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Effect of age on follicular dynamics of goat ovaries

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ABSTRACT

Reproductive aging is influenced by a gradual decline in both the quantity and quality of oocytes found within ovarian follicles. In mammals, fertility relies heavily on the health of oocyte reserves, the dynamics of follicular development, and the functioning of the ovaries. This study aimed to investigate the impact of age on the follicular dynamics of goat ovaries. A total of 40 ovaries of Black Bengal goats were obtained from a local slaughterhouse and sorted into four age groups: 6-12 months, 13-15 months, 18-21 months, and 22-24 months. Subsequently, the ovaries were treated, and follicles were assessed through visually and histologically. The findings revealed that nearly all parameters, including weight, length, and width, of both ovaries in the 18-21 months age group were insignificantly different ($p>0.05$) from the other age groups. Histologically, the number of normal primordial and primary follicles was also insignificantly higher ($p>0.05$) in goats aged 18-21 months compared to the other age groups. Conversely, the quantity of secondary follicles significantly increased ($p<0.05$) with age, but in terms of antral follicles, a quadratic trend was observed, where they increased up to a certain age and then declined. Additionally, the results showed that the number of degenerated primary and antral follicles was significantly higher ($p<0.05$) in the ovaries of 22–24-month-old goats, while the number of degenerated secondary follicles was insignificantly higher ($p>0.05$) compared to the remaining age groups. In conclusion, age had an effect on the follicular dynamics of goat ovaries, suggesting that ovaries from goats aged 18-21 months, sourced from slaughterhouses, hold potential for quality follicles and oocytes that can be valuable for further research.

Introduction

The goats are the first domesticated farm animals used for milk, meat, skin and hair production worldwide.Goats are distributed worldwide because they adapt to various environmental and climatic conditions (Aziz, 2010). There are about 1 billion goats in the world, out of which more than 90% of goats are found in developing countries. The majority of goats in the world are found in Asia (Utaaker *et al.*, 2021), among which in Bangladesh it is about 26.60 million goats (DLS, 2021). There is only one breed of goat in Bangladesh, which is referred to as the Black Bengal goat, that occupies the second position in the livestock sector. In Bangladesh, as the population is gradually increasing, goat production needs to be increased by improving reproductive efficiency to meet the need for additional animal protein. Production is directly related to reproductionand numerous factors can affect reproduction, such as repeat breeding (Yaginuma, 2019), quantity of dominant follicles (Gimenes *et al.*, 2009), early embryonic death and prolonged inter kidding duration (Sood *et al.*, 2017), higher circulating concentrations of estradiol, progesterone and insulin (Gimenes *et al.*, 2009). To solve these problems, modern technologies such as in vitro embryo production (IVEP), artificial insemination (AI) and multiple

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ovulation and embryo transfer (MOET) are used and these technologies requires a clear and sharp knowledge of female reproductive organ.

Ovary is a major organ of the mammalian reproductive tract that produces oocytes and releases estrogen and progesterone hormones (Hoque *et al.*, 2016). The ovarian cortex contains ovarian follicles at different phases of development or regression. Follicles are fluid-filled, blister-like structures that have developing oocytes. Therefore, evaluating ovaries and follicles are critical for successful in vitro production (IVP) of embryos (Khnadoker *et al.*, 2011).

Follicular dynamics during the estrous cycle of the goat were first reported by Ginther and Kot (1994). In sheep and goats, a vast reservoir of quiescent primordial follicles is developed during fetal development (Mariana *et al.*, 1991). The development of the follicle from the quiescent phase to the preovulatory phase takes around six months (Turnbull *et al.*, 1977; Cahill and Mauleon, 1980). Debora *et al.* (2019) studied the features of the goat ovary's luteal and follicular structures during the reproductive cycle at different ages and found that age is related to histomorphometric characteristics of the ovarian changes. Therefore, it is suggested that age has an influence on ovarian follicles. However, no research work was brought to the author regarding the goat ovaries and follicles evaluation by histological means in relation to the age of goat. Therefore, this study aims to elucidate the effect of age on the number and quality of ovarian follicles.

