



Anatomical and Histological Study of the Female Reproductive System of Green Freshwater Turtle (*Chelonia mydas*) During Breeding Season in Iraq

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ABSTRACT

Turtles are found in large numbers in Iraqi rivers, due to the availability of a suitable environment for reproduction and food. The breeding season begins from May to the end of October. The current study aimed to evaluate the anatomical and histological characteristics of the green freshwater turtle (*Chelonia mydas*) during the breeding season in Iraq. The samples included eight adult turtles within the age range of 11-14 years that were collected from Shatt Al-Hilla (Iraq) at month June. To investigate the reproductive system histological techniques and hematoxylin and eosin staining were used and before that the animals were anesthetized with chloroform. The results indicated two active ovaries and oviducts which fill the whole abdominal cavity. The mean weights of left and right ovaries and left and right oviducts in the turtles with average weights of 698 ± 0.05 g were 19.5 ± 0.01 g, 22 ± 0.022 g, 3.3 ± 0.05 g, and 4 ± 0.05 g respectively. The mean lengths of carapace, left ovary, right ovary, left oviduct, and right oviduct were 24 ± 0.08 cm, 15.9 ± 0.01 cm, 17 ± 0.04 cm, 13 ± 0.022 cm, 14 ± 0.056 cm. Anatomically the oviducts include the infundibulum, magnum, isthmus, uterus, and vagina. The infundibulum indicated a funnel-shaped membrane while the magnum was the muscular coiled long tube. The isthmus was shorter and less coiled than the magnum, the uterus appeared as the widest, thickest, and less coiled dark tube and swollen into the posterior to form a cyst-like part, and the vagina was muscular in structure. Histologically, the magnum and uterus were formed from mucosa, muscularis, and serosa. In both parts of the magnum and uterus, were branched crypt-like depressions that appeared devoid of sperm. The widespread distribution of this species in Iraqi rivers could be due to the activity of the ovaries and oviducts during the breeding season, which extends for 6 months.

Keywords: *Chelonia mydas*, Ovary, Oviduct, Turtle

INTRODUCTION

Various studies have extensively explored the morphology of the ovaries and oviducts of turtles, focusing on different aspects of the reproductive system (Cabral et al., 2011; Silva et al., 2011; Chaves et al., 2012; Firmiano et al., 2012). Some other researchers examined the morphology and functions of the female turtle's reproductive organs, offering comprehensive insights into breeding behavior and nesting (Peixoto et al., 2012; Souza et al., 2014).

In female turtles (*Podocnemis lewyana*), the reproductive system entailed two active ovaries (left and right), two oviducts, and suspensory ligaments (Yntema, 1981; Callebaut et al., 1997; Sánchez-Ospina et al., 2014). The ovaries have a long tubular shape with a thickness of 2 mm, width of 2 mm, and length of 20 mm. The reproductive system of marine turtles consists of two ovaries and oviducts with suspensory ligaments (Wyneken, 2001). The oviduct starts near the ovary and is divided into five regions (Girling, 2002). After ovulation, the corpus luteal is formed, and progesterone production occurs as in domestic mammals (Wyneken, 2001; Hafez and Hafez, 2004). A macroscopic study by Faillab et al. (2018) in sea green turtles (*Chelonia mydas*) indicated the medium length of 2.57 cm and width of 7.90 cm for ovaries.

Histologically, the ovaries are created from the cortex and medulla, having various follicles ranging from primordial oocytes to mature ova found in the cortex. The follicles, categorized based on size, undergo different stages of development, known as folliculogenesis, previtellogenesis, and vitellogenesis (small, medium, and large; Nainan et al., 2010; Pérez-Bermudez et al., 2012). In the hawksbill turtle (*Eretmochelys imbricate*), the size of follicles ranges from 19.2 to 24.9 μ m (Pérez-Bermudez et al., 2012). The medulla is composed of smooth muscle, blood vessels, fibroblasts, lymphoid tissue, and collagen fibers (Callebaut et al., 1997; Nainan et al., 2010; Pérez-Bermudez et al., 2012). There are differences in the size of ovaries considering the age of turtles and the follicles (Callebaut et al., 1997).

