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SUMMARY

Reproductive cycle of Small East African (SEA) goats in the tropics is characterized by a reduced fertility rate during the dry season. The reduced fertility rate has a negative impact on livestock sector development and the livelihood of rural communities. The current study was conducted to evaluate ovarian morphometric parameters and follicular atresia during dry and wet seasons. A total of 90 apparently healthy adult goats from Morogoro region in Tanzania brought for slaughter at Morogoro Municipal slaughterhouse were randomly selected for the study. Following the slaughter both left and right ovaries were collected for gross and histomorphological analysis. The results of morphometric analysis found that, length of right ovary was significantly higher than that of the left (p<0.05). Histological analysis revealed a significant increase in the number of atretic follicles during the dry season when compared to the wet season (p<0.05). These findings indicate that the reduced fertility rate in the SEA goat during the dry season could be contributed by an increased rate of follicular atresia.

Keywords: Follicular atresia, Morphometry, Ovary, Small East African goats

INTRODUCTION

The fertility of Small East African (SEA) goats is important in economic development. Maintaining a good reproductive performance needs clear and detailed information on the structure and reproductive patterns of the ovary (Fahad *et al*., 2019). Reports show that goat reproduction is cyclical and mostly influenced by photoperiod (Ungerfeld and Bielli, 2012). However, in the tropics, goat breeds such as SEA goats are regarded as continuous breeders due to minimal day length variation in the region compared to the temperate region (Alves *et al.*, 2018) and they can ovulate every month all the year round.

The fact that reproductive performance largely depends on the reserve of healthy oocytes and endocrine function of the ovary, understanding the seasonal ovarian morphological changes will contribute to our better understating of the reproductive physiology. Ovarian morphological changes can be contributed by follicular atresia, a known degenerative process in which oocytes in various stages of

development and growth are lost from the ovary. According to a report by Bari *et al.* (2012), atresia is the leading reason for diminished goat fertility. Studies of the gross and morphometry of the ovary have been shown to be one of the best approaches useful in describing the ovarian changes between seasons (Bijna *et al.*,2018). For example, high number of developed ovarian follicles was observed during winter compared to the summer season in temperate climate (Mm *et al.*, 2011; Bari *et al*., 2012).

In tropical environment, an increased number of ovarian follicles development was revealed in Sahelian goats during the wet season and decreased in the dry season (Jaji *et al.*, 2012). Despite of the importance of SEA goats, little is known on the morphological changes that occur in the ovary of the Small East African (SEA) goat between seasons and that can affect their reproductive performance. The current study aimed to reduce the knowledge gap on factors that can affect seasonal reproductive performance of SEA and contributes towards solutions to improve reproductive efficiency in this breed of goat.

MATERIALS AND METHODS

This study was granted ethical clearance Ref: SUA/DPRTC/R/186/27 by the Research Ethical Committee of Sokoine University of Agriculture. A total of 90 sexually matured adult female SEA goats aged between 1 and 2 years brought for slaughter in Morogoro Municipal slaughterhouse were utilized (45 goats in dry and wet seasons respectively). All healthy adult female animals were eligible to be included in the study. Age was determined during ante mortem examinations based on the dental formula as previously described (Eubanks , 2012). Samples of the ovaries were collected in both dry and wet seasons. According to meteorological data (Tanzania Meteorological Authority (TMA) , 2022), the dry season was in the months of August, September and October 2021. Wet season was in February, March and April 2022.

Immediately after animal slaughter, the abdomen was opened and the topographical position of ovaries were observed and noted. Both left and right ovaries were collected for gross and morphological studies including examination for the presence of either corpus haemorrhagicum or corpus luteum. Corpus haemorrhagicum and corpus luteum were identified on the basis of-morphological structure and coloration i.e., corpus haemorrhagicum was red while the corpus luteum was yellow.

