Effect of exogenous melatonin treatment on the reproductive characteristics and progeny of male rats exposed to different periods from light and darkness

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Summary

Light is an important environmental factor that controls the regulation of physiological functions of organisms. Melatonin is considered as one of the major hormones that play an important role in protecting body from many harmful effects and acts as a powerful anti-oxidant source to controlling the levels of antioxidant enzymes within the body. The main objectives of this work were to study the effect of 1) photoperiod changes and 2) exogenous melatonin hormone treatment on the sperm properties, body and testis weight, embryo characteristics, and concentration level of melatonin and testosterone in treated rat serum. This study has been conducted using thirty-six male rats and one hundred and twenty female rats. Animals were divided into six groups, each group containing six males and twenty female rats. Our results showed a significant influence of photoperiod changes on the melatonin and testosterone hormones concentration in rats serum, body weight only, while the other characteristics not affected. As for the effect of melatonin it has been found that the movement of sperm in GIII and IV, as well as embryos absorbed and newborns weight traits has been significantly affected, while the rest of the properties were not significantly affected by melatonin treatment.

Key words: Melatonin, Photoperiod, Embryo, Sperm properties

Introduction

Light and photoperiod changes from one season to another are considered as important environmental factors that affect the physiological processes in the animal, including the production of hormones and sperm by influencing the hypothalamus (Hotzel *et al.* 2003, Sogorescu *et al.* 2011, Rani and Kumar 2014).

Semen quality is important in the assessment of male fertility, sperm must be examined for each male where gives the reason for the lack of fertility. Many researchers have examined the relationship between fertility and semen characteristics and daily light cycle and they found conflicting results (Sellés *et al.* 2003, Popwell and Flowers 2004, Ruiz-Sánchez *et al.* 2006,

Broekhuijse *et al.* 2012, Petrocelli *et al.* 2015). Favre *et al.* (2012) reported that natural photoperiod induces seasonal changes in spermatozoa quality but not in serum testosterone concentrations. Photoperiod changes determine increased secretion of testosterone and testicular volume (Hotzel *et al.* 2003, Sogorescu *et al.* 2011 and 2012). The response to photoperiod was similar in different animals, such as sheep and goats that show variations in testosterone secretion and semen quality according to changes in long day and then light and darkness hours (Delgadillo and Chemineau 1992, Delgadillo *et al.* 2000, Lebouef *et al.* 2000, Sogorescu *et al.* 2011 and 2012). The percentage of normal spermatozoa was higher through February to July than during August to January (Axner and Linde 2007). Also, Blottner and Jewgenow (2007) found that there are significant differences between spring and autumn for the sperm count per testis and weight of testis. As mentioned before by Borg *et al.* (1993) and Kozdrowski and Dubiel (2004) reported that the sperm volume and concentrations were lowest in the spring, gradually increase in the summer and reach to the top values in late autumn season. Therefore, photoperiod changes are involved in regulating the reproductive cycles and are linked with functions in several mammals and avian (Misra *et al.* 2004, Hazlerigg and Wagner 2006, Dixit and Singh 2011). This is called photoperiodism (Rani and Kumar 2014).

Melatonin has been studied on a large scale during the past decades due to its physiological functions and medical importance. The chemical composition was identified after being isolated from the bovine pineal glands in 1956 (Lerner *et al.* 1959). Melatonin hormone is called sleep hormone, secreted by the pineal gland with rhythmically and is influenced by environmental photoperiod (Donmez *et al.* 2004), it is not only produced from mammalian organs and cells, but can be produced from non-mammalian species cells such as bacteria and edible plants (Lerner *et al.* 1959, Hardeland *et al.* 1995, Dubbels *et al.* 1995, Tilden *et al.* 1997, Stefulj *et al.* 2001, Rocha *et al.* 2015). Also, balsamic vinegars and dessert wines as example for grape product were found to contain melatonin (Vitalini *et al.* 2013). Many functions of melatonin are described in the past decades through numerous researches. Among these reported

