Temporal expression of factors involved in chromatin remodeling and in gene regulation during early bovine in vitro embryo development

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Abstract

Distinct epigenetic modification events regulate gene expression and chromatin structure during the period between the immature oocyte and the blastocyst. Throughout this developmental period, important methylation fluctuations occur on genomic DNA and histones. Finding single or combinations of factors, which are at work during this period is essential to understand the entire epigenetic process. With this in mind, we assessed the precise temporal expression profile, during preimplantation embryo development, of 15 key regulators involved in RNA, DNA or histone methylation, chromatin modification or silencing and transcription regulation. To achieve this, real-time RT-PCR was used to quantify the mRNA levels of ATF7IP, DMAP1, EHMT1, EHMT2, HELLS, JARID1A, JARID1B, JMJD1A, JMJD2A, LSD1, MeCP2, METTL3, PRMT2, PRMT5 and RCOR2, in the oocyte and throughout in vitro bovine embryo development. Our results demonstrate that all the 15 key regulators were present to different degrees in the developmental stages tested, and they can be divided into three different groups depending on their respective mRNA profile.

Introduction

The regulation of gene expression is a cellular process that controls the level and the interval of time in which genes are in a state of transcription or translation. This regulation also dictates the structure and function of the cell, and is fundamental to control differentiation, morphogenesis, and growth.

Different mechanisms are involved in the control of gene expression. By their diverse actions in eukaryotic cells, they can have a transcriptional, post-transcriptional, translational, or post-translational effect on gene activity. Epigenetics are involved in some of these processes and can be defined as the reversible heritable changes in gene function that occur during cell division without changes in nuclear DNA sequence. They include modifications that take place on genomic DNA such as methylation of cytosine residues, and post-transcriptional modifications on the tail domains of histones by methylation, acetylation, phosphorylation, ubiquitination or sumoylation (reviewed in Jenuwein & Allis 2001, Gill 2004, Peterson & Laniel 2004, Morgan et al. 2005).

DNA methylation is generally associated with transcriptional repression and is involved in genomic imprinting. This modification is active during the entire life of a cell, and it is essential for modeling and modulating the genome during gametogenesis and in the early stages of mammalian embryo development where epigenetic events are crucial (reviewed in Reik et al. 2001). Before oocyte growth, the methylation status of the primordial germ cell genome is completely blank, and as the oocyte grows, methylation in the female germ line is reinstated. Upon fertilization, the highly methylated state found in the matured oocyte will undergo a demethylation phase on most of its genome by a passive mechanism which depends on DNA replication (reviewed in Reik et al. 2001). By the time of implantation in the mouse, de novo methylation is initiated in the cell lineage that will form the inner cell mass and trophectoderm of the blastocyst, whereas in the bovine this begins at the 8- to 16-cell stage (reviewed in Dean et al. 2003). Genes from the DNA methyltransferase family (DNMT) have been linked to some of the methylation states found in the primordial germ cell and throughout embryo development until implantation.
(Maatouk et al. 2006; and reviewed in Trasler (2006). In recent years, the discovery of histone demethylases has shattered the long-held dogma that histone methylation was stable and irreversible. Even if LSD1, JHDM1, JMJD1A, JMJD2A and JMJD2C have all been classified as bona fide histone demethylases, thus far their presence and action in mammalian oocytes and early embryos have not been documented (Shi et al. 2004, Cloos et al. 2006, Klose et al. 2006, Yamane et al. 2006 and reviewed in Tsukada et al. 2006).

Abnormal epigenetic modifications, especially defects in methylation or demethylation, are associated with several syndromes and diseases (reviewed in Rodenhiser & Mann 2006). The probability of suffering from an alteration in imprinting patterns such as in Beckwith–Wiedemann syndrome, and other related epigenetic abnormalities is accentuated in embryos arising from assisted reproduction techniques (ARTs), or with manipulated gametes and embryos (reviewed in Gicquel et al. 2003, Rodenhiser & Mann 2006). In animals, the use of in vitro embryo culture is responsible for methylation status alteration of imprinted genes, resulting in a condition named the large offspring syndrome (Young et al. 2001). Even though the risk of complications for the incoming newborns is enhanced, these protocols are still being used in both animal and human ART.

