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Enzymes Involved in Epididymal Function of Corynorhinus Mexicanus Bat

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Abstract

In the *Corynorhinus mexicanus* four enzymes related to Reactive Oxygen Species (ROS) modulation are present in the cephalic and caudal region of the epididymides during the progress of the epididymis sperms and their maturation, but interesting to say the activity of the Super Oxide Dismutase (SOD) is not present or perhaps it is inhibited in both epididymal segments in the post testicular phase of the sperm storage function. The SOD is present during the period of maturation and during the transport of the sperms, but it is almost totally inhibited or not present in both epididymal segments during the storage, as well as an absence in the sperm cells SOD activity. It is interesting to observe that the Gamma Glutamyl Transpeptidase (GGT) and Glutathione PeroXidase (GPX) presents a complete pattern contrary to that of the SOD; its activity is low during the period of production and maturation of the epididymal sperms, but present an important activity in both segments of the epididymis during the storage phase. The activity of CATalase (CAT) is preserved relatively high during the whole reproductive cycle indicating its importance in the protection of the spermatic cells against the effect of Hydrogen peroxide (H_2O_2). However it is important to mention that its activity is significantly higher in the tail of the epididymis during the maturation and particularly during the storage process. In sperm from the cephalic and caudal region, the CAT activity reaches its highest levels at the beginning of September and the lowest at the end of the same month. In the epididiymal fluid the CAT activity shows a similar pattern in the cephalic region from that found for SOD and GGT/GPX in both regions. The redox balance associated with the microenvironment, through which the sperms pass, must be specific and differentially controlled to assure its adequate function.

Keywords: Epididymal sperm; Reactive Oxygen species; Superoxide dismutase; Gamma Glutamyl Transpeptidase; Glutathione peroxidase; Catalase; Bat reproduction

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Introduction

The reduction of the environmental temperature during the winter season provokes a reduction in the insect populations which are the basic food for most of the chiroptera species inhabiting in cold areas. For this reasons some species are forced to migrate to areas with more favorable climate conditions and only certain species are adapted to respond to

these changes executing a behavior of physiologic lethargy [1]. In the family's Vespertilionidae and Rhinolophidae, the reproduction season extends until the winter torpor period, when the lethargy has a marked influence on the reproductive physiology of the individuals, characterized by a temporary asyncrony among the sexual functions [2, 3].This asynchrony results in an unusual long period of storage of mature spermatozoa in the epididymides, which may extend for several months after the testes have totally regressed [3].

The prolonged epididymal spermatozoon storage becomes more interesting if we keep in mind that some of the most important physiological properties of mammalian spermatozoa that are necessary for fertilization develop gradually as they progress from the caput down to the cauda regions of the epididymides. These functional changes are known as epididymal maturation.

During their epididymal journey, spermatozoa are seriously at risk. Mammalian spermatozoa have been described as highly susceptible to the negative effects produced by ROS [4, 5]. Despite these adverse effects, production of regulated concentrations of ROS by the spermatozoa themselves and (or) by the epididymal environment is required during the epididymal maturation of the spermatozoa to achieve complete functional competence. Generation of adequate ROS levels has been related to important process in the development of coordinated spermatozoon movement [6, 7] and in the ability of undergoing spermatozoon capacitation [8].

Oxidative stress can be regarded as the consequence of an imbalance between ROS-generating systems and the presence and activities of antioxidant enzymes. For these reasons antioxidant strategies that protect spermatozoa during epididymal transit are of great importance in ensuring the capacity of these cells to fertilize the oocyte. In general, mammalian epididymal spermatozoa contain important enzymatic mechanisms. Some enzymes have been proposed as being important in this function: enzymes related to glutathione mainly GGT (EC 2.3.2.2) and GPX and their isozymes (GPx, E.C. 1.11.1.9; PHGPx; E.C.1.11.1.19) [9, 10]; SOD (E.C. 1.15.1.1) [11]; and CAT (E.C. 1.11.1.6) [12-14].

