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Histomorphological and immunohistochemical studies of the ovary during dry and rainy seasons in Small East African goats (*Capra hircus*)

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Abstract This study aimed to investigate the histomorphological and immunohistochemical changes in the ovaries of Small East African goats during the dry and rainy seasons. A total of 180 ovaries were subjected to analysis using hematoxylin and eosin staining and immunohistochemistry for desmin and smooth muscle actin as cytoskeletal components. The results demonstrated the presence of healthy and atretic primordial, primary, secondary, and antral follicles in the ovaries during the dry and rainy seasons. In the dry season, the proportions of healthy primordial, primary, secondary, and antral follicles were 236 (95%), 85 (97%), 25 (78%), and 6 (27%), respectively, whereas the atretic follicles accounted for 12 (5%), 3 (3%), 7 (22%), and 16 (73%). Conversely, during the rainy season, 157 (99%), 61 (98%), 48 (93%), and 31 (92%) were classified as healthy follicles, while 2 (1%), 1 (2%), 4 (7%), and 3 (8%) were categorized as atretic. There was a significant difference in healthy and atretic follicles between the dry and rainy seasons ($P < 0.05$). Immunohistochemical analysis revealed positive staining for both desmin and smooth muscle actin in the theca cells of secondary and antral follicles, cortical stroma, and tunica media of blood vessels in healthy and atretic follicles during dry and rainy seasons. However, staining was not observed in the granulosa cells and oocytes. Overall, this study indicates that most antral follicles undergo obliterated follicular atresia during the dry season.

Keywords: atresia, desmin, immunolocalization, ovary, smooth muscle actin

1. Introduction

The histomorphological structure of ovaries in different goat breeds has been extensively studied (Islam et al 2007; Teh et al 2018). Studies on the Malabari goat breed have shown that the ovaries consist of a cortex and medulla (Bijna et al 2018). The cortex contains primordial, primary, secondary, and antral follicles. Primordial follicles consist of an oocyte surrounded by a single layer of flattened granulosa cells. The primary follicle is characterized by cuboidal granulosa cells surrounding the oocyte. In the secondary follicle, the oocyte is surrounded by two or more layers of granulosa cells and theca cells. The antral follicle contains an oocyte surrounded by two or more layers of thecal and granulosa cells, along with an antrum. The medullary region consists of connective tissues, blood vessels, and nerves. Reports on the local Egyptian breed have shown seasonal histological changes in the ovary. During autumn, the ovaries contain numerous healthy follicles at different stages of development. However, in summer, the ovaries exhibit follicles with degenerated oocytes and abnormal arrangement of granulosa cells (Emara et al 2019). Ovaries have also been reported to exhibit two types of follicular atresia: obliterative and cystic. Obliterative atresia is characterized by early destruction of the follicular wall followed by the oocyte. This type of atresia is observed in antral follicles. Cystic atresia, on the other hand, involves the loss of the oocyte followed by destruction of the follicular wall. This type of atresia affects preantral follicles and is less common in goats (Ariyaratna and Gunawardana, 1997).

Previous studies on ovaries have demonstrated the presence of intermediate filaments in the surface epithelium, follicular cells, and smooth muscle cells (Van Nassauw et al 1989; Marettova and Maretta 2002). Desmin and smooth muscle actin are well-known cytoskeletal proteins that provide structural support and contribute to the development of smooth muscle cells (Paulin and Li 2004). Desmin and smooth muscle actin have been found in cortical and thecal cells in mammalian ovaries (Marc and Nassauw 2005), and their distribution changes during follicular development (Khan-dawood et al 1996; Salvetti et al 2004) and atresia (Van Nassauw et al 1989; Madekurozwa and Kimaro 2006), suggesting that the intermediate filaments desmin and vimentin are dismantled as follicles undergo atresia.

While desmin and smooth muscle actin have been immunohistochemically demonstrated in various cell types of several mammalian species, there is no information available for Small East African (SEA) goats. Therefore, the aim of this study was

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to investigate seasonal changes in the histological structure and immunolocalization of desmin and smooth muscle actin filaments in the ovaries of SEA goats.