To successfully develop into a healthy offspring, the growing and maturing mammalian oocytes must not only contain the appropriate epigenetic factors, but also need to accumulate mRNAs and proteins to allow the zygote to perform its first divisions until the embryonic genome is activated. After this maternal embryonic transition (MET), the embryo will become fully self-reliant by producing its own mRNAs and proteins. MET may occur at different times in different species: one- to two-cell stage in mouse (reviewed in Schultz 1993), four- to eight-cell stage in human (reviewed in Telford et al. 1990), and 8- to 16-cell stage in bovine (Kopecny et al. 1989). This major checkpoint is a crucial and necessary step during embryo ontogeny since it coincides with a developmental block observed within mammalian embryos cultured in vitro (reviewed in Memili & First 2000). Still, the individual genes or mechanisms responsible for such a transition are unknown.

Since unique and important epigenetic events occur during the period between the immature oocyte and the blastocyst, finding single or combinations of factors which are at work during this period is essential to understand the entire epigenetic process.

This is the first study that seeks to assess the precise temporal expression profile of 15 key regulators (Table 1) in oocytes and throughout early embryo development. The mRNA profiles were divided into three different groups depending on their respective appearance. To investigate the potential meaning of each gene profile, we have attempted to associate specific mechanisms or chromatin remodeling events that occur in somatic cells, oocytes and developing embryos. Since few studies have examined the role of regulatory genes in oocyte and preimplantation embryonic development, our results will expand the knowledge in this enigmatic field.

Materials and Methods

Unless otherwise stated, all materials were obtained from Sigma-Aldrich.

Oocyte recovery and in vitro embryo production

The procedures for oocyte recovery and in vitro embryo production have been described previously (Vigneault et al. 2004). Using this culture system, over 30% of oocytes usually developed into blastocysts. Briefly, cumulus–oocyte complexes (COCs) were harvested from bovine ovaries collected in a slaughterhouse. Throughout in vitro culture, oocytes or embryos were added to 50 μl droplets of media under mineral oil. COCs were matured in modified synthetic oviduct fluid (SOF) for 24 h. For in vitro fertilization, matured COCs were transferred and cultured in modified Tyrode lactate for 15–18 h. Following fertilization, putative zygotes were mechanically denuded by repeated pipetting, washed in PBS and transferred in groups of 25–30 to modified SOF medium for embryo development. The 2-, 4-, 8-, and 16-cell embryos were collected at 36, 48, 72, and 108 h post-fertilization respectively and morulae and blastocysts were collected after 6 and 8 days of development. All were washed thrice in PBS, collected in pools of 20, frozen and stored at −80 °C until RNA extraction. All oocyte and embryo pools used for RNA extractions were collected and analyzed in triplicates.

RNA extraction and cDNA preparation

As an external control, 10 pg exogenous GFP RNA containing a poly-A tail (Vigneault et al. 2004) was added to each pool of oocytes and embryos prior to RNA extraction. RNA extractions of the oocyte or embryo pools containing GFP RNA were then performed using the PicoPure RNA isolation kit (Arcturus, Molecular Devices Corporation, Mountain View, CA, USA) and directly used for cDNA preparation as previously described (Vigneault et al. 2004).

Quantitative PCR

Primers for all of the genes studied are listed in Table 2 and were designed using bovine sequences when possible, or from a consensus derived from human and mouse sequences. Real-time PCR was performed on a Lightcycler apparatus (Roche) using SYBR green incorporation as previously described (McGraw et al. 2003,
Briefly, for each gene, a standard curve consisting of purified PCR product was included in the run. Each of the PCR was performed using the equivalent of 0.5 oocyte/embryo, 0.25 µM of each primer, 3 mM MgCl₂, and 2 µl Master SYBR Green mix (Roche). The annealing temperatures for each gene are listed in Table 2. The real-time PCR product specificity was confirmed by the analysis of the melting curve given by the Lightcycler software (Roche). The PCR products were migrated on agarose gel electrophoresis and sequenced. GFP mRNA was used as an external control to account for experimental errors due to techniques involved or materials used for RNA extraction and RT. (McGraw et al. 2006, Vigneault et al. 2004).