functions, melatonin has anti-proliferative, anti-inflammatory and apoptotic properties (Perdomo *et al.* 2013). In addition, a study by Levine *et al.* (1990) found that count, concentration and motility of men sperm were significantly decreased during the longer days in summer. Peschke *et al.* (2006) reported important role for melatonin in metabolic deficiencies and glucose regulation. Furthermore, melatonin works to protect the testicular functions and spermatogenesis from many harmful effects of many disorders (Rocha *et al.* 2015). In addition, the roles of melatonin were attributed to its ability to minimize the oxidative stress by removing nitrogen species, free radicals and control the levels of oxidative enzymes (Hardeland *et al.* 1995, Lerner *et al.* 1959, Espino *et al.* 2010, Galano *et al.* 2011, Lampiao and Plessis 2013, Chabra *et al.* 2014). Noticeably, data indicated that melatonin administration causes a decrease in seminiferous tubules diameter, inhibition of spermatogenesis and reduction size of Leydig cells (Ooi and Ng 1989, Sogorescu *et al.* 2012). Many other functions of this hormone are described in the last decades. However, its overall impact on the physiological male reproductive system is still not well known (Rocha *et al.* 2015). The aim of this study was to determine the effect of photoperiod changes and treated with exogenous melatonin treatment on sperm characteristics, acquired body weight, testicle weight, serum melatonin and testosterone hormone level and embryos properties in groups of male and female rats.

Materials and Methods

This study was conducted at the Department of Zoology, Faculty of Science, King Saud University from 25 / 10 / 2015 to 24 / 1 / 2016.

Animals

In this study, thirty-six male (five males only used in mating process for each group) and one hundred twenty females from Albino Wister rat strain were used. Animals were obtained from Faculty of Pharmacy, King Saud University, and their weights ranged from 220 to 250 g for males and 180 to 200 g for females and all animal aged was about 12 weeks. Rats have been

bred in a special room; temperature was ranging from 22 to 24 °C. Water and feed were introduced ad libitum. Feed was purchased from the general organization for Grain Silos and Flour Mills, Riyadh, Saudi Arabia. The feed used in experiments was composed from 20% protein, 4% fat, 3.5% fiber, 0.6% phosphorus, 6.0% ash, 0.5% sodium chloride, 1.0% Calcium, 20.0 I.U vitamin A, 2.2 I.U vitamin D and 70.0 I.U vitamins C.

Experimental Design

Male rats were divided according to exposure to light and darkness cycle for ten weeks (Mukherjee and Haldar 2014) and treatment with melatonin by intraperitoneally injection for 4 weeks (Alabbassi *et al.* 2008) to 6 groups (G) as shown in table 1. Group I (GI) was considered as control group for groups III (GIII) and V (GV) to study the effect of light/darkness cycle. Also, GI, GIII and group V (GV) were considered as control for GII, group IV (GIV) and group VI (GVI), respectively, to study the effect of melatonin.

As for females, they has been maintained under lighting cycle consists of 12 hours light and 12 hours darkness from beginning the experiment until the tenth week for the regulation its reproductive cycle before mating. In the tenth week, four mature females were placed with one male from previous each groups in table (1) for six days to make mating. Insemination was detected daily, the insemination day was considered as a zero day of pregnancy. The fertilized females were divided into two groups*;* the first group has been sacrificed in the nineteenth day of pregnancy to determine the number of live and absorbed embryos and weights of live embryos. The second group was left until after the birth to determine the number of embryonic implantation and compare it with the number of live newborns, and fetus's weights.

Melatonin preparation

Melatonin (Melatonin powder, Sigma, USA, M5250) has been dissolved in pure sesame oil (Goldman 1991), which was obtained from the Plant Oil Kattouf Vallery, Jeddah, Saudi Arabia prior to injection directly. The dose used was prepared by dissolving 1 g of melatonin in

100 ml of sesame oil to obtain 20 mg/kg concentration. The volume of dose used was determined by using the following equation:

Volume required (ml) = Dose (mg/kg) X animal weight (Kg) / Melatonin concentration (mg/ml)

The dose of melatonin was injected between 7 and 8 am daily.

Experimental measurements

Testosterone and melatonin assay: Serum testosterone and melatonin were analyzed in blood samples collected at the end of experiment from males using testosterone Elisa Kit (Fisher Scientific, USA, Cat. No. 89-100-162) and rat melatonin Elisa kit (Fisher Scientific, USA, Cat. No. 50-155-280) respectively, as described in the manufacturer manual kit.