The collected ovaries were trimmed and weighed separately, using an electrical balance (Chong *et al.*, 2012). The ovaries were then measured in length (mm) and width (mm) using vernier calliper. The length was measured from cranial pole to caudal pole, whereas the width was measured transversely at the widest mid region of the ovary . Thereafter, ovarian tissue sections from cranial, middle and caudal pole were fixed by immersion in 10% buffered formalin for 48 hours pending processing. The fixed tissues were then dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were sectioned serially and stained with hematoxylin and eosin following standard procedures (Kiernan, 2016). At light microscopic level, every tenth section was examined and evaluated for number of healthy and atretic follicles. The mean length, width and weight were calculated and compared between left and right, as well as, between seasons using Analysis of Variance (ANOVA). A probability of 0.05 was considered significant.

RESULTS

It was generally observed that during both seasons the ovaries of the SEA goat were oval in shape and pale in color. They were positioned at the lateral margin of the pelvic inlet near the uterine tubes, covered by a broad ligament and mesovarium. Follicles at various stages of development as well as corpus haemorrhagicum or corpus luteum were observed on the surface of ovary (Figure 1). The evaluated morphometric parameters during the dry and wet seasons are summarized in Table 1. During the dry season, the mean (±SE) length of ovary was (9.76 ± 0.20 mm left, 10.28 ± 0.40 mm right), width was (7.76 ± 0.39 mm left, 7.76 ± 0.37 mm right) and weight was (1.91 ± 0.11 g left, 1.92 ± 0.12 g right). In the wet season, the left ovary measured mean (±SE) was 9.76 ± 0.20 mm in length, 7.76 ± 0.20 mm width and weighed 1.94 ± 0.13 g. The right ovary was 10.48 ± 0.39 mm length, 7.97 ± 0.40 mm width and weighed 1.94 ± 0.13 g.

