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Original article

Morphological assessment and characterization of uterine caruncles in Bengal goats

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ABSTRACT

This study aimed to evaluate and characterize the gross, histologic and ultrasonographic features of uterine caruncles in Bengal goats, an indigenous breed in Bangladesh. A total of 40 uteri were collected from sexually mature, pregnant and non-pregnant does obtained from local slaughter houses, preserved and processed in for gross morphological and histological characterization of uterine caruncles. Additionally, transabdominal ultrasonographic characterization of uterine caruncles were performed on randomly selected does at a commercial goat farm. Gross examination revealed multiple dome-shaped caruncles distributed along the endometrial surface, primarily arranged in four longitudinal rows. In both cyclic and non-cyclic goats, the average number of uterine caruncles was higher in the left uterine horn (55.85) compared to the right (54.42), with a consistent average of 4.30 rows observed in both horns. Histological examination of the caruncles revealed dense connective tissue, numerous blood vessels, and abundant uterine glands. The surface epithelium ranged from simple cuboidal to columnar. Morphometric analysis showed that the large caruncles were located in the mid-uterine horns. Transabdominal ultrasonography identified pregnancy in 7 of 20 does (35%) and visualized uterine cotyledons between days 32 and 40 of gestation. Measurement of cotyledons via ultrasound may serve as a reliable indicator of gestational age. These findings provide baseline data that may support reproductive research, enhance breeding management, and contribute to the assessment of reproductive performance and productivity of goats in Bangladesh.

Keywords: caruncles, goat, morphology, pregnancy, ultrasound, uterus

INTRODUCTION

Bangladesh's extensive farming system relies heavily on livestock. It makes up roughly 1.85% of the gross domestic product (GDP) overall, with a growth rate of 3.23% in fiscal year (FY) 2022-2023 (DLS, 2023). The majority of livestock in Bangladesh are small ruminants, particularly sheep and goats; approximately 80% of rural residents work in livestock farming (Siddiki *et al*., 2010). For many small farmers, particularly women, landless and marginal farmers who

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reside in remote areas and infrequently have other sources of income, small ruminant farming is a significant source of income (Choudhury *et al*., 2012). In Bangladesh, there are roughly 30.7 million small ruminants (sheep and goats), of which 26.9 million are goats (DLS, 2023). Goats are an important livestock resource with a well-established market, a shorter time to slaughter and higher productivity per unit of investment. Goats produce about 20,400 metric tons of meat annually or 25% of Bangladesh's total supply of red meat (Ferdous *et al*., 2011). Small ruminants also contribute significantly to the generation of foreign exchange earnings through the sale of skins and other by-products. In Bangladesh's rural areas, the smallholder traditional management system is used to raise the greatest number of goats. Because they can be swiftly slaughtered when revenue is needed, smallholder farmers greatly depend on their small ruminants for their livelihoods.

The uterus, a tubular hollow organ in the female reproductive system, serves as the site for fetal implantation and development during pregnancy. Goats have a bicornuate uterus composed of one uterine body, one cervix, and two uterine horns. According to Dyce *et al*. (2002) and Budras and Habel (2003), adult ruminants possess a Y-shaped, muscular, expandable bicornuate uterus with a small body and two long tapering horns connected caudally to the cervix and cranially to the uterine tubes. The uterus facilitates sperm transport, luteolysis, cyclicity regulation, and provides nourishment and protection to the embryo and fetus, aiding in their expulsion at delivery. In adult goats, the uterine epithelium changes from simple cuboidal to pseudostratified columnar (Singh and Prem Prakash, 1990). The uterine mucosa contains mushroom-shaped caruncles, gland-free areas, separated by intercaruncular spaces rich in endometrial glands (Atkinson *et al*., 1984; Gray *et al*., 2001). Ruminants exhibit synepitheliochorial placentation, where histotroph is secreted by intercaruncular glands, and caruncles serve as implantation sites. The placenta consists of fetal cotyledons and maternal caruncles, collectively called placentomes (Amoroso, 1952; Bazer, 1975; Gray *et al*., 2001; Igwebuike, 2009).

