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Epitranscriptomic modifications in embryonic development: insights into natural and ART-induced mechanisms and implications

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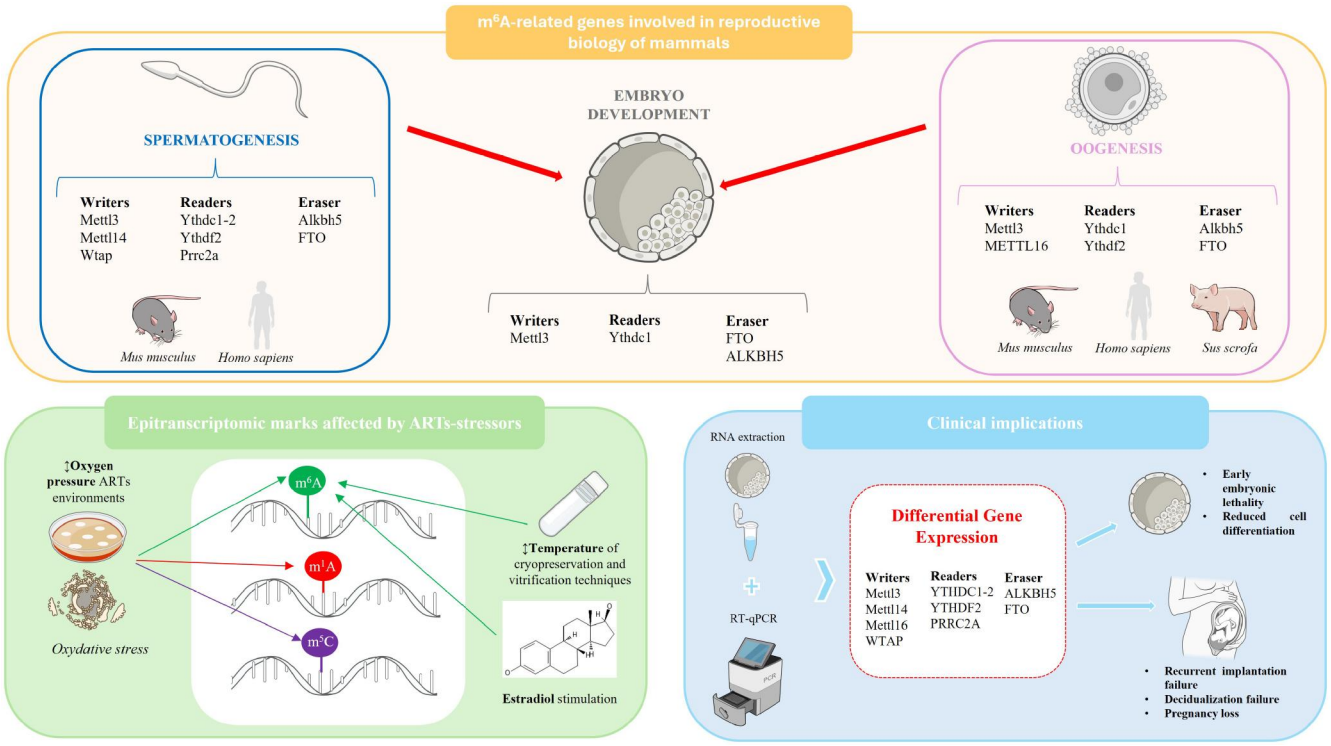
GRAPHICAL ABSTRACT



m^{6}A, a critical epitranscriptomic mark in gametogenesis and embryo development, is altered by ART procedures, with enzymatic machinery changes being detectable by RT-qPCR. 2D structure image of CID 5757 (estradiol, URL: https://pubchem.ncbi.nlm.nih.gov/compound/5757). Illustrations from NIAID NIH BioArt Source (https://bioart.niaid.nih.gov/).

ABSTRACT

BACKGROUND: Mammalian embryo development involves a complex process governed by multiple layers of cellular and molecular regulation mechanisms. ART is widely used around the world to assist fertility in humans, with ~12 million babies being born by ART in the last 40 years. These technologies are also used extensively for reproductive purposes in other mammalian species that have many analogies with human reproductive biology. Epitranscriptomic marks, including RNA modifications such as N6-methyladenosine (m^{6}A) and N1-methyladenosine (m^{1}A), modulate gene expression during gametogenesis and embryo development, and their dynamics are regulated by genes encoding m^{6}A writers (METTL3, METTL14, and WTAP), readers (YTHDF2, YTHDC1-2, and PRRC2A), and erasers (ALKBH5 and FTO). However, the impact of ART on these epigenetic modifications remains poorly understood.

OBJECTIVE AND RATIONALE: This narrative review explores the role of epitranscriptomic modifications in both naturally and ART-conceived embryos. It examines how RNA modifications regulate gametogenesis and early embryonic development and how ART-induced cellular stress might perturb these regulatory layers, potentially affecting gametogenesis, embryo competence, and offspring health. Understanding the interaction between ART and epitranscriptomic regulation is crucial for optimizing ART procedures and safeguarding offspring health.

SEARCH METHODS: The PubMed and Scopus literature databases were utilized to search for peer-reviewed articles and reviews using terms such as 'epitranscriptomic', 'RNA modification', 'gametogenesis', 'embryo development', 'mammalian development', 'in vitro fertilization', 'ART', and 'assisted reproductive technologies' in combination or individually. All relevant publications until the current year have been critically evaluated and discussed.

OUTCOMES: Epitranscriptomic modifications, particularly m^{6}A, have emerged as key regulators of RNA metabolism during gametogenesis and early embryo development. Evidence from both human and animal studies indicates that ART-related stressors, such as oxidative imbalance, hormonal stimulation, and cryopreservation, can disturb RNA methylation at the epitranscriptomic marks m^{1}A and 5-methylcytosine by modulating the expression and activity of m^{6}A writers, erasers, and readers, independently of global transcriptional changes. These alterations can affect embryo competence, placental function, lineage specification, and subsequent offspring development. Moreover, m^{6}A-associated factors participate in stress adaptation and developmental signalling beyond their canonical methylation activity. Collectively, these findings underscore the remarkable sensitivity of the embryonic transcriptome to *in vitro* manipulation and highlight epitranscriptomic marks as both predictive biomarkers and mechanistic targets for improving the safety, efficacy, and long-term outcomes of assisted reproduction.

WIDER IMPLICATIONS: Understanding how ARTs influence the epitranscriptome and its downstream effects is crucial for improving reproductive outcomes. *In vitro* manipulation, fertilization, and embryo culture can influence RNA regulation in gametes, causing reduced cell differentiation, and, in early embryos, contributing to recurrent implantation failure, decidualization failure, and pregnancy loss. This review aims to share with the scientific community insights into the critical role of epitranscriptomic modifications during gametogenesis and embryogenesis, as well as the potential consequences of *in vitro* procedures, to guide safer and more effective ART practices.

REGISTRATION NUMBER: N/A.

Keywords: epitranscriptomics / RNA modification / gametogenesis / embryogenesis / development / *in vitro* fertilization / embryo culture / assisted reproductive technologies / offspring health

Introduction

Background on ART

ARTs represent one of the most significant paradigm shifts in infertility treatment over the past century, offering a diverse array of interventions to facilitate conception for individuals and couples facing reproductive challenges. ART are defined as all interventions that include the collection of gametes (e.g. through ovarian stimulation and/or the surgical removal of oocytes), *in vitro* manipulation, and reproduction techniques such as IVF, ICSI, trophectoderm biopsy, and preimplantation genetic testing (PGT), as well as embryo cryopreservation. According to a recent report by the World Health Organization (WHO), the infertility prevalence has been estimated as 17.8% in high-income countries, while in low- and middle-income nations, it is estimated to be 16.5% (World Health Organization, 2023). Against this backdrop, the use of ARTs has surged in recent years, reflecting the technological advancements and mounting acceptance among individuals grappling with fertility issues (Adamson *et al.*, 2025).

Nevertheless, there is increasing interest in recent years regarding the long-term impact of ARTs on the health and well-being of the resulting offspring (Briana and Malamitsi-Puchner, 2022; Kim *et al.*, 2022b).

Prevalence of ART use and technological advancements

Since the births of Louise Joy Brown in 1978 and Alastair MacDonald in 1979, the first girl and boy conceived via IVF, respectively (Edwards, 1980), almost 13 million infants are estimated to have been born from ART in the last 40 years (Adamson *et al.*, 2025). An exponential growth of live-birth ART-conceived children has been observed (from 200 000 in the year 2000 to almost 1 400 000 ART-conceived infants born in 2018) (Adamson *et al.*, 2025). According to the last annual report of the European Society of Human Reproduction and Embryology (ESHRE), in 2021, a total of 1 103 633 ART treatment cycles were reported across 37 European countries, where 14% involved IVF, 38% ICSI, 33% frozen embryo replacement, 7.1% PGT, 7.2% oocyte donations, 0.04% *in vitro* maturation (IVM) of oocytes, and 0.5% of cycles used frozen oocyte replacement (ESHRE Annual Report, 2024). While the number of ART procedures has risen, 196 777 IUI treatments were also carried out. Of these, one-quarter used donor semen, and the rest used semen from their partner. Moreover, a total of 28 768 fertility preservation interventions (including oocyte, ovarian tissue, semen, testicular tissue, and embryo banking) were reported from 15 countries (ESHRE Annual Report, 2024). Recently, ARTs have integrated robotics and artificial intelligence (AI), marking the beginning of a transformative era in reproductive medicine (Chow *et al.*, 2021; The Lancet Digital Health, 2023). Particularly, the applications of AI in ART procedures have demonstrated promising results in improving the efficiency of the evaluation of embryo viability (VerMilyea *et al.*, 2020). AI algorithms allow factual analyses of embryo images, standardizing embryo scoring for the selection of viable embryos for ET, but also sperm classification, oocyte quality assessment, and protocol optimization (Hanassab *et al.*, 2025).

Increasing concern about the potential impact of ART

Although ARTs are accepted as a safe method of conception (Spaan *et al.*, 2019; Elhakeem *et al.*, 2023; Laugesen *et al.*, 2023), evidence suggests that ARTs are variably connected to a spectrum of obstetric and perinatal complications compared to pregnancies after natural conception (Hargreave *et al.*, 2019; Chambers *et al.*, 2021; review by Pinborg *et al.*, 2023). Several studies have reported an increased prevalence of major congenital heart defects (CHDs) in children conceived through ART, with the absolute risk being modest and partly associated with multiple pregnancies (Sargisian *et al.*, 2024; Wei *et al.*, 2024). Although it remains some controversial whether these adverse effects are caused by ART procedures *per se* or related to the population seeking ART, the evidence shows that, even excluding multiple pregnancy confounders, infants born after ART have slightly elevated risks compared to their naturally conceived (NC) peers (Zhao *et al.*, 2020).

These findings are consistent across large cohort studies and meta-analyses, and recent epidemiological studies have reported an increased incidence of perinatal and postnatal complications in children conceived by ART (Landsverk *et al.*, 2025; Li *et al.*, 2025a; Song *et al.*, 2025), with a less pronounced risk of adverse perinatal outcomes in pregnancies when ART was used for male infertility than for female infertility (Magnus *et al.*, 2025). Nonetheless, some studies have not found significant differences in the incidence of congenital anomalies between ART and NC infants, especially when analysing certain hospital-based populations (Xiong *et al.*, 2022).

While embryos exhibit remarkable developmental plasticity that enables them to adapt to a range of environmental variations (temperature, pH, oxygen, and media composition), such as those that occurs during ART, this adaptive capacity is not without consequence (Garcia-Dominguez *et al.*, 2020). Embryos can compensate for certain physical, chemical, and genetic challenges during *in vitro* culture (IVC), but these adaptations may be accompanied by alterations in epigenetic programming, particularly in the methylation of imprinted genes that may lead to the development of imprinting disorders, like Beckwith–Wiedemann syndrome (Chen *et al.*, 2014), Angelman syndrome, Prader–Willi syndrome, and Silver–Russell syndrome (Cocchi *et al.*, 2013; Hattori *et al.*, 2019; Henningsen *et al.*, 2021). Nonetheless, some studies have shown that ART-related epigenetic modifications identified at birth tend to diminish by adulthood, and there is no clear evidence that they have an impact on health or development (Novakovic *et al.*, 2019; Håberg *et al.*, 2022).

