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Bahareh AlijaniDepartment of Biology, Jahrom
branch, Islamic Azad University,
Jahrom, Iran**Farangis Ghassemi**Department of Biology, Jahrom
branch, Islamic Azad University,
Jahrom, Iran

Anatomy and histology of the heart in Egyptian fruit bat (*Rossetus aegyptiacus*)

Bahareh Alijani and Farangis Ghassemi

Abstract

This study was conducted to obtain more information about bats to help their conservation. Since 5 fruit bats, *Rossetus aegyptiacus*, weighing 123.04 ± 0.08 g were captured using mist net. They were anesthetized and dissected in animal lab. The removed heart components were measured, fixed, and tissue processing was done. The prepared sections ($5 \mu\text{m}$) were subjected to Haematoxylin and Eosin stain, and mounted by light microscope. Macroscopic and microscopic features of specimens were examined, and obtained data analyzed by ANOVA test. The results showed that heart was oval and closed in the transparent pericardium. The left and right side of heart were different significantly in volume and wall thickness of chambers. Heart was large and the heart ratio was 1.74%. Abundant fat cells, intercalated discs, and purkinje cells were observed. According to these results, heart in this species is similar to the other mammals and observed variation, duo to the high metabolism and energy requirements for flight.

Keywords: Heart, muscle, bat, flight, histology

1. Introduction

Bats are the only mammals that are able to fly^[1]. Due to this feature, the variation in the morphology and physiology of their organs such as cardiovascular organs is expected^[2, 3]

Egyptian fruit bat (*Rossetus aegyptiacus*) belongs to order megachiroptera and it is the only megabat in Iran^[4]. Bats help to pollination and dispersing seeds via feeding on fruits, nectar and pollen^[5], so they have important role in ecosystem, but unfortunately, the population of this useful animals is declining recently^[4].

The heart of several species of Microchiroptera was observed under light and electron microscope^[3, 6, 7], and in the megabat by^[8, 9] was studied.

There is large relative between heart and body size in mammals^[9], and bats due to the their unique movement and activity in mammal have high metabolism and energy requirement^[11]. Therefore, to facilitating the transfer of materials in the body and better efficiency, this system may be changed^[9, 10].

^[11] was studied the relationship between heart rate and the rate of oxygen consumption, and the ratio of heart and lung size in comparing to the body size in flying bird is greater than this ratio in waking birds. Even there is difference between exercise mice and otherwise^[12].

Pervious study showed that density of muscle fibers (myofibrils) in a species of small-sized bat was lower than hamster and mouse, but the density of fat cells in bat was much more than the other^[13]. The relation the size of heart and body mass of some insectivore and frugivore bats was shown in previous studies^[2, 9].

There is a little literature on the anatomy of this large order of mammals, and more information about behavior, physiology and anatomy of them is effective help to their conservation. So in present study, macroscopic and microscopic structure of the heart in *R. aegyptiacus* was examined.

2. Materials and Methods

In this study, 5 Egyptian fruit bat (*R. aegyptiacus*) accordance to the taxonomic characteristics as their large size (Fig 1) were captured using mist net from Zorok Cave. This cave is located in $28^{\circ} 45' \text{N}$ and $53^{\circ} 59' \text{E}$ with 1500 m height, in southern of Iran.

The captured bats were transferred to the animal laboratory of Jahrom University in the cotton bags, anesthetized with ether, weighted and removed their heart. All the experimental Procedures, in compliance with regarding the National Institute of Health for using the

Correspondence**Farangis Ghassemi**Department of Biology, Jahrom
branch, Islamic Azad University,
Jahrom, Iran

Laboratory animals. The removed hearts were weighed and measured immediately, then washed with diluted water and fixed in formalin (10%). After 2 weeks, the samples were placed in tissue processor for section preparation automatically. The serial sections (5 μ thickness) were prepared. The transverse and longitudinal sections were subjected to Haematoxylin and Eosin (H&E) stains, mounted on binocular microscope (10x and 40x) and stereomicroscope, and micrographs were taken with digital camera. Obtained data analyzed by SPPS (20) and ANOVA test.

