Received: 29.03.2026 | Revision: 24.04.2026 | Accepted: 05.05.2026 | Published: 12.09.2026

Veterinary Sciences

doi: 10.31210/spi2026.29.02.21
UDC 611.65:591.134.1-019-053.2:636.7
ORIGINAL ARTICLE

Scientific Progress & Innovations
ISSN: 2786-8842 (Print)
2786-8850 (Online)

29 (2)
2026

Developmental and histochemical study of the reproductive system in female dogs during the postnatal period

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Citation: Ahmed, N. M., Younis, N. A. S., & Naser, R. A. A. (2026). Developmental and histochemical study of the reproductive system in female dogs during the postnatal period. *Scientific Progress & Innovations*, 29(2), 141–149. doi: 10.31210/spi2026.29.02.21

The early postnatal organogenesis of the canine female reproductive tract marks a critical phase of morphofunctional and histochemical remodeling. This study targeted the biological characterization of age-dependent tissue development in the ovaries, uterus, and oviducts of female canine puppies between the 3rd and 8th weeks of postnatal life. Experimental procedures were executed using healthy female puppies divided into two age-matched cohorts (n=3). Excised tissue samples were fixed in 10 % neutral buffered formalin, embedded in paraffin, sectioned at 4–7 µm, and subjected to hematoxylin-eosin, Alcian blue (pH 2.5), and Periodic acid–Schiff (PAS) staining. Morphometric processing was performed using the Fiji ImageJ software, and mean differences were statistically evaluated via the independent samples Student t-test using SPSS. Histochemical evaluation confirmed the physiological initiation of endometrial secretory activity, as marked by the transition of neutral mucopolysaccharides within the uterine glands from a completely negative profile at 3 weeks to a distinct weak positive reaction by the 8th week of age. Morphometrically, the uterine wall exhibited highly significant vertical cellular hypertrophy of the luminal lining, manifested by a 5.2-fold increase in epithelial height from 5.50±0.93 µm to 28.50±2.91 µm (P=0.0001). Concurrently, intense longitudinal organ elongation resulted in a significant 39.4 % reduction in total uterine wall thickness from 447.40±53.45 µm to 271.30±40.45 µm (P=0.0303) and a synchronized 39.3% reduction in total oviductal wall thickness from 220.00±29.74 µm to 133.50±4.71 µm (P=0.0207). While the primary inner oviductal luminal epithelium maintained strict cytological stability between the 3rd and 8th weeks (10.00±1.76 µm versus 11.90±1.53 µm, respectively; P=0.4407), the functional ovarian cortex exhibited active folliculogenesis, characterized by a statistically significant 23.6 % linear expansion of the primordial follicle diameter from 27.50±1.20 µm to 34.00±1.85 µm (P<0.05). These quantitative relative and absolute indices establish definitive baseline criteria for mammalian reproductive histogenesis and provide critical reference profiles for evaluating developmental pathology in canine veterinary medicine.

Keywords: endometrial maturation, histomorphometry, neutral mucins, ovarian cortex, uterine development, canine female puppies.

Розвиток та гістохімічне дослідження репродуктивної системи самок собак у постнатальний період

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Ранній постнатальний органогенез репродуктивної системи ссавців закладає критичну морфофункціональну основу для подальшого статевого дозрівання та фертильності. Дане дослідження присвячене характеристиці вікових закономірностей розвитку тканин яєчників, матки та яйцепроводів самок цуценят у хронологічному інтервалі між 3-м та 8-м тижнями постнатального періоду. Експерименти проводили на клінічно здорових тваринах, розділених на дві вікові групи. Зразки органів фіксували в 10 % нейтральному формаліні, заливали в парафін, нарізали зрізи товщиною 4–7 мкм та фарбували гематоксилін-еозином, алціановим синім (pH 2,5) та піддавали ШІК-реакції. Морфометрію шарів тканин виконували в програмі Fiji ImageJ з подальшим статистичним аналізом даних за допомогою двовибіркового t-критерію Стьюдента в пакеті програм SPSS. Гістохімічно встановлено початок функціонального дозрівання ендометрію, що виражалося у переході секреції нейтральних мукополісахаридів у маткових залозах від абсолютно негативної реакції у 3 тижні до слабкопозитивної у 8-тижневому віці. Морфометрично зафіксовано високозначущу вертикальну клітинну гіпертрофію епітелію слизової оболонки матки, висота якого збільшилася у 5,2 раза – з 5,50±0,93 мкм до 28,50±2,91 мкм (P=0.0001). Одночасно з цим поздовжнє видовження органів призвело до фізіологічного стоншення шарів, зокрема: товщина стінки матки зменшилася на 39,4 % – з 447,40±53,45 мкм до 271,30±40,45 мкм (P=0.0303), а товщина стінки яйцепроводу скоротилася на 39,3 % – з 220,00±29,74 мкм до 133,50±4,71 мкм (P=0.0207). На тлі повної цитологічної стабільності епітелію слизової оболонки яйцепроводу між 3-м та 8-м тижнями (10,00±1,76 мкм проти 11,90±1,53 мкм відповідно; P=0.4407), у корі яєчника спостерігався стабільний фолікулогенез із закономірним лінійним збільшенням діаметра примордіальних фолікулів на 23,6 % – з 27,50±1,20 мкм до 34,00±1,85 мкм (P<0.05). Отримані якісні та кількісні показники мають суттєву практичну цінність для ветеринарної морфології та ембріології, оскільки виступають еталонним віковим нормативом для діагностики вроджених патологій розвитку та ендокринних порушень у сук.

