RESEARCH REPORT
ANIMAL SCIENCE

Title: Effects of melatonin feeding before and following breeding in mature gilts and primiparous sows to reduce failures in estrus expression and pregnancy establishment associated with seasonal infertility in summer and fall **– NPB #14-081**

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Industry Summary

The data on seasonal declines in fertility that result in reduced numbers of pigs available for market the following summer originates from estrus failures and delays in gilts and primiparous (P1) sows, reduced conception and farrowing rates. Research suggests that changing photoperiod determines the seasonality of reproduction by melatonin modulation of the function of hypothalamic-pituitary axis. The aim of this study was to determine if extending the duration of the nighttime increase in melatonin during summer and early fall would improve fertility similar to periods of higher fertility in winter and spring. Gilts and P1 sows were given oral melatonin once daily beginning in proestrus and extending into early gestation, coinciding with the periods of the follicular phase, corpus luteum formation, pregnancy recognition and embryo survival. The experiment (Expt.) was conducted at a 6,500 sow, breed to wean farm in 12 sequential replicates from Jun to Sep. In Expt. 1a, gilts (n=420) that had expressed a second heat-no-serve (HNS), were assigned by weight to receive either Melatonin (MEL, 3 mg once daily) or Control (CON) in a syrup solution at 1400 h for 3 wk starting 1 wk before insemination. In Expt. 1b, P1 sows (n=470) were assigned by lactation length and backfat to receive the 3 wk treatment starting approximately 1 wk before expected estrus after weaning. Season was classified in sequential 4 wk intervals as mid-summer, late summer, and early fall. In Expt. 1a there was no effect of treatment (P>0.10) on age at HNS (203 d) and cycle length (22.6 d). However, there was an effect of treatment (P=0.03) on number of follicles (MEL 14.6 and CON 13.1) and an effect of season (P< 0.005) for gilts expressing estrus within 23 d after HNS (mid-summer (61.3%), late summer (74.9%) and early fall (80.3%)). There was no effect of treatment or season (P>0.10) on farrowing rate (80.0%) or born alive (12.8). In Expt. 1b, MEL treatment did not affect number of follicles or wean to estrus interval (8.2 d), but MEL reduced estrus (P=0.03) within 7 d (73.5%) compared to CON (82.0%). Return to estrus was affected by season with rates greater in mid-summer (24.6%) than in late summer (11.6%) and early fall (7.5%). There was no effect of treatment on farrowing rate (83.0%), but an effect of season (P=0.001), with farrowing rate lower in mid-summer (73.6%) than late summer (85.9%) and early fall (89.5%). Total born was not affected (P>0.10) by treatment or season (13.0). In conclusion, season affected follicle development and estrus expression in gilts but surprisingly did not have any effect on farrowing rate or litter size. In P1 sows, season was associated with lower numbers of follicles and reduced

conception and farrowing rate, but without effect on estrus expression or litter size. Melatonin treatment in summer and fall increased numbers of follicles in gilts, but had no effect on expression of estrus, farrowing rate or litter size. In P1 sows, Melatonin treatment had no effect on number of follicles but did reduce expression of estrus, and did not affect farrowing rate or litter size. These results identify the complex effects of season on reproductive function and fertility in gilts and parity 1 sows. These two parity groups are critical as they comprise ~33% of the sows in the breeding herd and directly relates to measures of productivity, culling and longevity. The evidence in the present study supports previous literature indicating that exogenous Melatonin can affect pig reproduction and demonstrates effects on follicle development and estrus measures. However, positive or negative responses to Melatonin treatment depended upon the physiological stage of maturity and perhaps metabolic state since gilts and P1 responded differently. Further research should be performed to identify the underlying physiology behind seasonal infertility and the Melatonin effects as these impact reproductive physiology and fertility, but it is not clear how.

Keywords:
Melatonin; Seasonal infertility; Parity; Sows; Gilts.