In addition, fertile breeding cannot be determined because natural breeding dates in most goat flocks are either unrecorded or not observed. Moreover, precise data regarding the gestational stage would be helpful in tracking does in the near future (Doize *et al*., 1997). From an economic perspective, it is especially crucial to identify goat pregnancy early and precisely because doing so allows breeding technology to be scheduled and the flock to be divided into pregnant and non-pregnant goats (Yotov *et al*., 2005). Early pregnancy diagnosis with ultrasonography would enable culling or rebreeding of barren does, as it is a dependable technique (Doize *et al*., 1997). Pregnancy in small ruminants can be accurately, quickly and safely diagnosed with B-mode ultrasonography. Approaches such as transrectal or transabdominal could be utilized with about 100% accuracy (Karen *et al*., 2006; Abdelghafar *et al*., 2007). Maintaining fetal health and gestation depends on the normal growth and development of the ruminant uterine caruncle (Zhou *et al*., 2023). In ruminants, the placentome is a close interaction between the uterine caruncles and the chorionic cotyledon (Igwebuike, 2009). A specialized areas called the placentomes allows the fetal and maternal bloodstreams to exchange nutrients and metabolites hemotrophically (Igwebuike and Ezeasor, 2013).

In goats, measurement of cotyledon via transabdominal ultrasonography can assist in estimating gestational age. However, in ewes, cotyledon size shows a weak correlation with gestational age. Therefore, observation of gross morphology, histology and transabdominal ultrasonography of uterus especially the uterine caruncles can be a great source for the identification of cyclic, non-cyclic and pregnant goats. On the other hand, the diseases and disorders of animals are the biggest obstacle to the growth of livestock in our country (Islam *et al*., 2001). This study aims to contribute to the understanding of normal versus pathological reproductive conditions in goats, potentially

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supporting improved reproductive management and productivity.

MATERIALS AND METHODS

A total of 40 fresh reproductive tracts from female goats were collected from local slaughter houses located at Town hall and Krishimarket, Mohammadpur, Dhaka-1207 and transported directly to the laboratory within four hours post-slaughter.

Processing of female reproductive tract

Each reproductive tracts were skinned, and the uterus, including horns, body, and cervix, was carefully isolated and trimmed of excess tissue. The right and left uterine horn were identified based on the presence of corpus luteum on the ovaries. Tissues intended for histological examination were fixed in 10% neutral buffered formalin.

Histological observation

Tissue processing

Morphological analysis was conducted to examine the structural organization of uterine caruncle tissues using histological techniques. Uterine caruncles were dissected and fixed in 10% neutral buffered formalin for 18-24 hours. Following fixation, tissues were washed in distilled water for 10 minutes and then dehydrated in a graded ethanol series (70%, 80%, 90% for 2 hours each, 95% overnight, and 100% for 1 hour in three changes). Dehydrated tissues were then cleared in xylene (three changes, 30 minutes each), infiltrated with paraffin, embedded, and cooled to prepare for sectioning and staining.

Hematoxylin and Eosin (H&E) Staining

For H&E staining, paraffin-embedded tissue sections were first deparaffinized in xylene (three changes, 5 minutes each), then rehydrated through a descending ethanol series: 100% ethanol (three changes, 5 minutes each), followed by 95%, 90%, and 80% ethanol for 1 minute each, and 70% ethanol for 5 minutes. Sections were rinsed under running tap water for 10 minutes, stained with hematoxylin for 30 seconds, and rinsed again for 10 minutes. Differentiation was performed with a single dip in 1% acid alcohol, followed by a 2-minute rinse in water. Sections were then counterstained with eosin for 5 minutes and 30 seconds. Dehydration was performed using 70%, 80%, and 90% ethanol (two dips each), 95% ethanol for 1 minute, and 100% ethanol (three changes, 2 minutes each). Finally, sections were cleared in xylene (three changes, 5 minutes each) and mounted with a coverslip for microscopic examination.

Microscopic observation

The stained slides were examined under a light microscope at 4x, 10x, and 40x magnifications. This enabled the histological differentiation between cyclic and non-cyclic uterine caruncles.

Characterization of gravid uterine cotyledons using trans-abdominal ultrasonography

Pregnancy was diagnosed using a real-time B-mode ultrasound scanner with a 3.5 MHz probe and ultrasonic gel. Goats were fasted for 12 hours prior to scanning, which was performed on a fleece-free inguinal area while the animals stood restrained. The hind legs were flexed for proper probe placement, with gel applied to ensure good contact between the probe and skin.

