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Histomorphology Of One-Humped Dromedarian Uterus during three Pre-Natal Developmental periods

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ABSTRACT

Camelus dromedarius (Dromedary or one-humped camel) belonging to the genus *Camelus*, is recently receiving great research attention due to the increasing importance of this species as model for scientist in various discipline of biological science including reproduction. This study was aimed to evaluate histomorphological horizons during uterine development at three pre-natal stages in dromedary. Twenty-two fetuses were used to study the uterine horns, uterine body and uterine cervix histomorphological horizons in three trimesters of the prenatal development. Our results revealed that the uterine horns typically had three-layered tunics, which became more distinct across the trimesters, with pseudostratified epithelial lining. In the first trimester, the right and left uterine horns had mescenchyme cells widely distributed within the connective tissue stroma. The second trimester had a slight indentation of future endometrial glands on the right uterine horn while the left horn had a distinct invagination on the epithelial lining. The organization of canalized endometrial glands at the third trimester were marked and more on the left than the right uterine horn with both primary and secondary longitudinal folds. Our findings are similar to those of other mammals in developmental strides. Our results provide new information on the histological features of the uterus in female *Camelus dromedarius*, an insight to reproductive management of this species of mammals.

Key words: Dromedary, Histomorphology, developmental horizons, Prenatal, Uterus,

INTRODUCTION

Dromedary also called one-humped camel (*Camelus dromedarius*) is a member of the two species that belongs to the genus *Camelus*; the other being the Bactrian, also called two-humped camel (*Camelus bactrianus*)[1]. Reports have shown that the *Camelus dromedarius* has over the decades been a very useful animal to humans[2], and have therefore been described as a strategic animal for the exploitation of the desert and abhorrent arid including the semiarid lands[3]. In Nigeria, and especially in the northern part, camel is currently being rated among the major sources of animal protein[4]. Also, camel play great roles in the social life and economy of nomadic tribes providing their major source of food security[2, 5]. Other usefulness of camel includes its role as means of transportation[2, 6], source of wool[2] and in races and tourism[7] among others. Interest on the reproductive efficiency of *Camelus dromedarius* is recently receiving greater attention, targeted to better performance[1-2, 6, 8-9]. Reports have previously shown that the reproductive performances of *Camelus dromedarius* is relatively lower compared to other domestic animals[1-2, 10], and due mainly to the many complexities in their genetic make-up and environmental constraints[11], characterized by longer gestation periods, late reproductive perception, longer calving periods and mortality in the young class of this animals[2, 7]. In Africa generally, *Camelus dromedarius* forms a very important part of their livelihood and critical in their economy development, contributing significantly to food security[2, 12] hence the importance of their reproductive performance.

Studies have focused on anatomical descriptions of the adult females genital organs[13-15], including the ovaries and uterus[16] in camels. Also, anatomical studies on the female prenatal reproductive system especially the ovaries have been documented by Abdel-Elrazik et al.[17] and Oyelowo *et al.*[18]. There is a dearth of information on the pre-natal developmental histomorphological horizons and organization of the uterus of dromedary (*Camelus dromedarius*). Adult uterine function could be affected by these development horizons and organisation, hence this study.

MATERIALS AND METHODS

Samples collection, sexing and aging of foetuses: This study received ethical approval from the University of Abuja Ethics Committee for Animal Use (UAECAU/2022/0009) and in accordance with ethical standard of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023) and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

A total of twenty-two fetuses were sourced as accidental findings from uteri of slaughtered pregnant Camels at the Sokoto and Kano Municipal abattoirs over a period of six months were used for this study. The reproductive tracts of slaughtered female camels were thoroughly examined and palpated for presence of fetuses by veterinarians. Gravid uterus were exteriorized and fetuses were removed. All samples were located at the left uterine horns[18]. Grossly, there uterine horn ran from the oviduct to the bifurcation into the uterus. The female fetuses were then identified by the absence of an anogenital ridge[2]. The fetuses were then aged using the formula; X = CVRL (cm) + 29.66/0.366. Where: X = Age in days, and CVRL = Crown Vertebral Rump Length measured in centimeters[18], and grouped accordingly into trimesters for histological developmental horizons description. The aged fetuses were then assigned into the three (first, second and third) trimester periods of *Camelus dromedarius* pregnancy. Specifically, six were at the first trimester, nine at the second trimester and seven at the third trimester.

