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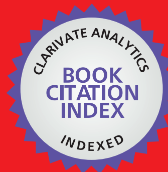

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Epigenetic Defects Related Reproductive Technologies: Large Offspring Syndrome (LOS)

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1. Introduction

Assisted reproductive technologies (ART), such as somatic cell nuclear transfer (SCNT) and *in vitro* fertilization (IVF), have been used to produce genetically superior livestock. Currently, embryos from IVF are commercially available from public or private corporations. However, calves derived by ART techniques frequently suffer with pathological changes in the fetal and placental phenotype, the so-called large offspring syndrome (LOS), and this has significant consequences for development both before and after birth (Behboodi et al., 1995; Constant et al., 2006; Wilmut et al., 2002; Young et al., 1998).

Although the etiology of LOS is not fully understood, these abnormalities may arise from disruptions in expression of developmentally important genes, in particular imprinted genes (Abu-Amero et al., 2006; Amor & Halliday, 2008; Angiolini et al., 2006; Coan et al., 2005; Fowden et al., 2006; Hitchins and Moore, 2002). Genomic imprinting is an important epigenetic mechanism in mammalian development, and is thought to influence the transfer of nutrients to the fetus and the newborn from the mother (Reik & Walter, 2001). Indeed, many imprinted genes are involved in fetal and placental development. Moreover, these imprinting defects cause various developmental disorders in humans, such as Beckwith–Wiedemann syndrome (BWS) (OMIM:130650), Russell–Silver syndrome (OMIM 180860), and Prader–Willi/Angelman syndrome (OMIM 105830) (Enklaar et al., 2006; Horike et al., 2009; Horsthemke & Wagstaff, 2008; Weksberg et al., 2003, 2005). In ruminants, the current study suggests that ART techniques, particularly *in vitro* culture of preimplantation embryos, have been associated with aberrant imprinted gene expression (Tveden-Nyborg et al., 2008). However, the exact mechanisms that lead to aberrant genomic imprinting after ART remain unknown.

The most likely explanation for the aberrant genomic imprinting in SCNT and IVF cattle may be failures in epigenetic reprogramming and/or maintenance (Bertolini et al., 2002; Beyhan et al., 2007; Blelloch et al., 2006; Everts et al., 2008; Hashizume et al., 2002; Herath et al. 2006; Hochedlinger et al. 2006; Oishi et al. 2006; Pfister-Genskow et al., 2005; Smith et al.,

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2005; Somers et al., 2006). Genome-wide epigenetic reprogramming in germ cells is essential in order to reset the parental-origin specific marking of imprinted genes. DNA methylation is one of the most important epigenetic marks for the allele-specific silencing of imprinted genes, and its genome-wide profiles undergo drastic changes during gametogenesis (Dupont et al., 2009; Arnaud & Feil, 2005; Bao et al., 2000). Indeed, the genome-wide DNA methylation patterns of the parental genomes are erased and a new methylation pattern is established by *de novo* methylation during gametogenesis (Arnaud & Feil, 2005; Bao et al., 2000). Therefore, the failures of epigenetic reprogramming could lead to loss of imprinting for many but not all imprinted genes (Reik & Walter, 2001).

A few reports to date have described the aberrant expression of imprinted genes in LOS animals produced by ART techniques. Interestingly, LOS phenotypes are reminiscent of BWS in humans, a loss-of-imprinting pediatric overgrowth syndrome associated with congenital malformations and tumor predisposition (Amor & Halliday, 2008; DeBaun et al., 2003; Maher et al., 2003; Maher, 2005; Manipalviratn et al., 2009; Shiota & Yamada, 2005, 2009). Because the majority of sporadic BWS patients show loss of DNA methylation at KvDMR1, which may function as an imprinting control region (ICR) on the *KCNQ1OT1*/*CDKN1C* domain (Mitsuya et al., 1999; Weksberg et al., 2003, 2005), it is possible that LOS is related to the loss of DNA methylation at KvDMR1, leading to diminished expression of *Cdkn1c*.

In this chapter we highlight some of the epigenetic defects identified in SCNT and IVF cattle and discuss the potential role that imprinted genes may play.

2. Assisted Reproductive Technologies (ART) and Large Offspring Syndrome (LOS)

LOS calves were first described by Willadsen et al. (1991) following ART technique; the fusion of blastomeres from embryos and enucleated eggs. Since then, oversized neonates and fetuses born after various manipulations of the embryo have been reported not only in calves, but also in sheep (Wilmut et al., 1997, 2002) and mouse (Eggan et al., 2001; Fernández-Gonzalez et al., 2004; Wakayama et al., 1998). Up to 40% of SCNT-derived full-term calves and lambs have LOS, which is characterized by large size at birth, enlarged umbilical cord, enlarged organs, hydrops of the fetus, lethargy, respiratory distress, muscle fiber composition, cerebellar dysplasia and skeletal and facial malformations (Chavatte-Palmer et al., 2002; Constant et al., 2006; Fletcher et al., 2007; Loi P et al., 2006; Maxfield et al., 1997; Schmidt et al., 1996; Walker et al., 1996; Young et al., 1998). Also, it is well known that in high frequency of LOS is also frequently observed in calves that developed from *in vitro* maturation (IVM) and IVF-derived embryos (Behboodi et al., 1995; Reichenbach et al., 1992; Bertolini et al., 2004).

The most remarkable feature of LOS is large size at birth. Increases in birth weight vary widely; twice the normal birth weight is not uncommon (Young et al., 1998). In our experiments, all calves derived by SCNT (n=7) and IVF (n=2) were shown to be a large size at birth, 1.3 to 2.3 times the normal birth weight. Enlarged umbilical cord was found in almost all of the calves (five of SCNT-derived and two of IVF-derived) (Fig.1), though abnormality of organs was found only in one SCNT-derived calf in our cases.

Fig. 1. Phenotype of LOS calf. Left, normal Japanese black calf produced by artificial insemination (body weight at birth: 27kg). Right, LOS Japanese black calf with enlarged umbilical cord produced by SCNT (body weight at birth: 51kg).

Placental anomalies, such as a reduced number of placentomes and increased weight of placentomes, lack of placental vascular development, reduced vascularization and poorly developed caruncles were also observed in all LOS cases in SCNT and IVF animals, and are thought to be associated with a high mortality rate and some fetal abnormalities (Bertolini & Anderson, 2002; Chavatte-Palmer et al., 2002; Constant at al., 2006; De Sousa et al., 2001; Hashizume et al., 2002; Hill et al., 2000, 2001).

While some investigations have previously suggested that reprogramming errors of the donor nucleus following SCNT could affect the fetal and placental development, the etiology of LOS remains unknown (Bertolini et al., 2002; Beyhan et al., 2007; Blelloch et al., 2006; Everts et al., 2008; Hashizume et al., 2002; Herath et al., 2006; Hochedlinger et al., 2006; Oishi et al., 2006; Pfister-Genskow et al., 2005; Smith et al., 2005; Somers et al., 2006).

Marques et al. (2004) have previously reported that paternal-allele-specific DNA methylation of the *H19* gene was significantly disrupted in spermatozoa from oligozoospermic patients. Although this result strongly suggests that transmission of paternal imprinting errors could affect embryo development, it is not likely that imprinting defects are associated with abnormal spermatogenesis in cattle, since commercially available sperms from healthy bulls are used for IVF.

