UNIVERSITI PUTRA MALAYSIA

HISTOLOGY OF THE OVARY OF THE LESSER SHORT-NOSED FRUIT BAT (*Cynopterus brachyotis*)

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HISTOLOGY OF THE OVARY OF THE LESSER SHORT-NOSED FRUIT BAT

(*Cynopterus brachyotis*)

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Histology of the Ovary of the Lesser Short-Nosed Fruit Bat (*Cynopterus brachyotis*)" by Nicole Tan Han Xin and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project (FYP).

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DEDICATION

I wish to dedicate this Final Year Project to all my friends, family and bat lovers out there who gave me continuous support to finish this study.

ACKNOWLEDGMENTS

First and foremost I would like to express my greatest gratitude to my supervisor, Dato' Dr. Tengku Azmi Tengku Ibrahim for the tremendous support and guidance throughout this study. Thank you so much Dato' for dedicating your precious time, effort, patience and encouragement that you have given me. It has definitely kept me motivated to do my best to complete this study.

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LIST OF ABBREVIATION

A	Antrum
AF	Atretic follicle
C	Cortex
CL	Corpus luteum
CO	Cumulus oophorus
CR	Corona radiata
CT	Connective tissue
Cv	Cervix
DF	Degenerating follicles of various stages
DPF	Degenerated primary follicles
F	Follicles of various development stages
FC	Follicular cells
FF	Follicular fluid
GE	Germinal epithelium
H&E	Hematoxylin & Eosin
M	Medulla
O	Oocyte
Ov	Ovaries (paired)
OvD	Oviduct
PF	Primary follicle
TA	Tunica albuginea

TC	Thecal cells
UH	Uterine horns
VPF	Viable primary follicle
ZP	Zona pellucida

ABSTRAK

Abstrak kajian projek yang dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Putra Malaysia sebagai memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Tahun Akhir

HISTOLOGI OVARI KELAWAR CECADU PISANG (*Cynopterus brachyotis*)

Oleh

Nicole Tan Han Xin

2020

Penyelia: Dato' Dr. Tengku Azmi bin Tengku Ibrahim

Pada mamalia apabila terdapat korpus luteum berfungsi dalam satu ovari semua folikel dalam ovari tersebut dan juga dalam ovari kedua kekal tidak aktif. Berlainan bagi kelawar cecadu pisang (*C. brachyotis*), sejenis mamalia terbang; apabila terdapat korpus luteum dalam ovari tanduk uterus gravid, pada masa yang sama folikel di pelbagai peringkat pembentukan dalam ovari tanduk uterus tidak gravid (Gopalakrishna, 1955). Berasaskan di atas penyelidikan ini adalah bertujuan mengkaji struktur histologi ovari mamalia ini dan mendalami kepentingan apabila terdapat serentak korpus luteum dan folikel dalam kelawar bunting. Enam ekor kelawar, tiga daripadanya bunting telah diperangkap di dusun buah-buahan berhampiran Kolej 17, Universiti Putra Malaysia. Ekoran laparoskopi ovari-ovari yang diperolehi telah diproses untuk penelitian mikroskopi cahaya. Pada hirisan ovari tanduk uterus tidak gravid terdapat ratusan,

mungkin ribuan folikel primer memenuhi hampir seperempat parenkima ovari tersebut. Namun demikian kebanyakan folikel ini didapati menjanarosot terbukti daripada piknosis nukleus selsel folikel mengelilingi oosit. Juga dalam ovari ini terdapat folikel sekunder dan tertiari tetapi kebanyakan folikel ini adalah atretik. Pada ovari tanduk uterus gravid hampir keseluruhan parenkima ovari dipenuhi oleh tisu lutea kecuali di periferi ovari terdapat beberapa folike atretik. Tiga inferensi dapat dirumus daripada kajian ini. Pertama, struktur histologi dan pembentukan folikel dalam ovari mamalia terbang ini tidak banyak berbeza berbanding dengan yang terdapat pada mamalia penghuni bumi. Pemerhatian ketara daripada kajian struktur histologi ovari kelawar ini dan ini belum pernah dilaporkan hingga kini ialah tisu lutea mengisi hampir keseluruhan parenkima ovari. Kedua, folikel atretik yang banyak terdapat dalam ovari tanduk uterus tidak gravid mungkin disebabkan aras progesteron yang tinggi dalam kitaran yang dirembes oleh korpus luteum dalam ovari tanduk uterus gravid. Progesteron menyekat folikel daripada menjalani ovulasi dan seterusnya folikel-folikel tersebut menjanarosot. Dengan penjanarosotan folikel ini kehadiran bersama korpus luteum dan folikel tidak mempunyai sebarang kepentingan kepada kelawar bunting. Akhir sekali, dalam keadaan biasa ekoran parturisi korpus luteum akan membentuk korpus albikans. Dengan keseluruhan parenkima ovari tanduk uterus gravid dipenuhi oleh korpus luteum dan membentuk korpus albikans, ovari ini tidak akan berfungsi semula selepas parturisi. Bagi ovari kedua, selepas ovulasi dan pembentukan korpus luteum, akan juga membentuk korpus albikans ekoran parturisi. Ovari ini juga tidak akan berfungsi selepas parturisi. Berasaskan struktur histologi korpus luteum dan perubahan struktur kepada korpus albikans pada kedua-dua ovari tertimbulnya kemungkinan kelawar cecadu pisang menghasilkan hanya dua ekor anak sepanjang hayatnya.

Kata kunci: *ovari, primer folikel, folikel atretik, korpus luteum. korpus albikans.*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Poject (FYP).

HISTOLOGY OF THE OVARY OF THE LESSER SHORT-NOSED FRUIT BAT (*Cynopterus brachyotis*)

By:

NICOLE TAN HAN XIN

Supervisor: Dato' Dr. Tengku Azmi Tengku Ibrahim

In mammals with the presence of a functional corpus luteum in one ovary all follicles in that ovary and in the second ovary will remain quiescent. The situation is different in the pregnant lesser short-nosed fruit bat (*Cynopterus brachyotis*). In this flying mammal when there is a functional corpus luteum in the ovary of the gravid uterine horn there are follicles at different stages of development in the ovary of the non-gravid uterine horn (Gopalakrishna, 1955). Based on the above the aim of the present study was to examine the histological structure of the ovary in this mammalian specie and delve into the significance of concurrent occurrence of functional corpus luteum and developing

follicles in the ovaries of the pregnant fruit bat. Six female bats, three being pregnant were caught by trapping in the fruit plantation close to the 17^{th} College, Universiti Putra Malaysia. Following laparoscopy the ovaries collected were processed for light microscopy. Histological sections of ovaries of non-gravid uterine horn revealed hundreds or probably thousands of primary follicles occupying almost a quarter of the ovarian parenchyma. The majority of these follicles were however degenerated as evidenced by pyknosis of follicular cell nuclei surrounding the oocytes. There were also secondary and tertiary follicles in the ovarian parenchyma but most of these follicles were atretic. In the ovary of the gravid uterine horn the entire ovarian parenchyma appeared to be occupied by luteal tissues except for some of atretic follicles at the periphery of the ovary. Three inferences could be drawn from the present study. Firstly, the histological structure and development of follicles in the ovary of this flying mammal were similar to those in other ground dwelling mammals. However a significant observation from this study and not reported in any other mammal until to date was the corpus luteum which occupied almost the entire ovarian parenchyma. Secondly, the numerous atretic follicles in the ovary of non-gravid uterus could be due to their inability to ovulate. Circulating progesterone secreted by the corpus luteum in the ovary of the gravid uterine horn prevented their ovulation and subsequently these follicles degenerate. With the degeneration of the follicles the concurrent occurrence of functional corpus luteum and developing follicles did not appear to have any significance in the pregnant fruit bat. Lastly, under normal circumstances, following parturition corpus luteum will involute and assume the structure of corpus albicans. The large corpus luteum which occupy almost the entire ovary of the gravid uterine horn will, following the birth of pups develop into a corpus albicans and the ovary will no longer be functional. As for the second ovary it will ovulate and form a corpus luteum; with ensuing pregnancy and following parturition the corpus luteum will also develop into a corpus albicans and the ovary will also become nonfunctional. Based on the histological structure of the corpus luteum and its development into a corpus albicans following parturition is there a possibility that the lesser short-nose fruit bat produces only two pups in its entire life time.

