# RESEARCH ARTICLE

# HISTOLOGY OF THE ONE-HUMPED CAMEL'S UTERUS AND VAGINA DURING THE FOLLICULAR AND LUTEAL PHASES OF THE OESTROUS CYCLE

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# **ABSTRACT**

The dromedary camel is one of the domestic animals that have not received adequate scientific attention. Unlike other domestic animals, the dromedary camel does not ovulate spontaneously until after a successful mating. Therefore, present study seeks to evaluate the histological features of the vagina and the uterus of dromedary camel during follicular and luteal phases of the oestrous cycle. A total of 86 one-humped camels were included, and their blood samples were collected for progesterone assay. The progesterone assay enabled us to divide the camels into the follicular and luteal phase groups, according to progesterone concentration standards for luteal and follicular phase of the oestrous cycle. In the specimen collected during the follicular phase, the mean serum progesterone level was 0.89±16 ng/ml, while its level was 1.61±0.81 ng/ml in the specimen during the luteal phase. Animals with P4 values <1 ng/ml (n=51, mean of  $0.89 \pm 0.16$  ng/ml) and those with P4 values >1 ng/ ml (n=35, mean of 1.61  $\pm$  0.81 ng/ml) were considered to be in follicular and luteal phase, respectively. Vaginal and uterine tissue samples were collected from all members of both groups. The histological features observed in the uterus during the follicular phase are presented with a layer of simple columnar epithelium with fewer elastic epithelial cells. In the uterine lamina propria, there are several visible tubular glands. Blood vessels are conspicuous and clogged. The inner circular and outer longitudinal layers of the muscle bands are thick, with numerous interstitial spaces. There are more intercellular gaps in the vagina than in the uterus. On the other hand, in the sample collected during the luteal phase, uterine tissue is consisted of simple columnar epithelium. The glands in the lamina propria are more coiled and tubular. Luteal phase is also characterized by the presence of congested blood vessels that are thick in appearance. Thick and clogged blood arteries in the uterus have also been observed. The uterine muscle layer consists of separate inner circular and outer longitudinal layers. It is also thicker than the one observed in the follicular phase. This detailed histological study of vagina and uterus will not only help to understand the reproductive status of camel, but it will also assist in pathological evaluation of the reproductive tract.

Keywords: Histology, one-humped camel, uterus, vagina

# INTRODUCTION

Camel is one of the most important animal species that is known to be adapted to arid and hot environments (Schwart, 1992; Beheiry, 2016). For centuries, the camel has been a very important animal in the desert region, because of its ability to provide milk, meat, and transport in harsh dry conditions. The improvement of the productivity of camel in Nigeria has necessitated the need for basic research on this animal species.

In the field of scientific research, the camel is one of the most neglected domestic animal species, which has not received adequate attention (Beheiry, 2016). This neglect could possibly be linked to poor nutrition and husbandry in arid and tropical areas of Africa and Asia (Sohail. 1983; Behery, 2016). Therefore, literature on the female reproductive tract of camels has been scantily evaluated compared to other mammalian species such as cows (Majama et al., 2018). Dromedary camels can also be found in arid regions of the Middle East, Northern India, and Africa, including the Sahara Desert. They have also been introduced to central Australia's arid regions, where wild populations still exist (Nowak 1991; Wilson, 1984). Some camels from Mali, Niger, and Chad were recorded in the southern Sahel states, particularly Upper Volta, Nigeria, and Cameroon. Pastoral groups in Sokoto, Katsina, Kano, and Borno, according to Ribadu (1988), own the majority of camels in Nigeria. Camels (Camelus dromedarius) are widely seen in Nigeria's northern borderlands, usually herded alongside cattle, sheep, goats, and donkeys, mingling freely at watering pools and marketplaces (El-Yuguda et al 2010; Markemann and Zarate 2010; Majama et al., 2023). Due to their sensitivity to cold and humidity, dromedary camels prefer desert environments with a long dry season and a short wet season (Nowak 1991). According to Khanvilkar (Khanvilkar et al., 2009), the female camel can breed as early as 2-5 years of age, and as late as 30 years of age. However, according to Marai (Marai et al., 2009), breeding can occur as early as 2-5 years of age. When compared to other ungulates, this animal has a distinct estrous

