THE HIGH FERTILITY CYCLE: A PARADIGM SHIFT IN MANAGEMENT OF REPRODUCTION IN LACTATING DAIRY COWS

By

Emily Louise Middleton

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Animal Science – Master of Science

2019

ABSTRACT

THE HIGH FERTILITY CYCLE: A PARADIGM SHIFT IN MANAGEMENT OF REPRODUCTION IN LACTATING DAIRY COWS

By

Emily Louise Middleton

Timely pregnancies are essential to reproductive success on dairy farms. The primary objective of this thesis was to demonstrate the relationship between previous calving interval and body condition change during the 1st 30 days in milk (DIM) and their relationship to subsequent fertility and health. Cows that became pregnant before 130 DIM had a greater chance of maintaining or gaining body condition during the 1st 30 d of the subsequent lactation. Cows that maintained or gained body condition during the 1st 30 DIM had a greater chance of pregnancy at 1st AI and reduced chance of pregnancy loss 35 to 60 d post-AI. This improved chances of becoming pregnant by 130 DIM again. We refer to this relationship as the "high fertility cycle". Cows that lost less body condition during the 1st 30 DIM also experienced fewer periparturient disorders. In order to maximize chances for pregnancy by 130 DIM it is critical to detect non-pregnant cows as early as possible and utilize a timely resynchronization program for re-insemination. The second objective of this thesis was to develop an early and highly accurate method to diagnose non-pregnancy. We utilized two within cow samples of PSPB taken before and after the time of embryonic attachment to diagnose non-pregnancy with 100% accuracy at 24 d post-AI. The third objective of this thesis was to use this early non-pregnancy diagnosis method and a short resynchronization protocol in an attempt to create timely pregnancies. We utilized CIDRs to prevent spontaneous ovulation and synchronize cows for re-insemination at 35 d post-AI. The control group was re-inseminated 42 d post-AI. The cows re-inseminated by 42 d post-AI had greater PR/AI and a greater percentage pregnant by 130 DIM.

ACKNOWLEDGMENTS

My heart is filled with gratitude towards everyone that has helped me to complete this Master's degree. First and foremost, a big thanks to my major professor Dr. Pursley. Thank you for challenging me and fostering my love for science. I am forever grateful for your mentorship. I would also like to thank my wonderful lab mates: Dr. Bob Vlietstra, Thainá Minela, Alisson de Mota Santos, Megan Ahearne, and Emmy Schuurmans. I benefitted from the different perspectives and advice each person had to offer. It truly does take a team to accomplish great things. Thank you, Dr. Jose Cibelli, Dr. Jen Roberts, and Dr. Jason Rowntree, for offering your time and guidance serving on my committee. I would also like to acknowledge the Animal Science department at Michigan State University for its support.

This project would not have been possible without funding from the Michigan Alliance for Animal Agriculture and the Michigan State University College of Agriculture and Natural Resources Undergraduate Research Program. A special thanks is due to Ken and Kerry Nobis and all the employees at Nobis Dairy Farm (St. Johns, MI) for their assistance and use of their cows. Their support for our research is admirable. Thank you to Parnell Animal Health (Overland Park, KS) for donating the GONAbreed® and estroPLAN® and to Zoetis (Florham Park, NJ) for donating the Eazi-Breed™ CIDR®s used in my research. Also, thank you to the many interns that have assisted me with data collection.

Last but far from least, I would like to thank my family for their support and always encouraging me. I also want to say a special thanks to my boyfriend, Matt, for his support, his patience and for listening to many practice presentations. Thank you once again to everyone who has helped me complete my Master's degree. I am truly appreciative to each and every one of you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

AI artificial insemination

BCS body condition score

BLV bovine leukemia virus

bST bovine somatropin

CI calving interval

CIDR controlled internal drug release

CL corpus luteum/corpora lutea

cpm counts per million

CV coefficient of variation

d day(s)

DIM days in milk

ELISA enzyme-linked immunosorbent assay

g gram

GnRH gonadotropin releasing hormone

h hours(s)

kg kilogram

LH luteinizing hormone

µg microgram

mg milligram

min minute

µL microliter

mL milliliter

mm millimeter

M305M 305-d mature equivalent milk

ng nanogram

nm nanometer

NPV negative predictive value

OD optical density

PAGs pregnancy-associated glycoproteins

$PGF_{2\alpha}$ prostaglandin $F_{2\alpha}$

PPV positive predictive value

PR/AI pregnancy per artificial insemination

PSPB pregnancy-specific protein B

P_4 progesterone

RP retained placenta

SEM standard error of the mean

TMR total mixed ration

US ultrasound

wk week (s)

vol volume

CHAPTER 1

INTRODUCTION

Reproductive success on dairy farms has a direct impact on profitability (Britt, 1985). Time to pregnancy influences reproductive success on dairy farms. Multiple studies over the years have shown that 12 to 13-month calving intervals are the most profitable (Louca and Legates, 1967; Dijkhuizen et al., 1984; Meadows, 2005). Pregnancies after approximately 130 DIM were no longer profitable (Giordano et al., 2011).

Cows that take more time to become pregnant have extended calving intervals. Extended calving intervals allow cows to become over-conditioned and calve at greater body condition scores (Ruegg and Milton, 1995). Cows with greater body condition at parturition experience greater body condition loss after parturition (Butler and Smith, 1989; Ruegg and Milton, 1995; Roche et al., 2007). Studies have shown that body condition loss is associated with lower PR/AI at 1st AI (Domecq et al., 1997; Moreira et al., 2000; Santos et al., 2009; Carvalho et al., 2014) and increased pregnancy loss (López-Gatius et al., 2002; Santos et al., 2009). There is also a relationship between body condition loss and postpartum health events (Ruegg and Milton, 1995; Gillund et al., 2001; Berry et al., 2007b). Cows that experience postpartum health events have been reported to have lower PR/AI (Gröhn and Rajala-Schultz, 2000; Ribeiro et al., 2013). There may be some association between time to pregnancy in one lactation and fertility and health in the next lactation, but it has not yet been demonstrated.

Reproductive management tools that control time to insemination, maximize PR/AI, and reduce re-insemination intervals are necessary for creating timely pregnancies (Pursley et al., 1997; Fricke, 2002; Giordano et al., 2013). Ovsynch (GnRH, 7 d – $PGF_{2\alpha}$, 2 d – GnRH, 16 h – AI) was the first protocol that synchronized ovulation and allowed producers to control time to insemination (Pursley et al., 1995; Pursley et al., 1997). Years of research have shown that stage of the estrus cycle, size and age of the dominant follicle, hormonal concentrations, and luteolysis

influence the success of the Ovsynch protocol (Vasconcelos et al., 1999; Martins et al., 2011; Wiltbank and Pursley, 2014). The addition of a second injection of $PGF_{2\alpha}$ (Brusveen et al., 2009) and presynchronization programs (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008) have worked to optimize these factors and have improved PR/AI to 1st AI dramatically. Nearly 50% of all cows still fail to conceive to 1st AI though and require a re-insemination (Bello et al., 2006; Souza et al., 2008; Strickland et al., 2010). In order to achieve a timely pregnancy, cows need to be re-inseminated as soon as possible which requires an early non-pregnancy diagnosis.

Non-pregnancy diagnoses rely on practical methods that are inexpensive and can be performed early with high accuracy (Giordano et al., 2013; Fricke et al., 2016). Ultrasound can be used to diagnose pregnancy directly at 26 to 33 d post-AI with 98% accuracy (Pieterse et al., 1990). PAGs and PSPB can be assayed in blood or milk samples for an indirect pregnancy diagnosis.

Serum levels of PSPB begin to increase in pregnant lactating cows around 22 d post-AI (Arnold et al., 2012). Due to variability between cows, PSPB samples are taken 28 to 35 d post-AI to diagnose pregnancy (Zoli et al., 1992; Humblot et al., 2001). PSPB samples taken at this time are 98% accurate at diagnosing pregnancy (Sasser et al., 1986; Piechotta et al., 2011). Martins et al., (2018) obtained a 98% accurate pregnancy diagnosis at 23 d post-AI with two PSPB samples. PSPB samples were taken on d 20 and 23 post-AI, and cows were diagnosed pregnant if there was a 28% or greater increase in serum PSPB levels (Martins et al., 2018). The percent change in serum levels of PSPB could provide a novel, robust method of diagnosing non-pregnancy earlier than any other method with very high accuracy.

Re-insemination strategies can be challenging due to variability in the stage of the estrous cycle at non-pregnancy diagnosis and the need for a short re-insemination interval (Silva et al.,

2007). Successful re-insemination strategies reduce re-insemination intervals while still producing acceptable PR/AI (Giordano et al., 2011; Green et al., 2011).

Relying on estrus detection alone is not an efficient re-insemination strategy (Pursley et al., 1997; Fricke, 2002; Giordano et al., 2012a). Hormonal resynchronization protocols control time to AI more effectively (Pursley et al., 1997; Fricke, 2002). Two common resynchronization protocols involve administering the first GnRH of Ovsynch a week before non-pregnancy diagnosis to all cows (Fricke, 2002; Fricke et al., 2003; Sterry et al., 2006) or initiating Ovsynch with GnRH and a CIDR after a non-pregnancy diagnosis (Chebel et al., 2006; Lima et al., 2009; Dewey et al., 2006; Bilby et al., 2013). CIDRs provide supplemental P_4 (Macmillan et al., 1991l Macmillan and Peterson, 1993; Xu and Burton, 2000) and are used to improve synchrony (Lima et al., 2009; Bilby et al., 2013) and to prevent spontaneous ovulations (Sirois and Fortune, 1990; Chebel et al., 2006; Bilby et al., 2013). CIDRs have been reported to increase PR/AI too, but the benefit may be greater for cows with low circulating P_4 or no CL (Chebel et al., 2006; Dewey et al., 2010; Bilby et al., 2013). CIDRs are usually removed on the day of the $PGF_{2\alpha}$, but removing the CIDR on this day may allow a premature ovulation, especially in cows with low circulating P_4 or no CL. It has not yet been determined if removing the CIDR on the day of $PGF_{2\alpha}$ or the day after is more optimal in terms of PR/AI.

There are three critical components to reproductive management programs: 1st AI, pregnancy diagnosis, and re-insemination of non-pregnant cows. The success and timing of these three components collectively influence time to pregnancy in lactating dairy cows. The objectives of the studies in this thesis are: 1) to demonstrate the importance of timely pregnancies in lactating dairy cows from the standpoint of health and fertility in the subsequent lactation, 2) to develop a very accurate early non-pregnancy diagnosis method and 3) to use this

early non-pregnancy diagnosis method and a short resynchronization protocol in an attempt to create timely pregnancies.

Chapter 2 reviews the literature that has laid the groundwork for the importance of timely pregnancies, factors that limit reproductive success, and how reproductive management tools can be used to create timely pregnancies. Chapter 3 describes the key study of this thesis that demonstrated the relationship between time to pregnancy in one lactation and body condition changes, health, and fertility in the subsequent lactation. In addition, the study also addressed the relationship between body condition change, fertility, and health in primiparous cows. Chapter 4 describes a study where two within cow PSPB samples taken before and after the time of embryonic attachment, at d 17 and d 24 post-AI, were used to diagnose non-pregnancy with 100% accuracy at 24 d post-AI. Chapter 5 describes a study that used an early non-pregnancy diagnosis at 24 d post-AI and a short resynchronization protocol to re-inseminate cows by 35 d post-AI. This study also determined whether it was better in terms of PR/AI to remove CIDRs on the day of the $PGF_{2\alpha}$ or the day after. Chapter 6 provides conclusions for this thesis in a lay article format that will be re-formatted and submitted for publication to an industry magazine.

CHAPTER 2

REVIEW OF LITERATURE

THE IMPORTANCE OF TIMELY PREGNANCIES IN LACTATING DAIRY COWS

Successful reproductive management on dairy farms is essential for profitability (Britt, 1985). Pregnancy renews and initiates lactation (Lucy, 2001). Reproductive failure decreases the number of replacements produced, milk yield, and genetic progress (Congleton and King, 1984; Gröhn and Rajala-Schultz, 2000). The monetary value of pregnancy and cost of DIM spent non-pregnant vary and are ultimately herd-specific (Groenendaal et al., 2004; De Vries, 2006). In general, greater DIM spent non-pregnant and longer calving intervals are less profitable and hinder reproductive success (Plazier et al., 1997; Groenendaal et al., 2004).

Additional datasets suggest that time to pregnancy also influences reproductive success on dairy farms (Louca and Legates, 1967; Dijkhuizen et al., 1984; Meadows, 2005). The literature is in agreement that 12 to 13-month calving intervals are the most profitable (Louca and Legates, 1967; Dijkhuizen et al., 1984; Meadows, 2005). Meadows (2005) concluded that cows cannot be non-pregnant for more than 115 DIM to maintain a 13-month calving interval. Giordano et al., (2011) determined with a Markov chain simulation model that cows need to become pregnant around 130 DIM from a profitability standpoint. Pregnancies after this point began to lose monetary value (Giordano et al., 2011). Data from these studies show that in order to be profitable, cows must become pregnant before 130 DIM (Meadows, 2005; Giordano et al., 2011). Therefore, reproductive success depends on a cow attaining a timely pregnancy and maintaining that pregnancy until parturition.

Time to pregnancy directly effects calving interval length. Aside from profitability, extended calving intervals can influence a cow's body condition. Body condition scoring is a reliable, subjective measure of fat stores assessed independently of body weight or frame size (Wildman et al., 1982; Edmonson et al., 1989; Otto et al., 1991) Body condition scoring is a

useful tool for monitoring the nutritional state of dairy cattle and a key indicator of cow health (Wildman et al., 1982; Hady et al., 1994). It is normal for dairy cows to go into a negative energy balance after parturition and lose body condition (Butler and Smith, 1989; De Vries and Veerkamp, 2000). Many studies have depicted body condition profiles in lactating dairy cows as mirror images of lactation curves (De Vries et al., 1999; Berry et al., 2006; Roche et al., 2007). Body condition is at nadir during peak lactation (Butler et al., 1981; Berry et al., 2006; Roche et al., 2007). The duration and magnitude of negative energy balance varies (Butler et al., 1981; Ruegg and Milton, 1995; Pryce et al., 2001). As cows go further into lactation, milk production plateaus or decreases and cows begin to gain body condition (Berry et al., 2006; Roche et al., 2007). Cows with extended calving intervals spend more time in lactation and may become over-conditioned as a result (Ruegg and Milton, 1995). Over-conditioned cows tend to lose a greater amount of body condition after parturition (Butler and Smith, 1989; Ruegg and Milton, 1995; Roche et al., 2007).

It has been well demonstrated that low body condition and/or a loss of body condition postpartum results in lower PR/AI at 1st AI (Domecq et al., 1997; Moreira et al., 2000; Santos et al., 2009). Carvalho et al., (2014) observed significant differences in PR/AI between cows that had lost body condition compared to cows that had maintained and cows that had gained body condition. Some studies have found that chances for pregnancy loss increased with body condition loss (López-Gatius et al., 2002; Santos et al., 2009). The risk for pregnancy loss is greatest during early pregnancy (Vasconcelos et al., 1997; Santos et al., 2004). Carvalho et al., (2014), however, did not find a relationship between pregnancy loss and body condition loss.

On average, cows resume cyclicity about 10 d after energy balance starts to return to zero (Butler et al., 1981). Butler et al., (1981) observed a negative correlation between energy balance

and days to first ovulation. Cows with greater negative energy balances took longer to ovulate for the first time after parturition (Butler et al., 1981). Another study showed that greater negative energy balances increased the chances of having lower progesterone during the 2nd and even 3rd estrous cycles (Villa-Godoy et al., 1988). Britt (1992) hypothesized that follicles grown under adverse conditions, like negative energy balance or postpartum disease, would be compromised. He hypothesized that the compromised follicles would produce inferior oocytes and the resulting CL would secrete less progesterone (Britt, 1992). Carvalho et al., (2014) provided some support for the Britt (1992) hypothesis by observing a greater percentage of degenerated embryos 7 d post-AI from cows that had lost the greatest amount of body weight 21 d after parturition. In that study there was a 6 to 7-week span from the time of body weight loss to embryo development. Negative energy balance may create residual negative effects on fertility.

The incidence of postpartum health events and body condition loss during early lactation are closely related (Ruegg and Milton, 1995; Gillund et al., 2001; Berry et al., 2007b). It is not clear whether body condition loss causes postpartum health issues or if postpartum health issues cause body condition loss. One postpartum disease may predispose individual cows to other diseases (Dohoo and Martin, 1984). Postpartum health events have been shown to negatively affect reproductive success in some studies (Gröhn and Rajala-Schultz, 2000; Ribeiro et al., 2013). Other studies, however, have shown no relationship between postpartum health events and PR/AI (Domecq et al., 1997; Heuer et al., 1999) or pregnancy loss 38 to 90 d post-AI (López-Gatius et al., 2002). Body condition loss and its effects on reproductive success can hinder achieving timely pregnancies.

Interestingly, not all cows experience postpartum negative energy balance and the associated body condition loss. Britt (1992) described two groups of cows: high body condition and low body condition. The low group had greater body condition than the high group at parturition and then lost more condition during the first 5 weeks postpartum. The high group had less body condition than the low group at parturition, but then maintained or gained body condition during the first 5 weeks postpartum. De Vries and Veerkamp (2000) found that 18% of the cows in their study did not lose any body condition. Carvalho et al., (2014) conveyed a considerably higher proportion where 58% of cows actually maintained or gained body condition during the first 21 d postpartum. Cows that maintained or gained body condition during the first 21 d postpartum had a significant increase in PR/AI and reduced DIM to pregnancy compared to cows that lost body condition (Carvalho et al., 2014).

Additionally, there are differences between parities regarding postpartum body condition change and reproductive success. Berry et al., (2006) found that 1st parity cows had the greatest body condition scores at parturition, but 3rd parity cows lost the most body condition during the first 60 DIM. Roche et al., (2007) reported 1st parity cows to have the greatest body condition scores at parturition and the lowest body condition scores at 240 DIM. Ruegg and Milton (1995) found no overall relationship between parity and body condition loss, but primiparous cows gained body condition at a slower rate than multiparous cows. De Vries et al., (1999) concluded that there were differences in energy balance profiles between primiparous and multiparous cows; where primiparous cows had smaller energy deficits in early lactation.

Primiparous cows generally have greater PR/AI than multiparous cows (Gröhn and Rajala-Schultz, 2000; Santos et al., 2009; Carvalho et al., 2014). One study has also reported lower rates of pregnancy loss in primiparous cows (Santos et al., 2009). Other studies have

contradicted this finding though, reporting no difference in pregnancy loss rates between primiparous and multiparous cows (Yousuf et al., 2016; Martins et al., 2017; Martins et al., 2018). Carvalho et al., (2014) found that like multiparous cows, primiparous cows that maintained or gained body condition during the first 21 DIM had greater PR/AI. However, Domecq et al., (1997) found that body condition loss negatively affected PR/AI in multiparous cows only. The relationship between body condition change, health and reproductive success in 1st parity cows is unclear.

Low body condition and/or body condition loss has been reported to negatively impact reproductive success (Domecq et al., 1997; Moreira et al., 2000; Santos et al., 2009). Cows that are over conditioned at parturition appear to lose more body condition (Butler and Smith, 1989; Ruegg and Milton, 1995; Roche et al., 2007). It is reasonable to believe that cows with extended calving intervals would be over conditioned at calving (Ruegg and Milton; 1995; Berry et al., 2006; Roche et al., 2007). Thus, experiencing greater body condition loss and reduced reproductive success in the next lactation. The relationship between time to pregnancy in one lactation and body condition change, health and fertility variables in the subsequent lactation needs to be better understood. The relationship between body condition change, health and reproductive success also needs to be better understood in primiparous cows. Controlling time to 1st AI, maximizing PR/AI at 1st and subsequent AI, and utilizing an early non-pregnancy diagnosis method to re-inseminate non-pregnant cows as soon as possible are critical to creating timely pregnancies (Pursley et al., 1997; Fricke, 2002; Giordano et al., 2013).

CREATING TIMELY PREGNANCIES: 1st AI SYNCHRONIZATION PROGRAMS

The first step to achieving a timely pregnancy is controlling time to 1st AI. This was made possible for the first time when Ovsynch was developed (Figure 2.1; Pursley et al., 1995; Pursley et al., 1997). Ovsynch allowed producers to control time to insemination by synchronizing ovulation of the dominant follicle with GnRH and $PGF_{2\alpha}$ (Pursley et al., 1995; Pursley et al., 1997). When compared to breeding to an estrus, cows that received Ovsynch for 1st AI were inseminated earlier in lactation and time to 1st AI was less variable (Pursley et al., 1997).

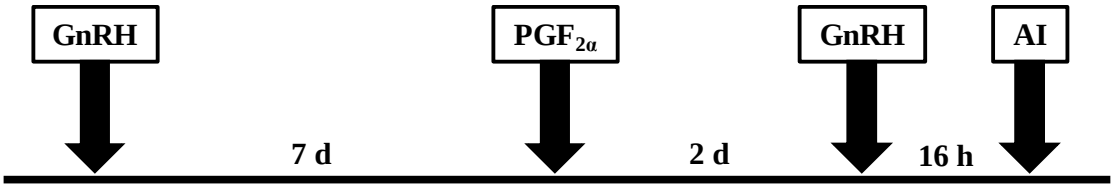

Figure 2.1 A schematic representation of the Ovsynch protocol.

