Review Articles

The importance of epigenetics in embryonic development and reproductive biotechnology

Tuempong Wongtawan*

Department of Pre-clinic and Applied Animal Science, Faculty of Veterinary Science, Mahidol University, Salaya campus, Puthamonthol, Nakhonpathom, Thailand, 73170

***Corresponding author, E-mail address: tuempong.wan@mahidol.ac.th**

Abstract

It is well established that epigenetics and chromatin modifications are primary factors that can govern gene activity and nuclear architecture. They are also proven to be essential for normal embryonic development and cell differentiation. One important step during mouse development is the establishment of epigenetic reprogramming which is believed to be vital for normal growth and development, however; the mechanism is still poorly understood. Creating embryo using reproductive biotechnology such as IVM/IVF, ICSI and nuclear transfer may cause abnormal epigenetic pattern. This review summarise the role and the importance of epigenetic in embryonic development as well as reproductive biotechnology from research literature mainly in mouse used as a model for mammalian development.

Keywords: Epigenetics, Development, Reproductive Biotechnology

ความสำคัญของสภาวะเหนือพันธุกรรม (Epigenetics) ในการเจริญเติบโตของตัวอ่อน และเทคโนโลยีทางการสืบพันธุ์

เติมพงศ์ วงศ์ตะวัน

ภาควิชาปรีคลินิค และสัตวศาสตร์ประยุกต์ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล วิทยาเขตศาลายา อ.พุทธมณฑล จ.นครปฐม ประเทศไทย 73170
***ผู้รับผิดชอบบทความ E-mail address:tuempong.wan@mahidol.ac.th**

บทคัดย่อ

ปัจจุบันการศึกษาค้นคว้าพบว่า สภาวะเหนือพันธุกรรม (Epigenetics) หรือที่อาจเรียกว่า พันธุศาสตร์ด้านกระบวนการเหนือพันธุกรรม หรือการควบคุมแบบเหนือพันธุกรรม ซึ่งเป็นการเปลี่ยนแปลงของเส้นใยโครมาตินนั้นในนิวเคลียสของเซลล์นั้นมีผลต่อการทำงานของยีนต์ และโครงสร้างของนิวเคลียส นอกจากนี้ยังพบว่า สภาวะเหนือพันธุกรรมนั้นมีความสำคัญอย่างมากในการควบคุมการเจริญเติบโตของตัวอ่อน และการเปลี่ยนแปลงทางด้านรูปร่างและการทำงานของเซลล์ ในระยะหนึ่งของการเจริญเติบโตจะเกิดขบวนการที่เรียกว่าการลบล้างสภาวะเหนือพันธุกรรมเก่าเพื่อจัดตั้งสภาวะเหนือพันธุกรรมใหม่ (Epigenetic reprogramming) อย่างไรก็ตามขบวนการนี้ยังไม่เป็นที่เข้าใจกันนักนอกจากนี้ยังพบว่าการสร้างตัวอ่อนด้วยขบวนการทางห้องทดลองไม่ว่าจะเป็น การเลี้ยงตัวอ่อนในหลอดแก้ว การปฏิสนธิในหลอดแก้ว การฉีดตัวอสุจิเข้าในไข่ ล้วนแต่สามารถไปรบกวนสภาวะเหนือพันธุกรรมได้ ในบทความนี้จะรวบรวมข้อมูลที่เกี่ยวกับความสำคัญสภาวะเหนือพันธุกรรมในการเจริญเติบโตของตัวอ่อน และผลเสียที่อาจเกิดขึ้นได้จาการใช้เทคโนโลยีทางการสืบพันธุ์

คำสำคัญ : การเจริญเติบดตของตัวอ่อน สภาวะเหนือพันธุกรรม เทคโนโลยีชีวภาพทางการสืบพันธุ์

1. Introduction to Epigenetics

Historically, the word "epigenetics" was coined by C. H. Waddington in 1942 as a portmanteau of the words "genetics" and "epigenesis" (Waddington 1942). Presently, epigenetics generally means "the study of heritable changes in genome function that occur without an alteration in DNA sequence" (Bird 2007;Goldberg et al. 2007). This may include the study of inherited gene expression from one cell to its descendants, alteration of gene expression during cell differentiation, and how environmental factors can induce the change of gene activities (The-Epigenome-Network-of-Excellence 2009). There are many implications of epigenetic research for agriculture and for human biology and disease, which bear on our understanding of stem cells, cancer, development and ageing (Laird 2005; Roloff and Nuber 2005; Srebro 1993).

