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ORIGINAL ARTICLE

The impact of melatonin on the sleep patterns of women undergoing IVF: a double blind RCT

Shavi Fernando [1,3,5,*], Sarah Nichole Biggs[2,3], Rosemary Sylvia Claire Horne[2,3], Beverley Vollenhoven[1,4,5], Nicholas Lolatgis[4], Nicole Hope[4], Melissa Wong[4], Mark Lawrence[4], Anthony Lawrence[4], Chris Russell[4], Kenneth Leong[4], Philip Thomas[4,5], Luk Rombauts[1,4,5], and Euan Morrison Wallace[1]

[1]Departments of Obstetrics and Gynaecology [2]Paediatrics, Monash University, Wellington Rd, Clayton, Victoria 3800, Australia [3]Hudson Institute of Medical Research, 27-31 Wright st, Clayton, Victoria 3168, Australia [4]Monash IVF, 7/89 Bridge rd, Richmond, Victoria 3121, Australia [5]Monash Women's, Monash Health, 246 Clayton Rd, Clayton 3168, Victoria, Australia

*Correspondence address. The Ritchie Centre, Department of Obstetrics and Gynaecology, Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia. Tel: +61-3-95946666; Fax: +61-3-85457299; E-mail: shavi.fernando@monash.edu orcid.org/0000-0001-9160-0084

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STUDY QUESTION: Does melatonin result in a dose–response effect on sleep quality and daytime sleepiness in women undergoing IVF?

SUMMARY ANSWER: Melatonin, even when given at high doses twice per day, does not cause significant daytime sleepiness or change night time sleep quantity or quality.

WHAT IS KNOWN ALREADY: Melatonin is being increasingly used as an adjuvant therapy for women undergoing IVF owing to its anti-oxidative effects. It is widely considered to be sedative but there are scant objective data on the effects of melatonin on sleep in the setting of IVF.

STUDY DESIGN SIZE, DURATION: The study was a double-blind placebo-controlled randomized trial of 116 women recruited between September 2014 and September 2016.

PARTICIPANTS/MATERIALS, SETTING, METHOD: Women who were undergoing their first cycle of IVF at private IVF centers were recruited into the RCT and randomized to receive either placebo, 2 mg, 4 mg or 8 mg of melatonin, twice per day (BD) from Day 2 of their cycle until the day before oocyte retrieval. Each participant wore an accelerometer that provides an estimate of sleep and wake activity for up to 1 week of baseline and throughout treatment (up to 2 weeks). They also kept sleep diaries and completed a Karolinska sleepiness score detailing their night time sleep activity and daytime sleepiness, respectively.

MAIN RESULTS AND THE ROLE OF CHANCE: In total, 116 women were included in the intention-to-treat analysis (placebo BD ($n = 32$), melatonin 2 mg BD ($n = 29$), melatonin 4 mg BD ($n = 26$), melatonin 8 mg BD ($n = 29$)). There were no significant differences in daytime Karolinska sleepiness score between groups ($P = 0.4$), nor was there a significant dose–response trend (β=0.05, 95% CI −0.22–0.31, $P = 0.7$). There were no differences in objective measures of sleep quantity or quality, including wake after sleep onset time, sleep onset latency, and sleep efficiency before and after treatment or between groups. There was an improvement in subjective sleep quality scores from baseline to during treatment in all groups, except 8 mg BD melatonin: placebo (percentage change −13.3%, $P = 0.01$), 2 mg (−14.1%, $P = 0.03$), 4 mg (−8.6%, $P = 0.01$) and 8 mg (−7.8%, $P = 0.07$).

LIMITATIONS, REASONS FOR CAUTION: As this was a subset of a larger trial, the melatonin in ART (MIART) trial, it is possible that the sample size was too small to detect statistically significant differences between the groups.

WIDER IMPLICATIONS OF THE FINDINGS: While this study suggests that melatonin can be used twice per day at high doses to achieve sustained antioxidation effects, with the reassurance that this will not negatively impact daytime sleepiness or night time sleep habits, the sample size is small and may have missed a clinically significant difference. Nevertheless, our findings may have implications not only for future studies of fertility treatments (including meta-analyses), but also in other medical fields where sustained antioxidation is desired.

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TRIAL REGISTRATION DATE: 27/11/2013

DATE OF FIRST PATIENT'S ENROLMENT: 1/9/2014

Key words: melatonin / sleep / infertility / ART / IVF / oxidative stress / antioxidant / oxygen scavenger

WHAT DOES THIS MEAN FOR PATIENTS?

This paper asks whether melatonin has an impact on sleep and sleepiness in women who are going through IVF. It reports on a trial carried out in Australia where women having IVF were given melatonin at different doses twice a day during the first part of their IVF cycle. Melatonin is sometimes given to women having IVF as one in three women with fertility problems experience disturbed sleep and it has been suggested this could have a negative impact on IVF outcomes. Melatonin is also used as an antioxidant for women having IVF with the aim of possibly improving egg quality.