### Statistical analysis

The level of mRNA for each gene subjected to statistical analysis was normalized using the GFP external control (Vigneault et al. 2004, McGraw et al. 2006). The value obtained for each gene, within each pool of cDNA, was divided by the value obtained for GFP in the same cDNA pool. Data are presented as mean ± S.E.M. Statistically significant differences in mRNA levels between each developmental stage were calculated by protected ANOVA (SAS Institute, Cary, NC, USA), and treatment and replicate were included in the model. Differences were considered statistically significant at the 95% confidence level (P<0.05).
Groups (Figs 1–3). The first group (Fig. 1) represents genes that are maternally stored in abundance, showed low levels around the MET stage and regained in the blastocyst at least 50% of the relative mRNA levels initially found in germinal vesicle (GV) or metaphase II (MII) oocytes. The second group (Fig. 2) consists of genes with elevated maternal transcripts in the oocyte stages, decreased intensity during the subsequent cleavage and lower relative mRNA levels in the blastocyst compared with values obtained in GV or MII oocytes. As for the final set of genes (Fig. 3), they did not reach their highest relative mRNA levels in the oocyte stages like the two previous groups, but rather during or after MET.

In the first group (Fig. 1), every gene (HELLS, JMD1A, JMD2A, LSD1, METTL3 and PRMT5) exhibited a similar expression pattern. Their levels were highest in the oocyte and tended to decrease during the first few cell divisions until the eight-cell stage, where they reached significantly lower amounts. In the subsequent cell divisions, the levels remained stable until the blastocyst stage, where the levels were significantly higher than at the eight-cell stage. The mRNA encoding HELLS, LSD1 and JMD1A not only showed a similar pattern, but also similar significant changes during embryo development. These three transcripts were abundant in the oocyte and showed their first significant reduction in the four-cell embryo. From the four-cell to the morula stage, no significant changes in mRNA transcripts were noted, but a significant increase was detected in the blastocyst.

Table 2 Primers used for RT-PCR experiments.

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<th>Gene</th>
<th>Primer sequences</th>
<th>Accession number</th>
<th>Length of PCR product (bp)</th>
<th>Tm (°C)</th>
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<td>ATF7IP</td>
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<td>BC053625</td>
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Results

Using an RNA expression database that profiles gene expression by microarray in an extensive range of mouse and human tissues (Su et al. 2002), in parallel with gene ontology (GO) terms association, we have selected genes that could potentially be involved in epigenetic transformations. To be selected, potential genes had to be present in the oocyte or blastocyst and record one GO designation related with RNA, DNA or histone methylation, chromatin modification or silencing and transcription regulation. Fifteen genes (listed in Table 1) that met these criteria were selected and their mRNA transcripts were studied and profiled in bovine oocytes and preimplantation embryos. Genes with these GO apppellations could play crucial roles during oocyte and embryo development.

Our results demonstrate that all the 15 regulation factors examined were present to different extents in each of the developmental stages observed (from the immature GV oocyte to the blastocyst stage). To our knowledge, this is the first study to precisely profile these mRNA transcripts in the oocyte and early mammalian embryo development (except for MeCP; Kantor et al. 2003, Huttriss et al. 2004). Based on the mRNA expression patterns obtained for each gene throughout embryo development, profiles were classified in three different groups (Figs 1–3). The first group (Fig. 1) represents genes that are maternally stored in abundance, showed low levels around the MET stage and regained in the blastocyst at least 50% of the relative mRNA levels initially found in germinal vesicle (GV) or metaphase II (MII) oocytes. The second group (Fig. 2) consists of genes with elevated maternal transcripts in the oocyte stages, decreased intensity during the subsequent cleavage and lower relative mRNA levels in the blastocyst compared with values obtained in GV or MII oocytes. As for the final set of genes (Fig. 3), they did not reach their highest relative mRNA levels in the oocyte stages like the two previous groups, but rather during or after MET.


