

**EVALUATION OF THE MORPHOLOGY OF THE FEMALE
REPRODUCTIVE SYSTEM OF THE GRASSCUTTER**

(Thryonomys swinderianus, Temminck 1827)

BY

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DEDICATION

This M.Sc research work is dedicated to my mother, Mrs. Monica Mary Onoja (õMummy Chinaö), my elder brother Mr. Lawrence Itodo Onoja and Mr. Andrew A. Odamah, all of blessed memory. Though you all encouraged me to remain steadfast during the course of this research, death has made you not to see the fruit of your labour of love upon me. Until we meet at the foot of our creator to part no more, adieu!!!

CERTIFICATION

ONOJA, BENEDICT ONU, a postgraduate student of the Department of Veterinary Anatomy with registration number **PG/M.Sc/06/41426**, has satisfactorily completed the requirements for the award of the Degree of Master of Science in Veterinary Anatomy, University of Nigeria Nsukka.

This dissertation embodies the results of my original research work that has not been submitted in part or in full for any degree or diploma of this University or any other University.

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ABSTRACT

Fifteen female grasscutters, obtained from David Mark Farms, Otukpo, Benue State, Nigeria, were used for the study. They were randomly assigned to 3 groups of 5 each according to their ages; 1-3 months, 4-6 months and 7-9 months. They were acclimatized for 14 days. The live weights of the grasscutters were obtained. They were then euthanized with gaseous chloroform in an air-tight container. The female reproductive system (ovaries, uterine tubes, uterine horns, uterus duplex, cervix and vagina) were carefully dissected out and observed for gross features. The morphometry was also obtained. Each segment was then fixed in 10% Bouin's solution and 10% neutral-buffered formalin of equal volume for 24 hours and processed routinely for light microscopy. Photomicrographs of the sections were taken using Moticam MC 2001 digital camera. The data were subjected to one way analysis of variance. Variant means were separated using Duncan's multiple range test. Significance was accepted at $p < 0.05$. Grossly, the ovaries were spindle-shaped at 1-3 months, oval at 4-6 months and round at 7-9 months. In all the age groups studied, the uterine horns had a firm consistency on palpation, the uterus presented the appearance of a double uterus, the cervix had a rubber-stopper consistency on palpation, and the vagina had a soft consistency on palpation. The weight and length of each segment of the female reproductive system of the grasscutter increased as the animal attained puberty. Thus, the organ sizes and lengths may not be pre-determined pre-natally, but increased proportionately as the animal attained puberty. There were significant ($p < 0.05$) variations between the means of the relative weight and length of the ovaries, uterine tubes, uterine horns, uterus duplex, cervix and vagina of all the age groups. Histomorphologically, the oocyte was peripherally located in the Graafian follicle as well as corpora haemorrhagica and lutea in the cortex at 7-9 months. An intensely eosinophilic vaginal plug that was absent in the lumen of the vagina at 1-3 and 4-6 months, was very conspicuous at 7-9 months. The female grasscutter could thus be said to attain puberty at 7 months of age.

CHAPTER ONE

2.0

INTRODUCTION

Grasscutter (*Thryonomys swinderianus*, Temminck 1827) is a wild herbivorous rodent of the Sub-Order Hystricomorpha, found only in Africa (Baptist and Mensah, 1986). Rodents (which the grasscutter is a part of) comprise the largest and most diverse group of mammals with over 1,700 different species (Besselsen, 2002; Annor and Djang-Fordjour, 2006).

In Nigeria where grass provides its main habitat and food, it is commonly called ògrasscutterö. The Igbos of Eastern Nigeria call it ònchiiö, Hausas of Northern Nigeria call it òjawjiö or òjebjiö (depending on the dialect spoken), Yorubas of Western Nigeria call it òewujuö or òoyaö (also depending on the dialect spoken), while the Idomas of North-C 1 1 Nigeria (precisely Benue State) call it òbijeö. The Igalas of Kogi State call it òemiö. Other parts of Africa particularly South Africa, where it is closely associated with cane fields, it is called ògreater cane ratö or simply òcane ratö.

French-speaking African countries call it òagoutiö, which means òanimal from the bushö (Ismail, 2008). Its distribution is therefore determined by the availability of adequate or preferred grass species for food (National Research Council-NRC, 1991; Songhai Centre, 2009). Annor *et al.* (2008) reported that grasscutters fed on the leafy portion of guinea grass had better growth performance than those fed on the stem.

Generally, *grasscutters* are of two species; the larger species (*Thryonomys swinderianus*, Temminck 1827) as found in Southern Nigeria and the smaller species (*Thryonomys gregorianus*) as found in Northern Nigeria. The larger species are the ones under study in this research. The females (does) have narrow heads that taper rostrally. The tails of does are short and their bodies are covered with coarse, bristly and even spiny hairs like that of the porcupine

(Mensah and Okeyo, 2005). The doe is small in size at maturity weighing 5-7kg. The female grasscutter breeds all-year-round with peak incidence of pregnancy between the months of June and July (the months of greatest rainfall). Grasscutters have many predators including humans, leopards, python and mangoose (Raymond, 2000).

1.1 STATEMENT OF PROBLEM

- ❖ There are documented scientific reports on the nervous system (Byanet *et al.*, 2008b), gastrointestinal tract (Byanet *et al.*, 2008a; Nwaogu and Adibe, 2010a), skeletal system (Byanet and Nwaogu, 2010) and the respiratory system (Ajayi *et al.*, 2009; Nwaogu and Aghaebita, 2010b).
- ❖ Paucity of scientific information on the reproductive system of the doe, which could have facilitated its maintenance in captivity and help breeders expand their small scale to large scale necessitated this research (Hirakawa, 2001; Adu and Yeboah, 2002).
- ❖ Dependence on wild stock as source of grasscutter meat has led to indiscriminate bush-burning, destruction of forest reserves, environmental pollution, destruction of the ecosystem by depletion of the ozone layer (Mensah *et al.*, 1992b; Yeboah and Adamu, 1995).
- ❖ Though grasscutter has high protein content (Jori *et al.*, 1995; Addo, 1998), little is known about the fecundity.

1.4 JUSTIFICATION

The justifications for this research are:

- a. obtaining baseline data of the doe's reproductive system for researchers and breeders.

- b. being a white meat, the grasscutter meat has low cholesterol levels (Addo *et al.*, 2002).
- c. fish has lower protein content (Ehizibolo *et al.*, 2007) than the grasscutter. Thus, the grasscutter contributes significantly in the protein content of the human diet.
- d. the pancreas have high insulin concentration (Ismail, 2008), and so could be employed in managing diabetes mellitus.
- e. the grasscutter meat has no religious sentiments (Ismail, 2008).
- f. conservation and prevention of extinction of grasscutter (Adu *et al.*, 1999; Adu, 2005) .
- g. source of income and employment. In most countries of the African sub-region, grasscutter meat fetches more money than beef (The Nation, 2010).
- h. derivation of pleasure from keeping the grasscutter as pets.
- i. source of fur and skin.

1.3 OBJECTIVES

1.3.1 MAIN OBJECTIVE

The main objective of this study is to investigate the gross morphology, morphometry and histomorphology of the female reproductive system of the grasscutter at different ages.

1.3.2 SPECIFIC OBJECTIVES

The specific objectives of this study are:

- a.** to study the gross morphology, morphometry and histomorphology of the reproductive system of the female grasscutter like: ovaries, uterine tubes, uterine horns, uterus, cervix and vagina.
- b.** to correlate the anatomical findings with the female reproductive pattern of the grasscutter.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GRASSCUTTER TAXONOMY

Kingdom-----*Animalia*

Phylum-----*Chordata*

Class-----*Mammalia*

Order-----*Vertebrata*

Sub-Order-----*Hystricomorpha*

Family-----*Thryonomyidae*

Genus-----*Thryonomys*

Species-----*Thryonomys swinderianus* (Temminck, 1827)

(Courtesy: <http://www.eol.org/pages/326380>)

2.2 GRASSCUTTER DENTITION

2.2.1 TEMPORARY DENTITION

I=1/1; C=0/0; P=2/2; M=1/1

Total=16

Where; I=Incisors; C=Canines; P=Premolars; M=Molars

2.2.2 PERMANENT DENTITION

I=1/1; C=0/0; P=2/2; M=2/2; Total=20

Where; I=Incisors; C=Canines; P=Premolars; M=Molars

2.3 DETECTION OF OESTROUS CYCLE IN DOES

The does should be monitored for manifestation of periodic vaginal membrane perforation phenomenon, which is a sign of oestrous cycling and sexual maturity in Hystricomorph rodents (Weir, 1974). The does should be placed on dorsal recumbency and the perineum examined for intermittent rupture and restoration of the vaginal closure membrane. The oestrous cycle length is defined as the interval between the first day of spontaneous vaginal membrane perforation (evidenced by patent vagina or secretion-sealed opening), through the period of vaginal closure by the epithelial membrane, to the day before the next perforation (Weir, 1974).

2.4 PREGNANCY DIAGNOSIS IN DOES

The following methods could be used to diagnose pregnancy in does:

1. If the doe is pregnant, the vagina will have pregnancy-associated intermittent bleeding. Weekly abdominal palpation can also be carried out (Addo, 2002).
2. Pregnant does would have sealed vagina with an epithelial membrane (vaginal plug) 60 days post mating. Does with unplugged vagina 100-110 days post mating could be considered as not being pregnant.
3. During grasscutters' resting period of 1pm daily, a pregnant doe would be found lying on her abdomen.
4. There is marked increase in the weight of pregnant does.
5. There would be marked protrusion of the abdomen of does 3 months post mating if pregnant.
6. A cotton bud could be passed into the vagina of the suspected doe and drawn out to observe any of the following changes:
 - a. If the slime on the cotton bud is white, elastic and jelly-like, it indicates the onset of pregnancy (Ismail, 2008).

- b. Yellow, elastic and jelly-like slime indicates normal pregnancy (100% pregnancy)- Ismail (2008)
- c. Black, elastic and jelly-like slime indicates partial abortion (Ismail, 2008).
- d. Pure red, elastic and jelly-like slime indicates complete abortion (Ismail, 2008).

2.5 GESTATION PERIOD IN DOES

This is computed from the day of the appearance of the mating sign to the day of parturition (kindling). It is usually about 154 plus or minus 2 days (approximately 5 months). Proper records should be kept of the mating day for accurate calculation of the date of parturition. Wider pens should be used for does in gestation. Two pregnant does could be housed together.

2.6 IMMINENT PARTURITION IN DOES

Towards parturition (kindling), does become restless, uninterested in their surrounding, present bristling fur, develop difficulty in movement and may present a òpenguin postureö (walking on her hind limbs with the forelimbs raised) when parturition is imminent (Addo, 2002). At parturition, does would usually prefer a calm, quiet and secluded area. So, kindling boxes should be provided for them 2 weeks to the expected date of their kindling. This is to avoid stress to the does which could lead to stillbirth. Care must be taken not to unnecessarily agitate the does during this period (parturition).

Under good management, fertility and viability rate of over 85% could be recorded. The young ones each could weigh up to 129g at kindling. Extreme diligence should be applied especially when two mothers (does) with their litters are housed in the same pen. Cannibalism (eating the young ones) by the other doe could occur. If this occurs, the cannibalistic doe should be removed along with her litters.

2.7 OOGENESIS

Frandsen and Whitten (1981) reported that in contrast to the male sex cells which form four spermatozoa from each primary sex cell, the maturation of the female sex cell results in only one mature ovum (or ootid). Three other rudimentary female sex cells called polar bodies or polocytes are also produced. Usually, the division that produces the first polar body is meiotic in nature. That is, it is a reduction division in which the chromosome number is reduced to half the original number. The ovum then undergoes mitotic division to split off the second polar body. The first polar body may also divide mitotically or amitotically before the polar bodies degenerate. In most animals, the first polar body is arrested in the ovary, while the second polar body is given off after ovulation occurs.

2.8 OESTROUS CYCLE

Domestic female animals come into heat at fairly regular intervals that differ rather widely between species. This interval from the beginning of one heat period to the beginning of the next is called the oestrous cycle. It is controlled directly by hormones from the ovary and indirectly by hormones from the cranial lobe of the pituitary gland (Frandsen and Whitten, 1981).

2.8.1 PROESTRUS

Frandsen and Whitten (1981) pointed out that under stimulation of Follicle Stimulating Hormone (FSH) from the cranial lobe of the pituitary and some Luteinizing Hormone (LH), the ovary produces increasing quantities of oestrogens. These oestrogens cause increased development of the female reproductive tract.

2.8.2 OESTRUS

Oestrus is the period of sexual receptivity in the female which is determined largely by circulating oestrogen level. During or shortly after this time, ovulation occurs due to a decrease in

the FSH and an increase in the LH levels. Queens, rabbits, minks and ferrets are called reflex-ovulators because ovulation occurs only after coitus with the male (Franson and Whitten, 1981).

2.8.3 METOESTRUS

Metoestrus is the postovulatory phase during which the corpus luteum functions. During this period, there is a decrease in oestrogen and an increase in progesterone formed by the ovary.

2.8.4 DIOESTRUS AND ANOESTRUS

Dioestrus is a relatively short period of quiescence between oestrous cycles in polyestrous animals. Anoestrus is a longer period of quiescence between the breeding seasons. In the bitch, even though unmated, the corpus luteum persists throughout a period equal to the normal gestation period (Franson and Whitten, 1981).

2.9 EMBRYOGENESIS OF THE FEMALE REPRODUCTIVE TRACT

Although the sex of an embryo is determined chromosomally at fertilization, an undifferentiated stage of development initially occurs, in which the primordia of both male and female genital organs are present (McGeady *et al.*, 2006). Depending on the genetically determined sex of the individual, the genital organs appropriate for that sex develop, while the genital organs for the other sex regress. Sexual identity is not confined solely to the reproductive organs, but is evident also in other anatomical features and in physiological and behavioural characteristics (McGeady *et al.*, 2006).

2.9.1 PRIMORDIAL GERM CELLS

Primordial germ cells, which eventually populate the undifferentiated gonad, can be detected in the epiblast at an early stage in embryological development. These cells, which migrate through the primitive streak and then to the yolk sac and allantois, move along the wall of the hindgut to the genital ridge---a structure destined to become the undifferentiated gonad. In

mammals (grasscutter being among), primordial germ cells arrive at their site of differentiation by an active migration. In Avian species, they reach the genital ridge via the bloodstream.

It has been suggested that primordial germ cells may be attracted to the genital ridge by chemotaxis (McGeady *et al.*, 2006). They further stated that primordial germ cells can be detected in the genital ridge by day 18 in pigs, day 21 in dogs, day 22 in sheep and by day 28 in cattle and humans. Primordial germ cells divide mitotically during migration to the developing gonads. Soon after entering the primordial gonad, the germ cells become enclosed in specific germ cell compartments---seminiferous cords in the male and primordial follicles in the female embryos, respectively. Both the proliferation and differentiation of primordial germ cells in these particular locations are strongly influenced by locally secreted soluble factors.

Only germ cells which reach the undifferentiated gonad differentiate and survive. Most germ cells outside the gonadal region undergo apoptosis (cell death). Some which survive outside this region may form germ cell tumours referred to as teratomata. Due to the fact that these abnormal structures are composed of the elements of the three embryonic germ layers, they may contain highly differentiated tissues such as skin, hair, cartilage and teeth (McGeady *et al.*, 2006).

2.9.2 UNDIFFERENTIATED STAGE OF THE GONADAL FORMATION

Even though the origin of the somatic gonadal cells is unresolved, three cellular sources have been proposed: local mesenchymal cells, coelomic epithelium and cells derived from the mesonephric tubules. It is proposed that mesonephric cells from the degenerating mesonephric tubules invade the presumptive gonadal tissue and are the principal cells contributing to the gonadal primordia. Some cells contributing to the gonadal primordia may be derived from the coelomic epithelium and also from the underlying mesenchyme. Following proliferation of coelomic epithelium and underlying mesenchyme, gonadal primordia develop as bilateral ridges.

These ridges develop medial to the mesonephros and project into the coelomic cavity where they become covered by coelomic epithelium and extend from the thoracic to the lumbar region. The outline appearance of the gonadal ridges precedes the arrival of the primordial germ cells to the area. The undifferentiated gonad consists of primordial germ cells and the mesenchymal cells. The invading mesonephric cells and the mesonephric tubules form a tubular network called the rete system which consists of extra-gonadal cords, connecting cords and intra-gonadal cords.

During development, as a consequence of proliferation in its mid-region, the developing gonadal ridge assumes a globular appearance. It remains attached to the mesonephros by a fold of mesothelium. Due to their morphological similarity, it is not possible to distinguish male from female primordial gonads at an early stage of development using histological methods. However, using modern molecular techniques, the sex of an embryo can be reliably confirmed at an early stage of development (McGeady *et al.*, 2006).

2.9.3 DIFFERENTIATION AND MATURATION OF THE OVARIES

Although the origin of the sex cords is not certain, in genotypic females, it is probable that they were derived from the mesothelial cells surrounding the gonads. The sex cords form irregular recognizable structures into which germ cells become incorporated (McGeady *et al.*, 2006). Following the breakdown of the sex cords, germ cells undergo a period of enhanced mitotic activity in the developing ovaries. Irrespective of the duration of oogonia mitosis within a species, in the majority of mammals, it ceases before or shortly post partum.

As individual oogonium completes its period of mitotic activity, it becomes surrounded by a layer of squamous somatic cells of mesothelial origin termed follicular cells. A germ cell enclosed in a basal lamina and surrounded by follicular cells constitutes a primordial follicle. The follicular cells induce the enclosed oogonium to enter the prophase of Meiosis 1. At this stage, the germ cells which are referred to as primary oocytes, undergo a prolonged resting or dictyate stage. Although

some maturation of primary oocytes may occur, these germ cells do not progress to the tertiary stage of development until stimulated by gonadotrophic hormones at the onset of puberty (McGeady *et al.*, 2006).

Following the advent of puberty, recurring cyclical stages of follicular maturation occur in response to gonadotrophic hormones. As folliculogenesis proceeds, the squamous follicular cells which become cuboidal, form stratified layers and are referred to as granulosa cells. Female mammals (does inclusive) have their full complement of primary oocytes before or shortly after birth. In the ovary, germ cell proliferation and follicular development are confined to the peripheral areas of the developing gonad. By the end of this developmental period in domestic species (except horses), the ovaries consist of a dense cortical area which contains the follicles, and a less dense central medullary area composed of degenerating intra-gonadal tubules, the rete ovarii (McGeady *et al.*, 2006).

McGeady *et al.* (2006) further explained that in cattle, sheep and pigs, follicles are randomly distributed in the cortex while in dogs and cats, they occur in clusters. In horses, follicular development is concentrated in the central area (medulla) while the non-follicular area is located peripherally in the cortex. In mammals, a high percentage of oogonia and primary oocytes undergo degenerative changes referred to as atresia during their pre- and post-natal lives.

2.9.4 DIFFERENTIATION OF THE MAMMALIAN FEMALE DUCT SYSTEM

The primordia of the paramesonephric ducts arise from the intermediate mesoderm lateral to the cranial ends of the mesonephric ducts. Initially, grooves which form in the coelomic epithelium give rise to paramesonephric ducts which move deeper into the mesenchyme adjacent to the related mesonephric ducts. The cranial portions of the paramesonephric ducts form the uterine tubes while the caudal portions of the ducts give rise to the horns and body of the uterus. At their cranial aspects, the uterine tubes remain open and communicate with the coelomic cavity. Postnatal, this

communication persists from the peritoneal cavity to the exterior (McGeady *et al.*, 2006). At first, the portions of the ducts which are closed elongate caudally, lateral to the mesonephric duct.

Close to the urogenital sinus, each duct occupies a position ventral to the mesonephric duct and fuses in the mid-line with its corresponding duct from the opposite side. The closed ends of the fused ducts continue to grow caudally and make contact with the urogenital sinus. Here, it induces cellular proliferation of the endoderm of the urogenital sinus and the formation of vaginal plate. Differences observed in the final anatomical arrangement of uteri in different species can be attributed to the relative positions of their primordial structures and the extent to which fusion occurs. In rodents (grasscutters inclusive), fusion is confined solely to the outer portions of the walls of the ducts while the lumina remain distinct. This results in a separate opening for each uterine lumen into the vagina, as stated by McGeady *et al.* (2006). They stated further that in domestic species, the caudal ends of the ducts fuse.

Subsequently, the medial fused walls atrophy resulting in the formation of a single tube, the body of the uterus, which has a single opening into the vagina. Those portions of the ducts cranial to the region of fusion remain distinct and are the primordia of the horns of the uterus and uterine tubes. Thus, in domestic animals, the uterus which consists of two horns and a body is referred to as a bicornuate uterus. In cattle, the primordia of the paramesonephric ducts appear at approximately the 34th day of gestation, and fuse with the urogenital sinus at approximately 50th day. In primates, including humans, extensive fusion of the paramesonephric ducts occurs with associated atrophy along the medial line of fusion resulting in the formation of a large uterine body termed a uterus simplex.

The vagina is derived from both the vaginal plate and the fused ends of the paramesonephric ducts. Subsequently, cannulation of these fused structures occurs forming the lumen of the vagina. Initially, the lumen of the vagina is separated from the urogenital sinus by a thin membrane, the

hymen, which subsequently breaks down. In domestic animals, persistence of hymen remnants is less evident than in primates. The caudal portion of the urogenital sinus forms the vestibule. Epithelial buds, which arise from the primitive urethra and definitive urogenital sinus, form the urethral and vestibular glands. These glands are the female homologues of the prostate and bulbo-urethral glands in the male embryo (McGeady *et al.*, 2006). Apart from some remnants of the excretory tubules and a small portion of the mesonephric duct, the female mesonephric system atrophies.

The cranial remnants of the mesonephric tubules form the epoophoron. The mesonephric tubules caudal to the developing gonad become the paroophoron, and the remainder of the mesonephric duct usually degenerates. Occasionally, a caudal portion of the duct persists as Gartner's duct (G-spot in humans) which may form a cyst in the vaginal wall (McGeady *et al.*, 2006). They further affirmed that in Avian, the left gonad and its associated duct in genotypic female embryos continue to develop into functional structures. The right gonad and its associated duct remain rudimentary. The left paramesonephric duct gives rise to the different regions of the female reproductive tract from the ovary, through the uterine tubes, magnum, isthmus, uterus, vagina and cloaca.

2.9.5 FORMATION OF THE GENITAL FOLD

The urogenital system, which develops retro-peritoneally, bulges into the peritoneal cavity. With the degeneration of the mesonephros, the gonads and genital ducts become suspended by thin folds of peritoneum. The caudal portions of the genital ducts meet and fuse in the mid-line. Fusion of their associated peritoneal folds forms the genital fold. In the female, this broad sheet of peritoneum is referred to as the broad ligament of the uterus. It is composed of three segments assigned names according to the structures which they support: the mesovarium which suspends the ovaries, the mesosalpinx which suspends the salpinx (uterine tubes), and the mesometrium which suspends the uterus. In the male, that part of the genital fold which suspends the testes is termed the

mesorchium. The portion that suspends the ductus deferens is the mesoductus deferens (McGeady *et al.*, 2006).

2.9.6 EXTERNAL GENITALIA

During the undifferentiated phase of sexual development in the embryo, mesenchymal cells from the primitive streak migrate to the region around the cloacal membrane and form two elevated folds, the cloacal folds. These folds fuse ventrally to form the genital tubercle. Later in development, as a consequence of the formation of the urorectal septum, the cloacal membrane is subdivided into anal and urogenital membranes. The anal and urogenital membranes subsequently breakdown allowing communication between the rectum and the urogenital sinus to the exterior.

McGeady *et al.* (2006) reported that endodermal cells from the urogenital sinus proliferate and grow into the mesoderm of the genital tubercles forming the urethral plate. They further reported that cloacal folds are also divided into the anal folds dorsally and the urogenital folds ventrally. Proliferation of mesoderm lateral to each urogenital fold forms elevations which are termed the genital (labio-scrotal) swellings.

2.9.7 DIFFERENTIATION OF THE EXTERNAL GENITALIA

McGeady *et al.* (2006) revealed that in the female embryo, the vestibule arose from the caudal end of the urogenital sinus. The urogenital folds which did not fuse developed into the labia of the vulva. The genital tubercle which was located on the floor of the vestibule gave rise to the clitoris which is covered by the labia at the point where these structures met ventrally.

2.9.8 INFLUENCE OF HORMONES ON DEVELOPMENT OF GENITAL DUCTS AND EXTERNAL GENITALIA

McGeady *et al.* (2006) reported that in the female, under the influence of oestrogens, the paramesonephric ducts develop and differentiate into uterine tubes, uterus and the cranial portion of the vagina. During differentiation, oestrogens act on the external genitalia inducing the formation of

the clitoris, caudal portion of the vagina, vestibule and vulva. Although the sites of oestrogen secretion are not definitively established, it is proposed that both maternal and foetal tissues secrete this hormone.

2.9.9 SEXUAL DIFFERENTIATION, ASSOCIATED BRAIN FUNCTION AND SUBSEQUENT SEXUAL BEHAVIOUR AT PUBERTY

McGeady *et al.* (2006) reported that sex hormones influenced the development of regions of the brain associated with sexual behavior. In female mammals, the hypothalamic nuclei of the brain regulate the rhythmical secretion of gonadotrophic hormones at puberty, which ultimately results in the oestrous cycle. Although oestradiol is produced by foetal ovaries, it does not cross the blood-brain-barrier, because it is bound by α -foetoprotein. Accordingly, oestradiol produced by the female foetus does not inhibit the post-pubertal cyclical secretion of gonadotrophic hormones.

2.10.10 OVARIAN MIGRATION

In females, some intra-abdominal migration of the ovary occurs in particular species. In dogs and cats, McGeady *et al.* (2006) reported that the ovaries occupied a position in the sub-lumbar region caudal to the kidneys. They also reported that the ovaries of mares migrate to a location mid-way between the kidneys and the pelvic inlet. McGeady and his colleagues further reported that in cattle and pigs, migration of the ovaries were more pronounced and occupied a position at the pelvic inlet. The position of the gubernaculum between the ovary and paramesonephric duct forms the proper ligament of the ovary. The remainder of the gubernaculum formed the round ligament of the uterus which occupied a position in the mesometrium. They found out that in bitches, the round ligament, which is a prominent structure, enters the internal opening of the inguinal canal and may predispose to inguinal herniation.

2.11 GROSS STUDIES OF THE FEMALE REPRODUCTIVE SYSTEM

The female reproductive system consists of two ovaries (the essential reproductive glands in which the ova are produced); two uterine tubes (which convey the ova from the ovary to the uterine horns); the uterine horns (which convey the ova from the uterine tubes to the uterine body); the uterine body (in which the ovum develops); the cervix (which serves as the neck of the uterine body) and the vagina (a dilatable passage through which the male penis penetrates into the female reproductive system, and by which also the foetus is expelled from the uterine body).

2.10.1 OVARY

The ovaries of a bitch are small, elongated (oval) in outline and flattened. The average length is a little less than 2cm. Each ovary is commonly situated a short distance (1-2cm) caudal to or in contact with the caudal pole of the corresponding kidney. It thus lies opposite the 3rd or 4th lumbar vertebra, or about half-way between the last rib and the crest of the ilium (Sissons and Grossman, 1966). They explained further that the right ovary lies between the right part of the duodenum and the lateral abdominal wall. The left ovary being slightly caudal to its right counterpart, is laterally related to the spleen. According to them, each ovary is concealed in a peritoneal pouch (the bursa ovarii). The ovaries are suspended by mesovarium and ligament of the ovary. The surface of the ovary presents projections due to the projecting follicles from the interior.

Frandsen and Whitten (1981) asserted that in most species, the ovaries are somewhat almond-shaped and cream-coloured. However, in the mare, the ovaries had a bean shape due to the presence of a definite ovulation fossa (an indentation in the attached border of the ovary). They emphasized further that the ovaries of the sow usually appeared lobulated because of the presence of numerous follicles, corpora lutea, or both.

2.10.2 UTERINE TUBES

According to Frandson and Whitten (1981), the uterine tubes are paired convoluted cream-coloured tubes measuring about 5-8cm in the bitch, and conduct the ova from each ovary to the respective horn of the uterus. The uterine tubes are suspended by mesosalpinx and also serve as the usual site for fertilization of the ova by spermatozoa. The portion of this structure (uterine tube) adjacent to the ovary is expanded to form a funnel-like structure called the infundibulum. The fringe-like margin of the infundibulum is called the fimbria. This fimbria appears to take an active part in ovulation, at least to the extent of partially or completely enclosing the ovary, and directing the ovum into the abdominal opening of the uterine tube. In most subjects, the uterine tubes are concealed by fat deposits, with the terminal part being usually visible (Dyce *et al.*, 2002).