3. Results

1.3 Macroscopic study

The heart in *R. aegyptiacus* is oval shape and had dark red color. It is located in the ventral half of thoracic cavity between the planes of 4th to 9th ribs (Fig 2). Heart apex is narrower than base, the apex slightly turned to the left (45°), as the greater part of the heart lied on the *left* side of the median plane (Fig 2). Its left ventricular border contacted with the left side of the diaphragm close to the median plane, and it is enclosed by the fibro-serous pericardium which its parietal layer attached dorsally to large vessels at the base, and is continued ventrally as sternopericardial ligament (Fig 2). The inferior end of this ligament is thick, because a mass of fat was in the apex (Fig 3A). The mass of fat is seen in

interconnected lobes, right and left atria and ventricles, also around the great vessels and in the coronary grooves (Fig 3). There are the fibrous rings around and between the valves as heart skeleton (Fig 5). Both the right and left surface is convex. Auricles are triangular with jagged edges and right auricle is greater than left one (Fig. 3). The coronary groove filled with mass of fat, the atrioventricular groove, interventricular groove, and paraconal groove are present (Fig 3). Obvious anterior and posterior interventricular vein are observed in the both surface of ventricles (Fig 3).

Although the wall of two ventricle are very thick, but there are significant difference ($P < 0.05$) between right and left ventricular volume and wall thickness (Figs 4, 5A and Table 1). The wall of right ventricle is smoother than wall of left ventricle. Although atria volume are been seen different, but due to the low thickness they were not measured. It is composed of the sinus venarum with smooth wall, auricle with pectinate muscle and atrium proper (Fig 5). The sinus venosus is a small triangular shaped chamber in right atrium and received blood from right and left superior vena cava, but conus arteriosus was large and muscular. The left and right pulmonary veins entered to left atrium via two separated openings (Fig 5). The ratio of body weight to heart weight was 1.74% (Table 1). The measurements of heart were documented in Table 1.



Fig 1: *Rossetus aegyptiacus*



Fig 2: Topography of the heart in *R. aegyptiacus*

1: Base 2: Apex 3: Paraconal intrententricular 4: left ventricle 5: Right ventricle 6: Left anterior vena cava 7: Left & right innominate artery 8: Posterior vena cava 9: Dorsal aorta 10: Liver 11: Rib



Fig 3 A: Anterior surface

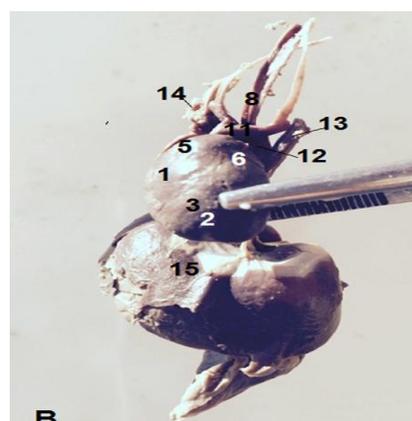


Fig B: Posterior surface

1: Left ventricle 2: Right ventricle 3: Anterior (A) & posterior (B) interventricular vein 4: ventricular apex 5: Left auricle (A) & atrium (B) 6: Right auricle (A) & atrium (B) 7: pulmonary artery 8: Ascending aorta 9: pulmonary vein 10: pulmonary trunk 11: Transverse aorta 12: Descending aorta 13: Right cranial vena cava 14: Left cranial vena cava 15: liver

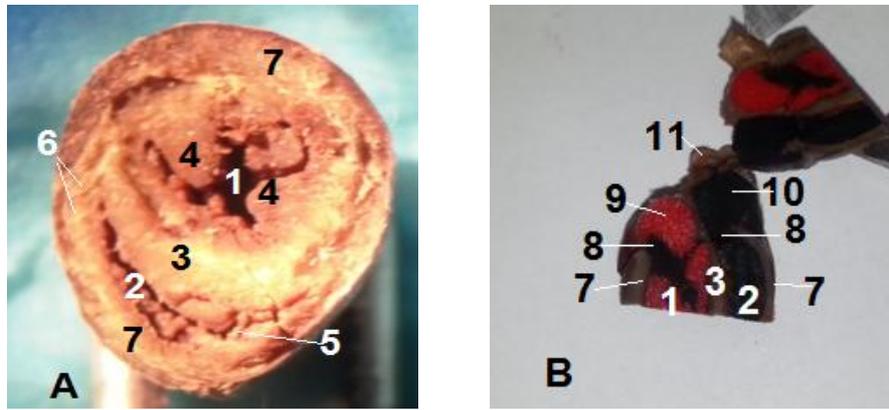


Fig 4: The heart chambers in *R. aegyptiacus* A: Left side (X.S) B: Right side (L.S)

