

THESIS

THE ASSOCIATION OF LUNAR PHASES WITH PREGNANCY AT FIRST ARTIFICIAL
INSEMINATION OF DAIRY COWS

Submitted by

Margaret Schatte

Department of Animal Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2024

Master's Committee:

Advisor: Temple Grandin

Co-Advisor: Pablo Pinedo

Kayleigh Keller

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ABSTRACT

THE ASSOCIATION OF LUNAR PHASES WITH PREGNANCY AT FIRST ARTIFICIAL INSEMINATION OF DAIRY COWS

Myths and old farming legends have circulated the belief that the full moon affects livestock behavior and reproduction. To assess this association in dairy cattle, 13,558 records from 2019 to 2021 at an organic dairy farm in Colorado were analyzed. These records included lactation number, artificial insemination date (AI date), and pregnancy result. AI date was categorized into season and lunar phases. Lunar phases were separated into four equal categorizations: new moon, first quarter, full moon, and third quarter. The primary objective of this study was to identify any associations between the lunar phases and PAI1 (pregnancy rate at first AI). The secondary objective of this study was to use logistic regression to specify which phases had the lowest and greatest PAI1 while accounting for other effects on fertility. Logistic regression was used to complete this by comparing the pregnancy result of lunar phases while accounting for lactation number and season, which are known to affect pregnancy rate. The 4 lunar phase categorizations did have an association on PAI1 ($p < 0.05$). The new moon phase resulted in the lowest PAI1 at 35.3% while the third quarter was the highest at 38.3%. Estimated marginal means were explored to identify the seasonal effect on pregnancy and found that winter had the highest probability of pregnancy and summer had the lowest. This analysis of 3 years of records provides evidence that the week of the new moon is the least probable week out of the month for pregnancy after first AI to occur, while the week before and week of the full moon are the most probable days for pregnancy after first AI to occur within the lunar cycle.

ACKNOWLEDGEMENTS

Thank you to my family for supporting me in my academic endeavors and to my committee of mentors and peers that have helped me gain experience and become a more educated individual. I am appreciative of Temple Grandin for her mentorship, Pablo Pinedo for his advice and resources that made this project happen, and Kayleigh Keller for her advice and guidance. Thank you to the farmers/ranchers that allowed access to the records of dairy cows, without them this project would not be possible. Lastly a special thank you to my instructors and peers not listed in my committee that have supported me throughout this process.

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CHAPTER 1

PREFACE

Pregnancy at first AI (Artificial Insemination) or PAI1 is a difficult factor to increase and in the past the field of dairy focused on maximizing milk production. In maximizing milk production there has been a genetic trade off of other traits such as reproductive efficiency (Lucy, 2001; Pinedo and Velez, 2019). Many technologies have been developed to improve this; such as Artificial Insemination (AI), estrous synchronization hormones, sexed semen, embryo transfer (ET), and production of estrus detection tools (Allrich, 1993; Moore and Hasler, 2017; Roelofs et al., 2010; Stevenson and Britt, 2017; Thatcher, 2017; Vishwanath, 2003). In addition to these technologies researchers have identified environmental and individual components that can affect reproductive efficiency (Ferguson and Skidmore, 2013; Stevenson and Britt, 2017; Temesgen, 2022). The acknowledgement of these trends has allowed producers to manage for these effects such as adjusting diet to ensure a cow reaches ideal body condition score (BCS) in order to better conceive and produce a calf.

This thesis investigates the question: does the lunar cycle influence the ability for cows to get pregnant? This question stems from myths and legends of the lunar cycle correlating to erratic behavior and physiological effects such as untimely parturition. In fact, the word ‘lunatic’ originates from the Latin word for moon, relating to erratic behavior often associated with the full moon (Oxford Advanced American Dictionary). These legends have circulated throughout many different societies and many different periods of history. In some tales of recorded history Native Americans believe the different phases of the moon represent different entities such as the full moon being a hero and vanquishing dark spirits (Boas, 1914). In other cultures, the moon is

seen as a goddess of fertility (Giumlia-Mair, 2008). While exact beliefs of the moon may vary from culture to culture, researchers have been increasingly interested in this effect. Current findings have a range of effects on human and animal behavior and reproduction. Due to the limited publications in lunar effects on cattle, other animal species including humans should be added to the search to better interpret the effects of the lunar phases. This review will expand on current findings on animal behavior and reproduction to investigate whether there is a measurable effect.

Purpose

The purpose of this study is to identify the potential associations of lunar phases on pregnancy rate within a sample group of certified organic dairy cows.

Hypothesis

The hypothesis is that increased lunar illumination, such as the full moon, affects reproductive efficiency positively while the absence of it will decrease efficiency.

CHAPTER 2 LITERATURE REVIEW

This review will delve into the specifics of how reproduction occurs in cows and the specifics of the lunar cycle and its effects on the environment and living organisms. These topics are important to better understand the difficulty in getting a herd of cattle pregnant. Understanding this difficulty can help animal experts develop new technologies and management strategies revolutionize the reproductive industry like AI and synchronization protocols have done.

Lunar Cycle Myths and Evidence Based Effects:

Lunar Cycle:

The lunar cycle has little to no known effect on reproduction and behavior in animals. Despite this there are many other scientists that have looked at the cycle in different aspects, including astrophysicists. This topic has been researched extensively and has found many effects on the environment including moonlight intensity, water/tide levels, temperature, food availability, oxygen levels, and magnetic fields are proven to be influenced by this cycle (Andreatta and Tessmar-Raible, 2020). Further evidence suggests an influence of the lunar cycle on mechanisms such as calcium flux, melatonin disruption, magnetite and cryptochromes (Bevington 2015).

Looking closer at what this cycle is, it is the orbit of the moon around the earth and while the moon does not produce light of its own instead what is visible is the reflection of the sun's light. This illumination of the sun causes different shapes/shades of light reflected from the moon's surface. Based on the moon's position and angle from earth to the sun it creates different 8 unique phases. In the northern hemisphere, these phases go in order from the new moon, waxing crescent, first quarter, waxing gibbous, full moon, waning gibbous, third quarter, waning

crescent and repeat back to the new moon. This cycle lasts about 29.5 days in length and each waxing/waning crescent/gibbous lasts about 5-7 days whereas the new moon, first quarter, full moon, and third quarter only lasts 1 day each.