2.10.3 UTERINE HORNS

The uterine horns are long narrow structures measuring about 12-15cm long in medium-sized dogs (Sissons and Grossman, 1966). They found out that the uterine horns were of uniform diameter, cream-coloured, nearly straight and lay entirely within the abdomen. They also found out that the uterine horns diverged from the body of the uterus in a form of V-shape towards the kidneys.

2.10.4 UTERINE BODY

The uterine body (corpus) is largest in the mare, less extensive in the cow and sheep, and small in sow and bitches. Superficially, the uterine body of the cow appears relatively larger than it actually is, because the caudal parts of the horns are bound together by the intercornual ligament (Frandson and Whitten, 1981). Dyce *et al.* (2002) reported that the uterus lay mainly dorsal to the small intestine with a very short body of about 2-3cm long in bitches. They reaffirmed that the uterine body was near the pubic brim, but may be abdominal or pelvic in position, and was cream-coloured.

2.10.5 CERVIX

Sissons and Grossman (1966) reported that the cervix was the neck of the uterus with very short diameter of 1cm and had a thick muscular coat. They reported that dorsally, there was no line of demarcation between the uterus and the cervix, but the cervix uteri was much thicker than the vagina. Ventrally, they reported that the cervix formed a cylindrical projection which lay in the depression of the vaginal wall. In a separate work, Frandson and Whitten (1981) established that in reality, the cervix was a heavily smooth-muscled sphincter. It is tightly closed except during oestrus (heat) or at parturition with good vascularization to make it appear red in colour.

They reiterated that during oestrus, the cervix relaxed slightly, permitting spermatozoa to enter the uterus so as to swim up the uterine tubes for fertilization of the ova. During this time (oestrus) still, it is not uncommon for some mucus to be discharged from the cervix and expelled from the vulva. Frandson and Whitten (1981) affirmed that in ruminants, and to some extent swine, the inner surface of the cervix was arranged in a series of circular ridges (or rings), sometimes called annular folds. Though the cervix of the mare was relatively smooth, it projected quite a distance caudally into the vagina.

2.10.6 VAGINA

Dyce *et al.* (2002) reported that the vagina was a very long structure (12cm), extending horizontally through the pelvis before moving ventrally beyond the ischial arch to join the vestibule. Apart from the prominent dorsomedian fold that continued the cervix for a short distance, the interior of the undistended organ (vagina) is obstructed by the irregular folds into which the wall naturally falls. The vagina was observed to be cream-coloured. In an earlier report, Frandson and Whitten (1981) described the vagina as that portion of the birth canal that is located within the pelvis, between the cervix of the uterus cranially and the vulva caudally.

They stated further that the vagina also serves as a sheath for acceptance of the male penis during copulation, the act of breeding or service. They added that the fornix of the vagina was the angle or reflection formed by the projection of the cervix into the vagina. The fornix may form a complete circle around the cervix (as in the mare), or may be completely absent (as in the sow), in which the caudal end of the cervix is continuous with the vagina. In ruminants, only a dorsal fornix is well marked.

2.12 HISTOLOGY OF THE FEMALE REPRODUCTIVE SYSTEM

Among domestic animals, the female reproductive tract is composed internally of two ovaries, two uterine tubes, two uterine horns, uterus, cervix and vagina. Collectively, these components receive the male gametes (spermatozoa) and facilitate their transportation as well as produce and transport the female gametes (ova) for fertilization. After fertilization, the conceptus of mammals continues to develop within the tract until birth (Don, 2007).

2.11.1 OVARIES

Among domestic species, the ovary is oval to round and can vary in appearance and size according to the different points along the cyclic production of ova, otherwise known as the reproductive cycle. Ovaries are paired in mammals and involved in both endocrine activity and ova production (Don, 2007). The outer layer of the ovary is called the cortex while the inner layer is called the medulla.

2.11.1.1 CORTEX

Bacha and Bacha (2000) revealed that a simple squamous or cuboidal epithelium, germinal epithelium, often missing from histological preparations, covers the cortex of the ovary. Beneath, the epithelium is a layer of dense connective tissue, the tunica albuginea. They emphasized that a cortical stroma, containing ovarian follicles in various stages of development lay internal to the tunica albuginea. In bitches and queens, but ordinarily not in other domestic mammals, cords of

epithelioid cells called interstitial glands occur throughout the stroma. The epithelioid cells are derived from the theca interna of atretic pre-antral follicles. There are four basic follicles at various stages of development in the cortex as shown below:

1. PRIMORDIAL FOLLICLES

Bacha and Bacha (2000) explained that primordial follicles were the least developed and most numerous follicles of the ovary. They lie just beneath the tunica albuginea with each consisting of a primary oocyte surrounded by a layer of simple squamous follicular cells. In response to periodic hormonal stimulation, growth is initiated in some of the primordial follicles.

2. PRIMARY FOLLICLES

Don (2007) reported that on reaching sexual maturity, individual primordial follicle will continue to develop into primary follicle (pre-antral or uni-laminar). He said, the primary oocyte grows larger, resulting in multiple Golgi complexes, extensive ribosomal endoplasmic reticulum, polysomes and further proliferation of mitochondria. The oocyte would increase its size by two or more times. The follicular cells become distinctly cuboidal to low columnar, but still comprising a single layer.

3. SECONDARY FOLLICLES

Don (2007) continued in his explanation that with further development of the primary follicle, the encircling follicular cells divide to give rise to a stratified epithelial lining. This results in a multi-laminar follicle which is still pre-antral and called secondary follicle. The follicular cells at this stage are called granulosa cells. The zona pellucida, an amorphous material produced by the primary follicles, separates the granulosa cells from the primary oocyte.

4. TERTIARY FOLLICLES

As granulosa cells continue to proliferate and a large spherical follicle takes shape, the tertiary follicle is formed. Spaces between granulosa cells arise forming the liquor folliculi (a fluid

of plasma origin in part, consisting of glycosaminoglycans and steroid-associated protein). The spaces, which become clefts, soon fuse into a single large cavity, the antrum. Thus, the tertiary follicle is sometimes referred to as antral follicle or Graafian follicle (Don, 2007). He reiterated that as the primary oocyte is displaced off to one side by further development of the antrum, the surrounding layers of granulosa cells become the cumulus oophorus. The granulosa cells immediately adjacent to the oocyte increase in their height and become radially oriented around the oocyte, resulting in the formation of the corona radiata, which support the primary oocyte. Ordinarily, each mature tertiary follicle contains a single primary oocyte. Some animals like carnivores, sows and ewes (polytochous animals) may contain as many as six oocytes. Mature follicles vary widely in size. They may be 2mm in diameter in the bitch and queen, 15mm in cow, and as large as 70mm in the mare. Maximum size is reached just prior to ovulation.

Bacha and Bacha (2000) explained that further development of the tertiary follicle will produce mature follicles that can be ovulated for fertilization by the spermatozoa. They elaborated that following ovulation, a yellow pigment (lutein) is formed by the luteal cells of the ewes, nanny goats and sows. Luteal cells produce progesterone for maintaining pregnancy if conception was achieved. Regression of the corpus luteum occurs during late dioestrus, leaving a scar tissue called the corpus albicans. They pointed out that although many primordial follicles begin the process of growth and differentiation, few become mature. Majority undergo a degenerative regression called atresia. The oocyte and membrana granulosa degenerate first, then cells of the theca interna hypertrophy, as well as the zona pellucida becoming swollen. Eventually, the entire follicle is resorbed.

2.11.1.2 MEDULLA

The medulla consists of richly vascularized loose connective tissue which lies medial to the ovarian cortex in the central area of the ovary. In the mare, the medullary tissue is contrary to the norm unlike many mammals. Here (in the mare), the medulla is peripherally located while the

cortex is centrally located. Channels, lined by a cuboidal epithelium called rete ovarii, are conspicuous components of the medulla in carnivores and ruminants. Hilus cells (groups of epithelioid cells) may be found close to the rete ovarii in the region of the hilus in some animals (Bacha and Bacha, 2000).

2.11.2 UTERINE TUBES

The uterine tubes (also called Fallopian tubes or oviducts) are paired convoluted tubes that conduct the ova from each ovary to the respective horn of the uterus. It also serves as the usual site for fertilization of ova by spermatozoa (Frandsen and Whitten, 1981). There are three sub-divisions of the uterine tubes as follows:

2.11.2.1 INFUNDIBULUM

This is the funnel-like portion of the uterine tube that lies closest to the ovary. Its most cranial portion forms a radiating fringe of projections called the fimbriae that lie next to the ovary. The fimbriae function in enhancing the capture of the oocyte after leaving the ovary and directing it into the uterus. At the distal end of the infundibulum, the fimbriae coalesce into a single tubular structure (Don, 2007).

2.11.2.2 AMPULLA

Don (2007) pointed out that the ampulla is the distal end of the fimbriae. He said the ampulla forms the thin-walled portion of the uterine tube that contains numerous mucosal and sub-mucosal folds, and is the region where fertilization most likely occurs.

2.11.2.3 ISTHMUS

The isthmus is the caudal continuation of the ampulla which has a thicker muscular wall than that of the ampulla as well as fewer and lesser branched mucosal and sub-mucosal folds (Don, 2007). Furthermore, Don (2007) mentioned that histologically, uterine tubes of many species are internally lined by a simple columnar epithelium. In the cow and sow, the internal lining consists of

pseudo-stratified columnar epithelia. Typically, he said, the heights of the epithelial cells shorten as the uterine tubes progress towards the uterus. Whether simple columnar or pseudo-stratified columnar, these epithelial cells can possess kinocilia and microvilli. Don (2007) continued his explanation that ciliated cells are especially prevalent within the infundibulum, where they facilitate the movement of the ova into the uterine tubes.

This movement, he said, is likewise contributed to by the well-ciliated cranial portion of the ampulla. The cilia of the epithelia that line the uterine tube beat in unison unlike that seen in the respiratory epithelia, causing the ovum to be propelled to the uterus. During ovulation, the fimbriae are able to extend onto the ovary to help collect and transport the soon-to-be-released oocyte. This is due to contraction of the smooth muscle of the infundibulum as a result of engorgement and distension of the infundibular veins. In addition to ciliated columnar cells, non-ciliated columnar cells exist within the epithelial lining. Although having some microvilli, these cells are believed to be secretory and provide nutritional support for the gametes moving through this portion of the reproductive tract. These secretions help the maturation process of spermatozoa known as capacitation---only then are spermatozoa able to fertilize ova. As oocytes are released during ovulation, the non-ciliated columnar cells become metabolically more active and typically increase in their height, being sometimes referred to as peg cells.

2.11.3 UTERUS

This is the structure that receives the fertilized ovum or conceptus directed by the uterine tubes. The uterus is the site of implantation and subsequent foetal development. It is also the site of deposition of semen in the mare (Don, 2007). He went further and said that the uterus is composed of three regions: a pair of uterine horns as in most domestic species that guide conceptus to the principal structure (body of the uterus), which is the site of implantation and finally the neck of the uterus (cervix), where semen is deposited in the sow. Since the uterine horns and the body of the uterus are histologically similar, they will be discussed together.

2.11.4 UTERINE HORNS AND BODY

The uterine horns and body have a wall composed of the endometrium, myometrium and perimetrium.

2.11.4.1 ENDOMETRIUM

Don (2007), in his research, reported that the endometrium forms a tunica mucosa and sub-mucosa that surrounds the luminal cavity. Internally, it contains a simple columnar epithelium in canine, feline and equine species. Areas of pseudo-stratified columnar epithelia in porcine and ruminants also occur. Small patches or isolated areas of simple cuboidal epithelia also can exist. The height of the epithelia and degree of cytoplasmic development among the epithelial cells will vary cyclically according to the stage of oestrus.

He reported further that the epithelium is invaded with numerous glands surrounded by variably developed vasculature and connective tissue elements. This area is said to be the functional zone. Within the uterine horns of ruminants are regions of the lamina propria-submucosa called caruncles. Caruncles can appear as round (cow) or cup-shaped folds (ewe) that lack any tubular glands, but are well vascularized. These structures form the eventual sites of the maternal placenta, where the maternal tissues contact the extra-embryonic membranes.

2.11.4.2 MYOMETRIUM

The myometrium comprises the tunica muscularis of the uterine horns and body. Most of it is composed of a thick inner layer of smooth muscle, which is externally covered by a comparatively thin outer layer of smooth muscle. The inner layer is oriented in a circular direction and can contain the basal portions of the endometrial glands (Don, 2007). He explained further that deep within this layer, as it blends with the outer layer, the muscle fibres intermesh with a vascular layer sometimes referred to as stratum vasculare of the myometrium. During pregnancy, the myometrium undergoes pronounced development--- the smooth muscle cells hypertrophy up to 10 times their non-gravid length, due to the elevated oestrogen levels. The absence of oestrogens leads

to their atrophy. He said, in addition to smooth muscle hypertrophy, new smooth muscle cells are formed. The smooth muscle cells are arranged in bundles that are attached to one another by thin sheaths of connective tissue that consists of fibrocytes, histiocytes, mast cells, collagen and elastic fibres.

2.11.4.3 PERIMETRIUM

The perimetrium forms the tunica serosa for the uterine horns and the body of the uterus. It is composed mainly of loose connective tissue lined externally by a simple squamous epithelium. Small blood and lymph vessels as well as nerve fibres occur in this layer. In the gravid animal, smooth muscles occupy most of this layer (Don, 2007).

2.11.5 CERVIX

In the work done by Don (2007), he explained that the uterus ends caudally in the cervix (neck of the uterus) which extends into the vagina. He reiterated further that during pregnancy, this portion of the uterus becomes valve-like and seals off the uterus from the vagina, protecting the developing foetus from harmful agents. It generally lacks glands within the lamina propria-submucosa, but can possess a mucus-producing epithelium that is especially laden with goblet cells and other mucus-producing cells within ruminant species. The lamina propria-submucosa consists of dense irregular connective tissue. They form major folds that are longitudinally arranged in secondary and tertiary elevated folds, each lined by simple columnar epithelium. A well developed tunica muscularis and elastic fibres, extending from the uterine horns and body are present here (in the cervix). They help in returning the cervix to its normal or near-normal shape post partum.

2.11.6 VAGINA

This is a muscular tube that connects the uterus via the cervix to the vestibule. It receives the male copulatory organ for the deposition of spermatozoa. This could eventually lead to fertilization of the ova in the uterine tubes. For the most part, the epithelial lining of the mucosa consists of a non-keratinized stratified squamous epithelium. In most species, the mucosa and sub-mucosa form

low folds or ridges that lie along the longitudinal axis of the vagina (Don, 2007). He emphasized that mucous secretions found here (vagina) originally arose from the cervix and increase in amount during oestrus, concomitant with the increased thickness of the epithelium.

He also contributed that in the cow, mucus can be formed by clusters of goblet cells within the cranial portion of the vaginal mucosa. The lamina propria-submucosa is surrounded by tunica muscularis (composed of an inner circular layer and an outer longitudinal layer), a thin third layer of inner longitudinal layer in the bitch and sow, and lacks glands.

CHAPTER THREE

4.0 MATERIALS AND METHODS

3.1 ANIMAL SOURCE

Ten female grasscutters of 1-9 months of age were purchased from David Mark Farms, Otukpo, Benue State, Nigeria. Another five female grasscutters of 1-9 months of age were also purchased from Valentine Farms in Nsukka, Enugu State, Nigeria. Their ages were ascertained from the farm records. The grasscutters were provided vitamin preparations prior to their transport from the source of purchase. This was to boost their immunity and make them withstand the stress of the journey that could lead to morbidity and mortality (Adenkola *et al.*, 2009). They were then transported in metallic cages measuring 50cm long, 45cm high and 30cm wide to Veterinary Anatomy Animal House, University of Nigeria, Nsukka, the experimental site.

3.2 ANIMAL MANAGEMENT

On reaching the experimental site, the grasscutters were fed elephant grass (*Pennisetum purpureum*) and water in concrete drinkers measuring 10 inches long, 7 inches wide and 4 inches deep (Ismail, 2008). The grasscutters were fed at least thrice daily; morning and evening forage of elephant grass and an interjecting lunch of commercially prepared pelleted feed to provide concentrates.

The grasscutters were examined thoroughly for any abnormal behaviour and clinical signs of systemic infection for appropriate treatment. Faecal sample was collected per rectum and put in a sterile sample bottle. Blood sample was also collected from the cephalic vein of the forearm and put in a sterile bottle containing heparin. The faecal sample was then sent to the Veterinary Parasitology laboratory of University of Nigeria, Nsukka, for helminth ova evaluation; while the blood sample was sent to the Clinical laboratory of the Veterinary

Teaching Hospital, University of Nigeria, Nsukka for blood parasite evaluation immediately. They were apparently healthy as no helminth ova nor blood parasites were observed from the faecal and blood sample analyses.

The grasscutters were housed at an ambient temperature of 25-30°C and humidity of 60-80%. The grasscutters were also kept warm by provision of kerosene stove fireplace in their pen during cold weather (Eben, 2004).

3.3 GROSS STUDIES

The gross studies of the grasscutters were carried out in the Veterinary Anatomy Gross Laboratory, University of Nigeria, Nsukka. Before the grasscutters were sacrificed, they were examined again to ascertain that they were clinically healthy and were found apparently healthy. They were sedated for 5 minutes in an air-tight container measuring 40cm × 25cm into which 10mls of liquid chloroform on a hand towel had been put. Each grasscutter to be sedated was effectively manipulated from its cage and put into the air-tight container (Ismail, 2008).

Each grasscutter after being sedated was weighed using a weighing scale of a maximum sensitivity of 20 kg to obtain the live body weight in grams (g). The grasscutters were returned into the air-tight container and allowed to stay for 10 minutes for euthanasia to take place. The female reproductive system of the grasscutter was approached after euthanasia by placing the animal on dorsal recumbence. An incision was made from the xiphoid cartilage to the pelvic brim along the ventral mid-line. Care was taken not to incise too deep at a time.

The skin, abdominal muscles and peritoneum were incised sequentially. The first abdominal viscera that was encountered immediately after the peritoneum was the gastrointestinal tract. The gastrointestinal tract was carefully displaced laterally to expose the female reproductive system comprising the ovaries, uterine tubes, uterine horns, uterine body, cervix and vagina. Photographs of the female reproductive system were taken in-situ before the

system was harvested. After harvesting, the female reproductive system was again photographed. The weight of the entire female reproductive system was obtained in grams using Analytical Mettler Balance MCT 500 with a sensitivity of 0.00001 g. The length of the entire female reproductive system was also obtained in centimeters (cm) using meter rule, flexible thread and vernier calipers. The weight and length of each segment were also obtained in grams and centimeters, respectively, according to the method described by Codon and Casanave (2009). Each of the segments of the female reproductive system was then preserved in 10 % neutral buffered formalin and 10 % Bouin's solution {300 mls of saturated aqueous solution of picric acid, 100 mls of formalin and 20 mls of glacial acetic acid as described by Drury *et al.* (1976) } in a ratio of 1:1 for 24 hrs. After 24 hrs, the segments were transferred into 70 % Alcohol for preservation before histological processing (Ezeasor, 1984).

3.4 HISTOLOGICAL STUDIES

The histological studies were carried out in the Veterinary Anatomy Histotechnique Laboratory, University of Nigeria, Nsukka. Each segment of the female reproductive system was cut into blocks. Physiological saline was sprinkled on the segments as they were being cut into blocks to prevent dehydration. The tissue blocks were dehydrated in series of ascending ethanol concentrations (70 % - 100 %). They were placed in tissue cassettes, processed and embedded in paraffin. Sections of about 5-6 μ m thickness were made using Jung Rotary Microtome model 32339, mounted on glass slides, dewaxed in xylene and hydrated in series of descending ethanol concentrations (100 % - 70 %).

The sections were routinely stained with Haematoxylin and Eosin. The stained slides were examined under light microscope using Olympus Binocular Microscope and appropriate slides were selected. Photomicrographs of observed slides were taken using Moticam MC 2001 1.3 Megapixels USB 3.0 digital camera attached to the light microscope and captured onto a laptop.

3.5 STATISTICAL ANALYSIS

The weight and length of each segment of the female reproductive system among the different age groups were expressed as Means, plus or minus Standard Error of the Means (Mean \pm SEM). The data were analyzed using one-way analysis of variance (ANOVA). Variant means were separated using Duncan's Multiple Range Test. Significance was accepted at $p < 0.05$. Statistical Package for Social Sciences (SPSS) Version 15 was used to analyze the data.

CHAPTER FOUR

4.0 RESULTS

4.1 GROSS ANATOMY AND MORPHOMETRY

4.1.2 OVARIES

4.1.1.1 LEFT OVARY

In all the age groups studied (1-3, 4-6 and 7-9 months), the left ovaries of the grasscutters were located caudo-ventral to the left kidneys between the 3rd and 4th lumbar vertebrae in the upper left quadrant of the abdominal cavity. The mesovarium separated them from the left kidneys. They appeared as cream-coloured structures in all the age groups and formed the most cranial part of the female reproductive system. The left ovary was spindle-shaped at 1-3 months, oval at 4-6 months and round at 7-9 months of age (Plates 1, 2 and 3). The Mean Organo-Somatic Indices (MOSI) of the left ovaries of the grasscutters at 1-3 months (0.51 ± 0.08) were significantly less ($p < 0.05$) than those at 4-6 months (1.09 ± 0.11), and these were also significantly less ($p < 0.05$) than those at 7-9 months (1.83 ± 0.14) of age (Table 1). The Mean Relative Lengths (MRL) of the left ovaries at 1-3 months (1.74 ± 0.04) were significantly less ($p < 0.05$) than those at 4-6 months (2.02 ± 0.05), and these were also significantly less ($p < 0.05$) than those at 7-9 months (2.38 ± 0.08) of age (Table 2).

4.1.1.2 RIGHT OVARY

In all the age groups studied (1-3, 4-6 and 7-9 months), the right ovaries of the grasscutters were located deeper to the right kidneys between the 2nd and 3rd lumbar vertebrae in the upper right quadrant of the abdominal cavity. The mesovarium separated them from the right kidneys in all the age groups. The right ovaries also appeared cream-coloured in all the age

groups studied, and constituted the most cranial part of the female reproductive system on the right side of the animal. The right ovary, like its left counterpart, was spindle-shaped at 1-3 months, but oval, both at 4-6 months and 7-9 months (Plates 1, 2 and 3). Moreover, the MOSI of the right ovaries of the grasscutter at 1-3 months (0.25 ± 0.06) were significantly less ($p < 0.05$) than those at 4-6 months (0.81 ± 0.10), and these were also significantly less ($p < 0.05$) than those at 7-9 months (1.35 ± 0.07) of age (Table 1). The MRL of the right ovaries at 1-3 months (1.45 ± 0.01) were not significantly different ($p > 0.05$) from those at 4-6 months (1.79 ± 0.07), but these were significantly less ($p < 0.05$) than those at 7-9 months (2.13 ± 0.03) of age (Table 2).

4.1.2 UTERINE TUBES

4.1.2.1 LEFT UTERINE TUBE

In all the age groups studied (1-3, 4-6 and 7-9 months), the left uterine tubes of the grasscutters appeared as long cream-coloured and tortuous structures embedded within the mesosalpinx, being tightly wrapped around both the caudal poles of the left ovaries and the cranial one-third of the left uterine horns (Plates 1, 2 and 3). Thus, the caudal poles of the left ovary and the cranial one-third of the left uterine horn appeared as segments of a single tube. The MOSI of the left uterine tubes of the grasscutters at 1-3 months (2.32 ± 0.06) were found to be significantly less ($p < 0.05$) than those at 4-6 months (3.26 ± 0.09), and these were also significantly less ($p < 0.05$) than those at 7-9 months (3.95 ± 0.12) of age (Table 1). Correspondingly, the MRL at 1-3 months (11.24 ± 0.08) were significantly less ($p < 0.05$) than those at 4-6 months (13.37 ± 0.12), and these were as well, significantly less ($p < 0.05$) than those at 7-9 months (16.58 ± 0.15) of age (Table 2).

4.1.2.2 RIGHT UTERINE TUBE

In the same pattern, in all the age groups studied (1-3, 4-6 and 7-9 months), the right uterine tubes of the grasscutters appeared as long cream-coloured and tortuous structures embedded within the mesosalpinx, being tightly wrapped around both the caudal poles of the right ovaries and the cranial one-third of the right uterine horns (Plates 1, 2 and 3). Thus, the caudal poles of the right ovary and the cranial one-third of the right uterine horn appeared as segments of a single tube. The MOSI of the right uterine tubes of the grasscutters at 1-3 months (2.17 ± 0.04) were significantly less ($p < 0.05$) than those at 4-6 months (3.08 ± 0.08), and these were also significantly less ($p < 0.05$) than those at 7-9 months (3.77 ± 0.10) of age (Table 1). The MRL at 1-3 months (10.77 ± 0.05) were not significantly different ($p > 0.05$) from those at 4-6 months (11.31 ± 0.11), but these were significantly less ($p < 0.05$) than those at 7-9 months (14.98 ± 0.14) of age (Table 2).

4.1.3 UTERINE HORNS

4.1.3.1 LEFT UTERINE HORN

In all the age groups studied (1-3, 4-6 and 7-9 months), the left uterine horns of the grasscutters appeared cream-coloured and extended from the left uterine tubes to the left part of the uterine bodies. They lay on the left side of the abdominal cavity in all the three age groups (Plates 1, 2 and 3). The MOSI of the left uterine horns of the grasscutters at 1-3 months (2.41 ± 0.02) were significantly less ($p < 0.05$) than those at 4-6 months (3.44 ± 0.04), and these were significantly less ($p < 0.05$) than those at 7-9 months (3.98 ± 0.05) of age (Table 1). In a similar fashion, the MRL showed that the left uterine horns of the grasscutters at 1-3 months (5.50 ± 0.03) were significantly less ($p < 0.05$) than those at 4-6 months (5.98 ± 0.06), and these were also significantly less ($p < 0.05$) than those at 7-9 months (6.94 ± 0.10) of age (Table 2).

4.1.3.2 RIGHT UTERINE HORN

In all the age groups studied (1-3, 4-6 and 7-9 months), the right uterine horns of the grasscutters appeared cream-coloured and extended from the right uterine tubes to the right part of the uterine bodies. They lay on the right side of the abdominal cavity in all the three age groups (Plates 1, 2 and 3). The MOSI of the right uterine horns of the grasscutters at 1-3 months (2.20 ± 0.06) were significantly less ($p < 0.05$) than those at 4-6 months (3.09 ± 0.04), and these were also significantly less ($p < 0.05$) than those at 7-9 months (3.79 ± 0.08) of age (Table 1). The MRL of the right uterine horns of the grasscutters at 1-3 months (5.34 ± 0.02) were not significantly different ($p > 0.05$) from those at 4-6 months (5.69 ± 0.08), but these were significantly less ($p < 0.05$) than those at 7-9 months (6.79 ± 0.09) of age (Table 2).

4.1.4 UTERUS DUPLEX

Studies on the grasscutter at 1-3, 4-6 and 7-9 months of age, showed that the uterine body was cream-coloured and formed the meeting points of the left and right halves of the female reproductive system at the mid-line of the abdominal cavity. It was situated superficial to the ileum and exhibited a Y-shaped appearance where it received the left and right uterine horns cranially and the cervix caudally. The uterine body in all the three age groups received the left and right uterine horns separately and maintained a partition between them. This presented the appearance of a double uterus, and thus the nomenclature, uterus duplex (Plates 1, 2 and 3).

The uterus duplex was firm on palpation. As shown in Table 1, the MOSI of the uterine body of the grasscutter at 1-3 months (2.48 ± 0.07) were significantly less ($p < 0.05$) than those at 4-6 months (3.97 ± 0.04), and these were significantly less ($p < 0.05$) than those at 7-9 months (4.53 ± 0.08) of age. As can also be seen from Table 2, the MRL at 1-3 months (4.89 ± 0.03) were significantly less ($p < 0.05$) than those at 4-6 months (5.55 ± 0.07), and these were also significantly less ($p < 0.05$) than those at 7-9 months (6.62 ± 0.08) of age.

