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Coenzyme Q_{10} levels in pigeon (*Columba livia*) spermatozoa

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Abstract **Aim:** To assess the CoQ_{10} levels in pigeon spermatozoa and to verify their possible correlation with spermatic kinetic parameters. **Methods:** In pigeons the sperm motility percentage (MOT%), mean linear velocity (VCM) and morphology were determined in ejaculated semen. In addition intracellular CoQ_{10} concentrations were also detected with a HPLC method. **Results:** Intracellular CoQ_{10} levels demonstrated wide individual variations, averaging 4.85 ¡À2.31 (SD) ng/10^6 spermatozoa. Statistical analysis showed a positive correlation of the substance with the sperm concentration (r= 0.63; P<0.05) and with the VCM (r=0.66; P<0.05), and a negative correlation with the MOT% (r= -0.78; P=0.01). No correlation was found between the CoQ_{10} concentration and the percentage of normal spermatozoa. **Conclusion:** Our results suggest a possible role for CoQ_{10} as a "fertility marker" in pigeons, which may be employed to monitor the pharmacological effects of cytostatic substances often used to reduce the pigeon fertility in urban environment.*(Asian J Androl 2002 Mar; 4: 73-76)*

1 Introduction

Coenzyme Q_{10} (CoQ_{10}), an integral redox and proton translocating component of the mitochondrial respiratory chain, also known as ubiquinone for its wide diffusion throughout mammalian tissues [1], plays a key role in energy metabolism and has potent antioxidant properties for cellular membrane integrity [2]. Biosynthetic machinery for CoQ is present at remarkably high levels in testis [3] where ubiquinone could cover important functions for its metabolic and antioxidant properties. In fact a large amount of mitochondria are present in spermatozoa, in which the motile activity requires a high energy expenditure; in addition the sperm, for its high contents of unsaturated fatty acids in its membrane, is particularly exposed to peroxidative stress due to the action of reactive oxygen species (ROS). These reactive molecules caused a loss of sperm motility and decreased the capacity for sperm-oocyte fusion [4,5]; they also affect the sperm axoneme as a result of ATP depletion[6] and inhibit mitochondrial functions and synthesis of DNA, RNA and proteins [7].

Recently, studies started to define the CoQ_{10} activity in male fertility, suggesting a possible role for this molecule as a possible fertility marker. Higher levels of intracellular CoQ_{10} were found in asthenozoospermic and teratozoospermic patient than in normal subjects [8]. Moreover, ejaculates from fertile individuals had lower CoQ_{10} concentrations than those from the infertile patients [9]. A study on stallion semen has confirmed a negative correlation between the intracellular levels of CoQ_{10} and the sperm motility [10]. Our previous research on pheasant semen has shown the presence of CoQ_{10} in this species [11], highlighting the specific role of this molecule also

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in avian reproduction.

The present study was designed to assess the CoQ_{10} levels in pigeon spermatozoa, at an aim to help clarifying its relationship with the spermatic kinetic parameters. This important antioxidant molecule may also provide a useful means to reduce the pigeon fertility in urban environment.

2 Materials and methods

2.1 Animals and routine sperm observation

Semen was collected by massage [12] from 11 male pigeons (2-3 years of age), caged individually according to the Italian Law about animal care (D.Lg. 116/92) and fed with tap water and a commercial ration ad libitum. For morphology determination, 10 μL of semen diluted 100 times with a saline buffer containing formalin [13] were examined under a phase contrast microscope, evaluating the shape of 100 cells. The kinetic parameters, the mean spermatozoa motility (MOT in %) and mean linear velocity (VCM in μm/s), were determined in semen samples diluted with Tyrode solution containing BSA 0.5% as previously described [14] using the semiautomatic system for computerised videomicrography "Cell-Count" (Motion Analysis Corp., USA).

2.2 CoQ_{10} determination

The CoQ_{10} intracellular concentration was detected by using a HPLC-UV (275 nm) technique [15]. Spermatozoa were washed twice with physiological solution by centrifugation (15 min at 1000×*g*) and the pellet was reconstituted to 1.5 mL. An aliquot of washed spermatozoa, diluted with Jasko solution, was employed for sperm concentration determination by Thoma-Zeiss chamber. One mL of the sample was extracted with 5 mL of acetone and after centrifugation it was dried on Argon flux at 37¡æ The sample was then dissolved in 50 μL of ethanol and a 10 μL aliquot was injected into the HPLC system with Nucleosil 100-5 C18 250×46 mm column, utilising a mobile phase methanol-ethanol (60:40) with a rate flux of 1.1 mL/min. CoQ_{10} concentrations were detected by using Coenzyme Q_8 as internal standard and expressed as ng/10^6 spermatozoa.

