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Abstract

Embryonic genome activation (EGA) is a fundamental developmental transition during which control of early embryogenesis shifts from maternally deposited transcripts to transcription from the embryonic genome. This process is essential for developmental competence and successful progression through preimplantation stages. Although EGA is conserved across mammals, its timing and regulatory mechanisms differ markedly between species. In this review, we summarize current knowledge of the molecular, epigenetic, and chromatin-based mechanisms governing EGA in human, mouse, porcine, and bovine embryos. We highlight the roles of key transcription factors, including the *DUX* family and paired-like (PRD-like) homeobox genes; maternal transcript clearance pathways; DNA methylation remodeling; histone modification dynamics; and chromatin accessibility. Comparative analysis reveals conserved regulatory themes and species-specific strategies that reflect distinct developmental programs. We further discuss how disruptions of EGA lead to developmental arrest and compromised embryo viability, with implications for assisted reproductive technologies (ART). Additionally, bioinformatic tools were used to analyze gene interactions and the biological processes involved. Understanding the coordinated regulation of EGA provides valuable insights into early embryonic development and offers potential molecular and epigenetic biomarkers for assessing embryo quality and improving outcomes in ART.

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Keywords: Embryonic genome activation, Molecular mechanisms, Epigenetic regulations, Species-specific differences

1. Introduction

Mammalian embryogenesis begins immediately after the fusion of male and female gametes, resulting in the formation of a totipotent zygote capable of giving rise to all cell types of the developing organism. This totipotent state is established through extensive epigenetic remodeling and transcriptional reprogramming, which enable the embryo to transition from reliance on maternally deposited transcripts and proteins to the transcription driven by its own genome [1, 2]. Early cleavage divisions produce the morula, a compacted cluster of cells characterized by the establishment of the first cell–cell junctions. This stage is followed by blastocyst formation, during which the first signs of lineage specification become evident, including the inner cell mass, which gives rise to the epiblast and hypoblast, and the trophectoderm, which contributes to placental formation [3]. The proper execution of these events is essential for successful implantation and pregnancy, as errors can lead to developmental arrest or early pregnancy loss [4, 5].

A critical event during early development is embryonic genome activation (EGA), also known as zygotic genome activation (ZGA), which marks the transition from maternal to embryonic control of gene expression. EGA begins with a minor wave of low-level transcription, followed by a major wave characterized by large-scale activation of embryonic genes, thereby establishing the foundation for preimplantation development and the first lineage decisions [6]. Failures in EGA, due to improper maternal transcript clearance, impaired transcription factor activity, or disrupted epigenetic remodeling, are associated with developmental arrest and reduced embryo viability [7, 8]. In mammals, key transcription factors coordinate the activation of the embryonic genome. In mice, these include double homeobox (*DUX*) and developmental pluripotency-associated protein 2 and 4 (*DPPA2/4*), while in humans, krüppel-like factor 17

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(*KLF17*) and *DUX4* play similar roles. These factors act in conjunction with chromatin remodeling complexes, RNA polymerase II preconfiguration, and histone modifications [9]. Additionally, microRNAs and RNA-binding proteins facilitate the clearance of maternal transcripts, ensuring a precise temporal and spatial progression from maternal to embryonic control of development [10].

The timing of EGA differs substantially among mammalian species. In mice, minor ZGA occurs during the S phase of the one-cell embryo, while major ZGA is completed by the mid-to-late two-cell (2C) stage [11]. In humans, major EGA occurs at the four- to eight-cell stage, while in bovine embryos, it occurs between the eight- and sixteen-cell stages, followed by blastocyst formation [12, 13]. These temporal differences reflect species-specific variations in cell-cycle length, maternal transcription composition, and the localization and activity of RNA polymerase II, as well as differences in epigenetic landscapes such as tri-methylation of histone H3 lysine 4 (H3K4me3) and histone H3 lysine 27 (H3K27me3) dynamics [14]. Although some transcriptional and epigenetic regulatory mechanisms are conserved across mammals, species-specific recruitment of maternal factors, transcription complexes, and chromatin modifiers underscores the need for comparative analyses to better understand developmental competence.

EGA is indispensable for establishing developmental competence. In mouse embryos, disruption of maternal-effect genes, cyclins, chromatin remodelers, and transcription factors results in delayed or failed EGA and developmental arrest [15]. In bovine embryos, inhibition of transcription at the eight-cell stage prevents further development, demonstrating the reliance of development on precise genome activation. In humans, defects in EGA contribute to poor outcomes in *in vitro* fertilization (IVF), including reduced blastocyst formation and early pregnancy loss. Maternal microRNAs, including miR-21, miR-130a, and miR-212, contribute to

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the participate in targeted degradation of maternal transcripts [16, 17], while histone modifications and DNA methylation establish the chromatin environment necessary for embryonic transcription. Broad *H3K4me3* domains are remodeled into sharp peaks during ZGA in mouse and human embryos, illustrating a conserved yet species-specific epigenetic regulation.

Understanding EGA is critical for assisted reproductive technologies (ART), including IVF and intracytoplasmic sperm injection, because *in vitro* conditions can influence the timing and efficiency of genome activation. Variability in embryo quality during ART is often associated with delayed or incomplete EGA, which can affect subsequent developmental milestones and implantation success. Animal models, particularly mouse and bovine embryos, provide valuable systems for studying these processes because of ethical and practical limitations associated with human embryo research. *In vitro* models, including embryonic stem cells, induced pluripotent stem cells, mouse 2C-like cells, and human eight-cell-like cells, recapitulate key aspects of genome activation and enable mechanistic studies of transcriptional networks, epigenetic remodeling, and retrotransposon activation [18]. These models provide insights into the molecular determinants of embryo competence and may contribute to the optimization of ART outcomes.

In summary, mammalian preimplantation development is orchestrated through a tightly regulated transition from maternal to embryonic control, culminating in embryonic genome activation, which is essential for developmental competence. Species-specific differences in the timing and regulation of EGA highlight the importance of comparative studies to elucidate conserved and divergent regulatory mechanisms. Precise activation of the embryonic genome depends on coordinated maternal transcript clearance, transcription factor recruitment, RNA polymerase II preconfiguration, and epigenetic remodeling, ensuring proper progression through

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early embryonic stages [19]. Understanding these molecular processes is critical for improving ART, enhancing embryo selection, and informing future applications in stem cell biology. Therefore, this review compares the molecular and epigenetic mechanisms regulating EGA across mammalian species and highlights how failures in these processes lead to developmental arrest during early embryogenesis.

2. Molecular Basis of Embryonic Genome Activation

During ZGA, regulatory control of early embryonic development progressively shifts from maternally deposited transcripts and proteins to newly synthesized products of the embryonic genome. This transition is known as the maternal-to-zygotic transition [20]. This developmental stage is largely governed by epigenetic regulation of gene expression in the totipotent embryo and represents a critical window during which major biological processes occur [21]. Numerous maternal genes are expressed from fertilization until the blastocyst stage, after which development becomes controlled by zygotic genes. Among the earliest transcription factors is *Dux*, which is expressed specifically at the 2C stage in mice and functions as the core activator of 2C genes such as zinc finger and SCAN domain-containing 4 (*Zscan4*), preferentially expressed antigen in melanoma family (*Pramef*), and lysine demethylase 4E (*Kdm4e*).