3. ART culture may cause epigenetic changes

ART-derived animals can severely influence fetal growth, resulting in LOS, and any disturbance during germ cell development or early embryogenesis has the potential to alter epigenetic reprogramming and/or maintenance (Dupont et al., 2009). The birth of LOS was initially thought to associate with the procedure of ART but it is now recognized that enhanced fetal growth can also result from *in vitro* culture of oocytes or embryos (Behboodi et al., 1995; Farin et al., 2004; Farin & Farin, 1995; Maxfield et al., 1997; Smith et al., 2009; Walker et al., 1996).

Very limited information is currently available on the effects of *in vitro* culture; IVM, IVF or SCNT and *in vitro* development (IVD) on the establishment of imprinting in oocytes or embryos. The influences of *in vitro* culture on the epigenetic changes are investigated mainly in mouse. The culture medium influences the kinetics of embryo cleavage and embryo morphology up to the blastocyst stage, and can affect the imprinted expression of the *H19* gene as well as the DNA methylation status of ICR1, controlling its imprinted manner (Fauque et al., 2007). The presence of serum in culture medium for preimplantation embryos can influence the regulation of multiple growth-related imprinted genes and lead to aberrant fetal growth and development (Khosla et al., 2001). Some researchers reported that ammonium accumulates in culture medium have been linked to aberrant imprinting of *H19* and *Igf2r* (Gardner et al., 2005; Kerjean et al., 2003), however, other researchers have refuted these suggestion that follicle culture system under high ammonia levels showed normal DNA methylation patterns at regulatory sequences of *Snprn*, *Igf2r* and *H19* (Anckaert et al., 2009a, 2009b). Mineral oil, which is widely used in *in vitro* culture, has also been associated with delayed nuclear maturation and reduced development capacity in pig IVM (Shimada et al., 2002). Oil overly extracts steroid hormones in culture medium and reduces steroid hormone level by 55-70% (Anckaert et al., 2009b). Reduced steroid hormones, estrogens or xenobiotic substances with estrogenic effects in culture medium may interfere with normal imprinting establishment (Ho et al., 2006).

4. LOS in animals is reminiscent of BWS in human

The phenotypes of LOS in animals, such as large size at birth, enlarged umbilical cord and enlarged organs, are reminiscent of BWS in human. Therefore, LOS is speculated to occur primarily as the result of the misregulation of BWS-associated imprinted genes (Fig.2), while the genomic regions associated with LOS have not yet been determined. BWS is associated with epigenetic alterations at either one of two imprinting control regions on human chromosome 11p15.5, ICR1 and KvDMR1 (Enklaar et al., 2006; Ideraabdullah et al., 2008; Delaval et al., 2006; Mitsuya et al., 1999; Smith et al., 2007; Weksberg et al., 2003, 2005; Owen & Segars, 2009). The domain controlled by ICR1 includes the paternally expressed insulin-like growth factor 2 (*IGF2*) and the maternally expressed *H19* genes (Thorvaldsen et al., 1998; Owen & Segars, 2009). *IGF2* is known to be involved in regulation of fetal growth and development (Guo.et al., 2008). *H19* is also associated with embryogenesis and fetal growth in mouse (Pachnis et al., 1984), human (Goshen et al., 1993), and sheep (Lee et al., 2002). Several studies have shown that epigenetic alterations in the *Igf2/H19* domain are associated with LOS in cattle, sheep, and mice produced by ART techniques (Curchoe et al., 2009; DeChiara et al., 1991; Doherty et al., 2000; Khosla et al., 2001; Li et al., 2005; Moore et al., 2007; Yang et al., 2005; Young et al., 2000, 2003; Zhang et al., 2004). On the other hand, the domain controlled by KvDMR1 contains several maternally expressed genes including *CDKN1C*, that encodes a cyclin-dependent kinase inhibitor belong to the CIP/KIP family (Yan et al., 1997; Fitzpatrick et al., 2002; Horike et al., 2000). KvDMR1 is a maternally methylated CpG island and includes the promoter of a paternally expressed non-coding RNA (*KCNQ1OT1*) (Beatty et al., 2006; Mitsuya et al., 1999;). Interestingly, previous studies revealed that KvDMR1 is demethylated in about half of the individuals affected by BWS, and this is associated with the biallelic expression of *KCNQ1OT1* and subsequent repression of *CDKN1C* (Higashimoto et al., 2006; Lee et al., 1999; Mitsuya et al., 1999; Owen & Segars, 2009). Thus, while the *Igf2-H19* and *Cdkn1c-Kcnq1ot1* gene pairs are good LOS candidates,

the phenotypic similarities between LOS and human BWS remain suggestive and deregulation of imprinting remains a plausible candidate mechanism for LOS.