Epitranscriptomics and post-transcriptional gene regulation mechanisms

Beyond the study of the DNA sequences and the epigenetic regulatory processes that modulate gene expression (such as DNA methylation, histone modifications, and chromatin remodelling) (Al Aboud *et al.*, 2025), there is also a framework of molecular mechanisms that influence gene expression regulation at the transcript level. 'Epitranscriptomics' is the field of molecular biology that studies the intricate network of post-transcriptional molecular regulatory mechanisms and structural modifications of RNAs that do not involve changes to the sequence itself.

Variations in epitranscriptomic patterns have been found to have a role in mammalian female infertility (Zheng et al., 2023), in mammalian embryo development by the regulation of the maternal-to-zygotic transition (MZT) (Ivanova et al., 2017; Sui et al., 2020), in semen quality (Li et al., 2023a), and in embryonic pluripotency (Geula et al., 2015).

The regulation of gene expression mediated by RNA modifications occurs in many ways, depending on the type of epitranscriptomic mark involved. It is involved in different biological processes, such as cell differentiation (Batista et al., 2014), sex determination (Haussmann et al., 2016), and stress responses (Carlile et al., 2014). More than 170 RNA modifications have been observed in coding and noncoding RNAs in organisms spanning all kingdoms (Crain and McCloskey, 1996; Sanoudou et al., 2022; Cappannini et al., 2024). The most extensively researched RNA modifications include N1-methyladenosine (m^1A), N6-methyladenosine (m^6A), N6,2′-O-dimethyladenosine, 5-methylcytosine (m^5C), 5-hydroxymethylcytosine, pseudouridine (Ψ) adenosine-to-inosine (A-to-I) editing (Roundtree et al., 2017; Walkley and Li, 2017), and, to a lesser degree, also N4-acetylcytidine (ac^4C) (Chen et al., 2022) and 2′-O-methylation (2′-O-me) (Häfner et al., 2023).

The regulatory mechanisms of RNA modifications control multiple aspects of RNA molecular structure and function, ultimately affecting gene expression post-transcriptionally (Bove et al., 2024). Incredible progress has been made in identifying the roles of epitranscriptomic modifications in female reproductive biology. In particular, epigenetic RNA modifications have been identified as playing a role in the development of infertility-related conditions such as polycystic ovary syndrome, premature ovarian insufficiency (POI), and endometriosis (review by Xiang et al., 2025).

Most of the data related to the RNA modification sequencing in *Homo sapiens* and *Mus musculus* (Boileau et al., 2025) and ineubacteria (Cappannini et al., 2024) are stored in user-friendly, searchable databases.

Enzymatic machinery of N6-methyladenosine: writers, readers, and erasers

The epitranscriptomic landscape is dynamically regulated by specialized proteins (writers, erasers, and readers) that modulate RNA modifications, notably N6-methyladenosine (Fig. 1). The methylation of adenosine (A) to m^6A occurs at the nucleotide level of adenosine during the transcription process (Sendinc and Shi, 2023). It is catalysed by a multicomponent methyltransferase complex (m^6A-MTC), comprising two subcomplexes: MAC and MACOM. MAC is a methyltransferase 3 (METTL3) and methyltransferase 14 (METTL14) heterodimer, where METTL3 transfers the methyl group from S-adenosyl-L-methionine to target transcripts, and METTL14 enhances substrate recognition and catalytic efficiency (Batista et al., 2014; Yoon et al., 2017).

MACOM includes Wilms' tumour 1-associating protein (WTAP) (Gong et al., 2025), RBM15/15B (Wang et al., 2021b), KIAA1429 (or VIMRA: vir-like methyltransferase associated) (Yue et al., 2018), ZC3H13 (Knuckles et al., 2018), and HAKAI26 (Bawankar et al., 2021), and confers specificity by anchoring MAC to RNA. Independently, METTL16 mediates methylation at select sites (e.g. MAT2A 3′-untranslated region (UTR) hairpins and U6 snRNA) as a monomeric enzyme (Flaherty et al., 2025).

All these m^6A marks are recognized by readers, including YTH domain-containing proteins (YTHDF1-3 and YTHDC1-2), insulin-like growth factor 2 mRNA-binding proteins (IGF2BP1–3), and heterogeneous nuclear ribonucleoproteins (HNRNPs) (Wang et al., 2014; Xu et al., 2014; Hsu et al., 2017; Liu et al., 2017; Huang et al., 2018; Zhu et al., 2023). These interactions modulate mRNA stability, splicing localizing, and translation.

As a reversible and dynamic RNA modification, m^6A is removed by erasers Fe^{2+}-dependent and 2-oxoglutarate-dependent oxygenases, FTO (Fat Mass and Obesity-associated protein), and ALKBH5 (AlkB Homolog 5) (Kaur et al., 2022). m^6A sites are non-randomly distributed within the conserved DRACH motif (D = G, A or U; R = A or G; H = A, C or U; and A = m^6A), which differs among cells, tissues, and organisms (Wei et al., 2018; Liu et al., 2020a). An overview of the N6-methyladenosine molecular component machinery and the biochemical sequence of the adenosine methylation is shown in Fig. 1.

Methods

The scientific literature was reviewed using PubMed and Scopus, focusing on the last 10 years due to the observed increase in publications using the term 'epitranscriptomics' during this period (Supplementary Fig. S1). Keywords such as 'epitranscriptomic', 'RNA modification', 'ncRNA', 'gametogenesis', 'embryo development', 'mammalian development', 'in vitro fertilization', 'ART', and 'assisted reproductive technologies' were used individually or combined. Studies examining the relationship between epitranscriptomic alterations, gamete competence, embryonic development, and ART in humans and other mammals were manually extracted for relevant information. To ensure terminology consistency, the International Glossary on Infertility and Fertility Care of 2017 was used, given the variability in ART terminology across species (Zegers-Hochschild et al., 2017). Inclusion criteria focused primarily on the data range from 2015 to 2025 (from January to October), investigating epitranscriptomic modifications in embryo development related to ART. However, additional relevant studies outside this timeframe were included if identified through complementary strategies. Supplementary Fig. S2 highlights only those studies specifically addressing how epitranscriptomic alterations impact mammalian gametogenesis and embryo development.

Role of the epitranscriptome in gametogenesis and embryonic development

Maternal RNA modifications: oocyte competence and follicular development

The N6-methyladenosine RNA modification plays a key role in mammalian gametogenesis (Hu et al., 2020), embryo development (Wang et al., 2023), pregnancy, and placental functions (Meng et al., 2019; Wu et al., 2024).

In mice, through oocyte maturation, RNAs accumulate extensive chemical modifications, including the widespread introduction of N6-methyladenosine in the transcriptome of oocytes and early embryos. m^6A regulates cytoplasmic maturation (i.e. progression of meiosis, spindle formation, and chromosome movement) in murine oocytes but lacks a defined role in nuclear maturation (Sui et al., 2020). Single-cell mapping revealed m^6A profiles and transcriptomes in murine oocytes and preimplantation embryos, clustering germinal vesicles (GVs) and metaphase II (MII) and separating them from the other stages (zygotes, two-cell, eight-cell embryos, and blastocysts) (Yao et al., 2023). Notably, widespread m^6A modifications occurred on transcripts inherited from the mother that are destined for degradation after fertilization, as well as on genes activated during embryonic genome activation (EGA) (Yao et al., 2023). In another study (Wang

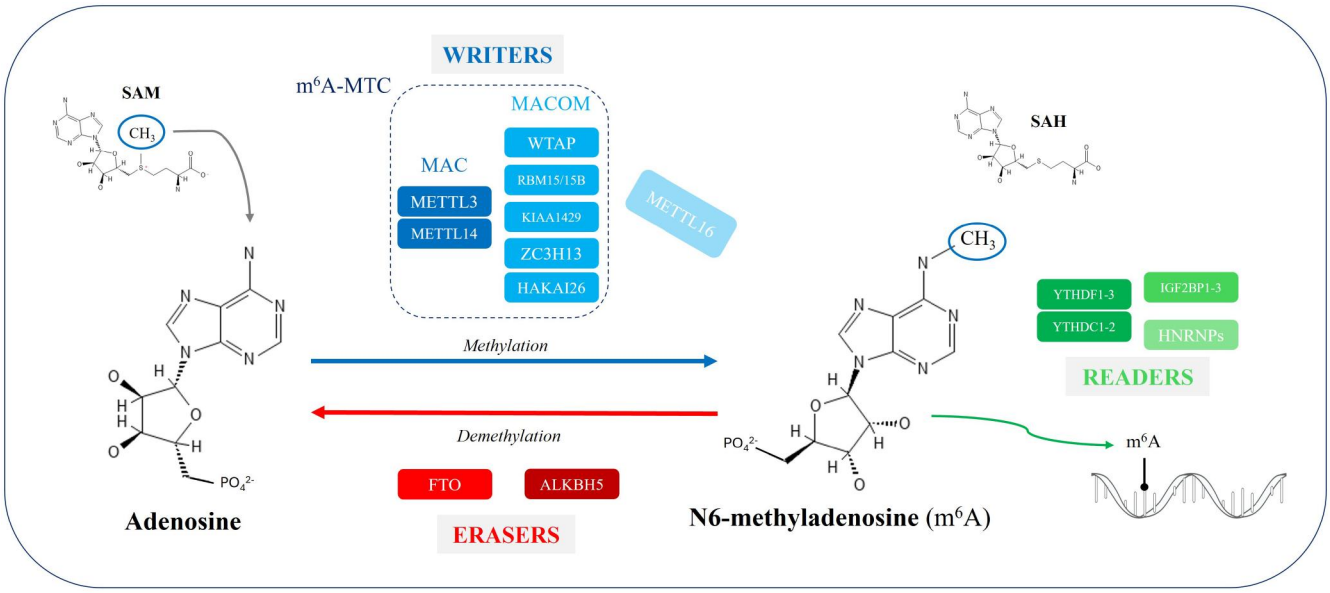


Figure 1. m^{6}A enzymatic machinery components. In blue, green, and red are represented the m^{6}A writers, readers, and erasers, respectively. A different shade of the same colour represents a polymeric molecular complex or subfamily members of the m^{6}A enzymatic machinery. m^{6}A-MTC, m^{6}A multicomponent methyltransferase complex; Mettl3, methyltransferase 3; Mettl14, methyltransferase 14; SAM, S-adenosyl-L-methionine; SAH, S-Adenosylhomocysteine; WTAP, Wilms' tumour 1-associating protein; RBM15/15B, RNA-binding Motif Protein 15/15B; ZC3H13, zinc finger CCCH domain-containing protein 13; Mettl16, methyltransferase 16; YTHDF1-3, YTH domain-containing protein DF1-3; YTHDC1-2, YTH domain-containing protein DC1-2; IGF2BP1–3, insulin-like growth factor 2 mRNA-binding proteins 1–3; HNRNPs, heterogeneous nuclear ribonucleoproteins. 2D structure image of CID 34755 (S-Adenosylmethionine, URL: https://pubchem.ncbi.nlm.nih.gov/compound/34755), CID 439155 (Adenosylhomocysteine, URL: https://pubchem.ncbi.nlm.nih.gov/compound/439155), CID 60961 (Adenosine, URL: https://pubchem.ncbi.nlm.nih.gov/compound/60961), and CID 102175 (N6-Methyladenosine, URL: https://pubchem.ncbi.nlm.nih.gov/compound/102175).

et al., 2023), m^{6}A marked ~20% of gene transcripts (10 947 out of 55 335) in at least one developmental stage, with 43–57% of highly expressed genes per stage marked (Wang *et al.*, 2023). A dramatic m^{6}A shift occurred between the zygote and the two-cell stage, where the two-cell embryo presented 2356 transcripts that gained m^{6}A and 2084 transcripts that lost m^{6}A compared with the zygote (Wang *et al.*, 2023) (Table 1). This phenomenon was observed at the embryo development stage when the EGA occurs, suggesting a possible role of this epitranscriptomic modification in the activation of the zygotic genome. In *Mettl3* knockout mice, the absence of m^{6}A leads to abnormal maternal RNA clearance and the production of low-quality oocytes that stop developing at the one-cell stage (Yao *et al.*, 2023). This is likely due to the accumulation of DNA damage in oocytes, and the *Mettl3* knockout mice is also defective in follicle development, displaying an abnormal ovulation (Mu *et al.*, 2021). Several transcripts associated with oocyte meiosis (*Itsn2*, *Spire1*, *Myt1*, *Pias1*, and *Cdc42bpa*) and DNA repair-associated transcripts (*Brca1*, *Ercc6l*, *Palb2*, *Brca2*, and *Mcm9*) are potentially regulated by METTL3 and IGF2BP2/3 via m^{6}A modification (Mu *et al.*, 2021).

m^{6}A readers are also important for female fertility. In mouse, YTHDF2 is required to produce MII oocytes that sustain early zygotic development (Ivanova *et al.*, 2017), while loss of YTHDC1 is associated with alternative polyadenylation (altering the length of the 3'-UTR) and alternative splicing defects in oocytes, resulting in maturation arrest (Kasowitz *et al.*, 2018).