Melatonin:

Melatonin is often known as the hormone responsible for sleep. What is less known is the hormone's influence on reproduction. In fact, melatonin supplements are sold in stores. These gummies are advertised as help for falling asleep and staying asleep. However, it has many other effects on the body, humans and other species. It was originally discovered in amphibious melanocytes which is where the word melatonin originated (Lerner et al., 1958). Its function was to be activated in darkness and lighten the color of the frog, toad, or fish skin color. The world of research and understanding of melatonin has only grown since that finding. In animals it is produced in the pineal gland and can be found in many other tissues. Most recently the hormone has been found in reproductive tissues such as granulosa cells, placenta, ovaries, oocytes, and testes in multiple different animal species (Auld et al., 2017; Li et al., 2023; Olcese, 2020; Paterson and Foldes, 1994; Tosini et al., 2014). Seasonal breeders such as sheep, goats, and horses are influenced heavily by this hormone as it helps signal when the days are getting shorter and longer (Giannetto et al., 2020). Specifically, it is the pineal gland and a combination of other parts of the brain and eyes that recognize light intensity. The pineal gland uses circulating amino acid L-Tryptophan and converts it into serotonin by tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase. This process then uses serotonin's N-acetylation, catalyzed by Nacetyltransferase, and its methylation by hydroxyindoleO-methyltransferase to produce melatonin (Zhdanova and Tucci 2003). Melatonin then enters the bloodstream and moves throughout the body. Thanks to its amphiphilic nature it can enter any bodily fluid or cell. This

means it can have many functions throughout the entire body. While researchers may never truly understand every function of melatonin, the main functions found currently published can be separated into 3 categories: behavior, reproduction, and thermoregulation.

Behavior

Melatonin can slow heart rate, cause sluggish reaction times, and reduce overall motor skill function (Dawson and Encel 1993). Melatonin controls the expression of certain genes responsible for circadian rhythm. In mice, those more proficient at synthesizing melatonin were found to have accelerated activity on the wheel and other enrichment activities in the 6 hours following the onset of darkness. The group of mice that had greater proficiency of synthesizing melatonin was significantly lower in body weight, gonadal weight and reproductive performance (Zhang et al., 2021).

Reproduction

Melatonin does appear to influence reproduction. However, that effect is not fully understood, especially across species. In humans melatonin levels decrease as a woman undergoes menopause. This is thought to contribute to restlessness and problems sleeping for women with menopause (Reiter 1998). In other animal species, specifically those with breeding seasons such as sheep, melatonin is one of the most essential hormones for fertility. “In one study, luteinizing hormone (LH) secretion peaks before ovulation appeared 2–3 times more often at night than during daylight hours, whereas melatonin secretion peaked at night” (Li et al 2023). In dairy cows specifically, light or inhibition of melatonin can increase prolactin levels (increasing milk output).

Thermoregulation

Melatonin slows down heart rate and decreases core temperature. Nightmares, night sweats, and hot flashes are thought to be effects of the absence of melatonin. (Dawson and Encel 1993). While there are many pathways at play, the summary of melatonin is that it puts the body on 'auto-pilot' while sleeping. This 'auto-pilot' allows for the body to rest and refresh itself while also maintaining a good environment by flushing out harmful effects. Melatonin 'refreshes' the body by upregulating antioxidant enzymes, increasing immune system responses, creating ATP for the body to use once awake, and signals the body for preparation for reproduction. Melatonin 'flushes out' harmful effects by removing excess free radicals, excess Ca^{2+} , and prevents the mtPTP from being left open. The mtPTP is the opening that allows Ca^{2+} ions from the mitochondria to the cell and if used excessively can lead to cell death. Melatonin is eventually ended by the liver by being converted to 6-hydroxymelatonin by the P450-dependent microsomal mixed-function oxidase enzyme system. This is then either excreted out of the body through urine/feces or converted to N Acetyl-5-methoxy kynurenine and used in the central nervous system (Zhdanova and Tucci 2003).

Human Reproduction:

Throughout history, legends have been created and passed down about the moon. Some legends call the moon a goddess of fertility which begs the question is there any basis to this legend (Giumlia-Mair, 2008)?

Menstruation

To shed some light on the lunar cycle and its effect on reproduction a review was completed and found quite an extensive collection of publications on the topic. In the fall of 1977, 312 women were selected for an observational study to better understand menstruation. These women had a menstruation cycle approximately 29.5 days in length. A pattern was found for menstruation to begin in the light half of the month and ovulation to occur in the dark half of the month (Cutler, 1980). 29.5 ± 1 days was found by several studies to be the most fertile of menstrual cycle lengths (Vollman 1968, 1970, 1977; Treloar et al. 1967). In a multiyear study (1976-1977, 1979, and 1983) of 229 women with menstruation cycles approximately 29.5 days in length, a kernel density evaluation was plotted to show a trend of menstruation cycles likely to begin on or around the full moon (Cutler et al., 1987). In a different population of 826 females from ages 16-25, 28% of the population menstruated during the new moon (Law, 1986). No pattern was found for women with menstruation cycles longer or shorter than 29.5 days in length (Cutler, 1980). No pattern was found between the menstruation cycle and the lunar cycle in 529 women. However, there appeared to be a trend between sleep quality where better sleep occurred in women whose menstruation cycles began in the light half of the lunar cycle (Komada et al., 2021). Wehr and Helfrich-Förster proposed the idea that current aggregate analysis' had led to false negative findings and longitudinal observations would better identify the effects (2021). Based on Wehr and Helfrich-Förster recent findings it calls into question any absence of trend and points towards synchronization of menstruation of any type of lunar cycle or phase.

