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Gene expression study of key regulators of germ layer formation in chicken embryos using RT-PCR technique

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Abstract

Objective

Various studies have shown that the PRDM14 gene plays a very important and fundamental role in the early development of the chicken embryo and the formation of the germ layer. The aim of this study was to investigate the expression pattern of the PRDM14 gene in early embryogenesis and also to investigate its evolutionary role. This work also seeks to simplify the identification of major transcription factor families active in the first five days of development.

Materials and methods

For comparison of PRDM14 and other members of the PRDM family, we used a phylogenetic analysis. Comparative expression profiling was also conducted to track gene activity during stages E1-E5, which represent the earliest and most sensitive phases of chicken embryonic development. Public genomic datasets and annotated gene families were used to identify transcription factors and determine their distribution across the genome.

Results

The analysis showed that PRDM14 and other PRDM family genes follow an evolutionary divergence pattern similar to that of other vertebrates. The stages E1-E5 were identified as the

most crucial in embryogenesis, showing major shifts in gene expression. Among the studied chicken embryos, 1097 transcription factors were observed. These genes did not have random distribution, but they have significant genomic structure pattern. Transcription factor families such as bHLH, Zf-C2H2, and Homeobox were also detected in this study. They play important role in early development. 60 amino acids make up Homeobox proteins. These proteins are essential for regulating many developmental processes. ISL1, MKX, NANOG, and CDX2 are key transcription factors identified and classified into the Homeobox and bHLH groups. These factors are involved in cell fate determination, somatic lineage formation, and pluripotency. At days 4 and 5, the bHLH group of transcription factors showed high expression. These factors were found to play an important and essential role in the nervous system and germ cell development.

Conclusion

This study shows that the early stages of embryogenesis involve coordinated activity of major transcription factor families. The structured expression pattern and early activation of PRDM14 suggest its essential role in guiding cell identity and developmental pathways in chicken embryos.
Keywords: chicken embryos, embryonic development, germ cells, Homeobox, PRDM14

Paper Type: Research Paper.

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Introduction

Livestock production in general and domestic chicken production in particular plays a vital socio-economic role for people living in low-income countries of Africa and Asia (Mohamadinejad et al. 2024; Khezri et al., 2025; Mohammadabadi et al., 2025a). Domestic chickens are widely distributed avian species around the world, due to their short generation interval and adaptability in a wide range of agro ecologies (Mohammadifar and Mohammadabadi,

2017; Khabiri et al. 2025; Mohammadabadi et al., 2025b). The domestic chickens provide high quality protein and income for the poor rural households and are the most widely kept livestock species in the world (Mohammadabadi et al. 2010; Mohammadifar and Mohammadabadi, 2018; Mohammadabadi et al., 2024). This is because chickens have many valuable traits. Some of these important traits include adaptability to harsh environments, ability to use poor quality feeds, and resistance to disease (Shahdadnejad et al., 2016; Khobiri et al., 2023; Mohammadabadi et al., 2025c). The RT-PCR technique has a high ability to accurately measure messenger RNA (mRNA) levels for specific genes. One of these important and practical applications is its high sensitivity in studying gene expression in vertebrates (Khodari et al., 2025; Mohammadabadi et al., 2025a). Although significant progress has been made in the development and optimization of RT-PCR, much research is still needed to understand the basic concepts related to its application in gene expression studies (Khobiri et al., 2025; Mohammadabadi et al., 2025b). Therefore, much research is being conducted worldwide on the measurement of stable mRNA transcripts in the developing embryo. RT-PCR enables researchers to see how certain genes are expressed, which provides more information on how they are expressed in critical stages of germination development (Khabiri et al. 2023; Mohammadabadi et al., 2025c). This method is extremely useful for learning how different types of cells grow and how their differentiation is controlled. This helps researchers understand what role-specific genes play in these complex processes (Mohamadinejad et al. 2024; Mohammadabadi et al., 2024; Mohammadabadi et al., 2025a). One of the most important genes is the transcript factor PRDM14. It is a member of the PRDM protein family and consists of an amine terminal PR domain and a variable number of carboxy terminal zinc fingers (Okuzaki et al., 2019). PRDM14 is important to mark primordial germ cells (PGC) in humans and other vertebrates. It is also important to regulate gene activity and maintain the natural ability of stem cells to differentiate to any type of cell. Recent investigations in avian embryology have demonstrated that PRDM14 exhibits intense activity during the earliest stages of chicken embryonic development, after which its expression becomes restricted to the mature neural plate and migrating primordial germ cells (PGCs). As development progresses, PRDM14 expression is later confined to differentiated adult testes (Glover, 2013). Functionally, this transcriptional regulator is known to preserve cellular pluripotency by counteracting fibroblast growth factor 4 (FGF4)-induced differentiation and maintaining a low level of global DNA methylation (Hagihara et al., 2020). In birds, PRDM14 expression is unique to PGCs, where it plays a pivotal role in sustaining their proliferation and is thought to be indispensable for germ cell specification and maturation (Lee et al., 2016). PRDM14 has been shown to perform a variety of functions in mouse models. Some of its roles include repressing somatic gene expression programs, reprogramming the genome to make it more flexible, and maintaining germ cell