The m^{6}A demethylase ALKBH5 has been identified as a key regulator of oocyte maturation, primarily through its role in ensuring the timely degradation of maternal RNAs (Bai *et al.*, 2023). Experimental deletion of ALKBH5 in mouse models leads to significant disruptions in meiotic progression and results in female infertility, underscoring the importance of ALKBH5-mediated m^{6}A removal in ensuring a correct maternal RNA turnover and oocyte meiotic division (Bai *et al.*, 2023) (Table 1). Thus, writers, readers, and erasers are all important for female fertility in the murine model.

m^{6}A-methylated transcripts also accumulate in the cytoplasm of oocytes during meiotic maturation in larger mammals, such as pigs (Zhang *et al.*, 2022). Notably, m^{6}A enrichment near the stop codon within 3'-UTRs has been linked to alterations in polyadenylation site selection, which may influence mRNA stability and processing in porcine oocytes (Cao *et al.*, 2020). Additionally, granulosa cells exhibit levels of m^{6}A methylation, with a notably higher abundance in smaller follicles (<3 mm) compared to larger ones (>5 mm) (Cao *et al.*, 2020). This differential distribution suggests that m^{6}A may contribute to the regulation of follicular development by modulating the expression of transcripts involved in key processes such as steroidogenesis and folliculogenesis, including in swine species.

In humans, granulosa cells from women aged between 40 and 50 years exhibit a higher number of m^{6}A-methylated genes compared to those from younger women, with an enrichment in the FoxO signalling pathway, adherens junction, and regulation of actin cytoskeleton (Liu *et al.*, 2022). This increase in m^{6}A is primarily attributed to a reduced expression of the m^{6}A demethylase FTO alongside an upregulated expression of the m^{6}A methyltransferase METTL16 (Hu *et al.*, 2025).

Regarding the human m^{6}A reader YTHDC2, its expression peaks in oogonia cells that are not yet fully meiotic, highlighting that its functional role likely begins before and continues through the transition into meiosis. Notably, pathogenic variants in this gene have been associated with POI, specifically presenting as primary amenorrhoea and absent puberty in affected individuals, indicating a critical role for YTHDC2 in early ovarian development and meiotic cycle entry (McGlacken-Byrne *et al.*, 2022). In women with diminished ovarian reserve undergoing

Table 1. Effect of m^6A enzymatic machinery alterations on female fertility in humans and other mammals.

Reproductive cell	Enzyme/ epitranscriptomic mark	Species	Alteration	Phenotype	Reference
Granulosa cells (GCs)	m^6A	*Homo sapiens*	(↑)m^6A	Ovarian ageing (OA)	(Liu *et al.*, 2022)
Follicular cells (follicular fluid)			(↓)m^6A	Diminished ovarian reserve (DOR)	(Li *et al.*, 2023b)
Endometrial tissue			(↑)m^6A	Recurrent implantation failure (RIF)	(Wang *et al.*, 2024)
Cumulus-oocyte complexes (COCs)		*Sus scrofa*	(↑)m^6A	Meiotic maturation progression	(Zhang *et al.*, 2022)
Granulosa cells			(↑)m^6A	(↕)Follicle development, (↕)gene expression(steroidogenesis), (↕)granulosa cell proliferation, (↕)follicular development	(Cao *et al.*, 2020)
Germinal vesicle (GV) and metaphase II (MII) oocytes, and early embryos		*Mus musculus*	m^6A	Maternal-to-zygotic transition (MZT)	(Wang *et al.*, 2023)
	WRITERS				
GV oocytes	METTL3	*Mus musculus*	*Mettl3* KD	(↓)oocyte maturation, (↓)mRNA translation, (↕)ZGA	(Sui *et al.*, 2020)
GV oocytes/blastomeres of cleavage-stage embryos			*Mettl3*^{Gdf9} cKO	(↓)oocyte quality, (↕)ZGA, (↑)YTHDF3 and (↓) YTHDF1 (MII oocytes)	(Yao *et al.*, 2023)
GV oocytes			*Mettl3*^{Gdf9} cKO	(↓)DNA integrity, (↓) follicle development, (↕)ovulation, (↕)gene expression (oocyte meiosis-associated transcripts, DNA repair-associated transcripts)	(Mu *et al.*, 2021)
Mouse embryonic stem-cells (mESCs) and blastocysts			*Mettl3* KOMP2	(↑)developmental pausing	(Collignon *et al.*, 2023)
			Mettl3 KO	(↕) heterochromatin organization	(Xu *et al.*, 2021)
				(↓) stem cell lineage differentiation	(Batista *et al.*, 2014)
Preimplantation epiblasts and naïve embryonic stem cells (ESCs)				Block of naïve state termination, restricted lineage priming, (↑)early embryonic lethality	(Geula *et al.*, 2015)
Mouse embryonic stem-cells			*Mettl3*^{cKO}	Implantation failure	(Zheng *et al.*, 2023)
Porcine induced pluripotent stem cells (piPSCs)		*Sus scrofa*	*Mettl3* KD	(↕)self-renewal, (↑)lineage differentiation, (↕)JAK2 and SOCS3 protein expression, (↓)KLF4 and SOX2	(Wu *et al.*, 2019)
Human embryonic stem-cells (hESCs)		*Homo sapiens*	*Mettl3* KO	(↓)stem cell lineage differentiation	(Batista *et al.*, 2014)
Human endometrial stromal cells			*Mettl3* KD	Decidualization failure	(Zheng *et al.*, 2023)
Human ovarian granulosa cells (KGN)	METTL16		(↑)METTL16	Ovarian ageing (OA)	(Hu *et al.*, 2025)
	READERS				
GV and MII stage oocytes	YTHDF2	*Mus musculus*	*Ythdf2*^{mCKO}	(↓)oocyte competence and early zygotic development	(Ivanova *et al.*, 2017)
GV and MII stage oocytes	YTHDC1		*Ythdc1*$^{fl/-}$ *Ddx4-Cre* (cKO)	Oocyte maturation arrest, sterility	(Kasowitz *et al.*, 2018)
Mouse embryonic stem cells			*Ythdc1*-KO	Cellular reprogramming to a 2C-like state	(Liu *et al.*, 2021)
Human foetal ovary	YTHDC2	*Homo sapiens*	Variants c. 2567C>G, p. P856R	Primary ovarian insufficiency (POI)	(McGlacken-Byrne *et al.*, 2022)

(continued)

Table 1. Continued

Reproductive cell	Enzyme/ epitranscriptomic mark	Species	Alteration	Phenotype	Reference
	ERASERS				
GV oocytes	ALKBH5	*Mus musculus*	*Alkbh5* KO	Oocyte maturation arrest, sterility	(Bai *et al.*, 2023)
Mouse embryonic stem cells	FTO		*FTO* KO	(↕)chromatin state and gene expression	(Wei *et al.*, 2022)
Human ovarian granulosa cells	FTO	*Homo sapiens*	*FTO* KD	Ovarian ageing (OA)	(Hu *et al.*, 2025)
Human ovarian granulosa cells	ALKBH5, FTO		(↑)m^6A	(↓)*FTO*	(Ding *et al.*, 2018)
Chorionic villi	FTO		(↓)*FTO*	Pregnancy loss, (↕)immune tolerance, (↕) maternal-foetal angiogenesis	(Qiu *et al.*, 2021)

GV, germinal vesicle; KD, knockdown; ZGA, zygotic genome activation; cKO, conditional knockout; mCKO, maternal conditional deletion; MII, metaphase II; KO, knockout; KOMP2, Knockout Mouse Phenotyping Program; ↑, upregulation/increase; ↓, downregulation/decrease; ↕, dysregulation.

ARTs, metabolomic profiling of follicular fluid revealed significantly decreased N6-methyladenosine levels compared to controls (Li et al., 2023b). Furthermore, in the case-controlled study (Ding et al., 2018a), the level of m^{6}A was significantly higher (170%) in the POI patients compared to the control group (tubal occlusion).

Additionally, the mRNA level of *FTO* was significantly lower than that of *ALKBH5* (55%), but the protein expression of FTO was only decreased by 52% in the POI group compared to ALKBH5 protein expression (Ding et al., 2018).

All these observations collectively underscore the importance of a precise and specifically balanced RNA modification, the N6-methyladenosine, for proper oocyte development and reproductive potential in different mammalian species, including humans (Table 1).

Paternal RNA modifications and male fertility

In mammalian spermatogenesis and male fertility, m^{6}A plays a pivotal regulatory role. Deletion of *Mettl3* or *Mettl4* in mouse germ cells eliminates m^{6}A marks and depletes spermatogonia stem cells due to dysregulation of transcripts essential for stem cell fate (Lin et al., 2017; Xu et al., 2017). Similarly, *Wtap* deletion in murine Sertoli cells impairs spermatogenesis (Jia et al., 2020). Furthermore, disruption of m^{6}A readers YTHDC2, YTHDF2 (Bailey et al., 2017; Hsu et al., 2017; Qi et al., 2022; Bailey and Fuller, 2024; Tian et al., 2024), and PRRC2A (Tan et al., 2023) compromises spermatogenesis and sperm function, leading to infertility in mice, where YTHDF2 facilitates clearance of m^{6}A-modified transcripts (e.g. metallopeptidase), promoting spermatogonial adhesion and proliferation (Huang et al., 2020) (Table 2).

Erasers such as ALKBH5 also influence sperm competence in mice (Zheng et al., 2013; Tang et al., 2018). Deletion of *Ythdc1* results in complete germ cell loss and a Sertoli-cell-only phenotype (Kasowitz et al., 2018).

Another modification, ac^{4}C, catalysed by *N*-acetyltransferase 10 (NAT10), contributes to spermatogonia differentiation. In mice, *Nat10* deletion disrupts meiotic entry, chromosome synapsis, recombination, and DNA repair during meiosis (Chen et al., 2022) (Table 2).

An increased m^{6}A content has been identified as a risk factor for human asthenozoospermia as well. METTL3 play key roles in increasing m^{6}A contents in sperm RNA, and this is negatively correlated with sperm motility and positively correlated with sperm immobility (Yang et al., 2016).

Meanwhile, the human m^{6}A reader YTHDC2 (Tian et al., 2024) and the eraser FTO (Landfors et al., 2016) alter spermatogenesis and sperm competence.

All this evidence highlights the pivotal role of N6-methyladenosine and its enzymatic machinery components in mammalian spermatogenesis and male fertility, with other epitranscriptomic biomarkers such as ac^{4}C contributing to a lesser degree (Table 2).