Ключові слова: дозрівання ендометрію, гістоморфометрія, нейтральні муцини, кора яєчника, розвиток матки, самки цуценят.

Бібліографічний опис для цитування: *Ахмед Н. М., Юніс Н. А. С., Насер Р. А. А.* Розвиток та гістохімічне дослідження репродуктивної системи самок собак у постнатальний період. *Scientific Progress & Innovations*. 2026. № 29 (2). С. 141–149.

Introduction

At birth, the morphologically indifferent reproductive system undergoes phenotypic sexual differentiation driven by the expression of the sex-determining region Y (SRY) gene on the Y chromosome [1]. The internal genital tracts of males and females are primarily derived from the paired mesonephric (Wolffian) and paramesonephric (Müllerian) ducts, respectively [2]. The vertebrate urogenital complex, comprising the kidneys, gonads, and urinary and reproductive tracts, initiates development during gastrulation via the differentiation of the intermediate mesoderm [3]. The formation of the renal tubules, testes, and female reproductive tracts involves extensive embryonic tissue proliferation and mesenchymal-to-epithelial transition. Prior to phenotypic sexual differentiation, mammalian embryos characteristically possess both pairs of genital ducts. In the male embryo, the Wolffian ducts differentiate into the epididymides, vasa deferentia, and seminal vesicles. Conversely, the Müllerian ducts develop adjacent to the mesonephric structures, subsequently giving rise to the oviducts, uterus, cervix, and cranial vagina [4].

Postnatal development of the female reproductive organs represents a continuous physiological progression intricately linked to prenatal organogenesis, which systematically extends through puberty until the attainment of full sexual maturity [5]. In heifers, a substantial increase in uterine weight, closely correlated with circulating gonadal hormone profiles, is observed up to the sixth month of life, after which the developmental growth rate typically plateaus [6]. Comparable postnatal macro-morphological changes, including distinct fluctuations in vaginal length and weight, have been documented in West African dwarf goats [7], while age-dependent histological modifications of the vaginal wall with maturity have been described in domestic pigs [8]. Furthermore, specific gross anatomical variations exist among distinct mammalian orders; for instance, the female guinea pig characteristically lacks a distinct vestibule at the caudal terminus of the reproductive tract, with the external vaginal orifice directly marking the functional termination of the vagina [9]. Unlike males, which maintain active spermatogenesis throughout their entire reproductive lifespan, mammalian females possess a finite, non-renewable pool of primordial oocytes established strictly at birth [5].

The aim of the study

The aim of the current study was to investigate the postnatal macro-morphological, histological, and histochemical development of the female reproductive system in canine puppies during the early postnatal period. Specifically, this work aimed to provide a comparative quantitative and qualitative evaluation of structural and morphometric alterations in the ovaries, uterus, and oviducts between the third and eighth weeks of age, establishing a baseline for understanding tissue remodeling and early organogenesis in canine females.

Materials and methods

Animal grouping and tissue collection

A total of six healthy female canine puppies were obtained from local veterinary clinics and divided into two age-matched experimental groups: a 3-week-old group (n=3) and an 8-week-old group (n=3). Prior to tissue sampling, the somatic body weight of each animal was determined using an electronic precision balance, and somatic parameters were standardized according to established mammalian developmental indices.

To ensure humane tissue harvesting and complete compliance with ethical standards, the animals were subjected to a standardized pre-euthanasia sedation and deep anesthesia protocol utilizing a combination of diazepam, propofol, and ketamine hydrochloride [10]. Following the confirmation of complete loss of consciousness and reflex activity, euthanasia was performed, and the female reproductive tracts were immediately excised.

Tissue specimens from the ovaries, uterine horns, uterine body, and oviducts were carefully isolated and thoroughly rinsed in 0.9 % physiological saline solution to remove excess erythrocytes. The collected specimens were then systematically fixed in 10 % neutral buffered formalin for 48 hours at room temperature for subsequent histological and histochemical investigations.

Routine histological and histochemical processing

The study was conducted at the Department of Anatomy, Faculty of Veterinary Medicine, University of Diyala, using tissue specimens collected from healthy female puppies during the period from March to September 2025. Following fixation, the tissue samples underwent automated dehydration using a series of ascending ethanol concentrations (50 %, 70 %, 80 %, 90 %, and 100 %) for two hours per station. The dehydrated specimens were cleared in xylene for two changes of one and two hours, respectively, and subsequently infiltrated with molten paraffin wax at 58–60°C for two hours.