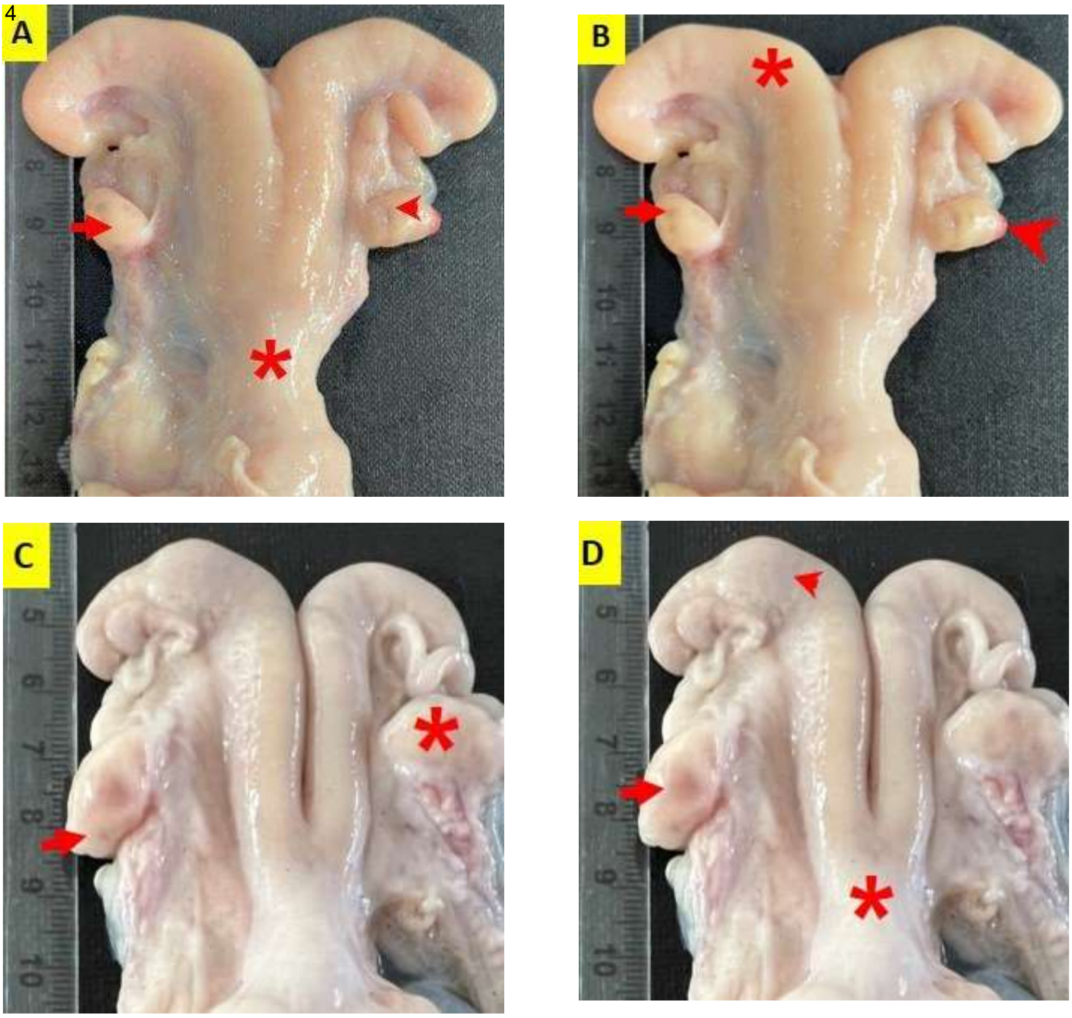

Figure 1. Photographs of reproductive tract from Small East African goat during wet season (**A** and **B**), dry season (**C** and **D**). **A**: Left ovary (arrow), right ovary (arrowhead) and uterine body (asterisk). **B**: Note presence of developing follicles (arrow), uterine horn (asterisk) and corpus haemorrhagicum (arrowhead). **C**: Left ovary (arrow) and right ovary (asterisk). **D:** Note the presence of developing follicles (arrow), uterine horn (arrowhead) and uterine body (asterisk).

Table 1. Morphometric parameters (length, width and weight) of Small East African goat ovaries during the dry and wet seasons.

Parameters (mean ± SE)	Ovary position	Seasons	
		Dry (n=45)	Wet (n=45)
Length (mm)	Left	9.76 ± 0.20^{a}	9.76 ± 0.28^{a}
	Right	10.28 ± 0.28^{b}	10.48 ± 0.39^{b}
Width (mm)	Left	7.76 ± 0.39^{a}	7.76 ± 0.42^{a}
	Right	7.76 ± 0.37^{a}	7.97 ± 0.40^{a}
Weight (g)	Left	1.91 ± 0.11^{a}	1.91 ± 0.13^{a}
	Right	1.92 ± 0.12^{a}	1.94 ± 0.13^{a}

SE: Standard Error, Values in the same column and row of each parameter (length, width, weight) with different letters in the superscript were significantly different ($p<0.05$), while number with the same letter did not differ significantly.