Free radicals and Reactive Oxygen Species

All the atoms and molecules are formed by small well-known particles such as subatomic particles; on one hand, the protons and neutrons, and on the other, the electrons that turn around the core through "orbitals".

Something that should be taken into account is that, you can never have more than two electrons in the same orbital, in this way, the global distribution of electrons of an atom or molecule is the sum of many similar orbitals. When an orbital field is full the pair of electrons should turn anti-clockwise (spin), to eliminate its magnetic field, but this doesn't always happen, when in an orbital there is an electron it is said that it doesn't match up, and in this, when an atom or molecule has one or more unpaired electrons it receives the name of free radical [15].

The molecular oxygen (O_2) is a bi radical, having two unpaired electrons of parallel spin. The molecules that are not radical possess electron pairs with opposed spines, and in this way, both electrons can be accepted. However, in the particular case of oxygen this is possible but a little rare, because the reactions which involve oxygen are regularly univalent, accepting a sole electron. The product of this univalent reduction is that called superoxide (O_2^-), a molecule that alone generates another ROS, the H_2O_2, is the last, for on the other hand, it is not a free radical it is considered also part of ROS, because it is produce fundamentally by the divalent reduction in value of O_2 and will give it origin another free radical capturing an electron and a proton, other ROS principals, that named ion hydroxyl (OH^-).

The O_2^- is produced in vivo constantly in small quantities, mainly from the mitochondrial respiratory chain, because contains various redox centers which take the oxygen electrons, constituting in this way the principal sources of O_2^- in most of the tissues [16].

The produced ROS in this way are usually related with toxic products and are involved in the cause and sicknesses and ageing [17]. Some cells have other mechanisms for producing ROS with physiological purposes, such as the phagocytic leukocytes which contain a type of membranal enzyme called: NADPH oxidase, the same that O_2^- produces in phagocytic blisters during "the reaction of death" [18, 19]. However, among the cells involves in the reproductive aspects and that produce ROS with physiological ends we find spermatozoa.

Reactive Oxygen Species and sperm cells

Many years ago it has been shown that sperm of different species can produce H_2O_2 by an oxidase that acts on the aromatic L-amino acids. Later [20] proved that rabbit spermatozoids are capable of producing H_2O_2 by dismutation of O_2^- resulting from the activity of the SOD [21].

On the other hand has been observed that, the extracellular addition of NADPH can pioneer the sperm capacitation due to H_2O_2 formation that has related with the tyrosine phosphorylation, and that the production of O_2^- can be maintained with the addition of NADPH or NADH, and interrupted by flavoproteins inhibitors, in the same way as what happen with NADPH oxidase in leukocytes, observing more that this production was not affected by mitochondrial inhibitors or by diaphorases (enzymes which can be found mainly in: cytosol and spermatozoa membranes, transferring electrons from NADH or NADPH to an electronic acceptor similar to 2,6-diclorofenol indophenol, the same which play an important part in the ROS formation), and the effect of NADPH was localized in the membranal fraction [22].

These observations were also corroborated even more by the NADPH membrane oxidase activity that later on is revalidated by the work of [23] which informs that the gene of NADPH oxidase, member of the NOX5 family, is expressed in primary spermatocytes of humans. However, the appearance of this enzyme has not been confirmed in mature spermatozoa.

In this way, the production of H_2O_2 by spermatozoa, is not only the result of the NADPH oxidase activity, because it has been confirmed that it depends also in a big way on the mitochondrial activity [8], and inclusive, it has shown that what has been reported about the rat epididymal sperm, where the NADPH oxidase activity occurs as the primordial source of ROS, is in reality the product of the Cytochrome-B5 reductase activity localized in epithelial cells which could have infected the spermatic suspension [24, 25]. This finishes with the majority of the evidences concerning the production of ROS given that the NADPH oxidase activity in rodents epididymal sperm, and centers the attention on the mitochondria as the principal sources of ROS.