Scientific Abstract:

Effects of feeding melatonin during proestrus and early gestation in gilts and P1 sows to minimize the effects of seasonal infertility

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Seasonal infertility is associated with heat stress and changing photoperiod in summer and fall and is thought to be the cause of delayed puberty, increased wean to estrus interval, pregnancy failure, and reduced litter size. Research suggests that changing photoperiod determines the seasonality of reproduction by modulation of the function of hypothalamic-pituitary axis and ovary. The aim of this study was to determine if extending the duration of the nighttime increase in melatonin during summer and early fall would reduce the incidence of seasonal infertility. Gilts and parity 1 (P1) sows were given oral melatonin once daily beginning in proestrus and extending into early gestation, coinciding with the periods of the follicular phase, corpus luteum formation, pregnancy recognition and embryo survival. The experiment (Expt.) was conducted at a 6,500 sow, breed to wean farm in Illinois, USA. Expt. 1a and 1b were performed in 12 sequential replicates from Jun to Sep. In Expt. 1a, only gilts (n=420) that had expressed a second heat-no-serve (HNS), were assigned by weight to receive either Melatonin (MEL, 3 mg once daily) or Control (CON) in a syrup solution at 1400 h for 3 wk starting 1 wk before insemination. In Expt. 1b, P1 sows (n=470) were randomly assigned by lactation length and backfat to receive treatment for 3 wk starting approximately 1 wk before estrus expression after weaning. Data were subjected to ANOVA with binary responses variables analyzed using PROC GENMOD and continuous response measures analyzed using PROC MIXED for the main effects of treatment, season and their interaction using SAS 9.4. Season was classified in sequential 4 wk intervals as mid-summer, late summer, and early fall. In Expt. 1a there was no effect of treatment (P>0.10) on age at HNS (203 d) and cycle length (22.6 d). However, there was an effect of treatment and season (P=0.03) for number of follicles (MEL 14.6 and CON 13.1) but only an effect of season (P< 0.005) for gilts expressing estrus within 23 d after HNS (mid-summer (61.3%), late summer (74.9%) and early fall (80.3%)). There was no effect of treatment or season (P>0.10) on return to estrus (RE, 9.2%), conception rate (CR, 84.5%), farrowing rate (FR, 80.0%), total born (TB, 13.6) or born alive (12.8). In Expt. 1b, MEL treatment did not affect follicle number (15.4) and wean to estrus interval (8.2 d), however there was an effect of treatment (P=0.03) on females expressing estrus within 7 days for MEL (73.5%) compared to CON (82.0%). Season did affect (P=0.002) follicle number and RE (14.6%) following P1 insemination (P=0.0003) with mid-summer (24.6%) higher than late summer (11.6%) and early fall (7.5%). There was no effect of treatment on CR (88.3%) and FR (83.0%), but there was an effect of season (P=0.001), with mid-summer (73.6%) lower than the other seasons (late summer 85.9% and early fall 89.5%). Total born was not affected (P>0.10) by treatment or season (13.0). In conclusion, seasonal effects were apparent for gilt follicle development and

estrus expression and for P1 sows on return to estrus following breeding. In gilts, MEL increased follicle number but without effect on fertility. In P1 sows, MEL reduced estrus expression within 7 d after weaning. These results suggest that the dose and duration of MEL treatment may interact with the physiological status of the female to have positive or negative effects on reproduction.

Introduction

The seasonal decline in pig production has been documented in the US for sows farrowed and pigs marketed with the annual decline noted in the 3rd quarter (July-September). The seasonal reduction in numbers of market pigs actually originates from lower reproductive fertility starting in summer and continues into fall with delayed puberty, longer wean to service intervals, higher rates of pregnancy failures and leads to fewer litters produced winter and pigs reaching market in the next summer. These failures may be explained based on the seasonality of reproduction in pigs. As with many seasonal breeders, breeding occurs in late fall during short daylengths and allows farrowing in early spring when temperatures and access to food favor piglet survival. While the seasonal infertility pattern is clear, the cause of the problem is confounded by the effects of photoperiod and high temperature. The reasons for estrus and pregnancy failures during this time period while seemingly quite different may in fact be related and linked to both photoperiod and heat stress. The seasonal effects appear to alter hormones that regulate follicle development, egg maturation, normal ovulation, corpora lutea formation and production of progesterone. Summer [1, 2] and autumn [3, 4] are associated with seasonal infertility with delayed age at 1st service in gilts and longer wean to estrus intervals in P1 sows. That gonadotropins are limiting in these animals is supported by the fact that more animals can be induced into estrus in summer and fall when treated with PG600 [5-7]. That both photoperiod and temperature play a role in fertility is supported by Iida and Koketsu (2013] who evaluated herd data and reported that age at 1st service increased with the number of days above 25°C and decreased as daylength shortened. While much of the data is in general agreement, discrepancies may occur as a result of differences in season classification, year of study, location, and whether data from herd records or controlled studies were analyzed.

