Rapid Communication: Maternal melatonin implants improve fetal oxygen supply and body weight at term in sheep pregnancies¹

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ABSTRACT: Fetal hypoxia, resulting in oxidative stress in pregnancies, contributes to reduced fetal growth. Melatonin, a potent antioxidant, has been associated with improved oxidative status. Maternal oral melatonin supplementation in sheep from day 50 of gestation ameliorates the consequences of fetal growth restriction in sheep. In rats, melatonin supplementation increases fetal weight via improved placental efficiency and reduction of oxidative stress. The objective of this study was to evaluate whether melatonin supplementation of single (S)- or twin-bearing (T) ewes using either 0 (0MEL), 1 (18-mg MEL), or 2 (36-mg MEL) slow release 18-mg melatonin implants (Regulin) from 100 to 140 d of pregnancy ($n = 8$ per group) influenced fetal oxygen supply and fetal weight. Fetal umbilical vein blood samples were collected at P140 and partial pressure of oxygen (PO_2) and hemoglobin saturation by oxygen (SatHb) measured. The placenta from each fetus was excised and placentomes individually weighed and typed (A–D). Pregnancy rank, sex of the fetus, number of implants, and their interaction on fetal weight, blood gases, and placentome weight were analyzed using ANOVA. A 22% and 14%

increase ($P < 0.05$) in body weight was obtained in 36- and 18-mg MEL compared with 0 MEL twin male fetuses, respectively, but no treatment effects were observed in singletons or females from twin pregnancies. Fetuses from ewes receiving 36-mg MEL had an 18% to 20% increase in cord PO_2 ($P < 0.05$) compared with 18-mg MEL and 0MEL fetuses, which in turn did not differ. Fetal weight was positively correlated with PO_2 ($r = 0.37$; $P = 0.02$), SatHb ($r = 0.26$; $P = 0.03$), and O_2 content ($r = 0.236$; $P = 0.048$). No treatment effect on placentome average weight, total placentome weight per fetus or per ewe, nor total number of placentomes per fetus was observed. However, placentae from 36-mg MEL fetuses had a greater proportion of Type C ($P < 0.05$) than 0MEL and 18-mg MEL ewes, and tended to have a lower proportion of Type A ($P = 0.1$) and a greater proportion of Type D ($P = 0.06$) placentomes, compared with 0MEL ewes. These results indicate that maternal melatonin implants, independently of sex, improve oxygen supply to the fetus, which could potentially improve lamb vigor at birth. In addition, melatonin can increase fetal weight of twin males, by improving placental adaptation and fetal blood oxygenation.

Key words: fetal growth, melatonin, ovine gestation, placenta

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INTRODUCTION

Fetal growth restriction, resulting in low birth weight, is one of the most important factors, associated with lamb mortality at birth (Alexander, 1974). Several sheep models have been developed to better understand the relationship between fetal growth restriction with maternal nutrition, hypoxia, and fetal number (Wallace et al., 2002; Regnault et al., 2003; Wu et al., 2004). From some of these studies, the use of melatonin supplementation to overcome fetal growth restriction in sheep has arisen as a potential intervention strategy; however, no studies have yet covered the use of melatonin from a practical point of view for farmers. Maternal daily oral supplementation with melatonin from day 50 of gestation has been associated with changes in uteroplacental hemodynamics, negating the consequences of fetal growth restriction in sheep (Lemley et al., 2012). Furthermore, melatonin improves fetal oxygenation in vivo, reducing fetal hypoxemia in sheep singleton fetuses when administered i.v. to the ewes (Tare et al., 2014). In other animal species, melatonin has been shown to exert a positive effect in fetal growth, as shown in rats, where oral melatonin improves placental efficiency and birth weight and upregulates placental antioxidant enzymes (Richter et al., 2009). The aim of this experiment was to investigate the effect of different doses of melatonin on fetal oxygen supply and growth. The relative contribution of 1 or 2 commercial melatonin implants administered to single- or twin-bearing ewes from days 100 to 140 of pregnancy was evaluated. We hypothesized that slow release maternal melatonin implants would result in an improvement of feto-placental blood perfusion, resulting in a better fetal oxygen supply, increasing fetal growth in sheep pregnancies in a dose-responsive manner.

