



Morphological and Histological Analysis of the Uterus in Adult Female Goats for Understanding Reproductive Health and Function

Rusul Hazim Al-darajee ^{1*}, Masarat S. Al-Mayahi ¹

Abstract

Background: Optimizing goat reproduction is essential for productivity, influenced by factors like climate and breed. The uterus, crucial for fertilization and parturition, lacks detailed study despite its significance. This study aimed to identify and distinguish variations in the histomorphological and histochemical characteristics of the uterus in female local goats. **Methods:** Ten samples of female local goats were purchased from various butchers and abattoirs in Al-Anbar and Al-Falluja during October 2022. Uterine samples were fixed in 10% neutral buffered formalin and Bouin's solution for histochemical staining. Sections of 6 µm thickness were stained with hematoxylin-eosin, Masson's Trichrome, Alcian blue (pH 2.5), and Periodic acid-Schiff (PAS) stains. **Results:** Gross examination revealed a bicornuate uterus with distinct anatomical features, including short body and long horns tapering and lying parallel to each other. Microscopic analysis exposed significant structural alterations in the uteruses of adult goats, particularly in the development of endometrial glands and the thickness of the myometrium. Histochemical staining showed intense PAS reactivity in

the endometrium. **Conclusion:** These findings showed valuable insights into goat reproductive anatomy, comparable to other ruminants, and shed light on uterine functionality. The absence of caruncles in the cranial uterine horn and mucopolysaccharides presence underscore significant reproductive mechanisms. This study contributes to understanding goat reproductive physiology, with implications for breeding and reproductive health management.

Keywords: Goat reproduction, Uterus morphology, Histomorphological analysis, Histochemical staining, Reproductive health

Introduction

The goat is considered one of the essential farm animals, playing a significant role in the economies of various countries, including Iraq. Goats contribute to agricultural productivity through their ability to provide milk, meat, and skin for leather and fibers (Ali and Ibrahim, 2019; Eesa, 2016). Additionally, goats serve as valuable subjects for study and biological research.

Reproduction in goats is of paramount importance to caretakers due to their high productivity and capacity to produce numerous offspring per breeding cycle (Amin, 2009; Jansen and van den Burg, 2004). Various factors influence goat reproduction, including climate, physiological condition, breed, photoperiodicity, breeding method, and the presence of a buck (Poyam et al., 2011). The mating season typically begins in autumn and extends into winter, while anestrus occurs during spring and summer (Brunet et al., 2011). Analysis of slaughterhouse samples provides valuable

Significance | The study on goat uterine morphology and histology offers insights into reproductive health and breeding management for sustainable agriculture.

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insights into reproductive organ anomalies (Palmieri et al., 2011), which are crucial for diagnosing, managing, and treating infertility and low production (Ogunbodede et al., 2014).

Goats are known to be seasonal breeders, with their breeding system influenced by factors such as photoperiod (Fatet et al., 2011; Muhson and Dawood, 2023). Typically, the breeding season for goats begins in the fall and ends in winter, with anoestrus prevailing in the spring and summer (Brunet et al., 2011).

The female reproductive system of goats comprises ovaries, uterine tubes, uterus, and vagina, all of which contribute to the fertility capacity of the females (Alwan, 2014; Hussin, 2011; Reece and Rowe, 2017). A detailed description of the goat uterus reveals it to be the site of fertilization and fetal development, consisting of a body, cervix, and two horns. Different species exhibit variations in the proportions of these uterine components, with the mare having the largest body, followed by the cow and sheep with less extensive bodies, and the sow and bitch with smaller bodies.

Additionally, the uterus secretes or transports crucial components essential for embryo viability and plays a significant role in regulating the estrus cycle during development (Filant and Spencer, 2014; Kumar et al., 2013). Moreover, it serves as a pathway for fetus delivery during parturition (Budras and Habel, 2003; Spencer et al., 2005). Uteruses are vital organs for the health of future offspring and for reproduction. Spencer et al. (2012) describe the uterus's vital role in reproductive processes, including the generation of PGF₂, a luteolysin important for ovarian cyclicity in local animals, spermatozoa transportation, storage, and maturation, preservation of an optimal environment for embryo growth and development, and transportation of the conceptus during childbirth.

