ARTERIAL SUPPLY AND HISTOLOGY OF THE FEMALE REPRODUCTIVE ORGANS OF THE AFRICAN LION

by
Dr. Marthinus J. Hartman BVSc (Hons)

Submitted in partial fulfilment of the requirements for the MSc degree

in the
Faculty of Veterinary Science
University of Pretoria

April 2013

DECLARATION

I, Marthinus J Hartman do hereby declare that the research presented in this dissertation, was conceived and executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor any part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other University.

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Marthinus J Hartman

Date: ……………………………………………..

Research conducted in the Section of Anatomy
Department of Anatomy and Physiology
Faculty of Veterinary Science
University of Pretoria

Supervisor:

Professor Hermanus B Groenewald BVSc PhD
Section of Anatomy
Department of Anatomy and Physiology
Faculty of Veterinary Science
University of Pretoria

To my lovely wife Suenette for her love and support and to my dear children M.J., Erik and Niel for their unconditional love and sacrifices to the cause of my career.

Quote

Every great success begins with a dream. Always remember you have within you the strength and the ability to fulfil your dream if only you can find the courage and the passion.

Modified: Harriet Tubman

Acknowledgements

My sincere appreciation to the following people for their contributions in empowering me to complete this dissertation:

My parents Thinus and Elna for providing me with huge support throughout my life and supplying all the basic life skills and principles that have lead to success. You have played the most important role in my life and I will forever be grateful for the impact you have made on me as a child and as a person. Thank you for the example you have set in our home and the confidence you have instilled in me.

To my wife lovely Suenette for your love and continued support for my career. I do believe that this will bear some fruit in our future together.

Our sons M.J., Erik and Niel for trusting me to guide your lives and our destiny as a family. At times my career does claim some of the time I owe to you as my children and I can only hope that there will be some reward for you in time to come. You are the most precious assets in our lives and will always be.

Professor Herman Groenewald for allowing me to conduct this research in your department and for your valuable guidance and time during this study.

Mr. Paul Carnall of Advanced Materials Technology (Pty) Ltd., Kempton Park, South Africa for supplying technical assistance.

Wilma Olivier for your kind assistance with the logistical challenges.

Charmaine Vermeulen for being so patient and precise while performing the photography.

Adam Flink and Mishack Mothemane for your help and assistance with the lions in the Anatomy halls.

To my saviour Jesus Christ for blessing my research.

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None

Summary

Arterial supply and histology of the female reproductive organs of the African lion (*Panthera leo*).

Marthinus J. Hartman marthinus.hartman@up.ac.za

This masters project was undertaken to have a better knowledge of the female lion reproductive tract and to equip the author for future surgical studies on this organ system. The objectives of this study were to describe the arterial supply and histology of the female reproductive organs of the African lion.

The reproductive organs of three embalmed cadavers and two fresh carcasses from three-year-old known aged nulliparous lionesses weighing between 120 kg and 140 kg were studied. The project was approved by the Animal Use and Care Committee and Research Committee of the University of Pretoria (protocol number V038-09).

The arterial supply of the reproductive organs was studied and described *in situ* and after removal and histology was subsequently performed. A novel technique in Veterinary anatomy involving the maceration of a silicone cast was used in the two fresh carcasses and all five specimens were incorporated in the comparative and arterial studies. Histology was performed on organs from the three embalmed cadavers.

The anatomical information obtained during this study was subsequently applied in a surgical study on sixteen lionesses using laparoscopy to perform laparoscopic ovariectomy and salpingectomy. The availability of these two laparoscopic procedures subsequently led to a wider interest to its application in population control of lions in the smaller national parks of South Africa.

Key terms

African lion, *Panthera leo*, silicone cast, arterial supply, histology, reproductive organs

Chapter 1
Silicone cast *in situ*: A technique to study the arterial supply of the female reproductive organs of the African lion

1. Introduction

This chapter of the dissertation was based on a human study by Aultman and co-workers in 2003. In this study a process describing four stages was advocated that included the preparation by which organs were freshly harvested from the cadaver and all vessels rinsed with warm water. Specimens were then placed in a running water bath overnight before injection of a silicone preparation the next day. The water remaining in the vessels was flushed out using compressed air before injection. If injection was planned for a later day, the specimens were placed in a 3-5% formaldehyde solution. Silicone injection followed where specimens are injected with an E RTV Silicone (Dow Corning, Midland, MI, USA) at room temperature. The silicone was coloured red or blue with Biodur E 20 (Biodur, Heidelberg, Germany); RTV Silicone Rubber Curing Agent was added at a 1:10 ratio to the silicone polymer immediately prior to injection. The curing process ensued where the silicone was allowed 24-48 hours at room temperature to harden. The last process was maceration by which specimens were placed in a freshly prepared 10% potassium hydroxide (Potassium Hydroxide Flakes, Fisher scientific, USA) solution for 5-7 days and then left in boiling water for 8-12 hours to detach tissues from the polymer. Thereafter specimens were placed in 5% hydrogen peroxide for about 2 hours to complete the removal of residual tissues. Casts were then rinsed in running water overnight[1]. The result of this study was that the casts were of excellent quality in terms of flexibility, clarity of details and anatomical accuracy. Specimens' sturdiness was assessed in terms of flexibility during regular handling and stretching.

The aim of this study was to describe a novel technique in Veterinary medicine using silicone to provide a flexible cast which could depict and aid in describing the arterial supply to the female reproductive organs of the African lion *in situ*.

2. Justification

2.1 Literature review

Currently there is no description of the arterial supply of the female reproductive organs of the African lion available in the literature.

Although the Italian Guido da Vigevano crudely depicted neuro-anatomical descriptions as early as 1345[2] the use of casts to make accurate anatomical descriptions has progressively evolved since the late fifteenth or early sixteenth century when Leonardo da Vinci was one of the first anatomists to use wax injection of ovine brain ventricles[3]. Gunther von Hagens is a German anatomist who invented the technique for preserving biological tissue specimens called plastination using various products during the late twentieth century[4-6]. The preparation of organ silicone casts in human gross anatomy to study the trachea and bronchi[7] and its associated vasculature[8] and the chambers of the heart and its great vessels[9] was done since 1992. In 1995 a resin cast with E20 red was used to study anatomical cavities[10] and the comparative use of gelatine and silicone in human brain ventricles was done in 2000[11].

A more comprehensive study to evaluate the enhancement of the quality and durability of silicone specimens of the human heart, tracheobronchial tree and brain ventricles was done. In this study a process describing four stages was advocated that included harvesting and preparation of organs, silicone injection, curing and maceration[1]. The sequence in which plastination chemicals are added can improve stability of the silicone impregnation mixture at room temperature[12] however a cold-temperature technique has also been described[13]. Recently a silicone cast of the uterine lumen of a mare was reported[14] and the preservation of organs using an alkyd resin has been used in various species[15].

In order to perform laparoscopic sterilization of the African lioness (*Panthera leo*) a sound knowledge of the arterial supply of the female reproductive tract is required.

In this study two fresh carcasses were used to perform a silicone study of the arterial supply to the female reproductive organs. A local company in South Africa was sourced to supply silicone materials, red pigment and equipment[16]. The project was approved by the Animal Use and Care Committee and Research Committee of the University of Pretoria (protocol number V038-09).

2.2 Problem statement

There is no description of the arterial supply to the female reproductive organs of the African lion available. A reliable technique to depict the fine arterial supply to an organ system *in situ* and in cast will aid in describing this aspect of the anatomy.

To perform laparoscopic sterilization of the lioness a sound knowledge of the arterial supply of the female reproductive tract is also required.

2.3 Research objectives

The purpose of this study was to develop an additional technique to study the arterial supply of the female reproductive organs in the African lion.

2.4 Hypothesis

Silicone casting can provide a reliable technique to aid in the study of the arterial supply to organs *in situ* and in cast.

2.5 Benefits

Provide an additional method to study and describe the arterial supply of the female reproductive organs of the African lion which will provide background knowledge for future reference during laparoscopic surgery performed on the described organs.

3. Materials and Methods

3.1 Sample population

Two nulliparous lionesses aged between 24 and 36 months weighing 118.6 kg and 122.0 kg respectively were used. They were captured and transported to the Department of Anatomy and Physiology, Faculty of Veterinary Science, Onderstepoort, South Africa under sedation and constant monitoring.

3.2 Procedure

A jugular catheter was placed and intravenous fluids were administered at a rate of 10 ml/kg/hr. Heparin sodium 5000IU/ml (Fresenius 5ml/vial) at a dose of 75 Units/kg (1.8ml) was injected intravenously through the catheter situated in the external jugular vein. 3L Ringer's lactate was used to flush the entire cardiovascular system through. After approximately 2L of Ringer's lactate had run in a metal trocar and cannula was placed in the contralateral carotid artery by means of a cut down technique. While under anaesthesia the lionesses were bled out into a trough until the heartbeat stopped. Euthanasia was therefore effected without recovery from anaesthesia, while patients were still in plane two of anaesthesia. Both lionesses were ear marked with numbered yellow Aussie ear tags (Milborrow).

An incision was made through the abdominal wall at the lateral border of the rectus abdominus muscle. Two additional incisions extending from this paramedian incision to the lateral border of the epaxial muscles directly caudal to the thirteenth rib as well as cranial to the tensor fascia lata muscle were completed. The lateral abdominal wall was reflected dorsally using this technique bilaterally. Since this was a fresh specimen without rigor mortis it was possible to dorsally reflect all the layers of the lateral abdominal wall. Because the caudal vena cava is situated to the right and ventral of the aorta, the aorta was approached from the left and was ligated immediately cranial to the renal arteries in order to secure preservation of bilateral origins of the ovarian arteries.

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The caudal mesenteric artery was ligated where it was readily accessible in the mesocolon to prevent silicone from being lost to the arterial supply to the large and small intestine. No other dissection was done in order not to lacerate any blood vessels resulting in seepage of silicone product.

3.3 Preparation of the product

One kilogram of Mold Max 30 silicone[16] was used. Ten percent (100 grams) parts by weight silicone thinning fluid should be added **(Figure 1)** to one kilogram of silicone and stirred thoroughly for 5 minutes using a plastic spatula **(Figure 2)**. To save silicone 500 grams of this composition was measured into a 1 kg plastic bucket using a gram scale. The other 500 grams was stored for later use.

Figure 1   Weigh 10% silicone thinning fluid.

Figure 2   Add thinner to silicone and stir thoroughly for 5 minutes.

15 Millilitres of red silicone pigment was added and again stirred thoroughly for 5 minutes **(Figures 3 and 4).**

Figure 3   Red silicone pigment added.

Figure 4   Stirred again for 5 minutes.

Ten percent (50 grams) parts by weight of Mold Max 30 STD catalyst was added to 500 grams of silicone preparation and stirred thoroughly for 5 minutes **(Figure 5)**.

Figure 5   Mold Max 30 STD catalyst.

Once the catalyst is added to the silicone preparation curing will initialise within 60 minutes. The silicone preparation was now decanted into a 5L plastic bucket and again stirred thoroughly for 5 minutes **(Figure 6)**.

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Figure 6   Silicone preparation into a 5 litre bucket.

It was then placed in the vacuum degassing chamber and vacuumed using a dual 3 minute cycle at -0.86 Bar (-28mmHg). The red lever should be turned to 90⁰ during the vacuum cycle **(Figure 7)**.

Figure 7   Vacuum degassing chamber.

The silicone started to display air bubbles on its surface, rose almost to the top of the bucket and then automatically collapsed **(Figures 8 and 9)**.

Figure 8   First vacuum cycle.

The red lever should be returned to 180⁰ to release the vacuum.

Figure 9   Second vacuum cycle.

The silicone applicator consisted of a handgun, a 400 ml twin chamber cartridge with two plungers and their black plastic seals and a static mixing nozzle **(Figure 10)**.

Figure 10   Silicone applicator.

The silicone preparation was poured in a very thin stream into a 400 ml twin chamber cartridge from a one metre height to eliminate any further small air bubbles **(Figure 11)**.
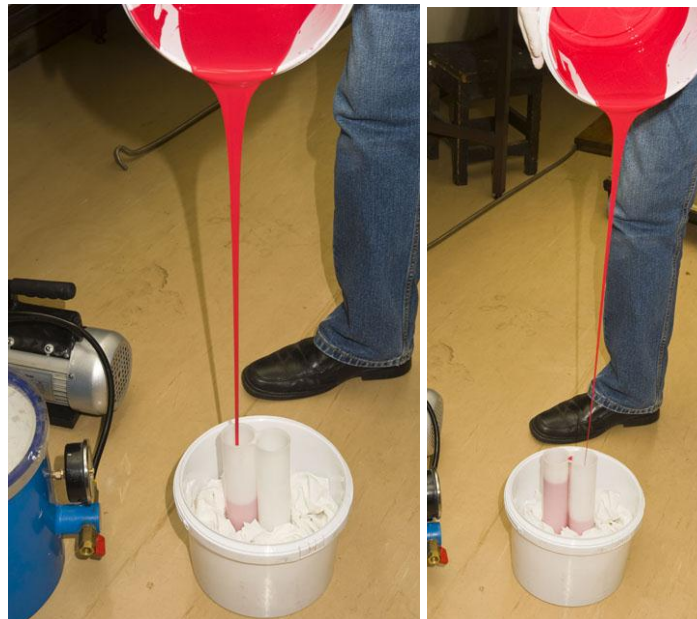
Figure 11   Silicone poured into the cartridge.

Two plungers were inserted into the cartridge without their black plastic seals to allow excess air to escape **(Figure 12)**.

Figure 12   Seals removed from plungers.

The seals were inserted as soon as silicone appeared through the escape ports **(Figure 13)**.
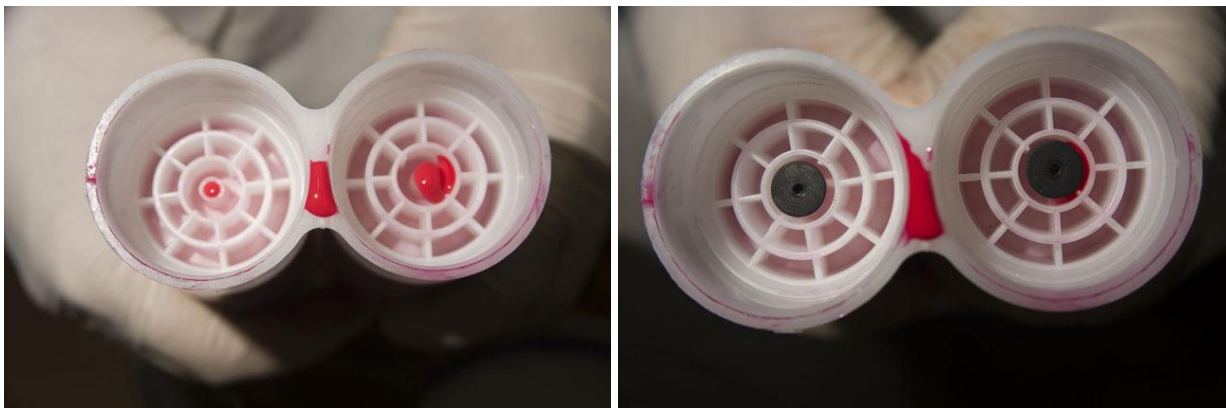
Figure 13   Seals replaced into plungers.

The seal of the static mixing nozzle was broken and the nozzle screwed on to the tip of the cartridge **(Figure 14)**.
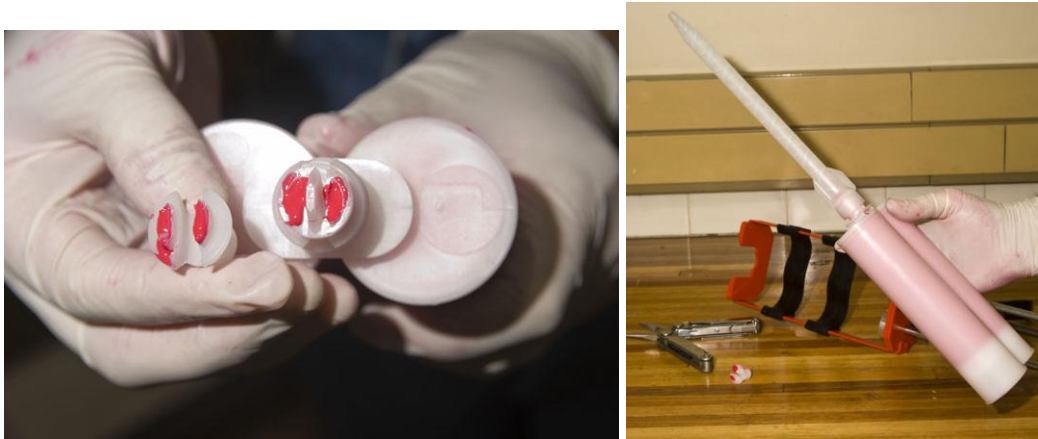
Figure 14   Static mixing nozzle seal broken and screwed on.

The cartridge was inserted into the dispensing handgun **(Figure 15)**.

Figure 15   Cartridge inserted into handgun.

The static mixing nozzle was primed to expel air **(Figure 16)**.

Figure 16   Static mixing nozzle primed.

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The total preparation time was 35 minutes.

The aorta was transected immediately caudal to the ligature that was placed earlier and the caudal part of the abdominal aorta was secured to the tip of the dispenser of the silicone gun using two 0 nylon ligatures **(Figure 17)**.
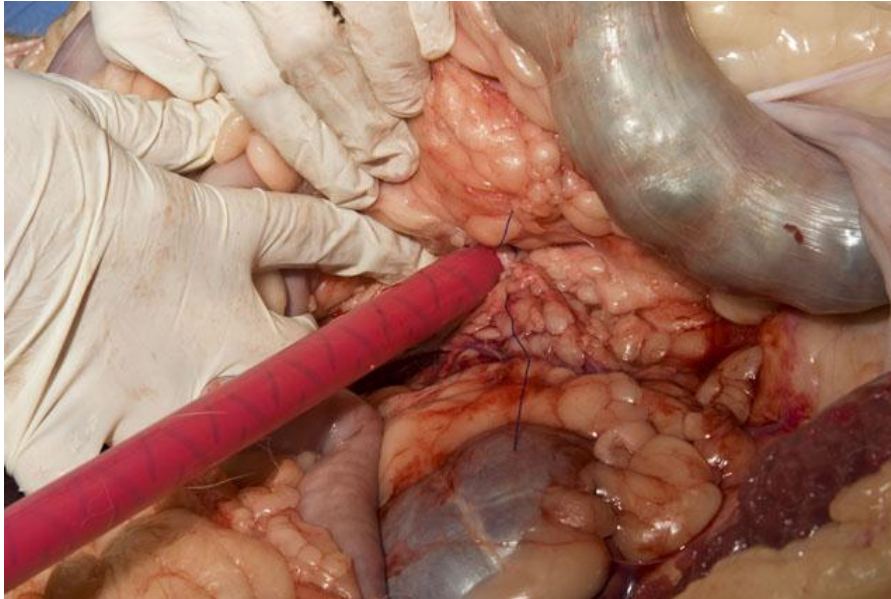
Figure 17   Silicone dispenser secured to the abdominal aorta.

Prepared Mold Max 30 silicone with red silicone pigment was injected by hand until the silicone appeared in an incision made in the plantar aspect of the metatarsus.

The silicone gun with static mixing nozzle was left attached to the aorta to retain silicone intra vascularly during the curing process.

This study was done in June during the winter months of South Africa. However the anatomy hall where the cadavers where stored overnight was heated to room temperature to facilitate curing of the silicone cast which is ideal at 23°C. Approximately 400 ml of silicone was used per lioness.

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3.4 Maceration

After 21 hours of curing and hardening of the silicone in a natural position the organ system was removed from the cadaver. After about 24 hours putrefaction became evident. Ostectomies of the pelvic floor were done at the lateral borders of the obturator foramina and through the lateral border of the ramus of the ischial bone **(Figures 18)** using an oscillating Plaster of Paris saw.
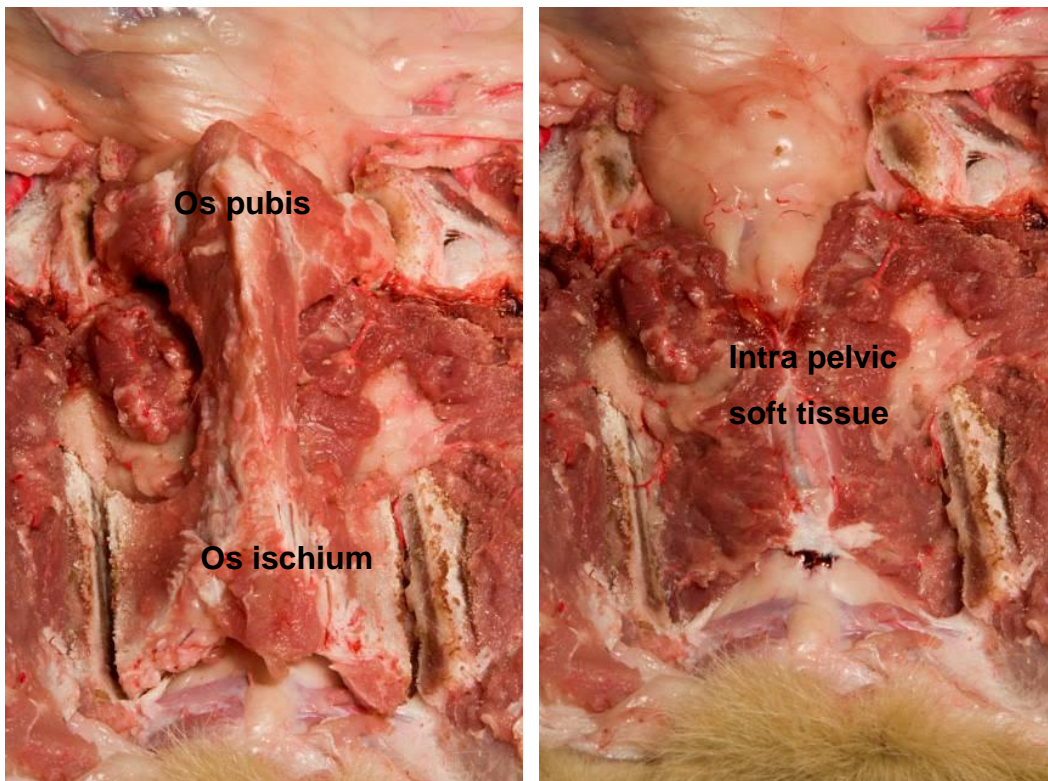
Figure 18   Pelvic floor osteotomised and removed.

The entire pelvic floor was removed and the entire content of the pelvis including the rectum, musculature and fat was removed. The intra pelvic arteries including the entire length of bilateral internal pudendal arteries were preserved. After removing the reproductive tract the organs were placed into 10% potassium hydroxide (KOH) in distilled water.

After eight days of maceration in lioness one and six days in lioness two the KOH was decanted and replaced with freshly made 10% KOH **(Figure 19)**.

Figure 19   Day 8 of maceration.

This KOH was however constituted using tap water instead of distilled water which was found to be ineffective due to precipitation of the KOH and resulted in the solution to become milky and ineffective. Since no maceration was noted by this solution within the following three days a new KOH solution was constituted using distilled water. This solution once again was effective and showed no signs of precipitation. After two more weeks the KOH was replaced with fresh solution **(Figure 20)**.

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Figure 20   Day 21 of maceration.

At this stage (three weeks after start of the maceration process) all the tissues were macerated apart from the tissue around the vulva area which was originally very bulky.

Another two weeks later (five weeks after start of maceration) some gelatine like tissue was still present in the area of the vulva. However at this stage it was decided to place the casts in a running water bath to rinse off the remaining tissue. This had good results in that the remaining tissue disintegrated into smaller pieces through the hydrodynamic forces of the running tap water within one hour. In areas where the tissue remnants were thick, gentle digital manipulation was applied and together with running water was effective in dislodging remaining tissue. The casts were boiled in water for thirty minutes until al tissue remnants were coagulated and removed. The casts were then placed in 6% hydrogen peroxide for two hours. Some very small remnants had to be gently teased off with a Treeve's dressing forceps. The casts were left in a running water bath overnight to yield a completely macerated cast of the arterial system. The arteries were then manually re-arranged in their natural position and suspended in a solution of glycerine, formalin and water inside a perspex container especially built to size. Cotton strands were used to keep the main arteries spatially arranged.

All used KOH was decanted into a 20L plastic container using a large funnel and was discarded by the University of Pretoria via standard procedures. Protective masks and gloves were used during the course of the process.

4. Results

Two silicone casts of good quality in terms of detail, flexibility and anatomical accuracy were yielded with even the fine arteries illustrated **(Figures 19 and 20)**. The casts endured handling well due good flexibility and appeared to provide a true reflection of the arterial supply of the female reproductive organs of the African lion (*Panthera leo*).

The arterial casts were severely entangled by the running water in the water bath. It was challenging and time consuming to re-arrange the arteries in their natural orientation after this process. Leaving the casts in a running water bath might be regarded as an unnecessary step in the authors' opinion. However once the casts were disentangled the silicone casts tended to adapt their own spatial arrangement by virtue of the memory in the silicone over time. The use of tap water instead of distilled water was found to be ineffective due to precipitation of the KOH which rendered the solution to become milky and ineffective.

In one cast the branches of the internal pudendal arteries distal to the vaginal arteries were unfortunately lost (Figure 19). However the branches of these arteries were preserved bilaterally in the subsequent cast (Figure 20).
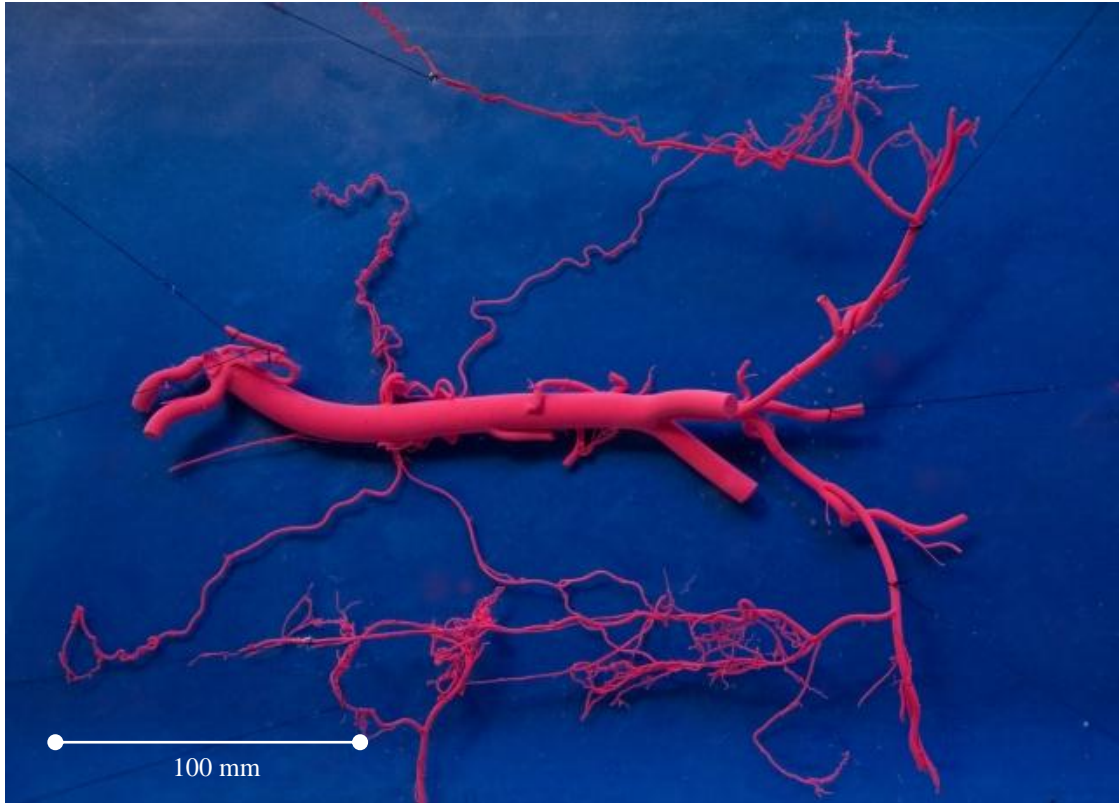
Figure 19   Silicone cast 1.
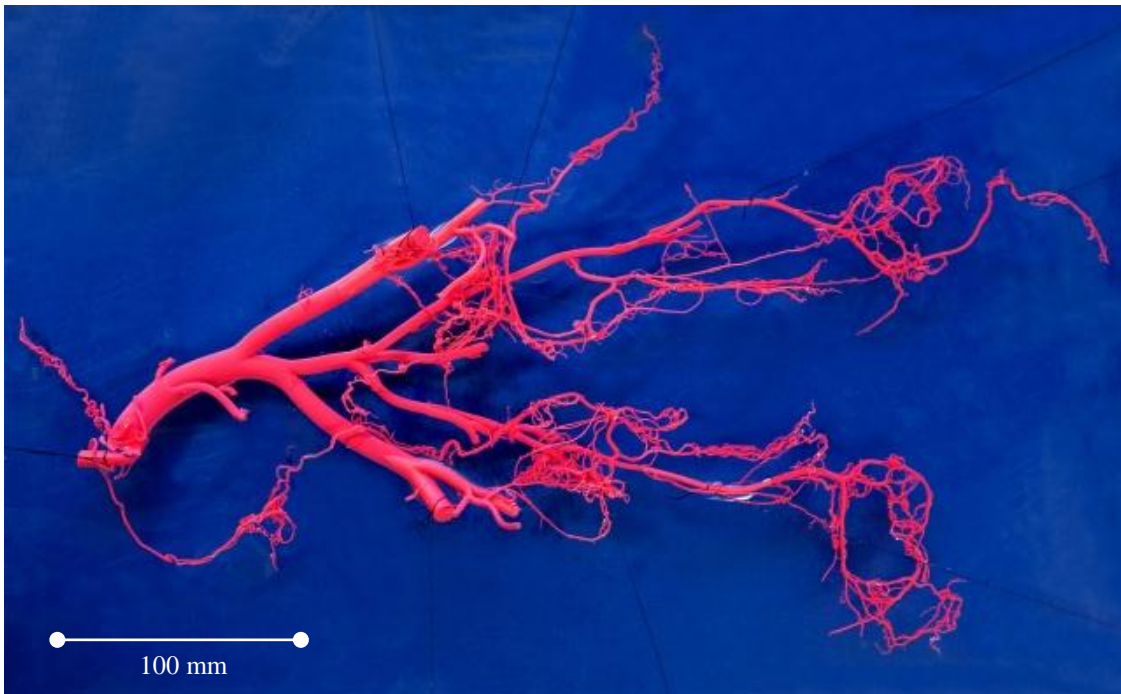
Figure 20   Silicone cast 2.