1: Left ventricle 2: Right ventricle 3: Inter ventricular septum 4: Papillary muscle (A) 5: Moderator band 6: Trabeculae carneae 7 ventricular wall 8: Right & left atrioventricular valve 9: Left atrium 10: Right atrium 11: Right auricle

Interventricular septum in heart of this species is very thick muscular partition (Figs 4, 5A) and the thickness of the left ventricular wall is 2.2 times thicker than right ventricle (Table 1). Prominent trabeculae carneae are seen in the internal surface of two ventricles especially left ventricle, and the cusps and chordae tendineae are very thick (Figs 4A, 5). Details of the chambers, atria and ventricles, in heart of this

species resemble those of typical mammals, but the chordae tendineae are very thick (Figs 5). Papillary muscles which are in the right ventricle, anterior, posterior and septal papillary, is larger and more than ones which in the left ventricle (Figs 4A, 5A). There are some tendinous moderator bands which are extend from septum to the wall of ventricle in both sides, near the apex, but there are more in the left side (Fig 4A, 5).

Table 1: The mean of heart measurements in *R. aegyptiacus*

Body weight (g)	Heart length	Heart width	Heart weight (g)	RV thickness	LV thickness	I. VS thickness	heart ratio
123.04±0.08	18.55±0.06	B: 13.20±0.0 M: 11.8±0.1 A: 8.6±0.0	B: 2.14±0.05	1.3±0.09	2.75±0.05	2.44±0.02	1.74%.

RV& LV: right & left ventricle heart ratio: Heart weight/body weight B: Base M: Middle A: Apex

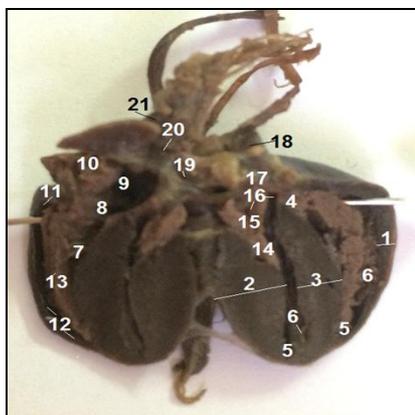


Fig 5: Anatomy of heart in *R. aegyptiacus*

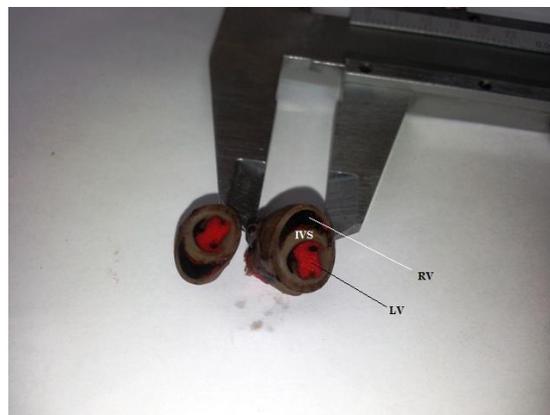


Fig 6: Topography of the vessel in *R. aegyptiacus*

1: Right ventricle 2: left ventricle 3: Interventricular septum 4: Membranous septum 5: Right and left ventricular apex 6: Moderator band 7: Septal papillary. m 8: Tricuspid valve 9: Right atrium 10: Auricle & pectinate muscle 11: R. coronary artery 12: Trabeculation 13: Posterior papillary muscle 14: anterior papillary muscle 15: chorda tendineae 16: Mitral valve 17: Left atrium 18: pulmonary vein 19: Dorsal aorta 20: anterior vena cava 21: Posterior vena cava

2.3 Microscopic study

The wall of heart includes three layers (epicardium, myocardium and endocardium) with different thickness in different regions of heart (Fig9 A, B) and table 1. The interventricular septum was very thick spatially in the left ventricle (Fig 7A). Three papillary muscle was seen in the right ventricle (Fig 7B) and two papillary muscle were in the left ventricle (Fig 8B). The visceral layer of pericardium, epicardium, is a simple squamous epithelium (mesothelium).

