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REVIEW ARTICLE

Coenzyme Q_{10} and male infertility

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ABSTRACT. We had previously demonstrated that Coenzyme Q_{10} [(CoQ_{10}) also commonly called ubiquinone] is present in well-measurable levels in human seminal fluid, where it probably exerts important metabolic and antioxidant functions; seminal CoQ_{10} concentrations show a direct correlation with seminal parameters (count and motility). Alterations of CoQ_{10} content were also shown in conditions associated with male infertility, such as asthenozoospermia and varicocele (VAR). The physiological role of this molecule was further clarified by inquiring into its variations in concentrations induced by different medical or surgical procedures used in male infertility treatment. We therefore evaluated CoQ_{10} concentration and distribution between seminal plasma and spermatozoa in VAR, before and after surgical treatment, and in infertile patients after recombinant human FSH therapy. The effect of CoQ_{10} on sperm motility and function had been addressed only through some *in vitro* experiments. In two distinct studies conducted by our group, 22 and 60 patients affected by idiopathic asthenozoospermia were enrolled, respectively. CoQ_{10} and its reduced form, ubiquinol, increased significantly both in seminal plasma and sperm cells after treatment, as well as spermatozoa motility. A weak linear dependence among the relative variations, at baseline and after treatment, of seminal plasma or intracellular CoQ_{10}, ubiquinol levels and kinetic parameters was found in the treated group. Patients with lower baseline value of motility and CoQ_{10} levels had a statistically significant higher probability to be responders to the treatment. In conclusion, the exogenous administration of CoQ_{10} increases both ubiquinone and ubiquinol levels in semen and can be effective in improving sperm kinetic features in patients affected by idiopathic asthenozoospermia

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INTRODUCTION

The role of oxidative stress in seminal fluid pathophysiology

Spermatozoa, like many other kinds of cells are susceptible to oxidative damage and reactive oxygen species (ROS) play a key pathogenetic role (1) in sperm dysfunction and male infertility. MacLeod et al. (2) reported the first original observation: spermatozoa incubated *in vitro* under high oxygen tension were subject to a loss of motility, which was reversed by the addition of catalase to the incubation medium. Thereafter a number of studies confirmed the importance of oxidative stress in male infertility, as recently reviewed (3, 4).

The high content of polyunsatured fatty acids (PUFA) within the spermatozoa plasma membrane and a low concentration of cytoplasmic scavenging enzymes make these cells highly susceptible to peroxidation in the presence of elevated levels of ROS in seminal fluid (5-7). Decosohexaenoic acid, with 6 double bonds per molecule, is particularly abundant (5); this characteristic is needed to maintain membrane fluidity for the fusion with oocyte membrane during fertilization (6-9). Due to peroxidative membrane damage, permeability is modified, with increased inflow of sodium and calcium and ATP depletion; consequent activation of Ca-dependent enzymes (proteases, phospholipases) with a cascade of proteins and lipid damage, also lead to enzyme inactivation, structural DNA alteration, and eventually cell death (10-12). PUFA pattern is modified during sperm maturation also outside the testis: the final composition is obtained during epididymal transfer in different animal species; in humans, the grade of unsaturation grows from the caput to the cauda of epididymis, indicating an active lipid metabolism (13-17). The importance of this district is underlined by the fact that the same region is the site of a leukocyte invasion; it has been demonstrated that the time of permanence in epididymus is greater in oligospermic patients and therefore the exposition to ROS is longer (18-20). Finally other characteristics that make spermatozoa fragile are: poorly compacted chromatin (21), frequency of DNA strand breaks (22), and the high probability of mitochondrial DNA fragmentation (23).

The source of ROS is related to both spermatozoa and infiltrating leukocytes in semen (11, 12, 24-28). There is a clear correlation between leukocyte concentrations and ROS levels (29) and between ROS, lipid peroxidation, and functional damage (8, 30). However, even if a clear detrimental effect on various spermatozoa functions has been reported in *in vitro* suspensions (31, 32), the situation cannot simply be applied to *in vivo* exposure in the male reproductive tract (20, 33).

