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Research Paper

Effect of melatonin supplementation on pregnancy outcome in Wistar-Kyoto and Sprague-Dawley rats

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Abstract: Although melatonin supplementation is known to influence numerous physiological functions, little is however known of its effects on pregnancy outcome. This study investigated the effects of melatonin supplementation on pregnancy outcome in Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats aged 12–13 weeks. Upon confirmation of proestrus, each female rat was housed overnight with a male of the same strain. On the next morning, following confirmation of mating (vaginal smear), WKY female rats were isolated into individual metabolic cages and given 0, 25, 50 or 100 mg/kg per day of melatonin in drinking water from day 1 of pregnancy to day 21 postpartum. SD females were given 0 or 100 mg/kg per day of melatonin. Maternal weight, duration of pregnancy, litter size, birth weight and body weight of pups up to day 42, and pup mortality were recorded. Data were analyzed using ANOVA for repeated measures. Compared to controls, maternal weight gain during pregnancy was significantly lower in melatonin-supplemented dams ($P < 0.01$). Litter size was significantly smaller in melatonin-supplemented dams ($P < 0.01$). Mean birth weight of pups was significantly lower only in pups of dams given 100 mg/kg per day of melatonin ($P < 0.001$). Mean body weight of pups of dams given melatonin was significantly lower than controls ($P < 0.01$). Pup mortalities were 9.5% and 21.6% in WKY dams given 25 and 100 mg/kg per day of melatonin respectively, and all pup deaths occurred after day 21 of weaning. The results suggest that melatonin supplementation during antenatal and postpartum period appears to adversely affect litter size, pup growth and mortality in WKY and SD rats. The precise mechanism causing the death is not clear.

Key words: melatonin; mortality; weight gain; pregnancy; pup growth

褪黑素对大鼠妊娠结局的影响

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摘　要：褪黑素能调节多项生理功能，但其对妊娠结局的影响所知甚少。本研究旨在探讨补充褪黑素对12~13周龄的Wistar-Kyoto (WKY)和Sprague-Dawley (SD)大鼠妊娠结局的影响。动情前期确认后，每只雌性大鼠与一只同品种雄性大鼠同笼饲养过夜，第二天阴道涂片确认交配后，雌性大鼠转移至单独的代谢笼，WKY大鼠每天给予含0、25、50 或100 mg/kg褪黑素的饮水，SD大鼠则每天给予含0或100 mg/kg褪黑素的饮水，从怀孕第一天持续至产后21天。记录母鼠体重、孕期、窝产仔数、仔鼠出生体重和出生后7~42 d体重及仔鼠死亡率，使用重复测量的方差分析对数据进行分析。结果显示，与对照组相比，褪黑素组母鼠孕期体重增长较小($P < 0.01$)，窝产仔数减少($P < 0.01$)，仔鼠平均出生体重仅在100 mg/kg褪黑素组显著降低($P < 0.001$)，出生后7~42 d仔鼠平均体重显著降低($P < 0.01$)。25 和100 mg/kg褪黑素组WKY仔鼠死亡率分别为9.5%和21.6%，且都发生在断奶21天后。以上结果提示，产前和产后补充褪黑素会严重影响WKY和SD大鼠窝产仔数、仔鼠生长和死亡率。

关键词：褪黑素；死亡率；体重增长；妊娠；幼崽生长
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Melatonin (N-acetyl-5-methoxytryptamine), a pineal gland hormone, has been shown to regulate sleep, immune system [1], reproductive function in seasonal breeders [2], and, more recently, to improve sperm quality in humans [3]. There is also evidence supporting its blood pressure lowering [4, 5], antioxidant [5–7] and pain modulating properties [8, 9].

Whilst numerous beneficial effects of melatonin supplementation have been reported, information on its adverse effects is scarce, except for a few isolated studies. Melatonin administration to adult rats has been found to (i) suppress LHRH release and disrupt normal estrus cycle and fertility [10], (ii) delay sexual maturation in female rats [10], and (iii) disrupt spermatogenesis in young male rats [11]. In the red deer subcutaneous implants of melatonin were found to inhibit mammary development during pregnancy [12]. In one of our recent studies investigating the effects of melatonin supplementation on the development of hypertension in SHR offspring, it was noted that while administration of melatonin in drinking water during pregnancy and up to postpartum day 21 had no effect on the survival or growth of SHR pups, it however caused significant mortality in pups of the Wistar-Kyoto (WKY) strain [13]. Neither the reason for the difference in its effects on WKY and SHR pups nor the cause of death in the WKY rats was immediately evident. Previous studies have revealed no significant toxic effects on either the dams or the pups following melatonin supplementation during pregnancy [14, 15]. Administration of 150 mg/kg per day of melatonin to Sprague-Dawley (SD) rats throughout gestation, for example, did not show any toxic effects of melatonin [16]. A recent review even concluded that treatment with melatonin during pre- and postnatal period might help reduce complications in the neonatal period and provide wide ranging health benefits [17]. The evident severe adverse effects in the WKY rats following maternal melatonin supplementation reported recently are therefore puzzling. It is unclear if the effects are unique to a particular strain of rat or just to a particular colony within the strain. This study therefore examined the effects of melatonin supplementation at doses of 25, 50 and 100 mg/kg per day during pregnancy on the duration of pregnancy, litter size and pup growth in WKY rats. To examine if the effect was strain specific, 100 mg/kg per day of melatonin was also similarly supplemented to age-matched pregnant SD rats.

