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Enhancing Reproductive Efficiency in Dairy Cattle Through Timed AI Programs and Genomic Predictions

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Author

melo, delaine braulio de

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A Review of the Current Literature Enhancing Reproductive Efficiency in Dairy Cattle Through Timed AI Programs and Genomic Predictions

By

DELAINE BRAULIO DE MELO
THESIS

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Approved:

Dr. Fabio Lima, Chair

Dr. Anna Denicol

Dr. Richard Pereira

Committee in Charge

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List of Abbreviations

AAM – automated activity monitoring

AI – artificial insemination

AIE – artificial insemination at estrus detection

BCS – body condition score

CL – corpus luteum

DF – dominant follicle

DIM – days in milk

E2 – estrogen

FSH – follicle-stimulating hormone

GCCR – genomic cow conception rate

GDPR – genomic daughter pregnancy rate

GnRH – gonadotropin-releasing hormone

G1 – first gonadotropin-releasing hormone treatment of the OvSynch protocol

HCR – heifer conception rate

High – P4 concentrations at Day 0 High (> 3ng/mL) of 6-day CoSynch plus P4 device program

ID – identification number

LH – luteinizing hormones

Long – long reproductive program

Low – P4 concentrations at Day 0 Low (≤ 3ng/mL) 6-day CoSynch plus P4 device program

NSFC – numbers of services for conception

PGF$_{2\alpha}$ – prostaglandin F$_{2\alpha}$

PL – pregnancy loss

P/AI – pregnancy per artificial insemination

P1 – pregnancy at the first service

P4 – progesterone

Q1 – highest genomic quartile group

Q4 – lowest genomic quartile group

RepP – reproductive programs

Short – short reproductive program

TAI – timed artificial insemination

TP1 – days from calving to the first service

VWP – voluntary waiting period

2CC– 100 µg of GnRH as gonadorelin hydrochloride

4CC– 200 µg of GnRH as gonadorelin hydrochloride

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Abstract

Recent advancements in the reproductive management of dairy cattle highlight the integration of multiple disciplines, including physiology, management, nutrition, genetics, economics, veterinary herd health, and production medicine, to improve reproductive performance. Generation of timely and cost-effective pregnancies is a key economic driver in dairy herds, influencing milk yield, income over feed cost, and culling decisions. Artificial insemination **(AI)** accelerates genetic progress, controls venereal diseases, and ensures safety. Estrous cycle synchronization programs, particularly timed AI **(TAI)**, have emerged as cost-effective solution to improve pregnancy rates and reducing estrus detection needs. Despite the development of numerous controlled breeding programs, a thorough understanding of estrous cycle physiology and follicular wave dynamics is essential. Different synchronization programs enhance insemination and pregnancy rates in dairy cows and heifers. Genetic selection for reproductive traits has improved breeding strategies by emphasizing both productive and reproductive traits. Improved understanding of hormonal influences, estrous synchronization, and genetic selection has significantly enhanced reproductive performance. The goals of this thesis are to: 1) review the literature on the current status of knowledge of factors impacting effective reproductive management strategies and hormonal manipulations in dairy cattle; 2) assess the impact of increasing the dose of GnRH at the beginning of a CIDR Synch to improve ovulation and pregnancy in dairy heifers, and 3) to compare key reproductive outcomes in two reproductive programs with variable estrus detection length followed by OvSynch and to assess their relationship with the fertility traits genomic prediction for daughter pregnancy rate **(GDPR)** and the genomic prediction for cow conception rate **(GCCR)** in lactating Holstein dairy cows.

Chapter 1 - Introduction

Reproductive management in dairy cattle has undergone significant changes, with researchers now deeply exploring the reproductive physiology of dairy cows and heifers. These improvements encompass a variety of disciplines integral to dairy farming, such as physiology, management, nutrition, genetics, economics, veterinary herd health, and production medicine. Integrative strategies are essential for achieving optimal fertility in lactating dairy cows and enhancing overall reproductive performance in herds. Aligning with industry standards and emerging market trends is critical for ensuring efficient reproductive management and sustainability in dairy cattle. (Thatcher and Santos, 2020).

Reproductive performance is a vital component of any livestock operation, with the success of dairy operations closely tied to economic outcomes. Failures in reproductive management can significantly impact crucial factors such as average milk yield, income over feed costs, and culling decisions, ultimately affecting the overall profitability of dairy herds. (Giordano et al., 2012b; Galvão et al., 2013). In the context of dairy farming, economic profitability is heavily reliant on enhancing the reproductive efficiency of dairy cows (Cabrera, 2014). A decline in fertility, which adversely affects milk production, can be attributed to various factors. These include inadequate estrous detection, suboptimal housing systems, herd size challenges, nutritional mismanagement, and reproductive diseases (Giordano et al., 2012b).

The productive lifespan of an adult cow is a crucial factor in dairy management. Optimizing this duration extends the most productive phase of their life cycle, enhancing overall farm efficiency and profitability. (Pecsok et al., 1994; Ferguson and Galligan, 1999). Therefore, it is essential to optimize pregnancy outcomes in dairy cattle to align their life cycle with the best predicted window for productive performance. The use of artificial insemination **(AI)** is known to accelerate genetic progress, control venereal diseases, and ensure the safety of cows and farm personnel compared to reproductive programs that rely on natural services (Champagne et al., 2002; Lima et al., 2009). Over the past 30 years, estrous cycle synchronization and timed artificial insemination **(TAI)** programs have emerged as pivotal strategies in dairy management. These programs have enabled farmers to achieve optimal reproductive performance, significantly enhancing conception rates.

Moreover, TAI has been identified as a cost-effective solution for managing reproduction, especially in high-producing dairy cows that exhibit reduced signs of estrus (Risco et al., 1998; Lima et al., 2010). Timed AI programs were developed to assist in obtaining pregnancies in groups of cows, either restricting the intervals during which estrus detection needs to be performed or eliminating the need for estrus detection **(ED)**. Although there are numerous TAI programs in beef and dairy herds, a thorough understanding of the physiology of the estrous cycle and follicular wave dynamics in particular, is necessary before attempting manipulation of the estrous cycle. (Colazo and Mapletoft, 2014).

A variety of estrus and ovulation synchronization programs have been designed and applied to improve insemination and pregnancy rates in dairy cows (Bisinotto et al., 2014; Borchardt et al., 2018; Stevenson et al., 2018) and dairy heifers (Lima et al., 2013; Silva et al., 2015; Colazo and Mapletoft, 2017; Karakaya-Bilen et al., 2019). Despite considerable progress in understanding the physiology of follicular development in cattle, there remain questions and pitfalls in controlling the normal growth of ovulatory follicles in high-producing dairy cows (Mohammadi et al., 2024). Physiological differences between cows and heifers have been widely reported in several studies. While a great percentage of heifers exhibit three follicular waves, dairy cows tend to have two follicular waves per cycle (Lindley et al., 2021). Moreover, discrepancies between the sizes of follicles and corpus luteum **(CL)** and serum steroid concentrations between dairy cows and heifers are another physiological difference that needs to be considered (Sartori et al., 2004). For example, lactating cows have greater steroid metabolism and clearance than dairy heifers (Sartori et al., 2004; Lopez et al., 2005a; Lopez et al., 2005b). Depending on the number of waves that occur in the cycle, the length of the cycle itself will vary, and the variable period of follicular turnover will require the use of different programs (Kasimanickam and Kasimanickam, 2021). Indeed, the latter is a major difference that has supported the use of specific programs for dairy heifers (Lima et al., 2011, 2013).

Another physiological aspect that impacts gonadotropin-releasing hormone **(GnRH)** induced ovulation at the beginning of estrous synchronization programs is the plasma concentration of progesterone **(P4)**. Studies have shown that higher P4 concentrations are consistently associated with lower plasma concentrations of luteinizing hormone **(LH)** in response to GnRH (Giordano et al., 2012a; Lima et al., 2013; Batista et al., 2017). The P4 environment is thus a crucial component of the hormonal landscape that determines the GnRH response and influences the ovulatory potential of the treated animal. Interestingly, it was reported that a double dose of GnRH (200 μg vs. the standard 100 μg) increased the plasma concentration of LH released (Giordano et al., 2012a). Therefore, optimizing hormonal treatments and understanding the underlying physiological mechanisms can significantly enhance reproductive performance in dairy cattle.

Selection for reproductive traits, such as genomic daughter pregnancy rate **(GDPR)** and heifer conception rate **(HCR)**, reshaped breeding strategies within the dairy community, underscoring the increasing emphasis on selecting for both productive and reproductive traits

(VanRaden et al., 2003; Council on Dairy Cattle Breeding, 2018). A positive correlation exists between cows in the highest quartiles for GDPR and the first AI service pregnancy success rates (Veronese et al., 2019; Lima et al., 2020), and also exhibit increased odds of estrous expression and lower odds of pregnancy loss **(PL)**, indicating broader genomic trait influences (Madureira et al., 2022).

The increased use of Timed AI programs addresses failures in ED (Chebel et al., 2010) and employs ovulation synchronization to facilitate AI without relying on visible estrus signs (McArt et al., 2010). However, concerns about extensive hormonal treatments have grown. Automated activity monitoring **(AAM)** systems enhance reproductive performance by detecting estrus through increased activity levels (Fricke et al., 2014a; Borchardt et al., 2024).

Higher GDPR scores correlate with more prolonged estrous expression (Lima et al., 2020), improving detection by visual observation or AAM systems, potentially leading to better reproductive outcomes.

Despite the substantial advancements observed in the past 2 decades of programs for synchronization of ovulation and timed AI, refining ED remains a major priority for dairy operations (Marques et al., 2020). Precisely detecting estrus plays a pivotal role in a successful and profitable dairy program. Inadequate or inaccurate detection of this crucial phase is often the root cause of suboptimal reproductive performance in dairy cows (Roelofs et al., 2010; Senger, 2010). Developing strategies to enhance estrous detection programs becomes exceedingly important given their widespread utilization.

Improved understanding of hormonal influences and estrous synchronization has enhanced reproductive performance. Genetic selection for traits like GDPR and GCCR has also improved fertility outcomes. However, refining estrous detection and combining it with TAI programs remains critical for maximizing reproductive efficiency and profitability.

Chapter 2 - Literature Review

1- Estrous Cycle

a. General Concepts

Dairy cows need to become pregnant and give birth to initiate lactation, and ideally, they should be able to conceive multiple times throughout their lives to maximize milk production, reduce the labor and costs involved in replacing departing herd members, and enhance profitability (Gordon et al., 2017). It is already known that female mammals are born with a finite number of ovarian follicles, and they progressively decrease during life, resulting in the end of their capability to conceive (Grieve et al., 2015). The commencement of puberty triggers the activation of these follicles, a crucial phase that not only determines the onset of reproductive capacity but also sets the foundation for their future productivity in the herd.

The estrous cycle (Figure 1) provides multiple opportunities for females to conceive during their reproductive years. The estrous cycle is predominantly regulated by intricate feedback mechanisms of several hormones, mainly from the hypothalamic-pituitary-ovarian axis (Roche, 1996). These feedback mechanisms include ovarian steroid hormones such as progesterone **(P4)** and estradiol **(E2)**, gonadotropin-releasing hormone **(GnRH)** from the hypothalamus, follicle-stimulating hormone **(FSH)** and luteinizing hormone **(LH)** from the anterior pituitary, and prostaglandin $F_{2\alpha}$ **($PGF_{2\alpha}$)** from the uterus (Roche, 1996).

The typical estrous cycle lasts approximately 21 days in mature cows and 20 days in heifers and is divided into four stages: estrus, metestrus, diestrus, and proestrus (Larson and Randlea., 2013). Estrus and proestrus are the stages that will comprise the follicular phase, while metestrus and diestrus form the luteal phase of the estrous cycle (Senger, 2010). Estrus is characterized by the initiation of sexual receptivity and the ovulation of the dominant follicle **(DF)** (Sartori et al., 2004). Metestrus spans 3 to 5 days, and this period is marked by the initial presence of the collapsed ovulated follicle that will give rise to the formation of the corpus hemorrhagicum, the structure that will become the functional corpus luteum (CL) that will produce a significant amount of P4 at the beginning of the diestrus (Larson and Randlea., 2013). The diestrus is defined by the lifespan of the CL, which lasts about 12 days. Early in diestrus, the CL experiences increases in P4 production, reflecting its maturing capabilities in steroid synthesis. Over time, as diestrus advances, P4 concentration stabilizes and maintains a steady state in the bloodstream (Larson and Randlea., 2013). During diestrus, $PGF_{2\alpha}$ will lyse the CL and initiate the next phase, proestrus. Proestrus lasts about 2 to 3 days, and during this stage, the CL continues to regress both structurally and functionally, accompanied by a significant increase in estradiol production by pre-ovulatory follicles. The increase in E2 triggers the onset of sexual receptivity, marking the beginning of the estrus stage. From the onset of luteolysis to the day before ovulation, a threefold increase in the maximal serum E2 has been estimated to occur. A study found that the peak serum E2 concentration before ovulation was higher in dairy heifers than in mature dairy cows (heifers = 11.3 pg/mL vs. cows = 7.9 pg/mL) (Sartori et al., 2004).

b. Timed AI programs

Understanding the physiological and endocrinological aspects of the estrus cycle opens vast opportunities for reproductive management and controlling the timing of estrus (Caraviello et al. 2006; Norman et al. 2009). Synchronization protocols have become a standard part of reproductive programs across the majority of dairy farms in the U.S. (Caraviello et al., 2006; Norman et al., 2009). Estrus synchronization significantly boosts economic returns by enhancing animal production efficiency, enhancing submission, optimizing insemination timing, and improving pregnancy rates across various management systems. Estrous synchronization programs can regulate follicular development, stimulate ovulation in non-cycling cattle, induce CL regression, and coordinate the timing of estrus and ovulation (Lucy et al., 2004).

The rate of estrus detection **(ED)** impacts the interval between calving and conception (Kinsel and Etherington, 1998) and the overall calving interval (Opsomer et al., 1996). The effectiveness of ED in lactating dairy cows is constrained by a combination of physiological and management factors (Lopez et al., 2004; Senger, 2010). Behavioral signs of estrus are key indicators for predicting ovulation and determining the optimal time for insemination. A study found the best results for good-quality embryos (67.6%) were achieved when AI was performed 24 to 12 hours before ovulation. Specifically, within this timeframe, the percentage of good embryos peaked at 89% when artificial insemination **(AI)** took place 16 to 13 hours before ovulation. (Roelofs et al., 2006). Traditionally, ED in cows was monitored through visual observation, which has been effective but becomes less practical and effective as herd sizes increase, often leading to estrus going unnoticed and subsequently reduced submission rates, extended intervals to breeding, and economic losses. Detection efficiency in dairy herds frequently falls below 50% (Van Vliet and Van Eerdenburg, 1996), and low detection rates could be attributed to management issues, with only 10% due to factors related to the cows themselves (Diskin and Sreenan, 2000).

The challenges in ED are compounded by the high variability in the duration and intensity of estrous signs among individual cows and the influence of various factors. For example, daily milk production significantly impacts the duration of estrus, with a correlation coefficient of $r = -0.51$ between milk yield and estrus duration (Wiltbank et al., 2006). This correlation might be due to the lower serum E2 concentrations observed on the day of estrus in high-yielding cows, potentially resulting from an increased metabolic clearance rate of steroid hormones (Lopez et al., 2004).

The OvSynch (Figure 2) program has revolutionized reproductive management in dairy cattle. This estrous synchronization program provided farmers an opportunity to control cows' ovarian functions, enabling timed AI **(TAI)** without the need for ED. The development of OvSynch significantly boosted the AI service rates and provided an invaluable tool for improving reproductive practices. To further enhance reproductive efficiency, many dairy farms have adopted additional synchronization protocols that were developed throughout the years (Fricke and Wiltbank, 2022).

The initial GnRH administration in the OvSynch protocol aims to trigger an LH surge and FSH release that can lead to the ovulation of a dominant follicle and the start of a synchronized follicular wave. The ovulation process results in the formation of the CL, which enhances the levels of circulating P4 during the development of the ovulatory follicular wave. The next step is administering $PGF_{2\alpha}$ seven days after the first GnRH treatment, causing luteolysis and a reduction in P4 concentrations, thus effectively synchronizing the start of proestrus. The second and final GnRH treatment in the OvSynch protocol is designed to synchronize ovulation, aligning the timing of AI optimally with ovulation to facilitate fertilization and the onset of pregnancy (Fricke and Wiltbank, 2022).