cycle. Unless the female is bred and has ovulated, the phases of the cycle outlined for animals with spontaneous ovulation (estrous and di-estrous) do not exist in Camelidae. There is simply a sequence of follicular waves with a highly fluctuating rhythm in the absence of mating (Tibary and Anouassi, 1997). Camels are known to be induced ovulators, with ovulation occurring after mating in the non-pregnant animal (Marie and Anouassi, 1986; Ayoub et al., 2003; Skidmore et al., 2005; Ghazi, 2007). During the camelid's estrous cycle, four distinct uterine activity phases-high, falling, low, and increasing-were noted by Al-Eknah (Al-Eknah et al. 1993). The level of progesterone in the camel cow's blood is determined by her reproductive status and age (Kamoun and Jemmali, 2014; Majama et al., 2018). The serum progesterone concentration in camel ranges from 0 to 0.41 ng/ml., with mean variations in the range of 0 to 0.380.04 ng/ml (Kamoun and Jemmali, 2014; Majama et al., 2018).

Srikandakumar et al., (2011) reported that the endometrial lining of the vagina consists of a single layer of columnar epithelium supported by a broad highly cellular connective tissue with simple tubular glands. These organs undergo tissue differentiation from prenatal, postnatal, and adult stages under varied physiological and hormonal influences.

Previous reports (Elias,1990; Srikandakumar et al., 2011) on the non-gravid uterus of an adult dromedary have been documented providing vivid gross descriptive features of the uterus in animals found in other parts of the world. The morphometric characteristics of the one-humped camel's reproductive system have been documented (Ribadu, 1988), with scarce information on histological features of the uterus and vagina of the dromedary camels during different reproductive phases. Determining the histological differences of the uterus and vagina in camels during the follicular and luteal phase was the goal of this investigation.

# **MATERIALS AND METHODS**

#### **Materials**

A total of 86 samples of blood and intact genitalia were collected from one-humped female camels brought for slaughter at the Maiduguri metropolitan abattoir. Jugular venipuncture was used to obtain blood samples before slaughter, which were then transferred into sterile, clean vacutainer tubes.

#### Methods

The genitalia were collected as soon as the animals were slaughtered, and put into a clean plastic container. They were urgently transported to the Department of Veterinary Anatomy Postgraduate Research Laboratory, located at the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria, as previously described (Jaji et al. 2012; Gazali et al. 2023). Serum was extracted from the blood specimens by centrifugation at 2000–3000 RPM for 10 minutes, and it was then kept at -80 °C until it was utilized for the progesterone assay.

# Progesterone assay (P4)

The Camel Progesterone (PROG) ELISA Kit (My Bio Science, USA) was used for the P4 assay. This assay was based on the Biotin double antibody sandwich technology, and was carried out according to the manufacturer's instruction. The results of the serum P4 profiles for all the camels examined ranged from 0.6 to 4.6 ng/ml. Those animals with the P4 values of < 1 ng/ml (n=51, mean of  $0.89 \pm 0.16$  ng/ml) and those with values of > 1 ng/ml (n=35, mean of 1.61  $\pm$  0.81 ng/ ml) were considered to be in their follicular and luteal phases, respectively, as previously described (Kamoun & Jemmali, 2014). Using this method, we divided the specimen of 86 camels into two groups: the follicular phase group (N=51), and the luteal phase group (N=34).

# Tissue preparation for histology

About 30-40 mm of the vaginal and uterine tissue was collected from each of the 86 intact genitalia. The tissue was fixed in Bouins solution for 72 hours, washed in running tap water to remove the fixatives, and then treated with ascending

grades of ethyl alcohol (70%, 90%, and 100%) for dehydration. After dehydrating the tissue, the alcohol was removed and subsequently treated with xylene, and finally embedded with paraffin wax at 63 degrees. The tissue blocks were then mounted on wooden chocks, cooled, and cut at 5µm thickness. The tissue sections were then flattened on warm water at 45° C and placed on glass slides, smeared with egg albumin, and dried in the oven at 45° C. The sections were then stained with H&E for histological examination using a light microscope (Drury et al, 1976).