The present study aimed to investigate the histomorphological and histochemical structures of the uterus (uterine horns and uterine body) in adult female goats, providing a better understanding of data on the morphology, macromorphometric, and micromorphometric aspects of the uteruses in adult goats.

Materials and Methods

Sample collection:

To conduct this study, ten samples of female local goats were acquired from Iraqi butchers and abattoirs in October 2022, with options available in the cities of Al-Anbar and Al-Falluja. The goats had body weights ranging from 20 to 22 kg and ages ranging from eight months to two and a half years. A midline abdominal incision was performed by the butcher to expose the tissues within the peritoneal cavity. Subsequently, the samples were transported to the departments of anatomy and histology in veterinary medicine.

Sample Preparation:

The uterus, including its various areas such as the horns, body, and cervix, was dissected out and trimmed of extraneous tissues. Macro morphometric measurements, such as the weight and length of the

uterus (including uterine horns, body, and cervix), were determined using a digital vernier caliper and a sensitive weighing balance, and the results were listed in a table. Subsequently, all parts of the uterus were washed with normal saline and then immersed in 10% neutral buffered formalin for 72 hours.

Tissue Processing:

To prepare for histochemical staining, a few samples were fixed using Bouin's solution. Following fixation, the samples underwent dehydration through a series of ascending concentrations of ethyl alcohol (70%, 80%, 90%, and 100%), each for 2 hours, and were then cleared with xylene for 30 minutes. Subsequently, the samples were permeated with paraffin wax (58–60 °C) and embedded in paraffin blocks (Al-Saffar and Al-Ebbadi, 2019; Al-Saffar and Almayahi, 2019; Reshag and Khalaf, 2021). Sections of six microns thickness were obtained using a rotary microtome.

Histochemical:

Both familiar and specific stains were utilized to stain the tissue sections. For instance, the Hematoxylin and Eosin (H&E) stain was applied following these steps: deparaffinization for 10 minutes, rehydration with alcohol (starting with 95% alcohol and then 70% alcohol), washing with distilled water, staining with hematoxylin solution for 8-10 minutes, washing with tap water, differentiation with 1% alcohol, further washing, counterstaining with eosin Y, dehydration, clearing with xylene, and finally mounting using a xylene-based mounting medium.

The Masson Trichrome (MTC) staining procedure involves several steps: first, deparaffinizing and rehydrating the sample using a series of alcohol concentrations (100%, 95%, and 70%). Then, the sample is washed with distilled water and fixed in Bouin's solution for 1 hour, followed by rinsing with tap water. Next, the color and stain are removed by using iron hematoxylin for 5-10 minutes, followed by rinsing with tap water. The sample is then immersed in distilled water for 10 minutes and stained with Biebrich fuchsin solution for 10-15 minutes, followed by rinsing with distilled water. Phosphomolybdic acid is applied to distinguish the sample, and after 10-15 minutes, when the collagen is no longer red, it is transferred to the aniline blue solution. The sample is rinsed with distilled water and 1% acetic acid for 5-10 minutes, followed by rinsing for 2-5 minutes with distilled water. Moisture is removed using 95% ethyl alcohol, and finally, the sample is immersed in xylene for clarity and mounted in a resinous medium.

For Alcian blue (AB) (pH 2.5) stain, the sections are immersed in distilled water and stained with AB for 15 minutes, washed in tap water for 2 minutes, and rinsed in distilled water. The sections are then stained with Hematoxylin for 1 minute, followed by differentiation using acid alcohol and staining nuclei with eosin and hematoxylin in Scott's tap water. After washing, dehydrating, cleaning, and mounting, the process is complete.

