Investigating Urocanic acid Concentration in Relation to Seasonal Subfertility in the Sow Model

Jessica Gold

School of Life and Environmental Sciences

The University of Sydney

The following thesis was submitted to complete the written portion the degree: “Master of Philosophy in Research” at the University of Sydney in 2024.

I certify that all intellectual contents in this these are either the product of my own work or have been correctly referenced. All the assistance received in conducting the research contained within this thesis have been acknowledged in the "Acknowledgements" section below

Acknowledgements

I thank Professor Claire Wade (the University of Sydney) for all her help, guidance, reviews, and diligent supervision. I thank Associate Professor Roslyn Bathgate (the University of Sydney) for her help in acquiring the skin tape samples from the University of Sydney owned swine farm in Camden, and for keeping track of and communicating the reproductive status of the sampled sows used in each month. I thank Honours Student Chelsea Moss (the University of Sydney) for her help in acquiring the skin tape samples and her information on the layout of the sheds, including light exposure, from the University of Sydney owned swine farm in Camden NSW. I thank Associate Professor Anne Kwan and Research Associate Rezwan Siddiquee (the University of Sydney) for allowing me to use and instructions on use of their high performance liquid chromatography equipment. I thank Thomas White for his guidance in determining, coding, and understanding the correct statistical methods.

Contents

Abbreviations

Urocanate hydratase (UROC1).

Urocanic acid (UCA).

Histidine deaminase pathway (HDP).

Gamma-aminobutyric acid (GABA).

Ultraviolet (UV).

Follicle-stimulating hormone (FSH).

Luteinising hormone (LH).

Pars tuberalis (PT).

Circadian Locomotor Output Cycles Kaput (CLOCK).

Basic helix-loop-helix ARNT like 1 (Bmal1).

Period circadian regulator 1 (Per1).

Period circadian regulator 2 (Per2).

Cryptochrome circadian regulator 1 (Cry1).

Cryptochrome circadian regulator 2 (Cry 2).

Suprachiasmatic nucleus (SCN).

4-imidiazole 4-propionic acid (IPA).

Oxidative stress response transcription factor (SKN-1).

Opsin–5 (OPN5).

Amino acids (AAs).

Generalised Additive Mixed Model (GAMM).

Abstract

Reproductive success is critical to the continuity of supply by the Australian pork industry. Seasonal fluctuations limit the reproductive success of swine, therefore causing a reduction in the potential maximum revenue of the Australian pork industry. Researchers at the University of Sydney identified a strong positive correlation between a variant in the gene for the enzyme *urocanate hydratase* (UROC1) and seasonal fertility differences between wild and domesticated canids. *Urocanate hydratase* acts on *trans*- urocanic acid (UCA) in the histidine deaminase pathway (HDP). *Trans*- UCA can be photoisomerized into *cis*- UCA by ultraviolet B (UVB) light. The HDP produces a reproductive neurotransmitter, glutamate, as an end product. *Cis*- UCA is thought to be involved in the activation of 5-HT_2A serotonin receptors. This study aims to identify a relationship between the activity of the HDP as impacted by UROC1 expression and the circannual rhythm of commercially housed sows. The concentrations of *trans*- and *cis*- UCA were calculated from samples taken from the epithelium of 29 commercially housed sows from a University of Sydney owned farm in Camden, New South Wales, using cello tape. The results of this study show clearly that there are circannual rhythms in *trans*- and *cis*- UCA concentrations, but a clear connection with an oestrus status was unable to be observed. The concentrations of both isomers of urocanic acid peaked between December and March during a period when sow fertility is typically reduced. Across all reproductive statuses measured, pregnant sows displayed a significant positive relationship with *trans*- UCA ($Pr(>|t|) = 0.0006$), and lactating sows showed a significant positive relationship with *cis*- UCA ($Pr(>|t|) = 0.0048$). Given that *trans*- UCA is implicated in the HDP, and *cis*- UCA has been shown to activate serotonin 5-HT_2A receptors, then presumably, *trans*- UCA induced HDP activity influences lactation and *cis*- UCA activated serotonin receptors influence pregnancy.

Introduction/Background

In 2015, it was estimated that the Australian pork industry contributed $5.2 billion to the domestic Australian economy and supported 36,000 jobs (Australian Pork Limited, 2020). Reproductive success is critical to the continuity of supply of the Australian pork industry. Sows have a gestation

length of 111-120 days (approximately four months), and their offspring reach market weight at 5–6 months of age (Peltoniemi & Virolainen 2006). Reduced reproductive success is often observed throughout the warmer months, resulting in a corresponding seasonal decrease in fresh pork production and availability during spring and early summer, when there are fewer than the yearly average number of pigs at a sufficient weight for retail sale (see Figure 1). The semi-seasonal fertility demonstrated by domesticated swine is thought to be a remnant of the reproductive seasonality of their wild swine ancestors that were strictly seasonal, short-day breeders (Love et al. 1993). The reduced reproductive success in swine in summer is referred to as summer subfertility and is a form of seasonal fertility.

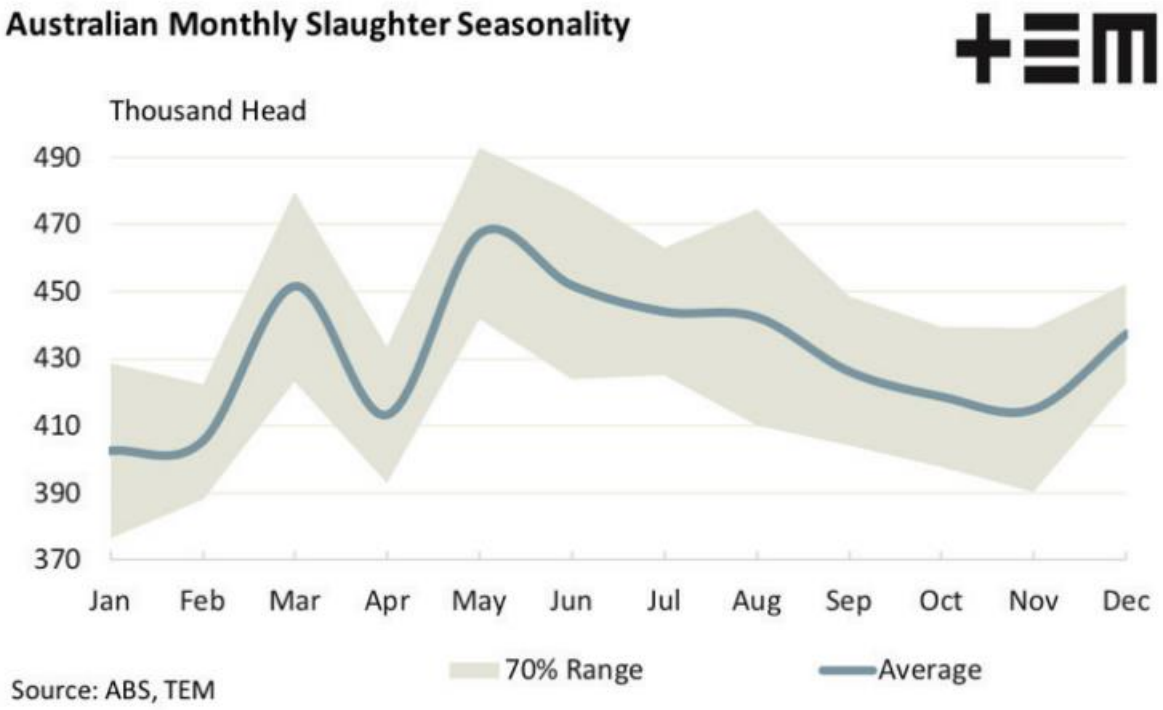


Figure 1. The above Figure has been taken from the State of Industry Report by Australian Pork limited (2021). It displays an increase in the number of pigs slaughtered during late autumn and early winter. A sharp increase occurs during March, followed by another sharp increase during May. After May, as mid spring approaches, the number of pigs slaughtered decreases until November, where slaughter starts to increase again (Australian Pork Limited, 2021).

By reducing the effects of seasonal subfertility on domesticated swine, annual swine production would increase in consistency and the availability of fresh pork would be ensured by providing a

constant supply of pigs at finishing weight for market annually. Therefore, addressing summer subfertility presents an opportunity to improve the economic growth of the Australian pork and swine industry.

To mitigate the negative effect of semi-seasonal fertility in swine, swine farmers employ multiple methods of oestrus control, such as photoperiod manipulation, melatonin administration and hormone treatment. The most widely applied method of oestrus control is the control of photoperiod (Chemineau et al. 2007). Artificial insemination facilities using artificial photoperiods (i.e., day–night cycles) have recorded a 40% increase in the production of sperm per male per year without any seasonal variation in sperm quality (Delgadillo et al. 1993). Given that swine are semi-seasonal short-day breeders, an increased dark phase to light phase ratio can be employed to simulate winter environmental lighting conditions and reduce the negative effect of summer environmental stimuli on the reproductive success of swine (Claus et al. 1985).

Currently, environmental lighting conditions are believed to affect seasonal reproduction via the control of melatonin production (Delgadillo *et al.* 1993). In short-day breeders, the increase in melatonin production induced by long nights is thought to be stimulatory of reproductive behaviours. Directly administering melatonin without altering light exposure has been shown to increase the length of oestrus and embryo survival (Arend & Knox 2021). The administration of melatonin in short-day breeding production animals has also been shown to increase reproductive success (Chemineau et al. 1996). Melatonin is commonly administered in the wool and lamb industry, but it is not commonly used in the pork industry. However, research has revealed that melatonin use has positive effects on swine production (Arend & Knox 2021).

The biochemicals that are commonly administered to counteract summer subfertility in swine are associated with the downstream effects of melatonin. Follicle-stimulating hormone (FSH) and luteinising hormone (LH) are used to synchronise oestrus by initiating oestrus in noncycling gilts and sows (Pork Information Gateway 2012). Administered FSH stimulates the growth of new follicles within the ovary and constitutes the follicular stage of the female reproductive cycle (Roy Homburg

2014). An increase in LH causes the mature follicle to burst and release a gamete, forming a corpus luteum from the follicle (François et al. 2017). Progesterone, which is produced by the follicle, is also used to synchronise sows and gilts by inhibiting FSH production and encouraging the release of LH, therefore resetting the reproductive hormones within the breeding herd (Homburg 2014; Samani et al. 2023). Better understanding of the genetic control of seasonal subfertility may enable managers to breed non-seasonal pigs, alleviating the need for managerial solutions to the problem.

Circannual changes in reproductive success

Seasonal fertility is a circannual rhythm that takes the form of seasonal oscillations in breeding behaviour caused by changes in environmental stimuli such as temperature, nutrition and light (Gwinner 1977; Körtner & Geiser 2000; Küller et al. 2006; Jong-Min Woo & Teodor T Postolache 2008; Soh et al. 2009; Ely et al. 2013). Entrainment is a scenario in which two oscillations or patterns align. Animals that experience seasonality are entrained because their behaviours and annual environmental oscillations are closely related (Jiménez et al. 2022).

Changes in behaviour due to 24-hour cyclic changes in ultraviolet (UV) light exposure and temperature are referred to as circadian rhythms (Zee et al. 2013). These rhythms change over a roughly 365-day time period as environmental stimuli vary in intensity and availability, creating circannual rhythms (Johnsson 2008). Circannual rhythms contribute to phenomena such as migration, hibernation and seasonal fertility (Gwinner 1977; Körtner & Geiser 2000). Circadian rhythms are most obvious at high latitudes, resulting in the inhabitants of high latitudes evolving higher degrees of seasonal behaviour (Chemineau et al. 2008; Dardente et al. 2016). Along with the effect of environmental stimuli on circannual and circadian rhythms, environmental stimuli entrain seasonal fertility. In wild swine, entrainment serves to give offspring the best chance of survival by farrowing during an annual period of high nutritional availability (Swaab et al. 1996; Chemineau et al. 2008; Dardente et al. 2016).

In addition to environmental stimuli, Circadian Locomotor Output Cycles Kaput (CLOCK) genes provide a method to control circadian and circannual rhythms (Rijo-Ferreira & Takahashi 2019).

CLOCK genes are genes that exist within a transcriptional network that oscillates within a period of approximately 24 hours (Rijo-Ferreira & Takahashi 2019). The CLOCK-gene-driven transcriptional network is the underlying mechanism of circadian rhythms. However, fine-tuning via other methods of control occurs after the expression of the CLOCK genes (Kojima et al. 2011).

The pineal gland is an organ of great importance in the control of circadian and circannual rhythms. Within the pineal gland, there is a region known as the pars tuberalis (PT), where CLOCK genes are present within specialised agranular cells (Lincoln et al. 2003). Agranular PT cells possess melatonin (mt1) receptors and express the following genes: basic helix-loop-helix ARNT like 1 (Bmal1), CLOCK, period circadian regulator 1 (Per1), period circadian regulator 2 (Per2), cryptochrome circadian regulator 1 (Cry1), and cryptochrome circadian regulator 2 (Cry 2) (Lincoln et al. 2003). This provides a molecular base for melatonin-initiated circannual rhythm generation. Period genes (Per1 and Per2), are expressed during the early light phases, whereas cryptochrome genes (Cry1 and Cry2) are expressed during dark phases (Lincoln et al. 2003). The above explanation of how agranular melatonin-receptive PT cells express CLOCK genes, and season-based research on hamsters and sheep indicate that circadian CLOCK gene expression in the PT is entrained by photoperiod via melatonin signals (Lincoln et al. 2003).

UV, melatonin and the circadian response

Circannual rhythms and seasonal fertility are believed to be regulated by the production of melatonin during dark circadian periods (night) and melatonin's effect on the expression of cryptochrome and period genes in agranular PT cells (Loudon 1994; Cassone & Yoshimura 2022). Under commercially housed conditions, rapid changes in the photoperiod within an enclosed space can alter the concentration of blood melatonin within one week (Klupiec et al. 1997; Tast et al. 2001b). A decrease in light exposure to the retina signals the pineal gland to produce melatonin, which regulates the animal's seasonal attributes, such as reproductive fertility, via the melatonin pathway (Peltoniemi & Virolainen 2006; Yoshimura 2010).

The initiation of the melatonin pathway requires light to affect the suprachiasmatic nucleus (SCN), which signals the pineal gland. The stimulation of the SCN by changes in the photoperiod initiates a melatonin release, which signals the pineal gland to release gonadotrophin-releasing hormone (GnRH) (Peltoniemi & Virolainen 2006; Yoshimura 2010). Long nights (the dark phase) induce increased levels of melatonin. Environmental light with short wavelengths associated with daylight (the light phase) suppresses dark-phase melatonin production (Lockley et al. 2003). Decreasing wavelengths drastically suppress melatonin production, with wavelengths of 460 nm producing twice the level of suppression as wavelengths of 555 nm (Lockley et al. 2003). An increase in lux initiated by the light phase has no recorded effect on the melatonin response in domesticated pigs (Tast et al. 2001a). Therefore, the changes in wavelength associated with the light phase alone, not the intensity of light, as measured in lux, suppress the production of melatonin.

Melatonin and its downstream counterparts interact with receptors in the hypothalamus, and these interactions initiate the production of GnRH (Peltoniemi & Virolainen 2006). GnRH stimulates the pituitary gland to produce the gonadotrophins FSH and LH (Peltoniemi & Virolainen 2006). FSH leads to the follicular phase of the female reproductive cycle while LH results in the luteal phase and the breeding period in said cycle (Peltoniemi & Virolainen 2006; François *et al.* 2017). The melatonin pathway is shared across multiple mammals (Lincoln 1992; Peltoniemi & Virolainen 2006). The key areas of the brain related to this process are shown below (Figure 2.).

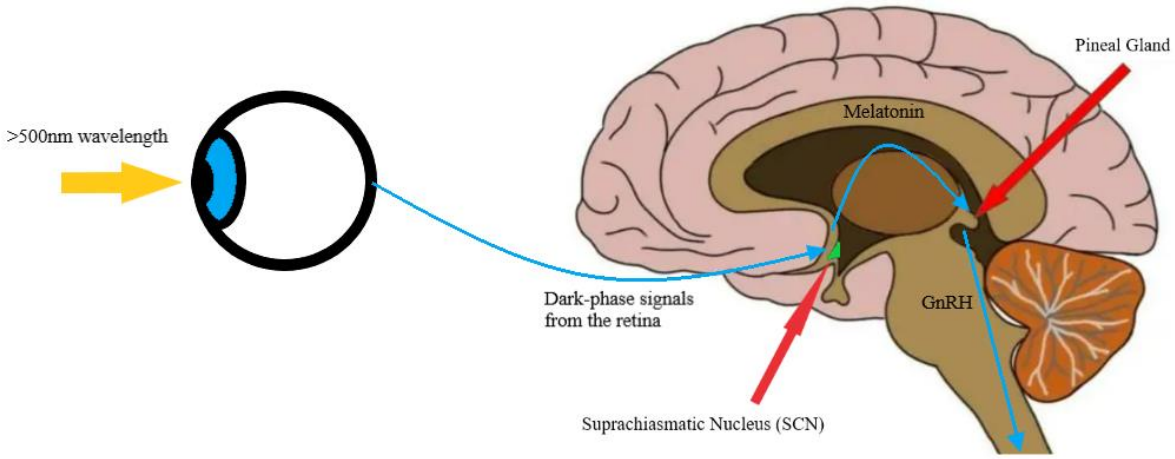


Figure 2. The pathway by which light with wavelengths greater than 500 nm that are detected through retinal neurons reduces melatonin inhibition in the SCN, increasing melatonin production and interacting with the pineal gland. The increase in melatonin production is expected to either increase GnRH in short-day breeders or inhibit it in long-day breeders. This image was modelled after the melatonin pathway as described in (Charles H. Emerson 2020).

The dark phase of the photoperiod induces the production of melatonin, controlling reproductive seasonality through two mechanisms. The first is to synchronise the phases of reproductive and gonadal development with external stimuli from the environment by providing the local population with a shared stimulus that is correlated with an increase in food availability and quality (Chemineau et al. 2008). Secondly ,it synchronises the reproductive cycle with others of the same species to increase mate availability (Chemineau et al. 2008).

Surprisingly, research on ewes and wolves has revealed that when the pineal gland is removed, seasonal fertility continues to be expressed, indicating that the pineal–melatonin pathway is not the only cause of seasonal fertility (Bittman et al. 1983; Asa et al. 1987). This finding reveals that there is a seasonal fertility knowledge gap that is open for further investigation.

Identification of the histidine–deaminase pathway as a potential circannual modulator

In mammals, seasonal variations in fertility are believed to be primarily regulated by the effect of the photoperiod on melatonin production (Loudon 1994; Cassone & Yoshimura 2022). However, the results of unpublished work conducted at the University of Sydney indicate that a different pathway initiated by environmental signal mechanisms may contribute to seasonal fertility in mammals. Researchers at the University of Sydney conducted a GWAS and identified an association between a variant in the gene for the enzyme *urocanate hydratase* (UROC1) and seasonal fertility differences between wild and domesticated canids.