Several epigenetic programs, including DNA methylation and demethylation, chromosome remodeling, and histone modification, together with transcriptional mechanisms, are involved in EGA. After fertilization, the paternal genome undergoes rapid ten-eleven translocation 3 (TET3)-mediated active demethylation, while the maternal genome primarily undergoes passive demethylation owing to replication-dependent dilution caused by nuclear exclusion of DNA methyltransferase 1 (*DNMT1*) [22]. Although active paternal demethylation is not strictly required

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for viability, these asymmetric reprogramming events help establish an open chromatin state that facilitates transcriptional activation during EGA. Notably, transient nuclear entry of *Dnmt1* at the eight-cell stage preserves imprinted regions amidst global demethylation, ensuring that essential epigenetic marks are retained while the embryo resets its genome for EGA [22]. In mice, global DNA methylation patterns are established after embryo implantation and subsequently maintained across most developing cell lineages [23]. The loss of DNA methylation in *Dnmt1*-deficient embryos leads to repression of the germline gene program. Additionally, genes specifically expressed in 2C embryos and 2C-like embryonic stem cells, such as *Zscan4* family genes, transmembrane protein 92 (*Tmem92),* 2C stage variable transcript 1 and 3 *(Tcstv1/3),* eukaryotic translation initiation factor 1A-like (*Eif1a*), and ubiquitin-specific peptidase 17-like (*Usp17*), are highly derepressed in *Dnmt1* knockout embryos, indicating the critical role of *Dnmt1* in maintaining totipotency (Table 1) [23]. Proper establishment and maintenance of DNA methylation landscapes are therefore fundamental for the initiation and progression of EGA. A study exposing embryos to *in vitro* conditions at different cleavage stages demonstrated that disruption of normal DNA methylation patterns can adversely affect gene transcription during EGA [24]. These alterations include aberrant imprinting of paternally expressed gene 3 (*PEG3*), insulin-like growth factor 2 receptor (*IGF2R*), and growth factor receptor-bound protein 10 (*GRB10*), as well as dysregulated methylation of genes involved in solute transport, zinc-finger proteins, and extracellular matrix components such as collagen type 4 alpha 1 chain (*COL4A1*) [24].

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Moreover, the *PRAMEY* gene contributes to proper epigenetic regulation during early embryonic development by influencing histone methylation dynamics, particularly H3K27me3, thereby supporting normal developmental progression [25]. In parallel with these chromatin-based mechanisms, the onset of EGA is also accompanied by major remodeling of the repeatome, during which specific retrotransposons, including long interspersed nuclear elements and endogenous retrovirus-like element class L/K, become activated and enriched in accessible chromatin regions [26, 27]. These repetitive elements not only respond to pioneer transcription factors such as *DUX* but are increasingly recognized as functional regulatory modules that provide alternative promoters, enhancers, and transcription factor binding sites essential for early embryonic transcription [28]. Disruptions in these epigenetic processes ultimately impair embryo quality and reduce developmental competence, highlighting the high sensitivity of EGA to environmental and molecular disturbances. Additionally, 59 genes, including *TRIM49*, *ZSCAN4*, *DUX4*, *LEUTX*, *TRIM28*, *DNMT1*, *PRDM14*, *CBX5*, *SETDB1*, *NR5A2*, *TET1*, *PRAMEY*, and *KDM5A*, identified from previously published studies were analyzed using the ClueGO plugin in Cytoscape to determine enriched Gene Ontology (GO) biological processes. The analysis revealed significant enrichment of biological processes associated with early human embryonic development, including regulation of gene expression, DNA methylation and demethylation, chromatin organization, and histone modification (Figure 1).

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3. Timing and Species-specific Differences

EGA represents a complex, multi-layered developmental transition during which control of gene expression shifts from maternally deposited transcripts to zygotic transcription. Although chromatin remodeling is a central feature of this process, EGA is orchestrated through the coordinated action of transcription factor activation, DNA methylation reprogramming, dynamic histone modifications, changes in chromatin accessibility, genome reorganization, and maternal RNA clearance. Importantly, both the timing and the relative contribution of these mechanisms vary across species, reflecting distinct developmental strategies.

In humans (*Homo sapiens*), major EGA occurs later, predominantly between the four- and eight-cell stages, and is characterized by the gradual and coordinated activation of multiple regulatory mechanisms. Integrated single-cell RNA-sequencing and assay for transposase-accessible chromatin using sequencing analyses have revealed progressive transcriptional activation accompanied by chromatin accessibility remodeling [29]. A central regulator of human EGA is *DUX4*, which activates early embryonic genes and endogenous retroelements [30]. Unlike in mice, human embryos exhibit a global reduction in H3K4me3 during EGA, suggesting that transcriptional activation relies more heavily on chromatin remodeling and enhancer activation rather than promoter priming [31, 32]. DNA methylation dynamics further distinguish human EGA. Although global methylation levels remain relatively stable during early cleavage stages, locus-specific demethylation at regulatory regions correlates with transcriptional activation [8, 33]. Emerging evidence also highlights the role of maternal RNA decay pathways, including deadenylation and zygotically expressed RNA-binding proteins, in facilitating the maternal-to-zygotic transition [34, 35]. Figure 2 illustrates DUX4-interacting genes identified using the

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184 STRING (version 12.0) database in human embryos. This interaction network provides a
185 framework for future studies aimed at elucidating the molecular roles of these genes during EGA.
186 Together, these findings indicate that human EGA is governed by tightly coordinated
187 transcriptional, epigenetic, and post-transcriptional mechanisms.

188 In the mouse (*Mus musculus*), EGA occurs rapidly, with transcription initiating shortly
189 after fertilization and major activation occurring at the 2C stage. Recent single-cell transcriptomic
190 studies have demonstrated that transcription begins earlier than previously appreciated [11, 36].
191 This early transcriptional activation is driven by the double homeobox transcription factor *Dux*,
192 which induces cleavage-stage genes and repetitive elements [37]. *Dux* activation is primed by
193 maternally supplied *Dppa2* and *Dppa4*, which establish transcriptional competence before major
194 EGA [38, 39]. Concomitant with transcriptional activation, mouse embryos undergo extensive
195 epigenetic reprogramming. Genome-wide DNA demethylation, largely mediated by passive
196 demethylation and TET3-dependent oxidation, creates a permissive transcriptional landscape [40,
197 41]. In parallel, histone modification landscapes are rapidly restricted: promoter-associated
198 H3K4me3 domains expand during EGA, while repressive marks such as H3K9me3 remain low,
199 thereby facilitating transcriptional plasticity [42]. These epigenetic changes occur within a highly
200 relaxed chromatin architecture characterized by the delayed establishment of topologically
201 associating domains [43, 44]. Within this dynamic transcriptional and epigenetic landscape, key
202 regulatory factors involved in EGA have been identified in previous studies and were analyzed
203 using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, v12.0). This
204 protein–protein interaction analysis was used to explore potential functional relationships among
205 EGA-associated regulators approach can help future studies better understand their roles and how
206 they function together during EGA (Figure 3).

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Porcine (Sus scrofa domesticus) embryos provide an informative comparative model for understanding mammalian EGA because they exhibit a distinct pattern of genome activation that occurs primarily during the early cleavage stages. In pigs, minor transcriptional activity can begin during the one- to two-cell stages, whereas major EGA occurs predominantly during the transition from the two- to four-cell stage with substantial transcriptional remodeling continuing through the eight-cell stage [45, 46]. Transcriptomic studies using in vitro fertilized (IVF) and parthenogenetically activated embryos have identified extensive changes in genes expression during this transition, including *EIF1A*, *ZSCAN4*, *DPPA2*, *SIRT1*, *DUXA*, *LEUTX*, *KLF17*, *ELF4*, and *TBX3* [45-49]. Among these factors, *ZSCAN4* has been implicated in porcine ZGA based on functional studies in parthenogenetic embryos, in which *ZSCAN4* depletion reduced ZGA-associated gene expression and impaired embryonic development [47]. Epigenetic regulation is also closely coupled to porcine EGA. Histone-modifying enzymes such as KDM5B and KDM5C are transiently increased around genome activation, and their attenuation alters H3K4/H3K9 methylation, delays embryonic transcription, and compromises development to the blastocyst stage [50]. Similarly, dynamic changes in H3K4me3 and H3K27me3 accompany the transition from maternal to embryonic control, highlighting the importance of chromatin remodeling during porcine EGA [51]. Recent studies have further implicated *DUXA* and *LEUTX* as important regulators of porcine EGA. However, the functional evidence for *DUXA* was obtained primarily using porcine SCNT embryos [52], whereas *LEUTX* was examined in fertilized embryos for its transient expression during EGA and functionally evaluated in porcine SCNT embryos, where its overexpression improved EGA and preimplantation development [53]. Thus, these findings provide evidence that DUXA- and LEUTX- associated regulatory mechanisms may contribute to porcine EGA, while their functional roles should be interpreted in the context of the specific

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embryo models used. Collectively, current evidence supports the pig as a valuable model for investigating the molecular and epigenetic mechanisms governing the maternal-to-embryonic transition and for identifying conserved and species-specific features of mammalian EGA.