The Ovsynch protocol (Figure 2.1) is initiated at random stages of the estrus cycle (Pursley et al., 1995). The initial GnRH induces a LH surge to ovulate the dominant follicle and induce a new follicular wave (Pursley et al., 1995; Nation et al., 2000; Silvia et al., 2002). If animals respond to the initial GnRH there will be a new dominant follicle on the day of the $PGF_{2\alpha}$. The $PGF_{2\alpha}$ will then regress CL from the previous ovulation (Pursley et al., 1995). Studies have shown that CL need to be at least 5 days old to respond to the luteolytic effects of $PGF_{2\alpha}$ (Howard and Britt, 1990; Pursley et al., 1995; Levy et al., 2000). The final GnRH induces another LH surge to ovulate the new dominant follicle. If cows responded to the first GnRH, the new dominant follicle would be 8 d old. Approximately 96% of cows with follicles 5 to 9 d old will respond to a GnRH induced LH surge (Vasconcelos et al., 1999). Ovulation occurs 24 to 32

h after the final GnRH (Pursley et al., 1995). The 16 h interval between the final GnRH and AI optimizes the timing between ovulation and insemination and maximizes PR/AI (Pursley et al., 1998). The first Ovsynch field trial reported 37% PR/AI (Pursley et al., 1997). A metanalysis showed no difference in PR/AI between Ovsynch and estrus, but overall pregnancy rates are improved by utilizing Ovsynch because time to insemination was controlled (Raibee et al., 2005). Modifications to the Ovsynch protocol have since dramatically improved PR/AI in lactating dairy cows (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008).

Limiting factors to the success of the Ovsynch protocol include: stage of the estrous cycle at the 1st GnRH, quality of the dominant follicle, circulating concentrations of progesterone, and luteolysis (Vasconcelos et al., 1999; Martins et al., 2011; Wiltbank and Pursley, 2014). There are differences in the success of the Ovsynch protocol depending on the stage of the estrous cycle when the first GnRH is administered (Vasconcelos et al., 1999). Vasconcelos et al., (1999) showed that 96% of all cows on d 5 to 9 of the estrous cycle ovulated to the first GnRH. Bello et al., (2006) found that a greater portion of cows ovulated to the first GnRH of Ovsynch on d 6 of the cycle when compared to d 4 and 5. Cows that ovulated to the first GnRH had higher synchronization rates than cows that did not ovulate to the first GnRH (Vasconcelos et al., 1999; Bello et al., 2006). Cows are considered to be completely synchronized if P_4 is high before the injection of $PGF_{2\alpha}$, P_4 is low on the day of the final GnRH, and the dominant follicle(s) ovulates in response to the final GnRH (Giordano et al., 2012b). Cows that are completely synchronized yield greater PR/AI than cows that are not completely synchronized (Bello et al., 2006; Giordano et al., 2012b). Response to the 1st GnRH is critical to the success of the rest of the Ovsynch protocol and resulting PR/AI (Vasconcelos et al., 1999; Bello et al., 2006; Cerri et al., 2009).

The size of the dominant follicle may be an indicator of its maturity (Bello et al., 2006; Perry et al., 2007). More mature follicles with extended periods of dominance produce poor quality embryos (Cerri et al., 2009). Larger ovulatory follicles had lower PR/AI and greater pregnancy loss than smaller ovulatory follicles in a study by Vasconcelos et al., (1999). Bello et al., (2006) and Perry et al., (2007) suggested there is a quadratic relationship between fertility and ovulatory follicle size. Ovulatory follicles close to 16 mm in diameter resulted in the greatest PR/AI for lactating dairy cows (Bello et al., 2006). Older follicles also have reduced ovulation rates (Vasconcelos et al., 1999). Second wave dominant follicles have been reported to result in greater PR/AI than first wave dominant follicles (Bisinotto et al., 2010; Denicol et al., 2012). It is unclear if differences in fertility are a result of the follicle itself or the low P_4 environment during the first follicular wave (Bisinotto et al., 2010).

Low circulating concentrations of P_4 during follicular development allows for a higher frequency of LH pulses which can create large dominant follicles with reduced fertility (Ireland and Roche, 1982; Savio et al., 1993b; Cerri et al., 2009; Cerri et al., 2011a). Conversely, high P_4 during follicular development, as measured on the day of the $PGF_{2\alpha}$, increases the probability of pregnancy (Bello et al., 2006; Martins et al., 2011; Denicol et al., 2012). Low P_4 on the day of the $PGF_{2\alpha}$ has been reported to decrease response to $PGF_{2\alpha}$ and increase chances of incomplete CL regression (Martins et al., 2011; Wiltbank et al., 2015). Cows with low P_4 during ovulatory follicle development have been reported to have greater rates of pregnancy loss compared to cows with high P_4 (Wiltbank et al., 2012; Martins et al., 2018). Low circulating concentrations of P_4 during ovulatory follicle development have also been associated with a higher rate of double ovulations (Cerri et al., 2011a; Cerri et al., 2011b; Martins et al., 2018). Double ovulations are undesirable on dairy farms because they lead to twinning. Twin pregnancies, especially

ipsilateral twins, are associated with higher rates of pregnancy loss (López-Gatius et al., 2002; Echternkamp et al., 2007; Martins et al., 2018). Cows that do carry twins to term often experience higher incidences of periparturient health events which can negatively affect milk production and reproductive performance (Nielen et al., 1989). Greater circulating concentrations of P_4 before luteolysis are important for fertility (Bello et al., 2006; Martins et al., 2011; Denicol et al., 2012).

It is equally as important for P_4 to rapidly decline after the injection of $PGF_{2\alpha}$. $PGF_{2\alpha}$ causes luteolysis which is characterized by regression of the CL. Regressing the CL causes a decrease in circulating concentrations of P_4 (McCracken et al., 1999). Souza et al., (2007) used a cutoff of 0.5 ng/mL to assess luteolysis where cows with $\leq$ 0.5 ng/mL of P_4 were considered to have full CL regression. Lack of complete CL regression has been reported to occur 10 to 25% of the time with the Ovsynch protocol (Brusveen et al., 2009; Martins et al., 2011; Wiltbank and Pursley, 2014). Incomplete luteolysis reduces PR/AI (Moreira et al., 2000; Souza et al., 2007; Martins et al., 2011). To overcome this issue, Brusveen et al., (2009) added an additional $PGF_{2\alpha}$ to the Ovsynch protocol 1 d after the original $PGF_{2\alpha}$. The extra $PGF_{2\alpha}$ increased luteal regression by 11% (Brusveen et al., 2009). No improvements in PR/AI were observed in this study, but Wiltbank et al., (2015) reported 10% more pregnancies when cows were treated with an extra $PGF_{2\alpha}$ during both the Double-Ovsynch and Ovsynch protocol. In a review on timed-AI programs, Wiltbank and Pursley (2014) determined that adding an additional $PGF_{2\alpha}$ after the original $PGF_{2\alpha}$ will increase PR/AI 3 to 5%.

Taking into consideration these limiting factors, researchers developed presynchronization protocols to ensure cows are at an optimum stage of the estrous cycle when the Ovsynch protocol is initiated. By initiating Ovsynch at a more optimum stage,

synchronization rates are improved which creates the ideal hormonal environment and yields greater PR/AI (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008). Presynch-11, G6G, and Double-Ovsynch are among the most commonly used presynchronization programs (Figure 2.2; Wiltbank and Pursley, 2014).

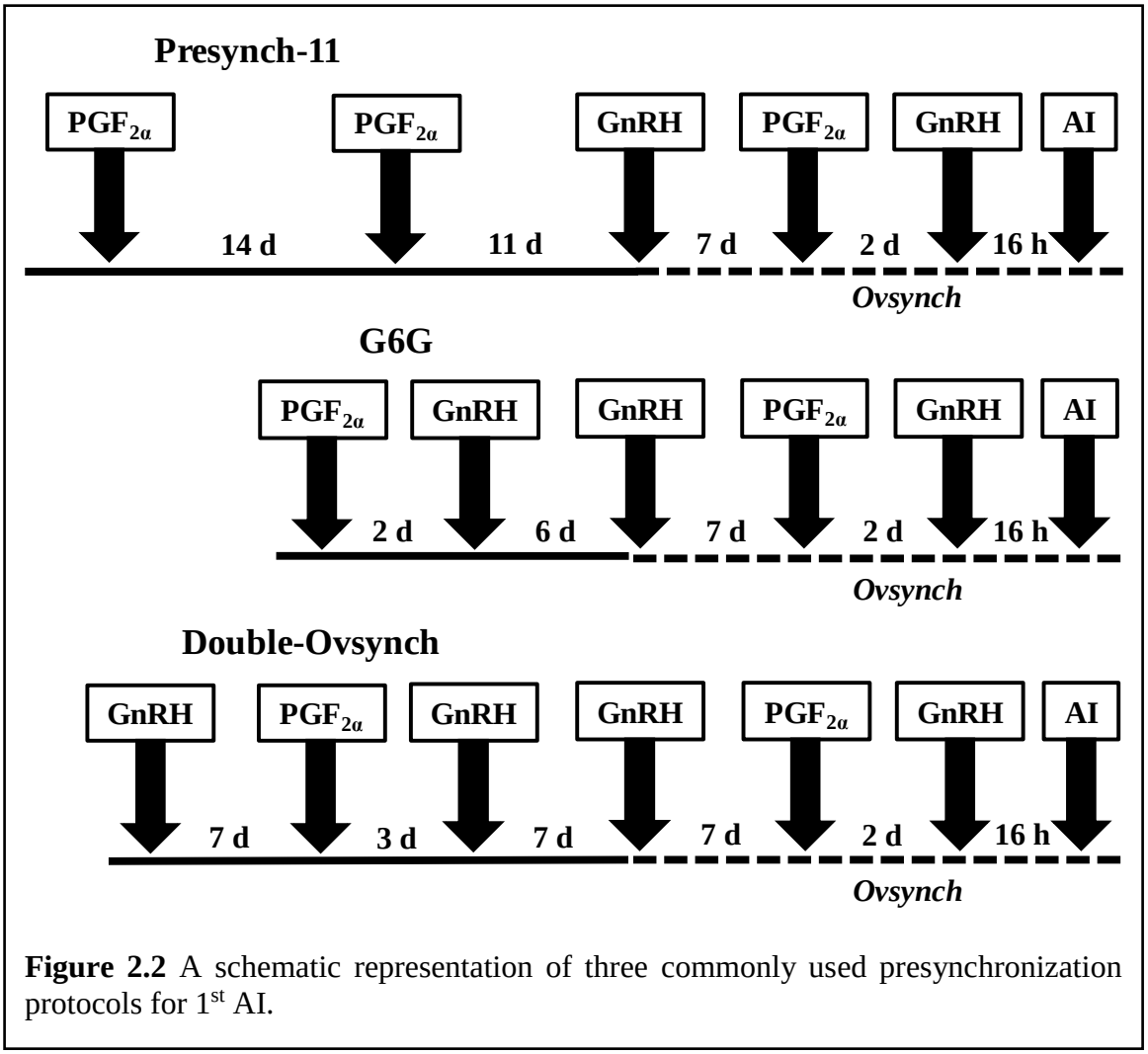

Figure 2.2 A schematic representation of three commonly used presynchronization protocols for 1st AI.

Briefly, Presynch-11 administers two injections of $PGF_{2\alpha}$ 14 d apart. The last $PGF_{2\alpha}$ is 11 d before the first GnRH of the Ovsynch protocol (Moreira et al., 2001; Galvão et al., 2007). The design intends to have cows between d 5 and 11 in the estrous cycle when the Ovsynch protocol

is initiated (Moreira et al., 2001). The original study reported 40.5% PR/AI (Galvão et al., 2007). A later study reported 49.2% PR/AI when cows were bred to Presynch-11-Ovsynch (Strickland et al., 2010). G6G administers a $PGF_{2\alpha}$ at a random point in the estrous cycle and then GnRH 2 d later. The Ovsynch protocol is initiated 6 d later at d 6 in the estrous cycle. The original study reported 50% PR/AI (Bello et al., 2006). Double-Ovsynch uses the Ovsynch protocol to pre-synchronize cows so that they are at d 7 of the estrous cycle when the Ovsynch for timed-AI is initiated. The original study reported 49.7% PR/AI (Souza et al., 2008). These protocols are often referred to as fertility programs because their use has significantly increased PR/AI in dairy herds (Wiltbank and Pursley, 2014).

The majority of dairy farms in the United States use hormonal synchronization and timed-AI programs (Caraviello et al., 2006). Fertility programs for 1st AI have dramatically improved PR/AI to 1st AI and ensure that all cows are inseminated in a timely matter (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008). This is a critical first step in creating timely pregnancies. Non-pregnant cows need to be quickly and accurately identified so they can also be re-inseminated in a timely matter (Fricke, 2002; Giordano et al., 2013).

CREATING TIMELY PREGNANCIES: EARLY NON-PREGNANCY DIAGNOSES

Non-pregnancy diagnoses are an essential part of reproductive management in dairy farms. Non-pregnancy can be diagnosed either directly or indirectly (Fricke et al., 2016). Direct methods that physically palpate or visualize the embryo/fetus include transrectal palpation and transrectal ultrasound. Indirect methods use quantitative or qualitative measures of conceptus-specific substances or hormones in maternal serum or milk (Fricke et al., 2016). The main considerations for utilization of one non-pregnancy diagnosis method over another are:

sensitivity, specificity, feasibility, cost, and time to diagnosis (Giordano et al., 2013; Fricke et al., 2016).

Sensitivity is the proportion of true positives (Altman and Bland, 1994). In terms of non-pregnancy diagnoses, sensitivity refers to the proportion of non-pregnant cows that were truly non-pregnant. A pregnant cow diagnosed as non-pregnant would be a false negative (Giordano et al., 2013). High sensitivity is critical to a non-pregnancy diagnosis because resynchronization protocols for non-pregnant cows can terminate pregnancy if a cow was misdiagnosed (Fricke et al., 2016). This incurs cost to the dairy (Fricke et al., 2016) and delays time to pregnancy (Fricke, 2002). Specificity is the proportion of true negatives (Altman and Bland, 1994). Specificity of a non-pregnancy diagnosis refers to the proportion of pregnant cows that were truly pregnant. A non-pregnant cow diagnosed as pregnant would be a false positive (Giordano et al., 2013). Although sensitivity has greater economic implications than specificity (Giordano et al., 2013), diagnosing non-pregnant cows as pregnant delays time to re-insemination and pregnancy (Fricke et al., 2016). Low sensitivity and specificity add to the initial cost of the non-pregnancy diagnosis (Giordano et al., 2013). Time to diagnosis is also critical. An early non-pregnancy diagnosis allows non-pregnant cows to be re-inseminated sooner (Fricke, 2002; Giordano et al., 2013). Thus, giving cows more chances to attain a timely pregnancy.

Transrectal palpation is the oldest method of non-pregnancy diagnosis (Fricke et al., 2016) and is performed by a trained veterinarian. The veterinarian palpates for four positive signs of pregnancy: the amniotic vesicle, fetal membrane slip, placentomes, and the fetus itself (Momont, 1990; Youngquist, 2006). The fetus, however, is not palpable until 65 days in gestation and placentomes are not palpable until 75 days in gestation (Youngquist, 2006). The accuracy of rectal palpation depends on the veterinarian's skill (Youngquist, 2006). Typically, a

skilled veterinarian can accurately diagnose pregnancy 30 to 35 d in gestation (Momont, 1990; Youngquist, 2006). Accuracy will increase as d in gestation increases.

Another method of non-pregnancy diagnosis is transrectal ultrasound. Pregnancy is confirmed by visualizing nonechodense fluid in the uterine lumen, presence of a CL on the corresponding ovary, and a viable embryo with a heartbeat (Romano et al., 2006). Pregnancy diagnosis with ultrasound again depends on the skill of the technician, but can be done with 97.7% sensitivity and 87.8% specificity from 26 to 33 d post-AI (Pieterse et al., 1990). It is recommended to not use ultrasound for non-pregnancy diagnosis until 30 d post-AI to maximize accuracy (Fricke et al., 2016).

Blood and milk samples that assay for PAGs can also be used to diagnose pregnancy indirectly (Fricke et al., 2016). PAGs are enzymatically inactive members of the aspartic proteinase gene family produced in the placenta of ruminants (Xie et al., 1991). There are 22 different PAGs in cattle that vary in temporal and spatial expression during pregnancy (Green et al., 2000; Prakash et al., 2009). Often, indirect methods of non-pregnancy diagnosis cannot provide diagnoses until samples are analyzed in a laboratory. The lag time prevents producers from making management decisions immediately (Fricke et al., 2016). However, utilizing PAGS and similar indirect methods provides an opportunity to perform non-pregnancy diagnoses with limited handling by utilizing milk samples (Fricke et al., 2016). At 32 d post-AI ELISAs for blood and milk PAGs are 92% and 89% accurate, respectively (Ricci et al., 2015).

Before PAGs were well understood, Butler et al., (1982) discovered PSPB. Sasser et al., (1986) developed a radioimmunoassay for PSPB to detect pregnancy in cows. The trophectoderm of the embryo contains binucleated cells that produce PSPB. PSPB is released from these cells as the trophectoderm begins to attach to the uterine epithelium around 17 d in

gestation (Wooding, 1992; Roberts et al., 1996). PSPB then traverses the uterine epithelium and enters the maternal circulation where it becomes detectable by blood or milk samples (Sasser et al., 1986; Wooding, 1992).

Sasser et al., (1986) showed that PSPB was detectable in some but not all pregnant cows at 15 d post-breeding using blood samples. At 24 d post-breeding all cows had detectable PSPB and PSPB levels increased linearly throughout gestation. Arnold et al., (2012) demonstrated that levels of PSPB start to increase at d 19 post-AI in pregnant nulliparous heifers and d 22 post-AI in pregnant lactating cows. Studies have shown that due to variability between cows and assay error, PSPB tests for pregnancy diagnosis are the most accurate after 28 d post-AI (Zoli et al., 1992; Humblot, 2001). Concentrations of PSPB increase rapidly during the last 20 d of gestation and peak at parturition. After parturition, PSPB levels start to decrease (Sasser et al., 1986). PSPB is a large glycoprotein with a long half-life of about 7 to 8 d (Kiracofe et al., 1993). Levels of PSPB decrease after parturition and finally plateau at approximately 90 DIM (Kiracofe et al., 1993). Non-pregnant lactating cows > 90 DIM appear to have higher levels of PSPB than non-pregnant nulliparous heifers (Arnold et al., 2012). The reason for this has not been elucidated.

A commercially available ELISA assay for PSPB became available through BioPRYN in 2003 (Piechotta et al., 2011). Based on data from Kiracofe et al., (1993) non-pregnancy diagnoses with PSPB cannot be utilized until cows are 90 d postpartum. A single blood sample assayed for PSPB can diagnose pregnancy with 98.0% sensitivity and 97.1% specificity 28 to 35 d post-AI (Sasser et al., 1986; Piechotta et al., 2011). Some studies have reported relationships between serum levels of PSPB and pregnancy loss where lower values of PSPB were associated with a greater probability of pregnancy loss (Gábor et al., 2016; Martins et al., 2018).

Martins et al., (2018) demonstrated that two samples of PSPB could be used to diagnose pregnancy earlier than 28 d post-AI. Blood samples for PSPB were taken pre-attachment at d 16 post-AI and post-attachment at d 20, 23, and 28 post-AI. The authors showed that from d 16 to d 20 serum PSPB levels within cow were consistent regardless of pregnancy status, but from d 20 to d 23 serum PSPB levels increased within pregnant cows significantly. Cows that had an increase ≥ 28% in serum PSPB levels from d 20 to d 23 were diagnosed pregnant with 98% sensitivity and 97% specificity on d 23 post-AI. The standard used for comparison was a pregnancy diagnosis from a single PSPB sample at d 28 post-AI. Utilizing two blood samples allowed for an accurate and robust assessment of the change in serum PSPB levels within cow (Martins et al., 2018).

Early non-pregnancy diagnoses are critical to creating timely pregnancies. Non-pregnant cows need to be identified as soon as possible in order to be resynchronized for AI (Fricke, 2002; Giordano et al., 2013). It is also critical for a non-pregnancy diagnosis method to have high sensitivity and specificity (Fricke et al., 2016). Pregnant cows need to be diagnosed pregnant 100% of the time and the proportion of non-pregnant cows diagnosed pregnant needs to be kept to a minimum. There is potential to obtain an accurate non-pregnancy diagnosis earlier than any other method by using within cow PSPB samples before and after the time of attachment (Martins et al., 2018). An early non-pregnancy diagnosis may allow for the development of resynchronization protocols that inseminate cows earlier. This would give dairy cows more chances to achieve a timely pregnancy.

CREATING TIMELY PREGNANCIES: RE-INSEMINATION STRATEGIES

Re-insemination strategies are influenced by method and day of non-pregnancy diagnosis and management capabilities. Typically, cows are re-inseminated to either a detected estrus or to

a resynchronization protocol. Re-insemination strategies are challenging. There is a lot of variability between cows that limits the success of re-insemination such as: stage of estrous cycle at non-pregnancy diagnosis, asynchrony, and pregnancy loss (Silva et al., 2007). To attain a timely pregnancy, it is critical for re-insemination strategies to reduce re-insemination intervals while maximizing PR/AI.

