MORPHOMETRICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE OVARY OF SMALL EAST AFRICAN GOAT

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Veterinary Anatomy of Sokoine University of Agriculture, Morogoro, Tanzania.

By

Jackson Alex Ngou

Supervisors

Prof. Wahabu Hamisi Kimaro
Prof. Claudius David Luziga

Department of Veterinary Anatomy and Pathology,
College of Veterinary Medicine and Biomedical Sciences,
Sokoine University of Agriculture, Morogoro, Tanzania.

April, 2023

EXTENDED ABSTRACT

In the present study, the morphometrical and immunohistochemical changes in the ovaries of Small East African (SEA) goat during dry and wet seasons were investigated. In addition, the study describes seasonal morphological changes occurring in the ovary of this breed. The findings of this study provide the insight for improving reproductive efficiency in SEA goat. A total of 45 sexually mature female goats were utilized during each season of dry and wet. These animals were brought for slaughter at Morogoro Municipal slaughterhouse from various districts of Morogoro Region including Gairo, Kilosa and Mvomero. After slaughter, ovaries were collected and measured in length, weight and width. Thereafter, the collected ovaries were fixed in 10% buffered formalin and processed routinely for histomorphological and immunohistochemical analysis. Mouse and Rabbit Specific HRP/DAB IHC Detection Kit-Micro polymer (abcam, ab64261) was used for immunohistochemical study. The results revealed that the length of the right ovary was significantly higher than that of the left. In addition, four types of follicles (primordial, primary, secondary and antral follicles) were identified during both dry and wet seasons. Primordial follicles had a single layer of flat granulosa cells; primary follicles with one layer of cuboidal granulosa cells; secondary follicles having more than one layer of granulosa cells and antral follicles having an antrum with multiple layers of granulosa and theca cells. It was further observed that, follicular atresia occurred during all stages of follicular development. However, the antral follicles were the most affected type by atresia. The affected follicles were undergoing obliterative type atresia with primary follicular wall detachment. Furthermore, large number of atretic follicles was observed during the dry season than in the wet season. Positive immunostaining for desmin and smooth muscle actin was observed in the theca cells of secondary and antral follicles as well as in the tunica media of ovarian blood vessels. The staining intensities were varied between the healthy and atretic follicles. The atretic follicles stained lighter than the healthy follicles. The results of this study have shown that SEA goat ovaries undergo follicular dynamics with increased rate of follicular atresia during dry season. Furthermore, based on immunohistochemical results, the distribution of desmin and smooth muscle actin varied during stages of follicular development and atresia. The dry season has bad effects on folliculogenesis as it lowers the number of follicles and causes many follicles to be atretic. Based on the fact that, during the dry season there is scarcity of feeds and increased heat which could affect follicular development. This study recommends improvement in nutritional requirements of the SEA goat by providing feed supplements during the dry seasons.

Key words: Follicular atresia, Histology, Immunolocalization, Morphometry, Small East African goats.

MUHTASARI JUMUISHI

Katika utafiti huu, mabadiliko ya kimofometriki na ya immunohistokemikali katika ovari ya mbuzi wadogo wa Afrika Mashariki wakati wa kiangazi na masika yalichunguzwa. Aidha, utafiti unaeleza mabadiliko ya kimofolojia ya msimu yanayotokea katika ovari ya mbuzi wa aina hii. Matokeo ya utafiti huu yanatoa ufahamu wa kuboresha ufanisi wa uzalishaji katika mbuzi hao. Jumla ya mbuzi jike 45 waliofikia umri wa kupandwa walitumiwa katika kila msimu wa kiangazi na masika. Wanyama hao waliletwa kwa ajili ya kuchinjwa katika machinjio ya Manispaa ya Morogoro kutoka wilaya mbalimbali za mkoa wa Morogoro zikiwemo Gairo, Kilosa na Mvomero. Baada ya kuchinjwa, ovari zilikusanywa na kupimwa kwa urefu, uzito na upana. Baada ya hapo, ovari ziliwekwa katika kemikali aina ya formalin (10%) na kuandaliwa kwa uchunguzi wa histomorpholojia na immunohisto kemikali. Kifaa maalum kiitwacho Mouse and Rabbit Specific HRP/DAB IHC Detection Kit-Micro polymer (abcam, ab64261) kilitumika kwa uchunguzi wa immunohistokemikali. Matokeo yalionesha kuwa urefu wa ovari ya kulia ulikuwa mkubwa zaidi kuliko ule wa kushoto. Aidha, aina nne za follicles (primordial, primary, secondary and antral follicles) zilipatikana wakati wa msimu wa kiangazi na masika. Follicles za awali (primordial) zilikuwa na safu moja ya seli za granulosa. Follicles za msingi (primary) zilikuwa na safu moja ya seli za granulosa aina ya cuboidal; follicles za upili (secondary) zilikuwa na zaidi ya safu moja ya seli za granulosa. Follicles za utatu (antral) zilikuwa na safu nyingi za seli aina ya granulosa na theka pamoja na tundu la antral. Aidha, ilionekana zaidi kuwa, atresia ya follicle ilitokea wakati wa hatua zote za maendeleo ya follicle. Hata hivyo, follicle aina ya antral ilikuwa imeathiriwa zaidi na atresia kuliko aina zingine. Follicles zilizoathiriwa zilikuwa zinapitia atresia ya aina ya obliterative iliyoathiri na kubomoa ukuta wa msingi wa follicle. Zaidi ya hayo, idadi kubwa ya follicles zenye atresia zilionekana zaidi wakati wa msimu wa kiangazi kuliko masika. Kinga chanya kwa desmin na actini ya misuli laini ilionekana katika seli za theka za follicle ya upili na utatu pamoja na tunica media ya mishipa ya damu ya ovari. Muonekano huo wa kinga chanya ulikuwa tofauti kati ya follicles zenye afya na na zisizo na afya (zenye atresia). Follicles zenye atresia zilishika rangi kiasi kuliko follicles zenye afya. Matokeo ya utafiti huu yameonyesha kuwa ovari za mbuzi wadogo wa Afrika mashariki hupitia mabadiliko ya ukuaji wa follicles yanayoambatana na kasi ya kuongezeka kwa atresia ya follicles wakati wa kiangazi. Zaidi ya hayo, kulingana na matokeo ya immunohistokemikali, usambaaaji wa desmin na actin ya misuli laini ulitofautiana katika hatua za ukuaji wa follicles na atresia. Msimu wa kiangazi una athari mbaya katika kutengenezeka kwa follicles kwani hupunguza idadi ya follicles na husababisha follicles nyingi kuwa na atresia. Kulingana na ukweli kwamba, wakati wa kiangazi kuna uhaba wa malisho na kuongezeka kwa joto ambayo inaweza kuathiri ukuaji wa follicles. Utafiti huu unashauri uboreshaji wa mahitaji ya lishe ya mbuzi wa jamii hii kwa kutoa virutubisho vya malisho wakati wa kiangazi.

Maneno muhimu: Follicular atresia, Histology, Immunolocalization, Morphometry, Mbuzi wadogo wa Afrika Mashariki.

iv

DECLARATION

I, **Jackson Alex Ngou**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and it has neither been submitted nor being concurrently submitted in any other institution.

J. Ngou

Jackson A. Ngou
(MSc. Candidate)

18 · 04 · 2023

Date

The above declaration is confirmed by;

Prof. W. H. Kimaro (PhD)
(Supervisor)

18 · 04 · 2023

Date

Prof. C. D. Luziga (PhD)
(Supervisor)

18 · 04 · 2023

Date

LIST OF PUBLISHED PAPERS/MANUSCRIPTS

COPYRIGHT

ACKNOWLEDGEMENTS

First and foremost, I thank God Almighty who kept me in good health throughout my studies. I acknowledge and thank my supervisors, Prof. W.H. Kimaro and Prof. C.D Luziga from the Department of Veterinary Anatomy and Pathology for their close supervision, guidance, discussion, constructive criticism, encouragement, and extra time they spent with me to ensure accomplishment of this dissertation. I would like to acknowledge Sokoine University of Agriculture through the Office of Deputy Vice-Chancellor Academic, Research and Consultancy for the financial support. I am grateful to Mr. M.O. Mwangalimi from the Department of Veterinary Anatomy and Pathology for his technical assistance during tissue collection and processing. Last, but not least, I would like to thank all staff members in the Department for their support during my study.

DEDICATION

This dissertation is dedicated to my father Alex Ngou and my mother Clementina Mgululi as well as my wife Anitha Ngesi and all who devoted their time and resources to make this work successful. May the God Almighty bless them.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AVR	Applied Veterinary Research
DAB	3, 3′-Diaminobenzidine
DPX	Dibutylphthalate Polystyrene Xylene
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
HRP	Horse Radish Peroxidase
IHC	Immunohistochemistry
LH	Luteinizing Hormone
PBS	Phosphate-Buffered Saline
RT	Room Temperature
SE	Standard Error
SEA	Small East African
SMA	Smooth Muscle Actin
TVJ	Tanzania Veterinary Journal

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

The histological and morphometrical variations of the ovaries in goat have been described in several studies (Haque *et al*., 2016; Bijna *et al*., 2018; Emara *et al*., 2019). Significant difference has been observed in the length of right ovary when compared to the left in Malabari goat (Bijn*a et a*l., 2018). In addition, the right ovary of Black Bengal goat was reported to be heavier than the left (Haqu*e et* al., 2016). Furthermore, in tropical environment, an increased number of ovarian follicles development was revealed in Sahelian goats during wet season and decreased in dry season (Jaj*i et al*., 2012). Two types of follicular atresia (obliterative and cystic) have been reported in goats (González-Vall*e et* al., 1998). They were reported in Batu goats (Ariyaratna and Gunawardana, 1997), whereas, only the obliterative types have been encountered in short-haired goats (Vlčkov*á et* al., 2012). However, there is no report showing the types of atresia that occur in Small East African (SEA) goats.

It is well established that cytoskeleton desmin and smooth muscle actin (SMA) play an important role in the structural support and development of smooth muscle cells. They have been found in cortical and thecal cells in ovaries. The major role of the cytoskeletal elements in the ovaries is to provide a structural framework (Paulin and Li, 2004). There is a difference in staining intensity and distribution of the cytoskeletal elements during follicular development and atresia. Although the distribution of cytoskeleton in the ovarian follicles of various goat breed has been studied, no information is available in the SEA goat. The present study was undertaken to examine the seasonal morphological changes in the ovary of SEA goat. In addition, the immunohistochemical localization of desmin and SMA in ovarian follicles was described. The findings of this study are crucial in dealing with infertility problems and their solutions to improve fertility rate in SEA goats.

1.2 Gross Morphology and Topography of the Ovary

Goat ovaries are paired, firm, round-oval or almond-shaped organs. They are positioned on each side of the pelvic cavity in the sub-lumbar area (Haque *et al.,* 2016). Measure 9.76-15 mm long, weigh 0.8-2 grams, and have an attached border to the mesovarium and a free border opposite to the attached border, as well as a tubal end connected to the uterine tube (Bari *et al*., 2018). Blood vessels, lymphatic vessels, and nerves enter and leave the ovary via the broad ligament, suspensory ligament, and mesovarium (Kumar *et al.,* 2020).