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5. Discussion

We found that silicone can be used to produce fine arterial casts of accurate anatomic detail using an organ system *in situ*. It was then possible to remove the organ structure from a complex location in the body, complete the maceration process and yield a cast that resembles the original arterial supply.

This technique provided the ability to infiltrate the fine arterial supply of the female reproductive organs. It was possible to depict the spatial arrangement of the arteries of these visceral and intra pelvic organ system in its natural position by allowing the cast to mature inside the carcass. Up to now different techniques used to illustrate vascular anatomy made use of loose organs mainly[1;4;10;17]. The flexibility of silicone casts allow their removal, after curing, from complex locations in the body without damage compared to resin casts[4;5;10;18]. Silicone casts like the ones produced in this study can be used as teaching aids[1;19] as library specimens[20] and in research[21].

This is the first study in Veterinary medicine describing the use of a silicone product to study fine arterial supply to any organ system.

6. Conclusion

Two silicone casts that supply accurate anatomical detail of the fine arterial supply of the female reproductive organs were produced. The casts were of excellent quality in terms of detail and anatomical accuracy with even the fine arteries infiltrated. The cast endured handling well and showed good flexibility. Silicone can be used in abdominal organs *in situ* to illustrate study and anatomically describe fine arterial supply. This method prevents leakage of silicone from severed vessels should the organs be removed prior to casting, and it could possibly be used for other organ structures.

This technique was used in chapter two of this dissertation to compare itself with two other techniques namely direct observation and transillumination in describing the arterial supply to the reproductive organs of the African lion.

Chapter 2
Arterial supply of the female reproductive organs of the African lion

1. Introduction

The female reproductive system of the African lion consists of bilateral ovaries suspended by the suspensory ligaments cranially and connected to the cranial tips of the uterine horns by the proper ligaments caudally. The bilateral uterine tubes are contained by a short mesosalpinx that originate from the mesovarium at the mesovarial margin of the ovaries. A bicornuate uterus is suspended bilaterally by the mesometria of which the lateral layers give rise to a separate fold which contain the bilateral round ligaments. The cervix, vagina and vaginal vestibule form the terminal part of the reproductive system[25].

In the lioness the kidneys are located far caudally in relation to the thirteenth ribs. The suspensory ligament is very well developed. It originates in a fan-like manner from the dorso-lateral abdominal wall lateral to the kidney extending up to a few centimetres cranial to the kidney. The proper ligament of the ovary as well as the round ligament is well developed. The round ligament inserts on the medial femoral fascia. The left ovary is bigger than the right and the ovarian bursa has a short mesosalpinx that does not cover any part of the ovary and the fimbriae extended the entire length of the ovary. The urethral tuberculum as well as the urethral crest are well developed. The left uterine horn is longer than the right. The uterine tube opens directly into the tip of the uterine horn and not onto a papilla[25]. The left ovarian artery originates from the aorta between 10.0 and 11.8 cm caudal to that of the ipsilateral phrenico-abdominal artery and between 3.9 and 5.6 cm cranial to the deep circumflex iliac artery. The right ovarian artery originates between 9.8 and 12.3 cm caudal to the phrenico-abdominal artery and between 2.7 and 3.9 cm cranial to the deep circumflex artery[25].

The purpose of this study chapter was to provide an accurate and detailed description of the arterial supply of the female reproductive organs of the African lioness. The addition of this description to existing morphological information would complete the anatomical description of this organ system except for the venous drainage and nerve supply.

The silicone technique described in Chapter 1 was also used to compare its ability to identify arterial supply to an organ system, in this instance the reproductive organs of the African lioness, to that of direct observation and transillumination.

2. Justification

2.1 Literature review

The distribution of the ovarian artery has been studied by four methods:

a) direct observation of the vascular pattern;[22]

b) using a tissue clearing technique following latex injection;[22] and

c) tissue corrosion following methyl methacrylate injection[22] and transillumination.[23]

d) more recently BIODUR E 20 BLAU® has been used to depict the arterial system of the reproductive organs.[24]

The female reproductive system of the African lioness was described during a morphological study[25]. In this study the left ovarian artery originated from the abdominal aorta 10.60 cm caudal to the ipsilateral phrenicoabdominal artery and 4.57 cm cranial to the deep circumflex iliac artery. Similarly the right ovarian artery originated 11.17 cm caudal and 3.27 cm cranial to the respective arteries[25].

No comprehensive arterial descriptions exist in wild carnivores. In particular no reports on the reproductive organs in this species have been published. Due to this lack of reproductive vascular information, the literature was searched for applicable anatomical studies of other species and especially the dog and cat were used as reference species for this study. The arterial supply of the reproductive organs of the dog[26], cat[27], donkey[24], horse[28] and various laboratory animals[29-31] has been well described. Production animal species were not included in this search.

2.2 Problem statement

There is no description of the female reproductive organs of the African lion available. To perform laparoscopic sterilization of the lioness a sound knowledge of the arterial supply of the female reproductive tract is required. Various techniques to describe the arterial supply to organs have been developed. However no comparisons between these techniques have been made.

2.3 Research objectives

The purpose of this study was to accurately describe the arterial supply of the female reproductive organs in the African lion using the three techniques described in the previous chapter and to compare the silicone technique to direct observation and transillumination in its ability to describe arterial supply.

2.4 Hypothesis

The arterial supply of the female reproductive organs of the African lion will resemble that of the domestic cat, with a few possible differences especially with regard to size and the silicone technique would be more effective in demonstrating the arterial supply to the female reproductive organs of the African lion.

2.5 Benefits

A complete description of the arterial supply of the female reproductive organs of the African lion will provide background knowledge for future reference during laparoscopic surgery performed on the described organs. To determine the best technique for studying the arterial supply to the reproductive organs and also to determine whether the reliability of the techniques would vary between different anatomical locations.

3. Materials and Methods

3.1 Sample population

The three formalin embalmed cadavers as well as the two fresh carcasses were used in this part of the study.

3.2 Procedures

The arteries of loose reproductive organs harvested from three formalin embalmed cadavers were first studied by direct observation in an anatomy hall and then studied illuminated in front of a theatre light **(Figure 21)**. Arteries were identified and their visibility was recorded in tabular format **(Tables 1-4)**. Images were catured using a Canon EOS 5D camera with a Canon EF 28-135 mm lens for large objects and EF 100 mm f/2.8 macro USM lens for close up images.

In two fresh cadavers the arteries were studied after injection of red pigmented silicone prepared according to the process described in chapter one. The lateral abdominal wall was reflected dorsally bilaterally and the arteries were studied and recorded with the organs *in situ* and after removal **(Tables 1-4)**.

Figure 21   Theatre light.

4. Results

The reproductive organs were supplied by three main arteries and their respective branches: the ovarian artery; the vaginal artery and the internal pudendal artery.

4.1 Ovarian artery

The ovarian artery **(Figure 22)** gave rise to branches supplying the suspensory ligament and its mesovarial part **(Figures 23, 24 and 25)**, the cranial (tubular) pole of the ovary **(Figure 26)**, the caudal (uterine) pole of the ovary **(Figure 26)** and a branch that anastomosed with the uterine artery **(Figures 27 and 28)**. It also gave off smaller branches that supplied the mesometrium **(Figure 23)**. The ovarian artery therefore displayed a five divisional character.
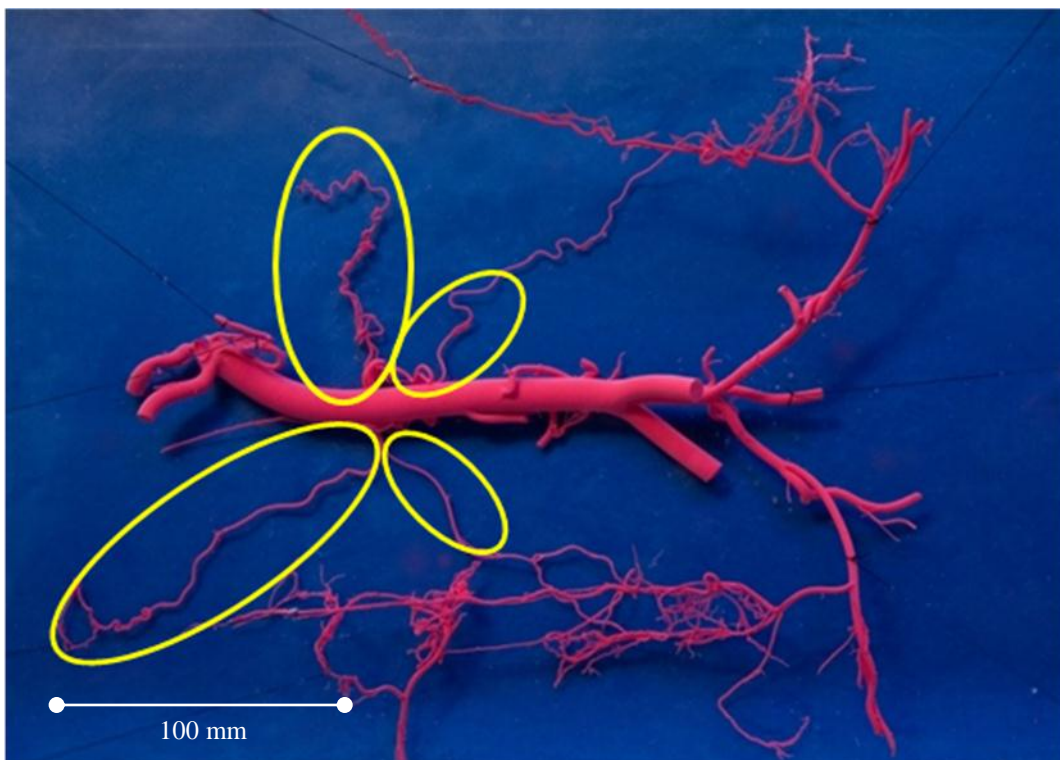
Figure 22　　The ovarian artery (yellow ovals).

Small ovals indicate the anastomotic branch between the ovarian artery and the uterine artery.
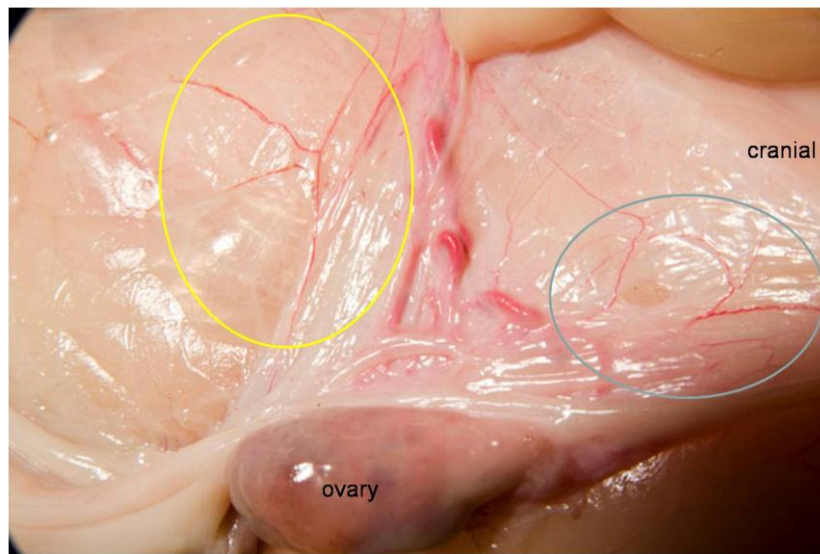
Figure 23   Small branches to the mesometrium (yellow oval) and cranial part of mesovarium (blue oval) and suspensory ligament.
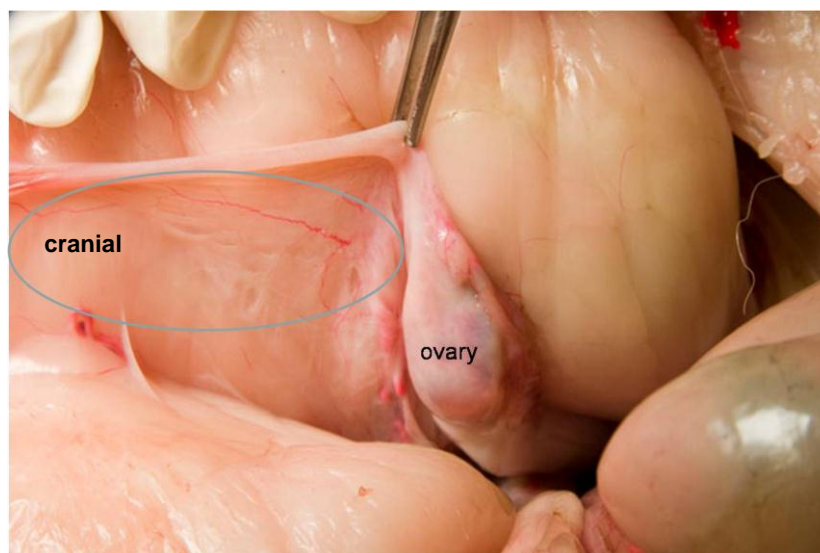
Figure 24   Supply to cranial part of mesovarium and suspensory ligament (blue oval).
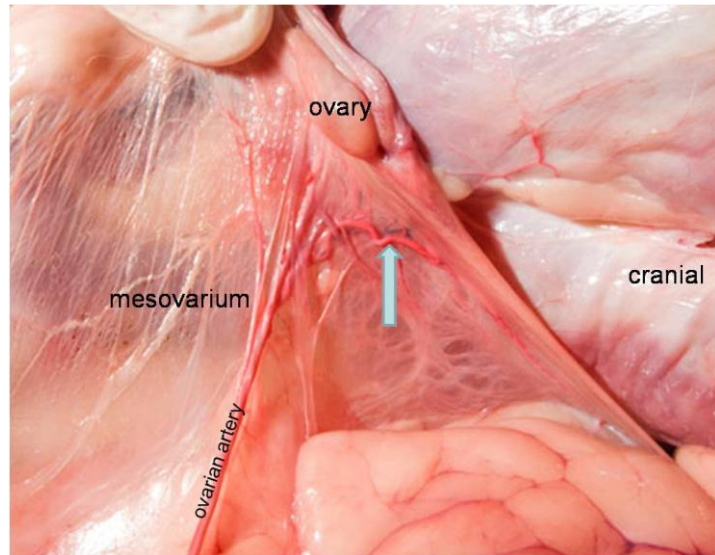
Figure 25   Supply to cranial part of mesovarium and suspensory ligament (blue arrow).
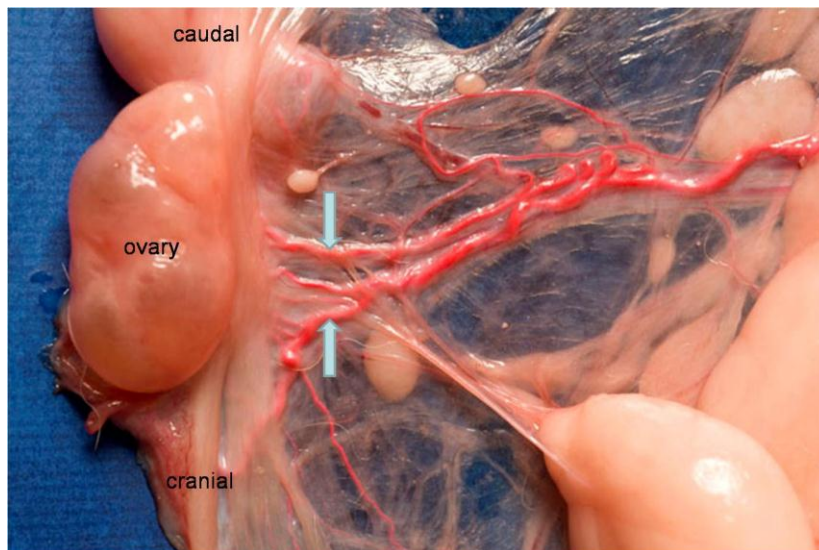
Figure 26   Branches of ovarian artery to cranial (tubular) pole and caudal (uterine) pole of ovary (blue arrows).
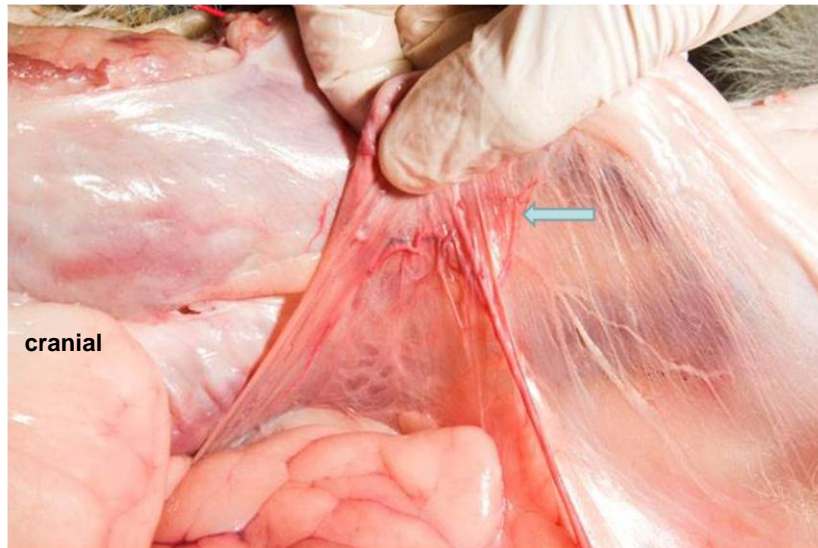
Figure 27   Anastomosing branch between ovarian and uterine arteries (blue arrow).

Figure 28   Anastomosing branch between ovarian and uterine arteries (red line).

The deep circumflex iliac artery had a little arterial branch supplying the mesovarium and which anastomosed with the ovarian arterial branch to the suspensory ligament **(Figures 29, 30 and 31)**.
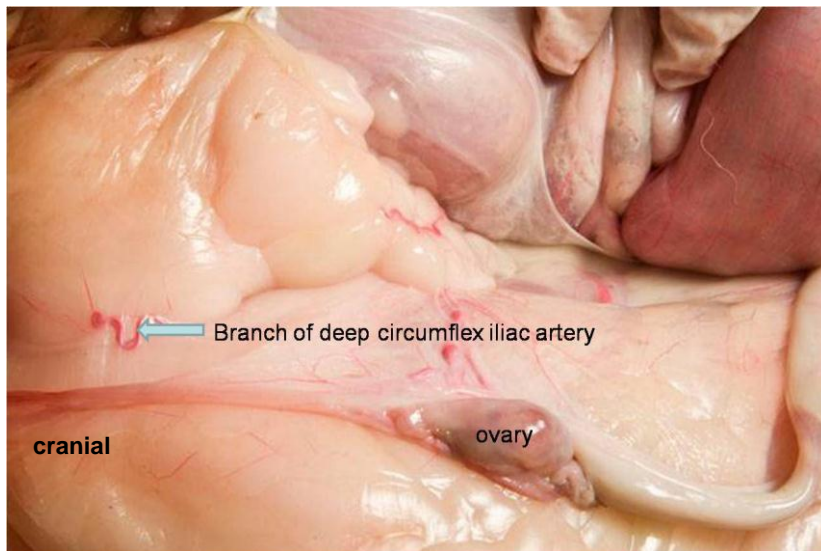
Figure 29   Deep circumflex iliac artery.
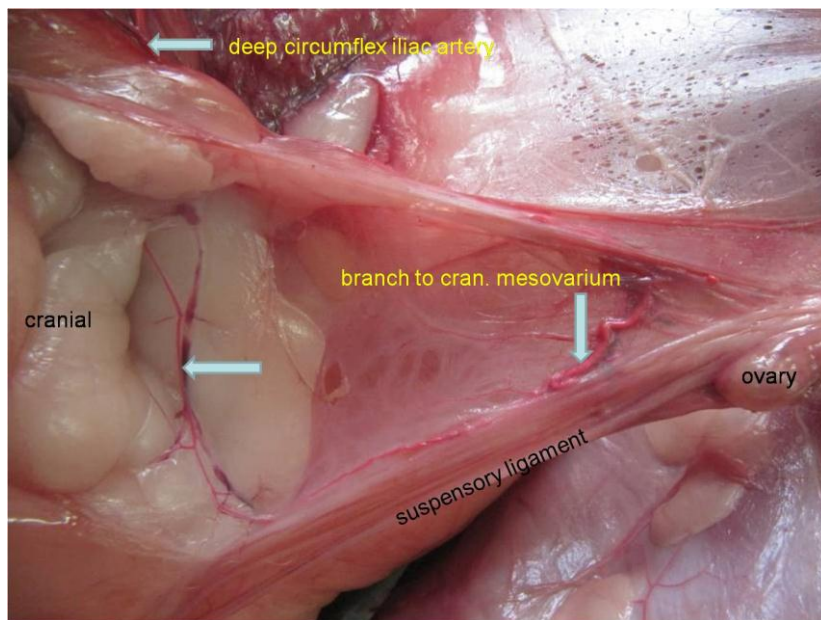
Figure 30   Anastomosis between ovarian and deep circumflex iliac arteries (unlabelled blue arrow) dissected.

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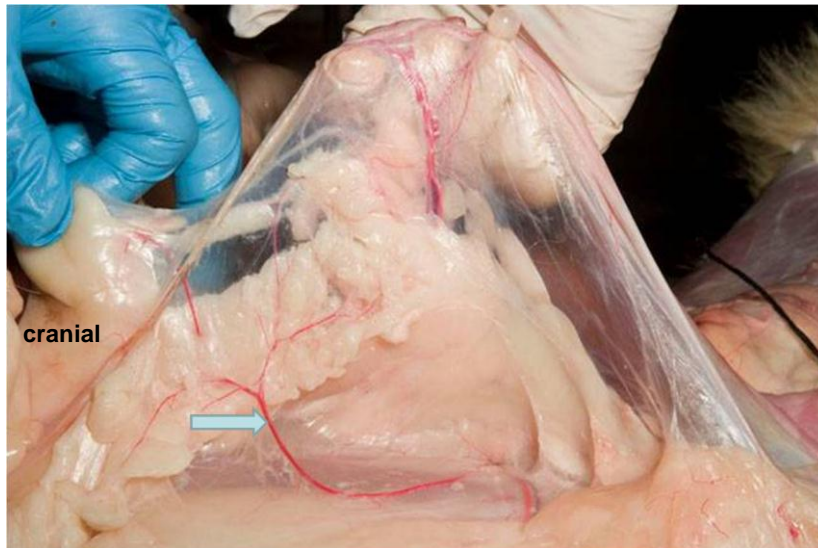
Figure 31   Anastomosis between ovarian and deep circumflex iliac arteries (blue arrow) dissected.

4.2 Vaginal artery

The vaginal artery **(Figure 32)** arising from the internal pudendal artery, gave rise to the uterine artery **(Figures 33 and 34)**, the caudal vesicular artery **(Figures 33 and 34)**, a branch of the caudal vesicular artery onto the distal ureter, a branch of the caudal vesicular artery onto the distal urethra, and ultimately terminated by supplying the vagina **(Figure 35)**. The branch of the caudal vesicular artery onto the ureter, coursed on the medial aspect of the ureter.
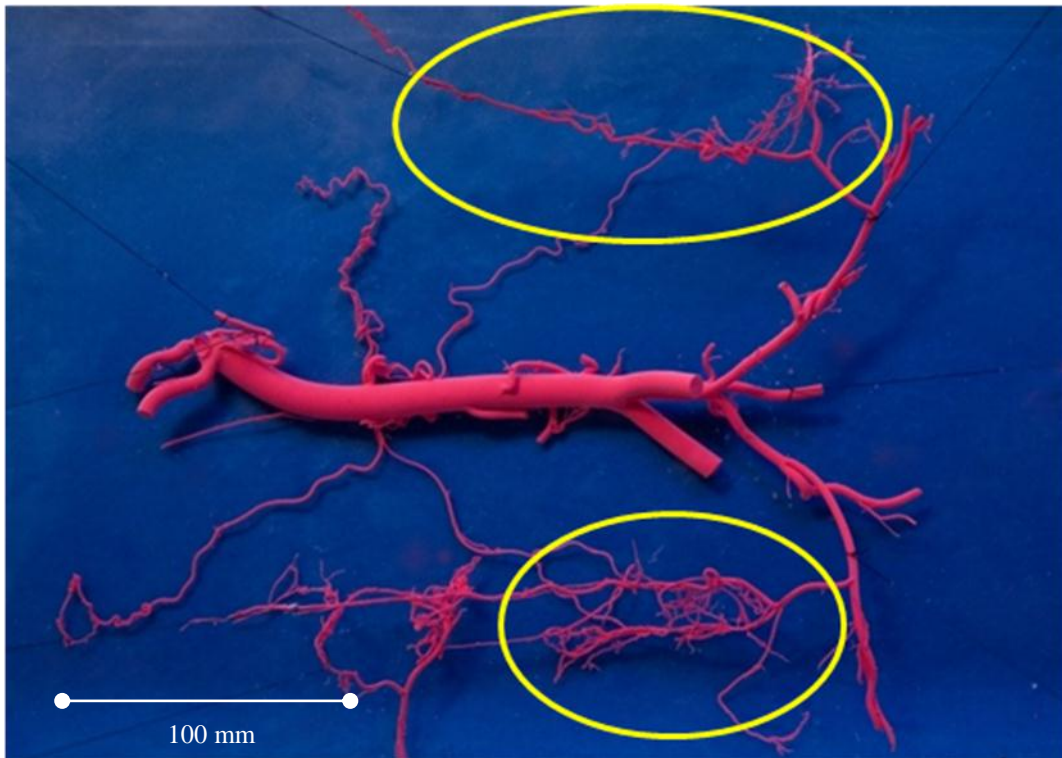
Figure 32   The vaginal artery and its branches (yellow ovals).

Note the anastomoses between the urethral branches and caudal vesicular artery in the bottom

circle.
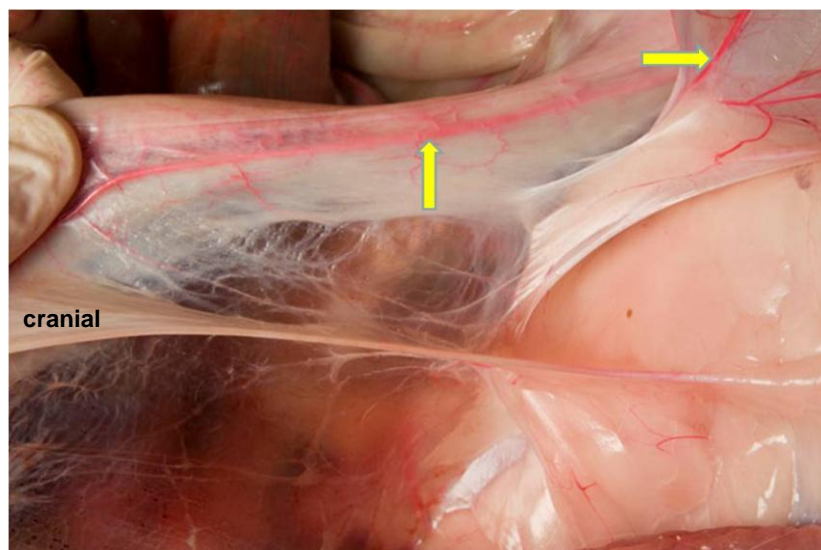
Figure 33   Uterine artery (up arrow) and caudal vesicular artery (right arrow).
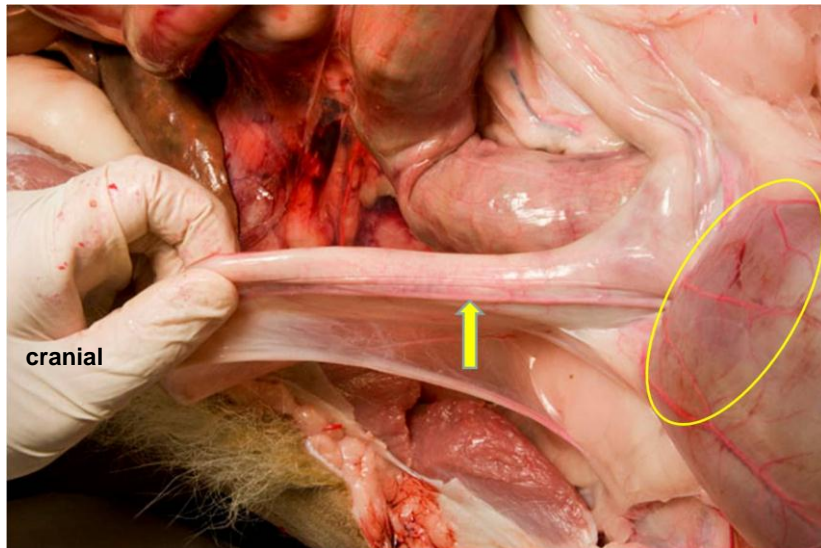
Figure 34   Uterine artery (arrow) and caudal vesicular artery (oval).

