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Anatomical and Histological Characteristics of the Female Reproductive System in the Arabian Sand Gazelle (*Gazella marica*) in Jordan

Abdulhakeem Eljarah [1#]*, Hebah Alaeddin Aboomer [1#] and Mohammad Borhan Al-Zghoul [2]

[1]Department of Clinical Veterinary Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Jordan
[2]Department of Basic Veterinary Medical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Jordan
[#]These authors contributed equally to this work
***Corresponding author:** eljarah@just.edu.jo

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ABSTRACT

Arabian sand gazelle (*Gazella marica*) is listed as a vulnerable species. Few studies exist on the anatomy and histology of its reproductive system. The purpose of this work is to fill this gap by providing comprehensive characterization of female reproductive anatomy and histology and establishing baseline data for various areas of the reproductive tract. The results showed that the ovary is composed of a simple squamous to cuboidal epithelium, forming the germinal epithelium, where follicles are arranged in the cortical region of the ovary. The oviduct is characterized by a simple columnar, highly folded epithelium with decreasing diameter as it reaches its end. Peripheral gland-like structures are seen along the extension of the oviduct epithelium, more often in the infundibulum than in the isthmus. The uterus is bicornuate, similar to the bovine uterus, but with a deep groove between the uterine horns reaching the cervix. Caruncles of the uterus are pigmented and found only in uterine horns, suggesting a cotyledonary placenta. The endometrium exhibits an abundance of coiled simple tubular glands in both the uterine horns and the uterine body. Three cervical folds in the cervix exhibit incomplete encirclement that lacks a fully formed ring structure, facilitating the introduction of semen catheters. The cervical epithelium is mainly composed of secretory cells. The structure of the reproductive tract in Arabian sand gazelle is similar to that of domestic ruminants, such as cows, sheep, and goats, and wild small ruminants, with few discernible variations.

Key words: Gazelle; Anatomy; Female reproduction; Histology; Uterus; Wildlife conservation

INTRODUCTION

The Arabian sand gazelle (*Gazella marica*), or Alreem, as it is locally called, is a wild desert antelope of the Bovidae family (Wacher et al. 2011). According to the International Union for Conservation of Nature (IUCN), the Arabian sand gazelle is listed as a vulnerable species in its natural habitat of the desert lands of the Arabian Peninsula, Jordan, Syria, and Iraq (IUCN SSC Antelope Specialist Group 2017). It has been estimated that there were fewer than 50 of these animals in the wild in Jordan (Eid et al. 2020).

Because of the vulnerability of the Arabian sand gazelle, many populations are kept in protected areas or reserves as captive animals. They are also found in several national parks (Soares et al. 2021). The survival of the species is influenced by environmental factors: group size and social mobility are greatly affected by drought conditions (Cunningham and Wronski 2011). As predicted by habitat suitability models, climate change is an additional threat because it may affect the availability of resources to these species (Özcan et al. 2022). Animals in captivity face additional health risks, such as respiratory diseases. Such threats confirm the importance of ongoing efforts to protect this vulnerable species (Ali et al. 2024).

Reintroduction projects have tried to reintroduce these creatures to the wild. Understanding the anatomy and histology of the female reproductive system in the Arabian sand gazelle is clinically important, which is underscored when we link it to long-term mortality monitoring studies conducted on sand gazelles during the period 1988-2012. Investigations into mortality events revealed a noteworthy association with reproductive factors, such as birth-related causes, which accounted for 21.3% of all mortalities (Soares et al. 2021).

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Saatoglu et al. (2019) presented new insights on the genetic diversity of Arabian sand gazelle, emphasising the importance of conservation strategies to preserve groups. Investigations by Uztemur and Orman (2024) regarding this species' growth and physical development have further enhanced understanding of their health and management in different settings. Their behavioral medicine studies have revealed key aspects of gazelle resting patterns, offering important implications for improving breeding programs and reintroduction projects.

Information in the literature is limited regarding the anatomy and histology of the reproductive system of *G. marica*; until recently, only one study provided some anatomical dimensions of the female reproductive system in this species (Abdulhamza et al. 2022). Demircioglu et al. (2021) provided three-dimensional models of the pelvic cavity of *Gazella subgutturosa* using computed tomography to give visual representation of the position of reproductive organs, including the birth canal. Our work aims to provide a comprehensive characterisation of reproductive anatomy and histology and to establish baseline data for the dimensions of different segments of the reproductive tract of the Arabian sand gazelle. This information will make the diagnosis of various abnormalities in these organs easier, thus facilitating the propagation and conservation of this vulnerable species.