Body weight: The body weight of rats was taken after six weeks from experiment start to study the effect of lighting cycle on body weight and at the end of experiment and treatment with melatonin, the body weight was taken again to study the effect of treatment with melatonin on the body weight.

Testes weight: The testes weight of male rats exposed to lighting cycle and treatment with melatonin was taken at the end of experiment.

Pregnancy rate: The pregnancy rate was calculated by the number of pregnant females at the end of the experiment for each male

Number and weight of embryos and newborns: The number and weight of embryos was determined after sacrificing pregnant females at nineteenth day of gestation. As for the newborns, the number and weights were taken after the birth.

Sperm collection and analysis

After treatment throughout the experiment, sperms were collected from all male rats by removed tail epididymis from sacrificed males to assess the motility, total number and live and dead of sperm using semen analyzer, HAMILTON THORNE IVOS (Sharon *et al.* 2015).

Statistical analysis

All data was analysis using paired sample t-test and one way analysis of variance (ANOVA). Treatment means were compared using Duncan test at $P \leq 0.05$ significant level. Analyzed data were expressed as mean ± SE.

Results

Effect of light/darkness cycle

Lighting cycle had a significant effect ($p \leq 0.05$) on the body weight, where the mean value was greatest in the GV (129.0 ± 13.8) at 18 h light and lowest in GI (73.2 ± 14.5) at 12 h light. On the other hand, it does not have any significant effect on the testes weight (table 2). Similarly, for characteristics of sperm (table 3) and embryos, pregnancy rate and implantation sites (Fig. 1), it was found that the photoperiod changes have no significant effect.

Moreover, table (4) showed that the change in lighting period leads to increase in the concentration level of melatonin hormone in rat blood serum from 2.4 ± 0.2 in GI to 44.5 ± 3.5 in GIII and then decreased to 33.0 ± 8.0 in GV with increasing lighting period (18 h light: 6 h darkness). While, the testosterone concentration level was not different significantly with decrease the light period from 12 h in GI to 6 h light in GIII, but decreased to 2.6 ± 0.1 with maximum period of light in GV (18 h light). Then, the concentrations of hormones were increased with decrease the light period and down with increase the light period. In addition to, results in Fig. 1 showed that there is no any significant difference between GI, GIII and GV concerning with embryo and newborn characteristics and implantation sites number.

Effect of melatonin

Body and testes weight were not significant at $p \leq 0.05$ between all experimental groups (Fig. 2). Similar results were obtained for sperm characteristics as showed in Fig. (3A), when comparing GI with GII and GV with GVI. As the same results, were found between GIII and GIV for mean values of total number sperm and dead sperm (Fig. 3A). On the other hand,

significant difference ($p \leq 0.05$) between the GIII and GIV was found with respect to normal and abnormal sperm movement trait (Fig. 3B). Melatonin treatment showed a positive impact on the melatonin and testosterone hormones level in the blood serum as showed in table (5), where very highly significant difference was found between the GI and GII, GIII and GIV, GV and GVI, with the exception of melatonin level between GV and GVI. Figure (4A, B and C) showed that embryos characteristics and implantation site traits were not significantly affected by treatment with melatonin. While, the mean values of the life new born weight and absorbed embryos characteristics showed significant differences between GI and GII (Fig. 4A).

Discussion

The results of this study showed that the sperm, embryos and newborns characteristics, testis weight and implantations sites were not affected by changing photoperiods. While, body weight increased in the third and fifth groups. However, we have found an increase in the total number of sperm, pregnancy rate, number and weight of live newborns in the third group, but this increment was not significant when compared to GI. The same result when compared the GV with GI for pregnancy rate, live newborns and the normal sperm movement. The effect of photoperiod and seasonal variations on reproductive in different animals was studied by many researches (Delgadillo *et al.* 1993, Kozdrowski and Dubiel 2004, De Ambrogi *et al.* 2006, Enciso *et al.* 2006, López-Fernández *et al.* 2007, Knecht *et al.* 2013, Petrocelli *et al.* 2015, Lourdes *et al.* 2015). Earlier works on F344 and BN male rats showed decrease in reproductive and body mass by short photoperiod (Heideman and Sylvester 1997, Lorincz *et al.* 2001). Also, Olayaki *et al.* (2008) showed that there was significant reduction in reproductive organ mass, viability, counts, and sperm motility when rats were exposed to short photoperiod. On the other hand, Ben Saad and Maurel (2002) reported that short photoperiod has an improvement effect on male reproductive characteristics in wild rabbits. Moreover, long photoperiod resulted in non-significant increase in sperm properties, but it increased the testis weight (Olayaki *et al.* 2008).

