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Research article

A comparative histological study of nervous system development in chickens during 24 and 48 hours of incubation

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ABSTRACT

This study investigated the early development of the central nervous system (CNS) in chicken embryos, comparing morphological and histological changes between 24 and 48 hours of incubation. A total of 150 chicken egg samples were randomly divided into three equal incubation groups (n=50 per group). To ensure baseline uniformity, initial egg weights were analyzed using one-way ANOVA, which revealed no statistically significant differences between the cohorts ($p > 0.05$). Following incubation for 1-16,24, and 48 hours, embryos were extracted, fixed in 10% neutral buffered formalin, processed histologically, and stained with Harris hematoxylin and eosin (H&E). Morphological and microscopic examinations demonstrated that after 24 hours of incubation (Hamburger-Hamilton stages HH8–HH9), the embryos were at the neural plate stage, characterized by an open neural groove and elevating neural folds. By 48 hours of incubation (stages HH12–HH13), primary neurulation was completed, showing a fully closed neural tube separating from the surface ectoderm, alongside primary brain vesicle differentiation and adjacent somites. Ocular micrometer measurements showed a significant increase ($p \leq 0.05$) in neural tube length and width by 48 hours. It was concluded that the 24 to 48-hour incubation window represents a critical phase for primary neurulation and early brain regionalization governed by intrinsic genetic programs rather than external egg metrics.

1. Introduction

The avian embryo, particularly the chicken (Gallus gallus domesticus), serves as one of the most vital and classic model organisms in developmental biology due to its accessibility, rapid development, and structural similarities to mammalian embryogenesis Bednarczyk et al. (2021). The early development of the central nervous system (CNS) is one of the most complex and dynamic morphogenetic network established during early life (Roy et al., 2025). The foundational process governing this development is neurulation, through which the primitive ectoderm is specified and transformed into a functional neural tube, laying the structural groundwork for the future brain and spinal cord (Choi et al., 2022; van der Spuy et al., 2023). The initiation of the CNS begins when the

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embryonic blastoderm differentiates into distinct germ layers: the surface ectoderm and the neuroectoderm (Sinowatz, 2010). This process is highly regulated by underlying signaling networks, including embryonic inductive signals from the notochord and somatic mesoderm, which induce the thickening of the overlying ectoderm to form the neural plate (Karfunkel, 1974). As development progresses, the lateral borders of the neural plate elevate to form the neural folds, while the central axis invaginates to create the neural groove (Karfunkel, 1974). The continuous medial migration and subsequent fusion of these neural folds along the dorsal midline establish the hollow neural tube (Karfunkel, 1974; Ravi et al., 2021). This primary neurulation process transitions the embryo through distinct morphological milestones classified globally under the Hamburger–Hamilton (HH) staging system (Hamburger & Hamilton, 1992) .

The newly formed neural tube subsequently undergoes precise regionalization and neural diversity patterning (Catela, 2020). The brain and spinal cord are part of the central nervous system (CNS) (Thau et al., 2019), containing many inter-connected neurons involved in movement, sensing, and automatic processes (Weber et al., 2017). The anterior portion of the tube expands rapidly to give rise to the three primary brain vesicles: the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain), which further divide into structures like the telencephalon, diencephalon, metencephalon, and myelencephalon (Radhakrishnan & Anand, 2016). Concurrently, the caudal portion of the neural tube elongates to form the spinal cord (Diez del Corral & Morales, 2017). This caudal growth is synchronized in space and time with body elongation and the segmentation of adjacent paraxial mesoderm into somites, which flank the developing spinal cord and contribute to sensory and motor gangliogenesis (Diez del Corral & Morales, 2017). This intricate morphological progression is highly sensitive to genetic, mechanical, and environmental cues (van der Spuy et al., 2023) .

Despite the extensive body of literature focusing on avian organogenesis, a significant research gap remains regarding the precise localized histological quantification and synchronization of early primary neurulation phases within specific windows of the incubation period particularly encompassing the critical timeline from 1 to 48 hours. While general timelines are established, detailed structural characterizations of the transition from an open neural groove to a completely closed segmented neural tube, combined with strict statistical tracking of baseline sample uniformity (such as initial egg weights), are often underrepresented in localized comparative studies.