4.3 Internal pudendal artery

Apart from the vaginal artery the internal pudendal artery also gave rise to the urethral artery **(Figure 35)**, the ventral perineal artery (which in turn gave rise to the dorsal labial artery), and the clitoral artery **(Figures 39 and 37)**. The clitoral artery was well developed and clearly visible but some dissection was required to demonstrate the ventral perineal and dorsal labial arteries.
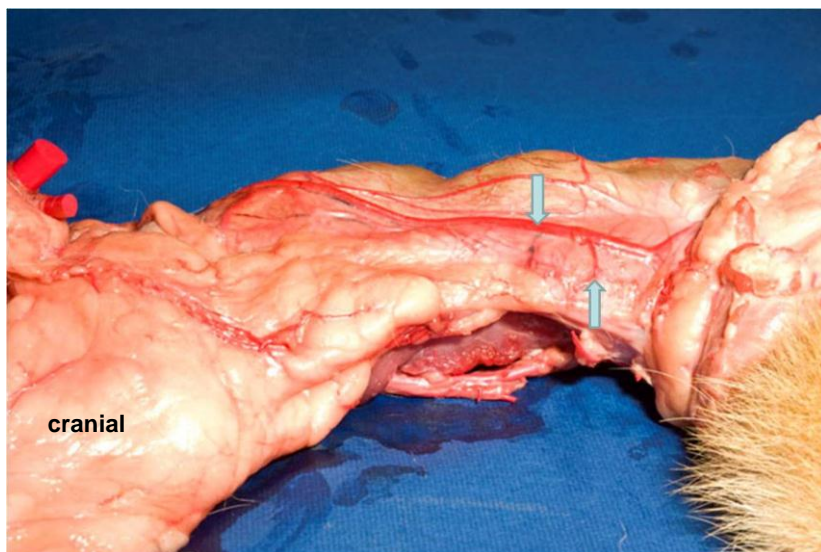
Figure 35   Internal pudendal artery (down arrow) and urethral artery (up arrow).
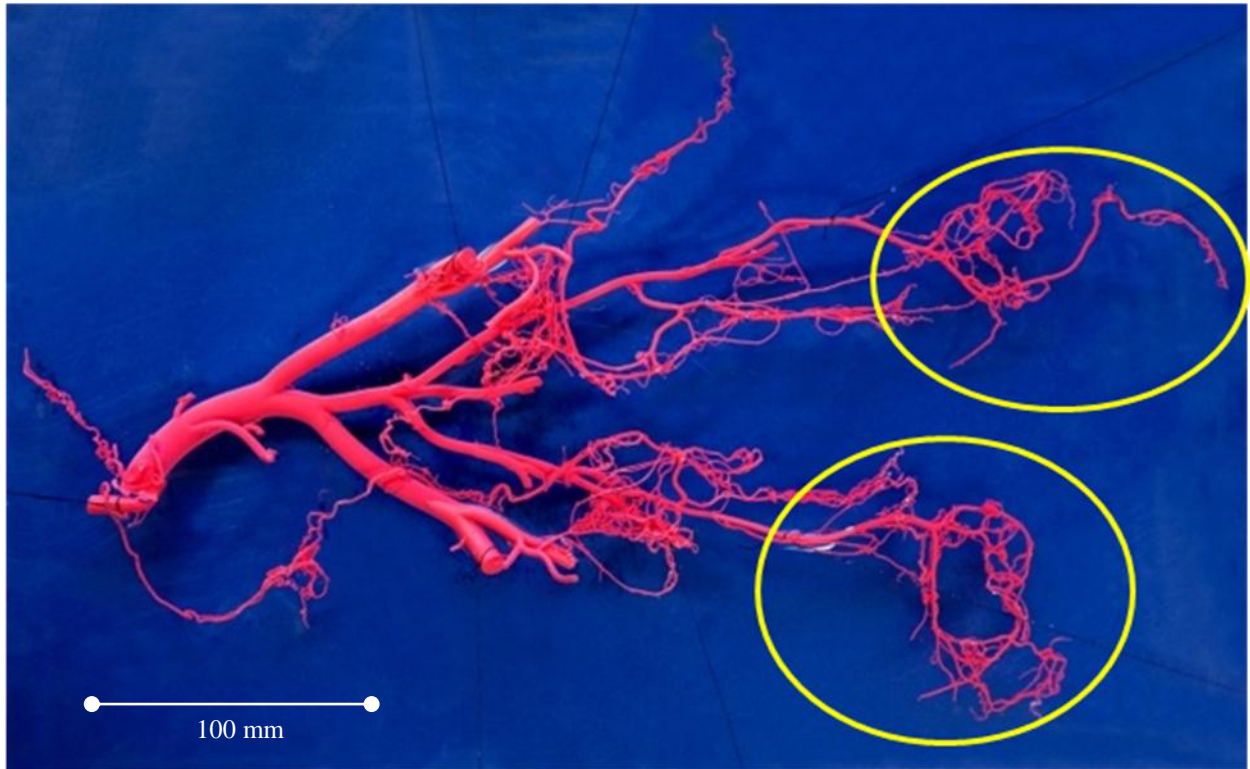
Figure 36   The vaginal artery and its branches (yellow ovals).
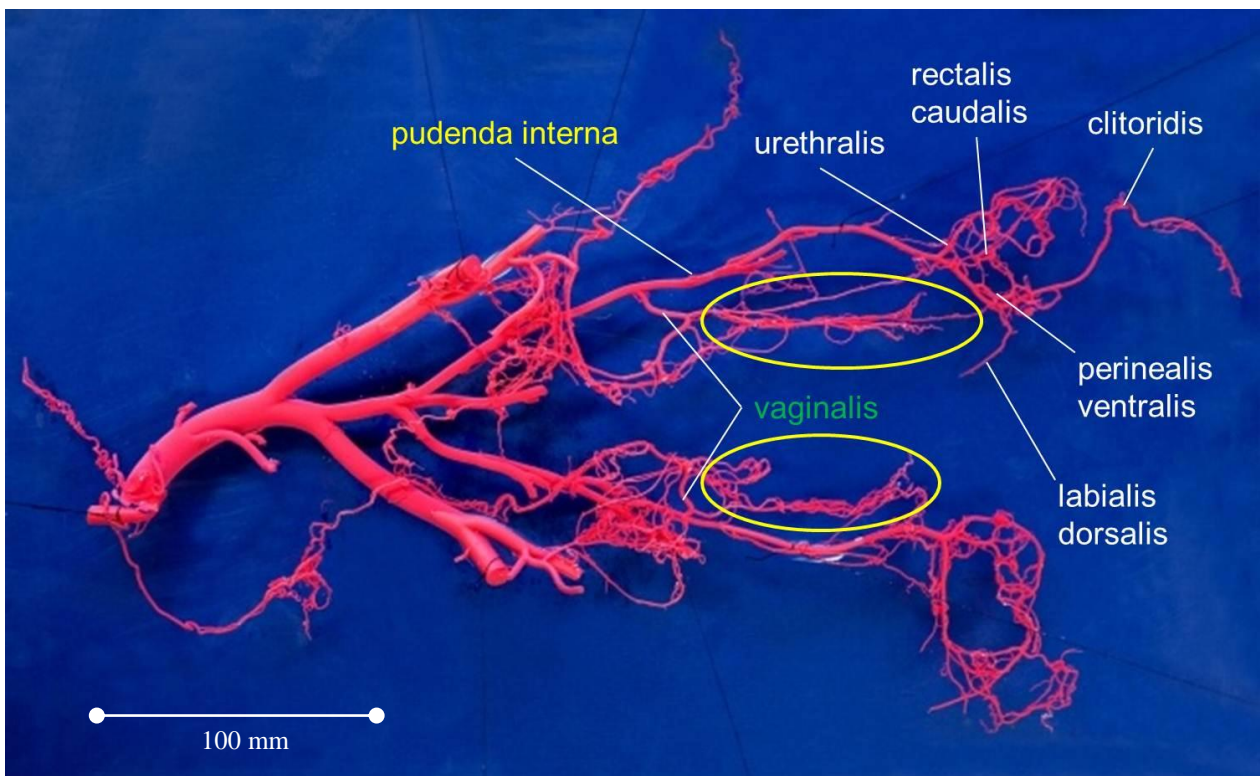
Figure 37   The vaginal artery and its branches.

Note the anastomoses between the vaginal artery and the caudal aspect of the internal pudendal

artery (yellow ovals).

4.4 Uterine artery

The uterine artery originated from the vaginal artery and supplied mainly the uterus and uterine horns and anastomosed with the ovarian artery. It also supplied small arterial branches to the broad ligament **(Figure 39)** and round ligament **(Figure 40)**.

4(i) Arterial supply to the mesometrium

Fine arterial supply to the mesometrium originated from the proximal part of the ovarian artery **(Figure 38)** as well as the anastomosing branch of the ovarian artery **(Figure 40)** and from the entire length of the uterine artery **(Figures 39 and 40)** including the proximal part of the uterine artery **(Figure 41)**. These arteries gave rise to a network of fine arterial supply to the mesometrium which extended into the fold of the round ligament **(Figures 41, 42 and 43)**. There were similar small branches originating from the proximal uterine artery extending caudally into the mesometrium **(Figure 41)**.
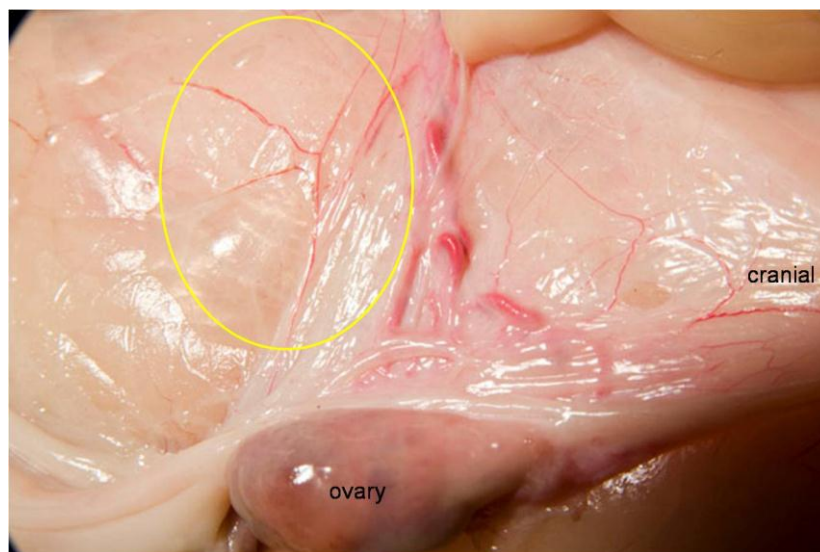
Figure 38   Branches from the ovarian artery (yellow oval).
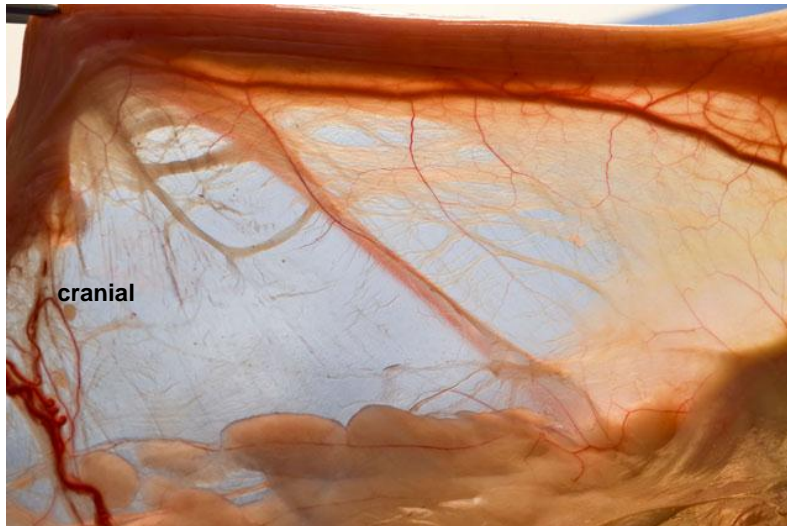
Figure 39   Branches from the ovarian artery, uterine artery and vaginal artery to the mesometrium.
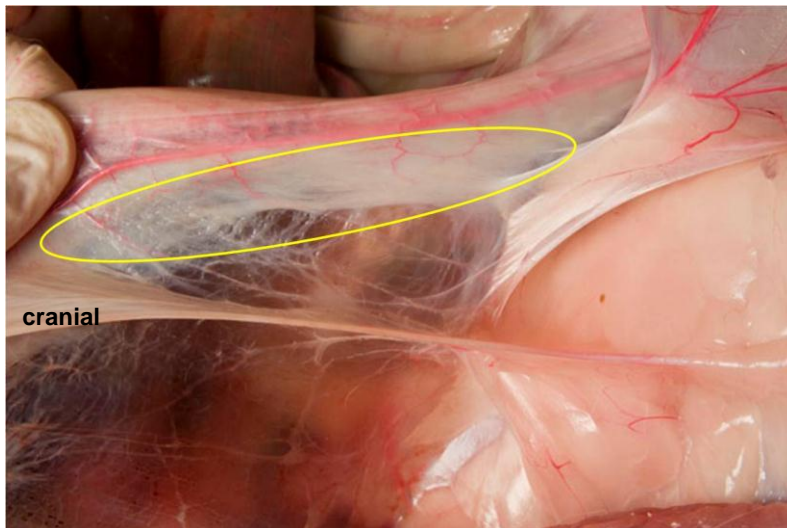
Figure 40   Branches from uterine artery running along the uterine horn (oval).
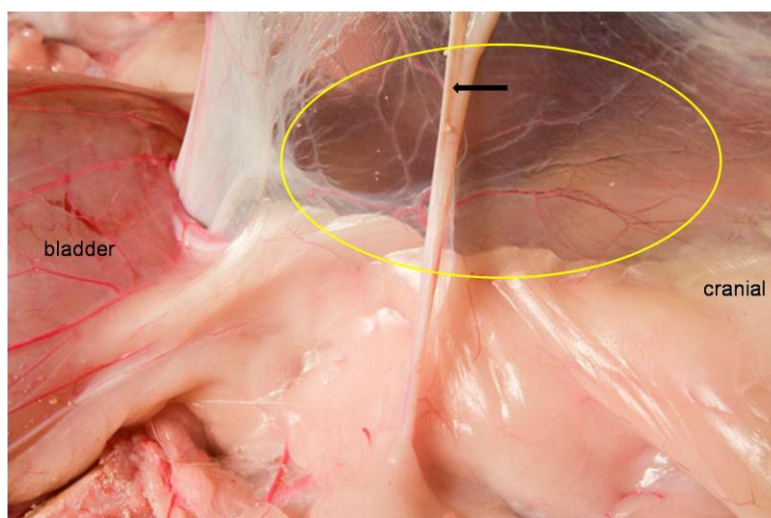
Figure 41   Branches from the proximal uterine artery (oval) and round ligament (arrow).

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4(ii) Arterial supply to the round ligament

Arterial supply to the round ligament originates from the proximal uterine artery **(Figure 41)** as well as from another source at its insertion which has to be determined **(Figures 42 and 43)**.
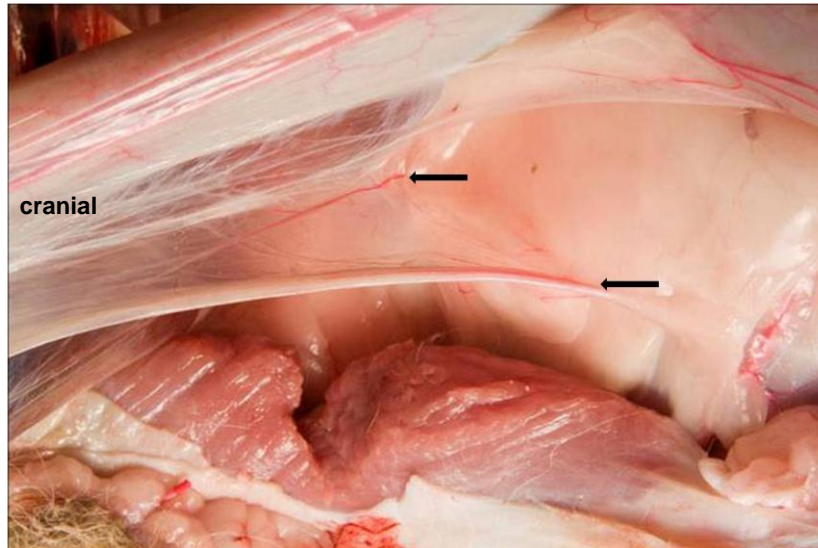
Figure 42   Supply to round ligament originate caudally
in the region of the inguinal canal (arrows).
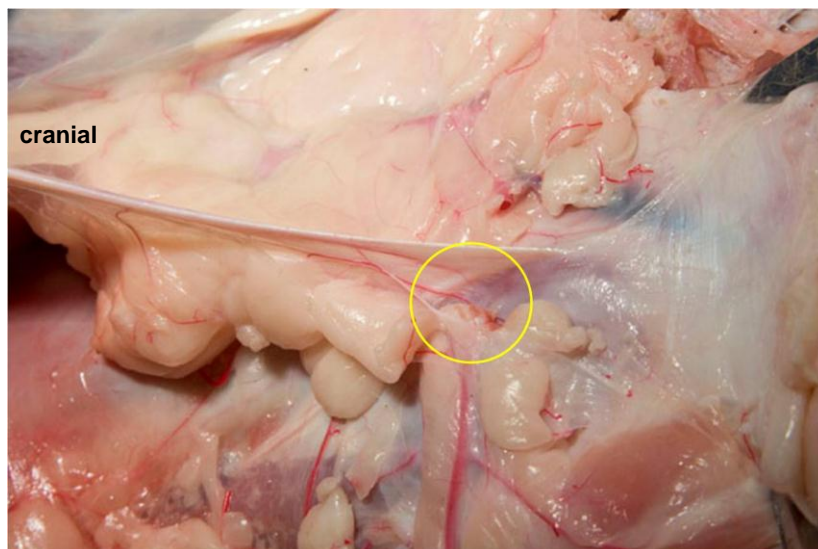
Figure 43   Supply to the round ligament originate caudally
in the region of the inguinal canal (yellow circle).

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4(iii) Summary

A detailed illustration of the arterial supply provided by two silicone casts **(Figures 44 and 45)**.
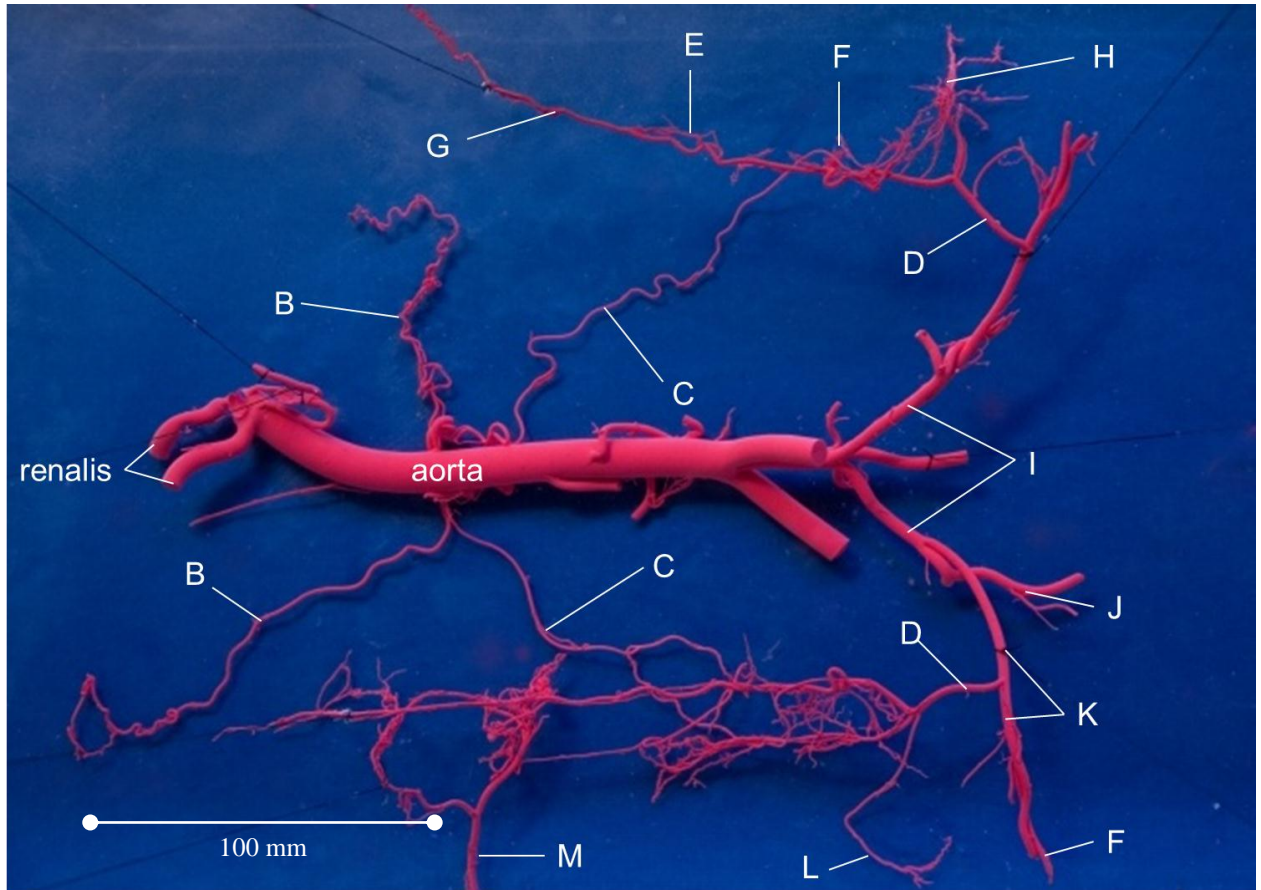
Figure 44   Silicone cast 1

Aorta, ovarian arteries (B), uterine arteries (C), vaginal arteries (D), branches of the vaginal artery to the ureter (E), urethra (F), caudal vesical artery (G), terminal part of the vaginal artery (H), internal iliac artery (I), caudal gluteal artery (J), internal pudendal artery (K), medial rectal artery (L) and deep circumflex iliac artery (M).

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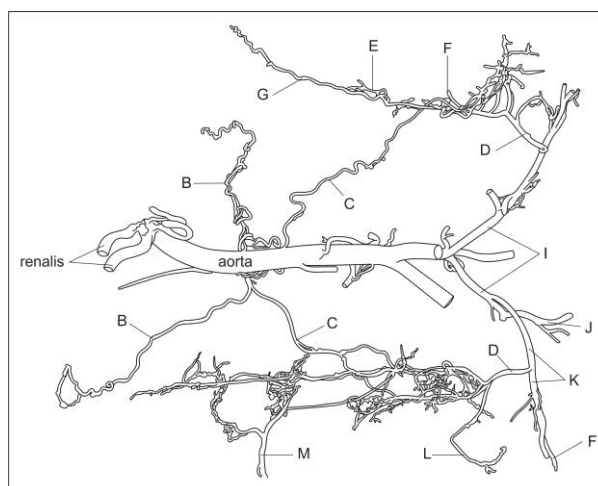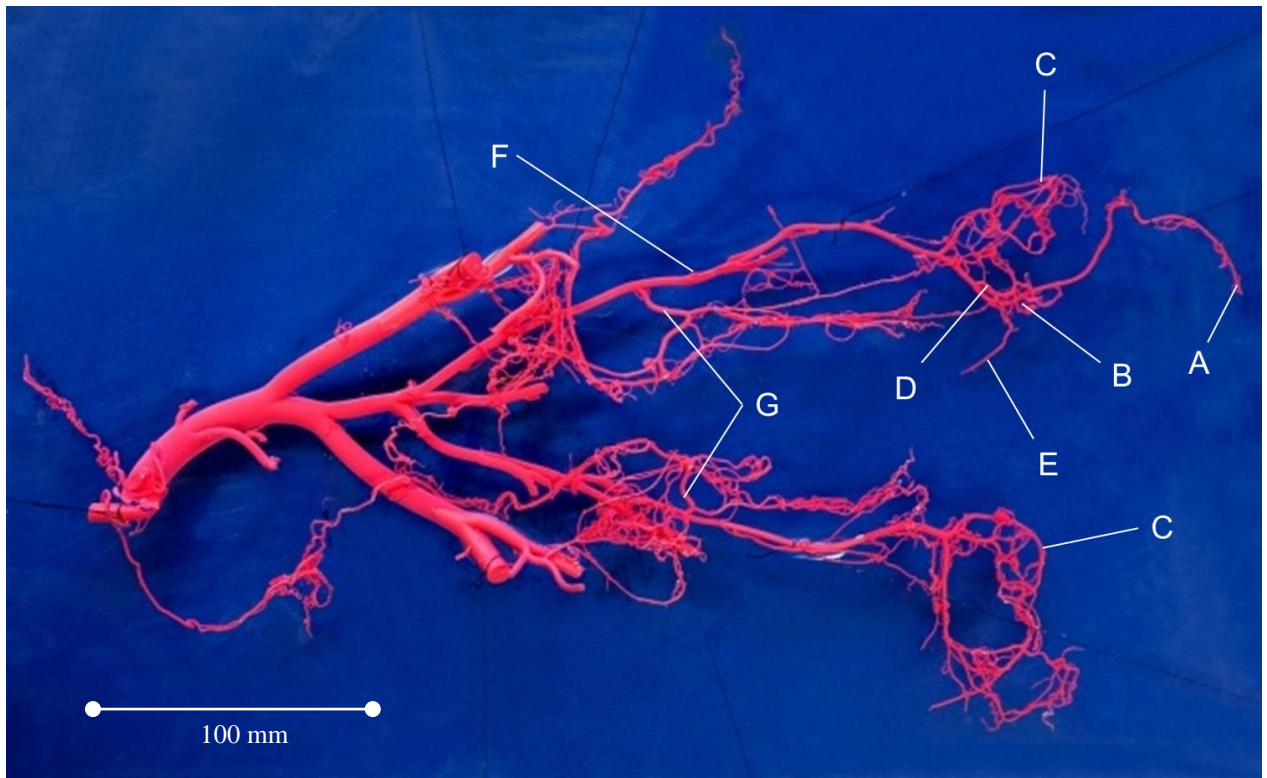
Figure 45   Silicone cast 2

Clitoral artery (A), caudal rectal artery (B), urethral arteries (C), ventral perineal artery (D), dorsal labial artery (E), internal pudendal artery (F) and vaginal arteries (G).
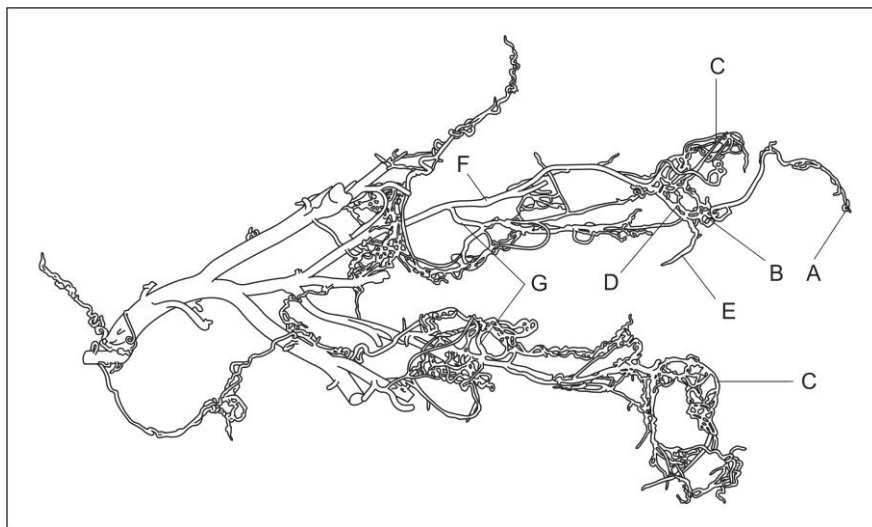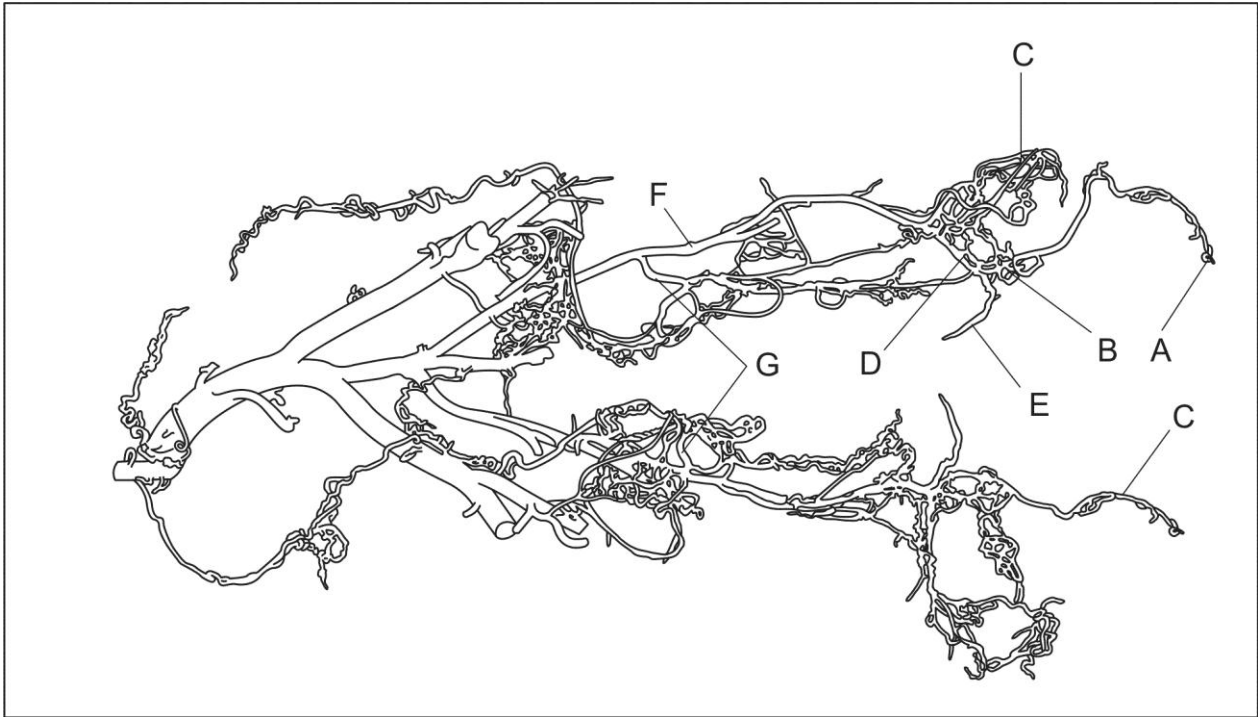
Illustration 1   Line drawing of the arterial supply to the female reproductive organs of the African lion

4(iv) Comparison of observational techniques

The abilities of the three techniques incorporated in the study were summarised **(Tables 1, 2, 3 and 4)**. In all of the tables direct observation was indicated by D, transillumination by T, the silicone technique by S, left by L and right by R.

