Fibre Interplay in the Pregnant Uterus of the Frugivorous Bat *Eidolon Helvum*

Samson Oludare Ayodeji Odukoya[1] Opeyemi Abimbola Odukoya[2] Stephen Taiye Adelodun[1]

[1] Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria
[2] School of Nursing, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria

Address for correspondence Samson Oludare Ayodeji Odukoya, PhD, Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria (e-mail: goshevnigeria@yahoo.com).

J Morphol Sci 2018;35:183–186.

Abstract

Introduction There is currently a lack of adequate information on the mating, reproduction and the reproductive anatomy of the frugivorous bat *Eidolon helvum.* We, therefore, investigated their uterus during the non-pregnant and pregnant states for adequate information and comparison between the two states to ascertain what histological adaptations occur during pregnancy in relation to collagen and elastic fibers.

Materials and Methods A total of 47 female bats (*E. helvum*) were used, out of which 24 were pregnant and 23 were not. They were harvested on the Obafemi Awolowo University Campus. The animals were carefully screened, assumed to be presumably healthy and then sacrificed by cervical dislocation. The left and right uterine limbs with the uterine body and part of the placenta were fixed in 10% formal saline, processed for paraffin embedding and sectioned at 5 μm with a rotary microtome. The sections were stained using Verhoeff-van Gieson stain to demonstrate collagen and elastic fibers.

Results The results showed that both uterine limbs were histologically active during pregnancy. The prevalent fiber was the elastic type during the non-pregnant state, and collagen type during pregnancy.

Conclusion We conclude that elastic fibers tend to obey Hooke's law during pregnancy in the uterus of the frugivorous bat *E. helvum*, which signifies a form of histomorphological response or adaptation to pregnancy in the uterus of this bat species.

Keywords
- *eidolon helvum*
- uterus
- fiber
- gravid
- non-gravid

Introduction

Bats are unmistakable, as no other mammal has true wings for flight. Globally, the order Chiroptera (from the Greek *Cheiro,* meaning hand, and *ptera,* meaning wing) is currently composed of 18 families, ~ 174 genera and 900 species, while some authors put it at 1,000 (Microchiroptera 825 and Megachiroptera 175) and some at 1,100 species accounting for ~ 20% of all mammals.[1,2] About 211 species live in Africa. Chiropterans are subdivided into two main suborders;

1. Megachiropterans (Old World fruit bats or flying foxes) and
2. Microchiropterans (any bat that is not a fruit bat).[1]

There are approximately 164 species of fruit bats, grouped into 41 genera in a single family, the Pteropodidae. The Microchiroptera is the larger of the suborders, consisting of 17 families, 133 genera and 743 species. All bats belong to the kingdom Animalia, phylum Chordata, subphylum Vertebrata, class Mammalia and order Chiroptera.

Bats, like humans, are mammals, having hairs and giving birth to living young and feeding them milk from mammary glands. They are most abundant in the tropics and comprise about one fifth of all mammal species.[3]

Each of the different species differs in its breeding mode, even a particular species may differ in its breeding habits in different climatic regions,[4,5] but they all have certain characteristics in common; from a review of the large volume of

received
March 14, 2017
accepted
August 15, 2018
published online
October 29, 2018

DOI https://doi.org/10.1055/s-0038-1675227.
ISSN 2177-0298.

Copyright © 2018 by Thieme Revinter Publicações Ltda, Rio de Janeiro, Brazil

License terms

work on the reproduction of bats, the following can be deduced:

First, bats breed during sharply defined seasons.[6–8] It is often recognized that reproductive periods of frugivorous bats are closely linked to and may be triggered by peaks of fruit abundance.[9–11]

Secondly, they exhibit the phenomenon of sperm storage and delayed fertilization and/or delayed implantation, sometimes due to alternation or intermittent periodic development of the male and female gonads per season.[8,12,13]

Third, the uterus was classified as bicornuate and symmetrical,[4,8,14] but a publication by Odukoya et al (2009) has reclassified it as partial septate and symmetrical.