Keywords: *Lesser short-nosed fruit bat, ovaries, degenerated primary follicles, atretic follicles, corpus luteum, corpus albicans.*

1.0 INTRODUCTION

Bats belong to the order Chiroptera. The lesser short-nose fruit bat, *Cynopterus brachyotis* is a common frugivorous bat species in Southeast Asia and is widely distributed in Malaysia (Ibrahim *et al.*, 2013). Bats are unique as they have a diverse breeding cycle. The breeding cycles being influenced by factors such as photoperiod, rainfall, food availability and temperature the onset of the reproductive cycle is very important as it increases survival of the young (Ibrahim *et al.*, 2013).

The ovary is a vital component of the reproductive system as it is the site for oogenesis, ovulation and formation of the corpus luteum. However in the bat during pregnancy the ovary of the non-gravid horn does not appear to remain quiescent but rather demonstrate all stages of folliculogenesis (Gopalakrishna, 1955). Studies on the histological structure of the ovary in several bat species including the *Miniopterus fraterculus* (Bernard, 1980), *Rossetus aegyptiacus* (Ghobadian and Ghassemi, 2015), *Dermanura cinerea* (Nivaldo B. Lima Junior *et al.*, 2017) have been reported; however no data have been published on the ovary of the *Cynopterus brachyotis*. Therefore a study on the histological structure of ovary of *C. brachyotis*, was undertaken with a view to understand the development of the various stages of the follicles – primary, secondary, tertiary – through to ovulation and formation of the corpus luteum. This study is also undertaken with a view to determine whether there are differences between folliculogenesis in the ovary of bat, being a flying mammal compared to that of its ground dwelling counterparts.

1.1 OBJECTIVE

In the lesser short-nosed fruit bat (*C. brachyotis*) there are both functional corpus luteum and developing follicles in the ovaries of gravid and non-gravid uterine horns respectively. The study focuses on the histological structure of the ovary in the fruit bat, a flying mammal and additionally delve into the significance of concurrent occurence of a functional corpus luteum and developing follicle in the pregnant fruit bat.

1.2JUSTIFICATION

In mammals with the presence of a functional corpus in an ovary all follicular structures in the said ovary and in the other ovary will remain quiescent. However in the lesser short-nosed fruit bat Gopalakrishna (1955) reported that although there was a corpus luteum in the ovary of a gravid uterine horn the ovary of the non-gravid uterine horn did not remain quiescent meaning there are follicles at various stages of development in this ovary. Hence this study investigates the significance of concurrent occurrence of functional corpus luteum and developing follicles in the pregnant fruit bat.

1.3 HYPOTHESIS

Ho= There are no differences in the histological structure of the primary, secondary and tertiary follicles and the corpus luteum in the fruit bat bats compared to other non-flying mammalian species.

HA= There are differences in the histological structure of the primary, secondary and tertiary follicles and corpus luteum in the fruit bats compared to other non-flying mammalian species.

2.0 LITERATURE REVIEW

2.1 BATS

Bats belong to the order of Chiroptera, which is derived from the terms *"Chiro"* and *"pteron"* which means "hand" and "wings" in modern latin. Chiropterans are divided into two suborders, Megachiroptera and Microchiroptera. The Megachiroptera suborder consists of a single family, which are the Pteropodidae, or also known as the Old World fruit bats. According to the IUCN/SCC Chiroptera Specialist Group (1992), they can be found throughout the Old World tropics and subtropics from Africa, Southern Asia to Australia and on islands in the Indian and Western Pacific Oceans. Microchiropterans on the other hand are the echolocating bats. They utilize and emit ultrasonic sounds through their mouths and noses for orientation, navigation and capture of food (IUCN/SCC Chiroptera Specialist Group, 2001). Microchiropterans are insectivorous in nature and are found in all parts of the world except the Arctic and Antartica. To this date, the Krau Wildlife Reserve (KWR) located in Pahang, Malaysia is home to at least 69 bat species has the highest diversity of bats recorded anywhere in the Old World (Kingston *et al.*, 2009).

According to Kingston *et al.* (2009), the lesser short-nosed fruit bat, the *C. brachyotis* is the most common and abundant of the *Cynopterus* species in Peninsular Malaysia. This species is also known as the dog-faced fruit bat as their appearance shows slight resemblance to dogs. They can be identified by several distinctive features, including having full white edges of the ear, large eyes and a greyish to yellowish brown torso, often with a coat of dark orange around the neck and shoulder region (Kingston *et al*., 2009). They also have a smaller bodice as compared to other *Cynopterus* species. Funakoshi *et al.* (1997) reported that this species is ubiquitous and inhabits a wide range of habitat including primary forests, mangrove, orchards, cultivated and even urban areas. As its name suggests, this species is frugivorous and consumes a wide variety of fruits, plants and also flower parts (Tan *et al*., 1998). Tan *et al*. (1998) reported that the *Ficus* spp., or also commonly known as figs, is one of the main food source as it bear fruits all year round. The fruit bats therefore play an important role as seed dispersers and plant pollinators (Ridley, 1930), which is vital in maintaining forest health.

2.2 REPRODUCTION OF THE LESSER SHORT-NOSED FRUIT BAT

Bats are said to be highly successful mammals being the only mammalian species that has the ability to fly. Furthermore, this species is unique as they also express great intraspecific diversity in terms of their ecology, dietary specialization and reproductive characteristics (Rasweiler and Badwaik, 2000). Studies have shown that they exhibit reproductive specializations in terms of morphology and cyclicity (Jerrett, 1979) of which some are notably similar yet some atypical in comparison to those of primates or commonly studied mammals. However although the bat species comes in second after rodents as the second largest order of mammals, fruit bats are said to have low reproductive rates and small litter size (Gaisler, 1989). While other small animals that adopt the 'live fast-die young' life strategy described by Promislow and Harvey (1990), bats have evolved for longevity with delayed sexual maturity. According to Crichton and Krutzsch (2000), the lesser short-nosed fruit bat, *C. brachyotis*, can live up to 20 to 30 years. Tuttle and Stevenson (1982) reported that delayed sexual maturity occurs in most bat species which may be as long as 5 years; however most species reached sexual maturity at 1 to 2 years of age. Findings from a study by Charnov (1993) suggests that the late age and delayed sexual maturity is a reproductive strategy to ensure bat has enough energy to support pregnancy, parturition and post-partum events.