Birthing and Fertility

Continuing in the theory of the lunar cycle and its connection to reproduction fertility and birthing frequency was reviewed and did have quite a collection of publications on the topic. Multiple studies found a peak in fertility around the 3rd lunar quarter (Criss and Marcum, 1981

and Guillon et al., 1986). The 3rd quarter of the lunar cycle is the phase between the full moon and new moon. In a study looking at both menstruation and ovulation women tended to ovulate during the dark phase of the lunar cycle (Cutler et al., 1980). Through these findings it can be concluded that ovulation was most likely to occur during the period of the lunar cycle going from light to dark.

However, the literature includes very mixed results about birth frequency and its response to the lunar cycle. One study found that more births between the last quarter and the new moon and fewer births in the first quarter of the moon cycle (Guillon et al., 1986). Yet another study found opposing results in the form of more frequent births around the full moon (Ghiandoni et al., 1998). Multiple studies found no statistical trend in births (Arliss et al., 2005, and Chakraborty, 2013). Through these findings it is likely a safe assumption that the lunar cycle has no statistically significant effect on human birth frequency.

Human Behavior:

As mentioned previously, there are long standing myths circulating that the full moon causes erratic behavior. To conclude if these myths had any substantial evidence behind it, current publications were reviewed and overall found some research supporting while others disproved the belief; The crime rate in three different towns was found to be significantly higher on days with the full moon (Thakur and Sharma, 1984). Accidents were found to be highest just before the full moon and lowest on the full moon (Alonso, 1993). Multiple analyses found no effect of the lunar cycle on behavior, behavior included homicides, other criminal offenses, mental hospital admissions, and psychiatric disturbances; crisis calls, panic attacks, anxiety or mood

disorders and suicidal thoughts (Rotton and Kelly, 1985 and Roy et al., 2017). These findings have mixed results and cannot conclude any clear answer.

Animal Reproduction:

Horses

Horses are long day seasonal breeders meaning they rely on the pineal gland to recognize the shortened duration of melatonin synthesis as the longer days begin in the spring (Cleaver et al., 1991). This light cycle is synthesized by breeders starting as early as November (Palmer and Guillaume, 1992). This begs the question, does lunar light play an effect on this in a natural environmental setting? To understand this an analysis was conducted through a stud book totaling to 9 years of data and 1,927 mating dates. This analysis found a peak of conceptions and conception rate just after the full moon (Kollerstrom and Power 2000). In an analysis of 4,891 thoroughbred mares over 14 years, estrus was found most likely to occur at the full moon and for a week after. Conception rate per coverings was highest at the full moon and 3.8% higher compared to the new moon. Breaking the data down further into 'nodes' where the ecliptic lunar orbit plane intersects. Low nodes were determined as the Sun-node angle between 0–45° where 1,780 coverings found no effect on conception rate. High nodes were determined as the Sun-node angle >45° where 1,646 coverings found the highest fertility percentage just after the full moon (Kollerstrom, 2004). These findings point strongly in the direction that the lunar cycle influences estrus and fertility in horses.

Sheep and goats

Like horses, sheep and goats are daylight breeders. However instead of breeding in the spring when daylight increases, sheep breed in the fall when daylight decreases (Matthews et al., 1993). This light effect begs the question, would lunar light have any influence on this? In a 2 year project looking at the lunar cycle effects on 2724 AIs moon phases and type of oestrus did appear to have an effect on fertility. Fertility rate was found to be highest at the new moon and lowest at the crescent phase (Palacios and Abecia, 2014). In a study looking at the effect of lunar phases on goat reproductive performance, goats were split into three groups based on the lunar phases on their date of birth. Goats born on the full moon phase exhibited estrus signs earlier, had greater progesterone at ages 26-27 weeks, and greater values of cervical mucus indicating earlier age of puberty onset (El-Darawany et al., 2021).

Cattle

While cattle are not daylight breeders melatonin, the major influential hormone on daylight breeders, does appear to play some roles on their reproductive mechanisms (Li et al., 2023). This then begs the question does the lunar cycle play a part in influencing reproduction? In a retrospective study examining 2,051 tropical brahman crossbred cows over a 6 year period, conception rate was found to be highest two weeks after the full moon. However this was not the only finding in this study, they also found a dramatic increase in pregnancy, estrus, and calving frequency in the new moon and full moon days of the lunar cycle (Aguirre et al., 2021). In an observational study examining calving in Holstein dairy cattle, they found a significant p-value of 0.0006 that deliveries were highest in frequency from the waxing gibbous to the full moon phases (Yonezawa et al., 2016). Overall it does appear that the lunar cycle has an association with reproduction in cows.

Cattle Reproduction (Non-Lunar Effects):

Agriculture is an essential work force. Cattle specifically are one of the few business commodities that rely on reproduction for financial success. Whether the product is beef or dairy a successful pregnancy is required to produce a calf crop, milk production, or replacement heifers. Therefore the process of reproduction should be researched and understood to construct ideal management practices.

The Estrous Cycle

1. Proestrus

Puberty begins with the maturation of the hypothalamus and the readiness of the individual's body (usually occurring at 45-50% mature liveweight) (Garcia-Muniz et al. 1998; Hancock et al., 2019; McNaughton et al. 2002). Once the hypothalamus reaches maturity Gonadotropin-Releasing Hormone (GnRH) is secreted. This hormone then stimulates the anterior pituitary gland to release Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). These hormones or gonadotropins are essential to the reproductive process as FSH stimulates ovarian follicle growth. As the follicle grows it secretes increasing amounts of estrogen. Estrogen then circulates throughout the plasma in the reproductive tract to prepare for ovulation and copulation. The period of time where estrogen and FSH continue to surge is known as proestrus and ends when the primary estrogen hormone, estradiol, reaches peak levels (Allrich, 1993).

2. Estrus

This new period is called estrus or heat and can be visually identified through mucus discharge of the vulva and behaviors such as sniffing the vulva of another cow, licking, flehmen, rubbing, restlessness, and most commonly shown are mounting behaviors including standing while being

mounted and mounting other cows. This peak of estrogen stimulates a surge of LH in the anterior pituitary which makes its way back to the follicle and triggers ovulation, or the release of the egg from the dominant follicle (Roelofs, 2010). (Allrich, 1993)(Thatcher, 2017). This preovulatory phase is the best indication for producers to decide when to breed as behaviors and other visual signs can be seen.