MATERIALS AND METHODS

Ethics Statement

This study was performed in agreement with the Guide for Care and Use of Laboratory Animals (Eighth Edition, National Research Council, National Institute of Health, USA). The protocol was approved by the Bioethics Review Committee of the Instituto de Investigaciones Agropecuarias (INIA, Ministry of Agriculture), Faculty of Veterinary and Animal Sciences, University of Chile, as well as by the Bioethics Advisory Committee of the Chilean National Commission for Scientific and Technological Research (CONICYT, Chile).

Animals and Experimental Procedure

This study was conducted at INIA Kampenaike Research Farm, 60 km north of Punta Arenas, Chile (Chilean Patagonia, Lat. 52°41'S Long. 70°54'W). Corriedale ewes (4–6 yr old; body weight: 62.8 ± 3.5 kg) from a commercial flock were synchronized using a CIDR (CIDR G, Pfizer, Chile) during 12 d, followed by 300 IU eCG (Novormon, Syntex, Argentine) i.m. injection. Mating was carried out using fertile-proved Suffolk rams with painted chests with a solution containing food-grade oil with colored earth. The exact day of service of the ewes was identified by daily visual inspection of ewe's colored rumps. Ultrasound examination was done 30 d after mating, where 24 single- and 24 twin-bearing ewes were selected. Weight and body condition scores (BCS, 1 to 5 scale; Russel et al., 1969) were similar for all groups (62.8 ± 1.8 and 1.8 ± 0.2 kg, respectively) at the start of the experiment. At 100 d of gestation, ewes were randomly assigned into 6 equal-sized groups ($n = 8$) according to pregnancy rank (Single: S; Twin: T) and number of melatonin implants (0: 0MEL; 1: 18-mg MEL; and 2: 36-mg MEL) to generate 6 experimental groups: single-bearing ewes with 1 implant (S-18 mg MEL); single-bearing ewes with 2 implants (S-36 mg MEL); twin-bearing ewes with 1 implant (T-18 mg MEL); single-bearing ewes with 2 implants (T-36 mg MEL) and a single- (S-0MEL) and twin-bearing (T-0MEL) control groups. Each melatonin implant contained 18-mg melatonin (Regulin, CEVA, Melbourne, Australia). Melatonin was placed at the base of the ear, according to manufacturer instructions. Animals were grazed under commercial grazing conditions in Patagonia on natural pasture (DM: 53.2%–64.6%; CP: 2.7%–4.9%; GE: 4.2–4.3 Kcal/g).

At 140 d of gestation, ewes were weighed and BCS recorded. Fetal samples were collected following cesarean section. Ewes received spinal anesthesia by injection of 2-mL 2% Lidocaine hydrochloride (Lidocalm, Drag Pharma, Chile) into the sacrococcygeal space. After the loss of the hind limbs reflexes, the ewe was placed on a surgical table, carotid arterial blood samples were obtained from the ewe for gases (1 mL) and melatonin concentration (3 mL) assessment, and a cesarean section was immediately performed. Once the corresponding uterine horn was excised, the umbilical cord was located and heparinized (Sodic Heparine, Sanderson, Chile) venous blood samples were collected for gases (1 mL) and melatonin (3 mL) measurements. Subsequently, fetuses were excised

and the ewe and fetus were euthanized by barbiturate overdose (Opet, Pro-Vet, Chile). Excess of fetal fluids were hand stripped and fetal weight and sex were recorded. Placentas were also extracted and weighted, and each placentome was dissected from the uterus, weighed individually, and classified morphologically as first described by [Vatnick et al. \(1991\)](#). This system classifies placentomes on the basis of their shapes (Types A to D). Type A placentomes are concave in shape with the maternal tissue completely surrounding the fetal tissue. In Type B placentomes, the fetal tissue begins to grow over the surrounding maternal tissue. Type C placentomes are flat and fetal tissue has begun to surround the maternal tissue. Finally, in Type D placentomes, fetal tissue surrounds completely the maternal tissue, resembling the cow's placentomes. For those placentomes with an "intermediate" type, they were classified into the more advanced type. The classification of placentome type of all placentae was performed by the same investigator to reduce individual variability.

Assessment of Maternal and Feto-Placental Oxygenation Status

Partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), hemoglobin concentration (Hb), Hb saturation by oxygen (SatHb), and pH value were determined using a IL Synthesis 25 gas analyzer (Instrumentation Laboratory, Lexington, MA), adjusted to ovine body temperature ([Merck, 2018](#)).