This result disagree with Petrocelli *et al.* (2015), who showed that long photoperiod has significantly effect on the sperm concentration. Also, this result disagreed with results of Bagher *et al.* (2014) who mentioned that melatonin could improve the sperm motility. These results agreed with previous reports by Ciereszko *et al.* (2000), Sancho *et al.* (2004) and (2006). Moreover, results of many studies agreed with our results in this study. It was reported that melatonin has no effect on sperm properties as these studies have shown that there is no relationship between the number of sperm and melatonin treatment (Casao *et al.* 2010, da Silva *et al.* 2011, Martín-Hidalgo *et al.* 2011, Ortiz *et al.* 2011). The body weight was higher during spring season than other seasons and plasma testosterone was higher during autumn and summer than during winter and spring (Lourdes *et al.* 2015). On the other side, our results agreed with Karakas and Gündüz (2003) and Bagher *et al.* (2014) who found that melatonin had no effect of the body weight. Although, there are some works indicated the melatonin may be able to affect energy intake and showed a decrease in the body weight (Marcon *et al.* 2008). The results of our study may be explained due to the increase melatonin in the rat serum in the third and fifth groups, and increased testosterone in the first and third groups only. Melatonin works as regulators for reproductive function in mammals through inhibitory action on melatonin receptors in the anterior pituitary gland, reproductive organs, and hypothalamus as described by Zemkova and Vanecek (1997), Balik *et al.* (2004), Soares *et al.* (2003) and Frungier *et al.* (2005). In addition, Heterosis may be affecting the reproductive characteristics (Smital 2009). The current results showed that serum testosterone was significantly higher in GII and GVI when compared with their control, while, the concentration of the same hormone was reduced significantly in GIII. Sarbast *et al.* (2013) and Kus *et al.* (2002) supported these results. The difference in the concentration of testosterone may refer to the relationship between the melatonin, photoperiod and gonadotropin releasing hormone (GnRH) secreted from pituitary gland. Where, secretion of Luteinizing hormone (LH) and GnRH was affected by melatonin administration (Valenti *et al.* 1997 and 1999).

Conclusion

The present study indicated that the light/darkness cycle affect the body weight and melatonin and testosterone hormones, but not have any significant effect on the sperm and embryos characteristics, testes weight, pregnancy rate and implantation sites. Moreover, treatment with exogenous melatonin resulted in increase of melatonin and testosterone hormones concentration in case exposed rats to 12 and 18 h light only, but not significantly affect all other characteristics.

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Table 1. Groups of treated and non-treated animals, light/darkness cycle and melatonin injection

Groups (G)	Treatment Beginning	GI	GII	GIII	GIV	GV	GVI
Treatment		Control					
12 h light/12 h darkness	First week to Tenth week						
(Natural photoperiod)		√	√	_	_	_	_
6 h light/18 h darkness	First week to Tenth week						
(Short day)		_	_	√	√	_	_
18 h light/6 h darkness	First week to Tenth week						
(Long day)		_	_	_	_	√	√
Melatonin	Seventh week for 28 days						
(20 mg/kg)		_	√	_	√	_	√
Sesame Oil (0.3 – 0.4 ml)	Seventh week for 28 days						
(Melatonin Solvent)		√	_	√	_	√	_

√ = **Treated** **-- = non-treated**

Table 2. Effect of light/darkness cycle on the acquired body and testes weight in groups non-treated with melatonin

Traits / Treatment groups	acquired body weight	Acquired testes weight
Group I (GI)	73.2 ± 14.5 [a]	1.77 ± 0.0
Group III (GIII)	112.8 ± 8.3 [b]	1.78 ± 0.1
Group V (GV)	129.0 ± 13.8 [b]	1.75 ± 0.0

* Mean values within the same column with different superscripts (a, b) differ significantly ($p \leq 0.05$).

