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Analysis of imprinted messenger RNA expression in deceased transgenic cloned goats

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ABSTRACT. Genomic imprinting is an important epigenetic mechanism that has vital effects on fetal growth and development. We observed the differences in four tissues (heart, spleen, liver, and kidney) from dead transgenic cloned goats using hematoxylin and eosin (H&E) staining. Eight imprinted genes in the tissues of dead transgenic cloned and normal goats were analyzed using reverse transcription polymerase chain reaction. H&E staining results from the abortion group indicated the lack of obvious morphological changes in heart and spleen tissues, while inflammatory cell infiltration and glomerular nephritis characteristics were observed in liver and kidney tissues, respectively. Compared to the control group, *CDKN1C*, *H19*, *IGF2R*, and *SNRPN* were significantly ($P < 0.05$) overexpressed in the heart tissue of the abortion group, while *XIST* was significantly reduced. In the liver tissues, *CDKN1C* and *DLK1* expression decreased, while *GNAS*, *H19*, *IGF2R*, *PEG3*, and *XIST* expression increased significantly. In the spleen tissues, *DLK1* expression increased, while *GNAS*, *H19*, *IGF2R*, *PEG3*, *SNRPN*, and *XIST* expression decreased. In the kidney tissues, *CDKN1C*, *DLK1*, *GNAS*, *IGF2R*, and *PEG3* expression increased, while *H19* and *XIST* expression decreased. The overall expression of imprinted genes was abnormal in different tissues of transgenic cloned goats, and the

degree of abnormal genomic imprinting was more severe in the abortion group compared to the death and control groups. These results suggest that abnormal expression of imprinted genes may cause developmental defects in transgenic cloned goats. Moreover, abnormal epigenetic modifications may affect the reprogramming of transgenic donor cells.

Key words: Imprinted gene; Transgenic cloned goat

INTRODUCTION

The cloning of livestock animals by somatic cell nuclear transfer (SCNT) has been successfully achieved in a wide range of mammals, including a number of domestic species. However, there is a high incidence of abnormalities in SCNT clones, including low birth rate, placental dysfunctions, and large offspring syndrome (LOS), which collectively appear to be a great barrier to efficient cloning (Constant et al., 2006; Yang, et al., 2007; Bischoff et al., 2009).

The incomplete reprogramming of donor somatic cell nuclei, leading to aberrant (or even a lack of) expression of some developmentally important genes, has been implicated as a primary reason for this low efficiency. Genomic imprinting is an epigenetic mechanism that greatly impacts normal mammalian development.

Imprinted genes play important roles in embryo survival and postnatal growth regulation. Cyclin-dependent kinase inhibitor 1C (*CDKN1C*) is a potent inhibitor of several G1 cyclin/CDK complexes, and its overexpression leads to cell-cycle arrest in the G1 phase (Madhavan et al., 2010). Drosophila-like homolog 1 (*DLK1*) is a transmembrane signal protein with key roles in differentiation, and it controls several cell-differentiation processes throughout the embryonic and adult life cycles (Oczkowicz et al., 2010). Guanine nucleotide binding protein, alpha stimulating (*GNAS*) complex locus is a complex imprinted gene, which is required for normal fetal growth and development, and this transcript affects placental functions (Richard et al., 2013). *H19* and insulin-like growth factor-2 receptor (*IGF2R*) are among the best-studied imprinted genes involved in fetal growth regulation, and both are essential for normal development (Yang et al., 2005). The imprinted gene, paternally expressed gene 3 (*PEG3*), alters growth and development and regulates apoptosis (Jiang et al., 2010). *SNRPN* (small nuclear ribonucleoprotein polypeptide N) is a member of a gene family that encode proteins involved in pre-mRNA splicing and alternative RNA processing, which has been extensively studied due to correlations with cell differentiation, proliferation, and embryogenesis (Suzuki et al., 2009; Couldrey and Lee, 2010; Wang et al., 2012). X-inactive specific transcript (*XIST*), a long non-coding RNA, plays an important role in early embryonic development (Matoba et al., 2011).

Several studies of imprinted genes have been conducted using the pre-implanted embryos (Wrenzycki et al., 2004), placentas (Su et al., 2011), and fetuses (Ogawa et al., 2003) of cloned animals (e.g., mice, calves, and swine). However, there are limited studies that utilized transgenic cloned goats, especially the internal organs of these animals. Moreover, abnormal expression of imprinted genes in critical organs may cause the death of transgenic cloned animals. The objective of the present study was to investigate the expression of eight imprinted genes (*CDKN1C*, *DLK1*, *GNAS*, *H19*, *IGF2R*, *SNRPN*, *PEG3*, and *XIST*) in the heart, liver, spleen, and kidneys of transgenic cloned and normal goats to reveal possible genetic causes of death in transgenic cloned goats.

