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The Influence of Hen Age on Hatching Egg Quality and Embryonic Development of Sentul Chickens

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ABSTRACT

Sentul chicken is a local Indonesian chicken primarily raised for meat production. One crucial factor affecting egg quality and embryo development is hen age. This study aims to examine the influence of hen age on the external and internal quality of Sentul chicken hatching eggs, as well as the early development of the embryo. Eggs from 41-week-old and 78-week-old hens were compared across exterior and interior quality, nutrient composition, and embryonic development. Data analysis was performed using a descriptive method for hatching egg quality and a t-test for embryonic development. Results revealed that eggs from older hens (78 weeks) were significantly ($P < 0.05$) heavier, had a higher egg shape index, and exhibited higher albumen and yolk indices, as well as a higher Haugh unit (HU). Nutrient analysis showed an increase in protein and fat content in older hens' eggs, alongside a reduction in water content. However, embryonic development assessed by primitive streak and somite formation showed no significant differences ($P > 0.05$) between the age groups. These findings highlight the positive influence of hen age on egg quality and embryonic development, potentially enhancing hatchability and chick development. Further research is, however, needed to observe long-term effects of hen age on embryo development and post-hatch performance.



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1. Introduction

The local chicken breed is one of Indonesia's valuable genetic resources, possessing significant potential to be developed to meet the country's animal protein requirements. Indonesia has 32 local chicken breeds (Nataamijaya 2000; Nuraini *et al.* 2018), each with distinct physical and morphological characteristics unique to their regions of origin, and

each with its advantages (Nuraini *et al.* 2018). Among these, the Sentul chicken, originating from Ciamis, West Java, is recognized as a productive dual-purpose chicken (Hidayat and Sopiyan 2010).

The genetic potential of chicken breeds can be fully realized by ensuring high egg quality, which is crucial for hatchability and overall production efficiency. It is generally assessed through external and internal characteristics. Externally, egg quality criteria consist of shell cleanliness, shell integrity, and shell texture and shape. Eggs should be free from stains, dirt, and foreign materials. Clean egg shells reduce the

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risk of bacterial contamination. A strong, uncracked shell is essential for protecting the egg's contents and preventing microbial entry. The eggshell should have a uniform texture without ridges or rough areas, and the egg should be oval-shaped, with one end slightly larger than the other.

Internal egg quality criteria include the quality of albumen (egg white), yolk quality, and air cell size. High-quality eggs have a firm and thick albumen that holds the yolk centered. The viscosity of the albumen is a key indicator of freshness. The yolk should be round and firm, maintaining its position in the center of the egg. A strong vitelline membrane prevents yolk flattening and mixing with the albumen. A smaller air cell indicates a fresher egg. As eggs age, the air cell enlarges due to moisture and carbon dioxide loss through the shell's pores.

Previous studies indicated that these traits are strongly influenced by hen age (Zita *et al.* 2013; Moreki and Gabanakgosi, 2014; Wistedt *et al.* 2019). As hens grow older, yolk index and size also increase. At the same time, albumen quality, shell strength, and thickness decrease. Older hens tend to lay eggs with weaker structure (Wistedt *et al.* 2019) and lower hatchability (Joyner *et al.* 1987; Wistedt *et al.* 2019). The rate of egg production decreases as the hen's age increases. The rate of egg production decreases as the hen ages, and the egg shell also becomes thinner as the hen ages. Hens of different ages produce eggs with varying nutrient contents for hatching (Peebles *et al.* 2001).

The quality of hatching eggs also has a direct impact on embryonic development. Early embryonic development begins with blastulation, followed by gastrulation and the formation of somites. These somites, which start to appear around 10 hours of incubation (Bellairs and Osmond 2005) and reach seven pairs by 33 hours, are essential for vertebral column and skeletal development (Hamburger and Hamilton 1951; Huettnner 1957; Pourquie 2004; Bellairs and Osmond 2005). Poor somite formation may lead to developmental abnormalities and reduced chick viability.

Hen age can therefore influence the quality of the hatching egg and, subsequently, embryonic development. However, studies on these aspects in Sentul chickens are still limited. This study is purposed to assess hatching egg quality and embryo development in Sentul chickens at two age groups:

41 weeks (middle production period) and 78 weeks (late production period). The results are expected to help breeders select better eggs for hatching. This can improve hatchability and support sustainable local chicken production.