2. Materials and Methods

2.1. Study area

The study was conducted at Morogoro Municipal abattoir, located at 6.8157° S, 37.6657° E, with an elevation of 500 to 600 meters above sea level. Warm and cool temperatures ranging between 27 and 33.7 °C in the dry season and 14.2 and 21.7 °C in the rainy season are experienced in the municipality. The area has a subhumid tropical climate with a bimodal rainfall pattern characterized by two rainfall seasons. The mean annual rainfall is 870 mm, and the total annual evapotranspiration is approximately 1300 mm. The rainy season is divided into short rains (November to December; mean of 250 mm) and long rains (February to May; 620 mm). The dry months of the year are June, July, August, September, and October (Tanzania Meteorological Authority 2021).

2.2. Animals as a source of specimens

The collection of ovaries from Small East African goats during the dry and rainy seasons was the focus of the study. The active visits were conducted during August and September, the peak of the dry period, and from February to April, during the rainy season in 2022. The slaughterhouse received animals from the Kilosa, Mvomero, and Gairo districts of the Morogoro region. Most of the goats slaughtered during the study period were Small East African goats and had body condition scores ranging from 3.0 to 4.0 on a scale of 1 to 5. Ninety sexually mature healthy adult Small East African goats aged 1-2 years, brought for slaughter at the slaughterhouse, were randomly selected. The age of the animals was estimated through dental formula examination. After *ante mortem* examination, 45 healthy animals were selected for inclusion in the study for each season.

2.3. Tissue sampling and processing

Following slaughter, sections of ovarian tissue from the cranial, middle, and caudal poles of both left and right ovaries were collected and placed in 10% buffered formalin for fixation. The ovaries were then transported to the histology laboratory at Sokoine University of Agriculture for further fixation at room temperature for two days, following the described method by Slaoui and Fiette (2011) with minor modifications. The fixed tissues were divided into portions (cranial pole, middle, and caudal pole), dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax. Tissue blocks were prepared, and 5 µm thick sections were cut using a rotary microtome (Baird and Tatlock (London) Ltd; England) to produce tissue sections. Some sections were used for hematoxylin and eosin (H & E) staining, while others were used for immunohistochemistry.

2.4. Immunohistochemistry

The tissue sections were deparaffinized and rehydrated, followed by incubation with hydrogen peroxide block (ab64261) at room temperature (RT) for 10 minutes and washing (3 x 5 minutes) in PBS. Sections were then incubated with protein block (ab64261) at RT for 10 minutes. Next, the sections were incubated with rabbit polyclonal alpha-smooth muscle (ab5694) actin and desmin (ab15200) primary antibodies overnight in a dark, humid chamber at 4 °C, diluted at 1:200 in PBS. For the negative control, PBS was applied in place of the primary antibodies. The sections were washed (3x15 minutes) in PBS and incubated with goat anti-rabbit HRP conjugate micropolymer (ab64261) for 60 minutes at RT. Afterward, the sections were washed (3x15 minutes) in PBS before being incubated for 3-5 minutes with 50X DAB chromogen solution (ab64261) to visualize the binding sites. The reaction was stopped by rinsing the sections in water for 10 minutes, followed by dehydration through a series of ethanol solutions (70%, 95% I, 95% II, absolute I, and absolute II), clearance, and mounting using a mixture of distyrene (polystyrene), a plasticizer (tricresyl phosphate), and xylene (DPX). The binding sites were evaluated using an Olympus BH-2 microscope equipped with an Olympus camera for image capturing. The relative immunostaining intensities of desmin and smooth muscle actin were categorized as negative, weak, moderate, and strong.

2.5. Statistical analysis

At the light microscopic level, every tenth section from the serial sections was examined and evaluated for the number of healthy and atretic follicles. The collected data were stored in Microsoft Excel for analysis. Mean values were calculated and compared between follicles and seasons using one-way analysis of variance (ANOVA). A probability of 0.05 was considered significant.

3. Results

3.1. Gross observation

The shape of the ovaries of the Small East African goats was oval, and they exhibited a pale color. They were situated on each side of the pelvic cavity in the sublumbar area. Measurements revealed a length ranging from 9.76 to 10.07 mm, a width ranging from 7.76 to 7.97 mm, and a weight ranging from 0.8 to 2 grams.