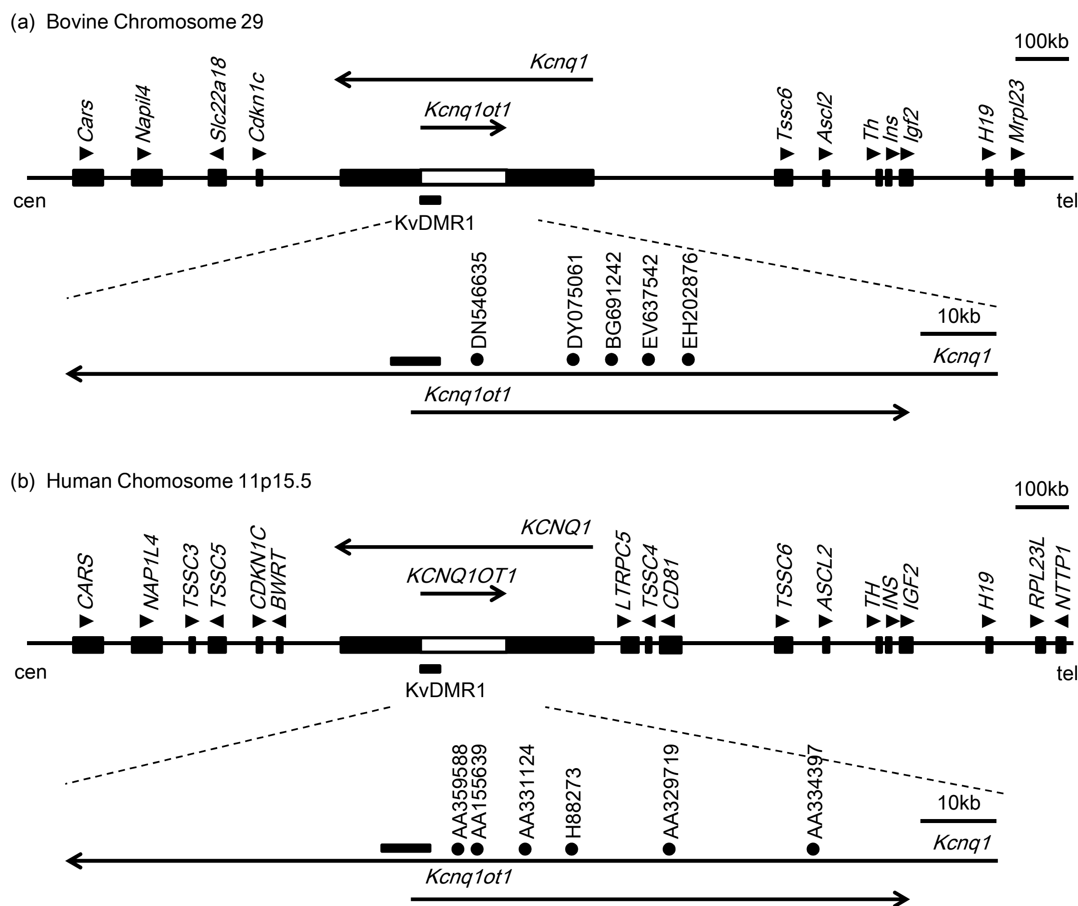

Fig. 2. Physical map of imprinting clusters in (a) bovine chromosome 29 and bovine KvDMR1, and (b) human chromosome 11p15.5 and human KvDMR1. Previously identified genes or transcripts (boxes) are drawn approximately to scale. Transcriptional orientation is indicated by arrows and arrowheads. Five and six expressed sequence tags of bovine and human are indicated by filled circle.

5. Assessment of the risk of imprinting defects in cattle born following ART

To assess of the risk of imprinting defects in cattle produced by SCNT and IVF, we analyzed DNA methylation status of the *Cdkn1c* promoter region, KvDMR1 and ICR1, and three promoter regions of other imprinted genes; *Peg1/Mest*, *Klf14* and *Gtl2* using CpG methylation sensitive restriction enzymes and bisulfite sequencing (Hori et al., 2010).

Since the use of two restriction enzymes with complementary methylation sensitivities, HpaII and McrBC, is unsurpassed as a simple, rapid method for the analysis of methylation status (Yamada et al., 2004), the HpaII–MspI–McrBC PCR assay is used for screening. HpaII and MspI recognize the CCGG sequence, but HpaII digestion is inhibited by CpG methylation at the internal cytosine while MspI is not. McrBC cleaves DNA containing a methylated cytosine and does not act upon unmethylated DNA (Fiona et al., 2000; Panne et al., 1999). In the case of a fully methylated sequence, amplification would be obtained only

from the HpaII-digested template. In contrast, an unmethylated sequence is digested only with HpaII but not with McrBC, and hence amplification would be obtained only from the McrBC-digested DNA. If the target sequence is differentially methylated, such as the imprinting control region, amplification will be obtained from both HpaII- and McrBC-digested DNA. Digestion profiles visualized by PCR amplification from the main organs of seven SCNT-derived and two IVF-derived calves were compared with those of three artificial insemination-derived calves. Lastly, the HpaII–MspI–McrBC PCR assays revealed aberrant KvDMR1 hypomethylation in two of seven SCNT-derived and one of two IVF-derived calves. For other imprinting control regions such as ICR1, *Peg1/Mest* and *Gtl2* promoter, PCR amplification was obtained from both HpaII- and McrBC-digested DNA from all samples, indicating that this region is differentially methylated in both normal and SCNT- and IVF-derived calves (Fig. 3). For the *Cdkn1c* and *Klf14* promoter, PCR amplification was obtained only from the McrBC-digested DNA, as indicating that both maternal and paternal alleles are unmethylated in all samples. In addition, bisulfite sequencing analyses were demonstrated to confirm the results obtained by HpaII–MspI–McrBC PCR analyses. Bisulfite sequencing is widely recognized to be the gold standard technique to analyze CpG methylation. Finally, these bisulfate sequencing analyses showed strong concordance with the HpaII–MspI–McrBC PCR results.

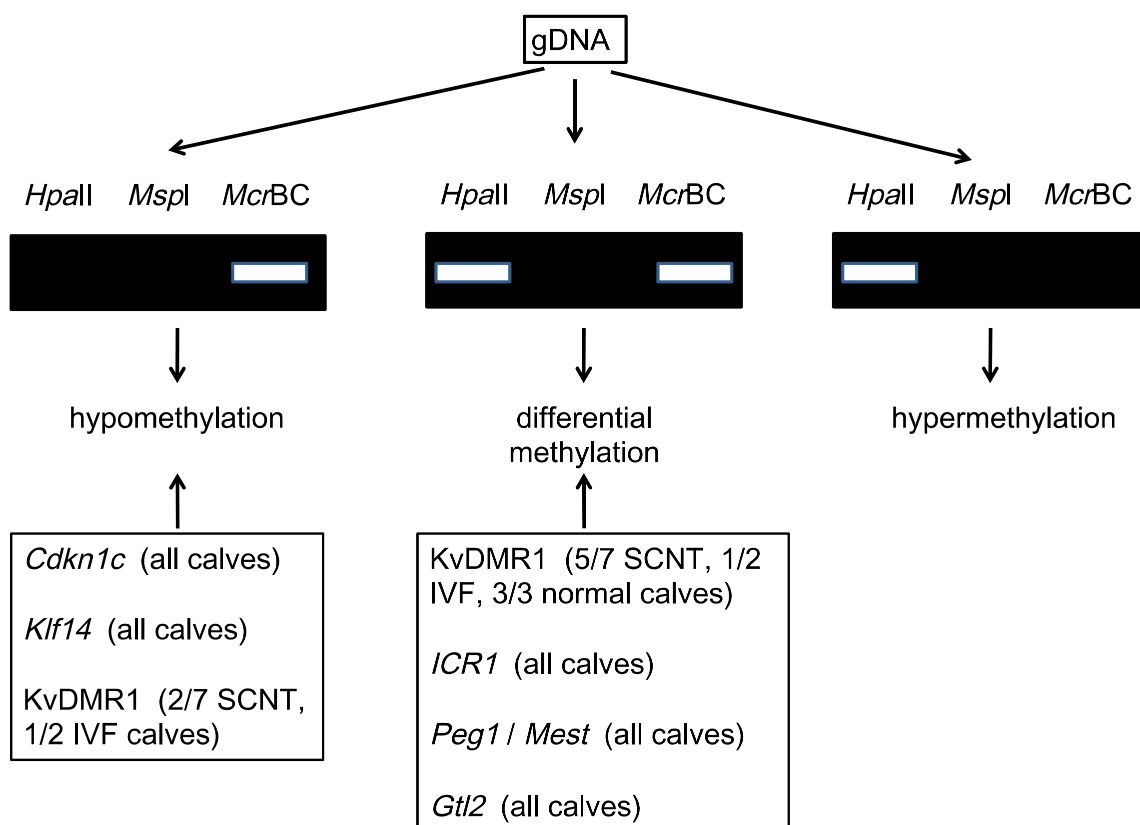

Fig. 3. A schematic gel pattern of HpaII-MspI-McrBC PCR products in hypomethylation, differentially methylation and hypermethylation cases and HpaII-MspI-McrBC PCR results of the selected six genes; Cdkn1c, Klf14, Peg1/Mest, KvDMR1, ICR1 and Gtl2 from seven SCNT-derived, Two IVF-derived and three normal calves.