+ = Slight visibility

++ = Good visibility

+++ = Very good visibility

++++ = Excellent visibility

- = Not visible

Table 1   Branches of the ovarian artery.

Lioness		*Pars cranialis* of the mesovarium		*Extremitas tubaria*		*Extremitas uterina* and tip of uterine horn		Anastomosis with *A. uterina*	
		L	R	L	R	L	R	L	R
1	D	+	+	++	++	+	+	++	++
	T	++	+	+++	+++	+	+	+++	+++
2	D	-	-	++	++	+	+	+	++
	T	-	-	++	++	+	+	++	+++
3	D	-	+	-	++	-	++	++	++
	T	+	++	-	+++	+	+++	+++	+++
4	S	++++	++++	++++	++++	++++	++++	++++	++++
5	S	++++	++++	++++	++++	++++	++++	++++	++++

Transillumination could in some instances improve visibility but not in all areas.
In areas where more connective tissue or fat was present a reduction in visibility was evident for both direct observation as well as transillumination.

Table 2   Branches of the vaginal artery.

Lioness		A. vaginalis		A. uterina		A. vesica caudalis		Branch of A.vesica caudalis to the distal urethra		Branch of A. vesica caudalis onto the ureter		Terminal part of A. vaginalis	
		L	R	L	R	L	R	L	R	L	R	L	R
1	D	++		++	+	++		+		-	-	+	
	T	++		+++	+	++		+		+	+	+	
2	D	+++		-	-	++		+		+	-	+	
	T	+++		+	+	++		+		++	+	+	
3	D	Destr		+	-	+		+		-	+	+	
	T	Destr		++	+	++		+		-	++	+	
4	S	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
5	S	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

All arteries on right hand side of embalmed cadavers were lost due to sagittal splitting of pelvis. Transillumination did not significantly improve visibility of arteries over direct observation, especially in areas where a lot of connective tissue or fat was present.

Table 3   Branches of the internal pudendal artery other than the vaginal artery.

Lioness		A. urethralis		A.perinealis ventralis		R. labialis dorsalis		A. clitoridis	
		L	R	L	R	L	L	R	L
1	D	+		+++	+++	+++	+++	++	++
	T	+		+++	+++	+++	+++	++	++
2	D	-		+++	+++	+++	+++	+++	+++
	T	+		+++	+++	+++	+++	+++	+++
3	D	+		+++	+++	+++	+++	++	++
	T	+		+++	+++	+++	+++	++	++
4	S	++++	++++	++++	++++	++++	++++	++++	++++
5	S	++++	++++	++++	++++	++++	++++	++++	++++

There was no difference in visibility between direct observation and transillumination.

Table 4   **Broad ligament and round ligament.**

Lioness		Fine arterial supply to the mesometrium		Fine arterial supply to the separate fold of round ligament	
		L	R	L	R
1	D	++	++	+	+
	T	+++	+++	++	++
2	D	++	++	++	+
	T	+++	+++	+++	++
3	D	++	++	+	++
	T	+++	+++	++	+++
4	S	++++	++++	++++	++++
5	S	++++	++++	++++	++++

Transillumination significantly improved visibility of arterial structures especially in areas where less connective tissue or fat was present.

Visibility of the ovarian artery and its branches were evaluated: The branch supplying the suspensory ligament and its mesovarial part was only slightly visible; the branch to the cranial (tubular) pole of the ovary showed good visibility, the branch to the caudal (uterine) pole of the ovary was only slightly visible and a branch that anastomosed with the uterine artery showed good visibility **(Table 1)**. In areas where more connective tissue and/or fat were present a reduction in visibility was evident for both direct observation as well as transillumination with the latter tending to improve vascular visibility.

The vaginal artery arising from the internal pudendal artery gave rise to the uterine artery which showed huge variability in its visibility and the caudal vesicular artery showed good visibility. Branches of the caudal vesicular artery onto the distal ureter and the distal urethra and the termination of the vaginal artery at the vagina were only slightly visible **(Table 2)**. In areas where a lot of connective tissue or fat was present, transillumination did not improve visibility of arteries over direct observation.

Apart from the vaginal artery the internal pudendal artery also gave rise to the urethral artery, the ventral perineal artery (which in turn gave rise to the dorsal labial artery), and the clitoral artery. The clitoral artery was well developed and clearly visible but some dissection was required to demonstrate the ventral perineal and dorsal labial arteries **(Table 3)**.

With regards to the arterial supply to the mesometrium and the fold of the round ligament transillumination clearly improved visibility of the fine arterial structures in areas where less connective tissue or fat was present over direct observation **(Table 4)**.

In some areas soft tissue dissection was necessary to expose the relevant arteries.

5. Discussion

The ovarian artery originated from the abdominal aorta between the phrenico-abdominal and deep circumflex iliac arteries and gave rise to four main branches in the lioness. Only three of these branches have been well described in the dog[26;28] and cat[27]. Similarly smaller branches from the ovarian artery supplying the broad ligament, clearly observed in this study, have not been well described in either species. The anastomosis between the ovarian and uterine arteries did occur in the lioness as in the dog and cat[26-28] however anastomosis could only be demonstrated well in two of four arteries. An anastomosis between the deep circumflex iliac artery and a branch of the ovarian artery was found in one specimen **(Figures 30 and 31)** which has not been described in the reference species.

The vaginal artery was found to be similar to that of the dog[26]. Anastomosis between the urethral branches and caudal vesicular artery was present similar to that of the dog **(Figure 32)** however anastomosis between the vestibular artery and caudal vestibular branches from the terminal part of the vaginal artery could not be demonstrated in the lioness[26]. Additional anastomosis between the vaginal artery and caudal part of the internal pudendal artery was found in this study **(Figure 37)** that has not been described in the dog or cat.

The internal pudendal artery gave rise to branches supplying the reproductive organs similar to that of the dog and cat[26;27].

Fine arterial supply to the mesometrium originated from the ovarian, uterine and vaginal arteries **(Figure 39)** and was clearly depictable in the lioness. However this aspect has not been well described in the reference species similar that of the round ligament.

A short trunk in caudal abdominal aorta between the bilateral external iliac and internal iliac arteries was present in both specimens which has not been described in the dog or cat[26;28]. The arterial supply of the round ligament originated from its area of insertion on the proximal aspect of the medial femoral fascia and needs to be investigated further.

Transillumination[23;24] significantly improved visibility of arterial supply over direct observation only in areas where minimal fat or connective tissue was present. The silicone technique could depict all arterial supply up to fine arterial level.

6. Conclusion

The arterial supply of the reproductive organs of the African lioness resembles that of the domestic cat and dog with some minor differences with regards to anastomoses. Description of the arterial supply to the suspensory ligament, cranial part of the mesovarium and the mesometrium has been improved in this study compared to literature available on the dog and cat. The silicone technique was superior to direct observation and transillumination in depicting arterial supply in all regions of the reproductive organs.

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Chapter 3
Histology of the female reproductive organs of the African lion

1. Introduction

In this study three nulliparous lionesses of similar age were used. This Masters project was undertaken to have a better knowledge of the female lion reproductive tract and to provide a histological basis from which some reproductive and behavioural characteristics of the African lion could be explained.

2. Justification

2.1 Literature review

The female reproductive system of the African lioness was described during a morphological study (Hartman et al) and the arterial supply to these organs was described in the previous chapter.

Currently few comprehensive histological studies exist in wild carnivores. In particular, no reports on the reproductive organs in these species have been published. Due to this lack of reproductive information, the literature was searched for applicable histological studies of other species and the dog and cat were used as only reference species for this study. The histology

of the connective and supportive tissue[32], muscle[33] and that of the female reproductive system[34] of the dog and cat has been well described. Follicular atresia has not been described in the domestic dog and cat and therefore description the human literature was searched[35]. Included in the splanchnology of reproductive organs are five different ligaments namely the suspensory, proper, broad, round and intercornual ligament. However available histological descriptions lack detail on these ligaments even in the dog and cat.

The uterine tube extends from a point in the region of the ovary to the tip of the uterine horn and consists of three segments; the infundibulum (a large funnel-shaped part), the ampulla (a thin walled part distal to the infundibulum) and the isthmus (a narrow muscular segment joining the uterus). The epithelium of the uterine tube is simple columnar or pseudostratified columnar with ciliated and non-ciliated cells. The ciliated cells are more in number and only the nonciliated cells possess a secretory function. These secretions nourish the ovum and zygote. The mucosa-submucosa of the ampulla is highly folded with primary, secondary and tertiary folds. In the isthmus these folds gradually decrease both in number and complexity towards the uterine junction to such an extent that only a few primary folds remain at the isthmus-uterine junction. The tunica muscularis consists mainly of circular smooth muscle bundles but few longitudinal and oblique bundles also exist[34].

The infundibulum has finger like projections called fimbriae which have the ability to move over the surface of the ovary due to smooth muscle contractions in order to facilitate transport of the oocyte into the infundibulum. The cilia of the infundibula epithelial cells in turn transport the oocyte into the ampulla where a combination of biliary propulsion and muscle contraction is responsible for its transportation. Fertilisation takes place in the caudal part of the ampulla. Smooth muscle contractility is the only propulsive force in the isthmus and therefore the muscle layer is most prominent in this part of the uterine tube. Muscular contractions of the uterus and uterine tube are responsible for transportation of sperm to the caudal ampulla for purposes of fertilisation and not the innate motility of the sperm itself[34].

The ovary consists of an outer cortex and inner medulla and is supplied by blood vessels, lymphatics and nerves. The cortex of the ovary is a broad peripheral zone that may contain various structures including primordial, primary, secondary, tertiary (Graafian) and atretic follicles as well as corpora lutea, rubra, albicantia and regressum. These structures are embedded in a loose connective tissue stroma in the cortex. The ovary is covered by a low cuboidal surface epithelium (germinal epithelium) and a thick connective tissue layer, the tunica albuginea, is situated immediately beneath the surface epithelium. The ovarian follicle consists of an oocyte surrounded by specialized epithelial cells. As the follicle develops specialised stroma cells surround the follicle while a fluid-filled cavity develops among the epithelial cells.

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The primordial follicle consists of the primary oocyte (± 20μm in diameter (ø)) surrounded by simple squamous epithelium. These primordial follicles arise prenatally from internal epithelial cell masses and in some species also postnatally by mitosis. The primordial follicle (± 40μm ø) develops as the primary oocyte goes through various stages of development. A single layer of flat follicular cells, which rests on a basal lamina, simultaneously forms around the oocyte.

Primary follicles consist of the primary oocyte surrounded by simple cuboidal epithelium. The primary oocytes begin the first meiotic division before birth, but the then remain dormant until after puberty. Only a small proportion of primary oocytes will ovulate during a lifetime, the rest will regress[34].

Secondary follicles consist of the primary oocyte surrounded by stratified epithelium of granulosa cells (polyhedral follicular cells) arranged in multiple layers. The development of a glycoprotein layer, the zona pellucida produced by the granulose cells, around the oocyte is a trademark of the secondary follicle. Oocyte microvilli and cytoplasmic extensions of the granulose cells penetrate this zona pellucida from either side to make some contact. Now some fluid-filled clefts will form among the granulosa cells and in the late secondary follicle a vascularised multilaminar layer of spindle-shaped cells (theca cells) form around the granulosa cell layer[34].

Tertiary (Graafian) follicles consist of the primary oocyte surrounded by stratified epithelium of granulosa cells, in turn surrounded by the theca, and a fluid filled cavity (the antrum) which develop among the granulosa cells. The tertiary follicle is characterised by this antrum which is formed through the coalescence of multiple small clefts amongst the granulosa cells and contains follicular liquor. The oocyte is positioned in an accumulation of granulosa cells (cumulus oophorus) and the granulosa cells in the immediately surrounding of the oocyte become columnar and radially orientated (corona radiata). The corona radiate provide nutrients to the oocyte. In tertiary follicles the granulosa cells forms a parietal follicular lining called the stratum granulosum which are polyhedral in shape. The basal layer however may be columnar. The granulosa cells produce the follicular liquor[34].

In tertiary follicles the theca differentiate into two layers; the theca interna and theca externa. The theca interna cells are spindle shaped and this layer is supplied with capillaries by the blood vessels of the theca externa. The theca externa consists of a thin layer of loose connective tissue with fibrocytes and surrounds the theca interna. A mature follicle by definition is a tertiary follicle just before ovulation. Near ovulation the primary oocyte completes the first meiotic division and become a secondary oocyte. The second meiotic division follows but arrest in metaphase and will only complete once fertilisation takes place. After fertilisation the second division complete and the oocyte becomes an ovum. The ovum becomes a zygote when the male and female chromosomes come together[34].

Ovulation takes place secondary to an increase in volume of liquor and weakening of the follicular wall. Increase in liquor volume secreted by the granulosa cells occur due to an increase in capillary blood pressure and permeability during oestrus. Weakening of the wall is caused by luteinising hormone (LH) that stimulate the production of prostaglandin F_2 which in turn release collagenases from follicular cells. Collagenases digest the follicular wall which also causes the release of proteins causing an inflammatory response with histamine release. All of these processes degrade the connective tissue of the follicular wall and the ground substance of the cumulus oophorus. Eventually the follicle will rupture at the thin transparent surface area called the stigma. As ovulation takes place the oocyte is simultaneously released from the now weakened cumulus oophorus. Most domestic animals will ovulate spontaneously, however the queen's ovulation is induced by copulation[34].

A corpus haemorrhagicum is a blood filled antrum that develops after ovulation due to rupture, collapse and shrinking of the follicle and severe folding of the follicular wall. The increased vascularisation and permeability of the capillaries into the granulosa cell layer from the theca interna leads to haemorrhage into the ruptured follicle.

Luteinisation is the process by which the granulosa and theca cells transform into luteal cells. The luteal cell population consists of two types of cells. Large luteal cells originate from the granulosa cells and small luteal cells from the theca interna cells. Growth of the corpus luteum results initially from mitosis of both types of cells but later mainly from hypertrophy of the large luteal cells. The small luteal cells make up a minor part of the corpus luteum and occupy mainly trabecular and peripheral areas. The two luteal cell types eventually become mixed in the corpus luteum and later on are difficult to distinguish.

Luteal cell regression, characterised by large lipid droplets and crystalloid inclusions, takes place in late dioestrus and also involves the condensation of lutein pigment. This results in a reddish discoloured appearance of the regressing corpus luteum which leads to the term corpus rubrum. Fribrosis and resorption of most of the corpus luteum follow which leaves a connective tissue scar called the corpus albicans. In older ovaries an abundance of these scars are to be expected.

Atresia is the process by which follicles regress at some stage during their development and do not undergo ovulation. Vesicular (antral) follicles may undergo regressive changes (atresia) at any time before reaching maturity. Many more follicles undergo atresia than ever attain maturity. Atresia of large follicles is gradual but the series of changes can be followed by noting follicles at different stages in the atretic processes. Nuclear pyknosis and chromatolysis in the follicular walls are molecular signs of atresia. During this process the basal lamina of the granulosa layer will also thicken and hyalinise and becomes the glassy membrane. During the process of atresia, prominent interstitial endocrine cells are formed. In antral follicles these cells arise from epitheloid theca interna cells and from hypertrophied granulosa cells in preantral

follicles. These interstitial endocrine cells are polyhedral and epitheloid and contain lipid droplets[34]. The process of atresia is better described in the human literature[35].

Very early atretic changes are indicated by an intact theca interna and membrana granulosa but some cells of the latter are beginning to slough into the antrum which still contains follicular fluid. Disruption of the cumulus oophorus takes place and degeneration of the ovum advances. Sometimes the remnant ovum, surrounded by a swollen zona pellucida can be seen free in the antrum[35].

Advanced early atresia is characterised by hypertrophy of the epitheloid cells of the theca interna but the membrana granulosa is no longer present; all of its cells have sloughed off and have been resorbed. The basement membrane between these two layers has now thickened, now called the hypertrophied glassy membrane. Loose connective tissue now grows in from the stroma and partially fills the reduced antral cavity in which follicular fluid is still present.

With moderate atresia, stroma replaces the theca interna cells the hypertrophied glassy membrane becomes thicker and loose connective tissue with small blood vessels completely fills the former antrum[35].

Finally with late atresia, the loose connective tissue is also replaced by stroma, but the hypertrophied glassy membrane remains for some time as the only indication of the former follicle[35]. In humans the glassy membrane also folds together with hypertrophy[34] similar that of the cow.

The medulla is the inner area of the ovary that contains nerves, many blood vessels and lymph vessels. It also contains loose connective tissue and strands of smooth muscle that are continuous with those in the mesovarium. Solid cellular cords or networks of irregular channels called the retia ovarii are also located in the medulla. These channels are lined by cuboidal epithelium that might differentiate into follicular cells under certain circumstances.

The anatomy and histology of the uterotubal junction of various mammalian species has been investigated and compared[26;36]. The uterotubal junction or uterine ostium is an important physiologic sphincter for the passage of sperm up the uterine tube as well as the passage of blastocysts down the tube[36]. During a preceding morphological study by the author various follicle-like structures were found on the ovaries of three three-year-old lionesses. It was also noticed during this study that the suspensory ligaments displayed the ability to contract and relax.

The uterus consists of bilateral horns (cornuae) an unpaired body (corpus) and a neck (cervix). Its wall consist of three layers namely the endometrium, myometrium and perimetrium[26].

The endometrium consists of a superficial (functional) layer and a thin deeper layer (basal zone). The functional layer arises from the basal zone mainly during pregnancy and degenerates again afterwards. Its surface epithelium is simple columnar in nature. This

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functional layer comprise of the epithelial layer, the subepithelial superficial part and a deep part. The height and structure of the simple columnar epithelium vary according to the stage of the reproductive cycle. The supepithelial superficial part is a richly vascular loose connective tissue layer which contains a variety of tissue and blood cells. The deep part also consists of loose connective tissue but is less vascular and cellular than the superficial part. The basal zone is rather inconspicuous. Tubular glands that are lined by simple columnar glandular epithelium are present throughout the endometrium. The epithelium includes secretory and non-secretory ciliated cells. The myometrium consists of thick inner circular layer and longitudinal outer layer of smooth muscle cells and a vascular layer (stratum vasculare) separates these two layers. The perimetrium consists of loose connective tissue, which contains some smooth muscle cells, covered by the peritoneal mesothelium[34].

The cervix (neck of the uterus) is thick-walled, muscular and rich in elastic fibres. The mucosa-submucosa forms prominent folds and, in the dog and cat, secondary and tertiary folds. Uterine glands are absent from the cervix. The cervical epithelium is simple columnar and contains many mucigenous cells including goblet cells. A small proportion of ciliated epithelial cells occur in some species. The propria-submucosa consists of dense irregular connective tissue and in bitches venous plexuses are present in its deeper parts. The tunica muscularis consists of inner circular and outer longitudinal smooth muscle layers and elastic fibres are prominent in the circular layer. The tunica serosa consists of loose connective tissue covered by mesothelium[34].

The vagina in the dog and cat is mostly lined by a stratified squamous epithelium. The tunica muscularis consists of three layers: a thick inner circular smooth muscle layer; a thin outer longitudinal smooth muscle layer and; thin layer of longitudinal smooth muscle inside the thick circular layer. The smooth muscle of the thick inner circular layer is separated into bundles by connective tissue. The tunica serosa of the vagina cranially is a typical serosa of loose connective tissue covered by mesothelium and caudally it is covered by a tunica adventitia of loose connective tissue only. This distinction is created by the peritoneal excavations of the rectogenital and vesicogenital pouches on the dorsal and ventral aspects of the vagina respectively[34].

2.2 Problem statement

There is no histological description of the female reproductive organs of the African lion available.

2.3 Research objectives

The purpose of this study was to accurately describe the basic histological characteristics of the female reproductive organs in the African lion.

2.4 Hypothesis

The histology of the female reproductive organs of the African lion will resemble that of the domestic dog and cat.

2.5 Benefits

A complete description of the histology of the female reproductive organs of the African lion will provide background knowledge for future reference during:
- i) histopathology performed on the described organs and
- ii) future histology studies on the female reproductive organs.

3. Materials and Methods

3.1 Sample population

Three formalin embalmed cadavers of free ranging lionesses, prepared with 4% formalin, were used in this part of the study.

3.2 Collection of samples

The female reproductive organs were harvested and stored in 10% formalin.

3.3 Histology

Tissue samples were collected and fixed in formaldehyde 10% for a prolonged period, processed by routine histological methods and embedded in paraffin wax according to annexure A. Sections of 5 µm thick were cut in the transverse plane of all components the organ system according to **Annexure A** and stained with haematoxylin and eosin (H&E). The sections were examined using an Olympus BX63 light microscope with bright field illumination. Images were captured using an Olympus DP72 camera. The software package cellSens Dimension was used and its sharpening filter, brightness and contrast enhancement functions were applied where deemed necessary.

The histological sections were examined. Appropriate areas of interest were identified and photographed under 40x (200µm), 100x (100µm), 400x (20µm) and 1 000x (10µm) magnifications (microbar measurements indicated in brackets) after filter sharpening was done and the microbar information was burnt into the image. In some areas 200x (50µm) magnification was required to provide better spatial orientation. The images were studied and annotated appropriately.

53

4. Results

4.1 Suspensory ligament

The cranial aspect of the suspensory ligament at its area of origin consisted entirely of smooth muscle and some connective tissue **(Figures 46-48)**.
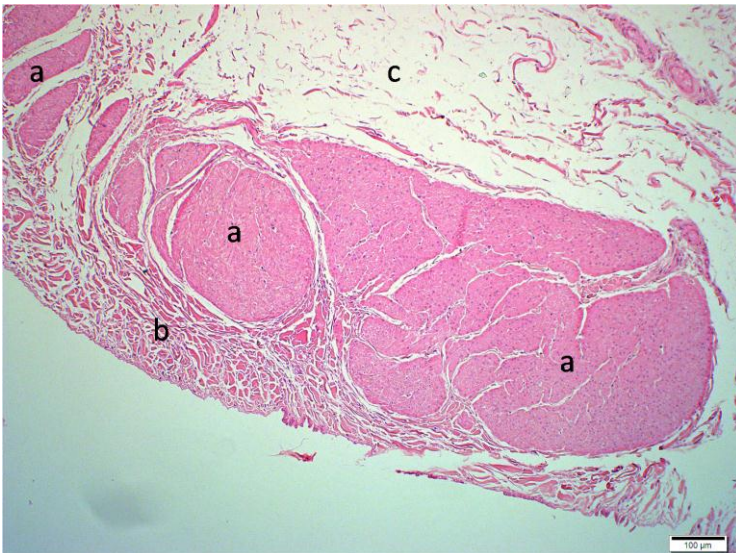
Figure 46   Suspensory ligament.

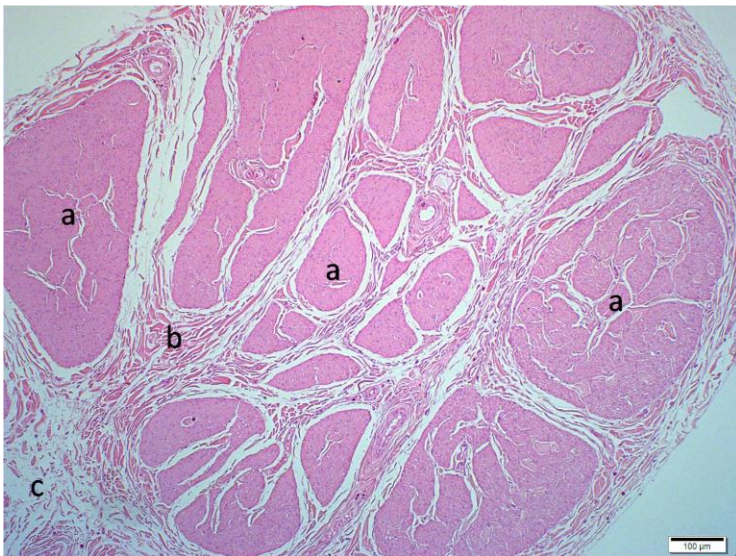Smooth muscle (a), dense connective tissue (b), loose connective tissue (c).
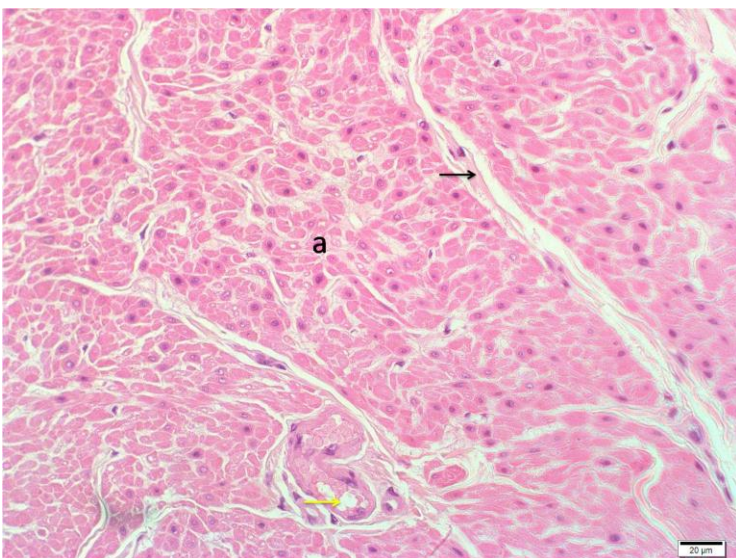
Figure 47   Suspensory ligament.

Smooth muscle (a), fibrocytes (black arrow), and arteriole (yellow arrow).
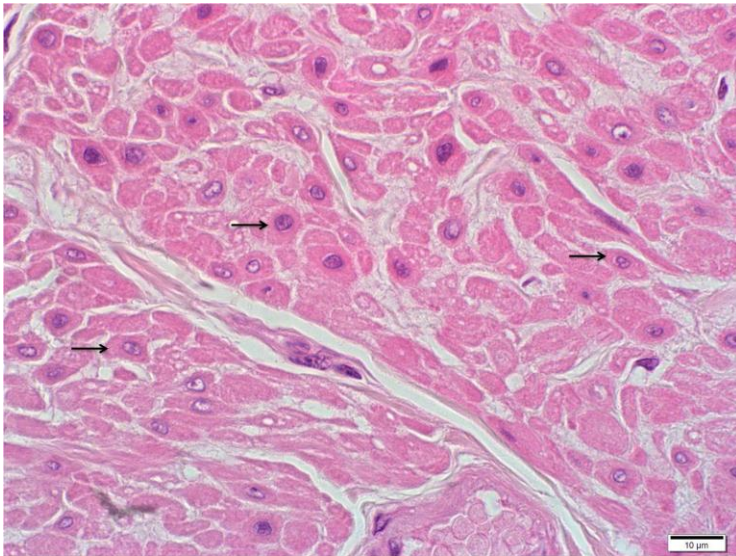
Figure 48   Suspensory ligament.
Smooth muscle cells (arrows).

