

## REVIEW

# Current status of genome-wide epigenetic profiling of mammalian preimplantation embryos

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**Abstract**

**Background:** Genome-wide information on epigenetic modifications in mammalian preimplantation embryos was an unexplored sanctuary of valuable research insights protected by the difficulty of its analysis. However, that is no longer the case, and many epigenome maps are now available for sightseeing there.

**Methods:** This review overviews the current status of genome-wide epigenetic profiling in terms of DNA methylome and histone modifications in mammalian preimplantation embryos.

**Main findings:** As the sensitivity of methods for analyzing epigenetic modifications increased, pioneering work began to explore the genome-wide epigenetic landscape in the mid-2010s, first for DNA methylation and then for histone modifications. Since then, a huge amount of data has accumulated, revealing typical epigenetic profiles in preimplantation development and, more recently, changes in response to environmental interventions.

**Conclusions:** These accumulating data may be used to improve the quality of preimplantation embryos, both in terms of their short-term developmental competence and their subsequent long-term health implications.

**KEYWORDS**

DNA methylation, epigenome, histone modification, preimplantation embryo

## 1 | INTRODUCTION

The explanation of “epigenetics” provided by Conrad Waddington (1905–1975), who originally introduced this term derived from the Aristotelian word “epigenesis,” is “a suitable name for the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being”.<sup>1</sup> Although he first introduced the term epigenetics in the 1940s, before it was announced that DNA exists as a double helix, his explanation literally supposed the underlying unknown mechanisms acting “upon” (i.e., epi-) the genes that materialize the gene–phenotype interactions.

As time has passed, scientists have revealed the nature of the hereditary molecular mechanisms that act “upon” DNA sequences, and control gene expression patterns in development, and thus the term “epigenetics” has been recognized as “the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence”.<sup>2</sup> Now, we generally accept that the formerly unknown mechanisms acting upon the genes (or genome elements) broadly comprise the DNA methylation, histone modifications, and noncoding RNA.<sup>3</sup>

The genome-wide epigenetic modification of preimplantation embryos had long been an unexplored sanctuary for researchers with

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critical value for several reasons. First, preimplantation embryos are the only totipotent cell population that serve as the primordium of all tissues. Second, their epigenetic modifications seemed to dynamically change throughout preimplantation development. Third, epigenetic modifications are also altered by surrounding environmental factors, and, therefore, fourth, the epigenetic modifications marked in this period can be an embryonic blueprint that defines embryo quality in terms of their developmental competence and long-term consequences in health and diseases.<sup>4</sup> What protected this sanctuary of potential research resources was the small number of cells in the preimplantation embryos, making their epigenomic analysis difficult because the analysis conventionally required the large number of cells. However, with the increasing sensitivity of epigenomic analysis methodologies (reviewed in<sup>5-8</sup>), pioneering researchers began to explore this sanctuary in the mid-2010s, and now, almost 10 years later, the number of epigenome maps drawn is exploding (Tables 1 and 2). The epigenome of preimplantation embryos is thus no longer a sanctuary, and we are free to go sightseeing there. This review briefly summarizes the current status of genome-wide epigenomic analysis of the DNA methylome and histone modifications in mammalian preimplantation embryos.

## 2 | DNA METHYLATION

### 2.1 | Exploring the genome-wide DNA methylation landscape of preimplantation embryos

In mammals, most DNA methylation occurs at the cytosine base in the dinucleotide sequence 5'CpG3' (abbreviated as CpG).<sup>9</sup> Several early studies implemented the reduced representation bisulfite sequencing (RRBS) method, which targets only CpG-rich regions of the genome in mice,<sup>10-12</sup> humans,<sup>12,13</sup> and bovines,<sup>14</sup> and these efforts were followed by whole-genome bisulfite sequencing (WGBS)-based studies (Table 1). The first WGBS-based DNA methylome of mammalian preimplantation embryos was reported in 2011 by Kobayashi et al., who analyzed mouse blastocysts together with data on oocytes and sperm,<sup>15</sup> and a subsequent report by Wang et al. extended the analysis to the cleavage stages (2- and 4-cell).<sup>16</sup> These studies confirmed, at a single-base resolution level, that the average methylation level of genomic DNA is higher (80%–90%) in sperm than in oocytes (40%–54%) and that the methylation level decreased after fertilization. The methylation levels were relatively stable during the cleavage stage and then further decreased toward

TABLE 1 Examples of genome-wide DNA methylome studies in mammalian preimplantation embryos.