To address this gap, this study aims to elucidate the precise timing and morphological transitions of early central nervous system development in chicken embryos during three critical milestones: 1-16 hours, 24 hours and 48 hours of incubation. By utilizing detailed micro-anatomical and histological cross-sections, this comparative analysis seeks to define the definitive structural transitions from the open neural fold stage to primary brain vesicle differentiation, establishing a baseline reference for avian neuroembryology.

2.1 Source of Eggs and Pre-Incubation Quality Control

A total of 150 fertilized chicken (*Gallus gallus domesticus*) eggs were obtained from local commercial markets in Al-Kut city, Wasit Governorate. To mitigate confounding factors inherent to market-sourced samples such as unknown avian breeds/strains, varying storage durations, and unmonitored ambient pre-incubation conditions strict morphological and quality screening criteria were implemented. Prior to incubation, all eggs were individually weighed to establish a baseline of uniformity and candle-inspected using a high-intensity LED light source to verify eggshell integrity and the absence of internal hairline cracks or microstructural anomalies.

2.2 Experimental Group Allocation and Incubation Conditions

The 150 screened eggs were randomly and equally divided into three experimental cohorts, assigning exactly 50 eggs to each treatment group based on the designated developmental timelines:

Group 1 (1–16 Hour Cohort): n = 50 eggs designated for early-stage incubation, encompassing critical developmental intervals between 1 and 16 hours to capture initial blastoderm differentiation and primitive streak formation.
Group 2 (24-Hour Cohort): n = 50 eggs designated for 24 hours of incubation to monitor early neurulation and initial somitogenesis.
Group 3 (48-Hour Cohort): n = 50 eggs designated for 48 hours of incubation to evaluate advanced neural tube closure and brain vesicle regionalization.
All eggs were incubated concurrently within a single digital forced-draft laboratory incubator to ensure completely identical environmental exposures. Incubation parameters were continuously monitored and rigorously maintained at a constant temperature of $(37.5 \pm 0.1)°C$ and a relative humidity range of 60%–70%. Automatic turning of the eggs through a 90° arc (45° longitudinal tilt to each side) was executed automatically every two hours to prevent premature embryonic adhesion to the inner shell membrane.

2.3 Embryo Extraction, Isolation, and Fixation

At the conclusion of each respective incubation timeline (1–16, 24, and 48 hours), embryos were recovered using a standardized surgical fenestration technique (the window-opening method). The eggshell was positioned horizontally, and a small aperture was carefully introduced into the blunt end over the air cell using sterile surgical scissors and forceps. The superficial shell and shell membranes were removed to expose the blastoderm. The entire blastoderm containing the developing embryo was gently lifted from the yolk surface using a perforated paper ring carrier to maintain tissue orientation and prevent mechanical distortion. For the early 1–16 hour cohort, due to the delicate and thin nature of the blastoderm, embryos were carefully isolated under stereomicroscopic guidance. The isolated embryo was immediately transferred into a warm, sterile physiological saline solution (0.9% NaCl) to wash away remaining yolk debris. Due to the early developmental status across these successive incubation windows, the neural and pre-neural structures were deeply integrated within the primary germ layers and adjacent paraxial mesoderm; hence, the entire intact embryo rather than isolated tissues was preserved to maintain anatomical integrity. The specimens were fixed in a 10% neutral buffered formalin solution; the fixation duration was optimized based on embryonic stage, lasting 2 to 4 hours for the 1–16 hour cohort, and extending up to 12 hours for the 24-hour and 48-hour embryos to ensure optimal tissue cross-linking while preventing structural brittleness.

2.4 Sample Attrition and Processing Flow

Of the initial 150 eggs, successful embryonic recovery and optimal histological integrity were achieved in 120 samples, representing a total recovery rate of 80%:

- **Group 1 (1–16 Hour Cohort, n = 50):** 40 embryos were successfully recovered and processed (with 10 discarded due to infertility, early blastoderm arrest, or extraction damage).

- **Group 2 (24-Hour Cohort, n = 50):** 40 embryos were successfully recovered and processed (with 10 discarded due to early mortality, developmental arrest, or extraction artifacts).

- Group 3 (48-Hour Cohort, $n = 50$): 40 embryos were successfully recovered and processed (with 10 discarded due to late-stage mortality or structural tissue damage during isolation).

For each experimental group, 30 fully intact, undamaged embryos were selected for comprehensive morphometric analyses, and 10 representative embryos per group were randomly allocated to undergo serial transverse sectioning and definitive histological evaluation.