Fourth, bats generally have a single parturition of one young per year.[15,16] Some have two parturitions per year or twins, some have triplets, like the hairy-tailed bat, while *Artibeus litaratus* breeds throughout the year.[8,15,17,18]

The aim of this study was to investigate the uterus of these bats during the non-pregnant and pregnant states for adequate information and comparison between the two states to ascertain what histological adaptations occur during pregnancy in relation to collagen and elastic fibers.

The African straw-colored fruit bat or African palm tree fruit bat is present in Africa, Asia (the Arab Peninsula), in the southwest of the Arab Peninsula.[19] In Africa, they form large colonies in widely scattered locations across the central belt of Africa. Major roost sites are known at Jinja and Kampala, in Uganda; Ile-Ife and Lagos, in Nigeria; Accra and Nli Falls, in Ghana; Abidjan, in Ivory Coast; Dar-es-Salaam, in Tanzania.[20] Others include Kahuzi-Biega National Park, in the Democratic Republic of Congo.[21]

The males are slightly larger than the females. The head-and-body length is reported to be between 143 and 215 mm. The weights ranges between 230 and 350 g. The wings are large and narrow, allowing the bats to fly long distances and not expend as much energy trying to flap them a lot. The head is large and pointed with large eyes and no white facial markings.[22]

The wingspan of the *E. helvum* averages 3 feet (a meter) and they have fox-like facial features (like all other Megachiropterans). The hair on their body and head is straw colored, but their wings, snout and ears have a dark brown color.[23]

Much has been published on the ovaries and ovarian cycle in this bat species.[8] The uterus was classified as bicornuate, but a more recent work by Odukoya et al (2009) has shown that it is a partial septate type of uterus, and also published much on the structural anatomy and the histoarchitecture of the organ [24]; therefore, this study only attempted to study its histological adaptations during pregnancy in relation to fibers integrity.

Materials and Methods

A total of 47 female bats were used; they were harvested on the Obafemi Awolowo University Campus at different times to assess the different stages in their reproductive cycle. The bats were brought into the department and kept in cages in the Animal Holdings of the Department to acclimatize for at least 24 hours before sacrifice. The bats were fed overnight with ripe bananas and water. The animals were carefully assessed, screened and assumed to be presumably healthy.

The animals were sacrificed by cervical dislocation after intramuscular pentobarbital sodium (40 mg/kg b/w) administration. Abdominopelvic incisions were made to expose and excise their uteri. These uteri were immediately washed in physiological saline and blotted dry using filter paper before being weighted on a Mettler Toledo sensitive weighing balance (Mettler-Toledo, LLC, Columbus, OH, USA).

Histological Procedures

Paraffin Tissue Processing

The uteri were immediately fixed in a 10% formal saline solution limiting the postmortem time to $\sim$ 2 minutes. Tissues were fixed for period of 24 hours before further processing. The tissues were dehydrated with graded alcohol, cleared in xylene and infiltrated with molten paraffin wax. The tissues were embedded in paraffin wax and sectioned at a thickness of 5 µm using a rotary microtome. The sections were placed on glass slides and dried at 37°C until they were ready for the staining procedures.

Verhoeff-Van Gieson Staining Procedure

The sections were dewaxed in xylene and rehydrated, and then stained for elastic and collagen fibers using the modified differential Verhoeff-van Gieson method according to Verhoeff, 1908. Elastic fibers stained black, while collagen fibers stained red, and muscle stained yellow.

Results

The fiber type is a mixture of both elastic and collagen fibers, and the predominant fiber being the elastic type in the non-pregnant state. During pregnancy, however, there is a gradual transition of the fiber type from elastic to predominantly collagen fibers. After parturition, as the integrity of the uterine muscularis returns to normal, the elastic fibers are also gradually regenerated, and once again become predominant. ►**Figs. 1**, **2**, **3**, **4**, **5**, **6**, **7**

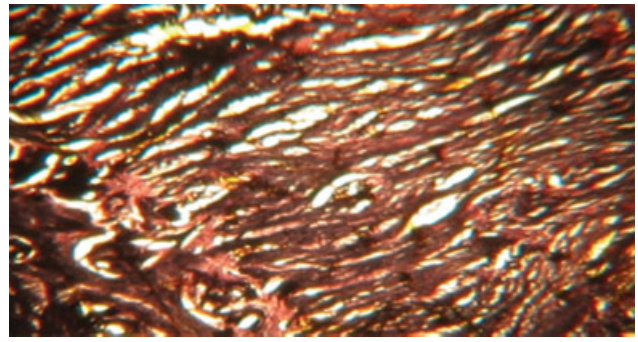

Fig. 1 The uterine muscularis in the non-pregnant state, showing an admixture of collagen (red) and elastic (brown/black) fibers. Verhoeff-van Gieson stain, (X 400).

