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Full Length Research Paper

Comparative biochemical studies of the pregnant and non pregnant uterine limbs of the frugivorous bat, *Eidolon helvum*

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Biochemical differences existing in the two uteri limbs during the pregnant state in the fungivorous bat *(Eidolon helvum)* were investigated. A total of twenty female pregnant bats were studied. They were harvested on the Obafemi Awolowo University Campus at different times to assess the different stages in their gestation and uterine cycle. The animals after being carefully assessed screened and confirmed to be presumably healthy were sacrificed by cervical dislocation. Abdominopelvic incisions were made on them to expose and excise their uteri. The pregnant and non pregnant uteri were divided into four parts; the right uterine limb, the left uterine limb, the placenta, and the uterine body. The different parts were assayed for their total protein concentrations, Lactate dehydrogenase (LDH) and Glucose-6-Phosphate dehydrogenase (G-6-PDH) activities. This study has shown that the uterine limbs actively utilized different metabolic pathways according to the functions they subserve during pregnancy. The pregnant limb utilizes the Embden Meyerhof pathway more while the non pregnant limb utilizes the Hexose Mono Phosphate Shunt actively.

Key words: *Eidolon helvum*, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, total protein, uterus.

INTRODUCTION

The African Straw Coloured Fruit Bat or African palm tree fruit bat is present in Africa, Asia (the Arab Peninsula), in the southwest of the Arab Peninsula (Donald et al., 2005). The males are slightly larger than the females. The head and body length is reported to be between 143 and 215 mm. Weights range 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 (Happold, 1987). The classification of *Eidolon helvum* is as shown in Table 1. Every living cell is known to undergo metabolic pro-cesses. This is carried out via different conventional pathways involved in the breakdown of glucose to release energy in the form of ATP which the organisms utilize for its daily activities (Ofusori et al., 2007). Since only one of the uterine limbs is involved in pregnancy in *E. helvum*, there is anticipation that the two uterine limbs might not be utilizing the same pathways for their metabolic processes

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Table 1. Showing the scientific classification of fruit eating bat (*Eidolon helvum*)

CLASSIFICATION	
Kingdom	**Animalia**
Phylum	Chordata
Subphylum	Vertebrata
Class	Mammalia
Order	Chiroptera
Suborder	Megachiroptera
Family	Pteropodidae
Species	*Eidolon helvum* (Kerr, 1792)

during gestation, hence the need to investigate the conventional pathways adopted by the two uterine limbs using maker enzymes such as LDH and G6PDH. LDH converts pyruvate into lactic acid during anaerobic metabolism while G-6-PDH converts glucose-6-phosphate to 6-phosphogluconic acid during direct oxidative pathway of carbohydrate metabolism. Information on the mating system of these animals is somewhat scanty. Pepe and Yochim (1971) showed that follicular phase of the estrous cycle is a period of increased hyperplasia and increased synthetic activity in the endometrium G6PDH. Recently Odukoya (2008) found from their investigation that the two uterine limbs are different histologically during pregnancy. Mating occurs in colonies from April - June. The reproductive cycle responds to rainfall and allows weaning of young to proceed at the time of greatest food availability. Pairs breed when the dry season begins. There is a delay in the implantation of the embryo in most, but not all, populations. The gestation period typically lasts 9 months, but the embryo only takes 4 months to develop. In populations without delayed implantation, births occur just 4 months after mating (Nowak, 1997; Smithers, 1983).

The young are born in February and March. Female gives birth to a single offspring that weighs 50 g at birth (Nowak, 1997). The straw coloured fruit bat has female parental care, like all other mammals. The female nurses her offspring until it is ready to forage on its own. In this species, young are not able to fly at birth and so are considered altricial. Although females give birth to their young in large colonies, there are no reports of cooperative care of young, or of paternal involvement in care of offspring (Ruiz, 2002; Nowak, 1997; Mutere, 1980).

Previous investigation by this same author has shown that pregnancy in the Eidolon helvum can either be in right or left uterine limbs (Odukoya, 2008). We therefore wish to continue our investigation by verifying the activities of Lactate dehydrogenase, Glucose-6-Phosphate dehydrogenase and total protein concentrations in the right uterine limb, the left uterine limb, the placenta and the uterine body during the pregnant state.