1.3 Histological Organization of the Ovary

Histologically, the ovary is conveniently divided into cortex and medulla. The cortex contains ovarian follicles at different stages of development and regression. These follicles include primordial, primary, secondary and antral follicles. The granulosa and theca cells in the healthy follicles are well organized surrounding an oocyte. Primordial follicles are clustered in peripheral zone of the cortex just beneath the tunica albuginea. Each primordial follicle has an oocyte surrounded by a single layer of flat granulosa cells (Salha *et al*., 1998). The oocyte in a primary follicle is surrounded by cuboidal granulosa cells. The oocyte in a secondary follicle is surrounded by a zona pellucida, stratified epithelium of granulosa cells and undifferentiated theca cells (Gupta *et al.*, 2007). The antral follicle contains an oocyte surrounded by zona pellucida, antrum, multiple layers of granulosa cells and theca cells layers (Dickinson *et al.,* 2010). Medullary region of the ovary is well-supplied with lymphatic and blood vessels (Kirbaş Doan *et al.,* 2019).

1.4 Atretic Follicles

Follicular atresia is a degenerative process in which oocytes in various stages of development and growth are lost from the ovary, and it is the leading reason for diminished goat fertility (Alam *et al.,* 2002). Pyknosis and chromatolysis are two of the most visible indicators of atresia in follicular wall cells (Sharma, 2000). The glassy membrane is formed when the basal lamina of the granulosa cell layer folds, thickens, and hyalinizes during atresia. Atretic small follicles are eventually absorbed. Scars may remain after atresia of large follicles that is presented by small fibrous tissue (Matsuda *et al.,* 2012). The oocytes frequently degenerate before the follicular wall in ruminant primary and secondary follicles (preantral follicles) atresia, but the follicular wall degenerates before the oocytes in antral follicles atresia (Rodgers and Irving-Rodgers, 2010).

Atresia is a hormonally regulated apoptotic process that is primarily dependent on granulosa cell death. Follicle-stimulating hormone (FSH), which increases follicle growth, inhibits follicular atresia. When a follicle matures, it produces estrogen, which suppresses FSH secretion at high levels (Lin and Rui, 2010). The fundamental mechanism of follicular atresia is thought to be granulosa cell apoptosis (Zhou *et al*., 2019). Atretic alterations in ruminant follicles can lead to the formation of two types of atretic follicles, which are obliterative and cystic atretic follicles (Hatzirodos *et al*., 2014). The granulosa and theca cell layers in obliterative atresia infold, enlarge, and expand inward to occupy the antrum (Irving-Rodgers *et al.,* 2001). The small follicles are invaded by fibroblasts and macrophages in this type of atresia. The obliterative type of atresia is further subdivided into basal atresia and antral or apical atresia based on where cells die first in the granulosa cells layer. Early degeneration or destruction of the layers of granulosa cells nearest to the antrum characterizes the apical atresia, while the most basal cells stay intact until later stages (Irving-Rodgers *et al*., 2001). Numerous pyknotic nuclei are first seen in the most antral layers and the antrum near the membrane granulosa in this form of atresia (Wang *et al.,* 2010). This is the typical description of atretic follicles, which can be found in antral follicles, including non-ovulating dominant follicles, in many species (Chu *et al*., 2018). The most basal layer of granulosa cells is destroyed first in basal atresia, whereas the cells in the most antral layers remain associated with each other and largely healthy until later stages of atresia (Hatzirodos *et al.,* 2014). Macrophages frequently pierce the follicular basal lamina of basal atretic follicles, phagocytizing dying basal granulosa cells (Logothetopoulos *et al.,* 1995). The granulosa and theca cell layers may atrophy in cystic atresia, or simply the granulosa cell layer may atrophy while the theca layer luteinizes, becomes fibrotic, or hyalinizes surrounding the antrum (Makarevich *et al.,* 2018). The atretic process causes cavity formation in cystic atresia of large follicles (Ariyaratna and Gunawardana, 1997).

The gap between zona pellucida and ooplasmic contents, as well as a decrease in follicular diameter, are the characteristic features in atretic follicles at an early stage of atresia (Irving-Rodgers *et al*., 2002). This stage is marked by a disorder of the oocytes nucleus and cytoplasm. The folding of the oocyte wall and dissolving of the zona pellucida is observed as the follicles advance in atresia (Sharma and Bhat, 2014). Presence of a gap between the zona pellucida and the ooplasmic contents is a distinguishing feature of atretic follicles in the ruminant animal ovary. Most significant function of follicular atresia in ruminant reproduction is to limit the number of follicles that can be sustained for maturation and ovulation (Zhou *et al*., 2019). At the molecular level, the mechanism that causes and regulates follicular atresia is unknown (Vlková *et al.,* 2012). Age, stage of the reproductive cycle, hormones, light, temperature, diet, irradiation and toxins such as biocides, all affect follicular atresia (Townson and Combelles, 2010).

1.5 Cytoskeleton

The cytoskeleton is a system of an interconnected network of regulatory proteins and filamentous polymers in the cytoplasm of the eukaryotic cell (Mullins and Francisco, 2014). The cytoskeletal components include intermediate filaments desmin and smooth muscle actin. These intermediate filaments are mid-sized with a diameter of 5-10nm. They are very stable structures forming the true skeleton of the cell. Intracellular transport, adhesion and motility are all regulated by the cytoskeleton (Madekurozwa and Kimaro 2006). The shape of the cell is determined by meshworks and changing arrays formed by actin filaments, which also mediate cell cleavage during mitosis (Xiao *et al.,* 2016). For cell mobility, they also make cilia and flagella. Intermediate filaments are responsible for anchoring and positioning the nucleus within the cell, as well as providing the cell with its elastic qualities and resistance to tension. Due to these crucial roles of the cytoskeletons in the cell, alterations in cytoskeletal components are normally correlated to several diseases, such as cancer (Verde *et al*., 2021).

1.6 Oogenesis in Goat

In most animals, oogenesis begins before or at birth but is arrested at the prophase phase of the first meiotic division until puberty, when the process is allowed to continue (Forcada *et* al., 2006). The primordial (primitive) germ cells move from the yolk sac to the gonad of a genetic female animal during the early embryonic period and differentiate into oogonia. The oogonia undergo several mitotic divisions, resulting in thousands to a few million of oogonia, but many of these undergo regression at this or later stages.

At or just before birth, the oogonia enters the prophase of the first meiotic division. At this stage, they are known as primary oocytes (Hurk and Telfer, 2000). However, completion of the first meiotic division does not take place until the female animal reaches sexual maturity. The meiotic process is arrested at the diplotene stage (at the primary oocyte level). At puberty, the primary oocyte completes the first meiotic division, but the cell division is unequal, one of the resulting cells being larger than the other. The cell that retains the bulk of the cytoplasm is the secondary oocyte. Under the influence of luteinizing hormone, the vesicular follicle then ruptures to release the secondary oocyte.
The released secondary oocyte is covered by zona pellucida which in turn is surrounded by granulosa cells. Oocyte quality is affected by nutritional and metabolic changes, particularly lipid metabolism (Bradley and Swann, 2019). The energy balance or particular nutrients can influence folliculogenesis and ovulation by acting on the hypothalamo-pituitary system or directly on the ovary (Dalbies-Tran *et al*., 2020). These changes can have a deleterious influence on folliculogenesis and oocyte maturation by altering the expression of genes involved in meiosis and steroidogenic pathways. Pyruvate is the predominant energy source oxidized in follicles, where both glycolysis and mitochondrial pyruvate oxidation occur and pyruvate is required to meet the oocyte's

metabolic requirements for meiosis to resume (Sutton-Mc Dowall *et al*., 2010). An ovulation is also linked to nutrients availability in the diet. Glucose is required for meiotic maturation and defining the developmental competence of oocytes in the ovaries before ovulation (Dalbies-Tran *et al.,* 2020). Some studies have described the importance of amino acids to the oocyte, such as their effect on ovulation or oocyte quality (Dupont *et al.,* 2014). Once fertilized, the oocyte ability to cleave is linked to the transport and composition of amino acids in the oocyte (Sinclair *et al*., 2008). Cumulus-oocyte complex quality measures in cows have also been proposed using glycine and alanine from the follicular fluid. Finally, amino acid deprivation alters rapamycin signaling mechanistic goal, impairing folliculogenesis and oogenesis (Collado-Fernandez *et al.,* 2012).

Lipids also play a crucial role in female animal fertility as reported by Bradley and Swann (2019). Indeed, the absence of granulosa cells around the oocyte in the bovine, affects its growth and maturation rate by modifying oocyte lipid metabolism, which is one of the primary activities impaired by their unavailability (Reverch*on et* al., 2014). Lipid metabolism is one of the biological functions that are significantly impacted when studying the transcriptome of ewe follicles at different stages of early folliculogenesis (Bonnet *et al*., 2015). Furthermore, fatty acid oxidation in cumulus cells is necessary for oocyte maturation in various mammalian species, including the mouse, cow, and pig (Collado-Fernandez *et al.,* 2012). Cumulus cells collect lipids from the follicular fluid and store them in lipid droplets to protect the oocyte from lipotoxicity (Auclair *et al.,* 2013).

1.7 Oestrus cycle in Goats

In goats, the oestrus cycle entails a series of hormone cascades that cause morphological changes in the female reproductive tract in preparation for pregnancy. Goats have a 21-day oestrus cycle, with oestrus lasting between 24-48 hours and ovulation occurring 24 hours following the commencement of oestrus (Edwards, 2020). The luteal phase (12–17 days; met-estrus and di-estrus) and the follicular phase (2–4 days; pro-estrus and estrus) are the two distinct phases that make up the cycle. The luteal phase begins after ovulation when the corpus luteum (CL) is formed and the follicular phase begins when the corpus luteum (CL) dies (luteolysis) and ends with ovulation (Shelton, 1978). Final maturation and ovulation of the ovulatory follicle occur during the follicular phase, resulting in the release of an oocyte into the oviduct, ready for fertilization to form an embryo (Bazer, 2019). The growth and survivability of the offspring, the post-partum anestrus period, cyclic activity, fertilization rate, ovulation rate and the length of the breeding season all influence goat reproduction efficiency (Alves *et al*., 2018). Different criteria, such as a newborn goat live weight and the length of the reproductive cycle, the kidding interval, the kidding rate, and the weaning rate, can be used to determine reproductive efficiency (Fatet *et al*., 2011). The oestrus cycle is well-organized, with hormonal sequences directing blood flow to specific tissues. As the amount of progesterone hormone decreases at the end of the oestrus cycle, gonadotropin-releasing hormone (GnRH) is released with a high frequency. The anterior pituitary gland produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to start the development of ovarian antral follicles (Gómez-Brunet *et al*., 2012). Selection, recruitment, dominance, and atresia are all stages in the development of antral follicles. Follicles that have been recruited begin to grow and produce a small amount of estradiol hormone. Follicles that do not die due to atresia (cell death) are selected. An additional one to two follicles are selected for dominance (Ramos *et al*., 2018). Non-dominant follicles undergo atresia, whereas dominant follicles release a cumulative amount of estrogen that acts on the hypothalamus to cause inhibin secretion, preventing FSH production from the anterior pituitary gland (Alves *et al.,* 2018). The hypothalamus will be stimulated to secrete GnRH, causing the anterior pituitary gland to secrete LH, causing the dominant follicle(s) to ovulate after the estrogen feedback control mechanism reaches threshold levels with a minimal concentration of circulating progesterone (Fatet *et al.,* 2011; Sahu *et al*., 2017).

