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Epigenetics, embryo quality and developmental potential

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Abstract

It is very important for embryologists to understand how parental inherited genomes are reprogrammed after fertilization in order to get good quality embryos that will sustain further development. In mammals, it is now well established that important epigenetic modifications occur after fertilization. While gametes carry special epigenetic signatures, they should attain embryo-specific ones, some of which are crucial to produce healthy embryos. Indeed, it appears that proper establishment of the different epigenetic modifications and subsequent scaffolding of the chromatin are crucial steps during the first cleavages. This “reprogramming” is promoted by the intimate contact between the parental inherited genomes and the oocyte cytoplasm after fusion of the gametes. This review aims at introducing two main epigenetic players: histone post-translational modifications and DNA methylation as well as the importance of their role during early embryonic development.

Supplementary keywords: chromatin, development, reprogramming, environment, histone post-translational modifications, DNA methylation

Running head: Epigenetics and embryo quality

Introduction: epigenetics and chromatin compaction

Epigenetics is currently defined heritable changes of genomic activities that occur without change in the DNA sequence (Goldberg *et al.*, 2007). These epigenetic changes orchestrate chromatin remodelling and dictate heritable patterns of gene expression. Epigenetic control is mainly achieved by chemical modifications, which can be propagated through mitosis, and in some cases through meiosis (Bonasio *et al.*, 2010). These changes include DNA methylation/hydroxymethylation and post-translational modifications of histone tails (e.g. acetylation, phosphorylation, methylation....) as well as chromatin structure and nuclear architecture (Bernstein *et al.*, 2007; Schneider and Grosschedl, 2007).

DNA methylation in mammalian cells is mostly found on Cytosines from CpG dinucleotides. DNA methyl-transferases (DNMT) are the enzymes responsible for this modification, and are divided in two groups: the maintenance DNMT1, which insures that DNA methylation patterns are copied on newly-synthesized DNA during replication; and the de novo DNMT 3A and 3B, which establish methylation on new regions (Jin *et al.*, 2011). The opposite reaction, demethylation, can occur in two ways: 1) passive demethylation results from the absence of DNMT 1, leading to a progressive loss of methylation through cellular divisions; 2) an active mechanism that has long remained elusive as no DNA demethylase could be identified. However, the discovery of 5-hydroxymethylcytosine (5-hmeC), which is obtained through 5-meC oxidation, in embryonic stem cells (ESC) has provided new insights in the field (Tahiliani *et al.*, 2009; Ito *et al.*, 2010). This reaction is catalyzed by enzymes of the Ten Eleven Translocation (TET) family, that can further oxidize 5-hmeC into 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) (He *et al.*, 2011; Ito *et al.*, 2011). 5-fC and 5-caC can then be removed by Thymidine-DNA Glycosylase (TDG) mediated excision (He *et al.*, 2011; Maiti and Drohat, 2011; Zhang *et al.*, 2012).

On the other hand, N-terminal histone tail, which emanates out of the nucleosome, the fundamental unit of the chromatin, can be targeted by enzymes that allow post-translational modifications on various residues: lysine residues can for example be either acetylated, methylated or coupled to ubiquitin (Peterson and Laniel, 2004; Kouzarides, 2007). Moreover, some residues can be modified several times (eg. di- or tri-methylated). There are several “chromatin-modifying” enzymes such as histone acetylases (HATs) and deacetylases (HDACs) that coordinate acetylation/deacetylation; the histone kinase family and phosphatases acting on phosphorylation/dephosphorylation or the histone methyltransferases (HMTs) and lysine demethylases (KDMs) able to add/remove methyl groups (Peterson and Laniel, 2004; Tsukada *et al.*, 2006; Black *et al.*, 2012). Among histone post-translational modifications, acetylation has the highest potential to “open” chromatin, since it neutralizes the basic charge of lysine residues, decreasing their binding to DNA (Hasan and Hottiger, 2002). Acetylation is therefore often associated to gene expression, whereas histones in condensed chromatin areas are relatively hypoacetylated but marked by the presence of methylation such as histone H3 lysine 9 trimethylation (H3K9me3) (Schneider and Grosschedl, 2007).