MATERIALS AND METHODS

Ethical approval

All procedures and protocols involving animal cadavers were reviewed and approved by the Deanship of Academic Research, Jordan University of Science and Technology and the Institutional Animal Care and Use Committee (545/2023).

Animals

The reproductive tracts (n=7) of female sand gazelles, aged 2.5 to 4 years and weighing 15 to 20kg, were collected from animal cadavers during necropsy during the nonbreeding season (anestrous). Dead animals were obtained from Shaumari Wildlife Reserve located in the northeastern part of Jordan (Azraq, Jordan, latitude 31°50′N). The animals were kept in enclosures and closely monitored, and animal death for any reason at the reserve was usually investigated. Dead animals were collected and necropsied. Additionally, some injured or diseased animals with poor prognoses due to fighting or disease were usually euthanised and necropsied.

Gross anatomy of the female reproductive system

Before necropsy, the morphology of the females' external genitalia was observed and recorded. During necropsy, the reproductive tracts were examined, and their orientation and relationships were observed. They were dissected and preserved in formalin-buffered saline for additional measurements and characterisation. Before fixation in formalin, different segments of the tract, i.e., the vagina, cervix, uterine horns, body, and ovaries, were collected and measured via a digital caliper. Ovarian lengths were measured from pole to pole. The cervical length was measured as the distance between the external and internal ostia. The width of each uterine horn was taken at the level of the intercornual ligament. The presence or absence of follicles, corpora hemorrhagica, corpora lutea, and corpora albicantia in the ovaries was recorded.

Microscopic anatomy of the female reproductive system

Whole specimens from various locations of the reproductive tract were used for histological sections. The samples were preserved in a neutral buffered 10% formaldehyde solution. Routine paraffin-embedding techniques were used to prepare the fixed tissues, which were then cut into 5mm pieces. Standard hematoxylin and eosin and Masson's trichrome stains were used to examine the general histological features according to the protocol described by Bancroft and Gamble (2007).

Following sample collection, each reproductive tract was carefully dissected from the surrounding tissues. The tracts were examined for the size and shape of their various components via an Optika light microscope (B-383PLi) attached to an Optikam PRO6 digital camera (C-P6) using Optika PROView software (version x64, 4.11.20805.20220506). Calibration was performed on a scale ruler (0.1mm or 0.01mm per division) with a microscope stage micrometer calibration slide. Specific measurements of the ovaries, oviducts, uterus, cervix, and vagina were recorded.

Statistical analysis

Statistical analyses were performed via Microsoft Excel. Data calculations included measurements of the mean, range, and standard deviation; t-tests were performed, assuming that differences with a probability level (P) of 0.05 or less were significant.

RESULTS

The Ovaries

Gross Anatomy

The ovaries were almond-shaped and slightly flattened on their surfaces, measuring on average 0.946 x 0.579 x 0.265cm for the right ovaries and 0.799 x 0.564 x 0.310cm for the left ovaries (Table 1). The mean length and height of the right ovary differed significantly from those of the left ovary ($P<0.05$), whereas there was no significant variation in the width of the ovaries at the 0.05 significance level.

Table 1: Macroscopic measurements (cm) of the ovaries in the Arabian sand gazelle measured using a digital caliper

Organs	Mean±SD	Range
Right ovary		
Length	0.946+0.219	0.703-1.13
Width	0.579+0.113	0.493-0.707
Hight	0.265+0.015	0.252-0.281
Left ovary		
Length	0.799+0.212	0.589-1.013
Width	0.564+0.204	0.393-0.789
Hight	0.310+0.028	0.281-0.336

Microscopic Anatomy

A single unbroken layer of simple squamous to cuboidal epithelium surrounded the ovaries, supported by a thin basal membrane known as the "germinal epithelium" (Fig. 1). The ovarian cortex ranged in width from 0.17 to 0.42mm and was slightly thickened at the site of larger follicles (Fig. 1B), whereas the medulla ranged from 1.85 to 2.49mm.

