Histological Study of Post-hatching Femoral Bone Maturation in Goose (Anser anser): Emphasis on Sexual Dimorphism

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***Abstract*—** Sex-specific histological changes during post-hatching skeletal maturation remain poorly understood in domestic geese. The study aimed to investigate the microstructural characteristics of femoral bone associated with sexual size dimorphism in domestic goose (*Anser anser*), where males typically exhibit greater body mass than females. To achieve this, femoral bone samples were collected from 18 domestic geese (9 males, 9 females) at 1, 6, and 24 weeks post-hatching (n = 3 per sex per age group). After standard histology using hematoxylin and eosin alongside Masson trichrome stains, we determined three parameters: Active Cartilaginous Zone Thickness (ACZT), Cortical Bone Thickness (CBT), and Total Bone Diameter (TBD). Data were statistically analyzed using an independent samples t-test to compare between sexes. At 1-week post-hatching, males showed significantly higher values for both ACZT (1499.34 ± 66.2 vs. 1346.84 ± 32.4 µm, P = 0.023) and CBT (429.92 ± 4.76 vs. 413.74 ± 6.79 µm, P = 0.023). By 6 weeks, the ACZT difference had disappeared (P = 0.745), whereas CBT remained significantly higher in males (1093.35 ± 18.5 vs. 939.26 ± 69.7 µm, P = 0.020). At 24 weeks (adulthood), males exceeded females in all three parameters: CBT (1383.88 ± 10.4 vs. 1281.28 ± 14.7 µm, P = 0.001), ACZT (210.58 ± 5.22 vs. 172.77 ± 4.09 µm, P = 0.001), and TBD (8199.13 ± 114.1 vs. 7739.90 ± 133.6 µm, P = 0.011). The histological analysis confirmed that male cortices were significantly thicker, with a slightly thicker growth plate remnant, than those of females. In this way, the phenomenon of sexual dimorphism in goose femoral histology appears at the age of one week after hatching and is accentuated with age. This finding suggest sex-specific trajectories in appositional baseline histological data for future studies of avian development.

***Keywords* —** Sexual dimorphism, domestic goose, femoral histology, cortical thickness, growth plate.

INTRODUCTION

Sexual dimorphism; domestic goose; femoral histology; cortical thickness

The avian skeletal system is structurally distinct, in that it combines the mechanical strength to support the body with the lightness required for flight. This balance is attained by clear structural modifications, such as thin cortical walls, the existence of internal air cavities, and high rates of bone remodeling (1,2). In addition to its supportive role, the role of the bone tissue in birds is to perform some essential metabolic functions, most notably, the regulation of the balance of calcium and phosphorus in the blood, which is an essential process and directly linked to the ability of the bird to fly and reproduce (3). This dynamic nature of bone is manifested through histological studies, which demonstrate the structure of the bone cells, the stages of cartilage-to-bone replacement, and the changes that occur in these tissues at various stages of growth (2,4).

Within this context of avian bone development, sexual size dimorphism is long established in the Anseriformes, where male geese tend to be larger than females in body mass and skeletal size (7,8,9). While there are extensive bone maturation studies in precocial birds (10) and avian skeletons, the majority of these studies did not use direct histological analysis of cortical bone or growth plate cartilage but instead used external morphometrics or radiography. This represents a notable lack of microstructural data, given that bone is a dynamic tissue that constantly responds to mechanical loading, hormones, and genetics (1,3). Specifically, during the post-hatching period, the heavier body mass of males exerts greater mechanical stress on weight-bearing bones to support movement (11), while sex steroids critically regulate both cortical bone accumulation and growth plate dynamics (12).

As a result of this consistent size disparity between the sexes, it is logical to assume that there are some microstructural differences: perhaps a thicker cortex, a more enduring growth plate, or even different patterns of bone remodeling. Based on the above, we formulated three hypotheses. First, males would exhibit greater cortical bone thickness (CBT) and a thicker active cartilaginous zone (ACZT) than females. Second, these histological differences would emerge early, specifically within the first week post-hatching. Third, the dimorphism would become more pronounced with advancing age. Identifying such

tissue-level markers has two practical implications: (1) enabling sex identification of goose remains in archaeological contexts, and (2) facilitating early sex selection in goose farming, where males are often preferred for meat production.

herefore, we compared male and female domestic geese at 1-, 6-, and 24-weeks post-hatching, measuring CBT, ACZT, and TBD to determine the timing and pattern of histological sexual dimorphism.