Presynchronization strategies were developed based on findings that initiating an OvSynch protocol between days 5 to 9 of the estrous cycle results in increased P/AI compared to starting earlier or later in the cycle (Vasconcelos et al., 1999). The initial presynchronization strategy tested involved administering two PGF2α treatments 14 days apart, with the second $PGF_{2\alpha}$ treatment occurring 12 days before the first GnRH treatment of an OvSynch protocol. The Presynch-

OvSynch (Figure 2) is an advanced strategy, and in a study, P/AI increased from ~36% to ~47% when compared to the standard OvSynch protocol (Moreira et al., 2001). One approach with the Presynch-OvSynch is to perform ED after the second $PGF_{2\alpha}$ of the Presynch (Fricke, 2020) However, studies showed that inseminating cows showing estrus after the second $PGF_{2\alpha}$ treatment of a Presynch-OvSynch protocol decreased P/AI compared to letting all cows complete the protocol and undergo TAI (Gumen et al., 2012; Borchardt et al., 2017). The authors of this study indicate that the reported reduction in P/AI might be attributed to the removal of cycling cows from the TAI program, thereby undermining the benefits of presynchronization (Borchardt et al., 2017). However, adjusting the interval between the second $PGF_{2\alpha}$ treatment and the start of the OvSynch protocol from 14 to 11 days has been shown to increase the ovulatory response to the initial GnRH treatment and boost P/AI by ~ 7 percentage points when cows receive TAI (Galvão et al., 2007).

Albeit the Presynch-OvSynch became a popular program among dairy farmers, one of its limitations is that noncycling cows do not benefit from the presynchronization treatment with $PGF_{2\alpha}$. Alternatively, the Double-Ovsynch (**DO**) program (Figure 2) was developed to improve ovulation and synchronization in both cyclic and anovular cows, which ultimately led to greater P/AI than the Presynch-OvSynch program (Souza et al., 2008). Albeit the DO has led to dairy farmers achieving high P/AI, other challenges remained when attempting to optimize the protocol. For example, the newly formed CL induced by the initial GnRH treatment in the OvSynch protocol can be resistant to regression by a single dose of $PGF_{2\alpha}$, with studies showing incomplete regression in 10 to 25% of cows (Brusveen et al., 2009; Martins et al., 2011; Wiltbank and Pursley, 2014). The increase in luteolysis translated into an increase in P/AI, particularly notable in multiparous cows (Wiltbank et al., 2015; Borchardt et al., 2018).

High-producing lactating dairy cows are affected by an anovulatory condition involving follicle growth without resulting in an ovulation event (Wiltbank et al., 2002). The proportion of cows that continue to be anovular during the voluntary waiting period (**VWP**), which is generally staged between 50 to 65 days in milk (**DIM**), was reported to be an average of 23.3%, ranging from 15% up to 50% and represent 20 to 30% of cows receiving TAI for the first service (Bamber et al., 2009; Santos et al., 2009). Anovulation has been associated with a decreased probability of conception, reported at 20% in one study (Gumen et al., 2012) and 6 to 10% in another (Walsh et al., 2007), with similar effects whether insemination followed by ED or a synchronization program. Anovulation is also linked to increased pregnancy losses (**PL**) (Santos et al., 2004; Gumen et al., 2012).

Anovular cows lack a CL and do not respond to the first two $PGF_{2\alpha}$ treatments, leading to the initiation of the OvSynch protocol in a low progesterone environment and resulting in lower P/AI to TAI (Wiltbank and Pursley, 2014). In these cases, follicles might develop but either fail to reach the necessary size or responsiveness needed for ovulation. The reproductive cycle remains incomplete without any follicle maturing enough to release an oocyte and subsequently transform into a CL (Wiltbank et al., 2002, 200). The DO protocol is consistently acknowledged as one of the top-performing protocols, significantly enhancing overall pregnancy per artificial insemination **(P/AI)** and improving P/AI for anovular cows (Souza et al., 2008). Administering the comprehensive series of treatments involved in DO can be beneficial, and various studies have demonstrated that the P/AI of the DO is usually greater than in cows receiving Presynch-OvSynch (Fricke and Wiltbank, 2022).

Lactating cows submitted to a presynch plus 5-day protocol without a CIDR outperformed a similar 7-day protocol in terms of pregnancies, with authors attributing the improvement to a reduced period of follicle dominance (Santos et al., 2010). Cows receiving the 5-day program with two $PGF_{2\alpha}$ doses on days 5 and 6 had greater P/AI compared with those receiving OvSynch. The follicular steroidogenic potential may be altered with longer or shorter periods of antral follicle development. Intrafollicular fluid concentrations of E2 increased exponentially with follicle diameter in both cows (Reyes et al., 2006) and heifers (Nishimoto et al., 2009).

c. Nuances in dairy heifers

The cost of raising heifers is the second largest and equals 15% to 20% of the total cost of milk production, shared among the cost factors, following feed costs (Tozer and Heinrichs, 2001). The time point of breeding heifers is pivotal, and management is primarily based on age but also influenced by growth during the rearing period (Wathes et al., 2014). The efficient breeding programs developed are essential to reduce the age of first parturition in dairy heifer (Silva et al., 2015). Understanding the developmental stages and physical characteristics of heifers is crucial to optimally timing artificial insemination (AI), which focuses on the physiological differences of heifers to properly adjust the strategic breeding practices to optimize the reproductive and economic results of dairy operations.

A primary physiological difference between heifers and cows is the follicular wave pattern of both, and each wave involves the emergence, selection, and dominance of follicles, culminating in either atresia or ovulation of the dominant follicle (Sirois and Fortune, 1988; Sartori et al., 2004). For instance lactating dairy cows have 2 to 3 waves of follicular during the estrous cycle (Lucy et al., 1992; De La Sota et al., 1993), and about 80% (78.6 to 83.3%) of them have two follicular waves (Sartori et al., 2004; Wolfenson et al., 2004). An association of a higher milk yield was reported in cows with 2-wave estrous cycles (Bleach et al., 2004). On the other hand, during the estrous cycles, more than 40%. of dairy heifers exhibit three follicular waves (Sirois and Fortune, 1988; Kulick et al., 2001; Sartori et al., 2004). The intervals of days separating each follicular emergence in heifers with three follicular waves are days 2, 9, and 16 of the cycle. This timing could potentially shorten the optimal window available for these heifers when treated with GnRH for follicles to achieve ovulation. (Savio et al., 1988; Sirois and Fortune, 1988; Sartori et al., 2004). This aspect is crucial for synchronizing and optimizing breeding protocols to enhance reproductive outcomes.

Previous studies have shown that the 5-day timed AI protocol developed leads to P/AI rates above 59% for the first insemination and above 55% for the second insemination in Holstein and crossbred dairy heifers (Rabaglino et al., 2010; Lima et al., 2011, 2013). The success of this protocol is attributed primarily to the higher number of pregnancies achieved within a fixed period and the reduction in the age at first calving, which collectively enhance the overall reproductive efficiency. A comparison between heifer breeding programs shows that TAI is more economically beneficial than relying on visual ED and subsequent AI. This is primarily due to the greater number of pregnancies achieved within a fixed period and the reduced age at first calving (Silva et al., 2015).

Albeit for heifers, timed AI programs, specifically the 5-day CoSynch protocols, are widely recognized as the gold standard for producing superior P/AI rates compared to other protocols (Masello et al., 2019), the initial ovulatory response to synchronization programs in dairy heifers tends to be lower—ranging from 15% to 35%—compared to that in dairy cows, which lies between 50% and 60% (Pursley et al., 1995; Sartori et al., 2004; Bello et al., 2006; Rabaglino et al., 2010; Lima et al., 2013; Lauber et al., 2021). This discrepancy can be potentially due to follicular dynamics and P4 secretion variations, affecting the efficacy of synchronization treatments (Sirois and Fortune, 1988; Sartori et al., 2004; Lima et al., 2013). This variation in the initial ovulatory response between heifers and dairy cows suggests that there is room for improvement in aligning the first ovulation with the timing of TAI program in dairy heifers. Further studies are needed to better understand and enhance the synchronization protocols, focusing on the unique follicular dynamics and P4 concentrations in heifers.

d. Ovulation responses at the beginning of Estrous Synchronization Programs

Advances in the understanding of the reproductive biology of dairy cows provide the ability to manipulate follicle growth and the luteal lifespan and have created opportunities to optimize fertility while ensuring effective insemination timing (Moreira et al., 2001; Souza et al., 2008).

The ovulatory response to the first GnRH injection in the OvSynch protocol **(G1)** is crucial for achieving successful synchronization outcomes (Vasconcelos et al., 1999). Despite its widespread use, the OvSynch program has limitations in effectively synchronizing follicles to growth. Ovulation in response to G1, which is given at random stages of the estrous cycle, typically occurs in only 50% to 60% of cases (Vasconcelos et al., 1999; Bello et al., 2006; Galvão and Santos, 2010). The ovulatory response to the G1 administration in timed AI protocols not only enhances synchronization through better control of the emergence of new follicular waves. A study revealed that ovulation to G1 synchronized follicular wave emergence within 1.5 for heifers to 2.1 d for cows after injection (Pursley et al., 1995). This event was key to coordinating a functional dominant follicle at the time of $PGF_{2\alpha}$ and subsequent final GnRH of OvSynch.

Ovulation in response to the first GnRH treatment is positively correlated with P/AI (Bisinotto and Santos, 2012). Furthermore, cows that ovulate following the G1 treatment have a higher likelihood of becoming pregnant compared to cows that do not ovulate in response to G1 (Bello et al., 2006; Chebel et al., 2006; Galvão et al., 2007).

Furthermore, the benefit of cows ovulating to G1 (of the breeding OvSynch) of a DO program is the formation of a new accessory CL, which increases circulating P4 concentrations during the development of the ovulatory follicle (Giordano et al., 2013; Carvalho et al., 2015). This increase in P4 concentrations has been associated with greater oocyte and embryo quality in lactating dairy cows (Cerri et al., 2011; Rivera et al., 2011).

When P4 concentrations are high (greater than 3 ng/mL), the ovulatory response decreases. This decrease occurs because high levels of P4 negatively affect the GnRH-induced LH surge, which in turn reduces the ovulatory response. (Giordano et al., 2012a). Giordano et al., 2013 compared the effect of treatment with 200 versus 100 µg of GnRH on ovulatory response and P/AI, and results showed that cows treated with the double dose 200 µg of GnRH at G1 had a greater ovulatory response than cows treated with the standard dose 100 µg (66.6% vs. 57.5%), and greater P/AI than cows that did not ovulate.

Different studies have shown that higher P4 concentrations were consistently associated with lower LH responses to GnRH in both beef and dairy heifers and cows (Colazo et al., 2008; Lima et al., 2013; Batista et al., 2017). In this regard, increasing the GnRH dose improved LH release and ovulatory responses for dairy cows and heifers (Lima et al., 2022; Valdés-Arciniega et al., 2023; Colazo et al., 2023).

2- Recent progress in estrus detection

Effective estrus detection is vital for reproductive management on dairy farms. Low detection rates negatively impact fertility, prolong calving intervals, increase heifer replacements, and slow genetic progress, leading to significant economic losses (Zebari et al., 2018). Moreover, undetected or falsely detected estrus activity escalates costs related to artificial insemination and feed (Reith and Hoy, 2018). Detection efficiency in dairy herds is often below 50% (Van Vliet and Van Eerdenburg, 1996). Despite poor reproductive performance being the primary reason for culling, few cows are deemed infertile. Management factors account for about 90% of low detection rates, with only 10% attributed to the cows themselves (Diskin and Sreenan, 2000). Nevertheless, the expansion of large dairy herds and year-round calving patterns in the dairy farming industry significantly hinders visual estrus observation (Dobson et al., 2008).

Moreover, a marked decline in estrus duration over the past 50 years, along with factors such as increasing cow age, higher milk production, and challenging environmental conditions (e.g., elevated ambient temperatures, uncomfortable housing, and flooring), adversely affects both the length and intensity of estrus expression which contribute to low estrus detection rates (Rutten et al., 2014). A study showed that the duration of cows displaying estrus behaviors varies depending on the detection method, as well as the housing system and floor type (Reith and Hoy, 2018). For instance, dairy cows housed on concrete flooring had less mounting activity and 4-hour shorter estruses compared with counterparts housed on dirt surfaces (Britt et al., 1986; Vailes and Britt, 1990).

High-production cows may have a reduction in their estrous behavior compared to nulliparous heifers due to the higher rate of estrogen metabolism, which is the hormone responsible for triggering estrous behavior (Sartori et al., 2004). Thus, visual direct observation of estrus has become less effective, requiring auxiliary methods for its identification (Roelofs et al., 2010). Studies using an automated activity monitoring **(AAM)** device demonstrated over again that higher-producing cows had less intense and shorter estrus despite not differing in E2 concentration from lower-producing cows. Nonetheless, a higher activity peak was associated with greater plasma E2 concentration (Madureira et al., 2015; Marques et al., 2020). The development of automated technology that can help identify the beginning of estrous behavior also opens new doors for adjusting the insemination schedule (Walker et al., 1996; Nebel et al., 2000).

3- Fertility traits

Over the past years, a revolution in the reproductive performance of lactating dairy cows happened; while days open increased steadily from 1955 to 2000, there was a dramatic decrease in days open from 2000 to 2010 without a corresponding rise in the genetic trend for daughter pregnancy rate (Fricke and Wiltbank, 2022). The introduction of genetic and genomic selection for reproductive traits, such as daughter pregnancy rate **(GDPR)** in 2003, marked a significant shift in breeding program strategies by emphasizing the importance of selecting for both productive and reproductive traits simultaneously (VanRaden et al., 2003; Council on Dairy Cattle Breeding, 2018). Additionally, the development and widespread adoption of fertility programs have been major driving factors behind this improvement (Carvalho et al., 2018; Fricke and Wiltbank, 2022).

Implementing a reproductive management strategy that P/AI by 130 days in milk **(DIM)** is an effective approach to positioning the herd within a high fertility cycle (Middleton et al., 2019). This strategy ensures that cows are bred at an optimal time postpartum, enhancing overall fertility rates and improving the productivity and health of the dairy herd. Programs like DO and G6G for first timed AI has proved to increase the AI services rate and increase P/AI beyond levels achieved through AI based on ED (Fricke and Wiltbank, 2022).

Simultaneously with the use of fertility programs, the dairy community has increasingly used genomic traits for fertility to enhance breeding strategies and improve herd performance (Council on Dairy Cattle Breeding, 2018). The hazard of pregnancy in lactating cows encompasses a multifaceted matter, influenced by various factors, including health status (Ribeiro et al., 2016), the resumption of ovarian cycles postpartum (Chebel et al., 2010a), the accuracy and efficiency of estrus detection (Chebel et al., 2006), and the probability of achieving pregnancy following insemination.

Advancements in genomic tools have enhanced the potential of using GDPR as a selection criterion, improving the reliability of fertility trait predictions (Wiggans et al., 2011). These genomic advancements have not only doubled the annual rates of genetic gain for production traits but have also resulted in a 3- to 4-fold increase for fitness traits, including female fertility (García-Ruiz et al., 2016). There is a positive correlation between GDPR and the success rate of first-service pregnancies (Veronese et al., 2019). Although GDPR and GCCR are strongly correlated (Chebel and Veronese, 2020), GDPR may more significantly affect estrus characteristics, enhancing the accuracy of breeding decisions and reproductive management success.

In summary, fertility programs have been directly linked to increases in P/AI, while genomic selection has contributed to the rise in cow conception rates over the past years. It is challenging to pinpoint which factor has had a more significant impact on these improvements. Additionally, other management factors can influence the increase in cow conception rates. However, it is evident that strategies involving first AI, coupled with presynchronization, have played a substantial role in this enhancement (Fricke and Wiltbank, 2022).