To determine whether hypomethylation at KvDMR1 was linked to the aberrant expression of *Kcnq1ot1*, *Cdkn1c*, *Igf2*, or *H19*, we performed RT-PCR analysis on samples from two SCNT- and one IVF-derived calves, which showed hypomethylation status at KvDMR1, and compared gene expression patterns with those of a normal calf. In comparison to the normal calf, *Kcnq1ot1* transcript levels were increased in three ART-derived calves (two SCNT and one IVF derived calves), whereas the *Cdkn1c* transcript levels were reduced. No significant differences between three ART-derived calves and the normal calf were detected in *H19* or *Igf2* expression (Fig. 4(a)). The putative epigenetic regulation at *Kcnq1ot1*/*Cdkn1c* and *Igf2*/*H19* domains of normal and LOS cattle is shown in Fig.4 (b). These findings are consistent with the epigenetic alteration in the *Kcnq1ot1*/*Cdkn1c* domain of human chromosome 11p15.5 that has been observed in 50-60% of BWS patients. The biallelic expression of *Kcnq1ot1* and diminished expression of *Cdkn1c* observed in NT- and IVF-derived calves suffering with LOS in this study suggest that aberrant imprinting of the bovine *Kcnq1ot1*/*Cdkn1c* domain may contribute to LOS calves derived from ART techniques.

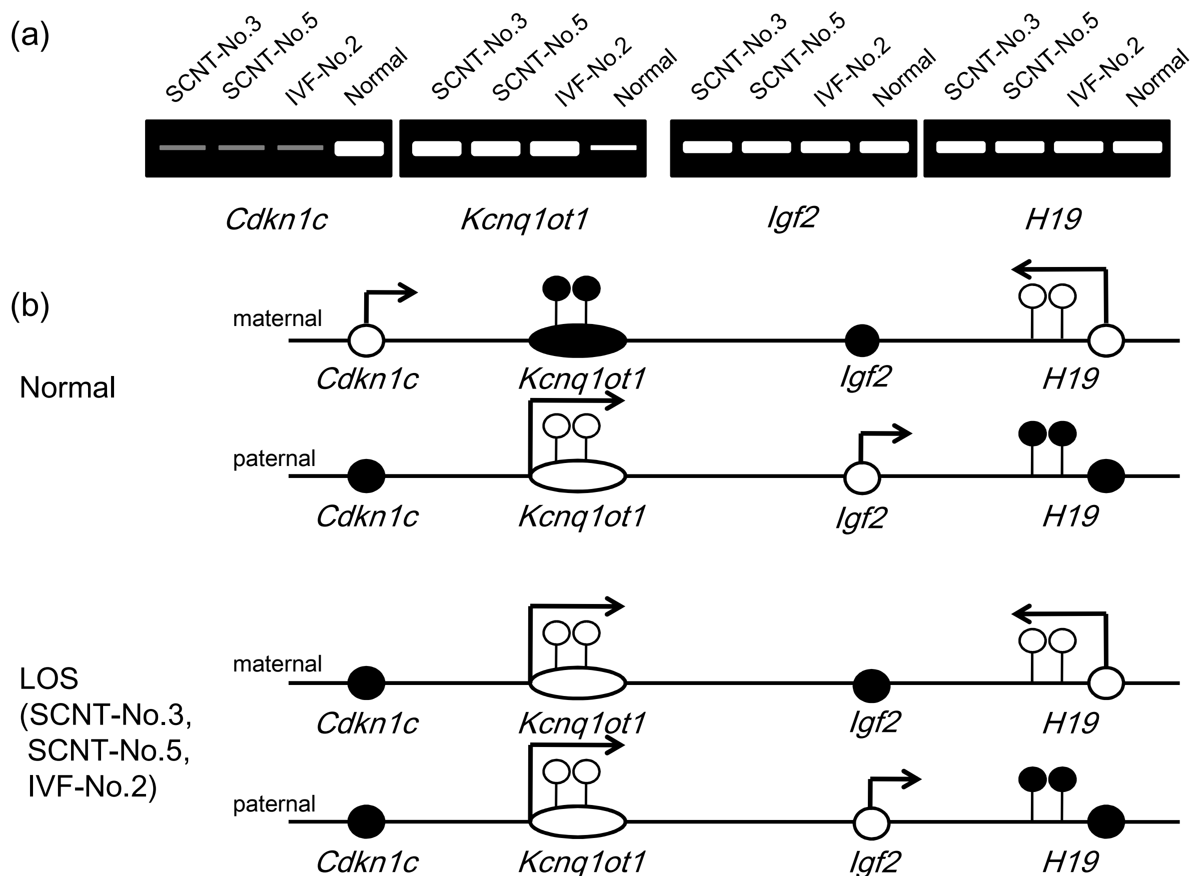

Fig. 4. (a) Scheme of RT-PCR amplification of *Cdkn1c*, *Kncq1ot1* and *H19* from SCNT-No.3 and 5, IVF-No.2 and normal cattle. (b) Putative epigenetic regulation at *Kcnq1ot1*/*Cdkn1c* and *Igf2*/*H19* domains of normal and LOS cattle. Transcription is indicated by arrows. Open and filled lollipop indicate unmethylated and methylated CpG site of KvDMR1 and ICR1.

6. Consideration and prospects

ART-derived embryos, particularly in the cow and sheep, can severely influence fetal growth, resulting in LOS. Disruptions in expression of developmentally important genes, in particular imprinted genes, were found in ART animals, suggesting that any disturbance during germ cell development or early embryogenesis may lead to altering of epigenetic changes. Aberrant gene expression is thought to associate with not only the procedure of ART, asynchronous embryo transfer or progesterone treatment but also *in vitro* culture of embryos.

The phenotypes of LOS are reminiscent of BWS in humans, an overgrowth syndrome associated with congenital malformations and tumor predisposition. Half of sporadic BWS cases show loss of DNA methylation at KvDMR1, which may function as an ICR on the *Kcnq1ot1*/*Cdkn1c* domain. Therefore we examined DNA methylation status of the bovine KvDMR1 in ART cattle. Abnormal hypomethylation status at an imprinting control region of *Kcnq1ot1*/*Cdkn1c* domain was observed in two of seven SCNT-derived calves and one of two IVF-derived calves. Moreover, abnormal expression of *Kcnq1ot1* and *Cdkn1c* were observed by RT-PCR analysis. There are very few papers which report KvDRM1 in ART-derived cattle. Coulrey and Lee (2010) reported hypomethylation of KvDMR1 in mid-gestation bovine fetuses produced by SCNT. Imprinting disruption of KvDMR1 and aberrant expression of *Kcnq1ot1* and *Cdkn1c* identified in SCNT and IVF calves may contribute to LOS in animals conceived using ART techniques. Our findings and those of Couldrey and Lee (2010) suggest that ART techniques might induce an increased risk of epigenetic defects, such as hypomethylation of KvDMR1, because epigenetic changes can be caused by embryo culture itself or the constituents of the culture medium. In humans, a significant deficit in DNA methylation at *Kcnq1ot1* in matured oocytes from stimulated cycles matured in *in vitro* culture (Khoueiry et al., 2008). This paper suggested that hyperstimulation likely recruits young follicles that are unable to acquire imprinting at KvDMR1 during the short *in vitro* maturation process. In cattle, it is unknown whether hyperstimulation is associated with acquiring imprinting at KvDMR1 of oocytes. A more thorough understanding of the stability of DNA methylation will be important for the continued safeguarding of ART techniques.