Characterization of gravid uterine cotyledons and confirmation of pregnancy

Following mating or artificial insemination, the selected goats were scanned twice weekly from day 30 to day 40 to identify the earliest day of pregnancy detection. Pregnancy was identified by imaging the apparent conceptus (anechoic, elongated structure) within the uterine fluid. The accuracy of the pregnancy diagnosis was confirmed by comparing ultrasonographic findings with actual kidding. A total of 20 observations were recorded, among which 7 pregnant goats were used for the assessment of fetal growth features. The timing of placentome appearance was also investigated.

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Data analysis

Numerical data that were recorded had been stored in MS Excel spread sheet and descriptive statistics (percentages) was computed. The results were expressed as mean ± standard error (SE) and were analyzed using nonparametric methods. Nonparametric methods are statistical techniques that do not require data to follow a specific distribution, making them useful for small samples or non-normal data.

RESULTS

Gross morphology of uterine caruncles

The bicornuate uterus of the goat comprises symmetrical, convex uterine horns attached to a relatively short uterine body, with the coiled horns positioned ventrally. In non-cyclic goats, uterine caruncles were regularly spaced and arranged longitudinally in three to four rows (Figure 1A) while in cyclic goats, they appeared in three to five rows (Figure 1 B). The uterine caruncles of cyclic goats exhibited greater tonicity compared to those of non-cyclic goats. Numerous prominent mucosal thickenings, oval to quadrilateral-shaped (caruncles) were visible in both groups (Figure 1 A, B). Caruncles were irregularly arranged and less conspicuous in the cranial third of the uterine horns but appeared more regularly arranged and numerous in the middle and caudal thirds, as well as in the uterine body. Uterine mucosal folds were also observed between caruncles. In non-cyclic goats, caruncles were relatively small and creamy in appearance (Figure 1 A) whereas in cyclic goats they were more prominent and varied in color from pale to pinkish (Figure 1 B).

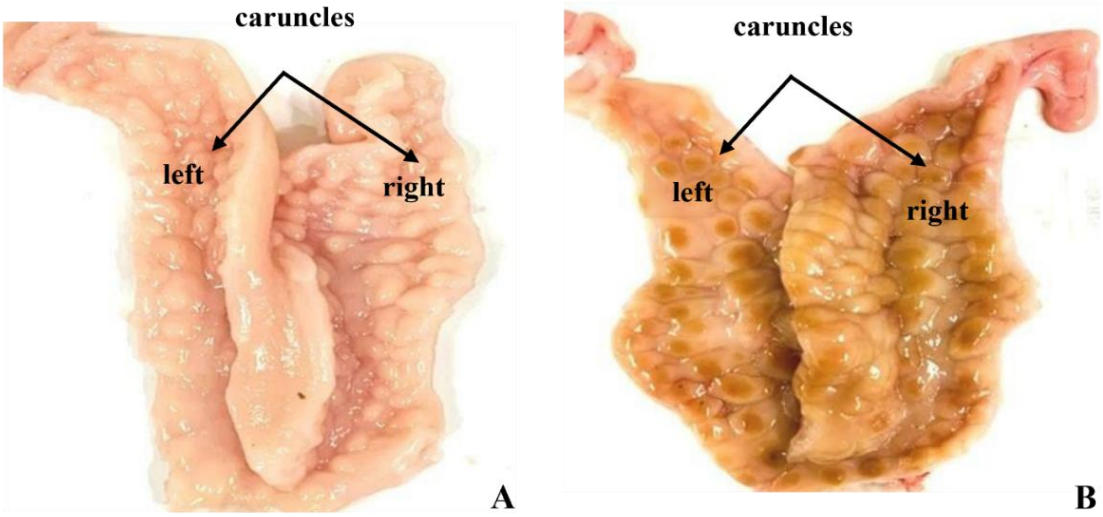

Figure 1 Gross morphology of uterine caruncles in A) non-cyclic and B) cyclic goats. Arrows indicate uterine caruncles.