4- Target reproductive programs

A new approach, known as targeted reproductive management, involves identifying subgroups of cows that require specific reproductive management strategies to achieve better reproductive performance (Giordano et al., 2022). The expectation is that by optimizing the management of subgroups of cows, greater gains in efficiency and performance can be achieved compared to applying a single management strategy to the entire herd (Rial et al., 2022). As technology continues to advance, incorporating traditionally unused AAM data, such as the occurrence of estrus within the VWP, into targeted reproductive management could enhance the sustainability and profitability of dairy farms (Rial et al., 2022; Gonzalez et al., 2023).

Programs that rely primarily on AI at estrus (**AIE**) provide cows with more opportunities and time to be inseminated during estrus, incorporating TAI to ensure timely insemination of cows not detected in estrus (Giordano et al., 2022). These programs highly depend on the cows' ability to express estrous behavior and conceive if inseminated at ED (Tenhagen et al., 2004). Conversely, programs that rely exclusively or almost exclusively on synchronization of ovulation protocols for insemination take advantage of TAI to tightly control the timing of AI, increase the fertility of AI services, or both (Moreira et al., 2001; Souza et al., 2008).

Assessing anovulation before the end of the VWP is labor-intensive, requiring multiple examinations either through circulating P4 measurements or by visualizing a CL using transrectal ultrasound (Lucy, 2019). However, recent advancements in technology have led to the development of AAM systems, which provide valuable insights into the reproductive status of dairy cows (Borchardt et al., 2024). These systems enable early ED, monitor cow health, and support data-driven decision-making, thereby improving conception rates, reducing calving intervals, and optimizing herd productivity (Borchardt et al., 2024).

Several studies demonstrate a positive correlation of cows in the highest groups of genomic traits like GDPR had a greater percentage of cows AI at ED (Lima et al., 2020; Rial et al., 2022). (Sitko et al., 2023) report that cows in the genomic high fertile group had greater P/AI to first service, became pregnant faster, and were more likely to be pregnant by 200 DIM than cows in the low fertility group. Overall, these findings indicate a favorable association between genetic merit for fertility traits and enhanced reproductive outcomes in dairy cows. More research is needed to explore the potential advantages of using tailored management strategies to better align genomic traits with cows bred through AIE and TAI, thereby maximizing fertility for AI services.

CHAPTER 3: Effect of 200 µg of gonadorelin hydrochloride at the first GnRH of a CIDR Synch program on ovulation rate and pregnancies per AI in Holstein heifers.

The initial ovulatory response during synchronization programs is often low in dairy heifers, largely due to follicular dynamics and hormonal dynamics. Specifically, the progesterone concentration **(P4)** at the time of the first GnRH treatment in a breeding program can influence the LH response, often resulting in a suboptimal ovulatory response. The objective of this study was to determine the effect of the highest label dose 200 µg (100 µg vs. 200 µg) of GnRH (50 µg gonadorelin hydrochloride per mL; Factrel®; Zoetis Inc. Madison, NJ) at the first GnRH of a 6-day CoSynch plus P4 device program on ovulatory response and pregnancy per AI **(P/AI)** in first service in Holstein heifers. A total of 1308 Holstein heifers were randomly allocated at the beginning of a 6-day CIDR-Synch program, Day 0, to receive either i.m. treatment of 100 µg (2CC, n = 655) or 200 µg (4CC, n = 653) of GnRH. Also, at Day 0, heifers received an intravaginal insert with 1.38 grams of P4 (Eazi-Breed CIDR® Cattle Insert; Zoetis Inc., Madison, NJ). On Day 6, the insert was removed, and i.m. treatment of 25 mg of $PGF_{2\alpha}$ (12.5 mg dinoprost tromethamine/mL; Lutalyse® HighCon Injection Zoetis) was administered. On Day 7, a second i.m. treatment of 25 mg of $PGF_{2\alpha}$ was given, followed on Day 9 by concurrent i.m. treatment of 100 µg of GnRH and timed AI **(TAI)**. A subset of 396 heifers had their ovaries scanned to evaluate ovulatory response, and blood samples were collected to measure the serum concentration of P4 at Day 0 and Day 6 of the study. The P4 concentrations at Day 0 were categorized as Low (≤ 3ng/mL) or High (> 3ng/mL). The ovulatory response was greater for heifers receiving 4CC than 2CC at Day 0 (54.7% vs. 42.8%). The ovulatory response was greater for Low P4 than High P4 at Day 0 (54.3% vs. 37.8%). However, there was not an interaction between treatment and P4 concentrations (Low P4 2CC = 48.6% vs. High P4 2CC = 30.0%; Low P4 4CC = 60.0% vs. High P4 4CC = 45.5%). The

ROC curve analysis indicates that P4 concentrations at Day 0 treatment could predict the ovulatory response, although the area under the curve was only 0.6. As expected, heifers that ovulated had increased P/AI (No = 55.6% vs. Yes = 67.7%); however, there was no effect of treatment on P/AI (2CC = 63.3% vs. 4CC = 59.6%), nor interactions between treatment and ovulation and treatment and P4 (HIGH vs LOW) for pregnancy outcomes. In summary, P4 concentration and increasing the dose of GnRH at Day 0 positively impacted ovulatory response in Holstein heifers. However, there was no interaction between treatment and P4 on ovulation and no subsequent impact of GnRH dose on P/AI.

INTRODUCTION

Synchronization of ovulation has been widely recognized as an effective method for optimizing pregnancy outcomes in heifers, with studies supporting its benefits in obtaining high pregnancy rates in a cost-effective manner. (Lima et al., 2011; Lima et al., 2013; Silva et al., 2015). However, the initial ovulatory response during synchronization programs in dairy heifers is usually lower (15 to 35%) than in dairy cows (50 to 60%) (Pursley et al., 1995; Sartori et al., 2004; Bello et al., 2006; Rabaglino et al., 2010; Lima et al., 2013; Lauber et al., 2021) often due to variations in follicular dynamics and progesterone **(P4)** secretion (Sirois and Fortune, 1988; Sartori et al., 2004; Lima et al., 2013). Dairy cows tend to have predominantly two follicular waves (Bleach et al., 2004; Sartori et al., 2004; Burns et al., 2005), but there have been studies reporting the existence of three follicular waves in more than 40% of the dairy heifers (Sirois and Fortune, 1988; Kulick et al., 2001; Sartori et al., 2004). Heifers exhibiting a pattern of three follicular waves experience intervals of approximately seven days between waves, with the first, second, and third waves initiating around day 2, 9, and 16, respectively. This, in turn, could reduce the window of time for follicles to ovulate when these heifers are treated with GnRH (Savio et al., 1988; Sirois and Fortune, 1988; Sartori et al., 2004).

Another physiological aspect that has been shown to impact GnRH-induced ovulation at the beginning of the estrous synchronization programs is the plasma concentration of P4. Indeed, findings from previous studies demonstrated a higher P4 concentration is consistently associated with lower plasma concentrations of LH in responses to GnRH in beef heifers (Colazo et al., 2008; Batista et al., 2017), dairy heifers (Lima et al., 2013), and dairy cows (Giordano et al., 2012a). Hence, the P4 milieu is an integral component of the hormonal landscape that determines the GnRH response, thereby influencing the ovulatory potential of the treated animal. Indeed, the previous study in dairy heifers demonstrated that heifers in the Low Progesterone group had higher LH release from 45 to 120 minutes after GnRH treatment and higher ovulatory response (48.4% vs. 19.0%) when compared to heifers in High Progesterone group (Lima et al., 2013).

An approach to mitigate the reduced LH release in lactating dairy cows with high progesterone was increasing the dose of GnRH in the estrous synchronization program (Giordano et al., 2012a). Interestingly, it was reported that when a double dose of GnRH of the current standard estrous synchronization dose (200 µg vs. 100 µg) was administered, it increased the plasma concentration of LH released (Giordano et al., 2012a). The translational potential of increased LH release with a double dose of GnRH for estrous synchronization was further assessed in other studies revealing an improvement in the ovulatory and pregnancy responses in dairy cows (Lima et al., 2022; Valdés-Arciniega et al., 2023). A recent study on dairy heifers demonstrated that those receiving a double dose of GnRH at the beginning of the program exhibited significantly higher ovulatory responses (Colazo et al., 2023).

Consequently, continuous refinement of synchronization protocols and integration of recent advances in reproductive management is crucial for maximizing the effectiveness of synchronization of ovulation in dairy heifers. The low GnRH-induced ovulatory response of dairy heifers at the beginning of estrous synchronization programs, combined with the negative impact of progesterone on LH release and ovulation suggests that doubling the dose of GnRH offers an opportunity to improve pregnancy outcomes. Therefore, we hypothesized that administering a double dose of gonadorelin hydrochloride (200 µg vs. 100 µg) at the first GnRH injection in a 6-day CIDR Synch program would enhance ovulatory response and first service pregnancies per AI in Holstein heifers. The study objectives were to evaluate the impact of a high dose of gonadorelin hydrochloride on ovulation rate and pregnancy outcomes in Holstein heifers following the 6-day CIDR Synch program.

MATERIALS AND METHODS

All experimental procedures carried out in this study received an ethical review by the Institutional Care and Use Committees of the University of California, Davis (Protocol# 22792).

Heifers, Diets, and Housing

A total of 1,308 nulliparous Holstein heifers received their first insemination, with an average age of 15.2 months (ranging from 14 to 17 months), were included in the study conducted between February and May 2022, and were housed in a cattle feeder located in Wellington, Nevada. They had unrestricted access to water and twice daily access to a TMR ration formulated to meet or exceed the nutritional requirements of Holstein heifers weighing 360 kg and gaining

0.8 kg/day (NRC, 2001). Throughout the administration of hormonal treatments, insemination, and pregnancy examination, heifers remained in the same pen until the last pregnancy diagnosis.

Experimental Design and Treatments

Day 0 was defined as the enrollment day of heifers in the study. At Day 0, heifers were evaluated and had their body condition score **(BCS)** assessed using the previously established scoring system for dairy cattle (2004 Elanco Animal Health, Body condition scoring for dairy replacement heifers, adapted from Ferguson et al., 1994). On Day 0, heifers were ranked in ascending order based on their genomic prediction of daughter pregnancy rate **(GDPR)**. Subsequently, at Day 0, they were randomly assigned to receive either **(2CC)** 100 µg (n = 655) or **(4CC)** 200 µg (n = 653) of GnRH as gonadorelin hydrochloride (50 µg gonadorelin hydrochloride per mL; Factrel®; Zoetis Inc. Madison, NJ). Also, at Day 0, heifers received a CIDR insert with 1.38 grams of P4 (Eazi-Breed CIDR® Cattle Insert; Zoetis Inc., Madison, NJ). The CIDR insert was removed six days later on Day 6, and concurrently, heifers received a 25 mg i.m. injection of prostaglandin F2 alpha ($PGF_{2\alpha}$) as 25 mg of dinoprost tromethamine (12.5 mg dinoprost/mL; Lutalyse® HighCon Injection Zoetis Inc., Madison, NJ). Heifers then received, on Day 7, a second dose of $PGF_{2\alpha}$ 24 hours after CIDR removal. Two days later, on Day 9, all heifers received a second dose of 100 µg of GnRH concurrent with timed AI and were performed by three technicians with sexed sorted semen: Holstein (n = 999), Crossbred (n = 152), Simental (n = 80) and Jersey (n = 77). (Figure 3; Table 1).

Ultrasonography of Ovaries and Evaluation of Ovulatory Responses

Each week, a cohort of 100 heifers was selected for the study, and from this group, 30 underwent ovarian evaluations and blood sample collections, totaling 396 heifers by the end of the study. These procedures were repeated on Day 6. Ovarian evaluations were performed using the Easi-Scan Go Veterinary Ultrasound Scanner (IMV Imaging, Rochester, MN). Presence of corpus luteum **(CL)** larger than 14 mm and follicles equal to or greater than 10 mm in diameter were documented. Ovulation at the start of the timed AI **(TAI)** program was assumed if a heifer had a follicle equal to or greater than 10 mm on Day 0, and a newly formed CL was observed in the same ovary on Day 6. Heifers with follicles smaller than 10 mm on study Day 0 but with a new CL on study Day 6 were considered to have ovulated prior to study Day 0.

Blood Sampling and Analysis of Progesterone Concentrations

Blood samples were taken from 396 heifers, which were from the same subset previously mentioned in the ultrasonography examination section. Blood was drawn on study Day 0 and Day 6 by puncturing the median coccygeal vein or artery, using evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) containing K2 EDTA for plasma separation. Immediately after collection, samples were placed on ice and refrigerated until transported to the laboratory. Upon arrival at the laboratory, blood tubes were centrifuged at 2,000 × g for 15 minutes at 5°C, and a 2 mL plasma aliquot was frozen at -80°C for later analysis. The P4 was analyzed using the competitive enzyme immunoassay of (Munro and Stabenfeldt, 1984), as previously described in (Gomez et al., 2018). The average sensitivity of the assay was 1.17 ng/mL, calculated at 2 standard deviations below the mean counts per minute at maximum binding. All samples were analyzed in duplicate, and the intra-assay coefficient of variation averaged 10.7%. The inter-assay coefficients of variation were 10.9%. Heifers were categorized based on the plasma concentration of P4. Heifers with plasma P4 concentration greater than 3 ng/mL were classified as High, while heifers with plasma P4 concentration lower or equal to 3 ng/mL were classified as Low. The rationale for using the 3.0 ng/ml was twofold. First, a previous study in dairy heifers from our group indicated that a concentration of P4 >3.5 ng/ml at the time at GnRH might compromise LH release and ovulation in dairy heifers (Lima et al., 2013a). Furthermore, a study demonstrated that cows with high P4 concentration at the time released more LH when receiving 200 vs. 100 ug of gonadorelin diacetate tetrahydrate(Giordano et al., 2012a).

Pregnancy Diagnosis and Calculation of Pregnancy per AI and Pregnancy Loss

Pregnancy was diagnosed at 37 days post-TAI through transrectal ultrasonography of the uterus using a portable ultrasound device with a 7.5-MHz transrectal probe (Easi-Scan; BCF Technologies USA Ltd. LLC, Rochester, MN). The presence of an amniotic vesicle with a visible embryo heartbeat was used as a criterion to determine pregnancy. Heifers confirmed pregnant on Day 37 underwent another transrectal palpation of uterine contents around 79 days post-AI. The pregnancy rate per AI **(P/AI)** was determined by dividing the number of pregnant heifers on days 37 or 79 by the total number of AI recipients. Pregnancy loss was diagnosed as heifers being pregnant at 37 days post-AI but then non-pregnant at 79- or 177-days post-TAI. Pregnancy loss between 37 and 79 days and between 37 and 177 days were calculated by dividing by the number of heifers identified as nonpregnant on Day 79 or 177 by heifers diagnosed pregnant at 37 post-TAI, respectively.

Statistical Analyses

Power analyses were performed to calculate sample sizes using G Power 3 (Universität Düsseldorf). Sample sizes were calculated for the study to allow sufficient power to detect a difference of seven percentage units in P/ AI between treatments [α = 0.05; β (the probability of a type II error) = 0.20; One-tailed test]. The expected P/AI for the first AI was combined for heifers receiving a CoSynch plus progesterone device program is 58% based on previous studies (Lima et al., 2013, Lima et al., 2011). Under these assumptions, a minimum of 597 experimental units per treatment were deemed necessary to test our hypotheses. Additional heifers were added to increase the statistical power and reduce the change of type II error. Also, sample sizes were calculated for the study to allow sufficient power to detect a difference of fifteen percentage units in ovulation response between treatments [α = 0.05; β (the probability of a type II error) = 0.20; one-tailed test]. The expected ovulation for the first AI combined for heifers receiving a CoSynch plus progesterone device program was between 34.4% and 35.4% based on previous studies (Lima et al., 2013, Lima et al., 2011). Under these assumptions, a minimum of 134 experimental units per treatment were deemed necessary to test our hypotheses. Because of potential attrition, additional heifers were added to both treatments.