For Periodic acid schiff (PAS) stain, the slides are deparaffinized and rehydrated in distilled water, treated with 0.5% Periodic acid for 5 minutes, and rinsed with distilled water. Schiff reagent is applied to the slides, followed by microwaving for 45-60 seconds. After washing in tap water and counterstaining with hematoxylin for 3 minutes, the slides are rinsed in tap water, treated with blue hematoxylin, and rinsed in distilled water. Finally, the slides are dehydrated with alcohol, cleared, and coverslipped. These staining techniques are essential for identifying secretion cells of acidic and neutral mucopolysaccharides in histological samples, with histological slides photographed using the USB 2.0 digital copy system (Scope Image 9.0) equipped with image processing software.

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Two-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. Also independent t test was used to identify the significant difference between two groups. $P < 0.05$ is considered statistically significant.

Results:

Morphological results:

Uterine horn:

The gross examination of the present research revealed a bicornuate uterus with a Y-shaped structure, consisting of two separate uterine horns, a body, and a single cervix, varying in color from reddish-pink to pale gray (Fig. 1). Additionally, the uterine horn of the studied goat exhibited twisting and longer diameters compared to the consistent uterine tubes, identified as the right and left long tubes extending from the uterotubular ram's horn. Starting with a narrow tube at its junction with the uterine tubes, the horn progressively enlarged in diameter in a corkscrew manner resembling a ram's horn (Fig. 1). A ventral and dorsal translucent sheet, the intercornual ligament, connected the two horns at the caudal third of the uterus as they accessed the body of the uterus (Fig. 2). The horns began as narrow tubes at their junction with the uterine tubes and gradually increased in diameter as they spiraled caudally, resembling rams' horns. Morphometrical measurements such as lengths and weights were recorded in Table 1, illustrating the lengths and weights of the uterine horns and body of adult goats. For instance, the length of the uterine horns was right 11.52 ± 0.88 cm and left 11.48 ± 0.43 cm, with corresponding weights of right 15.27 ± 1.96 gm and left 16.79 ± 3.05 gm. It was observed that there were no significant differences between the right (R) and left (L) locations for all organs studied, indicating consistency in measurements across both sides.

Uterine body:

According to the findings, the uterine body is relatively short and connects with a stiff and fibrous cervix, significantly differing in

consistency from the rest of the uterus. The internal uterine horns exhibit bifurcation, with two openings from the uterine body to the horns (Fig. 3). The body of the uterus extends cranially from the tips of entry of the duplex uterine horns to the compressed cervix caudally, constituting only a small portion of the overall uterus. Caruncles, elevated fleshy thickenings of the mucosa with an oval to quadrilateral form, are present internally within the horns and the body of the uterus (Fig. 4). 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 (Fig. 5). Those of the uterine body are fewer in number and arranged irregularly, resembling mushrooms with long, spherical prominences (Fig. 6). The uterus exhibits a short, well-developed body, with a length of 3.33 ± 0.42 cm and weight of 3.37 ± 0.41 gm. In adult goats, the lengths and weights of their left and right uterine horns displayed statistically significant increases ($P < 0.05$) (Table 1). Morphometrical measurements such as lengths and weights of the uterine body were detailed in Table 1, with the length of the uterine body measured as 3.33 ± 0.42 cm and the weight as 3.37 ± 0.41 gm.

Histology of Uterus (uterine horns and uterine body):

Microscopic examination of the uterus of adult female goats revealed three distinct parts: uterine horns, body, and cervix, each comprising three different tunics: endometrium, myometrium, and perimetrium, observed from internal to external layers. The histological architecture of the uterine horn and body were comparable, with several caruncles protruding above the mucosal surface within their lumens. These caruncles were divided from each other by intercaruncular areas projecting into folds separated by slender grooves (mucosal folds) (Fig. 6). In the present study, the uterine wall is composed of endometrium, myometrium, and perimetrium (Fig. 6, 7). The endometrium comprised simple columnar to pseudostratified columnar epithelium (Fig. 8), consisting of two structurally distinct zones: the functional zone, a superficial layer shed during estrus, and the basal zone, a thin, deep layer remaining after shedding of the functional layer (Fig. 9). In contrast, Agrawal and Laloraya (1978) observed a thicker perimetrium during the follicular phase in mammals but failed to discover elastic fibers. The tunica serosa exhibited a low level of PAS reactivity, indicating a low level of bound lipids and carbohydrates. Similar results were reported by Rajput (1995) with Gaddi sheep, where blood vessel intima exhibited Alcian blue reactivity, and a very weak response to cholesterol was observed.

