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# Morphometric and histological study of reproductive organs of indigenous ewes

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## ABSTRACT

Morphometric and histochemical studies provide valuable insights into the structural and functional characteristics of reproductive organs of animals. The experiment was conducted with the aim to evaluate the morphometry of ewe reproductive organs in relation to age and to investigate the effect of age on the histological structure of the ovary, oviduct and uterus. From 9 (nine) reproductive organs of ewe 18 ovaries, 18 oviducts, 18 horns of uterus and 9 uterine bodies were obtained from the local abattoir of Mymensingh municipality, Bangladesh and then categorized based on age (confirmed through dentition pattern and butcher's report) into two categories: 1-2 years and 2.5-4 years. In the morphometric study, the weight, length and width of ovary, oviduct, body of the uterus, and uterine horns were significantly higher ($p<0.05$) in ewes aged 2.5-4 years, except for the weight of the ovary and the length and width of the oviduct, which did not differ significantly ($p>0.05$). In the histological study of ovary, the number of normal primordial, primary, secondary, and antral follicles were non-significantly ($p>0.05$) higher in ewes at the age of 2.5-4 years than 1-2 years. In case of thickness of layer in oviduct, the serosa and mucosal folds were non-significantly ($p>0.05$) thicker in the older age compared to the younger group. However, the muscularis layer was significantly ($p<0.05$) thicker in the older age group (46.42±5.70 µm) than in the younger group (30.97±3.91 µm). In the uterine horn, all assessed layers (perimetrium, myometrium, and endometrium) increased with age. Significant differences were found in the perimetrium and myometrium, but not in endometrial thickness. Within the body of the uterus, all layers were thicker in the older age group, with highly significant differences observed in the myometrium (982.46±8.93 µm vs. 648.50±9.75 µm) and endometrium (1174.79±54.1 µm vs. 697.41±63.0 µm), while the perimetrium also showed a significant increase (43.89±5.73 µm vs. 23.51±3.71 µm). These findings indicate that age significantly influences morphometric characteristics and histological structure of ewe's reproductive organ, which may have implications for reproductive performance and productivity.



## Introduction

Sheep (*Ovis aries*) are ruminant mammals of the order Artiodactyla and the family Bovidae. They were among the first animals domesticated by humans and are now distributed worldwide. It is an important farm animal in Bangladesh which is considered important for its versatile production profile and valuable contribution like meat, industrial raw products such as wool, fiber and manure. Sheep meat is very rich in protein, energy and fat (Junkuszew *et al*., 2020). Sheep are gregarious small ruminants that primarily

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depend on natural grasses found on fallow land, roadsides, crop fields, and canal banks (Sultana *et al*., 2011). It can endure extended drought conditions and limited feed availability (Rakib *et al*., 2022). Most of the sheep in Bangladesh are indigenous, with few crossbreds and are capable of bi-annual lambing and multiple births (Bhuiyan, 2006). Sheep is preferred when smallholders are unable to raise cattle and other large animals. They are well suited for meat production and can be readily sold to generate immediate income.

Having one kid per year is the primary objective of farmers and profitable livestock production depends on successful and regular reproduction of the animals. In this sense, reproductive efficiency is a key component in getting the most outcome from the animal. But a number of reproductive diseases are impeding the animal productivity. Thus, maintaining excellent reproductive health also heavily depends on disease control. Animal reproduction may be halted by any anatomical, histological or functional problems in the reproductive system (Khaton *et al*., 2015). In the end, the species' whole economic tract might be affected. Furthermore, compared to all other animals in the world, the reproductive physiology of sheep is least understood according to age. Despite being sheep is a vital species of livestock. From an economic perspective, reproductive performance in small ruminants is crucial, as it influences the number of offspring produced each year (Greyling, 2000).

An animal's ability to reproduce depends on its genital organs' normal structure and functionality (Siddiqui *et al*., 2005). The biometry of genital tracts of the female reveals the overall wellbeing of animals. The knowledge of the biometrical status of female genital tract is essential to perform artificial insemination, pregnancy diagnosis and dealing with the infertility problems (Kunbhar *et al.,* 2003) and for treatment. Modern reproductive technologies, such as in vitro fertilization (IVF) and artificial insemination (AI), require a thorough understanding of female reproductive biometry. For successful in vitro embryo production (IVP), careful evaluation of the ovaries and efficient collection and grading of oocytes are essential. Different types of factors influence the quality of reproductive organs of animals such as season, age of animal, breed, body condition score, reproductive status etc. (Emara *et al*., 2019; Akhtar *et al*., 2023; Ibrahim *et al*., 2012; Shathi *et al*., 2022). But limited research work (Shehan *et al*., 2019) was come attention into the author regarding the sheep reproductive organs evaluation by morphologically and histologically but there is no direct research considering age. Therefore, this study was undertaken to know morphometric changes of ewe's reproductive organs according to age and to observe how age affect the number and developmental status of ovarian follicles and the thickness of oviduct and uterus histologically.