4.1.7 CERVIX

In all the three age groups studied (1-3, 4-6 and 7-9 months), the cervix of the grasscutter was observed to be cream-coloured and with firm consistency like a rubber stopper on palpation. The cervix was situated between the uterine body cranially and the vagina caudally. It lay deep to the urinary bladder within the lower abdominal cavity (Plates 4, 5 and 6). The MOSI of the cervix of the grasscutters at 1-3 months (4.07 ± 0.09) were significantly less ($p < 0.05$) than those at 4-6 months (4.52 ± 0.08), and these were also significantly less ($p < 0.05$) than those at 7-9 months (4.96 ± 0.06) of age (Table 1). In addition, it was noted in Table 2 that the MRL of the cervix of the grasscutter at 1-3 months (2.78 ± 0.02) were not significantly different ($p > 0.05$) from those at 4-6 months (2.98 ± 0.08), but these were significantly less ($p < 0.05$) than those at 7-9 months (4.24 ± 0.11) of age.

4.1.8 VAGINA

In all the three age groups studied (1-3, 4-6 and 7-9 months), the vagina of the grasscutter was seen as a cream-coloured structure and situated caudal to the cervix. It was softer than the cervix on palpation. The vagina was obscured *in-situ* by the urinary bladder within the pelvic cavity. The vagina formed the most caudal segment of the female reproductive system of the grasscutter in all the age groups (Plates 4, 5 and 6). The MOSI of the vagina of the grasscutter at 1-3 months (3.36 ± 0.01) were significantly less ($p < 0.05$) than those at 4-6 months (3.63 ± 0.03), and these were also significantly less ($p < 0.05$) than those at 7-9 months (3.99 ± 0.07) of age (Table 1). As shown in Table 2, the MRL of the vagina of the grasscutter at 1-3 months (3.94 ± 0.03) were not significantly different ($p > 0.05$) from those at 4-6 months (4.09 ± 0.09), but were significantly less ($p < 0.05$) than those at 7-9 months (4.79 ± 0.12) of age.

4.2 HISTOMORPHOLOGY

4.2.1 OVARY

A transverse section of the ovarian cortex of the grasscutter at 1-3 months of age showed that the external surface of the organ was lined by tunica serosa composed of loose connective tissue and flattened epithelial cells. Underlying the tunica serosa was a layer of dense connective tissue called tunica albuginea, containing some collagen and elastic fibres. Beneath the tunica albuginea was the cortical parenchyma which showed presence of small oval primordial follicles.

The primordial follicles were composed of flattened follicular cells surrounding an oval primary oocyte. They were observed lying side by side in pairs (Plate 7). A larger follicle than the primordial follicle, called unilaminar primary follicle, was also observed in the ovarian cortex having a single layer of cuboidal cells surrounding an oval primary oocyte (Plate 7). Some primordial follicles were also observed to be arranged in a nest of cells (Plate 8).

Similarly, at 4-6 months of age, a transverse section of the ovarian cortex showed that the tunica serosa was composed of loose connective tissue and squamous epithelial cells lining the external surface of the ovary. Beneath the tunica serosa was the tunica albuginea composed of dense connective tissue with many collagen and elastic fibres. Underneath the tunica albuginea was the cortex composed of ovarian follicles at different stages of development. In addition to the primordial follicles and unilaminar primary follicles that were also observed at this age, a multilaminar primary follicle (a spherical and much larger follicle than the previous two) was noticed. It had many layers of cuboidal cells arranged around the periphery of the cells surrounding the primary oocyte (Plate 9).

In like manner, a transverse section of the ovarian cortex at 7-9 months of age showed

that the tunica serosa was composed of loose connective tissue and squamous epithelial cells lining the external surface of the organ. Beneath the tunica serosa was the tunica albuginea which was composed of dense connective tissue and numerous collagen as well as numerous elastic fibres. Underneath the tunica albuginea was the ovarian cortex that presented follicles at various stages of development (Plate 10).

Two spherical secondary follicles were particularly observed in the cortex. One of the secondary follicles had several layers of granulosa cells surrounding the centrally located oocyte without an antrum, while the second secondary follicle had several layers of granulosa cells rearranged to form the antrum (Plate 10). In addition, a higher magnification of the secondary follicle at 7-9 months of age revealed the presence of an oval nucleus within the centrally located oocyte that was surrounded by a circular eosinophilic material called the zona pellucida. The cuboidal granulosa cells were rearranged into inner concentric layers of cells called theca interna, and outer concentric layers called theca externa. A pear-shaped cavity (antrum) appeared within the granulosa cells containing follicular fluid (Plate 11).

Still at 7-9 months of age, two Graafian (tertiary) follicles were observed. The first showed granulosa cells that were further rearranged into layers of radially appearing cuboidal follicular cells dorsal to the centrally located oocyte called corona radiata. The granulosa cells ventral to the oocyte formed a germinal hillock called cumulus oophorus. At this point, the pear-shaped antrum had coalesced further to form a semi-lunar cavity dorsal to the corona radiata. The second Graafian follicle was observed to have a peripherally located oocyte (Plate 12). Petechiation which signified development of the corpus haemorrhagicum, was observed on the remnant of the granulosa cells and within the antral follicle of another Graafian follicle that had a peripherally located oocyte (Plate 13). A yellow body called corpus luteum was also observed in the ovarian cortex and showed the presence of a localised blood clot within the wall of the follicle without an apparent presence of an oocyte (Plate 14).

An indentation was also observed on the external surface of the corpus luteum (Plate 14). It was made up of the remnants of the granulosa cells, remnants of the cumulus oophorus and antral fluid, and lacking an oocyte (Plate 14). Further examination of the ovarian cortex at 7-9 months of age, revealed the presence of developing follicles, degenerating follicles as well as atretic follicles (Plate 15).

4.2.3 TUBULAR ORGANS

The tubular organs of the female reproductive system of the grasscutter were the uterine tubes, uterine horns, uterine body, cervix and the vagina. The walls of these tubular organs were composed of several tunics. Typically, in sequence from the lumen to the exterior, were 4 tunics (layers): the tunica mucosa lined by epithelium, lamina propria-submucosa, tunica muscularis and tunica serosa and/or tunica adventitia. Some of the tubular organs had 3 tunics. The lamina muscularis mucosae that separates the lamina propria from the submucosa was conspicuously absent in the female reproductive system of the grasscutter. So the lamina propria did not have a distinct boundary from the submucosa.

4.2.3.1 UTERINE TUBES

A transverse section of the uterine tubes at 1-3 months of age revealed that the uterine tubes exhibited the characteristics of a typical tubular organ as it had 4 layers in its wall (Plate 16). The epithelium was composed of pseudostratified columnar epithelium lining the tunica mucosa. The tunica mucosa had finger-like apical cilia and mucosal folds that were so strongly convoluted that an appearance of a labyrinth was presented. The lamina propria-submucosa had narrow papillae projecting from it into the mucosal folds. The lamina propria-submucosa had loose connective tissue and folds, but no glands were observed in it. The tunica muscularis was made up of inner circular and outer longitudinal smooth muscles. The tunica serosa was composed of loose connective tissue externally lined by simple squamous epithelium. A thick

suspensory ligament was also observed (Plate 17).

Likewise, a transverse section of the uterine tubes at 4-6 months of age, revealed a typical tubular organ, possessing 4 layers in its wall. The microscopic architecture observed was similar to that observed at 1-3 months of age (Plates 18, 19 and 20). Furthermore, a transverse section of the uterine tubes at 7-9 months of age also reflected a typical tubular organ as it possessed 4 layers in its wall (Plates 21, 22 and 23). The histomorphology of the structures was similar to those at 1-3 and 4-6 months of age, except that the apical cilia appeared longer at 7-9 months of age than those of both 1-3 and 4-6 months of age.

4.2.3.2 UTERINE HORNS

The uterine horns had a slight modification from the uterine tubes in the number of layers in its wall and the terminologies used in describing them. The tunica mucosa and tunica submucosa were together referred to as the endometrium, tunica muscularis as myometrium, while the tunica serosa was referred to as perimetrium. Therefore, the wall of the uterine horns was composed of 3 major layers (endometrium, myometrium and perimetrium) as opposed to the 4 of a typical tubular organ.

A transverse section of the uterine horns at 1-3 months of age showed that the endometrium was lined by simple columnar epithelium (Plate 24). Within the endometrium, were acini of endometrial glands (Plate 25). The myometrium was composed of inner circular and outer longitudinal smooth muscles interwoven with a vascular bed called stratum vasculare. The perimetrium was composed of loose connective tissue lined by simple squamous epithelium with corrugated appearance (Plate 26).

A transverse section of the uterine horns at 4-6 months of age revealed a similar architecture with that at 1-3 months. Simple columnar epithelial cells lined the endometrium

(Plate 27). There was also presence of endometrial crypts at 4-6 months of age (Plate 28). Similarly, a transverse section of the uterine horns at 7-9 months of age revealed that the endometrium was lined by pseudostratified columnar epithelium (Plate 29). The endometrium was studded with numerous connective tissue cells and acini of endometrial glands. The myometrium had layers of inner circular and outer longitudinal smooth muscles that were intermeshed with blood vessels within the stratum vasculare. The perimetrium was composed of loose connective tissue lined by simple squamous epithelium with corrugated appearance (Plate 30).

4.2.3.3 UTERUS DUPLEX

The uterus duplex was also composed of 3 layers in its wall like that of the uterine horns. The layers were the endometrium, myometrium and perimetrium. The transverse section of the uterus duplex at 1-3 months of age revealed that the endometrium was lined by simple columnar epithelium (Plate 31). No apical cilia were observed on the endometrium. The endometrium had scanty acini of endometrial glands as well as collagen fibres, elastic fibres and blood vessels forming a loose connective tissue. The myometrium was composed of inner circular and outer longitudinal smooth muscles intermeshed with the stratum vasculare. The perimetrium had loose connective tissue lined by simple squamous cells and appeared corrugated (Plate 32).

Similarly, a transverse section of the uterine body at 4-6 months of age showed that the endometrium was lined by simple columnar epithelium (Plate 33). The endometrium was thrown into endometrial folds. Collagen fibres, elastic fibres, endometrial glands and blood vessels made up a dense connective tissue which were observed within the endometrium. Secretory products from the acini of endometrial glands were observed on the epithelial surface within the endometrial canal (Plate 33). The myometrium was composed of inner circular and outer longitudinal smooth muscles intermeshed with the stratum vasculare (Plates 33 and 34).

The perimetrium was composed of loose connective tissue lined by simple squamous epithelium. Corrugated appearance characterized the perimetrium (Plate 34).

Similarly, a transverse section of the uterus duplex at 7-9 months of age showed that the endometrium was lined by simple columnar epithelium (Plate 35). The endometrium was thrown into endometrial folds. Acini of endometrial glands with cuboidal cells were observed in the endometrium. Numerous collagen fibres, elastic fibres, blood vessels and connective tissue cells made up the dense connective tissue which was also observed within the endometrium. Secretory products from the acini of endometrial glands were observed on the surface of the epithelium (Plate 36). The myometrium was composed of inner circular and outer longitudinal smooth muscles intermeshed with stratum vasculare (Plate 37). The perimetrium was composed of loose connective tissue with simple squamous epithelium lining the external surface (Plate 37). The perimetrium presented a corrugated appearance.

4.2.3.4 CERVIX

At 1-3 months of age, the cervix possessed the 4 typical layers of tubular organs in its wall. The tunica mucosa (endocervical mucosa) was lined by simple columnar secretory epithelium (Plate 38). The endocervical mucosa had invaginations called endocervical crypts which were also lined by simple columnar epithelium (Plate 38). The endocervical mucosa exhibited endocervical folds on the surface of the mucosa (Plate 39). Beneath the endocervical mucosa was a thick layer of dense irregular connective tissue, the lamina propria-submucosa, which was made up of collagen fibres, elastic fibres, blood vessels, connective tissue cells and scanty acini of cervical glands. The tunica muscularis was composed of patches of smooth muscles as well as elastic fibres (Plate 39). The tunica serosa had loose connective tissue and some smooth muscles which were externally lined by simple squamous epithelium (Plate 39).

Furthermore, microscopic architecture of the cervix at 4-6 months of age (Plate 40) was

similar to that at 1-3 months of age, except that the cervical crypts were deeper at 4-6 months than at 1-3 months of age (Plate 40). The lamina propria-submucosa visually possessed more acini of cervical glands at 4-6 months than at 1-3 months of age. The acini of cervical glands were made up of cuboidal cells. The tunica muscularis also possessed thicker layers of inner circular and outer longitudinal smooth muscles at 4-6 months than at 1-3 months of age (Plates 40 and 41). Mucosal cells were present on the external lining (Plate 42).

Similarly, the cervix of the grasscutter at 7-9 months of age (Plate 43) showed that the lumen (endocervical canal) was filled with numerous simple columnar and polyhedral secretory epithelial cells lining the endocervical mucosa. The endocervical mucosa exhibited cervical crypts and endocervical folds (Plate 44). The simple columnar secretory epithelium was also observed lining the cervical crypts. The lamina propria-submucosa was made up of very thick dense irregular connective tissue composed of numerous collagen fibres, elastic fibres and numerous acini of cervical glands (Plate 44). The tunica muscularis was composed of very thick layers of inner circular and outer longitudinal smooth muscles (Plates 44 and 45). The external lining had numerous invaginations of mucosal cells (Plate 45).

4.2.3.5 VAGINA

Examination of the vagina of the grasscutter at 1-3 months of age showed that the 4 layers typical of tubular organs were present in its wall. The tunica mucosa was lined by non-keratinized stratified squamous epithelium (Plate 46). The tunica mucosa exhibited vaginal folds. Beneath the tunica mucosa was a dense connective tissue, the lamina propria-submucosa, which was made up of collagen fibres and elastic fibres. Narrow papillae projected into the mucosal folds from the lamina propria-submucosa. The tunica muscularis was composed of inner circular smooth muscles that were separated from the outer longitudinal smooth muscles by numerous blood vessels (*area vasculosa*). The tunica serosa was composed of loose

connective tissue that was externally lined by simple squamous epithelium (Plate 47).

Similarly, examination of the transverse section of the vagina at 4-6 months of age (Plate 48) showed almost the same histomorphology as that at 1-3 months of age, except that the tunica muscularis exhibited a thick blend of the inner circular and outer longitudinal smooth muscles on the cranial end of the vagina. Thus, the two layers of smooth muscles were indistinguishable at that point. At the caudal end, the tunica muscularis exhibited distinct inner circular and outer longitudinal smooth muscles (Plate 49). The last tunic in the vaginal wall was composed of a mixture of 2 tunics. The first was tunica serosa at the cranial end and composed of loose connective tissue lined externally by simple squamous epithelium. The second was tunica adventitia at the caudal end which was composed mainly of areolar connective tissue without a definite epithelial lining externally (Plates 49).

Likewise, examination of the longitudinal section of the vagina at 7-9 months of age showed that the vaginal wall was composed of 4 typical layers. The tunica mucosa was lined by non-keratinized stratified squamous epithelium (Plate 50). The tunica mucosa exhibited deep invaginations called vaginal crypt lined also by non-keratinized stratified squamous epithelium (Plate 50). Further examination of the transverse section of the vagina at 7-9 months showed a very conspicuous pear-shaped intensely eosinophilic structure called vaginal plug within the vaginal canal (Plate 51). A dense connective tissue layer, lamina propria-submucosa, was composed of collagen and elastic fibres as well as blood vessels (Plate 52). The tunica muscularis was composed of thick inner circular and outer longitudinal smooth muscles. The tunica serosa was composed of loose connective tissue and an external surface lined by simple squamous epithelium (Plate 52).

TABLE 1: MEAN ORGANO-SOMATIC INDICES \pm SEM OF EACH ORGAN**COMPRISING THE FEMALE REPRODUCTIVE SYSTEM OF THE****GRASSCUTTER**

ORGANS COMPRISING THE FEMALE REPRODUCTIVE SYSTEM	AGES (MONTHS)		
	GROUP A (1-3 MONTHS)	GROUP B (4-6 MONTHS)	GROUP C (7-9 MONTHS)
Left Ovary	0.51 \pm 0.08 ^a	1.09 \pm 0.11 ^b	1.83 \pm 0.14 ^c
Right Ovary	0.25 \pm 0.06 ^a	0.81 \pm 0.10 ^b	1.35 \pm 0.07 ^c
Left Uterine Tube	2.32 \pm 0.06 ^a	3.26 \pm 0.09 ^b	3.95 \pm 0.12 ^c
Right Uterine Tube	2.17 \pm 0.04 ^a	3.08 \pm 0.08 ^b	3.77 \pm 0.10 ^c
Left Uterine Horn	2.41 \pm 0.02 ^a	3.44 \pm 0.04 ^b	3.98 \pm 0.05 ^c
Right Uterine Horn	2.20 \pm 0.06 ^a	3.09 \pm 0.04 ^b	3.79 \pm 0.08 ^c
Uterus Duplex	2.48 \pm 0.07 ^a	3.97 \pm 0.04 ^b	4.53 \pm 0.08 ^c
Cervix	4.07 \pm 0.09 ^a	4.52 \pm 0.08 ^b	4.96 \pm 0.06 ^c
Vagina	3.36 \pm 0.01 ^a	3.63 \pm 0.03 ^b	3.99 \pm 0.07 ^c

Different Superscripts ^{abc} in a Row Indicate Significant Difference at **p < 0.05**

TABLE 2: MEAN LENGTH \pm SEM OF EACH ORGAN COMPRISING THE FEMALE REPRODUCTIVE SYSTEM OF THE GRASSCUTTER RELATIVE TO THEIR CROWN-RUMP LENGTH

ORGANS COMPRISING THE FEMALE REPRODUCTIVE SYSTEM	AGES (MONTHS)		
	GROUP A (1-3 MONTHS)	GROUP B (4-6 MONTHS)	GROUP C (7-9 MONTHS)
Left Ovary	1.74 \pm 0.04 ^a	2.02 \pm 0.05 ^b	2.38 \pm 0.08 ^c
Right Ovary	1.45 \pm 0.01 ^a	1.79 \pm 0.07 ^a	2.13 \pm 0.03 ^b
Left Uterine Tube	11.24 \pm 0.08 ^a	13.37 \pm 0.12 ^b	16.58 \pm 0.15 ^c
Right Uterine Tube	10.77 \pm 0.05 ^a	11.31 \pm 0.11 ^a	14.98 \pm 0.14 ^b
Left Uterine Horn	5.50 \pm 0.03 ^a	5.98 \pm 0.06 ^b	6.94 \pm 0.10 ^c
Right Uterine Horn	5.34 \pm 0.02 ^a	5.69 \pm 0.08 ^a	6.79 \pm 0.09 ^b
Uterus Duplex	4.89 \pm 0.03 ^a	5.55 \pm 0.07 ^b	6.62 \pm 0.08 ^c
Cervix	2.78 \pm 0.02 ^a	2.98 \pm 0.08 ^a	4.24 \pm 0.11 ^b
Vagina	3.94 \pm 0.03 ^a	4.09 \pm 0.09 ^a	4.79 \pm 0.12 ^b

Different Superscripts ^{abc} in a Row Indicate Significant Difference at $p < 0.05$

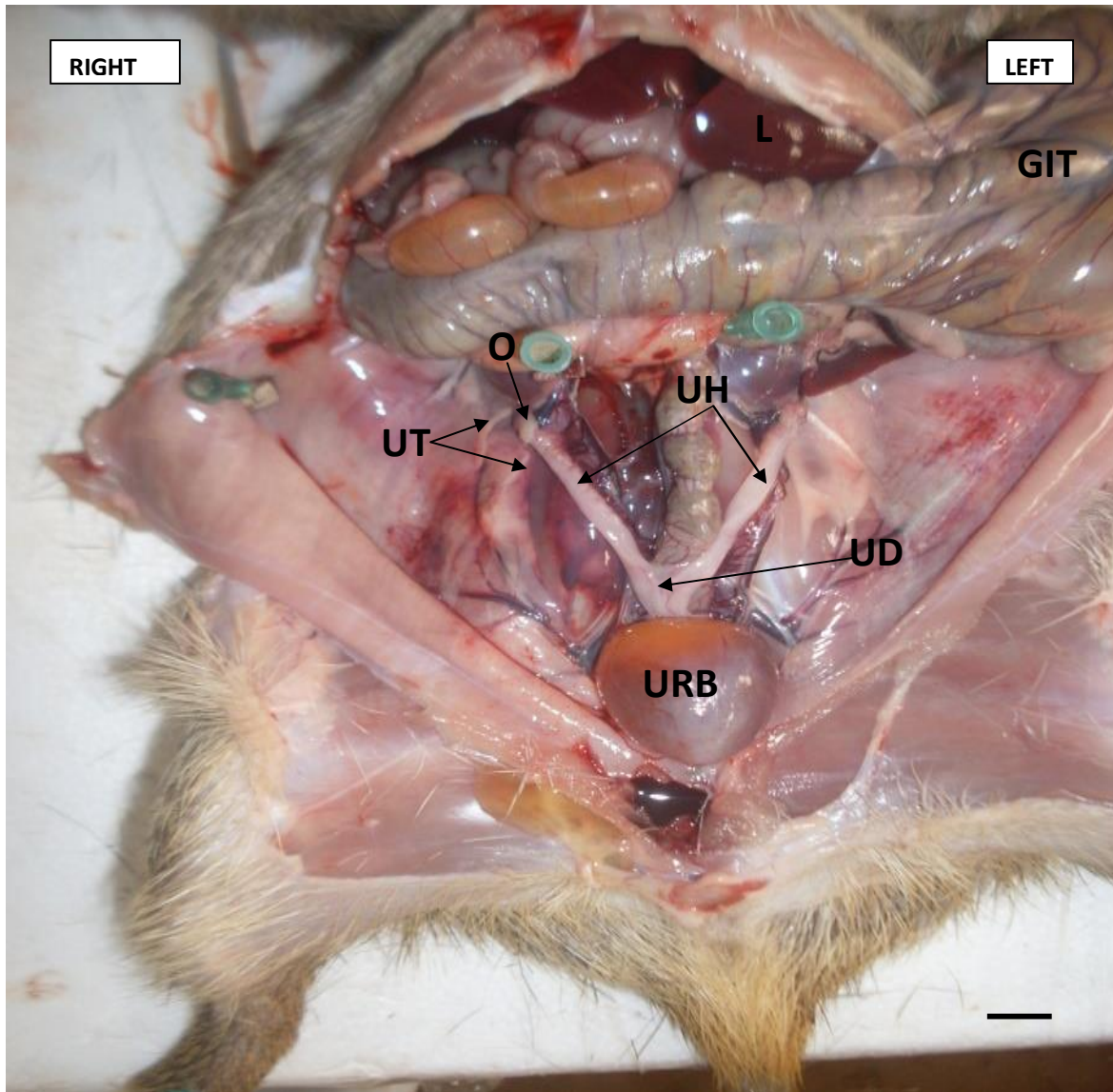


Plate 1: Abdominal Cavity of the Grasscutter at 1-3 Months showing the Ovary (O); Uterine Tube (UT) teased away from the Ovary and Uterine Horns (UH) where they had wrapped around; Uterus Duplex (UD); Liver (L); Urinary Bladder (URB) *in-situ*; Note that the Gastrointestinal Tract (GIT) was laterally displaced to expose the Female Reproductive System. Scale Bar =1cm.

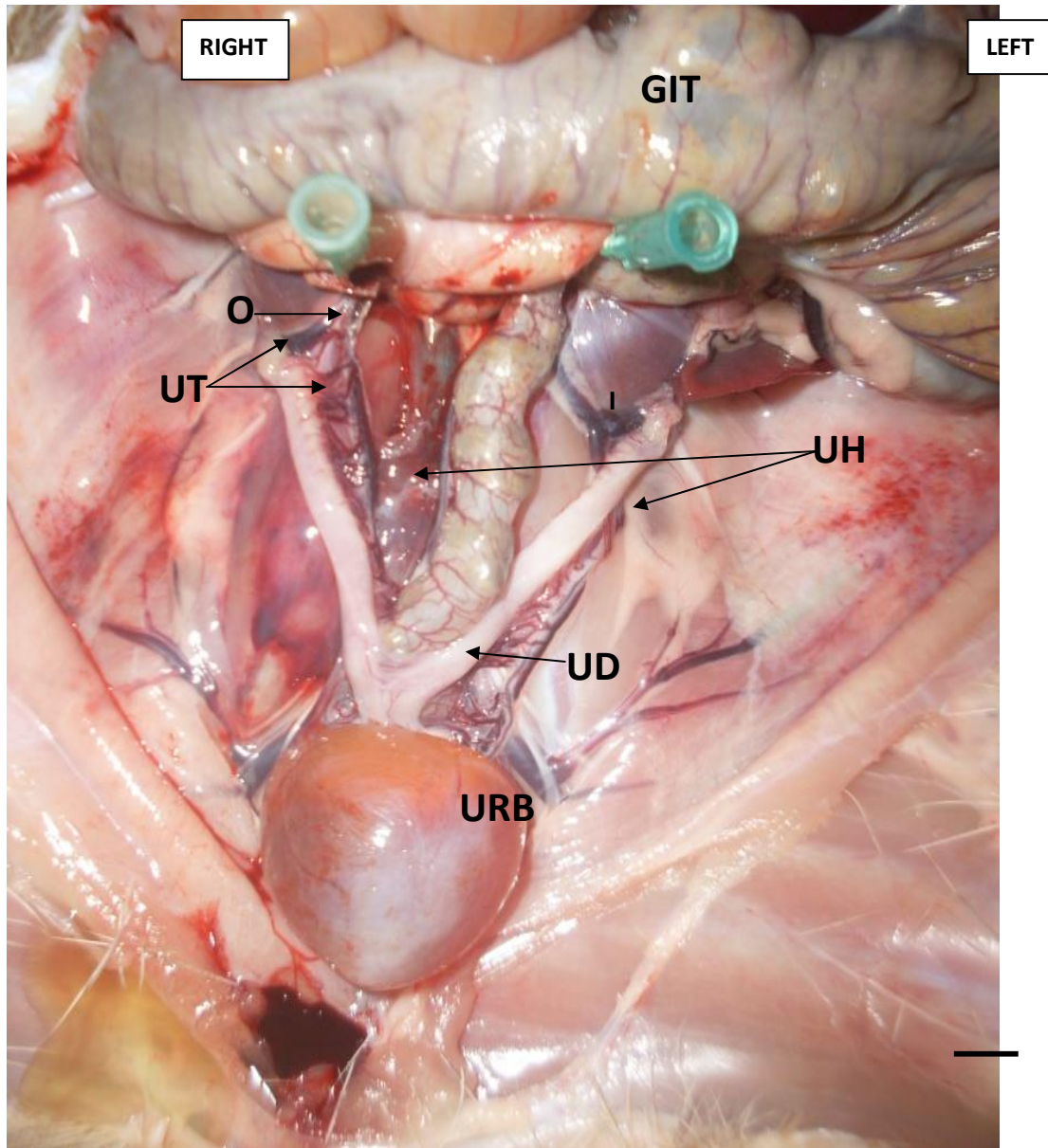


Plate 2: Abdominal Cavity of the Grasscutter at 4-6 Months showing the Ovary (O); Uterine Tube (UT) teased away from the Ovary and Uterine Horn (UH) where they had wrapped around; Uterus Duplex (UD); Ileum (I); Urinary Bladder (URB) *in-situ*; Note that the Gastrointestinal Tract (GIT) was laterally displaced to expose the Female Reproductive System. Scale Bar =1cm.

