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Minireview

Conservation of DNA Methylation Programming Between Mouse and Human Gametes and Preimplantation Embryos[1]

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ABSTRACT

In mice, assisted reproductive technologies (ARTs) applied during gametogenesis and preimplantation development can result in disruption of genomic imprinting. In humans, these technologies and/or subfertility have been linked to perturbations in genomic imprinting. To understand how ARTs and infertility affect DNA methylation, it is important to understand DNA methylation dynamics and the role of regulatory factors at these critical stages. Recent genome studies performed using mouse and human gametes and preimplantation embryos have shed light onto these processes. Here, we comprehensively review the current state of knowledge regarding global and imprinted DNA methylation programming in the mouse and human. Available data highlight striking similarities in mouse and human DNA methylation dynamics during gamete and preimplantation development. Just as fascinating, these studies have revealed sex-, gene-, and allele-specific differences in DNA methylation programming, warranting future investigation to untangle the complex regulation of DNA methylation dynamics during gamete and preimplantation development.

comparative reproduction, DNA methylation, gamete biology, genomic imprinting, preimplantation embryo

INTRODUCTION

Assisted reproductive technologies (ARTs) are fertility treatments used by infertile/subfertile couples to assist with conception of their biological children. In developed countries, these treatment modalities account for up to 4.5% of births [1]. Although generally considered safe, ART pregnancies exhibit increased risk for preterm birth, low birth weight, intrauterine growth restriction [2–10], and have been linked to genomic imprinting disorders, including Beckwith-Wiedemann syndrome [11–16], Angelman syndrome [15, 17–20], and Silver-Russell syndrome [16, 21–27]. One explanation for the increased risk for perinatal complications in ART children is that ARTs are used during critical epigenetic programming phases that occur during gametogenesis and preimplantation development. To understand the factors that contribute to ART- and/or subfertility-related disruption of genomic imprinting, as well as global DNA methylation during gamete and preimplantation development, it is necessary to understand the epigenetic events occurring at these critical stages. Recent genome-wide and genome-scale studies on DNA methylation have shed light onto the three phases of epigenetic programming that are orchestrated during mouse and human development. The first erasure phase and the acquisition phase are staged during gamete development. The second phase of DNA methylation erasure occurs during preimplantation development, throughout which imprinted methylation is maintained. Here, we provide a comprehensive review detailing the current state of knowledge regarding global and imprinted DNA methylation dynamics during mouse and human primordial germ cell (PGC), gamete, and preimplantation development.

GENOMIC IMPRINTING CONSERVATION BETWEEN MOUSE AND HUMAN

Genomic imprinting is an epigenetic phenomenon whereby gene expression is restricted to one parental allele [28]. Imprinted genes often reside in clusters that are regulated by a germline CpG island differentially methylated region (gDMR) (reviewed in [29]). A subset of gDMRs have been identified as imprinting control regions (ICRs), since experimental or congenital gDMR deletions cause loss of imprinted gene expression [30]. In the mouse, there are 24 known imprinted gDMRs: 21 are maternal in origin, where methylation is acquired during oogenesis, while 3 are paternal in origin, where methylation is acquired during spermatogenesis [29]. Of the 24

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TABLE 1. Imprinted gDMRs in mouse and humans.

Domain	Mouse gDMR	Mouse ZFP57 motifs 1, 2	Mouse ZFP57 binding	Mouse TRIM28 binding	Human gDMR	Human ZFP57 motifs 1, 2	Human TRIM28 binding	Human reference
Common domains								
Glt2/GTL2 (MEG3)	Yes	10, 4	Yes	Yes	ID	1, 0	Yes	[112, 182]
Gnas/GNAS	Yes	14, 7	Yes	Yes	Yes	5, 2	Yes	[186–187]
Gpr1/GPR1	No*	3, 0	Yes	Yes	Yes	0, 1	No	[193]
Grb10/GRB10	Yes	4, 0	Yes	Yes	Yes	8, 6	Yes	[192]
H13/HM13 (MCTS2)	Yes	3, 0	Yes	Yes	Yes	0, 1	Yes	[194]
H19/H19	Yes	6, 0	Yes	Yes	Yes	12, 10	Yes	[177–179]
Igf2r/IGF2R	Yes	8, 1	Yes	Yes	Yes	0, 2	Yes	[188]
Inpp5f_v2/INPP5F_V2	Yes	6, 0	Yes	Yes	Yes	4, 1	Yes	[194]
Kcnq1ot1/KCNQ1OT1	Yes	3, 2	Yes	Yes	Yes	6, 4	Yes	[109, 180]
Nap1l5/NAP1L5 (HERC3)	Yes	5, 2	Yes	Yes	ID	6, 3	Yes	[194]
Nnat/NNAT (BLCAP)	Yes	4, 1	Yes	Yes	Yes	5, 2	Yes	[189]
Peg1/PEG1 (MEST)	Yes	12, 3	Yes	Yes	Yes	8, 7	Yes	[65, 108, 183]
Peg10/PEG10	Yes	3, 3	Yes	Yes	Yes	0, 2	Yes	[190, 191]
Peg3/PEG3	Yes	8, 4	Yes	Yes	Yes	11, 5	Yes	[182, 184, 185]
Plagl1/PLAGL1	Yes	6, 1	Yes	Yes	Yes	6, 7	Yes	[106, 110, 183]
Snrpn/SNRPN	Yes	10, 4	Yes	Yes	Yes	3, 6	Yes	[113, 175, 181]
Trappc9/TRAPPC9	Yes	5, 2	Yes	Yes	Yes	9, 0	Yes	[195]
Mouse specific								
AK008011	Yes	4, 0	Yes	Yes	—	—	—	—
Cdh15/CHD15	Yes	2, 0	Yes	Yes	No	2, 3	Yes	[199]
Commd1/COMMD1	Yes	2, 2	Yes	Yes	No CpGi	No CpGi	No CpGi	[197]
Fkbp6/FKBP6	No*	3, 2	Yes	Yes	ID	2, 0	Yes	Unknown
Impact/IMPACT	Yes	5, 3	Yes	Yes	No	1, 4	No	[198]
Rasgrf1/RASGRF1	No*	9, 1	Yes	Yes	No CpGi	No CpGi	No CpGi	[196]
Slc38a4/SLC38A4	Yes	0, 0	No	No	ID	1, 2	Yes	Unknown
Human specific								
Agbl3/AGBL3	No	1, 3	No	No	Yes	0, 2	Yes	[31]
Aim1/AIM1	No	0, 3	No	No	Yes	0, 7	Yes	[31]
Ano8/ANO8	No	1, 1	No	No	Yes	4, 9	Yes	[33]
C6orf47	—	—	—	—	Yes	1, 2	Yes	[33]
Ccdc71l/CCDC71L	No	2, 9	No	No	Yes	2, 11	Yes	[34]
Dcaf10/DCAF10	No	1, 5	No	Yes	Yes	0, 8	Yes	[31]
DIRAS3	—	—	—	—	No*	1, 0	No	[209–211]
Dnahc7b/DNAH7	No	1, 1	No	Yes	Yes	0, 0	No	[33]
DNM1P35	—	—	—	—	Yes	0, 5	Yes	[33]
Dnmt1/DNMT1	Yes	0, 1	No	Yes	No*	2, 3	Yes	[31, 102]
ERLIN2	No CpGi	No CpGi	No CpGi	No CpGi	Yes	0, 1	No	[31]
EXD3	—	—	—	—	Yes	1, 6	Yes	[33]
Fam196a/FAM196A	No	3, 2	No	Yes	No	5, 5	Yes	[31]
Fam20a/FAM20A	No	1, 3	No	No	Yes	2, 4	Yes	[31]
FAM50B	No	2, 1	No	Yes	Yes	3, 0	Yes	[31]
Glis3/GLIS3	No	0, 3	No	Yes	Yes	2, 5	Yes	[31]
Htr5a/HTR5A	ID	1, 1	No	No	ID	2, 1	Yes	[31]
Igf1r/IGF1R	ID	0, 0	No	No	Yes	0, 1	Yes	[31]
L3mbtl/L3MBTL	Yes	1, 0	No	No	Yes	2, 3	Yes	[200–203]
Lin28B/LIN28B	No	0, 9	No	No	ID	1, 1	No	[31]
LINC00467	—	—	—	—	Yes	1, 11	Yes	[33]
Mccc1/MCCC1	ID	0, 1	No	No	Yes	1, 1	Yes	[31]
MIR512	—	—	—	—	Yes	5, 2	Yes	[31]
N4bp2l1/N4BP2L1	No	2, 4	No	No	Yes	2, 7	Yes	[31]
Nhp2L1/NHP2L1	No	1, 5	No	No	ID	2, 1	Yes	[31]
Pde4d/PDE4D	No	2, 2	No	No	Yes	1, 5	Yes	[31]
Plg/PLG	No CpGi	No CpGi	No CpGi	No CpGi	No	2, 2	Yes	[33]
PPIEL	—	—	—	—	ID	0, 1	Yes	[31]
Prmt2/PRMT2	No	0, 0	No	No	No	0, 2	Yes	[33]
RAPGEF5	No CpGi	No CpGi	No CpGi	No CpGi	Yes	1, 0	Yes	[33]
Rasgrf1/RASGRF1	No	1, 3	No	No	Yes	2, 4	Yes	[33, 34]
Rb1/RB1	No CpGi	No CpGi	No CpGi	No CpGi	Yes	1, 1	Yes	[31, 204, 205]
Rgma/RGMA	No	1, 3	No	No	No	0, 6	Yes	[31]
Rhobtb3/RHOBTB3	No	0, 8	No	No	Yes	5 ,3	Yes	[34]
Scin/SCIN	No	1, 0	No	No	Yes	1, 1	Yes	[34]
Sept4/SEPT4	No CpGi	No CpGi	No CpGi	No CpGi	Yes	1, 0	Yes	[33]
Sncb/SNCB	No	2, 2	No	Yes	Yes	1, 4	Yes	[34]
St8sia1/ST8SIA1	No	1, 2	No	No	Yes	0, 6	Yes	[34]
Thap3/THAP3	No	0, 3	No	No	Yes	0, 4	Yes	[33]
Trp73/TP73	No	1, 1	No	No	No	5, 16	Yes	[208]
Ttc39a/TTC39A	No	0, 5	No	No	Yes	1, 6	No	[34]
Wdr27/WDR27	No CpGi	No CpGi	No CpGi	No CpGi	ID	1, 0	No	[31]
Wrb/WRB	No CpGi	No CpGi	No CpGi	No CpGi	Yes	0, 0	Yes	[31]
Wt1/WT1	No	0, 1	No	No	No	1, 2	Yes	[206, 207]