Bovine (*Bos taurus*) embryos display EGA timing and regulatory features that closely resemble those observed in humans, with major genome activation occurring at the eight- to sixteen-cell stages. Transcriptomic profiling studies have demonstrated robust induction of zygotic transcripts at this stage [28, 54]. Similar to that of other mammals, bovine EGA involves transient expression of *DUX* family transcription factors, particularly *DUXA*, with enrichment of *DUX*-binding motifs in newly accessible chromatin regions. In addition, PRD-like homeobox transcription factors, including *LEUTX* and TPRX, are strongly upregulated and are thought to function downstream of *DUX* signaling pathways (Figure 4) [55, 56]. Epigenetically, bovine embryos undergo progressive DNA methylation remodeling and histone modification reprogramming, including reductions in H3K4me3 and dynamic changes in H3K27ac at regulatory regions, mirroring patterns observed in human embryos [57]. These events are accompanied by the gradual establishment of chromatin accessibility and three-dimensional genome organization, suggesting a slower yet highly coordinated EGA process.

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4. Future Directions and Conclusion

EGA represents a decisive developmental checkpoint during which embryonic viability is established through precise coordination of transcriptional activation, epigenetic reprogramming, and chromatin remodeling. All mammals require genome activation for early embryonic development; however, the timing, molecular drivers, and regulatory architectures of this process vary considerably among species. Rather than reflecting a single universal paradigm, these

variations highlight the importance of interpreting embryonic development within species-specific regulatory systems. Accordingly, this review summarizes current knowledge of transcriptional, epigenetic, and chromatin-based mechanisms that control EGA in human, mouse, porcine and bovine, highlighting shared regulatory themes and biologically meaningful variations.

Importantly, the mechanistic insights summarized in this review support the emerging concept that EGA-associated molecular features may serve as informative biomarkers of embryonic developmental competence. Regulatory pathways and genes consistently associated with successful genome activation, including species-specific ZGA transcription factors, chromatin remodelers, and epigenetic modifiers, may serve as informative markers of transcriptional readiness during early development. The functional state of the embryonic genome is reflected in dynamic changes in histone modification, including redistribution of H3K4me3, increased enrichment of the activation-associated mark H3K27ac, and regulated repression of H3K9me3-marked heterochromatin. When considered together with transcriptional activity, these epigenetic features provide a mechanistic framework for evaluating embryo quality.

From a clinical perspective, identifying EGA-related genes and epigenetic signatures holds considerable promise for improving diagnostic strategies in ART. Molecular evaluation of key genome activation regulators may help distinguish embryos with intact transcriptional programs from those prone to developmental arrest, implantation failure, or epigenetic instability. Furthermore, the recognition that different species—and even individual embryos—employ distinct regulatory strategies highlights the need for tailored assessment approaches and culture conditions rather than uniform benchmarks. Therefore, continued integration of comparative embryology, single-embryo multi-omics, and translational research will be essential for refining EGA-based biomarkers and translating fundamental insight into clinically meaningful advances in

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ART. Additionally, bioinformatics tools can be used to predict molecular structures and explore gene regulatory networks. These approaches help researchers identify potential interacting partners and better understand their biological roles. Future studies integrating stage-specific embryonic transcriptomic and epigenetic datasets with large-scale functional genomic resources, including Encyclopedia of DNA Elements (ENCODE), Model Organism Encyclopedia of DNA Elements (modENCODE), and Functional Annotation of Animal Genomes (FAANG), may further facilitate the identification of conserved and species-specific regulatory mechanisms underlying EGA.

Collectively, recent studies demonstrate that EGA is not a single molecular event but rather a multifactorial developmental transition involving transcription factor activation, DNA methylation remodeling, histone modification dynamics, chromatin architecture reorganization, and maternal RNA clearance. Although these mechanisms are broadly conserved, their relative timing and regulatory hierarchy differ substantially among species. For example, mice rely on rapid transcriptional activation within a permissive epigenetic environment, whereas humans and bovine embryos exhibit more gradual and multilayered regulatory programs. Understanding these mechanistic differences is essential for accurate cross-species comparisons and for selecting appropriate model systems in developmental and reproductive biology research.

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List of Abbreviations

ART: Assisted reproductive technologies

EGA: Embryonic genome activation

GO: Gene ontology

IVF: *In vitro* fertilization

PRD-like: Paired-like homeobox genes

STRING: Search Tool for the Retrieval of Interacting Genes/Proteins

TET3: Ten-eleven translocation 3

ZGA: Zygotic genome activation

2C: Two-cell

References

[1] J. Taubenschmid-Stowers, M. Rostovskaya, F. Santos, S. Ljung, R. Argelaguet, F. Krueger, J. Nichols, W. Reik, 8C-like cells capture the human zygotic genome activation program in vitro, Cell stem cell 29(3) (2022) 449-459. e6.https://doi.org/10.1016/j.stem.2022.01.014.

[2] L. Llobat, Pluripotency and growth factors in early embryonic development of mammals: a comparative approach, Veterinary sciences 8(5) (2021) 78.https://doi.org/10.3390/vetsci8050078.

[3] Y. Hu, Y. Wang, Y. He, M. Ye, J. Yuan, C. Ren, X. Wang, S. Wang, Y. Guo, Q. Cao, Maternal KLF17 controls zygotic genome activation by acting as a messenger for RNA Pol II recruitment in mouse embryos, Developmental Cell 59(5) (2024) 613-626. e6.https://doi.org/10.1016/j.devcel.2024.01.013.

[4] K. Sfakianoudis, E. Maziotis, E. Karantzali, G. Kokkini, S. Grigoriadis, A. Pantou, P. Giannelou, K. Petroutsou, C. Markomichali, M. Fakiridou, Molecular drivers of developmental arrest in the human preimplantation embryo: a systematic review and critical analysis leading to mapping future research, International journal of molecular sciences 22(15) (2021) 8353.https://doi.org/10.3390/ijms22158353.

[5] A. Korkidakis, A.Y. Albert, I. Jiang, J. Terry, F.F. AbdelHafez, M.A. Bedaiwy, The Clinical Significance of Embryonic Chromosomal Errors in Recurrent Pregnancy Loss: an Analysis of 1107 Miscarriages, Reproductive Sciences 30(10) (2023) 3019-3026.https://doi.org/10.1007/s43032-023-01239-3.

[6] G.E. Jarvis, Early embryo mortality in natural human reproduction: What the data say, F1000Research 5 (2017) 2765.https://doi.org/10.12688/f1000research.8937.2.

[7] J. Zhang, J. Lv, J. Qin, M. Zhang, X. He, B. Ma, Y. Wan, Y. Gao, M. Wang, Z. Hong, Unraveling the mysteries of early embryonic arrest: genetic factors and molecular mechanisms, Journal of Assisted Reproduction and Genetics 41(12) (2024) 3301-3316.https://doi.org/10.1007/s10815-024-03259-7.