## Methods and Materials

### Collection of reproductive organs

A total of nine (9) reproductive organs of ewe were collected from the local abattoir of Mymensingh municipality during October 2023 to September 2024. The organs were collected based on two age groups 1-2 years and 2.5-4 years and the experimental samples' approximate age was determined by the butcher and validated using their dentition pattern. Following collection, the organs were carried to the Reproductive Biotechnology Laboratory at the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, using a thermoflask containing 0.9% saline solution. Upon arrival, the reproductive organs were dissected, cleaned, trimmed, and placed in sterilized Petri dishes, and then thoroughly washed with physiological saline before subsequent procedures.

### Morphometric evaluation

For morphometric measurement, the length and width of the different organs were taken with the help of electronic digital calipers and the weight was taken using a digital balance (Mahzabin *et al*., 2020) (Precisa, XB-220A, Switzerland). The length of ovary was taken along the excision from the ovarian ligament and width as the greatest line perpendicular to the length line. The length of oviduct was measured from the top of the fimbria to the tubal-uterine horn junction and width same as ovary. Each uterine horn was incised along its dorsal surface to expose its lumen from the oviduct tubal junction to the bifurcation of the body of the uterus. The length of uterine horn was taken from the internal bifurcation to the apex of horn and the length of the uterine body was taken from its bifurcation to the internal os of the cervix and width as like ovary (Akhtar *et al*., 2023).

### Histological analysis

For histological study 18 ovaries, 18 oviducts, 18 horns of uterus and 9 uterine bodies were used. Each ovary was cut into 4–6 pieces using a surgical blade and fixed in Bouin's solution for 24

hours. The fixed tissues were then dehydrated through a graded alcohol series: 70% alcohol for 12 hours, followed by 80%, 90%, and 100% alcohol (each for 1 hour, with the absolute alcohol step repeated three times). After dehydration, the tissues were cleared in xylene-first in a 1:1 mixture of xylene and alcohol for 1 hour, then in pure xylene for another hour.

The cleared samples were subsequently infiltrated with a 1:1 mixture of paraffin and xylene, and finally embedded in molten paraffin. The paraffin blocks were left to harden at room temperature for 24 hours and then trimmed using a surgical blade. From each ovarian block, sixteen sections (8 µm thick, taken at every 11th–12th interval) were prepared using a rotary microtome (Leica RM 2125RT). Each section was mounted on a glass slide with a drop of water and air-dried at room temperature. The slides were then stained with hematoxylin and eosin (H&E) and permanently mounted with a cover slip using DPX mounting medium (Akhtar *et al*., 2023). From the serial sections, each and every section was observed with the help of a light microscope for identification of follicles and granulose cells. The follicles were counted from the top and left to right fields, then bottom and right to left fields to avoid double counting. The follicles were classified into four categories according to the number and arrangement of granulosa cell layers (Sarker *et al*., 2015). The histological changes in thickness of cell layers of different organs (oviduct, horn of uterus and body of uterus) were observed under microscope (10×10X) and the thickness was measured in micrometer with the help of Image J software.

**Statistical analysis**

All data were analyzed using GraphPad Prism software (version 5.0). Result is presented as mean ± standard error of the mean (SEM). Differences between groups were assessed using an independent sample t-test. A probability value of $p<0.05$ was considered statistically significant.

## Results

**Morphometric measurements of different reproductive organs of sheep**

From nine (9) reproductive tracts, 18 ovaries, 18 oviducts, 18 uterine horns, and 9 uterine bodies were obtained. Of these, 5 reproductive organs were from the 1-2 years age group and 4 reproductive organs were from the 2.5-4 years age group. Biometry of ewe reproductive organs of two different age groups was evaluated in terms of weight, length and width.