TABLE 1. *Continued.*

Domain	Mouse gDMR	Mouse ZFP57 motifs 1, 2	Mouse ZFP57 binding	Mouse TRIM28 binding	Human gDMR	Human ZFP57 motifs 1, 2	Human TRIM28 binding	Human reference
Zc3h12c/ZC3H12C	No	2, 7	No	Yes	Yes	2, 11	Yes	[31]
Zdbf2/ZDBF2	No	1, 5	No	No	No	6, 0	No	[31]
Zfat/ZFAT	No CpGi	No CpGi	No CpGi	No CpGi	Yes	4, 2	Yes	[31]
Zfp191/ZNF396	No	1, 5	No	No	Yes	2, 3	Yes	[31]
Zfp90/ZFP90	No	0, 0	No	Yes	Yes	1, 4	Yes	[33]
ZNF331 DMR1	—	—	—	—	Yes	0, 4	Yes	[31]
ZNF331 DMR2	—	—	—	—	Yes	0, 1	Yes	[32]
ZNF597	—	—	—	—	ID	1, 1	No	[31]
ZNF833P	—	—	—	—	Yes	1, 1	No	[33]

mouse gDMRs, 17 exhibit differential methylation in human gametes and/or tissues (Table 1). For the remaining seven mouse gDMRs, one has no human orthologue, while six have not been fully ascertained (Table 1). Conversely, 153 additional human DMRs have recently been identified through genome-wide methylation analysis, although many require validation for gametic-origin differential methylation [31–34] (see Table 1, which includes only those with validated DMRs). Additional studies are required in the mouse to determine whether they also possess potential species-specific gDMRs. Overall, a core of gDMR locations and their associated imprinted domains are generally well conserved, although there is some species specificity, which requires further investigation.

DNA METHYLATION ERASURE DURING MOUSE PGC DEVELOPMENT

The first phase of epigenetic programming is DNA methylation erasure. Here, previous parental DNA methylation marks are removed in sexually uncommitted PGCs. In the mouse, global 5-methylcytosine (5mC) loss occurs in two distinct waves. In stage I, DNA methylation erasure is initiated at Embryonic Day 8.0 (E8.0) [35–37]. Global 5mC levels progressively decline in a passive, replication-dependent manner to E9.0, reducing global methylation levels to ~30% [36, 38, 39] (Fig. 1). Although the maintenance methyltransferase *Dnmt1* remains highly expressed at these stages, its recruitment cofactor *Uhrf1* (ubiquitin-like with PHD and ring finger domain 1; *Np95*) is not, likely accounting for methylation dilution [40]. Stage II methylation erasure produces a further decline in 5mC levels between E10.5 and E13.5. Here, erasure occurs via active demethylation, resulting from ten–eleven translocation (TET) 1 and 2 oxidation of 5mC to the intermediate 5-hydroxymethylcytosine (5hmC) [41–44]. Levels of 5hmC peak at E11.5. The base excision repair pathway may also have a role in active demethylation, involving activation-induced cytidine deaminase and thymine-DNA glycosylase (TDG) [44–47]. At E13.5, 5mC and 5hmC decline to their lowest levels [35, 38, 39, 42, 45], representing the epigenetic ground state of the germline genome [48].