MATERIAL AND METHODS

Production of transgenic goats and sample collection

Deceased human lactoferrin (*hLF*) transgenic cloned goats were collected from previous studies that produced the goats using SCNT (Wan et al., 2012). Briefly, goat fibroblast cells from the ears of three-month-old goats were used to produce donor somatic cells harboring *hLF* for SCNT. Culture and passage of donor cells, oocyte collection and enucleation, nuclear transfer, oocyte activation, embryo culture, and embryo transfer were carried out in order (Wan et al., 2012). Normal goats of the same age, produced using conventional reproduction, were used as controls.

The transgenic cloned goats and three normal goats, with the same genetic background, were cultivated under the same husbandry conditions. The deceased transgenic goats were divided into two groups: 1) the abortion group included goats that died before the expected birth date, including four transgenic cloned goats; 2) the born dead group (D) included animals that died in 3 days after birth, including three transgenic cloned goats. The normal group (N) included three newborn normal goats produced via conventional reproduction, which is the control group.

Tissue collection

The tissues were collected immediately after the death of transgenic cloned goats and after the killing (overdose with barbiturate) of normal control goats. Some tissues were quickly frozen in liquid nitrogen and stored at -80°C. Other tissues were fixed in 4% formaldehyde for 24 h, and were then stored in 75% alcohol. Fixed tissues were analyzed for structural organization, whereas tissues in nitrogen were analyzed for the expression of imprinted gene mRNA. Animal handling and experimentation were in accordance with the National Research Council's publication "Guide for the Care and Use of Laboratory Animals", and the methods used were approved by the Institutional Animal Care and Use Committees at Nanjing Agricultural University.

Hematoxylin and eosin (H&E) staining

After fixation, tissues were embedded in paraffin, sectioned serially at 6 mm, and mounted on slides. Sections were deparaffinized in xylene, and then rehydrated in a series of graded ethanol concentrations before staining with H&E.

RNA isolation

Total RNA was extracted from the different tissues using the RNAprep Pure Tissue Kit (Tiangen, Beijing, China), according to the manufacturer protocol. RNA quality was confirmed using ratios of A_{260}/A_{280} and A260/A230 (Nanodrop, all between 1.8 and 2.0) and automated electrophoresis (BioRad Experion), which was based on the presence of three clear ribosomal RNA bands.

cDNA preparation

Reverse transcription was conducted using Transcriptor First Strand cDNA Synthesis Kit (Roche, USA). First, the template-primer mixture [1 µg total RNA, 1 µL Anchored-oligo(dT)18

Primer, 1.5 µL random hexamer primer, and up to 13 µL RNase-free dH_2O] was prepared. Second, the tube containing the template-primer mixture was heated for 10 min at 65°C to denature the secondary RNA structure. Third, 7 µL of the remaining components of the RT mix were added to the template-primer mix (4 µL 5X RT buffer, 0.5 µL Protector RNase Inhibitor, 2 µL Deoxynucleotide Mix, and 0.5 µL Transcriptor Reverse Transcriptase). Lastly, the RT reaction was incubated for 10 min at 25°C, followed by 30 min at 55°C, and the Transcriptor Reverse Transcriptase was then inactivated by heating the reactions for 5 min at 85°C.

Quantitative real-time PCR

The expression levels of the eight imprinted genes were quantified using an ABI7300 real-time PCR detection system (Applied Biosystems, Foster City, CA, USA) with the FastStart Universal SYBR Green Master (Rox) (Roche, USA). The 12.5-µL reaction mixture contained 6.25 µL SYBR Green Master, 0.5 µL PCR forward and reverse primers (10 µM), 4.75 µL dH_2O, and 1 µL cDNA template. The PCR conditions were as follows: 95°C for 10 min; 40 cycles of 95°C for 15 s, annealing for 30 s (annealing temperatures for each pair of primers are listed in Table 1), and 72°C for 30 s. Melting curves were generated after amplification to check the specificity of the amplified product. The transcripts of nine imprinted genes were quantified using three replicates, and the quantification was normalized to an endogenous RNA control (the house-keeping gene *GAPDH*). The negative control was analyzed without a DNA template in the PCR mix.

Table 1. Primers for quantitative real-time polymerase chain reaction.