1 METHODS

Twenty-four female WKY rats, aged 12–13 weeks, were housed in cages at room temperature with a 12/12 hours light/dark cycle and given *ad libitum* excess to food and water. Proestrus was confirmed through a vaginal smear, and after confirmation of proestrus, each female was housed overnight with a fertile male of the same strain (for 12 h). Mating was confirmed by a positive sperm smear on the following morning. This was marked as pregnancy day 1 (PD 1). Following mating, females were isolated to individual metabolic cages. WKY dams were randomised into 4 groups, with 6 dams per group. From day 19 of pregnancy the cages were inspected for birth every day from 9:00 to 11:00 h, and 16:00 to 18:00 h. The 24-hour period within which the dams delivered their pups was designated as postnatal day 1 (PND1). Group 1 (control group) received tap water, whereas groups 2, 3 and 4 received 25, 50 and 100 mg/kg per day of melatonin (Sigma Chemicals Co., USA) respectively in drinking water during pregnancy and up to day 21 postpartum. In a separate study, twelve SD dams were similarly prepared and randomised into 2 groups with 6 dams per group. Group 1 received just tap water, whereas group 2 received 100 mg/kg per day of melatonin. For the preparation of melatonin, 100 mg of melatonin was first dissolved in 15 mL of 20% ethanol and then made up to 100 mL with deionised tap water. For controls, 20 mL of 20% ethanol was made up to 100 mL with deionised tap water. From our previous experiences, we noted that water intake though increases from the pre-pregnancy state, it however remains relatively stable over the duration of pregnancy; ranging between 24 and 27 mL per day [5]. Consequently, based on an average intake of 25 mL per day, a stock melatonin solution containing 4 mg/mL was prepared daily, which was then diluted further with deionised tap water to 2 mg/mL and 1 mg/mL solutions to deliver 100, 50 and 25 mg/kg per day of melatonin respectively. As fluid intake increases postpartum, the dilution of melatonin containing drinking water was also adjusted accordingly. To avoid degradation by light, all drinking containers were kept wrapped in aluminium foil at all times.

All dams were weighed every four days during gestation and on PND 1. Volume of water consumed was recorded every four days using metabolism chambers to confirm the dosage of melatonin. Our earlier studies revealed that although water consumption increases

during pregnancy when compared to before pregnancy, it however remains generally stable for the duration of pregnancy [5]. Based on this, we therefore only measured water intake every four days in the rats throughout the period of study. Gestation period (in days) and litter size was recorded, and the litter was weighed individually at birth and then once every week until the age of 6 weeks.

Following either the death of the animal during the period of study or euthanization upon completion of the study period, external examination of the animal was performed for any physical abnormalities. The whole animal was immediately stored immersed in 10% formalin for histological studies at a later date. The liver, gastrointestinal tract (GIT), and kidneys were removed and processed in a tissue processor for paraffin embedding. The tissues were made into blocks, sectioned into 3–5 micron thick sections, and then stained with hematoxylin and eosin. The stained sections were examined under light microscopy. Data were analyzed using ANOVA for repeated measures, and if significant then a *post hoc* Tukey test was performed. Student's *t* test was performed where appropriate, and data are presented as mean ± SEM. All statistical tests used were those contained in the Statistical Package for the Social Sciences (SPSS) software version 15.

All animals used in the study were supplied by the animal care and breeding facility at Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia. The study was approved by the Animal Care and Users Committee, Universiti Teknologi MARA.

2 RESULTS

No significant differences were evident in fluid intake between the various groups during pregnancy (Table 1). Water intake ranged between 24 and 26 mL per day, delivering the prescribed dosage of melatonin. Water intake during the postpartum period was significantly higher when compared to that during pregnancy ($P < 0.01$; Table 1).