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8. References

Abu-Amero, S.; Monk, D.; Apostolidou, S.; Stanier, P. & Moore, G. (2006). Imprinted genes and their role in human fetal growth. *Cytogenetic and genome research* 113, 262–270.

Amor, DJ. & Halliday, J. (2008). A review of known imprinting syndromes and their association with assisted reproduction technologies. *Human Reproduction* Vol.23 (No.12): 2826–2834.

Anckaert, E.; Adriaenssens, T.; Romero, S.; Dremier, S. & Smitz, J. (2009). Unaltered imprinting establishment of key imprinted genes in mouse oocytes after in vitro follicle culture under variable follicle-stimulating hormone exposure. *The International journal of developmental biology* Vol.53 (No.4): 541-548.

Anckaert, E.; Adriaenssens, T.; Romero, S. & Smitz, J. (2009). Ammonium accumulation and use of mineral oil overlay do not alter imprinting establishment at three key imprinted genes in mouse oocytes grown and matured in a long-term follicle culture. *Biology of reproduction* Vol.81 (No.4): 666-673.

Angiolini, E.; Fowden, A.; Coan, P.; Sandovici, I.; Smith, P.; Dean, W.; Burton, G.; Tycko, B.; Reik, W.; Sibley, C. & Constância, M. (2006). Regulation of placental efficiency for nutrient transport by imprinted genes. *Placenta* Vol.27 (Suppl. A): S98–S102.

Arnaud, P. & Feil, R. (2005). Epigenetic deregulation of genomic imprinting in human disorders and following assisted reproduction. *Birth defects research. Part C, Embryo today : reviews* Vol.75 (No.2): 81-97.

Bao, S.; Obata, Y.; Carroll, J.; Domeki, I. & Kono, T. (2000). Epigenetic modifications necessary for normal development are established during oocyte growth in mice. *Biology of reproduction* Vol.62 (No.3): 616-21.

Beatty, L.; Weksberg, R. & Sadowski, PD. (2006). Detailed analysis of the methylation patterns of the KvDMR1 imprinting control region of human chromosome 11. *Genomics* Vol.87 (No.1): 46–56.

Behboodi, E.; Anderson, G.B.; BonDurant, RH.; Cargill, SL.; Kreuscher, BR.; Medrano, JF. & Murray, JD. (1995). Birth of large calves that developed from in vitro-derived bovine embryos. *Theriogenology* Vol.44 (No.2): 227–232.

Bertolini, M. & Anderson, GB. (2002). The placenta as a contributor to production of large calves. *Theriogenology* Vol.57 (No.1): 181-l87.

Bertolini, M.; Beam, SW.; Shim, H.; Bertolini, LR.; Moyer, AL.; Famula, TR. & Anderson, GB. (2002) Growth, development, and gene expression by in vivo- and in vitro-produced day 7 and 16 bovine embryos. *Molecular reproduction and development* Vol. 63 (No.3): 318–328.

Bertolini M, Moyer AL, Mason JB, Batchelder CA, Hoffert KA, Bertolini LR, Carneiro GF, Cargill SL, Famula TR, Calvert CC, Sainz RD, Anderson GB. (2004). Evidence of increased substrate availability to in vitro-derived bovine foetuses and association with accelerated conceptus growth. *Reproduction* Vol.128 (No.3): 341-354.

Beyhan, Z.; Forsberg, EJ.; Eilertsen, KJ.; Kent-First, M. & First, NL. (2007). Gene expression in bovine nuclear transfer embryos in relation to donor cell efficiency in producing live offspring. *Molecular reproduction and development* Vol. 74 (No.1): 18–27.

Blelloch, R, Wang Z.; Meissner, A.; Pollard, S.; Smith, A. & Jaenisch, R. (2006). Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the dono nucleus. *Stem Cells* Vol.24 (No.6): 2007–2013.

Chavatte-Palmer, P.; Heyman, Y.; Richard, C.; Monget, P.; Le Bourhis, D.; Kann, G.; Chilliard, Y.; Vignon, X. & Renard, JP. (2002). Clinical, hormonal, and hematologic characteristics of bovine calves derived from nuclei from somatic cells. *Biology of reproduction* Vol.66 (No.6): 1596–1603.

Coan, PM.; Burton, GJ. & Ferguson-Smith, AC. (2005). Imprinted genes in the placenta – a review. *Placenta* Vol.26 (Suppl. A): S10–S20.

Constant, F.; Guillomot, M.; Heyman, Y.; Vignon, X.; Laigre, P.; Servely, JL.; Renard, JP. & Chavatte-Palmer P. (2006). Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. *Biology of reproduction* Vol.75 (No.1): 122–130.

Couldrey, C. & Lee, RS. (2010). DNAmethylation patterns in tissues from midgestation bovine fetuses produced by somatic cell nuclear transfer show subtle abnormalities in nuclear reprogramming. *BMC developmental biology* Vol.10: 27.

Curchoe, CL.; Zhang, S.; Yang, L.; Page, R. & Tian, XC. (2009). Hypomethylation trends in the intergenic region of the imprinted IGF2 and H19 genes in cloned cattle. *Animal reproduction science* Vol.116 (No.3-4): 213–225.

DeBaun, MR.; Niemitz, EL. & Feinberg, AP. (2003). Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *American journal of human genetics* Vol.72 (No.1): 156–160.

DeChiara, TM.; Robertson, EJ.; Efstratiadis, A.; (1991). Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* Vol.64 (No.4): 849–859.

Delaval, K.; Wagschal, A. & Feil, R. (2006). Epigenetic deregulation of imprinting in congenital diseases of aberrant growth. *Bioessays* Vol.28 (No.5): 453–459.

De Sousa, PA.; King, T.; Harkness, L.; Young, LE.; Walker, SK. & Wilmut, I. (2001). Evaluation of gestational deficiencies in cloned sheep fetuses and placentae. *Biology of reproduction* Vol.65 (No.1): 23–30.

Doherty, AS.; Mann, MR.; Tremblay, KD.; Bartolomei, MS. & Schultz, RM. (2000). Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biology of reproduction* Vol.62 (No.6): 1526–1535.

Dupont, C.; Armant, DR. & Brenner, CA. (2009). Epigenetics: definition, mechanisms and clinical perspective. *Seminars in reproductive medicine* Vol.27 (No.5): 351-357.

Eggan, K.; Akutsu, H.; Loring, J.; Jackson-Grusby, L.; Klemm, M.; Rideout 3rd, WM.; Yanagimachi, R. & Jaenisch, R. (2001). Hybrid vigor, fetal overgrowth, and viability of mice derived by nuclear cloning and tetraploid embryo complementation. *Proceedings of the National Academy of Sciences of the United States of America* Vol.98 No.11): 6209–6214.

Enklaar, T.; Zabel, BU. & Prawitt, D. (2006). Beckwith-Wiedemann syndrome: multiple molecular mechanisms. *Expert reviews in molecular medicine* Vol.8 (No.17): 1–19.