In comparison to the whole genome, DNA methylation erasure at imprinted gDMRs is delayed. Onset of erasure begins after E9.5 and is complete at or after E13.5 [37, 38, 49–51]. More specifically, of the 18 maternal gDMRs and 3 paternal gDMRs analyzed, erasure of imprinted methylation has been nearly completed at *Peg10*, *Mest*, *Peg3*, *Kcnq1ot1*, *Grb10*, *Zrsr1*, and *Impact* (~20% methylation or less) and completed at the remaining gDMRs [51]. Current studies to date for imprinted gDMR methylation loss in PGCs indicate roles for both passive and active demethylation. Passive replication-dependent demethylation, beginning at E9.5 [50] (Fig. 1), is supported by repression of *Uhrf1* [40]. By comparison, active demethylation occurs through TET1 conversion of 5mC to 5hmC commencing at E10.5 [41, 49, 52]. There is little-to-no evidence for demethylation through the base excision repair pathway at imprinted gDMRs [45, 49, 50].

DNA METHYLATION ERASURE DURING HUMAN PGC DEVELOPMENT

Prior to comparing the erasure of DNA methylation in the mouse and human, it is important to correlate developmental time points. PGC development takes place between E6.25 and E13.5 in the mouse, with PGC development occurring during Weeks 2–9 of gestation in humans [53–56]. More specifically, PGC migration and colonization of the developing genital ridge occurs between E8 and E10.5 in mouse, which corresponds to ~3- to 5-wk gestation in humans [53, 57]. Mouse PGCs at E11.5–E12.5 were most similar to Week-7 to -9 human PGCs [53]. At E13.5 in mouse and after Week 9 in human, germ cell sexual differentiation produces oogonia and prospermatogonia in female and male gonads, respectively [53, 54, 58]. To study earlier stages of PGC development, human PGC-like cells have been generated from embryonic stem (ES) cells and are representative of E6.5–E7.5 premigratory mouse PGCs [53].

Three recent publications examining genome-wide DNA methylation have increased our understanding of the erasure phase in human PGCs [53, 59, 60]. Male PGCs exhibit low methylation levels at Weeks 7–8 (20%–30%), decreasing to the lowest levels at Weeks 9–13 (4%–12%) [53, 59] (Fig. 1A), with contrasting data suggesting maintenance of low levels (6.5%) [59] or initiation of de novo methylation (41.5%) [60] by 19-wk gestation. In females, Week-5.5 PGCs exhibit low methylation levels (16%). By Week 7, DNA methylation decreases to its lowest levels (~5%), where it is maintained through to Week 11 [53, 59] (A in Fig. 1A), albeit a third study reported elevated DNA methylation levels still present in Week-8 PGCs (38%) [60] (B in Fig. 1A). In female 16- to 17-wk germ cells, DNA methylation levels remain low (11%–17%) [59, 60]. These results are consistent with previous data, where analyses of 4- to 21-wk PGCs from fetal ovaries and testes revealed that 5mC staining levels are low to below detection [61–63]. Overall, these results indicate that two waves of methylation erasure occur in humans, as in mouse, with stage I methylation erasure likely initiating prior to Week 5.5 [53] and stage II methylation erasure occurring during Weeks 7–10 (Fig. 1A). DNA methylation analysis of two germ cell genes in cultured human PGC-like cells indicates that the stage I erasure phase is initiated in premigratory nascent germ cells, although this requires more extensive confirmatory studies on Week-2 to -7 samples [53, 64].

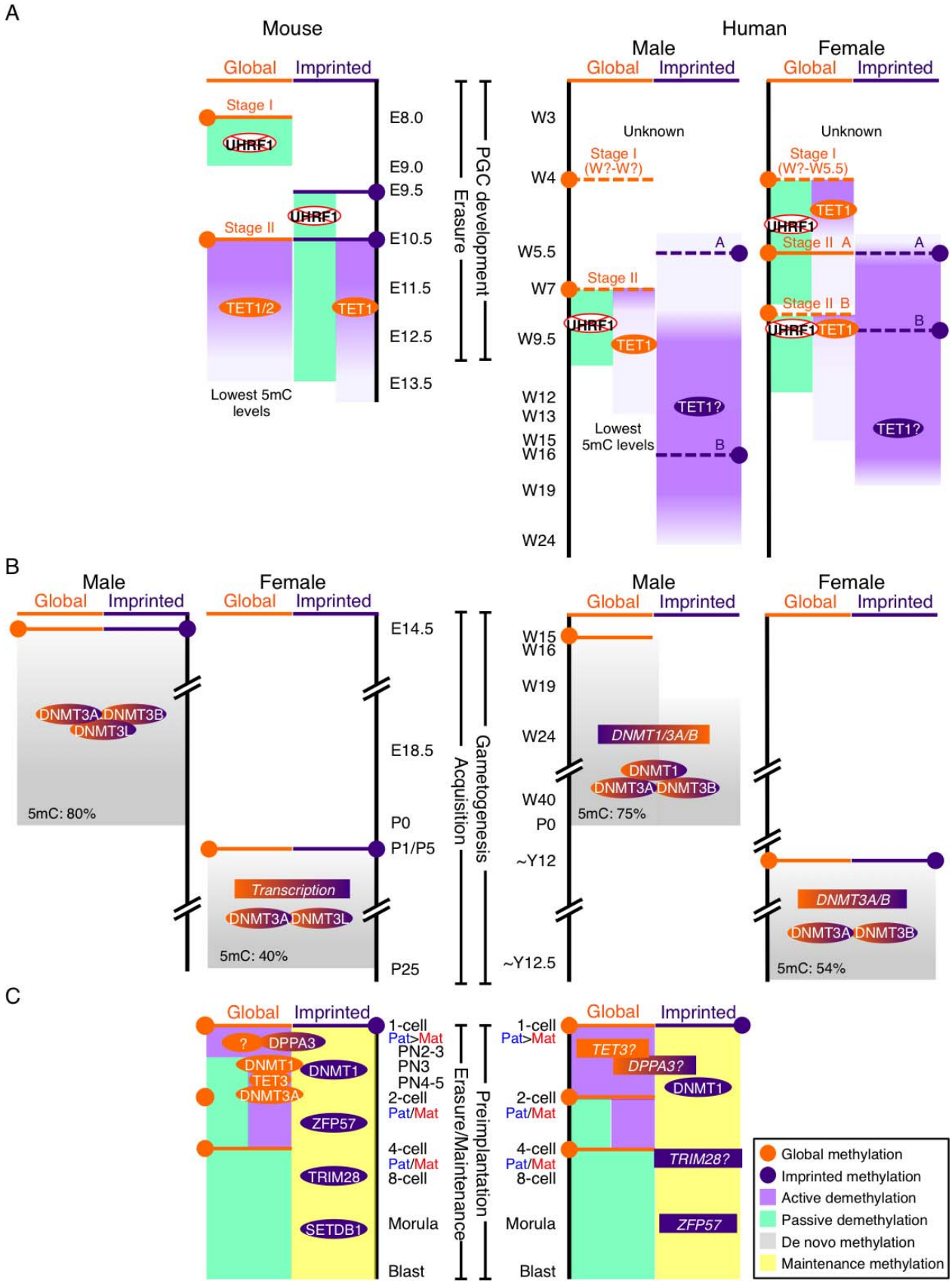

FIG. 1. Timeline of DNA methylation dynamics during mouse and human PGC development, gametogenesis, and preimplantation development. Stages of DNA methylation (**A**) erasure (PGC development), (**B**) acquisition (gametogenesis), and (**C**) erasure/maintenance (preimplantation development) are outlined for mouse and human, both globally and at imprinted gDMRs. Products involved in regulation of DNA methylation dynamics at each stage are shown in circles (protein) or rectangles (transcripts). Orange color, global methylation dynamics; purple color, imprinted methylation dynamics; dotted lines, unknown or conflicting data (denoted as A and B in **A**). GC, germ cells; Mat, maternal genome; Pat, paternal genome; W, week.