Genes	Primer sequence (5'-3')	Product size (bp)	Sequence accession No.	Annealing temperature (°C)
CDKN1C	F: GCCCATCTAGCTTGCAGTCTCT R: CAGACGGCTCAGGAACCATT	112	NM_001142510	56.0
DLK1	F: GTGACCAGTGCGTGACCTTT R: GCAGGTCTTGTCCATGAAGC	454	AY360448	54.0
GNAS	F: GAAGGACAAGCAGGTCTACC R: GACCATGTTGTAGCTGCTG	675	AY376066	60.0
H19	F: GGACTGGAACTTGGACTTCTTCA R: TGGTGTGGGTCTTCCGTTC	133	AY091484	55.5
IGF2R	F: CCAGCGTCTGTGACTTCGTG R: CCATCCTTGCAGCCTCCTTC	220	DQ666954	60.0
PEG3	F: CGCCAAAGTCAGGGAGAG R: CTTAACTGCCAGGACACC	150	AY427787	60.0
SNRPN	F: GGGACCGTTTACACTTGAGAC R: GGAAATCCACCACAGGTACT	153	NM_174463	60.0
XIST	F: AACCTCACGCCATTCCTCTG R: GGGTAGGTGTTCCTCTTGAG	226	AF104906	56.0
GAPDH	F: CGACTTCAACAGCGACACTCAC R: CCCTGTTGCTGTAGCCCAATTC	118	NM_001034034	58.0

F = forward; R = reverse.

The RT-PCR results are presented as C_T values (mean ± SD), where C_t was defined as the threshold PCR cycle number at which the amplified product was first detected. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to quantify the relative mRNA levels using the following formula:

$$\Delta C_t = C_{t\ (target\ gene)} - C_{t\ (GAPDH)}, \text{ and } \Delta\Delta C_t = \Delta C_{t\ (sample)} - \Delta C_{t\ (control)}$$

The relative mRNA levels were calculated as $2^{-\Delta\Delta Ct}$.

Data and statistical analysis

Data were reported as means ± SEM, and the gene expression levels were analyzed using SPSS Statistics 17.0. Differences were considered significant at $P < 0.05$.

RESULTS

Tissues morphology of transgenic cloned goats

Representative images of heart, liver, spleen, and kidney for each group are shown in Figures 1-4. No apparent veterinary developmental abnormalities were found in hearts and spleens of each group (Figures 1 and 3). However, compared to the liver tissues of the N group (Figure 2A), inflammation cells were present in livers of the D and abortion groups (Figure 2B and C). Furthermore, compared to the kidney tissues of the N group (Figure 4A), there were no apparent structural changes in kidneys of the D group (Figure 4B), but glomerular nephritis characteristics were observed in the kidneys of the abortion group (Figure 4C).

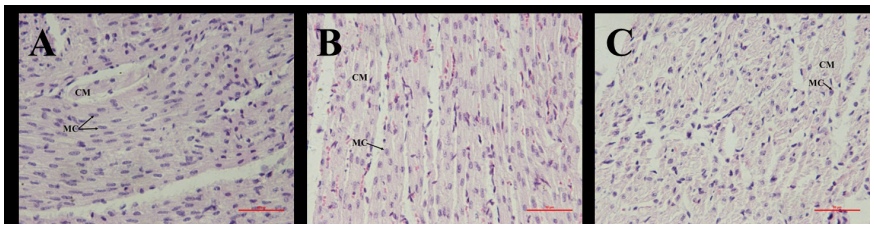

Figure 1. Cardiac morphology of transgenic cloned goats by H&E staining. **A.** normal goat; **B.** postnatal death group; **C.** abortion group; CM = cardiac muscle; MC = myocardial cell.

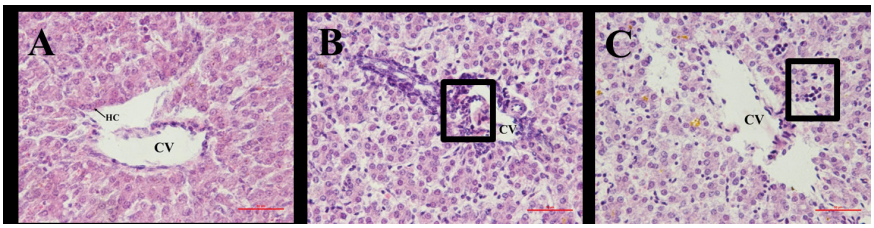

Figure 2. Hepatic morphology of transgenic cloned goats by H&E staining. **A.** normal goat; **B.** postnatal death group; **C.** abortion group; HC = hepatocytes; CV = central veins; black box highlights labeled inflammatory cells.