identity. This highlights its conserved function in vertebrates. In addition, PRDM14 has been shown to function in concert with other key growth regulators. Members of the Homeobox and basic Helix-Loop-Helix (bHLH) protein families appear to interact with PRDM14 during the early morphogenetic stages of chicken embryogenesis (Lambert et al., 2018). Recent molecular analyses have confirmed the co-localization of PRDM14 and bHLH proteins within the ectoderm, neural plate, and PGCs of both in vivo embryos and cultured primordial germ cells. These findings emphasize their synergistic contribution to PGC survival, proliferation, and differentiation in avian species (Choi et al., 2021). RT-PCR is a fundamental technique for assessing the transcriptional activity of genes that govern germ layer formation in chicken embryos and can be used to characterize the temporal regulation of such genes (Jacob et al., 2018). Therefore, it can be used to investigate dynamic changes in regulatory genes such as PRDM14. By tracking these changes over time, it is possible to identify the molecular mechanisms that drive germ cell formation and lineage specification in birds. The aim of this study was to use RT-PCR to understand how genes involved in germ layer formation are activated in chicken embryos.

Materials and methods

Embryo collection, isolation, and RNA extraction: Fertilized eggs were obtained from the Faculty of Veterinary Medicine, Basra University. The eggs were maintained under controlled environmental conditions using a fully automated incubator. The temperature was set at 37.5°C and the relative humidity was set at 65% to ensure optimal embryo development. The incubator automatically turned the eggs every six hours during the incubation period, as this is essential to simulate natural incubation and promote uniform embryogenesis. Embryonic development was assessed at 24, 48, 72, 96 and 120 hours after incubation. These times correspond to days 1 to 5 of development. After collection, they were numbered according to the development stage. To increase the reproducibility and reliability of the test results, three biological replicates were used for each sample. Total RNA was extracted using the TRIzol reagent kit (Invitrogen, Carlsbad, USA) according to the manufacturer's recommended protocol. An Agilent 2100 Bioanalyzer (Agilent Technologies, USA) was used to assess the quality of the extracted RNA. A NanoDrop 2000 spectrophotometer was used to assess the concentration and purity of the extracted RNA. High-purity RNA samples were used in the following.

Preparation of primers: First, a sequencing library was created. Then, complementary DNA (cDNA) was prepared according to the manufacturer's protocol (Gene-Denovo Biotechnology) (Zhang et al., 2018). Finally, sequencing was performed. Oligo(dT) beads were used to separate mRNA from total RNA. DNA polymerase I, RNase H, and deoxynucleotide triphosphate were used to synthesize the second-strand complementary RNA. Then, cDNA was

purified. Subsequently, residual RNA fragments were removed and the cDNA ends were repaired. Then, an adenine overhang was added. Finally, Illumina sequencing adapters were ligated to the cDNA fragments. Electrophoresis was used to select adapter-ligated products (Figure 1). This was done based on size. PCR was then used for amplification. fastp (version 0.18.0) was used to qualitatively filter the raw reads. Reads containing more than 10% ambiguous nucleotides were removed. Gene expression levels were then measured. Genes with a false discovery rate (FDR) < 0.05 were considered as differentially expressed genes. The ggplot2 package was used for hierarchical clustering of differentially expressed genes (Table 1). To identify transcription factors in the list of differentially expressed genes (DEGs), the method presented by Ren et al. (2019) was used. The STRING database (Hwang et al., 2018) was used to draw protein-protein interaction networks. In these steps, the focus was on transcription factor-related interactions. Cytoscape software (Kawaguchi et al., 2019) was used for network analysis. Validation of expression data was performed using quantitative real-time polymerase chain reaction (qRT-PCR).

