Coenzyme Q: Potentially Useful Index of Bioenergetic and Oxidative Status of Spermatozoa

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The concentration of coenzyme Q10 (CoQ10), a key intermediate of the mitochondrial respiratory chain, was determined in spermatozoa of 13 fertile subjects, 8 potentially fertile patients, and 12 infertile patients. CoQ10 concentrations were significantly higher (P < 0.001) in infertile patients than in fertile and potentially fertile subjects. The difference between potentially fertile and fertile subjects was also significant (P < 0.001). We propose that a decrease in consumption of CoQ10 in both fertile and potentially fertile populations is due to an autoregulatory mechanism of ATP production.

Indexing Terms: fertility/varicocele/male reproductive system/mitochondrial respiratory chain

Evaluation of male infertility includes morphologic and functional examination of seminal fluid. The characteristics usually examined are cell concentration, motility, and morphology. The percentage of immature cells is higher in infertile than in fertile subjects. From these determinations, fertility indices have been proposed. The popular index of Pag and Houlding (1) combines cell count, motility, and morphology. However, no physical index reliably predicts fertility (2). For this reason, many authors (3, 4) have tried to find a biochemical index of fertility; thus far, however, their efforts have been unsuccessful, probably because many metabolic aspects of sperm cells remain obscure.

The most studied biochemical component of sperm has been ATP; surprisingly, no significant difference in its concentration is found between fertile and infertile men (5, 6).

We have been interested in the role of coenzyme Q10 (CoQ10) as an electron carrier in the mitochondrial respiratory chain. The antioxidant activity of CoQ10, and its close relation with vitamin E, have been clearly demonstrated (7, 8). The aim of this study was to compare CoQ10 activity in sperm cells with other seminal fluid indices.

Materials and Methods

Papanicolaou stain was from Sigma Chemical Co. (St. Louis, MO). All HPLC solvents were from Carlo Erba (Milan, Italy).

The population studied included 13 fertile subjects (mean age 28.3 ± 9), 8 potentially fertile patients (mean age 28.5 ± 6), and 12 infertile patients affected by unilateral varicocele (mean age 31.4 ± 7). The diagnosis of infertility for these patients was formulated according to the criterion of the World Health Organization: A subject is considered infertile if after at least 12 months of attempts with a partner of ascertained fertility he has been unable to fecundate.

Seminal fluids were obtained by masturbation, after the informed consent of the subjects, and analyzed immediately after liquefication, which usually required 15–30 min. The total volume obtained was divided into two portions, the first to estimate cell concentration, motility, and morphology, and the second to measure total CoQ10. The first three characteristics were determined with the videomicrographic technique of Overstreet et al. (9) integrated with a computer system (Computer Assisted Semen Analysis; Cell Soft, Cryo Res, NY). CoQ10 was measured as described previously.5 The pellet was washed with isotonic saline (9 g/L NaCl) and extracted three times with 4 mL of acetone. The pooled extracts were evaporated to dryness, and the residues were redissolved in 100 µL of absolute ethanol. We injected 20 µL of this solution into the HPLC (Beckman Gold; Beckman Instruments, Fullerton, CA) under the following operating conditions: an Ultrasphere ODS 150 × 4.6 mm column, ethanol:methanol (70:30, by vol) mobile phase, isocratic elution, and photometric detection at 275 nm. CoQ8 as internal standard and CoQ10 as external standard were used to calculate the concentration of CoQ10 from the areas of the corresponding peaks in the sample chromatograms.

Cell concentration, motility, and morphology data were used for the spermograms according to Dickerman et al. (10).

Statistical calculations were performed with Student’s t-test for unpaired data and nonlinear regression analysis.

Results

Figure 1 shows the data on motility and morphology. From these data the subjects were classified as fertile, potentially fertile, and infertile according to the scatterplots of Dickerman et al., in which motility and morphology are plotted vs cell count. For each cell