Everts, RE.; Chavatte-Palmer, P.; Razzak, A.; Hue, I.; Green, CA.; Oliveira, R.; Vignon, X.; Rodriguez-Zas, SL.; Tian, XC.; Yang, X.; Renard, JP. & Lewin, HA. (2008). Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. Aberrant gene expression patterns in placentomes are associated with phenotypically normal and abnormal cattle cloned by somatic cell nuclear transfer. *Physiological genomics* Vol.33 (No.1): 65-77.

Farin, CE.; Farin, PW.& Piedrahita, JA. (2004). Development of fetuses from in vitro-produced and cloned bovine embryos. *Journal of animal science*. Vol.82 E-Suppl: E53-62.

Farin, PW. & Farin, CE. (1995). Transfer of bovine embryos produced in vivo or in vitro : survival and fetal development. *Biology of reproduction* Vol.52 (No.3): 676-682.

Fauque, P.; Jouannet, P.; Lesaffre, C.; Ripoche, MA.; Dandolo, L.; Vaiman, D. & Jammes, H. (2007). Assisted Reproductive Technology affects developmental kinetics, H19 Imprinting Control Region methylation and H19 gene expression in individual mouse embryos. *BMC developmental biology* Vol.7: 116.

Fernández-Gonzalez, R.; Moreira, P.; Bilbao, A.; Jiménez, A.; Pérez-Crespo, M.; Ramírez, MA.; Rodríguez De Fonseca, F.; Pintado, B. & Gutiérrez-Adán, A. (2004). Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. *Proceedings of the National Academy of Sciences of the United States of America* Vol.101 (No.16); 5880–5885.

Fiona, JS.; Daniel, P.; Thomas, AB. & Elisabeth, AR. (2000). Methyl-specific DNAbinding by McrBC, amodification-dependent restriction enzyme. *Journal of molecular biology* Vol.298 (No.4): 611–622.

Fitzpatrick, GV.; Soloway, PD. & Higgins, MJ. (2002). Regional loss of imprinting and growth deficiency in mice with a targeted deletion of KvDMR1. *Nature genetics* Vol.32 (No.3), 426–431.

Fletcher, CJ.; Roberts, CT.; Hartwich, KM.; Walker, SK. & McMillen, IC. (2007). Somatic cell nuclear transfer in the sheep induces placental defects that likely precede fetal demise. *Reproduction* Vol.133 (No.1): 243–255.

Fowden, AL.; Sibley, C.; Reik, W. & Constancia, M. (2006). Imprinted genes placental development and fetal growth. *Hormone research* Vol.65 (Suppl. 3): 50–58.

Gardner, DK. & Lane, M. (2005). Ex vivo early embryo development and effects on gene expression and imprinting. *Reproduction, fertility, and development* Vol.17 (No.3): 361-370.

Goshen, R.; Rachmilewitz, J.; Schneider, T.; de-Groot, N.; Ariel, I.; Palti, Z. & Hochberg, AA. (1993). The expression of the H-19 and IGF-2 genes during human embryogenesis and placental development. *Molecular reproduction and development* Vol.34 (No.4): 374-379.

Guo, L.; Choufani, S.; Ferreira, J.; Smith, A.; Chitayat, D.; Shuman, C.; Uxa, R.; Keating, S.; Kingdom, J. & Weksberg, R. (2008). Altered gene expression and methylation of the human chromosome 11 imprinted region in small for gestational age (SGA) placentae. *Developmental biology* Vol.320 (No.1): 79-91.

Hashizume, K.; Ishiwata, H.; Kizaki, K.; Yamada, O.; Takahashi, T.; Imai, K.; Patel, OV.; Akagi, S.; Shimizu, M.; Takahashi, S.; Katsuma, S.; Shiojima, S.; Hirasawa, A.; Tsujimoto, G.; Todoroki, J. & Izaike, Y. (2002). Implantation and placental development in somatic cell clone recipient cows. *Cloning and stem cells* Vol.4 (No.3): 197–209.

Herath, CB.; Ishiwata, H.; Shiojima, S.; Kadowaki, T.; Katsuma, S.; Ushizawa, K.; Imai, K.; Takahashi, T.; Hirasawa, A.; Takahashi, S.; Izaike, Y.; Tsujimoto, G. & Hashizume, K. (2006). Developmental aberrations of liver gene expression in bovine fetuses

derived from somatic cell nuclear transplantation. *Cloning Stem Cells* Vol.8 (No.1): 79–95.

Higashimoto, K.; Soejima, H.; Saito, T.; Okumura, K. & Mukai, T. (2006). Imprinting disruption of the KCNQ1OT1/CDKN1C domain: the molecular mechanisms causing Beckwith-Wiedemann syndrome and cancer. *Cytogenetic and genome research* Vol.113 (No.1-4): 306–312.

Hitchins, MP. & Moore, GE. (2002). Genomic imprinting in fetal growth and development. *Expert reviews in molecular medicine* Vol.4 (No.11): 1–19.

Hill, JR.; Burghardt, RC.; Jones, K.; Long, CR.; Looney, CR.; Shin, T.; Spencer, TE.; Thompson, JA.; Winger, QA. & Westhusin, ME. (2000). Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biology of reproduction* Vol.63 (No.6): 1787–1794.

Hill, JR.; Edwards, JF.; Sawyer, N.; Blackwell, C. & Cibelli, JB. (2001). Placental anomalies in a viable cloned calf. *Cloning* Vol.3 (No.2): 83–88.

Ho, SM.; Tang, WY.; Belmonte de Frausto, J. & Prins, GS. (2006). Developmental exposure to estradiol and bisphenol A increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. *Cancer research* Vol.66 (No.11): 5624-5632.

Hochedlinger, K. & Jaenisch, R. (2006). Nuclear reprogramming and pluripotency. *Nature* Vol.441 (No.29): 1061–1067.

Hori N, Nagai M, Hirayama M, Hirai T, Matsuda K, Hayashi M, Tanaka T, Ozawa T, Horike S. (2010). Aberrant CpG methylation of the imprinting control region KvDMR1 detected in assisted reproductive technology-produced calves and pathogenesis of large offspring syndrome. *Animal reproduction science* Vol.122 (No.3-4): 303-312.

Horike, S.; Ferreira, JC.; Meguro-Horike, M.; Choufani, S.; Smith, AC.; Shuman, C.; Meschino, W.; Chitayat, D.; Zackai, E.; Scherer, SW. & Weksberg, R. (2009). Screening of DNA methylation at the H19 promoter or the distal region of its ICR1 ensures efficient detection of chromosome 11p15 epimutations in Russell-Silver syndrome. *American journal of medical genetics. Part A* Vol.A149 (No.11): 2415–2423.

Horike, S.; Mitsuya, K.; Meguro, M.; Kotobuki, N.; Kashiwagi, A.; Notsu, T.; Schulz, TC.; Shirayoshi, Y. & Oshimura, M. (2000). Targeted disruption of the human LIT1 locus defines a putative imprinting control element playing an essential role in Beckwith-Wiedemann syndrome. *Human Molecular Genetics* Vol.9 (No.14): 2075–2083.

Horsthemke, B. & Wagstaff, J. (2008). Mechanisms of imprinting of the Prader-Willi/Angelman region. *American journal of medical genetics. Part A* Vol.146A (No.16): 2041–2052.