This epigenetic information is not uniform, but is applied regionally, and it signals or preserves local activity status, such as gene transcription or silencing. Epigenetic modifications are indeed found at functionally distinct regions of the genome (coding regions, promoters and enhancers) where they function as “on/off” switches which can modulate chromatin structure and drive gene expression. Whereas genes provide the framework for the manufacture of RNAs and proteins, chromatin structure and nuclear architecture control the accessibility of proteins to the DNA, especially transcription factors and RNA polymerase, and thereby gene expression (Schneider and Grosschedl, 2007). Moreover, as epigenetic modifications can modulate the contacts between the nucleosomes and the chromatin, they can lead to either compaction or relaxation of the DNA fiber. They can therefore not only “open/close” regions for transcription

but also play essential roles in DNA repair, DNA replication and chromosome condensation during mitosis (Peterson and Laniel, 2004; Kouzarides, 2007); (Schneider and Grosschedl, 2007). Finally, epigenetic modifications also participate in the distinction of the two main types of chromatin domains: euchromatin, which is gene-rich, lightly packed, and heterochromatin, known as being gene-poor, which is tightly packed. Euchromatin is more accessible to the transcriptional machinery thanks to its “open state” configuration; whereas heterochromatin, has a more “repressive” chromatin structure and contains mainly silent transcriptional genes (Grewal and Moazed, 2003; Grewal and Jia, 2007; Jost *et al.*, 2012). In mammals, the degree of heterochromatin clustering varies with cell type, cell cycle phase and differentiation stage (Alcobia *et al.*, 2000; Bartova and Kozubek, 2006). Within the nucleus of interphase somatic cells for example, heterochromatin located at the centromeric and pericentromeric regions from different chromosomes gather into foci which form very compact clusters, intensely stained by DNA dyes (Alcobia *et al.*, 2000; Guenatri *et al.*, 2004). It should be noted that epigenetic modifications often recruit non-histone proteins such as the CBX (ChromoBoX) or MBD (Methyl-CpG-binding domain) proteins, which mediate downstream chromatin compaction and accessibility (Kutateladze, 2011). In somatic cells, pericentric heterochromatin is for example characterized by trimethylation of the histone H3 at Lysine 9 which recruits ChromoBox protein homolog 1 (CBX1 or Heterochromatin Protein 1) (Festenstein *et al.*, 2003; Hiragami and Festenstein, 2005).

The whole epigenetic information is termed the epigenome. Unlike the genome, the epigenome is highly variable between cells of each individual and fluctuates in time according to environmental conditions even within a single cell. There are at least as many epigenomes as there are cell types. It is therefore widely thought that epigenetics play key role in cellular identity (Bernstein *et al.*, 2007; Keenen and De La Serna, 2009; Roper and Hemberger, 2009).

Epigenetics in preimplantation embryos

Research in recent years has shown that epigenetic changes play a key role in embryonic development. Indeed, in mammals, the first stages of development are the siege of important genome rearrangements and epigenetic modifications, which are thought to play a very important part in Embryonic Genome Activation (EGA). After fertilization, the genome of the newly formed embryo is transcriptionally silent and its development is sustained by maternally inherited RNA and proteins. The embryonic genome will then take over progressively: in most species a first “minor” activation will take place very early, by the end of the 1-cell stage (Christians *et al.*, 1994; Bouniol *et al.*, 1995; Memili and First, 1999). Rapid increase in the synthesis of transcripts known as “major activation” then follows and the embryonic contribution becomes more important than the maternal one. The timing of this major activation varies between mammalian species: mouse appears an exception, as it occurs very early (at 2-cell stage) (Flach *et al.*, 1982; Nothias *et al.*, 1995), while in most other mammals major EGA starts later: at 4-cell stage in human (Braude *et al.*, 1988), 8-cell stage in bovine and rabbit (Camous *et al.*, 1984; Pacheco-Trigon *et al.*, 2002), and 16-cell stage in sheep embryos (Crosby *et al.*, 1988). Importantly, failure to activate properly the embryonic genome leads to developmental arrest (Flach *et al.*, 1982; Bolton *et al.*, 1984).