2.5 Histological Processing and Microtomy

Following adequate fixation, the fixed entire embryos underwent dehydration through a progressively ascending gradient of ethyl alcohol concentrations (70%, 80%, 95%, 99%, and 100% absolute ethanol). The dehydrated tissues were cleared in two sequential changes of pure xylene for one hour each. Infiltration and embedding were performed in high-purity paraffin wax ($56 - 58\ ^{\circ}C\ melting\ point$) via two distinct changes lasting two hours each. The embryos were precisely oriented along their craniocaudally axis within paraffin embedding blocks. Serial transverse sections were cut at a standardized thickness of $5mm$ using an electrical rotary microtome. The ribboned sections were mounted onto glass slides, dewaxed in xylene, and rehydrated through a descending series of ethyl alcohol (100% down to 70%) before being rinsed in distilled water. The sections were stained with Harris hematoxylin and eosin (H&E), cleared, and permanently mounted using DPX (Dextrin Plasticizer Xylene) mounting medium.

2.6 Morphometric and Statistical Analysis

Histological slices and macro-anatomical views were examined under a compound digital light microscope. Morphometric measurements of the developing nervous system specifically the length and width of the neural tube/canal were quantified using a pre-calibrated ocular micrometer scale. To ensure anatomical reproducibility, measurements were systematically restricted to two specific anatomical markers:

The Cranial Region: At the level of the primary brain vesicles (prosencephalon/mesencephalon).

The Caudal Region: At the level of the first three pairs of fully differentiated somites.

All quantitative data collected from these morphometric evaluations and baseline weight assessments were structurally organized and analyzed using IBM SPSS Statistics software (Version 26). The sample size structure and replication framework for the statistical calculations were rigorously defined. For the baseline uniformity analysis, a total of $N = 150$ observations ($n = 50$ individual eggs independently weighed per incubation group) were evaluated to assess pre-incubation mass homogeneity. For the morphometric neural tube analysis, a total of 20 embryos ($n = 10$ randomly assigned embryos per incubation group) underwent definitive micro-anatomical measuring. For each individual embryo, measurements were taken across 5 independent, non-overlapping serial transverse sections per anatomical region (cranial and caudal levels), yielding a baseline of 50 technical replicates per incubation group. All morphometric trials were performed in triplicate by the same investigator to calculate an internal mean value and minimize inter-assay variance. To determine the statistical significance of differences between the independent incubation groups (24-hour and 48-hour cohorts), a One-Way Analysis of Variance (ANOVA) was executed. Prior to running the parametric ANOVA models, the underlying statistical assumptions were rigorously evaluated:

1- Normality of Data: Verified using the Shapiro-Wilk test ($p > 0.05$), confirming that the datasets followed a normal distribution.

2- Homogeneity of Variances: Assessed using Levene's test (p > 0.05), ensuring homoscedasticity across the compared cohorts.

Once these baseline parametric criteria were satisfied, the ANOVA model was applied, combined with Fisher's Least Significant Difference (LSD) post-hoc test for pairwise comparisons between the developmental groups to pinpoint specific significant variations. Statistical significance across all mathematical tests was strictly defined at a probability level of $p > 0.05$. In accordance with standard biostatistical conventions, results yielding a probability value greater than 0.05 (p > 0.05) were defined as statistically non-significant, confirming the absence of true variance (such as in baseline egg weights). Conversely, results yielding a probability value less than or equal to 0.05 ($p \leq 0.05$)were designated as statistically significant, indicating verifiable morphological transformations during the primary neurulation period.

3. Results and Discussion

3.1 Baseline Uniformity

Statistical analysis via one-way ANOVA confirmed that the initial mean total weight of the eggs at the start of the experiment was 61.67 g (Table 1). The variance in egg weights between the designated 16–24 hours group (61.16 g) and the pre-incubation baseline was statistically non-significant (p > 0.05), satisfying the homoscedasticity criteria and confirming that all embryos began development within a uniform physical environment, consistent with the standard egg weight ranges reported by Aygün and Olgun (2019).

3.2. Evaluation of Egg Weights and Embryonic Parameters

The physical environment and attributes of the egg play a supportive role during early embryogenesis, as the egg contents provide the structural and energetic substrate required for tissue differentiation. Based on the statistical data generated in this study (Table 1), the relationship between egg weight parameters and early neural development was analyzed.

