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Melatonin Administration Alters Semen Quality in Healthy Men

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ABSTRACT: The role of melatonin in the regulation of reproduction in humans is unknown. We conducted a 6-month, double-blind, crossover study of a daily treatment dose of 3 mg melatonin or placebo given orally at 1700 hours in 8 healthy men. Semen quality (concentration, motility, and morphology), serum and seminal plasma 17-β-estradiol (E_2), testosterone, melatonin, and serum gonadotropin levels were determined every 3 months throughout the study. In 6 men, there was no change in semen quality or in serum and seminal plasma hormone levels during the study period. In 2 men, during the melatonin treatment period, sperm concentration decreased to 3×10^6/mL and 12×10^6/mL, and motility declined to 32% and 30%. These coincided with a decline in seminal plasma and serum E_2 levels and with an increase in testosterone:E_2 ratios. Six months after the cessation of melatonin, sperm concentration and motility were normal in 1 man but remained abnormal in the other one with a still elevated testosterone:E_2 ratio. Serum gonadotropin levels were unchanged during the study in all 8 men. Our preliminary observations suggest that long-term melatonin administration is associated with decreased semen quality in a number of healthy men, probably through the inhibition of aromatase at the testicular level.

Key words: Estradiol, seminal plasma, sperm analysis, testosterone.

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The impact of melatonin in human reproduction is still questionable in spite of data suggesting that melatonin and the reproductive hormones are interrelated (Brzezinski et al, 1988; Luboshitzky et al, 1996). Currently, it is accepted that in mammals melatonin acts by influencing gonadotropin-releasing hormone (GnRH) secretion (Sizonenko and Aubert, 1986), although a direct effect on the testis was also suggested (Persengiev and Kehajova, 1991). The effect of exogenous melatonin on pituitary-gonadal hormones in men has been studied during the last 25 years and has revealed no effect on basal morning hormone levels (Nordlund and Lerner, 1977; Wright et al, 1986; Seabra et al, 2000; Siegrist et al, 2001) or on the nocturnal pulsatile secretion of hormones (Luboshitzky et al, 2000).

However, several studies provided evidence that melatonin may have an effect on sperm in humans. Melatonin is present in human semen (Bornman et al, 1989), and melatonin binding sites were demonstrated in human spermatozoa (Van Vuuren et al, 1992). Melatonin in concentrations of 150–450 pg/mL was reported to have an inhibitory effect on sperm motility in vitro (Irez et al, 1992). In addition to melatonin, estrogen may have a role in human spermatogenesis.

Recent data have suggested a role for estrogen in males in general and within the reproductive system in particular (O'Donnell et al, 2001). Support for this concept is provided by the findings of estrogen receptors and aromatase in human testis (Inkster et al, 1995) and by the demonstration of abnormal semen analysis in men affected with congenital estrogen deficiency (Carani et al, 1997). Estrogen receptor-alpha knockout mice were infertile presumably as a result of abnormal fluid resorption in the epididymal efferent ducts (Hess et al, 1997). Aromatase-deficient mice were also infertile but had no efferent duct abnormalities, suggesting a direct action of estrogen on germ cell development (Robertson et al, 1999). These data may imply that locally produced estrogen, or the balance between androgen and estrogen action, is important in spermatogenesis (O'Donnell et al, 2001).

We conducted a study to determine the effects of long-term melatonin administration in healthy men on semen production and on pituitary-gonadal hormone secretion as indicated by the concentrations of gonadotropins and gonadal steroid hormones in serum and seminal plasma.

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Materials and Methods

Participants

Eight men, aged 23.4 plus or minus 1.2 years, who were paid volunteers participated in the study. All were in good health, within plus or minus 10% of ideal body weight, and not receiving any medication. None had a history of cryptorchidism, varicocele, infection, or testicular trauma. Physical examination at the starting point of the study revealed normal secondary sex signs and testicular volumes as measured with a Prader orchidometer (normal value for adult testicular volume: >15 mL). Varicocele and gynecomastia were absent in all participants. None of the subjects experienced any disease or fever during the study period.

The study was approved by the appropriate local Human Subjects committees. All participants gave their informed consent before the start of the study.