Ideraabdullah, FY.; Vigneau, S. & Bartolomei, MS. (2008). Genomic imprinting mechanisms in mammals. *Mutation research* Vol.647 (No.1-2): 77–85.

Kerjean, A.; Couvert, P.; Heams, T.; Chalas, C.; Poirier, K.; Chelly, J.; Jouannet, P.; Paldi, A. & Poirot, C. (2003). In vitro follicular growth affects oocyte imprinting establishment in mice. *European journal of human genetics* Vol.11 (No.7): 493-496.

Khosla S, Dean W, Brown D, Reik W, Feil R. (2001). Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biology of reproduction* Vol.64 (No.3): 918-926.

Khoueiry, R,; Ibala-Rhomdane, S,; Méry, L,; Blachère, T,; Guérin, JF,; Lornage, J. & Lefèvre, A. (2008). Dynamic CpG methylation of the *KCNQ1OT1* gene during maturation of human oocytes. *Journal of medical genetics* Vol.45 (No.9): 583-588.

Khosla S.; Dean, W.; Brown, D. Reik, W. & Feil, R. (2001). Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biology of reproduction* Vol.64 (No.3): 918–926.

Lee, MP.; DeBaun, MR.; Mitsuya, K.; Galonek, HL.; Brandenburg, S.; Oshimura, M. & Feinberg, AP. (1999). Loss of imprinting of a paternally expressed transcript, with antisense orientation to KVLQT1, occurs frequently in Beckwith-Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. Proc. *Proceedings of the National Academy of Sciences of the United States of America* Vol.96 (No.9): 5203–5208.

Lee, RS.; Depree, KM. & Davey, HW. (2002). The sheep (Ovis aries) H19 gene: genomic structure and expression patterns, from the preimplantation embryo to adulthood. *Gene* Vol.301 (No.1-2): 67-77.

Li, T.; Vu, TH.; Ulaner, GA.; Littman, E.; Ling, JQ.; Chen, HL.; Hu, JF.; Behr, B.; Giudice, L. & Hoffman, AR. (2005). IVF results in de novo DNA methylation and histone methylation at an Igf2-H19 imprinting epigenetic switch. *Molecular human reproduction* Vol.11 (No.9): 631–640.

Loi, P.; Clinton, M.; Vackova, I.; Fulka, J Jr.; Feil, R.; Palmieri, C.; Della Salda, L. & Ptak, G. (2006). Placental abnormalities associated with post-natal mortality in sheep somatic cell clones. *Theriogenology* Vol.65 (No.6): 1110–1121.

Maher, ER.; Brueton, LA.; Bowdin, SC.; Luharia, A.; Cooper, W.; Cole, TR.; Macdonald, F.; Sampson, JR.; Barratt, CL.; Reik, W. & Hawkins, MM. (2003). Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). *Journal of medical genetics* Vol.40 (No.1): 62–64.

Maher, ER. (2005). Imprinting and assisted reproductive technology. *Human Molecular Genetics* Vol.14: R133–R138.

Manipalviratn, S.; DeCherney, A. & Segars, J. (2009). Imprinting disorders and assisted reproductive technology. *Fertility and sterility* Vol.91 (No.2): 305–315.

Marques, CJ.; Carvalho, F.; Sousa, M.& Barros, A. (2004). Genomic imprinting in disruptive spermatogenesis. *Lancet* Vol.363 (No.9422): 1700-1702.)

Maxfield, EK.; Sinclair, KD.; Dolman, DF.; Staines, ME. & Maltin, CA. (1997). In vitro culture of sheep embryos increases weight, primary fibre size and secondary to primary fibre ratio in fetal muscle at day 61 of gestation. *Theriogenology* Vol.47: 376.

Mitsuya, K.; Meguro, M.; Lee, MP.; Katoh, M.; Schulz, TC.; Kugoh, H.; Yoshida, MA,; Niikawa, N,; Feinberg, AP,; Oshimura, M. (1999) LIT1, an imprinted antisense RNA in the human KvLQT1 locus identified by screening for differentially expressed transcripts using monochromosomal hybrids. *Human Molecular Genetics* Vol.8 (No.7): 1209–1217.

Moore, K.; Kramer, JM.; Rodriguez-Sallaberry, CJ.; Yelich, JV. & Drost, M. (2007). Insulin-like growth factor (IGF) family genes are aberrantly expressed in bovine conceptuses produced in vitro or by nuclear transfer. *Theriogenology* Vol.68 (No.5): 717–727.

Oishi, M.; Gohma, H.; Hashizume, K.; Taniguchi, Y.; Yasue, H.; Takahashi, S.; Yamada, T. & Sasaki, Y. (2006). Early embryonic death-associated changes in genome-wide gene expression profiles in the fetal placenta of the cow carrying somatic nuclear-derived cloned embryo. *Molecular reproduction and development* Vol.73 (No.4): 404–409.

Owen, CM. & Segars, JH Jr. (2009). Imprinting disorders and assisted reproductive technology. *Seminars in reproductive medicine* Vol.27 (No.5): 417-28.

Pachnis, V.; Belayew, A. & Tilghman, SM. (1984). Locus unlinked to alpha-fetoprotein under the control of the murine raf and Rif genes. Proceedings of the National Academy of Sciences of the United States of America Vol.81 (No.17): 5523-5527.

Panne, D.; Raleigh, EA. & Bickle, TA. (1999). The McrBC endonuclease translocates DNA in a reaction dependent on GTP hydrolysis. *Journal of molecular biology* Vol.290 (No.1): 49–60.

Pfister-Genskow, M.; Myers, C.; Childs, LA.; Lacson, JC.; Patterson, T.; Betthauser, JM.; Goueleke, PJ.; Koppang, RW.; Lange, G.; Fisher, P.; Watt, SR.; Forsberg, EJ.; Zheng, Y.; Leno, GH.; Schultz, RM.; Liu, B.; Chetia, C.; Yang, X.; Hoeschele, I. & Eilertsen, KJ.(2005). Identification of differentially expressed genes in individual bovine preimplantation embryos produced by nuclear transfer: improper reprogramming of genes required for development. *Biology of reproduction* Vol.72 (No.3): 546–555.

Reichenbach, HD.; Liebrich, J.; Berg, U. & Berm, G. (1992). Pregnancy rates and births after unilateral or bilateral transfer of bovine embryos produced in vitro. *Journal of reproduction and fertility* Vol.95 (No.2): 363-370.

Reik, W. & Walter, J. (2001). Genomic imprinting: parental influence on the genome. Nature reviews. Genetics No.1: 21-32.

Schmidt, M.; Greve, T.; Avery, B.; Beckers, JF.; Sulon, J. & Hansen, HB. (1996). Pregnancies, calves and calf viability after transfer of in vitro produced bovine embryos. *Theriogenology* Vol.46 (No.3): 527–539.

Shimada, M.; Kawano, N. & Terada, T. (2002). Delay of nuclear maturation and reduction in developmental competence of pig oocytes after mineral oil overlay of in vitro maturation media. *Reproduction* Vol.124 (No.4): 557-564.

Shiota, K. & Yamada, S. (2005). Assisted reproductive technologies and birth defects. *Congenital anomalies* Vol.45 (No.2): 39–43.