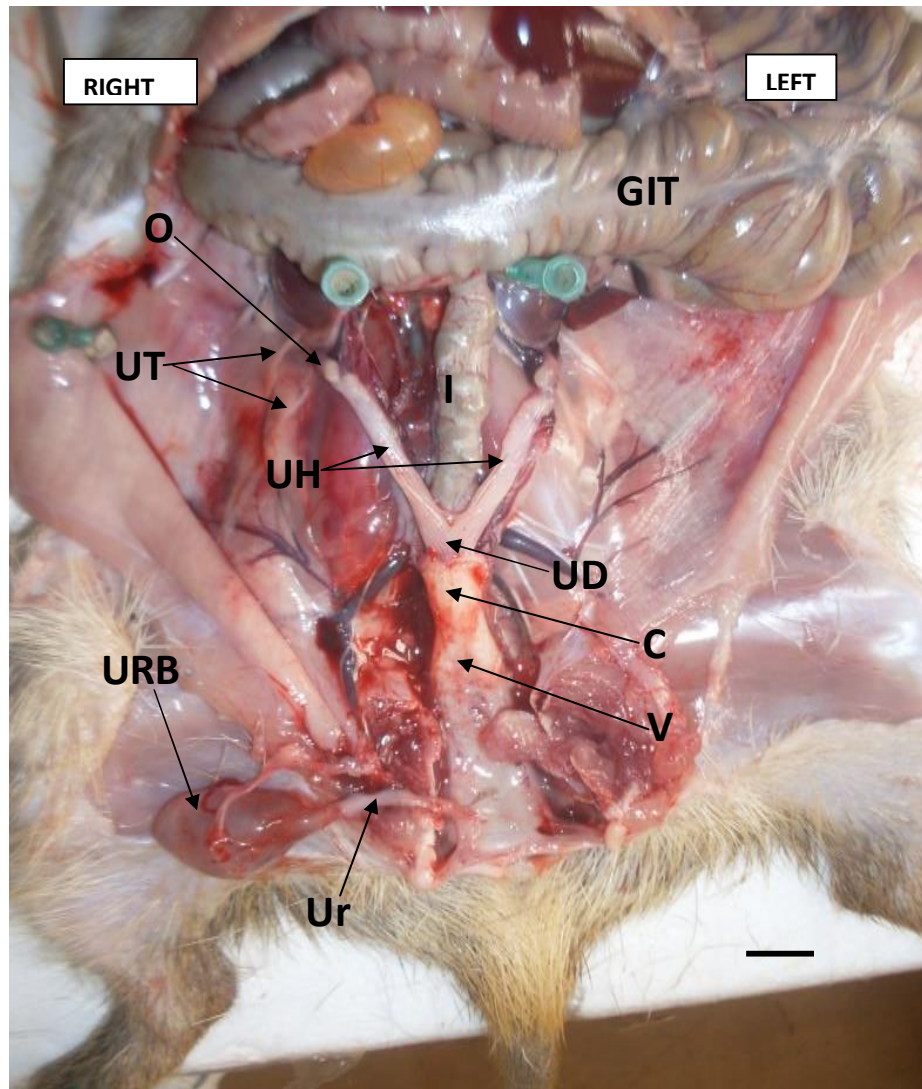


Plate 3: Abdominal Cavity of the Grasscutter at 7-9 Months showing the Ovary (O); Uterine Tube (UT) teased away from the Ovary and Uterine Horns (UH) where they had wrapped around; Uterus Duplex (UD) *in-situ*; Note also that the Urinary Bladder (URB) and Urethra (Ur) were carefully laterally displaced to expose the Cervix (C) and the Vagina (V). Observe the Ileum (I); Liver (L); Gastrointestinal Tract (GIT). Scale Bar =1cm.

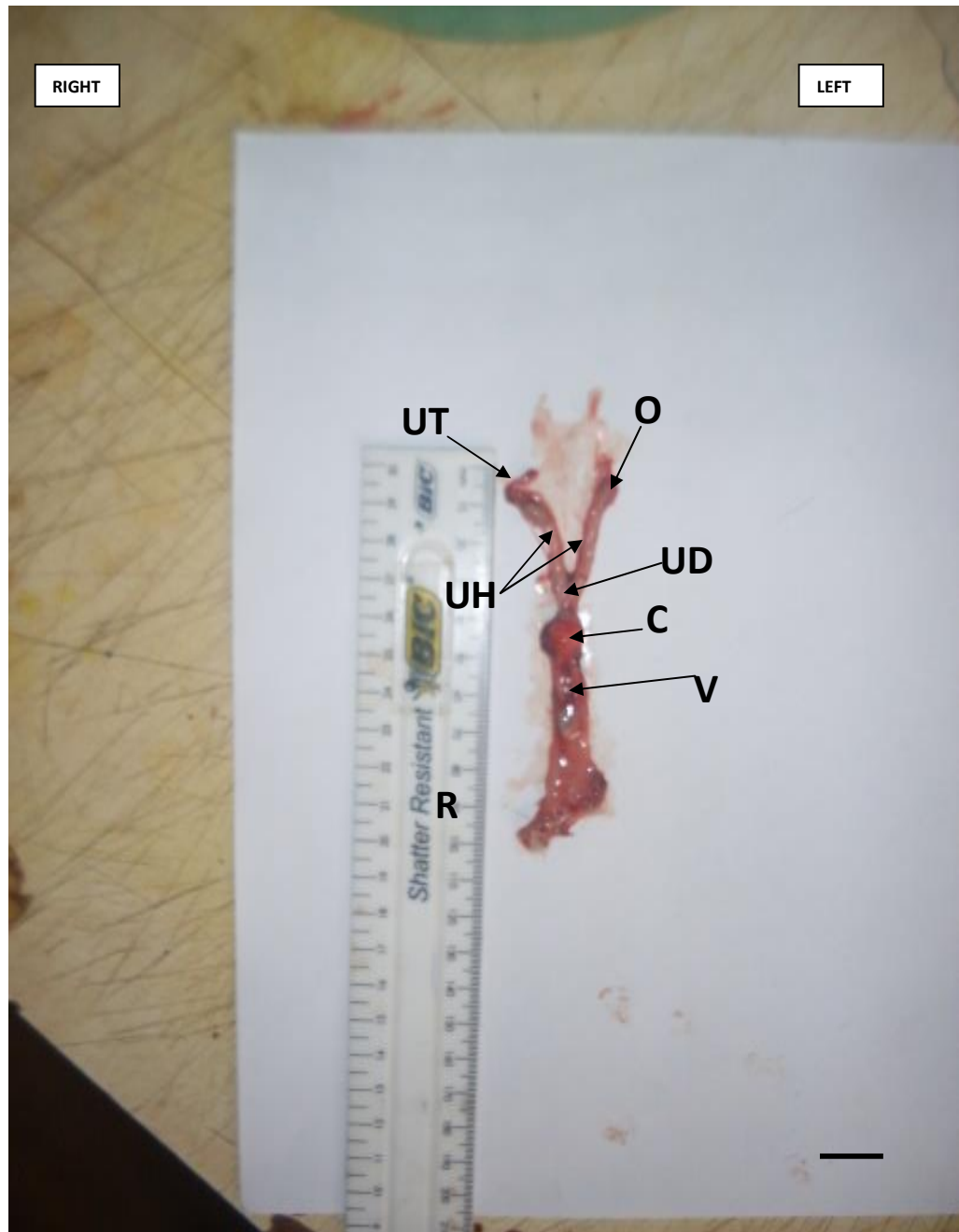


Plate 4: The Female Reproductive System of the Grasscutter at 1-3 Months showing the Ovary (O); Uterine Tube (UT) tightly wrapped around the Ovary and Uterine Horns UH); Uterus Duplex (UD); Cervix (C); Vagina (V); Metre Rule (R). Scale Bar =1cm.

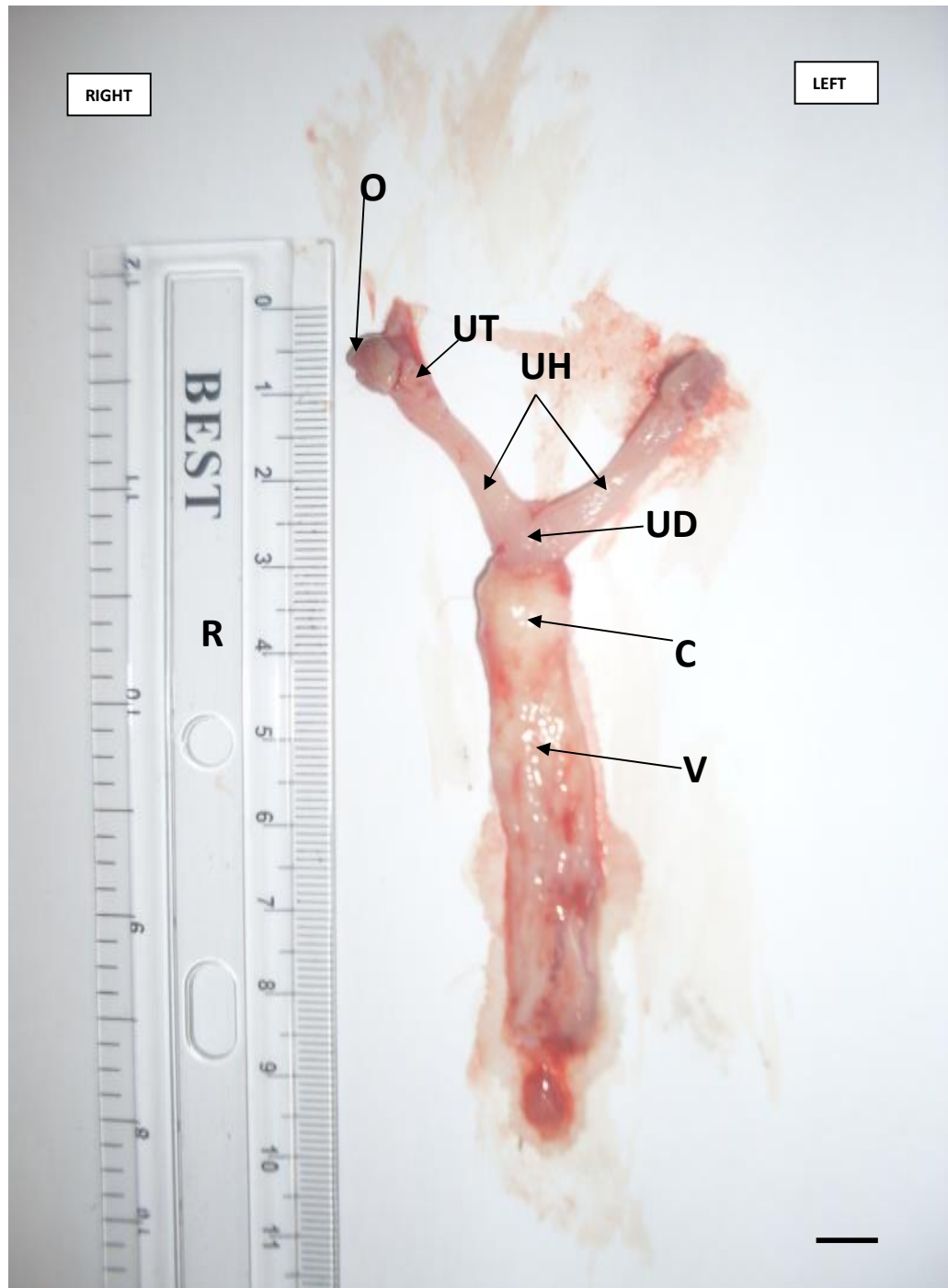


Plate 5: The Female Reproductive System of the Grasscutter at 4-6 Months showing the Ovary (O); coiled-up Uterine Tube (UT); Uterine Horns (UH); Uterus Duplex (UD); Cervix (C); Vagina (V); Metre Rule (R). Scale Bar =1cm.

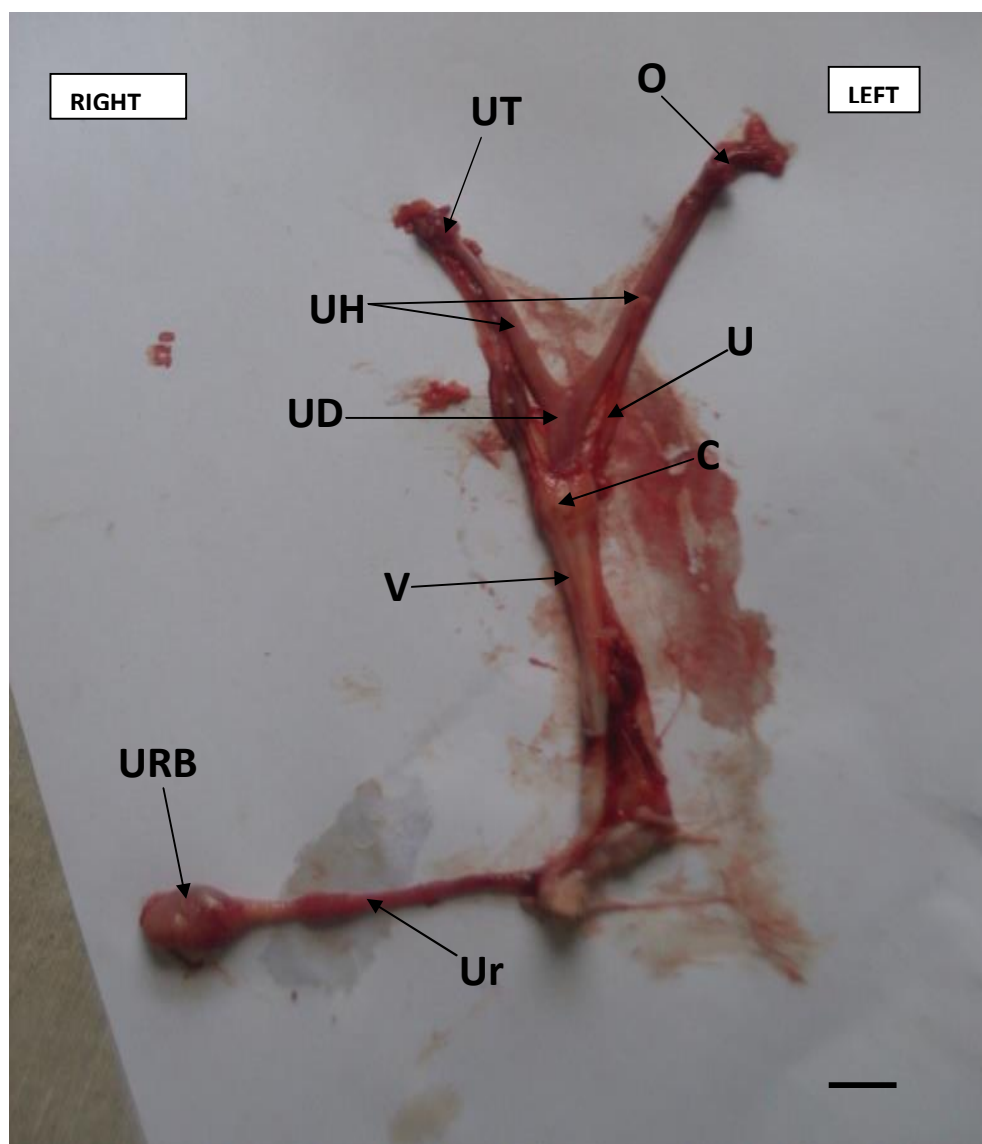


Plate 6: The Female Reproductive System of the Grasscutter at 7-9 Months showing the Ovary (O); Uterine Tube (UT) wrapped around the Ovary and cranial one-third of the Uterine Horns (UH); Uterus Duplex (UD); Cervix (C); Vagina (V); Ureter (U); Urinary Bladder (URB); Urethra (Ur). Scale Bar =1cm.

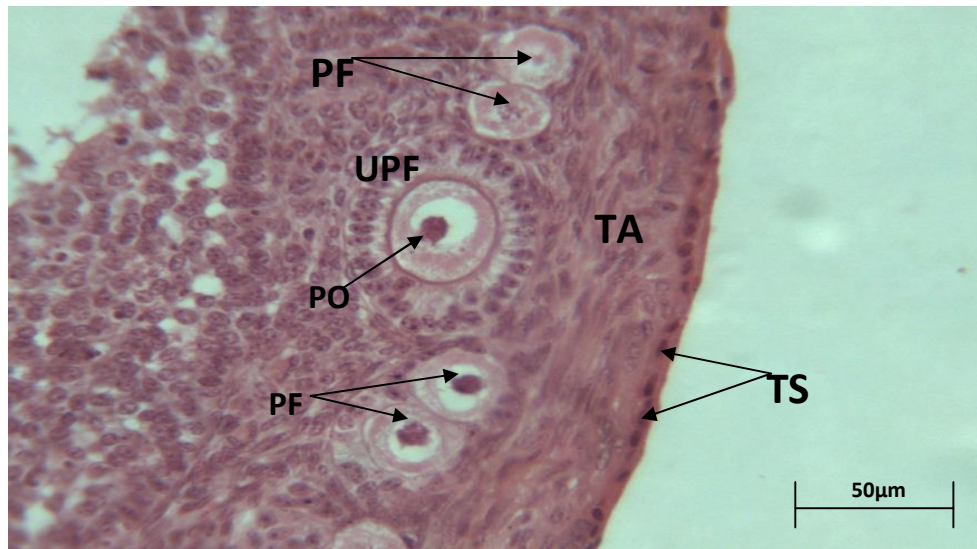


Plate 7: Transverse Section of the Ovarian Cortex at 1-3 months showing the Tunica Serosa (TS); Tunica Albuginea (TA); Pairs of Primordial Follicle (PF); Unilaminar Primary Follicle (UPF); Primary Oocyte (PO). x400. H & E.

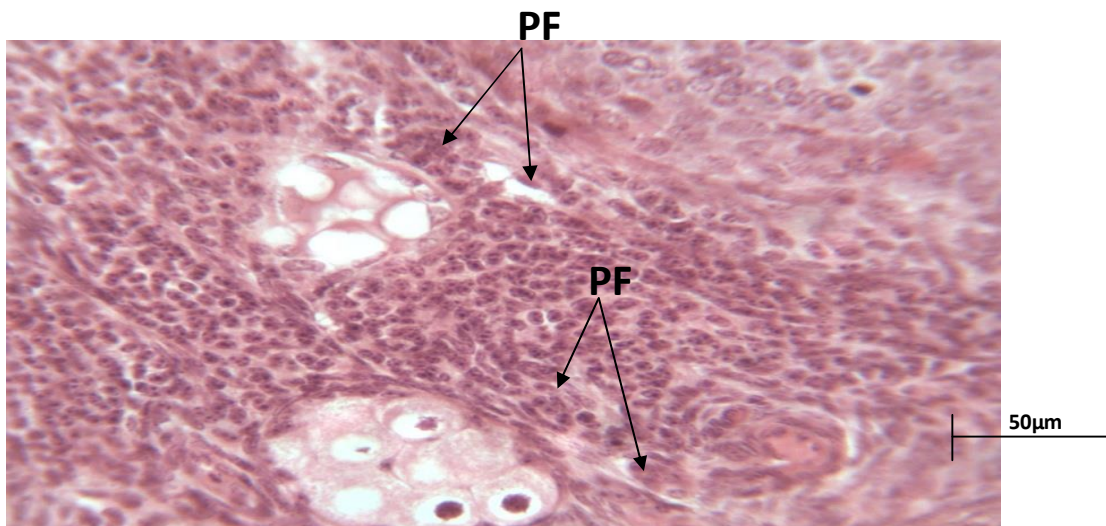


Plate 8: Section of the Ovarian Cortex at 1-3 months showing a nest of Primordial Follicles (PF). x400. H & E.

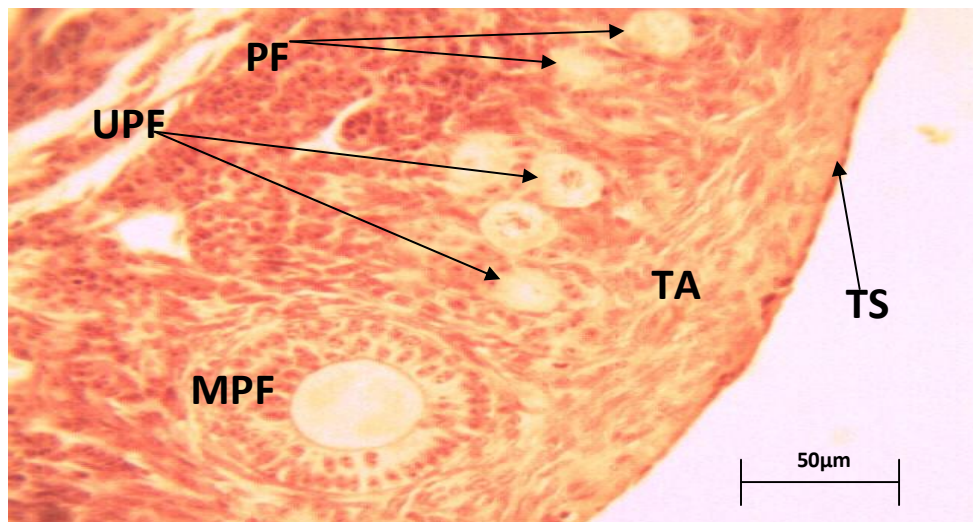


Plate 9: Transverse Section of the Ovarian Cortex at 4-6 Months showing the Ovarian Follicles at Different Stages of Development; Tunica Serosa (TS); Tunica Albuginea (TA); Primordial Follicle (PF); Unilaminar Primary Follicle (UPF); Multilaminar Primary Follicle (MPF). x100. H & E.

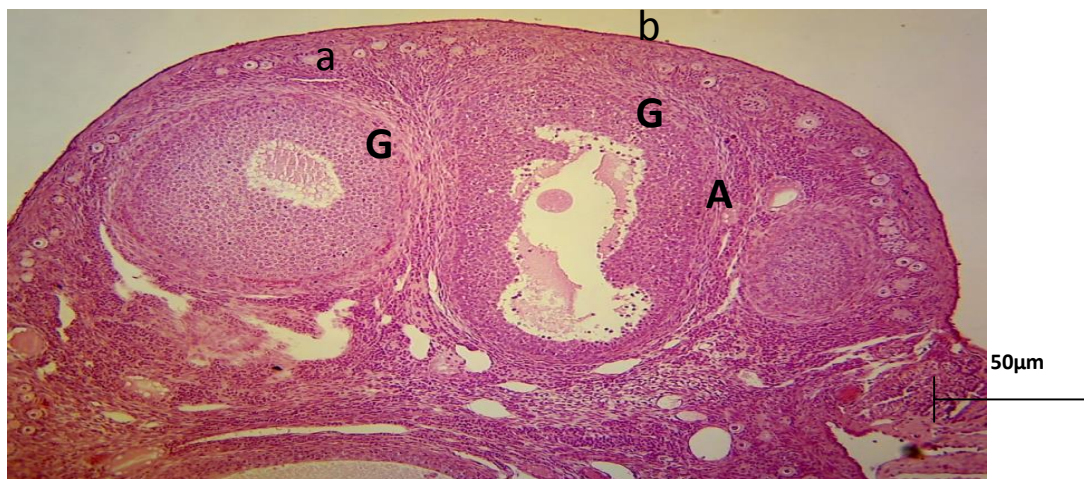


Plate 10: Section of the Ovarian Cortex at 7-9 Months showing two Secondary Follicles; (a) Granulosa Cells (G) around the Oocyte without Antrum. (b) Granulosa Cells (G) with an Antrum (A). x100. H & E.

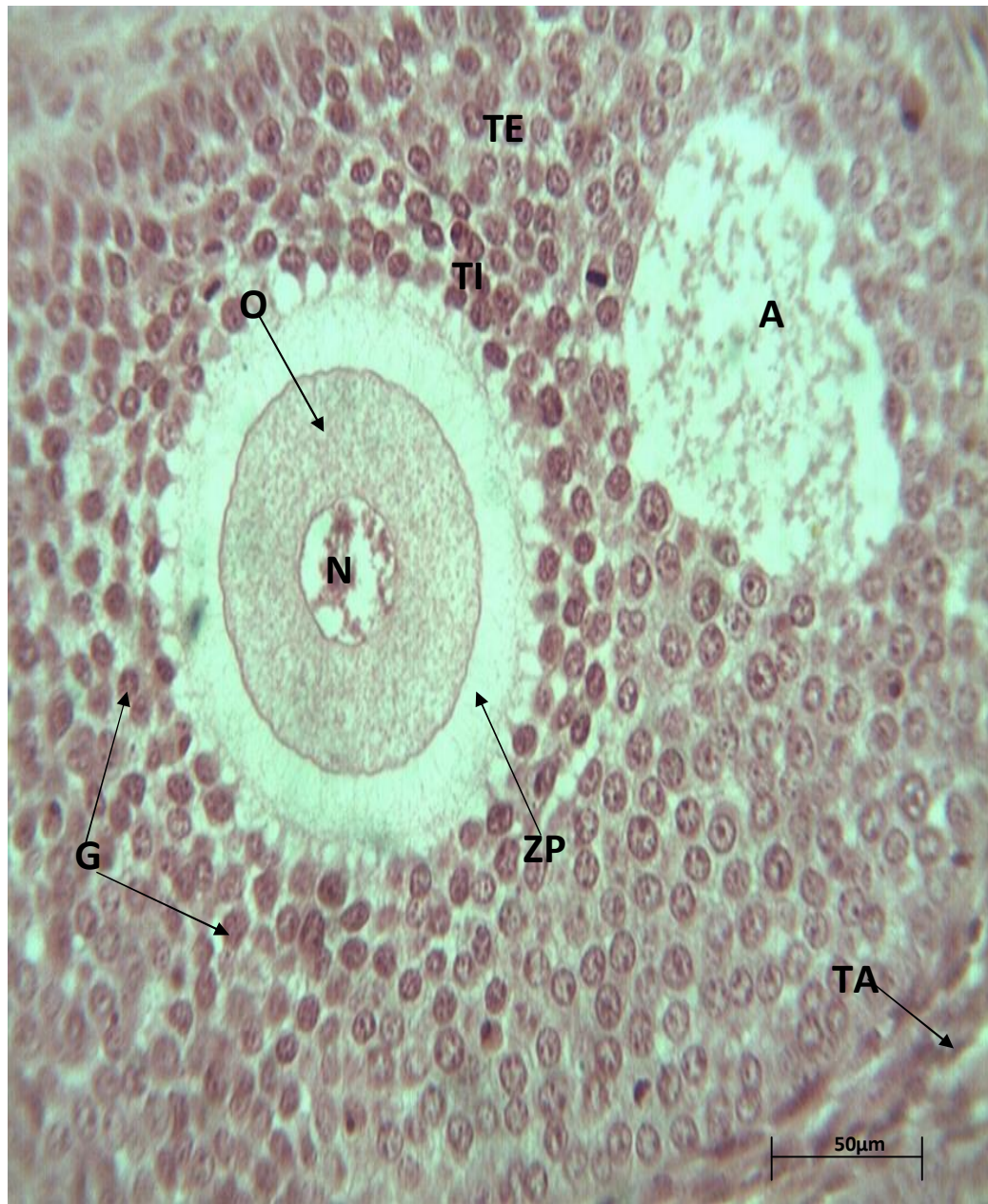


Plate 11: Transverse Section of the Ovarian Cortex at 7-9 Months showing a Secondary Follicle: Antrum (A); Centrally located Oocyte (O); Nucleus (N) of the Oocyte; Zona Pellucida (ZP); Multi-layered Granulosa Cells (G); Tunica Albuginea (TA). Note the Theca Interna (TI); Theca Externa (TE). x400. H & E.