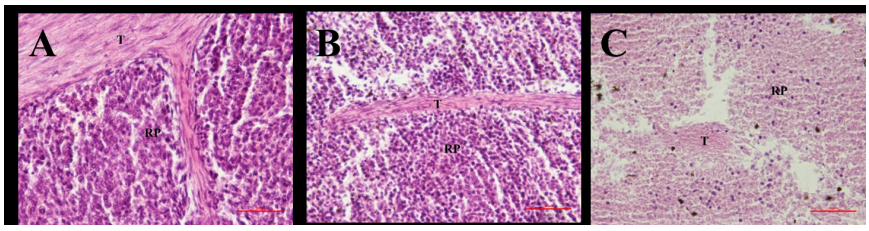

Figure 3. Splenetic morphology of transgenic cloned goats by H&E staining. **A.** = normal goat; **B.** postnatal death group; **C.** abortion group; T = trabecular; RP = red pulp.

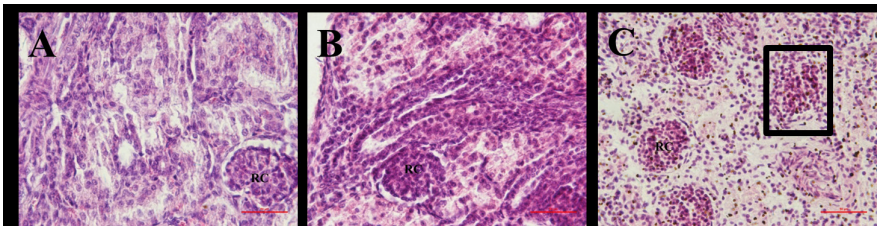

Figure 4. Renal morphology of transgenic cloned goats by H&E staining. **A.** normal goat; **B.** postnatal death group; **C.** abortion group; RC = renal corpuscle; black box highlights labeled capsular space deficiency.

Relative expression levels of imprinted genes in the heart

The expression levels of *CDKN1C*, *H19*, and *IGF2R* in the abortion group were significantly higher than that of the normal group (Figure 5A, D, and E; $P < 0.01$, $P < 0.01$, and $P < 0.05$, respectively) and the D group (Figure 5A, D, and E; $P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). Moreover, notably significant differences were detected between the *SNRPN* expression levels of normal and dead transgenic cloned goats. Specifically, the *SNRPN* expression level of the abortion group was higher than that of the N group, but the level of the D group was markedly lower than the N group (Figure 5G, $P < 0.01$). Moreover, the *XIST* expression levels of the abortion and D groups were significantly lower than the N group (Figure 5H, $P < 0.01$). No significant differences were detected between the normal goats and death transgenic cloned goats regarding the expression levels of the remaining imprinted genes (Figure 5B, C, and F; $P > 0.05$).

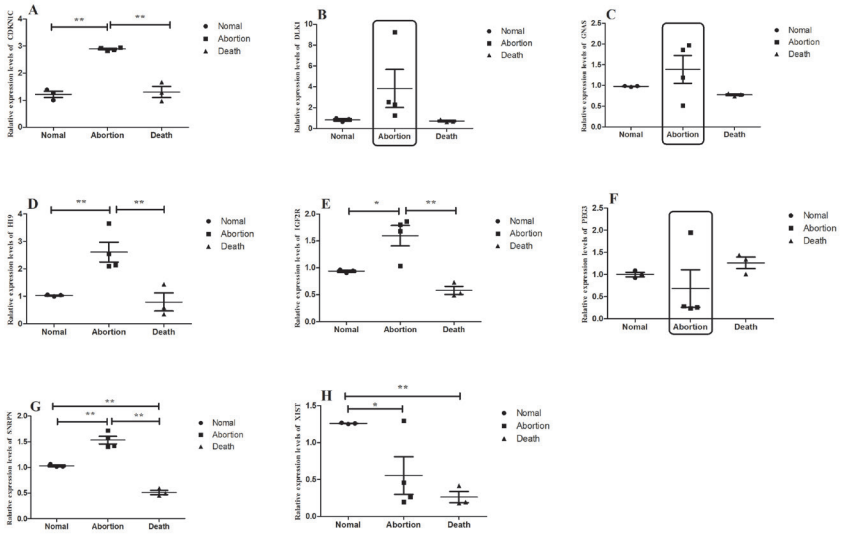

Figure 5. Relative expression levels of imprinted genes in heart tissues. **A. B. C. D. E. F. G. H.** Relative *CDKN1C, DLK1, GNAS, H19, IGF2R, PEG3, SNRPN,* and *XIST* expression levels in heart tissues of different groups, respectively. Normal = control group of normal goats; Abortion = abortion group of transgenic cloned goats; Death = postnatal death group of transgenic cloned goats. Sections surrounded with the black pane represent significant individual differences in the expression of imprinted genes, but there were no significant differences between the normal and abortion groups. **Remarkably significant differences ($P < 0.01$). *Significant differences ($P < 0.05$).