No significant difference was evident in mean body weight between the controls and the four experimental WKY groups on day 1 of pregnancy (PD1) except for the group of dams assigned to receive 25 mg/kg per day of melatonin, which had a slightly higher body weight (Table 2). On day 20 of pregnancy (PD20) mean body weight was only significantly lower in dams receiving 50 mg/kg per day of melatonin when compared to that in controls, but there was no difference in mean body weight between controls and the remaining melatonin treated groups. However, mean percentage change in overall body weight during pregnancy was significantly lower in melatonin supplemented WKY dams ($P < 0.001$) compared to that in controls. Mean body weight on PND1 was also significantly lower in WKY dams receiving melatonin when compared to the controls ($P < 0.01$). The mean difference in peri- and postpartum body weight was significantly greater in melatonin supplemented WKY dams ($P < 0.001$) except for dams receiving 50 mg/kg per day of melatonin. Percentage gain in actual maternal weight during pregnancy, which is the difference in body weight on PND1 and PD1, was significantly lower in melatonin supplemented

Table 1. 24-hour water intake during pregnancy and postpartum period in rat (mL/day)

	WKY (control)	WKY (25 mg/kg per day melatonin)	WKY (50 mg/kg per day melatonin)	WKY (100 mg/kg per day melatonin)	Sprague-Dawley (control)	Sprague-Dawley (100 mg/kg per day melatonin)
Pregnancy (d)						
4	24.8 ± 2.1	25.6 ± 1.1	23.8 ± 2.4	25.8 ± 1.1	26.8 ± 1.9	24.6 ± 1.8
8	26.1 ± 2.3	24.2 ± 2.2	26.3 ± 2.3	24.5 ± 2.7	25.1 ± 1.2	26.1 ± 1.4
12	25.5 ± 1.6	25.9 ± 1.7	25.4 ± 1.8	26.5 ± 1.9	25.1 ± 1.1	25.2 ± 0.9
16	26.3 ± 2.4	25.3 ± 1.8	24.9 ± 1.8	26.2 ± 1.8	25.3 ± 1.3	25.6 ± 1.1
Postpartum (d)						
24	28.3 ± 1.1	28.6 ± 1.2	29.1 ± 1.7*	28.4 ± 1.5	28.7 ± 1.3	29.3 ± 0.9*
28	30.2 ± 2.6**	29.6 ± 0.7*	28.2 ± 1.9	30.3 ± 2.1**	29.9 ± 1.5	30.2 ± 0.7**
32	31.4 ± 1.2**	30.3 ± 1.4**	32.4 ± 1.7**	30.4 ± 0.9**	31.4 ± 1.2**	31.4 ± 1.2**
36	33.2 ± 2.2**	34.8 ± 3.2**	33.6 ± 2.1**	34.2 ± 2.6**	33.8 ± 2.3**	35.2 ± 2.8**
40	40.2 ± 1.6**	39.2 ± 2.9**	38.8 ± 2.2**	41.7 ± 3.9**	41.8 ± 1.8**	40.6 ± 2.3**

$^{*}P < 0.05$, $^{**}P < 0.01$ *vs* pregnancy.

dams compared to that in controls ($P < 0.05$).

No significant difference was evident in mean body weight between the two groups of SD rats on PD1 (Table 2). However, mean body weight on PD20 ($P < 0.05$), mean percentage change in overall body weight ($P < 0.001$), body weight on PND1 ($P < 0.001$) and actual maternal weight gain during pregnancy (PND1–PD1) ($P < 0.001$) were significantly lower in melatonin supplemented SD dams compared to those in controls.

Litter size was significantly smaller in all melatonin treated WKY dams compared to that in controls ($P < 0.001$; Table 3). The number of pups was 66, 42, 47 and 37 in the controls, 25, 50 and 100 mg/kg per day of melatonin treated dams respectively. The number of pups that died was 0, 4, 0, and 4 in the controls, 25, 50 and 100 mg/kg per day of melatonin treated dams respectively. Death occurred between the age of 28 and 42 days.

Litter size was slightly but significantly smaller in melatonin treated SD rats when compared to that of SD controls ($P < 0.05$) (Table 3). No deaths were recorded in the SD pups over the duration of the study.

The pattern of change in body weight of pups of WKY dams supplemented with melatonin is presented in Fig. 1. Mean body weight of pups of dams supplemented with 100 mg/kg per day of melatonin was lower than that in controls at birth and remained lower throughout the study period ($P < 0.05$ and 0.01, ANOVA). At the age of 28, 35 and 42 days mean body weight of pups of all dams supplemented with melatonin was significantly lower than that of controls ($P < 0.01$ and $P < 0.001$, ANOVA).