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compared with the previous embryo stage suggesting a more active transcription state. The METTL3 and JMJD2A transcripts were comparable in the developing embryo. The first significant loss of maternal transcripts was observed in the two-cell embryo, and then a significant decrease was also seen during the following cell division at the four-cell stage. No significant changes were observed from the four-cell to the morula stages, but blastocyst levels were different from the morula for METTL3 and from the 16-cell embryo for JMJD2A. Of all the genes in the first group, only PRMT5 displayed a significant reduction of transcripts between the GV and the MII oocytes. Even if the PRMT5 mRNA tended to increase in the 16-cell and blastocyst stages, it was the only member of the first group that did not show any significant differences in the period between the MII oocyte stage and the blastocyst. With the exception of JMJD2A and METTL3, no significant differences were found between transcripts in the GV oocyte and blastocyst. These genes showed accumulated mRNA in the GV oocyte, and then lost most of the transcripts during subsequent cleavage stages. However, the embryo reinitiated transcription in or around the blastocyst stage to reach levels comparable with those observed in the oocyte.

In the second group (Fig. 2), the expression patterns of DMAP1, EHMT1, EHMT2, PRMT2, JARID1A and RCOR2 were relatively comparable with the genes found in group 1. They also had their highest relative levels peaking in GV or MII oocytes and their lowest levels around the MET embryo stages. However, relative transcript levels observed at the blastocyst stage did not reach at least 50% of the original levels measured in the GV oocyte (DMAP1, EHMT2, PRMT2, RCOR2) or MII oocyte (EHMT1, JARID1A). In addition, some genes showed a similar pattern and comparable significant changes during embryo development. The transcript levels for EHMT1 and RCOR2 remained stable and elevated during the stages comprised between the oocyte and the four-cell embryo, and a major decrease in levels was measured during the MET period. Despite the fact that a significant up-regulation was observed between the eight-cell embryo and the blastocyst, the levels remained relatively low. The accumulated maternal levels observed for DMAP1 (in GV oocytes) and EHMT2 (in GV and MII oocytes) were not reached in any other development stage. During oocyte maturation, DMAP1 mRNA decreased markedly to remain stable during the entire development, even if reductions were also assessed from the two- to the eight-cell stage and morula embryo. As for EHMT2, significant decreases were measured after maturation, in the two- and four-cell embryo. The EHMT2 mRNA transcripts were nearly eliminated in the eight-cell embryo and a significant

**Figure 1** Quantification of HELLS, JMJD1A, JMJD2A, LSD1, METTL3 and PRMT5 mRNA in bovine oocytes and early embryos using RT-PCR. Each developmental stage was analyzed in triplicate using 0.5 oocyte or embryo per reaction. The relative mRNA levels shown represent the quantity of transcript corrected with the GFP value obtained for each pool. The highest level was attributed the relative value of 100. Shown is the relative mRNA abundance (mean ± S.E.M.). Different letters indicate a significant difference of relative mRNA abundance (P<0.05).

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increase was measured between the eight-cell and blastocyst stages. During the first three stages examined, the mRNA transcripts for \( \text{JARID1A} \) remained constant except for a relative increase during the maturation period. A significant decrease was observed between the MII oocyte and the four- and eight-cell embryos, although no significant distinction could be established between the four-cell and the blastocyst embryos. The stored maternal transcript of \( \text{PRMT2} \) diminished significantly during maturation, and remained stable until MET, where they declined again to remain steady until a significant up-regulation was observed in the blastocyst. These genes are probably needed during the very first cleavage of embryo development, even if their mRNA is still present at different degrees in the later stages of preimplantation.

The three members (\( \text{ATF7IP}, \text{MeCP2} \) and \( \text{JARID1B} \)) comprising the third group displayed various mRNA patterns not seen in the two other groups. For \( \text{ATF7IP} \), the levels in the oocyte through the four-cell stage were low and very similar, and the levels were significantly up-regulated during MET and reached their highest relative level. In the case of \( \text{MeCP2} \), levels tended to be stable during all development stages, but the lowest relative level was found in the eight-cell embryo, which was significantly lower than in the blastocyst. In human tissues, \( \text{MeCP2} \) was also detected in the GV oocyte, but was sporadically present in the cDNA prepared from single MII oocyte, four-cell embryo and blastocyst (Huntriss et al. 2004). In the mouse oocyte, 8- and 16-cell embryos and blastocyst, no \( \text{MeCP2} \) mRNA was detected (Kantor et al. 2003). The mRNA levels for \( \text{JARID1B} \) were significantly lower in the oocyte and in the first cleavage stages compared with the eight-cell embryo. Later in development, the relative levels increased and were higher than levels observed in the eight-cell embryo. The profiles for the third group, especially for \( \text{ATF7IP} \) and \( \text{JARID1B} \), indicate that these genes could be more important in the embryo during or after MET.