To counteract the potentially hazardous effects of oxidative stress, spermatozoa and seminal plasma are en-

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dowed with a number of protective antioxidant systems. Spermatozoa possess a low amount of cellular ROS defence systems such as catalase, superoxide dismutase, glutathione peroxidase, and vitamin E. In contrast, seminal plasma is well endowed with an antioxidant buffer capacity (34, 35). This protective system includes chain-breaking antioxidants capable of counteracting oxidant radicals thus blunting the propagation of free radical chain reactions.

Some studies have shown that infertile men have an impaired seminal plasma non-enzymatic antioxidant capacity, suggesting that decreased total antioxidant capacity may exert a pathogenetic role in male infertility (36, 37). Oxidative stress has been shown to be associated with alteration in seminal characteristics, such as severe oligospermia and asthenozoospermia (1, 7, 25, 29), but also in normozoospermic infertile men this mechanism may underlie an unexplained infertility, otherwise attributed to female factors (38).

Additional studies have demonstrated that the levels of spermatozoal reactive oxygen species are higher in men with varicocele (VAR) than in normal controls, suggesting that sperm dysfunction in VAR patients may, in part, be related to oxidative stress (39). VAR patients represent an interesting study model since they exhibit an enhanced ROS generation and high levels of nitric oxide, which are directly related to the amount of ROS generated (40). Infertile subjects with a history of smoking exhibit higher ROS levels than non-smoking infertile subjects (41). Men with chronic prostatitis or prostatodynia have signs of seminal oxidative stress, whether or not leukocytospermia is present (42).

Furthermore, other pathophysiology mechanisms may be indirectly related to oxidative stress, such as the interaction with cytokines in mediating the effects of phlogosis (43, 44), the modification of nitric oxide effects in relation to the redox state of cells (45), and Fas-dependent apoptosis (3, 46, 47). Finally, various models have been introduced to explore the protective role of different antioxidants *in vitro* and some differences can be found among the pattern of protective effects exerted by specific enzymatic or non-enzymatic molecules (48).

COENZYME Q_{10}

Biological roles and levels in seminal fluid

Following its discovery in 1957, Coenzyme Q (CoQ_{10} in humans) studies particularly addressed its key role in mitochondrial bioenergetics; later studies demonstrated its presence in other subcellular fractions and in plasma, and deeply investigated its antioxidant role. These two roles constitute the basis on which research supporting the clinical use of CoQ_{10} is founded. Also at the inner mitochondrial membrane level, Coenzyme Q is recognized as an obligatory co-factor for the function of uncoupling proteins and a modulator of the transition pore (49). Furthermore, recent data reveal that CoQ_{10} affects expression of genes involved in human cell signaling, metabolism, and transport (50) and some of the effects of exogenously administered CoQ_{10} might be due to this property.

Both the bioenergetic and the antioxidant roles of CoQ_{10} suggest a possible involvement in male fertility: on the one hand, it is known that a large number of mitochondria are present in spermatozoa, the motility of which requires a high energy expenditure (51); on the other hand, as shown in the previous paragraph, the protection of membranes from oxidative stress could play a role in preserving sperm integrity; moreover, the biosynthetic machinery for CoQ is present at remarkably high levels in rat testis (52).

The first analytical data on CoQ_{10} levels in seminal fluid were produced by our group (53) showing that CoQ_{10} was assayable in total seminal fluid and in seminal plasma; its levels showed a good correlation with sperm count and motility, except in the population of VAR patients, in whom the correlation with sperm motility was completely lacking (Table 1). In this study, the sample included 60 subjects (21 patients with normozoospermia, 15 patients with azoospermia or oligozoospermia, 2 patients with germ-free genital tract inflammation, and 22 subjects with VAR, 7 of whom presented oligo-azoospermia). Moreover, in the VAR patients a significantly higher proportion of total CoQ_{10} was present in seminal plasma when compared with normal subjects or other infertile patients without VAR (the ratio plasma/seminal fluid Q_{10} was 69±7.1% *vs* 41.2±5.6%, $p<0.01$, respectively). In all patients, standard semen analysis was performed, assessing semen parameters, including ejaculate volume, sperm count, percent motility and sperm morphology, according to World Health Organization (WHO) classification (54). Semen specimens were analysed within 1 h from collection. All patients were clinically examined and the diagnosis of VAR was confirmed by Color-Doppler sonography (55) in 22 of them. These data were also confirmed in a larger series of patients (48, 56, 57).