The recruitment and growth of follicles occur in waves, with the dominant follicle ovulation occurring during periods of higher estrogen and lower progesterone levels. The ovulatory follicle is produced by the last of three to four follicular waves in the estrus cycle of a goat (Haque *et al.,* 2016). The ovulated follicle is changed to a corpus hemorrhagicum, which undergoes luteinization forming corpus luteum. Luteinization process involves converting follicular cells into luteal cells. The corpus luteum (CL) secretes progesterone which is necessary for pregnancy survival (Fatet *et al.,* 2011). Five days post ovulation, the corpus luteum begins to release progesterone in a cumulative amount until the highest production level is reached. In goats, the CL functions for an average of sixteen days, through which waves of follicle carry on the recruitment and advancement of follicles. The ovulation process is inhibited in ultimate corpus luteum function due to the negative feedback control mechanism of progesterone on GnRH production from the hypothalamus. At sixteen days after oestrus in goats, the prostaglandin F2 Alpha (PGF2α) is secreted from the uterus of non-pregnant goats to lyse the CL (Alves *et al*., 2018). As progesterone levels fall, its inhibitory effect on gonadotropin release is removed and a new oestrus cycle begins (Fatet *et al.,* 2011).

1.8 Goats Cyclicity

Goats go through seasonal phases of active cyclicity. This estrus seasonality prevents newborn kids from being born in months with small survival rates. Deviations in hormone receptors and photoperiod determine the regular breeding season of goats (Ungerfeld and Bielli, 2012; Alves *et al*., 2018). The length of the day affects the hormonal activators that cause the goat to develop a new active cyclicity. To trigger the production of luteinizing hormone in the anterior pituitary gland, the hypothalamus must be stimulated to secrete the required concentrations of GnRH (Bari *et al.,* 2012).

A goat can go through follicular growth during seasonal anestrus, but because GnRH surges do not occur, sexual behavior and ovulation will also fail to occur. Variations in the ability of GnRH neurons to respond to positive feedback from estradiol lead GnRH surges to drop sharply throughout seasonal anestrus (Gómez-Brunet *et al*., 2012). The rise of estradiol hormones during the follicular development period of the estrus cycle will not occur due to the small concentration of GnRH pulses. Breeding and anestrus phases are more likely to be triggered by the presence of good nutrition in the tropics, where there are no obvious seasonal changes in day duration (Minister *et al*., 2020).

1.9 Problem Statement and Study Justification

In all female vertebrates, the ovary is a vital reproductive organ (Forcada *et al.,* 2006). It is responsible for the production of sex hormones such as estrogen and progesterone as well as the formation of an ovum. The ovarian follicles secrete estrogens which regulate secondary sex characteristics development in a female animal (Slides and Turner, 2011). The corpus luteum produces progesterone, which is crucial for the continuation of pregnancy (Fatet *et al*., 2011).

Photoperiod has a big influence on goat reproduction in the temperate region. Due to the small day length change in the tropical region compared to a temperate one, tropical goat breeds such as Small East African (SEA) goats are continuous breeders and can ovulate each month throughout the year (Alves *et al.,* 2018). The pathology, physiology and morphology of small ruminant reproductive organs have been reported in several studies (Rahman *et al.,* 2006). Increased number of ovarian follicular development in wet season and a decrease in the number of the follicles during dry season have been demonstrated in Sahelian goat ovary in Nigeria by Jaji *et al.* (2012). In temperate region, there is a higher number of developed follicles in the winter compared to the summer season (Bari *et al.,* 2012).

The fertility performance of SEA goat is critical in improving the livelihood of goat keepers. Clear and complete knowledge of the structure and reproductive patterns of the ovary is required to maintain optimum reproductive performance (Fahad *et al.,* 2019). Despite their relevance, little information is available about the Small East African (SEA) goat ovary. Seasonal ovarian morphological variation in SEA goats is poorly understood. Therefore, the goal of this study is to determine the morphological changes in the ovaries of SEA goats during dry and wet seasons. The findings of this research will lay the groundwork for improving reproductive performance in SEA goat.

1.10 Objectives

1.10.1 General objective

The overall goal of this study was to establish the histological and morphometrical variation of the ovary in Small East African (SEA) goats during dry and wet seasons.

1.10.2 Specific objectives

Specifically, the study intended:

i. To compare morphological changes in the ovary between dry and wet seasons.
ii. To determine the histological changes in the ovary during dry and wet seasons.
iii. To localize intermediate filaments (desmin) and smooth muscle actin in the ovary during dry and wet seasons using immunohistochemical techniques.
iv. To characterize types of atresia and compare between dry and wet seasons.

1.11 Research Hypotheses

i. The Small East African (SEA) goat ovarian histological structure does not change with seasons.
ii. There is no ovarian morphometrical changes in SEA goat during dry and wet seasons.

1.12 Research Questions

i. Does ovarian histological structure in Small East African (SEA) goat vary with seasonal changes?
ii. Does seasonal changes cause any variation in SEA goat ovarian morphometry?
iii. What type of ovarian follicular atresia occur in SEA goat ovary?
iv. In which season does the ovarian follicular atresia occur most?

1.13 General Methodology

In total, 90 adults sexually matured female Small East African (SEA) goats between the ages of 1 and 2 years from Morogoro region in Tanzania brought for slaughter at Morogoro Municipal slaughterhouse were utilized in the study. Animal's age was estimated based on dental examination. Ovarian samples were collected during the dry season (August, September and October 2021) and wet season (February, March and April 2022). Forty-five adult female goats were utilized during each season. Animals were selected based on ante-mortem findings. After animal slaughter, the abdomen was opened to observe and note the topographical position of ovaries. The presence of either corpus haemorrhagicum or corpus luteum was noted. They were identified according to coloration such as red and yellow.

Both left and right ovaries were collected for morphometrical and immunohistochemical studies. The collected ovaries were trimmed and weighed separately, using an electrical balance. The width was measured transversely at the widest part of the ovary, while the length was measured from cranial pole to caudal pole using vernier calliper (Bijn*a et* al., 2018). The mean length, width and weight were determined using Analysis of Variance (ANOVA) and compared between left and right, as well as between seasons.

Ovarian tissue samples from the cranial, middle and caudal poles were immersed in 10% buffered formalin for 48 hours. The fixed tissues were then processed routinely and sectioned serially. Standard procedures were used to stain sections with hematoxylin and eosin (Bari *et al.,* 2012). The immunohistochemical staining was carried out on ovarian sections using Mouse and Rabbit Specific HRP/DAB IHC Detection Kit (abcam, ab64261). Every tenth section was examined and evaluated for number, morphology and immunostaining of healthy and atretic follicles. Selected areas of the observed slides were photographed using Moticam Pro 205A Camera mounted on an Olympus BH-2 light microscope.

1.14 Dissertation Organization

The present dissertation follows the Guidelines for preparation and submission of dissertations/ thesis/ research reports/ research papers and other scientific publications of Sokoine University of Agriculture, 5th Edition, 2022. Chapter one contains the introduction, literature review, problem statement and study justification, study objectives, study hypotheses, study questions, general methodology as well as the dissertation organization. Chapter two contains a paper that is published in peer reviewed scientific journal; the paper presents findings on Cross Sectional Study of Small East African Goat Ovarian Morphology During Wet and Dry Seasons. Chapter three contains a manuscript submitted in peer reviewed scientific journal, the manuscript presents findings on Histomorphological and immunohistochemical changes of the Small East African (SEA) goat ovary during dry and wet seasons. In addition, chapter four addresses the general discussion and chapter five presents the conclusion and recommendations of the study.

CHAPTER TWO

PAPER I

The material contained in this chapter has been published in Tanzania Veterinary Journal (https://dx.doi.org/10.4314/tvj.v37i1.1).

[1]Cross Sectional Study of Small East African Goat Ovarian Morphology During Wet and Dry Seasons

J. A. Ngou [*] ***and W. H. Kimaro***

Department of Veterinary Anatomy and Pathology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3016, Morogoro, Tanzania.

[*]*E-mail: jackson.ngou@sua.ac.tz*

Article timeline

Submitted: 16-08-2022, Revised: 15-10-2022, Accepted: 04-01-2023, Published: 21-01-2023

Tanzania Veterinary Journal Vol. 367(1) 2022 ***https://dx.doi.org/10.4314/tvj.v37i1.1***

2Cross Sectional Study of Small East African Goat Ovarian Morphology During Wet and Dry Seasons

J. A. Ngou **and** ***W. H. Kimaro***

Department of Veterinary Anatomy and Pathology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3016, Morogoro, Tanzania.

*E-mail: jackson.ngou@sua.ac.tz

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.

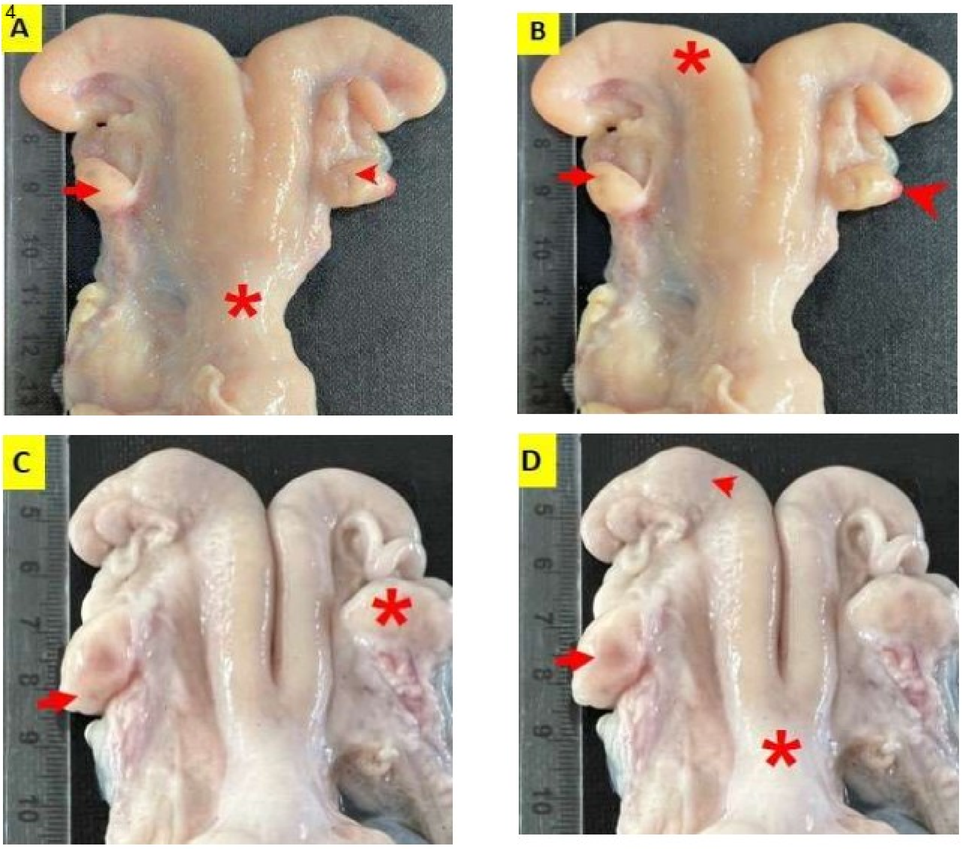

Figure 1. Photographs of reproductive tract from Small East African goat during wet season (**A** and **B**), dry season (**C** and **D**). **A**: Left ovary (arrow), right ovary (arrowhead) and uterine body (asterisk). **B**: Note presence of developing follicles (arrow), uterine horn (asterisk) and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