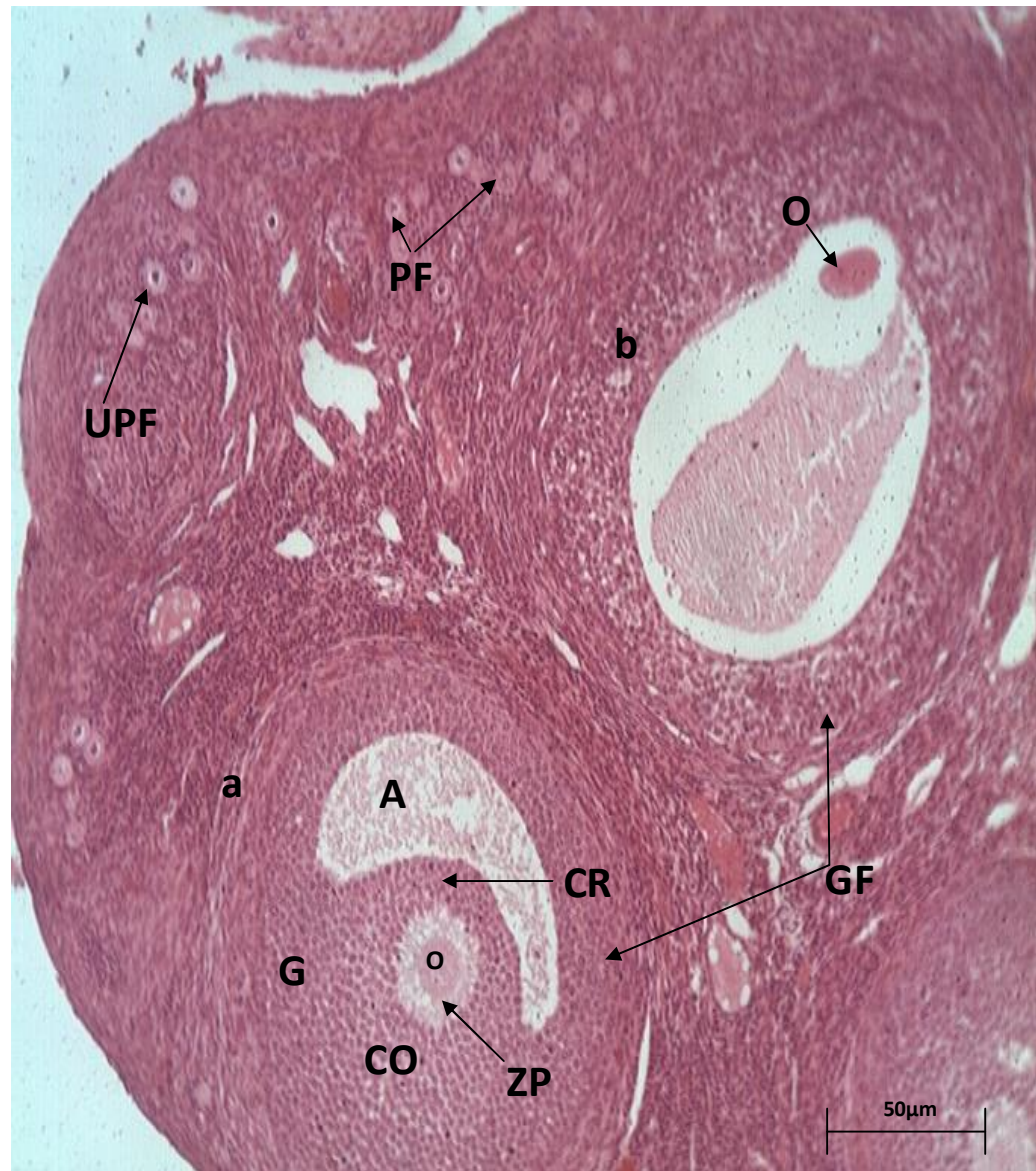


Plate 12: Ovarian Cortex at 7-9 Months showing Different Stages of Follicular Development;

Primordial Follicle (PF); Unilaminar Primary Follicle (UPF); Graafian Follicle (GF); (a) Antrum (A); Corona Radiata (CR); Cumulus Oophorus (CO); Zona Pellucida (ZP); Granulosa Cells (G); Oocyte (O) at the centre of the ovary. (b) Oocyte (O) at the periphery of the ovary. x100. H & E.

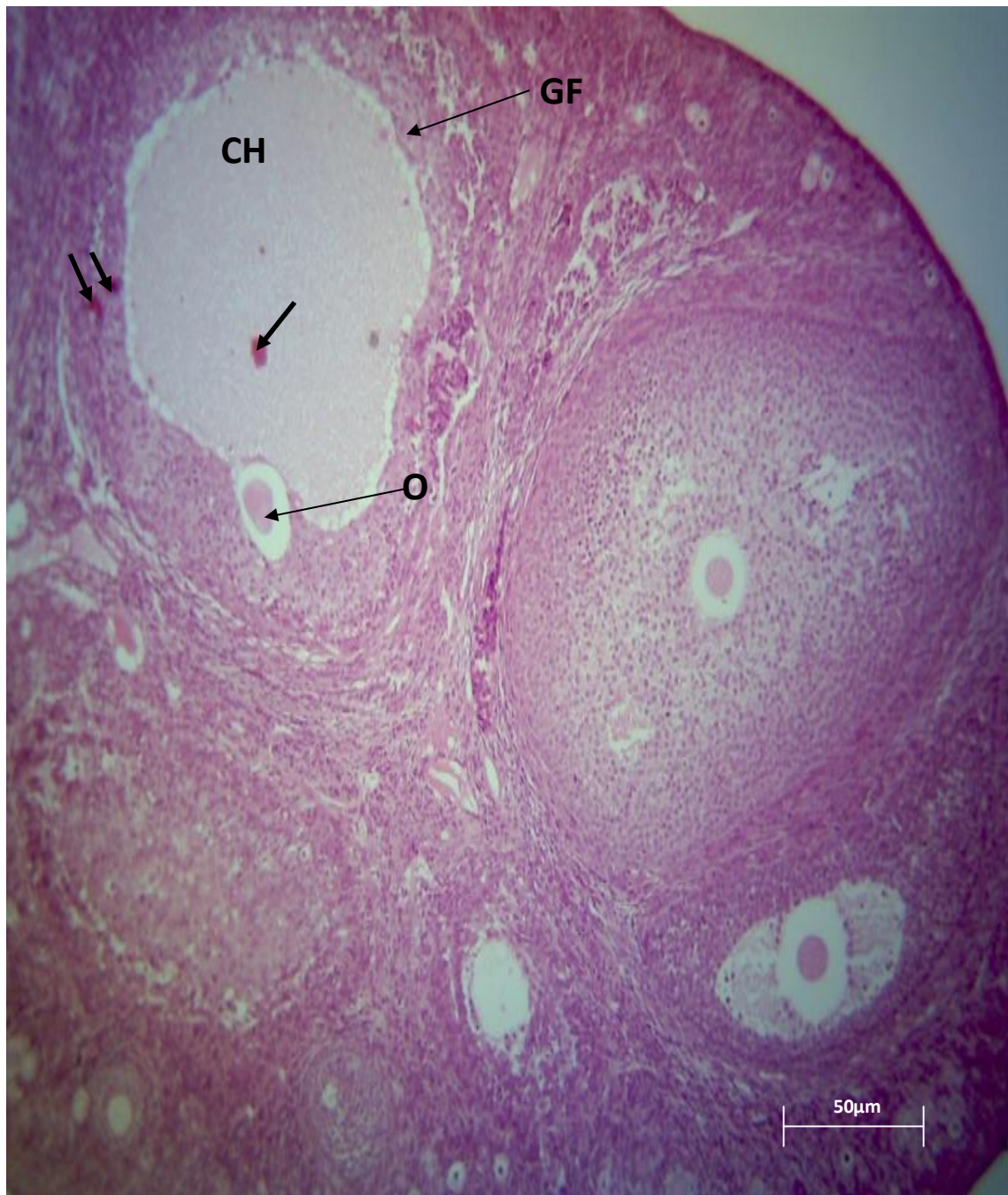


Plate 13: Transverse Section of the Ovarian Cortex at 7-9 Months showing the Graafian Follicle (GF); Corpus Haemorrhagicum (CH) as indicated by Petechiation (Arrows). Note the Oocyte (O) at the periphery of the follicle. x100. H & E.

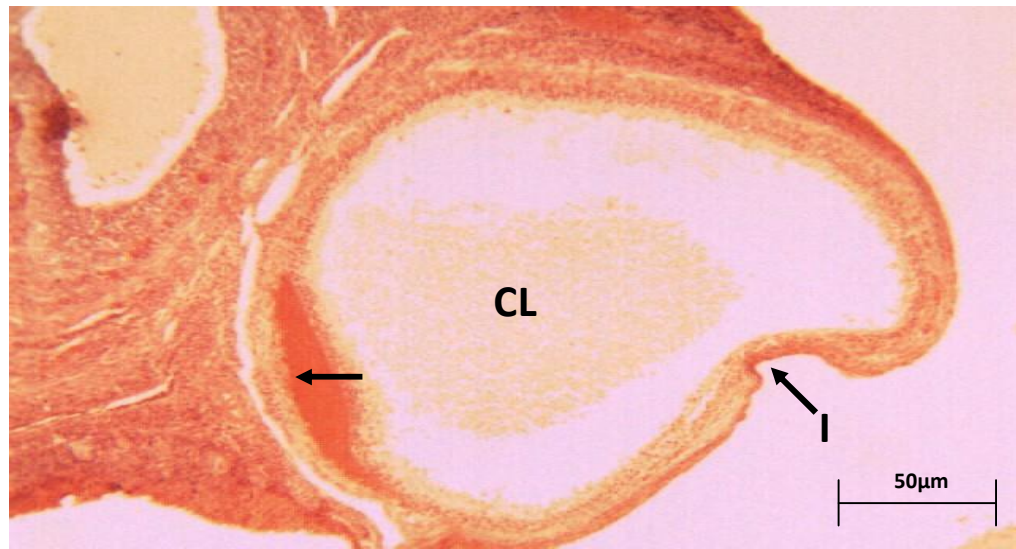


Plate 14: Section of the Ovarian Cortex at 7-9 Months showing the Corpus Luteum (CL) indicated by blood clot (Arrow) within the inner wall of the Graafian Follicle. Note the Indentation (I) on the Ovarian wall. x400. H & E.

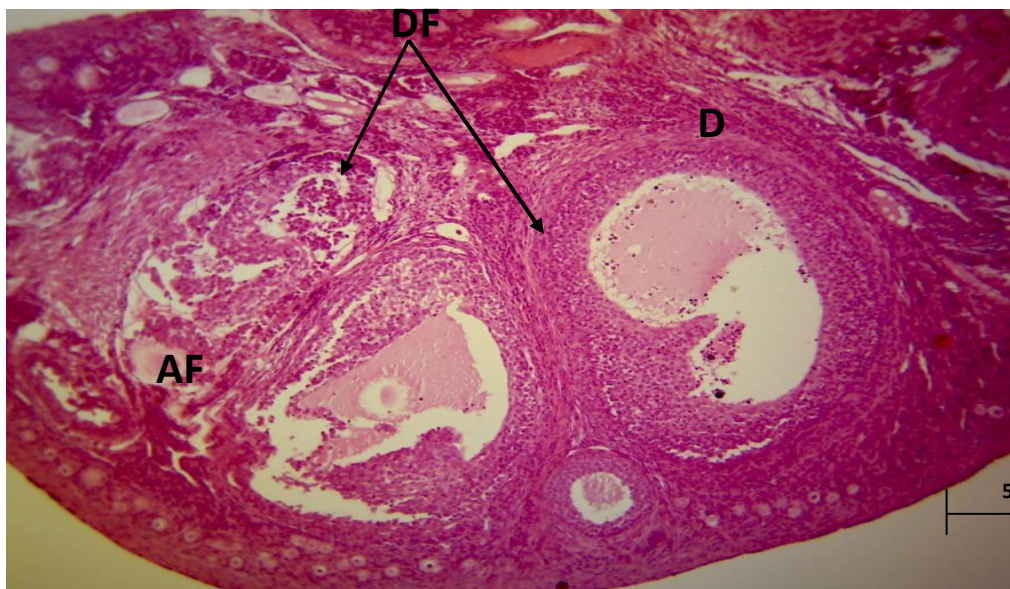


Plate 15: Section of the Ovarian Cortex at 7-9 Months showing Developing Follicles (D); Degenerating Follicle (DF); Atretic Follicle (AF). x100. H & E.

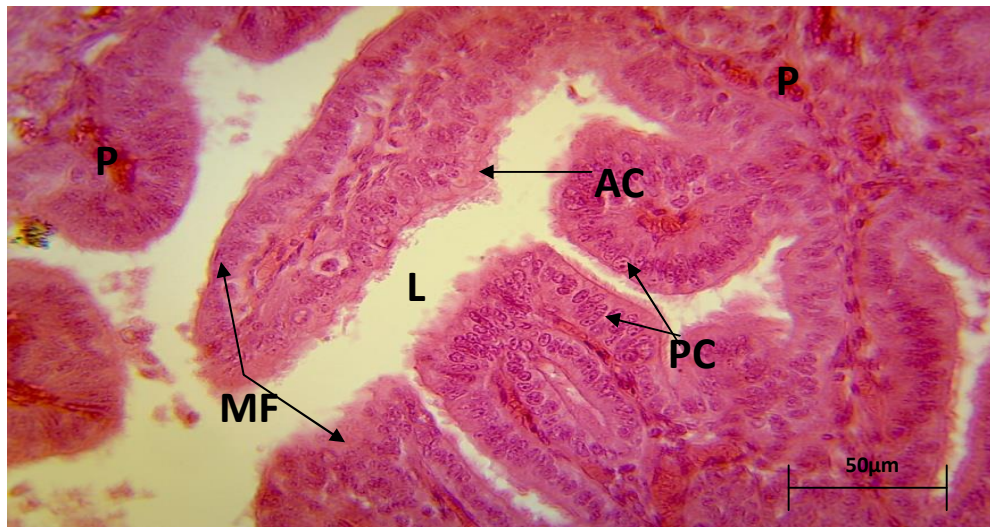


Plate 16: Transverse Section of the Uterine Tube at 1-3 Months showing the Lumen (L) lined by Pseudostratified Columnar Epithelium (PC) with Apical Cilia (AC). Note the Lamina propria-submucosa projecting narrow Papillae (P) into the Mucosal Folds (MF). x400. H & E.

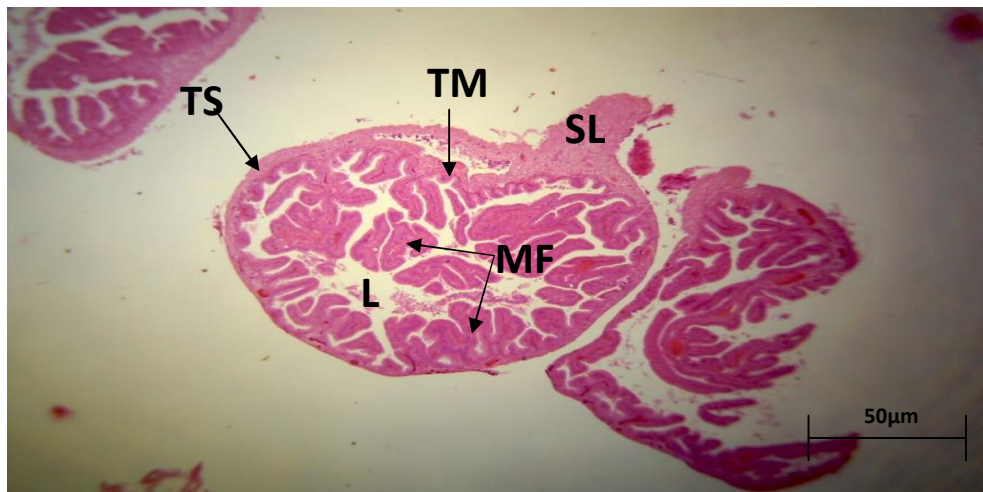


Plate 17: Transverse Section of the Uterine Tube at 1-3 Months showing the Lumen (L); highly convoluted Mucosal Fold (MF) presenting a Labyrinth; Tunica Muscularis (TM); Tunica Serosa (TS); Suspensory Ligament (SL). x40. H & E

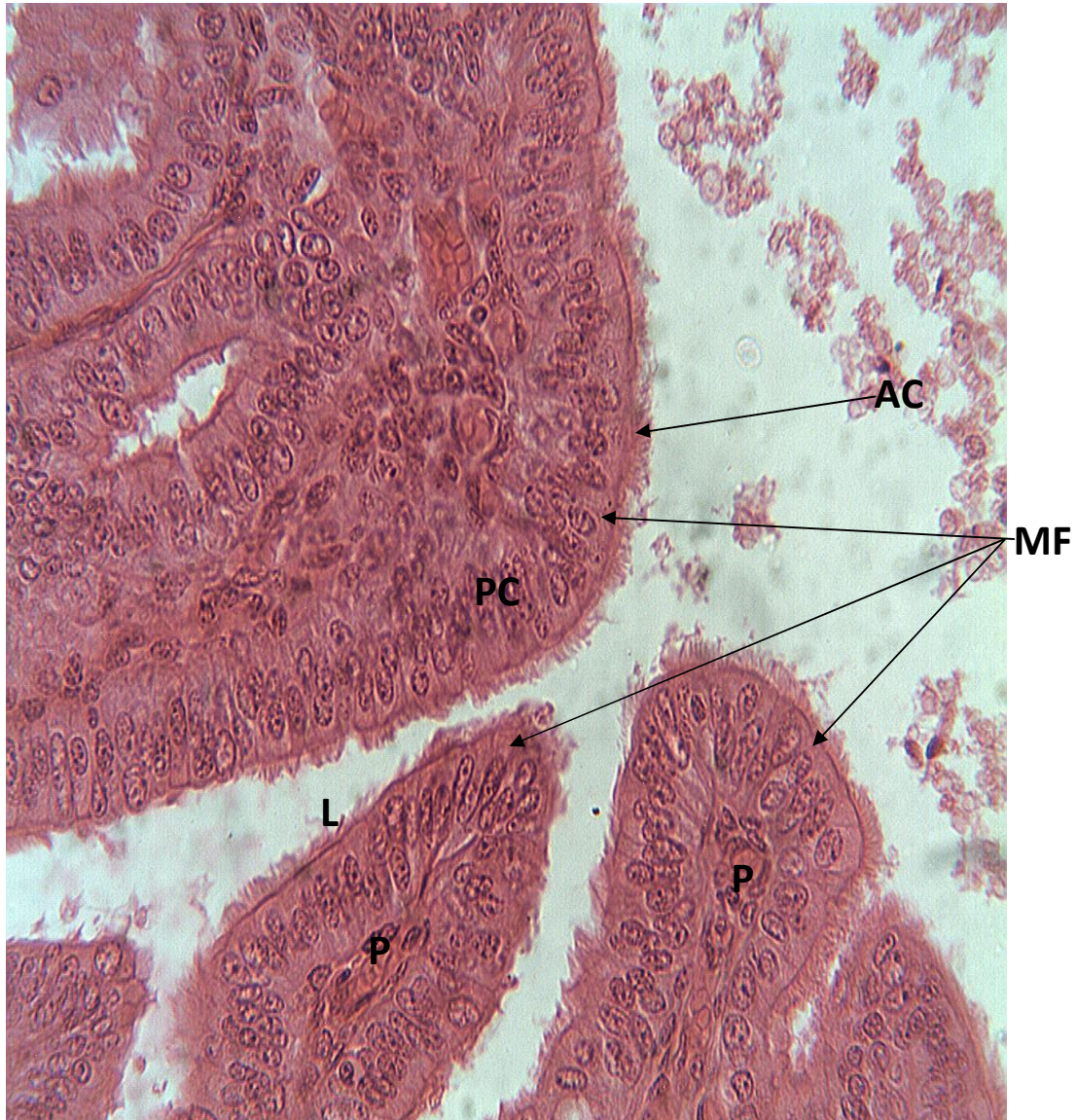


Plate 18: Transverse Section of the Uterine Tube at 4-6 Months showing the Lumen (L) lined By Pseudostratified Columnar Epithelium (PC) with Apical Cilia (AC). Note the Lamina propria-submucosa projecting narrow Papillae (P) into the Mucosal Folds (MF). x400. H & E.

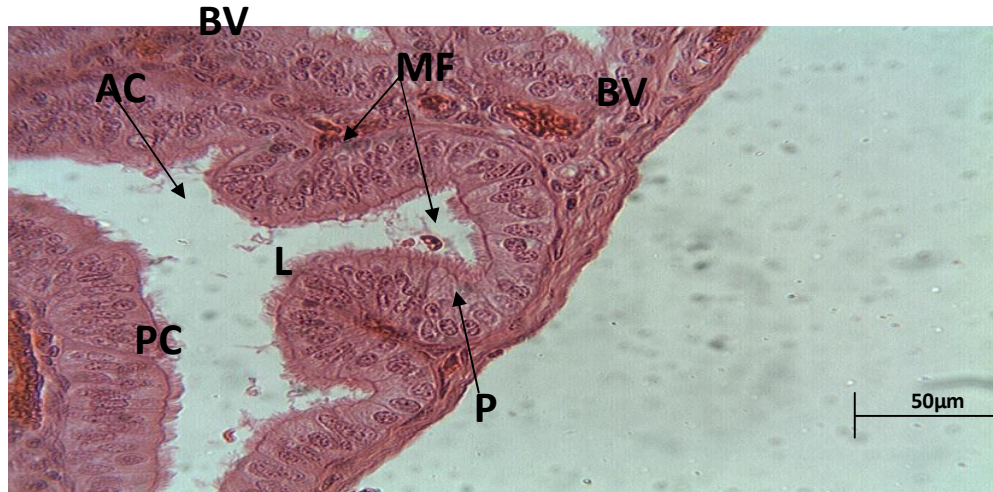


Plate 19: Transverse Section of the Uterine Tube at 4-6 Months showing the Lumen (L) Lined by Pseudostratified Columnar Epithelium (PC) with Apical Cilia (AC); Blood Vessels (BV). Note the Lamina propria-submucosa projecting narrow Papillae (P) into the Mucosal Folds (MF). x400. H & E.



Plate 20: Section of the Uterine Tube at 4-6 months showing the Lumen (L); highly convoluted Mucosal Fold (MF); Tunica Muscularis (TM); Tunica Serosa (TS); Suspensory Ligament (SL). Note the Lamina Propria projecting narrow Papillae (P) into the mucosal folds. x100. H & E.

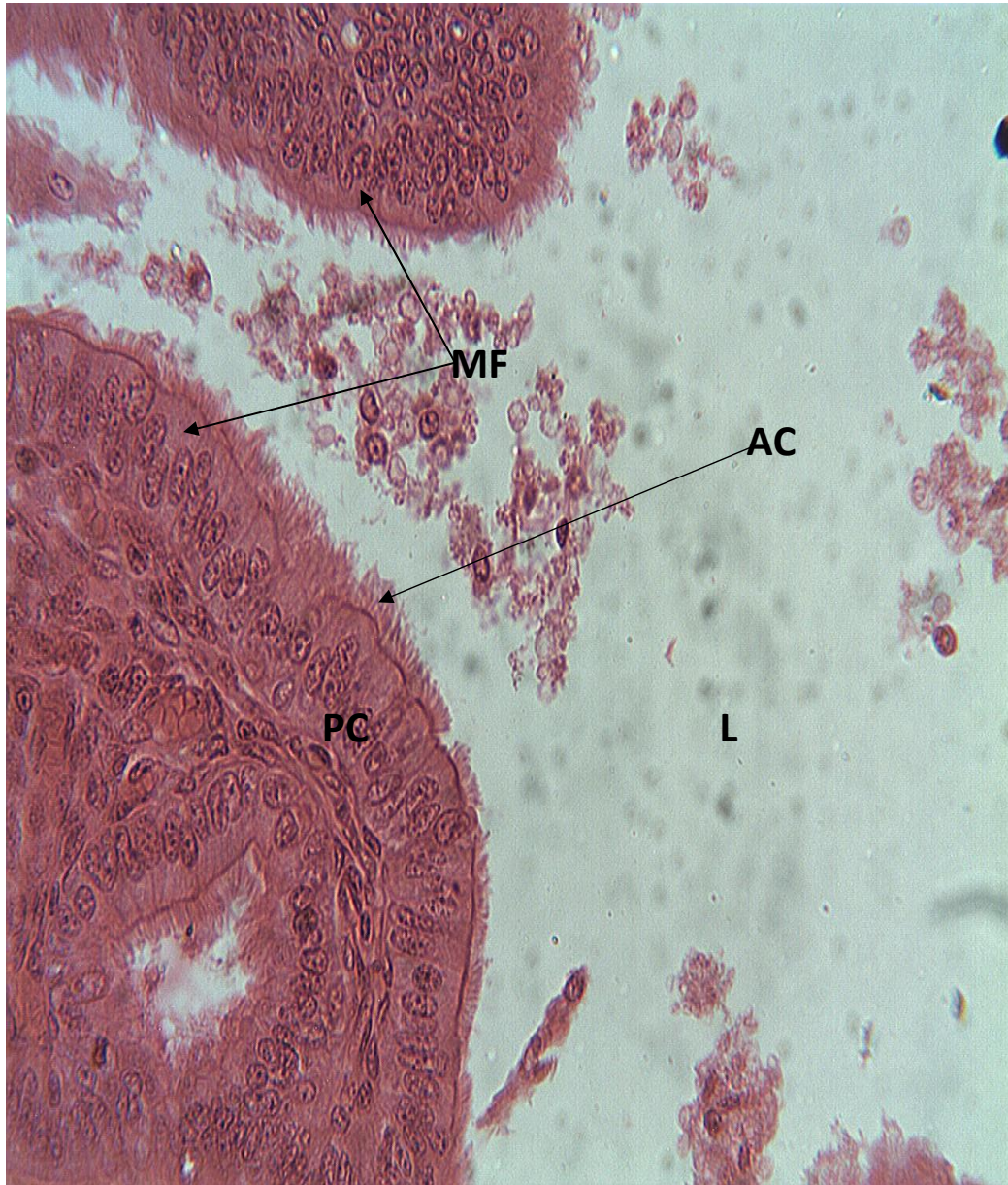


Plate 21: Transverse Section of the Uterine Tube at 7-9 Months showing the Lumen (L) lined by Pseudostratified Columnar Epithelium (PC); Apical Cilia (AC); Mucosal Folds (MF).
x400. H & E.

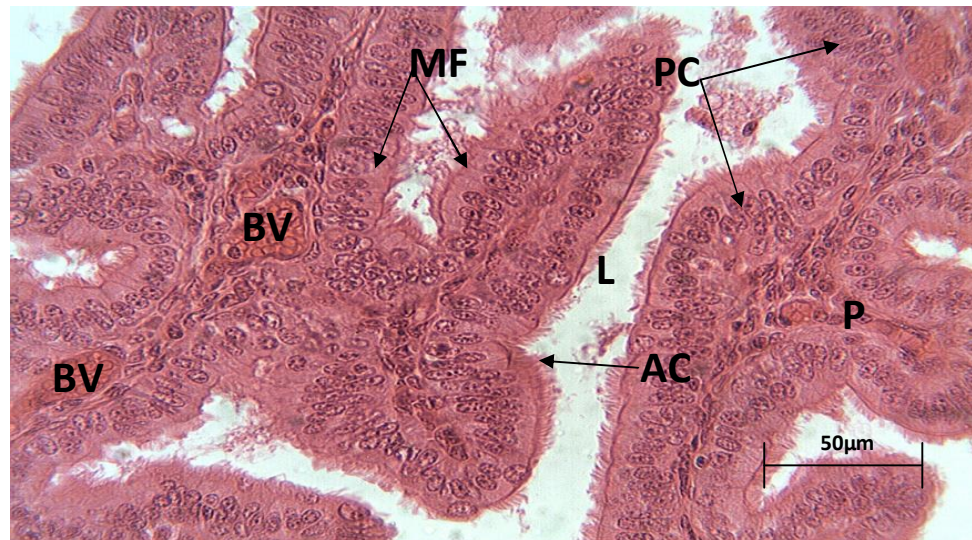


Plate 22: Transverse Section of the Uterine Tube at 7-9 Months showing the Lumen (L) lined by Pseudostratified Columnar Epithelium (PC) ; Apical Cilia (AC); Blood Vessels (BV). Note the Lamina Propria-submucosa projecting narrow papillae (P) into the Mucosal Folds (MF). x400. H & E.



Plate 23: Section of the Uterine Tube at 7-9 Months showing the Lumen (L); highly convoluted Mucosal Fold (MF); Lamina Propria-submucosa (LP); Tunica Serosa (TS); Suspensory Ligament (SL). x100. H & E.



Plate 24: Transverse Section of the Uterine Horn at 1-3 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Epithelium (SC); Endometrium (E). x400. H & E.