2. Materials and Methods

This study was approved by the Animal Ethics Committee at the School of Veterinary Medicine and Biomedical Sciences of IPB University in Bogor, Indonesia (ID Code: 249 KEH/SKE/IX/2024). The research was conducted from March to September 2024. Egg incubation was carried out at the Hatching Laboratory, Department of Poultry Production Science, Faculty of Animal Science, IPB University. Observations of both external and internal egg quality, as well as proximate analysis, were conducted at the Feed Science and Technology Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University. Nutrient analysis of the hatching eggs was conducted at the Biotechnology Center Laboratory of IPB University. Embryo development was observed at the Embryology Laboratory, School of Veterinary Medicine and Biomedical Science, IPB University.

2.1. Incubator and Hatching Egg Preparation

The AGR-TT1056 automatic hatching machine was cleaned and disinfected before use. The incubator equipment, including the thermometer, thermostat, heating lamp, and water tray, was checked for functionality. Fumigation was performed for 15 minutes to minimize microbial contamination. The study sample included 126 hatching eggs (63 from 41-week-old hens and 63 from 78-week-old hens), sourced from PT WUG in Bogor, West Java. Only clean and intact hatching eggs were selected for incubation. The incubator was operated for 24 hours to stabilize the temperature and humidity level.

2.2. Egg Quality Analysis

2.2.1. External Egg Quality

The external parameters observed included egg weight (measured using a digital scale) and egg index ($\text{width/length} \times 100$). Eggshell thickness was measured at the pointed end, blunt end, and equator using a Mitutoyo micrometer after the eggshell membrane was removed. The values were averaged. Eggshell weight

was calculated by weighing the shell using a digital scale. Shell strength was measured using an eggshell strength tester (RH-DQ200).

2.2.2. Internal Egg Quality

The internal quality parameters were observed by SNI 01-3926-2008 (National Standardization Agency 2008). The albumen index was calculated by comparing the albumen height and diameter of the thick albumen. The yolk index was calculated by dividing the is calculated by dividing the yolk height by its average diameter and then multiplying by 100. The yolk colour score was scored using a yolk colour fan. The Haugh Unit (HU) was determined using the formula: $HU = 100 \log (H + 7.685 - 1.7 W^{0.37})$, where H represents albumen height (mm) and W represents egg weight (g).

2.3. Nutritional Composition of Sentul Chicken Hatching Eggs

The nutrient composition of combined albumen and yolk was analyzed using the method outlined by the Association of Official Analytical Chemists (AOAC 2005).

Water Content (%): A 1 g sample was dried in an oven at 105°C for 8 hours to determine the moisture content, and the percentage was calculated as: $\{(\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}\} \times 100$

Ash Content: A 1 g sample was incinerated in a porcelain dish at 600°C for 4 hours. Ash content (%) was calculated as: $(\text{Ash weight} / \text{Sample weight}) \times 100$.

Fat Content: A 2 g sample was wrapped in filter paper, placed in a Soxhlet flask, and extracted for 6 hours using 150 mL of hexane as the solvent for fat extraction. The extracted fat was then dried at 100°C for 1 hour. The fat content (%) was calculated as: $\{\text{Extracted fat weight} / \text{Sample weight}\} \times 100$.

Protein Content: A 0.25 g sample was digested in a 100 ml Kjeldahl flask with 0.25 g selenium and 3 mL concentrated H_2SO_4 until the solution became transparent (approximately 1 hour). After cooling, 50 mL of distilled water and 20 ml of 40% NaOH were added before distillation. The distillate was collected in an Erlenmeyer flask containing a mixture of 10 ml of 2% H_3BO_3 and two drops of Bromocresol Green-Methyl Red indicator, and then titrated with 0.2 N HCl. Protein content (%) was calculated as: $\{(\text{Volume HCl} \times \text{Normality HCl} \times 6.25 \times 14 \times 0.001) / \text{Sample weight}\} \times 100$.

