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Effect of melatonin or maternal nutrient restriction on vascularity and cell proliferation in the ovine placenta

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ABSTRACT

Previously we reported increased umbilical artery blood flow in ewes supplemented with melatonin from mid- to late-pregnancy, while maternal nutrient restriction decreased uterine artery blood flow. To further unravel these responses, this study was designed to assess placental cell proliferation and vascularity following supplementation with melatonin or maternal nutrient restriction. For the first experiment, 31 primiparous ewes were supplemented with 5 mg of melatonin per day (MEL) or no melatonin (CON) and allocated to receive 100% (adequate fed; ADQ) or 60% (restricted; RES) of their nutrient requirements from day 50 to 130 of gestation. To examine melatonin receptor dependent effects, a second experiment was designed utilizing 14 primiparous ewes infused with vehicle, melatonin, or luzindole (melatonin receptor 1 and 2 antagonist) from day 62 to 90 of gestation. For experiment 1, caruncle concentrations of RNA were increased in MEL–RES compared to CON–RES. Caruncle capillary area density and average capillary cross-sectional area were decreased in MEL–RES compared to CON–RES. Cotyledon vascularity was not different across dietary treatments. For experiment 2, placental cellular proliferation and vascularity were not affected by infusion treatment. In summary, melatonin interacted with nutrient restriction to alter caruncle vascularity and RNA concentrations during late pregnancy. Although melatonin receptor antagonism alters feto-placental blood flow, these receptor dependent responses were not observed in placental vascularity. Moreover, placental vascularity measures do not fully explain the alterations in uteroplacental blood flow.

1. Introduction

Melatonin is a neurohormone produced from the amino acid tryptophan and is secreted from the pineal gland on a diurnal rhythm depending on environmental lighting conditions. Melatonin has been found to regulate seasonal reproduction in a variety of animals including the ewe (Yellon and Longo, 1987; Monroe and Watts, 1998). Additionally, melatonin is unique in that it is one of the few maternal hormones that is able to cross the placenta unaltered (Torres-Farfan et al., 2008). Previous studies have associated melatonin supplementation with stimulating the secretion of progesterone (Forcada et al., 2006) and increasing the release of gonadotropins from the pituitary

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in the ewe (Aleandri et al., 1996). Moreover, removal of the pineal gland in pregnant rats has been linked to an elevated incidence of spontaneous abortions (Sandyk et al., 1992).

Adequate maternal nutrition during gestation is essential for the proper growth and survival of the developing conceptus. During gestation, the placenta is responsible for the transfer of nutrients and wastes between the dam and fetus. Studies have shown that when pregnant ewe lambs are nutrient restricted during various periods of gestation, lamb birth weight is reduced (Swanson et al., 2008; Meyer et al., 2010), umbilical artery blood flow is decreased (Lemley et al., 2012), and umbilical vascular resistance is increased (Lekatz et al., 2013). Therefore, adequate nutrient intake during gestation is essential for the proper growth and development of the fetus.

Similar to previous reports we have shown a decrease in uterine artery blood following maternal nutrient restriction during the last two-thirds of pregnancy (Lemley et al., 2012). In the same study, supplementing ewes with melatonin through the feed increased umbilical artery blood flow compared to non-supplemented ewes. In addition, chronic infusion of melatonin into the mesometrial region of the gravid uterus during mid-gestation (Lemley et al., 2013b) increased fetal abdominal girth, fetal aorta blood flow, and umbilical artery blood flow. These results led us to hypothesize that supplementation with melatonin during mid-gestation would increase fetal placental vascularity, thereby allowing for the increased umbilical artery blood flow. Conversely, maternal nutrient restriction during the last two-thirds of gestation would decrease maternal placental vascularity, thereby allowing for the decreased uterine artery blood flow. The hypothesis was tested through two separate studies. First, we determined placental cell proliferation and vascularity in ewes that were supplemented with or without dietary melatonin during nutrient restricted from mid- to late-gestation. Secondly, we determined the effects of chronic in vivo infusions of vehicle, melatonin or melatonin receptor antagonist on placental cell proliferation and vascularity during mid-gestation.