Categorical data were analyzed by logistic regression using PROC GLIMMIX of SAS version 9.4 (SAS/ STAT; SAS Institute Inc., Cary, NC), fitting a binary distribution Backward stepwise logistic regression models were used, and variables were continuously removed from the models by the Wald statistic criterion when P > 0.10. The Akaike information criterion **(AIC)** was used to select the final model variables (stepwise elimination approach). The model with the lowest AIC was used. Descriptive statistics were utilized to assess the pre-enrollment equivalence between treatment groups, for continuous variables such as average age, BCS, and GDPR were performed using PROC MEANS. Moreover, categorical variables, including age in months categories, BCS categories (Low from ≤ 2.75, Medium = 3, and ≥3.25, and GDPR categories (Q1 = Lowest GDPR and Q4 = Highest GDPR) were analyzed using PROC FREQ to report proportions. The GLIMMIX procedure of SAS was also utilized to compare 2CC and 4CC enrolled heifers. The study's models for P/AI and pregnancy loss included the effects of treatment, sire, AI technician, and their interactions. Continuous data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS/ STAT; SAS Institute Inc., Cary, NC), with models fitting a Gaussian distribution. Logistic regression and receiver operating characteristic **(ROC)** curves were used to generate a threshold of P4 to predict ovulation and pregnancy. Additional models were also performed to assess the impact of P4 at Day 0 on pregnancy and ovulation, with models including treatment effects, categorized P4 on d0 (High > 3 ng/mL and Low ≤ 3 ng/mL), and their interactions. Differences with $P \leq 0.05$ were considered significant, and those with $0.05 < P \leq 0.10$ were considered tendencies.

RESULTS

Descriptive Statistics

The mean and distribution by month age for heifers, the mean and distribution BCS, and the mean and distribution GDPR did not differ for heifers enrolled in the 2CC and 4CC treatments (Table 1). For ovarian characteristics on Day 0 of the study, the presence of one ($P = 0.14$) or multiple ($P = 0.82$) follicles, the number of follicles ($P = 0.59$), and the size of the largest follicle ($P = 0.23$) were not different for heifers in 2CC or 4CC treatments (Table 2). The presence of one ($P = 0.65$) or multiple CLs ($P = 0.86$), the number of CLs ($P = 0.87$), and the size of the largest CL ($P = 0.36$) were not different at Day 0 for heifers in 2CC or 4CC treatments (Table 2). The concentrations of P4 at Day 0 tended to be lower (*P* = 0.06) in the 2CC compared to the 4CC treatment (Table 2).

Ovulatory Responses

Heifers receiving 4CC treatment had a greater ovulatory response (*P* = 0.01) than heifers receiving 2CC (Figure 4A). Only a small fraction of heifers without follicles equal to or larger than 10 mm at the beginning of the study at (Day 0) had new CL at Days 6 (n = 5, 2CC = 2, and 4CC = 3) at Day 6 indicating very few heifers potentially had ovulation occurring before treatment was administered. Heifers in the Low P4 group had greater (*P* < 0.001) ovulatory response than heifers in the High P4 group (Figure 4). However, no interactions were observed between treatment and the P4 group (*P* = 0.26) for ovulatory response (Figure 4). Moreover, ROC curve analyses were conducted to assess the predictive ability of P4 concentrations during Day 0 for the ovulatory response. The analysis revealed that P4 concentrations could predict ovulation; however, the area under the curve was only 0.6 (Figure 5), indicating a moderate prediction.

Ovarian Responses on Day 6 of the Study

For ovarian responses, a trend was observed (*P* = 0.09) for the presence of follicles, with fewer heifers in the 4CC treatment tending to have follicles ≥ 10 mm when compared to the 2CC treatment (Table 3). The presence of multiple follicles (*P* = 0.79), the number of follicles (*P* = 0.52), and the size of the largest follicle (*P* = 0.86) were not different for heifers in the 2CC and 4CC treatments (Table 3). The presence of CL was not different (*P* = 0.39) between treatments (Table 3). A trend was observed for the presence of multiple CLs (*P* = 0.07), with more heifers in the 4CC treatment tending to have multiple CLs. Also, the heifers in the 4CC treatment tended to have more CLs ($P = 0.07$) than heifers in the 2CC treatment. The size of the largest CL ($P = 0.36$) was not different between treatments (Table 3). The P4 concentrations on Day 6 of the study did not differ between the treatments (Table 3).

Pregnancy Outcomes

Pregnancy rates at 37 ($P = 0.58$), 79 ($P = 0.68$), and 177 ($P = 0.61$) days post-TAI were not different between heifers receiving 2CC and 4CC treatments (Table 4). There were effects of sire ($P < 0.001$) and technician ($P < 0.05$) on Day 37, 79, and 177 post-TAI, but there were no interactions between treatment, sire, and technician at Day 37 ($P = 0.74$), 79 ($P = 0.65$), and 177 ($P = 0.43$). Also, pregnancy losses between Days 37 and 79 ($P = 0.33$) and between 37 and 177 ($P = 0.96$) days post-TAI were not different between heifers receiving 2CC and 4CC treatments (Table 4).

For the subset group of heifers evaluated based on ovarian dynamics ($P = 0.45$; Figure 6A) and P4 ($P = 0.75$; Figure 6B), there were no differences in P/AI at 37 post-TAI between 2CC and 4CC treatments, and there were no interactions ($P = 0.75$) between treatment and P4 groups (Figure 6C).

For the subset of heifers with ovulation data, heifers that ovulated had greater ($P = 0.01$) P/AI at 37 post-TAI than heifers that failed to ovulate (Figure 6D), but no interactions ($P = 0.54$) between treatment and ovulation were found (Figure 6E).

DISCUSSION

As anticipated, heifers treated with the doubled GnRH dose displayed an increase in the ovulatory response at the first GnRH treatment of the program, and heifers categorized as Low P4 plasma concentrations at Day 0 had higher ovulatory response than heifers in High P4 group. However, contrary to expectations, the improved ovulatory response at the beginning of the program did not translate into improved pregnancy per AI. While the P4 concentration at the beginning of the program predicted the ovulatory response and heifers that ovulated had higher pregnancy rates, no interactions between ovulation treatment or P4 and treatment were present for pregnancy outcomes at either Day 79 or 177 post-AI.

The increase in ovulatory response of heifers in the 4CC treatment aligned with results from a recent study on dairy heifers that compared the use of a double dose of GnRH at the beginning of a 5-day CO-Synch protocol, reporting an increase from 27.9% to 51.8% in heifers receiving 200 µg when compared to heifers receiving 100 µg (Colazo et al., 2023). The improved ovulatory response is consistent with previous studies conducted in Holstein dairy cows receiving 200µg vs. 100 µg at the beginning of the breeding OvSynch of a double OvSynch program. Ovulatory response increased from 69.4% to 81.2% (Martinez et al., 2021), and from 65.0% to 81.3%, for cows treated with 200 µg compared to 100 µg (Martinez et al., 2021; Valdés-Arciniega et al., 2023). Previous studies reported a GnRH, dose-dependent LH release response in dairy cows (Souza et al., 2009; Giordano et al., 2012a), consistent with ovulatory responses observed in the current studies. Thus, the premise that there is a GnRH dose-related response for ovulation was confirmed herein as previously described (Colazo et al., 2023).

As expected, progesterone concentrations on Day 0 influenced ovulatory response such that heifers in the Low P4 group had an increase in ovulatory response, but there was no interaction with treatment. The lack of interaction suggests that the magnitude of response to treatment was not sufficient to influence responses observed in ovulation between progesterone groups differently. An increase in the ovulatory response for heifers in the Low P4 group from 19.0% to 48.4%, when compared to the High P4 group, was similar to results previously reported (Lima et al., 2013). In that study, heifers in the high progesterone group had a mean plasma concentration of progesterone of 7.35 ng/mL, which was slightly higher than the mean concentration observed in the current study (6.57 ng/mL), but that is unlikely to be sufficient to explain the differences in the magnitude of ovulatory response (Lima et al., 2013). Previous studies have found differences in the magnitude of the responses to GnRH in high and low P4 environments, consistently demonstrating an increased ovulatory response in animals within the low P4. For instance, beef heifers (Colazo et al., 2008; Dias et al., 2010) and dairy cows showed a notable increase in ovulatory response for those animals with low P4 (Giordano et al., 2012a; Stevenson, 2016). Past research has suggested that progesterone can reduce the expression of GnRH receptors and thereby pituitary sensitivity to GnRH (Nett et al., 2002; Rispoli and Nett, 2005; Stevenson and Pulley, 2016). Indeed, progesterone concentration was a significant (if only moderate) predictor for ovulation but was insufficient to translate into improved pregnancy outcomes as anticipated, suggesting that other physiological factors play a role in dairy heifers subjected to estrous synchronization programs.

As anticipated, heifers ovulating at the beginning of the synchronization program had increased P/AI , but surprisingly, the benefits of the increased ovulatory response achieved by the double dose of GnRH did not result in significant differences in P/AI at Days 37, 79, and 177 post AI. The positive association between ovulation at the beginning of the synchronization program and pregnancy outcomes is consistent with studies in dairy cows and heifers (Colazo et al., 2008;

Giordano et al., 2012a; Lima et al., 2013; Bisinotto et al., 2015). Nonetheless, contrary to our hypothesis, the greater ovulatory response induced by the double dose of GnRH failed to improve pregnancy outcomes, contrasting the findings of a similar recent study (Colazo et al., 2023). In that previous study, heifers receiving 200 μg of GnRH had increased P/AI at 30 days post-AI (75.9% vs. 63.1%) when compared to animals receiving 100 μg (Colazo et al., 2023). These disparities in pregnancy outcomes may potentially be attributed to the differences between the protocols used. In the current study, heifers stayed with the progesterone device for six days, while in the previous study, they kept the progesterone device for only 5 days. Thus, in the current study, heifers had follicles that were under a longer period of dominance when the P4 device was removed, which could have made them more susceptible to express estrus earlier, as indicated by the previous studies (Colazo et al., 2023; Gobikrushanth et al., 2023). In that study, the researchers revealed that increasing the dose of GnRH from 100 μg to 200 μg reduced the interval from device removal to estrus from 61.0 to 54.3 hours in the heifers ovulating after GnRH treatment(Colazo et al., 2023). Therefore, it is possible that in the current study, the additional day available for follicle development increased the percentage of heifers expressing estrus too early and in an asynchronous manner, relative to the insemination time when receiving a higher dose of GnRH, which might have offset the benefits from the increased ovulatory response in heifers receiving 200 μg of GnRH. Another difference was that the increase in ovulatory response in the previous study was larger (From 27.9% to 51.8%) than in the current study, potentially contributing to the effects of pregnancy observed in their study.

Therefore, it is reasonable to surmise that the lack of improved reproductive outcomes in heifers receiving 200 μg of GnRH in the current study might be a result of the increased percentage of heifer expressing estrus sooner after P4 removal (e.g., < 48 hours) that led to an asynchronous ovulation relative to semen availability, ultimately offsetting benefits of the improved ovulation induced by higher dose of GnRH. Even though the results support the importance of ovulation in influencing the outcomes of the timed AI program, it is important to acknowledge that pregnancy rates are influenced by broader and more complex factors such as length of follicle dominance and synchrony between estrus expression, ovulation, and semen availability to support successful fertilization.

CONCLUSION

Overall, the findings of this study indicate that increasing the dose of GnRH from 100 µg to 200 µg at Day 0 of the 6-d CIDR synchronization program enhances the ovulatory response in Holstein heifers. However, despite this improvement in ovulation, no effects were observed on pregnancy outcomes. These results have practical implications for reproductive management strategies in dairy heifers, suggesting that increasing the GnRH dose alone may not be sufficient to improve overall pregnancy. Ultimately, comprehending the complexity of factors influencing pregnancy outcomes in heifers is needed, highlighting the necessity to consider multiple variables when evaluating and predicting pregnancy rates.

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CHAPTER 4: Impact of genomic prediction of daughter pregnancy rate and cow conception rate in two reproductive programs combining estrus detection and timed AI in dairy cows.

Genomic prediction of daughter pregnancy rate **(GDPR)** and cow conception rate **(GCCR)** are fertility traits developed to help improve selection for reproductive performance. Although these traits might share similar numerators in the U.S. national population and overlap considerably, the denominator varies, and programs with different strategies combining estrus detection and timed AI might experience different associations with these traits. The objectives were to assess the reproductive responses: 1) days from calving to first service **(TP1)**, 2) AI at estrus detection **(AIE)**, 3) pregnancy at the first service **(P1)**, 4) pregnancy loss for the first service **(PL)**, and 5) number of services to conception **(NSFC)** in two reproductive programs **(RepP)** combining variable estrus detection **(ED)** length and timed AI **(TAI)** and their relationship of GDPR and GCCR. Holstein dairy cows from a single dairy farm were randomly allocated to RepP Short (n = 982) or Long (n = 942). In the Short RepP, cows were enrolled in a Presynch-OvSynch ($PGF_{2\alpha}$ at 36 ± 3 and 50 ± 3 DIM followed by ED and AI from 50 ± 3 to 62 ± 3; or for cows not showing estrus, GnRH at 62 ± 3, $PGF_{2\alpha}$ at 69 ± 3, GnRH at 71 ± 3 and TAI at 72 ± 3 DIM). In the Long RepP, cows received a $PGF_{2\alpha}$ at 50 ± 3 followed by AI at ED at 82 ± 3, and cows not detected in estrus by this date were enrolled in the OvSynch program (GnRH at 82 ± 3, $PGF_{2\alpha}$ at 89 ± 3, GnRH at 91 ± 3 and TAI at 92 ± 3 DIM). Data for GDPR and GCCR were categorized into quartiles (Q1 to Q4). Statistical analyses included logistic regression used for AIE, P1, and P1; Poisson regression for the NSFC; and linear regression for TP1. Pregnancy outcomes were modeled with average days to first service, number of cows bred after estrus detection, and P/AI variances at days 32 and 88 post-AI as dependent variables, and RepP, GDPR quartiles **(qGDPR)**, GCCR quartiles **(qGCCR)**, and parity as independent variables. Genomic evaluations included AIE, P1, PL, NSFC, and TP1 as dependent variables, with RepP and qGDPR for GDPR and RepP and qGCCR for GCCR as independent variables. Time to pregnancy was analyzed using Cox's proportional hazard model, adjusting for parity. The Short RepP had a shorter TP1 (Short = 64.3 vs. Long = 72.1) and fewer NSFC than the Long RepP (Short = 2.9 vs. Long = 3.1). The Long RepP had a higher AIE (Short = 45.2% vs. Long = 73.2%). The P1 tended (*P* = 0.09) to be greater in the Short than the Long RepP (Short = 33.7% vs. Long = 30.0%). There was a positive relationship between the GDPR and GCCR highest quartile (Q4) for TP1, AIE, P1, and NSFC compared to the lowest quartile (Q1). Interactions between RepP and GDPR were present for TP1, AIE, and P1, but no interactions were observed between RepP and GCCR. Cows in the Short program had a shorter median interval from calving to pregnancy of 113 days, while those in the Long program had a median interval from calving to pregnancy of 124 days. Cows in the highest quartiles for GDPR and GCCR showed a reduced interval of pregnancy and a higher pregnancy hazard ratio of up to 300 DIM compared to those in the lowest quartiles for both GDPR and GCCR. In summary, the current study revealed that cows enrolled in Long RepP that relied on longer ED interval had lower reproductive outcomes than Short RepP, and cows ranked in the highest GDPR and GCCR quartile had better reproductive outcomes independent of the RepP program used.

INTRODUCTION

In recent years, the dairy community has increasingly used genomic traits for fertility to enhance breeding strategies and improve herd performance (Council on Dairy Cattle Breeding, 2018). The hazard of pregnancy in lactating cows encompasses a multifaceted matter, influenced by various factors, including health status (Ribeiro et al., 2016), the resumption of ovarian cycles postpartum (Chebel and Santos, 2010), the accuracy and efficiency of estrus detection (Chebel et al., 2006), and the probability of achieving pregnancy following insemination.

The inception of genetic and genomic selection for reproductive traits, including daughter pregnancy rate **(GDPR)** in 2003, marked a shift in breeding program strategies, recognizing the importance of simultaneously selecting for both productive and reproductive traits (VanRaden et al., 2003; Council on Dairy Cattle Breeding, 2018). While GDPR quantifies the likelihood of a bull's daughter becoming pregnant after calving, cow conception rate **(GCCR)** evaluates the probability of pregnancy following artificial insemination **(AI)** in a bull's daughter. Although these fertility traits share similar numerators in the US national population and overlap considerably, the denominator varies, and programs with different strategies combining estrus detection and timed AI might experience different associations with these traits. For instance, a study assessing the relationship of GDPR and GHCR in dairy heifers indicated that positive associations with the hazard of estrus only occurred in the former leading the authors to suggest that those two traits might impact pregnancy through different mechanisms (Veronese et al., 2019a).