Morphometry of uterine caruncles

The average number of uterine caruncles was higher in the left uterus compared to the right in both cyclic and non-cyclic goats (Figure 2), although the average number of caruncular rows was similar on both sides (Figure 3). The arrangement of caruncles within rows varied slightly among individuals. Most goats had three to four rows, while a few exhibited five rows with comparatively fewer caruncles (Figure 4). In the right uterine horn, the highest average number of caruncles was observed in the second row (12.72), while the lowest was in the fifth row (4.92). In the left uterine horn, the highest average was found in the first row (14.2) and the lowest in the fifth row (2.85)

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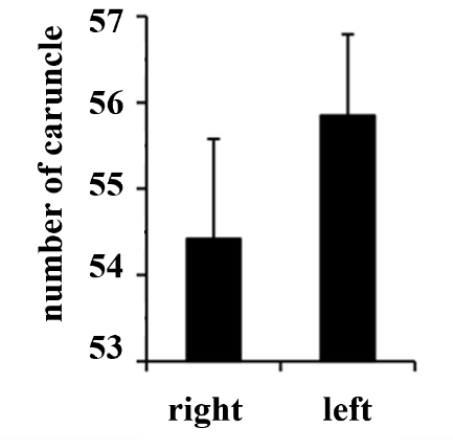

Figure 2 Average number of caruncles in the right and left uterine horns of goats.

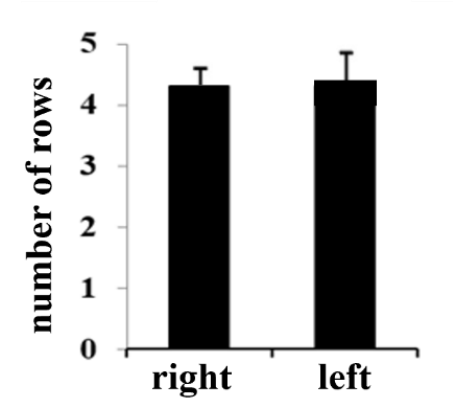

Figure 3 Average number of caruncle rows in the right and left uterine horns of goats.

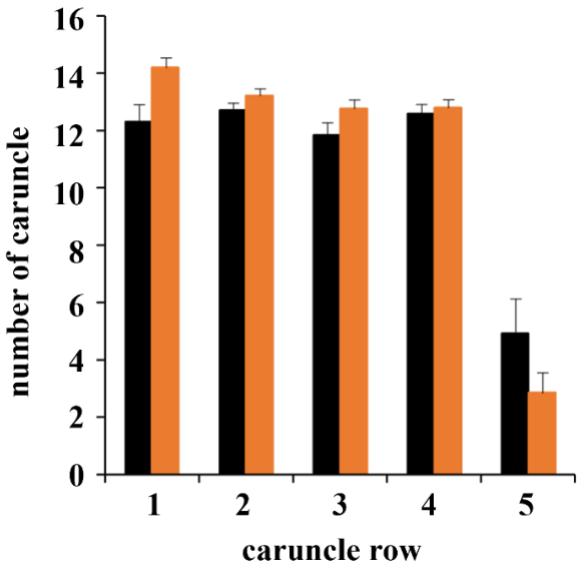

Figure 4 Arrangement of caruncles in rows in the right uterine horn (black bars) and the left uterine horn (orange bars) of goats.

Histomorphology of uterine caruncles

Histological examination showed similar structural features in both the uterine horns and the uterine body. The caruncles appeared as dome-shaped, glandless structures composed of dense connective tissue. In cyclic does (Figure 5B), the caruncles exhibited a more prominent vascular network compared to those of non-cyclic does (Figure 5A). Multiple caruncles projected above the endometrial surface, separated by intercaruncular areas containing mucosal folds (Figure 5C). Endometrial glands were observed as epithelial buds and invaginations extending from the luminal epithelium into the underlying connective tissue, with a higher concentration of glands in the intercaruncular regions (Figure 5D).