Mean birth weight of the pups of SD dams supplemented with melatonin during gestation was signifi-

Table 2. Ante partum and postpartum body weight in melatonin supplemented and control dams

	Mean body weight (g) on PD1	Mean body weight (g) on PD20	Overall weight gain during pregnancy (%) [(PD20 – PD1)/PD1]	Mean body weight (g) on PND1	Difference in weight between PD20 and PND1 (%)	Maternal weight gain during pregnancy (%) [(PND1 – PD1)/PD1]
			WKY rats			
n	6	6	6	6	6	6
Control	171.16 ± 6.93	260.66 ± 1.30	52.3 ± 1.3	231.00 ± 2.29	11.4 ± 1.6	34.9 ± 1.1
25 mg/kg per day melatonin	188.16 ± 5.60*	265.00 ± 3.65	40.8 ± 1.9***	214.00 ± 3.45***	19.2 ± 1.1***	13.7 ± 1.8***
50 mg/kg per day melatonin	170.83 ± 3.73	255.00 ± 2.84*	45.2 ± 0.9***	220.50 ± 2.92**	13.5 ± 1.3	29.3 ± 1.2*
100 mg/kg per day melatonin	184.16 ± 7.42	257.00 ± 4.03	39.6 ± 1.4***	206.83 ± 5.26***	19.5 ± 0.8***	12.1 ± 2.3***
			Sprague-Dawley rats			
n	6	6	6	6	6	6
Control	203.58 ± 10.90	297.11 ± 15.46	45.9 ± 3.0	254.31 ± 6.65	14.4 ± 1.6	21.1 ± 1.5
Melatonin	195.16 ± 6.56	261.45 ± 2.82*	33.9 ± 1.4***	230.06 ± 2.46***	12.0 ± 0.9	16.1 ± 1.8***

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ *vs* their respective controls. PD: Pregnancy day; PND: Postnatal day.

Table 3. Litter size per dam of controls and melatonin supplemented WKY and Sprague-Dawley dams

Group	Mean litter size per dam (*n*)		
	At birth	22-day old	42-day old
	WKY rats		
Control	11.0 ± 0.57	11.0 ± 0.57	11.0 ± 0.57
25 mg/kg per day melatonin	7.0 ± 1.15***	7.0 ± 1.15***	6.33 ± 1.02***
50 mg/kg per day melatonin	7.8 ± 0.69***	7.8 ± 0.69***	7.8 ± 0.69***
100 mg/kg per day melatonin	6.16 ± 1.07***	6.16 ± 1.07***	5.5 ± 1.05***
	Sprague-Dawley rats		
Control	9.16 ± 0.57	9.16 ± 0.57	9.16 ± 0.57
Melatonin	8.16 ± 0.22$^{\#}$	8.16 ± 0.22$^{\#}$	8.16 ± 0.22$^{\#}$

$^{***}P < 0.001$ compared to WKY controls; $^{\#}P < 0.05$ compared to Sprague-Dawley controls.

cantly lower than that in controls and remained consistently lower throughout the study period (Fig. 2; $P < 0.001$). There was no pup mortality in SD rats.

No significant differences were evident in the duration of pregnancy between melatonin treated and untreated WKY rats or SD rats (Table 4). Mean birth weight was significantly lower in WKY pups of dams given 100 mg/kg per day of melatonin when compared to that of pups of untreated WKY dams ($P < 0.001$). Similarly, mean birth weight was also lower in SD pups of dams treated with 100 mg/kg per day of melatonin when compared to that of pups of SD controls (Table 4).

Macroscopic examination of the pups revealed no obvious defects in the physical appearance of the liver, GIT or the kidney apart from them being slightly smaller in size than those in age-matched controls. Histologi-

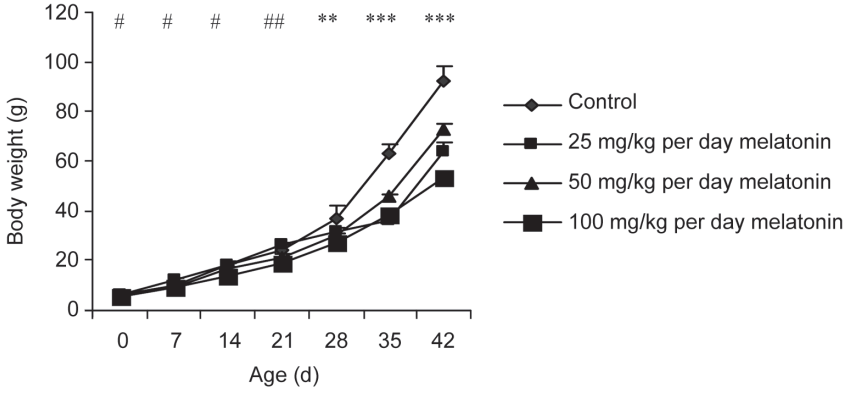

Fig. 1. Body weight changes of pups of controls and melatonin supplemented WKY dams. $^{**}P < 0.01$, $^{***}P < 0.001$ when all melatonin treated groups were compared to controls (ANOVA). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ when 100 mg/kg per day of melatonin treated rats were compared to controls.