Reproduction in bats is different compared to that in other mammals and hibernation appears to be a major influence on the reproductive patterns of bats (Oxberry, 1979). For non-hibernating bats it has been reported that these bats exhibit three different breeding patterns, namely aseasonal polyestrous, seasonal polyestrous and seasonal monoestrous (Jerrett, 1979). Reproductive patterns for hibernating bats on the other hand are categorized into two patterns - Type 1 general reproductive pattern (Wimsatt, 1944a) and Type 2 pattern. Demonstration of Type 1 and Type 2 reproductive patterns are resultant to modification of the reproductive physiology due to hibernation which include a delay between copulation and ovulation or between fertilization and implantation (Oxberry, 1979).

A recent study by Ibrahim *et al*. (2013) concluded that the lesser short-nosed fruit bat, *C. brachyotis* are asesonally polyestrous, meaning they do not have a specific breeding season. As pregnant female lesser short-nosed fruit bats were successfully captured every month and the captures peaked during rainy season the breeding patterns of this bat species is said to be associated with seasonality of rainfall and food availability (Racey *et al.*, 2000).

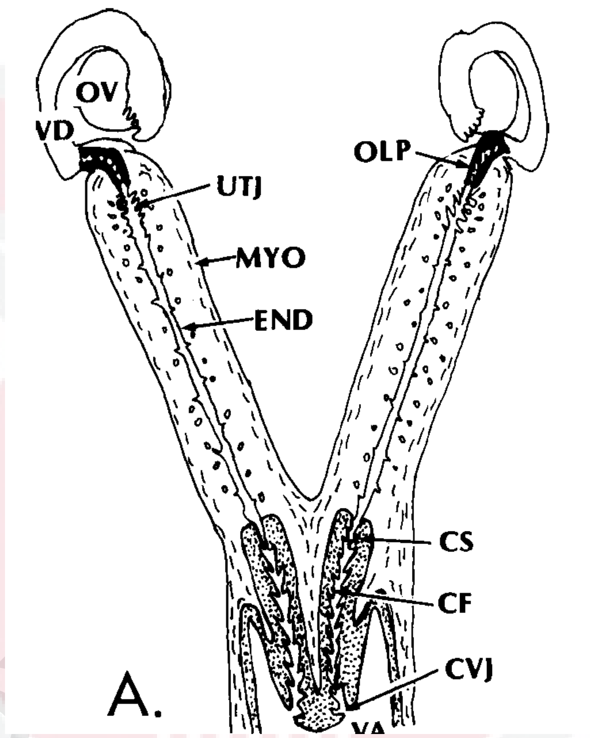
The gestational period of bats varies among species (Gopalakrishna and Badwaik, 1993b) which could also differ within species due factors such as hibernation (Racey and Swift, 1981). The Jamaican fruit bat, *Artibeus jamaicensis*, has a gestational period of 3-4 months that could extend to 7 months due to prolonged embryonic diapause of 2.5 months (Jerrett, 1979). According to Nowak (1997) the female lesser short-nosed fruit bats reached sexual maturity at 5 to 6 months of age and have a gestational period of 120 days. While there is no concrete data on the weaning period of the neonate, Kingston *et al*. (2009) reported that the pup will be carried by the mother for months. Nowak (1997) recorded that the weaning age for the pups of *C. sphinx* is about 40-45 days which is most likely similar to that of *C. brachyotis*. The latter is monotocous, which means it only produces one offspring or pup in each pregnancy. Rasweiler and Badwaik (2000) reported that the infant size is considerably large when compared to non-flying mammals that are of the body size of a bat.

2.3. ANATOMY OF THE REPRODUCTIVE SYSTEM OF THE FEMALE LESSER SHORT-NOSED FRUIT BAT (*C. brachyotis*)

2.3.1 THE REPRODUCTIVE TRACT

A study by Hood (1989) describes the gross anatomy of the reproductive tract of pteropodid bats possesses a primitive duplex uterine morphology when compared the uteri of other Cynopterus species. *C. horsefieldii* and *C. sphinx* appeared to possess similarities. They have Y-a shaped uterus with long uterine horns and a short common uterine body. The cervical canals is unique as they open independently into the lateral fornices of the vagina by way of an enormous portio vaginalis that fills most of the canial end of the vagina. The cervicovaginal junction is also described to be a distinctly complex structure (Figure 1).

Figure 1: Schematic diagram of the longitudinal section of the reproductive system of the female fruit bat C*ynopterus brachyotis* (Hood,1989).



2.3.2 THE OVARY AND CORPUS LUTEUM (CL)

Morphologically, the ovaries of the bat are paired, whitish ovoid structures, covered by a layer of connective tissue bursa as in other mammalian species (Krishna and Bhatnagar, 2011). However, as a flying mammal, bats are noted to exhibit various unique ovarian characteristics that are unusual from other mammalian species. According to Priedkalns and Leiser (2006), primordial follicles are located in the outer cortex of the ovary and are evenly distributed in cows and sows and clustered in bitches. However this is not entirely represented in bat species. In one of the phyllostomid bat, *G. soricina*, it was reported that all primordial follicles are restricted to the medial side of the ovary, and ovulation has always occurred in this zone. While there are no data on the distribution of primordial follicles in *C. brachyotis*, similar ovarian follicle distribution was noted in other pteropodids (megachiropterans), including *Pteropus sp*., *Ptenochirus jagori* and *Otopteropu cartilagonodus*. (Heideman and Powell, 1998; Pow and Martin, 1994, 1995; Heideman et. al., 1993). This unique ovarian characteristic may be a novel model in determining local factors that allows long-term oocyte storage, recruitment of the oocytes into developing follicles and follicular rupture.

What is unique about the reproduction of the female bats is that during pregnancy, cycling activity of the ovary is evident where folliculogenesis is observed in the ovary of the non-pregnant horn. The contra-lateral ovary does not remain quiescent during a pregnancy (Gopalakrishna, 1955). Several differences in the ovarian morphology and follicular development were observed in bats species. Firstly, the thecal and interstitial cells that are usually present in the ovarian cortex varies in terms of type and abundance (Rasweiler and Badwaik, 2000). These cells are abundant in many vespertilinoid bats, however may be indistinct in noctilionid bats (Krishna and Bhatnagar, 2011). Partial or complete eversion of the corpus luteum (CL) is also observed in certain Emballonirodid and Rhinolophodid bat species. Differences in the growth rate, structure, development, functional state and the mode of regression of the CL are noted also in different bat species. (Gopalakrishna and Badwaik, 1988). It was suggested that the extension of the eversion may be dependent on the functional state of the CL, however, there is still lack of information to date.

3.0 MATERIALS AND METHOD

3.1 ETHICAL APPROVAL

This study was approved by the Institutional Animal Care and Use Committee Universiti Putra Malaysia, UPM/IACUC/AUP-R094/2016.

3.2 BAT TRAPPING

Bat trapping was carried out in the fruit plantation adjacent to the 17th college, Universiti Putra Malaysia (UPM). Two mist nets were set strategically along the fruiting trees at dusk (Figure 2) and the nets were examined at 6:30a.m. - 7:00a.m. in the following morning. Female bats trapped in the net were identified and placed individually in a pouch. A total of six female lesser short-nosed fruit bats were captured and three of which were pregnant.

Figure 2: Bat trap using mist nets set up along fruiting trees in the fruit plantation adjacent to 17th college UPM at dusk.

