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

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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

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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**

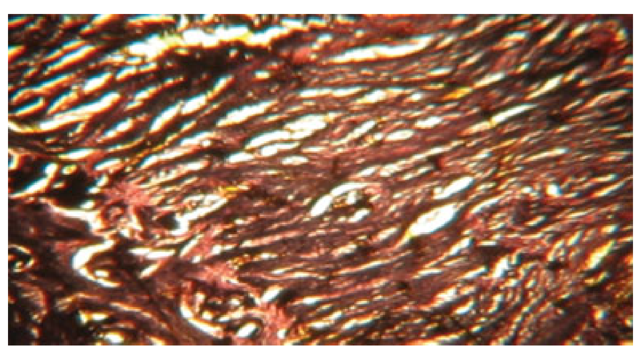

Fig. 1 The uterine muscularis in the non-pregnant state, showing an admixture of collagen (red) and elastic (brown/black) fibers. Verhoeff-van Gieson stain, (X 400).

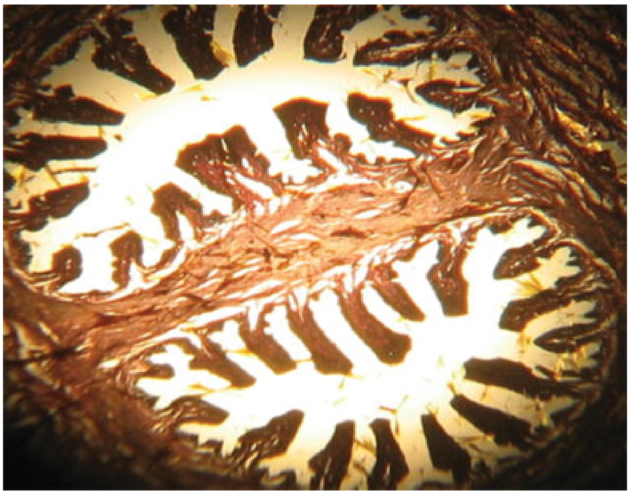

Fig. 2 Cross section of the uterine body during the non-pregnant state, showing an admixture of elastic and collagen fibers, but predominantly elastic fibers. Verhoeff-van Gieson stain, (X 200).

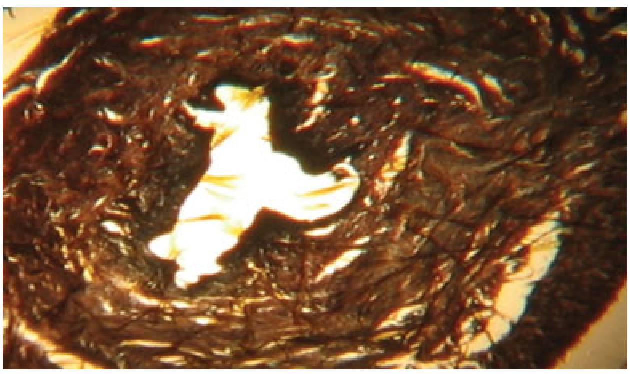

Fig. 3 Cross section of the non-pregnant limb at the initial stage of pregnancy showing the deeply stained, predominant elastic fibers. Verhoeff-van Gieson stain, (X 250).

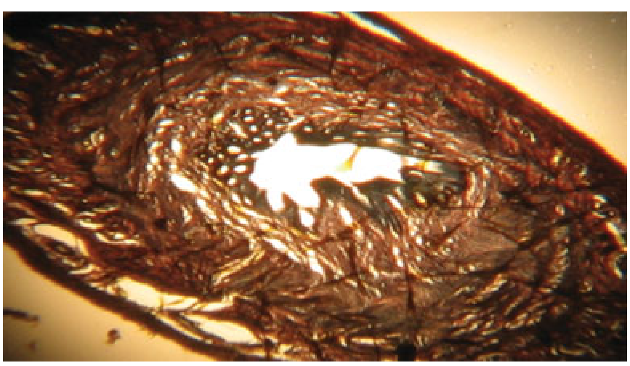

Fig. 4 Cross section of the pregnant limb at the initial stage of pregnancy showing more elastic fibers than collagen fibers. The pregnant limb showed more collagen fibers than both the non-pregnant limb and the non-pregnant uterus. Verhoeff-van Gieson stain, (X 200).

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

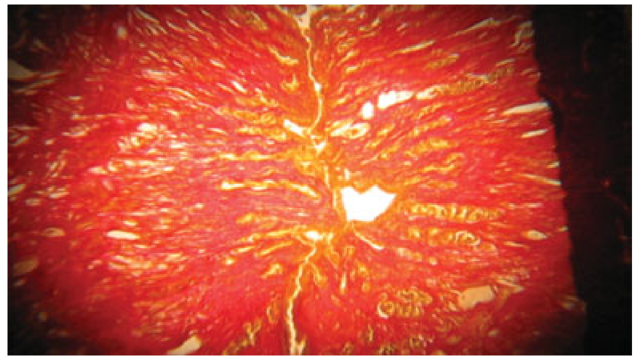

Fig. 5 The fibers type in the non-pregnant limb completely changed from predominantly elastic to exclusively collagen fibers (bright red stain) type during pregnancy. It shows no trace of elastic fibers. Verhoeff-van Gieson stain, (X 200).

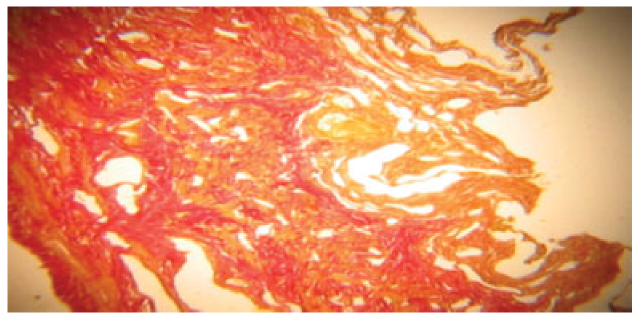

Fig. 6 The pregnant limb at an advanced stage in pregnancy shows a transition from the predominant elastic fibers to exclusively collagen fibers, and more smooth muscle fibers stained yellow. Verhoeff-van Gieson stain, (X 250).

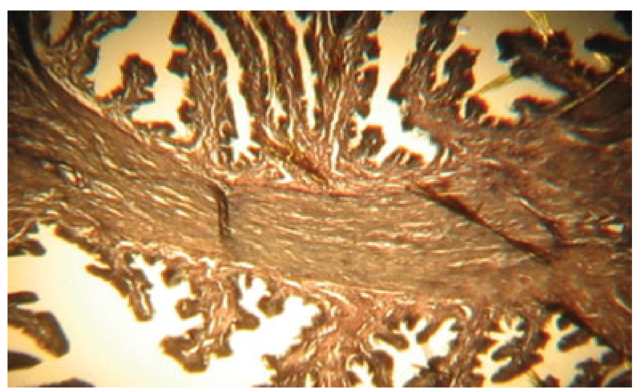

Fig. 7 The uterine body after parturition displaying elastic fibers regeneration. It depicts a fine distribution of both elastic and collagen fibers. Verhoeff-van Gieson stain, (X 250).

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.

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