Table 3. Effect of light/darkness cycle on the sperm characteristics in groups non-treated with melatonin

Traits / Treatment groups	Total number of sperm/ml X 10^6	Normal movement type	Abnormal movement type	Dead sperm
Group I (GI)	1146.7 ± 69.3	309.3 ± 33.9	478.7 ± 66.0	326.2 ± 23.4
Group III (GIII)	1454.7 ± 169.0	226.4 ± 58.3	419.5 ± 97.4	289.6 ± 35.4
Group V (GV)	1067.3 ± 132.0	286.0 ± 32.7	345.4 ± 59.6	318.4 ± 102.3

- Mean values comparison within the same column at $p \leq 0.05$

Table 4. Effect of light/darkness cycle on the melatonin and testosterone hormones level (pg/ml) in the serum

Traits / Treatment groups	Melatonin hormone	Testosterone hormone
Group I (GI)	2.4 ± 0.2 [a]	5.4 ± 0.2 [b]
Group III (GIII)	44.5 ± 3.5 [b]	5.6 ± 0.2 [b]
Group V (GV)	33.0 ± 8.0 [b]	2.6 ± 0.1[a]

- Mean values within the same column with different superscripts (a, b) differ significantly ($p \leq 0.05$).

Table 5. Effect of exogenous melatonin treatment on the melatonin and testosterone hormones level (pg/ml) in the serum

Traits / Treatment groups*	Melatonin hormone	Testosterone hormone
Group I (GI)	2.4 ± 0.2 [a]	5.4 ± 0.2 [a]
Group II (GII)	48.8 ± 6.7 [b]	8.7 ± 0.2 [b]
Group III (GIII)	44.5 ± 3.5 [b]	5.6 ± 0.2 [b]
Group IV (GIV)	20.9 ± 1.5 [a]	2.6 ± 0.2 [a]
Group V (GV)	33.0 ± 8.0	2.6 ± 0.1[a]
Group VI (GVI)	37.2 ± 4.1	7.9 ± 0.3 [b]

- Mean values within the same column with different superscripts (a, b) differ significantly ($p \leq 0.05$).