Arguably, estrus detection generates the shortest re-insemination intervals. With estrus detection cows receive a non-pregnancy diagnosis and re-insemination simultaneously. Although using estrus detection seems highly efficient, there are several factors that reduce the efficiency of estrus detection as a re-insemination strategy. The first limiting factor is that the efficiency of estrus detection on most dairy farms is ≤ 50% (Senger, 1994; Washburn et al., 2002). Cows that are not detected in estrus will not be inseminated. Technology like pedometers or rumination collars have been developed to aid with estrus detection. These technologies have improved estrus detection rates but are costly for producers to implement (Senger, 1994). The second limiting factor is the variability in return to estrus within cows (Remnant et al., 2015). Remnant et al., (2015) showed that insemination intervals ranged from 18 to 28 days in 90% of animals leaving the remaining 10% somewhere beyond that range. Thirdly, the percentage of anestrous dairy cows in the United States has been reported to range from 0 to 38% (Lucy, 2001). These cows will not show an estrus. Lastly, cows with high milk production have been reported to have a shorter duration of estrus than cows with lower milk production (Lopez et al., 2004). For high milk producers, this decreases the probability of being inseminated to an estrus (Lopez et al., 2004).

Pursley et al., (1997) showed that using a synchronization program for 2nd and 3rd AI reduced the median days to pregnancy by 19 d compared to estrus detection. An economic

simulation showed that 100% timed-AI programs outperformed 100% estrus detection programs (Giordano et al., 2012a). However, farms with low PR/AI could benefit from incorporating estrus detection into a timed-AI program (Giordano et al., 2012a). Clearly, estrus detection is not efficient enough to be used as the sole re-insemination strategy and there is a need to control days to insemination. This is possible through the use of hormonal resynchronization protocols (Fricke, 2002).

The variability in stage of the estrous cycle after a non-pregnancy diagnosis poses a significant challenge to the success of resynchronization protocols (Silva et al., 2007). The average estrous cycle for dairy cows is 22.9 ± 0.7 d (Sartori et al., 2004). Cows with atypical cycles, however, have an average estrous cycle of 29.3 ± 1.1 d (Sartori et al., 2004). Lack of synchronization during the 1st AI protocol increases the variability in stage of the estrous cycle even more (Silva et al., 2007). Cows that were pregnant but experienced embryonic loss ≥ 16 d in gestation will have a prolonged luteal lifespan and thus return to estrus later than expected (Northey and French, 1980; Van Cleeff et al., 1991; Humblot, 2001). This variability makes it more difficult to initiate Ovsynch during mid estrous cycle (Vasconcelos et al., 1999). To overcome this issue, it seems logical to use a presynchronization program similar to 1st AI, but the length of these programs creates too long of re-insemination intervals (Wiltbank and Pursley, 2014). Shorter protocols are more ideal for an aggressive re-insemination program.

Most resynchronization programs use Ovsynch (Pursley et al., 1995), or some variation of Ovsynch. One variation involves administering the first GnRH of Ovsynch a week before non-pregnancy diagnosis. All cows regardless of pregnancy status would receive GnRH. At non-pregnancy diagnosis non-pregnant cows would receive $PGF_{2\alpha}$ and then finish the protocol. Although all pregnant cows receive an unnecessary injection of GnRH, non-pregnant cows are

re-inseminated one week earlier compared to an Ovsynch protocol initiated on the d of the non-pregnancy diagnoses (Fricke, 2002; Fricke et al., 2003). Fricke et al., (2003) and Chebel et al., (2003) both showed that administering GnRH before non-pregnancy diagnosis does not induce iatrogenic embryonic loss in pregnant cows. This resynchronization strategy yielded similar PR/AI to Ovsynch (Fricke et al., 2003). There is some evidence though that the d the first GnRH is administered affects PR/AI. Fricke et al., (2003) showed that initiating Ovsynch 19 d post-AI produced fewer PR/AI than initiating Ovsynch 26 or 33 d post-AI. Sterry et al., (2006) found that initiating Ovsynch 26 d post-AI yielded fewer PR/AI than initiating Ovsynch 33 d post-AI.

Another modification of Ovsynch used for resynchronization is the incorporation of CIDRs. CIDRs are controlled internal drug releasing devices that are used to supplement P_4 (Macmillan et al., 1991; Macmillan and Peterson, 1993; Xu and Burton, 2000). The purpose of CIDRs are to improve synchrony (Lima et al., 2009; Bilby et al., 2013) and to prevent spontaneous ovulations (Sirois and Fortune, 1990; Chebel et al., 2006; Bilby et al., 2013). Some studies have reported that using CIDRs in the Ovsynch protocol can increase PR/AI (Chebel et al., 2006; Dewey et al., 2010). Although this benefit may be greater for cows with low circulating P_4 at the initiation of the protocol or for cows with no CL (Bilby et al., 2013).

The P_4 from the CIDR suppresses LH pulses and prevents cows from ovulating prematurely (Sirois and Fortune, 1990; Chebel et al., 2006; Bilby et al., 2013). When used in an Ovsynch protocol, CIDRs are usually inserted on the day of the first GnRH and then removed on the day of the $PGF_{2\alpha}$ (Chebel et al., 2006; Dewey et al., 2010; Bilby et al., 2013). If a cow has low circulating P_4 or no CL on the d of the $PGF_{2\alpha}$, removing the CIDR may allow an LH surge to cause an ovulation before the final GnRH and AI. It is not clear if removing the CIDR on the day of the $PGF_{2\alpha}$ or the d after is better for PR/AI.

Some researchers have developed more intricate resynchronization strategies that attempt to maximize PR/AI by basing the protocol off of the presence of a CL at non-pregnancy diagnosis (Giordano et al., 2016; Kelley et al., 2016). Giordano et al., (2016) administered a GnRH 32 d post-AI to all cows. Non-pregnancy diagnosis was performed with ultrasound 39 d post-AI. At non-pregnancy diagnosis cows with a CL ≥ 15 mm received $PGF_{2\alpha}$ per the Ovsynch protocol and were re-inseminated 42 d post-AI. Cows that either had no CL, a CL < 15 mm, or were considered cystic either went on an Ovsynch+P_4 or a PreG-Ovsynch protocol. These cows were considered to have suboptimal fertility due to lack of P_4. The Ovsynch+P_4 treatment initiated Ovsynch on the d of the non-pregnancy diagnosis with the addition of a CIDR. Cows in the Ovsynch+P_4 treatment were re-inseminated 49 d post-AI. The PreG-Ovsynch treatment administered another GnRH on the d of the non-pregnancy diagnosis then 7 d later cows started the Ovsynch protocol. Cows were in the PreG-Ovsynch treatment were re-inseminated 56 d post-AI. The Ovsynch+P_4 and PreG-Ovsynch treatments produced similar PR/AI as the cows in the Ovsynch group.

Kelley et al., (2016) inserted a CIDR into all cows 13 d post-AI and then removed it on d 20 post-AI. All cows were scanned with ultrasound on d 13 and d 20 post-AI. Cows that did not have a CL ≥ 15 mm, or had a CL that decreased ≥ 10 mm from d 13 to d 20, and had a follicle ≥ 12 mm on d 20 received an injection of GnRH and were re-inseminated on d 21 post-AI. All other cows were resynchronized with Ovsynch. PR/AI were similar to Ovsynch and the re-insemination interval was shortened, but only 45% of non-pregnant cows were actually re-inseminated with this strategy. These resynchronization strategies take a more individualized approach than traditional synchronization programs. PR/AI appear to be similar to PR/AI with Ovsynch (Giordano et al., 2016; Kelley et al., 2016). The complexity of these protocols may not

always be feasible for producers though. This type of resynchronization strategy also requires the use of ultrasound for all non-pregnancy diagnoses.

Hormonal resynchronization protocols can shorten re-insemination intervals and increase the chances for timely pregnancies (Fricke, 2002). The dairy industry needs short, simple, and effective resynchronization protocols. Shortened resynchronization strategies are only effective if they result in acceptable PR/AI (Green et al., 2011). CIDRs can improve synchrony and benefit PR/AI for cows with low P_4 (Chebel et al., 2006; Dewey et al., 2010; Bilby et al., 2013), but the optimal day to remove CIDRs needs to be determined. The development of early non-pregnancy diagnosis methods may create new opportunities for shorter resynchronization protocols.

SUMMARY

Timely pregnancies are economically important (Dijkhuizen et al., 1984; Meadows, 2005; Giordano et al., 2011). Body condition loss during early lactation can reduce PR/AI (Domecq et al., 1997; Moreira et al., 2000; Santos et al., 2009). Thus, extending time to pregnancy and resulting calving intervals (Pryce et al., 2001; Carvalho et al., 2014). Body condition loss is also associated with postpartum health events (Ruegg and Milton, 1995; Gillund et al., 2001; Berry et al., 2007b). The relationship between time to pregnancy in one lactation and physiological changes in regards to body condition, fertility and health in the next lactation needs to be better understood. It is also important to establish the time frame for a timely pregnancy and manage cows in a way that maximizes chances for pregnancy during this period.

Achieving timely pregnancies will depend on reproductive programs with high PR/AI and short re-insemination intervals. Early non-pregnancy diagnoses play an important role in reducing re-insemination intervals. Within cow PSPB samples (Martins et al., 2018) have the potential to diagnose non-pregnancy in lactating dairy cows earlier than any other method.

Further research is needed to develop a more accurate early non-pregnancy diagnosis method. The use of fertility programs like Presynch-11, G6G, and Double Ovsynch have increased PR/AI at 1st AI (Moreira et al, 2001; Bello et al., 2006; Souza et al., 2008), but are not ideal for 2nd + inseminations (Wiltbank and Pursley, 2014). There is a need for early resynchronization protocols to be developed that reduce re-insemination intervals and maximize PR/AI. Combining the use of an early non-pregnancy diagnosis and a short resynchronization protocol can give cows more chances to attain a timely pregnancy.

CHAPTER 3

THE HIGH FERTILITY CYCLE: HOW TIMELY PREGNANCIES IN ONE LACTATION MAY LEAD TO LESS BODY CONDITION LOSS, FEWER HEALTH ISSUES, GREATER FERTILITY, AND REDUCED EARLY PREGNANCY LOSS IN THE NEXT LACTATION

A version of this manuscript has been accepted for publication in the Journal of Dairy Science on January 23rd, 2019

E. L. Middleton, T. M. Minela, and J. R. Pursley

Department of Animal Science

Michigan State University

ABSTRACT

Body condition loss during early lactation varies amongst cows in the herd and is associated with future health and reproductive outcomes. The objective of this study was to gain a greater understanding of the relationship between previous calving interval and body condition change during the 1st 30 days in milk (**DIM**) and their relationship to subsequent fertility and health variables, and sex ratio at birth. Dry cows and heifers (n = 851) from a single farm entered the study approximately 25 days prior to due date. They were evaluated and given a body condition score on a 1 to 5 scale with 1/10-point increments weekly until parturition. Body condition score was assessed within 1 week of parturition then again 27 to 33 DIM. Previous calving interval, gestation length, periparturient health events (giving birth to twins, dystocia, retained placentas, ketosis, metritis, and displaced abomasum), sire net merit $, and milk data were utilized for each cow as recorded in PCDART (DRMS; Raleigh, NC) by the herd managers. Longer previous calving intervals were related to greater body condition at parturition and body condition loss during the 1st 30 DIM. Cows with a calving-to-pregnancy interval shorter than 130 d had a 75% greater proportion of cows that maintained or gained body condition during the 1st 30 DIM compared to cows with calving-to-pregnancy intervals longer than 130 days. Multiparous cows that maintained or gained body condition (n = 144) had greater PR/AI following 1st service compared to cows that lost body condition (n = 577) during the first 30 DIM when health events were both considered or removed. When cows with health events were considered, multiparous cows that maintained or gained body condition had a greater percentage pregnant by 130 DIM (67 vs 55%; n = 522) compared to cows that lost body condition. Cows that lost body condition during the 1st 30 DIM regardless of health events experienced greater pregnancy loss (n = 224) between 35 and 60 d after 1st AI (0.0 vs. 6.7%)

compared to cows that maintained or gained body condition (n = 69) during that period. Based on data in this study from a single herd, maintaining a cycle of pregnancy prior to 130 DIM may reduce the amount of body condition lost after the next parturition, enhance subsequent PR/AI, and reduce the possibility of early pregnancy loss. We refer to this phenomenon as the "high fertility cycle."

INTRODUCTION

Physiological changes in early lactation can alter subsequent fertility of lactating dairy cows (Carvalho et al., 2014). It is common for dairy cows to lose body condition after parturition. Multiple studies demonstrated that low, and/or loss of, body condition creates fewer pregnancies per AI (**PR/AI**) at first service (Domecq et al., 1997; Moreira et al., 2000; Santos et al., 2009). Furthermore, Carvalho et al., (2014), reported a significant increase in PR/AI if cows gained or maintained body condition during the first 3 wk postpartum.

Cow health affects reproductive success (Ribeiro et al., 2013). Body condition is a key indicator of cow health and a useful tool in monitoring the nutritional state of dairy cattle (Heuer et al., 1999). Changes in body condition in early lactation are associated with postpartum health events such as: twinning, dystocia, retained placenta (**RP**), ketosis, metritis, and displaced abomasum (Ruegg and Milton, 1995; Gillund et al., 2001; Berry et al., 2007b). Dohoo and Martin (1984) reported the presence of a single clinical disease may predispose cows to other diseases in current and future lactations, particularly mastitis and metabolic diseases.

Pryce et al., (2001) indicated that poor reproductive performance and low body condition may extend the length of calving interval (**CI**). A cow's body condition during the 1st 30 DIM can be used as an indicator of CI (Pryce et al., 2000). Calving interval is an important

reproductive variable with economic consequences for dairy cows. The ideal CI for most cows was determined to be 12 to 13 months (Morris, 1971). Body condition score profiles are mirror images of lactation curves (Berry et al., 2006; Roche et al., 2007), so it is reasonable to believe that cows with an extended CI may become over-conditioned resulting in a greater loss of body condition following the next parturition (Ruegg and Milton, 1995; Heuer et al., 1999) and an increased mortality risk (Shahid et al., 2015).

There is a need for a greater understanding of the association between previous CI, BCS, fertility and health in the next lactation. The main objective of this study was to gain a greater understanding of the association between previous calving interval on body condition change during the 1st 30 DIM and their relationship to subsequent fertility and health variables and sex ratio at birth. We hypothesized that time to pregnancy in the previous lactation will be associated with body condition change, health and fertility variables in the subsequent lactation. A secondary objective was to describe how body condition changes and other measurements in 1st parity cows were associated with fertility and health variables.

MATERIALS AND METHODS

Cows, Housing and Materials

This trial was conducted from March 2016 to June 2017 on a commercial Holstein dairy farm (Nobis Dairy Farm, St. Johns, MI, USA). The farm milked approximately 1,000 dairy cows three times a day with daily average milk production of 42 kg/cow/day. Lactating cows were fed a TMR once a day with free access to feed and water and were housed in a 4-row free-stall barn with sidewall curtains and fans. The TMR consisted of corn, wheat, and alfalfa silages, and corn-soybean meal-based concentrates formulated to meet nutrient recommendations for lactating

dairy cows (NRC, 2001). There were three basic lactating cow diets fed to 1) early lactation, 2) 1st parity, and 3) multiparous cows. Dry cows were fed two diets: 1) cows entering the dry period and 2) cows within 3 weeks of their calving date. Both dry cow diets consisted of primarily corn silage but with grass hay when entering the dry period, and straw plus concentrates in the close-up ration. Cows entering 3rd + lactations and any cows with twins received calcium supplementation at calving. The Institutional Animal Care and Use Committee at Michigan State University approved all animal handling and procedures.

Experimental Design

Heifers prior to 1st calving were included in this study even though the primary objective of this study dealt with multiparous cows. Thus, dry cows following 1st and subsequent lactations and heifers (n = 851) entered the study approximately 25 days prior to due date. Cows and heifers were evaluated and given a body condition score (**BCS**) on a 1 to 5 scale with tenth of point increments weekly until parturition. This scale was adapted from the 0.25 increments described by Edmondson et al., (1989). The BCS within 1 week of parturition was used in analyses. All cows were then assessed another BCS on the same scale at 27 to 33 days in milk (**DIM**; n = 787). All cows on this farm received 1st timed-AI 75-81 DIM using the farm managed G6G/Ovsynch program as follows: $PGF_{2\alpha}$, 2 d – GnRH, 6 d - GnRH, 7 d - $PGF_{2\alpha}$, 56 h - GnRH, 16 h - AI. Cows were diagnosed for pregnancy 35 d post-AI by the herd veterinarian using ultrasound. If diagnosed not-pregnant, cows received AI 22 d later following retreatment with G6G/Ovsynch. An additional pregnancy diagnosis was performed 60 d post-AI by the herd veterinarian using ultrasound. Fetal sex was determined (Curran, 1992) 60 to 66 d post-AI (n = 493) using transrectal ultrasonography (SonoSite MicroMaxx 10-5 MHz linear array probe; Bothwell, WA). Pregnancy was re-confirmed using a pregnancy associated glycoprotein (**PAG**)

ELISA assay 120 d post-AI and, if pregnant, again 188 d post-AI (AntelBio, NorthStar Cooperative DHI Services, Grand Ledge, MI). Fetal sex was determined with ultrasound at 60 to 66 d post-AI in all cows. However, analyses of the association between body condition loss during the 1st 30 DIM and sex ratio utilized calving information, and fetal sex at 60 to 66 d post-AI in cows that left the herd prior to parturition. Only cows with singletons were included in sex ratio analyses (n = 466; n = 400 with calving records and n = 66 with fetal sexing).

Previous CI, gestation length, periparturient health events, including giving birth to twins, dystocia, RP, ketosis, metritis, and displaced abomasum, sire net merit $, and milk data were utilized for each cow as recorded in PCDART (DRMS; Raleigh, NC) by the herd managers. Milk production information was collected every two weeks (NorthStar Cooperative DHI Services; Grand Ledge, MI). Milk production information nearest 30 and 60 DIM were chosen for analyses. The presence of bovine leukemia virus (**BLV**) and Johne's disease was determined by an ELISA assay (AntelBio, NorthStar Cooperative DHI Services, Grand Ledge, MI).

Statistical Analyses

All information was recorded in an Excel spreadsheet for organization before statistical analysis. There was a total of n = 851 primiparous (34%) and multiparous cows (66%) that received a body condition score at calving. There were n = 160 cows that left the herd following 1st BCS. Of these, n = 64 cows did not receive a second BCS, n = 20 did not receive 1st AI and n = 76 left the herd following 1st AI. Over half (52.50%) of the 18.4 % of cows that were culled or died did so before 1st AI at 75 to 81 DIM. This distribution agrees with hazards calculated by De Vries et al., (2010) where hazards peaked at 10 to 30 DIM and were at the lowest around 70 DIM. Hazards of culling gradually increased after that point (De Vries et al., 2010). Some analyses related to only pregnancy outcomes removed cows with health events (n = 126). When

analyzing data for net merit $ there were only n = 695 cows with that information in PCDART. Milk fever was not considered in analyses due to only n = 4 cows being treated for this periparturient disorder during this study.

Binomial variables were analyzed using a generalized linear mixed model fitted with the GLIMMIX procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). All models considered class of body condition change or body condition score at calving, or body condition score at 30 DIM, parity and their interactions as fixed effects, and season (month of calving), sire net merit $, health events, and 30 and 60 DIM milk production as random effects. Non-significant interactions were then removed in a stepwise fashion from the model. Only random variables with $P < 0.20$ remained in the model. Predicted probabilities of pregnancy were computed using the LOGISTIC procedure of SAS. In some cases, season and health events were considered fixed effects. Selected analyses utilized Cochran-Mantel-Haenszel chi-square analyses using the FREQ procedure of SAS for the effect class of body condition change on pregnancy outcomes. Survival curve analyses were performed using the LIFETEST procedure of SAS. Log rank P values were utilized for significance.

Continuous variables were analyzed using the CORR procedure of SAS. These included univariate analyses of the relationship between previous calving interval and body condition at calving, previous calving-to-pregnancy and subsequent gestation length.

Gestation lengths that were shorter than 250 d were removed from any analyses relating to gestation length. The percentage of single male calves was compared by the chi-square goodness-of-fit test to an expected population value of 54% obtained from a study with 12,325 calves born to multiparous Holstein-Friesian cows (Ryan and Boland, 1991).

RESULTS AND DISCUSSION

The Britt Hypothesis (Britt, 1992) states that cows undergoing body condition loss in early lactation have lower progesterone and poorer fertility compared to cows that maintained or gained body condition. Data (Britt, 1992) supporting this hypothesis delineated two groups of cows that either lost body condition or maintained, and to some extent gained, body condition following parturition. This was clearly the case in the current study although there was a greater proportion of cows that lost body condition compared to Britt, (1992). Percent of cows that lost body condition (**LBC**; 80%; n = 629) was greater (P = 0.001) than cows that maintained or gained (**MGBC**; 20%; n = 158) body condition during the 1st 30 DIM in the current study. Outcomes from this study in this single herd of cows describe how cows that become pregnant early in lactation have a greater likelihood of maintaining or gaining body condition early in lactation vs. cows that become pregnant later in lactation. This in turn increased chances of pregnancy per AI at 1st service and reduced post-partum health issues and early pregnancy loss (shown later). We refer to this as the "high fertility cycle."