Urocanate hydratase is a strong potential candidate gene that may affect seasonal fertility for multiple reasons. First, in the canids used for mapping, it was the only gene in the mapped region to have a nonsynonymous single nucleotide polymorphism with significant constraint (Wade pers. comm). Secondly the encoded protein (i.e., the enzyme *urocanate hydratase*) is photoactivated by light and is optimally activated by wavelengths between 275–280 nm (Hug & Roth 1972). Temperatures

approaching 30°C led to increased *urocanate hydratase* activity, indicating that this activity can be influenced by external stimuli. However, the Hug and Rosh (1972) study was performed on removed rat livers, meaning *urocanate hydratase* was exposed to external temperatures before analysis (Hug & Hunter1974; Hug & Roth 1972). *Urocanate hydratase* is also expressed in the brain, testes, and ovaries (Weizmann Institute of Science, 2022). The average body temperature is considered to be 37°C with variations of up to 0.5°C (Walker 1990). UCA is one of the initial substrates in the histidine deaminase pathway (HDP; see Figure 3). The HDP converts the amino acid histidine into the neurotransmitter glutamate via multiple biochemical reactions (Hall 1952; Kolenbrander & Berg 1967). Thus, although there is potential for temperature to alter HDP activity, glutamate, gamma-aminobutyric acid (GABA) and GnRH production by directly affecting *urocanate hydratase* activity, the stability of the internal temperatures of tissues expressing *urocanate hydratase* activity shows that the possibility is unlikely.

The substrate of *urocanate hydratase*, UCA, is isomerised from its *trans* to its *cis* form within five minutes of exposure to ultra-violet B (UVB) light (Norval 2001). *Urocanate hydratase* acts only on *trans*- UCA and not the *cis*- isomer, hydrating trans-UCA into 4-imidiazole 4-propionic acid (IPA). The UVB light reduces the concentration of *trans*-UCA and therefore its availability as a substrate for *urocanate hydratase* (Garssen et al. 1999). UCA was originally identified in canine urine samples (Gibbs & Norval 2011; Safran et al. 2010. However, it is also found in mammalian livers and brains, and both UCA isomers are found in the epithelium (Safran et al. 2010; Gibbs & Norval 2011).. UCA isomers are thought to accumulate in the epithelium because skin cells are unable to convert *trans*-UCA to IPA due to a lack *urocanate hydratase* expression or presence in the stratum corneum (Eckhart, 2016)

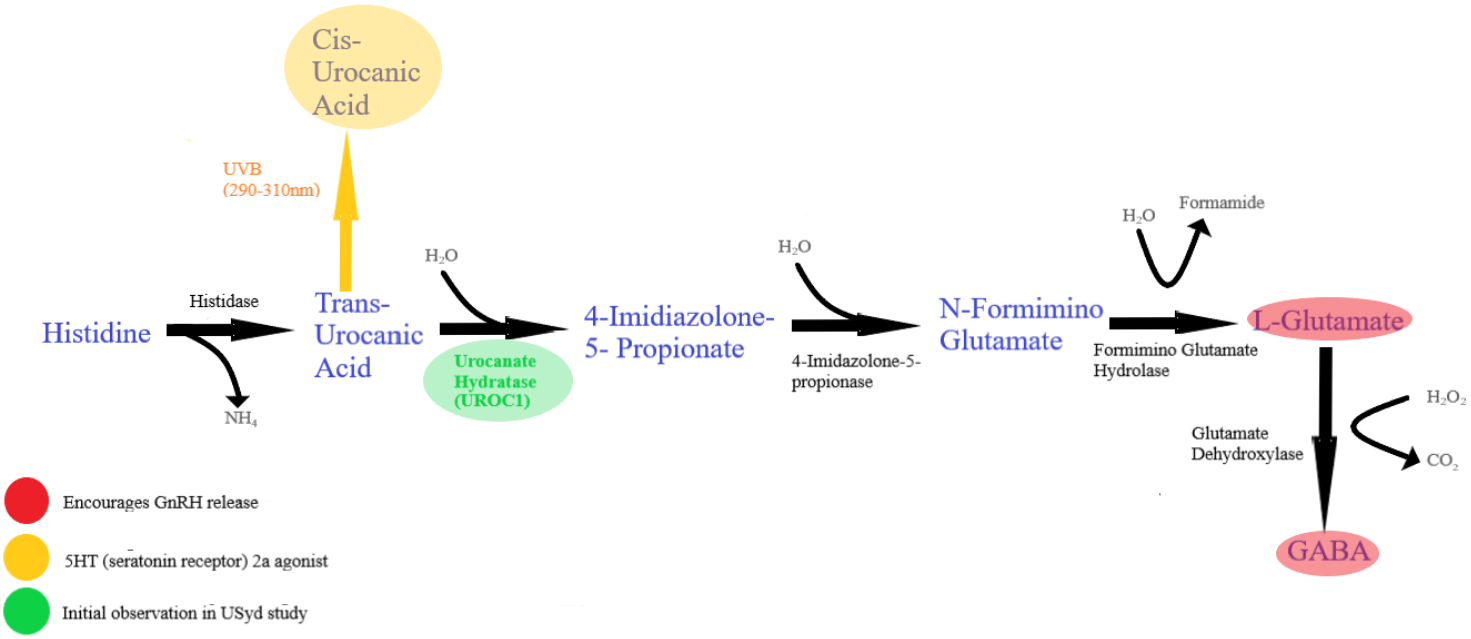


Figure 3. A flow chart displaying the histidine deaminase pathway and its associated enzymes, substrates and products of interest. GABA is not a part of this pathway. However, its importance in reproductive biochemistry and its anabolism from glutamate justifies its placement in this figure (Maffucci & Gore 2009).

The HDP produces two end-of-pathway biochemicals: the neurotransmitter glutamate and the isomerised form of *trans*-UCA, *cis*-UCA (Kohlmeier 2015).

Glutamate and GABA are neurotransmitters that may impact the expression of GnRH (Iremonger et al. 2010; Maffucci & Gore 2009). GnRH initiates seasonal breeding and oestrus by initiating the release of gonadotrophins (Dhandapani & Brann 2000). *Cis*- UCA is an agonist of the serotonin [5-hydroxytryptamine] $(5\text{-HT})_2$A receptors, meaning that *cis*- UCA activates 5-HT_2A receptors in place of serotonin (Walterscheid et al. 2006). Serotonin has been shown to act as a neuromodulator for sexual inhibitory pathways by reducing the sexual excitatory effects of dopamine and norepinephrine (Croft 2017). Evidence suggests that the activation of 5-HT_2A receptors reduces male sexual arousal and increases female sexual behaviour, providing a possible neurobiological method through which UCA availability and UROC1 activity may affect seasonal fertility (Popova & Amstislavskaya 2002; Nedergaard et al. 2004),

The HDP has multiple elements that react to light or temperature. Light (specifically UVB, which is characterised as having a wavelength from 280–315 nm) affects the relative concentrations of *trans*-

UCA, *cis*- UCA, 4-imidiazole 5-propionic acid and *urocanate hydratase* (Hassall & Greenberg 1971; Kammeyer et al. 1995; Garssen *et al.* 1999; Hug et al. 1999). Heat also affects *urocanate hydratase* activity (Hug & Hunter 1974). Changes in the relative concentrations of *trans*- UCA, 4-imidiazole 5-propionic acid and *urocanate hydratase* affect reproductive behaviour by influencing final glutamate concentrations and GnRH release (Kuo et al. 2014).

The immediate downstream product of *urocanate hydratase* acting on *trans*-UCA is 4-imidazole-5-propionate (IPA). Solidified 4-imidazole-5-propionate has been shown to be susceptible to light, turning into a reddish-brown substance upon light exposure. However, there is no evidence that biological 4-imidiazole 5-propionate is affected by light exposure (Hassall & Greenberg 1971). The IPA initiated activation of oxidative stress response transcription factor (SKN-1) genes has been linked to a decreased survival of heat stress, providing evidence that an increase in high environmental temperature exposure capable of inducing heat stress may be related to a decrease in IPA, minimising the available pool of IPA for HDP metabolism (Frankino, 2022).

Circannual entrainment modulation of seasonality by UV

Over the course of a year, the day–night ratio changes and, therefore, so does the duration of UV exposure per day (photoperiod). In winter, the day–night ratio decreases due to the increased length of the dark phase, resulting in a decrease in UV exposure. Data provided by the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) shows that the UV index also reduces during winter (Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) 2023). The opposite occurs leading into summer, with an increased day–night ratio and an increased recorded ARPANSA UV index (Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) 2023).

The most studied and accepted environmental factor that affects seasonal entrainment is the change in the day–night ratio on the concentration of melatonin and its resulting effect on the SCN (Loudon 1994; Cassone & Yoshimura 2022). The SCN has been shown to be a crucial neurological location for photoperiod detection and effects (Chen et al. 2020). The SCN maintains a basic circadian rhythm

using CLOCK genes, the expression of which is controlled by the external photoperiod and seasonal phase shift (Coomans et al. 2015).

Along with the SCN, the pituitary gland causes hormonal changes and acts as a centre for entrainment control. Melatonin affects the circannual rhythm of the pituitary gland through the SCN. However, there are multiple additional potential pathways in which photoperiod influences the input for the pituitary gland (Peltoniemi & Virolainen 2006; Yoshimura 2010; Chen *et al.* 2020). These include neural receptors that have been identified in Opsin–5 (OPN5) positive neurons in avians which act as deep-brain photoreceptors (Nakane et al. 2010). These receptors, which are also found in mammals in addition to birds, reach the pituitary gland, providing an alternate method for photoperiod to create neuroendocrine changes (Chen et al. 2020). GABA and glutamate, which are both produced by the HDP, are important neurotransmitters for the activity of the pituitary gland (Maffucci & Gore 2009).

Glutamate is produced by the HDP from *trans*-UCA, and can be converted to GABA through *glutamate dehydroxylase* (Kohlmeier 2015). Glutamate and GABA concentrations have potential to be altered through the photoisomerization of *trans*- UCA according to the photoperiod and UVB exposure. Changes in glutamate and GABA cause circannual rhythms in the hormonal release of the pituitary gland, resulting in environmental entrainment.

Modulation of seasonality by temperature

Temperature is highly correlated with the UV index. Therefore, as the UV index decreases during winter, the temperature decreases (Australian Government Bureau of Meteorology 2023). An increase in temperature can increase nutrient availability and decrease energy expenditures required for homeostasis, making it an important environmental factor to entrain with seasonal behaviour (Chmura & Williams 2022). However, if temperatures do not drop below 21°C for three to six hours per day, heat stress can occur because the animal does not have the opportunity to lose accumulated heat from the light phase, leading to a decrease in appetite and feed intake (Silanikove, 2000). Evidence from fish suggests that temperature can impact seasonal reproduction via its role as a metabolic driver (Dodson et al. 2013). In mammals, environmental temperature is perceived through neurons in the

skin that express genes in the transient receptor potential family (Chmura & Williams 2022). Another viable mechanism by which temperature affects seasonal fertility resides in a section of the hypothalamus called the pre-optic area. The pre-optic area regulates the melatonin pathway via temperature detection (Caro et al. 2013). Research into voles has shown that an increase in temperature from 8°C to 20°C is correlated with a decrease in GnRH production (Kriegsfeld et al. 2000). GnRH has been found to increase reproductive behaviour (Iremonger et al. 2010; Maffucci & Gore 2009). The negative correlation between GnRH production and increased temperature suggests that temperature can play a role in reproductive control.

Modulation of seasonality by nutrition

During spring, many grasses and other foraging plants begin to enter a growth phase. The new shoots produced during this phase have elevated digestibility and protein content compared to the main fibrous structure of the plant (NSW Department of Primary Industries 2023). The digestibility and protein content of the plant decreases during flowering, and drops further after flowering when the plant becomes increasingly fibrous (NSW Department of Primary Industries 2023). The catabolism of the increased protein presence and digestibility in the growth phase of vegetation provide the raw amino acids (AAs) required for cell proliferation and protein development. Of these AAs, histidine, the initiator of the HDP and precursor to glutamate and GABA, is present (Kohlmeier 2015; Schousboe et al. 2014). Thus, it is possible that histidine is more available during spring due to the increase in available digestible protein sources in the form of either growth phase vegetation or an increase in herbivore prey due to seasonal birth rates.

Metabolic rate and nutrition have both been shown to modulate biological clock functions in peripheral tissues (peripheral clocks) independently of photoperiod (Froy 2007; Lamia et al. 2008). Research on mice with a liver-specific deletion in the Bmal1 gene demonstrated that the liver also plays a vital role in nutritional control over biological clocks (Lamia et al. 2008). *Urocanate hydratase*, which is ultimately dependent upon histidine, has been recorded to be most active in the liver, providing an avenue for *urocanate hydratase* activity and, by proxy, enables the HDP to

contribute to the control of peripheral and circannual biological clocks (Weizmann Institute of Science 2022).

Aims of the current research

In the current body of work, skin-tape samples from commercially housed sows were used to measure *trans*- and *cis*-UCA concentrations in the epithelium. It is proposed that *trans*- UCA concentrations can be used as an indication of UROC1 activity. Our hypothesis is that *cis*- UCA concentrations may increase 5-HT_2A serotonin receptor activity, leading to a decrease in male sexual behaviour and an increase in female sexual arousal. Using measurements of UCA isomers, we explored the potential relationships between the activity of the HDP impacted by *urocanate hydratase* expression and reproductive statuses of commercially housed sows over a full annual period.

Methods

We investigated the environmental variables of temperature, UV index, light exposure, and reproductive status to determine whether any have a correlation with *trans*- or *cis*- UCA. UVB exposure isomerises the *trans*- isomer to the *cis*- isomer of UCA (Kammeyer *et al.* 1995; Garssen *et al.* 1999; BioSynthesis 2017). Increased UV index and natural light exposure are expected to decrease *trans*- UCA and increase *cis*- UCA. Increased *trans*- UCA promotes increased glutamate production by increasing its availability for metabolism by the HPD. Increasing *cis*- UCA concentrations is proposed to result in increased 5-HT_2A receptor activation. Both neurotransmitters (i.e., glutamate and *cis*- UCA) have the potential to affect reproductive behaviour. Therefore, reproductive status was expected to be positively correlated to UCA isomer measurements. Given that *cis*- UCA-activated 5-HT_2A receptors increase female sexual behaviour (Popova & Amstislavskaya 2002; Nedergaard *et al.* 2004) glutamate-initiated GnRH release, seasonal breeding, and oestrus (Dhandapani & Brann 2000), sows experiencing oestrus are expected to display higher concentrations of *trans*- UCA and *cis*-UCA than those in other reproductive states.

Animals and environmental variables

Full-grown, commercially housed sows (N = 30) at a University of Sydney owned swine production farm in Camden were sampled monthly over a 12-month period by experienced University of Sydney personnel according to approved animal ethics (2021/2015) protocols. The number of sows assayed was predicted to provide sufficient statistical power and data saturation to detect *urocanate hydratase* activity and annual behavioural patterns across months. To detect the relative abundances of *cis*- and *trans*- UCA in the epithelium, dermal cello tape samples were taken by experienced technical staff. Animals used in this analysis were housed in different conditions according to reproductive capacity and status. Three main areas were used for housing. The "Main Shed" houses sows that were in the luteal phase (sows in the luteal phase were categorised as not pregnant, lactating, or in pre/full oestrus) or pre-oestrus phase of their reproductive cycle, and gilts that had reached 100kg. The "Main Shed" was fitted with light emitting diode (LED) bulbs, which remained on for seven days a week at 24 hours each day. The LED bulbs did not emit any UV radiation. The "Dry Sow Shed" housed pregnant sows for 90% of their gestation period and had access to natural light through large openings on parallel sides of the shed. The "Finisher Shed" housed sows in the last 10% of their gestation period, sows that were lactating, and gilts that weighed from 30-100kg. The "Finisher shed" had access to limited natural light through two small windows.

The ethical approval for the skin-tape method was granted by the University of Sydney Animal Ethics Committee under the approval number 2022/2173. The sow housing followed the NSW department of Primary Industries Animal Welfare Code of Practice

The UV index data was acquired by manually copying the UV index values from the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) into an Excel spreadsheet using the measured daily UV index level. The UV index level was used because the difference between the highest and lowest monthly average value of the year was factor of 5.77 (a UV index increase from 1.21 – 7.00 on the UV index scale). This was a higher difference than that of UV exposure duration (ARPANSA 2023). The periods of exposure per day may have also been limited regardless of the time of year because of the location of the windows in the "Dry Sow Shed" and "Finisher Shed". For every

day within a month between 9 am and 3 pm, the UV index values were used to find the daily median (ARPANSA 2023). UV index values were taken between 9 am and 3 pm to negate the effect of outliers from low-light periods (i.e., dusk and dawn). The median was taken for each day because spikes in UV exposure would have altered the average daily value. The resulting daily median was averaged across the month to get the final monthly UV index level.

The monthly minimum and maximum temperatures were taken from the Australian Government Bureau of Meteorology and used to calculate the monthly temperature average (Australian Government Bureau of Meteorology 2023). The monthly temperature average was then added to the Excel sheet containing the average daily median UV index data.

Skin samples and assays

The back of the ear was washed and scrubbed with 70% ethanol to remove any potential debris. Three tape samples were taken from the same marked and washed area on each sow by applying a strip of cellophane tape (referred to as cello tape), applying pressure, and removing the strip. The initial cello tape strip was used to remove any remaining detritus. A second tape sample was used for data acquisition and a third tape sample was taken as a backup. Each tape sample was folded in half and covered in foil to prevent UCA photoisomerization. The samples were stored in the dark at room temperature until they were processed.

Preparation of skin samples

The UCA extraction process was modified from that used by Tateda et al. (2001) to improve the ability to detect low concentrations of *trans*- and *cis*- UCA by increasing the time each tape sample spent agitated in solution. For each cellophane tape sample, a 1.5-ml tube was labelled with the sample identifier and wrapped in aluminium foil to exclude light. Next, 0.9 ml of a 0.1-M KOH solution was added to each foil-wrapped tube. Each cellophane tape sample was then unwrapped, and a pair of fine-nosed tweezers and scissors were used to cut a 1.5 cm by 1.5 cm square from each sample. The fine-nosed tweezers and scissors were cleaned with 70% ethanol between sample cuts.

Each cut sample was placed into its respective foil-covered tube, ensuring the clean (non-adhesive) side of the tape was against the wall of the tube and that the adhesive (skin-sample-containing) surface was facing the KOH solution to increase isomer-containing surface area contact with the KOH solution. The tubes were placed on an automatic shaker, ensuring that the adhesive surface of the tape faced upwards. The samples were taped down and shaken on the maximum setting for two hours to thoroughly dissolve all available UCA isomers into the solution.

After agitation, the tubes were removed and 0.3 ml of a 0.38M HCl solution was added to each one to neutralise the KOH solution. The tubes were then taped to an automatic shaker with the adhesive side of the tape sample facing upwards and shaken on high speed for a further two hours to further confirm that all UCA isomers were dissolved into the solution. The solutions from the processed samples were filtered through a 0.22nm nylon syringe filter according to supplier instructions before being stored at -20°C prior to HPLC.

Preparation of standards for UCA

Standard solutions were used to create a calibration curve that allowed the absorption data from the HPLC to be converted to concentrations of relevant chemicals in the plasma and skin samples.

Standard solutions of UCA were made with concentrations of 1mg/ml, 0.1mg/ml, 0.01mg/ml, 1µg/ml and 0.1µg/ml. New 1mg/ml, 0.1mg/ml, 0.01mg/ml, 1µg/ml and 0.1µg/ml standards of UCA were made each month that samples were processed to re-calibrate the standard curve produced.