**Table 1. Measurements of ovary, oviduct, horn of uterus, and body of uterus from ewes (Mean ± SE)**

| Reproductive organs | Parameter | Age groups | | p-value |
|---|---|---|---|---|
| | | 1-2 years (10) | 2.5-4 years (8) | |
| **Ovary** | Weight (g) | 0.56±0.17 | 0.79±0.06 | 0.21 |
| | Length (cm) | 0.99±0.05$^b$ | 1.28±0.08$^a$ | 0.01 |
| | Width (cm) | 0.59±0.04$^b$ | 0.79±0.05$^a$ | 0.003 |
| **Oviduct** | Weight (g) | 0.33± 0.04$^b$ | 0.51± 0.06$^a$ | 0.03 |
| | Length (cm) | 14.91 ± 0.71 | 17.55 ± 1.01 | 0.05 |
| | Width (cm) | 0.22± 0.01 | 0.24± 0.01 | 0.33 |
| **Horn of uterus** | Weight (g) | 2.28±0.34$^b$ | 5.96±0.76$^a$ | 0.001 |
| | Length (cm) | 3.73±0.41$^b$ | 6.88±0.57$^a$ | 0.001 |
| | Width (cm) | 0.79±0.03$^b$ | 1.05±0.09$^a$ | 0.031 |
| **Body of uterus (5 and 4)** | Weight (g) | 5.33±0.38$^b$ | 12.82±1.38$^a$ | 0.01 |
| | Length (cm) | 3.69±0.26$^b$ | 6.56±0.60$^a$ | 0.01 |
| | Width (cm) | 1.87±0.14$^b$ | 2.45±0.07$^a$ | 0.01 |

*Values in parentheses indicate the number of observations; SE indicates standard error; a,b superscripts within the same row differ statistically significant ($p<0.05$) and p-value >0.05 indicate non- significant effect.*

In the ovary, the weight was non-significantly ($p>0.05$) higher in the age group of 2.5-4 years (0.79±0.06 g) compared to 1-2 years (0.56±0.17 g). On the other hand, length (0.99±0.05 and 1.28±0.08 cm) and width (0.59±0.04 and 0.79±0.05 cm) was significantly ($p<0.05$) increased in the age group of 2.5-4 years compared to 1-2 years, respectively. In contrast, weight of the oviduct (0.33±0.04 g and 0.51±0.06 g) differ significantly between age groups, whereas the length (14.91±0.71 cm and 17.55± 1.01 cm) and width (0.22±0.01cm ,

0.24±0.01cm) of the oviduct differ (p<0.05) non- significantly between age group .The weight, length, and width of the uterine horn differ highly significantly (p<0.05) between age groups. Similar pattern was also observed in case of body of uterus (Table 1).

**Histological study of ovary**

In the histological examination of the ovary, different types of normal follicles were enumerated (Figure 1). The results indicated that the number of primordial follicles was non-significantly (p>0.05) higher in 1-2 years ewes ovaries (68.80 ± 15.07) than ovaries of 2.5-4 years (57.75 ± 11.43). The primary and antral follicles also showed a similar trend. In contrast, the number of secondary follicles was non-significantly (p>0.05) higher in 2.5-4 years ewes ovaries (1.62 ± 0.53) than ovaries of 1-2 years (1.60 ± 0.37) (Table 2).

