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# Age-related changes in testicular histological structure, testosterone level, libido and semen traits in male Coturnix quail

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## Abstract

To investigate the effect of age in male quail on testicular weight and histology, sexual libido and semen characteristics, a study was performed on 100 quails at 10, 16, 22, 28 and 34 weeks of age. The body and testicular weights were significantly ($p < .05$) higher at 16 and 22 than at 28 weeks of age. The circumference and diameter of the seminiferous tubules were significantly ($p < .05$) higher at 28 and 34 than at 10 and 16 weeks of age. Histological evaluation of testicular slices revealed advanced and effective seminiferous tubes as early as 10 weeks, while spermatogenic activity peaked at 16 weeks of age. The highest semen volume, sperm motility and sperm concentration were observed at weeks of age and then decreased gradually with age. At the same time, the testosterone level and libido were significantly ($p < .05$) higher at 22 than at 10 weeks of age. Furthermore, the age was positive ($p < .05$), correlated to seminiferous tubule circumference and diameter, and negative ($p < .05$), correlated to sperm concentration. In conclusion, the age of quail markedly affected the testicular histological structure, libido, testosterone level and semen characteristics.

KEYWORDS
age, male Coturnix quail, semen characteristics, testicular histological

## 1 | INTRODUCTION

Old age reverses reproductive success in all bird species and other species in the animal kingdom. The decline in commercial chicken reproduction in old age is attributed to decreased egg production, fertility and hatchability of viable and fertile eggs. This reduction in egg production begins to occur once hens peak in production (Abd El-Hack et al., 2019; Abdelnour et al., 2020; Nour et al., 2021). The effect of age on testicular histological structure and semen characteristics in Japanese quail is an important topic in reproductive biology research (Molele et al., 2021). The Coturnix quail, also known as the Japanese quail, is a widely used avian model for studying reproductive physiology due to its short reproductive cycle and high fertility rates (Abd El-Hack et al., 2016; Abou-Kassem et al., 2020; Ball & Balthazart, 2010; Dosoky et al., 2021; Hussein et al., 2019; Taha et al., 2019).

There are noticeable variations in the histological structure of the quail testes at different ages. The testes consist of seminiferous tubes responsible for spermatogenesis and interstitial tissue that produces hormones (Gonzalez-Moran & Soria-Castro, 2010). With advancing age, the seminiferous tubules undergo structural modifications such as reduced diameter and increased connective tissue deposition (Ashour et al., 2022). These changes can lead to a decline in spermatogenesis and affect semen characteristics.

Roshdy (2021) suggested that semen characteristics were affected by age. Older male quail exhibits decreased sperm motility, viability and concentration compared to younger individuals. This decline in sperm quality can be attributed to the structural changes occurring within the testes. Testosterone is a crucial hormone in spermatogenesis and semen production (Sedqvar et al., 2008). The testosterone levels are decreased in advanced age, which can impact sperm production and semen quality (Biswas et al., 2007).

It is hypothesized that age can affect testicular weight and histology, sexual libido and semen characteristics of male quail. Understanding these age-related changes in testicular histological structure and associated semen characteristics is essential for optimizing breeding strategies and improving reproductive efficiency in coturnix quail farming. Therefore, this study aimed to investigate the effect of age on testicular weight and histology, sexual libido and semen characteristics in male quail.

## 2 | MATERIALS AND METHODS

The authors adhere that procedures imposed on the animals were carried out according to the Directive 2010/63/EU of the European Parliament and the Council of 22 September 2010 on protecting animals used for scientific purposes. Authors also adhere to the EU regulations on feed legislation, such as regulation EC No 767/2009 of the European Parliament Council of July 13, 2009. The Authors' Institution Ethics Committee approved the study at the Animal Production Department, Faculty of Agriculture, Suez Canal University, Egypt, for animal studies, and care was taken to minimize the number of animals used.

