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Special Article – Male Fertility

Protective Effect of Coenzyme Q_{10} in Mouse Testes Infected with *Staphylococcus epidermidis*

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Abstract

The abundant presence of saprophytes microorganisms in microbiological cultures has acquired interest in the study of infectious pathologies. The protective effect of coenzyme Q_{10} in mice testes infected with *Staphylococcus epidermidis* was evaluated. Sperm characteristics and testicular histology of male mice (NMRI) infected with *S. epidermidis* and infected and co-treated with coenzyme Q_{10} were studied. Sperm density, motility and morphology of epididymis, as well as testicular weight and histology were evaluated at 21 days post-treatment. Sperm abnormal forms and higher banana form and vacuolization in the seminiferous epithelium were observed in infected mice. The morphologic alterations were reduced after the treatment with coenzyme Q_{10} in co-trated group. Coenzyme Q_{10} may be an important therapeutic option in the reversal of sperm damage as potent antioxidant, in the restoration of reproductive tract epithelium and probably in infertility in mice infected with *S. epidermidis*.

Keywords: Coenzyme Q_{10}; *Staphylococcus epidermidis*; Male accessory glands; Coagulase-negative Staphylococci

Introduction

Genital tract infections have been associated to infertility in many couples. The most studied microorganisms responsible among the causes of infectious infertility are *Chamydia trachomatis, Mycoplasma hominis, Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Treponema pallidum* among others [1]. The impact of bacterial infection on seminal parameters are leukocyte production, with reduction of sperm density, normal forms and motility [2,3]. The aforementioned microorganisms have been associated with reduction of seminal characteristics; however, no specific changes have been defined in semen with high concentrations of Gram positive cocci. The frequency of Coagulase-negative Staphylococci (CoNS) in seminal samples has broad frequency ranges [4,5]. CoNS are considered saprophytes microorganisms of male urethra and are observed with higher frequency in semen of infertile men. A study showed associated the concentration of CoNS 10^4CFU/mL in semen with increased round cells (specially seminiferous epithelial cells instead leukocytes) and abnormal sperm forms (microcephalic) [6]. Another study showed that the same species of CoNS isolated in semen from infertile patients inoculated in testicles of rodents led to detachment of cells of the seminiferous epithelium and increase of compacted heads with smaller size and with lower acrosomal volume probably associated to oxidative stress [7].

A study demonstrated that species such as *Enterococcus faecalis*, *Staphylococcus aureus* and CoNS were observed in higher frequency in teratozoospermic men, but the finding have not been supported by statistical significance [3]. These observations make a point of debate that a saprophyte microorganism can cause damage to the male genital tract [8].

Staphylococcus epidermidis is the most frequent specie of CoNS, probably it has a beneficial role in the balance of the urethral microbiota of many epithelia to avoid the proliferation of harmful bacteria like *S. aureus*, so that, in special conditions *S. epidermidis* can behave as a nosocomial pathogenal [9]. In semen samples with *S. epidermidis* an increased apoptosis and ultrastructural changes have been observed [10]. The pathogenicity of *S. epidermidis* is due to the ability to produce adhesion factors, toxins, hemolysin, leucocidins and enterotoxins. Also, the formation of biofilms can inhibit the main mechanisms of defense in the host by means of production of protective surface polymers, exoenzymes and other cytolytic agents [9,11].

The mechanism that allows the conversion of a resident microbiota such as *S. epidermidis* to pathogen microorganism is difficult to explain [12]. A probable cellular mechanism in the host known as "extracellular ATP" (eATP) has been proposed, it may be an important physiological mediator of signaling associated to the conversion of opportunistic to pathogenic pro-inflammatory bacteria, producing a chronic infections [13]. The mechanism eATP has been associated with Reactive Oxygen Species (ROS) production and other extracellular signals involved in the pathophysiological response [14,15], so that, oxidative stress is a mechanism that can trigger the damage in the genital tract in presence of infections or inflammations [16]. ROS play an important role in male infertility; its levels of higher in semen of infertile men and the antioxidants could play an important role in protecting spermatozoa. Antioxidants have improved sperm density and normal forms in males with idiopathic oligoasthenoteratozoospermia [17].

