

Biometry with Quantitative Analysis of Caruncle Distribution in Uterus of Black Bengal Goats (*Capra hircus*)

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With 2 tables & 6 figures

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Abstract

This study aimed to provide basic information regarding the anatomical and histological structure of the uterus and to establish a baseline quantitative analysis of the distribution of caruncles in the different parts of the uterus of Black Bengal Goat (BBG). A total of 64 reproductive organs of female goats were collected from a local slaughterhouse and biometric parameters of different segments of the uterus. The mean length of the right and left uterine horns were 14.90 ± 3.47 cm and 14.79 ± 3.80 cm, respectively. The mean width of the right and left uterine horns were 2.45 ± 0.55 cm and 2.43 ± 0.57 cm, respectively. The lengths of the right and left of the caruncle-free part were 6.46 ± 1.90 cm and 6.45 ± 1.91 cm, respectively. The caruncle-free area was related to the length

of the right ($P=0.0000$) and left ($P=0.0000$) horns of the uterus. The average of the caruncle number in the right and left horns were 65.40 ± 8.81 and 64.93 ± 8.20 , respectively. The quantity of caruncle was strongly influenced by the length of the right ($P=0.0001$) and left ($P=0.0003$) horns of the uterus. The caruncles per row in the right and left horns were 17.72 ± 0.35 and 17.59 ± 0.52 , respectively. The length, width, and number of caruncles in the body of the uterus were 2.71 ± 0.55 cm, 3.34 ± 0.56 cm, and 6.71 ± 1.32 , respectively. The length, width, and number of annular rings in the cervix were 3.54 ± 1.07 cm, 1.81 ± 0.62 cm, and 4.15 ± 0.76 , respectively. Microscopically, the uterine glands were absent in the caruncle areas. The caruncles were circumscribed thickening in the endometrium of the uterine horn and body except

caruncle free area. Histologically caruncle's free area was composed similarly to the uterine horn and body with absence of the caruncles. The present study found that a comparatively less quantity of glands was present in the cervix. The length, width, caruncle-free part, quantity of caruncle, and caruncle per row were apparently higher values in the right uterine horns but statistically non-significant. All the findings mentioned above have established the baseline for the parameters of different segments of the uterus with quantitative analysis of the caruncles of the BBG and may be utilized by physiologists, gynecologists, embryo-transfer technologists, animal breeders, and other related workers for the development of goat production.

Keywords: Black Bengal goat, uterus, biometry, distribution, caruncle.

Introduction

One of the most effective ways to help the poorest people in Bangladesh escape poverty and increase their earnings is through goat-rearing (Prank et al., 2023). Goats are a valuable livestock resource in Bangladesh, especially the Black Bengal goats (BBG). The BBG is the most well-known goat breed in Bangladesh and is famous for its unique characteristics such as early sexual maturity, high fertility, and prolificacy (Apu et al., 2023). In the year 2022-23, there were about 26.9 million goats in Bangladesh of which about

90% were Black Bengal goats (BBS, 2019; DLS, 2022). Its skin is also highly valued in the global market due to its special qualities of yielding fine leather that is low in weight and fine in texture (Haque et al., 2016). Small ruminant meat is acceptable to all segments of society regardless of religion, especially goat meat has a ready market due to the heavy demand (Kumar et al., 2018). Reproductive performance is economically significant in small ruminants because of its impact on the quantity of offspring produced per year (Greyling, 2000). To maintain an optimal reproduction performance a clear understanding of the reproductive organs of small ruminants is essential. The female's reproductive tract biometry provides information about the animal's general health (Kunbhar et al., 2003). The female genital system consists of two ovaries, two uterine tubes (also known as oviducts or fallopian tubes), a uterus, a vagina, and vulva. The ampulla, infundibulum, and isthmus are the three parts of the oviduct. The uterus is made up of two horns, a body, and a cervix. The vagina connects the uterus with the vulva. Vestibules, clitoris, and two labia make up the vulva (Kumar et al., 2018). The uterus has caruncles inside it that are thought to be gland-free, and there are a lot of microscopic endometrial glands in the intercaruncular spaces (Gray et al., 2001). The normal growth and development of the caruncles in the ruminant uterus are crucial to maintaining gestation and fetus health (Zhou et al.,

