Research Article

Age-Related Testicular Development in Turkeys Reared in Tropical Conditions: A Morpho-Histological and Morphometric Study for Maturity Assessment

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Abstract | Understanding age-related testicular maturation in turkeys is essential, as information on reproductive development under tropical conditions remains limited and may affect effective breeding management. This study investigated age-related testicular development in turkeys reared in a tropical environment using morphometric and histomorphometric approaches. Thirty male turkeys were divided into five age groups (8, 16, 24, 32, and 40 weeks; n = 6 per group). We examined testes for macroscopic parameters including weight, length, and circumference, while histomorphometric analysis evaluated seminiferous tubule diameter, epithelial height, lumen diameter, intertubular distance, and germ-cell composition. Testicular weight, size, and gonadosomatic index (GSI) increased significantly with advancing age ($p < 0.01$), with the most rapid growth observed between 24 and 40 weeks. The left testis was consistently heavier than the right across all ages, reflecting normal avian testicular asymmetry. Histomorphometric findings demonstrated progressive enlargement of seminiferous tubules, thickening of the germinal epithelium, and a reduction in interstitial tissue with age. Early age groups (8–16 weeks) were characterized by seminiferous tubules predominantly containing spermatogonia and primary spermatocytes, whereas turkeys aged 32–40 weeks displayed a complete spermatogenic series with abundant spermatids and luminal spermatozoa, indicating sexual maturity. These results demonstrate that testicular maturation in turkeys is characterized by coordinated increases in organ size, seminiferous tubule expansion, and enhanced spermatogenic activity. This study provides essential baseline data on testicular development in turkeys under tropical conditions and supports improved reproductive management and breeding strategies.

Keywords | Breeding management, Histomorphometry, Spermatogenesis, Tropical conditions, Turkey reproduction

Received | December 08, 2025; **Accepted** | January 25, 2026; **Published** | February 06, 2026
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Citation | Masyitha D, Akmal M, Wahyuni S, Gholib G (2026). Age-related testicular development in turkeys reared in tropical conditions: A morpho-histological and morphometric study for maturity assessment. Adv. Anim. Vet. Sci., 14(2):443-451.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.2.443.451
ISSN (Online) | 2307-8316

INTRODUCTION

Global demand for poultry meat has risen significantly in recent decades, including in Indonesia (Windhorst, 2017; Sari *et al.*, 2021). While chicken and duck remain the dominant sources of poultry meat in the country, turkey (*Meleagris gallopavo*) production has gained attention as an alternative source of high-quality meat (Al-Baidhani and Al-Qutaifi, 2021). Turkey meat contains a high protein content (30.5–34.3%) with moderate fat levels (7.5–11.5%)

and a favorable nutritional profile, supporting its potential contribution to food security and dietary diversification (Solaesa *et al*., 2024). In addition, turkeys exhibit efficient growth performance, with males and females reaching approximately 1.6 and 2.0 kg at three months of age and 2.5–4.0 and 5.0–6.0 kg at 4–6 months, respectively, corresponding to typical market age (Safiyu *et al*., 2019). Despite these advantages, turkey production in Indonesia remains limited, partly due to a lact of knowledge regarding male reproductive development, particularly the timing of sexual maturity.

The testes play a central role in male reproductive function through spermatozoa production and testosterone synthesis (Akmal *et al*., 2019), the principal androgen for regulating spermatogenesis and secondary sexual characteristics (Kiezun *et al*., 2015). Testicular development involves coordinated structural and functional changes, including increases in testicular weight, seminiferous tubule diameter, epithelial height, and germ cell proliferation (Li *et al*., 2024). In avian species, germ cell proliferation progresses from spermatogonia to primary spermatocytes and subsequent spermatogenic stages, a process that depends on close interactions between germ cells and Sertoli cells and is tightly regulated by gonadotropins and androgens (Parvez *et al*., 2023). These parameters are widely recognized as reliable indicators of sexual maturation in birds.

Avian testicular development begins during embryogenesis with the formation of the genital ridge and gonadal differentiation, followed by post-hatch growth characterized by progressive histomorphological changes in the seminiferous epithelium (Olea *et al*., 2018). During juvenile and prepubertal stages, seminiferous tubules enlarge, the germinal epithelium thickens, and spermatogenic activity gradually increases until full reproductive competence is achieved at puberty (Mfoundou *et al*., 2022). The timing and rate of these developmental processes are influenced by genetics, nutrition, and environmental factors such as temperature and photoperiod, factors particularly relevant under tropical production systems (Stephens and Johnson 2020).