MATERIALS AND MTHODS

Sample collection and preparation

Eighteen domestic geese (*Anser anser domesticus*); 9 males, 9 females) of the local breed were allocated to three post-hatching age groups: 1 week, 6 weeks, and 24 weeks (n = 6 per group, with equal sex distribution, resulting in n = 3 per sex within each age cohort). Eggs were obtained from a bird sanctuary in Karbala province, Iraq, and incubated under standard conditions (37.5–38°C, 55–65% relative humidity) in a locally manufactured incubator following (7). After hatching, all geese were raised together under identical environmental conditions in clean, well-ventilated brooding cages (week 1) before transitioning to a semi-intensive diurnal grazing system (from week 3 onward) with necessary vaccinations (13). They were provided with commercial balanced starter and grower feed and water ad libitum. Live body weight was not recorded as the study focused strictly on microstructural parameters. Prior to euthanasia, the sex of each bird was initially determined using the cloacal vent sexing method by gently inverting the bird and applying mild digital pressure around the vent to evert the cloaca. Euthanasia was performed at the designated ages by intravenous administration of an overdose of sodium pentobarbital (150mg/kg), a method approved by (14). Intravenous sodium pentobarbital overdose is the fastest and most reliable euthanasia method in birds (15). Following euthanasia, the sex was definitively verified post-mortem via direct visual macroscopic examination of the exposed gonads (testes or ovaries). The right femur was carefully dissected, freed from all adherent soft tissues, and fixed in 10% neutral buffered formalin for 72 hours according to (16). The small sample size (n = 3 per sex/age group) represents a study limitation necessitated by the labor-intensive nature of bone histology, which may constrain the statistical power regarding sexual dimorphism. The experimental protocol was officially reviewed and approved by the Scientific Committee of the Department of Anatomy, Histology and Embryology, College of Veterinary Medicine, Al-Muthanna University, Iraq.

Decalcification and histological processing

Decalcification was performed using a 2.5 percent ethylenediaminetetraacetic acid (EDTA) solution. The decalcification period lasted 5 days for the 1-week-old samples, 10 days for the 6-week-old samples, and 20 days for the adult (24-week-old) samples. The decalcification endpoint was determined by the ability to easily penetrate the bone with a fine needle (16,17). After that, the specimens were rinsed with running tap water until the EDTA was removed. Dehydration of specimens was then carried out in a graded series of ethanol (70%, 80%, 90%, and absolute ethanol), followed by clearing with xylene. The specimens were then sectioned into distinct anatomical blocks representing the mid-diaphysis and the distal epiphysis of the femur. These blocks were embedded individually in paraffin wax (melting point 58-60 °C). The solidified wax blocks were sectioned longitudinally along the mid-sagittal plane at 5-6 µm thickness with a rotary microtome (16). Staining procedures included hematoxylin and eosin (H&E) to perform general histological analysis, and Masson trichrome to evaluate bone matrix maturity and differentiate between mineralized bone, osteoid, or cartilaginous matrix (18,19). Histological procedures were carried out in the Postgraduate Laboratory, College of Veterinary Medicine, Al-Muthanna University, during the academic year 2026.