Histological Examination of the uterine tissue specimen: The protocol for histological preparations of all samples was according to the methods of Usende *et al.* [19-20] and Igwenagu *et al.* [21] using the normal standard paraffin method of embedding tissue. Briefly, approximately 4–6 mm of proximal, middle and distal regions of uterine tissues were collected. Collected tissues were then dehydrated in graded (increasing) concentrations of ethanol before they were cleared in xylene and embedded in paraffin. Serial sections of 6μm thickness were cut and carefully collected on coated slides for staining with Haematoxylin and Eosin (H&E) and Period Acid Schiff (PAS) for light microscopy[19, 21]. Both H&E and PAS stained slides were viewed and age dependent histological descriptions of the uterus were carried out and photomicrographs taken with Amscope 40X-2500X Digital binocular photomicroscope fitted with a 10MP microscope digital camera (Amscope MU10003B, 10 MP Camera, Version 3.7 software, China).

RESULTS

The uterus of the one humped camel (*Camelus dromedaries*) during the three prenatal development periods studied are grossly divided into three segments; 1) the uterine horn, 2) the body of the uterus, and 3) the uterine cervix.

Histomorphological observations occurring frequently in the uterine horns in the different prenatal stages of uterus development of one humped camel: A transverse section of the uterine horns of the one humped camel showed an oval to round lumen in the three developmental stages studied. Typically, the uterine horns had three tunics (layers) consisting of the endometrium, myometrium and perimetrium; and the endometrium was lined by epithelium. In the 1st trimester (37.44±7.10 days), the endometrial lining of the uterine horn was pseudostratified columnar (Fig. 1 A and B) with no invaginations. However, in the 2nd trimester (71.81±3.81 days), the pseudostratified columnar epithelium began to show sign of invaginations to form the endometrial glands (Fig. 1 C and D). These invaginations were marked and increased in number on the left horn (Fig. 1 E and F) than the right horn (data not shown). Also, the lamina propria in the 2nd trimester showed numerous fibroblasts; a feature that was rarely seen in the first trimester. Concerning the myometrium, prominent spindle shaped cells/fibers were observed in the developing tunica muscularis of the uterine horn of the one humped camel studied in the three developmental periods. In the 3rd trimester, the uterine horns of the developing one humped camel presented numerous endometrial glands compared to the 2nd trimester. These endometrial glands appeared to also increase in size in the 3rd trimester (109.13±10.21 days) compared to the 2nd trimester (Fig. 2 A and B). Interestingly, the left horn had more endometrial glands (Fig. 2 B and C) compared to the right horn. The submucosa had numerous fibroblasts within the loose connective tissue. Also, this submucosa appeared wider in the right than the left horn. When stained with PAS, the connective tissue stroma appeared distinctly spindle-like in shape. Generally, the muscular myometrium was thicker in the right horn than in the left horn. Moreover, the vascular layer was distinct at this stage of development (3rd trimester), and more prominent in the left than the right horn.

Histomorphological observations occurring frequently in the uterine body (corpora uteri) in the different prenatal stages of uterus development of one humped camel: The bipartite uterine body (corpora uteri) increased in size with increase in age of the foetuses (i.e, from 1st to 3rd trimester). Histologically, the inter-cornual septum is the point of the bipartition, and this was noticed from the 1st to the 3rd trimester. The uterine corpus of

the one humped camel in the 1st trimester uniquely presented a pseudostratified endometrial lining without invagination into the lamina propria (Fig. 3 A and B). The lamina propria had numerous fibroblasts. In addition, there was distinct demarcation between the endometrium and myometrium. The endometrial stroma lies underneath the epithelium and appeared densely organised. The outer longitudinal muscle layer of the corpora uteri joined the inner circular muscle layer closely, and wider than seen in the 2nd trimester (Fig. 3 C and D). Also, notice was a thin vascular layer, with few arterioles and venules.