The infiltrated tissues were embedded into paraffin blocks using a tissue embedding center. Serial sections were cut at a thickness of 5–7 µm for routine histology using a rotary microtome, mounted onto glass slides, and maintained in an incubator at 37°C for 24 hours prior to morphological staining. The fundamental parameters of tissue block sectioning and early slide incubation protocols were adapted from established comparative non-mammalian and avian upper digestive tract histomorphological models [11].

For routine morphological examination and histopathological evaluation, tissue sections from the uterine horns, body, and ovarian cortex were prepared, cleared, and stained according to standard laboratory protocols and textbooks on universal histotechniques [12]. Structural interpretations and early zonal cell identification maps were comparatively evaluated against documented reproductive histogenesis profiles of adult and pregnant domestic cats (*Felis catus*) [13]. Serial sections were also cut at 4 µm for specific histochemical evaluations. Histometrical and morphometric measurements of the uterine layers,

epithelial heights, total wall thicknesses, and primordial follicle diameters were performed using the Fiji ImageJ image analyzer software system, implementing automated pixel-to-micrometer calibration curves historically utilized in avian intestinal mucosal morphometry [14].

Histochemical characterization of secretory profiles was performed using the Periodic acid–Schiff (PAS) test to identify the expression of neutral polysaccharides and mucins within the developing genital tract and uterine glands, utilizing enzymatic and cellular degradation control methods established for complex intracellular carbohydrate screening [15]. Concurrently, Alcian blue (AB) staining at pH 2.5 was utilized to verify the presence and distribution of acidic mucopolysaccharides (mucins). Photomicrographs were captured using an Olympus digital microscope camera (Olympus, Japan).

Statistical analysis

All biometric and morphometric data were subjected to statistical analysis using the Statistical Package for the Social Sciences (SPSS) software program [16] to determine the significance of differences in study parameters between the age groups. Data were expressed as Mean ± Standard Error (SE). To evaluate the significance of variations between the two independent experimental groups (3 weeks versus 8 weeks), a two-tailed independent-samples Student's t-test was utilized. Differences were considered statistically significant at $P<0.05$ and highly significant at $P<0.01$.

Results and discussion

Histological features at three weeks of age

Histomorphological evaluation of the female reproductive system in 3-week-old canine puppies revealed distinct tissue architecture in both the ovaries and the uterus. The ovarian cortex characteristically contained numerous clusters of primordial follicles nested within the connective tissue stroma. While individual follicles exhibited normal developmental features, signs of physiological atresia accompanied by localized collagen bundles and active fibroblasts were observed. Each primordial follicle was lined by a single layer of simple squamous follicular cells. Select oocytes within these follicles possessed distinct, spherical basophilic nuclei surrounded by a well-defined cytoplasm, whereas others exhibited nuclear regression or appeared anucleated under light microscopy. The ovarian surface was delimited by a continuous germinal epithelium composed of simple cuboidal cells, supported externally by a tunica albuginea capsular layer rich in collagen fibers.

Histological analysis of the uterine wall utilizing hematoxylin and eosin (H&E) staining demonstrated an active development of the mucosal layer. The endometrium contained multiple developing uterine glands lined by simple cuboidal epithelium, alongside newly formed glandular structures surrounded by primitive squamous cells. The lamina propria was characterized by loose connective tissue containing abundant fibroblasts. Distinct aggregations of adipocytes were identified surrounding the periphery of the uterine glands, and prominent neocapillarization was evident throughout the stromal layer (***Fig. 1***).

The endometrial mucosa was arranged into numerous complex folds lined by a prominent simple cuboidal epithelium. The functional zone of the lamina propria hosted primitive, newly formed tubular uterine glands within a matrix of loose connective tissue. Due to the extensive branching and projections of these endometrial folds, the uterine lumen characteristically assumed a distinctive stellate appearance.