3.3 Mass Reduction via Evaporation

By 48 hours of incubation, the mean total egg weight exhibited a slight reduction to 60.54 g and the weight of the contents (yolk and albumen) was measured at 55.10 g (Table 1). Statistical analysis showed that these early variations in total egg weight and content weight were mathematically highly consistent, as evidenced by low coefficients of variation ($C.V = 2.90\%\ and\ C.V = 4.40\%, respectively; p \leq 0.001; LSD = 1.83\ and\ LSD = 2.58$). This physiological reduction in mass is attributed to natural water loss and fluid evaporation through the eggshell pores, driven by the constant incubator temperature of 37.5°C and environmental airflow, a thermodynamic phenomenon extensively documented by Henderson (1950) and Rahn and Ar (1974).

Table 1: Morphometric Parameters, Egg Components, and Embryonic Growth Measurements Across Successive Incubation Intervals (n = 150)

	Total weight of eggs (g)	**Shell weight (g)**	**Weight of contents (yellow and white) (g)**	**Embryonic weight (g)**	**Length of the neural tube (mm)**	**Width of the neural tube (mm)**
1-16 hours	61.67	5.50	57.17	0.00	-	-
16-24 hours	61.16	5.43	56.86	0.001	0.6	0.5

48 hours	60.54	5.44	55.10	0.01	1.2	1.5
LSD Value	1.83	-	2.58	0.001	0.12	0.24
C.V	2.90	9.80	4.40	-	-	-
P- value	$P \leq 0.001$	0.969(NS)	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$

Abbreviations used in Table 1: g: Grams; mm**:** Millimeters; **LSD:** Least Significant Difference; **C.V:** Coefficient of Variation; **NS:** Non-Significant (p > 0.05); **—:** Not applicable.

Note: *Data are presented as group means. Statistical variation and dispersion were evaluated using the Coefficient of Variation (C.V%) and Least Significant Difference (LSD) as detailed above, indicating homogenous sample variance (p > 0.05).*

3.4. Morphological and Histological Analysis of CNS Development

A. The 24-Hour Incubation Cohort (Primary Neurulation Initiation)

The macro-anatomical and microscopic examinations of the chicken embryos fixed after 24 hours of incubation revealed that the embryos had reached the definitive neural plate and early neural groove stages. Rather than relying strictly on chronological incubation time, individual embryos were rigorously assigned to the Hamburger–Hamilton (HH) system as stages HH8–HH9 based on clear morphological criteria: the presence of an open but distinct neural groove at the cranial midline, and the early segmentation of paraxial mesoderm showing 4 to 7 pairs of visible somites.

Histological cross-sections stained with Harris hematoxylin and eosin (H&E) provided detailed micro-anatomical evidence of primary neurulation (Figure 1). The neuroectoderm exhibited marked cell proliferation, transforming into a dense, pseudo-stratified columnar neuroepithelium that was significantly thicker than the adjacent surface ectoderm. The lateral margins of this specialized neural plate were observed to bend dorsally, establishing elevated neural folds. This in folding created a prominent central depression, identified as the neural groove (NG). At this stage, the neural tube (NT) remained completely open dorsally, communicating directly with the amniotic cavity. The surrounding mesoderm (M) appeared as a loosely organized mesenchymal network flanking the invaginating neural structures, providing the mechanical support necessary for subsequent fold migration.

These findings directly align with the foundational observations of Karfunkel (1974), who demonstrated that the initial elevation of neural folds is driven by intracellular microfilament contraction and localized cell shape changes within the neuroepithelium. Furthermore, our observations match the structural descriptions by van der Spuy et al. (2023), confirming that during the 18–24 hour incubation window, multiple cellular and mechanical processes must tightly coordinate in space to successfully convert the flat neural plate into a transitional groove before fusion occurs.

B. Transitional Phasing and the 48-Hour Incubation Cohort (Neural Tube Closure and Regionalization)

As the incubation progressed, transitional stages within the 48-hour cohort captured the continuous medial migration, transitional cross-sections (Figures 2 and 3) captured the continuous medial migration and progressive interlocking of the neural folds along the craniocaudally axis. This transitional phasing is structurally justified as it traces the dynamic wave of primary neurulation closure, which initiates in the future hindbrain region and spreads both anteriorly and posteriorly.