Regulation of early embryonic development

In mammalian cells, N6-methyladenosine RNA modification is the most common modification of messenger RNA. Its regulatory pathways have been shown to regulate embryonic stem cell (ESC) differentiation in mice by modifying the dosage of key mRNA by the reader proteins YTHDF2 (Ivanova et al., 2017) and/or YTHDC1 or transposable element RNAs (Liu et al., 2021), and the methyltransferase METTL3 (Batista et al., 2014; Xu et al., 2021), which also regulate developmental pausing in mouse blastocysts and ESCs (Collignon et al., 2023) (Table 1).

The mouse model has helped elucidate the fundamental functions of m^{6}A in early embryogenesis. Its high genetic and functional similarity to humans allows it to replicate many epitranscriptomic changes observed in human tissues. Despite this, species-specific differences exist; for example, recent studies have reported differences in m^{6}A patterns between mouse and human around the timing of EGA (Li et al., 2025b). In mice, during the early stages of stem cell differentiation, m^{6}A leads the timely downregulation and reduction of stemness and pluripotency factors, while also promoting the upregulation of differentiation genes (Geula et al., 2015). Another example is given by the writer *Mettl3*; the progesterone receptor (*Pgr*) promoter-mediated deletion of the *Mettl3* leads to complete infertility in the mouse, due to a failure in the embryo implantation and decidualization (Zheng et al., 2023). The main cause is related to a decreased *Pgr* expression, since METTL3-mediated m^{6}A modification in the 5′-UTR of *Pgr* mRNA is essential for its efficient translation (Zheng et al., 2023). The global number of m^{6}A-marked genes decreases from the GV stage to the zygote, when murine embryo genome activation takes place, and a dramatic shift in m^{6}A status is observed between the zygote and two-cell embryo stages, with ~50% of genes (2356/4579) gaining m^{6}A and 45% of genes (2084/4579) losing m^{6}A (Wang et al., 2023). m^{6}A modifications can be recognized by the reader protein YTHDF2 to mediate degradation of different types of methylated RNA, including the ones that are subject to the EGA (Wang et al., 2014).

Also, the eraser FTO has an important role in early murine embryonic development. This latter mediates m^{6}A demethylation of long-interspersed element-1 (LINE1) RNA in mouse embryonic stem cells (mESCs), regulating LINE1 RNA abundance and the local chromatin state, carrying out regulatory roles in gene expression during both mouse oocyte and embryonic development (Wei et al., 2022) (Table 1).

In the swine species, the YTHDF1/YTHDF2 coordinated activity is involved in METTL3-m^{6}A-mediated maintenance of the pluripotent state in porcine-induced pluripotent stem cells, by elevating JAK2 levels and reducing SOSC3 expression (Wu et al., 2019) (Table 1) (Fig. 2).

Again, all these observations show that m^{6}A epitranscriptomic mark is an essential element in mammalian early embryonic development.

X-chromosome inactivation

X-chromosome inactivation (XCI) is a fundamental epigenetic mechanism in mammals that ensures dosage compensation between XX females and XY males, as well as playing a crucial role in the initiation and maintenance of XCI through epitranscriptomic regulation, particularly via N6-methyladenosine. At the core of this mechanism lies the long non-coding RNA XIST, which coats the future inactive X chromosome and recruits the machinery that silences it. The site-specific deposition of m^{6}A on XIST is mediated by the METTL3/METTL14 methyltransferase complex and directed by the RNA-binding proteins (RNABPs) RBM15 and RBM15B, with the participation of WTAP. YTHDC1 acts as the key nuclear m^{6}A reader required for XIST-mediated X-chromosome silencing. YTHDC1 preferentially binds to m^{6}A residues on XIST, and this recognition is essential for XIST to effectively repress transcription during X-inactivation. Depletion of YTHDC1 or disruption of m^{6}A on XIST impairs transcriptional repression, whereas artificial tethering of YTHDC1 restores silencing capacity. This highlights the vital role of the m^{6}A–YTHDC1 interface in XCI-mediated transcriptional silencing (Patil et al., 2016). However, a more recent study suggests that, although m^{6}A modification and its associated enzymatic machinery play a role in

Table 2. Effect of m^6A enzymatic machinery alterations on male fertility in humans and other mammals.

Reproductive cell	Enzyme	Species	Alteration	Phenotype	Reference
	WRITERS				
Undifferentiated spermatogonium	METTL3, METTL14	*Mus musculus*	*Mettl3* cKO, *Mettl14* cKO (Stra8)	(↕)spermatogonia differentiation	(Lin *et al.*, 2017; Xu *et al.*, 2017)
Spermatozoa	METTL3, METTL14	*Homo sapiens*	(↑)m^6A content (↑)*METTL3*, *METTL14*	Asthenozoospermia, (↓)sperm concentration	(Yang *et al.*, 2016)
Sertoli cells	WTAP	*Mus musculus*	*Wtap* cKO	(↕)spermatogonia stem cell maintenance, (↓) sperm cell concentration, sterility	(Jia *et al.*, 2020)
	READERS				
Spermatogonium cell	YTHDC1	*Mus musculus*	*Ythdc1* KO (DDX4)	Absence of germ cells (Sertoli-cell-only phenotype)	(Kasowitz *et al.*, 2018)
Spermatocyte cell	YTHDC2		*Ythdc2* KD	Smaller testes, (↓)cell viability, (↕) impaired maturation	(Hsu *et al.*, 2017)
	YTHDC2		*Ythdc2* KD	Lack of mature spermatozoa in the epididymis, (↕)mitosis-to-meiosis transition of germ cells	(Bailey *et al.*, 2017)
	YTHDC2		*Ythdc2* cKO	(↕)meiotic progression	(Bailey and Fuller, 2024)
Spermatocyte cell	YTHDC2	*Homo sapiens*	Homozygous missense variant (c.962G>C, p. Arg321Thr) *YTHDC2*	Non-obstructive azoospermia (NOA)	(Tian *et al.*, 2024)
Spermatogonium cell	YTHDF2	*Mus musculus*	*Ythdf2* KD	(↓)cell cycle, (↓)cell proliferation, (↕)cell adhesion	(Huang *et al.*, 2020)
Metaphase-stage spermatocytes cell	PRRC2A		*Prrc2a* KO	XY asynapsis, spindle disruption, arrest at metaphase I, chromosomal disorders (impaired sex chromosome inactivation)	(Tan *et al.*, 2023)
	ERASERS				
Metaphase-stage spermatocytes, spermatocyte, and round spermatid cells	ALKBH5	*Mus musculus*	*Alkbh5* KO	(↕) spermatogenesis	(Zheng *et al.*, 2013; Tang *et al.*, 2018)
Spermatozoa	FTO	*Homo sapiens*	FTO infertility-associated variant, two missense mutations	Infertility	(Landfors *et al.*, 2016)

KO, knockout; cKO, conditional knockout; KD, knockdown; Stra8, stimulated by retinoic acid 8; DDX4, DEAD-box helicase 4; ↑, upregulation/increase; ↓, downregulation/decrease; ↕, dysregulation.

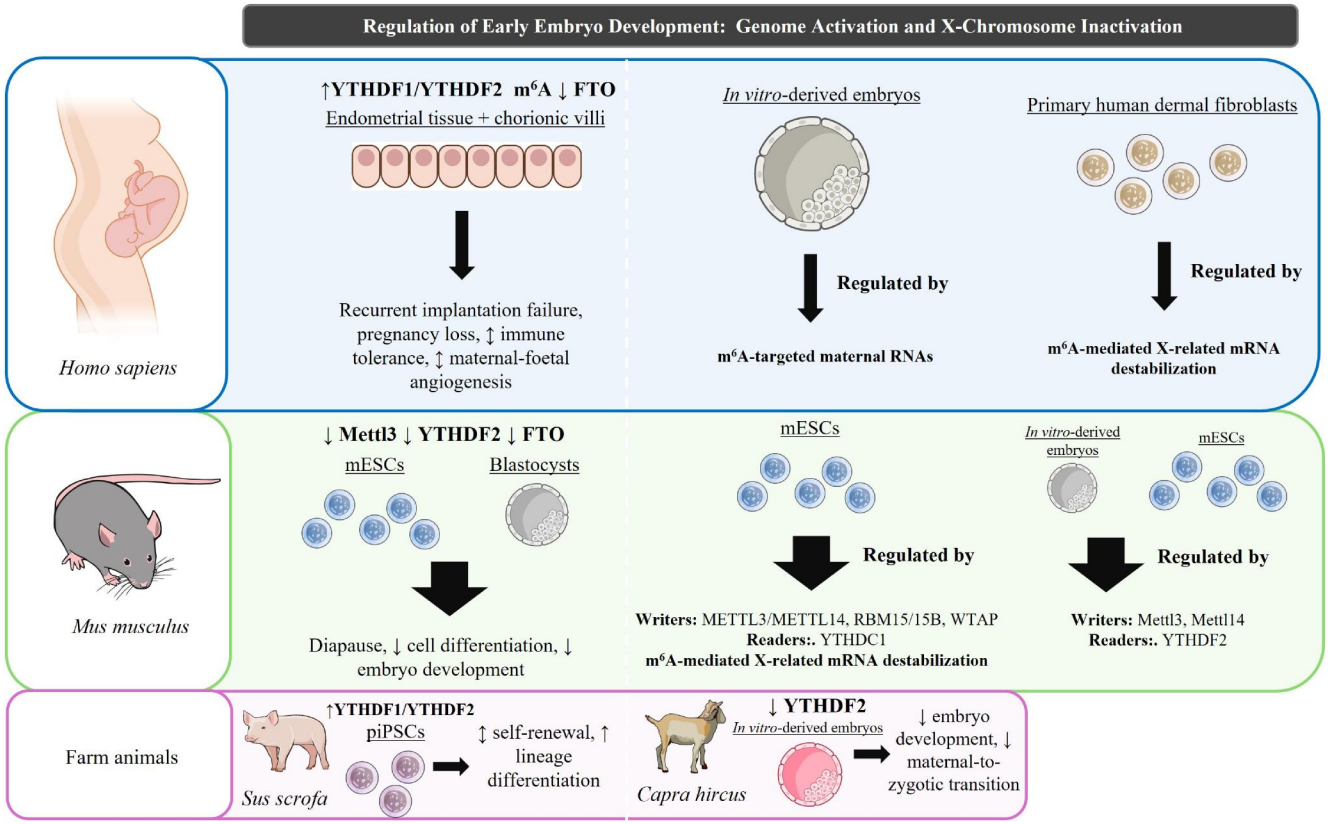


Figure 2. Roles of m^{6}A enzymatic machinery components in early embryo development, embryonic genome activation, and X-chromosome inactivation. mESCs, mouse embryonic stem cells; iPSCs, porcine induced pluripotent stem cells. Illustrations from NIAID NIH BioArt. Source (https://bioart.niaid.nih.gov/).

XIST-mediated silencing, they are auxiliary rather than core factors in this process. The dominant drivers of XIST function include SPEN, PCGF3/5-PRC1, and other key chromatin regulators that mediate the majority of gene repression during XCI (Nesterova *et al.*, 2019) (Fig. 2).

Additionally, m^{6}A extends beyond the XIST lncRNA and the initiation phase of XCI. X-chromosomal mRNAs harbour significantly fewer m^{6}A modifications than their autosomal counterparts due to the depletion of m^{6}A consensus motifs (GGACH). This results in higher mRNA stability for X-chromosomal transcripts compared to autosomal ones. Functionally, the acute depletion of m^{6}A leads to the selective stabilization of autosomal transcripts, thereby disturbing the balance of gene dosage between the X chromosome and the autosomes. These observations have been validated across multiple murine and human cell lines and tissues, revealing that m^{6}A-mediated mRNA destabilization is a conserved mechanism in X-to-autosome dosage compensation in mammals (Rücklé *et al.*, 2023) (Fig. 2).

