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Epigenetic programming of thermal resilience in poultry: From embryonic heat conditioning to *trans*-generational inheritance

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Abstract
Heat stress is a major challenge to poultry production, adversely affecting growth, reproduction, immunity and physiological homeostasis. Embryonic thermal conditioning has emerged as a potential strategy for improving thermal resilience through developmental programming. This review examines the epigenetic mechanisms underlying this adaptation, focusing on DNA methylation, histone modifications and non-coding RNAs. It also discusses the roles of heat-shock proteins, hypothalamic and neuro-endocrine regulation, oxidative stress, mitochondrial function and immune responses. Particular attention is given to maternal and paternal contributions to offspring thermal responses and the possible involvement of germ-line epigenetic modifications. The implications of thermal conditioning for growth performance, hatchability and poultry productivity are evaluated. Despite promising findings, variations in conditioning protocols, limited functional validation and insufficient multigenerational evidence remain important research gaps. Integrated multi-omics approaches and long-term studies are needed to clarify the mechanisms of thermal memory and support the development of effective strategies for heat-resilient poultry production.

Keywords: Poultry, Heat stress, Programming, DNA methylation, Histone modifications, Micro RNAs, Thermal resilience

1. Introduction

Heat stress is a major environmental challenge affecting poultry health, productivity and welfare, particularly in tropical and subtropical regions. Modern commercial poultry breeds, selected for rapid growth and high production efficiency, are particularly vulnerable to elevated environmental temperatures because of their high metabolic heat production and limited capacity for heat dissipation. Prolonged exposure to high temperatures disrupts physiological homeostasis, reduces feed intake, impairs nutrient utilization and negatively affects growth, reproductive performance and immune function (Lara *et al.*, 2013; Wasti *et al.*, 2020) [1, 2]. At the cellular level, heat stress stimulates heat-shock protein expression, alters endocrine activity and increases oxidative stress, resulting in disturbances in metabolic and immune regulation (Akbarian *et al.*, 2016) [3]. Recent research indicates that thermal resilience may be influenced not only by genetic selection and post-hatch management but also by environmental conditions experienced during embryonic development. Embryonic heat conditioning involves controlled exposure to elevated incubation temperatures during specific developmental stages to improve subsequent thermal adaptation. Such manipulation can modify thermoregulatory responses, metabolic activity and heat-shock protein expression, potentially improving post-hatch tolerance to high ambient temperatures (Al Amaz *et al.*, 2024) [4]. These findings have highlighted embryonic development as an important period for establishing physiological responses to environmental stress.

The molecular mechanisms underlying this developmental adaptation are increasingly associated with epigenetic regulation. Epigenetic modifications, including DNA methylation, histone modifications and non-coding RNA-mediated regulation, influence gene expression without altering the underlying DNA sequence. These mechanisms may regulate genes involved in heat-shock responses, oxidative defense, immune function and thermoregulation. Experimental evidence from chickens has demonstrated that embryonic heat conditioning can induce changes in DNA methylation and gene expression associated with improved thermal and immunological resilience in offspring (Rosenberg *et al.*, 2022) [5]. Similarly, maternal heat stress has been associated with alterations in embryonic DNA methylation patterns and gene expression, suggesting that parental environmental conditions may influence early developmental programming (Karami *et al.*, 2025) [6]. Recent investigations have further extended this concept towards paternal contributions to thermal adaptation. Ravi *et al.* (2026) [7] reported improved thermal resilience in offspring of heat-conditioned male

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chickens and identified miR-210a as a potential regulator of this response. These findings provide emerging evidence that epigenetic mechanisms may contribute to the transmission of thermal adaptation-associated characteristics across generations. However, the distinction between developmental programming, intergenerational effects and true *trans*-generational inheritance remains important; as the stability and mechanisms of inherited epigenetic modifications have not yet been fully established. The present review therefore examines the molecular mechanisms underlying epigenetic programming of thermal resilience in poultry, focusing on embryonic heat conditioning, developmental thermal memory and emerging evidence of intergenerational and *trans*-generational inheritance. It also identifies current research limitations and future opportunities for applying epigenetic knowledge to improve poultry adaptation under increasing climatic challenges.