2023). The uterine caruncles and chorionic cotyledon in ruminants have a close relationship that results in the placentome (Igwebuike, 2009). Placentomes are specialized regions that facilitate the hemotrophic exchange of nutrients and metabolites between the mother's and the fetus' bloodstreams (Igwebuike & Ezeasor, 2013). Moreover, it is crucial to understand the biometric properties of female genitalia to diagnose pregnancies, manage infertility issues, and execute artificial insemination (Kumar et al., 2004). Successful in vitro fertilization (IVF), placental efficiency, cotyledon efficiency, and evaluation of placentation requires a good knowledge of uterus biometry (Carter, 2019; Cognie et al., 2003; Ocak et al., 2015). Although several studies have been done about the female genital system of BBG, little attention has been made specifically details investigating the biometry and distribution of caruncle in the different parts of the uterus. Therefore, the goal of this study is to provide basic information regarding the anatomical structure of the uterus and to establish a baseline quantitative analysis of the distribution of caruncles in the different parts of the uterus of BBG.

Materials and methods

Animal selection and sample collection

A total of 64 reproductive organs of female BBG were collected from Riaz Uddin Bazar slaughterhouse in the

Chattogram district of Bangladesh from November 2023 to January 2024. Adult BBGs were selected by phenotypical characteristics and dentition. The goats contained a minimum of two permanent teeth present the goat's reproductive organs were collected. The samples were taken in plastic bags and transported to the laboratory in the Department of Anatomy and Histology, Chattogram Veterinary and Animal Sciences University within 40 minutes.

Measurement and sample processing

The uterus was washed with clean water and the adipose tissue or other surrounding was removed by dissection. The length and width were measured with the aid of a scale. Then the uterus was opened by the surgical blades in the longitudinal direction and counting of the caruncles number in the different parts of the uterus and annular rings of the cervix. The samples were collected from the caruncle-free area of the uterus, the horn, the body, and the cervix for histological examination. The specimens were cut and fixed by immersion in Bouin's fluid for 48 hours. The specimens were dehydrated in gradually increasing concentrations of alcohol, cleaned by xylene, infiltrated, and embedded in paraffin. Then microscopic sections were cut at 6µm thickness using a sliding microtome (Leica SM2010R V1.2 English-09/2008 Sliding Microtome, Germany) with the help of a disposable Carbon blade. The water bath was set at 60°C temperature.

Slides were labelled with a diamond pencil as labelled with a tag. Gelatine was used as an adhesive to attach the section to a glass slide. Then the slide was dried in air for 12 hours before staining. The tissues were stained with haematoxylin and eosin according to the standard protocol (Cardiff et al., 2014) for visualization under the light microscope.

Histomorphometry

To obtain histomorphometries, a microscopic examination of the different parts of the uterus and photomicrographs were taken using a photomicroscope (Am Scope Trinocular Compound Microscope with 1.3 MP Camera, Model T490 B-MT) and AmScope image measuring software (x86, 3.7.3036 version).

Data analysis

At first, all data obtained from this study were entered and stored in Microsoft Excel Professional 2020 (Microsoft Corporation, USA). Then data were transferred to statistical software, STATA-16 (STATA Corp., Texas, USA) to perform statistical analysis. Unpaired sample t-test to compare means of different variables between two groups. A p-value of equal to or less than 0.05 ($P \leq 0.05$) was considered significant for this test. Results were expressed as arithmetic mean \pm standard deviation (Mean \pm SD).

Results and Discussion

In this study, the right and left uterine horns extended from the uterotubal junction with the fallopian (uterine) tubes and ran caudally to join the body of the uterus. A small section of the uterus is made up of the body, extending from the cranial places of entry of the paired uterine horns to the firm, constricted cervix caudally (Fig. 1). The mean length of the right and left uterine horns were 14.90 ± 3.47 cm and 14.79 ± 3.80 cm, respectively (Table 1). The result was supported by (Adigwe & Fayemi, 2005) who reported that the mean lengths of the uterine left and right horns were 14.43 ± 0.94 and 14.17 ± 1.33 cm, respectively in the red Sokoto goats. Closely similar result was further reported by (Kumar et al., 2018) the mean length of the horn's right and left sides were 14 ± 0.61 cm and 12.5 ± 0.63 cm respectively in the BBG. Comparatively, a lower value was reported by (Gupta et al., 2011). The length of uterine horns in the BBG was within (10-12 cm) as reported by (Sisson & Grossman, 1947) in small ruminants. There were no statistically significant differences ($P > 0.05$) in the length of the right uterine horn from that of the left uterine horn (Table 1). The right uterine horns were apparently larger than the left but statistically insignificant. Contrastingly, (Uddin et al., 2021) reported that the length of the right horn was significantly ($P \leq 0.05$) higher than the left horn in the 19-24 m age group.