Study Protocol

At the first screening visit (baseline), fasting (0800 hours) serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, 17-β-estradiol (E_2), melatonin and a semen analysis (performed after 4 days of abstinence) were determined in all subjects. Eligibility for the study was determined if semen analysis was normal according to previous World Health Organization guidelines (1993) and if serum gonadotropin–gonadal steroid hormone levels were normal. The study was a double-blind, placebo-controlled, crossover design. Subjects were given placebo or melatonin for 3 months and then had a 2-week washout period, after which they received melatonin or placebo for an additional 3 months. The purpose of the washout period was to avoid any possible effect of the medication given in the first 3 months on parameters determined in the second 3-month treatment period. Reevaluation was performed 3 and 6 months after the cessation of all medication (recovery period). Semen and blood samples were obtained every 3 months throughout the study for the determination of semen analysis and serum and seminal plasma concentrations of testosterone, E_2, and melatonin, as well as serum LH and FSH levels. Samples were collected in the morning between 0800 and 1000 hours.

Medications

Subjects were given placebo or 3 mg melatonin orally once a day at 1700–1800 hours. The commercial preparation used in this study (Melatone, Cardiovascular Research Ltd, Concorn, Calif) was previously shown to provide pharmacological serum concentrations (Luboshitzky et al, 2000). Subject compliance with medications was verified by counting the number of study drugs returned divided by drugs dispensed and by the measurement of serum melatonin levels every 2 weeks at 2000 hours and every 4 weeks at 2000 and 0800 hours. The placebo (starch) and melatonin were look-alike white capsules.

Semen Analysis

All semen samples were collected by masturbation after 4 days of abstinence and were brought to the laboratory within 1 hour of collection. Samples were analyzed for volume, sperm concentration, total sperm count, motility, and morphology. The percentage of morphologically normal spermatozoa was evaluated at a final magnification of 1000× using prestained slides (Boehringer, Mannheim, Germany). Semen analysis was performed as described by Jequier and Crich (1986), using an improved version of the Makler counting chamber (Sefi Medical Instruments Ltd, Haifa, Israel). Normal semen analysis data according to World Heath Organization guidelines (1993) are: 1) concentrations more than or equal to 20×10^6/mL, 2) motility more than or equal to 50%, and 3) normal forms more than or equal to 30%.

Hormone Measurements

Blood and semen samples were immediately separated and stored at −20°C until assayed. Serum LH and FSH levels were determined by immunoradiometric techniques (Biodata Diagnostics, Rome, Italy). Serum and seminal plasma testosterone and E_2 levels were determined by competitive immunoassay with the Immulite analyzer (Diagnostic Products Corp, Los Angeles, Calif). The inter- and intra-assay coefficients of variation were 7.1% and 6.4% for E_2 and 7.5% and 7.7% for testosterone. The sensitivity of E_2 and testosterone assays were 44 pmol/L and 0.3 nmol/L, respectively. Serum and seminal plasma melatonin levels were determined by radioimmunoassay (Buhlman Laboratory, Albschwill, Switzerland) with an assay sensitivity of 1.3 pmol/L and intra- and interassay coefficients of variation of 4.9% and 8.3%, respectively. The immunoassays used were validated for the measurement of hormones in semen by the addition of standard as follows: melatonin 250 pmol/L, E_2 231 pmol/L, and testosterone 1.4 nmol/L. The percentage recoveries were 78% for melatonin, 76% for testosterone, and 70% for E_2. The same antisera were used for the determination of hormones in blood and seminal plasma. The antisera were highly specific for testosterone, E_2, and melatonin according to the manufacturer's instructions.

Statistical Analysis

Differences in the subjects' responses in sperm concentration, sperm motility, seminal plasma testosterone, E_2, and the testosterone:E_2 ratio, as well as the serum testosterone, E_2, and testosterone:E_2 ratio between placebo and melatonin (placebo-melatonin), were compared by an independent sample *t* test. Statistical significance was considered at *P* less than .05.