**Discussion**

The data presented here provide novel information in the early embryo development about 15 key regulators known or thought to be involved in the control of gene regulation. If we exclude \( \text{MeCP2} \), which had already been studied in human and mouse oocytes and embryos (Kantor et al. 2003, Huntriss et al. 2004), our study is the first to explore and precisely report these mRNA transcripts in oocytes and throughout preimplantation embryos. The profile obtained for each gene will help to trace a parallel between their presence in specific stages and different gene regulation processes such as RNA,
DNA or histone methylation, chromatin modification or silencing and transcription regulation. These key processes which are active at different stages during embryo development are still enigmatic. Our work will provide new information on whether these 15 genes are potentially involved in important mechanisms occurring during oocyte and embryo development.

**mRNA profiles observed**

One of the first noticeable characteristics common to all 15 transcripts analyzed is that none of the genes retained significantly stable and ubiquitous mRNA expression during the eight oocyte and embryo stages examined. In fact, three different profiles can be extrapolated from the quantitative results. The first collection of profiles included genes with higher expression in oocytes and blastocysts, with generally a minor presence at or around MET. This category of transcripts suggests three possible explanations for the high accumulation originating from the oocyte. The first is that mRNAs accumulated during oocyte growth to be transcribed into proteins during that period and levels detected could just be leftover mRNA. The second option is that they accumulated during oocyte growth to be transcribed during maturation or early cleavage stages to support the maternal zygotic transition until the embryo is competent to produce its own mRNA and be self-reliant. The third alternative could involve a combination of the two previous options. Then, after reaching reduced levels around MET, the elevated intensities that were re-established in the blastocyst are presumably required to support the embryonic pattern of epigenetic modifications needed for further development. The declines in maternal mRNA observed could imply deadenylation, degradation, protein translation or synthesis. These distinctive processes have all been linked with mRNA decay in eukaryotes (reviewed in Sachs 1993, Ross 1995, Paillard & Osborne 2003, de Moor et al. 2005). However, it is unlikely that the oocyte would store useless products, so it is likely that this decrease is related to protein production, although this remains to be demonstrated for each gene.

The second group also exhibited elevated maternal transcripts, with decreased intensity in the subsequent embryo stages. However, the relative mRNA levels in the blastocyst remained lower than those observed in oocytes. The potential significance of elevated maternal levels can be similar to that of the first group, but the lower mRNA levels measured in the post-MET period could indicate that for the most part, these genes are not as critical for epigenetic events leading to gene regulation in the blastocyst or that their impact is limited to the modification events occurring right after fertilization. The declines in maternal mRNA observed could imply deadenylation, degradation, protein translation or synthesis. These distinctive processes have all been linked with mRNA decay in eukaryotes (reviewed in Sachs 1993, Ross 1995, Paillard & Osborne 2003, de Moor et al. 2005). However, it is unlikely that the oocyte would store useless products, so it is likely that this decrease is related to protein production, although this remains to be demonstrated for each gene.

The third and final set of genes revealed the opposite, with transcripts reaching their peak during or after MET, suggesting that they either play a critical function in the embryonic pattern of expression or are only needed in the embryo.

To further explore the significance of these profiles, each gene was correlated to its involvement in specific mechanisms or chromatin remodeling events established in somatic cells, oocytes and embryos.