The mean width of the right and left uterine horns were 2.45 ± 0.55 cm and 2.43 ± 0.57 cm, respectively (Table 1). The result was supported by (Uddin et al., 2021) who mentioned that the mean widths of the right and left uterine horns were 2.80 ± 0.07 and 2.42 ± 0.07 cm at 19-24 months age of BBG. A similar result was reported by (Gupta et al., 2011) who found that the average width of the uterine horn was 2.50 ± 0.112 cm in the BBG. Comparatively lower value was mentioned by (Adigwe & Fayemi, 2005) in Sokoto goat of Nigeria. There were no statistically significant differences ($P > 0.05$) in the width of the right uterine horn from that of the left uterine horn (Table 1). According to (Uddin et al., 2021), the width of the right horn was significantly ($p \leq 0.05$) higher than the left horn.

Within the horn and body of the uterus, numerous caruncles were presented. The caruncles were regularly longitudinally arranged by four rows in the horn of the uterus and comparatively fewer numbers were irregularly arranged in the body of the uterus. The cranial part of the uterus was free of the caruncles. This portion is called the caruncles-free area (Fig. 1). The mean lengths of the right and left of the caruncle-free part were 6.46 ± 1.90 cm and 6.45 ± 1.91 cm, respectively. There were no statistically significant differences ($P > 0.05$) in the length of the right and left of the caruncle-free part (Table 1). However, the free part of the caruncle in BBG

was lower than that mentioned by (Suri et al., 2013) in Bakerwali Goat of India. It was further reported that the free part of the caruncle had higher values in the left one in 2-3 years goats but was statistically non-significant within the group. A similar result was also reported by (Abiaezute et al., 2017) who found the absence of caruncles in the cranial one-third of the uterine horns in WAD goats of all ages. To prevent the implantation of fetuses in the small, narrow, and spiral-shaped cranial portion of the uterine horn (Abd-Elnaeim, 2008). The caruncle-free area was related to the length of the right ($P=0.0000$) and left ($P=0.0000$) horns of the uterus (Fig. 3). The increased caruncle-free area along with increased the right and left horns of the uterus.

The means of the caruncle number in the right and left horns were 65.40 ± 8.81 and 64.93 ± 8.20 , respectively. There were no statistically significant differences ($P > 0.05$) in the number of caruncles in the right and left horns (Table 1). This result was supported by (Lyngset, 1968) who reported that the number of caruncles in the two uterine horns was 61.3 caruncles in the right and 60.7 caruncles in the left uterine horn. Basil et al., (1961) found that the average of 51.01 in the left horn and 51.14 in the right one. Further reported that the average number of caruncles and cotyledons were 102 and 80.47, respectively. It was very difficult to estimate the extent to

which all of the caruncles took part in the placentation at the stage of pregnancy (Basil et al., 1961). The quantity of caruncle was strongly influenced by the length of the right ($P=0.0001$) and left ($P=0.0003$) horns of the uterus (Fig. 2).

The means of the caruncles per row in the right and left horns were 17.72 ± 0.35 and 17.59 ± 0.52 , respectively. There were no statistically significant differences ($P > 0.05$) in the caruncles per row in the right and left horns (Table 1). A previous study found that the caruncles appear in four rows, as in the cow (Martin, 1912).

The means length, width, and number of caruncles in the body of the uterus were 2.71 ± 0.55 cm, 3.34 ± 0.56 cm, and 6.71 ± 1.32 , respectively (Table 2). A similar result in BBG was reported by (Uddin et al., 2021) who found that the length and width of the uterine body were 2.43 ± 0.08 cm and 3.42 ± 0.10 cm, respectively. The mean length of the uterine body in BBG was closely similar to the value reported by (Gupta et al., 2011; Suri et al., 2013) but lower than that by (Adigwe & Fayemi, 2005). The uterus's well-developed body was 1.59 ± 0.21 cm on average in length, with a range of 1.8 to 1.5 cm (Kumar et al., 2018). According to (Lyngset, 1968), the average number of caruncles in the uterine body was 4.71 ± 0.28 .

