Uterotubal Junction of the Bovine (Bos taurus) vs. the Dromedary Camel (Camelus dromedarius): Histological and Histomorphometry Analysis

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Article

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Abstract

This study compares the histomorphology differences of cattle uterotubal junction (CUTJ) and dromedary camels uterotubal junction papilla (CUTJP). UTJ were dissected from eight cows and twelve camels with dominant follicles, and processed for H&E staining for morphology, histology, and histomorphometry examination. The results showed that the CUTJP existed only in camels and was completely absent in cattle. Histologically, CUTJ appears with a star-shaped lumen, and the mucosa lined by a simple columnar epithelium containing ciliated and non-ciliated cells, superficial (SG), and deep glands (DG) were abundant in the submucosa. CUTJP is a fibrous conical structure, it has a pale yellowish color and 0.5 ± 0.2 cm height and 0.3 ± 0.1 cm width. CUTJP, the lumen is wider, and the mucosa showed large multiple folds lined with ciliated and non-ciliated pseudostratified columnar epithelium. The submucosa showed no endometrial glands. Lumen area, lumen epithelial height, luminal epithelial density, the thickness of the muscular layer, number of folds, folds height, epithelial height, fold area, and epithelial perimeter were higher in CUTJP (P < 0.001) than CUTJ. While the total endometrial area and glandular epithelial density were lower in UTJP (P < 0.001) than in CUTJ. In conclusion, the epithelial lining, absence of glands, and the thick layer of tunica muscularis might indicate that camel CUTJP could play a mechanical role in selecting spermatozoa and assisting the hatching of blastocysts during their passage through it.

Background

Camels have anatomical, physiological, behavioral, and reproductive peculiarities that make them differ from other mammals. Female dromedary camels present unique reproductive peculiarities compared with other domestic livestock, including induced ovulation\(^1\), spontaneous parthenogenetic development of oocytes\(^2,3\), the two uterine horns showed distinct luteolytic activity, resulting in the establishment of left horn pregnancies\(^4\). Early maternal recognition of pregnancy by Day 10, embryo migration from the right to left uterine horns for implantation\(^5\). In camelids, between 6- and 6.5-days post ovulation, the embryo reaches the uterus at the early hatched blastocyst stage, while cleavage and embryo development before the hatched blastocyst stage take place in the oviduct and hatches before it descends to the uterus is mandatory for the embryo to continue to develop\(^6\). Information on the mechanisms of the mechanical hatching of blastocyst before descending to the uterus is unknown in camels.

A functioning oviduct is required for gamete storage, maturation, fertilization, and early embryonic development\(^7\). The oviduct is a fibromuscular structure that connects the ovary to the uterus, it is elongated, and tubular\(^8\). The uterotubal junction (UTJ), which connects the uterine tube to the uterine horn, is the uterine part of the isthmus and/or the last few millimeters of the extramural isthmus, and the cranial tip of the uterine horn\(^9\). In mammals, the structure of both the oviduct and uterus showed great diversity in morphological and functional features\(^10\). There have been reports of species differences in the interface between the uterine tube (isthmus) and the uterine horn\(^11\). In the cow and sheep, the uterine tube simply opens into the uterine horn\(^11\). Whereas, in camelids, at the uterotubal junction, the isthmus
opened into the uterine horn with a distinct papilla (UTJP) toward the uterine side. The basic role, morphology, and description of UTJP are still unidentified in the camel. A microscopic papilla is present at the UTJ in cheetahs. In the mare, the uterine tube opens into the uterine horn with the ostium forming a barrier against ascending infection. The isthmus opens in dog and mouse by a slit-like ostium on a mound that projects into the uterine lumen. The UTJ has been proven to be a major barrier to the passage of sperm in dogs, cats, and pigs.

Changes in the uterotubal junction microstructure are required to facilitate specific functions for this region. Knowledge of these differences could provide scientific bases for the development of reproductive biotechnology, especially in a species like camel. Therefore, understanding the reproductive biology of the camel is essential, including an in-depth description of the anatomy and histology of the UTJP. This is the first study to investigate the histological and morphometric analysis of UTJP in dromedary camels to enhance our understanding of the role of this region in camels.

**Materials and methods**

**Ethical approval**

This experiment was approved by the Institutional Medical Research Ethical Committee, National Research Centre, Egypt (Project ID: 13050416). The work was conducted under the provision of relevant Egyptian laws, World Health Organization (WHO), and Good Medical and Laboratory Practice (GCP and GLP) guidelines. The experimental work was conducted during the period from Feb 2023 to April 2023. The whole work was conducted on slaughterhouse materials only.