The direct impact of microorganisms on the testis is not clearly established. In animal models the bacterial inoculation increases the leukocyte infiltration and reduces the spermatogenesis. These models have been applied to some bacteria whose pathogenicity ways are

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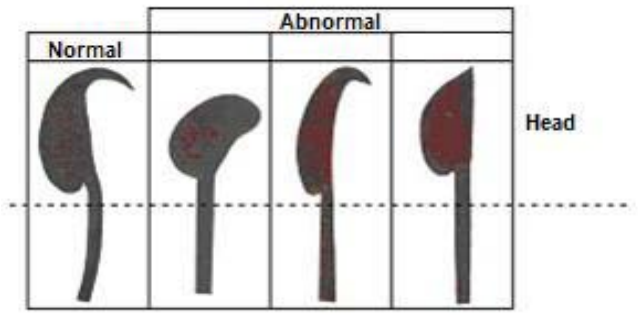

Figure 1: Sperm forms in mice (Authorized by Domínguez-Odio et al 2012).

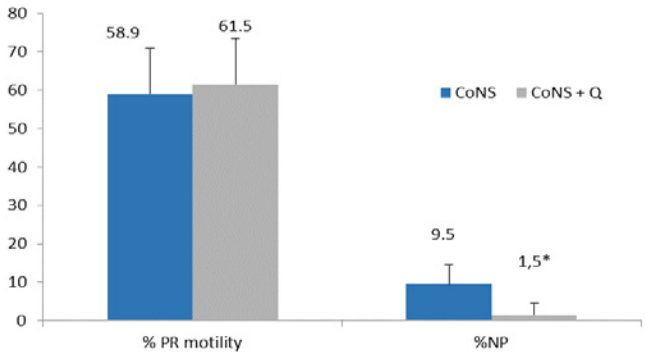

Figure 2: Percentage of sperm with progressive motility (PR) and non-progressive (NP).

best known that CoNS infection [18-20].The protective role of an anti-oxidative agent as CoQ in immunity was demonstrated. A study showed that flies defective in CoQ biosynthetic were more susceptible to bacterial and fungal infections, in mammals the evidences aren´t enough [21]. This study evaluates the protective effect of coenzyme Q_{10} on the reproductive tract of mice infected with *S. epidermidis*.

Materials and Methods

Two groups of male mice (30 days old) were infected on testicular mediastinum with 0.1mL of *S. epidermidis* dilution; 6 of them were treated orally with coenzyme Q_{10} (group CoNS+Q), the other animals (n=6) were treated with placebo (group CoNS). The protocol followed the guidelines of the bioethics code of the University of Los Andes for laboratory animals [22]. The number of animals (n) was determined probabilistically with a 5% error α, 95% confidence level and the respective value $p \leq 0.05\%$ [23]. Mice were weighed before and after treatment.

The inoculum was prepared from *S. epidermidis* obtained from a semen culture (10^6CFU/mL) of infertile men with normozoospermia. The bacterial inoculum was pre-incubated in Mueller Hinton broth (2.5mL) at 37°C for 6-8 hours, then it was diluted in physiological saline solution (0.85%) up to turbidity McFarland standard Nº 0.5 ($1.5x10^8$CFU/mL) [24].

The amount of 100mg contained into each capsule of coenzyme Q_{10} was diluted in 2.7mL of glycerin (100mg/3000µL). It was calculated on human dose (400mg/d) [25]. For an average of 28 grams in each mouse, a daily oral dose (160µg/5µL of the dilution) to 21 day was given, which were prepared 10 minutes prior to application. The

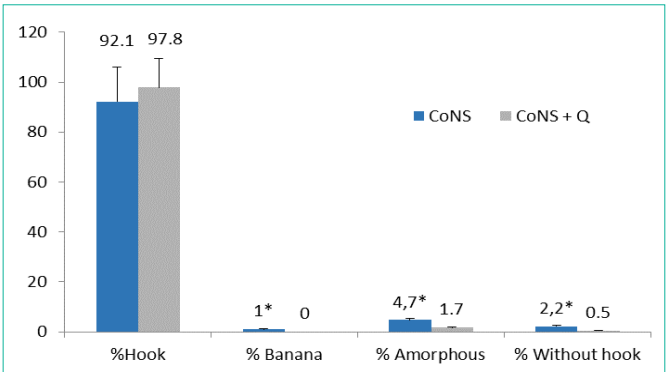

Figure 3: Percentage of sperm forms in group infected with *S. epidermidis* (CoNS) and infected with *S. epidermidis* + coenzyme Q (CoNS+Q).

group CoNS an oral dose of 5µL glycerol alone was given in the same period as in the other study group.

The animals were kept in polypropylene cages at an average temperature of 21±3°C for 3 weeks, and fed with commercial rat and sterilized water *ad libitum* in an environment of 12 hours of light and 12 hours of darkness. The animals were sacrificed with halothane [26,27]. The testes were removed, weighed and processed histologically into paraffin blocks, the sections were stained with hematoxylin eosin. The tubular compartments, the basal membrane of the tubules and the interstitial spaces were evaluated [28,29].