Relative expression levels of imprinted genes in the liver

Compared to the normal group, the expression levels of *CDKN1C* and *PEG3* were significantly higher in the abortion group (Figure 6A and F; P < 0.01 and P < 0.01, respectively), and the D group (Figure 6A and F; P < 0.05 and P < 0.01, respectively). However, the *GNAS* expression levels were significantly lower in the abortion and D groups (Figure 6C; P < 0.05 and P < 0.01, respectively).

The *DLK1* expression levels of the abortion group were significantly lower than the N and D groups (Figure 6B, P < 0.01). Contrarily, the *H19*, *IGF2R*, and *XIST* expression levels of the abortion group were significantly higher than those of the N group (Figure 6D, E, and H; P < 0.05, P < 0.05, and P < 0.01, respectively). No significant differences were detected between the *SNRPN* expression levels of normal and death transgenic cloned (Figure 6G, P > 0.05).

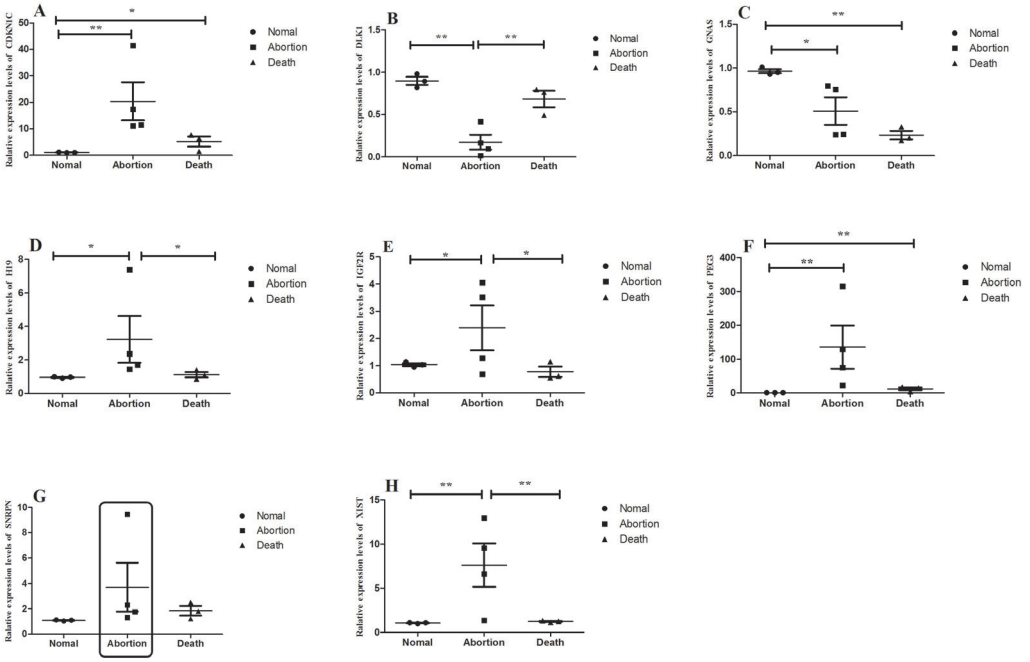

Figure 6. Relative expression levels of imprinted genes in liver tissues. **A. B. C. D. E. F. G. H.** Relative *CDKN1C, DLK1, GNAS, H19, IGF2R, PEG3, SNRPN,* and *XIST* expression levels in liver tissues of different groups; Normal = control group of normal goats; Abortion = abortion group of transgenic cloned goats; Death = postnatal death group of transgenic cloned goats. Sections surrounded with the black pane represent significant individual differences in the expression of imprinted genes, but there were no significant differences between the normal and abortion groups. **Remarkably significant differences (P < 0.01). *Significant differences (P < 0.05).

Relative expression levels of imprinted genes in the spleen

Compared to the normal group, the *DLK1* expression level was significantly higher in the abortion and D groups (Figure 7B, P < 0.01), but *GNAS* expression was significantly lower in the abortion group (Figure 7C, P < 0.01). Furthermore, the *H19*, *IGF2R*, *PEG3*, *SNRPN*, and *XIST*

expression levels were striking reduced in the abortion (Figure 7D-H; P < 0.01, P < 0.05, P < 0.01, P < 0.01, and P < 0.01, respectively) and D groups (Figure 7D-H; P < 0.01, P < 0.01, P < 0.01, P < 0.05, and P < 0.01, respectively) as compared to the N group. No significant differences were detected in *CDKN1C* expression levels (Figure 7A, P > 0.05).