The lining epithelium was characterized by multiple invaginations of uterine glands into the lamina propria submucosa, visibly filled with blood and dispersed throughout the underlying loose connective tissue containing lymphocytes (Fig. 10). These glands were lined by simple low columnar epithelial cells (Fig. 11). In the absence of lamina muscularis mucosae, the cellular connective

Table 1. The Gross measurement of the left and right uterine horns of a adult local goat (Mean ± SE)

Organ	Adult goat
Length of Uterine Horne (cm)	Right 11.52±0.88 Left 11.48±0.43
weight of Uterine Horne(gm)	Right 15.27±1.96 Left 16.79±3.05
Length of Uterus body(cm)	3.33±0.42
weight of Uterus body (gm)	3.37±0.41

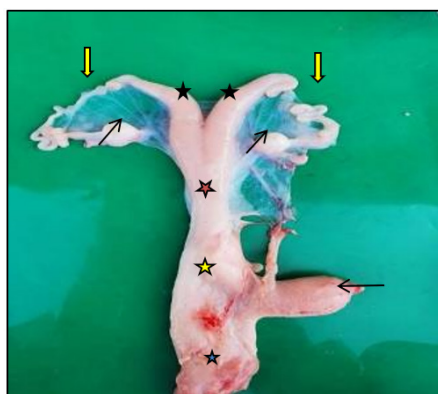


Figure 1. the anatomy of the female Reproductive system in adult goat: vagina (blue star), urinary bladder (orang arrow), cervix (yellow star), urinary body (red star), uterine horn (black star), oviduct (yellow arrow), ovary (red arrow)

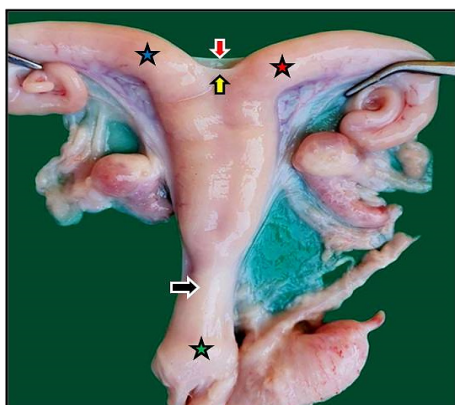


Figure 2. The parts of the uterus and intercornual ligaments (red & yellow arrows), blue star: right uterine horn, red star: left uterine horn, black arrow: uterine body, green star: cervix

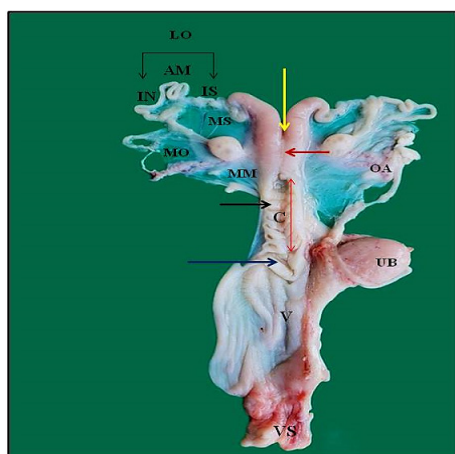


Figure 3. The female Reproductive system in local Iraqi goat. It showed left uterine horn (LUH), left oviduct (LO), isthmus (IS), ampulla (AM), infundibulum (IN), mesosalpinx (MS), mesovarium (MO), mesometrium (MM), ovarian artery (OA), left ovary (red star), cervix (C), vagina (V), vestibule (VS), urinary bladder (UB), dorsal intercornual ligament (yellow arrow) and external uterine bifurcation (red arrow)

