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Histomorphological Developmental Study of the Uterus in Local Awassi Sheep Fetuses During the Postnatal Period

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Abstract | This study aims to investigate the postnatal stages of the uterus in lambs of Awassi sheep. For this purpose, samples from sixteen female sheep were taken from uterus of healthy females and the age of the animals was determined using the formula of milk teeth and eruption age of teeth. At 2–3-month-old lamb, the uterus of the sheep musculo-membranous tubular organ with a thick wall large in size relatively composed of the long uterine body, two uterine horns and the uterine cervix which has (5-6) ring. Histologically, the wall consists of four layers: epithelium, submucosa, muscularis and tunica serosa layers respectively. The lumen irregular wide and the deepest portion of the intercarunacles area is expanded parallel to the luminal surface. At eight months postnatally, the lambs uterus was bicornuate and bipartite, a hollow musculomembranous tubular elongate cylindrical organ with a thick wall. It was composed of two uterine cornua which appeared as ram horns shape like structures, a large quadrilateral corpus uterus (body of the uterus) and a narrowing cervix uterus. Histologically, uterine wall at this stage exhibits more growth and development with advanced stage composed of three layer: endometrium (mucosa), myometrium (muscle) and perimetrium (serosa). The lumen of the horn star-shape while body was irregular in shape, because growth and development of the caruncle toward the center causes a narrowing of the lumen which the almost completely closed.

Keywords | Fetuses, Uterus, Prenatal, Sheep

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INTRODUCTION

Sheep are the most important and earliest domesticated species of livestock. The global sheep population currently stands at over one billion heads with 19 per cent located in Asia and Africa. There are more than 850 breeds of sheep in the worldwide (Rege and Gibson, 2003). There are three native sheep breeds and some sub breeds in Iraq due to random crossing with other breeds they are not considered purebred. Awassi sheep is a dominant type of sheep in Iraq and the most famous species; it is mainly raised for the production of meat, milk and wool, which used for sheep wool carpet (Al-Dabbagh, 2009; Mohsen *et al*., 2024). The female reproductive tract organs of mammals, including ovary, oviducts, uterus, and vagina, the uterus is bicornuate and body of the uterus is short, which formed by incomplete fusion of the caudal parts of the horns that lie side by side. Cranially, the two uterine horns, or cornea, have an inverted comma shape, held within the abdomen by the broad ligament, so that the ovaries come to lie adjacent to the bifurcation of the two corneas near the body of the uterus. The body of the

uterus is relatively short and poorly developed, while the uterine horns are longer and well developed. The uterine cervix is cylindrical with a thick wall situated between the uterine body and vagina containing cavity called cervical canal (Shehan *et al.*, 2019). Histologically, the uterine wall divided into; the endometrium, myometrium and an outer layer perimetrium (serosa). The endometrium of domestic animals consists of two epithelial cell types, luminal epithelium, glandular epithelium, stratified stromal compartments. The endometrium epithelium of they may be stratified or pseudostratified. Simple, branched uterine (endometrial) glands extend into the lamina propria. The myometrium consists of compact bundles of smooth muscle in different directions separated by thin strands of interstitial connective tissue with numerous blood vessels while; Perimetrium consider the outer serous coat of uterus composed of simple squamous epithelium supported by connective tissue rich in blood vessels and elastic fibers and continuous with the broad ligament (Shehan *et al.*, 2019). The female reproductive system originates from four sources: mesoderm, primordial germ cells, coelomic epithelium, and mesenchyme. Uterine development is initiated in the fetus and it is not completed until after birth. In females the mesonephric duct regresses and the cell of a parallel paramesonephric duct proliferates cranially forming oviduct, uterus, cervix and vagina while; the posterior ends of the paramesonephric ducts fused resulting in a bicornuate uterus and uterine body that opens into the vagina by a single cervix (Noden and de Lahunta, 1985). There are little information and research was available regarding the development of uterus at postnatal in the local Iraqi Awassi sheep, so this study was designed to provide a more complete quantitative description of the morphological and histomorphometric development of the uterus ewe postnatally.