[8] A.L. Wilkinson, I. Zorzan, P.J. Rugg-Gunn, Epigenetic regulation of early human embryo development, Cell Stem Cell 30(12) (2023) 1569-1584.https://doi.org/10.1016/j.stem.2023.09.010.

[9] X. Liu, Y. Wang, Y. Gao, J. Su, J. Zhang, X. Xing, C. Zhou, K. Yao, Q. An, Y. Zhang, H3K9 demethylase KDM4E is an epigenetic regulator for bovine embryonic development and a defective factor for nuclear reprogramming, Development 145(4) (2018).https://doi.org/10.1242/dev.158261.

[10] J.M. Cuthbert, Comparative Analysis of Small Non-Coding RNA and Messenger RNA Expression in Somatic Cell Nuclear Transfer and In Vitro-Fertilized Bovine Embryos During Early Development and Through the Maternal-to-Embryonic Transition, Utah State University, 2020.

[11] F. Aoki, Zygotic gene activation in mice: profile and regulation, Journal of Reproduction and Development 68(2) (2022) 79-84.https://doi.org/10.1262/jrd.2021-129.

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[12] B. Yaşar, N. Boskovic, T. Org, J. Kere, A. Kurg, S. Katayama, RNA sequencing of mRNA 5'-ends reveals regulators of bovine embryonic genome activation, BMC genomics 26(1) (2025) 910.https://doi.org/10.1186/s12864-025-12110-x.
[13] A.C. Perry, M. Asami, B.Y. Lam, G.S. Yeo, The initiation of mammalian embryonic transcription: to begin at the beginning, Trends in cell biology 33(5) (2023) 365-373.https://doi.org/10.1016/j.tcb.2022.08.008.
[14] M. Thool, P.K. Sundaravadivelu, S. Sudhagar, R.P. Thummer, A comprehensive review on the role of ZSCAN4 in embryonic development, stem cells, and cancer, Stem Cell Reviews and Reports 18(8) (2022) 2740-2756.https://doi.org/10.1007/s12015-022-10412-1.
[15] A. Graf, S. Krebs, M. Heininen-Brown, V. Zakhartchenko, H. Blum, E. Wolf, Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments, Animal reproduction science 149(1-2) (2014) 46-58.https://doi.org/10.1016/j.anireprosci.2014.05.016.
[16] X. Yang, H. He, P. Wang, Y. Wang, L. Wang, F. Yang, J. Li, H. Zhang, Role of miRNAs in Bovine Oocyte Maturation and Reproductive Regulation, International Journal of Molecular Sciences 26(7) (2025) 2828.https://doi.org/10.3390/ijms26072828.
[17] D. Salilew-Wondim, S. Gebremedhn, M. Hoelker, E. Tholen, T. Hailay, D. Tesfaye, The role of MicroRNAs in mammalian fertility: from gametogenesis to embryo implantation, International journal of molecular sciences 21(2) (2020) 585.https://doi.org/10.3390/ijms21020585.
[18] A. Golkar-Narenji, H. Gourabi, H. Eimani, Z. Barekati, A. Akhlaghi, Superovulation, in vitro fertilization (IVF) and in vitro development (IVD) protocols for inbred BALB/cJ mice in comparison with outbred NMRI mice, Reproductive medicine and biology 11(4) (2012) 185-192.https://doi.org/10.1007/s12522-012-0127-8.
[19] S. Wang, Z. Li, Z. Fan, T. Wang, T. Hu, R. Hu, Z. Liu, T. Wang, L. Wu, X. Zhang, Coordination of zygotic genome activation entry and exit by H3K4me3 and H3K27me3 in porcine early embryos, (2022).https://doi.org/10.1101/gr.276207.121.
[20] Y. Su, J. Kouranova, J. Shea, X. Cui, Z. Su, Single-cell RNA-seq Reveals Early Transcriptional Programs of the Maternal to Zygote Transition in Mice and Rats, bioRxiv (2024) 2024.09.30.615942.https://doi.org/10.1101/2024.09.30.615942.
[21] Q. Zhang, W. Li, P. Feng, Y. Liu, B. Chu, J. Zhao, Y. Li, Y. Zhang, J. Liu, Lnc5926 is essential for early embryonic development in goats through regulation of ZSCAN4 and EIF1AX, Theriogenology 180 (2022) 87-93.https://doi.org/10.1016/j.theriogenology.2021.12.020.
[22] D.M. Messerschmidt, B.B. Knowles, D. Solter, DNA methylation dynamics during epigenetic reprogramming in the germline and preimplantation embryos, Genes & development 28(8) (2014) 812-828.https://doi.org/10.1101/gad.234294.113.
[23] T. Dahlet, A. Argüeso Lleida, H. Al Adhami, M. Dumas, A. Bender, R.P. Ngondo, M. Tanguy, J. Vallet, G. Auclair, A.F. Bardet, Genome-wide analysis in the mouse embryo reveals the importance of DNA methylation for transcription integrity, Nature communications 11(1) (2020) 3153.https://doi.org/10.1038/s41467-020-16919-w.
[24] D. Salilew-Wondim, M. Saeed-Zidane, M. Hoelker, S. Gebremedhn, M. Poirier, H.O. Pandey, E. Tholen, C. Neuhoff, E. Held, U. Besenfelder, Genome-wide DNA methylation patterns of bovine blastocysts derived from in vivo embryos subjected to in vitro culture before, during or after embryonic genome activation, BMC genomics 19(1) (2018) 424.https://doi.org/10.1186/s12864-018-4826-3.
[25] C.H. Kern, Functional Roles of PRAMEY in Sperm Maturation, Sperm-Egg Binding, and Early Embryonic Development in Cattle, The Pennsylvania State University, 2025.
[26] Y. Xiang, H. Liang, The regulation and functions of endogenous retrovirus in embryo development and stem cell differentiation, Stem Cells International 2021(1) (2021) 6660936.https://doi.org/10.1155/2021/6660936.
[27] J.A. DiRusso, A.T. Clark, Transposable elements in early human embryo development and embryo models, Current opinion in genetics & development 81 (2023) 102086.