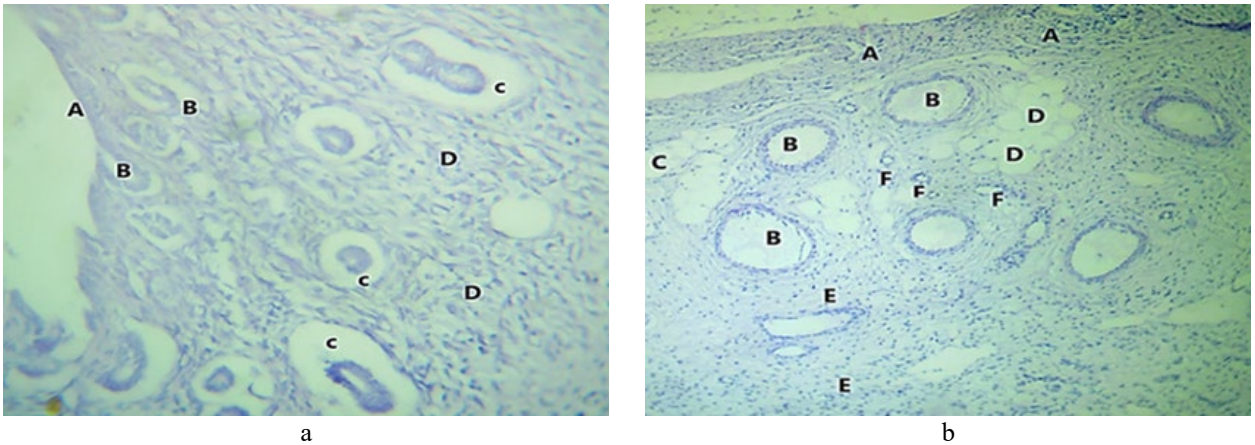

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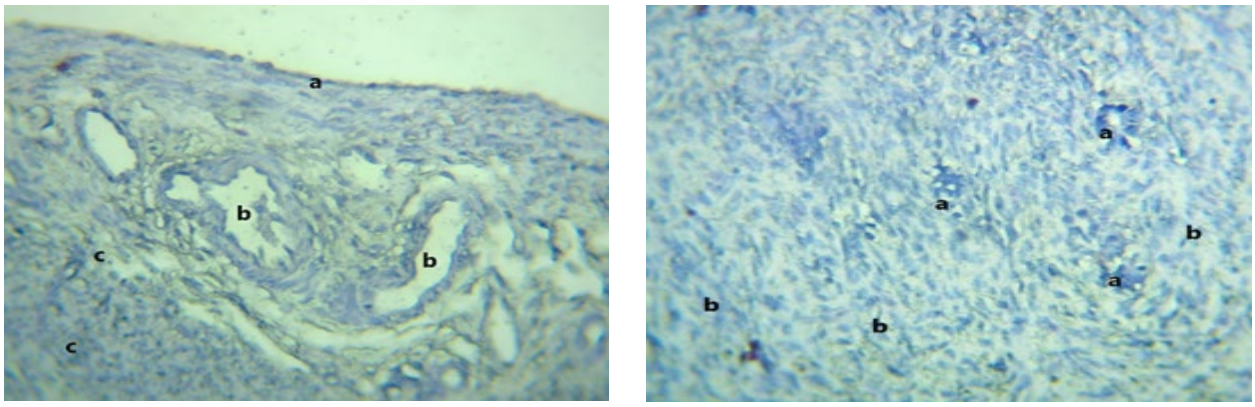
Fig. 1. Morphological features of the uterine wall in 3-week-old canine puppies (H&E staining).
a – endometrial mucosa under higher magnification showing the simple cuboidal luminal epithelium (A); developing primitive uterine glands (B); artifactual retraction spaces between the glandular epithelium and the basement membrane (C); and a dense population of stromal fibroblasts intermingled with migrating white blood cells (WBCs) within the lamina propria (D) (×40).
b – panoramic view of the endometrium demonstrating structural collagen bundles interwoven with active fibroblasts (A); well-differentiated uterine glands lined by simple cuboidal epithelium (B); newly formed primitive glandular nests (C); interstitial adipocyte/fat cell aggregations (D); loose connective tissue stroma infiltrated by residential WBCs (E); and prominent intramural neocapillarization (F) (×10).

Histochemical and comparative findings

Histochemical analysis using Alcian blue staining revealed that a substantial number of the developing uterine glands lacked detectable secretory products within their lumens. These glandular structures were structurally supported by dense collagen bundles

intermingled with a high density of active fibroblasts and scattered wandering white blood cells (WBCs) localized within the deeper regions of the endometrial stroma (***Fig. 2***).