The caudal aspect of the suspensory ligament close to its insertion consisted of a larger number of smooth muscle cell bundles associated closer together and some interposing connective tissue **(Figures 49 and 50)**.

Figure 49   Suspensory ligament.
Smooth muscle (a), dense connective tissue (b), loose connective tissue (c).

55
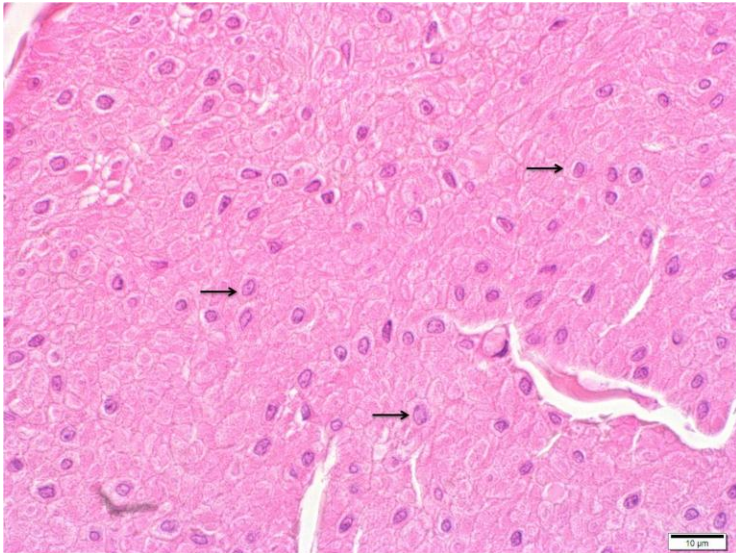
Figure 50   Suspensory ligament.
Smooth muscle cells (arrows).

4.2 Uterine tube

The uterine tube consisted of numerous fimbriae **(Figures 51-54)**, the infundibulum **(Figures 55)**, ampulla **(Figures 56-59)** and isthmus.
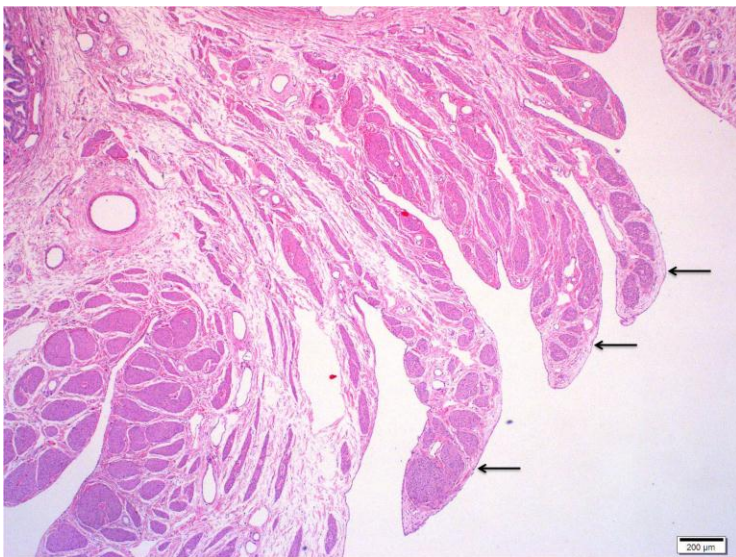
Figure 51   Fimbriae.
Indicated by arrows.
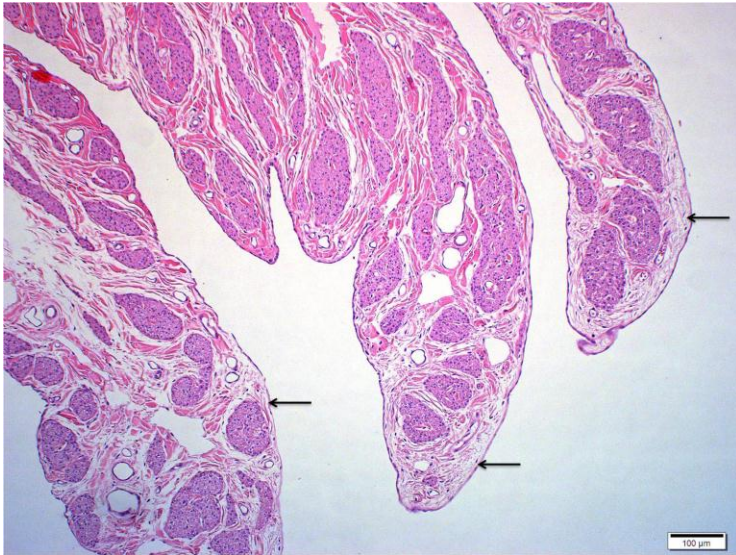
Figure 52   Fimbriae.

Indicated by arrows. The smooth muscle bundles are visible at this magnification.
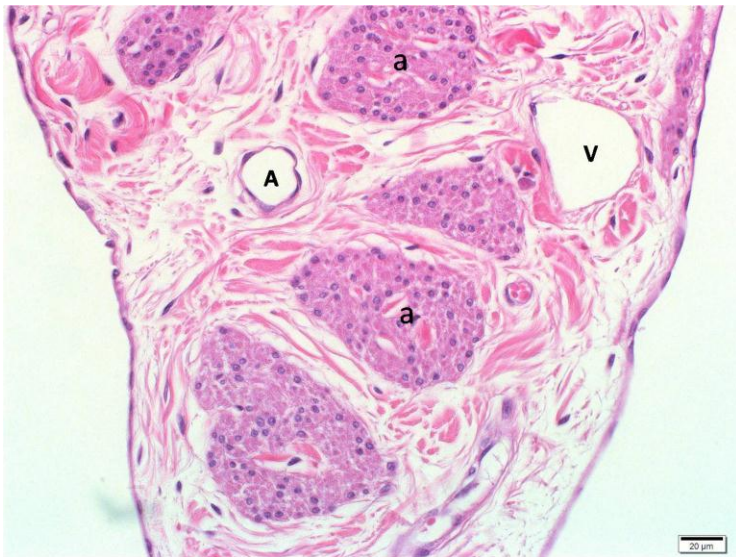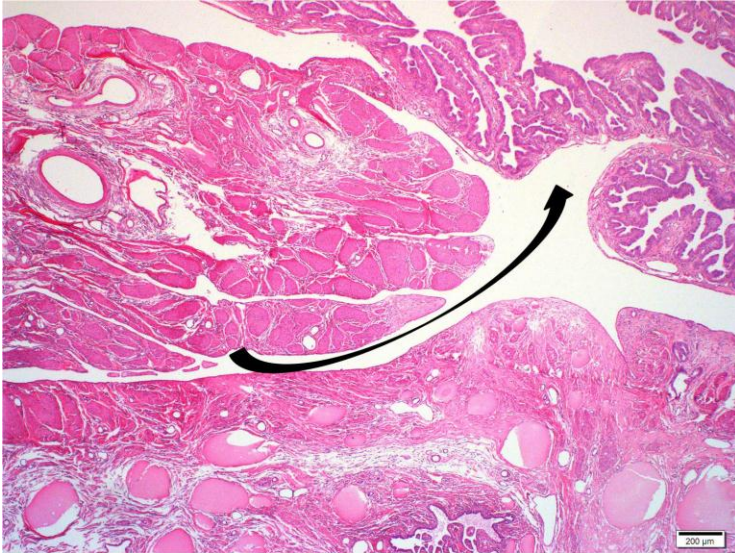

Figure 53   Smooth muscle of the fimbriae.

Smooth muscle (a), arteriole (A) and venule (V).
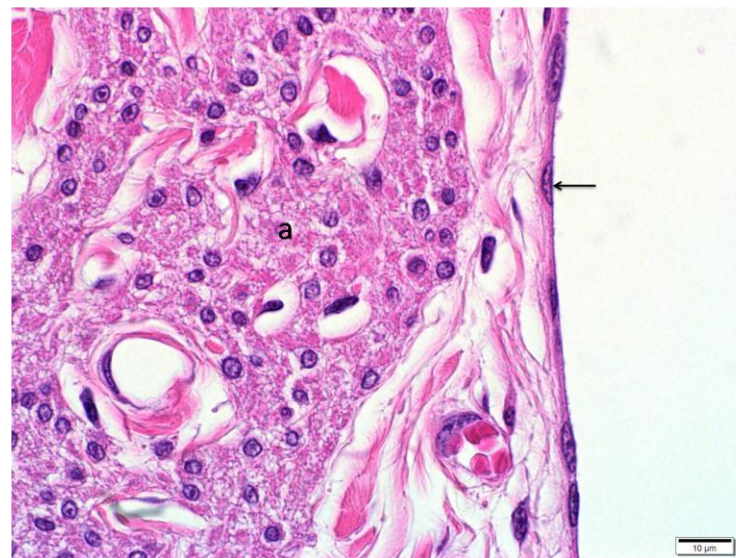
Figure 54   Smooth muscle of the fimbriae (a).

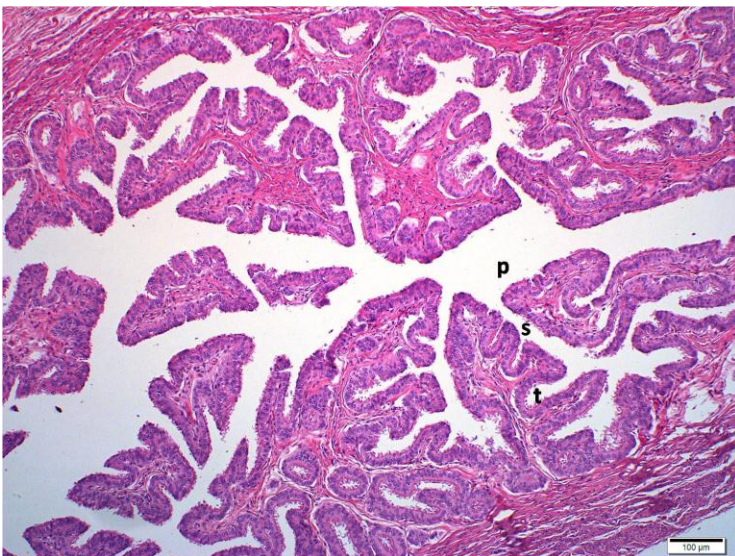Surface epithelium indicated by the arrow.

57

Figure 55   Infundibulum.

The transition of the fimbriae to the infundibulum is indicated by the arrow.
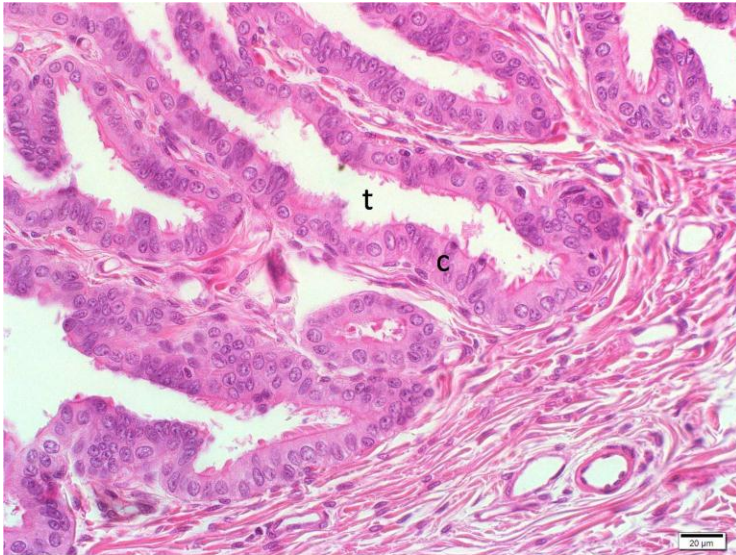

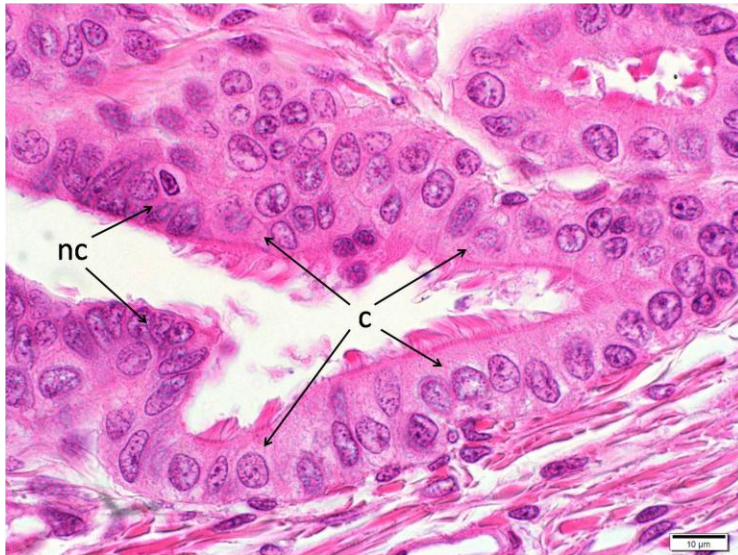**Figure 56**   Ampulla.

Indicated by the oval shapes.

Figure 57   Folds of the ampulla.

Primary (p), secondary (s) and tertiary (t) folds.

58

Figure 58   Ampulla.

Tertiary folds (t) and ciliated columnar epithelium (c).


Figure 59   Columnar epithelium of the ampulla.

Ciliated (c) and non ciliated (nc) columnar cells of the uterine tube.

4.3 Ovary

The ovary consisted of a cortex covered with surface epithelium **(Figures 60)** and medulla **(Figures 61)**.

4.3.1 Cortex

The cortex contained primordial **(Figures 61 and 62)**, primary **(Figures 63)**, secondary **(Figures 64-66)**, tertiary **(Figures 67-74)** and atretic follicles **(Figures 75-85)** at various stages.

59
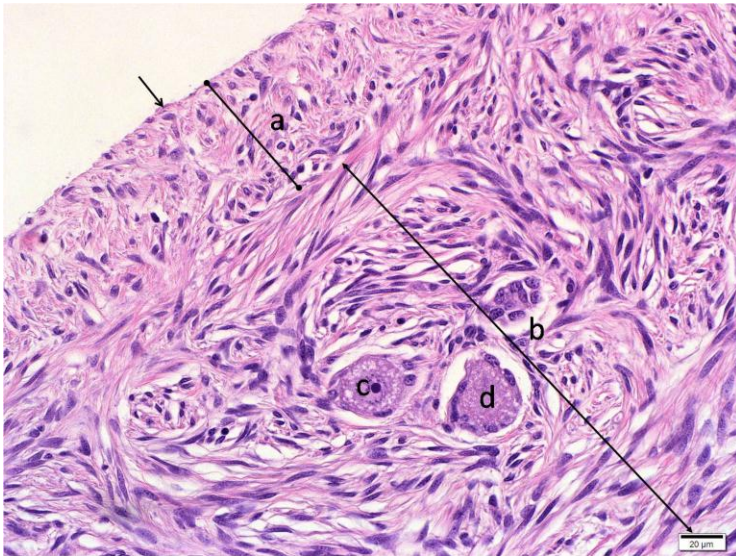
Figure 60   Ovarian cortex.

Surface epithelium (arrow), tunica albuginea (a) and cortex (b) of the ovary. Note the primordial follicle (c) which consists of a primary oocyte with its nucleolus surrounded by simple squamous epithelium and a primordial follicle undergoing transition to a primary follicle with cuboidal epithelium (d).

4.3.1 Primordial follicle
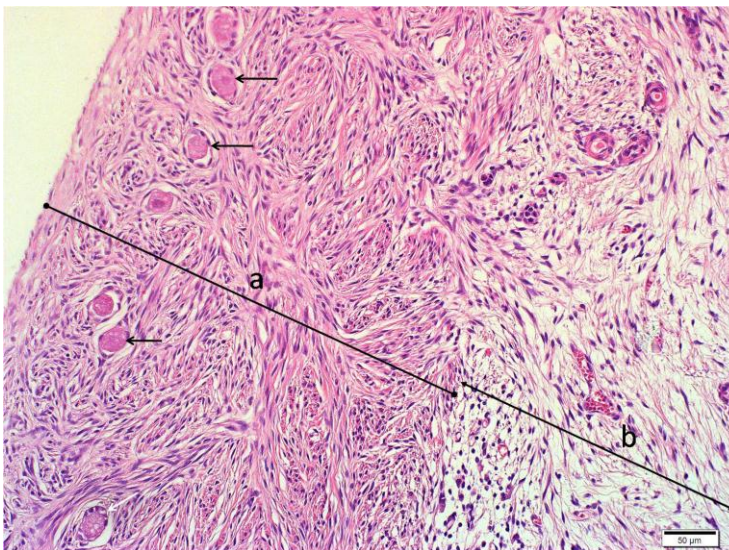

Figure 61   Ovarian cortex and medulla.

Ovarian cortex (a) and medulla (b). Several primordial follicles (black arrows) and a primary follicle (white arrow) may be noted in the cortex.

60
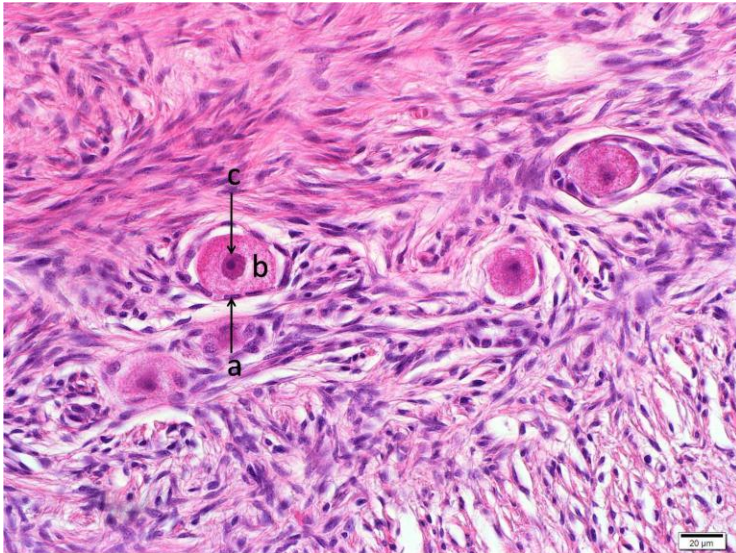
Figure 62   Primordial follicles.

Several primordial follicles surrounded by simple squamous epithelium (a) contain a primary oocyte (b) with its nucleus and nucleolus (c).
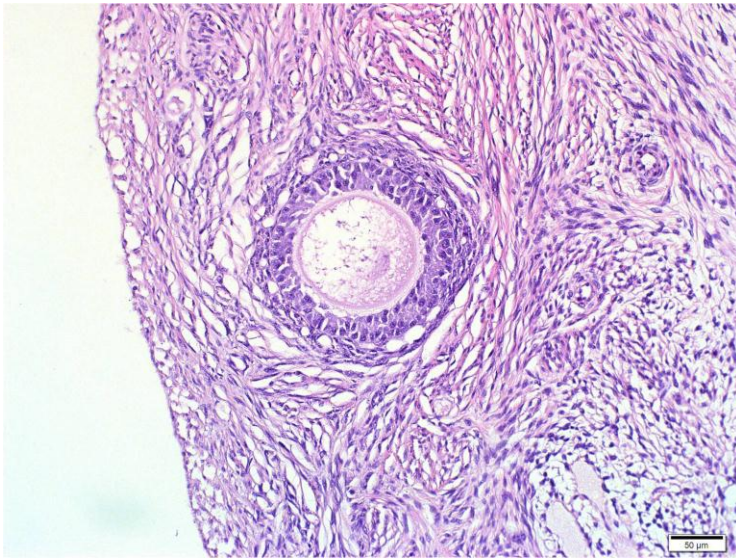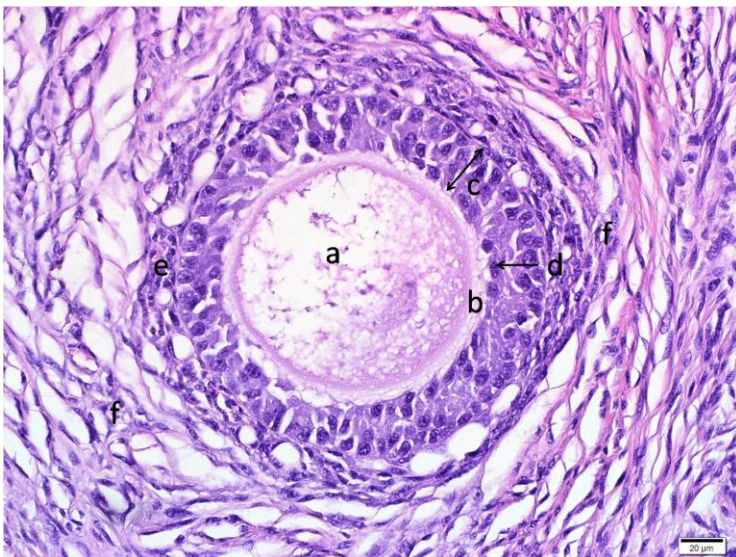
4.3.2 Primary follicle
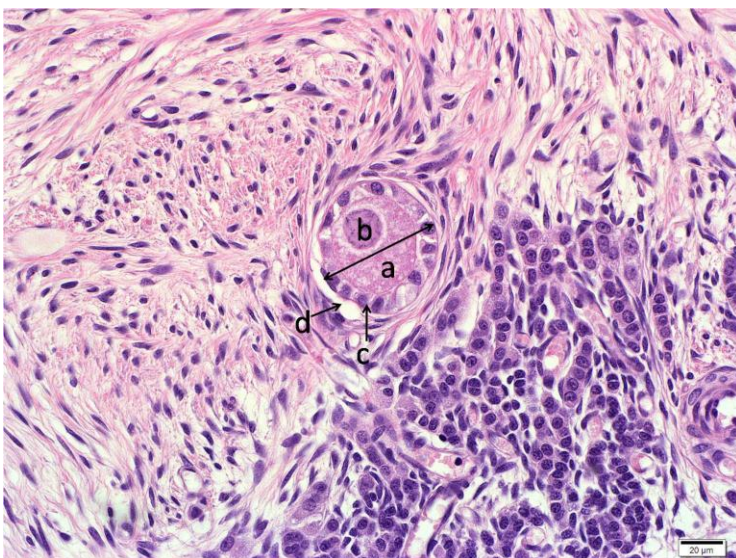
Figure 63   Primary follicle.

The primary follicle is surrounded by cuboidal epithelium (c) and contains a primary oocyte (a) with its nucleus (b) and an associated capillary (d).
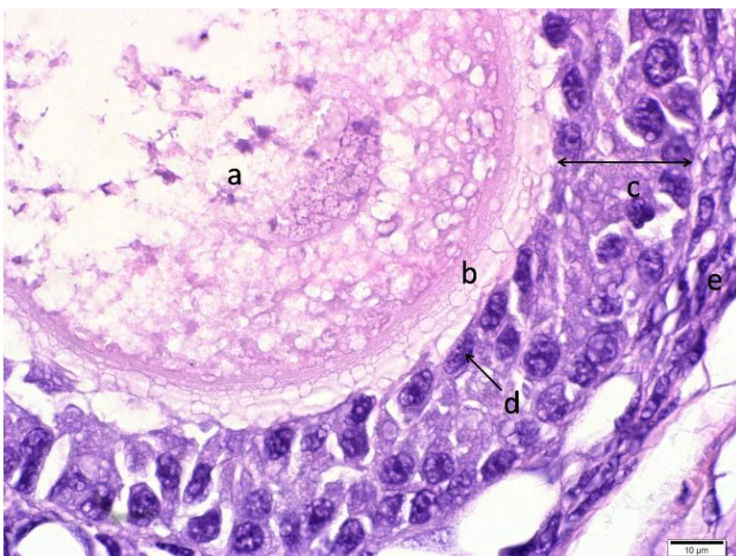
61

4.3.3 Secondary follicle

Figure 64   Secondary follicle.

Situated in the peripheral ovarian cortex.

Figure 65   Secondary follicle.

The secondary follicle contains a primary oocyte (a) with its nucleus surrounded by a distinct zona pellucida (b). A stratified epithelium of polyhedral cells (c) surrounding the zona pellucida, of which the inner layer of cells forms the corona radiata (d), which in turn is surrounded by the connective tissue cells of the theca interna (e) and theca externa (f).


Figure 66   Secondary follicle.

The secondary follicle and primary oocyte with nucleus (a), zona pellucida (b), stratified epithelium of polyhedral cells (c), corona radiata (d) and theca interna (e).

62

4.3.4 Tertiary follicle
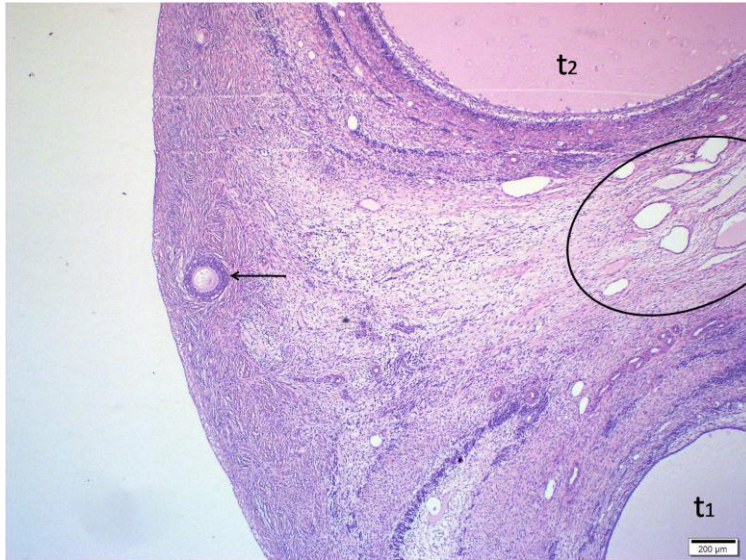
Figure 67   Tertiary follicles.

Two tertiary follicles (t1 and t2) with t2 showing very early atretic changes. Numerous interposing vascular structures (oval) and note the secondary follicle (arrow) in the peripheral cortex.
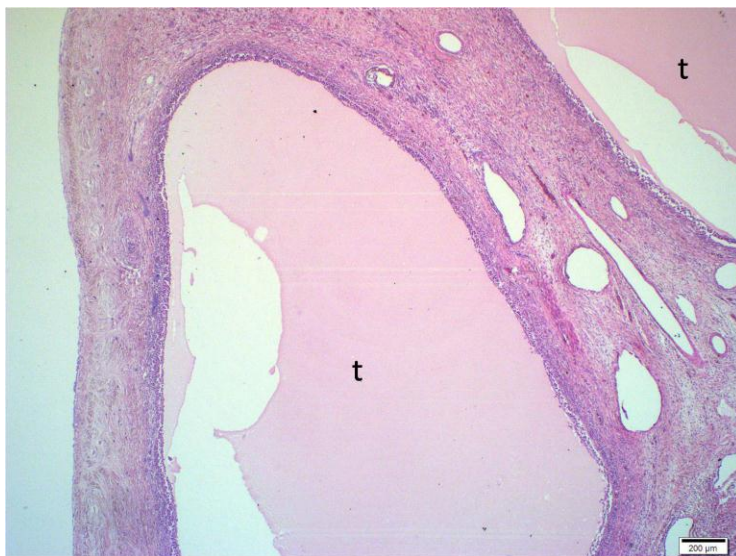
Figure 68   Tertiary follicle.

Two tertiary follicles (t) with numerous interposing vascular structures.
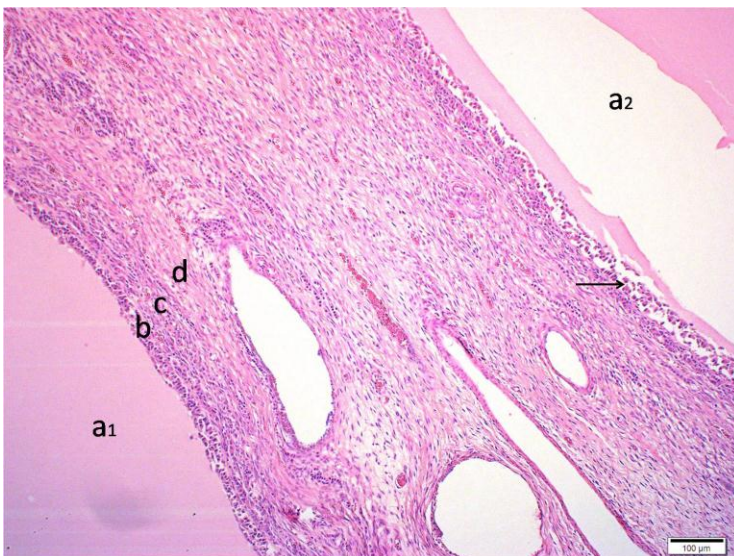
63

Figure 69   Tertiary follicle.

The antrum of the tertiary follicle (a) filled with follicular liquor and a primary oocyte (c) surrounded by the zona pellucida (d) and situated on the cumulus oophorus (b).

Figure 70   Polyovular follicle.

The second oocyte (c) was lost during sectioning.

Figure 71   Tertiary follicles.