Although histomorphological and morphometric approaches have been widely applied to assess testicular maturation in poultry (Mfoundou *et al*., 2022), age-related studies focusing specifically on turkeys reared under tropical conditions remain scarce. Previous studies have reported significant age-dependent increases in seminiferous tubule diameter and epithelial height in turkeys (Yahaya *et al*., 2017). However, comparable data from tropical environments are limited. This lack of region-specific data may hinder accurate breeding management decisions and ultimately affect reproductive efficiency. Therefore, the present study aimed to evaluate age-related testicular development in male turkeys reared under tropical conditions using histomorphological and morphometric analyses. The findings are expected to provide essential baseline data on post-hatch testicular maturation and to support improved breeding management and reproductive strategies in turkey production systems in tropical regions.

MATERIALS AND METHODS

Animals and husbandry

Thirty day-old turkey poults were obtained from a commercial turkey farm in North Sumatera, Indonesia, and reared under uniform management conditions until sampling. At sampling, turkeys were assigned to five age groups (n = 6 per group): 8, 16, 24, 32, and 40 weeks. The experimental design and sequential stages of the study are summarized in Figure 1.

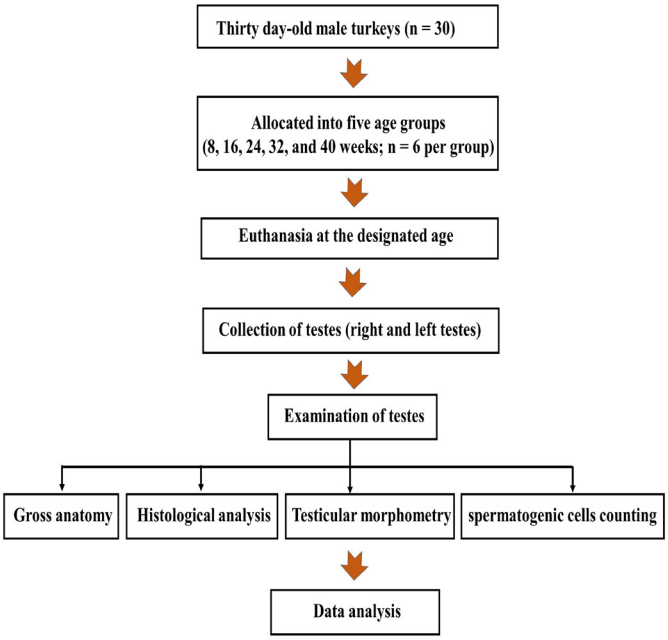

Figure 1: Flowchart illustrating the experimental design and sequential stages of the study.

The turkeys used in this study belonged to the bronze turkey line, a heritage-type strain widely reared across diverse production systems and recognized for its adaptability to both temperate and tropical environments. The turkeys were housed in an open-sided poultry house typical of tropical production systems. Turkeys were maintained in age-specific group pens with litter flooring under adequate space allowance, with each pen measuring 3×4 m^2 and housing six turkeys.

The turkeys were fed commercial chicken diets formulated according to growth phase. A starter diet was provided from day 1 until 8 weeks of age, followed by a grower diet from 9–40 weeks of age. The starter diet contained 22–24% crude protein, ≤12% moisture, ≥5% crude fat, ≤4% crude

fiber, ≤7% ash, 0.8–1.1% calcium, ≥0.5% phosphorus, and ≥2,900 kcal/kg metabolizable energy, with a maximum aflatoxin level of 40 µg/kg. The grower diet contained 19–20% crude protein, ≤12% moisture, ≥5% crude fat, ≤5% crude fiber, ≤7% ash, 0.8–1.1% calcium, ≥0.45% phosphorus, and ≥3,100 kcal/kg metabolizable energy, with a maximum aflatoxin level of 50 µg/kg. Feed and drinking water were provided ad libitum throughout the study. Environmental conditions were representative of a tropical climate, with ambient temperatures ranging from approximately 26 to 32 ˚C and relative humidity between 70 and 90% throughout the rearing period. Natural daylight predominated, resulting in a photoperiod of approximately 12–13 h of light per day, and no artificial environmental cooling was applied.

Anatomical observations

Prior to euthanasia, each turkey was weighed using a digital balance to obtain live body weight. Necropsy was then performed, followed by a ventral midline incision to expose the thoracic and abdominal cavities for in situ examination of the testes, as described previously (Mfoundou *et al*., 2022). Gross anatomical observations included the testicular position, shape, color, surface characteristics, and the spatial relationship with surrounding organs. The left and right testes were carefully excised, trimmed of connective tissue and fat, and weighed using a precision digital balance. Testicular length and circumference were measured using a vernier caliper and flexible measuring tape, respectively. The gonadosomatic index (GSI) of the testes was calculated using the formula described by Mohammadi *et al*. (2022): GSI = (weight of both testes/ body weight) x 100.