Melatonin is not stored easily in the body, so the researchers gave some women higher doses of melatonin as they wanted to see if this would make a difference to their sleep at night, and if it might make them feel sleepy during the day. They found that even at the highest dose, the melatonin did not cause significant daytime sleepiness. They also found that melatonin had no impact on sleep quality, quantity or daytime drowsiness at any dose. Although the women's night time sleep quality improved, the same thing happened to women who were given a placebo rather than melatonin.

This was quite a small study but it does suggest that women could take melatonin for its antioxidant qualities without it making them sleepy in the daytime, but also suggests that melatonin itself is not linked to better sleep in women going through fertility treatment.

Introduction

Women undergoing IVF for the treatment of infertility have increased levels of stress and associated sleep disturbance (Bjornsdottir *et al.*, 2014; Sanford *et al.*, 2014; Shibata *et al.*, 2014; Vitiello *et al.*, 2014). Anxiety is heightened and quality of life reduced in infertile patients (Sezgin *et al.*, 2016; Vitale *et al.*, 2016, 2017a, 2017b; Borghi *et al.*, 2017). Indeed, more than one in three infertile women have disturbed sleep (Lin *et al.*, 2014). Sleep disturbance may interfere with fertility through effects on the hypothalamic–pituitary axis and gonadotrophin regulation (Kloss *et al.*, 2015) or on immunity (Vgontzas *et al.*, 2004; Irwin *et al.*, 2006). It has also been hypothesized that sleep disturbance may have a negative effect on IVF outcomes (Goldstein *et al.*, 2017).

Melatonin has long been used in the general population to manage sleep dysfunction and improve sleep quality. Traditionally, oral melatonin has been used to improve sleep disturbance and better regulate sleeping patterns, particularly in those with insomnia (Garfinkel *et al.*, 1995; Cardinali *et al.*, 2012; Goldman *et al.*, 2014). It is also commonly used in travelers to manage 'jet-lag' (Herxheimer and Petrie, 2002) and by shift workers, as a means of shortening sleep onset latency and facilitating sleep phase shift (Xiang *et al.*, 2015; Sadeghniiat-Haghighi *et al.*, 2016). While not required to be labeled as a sedative, exogenous melatonin is largely believed to facilitate planned sleep onset as well as contribute to circadian regulation.

However, largely because of its additional capabilities as a potent oxygen scavenger and antioxidant enzyme inducer (Reiter *et al.*, 2016), melatonin is being increasingly prescribed for women undergoing IVF, sometimes in combination with other antioxidants such as myo-inositol (Lagana *et al.*, 2017), with the aim of reducing oxidative damage, thereby improving oocyte quality and subsequent pregnancy rates (Fernando and Rombauts, 2014). Therefore, the potential utility of melatonin in IVF treatments is two-fold – it may have a beneficial effect on sleep, but also, because of its potent antioxidant properties, it may protect oocytes and embryos from oxidative damage during the IVF process.

Melatonin has a short half-life when administered as a small single dose per day, therefore serum concentrations of melatonin decline rapidly and are unlikely to result in sustained antioxidation (Waldhauser *et al.*, 1984).

In addition, higher doses of melatonin are known to result in more sustained serum concentrations (Waldhauser *et al.*, 1984). In order to assess the effect of sustained antioxidation on IVF outcomes, the melatonin in ART (MIART) trial (Fernando *et al.*, 2014) was designed to assess twice daily melatonin dosing (at doses as high as 8 mg twice per day) on clinical pregnancy rates after IVF. A subset of patients was also asked to participate in a nested study to determine whether melatonin given in this manner would alter either daytime sleepiness or night time sleep quality and quantity in women undergoing IVF. We report the sleep and sleepiness findings here.

Materials and Methods

Participants

The MIART trial was a double-blind, randomized placebo-controlled multicenter trial of melatonin in women undergoing IVF (ACTRN12613001317785) (Fernando *et al.*, 2014). Eligibility criteria are listed in Table I. We have reported the full trial protocol previously (Fernando *et al.*, 2014). Briefly, participants were recruited from Monash IVF clinics in Melbourne, Australia between September 2014 and September 2016. Randomization was performed by blinded investigators using the minimization method by the factors age, BMI, parity and smoking status (Altman and Bland, 2005). Additional demographic information collected included etiology of infertility and night shift work. One hundred and twenty women (30 in each group) who were enrolled in the MIART trial, received one of four oral medications (placebo, 2 mg, 4 mg or 8 mg of sustained-release melatonin (which looked identical) twice per day (BD)). Trial medication was self-administered between 08:00 and 10:00 and then again between 20:00 and 22:00 from the first day of FSH administration on Day 2 of their menstrual cycle until the night before oocyte retrieval or, in the event of cycle cancellation, on the day of cancellation, whichever came earlier (8–14 days). Participants for this nested study were followed up until the completion of their oocyte retrieval. Medication compliance was assessed by a diary, in which participants recorded the dates and times that trial medication was taken, and by counting the number of capsules remaining after the trial period. All participants and investigators were blinded to the melatonin dose until the conclusion of the trial.