Relationships Between Previous Calving Interval and Body Condition Changes During the 1st 30 DIM

Longer previous calving intervals were related to greater body condition at parturition (Figure 3.1) and body condition loss during the 1st 30 DIM (Figure 3.2). Berry et al., (2006) demonstrated that BCS profiles are mirror images of lactation curves where cows start to gain body condition later in lactation when their milk production plateaus or decreases. Multiparous cows with longer previous calving intervals gained more body condition in late lactation and thus had greater body condition at calving.

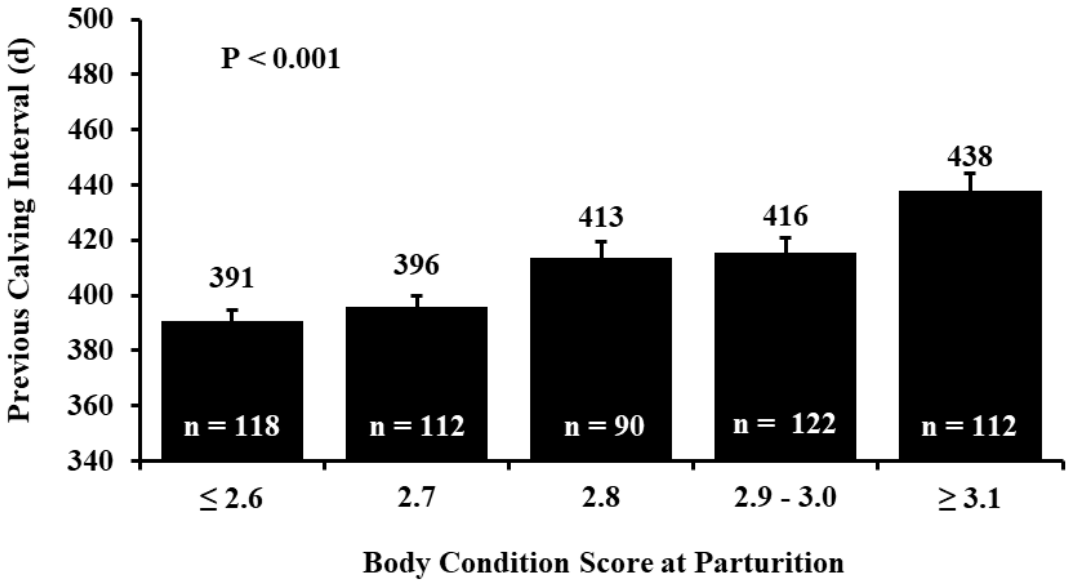

Figure 3.1 Relationship between average previous calving interval (d) for multiparous lactating Holstein dairy cows and body condition scores evaluated on a 1 to 5-point scale in tenths of points ≤ 1 wk prior to parturition. Data are shown as mean ± SEM.

Cows with a calving-to-pregnancy interval shorter than 130 days had a 75% greater chance to maintain or gain body condition during the next lactation compared to cows with calving-to-pregnancy intervals longer than 130 days (28 vs. 16%; P = 0.001; n = 475). It was clear in this herd that timing of pregnancy in one lactation may play an integral role in what happens with body condition at calving and subsequent changes during early lactation. Using 130 DIM as a reference aligns closely with Meadows (2005). That study determined that in order for individual cows to maintain a 13-month CI they cannot be open for more than 115 DIM. It seems logical to utilize fertility programs at 1st AI to control days to first service while ensuring high PR/AI (Bello et al., 2006; Souza et al., 2008) and an aggressive resynchronization program (Fricke, 2002; Giordano et al., 2013) to maximize a cow's chances to conceive before 130 DIM. Poor fertility at 1st and 2nd AI clearly extends calving intervals past 130 DIM and reduces dairy

farm profit (Dijkhuizen et al., 1984; Britt, 1985). Increasing chances for pregnancy for 1st and 2nd AI increase the chance that cows will calve at a body condition that reduces the chances for body condition loss early in lactation. This is the first step in the process of cows maintaining a high fertility cycle.

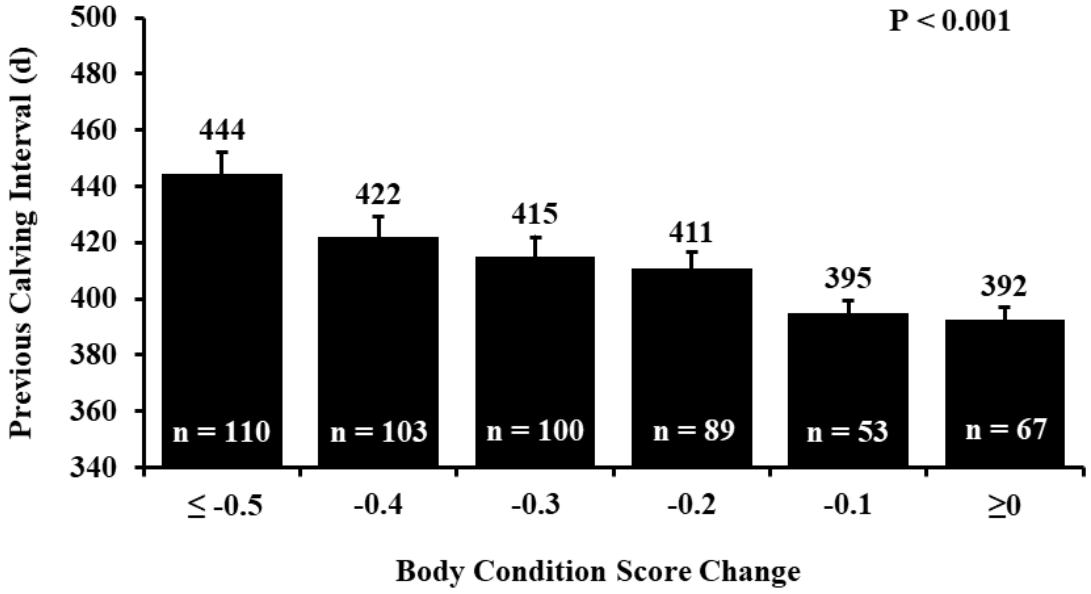

Figure 3.2 Relationship between average previous calving interval (d) and body condition change evaluated ≤ 1 wk prior to parturition and 27-33 d post-partum in multiparous lactating Holstein dairy cows. Data are shown as mean ± SEM.

Relationships Between Body Condition at Calving with Body Condition Changes During the 1st 30 DIM

Greater body condition at parturition resulted in greater loss of body condition during the 1st 30 DIM (n = 787; P < 0.01). Primiparous cows had greater body condition at parturition (3.01 ± 0.02 vs. 2.78 ± 0.01; n = 787; P < 0.01) and lost more body condition (-0.15 ± 0.01 vs. -0.11 ± 0.01; n = 787; P < 0.01) compared to multiparous cows. Ruegg and Milton (1995) and Roche et

al., (2007) also reported cows with greater BCS at parturition lost more condition. Berry et al., (2006) and Roche et al., (2007) both reported primiparous cows having greater body condition at calving than multiparous cows. Interestingly, 20% of the cows in this study did not experience body condition loss. This was similar to the 18% that De Vries and Veerkamp (1999) reported, but very low compared to the average of 58% for two herds in Carvalho et al., (2014). Interestingly, these herds were utilizing a fertility program (Double Ovsynch) for 1st AI but it wasn't clear if this had been part of the historical management of the herd. Herds in the Carvalho et al., (2014) study utilized bST. Both of these management strategies, if used in the previous 2 years, could have had an impact on body condition at parturition. It appears that one of these herds may have already been in a high fertility cycle for a long enough time to shift a greater % of cows to maintaining or gaining body condition during early lactation.

There was no relationship between class of body condition change and parity (n = 787; P = 0.29). Berry et al., 2006 found that third parity cows lost more body condition. Ruegg and Milton (1995) found no significant effect of parity on body condition change.

Cows that lost body condition had greater milk production at 30 DIM (n = 720; P = 0.01) and 60 DIM (n = 684; P = 0.001) compared to cows that maintained or gained body condition (Figure 3.3). The magnitude of body condition loss was positively associated with milk production at 30 (P = 0.02) but not 60 d (P = 0.51). Greater body condition loss has been previously associated with higher peak milk yields (Berry et al., 2007a). There may be tradeoffs from an economic perspective and it is not clear if they favor cows that maintained or gained body condition during the 1st 30 DIM that work their way into a high fertility cycle. Cows that maintain or gained would likely have fewer d in late lactation, less chance for being treated for

health issues and being culled. But, cows in the MGBC group may also have less peak milk based on milk production near 60 DIM (Figure 3.3).

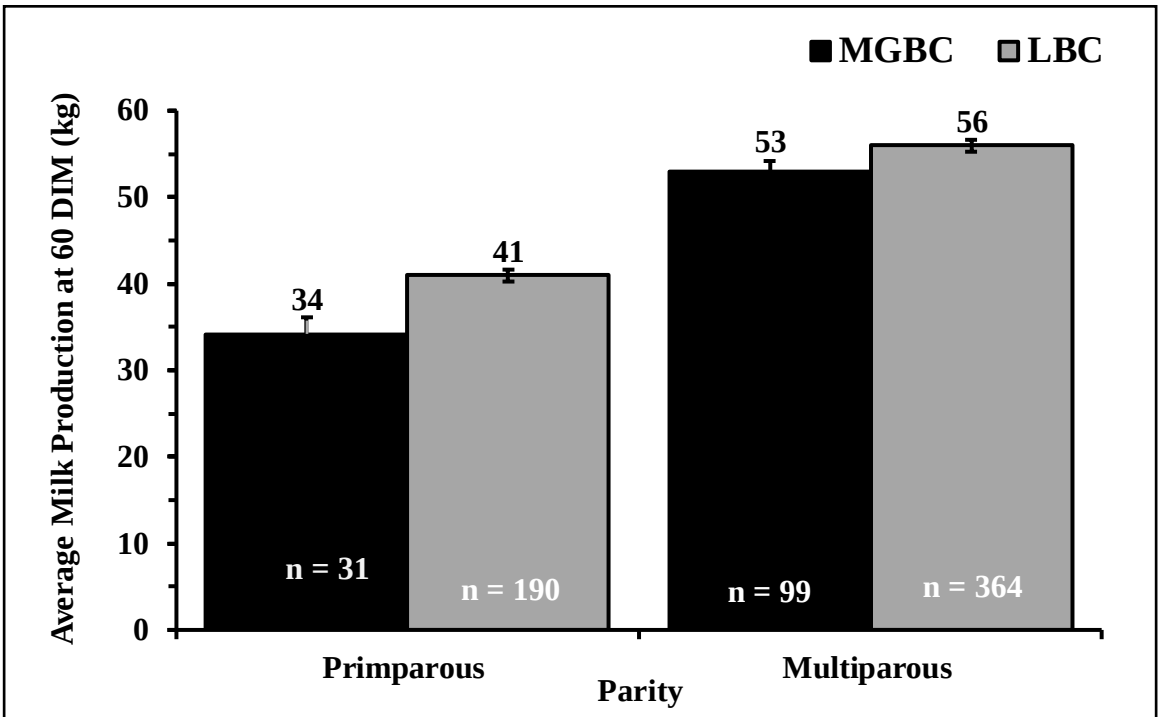

Figure 3.3 Relationship between primiparous and multiparous lactating Holstein dairy cows that maintained / gained (MGBC; n = 130) or lost (LBC; n = 554) body condition during the 1st 30 DIM and average milk production at 60 DIM (53 to 67 day range). Significance between body condition change classes and parity are as follows: Primiparous MGBC vs. Primiparous LBC (P = 0.004), Multiparous MGBC vs. Multiparous LBC (P = 0.03), Primiparous MGBC vs. Multiparous MGBC (P < 0.001), Primiparous LBC vs. Multiparous LBC (P < 0.001), Primiparous MGBC vs. Multiparous LBC (P < 0.001), Primiparous LBC vs. Multiparous MGBC (P < 0.001).

Also, cows that calved in the first four months of the year had a greater probability of losing body condition compared to cows that calved the rest of the year in both primiparous (n = 262; P = 0.002) and multiparous (n = 525; P = 0.02) cows. This relationship was likely the result of cold stress in Michigan during these months. Thus, there are clearly other factors that create

variability in body condition at parturition and body condition loss early in lactation that can negatively or positively affect the high fertility cycle.

There was a relationship between body condition change during the 1st 30 DIM and sire net merit $ (P = 0.05; n = 759) when only parity was in the model. Greater sire net merit $ increased the probability of maintaining and gaining body condition during the 1st 30 DIM. Primiparous cows had greater sire net merit values than multiparous cows (P < 0.001) but there was no interaction between class of body condition change and parity (P = 0.91).

Relationships Between Body Condition Change During the 1st 30 DIM and PR/AI in Dairy Cows

Multiparous cows that maintained or gained had greater PR/AI following 1st service compared to cows that lost body condition during the first 30 DIM when cows with health events were considered (P = 0.02; n = 577) or removed (n = 461; Figure 3.4) from the analyses. Multiparous cows that maintained and gained also had a greater percentage of cows that were pregnant by 130 DIM (64 vs 51%; P = 0.04; n = 334). Primiparous cows did not have this relationship when cows with health events were considered (P = 0.43; n = 144) or removed (n = 134; Figure 3.4) from the analyses. Removing cows with health events from this analysis reduces confounding in the relationship between body condition loss and fertility, and argues that body condition loss during the 1st 30 DIM may have a direct physiological impact on ovarian development or uterine involution at time of 1st AI. Carvalho et al. (2014) also detected a difference in PR/AI in favor of cows that maintained or gained body condition; although, the differences were substantially greater than they were in this study. This is likely due to a larger proportion of cows that actually maintained or gained body condition in the Carvalho et al., (2014) study. Santos et al., (2009), also reported reduced PR/AI when cows lost body condition.

Carvalho et al., (2014) found that the average calving-to conception interval was longer for cows that lost body condition than the cows that maintained or gained body condition. A reason for reduced fertility in the cows that lost body condition during the 1st 30 DIM could be related to the effect of negative energy balance on subsequent circulating concentrations of progesterone. Villa-Godoy et al., (1988) indicated that the longer the negative energy balance during the 1st 100 DIM, the greater the chances of having lower progesterone during the 2nd and 3rd estrous cycles. Cows at 1st AI in the present study would correlate to approximately the 3rd estrous cycle. It is possible that the cows that lost body condition during the 1st 30 DIM had lower average circulating concentrations of progesterone.

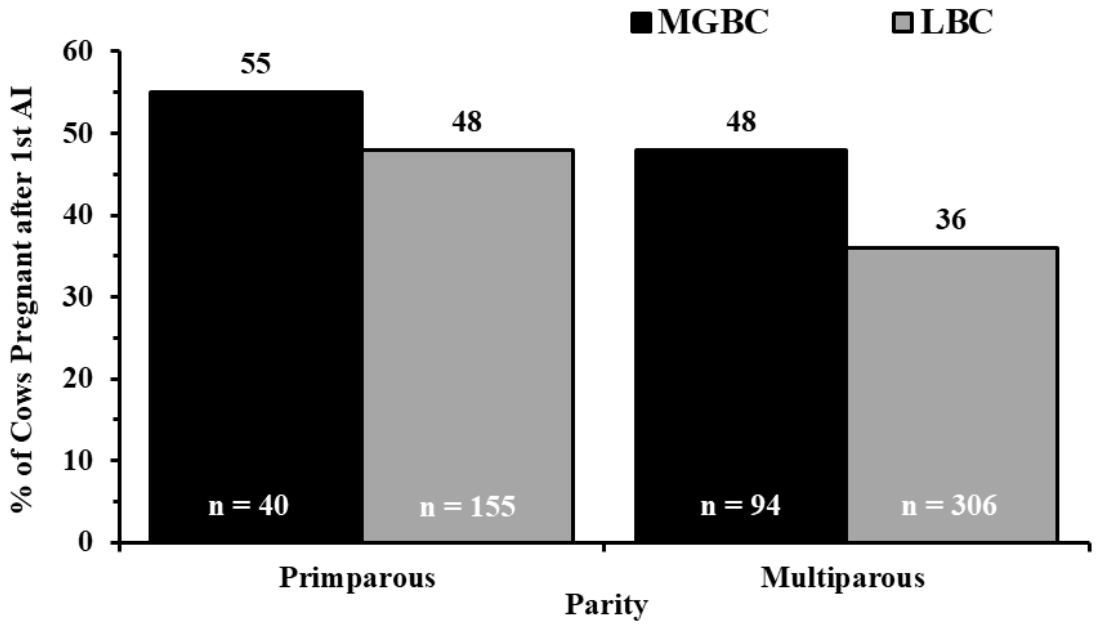

Figure 3.4 Percentage of primiparous and multiparous cows lactating Holstein dairy cows diagnosed pregnant 35 d after 1st AI that were classed into either maintained/gained (MGBC; n = 146) or lost (LBC; n = 500) body condition during the 1st 30 DIM. Cows that experienced at least 1 health event were not considered in the analyses. Significance between combinations of body condition change classes and parity are as follows: Primiparous MGBC vs. Primiparous LBC (P = 0.45), Multiparous MGBC vs. Multiparous LBC (P = 0.04), Primiparous MGBC vs. Multiparous MGBC (P = 0.44), Primiparous LBC vs. Multiparous LBC (P = 0.01), Primiparous MGBC vs. Multiparous LBC (P = 0.02), Primiparous LBC vs. Multiparous MGBC (P = 0.94).

Overall, primiparous cows (n = 274) had greater PR/AI than multiparous cows (n = 493) at 1st AI (Figure 3.4). Similarly, Carvalho et al., (2014) and Santos et al., (2009) also reported higher PR/AI for primiparous cows vs. multiparous cows. There was no difference in PR/AI for primiparous cows in MGBC vs. LBC groups, but multiparous cows had greater PR/AI in MGBC vs. LBC (Figure 3.4). Carvalho et al., (2014) reported a difference in PR/AI in multiparous cows that maintained, gained or lost body condition. This difference was also detected in primiparous cows. Domecq et al., (1997) concluded that loss of BCS contributed to conception failure in multiparous but not primiparous cows.

There was a linear relationship between body condition loss and PR/AI at 2nd (n = 213) and 3rd AI (n = 98) in cows that lost body condition but not in cows that maintained or gained. Cows with greater body condition loss during the 1st 30 DIM had reduced PR/AI at 2nd AI (P = 0.02) and 3rd AI (P = 0.03). Thus, it appears that the greater the % of cows that maintain or gain body condition during early lactation the greater the chances of pregnancy at 1st and subsequent AI. Overall, survival analysis indicated that DIM to pregnancy was influenced by class of body condition change during the 1st 30 DIM and parity (Figure 3.5). Cows that maintained or gained body condition became pregnant sooner in lactation compared to cows that lost body condition during the 1st 30 DIM. Once again, this appears to drive the high fertility cycle from lactation to lactation.

Relationships Between a Single Body Condition Score at Calving or 30 DIM on PR/AI

There was no relationship between body condition at parturition and 1st AI PR/AI (n = 721; P = 0.12). This agrees with results published by Gillund et al., (2001) and Pryce et al., (2001), but not Santos et al., (2009). In addition, there was no relationship between the BCS at 30 DIM and 1st AI PR/AI (P = 0.21; n = 721). Moreira et al., (2000) observed lower PR/AI in

cows with low BCS at the initiation of Ovsynch 63 ± 3 DIM. Typically, dairy cows in negative energy balance do not stop losing body condition until 50 to 100 DIM (Ruegg and Milton, 1995; Pryce et al., 2001).

There was no relationship between milk production at 30 (n = 664; P = 0.50) or 60 DIM (n = 638; P = 0.46) and 1st AI PR/AI. Month of parturition did not predict PR/AI at 1st AI (n = 767; P = 0.36). There was no overall relationship between sire net merit $ and 1st AI PR/AI (n = 695; P = 0.21).