In the 2nd trimester, the uterine body became wider than seen in the 1st trimester. At this stage of development, the endometrial lining was pseudostratified with invaginations into the lamina propria to form endometrial glands similar to what was observed in the uterine horn (Fig. 3 C and D). However, these glands were fewer in numbers and smaller in size when compared to the same stage of development (2nd trimester) of the uterine horn. The lamina propria was also wider in the 2nd trimester of the developing corpora uteri of one humped camel, bearing numerous fibroblasts compared to the 1st trimester.

In the 3rd trimester, the pseudostratified endometrial lining was fully invaginated into the lamina propria and the endometrial glands were completely formed. Although these fully formed glands were less in size and number compared to what was seen in the uterine horn at this period of development. Specifically, the uterine body at this stage of developed was wider than the 2nd and 1st trimester (Fig. 3 E and F). The lamina propria was wider in the 3rd trimester bearing numerous fibroblasts than seen in the 2nd trimester. The same pattern was seen in the inner circular muscular layer. The vascular layer in the 3rd trimester of the developing corpora uteri of one humped camel had few but well-developed arterioles and venules.

Histomorphological observations occurring frequently in the uterine cervix in the different prenatal stages of uterus development of one humped camel: Uterine cervix of the developing one humped camel at the 1st trimester had the uterine mucosa thrown into few primary longitudinal folds. The epithelial lining was simple columnar epithelium. At 2nd trimester, primary longitudinal folds were more, and numerous fibroblasts were present within the connective tissue of the lamina propria (Fig. 4 A and B). The stroma at this stage of development (2nd trimester) were irregularly shaped with abundant ground substances. Also, the inner circular layer of the myometrium was remarkably thick, while the vascular layer was wide with numerous arterioles, venules and lymphatics. There was absence of secondary longitudinal folds in the 1st and 2nd trimesters. The one humped camel uterine cervix at the 3rd trimester had both primary and secondary (few) longitudinal folds. The secondary folds were seen to be larger and wider than the primary. The vascular layers of the myometrium were wider than the 2nd trimester bearing more vessels (Fig.