Mechanistically, active demethylation may contribute to erasure of stages I and II methylation in human PGCs. In Week-4 to -9 female PGCs and Week-8 to -13 male PGCs, there are high TET1 protein levels, and in Week-4 to -11 female and male PGCs, BER pathways members are highly expressed [53, 59–61]. Consistent with this observation, 5hmC staining that is present in female Week-4 PGCs is depleted or absent in Week-7 to -10 female PGCs, as well as in Week-10 to -11 male PGCs [53, 59]. Genome-wide, 5hmC levels reach very low levels (~2%) in Week-10 male PGCs [59]. These data contrast with those of others [60, 61], where 5hmC foci are present in Week-7.5 to -13 PGCs. This may relate to the higher global 5mC levels in Week-8 to -9 female and male PGCs observed in this study. Passive demethylation may also contribute to erasure of stage II methylation, since UHRF1 and DNMT3A are absent in Week-4 to -9 female PGCs and Week 8- to -10.5 male PGCs [53, 60]. Passive dilution of 5mC and 5hmC is further supported by the lack of 5-formylcytosine (5fC), 5-carboxylcytosine (5caC), and TDG (Week-7 to -9 PGCs), although the specific stages of PGC development analyzed for 5fC and 5caC were not specified [53].

Current examination of erasure of DNA methylation at imprinted domains in PGCs has produced conflicting data regarding the timing of methylation erasure. Imprinted DNA methylation erasure has been reported to follow a similar pattern to global DNA demethylation. In female Week-5.5 PGCs, a subset of imprinted gDMRs contain 20%–40% 5mC methylation (note: only one parental allele is methylated; thus, the highest methylation levels expected are 50%), indicating that some imprinted gDMRs have begun methylation erasure [53] (A in Fig. 1A). Subsequent DNA demethylation continues, with imprinted gDMRs having 10%–20% methylation in Week-7 and -9 female and male PGCs. In Week-10 PGCs through to Week-16 female and Week-19 male germ cells, imprinted gDMRs possess low methylation levels [53, 59]. Having said this, there may be gDMR-specific erasure of DNA methylation, since the *IGF2R* and *PEG10* gDMRs were found to retain DNA methylation at least until Weeks 17–19 [53, 59].

In contrast to these studies, another group found delayed DNA methylation erasure at imprinted gDMRs in PGCs, occurring weeks after global demethylation [60, 61]. In male fetal germ cells, the *H19*, *GTL2*, *PEG3*, and *KCNQ1OT1* ICRs exhibit hypermethylation at 9 and 16 wk. After 16 wk, there is DMR-specific methylation loss, with a sharp reduction in 5mC methylation at the *PEG3* and *KCNQ1OT1* ICRs between 16 and 17 wk, a partial methylation loss at the *H19* ICR at 16–20 wk, while the *GTL2* ICR remains hypermethylated at 17–20 wk [61] (B in Fig. 1A). By 24 wk, fetal prospermatogonia possess unmethylated *H19* and *MEST* ICRs, indicating that erasure of imprinted methylation at these genes was completed within the 20- to 24-wk window [65]. In female PGCs, *H19*, *GTL2*, and *MEST* ICRs are hypermethylated, while the *KCNQ1OT1* ICR has been partially demethylated at Week 9.5 [61] (B in Fig. 1A). Methylation erasure of the *H19* ICR is completed by 14.5 wk, and is nearly complete for the *GTL2* and *KCNQ1OT1* ICRs by 16.5 wk [61]. While a decline in DNA methylation occurs at the maternally methylated *PEG3* gDMR in 16.5-wk PGCs, erasure of DNA methylation has not yet been completed [61]. These results are consist with genome-wide DNA methylation analysis, where the *H19*, *GTL2*, *PEG3*, and *KCNQ1OT1* ICRs exhibit partial methylation in 8- to 9-wk female/male PGCs and complete methylation loss in 16-/19.5-wk female/male germ cells [60]. This delayed methylation erasure at imprinted DMRs is consistent with that observed in mice.

Mechanistically, imprinted gDMR methylation loss in PGCs may occur by active DNA demethylation. Oxidation of 5mC to 5hmC was evident at *H19* and *GNAS* ICRs in human PGC-like cells, albeit at very low levels (2%–6%) [53], and at the *PEG3* DMR in 12- to 17-wk male and 14- to 16-wk female germ cells, although earlier PGCs were not assessed to determine when 5mC oxidation was initiated [61]. The role of passive demethylation has not been investigated.

Overall, by directly comparing human and mouse genome-wide and imprinted gDMR methylation data, human PGCs have comparable methylation erasure dynamics to that in mouse. Genome-wide methylation levels in Week-5.5 human and E10.5 mouse PGCs, and Week-7 to -9 human and E13.5 mouse PGCs, cluster together, respectively [53, 59]. Globally, for both mouse and human, DNA methylation erasure in PGCs produces the greatest global DNA methylation loss throughout development, returning to epigenomic ground state levels [48, 53, 59, 60]. For methylation erasure at imprinted gDMRs, it remains unclear how and when this process is occurring in humans. Notably, there were major differences in the dynamics of DNA methylation erasure between the three human genome-wide studies, which may be attributed to methodology, germ cell purity, read recovery, biological difference between human samples, and distinct erasure dynamics at specific imprinted gDMRs.

DNA METHYLATION ACQUISITION DURING MOUSE GAMETOGENESIS

Following erasure, the next phase of epigenetic programming is DNA methylation acquisition. In males, global DNA methylation acquisition commences in E14.5–E16.5 mitotically arrested fetal prospermatogonia, reaching 50% methylation levels by E16.5, and continues to rise through to the spermatogonia stage [51], where the highest global methylation levels are present during spermatogenesis [39, 51, 66, 67] (Fig. 1B). In mature sperm, ~80% of cytosines are methylated [67]. This pattern was recently confirmed in a genome-wide DNA methylation study, where, overall 5mC levels increased from 30% in E16.5 prospermatogonia to 76%, ~77%, and 79% in Postnatal Day 0.5 (P0.5) prospermatogonia, P7.5 spermatogonia, and adult spermatozoa, respectively [68]. In mature sperm, ~78%–90% of cytosines are methylated [68–71]. Mechanistically, DNA methylation acquisition occurs through the de novo DNA methyltransferases, DNMT3A and DNMT3B, and accessory protein, DNMT3L [72].