The mean length, width, and number of the annular rings in the cervix were 3.54 ± 1.07 cm, 1.81 ± 0.62 cm, and

4.15 ± 0.76 , respectively (Table 2). The length and width of the cervix recorded in this study were almost similar to the value reported by previous studies (Gupta et al., 2011; Talukder et al., 2015; Uddin et al., 2021). Contrastingly, (Adigwe & Fayemi, 2005) reported that the length and width of the cervix were 2.59 ± 0.61 cm and 1.07 ± 0.17 cm, respectively. The result was supported by (Dayan et al., 2010) who mentioned that the mean cervical folds in the cervix were 4.3 in the Angora goat. The ovine cervix is a fibrous tube whose lumen contains 3-7 annular folds or rings (Kershaw et al., 2005; Naqvi et al., 2005). Small ruminants have a complex anatomical cervix, which is a sphincter-like structure at the base of the uterus. It is made up of a fibromuscular canal with several tissue folds or rings. Breed-specific differences exist in the cervix's size and form, which can also be influenced by the female's age, history, and reproductive cycle (Dayan et al., 2010; Kershaw et al., 2005).

Histologically, the uterine wall (uterine horn and body) consists of three layers i.e., the mucosa and submucosa or endometrium, the tunica muscularis or myometrium, and the serosa or perimetrium. The endometrium was composed of the tunica mucosa and submucosa. The lining epithelium was simple columnar cells. The simple coiled, branched tubular glands were present throughout the endometrium. The uterine glands were absent in the

caruncle areas. The caruncles were circumscribed thickening in the endometrium of the uterine horn and body except caruncle free area. Histologically caruncle's free area was composed similarly to the uterine horn and body but with absence of the caruncles (Figure 5). The finding was in agreement with (Kumar et al., 2020) who have also described the uterine glands identified in the histological slides of the cranial part of the horn, middle, and base of the uterus. The numerous simple tubular branched uterine glands were located under the epithelium and these glands were very vascular and cellular containing reticular-like connective tissue (Nickel et al., 1979). These glands extend up to the inner circular muscle layer of tunica muscularis (Saleem, 2022). The caruncles were rich in fibroblasts and had an extensive blood supply (Gray et al., 2003). Myometrium consists of an inner circular and outer longitudinal layer of smooth muscle cells. These present findings were similar to the findings of (Saleem, 2022) who reported that the tunica muscularis was thicker in the uterine body as compared with the uterine horn. Myometrium was composed of a comparatively thick inner layer separated from each other by large arteries, veins, and lymph vessels. These blood vessels were especially large in the caruncle region (Dellmann & Brown, 1976). Perimetrium consists of loose connective tissue covered by the peritoneal mesothelium. This is in agreement with

(Kumar et al., 2020) who reported that the peritoneum was attached to the outer longitudinal muscle layer and composed of a thin layer of elastic fibers, vessels, and nerves.

The cervix of the uterus was a comparatively thick muscular wall, and rich in elastic fibers. The mucosa and submucosa form high primary folds with low secondary folds. The lamina mucosa was a simple columnar epithelium with many goblet cells (Figure 6). The finding was supported by Abiaezute et al., (2021) who reported that the cervix of the West African Dwarf (WAD) goat presented longitudinal cervical mucosal folds (primary folds) from which secondary and sometimes tertiary cervical folds extended from into the lumen. It was further reported that the tunica mucosa/submucosa formed cores of the cervical mucosal folds. The present study found that a comparatively less quantity of glands was present in the cervix. This result was supported by (Salih & Abass, 2014) who mentioned that simple tubular and branched glands were found in the cervix uteri (in the surface epithelium toward lamina propria) of Black goats in Iraq. Another previous study supported this finding with (Joshi et al., 1976). The cervical mucus was produced by the cervical glands in addition to the surface epithelium of the cervical mucosal lining (Salih & Abass, 2014). However, this finding was disagreed with Abiaezute et al., (2021); Dellmann & Brown, (1976). Abiaezute et al.,

(2021) reported that cervical glands were not observed in the cervix of the WAD goat. It was further reported that the cervical epithelial cell glands were fully developed with highly secretory activities in adult WAD goats. The tunica muscularis consists of an inner circular and outer longitudinal layer of smooth muscles. These present findings were similar to the findings of Samuelson, (2007). The tunica serosa consists of loose connective tissue. This is in agreement with Fuchs et al., (1996) the tunica serosa is composed of a loose connective tissue framework with blood vessels and lined by simple squamous epithelium.