3.3 SAMPLE COLLECTION AND PROCESSING FOR LIGHT MICROSCOPY

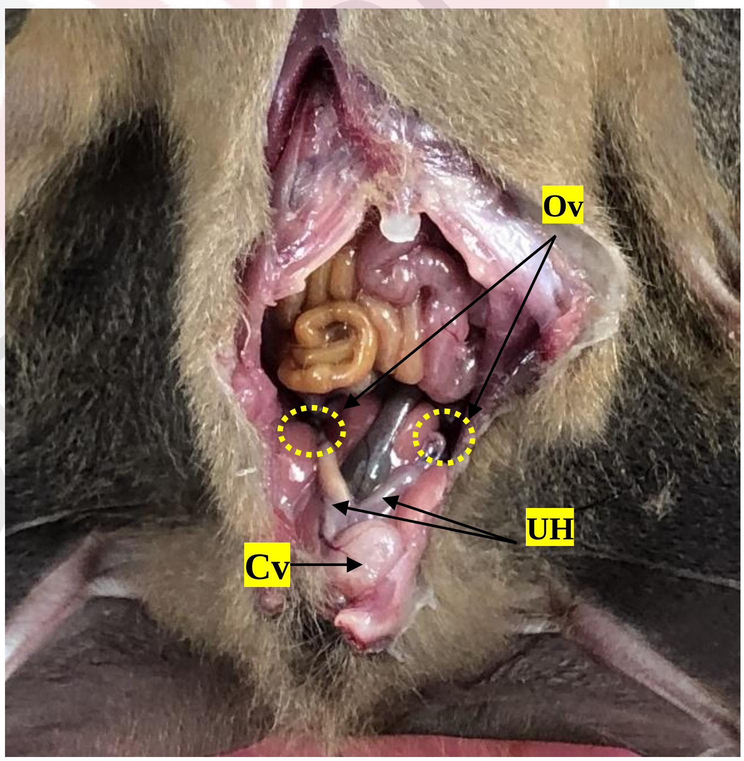
The captured bats were sedated with injectable ketamine-xylazine and euthanized via intracardiac exsanguination. A mid-line abdominopelvic incision was made to expose the abdominal organs and both ovaries identified and excised. Six ovaries from the gravid and non-gravid uterine horns of the pregnant bats were obtained for light microscopy. The ovaries were fixed overnight in 10% formalin and the following morning the samples were trimmed under the dissecting microscope to remove surrounding connective tissues. The samples were then transferred into cassettes and placed in the first beaker of the Histokinette for sample processing. The following morning the samples were positioned and embedded in liquid paraffin and cooled. Samples in paraffin blocks were sectioned 4-5 µm using the rotary microtome and floated on a water bath at 45ºC. The ribbons were dished onto glass slides, dried and stained with hematoxylin and eosin (H&E). The preparation was covered with a few drops DPX and covered with a cover slip.

4.0 RESULTS

4.1 Gross Anatomy of the Reproductive System of a Female Lesser Short-Nosed Bat.

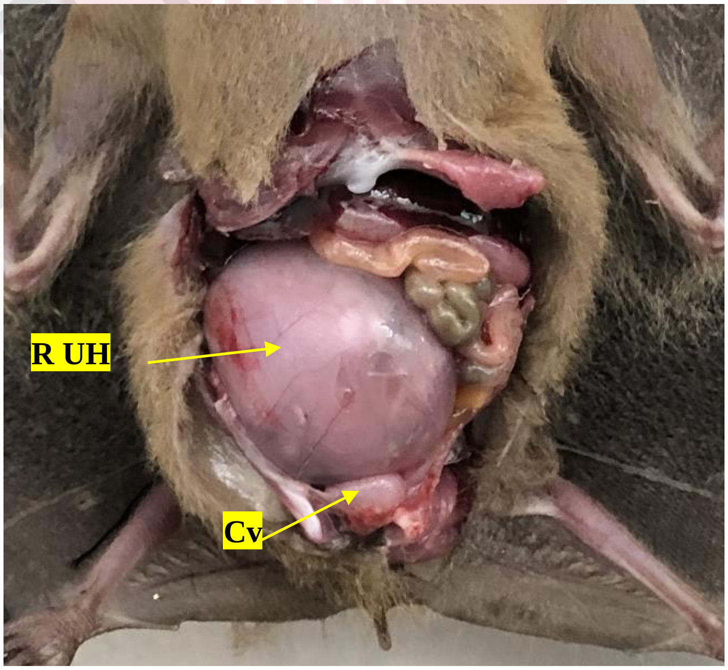
The gross anatomy of the reproductive system of the female lesser short-nosed bat *(C. brachyotis)* is as shown in Figure 3.

Figure 3: Gross anatomy of the reproductive system of the female fruit bat *(C. brachyotis)*. **Ov**= Ovaries (paired), **UH**= Uterine horns, **Cv**= Cervix.



The reproductive system of the female lesser short-nosed fruit bat comprised of a pair of ovaries and oviducts, the uterine horns, the cervix and vagina. The paired ovoid ovaries appeared whitish were enclosed in the highly coiled oviduct which in turn was surrounded by a layer of connective tissue. The Y-shaped uterine horn was connected to a short uterine body and finally to a whitish dense and thick cervix. Upon laparoscopy of the pregnant fruit bat there was only one uterine horn that was gravid and all 3 pregnant bats carried their fetuses on the right uterine horn. The uterine horn housing the growing fetus was markedly expanded and occupied almost the entire abdominopelvic cavity, as shown in Figure 4.

Figure 4: Anatomy of the reproductive system of a pregnant fruit bat with a fetus in the right uterine horn displacing the abdominal viscera to the left of the abdominal cavity. **R UH**= Right uterine horn, **Cv**= Cervix.



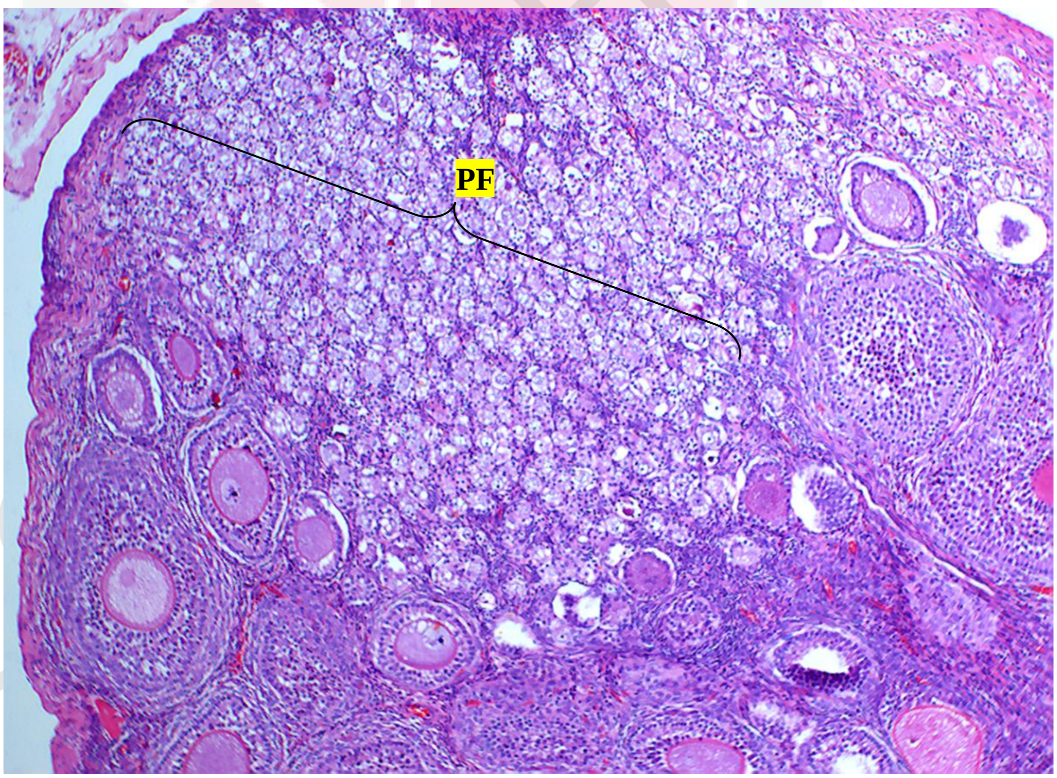
4.2 Histological Structure of Ovary of Non-Gravid Uterus.