Epigenetic modifications can be subdivided into four major categories, most of which are interdependent. First is the modification of DNA called DNA methylation which occurs at cytosine residues in CpG dinucleotides. (Bird 2002). Second are the modifications of core histones, in particular methylation of specific residues in the histone tails. (Kimura et al. 2005;Shilatifard 2006). Third, variant histones such as protamines or macro-H2A have been shown to correlate with epigenetic states in the mouse. (Henikoff and Ahmad 2005). Fouth, the Polycomb (PcG)and Trithorax (TrxG) group of proteins (Bird 2007). Finally, the group of heterochromatin protein 1 (HP1) which is strongly associate with gene regulation and heterochromatin formation. (Jones et al. 2000; Li et al. 2002).

1.1 Histone Methylation

Methylation of histones can occur on lysine (K) or arginine (R) residues by histone methyltransferases (HMTs). Conversely, demethylases work in the opposite way, as they remove methyl groups from these residues. Histonelysine residues can be mono-(me), di-(me2), or trimethylated (me3), and this has a decisive influence on gene and chromatin functions (Li et al. 2007). Modifications of histone lysine can act as either repressive or active marks. For example, methylations of H3K4, H3K36 and H3K79 have been highly correlated with transcriptional activation, whereas methylations of H3K9, H3K27 and H4K20 are associated with repressive chromatin states (Brinkman et al. 2006; Kourmouli et al. 2005; Peters et al. 2003; Schotta et al. 2004; Sims et al. 2006; Vakoc et al. 2006). A combinationof histone lysine modifications can occur; this system can separate chromatin or the chromosome into different areas. For instance, H3K27me3 combines with H3K9me2 and H4K20me1 to organise chromatin into facultative heterochromatin such asthe imprinted regions of the inactive X chromosome whereas H3K9me3, H4K20me3 and H3K27me1 together could lead chromatin to form constitutive heterochromatin (Brinkman et al. 2006; Kourmouli et al. 2005; Peters et al. 2003; Schotta et al. 2004; Sims et al. 2006; Vakoc et al. 2006).

1.2 Histone Variants

Many variant histones can be seen as polymorphisms of the major canonical form and just assembled into chromatin (Henikoff and Ahmad 2005). However, other variants are distinct from the canonical form, either in mechanism or function (Henikoff and Ahmad 2005). The centromeric chromatin protein named CENP-A is an H3 variant, and it is vital for chromosome segregation and centromeric heterochromatin formation (Palmer et al. 1991). Histone H3.3 is similar to canonical H3 and, it can be methylated and is correlated with active transcription (Hendzel and Davie 1990; McKittrick et al. 2004). H2AZ has been found in heterochromatin and it can interact with HP1α (Fan et al. 2004).One study of H2AZ genetically altered mice showed that embryos die during implantation, suggesting that it has a critical role in early development (Faast et al. 2001). MacroH2A is enriched in regions of the mammalian inactive X chromosome that is associated with facultative heterochromatin, Xist RNA and H3K27me3 (Chadwick and Willard 2001; Chadwick and Willard 2004).

1.3 DNA Methylation

DNA methylation is associated with chromatin structure and transcriptional repression and occurs mainly at the fifth position of cytosine (5mC) in the dinucleotide CpG. In humans CpGs occupy about 1% of the genome and more than 70% of CpGs are methylated (Ehrlich et al. 1982). They correspond to approximately 40% of promoters in mammals (Fatemi et al. 2005) and about 70% in humans (Saxonov et al. 2006). CpG islands are usually nonmethylated at genes except in imprinted genes, the female X chromosome, germ line specific genes and tissue specific genes (Bird 1986). The expression of genes associated with these regions is correlated with methylation status (Song et al. 2005). The majority of the TDMs appear to be associated with 5' promoter CpG islands and may have important roles in establishing or maintaining gene silencing during or after tissue differentiation (Song et al. 2005). Notably, the majority of DNA methylation occurs in repetitive sequences which make up approximately 40% of the mammalian genome (Goodier and Kazazian 2008;Yoder et al. 1997). The role of DNA methylation may be to inactivate repetitive sequences such as transposable element to maintain genomic stability (Goodier and Kazazian 2008).