Seasonal pregnancy failures are identified by increased regular and irregular returns and sows diagnosed as pregnant but failing to farrow [9]. Parity 1 sows bred in Jul.-Sep. are at greatest risk for reproductive failure [2, 10]. A strong argument exists for the effects of seasonal infertility attributed to photoperiod [9] with minimal effect of light intensity [11, 12] and comes from observations that many periods of reproductive failure do not coincide with high temperatures. Duration of daylength is reported to be the primary cue for seasonality in the pig [9]. Over a 5-year period, farm data from 4 regions in France with differences in numbers of hot days (≥25°C), showed seasonal infertility in all regions but with photoperiod as the only common feature across all regions [13]. Photoperiod acts through melatonin in pigs melatonin release at night occurs with as little as 40 lx of light intensity. Tast et al., (2001] reported that the wild and domestic pig show similar patterns of duration of nighttime melatonin release in response to changes in lighting and season. In the pig, plasma melatonin increases 2 h after dark and remains elevated based on the duration of daylight [15]. Data on supplemental melatonin in the pig is limited but Love et al., (1993] reported positive effects of melatonin on pig fertility and Paterson et al., (1992] fed melatonin once daily in the afternoon for 7 days and doubled the number of gilts reaching puberty. Diekman et al., (1991] also reported gilts fed melatonin daily from Sep.-Dec. showed reduced age at puberty. Most studies agree that the dose of melatonin (1-5 mg) has little effect on fertility, while the pattern of melatonin delivery will impact the duration of the nighttime melatonin rise. In fact, continuous melatonin delivery from implants were not effective for advancing puberty [16, 18] and the daily melatonin feeding in afternoon is used to simulate the longer dark phase in the fall. Data also indicate another important role for melatonin and Shi et al., (2009] reported that melatonin is present in pig follicles and Kang et al., (2009] reported melatonin receptors the granulosa cells of the pig follicle as well as cumulus cells surrounding the egg. Further, when melatonin was added to *in-vitro* culture media for only few days, egg maturation, fertilization and embryo development were improved. Together, these data provide strong evidence for a local and rapid effect of melatonin acting directly at the level of the follicle and egg.

Evidence also supports photoperiod involvement in seasonal pregnancy failure as a result of low progesterone during pregnancy [9]. One study showed a 10% reduction in the progesterone of gestating sows in early fall which then

progressed to 50% lower in late fall when compared to other seasons [21] while Wrathall et al., (1986] reported lower progesterone on days 28 and 80 for sows gestating in summer. Further, early pregnancy failure is associated with low progesterone at critical times of pregnancy establishment, on days 13-14 and days 20-25 after breeding [23, 24]. Although the reason for the low progesterone is not known, some have suggested that LH support for the CL may be limiting. However, it is also possible that the low progesterone originates from problems in follicle development, ovulation, corpora lutea formation (CL). Support for this theory comes from Bertoldo et al., (2012] who reported that progesterone was lower in the small and large follicles of sows in summer compared to winter. Kermabon et al., (1995] reported that sows exposed to long photoperiods in gestation and lactation showed an increased proportion of sows showing delayed return to estrus and increased LH receptor mRNA but not LH receptors at time of weaning. The authors concluded that these results indicated a direct effect of daylength on ovarian gene transcription. Further, *in-vitro* models have reported fewer blastocysts form from the eggs obtained from the follicles of pigs in summer when compared to those obtained in winter. Collectively, these data suggest an effect of daylength and melatonin on ovarian function, resulting altered follicles, estrus, progesterone, embryo survival, and pregnancy rates.