Subepicardium was thick (Figs 9A, B), and include abundance adipose cells (Fig 9D), vessels and abundance purkinje cells which myofibers were limited to the periphery of them (Fig 9F). Myocardium with different thickness include muscle cells which attached by many intercalated disks (Fig 9C), and in the left base of ventricle was the thickest region (Fig 3). The large lymphatic tissue was observed close the large vessel (Fig 10), and the topography of the large vessels was shown in the Fig 6.

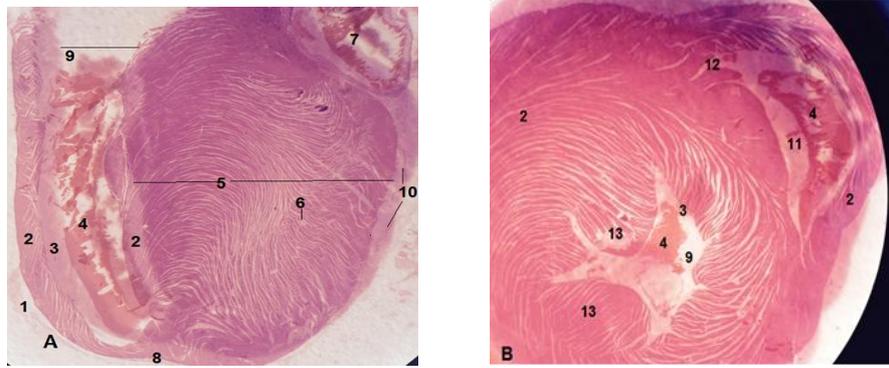


Fig 7 A: L.S & **B:** X.S of Heart chambers in *R. aegyptiacus* by stereomicroscope

1: Pericardim 2: Myocardium 3: Endocardium 4: Erythrocyte 5: Interventricular septum 6: Purkinje cell 7: Aorta 8: Apex 9:Ventricular cavity 10: Elastic fiber 11: Atrium 12: Valve 13: Papillary muscle

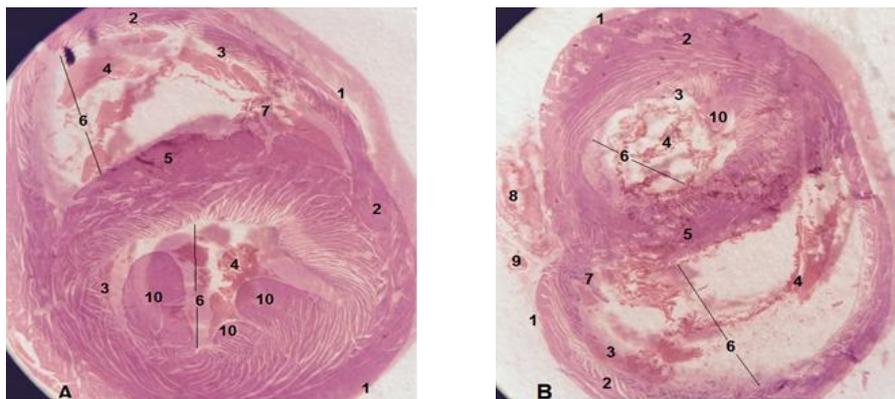
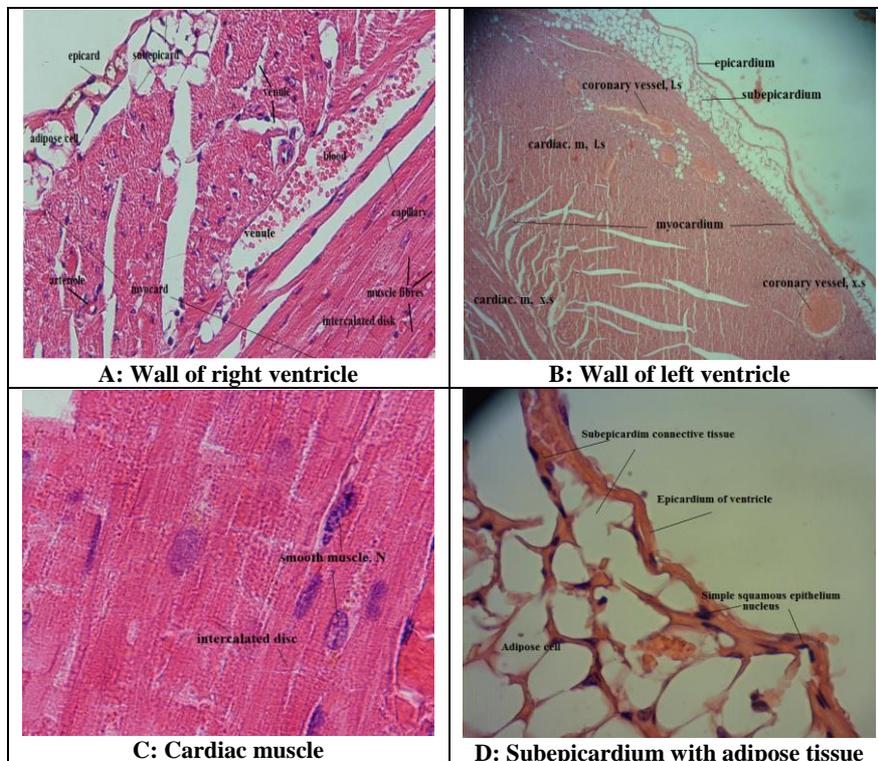