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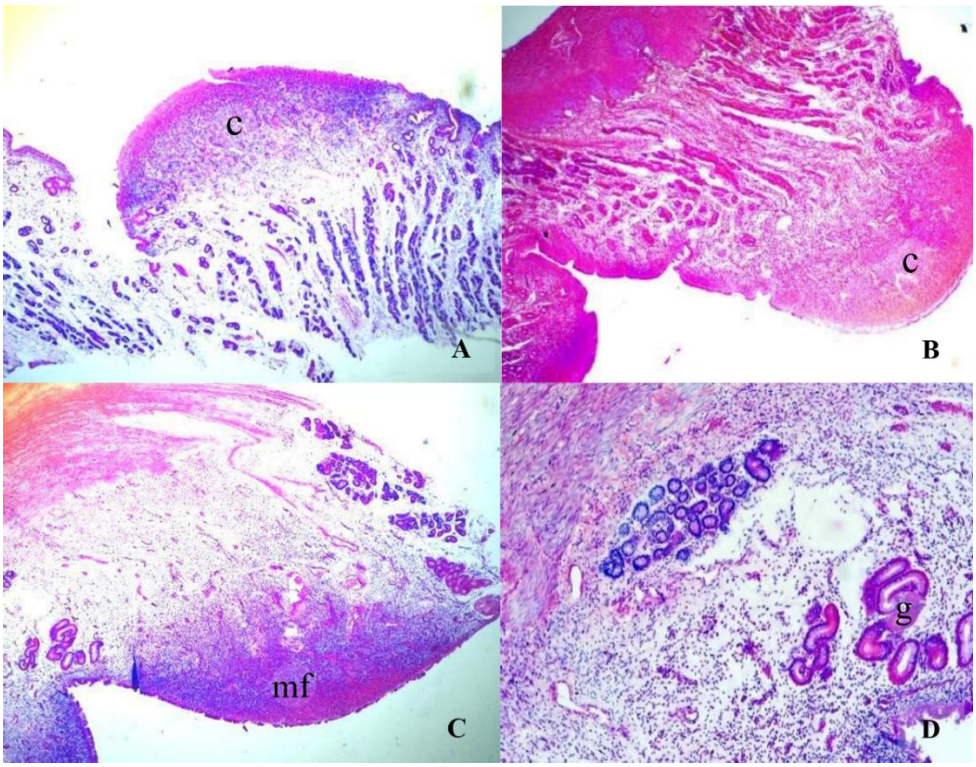

Figure 5 Histomorphology of uterine caruncles in goats; A) non-cyclic; B) cyclic; C) mucosal folds (mf); D) uterine glands (g); (H&E staining).

Trans-abdominal ultrasonography

Out of the 20 goats scanned, 7 (35%) were identified as pregnant based on the detection of cotyledons, while 13 (65%) were classified as non-pregnant (Table 1). Placentomes were first observed via ultrasonography at approximately 34.4 ± 0.42 days of gestation. Uterine cotyledons were detectable etween 32 and 40 days of pregnancy (Figure 6 A, B).

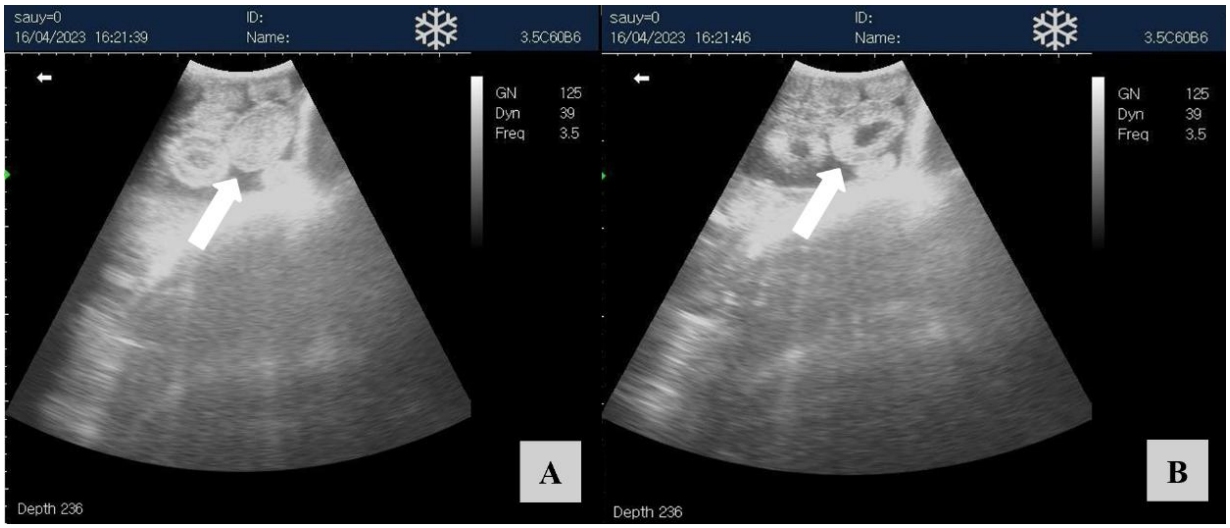

Figure 6 Transabdominal ultrasonography images of uterine cotyledons in pregnant goats; A) 32-34 days gestation and B) 35-40 days gestation; arrows indicate uterine cotyledons