Actigraphy

Sleep quantity and quality were assessed using the Phillips Actiwatch2® (Philips Respironics, Pittsburg, PA, USA), an accelerometer that provides an estimate of sleep and wake activity based on activity level thresholds. Participants were asked to wear the watch all day and night except while bathing or swimming. They were asked to wear the watch for up to one week before ovarian stimulation commenced (baseline) and for up to 16 days during their treatment cycle, until the day of oocyte retrieval (treatment).

Actiwatches® were worn on the non-dominant wrist and configured for individual patients using 1 min epochs (activity measurement intervals) and medium sensitivity (activity threshold = 40 activity counts). Sleep onset and offset was identified after 10 min of inactivity and activity, respectively (Ancoli-Israel *et al.*, 2015). An action marker button was pressed by the participant when she put the watch on, took it off, just before falling asleep and just before getting out of bed in the morning. This allowed for validation of diary and actigraphy recordings.

Actigraphy analysis was performed using the validated Phillips Respironics Actiware® System version 6 (Philips Respironics, Pittsburg, PA, USA) (Cellini *et al.*, 2013). The sleep period was defined as the period of time from when the participant went to bed until the time they got out of bed and was manually scored based on event marker and sleep diary reports.

Data were compared separately for weeknights (Sunday–Thursday) and weekend nights (Friday and Saturday) (Biggs *et al.*, 2016).

Sleep diary

Participants were asked to maintain a sleep diary during the baseline and treatment periods to record bed time, bed time activity, sleep time, wake time and pertinent subjective measures of sleep including a perceived sleep quality ranking (from 1 = 'very good' to 4 = 'very poor'). They were also asked to record daytime sleepiness using the Karolinska sleepiness score (ranging from 1 = 'extremely alert' to 9 = 'extremely sleepy/fighting sleep') (Reyner and Horne, 1998). To allow for comparisons of daytime sleepiness, participants were asked to report their Karolinska sleepiness score at midday every day while taking the trial medication (Reyner and Horne, 1998).

Of the 120 patients, four declined actigraphy (leaving 116 for the intention-to-treat (ITT) analysis) and a further three patients withdrew from the trial before commencing trial medication. Twenty-seven patients were removed for the per-protocol analysis because of incomplete data or if night shifts were worked during the study (Fig. 1). Complete data (including all subjective and objective measures) were available from 89 women (placebo BD, $n = 25$; melatonin 2 mg BD, $n = 22$; 4 mg BD, $n = 17$; 8 mg BD, $n = 25$) for per-protocol analysis (Fig. 1).

Outcome measures

The major concern for participants in the MIART trial was the effect of melatonin on their daytime sleepiness, therefore the primary outcome of the present study was a subjective measure of daytime sleepiness measured on the Karolinska sleepiness scale (Horne and Biggs, 2013). Secondary outcome variables included a subjective ranking of sleep quality, sleep onset latency (time between going to bed and first epoch of sleep), wake after sleep onset (WASO) expressed as both the number of awakenings and the total time awake during the night, total sleep time (time between first and last epoch of sleep, less total time awake after the first sleep epoch), and sleep efficiency (total sleep time/time in bed expressed as a percentage).

Statistical analysis

Statistical analysis was performed using SPSS version 22 (IBM, Armonk, NY, USA). All participants were analyzed in the group that they were originally assigned to. All data were checked for outliers and were normally distributed. Baseline categorical demographics were compared using Chi-square or Fisher's exact tests where required. Continuous data were compared using ANOVA. Daytime sleepiness scores and sleep quality scores were tested for normality and Student's *t*-tests were used to compare means between placebo and any dose of melatonin. Repeated measures ANOVA (group × time) was conducted to determine group differences in subjective measures of sleep parameters between baseline and treatment. Linear regression was used to assess subjective daytime sleepiness and night time sleep quality ratings for any dose–response relationship with increasing melatonin treatment.

Linear mixed model analyses were conducted to determine changes in actigraphy outcomes between groups over time. Actigraphy-measured sleep outcomes were the dependent variables. 'Time' (baseline versus treatment, or 'treatment status') was entered as the repeated measure. 'Group' was entered as the fixed effect and subject code as the random effect and the interaction of group by treatment status (Group × Time) was determined. As there were no baseline differences in covariates, such as age and BMI, none were entered into the model.

Table I Eligibility criteria for women undergoing IVF in a double blind RCT of the impact of melatonin on sleep patterns.