Microscopic analysis of the oviducts reveals distinct layers, as described by Palmer and Guillette (1988). The mucosa is composed of ciliated epithelium and connective tissue containing glands. The muscularis layer consists of

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smooth muscle, providing the necessary contractile function. The outermost layer, known as the serosa, forms the protective outer covering of the oviducts. Therefore, the current study aimed to evaluate the anatomical and histological characteristics of the green freshwater turtle (*Chelonia mydas*) during the breeding season (June) in Iraq.

METHODS AND MATERIALS

Ethical approval

The current study was conducted according to the ethical guidelines of the Department of Anatomy and Histology, college of Veterinary Medicine, Al-Qasim Green University, Babylon, Iraq (No: 2023, 8/9/2023).

Study animals

In the current study, eight female adult turtles (*Chelonia mydas*) were used as the samples. The turtles had an average weight of 698 grams and an age range of 11-14 years and were collected from Shatt Al-Hilla (Iraq) at month June 2023. Data on length straight carapace length (SCL) was measured from the middle of the nuchal notch to the posterior-most tip of the caudal peduncle and weights were systematically recorded for analysis. Anesthesia, using chloroform in a closed chamber, was administered to facilitate the removal of the plastron (MacLean et al., 2008).

Dissection process

The dissection process involved a precise cut through the neck skin, lateral extension, and circumferential cutting around the axillary region near the plastron. The incisions followed the seam created by the marginal and inframarginal cuts. The methodology employed in this study was based on a study by Wyneken (2001).

Macroscopic study histological examination

For the macroscopic study, data on the ovary and oviducts of the turtles, including shape, location, length, and weight, were recorded. Additionally, specific details about parts of the oviduct were recorded. Histological examination focused on the ovary, magnum, and uterus. In this regard, formalin 10% was first used to fix the samples, followed by dehydration, clearing, embedding, sectioning (5-7 μ m), and mounting. The sections were stained by Haematoxylin and Eosin (Suvarna et al., 2012).

RESULTS

Anatomical results

In breeding season which extends from May to the end of October, the reproductive system of Iraqi green freshwater turtle is composed of two active ovaries and two active oviducts. The ovaries occupied the whole abdominal cavity on both the left and right sides, extending from the liver cranially to the end of the coelomic cavity caudally. The ovaries had the shape of a cluster of grapes. Within the ovaries, follicles of varying sizes representing different stages of growth (Figure 1).

The mean weights of the left and right ovaries were recorded as 19.5 ± 0.01 gm and 22 ± 0.022 gm, respectively. Accordingly, the mean weights of the left and right oviducts were 3.3 ± 0.05 and 4 ± 0.05 gm, respectively. Regarding the length, SCL was measured as 24 ± 0.08 cm. The lengths of the left and right ovaries were 15.9 ± 0.01 cm and 17 ± 0.04 cm, respectively, while the lengths of the left and right oviducts were 13 ± 0.022 cm and 14 ± 0.056 cm, respectively (Tables 1 and 2). Both left and right oviducts exhibited a highly convoluted tube structure, comprising four distinct parts. The infundibulum, characterized by a very transparent and thin funnel membrane, was divided into two segments including a thin, flattened, diaphanous wall known as the funnel and another thick wall termed the tubular part. The magnum, a long and curvy tube with a white appearance, displayed a robust blood vessel supply on its external surface. This section featured a large diameter, a long and coiled portion, and a thick wall (Figure 2). The isthmus, shorter and less coiled than the magnum, was followed by the uterus.

The uterus, the widest and thickest segment, presented as a dark tube, swelling posteriorly to form a cyst-like part. Its thickness was comparable to that of the magnum, and it seamlessly transitioned into the vagina. The vagina, a muscular and short segment, led to the cloaca, completing the reproductive anatomy (Figure 2).

Histological results

In the histological examination, the outer layer of the cortex was observed to be composed of cuboidal cells, forming the epithelium. Within the cortex, both mature and immature follicles were identified. The oocytes within these follicles exhibited a central rounded nucleus with bushy chromatin, surrounded by cytoplasm in a fibrillar morphology. However, visualization of the medulla of the ovaries proved to be challenging (Figure 3).