DNA methylation

During these early stages of development, DNA methylation changes drastically: while most of the paternal genome is rapidly and actively demethylated, methylation of the maternal genome is lost progressively (Mayer *et al.*, 2000; Oswald *et al.*, 2000; Santos *et al.*, 2002),

(Rougier *et al.*, 1998). Upon fertilization, the genomes of maternal and paternal origin form two distinct haploid pronuclei (PN) that remain physically separated up to the first mitosis (Adenot *et al.*, 1997). During the first cell cycle in the mouse, the paternal PN undergoes active DNA demethylation but the maternal PN resists demethylation (Santos *et al.*, 2002). This striking asymmetry between the two parental genomes is lost as the maternal genome later undergoes passive demethylation, in the absence of the maintenance methyltransferase DNMT1 during the replication phases of the subsequent cell cycles (Cardoso and Leonhardt, 1999).

This discrepancy between the two parental genomes has clearly been demonstrated in mouse but is less evidenced in other species (Abdalla *et al.*, 2009). Studies have indeed been led in many mammalian species, giving more contrasted results suggesting that DNA methylation dynamics were not fully conserved. We, for example, showed that paternal genome rapid demethylation does not take place in sheep or rabbit one-cell embryos (Beaujean *et al.*, 2004a; Young and Beaujean, 2004; Reis Silva *et al.*, 2011) and that sheep, rabbit and monkey embryos are not fully demethylated during preimplantation development (Beaujean *et al.*, 2004b; Yang *et al.*, 2007; Reis e Silva *et al.*, 2012). Our work emphasizes the importance of proper staging of the embryos to establish a real kinetics and the importance of image acquisition/quantification (Beaujean *et al.*, 2004b; Reis Silva *et al.*, 2011; Salvaing *et al.*, 2012). It corroborates the work from Park and colleagues on bovine and rabbit embryos (Park *et al.*, 2007). In fact, in both species, an initial decrease in 5-meC staining is observed but is followed by an increase in the paternal PN at the end of the 1-cell stage (Park *et al.* 2007; Reis e Silva *et al.* 2011). Similarly, while very few studies are available on human embryos, they agree that there is limited demethylation of the paternal PN at the 1-cell stage (Beaujean *et al.*, 2004a; Fulka *et al.*, 2004), and limited demethylation over the next cleavages (Fulka *et al.*, 2004; Santos *et al.*, 2010). Finally, we and others also pointed out recently that DNA demethylation of the paternal pronucleus of mouse embryos is not as complete as the initial studies suggested (Li and O'Neill, 2012; Salvaing *et al.*, 2012): in particular, it remains particularly strong on heterochromatin regions located at the periphery of the nucleoli.

The mechanism of this active demethylation has long remained elusive, but recent breakthroughs have been made with the finding that TET proteins can convert 5-meC to 5-hmeC. In mouse embryos, the first studies emphasized the complementarity observed between 5-hmeC and 5-meC stainings during the first cell cycle (Gu *et al.*, 2011; Iqbal *et al.*, 2011; Ruzov *et al.*, 2011; Wossidlo *et al.*, 2011). Indeed, following active demethylation of the paternal genome, 5-hmeC seems to replace 5-meC and increases. 5-hmeC is then present during all mouse preimplantation development (Iqbal *et al.*, 2011; Ruzov *et al.*, 2011). It has been suggested to be lost passively (Inoue and Zhang, 2011) or by further oxidation in 5-fC and 5-caC which are detected at least until the 4-cell stage (Inoue *et al.*, 2011). However, we demonstrated that in 1-cell mouse embryos, 5-meC/5-hmeC stainings are not 100% complementary and that 5-hmeC is not a simple intermediate in an active demethylation process; it could play a role on its own during early development (Salvaing *et al.*, 2012). Indeed, several lines of evidence indicate that the role of DNA methylation derivatives cannot be reduced to intermediates in the demethylation process. In particular, in ES cells, 5-hmeC plays a role in the regulation of transcriptional activity and seems mainly associated with “open” chromatin structures (Ficz *et al.*, 2011; Pastor *et al.*, 2011; Song *et al.*, 2011; Williams *et al.*, 2011; Wu *et al.*, 2011; Xu *et al.*, 2011; Kubiura *et al.*, 2012). Remarkably, we have shown the existence of a peak of 5-hmeC in both PN coinciding with the minor EGA in mouse (Salvaing *et al.*, 2012) although activation of LINE-1 (long interspersed nucleotide element 1) and ERVL (endogenous retroviruses class III) transposable elements, that occurs in both paternal and maternal genomes in 1-cell mouse embryos, does not seem to be mediated by 5-meC oxidation (Inoue *et al.*, 2012).