Detection of UCA isomers by HPLC

HPLC was conducted using an Agilent Technologies 1200 infinity series HPLC unit comprising a G1379B degasser, a G1312A binary pump, G1315D Diode-array detector (DAD), and a stainless steel ProteCol HPLC column (C18G x 5 um particle size x 120 Å pore size, 250 mm length x 4.6 mm ID). The same instrumentation was used to detect UCA and 4-imidiazole 5-propionic acid in all samples. The assay process was initiated and controlled using Agilent Chemstation online (Agilent Australia 2022). The mobile used was a 25mM citrate buffer (pH 3.2) and acetonitrile at a ratio of 88:12, to

which 3mM of sodium octane sulphonate was added (according to https://www.aatbio.com/resources/buffer-preparations-and-recipes/citrate-buffer-ph-3-to-6-2). The ProteCol column temperature was maintained at room temperature and a flow rate of 1.0 ml/minute. The injection volumes for both the standards and the samples were 50ul due to the size of the available syringes, to account for the liquid retained in the syringe and to prevent air bubbles from being introduced to the HPLC. Any excess injected was automatically removed by the HPLC unit.

The detection of both UCA isomers was performed at a light wavelength frequency of 267nm for all samples. This wavelength was determined by other researchers to be the optimal wavelength at which to detect the *trans*- and *cis*- isomers of UCA using a stainless steel ProteCol HPLC column (Zare et al. 2012). The optimal wavelength for the detection of 4-imidiazole 5-propionic acid was identified through experimentation. The wavelength was altered by +/- 5nm starting from 200nm. The wavelength that provided the highest detection peak using the same concentration of IPA was identified and used for the remainder of IPA samples.

The spectrograph results from HPLC were generated using Agilent Chemstation offline, where they were exported as both PNG and Excel files containing the run time and light absorption at wavelength frequencies of 267nm and 225nm into lab archives (Agilent Australia 2022). Standards were run each day HPLC took place to identify the time of absorption for IPA, *cis*- or *trans*- UCA, depending on which samples were being analysed using HPLC that day. The heights of the absorption peak of the times corresponding to the detection of the standards (i.e., *trans*- UCA, *cis*- UCA, IPA) were isolated. The absorption peak heights were corrected using the baseline of the spectrograph by subtracting the average of 20 spreadsheet cells at the flattest time of the spectrograph that was located close to the peak. A standard curve was made each month for *trans*- UCA, *cis*- UCA and IPA when applicable. The standard curve was produced by plotting concentrations of 0.01mg/ml, 1µg/ml and 0.1µg/ml for *trans*- and *cis*- UCA on a scatterplot in Excel. We set the intercept as 0,0 and fitted the data as a straight line. The same was done for IPA using concentrations of 0.1mg/ml, 0.01mg/ml, and 1µg/ml. The standard curve correlated to the appropriate month of HPLC was used to calculate the concentration of IPA, *cis*- UCA or *trans*- UCA in solutions from their peak heights.

Statistical analysis

The sow sample number, its corresponding *cis*- UCA, and skin *trans*- UCA concentrations, monthly average localised temperature (Australian Government Bureau of Meteorology 2023), monthly average localised UV index (Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) 2023), monthly individual sow housing area within the facility and monthly individual sow reproductive status were imported into Rstudio for statistical analysis.

Generalised Additive Mixed Model (GAMM)

After importing the data into Rstudio, an R-markdown file was created and the mgcv, lme4, ggplot2 and patchwork packages were downloaded. The data were then imported into Rstudio, and the months were placed in chronological order and converted from categorical to numerical values. This enabled us to produce a time-based scatterplot using the ggplot package with the month as the x-axis and either *trans*- or *cis*-UCA concentration as the y-axis for preliminary visual analysis and to identify any extreme outliers, which we classified as concentrations over three times the second highest concentration value. The outliers (i.e., those with values of > 1.e-03 mg/ml/cm^2 for *cis*- UCA and > 0.002 mg/ml/cm^2 for *trans*- UCA) were then removed before a model was applied.

A generalised additive mixed model (GAMM) was chosen to determine whether there were any annual patterns in *trans*- and *cis*- UCA for two reasons. Firstly, the GAMM is not limited to linear relationships and can detect nonlinear patterns in the data. Secondly, as an additive model, the GAMM accounts for samples taken from the same pig each month and works on an individual sow level, as opposed to a population level. We used the following models to calculate *trans*- UCA and *cis*- UCA concentrations:

$$Trans\ UCA\ concentration = month + reproductive\ status + pig\ ID$$

$$Cis\ UCA\ concentration = month + reproductive\ status + pig\ ID$$

The UV index from ARPANSA and the average monthly temperature from the Australian Bureau of Meteorology were confounding variables found to have strong positive correlations with the month, and as a result were removed from the model. The month variable accounts for changes in temperature and UV index. The reproductive status of commercially housed sows was partially tied to

the housing location on the farm. Lactating sows were primarily kept in the finisher shed (two small windows on one wall present, allowing minimal natural light on one side). Sows in the luteal phase were located in the main shed (no window present, resulting in the least natural light exposure of all sheds). Pregnant sows are found in the dry sow shed (one large window on one wall, with two smaller windows on the parallel wall, letting in the most natural light of the three sheds). This correlation between reproductive status means that location can serve as a confounding variable. Only reproductive status was included in the GAMM model as a partial representative of on-farm location because of this confounding correlation between location and reproductive status.

A quantile-quantile (QQ) plot was produced for both models to assess whether the residuals followed a normal distribution. Deviations from the straight line in the QQ plots were indicative of non-normality in residuals. As an additional check for normality and to test the assumption that residuals have a mean value of zero, a histogram of residuals was produced for each model. A bell curve was indicative of normality, and a peak of close to zero was indicative that the residuals had a mean of zero. To test the assumption that the model's predicted values align with the observed values, a response vs fitted values plot was produced for both models. The forming of values along a straight line was indicative of the model's predicted values aligning with the observed values. Lastly, a residuals versus linear predictor plot was produced for both models to assess the validity of the model's assumptions. A grouping of points along the horizontal axis of 0 would indicate that the model's assumptions were valid.

Once the assumptions had been tested and met, each model was used to make a smooth plot and a multiple-line plot containing individual values with the month as the x-axis and either *trans*- UCA or *cis*- UCA as the y-axis to visually represent the annual patterns of *trans*- and *cis*- UCA. The summary function was then used to identify the p-value of the month and pig ID variables in both models, where a value of < 0.05 was considered to be significant. The summary function was also used to find the Pr(>|t|) value of each reproductive category, and a value of < 0.05 was considered to be significant.

Individual pig analysis

Following significance testing of the GAMM model, line graphs were made for each individual sow with 12 months of *trans*- and *cis*- UCA data using ggplot, with the month as the x-axis and UCA isomer (either trains- or *cis*- UCA) concentration as the y-axis. The theme_minimal() function was used to display the individual graphs for *trans*- and *cis*- UCA. Individual *trans*- and *cis*- UCA graphs were separated into groups depending on the number of UCA isomer peaks in them and the month in which they occur. A grid of individual sow reproductive status was produced in Excel with sow ID as the y-axis and month as the x-axis. The reproductive statuses were pregnant, lactating, luteal, oestrus, pro-oestrus and farrowing. The reproductive grid was then separated by pig ID to match the individual graph groups. The reproductive grid for each group was then used to determine how many sows in the group fell into statistically significant reproductive statuses each month. The number of sows in a statistically significant reproductive status each month and the average combined *trans*- or *cis*-UCA concentration of each individual graph group were then plotted on line graphs, and the peaks of each graph were cross-referenced with each other to identify positive or negative relationships.

Results

UCA concentrations by month and reproductive status as measured by skin tape sample

Generalised Additive Mixed Model (GAMM) Scatterplots of the raw *trans*- and *cis*- UCA concentration data were produced to identify outliers to exclude from further analyses in the data (as shown below in Figure 4). Three outliers were identified in the UCA concentration datasets. The two outliers that were present in the *cis*- UCA dataset were from sow 293 in September and sow 233 in October. In the *trans*- UCA dataset, sow 266 in January was the only outlier. The reasons for these three outliers were not identified based on the available data.

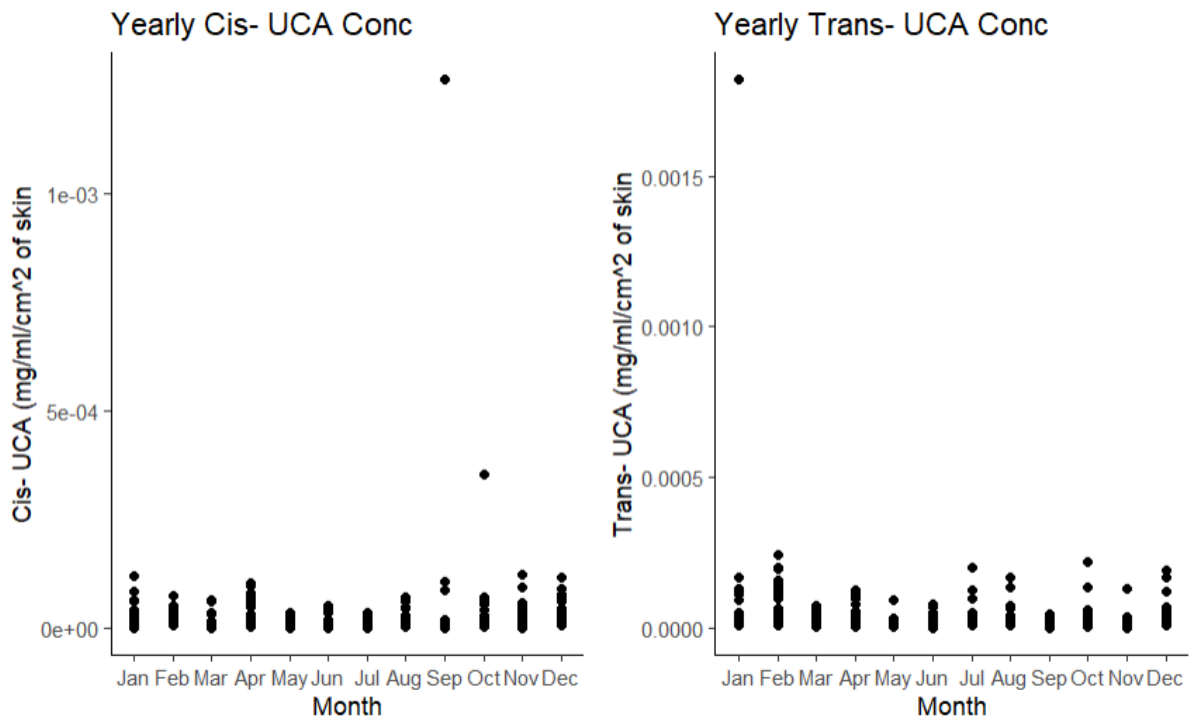


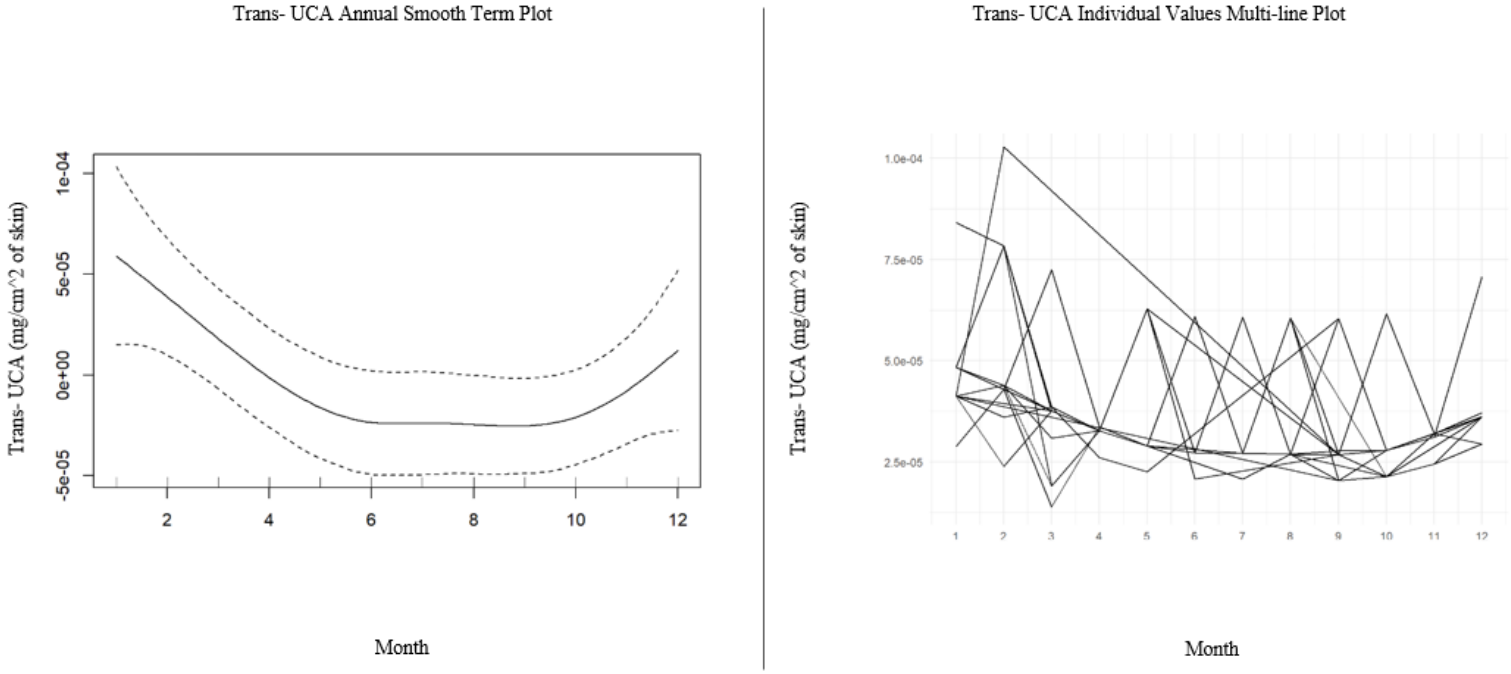
Figure 4. The left scatterplot depicts the raw *cis*- UCA (mg/ml/cm^2 of skin) plotted against the month variable from January to December with a > 1.e-03 outlier in September and a > 0.015 outlier in October. The scatterplot on the right depicts the raw *trans*- UCA (mg/ml/cm^2 of skin) plotted against the month variable from January to December with a single > 0.002 outlier in January.

Trans- UCA

The four assumptions of residual normality, a residual mean of zero, predicted values aligning with observed values and the validity of the previous assumptions were tested in the *trans*- UCA GAMM model. All data quality measures confirmed normality. A response versus fitted values plot showed that the points clustered around a horizontal axis with a y value of close to zero, meaning the model's predicted values aligned with the observed values. The residual versus linear predictor plot had clustered points along the horizontal axis close to zero, indicating that the *trans*- UCA GAMM model had valid assumptions.

The *trans*- UCA GAMM model was transformed into a smooth term graph to address the annual changes in population *trans*- UCA concentrations. Sow *trans*- UCA was below the mean in the months from May to November and above the mean in the months from December to April (as shown in Figure 5.).

Figure 5. The left *trans*- UCA smooth term plot is a visualisation of the *trans*- UCA (mg/ cm^2 of skin) predicted using the GAMM model (including standard error) as a function of the month. The average *trans*- UCA values follow the solid black line, and the standard error is shown as the dotted black lines above and below the average. The *trans*- UCA individual values multi-line plot on the right side is a visualisation of the observed values for individual sows over 12 months. The month variable along the x-axis has a numerical value of 1–12 based on the corresponding month.



The significance levels of individual model variables for the *trans*- UCA GAMM model are shown in Table 2. The model shows that *trans*- UCA was significantly affected by month (Pr(|t|) of < 0.001), as shown in Table 2, and the lactation reproductive status (Pr(|t|) of < 0.001), as shown in Table 1. All other variables and reproductive statuses had no statistical significance (Pr(|t|) of > 0.05)

Table 1. The significance of reproductive status on *trans*- UCA concentrations as measured by skin tape samples using a GAMM model.

Reproductive Status	Estimate	Standard Error	t Value	Pr(>|t|)	Significance

Farrowing	-7.9^{e-06}	1.5^{e-04}	1.238	0.216	None
Luteal	3.1^{e-04}	9.0^{e-05}	-0.053	0.957	None
Lactating	9.8^{e-05}	8.1^{e-05}	3.432	0.0006	High
Oestrus	2.3^{e-05}	9.4^{e-05}	0.243	0.223	None
Pro-oestrus	1.4^{e-04}	9.2^{e-05}	1.531	0.126	None
Pregnant	1.3^{e-04}	$8,1^{e-05}$	1.648	0.100	None

Table 2. The significance of Month and Pig ID on *trans*- UCA concentrations as measured by skin tape samples using a GAMM model.

Variable	Reference degree of freedom	F Value	P-Value	Significance
Month	9	1.416	0.005	Medium
Pig ID	1	0.000	0.953	None

Cis- UCA

The four assumptions of residual normality, a residual mean of zero, predicted values aligning with observed values, and the validity of the previous assumptions were tested in the trans- UCA GAMM model. All data quality measures confirmed normality. The response versus fitted values plot showed the points clustered around a horizontal axis with a y-axis value of close to zero, indicating that the model's predicted values align with the observed values. The residuals in the residual versus linear predictor plot were clustered along the horizontal axis where residuals have a value of zero, indicating that the *cis*- UCA GAMM model had valid assumptions.

We transformed the *cis*- UCA GAMM model into a smooth term graph to address the annual changes in population *cis*- UCA concentrations. The average was shown as a solid black line, and the standard error was displayed as two dotted black lines on either side of the average line. Sow *cis*-UCA was below the mean in the months from April to September and above the mean in the months from October to March (as shown in Figure 6).

Figure 6. The left *cis*- UCA smooth term plot is a visualisation of the *cis*- UCA (mg/cm^2 of skin) predicted by the GAMM model (including standard error) as a function of the month. The average *cis*- UCA values follow the solid black line, and the standard error is shown as the dotted black lines above and below the average. The *cis*- UCA individual values multi-line plot on the right is a visualisation of the observed values for individual sows over 12 months. The month variable along the x-axis has a numerical value of 1–12 based on the corresponding month.