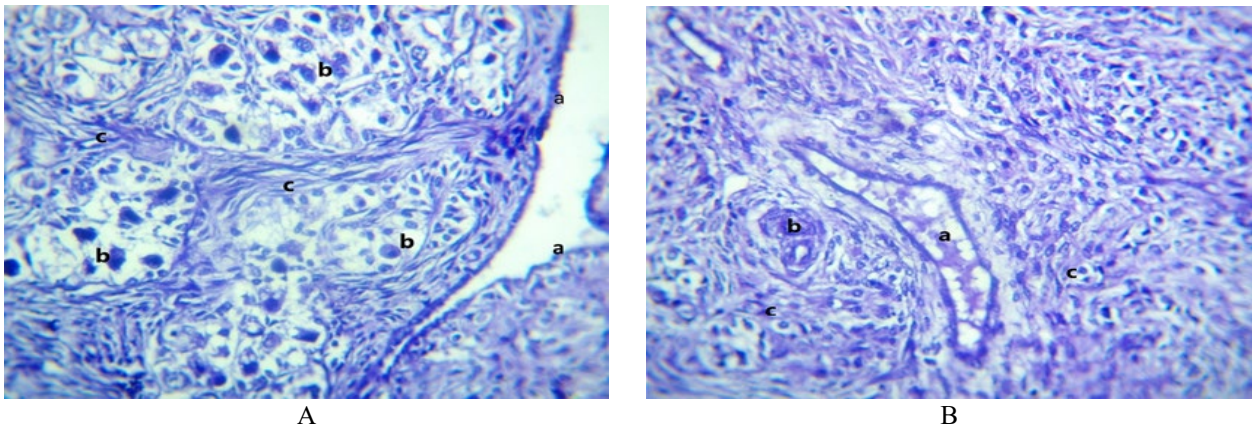


A B

Fig. 2. Histochemical features of the uterine wall in 3-week-old canine puppies (Alcian blue staining).
A – panoramic view of the deep endometrium showing the simple cuboidal luminal epithelium (a); elongated, branching uterine glands (b) supported by prominent perifollicular collagen bundles; and a highly cellular stroma containing numerous active fibroblasts intermingled with migrating white blood cells (c) (×10).
B – higher magnification of the broad endometrial stromal layer demonstrating newly formed, primitive uterine glands (a) characterized by a low cuboidal lining and distinctly narrowed, empty lumens; randomly distributed stromal fibroblasts and collagen bundles interwoven with dense inflammatory cell infiltrates (b) (×40).

At this developmental stage, the endometrial layer appeared significantly broader, characterized by a random distribution of primitive, small uterine glands. These newly formed glands were lined by a low cuboidal epithelium and possessed distinctly narrowed lumens.

When subjected to Periodic acid–Schiff (PAS) staining to detect mucus acid polysaccharides, the uterine glands of the 3-week-old canine puppies exhibited a completely negative histochemical reaction, demonstrating an absence of neutral mucins at this age. Concurrently, Alcian blue staining of the ovarian tissue sections at the same developmental stage highlighted a distinct simple low cuboidal germinal epithelium covering the ovarian surface. The subepithelial cortical zone contained well-defined clusters of primordial follicles characterized by spherical basophilic nuclei. These follicular cell nests were demarcated and encapsulated by prominent structural strands of collagen fibers (***Fig. 3***).



A B

Fig. 3. Histochemical characteristics of the ovary and uterus in 3-week-old canine puppies.
A – ovarian cortex highlighted by Alcian blue staining showing the thin simple low cuboidal germinal epithelium (a) on the outer surface; distinct groups of nests containing primordial follicles (b) with spherical basophilic nuclei; and thick structural bands of collagen fibers (c) compartmentalizing the follicular nests (×40).
B – endometrial matrix subjected to Periodic acid–Schiff (PAS) staining demonstrating intense PAS-positive reaction in the basement membrane of the luminal epithelium (a); a newly formed branching uterine tubular gland (b) with a well-defined basement membrane; and residential stromal fibroblasts within the supporting connective tissue (c) (×40).

Ovarian and uterine histomorphology at eight weeks of age

Histomorphological and histochemical evaluation of the female reproductive organs in 8-week-old canine puppies demonstrated advanced tissue differentiation. Under Periodic acid–Schiff (PAS) staining, the surface of the ovarian cortex was found to be lined by a well-defined simple cuboidal germinal epithelium. The peripheral zone of the cortex contained prominent clusters of developing primordial follicles,

each enveloped by a single layer of simple squamous follicular cells. The cortical stroma exhibited dense networks of collagen fibers intermingled with a high density of active fibroblasts and localized white blood cell (WBC) infiltrates within the deeper cortical regions (***Fig. 4***).