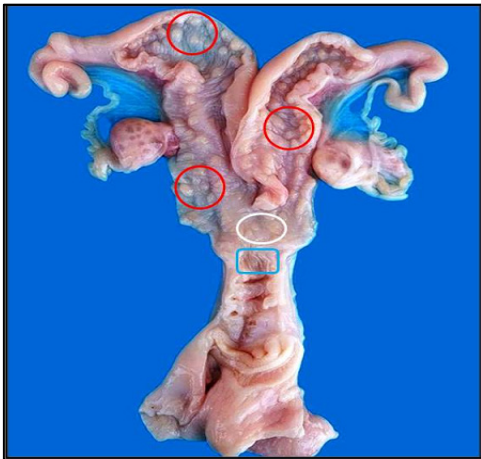


Figure 4. shows the red circle: the caruncles in the uterine horns, the white circle: the caruncles in the uterine body, blue square: small folds ; in Iraqi local goat.

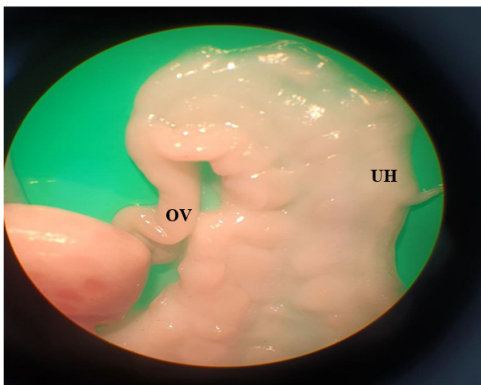


Figure 5. The picture show: ovary (red star), oviduct (ov), caruncles (blue circle), uterine horn(UH).

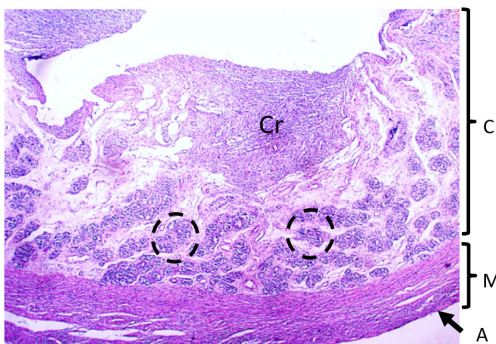


Figure 6. show the microstructure of the mature left uterine horn: endometrium (C),myometrium (M),perimetrium(A),uterine glands (dashed circle),caruncle (Cr) 40X, H&E

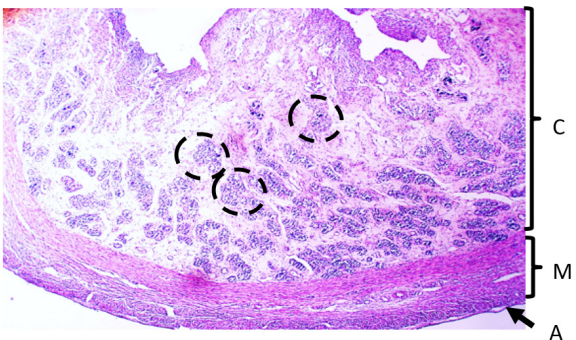


Figure 7. show the microstructure of the mature left uterine horn: endometrium (C),myometrium (M),perimetrium (A),uterine glands (dashed circle), 40X, H&E

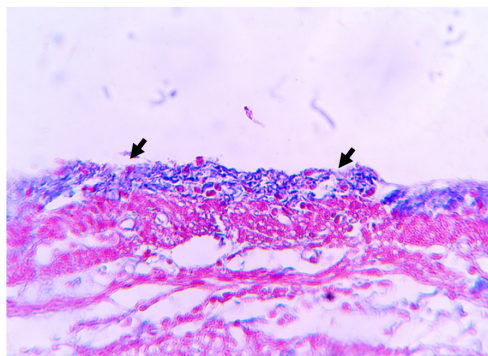


Figure 8. show the microstructure of the premature left uterine horn (mucosa) : uterine horn epithelium (pseudostratified columnar type), (arrows), 400X, H&E