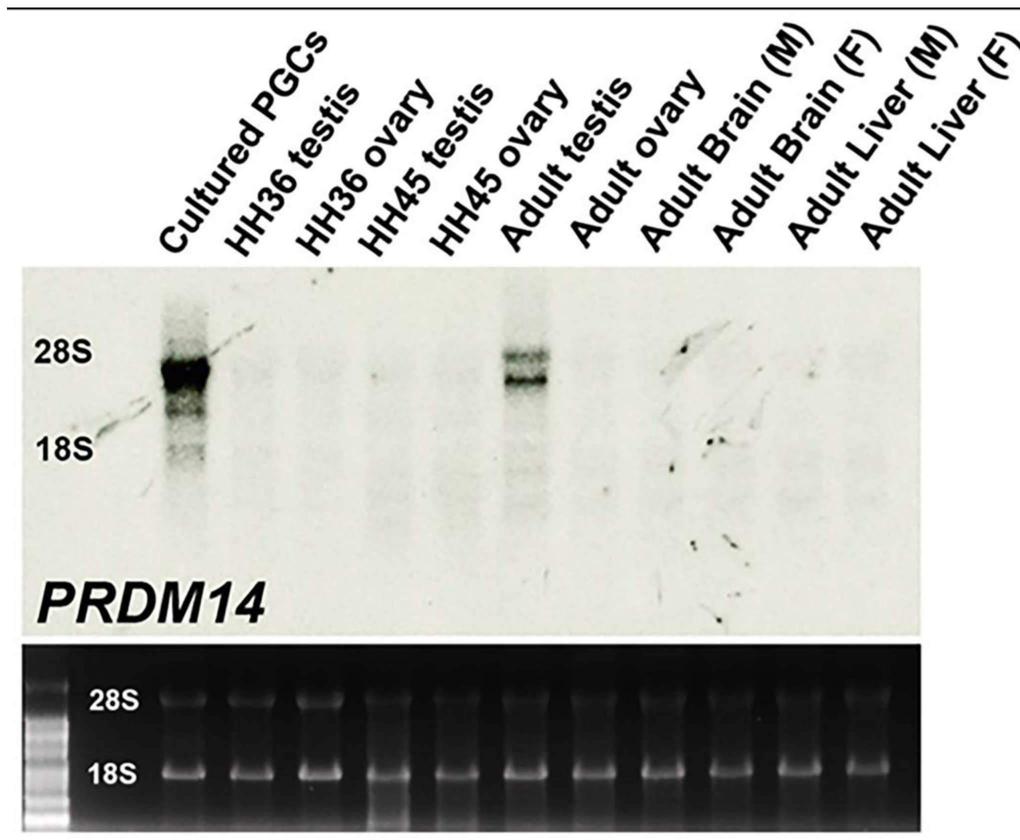

Figure 1. Gene expression of PRDM14 gene and PRDM family proteins

Results and discussion

Polymerase chain reaction (PCR): The RNeasy kit (Qiagen) was used to extract RNA. For synthesis of cDNA, AMV Reverse Transcriptase (Promega) was used. Specific forward and reverse primers were used to perform qRT-PCR. Relative expression was determined using the comparative ΔΔCt technique, as described in previous studies (Lambert et al., 2018; Kawaguchi et al., 2019). Immunofluorescence analysis was then performed on cells that were immediately fixed, with all washing and incubation steps carefully controlled. The strains were then cultivated from chicken primary germ cells in a serum-supplemented medium, either with or without STO feeder cells. For the PRDM14 genetically engineered chicken strains, between 5,000 and 7,000 targeted primary germ cells, both males and females, were injected into the dorsal aorta of HH embryos. At stage 16+, after the preservation of embryos and their paraffin embedding, the embryos were cultured under vibratory incubation until hatching, two cohorts of primary germ cells were identified. All six chicks were successfully hatched from a total of six chicks (Chua et al., 2022). Transcriptional data from PRDM14 transgenic embryos and PRDM family genes were investigated. Then high-quality reads were aligned to the chicken reference genome (GalGal6). For assessing the gene expression of the PRDM protein family, RNA-Seq data from poultry cell lines (PGCs) were used. Primary germ cells (PGCs) were subsequently cultivated from the ectodermal stage (Al-Naama et al., 2020). The procedures and methods were executed in accordance with the manufacturers' guidelines and many prior studies. A homolog of the avian PRDM14 gene was identified in the ectoderm and primordial germ cells (PGCs). The chicken PRDM14 gene has seven exons that encode a 486-amino acid protein including a PR domain and six C-terminal C2H2 zinc fingers, akin to other vertebrate homologs (Okuzaki et al., 2019). The sequences in this study showed few deviations from the reference genome. Phylogenetic analysis revealed that PRDM14 is evolutionarily related to other members of the PRDM family. PRDM14 in chickens was found to be highly similar to its counterparts, which is consistent with results from other vertebrates (Glover, 2013; Al-Naama et al., 2020; Wu & Guan, 2021).