* Comparisons between each sequence two groups

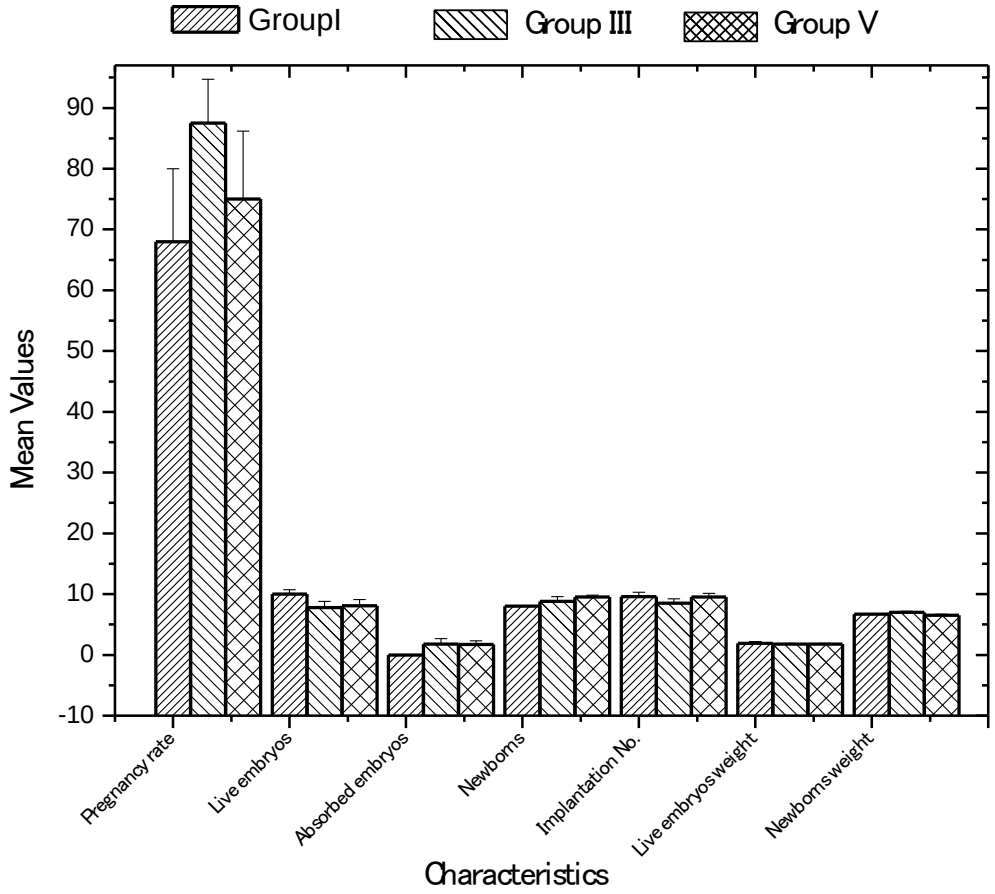

Fig. 1. Effect of light/darkness cycle on the embryos characteristics and implantation sites after mating with experimental males

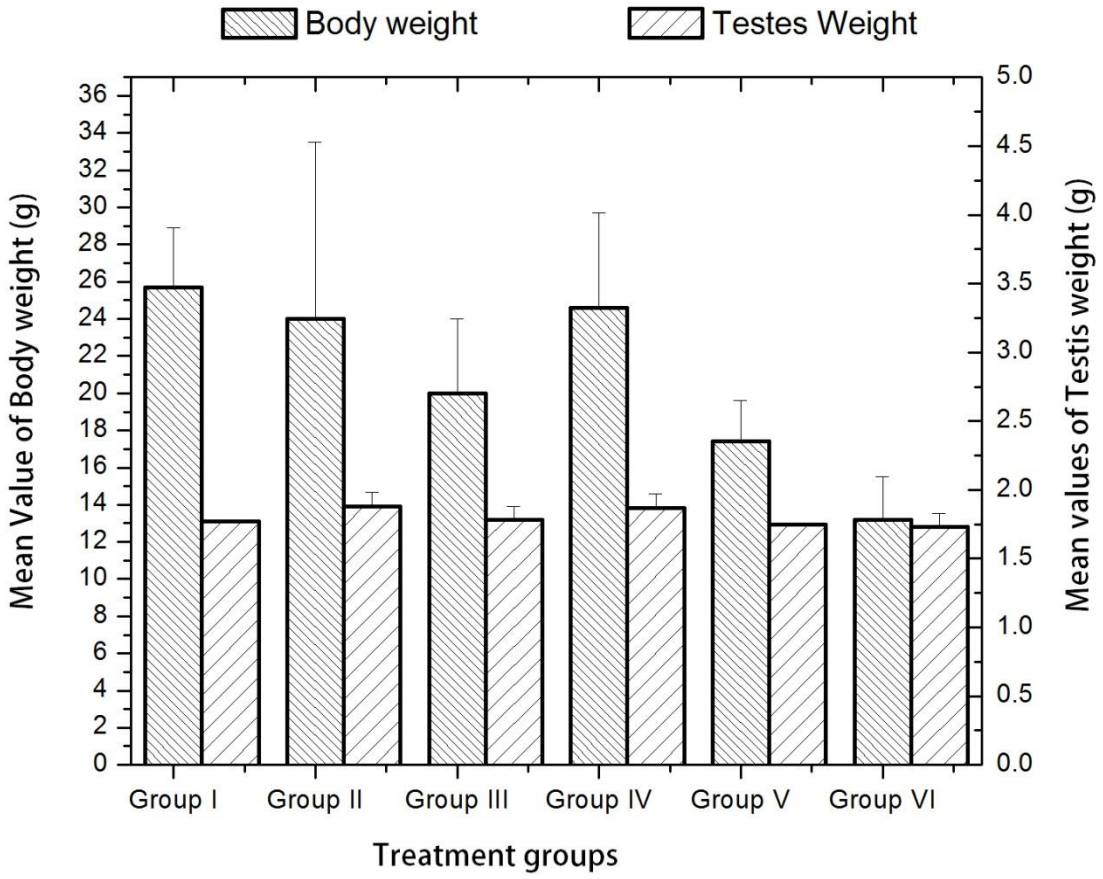

Fig. 2. Effect of exogenous melatonin treatment on the acquired body and testes weight (g) compared with untreated groups for each light/darkness cycle alone.