Considering the histological structure of the magnum and uterus, both organs were found to be comprised of mucosa, muscularis, and serosa layers, arranged from internal to external. The mucosa in both the magnum and uterus featured multiple longitudinal folds lined by the ciliated secretory epithelium of the pseudostratified columnar type. Notably, the mucosal folds in the uterus appeared narrower and taller, compared to those in the magnum. Some folds in both the magnum and uterus exhibited a leaf-like appearance. There are branched crypt-like depressions lined by cuboidal cells, the crypts see a lot at the caudal part of the oviduct (Figures 4, 5).

Table 1. The mean weight of Iraqi green freshwater turtle (*Chelonia mydas*) ovary, and oviduct

Anatomical parameters	Mean ± SD (gr)
Turtles weight	698±0.05
Left ovary	19.5±0.01
Right ovary	22±0.022
Left oviduct	3.3±0.05
Right oviduct	4±0.05

Table 2. The mean length of Iraqi green freshwater turtle (*Chelonia mydas*) ovary, and oviduct

Anatomical parameters	Mean ± SD (cm)
Turtles (carapace)	24±0.08
Left ovary	15.9±0.01
Right ovary	17±0.04
Left oviduct	13±0.022
Right oviduct	14±0.056

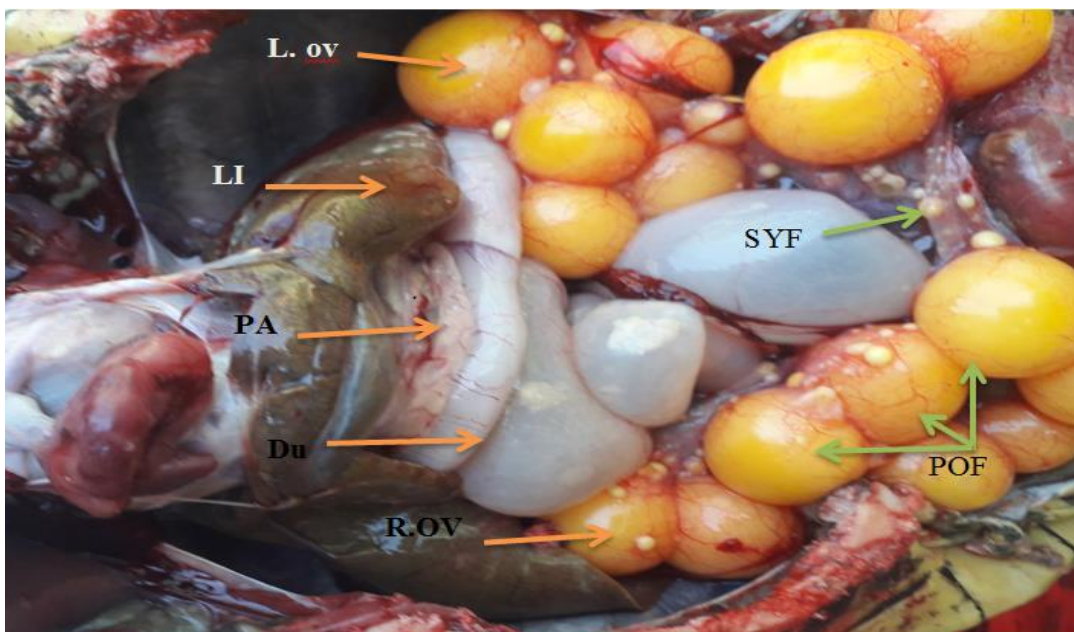


Figure 1. Anatomy of the reproductive system of Iraqi green freshwater turtle in the breeding season. L. OV: Left ovary, R. OV: Right ovary, LI: Liver, PA: Pancreas, DU: Duodenum, POF: Pre-ovulatory follicles, SYF: Small yellow follicles



Figure 2. Anatomy of the reproductive system of Iraqi green freshwater turtle in the breeding season. IN: Infundibulum, IS: Isthmus, MG: Magnum, UT: Uterus, OV: Ovary