Species	Author	Year published	PMID	Ref.	Data deposited	Stage in preimplantation development
Mouse 🐭	Kobayashi et al.	2012	22242016	15	DRA000484	OO SP BL
Mouse 🐭	Wang et al.	2014	24813617	16	GSE56697	OO SP 2C 4C BL IC
Mouse 🐭	Wang et al.	2018	29686265	46	GSE97778	1C 2C 4C 8C MO IC TE
Mouse 🐭	Gao et al.	2018	30146410	63	GSE108711	1C 2C 4C IC TE (Nuclear transfer embryos only)
Mouse 🐭	Matoba et al.	2018	3033120	59	GSE112546	BL
Mouse 🐭	Au Yeung et al.	2019	30943408	64	GSE112320	OO 2C BL
Mouse 🐭	Yu et al.	2019	31060426	26	PRJNA495861	1C 8C
Mouse 🐭	Wang et al.	2021	33623021	65	GSE136718	4C 8C
Human 👤	Guo et al.	2014	25079557	13	GSE49828	IC
Human 👤	Okae et al.	2014	25501653	18	DRA003802	OO SP BL
Human 👤	Li et al.	2017	29037989	29	CRA000114	OO SP 2C 8C MO IC
Human 👤	Li et al.	2018	30109120	17		
Human 👤	Zhu et al.	2018	29255258	19	GSE81233	OO SP 1C 2C 4C 8C MO BL IC TE
Human 👤	Leng et al.	2019	31588047	66	GSE133856	2C 4C 8C
Human 👤	Li et al.	2020	32864223	67	GSE114771	1C 8C
Human 👤	Olcha et al.	2021	33589136	27	Not deposited	IC TE
Human 👤	Yang et al.	2021	33846747	28	Protected as private information	TE
Monkey 🐒	Gao et al.	2017	28233770	21	GSE60166	OO SP 1C 2C 8C MO IC
Bovine 🐮	Duan et al.	2019	31191619	20	GSE121758	OO SP 2C 4C 8C 16C
Bovine 🐮	Ivanova et al.	2020	32393379	23	GSE143850	OO SP 2-4C 8-16C MO BL
Sheep 🐑	Zhang et al.	2021	35003207	22	GSE190746	OO 8C 16C MO BL
Porcine 🐷	Ivanova et al.	2020	32393379	23	GSE143850	OO SP 2-4C 8-16C MO BL

Note: RRBS-based reports and data for which papers have not yet been published are not listed. Studies with only gamete data are also not listed. Abbreviations: BL, blastocyst; IC, inner cell mass; MO, morula; nC, n-cell stage embryos; OO, oocyte; SP, sperm; TE, trophectoderm.

TABLE 2 Examples of genome-wide histone modification studies in mammalian preimplantation embryos.