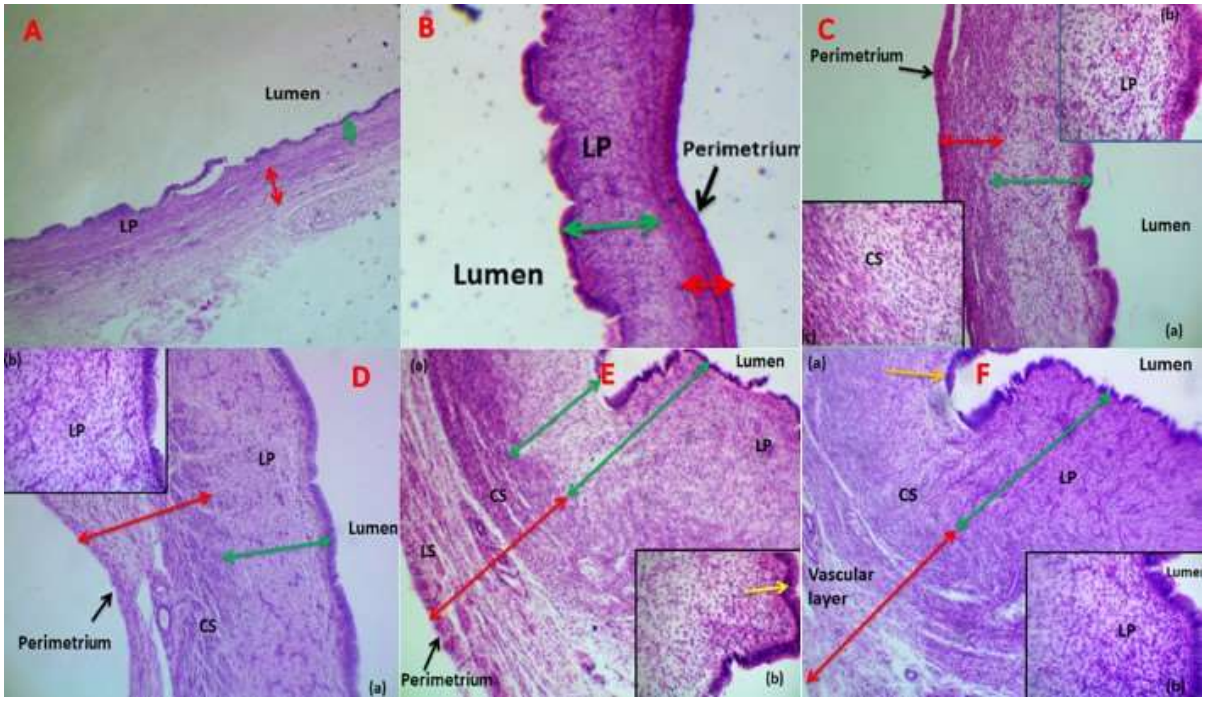

Figure 1: A-Photomicrograph in cross section of first trimester *Camelus dromedarius* right horn indicating myometrium (red arrow), endometrium (green arrow), and LP- lamina propria PAS x40. **B**-Photomicrograph in cross section of first trimester *Camelus dromedarius* left horn indicating myometrium (red arrow), endometrium (green arrow), and LP- lamina propria H & E x40. **C**-Photomicrograph in cross section of second trimester *Camelus dromedarius* right horn indicating myometrium (red arrow), endometrium (green arrow), CS- circular smooth muscle and LP- lamina propria (a) H & E x 40 (b) H & E x 400 (c) H & E x 400. **D**: Photomicrograph in cross section of second trimester *Camelus dromedarius* right horn indicating myometrium (red arrow), endometrium (green arrow), CS- circular smooth muscle and LP- lamina propria (a) PAS x 40 (b) PAS x 400. **E**-Photomicrograph in cross section of second trimester *Camelus dromedarius* left horn indicating myometrium (red arrow), endometrium (green arrow), epithelium (yellow arrow), CS- circular smooth muscle, LS- longitudinal smooth muscle and LP- lamina propria (a) H & E x 40 (b) H & E x 40. **F**-Photomicrograph in cross section of second trimester *Camelus dromedarius* left horn indicating myometrium (red arrow), endometrium (green arrow), endometrial gland (orange arrow), CS- circular smooth muscle and LP- lamina propria (a) PAS x40 (b) PAS x400.