Inclusion criteria	Undergoing first cycle of IVF or ICSI Aged between 18 and 45 years BMI between 18 and 35 kg/m^2 Undergoing a GnRH antagonist cycle (without OCP or Provera scheduling)
Exclusion criteria	Current untreated pelvic pathology – moderate to severe endometriosis, submucosal uterine fibroids/polyps assessed by the treating specialist to affect fertility, pelvic inflammatory disease, uterine malformations, Asherman's syndrome and hydrosalpinx Currently enrolled in another interventional clinical trial Concurrent use of other adjuvant therapies (e.g. Chinese herbs, Co-enzyme Q10, acupuncture) Current pregnancy Malignancy or other contraindication to IVF Autoimmune disorders Undergoing PGD Hypersensitivity to melatonin or its metabolites Concurrent use of any of the following medications Fluvoxamine Cimetidine Quinolones and other CYP1A2 inhibitors Carbemazepine, rifampicin Zolpidem, zopiclone and other non-benzodiazepine hypnotics Inability to comply with trial protocol

OCP, oral contraceptive pill; CYP1A2, Cytochrome P450 1A2.

Sample size was limited by the MIART study and, for this reason, precision estimates have been included in the presentation of results where possible.

Analysis was performed using both per-protocol and ITT principles. Results presented are based on the ITT principle, including all 116 patients who received objective sleep monitoring, unless otherwise stated. A value of $P < 0.05$ was considered statistically significant.

Ethics

This study was registered with the Australian and New Zealand Clinical Trials Register (ACTRN12613001317785). Human research ethics approval was obtained from the Monash Health HREC (13402B), Monash Surgical Private Hospital HREC (14107), Monash University HREC (CF14/523-2014000181) and Epworth HealthCare HREC (634-34). Written informed consent was obtained from all participants prior to enrollment on the trial.

Results

There were no significant differences between the groups in baseline demographics including age, BMI, parity, night shift work, smoking status or etiology of infertility (Table II). Medication compliance was above 95% in each group with no statistically significant differences between groups. The mean (SD) number of days of baseline and treatment recording was 4.7 (2.3) and 10.4 (2.3), respectively, and did not differ between groups. There was no difference between groups in the proportions of weekend nights included (Table II).

Daytime sleepiness scores are summarized in Fig. 2. The Karolinska sleepiness scores were not available for three patients who withdrew prior to commencing trial medication (Fig. 1). In addition, three patients did not record their Karolinska sleepiness score, leaving 110 with recorded Karolinska daytime sleepiness scores. The mean (SD) Karolinska score for these women was 4.21 (1.54), indicating that most women scored as 'relatively alert' for daytime sleepiness. There were no statistically significant differences between groups ($P = 0.4$). There was also no evidence of a dose–response trend between escalating melatonin dose and daytime sleepiness ($\beta = 0.05$, 95% CI −0.22–0.31, $P = 0.7$) (Fig. 2). There was no difference when comparing any dose of melatonin with placebo (Mean difference −0.3, 95% CI −0.9–0.4, $P = 0.4$).

Table III summarizes sleep quality scores. There were no differences in sleep quality between groups and no dose–response trend for sleep quality during treatment ($\beta = 0.05$, 95% CI −0.02–0.13, $P = 0.2$). Sleep quality did improve significantly from baseline to during treatment in all groups, except 8 mg BD melatonin: placebo (percentage change −13.3%, $P = 0.01$), 2 mg (percentage change −14.1%, $P = 0.03$), 4 mg (percentage change –8.6%, $P = 0.01$) and 8 mg (percentage change –7.8%, $P = 0.07$).

Table IV shows the average objective measures of sleep parameters at baseline and during treatment. Women in all melatonin groups got out of bed significantly earlier than participants receiving placebo ($P < 0.05$). However, this did not follow a dose–response pattern. In addition, there were no significant differences in time to bed, time in bed, total sleep time, onset latency, sleep efficiency or WASO either between groups or from baseline to during treatment. There were also no dose-dependent effects from baseline to treatment.

In all analyses, there were no significant differences between the ITT and per-protocol analyses.

Discussion

Here we report sleep outcome data from the first double-blind placebo-controlled randomized trial designed to measure the impact of different doses of oral melatonin on IVF outcome. We have shown that at doses as high as 8 mg BD there is no effect of melatonin on either subjective measures of sleep quality or daytime sleepiness or on objective measures of sleep quality and quantity.