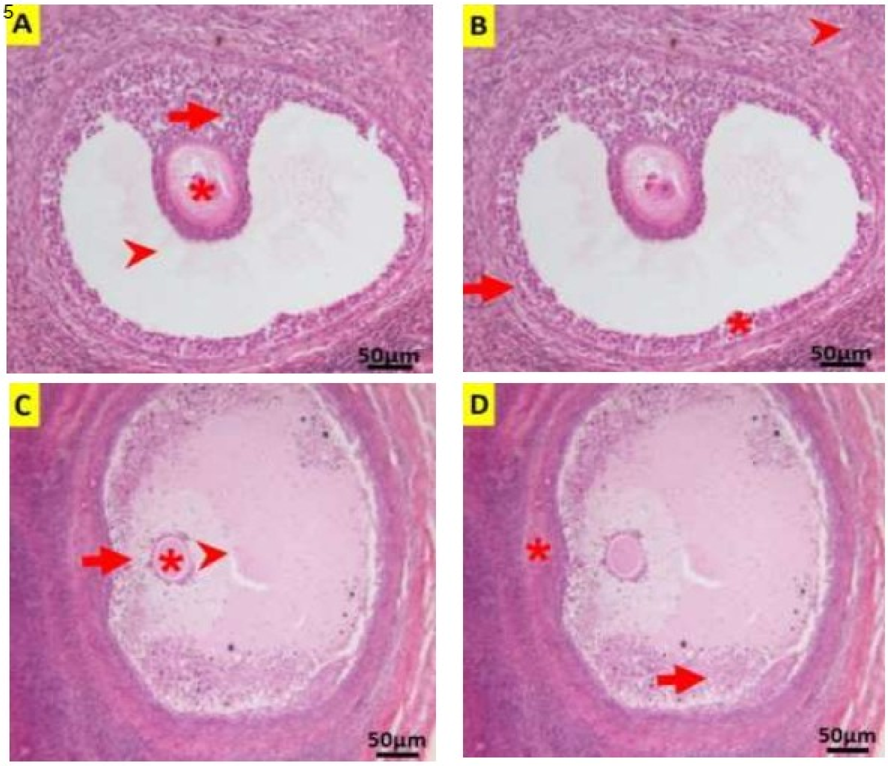

Figure 2. Photomicrographs of the Small East African goat ovary showing healthy antral follicle (**A** and **B**), atretic antral follicle (**C** and **D**). **A**. Oocyte (asterisk); cumulus oophorus (arrow) and antrum (arrowhead). **B**. Note a well-organized granulosa cells layers (asterisk); thecal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

ACKNOWLEDGEMENT

The authors acknowledges Sokoine University of Agriculture for funding and technical support received from O. Mwangalimi.

CONFLICT OF INTEREST

The authors declare no competing interests.

REFERENCES

Adjassin, J.S., Assani, A.S., Bani, A.A., Sanni Worogo, H.S., Adégbeïga Alabi, C.D., Comlan Assogba, B.G., Virgile Azando, E.B., and Alkoiret, I.T. Impact of heat stress on reproductive performances in dairy goats under tropical sub-humid environment. *Heliyon*, 8 (2), 2022.

Alves, N.G., Torres, C.A.A., Guimarães, J.D., Moraes, E.A., Costa, P.B., and Silva, D.R. Ovarian follicular dynamic and plasma progesterone concentration in Alpine goats on the breeding season. *Arq Bras Med Vet Zootec*, 70 (6), 2017–2022, 2018.

Asad, L., Rahman, A., Hossain, M., and Akter, M. Ovarian category, follicles and oocytes analysis of goat ovaries in view of in vitro production of embryos. *Int J Anim Resour*, 1 (1), 27–34, 2016.

Bari, M., Kabir, M., Sarker, M., Khan, A., and Momiruzzaman, M. Morphometric analysis of ovarian follicles of Black Bengal goats during winter and summer season. *Bangladesh J Anim Sci*, 40 (1–2), 51–55, 2012.

Bijna.M, Karthiayini K., Lucy, K.M. Morphometry and histology of ovaries in Malabari goats. *Int J Sci Res*, 7 (1), 291–293, 2018.

Chong, W.C., Wu, R., and Tu, Y. A Study on Tissue Processing. *Int J Innov Interdiscip Res Issue*, 3 (April), 2012, 2012.

Dadok, F., Islam, M.S., Rahman, A.N.M.I., Alam, M.J., Kabir, M.E., and Amy, B.F. Morphometric assessment of oocytes recovered from ovaries of Black Bengal goat, 13 (12), 40–44, 2020.

Emara, S., Hassanin, A., and Elnagar, H. Histological and histochemical changes of the ovary of goat during autumn and summer seasons. *J Curr Vet Res*, 1 (2), 47–54, 2019.

Eubanks, D.L. Dental considerations in cows and goats. *J. Vet. Dent.*, 29 (3), 200–203, 2012.

8
Fahad, T.A., Waheed, Z., and Kareem, D.A. Study of some abnormalities in the reproductive system of ewes in Basra province. *IOP Conf Ser Mater Sci Eng*, 571 (1), 2019.

Friedman, E., Voet, H., Reznikov, D., Dagoni, I., and Roth, Z. Induction of successive follicular waves by gonadotropin-releasing hormone and prostaglandin F2α to improve fertility of high-producing cows during the summer and autumn. *J Dairy Sci*, 94 (5), 2393–2402, 2011.

Islam, M.R., Khandoker, M.A., Afroz, S., Rahman, M.G., and Khan, R.I. Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of in vitro production of embryos. *J Zhejiang Univ Sci B*, 8 (7), 465–469, 2007.

Jaji, A., Buduwara, R., Akanmu, A., Zachariah, M., Luka, J., and Gambo, B. Pregnancy related biometric changes in the ovaries and uterus of the sahelian goat. *Sokoto J Vet Sci*, 10 (1), 18–21, 2012.

Jordan, E.R. Effects of heat stress on reproduction. *J Dairy Sci*, 86 (1), 104–114, 2003.

Kiernan, J.A. Histological and Histochemical Methods: Theory and Practice. *Folia Histochem Cytobiol*, 54 (1), 58–59, 2016.

Kırbaş Doğan, G., Kuru, M., Bakır, B., and Karadağ Sarı, E. Anatomical and histological analysis of the salpinx and ovary in Anatolian wild goat (Capra aegagrus aegagrus). *Folia Morphol*, 78 (4), 827–832, 2019.

Mm, A., Ad, G., and Das, A. Biometry of female genital organs of Black Bengal goat. *Int J Nat Sci*, 1 (1), 12–16, 2011.

Ozawa, M., Tabayashi, D., Latief, T.A., Shimizu, T., Oshima, I., and Kanai, Y. Alterations in follicular dynamics and steroidogenic abilities induced by heat stress during follicular recruitment in goats. *Reproduction*, 129 (5), 621–630, 2005.

Pramod, R.K., Sharma, S.K., Singhi, A., Pan, S., and Mitra, A. Differential ovarian morphometry and follicular expression of BMP15, GDF9 and BMPR1B influence the prolificacy in goat. *Reprod Domest Anim*, 48 (5), 803–809, 2013.

Tanzania Meteorological Authority (TMA) Statement on the Status of Tanzania Climate in 2021, 3, 2022. https://www.meteo.go.tz/.

Ungerfeld, R. and Bielli, A. Seasonal and social factors affecting reproduction. *Encycl life Support Syst*, 15, 2012.

Wilson, S.J., Marion, R.S., Spain, J.N., Spiers, D.E., Keisler, D.H., and Lucy, M.C. Effects of Controlled Heat Stress on Ovarian Function of Dairy Cattle. 1. Lactating Cows. *J Dairy Sci*, 81 (8), 2124–2131, 1998.

CHAPTER THREE

MANUSCRIPT II

Histomorphological and immunohistochemical changes in the ovary of the Small East African (SEA) goat during dry and wet seasons

J. Ngou[1*], W. H. Kimaro[1], C. Luziga[1]

Department of Veterinary Anatomy and Pathology, College of Veterinary Medicine and Biomedical Sciences P.O. Box 3016, Sokoine University of Agriculture, Morogoro, Tanzania.

*E-mail:jackson.ngou@sua.ac.tz.

The material contained in this chapter has been submitted to *Applied Veterinary Research Journal*

ABSTRACT

The objective of this study was to examine the morphological changes in the ovary of the Small East African (SEA) goat during dry and wet seasons. Analysis was performed on 180 ovaries using Hematoxylin and Eosin and immunohistochemistry for desmin and smooth muscle actin as cytoskeletal elements. The results revealed that during both dry and wet seasons, ovaries contained healthy and atretic primordial, primary, secondary and antral follicles. An oocyte in primordial follicles were surrounded by a layer of flat granulosa cells while in primary follicles, the oocyte was encircled by a layer of cuboidal granulosa cells. Follicular wall of secondary follicles was composed of granulosa cell and undifferentiated thecal cell layers. The antral follicles contained an oocyte surrounded by zona pellucida, multiple layers of granulosa cells, differentiated theca interna and externa cells. Atretic antral follicles were obliterated, characterized by disorganized granulosa cells, degraded oocyte and abnormal arrangement of cumulus oophorus. In dry season, healthy primordial, primary, secondary and antral follicles were 236 (95%), 85 (97%), 25 (78%) and 6 (27%) respectively, while atretic were 12 (5%), 3 (3%), 7 (22%) and 16 (73%). In wet season, 157 (99%), 61 (98%), 48 (93%) and 31 (92%) were healthy while 2 (1%), 1 (2%), 4 (7%) and 3 (8%) were atretic. The number of healthy and atretic follicles in dry and wet seasons differed significantly (p <0.05). Positive staining for both desmin and smooth muscle actin in healthy and atretic follicles, in dry and wet seasons was detected in theca cells of secondary and antral follicles, cortical stroma and tunica media of blood vessels but was not detected in granulosa cells and oocyte. The present study indicates that during dry season, most antral follicles undergo obliterative follicular atresia.

Keywords: Atresia, Desmin, Histology, Immunolocalization, Smooth Muscle Actin

INTRODUCTION

Several studies have been performed on the histomorphological structure of ovaries in various goat breeds (Islam et al 2007; Teh et al 2018). Studies in Malabari goat breed showed that, the ovaries were composed of cortex and medulla (Bijna et al 2018). The cortex contained primordial, primary, secondary and antral follicles. Primordial follicles were comprised of an oocyte surrounded by a single layer of flattened granulosa cells. Primary follicle contained cuboidal granulosa cells surrounding the oocyte. In the secondary follicle, an oocyte was surrounded by two or more layers of granulosa cells and theca cells. The antral follicle contained oocyte surrounded by two or more layers of thecal cells and granulosa cells and an antrum. The medullary region was composed of connective tissues, blood vessels and nerves. Reports on Egyptian local breed showed seasonal histological changes occurred in the ovary. During autumn, the ovaries contained abundant healthy follicles at different stages of development. But in summer, the ovaries contained follicles with degenerated oocytes and abnormal arrangement of granulosa cells (Emara et *a*l 2019). Ovaries are also reported to contain follicular atresia of two types: obliterative and cystic. The obliterative type of atresia is featured by the early destruction of follicular wall followed by the oocyte. This type of atresia is observed in antral follicles. Cystic atresia is characterized by the loss of oocyte followed by follicular wall destruction. This type of atresia affects pre antral follicles and it is less common in goat (Ariyaratna and Gunawardana 1997).

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

In ovaries, desmin and SMA have been immunohistochemically demonstrated in various cell types of few mammalian species. However, little is known about the goat ovary and in particular no information on Small East African (SEA) goats. The aim of this study was therefore to investigate seasonal changes on histological structure and immunolocalization of desmin and SMA filaments in the ovary of SEA goat.