Materials and Methods

Experimental site

The experiment was conducted at the Reproductive Biotechnology Laboratory under the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh.

Animals used

Twenty adult female Black Bengal goats were used in this research work, which were slaughtered at the local slaughterhouse of Ganginarpar, Mymensingh. The samples were collected from December 2021 to April 2022. The ovaries were separated and collected from the reproductive tract according to age. Forty (40) goat ovaries were randomly collected from the local slaughterhouse of Mymensingh municipality in Bangladesh.

Dentition

The approximate age of the experimental samples was assured by the butcher and confirmed through dentition (Mitchell, 1982). The age of slaughtered Black Bengal goats was divided into four groups as 6 to 12 months, 13 to 15 months, 18 to 21 months and 22 to 24 months. For each range of age, the sample number was 10.

Collection and transportation

The collected ovaries were transferred to the Reproductive Biotechnology Laboratory Department of Animal Breeding and Genetics, Bangladesh Agricultural University, in a thermo flask containing 0.9% normal saline solution and kept at 25°C. The ovary was trimmed and put into petridishes that have been sterilized in the laboratory and rinsed thoroughly with physiological saline before further processing.

Morphometric evaluation

Length (distance between anterior and posterior pole) and width (distance between anterior and posterior pole) of the ovary were recorded with the help of digital slide calipers (Stainless Harded, China), and weight was recorded by using a digital weighing balance (Unilab Instruments, USA). The ovaries were observed with the naked eyes and the follicles were graded into two groups: small follicles (2 to 5 mm) and large (more than 5 mm) based on the diameter of the follicle.

Histological procedure

For histological work, the ovary was cut into 2 to 4 parts with a surgical blade and then fixed in Bouin's solution for 24 hours. All the pieces were dehydrated in ascending graded of alcohol (70% alcohol for 12 hours, 80% alcohol for 1 hour, 90% alcohol for 1 hour and 100% alcohol for 1 hour for three times) and cleared with xylene (At first immerse in 50% xylene and 50% alcohol for 1 hour, then transferred to 100% xylene for 1 hour). The cleared samples were transferred to 50% paraffin and 50% xylene and embedded with melted paraffin. Then, the paraffin blocks were kept at room temperature for 24 hours for hardening and trimmed with the assistance of a surgical blade. Five µm thick sections (every 11 to 12th section) were prepared by using rotary microtome machine (Leica RM 2125RT). Every section was placed upon a glass slide containing water drops and dried at room temperature. The slides were stained with hematoxylin and eosin (H-E). Finally, the stained sections were permanently mounted with a cover slip using DPX mounting reagent (Alam *et al.*, 2014).

Microscopic observations

From the serial sections, each representative section was observed with the assistance of a light microscope to identify follicles and granulosa cells. The follicles were counted from the top and left to right fields, then bottom and right to left fields, according to Hoque *et al.* (2021). Double

counting was avoided. Four categories were created based on the quantity and shape of granulosa cell layers in the follicles as i) Primordial follicle with a single layer of flattened granulosa cells surrounding the oocyte, ii) Primary follicle with a single layer of cuboidal granulosa cells, iii) Secondary follicle with two or more layers of granulosa cells but no antrum, iv) Antral follicle having an antral cavity with multiple granulosa cell layers (Sarker *et al.*, 2015). Follicles were considered degenerated if they contained oocytes with pyknosis, large vacuoles, condensed cytoplasm, disappearing of nuclear membranes, shrinkage of the nucleus, swollen granulosa cells, or loss of granulosa cells. The granulosa layer for follicular degeneration was considered the main criterion.

Statistical analysis

The data was compiled, tabulated and analyzed based on the objectives of the study. Results were given as mean ± SE. ANOVA was performed to observe the significant differences among the mean values. Duncan's Multiple Range Test (DMRT) was performed to observe differences of mean values for ovaries in different age groups. Correlation of different follicles was carried out using SPSS (Statistical Package for Social Sciences) software version 23.