The significance levels of the individual variables for the *cis*- UCA GAMM model are reported in Table 4. The model shows that *cis*- UCA was significantly affected by month (Pr(|t|) of < 0.001), as shown in Table 4, as well as the pregnant reproductive status (Pr(|t|) of < 0.001), as shown in Table 3. All other variables and reproductive statuses had no statistical significance (Pr(|t|) of > 0.05)

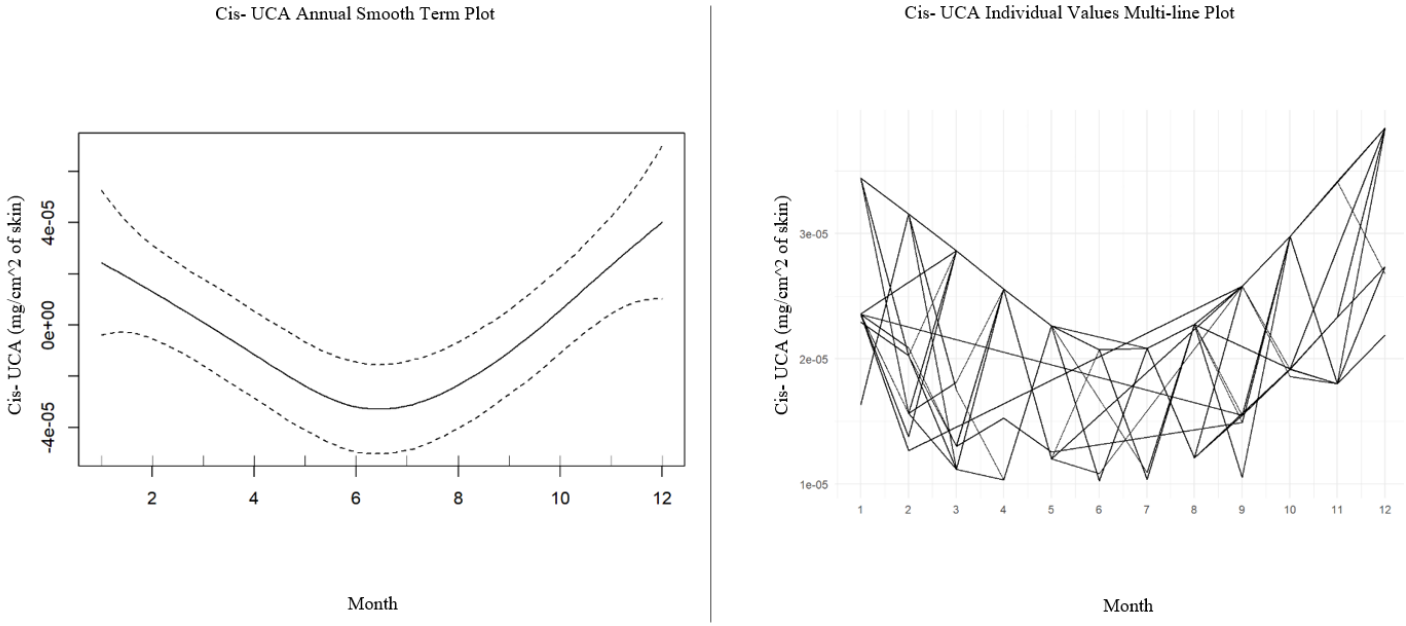


Table 3. The significance of reproductive status on *cis*- UCA concentrations as measured by skin tape samples using a GAMM model.

Reproductive Status	Estimate	Standard Error	t Value	Pr(>|t|)	Significance
Farrowing	5.5^{e-05}	1.1^{e-04}	0.509	0.611	None
Lactating	9.5^{e-05}	5.8^{e-05}	1.634	0.103	None
Luteal	1.0^{e-04}	5.6^{e-04}	1.792	0.0742	None
Oestrus	5.5^{e-05}	6.4^{e-05}	0.861	0.389	None
Pro-oestrus	6.9^{e-05}	6.1^{e-05}	1.141	0.254	None

Pregnant	1.6^{e-04}	5.7^{e-05}	2.842	0.0048	Medium

Table 4. The significance of Month and Pig ID on *cis*- UCA concentrations as measured by skin tape samples using a GAMM model.

Variable	Reference degree of freedom	F Value	P-Value	Significance
Month	9	1.916	0.0003	High
Pig ID	1	0.087	0.436	None

Analysis by individual sow

To determine the direction of the relationship between *trans*- UCA and lactation, individual month versus *trans*- UCA concentration line graphs were produced and revealed three groups. Group A consisted of sows that exhibited two midyear periods of elevated *trans*- UCA. Group B consisted of sows that exhibited a single midyear period of elevated *trans*- UCA. Group C consisted of sows that did not fit into groups A or B. The sows in groups A and B had midyear peaks and a strong positive correlation between *trans*- UCA and lactation. Within Group A, the February and July peaks in average *trans*- UCA concentration positively correlated with February and July peaks in the number of sows lactating per month. Within Group B, the June peak in average *trans*- UCA concentration was positively correlated with the June peak in the number of sows lactating per month. These findings provided strong evidence to support the positive association between lactation and increased *trans*

UCA concentrations.

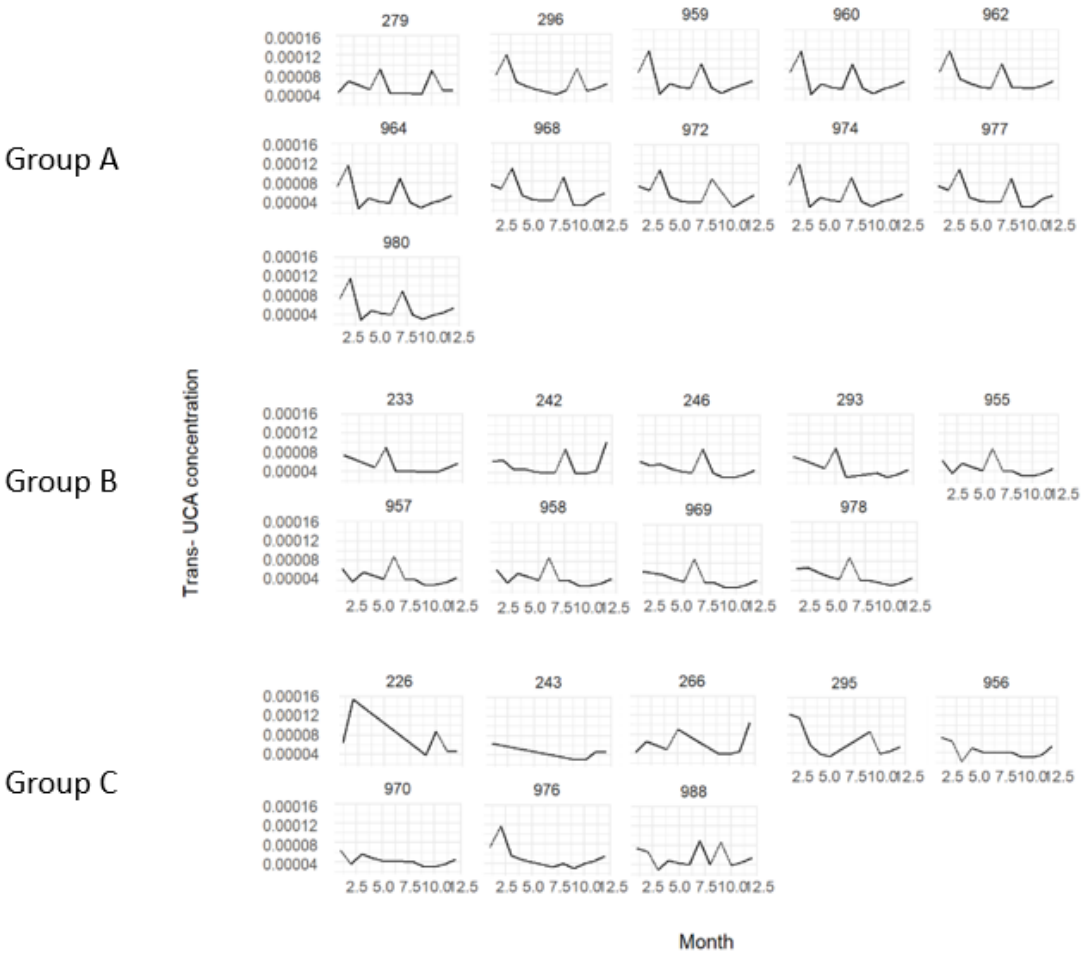


Figure 7. Three compact grids with the month along the x-axis and t*rans*- UCA (mg/ml in 1.5 cm^2 of skin) along the y-axis displaying a line graph for each sow in the study that had a *trans*- UCA measurement for all 12 months. Group A consists of sows that had two midyear peaks.in *trans*- UCA concentration. Group B consists of sows that expressed one midyear peak in *trans*- UCA concentration, and Group C consists of sows that did not fit into any of the other groups.

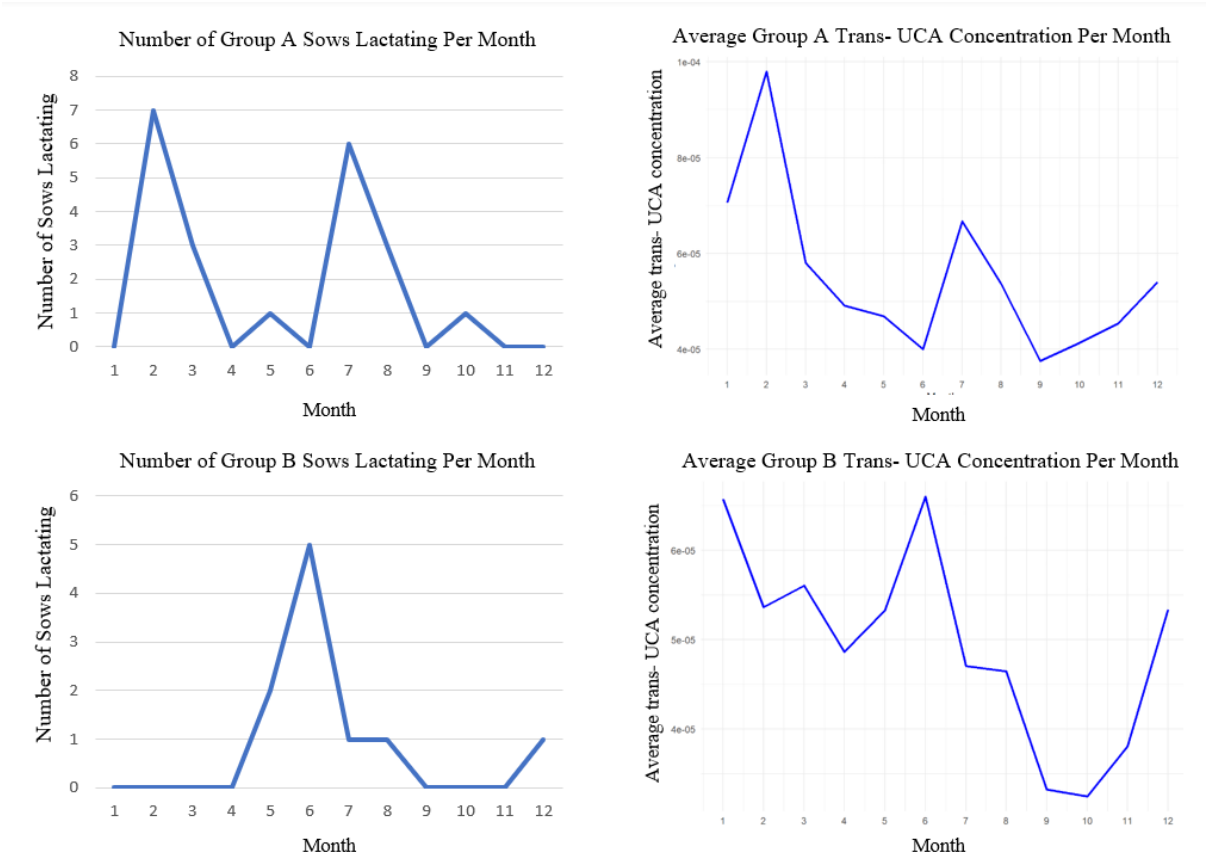


Figure 8. The top row displays the number of lactating sows and the average t*rans*- UCA concentration per month for Group A. Within Group A, there were peaks in February and July in both the number of lactating sows per month and the average *trans*- UCA. The bottom row displays the number of lactating sows and the average *trans*- UCA concentration for Group B. Within Group B, there was a peak in both the number of lactating sows per month and the average *trans*- UCA concentration in June.

To determine the direction of the relationship between *cis*- UCA and pregnancy, individual month versus *cis*- UCA concentration line graphs were produced and showed two groups. Group A consisted of sows with two midyear periods of increased *cis*- UCA concentration. Group B consisted of sows that do not fit into Group A. The sows in Group A had midyear peaks with a strong positive correlation between *cis*- UCA concentration and the first month of pregnancy. Within Group A, the April/May and August peaks of average *cis*- UCA concentration were found to be positively correlated with the April/May and August peaks in the number of sows pregnant per month. These findings provided strong evidence to support the significance between pregnancy and *cis*- UCA concentrations.

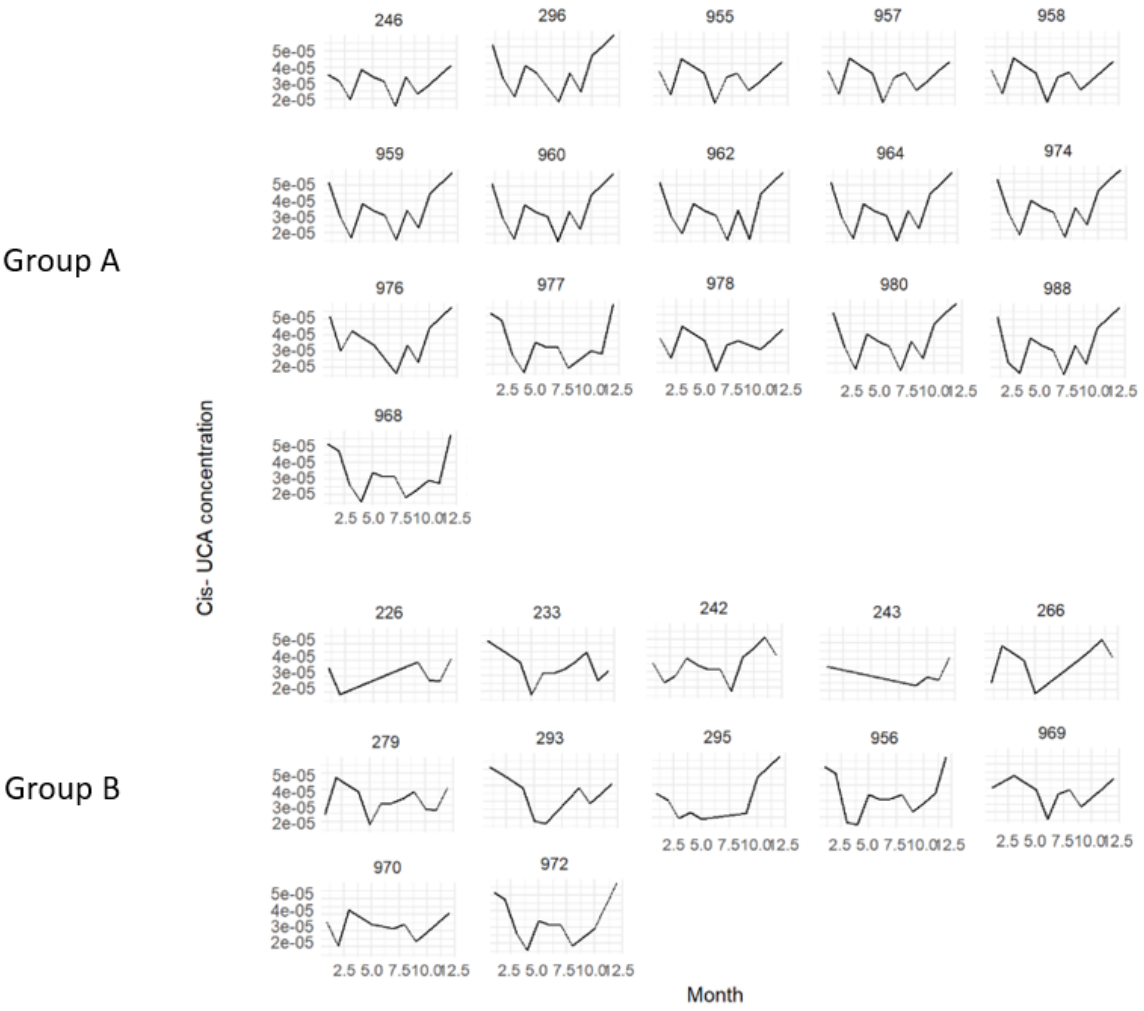


Figure 9. Two compact grids with the month along the x-axis and *cis*- UCA (mg/ml in 1.5 cm^2 of skin) along the y-axis displaying a line graph for each sow in the study that had a *trans*- UCA measurement for all 12 months. The month variable along the x-axis has a numerical value of 1–12 corresponding to the month of the year. Group A consisted of sows that had two midyear peaks. Group B consisted of sows that did not fit into the other groups.

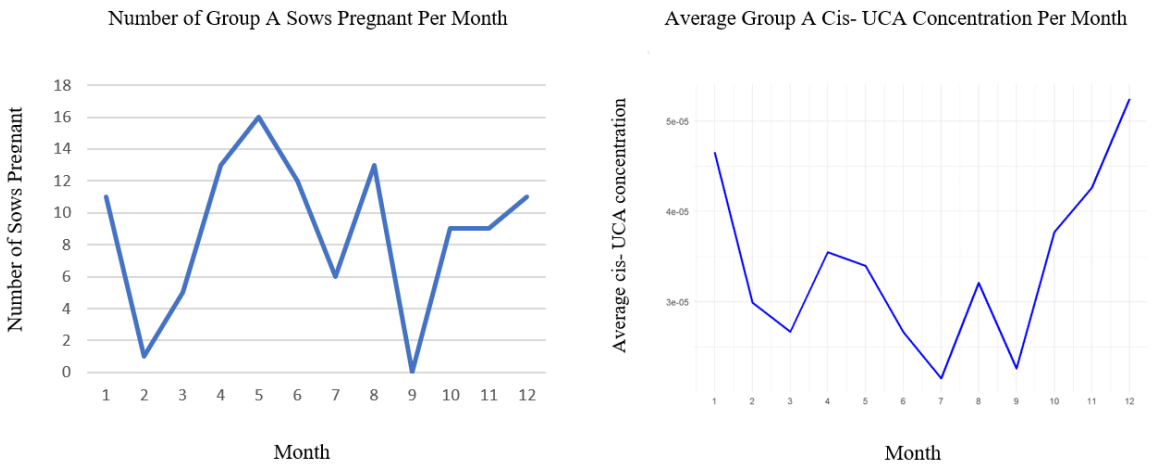


Figure 10. The top row displays the number of sows lactating and the average *trans*- UCA concentration per month for Group A. Within Group A, there is a peak in February and July in both the number of lactating sows per month and the average skin *trans*- UCA concentration. The bottom row displays the number of sows lactating and the average *trans*- UCA concentration for Group B. Within Group B, there is a peak in both the number of lactating sows per month and the average *trans*-UCA concentration in June.

Discussion

Annual patterns in trans- and cis-UCA

Trans- UCA

A circannual rhythm in the concentrations of *trans* -UCA with a decrease in *trans*- UCA concentrations from December to February, indicating a decrease in the pool of *trans*- UCA available for urocanate hydratase to metabolise into substrates for the HDP, and an increase from May to October was expected. However, the smooth plot for *trans*- UCA indicates that there was a decrease in *trans*- UCA from May to October and an increase from December to February. The cause of an inverse of the expected circannual pattern in *trans*- UCA is currently unknown. Investigation into the trajectories of monthly variables such as UV index, temperature and UV exposures may provide insight into the reasons for the changes in observed *trans*- UCA.

Temperature has no recorded effect on *trans*- UCA. There is some evidence of temperature affecting *urocanate hydratase*, which converts *trans*- UCA into IPA. However, that enzyme is located in the liver, brain, testes and ovaries, where the internal body temperature remains relatively constant at

37°C +/- 0.5°C (Hug & Hunter 1974; Hug & Roth 1972; Walker 1990). The sows in this study were fed an annually consistent pool of histidine, which is a precursor to *trans*- UCA, with the exception of sows in lactation, which were fed ad-libitum. There is a great increase in the number of pigs in lactation from February to March and May to August. Only the February to March time period matches the observed increase in *trans*- UCA concentration, suggesting that lactation cannot fully explain the observed circannual changes in *trans*- UCA concentration and its availability for *urocanate hydratase* and HDP metabolism.