Embryonic development in chicken embryos under study: The research elucidated the categorization of expressed transcription factors using RNA-Seq data from chicken embryos from day 1 to day 5, during which gastrulation and organogenesis occurred in vitro following oviposition. Somite formation was observed during the first five days of incubation (Figure 2), which aligns with previous work (Ayers et al., 2015; Wu & Guan, 2021). Consequently, stages E1-E5 are regarded as the pivotal phase in embryonic development. The comparative analysis of gene expression patterns across successive stages demonstrated that the up regulation of genes is

the primary molecular process. The gene expression profile exhibited substantial and distinct changes throughout the transition from Em2d to Em3d (Figure 3).

Table 1. A list of the primer sequences used in qRT-PCR

Gene	Primer	Accession No.	Product length (bp)	Annealing temperature (°C)
SOX10	F: TCTGAAGACCACCACTGCCTCTC R: CTTGACCTTGCCCATCTCTCCATTC	NM_204792.2	99	60
CDX2	F: AAACCAGGACGAAGGACAAATACCG R: GGTGATATAGCGGCTGTAGTGGAAC	NM_204311.2	92	60
ISL1	F: TCCGAGGGTCATCAGGGTTTGG R: TGGGTTGCTGCTGCTGAAGTTG	NM_205414.2	92	60
PAX6	F: CTATCCCGATGTGTTTGCGAGAGAG R: CTGGGAGTGTTGCTGGCTTGTC	NM_205066.2	150	61
SOX2	F: AAACCAAGACCCTGATGAAGA R: ATCCCATAGCCTCCGTTG	NM_205188.3	175	61
OLIG2	F: GCTTCAAGTCCTCGTCGTCGTC R: CGGCTCCGTCATCTGCTTCTTG	NM_001031526.1	80	61
SOX9	F: GCTGTGGAGGCTGCTGAATGAG R: CCTGCGTGGTTGGTACTTGTAGTC	NM_204281.2	112	60
MKX	F: GAACACAGTCAGGCAACCAGACC R: CCATCTTCAGAGCACGAGTCATCAC	XM_015282064.4	120	60
ZBTB16	F: TCCACCGCAACAGTCAGCATTAC R: CGTAGAGCAGGTCATCCAAGTCTTC	NM_001321488.2	126	61