MATERIALS AND METHODS

Ethical statement

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

Study area

The current study was conducted at Morogoro region, Tanzania. This study was designed to cover both dry and wet seasons of the year identified based on meteorological data from Tanzania Meteorological Authority. The dry season is observed in the months of August, September and November 2021 while the wet season in February, March and April 2022 (Tanzania Meteorological Authority, 2021).

Animals and source of specimens

The study was designed to collect ovaries from Small East African (SEA) goat during dry and wet seasons. Active visit was done during August and September where dry period is at peak and from February to April during rainy season in 2022. The slaughterhouse received animals from Kilosa, Mvomero and Gairo districts of Morogoro region. Majority of goats slaughtered during the study period were SEA goat, and mostly were in body condition scores of from 3.0 to 4.0 (under 1 – 5 BCS scale). Transportation means of animals from auction to the slaughterhouse was by vehicles and trekking. A total of 90 sexually mature female healthy adult SEA goats aged 1-2 years brought for slaughter at the slaughterhouse were randomly selected. The age of the animals was estimated using dental formula examination. After ante mortem examination, for each season 45 healthy animals were selected for inclusion in the study.

Tissue sampling and processing

After slaughter, left and right ovaries were dissected, and fixed in 10% buffered formalin. The ovaries were transported to Sokoine University of Agriculture histology laboratory to continue with fixation for 2 days at room temperature and then processed as previously described by Slaoui and Fiette (2011) with minor modifications. The fixed tissues were dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Tissue blocks were prepared and cut at 5 μm thick using rotary microtome (Baird and Tatlock (London) Ltd; England] to produce tissue sections. Some sections were used for Haematoxylin and Eosin (H & E) staining and others for immunohistochemistry study.

Immunohistochemistry

Tissue sections were deparaffinized and rehydrated followed by incubation for 10 min with hydrogen peroxide block (abcam, ab64261, supplied ready to use) at room temperature (RT) to inhibit endogenous peroxidase activity, then washed (3 x 5 min) in Phosphate-Buffered Saline (PBS). Sections were then incubated with protein block (abcam, ab64261, supplied ready to use) for 10 min at RT to block non-specific background binding. Tissue sections were then incubated with rabbit polyclonal to alpha smooth muscle (ab5694) actin and desmin (ab15200) primary antibody overnight in a dark, humid chamber at 4°C diluted at a ratio of 1:200 in PBS. For negative control, PBS was applied in place of the primary antibodies. Sections were then washed (3x15min) in PBS followed by incubation with goat anti-rabbit HRP conjugate micro-polymer (abcam, ab64261, supplied ready to use) for 60 min at RT. Sections were then washed (3x15min) in PBS before incubation for 3-5 min with 50X 3, 3′-Diaminobenzidine (DAB) chromogen solution (abcam, ab64261, supplied ready to use) to visualize binding sites, then rinsed in water for 10 min to stop reaction followed by dehydration through a graded ethanol series, clearance and mounting by a mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene (DPX). Binding sites were evaluated using Olympus BH-2 microscope fitted with Olympus camera for image capturing. The relative immunostaining intensities of desmin and SMA were categorized as negative, weak, moderate and strong.

Statistical analysis

At light microscopic level, every tenth section was examined and evaluated for number of healthy and atretic follicles. The data collected were stored in Microsoft Excel for analysis. The 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.

RESULTS

Histological examination findings of healthy and atretic follicles in Small East African (SEA) goats during dry and wet seasons

Both healthy and atretic follicles were found in histological examination of ovarian tissue sections during each season (dry and wet). Healthy primordial follicles were composed of an oocyte surrounded by a single layer of flat granulosa cells. The follicles occurred singly, however, a cluster of two or more follicles were occasionally seen. The wall of primary follicle was formed by one layer of granulosa cells that encircled the oocyte. At this follicular size, granulosa cells were cuboidal in shape. In the secondary follicles, the follicular wall surrounding the oocyte was composed of cuboidal granulosa cell and undifferentiated thecal cell layers. The antral follicles were composed of an oocyte surrounded by zona pellucida, multiple layers of granulosa cells, differentiated theca interna and externa cells layers. A fluid filled antrum was observed in these follicles. In addition, the granulosa cells which surrounded the oocyte are called corona radiata while others become cumulus oophorus forming a stalk of cells in one point (Figure 1: A-D). Antral follicles were obliterated, characterized by disorganized granulosa cells, degraded oocyte and abnormal arrangement of cumulus oophorus. The granulosa and theca interna varied in degrees of cellular disruption. The atretic follicle was detached from the stroma. Disruptions and total follicular obliteration and connective substitution were seen at advanced stages of atresia (Figure 2: A-D).

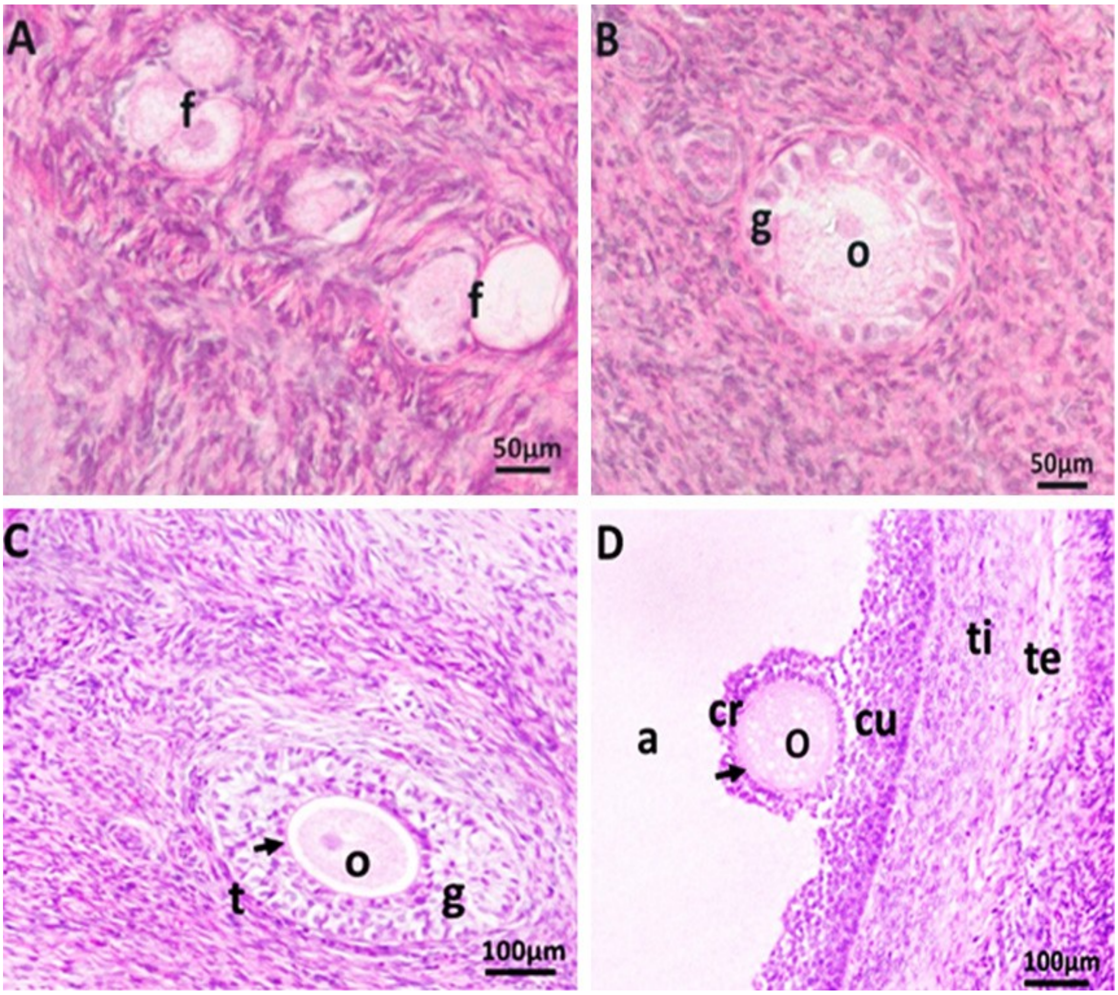

Figure 1: H & E stained photomicrographs of the SEA goat ovary showing healthy follicles at various developmental stages. (A) Cluster of primordial follicle **f**. (B) Primary follicle with an oocyte **o** surrounded by layer of granulosa cells **g**. (C) Secondary follicle with an oocyte **o** surrounded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

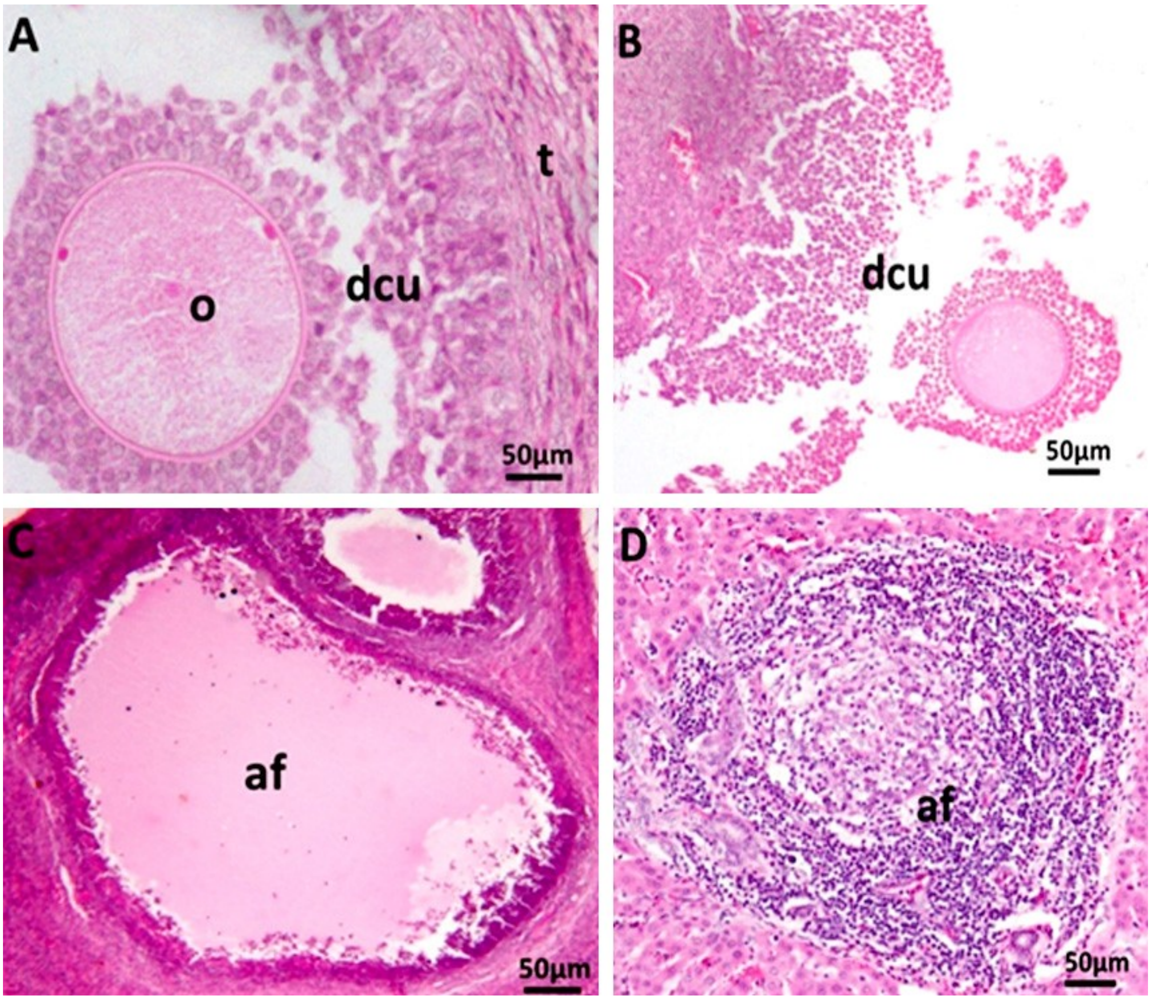

Figure 2: H & E stained photomicrographs of the SEA goat ovary showing atretic follicles. (A) Atretic antral follicle having degenerated oocyte **o**, abnormal arrangement of cumulus oophorus **dcu**, hypertrophied and detached theca layer **t**. (B) Atretic antral follicle with abnormal arrangement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

Histomorphological changes occurring in healthy and atretic follicles in SEA goats during dry and wet seasons

Ovaries collected in dry and wet seasons were evaluated in terms of histomorphological changes. The number of healthy and atretic antral follicles found in dry season differed significantly (*p* <0.05) from those observed during wet season. However, differences in the number of healthy and atretic primodial, primary and secondary follicles between dry and wet seasons were not statistically significant (Table 1).