Embryonic genome activation

Maternal factors represented by the oocyte cytoplasm components originally regulate the initial embryo development, whereas the embryonic nuclear genome is quiescent. Later, the EGA occurs, during which the embryo begins to transcribe its own genome. EGA is a key component of a broader process called the MZT. The MZT encompasses not only the onset of embryonic transcription but also the systematic degradation of maternal RNAs and proteins. EGA is initiated by maternal mRNAs and proteins that are present during the period of embryonic genome quiescence after fertilization, followed by a gradual switch to the activation of the embryonic genome, accompanied by the clearance of maternal RNAs and proteins (e.g. the enzyme RNA polymerase II) in vertebrates, with a species-specific delay after fertilization (Jukam *et al.*, 2017).

Loss of *Mettl3* in murine oocytes impairs maternal mRNA clearance, disrupting MZT and zygotic genome activation (Sui *et al.*, 2020; Wu *et al.*, 2022). EGA is tightly controlled by epigenetic mechanisms, including global DNA demethylation, re-establishment of histone modifications, and chromatin remodelling. In addition, epitranscriptomic modifications such as N6-methyladenosine have emerged as additional regulators of mRNA splicing, translation, and stability during EGA. m^{6}A marks display dynamic deposition and removal across transcripts in fertilized oocytes and early embryos. In mice, maternal RNA clearance depends on m^{6}A levels and recognition by YTHDF2. Depletion of *Mettl3*, *Mettl14*, or *Ythdf2* leads to persistent maternal RNA, EGA failure, and developmental arrest at the two-cell stage (Wu *et al.*, 2022). YTHDF2 deletion in mice results in reduced developmental competence post-fertilization, highlighting its essential role during oocyte maturation in selectively degrading m^{6}A-modified transcripts to ensure proper transcriptome remodelling (Ivanova *et al.*, 2017). Similar roles for YTHDF2 have been reported in goats, with conserved m^{6}A motifs supporting a shared mechanism across mammals (Deng *et al.*, 2020) (Fig. 2).

In humans, m^{6}A function in early development remains less defined due to ethical constraints and limited availability of early-stage human embryos. Nevertheless, a recent single-cell picogram-scale m^{6}A RNA IP and sequencing (picoMeRIP-seq) study

characterized m^{6}A distribution during the human oocyte-to-embryo transition (Li *et al.*, 2025b). Both the number and percentage of m^{6}A+ genes decreased until fertilization, and then increased from the zygote to the blastocyst stage. Most of the m^{6}A-marked genes were protein-coding genes (ranging from 82 to 92%), whereas a small percentage corresponded to lncRNAs (5–12%), pseudogenes (2–6%), and other types of RNA species (<1%). Additionally, the m^{6}A peaks were significantly enriched near the stop codons, and exhibited the consensus motif GGACU across all early embryo stages (Li *et al.*, 2025b).

Although suboptimal embryos were used, limiting generalizability, cross-species comparisons with mouse data revealed both conserved regulatory mechanisms and key differences during EGA. Notably, only 7% of co-expressed genes during major EGA exhibited m^{6}A modification exclusively in human eight-cell embryos, compared to 36% that were uniquely modified in mouse two-cell embryos (Li *et al.*, 2025b). Human-specific genes displayed reduced m^{6}A occupancy (35%) at the eight-cell stage, while mouse-specific genes retained higher m^{6}A levels (>54%), peaking at 65% during the two-cell stage.

m^{6}A+ genes specifically expressed in humans or mice were significantly longer than unmodified genes across all early embryo stages. Interestingly, human m^{6}A+ homologous genes were significantly shorter than mouse m^{6}A+ homologous genes at the two-cell and eight-cell stages, respectively. This difference could be attributed to non-equivalent development timing between the mouse two-cell stage and the human eight-cell stage (Li *et al.*, 2025b). Gene ontology analysis revealed that different groups of genes exhibited distinct functions: at the one-cell and eight-cell stages, m^{6}A+ genes were predominantly associated with transcriptional regulation, whereas unmodified genes were primarily involved in basic metabolic processes. At the blastocyst stage, human-specific m^{6}A+ genes were found to contribute to cell differentiation (Li *et al.*, 2025b). Across all stages, m^{6}A+ genes were largely associated with transcription and embryonic development, as well as development-related signalling pathways, while unmodified genes were primarily involved in various metabolic pathways in human–mouse co-expressed genes.

Differences extended to retrotransposon regulation: humans preferentially targeted young subfamilies (HERVH, L1HS, and SVA), while mice focused on LINE-1 and MERVL elements, each with distinct positional preferences near long terminal repeats (Li *et al.*, 2025b). m^{6}A also interacts with miRNA in species-specific patterns, with co-occupancy on zygotic-decay genes in both species but divergent associations with maternal-decay and EGA genes. Preferential m^{6}A marking of maternal transcripts destined for degradation contrasts with lower modifications in transcripts with early embryonic function. Loss-of-function studies show that disrupting m^{6}A writers delays maternal mRNA clearance and EGA, with these findings being consistent across other mammals such as mice (Wu *et al.*, 2022) and goats (Deng *et al.*, 2020) (Fig. 2).

These results emphasize that m^{6}A-mediated post-transcriptional molecular and cellular regulation is a critical, evolutionarily conserved mechanism for the productive entry into embryogenesis in humans.

ART-associated stressors and epitranscriptomic modifications

Potential mechanisms linking ART-associated stressors to epitranscriptomic changes

ARTs are extensively utilized in both human reproductive medicine and animal husbandry. However, the competence of ART-derived embryos is lower than that of NC embryos, and this is thought to be due to the accumulation of environmental stressors during *in vitro* embryo culture (Gardner and Kelley, 2017). Nevertheless, various stressors and techniques inherent to ARTs (e.g. ovarian stimulation, the presence of reactive oxygen species, IVC under 20% oxygen, oocyte IVM, suboptimal culture media, IVF and ICSI, *in vitro* embryo culture, trophectoderm biopsy, or cryopreservation) modify the transcriptome and epigenome. Although the epigenetic consequences of ARTs have been thoroughly investigated, limited research has explored the impact of ART-related stressors on more recently identified epitranscriptomic modifications. The importance of RNA modifications, particularly m^{6}A, in regulating gene expression in cellular processes (such as the heat shock response, the DNA damage response, and developmental processes) is increasingly recognized (Meyer *et al.*, 2012; Zhou *et al.*, 2015; Xiang *et al.*, 2017; Zheng *et al.*, 2023). Emerging scientific evidence is revealing a critical relationship between cellular stress conditions, such as oxidative stress, hypoxia, thermal stress, and specific modifications in germ cells or embryo epitranscriptome.

In murine species, recent evidence suggests that m^{1}A and m^{5}C tRNA modifications are hypoxia-sensitive (He *et al.*, 2022). tRNA modifications are abundant and environmentally responsive, with alterations linked to impaired spermatogenesis and male reproductive health. Specifically, alterations in tRNA modifications like m^{1}A and m^{5}C destabilize RNAs and compromise mitochondrial function, impairing cellular energy production in mouse spermatids (He *et al.*, 2022). Additionally, the epitranscriptomic writer ALKBH8 has a critical role in regulating ROS levels, DNA integrity, and selenoproteins expression. These latter functions are implicated in stress responses, embryonic viability, development, and offspring health (Shrimali *et al.*, 2007; Anouar *et al.*, 2018). Reduced expression in *Alkbh8*-deficient mouse embryonic fibroblasts entailed elevated intracellular ROS levels, increased DNA damage, impaired proliferation, accelerated senescence, and an inability to upregulate selenoprotein levels in response to hydrogen peroxide (Endres *et al.*, 2015; Lee *et al.*, 2020).

On the other hand, in bovines, it has been observed that METTL7A improves IVF embryo competence by attenuating oxidative stress. *METTL7A* overexpression resulted in an increase in the blastocyst formation rate, the number of trophectoderm cells (~50% increase), and the TE/ICM ratio (Zhu *et al.*, 2025a). Also, low mitochondrial stress and superoxide levels were observed in bovine pre-implantation embryos after exogenous *METTL7A* overexpression (Zhu *et al.*, 2025a).

In humans, during IVF, the alteration of many exogenous factors may affect human embryo development, such as physical conditions like oxygen tension (Simon and Keith, 2008; Krock *et al.*, 2011) and temperature (Mirmonsef *et al.*, 2016), but also chemical components like pH (Aroyo *et al.*, 2007; Wang *et al.*, 2009). m^{6}A has been identified as one of the epitranscriptomic marks evoked by stress, and a close relationship has been proposed between this RNA modification and oxidative stress. p21 can induce oxidative stress and regulate cellular senescence through m^{1}A, m^{6}A, and m^{5}C RNA modifications. In humans, oxidative stress can alter m^{6}A levels, thereby affecting mRNA translation (Li *et al.*, 2017; Anders *et al.*, 2018).

All these mechanisms hold significant relevance for ARTs, where germ cells and embryos are frequently exposed to non-physiological conditions that may induce analogous stress responses, potentially undermining gamete quality and developmental competence.

Evidence of epitranscriptomic modifications in ARTs

Epitranscriptomic modifications provide a fresh perspective on the molecular basis of gametes and embryos in ART settings, with mounting evidence suggesting that many of these modifications respond dynamically to external stimuli.

Cryopreservation and vitrification are techniques commonly employed in ART and can induce osmotic shock, ice crystal formation, and oxidative stress. During cryopreservation, sperm are exposed to osmotic stress resulting from hypertonic media or osmotic changes during freezing. This can potentially induce apoptotic-like changes, affecting sperm survival and function (Hungerford *et al.*, 2023; Simonik *et al.*, 2025). In boars, cryopreservation dysregulated m^{6}A writers (METTL3 and METTL14), erasers (ALKBH5 and FTO), and readers (YTHDF2), altering mRNA methylation patterns (Qin *et al.*, 2021). Highly methylated mRNAs were enriched in metabolic pathways (e.g. *ATP5F1*, *LDHA*), and these changes correlated with reduced sperm motility and increased apoptosis (Qin *et al.*, 2021) (Table 3).

Vitrification was found to have a long-term effect on mouse embryos, reducing m^{6}A modification in blastocysts in both the cytoplasm of the inner cell mass and the trophectoderm (Chen *et al.*, 2025). At the same time, the mRNA levels of *Mettl3* and *Mettl14* were found to be downregulated, while the mRNA levels of *Ytdhc1* were found to be increased (Chen *et al.*, 2025) (Table 3).

In humans, both m^{6}A content and METTL3 expression were found to be significantly higher in asthenospermic patients, suggesting that m^{6}A dysregulation in human sperm can substantially impair motility. The enzymes responsible for the m^{6}A modification (METTL3, METTL14, FTO, ALKBH5, and YTHDF2) have been shown to influence the level of m^{6}A in sperm RNA (Yang *et al.*, 2016). A recent analysis of the whole transcriptome of human cryopreserved sperm (normospermic donor) revealed that the transcript of the eraser ALKBH5 was more abundant in cryopreserved sperm in comparison with non-cryopreserved sperm (Stigliani *et al.*, 2024).

In relation to the enzymatic m^{6}A molecular machinery, after oestradiol (E2) exposure in ART, elevated levels of the transcripts *YTHDC1* (due to the transcriptional regulation of *RXRA* and *RPL37*), *EIF3A*, *IGF2BP3*, and *PRRC2A* were detected in human preterm placentas, while a downregulation of *ALKBH5* was observed. Regarding the protein expression level, the authors found an upregulation of both YTHDC1, but also of RXRA and GTF2I in the same tissue. To investigate the molecular mechanism driving YTHDC1 expression in trophoblastic cells, the authors evaluated the possible transcription factors for YTHDC1 in the trophoblastic cellular lines HTR-8/SVneo and choriocarcinoma JAR cell line. It was shown that the protein factors YTHDC1, RXRA, and RPL37 were overexpressed.

In murine preterm placentas, oestradiol exposure (Li *et al.*, 2024a) enhances YTHDC1 expression via the transcriptional factor RXRA, promoting the translation of *RPL37* in an m^{6}A-dependent way, affecting indirectly the expression levels of specific proteins in the WNT and JAK/STAT signalling pathways (Li *et al.*, 2024a).