Damage caused by Reactive Oxygen Species to spermatozoa

The oxidative stress is produced when a cascade of intracellular events generate which can result in the adaptation or in the cell damage. In the first of these cases, it is necessary to find the positive regulation of the elements that form a part of this anti-oxidant defense system, this is, in an attempt to restore the oxidant/anti-oxidant balance of the cell. However in the second of these cases, the oxidative stress can provoke injury or inclusive death [26, 27].

From the first reports about the adverse effects of ROS to the spermatozoa [28], up to this date, a large amount of studies have been published to this respect, accepting totally the overproduction of ROS in a sperm suspension, this will be associated with problems in the sperm function and the subfertility, due overalls to the injury membranes (lipid per oxidation) and DNA oxidation [29, 33].

The exposition of human spermatozoa in ROS induces the loss of its motility, this event can be related directly with the lipid per oxidation [34], due probably to the changes of the permeability and integrity of the membrane and successively to the problems to maintain a flagella movement, added to this, the lipid peroxidation destabilizes other dependent sperm functions of the membranal integrity, among these are: the capacitation,

the acrosomal reaction and the fusion of the sperm with the oocyte [35, 36].

It has been observed in human and hamster sperm that the effect provoked by ROS to the DNA includes: oxidative damage in its nitrogenous bases (modification and/or the suppression), rupture of the DNA chain and chromosomal re-arrangement. These damages have been observed, in both types of DNA: nuclear and mitochondrial [37].

Antioxidant enzymes in the epididymis

The sperms obtained from the testicle are not capable of exhibiting progressive motility neither to capacitate, being they acquire this ability while they pass through the epididymis, this process is known as sperm maturation. Other relative changes in the maturation, include the termination of the nuclear condensation and changes in the expression and distribution of the sperm surface molecules [38, 39], events that occur during the time they remain in this organ.

The time taken for the sperms to cross the epididymis, have been studied in humans, labeling these cells with thymidine, where time was estimated between 1 and 21 days (Table 1) [40]. However, in the case of various bat species Vespertilionids and Rinolofidos these show a temporary asyncrony in the development and the function of the male reproductive organs [2,41]; where particularly, the spermatogenesis develops in summer mean while the maximum development of the sexual accessory glands, the libido and the mating occur in autumn; this asynchrony results in an unusually long period of sperm storage in the epididymis, this can extend for several months after the testicles have involved totally [3].

ROS plays an important part in the maturation regulation, being that while the sperms acquire the capacity to move, these suffer an amount of changes obtaining their fertilizing capacity [47]; this is, due to an increment in the AMPc synthesis and to the phosphorylation/dephosphorylation events that can be found principally in the proteins of the flagellum [22, 48, 49]. The tyrosine phosphorylation in the cells, are pioneered by the union of ligands with its receptor; many studios have suggested that the stabilization of the sperm structure during the epididymal maturation, is completed principally by the oxidation of the thioles groups during its transit throughout the epididymis [50, 51]. In the same manner, the capacity of the spermatozoids to suffer tyrosine phosphorylation also is increased during the transit of the sperms, from the cephalic region until the caudal region of the epididymis [52, 53].

Table 1. Storage sperm time (days) in the epididymis of different species

Specie	Time to storage sperm in epididymis (days)
Corynorhinus mexicanus	180
Human	4
Rhesus	11
Pig	7
Rat	11
Mouse	9
[42-46]	

In some cellular types the increase in the tyrosine phosphorylation was suggested as a result of the oxidation and direct activation of the kinase proteins [54, 55]. However, it has been observed that the active center of the phosphatase proteins contains a large amount of residues of cysteine for the phosphatase activity [56, 57], in this way, the inhibitory effects of the oxidants on the phosphatase activity has been considered the most probable mechanism and indirectly responsible for the tyrosine phosphorylation [57, 58]. And from there it is greatly important to maintain a balance between the cellular production of ROS and its destruction.