Table 1: Mean+SE number of healthy and atretic follicles per ovary during the dry and wet seasons. * Indicates significant differences between health and atretic follicles in dry and wet seasons (p<0.05). SE: Standard Error

Follicle	**Seasons**

Type	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*

Immunohistochemical localization of desmin and SMA in healthy and atretic follicles in SEA goats during dry and wet seasons

In both health and atretic follicles during dry and wet 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 wet seasons but moderate to weak in dry season. In health 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. In atretic follicles, staining was moderate in theca cells of secondary and antral follicles. Staining was not detected in the granulosa cells and oocyte of both healthy and atretic secondary and antral follicles. In addition, staining for desmin (Figure 3: A-D) varied from moderate to weak while that of SMA (Figure 4: A-D) was strong in both healthy and atretic follicles.

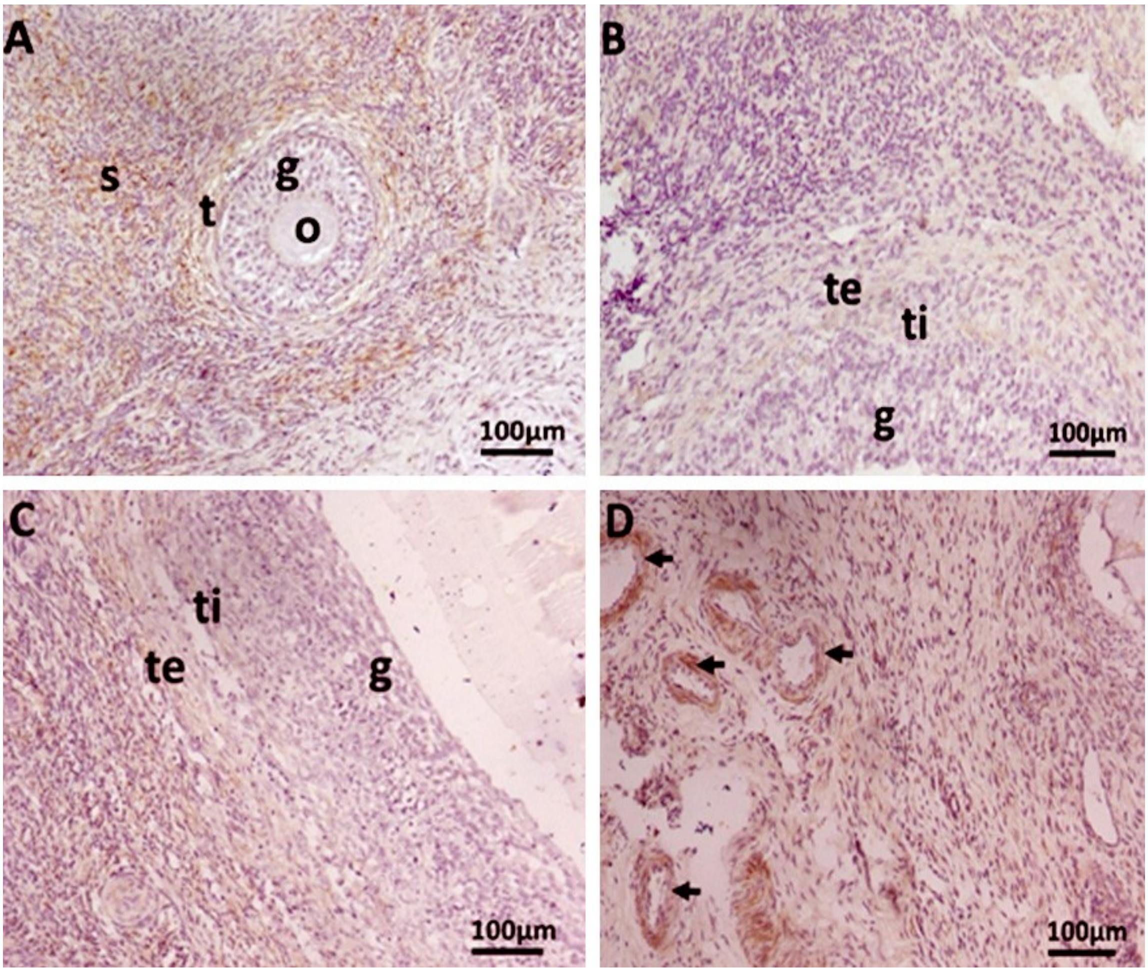

Figure 3: Immunohistochemical localization of desmin in the ovary of SEA goat. (A) Moderate immunoreactivity for desmin is seen in healthy secondary follicle within the theca cells **t** and cortical stroma **s** but is absent in granulosa cells **g** and oocyte **o**. Staining is moderate in cells of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

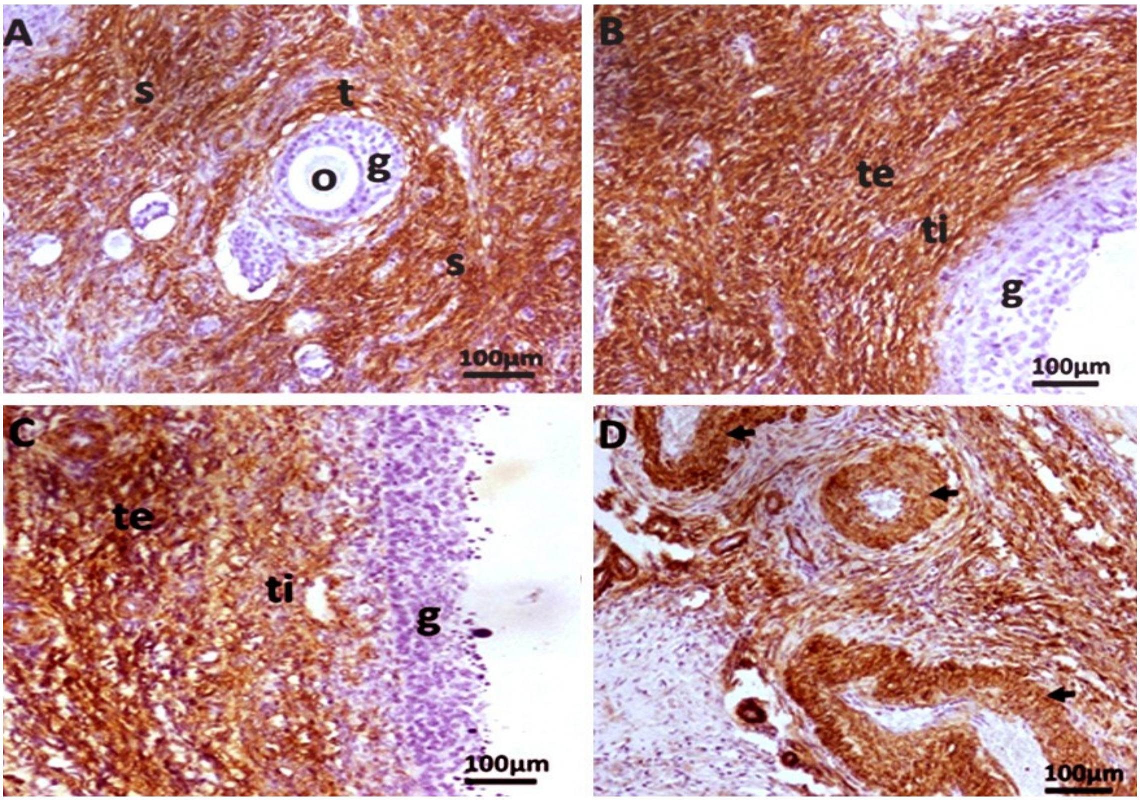

Figure 4: Immunohistochemical localization of SMA in the ovary of SEA goat. (A) Strong immunoreactivity for SMA is seen in healthy secondary follicle within the theca cells **t** and cortical stroma **s** but is absent in granulosa cells **g** and oocyte **o**. Staining is also strong in cells of theca interna **ti** and theca externa **te** healthy antral follicle (B). Moderate staining is seen in cells of theca interna **ti** and theca externa **te** of atretic antral follicle (C). The tunica media of blood vessels (**black arrows**) located in the ovarian parenchyma are also strongly stained (D). Scale bar: A-D: 100µm.

DISCUSSION

This study demonstrates histomorphological and immunohistochemical changes occurring in the ovary of Small East African (SEA) goat during dry and wet seasons. The findings of the current study showed that the histological morphology of healthy follicles in the SEA goat, are in line with description given to other breeds goat (Bari et al 2012; Emara et al 2019). In SEA goat, ovary composed of primordial, primary, secondary and antral follicles as documented in other breeds of goat such as the Egyptian goat (Emara et al 2019).

Based on the classification of atretic follicles in goats, only the obliterative type of atresia was observed in the present study. It was characterized by apical follicular wall detachment as seen in local goats in Sri Lanka (Ariyaratna and Gunawardana 1997). In dry season, large number of atretic follicles was observed compared to wet season.

This can be associated with insufficient gonadotrophins secretion caused by sub-optimal function of hypothalamo–hypophyseal–gonadal axis. Hormones are shown to play a pivot role in the development of summer anestrus in buffaloes as most of the buffaloes with aberrant reproduction during summer exhibit alterations in hormonal secretions (Das and Khan 2010).

Immunohistochemical staining on Small East African (SEA) ovaries in this study has localized the intermediate filament desmin at weak to moderate and Smooth Muscle Actin (SMA) at strong intensities in secondary follicles in theca cells; theca externa and interna of antral; cortical stroma and tunica media of blood vessels within ovarian parenchyma. It is known that desmin and SMA form an essential component of the cytoskeleton. Additionally, the intermediate filaments have a role in a number of biological processes include cell-to-cell adhesion, proliferation and differentiation (Helfand et al 2004). Occurrence of desmin and SMA in muscle bundles situated in the cortical stroma is thought to form a structural framework, which provides mechanical support to the ovary (Van Nassauw and Callebaut 1991). In addition to the stromal localization of desmin and SMA were also demonstrated in the tunica media of blood vessels in the ovary, where they play a role in the regulation of blood flow to the ovary. The observation of desmin and SMA in tunica media of blood vessels correlate well with findings reported in the ewe (Marettová and Maretta 2002) and cow (Wendl et al 2012). Moderate staining of desmin and strong of SMA in theca externa cells of healthy antral follicles indicated that they play a role in contraction of smooth muscle cells in the theca externa of antral follicles thereby initiating the process of ovulation (Madekurozwa *e*t al 2010). Atretic follicles showed reduced staining for desmin and SMA, suggesting that when follicles undergo atresia the intermediate filaments are dismantled.