3. Metestrus

Once ovulation begins the egg transforms into the Corpus Luteum (CL). This structure produces several hormones to help maintain the uterine environment including progesterone, prostaglandin, and Prostaglandin-F2 alpha (PGF2 α). (Hansel and Convey, 1983).

4. Diestrus

Lastly and most importantly for reproductive success is diestrus. This is the prolonged maturity of the CL and plateau of progesterone and prostaglandin hormones. This part can either continue on for the length of a pregnancy if fertilized. If not fertilized, or at the end of the pregnancy the CL will be activated to regress, likely by oxytocin (Hansel and Convey, 1983);).

Copulation and Insemination

Reproduction is an essential component to the entire dairy industry. Poor reproductive efficiency can result in a lower number of cows at their peak production period, delayed genetic progress, increased rates of culling, increased cost for bull management and/or increased costs in inseminations (Norman et al., 2009; Santos et al., 2010; Pinedo et al., 2010). Moreover, without reproduction the entire product (milk, beef, and replacement heifers) would not be possible.

In order to achieve pregnancy, the cow must be fertilized or given an Embryo Transfer.

Fertilization can occur multiple ways with current technology. The original way is copulation or

natural breeding where a bull mounts and deposits sperm within the uterus of the cow. This sperm must then make the journey to the egg. The alternative way to this is Artificial Insemination (AI). AI was developed centuries ago and has been commercially available for nearly 100 years now. This process begins with the collection of bull sperm, which is usually then evaluated and packaged into straws. These straws can then either be used immediately, refrigerated, or frozen. These straws are used by being placed through the vulva, vagina, cervix, and deposited directly into the back of the uterus with a guiding hand in the anal cavity (Moore and Hasler, 2017; Vishwanath, 2003). AI is the method of choice for most dairies as it allows for genetic trait selection, reduction of disease transmission, deposition closer to the egg for greater fertility, evaluation of sperm before insemination, and decreased/eliminated cost of bull maintenance (Lucy, 2001).

Fertility is the cow's ability to conceive or likelihood that the egg will be fertilized after copulation or insemination. This can be influenced by environmental and individual factors. Individual factors include genetics, health before adulthood, BCS, disease, age, number of previous calves, and days since previous lactation. Environmental factors include cow housing and comfort, nutritional management, management of the transition period, season, weather, and sanitation levels with the surrounding environment (Lucy, 2001; Lean et al., 2016).

Organic vs. Conventional

With the rising popularity of consumers wanting organic foods, producers have answered the call with increasing numbers of organic dairies and subsequent products (Dimitri and Nehring, 2022). Unfortunately, this product does not come easily as there are many more regulations in order to be certified organic. These dairy cows must have access to the outdoors, shade, fresh water,

exercise areas, proper sanitation, adequate ventilation, organic feed/hay, and direct sunlight year-round. Additionally, cows must be on grazing pasture for no less than 120 days of the year.

These requirements are just the start as there are many more prohibitions that separate certified organic from conventional dairies. Organic dairies cannot use drugs to promote growth, breeding hormones such as $PGF_{2\alpha}$, or use antibiotics without transferring the animal to conventional management (USDA). These challenges have caused some undesired effects unique to organic management; lower levels of milk production, smaller herd sizes, and decreased fertility (Pinedo & Velez, 2019).

CONCLUSION

Dairy cattle reproduction is a vast topic, and while many advancements have been made in the field, there are some areas still left unexplored. The lunar cycle and its effects on reproduction are one of those unexplored topics. The lunar cycle has many known effects including raising/lowering of tides, moonlight intensity, melatonin disruption, temperature, food availability, oxygen levels, calcium levels, and magnetic fields. With this many effects on the environment it begs the question, what effect does it have on an individual organism?

With only a few publications on the topic of the lunar cycle in dairy, a wider search to other large animal species was necessary. Subsequent findings concluded that the days following the full moon correlated to higher fertility in horses, sheep had greater fertility around the new moon, and goats born during the full moon had greater reproductive performance later in life. No trend was found for frequency of births in humans. However, cattle did appear to have a higher frequency of deliveries directly after the full moon. Most findings among the research of full moon effects were based upon human populations. These publications provided many different results for whether the lunar cycle affected behavior or birthing frequency. Therefore, no conclusion can be made about the effect of the lunar cycle on these factors in humans. The one factor that did in fact have a substantial trend in humans was menstruation and ovulation. Several studies found that women who have a menstruation cycle lasting about the same length as the lunar cycle (29.5 days) will often synchronize to ovulating towards the third quarter while menstruation tends to begin near the full moon, or the lightest part of the lunar cycle.

CHAPTER 3

THE ASSOCIATION OF LUNAR PHASES WITH PREGNANCY AT FIRST ARTIFICIAL INSEMINATION OF DAIRY COWS

INTRODUCTION

One imperfect statistic in the world of animal science is the pregnancy rate, or the percentage of animals in a group that become pregnant after being bred. This study will examine this in dairy cows using pregnancy rate after first AI (PAI1). Pregnancy rate in dairy cattle to average between 45 - 60% (Lamb et al., 2010, McWhorter et al., 2020, Moore and Hasler 2017, Norman et al., 2009, Norman et al., 2011, Temesgan et al., 2022), while beef pregnancy rate is often greater at 50 - 65% (Echternkamp and Thallman 2014, Bormann et al., 2006). This is measured using palpation or ultrasound after breeding occurs. Artificial insemination or AI is an important tool that over 80% of dairies use (Cozalo and Mapletoft, 2014).

The lunar cycle is about 29.5 days long, and can be recognized as 8 separate phases; new moon, waxing crescent, first quarter, waxing gibbous, full moon, waning gibbous, third quarter, and waning crescent (Arliss 2005). These phases are based on the position of the moon, earth, and sun. The moon reflects the sun's light differently during these phases. The moon is the brightest at the full moon and darkest during the new moon. This cycle is associated with menstruation and ovulation in humans (Criss and Marcum, 1981; Cutler, 1980; Guillon et al., 1986; Law, 1986; Wehr and Helfrich-Förster, 2021).