By the definitive 48-hour incubation milestone, all evaluated specimens had progressed to the neural tube stage, rigorously categorized as Hamburger–Hamilton stages HH12–HH13 (Hamburger & Hamilton, 1992). The morphological criteria confirming this assignment included the attainment of 16 to 19 pairs of somites, the complete dorsal fusion of the neural folds, and the clear macroscopic expansion of the anterior neural tube into distinct cerebral vesicles.

Histological profiles at 48 hours (Figures 4 and 5) demonstrated a completely closed, hollow neural tube (NT) completely separated from the overlying superficial ectoderm (SE), which had reunited at the midline to form the future epidermis. The centralized cavity, or lumen/neurocele (L), was well-defined and lined by a highly mitotic, multi-layered germinal neuroepithelium (NE). At the cranial level, our sections confirmed the advanced differentiation of the primary brain vesicles: the prosencephalon (forebrain), mesencephalon (midbrain), and the beginning of the rhombencephalon (hindbrain).

Laterally, the neural tube was flanked by paired, highly condensed blocks of paraxial mesoderm the somites (S) undergoing early differentiation (Figures 4 and 5). This strict topographical organization confirms that by 48 hours of incubation, the structural foundation of the central nervous system is completely established, and neural diversity patterning has commenced, supporting the basic anatomical structures defined by Thau et al. (2019) and Weber et al. (2017). These precise micro-anatomical milestones validate the evolutionary models of Hamburger and Hamilton (1992) and mirror the three-dimensional micro-CT imaging of secondary neurulation progression reported by Choi et al. (2022).

3.5. Quantitative Morphometric Analysis

Ocular micrometer measurements provided objective quantitative validation of the morphological growth observed across the three primary experimental groups.

During the initial 1–16 hour phase, embryonic measurements for the neural tube were non-applicable (0.0 mm) as the neural structures had not yet emerged. However, as development advanced, the neural tube length increased from an average of 0.6 mm at the 24-hour open-groove phase to 1.2 mm at the 48-hour closed-tube stage (Table 1). Similarly, the neural tube width exhibited a statistically significant expansion, widening from 0.5 mm at 24 hours to 1.5 mm at 48 hours of incubation ($p \leq 0.05; Table$ 1).

- **Neural Tube Width/Thickness:** Exhibited a statistically significant threefold increase ($p \leq 0.05$), expanding from 0.5 mm at 24 hours to 1.5 mm at 48 hours of incubation (Table 1).

Figure 1: Histological cross-section of a chicken embryo at 24 hours of incubation, corresponding to Hamburger–Hamilton stages HH8–HH9. The section illustrates the definitive neural groove (NG) flanked by elevated neural folds, with the central nervous system structures remaining completely open dorsally. The thickened walls consist of dense pseudo-stratified columnar neuroepithelium (NE), distinct from the adjacent surface ectoderm (SE) and supported by the underlying loose mesoderm (M). Scale bar represents 0.5 *mm*. Micrograph represents a typical observation replicated across multiple evaluated embryonic specimens (10X).