Species	Author	Year published	PMID	Ref.	Target	Data deposited	Stage in preimplantation development
Mouse 🐭	Wu et al.	2016	27309802	68	H3K27ac H3K27me3	GSE66390	2C
Mouse 🐭	Liu et al.	2016	27462457	47	H3K9me3	GSE70608	2C
Mouse 🐭	Dahl et al.	2016	27626377	31	H3K4me3 H3K27ac	GSE72784	OO 2C 8C
Mouse 🐭	Liu et al.	2016	27626379	32	H3K4me3 H3K27me3	GSE73952	OO 2C 4C 8C MO IC TE
Mouse 🐭	Zhang et al.	2016	27626382	33	H3K4me3	GSE71434	OO SP 1C 2C 4C 8C IC
Mouse 🐭	Zheng et al.	2016	27635762	41	H3K27me3	GSE76687	OO SP 1C 2C 8C IC
Mouse 🐭	Inoue et al.	2017	29089420	42	H3K27me3	GSE103714	MO
Mouse 🐭	Wang et al.	2018	29686265	46	H3K9me3 H3K4me3 H3K27me3	GSE97778	OO SP 1C 2C 4C 8C MO IC TE (H3K9me3) 1C (H3K4me3, H3K27me3)
Mouse 🐭	Matoba et al.	2018	30033120	59	H3K27me3	GSE112546	MO
Mouse 🐭	Inoue et al.	2018	30463900	44	H3K27me3	GSE116713	MO
Mouse 🐭	Xu et al.	2019	31040401	50	H3K4me3 H3K27me3 H3K36me3	GSE112835	OO 1C 2C 8C (H3K4me3, H3K27me3) OO SP 1C 2C 8C IC (H3K36me3)
Mouse 🐭	Xia et al.	2019	31273069	38	H3K4me3	GSE124718	OO 1C 2C 8C
Mouse 🐭	Chen et al.	2019	32064321	69	H3K4me3	GSE130115	MO TE
Mouse 🐭	Sankar et al.	2020	32231309	48	H3K9me3	GSE129735	2C
Mouse 🐭	Yang et al.	2021	33049217	55	H3K9ac	GSE143523	1C 2C MO
Mouse 🐭	Meng et al.	2020	33311485	56	H3K27me2	GSE134592	1C
Mouse 🐭	Mei et al.	2021	33821003	57	H2AK119ub1 H3K27me3	GSE153496	OO 1C 2C MO BL (H2AK119ub1) OO 2C MO (H3K27me3)
Mouse 🐭	Xiao et al.	2022	34709113	53	H3K27ac	GSE188298	MO
Mouse 🐭	Bai et al.	2022	35508139	60	H3K4me3 H3K27me3	GSE168274	MO IC TE
Mouse 🐭	Dang et al.	2022	35575026	54	H3K4me3 H3K27ac	GSE182555	2C
Mouse 🐭	Rong et al.	2022	35640597	58	H2AK119ub1	GSE154412	OO 1C 2C
Mouse 🐭	Rong et al.	2022	35640597	58	H2AK119ub1	GSE169199	OO SP 1C 2C 4C 8C IC
Mouse 🐭	Liu et al.	2022	35717671	70	H3K4me3 H3K27me3	GSE188590	MO
Mouse 🐭	Li et al.	2022	36167681	51	H3K27ac	GSE185653	OO SP 1C 2C 4C
Mouse 🐭	Wang et al.	2022	36215692	52	H3K27ac	GSE207222	OO 1C 2C MO
Rat 🐭	Lu et al.	2021	34818044	25	H3K4me3 H3K27me3	GSE163620	OO 1C 2C 4C 8C BL
Human 🧑	Zhang et al.	2019	30808660	71	H3K27me3	GSE123023	MO
Human 🧑	Xia et al.	2019	31273069	38	H3K4me3 H3K27me3 H3K27ac	GSE124718	OO 4C 8C IC (H3K4me3) OO 2C 4C 8C IC TE (H3K27me3) 8C IC (H3K27ac)
Human 🧑	Yu et al.	2022	35803225	49	H3K9me3	GSE176016	4C 8C MO BL IC TE
Bovine 🐮	Org et al.	2019	31765427	72	H3K4me3 H3K27me3	GSE103734	IC TE

(Continues)

TABLE 2 (Continued)

Species	Author	Year published	PMID	Ref.	Target	Data deposited	Stage in preimplantation development
Bovine	Ishibashi et al.	2021	33859293	73	H3K4me3	GSE161221	BL
Bovine	Lu et al.	2021	34818044	25	H3K4me3 H3K27me3	GSE163620	OO 4C 8C 16C BL
Bovine	Yamazaki et al.	2022	35083819	74	H3K27me3	GSE171701	BL
Bovine	Susami et al.	2022	35821505	61	H3K4me3 H3K27me3	zenodo.org/record/6002122	BL
Porcine	Lu et al.	2021	34818044	25	H3K4me3 H3K27me3	GSE163620	OO 2C 4C 8C BL (parthenotes)
Porcine	Bu et al.	2022	35868641	75	H3K4me3 H3K27me3	GSE163709	OO 1C 2C 4C 8C MO BL (H3K4me3) OO 2C 4C 8C MO BL (H3K27me3)

Note: Data for which papers have not yet been published are not listed. Studies with only gamete data are also not listed.

Abbreviations: BL, blastocyst; IC, inner cell mass; MO, morula; nC, n-cell stage embryos; OO, oocyte; SP, sperm; TE, trophectoderm.