The results of this study have measured the baseline for the dimensions of various segments of the uterus and quantitative analysis of the caruncles of the BBG in Bangladesh. This information will facilitate the diagnosis of different abnormalities of these organs more easily. More work on other breeds, like the Jamu-napari, cross-breed, and the causes of differences between breeds and species seems necessary for a better understanding of the reproduction in these animals.

Conclusion

The present study recorded the absence of caruncles in the cranial one-third of the uterine horn. The length, width, caruncle-free part, quantity of caruncle, and caruncle per row were apparently in higher values in the right

uterine horns but statistically non-significant which explains that maybe the right horns of the uterus were more active than the left. The number of caruncle and caruncle-free parts has significantly correlated with the length of both uterine horns. Microscopically, the uterine glands were absent in the caruncle areas. The caruncles were circumscribed thickening in the endometrium of the uterine horn and body except caruncle free area. Histologically caruncle's free area was composed similarly to the uterine horn and body but with the absence of the caruncles. The present study found that a comparatively few numbers of glands were present in the uterine cervix.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Table (1): The mean length, width, caruncle free part, caruncle number, and caruncle per row in each uterine horn of female BBG.

Horn of the uterus	Categories	Mean± SD	P value
Length (cm)	Right	14.90± 3.47	*
	Left	14.79± 3.80	
Width (cm)	Right	2.45± 0.55	*
	Left	2.43± 0.57	
Caruncle free part (cm)	Right	6.46± 1.90	*
	Left	6.45± 1.91	
Caruncle (no.)	Right	65.40± 8.81	*
	Left	64.93± 8.20	
Caruncle per row (no.)	Right	17.72± 0.35	*
	Left	17.59± 0.52	

* Non-significance (P > 0.05); ** Significance (P ≤ 0.05)

Table (2): The measurement of different parameters of the body of the uterus and cervix of the female BBG.

Body of the uterus	Mean± SD	Cervix	Mean± SD
Length (cm)	2.71± 0.55	Length (cm)	3.54± 1.07
Width (cm)	3.34± 0.56	Width (cm)	1.81± 0.62
Caruncle (no.)	6.71± 1.32	Annular ring (no.)	4.15± 0.76

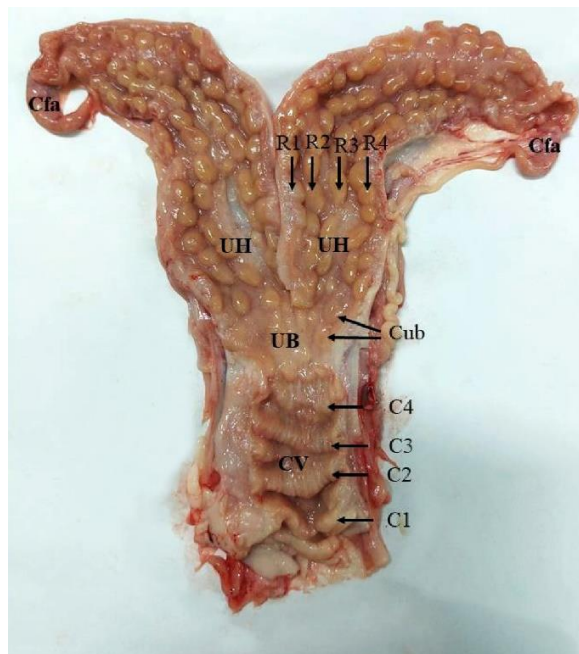


Fig. (1): Gross structure of the uterus of the BBG showing part of the cervix (CV), the body of the uterus (UB), the horn of the uterus (UH), the caruncle-free area (Cfa), and caruncle of the uterine body (Cub). C1, C2, C3, and C4 indicate the annular rings in the cervix, and R1, R2, R3, and R4 indicate the caruncle row in the horn of the uterus.