In contrast to male germ cells, E16.5 diplotene-stage female germ cells [54] remain globally hypomethylated [69]. Instead, acquisition of global de novo methylation is delayed until oocytes enter the growth phase [73] (Fig. 1B). By the time oocytes are at the germinal vesicle (GV) and mature metaphase-II (MII) stages, acquisition of DNA methylation is complete [69, 71, 73–75]. Thus, globally, ~40%–55% of cytosines are methylated in oocytes [69–71, 73]. Although CpG islands harbor comparable DNA methylation levels between oocytes (~11%) and sperm (~8%), oocytes lack the high levels of DNA methylation present within intergenic regions in sperm [69–71, 73]. DNMT3A and DNMT3L are indispensable for DNA methylation acquisition in female germ cells [69, 73]. Recently, CpG island DNA methylation acquisition in oocytes has been linked to transcription [76]. Transcription initiating from alternative transcriptional start sites throughout oogenesis is highly correlated with hypermethylated CpG domains in fully grown GV oocytes [76]. This occurs, in part, during transcription elongation, where disposition of histone 3 lysine 36 trimethylation (H3K36me3)

enhances DNMT3A activity [76–78]. More recent data point to a two-step process of de novo DNA methylation [79]. First, coincident with transcription is acquisition of H3K36me3, which marks gDMRs for de novo DNA methylation. This is followed by demethylation of H3K4me2, which, in the absence of its K4 demethylase KDM1B, blocks de novo DNA methylation.

For imprinted DNA methylation, acquisition occurs with similar timing to that of the whole genome. In the male germline, imprinted methylation acquisition at *H19*, *Gtl2*, and *Rasgrf1* has begun by E14.5, increasing progressively through to E18.5 in fetal prospermatogonia until completed in P0 mitotically arrested spermatogonia [51, 72, 80–84] (Fig. 1B). Interestingly, the two parental alleles undergo differential methylation acquisition, with de novo methylation initiating earlier (E14.5) on the previous paternally methylated *H19*, *Gtl2*, and *Rasgrf1* alleles than on the previous maternally unmethylated alleles (E16.5) [72, 83–85]. This differential acquisition indicates that some previous parental identity is retained in the absence of DNA methylation. *H19*- and *Gtl2*-imprinted methylation acquisition during spermatogenesis is dependent on DNMT3A and DNMT3L, while *Rasgrf1* additionally requires DNMT3B [67, 72, 80, 86–88].

In E16.5 female germ cells, imprinted gDMRs have low methylation levels [51]. DNA methylation acquisition at the *Snrpn*, *Igf2r*, *Peg1*, *Peg3*, *Kcnq1ot1*, *Zac1*, *Meg1*, and *Impact* gDMRs is delayed compared to male imprint acquisition, which occurs prenatally. Instead, DNA methylation is acquired during oocyte growth in a size-dependent manner from the primary to antral follicle stage, and is completed by the ovulated MII stage [82, 89–92] (Fig. 1B). In oocytes, allelic identity also influences DNA methylation acquisition. The previous maternally methylated *Snrpn*, *Zac1*, and *Peg1* alleles acquire DNA methylation beginning at P10, while the previous paternally unmethylated alleles initiate DNA methylation acquisition at P15 [72, 81, 83, 89, 92]. This indicates that epigenetic memory of parental identity is DNA methylation-independent. Expression of de novo methyltransferases, *Dnmt3A*, *Dnmt3B*, and *Dnmt3L*, occurs during 10–25 days postpartum, increasing coordinately with oocyte diameter [93] and DNA methylation acquisition [89]. However, imprinted DNA methylation establishment is dependent on DNMT3A and DNMT3L [82, 89, 90, 94–96], but not DNMT3B [96]. Consistent with this observation, impaired nuclear localization of DNMT3A and DNMT3L via *Hdac1*/*Hdac2* deletions, as well as *Sin3a* deletion, disrupt acquisition of imprinted methylation at the *Snrpn*, *Igf2r*, and *Peg3* ICRs in mutant growing oocytes [97]. Similar to global DNA methylation acquisition, imprinted DNA methylation acquisition at gDMRs within the oocyte is dependent on transcription through gDMRs, as shown for *Snrpn* [98], *Gnas* [99], and *Zac1*/*Plagl1* [76]. Here again, a two-step process of histone modifications may facilitate de novo DNA methylation at imprinted gDMRs, namely, acquisition of H3K36me3 during transcription followed by demethylation of H3K4me2 [79, 100]. This process may offer an explanation for differential acquisition of de novo DNA methylation on the previously methylated and unmethylated parental alleles. A lack of H3K4me2 at previously methylated maternal gDMRs may allow earlier de novo methylation than previously unmethylated paternal alleles, which require H3K4me2 removal. Further studies will be required to analyze H3K36me3 acquisition and H3K4me2 demethylation in an allele-specific manner.

DNA METHYLATION ACQUISITION DURING HUMAN GAMETOGENESIS

In humans, acquisition of DNA methylation also occurs differentially between spermatogenesis and oogenesis. Globally, in testes, the number of germ cells lacking 5mC staining between 15- and 40-wk gestation progressively decreases, with nearly all germ cells exhibiting DNA methylation at birth [63] (Fig. 1B). While these data are supported by one genome-wide DNA methylation study that reported initiation of de novo DNA methylation by 19 wk (41.5%) [60], a second investigation found that DNA methylation levels were still very low at this time (6.5%) [59]. Moreover, the timing of demethylation at imprinted gDMRs, which has been reported to occur at between 5.5 and 19 wk [53, 59] and 16 and 24 wk [60, 61] (Fig. 1A), overlaps with the gain of 5mC staining observed in germ cells [63] (Fig. 1B). Thus, genome-wide DNA methylation studies are needed on fetal germ cells to more accurately define the acquisition dynamics of DNA methylation. In mature sperm, cytosine methylation levels are reported to be 54%–75% [32, 69, 73, 101].

In contrast to male germ cells, female nongrowing germ cells remain unmethylated, both prenatally at 23-wk gestation and after birth at 3 yr of age [63]. Acquisition of cytosine methylation occurs during the growing oocyte stage, since GV/metaphase I (MI) and MII oocytes harbor ~50%–55% global DNA methylation levels [32, 101] (Fig. 1). While globally less methylated than sperm, which have higher DNA methylation levels at intergenic regions, oocytes (~17%) possess comparable DNA methylation levels at CpG islands to sperm (~12%) [102].