The increase in availability of *trans*-UCA may be caused by an increase in histidase due to a homeostatic response that provides an increased pool of *trans*- UCA to be isomerised to *cis*- UCA to cope with changes in the environment associated with the summer months, such as an increased UV index. Alternatively, an increase in the availability of *trans*- UCA can be attributed to changes in the diet of lactating sows. Further research into the *trans*- UCA concentration of sows fed consistent histidine throughout the year regardless of reproductive status can clarify the cause of the observed increases in *trans*- UCA during pregnancy.

Cis- UCA

It was expected that there would be a circannual rhythm in the concentrations of *cis*- UCA, including an increase in *cis*- UCA concentrations from December to February, indicating increased potential for activation of the 5-HT_2A receptors and a decrease in available *trans*- UCA for HDP metabolism, and a decrease from May to October. The smooth plot for *cis*- UCA indicates that there was an increase in *cis*- UCA from May to October and a decrease in *cis*-UCA from December to February. This aligns with our expectations because an increase in UV index in the Camden area during the beginning/end period of the year should increase the isomerisation of *trans*- UCA to *cis*- UCA, resulting an increase of *cis*- UCA as a proportion of total UCA.

Association of UCA with Reproductive Status

Trans- UCA

Trans- UCA is converted to IPA via the activity of *urocanate hydratase* before continuing down the HDP to form glutamate (Kohlmeier 2015). The end product of the HDP (glutamate) has been shown to regulate reproductive behaviour in mammals and was expected to perform the same role in swine. If our assumptions of glutamate contributing to the regulation of reproductive behaviour in swine is correct, we would expect *trans*- UCA concentration to correlate with oestrus and reproductive cycling in swine. As such, the positive correlations between *trans*- UCA, *urocanate hydratase* activity and lactation and their lack of correlation with oestrus were unexpected. The correlations between trans- UCA, urocanate hydratase activity and lactation indicate that there is a possibility for the remaining products of the HDP to provide some benefit to milk production. However, it is more likely that the high trans- UCA is due to extra feed. Investigating the activity of HDP-related biochemicals during lactation can provide some insight into any potentials reasons for the positive correlations between *trans*- UCA, *urocanate hydratase* activity and lactation that is not related to an increase in feed uptake during lactation.

Although there are no studies on the specific effects of *trans*- UCA on lactation, several other biochemicals in the HDP have been shown to be correlated with lactation, including histidine, IPA, the HDP enzyme formimino glutamate hydratase, and glutamate.

Histidine-deficient diets in dairy cows have been shown to have a negative effect on lactation by decreasing the digestibility of dry/organic matter and crude protein throughout the entire digestive tract, which decreases the nutrients available for milk production (Giallongo et al. 2017). The importance of histidine in healthy lactation is further supported by the positive effect of histidine supplementation in dairy cattle, which increases milk yield and milk true protein yield (Räisänen et al. 2023). Due to the positive effect of histidine on milk quality, our data support that there may be a potential increase in in histidine allocation to the HDP via an increased activity of histidase during lactation.

Although there was no information on the effect of 4-imidiazole-5-propionic acid specifically, researchers investigated the effect of propionic acid on lactation in dairy cows. Injecting propionic acid into the rumen of dairy cows has been shown to increase milk production and fat and protein yields (Rigout et al. 2003). The observed effect of propionic acid on lactation indicates that IPA, as a propionic acid, can increase the quality and quantity of milk produced during lactation. If propionic acid has a similar effect in nonruminants, then it would be expected that the production of IPA via the activity *of urocanate hydratase* is in increased demand during lactation.

The formimino group of formimino glutamate in the HDP is accepted by a folic acid coenzyme called formimino glutamate hydratase and incorporated into folic acid production (Berry et al. 1963). Folic acid is essential in the development of DNA, RNA and protein anabolism, making it an essential biochemical to produce during times of high anabolic function, such as lactation (O'Connor et al. 1997). The importance of formimino glutamate as a folic acid formimino group donor is supported by the folic acid deficiencies observed in pregnant women as pregnancy continues, as well as the accompanying increased uptake of intravenously-injected folic acid (Berry et al. 1963). The results of studies on swine revealed that folic acid administration increases milk quality and the performance of piglets, highlighting the importance of folic acid in development (O'Connor *et al.* 1997). There is ample evidence of the importance of folic acid in lactation. Formimino glutamate contributes the formimino chemical group to the production of folic acid through the folic acid coenzyme formimino glutamate hydratase (Berry et al. 1963; Kohlmeier 2015). Therefore, it stands to reason that the production of formimino glutamate via an increase in HDP and *urocanate hydratase* activity is likely to occur during lactation.

Glutamate is the most abundant amino acid in milk and milk glutamate concentrations increase with lactation up to 30–40 times that of glutamate concentrations found in plasma, according to human studies (Matsumoto et al. 2013; Koletzko 2018). In rats, glutamate levels in milk are 10 times higher than that in rat plasma (Matsumoto et al. 2013). The high concentrations of glutamate in both human and rat milk compared to their respective serum concentrations suggests that throughout mammals, glutamate is essential for the lactation of nutritionally appropriate milk and infant growth, providing

evidence that the HDP production of glutamate can contributes to the high concentrations of glutamate found in milk.

Multiple substrates of the HDP have been shown to increase the quality and quantity of milk production and have a positive impact on the survival of piglets, or are implicated in the production of important milk constituents (Giallongo *et al.* 2017). Despite this evidence for the importance of the HDP in producing constituents with positive effects on lactation, the ad libitum feed intake of sample sows during pregnancy is a large confounding variable. Further study is needed to determine if the increase in HDP activity indicated by the observed *trans*- UCA concentrations during pregnancy is due to the reproductive status, or the increased feed intake. To achieve this, future research can compare *urocanate hydratase* activity by measuring the levels of trans- UCA in the blood and/or skin between a group of swine in lactation, and a control group kept in a controlled environment with identical feed intake.

Cis- UCA

Cis- UCA was found to strongly peak in the first month of pregnancy, which similar to the *trans*-UCA result, was unexpected because previous research by the University of Sydney and the literature suggested that *cis*- UCA, serotonin and the 5-HT_2A receptor played role in controlling reproductive behaviour. It is possible that this increase in *cis*- UCA is due to the increased exposure to UV radiation as the dry sow shed where they are located is the most open to natural light out of the available locations as shown in Figure 11.

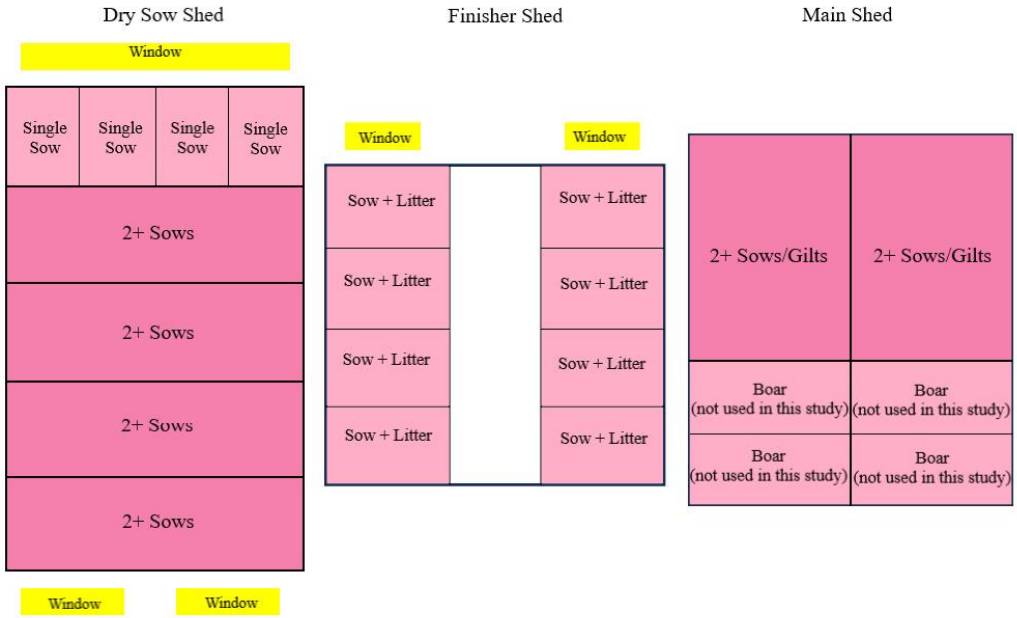


Figure 11. The pen layout for the "Dry Sow Shed", "Finisher Shed", and "Main Shed", including whether each pen is designed to hold a single sow/boar, multiple sows, or a sow and her litter. Each layout also includes the windows present in each shed. The "Dry Sow Shed" is exposed to the most natural light through its three large windows, followed by the "Finisher Shed" with two small windows, and the "Main Shed" is exposed to the least light.

Investigation into potential causes of the observed increase in *cis*- UCA focused on multiple studies investigating the role of serotonin and the 5-HT_2A receptor in pregnancy (Chanarin et al. 1963; Klempan et al. 2011). In humans, histidine metabolism is increased during pregnancy, indicating that there is an increased pool of *trans*- UCA for photoisomerization into *cis*- UCA. An increase in blood UCA (the study does not specify which isomer) uptake was also identified in pregnant human women, showing that UCA is in increased demand during pregnancy (Chanarin *et al.* 1963). These studies indicate that cis- UCA may have an increased production or absorption in pregnancy.

Cis- UCA is an agonist of serotonin 5-HT_2A receptors (Walterscheid et al. 2006). Studies on the effects of serotonin on pregnancy suggest that it plays an important role in placental function by regulating endocrine, paracrine and autocrine function (Klempan *et al.* 2011). This is further supported by the synthesis of serotonin in trophoblast cells (the outer layer of the blastocyst, which provides nutrients to the embryo) where serotonin acts as a trophoblast growth regulator via its

interactions with the 5-HT_2A receptor (Viau et al. 2009). Serotonin and the 5-HT_2A receptor have also been shown to regulate placental aromatase, which is a key enzyme that controls estrogen synthesis during pregnancy (Klempan et al. 2011). Lastly, 5-HT_2A receptors are associated with the remodelling of the myometrium (the muscular wall of the uterus) during pregnancy (Minosyan et al. 2007).

As shown above, serotonin and 5-HT_2A receptors both play important roles in the progression and regulation of tissue changes during pregnancy. Given that *cis* -UCA is an agonist of the 5-HT_2A serotonin receptor, the observed increase in *cis*- UCA during pregnancy, especially in the first term when rapid development is taking place, may be indicative of regulation of 5-HT_2A activity and an increase in the availability of free serotonin.

Studies in swine show that the reduction in successful pregnancies during summer is due to the disruption of a maternal recognition mechanism, and not to the structural integrity of tissue changes during pregnancy (Love et al. 1993). As such, the observed in cis- UCA during summer are not currently thought to affect summer sub-fertility in swine. In addition, given that an increase in *cis*-UCA is positively correlated with pregnancy, and pigs as semi-seasonal breeders have an increase in successful farrowing during winter when the UV isomerisation of *trans*- UCA to *cis*- UCA decreases, it is unlikely that *cis*- UCA contributes to seasonal fertility in sows. This is supported by the lack of significant correlation with oestrus, pre-oestrus, or the luteal phase with *cis*- UCA using a GAMM.

To determine whether detecting *cis*- UCA has a relationship with pregnancy independent of unique lighting conditions, further research needs to be done on the effect of *cis*- UCA administration on the progression and success of pregnancy. This research could be achieved in future studies by administering a model organism (such as mice) with low concentrations of *cis*- UCA, such as those found in the sow skin samples within this study (concentrations of e-05 or e-06) followed by observations of pregnancy success in the treated and control group.

Industrial Implications of UCA Results

UCA has been identified in mammalian species, including canids, hamsters, and humans (Mehler & Tabor 1953; Hart & Norval 2021). The UROC1 gene is thought to have originated in a common

ancestor of animals, which is supported by the presence of the UROC1 gene in multiple other animal classes, including avians, fish, clitellates and brachiopods (Safran *et al.* 2010; Fink *et al.* 2011; Hinrichs et al. 2016). The presence of the UROC1 gene in multiple animal classes indicates that *trans*-UCA has the potential to proceed through the HDP in most, if not all, animals. Evidence to support the application of *trans*- and *cis*- UCA for increased lactation and pregnancy analysis in multiple animal classes comes from the presence of 5-HT (including 5-HT_2A) receptors in bird and fish species (Deviche et al. 2008; Mager et al. 2012; Wood et al. 2013). The presence of multiple substrates and enzymes implicated in the HPD indicates that the HPD is present across mammals, avians and fish, showing that the results of this study can be applied to other species that have been domesticated for production purposes (Mehler & Tabor 1953).

Literature regarding the effect of HDP constituents on lactation suggests that elements of the HDP increase milk quality and yield (O'Connor *et al.* 1997; Rigout *et al.* 2003; Matsumoto *et al.* 2013; Koletzko 2018; Räisänen *et al.* 2023). This study identified a potential relationship between increased *trans*-UCA and *urocanate hydratas*e activity during times of lactation. As such, increases in trans-UCA concentrations may indicate lactation quality across mammalian animal species. Due to ad libitum feeding of pregnant sows during this study, further research into the potential relationship between *trans*- UCA concentrations and lactation must be initiated before *trans*- UCA has any commercial application in agriculture.

Cis- UCA plays important roles in the development of uterine muscle and placental and hormonal control during pregnancy due to its agonistic effect on the 5-HT_2A serotonin receptors (Minosyan *et al.* 2007; Viau *et al.* 2009; Klempan *et al.* 2011). This may provide a reason for the observation of a strong relationship between *cis*- UCA and pregnancy identified in this study. The relationship between *cis*- UCA concentration and pregnancy indicates that skin *cis*- UCA concentration measurement has the potential for use as a non-invasive pregnancy predictor. High *cis*- UCA concentrations in the skin would be indicative of confirmed pregnancy, enabling farmers to quickly and easily identify sows that have been successfully inseminated without the need for ultrasound or more invasive procedures. The continued presence of *cis*- UCA throughout pregnancy can be indicative of a successfully progressing

pregnancy. Alternatively, a drop-off of *cis*- UCA can be indicative of a failed or lost pregnancy, enabling farmers to alter feed allocation accordingly. Due to the different light exposure of sheds, and the allocation of sows to sheds depending on their reproductive further research into the potential relationship between *cis*- UCA concentrations and pregnancy must be initiated before *trans*- UCA has any commercial application in agriculture.

The evidence at this point does not support commercial use for pregnancy testing. Because of this, methods of UCA detection do not compete with current reproductive tests such as progesterone assays.

The Potential Effects of Climate Change on UCA Concentrations

Climate change has caused an increase in the average individual exposure to UV light and the average global temperature (Diffey 2004; Loarie et al. 2009). The predicted growing effect of climate change on these variables has the potential to alter *trans*- and *cis*- UCA concentrations because an increase in UV exposure can lead to an increase in the isomerisation of *trans*- UCA by UVB into *cis*- UCA (United States Environmental Protection Agency). This predicted increase in isomerisation can potentially lead to a decrease in milk quality due to a reduced pool of *trans*- UCA available for the remainder of the HDP and an increase in successful pregnancies due to high concentrations of *cis*- UCA.

The increase of carbon dioxide in the atmosphere since the industrial revolution has led to a greenhouse effect, resulting in an increase of global temperatures (Mitchell, 1989, Diffey 2004; Loarie et al. 2009). This increase in average global temperatures has the potential to increase nutrient availability and decrease energy expenditure for a greater annual period. The decrease in energy expenditure can result in a decreased feed uptake. An increase in global temperatures can also result in a greater chance of heat stress, which occurs if temperatures do not drop below 21°C for three to six hours per day (Silanikove, 2000). Heat stress leads to a decrease in appetite and feed uptake (Silanikove, 2000). Overall, the annual increase in global temperatures attributed to climate change and the greenhouse effect can cause a decrease in appetite, limiting the levels of histidine acquired

from the allocated feed and causing a decrease in HDP activity, *trans* UCA concentrations, and *cis* UCA concentrations, leading to a potential decrease in milk quality and successful pregnancies.

Limitations

There were some limitations within this study that may have affected the results. The number of sow samples per month (n) decreased over the course of the study due to culling. In some months, N was particularly low due to missing samples, or incorrect labelling. In addition, in accordance with the animal ethics protocols, this study used the minimum viable number of animals to achieve a statistically significant result. As such, we were unable to sample all available sows located on the Camden University of Sydney farm, decreasing our number of sows sampled per month (N).

A better indication of *trans*- UCA concentration than skin *trans*- UCA measurements could be obtained from the sow liver or brain, where the activity of *urocanate hydratase* is highest. However, this introduces new issues that make this method unsuitable (Weizmann Institute of Science 2022). Sampling the liver requires killing the sow, therefore removing it from an already reduced data pool. An alternative to this would be to source livers from pig mortuaries, taking note of when and under what conditions the pig was slaughtered. However, this would only allow one sample to be taken per sow, and the method for transporting and thawing livers may affect the concentrations of *trans*- UCA, *urocanate hydratase* and imidazole propionic acid in the samples because *urocanate hydratase* has decreased activity at temperatures below 30°C (Hug & Hunter 1974; Hug & Roth 1972). If we used this sampling method, then given that pigs are semi-seasonal, short-day breeders, give birth after 111-120 days (roughly four months) and are butchered at 5–6 months of age, there would be an expected decrease in the availability of sample livers during the summer months when there are fewer pigs at a sufficient finishing weight (Pork 2023).

The reproductive status of commercially housed sows was partially tied to the housing location on the farm. This correlation between reproductive status and lighting conditions means that location can serve as a confounding variable. Only reproductive status was included in the GAMM model as a partial representative of on-farm location. Research into the effect of multiple HDP constituents on

the quality of lactation supported the existence of correlations between *trans*- UCA availability, UROC1 expression, *urocanate hydratase* and HDP activity and lactation. Performing individual analysis on *cis*- UCA based on statistically significant reproductive status and research into the role of serotonin and serotonin 5-HT_2A receptors also strongly reveals the effect of *cis*- UCA on pregnancy. Further research on both the effects of trans- UCA on lactation, and cis- UCA on pregnancy without any of the aforementioned confounding variables would be mandatory for the relationships to be validated.

Regumate® was administered in four groups to reduce LH concentrations, resulting in a prolonged luteal phase for 18-21 days (Wang et al. 2018). This served to synchronise the oestrus in the four treated groups. The groups were of 11 sows and/or gilts in September 2022, four in October 2022, five in November 2022 and seven in January 2023. This may have affected some of the sows found in the pregnancy Group B who lack any clear pattern but does not seem to have affected the results of the Group A pregnancy group, which express two midyear peaks associated with pregnancy and one expected end of year peak that doesn't appear to have any correlation with reproductive status.

Future research

Further research into UROC1 expression and *urocanate hydratase* activity should be focused on *urocanate hydratase* activity through RNA expression in the liver and/or skin during pregnancy, lactation, the luteal phase, and the oestrus phase. An alternate path for studies into *urocanate hydratase* activity can include using the methods in this study for the analysis of UCA isomers in the skin, or usingquantification methods of a blood *trans*- UCA and blood IPA concentration during pregnancy, lactation, the luteal phase, and the oestrus phase. All sample animals in these proposed future studies should be kept in annually consistent housing and be provided an annually consistent feed intake independent of reproductive statuses. These conditions will untangle the reproductive statuses, feed conditions, and location.

Proposed research into the *cis*- UCA should be focused on the effect of *cis*- UCA on the progression and success of pregnancy through a comparison of the number of successful pregnancies compared

between a group of model organisms given venous injections of *cis*- UCA during pregnancy and a control group. Measuring the activity of serotonin 5-HT_2A before and after venous administration of *cis*- UCA during pregnancy can also investigate the effect of *cis*- UCA initiated 5-HT_2A receptor activation on successful pregnancies.