3.2. Histological observations

During each season (dry and rainy), histological examination of ovarian tissue sections revealed the presence of both healthy and atretic follicles. Healthy primordial follicles consisted of an oocyte surrounded by a single layer of flat granulosa cells. These follicles were predominantly solitary, although occasional clusters of two or more follicles were observed. The primary follicle displayed a wall formed by a layer of granulosa cells encircling the oocyte. At this follicular size, the granulosa cells exhibited a cuboidal shape. In the secondary follicles, the follicular wall surrounding the oocyte comprised cuboidal granulosa cells and undifferentiated thecal cell layers. The antral follicles, on the other hand, consisted of an oocyte surrounded by the zona pellucida, multiple layers of granulosa cells, and differentiated theca interna and externa cell layers. These follicles exhibited a fluid-filled antrum. Additionally, the granulosa cells surrounding the oocyte were referred to as corona radiata, while others formed cumulus oophorus, creating a stalk of cells at one point (Figure 1: A-D). Obliterated antral follicles were characterized by disorganized granulosa cells, degraded oocytes, and abnormal arrangement of cumulus oophorus. The granulosa and theca interna exhibited varying degrees of cellular disruption. Atretic follicles were detached from the stroma, and advanced stages of atresia exhibited disruptions, complete follicular obliteration, and connective substitution (Figure 2: A-D).