The antrum containing follicular liquor (a1) is bordered by a multilayered stratum granulosum (b), theca interna (c) and theca externa (d). Note the very early atresia of the granulosa cell layer with sloughing of the cells into the antrum (arrow) in a tertiary follicle (a2).
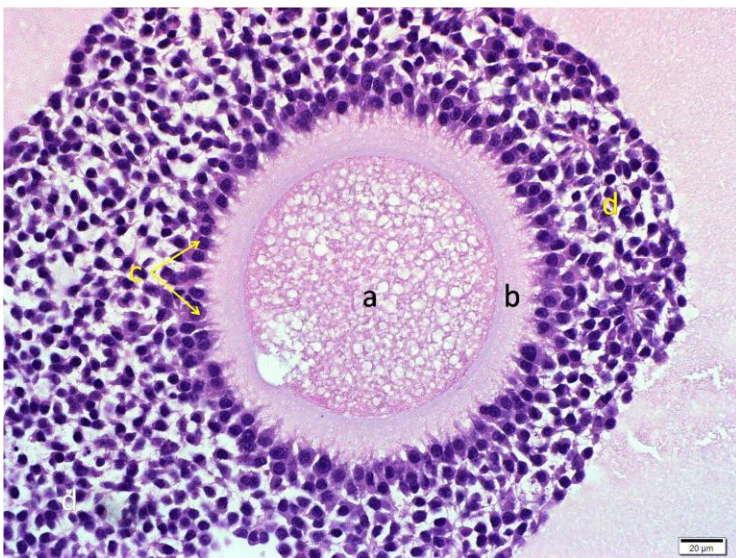
64

Figure 72 Tertiary follicular wall.

The antrum containing follicular liquor (a) is bordered by a multilayered epithelial layer, the stratum granulosum (b), theca interna (c) and theca externa (d).
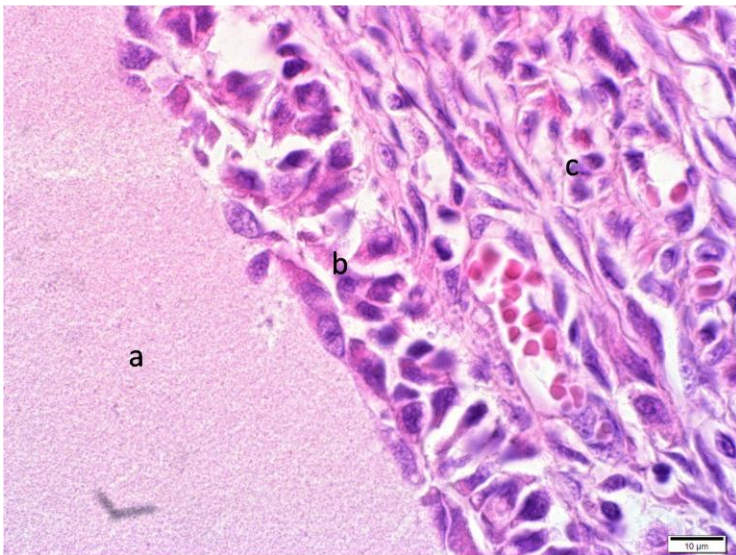
Figure 73 Tertiary follicular wall.

Antrum containing follicular liquor (a) stratum granulosum (b) and theca interna (c).
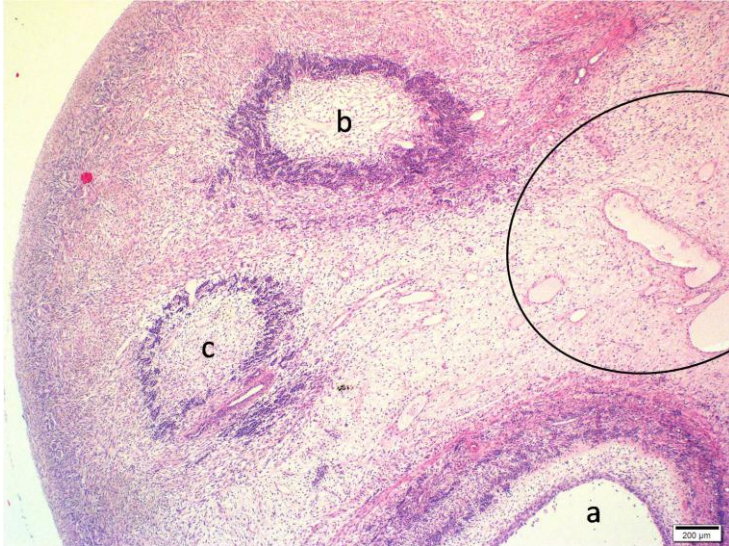

Figure 74 Primary oocyte.

A primary oocyte (a) surrounded by the zona pellucida (b), corona radiata (c) and cumulus oophorus (d). Note the cytoplasmic extensions of the granulosa cells, around the oocyte, penetrating the zona pellucida.

65

4.3.5 Atresia

The various stages of follicular atresia were studied and described **(Figures 75 – 86)**.

Figure 75   Atretic follicles.

Atretic follicles at varying stages of atresia. Very early (a) advanced early (b) and moderate atresia (c). The ovarian medulla is indicated by the oval.
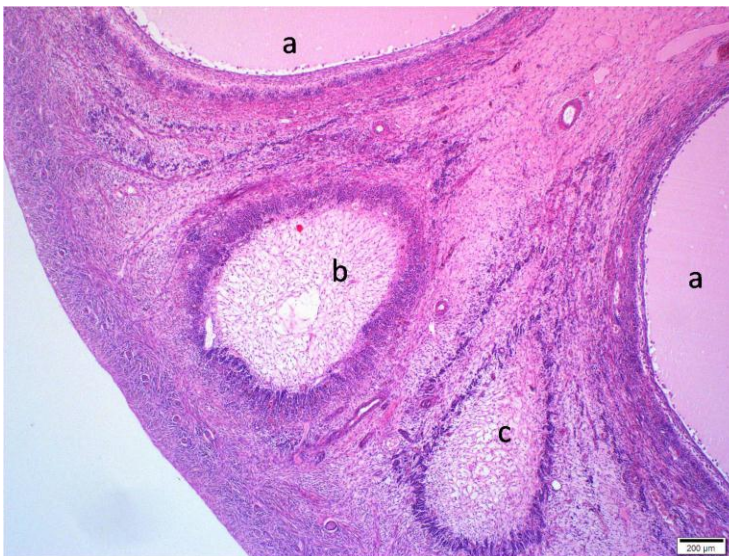
Figure 76   Atretic follicles.

Atretic follicles at varying stages of atresia. Very early (a) advanced early (b) and moderate atresia (c). The ovarian medulla is indicated by the oval.

Figure 77   Very early to advanced early atretic follicle.

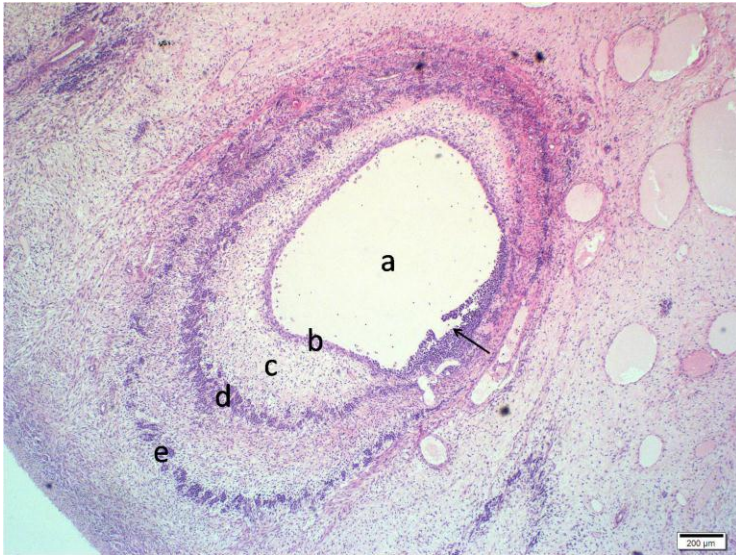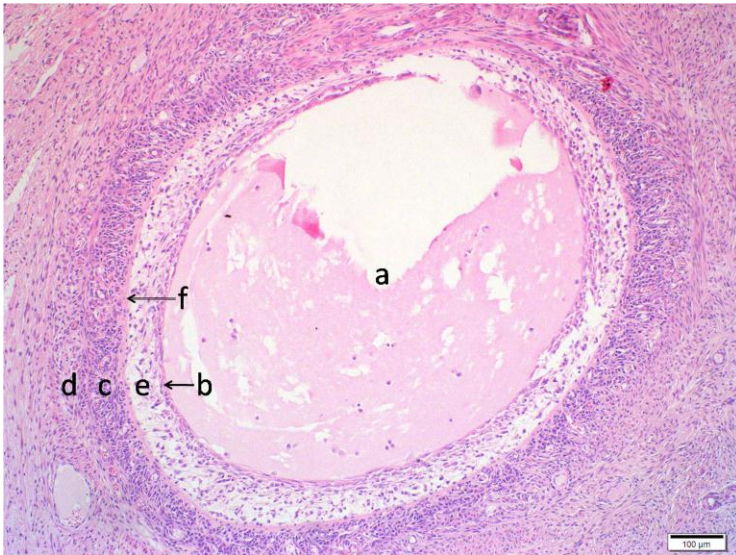The antrum (a) degenerating granulosa layer (b), loose connective tissue (c), theca interna (d) and theca externa (e). Loose connective tissue is growing in from the stroma while the granulosa cell layer is still present. Note the disruption of the cumulus oophorus (arrow) and sloughing of granulosa cells into the antrum.
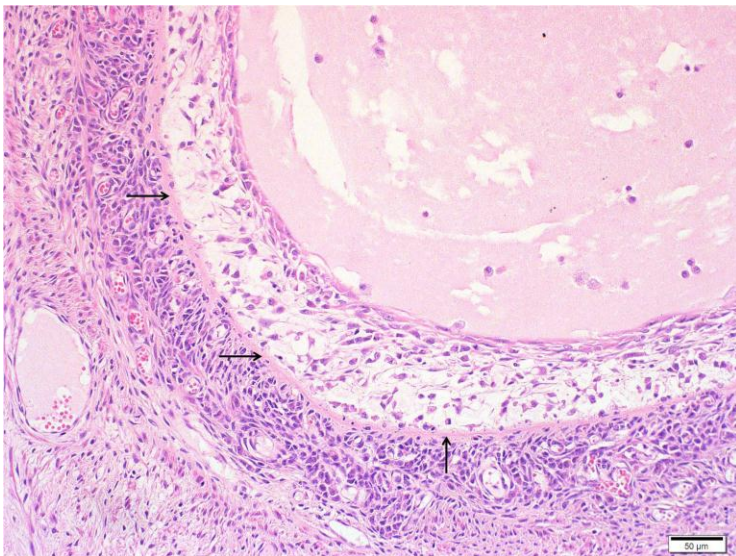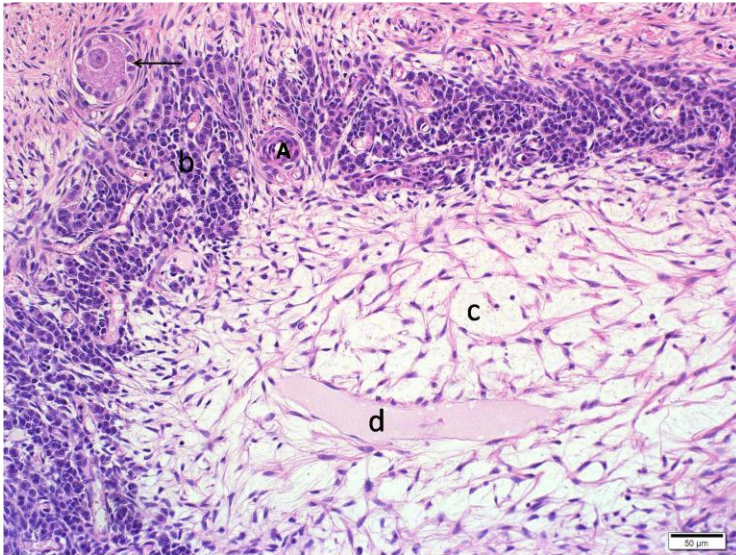

Figure 78   Very early to advanced early atretic follicle.

The antrum (a), degenerating granulosa layer (b), theca interna (c), theca externa (d) and loose connective tissue layer (e). Note the developing glassy membrane (f).

Figure 79   Very early to advanced early atretic follicle.

Note the glassy membrane (arrows) starting to hypertrophy.

Figure 80   Advanced early atretic tertiary follicle.

Hypertrophy, fibrosis and vascularisation of the theca interna (b), loose connective tissue cells (c) and remnant of the antrum with follicular liquor in the centre (d), note that the granulosa cell layer has degenerated completely. Arteriole (A) and incidental secondary follicle (arrow).
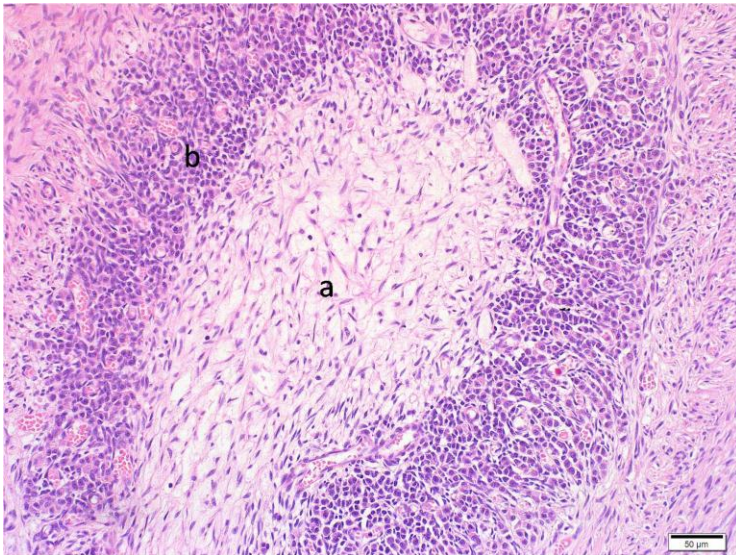
Figure 81   Moderate atretic follicle.

Hypertrophy, fibrosis and vascularisation of the theca interna (b) and loose connective tissue (a) with complete absorption of the granulosa cell layer and total loss of the antrum.
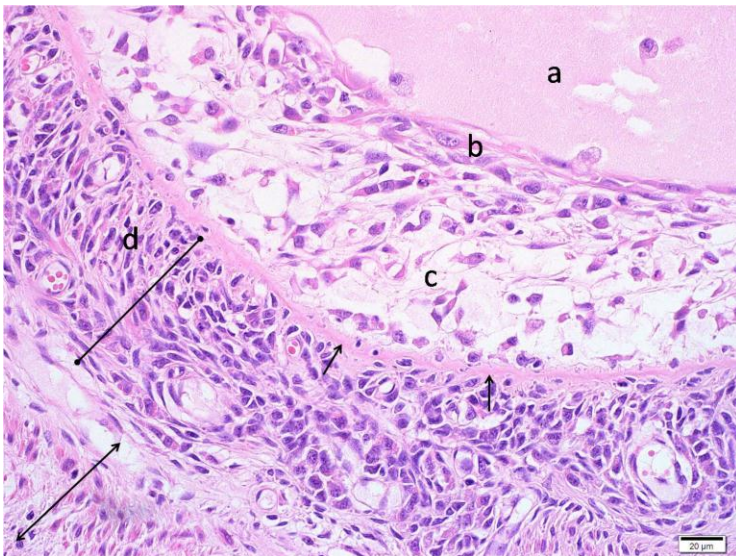
Figure 82   Atretic follicle.

The antrum (a), degenerating granulosa layer (b) and the fibrosing epitheloid theca layer producing interstitial endocrine cells (c). Note the early hypertrophy of the glassy membrane (small arrows), theca interna (d) and theca externa (double black arrow).

68
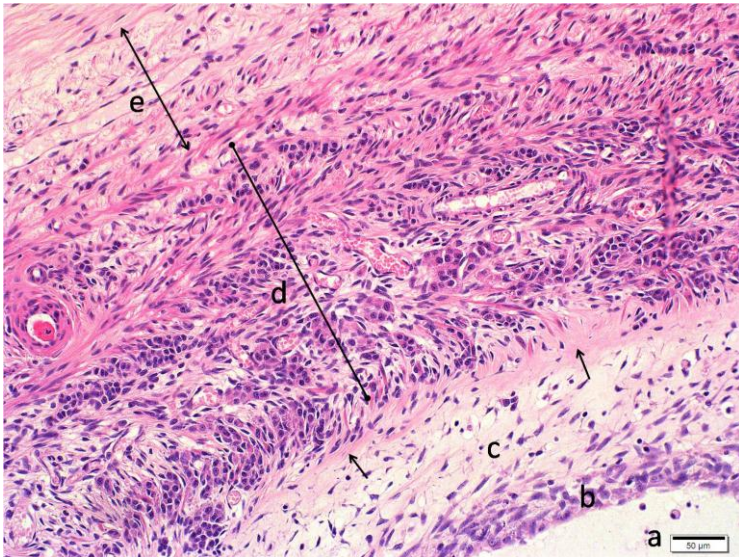
Figure 83   Atretic follicular wall.

The antrum (a), degenerating granulosa layer (b) loose connective tissue laid down by the stroma (c) fibrosis and vascularisation of the theca layer, hypertrophied glassy membrane (white arrows), theca interna (d) and theca externa (e). Note the tendency of layering by the epitheloid cells in the theca interna.
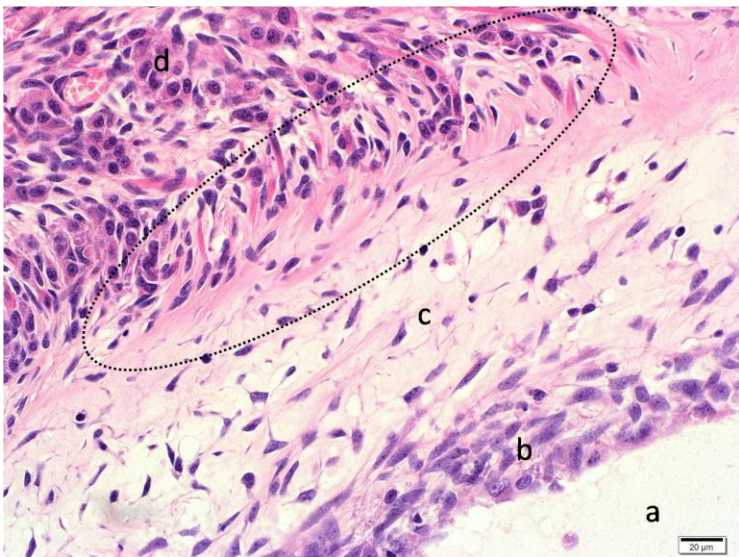
Figure 84   Loose connective tissue grow in from the stroma.

The antrum (a), degenerating granulosa layer with slight hypertrophy of the granulosa cells at this stage (b) and the fibrosing epitheloid theca interna (d). Loose connective tissue (c) grows in from the stroma through the glassy membrane (oval) to fill the antrum.
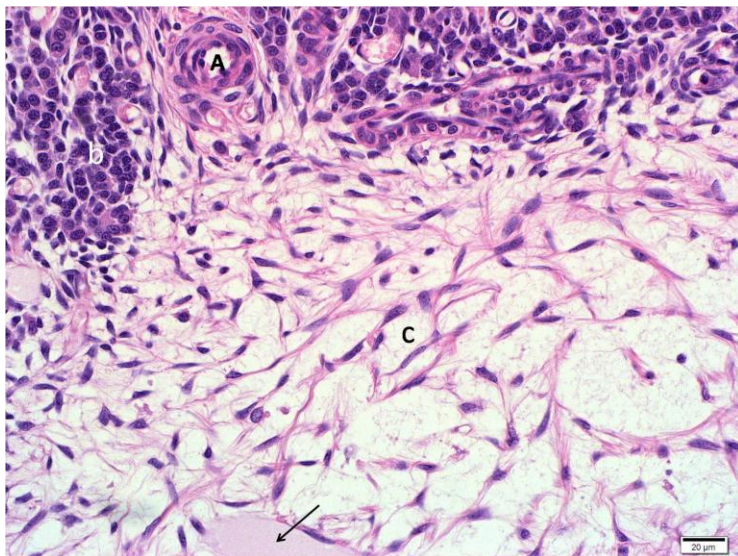
Figure 85   Advanced early atretic follicle.

Hypertrophied epitheloid cells of the theca interna (b) and loose connective tissue(c). Arteriole (A) and note the remnant of the antrum (arrow) with follicular liquor.
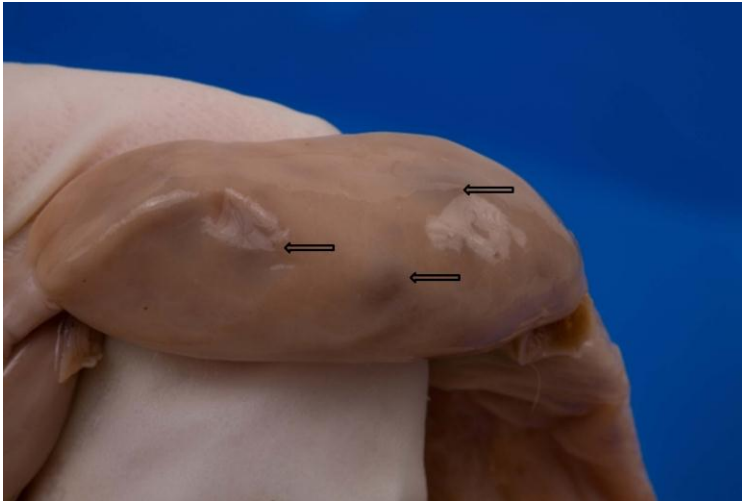
Figure 86   Macroscopic ovary

Note the tertiary (possibly atretic) follicles visible under the surface (tunica albuginea) of the follicle (open arrows).

4.3.6 Medulla

The medulla contained numerous blood vessels **(Figure 87)**.
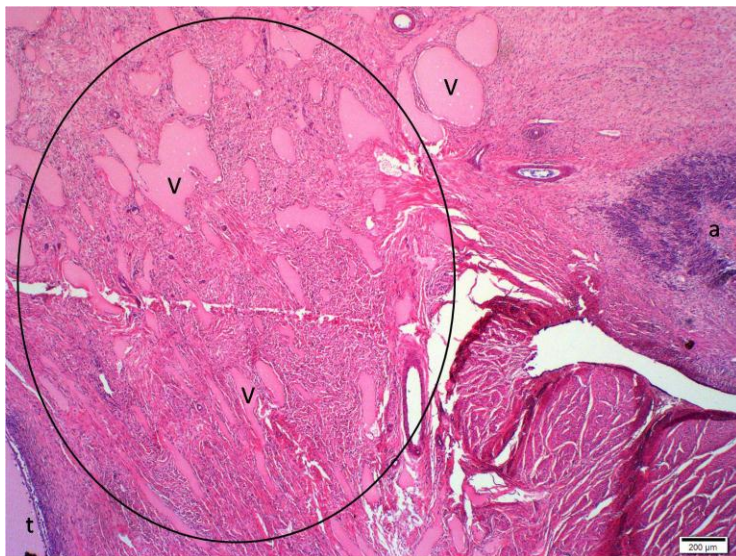
Figure 87   Ovarian medulla (oval).

Blood vessels (V), edge of a tertiary follicle (t) and a late atretic follicle (a).

4.3.7 Mesovarial margin

The mesovarial margin contained large bundles of smooth muscle cells **(Figure 88)**.
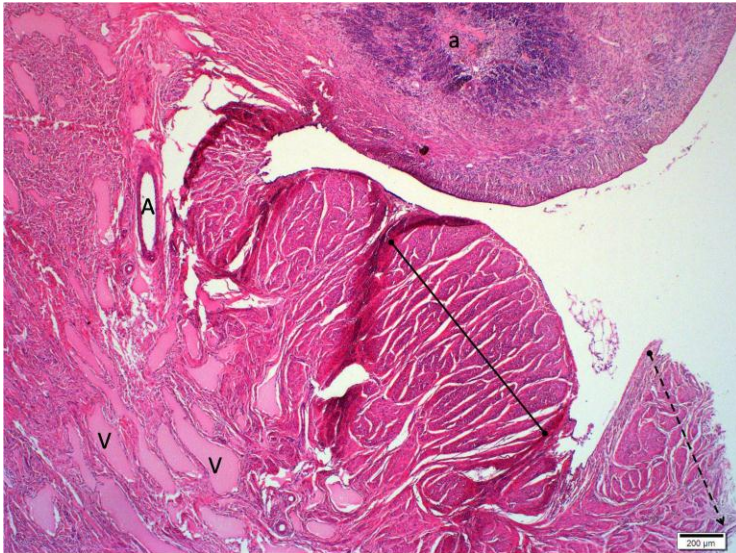
Figure 88   Mesovarial margin of the ovary (stippled line).

Note the numerous venules (V), arteries (A) and prominent smooth muscle (solid line). Late atretic follicle (a).

4.4 Round ligament

The round ligament cranially, at its origin from the tip of the uterine horn, consisted entirely of smooth muscle fibre bundles interposed with connective tissue **(Figures 89-92)**.

Figure 89   Round ligament cranial aspect.

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Figure 90   Round ligament cranial aspect. Consists of smooth muscle cells and connective tissue.
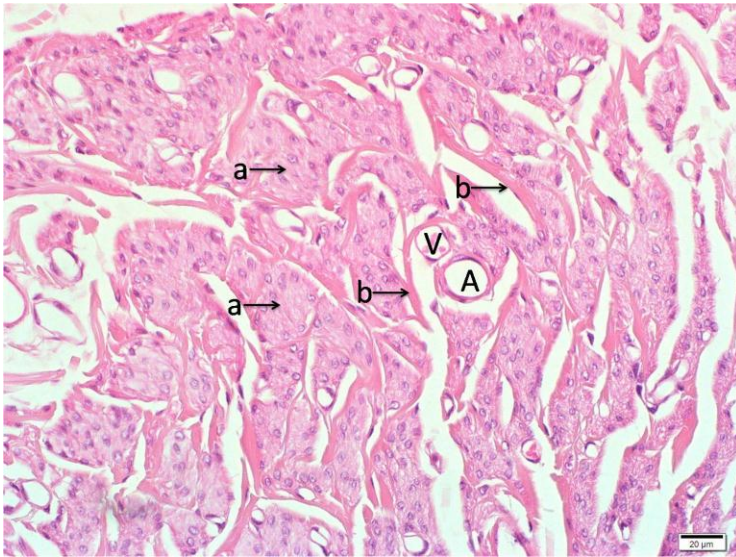
Figure 91   Round ligament cranial aspect.

Consists of smooth muscle cells (a) and connective tissue (b).   Venule (V), arteriole (A).
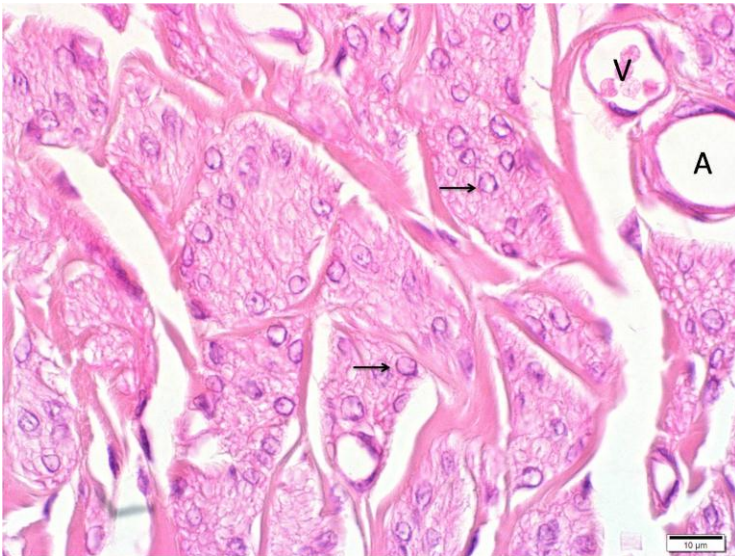
Figure 92   Round ligament cranial aspect.

Smooth muscle cells (arrows), arteriole (A) and venule (V). The size of the smooth muscle cells on cross section is highly variable due to the tapered shape of the cells.

Caudally near its insertion on the medial femoral fascia however it consisted of both smooth muscle and striated muscle constructed in two major bundles **(Figures 93-97)**.

72
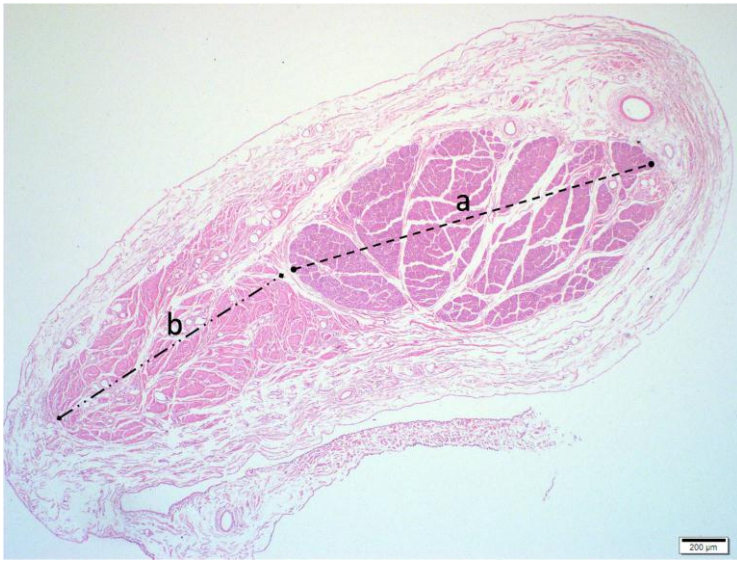
Figure 93   Round ligament caudal aspect.
Skeletal muscle (a) and
smooth muscle (b).