Finally, at the blastocyst stage, the first lineage specifications appear between inner cell mass (ICM) and trophoblast cells that will give rise to the fetus and to (parts of) the extra embryonic tissues, respectively (Wang and Dey, 2006). At that stage, DNA methylation is asymmetrically distributed between these two types of cells in most species showing high levels of 5-meC within the ICM (Fulka *et al.*, 2004; Santos *et al.*, 2010; Deshmukh *et al.*, 2011; Ruzov *et al.*, 2011; Reis e Silva *et al.*, 2012). Furthermore, DNA methylation changes appear to be necessary for the establishment of pluripotency (Farthing *et al.*, 2008) and are essential to sustain full term development (Li *et al.*, 1992; Tate *et al.*, 1996). On the other hand, knockdown of Tet1 in mouse pre-implantation embryos results in a bias towards trophoblast differentiation, suggesting a role of TET1 protein in ICM specification (Ito *et al.*, 2010). It was also recently shown in pig that TET1 and TET3 enzymes are both playing an important role during early embryogenesis, especially regarding the expression of NANOG at the blastocyst stage (Lee *et al.*, 2014).

Histone post-translational modifications

Histone post-translational modifications reinforce epigenetic asymmetry between the paternal and maternal genomes after fertilization. After fertilization, protamines inherited from the sperm are actively exchanged with oocyte inherited histones resulting in a rapid decondensation of the paternal genome (Adenot *et al.*, 1991; Heijden *et al.*, 2008). In mouse, this decondensation is clearly associated with a more rapid increase of histone acetylation in the paternal pronucleus (Adenot *et al.*, 1997; Heijden *et al.*, 2006; Santenard *et al.*, 2010). Conversely, the maternal genome is more methylated as compared to the paternal one: H3K4, H3K9 and H3K27 methylation are higher in the maternal PN, although histones of the paternal PN become progressively methylated during the first cell cycle (Lepikhov and Walter, 2004; Santos *et al.*, 2005; Yeo *et al.*, 2005). Similarly, in bovine, we observed that both H3K9me3 and H3K27me3 are asymmetrically distributed between the two inherited parental genomes, with specific targeting of the maternal genome as in mouse (Breton *et al.*, 2010; Pichugin *et al.*, 2010). Asymmetric staining of H3K27me3 has also been observed in pig (Bogliotti and Ross, 2012). On the other hand, we recently showed that H3K36me3 displays mostly undetectable and heterogeneous localization pattern throughout bovine pre-implantation development in contrast to mouse (Bošković *et al.*, 2012), indicating again that the dynamics of some epigenetic marks is not conserved between all mammalian species. Thereafter, histone modifications levels can be maintained up to the blastocyst stage, completely disappear or disappear and reappear later, usually after EGA (Beaujean, 2014).