2.3 Data processing

Statistical analysis was performed with the Pearson's Test and the ANOVA Test. $P<0.05$ was considered significant.

3 Results

The CoQ_{10} intracellular concentrations and the semen parameters in the pigeons are showed in Table 1. The intracellular CoQ_{10} levels exhibited a wide individual variation. Statistical analysis showed a positive correlation ($r= 0.63$, $P<0.05$) between the CoQ_{10} level and the sperm concentration (Figure1), a negative correlation ($r= -0.78$, $P=0.01$) between the CoQ_{10} level and the MOT (Figure 2) and a positive correlation ($r=0.66$, $P<0.05$) between the CoQ_{10} level and the VCM (Figure 3). No correlation was found between the percentage of normal spermatozoa and the CoQ_{10} concentration.

Table 1. CoQ_{10} intracellular levels and semen parameters in pigeons.

Pigeon	CoQ_{10} (ng/10^6 spermatozoa)	Normal form spermatozoa (%)	MOT (%)	VCM (μm/s)
1	3.00	72	75	76
2	6.96	82	53	108
3	5.72	87	62	106
4	0.69	81	96	67
5	2.69	88	72	75
6	4.77	86	58	72
7	3.47	92	53	58
8	7.68	85	54	78
9	7.50	86	64	95
10	3.77	89	76	88
11	7.05	72	54	122
Mean	4.85	84.56	5.18	85.91
S.D.	2.31	5.34	13.49	19.73

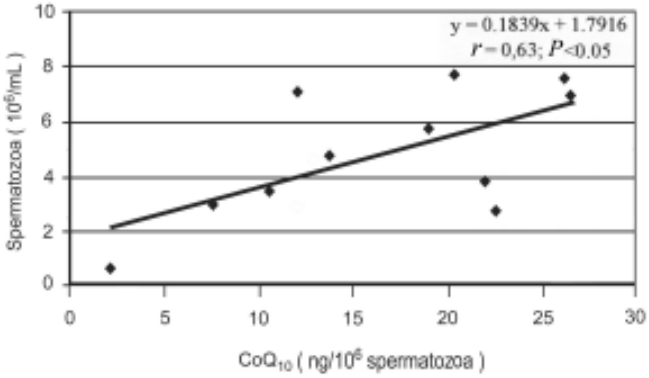

Figure1. Linear regression between intracellular CoQ_{10} level and sperm concentration.

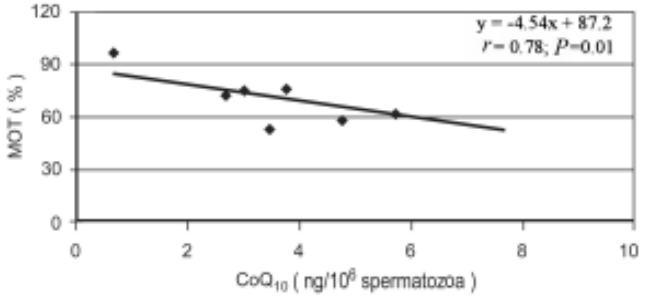

Figure 2. Linear regression between intracellular CoQ_{10} level and sperm motility (MOT).

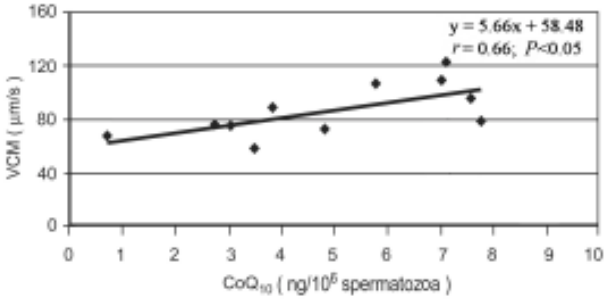

Figure 3: Linear regression between intracellular CoQ_{10} level and mean cellular velocity (VCM).