Histological preparations and histo-morphological analysis

Testes were fixed by immersion in 10% neutral buffered formalin (NBF) for 24 h and subsequently transferred to 70% ethanol for storage. Before processing, testes were cut into small pieces (approximately 0.5 × 0.5 × 0.5 cm). Testes were then dehydrated through an ascending graded ethanol series (70%, 80%, 90%, and absolute ethanol), cleared in xylene (two changes, 1 hour each), and infiltrated with molten paraffin wax before embedding in paraffin blocks, following standard histological procedures (Wahyuni *et al*., 2018). Paraffin-embedded tissues were sectioned at 4-µm thickness using a Leica RM2235 manual microtome (Leica Biosystems, Nussloch GmbH, Germany). Sections were mounted on glass slides, deparaffinized in xylene, rehydrated through descending ethanol concentrations, and stained with hematoxylin and eosin (H and E). Stained sections were dehydrated through an ethanol series, cleared in xylene, and mounted with Entellan®.

Testis and seminiferous tubule morphometry assessments

Histomorphometric measurements of testicular tissue were performed using a light microscope equipped with a digital camera and image-analysis software at 400× magnification, following established methods (Wahyuni *et al*., 2018). Several structural parameters were assessed. Tunica albuginea thickness (µm) was measured as the perpendicular distance from the outer fibrous capsule to its inner boundary in direct contact with the seminiferous tubules. Seminiferous epithelial (germinal epithelium) thickness (µm) was determined by measuring the distance from the basement membrane to the luminal border of the epithelium. Seminiferous tubule diameter (µm) was calculated as the mean of two perpendicular diameters from circular or nearly circular tubules in transverse orientation. Lumen diameter of seminiferous tubules (µm) was measured as the average of two perpendicular lumen diameters in cross-section. In addition, intertubular distance (µm) was assessed by measuring the connective tissue spacing between two adjacent seminiferous tubules. Measurements were taken from tubules meeting the criteria for proper transverse sectioning and were selected randomly to ensure representative sampling.

Quantitative assessment of spermatogenic cells (spermatogonia, primary spermatocytes, round spermatids, elongated spermatids, and luminal spermatozoa) was quantified in 10 randomly selected seminiferous tubules per sample. Only cells with clearly identifiable nuclei or characteristic morphology were included.

Data analysis

All statistical analyses were conducted using the individual turkey as the experimental unit. For each animal, ten seminiferous tubules were randomly selected from each testis, and the resulting values were averaged to obtain a single representative measurement per individual. These averaged values were used for all subsequent analyses, thereby preventing pseudo-replication. Before analysis, all quantitative data were tested for normality using the Shapiro–Wilk test, and the data were found to be normally distributed ($p>0.05$). Gonadosomatic index (GSI) data were analyzed using one-way analysis of variance (ANOVA) across age groups (8, 16, 24, 32, and 40 weeks), followed by Tukey's post hoc test for pairwise comparisons. Gross anatomy of testes, testicular histomorphometric parameters, and spermatogenic cell counts were analyzed using linear mixed-effects models (Gholib *et al*., 2025). In these models, age and testis side (right and left) were included as fixed effects, while individual identity was incorporated as a random effect to account for repeated measurements. Post hoc comparisons were adjusted using the Bonferroni correction. All data are presented as mean ± standard deviation (SD), and statistical significance was defined as $p < 0.05$.

RESULTS

Gross anatomy of testes

Turkey testes showed progressive age-dependent morphological changes (Figure 2). At 8 weeks, testes were small, oval, pale cream and soft, with smooth surfaces and no visible vascularization. Testicular weight ranged from 0.14 to 0.16 g. By 16 weeks, testes increased significantly in size (1.21–1.57 g), became elongated and firmer, and showed early signs of surface vascularization. At 24 weeks, testes were markedly enlarged (3.20–3.50 g), with visible vasculature and increased turgidity. The left testis was consistently larger and positioned more caudally than the right. Structural maturity was evident at 32 weeks, with testes occupying a substantial portion of the abdominal cavity, showing dense vascularization and glistening surfaces (8.36–9.57 g). Maximal development was observed at 40 weeks, characterized by spindle-shaped, firm, yellowish-cream testes with pronounced vascularization (10.18–11.23 g).