2. Heat stress and thermal adaptation in poultry

Heat stress develops when environmental heat load exceeds the bird's capacity to maintain body temperature through physiological and behavioral mechanisms. Chickens are particularly susceptible because of their feather coverage, absence of sweat glands and high metabolic heat production. Under elevated temperatures, birds increase respiratory evaporation through panting, enhance peripheral heat dissipation and reduce physical activity and feed consumption. Although these responses help maintain thermal balance, prolonged exposure can disturb acid-base regulation, nutrient metabolism and physiological homeostasis (Lara *et al.*, 2013; Mangan *et al.*, 2024) [1, 8]. Thermal adaptation involves coordinated physiological and biochemical responses that improve the ability of birds to tolerate elevated temperatures. Activation of the hypothalamic pituitary adrenal axis, alterations in thyroid hormone activity and increased expression of heat-shock proteins, particularly HSP_{70} and HSP_{90}, contribute to stress management and cellular protection. Antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase, also participate in controlling oxidative damage. However, prolonged heat exposure may exceed the capacity of these protective systems, resulting in mitochondrial dysfunction, intestinal barrier damage and impaired immune responses (Akbarian *et al.*, 2016; Wasti *et al.*, 2020) [3, 2].

Thermal tolerance also varies considerably among poultry genotypes. Indigenous chickens and certain heat-adapted genetic lines possess characteristics that support survival under high-temperature conditions, whereas rapidly growing commercial broilers may experience greater thermal challenges because of their higher metabolic heat production. Genetic differences in body size, feather characteristics, metabolic activity and stress-response pathways contribute to this variation. Consequently, genetic selection for thermo-tolerance, together with appropriate environmental and nutritional management, represents an important strategy for improving poultry resilience under changing climatic conditions (Schmidt *et al.*, 2025) [9].

3. Concept of epigenetic programming and thermal memory

Epigenetic programming refers to environmentally induced changes in gene expression without alterations in the DNA sequence. In poultry, embryonic thermal exposure may influence DNA methylation, histone modifications and non-coding RNA expression, thereby regulating genes associated with thermoregulation, metabolism and cellular stress responses (Bednarczyk *et al.*, 2021) [10]. Thermal memory represents the persistence of molecular changes following early temperature exposure, influencing responses to subsequent heat challenges. David *et al.* (2019) [11] reported alterations in H_{3K4me3} and $H_{3K27me3}$ histone marks in the chicken hypothalamus following embryonic thermal manipulation. Similarly, Vinoth *et al.* (2018) [12] demonstrated changes in DNA methylation and heat-shock protein expression. While the former study focused on chromatin modifications, the latter highlighted the involvement of DNA methylation in thermal adaptation.

Evidence of longer-term effects has also emerged from offspring studies. Rosenberg *et al.* (2022) [5] reported improved thermal and immunological resilience in offspring of heat-conditioned chickens, while Ravi *et al.* (2026) [7] identified miR-210a as a potential regulator of paternal transmission of thermal resilience (Table 1).

Table 1. Experimental studies on embryonic thermal conditioning and its effects on physiological, molecular and intergenerational thermal resilience in poultry

S. No.	Study/model	Thermal conditioning or experimental approach	Developmental stage/exposure	Parameters investigated	Major findings	Biological significance	References
1	Broiler chicken embryos	Intermittent elevated incubation temperature	Embryonic development	Thermo-tolerance, mortality and corticosterone	Improved thermo-tolerance and altered corticosterone response following heat challenge	Demonstrated the potential of embryonic conditioning	Piestun *et al.* (2008) [13]
2	Broiler chicken embryos	Thermal manipulation during incubation	Embryogenesis	Thermoregulation, thyroid hormones and development	Altered thermoregulatory development and endocrine responses	Indicated the importance of developmental timing	Piestun *et al.* (2009) [17]
3	Thermally manipulated broilers	Embryonic thermal manipulation	Embryogenesis and post-hatch assessment	DNA methylation and HSP expression	Altered methylation and expression of heat-shock-related genes	Provided evidence of epigenetic regulation	Vinoth *et al.* (2018) [12]
4	Chicken hypothalamus	Embryonic thermal manipulation	Embryogenesis	H_{3K4me3} and $H_{3K27me3}$ histone marks	Differential histone-mark distribution in hypothalamic regions	Demonstrated chromatin-level changes	David *et al.* (2019)
5	Broiler chickens	Embryonic thermal manipulation followed by heat challenge	Embryogenesis and post-hatch heat exposure	Antioxidant and oxidative-stress-related gene expression	Modified expression of genes involved in oxidative-stress responses	Suggested altered cellular stress regulation	Al-Zghoul *et al.* (2019) [18]
6	Broiler chickens	Embryonic thermal manipulation	Embryogenesis and post-hatch heat exposure	Cytokine and inflammatory gene expression	Altered expression of immune-related cytokines during heat exposure	Indicated modulation of immune responses	Saleh *et al.* (2019) [19]