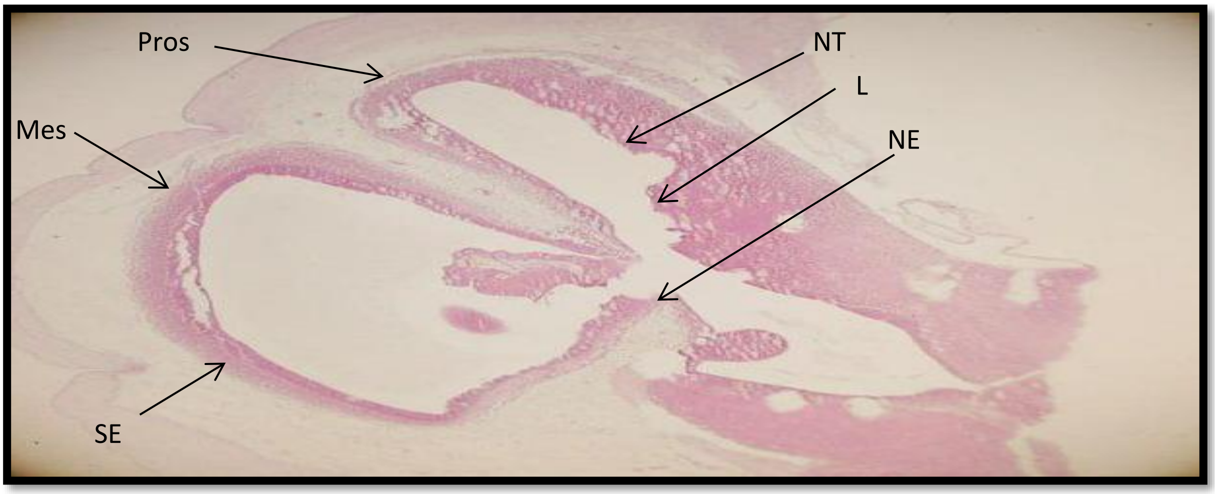

Figure 2: Histological cross-section through the cranial region of a chicken embryo at 48 hours of incubation, corresponding to Hamburger–Hamilton stages HH12–HH13. The section demonstrates advanced primary brain regionalization within a fully enclosed neural tube (NT) composed of thick neuroepithelium (NE) enclosing a prominent centralized lumen (L). Clear differentiation is evidenced by the structural expansion of the prosencephalon (Pros) and mesencephalon (Mes) compartments, surrounded by continuous protective layers of surface ectoderm (SE) and underlying structural mesoderm (M). Scale bar represents 1.5 *mm*. Micrograph represents a typical observation replicated across multiple evaluated embryonic specimens (10X).

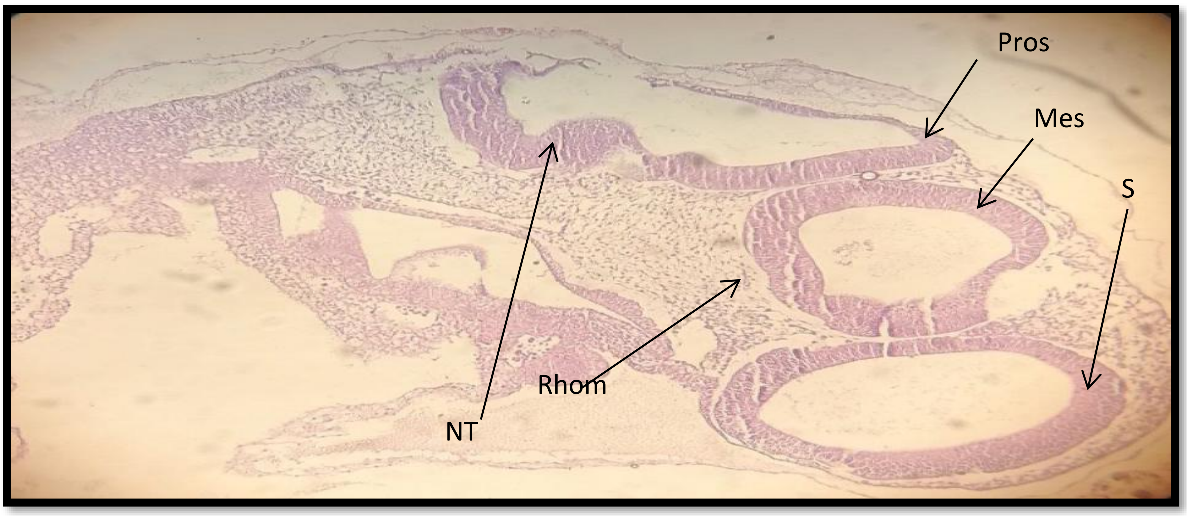

Figure 3: Histological oblique-longitudinal section of a chicken embryo at 48 hours of incubation, corresponding to Hamburger–Hamilton stages HH12–HH13. The section highlights advanced regionalization within the expanded neural tube (NT) infrastructure. Distinct cerebral subdivisions are clearly delineated, including the thickened neural walls of the prosencephalon (Pros), the midbrain vesicle or mesencephalon (Mes), and the lower expansive cavity of the rhombencephalon (Rhom), with the neural vesicles enveloped by loose connective networks of supporting mesoderm (M). Scale bar represents 1.5 mm. Micrograph represents a typical observation replicated across multiple evaluated embryonic specimens (10X).