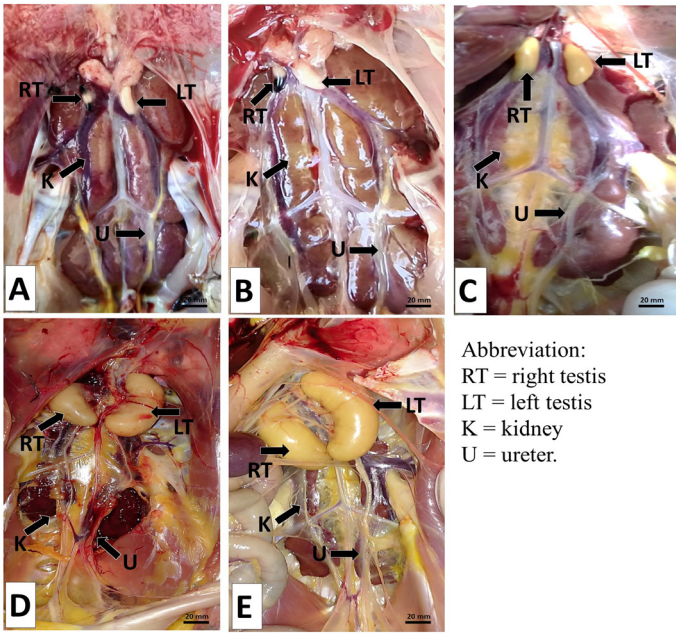

Figure 2: Macroscopic appearance of the testes in turkeys at 8 (A), 16 (B), 24 (C), 32 (D), and 40 (E) weeks of age.

Linear mixed-effects model analysis revealed significant age-related differences in testicular weight, length, and circumference ($p < 0.01$; Figure 3). The left testis was consistently heavier than the right across all age groups ($p < 0.01$). No significant left–right differences were observed in testicular length at any age ($p > 0.05$). Circumference differed significantly between sides only at 24 weeks ($p < 0.05$).

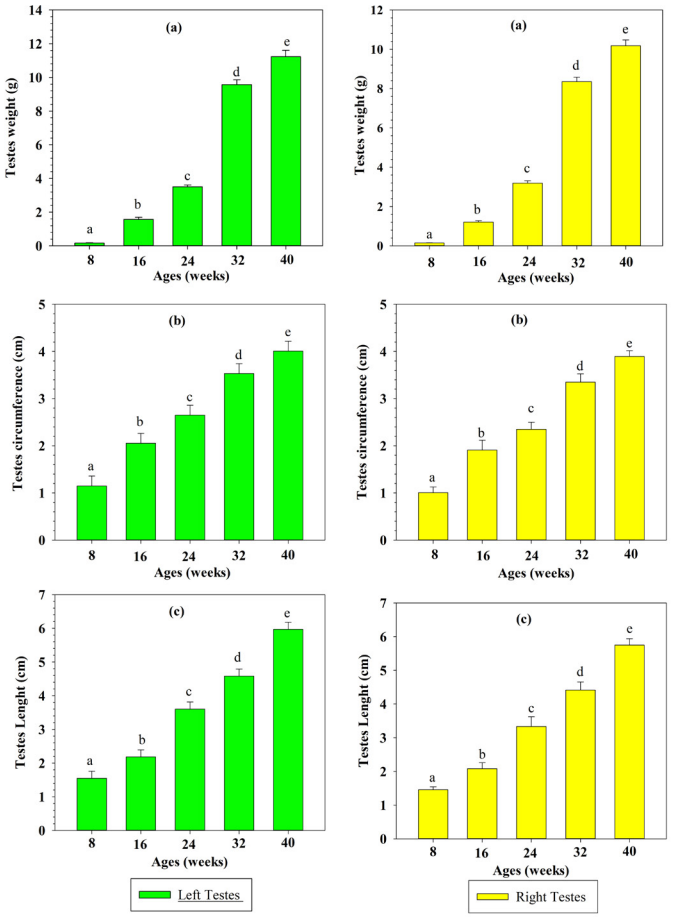

Figure 3: Left and right testis weight, circumference, and length in turkeys at different ages (mean ± SD). (A) Testis weight, (B) testis circumference, and (C) testis length. Different superscript letters (a–e) above the bars indicate significant differences among age groups ($p < 0.01$).

Gonadosomatic index (GSI)

Testicular weight, body weight, and GSI increased significantly with age ($p < 0.01$; Table 1). The lowest GSI was recorded at 8 weeks (0.001%), while the highest values were observed at 32 and 40 weeks (0.14%). No significant difference was detected between the 16- and 24-week groups or between the 32- and 40-week groups ($p > 0.05$).

Table 1: Testicular weight, body weight, and gonadosomatic index (GSI) of turkeys at different ages

Ages (weeks)	Left testis weight (g)	Right testis weight (g)	Both testis weight (g)	Body weight (g)	GSI (%)
8	0.17±0.02^{a}	0.15±0.01^{a}	0.16±0.02^{a}	830.83±80.39^{a}	0.001^{a}
16	1.57±0.12^{b}	1.20±0.08^{b}	1.20±0.21^{b}	1645.83±276.78^{b}	0.086^{b}
24	3.50±0.11^{c}	3.20±0.12^{c}	3.35±0.19^{c}	3605.83±807.49^{c}	0.097^{b}
32	9.57±0.28^{d}	8.36±0.23^{d}	8.96±0.68^{d}	6216.67±366.39^{d}	0.144^{c}
40	11.23±0.38^{e}	10.18±0.30^{e}	10.70±0.64^{e}	7491.67±380.09^{e}	0.143^{c}

^{a,b,c,d,e} Different superscripts in the same column indicated significant differences ($p<0.01$).