From a functional point of view, it is hypothesized that this asymmetry may be required for proper embryonic development, especially during the onset of the minor phase of EGA. The reprogramming of the paternal genome that becomes hyperacetylated and hypomethylated may indeed increase chromatin flexibility and allow the minor EGA at the end of the 1-cell stage (Aoki *et al.*, 1997; Bouniol-Baly *et al.*, 1997). Remarkably, the major satellites repeated sequences located in the pericentromeric regions are among the first sequences transcribed at that stage, especially the paternal ones, and are clearly required for further development (Probst *et al.*, 2010). Reprogramming of histone modifications is also involved in the major EGA (Beaujean, 2014). Increasing histone acetylation with a histone deacetylase inhibitor (HDACi), can for example stimulate global transcription by 60% in mouse embryos at major EGA (Aoki *et al.*, 1997). Reversely, inhibition of H3K4 demethylation induces aberrant expression of Oct4 and inhibits the cleavage to 4-cell (Shao *et al.*, 2008). Similarly, we showed that components of the PRC1 complex that binds H3K27me3, serve transcriptional functions during oogenesis that are essential for proper EGA and further development (Posfai *et al.*, 2012). Altogether it

appears that histone modifications are closely related with the formation of an “open” chromatin state allowing transcription and that they participate in the regulation of gene expression during preimplantation development.

Conversely, the enzymes “writing” and “erasing” these histone modifications from the genome are of prime importance: inactivation of KMT5A catalyzing methylation of H4K20, for example, induces early embryonic lethality prior to the 8-cell stage (Oda *et al.*, 2009). Similarly, after deletion of JMJD2C, the H3K9me3 demethylase, embryos stop developing before the blastocyst stage (Wang *et al.*, 2010). Similarly, in bovine, the activity of JMJD3, an enzyme with lysine-specific histone demethylase activity, is necessary for the reprogramming of H3K27me3 and for normal embryonic development (Canovas *et al.*, 2012).

At the blastocyst stage, some histone modifications have been found to differ between the ICM and the trophectoderm (TE) (Beaujean, 2014). In mouse, this is the case for histone H4/H2A serine 1 phosphorylation (H4/H2AS1P) which is more intense in the TE (Sarmiento *et al.*, 2004). Reversely, methylation of H3K27 presents a strong overall staining of the ICM while TE cells show a particularly intense staining concentrated on the inactive X chromosome (Erhardt *et al.*, 2003). However, species differences are again observed: the onset of X chromosome inactivation for example differs completely between mouse and human or rabbit embryos (Okamoto *et al.*, 2011). Remarkably, the presence of histone modifications differences between the ICM and TE is in line with the results from gene analysis by use of carrier ChIP (CChIP) in mouse embryos (Vermilyea *et al.*, 2009). In the ICM, where *Nanog* and *Oct4* are highly expressed, the promoters of both genes are enriched in H4K8ac and H3K4me3 whereas in the TE, where *Nanog* and *Oct4* are silent, their promoters are enriched in H3K9me2. The opposite epigenetic patterns are observed for *Cdx2* which is silent in the ICM and highly expressed in the TE. This relationship between histone modifications and gene expression has also been underlined by experiments targeting the corresponding regulatory enzymes. In the mouse blastocysts, upregulation of H3R26 methyltransferase CARM1 for example has been shown to induce the upregulation of the ICM pluripotency markers NANOG and SOX2 (Torres-Padilla *et al.*, 2007), whereas depletion of H3K27 lysine-specific demethylase KDM6B abrogates CDX2 and GATA3 expression in the nascent TE-lineage and leads to implantation failures (Saha *et al.*, 2013).

Nuclear Transfer as a model

The relevance of chromatin epigenetic modifications during preimplantation development has been sustained by studies on cloned embryos derived from nuclear transfer (NT).

Since Dolly the sheep, NT has been achieved in a variety of mammalian species (Rodriguez-Osorio *et al.*, 2012). NT has potential applications for human health (eg. the derivation of patient-specific stem cells; Tachibana *et al.*, 2013) and for agronomical purposes (eg. to reproducing superior livestock or conserve exotic and endangered species; Campbell *et al.*, 2007). It has also caught the interest of many groups for basic biological research. Despite the great achievement in cloning mammals using NT, the overall efficiency of cloning is quite low. It is believed that incomplete reprogramming of the donor nucleus, especially the incomplete re-establishment of the embryonic epigenetic patterns may be among the causes leading to developmental arrest before or soon after implantation (Ogura *et al.*, 2013; Long *et al.*, 2014).