While the photoperiod evidence is convincing, the effects of temperature or heat stress cannot be ignored and appear to exacerbate photoperiod sensitivity. In early studies, elevated temperatures were reported to reduce fertility [27] with Flowers and Day (1990] showing hot temperatures for extended periods suppressing gonadotropic hormones and reducing follicle development in gilts. d'Arce et al., (1970] reported that as number of hot days increased, so did the incidence of abnormal corpora lutea formation [29]. Early pregnancy failures and loss of embryos have been reported as a direct result of heat stress (>30°C) applied during early gestation [30-32]. Recently, genetic line differences were reported for upper critical temperature limits for farrowing rate and litter size [33]. Further, Bloemhof et al., (2013] examined herd records and noted a correlation of daytime temperature with reduced farrowing rate and litter size. In this study, they observed that heat stress three weeks before breeding influenced farrowing rate while heat stress in the week before until two weeks after breeding had effects on number of total born pigs. Heat stress is defined as the temperature above the set point, which affects physiological [35] and behavior patterns for thermoregulation. In pigs, heat stress can be complicated by the animal's ability to regulate heat loss through increased water consumption, standing or lying, and respiration rate. Further, heat production can be reduced through decreased feed consumption and reduced metabolic rate. Evidence for the pig's ability to adapt to heat stress comes from controlled studies where sows or gilts kept at 29°C [36], 30°C [12] or 32°C for extended periods had no effect on reproductive physiology or fertility [37] while changes in mechanisms for heat loss and production were observed. Thermal adaptation may occur through thyroid hormones and metabolic rate [38] and mechanisms involving behavioral changes to help animals lose heat. It is not clear whether this is equally true for diurnal and nocturnal fluctuations in temperature compared to exposure to a constant temperature. Lastly, surprisingly, there is limited data on evaporative cooling effects in swine, but in cattle, reports indicate reduction in mid-day temperatures by 2-7°F resulting in reduced respiration rates and rectal temperatures [39] and one study indicating and improvement in pregnancy rate [40].

Project Objectives

The objectives of this project were to test the effect oral melatonin administration for 7 days prior to 1st service and for 14 days following breeding on measures of fertility in gilts and weaned primiparous sows in summer and fall.

Materials and Methods

The use of animals for this experiment was approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign (#14081).

Experimental Design

These experiments were performed at a 6,500 sow breed-to-wean commercial research farm in western Illinois, USA, in 12 replicates each starting in the beginning of June through end of September of 2015. The farm was designed to allocate females in individual stalls from breeding until 35 days of gestation when they were moved to gestation group pens for further being moved to individual farrowing crates. Although experiments were completed during same time and using the

same treatment and dose, they were divided in Experiment 1a and 1b according to the category of animal used (P1 and gilts) and place where they were selected and moved in the first days of treatment. Treatment consisted of a 5 ml dose containing either 3 mg of Melatonin (Sigma-Aldrich, ≥98% pure, N-Acetyl-5-methoxytryptamine) diluted in ethanol or Control (only ethanol) in a syrup solution to be fed using an oral dosing gun, at 1400 h once daily.

Experiment 1a was conducted selecting genetic PIC Camborough® 1050 gilts (n= 420) in a gilt development unit (GDU) of the farm after the second estrus detected in the female using daily boar exposure. Each replicate of this experiment corresponded to the respectively week (Sunday to Saturday) of estrus detection. At approximately 203 days of age and approximately 10 days after second estrus gilts were weighed (average of 116 kg) and then assigned randomly by weight to receive either Melatonin (MEL) or Control (CON). A minimum weight target (100 kg) at the time of selection was established by the farm to make possible breeding gilts in the third estrus of their life. Gilts selected were allocated in individual stalls where they were organized intercalating MEL and CON treatment animals in the gestation barn in one of two gilt breeding lines. The treatment started 14 days after second estrus (approximately 7 days before next estrus) following 21 days of feeding the treatment at 1400 h. Gilts were checked individually for estrus expression twice daily in the mornings and in the afternoons using fenceline boar exposure with a mature boar and also applying backpressure. Once detected estrus, gilts were inseminated using cervical artificial insemination and moved to the gilts gestation line when at least 2 days after they were not standing in estrus anymore.