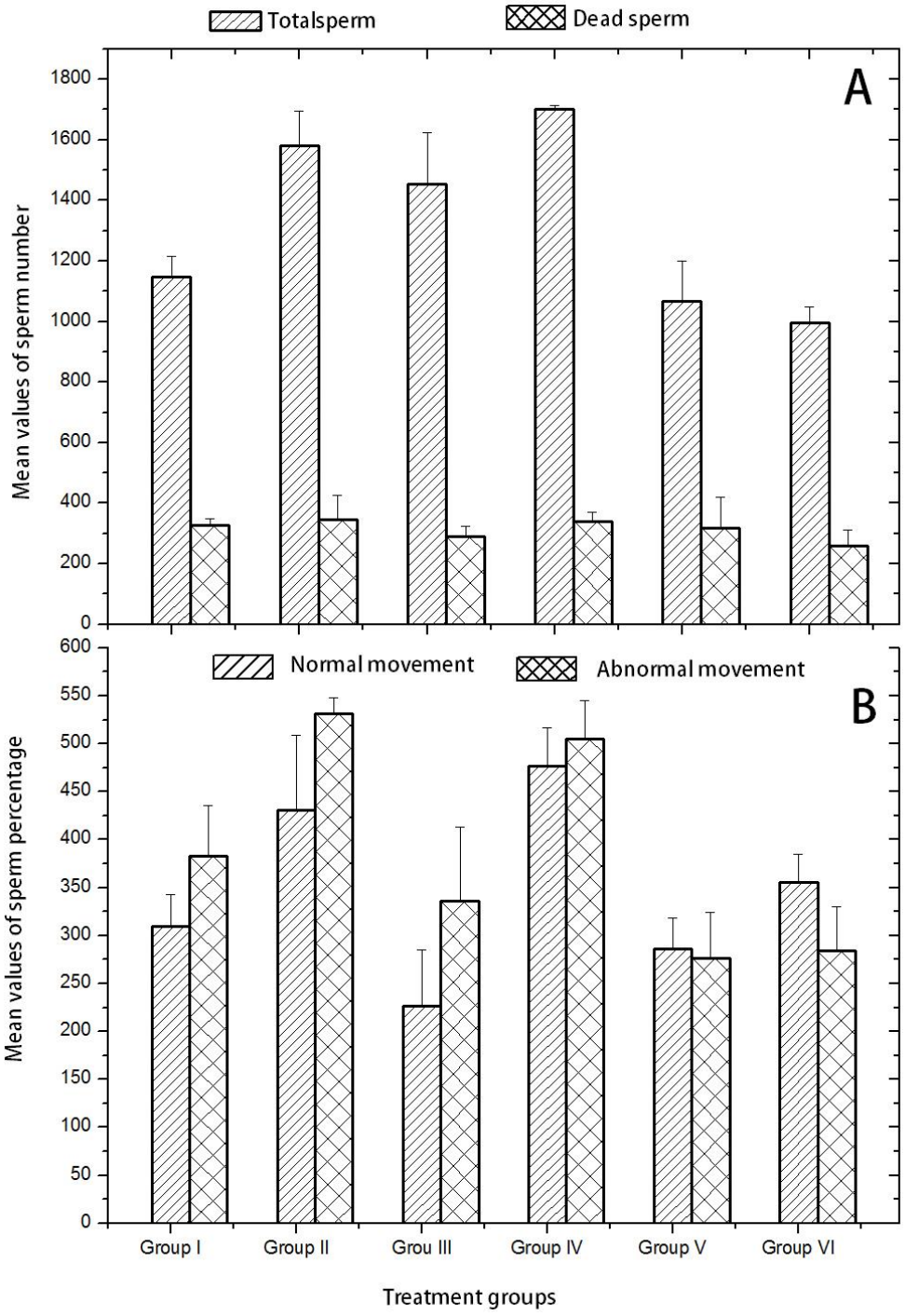

Fig. 3. Mean values of sperm characteristic in groups treated with exogenous melatonin compared to untreated groups in each light/darkness cycle alone.