### 2.1 | Birds

One hundred sexually matured male quails (*Coturnix coturnix japonica*) were reared in individual cages in the Poultry Research Station of Inshas of Animal Production Research Institute and used in the current study to evaluate their physiological traits at different ages (10, 16, 22, 28 and 34 weeks). Quail body weights ($n$ = 20/group) were measured at 10, 16, 22, 28 and 34 weeks to determine body weight variations.

### 2.2 | Semen collection and evaluation

Semen samples were collected at 10, 16, 22, 28 and 34 weeks of age. The female and male quail were placed in one cage. The male was taken directly before ejaculation during mating male with female. Before collecting semen, every male was softly restricted on the palm, and foam was taken out. The lumbar region was gently rubbed 3–4 times; a light press was provided to each side of the vent with the fingertips. A micropipette was used to collect the semen. The foam gland was carefully cleansed with wipes to avoid pollution with foam, faeces, and watery liquid. Five microlitres of semen from a single male was collected and mixed with warm normal saline (0.9% NaCl) at a rate of 1:10 in an Eppendorf tube. The total volume of 5 μL methylene blue liquid (50 mg % w/v) was added to the dissolved semen.

Semen traits were evaluated directly after collection in each semen at 10, 16, 22, 28 and 34 weeks by the same expert evaluator, as follows: Semen volume (SV) was calculated to the nearest 1 μL utilizing a graded collecting micropipette with rubber tubing and a mouthpiece into a small, calibrated tube, allowing SV to be precisely quantified to 20 μL. Sperm progressive motility (SM) was evaluated using a phase contrast microscope (400×). A drop of diluted semen (10 μL:100 μL) in warm normal saline (0.9% NaCl) was placed on a warm slide and spread by a cover slip. The haemocytometer detected sperm concentration (SC). A modified Methylene Blue Response Time (MBRT) assay was used to determine the metabolism of quail sperm, as explained by Shit et al. (2010). The upper layer was gently mixed before being wrapped with 0.2 mL mineral oil and incubated in the water bath at 37°C. Time was calculated.

### 2.3 | Sexual libido

The sexual libido time (SLT) of male quails ($n$ = 5/group) was assessed in terms of reaction time in seconds (s), beginning with the placement of the female inside the male's cage and ending until the male began to mount the female.

### 2.4 | Testosterone (T) levels measuring

Blood samples were collected from the jugular vein of male quails ($n$ = 5/group) into plastic tubes with heparin and centrifuged for 15 min at 3000 rpm, and plasma was kept at −20°C for further examination. Testosterone (T) levels were measured using ELISA kits produced by DiaMetra in Spello-Perugia, Italy. The sensitivity test was 0.07 ng/mL for testosterone.

### 2.5 | Testicular weight evaluation

The abdominal region of quails ($n$ = 5/group) was opened to investigate age-related changes in gonadal development and the testes were extracted after males were slaughtered directly. The weight of the testis was calculated separately with an electronic analytical balance. The relative weight of the testes to body weight was calculated.

### 2.6 | Histological and morphometric evaluation of seminiferous tubes

The left testicles ($n = 5$/group) were prepared for histological examination and general morphology changes. Briefly, testes were kept by formalin (10%) for 24 h. After dehydration with increasing concentrations of ethyl alcohol (70%–100%) and passing through two contents of xylol, the samples were paraffin-embedded and sectioned using a microtome (rotary) at 5 m. Then, the slides were run through a decreasing concentration (100%–70%) of ethylic alcohol and xylol. Haematoxylin and eosin staining were used to stain the histology slides. Histological development of seminiferous tubes in the left testis was evaluated from 10 to 34 weeks. To collect histomorphometric data, a photomicroscope was used to obtain 10 photomicrographs (5 at 10 and 5 at 40) randomly from the middle zone of cross-sections of each testis. The seminiferous tubules' diameters and circumferences were measured using ImageJ 1.52o software.

### 2.7 | Statistical analysis

SPSS's General Linear Model (GLM) technique was used to analyse the collected data. Duncan's novel multiple-range test was once used to discover mean differences. The differences were regarded as significant if $p$ was $<.05$. Correlation coefficients were calculated between some of the studied traits.