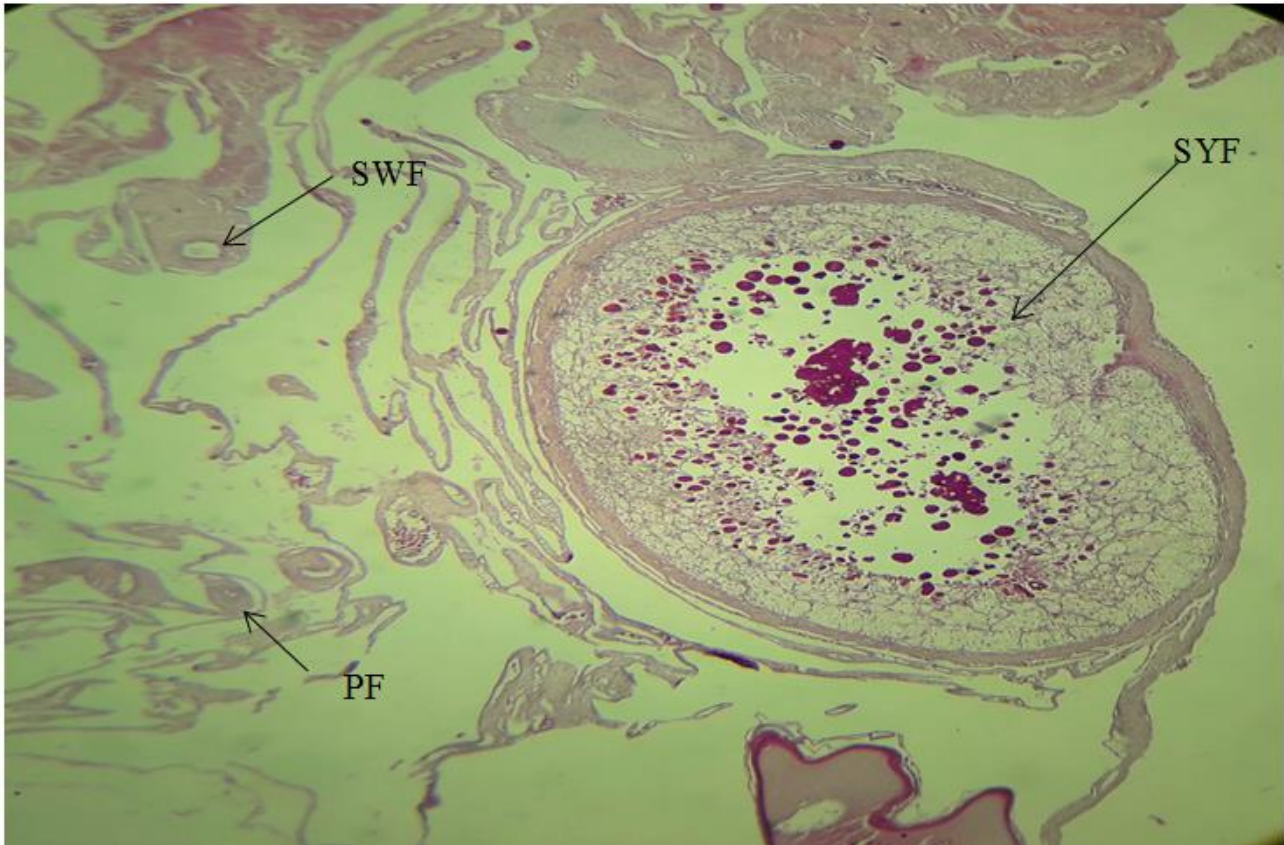


Figure 3. Histology of reproductive system of Iraqi green freshwater turtle in breeding season. SYF: Small yellow follicle, SWF: Small white follicle, PF: Primary follicle