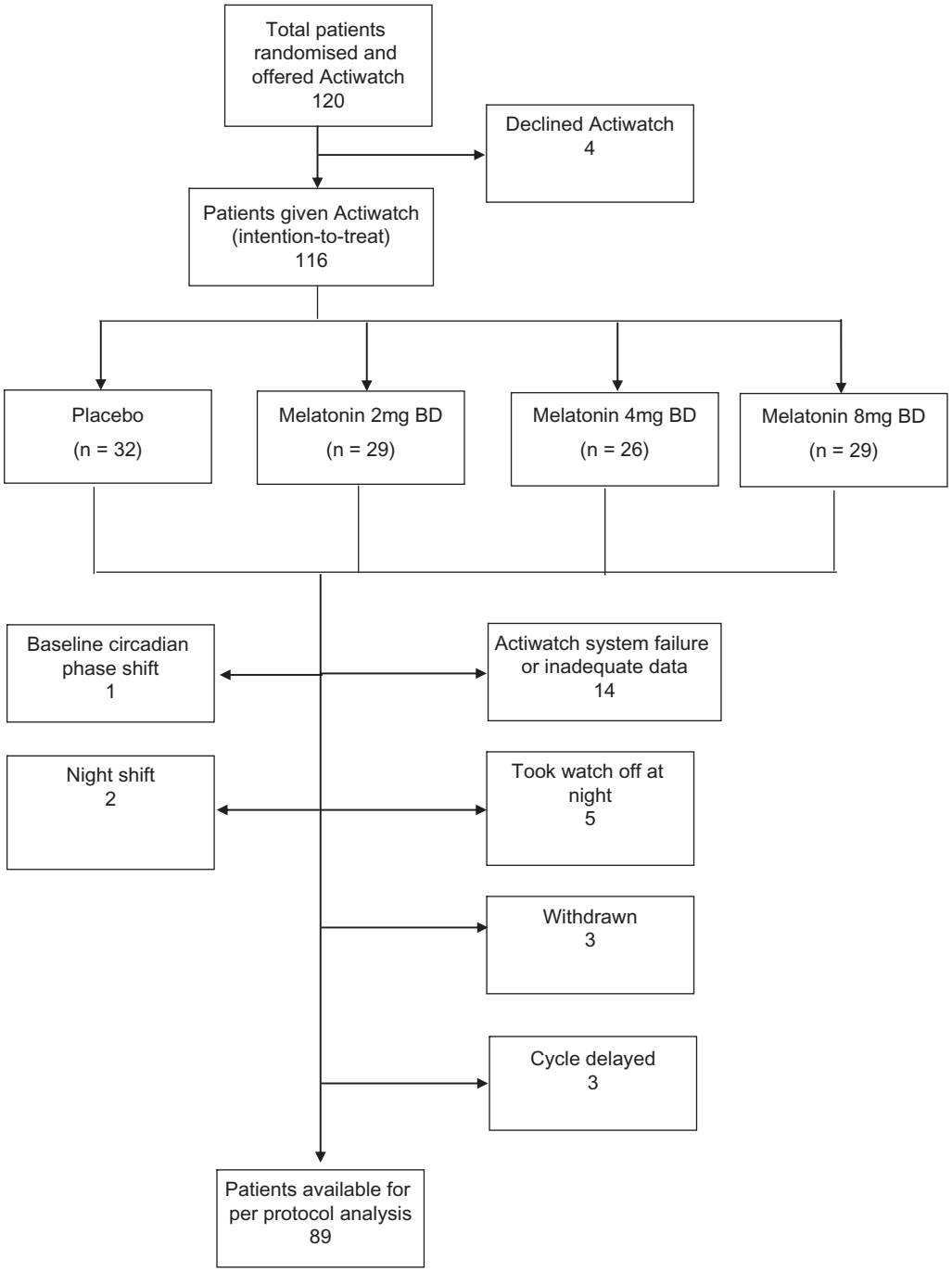

Figure 1 Recruitment flowchart for a RCT of the impact of melatonin on the sleep patterns of women undergoing IVF. Actiwatch is an accelerometer that provides an estimate of sleep and wake activity based on activity level thresholds. BD: twice per day.

A commonly used dose of melatonin during IVF treatment is 3–4 mg given once at night. Melatonin has a relatively short half-life (Waldhauser *et al.*, 1984). Given as a once per day dose, melatonin reaches peak serum concentrations within 1–3 h, which then decline over 8–12 h (Waldhauser *et al.*, 1984). It is likely that such a single daily dosing regimen does not achieve sustained antioxidant effects during the IVF cycle. This may explain why previous studies exploring the efficacy of melatonin in improving IVF success have been inconclusive (Gooneratne *et al.*, 2012). A more frequent dosing regimen, such as BD, would be expected to result in more sustained increases in systemic melatonin levels. This would, in turn, result in more sustained protection from oxidative stress. For this reason, in the MIART trial (Fernando *et al.*, 2014), we chose to examine administration BD in this dose-finding study, aiming to determine whether melatonin given in such a dosing regimen might improve

Table II Demographics of participants.