Since CoQ_{10} in seminal plasma did not correlate with lactic dehydrogenase (LDH) levels, we concluded that the amount of CoQ_{10} in plasma was most likely not due to spermatozoa damage and to a consequent release of CoQ_{10} from the cells. We hypothesized that seminal plasma CoQ_{10} levels reflect an interchange between cellular and extracellular compartments, with a pathophysiological meaning similar to serum CoQ_{10} values (58, 59); a relative deficiency or utilization of CoQ in sperm cells was therefore presumed to be present in VAR condition (60). We also hypothesized that the significantly higher percentage of CoQ_{10} in plasma found in VAR patients could reflect an altered compartment distribution: the intracellular, bioenergetic use of CoQ_{10} could be defective in these patients and there might be some release towards the plasma compartment.

Finally, we studied VAR patients after surgical repair; only a partial reversion was observed, since the ratio plasma to total CoQ_{10} decreased, but the correlation between total CoQ_{10} and motility was not restored (61). As

Table 1 - *Correlation between endogenous Coenzyme Q_{10} in whole seminal fluid and sperm parameters [from (53)].*

	Correlation CoQ_{10} *vs* sperm count	Correlation CoQ_{10} *vs* sperm motility
Total sample	R=0.504, $p<0.0005$	R=0.261, $p<0.05$
Varicocele patients	R=0.666, $p<0.0005$	R=0.008, ns

FSH seemed to be involved in the regulation of total antioxidant capacity of seminal plasma (62), in order to explore the physiological hormone control of seminal CoQ_{10} another trial was conducted in 13 oligoasthenozoospermic subjects, studied before and after 3 months of recombinant human FSH (225 UI/week) (63). Following FSH treatment, CoQ_{10} showed an increase, although not significant, in seminal plasma levels (0.035±0.010 *vs* 0.028±0.005 µg/ml).

CoQ_{10} and its redox status in seminal fluid

All these studies consider the levels of total CoQ_{10}, irrespective of its redox status. The first report on the assay of reduced and oxidized forms of CoQ_{10} was performed by our group (64). We showed a significant correlation between the reduced form (ubiquinol) and sperm count in seminal plasma, an inverse correlation between ubiquinol and hydroperoxide levels both in seminal plasma and seminal fluid, a strong correlation – using multiple regression analysis – between sperm count, motility, and ubiquinol-10 content in seminal fluid, and, finally, an inverse correlation between ubiquinol/CoQ_{10} ratio and the percentage of abnormal forms. These results suggest a possible role of ubiquinol-10 in inhibiting hydroperoxide formation in seminal fluid, as had previously been demonstrated for plasma lipoproteins (65). We also found a lower ubiquinol/CoQ_{10} ratio in sperm cells from idiopathic asthenozoospermic (IDA) patients and in seminal plasma from IDA and VAR-associated asthenozoospermic patients compared to controls (66). The important conclusion was that the QH_2/Q_{OX} ratio may be an index of oxidative stress and its reduction a risk factor for semen quality.

Administration of CoQ_{10} in male infertility

CoQ_{10} was first introduced as an ethical drug for heart failure patients, but its use has grown since its recognition as a food supplement aimed at improving cellular bioenergetics, counteracting oxidative stress and slowing down some age-related pathologies. Numerous clinical studies have shown its efficacy as an adjunctive therapy in cardiovascular and neurodegenerative diseases and in mitochondrial myopathies (67). The above-mentioned studies constitute a rationale which eventually led us to treat infertile subjects with exogenous CoQ_{10}.

Original studies on CoQ_{10} administration in unselected populations of infertile patients, showed an amelioration of the results in membrane integrity tests (swelling test) (68) and an improvement in seminal parameters in men with sperm pathology (69); however these studies did not report the endogenous CoQ_{10} levels in such patients.