Later studies may also focus on the idea that there is a *trans*- or *cis*- UCA threshold that initiates oestrus or other reproductive states, or that there exists a delay between *trans*- or *cis*- UCA concentrations. This can be a secondary objective of the proposed future *trans*- and *cis*- UCA studies as discussed previously in this section.

Conclusion

There is evidence of a seasonal cycle independent of reproductive status for skin *trans*- UCA and *urocanate hydratase* activity, with an increase from December to February before decreasing during winter. It is proposed that this is due to an increase in histidase activity from December to February that provides increased concentrations of *trans*- UCA for isomerisation into *cis*- UCA. *Cis*- UCA also shows seasonal cycles independent of reproductive status with an increase from December to March. This is likely due to an increased UV index during these months, resulting in an increased rate of isomerisation of *trans*- UCA to *cis*- UCA.

The results within this study do not support the notion that peaks in *urocanate hydratase* activity or UCA isomers immediately affect oestrus and anoestrus cycles in commercially housed sows. Although there was minimal evidence of seasonal fertility-based oscillations in *trans*- or *cis*- UCA and its associated pathways, we identified a positive relationship between *trans*- UCA and lactation. The ad libitum feeding of sows during lactations may have contributed to this association. However, due to the positive effect of the multiple biochemicals of the HDP on the quality of lactation in the literature, an increase in trans- UCA affecting lactation is possible.Further research without ad libitum feeding is mandatory to verify this.

We also identified a positive relationship between *cis*- UCA and pregnancy.Since the location and light exposure of sows was based on reproductive status; however it is not possible for this study to certify this relationship between cis-UCA and pregnancy. Despite lighting conditions being a confounding variable, literature investigating the effects of serotonin and 5-HT_2A serotonin receptors (of which *cis*- UCA is an agonist) on trophoblasts and myometrium development suggest there is still a possible link between an increase in *cis*- UCA and pregnancy. Further research in organism located in consistent lighting conditions is required to verify this link.

References

1. Agilent. (2022). *Chromatography Data Systems: OpenLab ChemStation*. Retrieved 25 March, 2024 from https://www.agilent.com/en/product/software-informatics/analytical-software-suite/chromatography-data-systems/openlab-chemstation
2. Arend, L. S., & Knox, R. V. (2021). Fertility responses of melatonin-treated gilts before and during the follicular and early luteal phases when there are different temperatures and lighting conditions in the housing area. *Anim Reprod Sci*, *230*, 106769. https://doi.org/10.1016/j.anireprosci.2021.106769
3. Asa, C. S., Seal, U. S., Letellier, M. A., Plotka, E. D., & Peterson, E. K. (1987). Pinealectomy or superior cervical ganglionectomy do not alter reproduction in the wolf (Canis lupus). *Biology of reproduction*, *37 1*, 14-21.
4. Australian Government Bureau of Meteorology. (2023). *Climate Data Online*. Retrieved 22 March, 2024 from http://www.bom.gov.au/climate/data/index.shtml
5. Australian Pork. (n.d.). *Stages of pork production*. Retrieved 21 March, 2024 from https://australianpork.com.au/about-pig-farming/stages-pork-production#:~:text=Once%20slaughter%20weight%20is%20achieved,yards%20towards%20the%20slaughter%20process
6. Australian Radiation Protection and Nuclear Safety Agency. (2023). *Ultraviolet radiation index*. Retrieved 25 March, 2024 from https://www.arpansa.gov.au/our-services/monitoring/ultraviolet-radiation-monitoring/ultraviolet-radiation-index
7. Berry, V., Booth, M. A., Chanarin, I., & Rothman, D. (1963). Urinary Formimino-glutamic Acid Excretion in Pregnancy. *British Medical Journal*, *2*(5365), 1103-1104. https://doi.org/10.1136/bmj.2.5365.1103
8. BioSynthesis. (2017). *What is Photoisomerization?* Retrieved 27 March, 2024 from https://www.biosyn.com/faq/What-is-Photoisomerization.aspx
9. Bittman, E. L., Karsch, F. J., & Hopkins, J. W. (1983). Role of the pineal gland in ovine photoperiodism: regulation of seasonal breeding and negative feedback effects of estradiol upon luteinizing hormone secretion. *Endocrinology*, *113*(1), 329-336. https://doi.org/10.1210/endo-113-1-329
10. Caro, S. P., Schaper, S. V., Hut, R. A., Ball, G. F., & Visser, M. E. (2013). The case of the missing mechanism: how does temperature influence seasonal timing in endotherms? *PLoS Biol*, *11*(4), e1001517. https://doi.org/10.1371/journal.pbio.1001517
11. Cassone, V. M., & Yoshimura, T. (2022). Chapter 43 - Circannual cycles and photoperiodism. In C. G. Scanes & S. Dridi (Eds.), *Sturkie's Avian Physiology (Seventh Edition)* (pp. 1183-1201). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-819770-7.00045-1
12. Chanarin, I., Rothman, D., & Watson-Williams, E. J. (1963). Normal formiminoglutamic acid excreton in megaloblastic anaemia in pregnancy studies on histidine metabolism in

pregnancy. *Lancet*, *281*(7290), 1068-1072. https://doi.org/https://doi.org/10.1016/S0140-6736(63)92109-3
13. Chemineau, P., Guillaume, D., Migaud, M., Thiéry, J. C., Pellicer-Rubio, M. T., & Malpaux, B. (2008). Seasonality of reproduction in mammals: intimate regulatory mechanisms and practical implications. *Reprod Domest Anim*, *43 Suppl 2*, 40-47. https://doi.org/10.1111/j.1439-0531.2008.01141.x
14. Chemineau, P., Malpaux, B., Brillard, J. P., & Fostier, A. (2007). Seasonality of reproduction and production in farm fishes, birds and mammals. *Animal*, *1*(3), 419-432. https://doi.org/10.1017/s1751731107691873
15. Chemineau, P., Malpaux, B., Pelletier, J., Leboeuf, B., Delgadillo, J., Deletang, F., Pobel, T., & Brice, G. (1996). Emploi des implants de mélatonine et des traitements photopériodiques pour maîtriser la reproduction saisonnière chez les ovins et les caprins. *INRAE Productions Animales*, *9*(1), 45-60.
16. Chen, J., Okimura, K., & Yoshimura, T. (2020). Light and Hormones in Seasonal Regulation of Reproduction and Mood. *Endocrinology*, *161*(9). https://doi.org/10.1210/endocr/bqaa130
17. Chmura, H. E., & Williams, C. T. (2022). A cross-taxonomic perspective on the integration of temperature cues in vertebrate seasonal neuroendocrine pathways. *Horm Behav*, *144*, 105215. https://doi.org/10.1016/j.yhbeh.2022.105215
18. Claus, R., Weiler, U., & Hahn, R. (1985). Photoperiod and Fertility in the Pig. In F. Ellendorff & F. Elsaesser (Eds.), *Endocrine Causes of Seasonal and Lactational Anestrus in Farm Animals* (pp. 119-130). Springer Netherlands. https://doi.org/10.1007/978-94-009-5026-9_14
19. Coomans, C. P., Ramkisoensing, A., & Meijer, J. H. (2015). The suprachiasmatic nuclei as a seasonal clock. *Front Neuroendocrinol*, *37*, 29-42. https://doi.org/10.1016/j.yfrne.2014.11.002
20. Croft, H. A. (2017). Understanding the Role of Serotonin in Female Hypoactive Sexual Desire Disorder and Treatment Options. *J Sex Med*, *14*(12), 1575-1584. https://doi.org/10.1016/j.jsxm.2017.10.068
21. Dardente, H., Lomet, D., Robert, V., Decourt, C., Beltramo, M., & Pellicer-Rubio, M. T. (2016). Seasonal breeding in mammals: From basic science to applications and back. *Theriogenology*, *86*(1), 324-332. https://doi.org/10.1016/j.theriogenology.2016.04.045
22. Delgadillo, J. A., Leboeuf, B., & Chemineau, P. (1993). Maintenance of sperm production in bucks during a third year of short photoperiodic cycles. *Reprod Nutr Dev*, *33*(6), 609-617. https://doi.org/10.1051/rnd:19930612
23. Deviche, P., Sabo, J., & Sharp, P. J. (2008). Glutamatergic stimulation of luteinising hormone secretion in relatively refractory male songbirds. *J Neuroendocrinol*, *20*(10), 1191-1202. https://doi.org/10.1111/j.1365-2826.2008.01771.x
24. Dhandapani, K. M., & Brann, D. W. (2000). The role of glutamate and nitric oxide in the reproductive neuroendocrine system. *Biochem Cell Biol*, *78*(3), 165-179.
25. Diffey, B. (2004). Climate change, ozone depletion and the impact on ultraviolet exposure of human skin. *Phys Med Biol*, *49*(1), R1-11. https://doi.org/10.1088/0031-9155/49/1/r01
26. Dodson, J. J., Aubin-Horth, N., Thériault, V., & Páez, D. J. (2013). The evolutionary ecology of alternative migratory tactics in salmonid fishes. *Biol Rev Camb Philos Soc*, *88*(3), 602-625. https://doi.org/10.1111/brv.12019
27. Ely, B. R., Sollanek, K. J., Cheuvront, S. N., Lieberman, H. R., & Kenefick, R. W. (2013). Hypohydration and acute thermal stress affect mood state but not cognition or dynamic postural balance. *Eur J Appl Physiol*, *113*(4), 1027-1034. https://doi.org/10.1007/s00421-012-2506-6
28. Emerson, C. H. (2024). *Pineal gland*. Retrieved 21 March, 2024 from https://www.britannica.com/science/pineal-gland
29. Fink, P., Pflitsch, C., & Marin, K. (2011). Dietary essential amino acids affect the reproduction of the keystone herbivore Daphnia pulex. *PLOS ONE*, *6*(12), e28498. https://doi.org/10.1371/journal.pone.0028498
30. François, C. M., Petit, F., Giton, F., Gougeon, A., Ravel, C., Magre, S., Cohen-Tannoudji, J., & Guigon, C. J. (2017). A novel action of follicle-stimulating hormone in the ovary promotes

estradiol production without inducing excessive follicular growth before puberty. *Sci Rep*, *7*, 46222. https://doi.org/10.1038/srep46222
31. Froy, O. (2007). The relationship between nutrition and circadian rhythms in mammals. *Front Neuroendocrinol*, *28*(2-3), 61-71. https://doi.org/10.1016/j.yfrne.2007.03.001
32. Garssen, J., Norval, M., Crosby, J., Dortant, P., & Van Loveren, H. (1999). The role of urocanic acid in UVB-induced suppression of immunity to Trichinella spiralis infection in the rat. *Immunology*, *96*(2), 298-306. https://doi.org/10.1046/j.1365-2567.1999.00698.x
33. Giallongo, F., Harper, M. T., Oh, J., Parys, C., Shinzato, I., & Hristov, A. N. (2017). Histidine deficiency has a negative effect on lactational performance of dairy cows. *J Dairy Sci*, *100*(4), 2784-2800. https://doi.org/10.3168/jds.2016-11992
34. Gibbs, N. K., & Norval, M. (2011). Urocanic Acid in the Skin: A Mixed Blessing? *Journal of Investigative Dermatology*, *131*(1), 14-17. https://doi.org/https://doi.org/10.1038/jid.2010.276
35. Gwinner, E. (2003). Circannual rhythms in birds. *Current Opinion in Neurobiology*, *13*(6), 770-778. https://doi.org/https://doi.org/10.1016/j.conb.2003.10.010
36. Hall, D. A. (1952). Histidine alpha-deaminase and the production of urocanic acid in the mammal. *Biochem J*, *51*(4), 499-504. https://doi.org/10.1042/bj0510499
37. Hart, P. H., & Norval, M. (2021). The Multiple Roles of Urocanic Acid in Health and Disease. *J Invest Dermatol*, *141*(3), 496-502. https://doi.org/10.1016/j.jid.2020.07.017
38. Hassall, H., & Greenberg, D. (1971). [151] The preparation and properties of 4 (5)-imidazolone-5 (4)-propionic acid. In *Methods in Enzymology* (Vol. 17, pp. 89-91). Elsevier.
39. He, H., Chen, R., He, J., & Wang, J. (2015). The effects of L-histidine on the light and perspiration stability of 4,4'-diamino-stilbene-2,2'-disulfonic acid-based fluorescent whiteness agents on cotton fabrics. *Textile Research Journal*, *85*(7), 775-782. https://doi.org/10.1177/0040517514557312
40. Hinrichs, A. S., Raney, B. J., Speir, M. L., Rhead, B., Casper, J., Karolchik, D., Kuhn, R. M., Rosenbloom, K. R., Zweig, A. S., Haussler, D., & Kent, W. J. (2016). UCSC Data Integrator and Variant Annotation Integrator. *Bioinformatics (Oxford, England)*, *32*(9), 1430-1432. https://doi.org/10.1093/bioinformatics/btv766
41. Homburg, R. (2014). *The Mechanism of Ovulation*. Retrieved 22 March, 2024 from https://www.glowm.com/section-view/heading/The%20Mechanism%20of%20Ovulation/item/289#
42. Hug, D. H., Dunkerson, D. D., & Hunter, J. K. (1999). The degradation of l-histidine and trans and cis-urocanic acid by bacteria from skin and the role of bacterial cis-urocanic acid isomerase. *Journal of Photochemistry and Photobiology B: Biology*, *50*(1), 66-73. https://doi.org/https://doi.org/10.1016/S1011-1344(99)00072-X
43. Hug, D. H., & Hunter, J. K. (1974). Effect of temperature on histidine ammonia-lyase from a psychrophile, Pseudomonas putida. *J Bacteriol*, *119*(1), 92-97. https://doi.org/10.1128/jb.119.1.92-97.1974
44. Jiménez, A., Lu, Y., Jambhekar, A., & Lahav, G. (2022). Principles, mechanisms and functions of entrainment in biological oscillators. *Interface Focus*, *12*(3), 20210088. https://doi.org/10.1098/rsfs.2021.0088
45. Johnsson, A. (2008). Light, circadian and circannual rhythms. *Solar radiation and human health*(5), 57-75.
46. Kammeyer, A., Teunissen, M. B. M., Pavel, S., Rie, M. A., & Bos, J. D. (1995). Photoisomerization spectrum of urocanic acid in human skin and in vitro: effects of simulated solar and artificial ultraviolet radiation. *British Journal of Dermatology*, *132*(6), 884-891. https://doi.org/https://doi.org/10.1111/j.1365-2133.1995.tb16943.x
47. Klempan, T., Hudon-Thibeault, A. A., Oufkir, T., Vaillancourt, C., & Sanderson, J. T. (2011). Stimulation of serotonergic 5-HT2A receptor signaling increases placental aromatase (CYP19) activity and expression in BeWo and JEG-3 human choriocarcinoma cells. *Placenta*, *32*(9), 651-656. https://doi.org/10.1016/j.placenta.2011.06.003
48. Klupiec, C., Evans, G., Love, R. J., & Kennaway, D. J. (1997). Clarifying plasma melatonin profiles in domestic pigs: a critical and comparative evaluation of two radioimmunoassay systems. *J Pineal Res*, *22*(2), 65-74. https://doi.org/10.1111/j.1600-079x.1997.tb00305.x

49. Kohlmeier, M. (2015). Chapter 8 - Amino Acids and Nitrogen Compounds. In M. Kohlmeier (Ed.), *Nutrient metabolism: structures, functions, and genes* (2nd ed., pp. 265-477). Academic Press.
50. Kojima, S., Shingle, D. L., & Green, C. B. (2011). Post-transcriptional control of circadian rhythms. *Journal of Cell Science*, *124*(3), 311-320. https://doi.org/10.1242/jcs.065771
51. Kolenbrander, H. M., & Berg, C. P. (1967). Role of urocanic acid in the metabolism of L-histidine. *Arch Biochem Biophys*, *119*(1), 110-118. https://doi.org/10.1016/0003-9861(67)90435-3
52. Koletzko, B. (2018). Glutamate Supply and Metabolism in Infants. *Ann Nutr Metab*, *73 Suppl 5*, 29-35. https://doi.org/10.1159/000494780
53. Körtner, G., & Geiser, F. (2000). The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. *Chronobiol Int*, *17*(2), 103-128. https://doi.org/10.1081/cbi-100101036
54. Kriegsfeld, L. J., Ranalli, N. J., Bober, M. A., & Nelson, R. J. (2000). Photoperiod and temperature interact to affect the GnRH neuronal system of male prairie voles (Microtus ochrogaster). *J Biol Rhythms*, *15*(4), 306-316. https://doi.org/10.1177/074873000129001413
55. Küller, R., Ballal, S., Laike, T., Mikellides, B., & Tonello, G. (2006). The impact of light and colour on psychological mood: a cross-cultural study of indoor work environments. *Ergonomics*, *49*(14), 1496-1507. https://doi.org/10.1080/00140130600858142
56. Kuo, J. R., Lin, S. S., Liu, J., Chen, S. H., Chio, C. C., Wang, J. J., & Liu, J. M. (2015). Deep brain light stimulation effects on glutamate and dopamine concentration. *Biomed Opt Express*, *6*(1), 23-31. https://doi.org/10.1364/boe.6.000023
57. Lamia, K. A., Storch, K. F., & Weitz, C. J. (2008). Physiological significance of a peripheral tissue circadian clock. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(39), 15172-15177. https://doi.org/10.1073/pnas.0806717105
58. Lincoln, G. A., Andersson, H., & Loudon, A. (2003). Clock genes in calendar cells as the basis of annual timekeeping in mammals--a unifying hypothesis. *J Endocrinol*, *179*(1), 1-13. https://doi.org/10.1677/joe.0.1790001
59. Loarie, S. R., Duffy, P. B., Hamilton, H., Asner, G. P., Field, C. B., & Ackerly, D. D. (2009). The velocity of climate change. *Nature*, *462*(7276), 1052-1055. https://doi.org/10.1038/nature08649
60. Lockley, S. W., Brainard, G. C., & Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *J Clin Endocrinol Metab*, *88*(9), 4502-4505. https://doi.org/10.1210/jc.2003-030570
61. Loudon, A. S. (1994). Photoperiod and the regulation of annual and circannual cycles of food intake. *Proc Nutr Soc*, *53*(3), 495-507. https://doi.org/10.1079/pns19940060
62. Love, R. J., Evans, G., & Klupiec, C. (2020). Seasonal effects on fertility in gilts and sows. *Journal of reproduction and fertility. Supplement*, *48*, 191-206.
63. Maffucci, J. A., & Gore, A. C. (2009). Chapter 2: hypothalamic neural systems controlling the female reproductive life cycle gonadotropin-releasing hormone, glutamate, and GABA. *Int Rev Cell Mol Biol*, *274*, 69-127. https://doi.org/10.1016/s1937-6448(08)02002-9
64. Mager, E. M., Medeiros, L. R., Lange, A. P., & McDonald, M. D. (2012). The toadfish serotonin 2A (5-HT(2A)) receptor: molecular characterization and its potential role in urea excretion. *Comp Biochem Physiol A Mol Integr Physiol*, *163*(3-4), 319-326. https://doi.org/10.1016/j.cbpa.2012.07.013
65. Matsumoto, T., Nakamura, E., Nakamura, H., Hirota, M., Gabriel, A., Nakamura, K.-I., Chotechuang, N., Wu, G., Uneyama, H., & Torii, K. (2013). Production of free glutamate in milk requires the leucine transporter LAT1. *American journal of physiology. Cell physiology*, *305*. https://doi.org/10.1152/ajpcell.00291.2012
66. Mehler, A. H., & Tabor, H. (1953). Deamination of histidine to form urocanic acid in liver. *J Biol Chem*, *201*(2), 775-784.
67. Minosyan, T. Y., Lu, R., Eghbali, M., Toro, L., & Stefani, E. (2007). Increased 5-HT contractile response in late pregnant rat myometrium is associated with a higher density of 5-HT2A receptors. *J Physiol*, *581*(Pt 1), 91-97. https://doi.org/10.1113/jphysiol.2007.129726