2.4. Observation of Embryo and Primitive Streak Development

Embryonic development was observed at 16 and 33 hours at a temperature of 38°C, following Schoenwolf's method (2001). At each embryo collection, 15 eggs per treatment group were cracked. The yolk was gently rolled into the embryo position. The embryo was collected using a racket-shaped filter paper. The vitelline membrane was cut around the filter paper, allowing the paper to be lifted with the attached embryo. The embryos were then rinsed in warm NaCl solution in a petri dish to remove excess yolk. The vitelline membrane was detached by gentle agitation.

Fixation was performed by gradually adding Bouin's fixative solution until the embryo was adequately submerged (up to 2/3 of the Petri dish's height). The fixed embryo was then stained with carmine dye for 24 hours, followed by sequential ethanol washes: 3 to 6 times of 70%, and twice of 95% and absolute, respectively, followed by xylol (three times) clearing at 15-30 minute intervals.

After staining, the next step was to prepare the slides. With the desired colour intensity, the stained embryos were mounted on a glass slide using Entellan and covered with a 22 × 22 mm cover glass. Further somite observations were conducted under an Olympus SZX7 microscope with 6× magnification. Images were captured using a digital camera connected to the microscope and laptop via Toup View software and analyzed using ImageJ version 1.54 g (NIH).

3. Results

3.1. Hatching Egg Quality of Sentul Chicken

3.1.1. Exterior Quality

The external characteristics of hatching eggs from Sentul chickens change with hen age. Eggs from 78-week-old hens were significantly heavier and had heavier shells ($P < 0.05$) than those from 41-week-old hens (Table 1). However, the egg index slightly decreased, indicating a change in egg shape with hen age. There were no significant differences ($P > 0.05$) in shell thickness and shell strength, suggesting that shell integrity remains stable despite hen aging.

3.1.2. Interior Quality

Interior egg quality improved with the age of sentul hens (Table 2). Eggs from older hens had significantly

higher albumen and yolk indices ($P<0.05$), indicating better structural quality. Additionally, yolk color and HU scores were significantly higher ($P<0.05$) in older hens compared to younger hens. These results suggest that eggs from older Sentul hens provide better internal quality for hatching purposes.

3.2. Nutrient Composition of Sentul Chicken Hatching Egg

Table 3 highlights the nutrient composition of hatching eggs from hens at 41 and 78 weeks of age. Eggs from older hens had a lower water content (70.88%) compared to eggs from younger hens (72.72%). Conversely, fat content increased from 10.49% to 12.38%, and protein content showed a slight increase from 12.89% to 13.67%. Ash content remained relatively consistent (1.54% vs. 1.47%). As hens aged, the nutritional profile of the eggs shifted. Eggs from 78-week-old hens had lower water content but higher fat and slightly higher protein contents than those from younger hens (41-week-old hens) (Table 3). The ash content remained consistent, indicating that the mineral content was unaffected by age. Overall, older hens produced eggs with a denser nutritional composition.

Table 1. Exterior quality of hatching eggs from Sentul Chicken at 41 and 78 weeks

Parameter	41 weeks ($\bar{x} \pm sd$)	78 weeks ($\bar{x} \pm sd$)	P-value
Egg weight (g)	50.83 \pm 2.17	53.06 \pm 3.17	0.002**
Egg index (%)	78.09 \pm 2.81	76.50 \pm 2.45	0.022*
Shell thickness (mm)	0.35 \pm 0.02	0.35 \pm 0.02	0.622 ^{ns}
Shell weight (g)	6.38 \pm 0.54	6.74 \pm 0.58	0.01*
Shell strength (N)	18.52 \pm 8.65	17.10 \pm 9.33	0.54 ^{ns}

Note: \bar{x} : mean; sd: standard deviation; p-value: significance of t-test between hens at 41 weeks and 78 weeks; *significantly different $p<0.05$; **: highly significantly different $p<0.01$; ns: not significantly different.