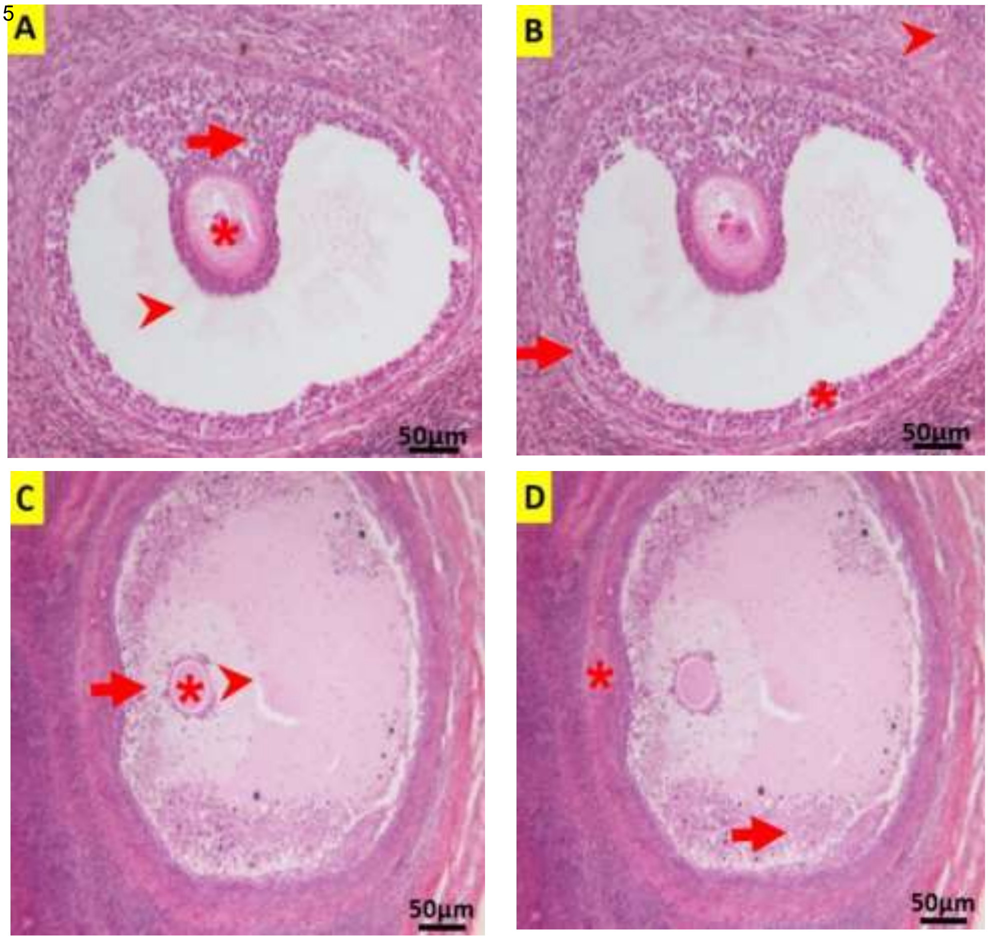

Figure 2. Photomicrographs of the Small East African goat ovary showing healthy antral follicle (**A** and **B**), atretic antral follicle (**C** and **D**). **A**. Oocyte (asterisk); cumulus oophorus (arrow) and antrum (arrowhead). **B**. Note a well-organized granulosa cells layers (asterisk); thecal layer (arrow) and stroma (arrowhead). **C**. Oocyte (asterisk), destructed cumulus oophorus (arrow) and antrum (arrowhead). **D**. Disorganized granulosa cells (arrow) and hypertrophied theca layer (asterisk).

Table 2. Mean±SE number of healthy and atretic follicles per ovary of the Small East African goat during the dry and wet seasons

Follicle Type	Seasons					
	Dry (n=45)			Wet (n=45)		
	Total	Healthy %	Atretic %	Total	Healthy %	Atretic %
Primordial	248	95[a]	5[a]	159	99[a]	1[a]
Primary	88	97[a]	3[a]	62	98[a]	2[a]
Secondary	32	78[a]	22[a]	52	93[a]	7[a]
Antral	22	27[a]	73[a]	34	92[b]	8[b]

Percentage values in the same row of each follicle type with different letters in the superscript were significantly different (p<0.05), while those with the same letter did not differ significantly

Seasonal morphometric characteristics of antral follicles

The ovaries of the SEA goat contained follicles at various stages of development. The observed follicles were primordial, primary, secondary and antral follicles.

Primordial follicles were characterized by a single layer of flat granulosa cells surrounding the oocyte. In the primary follicles, the oocyte was surrounded by a- single layer of cuboidal granulosa cells. Secondary follicles had two layers of cuboidal granulosa cells. The oocyte in the antral follicle was surrounded by multiple layers of cuboidal granulosa cells. In both seasons, the ovary contained both healthy and atretic antral follicles. The healthy antral follicle contained an oocyte which was surrounded by a zona pellucida followed by multiple layers of granulosa cells, an antrum and theca cells. The cumulus oophorus formed by granulosa cells was clearly observed (Figure 2A and 2B).

In atretic antral follicle, the oocyte was surrounded by multiple layers of disorganized granulosa cells, shrunken antrum and hypertrophied theca cells. In addition, in atretic antral follicles the granulosa cells forming cumulus oophorus were un-evenly dispersed and displayed pale cytoplasm (Figure 2C and 2D). The results of the observed changes in number of healthy and atretic follicles between seasons are summarized in Table 2. During the dry season, 95% of all counted primordial follicles were healthy (i.e., displayed well organized follicular wall), whereas 5% were atretic (vacuolated granulosa cells and dispersed cumulus oophorus). In addition, up to 97% healthy and 3% atretic primary follicles were observed. Furthermore, 78% healthy and 22% atretic secondary follicles were documented.