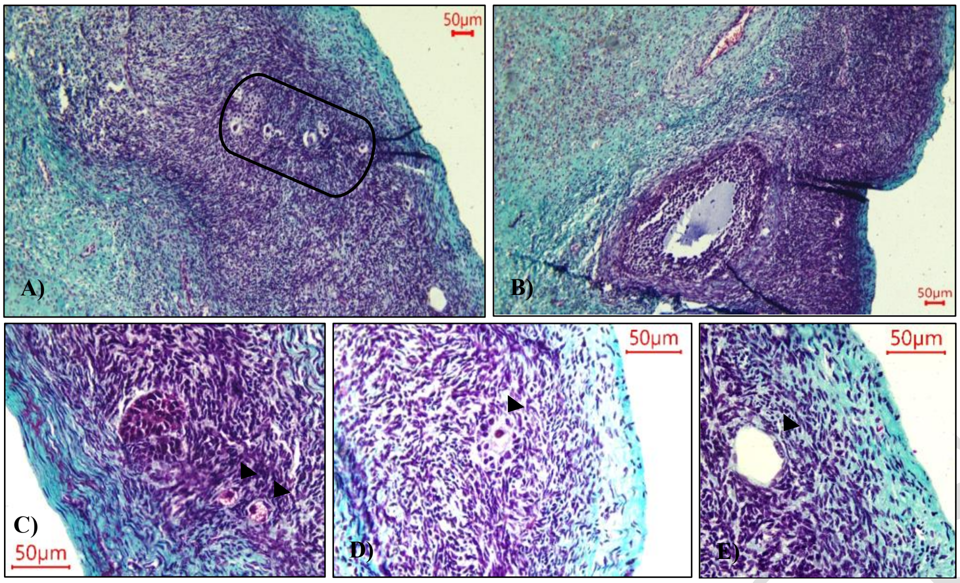

Fig. 1: Histological section of the ovary in the Arabian sand gazelle, Masson's trichrome stain. A) A nest of primordial follicles. B) Thickening of the ovarian cortex at the site of a large follicle. C) Primordial follicles. D) A primary follicle. E) An atretic follicle. Masson's trichrome stain.

The tunica albuginea was composed of collagenous fibres located beneath the germinal epithelium. It was 39µm deep, on average, ranging from 35µm to >137µm in a few areas. Immediately below the tunica albuginea, the ovary was subdivided into an outer zona parenchymatosa and an inner vascular layer. The stroma ovarii and follicles occupied the outer cortex.

Ranges of follicular sizes were recorded by their stage of development (Fig. 1); the number of follicles tended to be relatively low in adult gazelles, corresponding to animal maturity. Primordial follicles were located in the periphery of the zona parenchymatosa just beneath the tunica albuginea, forming nests (Fig. 1A) and were more abundant than other follicle types. As shown in Fig. 1C, the primordial follicles ranged in size from 17.319 to 24.988µm, whereas their oocytes measured 12.028 to 16.512µm in diameter. The primary follicles (Fig. 1D) measured 24.612 to 28.003µm, and their oocytes were 13.476 to 14.158µm. Many atretic follicles were observed (Fig. 1E). One secondary follicle that had undergone atresia was observed (Fig. 1B). No tertiary follicles were observed. Table 2 summarises the measurements of the oocytes and follicles.

Table 2: Arabian sand gazelle oocytes and follicles: size and diameter measured using slide micrometer under microscope

Follicle Type	Follicle Size Range (µm)	Oocyte Diameter Range (µm)
Primordial	17.319-24.988	12.028-16.512
Primary	24.612-28.003	13.476-14.158

The oviduct
Microscopic Anatomy

Samples were collected from the ampulla and isthmus of the oviduct (Fig. 2A and B), where the epithelium consisted primarily of two populations of ciliated and nonciliated cells that were simple columnar in type (Fig. 2C). The nonciliated cells predominated. The thickness of the epithelium was not uniform, ranging from 68.87 to 216.57µm. The abundance and distribution of these cells were not affected by the thickness of the epithelium. The muscular layer consisted of two layers of smooth muscle cells ranging in length from 633.71 to 1217.60µm in the ampulla and from 968.62 to 2057.95µm in the isthmus; the muscular layers of the ampulla were less defined than in the isthmus. The inner layer was circular muscle; the outer layer of the longitudinal muscle was thinner.

The ciliated cells were larger than the nonciliated and had peripherally located nuclei, and their cytoplasm stained faintly with H&E. In contrast, the cytoplasm of nonciliated cells was more heavily stained than that of ciliated cells, and their nuclei were more elongated (Fig. 2C).