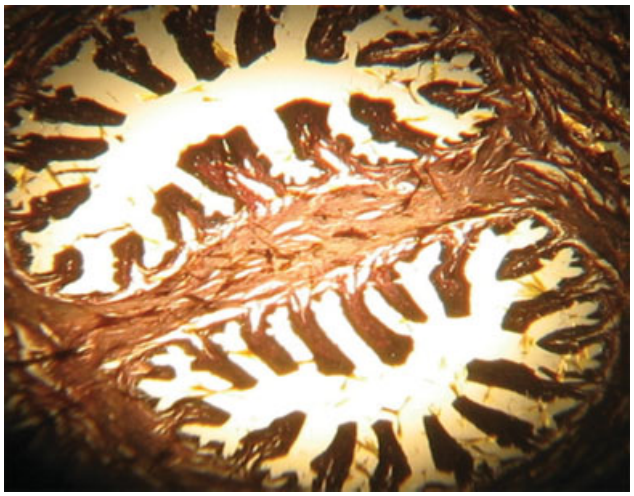

Fig. 2 Cross section of the uterine body during the non-pregnant state, showing an admixture of elastic and collagen fibers, but predominantly elastic fibers. Verhoeff-van Gieson stain, (X 200).

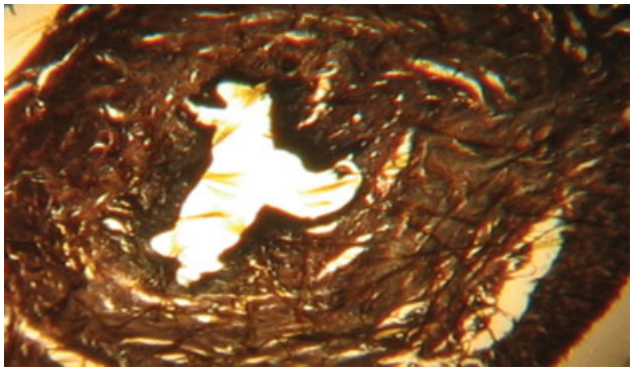

Fig. 3 Cross section of the non-pregnant limb at the initial stage of pregnancy showing the deeply stained, predominant elastic fibers. Verhoeff-van Gieson stain, (X 250).

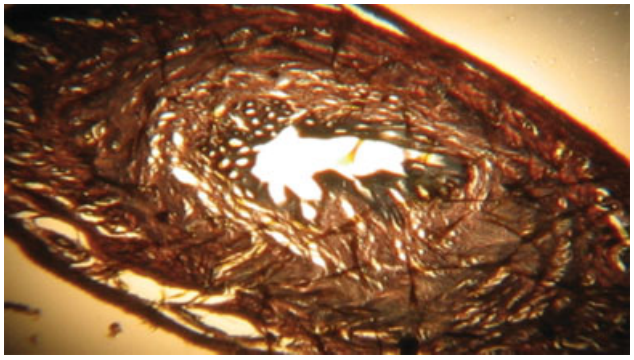

Fig. 4 Cross section of the pregnant limb at the initial stage of pregnancy showing more elastic fibers than collagen fibers. The pregnant limb showed more collagen fibers than both the non-pregnant limb and the non-pregnant uterus. Verhoeff-van Gieson stain, (X 200).

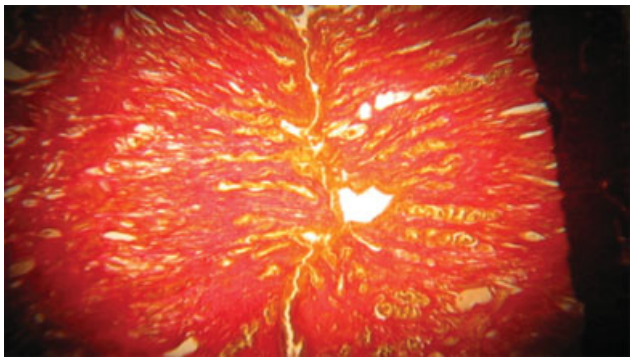

Fig. 5 The fibers type in the non-pregnant limb completely changed from predominantly elastic to exclusively collagen fibers (bright red stain) type during pregnancy. It shows no trace of elastic fibers. Verhoeff-van Gieson stain, (X 200).