Figure 5 shows a general view of the histological structure of the ovary of the non-gravid uterine horn of the lesser short nosed fruit bat in close association with the oviduct and surrounding layer of connective tissue of the ovarian hilum. The ovarian parenchyma in this mammalian species comprised of primary, secondary and tertiary follicles at various stages of development and degeneration.

Figure 5: General view of the histological structure of the ovary of the fruit bat in relation to the oviduct (**OvD**) and surrounded by a connective tissue (**CT**) of the ovarian hilum. The ovarian parenchyma comprised largely of primary follicles (**PF**), secondary and tertiary follicles (**F**) and atretic follicles. **C**= Cortex, **M**= Medulla. H&E, x40.

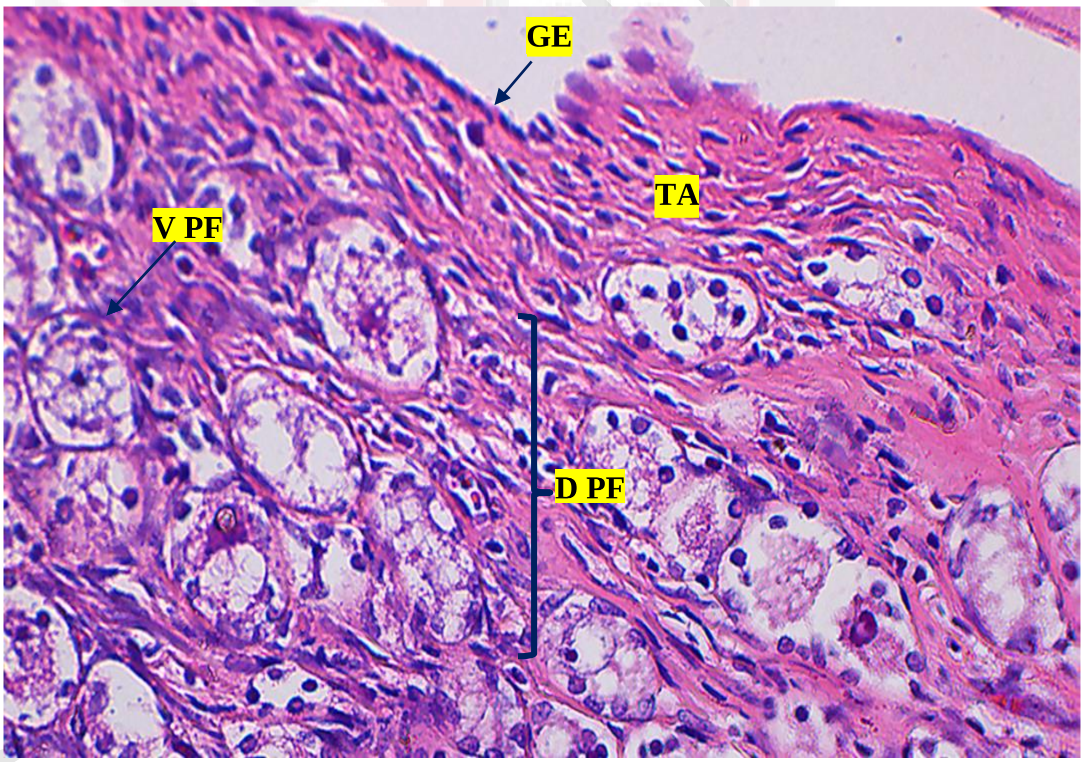
About a quarter of the ovarian parenchyma in this mammalian species was occupied by primary follicles. These primary follicles are however confined to one part of the ovarian parenchyma (Figure 6). Based on the volume of the ovarian tissue there must be hundreds or probably thousands of primary follicles in one ovary.

Figure 6: Part of the ovarian parenchyma occupied by hundreds or probably thousands of primary follicles (**PF**). H& E, x100.



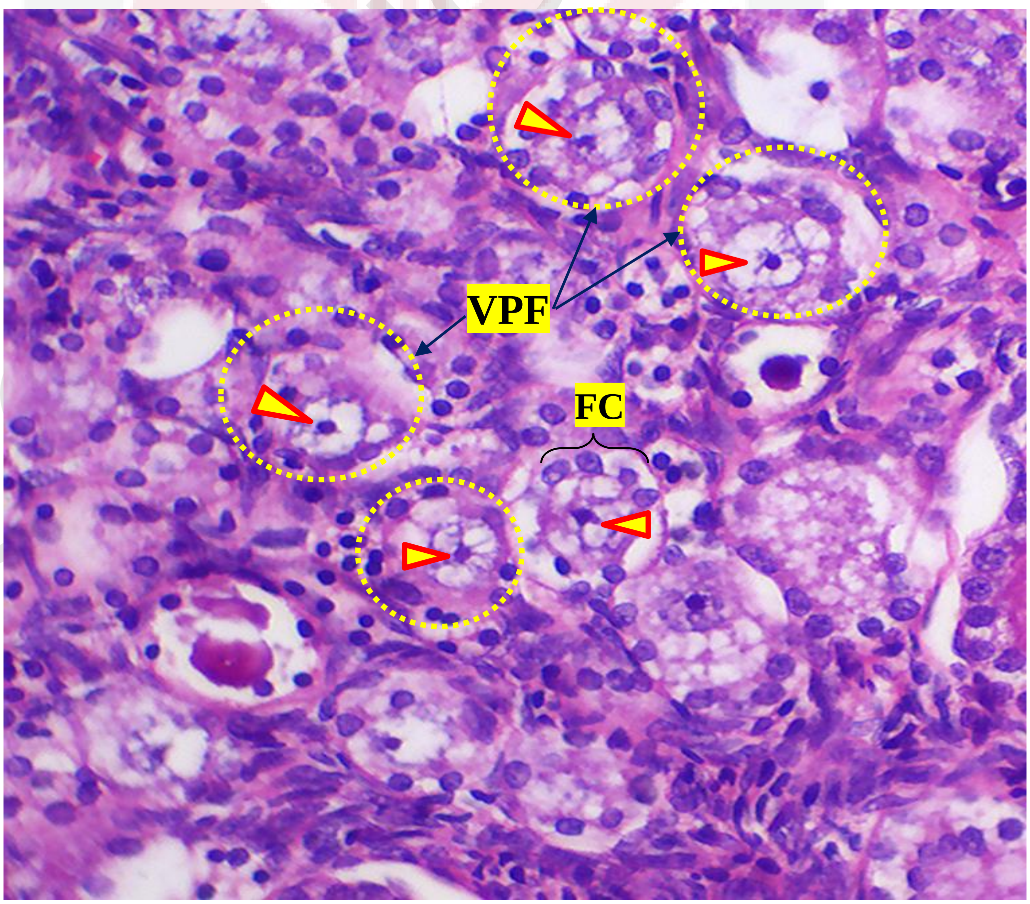
Under higher magnification it was observed that most of the primary follicles were degenerated as evidenced by pyknotic nuclei of the follicular cells surrounding the oocytes (Figure 7).