Lewin & Lavon (70) originally reported the effect of CoQ_{10} on sperm motility *in vitro*: a significant increase in motility had been observed in sperm obtained from asthenozoospermic men, incubated with exogenous CoQ_{10}, while no significant variation was reported in the motility of sperm cells from normal subjects. The same study also reports the effect of exogenous CoQ_{10} *in vivo*, in a group of patients with low fertilization rates, after *in vitro* fertilization with intracytoplasmatic sperm injection for male factor infertility. No significant changes were reported in most sperm parameters, but a significant improvement was noticed in fertilization rates after treatment with 60 mg/day for a mean of 103 days.

As CoQ_{10} is one of the compounds contributing to the total antioxidant buffer capacity of semen and its decrease could lead to an impairment of the system in counteracting oxidative stress (71), exogenous administration of CoQ_{10} could increase its content in semen and improve sperm cell function.

In order to investigate its potential therapeutic role, we first administered CoQ_{10} to a group of idiopathic asthenozoospermic infertile patients in an open uncontrolled pilot study (72). Twenty-two patients affected by idiopathic asthenozoospermia, were enrolled in the study. All subjects presented a clinical history of primary infertility of at least 3 yr. No female-related factor was apparently involved in sterility. Eligible patients had sperm count >20×10^6/ml, sperm motility (forward motility, class a and b, according to WHO 1999 criteria) (54) <50% at two distinct sperm analyses and normal sperm morphology >30%. Patients were given CoQ_{10} (PharmaNord, Denmark), 200 mg/day divided into two doses, for 6 months. Semen analysis, including computer-assisted sperm analysis and motility (C.A.S.A.) (73), CoQ_{10} and phosphatidylcholine (PC) assays, were performed at baseline and after 6 months of therapy. A semen analysis was further performed after 6 months from interruption of therapy (wash-out). CoQ_{10} levels were assayed in sperm cells and seminal plasma using a Beckman Gold HPLC System HPLC (Beckman Instruments, San Ramon, CA, USA) equipped with an electrochemical detector (EC, ESA 5100, Bedford, MA, USA) (65). PC was essentially determined according to Frei et al. (74).

An increase of CoQ_{10} was found in seminal plasma after treatment, the mean value rising significantly from 42.0±5.1 at baseline to 127.1±1.9 ng/ml after 6 months of exogenous CoQ_{10} administration ($p<0.005$). A significant increase of CoQ_{10} content was also detected in sperm cells (from 3.1±0.4 to 6.5±0.3 ng/10^6 cells; $p<0.05$). Similarly, PC levels increased significantly both in seminal plasma and sperm cells after treatment (from 1.49±0.50 to 5.84±1.15 µM, $p<0.05$; and from 6.83±0.98 to 9.67±1.23 nmoles/10^6 cells, $p<0.05$, respectively) (Table 2). Regarding semen, a significant difference was found in forward (class a+b) motility of sperm cells after 6 months of CoQ_{10} dietary implementation (from 9.13±2.50 to 16.34±3.43%, $p<0.05$) (72). The improvement of motility was also confirmed by means of computer-assisted determination of kinetic parameters. A significant increase of curvilinear velocity (VCL) (from 26.31±1.50 to 46.43±2.28 µm/sec, $p<0.05$), and linear velocity (VSL)

Table 2 - *Coenzyme Q_{10} and PC levels in seminal plasma and sperm cells, baseline and after treatment [from (72)].*

	Baseline	After treatment
CoQ_{10}, seminal plasma (ng/ml)	42.0±5.1	127.1±1.9*
CoQ_{10}, sperm cells (ng/10^6 cells)	3.1±0.4	6.5±0.3**
PC, seminal plasma (µM)	1.49±0.50	5.84±1.15**
PC, sperm cells (nmoles/10^6 cells)	6.83±0.98	9.67±1.23**

*After treatment *vs* baseline, $p<0.005$; **after treatment *vs* baseline, $p<0.05$.

(from 15.20±1.30 to 20.40±2.17 μm/sec, $p<0.05$) was found after treatment. No significant differences were found in sperm cell concentration and morphology (72). Although a direct correlation was not found (data not shown), a positive dependence (using the Cramer's index of association) was evident among the relative variations, baseline, and after treatment, of seminal plasma or intracellular CoQ_{10} content and of computer-assisted sperm analysis (C.A.S.A.) (VCL and VSL) kinetic parameters (Cramer's V=0.4637; 0.3818; 0.3467; 0.5148, respectively) (72). A significant reduction in sperm forward motility was reported after 6 months of wash-out (from 16.34±3.43 to 9.50±2.28%, $p<0.001$), while no significant differences were found in sperm cell concentration and morphology. In order to find out whether different responses were age-related, the relative variations (before and after treatment) of CoQ_{10} and PC content in seminal plasma and sperm cells, as well as forward motility were analysed, but no correlation was found.