Several hypotheses have been proposed to explain the mechanism of gene regulation by DNA methylation. The first possibility is that DNA methylation prohibits the binding of CpG specific transcription factors to promoters (Tate and Bird 1993). Second, the DNA methylation may alter nucleosome structure so that gene silencers may bind more effectively bind to promoter than the transcription machinery (Kass et al. 1997). The third possibility is that DNA methylation may recruit

specific factors that prevent the binding of transcription factors (Lewis et al. 1992). In mammalian cells, DNA methylation is maintained and established by three DNA methyltransferases (Dnmt).

1.4 Polycomb and Trithorax proteins

Polycomb proteins (PcG) are usually associated with gene silencing and they are essential to epigenetic maintenance and normal development in multicellular organisms (Ringrose and Paro 2004; Schwartz and Pirrotta 2007). PcG can be biochemically and functionally separated into two major multiprotein complexes, polycomb repressive complex 1 (PRC1) and PRC2 which is also known as initiation complex. Unlike PcG, thrithorax proteins (TrxG) are commonly linked to the general transcription process and gene activation, and they work as antagonists to PcG (Ringrose and Paro 2004; Schwartz and Pirrotta 2007). PcG and TrxG regulate many developmentally important genes (Boyer et al. 2006; Hanson et al. 1999) and also associate with X chromosome inactivation (Schwartz and Pirrotta 2007).

1.5 Heterochromatin Protein 1 (HP1)

In mammals, three isoforms of HP1 have been identified, HP1α, HP1β and HP1γ. These are associated with constitutive heterochromatin as well as some forms of facultative heterochromatin (Jones et al. 2000; Li et al. 2002). In somatic mammalian cells HP1α and HP1β are predominantly localised at pericentric heterochromatin where as HP1γ uniformly distributes at both heterochromatin and euchromatin (Dialynas et al. 2007; Fischle et al. 2005; Schmiedeberg et al. 2004; Vakoc et al. 2005). Notably HP1α, but not HP1β and HP1γ, remain associated with heterochromatin during cell division (Fischle et al. 2005; Schmiedeberg et al. 2004). Studies of their chromatin binding proteins showed that HP1α has a higher affinity for either chromatin or DNA than HP1β and HP1γ (Gilbert et al. 2003; Meehan et al. 2003; Remboutsika et al. 1999). Although all HP1 isoforms may be associated with gene silencing (Smallwood et al. 2007), HP1γ seems to be connected to transcription activation (Vakoc et al. 2005). Collectively this makes HP1α a more specific marker for constitutive heterochromatin and a true epigenetic mark.

2. Chromatin Modification in Mouse Development

In the mammalian reproductive system, the male and female gametes have very different chromatin organisations. In this chapter summarises the chromatin modifications during gamete development and postfertilization to postimplantation development.

2.1 Chromatin Modification in Early Germ Cell Development

Germ cells are the progenitors of gametes. Mouse primordial germ cells (PGC) begin to form at embryo day 7.5 (E7.5) in the posterior primitive streak and then migrate to genital ridge by day E11.5 (McLaren 2003). There they undergo global epigenetic and chromatin reprogramming. Most of the DNA methylation at imprinted genes and repeat sequences is removed in this process (Hajkova et al. 2008; Hajkova et al. 2002; Morgan et al. 2005). Some DNA demethylation occurs specifically at paternally inherited imprinted genes (Hajkova et al. 2002). Although the DNA methylation at repeat sequences is mostly removed, some retrotransposons such as LINE 1 and IAPs are resistant (Hajkova et al. 2002; Lane et al. 2003). The differential erasure of DNA methylation persists until new imprints are established (Hajkova et al. 2002; Lee et al. 2002).