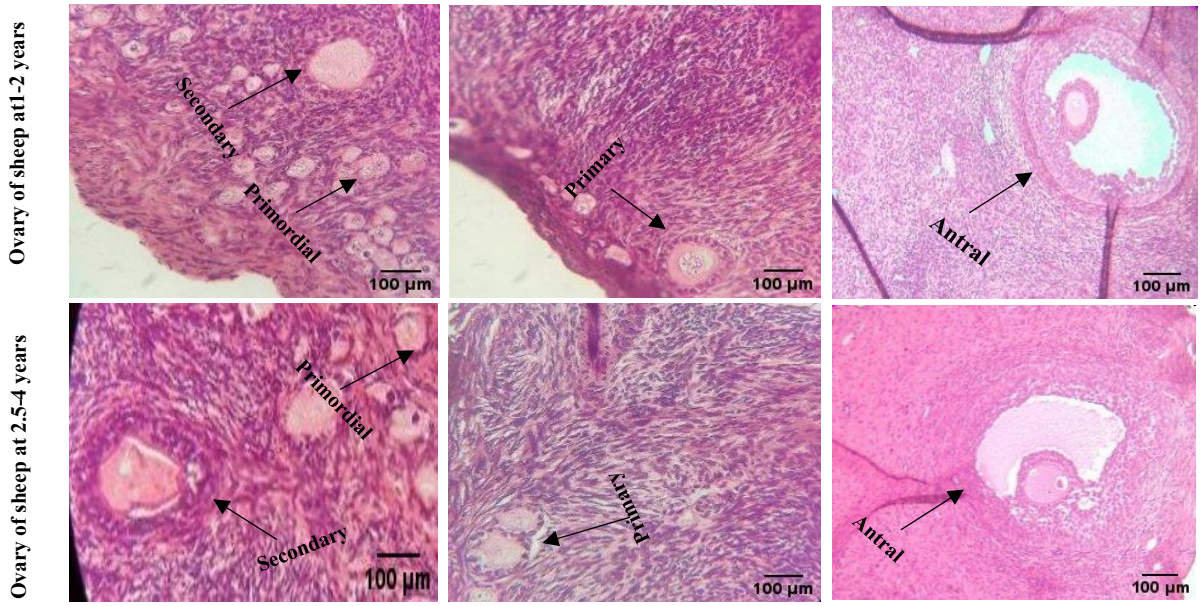


**Figure 1: Representative photomicrographs of paraffin sections of ewes ovaries stained with hematoxylin and eosin, and arrow indicates different types of normal follicles. Picture was captured at (10×10X). Scale bars represent 100 µm.**

**Table 2. Effect of age on histologically counted different types of normal follicles (Mean ± SE) in ewes ovaries**

| Type of follicles | Age groups | | p-value |
|---|---|---|---|
| | 1-2 years (10) | 2.5-4 years (8) | |
| **Primordial** | 68.80 ± 15.07 | 57.75 ± 11.43 | 0.567 |
| **Primary** | 6.60 ± 1.63 | 5.75 ± 2.27 | 0.766 |
| **Secondary** | 1.60 ± 0.37 | 1.62 ± 0.53 | 0.970 |
| **Antral** | 2.40 ± 0.31 | 2.38 ± 0.53 | 0.968 |

*Values in parentheses indicate the number of observations; SE indicates standard error; p-value >0.05 indicate non-significant effect.*

**Histological study of oviduct, horn of uterus and body of uterus**

Histological sections of the oviduct, uterine horn, and uterine body were examined under a microscope (10×10X), and the different tissue layers were identified and measured according to age (Figure 2). In the oviduct, the serosa and mucosal folds were thicker in the 2.5-4 years group compared to the 1-2 years group, although the differences were not statistically significant (p>0.05). The muscularis layer, however, was significantly thicker in the older age group (46.42±5.70 µm) than in the younger group (30.97±3.91 µm).

In the uterine horn, all assessed layers (perimetrium, myometrium, and endometrium) increased with age. Significant differences were found in the perimetrium (141.49±5.90 µm vs. 71.86±4.69 µm) and myometrium (391.52±26.27 µm vs. 268.38±10.61 µm), but the endometrial thickness increase was not statistically significant (889.95±54.68 µm vs. 676.42±71.86 µm).

Similarly, in the body of the uterus, all layers were thicker in the older age group, with highly

significant differences observed in the myometrium (982.46±8.93 µm vs. 648.50±9.75 µm) and endometrium (1174.79±54.1 µm vs. 697.41±63.0 µm), while the perimetrium also showed a significant increase (43.89±5.73 µm vs. 23.51±3.71 µm).

Overall, the data indicate that the thickness of most reproductive tract layers increases with age, with the muscular and endometrial layers showing the most pronounced age-related changes (Table 3).