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S. No.	Study/model	Thermal conditioning or experimental approach	Developmental stage/exposure	Parameters investigated	Major findings	Biological significance	References
7	Chicken hypothalamus	Embryonic thermal manipulation	Thermoregulatory development	*miR*-138, EZH2 and H3K27 methylation	*miR*-138 regulated EZH2 expression and histone methylation	Linked microRNA activity with hypothalamic development	Kisliouk *et al.* (2011) [15]
8	Chickens and their offspring	Embryonic heat conditioning	Parental conditioning and offspring assessment	DNA methylation, hypothalamic gene expression and immune responses	Reported improved thermal and immune resilience in offspring	Suggested intergenerational epigenetic effects	Rosenberg *et al.* (2022)
9	Heat-conditioned male chickens and offspring	Paternal embryonic heat conditioning	Parental conditioning and offspring assessment	Sperm DNA methylation and miR-200a	Identified epigenetic associations with paternal transmission of immune cross-tolerance	Highlighted a possible germline-mediated mechanism	Ravi *et al.* (2025) [16]
10	Heat-conditioned male chickens and offspring	Paternal heat conditioning	Parental conditioning and offspring assessment	miR-210a and hypothalamic regulation	Identified miR-210a-associated changes linked with offspring thermal resilience	Suggested a role for microRNA-mediated regulation	Ravi *et al.* (2026)

4. Embryonic heat conditioning: Developmental windows and physiological responses

Embryonic heat conditioning, commonly termed thermal manipulation (TM), involves exposing developing poultry embryos to a controlled increase in incubation temperature for a specific period. In broiler chickens, the commonly studied protocol involves increasing eggshell temperature to approximately 39.5 °C for 12 h/day between embryonic days 7 and 16, a period associated with the development of the hypothalamic pituitary thyroid and adrenal systems. The timing is important because the embryo's capacity to respond to thermal signals develops progressively; exposure that is too early, intense or prolonged may disturb normal development rather than promote adaptation (Piestun *et al.*, 2008; Al Amaz *et al.*, 2024) [13, 4].

The physiological effects of TM depend on exposure intensity and duration. Piestun *et al.* (2008) [13] found that intermittent exposure (12 h/day) improved thermo-tolerance and reduced cortico-sterone responses and mortality during subsequent heat challenge, whereas continuous exposure (24 h/day) reduced hatchability and impaired chick development. Similarly, Leão *et al.* (2024) [14] reported that increasing incubation temperature to 39 °C for 3 h on embryonic days 16–18 influenced post-hatch thermoregulation and HSP_{70} levels, while 24-h exposure reduced hatchability. These findings indicate that appropriately timed, moderate thermal exposure can support physiological adaptation, but excessive exposure may compromise embryonic development and hatch success.

5. Molecular mechanisms of epigenetic regulation

Embryonic thermal conditioning may influence long-term heat adaptation through coordinated changes in DNA methylation, histone modifications and non-coding RNA expression. These mechanisms regulate the accessibility of genes involved in thermoregulation, cellular protection, metabolism and stress responses without changing the underlying DNA sequence. Evidence from chicken studies indicates that these pathways interact rather than operate independently, although their precise contributions to persistent thermal resilience are still being investigated.

5.1 DNA methylation and demethylation

DNA methylation involves the addition of methyl groups to cytosine residues, mainly at CpG sites, through DNA methyl transferases (DNMTs). Promoter methylation is generally associated with reduced transcription, whereas demethylation can restore gene activity. In thermally manipulated chickens, Vinoth *et al.* (2018) [12] reported altered methylation in heat-shock protein (HSP) gene promoters alongside changes in their expression. Birds exposed to a later heat challenge showed lower HSP promoter methylation and higher expression in the non-conditioned group, whereas embryonically conditioned birds showed a different expression pattern, consistent with improved thermo-tolerance (Fig 1).