Recently Hajkovaet. al, (2008) reported dynamic changes in chromatin modifications during mouse early germ line development. Initially at E8.5 loss of heterochromatin marker H3K9me3 is observed despite the presence of histone methyltransferase G9a. Conversely, repressive histone mark H3K27me3, histone methyltransferase Ezh2 and active histone modifications such as H3K4me2, H3K4me3 and H3K9ac seem to increase as well as the symmetrical methylation of arginine 3 on H4 (H4R3me2s) and H2A (H2AR3me2s) (Hajkova et al. 2008). A massive remodelling of heterochromatin and chromatin

modifications is found at E11.5, when linker histone H1 and heterochromatin markers such as H3K9me3, H3K27me3, HP1α, HP1β, HP1γ, ATRX and M33 become undetectable or redistribute simultaneously with the loss of chromocentres (stained with DAPI) as well as nuclear enlargement (Hajkova et al. 2008). In addition, the active mark H3K9ac is also removed (Hajkova et al. 2008) and new arrangements or reappearance of chromatin modifications as well as heterochromatin are observed around E12.5 (Hajkova et al. 2008). The loss of many repressive marks and heterochromatin markers may suggest that the chromatin of germ cells may be in the loose and open state to allow resetting of the epigenetic and chromatin profile.

Although male germ cells enter a mitotic arrest at E13.5 that lasts until after birth, epigenetic and chromatin remodelling does not stop and seems to be dynamic (Yoshioka et al. 2009). A temporal and spatial reorganisation of chromocentres and heterochromatin marker H3K9me3 and HP1α has been found (Yoshioka et al. 2009).

2.2 Chromatin Modification in Oogenesis

During oocyte development, chromatin structure is globally altered, and most genes are transcriptionally silenced (Kageyama et al. 2007; Kim et al. 2003). Many marks for histone methylation, acetylation and DNA methylation significantly increase from primary oocytes to germinal vesicle stage (GV) (Kageyama et al. 2007). This global change in chromatin modification coincides with increased gene expression of both methyltransferases and acetyltransferases (Kageyama et al. 2007). Gene repressive as well as active histone marks seem to colocalise with heterochromatin regions (Kageyama et al. 2007). Moreover, somatic linker histone H1 is replaced by an oocyte specific H1 variant named H1Foo, which is abundant in lysines and have many potential sites for methylation and acetylation (Kimmins and Sassone-Corsi 2005). Taken together, it seems likely that epigenetic and chromatin modifications change take place during oogenesis in association with chromatin architecture but this does not seem to be related to global gene expression.

After meiosis, acetylation of all histones is reduced to undetectable level by HDAC (Kim et al. 2003). A study of deacetylation in meiotic oocytes showed that $p34^{cdc2}$ kinase activity and/or ATRX (A member of SWI/SNF family, a chromatin remodelling protein) are responsible for the activation of HDACs (Akiyama et al. 2004; De La Fuente et al. 2004).

2.3 Chromatin Modification in Spermatogenesis

Unlike the oocyte, sperm neither have cytoplasm nor contain paternal mRNA. The main function of sperm is to transfer paternal genomic information to the oocyte during fertilization, to form the zygote. During spermatogenesis, sperm chromatin undergoes dramatic changes, during which the histones are replaced by special proteins named protamines. This results in an extremely compact chromatin compared to somatic cell nuclei (Ward and Coffey 1991). However the histone replacement by protamines is incomplete; it has been shown that the proteins associated with DNA are approximately 85% protamine whereas 15% is histone and other protein (Gatewood et al. 1990; Oliva 2006; van der Heijden et al. 2006). Most histone modifications are significantly decreased or absent after spermatogenesis while the remaining modifications such as acetylation of H4K8 and K12 are transmitted to the zygote after fertilization (van der Heijden et al. 2006). The DNA methylation status at specific sites in three spermatogenesis-specific genes, Pgk-2, ApoA1 and Oct-3/4 is unmethylated in adult spermatogenic cells in the testis, but remethylated in mature spermatozoa in the vas deferens (Ariel et al. 1994). A sperm chromatin architecture study showed that the centromere are organized in a chromocentre, well positioned inside the nucleus while the telomeres forming dimers are positioned at the nuclear periphery (Oliva 2006). Additionally, several epigenetic modifiers are important for spermatogenesis, for example, disruption of Suv39h2 or G9a or Jmjd1a (H3K9-HMT) which are preferentially expressed

in the testis, results in infertility due to spermatogenetic failure (Okada et al. 2007; Peters et al. 2001; Tachibana et al. 2007)