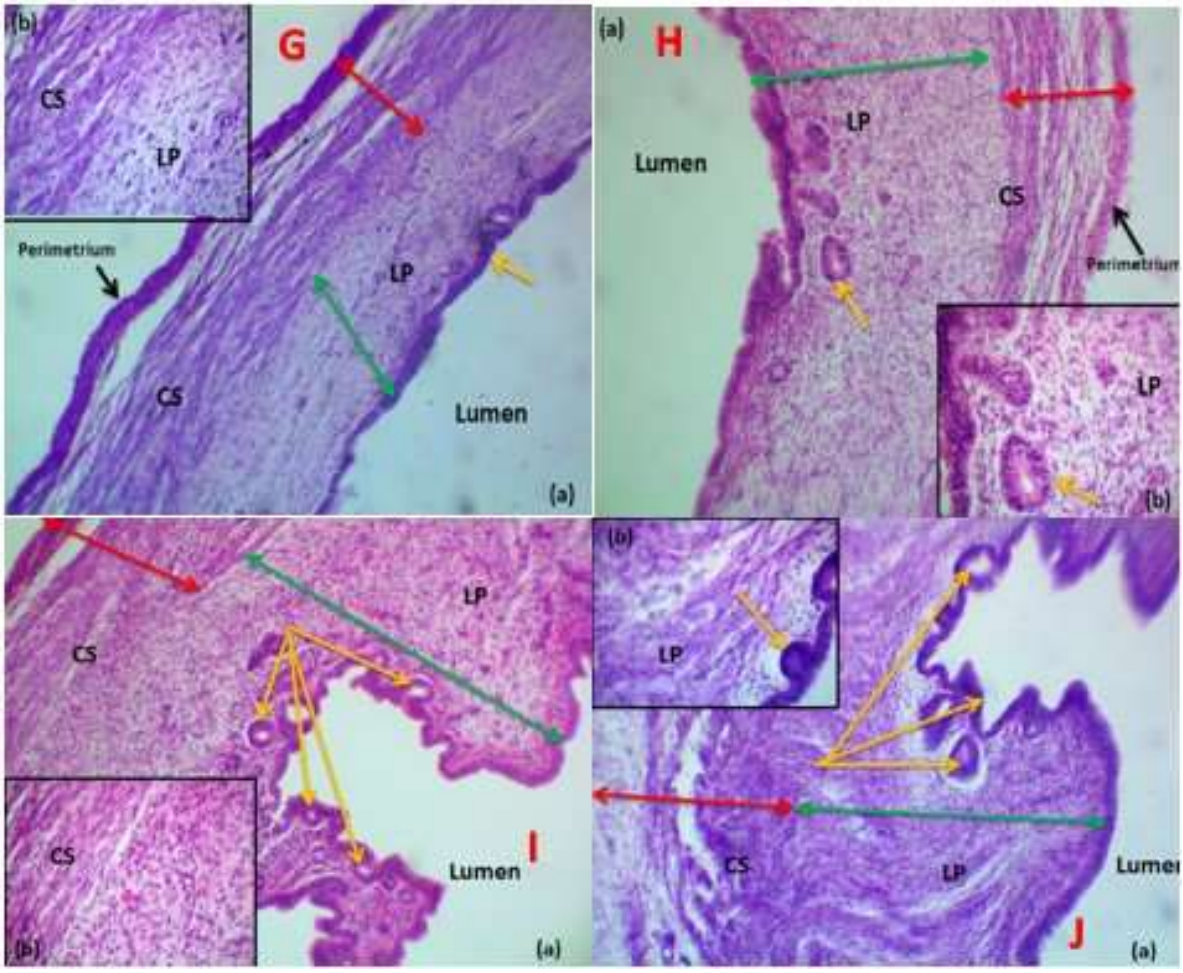

Figure 2: A-Photomicrograph in cross section of third trimester *Camelus dromedarius* right horn indicating myometrium (red arrow), endometrium (green arrow), epithelium (yellow arrow), CS- circular smooth muscle and LP- lamina propria (a) PAS x40 (b)PAS x400. B-Photomicrograph in cross section of third trimester *Camelus dromedarius* right horn indicating myometrium (red arrow), endometrium (green arrow), endometrial gland (orange arrows), CS- circular smooth muscle and LP- lamina propria (a) H & E x40 (b) H & E x400. C- Photomicrograph in cross section of third trimester *Camelus dromedarius* left horn indicating myometrium (red arrow) , endometrium (green arrow), endometrial gland (orange arrows), CS- circular smooth muscle and LP- lamina propria (a) H & E x40 (b)H & E x400. D- Photomicrograph in cross section of third trimester *Camelus dromedarius* left horn indicating myometrium (red arrow), endometrium (green arrow), endometrial gland (orange arrows), CS- circular smooth muscle and LP- lamina propria (a) PAS x40 (b) PAS x400