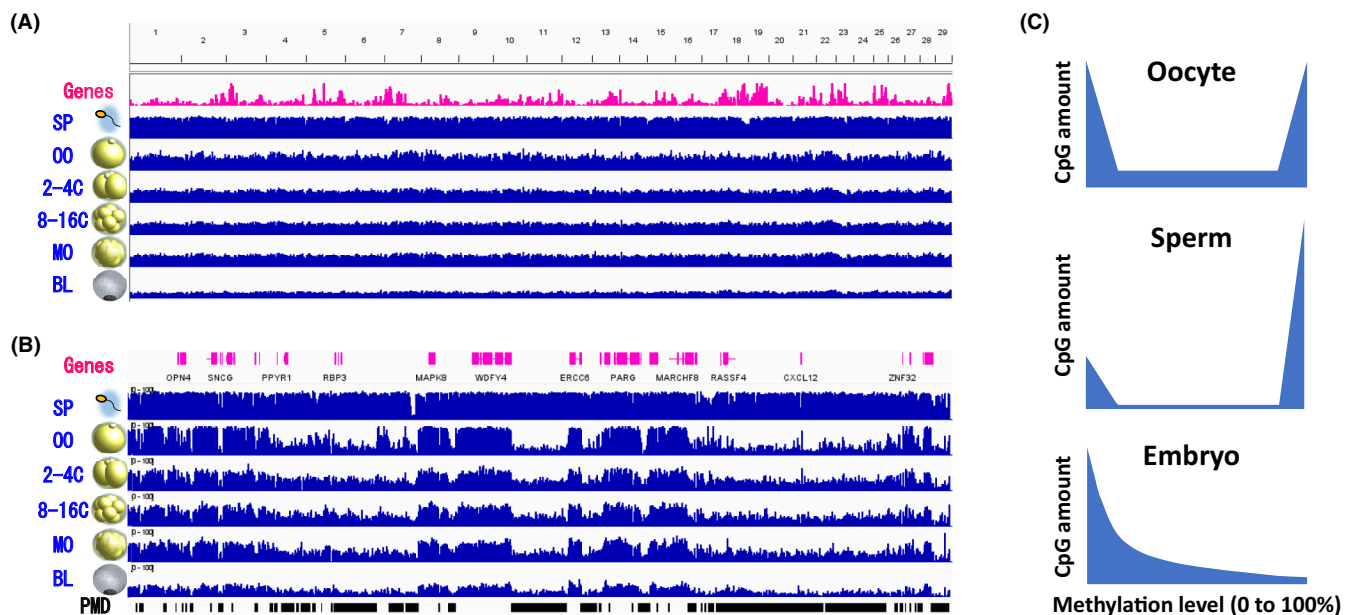


FIGURE 1 Characteristic features of DNA methylation dynamics during mammalian preimplantation development. (A, B) DNA methylation levels in bovine autosomes (chr1 to 29) (A) and a 5000kb-region (41 332 001–46 332 000) of chr28 (B) in gametes and during preimplantation development. The blue charts indicate the DNA methylation level (%) at each location, with the highest chart width and bottom of the track indicating 100% and 0%, respectively. The pink charts show where genes are. The horizontal black bars indicate partially methylated domains (PMDs) for oocytes calculated as regions with length > 10kb and average DNA methylation level < 40%. The figure was drawn with the Integrative Genomics Viewer (IGV)<sup>62</sup> using data from Ivanova et al.<sup>23</sup> (GSE143850). SP, sperm; OO, oocyte; 2–4C, 2–4-cell embryo; 8–16C, 8–16-cell embryo; MO, morula; BL, blastocyst. (C) Schematic histograms of CpG distribution by methylation levels in oocyte, sperm, and preimplantation embryos in mice and humans.

the blastocyst stage (~20%). The global dynamics of DNA methylation during preimplantation development were later revealed to be roughly conserved among mammalian species (Figure 1A,B). These changes in the DNA methylome accompany the following phenomena. The distribution of CpG methylation levels in oocytes and just after fertilization is roughly bimodal, with many either extremely high ( $\geq 90\%$ ) or low ( $< 10\%$ ) methylation regions and few intermediate

methylation (10%–90%) regions, and these distributions changes such that the regions of high methylation decrease; thus, the shape of the distribution changes from bimodal to a downward slope from low to high methylation regions (Figure 1C). Furthermore, these studies have revealed precise profiles of DNA methylation during this period, including (1) thousands of germline differentially methylated regions (gDMRs), half of which appear to be resistant to some

extent to global DNA demethylation, (2) almost entire hypermethylation in the sperm genome except at most CpG-rich regions, (3) oocyte-specific strong positive correlations between gene expression and gene-body DNA methylation levels, and (4) active demethylation from the gamete stage to the 4-cell embryo stage for both paternal and maternal genomes.<sup>15,16</sup>