2.4 Chromatin after Fertilization

At fertilization, the parental genomes are in different stages of the cell cycle and have different chromatinorganisations. The paternal chromatin has been delivered by sperm, and is mostly densely packaged with protamines rather than histones (Oliva 2006). By contrast,the maternal chromatin is arrested at metaphase II and is packaged with histones (van der Heijden et al. 2005). Upon fertilization, sperm nucleoprotamine is decondensed and rapidly replaced with new histones, after which modification may occur (van der Heijden et al. 2005). Maternal chromatin, however; already contains histones and has abundant with DNA methylation andchromatin modifications at fertilization (Santos et al. 2002; van der Heijden et al. 2006). Most types of histone methylation such as H3K4, H3K9, H3K27, H4K20 are already present in female chromatin whereas only H3K9me1, H3K4me1, H4K20me1 are found in male chromatin (Erhardt et al. 2003;Torres-Padilla et al. 2006;van der Heijden et al. 2005). After chromatin decondensation, active DNA demethylation occurs in male chromatin by an unknown mechanism (Mayer et al. 2000; Santos et al. 2002; Yamazaki et al. 2007); at the same time new histones are modified (Santos et al. 2005; van der Heijden et al. 2005) including H3K4me1, H4K20me1, and acetylation of H3 and H4 (Adenot et al. 1997; Kim et al. 2003; Santos et al. 2005; van der Heijden et al. 2005). Both the mechanism and the function of paternal genome demethylationare still unclear. It is hypothesised that the oocyte cytoplasm containsdemethylation factors that are specifically targeted to sperm chromatin (Beaujean et al. 2004b; Yoshida et al. 2006). Histone variant H3.3 is already present in the oocyte as a maternal factor, and then incorporates preferentially into the male pronucleus before gamete activation (Torres-Padilla et al. 2006). That paternal chromatin has DNA demethylation and fewer histone modifications may associate with the fact that zygotic gene transcription of paternal chromatin is higher and occurs earlier than in maternal chromatin (Aoki et al. 1997; Schultz 2002). Further more, both H3K79me2 and H3K79me3 are decreased soon after fertilization. The level of H3K79me2 is maintaineduntil morula stage whereas H3K79me3 is not detected throughout preimplantation (Ooga et al. 2008). Zygotic gene activation (ZGA), the critical event that direct the transition from maternal to embryonic control of development, occurs during the 2-cell stage in mice (Schultz 2002). At two cell embryo, H3K9me2, H3K9me3 and H4K20me3 are removed and remethylation occur at four-cell stage except for H4K20me3 which remethylation happen postimplantation (Wongtawan 2009; Wongtawan et al. 2011). A significant chromatin reorganisation also happens simultaneously at the two-cell embryo stage (Martin et al. 2006;Probst et al. 2007; Schultz 2002). For these reasons, it may be suggested that a highly permissive chromatin in 2-cell embryo allows access of the transcriptional machinery so as to activate zygotic gene expression.

2.5 Chromatin at Peri and Post implantation

Blastocyst is the stage during which the embryo prepares to implant in the uterine epithelium. There are two populations of cells at this stage; inner cell mass (ICM) which develops into the future postimplantation embryo, and trophectoderm (TE) which forms the placenta. It has been shown that DNA methylation, H3K4me3 and H3K27me3 are significantly higher in ICM than in TE, suggesting that they are essential to establish and maintain pluripotency and separate cell population (Erhardt et al. 2003; Santos et al. 2002; Wongtawan 2009) whereas H4K20me3 is reappeared specifically at mural trophectoderm (Wongtawan et al. 2011). Histone arginine methylation can also regulate pluripotency in the early mouse embryo. One study showed that higher levels of H3 arginine methylation predispose blastomeres to contribute to the pluripotent cells of the ICM (Torres-Padilla et al. 2007).

After implantation H4K20me3 is reappeared at postimplantation embryos day 11.5(E11.5) and locate specifically at pericentric heterochromatin after E13.5 and suggesting this marker may specific to mature chromatin in terminal differentiated cells (Wongtawan et al. 2011). Increase of H3K9me3 and H4K20me3 are also found in the neural tube of postimplantation embryos (E12.5) indicating that they may be associated with neural differentiation (Biron et al. 2004).