Histomorphometry of testes and seminiferous tubules

Histological evaluation revealed progressive maturation of testicular microarchitecture. Age-related changes included thickening of the tunica albuginea, enlargement of seminiferous tubules, increased germinal epithelium height, and expansion of the tubular lumen (Figure 4).

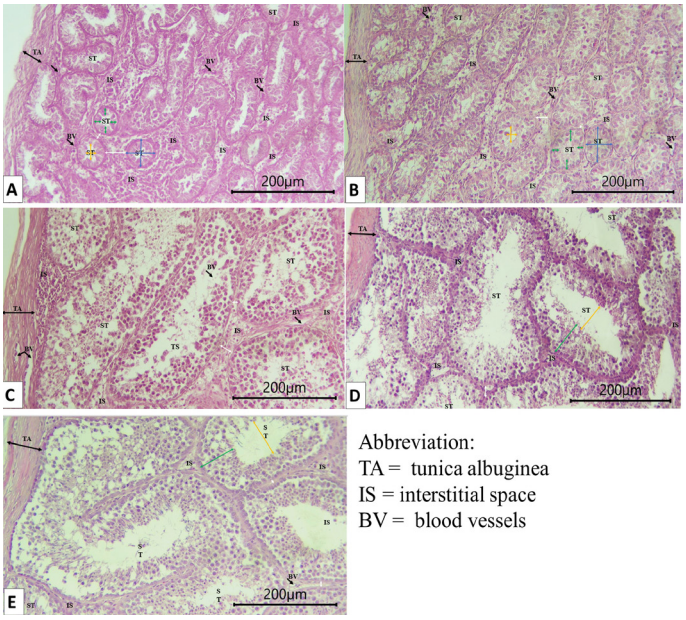

Figure 4: Histological micrographs of seminiferous tubules in turkeys at different ages: 8 weeks (A), 16 weeks (B), 24 weeks (C), 32 weeks (D), and 40 weeks (E) are shown at 100× magnification. The diameter of seminiferous tubules (blue arrows), thickness of the germinal epithelium (green arrows), diameter of the seminiferous tubule lumen (yellow arrows), and distance between seminiferous tubules (white arrows) are indicated.

Linear mixed-effects model analysis revealed a significant effect of age on all testicular histomorphometric parameters ($p < 0.01$; Table 2). Tunica albuginea thickness increased significantly from 8 to 16 weeks ($p < 0.01$) and remained relatively stable thereafter, with no significant differences between right and left testes ($p > 0.05$). Seminiferous tubule epithelium thickness and seminiferous tubule diameter showed a progressive age-related increase in both testes, with markedly higher values observed from 24 weeks onward ($p < 0.01$). For both parameters, the left testis consistently exhibited significantly greater values than the right testis across all age groups ($p < 0.01$). Seminiferous tubule lumen diameter increased significantly with age from 8 to 32 weeks, followed by a significant reduction at 40 weeks ($p < 0.01$). Significant laterality effects were detected at 16, 24, and 32 weeks ($p < 0.01$) but not at 8 and 40 weeks ($p > 0.05$). Intertubular distance decreased significantly from 8 to 32 weeks ($p < 0.01$) and subsequently increased at 40 weeks, with a significant difference between right and left testes observed only at 24 weeks.

Table 2: Mean±standard deviation of testicular histomorphometric parameters in turkeys across different age groups.

Ages (weeks)	Right testis	Left testis
a. Tunica albuginea thickness (µm)		
8	17.25 ± 0.98^{ax}	19.87 ± 1.11^{ax}
16	24.84 ± 3.34^{bx}	27.60 ± 3.71^{bx}
24	26.54 ± 2.85^{bx}	29.05 ± 4.44^{bx}
32	28.82 ± 1.91^{bx}	30.57 ± 4.53^{bx}
40	31.11 ± 6.02^{bx}	25.84 ± 1.08^{bx}
b. Seminiferous tubule epithelium thickness (µm)		
8	7.88 ± 0.29^{ax}	10.94 ± 0.32^{ay}
16	19.69 ± 1.07^{bx}	22.87 ± 1.16^{by}
24	33.65 ± 1.91^{cx}	38.75 ± 1.90^{cy}
32	41.15 ± 0.72^{dx}	45.89 ± 0.72^{dy}
40	61.57 ± 1.51^{ex}	67.06 ± 1.39^{ey}
c. Seminiferous tubule diameter (µm)		
8	36.72 ± 0.91^{ax}	48.90 ± 0.37^{ay}
16	51.17 ± 1.77^{bx}	65.83 ± 1.82^{by}
24	139.99 ± 15.84^{cx}	162.77 ± 5.66^{cy}
32	174.24 ± 6.99^{dx}	193.60 ± 3.82^{dy}
40	181.24 ± 9.01^{dx}	199.54 ± 5.71^{dy}
d. Seminiferous tubule lumen diameter (µm)		
8	7.15 ± 0.31^{ax}	8.39 ± 0.68^{ax}
16	18.45 ± 1.48^{bx}	24.28 ± 2.02^{by}
24	90.89 ± 10.00^{cx}	109.85 ± 7.00^{cy}
32	106.16 ± 4.26^{dx}	122.17 ± 3.64^{dy}
40	50.12 ± 4.44^{ex}	52.56 ± 1.04^{ex}
e. Intertubular distance (µm)		
8	18.48 ± 5.08^{ax}	16.06 ± 3.57^{ax}
16	13.42 ± 4.77^{bx}	13.37 ± 2.27^{bx}
24	7.07 ± 1.01^{cx}	5.48 ± 0.58^{cy}
32	4.15 ± 0.83^{cx}	3.55 ± 0.74^{cx}
40	7.52 ± 1.09^{cx}	5.58 ± 0.93^{cx}