The above-mentioned evidence underlines that the effects of ART-stressors on epitranscriptomic patterns in sperm and embryos of the major mammalian species have been studied, but there is a knowledge gap about the effects of ARTs on oocyte epitranscriptomic changes.

All the epitranscriptomic marks affected by ART-associated stressors in the above-mentioned studied mammalian species are indicated in the Table 3.

Biomarkers of epitranscriptomic changes

Identification of RNA modifications as potential indicators of embryonic health

In ART, identifying reliable biomarkers of embryonic health is central to improving embryo selection and clinical outcomes. Current indicators fall into three main categories: molecular, genetic, and non-invasive biomarkers. PGT detects chromosomal abnormalities and single-gene disorders, though with limitations for broader use (Yan *et al.*, 2021). Metabolic and proteomic profiling also show promise; proteomic signatures correlate with early developmental competence, providing insights into embryo quality at the early stages of preimplantation development (Zhu *et al.*, 2025b). Non-invasive approaches, like analysing proteins and metabolites secreted into the culture medium or from cumulus cells, are emerging (Zhang *et al.*, 2019; Martínez-Moro *et al.*, 2023). Moreover, recent research highlights the value of epigenetic and epitranscriptomic biomarkers, such as DNA methylation, histone modifications, and RNA methylation (e.g. m^{6}A), as sensitive modulators that reflect an embryo's response to environmental stressors and *in vitro* conditions during ART (Liu *et al.*, 2021, 2022).

Most of the epitranscriptomic indicators of embryonic health have been identified in laboratory rodents. A recent study in mice reported asymmetries in m^{6}A in two-cell embryos (Yao *et al.*, 2023), which would lead to an unequal developmental potential of the two blastomeres of the embryos (Wang *et al.*, 2018; Junyent *et al.*, 2024). In murine blastocysts, the m^{6}A writer METTL3 has been shown to play a critical role in regulating naïve pluripotency because Mettl3 knockout naïve mESCs exhibit a high level of methylation for pluripotency regulators, including *Nanog*, *Klf2*, and *Esrrb* (but not *Oct4*), giving the methylated transcripts a longer stability (Geula *et al.*, 2015). The absence of METTL16 in 16-cell murine embryos decreases the transcription of its target MAT2A, which encodes an S-adenosylmethionine synthetase. This leads to transcriptome-wide disruption in the 64-cell embryo stage, resulting in developmental arrest around the time of implantation (Mendel *et al.*, 2018). METTL14 is also critical for early embryonic development and is indispensable for proper post-implantation development. Deficiency of METTL14 leads to delayed development from embryonic Day 6.5 onwards, resulting in embryonic death due to obstructed ectodermal differentiation and impaired maturation processes essential for embryogenesis (Meng *et al.*, 2019). Depletion of the m^{6}A reader protein YTHDF2 in mice also causes lethality at late embryonic developmental stages, with embryos exhibiting compromised neural development. This affects the self-renewal and spatiotemporal generation of neural stem/progenitor cells (Li *et al.*, 2018). Conversely, the m^{6}A-YTHDF2-mediated decay of *Notch1* mRNA is essential for generating the earliest haematopoietic stem/progenitor cells during the endothelial-to-haematopoietic transition in mouse embryos (Zhang *et al.*, 2017). However, depleting the m^{6}A reader YTHDF3 in mESCs results in a loss of pluripotency, with accelerated expression of marker genes involved in the formation of the three germ layers. Furthermore, the loss of YTHDF1 leads to a dramatic impairment in the differentiation of cardiomyocytes (Wang *et al.*, 2021a).

In humans, the downregulation of m^{6}A eraser FTO in pregnancy loss at the level of the chorionic villi leads to the disruption of the immune tolerance and angiogenesis at the maternal–foetal interface in healthy women of an average age of 33 years (Qiu *et al.*, 2021). Alternatively, m^{6}A modifications play a critical role in clinical phenomena like recurrent implantation failure (RIF). In a recent study (Wang *et al.*, 2024), m^{6}A marks on long non-coding RNA in endometrial tissue from patients with RIF

Table 3. Reproductive cells/tissue affected by epitranscriptomic alteration according to ART-stressors, the molecular component involved, and mammal species.

ART-stressor	Epitranscriptomic mark	Species	Reproductive cell	Molecular alteration	Reference
Oxidative stress	N6-methyladenosine (m^6A)	*Homo sapiens*	/	mRNA translation	(Li *et al.*, 2017; Anders *et al.*, 2018)
	5-methylcytosine (m^5C)		/	mRNA translation	(Li *et al.*, 2017)
		Mus musculus	Spermatid cells	RNA stability, mitochondrial function, bioenergetic	(He *et al.*, 2022)
	N1-methyladenosine (m^1A)		Spermatid cells	RNA stability, mitochondrial function, bioenergy	(He *et al.*, 2022)
Vitrification	N6-methyladenosine		Blastocysts	(↓)*Mettl3*, *Mettl14*	(Chen *et al.*, 2025)
				(↑)Ytdhc1	
Cryopreservation	N6-methyladenosine	*Sus scrofa*	Sperm cells	(↓)METTL3, METTL14, ALKBH5, YTHDF2	(Qin *et al.*, 2021)
				(↑)FTO, ALKBH5	
		Homo sapiens		(↑)ALKBH5	(Stigliani *et al.*, 2024)
E2 exposure	N6-methyladenosine				
		Mus musculus (C57BL/6J mice)	Preterm placentas	(↑)YTHDC1	(Li *et al.*, 2024a)
		Homo sapiens	Placentas(ARTs-preterm)	(↑)*YTHDC1* (by transcriptional regulation of *RXRA* and *RPL37*), *EIF3A*, *IGF2BP3*, *PRRC2A*	
				(↑)YTHDC1, RXRA and GTF2I	
				(↓)*ALKBH5*	
			Trophoblastic (HTR-8/SVneo) and choriocarcinoma (JAR) cell lines	(↑)YTHDC1, RXRA and RPL37	

revealed that 1443 were significantly upregulated and 425 were significantly downregulated (Table 1). Functional enrichment analysis revealed that genes associated with differentially methylated lncRNAs were enriched in the p53 signalling pathway and in amino acid metabolism. lncRNAs are known to regulate both miRNAs and mRNAs, and the authors speculated that *LINC01152* may act as a molecular sponge for hsa-miR-6801-3p, thereby influencing LIF expression (Wang *et al.*, 2024). Considering that p53 is crucial for embryonic implantation through upregulation of uterine *LIF* transcription, m^{6}A might regulate the p53 signalling pathway and contribute to the pathogenic mechanism of RIF (Wang *et al.*, 2024). Therefore, aberrant m^{6}A methylation may disrupt the p53 signalling pathway, contributing to impaired LIF expression and the pathogenesis of RIF. Additionally, in humans, m^{6}A deficiency has been correlated to poor oocyte reservoirs and low blastocyst rates (Li *et al.*, 2023b), suggesting that it is a potential prognostic biomarker for ovarian response in IVF procedures and subsequently for the effect on embryonic health. While the clinical application of epitranscriptomic biomarkers is still in its early stages, advances in RNA modification mapping technologies (e.g. m^{6}A-seq or mass spectrometry) are rapidly improving our ability to profile these changes in low-input or single-cell samples, making their translation into ART settings increasingly feasible (Yao *et al.*, 2022; Zhang *et al.*, 2023).

All these data underline the importance of expression levels of m^{6}A RNA modification enzymatic components in keeping a proper embryo development.

Meanwhile, an *in silico* analysis comparing published single-embryo transcriptomic datasets from *in vivo* and *in vitro* conditions across three mammalian species (mouse, pig, and cow) identified differentially expressed genes involved in m^{6}A regulation (Heras *et al.*, 2016; Canovas *et al.*, 2017; Feuer *et al.*, 2017; Van Der Weijden *et al.*, 2021) (Fig. 3). Across all three species, there was a consistent trend towards the downregulation of several m^{6}A readers, mirroring the pattern observed for the m^{6}A writers. When differentially expressed, the latter were also predominantly downregulated. However, a subset of m^{6}A readers was found to be upregulated under *in vitro* conditions, suggesting that the m^{6}A machinery may be modulated in a species- or context-specific manner.

In bovine embryos, male blastocysts exhibited a greater number of m^{6}A-related transcriptomic alterations, consistent with their reported increased sensitivity to IVC conditions compared to females. This observation aligns with previous findings from our group and others, which documented more pronounced transcriptomic and epigenomic differences in male embryos under *in vitro* or suboptimal conditions. Interestingly, ontology enrichment analysis of differentially expressed genes in male bovine embryos using Metascape revealed that the top enriched term was 'R-HSA-8953854: Metabolism of RNA', comprising 121 out of 644 genes ($-\log_{10}$ *P*-value: 33.27). This highlights a possible link between RNA metabolism and sex-specific sensitivity to *in vitro* conditions.

Detection methods for epitranscriptomic biomarkers

As previously stated, m^{6}A epitranscriptomic modification represents the most prevalent internal modification of mRNA in mammals. Initially, the detection of m^{6}A was accomplished through biochemical methods, but the advancements in technology have led to the development of novel protocols that utilize sophisticated technologies to establish new standards for the detection

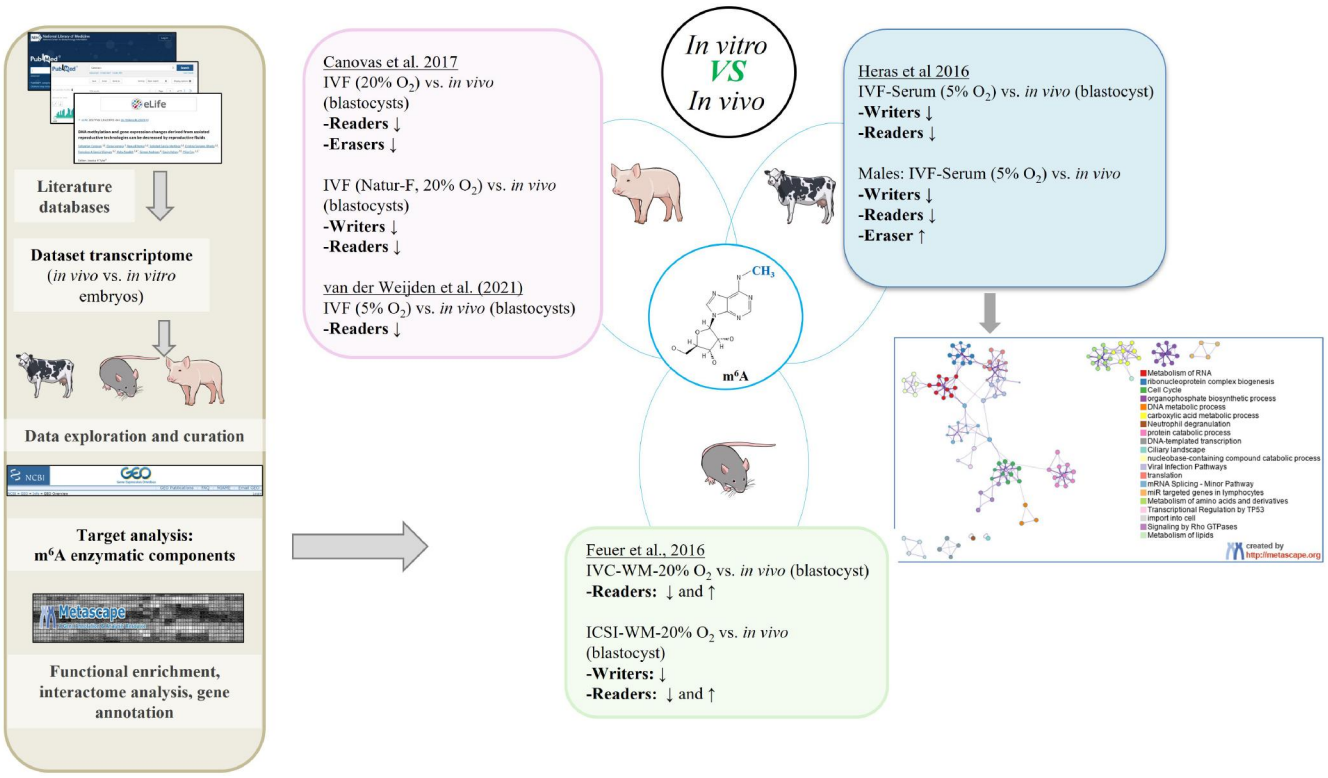


Figure 3. Comparative transcriptomic analysis of *in vivo* and *in vitro* embryos in mice, pigs, and cows, targeted to m^{6}A-related factors (writers, erasers, and readers), with functional enrichment and interactome networks identified through Metascape analysis. 2D structure image of CID 102175 (N6-Methyladenosine, URL: https://pubchem.ncbi.nlm.nih.gov/compound/102175). Illustrations from NIAID NIH BioArt Source (https://bioart.niaid.nih.gov/).