MATERIALS AND METHODS

This study was designed to describe the histomorphological and developmental characteristics of the uterus in local Awassi sheep fetuses (*Ovis aries*) at postnatal age were collected from the Najaf abattoir soon after were slaughtered. The sample are collection was conducted over one month from September 2024 to October 2024. The sheep samples at postnatal stage were distributed into two groups (eight samples at 2-3 neonatal and eight samples at 8-9 months lambs where the approximate age of the animals was determined depending on formula of milk teeth and eruption age of teeth using the following formula: [2(0\3 + 0\1 + 3\3)= 20], while the formula for the permanent teeth is [2(0\3 + 0\1 + 3\3 + 3\3)=32]. Moreover, the information attainable from the owner is regarded as a means to detect the exact age. The uterus of sheep lambs was fixed in 10% buffered formalin, dehydrated in a gradual series of alcohol, cleared in xylene and embedded in paraffin wax. The blocks were sectioned at thickness of 5-6 um using a rotary microtome. Histological sections were stained with Hematoxylin and Eosin, periodic acid Schiff, Van Gieson's, Alizarin blue and Trichrome Masons (Suvarna *et al.*, 2018; Mohassen and Al-Jebori, 2020). The sections were studied using an Olympus light microscope equipped with a camera, which was connected to a computer (Niyf and Al-Jebori, 2024).

RESULTS AND DISCUSSION

Observation of a 2–3-month-old lamb

The body weight of sheep lambs at (2-3) months was about (15000±316.38) grams. The uterus thickness was (7.60±0.291) mm (Tables 1, 2). The uterus appears pink-white, a Musculo-membranous tubular organ with a thick wall large in size composed of the long uterine body and two uterine horns that are attached to the broad ligament, connecting the uterine body to ovaries. The uterine cervix located in the abdominal cavity was small in size and had about (5-6) ring. The external opening is directly connected to the first fold as an integral part of it (Figure 1). The gross morphological parameters of the uterus showmen in (Tables 1, 2). The length of the body of the uterus was about (1.7±0.12) cm and the width of the body of uterus at the cranial end, middle and caudal end was found about 1.2±0.07 cm, 1.1±0.03 cm and 0.9±0.02 cm, respectively.

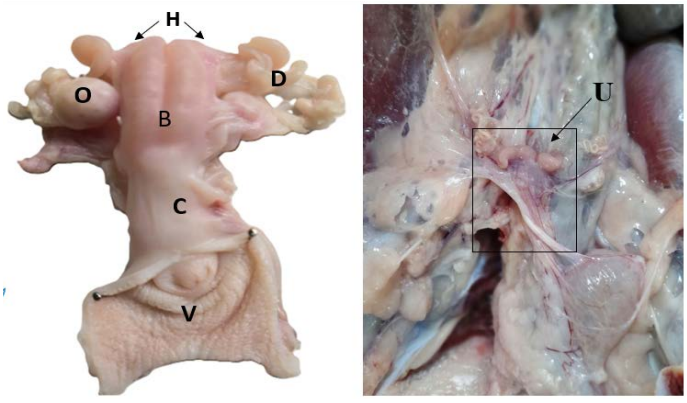

Figure 1: Photograph show at 2-3 months old location of (U) uterus in pelvic cavity and female reproductive system parts; (B) uterine body, (H) uterine, (C) uterine cervix, (V) vagina.

Histologically, the uterus wall consists of four layers: The epithelium, submucosa, muscularis and tunica serosa. The endometrial glands almost simple, tubular glands formed as invaginations of the endometrial lining epithelium, lined by columnar epithelium. The lumen of horn irregular wide and deepest portion of the intercarunacles areas expanded parallel with the luminal surface while; the lumen of body the lumen irregular narrowing due to mucosal folds almost close partially uterine lumen, very development numerous

structures called caruncles appear more extensive and clears than in uterine horn and the lumen of uterine cervix narrowing, semi close with numerous development folds with different sizes and length (Figure 2). The uterine wall of Kendra pada sheep at 4 months postnatal similar to the current study consists of four layers: Mucosa, submucosa, muscularis and serosa layer where the horns and body lined with pseudostratified columnar epithelium, the epithelium contain the columnar, basal goblet cells a reset on the basement membrane with mucosal folds closed relatively the uterine horn and body, the epithelium appear invagination into propria submucosa forming endometrial glands in some area, the endometrial glands more in the body than in horn of uterus (Sahu *et al*., 2017).