Values are presented as mean ± SD. Different superscript letters (a–e) within the same column indicated significant differences among ages, while identical letters indicated no significant difference ($p < 0.01$). Different superscripts (x–y) within the same row indicate significant differences between right and left testes ($p < 0.01$).

Spermatogenic cell development

At 8 and 16 weeks, seminiferous tubules contained mainly spermatogonia and occasional primary spermatocytes, with absent or narrow lumina. In contrast, at 24–40 weeks, seminiferous tubules showed well-defined lumina, increased numbers of germ cell layers, and the presence of spermatids and spermatozoa (Figure 5). Based on linear mixed-effects model analysis, the number of spermatogenic

cells varied significantly with age and testis side ($p < 0.01$; Table 3). Spermatogonia decreased from 8 to 16 weeks and increased progressively thereafter, with consistently higher values in the left testis. Spermatocytes increased markedly from 16 weeks and reached a plateau at 32–40 weeks. Round and elongated spermatids first appeared at 24 weeks and increased with age, while spermatozoa were detected only at 32 and 40 weeks, with significantly higher counts in the left testis ($p < 0.01$).

Table 3: Number of spermatogenic cells in turkey testes across different age groups.

Ages (weeks)	Right testis	Left testis
a. Number of spermatogonia		
8	8.81 ± 0.59^{ax}	11.39 ± 0.90^{ay}
16	2.11 ± 0.70^{bx}	4.40 ± 0.93^{by}
24	19.70 ± 3.98^{cx}	27.72 ± 3.08^{cy}
32	28.41 ± 2.83^{dx}	36.50 ± 3.56^{dy}
40	38.96 ± 2.74^{ex}	50.15 ± 3.37^{ey}
b. Number of spermatocytes		
8	0.01 ± 0.01^{ax}	0.02 ± 0.06^{ay}
16	11.03 ± 1.19^{bx}	14.64 ± 1.49^{by}
24	28.48 ± 4.63^{cx}	43.81 ± 12.41^{cy}
32	69.25 ± 2.30^{dx}	82.54 ± 4.13^{dy}
40	70.07 ± 5.57^{dx}	79.54 ± 5.65^{dy}
c. Number of round spermatids		
8	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
16	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
24	22.20 ± 4.20^{bx}	31.24 ± 7.83^{by}
32	109.66 ± 7.81^{cx}	121.08 ± 8.35^{cy}
40	101.13 ± 11.15^{cx}	116.93 ± 9.70^{cy}
d. Number of elongated spermatids		
8	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
16	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
24	11.51 ± 3.42^{bx}	15.85 ± 6.44^{by}
32	33.74 ± 3.51^{cx}	43.89 ± 3.86^{cy}
40	56.70 ± 6.16^{dx}	66.23 ± 3.90^{dy}
e. Number of spermatozoa		
8	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
16	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
24	0.00 ± 0.00^{ax}	0.00 ± 0.00^{ax}
32	19.91 ± 2.32^{bx}	28.81 ± 4.22^{by}
40	33.46 ± 2.34^{cx}	43.30 ± 5.52^{cy}

Values are presented as mean ± SD. Different superscript letters (a–e) within the same column indicate significant differences among ages, while identical letters indicate no significant difference ($p < 0.01$). Different superscripts (x–y) within the same row indicate significant differences between right and left testes ($p < 0.01$).