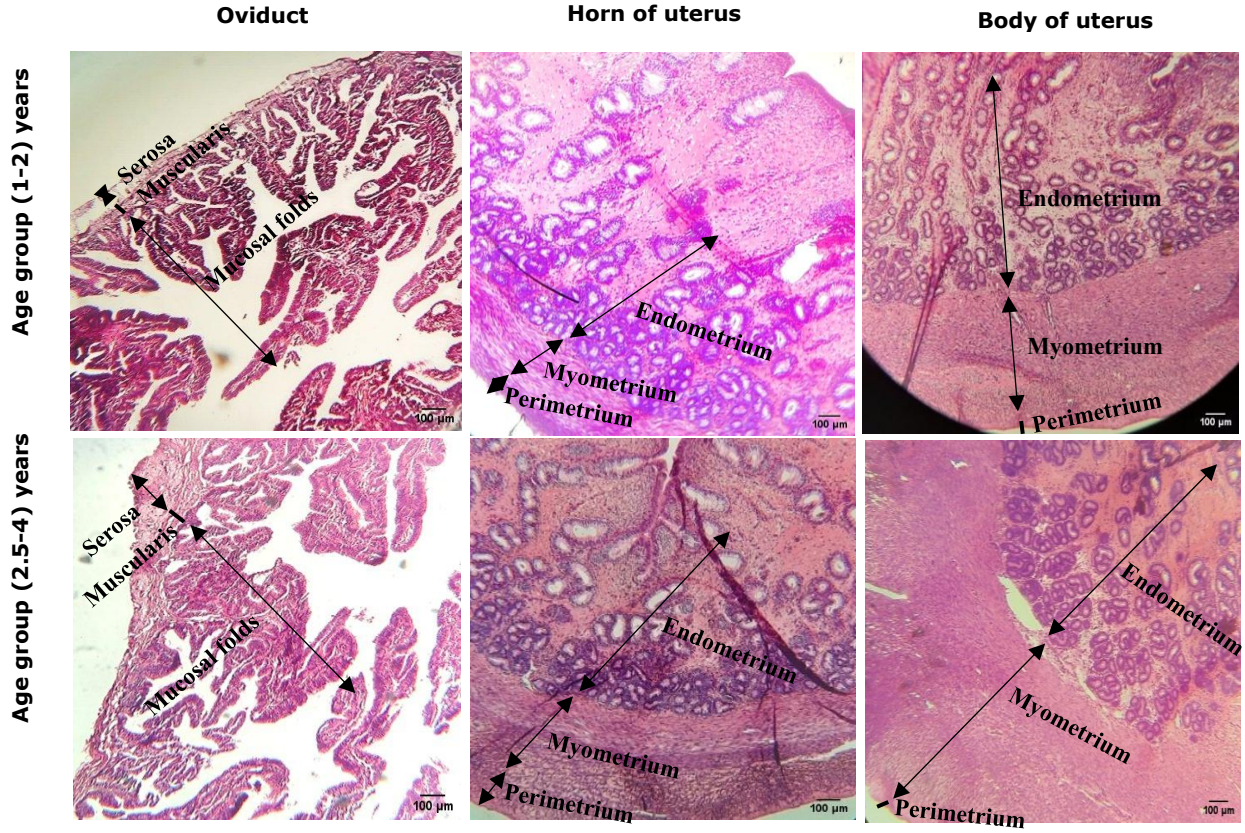


**Figure 2: Representative photomicrographs of paraffin sections of ewes oviduct, horn of uterus and body of uterus stained with hematoxylin and eosin, and showing thickness of different cell layers. Picture was captured at (10×10X). Arrows indicate cell layers. Scale bars represent 100 µm.**

**Table 3. Comparative measurements of thickness (µm) of different parts of oviduct, horn of uterus and body of uterus of ewes between two age groups (Mean±SE)**

| Organs | Parts | Age groups | | p-value |
|---|---|---|---|---|
| | | 1-2 years (10) | 2.5-4 years (8) | |
| **Oviduct** | Serosa | 50.40 ± 7.65 | 89.92 ± 19.22 | 0.088 |
| | Muscularis | 30.97 ± 3.91[b] | 46.42 ± 5.70[a] | 0.044 |
| | Mucosal folds | 479.33 ± 44.75 | 612.81 ± 69.88 | 0.133 |
| **Horn of uterus** | Perimetrium | 71.86±4.69[b] | 141.49±5.90[a] | <0.0001 |
| | Myometrium | 268.38±10.61[b] | 391.52±26.27[a] | 0.002 |
| | Endometrium | 676.42±71.86 | 889.95±54.68 | 0.239 |
| **Body of uterus** | Perimetrium | 23.51±3.71[b] | 43.89±5.73[a] | 0.009 |
| | Myometrium | 648.50±9.75[b] | 982.46±8.93[a] | <0.0001 |
| | Endometrium | 697.41±63.0[b] | 1174.79±54.1[a] | <0.0001 |

