peri- and post-implantation stage appearing to be the most critical period. However, Increase of these putative imprinting gene transcripts from day 14 to day 21 of gestation corresponds to the period of continued embryonic differentiation and attachment to the uterine surface epithelium. Further studies are necessary to determine the protein expression level of these imprinting genes in the conceptus during the critical period of peri- and post-implantation development in the pig.

## REFERENCES


Amarger, V., M. Nguyen, A. S. Van Laere, M. Braunschweig, C. Nezer, M. Georges and L. Andersson. 2002. Comparative sequence analysis of the INS-IGF2-H19 gene cluster in pigs. Mamm Genome. 13:388-398.

Bennett, G. L. and K. A. Leymaster. 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. J. Anim. Sci. 67:1230-1241.

Brannan, C. I., E. C. Dees, R. S. Ingram and S. M. Tilghman. 1990. The product of the H19 gene may function as an RNA. Mol. Cell Biol. 10:28-36.

Cheng, H. C., F. W. Zhang, C. Y. Deng, C. D. Jiang, Y. Z. Xiong, F. E. Li and M. G. Lei. 2007. ASCL2 gene expression analysis and its association with carcass traits in pigs. Asian-Aust. J. Anim. Sci. 20:1485-1489.

Constancia, M., M. Hemberger, J. Hughes, W. Dean, A. Ferguson-Smith, R. Fundele, F. Stewart, G. Kelsey, A. Fowden, C. Sibley and W. Reik. 2002. Placental-specific IGF-II is a major modulator of placental and fetal growth. Nature 417:945-948.

DeChiara, T. M., A. Efstratiadis and E. J. Robertson. 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345:78-80.

Efstratiadis, A. 1998. Genetics of mouse growth. Int. J. Dev. Biol. 42:955-976.

Geisert, R. D., J. W. Brookbank, R. M. Roberts and F. W. Bazer. 1982. Establishment of pregnancy in the pig: II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy. Biol. Reprod. 27:941-955.

Geisert, R. D. and J. V. Yelich. 1997. Regulation of conceptus development and attachment in pigs. J. Reprod. Fertil. Suppl. 52:133-149.

Goshen, R., J. Rachmilewitz, T. Schneider, N. de-Groot, I. Ariel, Z. Palti and A. A. Hochberg. 1993. The expression of the H-19 and IGF-2 genes during human embryogenesis and placental development. Mol. Reprod. Dev. 34:374-379.

Gries, L. K., R. D. Geisert, M. T. Zavy, J. E. Garrett and G. L. Morgan. 1989. Uterine secretory alterations coincident with embryonic mortality in the gilt after exogenous estrogen administration. J. Anim. Sci. 67:276-284.

Killian, J. K., C. M. Nolan, A. A. Wylie, T. Li, T. H. Vu, A. R. Hoffman and R. L. Jirtle. 2001. Divergent evolution in M6P/IGF2R imprinting from the Jurassic to the Quaternary. Hum. Mol. Genet. 10:1721-1728.

Latham, K. E. and R. M. Schultz. 2001. Embryonic genome activation. Front Biosci. 6:D748-759.

Lee, H. K., S. S. Lee, T. H. Kim, G. J. Jeon, Y. S. Shin, J. H. Han, B. H. Choi and I. C. Cheong. 2003. Detection of imprinted QTL for growth traits in pigs. Asian-Aust. J. Anim. Sci. 16:1807-1092.

Lee, M. Y., J. H. Lee and J. J. Kim. 2007. Evaluation of reciprocal cross design on detection and characterization of non-mendelian QTL in F2 outbred populations: I. parent-of-origin effect. Asian-Aust. J. Anim. Sci. 20:1805-1811.

Li, X. P., K. T. Do, J. J. Kim, J. Huang, S. H. Zhao, Y. Lee, M. F. Rothschild, C. K. Lee and K. S. Kim. 2008. Molecular characteristics of the porcine DLK1 and MEG3 genes. Anim Genet. 39:189-192.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25:402-408.

MacDonald, H. R. and R. Wevrick. 1997. The necdin gene is deleted in Prader-Willi syndrome and is imprinted in human and mouse. Hum. Mol. Genet. 6:1873-1878.

Perry, J. S. 1981. The mammalian fetal membranes. J. Reprod. Fertil. 62:321-335.

Piras, G., A. El Kharroubi, S. Kozlov, D. Escalante-Alcalde, L. Hernandez, N. G. Copeland, D. J. Gilbert, N. A. Jenkins and C. L. Stewart. 2000. Zac1 (Lot1), a potential tumor suppressor gene, and the gene for epsilon-sarcoglycan are maternally imprinted genes: identification by a subtractive screen of novel uniparental fibroblast lines. Mol. Cell Biol. 20:3308-3315.

Pusateri, A. E., M. F. Rothschild, C. M. Warner and S. P. Ford. 1990. Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on days 9 to 13 of gestation. J. Anim. Sci. 68:3727-3735.

Reik, W., W. Dean and J. Walter. 2001. Epigenetic reprogramming in mammalian development. Science 293:1089-1093.

Ross, J. W., M. D. Ashworth, A. G. Hurst, J. R. Malayer and R. D. Geisert. 2003. Analysis and characterization of differential gene expression during rapid trophoblastic elongation in the pig using suppression subtractive hybridization. Reprod. Biol. Endocrinol. 1:23-34.

Ruddock, N. T., K. J. Wilson, M. A. Cooney, N. A. Korfiatis, R. T. Tecirlioglu and A. J. French. 2004. Analysis of imprinted messenger RNA expression during bovine preimplantation development. Biol. Reprod. 70:1131-1135.

Schmidt, J. V., P. G. Matteson, B. K. Jones, X. J. Guan and S. M. Tilghman. 2000. The *Dlk1* and *Gtl2* genes are linked and reciprocally imprinted. Genes Dev. 14:1997-2002.

Surani, M. A., S. C. Barton and M. L. Norris. 1984. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 308:548-550.

Surani, M. A., R. Kothary, N. D. Allen, P. B. Singh, R. Fundele, A. C. Ferguson-Smith and S. C. Barton. 1990. Genome imprinting and development in the mouse. Dev. Suppl. 89-98.

Tilghman, S. M. 1999. The sins of the fathers and mothers:

genomic imprinting in mammalian development. Cell. 96:185-193.

University of Otago. 2006. The catalogue of imprinted genes and parent-of-origin effects in humans and animals. http://igc.otago.ac.nz/home.html/Acessed Dec. 1, 2006.

Young, L. E., A. E. Schnieke, K. J. McCreath, S. Wieckowski, G. Konfortova, K. Fernandes, G. Ptak, A. J. Kind, I. Wilmut, P. Loi and R. Feil. 2003. Conservation of IGF2-H19 and IGF2R imprinting in sheep: effects of somatic cell nuclear transfer. Mech. 120:1433-1442.