Animal species such as horses, insects, fish, and birds do have a proven association between the lunar cycle and reproductive behaviors (Kollerstrom and Power 2000; Kollerstrom, 2000; Zimecki 2006). Multiple studies have concluded that lunar phases do have an effect in cattle reproduction. Phases just before and during the full moon correlated to higher frequency of

calving in cows (Yonezawa et al., 2016). A separate study expanded on this in beef cows and found the full moon and new moon phases correlated to a spiked increase in calving frequency, estrus frequency, and pregnancy rate. To further examine pregnancy rate, researchers categorized the lunar cycle into 4 weeks: first quarter (week following new moon), second quarter (week before full moon), third quarter (week following full moon), and fourth quarter (week before new moon). The results concluded that the week after the new moon had the lowest pregnancy rate while the two weeks going from full to new moon (third and fourth quarter) had the highest pregnancy rate (Aguirre et al., 2021). Based on these findings, the expected hypothesis is to find that the days after the new moon will be lowest in PAI1 and the days after the full moon to be highest. In order to better understand the association of the lunar cycle with PAI1, other known effects must be taken into account in order to isolate the specific outcome in question.

The primary objective of this study is to identify any association between the lunar phases and PAI1 in dairy cows. The secondary objective of this study is to use logistic regression to specify which phases have the worst and greatest PAI1 while accounting for other effects. Identifying what phases associate with an increase or decrease in PAI1 suggests an influence of the lunar cycle on fertility but needs further exploration to discover the exact mechanisms. More research can provide a better understanding of the factors that may influence fertility and ultimately suggest different management practices to increase the pregnancy rate.

MATERIALS AND METHODS

ENVIRONMENT

This observational study used existing records from an organic dairy in Northern Colorado. Records were limited to cows, meaning no heifers were used. Per USDA guidelines organic dairy cows older than 6 months must have access to the outdoors and spend at least 120 days on grazing pasture. Therefore, these cows have regular exposure to the elements including temperature, daylight, and lunar cycle. Another requirement from the USDA for the product to be considered organic is access to shelter. This dairy provides cover from the elements through open walled but roofed barns with free stalls, so even in their covered housing they are exposed to temperature, daylight, and the lunar cycle. Cows are kept in groups to allow for normal social behavior such as estrus. Estrus detection is essential for organic dairies as no synchronization hormones are allowed (USDA). Cows are inseminated through deposition of thawed frozen semen to the uterus through straws by trained employees after estrus is detected.

DATA RECORDS

Observational records collected from a certified organic dairy were used in this study (see Appendix A). Data used included 72,353 records of cows subjected to artificial insemination (AI) from January 2019 to December 2021. Cows were inseminated through deposition of thawed frozen semen to the uterus through straws by trained employees after estrus was detected. Approximately 55 days after insemination, cows were rectally palpated to check for pregnancy. Records were limited to the first AI, meaning if the first insemination did not result in pregnancy at time of palpation they were recorded as not pregnant, and no further records were analyzed. Cows that repeated in years during this study were selected for only the first year recorded. For

example, if a cow appeared in all three years (2019, 2020, and 2021) only the first year (2019) was analyzed. These limitations narrowed the records from 72,353 to 13,558.

Records included cow ID, date AI, number of previous lactations, and outcome of pregnancy (pregnant or not pregnant). Date AI was then linked to two categories of lunar cycles (4 equal phases and 8 specific phases) and season. 4 phase categorization was sorted as new moon, first quarter, full moon, and third quarter as seen below:

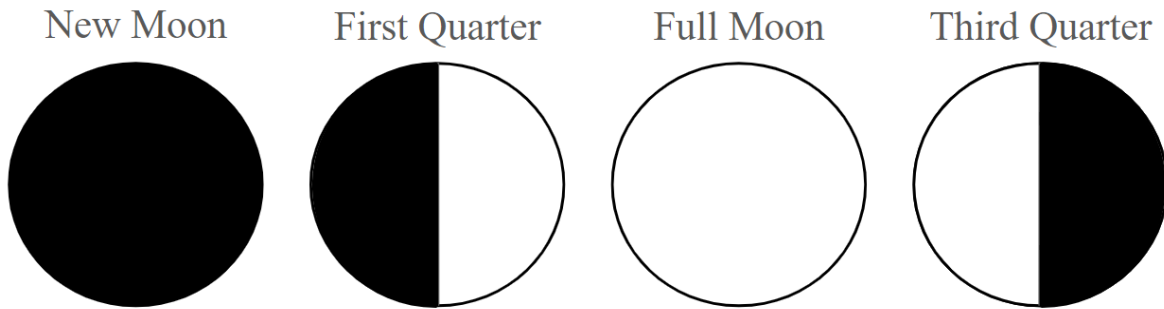


Figure 1: Depicts the moon shape appearing illuminated. Each phase begins on the day the moon appears in the following shape and ends when the next shape appears. Each phase is approximately 7.375 days or 5-9 days in length and repeats every 29.5 for the span of the study (2019-2021).

These 4 phases began on the day of the lunar phase (new moon, first quarter, full moon, and third quarter) and ended the day before the next lunar phase.

The 8 day categorization was sorted into new moon, waxing crescent, first quarter, waxing gibbous, full moon, waning gibbous, third quarter, and waning crescent. It is important to note that the new moon, first quarter, full moon, and third quarter phases are only one day long whereas the waxing/waning gibbous/crescent phases can vary between 5-8 days or roughly one week. The waxing phases refer to the period from new moon to full moon, or dark to light.

Waning refers to the opposite, full to new or light to dark. Crescent phases refer to the smaller shape of the light part of the moon where it is smaller than a half moon or from third quarter to

first quarter. Gibbous phases refer to the larger shape of the moon where it is larger than a half moon or from first quarter to third quarter.

Season of AI date was sorted into either Spring (March, April, May), Summer (June, July, August), Fall (September, October, November), or Winter (December, January, February).