4 Discussion

CoQ_{10} function in biological fluids and cells has been widely investigated in the recent years, highlighting its importance in the mechanism of electron transfer in the mitochondrial respiratory chain and in the neutralisation of O_2 reactive species [2]. Its favorable action has also been described in the cardiovascular [16] and other diseases [17, 18]. In the reproductive field, several studies have attempted to establish a link between CoQ_{10} and sperm quality and function, but the results appeared to be conflicting. CoQ_{10} concentration in human seminal fluid was, in fact, found to be in good correlation with sperm motility and sperm count but in regard to the intracellular levels, a trend towards an inverse correlation with the main sperm parameters was observed [15]. Angelitti et al. [9] have also observed an inverse correlation between the CoQ_{10} concentration and the sperm motility in human semen. Our previous studies have also indicated an inverse correlation between the intraspermic CoQ_{10} level and the sperm motility in humans [8] and horse [10], but a positive correlation between the sperm motility and the seminal plasma level of ubiquinone in rabbits [19]. The present finding showed a significant inverse correlation between the ubiquinone intranemaspermic level and the sperm motility in pigeons.

All these data seem to indicate a double and contrasting action of this molecule on the sperm quality: on the one hand an elevated seminal plasma concentration would improve the sperm motility; on the other hand poorly motile sperm had, paradoxically, a high intracellular CoQ_{10} level.

The positive correlation between the CoQ_{10} level in the seminal plasma and the sperm motility has a simple and logic explanation: consequent to its antioxidant property, the molecule could counteract ROS damage on the sperm membrane.

The explanation of the inverse correlation between the intracellular ubiquinone level and the sperm motility requires a more complex reasoning. First of all, because the spermatozoa are non-synthesising cells, we must expect that augmented CoQ_{10} levels are produced by spermatogenic undergoing spermatogenesis, probably in reply to a hyperoxidative environment. For this reason, intranemaspermic CoQ_{10} determination could have a great clinical relevance permitting to point out possible alterations of testicular environment that could lead to a reduced fertility. Nevertheless the augmented protection provided by ubiquinone, seems to be ineffective as the sperm motility is depressed. This phenomenon is not really surprising because CoQ_{10}, in its semiquinone form, can generate superoxide from oxygen and this, in turn, can result in oxidation of biomolecules [20]. The radical semiquinone is originated from the partial ubiquinol oxidation at least in two physiological cellular processes. Oxidation of ubiquinol is in fact an essential step in the proton motive Q-cycle, a mechanism by which the cytochrome bc_1 complex links electron transfer to proton translocation across the mitochondrial membrane in which this complex resides [21]. The generation of superoxide results from a leakage of the second electron of ubiquinol from its Q-cycle electron transfer pathway to interact with oxygen [22]. Another possible process leading to superoxide production is the regenerative cycle of vitamin E, a more powerful antioxidant whose action in semen has been extensively studied. A protective effect of this vitamin has been seen in human semen with a dose-dependent improvement in both the sperm motility and viability, accompanied by concomitant decrease in malondialdehyde, an end product of lipid peroxidation [23]. In addiction, a recent study showed protective role of vitamin E co-treatment against mercury induced male reproductive toxicity in mice [24].

In the regenerative cycle, the vitamin E is converted in the first place to its phenoxyl radical (tocopheroxyl radical) and is then reverted by the reduced forms of CoQ_{10} to its parent molecule [25]. The one electron reduction of tocopheroxyl radical by ubiquinol gives rise to semiquinone. This product can undergo disproportionation or alternatively it can reduce molecular oxygen to produce superoxide. The probability that disproportionation or superoxide production will result depends on a number of factors, including the relative concentration of semiquinone and oxygen. Thus, changes in the concentration of any reactants may shift the equilibrium and determine whether production of superoxide will dominate. In both processes CoQ_{10} increase favours superoxide production with negative effects on the sperm motility, but this detrimental action can be counteracted by superoxide dismutase, an antioxidant factor which greatly accelerates the dismutation of the superoxide anion into hydrogen peroxide [26] that is finally degraded into water and oxygen by catalase [27] and glutathione

peroxidase [28]. Spermatozoa having an efficient antioxidant system of defence will not suffer for this excessive superoxide production and will maintain a good motility with high linear velocity, as demonstrated by significant positive correlation existing between ubiquinone levels in pigeon sperm and VCM.

In conclusion, our data confirm the importance of CoQ_{10} also in pigeon reproduction, suggesting a possible role for this molecule as "fertility marker". High CoQ_{10} levels are in fact produced as a reply to a hyperoxidative testicular environment, but can exert a paradoxical effect if the antioxidant defence system of the cell is altered. The determination of CoQ_{10} intranemaspermic levels could become a useful tool for monitoring the effects on the reproductive system of cytostatic substances often utilized to reduce pigeon fertility in urban environment. It is in fact known that the chemotherapeutic treatment leads to an increase in free radical formation [29,30].

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