Assessment of Maternal and Feto-Placental Plasma Melatonin Concentration

After blood samples collection, plasma was harvested following samples centrifugation at $1200 \times g$ for 10 min and stored at -80°C until analysis. Maternal and fetal plasma melatonin concentrations were determined using a commercial radioimmunoassay kit (Melatonin direct RIA, RE 29301, IBL Hamburg, Germany) previously described for the specie ([Morris et al., 2008](#)).

Statistical Analysis

Comparisons among groups were done by analysis of variance, using PROC MIXED. Factors included in the model were pregnancy rank, treatment, sex of the fetus, and their interactions, with ewe as random effect in order to account for twin pregnancies. Melatonin concentrations were not

normally distributed and were therefore log transformed ($\log_e$). Means comparison was performed using LSMEANS/PDIFF option. The percentage of each placentome type was analyzed with a chi-square test using PROC MIXED prior arcsine square root transformation. Pearson correlation was determined for blood gases and fetal weight. Significant differences were considered when $P < 0.05$ and a trend to significance when $P < 0.10$. All statistical analysis were performed with SAS program ([SAS, 2006](#)).

RESULTS

Effects of Maternal Melatonin Implants on Maternal and Fetal Weight

Maternal weight and BCS and placental traits are presented in [Table 1](#). Maternal weight nor maternal BCS were different ($P > 0.05$) between treatments at 140 d of gestation. A trend ($P = 0.09$) for a greater maternal BCS was observed for single-compared with twin-bearing ewes. Fetal weight at 140 d of gestation was affected by the interaction ($P = 0.08$) among maternal treatment, pregnancy rank, and fetal sex. In this regard, no effects of treatment were observed in singleton fetuses, regardless of sex ($P > 0.05$), or when comparing twin females among treatments ($P > 0.05$). Conversely, significant effects were found in twin males in which the use of melatonin implants resulted in a greater body weight (4.10 ± 0.22 kg for 36-mg MEL and 3.86 ± 0.17 kg for 18-mg MEL) than in the control group 0MEL (3.38 ± 0.14 kg, $P < 0.05$ for both), without significant differences between both groups of treatment ($P > 0.05$). These increases in weight caused that 36-mg MEL twin male fetuses reached a similar weight to fetuses in all the singleton groups (male or females, treated or controls; $P > 0.05$ for all) and a higher weight than in all the other twin groups excepting 18-mg MEL. In the case of 18-mg MEL, the increase in weight of twin males supposed that they were only lighter than 36-mg MEL single males and females (4.54 ± 0.20 kg and 4.52 ± 0.23 kg, respectively; $P < 0.05$) and 0MEL male singletons (4.45 ± 0.23 kg, $P < 0.05$).

Effects of Maternal Melatonin Implants on Maternal and Fetal Melatonin Concentrations

A treatment effect ($P < 0.0001$) was observed for maternal melatonin plasma concentration, where 36-mg MEL ewes had the highest melatonin concentration (274.65 ± 38.17 pg/mL) compared

Table 1. Maternal, placentome traits, and fetal blood gases at day 140 of pregnancy from single- and twin-bearing ewes receiving either 0 (0 MEL), 1 (18-mg MEL), or 2 (36-mg MEL) melatonin implants from day 100 of pregnancy