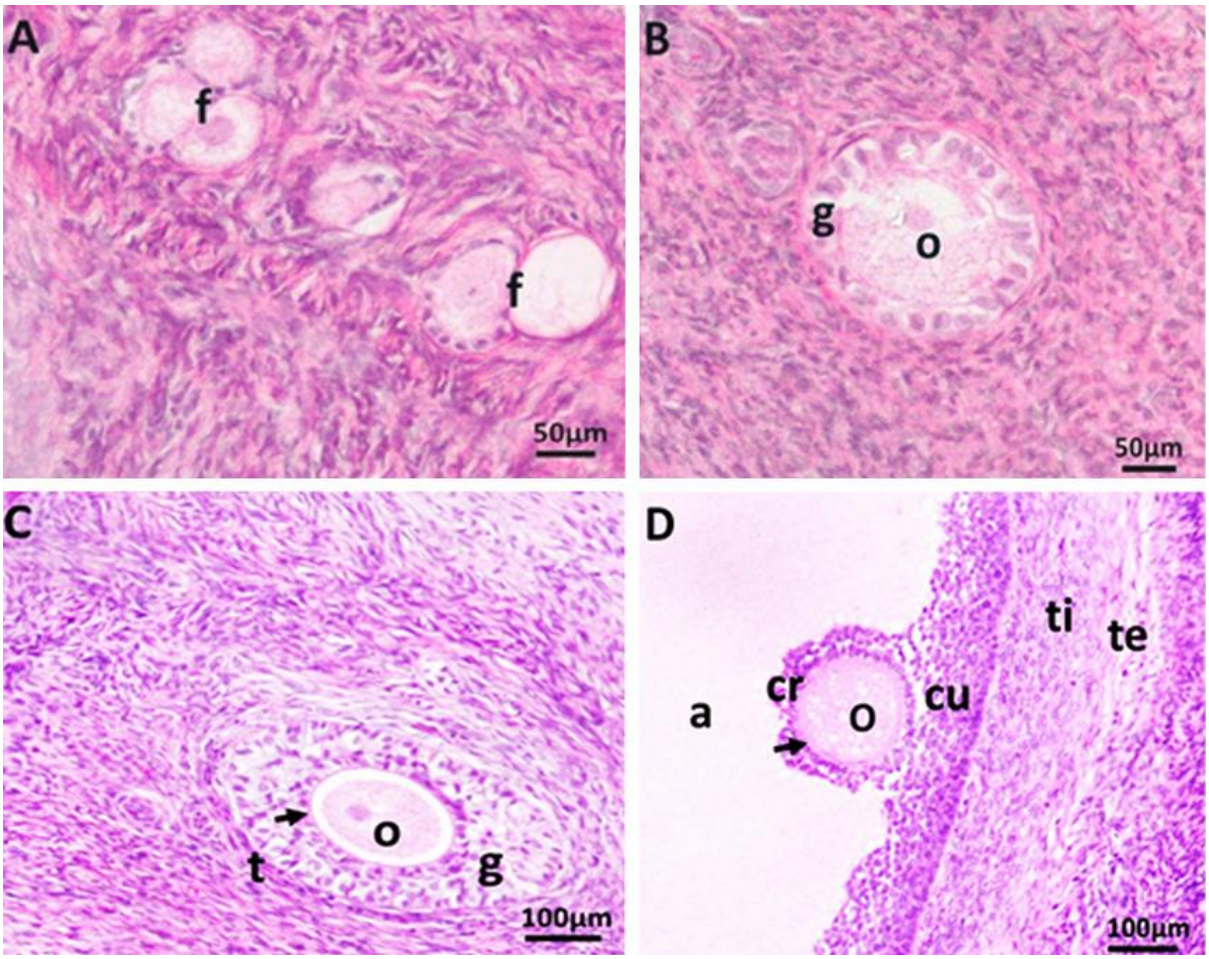

Figure 1 Cross section through Small East African goat ovary. Hematoxylin and eosin-stained photomicrographs showing healthy follicles at various developmental stages. (A) Cluster of primordial follicle **f**. (B) Primary follicle with an oocyte **o** surrounded by a layer of granulosa cells **g**. (C) Secondary follicle with an oocyte **o** surrounded by zona pellucida (**black arrow**), granulosa cells **g,** and undifferentiated theca cells **t**. (D) A healthy antral follicle is composed of an oocyte **o**, zona pellucida (**black arrow**), antrum **a**, corona radiata **cr**, theca internal **ti,** and external cells **te**. The oocyte lies in accumulating granulosa cells, the cumulus oophorus **cu**. **Scale bar: A & B: 50 µm; C & D: 100 µm**.

3.3. Micrometry

Histomorphological changes in the collected ovaries during the dry and wet seasons were assessed. A significant difference ($P < 0.05$) was observed in the number of healthy and atretic antral follicles between the two seasons. In the dry

season, a total of 248, 88, 32, and 22 primordial, primary, secondary, and antral follicles were identified, respectively. Among these, 236 (95%), 85 (97%), 25 (78%), and 6 (27%) primordial, primary, secondary, and antral follicles were categorized as healthy, while 12 (5%), 3 (3%), 7 (22%), and 16 (73%) were classified as atretic. In contrast, during the wet season, the total numbers of primordial, primary, secondary, and antral follicles counted were 159, 62, 52, and 34, respectively. Among these, 157 (99%), 61 (98%), 48 (93%), and 31 (92%) were identified as healthy, while 2 (1%), 1 (2%), 4 (7%), and 3 (8%) were categorized as atretic. However, no statistically significant differences were observed in the number of healthy and atretic primordial, primary, and secondary follicles between the dry and wet seasons.