Many studies in gene knock-out mice models have demonstrated that the epigenetic mechanisms are essential for development. A summary of phenotypes of gene knock-out mice is shown in Table 1

Table 1 epigenetic modifiers that are critical for mouse development (knock-out mice model).

Name	Function	Embryonic lethality	Extraembryonic tissue	References
Ehmt1	H3K9me	E9.5	Chrioallantoic fusion defect	(Tachibana et al. 2005)
Ehmt2/G9a	H3K9me	E8.5	Chorioallantoic fusion defect, small placenta, reduction of giant cell	(Tachibana et al. 2002; Wagschal et al. 2008)
ESET	H3K9me	E3.5-E5.5	Abnormal blastocyst	(Dodge et al. 2004)
Suv3-9h	H3K9me	E14.5	No data	(Peters et al. 2001)
Ezh2	H3K27me	E7	Amnion and chorion defect	(O' Carroll et al. 2001)
PRMT1	Arginine methylation	E6.5	Lack of amnion and ectoplacental cavity	(Pawlak et al. 2000)
Dnmt1	DNAme	E9.5	Chorioallantoic fusion defect	(Li et al. 1992)
Suv4-20h	H4K20me	E17-E19	Labyrinth trophoblast defect, small size of placenta	(Schotta et al. 2008) and (Schotta personal communication)
Dicer	RNAi	E9.5	No data	(Bernstein et al. 2003)
Dnmt3L	DNAme	E9.5	No labyrinth formation, less spongiotrophoblast, more trophoblast giant cell	(Arima et al. 2006)
Dnmt3b	DNAme	E15.5	No data	(Okano et al. 1999)
Eed	H3K27me	E9.5	Secondary giant trophoblast defect	(Faust et al. 1995; Wang et al. 2002)
Nsd1	H3K36me	E10.5	Lack of allantoids	(Rayasam et al. 2003)
HDAC1	deacetylase	E9.5	Lack of allantois formation	(Lagger et al. 2002)
Suz12	H3K27me	E10	Lack of chorion, amnion and ectoplacental cavity	(Pasini et al. 2004)
Dot1L	H3K79	E9.5-10.5	abnormal vascular morphology in yolk sac, severe cardiac dilation	(Jones et al. 2008)

3. Genomic Imprinting Controlled by Epigenetics

Genomic imprinting is a genetic phenomenon in which certain genes called imprinted genes are expressed in a parent-of-origin-specific manner. It is an inheritance process independent of classical Mendelian genetics, which describes the inheritance of traits as due to either dominant or recessive gene. Unlike Mendelian laws, in which both parental copies are equally likely to contribute to the phenotype or gene expression, imprinted genes expression depends only on which parent it was inherited from (Ideraabdullah et al. 2008; Swales and Spears 2005). For example, gene H19 is expressed only from the allele inherited from the mother whereas IGF-II is expressed from the allele inherited from the father (Ideraabdullah et al. 2008). Studies in many species indicate that imprinting is broadly conserved among placental mammals, including primates, rodents, ruminants and marsupials (Lucifero et al. 2006; Monk et al. 2006; Umlauf et al. 2004; Vu et al. 2006).

Many imprinted genes are found in clusters throughout the genome. Their gene expression is regulated by imprinting control regions (ICRs), which are marked by DNA methylation, histone modification, polycomb protein and non-coding RNA on one of two parental alleles (Delaval and Feil 2004; Ideraabdullah et al. 2008). These allelic epigenetic marks are established in either the female or male germ line, following the epigenetic reprogramming in the primordial germ cells. The parental imprinted genes escape from the global epigenetic reprogramming after fertilization, so that their epigenetic marks are maintained during preimplantation development (Ideraabdullah et al. 2008).

DNA methylation is arguably the most studied in epigenetically controlled imprinted genes. The expressed imprinted genes are not marked with DNA methylation while repressed imprinted genes are enriched for DNA methylation (Fowden et al. 2006). Not only DNA methylation, but also histone modifications play a vital role in the regulation of imprinted genes. DNA methylated ICRs are associated with H4K20me3 and H3K9me3, in contrast, the unmethylated allele has H3K4me2 and H3ac (Delaval et al. 2007).