The counting of antral follicles shows only 27% healthy and 73% atretic follicles. However, during the wet season, 99% of all assessed primordial follicles were healthy while only 1% were atretic. Additionally, 98% primary follicles were healthy and 2% were atretic. Furthermore, 93% secondary follicles were healthy while 7% were atretic. Approximately 92% of antral follicles were healthy. In this type only 8% were atretic.

DISCUSSION

The present study documents the morphometrical changes and follicular atresia in the ovary of the Small East African goats during the dry and wet seasons. Based on the findings of this study, the gross morphology and location of ovaries in SEA goat correspond to the earlier descriptions in other breeds of goats such as Black Bengal goats and Anatolian wild goats (Islam *et al.*, 2007; Kırbaş Doğan *et al.*, 2019). In SEA goat, ovaries were oval in shape and located in the pelvic inlet of the sub-lumbar region.

In the current investigation, the ovarian morphometric parameters revealed that there were no significant variations in the length, width and weight of the left ovary between dry and wet seasons. However, there was an increase in the afore-mentioned parameters in the right ovary as compared to the left ovary during both seasons.

The cause of his change was not established but is likely to be related to physiological changes. This argument is supported by earlier findings by Pramod *et al*.(2013) and Asad *et al.* (2016) which show that reproductive performance in the prolificacy goat depends on increased ovulation rate of the right ovary.

As shown in the results, the number of healthy and atretic ovarian follicles differed between seasons. The percentage number of follicles (primordial, primary, secondary and antral) was lower in the dry season than in the wet season. This finding suggests that the rate of follicular development was higher in the wet season than in the dry season. The high rate of follicular development could be due to the availability of nutrients. Nutrients have been shown to influence ovarian activity. According to Dadok*et al. (*2020) availability of nutrients during wet season improved ovarian steroidal activity and enhance folliculogenesis in Black Bengal goat. In the current study, atresia affected all types of ovarian follicles. However, there was a positive correlation between follicular size and the rate of atresia.

Large (antral) follicles were highly affected by atresia when compared to the small/developing follicles. In addition, the rate of atresia was higher in the dry season (73%) than wet season (8%). The presence of large number of atretic follicles during the dry season can be linked to the-

Insufficient gonadotrophins secretion caused by sub-optimal function of hypothalamo–hypophyseal–gonadal axis (Wilson *et al.* , 1998). Indeed, during the dry season goats are exposed to direct sunshine and thus high temperature which causes detrimental effect on ovarian folliculogenesis (Ozawa *et al.*, 2005).

Reports by Emara *et al*. (2019) and Adjassin *et al.* (2022) support this argument. According Emara *et al*. (2019) and Adjassin *et al.* (2022), a decreased rate of folliculogenesis was recorded in goat during high temperature. Furthermore, heat affects steroidogenic activities in the follicular cells. As reported in the cow, heat stress affected number of ovulated follicles and the size of oocyte. The report further showed that heat stress could have effect on steroidogenesis in the thecal cells, granulosa cells or both thecal and granulosa cells (Wilson *et al.*, 1998).
This could also be the case in SEA goat. In support of this argument studies by Jordan, 2003; Ozawa *et al.*, 2005; Friedman *et al.* 2011 confirm that heat stress affects the generation time of granulosa cells in goats. The findings of this study demonstrated the existence of ovarian morphometrical differences between dry and wet seasons.

The results of this study provide the basis for a more detailed longitudinal study to follow up ovarian changes in individual goats between seasons. However, the results of the current study should be interpreted with caution due to some limitations associated with the study including failure to examine the influence of functional corpus luteum and stage of estrus cycle on morphological changes of the SEA goat ovary and morphological study of SEA goat ovary relative to body size. Therefore it will be of interest if future studies will address these limitations.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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