Figure 7: Degenerated primary follicles indicated by pyknotic nuclei of the follicular cells around oocytes. **GE**= Germinal epithelium, **TA**= Tunica albuginea, **VPF**= Viable primary follicles, **DPF**= Degenerated primary follicles. H&E, x400.



Not all the primary follicles present in the ovary were degenerated. Some of the primary follicles appeared still viable. Viable primary follicle comprised of a large nucleus and prominent nucleolus surrounded by an extensive cytoplasm which in turn is surrounded by follicular cells (Figure 8).

Figure 8: A few viable primary follicles (**VPF**) among degenerated primary follicles. The VPF comprised of a large nucleus with extensive cytoplasm surrounded by follicular cells (**FC**). Arrowheads = nucleoli in the nuclei of viable primary follicles. H & E x400.



The development of secondary follicles from primary follicles is as shown in Figure 9. Secondary follicle comprised of a marked zona pellucida, corona radiata cells and follicular cells. Viability of secondary follicles is evidenced by the presence of mitotic figures amongst follicular cells. Absence of follicular fluid among the follicular cells is a criterion for the identification of a secondary follicle.

Figure 9: Secondary follicle is characterized by an oocyte (**O**) surrounded by the zona pellucida (**ZP)** and follicular cells (**FC**) and absence of follicular fluid amongst follicular cells. H&E, x400.

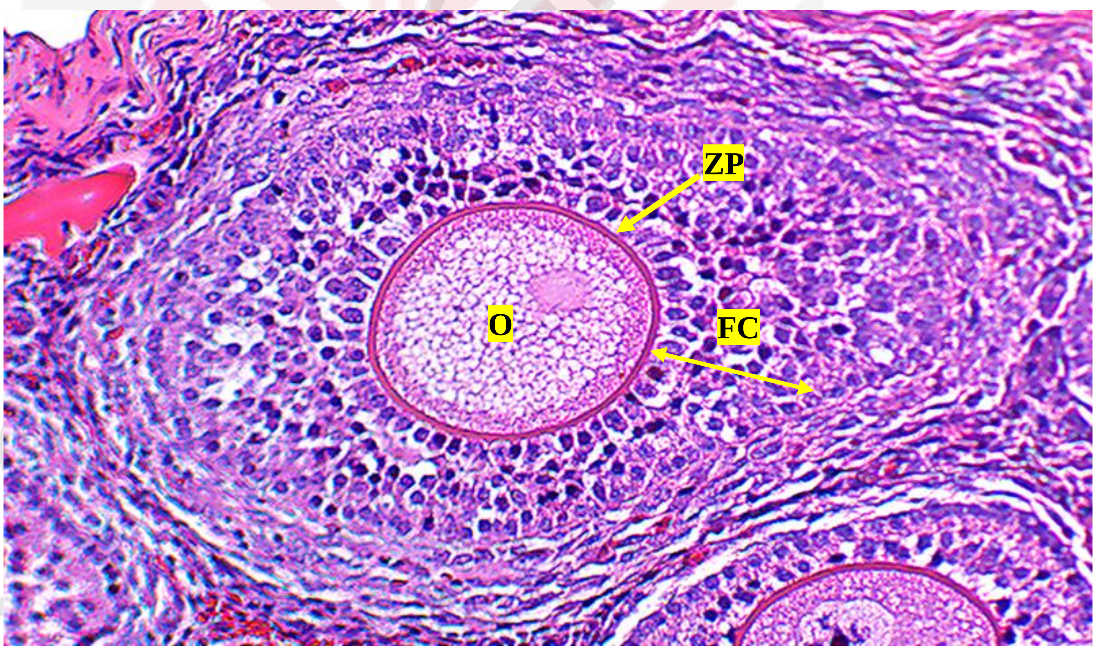


Figure 10: Early development of a tertiary follicle with the beginning of formation of follicular fluid (**FF**) in between the follicular cells. Tertiary follicle comprised of zona pellucida (**ZP**), corona radiata (**CR**) and follicular cells (**FC**). H&E, x400.