**Collection of the reproductive tract**

In the present study reproductive tract of eight cows at the proestrus phase (the presence of regressed CL and at least one dominant follicle) and the reproductive tract of twelve mature nonpregnant dromedary camels at the mature follicular phase (in which the ovary showed at least one dominant follicle with 1.3–1.7 cm in diameter). All samples were collected after the slaughter at a local abattoir (El Bassatein slaughterhouse, Cairo, Egypt). Immediately after collection, an incision was made along the dorsal surfaces of the left and right uterine horns of the reproductive tract of cows and dromedary camels, and the cow’s uterotubal junction (UTJ) and camel uterotubal junction papilla (UTJP) were separated from uterine tissue and the length and width of the UTJP were recorded. All measurements were made using a vernier caliper to the nearest mm.

**Histological examination**

For 48 h, cows UTJ and camel’s UTJP were fixed in 10% neutral buffered formalin at room temperature, then dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax. Tissue blocks were cut using a rotatory microtome into 5-µm sections and placed on glass slides. Sections were rehydrated through a series of decreasing concentrations of ethanol and stained with hematoxylin and
eosin stains. A light microscope was used to examine the slides, and photomicrographs from the selected tissues were taken using a digital camera.

**Morphometric analysis**

The sections cows UTJ and camels UTJP stained with H&E were examined using an Olympus research microscope CX41 (Olympus, Japan), and the digital photomicrographs sections were photographed at different magnifying powers with an Olympus SC100 digital CCD camera (Olympus, Japan). Fifteen captured images for the representative areas from cows UTJ and camel’s UTJP were exported and analyzed using Image J analysis software (ImageJ 1. x version). The threshold of the signals was set to identify the selected parameters and subtract the background noise (Figure 1). Different morphometric analysis parameters of uterine sections at the level of the uterotubal junction of camel and cow including luminal epithelium height (µm) from the base of the membrane to the tip of luminal epithelial cells, luminal area (µm) (Figure 1A). In addition, total endometrial area (mm²) was measured, letting calculation of stromal density, luminal epithelium density, and glandular epithelium density as a percentage of the total endometrium (Figure 1B). Moreover, the thickness of the muscular layer was determined. Gland duct numbers, the height of the epithelial lining (µm), density, perimeters, and areas of superficial (SG) and deep glands (DG) were assessed in different endometrial compartments. For camel’s UTJP morphometric parameters were estimated including the total number, height of epithelial cells from the base of the membrane to the tip of the epithelial cell, length, density, and area. Ten random measurements of each parameter of the selected areas were estimated per sample.

**Statistical analysis**

All statistical tests were carried out using GraphPad Prism 9 (GraphPad Software, Inc., CA, and USA). Differences between parameters were analyzed by unpaired Student’s t-test. All data are presented as means ± SEM.

**Results**

**Gross morphology**

The gross morphology of the cattle’s UTJ and camel’s UTJP is shown in Figure 2. The UTJP has existed only in camels and is completely absent in cows. In cattle, the oviduct opens directly into the uterine horn and the UTJ extends up to the first uterine caruncle (Fig. 2A, Arrow). While in dromedary camel the UTJP is a fibrous conical structure protruding from the base of the oviduct toward the tip of the uterine horn, it has a pale yellowish color and 0.5± 0.2 cm height and 0.3±0.1 cm width (Fig. 2B).

**Light microscopy**

Light microscopic examination revealed that the cattle’s UTJ and camel’s UTJP differ in structure, the size of the lumen, the shape of the mucosa, and the thickness of the tunica muscularis. In cross-section, the
cattle's UTJ appears with a star-shaped lumen that lies centrally and the mucosal layer forms low transversal ridges. Cattle's UTJ consists of the three layers of mucosa, myometrium, and serosa. The mucosa consists of primary folds and the submucosa is enriched with simple tubular glands opening directly to the lumen (Fig. 3A). The openings of the uterine glands are mostly situated in the furrows separating the transversal ridges. In cattle TUJ, the mucosa is lined by a simple columnar epithelium containing ciliated and non-ciliated cells (Fig. 3C). Whereas in cross-section in camel's UTJP consists of the three layers of mucosa, myometrium, and serosa. The lumen is wider, and the mucosa showed large numerous primary folds that branched forming secondary and sometimes tertiary folds (Fig. 3B). In camel's UTJP the mucosa is lined with ciliated and non-ciliated pseudostratified columnar epithelium (Fig. 3D). The sub mucosa showed no endometrial glands.