Morphometric measurements

All measurements were performed on digital images using Fiji software (an open-source distribution of ImageJ). To eliminate observer bias, evaluations were conducted in a strictly blinded manner, measuring five representative longitudinal sections per specimen across three distinct microscopic fields per section. The software was calibrated using a digital stage micrometer image captured under identical optical settings, and a standard scale bar was embedded into all micrographs. Three parameters were recorded; each measured three times per specimen and recorded in micrometers (µm). ACZT (Active Cartilaginous Zone Thickness) was defined as the combined thickness of the proliferative and hypertrophic zones measured at the distal epiphysis of the femur at week 1, and the residual hypertrophic zone remnant at weeks 6 and 24.due to progressive skeletal maturation and growth plate involution, this parameter captured the residual hypertrophic zone remnant. Although these represent distinct histological phases of epiphyseal maturation, they collectively reflect the age-associated decline in longitudinal growth potential. CBT (Cortical Bone Thickness) was measured at the mid-diaphysis and averaged from three equidistant points. Total Bone Diameter (TBD) was measured at the mid-diaphysis as the mean of three perpendicular measurements. All measurements followed the guidelines of (20,21). For each bird, measurements obtained from all sections and microscopic fields were averaged to produce a single representative value for ACZT, CBT, and TBD. Statistical analyses were performed using the individual bird as the experimental unit.

STATISTICAL ANALYSIS

Data were analyzed using IBM SPSS Statistics software for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Prior to analysis, the normality of quantitative data, including active cartilaginous zone thickness (ACZT), periosteal cortical bone thickness (CBT), and total bone diameter, was assessed within each age group using the Shapiro–Wilk test (22), supported by visual inspection of histograms and Q–Q plots. However, this normality assessment should be interpreted cautiously because of the limited sample size within each experimental group (n=3). All quantitative data were found to be normally distributed and are presented as mean ± standard deviation (SD). Sex-related differences within post-hatching age groups (1 week, 6 weeks, and

24 weeks) were analyzed using the independent samples t-test (23,24). A significance level of $P< 0.05$ was considered statistically meaningful for all tests, while $P<0.01$ was considered highly significant.

RESULT

Sex differences appeared early and followed distinct trajectories across post-hatching ontogeny (Table 1-3; Figures 1–4).

One week post-hatching

Males exhibited significantly greater ACZT (1499.34 ± 66.2 vs. 1346.84 ± 32.4 µm, P = 0.023; 11% larger; Table 1) and CBT (429.92 ± 4.76 vs. 413.74 ± 6.79 µm, P = 0.023; 4% thicker; Table 2) than females. In contrast, total bone diameter (TBD) did not differ between sexes (P = 0.722; Table 3).

Six week post-hatching

CBT dimorphism persisted and became more pronounced (1093.35 ± 18.5 vs. 939.26 ± 69.7 µm, P = 0.020; 16% thicker; Table 2). Conversely, there was no longer a difference between sexes in ACZT (P = 0.745; Table1), with TBD being similar (P = 0.653; Table 3). The week 1 to 6 differences of CBT (429.92 to 1093.35 µm) and ACZT (1499.34 to 291.16 µm) represented a 154.3% increase and 80.6% decrease, respectively, indicating a distinct physiological shift from longitudinal to appositional growth.

Twenty-four weeks post-hatching (adulthood)

Males surpassed females in all three parameters: CBT (1383.88 ± 10.4 vs. 1281.28 ± 14.7 µm, P = 0.001; 8% thicker; Table 2), ACZT (210.58 ± 5.22 vs. 172.77 ± 4.09 µm, P = 0.001; 22% thicker remnant; Table 1), and TBD (8199.13 ± 114.1 vs. 7739.90 ± 133.6 µm, P = 0.011; 6% larger; Table 3). The longitudinal growth potential was evidenced by the measurable remnant of the growth plate at 24 weeks (mean thickness 192 µm) of the geese.

Histological observations and maturation of trabecular bone

The qualitative results were in line with the quantitative measurements. At 24 weeks, male femur cortices were visibly thicker and more compact (Figures 3 and 4), always with cartilaginous remnants that were thicker (Figures 1 and 2). Early stage differences (weeks 1 and 6) did not display any obvious qualitative differences. The sex-specific differences in trichrome staining of the distal femoral epiphysis, as demonstrated by Masson, were that males had a mixed pattern with more red (mineralized matrix; Figure 1) and females had more blue (unmineralized osteoid; Figure 2).