For the acquisition of imprinted methylation during spermatogenesis, only studies involving testicular biopsies from adults are available. While one study reported that *H19* methylation had been acquired in 75% of DNA clones from adult spermatogonia (pool of three) and fully acquired in premeiotic spermatocytes [65], a second study analyzing 49 clones from a pool of 300 spermatogonia A cells had found that all clones were hypermethylated [103]. Furthermore, primary and secondary spermatocytes, round and elongating spermatids, and mature ejaculated spermatozoa are also fully methylated at *H19* [65, 103–107]. Therefore, for the most part, imprinted *H19* DNA methylation has been fully acquired in mitotically dividing adult spermatogonia, and is maintained in premeiotic, postmeiotic, differentiating, and mature sperm cells in the adult testes [65, 103]. Consistent with this finding, *DNMT1* and *DNMT3A* transcripts are evident in spermatogonia A, primary and secondary spermatocytes, round spermatids, and spermatozoa, and *DNMT3B* transcripts are present in spermatogonia A, primary spermatocytes, and round spermatids, while all three transcripts are absent in elongated spermatids. At the protein level, DNMT1, DNMT3A, and DNMT3B are present at all spermatogenic cell stages in the adult testes [103]. In contrast to mice, *DNMT3L* transcripts are not detected in any spermatogenic cells [103], indicating a lack of a role for this protein in human male imprinted methylation acquisition, although this requires further validation.

In comparison to spermatogenesis, acquisition of DNA methylation is delayed at imprinted domains during human oogenesis [108–113]. More specifically, oocyte size-dependent acquisition of DNA methylation occurs from primary to preantral/antral follicle stages at *PEG1*, *KCNQ1OT1*, and *PLAGL1* [108, 110] (Fig. 1B). While several studies have shown that imprinted DNA methylation acquisition at *SNRPN* and *KCNQ1OT1* is complete in the GV, MI, and MII stages of human oocyte development [111, 113, 114], one study reported

that DNA methylation is still progressively acquired during meiotic maturation, with *KCNQ1OT1* methylation levels increasing from 62%, 67%, and 90% in GV, MI, and MII oocytes, respectively [109]. In the female germline, *DNMT3A* transcripts have been detected from the primordial follicle stage to mature MII stage, while *DNMT3B* transcripts are present in secondary follicles and MII oocytes [115, 116] (Fig. 1). At the protein level, DNMT3A and DNMT3B localization is primarily cytoplasmic in GV, MI, and MII oocytes [115] (Fig. 1B). However, earlier stages have not been examined. As the majority of de novo methylation occurs during earlier stages, nuclear localization of DNMT3A and/or DNMT3B would be required at these stages for methylation acquisition. Finally, *DNMT3L* transcript [32, 101, 116, 117] and protein [115] have not been detected in human follicles and oocytes. This absence suggests divergence in its requirement for imprinted methylation acquisition compared to the mouse.

DNA METHYLATION DYNAMICS DURING MOUSE PREIMPLANTATION DEVELOPMENT

Preimplantation development represents the third epigenetic programming phase, where DNA methylation loss occurs globally through the zygote to blastocyst stages, albeit not to the epigenomic ground state level seen in PGCs. Following fertilization, there is active loss of DNA methylation globally in zygotes [71, 118] and two-cell embryos [70]. As the latter study did not analyze zygotes [70], active DNA methylation loss was hypothesized to occur at the one-cell stage, consistent with loss of global 5mC staining in the paternal pronucleus 4–6 h following in vitro fertilization [119, 120]. Based on 5hmC staining and DNA methylation analyses of *Tet3*-deficient zygotes, active demethylation of the paternal pronucleus occurs via TET3-mediated 5mC conversion to 5hmC [121–126]. Consistent with this, *Tet3* mRNA is more abundant than *Tet1* and *Tet2* transcripts in oocytes and zygotes [32, 124], and TET3 protein along with 5hmC levels are restricted to/overabundant in the paternal compared to maternal pronucleus [70, 121–123, 127]. Having said this, TET3 hydroxylation and the spike in 5hmC levels may be restricted to S-phase (pronuclear stage 3 [PN3]) [120], which occurs subsequent to initiation of DNA demethylation [118, 128], indicating a role for additional mechanisms in this initial demethylation event (Fig. 1C). In fact, abrogated 5hmC formation via small-molecule TET inhibitors or oocyte *Tet3* deletion had no effect on paternal 5mC loss in early PN3 zygotes [128]. Thus, additional mechanisms are likely involved in prereplicative active DNA demethylation of the paternal pronucleus [128]. In postreplicative PN3–PN4 zygotes, genome-wide CpG sites exhibited methylation loss both actively (TET3-dependent) and/or passively (replication-dependent) [126]. The latter includes repetitive elements, where DNA demethylation in the paternal pronucleus possessed hemimethylated CpG dinucleotides due to replication-dependent dilution, with minor replication-independent active demethylation [128, 129]. Interestingly, production of 5hmC by TET3 is linked to DNMT1 and DNMT3A in late-P4 zygotes, suggesting that de novo-methylated cytosines may be targets of hydroxylation [128]. Additional factors involved in paternal genome demethylation are the elongator complex proteins, ELP1, -3, and -4 [130], and gonad-specific expression gene, GSE, in PN3–PN5 zygotes [131], which require further investigation to determine their mechanistic action. Overall, evidence supports both active and passive pathways in paternal pronuclear demethylation.

In comparison to the paternal pronucleus, the maternal pronucleus is protected from 5mC demethylation. Protection from DNA demethylation is accomplished via maternal effect proteins, which are synthesized by the oocyte and required in the preimplantation embryo. In zygotes, the maternal effect protein developmental pluripotency associated factor 3 (DPPA3/Stella/PGC7) binds to maternal chromatin containing histone 3 lysine 9 dimethylation (H3K9me2), thereby inhibiting TET3 activity [132–134]. DPPA3 binding to chromatin may be dependent on the H3K9me2 methyltransferase protein, G9a/EHMT2, as well as on its heterodimeric partner, GLP/EHMT1, since their deletion in ES cells results in reduced DNA methylation at promoter regions [132, 135]. Despite this protection, active demethylation may lead to partial DNA methylation loss on the maternal genome, since low 5hmC levels are present in maternal pronuclei of zygotes [124, 136]. In support of this concept, haploid parthenogenetic embryos (only maternal genome) display pre-S-phase 5mC depletion 6 h postactivation [118, 128], and *Tet3*-deficient zygotes show impaired DNA demethylation on both paternal and maternal pronuclei [126].

After the first cleavage division, demethylation of the majority of the maternal genome is initiated in a passive, replication-coupled manner. Thus, DNA methylation loss of ~50% at each cell cycle leads to the lowest levels by the early blastocyst stage [119, 137, 138]. The absence of highly concentrated oocyte-specific DNMT1o in nuclei, except for at the eight-cell stage, and the presence of small amounts of the somatic DNMT (DNMT1s) in nuclei during preimplantation development are the contributing factors to passive DNA demethylation [88, 139, 140]. However, DNA methylation loss may not occur solely through replication dilution. A recent genome-wide, allele-specific study has documented 5mC oxidized derivatives, 5hmC and 5fC, in two- to four-cell embryos (5caC by immunofluorescence), identifying a role for active demethylation of the paternal and maternal genome at these stages [70]. Thus, passive replicative dilution of maternal DNA methylation may be delayed until the four-cell stage (Fig. 1C). However, the loss of paternal genomic 5hmC is controversial, as evidence has been presented for active BER pathways [120, 126, 141], as well as passive replication-dependent dilution [122, 123, 126, 127, 129]. For the latter, there is a progressive decline in asymmetric 5hmC, 5fC, and 5caC staining on the presumptive paternal metaphase chromatids from the two-cell to eight-cell stage, pointing to passive replication-dependent dilution of these oxidized derivatives [122, 123, 127]. Future studies are needed to uncover the mechanisms and dynamics of demethylation during preimplantation development.