Experiment 1b was performed selecting genetic PIC Camborough® 1050 parity 1 (P1) sows (n=472) and randomly assigning them by lactation length and back fat measurement in the farrowing rooms one week before weaning. Two days before weaning, animals started receiving either MEL or CON at 1400 h following 21 days. Replicates matched with sows that were weaned together in the subsequent week (Monday to Friday) after an average of 23 days of lactation length. At weaning, sows were moved from the farrowing rooms to the breeding room where they were placed in individual stalls intercalating MEL and CON treatments. Females were checked individually for estrus twice daily one in the morning and another in the afternoon using fence line boar exposure to a mature boar and backpressure test. Once detected estrus, animals were inseminated at onset estrus using post cervical artificial insemination at 24 h intervals until no longer standing and then moved to the gestation barn in individual stalls.

In both experiments animals received treatment during 21 days with the purpose of starting in the reproductive status of the follicular phase and following the luteal phase in the early gestation. Furthermore, at 35 days of gestation females were all ultrasounded to pregnancy check and all gestating sows were moved from individual gestation stalls into pens with a single electronic sow feeding station (ESF). In addition, all females that returned to estrus were recorded, re-inseminated and removed from trial from that point.

Real-Time Trans-Rectal Ultrasound

Every other replicate on a sub-population of gilts and P1 sows, trans-rectal ultrasound was performed and digital images were recorded when animals were in individual stalls. Trans-rectal ultrasound (Hitachi Aloka Medical, Ltd., Wallingford, CT) involved the use of an Aloka 500 V ultrasound with a 7.5 MHz linear array transducer attached to a PVC stabilizing rod and a tablet with Pinnacle program was used to record video images. The transducer was attached to the rod using tape and the rod was well lubricated before insertion into the rectum. All scanning were conducted between 8000 and 1200 h on day 7 of melatonin or control treatment with the purpose to match the estrus day. Both ovaries were digitally recorded for determination of the number and size of follicles assessment at a later time.

Ambient Light Intensity, Temperature and Humidity

Weekly ambient light intensity, temperature, relative humidity and wind speed were obtained in each replicate during the entire experiment period. Measurements were obtained at the pig level (approximately 0.5 m from floor) at three different places in the replicate row and an average was performed. Light intensity was measured using a Mini Environmental Quality Meter (Sper Scientific, Scottsdale, AZ) with an operational detection limit of 0 to 20,000 lx (accuracy range: ± 5%

+ 4d). Temperature and humidity readings were obtained using the same device reader with and limit detection of 10 to 95 ± 6% (accuracy 30 ~ 60RH otherwise ± 8%) for RH and ºC ambient limit detection of 0 to 50 (accuracy 0.1 ± 1.2 ºC).

Reproductive Measures

Reproductive measures were assessed for all gilts and sows in all replicates. Estrus detection was performed by farm technician although supervised by the researcher. Dates of second and third estrus were recorded to calculate the inter-estrus interval for gilts. For P1, dates of weaning and first estrus post weaning were recorded to determine time of estrus and wean-to-estrus interval. Number of services were recorded for both gilts and P1. Conception rates were determined by calculating animals that were pregnant or not pregnant using trans-abdominal real-time ultrasound by the farm staff at 30 ± 2 d after first insemination. Farrowing rate, total born pigs, total pigs born alive, stillborn and mummified fetuses were recorded for each gilt and P1 sow that remained in the experiment.

Statistical Analysis

Gilts and parity 1 sows data were collected and were analyzed using SAS (SAS Institute Inc., Cary, NC USA). Data were subjected to ANOVA with binary response variables analyzed using PROC GENMOD or GLIMMIX and with continuous response measures analyzed using PROC MIXED for significance ($P \leq 0.05$) of the main effects of treatment using F-Test. Differences between least squares means were identified using the Bonferroni adjustment for multiple comparisons using pairwise t tests. Binary data analyses for females in estrus, estrus within 7 days, return to estrus, conception and farrowing rates were performed using a binary distribution using a logit-link function. Least squares means were then back transformed to the observed scale for interpretation. Models for the reproductive response included the main effects of treatment and season and random effect of replicates (weeks). Other variables such as lactation length, body condition score and weight were included as covariates and removed when not significant. For environmental measures, models included the main effect of season and local of the farm, with week included as a random effect. The assumptions of the ANOVA were tested for normal distribution data using PROC UNIVARIATE and Levene's test was used to test the homogeneity of variances. When data could not meet the assumptions, they were transformed for analysis.