MATERIALS AND METHODS

Care of animal

Twenty pregnant female bats were harvested on the Obafemi Awolowo University Campus, Nigeria at different times between April and June, 2006 to assess different stages in their reproductive cycle. The bats were brought and kept in cages in the Animal Holdings of the Department of Anatomy and Cell Biology to acclimatize before sacrifice. The bats were fed with ripe bananas and water. The animals were carefully assessed, screened and confirmed to be presumably healthy. All animals were treated in accordance with the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health.

Excision of the abdominopelvic region

The animals were sacrificed by cervical dislocation after pentobarbital 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.

Biochemical assay techniques

The uteri from the animals were divided into four parts; the pregnant limb, the non-pregnant limb, the uterine body, and the placenta. The tissues were passed through a mincer. Two Molar phosphate buffer (PH 7.2) was added to the minced tissues and then homogenized for about I minute in a Potter Elvejhem Teflon-type tissue grinder, driven by a TRI-R instruments motor model K43. The homogenate was then put in a Gallenkamp bench centrifuge and centrifuged for 5 min at top speed to remove cell debris. The clear supernatant obtained was used for total protein estimation and enzyme activity determination.

Total protein estimation

The protein concentrations of the different parts were determined by the Biuret method of Gornall et al. (1949) using bovine serum albumin as standard protein. The absorbance was taken at 540 nm in the photo spectrometer and the mean absorbance values were used to calculate the total protein concentration in the different parts of the uterus and the placenta.

Lactate dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH)

Biochemical kits for lactate dehydrogenase assay were purchased from PPC Pharm-tec GmbH, Germany. The Biochemical kits for Glucose-6-Phosphate Dehydrogenase assay was purchased from BIOLABO Reagents, France. The measurements were made according to the manufacturer’s specifications to determine the activities of LDH and G-6-PDH in the different parts.

Statistical analysis

Values were reported as mean ± S.E.M and data were analyzed using student’s t-test using the statistical software STATISTICA VERSION 5. $P < 0.05$ was considered to indicate a significant difference between groups.

RESULTS

Biochemical analysis was carried out for lactate dehydrogenase, glucose-6-phosphate dehydrogenase and total protein estimation. The protein concentration was highest in the placenta and lowest in the uterine body. It was slightly higher in the non pregnant uterine limb than in the pregnant limb (Figure 1). LDH activity was highest in the placenta and lowest in the uterine body. The higher LDH activity observed in the non pregnant limb over the pregnant limb was rather insignificant statistically (Figure 2). G- 6-PDH activity was highest in the placenta and lowest in the uterine body, the activity was higher in the pregnant limb than in the non pregnant limb (Figure 3).

DISCUSSION

Generally, it was evident that more metabolic activities occurred in the placenta than in any other part of the uterus during pregnancy (Figure 1), this was clearly evident from the total protein estimation carried out, which was consistent with the highest rates of metabolic activities observed in the placenta throughout the studies. The pregnant limb utilized the Hexose-mono-phosphate Shunt more than the Embden Meyerhof pathway as a higher significant activity was observed in the pregnant limb for Glucose-6-phosphate dehydrogenase analysis (Figure 3).

The Hexose-mono- phosphate Shunt is a cytosolic process that serves to generate NADPH, which prevents oxidative stress and supports the synthesis of pentose (Rendall et al., 2005; Raupp et al., 2001; Gaskin et al., 2001; Mehta et al., 2000) while the Embden Meyerhof pathway is a catabolic pathway responsible for the generation of high energy molecules (ATP and NADH) as cellular energy sources, a part of aerobic and anaerobic respiration; production of pyruvate for the Citric Acid Cycle; and production of a variety of 6 and 3 carbon intermediate compounds, which may be removed at various stages in the process for other cellular purposes (Bustamante and Pedersen, 2005; Selig et al., 1997; Romano and Conway, 1996).