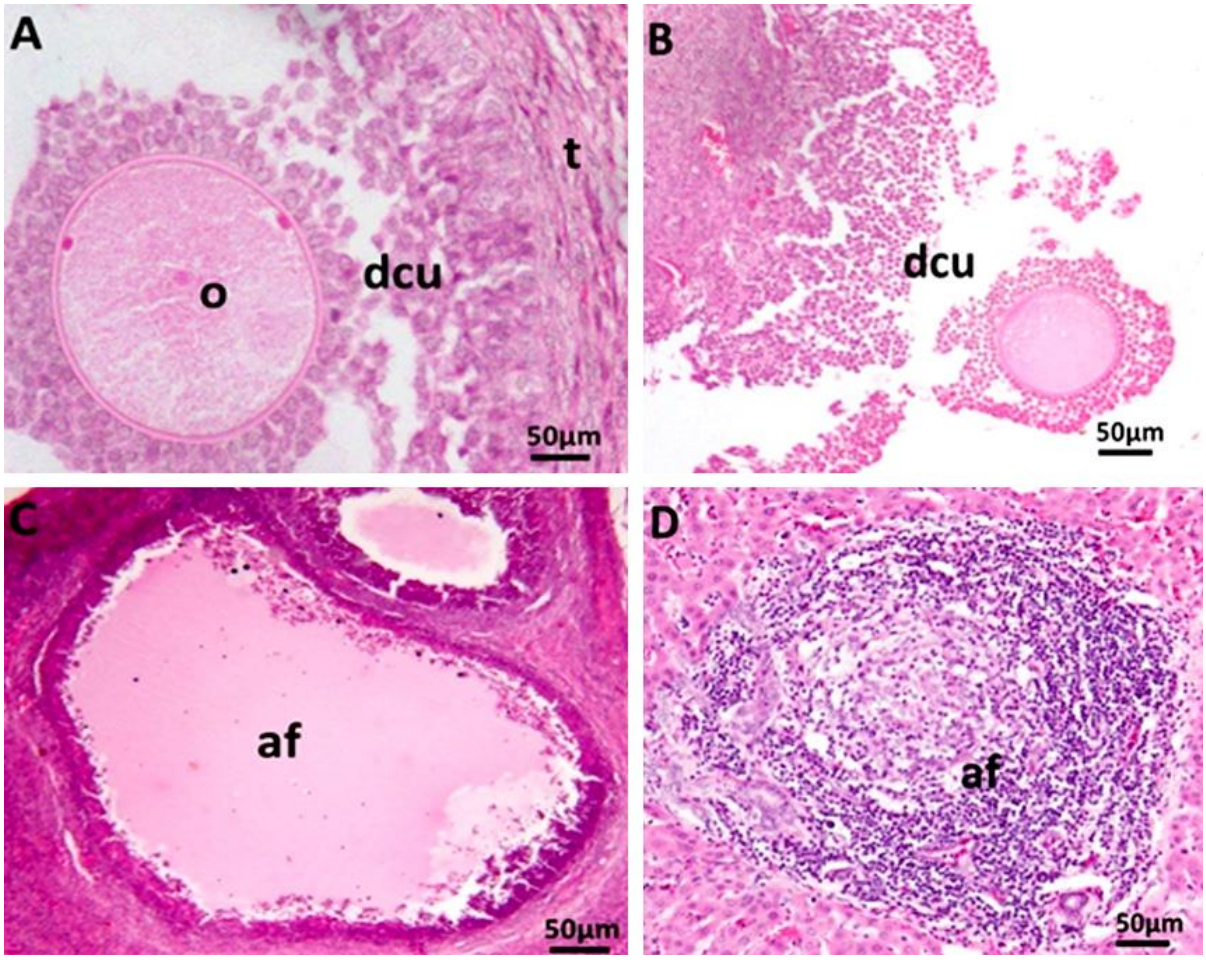

Figure 2 Cross section through Small East African goat ovary. Hematoxylin and eosin-stained photomicrographs showing atretic follicles. (A) Atretic antral follicle with degenerated oocyte **o**, abnormal arrangement of cumulus oophorus **dcu**, hypertrophied and detached theca layer **t**. (B) Atretic antral follicle with an abnormal arrangement of cumulus oophorus **dcu**. (C) Atretic follicle **at the** early stage of atresia characterized by an intact wall and fluid-filled follicle. (D) Atretic follicle **af** at an advanced stage of atresia characterized by wall degeneration, total follicle obliteration, and connective substitution. **Scale bar: A-D: 50 µm**.

Table 1 Mean±SE number of healthy and atretic follicles per ovary during the dry and wet seasons.

Follicle Type	Seasons									
	Dry (n=45)					Wet (n=45)				
	Total	Healthy	%	Atretic	%	Total	Healthy	%	Atretic	%
Primordial	248	236±0.9	95	12±0.3	5	159	157±0.5	99	2±0.4	1
Primary	88	85±0.5	97	3±0.3	3	62	61±0.8	98	1±0.0	2
Secondary	32	25±0.7	78	7±0.4	22	52	48±0.8	93	4±0.5	7
Antral	22	6±0.7	27*	16±0.8	73*	34*	31±0.8	92	3±0.4	8*

*Indicates significant differences between healthy and atretic follicles in dry and wet seasons ($P < 0.05$).
SE: Standard Error

3.4. Immunohistochemical localization of desmin and smooth muscle actin in healthy and atretic follicles during dry and rainy seasons

In healthy and atretic follicles during dry and rainy seasons, immunostaining for desmin and smooth muscle actin was detected in the cytoplasm of smooth muscle cells. Strong staining was observed in healthy follicles in rainy seasons, but moderate to weak staining was observed in the dry season. In healthy follicles, staining was specifically strong in secondary follicles in theca cells, theca externa, and interna of antral follicles; cortical stroma and tunica media of blood vessels within ovarian parenchyma. Staining was moderate in the theca cells of secondary and antral follicles in atretic follicles. Staining was not detected in the granulosa cells and oocytes of healthy and atretic secondary and antral follicles. In addition, staining for desmin (Figure 3: A-D) varied from moderate to weak, while that of smooth muscle actin (Figure 4: A-D) was strong in both healthy and atretic follicles.