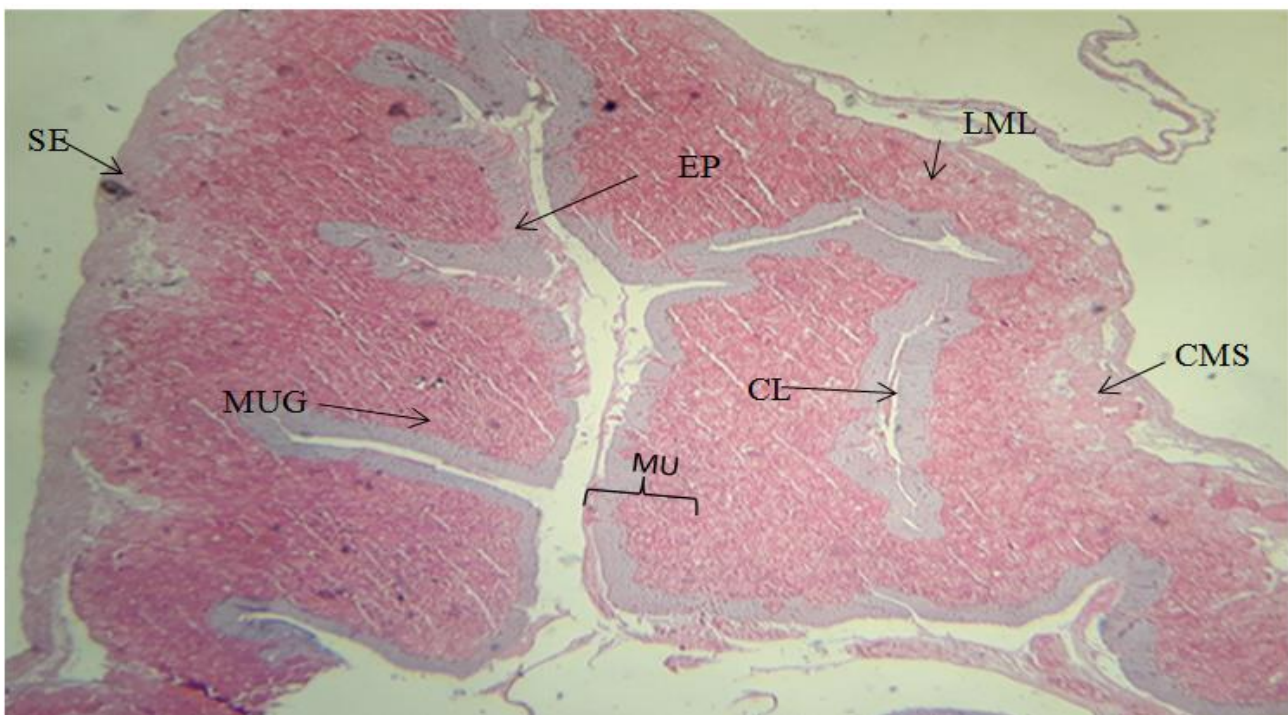


Figure 4. Histology of reproductive system of Iraqi green freshwater turtle in breeding season. MU: Mucosa, EP: Epithelium, CM: Circular muscular layer, LML: Longitudinal muscular layer, SE: Serosa, MUG: Mucosal gland, CL: Crept-like gland, H and E staining 10 X.