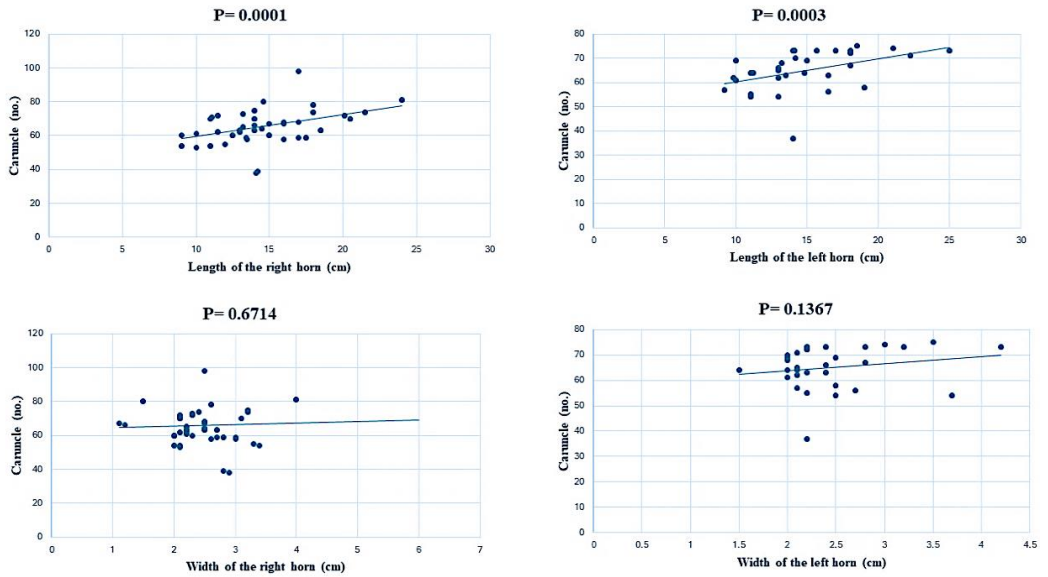


Fig. (2): Bivariate linear regression showing effects of length and width of the right and left uterine horn (cm) on the caruncle (no.) of the BBG (N=64).

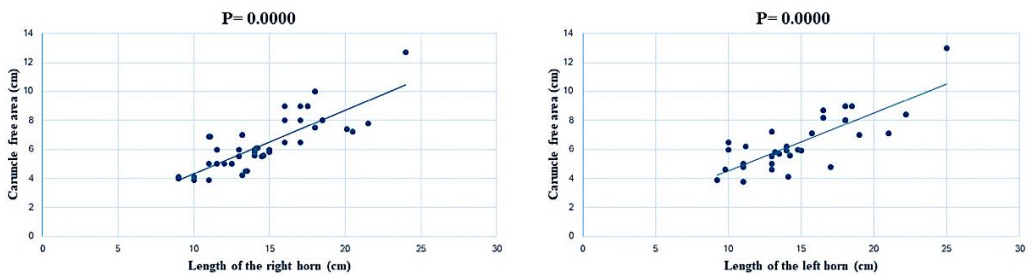


Fig. (3): Bivariate linear regression showing effects of length of the right and the left horn of the uterus (cm) on the caruncle-free area (cm) of the BBG (N=64).

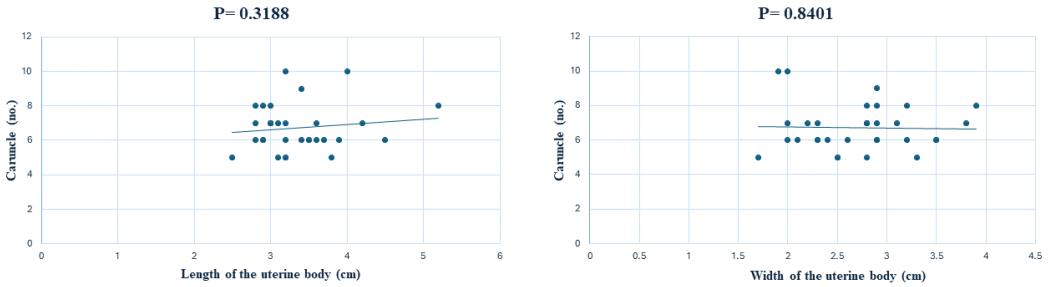


Fig. (4): Bivariate linear regression showing effects of length and width of the body of the uterus (cm) on the caruncle (no.) of the BBG (N=64)