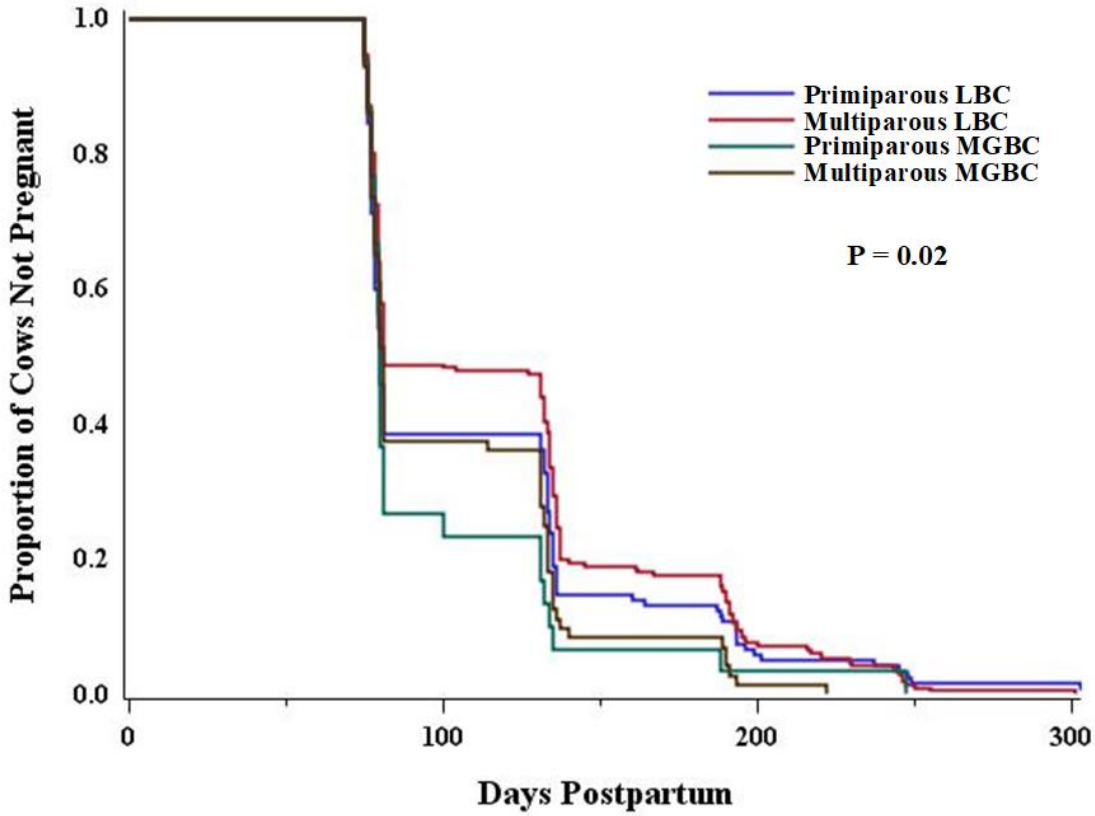

Figure 3.5 Survival curve estimates for the effect of body condition change on days to pregnancy for primiparous and multiparous lactating Holstein dairy cows (n = 435) that maintained / gained (MGBC) or lost (LBC) body condition during the 1st 30 DIM.

Relationship Between Body Condition Variables and Pregnancy Loss

Cows that lost body condition during the 1st 30 DIM experienced greater pregnancy loss between 35 and 60 d after 1st AI compared to cows that maintained or gained body condition during that period (Table 3.1) regardless whether cows with health events were considered. Carvalho et al., (2014) reported no difference in pregnancy loss rates from 40 to 70 d amongst classes of body condition change, but did report a greater chance for degenerated embryos for cows that lost more body condition during the 1st 21 DIM. López-Gatius et al., 2002 and Santos et al., (2009) both reported an increased risk of pregnancy loss associated with body condition loss. This relationship was not found for later gestation pregnancy diagnoses following 1st AI or for 2nd and greater AI regardless of time of pregnancy diagnoses (Table 3.1).

Of all cows that experienced pregnancy loss between 35 and 60 d after AI, 21 of 26 were multiparous cows. Santos et al., (2009) reported that multiparous cows were more likely to lose a pregnancy than primiparous cows although other studies indicate there is no difference between parities and pregnancy loss (Yousuf et al., 2016; Martins et al., 2017; Martins et al., 2018). There was no relationship between the BCS at 30 DIM and early pregnancy loss ($P = 0.50$; $n = 431$). These data continue to support the argument for a high fertility cycle. Cows that have more body condition loss in early lactation have a greater chance of pregnancy loss that eventually places these cows at greater risk of not being pregnant before 130 DIM.

	MGBC	LBC	P - value
Pregnancy Losses after 1st AI			
35 to 60 days post-AI[1], % (n / n)	0.0 (0 / 64)	8.2 (15 / 183)	0.02
61 to 119 days post-AI[1], % (n / n)	9.4 (6 / 64)	3.6 (6 / 168)	0.08
Greater than 120 days post-AI[1], % (n / n)	5.2 (3 / 58)	2.5 (4 / 162)	0.32
Pregnancy Losses after 2nd AI			
35 to 60 days post-AI[1], % (n / n)	3.1 (1 / 32)	7.8 (10 / 128)	0.35
61 to 119 days post-AI[1], % (n / n)	3.2 (1 / 31)	5.1 (6 / 118)	0.66
Greater than 120 days post-AI[1], % (n / n)	0.0 (0 / 30)	5.4 (6 / 112)	0.20

Table 3.1 Relationship of body condition change during the 1st 30 DIM that were classed as maintained or gained (MGBC; n = 146) vs. loss (LBC; n = 500) of body condition and pregnancy loss in dairy cows diagnosed pregnant at 35 d post-AI. Cows that experienced at least 1 health event were not considered in the analyses for 1st AI only. When cows with at least 1 health event were considered for 1st AI pregnancy losses, P values were: 35 to 60 days (P = 0.02), 61 to 119 days (P = 0.07) and > 120 days (P = 0.36). Twenty-one of 26 cows that lost pregnancies between 35 and 60 days after 1st and 2nd AI were multiparous cows. There was no effect of parity on pregnancy losses after 1st (P = 0.51) or 2nd AI (P = 0.46).

[1]Cows that were culled or died before pregnancy diagnoses at 60 d or 119 d post-AI, or from 120 d post-AI to parturition, were removed from this calculation.

Relationships of Body Condition Change During 1st 30 DIM and Periparturient Health Events

There was a significant relationship between body condition loss and the occurrence of at least one periparturient health disorder of the seven that were measured (RP, twins, dystocia, ketosis, displaced abomasum, pyometra/metritis) in both primiparous (n = 262; P = 0.02) and multiparous (n = 525; P = 0.003) cows. There was a relationship between the amount of body condition cows lost in the 1st 30 DIM and the chance for experiencing at least one health event (Figure 3.6). Cows that maintained or gained (n = 158) body condition had fewer single (6 vs.

13%; P = 0.02) and multiple (1 vs. 7%; P = 0.002) periparturient health events compared to cows that lost body condition (n = 629) during the 1st 30 DIM. However, the proportion of cows in the LBC group did not change when cows with at least one periparturient event was removed (80 vs. 77%; P > 0.10). Ruegg and Milton (1995) also reported a higher incidence of diseases in cows that lost body condition.

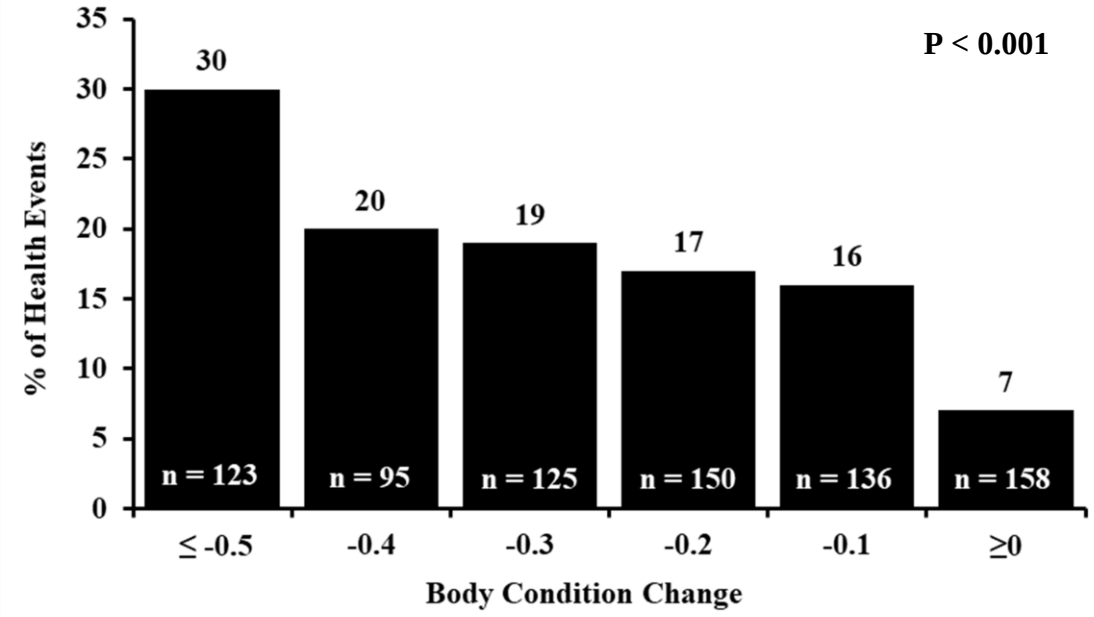

Figure 3.6 Percentage of primiparous and multiparous lactating Holstein dairy cows that experienced at least one periparturient health event within classes of body condition change during the 1st 27-33 d DIM. There was no difference in primiparous vs. multiparous cows in % of health events (P = 0.08).

Cows with RP and twins had greater chances for more body condition loss (n = 787; P = 0.05 and P < 0.01, respectively). Greater body condition loss was related to incidences of ketosis (n = 787; P = 0.003), displaced abomasum (P < 0.01), and metritis (P < 0.01). There was no relationship between dystocia and subsequent body condition loss (n = 787; P = 0.18), contrary to the findings published by Berry et al., (2007b). There was no relationship between the

presence of BLV and class of body condition change (n = 449; P = 0.55), or the occurrence of a periparturient health event (P = 0.17). Also, there was no relationship between presence of Johne's Disease and class of body condition change (n = 447; P = 0.09).

Cows with lower body condition at parturition had a greater predicted probability of twins (n = 787; P = 0.04). The twinning rate was 3.0% for primiparous and 7.0% for multiparous cows. There was no relationship between body condition at parturition and the incidence of at least one periparturient health event (n = 787; P = 0.54). In contrast, greater body condition at calving was associated with a greater risk of periparturient health events (Gillund et al., 2001). Gearhart et al., (1990) and Ruegg and Milton, (1995) did not find relationships between body condition at parturition and incidence of periparturient health events.

Higher body condition at parturition was not associated with greater incidences of dystocia and metritis (n = 787; P = 0.24 and P = 0.61 respectively). Berry et al., (2007b) did not find a relationship between dystocia and body condition at parturition. There was a relationship between body condition at 30 DIM and the occurrence of at least one periparturient health event (P = 0.001; n = 787). It seems logical that health events would have a negative consequence on body condition at 30 DIM, although, when disease was accounted for in the model it did not change the proportion of cows in the body condition change groups. Yet, one thing to consider in this study in this single herd of cows is the limited statistical power with only n = 787 cows of which only 19% having at least one health event.

There was a relationship between the birth of twins and chances for dystocia and displaced abomasum. Cows that gave birth to twins had a greater chance for dystocia (P < 0.01) and displaced abomasum (P = 0.002). Month of parturition predicted (P = 0.001) periparturient health events (n = 851). Cows that calved during the first 5 months of the year had a greater

chance of at least one periparturient health event. This coincides with the months that cows are more likely to lose body condition. Cows that experienced at least one periparturient health event had lower ($P < 0.001$) milk production at 30 DIM (41.9 ± 1.4 vs. 46.5 ± 2.5 SEM kg/cow/d; n = 719), but not ($P = 0.84$) at 60 DIM (45.8 ± 2.8 vs. 46.1 ± 1.6 SEM kg/cow/d; n = 684) compared to cows that did not have at least one periparturient health event.

There was no relationship in the occurrence of at least one periparturient health event and 1st AI PR/AI (n = 767; $P = 0.15$). There was no relationship between the presence of BLV or Johne's Disease and 1st AI PR/AI (n = 423; $P = 0.47$; n = 420; $P = 0.82$ respectively). Domecq et al., (1997), did not find a relationship between periparturient health events and PR/AI.

There was no relationship between the occurrence of a single periparturient health event and early pregnancy loss (n = 552; $P = 0.41$). López-Gatius et al., (2002) also did not detect a relationship between cow health and subsequent pregnancy loss between 38 and 90 d.

Relationship Between Previous Gestation Length, Body Condition and Fertility

There was a correlation between previous gestation length and body condition change but not body condition at parturition (n = 851; $P = 0.93$). Cows with longer previous gestation length had a greater probability (n = 724; $P = 0.01$) of body condition loss during the 1st 30 DIM. Longer gestation lengths were associated with lower PR/AI at 1st AI (n = 735; $P = 0.02$). Interestingly, there was a lack of a relationship between gestation length and chances for dystocia in singleton births (n = 731; $P = 0.85$). It was unclear as to why longer previous gestation lengths are associated with greater body condition loss and lower PR/AI at 1st AI but not greater instances of dystocia. There was no relationship between time of pregnancy in previous lactation and subsequent gestation length (n = 496; $P = 0.72$). Shorter previous gestation lengths were

associated with a higher incidence of periparturient health events (n = 771; P = 0.05), twin births (n = 771; P < 0.01) and greater chances for displaced abomasum (n = 771; P = 0.03).

Relationship Between Body Condition Loss During 1st 30 DIM and Subsequent Sex Ratio at Birth

Sex ratio at parturition following 1st and subsequent AI for singleton calves was 53% male and 47% female (n = 466). This was similar to previously reported secondary sex ratios (Ryan and Boland, 1991; Roche et al., 2006). When the MGBC and LBC groups were analyzed separately in cows that had both body condition scores (parturition and 30 DIM) there was a relationship in the LBC group between the extent of body condition that was lost and the subsequent sex of the calf (n = 346; P = 0.04). The probability of a male decreased as cows lost more body condition (Figure 3.7). In the MGBC group, there was a tendency for more male calves born as cows gained more body condition (n = 91; P = 0.12). There was no relationship between the occurrence of a single periparturient health event and the subsequent sex of calf (n = 466; P = 0.93). Trivers and Willard (1973) hypothesized that sex ratio at birth is influenced by maternal condition in species where reproductive success varies between sexes. In this case we have defined maternal condition in terms of the amount of body condition lost following parturition. This hypothesis has been applied to dairy cows (Roche et al., 2006). Cows with greater body condition at conception or cows that experienced less loss were viewed as being in greater maternal condition. These cows have a sex ratio at birth skewed towards male calves. Cows with low body condition at conception or greater body condition loss would have a sex ratio skewed towards female calves. Roche et al., (2006), found that body condition at conception did not influence sex ratio at birth, but cows that had less loss or gained body condition had a greater number of bull calves. Our data supported these findings and partially

support the Trivers-Willard hypothesis. Greater body condition loss was indicative of poor maternal condition and skewed the sex ratio at birth to fewer male calves born. There was very little homogeneity of body condition loss in the maintained and gained group of cows to evaluate for skewing of sex ratio.

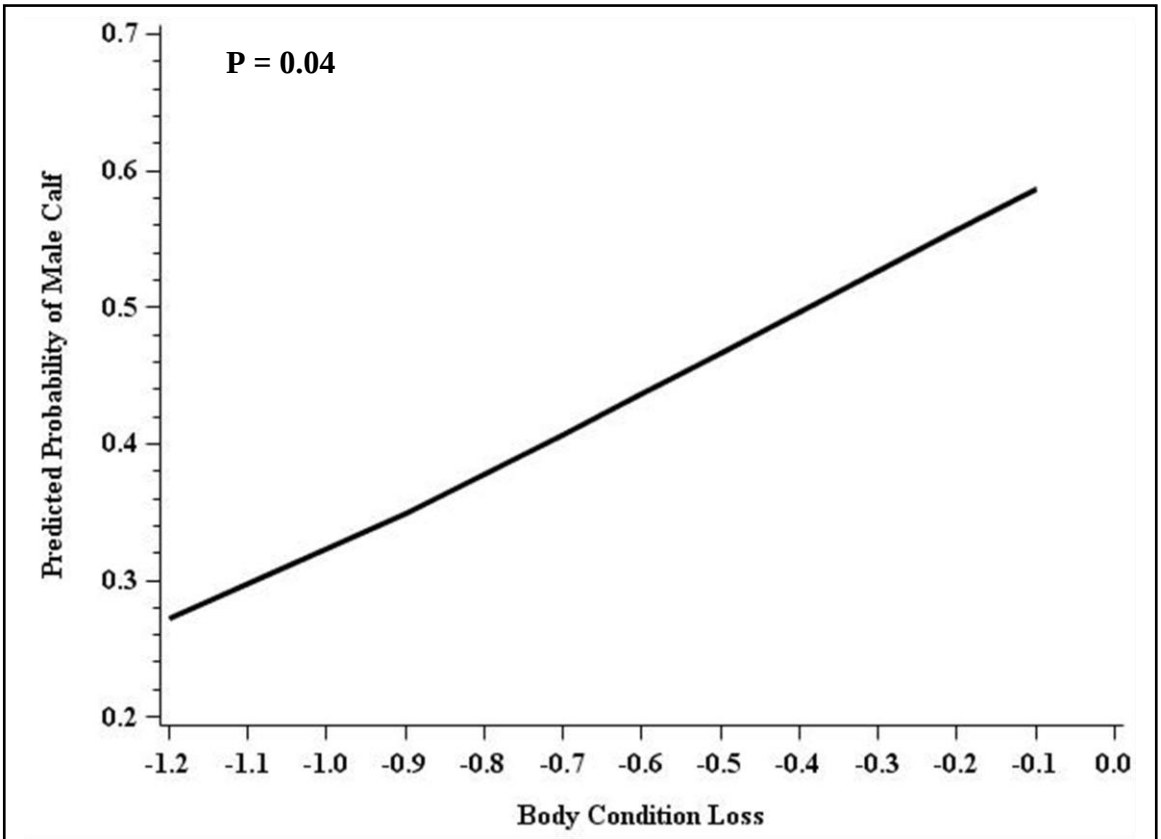

Figure 3.7 Predicted probability of a male calf based on extent of body condition lost from ≤ 1 wk of parturition to 27-33 d post-partum in only the group of primiparous and multiparous lactating Holstein cows that lost body condition (LBC; n = 346).

Summary

These data lead to a greater understanding of how length of previous lactations lead to variability in body condition at parturition, body condition change in early lactation, and

subsequent fertility in this single herd of cows. Cows that become pregnant before 130 DIM have a greater chance of maintaining or gaining body condition during the 1st 30 d of the subsequent lactation leading to a greater chance of pregnancy and a reduced chance of pregnancy loss from 35 to 60 d post-AI. This leads to greater chances of maintaining a cycle of pregnancy prior to 130 DIM. Based on data in this study, maintaining a cycle of pregnancy prior to 130 DIM will likely enhance PR/AI at 1st and subsequent AI and reduce the possibility of early pregnancy loss. We now refer to this potential phenomenon as "the high fertility cycle." Utilizing fertility programs at 1st AI and controlling time to subsequent inseminations can help accomplish this. But it is also critical to detect non-pregnant cows as soon as possible and ensure a timely resynchronized timed-AI to enable cows' chances to become pregnant prior to 130 DIM.

Reducing the chances for body condition loss at parturition also reduces the chances of periparturient health disorders. Although it did not appear that cows with health issues had reduced chances for pregnancy, maintaining a high fertility cycle may reduce peripartum health disorders. Finally, we repeated previously published data in dairy cows that partially support the Trivers-Willard hypothesis. Cows that maintained or gained body condition during the 1st 30 DIM appear to be more apt to pass along their genes through more male calves. Unfortunately, dairy producers only genetically select for females that of course have greater chances of being born from cows with more health issues. The relationship between cows that gain too much body condition during gestation and its impact on fetal development, or the impact of body condition loss on subsequent oocyte competence, is not well understood in dairy cows.

CHAPTER 4

SHORT COMMUNICATION: BLOOD SAMPLES BEFORE AND AFTER EMBRYONIC ATTACHMENT ACCURATELY DETERMINES NON-PREGNANT LACTATING DAIRY COWS AT 24 D POST-AI USING THE BIOPRYN TEST FOR PSPB

A version of this manuscript has been accepted for publication in the Journal of Dairy Science on

March 28, 2019

E. L. Middleton and J. R. Pursley

Department of Animal Science

Michigan State University

ABSTRACT

Early pregnancy diagnosis is critical to reproductive success on dairy farms. Reproductive success depends on cows becoming pregnant before 130 DIM and then maintaining that pregnancy. The earlier non-pregnant cows are identified the sooner they can be re-inseminated, thus reducing days to pregnancy. Assays for pregnancy-specific protein B (**PSPB**) and pregnancy associated glycoproteins (**PAGS**) can be used to diagnose pregnancy >28 d post-AI in lactating cows. The objective of this study was to determine if percentage change in serum levels of PSPB within cow from d 17 to 24 can be utilized to identify non-pregnant cows utilizing a commercially available assay. This study was performed on a large commercial dairy. Blood samples were taken at d 17 and 24 post-AI. The d 17 sample served as a baseline based on previous data. Cows with a 10% increase in serum PSPB levels from d 17 to 24 were considered pregnant. Lactating dairy cows (n = 206; 39% primiparous and 61% multiparous) were synchronized using G6G-Ovysnch. PSPB diagnosis was compared to the herd veterinarian's diagnosis via ultrasound on d 34. The sensitivity for a 10% cutoff as a non-pregnant diagnosis was 100% and the specificity was 93.58%. The positive predictive value was 93.27% and the negative predictive value was 100%. Low PSPB levels at d 24 were predictive of early pregnancy loss by 60 d post-AI. To our knowledge there is no other method that can diagnose non-pregnancy with 100% accuracy and predict pregnancy loss earlier than 24 d post-AI. Using comparative PSPB samples at d 17 and d 24 post-AI provides an accurate non-pregnancy diagnosis earlier than any other pregnancy diagnosing method.