#### **RESULTS**

### Serum Progesterone (P4) Assay

Table 1 displayed the results of the progesterone assay. During the follicular phase, the female camel's mean serum progesterone concentration was  $0.89 \pm 0.16$  ng/ml, and during the luteal phase, it was  $1.61 \pm 0.81$  ng/ml. Animals with P4 values < 1 ng/ml (n=51, mean of  $0.89 \pm 0.16$  ng/ml) and those with P4 values > 1 ng/ml (n=35, mean of  $1.61 \pm 0.81$  ng/ml) were considered to be in follicular and luteal phase, respectively, as previously described (Kamoun and Jemmali, 2014).

**Table 1** One-humped camel (*Camelus dromedarius*) from Maiduguri, Nigeria, serum progesterone (P4) levels during the follicular and luteal phase of the estrous cycle

Estrous Cycle Phases	Number of animals	P4 onc. (ng/ml)	
		$Mean \pm SD$	Range
Follicular phase	51	0.89±0.16	0.6-1.0
Luteal phase	34	1.61±0.81	1.1-4.6

# Histology

The histological features of the uterus and the vagina of the one-humped camel in the follicular and luteal phases are presented below in Figures 1 and 2.

Figure 1 presents the photomicrographs of the

uterine and vaginal tissue during the follicular phase. Throughout the follicular period, the epithelial layer of uterus is consisted of simple columnar epithelium with less elastic epithelial cells. The lamina propria shows numerous tubular glands. There are prominent and highly congested blood vessels. The muscular layer is thick and edematous, with collagen fibers showing distinct inner circular and outer longitudinal layers. The histological features of the vagina during the follicular phase are presented with stratified squamous epithelium which is more folded than the one in the uterus. The lamina propria of the vagina is observed to be devoid of glands but has few blood vessels scattered. The muscular layer

is thick and consisted of inner circular and outer longitudinal layers with more interstitial space. The intercellular spaces observed in the vagina are more spacious than those in the uterus.

Figure 2. exhibits the histological features of the uterus and the vagina during the luteal phase. The uterus is lined with simple columnar epithelium. The lamina propria shows more coiled and tubular glands. It is characterized by the presence of congested blood vessels which are thick in appearance. The muscular layer of the uterus is thicker during the luteal than in the follicular phase, with distinct inner circular and outer longitudinal layers. Histologically, during the luteal phase

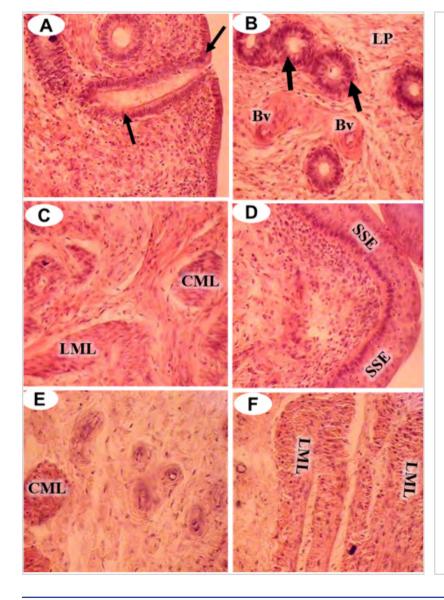


Figure 1 Photomicrograph of the uterine and vaginal tissues of the one-humped camel (Camelus dromedarius) during the follicular phase showing: (A) Uterus consisting of simple columnar epithelium with less elastic epithelial cells (thin black arrows), (B) Lamina propria (LP) of the uterus showing numerous tubular glands (thick black arrows), and congested blood vessels (Bv), (C) Myometriun showing thick musculature with distinct inner circular muscular layer (CML) and outer longitudinal muscular layer (LML), (D) Vaginal tissue showing stratified squamous epithelium (SSE) that is more folded than the uterus, (E) Vaginal lamina propria with scattered blood vessels (Bv) as shown in (B), (F) Vaginal muscular layer consisting of inner and outer longitudinal muscular layers (LML)

the vagina is characterized by the presence of stratified squamous epithelium with prominent blood vessels. There are more intercellular spaces observed in the lamina propria than in the tunica adventitia. The tunica muscularis of the vagina consists of inner circular and outer longitudinal layers with muscle bundles, reticular fibers, and scanty nerve fibers.