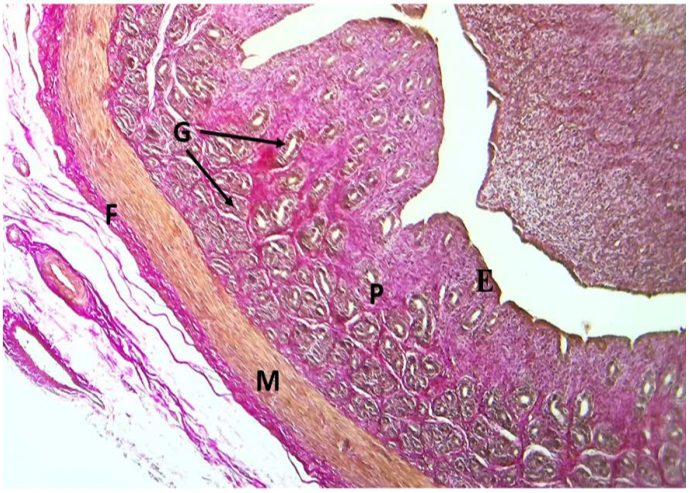

Figure 2: Histological cross section explains uterine horn layer at 2-3 months old; (E) tunica mucosa, (P) tunica submucosa, (M) tunica musculares, (F) tunica serosa (perimetrium), (G) gland (Van Gieson's. 4X).

First, the epithelium, submucosa and lamina propria together were known as the endometrium. The first layer the epithelium thicknesses less layer in uterine horn, composed of columnar epithelial tissue cellular layer without lamina propria and muscular mucosa that reset on visible basement membrane by P.A.S stain and not densely packed and contained elongated central large nuclei light in color and primitive goblet cells with large rounded macrophage suite beneath the epithelium layer while; the second layer, submucosa and lamina propria layer, found beneath epithelium directly comprised of loose connective tissue that location the uterine gland, composed of blood vessel and collagen fibres that also surrounds the endometrial gland and reticular fibres observed forming network around the glands. The connective tissue fibres are denser and more arranged. The invagination from epithelium into propria submucosa forming numerous endometrial glands. These endometrial glands were visible and more developed in the uterine horn at this stage (Figure 3).

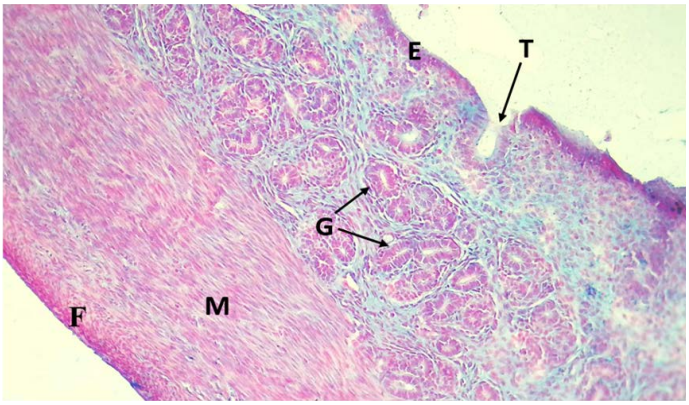

Figure 3: Histological cross section explain layer of uterine horn at 2-3 months old; (T) invagination, (E) epithelium, (M) musculares layer, (G) gland in tunica mucosa layer, (F) tunica serosa (allusion blue. 10X).

Third, the tunica muscularis is comprised of two layers: inner longitudinal and the outer circular layer of smooth muscles. The inner longitudinal layer in its inner third contained a very well-developed vascular layer (*Stratum vascular*), which was significantly developed, thick and composed of muscle bundle arranged in longitudinal form, Collagen fibers were present between the smooth muscle bundles. Reticular fibers were very few and formed a fine network in between the muscle bundles and contained blood vessels, nerves and round or longitudinal nuclei. The undifferentiated outer circular layer composed of bundles of smooth muscle with collagen fibers (Figure 3). The four, the tunicae serosa consist of a loose connective tissue layer covered by a mesothelial layer and comprised of collagen fibers, reticular fibers. The thickness of the tunica serosa of the uterine horn is less than that of the tunica muscular at this stage, connecting with the ligament (Figure 3). The previous studies in goats, at three months old, the lumen of uterus contains numerous projected called caruncles, separated from each other by the inter caruncular areas which were thrown into folds separated mucosal folds, the lamina propria and submucosa together extended to formed the core of caruncles and mucosal folds also noted at one week appear gland in endometrium, lining by pseudostratified columnar (Abiaezute, *et al*., 2018).