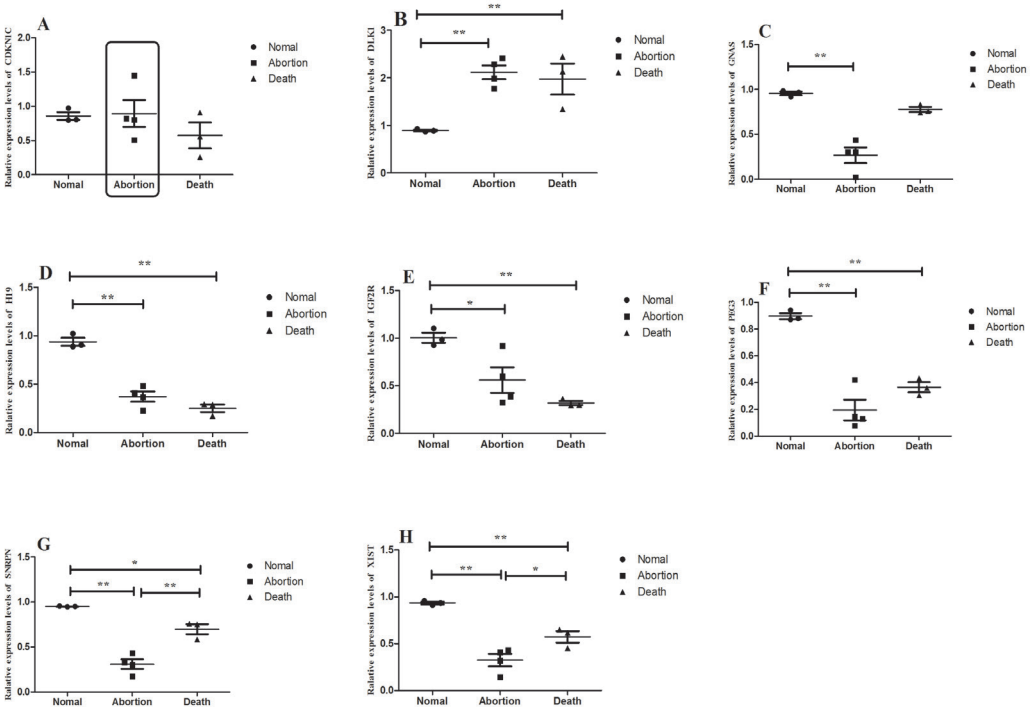

Figure 7. Relative expression levels of imprinted genes in spleen tissues. **A. B. C. D. E. F. G. H.** Relative *CDKN1C*, *DLK1*, *GNAS*, *H19*, *IGF2R*, *PEG3*, *SNRPN*, and *XIST* expression levels in the spleen tissues of different groups; Normal = control group of normal goats; Abortion = abortion group of transgenic cloned goats; Death = postnatal death group of transgenic cloned goats. Sections surrounded with the black pane represent significant individual differences in the expression of imprinted genes, but there were no significant differences between the normal and abortion groups. **Represents remarkably significant differences (P < 0.01). *Represents significant differences (P < 0.05).

Relative expression levels of imprinted genes in the kidney

In the abortion group, the *CDKN1C*, *DLK1*, *GNAS*, *H19*, *IGF2R*, and *PEG3* expression levels were visibly increased (Figure 8A-F; P < 0.01, P < 0.01, P < 0.01, P < 0.01, P < 0.05, and P < 0.05, respectively), whereas the *XIST* expression level was significantly reduced (Figure 8H, P < 0.01) as compared to the N group. Moreover, *PEG3* expression levels in the abortion group were discernibly lower than those of the N group (Figure 8F, P < 0.05). There were no significant differences in *SNRPN* expression (Figure 8G, P > 0.05).