## 3 | RESULTS

### 3.1 | Semen characteristics and testosterone profiles

Data analysis revealed significance ($p < .05$) of age on sexual libido time (SLT), Semen volume (EV), sperm motility (SM), methylene blue response time (MBRT) and sperm concentration (SC) (Table 1). The shortest TSL was recorded in males at 10 weeks, while the longest was at 22 weeks. Furthermore, the highest EV, SM and SC were obtained at 10 weeks of age. The lowest EV and SC were obtained at 34 and 28 weeks for SIM. Yet, MBRT was significantly increased at 16 and 28 weeks of age compared with other ages. In contrast, the testosterone hormone concentration gradually increased with age to peak at 22 weeks, then decreased at 28 weeks and again increased slightly at 34 weeks (Figure 1).

### 3.2 | Testicular weight and measurements

Testes development is extremely high between the 10th and 22nd week; it reduced lightly from the 28th to the 34th (Table 2). Body and testis weights were significantly ($p < .05$) lower in the 10th week of age. Except for the right testicle weight, the values of the other traits

TABLE 1 Testosterone levels, sexual libido and semen traits in male quail at 10, 16, 22, 28 and 34 weeks of age.

| Parameters | n/Group | Age (week) | | | | |
|---|---|---|---|---|---|---|
| | | 10 | 16 | 22 | 28 | 34 |
| Testosterone ($ng\,mL^{-1}$) | 5 | $0.49 \pm 0.081^{b}$ | $1.02 \pm 0.15^{ab}$ | $1.74 \pm 0.11^{a}$ | $0.81 \pm 0.06^{b}$ | $1.05 \pm 0.32^{ab}$ |
| Sexual libido (S) | 5 | $5.00 \pm 0.49^{b}$ | $6.07 \pm 0.886^{b}$ | $12.60 \pm 4.27^{a}$ | $8.75 \pm 1.56^{ab}$ | $8.83 \pm 0.991^{ab}$ |
| Semen volume (µL) | 3 semen samples were collected/quail from 5 quails | $20.27 \pm 1.00^{a}$ | $18.33 \pm 0.504^{ab}$ | $17.20 \pm 0.97^{b}$ | $18.67 \pm 1.03^{ab}$ | $16.83 \pm 0.49^{b}$ |
| Sperm motility (%) | | $77.33 \pm 2.063^{a}$ | $75.33 \pm 6.67^{ab}$ | $73.00 \pm 7.58^{ab}$ | $69.58 \pm 6.20^{b}$ | $74.58 \pm 7.39^{ab}$ |
| MBRT (S) | | $1.65 \pm 0.20^{b}$ | $2.64 \pm 1.04^{a}$ | $2.28 \pm 0.31^{ab}$ | $1.86 \pm 0.222^{b}$ | $1.700 \pm 0.13^{b}$ |
| Sperm concentration ($\times 10^{6}\,mL^{-1}$) | | $2244.00 \pm 10.90^{a}$ | $2220.00 \pm 18.21^{a}$ | $2209.60 \pm 93.75^{ab}$ | $2210.00 \pm 15.67^{ab}$ | $2140.00 \pm 15.37^{b}$ |

*Note*: [a,b]Means in each row with the same letter are not significantly different ($p \le .05$).

Abbreviation: MBRT, Methylene Blue Response Time (Sc).