Fig 8 A: Right & **B:** Left chambers in *R. aegyptiacus* by stereomicroscope

1: Epicardim 2: Myocardium 3: Endocardium 4: Erythrocyte 5: septum 6: cavity of ventricle and atrium 7: Valve 8: Pulmonary vein 9: Coronary vessel 10: Papillary muscle



A: Wall of right ventricle

B: Wall of left ventricle

C: Cardiac muscle

D: Subepicardium with adipose tissue

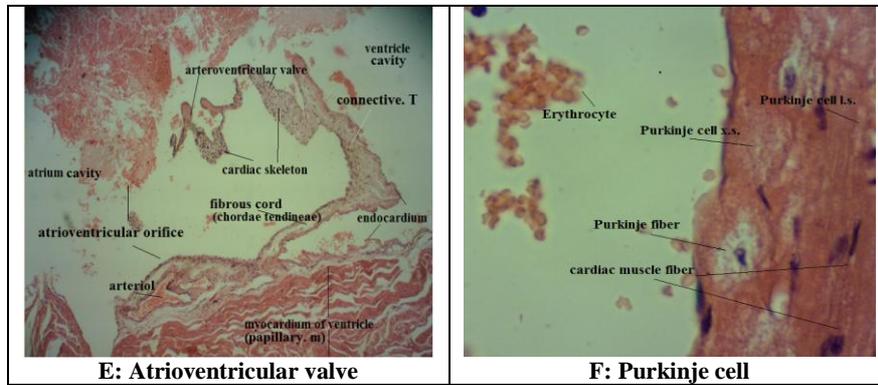


Fig 9: Heart tissue in *R. aegyptiacus*

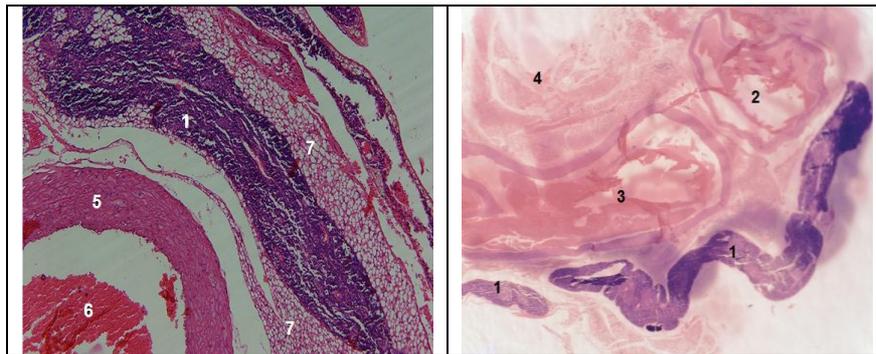


Fig 10: Lymphatic tissue around the large heart vessel in *R. aegyptiacus*

1: Lymphatic tissue 2: Posterior vena cava 3: Anterior vena cava 4: Right atrium 5: Media of aorta 6: Blood in the cavity of aorta 7: Adipose tissue

4. Discussion

Bats have a much higher metabolic rate than the non-flying mammals [14], so differences in morphology and physiology of their organs are expected. Although all of specimens were adult male, but the factors such as age, excess of fatty tissue or emaciation, even climate adaptation [15], and other ecologic condition [16] can't be ignored.