The epididymis were removed and placed in Eppendorf vials. Density and sperm morphology were measured from the right epididymis using the Dominguez morphological criteria with normal forms hooked, and abnormal forms: amorphous, banana-shaped and without a hook (Figure 1) [30] and sperm motility was evaluated from the tail of left epididymis [31]. The results were analyzed according to the multivariate, dichotomous, multifactorial design, through the Statistical Package for the Social Science (SPSS), version 17.0 to determine absolute frequencies.

Results

The body weight (grams) was similar between the groups CoNS and CoNS+Q after the treatment 32.2±1.7 vs 32.8±2.1, respectively. Testicular weight mean (left and right) did not show significant differences between the two groups.

Sperm density

No difference in sperm density was observed between the CoNS group ($650±222x10^6$ /mL) and CoNS + Q group ($669±93x10^6$ /mL) ($p \geq 0.05$). Increased non progressive motility was observed in the CoNS group, therefore, it was higher in the CoNS group ($p \leq 0.05$) (Figure 2).

Sperm morphology

The sperm forms banana, amorphous and without hook were higher in the CoNS group ($p \leq 0.05$) with respect to the CoNS+Q group (Figure 3).

Histological analysis

Figure 4 shows transverse sections three weeks after the inoculation with *S. epidermidis* with mild hyalinization in the basement membrane, (4a): vacuolations were observed in some

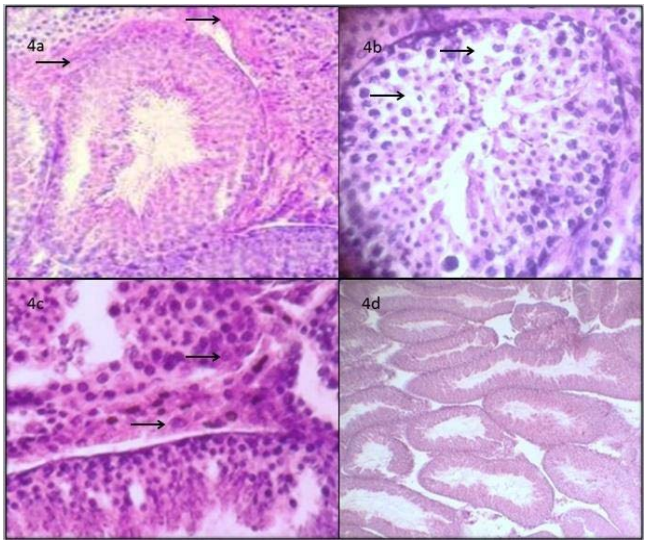

Figure 4: Group CoNS: testicular tissue 400X, transversal cortex with seminiferous tubule with mild hyalinization of the basement membrane (4a); some vacuolations on the epithelia of seminiferous tubules (4b); mild hypertrophy of the Leydig cells in sagittal cortex (4c); Group CoNS+Q 100X describes normal epithelium without alterations (4d).

seminiferous tubules; (4b): and mild hypertrophy of the Leydig cells in sagittal cortex; (4c): In the cross section of the testes of the CoNS+Q group, germinal epithelium, interstitial space and the tubular lumen showed greater histological integrity. Some tubules were observed with moderate detachment of the germinal cells in their most apical area (4d).

Discussion

The animal model shows the negative effect of *S. epidermidis* on male genital tract. Sperm changes have been observed in abnormal forms in infected animals. A possible teratozoospermia-inducing factor has been found in humans, when globular forms are increased in the semen of infertile men and are associated with increased ROS morphologic characteristics of the sperm cell are the outcome of highly complex cellular modifications occurring during spermatogenesis [32]. The presence of abnormal spermatozoa in mice suggests specific structural abnormalities related to spermatozoa production and/or maturation associated to ROS over production [33,34].

Oral administration of coenzyme Q_{10} during months of treatment has allowed improve the morphology of spermatozoa in infertile men, especially by reduction of ROS levels that alter the permeability of the plasma membrane and head forms of the spermatozoa [35]. Treatment of subclinical infections and secretory failure of male accessory glands can improve sperm physiology to achieve spontaneous pregnancies [36].

The histological findings suggest the need to prescribe coenzyme Q_{10} to maintain the functional integrity of the seminiferous tubule before irreversible damage is generated, such as tubular hyalinization, with a significant alteration in the quality of spermatogenesis [37,38].

Conclusion

In summary, *S. epidermidis* causes microcytosis in sperm. The use of coenzyme Q_{10} may be the most favorable therapeutic option before the application of antimicrobial drugs, since the uncontrolled use of antibiotics can eliminate the resident microbiota on male genital tract or presenting complications associated with multiresistance to antibiotics.

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