Results

The clinical characteristics of the subjects studied are given in Table 1. All 8 men were nonobese, had normal testicular volume, and had normal serum LH, FSH, and testosterone levels at baseline. The characteristics of all semen analysis data during the study are given in Figure 1. The 8 men studied were divided into groups according

Table 1. *Clinical characteristics of the study group**

Parameter	Subjects 1	2	3	4	5	6	7	8	Mean ± SD
Age (years)	22	26	23	23	22	23	24	24	23.4 ± 1.2
BMI (kg/m^2)	22.8	25.6	22.8	22.1	24.2	23.8	24.2	25.8	23.9 ± 1.2
Testicular volume (mL)†	23	22	24	22	21	25	24	24	23.1 ± 1.3

* BMI indicates body mass index.
† Testicular volume: values are the mean of right and left testicular volumes.

to their response to melatonin administration: nonresponders (n = 6) and responders (n = 2). A subject was defined as a responder if his sperm concentration and motility dropped during the melatonin treatment period. In 6 men (nonresponders), sperm concentration, motility, and morphology were unaltered during the study. In subjects 2, 5, and 8, sperm concentrations were higher during the melatonin treatment period, although all were within the normal range (Figure 1A). This increase in sperm concentration in these subjects was not associated with a similar change in sperm motility. In 2 men (responders), both sperm concentration and motility decreased to subnormal values during the melatonin treatment period. Although the 2 responders had, at baseline, lower sperm concentrations than the 6 nonresponders, the values were well within the normal range, as suggested by the World Health Organization guidelines (1993). Their sperm motility at baseline was similar to the values observed in the 6 nonresponders.

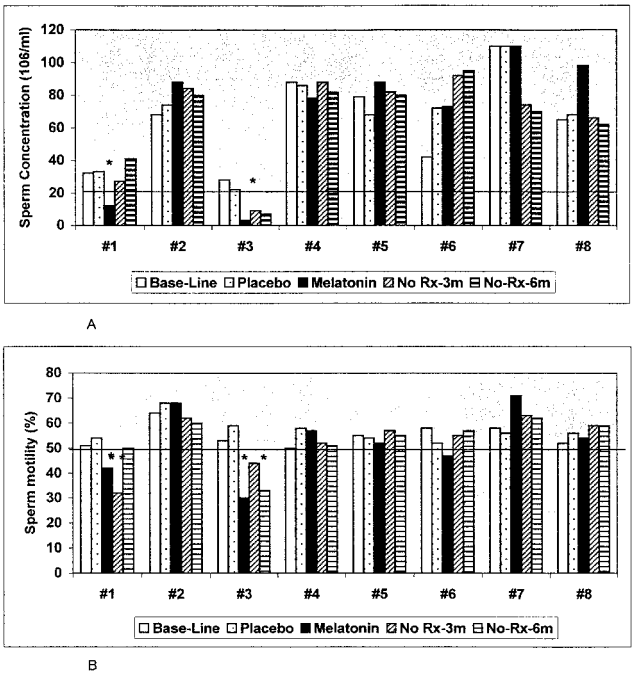

Figure 1. Sperm concentration **(A)** and motility **(B)** in 8 healthy men during 6 months of melatonin-placebo administration. The solid line represents the lower normal limit suggested by World Health Organization guidelines (1993). The sequence of medications was placebo followed by melatonin for subjects 1–4 and melatonin followed by placebo in subjects 5–8. All 8 subjects had a 2-week washout period after the first 3-month treatment period. Statistically different values are indicated with an asterisk.

In subject 1, sperm concentration reverted to normal 3 months after the cessation of melatonin, but motility normalized only 6 months after the cessation of melatonin. In subject 3, sperm concentration and motility remained subnormal 6 months after the cessation of melatonin. Sperm concentrations in subject 6 were higher 6 months after the cessation of all medications, and in subject 7, sperm concentrations were lower (Figure 1A).

Sperm morphology remained unaltered in all 8 men throughout the study. The percentage of normal forms (mean ± SD) in the 8 men was as follows: 62.6 plus or minus 3.5 at baseline, 63.0 plus or minus 2.2 during placebo, 61.4 plus or minus 4.0 during melatonin, 64.4 plus or minus 2.1 at 3 months of no treatment, and 63.2 plus or minus 2.4 at 6 months of no treatment.

Serum and seminal plasma melatonin levels were highly elevated during the melatonin treatment period, whereas serum gonadotropin levels were unchanged in all participants during the study (Table 2).

Serum and seminal plasma testosterone levels were unchanged in all 8 men during the study, whereas E_2 levels were decreased in subjects 1 and 3 during the melatonin treatment period. As a result, serum and seminal plasma testosterone:E_2 ratios were increased in the 2 responders (Figures 2 and 3).

Seminal plasma E_2 levels were three- to fivefold higher than serum hormone levels in all 8 men during the study.