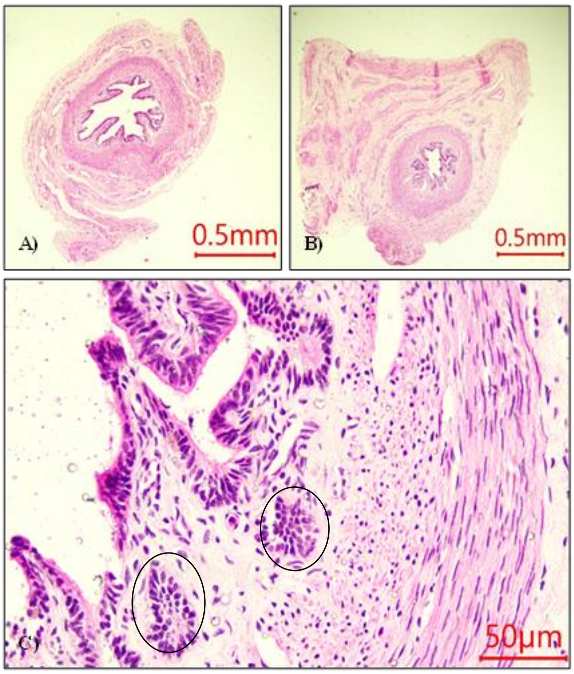

Fig. 2: Histological section of the the oviduct in the Arabian sand gazelle, H and E stain. A) The ampulla. B) The isthmus. C) The epithelium and muscular layers of the oviduct; simple columnar epithelium constituting of ciliated and non-ciliated cells; circles surround Peripheral gland-like structures. H and E stain.

All the oviducts exhibited only the primary fold type. Peripheral gland-like structures near the epithelial folds were observed in 100% of the oviducts (Fig. 2C); these ranged in length from 280.06 to 614.66µm. These structures can be classified as tubular glands, and they are formed by nonciliated epithelial cells with apparently the same cell arrangement as the epithelial surface. The folds were formed by simple columnar epithelium with a striated border.

Table 3: Measurements (cm) of the different parts of the uterus and cervix in the Arabian sand gazelle using a digital caliper

Organs	(Mean+SD)	Range
Width		
Right uterine horn	1.153+0.145	1.006-1.296
Left uterine horn	1.344+0.158	1.179-1.493
Uterine body		
Length	2.548+0.082	2.454-2.6
Width	2.565+0.316	2.357-2.929
Cervix		
Length	3.600+0.160	3.473-3.779
Diameter	1.785+0.070	1.705-1.837

The uterus

Gross Anatomy

The reproductive tract was located entirely within the pelvic cavity, and the uterus was bicornuate, consisting of two short uterine horns, a small uterine body, and a muscular cervix (Fig. 3). The uterine horns were separated, but they communicated to form a small chamber near the region of the cervix. After the uterine horns were cut, the caruncles were identified, as shown in Fig. 4A. These are the areas where the placenta attaches to the uterus.

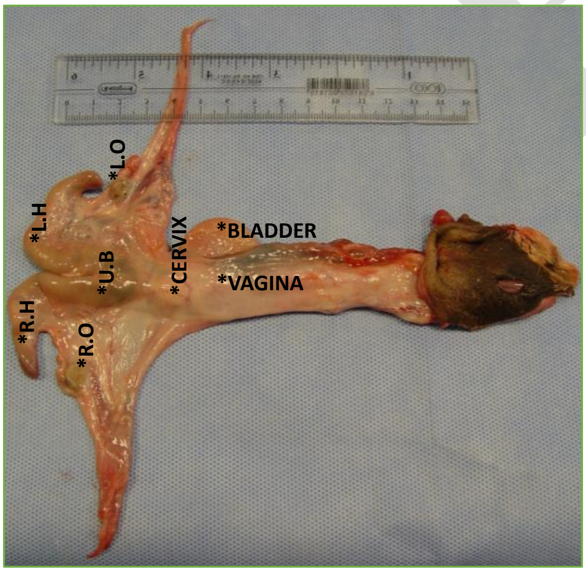

Fig. 3: Excised reproductive tract of the Arabian sand gazelle showing different parts of the reproductive system. L.H: left uterine horn, R.H: right uterine horn, U.B: uterine body, L.O: left ovary, R.O: right ovary.

Microscopic Anatomy

The uterus was composed of three distinct layers: the innermost mucosa, known as the endometrium; the middle muscular layer, referred to as the myometrium and the outermost serosa, also known as the perimetrium.

The endometrium, the innermost lining of the uterus, displayed an abundance of simple tubular glands in both uterine horns (Fig. 5A and B) and the uterine body (Fig. 7A and B). This layer in the uterine horns was thinner than that in the body of the uterus, ranging from 0.38 to 1.44mm for the uterine horns and from 5.06 to 8.64mm for the uterine body. The highly cellular nonglandular area in Fig. 5A) represents the caruncle.