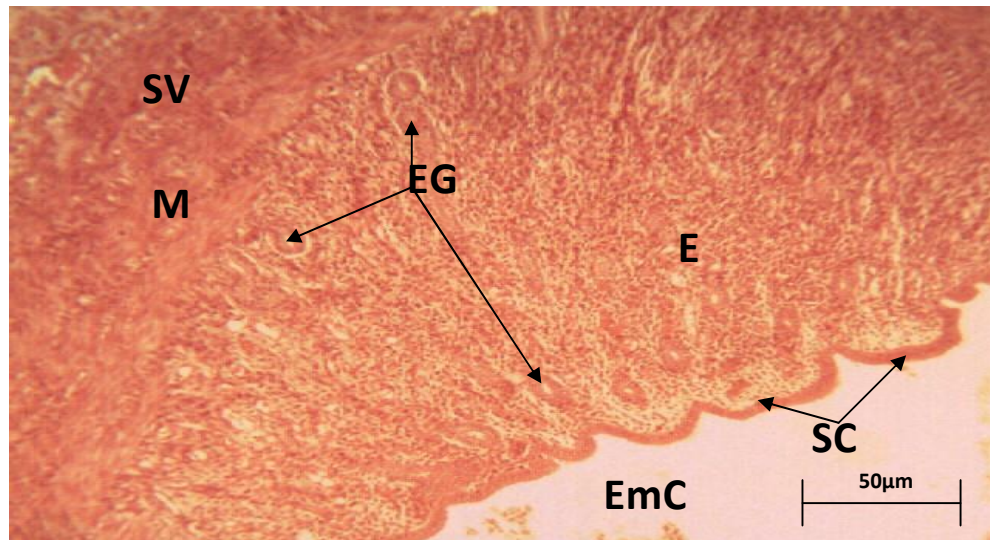


Plate 25: Transverse Section of the Uterine Horn at 1-3 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Epithelium (SC); Endometrium (E) containing Endometrial Glands (EG); Myometrium (M). Note the Stratum Vasculare (SV). x100. H & E.

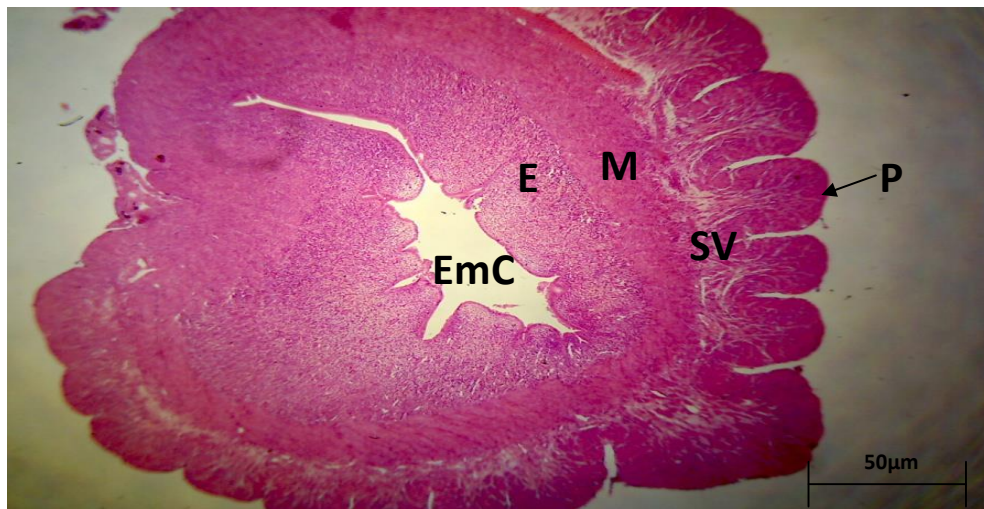


Plate 26: Transverse Section of the Uterine Horn at 1-3 Months showing the Endometrial Canal (EmC); Endometrium (E); Myometrium (M); Perimetrium (P). Note the Stratum Vasculare (SV). x40. H & E.



Plate 27: Transverse Section of the Uterine Horn at 4-6 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Epithelium (SC); Endometrium (E) containing Endometrial Glands (EG). x400. H & E.

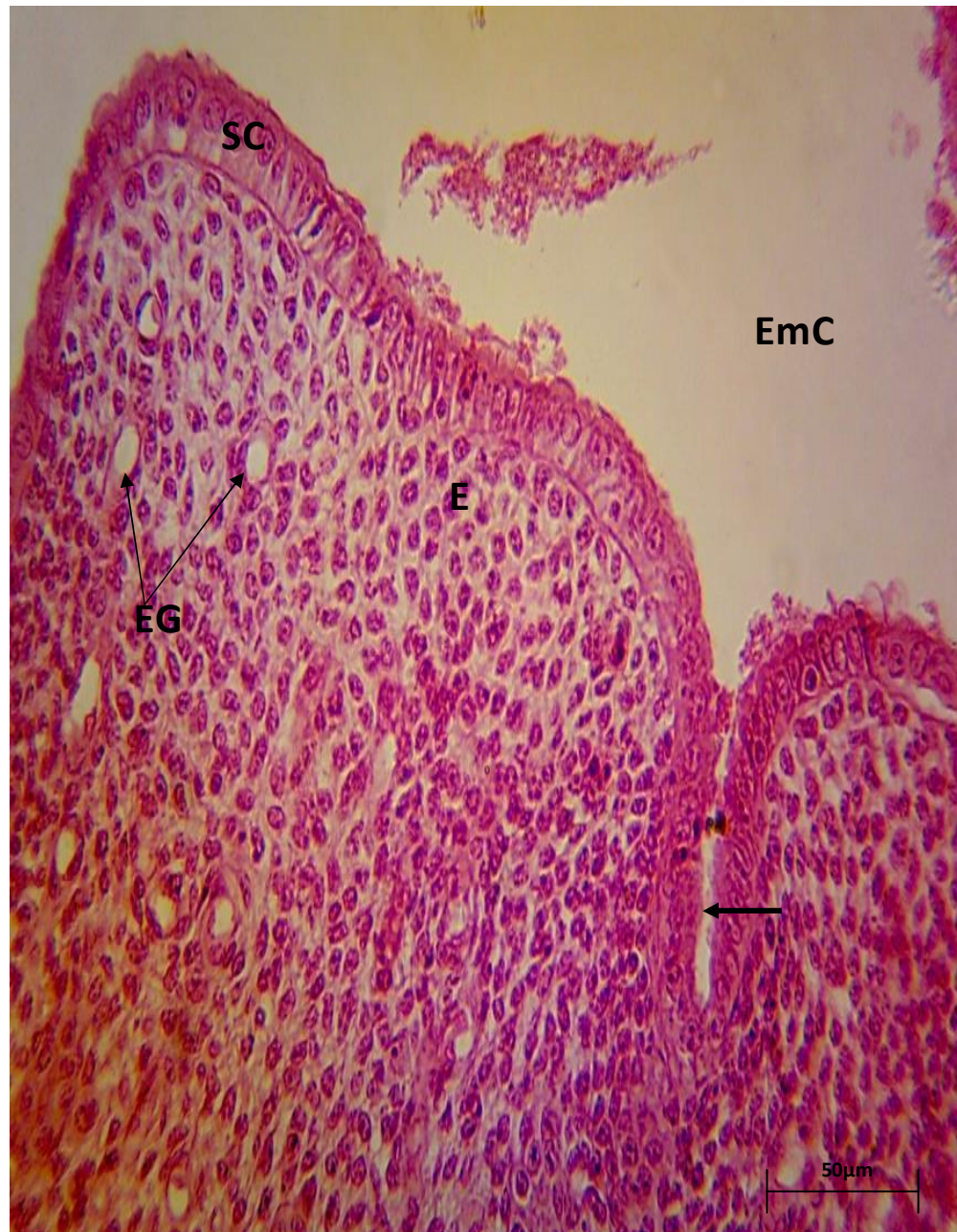


Plate 28: Transverse Section of the Uterine Horn at 4-6 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Epithelium (SC); Endometrium (E) containing Endometrial Glands (EG). Note the Endometrial Crypt (Arrow). x400. H & E.

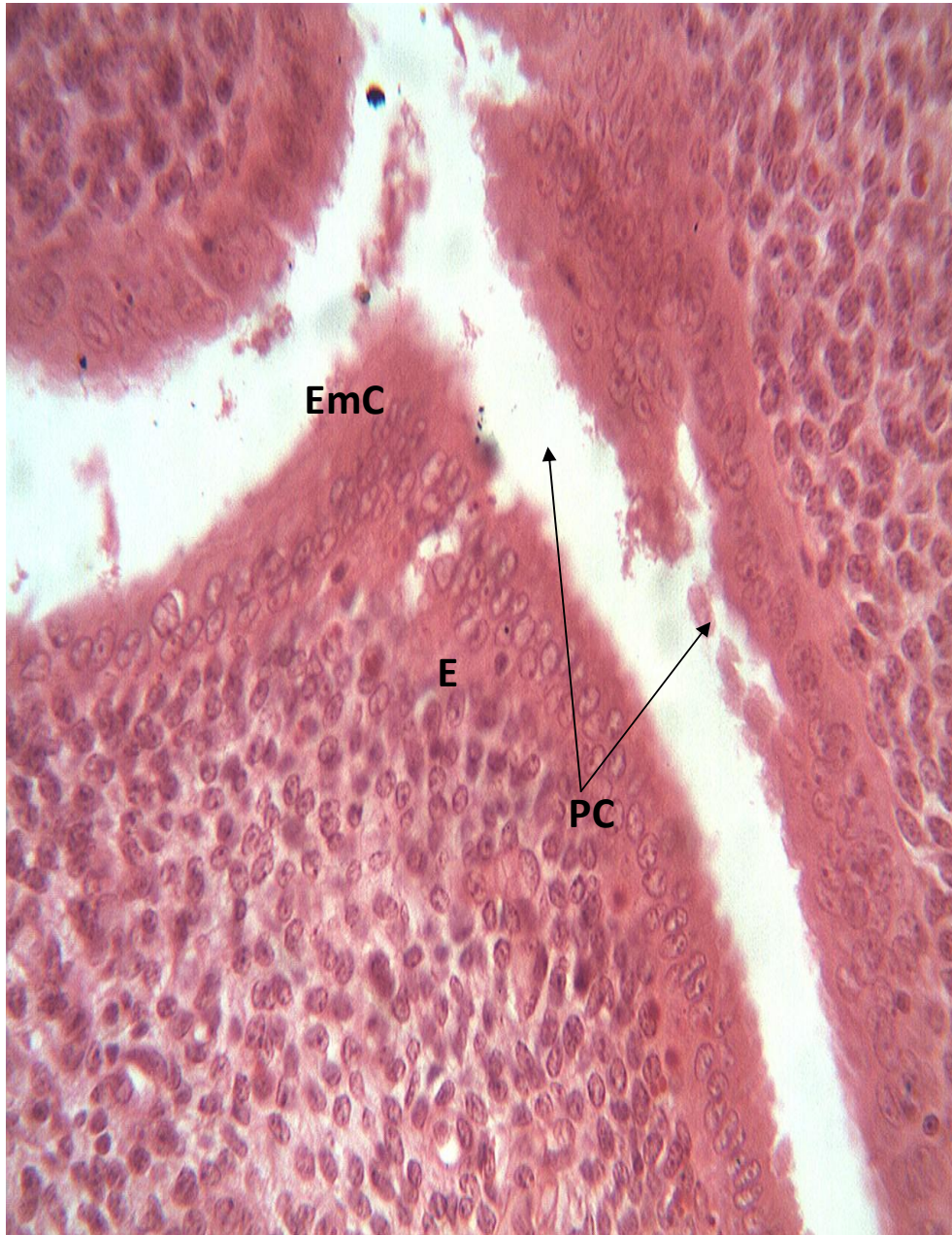


Plate 29 : Transverse Section of the Uterine Horn at 7-9 Months showing the Endometrial Canal (EmC); Endometrium (E) lined by Pseudostratified Columnar Epithelium (PC). x400. H & E.

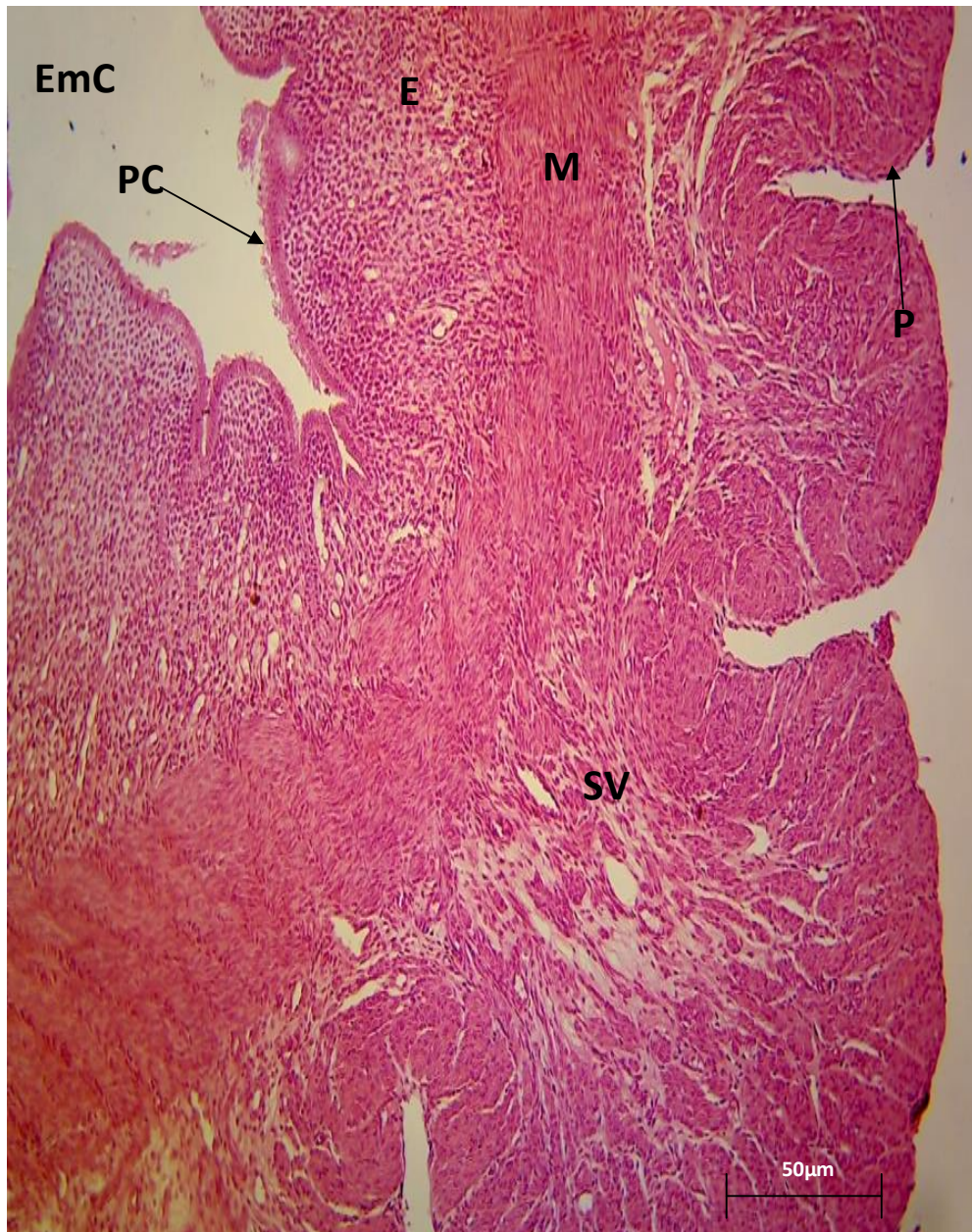


Plate 30: Transverse Section of the Uterine Horn at 7-9 Months showing the Endometrial Canal (EmC); Endometrium (E) lined by Pseudostratified Columnar Epithelium (PC); Myometrium (M); Perimetrium (P); Stratum Vasculare (SV).
x100. H & E.

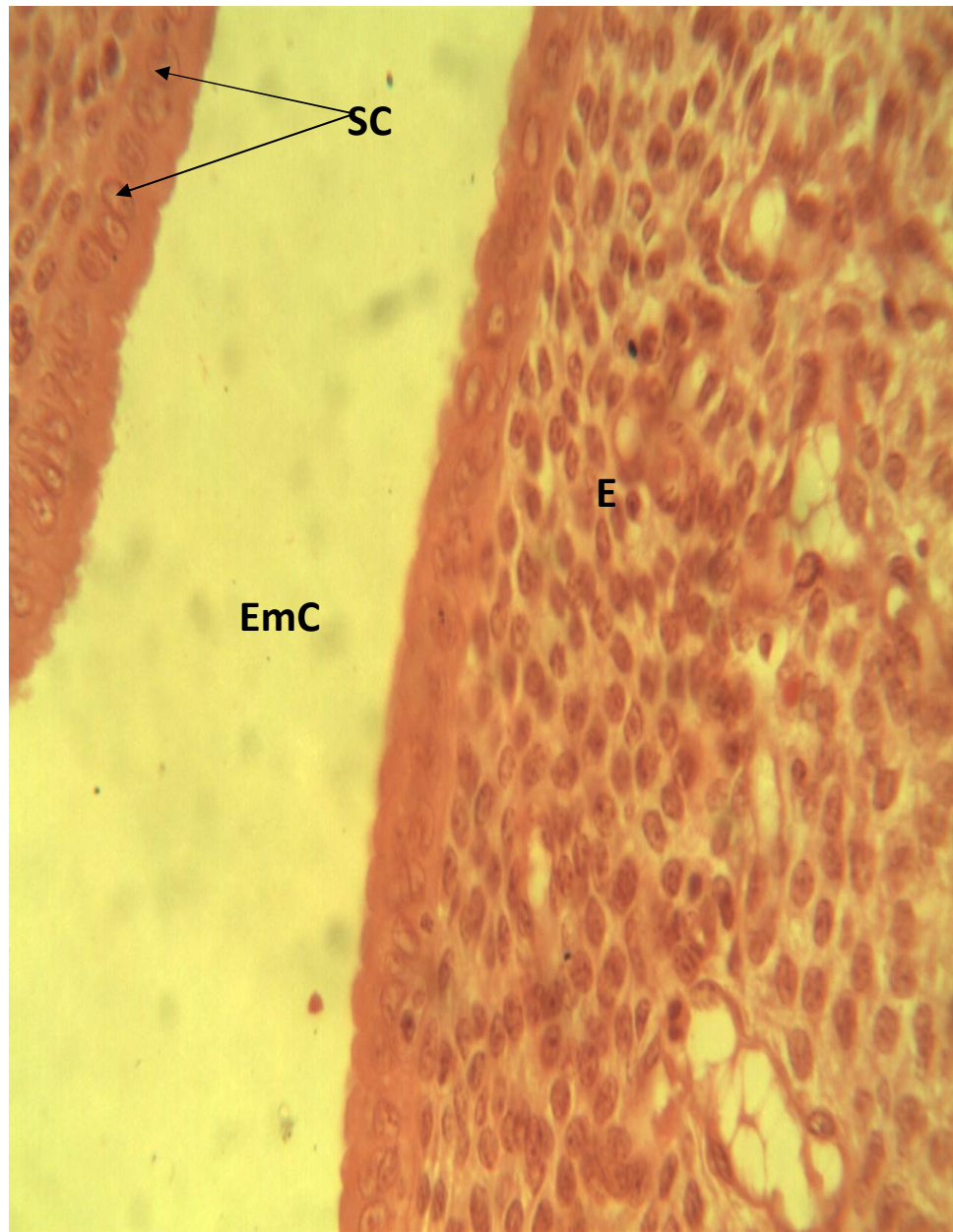


Plate 31: Transverse Section of the Uterus Duplex at 1-3 Months showing the Endometrial Canal (EmC); Endometrium (E) lined by Simple Columnar epithelium (SC).
x400. H & E.

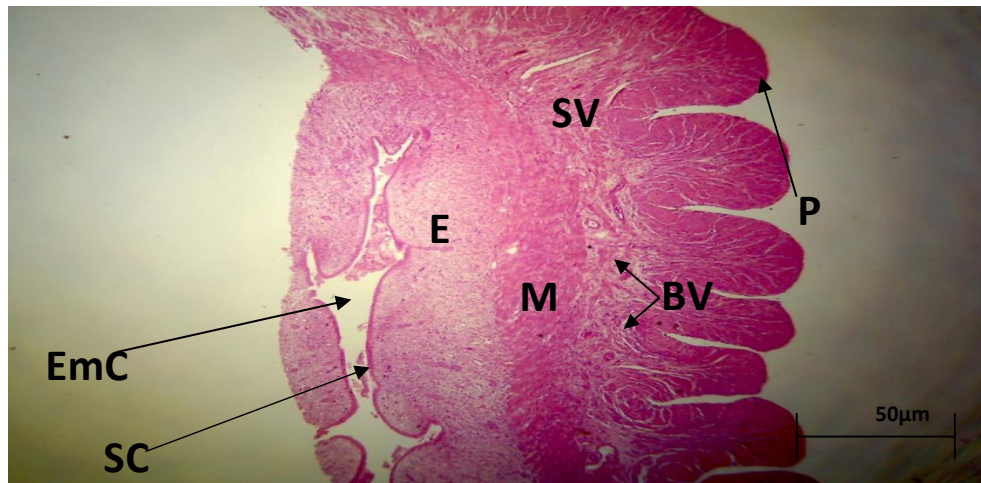


Plate 32: Transverse Section of the Uterus Duplex at 1-3 Months showing the Endometrial Canal (EmC); Endometrium (E) lined by Simple Columnar epithelium (SC); Myometrium (M); Stratum Vasculare (SV); Blood Vessels (BV); Perimetrium (P). x40. H & E.

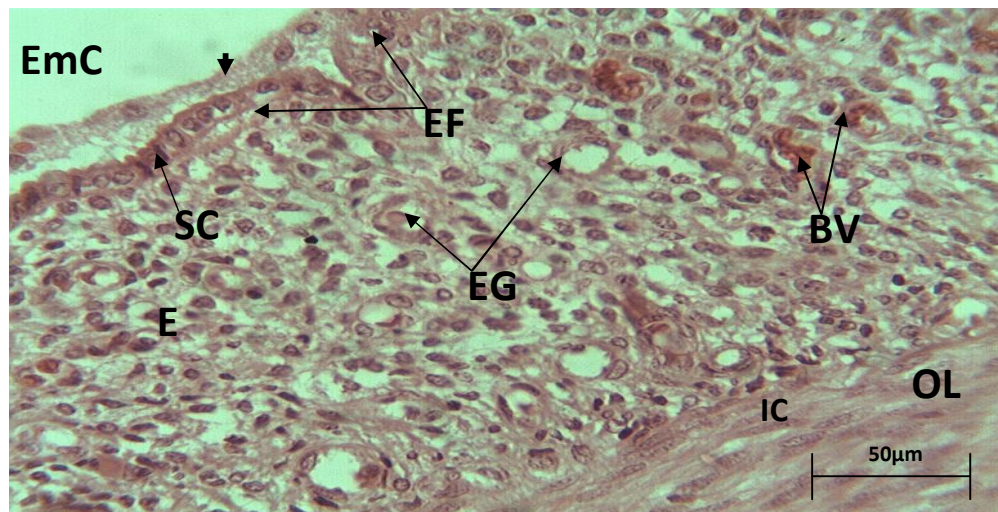


Plate 33: Transverse Section of the Uterus Duplex at 4-6 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Cells (SC); Endometrium (E); Endometrial Folds (EF); Endometrial Glands (EG) with its Secretion (Arrowhead); Blood Vessels (BV); Inner Circular (IC) and Outer Longitudinal (OL) Smooth Muscles. x400. H & E.

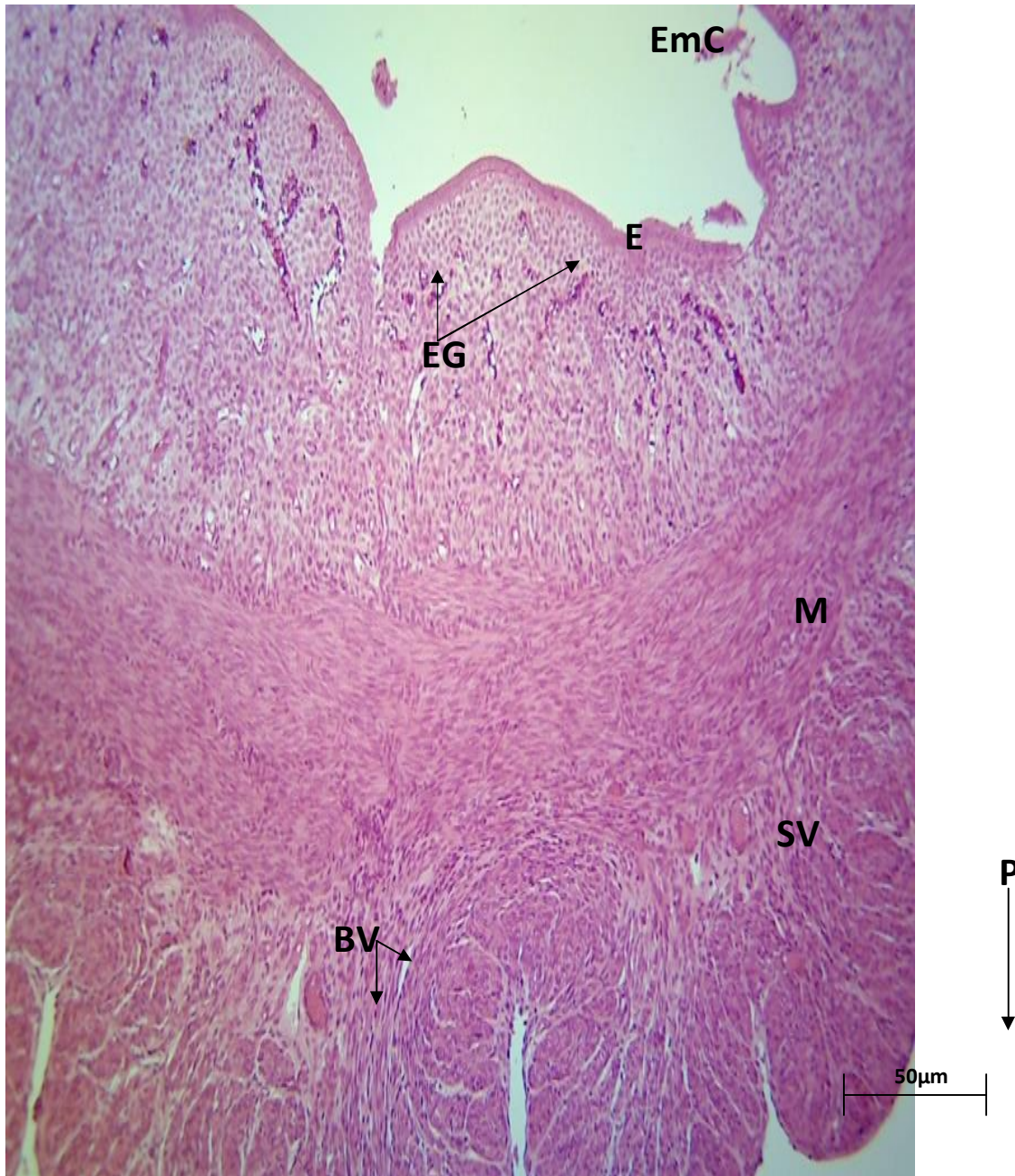


Plate 34: Transverse Section of the Uterus Duplex at 4-6 Months showing the Endometrial Canal (EmC); Endometrium (E) containing Endometrial Glands (EG); Myometrium (M); Stratum Vasculare (SV); Blood Vessels (BV); Perimetrium (P). x100. H & E.