Figure 94   Round ligament caudal aspect.
Skeletal muscle bundles.
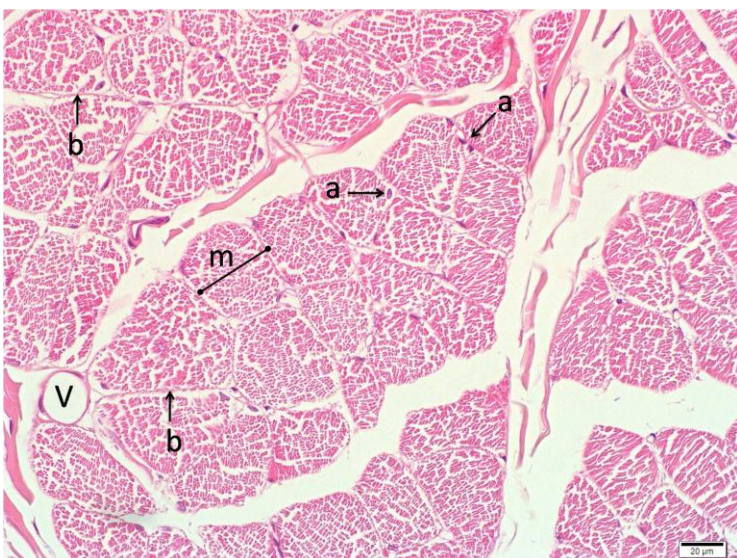
Figure 95   Round ligament caudal aspect.
Skeletal muscle myocytes (m) contain multiple
myofibrils and peripherally located nuclei (a)
and a surrounding cell membrane (b). Note the
venule (V).

73
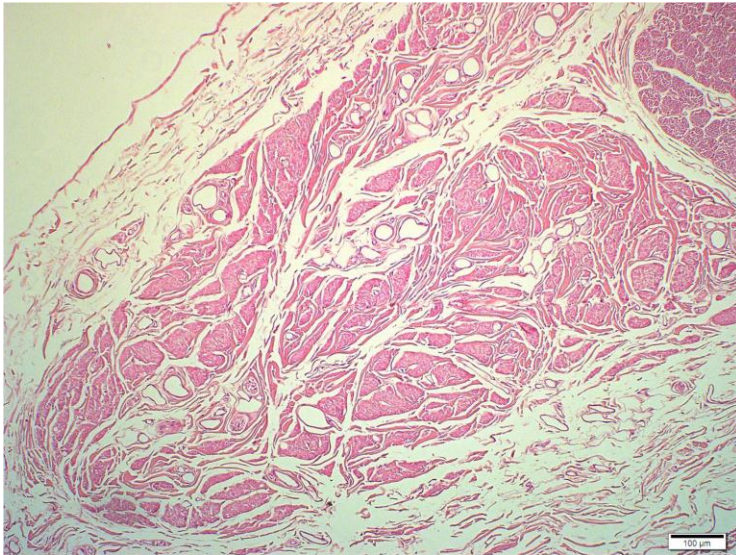
Figure 96   Round ligament caudal aspect.

Bundles of smooth muscle cells arranged in a
very vascular loose connective tissue.
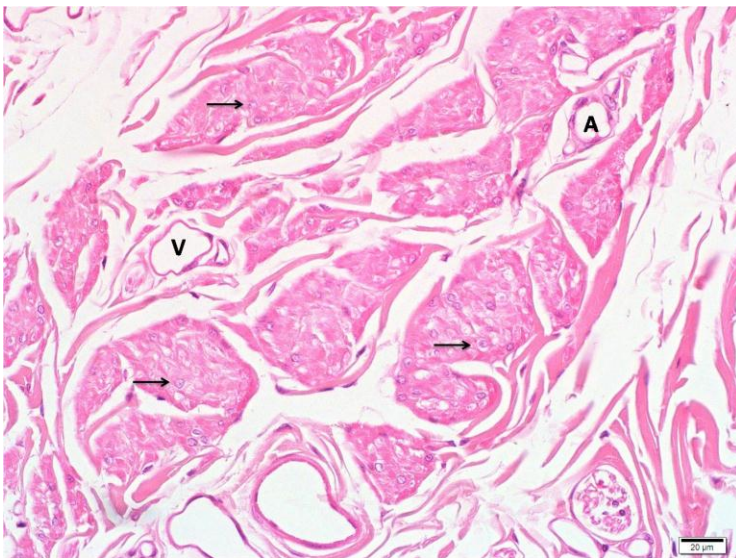
Figure 97   Round ligament caudal aspect.

Smooth muscle cells (arrows), arteriole (A)
and venule (V).

4.5 Uterus

4.5.1 Uterine horn

4.5.1.1 Tip of the uterine horn (cranial aspect)

The cranial **(Figures 98-101)** and caudal **(Figures 102-106)** aspects of the uterine horns
and the uterine body **(Figures 107-110)** were evaluated.

Figure 98 Endometrium, myometrium and perimetrium of the uterine horn (cranial aspect).

Prominent well developed longitudinal smooth muscle bundles (a) in the thick outer layer of the myometrium separated from a thinner inner layer of circular smooth muscle (c) by the vascular layer (b). Peritoneal mesothelium (black arrow) and endometrium (d) with tubular glands (yellow arrows).

Figure 99 Endometrium and myometrium of the uterine horn (cranial aspect).

Inner layer of the myometrium (c) and endometrium (d) with tubular glands (yellow arrows).
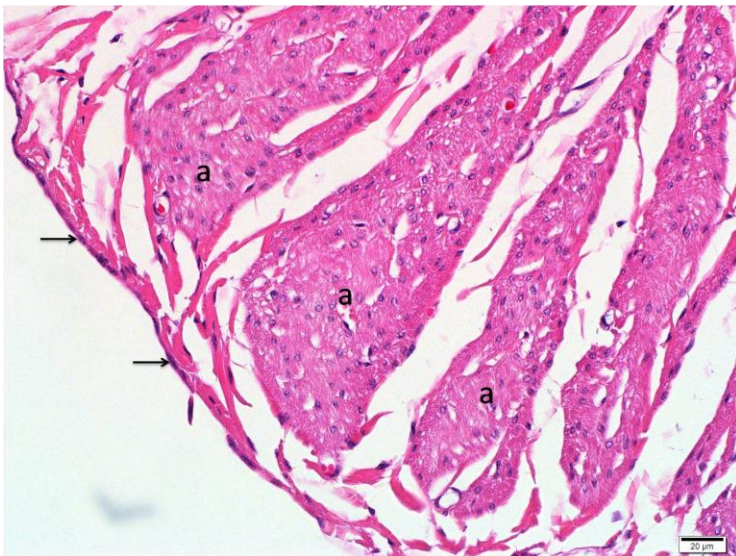
Figure 100 Perimetrium.

Longitudinal smooth muscle cell bundles (a) and peritoneal mesothelium (arrows).

75
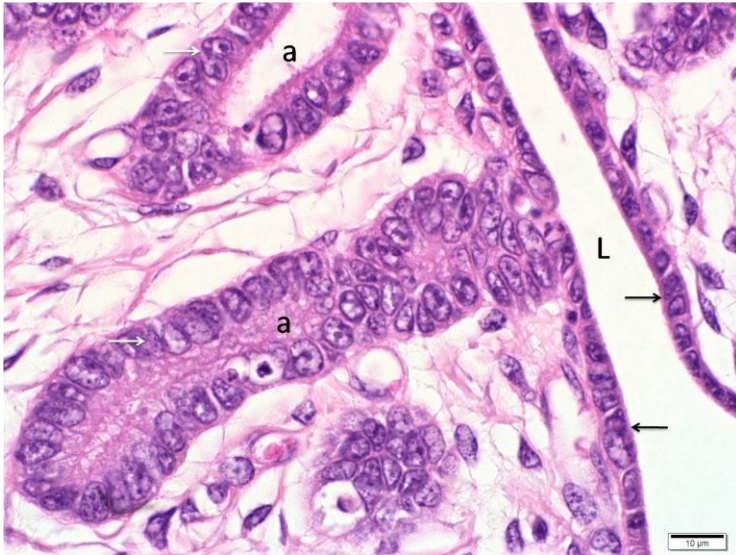
Figure 101 Uterine horn lumen and tubular glands (cranial aspect).

The tubular glands (a), lined by ciliated simple columnar glandular epithelium cells (white arrows), open into the uterine lumen (L) which is lined by simple columnar epithelium (black arrows).

4.5.1.2 Uterine horn immediately cranial to the uterine bifurcation (caudal aspect)

Figure 102 Endometrium, myometrium and perimetrium of the uterine horn (caudal aspect).

Longitudinal smooth muscle bundles (a) in the outer layer of the myometrium separated from the inner layer of circular smooth muscle (c) by the vascular layer (b). Peritoneal mesothelium (black arrow) and endometrium (d) with tubular glands (yellow arrows). Note that the two layers of the myometrium are of similar development in this area.

Figure 103   Endometrium of the uterine horn (caudal aspect).

Lumen of the uterine body (L) and endometrium (d) with tubular glands (yellow arrows).
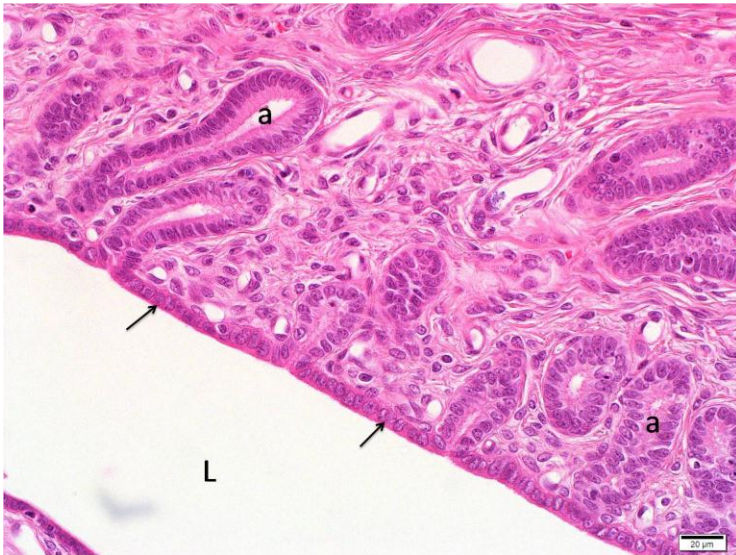
Figure 104   Uterine horn lumen and tubular glands (caudal aspect).

The tubular glands (a), lined by ciliated simple columnar glandular epithelial cells, open into the uterine lumen (L) which is lined by simple columnar epithelium (black arrows).
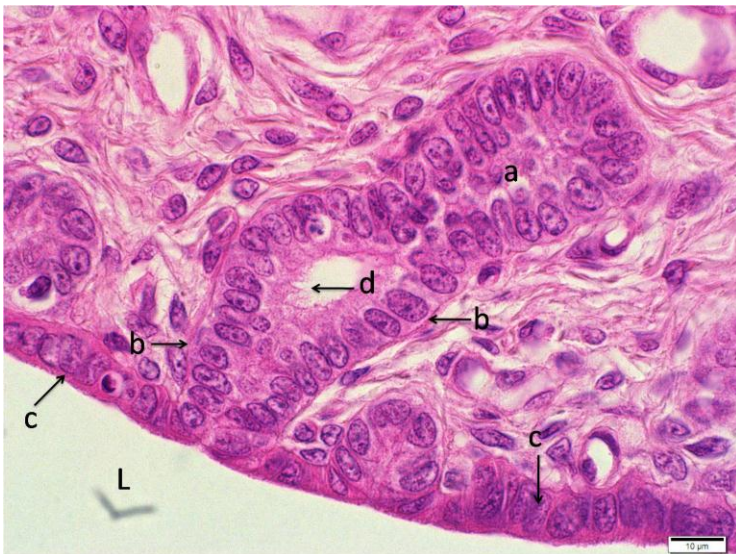
Figure 105   Uterine horn lumen and tubular glands (caudal aspect).

The tubular gland (a), lined by ciliated simple columnar glandular epithelial cells (b), open into the uterine lumen (L) which is lined by simple columnar epithelium (c). Note the cilia (d).

Figure 106   Tubular gland.

Ciliated secretory and non-secretory simple columnar glandular epithelial cells (arrows) line the tubular gland. Note the surrounding smooth muscle cells in longitudinal section.

4.5.2 Uterine body

The uterine body was evaluated **(Figures 112 – 115)**.
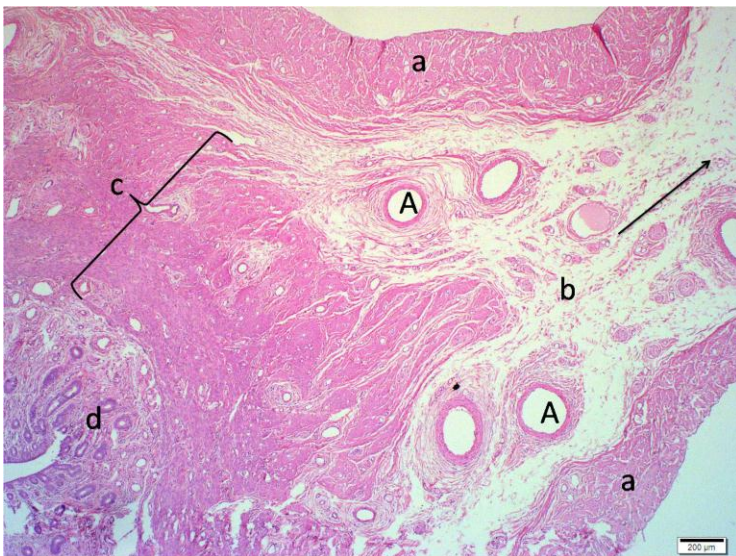
Figure 107   Endometrium, myometrium and mesometrium of the uterine body.

The outer layer smooth muscle (a) of the myometrium continues into the mesometrium, which also contains loose connective tissue (b) with large arteries (A). The thick inner layer of the myometrium (c) is now better developed than its outer longitudinal (a) counterpart. Endometrium (d) with tubular glands. The large arrow points towards the body wall.

78
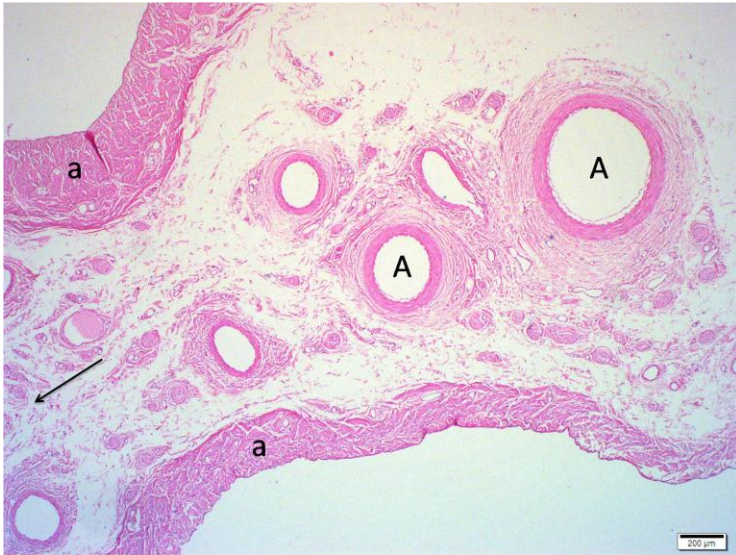
Figure 108   Mesometrium of the uterine body.

The outer layer smooth muscle (a) of the myometrium which is still well developed continues into the mesometrium. It also contains abundant loose connective tissue with large arteries (A). Large arrow points towards the uterine side.
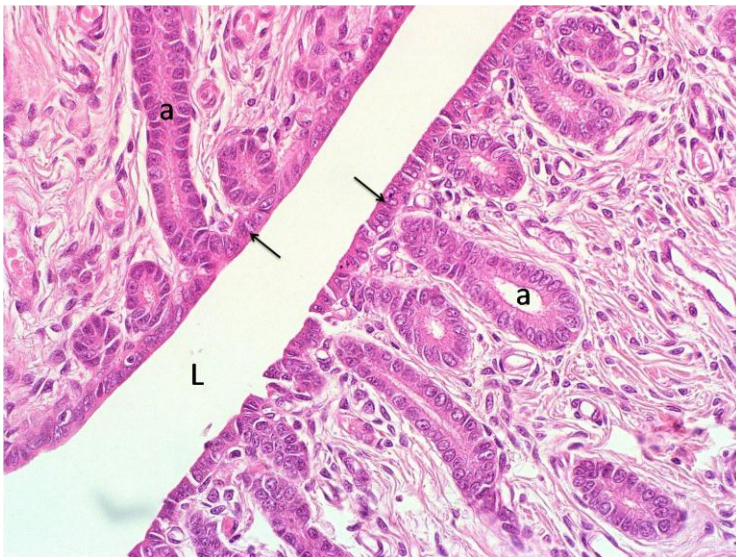
Figure 109   Uterine body lumen and tubular glands.

The tubular glands (a), lined by ciliated simple columnar glandular epithelial cells, open into the uterine lumen (L) which is lined by simple columnar epithelium (black arrows).
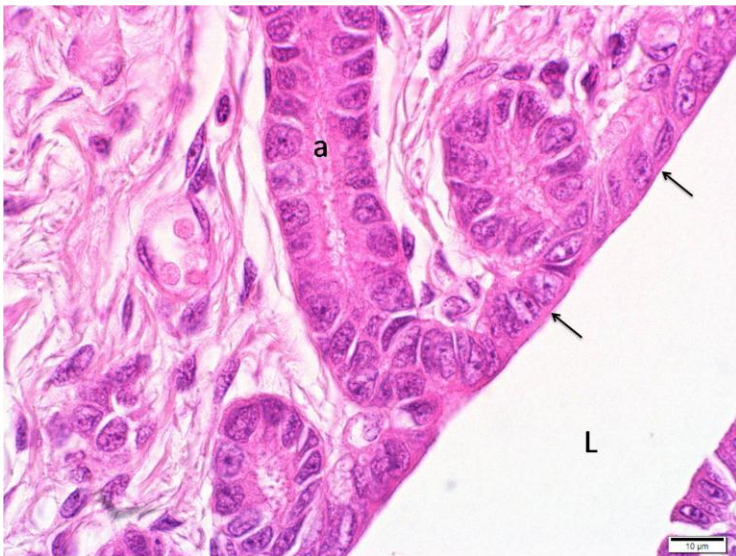
Figure 110   Uterine body lumen and tubular glands.

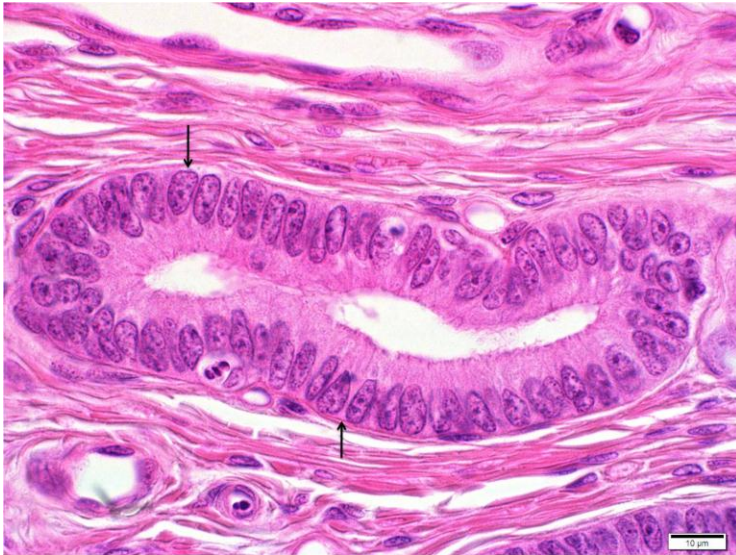The tubular glands (a), lined by ciliated simple columnar glandular epithelial cells, open into the uterine lumen (L) which is lined by simple columnar epithelium (black arrows).

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4.6 Cervix

The cervix was evaluated **(Figures 111-118)**.
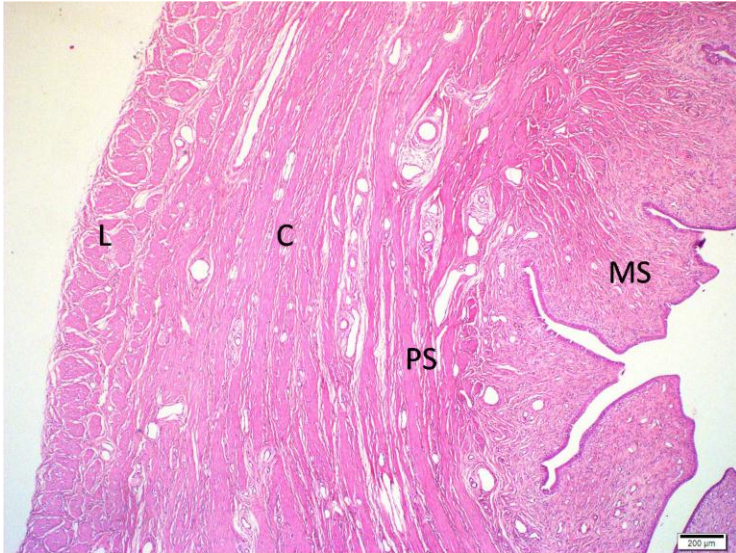
Figure 111   Cervical wall.

The tunica muscularis consists of an outer longitudinal smooth muscle layer (L) and an inner circular layer (C). The propria-submucosa (PS) is situated deep to the mucosa-submucosal folds (MS).
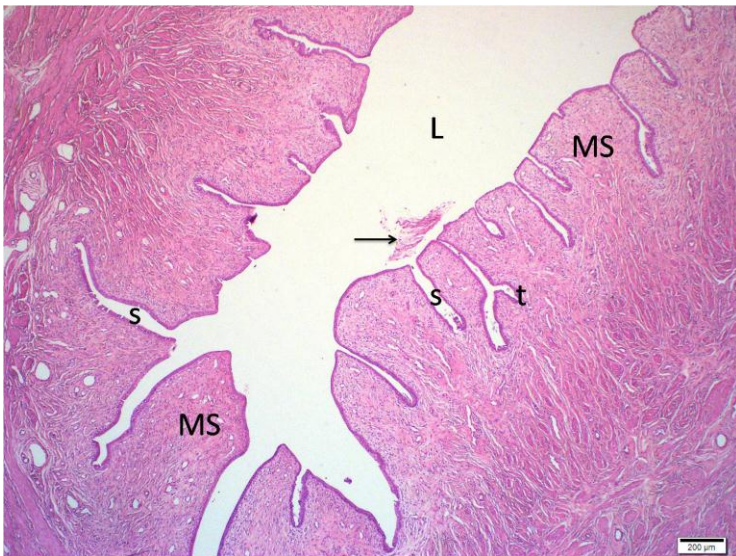
Figure 112   Cervical folds.

Secondary (s) and few tertiary (t) folds, situated between the mucosa-submucosal folds (MS), extend from the cervical lumen (L). Note the little mucus in the cervical lumen (arrow).

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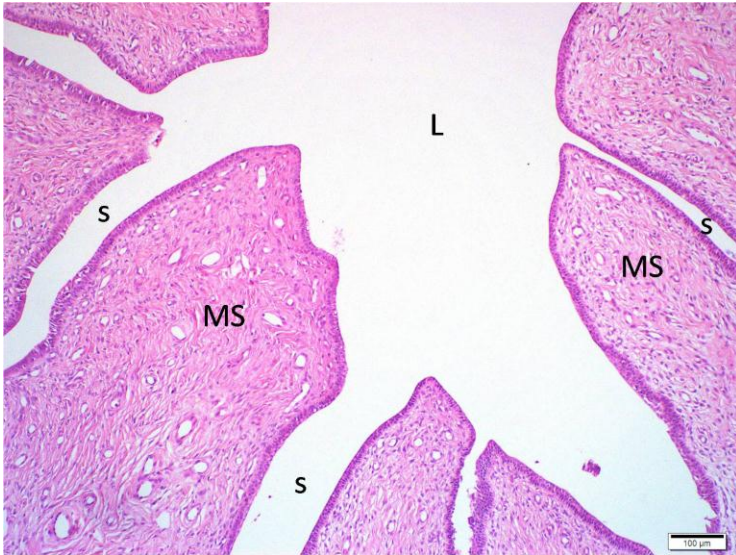
Figure 113   Mucosa-submucosal cervical folds.

Secondary (s) folds, situated between the mucosa-submucosal folds (MS), extending from the cervical lumen (L).
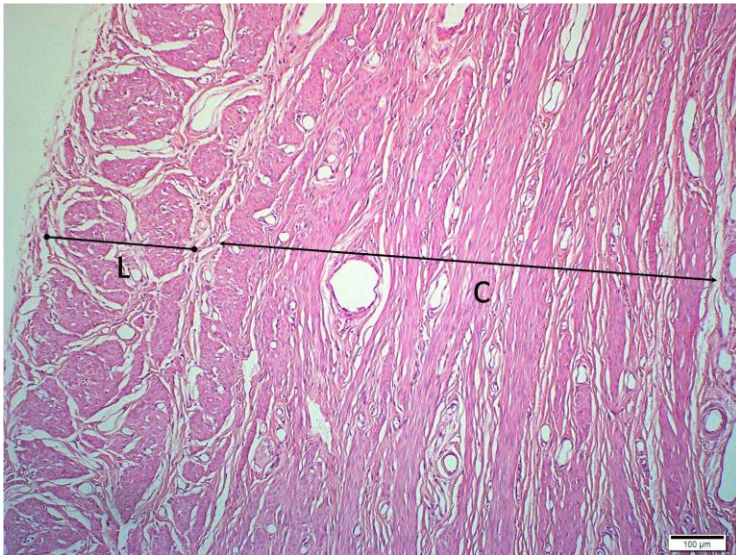
Figure 114   Tunica muscularis.

Outer longitudinal smooth muscle bundles (L) and an inner circular layer (C) make up the tunica muscularis of the cervix wall.
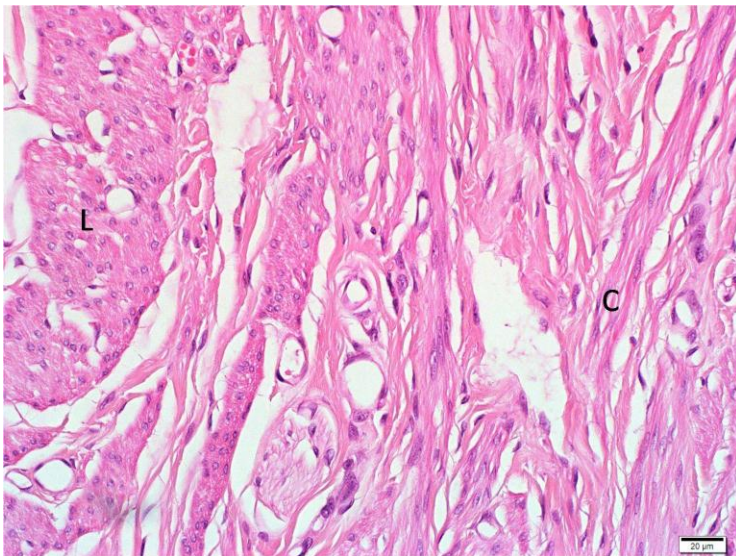
Figure 115   Tunica muscularis.

Outer longitudinal smooth muscle bundles (L) and inner circular layer (C) make up the tunica muscularis.

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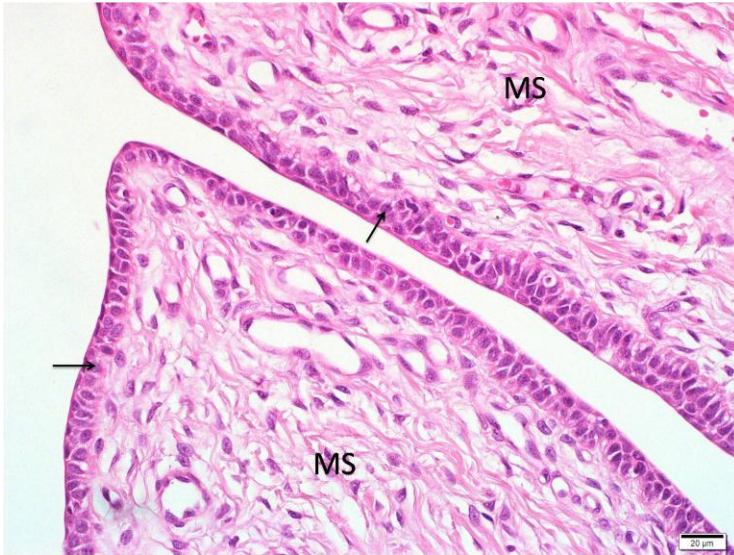
Figure 116   Epithelium.