Genome-wide evidence also supports the involvement of DNA methylation in thermal programming. Recent work by Ravi *et al.* (2026) [7] identified differential CpG methylation near microRNA genes in the hypothalamus of offspring from heat-conditioned male chickens, highlighting a possible link between paternal thermal exposure and offspring gene regulation. However, methylation changes at individual loci do not, by themselves, establish stable inheritance; their functional significance requires validation across generations.

5.2 Histone modifications and chromatin remodeling

Histone modifications regulate gene activity by changing chromatin structure and the accessibility of transcriptional machinery. Histone H3 lysine 4 trimethylation (H_{3K4me3}) is commonly associated with active transcription, whereas $H_{3K27me3}$ is generally linked to gene repression. David *et al.* (2019) [11] identified 785 differential H_{3K4me3} peaks and 148 differential $H_{3K27me3}$ peaks in the hypothalamus of thermally manipulated chickens. These changes involved genes associated with neurodevelopment, metabolism and gene regulation, suggesting that embryonic temperature exposure can establish persistent chromatin-level changes relevant to later thermal responses.

Chromatin remodeling proteins and histone-modifying enzymes may further connect these changes with transcriptional regulation. The recent identification of ARID5B and other chromatin-associated genes in the *miR*-210a regulatory network of heat-conditioned chickens provides additional evidence of interaction between non-coding RNAs and chromatin organization. Nevertheless, the causal contribution of individual histone marks to improved thermo-tolerance remains to be established experimentally.

5.3 MicroRNAs and other non-coding RNAs

MicroRNAs (miRNAs) are short, non-coding RNA molecules, generally 18–24 nucleotides long that regulate gene expression by binding to target mRNAs and inhibiting their translation or promoting their degradation. In chickens, *miR*-138 has been shown to regulate the histone methyl transferase EZH2 during the critical period of thermoregulation development. Increased *miR*-138 expression reduced EZH2 levels and H3K27 methylation, while experimental *miR*-138 administration disrupted normal thermoregulatory development, demonstrating that miRNA activity can influence thermal programming through histone-modifying enzymes (Kisliouk *et al.*, 2011) [15].

More recent studies have identified miR-200a and miR-210a as candidate regulators of heat-conditioning responses. Ravi *et al.* (2025) [16] associated miR-200a regulation and sperm DNA methylation with pa

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ternal transmission of immune cross-tolerance, while Ravi *et al.* (2026) [7] linked *miR*-210a expression and differential methylation near its regulatory region with offspring thermal resilience. These findings suggest that miRNAs may connect environmental exposure with gene regulation across generations. However, evidence for long non-coding RNAs and circular RNAs in embryonic thermal conditioning of poultry remains limited.