and quantification of m^{6}A in mRNA, as well as other chemical RNA modifications. The initial detection methods were employed in the field of microbiology and based on liquid chromatography (LC) (Gehrke and Kuo, 1989) and thin-layer chromatography (TLC) on cellulose plates (Grosjean *et al.*, 2007), but both procedures were time-consuming and not informative about RNA species or stoichiometry related to the modification. The mounting interest in this RNA modification has resulted in the development of more precise and sensitive qualitative and quantitative detection tools, including the ones used for mapping. Among these, there are mass spectrometry-based approaches paired to LC such as liquid chromatography-mass spectrometry (LC-MS) (Sarin *et al.*, 2018; Solivio *et al.*, 2018) and antibody-dependent methods (Table 4). Regarding the latter, there are several tools available for mapping the m^{6}A in the transcriptome, including crosslinking and immunoprecipitation (CLIP)-based methods (Ke *et al.*, 2015; Dziuba *et al.*, 2020; Linder *et al.*, 2015; Körtel *et al.*, 2021), as well as isoform-aware and low-input methods.

The antibody-based indirect detection tools use the next-generation sequencing to identify immunoprecipitated m^{6}A-mRNA fragments captured by highly specific anti-m^{6}A antibodies. The methylated sites are identified by peak calling, and the enriched sequence reads detect over input libraries with the power of massively parallel sequencing (m^{6}A-seq, m6A-seq2 and MeRIP-seq) (Dominissini *et al.*, 2012; Dierks *et al.*, 2021).

Furthermore, immunoprecipitation (IP) combined with the use of ultraviolet irradiation, which induces covalent crosslinks between RNA bases (particularly pyrimidines) with amino acid residues (for example, lysine, cysteine, and tyrosine), also allows the characterization of the RNABPs along with small fragments of RNA that are subsequently amplified and sequenced (CLIP-based methods).

On the other hand, the isoform-aware detection tool m^{6}A-level and isoform-characterization sequencing compares sequence reads from immunoprecipitated and methylation-free fractions, at a sub-saturated ratio of mRNA to anti-m^{6}A antibody (Molinie *et al.*, 2016; Yin *et al.*, 2022) (Table 4).

Conversely, the antibody-independent profiling methods encompass metabolic labelling methods (e.g. m6A-label-seq, DARTS-seq and scDARTS-seq) (Shu *et al.*, 2022; Meyer, 2019), profiling methods based on differential sensitivity of enzymes (e.g. TadA-assisted N6-methyladenosine sequencing—eTAM-seq, MAZTER-seq, m6A-REF-seq and eTAM-seq) (Garcia-Campos *et al.*, 2019), chemical labelling tools (GLORI) (Liu *et al.*, 2023; Shen *et al.*, 2024), locus-specific detection mapping platforms like site-specific cleavage, and radiolabelling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) (Liu *et al.*, 2013).

A third category of detection methods is represented by direct RNA sequencing, carried out on Oxford Nanopore platforms (Garalde *et al.*, 2018; Workman *et al.*, 2019). The ribonucleotide modification sites can be predicted directly from read-level electrical signals that are elaborated with software such as Nanopolish or Tombo (Ding *et al.*, 2020). At the moment, numerous software tools have been developed to predict RNA modifications based on nanopore sequencing data, such as MINES (Lorenz *et al.*, 2020), xPore (Pratanwanich *et al.*, 2021), DRUMMER (Price *et al.*, 2020), Penguin (Hassan *et al.*, 2021), and nanoDoc (Ueda, 2020) (Table 4).

The advantages and drawbacks of each m^{6}A detection technique have been widely discussed in a previous review (Moshitch-Moshkovitz *et al.*, 2024). Despite each detection tool for mapping and quantifying m^{6}A having its own advantages and disadvantages, the authors concluded that TadA-assisted N6-methyladenosine sequencing (eTAM-seq) (Xiao *et al.*, 2023) and picoMeRIP-seq (Li *et al.*, 2024b) are the most suitable methods for qualitative and quantitative analysis of m^{6}A in gametes and/or embryos in the correct sequence context.

eTAM-seq discriminates between unmethylated adenosine and m^{6}A via deamination and uses a variant of the tRNA deaminase, TadA8.20. It is highly sensitive, uses a small amount of biological sample, and provides an m^{6}A+ RNA mapping profile with single-nucleotide resolution and quantitative stoichiometric information for each site. However, performing an eTAM-seq requires the preparation of a negative control library (*in vitro* transcribed transcriptome) and a dedicated statistical model. Although it is a simple and cost-effective detection tool, it requires the expression and purification of the TadA8.20 enzyme, which is not commercially available.

On the other hand, picoMeRIP-seq, which was developed by adjusting the MeRIP-seq protocol, requires as little as 100 pg of input RNA, which is a quantity consistent with the RNA content of single cells. However, antibody cross-reactivity and low site detection specificity mean that the optimal antibody for each model organism and experimental condition must be empirically tested.

In general, the ideal approach would be easy to execute, not require specialized equipment, and rely on commercial or readily available reagents and components.

Advances in single-cell epitranscriptomic sequencing

Recent advances in single-cell RNA sequencing technologies have enabled the analysis of epitranscriptomic marks at single-cell resolution, most notably m^{6}A and A-to-I editing (review by Crespo-García *et al.*, 2024). However, a substantial challenge remains the need for highly sensitive and robust m^{6}A mapping methods that can be applied to primary cell types and precious samples such as human oocytes or early embryos. To date, only a limited number of techniques have been developed for this purpose, including epitranscriptome profiling (Kim *et al.*, 2021), m^{6}AISH-PLA (Ren *et al.*, 2021), scDART-seq (Tegowski *et al.*, 2022), scm^{6}A-seq (Yao *et al.*, 2023), and picoMeRIP-seq (Li *et al.*, 2024b).

Single-cell m^{6}A sequencing, such as scm^{6}A-seq, enables the simultaneous profiling of the m^{6}A methylome and the transcriptome in a single oocyte/blastomere from cleavage-stage embryos (Yao *et al.*, 2023). In comparison, picoMeRIP-seq stands out because it does not require the use of specialized instrumentation and demonstrates sufficient sensitivity to analyse single oocyte and embryos. This allows for the interrogation of the m^{6}A landscape in preimplantation embryos and human oocytes, facilitating the study of m^{6}A in relation to developmental defects and fertility (Li *et al.*, 2024b).

In the case of A-to-I editing, inosine residues, resulting from enzymatic deamination of adenosine, are recognized as guanosine after cDNA synthesis and PCR amplification. Thus, detection of these event traditionally relies on standard RNA-seq, followed by an A-to-G variant calling (review by Crespo-García *et al.*, 2024).

Notably, the application of scm^{6}A-seq allows for high-resolution mapping of m^{6}A at single-cell scale, providing valuable insights into the heterogeneity of epitranscriptomic regulation during early embryo development. More recently, methods allowing the simultaneous profiling of m^{6}A and gene expression at single-nucleus resolution have been introduced, enabling complex tissues and developmental contexts qualitative and quantitative epitranscriptomic analysis (Hamashima *et al.*, 2023).

Table 4. The most widespread detection methods for epitanscriptomic marks, according to the RNA modification and type of detection tool.

RNA modification	Detection principle		Method	References
N6-methyladenosine (m^6A)	Liquid chromatography (LC)		Liquid chromatography (LC)	(Gehrke and Kuo, 1989)
			Thin-layer chromatography on cellulose plates (TLC)	(Grosjean *et al.*, 2007)
	Mass spectrometry (MS)		Liquid chromatography-mass spectrometry (LC-MS)	(Sarin *et al.*, 2018; Solivio *et al.*, 2018)
	Antibody-dependent profiling methods	Transcriptome-wide mapping tools	m^6A-seq	(Dominissini *et al.*, 2012)
			m^6A-seq2	(Dierks *et al.*, 2021)
			MeRIP-seq	(Meyer *et al.*, 2012)
		Crosslinking and immunoprecipitation (CLIP) tools	m^6A-seq/MeRIP-seq	(Dziuba *et al.*, 2020)
			m^6A-CLIP/IP	(Ke *et al.*, 2015)
			miCLIP	(Linder *et al.*, 2015)
			miCLIP2 integrated with machine-learning model m^6Aboost	(Geula *et al.*, 2015; Körtel *et al.*, 2021)
		Isoform-aware and low input tools	m^6A-LAIC-seq	(Molinie *et al.*, 2016)
			Super-Low-Input M^6A sequencing (SLIM-seq)	(Yin *et al.*, 2022)
			picogram-scale M^6A RNA Immunoprecipitation and sequencing (picoMeRIP-seq)	(Li *et al.*, 2024b)
	Antibody-independent profiling methods	Metabolic labelling tools	m^6A-label-seq	(Shu *et al.*, 2022)
			DARTS-seq	(Meyer, 2019)
			single-cell DARTS-seq (scDARTS-seq)	(Tegowski *et al.*, 2022)
		Profiling methods based on differential sensitivity of enzymes	MAZTER-seq	(Garcia-Campos *et al.*, 2019)
			m^6A-REF-seq	(Chen *et al.*, 2022)
			TadA-assisted N6-methyladenosine sequencing (eTAM-seq)	(Xiao *et al.*, 2023)
		Chemical labelling tools	m^6A mapping by glyoxal and nitrite-mediated adenosine deamination (GLORI)	(Liu *et al.*, 2023; Shen *et al.*, 2024)
		Locus-specific detection mapping platforms	Site-specific cleavage and radiolabelling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET)	(Liu *et al.*, 2013)
	Direct RNA sequencing (DRS) with data extraction software	MINES		(Lorenz *et al.*, 2020)
		xPore		(Pratanwanich *et al.*, 2021)
		DRUMMER		(Price *et al.*, 2020)
Pseudouridine (Ψ or psi)		Penguin		(Hassan *et al.*, 2021)
Multiple RNA modifications		nanoDoc		(Ueda, 2020)

Integration of multi-omics approaches to correlate RNA modifications with developmental outcomes

Integrating multi-omics approaches has transformed our understanding of how molecular layers converge to regulate essential processes during embryonic development, such as organogenesis. Disruptions to this process can result in premature death and congenital anomalies, collectively known as developmental diseases or birth defects (Wen *et al.*, 2024).

Historical studies relied solely on genomics, but advances in technology have broadened the scope to include transcriptomics, epigenomics, proteomics, and metabolomics. This provides a more comprehensive understanding of the molecular mechanisms that drive development and the pathogenesis of congenital anomalies (Wagner *et al.*, 2023). Despite significant progress, direct multi-omics approaches that correlate epitranscriptomic changes with developmental outcomes are only just emerging, such as in the case of the male component (review by Podgrajsek *et al.*, 2024). However, recent studies have used single-cell multi-omics technologies, such as integrative single-cell transcriptomics paired with RNA modification profiling, to start mapping these relationships with unparalleled accuracy (Yao *et al.*, 2025). Notably, methods like C2T-seq enable the simultaneous analysis of the transcriptome, m^6A and cap modifications, poly(A) tail length, and gene expression in individual oocytes, providing valuable new resources for understanding early embryo development in both mouse and human (Yao *et al.*, 2025).