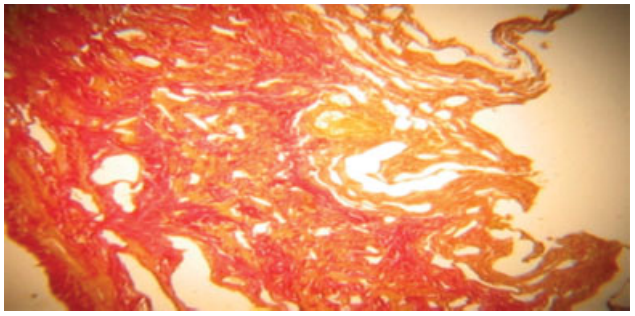

Fig. 6 The pregnant limb at an advanced stage in pregnancy shows a transition from the predominant elastic fibers to exclusively collagen fibers, and more smooth muscle fibers stained yellow. Verhoeff-van Gieson stain, (X 250).

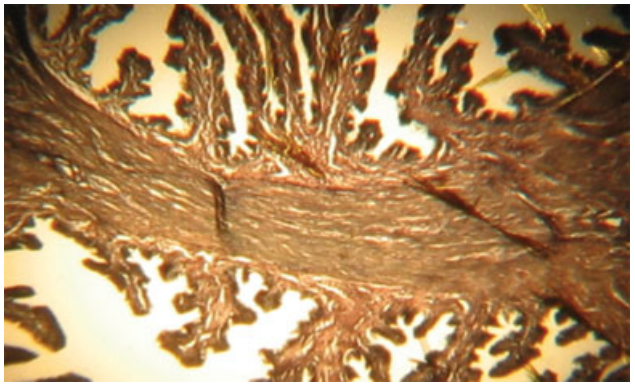

Fig. 7 The uterine body after parturition displaying elastic fibers regeneration. It depicts a fine distribution of both elastic and collagen fibers. Verhoeff-van Gieson stain, (X 250).

Discussion

This study has been able to show that there is interplay of elastic and collagen fibers during the non-pregnant and the pregnant states, specifically in the uterus of the *E. helvum*. During pregnancy, the elastic fibers, which are predominant during the non-pregnant state, are replaced by collagen fibers, which are able to withstand the stretch pressure exerted on the uterus. Hooke's law states that an elastic body will stretch and return to its normal state until the elastic limit is exceeded, and the yield point is reached when the elastic body can no longer return to its normal state, any further stretch at this point will take it to the breaking point.

It can thus be inferred that, during pregnancy, the elastic fibers are stretched beyond the elastic limit; they reached the yield point and degenerated, gradually giving way to the

collagen fibers that are well able to withstand the stretch pressure exerted on the uterus by the developing fetus, until parturition, when the elastic fibers are regenerated.

Conclusion

Elastic fibers tend to obey Hooke's law during pregnancy in the uterus of the frugivorous bat *E. helvum*, which signifies a form of histomorphological response or adaptation to pregnancy in the uterus of this bat species.

Acknowledgment

The authors wish to express their profound gratitude to the late sage Professor Ezekiel Ademola Caxton-Martins, who, before his demise, single-handedly supervised this work until its completion, and to Dr. Adeoye, of the Department of Histopathology at the University College Hospital, in Ibadan, Nigeria, for his contributions to the completion of this project.

