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Original Research Article

Reactive oxygen species production and antioxidant enzyme activity during epididymal sperm maturation in *Corynorhinus mexicanus* bats

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ABSTRACT

Prolonged sperm storage in the epididymis of *Corynorhinus mexicanus* bats after testicular regression has been associated with epididymal sperm maturation in the caudal region, although the precise factors linked with this phenomenon are unknown. The aim of this work is to determine the role of reactive oxygen species (ROS) and changes in antioxidant enzymatic activity occurring in the spermatozoa and epididymal fluid over time, in sperm maturation and storage in the caput, corpus and cauda of the bat epididymis. Our data showed that an increment in ROS production coincided with an increase in superoxide dismutase (SOD) activity in epididymal fluid and with a decrease in glutathione peroxidase (GPX) activity in the spermatozoa in at different time points and epididymal regions. The increase in ROS production was not associated with oxidative damage measured by lipid peroxidation. The results of the current study suggest the existence of a shift in the redox balance, which might be associated with sperm maturation and storage.

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1. Introduction

Males of some species of *Rhinolophidae* and *Vespertilionidae* families show a significant temporal asynchrony in sexual organs development and function [1]. While the development of testes and spermatogenesis take place in the summer, the maximum development of accessory sex glands and mating occur in the autumn. This asynchrony is due to the unusually long period of sperm storage in the cauda epididymis, which may extend over several months after spermatogenesis has ceased and the testes have completely retracted [2]. Twenty years ago it was proposed that the prolonged sperm storage was a natural consequence of physiological lethargy associated with hibernation [3]; however it is now known that several bat species inhabiting tropical regions do not hibernate and yet exhibit prolonged sperm storage in the epididymis [4]. Nevertheless, the reasons for the prolonged epididymal sperm storage are currently still unknown. This phenomenon is interesting because a number of key physiological sperm capabilities required for fertilization, such as the ability to move, to undergo capacitation, and ability to interact with oocyte zona pellucida, develop gradually as spermatozoa progress from the caput to the cauda region. These changes are species-specific and altogether are known as epididymal sperm maturation process. In most species, including humans, this process occurs over a period of 10–20 days [5].

Generally, sperm maturation takes place in the caput and corpus regions of the epididymis. By the time the spermatozoa reach the caudal region, they are already mature (*i.e.*, they eliminate the cytoplasmic droplet and are fully capable to achieve capacitation and acrosome reaction) [5]. However, the reproductive pattern of the vespertilionid bat, *Corynorhinus mexicanus* (G.M. Allen, 1916) is atypical, since sperm maturation requires an unusually long time. This implies longer sperm storage in the caudal region and it is very related to climatic and seasonal conditions. For example, sperm storage at the epididymis starts on September, while in October sperm production is suspended, but sperm still remain viable until the copulation season, which takes place in November [6,7]. A problem that sperm have to face during this process is ROS generation during their journey through the epididymis, which along with a decrease in antioxidant enzyme availability might alter spermatozoa structurally or functionally. It is known that mammalian spermatozoa are highly susceptible to ROS negative effects such as DNA, protein and lipid oxidation [8]. Oxidative damage may also reduce fertilization capacity and genetic integrity [8]. In humans, ROS generation and oxidative stress have been associated with decreased fertility or with infertility [8]. Despite these potentially adverse effects, regulated ROS production, mainly in the epithelium of the epididymal tubule [9], is necessary for sperm maturation. In fact, redox modulation is necessary to regulate phosphorylation and dephosphorylation events [10] required to activate flagella proteins involved in motility, as well as in oocyte fertilization [11].

Oxidative stress is defined as the imbalance between ROS production and the activity of antioxidant enzymes to eliminate them [12]. Hence, antioxidant enzymes that protect spermatozoa during their transit through the epididymis are essential to produce functional sperm. It has been shown that three enzymes participate in sperm redox regulation: glutathione peroxidase (GPX) [13], superoxide dismutase (SOD), and catalase (CAT) [14]. Since ROS are both necessary and harmful to spermatozoa, the redox balance in the epididymis duct must be tightly regulated. Therefore, the aim of the current study was to study the relationship between sperm ROS production and primary antioxidant defense system capability (*i.e.*, CAT, GPX and SOD), in order to determine sperm protection during the prolonged maturation time and the epididymal storage period in the bat *Corynorhinus mexicanus*.

2. Materials and methods

2.1. Chemicals and animals

Unless otherwise stated, chemicals were purchased from Sigma (St. Louis, MO, USA). Animal capture and handling were conducted in accordance with the guidelines of American Society of Mammalogists for the use of wild mammals in research [15]. To date, the bat *Corynorhinus mexicanus* is not included in any category of threatened animal species in the Mexican Official Standard or in the NOM-059-SEMARNAT-2010 for native wild species protection in Mexico [16]. This protocol was approved by the Ethics Committee of the Biology and Health Sciences Divisional Board (Universidad Autónoma Metropolitana-Iztapalapa in Mexico City). The capture and sampling were carried out in two consecutive years (2006–2007) in order to minimize the ecological impact of removing adult individuals from the study population.

Bats were captured inside a tunnel in the Mexican state of Tlaxcala, Central Mexico (19°37′14″ N, 98°02′02″ W; 3220 m altitude). All captures were done before the animals left the roost (12:00–16:00) using funnel-shaped basket traps. The body weight of each bat was determined using an Ohaus® portable electronic balance (±0.01 g), and forearm length was measured with a Vernier caliper (±0.1 mm). A total of 26 adult male bats were included in the study. Small groups of two or three animals were picked every fortnight during the full spermatogenesis-epididymal sperm maturing and storage period (July 31–October 30), which precedes the mating period [1].

To avoid any negative effects on sperm quality associated to aging, only 1–4 years old animals were included to the study. The criteria to classify the animals as adults were as follows: 1) Pelage color pattern, there is a color change during adulthood, and the brown pelage at the posterior base of the large pinna becomes paler than in the rest of the dorsum [17]; 2) average forearm length (41.0 mm, range 39.7–43.1 mm); 3) average body weight, which varied depending on the month, in September was 7.7 g ± 0.2 and in October was 7.3 g ± 0.2 [1,17]; 4) totally ossified epiphyses; 5) teeth with little to moderate wear [18–21]; 6) enlarged inguinal testes, which was mainly observed during full spermatogenesis in July–August; and 7) elongated epididymides with bulbous whitish caudal region extended into the interfemoral membrane during the sperm maturation and storage period [1]. The selected animals were transferred to the laboratory in cotton bags (kept wet), hydrated upon arrival and held for a period not exceeding 24 h.

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