Advancements in genomic tools have furthered the potential of using GDPR as a selection criterion, significantly improving the reliability of fertility trait predictions (Wiggans et al., 2011). Such genomic advancements have not only doubled the annual rates of genetic gain for production traits but have also seen a 3- to 4-fold increase for fitness traits, including female fertility (García-Ruiz et al., 2016). Heifers with lower GDPR rankings were less likely to enter estrus within 7 days post-$PGF_{2\alpha}$ treatment and experienced shorter durations of $PGF_{2\alpha}$-induced estrus than those in the higher GDPR quartile (Veronese et al., 2019a). Additionally, a positive correlation was found between GDPR and the success rate of 1st service pregnancies (Veronese et al., 2019a). Another study indicated that primiparous and multiparous cows in the highest quartile for GDPR had reduced time from calving to first AI and pregnancy, reduced number of services per conception, and increased P/AI for the 1st service and throughout lactation when compared to the lowest GDPR quartiles in cows bred using Presynch-OvSynch program for the first service (Lima et al., 2020). Madureira et al. (2022) showed that cows with higher GDPR scores exhibit increased odds of estrous expression at the time of insemination and lower odds of pregnancy loss, suggesting a broader influence of genomic traits on underlying physiological mechanisms linked estrus and pregnancy maintenance. A recent study indicated that cows with greater GDPR had greater intensity and duration of estrus independent of parity (Borchardt et al., 2024).

Although GDPR and GCCR show a strong correlation, the body of evidence from the literature indicates that GDPR may substantially affect estrus characteristics and potentially favors breeding programs relying more on estrus detection, while GCCR might be highly correlated with pregnancy outcomes in programs relying on timed AI. Thus, it was hypothesized that cows within the highest quartile for GDPR are more likely to be successfully inseminated during the estrus detection period, thereby favoring programs that rely on longer opportunities for ED. For cows in the highest quartile for GCCR, the positive pregnancy outcomes would be evident only in programs that rely on a shorter ED period followed by TAI. The objectives were to assess the reproductive responses in two reproductive programs (RepP) combining variable lengths for ED and TAI and their relationship with GDPR and GCCR.

MATERIALS AND METHODS

All animal procedures followed the recommendations of the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Cows, Diets, and Housing

The study was conducted between March and December 2017 on a commercial dairy farm in Merced, California. A total of 1,924 Holstein cows were housed in a free-stall barn and had unrestricted access to a TMR ration formulated to meet or exceed the nutrient requirements for a lactating Holstein cow producing 35 to 45 kg of milk/d with 3.5% fat and 3.2% true protein when DMI is 21 to 23 kg/d (NRC, 2001). Diets were fed to cows located in a group pen and adjusted according to refusals in each group.

Experimental Design and Treatments

Cows used in the current study were enrolled in two different reproductive management that included using the AIE followed by the OvSynch program. In the first RepP, with a shorter period of estrus observation **(Short)** cows were enrolled at 36 ± 3 DIM, receiving an i.m. injection of 5 mL of $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine, Lutalyse® Zoetis Inc., Kalamazoo, MI) followed by the second $PGF_{2\alpha}$ treatment administered 14 d later at 50 ± 3 DIM. At the second injection of $PGF_{2\alpha}$, cows' tailheads were painted daily with chalk to aid ED. Cows identified in estrus received AI on the same morning. Cows not observed in estrus after 12 d of the second $PGF_{2\alpha}$ treatment were enrolled in the OvSynch program. The protocol included an intramuscular injection of 2 mL of GnRH (50 µg of gonadorelin hydrochloride per mL; Factrel®; Zoetis Inc. Madison, NJ) followed by an intramuscular injection of $PGF_{2\alpha}$ 7 d later. A second intramuscular injection of GnRH was administered at 56 after the $PGF_{2\alpha}$, and TAI was performed 16 h later at 72 ± 3 DIM. If cows were detected in estrus after the $PGF_{2\alpha}$ treatment of the OvSynch protocol, then AI was performed the same day (Figure 7).

In the second program, with a longer period of estrus detection **(Long)**, cows were enrolled at 50 ± 3 DIM, receiving an intramuscular injection of $PGF_{2\alpha}$, and the cows' tails were painted daily with chalk, and those identified in estrus received AI on the same morning until 82 ± 3 DIM. Cows not observed in estrus by 82 ± 3 DIM were enrolled in the OvSynch. The protocol was the same for Short, and AI was performed at 92 ± 3 DIM (Figure 7). Cows were inseminated with conventional or sexed semen from a bull compatible with the farm's genetic management program (Figure 7).

Eligible cows were enrolled in one of the RepP based on odd or even ear tags that, in this particular farm, were placed in ascending order according to the date of birth. Thereafter, cows (n = 1924) were enrolled for each treatment with an odd ID number for treatment Short and an even ID number for the Long treatment group. Parity Short (primiparous = 378 and multiparous = 585), and Long (primiparous = 390 and multiparous = 569), GDPR and GCCR categories (**Q1** = Lowest quartile and **Q4** = Highest quartile), and semen type were analyzed after enrolment.

Pregnancy Diagnoses and Reproductive Outcomes

Pregnancy was diagnosed via transrectal palpation through transrectal ultrasonography of the uterus using a portable ultrasound device with a 7.5-MHz transrectal probe (Easi-Scan; BCF Technologies USA Ltd. LLC, Rochester, MN) at d 32 ± 3 after AI. Pregnancy was determined by the presence of an amniotic vesicle with a visible embryo heartbeat. Pregnant cows on d 32 ± 3 were re-examined for pregnancy via transrectal ultrasonography 5 weeks later (88 ± 3 d of pregnancy) to reconfirm pregnancy status.

For all evaluated pregnancy outcomes, traits were binary variables, coded as 1 if the cow became pregnant and 0 if not pregnant. Data on reproductive outcomes were collected from the on-farm software DairyComp 305 (Valley Agriculture Software, Tulare, CA).

Statistical Analyses

Statistical analyses were performed using JMP from SAS (JMP®, Version 17 Pro, SAS Institute Inc., Cary, NC). Data for GDPR and GCCR were categorized according to quartiles across all cows, with values grouped in ascending order from the lowest **(Q1)** to the highest **(Q4)** quartiles.

The evaluated variables included: 1) days from calving to first service **(TP1),** 2) AI at estrus detection **(AIE)**, 3) pregnancy at the first service **(P1),** 4) pregnancy loss for the first service **(PL),** and 5) number of services per conception **(NSFC)**. Binary outcomes such as AIE, P1, and PL were analyzed using logistic regression. NSFC was analyzed using Poisson regression, and TP1 was analyzed using linear regression.

The final model for overall pregnancy outcomes included the dependent variables of average days to first service, the number of cows bred after the estrus detection period in each group, and all variances for P/AI on day 32 and day 88 after AI. The independent variables in the model were reproductive programs **(RepP)**, quartile of GDPR **(qGDPR)**, quartile of GCCR **(qGCCR)**, and Parity.

For genomic evaluations of GDPR and GCCR, the final model included dependent variables (AIE, P1, PL, NSFC, TP1) and the effects of independent variables RepP and qGDPR for GDPR and RepP and qGCCR for GCCR and their respective interactions.

Time to pregnancy was analyzed by survival analysis using Cox's proportional hazard model with the PHREG procedure of SAS, incorporating parity. For time from calving to pregnancy, cows that did not become pregnant were censored at study exit or 300 DIM, whichever occurred first. The adjusted hazard ratio **(HR)** and respective 95% CI were calculated. The proportionality of hazards was assessed using ASSESS, PH, and RESAMPLE in the PHREG procedure. The LIFETEST procedure of SAS generated survival curves, least squares mean **(LSM)** ± standard error of the mean **(SEM)**, and median days to the event.

For all analyses, backward elimination of explanatory variables with $P > 0.10$ was used to select final models. All variables and their relevant interactions were considered significant if $P \leq 0.05$, while $0.05 < P \leq 0.10$ were considered tendencies.

RESULTS

Descriptive Statistics

Table 5 presents the results from the analysis of the reproductive programs (Short and Long) standardization, and includes variables evaluated that are related to reproductive performance, such as the number of lactations, parity percent, the 305-day mature equivalent (305ME), daily milk average, dairy wellness index **(DWP$)**, genomic net merit **(GNM$)**, genomic predicted transmitting abilities for milk **(GPTAM),** genomic predicted transmitting abilities for fat **(GPTAF)**, genomic predicted transmitting abilities for protein **(GPTAP)**, genomic daughter pregnancy rate **(GDPR)**, genomic cow conception rate **(GCCR)**, genomic breeding value for protein **(GBPI)**, genomic predicted lifespan **(GPL)**, genomic somatic cell score **(GSCS)**, genomic mastitis **(GMAST)**, and genomic lameness **(GLAME)** were assessed. Table 5 also provides information on the type of semen used (conventional and sexed). None of the parameters evaluated were significantly ($P < 0.05$) between the RepP (Table 5). The Spearman correlation coefficient between GGDPR and GCCR indicated a strong positive linear relationship between the two fertility traits of interest for the study (Figure 8).

Pregnancy outcomes

The TP1 for cows in the Short RepP was lower ($P < 0.01$) than in the Long RepP. Multiparous cows had lower TP1 ($P < 0.01$) than primiparous cows, but no interactions ($P = 0.21$) between RepP and parity were detected (Table 6). The NSFC was lower ($P = 0.02$) for the Short than the Long RepP, but no difference ($P = 0.12$) between parity and interactions between parity and RepP ($P = 0.20$) was observed (Table 6). The AIE was greater ($P < 0.01$) for cows in the Long than in the Short RepP. A tendency was detected for parity ($P = 0.09$), but no interactions ($P = 0.81$) between the RepP and parity were observed.

Cows in the Short RepP tended ($P = 0.09$) to have greater P/AI at day 32 post-AI than cows in the Long RepP, but no differences for RepP at day 88 post-AI ($P = 0.24$). There were no effects of parity on P1 at days 32 ($P = 0.59$) and 88 ($P = 0.48$) post-AI, and no interactions were detected between RepP and parity for P1 at days 32 and 88 post-AI (Table 6). There was no difference ($P = 0.31$) for PL between RepP, and no effect of parity ($P = 0.53$) or their interactions ($P = 0.86$) were observed.

Pregnancy outcomes based on GDPR quartiles analysis

Cows in both RepP groups that were in Q4 for GDPR had lower TP1 ($P < 0.01$) than cows in Q1 (Table 7), and an interaction between GDPR and the RepP ($P < 0.03$) was detected with the Long RepP having a linear decrease in days to the first breeding from Q1 to Q4, while the same trend was not observed for the Short RepP. In both programs, the NSFC was lower for cows in the highest Q4 for GDPR (*P* = 0.02) than in Q1, but no interactions (*P* = 0.67) between RepP and GDPR were observed (Table 7). Cows in Q4 for GDPR for both RepP had a higher AIE when compared to cows in Q1 (*P* < 0.01), and an interaction between GDPR and RepP was observed (*P* = 0.03) with the Long RepP having a linear increase in the proportion of cows bred in estrus Q1 to Q3, while the same trend was not observed for the Short RepP. The P1 was greater on days 32 (P = 0.02) and 88 (P = 0.01) post-AI for cows in Q4 for GDPR than for cows in Q1 in both RepP. Interactions between RepP and GDPR for P1 at day 32 post-AI were observed (*P* = 0.02), with cows in the two highest quartiles for GDPR (Q3 and Q4) having a greater P1 than cows in the lowest quartiles (Q1 and Q2), but the same trend was not observed for the Long RepP. Interactions between RepP and GDPR for P1 at day 88 post-AI were also observed (*P* = 0.02), with cows in the two highest quartiles for GDPR (Q3 and Q4) having a greater P1 than cows in the lowest quartiles (Q1 and Q2), but the same trend was not observed for the Long RepP. There were no differences (*P* = 0.15) amongst the GDPR quartiles in PL, nor there were interactions (*P* = 0.71) between GDPR quartiles and RepP (Table 7).

Pregnancy outcomes based on GCCR quartiles analysis

Cows in the highest quartile (Q4) for GCCR had shorter TP1 (*P* < 0.01) than cows in Q1 for GCCR. There were no interactions (*P* = 0.59) between RepP and GCCR for TP1. Cows in Q4 for GCCR, for both RepP, had lower (*P* < 0.01) NSFC than herd mates in Q1, but no interactions (*P* = 0.56) were detected between RepP and GCCR. Additionally, the AIE was greater (*P* < 0.01) for Q4 for GCCR than in Q1, and no interactions (*P* = 0.95) between GCCR and RepP were observed. The P1 in Q4 for GCCR was greater than in Q1 at day 32 (*P* < 0.01) and 88 days (*P* < 0.01) post-AI. There were no interactions between RepP and GCCR for P1 at 32 days (*P* = 0.64) and 88 days (*P* = 0.89) post-AI. There was no difference (*P* = 0.26) in PL for GCCR quartiles, nor were there interactions (*P* = 0.81) between GCCR and RepP (Table 8).

Time to pregnancy and proportion of pregnant cows by 300 days postpartum

The median interval from calving to pregnancy for cows in the Short RepP was shorter at 113 days [95% confidence interval (CI) = 106 to 117] than in the Long RepP, which was 124 days (95% CI = 118 to 132), as depicted in Figure 9. The hazard of pregnancy up to 300 DIM was 20% greater (HR = 1.20, 95% CI = 1.09 to 1.32, $P > 0.01$) for the Short than for the Long RepP (Figure 9).

Cows in the Q4 (Highest) for GDPR had a shorter median interval from calving to pregnancy of 101 days (95% CI = 95 to 113) than cows in the Q1 that had a median of 135 days (95% CI = 126 to 141), as depicted in Figure 10A. Similarly, for GCCR (Figure 10B), cows in Q4 had a median interval to pregnancy of 100 days (95% CI = 95 to 113), contrasted with a longer interval of 132 days (95% CI = 122 to 138) for cows in Q1. The adjusted pregnancy hazard up to 300 DIM was 39% greater for Q4 for GDPR than for Q1 (HR = 1.39, 95% CI = 1.22 to 1.59, $P < 0.01$). The adjusted pregnancy hazard up to 300 DIM was 35% greater for Q4 for GCCR than for Q1 (HR = 1.35 95% CI = 1.18 to 1.54, $P < 0.01$).

DISCUSSION

The major goal of the current study was to assess if the two reproductive programs, either being a typical short ED period or a program with prolonged ED, would lead to different reproductive performances and to evaluate the potential association of GDPR and GCCR were linked to these results. As stated in the hypothesis, we anticipated that cows within the highest quartile for GDPR are more likely to be successfully inseminated during the estrus detection period, thereby favoring programs that rely on longer opportunities for ED, while the highest quartile for GCCR the positive pregnancy outcomes would be only evident in programs that rely on shorter ED period follow by TAI. The findings indicated that the Short RepP resulted in a shorter time to pregnancy first service and pregnancy and a greater percentage of cows pregnant cows after the first service compared to the Long RepP. Cows in the highest quartiles for GDPR and GCCR had shorter intervals of time to pregnancy and higher pregnancy hazard ratios up to 300 DIM than those in the lowest quartiles. Nonetheless, the interaction between RepP and GDPR suggests that adopting different lengths of ED leads to variable trends of improvements with programs with longer ED intervals potentially benefiting more from genetic selection for this trait than GCCR, which lacked any interaction with RepP.