It is relevant to observe that the non pregnant limb was metabolically very active during pregnancy, actively utilizing the Embden Meyerhof pathway upon which the Pentose Phosphate pathway also depends as a source of metabolite, even more than the pregnant limb (Figure 2). This suggests that the non pregnant limb is some how involved in the maintenance of pregnancy.

Moreover, the glycolytic pathway continues to function actively under both aerobic and anaerobic conditions, ensuring maximum and continuous protection and care of the developing fetus. The Hexose-Mono-Phosphate Shunt is an alternative pathway to glycolysis, and its primary role is anabolic rather than catabolic despite the oxidation of glucose.

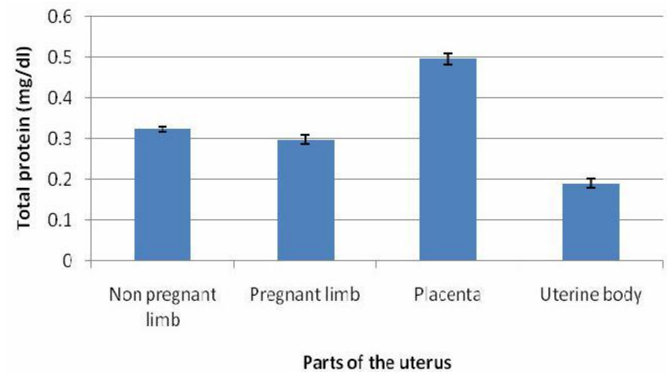

Figure 1. Total protein estimation (Mean ± S.E.M).

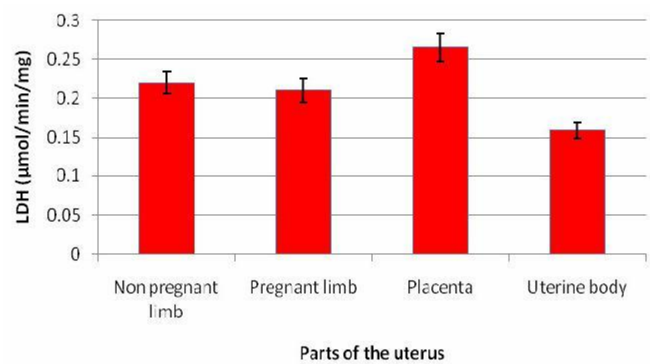

Figure 2. Lactate dehydrogenase (LDH). (Mean ± S.E.M).

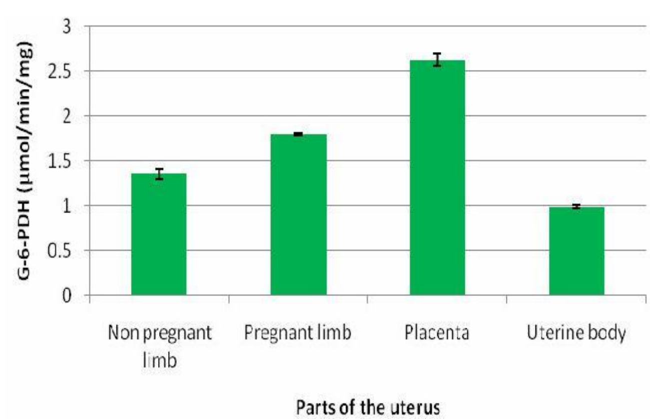

Figure 3. Glucose-6-phosphate (G-6-PDH) dehydrogenase (Mean ± S.E.M).

The anabolic process is directed at the production of the pentose sugars used as precursors of nucleotides which are monomers of nucleic acids. Nucleotides are the struc-tural units of DNA, RNA and Co-factors such as CoA, flavin adenine dinucleotide, flavin mononucleotide, ATP and NADP – having important role in cell metabolism and signaling, (Yamagashi and Shimabukuro, 2007). This implied that most of the activities going on in the pregnant limb were directed towards development, growth and cell differentiation in the fetus.

This study showed that the uterine limbs actively utilized different metabolic pathways according to the functions they subserve during pregnancy. The pregnant limb utilizes the Embden Meyerhof pathway more while the non pregnant limb utilizes the hexose mono phosphate shunt actively.

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