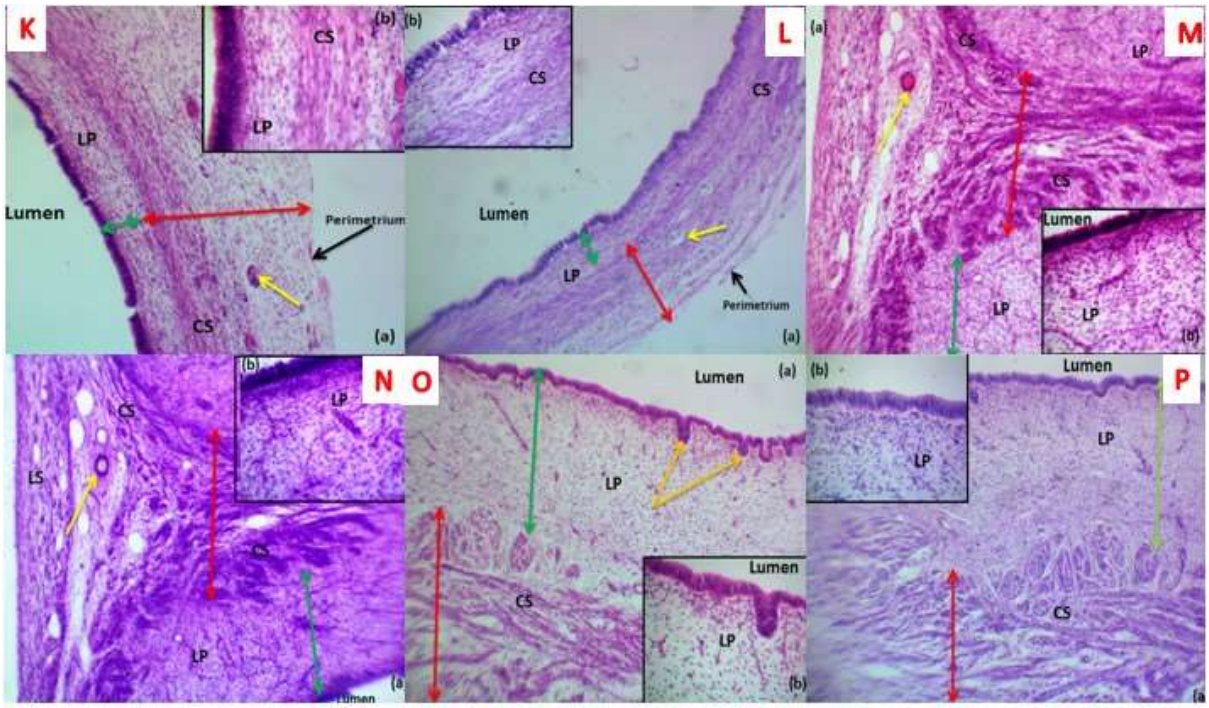

Figure 3: A-Photomicrograph in cross section of first trimester *Camelus dromedarius* uterine body indicating myometrium (red arrow), endometrium (green arrow), arteriole (yellow arrow), CS- circular smooth muscle and LP- lamina propria (a) H & E x40 (b)H & E x400. B-Photomicrograph in cross section of first trimester *Camelus dromedarius* body indicating myometrium (red arrow) , endometrium (green arrow), arteriole (yellow arrow), CS- circular smooth muscle and LP- lamina propria (a) PAS x40 (b) PAS x400. C-Photomicrograph in cross section of second trimester *Camelus dromedarius* uterine body indicating double myometrium (red arrow), endometrium (green arrow), venule (yellow arrow), CS- circular smooth muscle and LP- lamina proprias (a) H & E x40 (b) H & E x400. D- Photomicrograph in cross section of second trimester *Camelus dromedarius* uterine body indicating double myometrium (red arrow), endometrium (green arrow), venule (yellow arrow), CS- circular smooth muscle and LP- lamina propria (a) PAS x40 (b) PAS x400. E- Photomicrograph in cross section of third trimester *Camelus dromedarius* uterine body indicating myometrium (red arrow), endometrium (green arrow), endometrial glands (orange arrows), CS- circular smooth muscle and LP- lamina propria (a) H & E x40 (b) H & E x400. F- Photomicrograph in cross section of third trimester *Camelus dromedarius* uterine body indicating myometrium (red arrow) , endometrium (green arrow), CS- circular smooth muscle and LP- lamina prop(a) PAS x40 (b) PAS x400