For the most part, cows in the Short RepP achieved improved reproductive performance compared to those in the Long RepP. For instance, reproductive responses such as time to first service were by design anticipated to be different since a shorter period for ED followed by TAI was applied in the Short RepP when compared to the Long RepP. While, due to the longer period for ED, it was expected that the Long RepP would have a greater proportion of cows receiving AIE than the Short RepP. These two responses aligned to experimental design premises and allowed us to explore further if GDPR and GCCR interact with these responses differently. The tendency for cows enrolled in the Short RepP to achieve higher P1 than cows in the Long RepP might be explained by the fact that a high proportion of cows received a Presynch-OvSynch, a fertility program that synchronizes follicle development, luteolysis, and ovulation events, and when compared to cows receiving AI after estrus in controlled studies frequently resulted higher pregnancy per AI (Strickland et al., 2010; Gumen et al., 2012; Fricke et al., 2014b). Another factor is that TAI for cows in the Long Rep were not presynchronized despite being later in lactation, representing a random stage of the cycle that is less likely to result in ovulation and pregnancy (Vasconcelos et al., 1999; Moreira et al., 2001). Another aspect that could be considered is the fact that a higher proportion of cows receiving AI after ED in the Long RepP may result in higher odds of false positive ED, which has been shown to decrease overall pregnancy outcomes (Gowan et al., 1982).

Other reproductive responses assessed corroborated that cows in the Short RepP achieved improved reproductive performance compared to those in the Long RepP, notably having a shorter median interval from calving to pregnancy (113 vs. 124 days) and a 20% increase in the pregnancy hazard. It is worth noting that anovular cows might not potentially benefit from an extended period for estrus detection in the Long RepP. Anovulation negatively affects the reproductive efficiency of dairy farms by extending the calving intervals (Darwash et al., 1997). Previous research has observed that the prevalence of anovulation in dairy cows, measured around 60 DIM, spans from 15% to 50% (Moreira et al., 2001; Lopez et al., 2005a; Chebel et al., 2006). This condition can lead to prolonged intervals between calving and conception, thereby reducing the number of calves born per cow per year, impacting milk production, and ultimately decreasing farm profitability. Cows returning to cyclicity early after calving exhibit improved reproductive performance (Chebel et al., 2006; Santos et al., 2009; Galvão et al., 2010). In the current study, multiparous cows received AI earlier and tended to be more frequently inseminated at estrus than their primiparous counterparts, leading to shorter DIM at the first insemination. This finding is consistent with the findings by Lima et al. (2020), who showed that more multiparous than primiparous cows were bred after detecting estrus. Conversely, Madureira et al. (2015) found that when using an activity monitoring system, multiparous cows displayed a lower peak activity and shorter estrus durations compared to primiparous cows. Likewise, another study using activity monitoring for ED found more primiparous (75.3%) than multiparous cows (64.7%) receiving AI (Rial et al., 2022). The discrepancy between the current study, the study by Lima et al. (2020), and the study by Rial et al. (2022) may be because AIE was performed using an automatic detection in the latter study while the first two relied on tail chalking and might be more susceptible to false positives ED.

A positive correlation between GDPR and GCCR of 0.87 suggests that these traits overlap considerably, as anticipated, and need to be analyzed carefully before being used for targeted reproductive management in dairy cattle. The results of the current study corroborate that there is a positive relationship between the fertility traits GDPR and GCCR for pregnancy outcomes. For instance, cows within the highest quartile of GDPR and GCCR exhibited reduced time from calving to the first service, higher P/AI for the first service, and fewer services to pregnancy than the lowest quartiles. However, no differences in pregnancy loss for GDPR nor GCCR were present. Similarly, (Lima et al., 2020) also reported a consistent positive relationship between GDPR and pregnancy outcomes, encompassing both first-time and cumulative AIs in both multiparous and primiparous cows. Madureira et al. (2022) noted that elevated GDPR was linked to higher initial pregnancy rates per AI, improved ongoing pregnancy rates across multiple AIs, and a reduced risk of pregnancy loss. The main motives for analyzing the relationship of GCCR in the current study were the previous variable responses and suggested mechanisms by which GDPR and GHCR impact pregnancy (Veronese et al., 2019). Our study's additional focus on GCCR indicates that a model including these two traits impacts P/AI differently for the first service than when compared to only adding either of the traits. The finding that GCCR and GDPR are incorporated together in the models shifts the significance of the first service for programs with variable lengths for ED, which corroborates with the previous study's idea that the mechanisms by which these traits impact pregnancy might differ. Thus, the current findings suggest that the dual metric approach might provide a wider comprehensive assessment of reproductive strategies, potentially guiding more targeted interventions to optimize responses of interest.

Lima et al. (2020) demonstrated that the proportion of primiparous cows bred after estrus detection increased linearly with the increase in GDPR. Similarly, Rial et al. (2022) reported that using automated estrus alerts, cows in the highest tercile for GDPR experienced higher insemination rates at estrus detection than those in the medium and low tercile groups. Veronese et al. (2019)also contributed to understanding GDPR's impact by showing that heifers in the highest GDPR quartile had a shorter interval to detect estrus induced by $PGF_{2\alpha}$ and an increased hazard of estrus 7 days post-administration compared with heifers in the lowest quartile. Notably, while previous studies have predominantly focused on GDPR, our study also showed that GCCR is positively associated with the odds for estrus expression, followed by AI, and pregnancy outcomes in general, highlighting the need to investigate the role of GCCR further as a critical factor in reproductive strategies that incorporate estrus and timed AI.

CONCLUSION

The current study underscores that reproductive programs that have extended time for ED might lead to a negative impact on reproductive responses, such as the time to pregnancy and pregnancy per AI, and need to be carefully considered before its implementation. Furthermore, the current study highlights that these programs with variable estrus detection length before timed AI benefit from selection for fertility traits such as GDPR and GCCR with the former having interactions with these reproductive programs. While GCCR lacked interactions with reproductive programs assessed, this trait positively impacts all reproductive responses measured in both programs and as for GDPR, it needs further investigation to elucidate how it can impact pregnancy outcomes.

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List of tables

Table 1. Descriptive data representing the number of heifers (n), and the distribution within treatment groups. mean (±SE), and range for genomic prediction of daughter pregnancy rate (GDPR), average milk production in the first 2 tests (M1), and 305-d mature-milk equivalent (ME305) for the lowest (Q1), second (Q2), third (Q3), and highest (Q4) quartiles of primiparous and multiparous cows, and semen breed type.

	Treatments		*P*-value
	2CC	4CC	
Item			
Age in month			
14, % (n/n)	5.5 (36/655)	6.1 (40/653)	0.83
15, % (n/n)	71.3 (467/655)	72.3 (472/653)	
16, % (n/n)	21.4 (140/655	20.2 (132/653)	
17, % (n/n)	1.8 (12/655)	1.4 (9/653)	
Mean, months ± SEM	15.2 ± 0.5	15.2 ± 0.6	0.97
BCS groups			
Low, % (n/n)	7.0 (46/655)	6.4 (42/653)	0.91
Medium, % (n/n)	42.0 (275/655)	42.4 (277/653)	
High, % (n/n)	51.0 (334/655)	51.2 (334/653)	
Mean, BCS ± SEM	3.15 ± 0.1	3.15 ± 0.8	0.77
GDPR groups*			
Q1, % (n/n)	25.2 (154/610)	26.6 (162/608)	0.93
Q2, % (n/n)	26.6 (162/610)	26.2 (159/608)	
Q3, % (n/n)	23.4 (143/610)	22.2 (135/608)	
Q4, % (n/n)	24.8 (151/610)	25.0 (152/608)	
Total, % (n/n)	93.1 (610/655)	93.1 (608/653)	
Mean, GDPR ± SEM	0.37 ± 5.4	0.33 ± 5.3	0.63
Semen Breed Type			
Holstein, % (n/n)	75.1 (492/655)	77.7 (507/653)	0.46
Jersey, % (n/n)	5.7 (37/655)	6.1 (40/653)	
Crossbred, % (n/n)	12.2 (80/655)	11.0 (72/653)	
Simental, % (n/n)	7.0 (46/655)	5.2 (34/653)	

* Not all the heifers in the study had a GDPR, and those without were excluded from the analysis.

Table 2. Ovarian responses and progesterone (P4) concentrations at Day 0 of a CIDR Synch program**.**

	Treatment		*P*-value
	2CC	4CC	
Item			
Presence of follicle ≥ 10mm at Day 0, % (n/n)	96.9 (192/199)	93.9 (184/197)	0.14
Presence of multiple follicles ≥ 10mm at Day 0, % (n/n)	55.2 (106/192)	54.1 (100/185)	0.82
Number of follicles ≥ 10mm at Day 0, mm ± SEM	1.66 ± 0.05	1.70 ± 0.05	0.59
Size of the largest follicle at Day 0, mm ± SEM	13.9 ± 0.21	13.6 ± 0.23	0.23
Presence of CL at Day 0, % (n/n)	84.9 (168/199)	83.2 (163/197)	0.65
Presence of multiple CL at Day 0, % (n/n)	6.0 (10/169)	5.5 (9/164)	0.86
Number of CL at Day 0, mm ± SEM	1.06 ± 0.02	1.05 ± 0.02	0.87
Size of the largest CL at Day 0, mm ± SEM	23.4 ± 0.44	22.8 ± 0.41	0.36
P4 concentrations at Day 0, ng/mL ± SEM	4.80 ± 0.31	5.67 ± 0.36	0.06*
P4 concentrations distribution by group			
High, % (n/n)	58.8 (117/199)	66.5 (131/197)	
Low, % (n/n)	41.2 (82/199)	33.5 (66/197)	0.11

* CL presence and number are based on ultrasound.

Table 3. Follicular ovarian responses and progesterone (P4) concentrations at Day 6 of a CIDR Synch program**.**

	Treatment		*P*-value
	2CC	4CC	
Item			
Presence of follicle ≥ 10mm at Day 6, % (n/n)	99.5 (198/199)	97.5 (192/197)	0.09*
Presence of multiple follicles ≥ 10mm at Day 6, % (n/n)	56.6 (112/198)	55.2 (105/192)	0.79
Number of follicles ≥ 10mm at Day 6, mm ± SEM	1.70 ± 0.07	1.75 ± 0.05	0.52
Size of the largest follicle at Day 6, mm ± SEM	12.9 ± 0.18	13.0 ± 0.20	0.86
Presence of CL at Day 6, % (n/n)	95.0 (189/199)	92.9 (183/197)	0.39
Presence of multiple CL at Day 6, % (n/n)	29.6 (55/189)	38.3 (70/183)	0.07*
Number of CL at Day 6, mm ± SEM	1.30 ± 0.03	1.39 ± 0.04	0.07*
Size of the largest CL at Day 6, mm ± SEM	20.8 ± 0.42	19.9 ± 0.41	0.13
P4 concentrations at Day 6, ng/mL ± SEM	4.00 ± 0.25	4.00 ± 0.26	0.93
P4 concentrations distribution by group			
High, % (n/n)	53.3 (106/199)	47.2 (93/197)	
Low, % (n/n)	46.7 (93/199)	52.8 (104/197)	0.23

* CL presence and number are based on ultrasound.

Table 4. Pregnancy outcomes for heifers in the 2CC and 4CC treatments.

	Treatment		*P*-value
	2CC	4CC	
Item			
Pregnancy			
At 37 days, % (n/n)	60.6 (397/655)	58.8 (382/653)	0.58
At 79 days, % (n/n)	58.4 (378/647)	57.1 (371/649)	0.68
At 177 days, % (n/n)	58.1 (375/645)	56.5 (366/647)	0.61
Pregnancy Loss			
From 37 to 79 days, % (n/n)	2.8 (11/383)	1.7 (6/381)	0.33
From 79 to 177 days, % (n/n)	2.8 (11/383)	2.8 (11/381)	0.96

Table 5. Descriptive statistics for continuous data and their distribution between two reproductive programs (Short and Long timed AI programs).

	Reproductive Program		*P*-value
	Short	Long	
Item			
Number of cow, % (n/n)	51.1 (982/1924)	48.9 (942/1924)	0.20
Number of lactation, mean ± SEM	2.26 ± 0.04	2.19 ± 0.04	0.20
305ME[1], mean ± SEM	28202.1 ± 122.7	28271.5 ± 117.7	0.68
Primiparous enrolled, % (n/n)	49.8 (368/739)	50.2 (371/739)	0.39
Multiparous enrolled, % (n/n)	51.8 (614/1185)	48.2 (571/1185)	0.39
Milk daily average, mean ± SEM	82.5 ± 0.56	81.6 ± 0.56	0.25
DWP$[2], mean ± SEM	439.7 ± 6.48	445.3 ± 6.74	0.55
GNM$[3], mean ± SEM	352.4 ± 4.96	356.4 ± 5.03	0.57
GPTAM[4], mean ± SEM	318.1 ± 15.3	335.8 ± 15.9	0.42
GPTAF[5], mean ± SEM	23.2 ± 0.64	23.6 ± 0.65	0.69
GPTAP[6], mean ± SEM	16.4 ± 0.38	17.0 ± 0.41	0.23
GDPR[7], mean ± SEM	2.48 ± 0.05	2.54 ± 0.05	0.37
GCCR[8], mean ± SEM	2.92 ± 0.05	2.90 ± 0.05	0.79
GBPI[9], mean ± SEM	1987.8 ± 5.86	1992.3 ± 5.98	0.59
GPL[10], mean ± SEM	4.36 ± 0.64	4.41 ± 0.06	0.53
GSCS[11], mean ± SEM	2.85 ± 0.005	2.85 ± 0.005	0.79
GMAST[12], mean ± SEM	99.9 ± 0.16	99.9 ± 0.19	0.89
GLAME[13], mean ± SEM	100.6 ± 0.19	100.4 ± 0.16	0.53
Semen Type			
Conventional, % (n/n)	50.5 (719/1425)	49.5 (706/1425)	0.45
Sexed, % (n/n)	52.5 (82/199)	47.5 (66/197)	0.45

[1] 305ME: 305-day mature equivalent

[2] DWP$: daily milk average, dairy wellness index

[3] GNM$: genomic net merit

[4] GPTAM: genomic predicted transmitting abilities for milk

[5] GPTAF: genomic predicted transmitting abilities for fat

[6] GPTAP: genomic predicted transmitting abilities for protein

[7] GDPR: genomic daughter pregnancy rate

[8] GCCR genomic cow conception rate

[9] GBPI: genomic breeding value for protein

[10] GPL: genomic predicted lifespan

[11] GSCS: genomic somatic cell score

[12] GMAST: genomic mastitis

[13] GLAME: genomic lameness

Table 6. Reproductive parameters for Short and Long reproductive programs and Primiparous and Multiparous cows.

	Short		**Long**		*P*-value		
	Primiparous	Multiparous	Primiparous	Multiparous	RepP	Parity	RepP x Parity
TP1[1], n	64.6 ± 0.69	63.9 ± 0.53	73.5 ± 0.68	70.6 ± 0.55	< 0.01	< 0.01	0.21
NSFC[2], n	2.9 ± 0.1	2.9 ± 0.08	3.2 ± 0.1	2.9 ± 0.08	0.02	0.12	0.20
AIE[3], %	43.5 ± 2.5	46.8 ± 1.9	71.1 ± 2.5	75.4 ± 1.9	< 0.01	0.09	0.81
P1 at 32d[4], %	35.6 ± 2.5	31.8 ± 1.9	29.3 ± 24	30.8 ± 1.9	0.09	0.59	0.24
P1 at 88d[5], %	31.3 ± 2.4	27.6 ± 1.8	26.6 ± 2.3	27.2 ± 1.8	0.23	0.48	0.31
PL[6], %	9.5 ± 3.3	11.8 ± 2.5	9.5 ± 3.3	12.8 ± 2.5	0.31	0.53	0.86

[1] TP1 = Days from calving to first service.

[2] NSFC = Number of services per conception

[3] AIE = Cows artificially inseminated at estrus detection

[4] P1 at 32d = Pregnancy rate for the first service, 32 days after artificial insemination

[5] P1 at 88d = Pregnancy rate for the first service, 88 days after artificial insemination

[6] PL = Pregnancy loss for the first service

Table 7. Reproductive parameters for lowest (Q1), second (Q2), third (Q3), and highest (Q4) quartiles for genomic prediction for daughter pregnancy rate (GDPR).