In addition, microscopic examination of cattle's UTJ indicated that the tunica muscularis is thin and consists of inner circular and outer longitudinal muscles (Fig. 4A). While, in the dromedary camel, the tunica muscularis is double the thickness of that of cattle and consists of inner circular and outer longitudinal muscles (Fig. 4B).

**Morphometric analysis**

The morphometric analysis of camel's UTJP and cattle's UTJ showed significant differences between the two species (Table 1 and Figure 5). The morphometric analysis of camel UTJP and cattle UTJ showed significant differences in the lumen area which were higher in camel UTJP (P < 0.001) compared to cattle UTJ. Also, the thickness of the muscular layer was significantly (P < 0.001) higher in camel's UTJP than in cattle UTJ. The lumen epithelium density percentage was higher in camel’s UTJP (P < 0.001) compared to cattle’s UTJ. On the other hand, the total endometrial area and the glandular epithelial density was lower in camel UTJP (P < 0.001) compared to cattle UTJ. While there was no significant difference in stromal density percentage between camels and cattle.

Data presented in Table 2 and Figure 6 indicated that the morphometric analysis of the UTJ folds significantly differ between camels UTJP and cattle UTJ, higher number of folds were recorded in camel UTJP (P = 0.0025) compared to cattle UTJ. Additionally, the length of folds and epithelium height of the camel UTJP were greater than those of cattle UTJ (P = 0.0023, P < 0.001), respectively. Moreover, the perimeter and area of the folds were also higher in camels UTJP (P = 0.0299, P = 0.0448, respectively) compared to cattle UTJ.

The uterine glands were absent in camel’s UTJP, while in cattle, there were plenty of superficial (SG) and deep glands (DG), (Table 3 and Figure 7). The average number of uterine glands was 348.0 ± 8.643 per cross-section for both SG and DG. The epithelium height, perimeter, and area of SG were significantly (P < 0.001) higher than DG. However, the density of DG was higher (P < 0.001) than SG.
Discussion

There are species differences in the junction between the uterine tube (isthmus) and uterine horn, and the role played by the UTJ is affected by different factors, which greatly vary among mammals. In the present work, the cattle’s UTJ and camel’s UTJP were investigated by histological and histomorphometric examination as a fundamental knowledge for further biotechnological and clinical application. This study indicated that the morphology and histology within the cattle’s UTJ and camel’s UTJP differ depending on their functions.

In the present work, the UTJP exists in camels and is completely absent in cattle. Similarly, UTJP was previously reported in dromedary camel\cite{5,12-14}. However, the details regarding its morphology and morphometry are lacking. Dromedary camel's UTJP has a conical shape directed from the base of the oviduct toward the uterine lumine, it has a pale yellowish color, 0.5± 0.2 cm height, and 0.3±0.1 cm width. The luminal area, luminal epithelial density, and the thickness of tunica muscularis is higher in camel UTJP than that of cattle UTJ. Moreover, camels UTJP possess a higher number of uterine folds, high folds height, higher epithelium height, and higher fold area compared to cattle UTJ. This unique feature is attributed to the formation of primary, secondary, and occasionally tertiary folds. This difference compared with other mammals could be related to the regional function. In the dromedary camel, the function of the uterotubal junction papilla is lacking.

Furthermore, in cattle’s UTJ, the mucosa consists of primary folds lined with ciliated and non-ciliated simple columnar epithelium, and the submucosa enriched with simple tubular glands open directly into the lumen. These results are consistent with that previously reported in cattle\cite{21}. Several species indicate increased ciliogenesis under the influence of estradiol\cite{22}, and non-ciliated cells may have the ability to transform into ciliated cells following estradiol treatment\cite{23}. Whereas, in the dromedary camel’s UTJP, the mucosa consists of primary, secondary, and tertiary folds lined with ciliated and non-ciliated pseudostratified columnar epithelium, and the submucosa showed no endometrial glands. Similarly, the uterotubal junction in camel is lined by ciliated and non-ciliated pseudostratified columnar epithelium\cite{14}. In contrast, the oviduct of the camel is lined by ciliated and non-ciliated columnar epithelium\cite{13}. This variation could be related to regional differences in the or the reproductive status of the camels. Camels are induced ovulatory and follicular waves continue to develop during the breeding season. The height of the lining epithelium increased during the follicular wave in response to the high serum concentrations of estradiol\cite{1}. High estrogen levels caused an increase in cellular proliferation, uterine epithelial cell secretion, uterine blood flow, and endometrial vascular permeability\cite{24}.