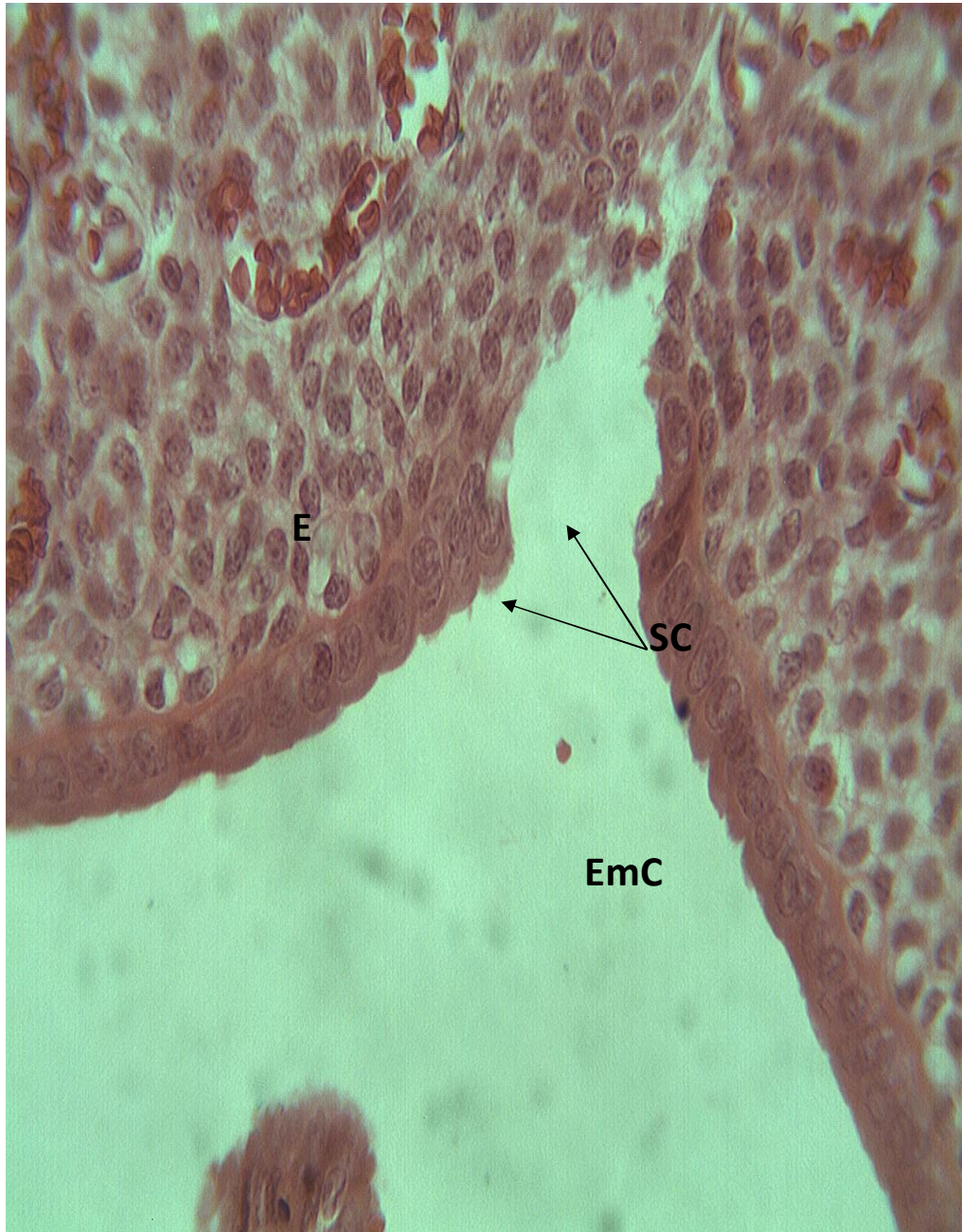


Plate 35 : Transverse Section of the Uterus Duplex at 7-9 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Cells (SC); Endometrium (E).
x 400. H & E.

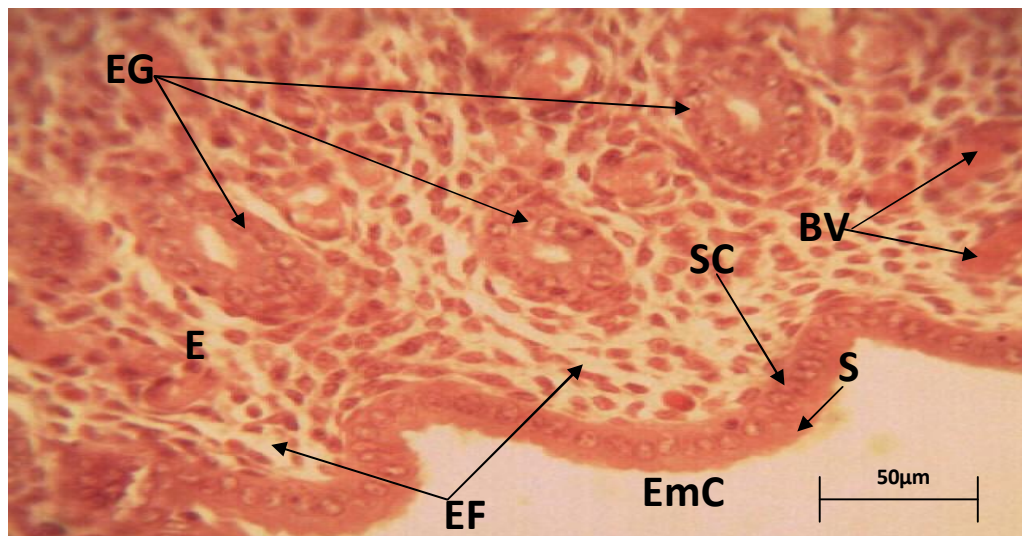


Plate 36: Transverse Section of the Uterus Duplex at 7-9 Months showing the Endometrial Canal (EmC) lined by Simple Columnar Cells (SC); Endometrial Folds (EF); Endometrium (E) containing Blood Vessels (BV) ; Endometrial Glands (EG). Note Glandular Secretion (S). x 400. H & E.



Plate 37: Transverse Section of the Duplex at 7-9 months showing the Endometrial Canal (EmC); Endometrium (E); Myometrium (M); Stratum Vasculare (SV); Blood Vessel (BV); Perimetrium (P). x100. H & E.

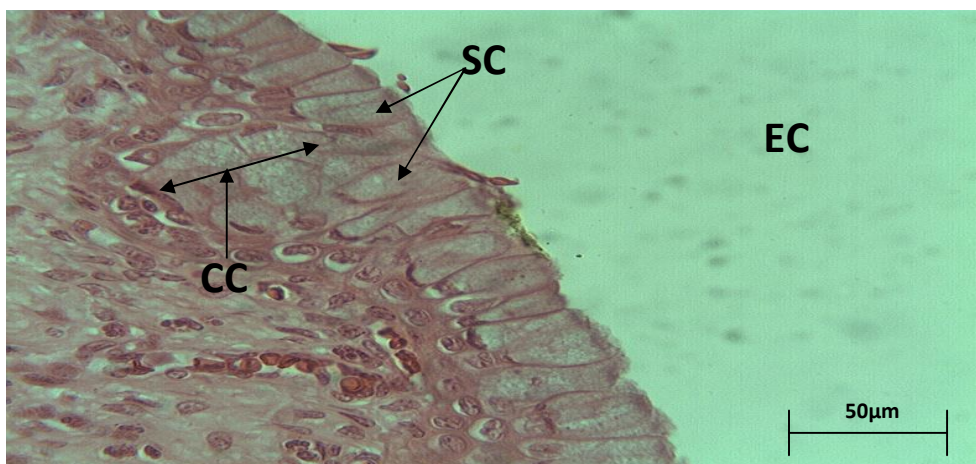


Plate 38: Transverse Section of the Cervix at 1-3 Months showing the Endocervical Canal (EC); Cervical Crypt (CC) Lined by Simple Columnar Secretory Epithelial Cells (SC). x400. H & E.

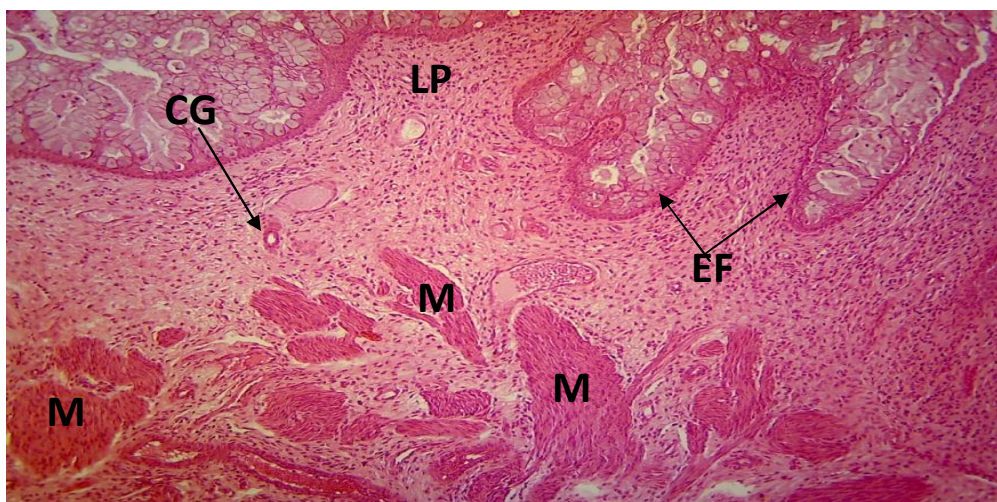


Plate 39: Section of the Cervix at 1-3 Months showing Simple Columnar Secretory Epithelial Cells (SC); Endocervical Fold (EF); Cervical Gland (CG) within the Lamina Propria-Submucosa (LP); Muscularis (M). x100. H & E.

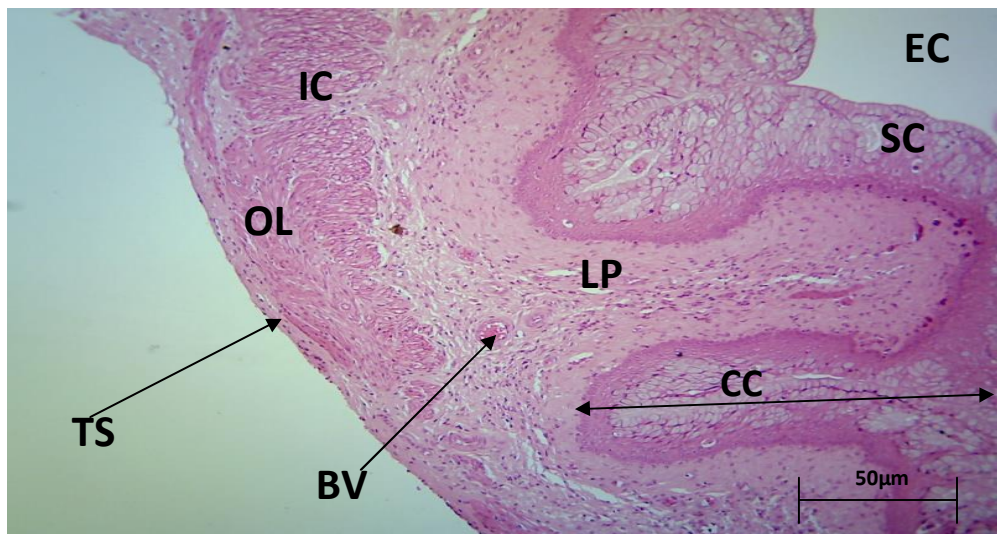


Plate 40: Transverse Section of the Cervix at 4-6 Months showing the Endocervical Canal (EC) lined by Simple Columnar secretory epithelial cells (SC); Cervical Crypt (CC); Lamina Propria-Submucosa (LP); Blood Vessel (BV); Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles; Tunica Serosa (TS). x100. H & E.

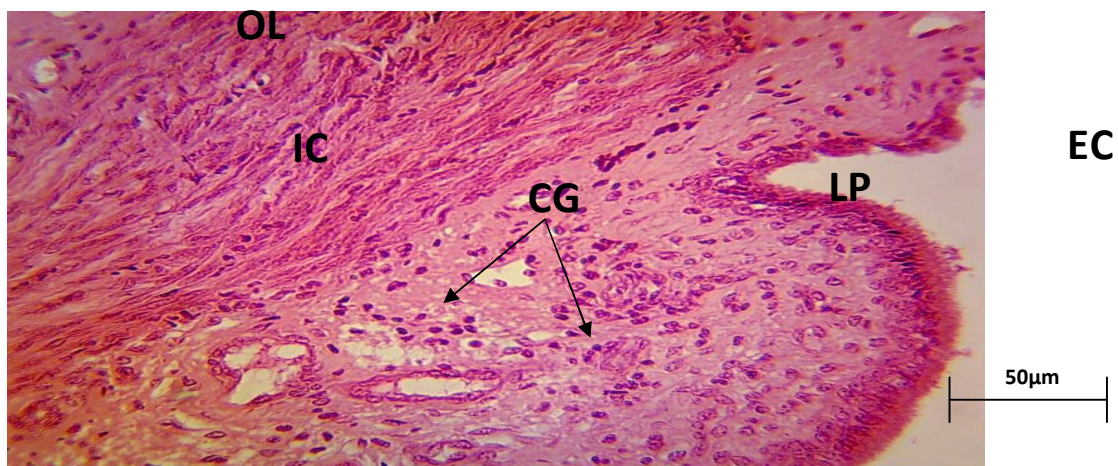


Plate 41: Section of the Cervix at 4-6 Months showing the Endocervical Canal (EC) lined by Simple Columnar secretory epithelial cells (SC); Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles. Note the Cervical Glandular acini (CG) within the Lamina Propria-Submucosa (LP). x400. H & E.

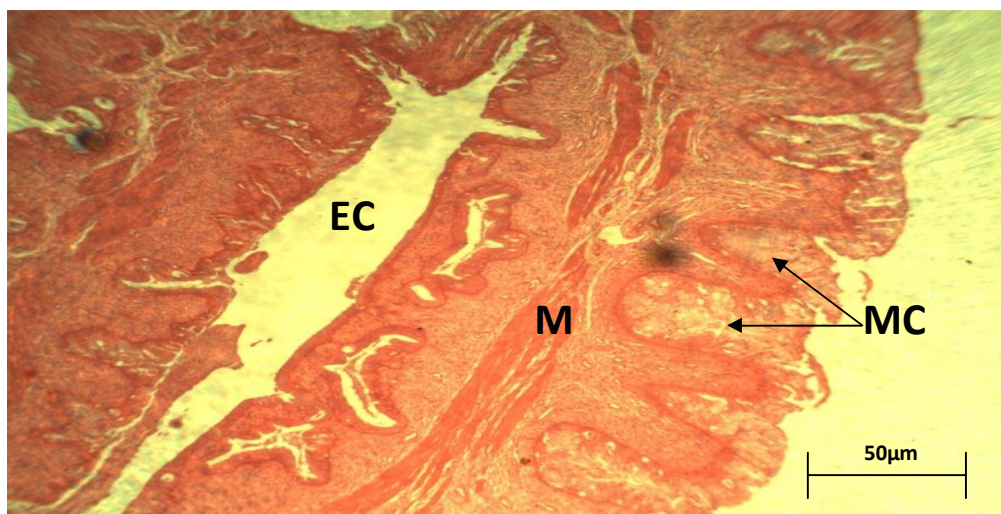


Plate 42: Section of the Cervix at 4-6 Months showing the Endocervical Canal (EC); Muscularis (M). Note the invagination on the external lining with Mucosal Cells (MC). x40. H & E.

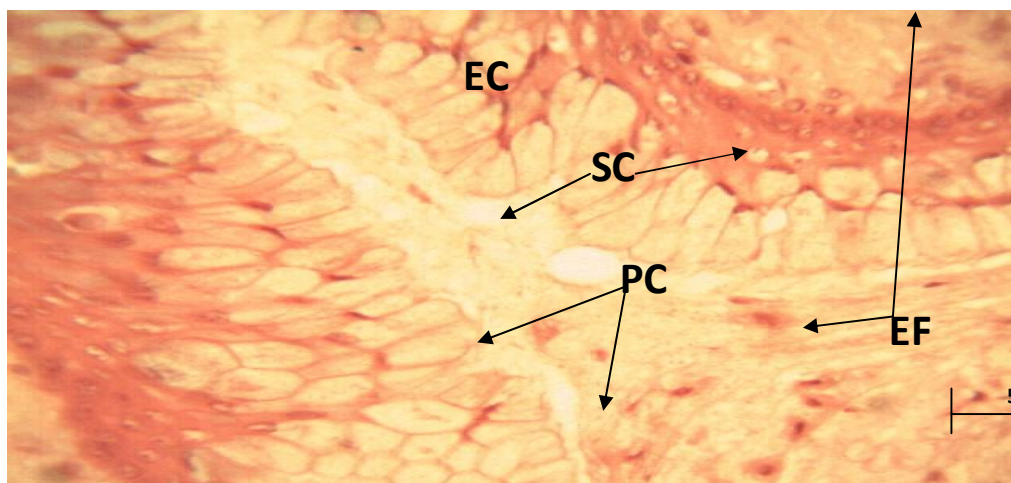


Plate 43: Transverse Section of the Cervix at 7-9 Months Showing the Endocervical Canal (EC) filled with numerous Simple Columnar secretory epithelial cells (SC); Polyhedral Cells secretory epithelial cells (PC) giving a Glassy Appearance. Note the Endocervical Folds (EF). x400. H & E.



Plate 44: Section of the Cervix at 7-9 Months showing the Endocervical Canal (EC); Cervical Crypts (CC) lined by Simple Columnar secretory epithelial cells (SC); Endocervical Fold (EF); Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles. Note the Cervical Glands (CG); Collagen Fibres (C) within the Lamina Propria-Submucosa (LP). x40. H & E.



Plate 45: Section of the Cervix at 7-9 Months showing the Endocervical Canal (EC); Lamina Propria-Submucosa (LP); Muscularis (M). Note that the dorsal aspect of the external lining is normal without mucosal cells, while the ventral aspect had invagination with Mucosal Cells (MC). x40. H & E.

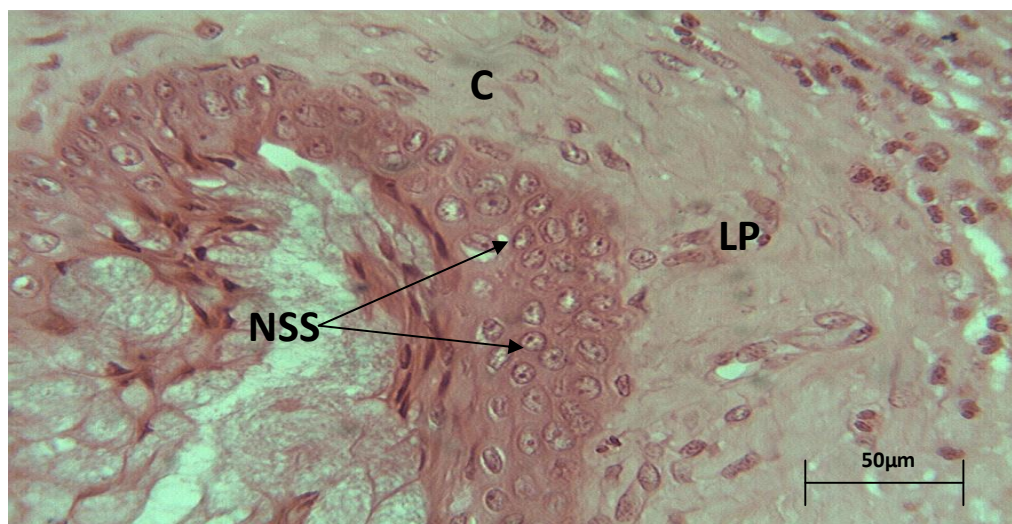


Plate 46: Transverse Section of the Vagina at 1-3 Months showing the Endovaginal mucosa lined by Non-keratinized Stratified Squamous epithelial cells (NSS). Note the Lamina Propria-Submucosa (LP) containing Collagen Fibres (C). x400. H & E.

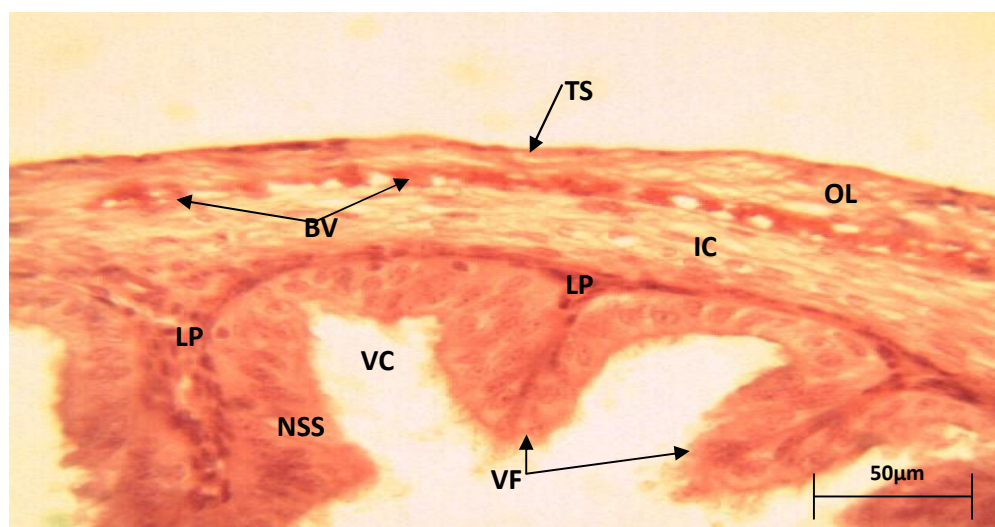


Plate 47: Transverse Section of the Vagina at 1-3 Months showing the Endovaginal Canal (VC); Endovaginal mucosa lined by Non-keratinized Stratified Squamous Epithelium (NSS); Vaginal Folds (VF); Lamina Propria-Submucosa (LP), Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles; Blood Vessels (BV); Tunica Serosa (TS). x400. H & E.

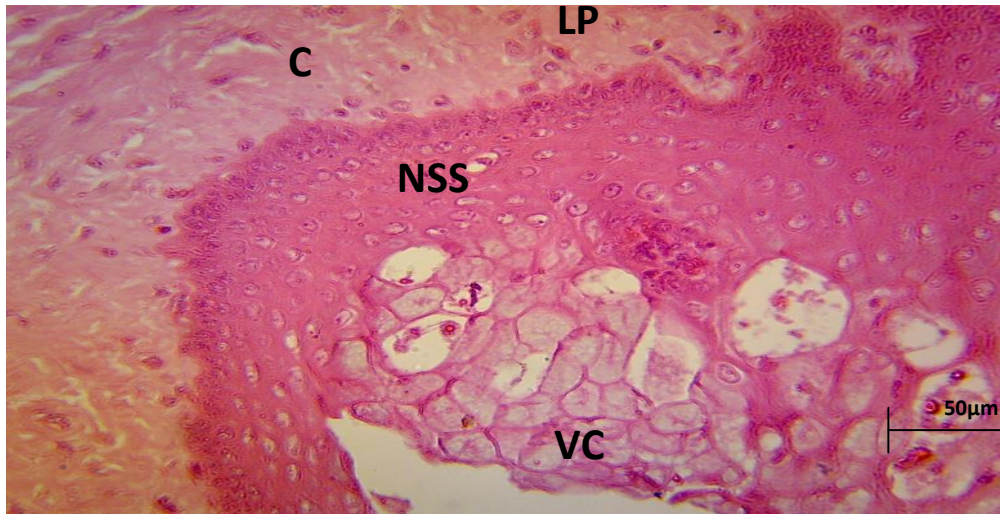


Plate 48: Transverse Section of the Vagina at 4-6 Months showing the Endovaginal Canal (VC) lined by Non-keratinised Stratified Squamous epithelial cells (NSS). Note the Lamina Propria-Submucosa (LP) containing Collagen fibres (C). x400. H & E.

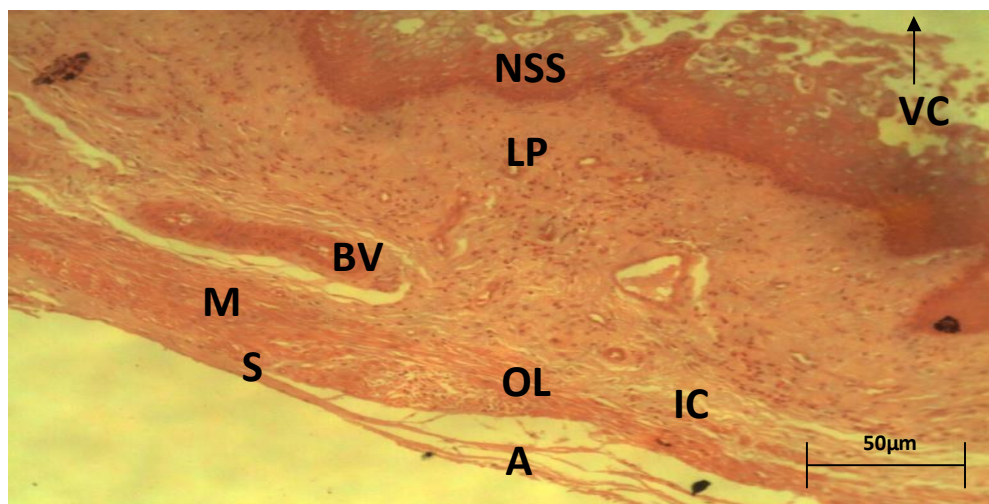


Plate 49: Transverse Section of the Vagina at 4-6 Months showing the Endovaginal Canal (VC) Lined by Non-keratinized Stratified Squamous epithelial cells (NSS). Lamina Propria-Submucosa (LP); Blood Vessel (BV); Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles; Serosa (S); Adventitia (A). x100. H & E.

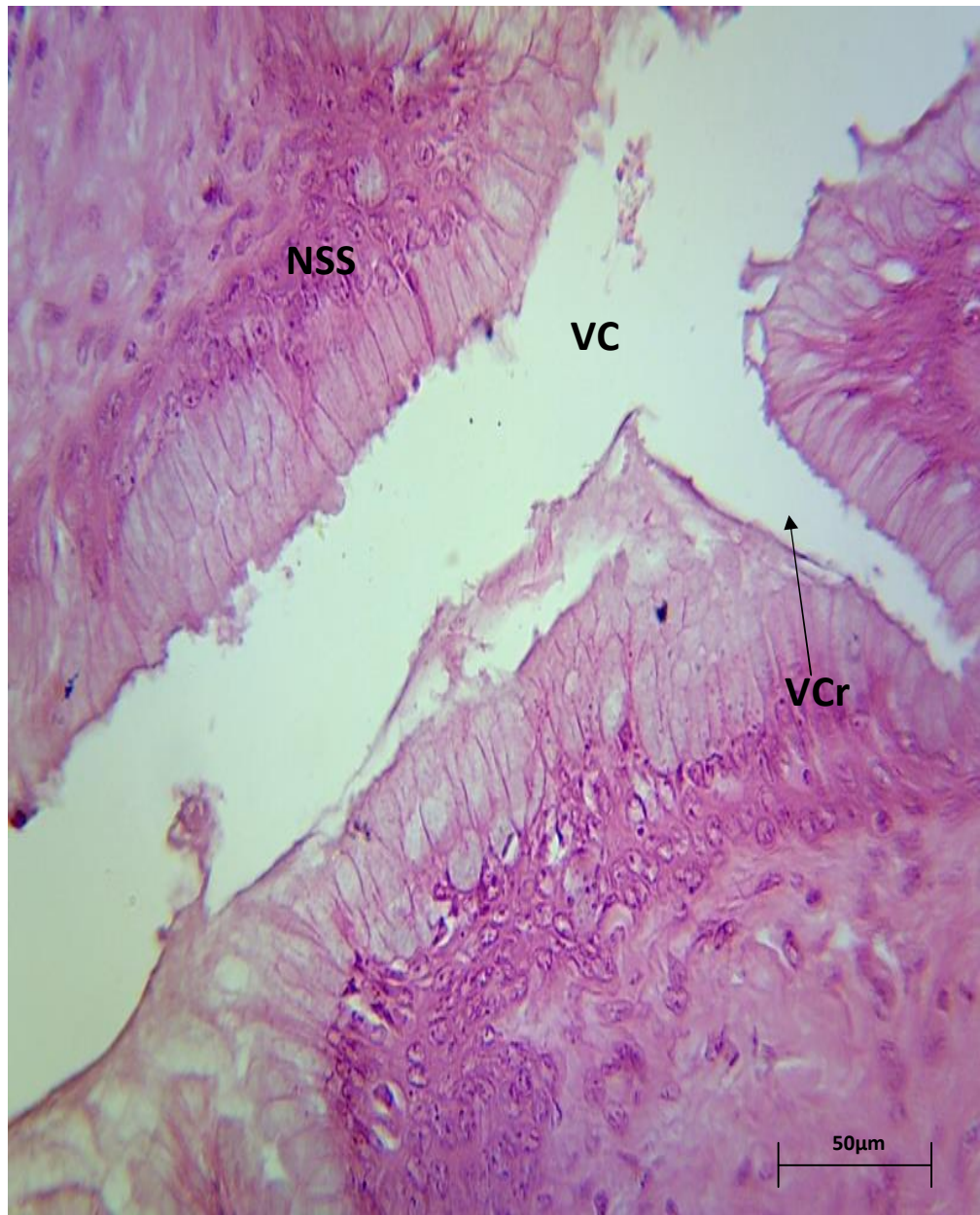


Plate 50: Longitudinal Section of the Vagina at 7-9 Months showing the Endovaginal Canal (VC) lined by Non-keratinized Stratified Squamous epithelial cells (NSS). Note the Vaginal Crypt (VCr). x100. H & E.