#### DISCUSSION AND CONCLUSION

The vagina and uterus are parts of the female reproductive system of the one-humped camel with very important role in fertilization. Unlike other domestic animals, the camel does not ovulate spontaneously. As a result, the luteal phase appears only following a successful mating (El Allal et al., 2017; Majama et al., 2018). Therefore, progesterone in high concentration can only occur during pregnancy in the camelids (Majama et al., 2018), what is consistent with the present study, as the progesterone concentration was higher during the luteal phase (0.1-1.0 ng/ml) than in the follicular phase (1.1-4.6 ng/ml).

Furthermore, since the cyclical patterns observed in other animals are not consistent with the Camelids, the histological features of the uterovagina have not been adequately described in this species (Ahmadi et al. 2005; Majama et al., 2018).

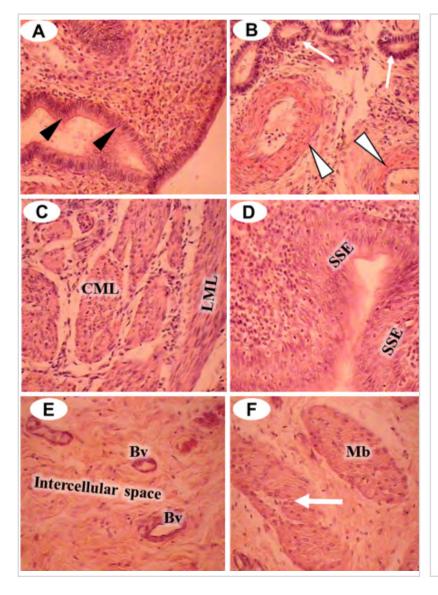


Figure 2 Photomicrograph of the uterine and vaginal tissues of the camel (Camelus dromedarius) during the luteal phase showing (A) Uterus characterized by simple columnar epithelium(black arrow heads), (B) Lamina propria with more coiled and tubular glands (thin white arrows) larger tubular glands with congested blood vessels (white arrow heads) (C) Myometrium showing thick muscular with distinct inner circular muscular layer (CML) and outer longitudinal muscular layer (LML), (D) Vaginal stratified squamous epithelium (SSE), (E) Vaginal lamina propria with intercellular space and blood vessels (Bv), (F) Tunica muscularis consisting of inner and outer longitudinal muscular bands muscle bundles (Mb) and scanty reticular nerve fibers (white arrow).  $H\&E \times$ 400

Present study revealed more prominent mucosal folds in the uterine epithelium during the follicular phase than in the luteal phase, as it was previously observed in the cow (Mokhtar, 2014). Similarly, the uterine tissue was characterized by simple columnar epithelium with less elastic epithelial cells during the follicular phase. The lamina propria revealed numerous glands that were tubular with prominent blood vessels. These differences could possibly be due to the varying secretion rates of ovarian hormones as were previously observed in bovine species (McDanie et al., 1968; Mokhtar, 2014), and camelids (Srikandakumar et al., 2011). The muscular layer was thick and edematous with distinct collagen fibers showing inner circular and outer longitudinal layers during this phase. The mucosa showed irregular raised longitudinal folds, which agrees with a previous report on Arabian camels (Srikandakumar et al., 2011).

During the luteal phase, there were no marked deviations in the histological features observed during the follicular phase, except that the glands became more coiled and tubular. The histological features of the vagina during the follicular phase were characterized by stratified squamous epithelial layers which were more folded than the uterus. These cyclic changes in the vagina and uterus showing hypertrophy and atrophy of their epithelial tissues could be due to the status of the endocrine function; as a result, the tissues may undergo some changes during the estrous cycle, as previously reported (Yániz et al., 2000; Mokhtar, 2014).