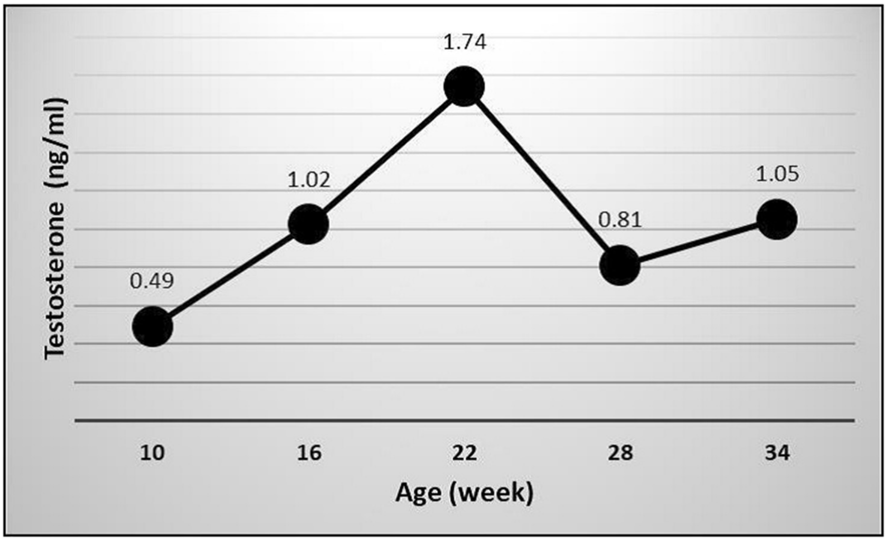


FIGURE 1 Variation of testosterone levels with age.

TABLE 2 Body and testicular weights of male quails at 10, 16, 22, 28 and 34 weeks of age.

| | | Age (week) | | | | |
|---|---|---|---|---|---|---|
| Parameters | *n*/Group | 10 | 16 | 22 | 28 | 34 |
| Body weight (g) | 20 | 232.20 ± 2.59[b] | 248.40 ± 0.65[a] | 249.40 ± 7.64[a] | 230.00 ± 2.01[b] | 237.50 ± 3.77[b] |
| Right testis weight (g) | 5 | 3.74 ± 0.12[ab] | 3.94 ± 0.15[a] | 3.95 ± 0.15[a] | 3.07 ± 0.19[b] | 3.20 ± 0.23[b] |
| Left testis weight (g) | 5 | 3.81 ± 0.12[ab] | 4.04 ± 0.11[a] | 3.87 ± 0.14[ab] | 3.35 ± 0.09[b] | 3.65 ± 0.24[ab] |
| Testes weight (g) | 5 | 7.55 ± 0.23[ab] | 7.99 ± 0.26[a] | 7.82 ± 0.28[a] | 6.41 ± 0.27[b] | 6.78 ± 0.45[ab] |
| Relative testicular weight (%) | 5 | 3.25 ± 0.09 | 3.21 ± 0.10 | 3.16 ± 0.17 | 2.80 ± 0.13 | 2.83 ± 0.15 |

*Note*: [a,b]Means in each row with the same letter are not significantly different ($p < .05$).

in the 34th week were considerably ($p < .05$) lower than comparable values recorded at 16, 22 and 28 weeks of age. The relative testicular weight did not differ significantly at different ages (Figure 2).

### 3.3 | Histological evolution of seminiferous tubes

A morphological parameter of seminiferous tubes (Figure 3) demonstrated increased seminiferous tube size and, more importantly, spermatogenesis efficacy from 10 weeks old. Spermatogenesis seems higher at 16 weeks. At 22 weeks, some huge and empty seminiferous tubule lumens and other lumens with reduced size and high spermatozoa concentration were recorded.

### 3.4 | Histomorphometry of the seminiferous tubes

Morphometric analysis of seminiferous tubes indicated that seminiferous tubules' circumferences and diameter increased significantly ($p < .05$) with advancing age (Figure 4). There are positive and significant correlations ($p < .01$) between age and seminiferous tubule circumferences and diameter in quail testes. The results explained a significant positive correlation between testosterone level and sexual libido time. In contrast, a significant negative correlation was detected between age and sperm concentration (Table 3).

## 4 | DISCUSSION

These data indicated that higher values of semen traits were obtained from 10 to 22 weeks compared with other ages. These findings agree with Farooq et al. (2020), who reported that most measures of sperm quality in Japanese quail declined with age. Sperm production peaks around week 16 and decreases as the birds approach 36 weeks of age.