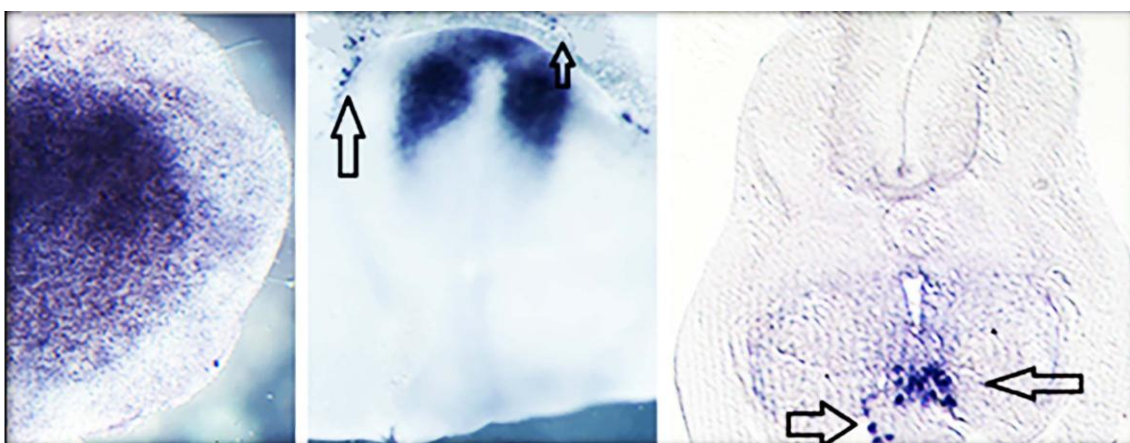

Figure 2. The embryonic development of chicken embryos and the gene expression of the PRDM14 protein in chicken embryos using situ hybridization. Arrowheads indicating the presence of primordial germ cells (PGCs). (Bar scale = 1 mm. D)

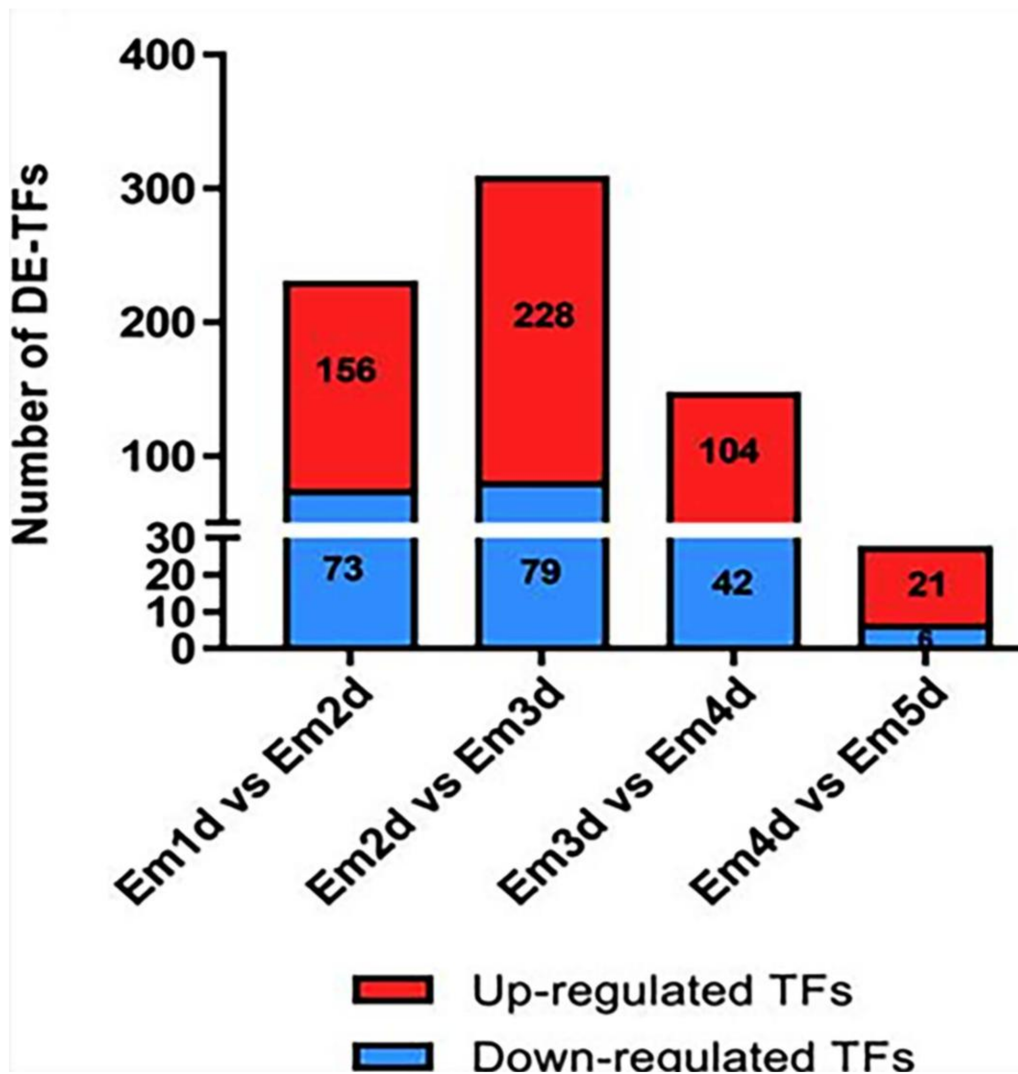

Figure 3. Transcription factors, namely DE-TFs for early stages of embryogenesis. A. External imaging: the avian genome's karyotype, the expression of transcription factors, the coding of specific transcription factors, and the link between transcription factor families. B. DE-TFs were identified during the change from Em1d to Em5d in the embryo. Red shows transcription factors that are up-regulated, while blue shows transcription factors that are down-regulated