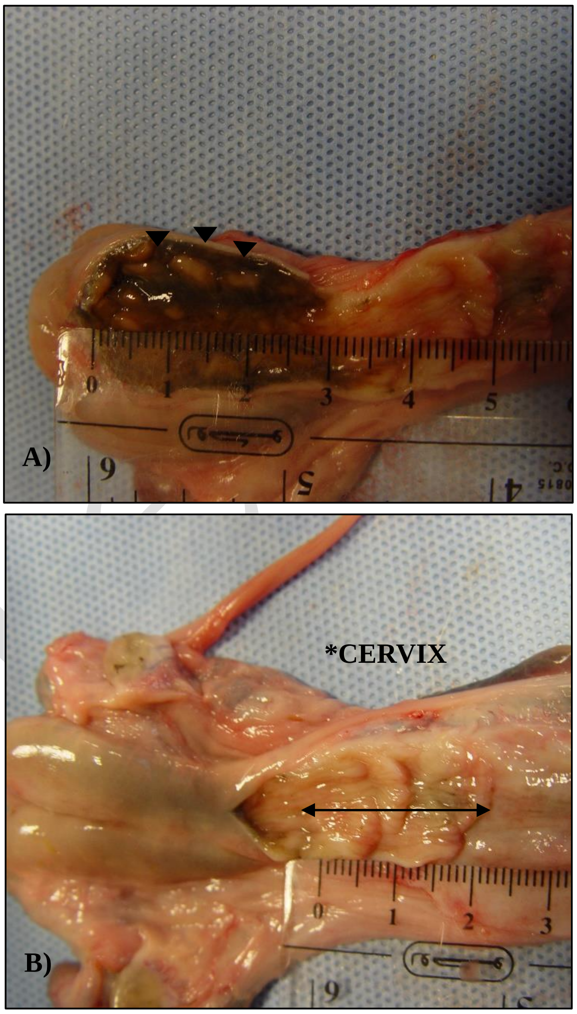

Fig. 4: Bisected reproductive tract of the Arabian sand gazelle showing its internal structure. A) Opened uterus of the Arabian sand gazelle showing the caruncles (arrowheads). B) The arrow points out the Arabian sand gazelle cervix which is composed of three cartilaginous rings; the cervical rings display incomplete encirclement thus facilitating the pathway of semen catheter.

The myometrium was composed of smooth muscle fibres. These muscular fibres were organised into both an inner circular and an outer longitudinal layer, ranging in length from 996.22 to 1000.99µm for the uterine horns and from 2946.50 to 8266.66µm for the uterine body.

The Cervix

Gross Anatomy

The uterine cervix was the lowest region of the uterus, forming a highly convoluted canal attaching the uterus to the vagina. In all gazelles it had a single opening connecting to both respective uterine horns and was composed of three thick-walled, closely spaced cartilaginous cervical rings. The relative position of these rings was difficult to locate by palpating the cervix from

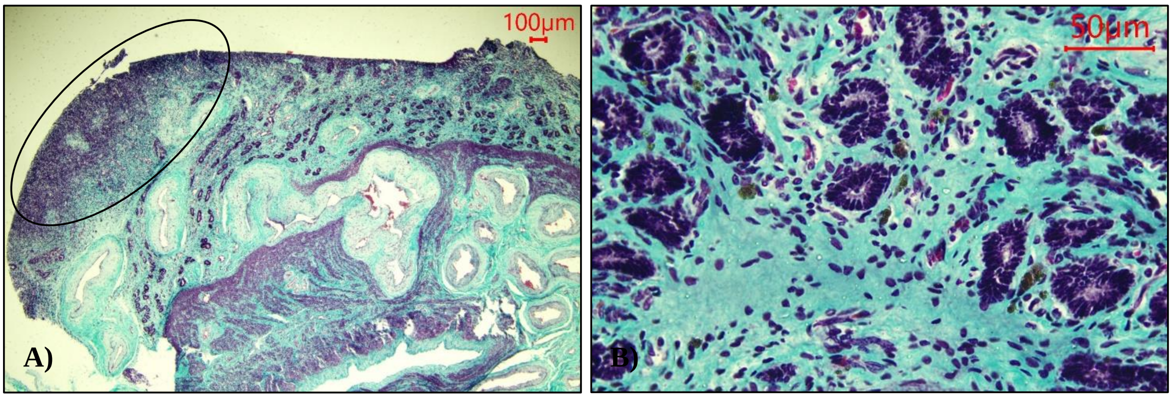

Fig. 5: Histological section of the uterine horn in the Arabian sand gazelle. Masson's trichrome stain. A) The endometrium displays a prominent abundance of simple tubular glands; circle surrounds a caruncle. B) The endometrial glands. Masson's trichrome stain.