In addition, in cattle’s UTJ, the tunica muscularis is thin and consists of inner circular and outer longitudinal muscles. While, in the dromedary camel, the tunica muscularis is nearly double the thickness of that of cattle and consists of inner circular and outer longitudinal muscles. The significance of the absence of uterine glands and the thick tunica muscularis of the camel’s UTJP is unknown. The varying thickness of the tunica muscularis between the two species suggests that it is related to the function of this region. Contractions of the tunica muscularis may cause stenosis of the utero-tubal junction resulting...
in restriction and regulation of the sperm passage towards the isthmus; or descent of oocyte and fertilized zygote to the uterus\textsuperscript{25}. Furthermore, the thick tunica muscularis might serve to provide the peristaltic and anti-peristaltic waves in the isthmus. Peristaltic contractions of these thick muscles together with the movement of the cilia could help in the passage of the early embryo toward the uterus\textsuperscript{26}. Therefore, the thick tunica muscularis camel’s UTJP might play a role in regulating the passage of sperm from the uterus to the oviduct. In addition, it could play a role in the hatching of blastocyst before it descends from the isthmus into the uterine lumine. In Llama, the hatching of the blastocyst in the oviduct is mandatory before they descend to continue to develop in the uterus\textsuperscript{6}. Therefore, the morphological, histological, and morphometric characteristics of the camel’s UTJP might play a significant role in selecting sperm before passage to the oviduct for fertilization, and hatching of the blastocyst might be a mechanical process that occurred during the passage of the blastocyst through the UTJP in dromedary camel.

Furthermore, in this work, in cattle, there were plenty of superficial (SG) and deep uterine glands (DG). The epithelium height, perimeter, and area of SG were significantly higher than DG. However, the density of DG was higher than SG. This increase in superficial endometrial glands might occur due to the reduction in uterine edema of the subepithelial connective tissue layer. Similar results were recorded in buffalo\textsuperscript{27}. In mammals, the UTJ acts as a reservoir for sperm storage in the preovulatory phase because it contains abundant uterine glands. After mating the sperm enters the uterus and aggregate at the terminals of the uterine horns, which clump together and adhere to the ciliated epithelium in the dog to conserve energy until ovulation and the ova entering the ampulla\textsuperscript{28}. Passage of motile sperm through the UTJ is regulated by both the physical constraints of the UTJ opening, resulting in only a privileged population of sperm, and molecular screening of the sperm\textsuperscript{28,29}. The ciliated epithelium of the UTJ has the capability of screening the proteins on the acrosome of the sperm and allowing the passage of selected sperm\textsuperscript{29}. In contrast, the total number of superficial and deep endometrial glands in cows was unchanged between the estrous cycle, but the area of the subepithelial connective tissue layer enlarged at the follicular phase\textsuperscript{30}. In our case, the samples were collected with unknown reproductive history, but it seems that it collected from cyclic animals during the follicular phase of the estrous cycle. In addition, the absence of uterine glands in camel’s UTJP requires further studies on the mechanism of sperm storage before ovulation and screening the different parts of the uterus to localize the area of sperm storage.

In conclusion, this study revealed several interesting morphological and histological features of the camel’s UTJP such as the epithelial lining, absence of glands, and the presence of a thick layer of tunica muscularis might indicate that this part of the oviduct plays a mechanical process in selecting spermatozoa and assisting of blastocyst hatching during their passage through it.

In Conclusion: Camel UTJP is a specialized structure found at the UTJ, several interesting morphological and histological features of the camel UTJP such as the epithelial lining, absence of uterine glands, and the thick tunica muscularis might indicate that this part of the oviduct plays mechanical process in selecting spermatozoa and assist of blastocyst hatching during their passage through it. Further
research is needed to fully understand the molecular mechanisms underlying the papillae function, which could have important implications for improving reproduction in camels.

**Declarations**

**Ethics approval and consent to participate.**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and material**

The data are available upon reasonable request from the corresponding author.

**Competing interests**

The authors declare no conflict of interest.

**Authors’ contributions**

ASSA conceived the study design, ASSA, SSS, ANM performed the experiments, ANM analyzed data, and ASSA wrote the manuscript. All authors read and approved the final manuscript.