Observation of an 8–9 month old lamb

The body weight of sheep lambs at (8-9) months is (37000±707.1) grams and the uterus weight about (18.2±03.58) (Tables 1, 2). The uterus was bicornuate and bipartite, large relatively comparative with other stages of development, pinkish to whitish, a hollow musculomembranous tubular elongate cylindrical organ with thick wall, composed of two uterine cornuae appear ram horns shape structures, large quadrilateral corpus uteri (body of uterus) and narrowing cervix uteri (neck of uterus) (Figure 4). The uterus was located in the pelvic cavity posterior-ventrally to the right and left kidneys, related

dorsally to rectum, ventrally to the urinary bladder and covered by the pelvic peritoneum at the level of 6th or 7th lumbar vertebrae. The all-gross morphological parameters of uterus mentioned in (Tables 1, 2). In cats, the uterus was a Y-shape, with a long uterine horn about 3-4 cm, in length and a short body 0.5 to 1 cm, suspended in the pelvic cavity by two ligaments, the broad ligament and the round ligament (Elbably *et al.*, 2020).

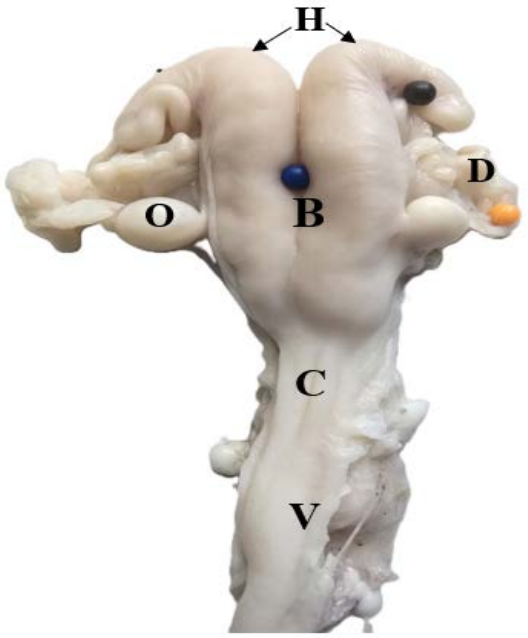

Figure 4: Photograph shows at 8 months old (H) uterine horns appear ram horns shape, large. (B) uterine body, narrowing (C) uterine cervix (V) vagina, (D) oviduct, (O) ovary.

The uterine horn and body contain numerous structures called caruncles, which are elevated fleshy thickenings of the mucosa with an oval to quadrilateral form, are present internally within the horns and the body of the uterus. The midpoint and caudal third of the uterine horns contain four rows of uniformly spaced caruncles. Caruncles become less noticeable and irregularly arranged towards the high point or cranial one-third of the horn. Those of the uterine body are fewer in number and arranged irregularly, resembling mushrooms with long, spherical prominences (Figure 5). In goats, the caruncles within the body of the uterus are irregularly arranged and less numerous. Between the caruncles were the uterine mucosal folds at (8) months (Abiaezute *et al.*, 2018). The uterine cervix has (5-6) long folds as ring with external and internal uterine opening, the first open caudally and internal open cranially, and the external opening is directly connected to the first fold as an integral part of it, and internal open connect with last cervix fold as an integral part of it (Figure 5). In camels, the length of the cervix is about (1.2±0.08) and the width (0.8±0.03) cm, in 8 months old sheep cervix had definite transverse folds (muscular annular rings) (Jaji *et al.*, 2022).