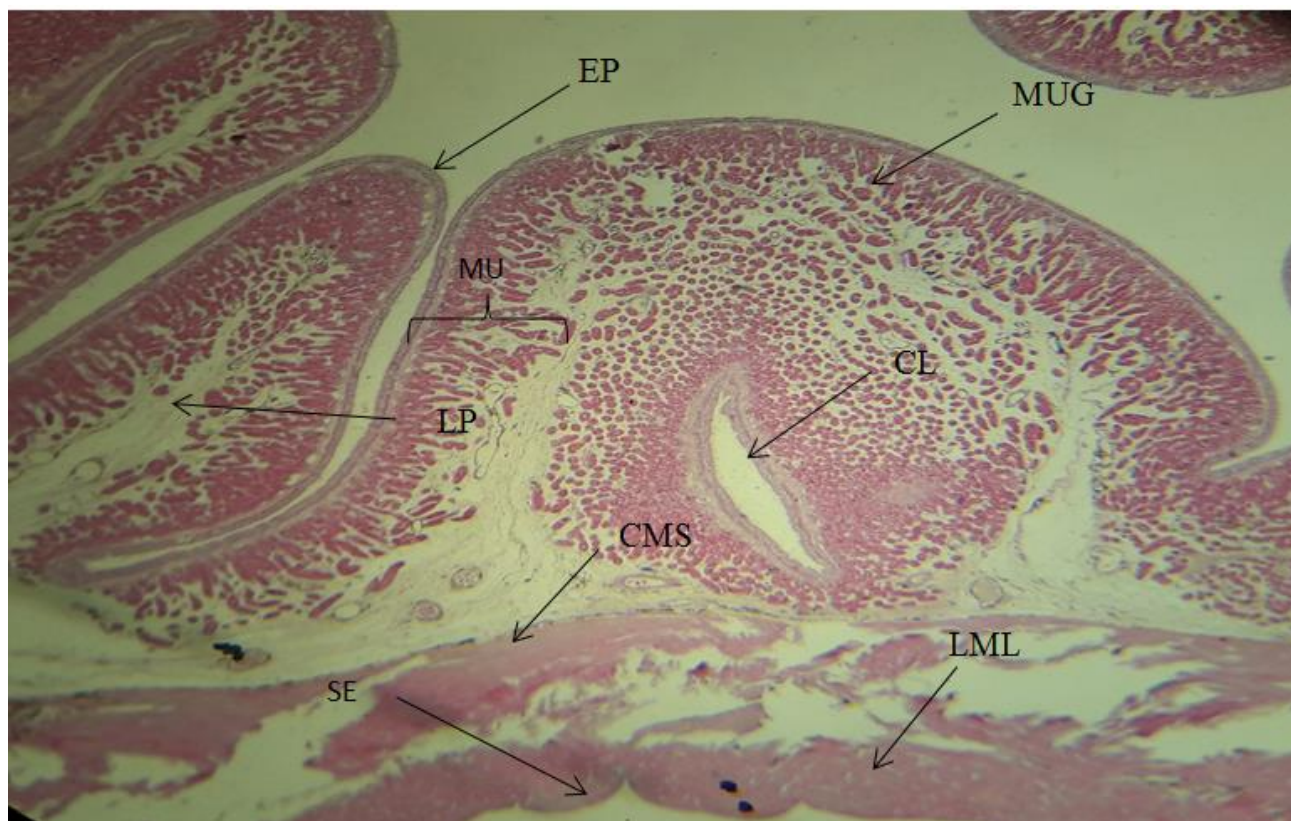


Figure 5. Histology of the reproductive system of Iraqi green freshwater turtle in the breeding season. MU: Mucosa, EP: epithelium, CM: Circular muscular layer, LML: Longitudinal muscular layer, SE: Serosa, MUG: Mucosal gland, CL: Crept-like gland, LP: Lamina propria, H and E staining 10 X.

DISCUSSION

The reproductive system of the female Iraqi green freshwater turtle (*Chelonia mydas*) during breeding season consists of two ovaries and oviducts. This observation aligns with findings from previous studies conducted on *Podocnemis lewyana* turtles by Yntema (1981), Callebaut et al. (1997), and Sánchez-Ospina et al. (2014). The findings revealed that ovaries occupied the entire abdominal cavity bilaterally, extending from the cranial aspect near the liver to the caudal end of the coelomic cavity. The ovaries exhibited a distinctive bunch-of-grapes morphology, featuring various sizes of follicles.

The mean weight of the left and right ovaries in female Iraqi green freshwater turtles during the breeding season was recorded as 19.5 ± 0.01 gm and 22 ± 0.022 gm, respectively. Concurrently, the mean SCL of the turtles was measured at 24 ± 0.08 cm. The mean lengths of the left and right ovaries were found to be 15.9 ± 0.01 cm and 17 ± 0.04 cm, respectively. These findings align with the results reported by Faillab et al. (2018) in *Chelonia mydas*, where the length of ovaries varied between 2.57 cm and 7.90 cm, and the width ranged from 0.14 cm to 1.14 cm. Interestingly, this study also corresponds with the observations made in *Podocnemis lewyana* turtles by Sánchez-Ospina et al. (2014), who found that the ovaries presented as extended tubular structures measuring 20 mm in length 2 mm in width, and 2 mm in thickness. Moreover, the length of the ovaries was found to be associated with the laying period, exhibiting elongation in more advanced stages.