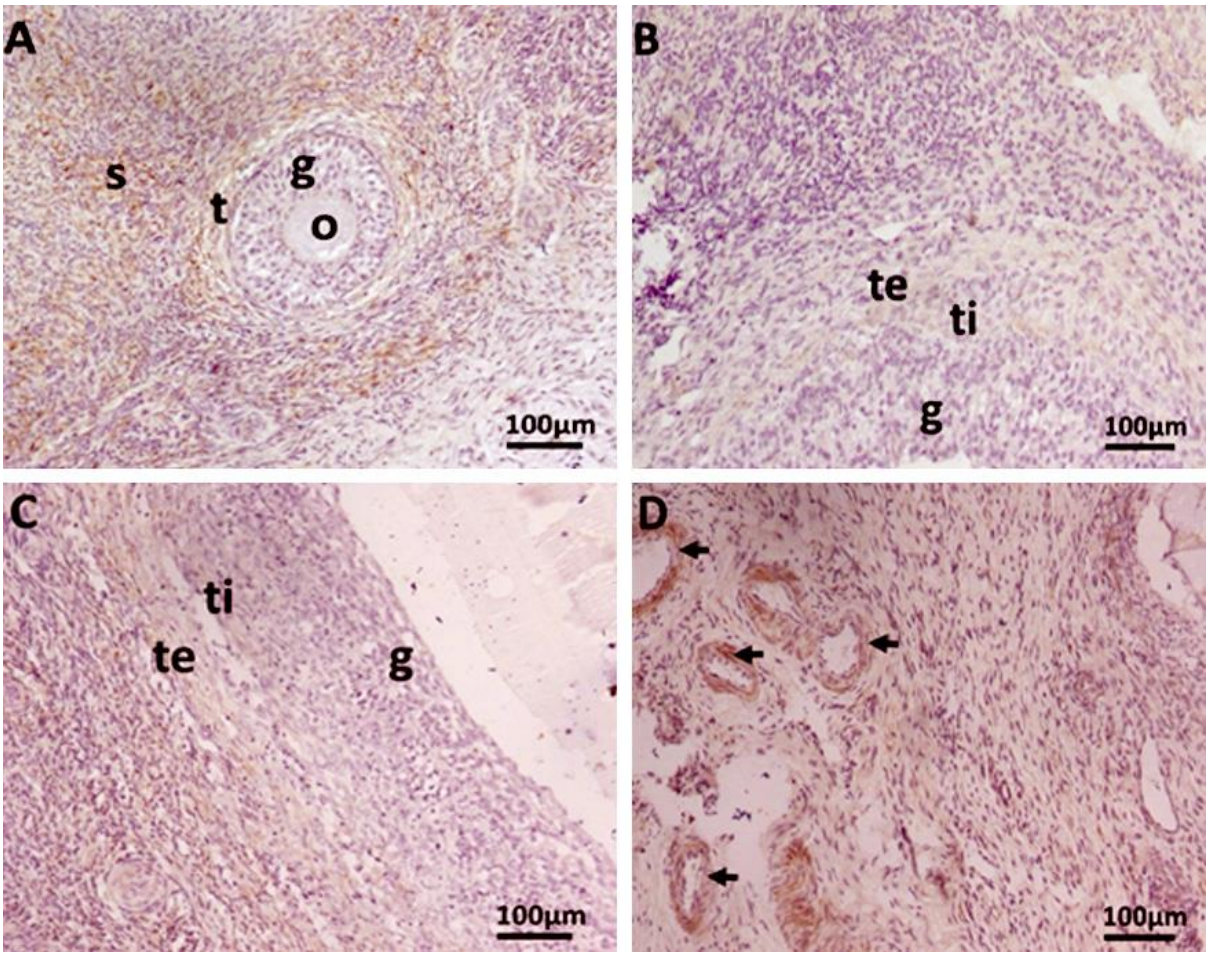

Figure 3 Immunohistochemical localization of desmin in the ovaries of Small East African goats. (A) Moderate immunoreactivity for desmin is seen in healthy secondary follicles within the theca cells **t** and cortical stroma **s** but is absent in granulosa cells **g** and oocytes **o**. Staining is moderate in cells of the theca interna **ti** and theca externa **te** of healthy antral (B). (C) Weak staining is seen in cells of the theca interna **ti** and theca externa **te** of atretic antral follicles. (D) The tunica media of blood vessels (**black arrows**) in the ovarian parenchyma are also moderately stained. **Scale bar: A-D: 100 µm.**