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**References**


Tables
Table 1
Morphometric analysis of camel’s UTJP and cattle’s UTJ (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Camel’s UTJP</th>
<th>Cattle’s UTJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal area (µm)</td>
<td>48706 ± 400.5***</td>
<td>23427 ± 385.1</td>
</tr>
<tr>
<td>Total endometrial area (density) (mm²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroma density (% of total endometrium)</td>
<td>46.89 ± 2.31</td>
<td>73.223 ± 2.78***</td>
</tr>
<tr>
<td>Luminal epith. density (% of total endometrium)</td>
<td>24.84 ± 0.69***</td>
<td>6.02 ± 0.47</td>
</tr>
<tr>
<td>The thickness of the muscular layer</td>
<td>393.30 ± 30.24***</td>
<td>178.30 ± 19.76</td>
</tr>
<tr>
<td>Glandular epith. density (% of total endometrium)</td>
<td>0.00</td>
<td>21.24 ± 0.8976***</td>
</tr>
</tbody>
</table>

*** Significantly differ between rows at P < 0.0001

Table 2
Mean ± SEM morphometric characteristics of the UTJ folds of cattle and camel.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Camel UTJP folds</th>
<th>Cattle UTJ folds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of folds</td>
<td>11.80 ± 1.594**</td>
<td>5.4 ± 1.140</td>
</tr>
<tr>
<td>Epithelium height (µm)</td>
<td>58.06 ± 4.033***</td>
<td>24.968 ± 4.094</td>
</tr>
<tr>
<td>Length of folds (µm)</td>
<td>276.0 ± 24.66**</td>
<td>153 ± 12.263</td>
</tr>
<tr>
<td>The perimeter of folds (µm)</td>
<td>995.7 ± 141.6*</td>
<td>561.062 ± 172.511</td>
</tr>
<tr>
<td>Folds area (µm)</td>
<td>30989 ± 5866*</td>
<td>15238 ± 4908</td>
</tr>
</tbody>
</table>

* Significantly differ between rows at P < 0.05
** Significantly differ between rows at P < 0.01
*** Significantly differ between rows at P < 0.001
Table 3
Mean ± SE morphometric measurements of endometrial glands in cattle's UTJ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Superficial Glands</th>
<th>Deep Glands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of uterine gland ducts</td>
<td>348.0 ± 8.643</td>
<td></td>
</tr>
<tr>
<td>Epithelium height (µm)</td>
<td>25.17 ± 2.007**</td>
<td>18.37 ± 0.8242</td>
</tr>
<tr>
<td>Gland duct density</td>
<td>24.43 ± 1.305</td>
<td>36.42 ± 1.233***</td>
</tr>
<tr>
<td>Gland duct perimeter (µm)</td>
<td>582.7 ± 20.96***</td>
<td>303.3 ± 11.50</td>
</tr>
<tr>
<td>Gland duct area (µm)</td>
<td>12854 ± 573.0***</td>
<td>4776 ± 275.8</td>
</tr>
</tbody>
</table>

** Significantly differ between rows at P < 0.001
*** Significantly differ between rows at P < 0.0001

Figures

Figure 1

Threshold ranges of the staining signal are set to identify the selected parameters and subtract the background noise of cross-sections at (A) bovine UTJ; (B) camel's UTJP.
Figure 2

Gross morphology of (A) bovine UTJ; (B) camel's UTJP (White arrow).
Figure 3

Photomicrograph showing the histological difference between cattle's UTJ and camel's UTJP; A) cross-section in cattle's UTJ showing star-like lumen and abundance of the uterine gland (Black arrow) (40x); B) camel's UTJP showing mucosal folds and muscular layer (40x); C) Ciliated (White arrow) and non-ciliated simple columnar epithelium lining mucosa of cattle's UTJ; D) Ciliated (Black arrows) and non-ciliated pseudostratified columnar epithelial cells lining mucosa of camel's UTJP (400x). H&E stain, scale bar A, B= 100 µm; and C, D= 25µm.
Figure 4

Photomicrograph showing the histological difference between cattle's UTJ and camel's UTJP A) muscular layer of cattle's UTJ (400x); B) muscular layer in camel's UTJP showed double the size of cattle's UTJ (400x). H&E (Scale bar A, B= 50 µm).
Figure 5

Morphometric analysis parameters of camel’s UTJP and cattle’s UTJ. a) Luminal epithelium height (µm); b) Luminal area (µm); c) Total endometrial area (mm²); d) Thickness of the muscular layer (µm); e) Stroma density (% of total endometrium); and f) Luminal epith. density (% of total endometrium).
Figure 6

Morphometric analysis uterine folds of camel and cattle UTJ. a) total number; b) epithelium height (µm); c) length (µm); d) Perimeter (µm); e) area (µm).
Figure 7

Morphometric measurements of endometrial glands in cattle’s UTJ. a) Epithelium height (µm); b) Gland duct density; c) Gland duct perimeter (µm); and d) Gland duct area (µm). ** Significantly differ between rows at $P<0.01$, *** Significantly differ between rows at $P<0.001$