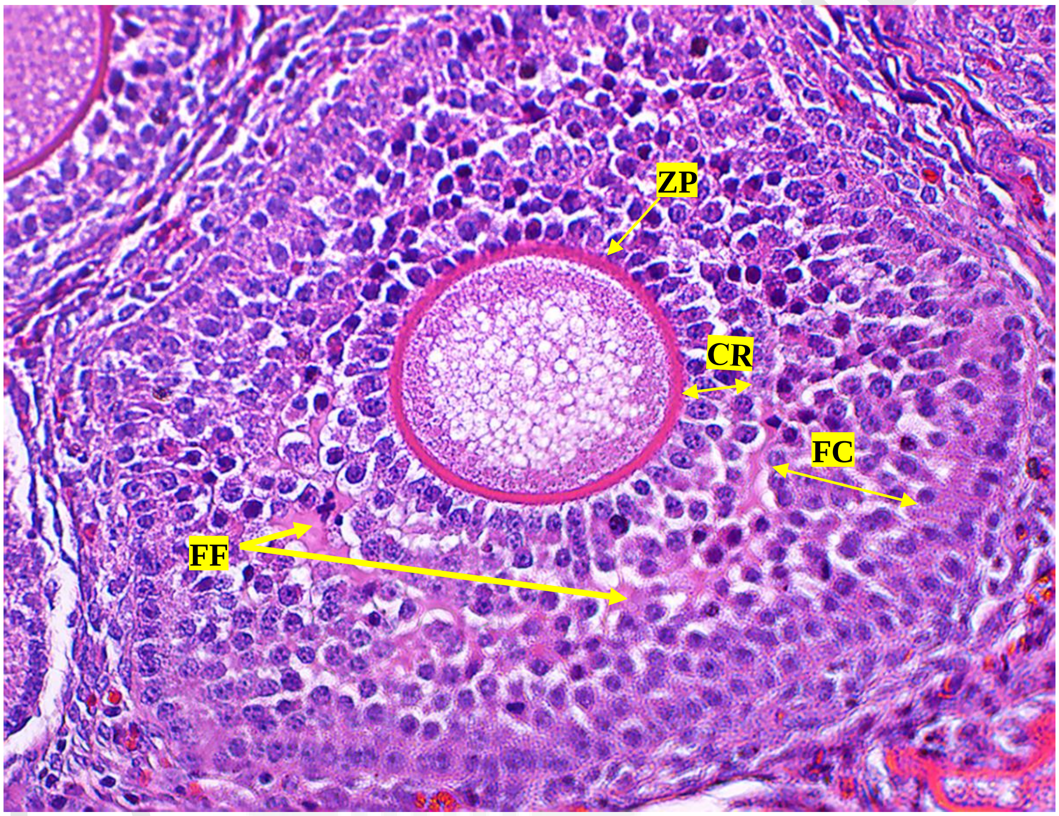
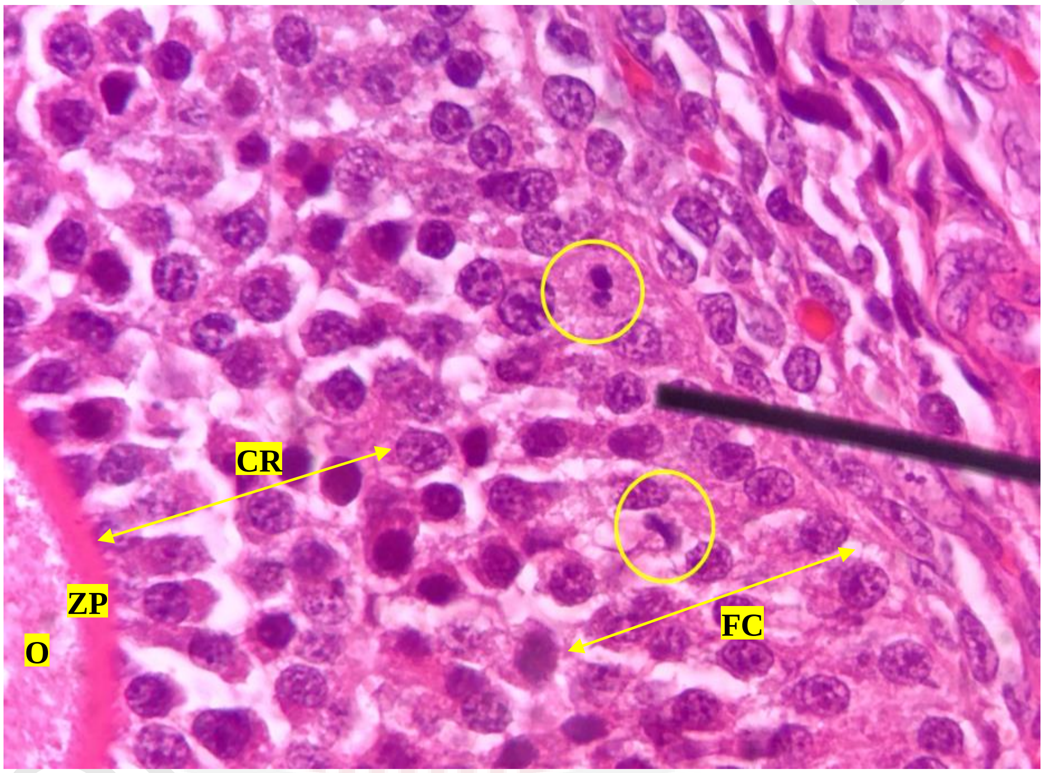
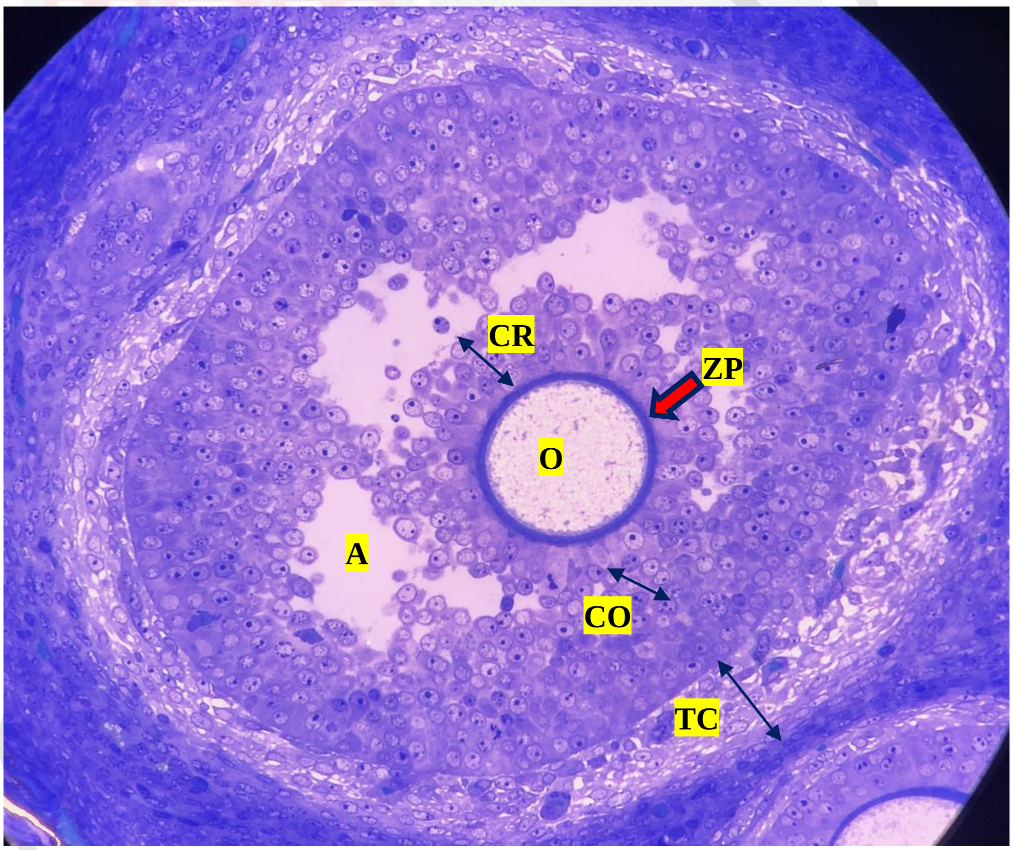


Figure 11: Viable tertiary follicle as evidenced by the mitotic figures at the anaphase stage (yellow circle) among the follicular cells. **O**= Oocyte, **ZP** = Zona pellucida, **CR** = Corona radiata, **FC**= Follicular cells. H&E, x1000.



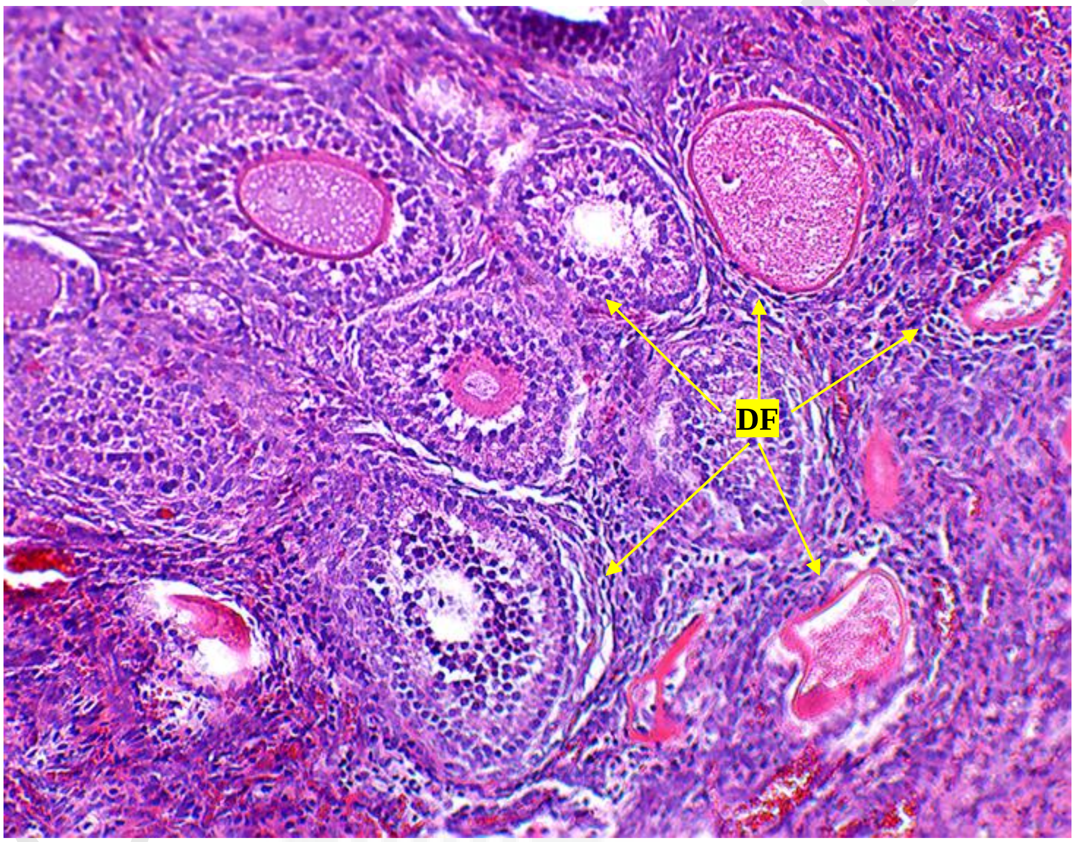
A fully developed tertiary follicle in the lesser short-nosed fruit bat is as shown in Figure 11 and 12. Tertiary follicles was identified by the presence of antrum amongst the follicular cells. Initially the antrum were present as isolated vacuoles amongst the follicular cells. However when more follicular fluid are produced by the follicular cells, the antrum coalesce separating the follicular cells into two layers – corona cells which surround the oocyte and peripheral follicular cells.