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Figure 3 Quantification of ATF7IP, MeCP2 and JARID1B mRNA in bovine oocytes and early embryos using real-time RT-PCR. Each developmental stage was analyzed in triplicate using 0.5 oocyte or embryo per reaction. The relative mRNA levels shown represent the quantity of transcript corrected with the GFP value obtained for each pool. The highest level was attributed the relative value of 100. Shown is the relative mRNA abundance (mean ± S.E.M.). Different letters indicate a significant difference of relative mRNA abundance (P < 0.05).
**Specific gene functions**

**RNA methylation**

In eukaryotic cells, pre-mRNAs have to undergo several modifications to excise the introns present prior to translation of the matured mRNA. Interestingly, this splicing process can be affected by the methylation of internal adenosine residues located within the 5′ cap and 3′ poly (A) tail (reviewed in Rottman et al. 1994). The mRNA N6-adenosine methyltransferase activity is generated by a large, mostly uncharacterized complex. METTL3 is one of the components found in this large enzymatic organization and is similar to other genes involved in methyltransferase activity (Bujnicki et al. 2002). The high levels of METTL3 present in the oocyte that decreased until the four-cell stage suggest possible mRNA degradation or protein synthesis. METTL3 may in fact be necessary between these stages for pre-mRNA processing, because of the minor gene activation occurring at the 1- through four-cell stages in bovine embryos (Memili & First 2000).

**Histone H3–K9 methylation**

The mRNA expression levels for EHMT1 and EHMT2 are similar in their general patterns, although levels of EHMT1 tended to remain constant during the oocyte to the four-cell stage before diminishing significantly at the eight-cell stage, whereas EHMT2 had significant drops after the MI and the two-cell stages. In fact, these similar patterns are not surprising because EHMT1 and EHMT2 are involved in a heteromeric complex critical for the mono- and di-methylation of H3–K9 in euchromatin (Tachibana et al. 2005). The di-methylation state of H3–K9 in mouse oocyte and zygote revealed that only the maternal histone H3 showed clear staining, whereas the male pronucleus remained unstained. This asymmetrical methylation is maintained even in the two-cell embryo and only becomes symmetrical in the four-cell stage indicating that it is needed for post-blastocyst events. Results from a suppression subtractive hybridization (SSH) revealed that ATF7IP was highly transcribed in eight-cell embryos (C.V., unpublished observations). ATF7IP forms a tight complex with SETDB1 that increases the methylase activity of SETDB1 and allows the conversion of di-methylated H3–K9 to the tri-methylated form, which hinders transcription from chromatin (Wang et al. 2003). The intensity of H3–K9 methylation in 16-cell bovine embryos increases compared with the four-cell stage suggesting a repressive state (Santos et al. 2003). However, in eight-cell bovine embryos, the general transcriptional state is permissive, although the presence of ATF7IP at that time suggests that with SETDB1, they could be involved in the repression of oocyte-specific genes when the embryonic pattern of gene expression is starting to take place.

Although the presence of MeCP2 mRNA in oocytes and embryos is only sporadic in human, while it is not even detected in the mouse (Huntriss et al. 2004), we clearly demonstrated that MeCP2 was present in all developmental stages tested. In our study, MeCP2 showed a relatively constant expression profile, except for the slight decrease at the eight-cell stage and the higher levels found in the blastocyst. MeCP2 can bind to DMNT1 (Kimura & Shiota 2003), which is absent in mouse oocyte and early embryo, however, they express an oocyte-specific form DNMT1o (Howell et al. 2001, Ratnam et al. 2002). Given that DNMT1o and DNMT1 are almost identical, could MeCP2 bind to DNMT1o? Although this possibility has not been documented, a stretch of 11 consecutive residues within the 64-amino acid region needed for MeCP2/DNMT1 association are different in DNMT1o (McGraw, unpublished observations), suggesting that their interaction might be compromised. MeCP2 is also associated with an unknown histone methyltransferase which targets histone H3–K9 (Fukus et al. 2003). A recent study suggests that MeCP2 is probably involved in the stabilization of transcriptional silencing patterns rather than regulating gene expression during cell differentiation (reviewed in Bacro et al. 2006). MeCP2 could be involved in many different mechanisms during embryo development, but high levels re-established after the eight-cell stage indicate that it is needed for post-blastocyst events.

**Histone H3–K4 methylation**

HELLS, which is crucial for normal embryogenesis, belongs to the SNF2 family, which is at the core of the SWI/SNF chromatin remodeling complex (reviewed in Muegge 2005). It controls hyper-methylation of H3–K4 in the tightly packed heterochromatin which is fundamental for the normal control of gene transcription (Yan et al. 2003). These specific histone H3 modifications are present within the maternal genome throughout all the zygotic
stages including the mature oocyte (Lepikhov & Walter 2004). Recently, HELS has also been associated with DNMT3A and DNMT3B suggesting a possible implication in the control of de novo methylation of DNA during embryogenesis (Zhu et al. 2006). The elevated HELS mRNA levels that we have found in the oocyte and its decrease right before MET could indicate translation of this transcript in response to H3–K4 methylation and de novo methylation found in the 8-to 16-cell bovine embryo (reviewed in Dean et al. 2001).