	Short				Long				*P*-value		
Item	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	RepP	GDPR	RepP x GDPR
TP1[1], n	65.6 ± 0.8	63.9 ± 0.8	64.5 ± 0.9	62.9 ± 0.8	74.9 ± 0.8	73.2 ± 0.9	69.8 ± 0.8	69.3 ± 0.9	< 0.01	< 0.01	0.03
NSFC[2], n	3.1 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	0.02	0.02	0.67
AIE[3], %	39.4 ± 2.9	47.6 ± 2.8	42.1 ± 3.1	53.4 ± 3.1	61.9 ± 3.0	72.5 ± 3.1	80.9 ± 2.9	79.0 ± 3.1	< 0.01	< 0.01	0.03
P1 at 32d[4], %	28.7 ± 2.9	27.1 ± 2.8	40.7 ± 3.1	38.0 ± 3.0	27.7 ± 2.9	31.9 ± 3.1	27.1 ± 2.9	34.8 ± 3.1	0.12	0.02	0.02
P1 at 88d[5], %	23.6 ± 2.8	23.0 ± 2.7	36.7 ± 3.0	33.8 ± 2.9	23.1 ± 2.9	28.8 ± 2.9	24.7 ± 2.8	32.1 ± 2.9	0.31	< 0.01	0.02
PL[6], %	17.6 ± 3.7	15.1 ± 3.8	10.0 ± 3.4	11.3 ± 3.4	16.4 ± 3.9	9.6 ± 3.8	8.9 ± 3.9	7.7 ± 3.7	0.29	0.15	0.92

[1] TP1 = Days from calving to first service.

[2] NSFC = Number of services per conception

[3] AIE = Cows artificially inseminated at estrus detection

[4] P1 at 32d = Pregnancy rate for the first service, 32 days after artificial insemination

[5] P1 at 88d = Pregnancy rate for the first service, 88 days after artificial insemination

[6] PL = Pregnancy loss for the first service

Table 8. Reproductive parameters for lowest (Q1), second (Q2), third (Q3), and highest (Q4) quartiles for genomic prediction for cow conception rate (GCCR)**.**

Item	Short				Long				*P*-value		
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	RepP	GCCR	RepP x GCCR
TP1[1], n	65.5 ± 0.8	63.9 ± 0.8	64.2 ± 0.8	63.2 ± 0.9	73.4 ± 0.8	72.6 ± 0.9	70.7 ± 0.8	70.5 ± 0.9	< 0.01	< 0.01	0.59
NSFC[2], n	3.2 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	0.03	< 0.01	0.56
AIE[3], %	39.2 ± 3.0	46.4 ± 2.9	46.6 ± 2.9	50.0 ± 3.1	67.2 ± 3.0	73.8 ± 3.1	76.6 ± 3.1	76.9 ± 3.1	< 0.01	< 0.01	0.95
P1 at 32d[4], %	27.9 ± 2.9	28.2 ± 2.9	36.3 ± 2.9	40.5 ± 3.0	26.1 ± 2.9	29.2 ± 3.0	30.1 ± 3.0	35.8 ± 3.1	0.17	< 0.01	0.64
P1 at 88d[5], %	23.3 ± 2.9	25.4 ± 2.8	30.5 ± 2.8	36.2 ± 2.9	22.4 ± 2.9	25.8 ± 3.3	26.8 ± 2.9	33.6 ± 2.9	0.39	< 0.01	0.89
PL[6], %	16.4 ± 3.9	10.0 ± 3.9	15.8 ± 3.3	10.6 ± 4.8	14.3 ± 4.1	11.8 ± 3.9	11.1 ± 3.8	6.1 ± 3.4	0.37	0.26	0.81

[1] TP1 = Days from calving to first service.

[2] NSFC = Number of services per conception

[3] AIE = Cows artificially inseminated at estrus detection

[4] P1 at 32d = Pregnancy rate for the first service, 32 days after artificial insemination

[5] P1 at 88d = Pregnancy rate for the first service, 88 days after artificial insemination

[6] PL = Pregnancy loss for the first service

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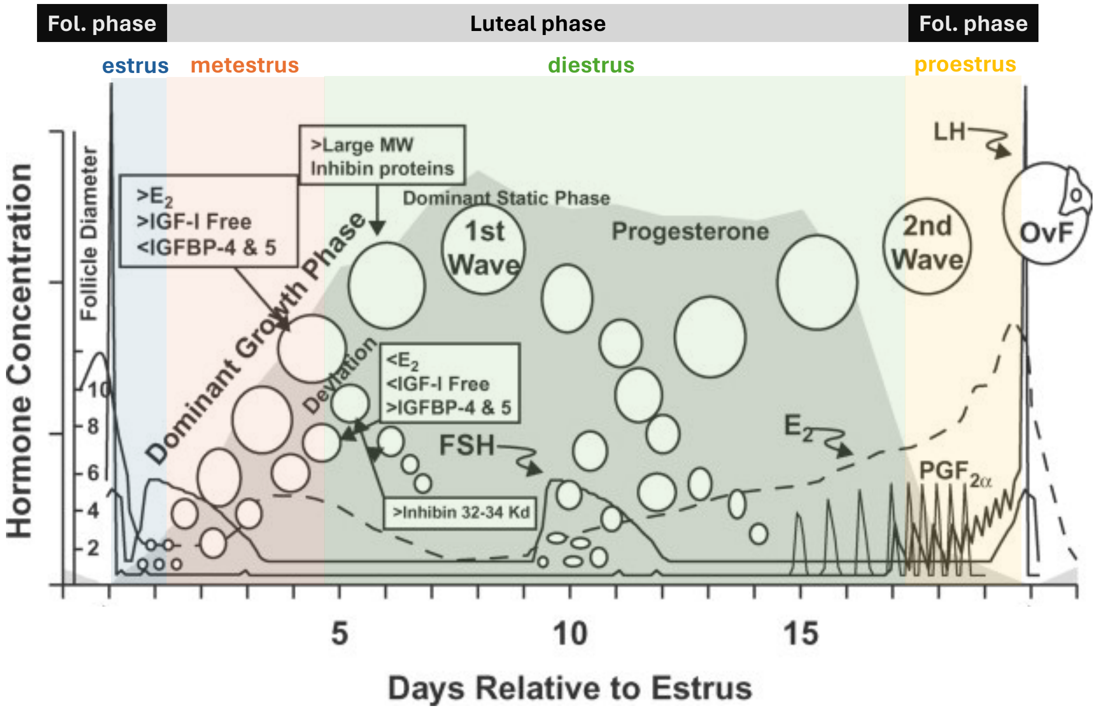

Figure 1. A schematic representation of the estrous cycle. Adapted from Moore and Thatcher, 2006.

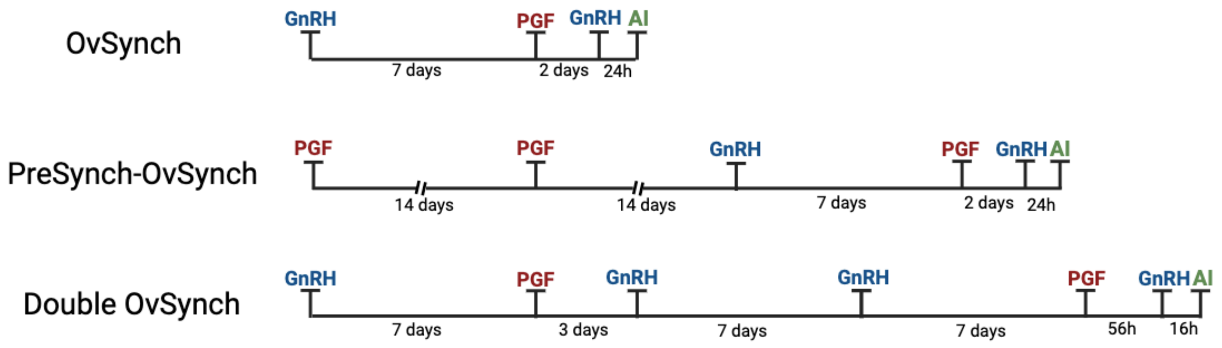

Figure 2. Schematic representation of three different Timed Artificial Insemination (TAI) protocols: Ovsynch, PreSynch-Ovsynch, and Double Ovsynch. Ovsynch; begins with a GnRH injection followed seven days later by a PGF injection. Two days after the PGF, another GnRH is administered, with artificial insemination (AI) 16 hours later. PreSynch-Ovsynch; initiates with two PGF injections spaced 14 days apart. Seven days following the second PGF, a GnRH injection is administered. This is followed two days later by another PGF injection and then 24 hours later by the final GnRH injection before AI. Double Ovsynch, starting with a GnRH injection, followed seven days later by a PGF injection, and three days after that, another GnRH injection. This sequence is repeated seven days after the second GnRH, a PGF injection is given, and 56 hours later, a third GnRH injection. Finally, AI is performed 16 hours after this last GnRH injection.

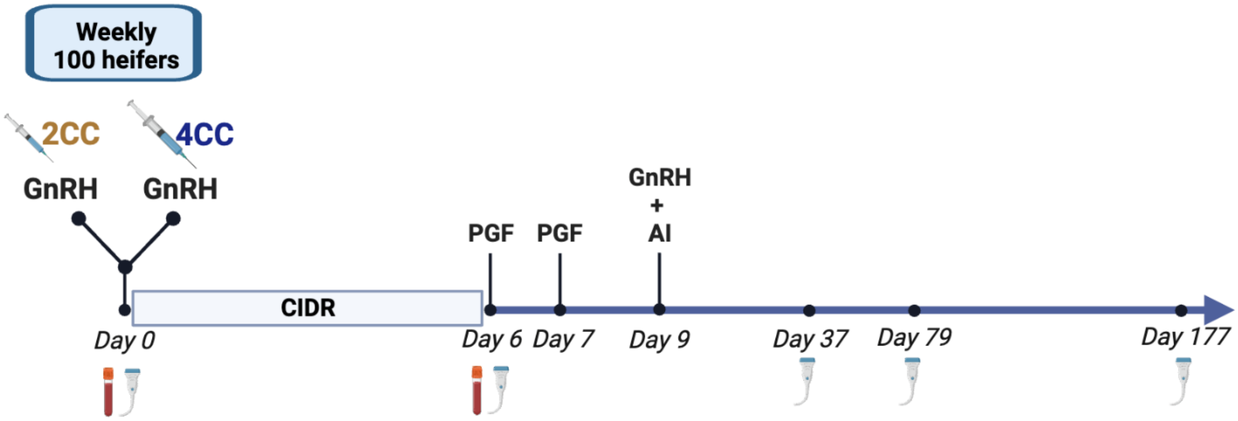

Figure 3: Schematic representation of experimental procedures: 1,308 Holstein heifers were enrolled in the study. BCS were recorded, and the heifers were ranked based on their GDPR. On Day 0, the heifers were then randomly assigned to two treatments, either a 100 µg dose (2CC = 655) or a 200 µg dose (4CC = 653) of GnRH (Day 0) as part of a CIDR Synch protocol. Additionally, on Day 0, the heifers were administered a CIDR and GnRH (Day 0). On Day 6, the CIDR was removed, and a shot of PGF was administered. On Day 7, a second shot of PGF was given. Finally, on Day 9, the heifers received 100 µg of GnRH and were artificially inseminated. A subset of 396 heifers underwent ovarian scanning, and blood samples were collected to measure the serum concentration of progesterone (P4) at GnRH (Day 0) and six days later.

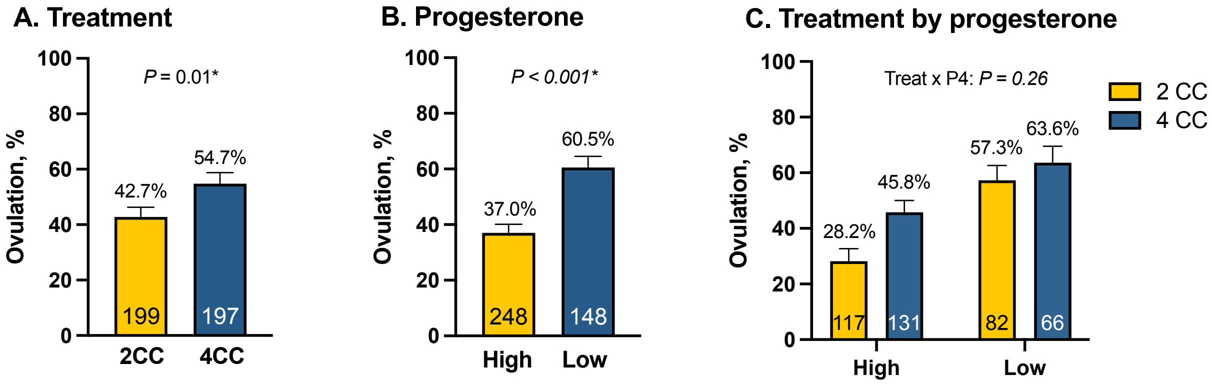

Figure 4. Bar graph illustrating the adjusted ovulatory response (A) in heifers in the 2CC and 4CC (100 µg vs. 200 µg) of GnRH (50 µg gonadorelin hydrochloride per mL; Factrel®; Zoetis Inc. Madison, NJ) at the first GnRH of a 6-day CoSynch plus P4 device program) treatments, (B) evaluation of ovulatory response when groups were divided into High and Low progesterone concentrations (High > 3.0 ng/mL vs. Low ≤ 3.0 ng/mL) at Day 0 of the study; and (C) comparison between treatment (2CC and 4CC) within each progesterone concentration (Low and High) at Day 0. Analyses were done from the subset group of heifers (n = 392), and the proportion is listed above the bar graph and standard, whereas the number of cows per treatment is within the bar at the bottom. Error bars represent the standard error of the mean.

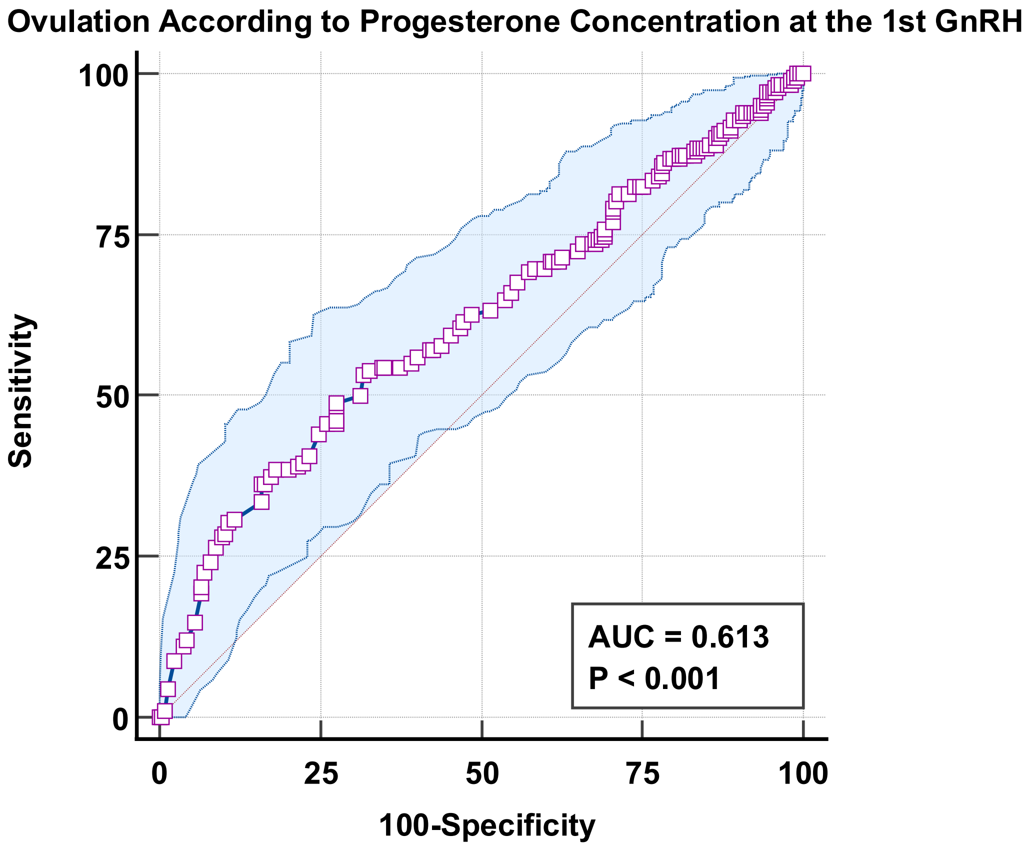

Figure 5. Evaluation of progesterone concentration at Day 0 as a predictor of ovulation at the beginning of the program/first GnRH of CIDR Synch protocol. AUC = Area under curve.