There are also genome-wide DNA methylome studies on human gametes and preimplantation embryos.<sup>13,17-19</sup> After the pioneering work by Okae et al.<sup>18</sup> on human gametes and blastocysts and by Zhu et al.<sup>19</sup> on single-cell-based analysis, Li et al.<sup>17</sup> published a follow-up result with the comparison to publicly available mouse data.<sup>16</sup> Notably, Li et al.<sup>17</sup> observed that the correlation between genic DNA methylation and expression levels in oocytes differs depending on the CpG density of the region. In addition, they also found species-specific imprinting control regions between humans and mice. Other species of economic or medical research importance have also been the subjects of DNA methylome studies.<sup>20-23</sup>

In addition, mammalian genomes have kilo- to mega-base scale regions with low average methylation levels called partially methylated regions (PMDs),<sup>24</sup> which are also observed in oocytes in gene-poor and transcriptionally inactive regions, as shown in [Figure 1B](#). The oocyte PMDs are inherited by preimplantation embryos.<sup>23,25</sup> The locations of PMDs are also related to where histone modifications occur, as described later.

## 2.2 | The possible use of DNA methylation for assessing embryo quality

The establishment of methodologies for investigating the genome-wide DNA methylome in preimplantation embryos has also enabled interventional and/or diagnostic studies aimed at diagnosing or improving embryo quality. For example, Yu et al. compared the genome-wide DNA methylome in mouse 1-cell zygotes and 8-cell embryos between natural mating-derived and superovulation-derived sources given the possible epigenetic alterations induced by assisted reproductive technologies (ARTs).<sup>26</sup> Thus, they traced the above-mentioned 'bimodal-to-slope' change in the distribution of CpG methylation; however, the top of the slope (lowest methylated CpG distribution) was much higher in superovulation-derived 8-cell embryos compared with the natural mating counterparts. This difference was also represented by the differentially methylated CpGs between natural mating- and superovulation-derived 8-cell embryos, with the trend of lower methylation (vs. natural mating) in superovulation being more substantial rather than higher methylation.<sup>26</sup> These results suggest that ART interventions alter genome-wide DNA methylation in preimplantation embryos, and it is necessary to examine whether these epigenetic changes have long-term effects on the development, health, and disease-related outcomes of the resulting fetuses and offspring. As already achieved in some "exploratory" studies, genome-wide DNA methylome could be analyzed using single embryos or, furthermore, small numbers of embryonic cells as portions of whole embryos.<sup>19,20</sup> Consequently,

these methodologies motivate the testing as a diagnostic method of embryo properties. Several studies have reported biopsies of human IVF-derived blastocysts and conducted WGBS to evaluate their DNA methylation profile.<sup>27-29</sup> For example, Yang et al.<sup>28</sup> found that genome-wide DNA methylation levels increased (1) in aneuploid embryos compared with euploid embryos and (2) as the maternal age increased. Li et al. reported the differences in methylation levels and their variation between morphologically high- and low-grade blastocysts. They also showed that high-quality embryos exhibited uniform methylomes, and the proportion of blastocysts with a methylation level falling within the reference range in different grades is correlated with the live birth rate for that grade.<sup>29</sup> These reports<sup>27-29</sup> also detected DNA methylation changes that reflected chromosome-specific ploidy variance. Although these changes are macroscopic and do not take full advantage of the comprehensiveness and high resolution of DNA methylome analysis, these studies anticipated the idea of using epigenetic modifications to diagnose embryo quality to increase the chance of live birth.

## 3 | HISTONE MODIFICATIONS

Epigenetic modifications of histones include methylation, acetylation, ubiquitination, and so on, in contrast with those of DNA, which is represented by methylation.<sup>30</sup> For the histone modifications in mammalian preimplantation embryos, the first three "exploratory" studies were published in the same issue of a journal in 2016 using a mouse model.<sup>31-33</sup> Since then, data on genome-wide profiles of many histone modifications in preimplantation embryos have accumulated ([Table 2](#)). Of these, trimethylation of lysine 4 and lysine 27 of histone H3 (H3K4me3 and H3K27me3, respectively) are the most well studied, as they were investigated in the three pioneering reports mentioned above.