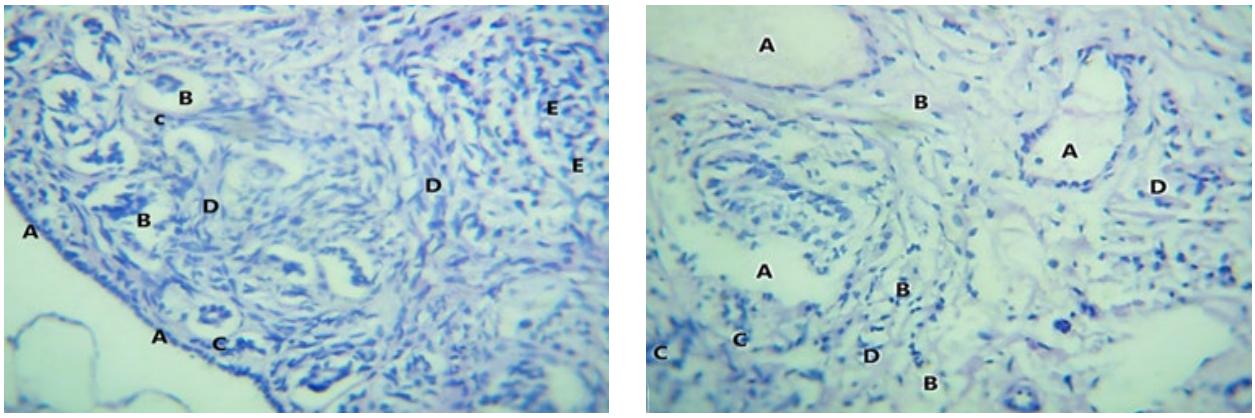


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Fig. 4. Histochemical and structural features of the ovary in 8-week-old canine puppies (PAS staining)
a – ovarian cortex demonstrating the continuous simple cuboidal germinal epithelium (A) on the outer surface; newly formed clusters of primordial ovarian follicles (B) containing basophilic nuclei and enveloped by a single layer of simple squamous follicular cells (C); dense collagen fiber bundles with fibroblasts (D); and localized white blood cell (WBC) infiltration (E) within the deeper cortical matrix (×40).
b – ovarian medulla exhibiting prominent medullary blood vessels characteristically devoid of erythrocytes (A); a delicate structural framework of collagen fibers (B) interspersed throughout the vascular network; newly formed blood capillaries (C); and focal accumulations of residential WBCs and tissue macrophages (D) (×40).

Examination of the ovarian medulla revealed numerous prominent blood vessels, which were characteristically devoid of erythrocytes and lined by a continuous layer of flattened endothelial cells. The medullary stroma was composed of a delicate framework of collagen fibers distributed among the vascular channels, presenting noticeable WBC infiltration, scattered macrophages, and residential fibroblasts aligned with the collagenous extracellular matrix. Concurrently, histochemical analysis of the uterine wall at 8 weeks of age demonstrated that the developing uterine glands exhibited a weak positive PAS reaction, indicating the initiation of neutral mucopolysaccharide and mucus secretion within the glandular epithelium at this developmental stage.

Statistical and morphometric analysis of postnatal development

Comparative statistical analysis of the biometric parameters across different postnatal stages demonstrated significant age-dependent development in female canine puppies. The body weight of the puppies significantly increased with age, with the 8-week-old group exhibiting a highly significant increase in mean body weight (3.016±0.08 g) compared to the 3-week-old group (2.128±0.11 g; P=0.0045).

Furthermore, an evaluation of the total weight of the female reproductive tract revealed a synchronized developmental progression. A highly significant increase in reproductive system mass was documented in the 8-week-old puppies compared to the 3-week-old animals, which presented a mean reproductive tract weight of 3.104±0.29 g (P=0.0004). These findings establish a strong positive correlation between somatic growth and the macro-morphological development of the reproductive organs during the early postnatal period (***Table 1***).

Table 1
Effect of postnatal age on body weight and total weight of the female reproductive system in canine puppies, Mean±SE

Age group	Body weight, g	Weight of female reproductive system, g
Week 3	2.128±0.110	1.214±0.130
Week 8	3.016±0.083	3.104±0.290
Significance	P=0.0045	P=0.0004

Note: statistically highly significant differences are observed between the age groups (P<0.01).

Morphometric analysis of the uterine tissue sections revealed substantial age-dependent remodeling of both the mucosal lining and the total wall thickness. The height of the uterine luminal epithelium demonstrated a highly significant increase associated with the postnatal advancement of the animals. In the 8-week-old group, the epithelial height reached a mean value of 28.50±2.91 µm, establishing a highly significant difference compared to the 3-week-old group, which presented a baseline epithelial height of 5.50±0.93 µm (P=0.0001).

Conversely, an inverse morphometric trend was documented regarding the total wall thickness of the uterus. The total uterine wall thickness was significantly greater in the 3-week-old puppies, presenting a mean value of 447.40±53.45 µm. In contrast, the 8-week-old group exhibited a significantly reduced wall thickness, averaging 271.30±40.45 µm (P=0.0303). This structural thinning of the uterine wall, occurring concurrently with the vertical hypertrophy of the luminal epithelium, reflects intensive longitudinal elongation and regional architectural reorganization of the myometrium and

endometrium during this specific phase of early postnatal organogenesis (***Table 2***).

Table 2

Effect of postnatal age on luminal epithelial height and total wall thickness of the uterus in canine puppies, Mean±SE

Age group	Epithelial height, µm	Total wall thickness, µm
Week 3	5.50±0.93	447.40±53.45
Week 8	28.50±2.91	271.30±40.45
Significance	P=0.0001	P=0.0303

Note: statistically significant difference (P<0.05); Statistically highly significant difference (P<0.01).

Morphometric investigation of the oviduct structures demonstrated architectural alterations in total wall thickness alongside structural stability in the mucosal lining. A statistically significant age-dependent decrease in the total wall thickness of the oviduct was documented. In the 3-week-old puppies, the total wall thickness averaged 220.00±29.74 µm, whereas a significantly reduced wall thickness of 133.50±4.71 µm was recorded in the 8-week-old group (P=0.0207). This variation represents localized tissue thinning and longitudinal stretching of the oviductal wall during early postnatal development.

In contrast, the height of the oviductal luminal epithelium remained highly stable between the two developmental stages. No statistically significant differences were observed in epithelial height between the 3-week-old and 8-week-old groups, with the morphometric values demonstrating statistical homogeneity (10.00±1.76 µm versus 11.90±1.53 µm, respectively; P=0.4407, NS; ***Table 3***).