Dependent variable	Rank		Treatment			P-values	
	Single	Twin	0 MEL	18-mg MEL	36-mg MEL	Rank	Trt
Maternal traits							
Maternal weight, kg	62.61 ± 1.13	64.83 ± 1.05	62.50 ± 1.32	65.07 ± 1.32	63.59 ± 1.37	0.16	0.39
Maternal BCS	1.61 ± 0.10	1.37 ± 0.09	1.49 ± 0.12	1.48 ± 0.12	1.50 ± 0.13	0.09	0.99
Placentome							
Average weight, g	5.57 ± 0.37	6.87 ± 0.32	6.38 ± 0.44	5.77 ± 0.42	6.51 ± 0.42	0.01	0.19
Total weight per fetus, g	429.28 ± 15.10	306.64 ± 13.05	362.68 ± 17.78	357.06 ± 17.01	384.59 ± 17.05	<0.01	0.25
Total weight per ewe, g	429.28 ± 21.46	618.16 ± 27.52	497.37 ± 31.77	522.76 ± 31.06	551.04 ± 27.68	<0.01	0.29
Total number per fetus	78.28 ± 2.07	45.91 ± 1.78	60.32 ± 2.44	63.88 ± 2.34	62.09 ± 2.32	<0.01	0.45
Number (%)							
Type A	49.17 ± 6.97	21.71 ± 6.27	49.10 ± 8.33 ^a	37.12 ± 7.93 ^{a,b}	20.10 ± 8.10 ^b	0.01	0.09
Type B	31.56 ± 5.79	45.50 ± 4.93	39.80 ± 6.78	38.46 ± 6.50	37.33 ± 6.48	0.05	0.67
Type C	12.21 ± 3.17	16.55 ± 2.84	8.15 ± 3.79 ^a	11.65 ± 3.60 ^b	23.35 ± 3.68 ^c	0.21	0.01
Type D	7.05 ± 5.26	16.73 ± 4.68	2.95 ± 6.26	13.47 ± 5.96	19.24 ± 6.07	0.18	0.19
Weight (g)							
Type A	4.68 ± 0.46	4.93 ± 0.38	5.56 ± 0.45	4.53 ± 0.50	4.33 ± 0.58	0.50	0.10
Type B	6.35 ± 0.54	6.44 ± 0.51	7.46 ± 0.65	5.65 ± 0.70	6.07 ± 0.63	0.94	0.14
Type C	6.72 ± 1.14	7.29 ± 0.94	6.75 ± 1.55	7.35 ± 1.12	6.92 ± 1.11	0.76	0.99
Type D	9.28 ± 1.71	9.90 ± 1.38	9.18 ± 2.30	9.75 ± 1.78	9.84 ± 1.54	0.76	0.78

Data are presented as means (predicted from the fitted statistical model) and average SEM, together with *P* value for pregnancy rank and treatment (Trt) effect.

^{a,b,c}Values with different superscripts in a same row differ (*P* < 0.05).

with 18-mg MEL ewes (106.21 ± 46.23 pg/mL) which, in turn, were greater than the 0MEL ewes, whereby the levels of melatonin in the plasma were below the detection limits of the assay. When analyzing the fetal melatonin plasma concentration, an interaction (*P* = 0.007) was observed between pregnancy rank and treatment. Twin 36-mg MEL fetuses had the highest melatonin concentration (108.85 ± 38.10 pg/mL) compared with all other melatonin-supplemented groups (S-18 mg MEL: 55.53 ± 16.21 pg/mL; S-36 mg MEL: 51.50 ± 13.24 pg/mL; T-18 mg MEL: 24.98 ± 16.21 pg/mL; *P* < 0.05), which, in turn, were greater than all control groups (S-0 MEL: 3.00 ± 13.24 pg/mL; T-0 MEL: 3.00 ± 14.80 pg/mL; *P* < 0.05), whereby the levels of melatonin in the plasma were below the detection limits of the assay. No sex effect was observed for fetal melatonin plasma concentration.

Effects of Maternal Melatonin Implants on Placenta Traits

The use of maternal melatonin implants resulted in no effect on placentome average weight, total placentome weight per fetus or per ewe, nor total number of placentomes per fetus. Only a pregnancy rank effect was observed (*P* < 0.05) for all these traits, with twins having a 22% greater average

individual placentome weight and a 44% greater total placentome weight per ewe. In contrast, twins had a 29% lower total placentome weight and a 41% lower total number of placentomes per fetus (Table 1). No effect of sex was observed for any placental traits.

The analysis of the effect of maternal melatonin implant on the percentage of placentomes, according to their type, showed that the use of 2 melatonin implants (36-mg MEL group) resulted in an increase (*P* = 0.01) in Type C placentomes compared with 18-mg MEL and both treated groups had greater Type C placentomes compared with 0MEL ewes. In addition, 36-mg MEL ewes showed a trend for a lower proportion of Type A (*P* = 0.09) compared with 0MEL but was similar to 18-mg MEL ewes, which did not differ from 0MEL ewes. No difference was observed for the distribution of Type B and Type D placentomes between all treatments. When comparing the effect of pregnancy rank on placentome distribution per type, single-bearing ewes had a 2-fold greater percentage (*P* = 0.01) of Type A placentomes compared with twin-bearing ewes. In contrast, twin-bearing ewes had (*P* = 0.05) a 44% greater proportion of Type B placentomes compared with single-bearing ewes. Finally, no differences were observed in the distribution of Type C or D placentomes between pregnancy ranks.