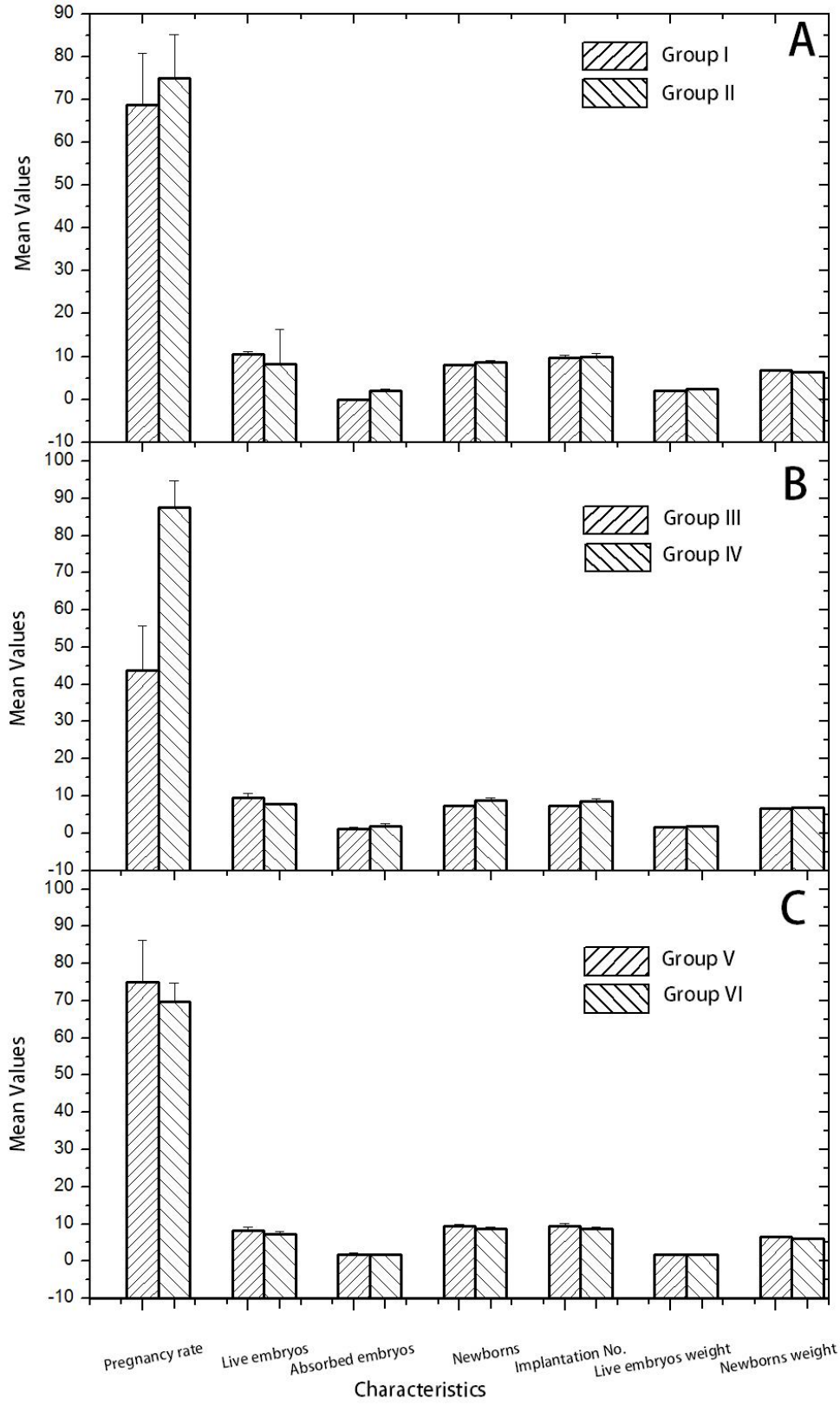

Fig. 4. Effects of melatonin injection on the embryo and newborns characteristics and implantation sites in groups for each light/darkness cycle alone.