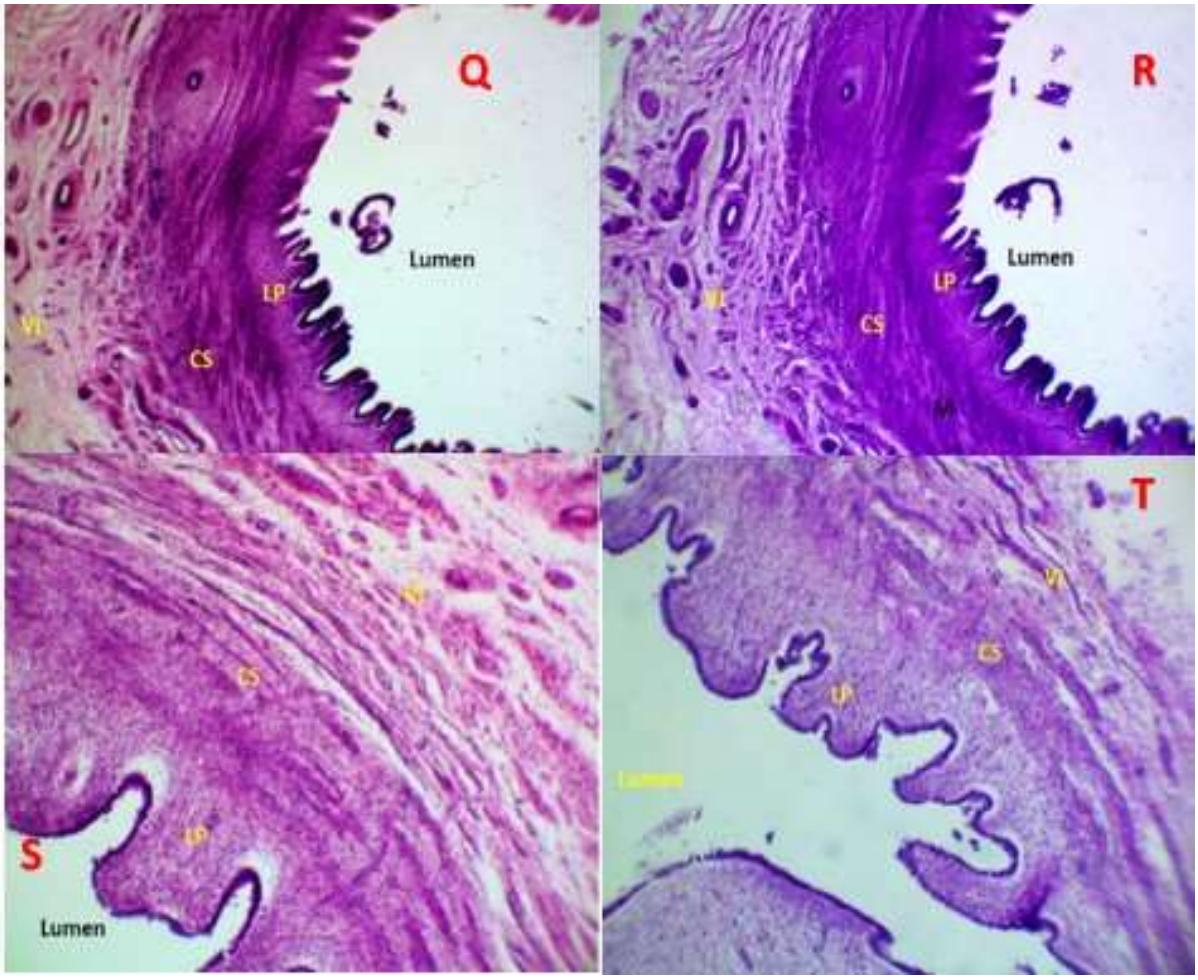

Figure 4: A-Photomicrograph in cross section of second trimester *Camelus dromedarius* uterine cervix indicating VL- vascular layer, CS- circular smooth muscle and LP- lamina propria H & E x40. B-Photomicrograph in cross section of second trimester *Camelus dromedarius* uterine cervix indicating VL- vascular layer, CS- circular smooth muscle and LP- lamina propria PAS x40. C- Photomicrograph in cross section of third trimester *Camelus dromedarius* uterine cervix indicating VL- vascular layer, CS- circular smooth muscle and LP- lamina propria H & E x40. D-Photomicrograph in cross section of third trimester *Camelus dromedarius* uterine cervix indicating VL- vascular layer, CS- circular smooth muscle and LP- lamina propria PAS x40

DISCUSSION

In this study, uterine horns of the left and right differed histologically, due to the abundant connective tissue deposit making the left horn thicker than the right horn[22]. The uterine horns were observed to bear a pseudostratified columnar epithelium at the first trimester similar to the findings of Bacha and Bacha[24]in ruminants and sows. The lamina propria has fibroblast and monocytes, which were similar to that reported in the works of Farouk *et al*.[25] in prenatal camels.