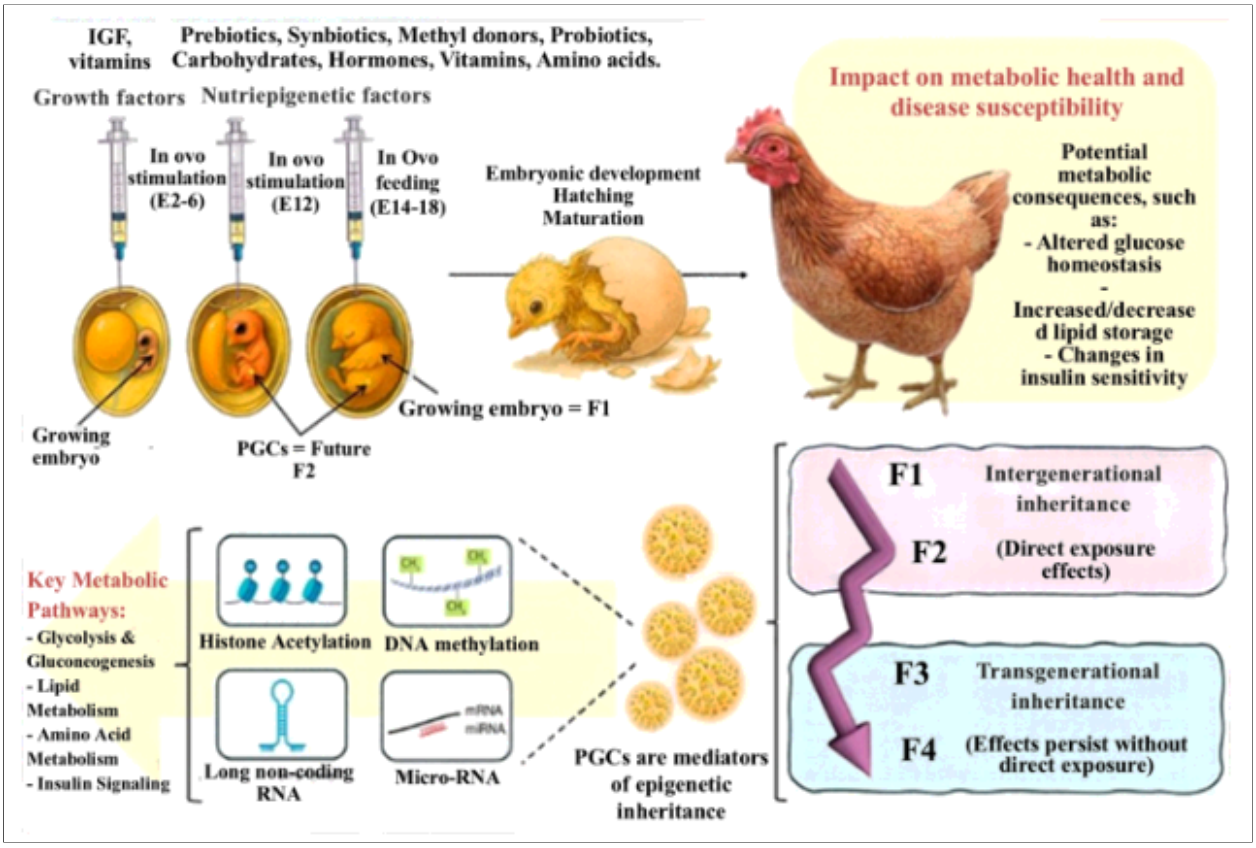


Figure 1. Conceptual framework of epigenetic programming of thermal resilience in poultry

5.4 Epigenetic regulation of heat-shock proteins

Heat-shock proteins, particularly HSP_{70} and HSP_{90}, protect cells by assisting protein folding, preventing protein aggregation and supporting the removal of damaged proteins. Their expression is regulated by heat-shock factors, especially HSF1, which activates heat-responsive genes. Epigenetic modifications can influence this response by altering promoter accessibility and transcriptional activity. In thermally manipulated chickens, Vinoth *et al.* (2018) [12] reported differences in HSP promoter methylation, promoter activity and gene expression between conditioned and non-conditioned birds during subsequent heat exposure.

Embryonic thermal manipulation has also been associated with altered post-hatch expression of HSP_{27}, HSP_{60}, HSP_{70} and HSP_{90}, together with changes in promoter methylation. Lower HSP expression in conditioned birds under later heat challenge may reflect reduced cellular stress rather than weakened protection; therefore, HSP expression should be interpreted alongside body temperature, oxidative damage and physiological performance. Overall, epigenetic regulation of HSP genes appears to contribute to thermal adaptation, although the relative roles of DNA methylation, histone modifications and non-coding RNAs require further functional investigation.

6. Hypothalamic and neuroendocrine programming of thermotolerance

The hypothalamus, particularly the preoptic anterior hypothalamus (PO/AH), plays a central role in detecting temperature changes and coordinating physiological responses. It regulates thermoregulatory behaviour, panting, peripheral heat dissipation and endocrine activity through interactions with the hypothalamic pituitary thyroid (HPT) and hypothalamic–pituitary–adrenal (HPA) axes. Embryonic thermal manipulation during development of these systems can modify their functional set points. Piestun *et al.* (2009) [17] reported reduced thyroid hormone concentrations and altered corticosterone profiles in thermally manipulated embryos, indicating changes in endocrine regulation and embryonic metabolic activity.