A stratified cuboidal epithelium (black arrows) covers the mucosa-submucosal folds (MS).
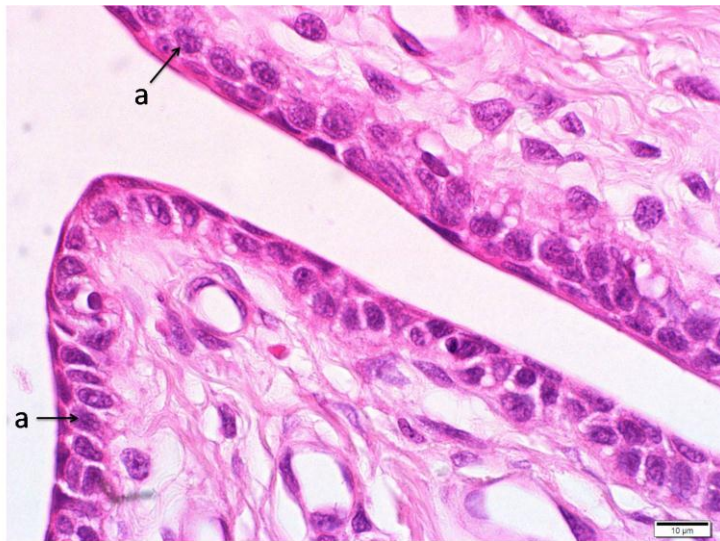
Figure 117   Epithelium.

Stratified cuboidal epithelium (a).

Figure 118   Epithelium.

The stratified cuboidal epithelium (a) in some areas was simple in nature (b).

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4.7 Vagina

The vagina was evaluated **(Figures 119-124)**.
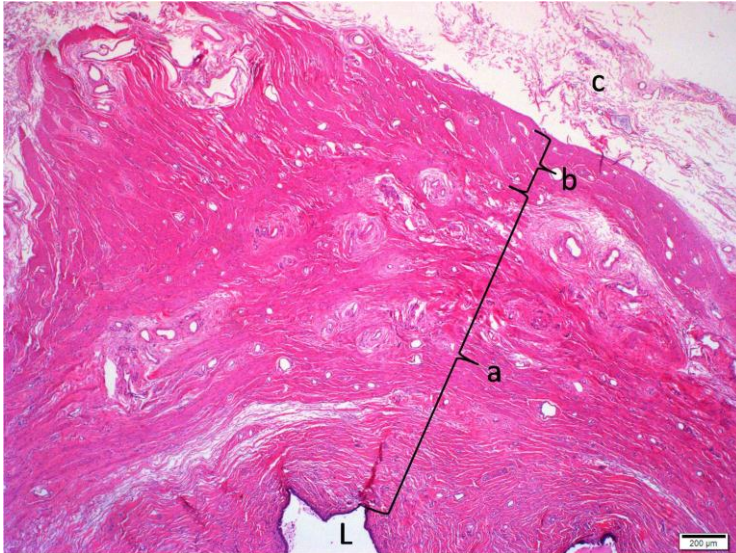
Figure 119   Vaginal wall.

The tunica muscularis consists of a thick inner circular (a) and a thin outer longitudinal (b) smooth muscle layer. The vagina caudally is covered by a tunica adventitia (c) consisting of loose connective tissue only. Lumen of the vagina (L).
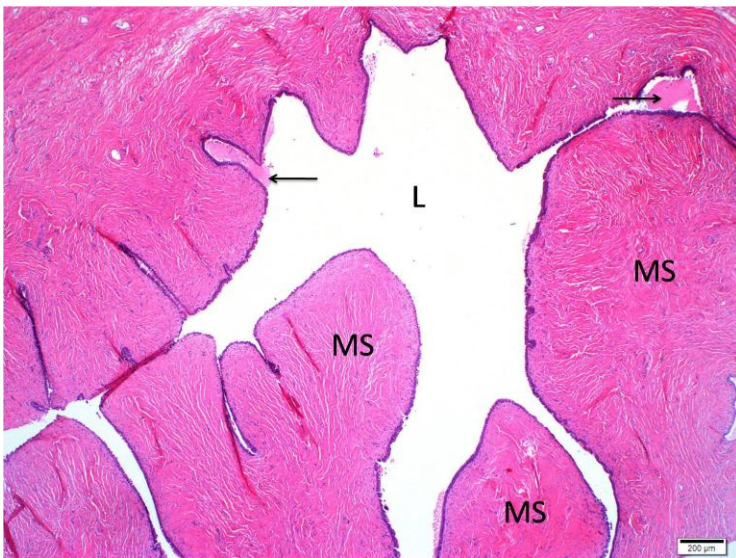
Figure 120   Mucosal-submucosal folds.

Longitudinal mucosal-submucosal folds (MS) of the vagina with some small amounts of mucus (arrow) in some areas of the vaginal lumen (L).

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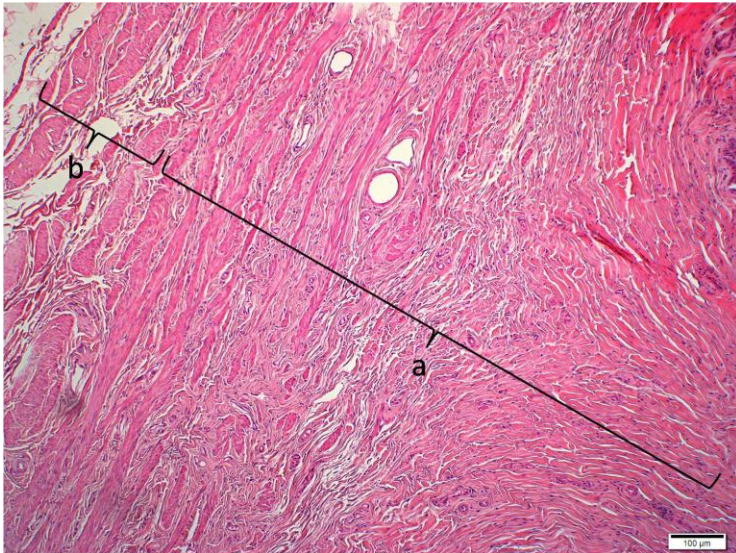
Figure 121   Tunica muscularis.

The thick inner circular (a) and a thin outer longitudinal (b) smooth muscle layer of the tunica muscularis.
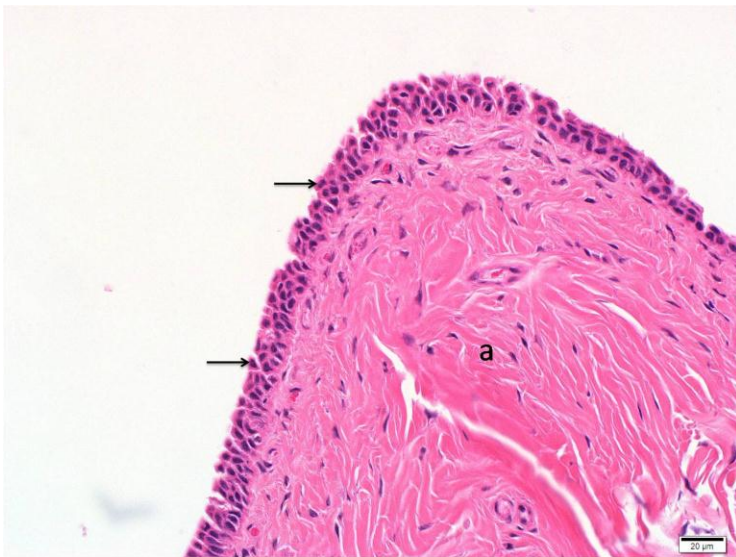
Figure 122   Epithelium.

Stratified cuboidal epithelium (black arrows) of the vagina. Circular smooth muscle (a) of the thick inner layer of the tunica muscularis.

Figure 123   Epithelium.

Stratified cuboidal epithelium (black arrows) of the vagina. Circular smooth muscle (a) of the thick inner layer of the tunica muscularis.

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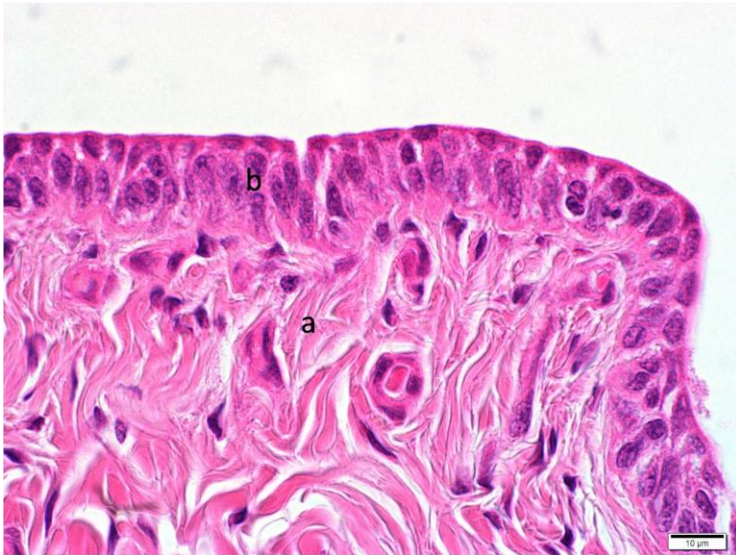
Figure 124   Epithelium.

Stratified cuboidal epithelium (b) and circular smooth muscle (a).

4.8 Vaginal vestibule

The vaginal vestibule was evaluated **(Figures 125-131)**.
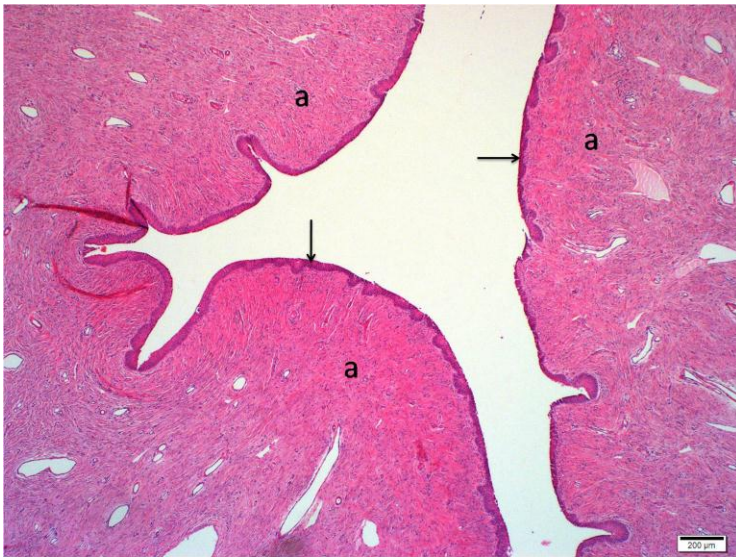
Figure 125   Vaginal vestibule.

Stratified cuboidal epithelium (arrows) and circular smooth muscle (a).

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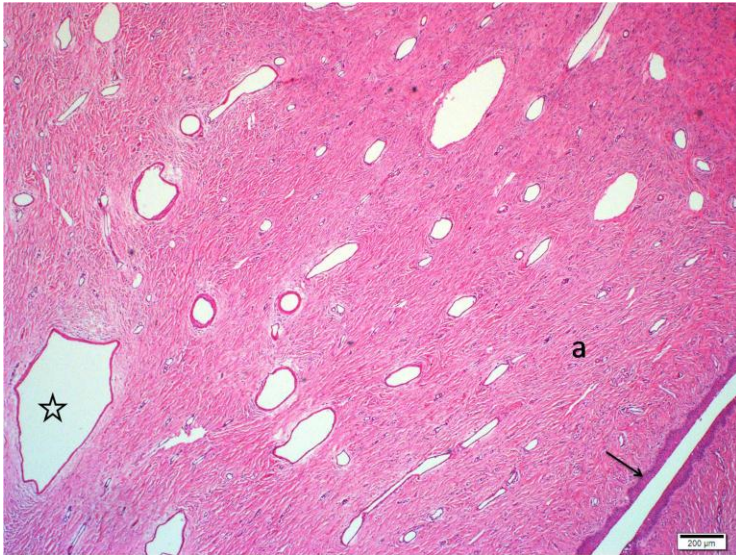
Figure 126   Vaginal vestibule.

Stratified cuboidal epithelium (arrow) and circular smooth muscle (a). The marker (star) corresponds with the marker in Figure 132.
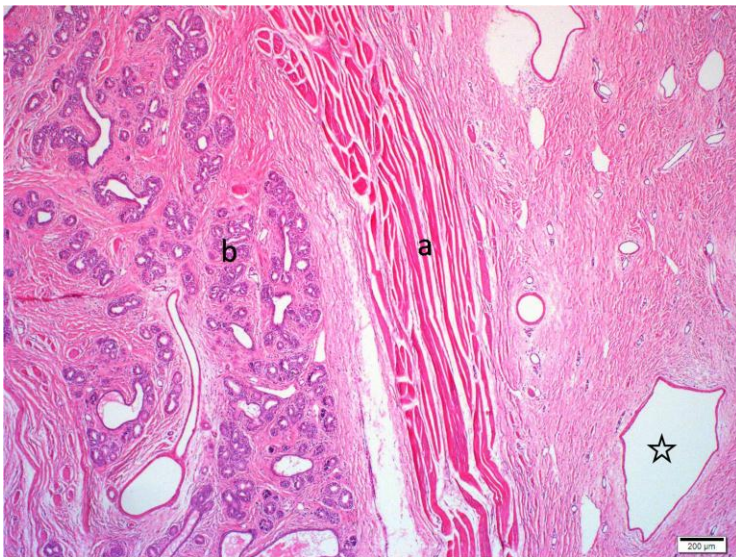
Figure 127   Vaginal vestibule.

Corpus spongiosum (b) and skeletal muscle in longitudinal section (a). The marker (star) corresponds with the marker in Figure 131.
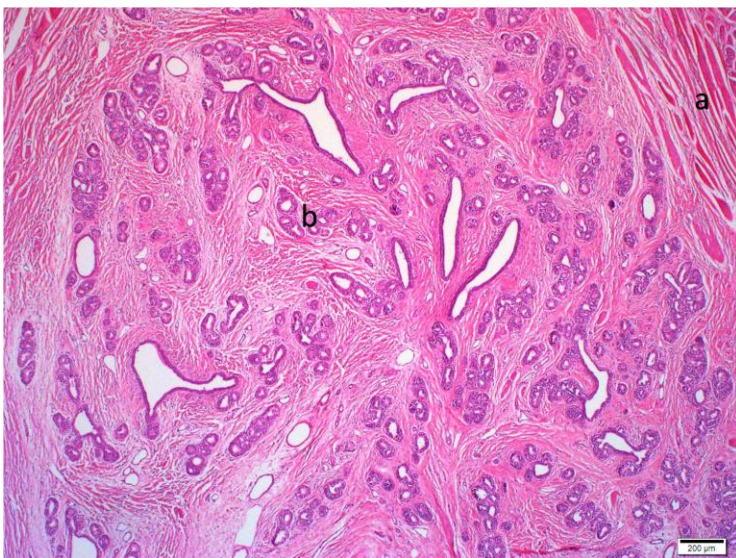
Figure 128   Vaginal vestibule.

Corpus spongiosum (b) and skeletal muscle in longitudinal section (a).

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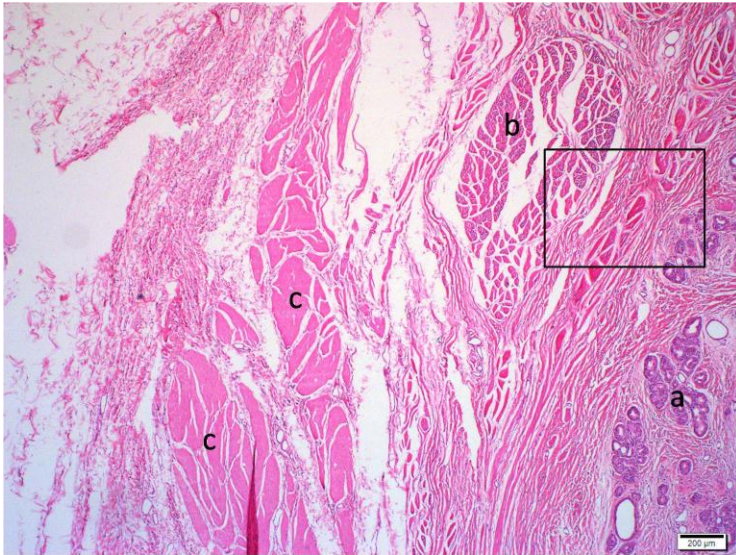
Figure 129   Vaginal vestibule.

Corpus spongiosum (a) skeletal muscle in cross section (b) and longitudinal smooth muscle (c). Figure 135 represents the content of the black square.
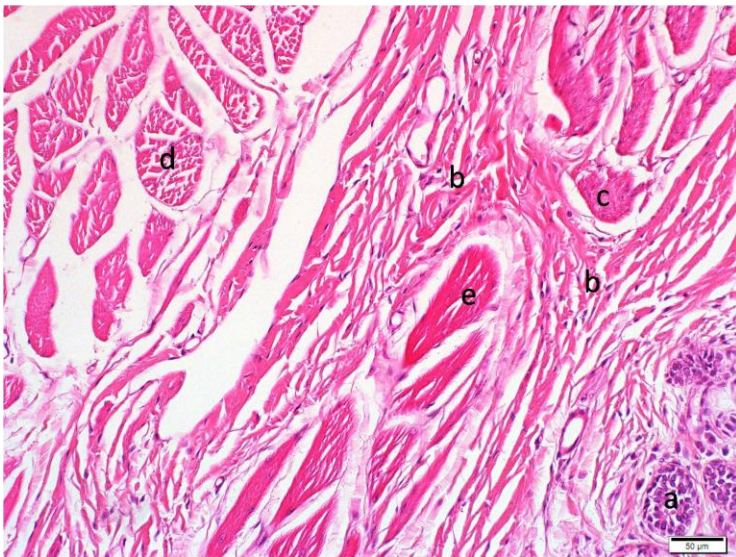
Figure 130   Vaginal vestibule.

Corpus spongiosum (a) smooth muscle in longitudinal section (b) and cross section (c) and skeletal muscle in cross section (d) and longitudinal section (e). This image represents content of the black square in Figure 134.
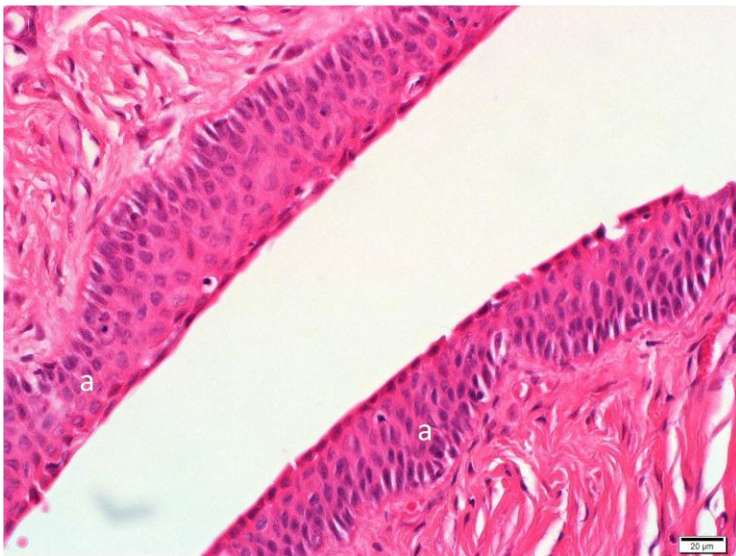
Figure 131   Epithelium.

Stratified cuboidal epithelium (a) of the vaginal vestibule. Circular smooth muscle of the thick inner layer of the tunica muscularis surrounds the epithelial layer.

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5. Discussion

5.1 Suspensory ligament

The suspensory ligament contains smooth muscle fibers similar to that of the cat[27]. This confirms the personal observation by the primary investigator during a previous morphological study where the suspensory ligament was witnessed to contract and relax directly post mortem.

5.2 Uterine tube

The uterine tube resembles that of the dog[26] and cat[37] and was compiled by the fimbriae with well-developed smooth muscle, infundibulum, ampulla and isthmus. The ampulla contained primary, secondary and tertiary folds[34].

5.3 Ovary

Numerous primordial and tertiary follicles were present in the ovaries of these three-year-old lionesses similar to the dog[34]. However in contrast few primary and secondary follicles were found. No signs of ovulation (corpora albicantia, rubrae or luteae) were detectable.

Atretic follicles, at varying stages from very early, advanced early to moderate atresia, were encountered readily in all ovaries as described in humans[35]. However follicular atresia in the domestic dog and cat has not been described. In very early atretic follicles the degeneration and sloughing of the granulosa cell layer and disruption of the cumulus oophorus and a swollen zona pellucida were also present[35]. In advanced early atresia hypertrophy of the epitheloid cells of the theca interna was prominent and loss of the membrana granulosa (complete sloughing and absorption) was obvious and thickening of the basement membrane between these two layers however was gradual with no clear distinction between the glassy membrane and its hypertrophied state. Loose connective tissue now grew in from the stroma, deposited between the degenerating granulosa layer and the hypertrophic theca, and partially filled the reduced antral cavity in which follicular fluid which was still present to varying degrees. Distinct layering by the epitheloid cells in the theca interna was also noticed during the advanced early stage of atresia. With moderate atresia stroma replaced the hypertrophied theca interna cells and loose connective tissue with small blood vessels completely filled the former antrum. The glassy

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membrane however was noticed to regress and disappear during moderate atresia as opposed to hypertrophy noticed in humans[35]. Late atretic follicles were difficult to find as they are well described in the human. Interstitial endocrine cells in the thecal layers were not noted in any of the sections and no scarring from completely atretic and resorbed follicles was noted. The presence of follicles of varying stages indicates that these lionesses have all reached puberty however ovulation has not occurred. The specific reason or cause for this ovulatory suppressive state has yet to be determined and possible factors that might influence this occurrence include the "Whitten (male) effect[38], the role the pheromones and the vomeronasal organ has on tonic levels of LH[39] and absence of coitus.

The feint follicle-like structures noticed on the surfaces of the ovaries during the preceding morphological study[25] were now confirmed to be tertiary or atretic tertiary follicles.

5.4 Round ligament

The presence of a prominent striated muscle component in the round ligament has not been demonstrated in any other species[26-28;34]. Since the muscle fibre type of skeletal muscle in the African lion has been determined as type IIx the fibre type of this striated muscle in the round ligament is expected to be of same the type[40].

5.5 Uterus

Smooth muscle contained in the perimetrium of the entire uterus was extremely well developed which is not noted in the dog[34] and in the cat smooth muscle has only been documented in the suspensory, proper and round ligaments[27]. The smooth muscle was best developed in the cranial aspect of the uterine horns and gradually decreased in size caudally into uterine body and cervical regions as the female reproductive approached the pelvic inlet topographically. The smooth muscle development continued into both layers of the mesometrium as well.

Considering the nature of smooth muscle development in the suspensory ligament, proper ligament, various regions of the uterus, mesometrium and cervix it should be possible for the lioness to suspend the intra-abdominal part of her reproductive organs firmly and close to her body wall under certain circumstances. We can expect this smooth muscle to contain adrenergic receptors and assuming that the lioness secretes cortisol during hunting she will, as primary hunter in the pride, still be able to kill large prey such as zebra, wildebeest and buffalo by decreasing the motion of her gravid uterus intra-abdominally. Considering the presence of well develop striated muscle bundles in the round ligament and the fact that the round ligament inserts firmly on the medial femoral fascia[25] it might even be possible for the lioness to

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voluntarily retract the tips of her uterine horns during hunting to increase stability of the gravid uterus.

5.6 Uterine body

No ciliated epithelial cells were noted in the body of the uterus as in the dog[34].

5.7 Cervix

The epithelium of the cervix was stratified cuboidal epithelium in nature rather than simple columnar as in the dog and cat. In some lesser areas the cuboidal epithelium was simple in nature rather than stratified[34].

No ciliated epithelial cells or goblet cells were noted in the stratified cuboidal epithelium in the cervices of these lionesses. Some mucous secretory activity was evidenced by the presence of small areas of coagulated mucus in the cervical lumen however. Since increasing amounts of mucous is produced during estrus the non-ovulatory state in these individuals could explain this finding. Very few tertiary folds were present compared to the dog[34].

5.8 Vagina

Flat longitudinal mucosal-submucosal folds extended throughout the length of the vagina as in the dog and the tunica muscularis was well developed. The epithelium of the vagina was stratified cuboidal epithelium in nature rather than a stratified squamous epithelium as in the dog[34]. The innermost thin longitudinal smooth muscle layer (third layer) was not visible in these lionesses as described in the dog[34].

5.9 Vaginal vestibule

The vestibular tunica muscularis was even better developed than in the vagina. The corpus spongiosum was particularly well developed.

The histology of the clitoris and vulva is not described in this study as it is poorly described in other texts[26;27;34].

6. Conclusion

The histology of the female reproductive organs of the African lion resembles that of the domestic dog[34] with some major differences with regards to the suspensory ligament, round ligament and outer layer of the myometrium.

The presence of striated muscle in the round ligament, together with its insertion on the medial femoral fascia[25], should enable the lioness to voluntarily manipulate her uterus. Interpreted with the presence of prominent smooth muscle development throughout the uterus and cervix will allow the lioness to suspend and stabilise her reproductive organs during hunting especially when pregnant.

The three-year-old free ranging lionesses in this study have reached puberty but no ovulation has occurred. The reason for this remains unclear. Lioness' ovaries of all ages should be investigated to determine at what stage captive and free-range lionesses will start to ovulate.

7. General Conclusion

The silicone technique described in chapter one assisted in the describing the arterial supply to the female reproductive organs of the African lion. This description prepared the primary investigator for a subsequent laparoscopic study on ovariectomy and salpingectomy in the African lioness[41]. The prominent smooth muscle development associated with the lion's female reproductive organs stimulates the idea of uterine suspension during pregnancy and anovulation and atresia of tertiary follicles in the young lioness has not been described in domestic carnivores. Social influences on this phenomenon in the lion pride needs to be investigated.

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	Sec tion	Lioness 1 S 421 - 12				Lioness 2 S 422 - 12				Lioness 3 S 423 - 12			
		Left		Right		Left		Right		Left		Right	
Suspensory ligament	1[a]	1SLL1	1-1	1SLR1	1-3	2SLL1	2-1	2SLR1	2-3	3SLL1	3-1	3SLR1	3-3
	2[b]	1SLL2	1-2	1SLR2	1-4	2SLL2	2-2	2SLR2	2-4	3SLL2	3-2	3SLR2	3-4
Uterine tube	1[c]	1UTL1	1-5	1UTR1	1-7	2UTL1	2-5	2UTR1	2-7	3UTL1	3-5	3UTR1	3-7
	2[d]	1UTL2	1-6	1UTR2	1-8	2UTL2	2-6	2UTR2	2-8	3UTL2	3-6	3UTR2	3-8
Ovary[e]	1[f]	1OVL1	1-9	OVR1	1-14	2OVL1	2-9	2OVR1	2-14	3OVL1	3-9	3OVR1	3-14
	2	1OVL2	1-10	OVR2	1-15	2OVL2	2-10	2OVR2	2-15	3OVL2	3-10	3OVR2	3-15
	3	1OVL3	1-11	OVR3	1-16	2OVL3	2-11	2OVR3	2-16	3OVL3	3-11	3OVR3	3-16
	4	1OVL4	1-12	OVR4	1-17	2OVL4	2-12	2OVR4	21-17	3OVL4	3-12	3OVR4	3-17
	5[g]	1OVL5	1-13	OVR5	1-18	2OVL5	2-13	2OVR5	2-18	3OVL5	3-13	3OVR5	3-18
Round ligament	1[h]	1RLL1	1-19	RLR1	1-21	2RLL1	2-19	2RLR1	2-21	3RLL1	3-19	3RLR1	3-21
	2[i]	1RLL2	1-20	RLR2	1-22	2RLL2	2-20	2RLR2	2-22	3RLL2	3-20	3RLR2	3-22
Uterine horn	1[j]	1UHL1	1-23	UHR1	1-25	2UHL1	2-23	2UHR1	2-25	3UHL1	3-23	3UHR1	3-25
	2[k]	1UHL2	1-24	UHR2	1-26	2UHL2	2-24	2UHR2	2-26	3UHL2	3-24	3UHR2	3-26
Uterine body	1[l]	1UB	1-27			2UB	2-27			3UB	3-27		
Cervix	1[m]	1CE	1-28			2CE	2-28			3CE	3-28		
Vagina	1[n]	1VG	1-29			2VG	2-29			3VG	3-29		
Vaginal vestibule	1[o]	1VV	1-30			2VV	2-30			3VV	3-30		

a   cranial aspect of suspensory ligament in area of origin
b   caudal aspect of suspensory ligament at insertion on the ovary
c   cranial half of uterine tube and insertion of suspensory ligament
d   caudal half of uterine tube, proper ligament and uterotubal junction
e   ovary was divided into six portions using five incisions, the caudal aspect of each protion was sectioned
f   cranial (tubular) pole of ovary plus insertion of the suspensory ligament
g   caudal pole of ovary + origin of the proper lig, two caudal portions in one tray with opposing cut margins facing the same direction
h   proximal aspect of round ligament at point of origin from uterine horn
i   distal aspect of round ligament where it inserts of the medial femoral fascia
j   cranial tip of uterine horn
k   uterine horn immediately cranial to the uterine bifurcation
l   mid uterine body
m   mid cervix
n   mid vaginal body
o   immediately caudal to the urethral tuberculum
Abbreviations in the table represent the anatomical discription in the left column and headings left or right.