Results

Morphometric measurements of ovaries and follicles

In this study, almost all parameters (weight, length and width) of both ovaries of all age groups were observed to be statistically similar (P>0.05). However, the age group 18 to 21 months was observed to be higher than those of other age groups (Table 1). In the left and right ovary, the percentage of small follicles was 32.24% and 34.22%, respectively, which was non-significantly (P>0.05) higher in the 18 to 21 months age group than others. Similarly, the proportion of large follicles (33.34%) of the left ovaries was non-significantly (P>0.05) higher in the same age group, but in right ovary, the large follicles (30.43%) for two groups 13 to 15 months and 18 to 21 months is same which is higher than the other age group (Figure 1).

Table 1. Morphology of ovaries from goats of different ages (Mean ± SE)

Ovary	Parameters	Age groups				Level of significance
		6 to 12 months (10)	13 to 15 months (10)	18 to 21 months (10)	22 to 24 months (10)	
Left (20)	Weight (g)	0.52±0.04	0.57±0.04	0.60±0.10	0.47±0.04	NS
	Length (cm)	1.25±0.96	1.28±0.95	1.38±0.88	1.21±1.15	NS
	Width (cm)	0.95±0.66	0.97±0.56	0.99±0.91	0.99±0.34	NS
Right (20)	Weight (g)	0.61±0.25	0.65±0.13	0.69±0.30	0.57±0.10	NS
	Length (cm)	1.22±0.87	1.29±0.88	1.37±0.95	1.36±0.69	NS
	Width (cm)	0.93±0.77	0.97±1.05	1.01±0.71	1.03±0.45	NS

Figure in the parenthesis indicates the number of observations; SE, standard error; NS, non-significant (P>0.05) effect on the parameters

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

Type of follicle	Number of normal follicles per ovary				Level of significance
	6 to 12 months	13 to 15 months	18 to 21 months	22 to 24 months	
Primordial	121.65±20.53	142.45±14.61	169.59±19.80	135.81±19.72	NS
Primary	9.41±1.42	13.14±1.89	16.29±±2.52	11.28±1.59	NS
Secondary	$2.63±0.54^{b}$	$3.04±0.40^{ab}$	$4.47±0.53^{a}$	$4.02±0.53^{a}$	*
Antral	$5.24±0.66^{a}$	$6.19±0.64^{a}$	$4.81±0.59^{a}$	$2.61±0.45^{b}$	*

SE, standard error; Values with different superscripts (a, b) at the same row differ significantly (P<0.05); NS, non-significant (P> 0.05) effect on the parameters