DISCUSSION

As displayed in Table 1, TiO2NPs at higher concentration (The early separation offers the earliest histological proofs of sexual dimorphism in the domestic goose femur, which is observable as early as one week after hatching. The male-biased cortical thickening (CBT) agrees with the known effects of gonadal steroids in birds, specifically, testosterone, which is hypothesized to stimulate periosteal apposition (25). Similar sex differences in cortical thickness have been observed in ostriches at 14 months of age (26); the ACZT differences at this age are indicative of early sex-specific control over the growth plate, which may be related to the initiation of gonadal maturation.

The ACZT of week 6 in females indicates that females increase the pace of skeletal senescence of longitudinal growth potential, shifting skeletal resources to reproductive maturation—a pattern that has been observed in other precocial birds (27, 28). This sex difference in growth plate dynamics may be related to estrogen that stimulates growth plate fusion in females (29) and testosterone in males, which likely enhances periosteal bone formation (25). Male cortical dominance is a biomechanical trait of bigger body mass in adulthood, beneficial in mate competition (9). Ongoing periosteal deposition creates structural capacity to handle greater body mass and combat needs (11), in line with sexual size dimorphism predicting male-male competition (7).

The continuity of all parameters supports irreversible sex-specific skeletal paths. Reflecting the structural transition of the parameters across ages, the thicker remnant of ACZT in males (22% difference) is a sign of either long-term chondrocyte activity or late epiphyseal closure and is probably an adaptation reaction to testosterone that is necessary to sustain the significantly higher level of male body mass needed to compete in the territory. The longitudinal growth potential extends even at ages far beyond those of other domestic birds normally regarded as skeletally mature (27).

The sex-specific differences in trichrome staining suggest that males exhibit more advanced trabecular maturation, likely due to sex-specific osteoblast activity and hormonal influences on bone turnover. The differences can be linked to the functional adjustments concerning adaptations related to mechanical loading and mineral metabolism (11); however, further studies are required to directly evaluate their biomechanical and physiological significance.

Table 1. Morphometric Comparison of the Active Cartilaginous Zone Thickness (ACZT) between Male and Female goose (*Anser anser*) at Different Post-Hatching Stages

Age Group	Gender	Mean-ACZT (µm)	T value	P value
1week	Male	1499.34±66.2*a*	3.57	0.023
	Female	1346.84±32.4*b*		
6week	Male	291.16±31.7*a*	0.349	0.745
	Female	284.39±11.1*a*		
24week	Male	210.58±5.22*a*	9.86	0.001
	Female	172.77±4.09*b*		

Note: Data are expressed as Mean ± SD (n = 3 per sex per age group). Means with different superscript letters (a, b) between genders within the same age group indicate a significant difference at ($P < 0.05$) according to the Independent Samples T-test.
ACZT: Active Cartilaginous Zone Thickness; **SD**: Standard Deviation

Table 2. Morphometric Comparison of the Cortical Bone Thickness (CBT) between Male and Female goose (*Anser anser*) at Different Post-Hatching

Age Group	Gender	Mean -CBT (µm)	T value	P value
1week	Male	429.92±4.76a	3.370	0.023
	Female	413.74±6.79b		
6week	Male	1093.35±18.5a	3.720	0.020
	Female	939.26±69.7b		
24week	Male	1383.88±10.4a	9.830	0.001
	Female	1281.28±14.7b		

Note: Data are expressed as Mean ± SD (n = 3 per sex per age group). Means with different superscript letters (a, b) between genders within the same age group indicate a significant difference at (P < 0.05) according to the Independent Samples T-test.
CBT: Cortical Bone Thickness; **SD**: Standard Deviation

Table 3. Morphometric Comparison of The Total Femoral Diameter between Male and Female goose (*Anser anser*) at Different Post-Hatching Stages

Age Group	Gender	Mean TBD - (µm)	T value	P value
1week	Male	1870.26±21.3*a*	0.381	0.722
	Female	1876.40±17.8*a*		
6week	Male	4812.67±106.1*a*	0.484	0.653
	Female	4766.96±124.3*a*		
24week	Male	8199.13±114.1*a*	4.52	0.011
	Female	7739.90±133.6*b*		