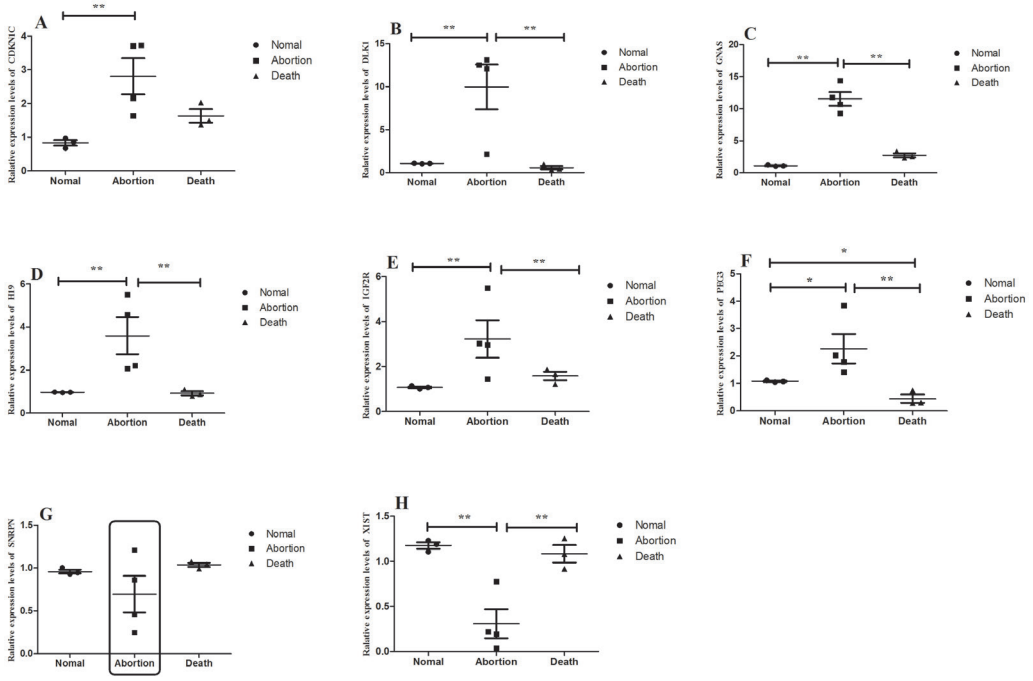

Figure 8. Relative expression levels of imprinted genes in kidney tissues. **A. B. C. D. E. F. G. H.** Represent the relative *CDKN1C*, *DLK1*, *GNAS*, *H19*, *IGF2R*, *PEG3*, *SNRPN*, and *XIST* expression levels in the kidney tissues of different groups; Normal = control group of normal goats; Abortion = abortion group of transgenic cloned goats; Death = postnatal death group of transgenic cloned goats. Sections surrounded with the black pane represent significant individual differences in the expression of imprinted genes, but there were no significant differences between the normal and abortion groups. **Remarkably significant differences ($P < 0.01$). *Significant differences ($P < 0.05$).

DISCUSSION

The possible negative long-term effects of cloning, as well as the high incidence of spontaneous abortion and abnormal birth observed in cloned animals, are causes for concern regarding attempts to clone animals for reproductive purposes. This study was performed to better understand why SCNT often results in a wide range of severe malformations such as neonatal edema, LOS, and immune system deficiencies. The examination of epigenetic information associated with imprinted genes during clonal development offers one method of addressing these questions.

Clear decreases of *CDKN1C* expression were observed in many types of malignancy, including hepatocarcinoma, esophageal cancer, and stomach carcinoma (Kavanagh and Joseph, 2011). Furthermore, abnormal expression of *GNAS* was associated with glucose-lipid metabolism disorder, and *GNAS* expression increased significantly in the livers of mice with hypercholesterolemia (Chen et al., 2005). Overexpression of *GNAS* promoted and aggravated hepatocarcinogenesis (Lambert et al., 2015). It was also demonstrated that high *PEG3* expression levels were closely associated with primary liver cancer and renal developmental defects (Ludgate et al., 2013; Shibata and Aburatani, 2014). In the present study, *CDKN1C* expression decreased, while *GNAS*

and *PEG3* increased significantly in the livers of the abortion and D groups. In addition, *GNAS* and *PEG3* expression increased significantly in the kidneys of the abortion group. The H&E staining results from the abortion group indicated that inflammatory cell infiltration and glomerular nephritis characteristics were observed in the liver and kidney tissues, respectively, and inflammation cells were also present in the livers of the D group. This result indicated that aberrant expression of these genes might contribute to organic developmental defects, which might lead to the abortion and death of transgenic cloned goats.

This study also found that some imprinted genes showed significant expression variation among different tissues within the same group. For instance, in the abortion group, *CDKN1C* exhibited higher expression in heart and kidney tissues but lower expression in liver tissues. Moreover, *DLK1* was more highly expressed in spleen and kidney tissues but lower expression in liver tissues. The expression of *GNAS*, *H19*, *IGF2R*, *PEG3*, *SNRPN*, and *XIST* also had similar situations. Similarly, in the death group, higher expression of *PEG3* was observed in the liver, but expression was lower in spleen and kidney tissues. These results were consistent with reports of gene expression in cloned piglets (Jiang et al., 2007). Lack of expression found in mouse research indicated that *CDKN1C* resulted in the excessive growth of the placenta and fetus, but its overexpression also induced fetal growth retardation and even death (Andrews et al., 2007; Tunster et al., 2011). The *DLK1* higher expression levels were proportional to the deterioration of hepatocarcinogenesis (Huang et al., 2007; Jin et al., 2008). *DLK1* knockout studies in mice also found evidence of growth retardation, skeletal deformities, and obesity (Lin et al., 2007). Higher *PEG3* expression could affect liver and kidney development in humans, and the homozygous deletion of *PEG3* in mice caused growth retardation and metabolic abnormalities (Li, 1999; Curley et al., 2005; Swaney et al., 2007; Champagne et al., 2009). These results indicated that aberrant expression (overexpression or low expression) of imprinted genes might cause abnormal growth and development of relevant organs. However, further research should be performed to examine the functional mechanisms of imprinted genes.