Identification of key genes involved in embryonic development for germ layer formation in chicken embryos: In chicken embryos, 1,134 transcription factors have been identified. In the present study 1,097 transcription factors were detected during early embryonic development in chickens. These genes are not randomly distributed throughout the genome; instead, they are topologically organized. Previous studies have shown that genes exhibiting a

certain expression pattern are in proximity (Ayers et al., 2015; Sybirna et al., 2020). This study's findings indicated that Homeobox, bHLH, and Zf-C2H2, three genes involved in embryonic development and protein synthesis, are among the most highly expressed transcription factors during early vertebrate embryonic development (Table 2). Zf-C2H2 constitutes the predominant element of several DNA-binding domains and contributes significantly to the diversity of encoded proteins that govern differentiation and development throughout the early embryonic stage, as shown by prior research (Tam & Zhou, 1996; Okuzaki et al., 2019). The Homeobox family, including around 60 amino acids encoded by Hox genes, functions as a crucial transcription factor family for developmental processes, owing to its significant functions in cell fate determination and differentiation (Hisey et al., 2021). Pivotal transcription factors, including ISL1, MKX, NANOG, and CDX2, involved in chicken embryo development, are part of the Homeobox family (Table 2). bHLH factors are associated with pluripotency and proliferation, influencing and regulating the destiny of somatic cells, especially neurons (Sidrat et al., 2020; Hisey et al., 2021).

Table 2. List of pivotal transcription factors discovered in the development of chicken embryos

Symbol	NCBI Gene ID	Chromosome	TF family
NANOG	100,272,166	1	Homeobox
CDX2	374,205	1	Homeobox
ISL1	396,383	Z	Homeobox
SOX2	396,105	9	HMG
OLIG2	428,612	1	bHLH
GATA6	396,390	2	zf-GATA
ZBTB16	458389	26	Zf-C2H2
MKX	771,284	2	Homeobox
THRB	396,431	2	THR-like
ZBTB15	419,759	24	Zf-C2

The cranial-caudal polarity and the polarity of individual cell groups inside the somite were evaluated via bHLH-type transcription factors. The research findings confirm that bHLH factors were notably expressed on days 4 and 5 and are primarily responsible for the comprehensive

development of the nervous system (Table 3). Moreover, 164 embryonic transcription factors were consistently and robustly expressed across all developmental stages, demonstrating their shared and essential role in growth and differentiation throughout the embryonic phase of chickens (Table 3).

Table 3. Validation of selected key transcription factors by RT-qPCR. Heatmaps were generated using qPCR and RNA-seq data for 16 selected genes. The heatmap illustrates the correlation between relative expression levels of RNA-seq and qPCR data, shown as $\log_2$ expression values normalized to the mean. Red signifies elevated gene expression levels, while blue denotes diminished gene expression levels with relation to the mean of all samples, respectively (ranging from +2.0 to −2.0). Statistical differences are determined by the False Discovery Rate (FDR) for RNA-Seq data and the P-value for quantitative PCR data ($P < 0.05$)

TF	Technology	Em1d	Em2d	Em3d	Em4d	Em5d	FDR RNA-seq/ *P* value qPCR			
							Em1d vs Em2d	Em2d vs Em3d	Em3d vs Em4d	Em4d vs Em5d
NANOG	RT-qPCR						1.11E-03	9.62E-06	—	—
	RNA-seq						8.71E-11	4.86E-13		
SOX9	RT-qPCR						2.81E-03	4.93E-05	5.79E-04	—
	RNA-seq						3.22E-04	1.48E-03	4.30E-10	
CDX2	RT-qPCR						1.14E-05	1.03E-06	2.50E-05	—
	RNA-seq						7.92E-04	2.51E-05	1.72E-11	
SMAD3	RT-qPCR						7.36E-05	—	8.72E-04	—
	RNA-seq						7.74E-23		1.94E-18	
SOX10	RT-qPCR						3.60E-04	8.54E-04	—	—
	RNA-seq						3.94E-11	3.76E-06		
PAX6	RT-qPCR						4.54E-05	8.00E-03	—	—
	RNA-seq						2.90E-16	6.70E-04		
SOX2	RT-qPCR						—	3.50E-03	—	—
	RNA-seq							2.07E-11		