Most semen parameters, especially sperm motility and kinetics measurements, were affected by age and decreased significantly from 8 to 16 Weeks and continued to decrease to 26 Weeks (Farooq et al., 2020). The sperm concentration in chickens increased from 24 to 39 weeks of age, then stayed consistent until dropping at 72 weeks of age (Cerolini et al., 1997; Kelso et al., 1997). The volume and concentration of semen in male broiler breeders decreased linearly with age (Zhang et al., 1999).

The testes' weights observed in our study are greater than those observed in quail at 52 days by Vatsalya and Kashmiri (2012) and Saqib et al. (2001) because these authors' weights were obtained from younger birds (52 days) than that used in our study (minimum

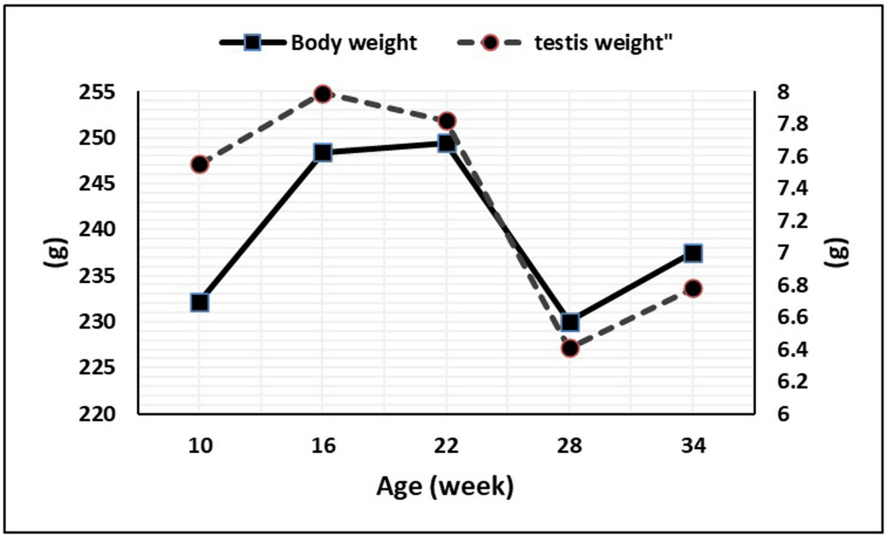


FIGURE 2 Body weight and testis weight variation with different ages.