4. Discussion

Histomorphological and immunohistochemical changes occurring in the ovaries of Small East African goats during dry and rainy seasons are demonstrated in this study. The results of the current study show that the histological morphology of healthy follicles in the Small East African goat corresponds to the description given for other breeds (Bari et al 2012; Emara et al 2019). The ovary of SEA goats is composed of primordial, primary, secondary, and antral follicles, as documented in other breeds, such as the Egyptian goat (Emara et al 2019).

Regarding the classification of atretic follicles in goats, only the obliterative type of atresia was observed in the present study. This type was characterized by apical detachment of the follicular wall, as observed in local goats in Sri Lanka (Ariyaratna and Gunawardana, 1997). A higher number of atretic follicles was observed during the dry season than during the wet season. This may be associated with insufficient secretion of gonadotropins caused by suboptimal function of the hypothalamo-hypophyseal-gonadal axis. Hormones have been shown to play a crucial role in the development of summer anestrus in buffaloes, as alterations in hormonal secretions are observed in most buffaloes with abnormal reproduction during the summer (Das and Khan 2010).

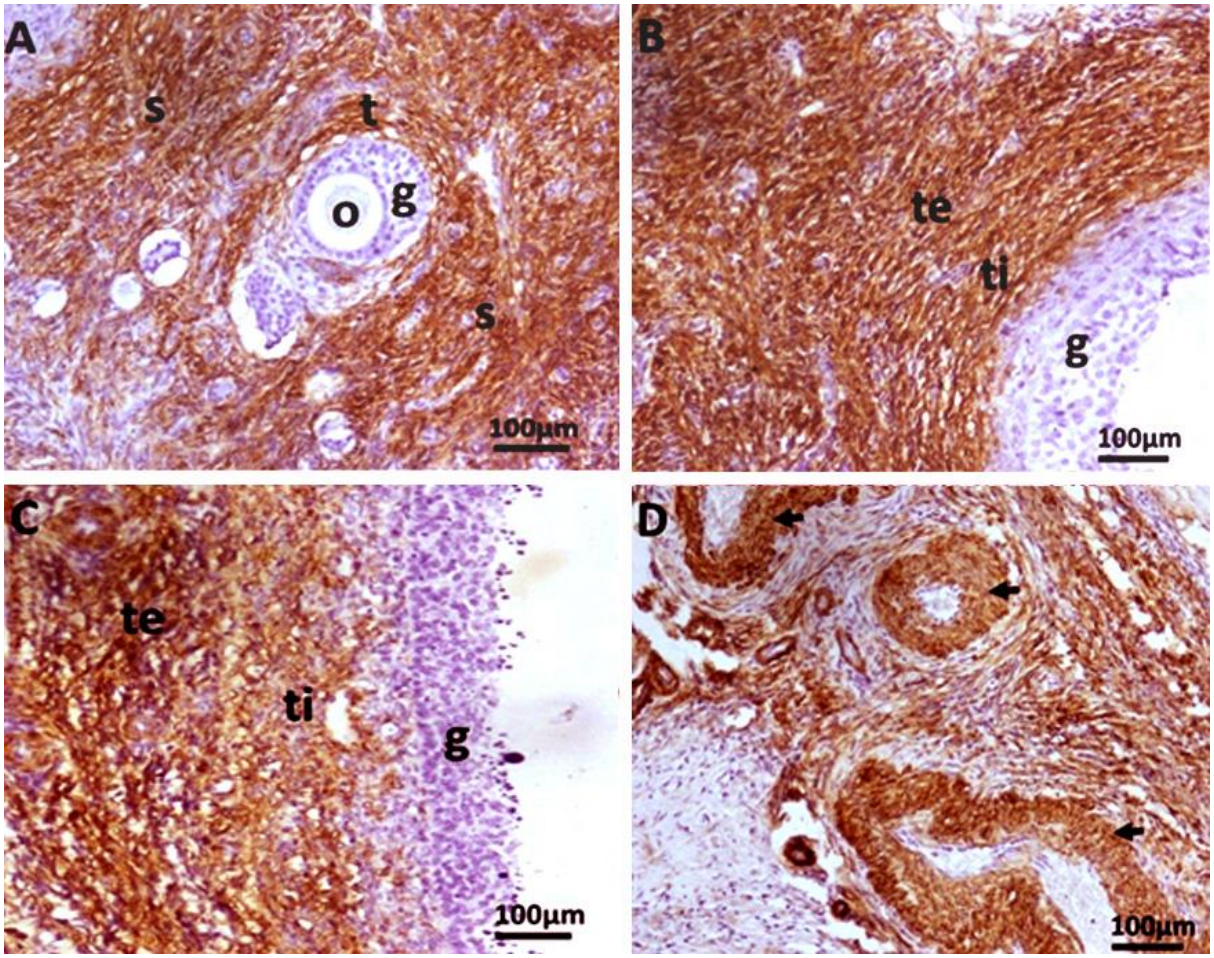

Figure 4 Immunohistochemical localization of smooth muscle actin in the ovaries of Small East African goats. (A) Strong immunoreactivity for smooth muscle actin is seen in healthy secondary follicles within the theca cells **t** and cortical stroma **s. Nevertheless, i**t is absent in granulosa cells **g** and oocytes **o**. Staining is also strong in cells of the theca interna **ti** and theca externa **te** healthy antral follicle (B). Moderate staining is seen in cells of the theca interna **ti** and theca externa **te** of the atretic antral follicle (C). The tunica media of blood vessels (**black arrows**) in the ovarian parenchyma are also strongly stained (D). **Scale bar: A-D: 100 µm.**

Desmin and smooth muscle actin are essential components of the cytoskeleton. Immunohistochemical staining of the ovaries localized the intermediate filament desmin at weak to moderate levels and smooth muscle actin at strong intensities in theca cells of secondary follicles, theca externa and interna of antral follicles, cortical stroma, and tunica media of blood vessels