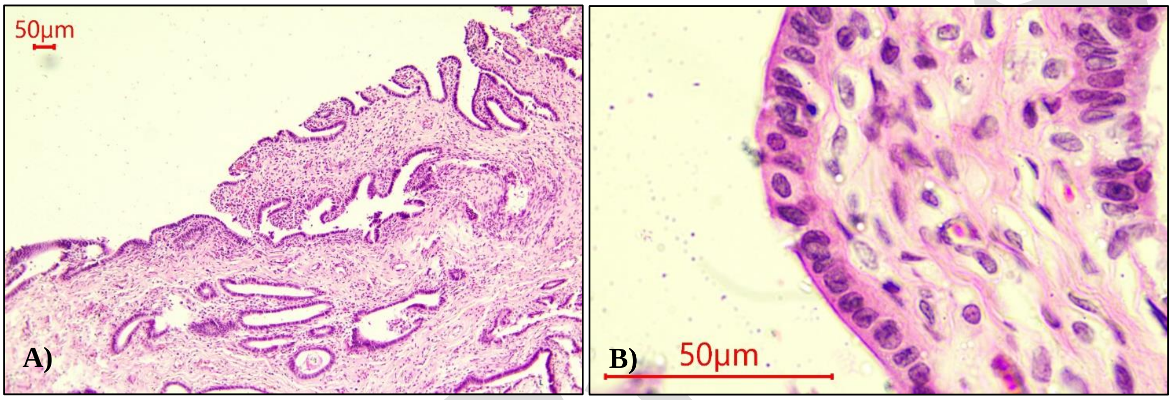

Fig. 6: Histological section of the cervix in the Arabian sand gazelle. H and E stain. A) The mucosa of the endocervix had many high, irregular, thin longitudinal folds, with primary and secondary folds. B) Simple columnar epithelium consisting of two populations of cells. H and E stain.

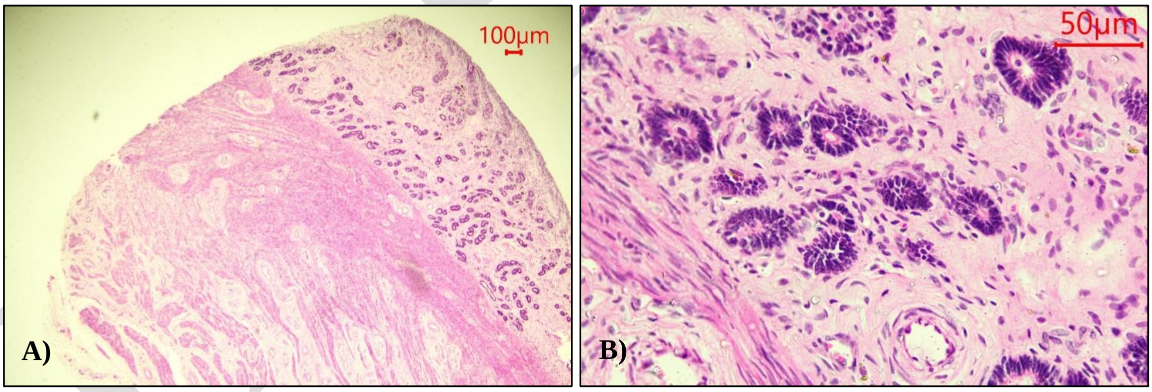

Fig. 7: A) Histological section of the uterine body in the Arabian sand gazelle. B) The endometrial glands in the uterine body. H and E stain.

the outside since the cervical rings display incomplete encirclement, resulting in the absence of a fully formed ring structure; this facilitates the pathway for the introduction of a semen catheter, as shown in Fig. 4B. All the cervical rings were oriented with the lips of the rings extending posteriorly. Measurements were taken by calculating the length from the most anterior portion of the longitudinal folds of the tissue on the inner surface to the most posterior portion of the last valve. The mean length of the cervix was 3.6cm in all age groups.

Microscopic Anatomy

The cervix was lined by a simple columnar epithelium, with some ciliated epithelial cells. The epithelium consisted of two types of cells. The first type had cytoplasm that stained lightly, whereas the second cell type stained heavily (Fig. 6B). The lamina propria mucosae consisted of loose connective tissue. In addition to the mucosal region, the submucosa had abundant mucus-secreting glands (Fig. 6A). The cervical folds were evident. The mucosa of the endocervix had many high, irregular, thin longitudinal folds, with primary and secondary folds. Layers of smooth muscle cells were observed, as evidenced by internal circular muscular fibres. The external muscle was formed by longitudinal fibres and transverse fibres.

The Vagina

The vaginal epithelial cells exhibited a stratified squamous epithelium ranging in depth from 0.04 to 0.29mm (Fig. 8), with an occasional presence of cornified cells. Histological examination of the vaginal epithelium did not reveal significant differences among the adult females nor unique distinguishing characteristics specific to the Arabian sand gazelle compared with those of other ruminant species.