Results

Environment Ambient Measures Areas of Gilts

During overall experiment 1a, natural light intensity was measured in the Gilt Developmental Unit (GDU) and within season appeared to be significant different ($P<0.0001$). Mid-Summer had 1125.6 ±18.5 Lux differing from Late-Summer (748 ±18.5 Lux) and Early-Fall (800.0 ±18.5 Lux). In the breeding room, light did not differ ($P>0.10$) among seasons (181.3 ± 127.2 Lux) however temperature did have a difference between all seasons ($P<0.0001$) being Mid-Summer the lowest temperature (22.4 ± 0.5 °C), Late-Summer the highest (25.3 ± 0.5 °C) and Early-Fall between the previous season (24.3 ± 0.5 °C). Similarly, the Relative Humidity (RH) differ among seasons following the same pattern as temperature, being Mid-Summer the lowest (68.8 ± 3.6 %), Late-Summer the highest (81.3 ± 3.6 %) and Early-Fall between the previous season (74.5 ± 3.6 %). Gestation luminosity (94.8 ± 67.8 Lux), temperature (24.3 ± 1.3 °C) and RH (72.5 ± 3.2 %) did not differ within season ($P>0.10$) (Table 1).

Environment Ambient Measures Areas of Parity 1

During overall experiment 1b temperatures (24.9 ± 1.2 °C) in the farrowing room where females were selected, did not indicate any statistically difference within seasons. In the weaned sow room where animals where checked for estrus and were inseminated, light differ within seasons ($P<0.0001$) being during Mid-Summer the higher intensity (57 ± 10.1 Lux) compared to Late-Summer (31.0 ± 10.1 Lux) and Early-Fall (30.0 ± 10.1 Lux). In the same room, temperature (27.1 ± 1.5 °C) and RH (81.4 ± 7.2 %) did not showed any difference within seasons ($P>0.10$). In the gestation room, measures of light showed to be significant higher ($P=0.03$) during Early-Fall (98.1 ± 11.1 Lux) than Mid-Summer (37.9 ± 11.1 Lux) and Late-Summer (46.6 ± 11.1 Lux). In the same place, temperature (23.8 ± 2.1 °C) and RH (69.6 ± 2.1 °C) were similar throughout seasons ($P>0.10$) (Table 2).

Reproductive Measures in Gilts

Treatment influenced follicle number (P=0.03) where Melatonin treatment averaged 14.6 ± 0.8 and Control 13.1 ± 0.8 while season also affected follicle number (P=0.03) as well. Gilts had greater number of follicles during Mid-Summer (14.9 ± 0.8), followed by the transition from late Summer (13.8 ± 0.8) and early Fall (12.8 ± 0.8). Inter estrus interval average was 23 d and the percentage of gilts that expressed the third estrus within 23 days was 72.2% with no significant difference (P≥ 0.10) between melatonin and control treatments. However, there was a statistical difference (P=0.005) for season, being the percentage of estrus within 23 days during mid-Summer (61.5 ± 4%) reduced compared to late Summer (74.8 ± 4%) and to early Fall (80.3 ± 5%). The returns to estrus corresponded to 9.2 % and did not have any treatment or season effect on its response. Conception rate (84.5 ± 4.3%) did not differ (P≥ 0.10) among treatments, seasons and neither their interaction. Farrowing rate (80.0 ± 4.9%) also did not differ statistically (P≥ 0.10) between melatonin (80.0%) and control (79.8%). Total born (13.6 ± 0.4), born alive (12.8 ± 0.4), stillborn (0.6 ± 0.1) and mummified fetuses (0.2 ± 0.1) did not differ (P≥ 0.10) among treatments, seasons and their interaction (Table 3).