After NT, the donor cell has to setback its differentiated state to a totipotent one by reprogramming its epigenetic memory. If the epigenetic reprogramming of the donor genome is not complete the appropriate embryonic genes will not be activated at the appropriate time

and in the appropriate lineage. Reversely, proper inactivation of somatically expressed genes is also required (Bui *et al.*, 2009). In line with these hypotheses, NT inefficiency seems correlated with the differentiation state of the donor cell (Heyman *et al.*, 2002; Ogura *et al.*, 2013). Neural stem cells for example can be more efficiently cloned than terminally differentiated neuronal cells (Blelloch *et al.*, 2006). We also observed that the induction of pluripotency in the donor cell (iPS induction) prior to their transfer in recipient oocytes allows a better reprogramming and gave much higher rates of live-born cloned mice (Liu *et al.*, 2012). NT therefore represents a unique opportunity to understand how the epigenome has to be reprogrammed to get good quality embryos that will further develop.

In the past 15 years, many studies pointed out abnormal epigenetic reprogramming in NT embryos. DNA methylation for instance remains much higher in cloned embryos than in normal fertilized embryos in many species (Kang *et al.*, 2001; Dean *et al.*, 2003; Beaujean *et al.*, 2004b; Yang *et al.*, 2007; Lan *et al.*, 2010; Yamanaka *et al.*, 2011a; Couldrey and Wells, 2013); Live-cell imaging studies on cloned mouse embryos showed that pericentromeric heterochromatin domains are particularly hypermethylated (Yamagata *et al.*, 2007). Moreover, the related enzyme, the methyltransferase DNMT1, was detected in cloned mouse embryos one stage earlier than in fertilized ones (Chung *et al.*, 2003). Remarkably, in bovine, knockdown of DNMT1 by small interfering RNA (siRNA) in NT embryos successfully induced DNA demethylation of the satellite I region and improved the developmental rate of these embryos to blastocysts (Yamanaka *et al.*, 2011b).

As epigenetic modifications are in close relation to chromatin flexibility, several groups, including ours, also investigated the consequences of these abnormal levels of various epigenetic marks on global nuclear organization. It was shown that heterochromatin can be remodeled into an embryonic-like pattern after NT in mouse, bovine and rabbit (Martin *et al.*, 2006; Merico *et al.*, 2007; Pichugin *et al.*, 2010; Yang *et al.*, 2013). However, a great number of cloned embryos presents abnormal heterochromatin redistribution. Interestingly, the percentage of such abnormalities will depend on the differentiation status of the nucleus transferred: ES cell nuclei undergo better remodeling after NT than cumulus cell nuclei (Maalouf *et al.*, 2009). Moreover, it seems that only the cloned embryos with a good nuclear reorganization manage to develop up to the blastocyst stage (Maalouf *et al.*, 2009). In mouse, we observed that H3S10P and H3K9me3S10P, two epigenetic modifications related to chromatin compaction, are the ones found on the part of the pericentromeric heterochromatin that is remodeled correctly, resembling exactly the embryonic heterochromatin configuration of naturally fertilized embryos. Conversely, H3K9me3 and its associated protein HP1 were detected in the perinuclear clumps of heterochromatin, making obvious the maintenance of the somatic epigenetic signature within these nuclear regions (Ribeiro-Mason *et al.*, 2012). Similarly, in bovine NT embryos derived from fibroblast cells, 42% of the NT embryos displayed premature compaction of pericentromeric heterochromatin with accumulation of H3K9me3 and HP1 at the 4-cell stage, one stage earlier than in fertilized embryos (Pichugin *et al.*, 2010). These results demonstrate that some specific epigenetic marks can be good candidates to evaluate chromatin reorganization following NT.