Another type of genomic imprinting is the imprinted X chromosome inactivation (XCI) which is found in female cells that have two X chromosomes (XX). XCI is a developmentally regulated process that causes one of the two X chromosomes to become transcriptionally silenced, thus equalising the expression of X-linked genes between male and female (Sado and Ferguson-Smith 2005;Thorvaldsen et al. 2006). In postimplantation embryos, either the parental or maternal X-chromosome has to be inactivated, which is referred as random XCI, whereas in the preimplantation embryo and extraembryonic lineages, XCI is paternally imprinted (Thorvaldsen et al. 2006). The imprinted XCI is marked by repressive epigenetic markers such as H3K27me3 (Erhardt et al. 2003) , H4K20me1, H3K9me2 (Erhardt et al. 2003) and DNA methylation (Sado and Ferguson-Smith 2005) whereas the active X chromosome is marked by active markers such as histone acetylation and H3K4me3 (Ideraabdullah et al. 2008).

It has been demonstrated that imprinted genes play vital roles in foetal and placental growth and development (Arnaud and Feil 2005; Fowden et al. 2006; Nafee et al. 2008). They affect the growth, morphology and nutrition by controlling nutrient demand and supply between foetus and placenta (Fowden et al. 2006). Abnormal development in foetus and placenta has been found in mice in which imprinted genes were genetically deleted or altered (see Table 1). Epigenetic dysregulation could also affect the foetal and placenta growth through malfunction of imprinted genes (see Table 2).

Table 2 Imprinted gene that affect embryo and placental development (Modified from Fowden et al. 2006)

Allele	Gene	Gene product	Knockout proportion of normal weight, %	
			Foetus	Placenta
Paternally expressed	Igf2	IGF II growth factor	50	60
	Igf2P0	IGF II growth factor	75	70
	Peg1	α/βhydrolase	87	86
	Peg3	Zinc finger transcription	80	72
	Ins $\frac{1}{2}$	Insulin	80	No result
	Slc 38a4	System A amino acid transporter	80	No result
Maternally expressed	H19	Non-coding RNA	130	140
	Igf2r	IGF II receptor	140	140
	Ip1	Cytoplasmic protein	100	140
	Grb10	Adaptor protein	146	130
	$p57^{kip2}$	Cyclin-dependent kinase inhibitor	100	140

4. Epigenetic Alterations by Reproductive Biotechnology

In the last decade, the use of reproductive biotechnologies such as hormonally induced ovulation, artificial insemination (AI), in vitro maturation of oocyte (IVM), in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have been proven to resolve infertility problems in humans (De Rycke et al. 2002; Horsthemke and Ludwig 2005), to increase production of farm animals and to save endangered species (Andrabi and Maxwell 2007; Paterson et al. 2003). However, the recent realisation of the critical importance of epigenetic information and its impact on health has focused attention on assisted reproductive technique (ART) births (De Rycke et al. 2002; Horsthemke and Ludwig 2005).

There is increasing evidence that invitro culture of preimplantation embryos may be associated with epigenetic alterations resulting in abnormal growth, phenotypic abnormalities, and developmental failure. One possible cause is an inappropriate use of culture medium. Khoslaet al. (2001) showed that mouse embryos culture in M16 plus FCS induce aberrant DNA methylation of imprinted genes causing repression of the H19 gene. Furthermore, culture of preimplantation ovine and bovine embryos frequently causes overgrowth and diverse developmental abnormalities during foetal and postnatal development and is related to abnormal pattern of DNA methylation at ICR controlling the imprinted IGF2R gene (Young et al. 1998). These aberrant phenotypes are named "large offspring syndrome" (Young et al. 2001; Young et al. 1998), which is the model for *Beckwith-Wiedemann syndrome* (BWS) in human. Recent studies in mouse also showed that *in vitro* mouse embryo culture can affect global DNA methylation patterns (Zaitseva et al. 2007) but not histone modifications such as acetylation of H4, methylation of H3K9, and phosphorylation of H3 serine 10 (Huang et al. 2007).