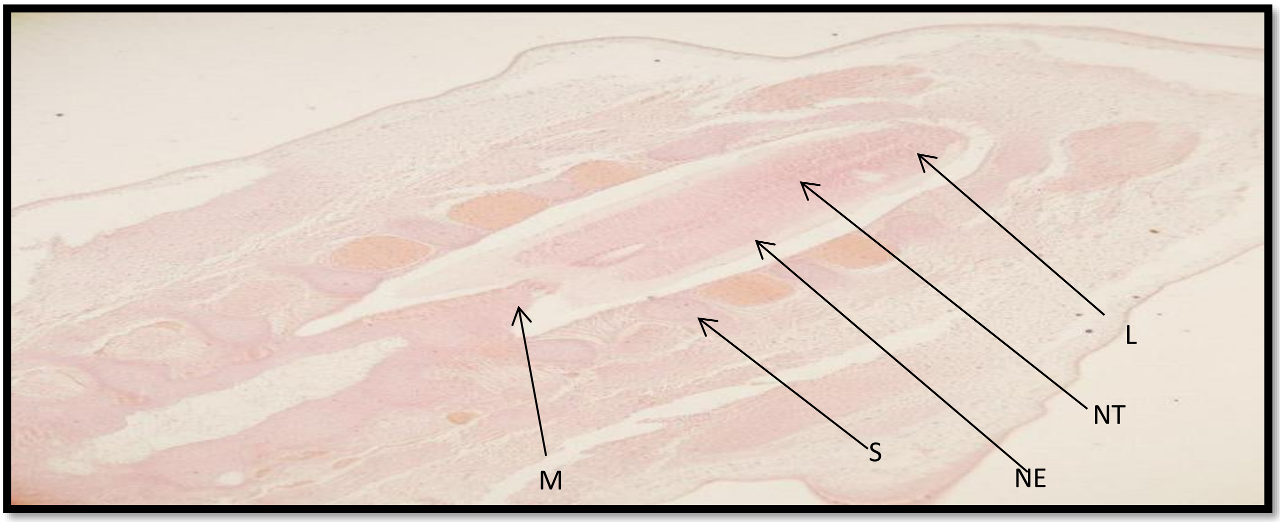

Figure 4: Histological cross-section through the mid-body axis of a chicken embryo at 48 hours of incubation, corresponding to Hamburger–Hamilton stages HH12–HH13. The micrograph captures the completed primary neurulation stage, showing a fully closed neural tube (NT) enclosing its elongated, vertical centralized lumen (L) composed of dense neuroepithelium (NE). Flanking the neural core are well-segmented, symmetric paired somites (S) undergoing early differentiation within the surrounding continuous mesoderm (M). Scale bar represents 1.5 mm. Micrograph represents a typical observation replicated across multiple evaluated embryonic specimens (4X).

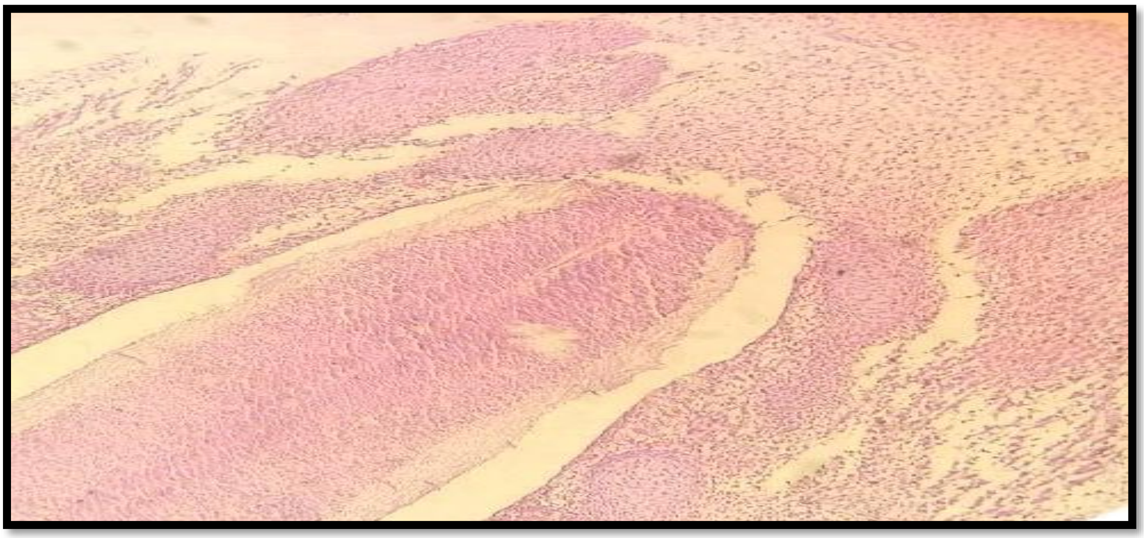

Figure 5: High-magnification histological cross-section focusing on the paraxial structures of a chicken embryo at 48 hours of incubation, corresponding to Hamburger–Hamilton stages HH12–HH13. The