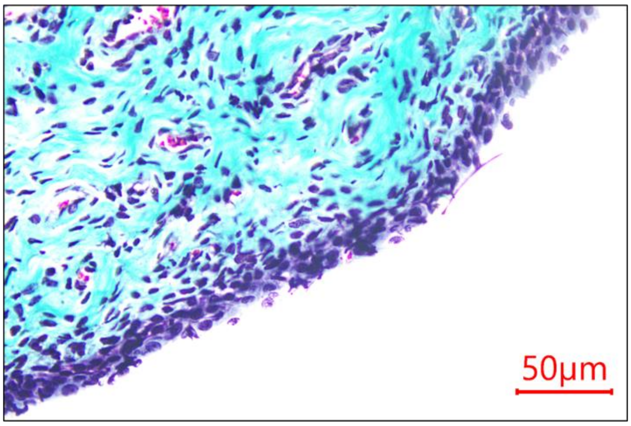

Fig. 8: Histological section of the vagina of the Arabian sand gazelle showing stratified squamous epithelium of the vagina. Masson's trichrome stain.

DISCUSSION

The degree of reproductive seasonality and expression of polyestrous behaviour can vary among ruminant species and between different breeds within a single species. Some breeds appear to be very seasonal, while others show few signs of it (Cosentino et al. 2023). The Arabian sand gazelles are considered seasonal animals, with females exhibiting estrous and anestrous seasons in the Arabian Peninsula (Sempere et al. 2001). Similarly, in Iraq, Radhi et al. (2021) concluded that *G. marica* is a seasonal species, with the primary breeding season taking place in the fall.

There is great variation in the structure and morphology of the reproductive system between mammals (Hradecky 1982; Kanagawa and Hafez 1973). The anatomy and histology of the cervix also can vary among different species of ruminants. For example, some species, such as the Hippotragini, demonstrate duplex uteri, in which the cervix is bifurcated, creating a physical separation between the uterine horns with no uterine body (Wolfe et al. 2010). In contrast, the cervix of domestic ruminants, including sheep and goats, does not have this bifurcation (Wolfe et al. 2010). This variation underscores the importance of characterising the reproductive anatomy of vulnerable Arabian sand gazelles.

Follicles are the primary functional units of the ovaries and are responsible for oocyte development and maturation (Shea 2008). They consist of an oocyte surrounded by granulosa cells and are classified into different stages based on their size and morphology (Groppetti et al. 2019). The growth and development of follicles are regulated by complex hormonal interactions, including follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Kawate 2004). The anatomy of follicles in domestic ruminants has been extensively studied, providing insights into their growth patterns and hormonal regulation (Kandiel et al. 2012). In the Arabian sand gazelle, each ovary is divided into an outer parenchymatous zone (cortex) and an inner vascular zone (medulla). This finding is in agreement with the structure of ovaries in all mammals, except in the mare, where these structures are reversed (Aughey and Frye 2001).

The degree of Müllerian duct fusion, which is species-specific and can be complete, partial, or incomplete, defines the gross anatomy of the uterus (i.e., simplex, bicornuate, or duplex). In rodents, Müllerian fusion is absent or limited, leading to the formation of a duplex uteri. In domestic animals, the Müllerian ducts fuse more posteriorly, resulting in a long uterus (pig) to a medium-length uterus (sheep and cow), which is bicornuate. In contrast, the Müllerian ducts of higher primates (including humans) fuse more anteriorly, resulting in the formation of a single simplex uterus (Spencer et al. 2005).

The Arabian sand gazelle has a bicornate uterus similar to the bovine uterus (Dyce et al. 2009). This uterine type was also found when Hradecky (1982) investigated other antelopes belonging to the subfamilies Alcelaphinae, Tragelaphinae, Reduncinae, Antilopinae, and Aepycerotinae; thus, their findings are strikingly similar to ours.

Dyce et al. (2009) described the disposition of uterine horns as varying from characteristically round in ruminants to straight and divergent in mares and bitches to array in intestine-like loops in sows. In our case, the *G. marica* had uterine horns that were characteristically round, similar to other ruminants.

The characteristic feature of the interior of the uterus of ruminants is the caruncles, which are the attachment sites of the foetal membranes during pregnancy. Each cotyledon and its associated caruncle form a separate unit or placentome, collectively constituting a placenta (Dyce et al. 2009). Thus, the placenta in the Arabian sand gazelle is classified as a cotyledonary placenta.

The uterine caruncles of the Arabian Desert gazelle are macroscopically similar to those of domestic ruminants. Nevertheless, some differences have been identified. Caruncles are distributed throughout the endometria of most antelopes (Hradecký 1983) but have been found only in the uterine horns of the Arabian sand gazelle, while the body of the uterus is free of them. The impala, four-horned antelope, and Indian blackbuck antelope have all been found to have similar features (Hradecký 1983). We expect placentomes to display convex shapes according to the caruncle's shape.