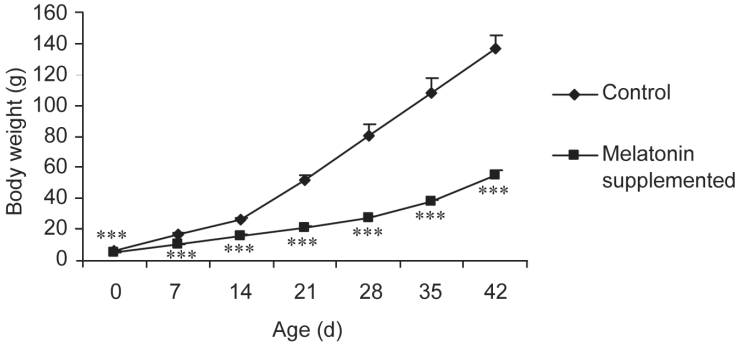

Fig. 2. Body weight changes of pups of controls and melatonin supplemented Sprague-Dawley dams. $^{***}P < 0.001$ compared to controls.

Table 4. Duration of pregnancy and mean birth weight of pups in melatonin treated and untreated WKY and Sprague-Dawley rats

	WKY (control)	WKY (25 mg/kg per day melatonin)	WKY (50 mg/kg per day melatonin)	WKY (100 mg/kg per day melatonin)	Sprague-Dawley (control)	Sprague-Dawley (100 mg/kg per day melatonin)
Mean duration of pregnancy (d)	21.66 ± 0.33	22.33 ± 0.45	22.50 ± 0.36	21.50 ± 0.22	21.83 ± 0.30	21.83 ± 0.30
Mean birth weight per pup (g)	5.70 ± 0.22	5.93 ± 0.13	6.18 ± 0.37	5.00 ± 0.23***	5.91 ± 0.03	5.60 ± 0.12$^{\#\#\#}$

$^{***}P < 0.001$ compared to WKY control; $^{\#\#\#}P < 0.001$ compared to Sprague-Dawley control.

cal examination of the liver, GIT and the kidney did not reveal anything of significance except in the kidney of one animal in the 25 mg/kg per day of melatonin group where areas of necrosis involving the tubules were evident, probably due to acute tubular necrosis secondary to ischaemia. However, it has to be added that most of the specimens were unfortunately partially autolysed, and their finer morphological details were obscured.

3 DISCUSSION

The major observations of the study are that melatonin supplementation during pregnancy to WKY and SD rats resulted in (i) lower gain in body weight by the dams during pregnancy, (ii) smaller litter size, (iii) lower rate of increase in body weight of pups after weaning, and (iv) mortality in some of the WKY pups.

Overall gain in body weight during pregnancy, when expressed as a percentage of body weight on PD1, was consistently lower in melatonin supplemented WKY and SD rats (Table 2). In general, maternal weight gain during pregnancy is primarily due to three main factors, which include the contribution by the developing feto-placental units, maternal fat deposition, and expansion in maternal extracellular fluid volume (ECFV). Litter size was consistently smaller in melatonin supplemented WKY and SD rats (Table 3) and the lower weight gain during pregnancy in melatonin supplemented dams could therefore be due to the lower number of developing fetuses. In addition, this could also be related either directly to the weight of the developing fetuses or indirectly through the impact of the number of developing fetuses on the maternal need for weight gain. A smaller number of pups could signal a lesser circulatory demand during pregnancy, together with lesser energy demand during lactation and therefore a lesser need for ECFV expansion and fat accumulation during pregnancy. A larger number of fetuses, on the other hand, could place greater circulatory and subsequent energy demands on the dam, consequently resulting in the need for a greater ECFV expansion and accumulation of body fat.