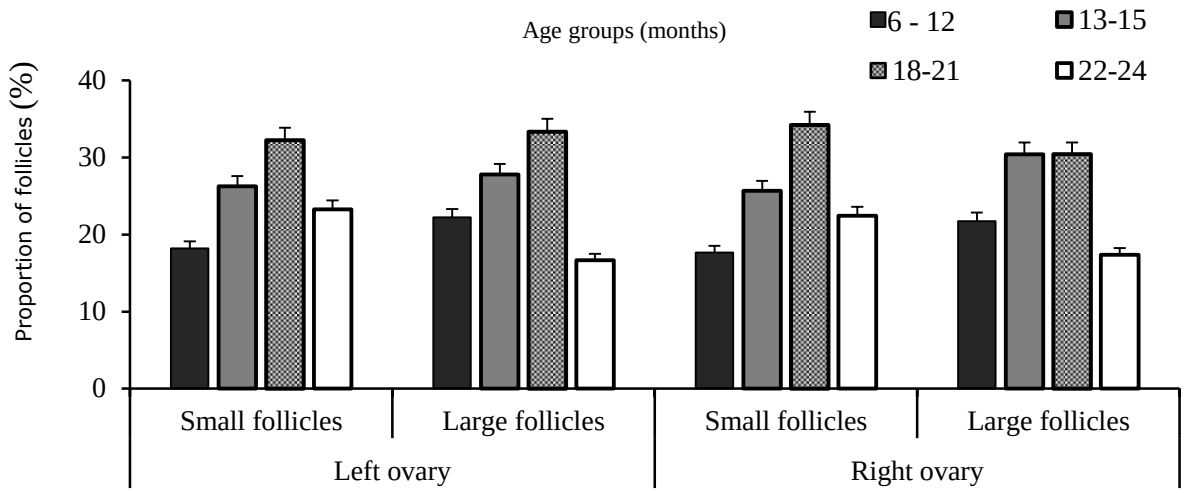

Fig 1: Different types of visible follicles (%) in ovary of goat at 6 to 12 months, 13 to 15 months, 18 to 21 months and 22 to 24 months ages.

Histological analysis of follicles

The number of primordial and primary follicles was found to be non-significantly (P>0.05) higher in 18 to 21 months aged goat ovaries (169.59±19.80) and (16.29±±2.52) than in ovaries of other age groups. The number of secondary follicles differs significantly (P<0.05) among the different age groups.

Table 3. Correlation between normal follicles number of goat ovaries

	Follicle			
	Primordial	Primary	Secondary	Antral
Primordial		0.990**	0.651^{NS}	0.062^{NS}
Primary			0.571^{NS}	0.169^{NS}
Secondary				0.713^{NS}

*** Significant at P<0.01 and NS, non-significant (P>0.05)*

Conversely, antral follicles number recorded significantly (P<0.05) higher in 13-15 months aged goat ovaries (6.19±0.64) than ovaries of 6-12 months (5.24±0.66), 18 to 21 months (4.81±0.59) and 22 to 24 months (2.61±0.45) age groups (Table 2). The histological section of the ovaries of age groups 6 to 12 months and 22 to 24 months are presented in Figure 2 to observe the differences in microscopic structure of different normal and degenerated follicles at very beginning and at the last age group because there are great difference in the quality and quantity of follicles of ovary.

It was observed that the number of primordial follicles had a strong positive linear relationship with primary follicles (P<0.01). Moreover, the number of secondary and antral follicles were also positively correlated with primordial follicles of different aged goat ovaries. Like primordial follicle, primary follicles number also positively correlated with secondary and antral follicles number of different aged goat ovaries. However, the secondary follicles number negatively correlated with the number of antral follicles (Table 3).

Table 4. Correlation between degenerated follicles number of goat ovaries

	Follicle		
	Primary	Secondary	Antral
Primary		0.992**	0.998**
Secondary			0.989*

*** Significant at P<0.01 and *significant at P<0.05*

Degeneration of ovarian follicles

The degeneration of primary, secondary and antral follicles of age groups 6 to 12 months and 22 to 24 months using photomicrographs are depicted in Figure 4 and the results of the follicular degeneration are compiled in figure 3. The number of degenerated primary follicles was found significantly (P<0.05) higher in 22 to 24 months aged goat ovaries (6.02±0.58) than ovaries of 6 to 12 months (3.15±0.49), 13 to 15 months (3.59±0.43) and 18 to 21 months (3.61±0.63) age groups. Conversely, the number of degenerated secondary follicles was found non-significantly (P >0.05) higher in 22 to 24 months aged goat ovaries (1.00±0.22) than ovaries of 6 to 12 months (0.79±0.19), 13 to 15 months (0.80±0.19) and 18 to 21 months (0.83±0.14) age groups.

Like primary follicle, degenerated antral follicles were also significantly differed (P<0.05) and it was found significantly higher in 22 to 24 months aged goat ovaries (4.27±0.56) than ovaries of other age groups. However, histological results of this study indicated that total numbers of degenerated follicles differ significantly (P<0.05) among different age groups (Figure 3).

In correlation, it was observed that the number of degenerated primary follicles were strongly positively (P<0.01) correlated with degenerated secondary and antral follicles number of different aged goat ovaries. Similarly, a significant positive linear relationship subsisted between the number of degenerated secondary and antral follicles (P<0.01) (Table 4).