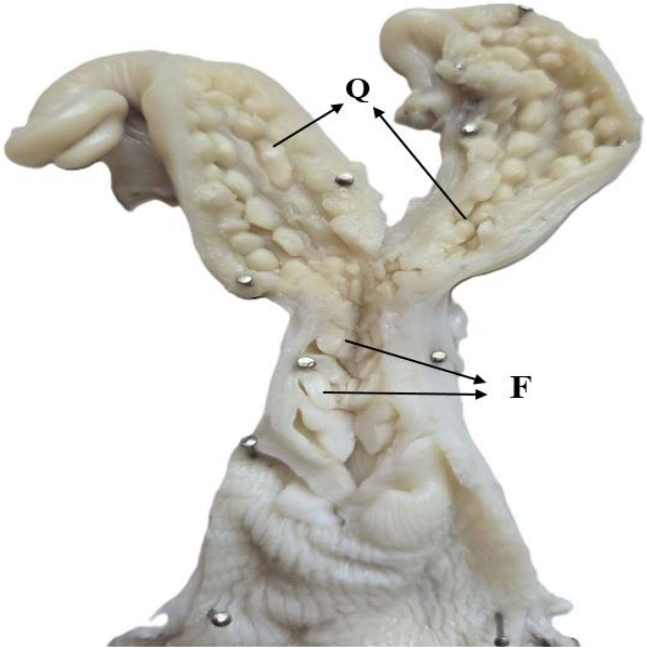

Figure 5: Photograph shows at 8 months old the internal uterine horns and body contain (Q) caruncles, (F) folds of uterine cervix.

Table 1: Weight and length of sheep uterus in different periods of development (postnatal stages).

Total length of uterus (mm)	Uterine cervix length (mm)	Uterine body length (mm)	Uterine horn length (mm)	Uterus weight (gram)	Parameter/ Day
59.3±1.324C	16.2±0.254C	12.1±0.363C	31±0.707C	7.60±0.291B	3 months
86±1.767D	23.6±0.509D	22±0.749D	40.4±0.509D	18.2±0.583C	4 months

Values represent mean ±S. E. Different capital letters indicate significant differences (P≤ 0.05) between various age groups after birth.

Table 2: Some morphometric parameters of the uterine horne, uterine body and uterine cervix in different periods of development (postnatal stages).

Uterine cervix thickness (mm)	Uterine cervix width (mm)	Uterine body thickness (mm)	Uterine body width (mm)	Uterine horn diameter (mm)	Parameter/ Day
5.520±0.259C	9.58±0.259C	6.46±0.186C	11.78±0.274C	5.540±0.188B	3 months
9.410±299D	12.18±0.239D	8.54±0.195D	17.6±0.753D	13.51±0.23C	4 months

Values represent mean ±S. E. Different capital letters indicate significant differences (P≤ 0.05) between various age groups after birth.

Histologically, from the current study revealed that the uterine wall, exhibit more growth and development consistent with advanced stage, The walls of both regions composed of three visible and well developed three layers: endometrium (mucosa)), myometrium (muscle) and perimetrium (serosa) (Figures 6, 7). The lumen of the horn star or X star-shape. At the same time, the body is irregular in shape, because growth and development of the caruncle toward the center causes a narrowing of the lumen of which is almost completely closed. In contrast, the lumen of the uterine cervix closed with numerous developmental folds of different sizes and length (Figure 6). In adult sheep, the wall of uterine body and horns consists of three zones: perimetrium, myometrium and endometrium. The endometrium epithelium lining may be stratified or pseudostratified, contain uterine glands that may be coiled, distributed into the lamina propria, and inter glandular lamina and basal lamina (Shehan *et al*., 2019).

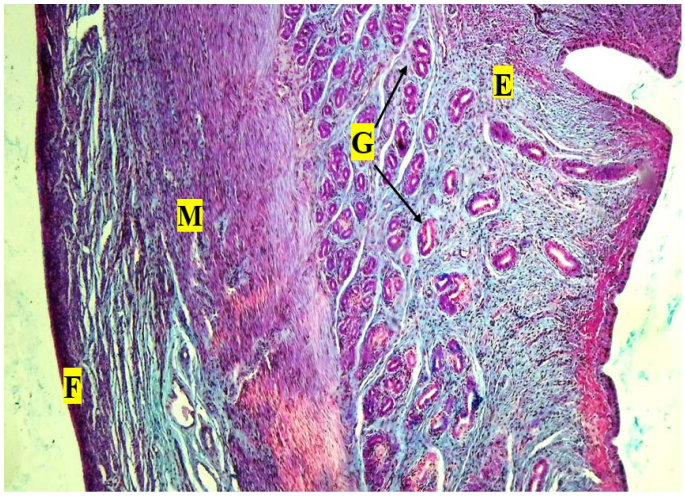

Figure 6: Histological cross section explain uterine body layer at 8 months old; (E) endometrium, (M) myometrium, (F) (perimetrium, (G) glands (allusion blue. 4X).