Genome-wide data have reported higher DNA methylation levels in the blastocyst than expected if subjected to passive demethylation [69]. This is attributed to maintenance methylation at oocyte gDMRs, imprinted gDMRs, and repetitive elements, which retain DNA methylation though preimplantation development. For imprinted gDMRs, several proteins have been identified that maintain/protect imprinted methylation during preimplantation development. In zygotes, maternally (*Peg1*, *Peg3*, and *Peg10*) and paternally (*H19* and *Rasgrf1*) methylated gDMRs are protected from TET3 demethylation of 5mC to 5hmC by maternally derived DPPA3 binding to H3K9me2 [132, 133] (Fig. 1C). After the one-cell stage, maternal and embryonic zinc finger protein (ZFP) 57 likely protects imprinted gDMRs from passive demethylation by binding to CpG methylation [142–144] and recruiting repressive complex machinery, which includes tripartite motif 28 protein (TRIM28), the H3K9me3 histone methyltransferase

SET domain bifurcated 1 (SETDB1), and DNMT1s/1o [139, 140, 142–152] (Fig. 1C). As studies involving ZFP57 have been performed in later-stage embryos (E11.5) and ES cells, future studies are required to validate this mechanism in preimplantation embryos. Overall, current evidence indicates that imprinted gDMRs are protected from both active and passive forms of demethylation during preimplantation development by DNA methylation protector proteins. Further investigations are also required to elucidate the mechanisms and dynamics of methylation maintenance at nonimprinted oocyte gDMRs and repetitive elements.

DNA METHYLATION DYNAMICS DURING HUMAN PREIMPLANTATION DEVELOPMENT

Preimplantation DNA methylation has not been as extensively analyzed in human embryos. Globally, the greatest drop in DNA demethylation during preimplantation takes place between gametes and zygotes, with a further decrease at the two-cell stage [101], providing support for active DNA demethylation. Shortly after fertilization, there is greater methylation loss [101], as well as low 5mC/high 5hmC staining on paternal compared to maternal pronuclei/metaphase chromosomes [153–156], indicating that active DNA demethylation is more robust on the paternal genome. The greater transcript abundance of *TET3* compared to *TET1* and *TET2* transcript levels in human oocytes [32, 69, 117] points to maternal TET3-dependent active DNA demethylation in resulting zygotes [32] (Fig. 1C). Since the maternal pronucleus harbors greater 5mC/lower 5hmC levels than the paternal pronucleus, at least a portion of the maternal genome must be protected from active demethylation. *DPPA3*, the active demethylation protective factor in mouse, is expressed in the human ovary [157], and, more specifically, shows high mRNA abundance in GV and MII oocytes [69, 117, 158], indicative of a role as a maternal effect-protective factor in human preimplantation embryos (Fig. 1C). However, its role in protection of maternal DNA methylation from active demethylation in human zygotes has not yet been determined. The presence of low 5hmC levels in maternal pronuclei of triploid and diploid zygotes suggests at least some role for active demethylation of the maternal genome [101, 159].

During cleavage divisions, DNA demethylation likely occurs though passive replication-dependent mechanisms. While one homologue of each chromosome set is much less methylated (likely paternal set), as determined by 5mC staining of metaphase chromosomes, both parental chromosomes undergo progressive demethylation in two-cell- to morula-stage human triploid and diploid preimplantation embryos [155, 159] (Fig. 1C). This contrasts with an earlier study, where reduction of 5mC staining is restricted to the four-cell-to-eight-cell transition [153]. Interestingly, metaphase chromosomes of two-cell- to morula-stage embryos display decreasing proportions of asymmetric 5mC staining of sister chromatids, consistent with passive replication-dependent dilution [155, 159]. By the blastocyst stage, 5mC staining begins to increase [153, 155, 159]. Genome-wide DNA methylation data reveal decreasing DNA methylation levels from cleavage-stages though to blastocyst-stage embryos, the inner cell mass, and trophoblast cells [101, 102]. Having said this, 5mC levels are retained at higher levels than expected: two-cell- (32%), four-cell- (31%), eight-cell- (33%), morula-stage (32%) embryos, and inner cell mass (ICM) of blastocysts (29%) [101]. This retention may be due to maintenance methylation at oocyte gDMRs, imprinted gDMRs, and repetitive elements [32, 101, 102]. Similar to 5mC staining, 5hmC staining on the presumptive paternal chromosomes displays the same asymmetric sister chromatid staining on metaphase chromosomes at the two-cell stage. Subsequently, the number of chromosomes with asymmetric 5hmC staining of sister chromatids is halved at each developmental stage (three cell to blastocyst), indicative of passive replication-dependent hydroxymethylation dilution, similar to the mouse [159]. Overall, dynamic demethylation of the paternal genome, as well as passive global hypomethylation during cleavage stages of preimplantation development, recapitulates the global demethylation dynamics seen in the mouse preimplantation embryo [69, 71, 73, 102] (Fig. 1C). A direct comparison of mouse and human data has revealed that global DNA methylation loss occurs with congruent kinetics [102].

In contrast to global DNA demethylation, imprinted gDMRs generally retain their differential methylation profiles during preimplantation embryo development. Genome-wide methylation studies of human gametes and preimplantation embryos indicate preservation of maintenance of DNA methylation at imprinted gDMRs [32, 69, 101] (Fig. 1C). The high abundance of *DPPA3* in human oocytes [69, 117, 158] suggests a conserved role for this protein in protecting imprinted gDMRs from active DNA methylation loss in zygotes. For maintenance during cleavage divisions, *DNMT1o* and *DNMT1s* transcripts are present throughout preimplantation development [115, 116]. DNMT1 (1o and 1s) proteins have been detected in human preimplantation embryos, with DNMT1s nuclear localization though Day 2 (~six cell) to Day 5 (late morula), indicating that DNMT1o localizes to the nuclear compartment from Day 1 to Day 7 [115]. Therefore, DNMT1o and DNMT1s may both play roles in maintaining DNA methylation at imprinted gDMRs in humans. With regard to the DNMT1 interacting partner, ZFP57, limited data exist for its role in human embryos. However, ZFP57 hexanucleotide recognition motifs [143, 160] are present within the majority of imprinted gDMRs [31, 143], albeit the number of sites in humans is generally at a decreased density compared to the mouse (Table 1). This suggests a conserved role for ZFP57 or related proteins in maintaining imprinted DNA methylation between mouse and human. To assess its function, mouse ES cells were transfected with human *ZFP57*. The mouse and human ZFP57 proteins are interchangeable in maintaining imprinted DNA methylation as well as binding to TRIM28 [161]. In line with this, in human ES cells, TRIM28 is recruited to the majority of human imprinted DMRs by KRAB-containing ZFPs (KRAB-ZFPs) [162, 163] (Table 1). Since DNMT1, ZFP57, and TRIM28 are maternal effect proteins in the mouse, their expression has been examined in human oocytes. *DNMT1* and *TRIM28* mRNA abundance are similar between human and mouse oocytes [32]. By comparison, human oocytes were reported to lack *ZFP57* transcripts [32, 117], with embryonic *ZFP57* expression commencing at the morula stage [117] (Fig. 1). This requires further validation, since *ZFP57* protein levels were not assessed. Overall, current evidence indicates that imprinted gDMRs are maintained during preimplantation development, with potential conservation of DNA methylation protector proteins that bar active and passive demethylation.