Moreover, aberrations of DNA methylation by unknown mechanisms during ART may cause abnormalities at imprinted genes. There are few clinical investigations of epigenetic errors that may arise during

early development caused by ART (De Rycke et al. 2002;Dean et al. 2005; Kelly and Trasler 2004), but they are thought to mimic certain genetic disorders. Firstly, the BWS is a pre/postnatal overgrowth syndrome associated with errors at the imprinting cluster. BWS is thought to be a result of the inappropriate epigenetic imprinting at specific loci on the maternal allele, such as inactivation of the cyclin-dependent kinase CDKN1C or defects of the H19 DMR. Secondly, *Angelman Syndrome (AS)* is caused by epigenetic alteration resulting in loss of function in the brain of the maternal copy of Ube3a.e. Thirdly, *Retinoblastoma,* a mutation in a tumour suppressor gene is through interactions with DNMT1 is associated with hypermethylation of the promoter region of RB. Finally, *ATR-X* syndrome is an X-linked syndrome characterized by severe mental retardation, reduced or absent speech, delayed developmental milestones, and also facial dysmorphism, α-thalassemia, and sexual dysgenesis, and is associated with DNA methylation defects in specific regions of the genome.

Advances in biotechnology such as nuclear transfer techniques (NT) have allowed the propagation of multiple genomic copies of an animal utilizing both embryonic and somatic cells as donors of genetic materials (Wilmut et al. 2002; Wilmut et al. 1997). The employment of NT has also provided a way for the control of genetic content and accurate genetic modification of animal species, thus providing a route for the study of gene function, the production of biopharmaceutical proteins (Schnieke et al. 1997), the modification of production traits or disease susceptibility (Wilmut et al. 2000), the preservation of endangered species (Gomez et al. 2004) and the creation of pluripotent stem cells to serve in patient-specific cell transplantation for treatment of degenerative disease (Yang et al. 2007b). Notwithstanding there are many advantages of NT, the success rate of reconstructive embryo to ES cell and full term offspring is extremely low (Wilmut et al. 2002). The majority of cloned animals die during pre-implantation and gestation due to developmental abnormalities (Wilmut et al. 2002). Epigenetic malfunction has been observed in several species of cloned embryos (Beaujean et al. 2004a; Santos et al. 2003; Yang et al. 2007a); this may produce inaccurate gene expression and lead to developmental failure (Blelloch et al. 2006; Eilertsen et al. 2007; Inoue et al. 2006; Vignon et al. 2002). For these reasons, successful cloning likely requires reorganisation of donor chromatin to a state compatible with embryonic development. The transferred genome must properly activate genes important for early embryonic development and also adequately suppress differentiation-associated genes that are transcribed in the original donor cell (Suzuki et al. 2006).

In order to these resolve epigenetic abnormalities, several studies have attempted to use epigenetic reagents to improve the developmental success rate of NT and round spermatid injection (ROSI). For example, TrichostatinA (TSA), the deacetylase inhibitor, can induce global histone acetylation in many cells. Treating mouse cloned embryos with TSA after nuclear implantation of specific cell types can significantly improve the development to blastocyst (Rybouchkin et al. 2006) and full term development (Kishigami et al. 2006a). Another study in ROSI shows that unusual remethylation of the paternal genome occurs after normal active demethylation, but using TSA can reduce the DNA remethylation of paternal chromatin (Kishigami et al. 2006b). Furthermore, studies in bovine nuclear transfer show that partial removal of DNA methylation by using either TSA or 5-aza-2'deoxycytidine (a DNA methyltransferase inhibitor) can increase blastocyst development of bovine cloned embryos (Enright et al. 2003).

5. Conclusion

Epigenetic and chromatin modifications are primary modulators of chromatin structure and nuclear organisation, which can control gene activities in unicellular organisms such as yeast, as well as in the

developmental programme of multicellular organisms. Although all cells in one organism have the same genome, each cell type has a different and unique epigenome, which may be unique at individual cell nuclei. Different signatures of epigenetic modifications can create diversity in different cell phenotypes and may also stimulate cell differentiation and development. Under normal circumstances the epigenome differentiates in an irreversible manner except during preimplantation and germ cell development. In these phases, epigenetic and chromatin modifications are extensively reprogrammed and improper environment and nutrition can cause epigenetic error in this state resulting in abnormal development.

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