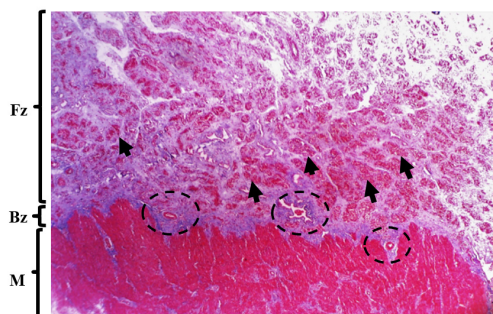


Figure 9. show the microstructure of the mature uterus (endometrium) : the functional zone (Fz),the basal zone (Bz), the myometrium (M) ,the coiled uterine glands (arrows), uterine arteries (dashed circle), 40 X, MTS (Masson's trichrome stain)

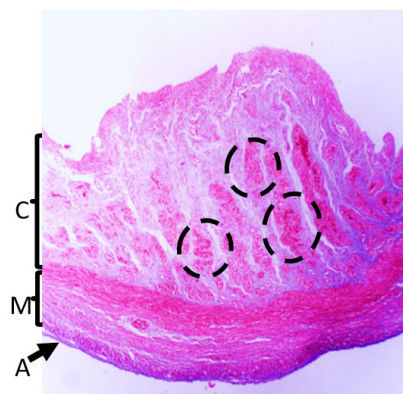


Figure 10. show the microstructure of the mature left uterine horn: endometrium(C) ,myometrium (M) , perimetrium (A) , uterine glands (dashed circle) , 40X, MTS

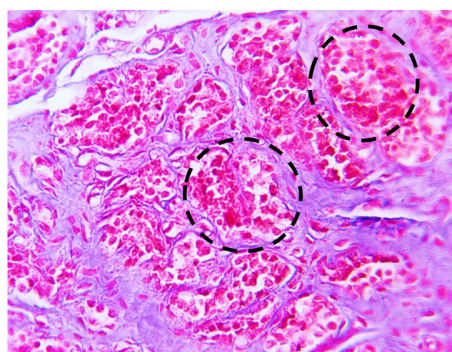


Figure 11. show the microstructure of the mature left uterine horn (mucosa) : uterine glands (dashed circle), 400X, MTS

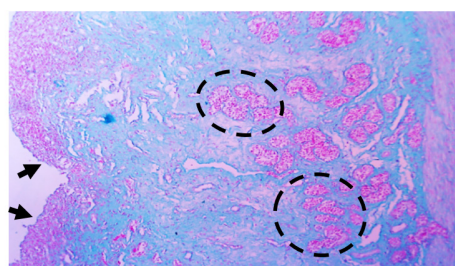


Figure 12. show the microstructure of the mature left uterine horn (mucosa) : uterine horn epithelium (pseudostratified columnar type), (arrows), uterine glands (dashed circle), both response intensely to PAS stain 100X, PAS\AB

tissue of the lamina propria mixed with the connective tissue of the submucosa. The endometrial glands were surrounded by loose connective tissue, primarily composed of collagen fibers, which gradually increased in proportion towards the tunica muscularis, forming the lamina propria submucosa (Fig. 11). The lamina propria submucosa contained densely spread glands in the deep zone and fewer in the superficial zone. The lining epithelium exhibited a positive periodic acid-Schiff (PAS) response in its basement membrane, suggesting the presence of mucopolysaccharides (Fig. 12). Conversely, significant PAS response was observed at the lining epithelium in goats by Joshi et al. (1983) and Roy and Saigal (1986). In the current investigation, the uterine horns and body were unaffected by mild lipid and cholesterol response experimentally at their highest and basal boundaries of the inner surface epithelium. Along the uterine horns at their free edges, the perimetrium was a fluffy layer of loose connective tissue enclosed by simple squamous mesothelium at their attached borders to the broad ligament, covered by adventitial connective tissue. Collagen fibers constituted the majority of the fibroarchitecture in the perimetrium, with fewer reticular and elastic fibers. The tunica serosa in the uterine horn and body exhibited greater thicknesses (Fig. 10).