Table 2. Interior quality of hatching eggs from Sentul Chickens group 41 week-old and 78 week-old hens

Parameter	41 weeks ($\bar{x} \pm sd$)	78 weeks ($\bar{x} \pm sd$)	P-value
Albumen index	0.052 \pm 0.017	0.063 \pm 0.018	0.01*
Yolk index	0.372 \pm 0.041	0.419 \pm 0.062	0.001*
Yolk color score	5.567 \pm 0.935	6.467 \pm 0.937	0.0004**
Haugh unit	68.246 \pm 6.937	75.525 \pm 8.305	0.0005**

Note: \bar{x} : mean; sd: standard deviation; p-value: significance of t-test between two groups of hen age (41 weeks and 78 weeks); *significantly different $p<0.05$; **: highly significantly different $p<0.01$; ns: not significantly different.

3.3. Development of the Primitive Streak and Somite in Sentul Chicken Embryos

Despite the differences in egg quality and nutrient composition, there were no significant differences ($P>0.05$) in early embryonic development between the two hen age groups. (Table 4) The length of the primitive streak after 16 hours and the somite pair count after 33 hours of incubation were statistically similar. This indicates that hen age did not affect the initial stages of embryogenesis in Sentul chickens.

4. Discussion

This study evaluated the quality of hatching eggs and the development of embryos in Sentul chickens across two age groups: hens at 41 weeks old, which corresponds to the middle production period, and those at 78 weeks old, indicating the late production period.

4.1. Hatching Egg Quality of Sentul Chicken

4.1.1. Exterior Quality

The results indicated that hen age significantly ($P<0.05$) affects the external traits of hatching eggs, particularly egg weight and shell weight. Eggs

Table 3. Nutrient composition of Sentul Chicken hatching eggs from 41-week-old and 78-week-old hens

Parameter	Water content	Ash content	Fat content	Protein content
	%			
41 weeks	72.72	1.54	10.49	12.89
78 weeks	70.88	1.47	12.38	13.67

Source: Results of the analysis of mixed egg white and yolk at the Central Biotechnology Laboratory, IPB University 2024

Table 4. Development of the primitive streak and somites in Sentul Chicken embryos from hatching eggs produced by 41-week-old and 78-week-old hens

Incubation Period	41 weeks ($\bar{x} \pm sd$)	78 weeks ($\bar{x} \pm sd$)	P-value
Primitive streak length 16 hours (mm)	1.18 \pm 0.36	1.21 \pm 0.43	0.869 ^{ns}
Somite number 33 hours (pairs)	9.58 \pm 2.14	9.35 \pm 2.64	0.797 ^{ns}

Note: \bar{x} : mean; sd: standard deviation; p-value: significance of t-test between two groups of hen age (41 weeks and 78 weeks); *significantly different $p<0.05$; **: highly significantly different $p<0.01$; ns: not significantly different.

produced by 78-week-old Sentul hens were heavier and had greater shell weight compared to those from 41-week-old hens (Table 1). This finding aligns with studies by Peebles *et al.* (2001), Campbell *et al.* (2003), and Rashid *et al.* (2013), which reported that older hens tend to produce larger eggs due to physiological changes in their reproductive systems. As hens mature, their reproductive organs develop, enabling them to produce larger eggs. Older hens have more developed ovaries and oviducts that support bigger yolks and albumen, as well as an increase in shell mass. Hormonal stabilization in older hens contributes to improved egg formation and size (Decuypere and Bruggeman 2007). Better access to nutrients further enhances their reproductive capabilities, resulting in larger eggs (Stephens and Johnson 2020). However, the slight decrease in egg index indicates a potential alteration in egg shape (Table 1), which may result from differences in reproductive tract elasticity as hens age. The oviducts of older hens become more elastic and perhaps more competent in forming eggs. This enhanced elasticity facilitates the formation of a more consistent shape during the egg-laying process (Stephens and Johnson 2020). The lack of significant changes in shell thickness and shell strength (Table 1) suggests that calcium deposition per unit area may not increase proportionally to the increase in shell thickness. Therefore, shell quality remains consistent, regardless of hen age, ensuring the structural integrity of the eggs. This supports previous findings that older hens lay eggs. However, the shell thickness may remain constant or slightly decrease, potentially affecting shell quality (Roberts 2004; Hamidu *et al.* 2007; Dunlop *et al.* 2016).