	Placebo BD *N* = 32	Melatonin 2 mg BD *N* = 29	Melatonin 4 mg BD *N* = 26	Melatonin 8 mg BD *N* = 29
Mean (SD) age (years)	35.3 (4.0)	35.0 (4.5)	35.8 (4.5)	35.9 (4.4)
Mean (SD) BMI (kg/m^2)	24.1 (4.6)	24.6 (4.0)	25.0 (4.8)	25.2 (4.0)
Parity				
0	25 (78.1)	25 (86.2)	21 (80.8)	22 (75.9)
≥1	7 (21.9)	4 (13.8)	5 (19.2)	7 (24.1)
Night shift work *N* (%)	1 (3.1)	1 (3.4)	2 (7.7)	2 (6.9)
Smoker *N* (%)	3 (9.4)	0 (0.0)	1 (3.8)	2 (6.9)
Etiology of infertility				
Endometriosis	4 (12.5)	5 (17.2)	5 (19.2)	2 (6.9)
PCOS	0 (0.0)	3 (10.3)	1 (3.8)	2 (6.9)
Tubal	6 (18.8)	6 (20.7)	3 (11.5)	2 (6.9)
Ovulatory	1 (3.1)	1 (3.4)	0 (0.0)	1 (3.4)
Male factor	5 (15.6)	11 (37.9)	7 (26.9)	12 (41.4)
Social	1 (3.1)	2 (6.9)	1 (3.8)	2 (6.9)
Idiopathic	16 (50.0)	9 (31.0)	14 (53.8)	10 (34.5)
Mean (SD) days of recording baseline*	4.0 (2.4)	4.7 (2.4)	4.9 (2.2)	5.3 (2.3)
Mean (SD) days of recording during treatment*	10.8 (1.9)	10.0 (2.3)	10.6 (2.9)	10.1 (2.2)
Friday or Saturday included in baseline *N* (%)*	14 (56.0)	16 (72.7)	16 (84.2)	22 (84.6)
Friday or Saturday included during treatment *N* (%)*	25 (100.0)	22 (100.0)	18 (94.7)	25 (96.2)
Sunday to Thursday included at baseline *N* (%)*	23 (92.0)	20 (90.9)	17 (89.5)	25 (96.2)
Sunday to Thursday included during treatment *N* (%)*	25 (100.0)	22 (100.0)	19 (100.0)	26 (100.0)

*Data available for 92 patients (89 with complete data and three with data eventually excluded: see Figure 1). All continuous variables reported as mean (SD); BD, twice per day; PCOS, polycystic ovary syndrome.

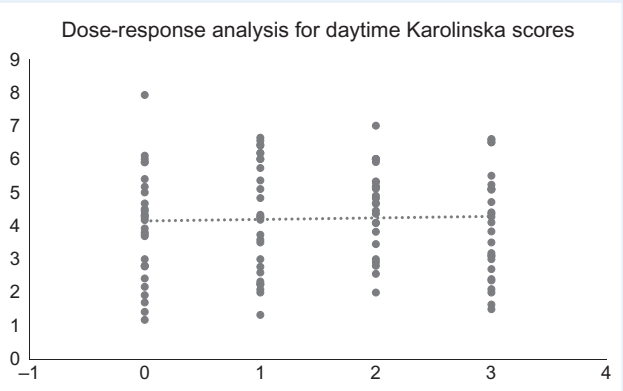

Figure 2 Dose–response analysis for daytime Karolinska sleepiness scores. There was evidence (n = 110) of a dose–response trend between increased melatonin dose and daytime sleepiness score (β=0.05, 95% CI −0.22–0.31, P = 0.7), and no differences in score when comparing any dose of melatonin with placebo (mean difference −0.3, 95% CI −0.9–0.4, P = 0.4).

IVF outcomes. As an *a priori* determined outcome, we also wished to explore the effect of such BD melatonin on sleep and daytime sleepiness because any beneficial effect on sleep in infertile women is likely to have a positive impact on patients (Goldstein *et al.*, 2017) and any negative effects on sleep quality or daytime sleepiness may restrict its use in future clinical trials, in this and other medical disciplines.

Several pharmacokinetic studies have addressed long-term night time dosing of melatonin as a treatment for sleep disorders in the elderly and infirm (Dawson *et al.*, 1998; de Castro-Silva *et al.*, 2010; Lemoine *et al.*, 2011; Gooneratne *et al.*, 2012). Such studies have tested doses ranging from 0.1 mg/nocte to 10 mg/nocte for up to 12 months (Zhdanova *et al.*, 2001; Bourne *et al.*, 2008). Only higher doses of melatonin resulted in a more persistently elevated circulating melatonin concentration and improved night time sleep (Zhdanova *et al.*, 2001). A recent meta-analysis of elderly patients with neurodegenerative disease found that melatonin improves subjective measures of sleep quality, as assessed by the Pittsburgh Sleep Quality Index, but has no effect on objective outcomes (Zhang *et al.*, 2016). For subjective outcomes, this meta-analysis included one trial with only eight participants. The effect of night time melatonin on sleep quality has also been examined in young people. In a trial of 21 adolescents who were given 1 mg melatonin in the evening, melatonin appeared to reduce daytime sleepiness and improve sleep quality (Eckerberg *et al.*, 2012). This trial did not assess morning doses of melatonin and the reduction in daytime sleepiness was attributed to improved sleep quality at night. In a meta-analysis of five studies involving 57 participants, Rossignol and colleagues (Rossignol and Frye, 2011) assessed the impact of melatonin (0.75–15 mg nocte for 14 days to 4 years) on the treatment

Table III Subjective measures of sleep quality between groups.