Mean plus or minus standard deviation of the difference between the placebo and melatonin treatments within the 2 groups showed a significant difference in the sperm concentration and motility, seminal plasma testosterone, E_2, and testosterone:E_2 ratio, as well as the serum E_2 and testosterone:E_2 ratio (Table 3).

Discussion

In this study, we examined the effects of melatonin on sperm production and seminal and blood hormone con-

Table 2. *Gonadotropin and melatonin concentrations in blood and seminal plasma during 6 months of melatonin-placebo administration in 8 healthy men**

Variable	Nonresponders (n = 6)†					Responders (n = 2)					
	Subject 1					Subject 2					
	B	P	M	R_1	R_2	B	P	M	R_1	R_2	
Seminal plasma melatonin (pmol/L)	8.1 ± 4.3	10.3 ± 4.3	>250	7.7 ± 3.0	8.2 ± 3.9	2.6	3.0	>250	5.5	6.0	3.4	7.2	>250	4.3	3.0
Serum LH (IU/L)	3.3 ± 1.2	3.8 ± 1.9	4.2 ± 1.9	4.6 ± 1.1	4.9 ± 1.6	4.9	3.7	4.2	3.8	4.7	1.7	2.5	2.7	2.5	2.7
Serum FSH (IU/L)	1.9 ± 1.1	2.3 ± 1.0	2.5 ± 1.0	2.4 ± 0.9	3.1 ± 1.2	1.1	1.2	1.2	1.5	2.9	3.5	3.0	3.4	3.5	1.2
Serum melatonin (pmol/L)	12.3 ± 6.0	8.1 ± 4.3	>450	9.8 ± 2.6	6.8 ± 2.4	6.8	6.4	>450	4.3	6.0	3.4	4.3	>450	10.2	7.2

* B indicates baseline; P, placebo; M, melatonin; R_1, no treatment at 3 months; R_2, no treatment at 6 months; LH, luteinizing hormone; and FSH, follicle-stimulating hormone.
† Data are mean ± SD.

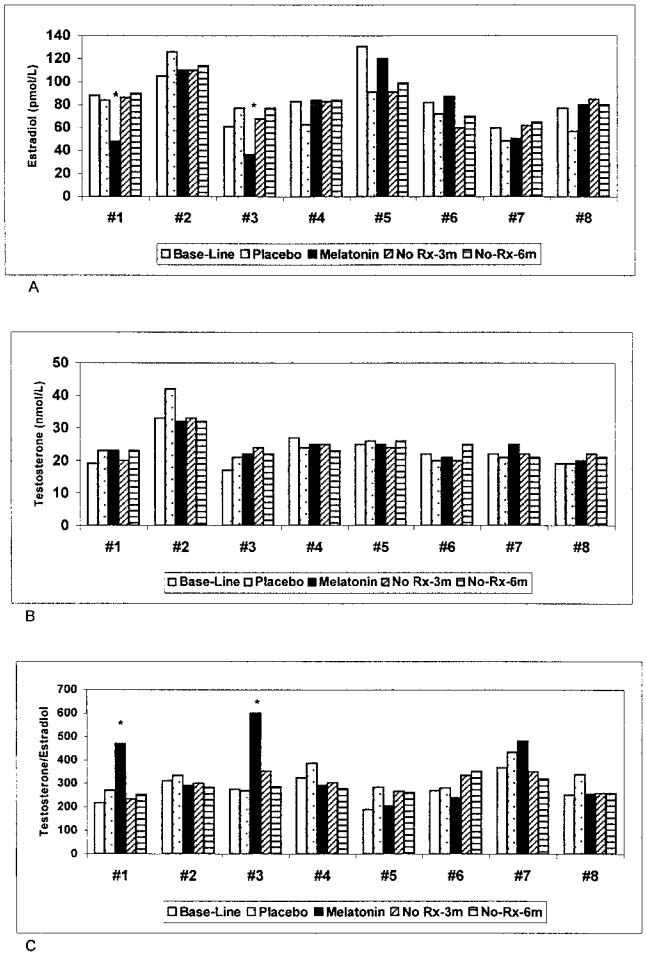

Figure 2. Serum 17-β-estradiol (E_2) **(A)**, testosterone **(B)**, and the testosterone:E_2 ratio **(C)** in 8 healthy men during 6 months of melatonin-placebo administration. The lower normal limit in men is E_2 77 pmol/L and testosterone 10 nmol/L. See legend to Figure 1 for order of medications. Statistically different values are indicated with an asterisk.

centrations. We found a decrease in sperm concentration and motility below the normal range in 2 of 8 men enrolled in a double-blind study of daily treatment. Moreover, in these 2 men, we observed a decrease in seminal and blood E_2 levels with a concomitant increase in testosterone:E_2 ratios. Since the sequence of medications in these 2 men was placebo followed by melatonin, we conclude that the decrease in semen quality was associated with melatonin administration.