Long-term effects are also reflected in hypothalamic gene expression. Rosenberg *et al.* (2022) [5] identified differential gene expression and DNA methylation in the anterior preoptic hypothalamus of offspring from heat-conditioned chickens, involving genes associated with stress responses and thermoregulation. More recently, Ravi *et al.* (2026) [7] reported increased baseline *miR*-210a expression in the PO/AH of heat-conditioned chickens and their offspring, suggesting a role for this regulatory pathway in thermal resilience. These findings indicate that embryonic conditioning may influence hypothalamic responsiveness through both neuroendocrine and epigenetic mechanisms.

7. Oxidative stress, mitochondrial function and immune regulation

Heat exposure increases cellular reactive oxygen species (ROS), which can damage lipids, proteins and DNA when antioxidant defences are insufficient. Embryonic thermal manipulation has been associated with altered expression of antioxidant-related genes, including SOD_2, GPX_2, CAT and NOX_4. Al-Zghoul *et al.* (2019) [18] observed reduced expression of several oxidative-stress-associated genes during subsequent acute heat exposure in thermally manipulated broilers, although antioxidant capacity and enzyme activities also varied. These results suggest that conditioning modifies the oxidative response rather than simply increasing antioxidant activity. Mitochondria are important in this process because they regulate cellular energy production and ROS generation; however, direct evidence connecting embryonic conditioning with persistent mitochondrial functional changes remains limited.

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Thermal conditioning may also modify immune responses by regulating cytokines and inflammatory signalling. Al-Zghoul *et al.* (2019) [19] reported altered splenic expression of interferons and interleukins during acute heat stress, indicating changes in immune-response dynamics. Rosenberg *et al.* (2022) [5] further demonstrated reduced inflammatory responses in offspring of heat-conditioned male chickens following lipopolysaccharide (LPS) challenge, accompanied by altered expression of $SOCS_3$ and heat-shock-related genes. Together, these findings suggest that thermal conditioning may promote coordinated regulation of oxidative and immune responses, although outcomes depend on the conditioning protocol and subsequent stress challenge.

8. Intergenerational and transgenerational transmission of thermal resilience

Embryonic heat conditioning may influence not only the exposed bird but also the thermal and immune responses of its offspring. Such effects may involve epigenetic information carried through germ cells, including sperm DNA methylation, regulatory non-coding RNAs and changes in chromatin organisation. Maternal and paternal contributions need to be distinguished because embryonic exposure can affect developing reproductive cells differently from later-life exposure of mature parents.

8.1 Maternal and paternal contributions

Rosenberg *et al.* (2022) [5] demonstrated that offspring of heat-conditioned male and female chickens exhibited improved thermal and immunological resilience. The study also identified a paternal contribution, supported by altered hypothalamic DNA methylation and gene expression in offspring. More recently, Ravi *et al.* (2025) [16] demonstrated paternal transmission of immune cross-tolerance using sperm from heat-conditioned males to produce offspring through artificial insemination of untreated hens. These findings suggest that paternal epigenetic mechanisms may contribute to the transmission of adaptive responses.

8.2 Germline epigenetic modifications

DNA methylation and non-coding RNAs are potential carriers of environmentally induced information through the germline. Ravi *et al.* (2025) [16] identified differential DNA methylation in sperm from heat-conditioned males and associated these changes with immune-related genes and *miR*-200a regulation in offspring. Similarly, Ravi *et al.* (2026) [7] identified *miR*-210a as a candidate regulator associated with paternal transmission of thermal resilience. These studies provide evidence linking sperm epigenetic profiles with offspring responses, although the stability of these modifications during fertilisation and early embryonic development requires further investigation (Table 2).

Table 2. Epigenetic and molecular mechanisms regulating thermal resilience in poultry