Plate 51: Transverse Section of the Vagina at 7-9 Months showing the Endovaginal Canal (VC) lined by Non-keratinized Stratified Squamous epithelial cells (NSS). Note the Conspicuous Vaginal Plug (VP) within the Endovaginal Canal. x400. H & E.

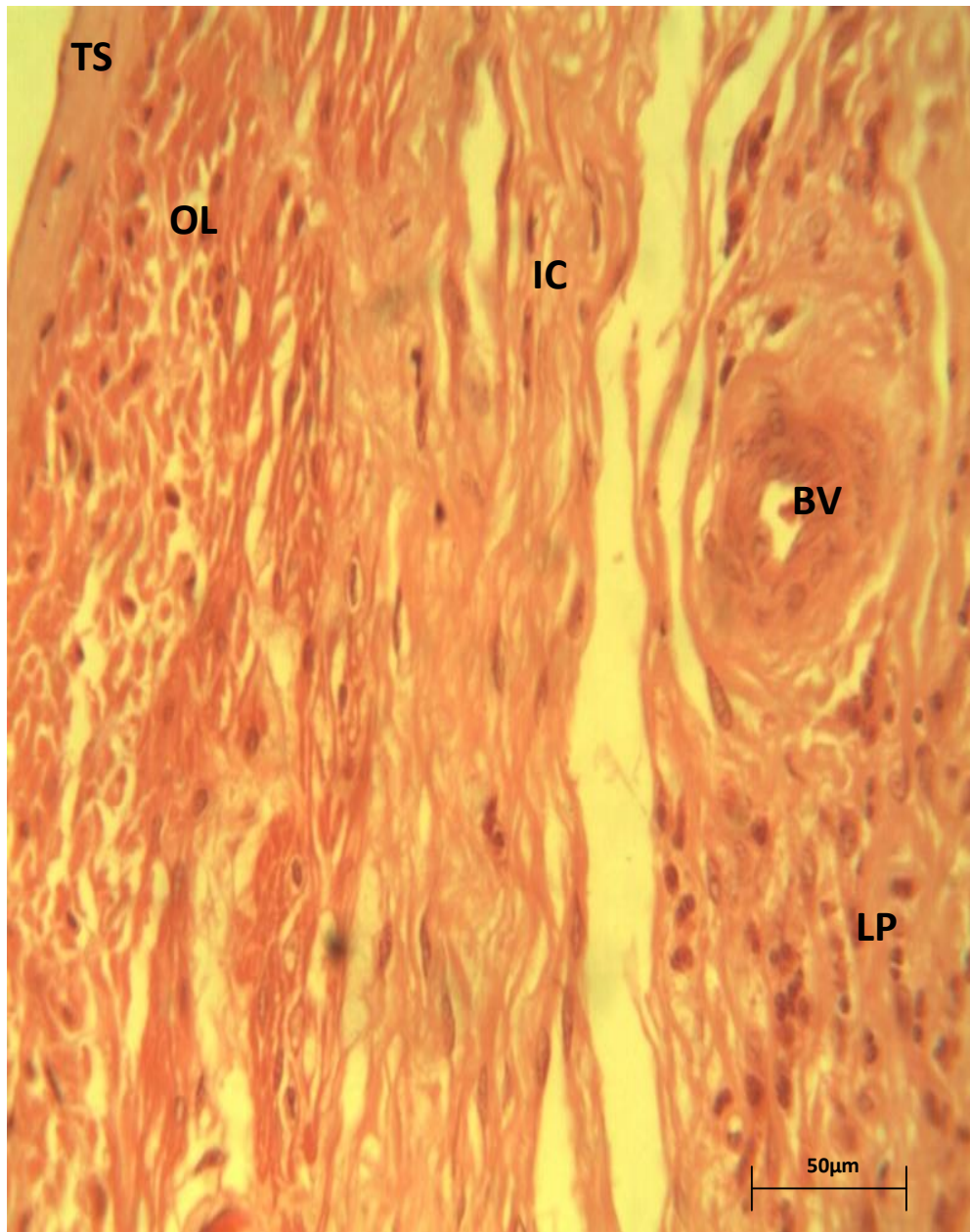


Plate 52: Transverse Section of the Vagina at 7-9 Months showing the Blood Vessel (BV) in the Lamina-Propria-Submucosa (LP); Inner Circular (IC) and Outer Longitudinal (OL) smooth muscles; Tunica Serosa (TS). x400. H & E.

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 GENERAL CONSIDERATION

The female reproductive system of the grasscutter (*Thryonomys swinderianus*) is composed of the left and right ovaries, left and right uterine tubes, left and right uterine horns, uterus duplex, cervix and vagina. Collectively, these components produce and transport the female gametes (ova) for fertilization, as well as receive the male gametes (spermatozoa) and facilitate their transportation. Post fertilization, the conceptus of mammals continues to develop within the tract until birth (Don, 2007).

5.2 OVARY

5.2.1 MORPHOMETRY

The morphometry of the ovaries in the present study showed a positive allometry in the MOSI and the MRL of the left and right ovaries of the grasscutter as the animal advanced in age from 1 to 9 months. This suggests that the contribution by weight and length of the left and right ovaries to the body weight of the grasscutter linearly increased with age; though the left may have contributed more to the body weight, being significantly higher than those of the right.

It may be postulated that the left ovary was more functional than its right counterpart. An earlier study on the ovary of the Japanese quails by Sonfada *et al.* (2006) showed that the MOSI and MRL of the ovaries were significantly increased as the quails advanced in age from 2 to 6 weeks post-hatch. This finding is in agreement with the current study.

5.2.2 POSITION OF THE OOCYTE WITHIN THE OVARIAN FOLLICLE

The results of the present study showed that the position of the oocyte within the ovarian follicle varied with age of the grasscutter; from a central location at 1-3 and 4-6 months of age, to a peripheral position at 7-9 months of age. The peripheral location of the oocyte at 7-9 months may be in preparedness for ovulation (extrusion) from the ovarian follicle as the animal attained puberty.

Bacha and Bacha (2000), explained that further development of the tertiary follicle in the dog, produced matured follicles that can be ovulated for fertilization by the spermatozoa. This is consistent with the current report. The result of the present study is also corroborated by that done on the brush-tailed porcupine ovary; in which it was reported that the cortex had follicular and luteal structures at different stages of development (Mensah *et al.*, 1992a; Jori *et al.*, 2002; Ozdemir *et al.*, 2005). Similarly, Don (2007) demonstrated that the primary oocyte was displaced off to one side by further development of the antrum in the dog, which is in consonance with the present study.

5.2.3 PRESENCE OF CORPUS HAEMORRHAGICUM IN THE OVARIAN CORTEX

The results of the present study also showed the presence of corpus haemorrhagicum in the ovarian cortex at 7-9 months of age, but this structure was absent at 1-3 and 4-6 months of age. Moreover, petechiation, probably due to rupture of the ovarian blood vessels was observed within the wall of the ovary without apparent presence of an oocyte at 7-9 months, but not at 1-3 and 4-6 months of age. Usually, a corpus haemorrhagicum (öbloody bodyö) is the earliest structure to form, peri- and post-ovulation (Don, 2007).

5.2.4 PRESENCE OF CORPUS LUTEUM IN THE OVARIAN CORTEX

The results of the current study demonstrated the presence of corpus luteum in the ovarian cortex at 7-9 months, but this structure was absent at 1-3 and 4-6 months of age. The

corpus luteum (yellow body) is a structure that develops after the formation of the corpus haemorrhagicum. The corpus luteum was made up of remnants of the granulosa cells, remnants of the cumulus oophorus and antral fluid without an oocyte in the follicle. There was an indentation on the ovarian wall, possibly indicating the point of ovulation of the oocyte from the Graafian follicle. The work of Bacha and Bacha (2000) in dogs agrees with this present finding.

Don (2007) reported that the corpus luteum usually produces progesterone which helps to maintain pregnancy if the oocyte is fertilized by the spermatozoa.

5.3 UTERINE TUBES

5.3.1 GROSS ANATOMY

The results of the present study showed the cream-coloured tortuous appearance of the uterine tubes in all the age groups of the grasscutter. Ali *et al.* (2009) reported that the oviduct of the African giant rat appeared convoluted around the mid-caudal aspect of the ovary. In their own report, Codon and Casanave (2009) opined that in the *Xenarthra (Chaetopractus villosus)*, the oviducts were paired tubes extending from the ovaries to the uterine horns. This is consistent with the current report.

Thus, being tortuous and wrapping around the ovaries and uterine horns by the uterine tubes for mechanical support and prevention of rupture, appear to be a natural mechanism by which the grasscutter perpetuates itself; as it was already endangered in the ecosystem.

5.3.2 MORPHOMETRY

The morphometry of the uterine tubes in the present study showed that there was a positive allometry in the MOSI and MRL of the left and right uterine tubes of the grasscutter as the animal advanced in age from 1 to 9 months. However, the MOSI and MRL of the left uterine tubes were significantly higher than those of the right at different age groups. This suggests that

the percentage contribution by weight and length of the left and right uterine tubes to the body weight of the grasscutter linearly increased with age; though the left may have contributed more to the body weight, being significantly higher than that of the right. Sonfada *et al.* (2006) reported that the MOSI and MRL of the Japanese quail oviducts were significantly increased as the quails advanced in age from 2 to 6 weeks post-hatch. This report is in agreement with the current postulation.

5.3.3 APICAL MODIFICATION ON THE MUCOSA OF THE UTERINE TUBES

The results of the present study showed that the uterine tubes had pseudostratified columnar epithelial lining with apical cilia. Non-ciliated columnar cells were absent on the mucosa of the uterine tubes of the grasscutter. The pseudostratified columnar epithelial type may assist in providing some mechanical support to the uterine tubes, while the ciliary action may serve to facilitate propulsion of oocyte through the uterine tubes, towards the uterus duplex. The present study is at variance with that of Joshi *et al.* (1977), who observed that the goat's fallopian tube was lined by both non-ciliated cells and ciliated cells. According to Don (2007) the uterine tubes of the cow and sow were lined by pseudo-stratified columnar epithelia, while that of the dog and cats were lined by simple columnar epithelia. Moreover, he said non-ciliated columnar cells existed within the epithelial lining and could be secretory and nutritional to the gametes. He reported further that such secretions may play important roles during capacitation of the spermatozoa within the female reproductive tract.

Furthermore, he explained that during ovulation, the non-ciliated columnar cells became metabolically more active, increasing in their height, being sometimes referred to as peg cells. This is at variance with the present study. It could therefore imply that the uterine tubes of the grasscutter had little secretory and nutritive functions on the gametes.

5.4 UTERINE HORNS

5.4.1 MORPHOMETRY

The morphometry of the uterine horns showed that the MOSI and MRL of the left and right uterine horns of the grasscutters linearly increased as the animal attained puberty. However, the MOSI and MRL of the left were significantly higher than those of the right at different age groups. The indication is that the contribution by weight and length of the left and right uterine horns to the body weight of the grasscutter linearly increased with age. However, the left uterine horn must have contributed more to the body weight, as it was significantly higher than the right.

5.4.2 NUMBER AND SIZE OF THE ENDOMETRIAL GLANDULAR ACINI OF THE UTERINE HORNS

The results of the present study showed that at 1-3 and 4-6 months of age, there were sparse and smaller endometrial glandular acini in the uterine horns; whereas at 7-9 months of age, they were numerous and larger. It may be inferred that the endometrial glandular acini at 1-3 and 4-6 months of age were sparse and smaller due to immaturity and non-cycling of the grasscutter.

However, the endometrial glandular acini at 7-9 months of age were numerous and larger because the animals may have attained puberty and started cycling. Saruhan *et al.* (2006) reported that the endometrium of the rat uterine horns contained sparse simple tubular glands. This strongly agrees with the present study.

5.5 UTERUS DUPLEX

5.5.1 GROSS ANATOMY

The findings in this study demonstrated the presence of a cream-coloured uterus duplex in all the age groups of the grasscutter. Since the ovum is released from the ovaries of the left and right sides of the grasscutter in an alternate manner, one side of the uterus duplex is usually being rested. This phenomenon may buttress the reason why grasscutters are known to rarely abort, but carry their pregnancies to term. Many researchers have reported the occurrence of a uterus duplex in other rodents like the laboratory rat, Mongolian gerbil, rabbit, African giant rat and mice (Hebel and Stromberg, 1976; Camilla *et al.*, 2001; Praag, 2003; Ali *et al.*, 2009; Claudio *et al.*, 2009; Ozdemir and Atalar, 2009)).

The findings of Claudio *et al.* (2009) that in the mice, there existed two cranial portions of the uterine body separated by a median septum and a single caudal undivided portion- the cervix, is in agreement with the present report on the grasscutter.

5.5.2 MORPHOMETRY

The results of the present study showed that the MOSI and MRL of the uterus duplex of the grasscutters progressively increased as the animal attained puberty. This suggests that the contribution by weight and length of the uterus duplex to the body weight of the grasscutter linearly increased with age.

5.5.3 NUMBER AND SIZE OF THE ENDOMETRIAL GLANDULAR ACINI OF THE UTERUS DUPLEX

The results of the present study showed that at 1-3 and 4-6 months of age, there were sparse and smaller endometrial glandular acini (simple tubular glands) in the uterine body.

However, at 7-9 months of age, there were numerous and larger endometrial glandular acini (coiled tubular glands) in the uterus duplex. The endometrial glands are known to be involved in the elaboration of secretory products that nourish the conceptus. Since the grasscutter may not have started cycling at 1-3 and 4-6 months of age, the glandular acini in the endometrium could be sparse and small, but numerous and larger at 7-9 months of age, probably due to cycling. Also, in all the age groups, there were openings of the endometrial glandular acini into the endometrium which were indicated by invagination on the surface of the endometrium; although they were deeper at 4-6 months of age than at 1-3 months, but deepest at 7-9 months of age.

Bazer (1975) reported that the uterine glands of ruminants synthesize and secrete a variety of substances collectively referred to as histotroph. Again, Don (2007) reported that numerous endometrial glands surrounded by variably developed vasculature and connective tissue elements were present in the dog. Also, Igwebuikwe (2012), reported that in addition to haemotrophic nutrition, the conceptus requirement for histotroph remains critical during late pregnancy in West African dwarf (WAD) goats. These reports are in line with the report of the present study.

5.7 CERVIX

5.7.1 GROSS ANATOMY

The results of the present study showed a rubber-stopper texture of the cervix in all the age groups of the grasscutter. The cervix had very firm consistency on palpation probably due to the abundance of smooth muscles in its wall. Sissons and Grossman (1966) reported that the cervix of the dog constitutes a thick muscular coat. However, Frandson and Whitten (1981) established that the cervix of the cow constitutes a smooth-muscle sphincter between the uterus and the vagina. According to these authors, the cervix is usually tightly closed except during oestrus (heat) or at parturition, and the inner surface of the cervix is arranged in a series of circular ridges (rings) called annular folds in ruminants and pigs.

The rubber-stopper texture of the cervix may help to mechanically seal-off the uterus during pregnancy, and so prevent abortion, which otherwise may result in foetal losses and economic wastage. The muscular nature of the cervix confers it with the capacity to dilate adequately without getting ruptured during kindling. The muscular texture of the cervix may also assist in closing up the uterus post partum, so as to mechanically prevent ascending infection due to opening of the uterus during parturition. The morphometry of the present study also showed that the MOSI and MRL of the cervixes of the grasscutters appreciably increased as the animal advanced in age. This suggests that the contribution of the weight and length of the cervix to the body weight linearly increased as the grasscutter attained puberty.

5.6.2 APICAL MODIFICATION ON THE ENDOCERVICAL MUCOSA

In the present study, it was observed that the endocervical mucosa of the cervix was lined by simple columnar secretory epithelium. Moreover, there were mucosal invaginations called endocervical crypts and endocervical folds in all the age groups of the grasscutter studied. Simple columnar secretory epithelium was observed at 1-3 and 4-6 months of age. While at 7-9 months of age, polyhedral secretory cells were found together with the simple columnar secretory epithelium. The endocervical crypts visually deepened with more convolutions of the endocervical folds as the animal attained puberty.

The columnar secretory and polyhedral cells may produce cervical secretion that is viscous and this may assist in forming cervical plug that seals off the uterine opening during pregnancy. In addition, this will mechanically prevent ascending infection into the uterus by invading microbes. During oestrus, the cervical secretion becomes serous; to permit the spermatozoa to travel up the uterine tubes to fertilize the oocyte. The cervical secretion is also important in lubricating the birth canal during kindling.

The endocervical crypts and endocervical folds may be useful in increasing the surface area of the cervix; especially during pregnancy and parturition when much tension is exerted on the cervix by the foetus. Saruhan *et al.* (2006) showed that primary and secondary longitudinal folds occurred throughout the endocervical mucosa of the rat, almost occluding the endocervical lumen. Don (2007) also stated that during pregnancy in the dog, the cervix acts like a valve and seals off the uterus from the vagina. He said this valve-like sealing-off protects the foetus from being aborted, or harmed by microbes. This agrees with the present study.

5.6.3 MUCOSAL CELLS ON THE *EXTERNUM* OF THE CERVIX

The *externum* of the grasscutter cervix exhibited numerous invaginations with mucosal cells in all the age groups studied. Visual estimation revealed that the invaginations increased both in number and depth as the animal attained puberty. This may be an adaptation to increase the surface area of the cervix to allow maximum stretch, both during pregnancy and kindling. The mucosal cells secrete mucus, which aid in lubricating the *externum* of the cervix. This prevents abrasion on the cervix, and subsequent maintenance of the integrity of the cervix, especially during the gestational period. By this, the elasticity of the cervix could be maintained so as to seal off the uterus duplex and help in safeguarding the developing foetus within the endometrium. The mucus secreted by the mucosal cells could also mechanically trap invading microbes within the peritoneal cavity, before other immune responses could be triggered by the body. The mucosal cells on the *externum* of the cervix can thus serve as the first line of defence to invading microbes within the peritoneal cavity.

Although there is dearth of information on this morphological modification of the cervix in other rodents and domestic animals, it is possible that occurrence of mucosal cells on the *externum* of the cervix of the female grasscutter, may enable the grasscutter to carry its foetuses

to term, without abortion. This may be important in the conservation and perpetuation of the already endangered grasscutter species.

5.7 VAGINA

5.7.1 MORPHOMETRY

The findings of the present study showed that the MOSI and MRL of the vagina of the grasscutters progressively increased as the animal attained puberty. This may suggest that the contribution of the weight and length of the vagina to the body weight increased linearly with attainment of puberty.

5.7.2 APICAL MODIFICATION ON THE TUNICA MUCOSA OF THE VAGINA

The findings of the present study showed that the tunica mucosa of the vagina was lined by non-keratinized stratified squamous epithelium. There were also exhibition of endovaginal crypts and folds on the tunica mucosa in all the age groups. Visual observation revealed that the endovaginal crypts deepened and the folds became more convoluted as the animal attained puberty. Since this segment of the female reproductive tract is in close proximity to the exterior, the type of epithelium found here would assist in withstanding abrasion.

The endovaginal crypts and folds would aid in increasing the surface area of the vagina for expansion; both during copulation by the male and kindling. Saruhan *et al.* (2006) reported that the epithelium of the vagina of the rat was of a non-keratinized stratified squamous type. Don (2007) also reported that the mucous secretion found in the vagina of dogs originated from the cervix. These reports agree with the present study.

5.7.3 PRESENCE OF VAGINAL PLUG IN THE VAGINAL LUMEN

In the present study, it was demonstrated that at 7-9 months of age, a very conspicuous pear-shaped and intensely eosinophilic structure, the vaginal plug, was present; but was absent both at 1-3 and 4-6 months of age of the grasscutters studied. Stockyard and Papanicolaou (1919) reported that the vaginal plug is made up of sloughed-off endometrium and endovaginal mucosa, as well as cervical and vaginal secretions. Although there are no secretory glands within the vagina, the cells of the thick non-keratinized stratified squamous epithelium become filled with glycogen before desquamation. The thin-walled veins of the endovaginal mucosa and muscular layers exude fluid into the epithelium. This greatly contributes to the formation of the vaginal plug along with secretions from the cervix and the sloughing off of the endometrium.

The vaginal plug is only present in the pubertal animal during anoestrus; and may prevent intromusion of the male penis; but breaks down during the grasscutter's periodic vaginal membrane perforation to permit copulation (Addo *et al.*, 2007; Anthony, 2010). This phenomenon is observed in hystricomorph rodents and can be used as a strong indicator for selecting breeders for use in captivity.

5.8 SUMMARY

In summary, the present study demonstrated that grossly, the uterine tubes of the grasscutter in all the age groups were long and tortuous. The uterine body exhibited a duplex state. The cervix in all the age groups had a rubber-stopper texture due to the well developed musculature. Morphometrically, the MOSI and MRL of the ovaries and all the organs comprising the female reproductive tract (uterine tubes, uterine horns, uterus duplex, cervix and vagina) increased significantly as the animal attained puberty; though the left side was significantly higher than the right side.

It can thus be inferred that as the grasscutter attained puberty, the weight and length of the various organs comprising the female reproductive system proportionately increased. Therefore, the size and length of the organs comprising the female reproductive system were not determined pre-natally. Furthermore, it can also be inferred that the left side of the female reproductive system was functionally more active than the right side.

Histomorphologically, it was observed from the present study that the oocyte was centrally located in the ovarian follicle at 1-3 and 4-6 months of age; but eccentric at the periphery of the ovarian follicle within the ovarian cortex at 7-9 months of age. Again, corpora haemorrhagica and lutea that were absent at 1-3 and 4-6 months of age, were present at 7-9 months of age in the ovarian cortex. On visual observation, the cilia on the tunica mucosa of the uterine tubes at 7-9 months of age, appeared longer than those at 1-3 and 4-6 months of age; thereby facilitating the movement of the oocyte via the uterine tube when released.

Subsequently, larger and more numerous coiled endometrial glandular acini were observed at 7-9 months of age than those at 1-3 and 4-6 months of age. Cervical crypts deepened and the cervical mucosal folds had more convolutions at 7-9 months of age than at 1-3 and 4-6 months of age. Similarly, the invaginations on the *externum* of the cervix increased in depth as the animal attained puberty; thereby enabling a greater surface area to be attained during pregnancy and kindling. Mucosal cells were also present within the invaginations, which aided lubrication of the cervix as well as mechanically trapping any invading microbe within the peritoneal cavity.

The present study showed that the tunica mucosa of the vagina was lined by non-keratinized stratified squamous epithelium in all the age groups; but better developed at 7-9 months of age. The vaginal crypts and folds also deepened and got more convoluted, respectively, as the animal attained puberty. The vaginal plug, a pear-shaped intensely eosinophilic structure, was observed in the lumen of the vagina at 7-9 months of age; but absent

at 1-3 and 4-6 months of age. The presence of the vaginal plug in the vaginal lumen is a strong indicator of puberty. Secretory products from the cells of the non-keratinized stratified epithelium, the endovaginal mucosa, the cervix and sloughed-off debris of the endometrium may assist in forming the vaginal plug.

5.9

CONCLUSION

It could be asserted that because of these indicators above, puberty in the female grasscutter was attained at 7 months of age. Breeders and researchers can avail themselves of this information and select their breeding stock of female grasscutters from 7 months of age. The actual origin and mechanism of formation of the vaginal plug is open to further research.

This is the first time a concise study of the gross, morphometry and histomorphology (at the level of light microscopy) of the ovaries, uterine tubes, uterine horns, uterus duplex, cervix and vagina of the grasscutter at different age groups (1-9 months of age) would be reported. It is hoped that breeders and researchers would gain from the information provided in this study, so as to minimize the dearth of information on the reproductive biology of the female grasscutter.

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APPENDIX I

ABSOLUTE WEIGHT OF EACH ORGAN COMPRISING THE FEMALE REPRODUCTIVE SYSTEM OF THE GRASSCUTTER AT DIFFERENT AGES

G	LO (g)	RO (g)	LUT (g)	RUT (g)	LUH (g)	RUH (g)	UD (g)	C (g)	V (g)	LW (g)	A (m)
1.	0.4	0.1	2.2	2.0	2.2	2.0	2.2	3.8	3.1	580	1
2.	0.5	0.2	2.3	2.1	2.3	2.1	2.3	3.9	3.2	600	2
3.	0.5	0.2	2.3	2.2	2.4	2.2	2.4	3.9	3.3	615	2
4.	0.6	0.3	2.4	2.3	2.5	2.3	2.6	4.1	3.4	640	3
5.	0.6	0.4	2.4	2.4	2.6	2.4	2.7	4.3	3.5	650	3
6.	0.7	0.6	3.1	2.8	3.2	2.8	3.8	4.3	3.5	670	4
7.	0.8	0.7	3.1	2.9	3.3	2.9	3.9	4.4	3.5	675	4
8.	0.9	0.8	3.2	3.0	3.4	3.0	4.0	4.5	3.6	695	5
9.	1.1	0.9	3.3	3.1	3.5	3.1	4.1	4.6	3.6	710	5
10.	1.2	1.0	3.4	3.2	3.6	3.2	4.2	4.7	3.8	760	6
11.	1.5	1.2	3.7	3.4	3.7	3.5	4.3	4.8	3.9	825	7
12.	1.6	1.3	3.7	3.5	3.8	3.6	4.3	4.8	3.9	840	7
13.	1.7	1.3	3.8	3.7	3.9	3.7	4.5	4.9	3.9	885	7
14.	1.9	1.4	4.0	3.9	4.0	3.8	4.6	5.1	4.0	970	8
15.	2.0	1.5	4.4	4.1	4.2	4.0	4.8	5.4	4.3	1,450	9

LEGEND

G: Grasscutter Nunber;

RO: Right Ovary;

RUT: Right Uterine Tube;

RUH: Right Uterine Horn;

C: Cervix;

LW: Live Weight;

g: grams;

LO: Left Ovary;

LUT: Left Uterine Tube;

LUH: Left Uterine Horn;

UD: Uterus Duplex;

V: Vagina;

A: Age;

m: months;

APPENDIX II

ABSOLUTE LENGTH OF EACH ORGAN COMPRISING THE FEMALE REPRODUCTIVE SYSTEM OF THE GRASSCUTTER AT DIFFERENT AGES

G	LO (cm)	RO (cm)	LUT (cm)	RUT (cm)	LUH (cm)	RUH (cm)	UD (cm)	C (cm)	V (cm)	CR (cm)	A (m)
1.	1.55	1.25	10.90	10.60	5.30	5.10	4.70	2.45	3.65	24.00	1
2.	1.65	1.35	10.95	10.65	5.35	5.20	4.75	2.50	3.70	25.25	2
3.	1.75	1.45	11.00	10.65	5.35	5.20	4.80	2.55	3.70	25.30	2
4.	1.85	1.55	11.15	10.70	5.45	5.30	4.85	2.70	3.80	25.70	3
5.	1.90	1.60	11.35	10.80	5.65	5.45	4.95	2.80	3.95	25.80	3
6.	1.95	1.65	13.10	11.10	5.75	5.50	5.35	2.85	4.05	26.10	4
7.	2.00	1.70	13.20	11.15	5.80	5.55	5.40	2.90	4.05	26.20	4
8.	2.05	1.85	13.35	11.20	5.85	5.60	5.45	2.95	4.10	26.45	5
9.	2.10	1.90	13.40	11.25	5.90	5.65	5.50	2.95	4.15	26.55	5
10.	2.20	1.95	13.50	11.45	6.05	5.80	5.65	3.05	4.20	26.85	6
11.	2.25	2.05	16.35	14.70	6.75	6.50	6.45	4.20	4.45	27.95	7
12.	2.35	2.10	16.40	14.75	6.80	6.55	6.50	4.30	4.55	28.15	7
13.	2.40	2.10	16.45	14.90	6.85	6.60	6.65	4.35	4.65	28.40	7
14.	2.50	2.20	16.55	15.10	6.95	6.80	6.85	4.50	4.90	30.20	8
15.	2.65	2.40	16.75	15.35	7.45	7.25	6.95	4.75	5.00	35.05	9

LEGEND

G: Grasscutter Nunber; **LO:** Length of Cranial to Caudal Pole of the Left Ovary;

RO: Length of Cranial to Caudal Pole of the Right Ovary;

LUT: Left Uterine Tube;

RUT: Right Uterine Tube;

LUH: Left Uterine Horn;

RUH: Right Uterine Horn;

UD: Uterus Duplex;

C: Cervix;

V: Vagina;

CR: Crown-Rump Length;

A: Age;

cm: centimeters;

m: months;