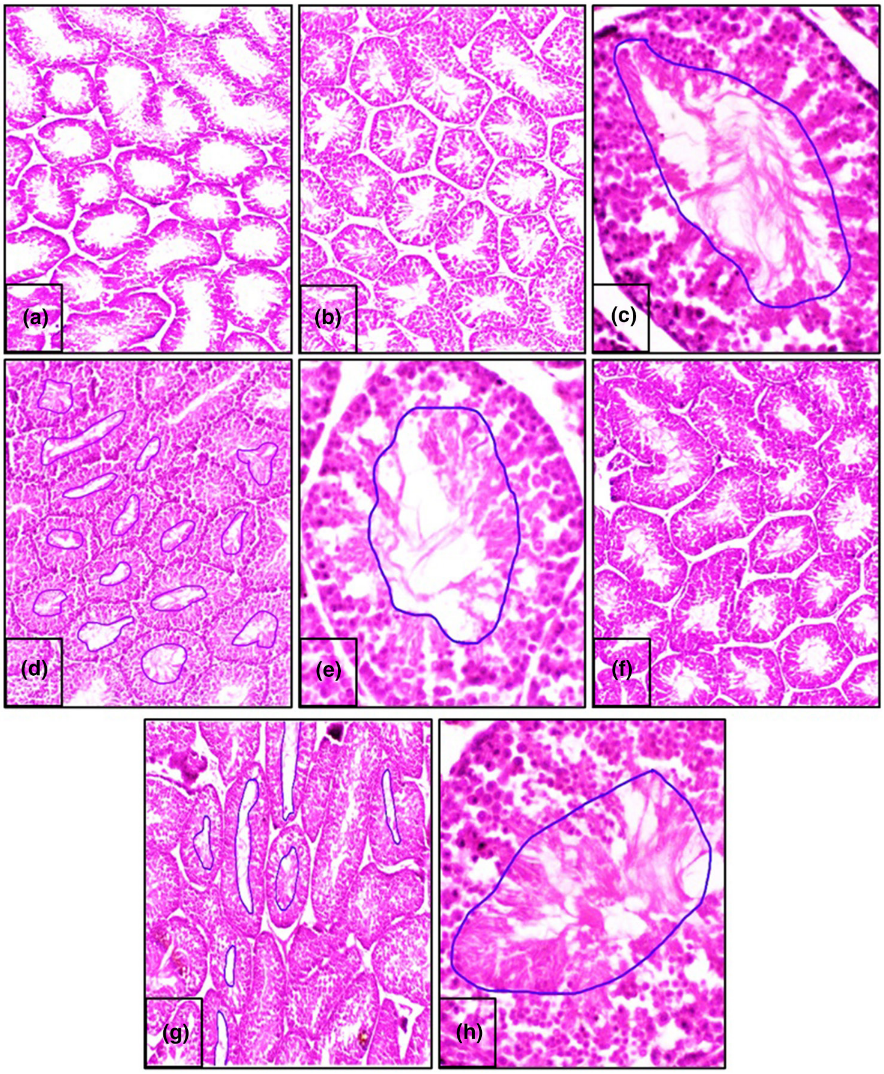


FIGURE 3 Histological development of seminiferous tubule in the left testis of quails ($n=5$/group) at different ages (10, 16, 22, 28, and 34 weeks). (a) Spermatogenesis is initiated by seminiferous tubules (Week 10, 100×). (b) The activity of seminiferous tubules (Week 16, 100×). (c) The presence of spermatozoa in the seminiferous tubule's lumen (Week 16, 400×). (d) Spermatogenic activity is generalized and pronounced (22 weeks, 100×). (e) Seminiferous tubule presenting large and relatively empty lumen (22 weeks, 400×). (f) At 28 weeks old, displaying reduced activity levels (100×). (g) Cross testis section at 34 weeks demonstrating high and low activity levels (100×). (h) Seminiferous tubule in 34 weeks showing pronounced spermatogenic activity (400×).

70 days). The testicular weight and size rise with age until puberty and increase during the breeding season, when maximum values are reached (Vatsalya & Kashmiri, 2012). Our study showed that the weight of the left testicle is usually greater than the weight of the right testicle. This agrees with other researchers' findings on roosters (Vatsalya & Kashmiri, 2012) and Mullard ducks (Denk &

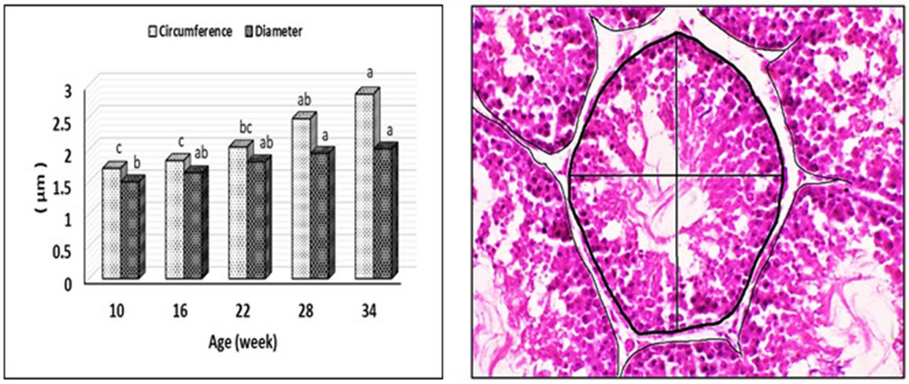


**FIGURE 4** Histomorphometrically evaluation of seminiferous tubule in the left testis of quails ($n = 5$/group) at different ages (10, 16, 22, 28 and 34 weeks). Means in each parameter with the same letter (a, b, c) are not significantly different at $p < .05$.

