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Melatonin implants do not alter estrogen feedback or advance puberty in gilts

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ABSTRACT

Puberty in pigs is often delayed during late summer and autumn, with long daylength the most likely cause. We hypothesised (1) that gilts born around the shortest day would have a later release from the negative feedback actions of estradiol than gilts born around the spring equinox and (2) melatonin treatment would result in an earlier release from estradiol negative feedback and advance the onset of puberty in gilts born around the spring equinox. We first determined the optimal number of estradiol implants required to monitor the release from estradiol negative feedback in ovariectomised gilts. Secondly we determined whether melatonin implants altered negative feedback in 4 cohorts of ovariectomised gilts born between the winter solstice and spring equinox, and in the following year whether melatonin altered the time of the first ovulation in 5 cohorts of intact gilts born between the winter solstice and spring equinox. Plasma LH and FSH increased between 126 and 210 d of age ($P < 0.001$) in each cohort (season), but there was no effect of cohort, melatonin treatment or interactions ($P > 0.05$). Age at first detection of elevated plasma progesterone in untreated, intact gilts decreased across the 4 cohorts ($P < 0.05$). Melatonin treatment of intact gilts failed to advance the age of puberty irrespective of their season of birth ($P > 0.05$). In conclusion, while we confirmed that estradiol sensitivity is decreased as gilts age, we failed to demonstrate any effects of season or melatonin on estradiol feedback or melatonin on puberty.

1. Introduction

Pigs have been intensively selected for high fertility and fecundity and the industry expectation is that this potential can be sustained throughout the year. However, seasonal infertility represents a considerable problem for pork farmers in that it reduces overall fertility and fecundity of herds and causes unpredictable variations in production. An important contributor to the variability in production is the seasonal variation in the age at which gilts reach puberty (Paterson et al., 1989, 1991; Paterson and Pearce, 1990). Based on a considerable amount of research into this phenomenon, it has been concluded that photoperiod and environmental temperature are the most likely primary influences, with housing conditions, pheromones and drinking water temperature also playing a role (Peltoniemi and Virolainen, 2006). In other domesticated farm animals with a similar gestation length to that of pigs

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(e.g., sheep and goats), it is well established that daylength (photoperiod) is the prime environmental variable controlling fertility and fecundity (Legan and Karsch, 1980; Foster, 1981). Reproductive seasonality does occur in domestic sows kept outdoors (Bassett et al., 2001) with anoestrus evident in late summer and autumn.

In pigs, sheep and other animals it has been shown that during the follicular phase the developing follicles secrete low levels of estradiol continuously which acts on the hypothalamus to maintain low frequency, high amplitude, pulsatile secretion of GnRH and pituitary secretion of luteinising hormone (LH). As the dominant follicle continues to grow and secrete increasing amounts of estradiol there is a gradual release from the negative feedback such that the frequency of LH pulses increases and their amplitude decreases until the estradiol levels achieve a threshold and cause the LH surge and ovulation to occur (Karsch et al., 1977). For animals that are capable of ovulating only during specific times of the year a model has been developed that can both determine the extent of the seasonality and provide a template for studying possible interventions. The model is based upon the understanding that release from the negative feedback actions of estradiol is a prerequisite for a preovulatory LH surge and ovulation to occur (Legan and Karsch, 1980), that in the absence of ovaries the LH and FSH secretion is maximal and that provision of continuous low levels of estradiol can suppress the gonadotropin secretion (Legan and Karsch, 1980; Berardinelli et al., 1984; Barb et al., 2010). When sheep are ovariectomised and implanted with continuous release estradiol implants, the negative feedback actions of estradiol on the hypothalamus of the ovariectomised animals are decreased and basal levels of LH are increased coincident with the seasonal onset of ovarian cyclicity in intact prepubertal or adult animals. Research into the downstream mechanisms driving the seasonality has highlighted the role of kisspeptin (Clarke and Caraty, 2013). The seasonal estradiol feedback model has not been systematically evaluated in pigs, but there is strong evidence that exposure to long photoperiod results in delayed onset of puberty in gilts not exposed to boars (Paterson and Pearce, 1990) suggesting that similar feedback processes are involved in the timing of puberty of pigs.

Melatonin has been shown unequivocally to be the key link between the photoperiod and the reproductive system of a wide range of animals from small rodents through to sheep and deer (Chemineau et al., 2008). Administration of melatonin through continuous release implants has emerged as a practical means of overcoming reproductive seasonality in sheep (Staples et al., 1992) but there have been limited studies addressing its use to alter the timing of puberty in pigs. Two previous studies (Paterson et al., 1992b; Diekman et al., 1997) concluded that treatment with melatonin implants could not overcome the seasonal inhibition of the attainment of puberty in domestic gilts, but they did not address whether there was an optimal time of year to commence treatment.

For the current study, we hypothesised that gilts born around the shortest day and developing during an increasing photoperiod would have a later release from the negative feedback actions of estradiol than gilts born around the spring equinox and that treatment with melatonin would result in an earlier release from the estradiol negative feedback and advance the onset of puberty in gilts born around the spring equinox. To addresses these hypotheses we first determined the number of estradiol implants that would allow us to monitor the gradual release from the negative feedback actions of the steroid in ovariectomised gilts. Secondly, we determined the effect of a continuous release melatonin implant on the timing of the release from the negative feedback actions of estradiol in ovariectomised gilts in 4 cohorts born between the winter solstice and spring equinox. Thirdly we determined the effect of melatonin implants on the time of the first ovulation in intact gilts in 5 cohorts born between the winter solstice and spring equinox.

2. Materials and methods

The studies were conducted at the University of Adelaide Pig Research Facility at Roseworthy, South Australia (Latitude: 34°31′59″S, Longitude: 138°43′59″E). All experiments were approved by the University of Adelaide Animal Ethics Committee and were conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Gilts used in all experiments were F2 generation (Large White/Landrace maternal line cross Terminal Sire Line (Duroc × Landrace)). Housing consisted of concrete, slatted pens in sheds with adjustable side blinds, and thus exposure to seasonal variations in day length.

2.1. Experiment 1

In February 2007, gilts (aged 126 d; $n = 22$) were bilaterally ovariectomised under isoflurane anaesthesia and immediately implanted sub-cutaneously at the incision site with 1 ($n = 6$), 2 ($n = 5$) or 4 ($n = 5$) Silastic® Medical Grade tubing (0.33 cm i.d., 0.46 cm o.d., 3 cm long; Dow Corning, North Ryde, NSW) implants containing crystalline estradiol (Sigma Chemical Co., St Louis, Missouri, U.S.A.), prepared according to (Karsch et al., 1973) or an empty implant ($n = 6$). After surgery the gilts were maintained without any boar contact in groups of 6 for 11 weeks in pens in a shed that exposed them to natural changes in ambient light and temperature. Blood was collected twice weekly by jugular venepuncture into heparinised vacutainers (10 mL, Becton Dickinson, North Ryde, NSW) and plasma removed and stored at −20 °C.

In a pilot study 2 weeks before the termination of the Experiment, 4 animals with 1 estradiol capsule and 4 with 4 estradiol capsules were implanted subcutaneously behind the ear with either 1 Regulin® melatonin implant (Staples et al., 1992) containing 18 mg melatonin (Ceva Animal Health, Glenorie, New South Wales). A further 4 animals with 2 estradiol capsules were implanted with 2 melatonin implants and blood samples collected during daytime from the contra lateral jugular vein 3 and 7 d later.

2.2. Experiment 2

The study was conducted on 4 cohorts of 126 d old gilts born in early July, early August, late August and