Furthermore, we found that expression disorders of imprinted genes were more severe in the abortion group than the born death group, and details are shown in Table 2. In the abortion group, five imprinting genes exhibited inordinate expression in the heart, and seven imprinting genes were abnormally expressed in the liver, spleen, and kidney. In the born dead group, there were two, three, six and one imprinted gene abnormally expressing in heart, liver, spleen and kidney tissues, respectively. These results indicated that there were relatively more developmental defects in the abortion transgenic cloned goats. Studies have shown that analysis of the RNA expression levels of imprinted genes is an important way to measure the degree of reprogramming in transgenic cloned embryos (Gong et al., 2012). Thus, significant aberrant expression of imprinted gene in the abortion group might reflect much lower reprogramming efficiency of transgenic cloned embryos. Aberrant expression of *H19*, *IGF2R*, and *XIST* was detected in heart, liver, spleen, and kidney tissues of the abortion group. *H19* is a sensitive imprinted gene, which contains a regulatory domain that is vulnerable to manipulation *in vitro* (Fauque et al., 2007). Moreover, *H19* controls the gene expression of the imprinted gene network by recruiting *MBD1*, which is involved in individual growth and development (Monnier et al., 2013). The results of previous studies showed that a CpG-island in intron 2 of *IGF2R* carries a maternal-specific methylation imprint that could act as an imprinting signal to maintain the expression of the maternal allele. Consistent with this result, genomic DNA methylation is necessary for *IGF2R* expression from the maternal allele (Wutz et al., 2001). Furthermore, *XIST* expression regulates DNA methylation of the 5' region of the CpG

island, which plays a key role in X chromosome inactivation (Zhao et al., 2011). These data indicate that DNA methylation acts as a key mechanism that regulates genomic imprinting. Consequently, imprinted gene expression disorders could reflect abnormal DNA methylation associated with the growth and development of transgenic cloned goats.

Table 2. List of significant differences in abortion and born death groups as compared to the normal group.

Tissues	Gene name	Significant difference A *vs* N	Significant difference D *vs* N
Heart	*CDKN1C*	**↑	No
	DLK1	No	No
	GNAS	No	No
	H19	**↑	No
	IGF2R	*↑	No
	PEG3	No	No
	SNRPN	**↑	**↓
	XIST	*↓	**↓
Liver	*CDKN1C*	**↑	*↑
	DLK1	**↓	No
	GNAS	*↓	**↓
	H19	*↑	No
	IGF2R	*↑	No
	PEG3	**↑	**↑
	SNRPN	No	No
	XIST	*↓	No
Spleen	*CDKN1C*	No	No
	DLK1	**↑	**↑
	GNAS	**↓	No
	H19	**↓	**↓
	IGF2R	*↓	**↓
	PEG3	**↓	**↓
	SNRPN	**↓	*↓
	XIST	**↓	**↓
Kidney	*CDKN1C*	**↑	No
	DLK1	**↑	No
	GNAS	**↑	No
	H19	**↑	No
	IGF2R	**↑	No
	PEG3	*↑	*↓
	SNRPN	No	No
	XIST	**↓	No

A = abortion group; D = born death group; N = normal group; **Remarkably significant difference ($P < 0.01$); *Significant difference ($P < 0.05$); up-arrow = increase; down-arrow = reduction.

In this study, all transgenic cloned goats were obtained using SCNT technology. Recent studies have suggested a link between the use of assisted reproductive techniques and an increase in normally rare imprinting disorders (Swales and Spears, 2005). In addition, the transfection, selection, and characterization procedures were requirements for the preparation of *hLF* transgenic donor cells. And these procedures might alter the gene expression profile of donor cells, which might influence reprogramming efficiency of donor cells (Zhao et al., 2009). Therefore, abnormal imprinting gene expression in the heart, liver, spleen, and kidney tissues of transgenic cloned goats might originate from the incomplete reprogramming of donor somatic cells, which might be associated with transgenic cloning technology. However, further studies on these effects are required.

In summary, our study showed alterations of imprinting gene expression in both the abortion and born death groups, and greater expression irregularities were detected in the abortion

group. Moreover, imprinting disorders in dead transgenic cloned goats may be due to the abnormal epigenetic modifications of donor cells during the reprogramming process. Increased knowledge of imprinted gene expression could provide clues to aid in our understanding of the molecular mechanisms associated with developmental defects in transgenic cloned goats, and it could also improve the low efficiency of transgenic technology in the future.

Conflicts of interest

The authors declare no conflict of interest.

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