STATISTICAL ANALYSIS

Sample size calculations were performed using the data analysis application SAS Power and Sample Size (release 9.4; SAS, Inst. Inc., Cary, NC). The sample size was calculated anticipating the potential positive impact of lunar phases that would result in improved fertility. Based on previous data, the anticipated proportion of pregnant cows in the CON group was set at 45% (Temesgen, 2022). Considering power = 80% and confidence = 95%, the number of cows required to show a significant difference between the groups was 392 cows per group, as determined by a two-sided hypothesis test.

Pregnancy status (yes or no) was analyzed using logistic regression with lunar phase as the independent variable of interest, and season and lactation number as adjustment factors. This was completed using R version 4.2.2. Additionally the two lunar phase categorizations were run separately to determine which specific phases and which weeks of the lunar cycle have the largest effect. The regression coefficients were exponentiated to obtain the odds ratios.

Estimated Marginal Means were used to compare the pregnancy rate among lunar phases and seasons.

$$f(z) = \frac{1}{1+e^{-z}}$$

The logistic function equation models the probability of a dependent variable belonging to a certain class. In this case pregnancy result after first AI is the dependent variable.

$$z = \beta_0 + \beta_1 \cdot x_1 + \beta_2 \cdot x_2 + \dots + \beta_n \cdot x_n$$

β_0 = intercept

β_1 = coefficient of predictor variable

x_1 = predictor variable (lunar phase)

The linear combination of predictors is the sum of variables and the intercept. This sum can then be used further into the logistic function equation to better understand the variable in question.

RESULTS

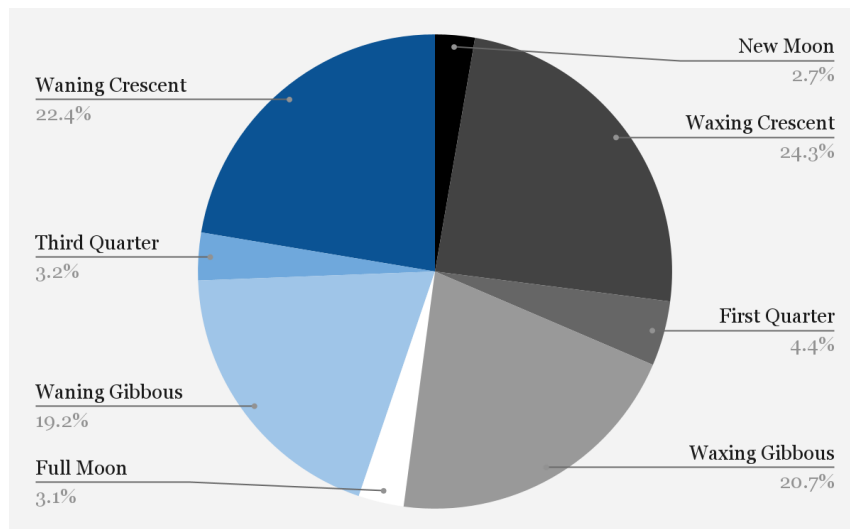


Figure 2: Distribution of AI dates. New moon, First Quarter, Full Moon, and Third Quarter only last one day in length each. Waxing and waning crescent and gibbous lasts approximately one week in length.

Estimated Marginal Means

Pregnancy Rate was calculated using estimated marginal means for the different lunar categorizations and for seasons. 4 Lunar phase categorization found that the week of the new moon had the smallest pregnancy rate and the week of the third quarter had the largest (as seen below in Figure 2).

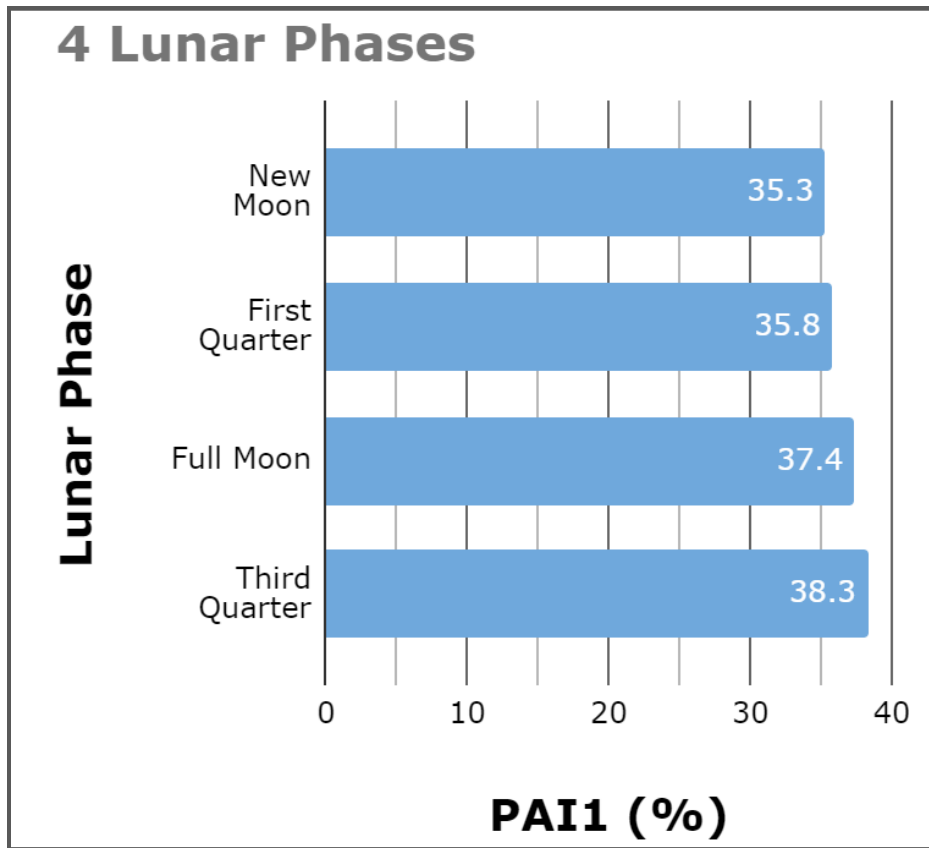


Figure 3: Average pregnancy rate for each week of the 4 lunar phase categorization.