SHORT COMMUNICATION

Reproductive success on a dairy farm depends on a cow becoming pregnant and maintaining the pregnancy until parturition. Data indicate that dairy cows need to become

pregnant before 130 DIM to be profitable (Giordano et al., 2011). In order to maximize chances for pregnancy by 130 DIM reproductive programs need to increase pregnancies per artificial insemination (**PR/AI**) at 1st AI and decrease re-insemination intervals. Fertility programs like Presynch-11, G6G, or Double-Ovsynch improved PR/AI at 1st AI (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008) but generally these programs are too long to use in a resynchronization program. Aggressive resynchronization strategies that reduce the re-insemination interval depend on early non-pregnancy diagnoses (Fricke, 2002; Giordano et al., 2013). Blood and milk samples that assay for pregnancy-associated glycoproteins can be used to diagnose pregnancy. Pregnancy-specific protein B (**PSPB**) can be measured in the maternal serum with a single sample taken between 28 to 35 d post-AI with 98% accuracy for diagnosing pregnancy (Sasser et al., 1986; Piechotta et al., 2011).

PSPB is produced in the binucleate cells of the trophectoderm of the embryo. As the trophectoderm begins to attach to the uterine epithelium PSPB is released via exocytosis and enters the maternal circulation (Wooding, 1992). Bovine placental attachment is believed to begin near d 17 of gestation (Roberts et al., 1996). Data from our laboratory indicated that serum levels of PSPB start to increase at d 22 post-AI in pregnant cows (Arnold et al., 2012), but is different between cows and heifers. Nulliparous heifers appear to initiate an increase in PSPB before lactating cows.

Martins et al., 2018, utilized blood sampling before and after increases in PSPB to determine pregnancy 23 d post-AI. Blood sampling at d 16 and 20, prior to an increase in PSPB, were quite homogeneous within cow. This allowed for an accurate assessment of within cow increases in PSPB utilizing two blood samples at d 20 and 23 post-AI. Pregnancy was determined using an increase of 28% or greater in serum levels of PSPB from d 20 to 23 post-AI.

Accuracy of pregnancy diagnosis had a sensitivity of 98% and specificity of 97% compared to a single PSPB determination at 28 d post-AI.

The objective of this study was to determine if percentage change in serum levels of PSPB within cow from d 17 to d 24 could be more accurate to identify non-pregnant cows. We hypothesized that within cow PSPB samples before and after the time of attachment could diagnose cows that are not pregnant with very high accuracy.

This study was conducted in November and December 2017 on a commercial Holstein dairy farm (Nobis Dairy Farm, St. Johns, MI, USA). The farm milked approximately 1,000 dairy cows three times a day with daily average milk production of 42 kg/cow/day. Cows were fed a total mixed ration once a day with free access to feed and water and were housed in a 4-row free-stall barn with sidewall curtains and fans. The total mixed ration consisted of corn, wheat, and alfalfa silages, and corn-soybean meal-based concentrates formulated to meet nutrient recommendations for lactating dairy cows (NRC, 2001). The Institutional Animal Care and Use Committee at Michigan State University approved all animal handling and procedures.

Weekly cohorts of lactating dairy cows (n = 206; 39% primiparous and 61% multiparous) were synchronized with G6G-Ovsynch and received timed AI on d 0. Cows ranged from 1st to 4th AI and were between 75 and 250 DIM. Blood samples for measurement of PSPB were collected from the coccygeal vein or artery by trained laboratory personnel on d 17 and 24 post-AI using Vacutainer tubes without anticoagulant (BD Vacutainer, Franklin Lakes, NJ). Following collection, samples were refrigerated at 4°C. The d 17 and d 24 samples for each cohort were mailed to a BioPRYN laboratory (West Michigan Veterinary Service, Coopersville, MI) on d 24 post-AI. This laboratory used a commercially available quantitative sandwich ELISA assay kit

(BioTracking LLC, Moscow, ID, USA) to measure serum concentrations of PSPB. Within cow samples on d 17 and 24 were assayed together on the same plate.

The BioPRYN assay is a sandwich ELISA in which rabbit anti-PSPB serum is coated to 96-well micro-titer plates to capture PSPB. Detector solution containing a primary antibody against PSPB is used as the detection antibody. Enhancer solution containing an enzyme (horseradish peroxidase-HRP) linked secondary antibody is used to detect the primary antibody from the Detector solution. The development of color occurs with the addition of 3,3',5,5', -Tetramethylbenzidine, the substrate for HRP. A fluoride stop solution is added to the reaction and optical density for each well was obtained from a plate reader with a filter wavelength of 650 nm. The assay provided a semi-quantitative analysis of samples using 4 standards on each plate (0.5, 1, 2, and 4 ng/mL). A curve was fitted to the standard wells on each plate using a linear least squares regression. This assay was validated with this standard curve with samples taken on d 28 post-AI or greater. The commercial laboratory that performed these analyses did not calculate sensitivity of the assay. PSPB results were reported as optical densities (**OD**) and received by email 2 d later.

The difference in serum PSPB levels was obtained by subtracting the basal d 17 serum level from the d 24 serum level. Percentage change in serum PSPB levels for each cow was calculated by dividing the difference in serum levels by the basal d 17 serum level and then multiplying by 100. All cows received pregnancy diagnoses on d 34 and 62 post-AI by the farm veterinarian using ultrasound (**US**).

All information was recorded in an Excel spreadsheet for organization before statistical analysis. Milk production data and pregnancy confirmation information was utilized for each cow as recorded in PCDART (DRMS; Raleigh, NC). The 305-d mature-equivalent milk

(**M305M**) was chosen for analyses. The d 17 PSPB OD, the d 24 PSPB OD, the difference in serum PSPB levels, and percentage change in serum PSPB from basal to d 24 post-AI were recorded as continuous variables. Pregnancy diagnoses and pregnancy loss diagnoses were recorded as binomial variables. Sensitivity, specificity, positive predictive value, negative predictive value and quartiles of PSPB OD values were analyzed using the FREQ procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). The veterinarian's pregnancy diagnosis on d 34 post-AI was the reference test. Sensitivity determined accuracy and measured the proportion of cows that were diagnosed non-pregnant by percentage change in serum PSPB from basal to d 24 post-AI and diagnosed non-pregnant by US. Specificity measured the proportion of cows that were diagnosed pregnant by percentage change in serum PSPB from basal to d 24 post-AI and diagnosed pregnant by US. The positive predictive value was the probability that the pregnancy diagnosis based off of percentage change in serum PSPB from basal to d 24 post-AI accurately identified cows that were pregnant. The negative predictive value was the probability that the non-pregnancy diagnosis based off of percentage change in serum PSPB from basal to d 24 post-AI accurately identified non-pregnant cows. The GLIMMIX procedure of SAS was utilized for evaluation of continuous data. Pearson correlation coefficients were calculated using the CORR procedure of SAS.

A 10% increase in PSPB OD values from d 17 to 24 was the factor utilized as a cutoff to identify non-pregnant cows. This conservative value was based on the lowest % increase in PSPB OD in cows (n = 102) diagnosed pregnant 34 d post-AI using US. Sensitivity, specificity, and positive and negative predicted values are described in Figure 4.1.

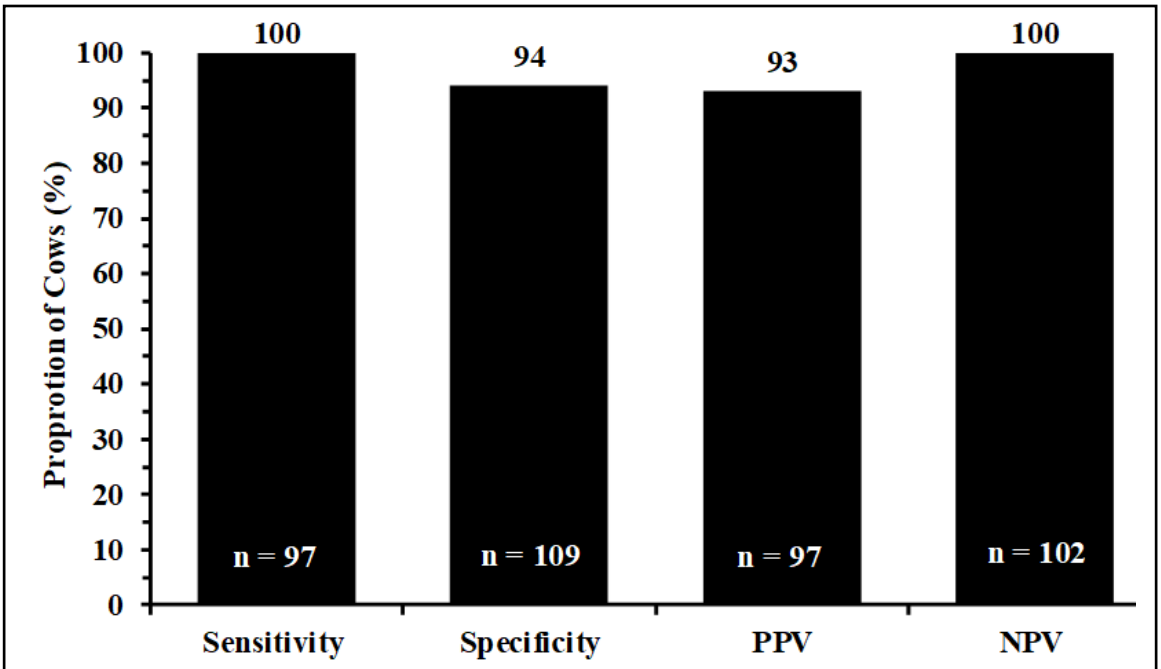

Figure 4.1 Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of a non-pregnancy diagnosis based on a decrease or less than 10% increase in serum levels of pregnancy-specific protein B (PSPB) from basal to d 24 post-AI in lactating dairy cows. Cows with a 10% or greater increase in serum levels of PSPB OD from basal to d 24 post-AI were diagnosed pregnant. The veterinarian's pregnancy diagnosis with ultrasound (US) on d 34 post-AI was the benchmark reference. Sensitivity was the proportion of cows diagnosed non-pregnant by US and by percentage change in serum PSPB OD. Specificity was the proportion of cows diagnosed pregnant by US and by percentage change in serum PSPB OD. PPV was the proportion of cows diagnosed pregnant by percentage change in serum PSPB OD that were actually pregnant**.** NPV was the proportion of cows diagnosed non-pregnant by percentage change in serum PSPB OD that were actually non-pregnant.

Mean PSPB OD values were 0.080 ± 0.001 SEM in non-pregnant cows and 0.083 ± 0.001 SEM in pregnant cows on d 17 post-AI. Mean PSPB OD values on d 24 were 0.082 ± 0.001 SEM in non-pregnant and 0.183 ± 0.005 SEM in pregnant cows. Pregnant cows ranged from a 13 to 306% increase with a mean increase of 122% ± 0.06. Cows (n = 104) with a decrease, or less than 10% increase, in serum PSPB OD from d 17 to 24 post-AI were determined non-pregnant and had 100% sensitivity and 94% specificity. Cows diagnosed non-

pregnant (n = 104) by US ranged from a 14% decrease to an 86% increase in serum PSPB OD from basal to d 24 with a mean increase of 2% ± 0.01 (Figure 4.2).

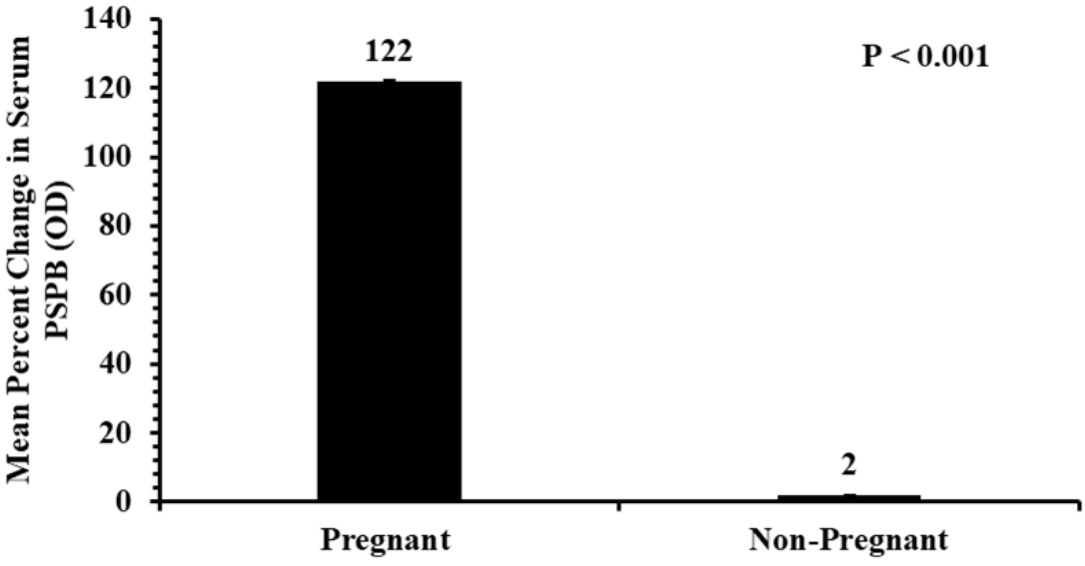

Figure 4.2 Mean percent change in serum pregnancy-specific protein B (PSPB) OD levels from d 17 to 24 post-AI in cows diagnosed pregnant (n = 102) and cows diagnosed non-pregnant (n = 104) via ultrasound. Data are shown as mean ± SEM.

There was a greater incidence of pregnancy loss between 34 and 62 d post-AI in cows with the lowest levels of PSPB OD on 24 post-AI (Table 4.1). Also, cows in the lowest quartile of percentage change of PSPB OD between d 17 and 24 (17 vs. 0 vs. 4 vs. 0% losses; n = 102) had the greatest pregnancy losses (P = 0.02).

Quartile	Range of serum PSPB (OD) levels on d 24 post-AI	Pregnancy Loss 24 to 34 d post-AI, % (n / n)	Pregnancy Loss 34 to 62 d post-AI, % (n / n)
Q1	(0.083 – 0.142)	18.52 (5 / 27)	15.00 (3 / 20)
Q2	(0.144 – 0.170)	3.57 (1 / 28)	7.41(2 / 27)
Q3	(0.172 – 0.213)	3.70 (1 / 27)	0.00 (0 / 25)
Q4	(0.213 – 0.328)	0.00 (0 / 27)	0.00 (0 / 25)
P	-	*0.01*	*0.01*

Table 4.1 Difference in percentages of pregnancy loss from 24 to 34 d post-AI and 34 to 62 d post-AI amongst quartiles of serum pregnancy-specific protein B (PSPB) OD levels on d 24 post-AI in cows diagnosed pregnant from the percent change in serum PSPB OD level from d 17 to 24 post-AI. Cows were considered to have undergone pregnancy loss from 24 to 34 d post-AI if diagnosed pregnant from the percent change in serum PSPB (OD) from basal to d 24 post-AI and then diagnosed non-pregnant via ultrasound on d 34 post-AI. Cows were considered to have undergone pregnancy loss from 34 to 62 d post-AI if confirmed pregnant at 34 d post AI via ultrasound and then diagnosed non-pregnant at the next confirmation 62 d post AI via ultrasound. Reduced numbers are a result of pregnancy loss 24 to 35 d post-AI and n = 2, n = 1, and n = 2 cows culled from Q1, Q3, and Q4 respectively before pregnancy confirmation 62 days post-AI.

The purpose of this experiment was to test if the BioPRYN commercial assay can be an accurate predictor of non-pregnancy. The commercial laboratory did not calculate the sensitivity of the assay, so it is unclear what the d 17 samples are measuring. There are several reasons why it seems likely that serum OD levels on d 17 are measuring PSPB and thus not an assay sensitivity problem. 1) Previous data (Arnold et al., 2012) indicated non-pregnant primi- and multiparous cows (0.095 ± 0.001 OD; n = 21) had greater OD levels of PSPB compared to non-pregnant nulliparous heifers (0.080 ± 0.001 OD; n = 8). 2) Data from Figure 4.3 indicate PSPB decreased from 1st to 4th AI (75 to 250 DIM). This was supported by a negative correlation (Pearson's R = -0.22) between DIM at insemination and d 17 post-AI PSPB OD levels in all

cows (n = 206). 3) There is low variability in the % change in PSPB from d 17 to 24 in non-pregnant cows (Figure 4.2; CV = .02) but high variability between cows on d 17 (OD values range from 0.069 to 0.1; CV = 0.08). 4) Sasser et al., 1986 reported that some cows, but not all, had detectable PSPB on d 15 post-breeding. 5) Even though data from Kiracofe et al., 1993, indicated that PSBP levels in beef cows decreased, it appeared, to near "basal" concentrations at 90 d postpartum, it was not clear why there are basal levels at that point.

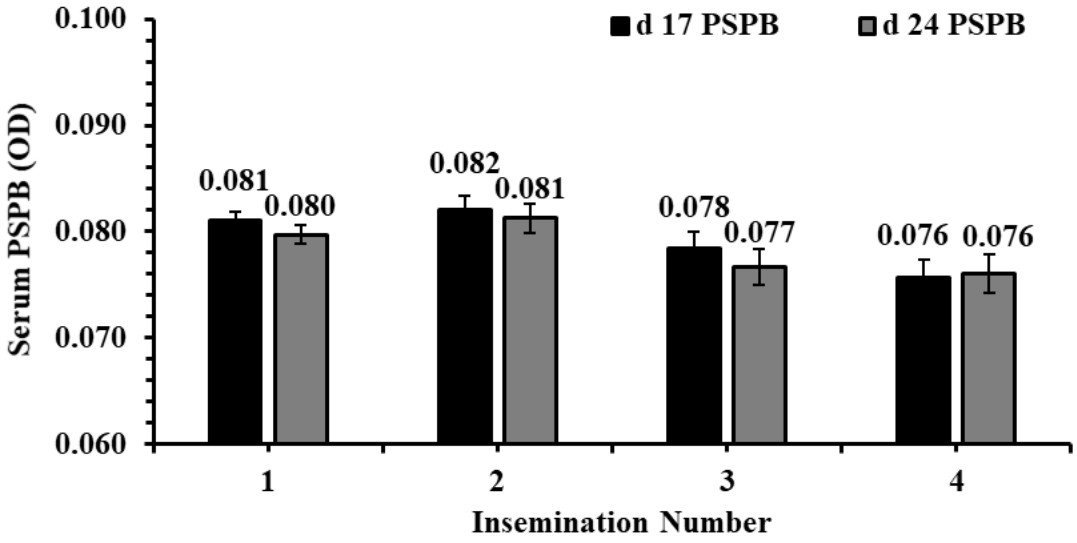

Figure 4.3 Serum pregnancy-specific protein B (PSPB) OD levels on d 17 post-AI and d 24 post-AI in non-pregnant cows at 1st insemination (n = 50), 2nd insemination (n = 20), 3rd insemination (n = 13), and 4th insemination (n = 12). Cows with a 5th insemination (n = 2) were excluded from the analysis. Data are shown as mean ± SEM. Significance between insemination numbers for serum PSPB OD levels on d 17 post-AI are as follows: 1st vs. 2nd (P = 0.52), 1st vs. 3rd (P = 0.13), 1st vs. 4th (P = 0.004), 2nd vs. 3rd (P = 0.07), 2nd vs. 4th (P = 0.003), and 3rd vs. 4th (P = 0.24). Significance between insemination numbers for serum PSPB OD levels on d 24 post-AI are as follows: 1st vs. 2nd (P = 0.34), 1st vs. 3rd (P = 0.12), 1st vs. 4th (P = 0.07), 2nd vs. 3rd (P = 0.04), 2nd vs. 4th (P = 0.02), and 3rd vs. 4th (P = 0.8). There was no effect of parity between 1st and 2nd + parity cows at d 17 (P = 0.39) and d 24 (P = 0.37). Days in milk ranges at 1st, 2nd, 3rd and 4th AI was 75 to 81, 131 to 137, 187 to 193, and 243 to 249.

There was a positive correlation (Pearson's R = 0.13) between d 17 post-AI PSPB OD levels and M305M (n = 206). There was no correlation between d 24 post-AI PSPB OD levels and M305M in pregnant cows (Pearson's R = 0.01; n = 109). Both López-Gatius et al., (2007) and Ricci et al., (2015) reported a negative relationship between milk production and PAG levels in pregnant cows. There was no relationship between M305M and pregnancy (P = 0.50; n = 206).