References

1 Kalko EKV. Bats. Mammal Directory of Bats – Online Edition. Accessed August, 1999
2 Pettigrew JD. John D. Pettigrew's Summary on Flying Primate Hypothesis. 2006www.wikipedia.org.
3 Riede K. Global register of migratory species – from global regional scales. Final Report of the R & D – Projekt: Federal Agency for Nature Conservation, 2004, 808 (05) 081:329
4 Marshall AJ. The breeding cycle in the equatorial bat (*Pteropus giganteus* of Ceylon). Proc. Linn. Soc. London 1947;159:103–111
5 Moghe MA. Development and placentation in the Indian fruit bat, *Pteropus giganteus* (Brunnich). Proc Zool Soc Lond 1951; 121:703–721
6 Heideman PD, Bhatnagar KP, Hilton FK, Bronson FH. Melatonin rhythms and pineal structure in a tropical bat, Anoura geoffroyi, that does not use photoperiod to regulate seasonal reproduction. J Pineal Res 1996;20(02):90–97
7 Mutere F. Eidolon helvum Revisited. Pp 145–150 in D. Wilson, A. Gardner, eds. *Proceedings of the Fifth International Bat Research Conference*1980, Lubbock, Texas, U.S.A.; Texas Tech. Press.
8 Okon EE, Opara MC, Caxton-Martins AE. A histological study of the ovarian cycle in the fruit bat, *Eidolon helvum. Proceedings of the Sixth International Bat Research Conference*1979, 47–55.
9 Bonaccorso FJ. Bats of Papua New Guinea. Conservation International Tropical Field Guide Series, Conservation InternationalWashington, D.C.1998, 489 pp.
10 Dinerstein E. Reproductive Ecology of Fruit Bats and the Seasonality of Fruit Production in a Costa Rican Cloud Forest. Biotropical 1986;18:307–318
11 Faria DM. A field study of reproductive interactions in Bosca's newts, *Triturus boscai. Amphib.-*. Reptilia 1995;16:357–374
12 Gopalakrishna A, Madhaven A. Survival of spermatozoa in the female genital tract of the Indian *vespertilionid bat, Pipistrellus* ceylonicus *chrysobrix (Wroughton)*.. Proc. Indian Acad. Sci. (B) 1971;73:43–49
13 Racey PA. Diagnosis of pregnancy and experimental extension of gestation in the pipistrelle bat, Pipistrellus pipistrellus. J Reprod Fertil 1969;19(03):465–474
14 Karim KB. Foetal membranes and placentation in the Indian leaf-nosed bat, *Hipposideros fulvus fulvus* (Gray). Proc Indiana Acad Sci 1972;76:71–78
15 Friederici P. Ecological Restoration of South Western Ponderosa Pine Forest. 2003Island PressWashington.
16 Gopalakrishna A, Choudhari PN. Breeding habits and associated phenomena in some Indian bats. Part 1. *Rousettus leschenaultil* (Desmarest) Megachiroptera. J Bombay Nat Hist Soc 1977; 59:1–57
17 Okia NO. Breeding in Franquet's bat, Epomops franquetti (Tomes), in Uganda. J Mammal 1974;55(02):462–465
18 Tamsitt JR, Valdivieso D. Reproductive cycle of the big fruit-eating bat, *Artibeus litaratus olfers*.. Nature 1963;198:104
19 Donald PF, Brooke M, De L, et al. Status of Raso Lark (*Alauda razae*) in 2003, with further notes on sex ratio, behaviour, and conservation. Bird Conserv Int 2005;15(02):165–172
20 Racey P. 8,000,000 fruit bats. National History Department, University of Aberdeen, Scotland. 2004www.kasanka.com.
21 Michael DD, Alvarez IM, Ocón OM, et al. Fibroblast growth factor-2 is expressed by the bovine uterus and stimulates interferon-tau production in bovine trophectoderm. Endocrinology 2006;147 (07):3571–3579
22 Happold DCD. The Mammals of Nigeria. New York: Oxford University Press; 1987.
23 Ruiz K. "*Eidolon helvum*" (On-line), Animal Diversity Web. Accessed September 07, 2002, at http://animaldiversity.ummz.umich.edu/site/accounts/information/Eidolon_helvum.html
24 Odukoya SOA, Adeeyo OA, Ofusori DA, et al. Partial Septate Uterus and Tubal Implantation: A Normal Phenomenon in the Eidolon helvum. Sci Res Essays 2008;4(01):13–117
25 Mcdonald P. Theory pertaining to low fertility. Paper presented at the IUSSP Seminar on International Perspectives on Low Fertility: Trends, Theories and Policies, Tokyo, March 2001, 21–23.