Effects of Maternal Melatonin Implants on Fetal Blood Gases

Maternal melatonin implants had an effect on venous cord blood PO_2 , where 36-mg MEL fetuses had greater venous PO_2 compared with 18-mg MEL and 0MEL fetuses. No treatment effect was evident for pCO_2 and SatHb ($P > 0.05$). A treatment effect ($P = 0.04$) on pH was also observed, where 0MEL fetuses had a higher pH compared with 18-mg MEL ($P = 0.01$), with 36-mg MEL no difference between other groups. A treatment effect ($P = 0.04$) was also observed for Hb where 36-mg MEL had a lower value compared with 0MEL and 18-mg MEL fetuses. In addition, a pregnancy rank effect was observed for PO_2 , SatHb, and O_2 content, where singletons had a greater concentration compared with twin fetuses (Table 2). No effects of sex and no treatment by birth rank and sex interactions were observed. Fetal weight was positively correlated with PO_2 ($r = 0.37$, $P = 0.02$), SatHb ($r = 0.26$, $P = 0.03$), and O_2 content ($r = 0.236$, $P = 0.048$).

DISCUSSION

In the present study, body weight of twin male fetuses increased as a result of the use of melatonin implants. The resulting outcome of using commercial melatonin implants placed in the ewe at 100 d of gestation, appears to be associated, at least in part, with an improvement in fetal oxygenation, potentially via placental adaptations.

Previous studies have indicated that melatonin could be associated with modification of behavior (Golombek et al., 1996), leading to conditions which could affect the normal grazing behavior of the animals. Although observing animal behavior

was not part of the present study, neither maternal weight nor maternal BCS was affected by melatonin treatments, suggesting maternal grazing behavior was not modified during the time of the trial. These results are in agreement with Lemley et al. (2012), where animals received an oral daily melatonin dose of $\sim 100 \mu\text{g/kg}$ body weight, resulting in no modification of animal behavior. In the present study, the implants used have a total of 18 mg of melatonin, which is a slowly release formulation; therefore, the daily concentration used per kg of body weight was lower than the 20 to 200 mg/kg body weight, which has been previously associated with behavior changes (Golombek et al., 1996) and reduced maternal weight gain (Jahnke et al., 1999), indicating that the use of commercial 18-mg melatonin implants in pregnant ewes results in no detrimental maternal effects in terms of grazing behavior.

The increase in fetal weight of male twins as an outcome of using melatonin implants in sheep from day 100 of gestation is a novel finding. There is limited information on the effect of melatonin in sheep during the last third of gestation and there is contradictory evidence regarding the impact of antenatal melatonin use, in terms of fetal growth when referring to other species. Using a model of developmental hypoxia in ewes kept in high altitude, González-Candia et al. (2016) found that a daily maternal oral doses of $10 \text{ mg/kg}^{-1}\text{day}^{-1}$ of melatonin during last third of gestation markedly decreased neonatal biometry and weight at birth. In contrast, different studies in rats using melatonin in doses of with $5 \mu\text{g/mL}$ in a maternal undernourished model (Richter et al., 2009), 10 mg/kg in a lipopolysaccharide-induced maternal restriction model (Chen et al., 2006), or $20 \mu\text{g/mL}$ in ischemia/reperfusion-induced oxidative placental damage

Table 2. Fetal blood gases at day 140 of pregnancy from single- and twin-bearing ewes receiving either 0 (0 MEL), 1 (18-mg MEL), or 2 (36-mg MEL) melatonin implants from day 100 of pregnancy