Note: Data are expressed as Mean ± SD (n = 3 per sex per age group). Means with different superscript letters (a, b) between genders within the same age group indicate a significant difference at (P < 0.05) according to the Independent Samples T-test.
TBD: Total Bone Diameter ; **SD**: Standard Deviation

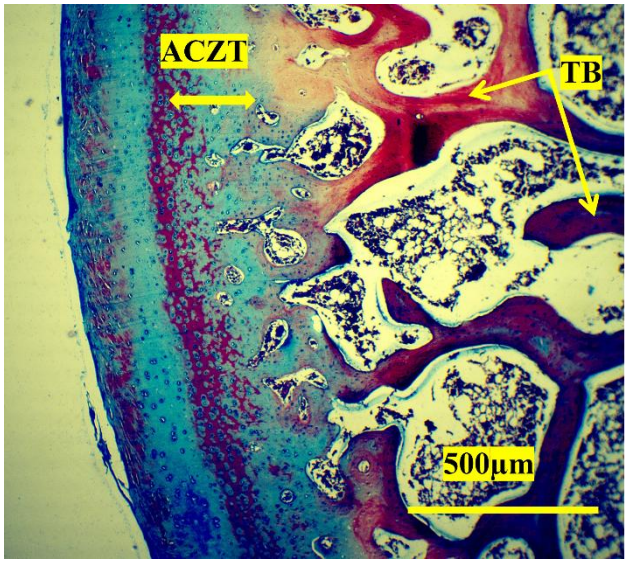

Figure 1. Histological sections of a femoral epiphysis in male goose (*Anser anser*) at 24 weeks post-hatching; ACZT:Active Cartilaginous Zone Thickness; TB: Trabecular Bone. (Masson's Trichrome, Scale bar = 500µm)

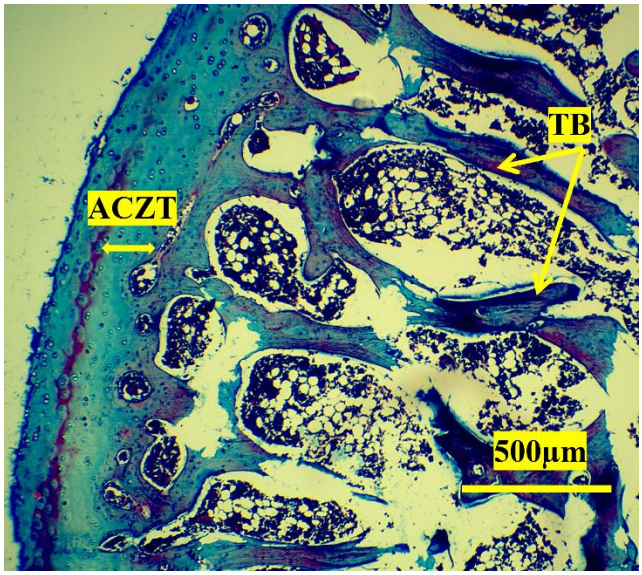

Figure 2. Histological sections of a femoral epiphysis in female goose (*Anser anser*) at 24 weeks post-hatching; ACZT:Active Cartilaginous Zone Thickness; TB: Trabecular Bone. (Masson's Trichrome, Scale bar = 500µm).