Table 3

Effect of postnatal age on luminal epithelial height, total wall thickness of the oviduct, and primordial follicle diameter in canine puppies, Mean±SE

Age group	Oviductal epithelial height, µm	Oviductal total wall thickness, µm	Primordial follicle diameter, µm
Week 3	10.00±1.76	220.00±29.74	27.50±1.20
Week 8	11.90±1.53	133.50±4.71	34.00±1.85
Significance	P=0.4407 (NS)	P=0.0207	P<0.05

Note: NS = non-significant (P>0.05). Statistically significant difference (P<0.05).

Concurrently, morphometric assessment of the ovarian tissue sections revealed steady growth dynamics in the early stages of oogenesis, with the mean diameter of the primordial follicles significantly expanding from 27.50±1.20 µm at 3 weeks to 34.00±1.85 µm at 8 weeks of age (P<0.05).

The canine female reproductive tract encompasses the paired ovaries, which serve as the primary oocyte reservoir, alongside the tubular genital ducts including the bi-cornuate uterus, a small uterine body, and the oviducts. During early postnatal ontogenesis, circulating gonadotropin and ovarian steroid levels characteristically exhibit low, irregular profiles, actively modulating both the biochemical microenvironment and structural properties of these developing tissue layers.

In the current study, morphometric and histochemical tracking of the canine reproductive organs revealed distinct chronological remodeling. At 3 weeks of age, the ovarian cortex contained prominent clusters of primordial follicles enclosed by supporting collagen bundles. While individual follicles presented normal morphology, signs of physiological atresia accompanied by nuclear regression were observed, with the primordial follicles exhibiting a negative histochemical reaction under PAS staining. These baseline cortical observations closely align with the developmental chronologies documented in historical canine studies [17]. Physiologically, while prenatal oogenesis establishes the initial oogonial pool, subsequent folliculogenesis occurs primarily postnatally. Prior to partus, the mammalian ovary hosts mitotic oogonial nests, whereas well-defined primordial follicles systematically become apparent within the first two to three weeks of postnatal life.

Concurrently, the uterine wall at 3 weeks of age demonstrated an active mucosal expansion, characterized by complex endometrial folds lined by a prominent simple cuboidal epithelium. The supporting lamina propria hosted primitive, newly formed tubular uterine glands within a loose connective tissue matrix, creating a distinctive stellate intraluminal appearance. These structural configurations are consistent with classical observations on early mammalian endometrial histogenesis [18]. In comparison to other domestic species, distinct inter-specific variations exist regarding the timeline of postnatal uterine organogenesis. In heifers, uterine development progresses continuously up to the sixth month of life before entering a relative resting phase. In domestic pigs, the uterine wall is characteristically devoid of differentiated uterine glands and exhibits a negative estrogen receptor-alpha profile at birth; however, intense glandular differentiation and stromal maturation are initiated within the first two weeks of postnatal life [19]. These comparative data highlight that pre-pubertal uterine growth dynamics, driven by structural tissue remodeling, reach peak intensity between the third and sixth months across distinct mammalian taxa.

Furthermore, the surge of circulating gonadal hormones during the late pre-pubertal phase exerts a profound structural impact on the mammalian uterine wall. These chronological tissue modifications closely correspond with reproductive research conducted on rodents [20]. This complex sequence of synchronized morphogenetic and cytodifferentiative processes establishes the primary foundation for definitive tissue functionality in maturity. In all female mammals, endometrial glands are fundamentally responsible for the synthesis, secretion, and transport of a complex histotrophic mixture of macromolecules required for pre-implantation embryo maintenance [21]. In addition to

serving as a crucial lubrication barrier, the intraluminal mucus hydrates the mucosal surface, prevents bacterial adhesion, and restricts pathogenic colonization, protecting the lining from localized chemical and physical harm.

In the current investigation, the functional initiation of this glandular activity was tracked utilizing PAS staining for neutral mucus polysaccharide secretion. The histochemical reaction was completely negative in the 3-week-old canine puppies, whereas a weak positive PAS reaction emerged within the uterine glands by the 8th week of postnatal life. This chronological onset of secretory maturation strongly agrees with historical baselines on early canine glandular differentiation [22]. In comparison to adult cyclical mammals, these early postnatal profiles represent the primitive stages of glandular development. During the luteal phase of the human menstrual cycle, the uterine luminal epithelium and glandular cells exhibit a robust increase in PAS-positive neutral carbohydrates and acidic mucin glycoproteins. Similarly, the intensity of the PAS reaction within the uterine epithelium of adult goats fluctuates dynamically from moderate to high density during proestrus, estrus, and diestrus, declining significantly during metestrus [23]. Conversely, in adult cows, both the glandular epithelial cells and the luminal lining characteristically lack carboxylated acid mucins. This comparative matrix underscores that the weak positive PAS reaction observed in our 8-week-old puppies marks the precise physiological transition toward active, mature mucopolysaccharide synthesis.