CONCLUSIONS

Regulation of DNA methylation dynamics during gamete and preimplantation development is complex. While a greater body of data exists for the mouse compared to the human, available data highlight striking similarities between these species. Overall, these studies demonstrated that mouse and human PGCs have comparable methylation erasure dynamics,

with similar stage-II methylation erasure events globally in mouse E10.5–E13.5 [41–44] and human 5- to 19-wk PGCs [53, 59, 60, 62, 63], and possibly similar stage-I DNA methylation erasure prior to E10.5 in mouse [35, 36, 38, 39, 164] and Week 5 in humans [53]. Globally, for both mouse and human, this erasure produces the greatest global DNA methylation loss throughout development, returning to epigenomic ground state [48, 53, 59, 60]. Further studies are required to delineate global human PGC methylation dynamics and mechanisms, including during fetal germ cell development prior to 5-wk gestation. For DNA methylation erasure dynamics at imprinted domains in humans, one group showed similar delayed DNA methylation erasure [60, 61], while other studies reported DNA methylation erasure initiating coincident with global erasure [53, 59]. In both cases, imprinted methylation erasure was more protracted than global erasure. Additional studies are required to resolve these differences, including investigations aimed at gene-specific imprinted methylation, employing allelic identification, in the human. Interestingly, human PGCs exhibited sex-specific and gene-specific erasure dynamics [60, 61]. Further studies are needed in the mouse to investigate similar sex-specific and gene-specific DNA methylation erasure.

Regarding methylation acquisition in gametes, the data presented point to spatial, temporal, and mechanistic conservation of global and imprinted methylation acquisition in sperm and oocytes between mouse and human [32, 69–71, 73, 101, 102]. Both species establish global DNA methylation profiles prenatally during spermatogenesis [51, 63] and postnatally during oocyte growth [63, 69, 71, 73–75], with maternal imprint acquisition occurring in an oocyte size-dependent manner [89, 91, 108, 110]. Mouse and human gametes possess *DNMT3A* and *DNMT3B* transcripts at similar levels in comparative oocyte analysis [32], with the presence of the corresponding protein products [115]. However, unlike the mouse [72, 165, 166], *DNMT3L* transcripts/protein have not been detected in human spermatogenic cells or oocytes [32, 115, 116], suggesting divergence in global and imprinted methylation acquisition. Investigations should also be aimed at the specific roles of DNMT3A and DNMT3B in human sperm and pre-GV methylation acquisition in oocytes.

In the preimplantation embryo, DNA methylation dynamics are more complex than expected. In zygotes, active DNA demethylation of the paternal genome by the TET family likely occurs in both species, with potential for roles at both paternal and maternal genomes [101, 121–125, 153–156]. Both mouse and human oocytes express elevated *Tet3/TET3* compared to *Tet1/TET1* and *Tet2/TET2*, in addition to expressing the protective *Dppa3/DPPA3* factor [32, 69, 117, 124]. The roles for these proteins in human zygotes remain to be elucidated. During cleavage divisions, DNA methylation and hydroxymethylation marks display an asymmetric chromatid localization, which are passively diluted through replication in both species [70, 122, 123, 127, 159]. However, a role for active demethylation also exists for both mouse and human, possibly in a stage-specific and sequence-specific manner. Mechanistically in the mouse, passive loss of DNA methylation during preimplantation development was attributed to DNMT1o exclusion from nuclei (except at the eight-cell stage) and low nuclear DNMT1s levels at all preimplantation stages [88, 139, 140, 148–150]. In humans, DNMT1o nuclear localization occurs throughout preimplantation, while nuclear localization of DNMT1s is restricted to nuclei of six-cell- to morula-stage embryos [115]. Notwithstanding this difference, it appears that DNMT1o and DNMT1s are present at sufficient levels to maintain imprinted methylation during mouse and human preimplantation development. Further research is required to delineate the functions of these DNMT1 isoforms. Furthermore, the role of ZFP57 and TRIM28 proteins in human oocytes and preimplantation embryos remains to be determined, as does the role of additional maternal effect proteins in maintaining imprinted DNA methylation in both mouse and human.

In the mouse, several additional proteins may have a role in the protection/maintenance of imprinted methylation. The H3K9me2 methyltransferases, G9a/EHMT2 and GLP/EHMT1, may protect imprinted methylation by blocking TET activity, as was seen in ES cells [135]. Also in ES cells, AFF3, an AF4/FMR2 family protein that acts as a scaffold protein in the super elongation-like 3 complex, was found to bind methylated DMRs, colocalizing with ZFP57, TRIM28, and DNMT1 in ES cells [167]. ATRX also localized to the methylated allele of imprinted DMRs in ES cells, and was required for H3K9me3 and H3.3 disposition at imprinted DMRs [168]. Finally, deletion of maternal lysine demethylase 1A, *Kdm1a*, producing a hypomorphic allele, caused aberrant gains and losses of imprinted methylation in rare surviving adults [169], pointing to a role in preimplantation development. In humans, mutation of the KH domain containing 3-like gene, *KHDC3L*, and the nucleotide-binding domain and leucine-rich repeat-containing receptor protein family members, *NLRP7* and *NLRP5*, caused loss of DNA methylation at maternally methylated DMRs, leading to multilocus imprinting disturbance/biparental complete hydatidiform moles [34, 170–175]. Although mice lack *Nlrp7*, they possess numerous reproduction-related *Nlrp* genes, including *Nlrp5*/*Mater* and *Nlrp2*. Mutations in *NLRP2* in two BWS siblings resulted in loss of maternal *KCNQ1OT1* methylation in one child and *KCNQ1OT1* and *PEG1* maternal methylation loss in the other [176]. Currently, it is not certain whether these mutations perturbed acquisition of imprinted methylation in oocytes or maintenance of imprinted methylation during preimplantation development. Further investigations are required to determine the mechanistic role of these proteins in mice and humans.

In summary, there are striking similarities in the regulation of DNA methylation dynamics during mouse and human gamete and preimplantation development, for which genome-wide studies have been invaluable in advancing our understanding. Moving forward, to untangle the complex yet fascinating regulation of DNA methylation programming, investigations need to focus on sex-, gene-, and allele-specific differences in DNA methylation dynamics to separate general versus specific programming events.

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