Interestingly, the egg index slightly decreased with age (Table 1), indicating a minor change in egg shape. However, Kusnawati *et al.* (2022) reported that 92-94-week-old hens of local chicken exhibited a slight reduction in egg shape that may be associated with reduced elasticity in the reproductive tract of older hens. However, despite the minor change in egg shape in this study, shell integrity, as measured by thickness and shell strength, was not significantly affected by hen age (Table 1). The average shell thickness remained at 0.35 mm for both age groups, within the acceptable range for good shell quality, as suggested by Soeparno *et al.* (2011). These findings imply that although older Sentul hens produce larger and heavier hatching eggs, the overall shell quality remains adequate for incubation purposes. The eggshell's structural robustness is

maintained, likely due to the good health and genetic quality of the hens.

4.1.2. Interior Quality

The interior quality of hatching eggs, which included the albumen index, yolk index, yolk color, and HU, showed significant improvements in eggs produced by 78-week-old hens compared to those from 41-week-old hens (Table 2). These parameters are critical indicators of egg freshness, nutritional value, and suitability for hatching. The albumen index, which reflects the firmness and the height of the thick albumen relative to its width, was significantly higher ($P < 0.05$) in older hens compared to younger hens. This suggests better egg freshness and protein quality, which may enhance hatchability.

Albumen degradation over time can occur due to increased alkalinity as carbonic acid breaks down (Bilalissi *et al.* 2022). However, eggs observed in this study were fresh hatching eggs, ensuring accurate measurements. Similarly, the yolk index, which measures yolk height relative to its diameter, was significantly higher ($P < 0.05$) in eggs from older hens (0.41 vs 0.37) (Table 2). A higher yolk index indicates a rounder and firmer yolk, which is characteristic of fresh eggs. The value observed in both age groups falls within the normal range (0.33–0.50), suggesting good freshness. This result is consistent with Zita *et al.* (2013), who reported that the yolk index is influenced by water movement from the albumen to the yolk.

Yolk color was also significantly deeper ($P < 0.05$) in eggs from older hens (Table 2). Yolk color is primarily influenced by the hen's diet, particularly the presence of pigments like carotenoids. A higher yolk color score in eggs from older hens may result from greater efficiency or dietary pigment deposition. A yolk color score of 6-8 is considered medium quality (Umar *et al.* 2001). The majority of eggs in this study (50%) fell within that range. Although yolk color does not directly affect hatchability, it can reflect nutritional composition and consumer preferences.

The Haugh Unit (HU) is a widely accepted indicator of interior egg quality that combines egg weight and height of the thick albumen. The HU value was also significantly higher ($P < 0.05$) in eggs from older hens (average > 75) (Table 2), classifying them as Grade A to AA based on Studelman and Conterill (1995). According to Putri *et al.* (2016), HU values of 80 or higher are associated with good hatchability. This study's supporting finding is that eggs from older hens may have good quality. The positive correlation between HU and egg freshness was

also endorsed by (Sari *et al.* 2012; Selim *et al.* 2018; Riawan *et al.* 2017). Overall, the interior quality of Sentul chicken hatching eggs increases with hen age, with eggs from 78-week-old hens exhibiting superior albumen firmness, yolk roundness, color intensity, and HU values. These enhancements may be advantageous for hatching success and chick viability.

4.2. Nutrient Composition of Sentul Chicken Hatching Egg

The nutrient composition of hatching eggs is a critical factor in supporting embryonic development and determining the nutritional quality of chicks. In this study, age-related differences in the nutrient content of Sentul chicken hatching eggs were observed, with older hens (78 weeks) producing eggs that had lower water content but higher levels of fat and protein, compared to eggs from younger hens (41 weeks) (Table 3). These findings are consistent with previous research on the influence of hen age on nutrient profiles of eggs (Hefflin *et al.* 2018; Marzec *et al.* 2019; Kowalska *et al.* 2021; de Juan *et al.* 2023).

Eggs from 78-week-old hens showed a decrease in water content (70.88%) compared to those from 41-week-old hens (72.72%) (Table 3). The reduction could be attributed to age-related physiological changes in the hens, affecting the deposition of water during egg formation. A lower water content contributes to higher nutrient concentration, potentially enhancing embryonic nutrition and egg quality.