The results of the present study showed that deletion of the PRDM14 gene in chickens mainly leads to gastrulation abnormalities, and this abnormality leads to the death of the chicken. This indicates that the PRDM14 gene can be introduced as a factor involved in the vital embryonic process of germ layer formation in bird embryos. Therefore, it can be said that this gene has an important and functional role in the early development of all vertebrates. Its expression in the ectoderm of humans and primates, as documented by previous research (Glover, 2013; Zhao et al., 2016), demonstrates its unequivocal role in the development of primitive streaks in

vertebrates. This study further indicates that the loss of PRDM14 leads to a reduction in the expression of pluripotency factors, including NANOG (Zhao et al., 2016). This transpires mostly via the control of epigenetic pathways and developmental signaling pathways, including FGF signaling.

The results of the present study showed that the reduction of PRDM14 levels in chicken primordial germ cells (PGCs) specifically leads to an increase in pERK levels. It has been suggested that this increase is necessary to promote the differentiation and development of PGCs into somatic lineages. However, to draw more definitive conclusions and recommendations, comprehensive studies on the molecular interaction between PRDM14 and fibroblast growth factor (FGF) signaling in chicken ectoderm are needed. It is possible that impaired or reduced response to FGF signals in the ectoderm of developing embryos could lead to a disruption in the establishment of local chemotaxis gradients essential for proper morphogenesis. Furthermore, loss of PRDM14 expression in chicken embryos could be associated with the failure to initiate primordial streak formation, a critical event in early. Previous findings further suggest that this disruption may be linked to an abnormal upregulation of the NODAL gene, observed exclusively in embryos deficient in PRDM14 (Choi et al., 2021).

These studies using a genetically deficient chicken model illuminate the specific involvement of PRDM14 in gastrulation. PRDM14 is critical for early embryonic development and survival of primordial germ cells (PGC) in chickens. The unique pattern of expression observed during embryogenesis, especially its presence in neural plate and ectodermal tissue, strongly suggests that it can also play a role in the development of the central nervous system, in addition to its known roles to maintain the pluripotent and conduct germ cell line obligation. This double expression pattern suggests that PRDM14 may still have an ancestral function related to neurogenesis in chickens, an evolutionary connection between neurogenesis and germline specification. Dysregulation of this gene could therefore alter or weaken germ cell regulatory mechanisms.

The results of this study suggest that the chicken can be used as a valuable comparative model to investigate the evolutionary trajectory of PRDM14 function in vertebrates. It is still unclear whether the neurogenic role of PRDM14 is an ancestrally conserved feature or a lineage-specific adaptation. Further cross-species analyses, particularly in other lineages of birds and reptiles, are needed to clarify this issue. The neural plate in zebrafish plays an important role in motor neuron specification and development. In this regard, the results of studies have shown that the PRDM14 gene is exclusively expressed in the neural plate during early embryogenesis, although no detectable expression in germ cells has been reported at these stages (Lee et al., 2016; Silva et al., 2018). This finding reinforces the hypothesis that PRDM14's original and perhaps

most conserved function lies in neurodevelopmental regulation (Zhao et al., 2016; Yu et al., 2019; Hagihara et al., 2020). Collectively, these findings expand our understanding of PRDM14's molecular and developmental roles but also raise compelling questions about how this transcription factor may have diversified functionally across vertebrate evolution, potentially shifting from a neural to a germline context, or vice versa.

Conclusion: This study found that a specific group of genes play acritical role in the early development of chicken embryos. The three primary protein-coding gene families that contribute significantly to important developmental processes were Homeobox, basic Helix-Loop-Helix (bHLH), and Zinc Finger-C2H2 (Zf-C2H2). These families oversee embryogenesis, protein synthesis, and transcription factor activity. Ensuring proper cell differentiation and tissue formation. The importance of this gene PRDM14 in this regulatory system is very significant. Because it is essential for the early development of the embryo. The formation of the three primary germ layers, namely ectoderm, mesoderm and endoderm, occurs during gastrulation. The molecular mechanisms governing this process have a profound influence on how the germ layers are formed. PRDM14 seems to affect lineage determination through transcription, as well as the spatial organization and movement of cells during gastrulation. It's vital to remember that not having enough PRDM14 or having less of it could cause major developmental difficulties and kill embryos. The main explanation for this result is issues with gastrulation. This suggests that the presence of this gene and its roles are crucial for maintaining embryo survival during this stage. It can be concluded that the function of PRDM14 at the molecular level is an important regulatory node in avian embryonic development.