Another epigenetic marker also attracted researchers' attention: H3K9 acetylation. Indeed, cloned embryos are hypoacetylated when compared to their in vivo counterparts (Wang *et al.*, 2007). As hyperacetylation of the paternal genome has been linked to chromatin remodeling after fertilization and to the onset of EGA (see above), this observation gave rise to a new method to improve the SCNT technique: the use of histone deacetylases inhibitors (HDACi). The use of HDACi, such as trichostatin A (TSA), has shown significant improvements in cloning efficiency for many species (Kretsovali *et al.*, 2012; Tachibana *et al.*, 2013). As

expected, HDACi causes hyperacetylation, therefore enhancing the acetylation state of the cloned embryos that nearly reach the acetylation levels of fertilized ones (Wang *et al.*, 2007; Iager *et al.*, 2008; Shi *et al.*, 2008; Cervera *et al.*, 2009; Yamanaka *et al.*, 2009). It also favors global chromatin reprogramming and nuclear reorganization. It was indeed shown to increase the levels of H3K4me2 (a marker of transcriptionally active chromatin) and reduce H3K9me3 (a marker of inactive chromatin) (Bui *et al.*, 2010). We also observed that TSA improves the structural remodeling of pericentromeric heterochromatin in mouse cloned embryos and that it dramatically increases the rate of full term development (10 times increase) (Maalouf *et al.*, 2009).

These results evidence a link between the developmental inefficiency of NT embryos and aberrant chromatin reprogramming. Remarkably, epigenetic modifiers such as HDACi are able to improve epigenetic reprogramming after NT and thereby improve the quality of the embryos obtained which are then able to reach development to term.

Conclusions and perspectives

It is now clear that epigenetic changes play a key role in embryonic preimplantation development but that it also contributes to the birth of healthy offspring. Determining what components are affected and to what extent alterations in the environment can cause disease and disturb normal development is a major challenge for future studies.

Environmental changes can indeed appose epigenetic marks on genes which are passed from one generation to the next (Feil and Fraga, 2011; Dupont *et al.*, 2012). Looking at the epigenetic modifications that may be caused by assisted reproductive technologies is therefore attracting much attention nowadays, not only in human but also in mouse (as a model) and in domestic animals (Urrego *et al.*, 2014). DNA methylation, especially with regards to imprinted genes, is a large matter of debate in human assisted reproductive technologies (ART) (van Montfoort *et al.*, 2012; El Hajj and Haaf, 2013). In contrast to embryos fertilized in vivo, in vitro fertilized (IVF) embryos are indeed exposed to in vitro culture medium. In mouse for example, 5-meC staining of 2-cell embryos revealed that suboptimal culture media can lead to developmental arrest (Shi and Haaf, 2002). It was also shown that the H3K4me3 level is significantly lower in the IVF embryos (Huang *et al.*, 2007; Wu *et al.*, 2012), inducing gene expression changes in the corresponding embryos (Fernandez-Gonzalez *et al.*, 2010). In ART, many groups also focus on oocyte vitrification or cryopreservation. After vitrification, acetylation abnormalities have been observed in the mouse: embryos derived from vitrified oocytes had abnormal acH4K12 patterns upon IVF (Suo *et al.*, 2010). Moreover, HDAC1 expression in these embryos was significantly lower than in the controls and this correlated with lower developmental rates (Li *et al.*, 2011). Again, epigenetics, embryo quality and developmental potential appear to be linked.

It should also be mentioned that in this review we introduced two main epigenetic players: histone post-translational modifications and DNA methylation. However, epigenetics also include small non coding RNAs: microRNAs (miRNA), small interfering RNAs (siRNA) and Piwi-interacting RNA (Bourc'his and Voinnet, 2010). In somatic cells, it has been shown that complementary pericentromeric transcripts which are processed to small RNAs, guide heterochromatin formation and establishment of a transcriptionally silent state (Muchardt *et al.*, 2002; Lu and Gilbert, 2007). Pericentromeric heterochromatin can be easily disrupted by RNase treatments (Maison *et al.*, 2002) and abnormal accumulation of centromeric transcripts

leads to impaired centromeric architecture and function in human cells (Bouzinba-Segard *et al.*, 2006). In mouse embryos, it has recently been suggested that transcripts generated by pericentromeric genomic repeats are also involved in heterochromatin clustering at EGA and that interference with this phenomenon results in developmental arrest (Probst *et al.*, 2010; Santenard *et al.*, 2010). The expression and potential roles of all the small non coding RNAs in reproduction will therefore probably require much investigations in the coming years (Hale *et al.*, 2014).

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