**Histone H4–R3 methylation**

PRMT5 is associated with many different proteins implicated in germ cell formation in drosophila (Anne & Mechler 2005, Gonsalvez et al. 2006) and in mouse (Ancelin et al. 2006). Its deletion in drosophila causes male infertility with defects in spermatogenesis. In mutant females, the first generation is fertile, but their descendants are sterile and agametic (Gonsalvez et al. 2006). Methylation of H4–R3 (Me[Arg3]H4) is one of the modifications triggered by PRMT5. This specific modification is present within the germinal vesicle of immature mouse oocytes and decreases dramatically in ovaulated oocytes to become undetectable in fertilized oocytes (Sarmento et al. 2004). It is also present in interphase blastomeres of the following stage embryos and in the blastocyst. The mRNA profile that we established for PRMT5 in bovine is relatively consistent with Me[Arg3]H4 modification found in the mouse oocyte and embryos, where higher levels seem to be found in GV oocyte. This modification mark is associated with gene expression (reviewed in Davie & Dent 2002) and its actual removal from the transcriptionally inactive mouse MI oocyte and fertilized egg suggests that PRMT5 may be important in germ cell formation, but also in gene expression in the oocyte and embryo.

Unlike PRMT5, PRMT2 substrate and effect are not fully known. However, it regulates factors that influence cell activation and programmed cell death (Ganesh et al. 2006) and it is also believed to be a coactivator of estrogen receptor alpha (ERα; Qi et al. 2002)). The mRNA profile for PRMT2 is quite similar to the pig ERα expression indicating that perhaps PRMT2 in the oocyte and embryo could affect ERα function (Ying et al. 2000).

**DNA methylation**

DMAP1 was the only transcript that was high in the GV oocyte and remained particularly low during all developmental stages. This protein binds to DNMT1 and HDAC2 to mediate transcriptional repression (Rountree et al. 2000). Mouse oocyte and early embryo are deficient in DNMT1, and the oocyte-specific form DNMT1o lacks nearly all of the N-terminal 120-amino acid region needed to bind with DMAP1 (Yoder et al. 1997, Mertineit et al. 1998, Howell et al. 2001, Ratnam et al. 2002). DMAP1 involvement and association in oocytes remain to be discovered.

**Histone demethylation**

JMJD1A exclusively targets mono- and di-methyl H3–K9 (Yamane et al. 2006) whereas JMJD2A efficiently targets both tri-methyl H3–K9 and H3–K36 for demethylation (Klose et al. 2006). Strong evidence supports the notion that JMJD2A could also be involved in mono- and di-methylation of H3–K9 (Klose et al. 2006). Similar general patterns of expression were revealed for both genes during development, except that JMJD1A decreased more rapidly after the first stages and increased in the blastocyst, whereas JMJD2A decreased constantly until the eight-cell stage and then steadily increased until the blastocyst stage. In bovine embryos, immunolocalization staining revealed that the H3–K9 di-methylation pattern is closely related to the DNA methylation pattern, with low H3–K9 di-methylation in the two-, four- and eight-cell embryo and higher levels in the morula and blastocyst stages (Santos et al. 2003). JMJD1A and possibly JMJD2a might be implicated in the reduction of H3–K9 methylation in pre-MET embryos.