Further analysis of the data revealed that the actual maternal weight gain was also lower in melatonin supplemented WKY and SD dams (Table 2). Actual maternal weight gain is the difference in body weight between PD1 and PND1. It refers to the weight gained by the dam excluding the feto-placental units. Increased fat deposition occurs during pregnancy, and this depends on increased food intake. Increased food and water intake are amongst some of the earliest behavioral changes that occur during pregnancy in the rat [18]. Any decrease in food intake during pregnancy could therefore compromise the expected weight gain. In addition, there is also evidence indicating that pineal gland and melatonin might be directly involved in the feeding behaviour of animals [19]. Pinealectomy, for example, has been found to increase food intake [20], whereas melatonin supplementation has been shown to decrease food intake in Wistar rats [21]. Food intake was also reportedly decreased in zebra fish exposed to melatonin in water and the decrease was associated with significant increase in genes codifying for leptin and melanocortin 4 receptor and a reduction in the expression of orexigenics like ghrelin and NPY [22]. Besides this, sleep promoting effects of melatonin and its agonists are also well documented [23, 24]. It is possible a combination of hypnotic and appetite suppressing effects of melatonin might have reduced food intake thereby contributing to a lower weight gain in melatonin supplemented dams in this study. Food intake was not estimated in this study and, it is therefore not possible to confirm whether decreased food intake had contributed to the lesser weight gain in melatonin supplemented dams. Nevertheless, the finding that total and actual weight gain were both lower in melatonin supplemented dams suggests that melatonin supplementation to WKY and SD rats during pregnancy results in lower maternal weight gain during pregnancy. This lower weight gain appears to be due to a lesser number of developing fetuses and a lower actual body weight gain, which might or might not be related to lower food intake. The 24-hour water intake during pregnancy was, however, not different between melatonin treated and untreated dams in this study (Table 1). Although water intake increased significantly during the postpartum period when compared to that during pregnancy in all the groups, there was no significant difference in water intake between melatonin treated and untreated dams during the postpartum period (Table 1). Postpartum increase in water intake in rats is well documented and is associated with lactation. The slightly higher water intake on day 40 (Table 1) might be due to water consumption by the litter approaching weaning.

Litter size was consistently smaller in melatonin supplemented dams (Table 3). Litter size depends on the number of ova produced, number of conceptus that implant successfully, and on the number of aborted fetus-

es over the duration of pregnancy. Melatonin supplementation to adult rats has been found to suppress LHRH release and disrupt normal estrus cycle and fertility [10]. Melatonin has also been found to inhibit ovulation in immature rats that had been induced to ovulate with pregnant mare serum. This effect was reversed by GnRH, indicating that melatonin inhibits ovulation by inhibiting the secretion of GnRH [25]. In contrast, chronic melatonin treatment has been shown to reverse continuous light-induced anovulatory syndrome in the rat [26]. Melatonin supplementation to pinealectomised rats had also been found to restore ovulation and rate of embryo implantation [27]. A study *in vitro* in mice also noted that melatonin supports fertilization and early embryo development after fertilization *in vitro* [28]. The reason for the conflicting findings is unclear, but it might be related to the dose of melatonin used and the duration of its supplementation. In this study, however, melatonin was only commenced on PD1, and it would have had little impact on the development of the ova or even on the number of ova released. It could, however, influence the number of conceptus that implant successfully and/or on the loss of fetuses over the duration of the pregnancy.

Birth weight of pups of dams receiving melatonin was not significantly different from that of the controls except for pups of WKY and SD dams given 100 mg/kg per day of melatonin (Table 4). Interestingly, maternal weight gain was also the lowest in dams receiving 100 mg/kg per day of melatonin when compared to those in the other melatonin treated dams (Table 2), implying that the effect might be dose dependent. Little has been published on this in the literature so far and much remains to be explored further.

The rate of weight gain in pups of dams treated with melatonin was also lower, and it remained so even after weaning and discontinuation of melatonin supplementation (Fig. 1 and 2). Body weight gain of rats during the suckling period has been found to be inversely related to litter size [29, 30]. Limited milk availability and inadequate maternal care have been attributed as causes of the slower growth of pups reared in larger litters [31]. In this study, however, the litter size of melatonin supplemented dams was smaller than that in the controls (Table 3), and litter size *per se* therefore does not appear to be the reason for the slower weight gain during suckling in these pups. Milk yield was not estimated in this study, and it is therefore not possible to conclude if inadequate milk supply and intake might have been responsible for the lower weight gain in pups of melatonin supplemented dams. Besides, the lower rate of weight gain continued even after weaning at day 21 of age, when the pups had access *ad libitum* to food and water. It therefore seems that the lower weight gain in these pups might be due to factors other than the availability of food alone. However, the role of lower milk supply during lactation and food intake after weaning cannot be excluded at this stage.