The left and right oviducts indicated a mean weight of approximately 3.3 ± 0.05 gm and 4 ± 0.05 gm, with corresponding lengths of 13 ± 0.022 cm and 14 ± 0.056 cm, respectively. These oviducts were observed to be highly convoluted and composed of four distinct segments, as also noted by Girling (2002). The four segments identified were the infundibulum, tubal uterine or magnum, isthmus, uterus, and vagina. The infundibulum, characterized by a very transparent and thin funnel membrane, was further divided into two parts including the funnel and the tubular part. The magnum was a long, wide, and curvaceous tube with a white appearance, exhibited a robust blood supply on its external surface. Notably, the magnum was distinguished by its large diameter, longest coiled portion, and thick wall, contributing to its vital role in the reproductive process.

Isthmus was shorter and less coiled than the magnum contrasts with the uterus, the widest, thickest, and less coiled dark tube that ended in the vagina. The uterus started as a tube and then posteriorly swollen in a cyst-like shape, sharing a thickness similar to that of a magnum. The vagina was a muscular, short, narrow, and straight segment, that leads to the cloaca.

Histologically, the epithelium is the outer layer of the cortex comprising cuboidal cells, and the cortex contains mature and immature follicles, supporting the findings of studies performed by Nainan et al. (2010) and Pérez-Bermudez et al. (2012). The oocyte inside the follicle has a central rounded poor nucleus and dense chromatin, the oocyte is encompassed by fibrillar cytoplasm, and the medulla of the ovaries is intractable to see due to the excessive activity of the cortex, as the process of laying eggs takes place simultaneously.

The magnum and uterus of the *Chelonia mydas* oviduct exhibit a three-layered structure, comprising mucosa, muscularis, and serosa from internal to external layers. The mucosa in both magnum and uterus consists of multiple longitudinal folds lined by ciliated secretory epithelium of pseudostratified columnar type. Interestingly, the mucosal folds in the uterus are narrower and taller than those in the magnum, with some folds displaying a leaf-like appearance. Both the magnum and uterus feature branched crypt-like depressions, lined by cuboidal cells, with a higher concentration observed at the caudal part of the oviduct. The lamina propria is densely populated with glands contributing to the production of albumin in the magnum and shell egg in the uterus. The number of glands increases significantly in the uterus, emphasizing its specialized role in reproductive processes. The tunica muscularis comprises circular muscle fibers as the inner layer and longitudinal smooth muscle fibers as the outer layer. Notably, the thickness of the muscle fiber layers intensifies from the magnum toward the end of the uterus. Finally, the serosa, forming a thin outer layer, consists of simple squamous cells underlining connective tissue.

CONCLUSION

The results of the current study indicate that the mean weights of left and right ovaries and left and right oviducts in the turtles were 19.5 ± 0.01 g, 22 ± 0.022 g, 3.3 ± 0.05 g, and 4 ± 0.05 g respectively. The mean lengths of carapace, left ovary, right ovary, left oviduct, and right oviduct were 24 ± 0.08 cm, 15.9 ± 0.01 cm, 17 ± 0.04 cm, 13 ± 0.022 cm, 14 ± 0.056 cm. Anatomically the oviducts include the infundibulum, magnum, isthmus, uterus, and vagina. The widespread distribution of this species in Iraqi rivers could be due to the activity of the ovaries and oviducts during the breeding season, which extends for 6 months. Therefore, more study needs to be conducted on green freshwater turtles during different seasons.

DECLARATIONS

Funding

The authors received no funding to perform the study.

Availability of data and materials

The data of the current study are available with a reasonable request from the corresponding author.

Competing interests

There are no conflicts of interest for the submission and publication of this study.

Ethical considerations

The current research has followed all ethics during collecting the data, writing the article, and revising the draft of the manuscript. The final edition of the article is prepared originally for review and publication in this journal.

Authors' contributions

Salim Salih Ali AL-Khakani and M.S.H. Simawy were responsible for the research article proposal, experiment design, explaining the findings, and article writing. Mustafa Fadhil, Amina Imad Jawad, Sabreen M. Al-Janabi handled the preparation of materials, funding acquisition, and data curation. Dunia M. Al-Rubaie and Ranin S Hamad contributed to the review and editing process. All authors confirmed the last edition of the article before processing in the journal.

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