Interestingly, the lamina propria was devoid of glands but with few scattered blood vessels. The muscular layer was observed to be thick and consisted of inner circular and outer longitudinal layers with more intercellular space. As observed during the follicular phase, there was also no marked difference in the histology of the vagina during the luteal phase; this could be due to limited activities of the vagina compared to the uterus during embryo nutrition and development. Djang et al., 1988, showed that the vaginal lumen was lined with many longitudinal folds. In conclusion, the changes in the vagina and uterus of dromedary

camel during follicular and luteal phases are similar to other domestic animals, though the luteal phase can only come following successful mating, which is remarkably different from other domestic species.

In conclusion, the progesterone levels observed throughout the oestrous cycle are very important in determining the reproductive status of camels. This detailed histological study of the vagina and uterus will not only help to understand the reproductive status of camel but will also assist in pathological evaluation of the reproductive tract.

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# **CONFLICT OF INTEREST**

According to the authors, there are no conflicts of interest that might be thought to compromise the objectivity of the research presented.

# **CONTRIBUTIONS**

Conceptualization and writing of the draft manuscript: YBM, writing and editing of the manuscript: MZ, HMBM, statistical analysis: LA and Supervision and critical review: HDK.

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# HISTOLOGIJA UTERUSA I VAGINE JEDNOGRBE KAMILE U FOLIKULARNOJ I LUTEALNOJ FAZI ESTRUSA

# **SAŽETAK**

Dromedar kamila je vrsta domaće životinje kojoj se ne posvećuje adekvatna znanstvena pažnja. Za razliku od ostalih domaćih životinja, dromedar ne ovulira spontano, već nakon uspješnog parenja. Stoga je cilj našeg istraživanja procijeniti histološke karakteristike vagine i uterusa kod dromedara za vrijeme folikularne i lutealne faze estrusa. U istraživanje je uključeno ukupno 86 jednogrbih kamila čiji su uzorci krvi prikupljeni za određivanje progesterona. Razina progesterona je omogućila podjelu kamila u dvije grupe, jednu u folikularnoj, drugu u lutealnoj fazi, a prema referentnim vrijednostima progesterona u lutealnoj i folikularnoj fazi estrusa. U uzorku prikupljenom u folikularnoj fazi, srednja vrijednost progesterona je iznosila 0.89±16 ng/ml, a u lutealnoj fazi 1±0.81ng/ml. Za životinje sa vrijednostima P4 < 1 ng/ml (n=51, srednja vrijednost od  $0.89 \pm 0.16$  ng/ml) i one sa P4 vrijednostima >1 ng/ml (n=35, srednja vrijednost od  $1.61 \pm 0.81$  ng/ml) je smatrano da se nalaze u folikularnoj, odnosno lutealnoj fazi. Uzorci vaginalnog i uterinog tkiva su uzeti od svih životinja iz obaju grupa. Uočene histološke karakteristike uterusa u folikularnoj fazi su predstavljene slojem jednostavnog cilindričnog epitela i manjim brojem elastičnih epitelnih ćelija. U uterinoj lamini propriji je vidljivo nekoliko tubularnih žlijezda. Krvni sudovi su uočljivi i začepljeni. Unutrašnji cirkularni i vanjski longitudinalni mišićni slojevi su zadebljani sa širokim intersticijalnim prostorom. Vagina ima više intercelularnog prostora nego uterus. S druge strane, u uzorku prikupljenom u lutealnoj fazi tkivo uterusa se sastojalo od jednostavnog cilindričnog epitela. Žlijezde u lamini propriji su vijugave i tubularne. Lutealna faza je također karakterizirana prisustvom kongestiranih krvnih žila koje su se doimale širim. U uterusu su uočene i proširene i začepljene arterije. Sloj uterinih mišića se sastoji od unutarnjeg cirkularnog i vanjskog longitudinalnog sloja koji su međusobno odvojeni. Ovaj sloj je deblji od sloja u folikularnoj fazi. Ovako detaljna histološka studija vagine i uterusa će biti od koristi ne samo u razumijevanju reproduktivnog statusa kamile, nego i u patološkoj evaluaciji reproduktivnog trakta.

Ključne riječi: Histologija, jednogrba kamila, uterus, vagina