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DISCUSSION

The gross morphology of the bicornuate uterus in goats showed typical features with caruncles arranged in rows, consistent with previous reports (Abiaezute *et al*., 2017; Shehan *et al*., 2019). The increased tonicity and prominence of caruncles in cyclic goats suggest a hormonal influence on uterine morphology. The irregular arrangement of caruncles near the cranial uterine horns may be an adaptative feature to prevent fetal implantation in narrow regions, similar to findings in West African Dwarf and Egyptian goats (Lyngset, 1971). Morphometric analysis revealed a higher number of caruncles on the left uterine horn, which may reflect functional asymmetry. The variation in caruncular rows and distribution contrasts with some earlier studies but aligns with species-specific adaptations. Although statistically non-significant within the group, the free portion of the caruncle showed greater values on the left side in goats aged two to three years. The absence of caruncles in the cranial third of the uterine horns in WAD goats across all age groups was also reported by Abiaezute *et al*. (2017) as a mechanism to prevent implantation in the narrow, spiral-shaped cranial region of the uterine horn, supporting the findings of Abd-Elnaeim (2008).

Histologically, the presence of mucosal folds and abundant endometrial glands supports the functional role of caruncles and inter-caruncular areas in implantation and nutrient secretion, as described by Abiaezute *et al*. (2017). In the caruncle regions, there were no uterine glands. Except in the caruncle-free area, the endometrium of the uterine horn and body thickened around the caruncles. The caruncles free area was histologically similar in structure to the body and uterine horn, but without the caruncles. These results were consistent with the study of Kumar *et al*. (2020), who also characterized the uterine glands found in the histological preparations of the middle, base, and cranial portions of the uterus.

Ultrasonographic detection of placentomes at approximately 34 days of gestation aligns with previous studies indicating placentome visibility between 31 and 50 days (Anwar *et al*., 2008; Ali *et al*., 2020). The observed pregnancy rate of 35% corresponds with the diagnosis capabilities of early trans-abdominal ultrasonography. However, the inability to measure placentome length was a limitation in accurately determining gestational age.

CONCLUSION

The study of uterine caruncles in Bengal goats revealed distinct structural features essential for reproductive function. According to the morphometric data, the average number of uterine caruncles was higher in left uterine horn compared to the right in both cyclic and non-cyclic goats, while the average number of caruncular rows was similar in both horns. Histologically, the caruncles are dome-shaped, glandless structures composed of dense connective tissue, abundant vasculature, and a surface epithelium ranging from cuboidal to columnar. Additionally, based on cotyledon observations, transabdominal ultrasonography was used to identify early pregnancy in goats. The size and morphology of the caruncles vary with reproductive status, enlarging notably during pregnancy to support placentome formation and fetal development. These findings provide valuable baseline data for reproductive biology, breeding management, and comparative analysis of caruncles in goats, contributing to improved reproductive efficiency. Furthermore, these results open up new possibilities for future research, such as the measurement of uterine caruncles to help determine precise gestational ages and improve goat productivity of in Bangladesh.

APPROVAL OF ETHICAL COMMISSION

This experiment was approved by the Institutional Ethics Committee of Sher-e-Bangla Agricultural University (SAU), Dhaka-1207, Bangladesh [Approval no.- SAU/ SUTH/25/53].

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AUTHORS' CONTRIBUTIONS

Hafsa Hossain (HH), Md Rashedul Islam (MRI), Maksuda Taslima (MT), Mozahidul Islam Tuser (MIT), Nurjahan Akter Juli (NAJ), Al Wasef (A), Mahfuzul Islam (MI), Jahagir Alam (JA).

HH, JA and MRI. Conceived and designed the experiments. HH, MIT, NAJ and MT. Performed the sampling of slaughterhouse goat ovaries. HH, JA, A and MRI. Performed the ultrasonography and histological investigations. HH, MI and MRI. Analyzed the data and wrote the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

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