In conclusion, ovarian follicles in the Small East African (SEA) goats were found to undergo a seasonal cycle of growth and atresia. During wet season the ovaries contained healthy follicles while in dry season contained many atretic antral follicles. The antral follicles undergo obliterative atresia characterized by the destruction of follicular cells closer to the antrum with the most basal follicular cells remaining intact. Furthermore, the results of the immunohistochemical study have shown that the distribution of intermediate filaments changes during follicular development and atresia and open the way to new studies on the effect of heat and nutrition on folliculogenesis in SEA goats.

ACKNOWLEDGEMENTS
Authors acknowledge financial support from Sokoine University of Agriculture. We also thank technical staff in the Department of Veterinary Anatomy and Pathology.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

CHAPTER FOUR

4.0 GENERAL DISCUSSION

The morphometrical and immunohistochemical changes occurring in the ovaries of the Small East African (SEA) goats were investigated in the current study. The findings of this study provide the insight for understanding of the morphological and immunohistochemical changes occurring in the ovaries during wet and dry seasons. It also highlights on the possible causes of the reduced reproductive performance in the SEA goat during dry season. Furthermore, the results on SEA goat ovarian morphometric parameters revealed that right ovary was longer than left. This finding proposes an increased physiological activity in the right ovary compared to the left ovary. Similar observation by Pramo*d et* al. (2013) that goat reproductive performance depends on increased ovulation rate of the right ovary.

Based on the findings of the current investigation, the percentage number of follicles (primordial, primary, secondary and antral) was lower in the dry season than in the wet season. This finding indicates that, the rate of follicular development was higher in the wet season than in the dry season. The high rate of follicular development in the wet season could be due to the availability of nutrients. This is in agreement to the findings by Dadok*et al. (*2020) that, availability of nutrients during wet season improved ovarian steroidal activity and enhance folliculogenesis in Black Bengal goat. The lower number of follicles recorded during the dry season could be caused by lack of nutrients and the increased ambient temperature. As stated in previous studies (De Rensis and Scaramuzzi, 2003, Emar*a et al.*, 2019), that during the dry season, goats are exposed to direct sunshine and high temperature causing detrimental effect on ovarian folliculogenesis.

The histomorphological findings of the current study show that Small East African (SEA) goat ovary composed of primordial, primary, secondary and antral follicles in line with description given to other breeds goat such as the Egyptian goat (Emara *et al.,*2019). Furthermore, in this study, follicular atresia was observed in all stages of follicular development. According the findings of the present study, it shows that SEA ovary undergo obliterative type atresia characterized by destructions of granulosa cells closer to the antrum while most basal positioned granulosa cells remain intact. This is in agreement with the findings reported by Ariyaratna and Gunawardana (1997) for the local goats found in Sri Lanka. In addition, the number of atretic follicles were higher in the dry season than in the wet season. This could be due to heat stress during the dry season. As reported in cow by Wilson *et al.* (1998) that, heat stress has effect on steroidogenesis in the thecal cells and granulosa cells. This could also be the case in SEA goat. In addition,), confirms that heat stress affects the generation time of granulosa cells in goats.

It was further revealed in this study that, cytoskeletons immunopositive staining was in theca cells layer and blood vessels. This location of desmin and smooth actin helps in initiating the process of ovulation and regulation of blood flow to the SEA goat ovary, in line to the findings reported in ewe by Marettová and Maretta (2002). There were no seasonal differences in staining intensities between the seasons, though the staining intensities was differed between the healthy and atretic follicle. For both desmin and smooth muscle actin, the intensities of immunopositive staining was generally reduced in the atretic follicles. According to the results of the present study, it appears that as follicles undergo atresia, the intermediate filaments are dismantled. Similar finding was also reported by Madekurozwa *et* al. (2010).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The current study has highlighted the morphometrical and histomorphological changes occurring in the ovary of Small East African (SEA) goat during wet and dry seasons. The ovaries of the SEA goat varied in size and number of follicles contained between seasons. In both wet and dry season, the right ovary was longer than left ovary. Both healthy and atretic follicles were observed during each season. Large number of atretic follicles were found in the dry season. In addition, SEA goat undergoes obliterative atresia featured by the destructions of granulosa cells near the antrum with the most basal granulosa cells remaining intact. Furthermore, the results of the immunohistochemical study have shown that the distribution and immunostaining of intermediate filaments changes during follicular development and atresia. The finding of this study suggests that ovaries of SEA goat undergo seasonal morphological changes which could be the cause of reduced fertility rate during dry season. The dry season has bad effects on folliculogenesis as it lowers the number of follicles and causes many follicles to be atretic.

5.2 Recommendations

Based on the fact that, during the dry season there is scarcity of feeds and increased heat which could affect follicular development. This study recommends improvement in nutritional requirements of the SEA goat by providing feed supplements during the dry seasons. In addition, SEA goat should be provided a good management system to reduce heat stress during the dry season. Government should establish the programs focusing on farm management, and investing in reproductive technologies such as estrus synchronization, artificial insemination and multiple ovulation and embryo transfer (MOET) to support goat rearing. The study also warrants further studies to ascertain the effect of feeds, heat stress and hormones on folliculogenesis in the SEA goat's ovaries in order to obtain higher reproductive performance with concurrent increase in the economy of goat keepers.

REFERENCES

Alam, M. H., Kabir, M. E., Sarker, M. B., Saha, B. K., Khan, R. I. and Moniruzzaman, M. (2002). Development and survivability of ovarian follicles of goat in different feeding systems *Bangladesh Journal of Animal Science* 43(3): 175–179.

Alves, N. G., Torres, C. A. A., Guimarães, J. D., Moraes, E. A., Costa, P. B. and Silva, D.R. (2018). Ovarian follicular dynamic and plasma progesterone concentration in Alpine goats on the breeding season. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 70(6): 2017–2022.

Ariyaratna, H. B. S. and Gunawardana, V. K. (1997). Morphology and Morphometry of Ovarian follicles in the goat. *Small Ruminant Research* 26: 123–129.

Auclair, S., Uzbekov, R., Elis, S., Sanchez, L., Kireev, I., Lardic, L., Dalbies-Tran, R. and Uzbekova, S. (2013). Absence of cumulus cells during in vitro maturation affects lipid metabolism in bovine oocytes. *American Journal of Physiology - Endocrinology and Metabolism* 304(6): 201-209.

Bari, F. Y., Ferdousy, R. N., Hasan, M. and Juyena, N. S. (2018). Gross and ultrasonographic morphometry of female reproductive tract in small ruminants. *Bangladesh Veterinary Journal* 52(1-4): 31–38.

Bari, M., Kabir, M., Sarker, M., Khan, A. and Momiruzzaman, M. (2012). Morphometric analysis of ovarian follicles of Black Bengal goats during winter and summer season. *Bangladesh Journal of Animal Science* 40(1–2): 51–55.

Bazer, F. W. (2019). Reproductive physiology of sheep and goats. *Animal Agriculture* 2: 199-209.

Bijna, M., Karthiayini, K. and Lucy, K. M. (2018). Morphometry and histology of ovaries in Malabari goats. *International Journal of Science and Research* 7(1): 291–293.

Bonnet, A., Servin, B., Mulsant, P. and Mandon-Pepin, B. (2015). Spatio-temporal gene expression profiling during in vivo early ovarian folliculogenesis: Integrated transcriptomic study and molecular signature of early follicular growth. *Public Library of Science* 10(11): 1–25.

Bradley, J. and Swann, K. (2019). Mitochondria and lipid metabolism in mammalian oocytes and early embryos. *International Journal of Developmental Biology* 63(3–5): 93–103.

Collado-Fernandez, E., Picton, H. M. and Dumollard, R. (2012). Metabolism throughout follicle and oocyte development in mammals. *International Journal of Developmental Biology* 56(10–12): 799–808.

Chu, Y. L., Xu, Y. R., Yang, W. X. and Sun, Y. (2018). The role of FSH and TGF-β superfamily in follicle atresia. *Aging* 10(3): 305–321.

Dadok, F., Islam, M.S., Rahman, A.N.M.I., Alam, M.J., Kabir, M.E. and Amy, B.F. (2020). Morphometric assessment of oocytes recovered from ovaries of Black Bengal goat. *IOS Journal of Agriculture and Veterinary Science* 13(12): 40–44.

Dalbies-Tran, R., Cadoret, V., Desmarchais, A., Elis, S., Maillard, V., Monget, P., Monniaux, D., Reynaud, K., Saint-Dizier, M. and Uzbekova, S. (2020). A Comparative Analysis of Oocyte Development in Mammals. *Cells* 9(4): 1–27.

De Rensis, F. and Scaramuzzi, R.J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow - A review. *Theriogenology* 60(6): 1139–1151.

Dickinson, R. E., Hryhorskyj, L., Tremewan, H., Hogg, K. and Axel, A. (2010). Europe PMC Funders Group Involvement of the SLIT / ROBO pathway in follicle development in the fetal ovary. *Reproduction* 139(4): 697–704.

Dupont, J., Reverchon, M., Bertoldo, M. J. and Froment, P. (2014). Nutritional signals and reproduction. *Molecular and Cellular Endocrinology* 382(1): 527–537.

Edwards, A. (2020). *T*he Estrous Cycle and Seasonality in Sheep and Goats. [https://www.lsuagcenter.com/profiles/bneely/articles/page1593707272607]. Site visited on 23/9/2021.

Emara, S., Hassanin, A. and Elnagar, H. (2019). Histological and Histochemical Changes of The Ovary of Goat During Autumn and Summer Seasons. *Journal of Current Veterinary Research*1(2): 47–54.

Fahad, T. A., Waheed, Z. and Kareem, D. A. (2019). Study of some abnormalities in the reproductive system of ewes in Basra province. *Conference Series: Materials Science and Engineering* 571(1): 10–21.

Fatet, A., Pellicer-Rubio, M. T. and Leboeuf, B. (2011). Reproductive cycle of goats. *Animal Reproduction Science* 124(3–4): 211–219.

Forcada, F., Abecia, J., Forcada, F. and The, J. A. (2006). The effect of nutrition on the seasonality of reproduction in ewes. *Reproduction Nutrition Development* 46(4): 355-365.

Friedman, E., Voet, H., Reznikov, D., Dagoni, I. and Roth, Z. (2011). Induction of successive follicular waves by gonadotropin-releasing hormone and prostaglandin F2α to improve fertility of high-producing cows during the summer and autumn. *Journal of Dairy Science* 94(5): 2393–2402.

González-Valle, F., Batista-Arteaga, M. and Gracia-Molina, A. (1998). Follicular atresia and LH concentrations during the follicular phase of the estrous cycle in the goat (*capra hircus*). Animal *Reproduction Science* 51(1): 23–30.

Gómez-Brunet, A., Santiago-Moreno, J., Toledano-Diaz, A. and López-Sebastián, A. (2012). Reproductive seasonality and its control in spanish sheep and goats. *Tropical and Subtropical Agroecosystems* 15(1): 30–39.