Single-cell transcriptomics remains the most advanced and broadly adopted approach, often integrated with functional genomics tools, such as the CRISPR/Cas9-based screening approaches, Perturb-seq, and CROP-seq (Datlinger *et al.*, 2017). The emergence of spatial multi-omics technologies allows the expansion of the mapping of interactions between single cells within intact tissues at a genome scale (Liu *et al.*, 2020b), preserving information about physical interactions within and between cells.

Implications for embryonic development and offspring health

Effect of epitranscriptomics changes on early embryogenesis, gene expression, and tissue differentiation

The epitranscriptomic signature has been demonstrated to play a critical role in the process of early embryogenesis, as well as in subsequent physiological processes. In the context of m^6A RNA modification, the role of *Mettl3* in female mice is of particular significance. *Mettl3* has been identified as a crucial factor in the efficient signalling of P4 during embryogenesis, as well as in cases of infertility resulting from embryo implantation failure and decidualization (Zheng *et al.*, 2023). In the murine model, PCR analysis revealed the presence of *Mettl14* knockout embryos until E6.5. These embryos exhibited growth retardation, aberrant morphology, and compromised post-implantation development of murine embryos. These defects observed likely result from an impaired naïve to primed epiblast (Meng *et al.*, 2019). The transition from the pluripotent naïve epiblast to a primed state is an essential event for mammalian embryo development after implantation (as can be measured by the presence of naïve and primed markers). Notably, the expression levels of numerous naïve markers (*Nr5a2*, *Klf2*, *Rex1*, and *Tfcp2l1*) in E5.5 *Mettl14*−/− mouse embryos were found to be elevated, while the primed markers (such as *Dnmt3b*, *Otx2*, and *Sox3*) exhibited a decrease (Meng *et al.*, 2019). In addition, NANOG was also expressed in a delimited region in the proximal posterior epiblast in normal E6.5 mouse embryos; conversely, the expression of the general pluripotency marker POU5F1 was not significantly altered (Meng *et al.*, 2019).

Conclusions and further directions

Summary of current knowledge

ARTs are essential for infertility treatment but influence the embryonic epitranscriptomic landscape, including the pivotal m^6A RNA modification that regulates RNA metabolism and developmental outcomes. Cross-species studies demonstrate that ART procedures perturb expression and activity of key m^6A machinery components, reshaping gene regulation during early embryo development. Current evidence indicates that m^6A machinery changes act as independent yet convergent contributors to ART outcomes, rather than reflecting broad gene expression alterations. ART-related stressors modulate m^6A writers, erasers, and readers via mechanisms such as post-translational modifications and RNA structural changes without global transcriptional impacts. For example, METTL3 phosphorylation by stress-activated kinases autonomously regulates its function (Sun *et al.*, 2020), and m^6A interaction with circular RNAs influences trophoblast development and pregnancy maintenance (Huang *et al.*, 2018; Cui *et al.*, 2023), reflecting a distinct regulatory layer of RNA metabolism responsive to ARTs conditions.

Animal models have elucidated fundamental roles of m^6A in gametogenesis, fertilization, and early embryogenesis, revealing conserved yet species-adapted epitranscriptomic dynamics. In mice, m^6A modulates critical processes such as follicle development, oocyte maturation, XCI, and MZT. In males, fertility strongly depends on m^6A-dependent regulation of spermatogonial stem cell fate and sperm function. Porcine studies complement this view by highlighting the role of m^6A in ooplasm transcript accumulation during meiosis and pluripotency maintenance in ESCs.

In humans, emerging evidence links m^6A dynamics to reproductive ageing and fertility: granulosa cells from older women exhibit enrichment of m^6A-methylated genes, while genetic variants in the m^6A reader YTHDC2 are associated with POI. Beyond ovarian cells, altered m^6A profiles in follicular fluid or sperm correlate with clinical ART outcomes, suggesting their potential as biomarkers of embryonic viability and fertility. Moreover, m^6A deficiency in humans is correlated with diminished oocyte reserve and lower blastocyst rate, indicating compromised oocyte quality, whereas elevated m^6A levels correlate with adverse reproductive outcomes such as miscarriage and RIF.

Collectively, these epitranscriptomic markers represent promising prognostic indicators for embryonic viability and may inform clinical decision-making in ARTs. However, comprehensive mechanistic studies and validation are necessary to fully realize their therapeutic and diagnostic potential.

Recap of the potential impacts of ART on epitranscriptomics

ART exposes germ cells and early embryos to diverse stressors, which can influence development. Epitranscriptomic marks are highly responsive to these environmental stimuli, dynamically modulating gene expression. For instance, non-physiological oxygen levels during IVF induce oxidative stress, a known modulator of embryonic development. The m^6A modification plays a central role in oxidative stress responses, partly through its interaction with p21, which also induces oxidative stress via m^5C RNA modification. In mice, hypoxia-sensitive tRNA modifications like m^1A and m^5C contribute to RNA stability and energy homeostasis during spermatid maturation in mice. Loss of the writer ALKBH8 worsens oxidative stress by impairing selenoprotein expression, highlighting epitranscriptomic control of redox homeostasis.

In bovine IVF embryos, *METTL7A* overexpression enhances development by mitigating oxidative and mitochondrial stress, improving blastocyst formation. Similarly, in mice, oestradiol increases expression of the m^{6}A reader YTHDC1 in preterm placentas, altering pathways essential for placental and embryonic development, such as WNT and JAK/STAT. Hormonal dysregulation, due to infertility or ART, may reshape the epitranscriptomic landscape and affect reproductive outcomes.

Cryopreservation and vitrification introduce additional stress through osmotic shifts, mechanical injury, and oxidative stress. In boar sperm, cryopreservation disrupts m^{6}A homeostasis, reducing sperm motility and viability via abnormal methylation of transcripts involved in energy metabolism (e.g. *ATP5F1* and *LDHA)* (Qin *et al.*, 2021). Vitrification also impacts murine embryos by modulating RNA m^{6}A levels at the blastocyst stage, suggesting lasting epitranscriptomic alterations.

In humans, m^{6}A dysregulation in sperm correlates with reduced motility, and regulatory enzymes adjust m^{6}A RNA content in response to cryopreservation (Stigliani *et al.*, 2024). Elevated YTHDC1 expression in both human and murine preterm placentas after ART with high E2 exposure highlights a conserved epitranscriptomic response across species (Li *et al.*, 2024a). In human ESCs, loss of the 2′-O-me at the 28S rRNA U3904 site promotes neuroectoderm differentiation via WNT pathway interaction with fragile X mental retardation protein, illustrating the role of RNA modifications in lineage specification.

Acknowledgment of gaps and limitations in the field

Despite emerging insights, the current literature remains limited by the scarcity of studies directly linking ART-induced epitranscriptomic alterations to human reproductive pathologies or long-term offspring health. Mechanistic insights into how these molecular changes drive disease processes are particularly scarce. The multifactorial interplay of genetic, environmental, and lifestyle factors further complicates causal inference. Studies confined to embryonic samples often neglect the dynamic *in vivo* context, limiting the predictive power of their findings. Key variables such as age, sex, geographic origin, and socioeconomic status are frequently underreported, despite their potential influence on epitranscriptomic profiles and developmental outcomes. Addressing these limitations demands well-designed, large-scale, longitudinal investigations integrating multi-omics approaches and rigorous control of confounding variables to advance personalized reproductive medicine.

Epidemiological data consistently show modest but significant associations between ART procedures and elevated risk of congenital cardiovascular, urogenital, and neurological anomalies in offspring (Zhang *et al.*, 2021; Lu *et al.*, 2022; Sargisian *et al.*, 2024; Wei *et al.*, 2024; Chen and Zhou, 2025). However, major mechanistic gaps persist regarding the epitranscriptomic pathways underlying these adverse outcomes. This is particularly relevant given the critical role of m^{6}A RNA methylation in processes such as cardiovascular formation, neurogenesis, synaptic plasticity, and reproductive system maturation, all of which are highly sensitive to oxidative stress and environmental perturbations typical of ART. For example, deficiency of the m^{6}A methyltransferase METTL3 has been linked to severe CHDs, including ventricular septal defects and pulmonary stenosis, through disruption of key cardiac transcription factor networks (Feng *et al.*, 2025). Nonetheless, direct evidence connecting ART-related stressors to epitranscriptomic alterations that drive embryonic malformations is still lacking. Elucidating these pathways could bridge epidemiological observations with molecular mechanisms, ultimately guiding the identification of novel biomarkers for embryo viability and potential therapeutic targets to mitigate ART-associated developmental risks.

Future research priorities

Several key research priorities warrant immediate attention to advance the field of epitranscriptomics in ARTs.

First, expanding our understanding of methylation-independent functions of m^{6}A machinery components is essential. Increasing evidence demonstrates that m^{6}A writers, erasers, and readers exert regulatory roles beyond canonical methylation, which may be crucial in reproductive cells and early embryos. For instance, the m^{6}A reader YTHDC1 has been shown to mitigate stress-induced senescence through m^{6}A-independent pathways (Zhang *et al.*, 2024), while YTHDF1 inhibits the mTOR complex 1 (mTORC1), a master regulator of metabolic stress and ageing, independently of methylation (Xu *et al.*, 2025). Similarly, the eraser FTO regulates WNT signalling bifurcation beyond its canonical demethylase activity (Kim *et al.*, 2022a). Elucidating these non-canonical mechanisms within reproductive contexts could uncover novel pathways influencing gamete and embryo health, thereby refining our understanding of ART-related epitranscriptomic effects.

Second, exploring therapeutic interventions targeting the RNA modification machinery offers promising avenues to mitigate ART-associated risks. Modulation of RNA modification enzymes has reversed disease phenotypes such as tumour growth using inhibitors targeting m^{6}A writers (METTL3), erasers (FTO), and readers (YTHDF2) (Dolbois *et al.*, 2021; Huff *et al.*, 2021; Liu *et al.*, 2021; Yankova *et al.*, 2021; Deng *et al.*, 2023). The E2/RXRA/YTHDC1/RPL37 signalling pathway identified in ART pregnancies (Li *et al.*, 2024a) is another potential target for preventing preterm birth. These advances herald the development of epitranscriptomic therapeutics tailored to improve ART outcomes. Additionally, RNA modifications that enhance RNA stability and translation efficiency, exemplified by pseudouridine (Ψ) and N1-methylpseudouridine (m^{1}Ψ) in COVID-19 mRNA vaccines (Wen *et al.*, 2024), highlight potential strategies for optimizing RNA-based reproductive interventions.

Finally, although epitranscriptomics in ART remains nascent and evidence limited, the foundational role of RNA modifications in gametogenesis, early development, and stress responses is clear. Bridging current knowledge gaps requires sustained, multidisciplinary research and transparent communication regarding the limitations and potentials of ART effects on the epitranscriptome. Clinical practice should prioritize informed consent and patient education on transcriptomic and epigenomic risk to support evidence-based decision-making (Matzuk and Lamb, 2008). Such collaborative efforts are key to advancing personalized reproductive medicine and improving ART safety and efficacy.

Supplementary data

Supplementary data are available at *Human Reproduction Update* online.

Data availability

The datasets were derived from sources in the public domain: Canovas *et al.* (2017) (https://doi.org/10.5061/dryad.n77r3); Heras *et al.* (2016) (https://doi.org/10.1186/s12864-016-2393-z); Van Der Weijden *et al.* (2021) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155043); and Feuer *et al.* (2017) (https://doi.org/10.1530/REP-16-0473). The derived data generated in this research will be shared on reasonable request to the corresponding author.

Authors' roles

A.M.V. contributed to conceptualization and design of this study, analysed and interpreted the data, and drafted the initial manuscript. S.C. conceptualized and designed this study and provided overall supervision and guidance throughout the study. S.C., E.R. H., and P.J.R. contributed to the analysis and interpretation of data, edited the manuscript, and provided feedback. All authors made substantial contributions, critically revised the manuscript, and approved of the final version.

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Conflict of interest

The authors declare no potential conflict of interest.

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