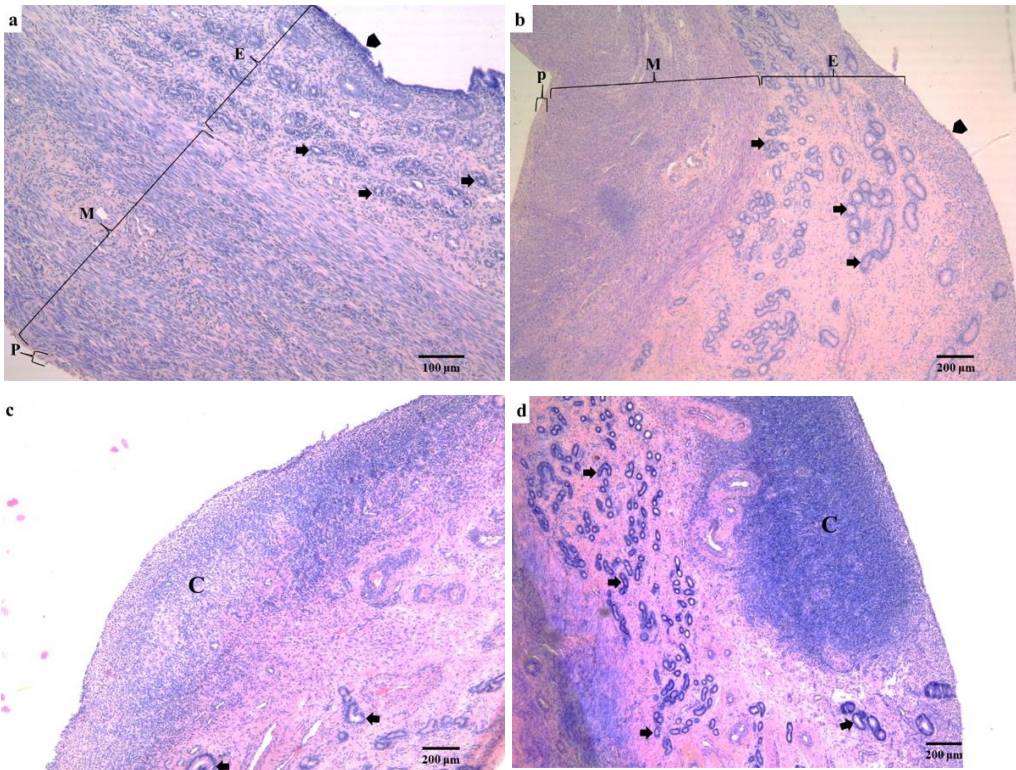


Fig. (5): Haematoxylin and eosin staining of the different segments of the uterus: a caruncle free-area, b & c) horn of the uterus, and d; body of the uterus) from the reproductive system of the BBG. The arrowhead and arrow in the images indicate where are the lining epithelium and uterine glands. Section through the uterus in Goats, E Endometrium, M Myometrium, P Perimetrium, and C Caruncle.

Scale for low magnification: 200 µm, for high magnification: 100 µm.

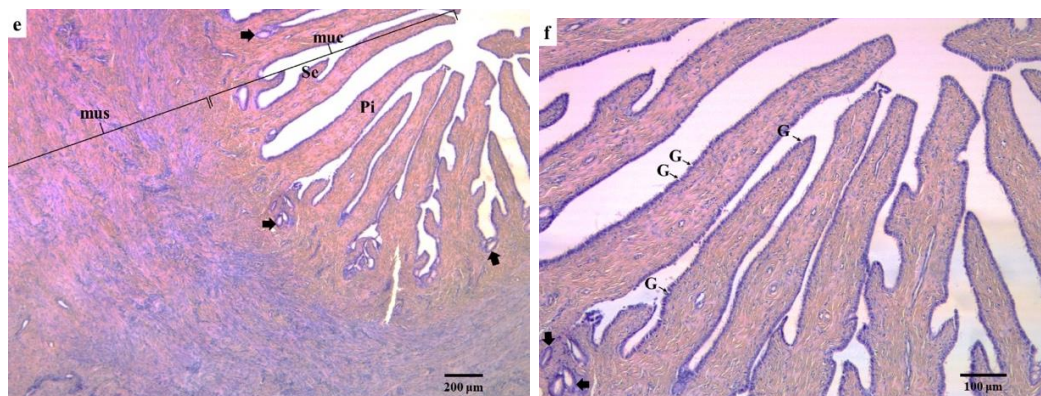


Fig. (6): Haematoxylin and eosin staining of the cervix of the uterus (e & f; cervix) from the reproductive system of the BBG. The arrowhead and arrow in the images indicate the lining epithelium and cervical glands. Section through the cervix in Goats, muc tunica mucosa- submucosa, mus tunica muscularis, Pi Primary mucosal folds, Se Secondary mucosal fold, G Goblet cell.

Scale for low magnification: 200 µm, for high magnification: 100 µm