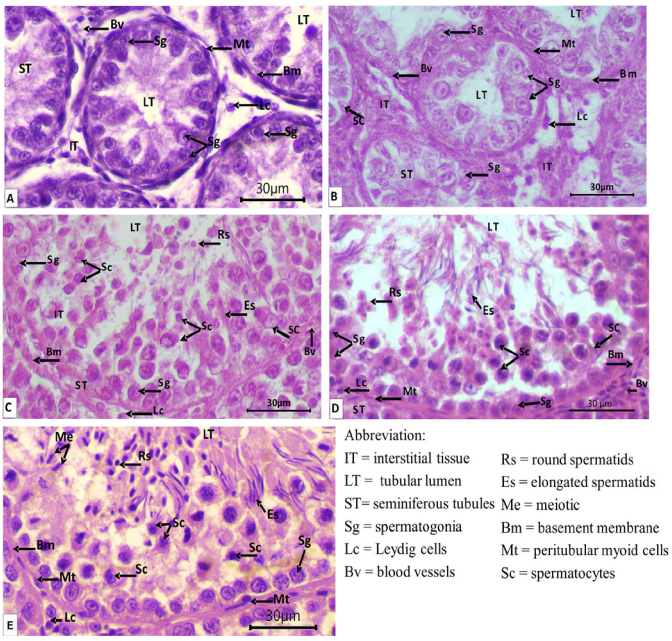

Figure 5: Histological appearance of seminiferous tubules (spermatogenic cells) in turkeys at different ages: 8 weeks (A), 16 weeks (B), 24 weeks (C), 32 weeks (D), and 40 weeks (E).

DISCUSSION

The present study demonstrates a clear age-dependent progression of testicular development in male turkeys, as reflected by changes in gross anatomy, gonadosomatic index, histomorphometry, and spermatogenic cell composition. Testicular growth accelerated markedly after 24 weeks of age, accompanied by structural maturation of seminiferous tubules, increased germinal epithelium height, lumen formation, and the appearance of mature spermatozoa. Collectively, these findings indicate that functional sexual maturity in male turkeys is achieved between 32 and 40 weeks of age.

Age-related increases in testicular weight, length, and circumference observed in this study are well-recognized indicators of reproductive maturity and sperm-producing capacity in poultry (Kouatcho *et al.*, 2015). The rapid enlargement of testes between 24 and 40 weeks corresponds with the pubertal growth phase reported in turkeys and other avian species, during which seminiferous tubules expand rapidly, and spermatogenesis becomes fully established (Parvez *et al.*, 2023). Similar associations between testicular biometry and reproductive potential have been linked to nutritional status, environmental conditions, and genetic background (Yahaya *et al.*, 2017; Yin *et al.*, 2023). Given the strong correlation between testicular size, sperm output, and semen quality (Sun *et al.*, 2019), these biometric parameters may serve as practical indicators for breeder selection and reproductive management in turkey production systems.

The progressive increase in gonadosomatic index (GSI) further reflects the trajectory of gonadal maturation in male turkeys. The marked rise in GSI between 16 and 32 weeks indicates the transition from prepubertal to sexually active stages, characterized by accelerated testicular growth and intensified spermatogenic activity. Comparable age-related GSI patterns have been reported in chickens and turkeys, where GSI increases sharply during puberty and stabilizes at sexual maturity (Abdul-Rahman *et al*., 2018; Ibrahim *et al*., 2022). The absence of significant differences between 32 and 40 weeks suggests stabilization of testicular function and attainment of a mature reproductive state, consistent with observations in other galliform birds (Oduwole *et al*., 2021).

Histomorphometric changes observed in this study further support the progression of testicular maturation. Early thickening of the tunica albuginea between 8 and 16 weeks has also been reported in chickens and quail and is considered important for providing mechanical support for subsequent seminiferous tubule expansion (Olea *et al*., 2018). The pronounced increase in seminiferous tubule diameter and germinal epithelium height from 24 weeks onward reflects intensified spermatogenic activity, as epithelial thickness is closely associated with germ cell proliferation and differentiation (Ni *et al*., 2019).

The increase in seminiferous tubule lumen diameter between 16 and 32 weeks indicates the transition from solid seminiferous cords to fully canalized tubules, a key event during pubertal onset in birds. The slight reduction in lumen diameter at 40 weeks may be attributed to increased epithelial thickness resulting from higher germ cell density, a pattern previously reported in mature roosters and ducks (Kareem *et al*., 2020). In parallel, intertubular distance exhibited a non-monotonic pattern, decreasing from 8 to 32 weeks due to parenchymal compaction driven by rapid tubule expansion, followed by an increase at 40 weeks. This rebound likely reflects post-pubertal structural reorganization, including relative expansion of the interstitial compartment and vascular components, as reported in mature avian and mammalian testes (Picut *et al*., 2015).