### 3.1 | Exploring the genome-wide H3K4me3 landscape of preimplantation embryos

H3K4me3 modification frequently accumulates at the promoters of active genes and is generally known as a histone modification associated with transcriptional activation.<sup>34</sup> There are also H3K4me3 not associated with transcriptional activation,<sup>35,36</sup> and their contribution to the specific three-dimensional architecture of the genome involving various chromatin remodeling factors, transcription factors, and DNA-cleaving enzymes, and so on has been reported.<sup>37</sup> These three-dimensional structures include not only those associated with transcriptional activation but also those associated with DNA recombination and repair.<sup>37</sup>

In 2019, Xia et al.<sup>38</sup> reported a comprehensive study of H3K4me3 and H3K27me3 methylome in human oocytes and preimplantation embryos using CUT&RUN. Based on the changes in H3K4me3 in humans through preimplantation development they revealed ([Figure 2](#)), let us look at the similarities and differences

with the previously reported murine cases. Human oocytes at the germinal vesicle stage exhibit strong and sharp (canonical) peaks at gene promoters, contrasting with mouse oocytes<sup>31,33</sup> showing a noncanonical (broad) pattern in PMDs regardless of gene proximity. These strong H3K4me3 at promoter regions in human oocytes, some of which are correlated with the expression of maternal factors (Figure 2A), further increase and become wider transiently at the 4-cell stage (termed as “priming H3K4me3”) (Figure 2B–D). Half of these promoters retain H3K4me3 and become preferentially activated at the 8-cell stage (Figure 2B,C), and the other half preferentially associated with developmental genes lose H3K4me3 and remain inactive upon zygotic genome activation (ZGA) (Figure 2D). Regarding the former half of promoters, given that ZGA occurs around the 8-cell stage in humans, the observed “priming H3K4me3” at the 4-cell stage may be linked to proper ZGA.<sup>38</sup> In contrast, murine promoter-associated canonical H3K4me3 at ZGA (the late 2-cell stage in mice) is established by the change from maternally inherited noncanonical (broad) H3K4me3 to the canonical (sharp) pattern.<sup>33</sup> The transient increase in H3K4me3 at the 4-cell stage in human embryos is also seen in distal (non-promoter) regions mainly at PMDs with a weaker magnitude compared with the promoter regions (Figure 2E). In addition, noncanonical (broad) oocyte H3K4me3 is also observed in oocytes of nonhuman mammals, including rat, pig, and bovine, and is resolved to canonical H3K4me3 after ZGA.<sup>25</sup>

### 3.2 | Exploring the genome-wide H3K27me3 landscape of preimplantation embryos

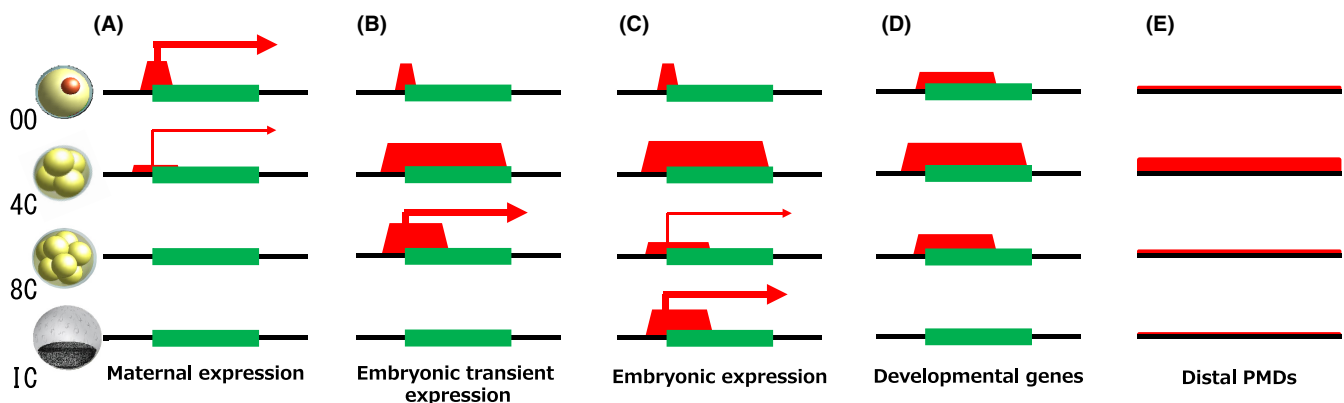
H3K27me3 deposition is catalyzed by Polycomb repressive complex 2 (PRC2), as other forms of H3K27 methylation (H3K27me1 and H3K27me2) are catalyzed.<sup>39,40</sup> H3K27me3 is generally considered a hallmark of PRC2-mediated gene silencing, which has a key role in preventing premature expression of developmental genes so as to achieve proper organismal development.<sup>40</sup>