Determining non-pregnancy with percentage change in serum PSPB from basal to d 24 post-AI was 100% accurate with a conservative percentage change cutoff that purposely included only non-pregnant cows. This favored accuracy of determining non-pregnant cows over pregnant cows. A quantitatively derived cutoff would not have allowed this conservative cutoff due to the variation in percentage change of pregnant cows. It is critical for an early non-pregnancy diagnosis method to not incorrectly diagnose pregnant cows as non-pregnant. Non-pregnant cows may be resynchronized with a $PGF_{2\alpha}$ which can cause termination of the pregnancy (Fricke et al., 2016). The lowest cutoff on d 24 for the current data set (0.1054 OD) was only 94% accurate in a much larger data set (n = 734 pregnant cows) in which samples were evaluated at the same laboratory with the same assay. This would mean that approximately n = 43 pregnant cows would have received $PGF_{2\alpha}$ and would possibly be aborted if utilizing a single OD cutoff from the current study. Approximately 7% of the cows diagnosed pregnant with percentage change in serum PSPB OD from d 17 to 24 post-AI were diagnosed non-pregnant by US 10 d later. This may be due to early pregnancy loss or inaccuracies utilizing the BioPRYN assay in this novel way. Cows that were diagnosed non-pregnant by the veterinarian on d 34 post-AI had a wide range of percentage differences (-14% to 86%) in serum PSPB OD levels from basal to d 24 post- AI. The 10% cutoff was conservative enough to not diagnose pregnant cows as non-

pregnant while limiting the proportion of cows falsely diagnosed pregnant. Utilizing the BioPRYN assay in this manner may allow for non-pregnant cows to be re-inseminated sooner and have more chances for pregnancy by 130 DIM.

Martins et al., 2018 also found a difference in serum levels of PSPB at d 23 and 28 post-AI between cows with versus without pregnancy losses between d 28 and 35 of gestation. There was no difference, however, in serum levels of PSPB at d 23 and 28 post-AI between cows with versus without pregnancy losses between d 35 and 56 in gestation (Martins et al., 2018). The inverse relationship between serum OD levels on d 24 and pregnancy loss agrees with Gábor et al., (2016). Pregnancy loss affects reproductive success on dairy farms (Fricke, 2002). Pregnancy loss rates are greatest during early pregnancy (Santos et al., 2004). The ability to identify cows at risk for losing a pregnancy could prove useful to producers and veterinarians.

In conclusion, using within cow PSPB samples at d 17 and d 24 post-AI provided a robust and very accurate determination of early non-pregnancy diagnosis. This could lead to development of synchronization methods for early re-synchronization of ovulation for timed-AI. It appears to be critical for cows to become pregnant before 130 DIM in order to maintain high fertility from lactation to lactation. Identifying non-pregnant cows as early as possible is important to allow for this process to happen. After further research, this method of early pregnancy diagnosis may eventually have the ability to alert producers and veterinarians to cows that are at risk for losing a pregnancy. To our knowledge there is no other method that can diagnose non-pregnancy with 100% accuracy. Utilizing this method of non-pregnancy diagnosis can detect non-pregnant cows earlier, allowing for the cows to be resynchronized and re-inseminated sooner, and ultimately decreasing DIM to conception, although, the labor and cost of two blood samples may not be practical for most dairy operations. In addition, a careful

revision of interpretation of results of this use of BioPRYN assay must be made before it is ready for field conditions.

CHAPTER 5

EFFECT OF A SHORT RESYNCHRONIZATION METHOD FOLLOWING EARLY PREGNANCY DIAGNOSIS ON FERTILITY OF LACTATING DAIRY COWS

Manuscript will be submitted for publication in Animal Reproduction Science

E. L. Middleton, A.M. Santos, T. Minela, and J.R. Pursley

Department of Animal Science

Michigan State University

ABSTRACT

Achieving pregnancy by 130 DIM is critical to the reproductive success of dairy farms. In order to maximize chances for pregnancy by 130 DIM, non-pregnant cows need to be resynchronized for re-insemination as soon as possible. Developing short resynchronization protocols can be challenging due to variability in ovarian dynamics. Controlled internal drug releasing devices (**CIDR**) provide supplemental progesterone (**P_4**). Incorporating CIDRs into the Ovsynch protocol can prevent premature ovulations, improve synchrony, and in some cases increase pregnancies per AI (**PR/AI**). Typically, CIDRs are inserted on the d the initial gonadotropin-releasing hormone (**GnRH**) is administered and removed the d the prostaglandin F2α (**$PGF_{2\alpha}$**) is administered. It is unclear, however, if PR/AI are better removing the CIDR on the day of the $PGF_{2\alpha}$ or the d after. The objective of this study was to utilize a $PGF_{2\alpha}$ - CIDR resynchronization protocol initiated with $PGF_{2\alpha}$ to re-inseminate non-pregnant lactating dairy cows by 35 d post-AI. A secondary objective was to determine if it was more optimal to remove CIDRs on the d of the $PGF_{2\alpha}$ or the d after. Lactating dairy cows (n = 834) diagnosed not-pregnant at 24 d post-AI were blocked by parity and AI service number and randomized into treatments. The treatment group had a 35-d re-insemination interval (**R35**) and the control group had a 42-d re-insemination interval (**R42**). R35 initiated Ovsynch with $PGF_{2\alpha}$ and a CIDR on d 28 post-AI. Cows then received another injection of $PGF_{2\alpha}$ 4 d later. GnRH was administered on d 6 of the protocol and all cows received timed-insemination 16 h later on d 7 of the protocol. R42 initiated Ovsynch with GnRH and a CIDR on d 32 post-AI. Cows then received an injection of $PGF_{2\alpha}$ 7 d later. GnRH was administered on d 9 and all cows received timed-insemination 16 h later on d 10 of the protocol. Within each group CIDRs were either removed on the d of or the d after $PGF_{2\alpha}$ creating four groups: R35 with CIDRS removed on d of $PGF_{2\alpha}$ (**RES35**), R35 with

CIDRs removed on the d after $PGF_{2\alpha}$ (**RES35+1**), R42 with CIDR removed on d of $PGF_{2\alpha}$ (**RES42**), and R42 with CIDR removed on d after $PGF_{2\alpha}$ (**RES42+1**). R35 had lower PR/AI and greater pregnancy loss 24 to 34 d post-AI than R42. R35 also had lower ovulation rates, larger average ovulatory follicles, and lower P_4 on the d of the $PGF_{2\alpha}$ than R42. Overall, higher PR/AI at 2nd AI indicate that R42 had a greater proportion of cows pregnant by 130 DIM than R35. R35 was not an effective resynchronization strategy when compared to R42. RES35 had larger average ovulatory follicles and a lower ovulation rate than RES35+1, but there was no difference in PR/AI between RES35 and RES35+1 nor RES42 and RES42+1. The d the CIDR was removed had no effect on PR/AI; therefore, it makes the most sense from a management standpoint to remove CIDRs the same day $PGF_{2\alpha}$ is administered.

INTRODUCTION

Timely pregnancies are essential for reproductive success on dairy farms (Dijkhuizen et al., 1984; Meadows, 2005; Middleton et al., 2019). Both Dijkhuizen et al., (1984) and Meadows (2005) concluded that 12 to 13-month calving intervals were the most profitable. Giordano et al., (2011) determined that from a profitability standpoint cows need to become pregnant around 130 DIM. Middleton et al., (2019) suggested that cows need to become pregnant by 130 DIM to be in a "high fertility cycle" that could reduce body condition loss and maximize fertility in the following lactation. Reproductive programs should decrease time to re-insemination and maximize pregnancies per artificial insemination (**PR/AI**) in order to create timely pregnancies. Synchronization of ovulation using gonadotropin-releasing hormone (**GnRH**) and prostaglandin F2α (**$PGF_{2\alpha}$**) controls time to insemination (Pursley et al., 1997). Fertility programs like Double-Ovsynch, G6G and Presynch-11 improved PR/AI at 1st AI (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008), but approximately 50% of all cows still fail to conceive (Bello et al.,

2006; Souza et al., 2008; Strickland et al., 2010). Aggressive resynchronization programs that reduce re-insemination intervals are necessary to maximize chances for pregnancy by 130 DIM.

Resynchronization strategies vary greatly depending on d of non-pregnancy diagnosis and management preferences. Variability in stage of the estrous cycle at non-pregnancy diagnosis, asynchrony, and pregnancy loss limit the success of resynchronization protocols (Silva et al., 2007). Fertility programs yield high PR/AI but are too long to use in an aggressive resynchronization program (Wiltbank and Pursley, 2014). Ovsynch (GnRH, 7 d - $PGF_{2\alpha}$, 56 h – GnRH, 16 h – AI; Pursley et al., 1995) is typically used to resynchronize cows (Fricke et al., 2003; Bilby et al., 2014; Giordano et al., 2016). One variation utilizes a controlled internal drug releasing (**CIDR**) device to supplement progesterone (**P_4**) between GnRH and $PGF_{2\alpha}$ (Macmillan et al., 1991; Macmillan and Peterson, 1993; Xu and Burton, 2000).

Using CIDRs in resynchronization protocols increased PR/AI (Chebel et al., 2006; Dewey et al., 2010), improved synchrony (Lima et al., 2009; Bilby et al., 2013), and prevented premature ovulation (Sirois and Fortune, 1990; Chebel et al., 2006; Bilby et al., 2013). The benefits of CIDRs in regards to PR/AI may be greater for cows with no corpus luteum (**CL**) or low circulating P_4 at the initiation of the resynchronization protocol (Bilby et al., 2013). CIDRs are usually removed on d of $PGF_{2\alpha}$ in the CIDR-Ovsynch protocol (Dewey et al., 2010; Bilby et al., 2013). This is convenient from a management perspective. Progesterone from the CIDR suppresses luteinizing hormone (**LH**) surges needed for ovulation (Nation et al., 2000; Silvia et al., 2002). The removal of supplemental P_4 on the d of $PGF_{2\alpha}$ may allow cows with no CL or low circulating P_4 to have an LH surge and ovulate before the final GnRH resulting in lower PR/AI. Yet, the optimal d to remove CIDRs relative to the final $PGF_{2\alpha}$ is not clear.

Accurate early non-pregnancy diagnoses are key to reducing re-insemination intervals (Fricke, 2002; Giordano et al., 2013). Pregnancy diagnoses by palpation or transrectal ultrasound can be performed 26 to 33 d (Pieterse et al., 1990). A single pregnancy-specific protein B (**PSPB**) sample can diagnose pregnancy 28 to 35 d post-AI (Piechotta et al., 2011). Martins et al., (2018) utilized the percent change in serum PSPB from d 20 to d 23 post-AI to diagnose non-pregnancy with 98% sensitivity and 97% specificity. Middleton and Pursley (2019) used the percent change between two PSPB samples taken before and after embryonic attachment to accurately diagnose non-pregnant cows at 24 d post-AI. Non-pregnancy diagnosis information was not available until 26 d post-AI using this method (Middleton and Pursley, 2019). Nevertheless, this non-pregnancy diagnosis method created an opportunity to shorten the re-insemination interval to 35 d without using one or multiple GnRH treatments prior to pregnancy diagnosis at d 32 post-AI (Fricke et al., 2003; Sterry et al., 2006).

The objective of this study was to utilize a $PGF_{2\alpha}$ - CIDR resynchronization protocol initiated with $PGF_{2\alpha}$ to re-inseminate non-pregnant lactating dairy cows by 35 d post-AI. Our second objective was to determine if it is more optimal to remove the CIDR on the d of $PGF_{2\alpha}$ or one d later. We hypothesized that utilizing a short resynchronization strategy initiated with $PGF_{2\alpha}$ 28 d post-AI would result in similar PR/AI compared to a traditional CIDR-Ovsynch program initiated 32 d post-AI; thus, creating an effective resynchronization protocol. We also hypothesized that removing the CIDR the d after $PGF_{2\alpha}$ would result in greater PR/AI than removing the CIDR on the d of $PGF_{2\alpha}$.

MATERIALS AND METHODS

Cows and Housing

This trial was conducted from January 2018 to October 2018 on a commercial Holstein farm (Nobis Dairy Farm, St. Johns, MI, USA). The farm milked approximately 1,000 dairy cows three times a day with daily average milk production of 42 kg/cow/d. Cows were fed a TMR once a day with free access to feed and water and were housed in a 4-row free-stall barn with fans and sidewall curtains. The TMR consisted of corn, wheat, and alfalfa silages, and corn-soybean meal-based concentrates formulated to meet nutrient recommendations for lactating dairy cows (NRC, 2001). All cows received 1st timed-AI 75-81 DIM using the G6G/Ovsynch program as follows: $PGF_{2\alpha}$, 2 d – GnRH, 6 d – GnRH, 7 d - $PGF_{2\alpha}$, 56 h – GnRH, 16 h – AI.

Experimental Design

This experiment was a 2 x 2 completely randomized block design. Weekly cohorts of lactating dairy cows (n = 834) diagnosed not-pregnant 24 d after previous AI were blocked by parity (1st, 2nd, and 3rd or greater) and AI service number (ranging from 1 service to 6) and randomized into one of four resynchronization protocols. Not-pregnant cows were re-enrolled in the study blind to previous treatment.

The treatment group had a 35 d re-insemination interval (**R35**) and the control group had a 42 d re-insemination interval (**R42**). Within each group CIDRs were either removed on the d of or the d after $PGF_{2\alpha}$ creating four groups: R35 with CIDR removed on d of $PGF_{2\alpha}$ (**RES35**), R35 with CIDR removed on the d after $PGF_{2\alpha}$ (**RES35+1**), R42 with CIDR removed on d of $PGF_{2\alpha}$ (**RES42**), and R42 with CIDR removed on the d after $PGF_{2\alpha}$ (**RES42+1**; Figure 5.1). Cows in the R35 treatment began the resynchronization protocol on d 28 post-AI with a CIDR and the

administration of $PGF_{2\alpha}$. Then 4 d later these cows received an additional administration of $PGF_{2\alpha}$ to induce luteolysis. Cows in the RES35 treatment had CIDRs removed on this d. The cows in the RES35+1 treatment had CIDRs removed d 5 of the protocol. All R35 cows received an injection of GnRH on d 6 to synchronize ovulation of the ovulatory follicle and were timed-inseminated 16 h later on d 7 of the protocol. Cows in the R42 treatment began the resynchronization protocol on d 32 post-AI with an injection of GnRH and 5 h later a CIDR. Then 7 d later these cows received an injection of $PGF_{2\alpha}$ to induce luteolysis. On this d the cows in the RES42 treatment had CIDRs removed. The cows in the RES42+1 treatment had CIDRs removed on d 8 of the protocol. All R42 cows received an injection of GnRH on d 9 to synchronize ovulation of the ovulatory follicle and were timed-inseminated 16 h later on d 10 of the protocol (Figure 5.1). No cows in this study received AI following a detected estrus.

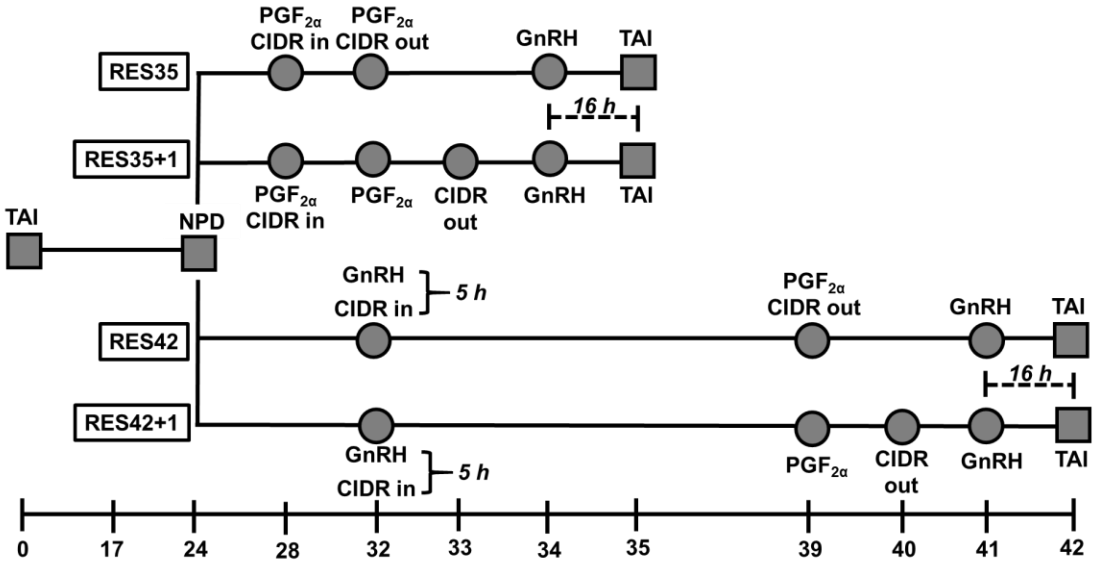

Figure 5.1 Schematic diagram of the treatments used. Non-pregnant cows were blocked by parity (1st, 2nd, and 3rd or greater) and AI service number (ranging from 1 service to 6) and randomly assigned into one of four treatments: 35 d re-insemination interval (R35) with the progesterone controlled internal drug release device (CIDR) removed on d of prostaglandin F2α ($PGF_{2\alpha}$; RES35), R35 with CIDR removed on the d after $PGF_{2\alpha}$ (RES35+1), 42 d re-insemination interval (R42) with CIDR removed on d of $PGF_{2\alpha}$ (RES42), and R42 with CIDR removed on the d after $PGF_{2\alpha}$ (RES42+1).

Trained laboratory personnel administered all injections with a 3 mL syringe and 20-gauge 3.8 cm needles in semimembranosus or semitendinosus muscles of cows. All $PGF_{2\alpha}$ treatments in this experiment used 25 mg of cloprostenol sodium (estroPLAN®; Parnell Animal Health, Overland Park, KS). All GnRH treatments used 100 μg of gonadorelin acetate (GONAbreed®; Parnell Animal Health, Overland Park, KS). All vaginal CIDRs (1.38 g of P_4, Eazi-Breed™ CIDR® Cattle Insert; Zoetis, Kalamazoo, MI) utilized were new. Blood samples for the measurement of PSPB and P_4 were collected from the coccygeal vein or artery by trained laboratory personnel using Vacutainer tubes without anticoagulant (BD Vacutainer, Franklin Lakes, NJ). Following collection, samples were refrigerated, transported to our laboratory, and stored in a refrigerator at 4 °C. Serum from the blood samples for P_4 was separated within 24 hours after collection by centrifugation at 2000 x *g* for 20 min at 4 °C and stored at -20 °C for later analyses. The Institutional Animal Care and Use Committee at Michigan State University approved all animal handling and procedures.

Pregnancy Diagnosis

Initial diagnosis of pregnancy was performed according to Middleton and Pursley (2019). Briefly, blood samples for measurement of PSPB for all cows were collected, as previously described, in weekly cohorts on d 17 and d 24 post-AI. The d 17 and d 24 samples for each cohort were mailed to a BioPRYN laboratory (West Michigan Veterinary Service, Coopersville, MI) on d 24 post-AI. This laboratory used a commercially available quantitative sandwich ELISA assay kit (BioTracking LLC, Moscow, ID, USA) to measure serum concentrations of PSPB. Within cow samples on d 17 and 24 were assayed on the same plate. PSPB results were reported as optical densities (**OD**) and received by email 2 d later. Pregnancy was diagnosed by the percent change in serum PSPB OD levels from d 17 to d 24. Cows with a 10% increase, or

greater, in serum PSPB OD from d 17 to 24 post-AI were diagnosed pregnant. Cows with a decrease or less than a 10% increase in serum PSPB OD from d 17 to 24 post-AI were diagnosed not-pregnant.

All cows diagnosed pregnant from the percent change in PSPB OD received additional pregnancy diagnoses on d 34 and 62 post-AI by the farm veterinarian using ultrasound. Pregnancy was confirmed by embryo presence with heartbeat. Cows were considered to have undergone pregnancy loss if they were diagnosed pregnant at 24 d post-AI and then diagnosed not-pregnant at any of the confirmations. Cows that experienced pregnancy loss were not enrolled in the experiment.

Evaluation of Ovarian Development

Ovaries were scanned and mapped for all cows (n = 834) on d of the final GnRH by transrectal ultrasound using a MyLab™ DeltaVET with a 6-10 MHz multi-frequency linear array probe (Esaote, Indianapolis, IN). Height and width of the largest cross-section of follicles with average diameter >8 mm and CL were measured using built-in calipers. CL with a fluid-filled central cavity also had the large cross-section of the cavity measured. Measurements of all follicles and CL were recorded in an ovarian map for each cow with date of examination. Ovaries were scanned with ultrasound again 4 d later to determine ovulation. Ovulation was characterized by the disappearance of a follicle(s), followed by detection of a newly formed CL on the same ovary. Ovulatory follicle(s) mean diameter was calculated by the average height and width of each follicle. The average size of ovulatory follicle was used for analyses. Ovulatory follicle size information was missing for n = 10 cows. Cows that did not ovulate were classified into one of two groups: (1) no dominant follicle or (2) no ovulation. Cows that were classified as no dominant follicle either did not have a follicle ≥10 mm on the d of GnRH or had a follicle ≥

10 mm that decreased in size and was accompanied by subordinate follicular growth. Cows that were classified as no ovulation had follicles ≥ 10 mm on the d of GnRH that either continued to grow or stayed the same size and was not accompanied by subordinate follicular growth.