Gupta, S. K., Prakash, A. and Ram, R. (2007). Histological study of tertiary follicles in the ovary of goat. *Indian Journal of Animal Sciences* 77(1): 48–50.

Hatzirodos, N., Irving-Rodgers, H. F., Hummitzsch, K. and Rodgers, R. J. (2014). Transcriptome profiling of the theca interna from bovine ovarian follicles during atresia. *Public Library of Science* 9(6): 15–40.

Haque, Z., Haque, A. and Quasem, M. A. (2016). Análisis morfológico y morfométrico del ovario de la cabra Black Bengal (Capra hircus). *International Journal of Morphology* 34(1): 13–16.

Hurk, R.V.D. and Telfer, E. E. (2000). Primate and bovine immature oocytes and follicles as sources of fertilizable oocytes. *Human Reproduction Update* 6(5): 457-474.

Irving-Rodgers, H. F., Mussard, M. L., Kinder, J. E. and Rodgers, R. J. (2002). Composition and morphology of the follicular basal lamina during atresia of bovine antral follicles. *Reproduction* 123(1): 97–106.

Irving-Rodgers, H. F., Van Wezel, I. L., Mussard, M. L., Kinders, J. E. and Rodgers, R. J. (2001). Atresia revisited: Two basic patterns of atresia of bovine antral follicles. *Reproduction* 122(5): 761–775.

Islam, M. R., Khandoker, M. A., Afroz, S., Rahman, M. G. and Khan, R. I. (2007). Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of in vitro production of embryos. *Journal of Zhejiang University Science* 8(7): 465–469.

Jaji, A., Buduwara, R., Akanmu, A., Zachariah, M., Luka, J. and Gambo, B. (2012). Pregnancy related biometric changes in the ovaries and uterus of the sahelian goat. *Sokoto Journal of Veterinary Sciences* 10(1): 18–21.

Kırbaş Doğan, G., Kuru, M., Bakır, B. and Karadağ Sarı, E. (2019). Anatomical and histological analysis of the salpinx and ovary in Anatolian wild goat (Capra aegagrus aegagrus). *Folia Morphologica (Poland)* 78(4): 827–832.

Kumar, M., Ray, S., Singh Vishen, A., Kumar Gupta, R., Singh, K. N. and Choudhary, P. K. (2020). Histological studies on the ovary and uterus of Black Bengal Goat. *Journal of Entomology and Zoology Studies* 8(2): 31–33.

Lin, P. and Rui, R. (2010). Effects of follicular size and FSH on granulosa cell apoptosis and atresia in porcine antral follicles. *Molecular Reproduction and Development* 77(8): 670–678.

Logothetopoulos, J., Dorrington, J., Bailey, D. and Stratis, M. (1995). Dynamics of follicular growth and atresia of large follicles during the ovarian cycle of the guinea pig: Fate of the degenerating follicles, a quantitative study. *The Anatomical Record* 243(1): 37–48.

Madekurozwa, M.C., Oke, B.O. and Akinloye, A.K. (2010). The immunohistochemical localization of desmin and smooth muscle actin in the ovary of the African giant rat during the oestrous cycle. *Journal of Veterinary Medicine Series C: Anatomia Histologia Embryologia* 39(1): 81–86.

Makarevich, A. V., Földešiová, M., Pivko, J., Kubovičová, E. and Chrenek, P. (2018). Histological characteristics of ovarian follicle atresia in dairy cows with different milk production. *Journal of Veterinary Medicine Series C: Anatomia Histologia*

Embryologia 47(6): 510–516.

Madekurozwa, M. and Kimaro, W. H. (2006). *A* Morphological and Immunohistochemical Study of Healthy and Atretic Follicles in the Ovary of the Sexually Immature Ostrich *(Struthio camelus) Anatomia Histologia Embryologia* 35: 253–258.

Marettová, E. and Maretta, M. (2002). Demonstration of intermediate filaments in sheep ovary. *Acta Histochemica* 104(4): 431–434.

Matsuda, F., Inoue, N., Manabe, N. and Ohkura, S. (2012). Follicular growth and atresia in mammalian ovaries: Regulation by survival and death of granulosa cells. *Journal of Reproduction and Development* 58(1): 44–50.

Minister, B., Mulalem, Z. and Hailai, H. (2020). Effect of nutritional flushing on reproductive and body weight performance of Abergelle goat does. *International Journal of Livestock Production*11(2): 68–71.

Mullins, R. D. and Francisco, S. (2014). Cell mechanics and the cytoskeleton. *Nature* 463(7280): 485–492.

Paulin, D. and Li, Z. (2004). Desmin: a major intermediate filament protein essential for the structural integrity and function of muscle. *Experimental Cell Research* 301: 1–7.

Pramod, R.K., Sharma, S.K., Singhi, A., Pan, S. and Mitra, A. (2013). Differential ovarian morphometry and follicular expression of BMP15, GDF9 and BMPR1B influence the prolificacy in goat. *Reproduction in Domestic Animals* 48 (5): 803–809.

Rahman, M. H., Chowdhury, E. H., Islam, A. and Islam, M. R. (2006). *Pathological-conditions in the reproductive tract of does at slaughter in Mymensingh district* 4(2): 265–271.

Ramos, A. F. and Silva, B. D. M. (2018). Hormonal Protocols in Small Ruminants. *Reproduction Biotechnology in Farm Animals* 2–18.

Reverchon, M., Ramé, C., Bertoldo, M. and Dupont, J. (2014). Adipokines and the female reproductive tract. *International Journal of Endocrinology* 1: 1-10.

Rodgers, R. J. and Irving-Rodgers, H. F. (2010). Morphological classification of bovine ovarian follicles. *Reproduction* 139(2): 309–318.

Sahu, S. K., Das, R. K., Sathapathy, S., Dehury, S., Mishra, U. K. and Dash, S. K. (2017). Gross, histological and histochemical studies on the ovary and oviduct of Kendrapada sheep (*Ovis aries*) at different age groups. *Journal of Entomology and Zoology Studies* 5(6): 2337–2342.

Salha, O., Abusheika, N. and Sharma, V. (1998). Dynamics of human follicular growth and in-vitro oocyte maturation. *Human Reproduction Update* 4(6): 816–832.

Sinclair, K. D., Lunn, L. A., Kwong, W. Y., Wonnacott, K., Linforth, R. S. T. and Craigon, J. (2008). Amino acid and fatty acid composition of follicular fluid as predictors of in-vitro embryo development. *Reproductive BioMedicine Online* 16(6): 859–868.

Sutton-McDowall, M. L., Gilchrist, R. B. and Thompson, J. G. (2010). The pivotal role of glucose metabolism in determining oocyte developmental competence. *Reproduction* 139(4): 685–695.

Sharma, R. K. and Bhat, R. A. (2014). Histomorphology of atretic follicles in rainbow trout from Kashmir. *Journal of Entomology and Zoology Studies* 2 (4): 21-26.

Sharma, R. K. (2000). Follicular atresia in goat: A review. *Indian Journal of Animal Sciences* 70(10): 1035–1046.

Shelton, M. (1978). Reproduction and Breeding of Goats. *Journal of Dairy Science* 61(7): 994–1010.

Slides and Turner (2011). Goat Reproduction. *Farm health online* (1)29: 1551–1572.

Townson, D. H. and Combelles, C. M. H. (2010). Ovarian Follicular Atresia. *Intechopen* 4(3): 25–36.

Ungerfeld, R. and Bielli, A. (2012). Seasonal and social factors affecting reproduction. *Encyclopaedia of Life Support Systems* 15(6): 53–65.

Verde, G.L., Artiola, V., Panzetta, V., Pugliese, M., Netti, P. A. and Fusco, S. (2021). Cytoskeleton Response to Ionizing Radiation*:* A Brief Review on Adhesion and Migration Effects *Biomedicines* 9: 1102-1120.

Vlčková, R., Sopková, D., Pošivák, J. and Valocký, I. (2012). Ovarian follicular atresia of ewes during spring puerperium. *Veterinary Medicine International* 1(3): 6–12.

Wang, W., Liu, H. L., Tian, W., Zhang, F. F., Gong, Y., Chen, J. W., Mao, D. G. and Shi, F. X. (2010). Morphologic observation and classification criteria of atretic follicles in guinea pigs. Journal of Zhejiang University: *Science B* 11(5): 307–314.

Wilson, S.J., Marion, R.S., Spain, J.N., Spiers, D.E., Keisler, D.H. and Lucy, M.C. (1998) Effects of Controlled Heat Stress on Ovarian Function of Dairy Cattle. 1. Lactating Cows. *Journal of Dairy Science* 81 (8): 2124–2131.

Xiao, Q., Hu, X., Wei, Z. and Tam, K. Y. (2016). Cytoskeleton Molecular Motors: Structures and their Functions in Neuron. *International Journal of Biological Sciences* 12(9): 1083-1092.

Zhou, J., Peng, X. and Mei, S. (2019). Autophagy in ovarian follicular development and Atresia. *International Journal of Biological Sciences* 15(4): 726–737.

STATEMENT OF RESEARCH ETHICAL APPROVAL

1. *This project has been considered and has been **Approved/~~Not Approved~~** by the Department/College Research and Publication Committee, Department/College/Unit

Click here to the name of the unit or delete this guiding text and print and write by hand

Signature: .... Name: A.M. Lupindu

Date: 18-01-2022

(Chairperson, Research & Publication Committee)

2. This project has been considered and has been **Approved/Not Approved** by the Ethical Committee, DPRTC

Signature: .... Name: D.G. NDOSSI
Date: 27-01-2022
(Chairperson, Ethics Committee, DPRTC)

3. This project has been considered and **Approved/Not Approved** by the Senate Research and Publication Committee (SRPC), Sokoine University of Agriculture

Signature: .... Name: PROF. ESRON D. KARIMURIBO
Date: 27/01/2022
(Chairperson, SRPC)

Director
Postgraduate studies, Research, Technology Transfer and Consultancy
Sokoine University of Agriculture
P. O. Box 3151, Morogoro
TANZANIA

Postal address:	Telephone:	Fax:	Telex:	e-mail Address:
P.O. Box 3151 Morogoro, Tanzania	+255 23 2640013	+255 56 4388	55308 UNIVMOG TZ	drpgs@suanet.ac.tz

* All special Projects (Undergraduate studies research be evaluated and approved by the Department/College Research and Publication Committee, Department/College/Unit and reported to REC/DPRTC. Only Applications from Postgraduate, Research Associates and Staff be forwarded to University wide REC

SECTION P: FOR OFFICIAL USE

(i) APPROVAL

Date received : Click here and arrow to enter a date. 27.01.2022.	Received by: Click here to type names. MARTHA E. MOSHI
Date of approval: Click here and arrow to enter a date. 27.01.2022. Name: PROF ESRON D. KARIMURIBO Title: DIRECTOR. Approving authority in capital letters (example: SRPC, Departmental /College/Centre R&PC Click here to enter the name of approving authority. Director Postgraduate studies, Research, Technology Transfer and Consultancy Sokoine University of Agriculture P.O. Box 3151, Morogoro	Approval reference number: Click here to enter number. SUA/DPRTC/R/186/27 Approval is valid from Click here and arrow to enter a date. 27.01.2022. To: Click here to enter a date. …………………………
*All undergraduate studies shall be evaluated and/or approved by the College/Centre R&PC and Reports submitted to the chair Research Ethics Committee, DPRTC	

(ii) NOT APPROVED

☐The applicant is required to revise the application by addressing reviewer's concerns (Reviwer's comments are provided to the applicant)

☐Other reasons (Describe briefly)