8 lunar phase categorization found the new moon and the period of time directly after the new moon had the lowest pregnancy rate (as seen in figure 3). The phases from the waxing gibbous to the waning crescent, or the days surrounding the full moon had the highest rate of pregnancy.

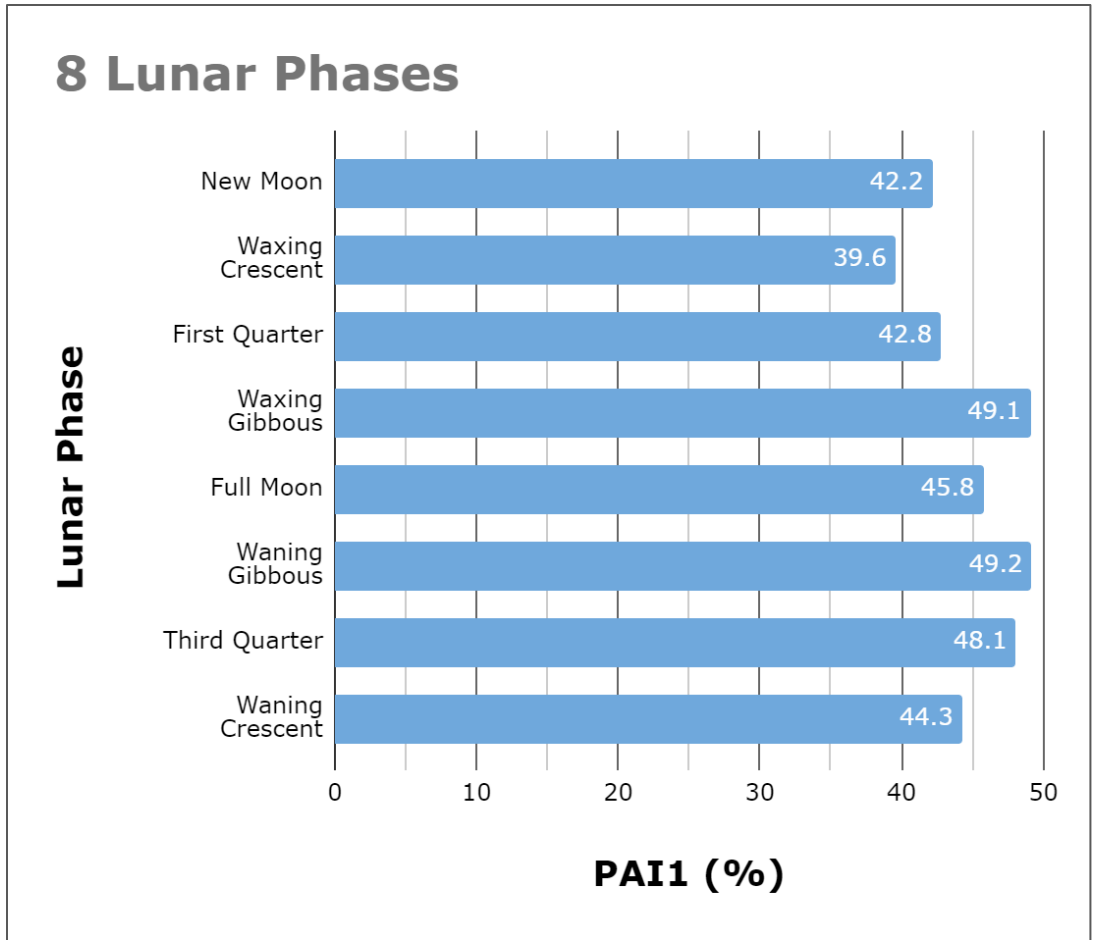


Figure 4: Average pregnancy rate for each phase of the 8 Lunar phase categorization

Seasonal results found lowest pregnancy rate during the summer season and highest in the winter (as seen in figure 4).