Of the components in charge of the regulation of ROS, the most important can be found, the enzymes: SOD, CATalase (CAT), and enzymes related to glutathione mainly gamma glutamyl transpeptidase and Glutathione PeroXidase (GPX).

Superoxide dismutase: The SOD is expressed at high levels in the epididymis, and doesn't vary significantly in its different regions [59]. In the caudal region of the epididymis, can be related with the protection of the sperms against the oxidative stress, and associated with the plasmatic membrane of the

epididymal sperms, being able to promote the production of H_2O_2 that would be participating in the maturation associated with the tyrosine phosphorylation [52].

In this way, the prolonged storage of sperm in bats appears to be more interesting if we take into account that the maturation develops gradually in a specific way, when the spermatozoa advance from the cephalic region until the caudal region of the epididymis; completing its maturation regularly, before reaching the caudal region, after that they are stored until ejaculation.

Catalase: The participation of CAT in the epididymis, is one of the most controversial, in that although it has shown expressions at very low levels in human and rat sperms [60, 62], this, is absent in rabbit sperm [20], mouse [21] and bull [14]; but it is present in the spermatic environment during its transit through the epididymis and in the ejaculation [14, 61, 63] finding that the expression patterns of this enzyme, involves not only the epididymal epithelium, but also other tissues of the masculine reproductive tract with the exception of the seminal blisters [11]. However, the expression of CAT mRNA is so low that its part in this enzyme is undervalued and as an anti-oxidant in these tissues.

Gamma Glutamyl Transpeptidase

GGT is present in the epididymis, principally in caput [64]. The catalytic activity of GGT is highest in the proximal epididymal regions and decreases toward the distal regions [65, 66].

Once the spermatozoa are formed during spermatogenesis and pass to the epididymis, they will carry on morphological and biochemical changes known as epididymal sperm maturation [67], i.e. the potential to acquire rectilinear and vigorous movement, to interact with zona pellucida and to fertilize the oocyte [52].

During sperm maturation, in addition to a major role as an antioxidant and in eliminating toxic compounds, GSH has been implicated in pro oxidation processes in various cells, via GGT dependent catabolism [68, 69]. Modulating effects of GSH catabolism have been observed on components of signal transduction pathways, such as those involving cell surface receptors and factors within the sperm cell itself and/or in the epididymis may control Sperm Protein Thiol (SPT) oxidation [68, 70].

In most mammals, epididymal sperm maturation takes place in a period not exceeding ten days, ending in the distal part of the corpus of the epididymis, before reaching the caudal region, which is responsible only for sperm storage (Table1) [42-46].

Glutathione peroxidase: From the GPX a large number of isoforms have been found, of these, those that are related with the epididymis are: GPX1, 3, 4 and 5 [71]; where GPX5 has been localized in the epithelium of the epididymis and in the sperms [72], have being secreted towards the epididymal lumen, where they can be found in a free form or associated with spermatozoa, this last, increasing as it advances from the cephalic region to the caudal region of the epididymis, remaining until the feminine reproductive tract [73-75].

The activity of GPX1 is related principally with the H_2O_2 regulation or terbutyl hydropexodide in total extracts of the epididymis, and GPX3 also is expressed in epididymis, although being in the cephalic region its expression very low, not like that in of the caudal region (principal place of expression in mouse), where remains in the cytosol of the epithelial cells. This activity being the dependent enzyme of androgens in the body region and tale of the epididymis, and independent from the cephalic region [76, 77].