Discussion:

Morphological uterus(uterine horns):

Typical gross findings revealed that goats possess a bicornuate uterus, characterized by highly expandable tubes with small bodies, similar to other documented types such as pigs (Nickel et al., 1979). However, it differs from animals like camels, which have bipartite uteruses. The goat uterus comprises three parts: two horns, a body, and a cervix, consistent with previous studies in ruminants (Budras and Habel, 2003; Dyce, 2002). The left and right horns measured an average of cm and cm, respectively. While these findings differ from Dyce (2002), they align with other studies (Banerjee, 2018; Frandson et al., 2009; Ommer, 1995; Pineda and Dooley, 2003; Sisson, 1914). Similar to the present results, the uterus of female donkeys exhibits a Y shape with diverging horns and a short body (Renner-Martin et al., 2009), while Khaton et al. (2015) recorded in cows a uterus with a Y-shaped hollow organ, anteriorly separated into two horns, similar to that of goats.

Uterine body:

The results indicate that the average length was cm. These findings align with research conducted by Banerjee (2018), Dyce (2002), Frandson et al. (2009), Getty and Sisson (1975), and Ommer (1995). However, they differ from the findings of Pineda and Dooley (2003), who reported that the length of the uterus could reach 10 to 12 cm. The cervix extended from the body, connected by the broad ligament.

Histology of uterus (uterine horns and uterine body):

The uterine horns, as documented by Bhattacharya and Saigal (1984), Singh and Prem (1990), Uppal and Roy (2002) in buffalo, and Poyam et al. (2011) in goats, exhibited an endometrium consisting of simple columnar to pseudostratified columnar epithelium. Similar findings were observed in buffalo by Sundaravadanan and Venkataswamy (1973) and Sweta et al. (1993), as well as in goats by Poyam et al. (2011), who reported pseudostratified columnar epithelium. The lining epithelium, characterized by multiple invasions of uterine glands, was reported similarly in other domestic animals by Dellmann (1998), in Gaddi goats by Suri and Sharma (2004), and in exotic species by Trautmann and Fiebiger (1957). The endometrial glands were surrounded by loose connective tissue, a feature also noted in goats by Singh and Prem (1990), buffaloes by Uppal and Roy (2002), and Gaddi goats by Suri and Sharma (2004), as well as described by Shalini (1997) in Gaddi goats. The myometrium, composed of inner circular and outer longitudinal sheets of smooth muscle fibers with numerous blood vessels interspersed between them, exhibited nearly identical thickness in both layers, consistent with research on other domestic animals by Dellmann (1998), Trautmann and Fiebiger (1957), and Uppal and Roy (2002). In contrast, Agrawal and Laloraya (1978) noted thicker perimetrium during the follicular phase in mammals but failed to detect elastic fibers. The tunica serosa showed low PAS reactivity, indicating a low level of bound lipids and carbohydrates, with similar findings reported by Rajput (1995) in Gaddi sheep. Blood vessel intima exhibited Alcian blue reactivity, while a weak response to cholesterol was observed.

Conclusion

The present study has highlighted notable similarities between the macroscopic and microscopic features of the uterus in local adult goats compared to both small and large ruminants. Specifically, the investigation revealed the absence of caruncles in the cranial one-third of the uterine horn and elucidated the characteristic histological layers of the uterine wall, namely the endometrium, myometrium, and perimetrium, along with the process of uterine gland formation. Additionally, the epithelium of the myometrium layer and uterine glands exhibited a robust reaction for PAS, indicating the presence of mucopolysaccharides that likely play a crucial role in providing nutrition for the developing fetus within the uterus.

Author contributions

R.H.A., M.S.A. conducted study design, analyzed data, wrote and drafted the manuscript.

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Authors were grateful to their department.

Competing financial interests

The authors have no conflict of interest.

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