ACCEPTED

[28] M.M. Halstead, X. Ma, C. Zhou, R.M. Schultz, P.J. Ross, Chromatin remodeling in bovine embryos indicates species-specific regulation of genome activation, Nature communications 11(1) (2020) 4654.https://doi.org/10.1038/s41467-020-18508-3.
[29] T. Wang, J. Peng, J. Fan, N. Tang, R. Hua, X. Zhou, Z. Wang, L. Wang, Y. Bai, X. Quan, Single-cell multi-omics profiling of human preimplantation embryos identifies cytoskeletal defects during embryonic arrest, Nature Cell Biology 26(2) (2024) 263-277.https://doi.org/10.1038/s41556-023-01328-0.
[30] V. Töhönen, S. Katayama, L. Vesterlund, M. Sheikhi, L. Antonsson, G. Filippini-Cattaneo, M. Jaconi, A. Johnsson, S. Linnarsson, O. Hovatta, Transcription activation of early human development suggests DUX4 as an embryonic regulator, BioRxiv (2017) 123208.https://doi.org/10.1016/j.gde.2023.102086.
[31] H. Yu, M. Chen, Y. Hu, S. Ou, X. Yu, S. Liang, N. Li, M. Yang, X. Kong, C. Sun, Dynamic reprogramming of H3K9me3 at hominoid-specific retrotransposons during human preimplantation development, Cell stem cell 29(7) (2022) 1031-1050. e12.https://doi.org/10.1016/j.stem.2022.06.006.
[32] R. Gao, S. Zeng, D. Yang, X. Li, W. Liu, Y. Gao, D. Bai, L. Zhang, C. Chen, Y. Kang, Resetting histone modifications during human prenatal germline development, Cell Discovery 9(1) (2023) 14.https://doi.org/10.1038/s41421-023-00519-1.
[33] C. Li, Y. Fan, G. Li, X. Xu, J. Duan, R. Li, X. Kang, X. Ma, X. Chen, Y. Ke, DNA methylation reprogramming of functional elements during mammalian embryonic development, Cell Discovery 4(1) (2018) 41.https://doi.org/10.1038/s41421-018-0039-9.
[34] M. Christou-Kent, M. Dhellemmes, E. Lambert, P.F. Ray, C. Arnoult, Diversity of RNA-binding proteins modulating post-transcriptional regulation of protein expression in the maturing mammalian oocyte, Cells 9(3) (2020) 662.https://doi.org/10.3390/cells9030662.
[35] Q.-Q. Sha, W. Zheng, Y.-W. Wu, S. Li, L. Guo, S. Zhang, G. Lin, X.-H. Ou, H.-Y. Fan, Dynamics and clinical relevance of maternal mRNA clearance during the oocyte-to-embryo transition in humans, Nature communications 11(1) (2020) 4917.https://doi.org/10.1038/s41467-020-18680-6.
[36] K.-i. Abe, S. Funaya, D. Tsukioka, M. Kawamura, Y. Suzuki, M.G. Suzuki, R.M. Schultz, F. Aoki, Minor zygotic gene activation is essential for mouse preimplantation development, Proceedings of the National Academy of Sciences 115(29) (2018) E6780-E6788.https://doi.org/10.1073/pnas.1804309115.
[37] D. Bosnakovski, M.D. Gearhart, S. Ho Choi, M. Kyba, Dux facilitates post-implantation development, but is not essential for zygotic genome activation, Biology of reproduction 104(1) (2021) 83-93.https://doi.org/10.1093/biolre/ioaa179.
[38] Z. Chen, Z. Xie, Y. Zhang, DPPA2 and DPPA4 are dispensable for mouse zygotic genome activation and pre-implantation development, Development 148(24) (2021) dev200178.https://doi.org/10.1242/dev.200178.
[39] M. Eckersley-Maslin, C. Alda-Catalinas, M. Blotenburg, E. Kreibich, C. Krueger, W. Reik, Dppa2 and Dppa4 directly regulate the Dux-driven zygotic transcriptional program, Genes & development 33(3-4) (2019) 194-208.https://doi.org/10.1101/gad.321174.118.
[40] M. Deng, G. Zhang, Y. Cai, Z. Liu, Y. Zhang, F. Meng, F. Wang, Y. Wan, DNA methylation dynamics during zygotic genome activation in goat, Theriogenology 156 (2020) 144-154.https://doi.org/10.1016/j.theriogenology.2020.07.008.
[41] K.N. Schulz, M.M. Harrison, Mechanisms regulating zygotic genome activation, Nature Reviews Genetics 20(4) (2019) 221-234.https://doi.org/10.1038/s41576-018-0087-x.
[42] X. Fu, C. Zhang, Y. Zhang, Epigenetic regulation of mouse preimplantation embryo development, Current opinion in genetics & development 64 (2020) 13-20.https://doi.org/10.1016/j.gde.2020.05.015.
[43] H. Zheng, W. Xie, The role of 3D genome organization in development and cell differentiation, Nature reviews Molecular cell biology 20(9) (2019) 535-550.https://doi.org/10.1038/s41580-019-0132-4.
[44] Z. Du, H. Zheng, B. Huang, R. Ma, J. Wu, X. Zhang, J. He, Y. Xiang, Q. Wang, Y. Li, Allelic reprogramming of 3D chromatin architecture during early mammalian development, Nature 547(7662) (2017) 232-235.https://doi.org/10.1038/nature23263.

ACCEPTED

[45] Z.-Q. Du, H. Liang, X.-M. Liu, Y.-H. Liu, C. Wang, C.-X. Yang, Single cell RNA-seq reveals genes vital to in vitro fertilized embryos and parthenotes in pigs, Scientific reports 11(1) (2021) 14393.https://doi.org/10.1038/s41598-021-93904-3.
[46] Y. Zhai, H. Yu, X. An, Z. Zhang, M. Zhang, S. Zhang, Q. Li, Z. Li, Profiling the transcriptomic signatures and identifying the patterns of zygotic genome activation–a comparative analysis between early porcine embryos and their counterparts in other three mammalian species, BMC genomics 23(1) (2022) 772.https://doi.org/10.1186/s12864-022-09015-4.
[47] X.-H. Li, M.-H. Sun, W.-J. Jiang, D. Zhou, S.-H. Lee, G. Heo, Z. Chen, X.-S. Cui, ZSCAN4 regulates zygotic genome activation and telomere elongation in porcine parthenogenetic embryos, International Journal of Molecular Sciences 24(15) (2023) 12121.https://doi.org/10.3390/ijms241512121.
[48] L. Shi, Y. Zhai, Y. Zhao, X. Kong, D. Zhang, H. Yu, Z. Li, ELF4 is critical to zygotic gene activation and epigenetic reprogramming during early embryonic development in pigs, Frontiers in Veterinary Science 9 (2022) 954601.https://doi.org/10.3389/fvets.2022.954601.
[49] X.-H. Li, S.-H. Lee, J.-D. Kim, G.-H. Lee, J.-m. Sim, X.-S. Cui, TBX3 is essential for zygotic genome activation and embryonic development in pigs, Microscopy and Microanalysis 31(1) (2025) ozae123.https://doi.org/10.1093/mam/ozae123.
[50] W.G. Glanzner, K. Gutierrez, V.B. Rissi, M.P. de Macedo, R. Lopez, L. Currin, N. Dicks, H. Baldassarre, L.B. Agellon, V. Bordignon, Histone lysine demethylases KDM5B and KDM5C modulate genome activation and stability in porcine embryos, Frontiers in cell and developmental biology 8 (2020) 151.https://doi.org/10.3389/fcell.2020.00151.
[51] Y. Gao, P. Hyttel, V.J. Hall, Regulation of H3K27me3 and H3K4me3 during early porcine embryonic development, Molecular Reproduction and Development: Incorporating Gamete Research 77(6) (2010) 540-549. https://doi.org/10.1002/mrd.21180Digital
[52] Z. Shi, Y. Yan, R. Zhu, X. Zhu, K. Hu, Y. Yue, W. Xu, M. Xuan, X. Gan, Z. Yang, DUXA regulates the early development of porcine cloned embryos, Theriogenology 247 (2025) 117563.https://doi.org/10.1016/j.theriogenology.2025.117563.
[53] K. Zhou, T. Wang, J. Zhang, J. Zhang, X. Liu, J. Guan, P. Su, L. Wu, X. Yang, R. Hu, LEUTX regulates porcine embryonic genome activation in somatic cell nuclear transfer embryos, Cell Reports 43(6) (2024).https://doi.org/10.1016/j.celrep.2024.114372.
[54] H. Ming, J. Sun, R. Pasquariello, L. Gatenby, J.R. Herrick, Y. Yuan, C.R. Pinto, K.R. Bondioli, R.L. Krisher, Z. Jiang, The landscape of accessible chromatin in bovine oocytes and early embryos, Epigenetics 16(3) (2021) 300-312.https://doi.org/10.1080/15592294.2020.1795602.
[55] B. Yaşar, N. Boskovic, M. Ivask, J. Weltner, E.-M. Jouhilahti, P. Vill, T. Skoog, Ü. Jaakma, J. Kere, T.R. Bürglin, Molecular cloning of PRD-like homeobox genes expressed in bovine oocytes and early IVF embryos, BMC genomics 25(1) (2024) 1048.https://doi.org/10.1186/s12864-024-10969-w.
[56] T.D. Lewin, A.A. Fouladi-Nashta, P.W. Holland, PRD-class homeobox genes in bovine early embryos: function, evolution, and overlapping roles, Molecular Biology and Evolution 39(5) (2022) msac098.https://doi.org/10.1093/molbev/msac098.
[57] F. Sotomayor-Lugo, N. Iglesias-Barrameda, Y.M. Castillo-Aleman, I. Casado-Hernandez, C.A. Villegas-Valverde, A.A. Bencomo-Hernandez, Y. Ventura-Carmenate, R.A. Rivero-Jimenez, The dynamics of histone modifications during mammalian zygotic genome activation, International Journal of Molecular Sciences 25(3) (2024) 1459.https://doi.org/10.3390/ijms25031459.
[58] V. Töhönen, S. Katayama, L. Vesterlund, E.-M. Jouhilahti, M. Sheikhi, E. Madissoon, G. Filippini-Cattaneo, M. Jaconi, A. Johnsson, T.R. Bürglin, Novel PRD-like homeodomain transcription factors and retrotransposon elements in early human development, Nature communications 6(1) (2015) 8207.https://doi.org/10.1038/ncomms9207.
[59] Z. Zou, C. Zhang, Q. Wang, Z. Hou, Z. Xiong, F. Kong, Q. Wang, J. Song, B. Liu, B. Liu, Translatome and transcriptome co-profiling reveals a role of TPRXs in human zygotic genome activation, Science 378(6615) (2022) abo7923.https://doi.org/10.1126/science.abo7923.