		Placebo BD N = 25	Melatonin 2 mg BD N = 26	Melatonin 4 mg BD N = 23	Melatonin 8 mg BD N = 26	β (95% CI)*	*P* for trend across groups*	Any Melatonin N = 75	Mean difference (95% CI)^	*P* value
Sleep quality score (mean+/−SD)	Baseline	2.25 (0.53)	2.21 (0.61)	2.22 (0.46)	2.23 (0.48)	−0.003 (−0.09–0.09)	0.9	2.22 (0.52)	0.03 (−0.21–0.26)	0.8
	Treatment	1.93 (0.49)	1.87 (0.45)	2.04 (0.38)	2.05 (0.40)	0.05 (−0.02–0.13)	0.2	1.99 (0.42)	−0.05 (−0.21–0.16)	0.6
Absolute change in sleep quality score (%)		−0.30 (−13.3)	−0.31 (−14.1)	−0.19 (−8.6)	−0.18 (−7.8)	0.05 (−0.04–0.14)	0.3	−0.22 (−9.9)	−0.08 (−0.32–0.16)	0.5
P value**		0.01	0.03	0.01	0.07	–	–	–	–	–

**Paired samples Student's *t*-test; *Linear regression for dose–response trend; ^Student's *t*-test. A lower score indicates better subjective sleep quality.

of sleep dysfunction in children with autism spectrum disorders. They found that night time melatonin improved sleep quality overall, increased sleep duration and shortened sleep onset latency. Taken together, these studies suggest that melatonin, when given at night, can be used to improve sleep. However, they provide no insights into the effect of melatonin on sleep and daytime sleepiness when the melatonin is given during the day.

In that regard, we found that daytime administration of melatonin, up to 8 mg BD, which would be considered a relatively high dose, affected neither subjective nor objective measures of sleep when compared with placebo. We did find that night time sleep quality significantly improved from baseline among almost all women, including women taking the placebo. The only group of women not to show a statistically significantly improved sleep quality was the group taking the highest dose of melatonin (8 mg twice daily). The reason for apparently improved sleep quality among the other three groups of women, including those taking the placebo, is not immediately apparent and was not expected. It is possible that a Hawthorne effect, where participants 'respond' solely because they are aware that they are being studied (McCambridge *et al.*, 2014), was at play. It is also possible that the relief of starting IVF treatment, in the hope or expectation of success, improved sleep quality. Sleep quality varies across the menstrual cycle (de Zambotti *et al.*, 2015; Mehta *et al.*, 2015) and that might also explain our findings because, by necessity, we compared sleep between the menstrual phase and late follicular phase. However, all of these possible explanations would apply equally to all groups of women.

Because of its circadian effects, most studies of melatonin have been performed in a 'sleep' setting, and therefore, dosing has been nocturnal. In our study, melatonin was administered BD, with the intention of sustaining its antioxidant affect throughout the day, without limiting it to the circadian night. Despite high doses given in the morning and evening, melatonin did not increase daytime sleepiness. This suggests that melatonin can be administered, even at relatively high doses, during the day without effecting daytime alertness. Because of its short half-life (Waldhauser *et al.*, 1984), this would allow for a more sustained anti-oxidant effect throughout the circadian cycle. This increases the dosing possibilities for the use of melatonin as an antioxidant to treat oxidative stress-related medical conditions (Esteban-Zubero *et al.*, 2017; Martinez-Campa *et al.*, 2017), with the reassurance that morning dosing does not increase daytime sleepiness. However, these findings should first be confirmed in further studies in these clinical contexts.

The strength of our trial is its randomized double-blind design and inclusion of a placebo group. This is particularly important when assessing subjective outcomes. We also assessed the effects of three different doses of melatonin with a view to likely future dosing regimens. If melatonin is shown to be effective, whether in IVF or for other indications such as fetal neuroprotection or as an adjuvant therapy for pre-eclampsia (Hobson *et al.*, 2013; Biran *et al.*, 2014; Miller *et al.*, 2014), this study provides supportive evidence that melatonin (at the doses tested) is unlikely to affect sleep quality or quantity when used in the short term. The analysis and comparison of both subjective and objective measures of sleep also minimizes the likelihood of bias.

A limitation of this study was the small sample size. The number of women in each group was determined by pregnancy rate, the primary outcome of the MIART trial (Fernando *et al.*, 2014), rather than by expected differences in sleep quality. It is possible that the sample size

Table IV Average objective measures of sleep quality/quantity by group at baseline and during treatment.