Spermatogenic cell dynamics observed in this study follow a typical avian maturation sequence. The predominance of spermatogonia and the absence of advanced germ cells at 8 and 16 weeks confirm prepubertal status, consistent with classical descriptions of avian testes prior to meiotic initiation (Akhtar *et al*., 2020). The marked increase in spermatogenic cells at 24 weeks indicates the onset of puberty, while the presence of abundant spermatids and spermatozoa at 32 and 40 weeks confirms full sexual maturity. Similar developmental timelines have been reported in chickens, ducks, and Japanese quail (Obeid *et al*., 2021; Mfoundou *et al*., 2022; Eldesoky *et al*., 2025). The transient decline in spermatogonia between 8 and 16 weeks likely reflects a shift in spermatogonial stem cell (SSC) dynamics during initiation of the first spermatogenic wave, followed by restoration of SSC self-renewal and niche stabilization after puberty (de Rooij, 2017).

Testicular maturation observed between 32 and 40 weeks in this tropical cohort is broadly consistent with findings from temperate regions. Noirault *et al*. (2006) reported that sexually mature turkeys under controlled photoperiods exhibited left and right testis weights of 34.52 ± 1.66 g and 19.36 ± 1.31 g, respectively, with seminiferous tubule diameters of approximately 205 µm (left) and 201 µm (right). The seminiferous tubule diameters observed in the present study at maturity fall within this reported range, suggesting that structural benchmarks of sexual maturity are largely conserved across environments, although minor differences in timing and magnitude may reflect regional influences such as photoperiod, temperature, and management practices.

Finally, the consistent dominance of the left testis across all age groups aligns with well-established patterns of avian reproductive asymmetry. Similar left-sided predominance has been reported in chickens, quail, ducks, and pigeons, where the left testis is typically larger and more metabolically active (Carvalho *et al*., 2015; Calhim and Montgomerie, 2015; Abdul-Rahman *et al*., 2018; Mizia *et al*., 2023). This asymmetry originates during early embryogenesis through left–right patterning pathways that confer enhanced growth potential to the left gonadal primordium and persists into postnatal life. At sexual maturity, the left testis in the present study exhibited significantly higher spermatozoa numbers than the right testis, indicating a disproportionate contribution to spermatogenic output. This finding suggests that left testis dominance in turkeys is not merely anatomical but functionally relevant, potentially reflecting differences in seminiferous tubule development, vascularization, endocrine responsiveness, or local microenvironment. Similar functional asymmetry has been reported in turkeys and other avian species and is considered an adaptive feature optimizing reproductive efficiency rather than an anatomical relic (Noirault *et al*., 2006; Sun *et al*., 2019).

CONCLUSION

Testicular development in male turkeys reared under tropical conditions shows clear age-related morphological and histomorphometric changes. Advancing age is associated with increased testicular size, gonadosomatic index, seminiferous tubule diameter, and germinal epithelium thickness, accompanied by progressive completion of spermatogenesis. Fully developed seminiferous tubules

with luminal spermatozoa were evident at 32–40 weeks, indicating attainment of sexual maturity. These findings provide baseline information on testicular maturation in turkeys under tropical conditions. Based on these findings, active breeding programs and semen collection in tropical turkeys should be initiated from 32 weeks of age, while testicular size at 24 weeks may serve as an early selection criterion for identifying prospective breeder males.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Faculty of Veterinary Medicine, Universitas Syiah Kuala, for providing laboratory facilities and technical support throughout this study. We also thank the staff of the Histology, Anatomy, and Physiology Laboratories for their assistance with animal handling, sample preparation, and data collection.

NOVELTY STATEMENT

This study presents the first integrative, quantitative analysis of age-related testicular development in male turkeys (*Meleagris gallopavo*) reared under tropical conditions in Indonesia. It provides tropical-specific reference data on testicular growth, gonadosomatic index, seminiferous tubule morphometry, epithelial maturation, and spermatogenic progression. Unlike previous studies from temperate regions, this work identifies 32–40 weeks of age as the critical window of functional reproductive maturity, supported by direct histological evidence of complete spermatogenesis and luminal spermatozoa. These findings provide novel, regionally relevant benchmarks to improve breeding selection and reproductive management in tropical turkey production systems.

AUTHORS' CONTRIBUTION

Dian Masyitha designed and performed the experiments, collected samples, and drafted the manuscript. Muslim Akmal contributed to the study design and supervised the histological analyses. Sri Wahyuni assisted with anatomical observations, sample processing, and data interpretation. Gholib Gholib conceived the study, performed data analysis, wrote, and revised the manuscript. All authors read and approved the final version of the manuscript.

Funding

The authors express their gratitude to the Universitas Syiah Kuala for funding this study through the Professor Research (grant no. 3/UN11.21/PT.01.03/PNBP/2020).

Ethics statement

All experimental procedures involving animals were conducted in accordance with the ethical guidelines approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala (approval number: 89/KEPH/XII/2020).

Generative AI and AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Conflict of interest

The authors have declared no competing interest.

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