Author Contributions

All authors contributed equally to the conception analysis and writing of this manuscript.

Data Availability Statement

All data supporting the findings of this study are publicly available.

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The authors have no acknowledgements to declare.

Ethical Considerations

The research was performed in accordance with the guidelines and approval of the Professional Ethics Committee, College of Veterinary Medicine, University of Basrah.

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بررسی بیان ژنی تنظیم‌کننده‌های کلیدی تشکیل لایه‌های زاینده در جنین‌های جوجه با استفاده از تکنیک RT-PCR

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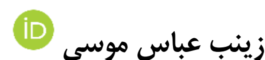
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چکیده

هدف: مطالعات مختلف نشان داده‌اند که ژن PRDM14 نقش بسیار مهم و بنیادی در رشد اولیه جنین جوجه و تشکیل لایه‌های زاینده دارد. هدف این مطالعه بررسی الگوی بیان ژن PRDM14 در مراحل اولیه تکوین جنینی و همچنین بررسی نقش تکاملی آن بود. این پژوهش همچنین به دنبال ساده‌سازی شناسایی خانواده‌های اصلی فاکتورهای رونویسی فعال در پنج روز نخست رشد جنینی است.

مواد و روش‌ها: برای مقایسه PRDM14 با سایر اعضای خانواده PRDM، از تحلیل فیلوژنتیک استفاده شد. همچنین، پروفایل‌گذاری مقایسه‌ای بیان ژن برای بررسی فعالیت ژنی در مراحل E1 تا E5 انجام شد که نمایانگر اولین و حساس‌ترین مراحل رشد جنین جوجه هستند. از مجموعه داده‌های عمومی ژنومی و خانواده‌های ژنی حاشیه‌نویسی‌شده برای شناسایی فاکتورهای رونویسی و تعیین توزیع آن‌ها در سراسر ژنوم استفاده شد.

نتایج: تحلیل‌ها نشان داد که ژن PRDM14 و سایر ژن‌های خانواده PRDM الگوی واگرایی تکاملی مشابهی با سایر مهره‌داران دارند. مراحل E1 تا E5 به‌عنوان حیاتی‌ترین مراحل تکوین جنینی شناسایی شدند و تغییرات عمده‌ای در بیان ژن در این دوره‌ها مشاهده شد. در بین جنین‌های بررسی‌شده، ۱۰۹۷ فاکتور رونویسی شناسایی شد. این ژن‌ها دارای توزیع تصادفی نبودند، بلکه الگوی ساختاری ژنومی معناداری داشتند. خانواده‌های فاکتور رونویسی مانند bHLH، Zf-C2H2 و Homeobox نیز شناسایی شدند

که نقش مهمی در رشد اولیه دارند. پروتئین‌های هومئوباکس از ۶۰ اسید آمینه تشکیل شده‌اند و برای تنظیم بسیاری از فرآیندهای تکوینی ضروری هستند. فاکتورهای رونویسی ISL1، MKX، NANOG و CDX2 شناسایی و در گروه‌های Homeobox و bHLH طبقه‌بندی شدند. این فاکتورها در تعیین سرنوشت سلولی، تشکیل دودمان‌های سوماتیک و پلوری‌پوتنسی نقش دارند. در روزهای ۴ و ۵، گروه bHLH بیان بالایی نشان داد که نقش اساسی در تکوین دستگاه عصبی و سلول‌های زایشی دارد.

نتیجه‌گیری: این مطالعه نشان می‌دهد که مراحل اولیه تکوین جنینی شامل فعالیت هماهنگ خانواده‌های اصلی فاکتورهای رونویسی است. الگوی بیان ساختاریافته و فعال‌سازی زودهنگام PRDM14 نشان‌دهنده نقش اساسی آن در هدایت هویت سلولی و مسیرهای تکوینی در جنین‌های جوجه است.

کلمات کلیدی: تکوین جنینی، جنین جوجه، سلول‌های زایشی، هومئوباکس، PRDM14

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