ACCEPTED

[60] E. Madissoon, V. Töhönen, L. Vesterlund, S. Katayama, P. Unneberg, J. Inzunza, O. Hovatta, J. Kere, Differences in gene expression between mouse and human for dynamically regulated genes in early embryo, PLoS One 9(8) (2014) e102949.https://doi.org/10.1371/journal.pone.0102949.
[61] L. Gao, Z. Zhang, X. Zheng, F. Wang, Y. Deng, Q. Zhang, G. Wang, Y. Zhang, X. Liu, The novel role of Zfp296 in mammalian embryonic genome activation as an H3K9me3 modulator, International Journal of Molecular Sciences 24(14) (2023) 11377.https://doi.org/10.3390/ijms241411377.
[62] A. Timofeeva, Y. Drapkina, I. Fedorov, V. Chagovets, N. Makarova, M. Shamina, E. Kalinina, G. Sukhikh, Small noncoding RNA signatures for determining the developmental potential of an embryo at the morula stage, International journal of molecular sciences 21(24) (2020) 9399.https://doi.org/10.3390/ijms21249399.
[63] G. Falco, S.-L. Lee, I. Stanghellini, U.C. Bassey, T. Hamatani, M.S. Ko, Zscan4: a novel gene expressed exclusively in late 2-cell embryos and embryonic stem cells, Developmental biology 307(2) (2007) 539-550.https://doi.org/10.1016/j.ydbio.2007.05.003.
[64] C. Zhang, M. Wang, Y. Li, Y. Zhang, Profiling and functional characterization of maternal mRNA translation during mouse maternal-to-zygotic transition, Science advances 8(5) (2022) eabj3967.https://doi.org/10.1126/sciadv.abj3967.
[65] W. Zhang, E. Walker, O.J. Tamplin, J. Rossant, W.L. Stanford, T.R. Hughes, Zfp206 regulates ES cell gene expression and differentiation, Nucleic acids research 34(17) (2006) 4780-4790.https://doi.org/10.1093/nar/gkl631.
[66] F.H. Biase, X. Cao, S. Zhong, Cell fate inclination within 2-cell and 4-cell mouse embryos revealed by single-cell RNA sequencing, Genome research 24(11) (2014) 1787-1796.http://www.genome.org/cgi/doi/10.1101/gr.177725.114.
[67] A. Graf, Analysis of genome activation in early bovine embryos by bioinformatic evaluation of RNA-Seq data, Universitätsbibliothek der Ludwig-Maximilians-Universität, 2015.
[68] B. Brinkhof, H.T. van Tol, M.J. Groot Koerkamp, F.M. Riemers, S.G. IJzer, K. Mashayekhi, H.P. Haagsman, B.A. Roelen, A mRNA landscape of bovine embryos after standard and MAPK-inhibited culture conditions: a comparative analysis, BMC genomics 16(1) (2015) 277.
[69] P.J. Hansen, P. Tríbulo, Regulation of present and future development by maternal regulatory signals acting on the embryo during the morula to blastocyst transition–insights from the cow, Biology of reproduction 101(3) (2019) 526-537.https://doi.org/10.1093/biolre/ioz030.
[70] W.A. Kues, S. Sudheer, D. Herrmann, J.W. Carnwath, V. Havlicek, U. Besenfelder, H. Lehrach, J. Adjaye, H. Niemann, Genome-wide expression profiling reveals distinct clusters of transcriptional regulation during bovine preimplantation development in vivo, Proceedings of the National Academy of Sciences 105(50) (2008) 19768-19773.https://doi.org/10.1073/pnas.0805616105.

ACCEPTED

Tables and Figures

Table 1: Reported genes expressed at different stages of early embryonic development in human, mouse, porcine and bovine embryos

	Two-cell stage	Four-cell stage	Eight-cell stage	Morula stage	Reference
Human	*CPHX1, ZSCAN4, ZSCAN5A, ZSCAN5B, ZSCAN5C, TPRXL, DUXA, DUXB, DPPA*	*ZSCAN4, KHDC1L, CPHX2, ZSCAN5A, ZSCAN5B, ZSCAN5C, DUXA, DUXB, TPRXL, NLRP2*	*ZSCAN4, LEUTX, TPRX, PRAMEFs, TRIM, DPRX, CPHX2, CPHX1, DUXB, DUXA, ARGFX, DNMT3L, POU5F1, TPRXL, KLF17, HIPK3, ZFP296*	*DNMT3L, POU5F1, TPRXL, NLRP2, NLRP7, ZFP296, ELF2, DLX4, SLC25A35, TMEM2, RPUSD2, GLUL, CPOX*	[1, 14, 55, 58-62]
Mouse	*Dux, Dppa2, Gm8300, Dppa4, Tbx3, Nlrp12, Gm13102, Pramel6, Pramel7, Zfp622, Obox, Otx2, Gsc, BB287469, Crx, Pitx1, Tmem92, Tcstv1, Gm5662, Gm2022, Tcstv3, Zfp352, Zscan4f, Zscan4d, Usp17l*	*Tbx3, Zfp296, Ccnt2, Zscan4, Tcstv1/3, Eif1a, Gm4340, Tdpoz1, Zfp352, Zfp206, Gm6594, Gm20449, Tctn1, Smad2, Cyfip1, Trim71, Acd*	*Nlrp12, Trim43a, Tbx3, H2afz, Zfp296*	*Bysl, Furin, Tsg101, Tbx3, Tead4, Pdcd2, Sall4, Zfp296,*	[11, 14, 15, 23, 60, 61, 63-66]
Porcine	*EIF1A/EIF1AX, SIRT1, DPPA2, KDM5B, KDM5C*	*ZSCAN4, EIF1A/EIF1AX, DPPA2, SIRT1, DUXA, LEUTX, KLF17, ELF4, TBX3, MYC*	*ZSCAN4, LEUTX, KLF17, ELF4, TBX3, POU5F1, SOX2, NANOG*	*POU5F1, SOX2, NANOG, CDX2, TEAD4, KLF17*	[45-53]

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Bovine	*CDX2, MYC, TBX3, SARS, IL18, CRABP1, ACO2, TXN2, SLC38A2, SLC25A3*	*KLF4, KLF17, H2AFZ, EPCAM, MLL2, SARS, IL18, CRABP1, ACO2, TXN2, SLC38A2, SLC25A3, YES1, AZI2, UBTFL1, KLHL28*	*SALL4, MYC, TBX3, KLF4, EIF3, DDX5, DHX8, DHX15, DHX38, LEUTX, TPRX3, ARGFX, DUXEIF2, EIF4, EIF5*	*NANOG, TSG101, TEAD4, FUZ, FOSL1, ELP2, FURIN, TFCP2L1, TPRX1, ARGFX, DUXA, SOX2, POU5F1*	[55, 67-70]