We also observed an increase in sperm concentration in 3 subjects during melatonin administration. These counts were within the normal range and were not associated with similar changes in sperm motility or with hormone concentrations. Therefore, these subjects were not regarded as melatonin responders. We attributed this isolated change in sperm concentration to the well-known variations between samples that exist in the same individual (World Health Organization, 1993).

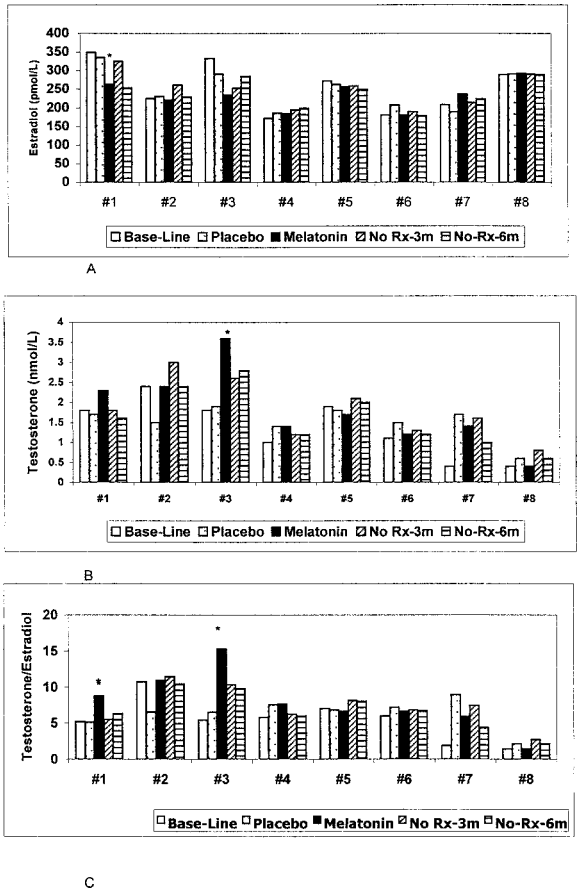

Figure 3. Seminal plasma 17-β-estradiol (E_2) **(A)**, testosterone **(B)**, and the testosterone:E_2 ratio **(C)** in 8 healthy men during 6 months of melatonin-placebo administration. See legend to Figure 1 for order of medications. Statistically different values are indicated with an asterisk.

Previously, Oosthuizen et al (1986) also reported a marked reduction in sperm motility during melatonin treatment. However, the authors did not specify the dose and duration of melatonin administration or the number of subjects studied. Melatonin may adversely affect sperm quality by inhibiting hypothalamic GnRH or pituitary-gonadotropin secretion, by directly affecting spermatozoa, or by changing gonadal steroid concentrations.

The possibility of melatonin action at the hypothalamic-pituitary level is less likely in view of the unaltered serum gonadotropin levels in the current study and their pulsatile secretory patterns in a previous report (Luboshitzky et al, 2000). A direct inhibitory effect of melatonin on sperm motility was suggested in several studies (Irez et al, 1992; Van Vuuren et al, 1992). It was suggested that melatonin inhibition might proceed by acting on sperm membranes or by binding to the tubulin of the sperm flagellum to decrease motility (Irez et al, 1992). The ability of melatonin to suppress experimentally induced lipid peroxidation in sperm membranes was studied in infertile men. Sperm incubated with melatonin showed a reduced rate of lipid peroxidation (Gavella and Lipovac, 2000).

A more plausible explanation is that by inhibiting epididymal and testicular aromatase, melatonin caused a decrease in locally produced E_2 and an increase in the androgen:estrogen balance, resulting in decreased sperm motility and concentration. E_2 given daily to male rats induced inhibitory effects on spermatogenesis and serum testosterone levels. When given with FSH, E_2 multiplied FSH effects on spermatogenesis up to 30 times of control values, suggesting a dual effect of E_2 in testicular maturation (Kula et al, 2001). A possible role for estrogen in human spermatogenesis has been suggested by the findings of estrogen receptors in the testis (Pentikainen et al, 2000) and aromatase activity within the Leydig cell cytoplasm of normal adult humans and in Sertoli cells (Inkster et al, 1995; Luconi et al, 2001). The concentrations of testosterone and dihydrotestosterone were significantly lower in seminal plasma from infertile patients than in that from normospermic men (Zalata et al, 1995).