The biometric and morphometric alterations documented in the current study underscore a synchronized progression of early postnatal reproductive development. The highly significant increase in somatic body weight (from 2.128±0.110 g to 3.016±0.083 g) and total reproductive tract mass (from 1.214±0.130 g to 3.104±0.290 g) between the 3rd and 8th weeks of life demonstrates a tight correlation between systemic somatic growth and reproductive organogenesis, which is consistent with early mammalian developmental indices [18]. These findings support the consensus that endogenous ovarian steroidogenesis exerts a primary regulatory influence on subsequent uterine and stromal modifications, while minor fluctuations in body mass play a secondary role. Furthermore, any early exposure to exogenous hormonal disruption, such as the administration of systemic steroids during the initial postnatal weeks, is known to induce severe structural and biochemical defects in endometrial maturation, permanently compromising future fertility [24].

Morphometrically, the highly significant increase in uterine luminal epithelial height (from 5.50±0.93 µm to 28.50±2.91 µm) observed in the 8-week-old group represents vertical cellular hypertrophy linked to active mucosal differentiation. Conversely, the total uterine wall thickness exhibited a significant reduction over the same period (decreasing from 447.40±53.45 µm to 271.30±40.45 µm). This structural pattern strongly agrees with historical observations on pre-pubertal mammalian uterine remodeling [25], reflecting intensive longitudinal elongation and mechanical stretching of the organ wall as the uterine horns expand during early ontogenesis.

A mirror morphometric trend was recorded within the tubular genital ducts. The total wall thickness of the oviduct decreased significantly from 220.00±29.74 µm at 3 weeks to 133.50±4.71 µm at 8 weeks (P=0.0207), driven by the same mechanism of regional longitudinal tissue expansion. Concurrently, the height of the oviductal luminal epithelium maintained statistical homogeneity (P=0.4407), proving that the functional inner lining remains cytologically stable during this rapid macro-morphological stretching phase. Synchronously, the mean diameter of the primordial ovarian follicles demonstrated a steady physiological enlargement, expanding significantly from 27.50±1.20 µm to 34.00±1.85 µm by the 8th week of age. This steady growth dynamic is compatible with established folliculogenesis profiles [26], indicating that early pre-antral follicle formation and initial oocyte expansion progress as an age-dependent physiological process, independent of external environmental or energetic variations, preparing the ovarian cortex for subsequent pubertal recruitment.

Conclusions

In conclusion, the early postnatal organogenesis of the canine female reproductive tract between the third and eighth weeks of life is driven by distinct, synchronized tissue remodeling characterized by highly targeted morphometric shifts rather than uniform growth. The functional maturation of the endometrium is confirmed by a histochemical transition of neutral mucopolysaccharides within the uterine glands from a completely negative reaction to a distinct weak positive profile under PAS staining. Morphometrically, the uterine wall exhibits a highly significant, more than 5-fold (5.2×) vertical hypertrophy of the luminal epithelium (P=0.0001). Concurrently, intense longitudinal organ elongation and regional tissue stretching result in a 39.4 % reduction in total uterine wall thickness (P=0.0303) and a 39.3 % reduction in total oviductal wall thickness (P=0.0207) during early tubular expansion. In contrast to the strict cytological stability maintained by the oviductal luminal epithelium (P=0.4407), the functional ovarian cortex demonstrates a steady, age-dependent folliculogenesis progression manifested by a 23.6 % linear expansion of the primordial follicle diameter (P<0.05), thereby establishing crucial relative biophysical and histochemical baselines for the evaluation of early reproductive development in canine females.

DECLARATIONS

Ethical Statement

The authors confirm that all experimental procedures, husbandry protocols, and studies involving biological objects were planned and conducted in strict accordance with the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986), Directive 2010/63/EU, national ethical norms, and international standards for the humane treatment of animals (NIH,

ARRIVE). The research protocol and experimental scheme were officially reviewed and approved by the Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq (Approval No. Vet Medicine 217 dated September 2025). To minimize animal stress, discomfort, and suffering, all handling, maintaining, and subsequent tissue sampling procedures involving canine subjects were executed in strict compliance with standard veterinary guidelines, recommendations of the American Veterinary Medical Association (AVMA), and current international principles of animal welfare.

Funding

This research received no external funding.

Conflict of Interest

The authors state that there is no conflict of interest.

Acknowledgements

The authors strongly acknowledge the Council of the College of Veterinary Medicine at Diyala University for their continuous support and for providing the necessary institutional facilities to successfully conduct this research project.

Declaration of AI and AI-assisted technologies

The authors declare that generative artificial intelligence (AI) and AI-assisted technologies were utilized strictly to improve the readability and language editing of the manuscript. No new content, clinical data, or scientific interpretations were introduced during this process. The authors have fully reviewed, verified, and revised all generated outputs and remain solely responsible and accountable for the accuracy, originality, and scientific integrity of all content within the manuscript. Furthermore, the AI technology was not utilized as a co-author and does not qualify for authorship.

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