68. Nakane, Y., Ikegami, K., Ono, H., Yamamoto, N., Yoshida, S., Hirunagi, K., Ebihara, S., Kubo, Y., & Yoshimura, T. (2010). A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(34), 15264-15268. https://doi.org/10.1073/pnas.1006393107
69. Nedergaard, P., Sanchez, C., & Mellerup, E. (2004). Different roles of 5-HT2A and 5-HT2C receptors in regulation of female rat paced mating behaviour. *Behav Brain Res*, *149*(2), 151-157. https://doi.org/10.1016/s0166-4328(03)00215-8
70. Norval, M. (2001). Chapter 5 - Effects of solar radiation on the human immune system. In P. U. Giacomoni (Ed.), *Comprehensive Series in Photosciences* (Vol. 3, pp. 91-113). Elsevier. https://doi.org/https://doi.org/10.1016/S1568-461X(01)80040-5
71. NSW Department of Primary Industries. (2023). *Nutritional Value of Native Grasses*. Retrieved 22 March, 2024 from https://www.dpi.nsw.gov.au/agriculture/pastures-and-rangelands/native-pastures/nutritional-value-of-native-grasses#:~:text=When%20maintained%20in%20the%20early,lower%20end%20during%20late%20flowering
72. O'Connor, D. L., Green, T., & Picciano, M. F. (1997). Maternal folate status and lactation. *J Mammary Gland Biol Neoplasia*, *2*(3), 279-289. https://doi.org/10.1023/a:1026388522182
73. Peltoniemi, O. A., & Virolainen, J. V. (2006). Seasonality of reproduction in gilts and sows. *Soc Reprod Fertil Suppl*, *62*, 205-218.
74. Peltoniemi, O. A. T., Tast, A., & Love, R. J. (2000). Factors effecting reproduction in the pig: seasonal effects and restricted feeding of the pregnant gilt and sow. *Animal Reproduction Science*, *60-61*, 173-184. https://doi.org/https://doi.org/10.1016/S0378-4320(00)00092-0
75. Popova, N. K., & Amstislavskaya, T. G. (2002). 5-HT2A and 5-HT2C serotonin receptors differentially modulate mouse sexual arousal and the hypothalamo-pituitary-testicular response to the presence of a female. *Neuroendocrinology*, *76*(1), 28-34. https://doi.org/10.1159/000063681
76. Pork Information Gateway. (2012). *Hormone Use in Swine Production and Worker Safety*. Retrieved 22 March, 2024 from https://porkgateway.org/resource/hormone-use-in-swine-production-and-worker-safety/
77. Räisänen, S. E., Lapierre, H., Price, W. J., & Hristov, A. N. (2023). Lactational performance effects of supplemental histidine in dairy cows: A meta-analysis. *J Dairy Sci*, *106*(9), 6216-6231. https://doi.org/10.3168/jds.2022-22966
78. Rigout, S., Hurtaud, C., Lemosquet, S., Bach, A., & Rulquin, H. (2003). Lactational effect of propionic acid and duodenal glucose in cows. *J Dairy Sci*, *86*(1), 243-253. https://doi.org/10.3168/jds.S0022-0302(03)73603-0
79. Rijo-Ferreira, F., & Takahashi, J. S. (2019). Genomics of circadian rhythms in health and disease. *Genome Med*, *11*(1), 82. https://doi.org/10.1186/s13073-019-0704-0
80. Safran, M., Dalah, I., Alexander, J., Rosen, N., Iny Stein, T., Shmoish, M., Nativ, N., Bahir, I., Doniger, T., Krug, H., Sirota-Madi, A., Olender, T., Golan, Y., Stelzer, G., Harel, A., & Lancet, D. (2010). GeneCards Version 3: the human gene integrator. *Database (Oxford)*, *2010*, baq020. https://doi.org/10.1093/database/baq020
81. Soh, N. L., Walter, G., Baur, L., & Collins, C. (2009). Nutrition, mood and behaviour: a review. *Acta Neuropsychiatr*, *21*(5), 214-227. https://doi.org/10.1111/j.1601-5215.2009.00413.x
82. Swaab, D. F., Van Someren, E. J., Zhou, J. N., & Hofman, M. A. (1996). Biological rhythms in the human life cycle and their relationship to functional changes in the suprachiasmatic nucleus. *Prog Brain Res*, *111*, 349-368. https://doi.org/10.1016/s0079-6123(08)60418-5
83. Tast, A., Love, R. J., Evans, G., Telsfer, S., Giles, R., Nicholls, P., Voultsios, A., & Kennaway, D. J. (2001). The pattern of melatonin secretion is rhythmic in the domestic pig and responds rapidly to changes in daylength. *Journal of Pineal Research*, *31*(4), 294-300. https://doi.org/https://doi.org/10.1034/j.1600-079X.2001.310402.x
84. United States Environmental Protection Agency. (n.d.). *Future of Climate Change*. Retrieved 28 March, 2024 from https://climatechange.chicago.gov/climate-change-science/future-climate-change#ref2

85. Viau, M., Lafond, J., & Vaillancourt, C. (2009). Expression of placental serotonin transporter and 5-HT 2A receptor in normal and gestational diabetes mellitus pregnancies. *Reprod Biomed Online*, *19*(2), 207-215. https://doi.org/10.1016/s1472-6483(10)60074-0
86. Walker, H. K., Hall, W. D., & Hurst, J. W. (1990). Clinical Methods: The History, Physical, and Laboratory Examinations. In. Butterworths.
87. Walterscheid, J. P., Nghiem, D. X., Kazimi, N., Nutt, L. K., McConkey, D. J., Norval, M., & Ullrich, S. E. (2006). Cis-urocanic acid, a sunlight-induced immunosuppressive factor, activates immune suppression via the 5-HT2A receptor. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(46), 17420-17425. https://doi.org/10.1073/pnas.0603119103
88. Wang, Z., Liu, B. S., Wang, X. Y., Wei, Q. H., Tian, H., & Wang, L. Q. (2018). Effects of altrenogest on reproductive performance of gilts and sows: A meta-analysis. *Animal Reproduction Science*, *197*, 10-21. https://doi.org/https://doi.org/10.1016/j.anireprosci.2018.08.035
89. Weizmann Institute of Science. (n.d.). *UROC1 Gene - Urocanate Hydratase 1*. Retrieved 21 March, 2024 from https://www.genecards.org/cgi-bin/carddisp.pl?gene=UROC1#expression
90. Woo, J. M., & Postolache, T. T. (2008). The impact of work environment on mood disorders and suicide: Evidence and implications. *Int J Disabil Hum Dev*, *7*(2), 185-200. https://doi.org/10.1515/ijdhd.2008.7.2.185
91. Wood, W. E., Roseberry, T. K., & Perkel, D. J. (2013). HTR2 receptors in a songbird premotor cortical-like area modulate spectral characteristics of zebra finch song. *Journal of Neuroscience*, *33*(7), 2908-2915. https://doi.org/https://doi.org/10.1523/JNEUROSCI.4291-12.2013
02. Yoshimura, T. (2010). Neuroendocrine mechanism of seasonal reproduction in birds and mammals. *Anim Sci J*, *81*(4), 403-410. https://doi.org/10.1111/j.1740-0929.2010.00777.x
93. Zare, D., Muhammad, K., Bejo, M. H., & Ghazali, H. M. (2012). Development and validation of an ion-pair chromatographic method for simultaneous determination of trans- and cis-urocanic acid in fish samples. *J Chromatogr A*, *1256*, 144-149. https://doi.org/10.1016/j.chroma.2012.07.083
94. Zee, P. C., Attarian, H., & Videnovic, A. (2013). Circadian rhythm abnormalities. *Continuum (Minneap Minn)*, *19*(1 Sleep Disorders), 132-147. https://doi.org/10.1212/01.CON.0000427209.21177.aa

Appendix

Table 5. All gathered commercially house sow data, excluding sows who had no reproductive status. The table includes the month of collection, trans- and cis- UCA, reproductive status, on farm location, average calculated UV index during the month of collection, average temperature during the month of collection and pig ID. Pig Id referred to the ear tag number of each pig.

pig_id	cis	trans	Repro status	House area	UV Index	temp	month
226	8.23E-06	4.08E-05	Luteal	Main shed	6.393548	22.6	Jan
233	8.16E-06	1.38E-05	Pregnant	Main shed	6.393548	22.6	Jan

242	1.54E-06	0.000168	Luteal	Main shed	6.393548	22.6	Jan
243	2.06E-05	0.000196	Luteal	Main shed	6.393548	22.6	Jan
246	2.72E-05	1.60E-05	Luteal	Main shed	6.393548	22.6	Jan
266	1.24E-05	0.00273	Oestrus	Main shed	6.393548	22.6	Jan
279	3.61E-06	5.24E-05	Oestrus	Main shed	6.393548	22.6	Jan
293	1.02E-05	7.47E-05	Pregnant	Main shed	6.393548	22.6	Jan
295	2.14E-05	1.49E-05	Lactating	Dry sow shed	6.393548	22.6	Jan
296	3.10E-05	2.30E-05	Pregnant	Dry sow shed	6.393548	22.6	Jan
955	9.87E-05	6.25E-05	Luteal	Dry sow shed	6.393548	22.6	Jan
956	6.45E-05	3.64E-05	Pregnant	Dry sow shed	6.393548	22.6	Jan
957	4.31E-05	3.04E-05	Luteal	Dry sow shed	6.393548	22.6	Jan
958	5.20E-05	3.49E-05	Luteal	Dry sow shed	6.393548	22.6	Jan
959	3.49E-05	7.21E-05	Pregnant	Main shed	6.393548	22.6	Jan
960	0.000182	0.000139	Pregnant	Main shed	6.393548	22.6	Jan
962	0.000126	0.000256	Pregnant	Main shed	6.393548	22.6	Jan
964	1.62E-05	1.93E-05	Pregnant	Main shed	6.393548	22.6	Jan
968	3.49E-05	3.44E-05	Pregnant	Main shed	6.393548	22.6	Jan
969	3.73E-05	2.15E-05	Luteal	Dry sow shed	6.393548	22.6	Jan
970	6.05E-05	3.84E-05	Luteal	Dry sow shed	6.393548	22.6	Jan
972	2.69E-05	1.60E-05	Pregnant	Dry sow shed	6.393548	22.6	Jan
974	9.46E-05	0.000191	Pregnant	Main shed	6.393548	22.6	Jan
976	2.73E-05	2.39E-05	Pregnant	Main shed	6.393548	22.6	Jan
977	2.29E-05	2.54E-05	Pregnant	Main shed	6.393548	22.6	Jan
978	2.63E-05	1.62E-05	Luteal	Dry sow shed	6.393548	22.6	Jan

980	1.38E-05	2.09E-05	Pregnant	Main shed	6.393548	22.6	Jan
988	4.75E-05	4.45E-05	Pregnant	Main shed	6.393548	22.6	Jan
226	1.90E-05	0.000154	Culled	Main shed	6.446429	22.85	Feb
233	1.07E-05	1.25E-05	Pregnant	Dry sow shed	6.446429	22.85	Feb
242	7.06E-05	6.91E-05	Pro-oestrus	Main shed	6.446429	22.85	Feb
246	3.01E-05	2.69E-05	Luteal	Dry sow shed	6.446429	22.85	Feb
266	5.51E-05	5.17E-05	Pregnant	Dry sow shed	6.446429	22.85	Feb
279	2.49E-05	2.74E-05	Pregnant	Dry sow shed	6.446429	22.85	Feb
293	3.57E-05	8.44E-05	Pregnant	Dry sow shed	6.446429	22.85	Feb
295	4.26E-05	0.000178	Lactating	Main shed	6.446429	22.85	Feb
296	3.86E-05	0.000209	Lactating	Main shed	6.446429	22.85	Feb
955	7.90E-05	6.22E-05	Oestrus	Main shed	6.446429	22.85	Feb
956	1.76E-05	2.27E-05	Pregnant	Main shed	6.446429	22.85	Feb
957	1.82E-05	2.37E-05	Oestrus	Main shed	6.446429	22.85	Feb
958	4.15E-05	4.83E-05	Oestrus	Main shed	6.446429	22.85	Feb
959	1.05E-05	0.000362	Lactating	Main shed	6.446429	22.85	Feb
960	4.40E-05	0.000216	Lactating	Main shed	6.446429	22.85	Feb
962	0.000113	0.000242	Lactating	Main shed	6.446429	22.85	Feb
964	1.44E-05	0.000196	Lactating	Main shed	6.446429	22.85	Feb
968	2.31E-05	3.39E-05	Pregnant	Main shed	6.446429	22.85	Feb
970	3.65E-05	3.46E-05	Oestrus	Main shed	6.446429	22.85	Feb
972	5.85E-05	3.84E-05	Pregnant	Dry sow shed	6.446429	22.85	Feb
974	2.00E-05	0.000303	Lactating	Main shed	6.446429	22.85	Feb
976	3.07E-05	0.000297	Lactating	Main shed	6.446429	22.85	Feb

977	2.66E-05	4.75E-05	Pregnant	Main shed	6.446429	22.85	Feb
978	3.17E-05	7.41E-05	Pro-oestrus	Main shed	6.446429	22.85	Feb
980	7.62E-06	9.90E-05	Lactating	Main shed	6.446429	22.85	Feb
988	7.82E-06	0.000146	Pro-oestrus	Main shed	6.446429	22.85	Feb
233	7.41E-06	1.13E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
242	3.77E-06	1.06E-05	Luteal	Dry sow shed	4.370968	21.8	Mar
246	5.48E-05	4.67E-05	Pro-oestrus	Dry sow shed	4.370968	21.8	Mar
266	4.76E-05	7.15E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
279	1.71E-05	1.50E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
293	2.06E-05	3.37E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
295	1.86E-05	9.82E-05	Pro-oestrus	Main shed	4.370968	21.8	Mar
296	5.56E-06	4.67E-05	Pro-oestrus	Main shed	4.370968	21.8	Mar
955	9.49E-05	8.59E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
956	1.68E-05	2.09E-05	Farrowing	Main shed	4.370968	21.8	Mar
957	2.64E-05	2.20E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
958	9.83E-05	8.98E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
959	1.53E-05	3.70E-05	Oestrus	Main shed	4.370968	21.8	Mar
960	1.30E-05	3.97E-05	Oestrus	Main shed	4.370968	21.8	Mar
962	3.95E-06	1.79E-05	Pro-oestrus	Main shed	4.370968	21.8	Mar
964	8.46E-06	2.09E-05	Oestrus	Main shed	4.370968	21.8	Mar
968	7.43E-06	0.00011	Lactating	Main shed	4.370968	21.8	Mar
969	1.07E-05	1.73E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
970	1.70E-05	3.71E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
972	4.34E-06	6.43E-05	Lactating	Main shed	4.370968	21.8	Mar

974	7.47E-06	2.64E-05	Oestrus	Main shed	4.370968	21.8	Mar
976	7.65E-06	1.45E-05	Pregnant	Main shed	4.370968	21.8	Mar
977	5.70E-06	1.49E-05	Lactating	Main shed	4.370968	21.8	Mar
978	9.89E-06	1.03E-05	Pregnant	Dry sow shed	4.370968	21.8	Mar
980	1.84E-06	1.12E-05	Oestrus	Main shed	4.370968	21.8	Mar
988	3.26E-06	1.08E-05	Oestrus	Main shed	4.370968	21.8	Mar
233	3.58E-06	9.61E-06	Pregnant	Dry sow shed	2.86	16.85	Apr
242	4.72E-05	4.67E-05	Pregnant	Finisher shed	2.86	16.85	Apr
246	0.0001	7.24E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
266	9.33E-05	0.00012	Pregnant	Dry sow shed	2.86	16.85	Apr
279	NA	NA	Pregnant	Dry sow shed	2.86	16.85	Apr
293	3.69E-05	7.08E-05	Pregnant	Main shed	2.86	16.85	Apr
295	4.88E-05	3.77E-05	Luteal	Dry sow shed	2.86	16.85	Apr
296	2.52E-05	2.53E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
955	0.000148	8.74E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
956	2.18E-05	0.000191	Pro-oestrus	Main shed	2.86	16.85	Apr
957	3.90E-05	4.67E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
958	0.000107	0.000146	Pregnant	Dry sow shed	2.86	16.85	Apr
959	0.000158	0.000151	Pregnant	Dry sow shed	2.86	16.85	Apr
960	2.85E-05	3.04E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
962	9.10E-05	7.58E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
964	7.94E-05	5.90E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
968	4.79E-06	2.50E-05	Pro-oestrus	Main shed	2.86	16.85	Apr
969	3.14E-05	2.84E-05	Pregnant	Dry sow shed	2.86	16.85	Apr

970	1.77E-05	1.88E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
972	4.46E-06	2.70E-05	Pro-oestrus	Main shed	2.86	16.85	Apr
974	0.000122	0.00017	Pregnant	Dry sow shed	2.86	16.85	Apr
976	1.26E-05	1.98E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
977	3.81E-06	3.44E-05	Pro-oestrus	Main shed	2.86	16.85	Apr
978	7.49E-05	7.55E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
980	7.79E-05	8.10E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
988	2.55E-05	1.55E-05	Pregnant	Dry sow shed	2.86	16.85	Apr
233	6.41E-07	7.89E-06	Lactating	Finisher shed	1.758065	11.75	May
242	1.61E-05	1.88E-05	Pregnant	Main shed	1.758065	11.75	May
246	1.34E-05	3.11E-05	Pregnant	Dry sow shed	1.758065	11.75	May
266	7.47E-06	4.62E-05	Lactating	Finisher shed	1.758065	11.75	May
279	1.93E-05	3.78E-05	Lactating	Finisher shed	1.758065	11.75	May
293	8.9E-06	2.16E-05	Lactating	Finisher shed	1.758065	11.75	May
295	5.33E-05	0.000138	Luteal	Main shed	1.758065	11.75	May
296	3.21E-05	2.77E-05	Pregnant	Dry sow shed	1.758065	11.75	May
955	2.09E-05	1.87E-05	Pregnant	Dry sow shed	1.758065	11.75	May
956	1.52E-05	1.29E-05	Pregnant	Dry sow shed	1.758065	11.75	May
957	1.49E-05	1.38E-05	Pregnant	Dry sow shed	1.758065	11.75	May
958	NA	NA	Pregnant	Dry sow shed	1.758065	11.75	May
959	1.20E-05	1.41E-05	Pregnant	Dry sow shed	1.758065	11.75	May
960	2.46E-05	1.53E-05	Pregnant	Dry sow shed	1.758065	11.75	May
962	4.19E-05	3.74E-05	Pregnant	Dry sow shed	1.758065	11.75	May
964	2.90E-05	3.65E-05	Pregnant	Dry sow shed	1.758065	11.75	May