Pup deaths occurred only after weaning, and this was evident in pups of WKY dams given 25 and 100 mg/kg per day of melatonin. No deaths occurred in pups of WKY dams given 50 mg/kg per day or in pups of SD dams given 100 mg/kg per day of melatonin (Table 3). The reason for the discrepancy between the WKY groups or between the WKY and SD groups is unclear. The effect of melatonin was somewhat less severe on every measured parameter in the dams and their pups in the group that was given 50 mg/kg per day of melatonin. It has also to be added here that the effect of melatonin supplementation on pup mortality was also less severe in this study when compared to our earlier published report where a dose of 10 mg/kg per day was used [13]. The reason for this is not apparent as the melatonin that was used was from the same stock and the WKY rats were also from the same breeding colony. The only difference was in the dose of melatonin used. Little can therefore be gleaned from this study to explain this difference apart from suggesting that it might indicate a biphasic effect of melatonin.

Macroscopic examination revealed no obvious defects in the physical appearance of the liver, GIT or the kidney apart from them being slightly smaller in size than those in age-matched controls. Histopathological examination of samples from the liver, GIT and kidney was limited by the presence of partial autolysis in most of the samples probably due to poor preservation of the tissues at the time of collection. Only in one animal evidence of renal tubular necrosis was noted, and it is not possible to extend this to all the animals that died or even used to explain the death or the poor rate of growth in pups of melatonin supplemented pups. The reason for the poor growth and death in some of the pups of dams given melatonin during pregnancy and lactation therefore remains uncertain. All that can be concluded from this study is that melatonin supplementation during pregnancy in rats could have serious adverse consequences on fetal and pup morbidity and mortality.

In conclusion, it appears that melatonin supplementation during pregnancy can have significant adverse effects on maternal weight gain during pregnancy. In addition, it can also affect the litter size and subsequent pup growth after delivery and weaning, and might even cause death in the offspring. The precise reasons for this remain unclear as milk yield, food intake and behavioural changes were not recorded in this study. Clearly, more studies are needed to examine the impact of melatonin supplementation on food intake, milk yield and nursing behaviour of the dams. In addition there is also a need for more detailed and well planned histological studies to ascertain the cause of death of pups following melatonin supplementation during gestation. It is possible that early exposure to high levels of melatonin compromises the development of some of the organs, which then affect the development of the pups, resulting in death in some ones. As this study was terminated when the pups were 6 weeks of age, longer term studies are therefore also needed to examine the full impact of melatonin supplementation on pup survivability.

REFERENCES

1 Zhang Z, Inserra PF, Liang B, Ardestani SK, Elliot KK, Molitor M, Watson RR. Melatonin, immune modulation and aging. Autoimmunity 1997; 26(1): 43–53.

2 Turek FW, Van Cauter E. Rhythms in reproduction. In: The Physiology of Reproduction, 2nd ed., Knobil E, Neill JD eds. New York: Raven Press, 1994, 487–540.

3 Ortiz A, Espino J, Bejarano I, Lozano GM, Monllor F, Garcia JF, Pariente JA, Rodriguez AB. High endogenous melatonin concentrations enhance sperm quality and short-term *in vitro* exposure to melatonin improves aspects of sperm motility. J Pineal Res 2011; 50: 132–139.

4 Scheer FA, van Montfrans GA, van Someren EJ, Mairuhu G, Buijis RM. Daily night time melatonin reduces blood pressure in male patients with essential hypertension. Hypertension 2004; 43(2): 192–197.

5 Lee SK, Sirajudeen KNS, Arunkumar S, Zakaria R, Singh HJ. Effects of antenatal, postpartum and post-weaning melatonin supplementation on blood pressure and renal antioxidant enzyme activities in spontaneously hypertensive rats. J Physiol Biochem 2011; 67: 249–257.

6 Reiter RJ, Paredes SD, Manchester LC, Tan DX. Reducing oxidative and nitrosative stress: a newly discovered genre for melatonin. Crit Rev Biochem Mol Biol 2009; 44: 175–200.

7 Hardeland R, Tan DX, Reiter RJ. Kynuramines metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. J Pineal Res 2009; 47: 109–124.

8 Onal SA, Inalkac S, Kutlu S, Kelestimur H. Intrathecal melatonin increases the mechanical nociceptive threshold in the rat. Agriculture 2004; 16: 35–40.

9 Shavali B, Ho B, Govitrapong P, Sawlom S, Ajjimaporn A, Klongpanichapak S, Ebadi M. Melatonin exerts its analgesic actions not by binding to opiod receptor subtypes but by increasing the release of beta-endorphin an endogenous opiod. Brain Res Bull 2005; 64: 471–479.

10 Rivest RW, Lang U, Aubert ML, Sizonenko PC. Daily administration of melatonin delays rat vaginal opening and disrupts the first oestrus cycles; Evidence that these effects are synchronized by the onset of light. Endocrinology 1985; 116: 779–787.