The fat content of eggs increased from 10.49% in younger hens to 12.38% in older hens. Fat may provide an essential energy source for the developing embryo, and this increase could be beneficial during incubation. However, excessive fat deposition may also affect the egg's overall quality and the embryo's development; thus, a balance is necessary. These values remain within the normal range (10.17%-12.72%) reported by Yuwanta (2010), indicating acceptable fat levels for hatching eggs.

A notable increase in protein content was also recorded with hen age, rising from 12.89% in younger hens to 13.67% in older hens (Table 3). Proteins play a crucial role in the formation of embryonic tissues and organs. Enhanced protein levels may support better growth and healthier chick development. The variation observed may be influenced by individual hen differences rather than feed quality, as both groups were maintained under the same management and nutritional conditions. The protein content of eggs in this study exceeded the typical protein range (10.6%-11.67%) reported by Yuwanta

(2010), suggesting that Sentul chickens may possess favorable genetic traits for producing protein-rich eggs.

The mineral content can influence the ash content in eggs (e.g., calcium, phosphorus, sodium) (Hefflin *et al.* 2018). The ash content in this study showed minimal variation between age groups, ranging from 1.4% to 1.54% (Table 3). These values are higher than the 0.75%-0.92% range reported by Yuwanta (2010), indicating that Sentul chicken eggs may have a relatively higher mineral concentration, which is beneficial for skeletal development during embryogenesis (Stephens and Johnson 2020).

The nutrient composition of Sentul chicken hatching eggs improves with hen age, particularly in terms of protein and fat content (Table 3), which are essential for supporting embryonic development. These age-related changes highlight the importance of considering hen age in breeding and incubation strategies to optimize chick viability and performance.

4.3. Development of the Primitive Streak and Somite in Sentul Chicken Embryos

The development of the primitive streak and somite is a crucial phase in early avian embryogenesis, laying the foundation for the body axis and musculoskeletal system. This study assessed the embryonic development of Sentul chicken embryos derived from 41-week-old and 76-week-old hens (Table 4), focusing on primitive streak formation at 16 hours (Figure 1) and somite formation at 33 hours of incubation (Figure 2).

4.3.1. Primitive Streak Development

The primitive streak is the first visible sign of gastrulation in the avian embryo and appears as a thickened structure at the posterior midline of the blastodisc. It facilitates cell migration and germ layer formation, which are essential for embryonic patterning (Bellairs and Osmond 2005). In this study, the primitive streak length averaged 1.18 ± 0.36 mm after 16 hours of incubation, with no significant difference between embryos from the two age groups of hens ($P > 0.05$) (Table 4). This measurement aligns with previous reports indicating that primitive streak development typically begins around 10 hours of incubation and reaches its full extension by 18 hours (Kochav *et al.* 1980; Lee *et al.* 2020).

Microscopically, embryos at this stage showed the characteristic structure of the area pellucid, area opaca, and marginal zone, with the primitive groove beginning to form (Figure 1). These structures are consistent with

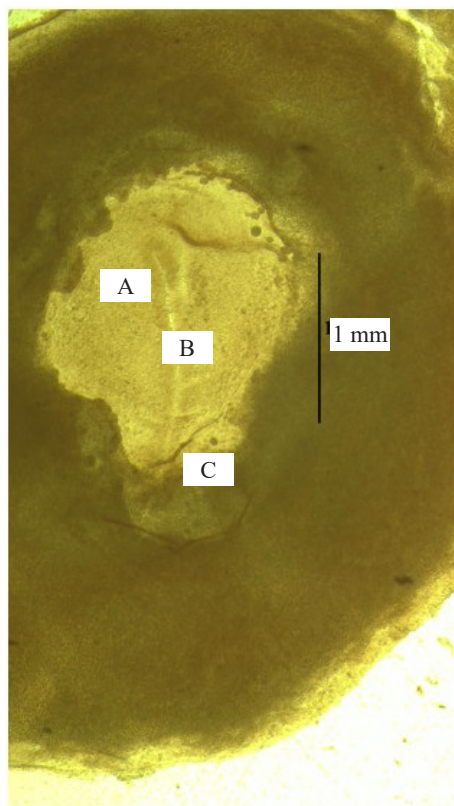


Figure 1. Primitive streak development at 16 hours (A) Area pellucida, (B) Primitive streak, (C) Area opaca of embryo chicken from 41 41-week-old

those described by Eyal-Giladi and Kochav (1976), reflecting normal progression of gastrulation.