Two other members of the jumonji family that also contain the jmjC domain found in demethylase genes (reviewed in Takeuchi et al. 2006) were assessed. Despite that no demethylation activity has yet been discovered for JARID1A and JARID1B, both have been implicated in transcription repression (Lu et al. 1999, Chan & Hong 2001). They displayed two different patterns of expression throughout preimplantation development. JARID1A had generally higher expression levels during the pre-MET period. Free JARID1A protein is considered to act as an inhibitor of differentiation, through the repression of transcription and its implication in a differentiation checkpoint (Benevolenskaya et al. 2005). In the same model, when forming a complex with RB1 in response to differentiation signals, JARID1A is converted to a stimulator of differentiation. The presence of the RB1/JARID1A complex is still unknown in the oocyte or embryo, although RB1 is present in mouse oocyte (Moore et al. 1996). In oocytes, JARID1A could possibly be involved in this inhibitor/activator complex to regulate differentiation. The profile of JARID1B was very intriguing; faint traces of JARID1B mRNA were detected until MET, when transcripts were up-regulated. In later mouse embryo stages, transcripts for JARID1B are present in developing organs, whisker follicles, teeth and limbs (Madsen et al. 2002). In the adult mouse JARID1B is only detected in two tissues: it is highly present in the testis and barely in the ovary. It is also highly expressed in breast cancer cells (Barrett et al. 2002). These results showing high expression in post-MET embryo suggest that JARID1B is important for embryo differentiation and progression, and that its localization and expression are stage specific.
RCOR2 belongs to the CoREST family, members of which interact with REST, a key regulator of neuronal gene expression (reviewed in Ballas & Mandel 2005). CoREST members are also known to form immunocomplexes with histone-modifying enzymes such as EHMT2 (Shi et al. 2003), LSD1 (Shi et al. 2004), HDAC1 and HDAC2 (Humphrey et al. 2001, You et al. 2001). Moreover, CoREST not only interacts with LSD1, but enhances its association with nucleosomes resulting in demethylation of H3–K4 (Lee et al. 2005). The mRNA profiles established for EHMT2 and LSD1 are very similar during the oocyte to eight-cell period and suggest a possible interaction between RCOR2-EHMT2 and/or RCOR2-LSD1. Since the low nucleosomal demethylation of H3–K4 is related to a repressive state of gene expression, the intensifying action of RCOR2 towards LSD1 demethylation activity could implicate this complex in the repression of specific genes during early embryo development.

**Risk of ART**

Transcriptional patterns from individual genes are altered during prolonged *in vitro* culture when compared with *in vivo* embryo development (reviewed in Lonergan et al. 2003). Other studies also noticed that the expression profiles of some genes, including DNA methylation transcripts, were aberrant in *in vitro* and nuclear transfer derived embryos when compared with their *in vivo* counterparts (reviewed in Wrenzycki & Niemann 2003). In *in vitro* derived embryos, if the expression variations take place within genes that are somehow involved in the regulation of transcription during embryo development, the incoming offspring could suffer from a variety of malformations or diseases (Young et al. 2001, reviewed in Gicquel et al. 2003, Rodenhiser & Mann 2006). Genes implicated in transcription regulation, especially methylation-related genes, are frequently associated to various disorders (reviewed in Arnaud & Feil 2005). All the 15 genes that we studied are implicated or are thought to be associated with methylation or demethylation complexes, thus their improper regulation during ART procedures could increase the risk of complications in the developing embryo. For instance, dysregulation of genes like HELLS during embryonic development could have severe impact on the *de novo* methylation of the genome which is reinstated during that period. Consequently, cross-examination of these and other chromatin remodeling genes with *in vivo*-derived embryos, would provide important information regarding epigenetic modifications implicated in the abnormal development frequently seen during ART.

**Concluding remarks**

The information obtained through this work highlight the fact that factors involved in chromatin remodeling and gene regulation are present to different degrees in the oocyte and early embryo development. For many of the genes studied, important maternal accumulation was observed in the oocyte and many are probably translated during the MET period. For some, expression was up-regulated in the blastocyst period, around the time that the embryonic pattern of epigenetics modification is implanted. We have also confirmed that genes responsible for methylation of certain recognized histone modifications are present during the preimplantation period. These modifications are often problematic in clones, thus the presence of the genes responsible for their maintenance gives possible clues for nuclear reprogramming abnormalities. Importantly, three novel histone demethylases have been linked with the oocyte and early embryos, a developmental period known for its important methylation fluctuations. The use of GO terms in database screening has revealed to be very useful when searching for related mechanisms of action in specific tissues. Although further characterization is required, this study has set firm foundations for the involvement and comprehension of specific chromatin remodeling and regulation genes in oocyte and embryo development.

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