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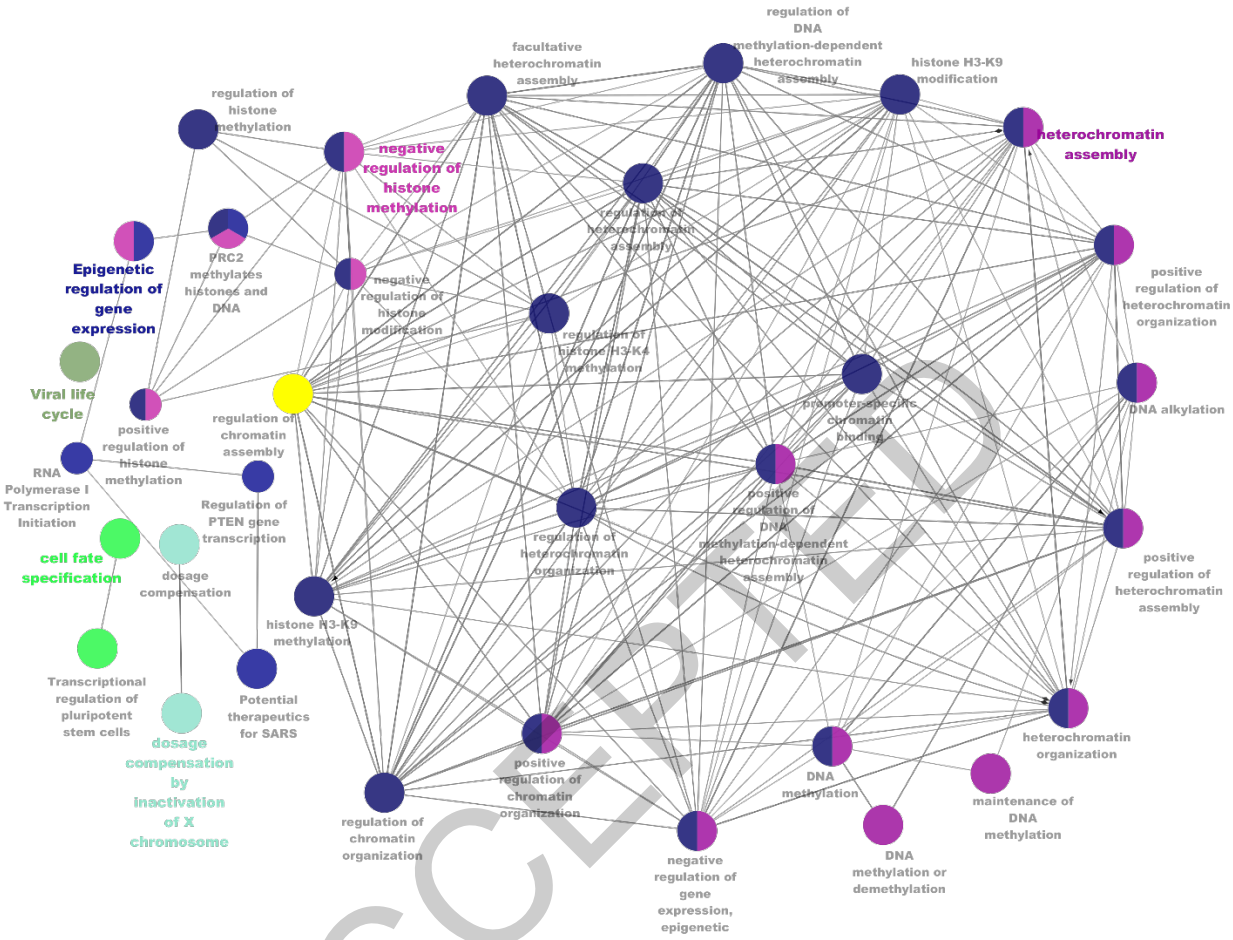


Figure 1. Interaction networks of enriched biological processes associated with genes involved in human embryonic development. The network was generated using the ClueGO (version 2.5.10) plugin in Cytoscape based on a curated set of human genes compiled from published studies. Significantly enriched GO terms are represented as nodes, while functional relationships between GO terms are depicted as edges. Node colors indicate functionally grouped GO terms as defined by ClueGO. The network highlights interconnected biological processes relevant to early human embryonic development, including regulation of gene expression, DNA methylation, chromatin organization, and histone modification.

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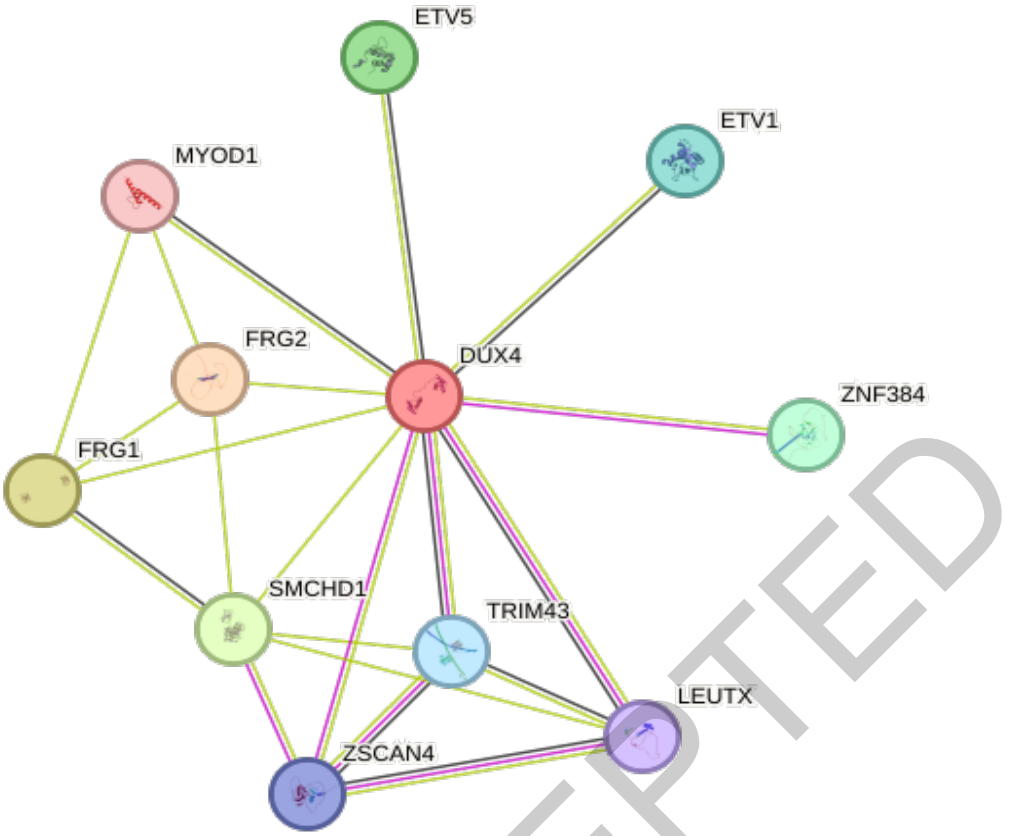


Figure 2. STRING-based interaction network of *DUX4* and associated genes during human EGA. The network was constructed using the STRING (version 12.0 database to visualize known and predicted interactions between *DUX4* and other genes implicated in early human embryogenesis. Nodes represent individual proteins, and connecting edges indicate functional associations between them. The network shows extensive interactions between *DUX4* and several embryonic transcription factors, suggesting its pivotal role in regulating early developmental gene expression.