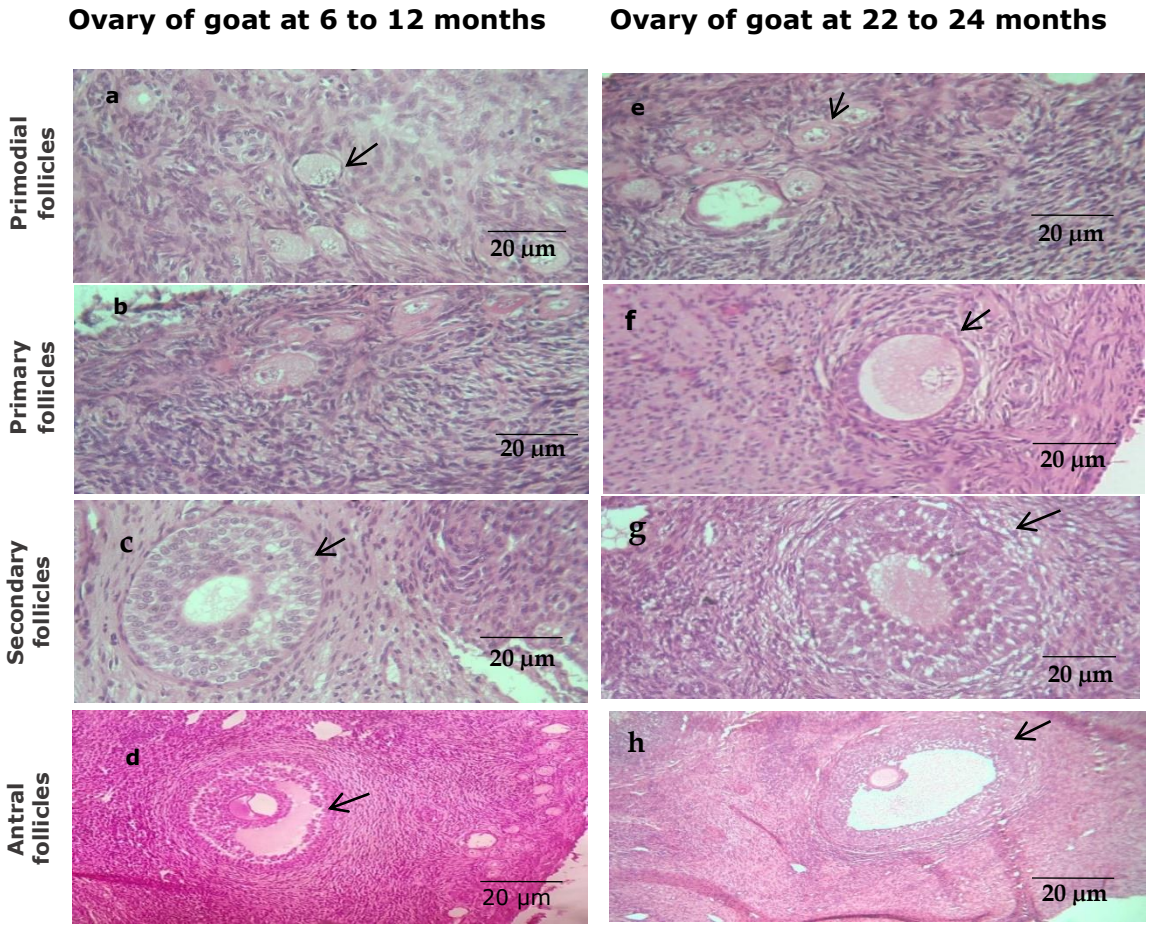

Figure 2. Histological sections of ovaries of 6 to 12 months and 22 to 24 months age groups. Arrows indicate follicles. Original magnification: **400X** (a, b, c, e, f & g) & **100X** (d & h) and the 20 µm scale bars provide a sense of proportion.