	Placebo BD		Melatonin 2 mg BD		Melatonin 4 mg BD		Melatonin 8 mg BD		Group *F*-value	Time *F*-value	Time × Group *F*-value
	Baseline (*N* = 23)	Treatment (*N* = 25)	Baseline (*N* = 21)	Treatment (*N* = 22)	Baseline (*N* = 18)	Treatment (*N* = 19)	Baseline (*N* = 25)	Treatment (*N* = 26)			
Bed time#	21:33 (4:39)	21:28 (4:35)	22:31 (0:50)	21:17 (4:45)	19:02 (8:12)	20:16 (6:55)	20:32 (6:07)	21:19 (4:22)	1.10	0.05	0.56
Get up time#	7:09 (1:07)	7:16 (0:44)	7:30 (0:49)	7:20 (0:48)	7:59 (1:40)	7:28 (1:04)	7:19 (1:00)	6:46 (0:55)	2.12	7.83[a]	2.87
Time in bed (hours)	8:33 (1:15)	8:50 (1:06)	8:59 (1:00)	8:58 (0:34)	8:57 (1:15)	8:40 (0:42)	8:52 (1:00)	8:33 (0:43)	0.48	0.91	2.43
Total sleep time (hours)	6:57 (1:03)	7:04 (0:52)	7:18 (0:54)	7:20 (0:36)	7:03 (0:54)	6:52 (0:32)	6:54 (1:35)	7:00 (0:41)	1.03	0.01	0.38
Onset latency (mins)	20.4 (17.6)	22.5 (14.0)	22.1 (19.4)	19.6 (10.9)	33.5 (23.3)	28.7 (13.1)	21.9 (15.9)	21.6 (14.6)	2.62	0.34	0.63
Sleep efficiency (%)	81.6 (8.9)	80.4 (5.4)	81.5 (6.2)	81.9 (4.5)	79.3 (6.1)	79.3 (5.1)	77.7 (16.7)	81.9 (4.8)	0.63	0.53	1.24
WASO (mins)	51.3 (23.9)	53.6 (22.6)	54.8 (19.5)	57.0 (20.7)	60.6 (21.9)	58.6 (17.7)	59.4 (26.7)	52.2 (14.5)	0.52	0.38	1.56

Linear mixed model analysis showing differences between treatment groups (Group), between baseline and treatment status (Time), and the interaction of group by treatment status (Group × Time); WASO: Wake after sleep onset; sleep efficiency: total sleep time/time in bed; Bonferroni for all comparisons; mins: minutes; #24-hour clock; [a]$P < 0.05$.

was too small to detect statistically significant differences between the groups. We sought to address this by accounting for the inter-individual differences through the mixed model analysis. The size of this study limits generalizability of the results; however, the data presented here would be appropriate for inclusion in future meta-analyses.

Actigraphy has been described as a viable and practical alternative to polysomnography in monitoring sleep disturbance (Hyde *et al.*, 2007; Marino *et al.*, 2013; Meltzer *et al.*, 2012). While this has the advantage that measurements can be taken in an environment more indicative of actual sleep conditions and is economical and minimally labor intensive, it is limited because assumptions are drawn based on the level of movement and its association with sleep, with limitations being more obvious in its specificity and in the detection of wakefulness (Meltzer *et al.*, 2012; Horne and Biggs, 2013; Marino *et al.*, 2013). We sought to overcome these limitations by the use of the device in conjunction with a detailed sleep diary (Wolfson *et al.*, 2003) and by including a period of 'calibration' (de Castro-Silva *et al.*, 2010; Horne and Biggs, 2013).

In summary, in this small randomized trial, we have shown that daytime dosing of melatonin in doses of 2 mg, 4 mg or 8 mg BD does not appear to affect night time sleep quality or daytime sleepiness in the population studied. While these findings are reassuring for the use of daytime melatonin, whether in future clinical trials in assisted reproduction or other conditions that might benefit from melatonin's antioxidant actions (Hobson *et al.*, 2013; Miller *et al.*, 2014), the limitations associated with our sample size must be considered when considering such trials.

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Authors' roles

S.F., S.N.B., R.S.C.H., E.M.W. and L.R. contributed to inception, concept and design, acquisition of data, data analysis and interpretation, drafting of the manuscript, critical revision of the manuscript and approval of the article. B.V., M.L., N.H., M.W., A.L., C.R., K.L., P.T. contributed to acquisition of data, drafting of the manuscript, critical revision of the manuscript and approval of the article.

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Conflict of interest

S.F., E.W., R.H., B.V., N.L., N.H., M.W., M.L., A.L., P.T., K.L. have nothing to declare. L.R. is a Minority shareholder in Monash IVF Group has unrestricted grants from MSD®, Merck-Serono® and Ferring® and receives consulting fees from Ferring®. S.N.B. reports consulting fees from Johnson & Johnson Consumer Inc®, outside the submitted work.

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