11 Lang U, Aubert ML, Rivest RW, Vinas-Bradtke JC, Sizonenko PC. Daily afternoon administration of melatonin does not irreversibly inhibit sexual maturation in the male rat. Endocrinology 1984; 115: 2203–2210.

12 Asher GW, Veldhuizen FA, Morrow CJ, Duganzich DM. Effects of exogenous melatonin on prolactin secretion, lactogenesis, and reproductive seasonality of adult female red deer. J Reprod Fertility 1994; 199: 11–19.

13 Singh HJ, Keah LS, Kumar A, Sirajudeen KN. Adverse effects of melatonin on rat pups of Wistar-kyoto dams receiving melatonin supplementation during pregnancy. Expt Toxicol Pathol 2012; 64: 751–752.

14 Cisternas CD, Compagnucci MV, Conti NR, Ponce RH, Vermouth NT. Protective effect of maternal prenatal melatonin administration on rat pups born to mothers submitted to constant light during gestation. Braz J Med Biol Res 2010; 43(9): 874–882.

15 Baka M, Uyanikgil Y, Ates U, Kultursay N. Investigation of maternal melatonin effect on the hippocampal formation of newborn rat model of intrauterine cortical dysplasia. Childs Nerv Syst 2010; 26: 1575–1581.

16 Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicol Sci 1999; 50(2): 271–279.

17 Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. J Pineal Res 2009; 46: 128–139.

18 Cripps AW, Williams VJ. The effect of pregnancy and lactation on food intake, gastrointestinal anatomy and the absorptive capacity of the small intestine in the albino rat. Br J Nutr 1975; 33(1): 17–32.

19 Nakagawa H, Nagai K, Kida K, Nishio T. Control mechanisms of circadian rhythm of feeding behaviour and metabo-

lism influenced by food intake. In: Suda, Hayashi O, nakagawa H, eds. Biological Rhythms and Their Central Mechanisms. Amsterdam: Elsevier-North Holland Press, 1979, 283–294.

20 Peschke D, Peschke E, Mess B. Circannual rhythm and increase of body weight and food intake in the young Wistar rat following pinealectomy and ganglionectomy. Neuroendocrinol Lett 1987; 9: 321–327.

21 Montano ME, Molpeceres V, Mauriz JL, Cruz BM, Gonzalez P, Barrio JP. Effect of melatonin supplementation on food and water intake in streptozotocin-diabetic and non-diabetic male Wistar rats. Nutr Hosp 2010; 25(6): 931–938.

22 Piccinetti CC, Migliarini B, Olivotto I, Coletti G, Amici A, Carnevali O. Appetite regulation: The central role of melatonin in *Danio rerio*. Horm Behav 2010; 58: 780–785.

23 Fisher SP, Davidson K, Kulla A, Sugden D. Acute sleep-promoting action of the melatonin agonist, ramelteon in the rat. J Pineal Res 2008; 45: 125–132.

24 Srinivasan V, Pandi-Perumal SR, Trahkt I, Spence DW, Poeggeler B, Hardeland R, Cardinali DP. Melatonin and melatonergic drugs on sleep: possible mechanisms of action. Int J Neurosci 2009; 19(6): 821–846.

25 Sorrentino S Jr. Ovulation of PMS-treatedn rats with gonadotrophin releasing hormone after phentobarbital and melatonin block. Neuroendocrinology 1975; 19(2): 170–176.

26 Trentini GP, Mess B, De Gaetani CF, Ruzsas C. Effect of melatonin on induction of ovulation in light induced constant estrous anovulatory syndrome and possible role of the brain serotoninergic system. J Endocrinol Invest 1978; 1(4): 305–310.

27 Dair EL, Simoes RS, Simoes MJ, Romeu LRG, Olivera-Filho RM, Haidar MA, Baracat EC, Soares JM. Effects of melatonin on the endometrial morphology and embryo implantation in rats. Fertil Steril 2008; 89(5): 1299–1305.

28 Ishikuza B, Kuribayashi Y, Murai K, Amemiya A, Itoh MT. The effect of melatonin on *in vitro* fertilisation and embryo development in mice. J Pineal Res 2000; 28: 48–51.

29 Chahoud I, Paumgartten FJR. Influence of litter size on postnatal growth of rat pups: Is there a rationale for litter-size standardization in toxicity studies? Environ Res 2009; 109: 1021–1027.

30 Galler JR, Turkewitz G. Variability of the effects of rearing in a large litter on the development of the rat. Dev Psychobiol 1975; 8: 325–331.

31 Agnish ND, Keller KA. The rationale for culling of rodent litters. Fundam Appl Toxicol 1997; 38: 2–6.