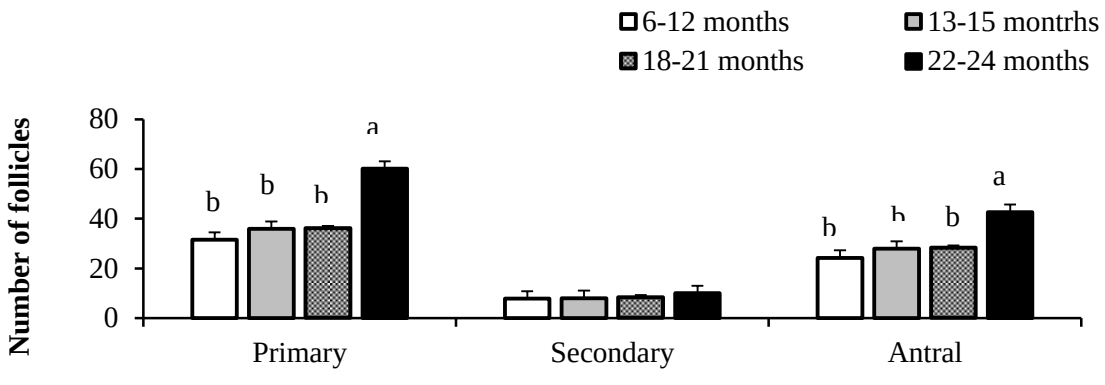

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Figure 3. Number of degenerated follicles in ovary of goat at different ages. Bars with different superscripts (a, b) differ significantly (P <0.05).