Dependent variable ¹	Rank		Treatment			P values	
	Single	Twin	0 MEL	18-mg MEL	36-mg MEL	Rank	Trt
Venous							
pO_2 , mm Hg	31.03 ± 1.53	24.39 ± 1.34	25.96 ± 1.65^a	25.53 ± 1.82^a	31.65 ± 1.81^b	0.01	0.05
SatHb, %	52.38 ± 3.57	38.46 ± 3.22	44.46 ± 3.91	40.00 ± 4.26	51.80 ± 4.30	0.01	0.17
pCO_2 , mm Hg	44.51 ± 1.29	43.98 ± 1.10	42.79 ± 1.37	45.35 ± 1.53	44.59 ± 1.51	0.72	0.61
pH	7.44 ± 0.01	7.43 ± 0.01	7.45 ± 0.01^a	7.42 ± 0.01^b	$7.43 \pm 0.01^{a,b}$	0.26	0.04
Hb, g/dL	10.01 ± 0.22	10.25 ± 0.40	10.33 ± 0.22^a	10.44 ± 0.25^a	9.62 ± 0.25^b	0.55	0.05
O_2 cont., mL/dL	7.12 ± 0.46	5.33 ± 0.38	6.10 ± 0.47	5.80 ± 0.55	6.78 ± 0.55	0.01	0.41

Data are presented as means (predicted from the fitted statistical model) and average SEM, together with P value for pregnancy rank by sex by treatment (Trt) interaction.

¹Partial pressure of oxygen = PO_2 ; hemoglobin saturation by oxygen = SatHb; partial pressure of carbon dioxide = pCO_2 ; hemoglobin = Hb; oxygen content = O_2 cont.

^{a,b,c}Values with different superscripts in a same row differ ($P < 0.05$).

and fetal growth restriction (Nagai et al., 2008), melatonin was capable to restore the fetal weight, negating the effects of fetal growth restriction. The results obtained in the present study indicate, for the first time to the best of our knowledge, that the effects of melatonin treatment differ depending on the sex of the offspring. The influence of fetal sex has been previously reported in the studies about fetal growth restriction and prenatal programming, as reviewed by Moritz et al. (2010) and Aiken and Ozanne (2013). Our study supports these findings, since it indicates that the effects from melatonin treatment on fetal development are driven by the sex and pregnancy rank of the offspring, with better outcomes in the case of twin males than females which warrants further research.

In the present study, fetuses from ewes receiving 2 melatonin implants (36 mg) had increased venous cord blood PO_2 , compared with fetuses of other groups. Fetal hypoxia, leading to oxidative stress, has been implicated as one of the potential factors contributing to fetal growth restriction (Robinson, 2017; Sultana et al., 2017). Previous reports have shown melatonin improved fetal oxygenation in vivo in sheep twin pregnancies by giving 0.25 mg h^{-1} i.v. from day 100 until term (Tare et al., 2014). A potential mechanism for increase oxygen supply to the fetus can be associated with increase umbilical blood flow, described in sheep after oral melatonin supplementation (Lemley et al., 2012), potentially via nitric oxide-dependent mechanisms (Thakor et al., 2010). Uteroplacental blood flow plays a role in fetal growth and development and efficiency of placental transport (Reynolds and Redmer, 1995). In the present study, blood flow was not measured; however, interestingly, 36-mg MEL fetuses showed a shift to a greater proportion of Type C placentomes. Expression of melatonin receptors have been described in the placenta and fetal tissues (Lee et al., 2003), and melatonin may alter caruncle vascularity and RNA concentrations during late pregnancy (Eifert et al., 2015). Placentome conversion from Types A to B, C, and D in ewes may be a functional adaptation to maintain normal nutrient delivery to the fetuses during maternal nutrient restriction (Vonnahme et al., 2006). Although we were not able to find any fetal sex effect on placental traits or blood gases concentrations, we hypothesize that melatonin drives changes in placentomes, resulting in improved transport of oxygen to the fetuses, thereby contributing to increased fetal growth. Whether the mechanisms driving these changes have a sexual dimorphism is part of ongoing studies.

Commercial melatonin implants, according to the findings in this research, may become a strategic option for producers to overcome the negative impacts for fetal growth of twin males resulting from pregnancies occurring when nutrient supply and quality is restricted as often observed in extensive grazing extensive systems. In addition, the potential correlation between changes in fetal oxygen supply as a result of melatonin supplementation on lamb vigor is an area of interest for future studies. This research contributes to generate novel information for further discussion of the potential role of melatonin in compromise fetal growth gestations in animals as well as in humans.

Conflict of interest statement. A.G.-B. is a member of the EU COST-Action BM1308 “Sharing Advances on Large Animal Models (SALAAM).”

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