This study indicates a significant improvement of kinetic features of sperm cells after 6 months of administration of CoQ_{10}, both on the basis of manual and computer-assisted evaluation. Moreover, these results constitute the first demonstration that exogenous administration of CoQ_{10} increases its levels in seminal plasma and in spermatozoa. The increment was important, especially in seminal plasma where post-treatment levels were 3 times higher than basal ones. Similar increases of CoQ_{10} concentration (2-3 times higher than baseline value) are commonly found in blood plasma after chronic administration of the quinone (75). As CoQ_{10} is a highly lipophylic molecule, we could reasonably hypothesize its diffusion through the phospholipid bilayer of cellular membranes, but we presently do not know whether transport from blood plasma to testicular and accessory male genital glands is passive or involves an active mechanism. Statistical analysis did not reveal any significant functional relationship among the therapy-induced variations of CoQ_{10} and kinetic parameters of spermatozoa, probably due to the low number of samples. Nevertheless, the good degree of association among these variables, according to Cramer's V index of association, supports the hypothesis of a pathogenetic role of CoQ_{10} in asthenozoospermia, according to previously reported data (66).

The first double-blind study

Following the pilot study, a placebo-controlled double blind randomized trial using 200 mg/day of CoQ_{10} in two administrations (Jarrow Formulas LA, USA) was carried out, involving 60 patients (76). The eligibility criteria were the same as in the pilot study. The study design was 1 month run in, 6 months of therapy (30 patients) or placebo (30 patients), and a further 3 months follow-up (controls at months T–1, T0, T+3, T+6, T+9). At various time points the following analysis were carried out: a) semen analysis at months T–1, T0, T+3, T+6, T+9, including C.A.S.A. at months T0, T+3, T+6, T+9 to evaluate modifications in semen parameters (21); b) CoQ_{10} and QH_2 in seminal plasma and sperm cells at months T0 and T+6 to evaluate any variations during therapy [CoQ_{10} levels were assayed in seminal plasma and sperm cells using a dedicated high performance liquid chromatography system with electrochemical detector (Shiseido Co. Ltd.)].

CoQ_{10} levels increased in seminal plasma after treatment, the mean value rising significantly from 61.29±20.24 at baseline to 99.39±31.51 ng/ml after 6 months of exogenous CoQ_{10} administration ($p<0.0001$). A significant increase of CoQ_{10} content was also detected in sperm cells (from 2.44±0.97 to 4.57±2.46 ng/10^6 cells, $p<0.0001$). Similarly, QH_2 levels increased significantly both in seminal plasma and sperm cells after treatment (from 31.54±10.05 to 51.93±16.44 ng/ml, $p<0.0001$; and from 0.95±0.46 to 1.84±1.03 ng/10^6 cells, $p<0.0001$, respectively) (76). No statistically significant modifications were found in the placebo group.

A significant improvement of sperm cell total motility (from 33.14±7.12 to 39.41±6.80%, $p<0.0001$) and forward motility (from 10.43±3.52 to 15.11±7.34%,

Table 3 - *Descriptive statistics of sperm kinetic variables at each timestep: mean, SD and p-value [from (76)].*