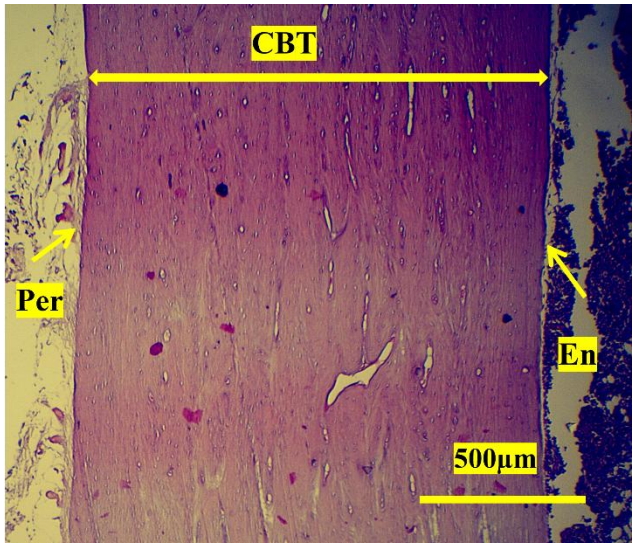

Figure 3. Longitudinal section of the femoral cortex in a male goose (Anser anser) at 24 weeks post-hatching; showing CBT: Cortical bone Thickness; Per: Periosteum; En: Endosteum. (H&E stain, Scale bar = 500µm).

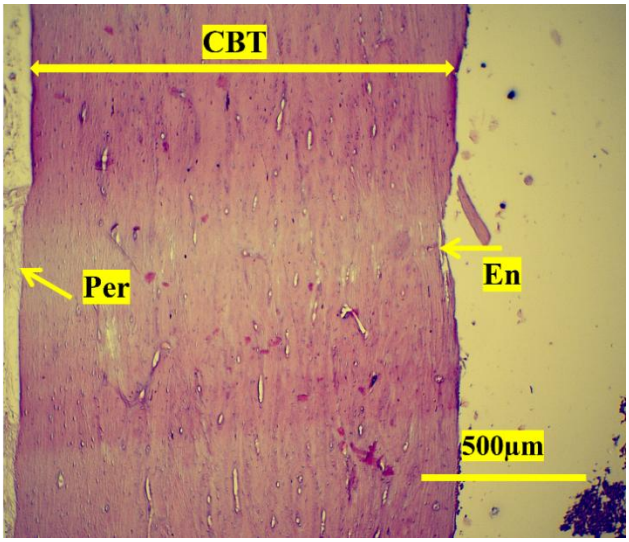

Figure 4. Longitudinal section of the femoral cortex in a female goose (Anser anser) at 24 weeks post-hatching; showing CBT: Cortical bone Thickness; Per: Periosteum; En: Endosteum. (H&E stain, Scale bar = 500µm).

Limitations and future directions

This study has several limitations. First, we did not measure circulating hormone levels, which would help establish causal links between gonadal steroids and the observed histological dimorphism. Second, we analyzed the femur only; it is not yet known whether sex differences are also similar in other skeletal parts (e.g., humerus, tibiotarsus). Third, the size of the sample, although balanced, was small. Future studies must: (1) utilize larger sample size to increase statistical power and ensure broader generalizability, (2) compare the concentration of plasma testosterone and 17b-estradiol with CBT and ACZT measurements, (3) compare results across many bones to establish whether dimorphism is site-specific or systemic, and (4) examine the embryonic origins of post-hatching histological divergence in earlier time points.

CONCLUSION

This paper confirms that sexual dimorphism in the femoral bone histology of the domestic goose (*Anser anser domesticus*) emerges as early as one week after hatching and escalates during adulthood. Males consistently exhibit markedly thicker in cortical bone (CBT) at all ages, whereas differences in active cartilaginous zone thickness (ACZT) appear early but equalize by six weeks of age, suggesting that longitudinal and appositional growth are regulated sex-specifically.

The longitudinal graded increase in cartilaginous depth in adult males suggests a potential delay in epiphyseal fusion or prolonged retention of transitional cartilaginous remnants compared to females, and this is a part of the established sexual size dimorphism patterns in Anseriformes. Moreover, qualitative assessments of trabecular bone via Masson trichrome staining at 24 weeks demonstrated a balance of red to blue in both sexes, with a preponderance of red in males and a higher percentage of blue in females, indicating a more advanced mineralization state in males despite continuous remodeling.

The results provide the first histological basis for sex-specific skeletal development in geese. Practically speaking, understanding this early-onset cortical dimorphism offers a valuable biological baseline that could inform sex-specific rearing strategies and post-mortem evaluations in goose farming, where males are usually desired for meat production.

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N/A

Conflict of Interest

The authors declare no conflict of interest.

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