Reproductive Measures in Parity 1 Sows

At the time that females were ultra-sounded, follicle number did not differ among treatments (15.4) but season did affect being during mid-Summer (13.1 ± 1.2) and Fall (14.5 ± 1.2) the lowest average comparing to the transition of late Summer (18.7 ± 1.2). The percentage of P1 sows that expressed estrus averaged in 92%, however 77.7% of overall P1 expressed a normal wean to estrus interval of 3 to 7 days. There was a significant difference (P=0.03) between melatonin treatment (73.5 ± 5%) and control (81.9 ± 5 %) for those P1 that expressed estrus in the normal interval. Return to estrus averaged in 14.5 % of total females inseminated and the only affect was season (P=0.0003), 24.6% of P1 returned estrus during mid-Summer compared to 11.6% and 7.5% of those inseminated during late Summer and early fall periods respectively. Conception rate (88.3 ± 3.8%) was not affected by treatment (P≥ 0.10) however it did differ per seasons (P=0.001). Animals that were bred during mid-Summer (81.1 ± 4.5%) had a lower CR compared to the transition from late Summer (89.6 ± 3.5%) and early fall (94.1 ± 3.4%). Similarly, farrowing rate while no statistical difference (P>0.10) among treatments (83.0 ± 4.5%), there was a significant difference among seasons (P=0.001). P1 inseminated during mid-Summer showed a lower percentage (73.6 ± 5.4%) of FR compared to late Summer (85.9 ± 4.5%) and early fall season (89.5 ± 4.5%). Total born (13.1 ± 1.3), born alive (12.2 ± 1.2), stillborn (0.5 ± 0.3) and mummified fetuses (0.2 ± 0.3) did not differ (P≥ 0.10) among treatments, seasons, and their interaction (Table 4).

Summary and Conclusions

In conclusion, season affected follicle development and estrus expression in gilts but surprisingly did not have any effect on farrowing rate or litter size. In P1 sows, season was associated with lower numbers of follicles and reduced conception and farrowing rate, but without effect on estrus expression or litter size. Melatonin treatment in summer and fall increased numbers of follicles in gilts, but had no effect on expression of estrus, farrowing rate or litter size. In P1 sows, Melatonin treatment had no effect on number of follicles but did reduce expression of estrus, and did not affect farrowing rate or litter size. These results identify the complex effects of season on reproductive function and fertility in gilts and parity 1 sows. These two parity groups are critical as they comprise ~33% of the sows in the breeding herd and directly relates to measures of productivity, culling and longevity. The evidence in the present study supports previous literature indicating that exogenous Melatonin can affect pig reproduction and demonstrates effects on follicle development and estrus measures. However, positive or negative responses to Melatonin treatment depended upon the physiological stage of maturity and perhaps metabolic state since gilts and P1 responded differently. Further research should be performed to identify the underlying physiology behind seasonal infertility and the Melatonin effects as these impact reproductive physiology and fertility, but it is not clear how.

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Table 1. Reproductive performance of gilts by treatment and season

	MID SUMMER		LATE SUMMER		EARLY FALL		SEM	Trt P-value	Season P-value
	CON	MEL	CON	MEL	CON	MEL			
N									
Follicle size	4.8	4.6	5.1	5.4	5.1	5.1	0.3	>0.10	>0.10
Follicle number	13.6^{xa}	16.3^{ya}	13.7^{xb}	13.9^{yb}	12.1^{xc}	13.5^{yc}	0.8	0.03	0.03
Inter estrus interval	24.3	23.6	23.0	21.8	21.4	21.9	0.7	>0.10	>0.10
Estrus within 23 days, %	64.4^{a}	58.6^{a}	72.5^{b}	77.2^{b}	85.0^{c}	75.5^{c}	5.8	>0.10	0.005
Return to estrus, %	14.6	10.1	11.7	7.6	7.7	3.6	4.0	>0.10	>0.10
Conception, %	82.1	83.5	81.5	84.7	86.0	89.2	4.3	>0.10	>0.10
Farrowing, %	75.3	81.4	80.5	72.9	83.7	86.0	4.9	>0.10	>0.10
Total born	14.0	13.9	13.4	12.8	13.8	13.5	0.4	>0.10	>0.10
Born alive	13.2	13.1	12.6	11.9	12.9	12.9	0.4	>0.10	0.08
Stillborn	0.6	0.6	0.5	0.7	0.6	0.4	0.1	>0.10	>0.10
Mummified	0.2	0.2	0.2	0.3	0.3	0.2	0.1	>0.10	>0.10