In this species, heart has been inscribed by lungs, closed to the ribs, and turned to the left side of body. Possibly this location of heart, aids to create more protection during flight. The large lungs and heart filled the small thoracic cavity, so heart mobility is restricted.

The presence of high adipose mass, abundant moderate bands and thick wall of heart cause heaviness of heart. [17] Concluded that exercise cause cardiac hypertrophy via increasing myocardial cell mass and wall tension myocardiocyte, and also they expressed that the weight of heart will lost, if exercise is not continued.

The size of heart in *R. aegyptiacus* is large since the body size of it is large (Fig 1, Table 1), but heart weight/body weight ratio in this species was larger (1.74%) than in non-flying mammals with the same size (about 0.6%) [9]. The heart ratio in active animals is higher than others, and small warm-blooded animals have much higher resting pulse rates than large ones [9]. It is obvious that warm-blooded animals needs to more quantities of energy at rest to maintain body temperature, because they loss heat through the body surface, and the small ones have higher resting pulse rates [17, 3]. In addition, cardiac work depends on expulsive volume and pressure, so bats with a relatively great cardiac output due to flight, have a large heart [18, 11]. Also a high heart ratio due to high heart rate was reported for bats spatially the smaller species.

A mass of fat in the apex of heart and its contact to

sternopericardium ligament may be support the heart when bat hung upside down or flight.

The cavity of right atrium is larger than left atrium. Previous study showed that increase in venous blood return to the heart during flight, affect on right side of right ventricle and atrium cavity. Large chambers in right side (table 1) are suitable for blood storage, and helps to the better and faster return of blood to the heart in flight and upside down resting in bats. Also smooth wall of right atrium (Fig 8) may reduce friction in the diastole [17]. Papillary muscles in the right ventricle, anterior, posterior and septal papillary muscle, is larger and more than left ventricle, anterior and posterior papillary muscle. Even though this finding was accordance to the some species of bat [19], but the number of every type of papillae is different in mammals [20, 21]. The papillary muscle in this species attach to mitral and tricuspid valves via the chordae tendineae [22]. The moderator bands are as the pathway for the passing of purkinje fibers and help to conduct impulses, so the presence prominent moderator band in the ventricles of this species is very important in cardiac output of them (Fig 7A). The relationship of this muscular band and purkinje fibers which lies on ventricular wall and interventricular septum aids them to high cardiac output [23, 24]. In addition thick myocardium of ventricle is important on strong systole during flight, and wide intrventricular septum help to rapid synchronize whole the heart.

Possibly large size of conus arteriosus aid to create strong contraction in ventricle, and also smooth wall of right ventricle reduce friction and facilitate to pump the blood to the lungs [6].

The abundant fat cells in subepicardium of ventricles (Fig 9A, B, D), is consistent with the findings of [2]. The excess fat accumulation in their heart functions as the main depot for fuel storage in the intense activity, hibernation and

thermoregulation spatially in small mammal [25]. Therefore, these fat cells related to high metabolism and adaptability of this species to fly. These cells provide energy for abundant purkinje cells (Fig 9F) that play important role in the cardiac beat. Many Purkinje cells were also observed in contact with the fat cells which provide energy for the cells that play a major role in the transfer of beats [26]. Bats have active life, and considering the high metabolism of this species and its high energy requirement, most of these changes are to ensure the adequate oxygen delivery to the tissues of the body [27, 28]. High density of capillaries in ventricle especially right side (Fig 9A) helps them to provide high energy for flight, predation and other action. Thin muscle fibers in the heart increases the strength of muscles and make them stronger to maintain rapid conduction of cardiac impulse [29], and the presence well developed cardiac conducting system and cardiac skeleton (Fig 9E) is necessary during flight [9]. The presence of many small veins with very thin walls, especially around the upper and lower large veins (Fig 9A), plays a basic role in receiving blood from the capillary network of various organs. The thick lymphatic tissue around large artery and veins (Fig 10) contributes to the lymph transfer largely.

5. Conclusion

According to the obtained results of this research, the structure of heart, especially left side, in Egyptian fruit bat follow the general plan of this system in other mammals, and some changes in the heart structure due to a greater need for oxygen in this species. Therefore, a better flow of blood is achieved with more volume of heart, more beat, and every factor that aid to better cardiac output. Understanding these differences and similarities enrich knowledge of wildlife to protect them.

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