2. Materials and methods

All protocols involving animals were approved by the North Dakota State University Intuitional Animal Care and Use Committee protocol #A10071 (experiment 1; dietary study) and protocol #A11061 (experiment 2; infusion study).

2.1. Animal management for dietary study

The animal management, breeding, and experimental design were previously published (Lemley et al., 2012). Nulliparous Western white face ewe lambs ($n = 64$) and two rams fitted with crayon marking harnesses were put on pasture with ad libitum access to hay and water. Mating was recorded daily at 12-h intervals. On day 28 of gestation, dams were transported to the Animal Nutrition and Physiology Center (ANPC, Fargo, ND, USA) with 12:12 h light–dark cycle with lights on at 07:00 and off at 19:00. At ANPC, pregnancy was determined during days 28 through 35 using a B mode ultrasound (model SSD-3500; Aloka America, Wallingford, CT, USA) fitted with a 7.5 MHz, linear transrectal probe. Thirty-two ewes carrying singletons were selected for the dietary study and individually housed in $0.91 \times 1.2\ m^2$ pens at ANPC for the duration of the study. One ewe was removed due to a perforated esophagus, which existed prior to dietary treatment; therefore a total of 31 ewes were treated for the study. On day 45 of gestation all animals were acclimated to a common pelleted diet consisting of 29% dehydrated beet pulp, 34% dehydrated alfalfa meal, 9% ground corn, 4% soybean meal, and 24% wheat middlings. The diet composition (% of dry matter) was 15.8% crude protein, 36.5% neutral detergent fiber, 20.8% acid detergent fiber, 1.11% calcium, 0.58% phosphorus, 7.8% ash, and 2.66 Mcal/kg of metabolizable energy. Ewes were also provided trace mineral salt blocks (4000 ppm Zn, 1600 ppm Fe, 1200 ppm Mn, 325 ppm Cu, 100 ppm I, 40 ppm Co; American Stockman, Overland Park, KS).

On day 50 of gestation, ewes were assigned to one of four treatment groups consisting of 5 mg of melatonin (MEL; Spectrum Chemical Mfg. Gardena, CA, USA) or no melatonin (CON) and supplied 100% (adequate diet; ADQ) or 60% (restricted; RES) of NRC recommendations (NRC, 2007) for the remainder of the study. The four resulting treatment groups consisted of CON–ADQ ($n = 7$), MEL–ADQ ($n = 8$), CON–RES ($n = 8$), MEL–RES ($n = 8$). Nutrient recommendations were set based on the mid- to late-gestational requirements for a 60 kg body weight pregnant ewe lamb. All ewes were fed and/or supplemented 5 h before the start of the dark cycle at 14:00 with melatonin enriched pellets. A melatonin solution was made by dissolving powdered melatonin in 95% ethanol at a concentration of 5 mg/ml. A day prior to feeding, 1 ml of the melatonin solution was applied to control pellets (100 g) and placed in a plastic bag to allow the ethanol to evaporate overnight at room temperature with no exposure to light (Lemley et al., 2012). The melatonin supplemented pellets (100 g) were fed to the MEL groups and were consumed within 5 min. Similarly, non-melatonin supplemented control pellets (100 g) were fed to the CON group. After the consumption of the melatonin treated pellets (MEL group) or non-treated pellets (CON group), the remainder of the control pellet was divided between all melatonin and control treated animals. Optimal feeding time (14:00; 5 h prior to scotophase) and dosage of melatonin (5 mg) was chosen based on a preliminary study examining 24 h serum concentrations of melatonin in non-pregnant cycling ewes following dietary melatonin supplementation (Lemley et al., 2012). Briefly, this feeding time and dosage resulted in an increased amplitude and duration of serum concentrations of melatonin in MEL versus CON ewes. In addition, scotophase concentrations of melatonin were increased at day 84 and 124 of pregnancy in MEL versus CON supplemented ewes, while nutrient restriction did not influence maternal concentrations of melatonin. Similarly, fetal concentrations of melatonin were increased at day 130 of pregnancy in fetuses from MEL versus CON ewes (Lemley et al., 2012).

On day 130 of gestation, ewes were weighed and euthanized with an overdose of sodium pentobarbital (Lemley et al., 2012, 2013a). Following euthanasia the gravid uterine