S. No.	Regulatory mechanism	Major molecular components	Epigenetic modification or regulatory process	Target pathways/biological functions	Potential effects on thermal resilience	Evidence status in poultry	References
1	DNA methylation	DNMT1, DNMT3A, DNMT3B and TET enzymes	Addition or removal of methyl groups at CpG sites	Gene transcription and cellular stress responses	Modulation of heat-responsive gene expression	Direct evidence reported	Vinoth *et al.* (2018) [12]; Rosenberg *et al.* (2022)
2	Histone methylation	H_{3K4me3}, $H_{3K27me3}$, EZH2	Histone methylation and chromatin regulation	Transcriptional activation or repression	Regulation of hypothalamic development and thermoregulatory genes	Direct evidence reported	David *et al.* (2019); Kisliouk *et al.* (2011) [15]
3	Histone acetylation	Histone acetyltransferases (HATs), histone deacetylases (HDACs)	Addition or removal of acetyl groups	Chromatin accessibility and transcription	Potential regulation of stress-response genes	Requires further validation in thermal conditioning	General epigenetic mechanism; poultry-specific evidence limited
4	MicroRNA regulation	miR-138	Post-transcriptional regulation of EZH2	Hypothalamic development and histone methylation	Influences development of thermoregulatory mechanisms	Direct experimental evidence	Kisliouk *et al.* (2011) [15]
5	MicroRNA regulation	miR-200a	Regulation of target mRNAs and association with sperm methylation	Immune-response pathways	Potential contribution to paternal transmission of immune resilience	Recent experimental evidence	Ravi *et al.* (2025) [16]
6	MicroRNA regulation	*miR*-210a	Post-transcriptional gene regulation	Hypothalamic and thermal-response pathways	Associated with offspring thermal resilience	Recent experimental evidence	Ravi *et al.* (2026)
7	Heat-shock protein regulation	HSF1, HSP_{27}, HSP_{60}, HSP_{70} and HSP_{90}	Transcriptional regulation and epigenetic changes at HSP-associated regions	Protein folding and cellular protection	Maintenance of protein stability during heat exposure	Direct evidence reported for HSP expression and methylation	Vinoth *et al.* (2018) [12]
8	Hypothalamic regulation	PO/AH, HPT axis and HPA axis	Developmental and epigenetic regulation of neuroendocrine pathways	Thermoregulation, thyroid activity and corticosterone response	Modification of physiological responses to heat	Physiological and molecular evidence reported	Piestun *et al.* (2009) [17]; Rosenberg *et al.* (2022)

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S. No.	Regulatory mechanism	Major molecular components	Epigenetic modification or regulatory process	Target pathways/biological functions	Potential effects on thermal resilience	Evidence status in poultry	References
9	Oxidative-stress regulation	SOD, CAT, GPx and ROS	Regulation of antioxidant-response pathways	Cellular redox balance and protection against oxidative damage	May reduce oxidative injury during heat exposure	Physiological and gene-expression evidence reported	Al-Zghoul *et al.* (2019) [18]
10	Immune regulation	ILs, IFNs, $SOCS_3$ and inflammatory mediators	Regulation of cytokine signalling and immune-related gene expression	Inflammatory responses and immune homeostasis	May improve immune tolerance under thermal stress	Direct experimental evidence reported	Saleh *et al.* (2019) [19]; Rosenberg *et al.* (2022)
11	Germline epigenetic regulation	Sperm DNA methylation and regulatory miRNAs	Transmission of epigenetic information through paternal germ cells	Offspring gene regulation and immune responses	Potential intergenerational transmission of thermal resilience	Emerging experimental evidence	Rosenberg *et al.* (2022); Ravi *et al.* (2025) [16]
12	Long non-coding RNA regulation	lncRNAs	Transcriptional and post-transcriptional regulation	Gene-expression networks and chromatin organisation	Potential involvement in long-term thermal adaptation	Insufficient direct evidence in poultry thermal conditioning	Further investigation required
13	Circular RNA regulation	circRNAs and miRNA interaction networks	miRNA sequestration and post-transcriptional regulation	Cellular stress and gene-expression pathways	Possible contribution to thermal-response regulation	Insufficient direct evidence in poultry thermal conditioning	Further investigation required
14	Mitochondrial regulation	Mitochondrial DNA, respiratory-chain proteins and ROS	Regulation of mitochondrial gene expression and redox signalling	ATP production and cellular energy metabolism	Potential maintenance of cellular energy balance under heat stress	Direct links with embryonic epigenetic conditioning remain	

8.3 Molecular evidence across generations

Molecular evidence supports the involvement of DNA methylation, microRNAs and chromatin organisation in inherited thermal responses. Rosenberg *et al.* (2022) [5] identified thousands of differentially methylated sites in offspring hypothalamic tissue, particularly in enhancer regions and CCCTC-binding factor (CTCF) binding sites. Their analysis linked methylation changes with genes involved in immune responses and protein-folding pathways, including HSP_{25} and $SOCS_3$. Ravi *et al.* (2025) [16] further associated sperm methylation patterns and *miR*-200a expression with paternal transmission of immune resilience, while Ravi *et al.* (2026) [7] implicated *miR*-210a in offspring thermotolerance. Collectively, these findings provide promising evidence of epigenetic transmission; however, additional multigenerational studies are required to establish stable transgenerational inheritance and its molecular mechanisms.