Shiota, K. & Yamada, S. (2009). Intrauterine environment-genome interaction and children's development (3): assisted reproductive technologies and developmental disorders. *The Journal of toxicological sciences* Vol.34 (Suppl.2), SP287–SP291.

Smith, AC.; Choufani, S.; Ferreira, JC. & Weksberg, R. (2007). Growth regulation, imprinted genes, and chromosome 11p15.5. *Pediatric research* Vol.61 (No.1): 43–47.

Smith, SL.; Everts, RE.; Sung, LY.; Du, F.; Page, RL.; Henderson, B.; Rodriguez-Zas, SL.; Nedambale, TL.; Renard, JP.; Lewin, HA.; Yang, X. & Tian, XC. (2009). Gene expression profiling of single bovine embryos uncovers significant effects of in vitro maturation, fertilization and culture. *Molecular Reproduction and Development* Vol.76 (No.1): 38-47.

Smith, SL.; Everts, RE.; Tian, XC.; Du, F.; Sung, L-Y.; Rodriguez-Zas, S.; Jeong, B-S.; Renard, JP.; Lewin, HA. & Yang, X. (2005). Global gene expression profiles reveal significant nuclear reprogramming by the blastocyst stage after cloning. *Proceedings of the*

National Academy of Sciences of the United States of America Vol.102 (No.49): 17582–17587.

Somers, J.; Smith, C.; Donnison, M.; Wells, DN.; Henderson, H.; McLeay, L. & Pfeffer, PL. (2006). Gene expression profiling of individual bovine nuclear transfer blastocysts. *Reproduction* Vol.131 (No.6): 1073–1084.

Thorvaldsen, JL.; Duran, KL. & Bartolomei, MS. (1998). Deletion of the H19 differentially methylated domain results in loss of imprinted expression of H19 and Igf2. *Genes & development* Vol.12 (no.23): 3693–3702.

Tveden-Nyborg, PY.; Alexopoulos, NI.; Cooney, MA.; French, AJ.; Tecirlioglu, RT.; Holland, MK.; Thomsen, PD. & D'Cruz, NT. (2008). Analysis of the expression of putatively imprinted genes in bovine peri-implantation embryos. *Theriogenology* Vol.70 (No.7): 1119-28.

Wakayama, T.; Perry, AC.; Zuccotti, M.;, Johnson, KR. & Yanagimachi, R. (1998). Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* Vol.394 (No.6691): 369–374.

Walker, SK.; Hartwich, KM. & Seamark, RF. (1996). The production of unusually large offspring following embryo manipulation: concepts and challenges. *Theriogenology* Vol.45: 111–120.

Weksberg, R.; Shuman, C. & Smith, AC. (2005). Beckwith-Wiedemann syndrome. *American Journal of Medical Genetics Part C* Vol.137C: 12–23.

Weksberg, R.; Smith, AC.; Squire, J. & Sadowski, P. (2003). Beckwith-Wiedemann syndrome demonstrates a role for epigenetic control of normal development. *Human Molecular Genetics* Vol.12, R61–R68.

Wilmut, I.; Beaujean, N.; de Sousa, PA.; Dinnyes, A.; King, TJ.; Paterson, LA.; Wells, DN. & Young, LE. (2002). Somatic cell nuclear transfer. *Nature* Vol.419 (No.6907), 583–586.

Wilmut, I.; Schnieke, AE.; McWhir, J.; Kind, AJ. & Campbell, KH. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* Vol.385 (No.6619): 810–813.

Willadsen, SM.; Janzen RE.; McAlister RJ.; Shea, BF.; Hamilton, G. & McDermand, D. (1991). The viability of late morular and blastocysts produced by nuclear transplantation in cattle. *Theriogenology* Vol.35 (No.1): 161-170.

Yamada, Y.; Watanabe, H.; Miura, F.; Soejima, H.; Uchiyama, M.; Iwasaka, T.; Mukai, T.; Sakaki, Y. & Ito, T. (2004). A comprehensive analysis of allelic methylation status of CpG islands on human chromosome 21q. *Genome research* Vol.14 (No.2): 247–266.

Yan, Y.; Frisén, J.; Lee, MH.; Massagué, J. & Barbacid, M. (1997). Ablation of the CDK inhibitor p57Kip2 results in increased apoptosis and delayed differentiation during mouse development. *Genes & development* Vol.11 (No.8): 973-983.

Yang, L.; Chavatte-Palmer, P.; Kubota, C.; O'Neill, M.; Hoagland, T.; Renard, JP.; Taneja, M.; Yang, X. & Tian, XC. (2005). Expression of imprinted genes is aberrant in deceased newborn cloned calves and relatively normal in surviving adult clones. *Molecular reproduction and development* Vol.71 (No.4): 431–438.

Young, LE.; Fairburn, HR. (2000). Improving the safety of embryo technologies: possible role of genomic imprinting. *Theriogenology* Vol.53 (No.2): 627-648.

Young, LE.; Schnieke, AE.; McCreath, KJ.; Wieckowski, S.; Konfortova, G.; Fernandes, K.; Ptak, G.; Kind, AJ.; Wilmut, I.; Loi, P. & Feil, R. (2003). Conservation of IGF2-H19

and IGF2R imprinting in sheep: effects of somatic cell nuclear transfer. *Mechanisms of development* Vol.120 (No.12): 1433–1442.

Young, LE.; Sinclair, KD. & Wilmut, I. (1998). Large offspring syndrome in cattle and sheep. *Reviews of Reproduction* Vol.3 (No.3): 155–163.

Zhang, S.; Kubota, C.; Yang, L.; Zhang, Y.; Page, R.; O'Neill, M.; Yang, X. & Tian, XC. (2004). Genomic imprinting of H19 in naturally reproduced and cloned cattle. *Biology of reproduction* Vol.71 (No.5): 1540–1544.

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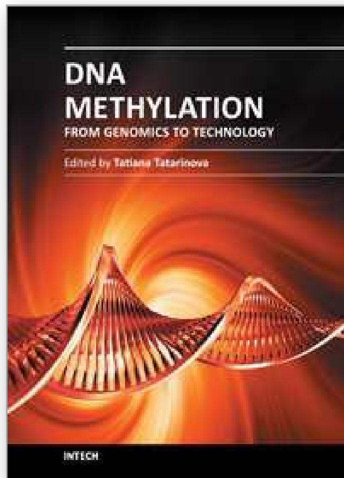

DNA Methylation - From Genomics to Technology

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Epigenetics is one of the most exciting and rapidly developing areas of modern genetics with applications in many disciplines from medicine to agriculture. The most common form of epigenetic modification is DNA methylation, which plays a key role in fundamental developmental processes such as embryogenesis and also in the response of organisms to a wide range of environmental stimuli. Indeed, epigenetics is increasing regarded as one of the major mechanisms used by animals and plants to modulate their genome and its expression to adapt to a wide range of environmental factors. This book brings together a group of experts at the cutting edge of research into DNA methylation and highlights recent advances in methodology and knowledge of underlying mechanisms of this most important of genetic processes. The reader will gain an understanding of the impact, significance and recent advances within the field of epigenetics with a focus on DNA methylation.

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