4.3.2. Somite Formation

Somites are segmented structures of mesoderm that give rise to the vertebral column, skeletal muscles, and dermis. The transition from a primitive streak to segmented somite is a hallmark of early vertebrate development, marking the transition from gastrulation to organogenesis. After 33 hours of incubation, embryos from both hen age groups had developed an average of 9.58 ± 2.14 somite pairs, with no significant difference between the two groups ($P > 0.05$) (Table 4). This finding suggests a normal rate of somitogenesis, which typically progresses at a rate of one somite pair every 90 minutes during the early stages (Hamburger and Hamilton 1951; Huettner 1957).

Interestingly, the number of somites observed in this study exceeds the expected count of seven somite pairs at 33 hours, as reported by Hamburger and Hamilton (1951) for stage 9 chick embryos. This discrepancy may be attributed to embryonic development occurring before incubation began, possibly during egg storage or

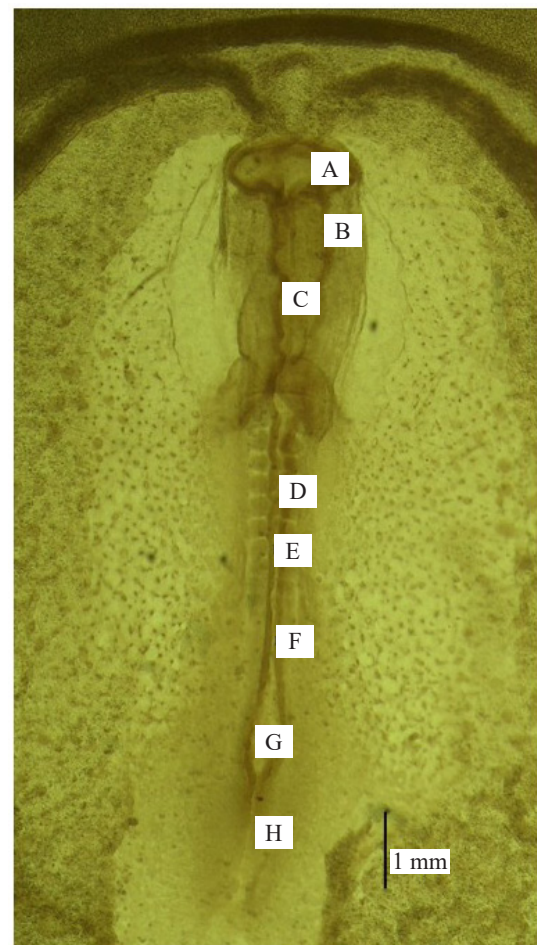


Figure 2. Somite development at 33 hours (A) Prosencephalon, (B) Mesencephalon, (C) Rhombencephalon, (D) Somite, (E) Neural tube, (F) Segmental plate mesoderm, (G) Hensen's node, (H) Primitive streak of an embryonic chicken from 41 weeks old

transport. Early signs of somite segmentation and head fold formation were visible by this time, further supporting the notion of slight pre-incubation development, as also reported by Fassenko *et al.* (2001), Nasri *et al.* (2019), Abioja *et al.* (2020), Bilalissi *et al.* (2022), and Tona *et al.* (2022). However, Zulfikar *et al.* (2024) also noted that embryos from 58-week-old hens developed an average of 11 ± 0.85 pairs, indicating that better hatching egg quality positively influences embryonic development.

The lack of significant differences ($P > 0.05$) in primitive length and somite number between embryos from younger and older hens (Table 4) suggests that hen age, under controlled feeding and management conditions, does not significantly affect these early stages of embryogenesis. The hens used in this study were genetically similar, provided with the same diets, and housed under uniform conditions. These factors likely

contributed to the consistency in embryonic outcomes. This study highlights the beneficial influence of hen age on hatching egg quality and embryonic development, potentially enhancing hatchability and chick growth after hatching. Nevertheless, additional research is necessary to examine the long-term effects of hen age on embryo development and chick performance after hatching.

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