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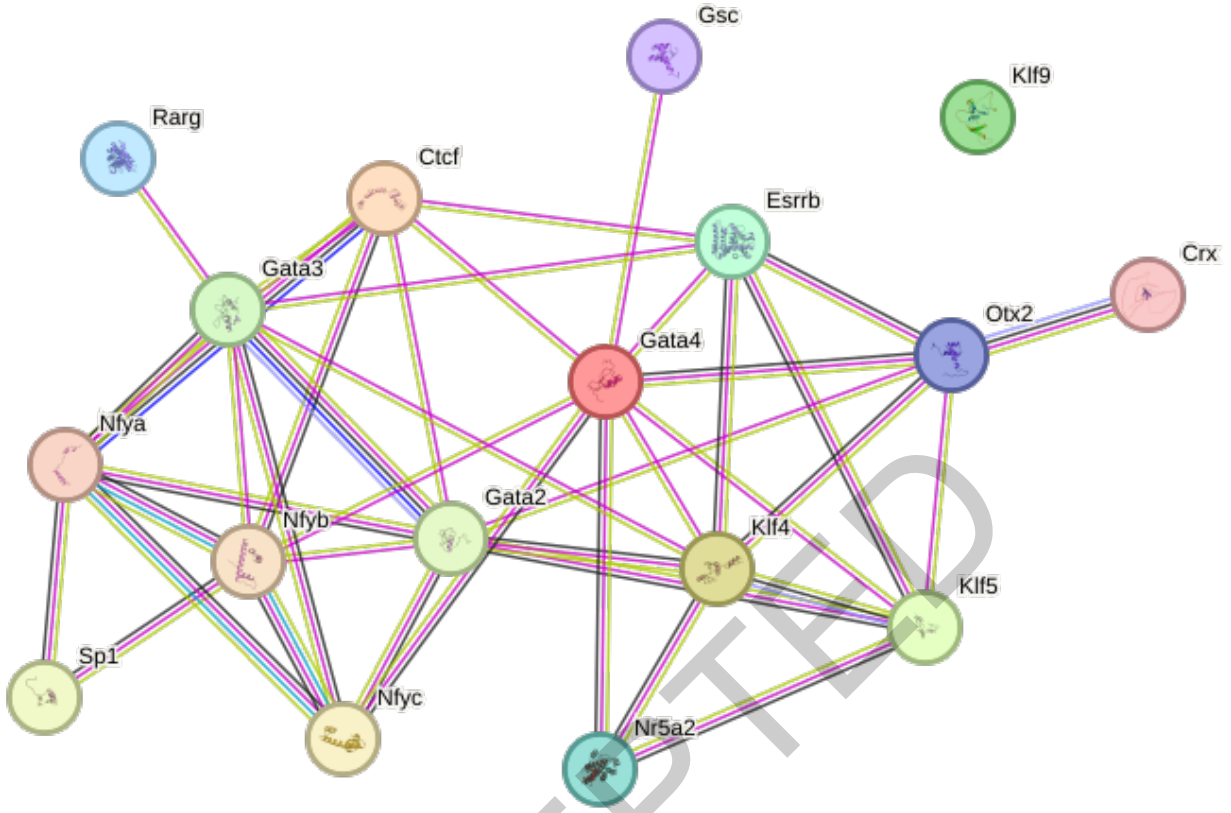


Figure 3. Interaction network of key regulatory factors involved in embryonic genome activation (EGA) in mouse, generated by STRING (version 12.0). The network illustrates interaction among transcription factors enriched during EGA. While edges indicated functional relationship between them. Nodes represent individual regulatory proteins, while edges indicate functional relationships between them. Different edge colors correspond to different kinds of supporting evidence used to predict the interactions.

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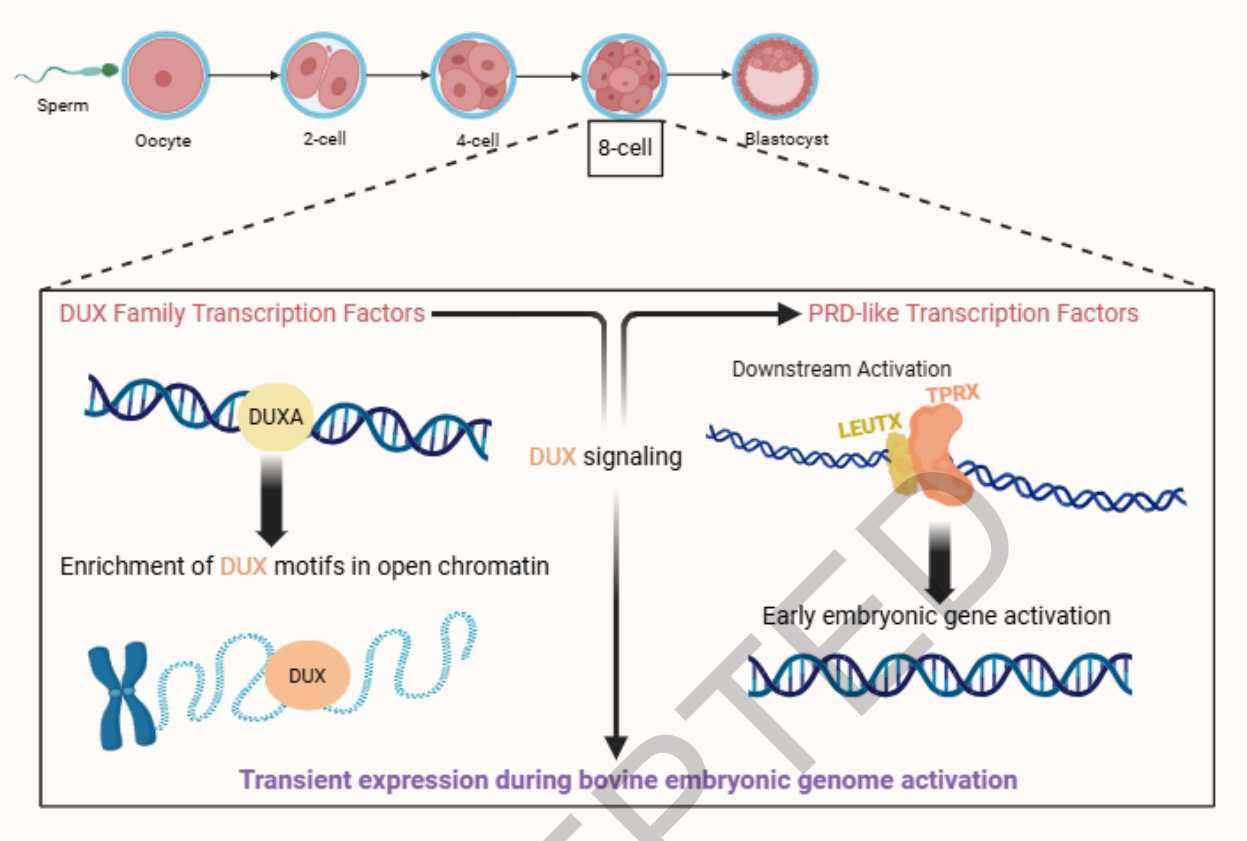


Figure 4. Role of *DUX* family transcription factors during bovine embryonic genome activation (EGA). Following fertilization, bovine preimplantation development progresses from the zygote to the blastocyst stage, with major EGA occurring at the eight-cell stage. At this stage, *DUX* family transcription factors such as *DUXA* are transiently expressed and function as upstream regulators of EGA. *DUX* proteins bind to their cognate DNA motifs enriched within open chromatin. *DUX* signaling subsequently induces PRD-like homeobox transcription factors, including *LEUTX* and *TPRX*, which function downstream to promote early embryonic gene activation. The figure was created using www.biorender.com.

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