968	NA	NA	Pregnant	Dry sow shed	1.758065	11.75	May
969	NA	NA	Pregnant	Dry sow shed	1.758065	11.75	May
970	1.50E-05	1.67E-05	Pregnant	Dry sow shed	1.758065	11.75	May
972	3.82E-05	3.45E-05	Pregnant	Dry sow shed	1.758065	11.75	May
974	3.87E-05	3.94E-05	Pregnant	Dry sow shed	1.758065	11.75	May
976	2.55E-05	1.65E-05	Pregnant	Dry sow shed	1.758065	11.75	May
977	1.14E-05	1.08E-05	Pregnant	Dry sow shed	1.758065	11.75	May
978	2.75E-05	2.42E-05	Pregnant	Dry sow shed	1.758065	11.75	May
980	1.06E-05	5.54E-06	Pregnant	Dry sow shed	1.758065	11.75	May
988	6.77E-06	5.92E-06	Pregnant	Dry sow shed	1.758065	11.75	May
206	4.09E-05	3.44E-05	NA	NA	1.213333	11.05	Jun
233	4.04E-06	8.91E-06	Pregnant	Main shed	1.213333	11.05	Jun
242	7.76E-05	0.000122	Pregnant	Dry sow shed	1.213333	11.05	Jun
246	1.48E-05	6.98E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
279	5.52E-05	5.13E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
293	3.02E-06	7.01E-06	Luteal	Main shed	1.213333	11.05	Jun
955	1.06E-05	4.25E-05	Lactating	Finisher shed	1.213333	11.05	Jun
956	0	0	Pregnant	Dry sow shed	1.213333	11.05	Jun
957	5.10E-06	2.24E-05	Lactating	Finisher shed	1.213333	11.05	Jun
958	5.82E-05	0.000106	Lactating	Finisher shed	1.213333	11.05	Jun
959	2.36E-05	3.20E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
960	1.17E-05	8.07E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
962	2.15E-05	3.41E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
964	1.53E-05	1.86E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun

968	3.14E-05	3.00E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
969	3.35E-07	6.23E-06	Lactating	Finisher shed	1.213333	11.05	Jun
972	2.10E-06	1.94E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
974	2.63E-05	3.27E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
977	6.67E-06	1.91E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
978	6.96E-05	4.98E-05	Lactating	Finisher shed	1.213333	11.05	Jun
980	4.18E-06	0.00002	Pregnant	Finisher shed	1.213333	11.05	Jun
988	1.58E-05	3.25E-05	Pregnant	Dry sow shed	1.213333	11.05	Jun
233	6.61E-06	2.02E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
242	5.15E-06	2.45E-05	Pregnant	Finisher shed	1.480645	11.4	Jul
246	6.03E-06	7.46E-05	Lactating	Finisher shed	1.480645	11.4	Jul
279	2.94E-05	4.78E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
296	1.64E-05	7.58E-05	Luteal	Finisher shed	1.480645	11.4	Jul
955	1.79E-05	2.50E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
956	8.40E-06	1.27E-05	Pregnant	Finisher shed	1.480645	11.4	Jul
957	4.06E-05	6.04E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
958	7.56E-06	1.80E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
959	1.05E-06	4.87E-05	Lactating	Finisher shed	1.480645	11.4	Jul
960	1.40E-05	0.000146	Lactating	Finisher shed	1.480645	11.4	Jul
962	2.79E-05	6.43E-05	Lactating	Finisher shed	1.480645	11.4	Jul
964	8.84E-07	1.95E-05	Lactating	Finisher shed	1.480645	11.4	Jul
968	NA	NA	Pregnant	Dry sow shed	1.480645	11.4	Jul
969	1.40E-05	2.67E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
970	2.23E-05	6.74E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul

972	1.32E-05	2.18E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
974	2.34E-05	0.000301	Lactating	Finisher shed	1.480645	11.4	Jul
976	5.19E-05	0.000193	Luteal	Finisher shed	1.480645	11.4	Jul
977	5.96E-06	1.28E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
978	8.95E-06	2.60E-05	Pregnant	Dry sow shed	1.480645	11.4	Jul
980	NA	NA	Lactating	Finisher shed	1.480645	11.4	Jul
988	1.58E-05	5.23E-05	Lactating	Finisher shed	1.480645	11.4	Jul
233	7.37E-06	1.14E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
242	1.88E-05	4.13E-05	Lactating	Finisher shed	2.009677	12.85	Aug
246	NA	NA	Pregnant	Dry sow shed	2.009677	12.85	Aug
279	3.85E-05	3.61E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
296	3.69E-05	0.000251	Pregnant	Dry sow shed	2.009677	12.85	Aug
946	9.08E-05	7.47E-05	NA	NA	2.009677	12.85	Aug
955	4.11E-05	3.26E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
956	2.76E-05	2.74E-05	Pregnant	Finisher shed	2.009677	12.85	Aug
957	2.95E-05	3.01E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
958	4.42E-05	3.98E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
959	6.72E-05	6.67E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
960	0.00011	0.00011	Pregnant	Dry sow shed	2.009677	12.85	Aug
962	3.21E-05	2.08E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
964	7.53E-05	9.99E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
968	2.92E-05	3.46E-05	Lactating	Finisher shed	2.009677	12.85	Aug
969	9.72E-06	1.36E-05	Pregnant	Finisher shed	2.009677	12.85	Aug
970	4.17E-05	4.47E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug

972	1.57E-05	2.62E-05	Lactating	Finisher shed	2.009677	12.85	Aug
974	0.000107	0.000201	Pregnant	Dry sow shed	2.009677	12.85	Aug
976	3.26E-05	4.73E-05	Pregnant	Main shed	2.009677	12.85	Aug
977	1.08E-05	2.46E-05	Lactating	Finisher shed	2.009677	12.85	Aug
978	9.41E-05	0.000103	Pregnant	Dry sow shed	2.009677	12.85	Aug
980	3.37E-05	5.69E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
988	1.74E-05	1.88E-05	Pregnant	Dry sow shed	2.009677	12.85	Aug
226	0.000134	6.38E-05	Pregnant	Dry sow shed	3.13	14.4	Sep
233	7.05E-06	5.82E-06	Pregnant	Dry sow shed	3.13	14.4	Sep
242	2.92E-06	1.83E-05	Pregnant	Main shed	3.13	14.4	Sep
243	0.000162	7.17E-05	Luteal	Dry sow shed	3.13	14.4	Sep
246	3.20E-06	2.69E-05	Luteal	Main shed	3.13	14.4	Sep
266	2.89E-05	1.71E-05	Pregnant	Dry sow shed	3.13	14.4	Sep
279	1.80E-05	1.06E-05	Pregnant	Dry sow shed	3.13	14.4	Sep
290	1.38E-05	4.53E-06	Pregnant	Main shed	3.13	14.4	Sep
293	0.001896	1.58E-05	Pregnant	Main shed	3.13	14.4	Sep
295	6.20E-06	1.58E-05	Lactating	Main shed	3.13	14.4	Sep
296	1.28E-05	4.65E-06	Lactating	Main shed	3.13	14.4	Sep
955	6.65E-06	2.61E-05	Luteal	Main shed	3.13	14.4	Sep
956	9.17E-06	5.70E-06	Luteal	Main shed	3.13	14.4	Sep
957	4.59E-06	1.21E-05	Luteal	Main shed	3.13	14.4	Sep
958	8.77E-06	1.31E-05	Luteal	Main shed	3.13	14.4	Sep
959	7.37E-06	4.88E-05	Luteal	Main shed	3.13	14.4	Sep
960	8.86E-06	2.50E-05	Luteal	Main shed	3.13	14.4	Sep

962	8.04E-06	5.81E-05	Pro-oestrus	Main shed	3.13	14.4	Sep
964	1.60E-05	5.02E-05	Luteal	Main shed	3.13	14.4	Sep
967	8.70E-06	9.94E-06	NA	Finisher shed	3.13	14.4	Sep
968	5.32E-06	7.73E-06	Luteal	Finisher shed	3.13	14.4	Sep
969	9.56E-06	4.69E-06	Luteal	Finisher shed	3.13	14.4	Sep
970	7.71E-06	7.28E-06	Luteal	Finisher shed	3.13	14.4	Sep
974	1.77E-05	6.60E-06	Luteal	Main shed	3.13	14.4	Sep
976	2.68E-05	2.59E-05	Luteal	Finisher shed	3.13	14.4	Sep
977	3.88E-06	3.57E-06	Luteal	Finisher shed	3.13	14.4	Sep
980	1.04E-05	7.36E-06	Luteal	Finisher shed	3.13	14.4	Sep
988	9.65E-07	7.95E-06	Lactating	Main shed	3.13	14.4	Sep
226	1.57E-05	6.52E-05	Lactating	Main shed	3.803226	17.25	Oct
233	0.000532	2.08E-05	Pregnant	Main shed	3.803226	17.25	Oct
242	2.95E-06	4.77E-05	Pregnant	Main shed	3.803226	17.25	Oct
243	3.93E-05	3.05E-05	Luteal	Dry sow shed	3.803226	17.25	Oct
246	2.22E-05	1.84E-05	Luteal	Dry sow shed	3.803226	17.25	Oct
266	4.00E-05	3.13E-05	Pregnant	Dry sow shed	3.803226	17.25	Oct
279	0.000108	0.000329	Lactating	Main shed	3.803226	17.25	Oct
290	1.16E-05	3.03E-05	Lactating	Main shed	3.803226	17.25	Oct
293	6.49E-06	3.00E-05	Luteal	Main shed	3.803226	17.25	Oct
295	8.29E-05	7.11E-05	Pregnant	Dry sow shed	3.803226	17.25	Oct
296	2.49E-05	2.08E-05	Pregnant	Dry sow shed	3.803226	17.25	Oct
955	2.96E-05	2.98E-05	Luteal	Finisher shed	3.803226	17.25	Oct
956	8.60E-06	8.60E-06	Luteal	Finisher shed	3.803226	17.25	Oct

957	4.10E-05	3.63E-05	Luteal	Finisher shed	3.803226	17.25	Oct
958	9.28E-05	9.17E-05	Luteal	Finisher shed	3.803226	17.25	Oct
959	4.28E-05	3.72E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
960	6.53E-05	5.53E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
962	8.97E-05	0.000205	Pregnant	Main shed	3.803226	17.25	Oct
964	1.99E-05	2.01E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
967	5.94E-06	8.06E-06	Anestrous	Finisher shed	3.803226	17.25	Oct
968	3.96E-05	3.20E-05	Luteal	Finisher shed	3.803226	17.25	Oct
969	1.10E-05	1.44E-05	Luteal	Finisher shed	3.803226	17.25	Oct
970	6.33E-05	6.09E-05	Luteal	Finisher shed	3.803226	17.25	Oct
972	1.99E-05	2.16E-05	Luteal	Finisher shed	3.803226	17.25	Oct
974	2.76E-05	3.16E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
976	4.33E-05	4.39E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
977	7.87E-06	8.32E-06	Luteal	Finisher shed	3.803226	17.25	Oct
978	1.49E-05	1.64E-05	Luteal	Finisher shed	3.803226	17.25	Oct
980	4.37E-05	3.28E-05	Pregnant	Finisher shed	3.803226	17.25	Oct
988	2.07E-05	1.40E-05	Pregnant	Dry sow shed	3.803226	17.25	Oct
226	3.18E-05	4.05E-05	Pro-oestrus	Main shed	6.516667	17.9	Nov
233	1.83E-05	2.01E-05	Pro-oestrus	Main shed	6.516667	17.9	Nov
242	2.71E-06	1.36E-05	Pregnant	Dry sow shed	6.516667	17.9	Nov
243	1.44E-05	2.67E-05	Pro-oestrus	Main shed	6.516667	17.9	Nov
246	5.02E-05	1.82E-05	Luteal	Dry sow shed	6.516667	17.9	Nov
266	2.51E-06	1.15E-06	Pregnant	Main shed	6.516667	17.9	Nov
279	4.76E-05	5.29E-05	Pro-oestrus	Main shed	6.516667	17.9	Nov

293	2.53E-05	3.12E-05	Luteal	Main shed	6.516667	17.9	Nov
295	4.64E-05	2.19E-05	Pregnant	Dry sow shed	6.516667	17.9	Nov
296	4.16E-05	1.83E-05	Pregnant	Dry sow shed	6.516667	17.9	Nov
955	2.75E-05	1.67E-05	Luteal	Finisher shed	6.516667	17.9	Nov
956	1.20E-05	5.69E-06	Luteal	Finisher shed	6.516667	17.9	Nov
957	1.84E-05	7.18E-06	Luteal	Finisher shed	6.516667	17.9	Nov
958	8.71E-05	5.77E-05	Luteal	Finisher shed	6.516667	17.9	Nov
960	0.000139	5.04E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
962	0.000187	0.000194	Pregnant	Main shed	6.516667	17.9	Nov
964	7.78E-05	3.50E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
967	1.14E-05	1.01E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
968	6.21E-05	2.97E-05	Pro-oestrus	Finisher shed	6.516667	17.9	Nov
969	1.05E-05	6.38E-06	Luteal	Finisher shed	6.516667	17.9	Nov
970	1.06E-05	1.06E-05	Luteal	Finisher shed	6.516667	17.9	Nov
974	6.83E-05	3.00E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
976	3.13E-05	1.24E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
977	1.48E-05	9.08E-06	Pro-oestrus	Finisher shed	6.516667	17.9	Nov
978	5.76E-06	5.12E-06	Luteal	Finisher shed	6.516667	17.9	Nov
980	3.97E-05	2.35E-05	Pregnant	Finisher shed	6.516667	17.9	Nov
988	8.77E-05	3.04E-05	Pregnant	Dry sow shed	6.516667	17.9	Nov
226	3.41E-05	0.000102	Luteal	Main shed	7.009677	19.6	Dec
233	1.50E-05	2.33E-05	Pro-oestrus	Main shed	7.009677	19.6	Dec
242	1.99E-05	7.52E-05	Lactating	Dry sow shed	7.009677	19.6	Dec
243	1.21E-05	2.19E-05	Luteal	Main shed	7.009677	19.6	Dec

246	2.39E-05	1.73E-05	Luteal	Dry sow shed	7.009677	19.6	Dec
266	4.32E-05	0.000253	Lactating	Main shed	7.009677	19.6	Dec
279	3.25E-05	0.000104	Luteal	Main shed	7.009677	19.6	Dec
293	2.61E-05	6.37E-05	Luteal	Main shed	7.009677	19.6	Dec
295	0.000117	8.64E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
296	2.93E-05	2.36E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
955	4.82E-05	5.08E-05	Luteal	Dry sow shed	7.009677	19.6	Dec
956	4.87E-05	4.31E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
957	3.19E-05	2.97E-05	Luteal	Dry sow shed	7.009677	19.6	Dec
958	9.35E-05	8.34E-05	Luteal	Dry sow shed	7.009677	19.6	Dec
959	5.90E-05	4.63E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
960	0.000135	0.000184	Pregnant	Dry sow shed	7.009677	19.6	Dec
962	0.000175	0.000289	Pregnant	Main shed	7.009677	19.6	Dec
964	3.46E-05	4.34E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
968	1.08E-05	1.58E-05	Pregnant	Finisher shed	7.009677	19.6	Dec
969	1.84E-05	1.63E-05	Luteal	Finisher shed	7.009677	19.6	Dec
970	1.42E-05	1.18E-05	Luteal	Finisher shed	7.009677	19.6	Dec
972	5.08E-05	3.32E-05	Pregnant	Finisher shed	7.009677	19.6	Dec
974	6.89E-05	0.000103	Pregnant	Dry sow shed	7.009677	19.6	Dec
976	1.85E-05	2.43E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec
977	1.46E-05	1.25E-05	Pregnant	Finisher shed	7.009677	19.6	Dec
978	0.000102	8.30E-05	Luteal	Dry sow shed	7.009677	19.6	Dec
980	1.25E-05	2.83E-05	Pregnant	Finisher shed	7.009677	19.6	Dec
988	6.12E-05	4.90E-05	Pregnant	Dry sow shed	7.009677	19.6	Dec

Table 6. The monthly pregnancy for all commercially housed sows from September 2022 – February 2023, excluding those that had less than 10 months' worth of entries. The y-axis consists of the pig ID, which referred to the ear tag number of each pig.

Pig ID	September 2022	October 2022	November 2022	December 2022	January 2023	February 2023
233	Pregnant	Pregnant	Pro-oestrus	Pro-oestrus	Pregnant	Pregnant
242	Pregnant	Pregnant	Pregnant	Lactating	Luteal	Pro-oestrus
246	Luteal	Luteal	Luteal	Luteal	Luteal	Luteal
266	Pregnant	Pregnant	Pregnant	Lactating	Oestrus	Pregnant
279	Pregnant	Lactating	Pro-oestrus	Luteal	Oestrus	Pregnant
293	Pregnant	Luteal	Luteal	Luteal	Pregnant	Pregnant
295	Lactating	Pregnant	Pregnant	Pregnant	Lactating	Lactating
296	Lactating	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
955	Luteal	Luteal	Luteal	Luteal	Luteal	Oestrus
956	Luteal	Luteal	Luteal	Pregnant	Pregnant	Pregnant
957	Luteal	Luteal	Luteal	Luteal	Luteal	Oestrus
958	Luteal	Luteal	Luteal	Luteal	Luteal	Oestrus
959	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
960	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
962	Pro-oestrus	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
964	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
968	Luteal	Luteal	Pro-oestrus	Pregnant	Pregnant	Pregnant
969	Luteal	Luteal	Luteal	Luteal	Luteal	Oestrus
970	Luteal	Luteal	Luteal	Luteal	Luteal	Oestrus
972	Luteal	Luteal	Oestrus	Pregnant	Pregnant	Pregnant
974	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
976	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
977	Luteal	Luteal	Pro-oestrus	Pregnant	Pregnant	Pregnant
978	Luteal	Luteal	Luteal	Luteal	Luteal	Pro-oestrus
980	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
988	Lactating	Pregnant	Pregnant	Pregnant	Pregnant	Pro-oestrus

Table 7. The monthly pregnancy for all commercially housed sows from March 2023 – August 2023, excluding those that had less than 10 months' worth of entries. The y-axis consists of the pig ID, which referred to the ear tag number of each pig.

Pig ID	March 2023	April 2023	May 2023	June 2023	July 2023	August 2023
233	Pregnant	Pregnant	Lactating	Pregnant	Pregnant	Pregnant
242	Luteal	Pregnant	Pregnant	Pregnant	Pregnant	Lactating
246	Pro-oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
266	Pregnant	Pregnant	Lactating	Lactating	Culled	
279	Pregnant	Pregnant	Lactating	Pregnant	Pregnant	Pregnant

293	Pregnant	Pregnant	Lactating	Luteal	Culled	
295	Pro-oestrus	Luteal	Luteal	Culled		
296	Pro-oestrus	Pregnant	Pregnant	Pregnant	Luteal	Pregnant
955	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
956	Farrowing	Pro-oestrus	Pregnant	Pregnant	Pregnant	Pregnant
957	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
958	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
959	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
960	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
962	Pro-oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
964	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
968	Lactating	Pro-oestrus	Pregnant	Pregnant	Pregnant	Lactating
969	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
970	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
972	Lactating	Pro-oestrus	Pregnant	Pregnant	Pregnant	Lactating
974	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
976	Pregnant	Pregnant	Pregnant	Pregnant	Luteal	Pregnant
977	Lactating	Pro-oestrus	Pregnant	Pregnant	Pregnant	Lactating
978	Pregnant	Pregnant	Pregnant	Lactating	Pregnant	Pregnant
980	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant
988	Oestrus	Pregnant	Pregnant	Pregnant	Lactating	Pregnant