A significant positive correlation was shown between the testosterone:E_2 ratio and prostatic size, suggesting the participation of estrogen in the regulation of prostatic growth (Shibata et al, 2000). In the present study, we have shown that seminal plasma E_2 levels were several-fold higher than serum hormone levels, suggesting intratesticular (or epididymis, prostate, seminal vesicle) estrogen

Table 3. *Mean ± SD of the difference between the placebo and melatonin treatments within the 2 groups of normal men studied**

	Nonresponders (n = 6)		Responders (n = 2)		*t* Test
	Mean	SD	Mean	SD	*P* value
Sperm concentration	−12.2	11.6	20.0	1.4	.01
Sperm motility	−0.8	7.1	20.5	12.0	.02
Seminal plasma E_2	−1.0	24.6	64.0	9.9	.01
Seminal plasma testosterone	0.0	0.5	−1.2	0.8	.04
Seminal plasma T:E_2	0.0	2.4	−6.2	3.7	.03
Serum E_2	−12.8	17.0	38.0	2.8	.007
Serum testosterone	0.7	4.8	−0.5	0.7	NS
Serum T:E_2	49.7	51.3	−265.0	94.0	.0007

* NS indicates not significant; E_2, 17-β-estradiol (pmol/L); and T, testosterone (nmol/L).

production or a means of increasing estrogen concentrations. Previously, Bujan et al (1993) also found elevated seminal plasma E_2 concentrations in infertile men. Melatonin concentrations and aromatase activity were determined in human seminal plasma and correlated with sperm density and motility. Aromatase activity was determined with an in vitro rat granulosa cell system. Aromatase activity was lower in azoospermic men, while melatonin was higher in azoospermic and oligospermic semen samples. These data suggested that low sperm production is associated with low aromatase activity and that melatonin may have an effect on sperm production and motility (Yie et al, 1991).

These findings may explain why 2 of the 8 men in our study exhibited decreased sperm production, as these 2 subjects had, at baseline, lower sperm concentrations than the other 6 nonresponders. Similarly, DeBleeker et al (1999) reported a case of painful gynecomastia and altered serum androgen:estrogen ratios in a man with amyotrophic lateral sclerosis who had been taking melatonin for several years. On stopping melatonin, symptoms resolved spontaneously.

It has become apparent, in several studies investigating the effects of melatonin on sleep, that there is great importance associated with the time of melatonin administration, especially in the late afternoon hours (Garfinkel et al, 1995). It was postulated that the time of sensitivity of melatonin receptors is between 1700 and 2000 hours. After an oral dose of 2–5 mg given in the afternoon, melatonin serum levels reached pharmacological concentrations within 1 hour and decreased progressively to basal physiological values within 8–12 hours (Guardiola-LeMaitre, 1997).

Currently, millions of people are using melatonin over protracted periods for several reasons, including scientifically unfounded indications such as cancer, anti-aging, and immunodeficiency syndrome (Reppert and Weaver, 1995). When suitably timed, the usual 2- to 3-mg dose of melatonin appears to be beneficial in alleviating symptoms of circadian-based sleep disorders, shift work, jet lag, and delayed sleep phase syndrome; also, it may act as a sleep-promoting agent in elderly insomniacs (Zhdanova and Wurtman, 1997). More recently, beneficial effects of melatonin were reported for the entrainment of sleep-wake cycles in blind people (Sack et al, 2000). Several studies have reported the long-term use of melatonin in children and adolescents with sleep disturbances for periods of up to 6 years (Jan and O'Donnell, 1996; Palm et al, 1997). However, potentially long-term side effects, as reported in the current study, have not been assessed in these studies.

Given the efficacy of melatonin in decreasing semen quality and E_2 levels identified in 2 of 8 men in our preliminary observation, we call for extra precaution when considering the long-term use of melatonin. Longer treatment of more subjects is required before any firm conclusions can be drawn regarding the magnitude of reproductive system inhibition by melatonin in normal men.

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