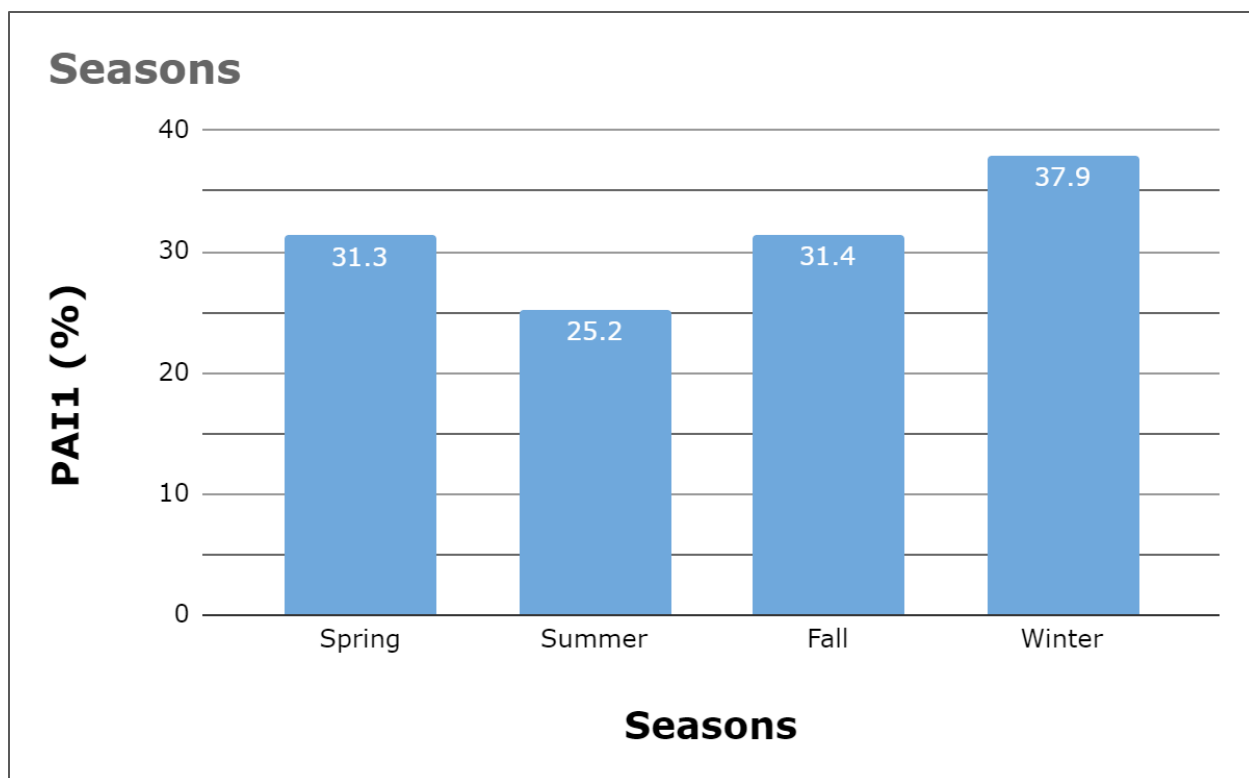


Figure 5: Average PAI1 rate in % for each season

Logistic regression

Based on previous literature the new moon likely would be lowest in pregnancy rate so the new moon was set as the main reference comparison for odds ratios (See figure 5). The week of the first quarter had the highest probability of pregnancy followed closely by the full moon, when adjusting for lactation number and season. The full moon had tendency to have a greater probability of pregnancy to occur than the new but the third quarter was the only statistically different result.

Lunar Phase	n	Odds Ratio	p-value	CI low	CI high
New Moon	3451	reference	reference	reference	reference
First Quarter	3308	1.017	0.742	0.920	1.124
Full Moon	3340	1.099	0.063	0.995	1.214
Third Quarter	3459	1.143	0.008	1.035	1.262

Figure 5: Odds Ratio comparative to the new moon within the 4 lunar phases categorization.

Within the eight phase categorization the new moon was not the lowest, instead it was the waxing crescent, or the days directly following the new moon (see figure 6). The highest odds ratio was in the third quarter at 1.46, meaning that a cow bred within the third quarter has 46% higher odds of becoming pregnant than a cow bred during the new moon. While the waning crescent and full moon trend larger than the new moon, they are not as significantly different as other phases. The waxing gibbous, waning gibbous, and third quarter are statistically different.

Lunar Phase	Odds Ratio	p-value	CI low	CI high
New Moon	reference	reference	reference	reference
Waxing Crescent	0.95	0.646	0.77	1.18
First Quarter	1.03	0.806	0.80	1.34
Waxing Gibbous	1.39	0.003	1.12	1.74
Full Moon	1.19	0.232	0.90	1.58
Waning Gibbous	1.32	0.013	1.06	1.64
Third Quarter	1.46	0.008	1.11	1.93
Waning Crescent	1.15	0.207	0.93	1.43

Figure 6: Odds Ratio comparative to the new moon within the 8 lunar phases categorization

CONCLUSION

These findings support the previous literature that pregnancy rate increases from the week after the new moon until the week before the new moon (Aguirre et al., 2021). Overall pregnancy rate was highest during the light half of the lunar cycle, or days surrounding the full moon, and the days directly after the new moon had the lowest pregnancy rate. This suggests the absence of lunar illumination during the lunar cycle affects the pregnancy rate of these cows negatively. Based on this result producers should be warned to breed directly after the new moon as this period of the lunar cycle has the lowest likelihood that the cow will become pregnant. The third quarter, or period between the full moon and new moon had the greatest pregnancy rate suggesting that the light of the lunar cycle, likely from the full moon occurring days previously, does indeed effect fertility positively. While the third quarter had the highest pregnancy rate, the phases from the waxing gibbous to the third quarter had more elevated levels of pregnancy rate compared to all other phases. This suggests that the days surrounding the full moon are the optimal time to breed as this is the most likely period of time during the lunar cycle which the cow will become pregnant.

DISCUSSION

Heifers were excluded from this study because previous publications have established that cows have an increased conception rate compared to heifers (Norman et al 2011). Therefore, excluding heifers eliminates another predictor variable that must be accounted for. Additionally, organic cows were selected for this study as there are no synchronization hormones that would affect the investigation of lunar cycle effects. This specific dairy only uses AI, meaning no bulls are around the cows at time of covering. This ensures that the cows can only become pregnant from the insemination date recorded.

Known influences of pregnancy rate include environmental and individual factors; cow housing and comfort, nutritional management, management of the transition period, season, weather, sanitation levels with the surrounding environment, genetics, health before adulthood, BCS, disease, age, number of previous calves, and days since previous lactation (Lucy, 2001; Lean et al., 2016; Sönmez et al., 2005). It can be incredibly difficult to determine all of these factors for one cow, let alone an entire sample size. Therefore, only factors previously recorded by the dairy were accounted for. In this case lactation number and season were the only recorded factors.

As no live animals were used, these records are dependent on the quality of the observers' observations. Additionally, only one dairy was used for obtaining records, so further research should explore the difference among different farms. As this dairy used only organic qualifications, meaning no synchronization protocols, and complete access to the outdoors, further research should look into the effect of shelters and timed AI around different lunar phases to expand on these findings.

Pregnancy was determined as the outcome of interest, since it is a binary variable (0=not pregnant, 1=pregnant) only a few statistical tests were appropriate for the data set. Logistic

Regression was chosen as it needed to account for multiple predictor variables and use a categorical variable as an outcome.

Statement of bias

The author(s) have no potential bias towards research or authorship. No live animals were used in this study, only records previously recorded were analyzed.

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APPENDIX A

#5679 - Lunar Effects on Conception Rate in Dairy Cattle

Protocol Information

Review Type	Status	Approval Date	Continuing Review Date
Designated Member Review	Approved	Apr 08, 2024	--
Expiration Date	Initial Approval Date	Initial Review Type	
--	Apr 08, 2024	Designated Member Review	

Feedback

Approval Comment

CSU IACUC has completed its review of this protocol and based on the information provided, an IACUC protocol is not required. Any additional changes to this project must be submitted as an amendment request for review to confirm the IACUC Waiver is still applicable, prior to implementation.
Thank you and good luck with your study.