In human preimplantation embryos, the manner in which the H3K27me3 modification changes also differs from that in mice.<sup>38</sup> The major features of the change are the strong modifications in oocytes at the promoter region and PMD as well as the global loss of these modifications at the 4- to 8-cell stage,<sup>38</sup> while H3K27me3 in mouse early embryos are persistent throughout preimplantation development except for the extensive loss of promoter H3K27me3 at developmental genes upon fertilization.<sup>41</sup> In addition, this persistent maternally inherited H3K27me3 in mice contributes to the DNA methylation-independent paternal-monoallelic expression of some genes (noncanonical imprinting) so far evidenced only in mice.<sup>42–44</sup> The patterns of change in each gene in human embryos are diverse, including (1) those that are unmodified throughout development (Figure 3A–C); (2) those in which modifications that disappeared at the 4 to 8-cell stage are restored thereafter (Figure 3D), which resembles the global change pattern; (3) those in which modifications occur only in the oocytes (Figure 3E); and (4) those in which modifications occur only in the blastocysts (Figure 3F). The associated genes in (1) include those expressed during one or all stages of preimplantation development, while those in (2) include many classical development-related genes such as homeobox genes. Modifications in (4) are thought to be related to cell lineage differentiation at the blastocyst stage.

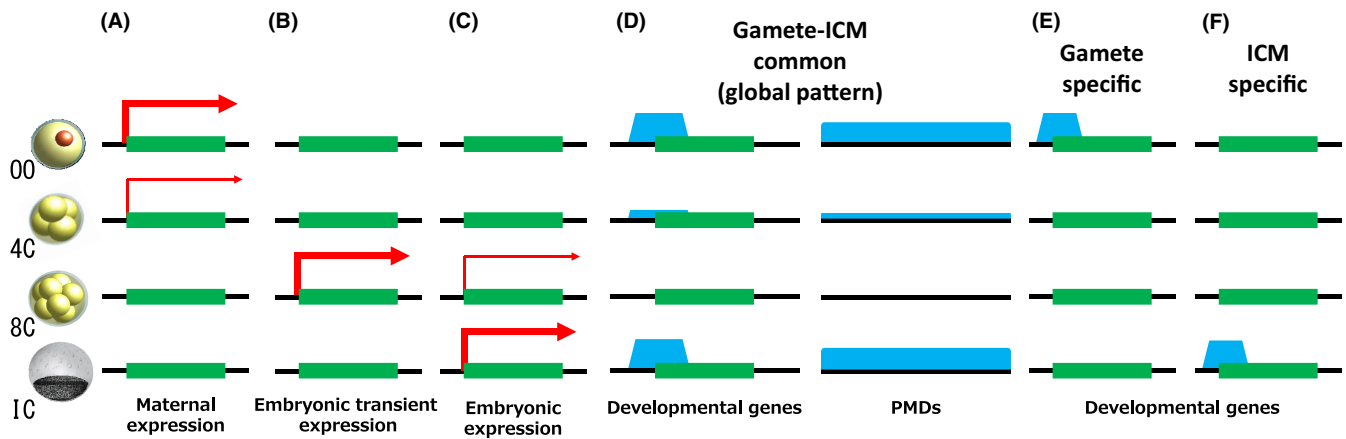
As in mice, distal (non-promoter) H3K27me3 in rat oocytes persists until the blastocyst stage, while promoter H3K27me3 in rat oocytes is reduced but is partially inherited, unlike mouse oocytes, which show complete loss upon fertilization. On the other hand, in cows and pigs, the global loss of H3K27me3 by the peri-ZGA stage is seen as in human, but their restoration toward the blastocyst stage is not seen in pigs.<sup>25</sup>

### 3.3 | Landscape of other histone modifications in preimplantation embryos

In addition to H3K4me3 and H3K27me3, for which there is a large body of data, there are several histone modifications for which



**FIGURE 2** Characteristic features of H3K4me3 dynamics during human preimplantation development. Green bars represent the transcription start to end sites of the genes. The location and amount of H3K4me3 are marked in red. Refer to the text for details, including the differences from other species. The figure is drawn based on reports by Xia et al.<sup>38</sup> and Lu et al.<sup>25</sup> Arrows and their thickness indicate gene expression and its level, respectively. OO, oocyte; 4C, 4-cell embryo; 8C, 8-cell embryo; IC, inner cell mass of blastocyst.