GPX4 or PHGPX are present principally in the epididymal sperms, in the head and middle piece of many, where it exhibits a thiol oxidase activity of protaminas, being larger in these coming from sperms obtained from the cephalic region that those obtained from the caudal region, thus like this, they contribute to the nuclear condensation during the transit trough the epididymis, being that, the compactation of the cromatin constitutes one of the main ways in the maturation of the epididymal sperms [10, 78]. These data suggest a primordial paper in the sperm maturation during the part of GPX in the

metabolism of hydroperoxides and thiol oxidation, to sum up with its anti-oxidant participation.

Experiments carried out in the rat epididymis show that the cellular synthesis of the prostaglandin is partially regulated by hydroperoxides and intracellular levels, for these reason, PHGPX would be participating in this regulation by means of the reduction of the same [79]. Extremely important activity in the regulation of the maturation.

Enzymes involved in epididymal function of *Corynorhinus mexicanus* bat

The C. mexicanus bat male, reveal a single yearly reproductive cycle, and a temporary asyncrony shows between the reproductive functions, in that have a long period of sperm storage in the epididymis of up to four months [41,42]. It is important to underline, in general for mammals, the time that the epididymal sperm storage lasts, around 11 days [40]. In this way, some species that have longer period of spermatic storage, like *C. mexicanus* have a peculiar phenomenon, in that the sperms prolong for several months (Figure 1).

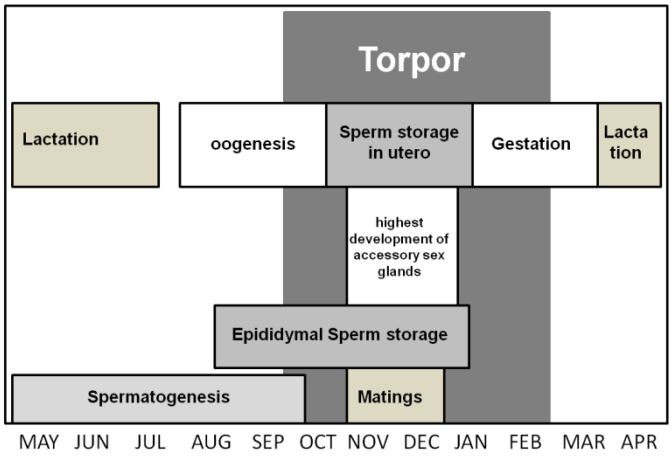

Figure 1: Major reproductive processes of *C. mexicanus* bat.

Initial intents to explain this important reproductive strategy, sustains the idea that the prolonged sperm storage could be a natural consequence of the descent of the corporal temperature in the hibernators. However, many tropical and sub-topical species exist in high areas that are not hibernating and they also present prolonged storage of sperms in the epididymis [80, 82]. In addition to this, some species of hibernating bats that store sperms, wake up regularly during this hibernation period in search of food [83] that implies dynamic changes in the metabolism and energy consumption that generate a great quantity of products among those are the ROS, same that take place inevitably during the physiologic processes that involve the oxygen consumption, normal in the aerobic organisms. However as has been said before, mammalian spermatozoa have been described as highly susceptible to the negative effects produced by ROS [4, 5].

Despite these adverse effects, production of regulated concentrations of ROS by the spermatozoa themselves and (or) by the epididymal environment is required during the epididymal maturation of the spermatozoa to achieve complete functional competence.

The production of ROS in the epididymal sperm of the *C. mexicanus* bat (Figure 2) indicates, in agreement with what has been said before this could be participating in the process of maturation of the spermatozoa in the cephalic region of the epididymis (Figure 2). However, the production of ROS remains in the spermatozoa obtained from the caudal region (Figure 2), even in the close dates from when the mating to place [84]. This could confirm the information reported by Cervantes and collaborators (2008), where they determined the presence of cytoplasmic drop and the sequential induction of capacitation and acrosomal reaction as indicated in maturation in sperms obtained from the different regions of the epididymis through the annual reproductive cycle of the *C. mexicanus* bat, where it can be found that, a high percentage of spermatozoa persists with cytoplasmic drop when arriving at the caudal region, including a low indication of capacitation and acrosomal reaction in spermatozoids obtained from the body that raises significantly during its stay in the tail; revealing that, the process of sperm maturation in the *C. mexicanus* bat, contrary to that reported in then general species of mammals, is completed in the caudal region of the epididymis, this could explain the necessity for the longer period storage of the epididymis.