The epithelia lining observed in second trimester with evidence of endometrial gland were similar to what was reported by Uppal *et al*.[26] in buffalo fetal uterus. Gray *et al*.[27] described development of the uterine glands as a complex process, though many details remain to be defined at different stages. The increased invaginations in the left horn than the right with numerous stroma cells fibroblasts, plasma cells and monocytes were all supported by findings of Fetaih *et al*.[28] and Albaghdady[13] in postnatal, but not prenatal camels as herein reported. The mesenchymal cells seen to be more densely packed towards the epithelium and was also similar to the findings of Uppal *et al*.[26] in uterus of fetal buffalo.

Moreever, the arrangement of muscles fibres within the myometrium were similar to the findings reported by Farouk *et al.*[25] in prenatal camels. Gray *et al*.[27] shed more insight as the derivatives of both inner circular and outer longitudinal smooth muscle were ductile mesenchymal cells and subperimetrial mesenchyme respectively. Prominent blood vessels were also noted at the outer longitudinal smooth muscle layer were similarly reported in uterus of fetal buffalo[26].

The abundant endometrial glands observed at the third trimester as compared to the second trimester were in agreement with the findings of Farouk *et al.*[25]. He reported an increase in number of canalized glands. In this study, the left uterine horn had numerous endometrial glands than the right uterine horn at second trimester. However, these maybe supported with the findings of Srikandakumar *et al*.[14], who reported 100% of fetuses implanted in the left uterine horn of adult camels.

The submucosa has numerous fibroblasts within the loose connective tissue with the submucosa wider on the left horn than on the right. The densely packed cells bearing stained nuclei and blood vessels at the basal epithelium noted in this study, were also reported on the uterine body of the fetal buffalo[26].

The connective tissue stroma observed to be distinct on the PAS stained tissues were noted to be spindle-like in shape with the muscular myometrium discovered to be thicker than those on the left horn. However, the vascular layer was noted to be slightly distinct, though observed to be more prominent on the left horn than on the right horn. Distinct vascular layer was also reported in fetal buffalo uterus[28].

It is pertinent to report that the bipartite uterine body separated by the inter-cornual septum histologically observed were similar to what was reported by Srikandakumar *et al*.[14] in adult female camels. However, the absence of invagination into the lamina propria on the epithelia of the uterine body of the first trimester were observed.

The findings on the uterine body of the second trimester uterine body with respect to the size and type of epithelial lining observed could be due to the fact that most fetuses are planted in the uterine, thereby to nurture to the fetuses. The histological arrangements on the uterine body and type of epithelia line, lamina propria with present of endometrial gland in third trimester as compared to the first and second trimester fetuses were not in accordance with the findings of Marai *et al*.[29] who documented

that uterine glands appear between 16-20 weeks of age of Egyptian water buffalo.

The findings on the uterine cervix of the first trimester was simple columnar epithelial lining similar to the findings of Porjoosh *et al*.[30] in adult female camel. The findings on the second trimester with primary longitudinal folds and the third trimester having both primary and secondary (few) longitudinal folds is also similar to the findings of Ernst *et al*.[31] in human fetal cervix that exhibited more branching glands at the late gestation period. The inner circular layer of the myometrium was remarkably thick, while the vascular layer was wide with numerous arterioles, venules and lymphatics on the second and third trimester. This was similar to the findings of Porjoosh *et al*.[30] in adult female camel who described the vascularisation of the cervix as a mechanism for rapid immunological reaction to bacteria.

CONCLUSION

In conclusion, we report here developmental changes in the uterus (comprising of the uterine horn, the uterine body and the uterine cervix) of the fetal *Camelus dromedarius* based on histomorphological observations and highlighted trimester-based differences in the development of these tissues. We also compared our findings with those of other mammals and noticed agreements in developmental strides. Our results provide new information on the histological features of the uterus in female *Camelus dromedarius*, an insight to reproductive management of this species of mammals, very critical for the continuity of life[32].

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