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concentration a reference range (mean ± SD) was obtained. We also made a first broad classification of the subjects into three main groups: the first, with a cell count ≤5 × 10^9 cells/L, was considered infertile; the second, with a cell count from 5.1 × 10^9 to 20 × 10^9 cells/L, was considered potentially fertile; and the third, with a cell concentration >20 × 10^9 cells/L, was reported as fertile. However, these criteria are not rigid. For a more complete classification, characteristics such as cell motility and morphology must be taken into account. In our classification a subject was included in the fertile population if all the characteristics examined were in the normal range. If one of these was out of the reference range for fertile subjects in the scatterplots of Dickerman et al., we classified the subject as potentially fertile or infertile, according to the value of the characteristic. CoQ10 concentrations (μg/10^9 cells, mean ± SD) in the three populations were: 1.9 ± 1.1 in the fertile group, 5.2 ± 1.1 in the potentially fertile group, and 11.4 ± 5.9 in the infertile group. The large difference (P < 0.001) between the fertile and infertile populations is evident. Also significant is the difference between the fertile and potentially fertile populations (P < 0.001) as well as between the infertile and potentially fertile populations (P < 0.02). These differences are not affected by the age differences between the fertile and infertile subjects because the decrease of tissue concentrations of CoQ10 in normal fertile subjects becomes significant only after age 60 years (11).

Figure 2 shows the correlation between cell concentrations of CoQ10 and the cell count. To find the line of best fit, we used a nonlinear correlation; the line shown has a good fit (P < 0.001). The relation of CoQ10 with cell motility is also represented, demonstrating an inverse, nonlinear correlation (P < 0.025). Fig. 3 shows a schematic representation of the autoregulatory mechanism of ATP concentration in spermatozoa.

**Discussion**

The large difference in CoQ10 concentration between the fertile and infertile population could result from either a greater uptake or a reduced consumption of ATP. From our data the second hypothesis seems more probable, although the first cannot be excluded a priori, as will be discussed later. If we consider the cell concentration of CoQ10, we observe an inverse correlation with cell number. As the cell number increases (Fig. 2), a fertile condition is progressively achieved (9, 10), resulting in an increased consumption of ATP.
All the cells and tissues examined thus far have exhibited a direct correlation between ATP and CoQ10 consumption (12, 13). Therefore, similar behavior in spermatozoa would not be unreasonable. Energetic metabolism in these cells proceeds mainly through anaerobic glycolysis, and the contribution of oxidative phosphorylation to the total ATP content is of secondary importance (14, 15).

ATP consumption in spermatozoa proceeds mainly through dynein ATPase, an enzyme responsible for cell motility. Dynein is a motor protein comprising three heavy chains (α, β, and γ) and localized mainly in the cytoplasmic vesicles, the microsomes, and the microtubules; ATPase activity is located in the β and γ chains (16). The ATPase inhibitor, sodium vanadate, also inhibits cell motility (17). In the presence of this inhibitor, the increase in the ATP/ADP ratio greatly reduces or blocks the flux of electrons through the mitochondrial respiratory chain, thus impairing oxidative phosphorylation as well as glycolysis. A similar situation could exist in infertile patients: Their reduced motility could result in an increased ATP/ADP ratio and a reduced rate of mitochondrial respiration. In contrast, the higher motility of normal spermatozoa leads to an increase in respiration rate and hence in oxidative phosphorylation. This autoregulatory mechanism (Fig. 3) could account for the invariance of ATP content in spermatozoa of fertile and infertile patients; however, in the latter, a reduced respiration rate could result in an accumulation of some intermediate substrates such as CoQ10. Our data clearly show the inverse correlation between CoQ10 and motility (Fig. 2, bottom).

Increased lipid peroxidation has been reported recently in spermatozoa of infertile patients (18). The antioxidant role of CoQ10 was established many years ago (19). In particular, increased CoQ10 concentrations are observed in cells exposed to peroxidative insults (e.g., irradiated platelets or sun-exposed skin) (20, 21). Hence an increased uptake of CoQ10 in spermatozoa from infertile patients could be triggered by the peroxidative events so as to meet the need of an increased antioxidant response. The significant differences in CoQ10 concentrations existing among the three groups suggest that this aspect may prove useful as a biochemical index of fertility. The importance of this index is evident if we consider that the current physical criteria for a correct classification of the subjects are not unequivocal and show some degree of subjectivity. Furthermore, the methods for assessing the various characteristics used for the diagnosis are tedious and time consuming, whereas only 15 min for each chromatographic run and 10 min for the batch extraction of all the samples are required to measure the CoQ10 concentration. We conclude that the use of CoQ10 measurement to augment traditional determinations could greatly reduce or eliminate subjectivity in making a correct diagnosis.

References