within the ovarian parenchyma. Additionally, intermediate filaments have roles in various biological processes, including cell-to-cell adhesion, proliferation, and differentiation (Helfand et al 2004). The presence of desmin and smooth muscle actin in muscle bundles in the cortical stroma is believed to form a structural framework that provides mechanical support to the ovary (Van Nassauw and Callebaut, 1991). Furthermore, the stromal localization of desmin and smooth muscle actin was also demonstrated in the tunica media of blood vessels in the ovary, where they play a role in regulating blood flow to the ovary. The observation of desmin and smooth muscle actin in the tunica media of blood vessels correlates well with findings reported in ewes (Marettová and Maretta, 2002) and cows (Wendl et al 2012). Moderate staining of desmin and strong staining of smooth muscle actin in theca externa cells of healthy antral follicles indicate their role in the contraction of smooth muscle cells in the theca externa, thereby initiating the process of ovulation (Madekurozwa et al 2010). Atretic follicles showed reduced staining for desmin and smooth muscle actin, suggesting that the intermediate filaments are dismantled when follicles undergo atresia.

5. Conclusions

A seasonal growth cycle and atresia were observed in the ovarian follicles of Small East African goats. Healthy follicles were present during the wet season, while the dry season showed a higher occurrence of atretic antral follicles. The atresia process in antral follicles involved the destruction of follicular cells closer to the antrum, while most basal follicular cells remained intact. Additionally, the results of the immunohistochemical study revealed changes in the distribution of intermediate filaments during follicular development and atresia, indicating the need for further investigations on the impact of heat and nutrition on folliculogenesis in Small East African goats.

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Ethical Considerations

Permission to carry out this study was granted by the Research and Ethical Committee of Sokoine University of Agriculture (Ref: SUA/DPRTC/R/186/27). Verbal consent was obtained from each traded stock owner after explaining the study's purpose and importance before data collection.

Conflict of Interest

The authors declare no conflicts of interest.

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