The observed pigmentation of the caruncles in nonpregnant animals, including nyalas, greater kudus, wildebeests, gemsboks, addaxes, roan antelopes, sable antelopes, and springbok, Kirk's dik-dik, red-fronted gazelle and sheep, suggests a link to previous pregnancies (Hradecký 1983). Els (1981) noted instances of brown to dark grey-black pigmentation at the tips (apices) of these caruncles. The source of this pigmentation appears to be associated with blood extravasates in the maternal-foetal attachment.

The anatomy of the reproductive system influences the distribution of pigmentation. Pigmentation has been observed in both horns of species such as nyalas and greater kudus, where a fully developed embryonic membrane occupies both horns of the uterus. In contrast, in antelopes of the subfamilies Hippotraginae and Alcelaphinae, where the embryonic membrane occupies only one uterine horn, caruncle pigmentation is limited to that specific horn (Hradecky 1982).

In rare instances in these latter antelopes, the caruncle displays pigmentation with varying intensities in both horns. This variation in intensity may be associated with the occurrence of two previous pregnancies, one in the left uterine horn and the other in the right uterine horn. These findings contribute to understanding the relationship between reproductive anatomy and permanent traces of previous pregnancies. Our exploration of the reproductive anatomy of the Arabian sand gazelle revealed the presence of this pigmentation in both horns in all the samples.

The uterine body is generally a rather small segment in domestic species; although the proportions vary, it is the largest in the mare. Our studies showed the mean length of the uterine body in the Arabian sand gazelle as 2.548±0.082cm, greater than the 2.0cm uterine body length published for small ruminants (Getty and Sisson 1953) and shorter than ≈3cm and ≈20cm, respectively, in cows and mares (Dyce et al. 2009).

The mean length of the cervix was 3.600±0.160cm. These values are shorter than the published values of 4cm, 10cm and 5.0 to 7.5cm for the respective organs in small ruminants, cows, and mares (Getty and Sisson 1953).

The uterus is lined with simple branched tubular endometrial glands that open into the lumen of the uterus. These glands are straight in carnivores and coiled in the mare, sow, and ruminant (Aughey and Frye 2001). Caruncles, areas of ruminants, lack glandular tissue and are highly cellular; our findings in Arabian sand gazelles are in agreement with those in other ruminants.

The presence of both circular and longitudinal smooth muscle layers in the myometrium of the Arabian sand gazelle uterus is consistent with the typical anatomy found in other bovids, except that the groove between the uterine horns is deeper and reaches the cervix.

A critical aspect of reproduction is the cervix, as it holds pivotal significance in understanding the mechanical insertion of an artificial insemination catheter. The complex anatomy of the cervix in small ruminants can pose challenges for reproductive procedures such as artificial insemination and embryo transfer (Intrakamhaeng et al. 2011; Kershaw et al. 2005). The multiple folds and rings limit the passage of an insemination pipette through the cervix, requiring cervical relaxation to facilitate the deposition of semen into the uterus (Leethongdee et al. 2021). It is inaccurate to attribute the vulnerability and decline in numbers of this species to the challenges in applying reproductive techniques, rather than to the lack of complete understanding of its reproductive morphology. Our results have shown that the cervix is composed of three closely spaced cartilaginous rings and that the cervical rings display incomplete encirclement, thereby resulting in the absence of a fully formed ring structure. Comparing the structure of these rings to those in other ruminants should facilitate the pathway for the introduction of a semen catheter in the Arabian sand gazelle.

Conclusion

In conclusion, the reproductive system of the Arabian sand gazelle is closely related to the anatomical and histological features of domestic ruminants but also presents certain unique characteristics. These findings suggest that the reproductive techniques used in domestic ruminants can be applied to this species. Gaining a comprehensive understanding of these reproductive characteristics and identifying their distinctive traits is of paramount importance in developing reproductive techniques, which is crucial for the successful propagation and conservation of this vulnerable species. As these findings serve as valuable aids in propagating the population of Arabian sand gazelles and controlling their reproduction, it is crucial to pursue further investigations. Future studies, supported by larger sample sizes whenever possible, are warranted to enable a more in-depth and comprehensive examination of the reproductive characteristics specific to this fascinating animal species.

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Authors' Contribution: AE and HA contributed equally to this work. HA and AE were responsible for the study's design and planning. They also oversaw sample collection, supervised laboratory work, and conducted formal data analysis. HA wrote the manuscript, and MBZ and AE contributed to its revision. All authors reviewed and approved the final version of the manuscript.

Data availability statement: The data that supports the findings of this study are available in the material of this article. There is no other supporting data.

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