Figure 12: Tertiary follicle identified by the antrum (**A**) amongst the follicular cells. **CO**= Cumulus oophorus, **CR**= Corona radiata, **O**= Oocyte, **ZP**= Zona pellucida, **TC**= Thecal cells. Resin embedded tertiary follicle stained with methylene blue, x400.



In the ovary of non-gravid uterine horn of the lesser short-nosed fruit bat although there were viable secondary and tertiary follicles, the majority of these follicles were atretic (Figure 13).

Figure 13: Degenerating secondary and tertiary follicles in the parenchyma of the ovary of the non-gravid uterine horn. **DF**= Degenerating follicles of various stages. H&E, x400.



4. 3 Histological Structure of Ovary of Gravid Uterus Horn.

Examination of the ovary of the gravid uterine horn showed that the entire parenchyma of the ovary was occupied by luteal tissues except for the few atretic follicles at the periphery of the ovary (Figure 14).

Figure 14: Ovary of the gravid uterus with almost the entire parenchyma of the ovary being occupied by luteal tissues except for a number of atretic follicles (arrowhead) at the periphery of the ovary. **AF**= Atretic follicles. H & E, x40.

5.0 DISCUSSION

The objective of the present study was to examine the histological structure of ovary in the lesser short-nosed fruit bat (*C. brachyotis*) a flying mammal and compare it with the histological structure of ovaries of ground dwelling mammals. Further the significance of occurrence of both functional corpus luteum and developing follicles in the pregnant fruit bat was also delved into. Towards both objective there was a need to examine the histological structure of ovaries of both non gravid and non-gravid uterine horns in the pregnant fruit bat.

The histological structure of ovaries of non-gravid uterine horn revealed the presence of hundreds or probably thousands of primary follicles localized in one part of the ovarian parenchyma. The majority of these follicles were however degenerated as evidenced by the pyknosis of follicular cells surrounding the oocytes. A similar situation occur in humans where thousands of primary follicles are present in both ovaries at birth; however only 300 to 400 of these follicles continue to grow and mature and ovulate to liberate the oocytes (Ham, 1969). The process involved in the selection of follicles for subsequent growth is however not understood (Priedkalns, 1976).

The development of follicles, from primary to secondary to tertiary in the ovary of non-gravid uterine horn in the fruit bat appeared to be very similar to that of other ground dwelling mammals. As such the histological structure of the primary, secondary and tertiary follicles in the flying and non-flying mammals are very similar. The similarity of histological structure of bats and mammalian ovaries contribute to the already existing knowledge that there are similarities in the histological structure of the lungs (Ojuolape *et al.*, 2016), gastrointestinal tract (Strobel *et al.*, 2015) and heart (Alijani *et al.*, 2016) of bats with those of other mammalian species.

Gopalakrishna (1955) reported that while there was a functional corpus luteum in the ovary of the gravid uterine horn in the pregnant fruit bat the ovary of the non-gravid uterine horn was not quiescent - there were follicular developments in the said ovary. The author however did not deliberate the significance for the concurrent occurrence of corpus luteum and developing follicles in the pregnant fruit bat. Neither did the author reported the large number of atretic follicles in the ovary of the non-gravid uterine horn as observed in the present study. Therefore the high level of progesterone in circulation produced by the functional corpus luteum in the ovary of the gravid uterine horn could be responsible for the inability of the follicles to ovulate and subsequently these follicles degenerate. The degenerate follicles could to a certain extent fulfill the second objective of this study. The concurrent occurrence of corpus luteum and developing follicles in the ovaries did not appear to have any significance in the fruit bat since the follicles did not ovulate. Rationale for the continuous development of follicles in the ovary of non-gravid uterine horn in spite of the presence of the corpus luteum in the gravid uterine horn is however not known.

A significant finding from the present study was the large corpus luteum in the ovary of the gravid uterine horn. A corpus luteum of similar histological structure has not been reported in any mammals until to date. Luteal tissue in the corpus luteum of the fruit bat appear to occupy almost the entire ovarian parenchyma except for some atretic follicles at the periphery of the ovary. In this respect the whole ovary is almost a corpus luteum which lead to an interesting possibility. In mammals, following parturition corpora lutea develop into corpus albicans, a scar tissue made up predominantly of collagen fibers and fibroblast. However in mammals the corpus luteum occupy only a small portion of the ovarian parenchyma. The corpus albicans will involute and primary, secondary and tertiary follicle occupying the remaining part of the ovarian parenchyma will resume normal developments and the ovary becomes functional again. In the fruit bat as the entire ovary is a corpus luteum almost the entire ovary will become a scar tissue and accordingly it will not be able to function again as an ovary. As for the tertiary follicle in the ovary of the non-gravid uterine horn it will also ovulate and form a corpus luteum. With ensuing pregnancy and parturition it will also assume the structure of a corpus albicans. From the foregoing is there a possibility that the lesser short-nosed fruit bat will only produce two pups during its entire life time.

6.0 CONCLUSION

It can be concluded from the present study that the histological structure and development of follicles in the fruit bat is similar to those of other mammals. This study report for the first time the structure of a corpus luteum which is markedly different from that of other mammals. Concurrent occurrence of both developing follicles and corpus luteum did not appear to have any significance in the pregnant fruit bat. The possibility that the fruit bat will only produce two pups during its life time though remote should not be ruled out as it is based on the unique histological structure of its corpus luteum and the sequence of natural events that takes place in a corpus luteum following parturition. However it is to be admitted that the above possibility is based on a very preliminary study using the ovaries of three pregnant fruit bats.

7.0 RECOMMENDATION

A larger sample size is recommended for a more comprehensive research of the corpora luteum to support or disprove the above possibility. The focus of future research should be on the identification of primordial follicles in the ovarian parenchyma. Should primordial follicles be present this entity will proliferate meiotically to produce primary, secondary and tertiary follicles for the resumption of normal function of the ovary thereby producing more than just two pups.

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