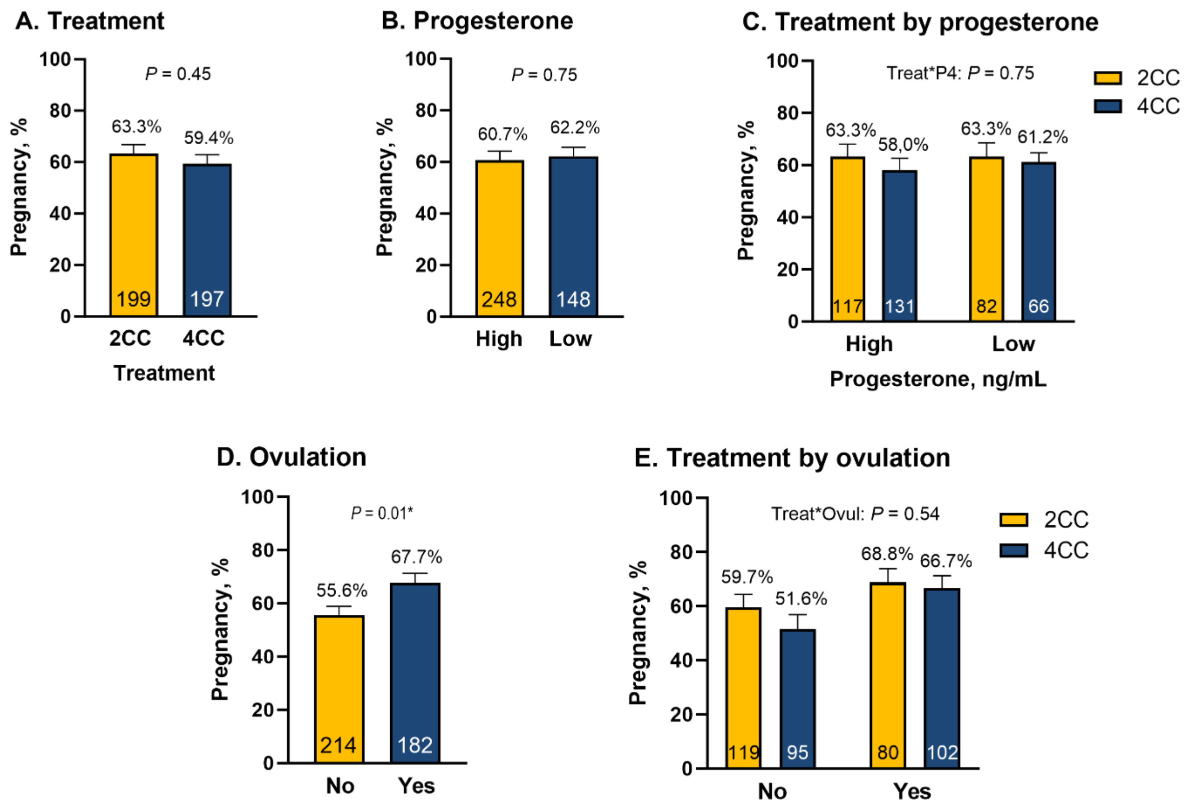

Figure 6. Bar graphs illustrate the proportion of heifer pregnant at Day 37 post-AI in the subset group according to treatment (A), progesterone group, (B), treatment by progesterone (C) ovulation at Day 0 of study (D), and interaction ovulatory response or not at Day 0 within each treatment group. The proportion of pregnant cows is listed on the bar graph at the top, whereas the number of cows per group is at the bottom. Error bars represent the standard error of the mean.

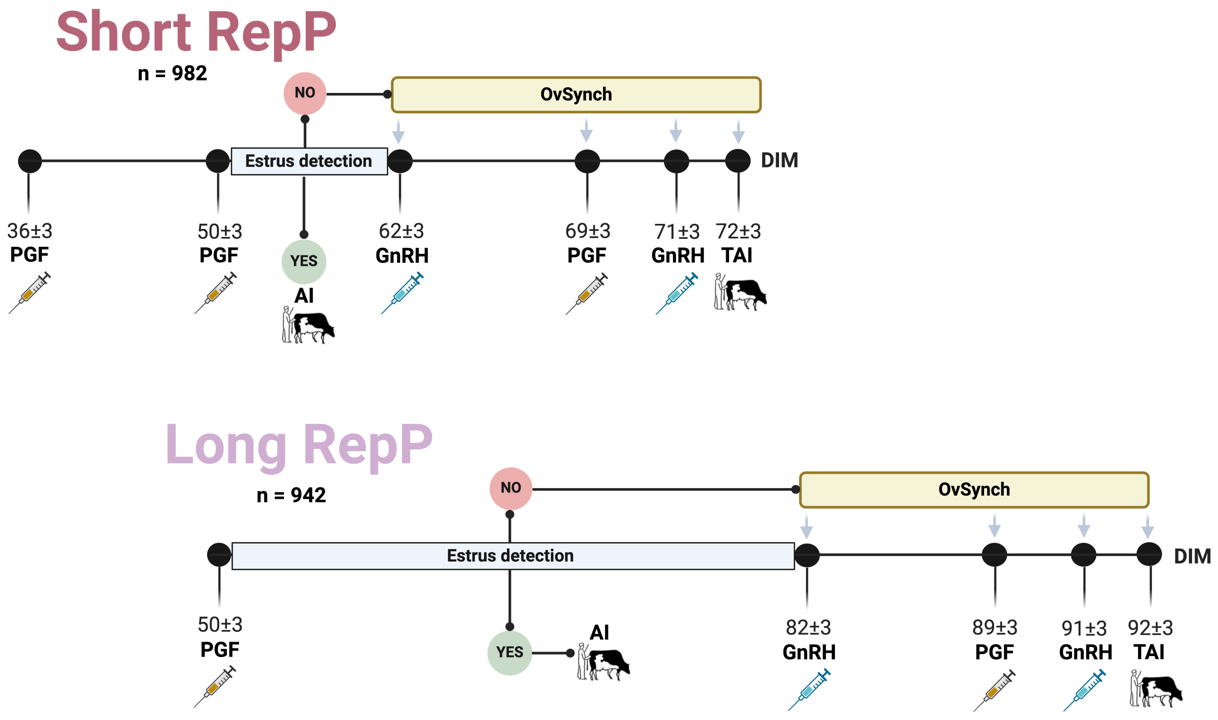

Figure 7: Timeline of study reproductive programs. A total of 1,924 Holstein dairy cows at 36 ± 3 days in milk (DIM), cows were allocated to one of two reproductive programs (RepP): 1) (Short RepP= 982 cows) PreSynch followed by 12 days of estrus detection and insemination and for cows not showing estrus 12 days later at 62 ± 3 an OvSynch (GnRH, followed by PGF2α after 7 days, a second GnRH dose 56 hours later, and timed AI (TAI)was started; or 2) (Long RepP = 942 cows) administration of $PGF_{2\alpha}$ program at 50 ± 3 DIM, followed by an extended estrus detection period of 32 days followed by OvSynch program starting at 82 ± 3 DIM for those not showing estrus.

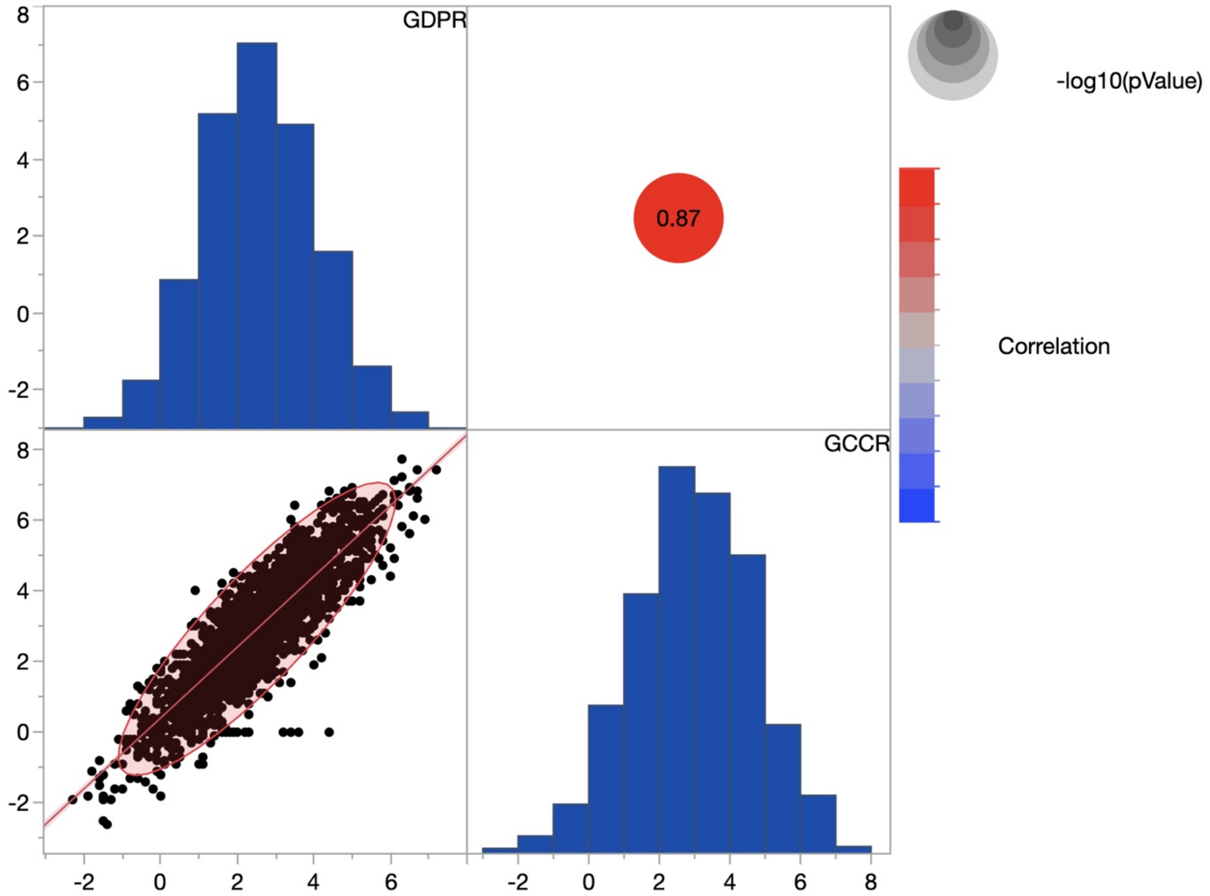

Figure 8. Correlation of genomic merit for daughter pregnancy rate (GDPR) and genomic merit for cow conception rate (GCCR) in Holsteins dairy cows.

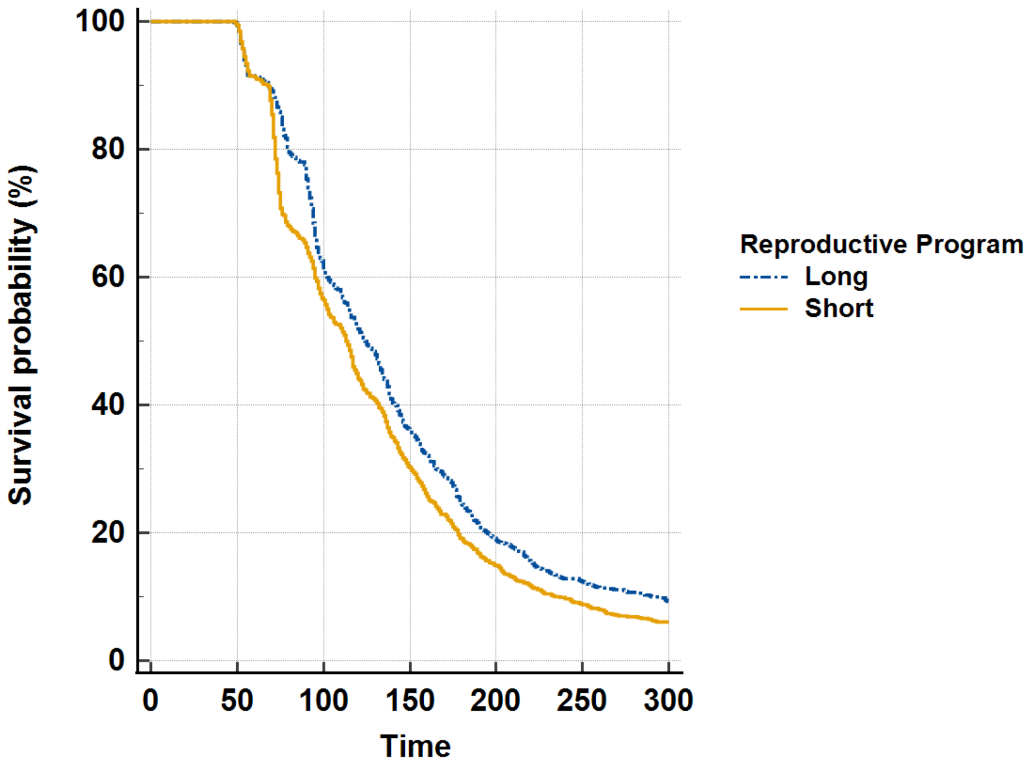

Figure 9. Survival curves for the proportion of nonpregnant cows by days postpartum for cows submitted in two different reproductive programs named Short or Long RepP in the first 300 days postpartum. The median interval to pregnancy for the Short and Long groups was 113 days (95% confidence interval [CI] = 106 to 117) and 124 days (95% CI = 118 to 132), respectively. The rate of pregnancy in the 300 days postpartum was greater (*P* > 0.01) for Short than Long (adjusted hazard ratio = 1.20; 95% CI = 1.09 to 1.32).

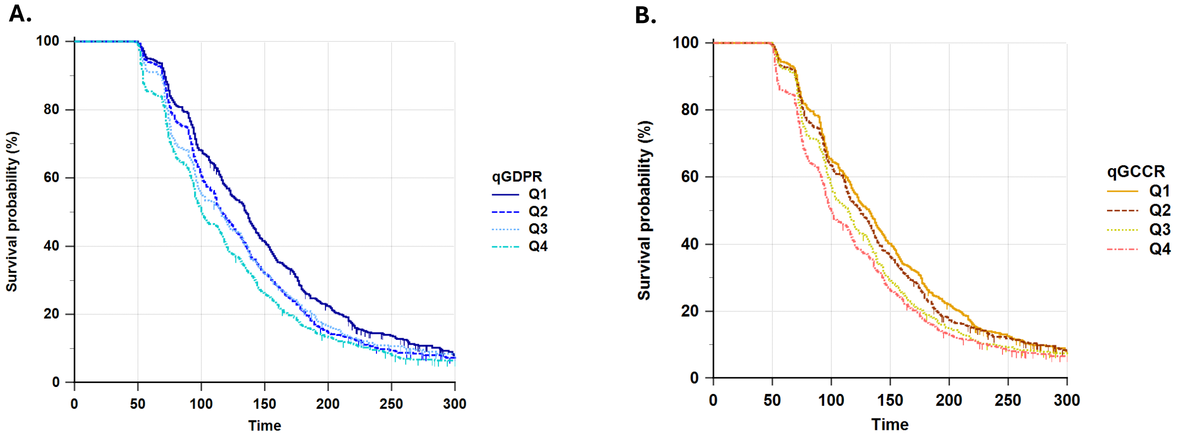

Figure 10. Survival curves for proportions of nonpregnant cows by days postpartum for cows in different quartiles for GDPR (Fig. A) and for GCCR (Fig. B) in the first 300 days postpartum. Median interval to pregnancy for GDPR for Q4 and Q1 was 101 days (95% confidence interval [CI] = 95 to 113) and 135 days (95% CI = 126 to 141), respectively. The rate of pregnancy in the 300 days postpartum was greater ($P = 0.05$) for Q4 than Q1 (adjusted hazard ratio = 1.39; 95% CI = 1.22 to 1.59). Median interval to pregnancy for GCCR for Q4 and Q1 was 100 days (95% confidence interval [CI] = 95 to 113) and 132 days (95% CI = 122 to 138), respectively. The rate of pregnancy in the 300 days postpartum was greater ($P = 0.05$) for Q4 than Q1 (adjusted hazard ratio = 1.35; 95% CI = 1.18 to 1.54).

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