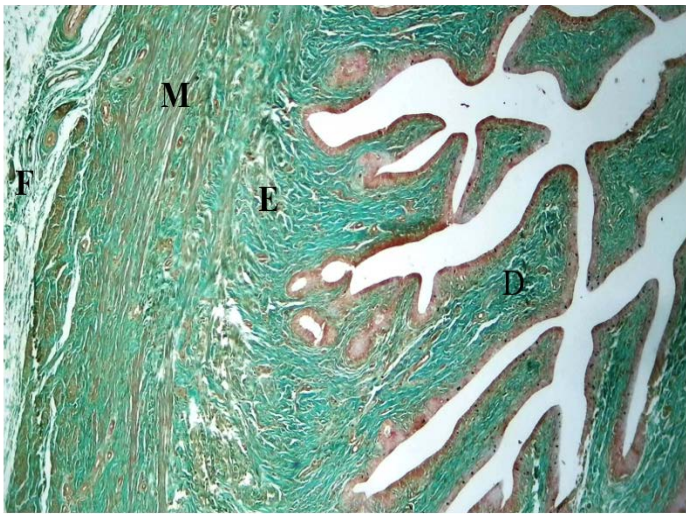

Figure 7: Histological cross section at 8 months old explain uterine cervix layer; (E) endometrium, (M) myometrium, (F) (perimetrium, (D) folds (Masson's trichrome stain. 4X).

The first, the endometrium of the uterine horn and body is composed of simple columnar epithelial tissue with single layer of cells and is not densely packed. It contains elongated, large nuclei that are light in color, where invagination of the epithelium inside the submucosa gives rise to numerous uterine glands. The endometrial glands multiple and vary in size, within sub-mucosa, almost simple, tubular glands formed as invaginations of the endometrial lining epithelium, lined by columnar epithelium with central elongated nuclei. The second layer, the myometrium layer comprises two layers: The inner longitudinal layer and developmentally primitive outer circular layer of smooth muscle. The inner longitudinal layer, in its inner third contained one vascular development layer (*Stratum vascular*), which was thick composed of muscle bundles arranged in longitudinal form, Collagen fibers were present in between the smooth muscle bundle. The third layer, the perimetrium layer thin contains loose connective tissue layer covered by mesothelial layer. Comprised of collagen fibres, reticular fibres connect areas with ligament that contain large blood vessels and nerves (Figures 6, 7). In goats, the uterine wall consists of three zone; inner mucosa, middle muscular, and outer serosa. The Endometrium lining by simple columnar epithelium, contain uterine glands, folds that close uteine lumen. The myometrium composed of two layers: The inner circular and outer longitudinal layer of smooth muscle. The perimetrium composed of thin layer of elastic fibres, containing muscle cells, blood vessels and lymph (Prank *et al*., 2024).

CONCLUSIONS AND RECOMMENDATIONS

All uterine biometric parameters (length, width, thickness) increased with the lambs' age, showing significant variation during postnatal development. At 2–3 months, the uterine wall consisted of four layers: epithelium, submucosa with lamina propria, muscular layer, and tunica serosa. The epithelium, particularly in the uterine horn, was composed of loosely packed columnar cells with large, light-staining central nuclei. By 8 months, the uterine wall showed three layers: endometrium (mucosa), myometrium (muscle), and perimetrium (serosa). The endometrium featuring simple columnar epithelium and numerous uterine glands formed by epithelial invaginations. Further studies using light and electron microscopy, as well as immunohistochemistry, are recommended to explore uterine development in different species and stages for comparative insights.

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NOVELTY STATEMENT

The present report and findings can advance several aspects of the uterine development and provide several markers to consider during practical assessment of animal health and production potential.

AUTHOR'S CONTRIBUTION

Mohammed Hamiza Hamid and Jafar Ghazi Abbas Al-Jebori confirm that they contributed equally to the paper.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The experimental procedures were approved by the Institutional Animal Ethics Committee and conducted at the Veterinary Medicine College, Al-Qassim Green University, Iraq. Ethical regulation of this work was assigned by the university research committee and adhered to the guidelines of the American Veterinary Medical Association.

Conflict of interest
The authors have declared no conflict of interest.

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