detailed micrograph demonstrates the tightly packed cellular arrangement of the neuroepithelium and the adjacent cellular somites (S) embedded within the protective sheets of the surrounding continuous mesoderm (M), highlighting early paraxial tissue differentiation. Micrograph represents a typical observation replicated across multiple evaluated embryonic specimens (n=150) (10X).

This statistically verifiable expansion ($p \leq 0.05$) quantitatively corroborates the rapid cell division occurring within the germinal neuroepithelium. This rapid thickening is mediated by the interkinetic nuclear migration of neural progenitor cells during the cell cycle, as previously detailed by Cayuso and Martí (2005). The significant growth in width and length represents the active accumulation of neuroepithelial mass required to support the emerging brain vesicles and spinal cord divisions (Diez del Corral & Morales, 2017; Radhakrishnan & Anand, 2016).

3.6 Correlation with Embryonic Growth

The data presented in Table 1 demonstrates that the actual embryonic weight undergoes a progressive increase from a nominal 0.000 g during the 1–16 hour interval to 0.001 g at 24 hours, reaching a measurable mass of 0.010 g at 48 hours due to rapid mitotic cell division. However, in agreement with Krzanowska (1959) and Schmidt et al. (2009), our statistical analysis confirms that there is no direct, statistically verifiable correlation ($p > 0.05$) between individual egg weights and the rate of early neural tube growth across this 1-to-48-hour developmental window.

As established by Wilson (1991), the correlation between egg size and embryonic weight is weakest during the first half of incubation and only becomes dominant approaching hatching. Thus, the rapid morphogenetic transitions observed in this study—such as the transition from an open neural groove (0.6 mm in length and 0.5 mm in width) to a regionalized, closed neural tube (1.2 mm in length and 1.5 mm in width)—are governed by intrinsic genetic programs and localized cell signaling pathways rather than being mathematically driven by the initial weight of the egg or the rate of incubation fluid loss. The gradual increase in embryonic weight is directly driven by rapid mitotic cell division and progressive tissue differentiation. This metabolic activity requires substantial energy expenditure, which the early embryo derives from the nutrients within the egg yolk. While this internal biochemical conversion increases the absolute mass of the embryo itself, the overall embryonic metabolism and respiration contribute to the subtle, progressive reduction in total egg mass through the respiratory loss of water vapor and carbon dioxide (CO_2) via the shell pores.

4. Conclusion

This comparative study provided precise histological and morphometric documentation of the critical transitional phases of primary neurulation in the chicken embryo (*Gallus gallus domesticus*) between 24 and 48 hours of incubation, demonstrating that the embryonic avian nervous system transitions rapidly from a rudimentary open ectodermal furrow into a fully enclosed, segmented, and regionalized neural tube. Structurally, the 24-hour incubation milestone (Hamburger–Hamilton stages HH8–HH9) was characterized by an open neural plate and an early prominent neural groove with elevated neural folds lined by a highly mitotic neuroepithelium, whereas by the 48-hour milestone (stages HH12–HH13), primary neurulation was successfully completed, evidenced by the total dorsal fusion of the neural tube, its complete segregation from the overlying surface ectoderm, and its advanced differentiation into the three primary brain vesicles (prosencephalon, mesencephalon, and rhombencephalon). This morphological progression was firmly supported by objective quantitative data, which revealed a highly significant, threefold expansion ($p \leq 0.05$) in neural tube width and a twofold increase in length as development advanced. Furthermore, while incubation under standard conditions induced a statistically verifiable reduction ($p \leq 0.001$) in total egg weight and content mass due to natural fluid evaporation through the shell pores, this biometric loss exhibited no direct statistical correlation ($p > 0.05$) with the embryonic neural growth rate, confirming that early neurulation is governed by intrinsic genetic programs and localized cell signaling pathways rather than being driven by external egg metrics. Ultimately, these definitive micro-anatomical findings establish a reliable, quantified structural baseline for early avian neuroembryology and localized developmental toxicology models.

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