*Values in parentheses indicate the number of observations; SE indicates standard error; a,b superscripts within the same row differ statistically significant (p<0.05) and p-value >0.05 indicate non- significant effect.*

## Discussion

Morphometric evaluation of the ovary, oviduct, and uterus is essential to understand the structural features of the reproductive system. These findings provide a basis for interpreting developmental variations across age groups. The weight, length and width of ovary, oviduct, body of the uterus, and uterine horns were significantly higher ($p<0.05$) in ewes aged 2.5-4 years, except for the weight of the ovary and the length and width of the oviduct. There is no research available on the morphometric changes in the reproductive organs of indigenous ewes in relation to age; however, such studies have been conducted in other species. Akhtar *et al*. (2023) reported that age affects the morphometric parameters of the goat ovary, which supports the findings of the present study. Uddin *et al*. (2021) and Rennak *et al*. (2024) reported that the size of most parts of the reproductive tract in goats increased with age. The study of Jahan *et al*. (2022) also supports the present study. Therefore, it can be stated that age exerts a significant influence on the morphometric parameters of sheep reproductive organs. With increasing age, hormonal activity, follicular development, and tissue growth become more pronounced, leading to larger ovaries, oviducts, and uterine structures. This natural maturation process explains why morphometric parameters differ significantly between younger and older ewe.

Histological examination of the ovary revealed that the numbers of primordial, primary, secondary, and antral follicles showed a non-significant decrease in the 2.5-4 years age group. This study supports the findings of Akhtar *et al*. (2023), who reported that in goat ovaries aged 22-24 months, the numbers of primordial and primary follicles decreased non-significantly, while the numbers of secondary and antral follicles decreased significantly. Comparable observations were made in humans by Gougeon (1998), who reported that the number of growing ovarian follicles declines with advancing age. The ovarian reserve decreases with age because follicles are steadily lost through atresia. Although primordial follicles continue to enter the growth phase, the loss of follicles becomes faster than their recruitment. Therefore, all types of follicles gradually decline as the animal gets older.

The histological structure of the oviduct consists of three layers: serosa, muscularis, and mucosal folds. In the present study, the thickness of the serosa and mucosal folds increased non-significantly, whereas the thickness of the muscularis increased significantly with the advancement of age in sheep. This finding substantiates the study of Lewis and Berardinelli (2001), who reported that in sheep, the thickness of the muscularis and submucosa, as well as the height of mucosal cells, increases from puberty to maturity. The present findings for the cell layers of uterus were similar with the finding of Sahu *et al*. (2017) and Shehan *et al*., (2019). With the advancement of age the thickness of uterine cell layers increase which is also in agreement with the finding of Mochow and Olds (1966). Overall, histological examination of the oviduct and uterus revealed that the thickness of cell layers varies with age, indicating that age plays a significant role in the structural development and functional capacity of these reproductive organs.

## Conclusion

The study showed that most morphometric parameters of the ovary, oviduct, and uterus were significantly higher in ewes aged 2.5–4 years compared to 1–2 years. Histologically, older ewes had slightly more follicles and significantly thicker cell layers in the reproductive tract. These findings provide baseline data that enhance understanding of age-related reproductive physiology in ewes.

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***Author's contribution***

**Shahnaj Sultana**: Design of the study, sample collection, methodology, investigation, writing-original draft, revision and drafting of the manuscript, final approval of the version to be submitted. **Mst. Mahomudha Akhtar:** Conceptualization, design of the study, methodology, investigation, analysis and interpretation of data, writing-original draft, revision and drafting of the manuscript, final approval of the version to be submitted, writing-review and editing. **Sakib Hossain, Afia Mahmuda Meem and Afiya Fairuz Lubaba**: Sample collection, Methodology, investigation, final approval of the version to be submitted. **Tanjin Alam:** Methodology, investigation,

writing-original draft, final approval of the version to be submitted. **M. A. M. Yahia Khandoker:** Conceptualization, investigation, revision and drafting of the manuscript, final approval of the version to be submitted.

## *Conflicts of interest*

The authors declare that there are no conflicts of interest involving any individual, organization, or aspect related to the content of this manuscript.

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### *Data Availability*

The data supporting the findings of this research will be provided upon authorization from the authors.

### *Compliance of ethical standards*

This study adheres to the ethical standards in Bangladesh regarding the handling of biological materials.

### *Consent for publication*

All authors have given their full consent for the publication of this research in the Bangladesh Journal of Animal Science.

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