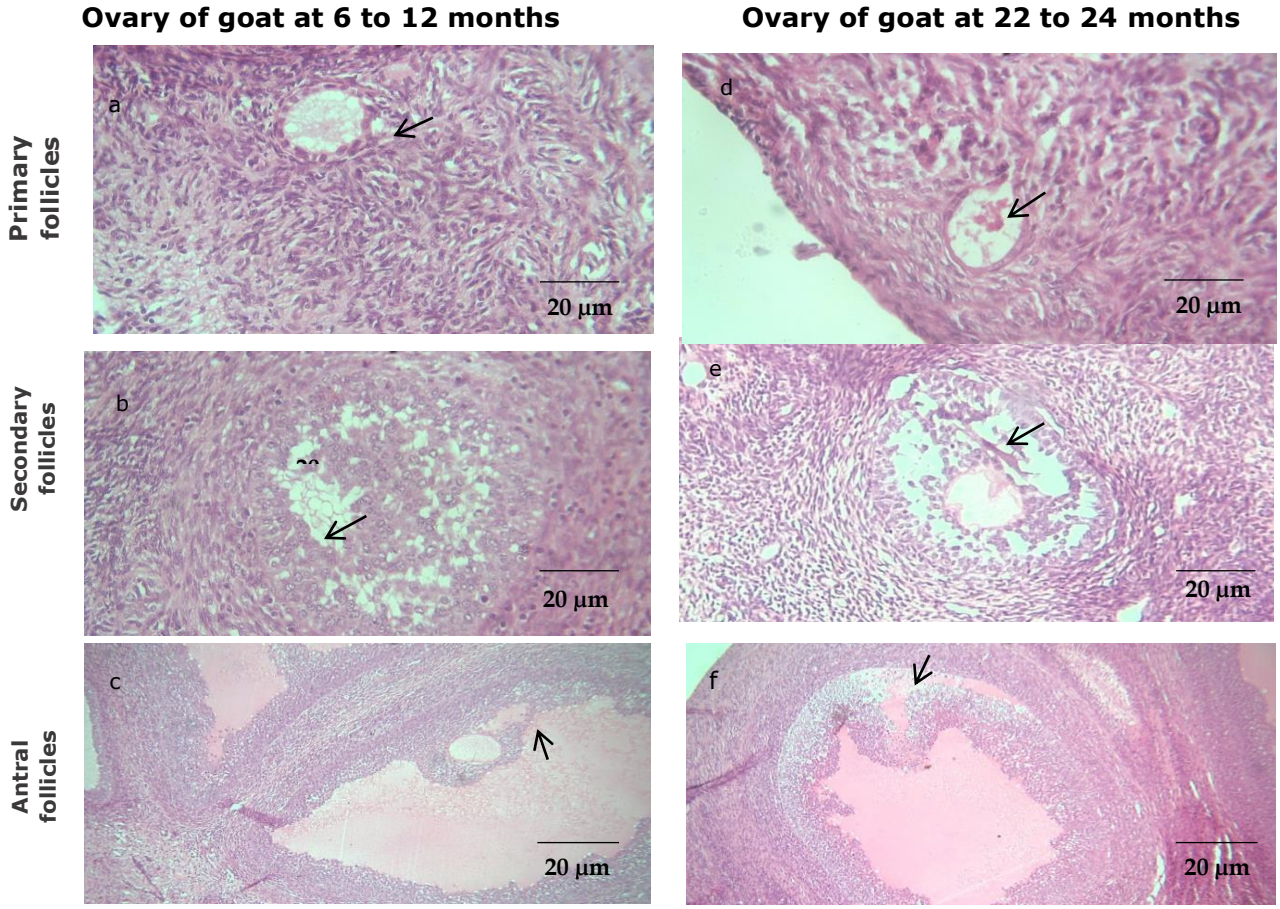

Figure 4. Histological section of ovaries 6 to 12 months and 22 to 24 months age groups showing the degeneration of follicles. Arrows indicate degeneration. Original magnification: **400X** (a, b, d & e) and **100X** (c & f) and the 20 µm scale bars provide a sense of proportion.

Discussion

Morphometric measurements of ovaries and follicles

The ovaries of mature goat were almond shaped. Morphology of ovary has influence on the quality and quantity of follicles within the ovary (Shathi *et al.*, 2022). The present findings for the weight of ovary were in concurring with the finding of Asad *et al.* (2016). However, a higher weight was reported by Hyacinth *et al.* (2016) and Mohammadpour (2007). The length of the ovary recorded in this study fall within the range of the results of Fernandez *et al.* (2020), Dogan *et al.* (2019) and Gupta *et al.* (2011). A higher length was reported by Sharma and Sharma (2004) in Gaddy goats of India, Mohammadpour (2007) in

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Iranian native goat and Adigwe and Fayemi (2005) in Maradi goat of Nigeria, respectively. Conversely, the width of left and right ovary is increased with the advancement of age which is similar to a study from Dogan *et al.* (2019), Bijna *et al.* (2016), Hyacinth *et al.* (2016) and Asad *et al.* (2016). The discrepancy in the parameters possively because of age, breed, parity, body weight, body condition score, fertility status, shrinkage of the endometrium and managemental factors variation. It was reported that the weight, length and width irrespective of left and right ovary found considerably higher in the age group of 18 to 21 months than that other groups. And this result supported by Chusman *et al.* (2009) where antral follicles were count between two age groups and observed that there was a quadratic effect of age such that antral follicle count increased until 5 years of age and decreased thereafter.

Based on the diameter of the follicles, they were graded into two categories such as follicles having 2 to 5 mm diameter and follicles having more than 5 mm diameter. Raghu *et al.* (2002) categorized the follicles of buffalo ovaries into 3 categories small (<3 mm diameter), medium (3 to 8 mm diameter) and large (>8 mm diameter) follicles which differs from the present study. The reason behind this possibly because of species variation. As regards to the number of different types of visible ovarian follicles, it was observed that number of 2 to 5 mm follicles in left and right ovaries was non-significantly (P>0.05) higher in 18 to 21 months aged goat than that of other age groups. Again, more than 5 mm follicles were found non-significantly (P>0.05) higher in 18 to 21 months aged goat than other age groups. This further implies that the number of visible follicles might increase until about 18 to 21 months of age and decreased thereafter. Similar type of studies was done in cattle. Kouamo *et al.* (2014) showed that follicular population and fertility of cattle decline with the increment of age.