No statistical difference (P>0.05) in the interaction of treatment and season

$^{a-c}$Means within a row with different superscripts means a significant difference (P<0.05) caused by season

$^{x-z}$Means within a row with different superscripts means a significant difference (P<0.05) caused by treatment

Table 2. Reproductive performance of Parity 1 sows by treatment and season

	MID SUMMER		LATE SUMMER		EARLY FALL		SEM	Trt P-value	Season P-value
	CON	MEL	CON	MEL	CON	MEL			
N									
Follicles size	5.7	5.9	4.8	5.3	5.4	5.2	0.3	>0.10	>0.10
Follicle number	13.0	13.1	18.2	19.1	12.6	16.4	1.2	>0.10	0.002
Wean to estrus interval	7.2	9.9	7.9	8.5	7.5	8.4	2.4	0.09	>0.10
Estrus within 7 days, %	76.9^{x}	72.2^{y}	82.0^{x}	71.9^{y}	86.8^{x}	76.5^{y}	4.8	0.03	>0.10
Return to estrus, %	21.5^{a}	27.7^{a}	11.4^{b}	11.8^{b}	8.4^{b}	6.5^{b}	4.5	>0.10	0.0003
Conception, %	86.3^{a}	75.9^{a}	88.5^{b}	90.6^{b}	92.6^{c}	95.6^{c}	3.8	>0.10	0.001
Farrowing, %	75.0^{a}	72.2^{a}	86.0^{b}	85.7^{b}	88.8^{b}	90.2^{b}	4.5	>0.10	0.001
Total born	12.6	13.5	13.6	12.7	12.6	13.2	1.3	>0.10	>0.10
Born alive	11.7	12.3	13.0	12.2	11.8	12.6	1.2	>0.10	>0.10
Stillborn	0.6	0.4	0.2	0.4	0.5	0.6	0.3	>0.10	0.07
Mummified	0.3	0.8	0.0	0.0	0.2	0.1	0.3	>0.10	0.09

No statistical difference (P>0.05) in the interaction of treatment and season

$^{a\text{-}c}$Means within a row with different superscripts means a significant difference (P<0.05) caused by season

$^{x\text{-}z}$Means within a row with different superscripts means a significant difference (P<0.05) caused by treatment

Table 3. Environmental measures on Gilts locations by season

	MID SUMMER	LATE SUMMER	EARLY FALL	SEM	P-value
GDU Light (Lux)	1125.6^{x}	748.0^{y}	800.0^{y}	18.5	<0.0001
Breeding Light (Lux)	187.4	138.5	217.9	127.2	>0.10
Breeding Temperature	22.4^{x}	25.3^{y}	24.3^{z}	0.5	<0.0001
Breeding Humidity (RH)	68.8^{x}	81.3^{y}	74.5^{xy}	3.6	0.02
Gestation Light (Lux)	56.1	173	55.4	67.6	>0.10
Gestation Temperature	26	23.6	23.2	1.3	>0.10
Gestation Humidity (RH)	77.4	71.1	69	3.2	>0.10

$^{x\text{-}z}$Means within a row with different superscripts means a significant difference (P<0.05) caused by season

Table 4. Environmental measures on Parity 1 sows locations by season

	MID SUMMER	LATE SUMMER	EARLY FALL	SEM	P-value
Farrowing Temperature	24.3	25.1	25.3	1.2	>0.10
Breeding Light (Lux)	57^{x}	31^{y}	30^{y}	10.1	<0.0001
Breeding Temperature	27.2	26.8	27.4	1.5	>0.10
Breeding Humidity (RH)	87	80	77.1	7.2	>0.10
Gestation Light (Lux)	37.9^{x}	46.6^{x}	98.1^{y}	11.1	0.03
Gestation Temperature	24.6	26	20.8	2.1	>0.10
Gestation Humidity (RH)	65.5	74	69.2	3.1	>0.10

$^{x\text{-}y}$Means within a row with different superscripts means a significant difference ($P<0.05$) caused by season