9. Implications for growth, reproduction and poultry productivity

Embryonic thermal manipulation (ETM) has potential applications in poultry production through improved thermotolerance, reduced mortality during heat exposure and maintenance of physiological performance. Its effects on growth and feed conversion, however, depend on the temperature, exposure duration, developmental stage, genotype and post-hatch environment. A 2024 study reported that thermal manipulation at 38.5 °C during embryonic days 12–18 improved hatchability and reduced hatching time, while a recent study found that moderate exposure protocols reduced mortality during post-hatch heat stress. Excessive exposure, particularly at higher temperatures and longer durations, may negatively affect feed conversion and growth.

The reproductive implications require more investigation. Although improved thermal resilience could potentially support the performance of birds maintained under hot conditions, direct evidence that embryonic conditioning improves adult fertility, egg production or semen quality remains limited. Therefore, ETM should currently be considered a promising strategy for improving heat resilience rather than an established method for enhancing reproductive output. Its practical value will depend on consistent hatchability, chick quality, growth performance and economic benefits under commercial conditions.

10. Research limitations and emerging technologies

Despite encouraging findings, research on epigenetic thermal programming in poultry has several limitations. Studies differ in incubation temperature, exposure duration, embryonic stage, chicken genotype and post-hatch heat-challenge conditions, making direct comparisons difficult. Moreover, many investigations focus on short-term physiological or gene-expression changes, while fewer examine persistent epigenetic modifications across multiple generations. The 2025 scientometric analysis by Zangeronimo and Alvarenga identified limited interconnection among research groups and highlighted the need for more integrated investigations.

Emerging molecular technologies can help address these gaps. Whole-genome bisulfite sequencing, RNA sequencing, small-RNA profiling and ATAC-seq can identify DNA methylation, transcriptional and chromatin-accessibility changes associated with thermal conditioning. Integrated multi-omics approaches, combined with functional validation using gene-silencing or gene-editing methods, may help establish causal relationships between epigenetic modifications and thermo-tolerance. Large-scale studies using commercial incubators, different genetic lines and controlled post-hatch heat challenges are also essential to assess practical applicability.

11. Future research perspectives

Future studies should establish standardized ETM protocols by comparing temperature intensity, exposure duration and developmental windows across broiler and layer genotypes. Longitudinal experiments should connect embryonic exposure with post-hatch growth, feed conversion, immune competence, reproductive performance and survival under natural or controlled heat stress. Particular attention should be given to separating temporary developmental responses from stable epigenetic changes. Multigenerational studies involving both maternal and paternal exposure are needed to determine whether

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DNA methylation and non-coding RNA changes persist through germ-cell development and fertilization. Integrating epigenomic, transcriptomic, metabolomic and physiological data may identify reliable biomarkers for selecting thermo-tolerant poultry. Finally, commercial-scale validation should evaluate hatchery feasibility, animal welfare, energy requirements and cost-effectiveness before ETM is recommended as a routine management practice.

12. Conclusion

Embryonic thermal conditioning represents a promising developmental approach for improving poultry thermo-tolerance under increasing environmental heat stress. Its effects involve coordinated changes in hypothalamic regulation, stress-response pathways, oxidative balance, immune function and epigenetic mechanisms, including DNA methylation, histone modifications and non-coding RNAs. Evidence of paternal effects in offspring further suggests that germ line-associated epigenetic mechanisms may contribute to the transmission of thermal resilience. However, variation among experimental protocols, limited functional validation and insufficient multigenerational evidence restrict its immediate application. Future research integrating molecular mechanisms with commercial production trials is necessary to establish reliable protocols and distinguish intergenerational effects from true *trans*-generational inheritance. Such progress could support the development of more heat-resilient poultry production systems.

CRediT author contributions

Ankita Siwach:- Literature search, Writing - original draft, Data analysis, Software, **Himanshu Malik:-** Conceptualization, Data analysis, **Anil Kumar**:-Resources, Supervision, Visualization,

Conflict of interests

The authors declare that they have no conflict of interest with contents of this article.

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