Generation of adequate ROS levels has been related to important process in the development of coordinated spermatozoon movement [6, 7] and in the ability of undergoing spermatozoon capacitation [8]. Hence the involvement of antioxidant enzymes. The anti-oxidant enzymes SOD, CAT, GGT and GPX are present in the cephalic and caudal region of the epididymis, being possibly more important in the modulation of ROS in the processes of maturation and storage of spermatozoa (Figure 2) [42], it can be observed that depending on the reproductive cycle phase, two different activity patterns of the anti-oxidant enzymes were observed: CAT is active during the whole yearly cycle, having its activity particularly high during the post testicular phase; the SOD activity shows itself higher during the phase which coincides with the transfer and maturation of spermatozoa, being almost totally absent or inhibited in both epididymal regions in the spermatic storage; GGT activity and the activity of GPX is low during the testicular phase and the spermatic maturation and high in both epididymal segments, during the spermatic storage period (Figure 2) [42].

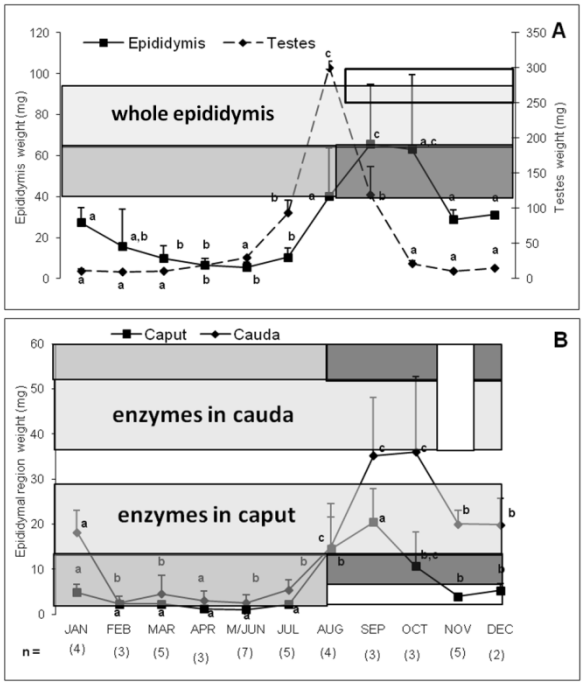

Figure 2: Testis weight (dashed line) and epididymal (solid line) (A); cephalic and caudal region (B)

from *C. mexicanus* bat; throughout their annual cycle, is marked with different colors the activity of different enzymes present in the epididymis (reported). Activity of CATalase (CAT, light gray); activity of Super Oxide Dismutase (SOD, dark gray); activity of Glutathione Peroxidase (GPX; black) and the activity of the Gamma Glutamyl Transpeptidase (GGT, white). Figure modified from Arenas-Ríos et al. 2005.

Conclusion

In addition to promoting sperm maturation and providing a place for sperm storage, the epididymis plays a role in the transport of spermatozoa along the duct and protects spermatozoa from harmful substances during its transport from the rete testis to the epididymal cauda. Many important tasks related to these processes appear to be under redox control. In *C. mexicanus*, redox equilibrium of the micro-environments associated with the milieus by which mammalian spermatozoa must progress during its transit throughout the epididymis seems to be specifically and differentially controlled in relation with the compartmentalization of epididymal functions. Our data on ROS related enzyme activities (GGT, GPX, CAT and SOD) stress the existence of a careful differentially regulated equilibrium between the activities of these enzymes in the cauda and in the caput epididymidis that seems to be specifically related to the precise maturation/storage function of the different epididymal regions.

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