**FIGURE 3** Characteristic features of H3K27me3 dynamics during human preimplantation development. Green bars represent the transcription start to end sites of the genes. The location and amount of H3K27me3 are marked in blue. Refer to the text for details, including the differences from other species. The figure is drawn based on reports by Xia et al.<sup>38</sup> and Lu et al.<sup>25</sup> Arrows and their thickness indicate gene expression and its level, respectively. OO, oocyte; 4C, 4-cell embryo; 8C, 8-cell embryo; IC, inner cell mass of blastocyst.

data sets are available for preimplantation development at least for one zygotic stage. For H3K9me3, which is known as a constitutive heterochromatin marker,<sup>45</sup> both mouse<sup>46–48</sup> and human<sup>49</sup> data are available. H3K36me3 has been reported in mice and implicated in the regularity of the DNA methylome, and along with other histone methylomes including H3K4me3 and H3K27me3.<sup>50</sup> H3K27ac, which is associated with chromatin accessibility, has been reported in mice<sup>31,51–54</sup> and humans.<sup>38</sup> H3K9ac and H3K27me2 were mapped by Yang et al. and Meng et al., respectively, in mouse preimplantation embryos.<sup>55,56</sup> H2AK119ub1 is formed by ubiquitination catalyzed by another PRC, PRC1,<sup>39</sup> and has been reported in mice in relation to maternally inherited and zygotically deposited H3K27me3.<sup>57,58</sup>

### 3.4 | The possible use of histone modification for embryo quality assessment

Here we discuss the prospects for interventional or diagnostic studies based on what can be learned from genome-wide histone modifications in preimplantation embryos.

Early ideas for using histone modifications as markers of embryo quality or as etiological factors for developmental abnormalities can be seen in studies that have focused on the low live birth rates and developmental abnormalities in somatic cell nuclear transfer (SCNT). Several researchers compared the genome-wide histone modification between IVF- and SCNT-derived preimplantation embryos; discussed the aberrant genomic regions in terms of histone modifications, specifically in SCNT embryos; and proposed a method to improve SCNT efficiency by correcting the aberrant histone modifications induced in the SCNT procedure.<sup>55,59</sup>

Embryo quality is also an important issue in more practical ARTs. Bai et al. compared H3K4me3 and H3K27me3 landscapes between natural mating-derived and IVF-derived cohort morulae and

blastocysts in mice and found that differential histone modification states existed in IVF embryos, especially represented by increased H3K4me3 modification in trophectoderm.<sup>60</sup> They further showed the increased H3K4me3 induced by IVF treatment reflected ectopically increased H3K4me3 and expression of the involved genes in subsequent extraembryonic ectoderm lineages.

In identifying histone modifications associated with embryo quality, genome-wide analysis using individual embryos rather than cohort analysis would be particularly useful because each individual embryo differs in developmental competence. Based on this idea, we proposed a method to analyze multiple embryos individually using bovine preimplantation embryos.<sup>61</sup> It is anticipated that the identification of useful epigenetic modifications will continue to progress for histone modifications, which will allow for the evaluation of embryo quality. If useful markers can be identified, they will enable quality control of embryos themselves and embryo production protocols and thus contribute to improved ART procedures.

## 4 | CONCLUSION

The current status of genome-wide epigenome analysis in mammals has been briefly reviewed. Researchers will continue to accumulate epigenomic data on early embryos produced under various conditions across a variety of species. The next important objective is linking these data to the improvement of embryo quality in reproduction. In this context, embryonic quality includes not only the short-term developmental potential of the embryo but also the long-term health and disease implications associated with the transmissibility of the epigenome.

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## CONFLICT OF INTEREST STATEMENT

The author declare no conflict of interest. The author's studies referred in this review (reference number 61,73,74) were conducted under the approval by the Animal Research Committee of Kyoto University and carried out in accordance with the Regulation on Animal Experimentation at Kyoto University.

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