	T–1		T0		T+6		T+9	
Sperm total motility	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo group	34.88	8.07	34.81	8.39	34.93	8.04	35.30	8.00
Treated group	32.96	7.19	33.14	7.12	39.41	6.80	32.93	6.33
p (mean of each group)	0.35		0.42		0.03		0.22	
p (mean of the treated group at each time)					<0.0001		<0.0001	
Sperm forward motility	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo group	9.96	4.26	10,.74	4.19	10.11	3.32	10.96	3.75
Treated group	10.17	3.77	10.43	3.52	15.11	7.34	10.07	3.21
p (mean of each group)	0.84		0.76		0.002		0.34	
p (mean of the treated group at each time)					0.0003		<0.0001	
Curvilinear velocity	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo group	28.59	4.71	28.21	5.41	28.30	4.74	28.99	5.20
Treated group	28.03	4.75	27.99	5.32	33.18	4.22	27.73	4.36
p (mean of each group)	0.66		0.87		0.0002		0.33	
p (mean of the treated group at each time)					<0.0001		<0.0001	
Straight progressive velocity	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo group	11.27	2.61	10.84	2.64	10.59	2.49	11.23	2.54
Treated group	10.86	2.62	10.76	2.63	13.13	2.86	10.72	2.40
p (mean of each group)	0.56		0.9		0.0009		0.43	
p (mean of the treated group at each time)					<0.0001		<0.0001	

p=0.0003) was observed in the treated group after 6 months (T+6) of CoQ_{10} administration. The improvement of sperm cell kinetic parameters was also confirmed after computer-assisted analysis, with an increase both in VCL (from 27.99±5.32 to 33.18±4.22 µm/sec, p<0.0001) and VSL (from 10.76±2.63 to 13.13±2.86 µm/sec, p<0.0001) after treatment (Table 3) (76). No statistically significant modifications in kinetic parameters were found in the placebo group. At the end of the treatment (T+6) the mean values of kinetic parameters in the treated group were significantly higher than those in the placebo group, confirming the above results. Interestingly, a weak linear dependence among the relative variations, baseline (T0) and after treatment (T+6), of seminal plasma or intracellular CoQ_{10} or QH_2 content and kinetic parameters was found in the treated group. Furthermore, a significant inverse correlation between baseline (T0) and T+6 relative variations of seminal plasma or intracellular CoQ_{10} or QH_2 content and kinetic parameters was also found in the treated group. In fact patients with a lower baseline value of motility and lower levels of CoQ_{10} had a statistically significant higher probability to be responders to the treatment. After wash out (T+9), sperm cells kinetic features (total and forward motility, VSL) were found to be significantly reduced in treatment groups when compared with month T+6. Improvement of the spontaneous pregnancy rate also suggests that this therapeutic approach is beneficial, although the patients' number was too small for statistical analysis.

CONCLUSIONS

Endogenous CoQ_{10} is significantly related to sperm count and motility, as one could expect considering its important cellular compartmentalization; furthermore, it appears to be one of the most important antioxidants in seminal plasma. Its presence in this compartment does not depend on sperm lysis, as it does not correlate with LDH (53); moreover, its distribution between intra- and extra-cellular compartments seems to be an active process, which is profoundly disturbed in VAR patients (56). CoQ_{10} levels in seminal plasma do correlate with sperm motility. It can be hypothesized that, in certain circumstances, the increased oxidative stress in sperm cells can somehow over-consume CoQ_{10} to the detriment of its bioenergetic role.

Improved sperm motility upon exogenous CoQ_{10} administration could be explained on the basis of the well-known involvement of CoQ_{10} in mitochondrial bioenergetics and of its widely recognized antioxidant properties. Regarding the first point, it is established that mitochondrial concentration of CoQ_{10} in mammals is close to its K_M, as far as NADH oxidation is concerned, therefore it is not kinetically saturating (77). In these conditions one might reasonably hypothesize that a small increase in mitocondrial CoQ_{10} leads to a relevant rise in respiratory velocity. The resulting improvement of oxidative phosphorilation might well affect sperm cells. Since low PC levels in semen were found to be related to a reduction of the phospholipid pool and to low antioxidant capacity (78), the increased PC content in semen after treatment might reasonably involve the restoration of scavenger equilibrium. Another possible reason for this finding is that increased levels of CoQ_{10} also need an appropriate, high concentration of a lipid carrier.

Thus, the administration of CoQ_{10} may play a positive role in the treatment of asthenozoospermia, probably related not only to its function in mitochondrial respiratory chain, but also to its antioxidant properties. The increased concentration of CoQ_{10} in seminal plasma and sperm cells, the improvement of semen kinetic features after treatment, and the evidence of a direct correlation between CoQ_{10} concentrations and sperm motility strongly support a cause/effect relationship. A deeper insight into these molecular mechanisms could lead to a greater knowledge of the so-called unexplained infertility.

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