**TABLE 3** Correlation coefficients between testicular measurements, testosterone level and semen characteristics of male quails ($n = 5$/group).

| | TTW | SLT | SV | SM | MBRT | SC | T | STC | STD |
|---|---|---|---|---|---|---|---|---|---|
| Age | −0.72 | 0.55 | −0.77 | −0.61 | −0.25 | −0.89* | 0.31 | 0.98** | 0.99** |
| TTW | | −0.10 | 0.11 | 0.61 | 0.67 | 0.49 | 0.33 | −0.80 | −0.72 |
| SLT | | | −0.74 | −0.58 | 0.14 | −0.38 | 0.88* | 0.40 | 0.62 |
| SV | | | | 0.36 | −0.28 | 0.79 | −0.78 | −0.65 | −0.77 |
| SM | | | | | −0.03 | 0.19 | −0.29 | −0.51 | −0.70 |
| MBRT | | | | | | 0.25 | 0.52 | −0.42 | −0.21 |
| SC | | | | | | | −0.29 | −0.90* | −0.83 |
| T | | | | | | | | 0.13 | 0.36 |
| STC | | | | | | | | | 0.95* |

Abbreviations: MBRT, Methylene blue reduction time; SC, Sperm concentration; SLT, Sexual libido time; SM, Sperm motility; STC, Seminiferous tubule circumference; STD, Seminiferous tubule diameter; SV, Semen volume; T, Testosterone; TTW, Total testis weight.
*$p \le .05$. **$p \le .01$.

Kampenaers, 2006). At week 22, the weight of the right testicle exceeded that of the left testicle. Similar observations were reported by Vatsalya and Kashmiri (2012) in quail for 3 weeks. This asymmetry in the growth and development of the testes is because of variation in the sensitivity of two testes to gonadal-stimulating substances (Norris & Lopez, 2011). This possible difference in cellular basis needs to be investigated (Norris & Lopez, 2011). Sertoli cells multiply rapidly, allowing rapid expansion of the seminiferous tubules and, subsequently, a gradual increase in the weight and size of the testes for up to 12 weeks (Denk & Kampenaers, 2006). The present study recorded that seminiferous tubules have a variety of shapes, although the majority are circular. At 34 weeks, the seminiferous tubule circumference significantly increased. A similar observation was recorded in the seminiferous tubule lumen circumference at 34 weeks. These disparities might be explained by weight and testicular measures, which vary greatly depending on species and strains of birds, as well as environmental factors such as the season. Testicular development increases during the mating season, including increasing seminiferous tubules' volume, diameter and length. Testicular regression is observed during the non-breeding season (Aire & Ozegbe, 2007; Norris & Lopez, 2011).

Testosterone develops the male genital system, secondary sexual characteristics and unique behavioural expression. Additionally, testosterone has a role in spermatogenesis. Our results showed that testosterone levels increased with increasing age until 22 weeks. These findings agree with several authors (Penfold et al., 2000) who reported that the testosterone level gradually increases during the mating season until reaching an average of 2.06 ng/mL. This is consistent with the results found between 16 and 22 weeks and may correspond to the end of the pubescent period.

It was concluded that the age significantly affected testicular histological structure, libido, testosterone level and semen characteristics of Coturnix quail. More research is needed to explore the underlying mechanisms and develop strategies for maintaining reproductive performance in old male quail.

## AUTHOR CONTRIBUTIONS

MR, AMH, HAK, SMS and MEM performed the experiments; AMH, HAK and SMS designed the experiments; MEAM, AAS, GS and VT analysed data and wrote the initial draft; all authors revised the manuscript.

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## CONFLICT OF INTEREST STATEMENT

None of the authors have any conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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