Histological analysis of follicles

Histological study indicated that ovary contains different stages of follicles such as primordial, primary, secondary and antral. In the present study, it was found that there was a quadratic effect of age on total follicle count of goat ovary. The number of primordial and primary follicles was found non-significantly (P >0.05) higher in 18 to 21 months aged goat ovaries than others. On the other hand, the number of secondary follicles was recorded significantly (P<0.05) increased with the advancement of age but the number of antral follicles increased until a definite age and decreased thereafter. Hormones, apoptosis of cells, and senility may be the main factors those responsible for this deflation of ovarian stock (Monniaux *et al.*, 2009). Cushman *et al.* (2009) researched on age related follicular development on cow and found that there was a quadratic effect of age such that antral follicles count increased until 5 years of age and decreased thereafter. Similarly, in the age of 5 years of cow Erickson (1966) began to observe a decrease in the number of primordial follicles. Ultrasonography detects less follicles in older Hereford cows (Malhi *et al.*, 2005; 2006) and decreased oocyte competence (Malhi *et al.*, 2007) when compared with their younger daughters. Those findings support the present study and similar findings in human ovarian follicular development according to age carried out by Gougeon (1998) and reported that the numbers of growing follicles decrease with aging, especially in women over the age of 40.

All follicles are not ovulated, some becomes degenerated. The follicular degeneration occurs when the follicles stop to grow. During degeneration, granulosa cells undergo apoptosis and replaced by fibrous material. The degeneration of follicles can be occurred in any stages of development of the follicles. In the current study the degeneration was observed in case of primary, secondary and antral follicles. It was found that the number of degenerated primary and antral follicles was significantly (P<0.05) higher in ovary of 22 to 24 months aged goat than other age groups but degenerated secondary follicles was non-significantly (P>0.05) higher in the same age group. Many factors are responsible for follicular degeneration among which one of the primary underlying mechanisms of follicular degeneration is thought to be granulosa cell apoptosis, which has been linked to five ligand-receptor systems that regulate cell death (Manabe, 2004; Matsuda *et al.*, 2005; 2008). It is widely accepted that apoptosis is the natural driving force behind follicle loss with aging (Tilly, 2001). Like apoptosis of cells, hormones also the factors responsible for this deflation of ovarian stock (Monniaux *et al.*, 2009). According to the present study, age is also factor that influence follicular degeneration. Similar findings were reported in human by Qiao *et al.* (2014) and May-Panloup *et al.* (2016) where they found that oocyte quality appears to relate to the decline in the quality of oocyte cytoplasm and increasing nuclear genome abnormalities occurring with age.

Conclusion

The present study showed that almost all morphometric parameters of ovary were non-significantly (P>0.05) higher in goat of 18 to 21

months age. Histologically, in case of normal follicles, the age had a quadratic effect, means it increased until a definite age and decreased thereafter. Results also revealed that the number of degenerated follicles increased with the advancement of age. Finally, findings of this study imply that the age has significant effect on the quality and quantity of follicles in goat ovary.

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Author's contribution

Mst. Mahomudha Akhtar: Conceptualization, design of the study, methodology, investigation, analysis and interpretation of data, writing-original draft, writing-review and editing. M. A. M. Yahia Khandoker: Conceptualization, design of the study, investigation, revision and drafting of the manuscript, final approval of the version to be submitted. Tasmina Akter: Conceptualization, design of the study, investigation, analysis and interpretation of data, revision and drafting of the manuscript, final approval of the version to be submitted.

Conflicts of interest

The authors declare that there is no conflict of interest of any person, company or any aspect of the impact of the manuscript.

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Data Availability

All the necessary data used in this research will be made available as per the authorization of the authors.

Compliance of ethical standards

This research complies with the ethical standard required for the research in Bangladesh in relation to the handling of biological material.

Consent for publication

All authors are fully agreed to publish this research in Bangladesh Journal of Animal Science.

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