Hormonal Assay

Blood samples for P_4 were collected on an unbiased subset of cows (n = 289) immediately before the final $PGF_{2\alpha}$ and the final GnRH. There was one blood sample missing for n = 5 cows, so there were n = 284 cows used in P_4 analyses. Serum concentrations of P_4 were analyzed with a radioactive immunoassay validated by Engel et al., (2008). Progesterone standards (0.02, 0.04, 0.10, 0.20, 0.50, 1.0, 1.5, and 2.0 ng / tube) and duplicate samples (50 µL) with 350 µL of assay buffer (0.1% gelatin, 0.05% sodium azide, 0.09% $NaH_2PO_4 \cdot H_2O$, 0.05% Na_2HPO_4, and 0.9% NaCl, pH 7.0) were incubated at 4°C for 20 h with 200 µL of P_4 antiserum (Assay Designs, Ann Arbor, MI; 1:550,000 vol / vol dilution). After incubation, 100 µL of [^{125}I] P_4 (adjusted to 20,000 cpm) was added to each tube and all tubes were incubated again at 4°C for 20 h. Then 100 µL of precipitated goat anti-mouse secondary antibody was added to separate bound and free P_4 and tubes were incubated for 15 min followed by centrifugation at 1,200 x *g* for 30 min. Supernatants were aspirated and the precipitates were counted in a gamma counter 1 min / tube. Cross reactivities of the antibody, as determined by the manufacturer, were 100% for P_4, 3.46% for 17-hydroxyprogesterone, 0.056% for deoxycorticosterone, 0.77% for corticosterone, and < 0.0001% for estradiol-17β, estrone, estriol, hydrocortisone, testosterone, 20alpha-doil, 5alpha-pregnane-2alpha, and danazol. All sample concentrations were determined in one assay. The interassay CV was 8.5%, the intraassay CV was 3.35% and the sensitivity was 0.2 ng / mL.

Statistical Analyses

All information was recorded in an Excel spreadsheet for organization before statistical analysis. Cows that lost their CIDRs (n = 24; R35 n = 10; R42 n = 14) were removed from the analyses. Binomial variables were analyzed using logistic regression with a generalized linear mixed model implemented with the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). The FREQ procedure of SAS was used to report the means of binomial proportions. Continuous variables were analyzed using a linear mixed model implemented by the MIXED procedure of SAS. The MEANS procedure of SAS was used to report means ± standard errors of continuous variables. The final model considered treatment (R35 and R42), parity (1st, 2nd, or 3rd and greater), and service (1st through 6th) as fixed effects. For analyses regarding d of CIDR removal treatments were RES35, RES35+1, RES42, and RES42+1. Two-way interaction of treatment and parity or service was only considered in the model if $P < 0.20$.

RESULTS

Effect of Treatment on Fertility Parameters

Over the course of this experiment n = 1,885 cows ranging from 1st through 6th insemination were diagnosed for non-pregnancy at 24 d post-AI. Pregnancies per AI at 1st AI was 49% and PR/AI for all re-inseminations across treatments was 39% (n = 1,885; $P < 0.0001$). Non-pregnant cows that were culled upon diagnosis or re-inseminated to an estrus were not enrolled in this study. R42 cows had higher ($P = 0.001$) PR/AI than R35 cows (42 vs. 31%; n = 810) at 24 d post-AI. Similarly, RES42 and RES42+1 both had higher PR/AI 24 d post-AI than RES35 and RES35+1 (Figure 5.2). There was no difference in PR/AI at 24 d post-AI between

RES35 and RES35+1 nor RES42 and RES42+1 indicating that the d the CIDR was removed had no effect on PR/AI (Figure 5.2).

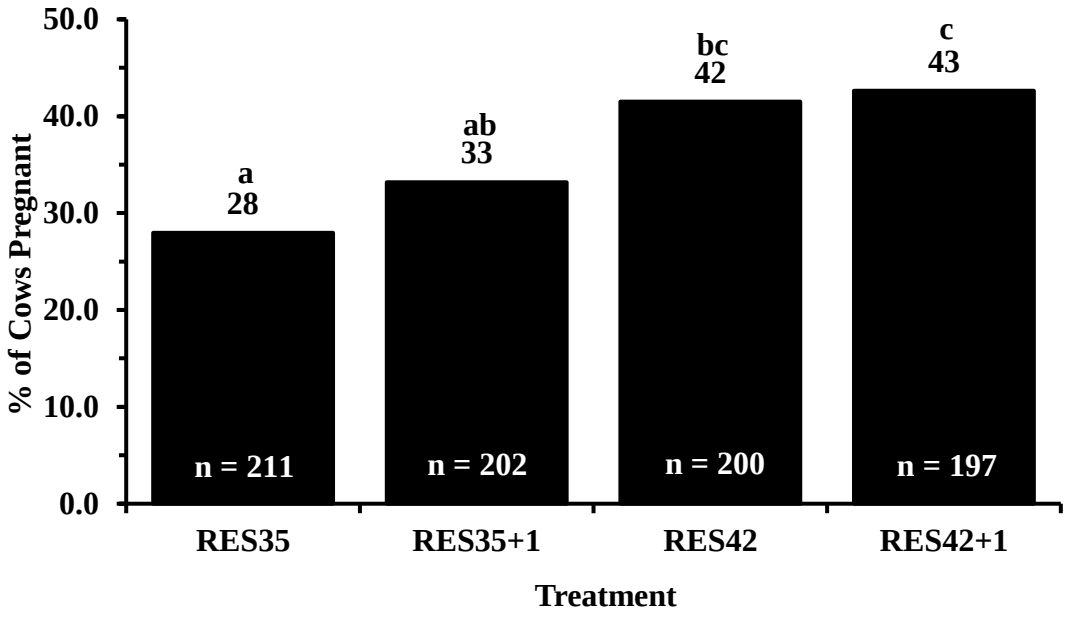

Figure 5.2 Effect of treatment on pregnancy per AI on d 24 post-AI. Pregnancy was diagnosed by utilizing the percent change in PSPB levels from d 17 to d 24 post-AI. RES35 = 35 d re-insemination interval (RI) with the progesterone controlled internal drug release device (CIDR) pulled on the d of $PGF_{2\alpha}$; RES35+1 = 35 d RI with CIDR pulled on the d after $PGF_{2\alpha}$; RES42 = 42 d RI with CIDR pulled on d of $PGF_{2\alpha}$; and RES42+1 = 42 d RI with CIDR pulled on the d after PGF2α. Different superscripts represent differences (a, b, c; $P < 0.05$). Significance between PR/AI and treatment are as follows: RES35 vs. RES35+1 ($P = 0.25$), RES35 vs. RES42 ($P = 0.004$), RES35 vs. RES42+1 ($P = 0.0002$), RES35+1 vs. RES42 ($P = 0.08$), RES35+1 vs. RES42+1 ($P = 0.05$), and RES42 vs. RES42+1 ($P = 0.82$).

Table 5.1 depicts an interaction between treatment and parity ($P = 0.15$) where differences in PR/AI were exacerbated in 2nd parity cows. There were differences in PR/AI between treatments at 2nd and 5th + inseminations, but not at 3rd or 4th insemination (Figure 5.3). R42 had greater PR/AI than R35 at 2nd AI and a tendency for greater PR/AI at 5th + AI (Figure 5.3). There was also a tendency ($P = 0.10$) for the month of insemination to influence PR/AI at

24 d post-AI where PR/AI was lower (P = 0.003) in May through August compared to the rest of the months (31 vs. 41%; n = 810). There was no interaction, however, between month of insemination and treatment on PR/AI 24 d post-AI (P = 0.84; n = 810).

Treatments	1st Parity	2nd Parity	3rd + Parity	Total	P-value
RES35, % (n/n)	31.6 (24/76)	27.3 (15/55)	25.0 (20/80)	28.0 (59/211)	0.36
RES35+1, % (n/n)	40.5 (30/74)	18.5 (10/54)	36.5 (27/74)	33.2 (67/202)	0.60
RES42, % (n/n)	46.6 (34/73)	47.2 (25/53)	32.4 (24/74)	41.5 (83/200)	0.08
RES42+1, % (n/n)	43.1 (31/72)	45.1 (23/51)	40.5 (30/74)	42.6 (84/197)	0.76
Total, % (n/n)	40.3 (119/295)	34.3 (73/213)	33.4 (101/302)	36.2 (293/810)	0.08
P-value	0.11	0.0004	0.07	0.002	-

Table 5.1 Effect of treatment on pregnancies per AI at 24 d post-AI amongst 1st, 2nd, and 3rd + parity cows. Pregnancy was diagnosed by utilizing the percent change in PSPB levels from d 17 to d 24 post-AI. RES35 = 35 d re-insemination interval with the progesterone controlled internal drug release device (CIDR) removed on the d of $PGF_{2\alpha}$; RES35+1 = 35 d re-insemination interval with CIDR removed on the d after $PGF_{2\alpha}$; RES42 = 42 d re-insemination interval with CIDR removed on d of $PGF_{2\alpha}$; and RES42+1 = 42 d re-insemination interval with CIDR removed on the d after PGF2α.

Pregnancy loss rates 24 to 34 d post-AI were greater for R35 cows than R42 cows (Table 5.2). Between 34 and 62 d post-AI though there was no difference in pregnancy loss rates between treatments (Table 5.2). Overall, there was no effect of treatment on total pregnancy loss from 24 to 62 d post-AI (Table 5.2). There was no effect of the d the CIDR was removed on pregnancy loss from 24 to 34 d post-AI (P = 0.11), pregnancy loss 34 to 62 d post-AI (P = 0.86), or total pregnancy loss from 24 to 62 d post-AI (n = 280; P = 0.43).

	R35	R42	P-value
Pregnancy Losses 24 to 34 d post-AI, % (n/n)	12.0 (15/125)	4.9 (8/164)	0.02
Pregnancy Losses 34 to 62 d post-AI, % (n/n)	3.7 (4/108)	5.4 (8/149)	0.55
Total Pregnancy Losses 24 to 62 d post-AI, % (n/n)	15.5 (19/123)	10.2 (16/157)	0.18

Table 5.2 Effect of treatment on pregnancy loss in cows diagnosed pregnant 24 d post-AI by utilizing the percent change in PSPB levels from d 17 to d 24 post-AI. R35 = cows re-inseminated 35 d post-AI with the progesterone controlled internal drug release device (CIDR) either removed on the d of or d after $PGF_{2\alpha}$ and R42 = cows re-inseminated 42 d post-AI with CIDRs either removed on the d of or d after $PGF_{2\alpha}$. Treatments were combined into R34 and R42 because there was no effect of the d the CIDR was removed on pregnancy loss from 24 to 34 d post-AI (P = 0.11), pregnancy loss 34 to 62 d post-AI (P = 0.86), or total pregnancy loss from 24 to 62 d post-AI (n = 280; P = 0.43).

Treatment Effects on Ovarian Responses

R35 cows had a lower (P = 0.04) ovulation rate to the final GnRH than R42 cows (91.8 vs. 95.5%; n = 810). This appeared to be a result of the low ovulation rates in the RES35 group. RES35 had a lower ovulation rate than the rest of the treatments (Table 5.3). There was no difference in ovulation rate amongst RES35+1, RES42, and RES42+1 (Table 5.3). The month of insemination had no effect on ovulation rate (P = 0.75; n = 810). There was no difference (P = 0.11) in multiple ovulation rates between R35 and R42 (18.2 vs. 14.0%; n = 758). The d the CIDR was removed also had no effect on multiple ovulation rates (Table 5.3). Overall, absence of a dominant follicle was the predominant reason cows in this study did not ovulate (P < 0.001; n = 52). The proportion of cows that did not ovulate because there was no dominant follicle present was greater for RES35 than the other treatments (Table 5.3). There was no effect of treatment on cows that had a follicle >10 mm on the d of GnRH but did not ovulate (Table 5.3).

R35 cows had larger ($P < 0.001$) average ovulatory follicles than R42 cows (17.51 ± 0.18 vs. 15.66 ± 0.13 mm; n = 748). RES35 had larger average ovulatory follicles than RES35+1, but there was no difference in average ovulatory follicle size between RES42 and RES42+1 (Table 5.3).

	RES35	RES35+1	RES42	RES42+1
Ovulation, % (n/n)	88.6[a] (187/211)	95.1[b] (192/202)	94.5[b] (189/200)	96.5[b] (190/197)
Multiple Ovulation, % (n /n)	19.3[a] (36/187)	17.2[a] (33/192)	12.7[a] (24/189)	15.3[a] (29/190)
No Ovulation[1], % (n/n)	1.4[a] (3/211)	1.5[a] (3/202)	1.0[a] (2/200)	0.5[a] (1/197)
No Dominant Follicle[2], % (n/n)	10.0[a] (21/211)	3.5[b] (7/202)	4.5[b] (9/200)	3.1[b] (6/197)
Average Ovulatory Follicle Size, mm ± SEM	17.9 ± 0.24[a]	17.2 ± 0.27[b]	15.4 ± 0.17[c]	15.87 ± 0.19[c]

Table 5.3 Effect of treatment on ovarian responses. RES35 = 35 d re-insemination interval with the progesterone controlled internal drug release device (CIDR) removed on the d of $PGF_{2\alpha}$; RES35+1 = 35 d re-insemination interval with CIDR removed on the d after $PGF_{2\alpha}$; RES42 = 42 d re-insemination interval with CIDR removed on d of $PGF_{2\alpha}$; and RES42+1 = 42 d re-insemination interval with CIDR removed on the d after PGF2α. Means and percentages within a row with different letters differ ($P \leq 0.05$).

[1] Cows that were classified as no ovulation had follicles ≥ 10 mm on the d of GnRH that either continued to grow or stayed the same size and was not accompanied by subordinate follicle growth.

[2] Cows that were classified as "No Dominant Follicle" either did not have a follicle ≥ 10 mm on the d of GnRH or had a follicle ≥ 10 mm that decreased in size and was accompanied by subordinate follicular growth.

Table 5.4 details the differences in P_4 on the d of the final $PGF_{2\alpha}$ between R35 and R42. R35 cows had lower P_4 on the d of $PGF_{2\alpha}$ than R42 cows (Table 5.4). There was no difference in levels of P_4 on the d of $PGF_{2\alpha}$ between RES35 and RES35+1 (3.6 ± 0.33 vs. 3.4 ± 0.32 ng / mL;

P = 0.63; n = 135) or between RES42 and RES42+1 (6.3 ± 0.49 vs. 5.5 ± 0.43 ng / mL; P = 0.24; n = 149). R42 had a greater proportion of cows with high P_4 (≥ 2 ng / mL) on the d of $PGF_{2\alpha}$ than R35 (Table 5.4). R42 cows with high P_4 (≥ 2 ng / mL) on the day of the $PGF_{2\alpha}$ had greater average P_4 than R35 cows in the high P_4 group (6.55 ± 0.34 vs. 4.45 ± 0.29; n = 222; P< 0.0001).

On the d of the final GnRH there was no difference in levels of P_4 between treatments (Table 5.4). There was no difference in levels of P_4 on the d of the final GnRH between RES35 and RES35+1 (1.2 ± 0.13 vs. 1.1 ± 0.15 ng / mL; P = 0.84; n = 135) or between RES42 and RES42+1 (1.1 ± 0.09 vs. 1.0 ± 0.12 ng / mL; P = 0.84; n = 149) either. R35 had a greater proportion of cows with high P_4 (≥ 2 ng / mL) on the d of the final GnRH than R42 (Table 5.4).

	R35	R42	P-value
Average P_4 on d of final $PGF_{2\alpha}$, ng / mL ± SEM	3.5 ± 0.23	5.9 ± 0.33	< 0.0001
P_4 ≥ 2.0 ng / mL on d of final $PGF_{2\alpha}$, % (n/n)	67.4 (91/135)	87.9 (131/149)	< 0.0001
Average P_4 on d of GnRH, ng / mL ± SEM	1.1 ± 0.10	1.1 ± 0.07	0.51
P_4 ≥ 2.0 ng / mL on d of GnRH, % (n/n)	14.8 (20/135)	6.7 (10/149)	0.04

Table 5.4 Treatment effects on fertility and ovarian parameters based on progesterone (P_4) levels from a subset of cows (n = 284) on the d of final $PGF_{2\alpha}$ and the d of the final GnRH. R35 = cows re-inseminated 35 d post-AI with the P_4 controlled internal drug release device (CIDR) either removed on the d of or d after final $PGF_{2\alpha}$ and R42 = cows re-inseminated 42 d post-AI with CIDRs either removed on the d of or d after final $PGF_{2\alpha}$. Treatments were combined into R35 and R42 because the d the CIDR was removed had no effect on circulating concentrations of P_4 on the d of the final $PGF_{2\alpha}$ or the d of the final GnRH. Differences in circulating concentrations of P_4 on the d of the final $PGF_{2\alpha}$ based on the d the CIDR was removed are as follows: RES35 vs. RES35+1 (P = 0.63) and RES42 vs. RES42+1 (P = 0.24). Differences in circulating concentrations of P_4 on the d of the final GnRH based on the d the CIDR was removed are as follows: RES35 vs. RES35+1 (P = 0.84) and RES42 vs. RES42+1 (P = 0.84).

Overall, cows with higher P_4 on the d of the final $PGF_{2\alpha}$ had smaller ovulatory follicles (P = 0.0002; n = 284). There was an interaction (P = 0.01), however, between the effects of treatment and class of P_4 (low vs. high) on the d of $PGF_{2\alpha}$ on average ovulatory follicle size (n = 274). R35 cows with high P_4 (≥ 2 ng / mL) on the d of the $PGF_{2\alpha}$ had larger average ovulatory follicles (P < 0.0001) than R42 cows with high P_4 (≥ 2 ng / mL) on the d of the $PGF_{2\alpha}$ (17.1 ± 0.39 vs. 14.9 ± 0.20; n = 215). There was no difference (P = 0.79) in average ovulatory follicle size between treatments when cows had low P_4 (< 2 ng / mL) on the d of $PGF_{2\alpha}$ (n = 59). Cows in the R42 treatment with low P_4 (< 2 ng / mL) on the d of $PGF_{2\alpha}$ had larger (P = 0.004) average ovulatory follicles than cows with high P_4 (≥ 2 ng / mL) on the d of $PGF_{2\alpha}$ (17.2 ± 0.31 vs. 14.9 ± 0.20 mm; n = 149). There was no difference (P = 0.76) in average ovulatory follicle size between cows with low P_4 (< 2 ng / mL) or high P_4 (≥ 2 ng / mL) within the R35 treatment on the d of $PGF_{2\alpha}$ (16.9 ± 0.49 vs. 17.1 ± 0.39 mm; n = 135).

Effect of Ovarian Responses on Fertility Parameters

There was no effect (P = 0.82) of average ovulatory follicle size or an effect of an interaction between average ovulatory follicle size and treatment (P = 0.84) on pregnancy at 24 d post-AI (n = 748). Similarly, there was no effect (P = 0.72) of average ovulatory follicle size or an effect of an interaction between average ovulatory follicle size and treatment (P = 0.33) on pregnancy loss between 24 and 62 d post-AI either (n = 270).

There was a tendency (P = 0.07) for R42 cows with low P_4 (< 2 ng / mL) on the d of $PGF_{2\alpha}$ to have greater PR/AI than R35 cows with low P_4 (< 2 ng / mL) on the d of $PGF_{2\alpha}$ (44.4 vs. 20.5%; n = 62). R42 also had greater (P = 0.02) PR/AI than R35 when cows had high P_4 (≥ 2 ng / mL) on the d of $PGF_{2\alpha}$ (42.8 vs. 27.5% n = 222). There was no difference (P = 0.40) in PR/AI between cows with high P_4 (≥ 2 ng / mL) or low P_4 (< 2 ng / mL) within the R35

treatment on the d of $PGF_{2\alpha}$ (27.5 vs. 20.5%; n = 135), nor any difference (P = 0.92) within the R42 treatment (42.8 vs. 44.4%; n = 149).

Cows with high P_4 (≥ 2 ng / mL) on the d of $PGF_{2\alpha}$ had greater (P = 0.04) pregnancy loss 24 to 62 d post-AI than cows with low P_4 (< 2 ng / mL) on the d of $PGF_{2\alpha}$ (12 vs. 0 %; n = 97). There was no interaction (P = 0.99) between the effects of treatment and class of P_4 on the d of $PGF_{2\alpha}$ and pregnancy loss 24 to 62 d post-AI (n = 97).

DISCUSSION

The primary objective of this study was to utilize an early non-pregnancy diagnosis at 24 d post-AI and a $PGF_{2\alpha}$ - CIDR resynchronization protocol initiated with $PGF_{2\alpha}$ (R35) to re-inseminate non-pregnant lactating dairy cows a week sooner than a traditional CIDR-Ovsynch resynchronization protocol (R42). We hypothesized that R35 and R42 would result in similar PR/AI making R35 an effective resynchronization protocol. Outcomes from this study indicate that R35 was not an effective resynchronization protocol, even though it shortened the re-insemination interval by 7 d. R35 was unsuccessful due to lower PR/AI and higher rates of pregnancy loss 24 to 35 d post-AI.

The resynchronization protocol was initiated 28 d post-AI for the R35 group. Sartori et al., (2004) reported that the average estrous cycle is 22.9 ± 0.7 d in cows. If a cow returned to estrous in 23 d, she would be at d 5 of the new cycle when R35 was initiated. If the R35 protocol was initiated with GnRH instead of $PGF_{2\alpha}$ less than 70% of the cows would respond (Bello et al., 2006), and the resulting 4 d old CL would not be responsive to the final $PGF_{2\alpha}$ (Howard and Britt, 1990; Pursley et al., 1995; Levy et al., 2000). Thus, it was not possible to obtain a 35 d re-insemination interval with a traditional CIDR-Ovsynch resynchronization protocol, so we

initiated R35 with an injection of $PGF_{2\alpha}$ and a CIDR instead of GnRH and a CIDR. Cows that had returned to estrus before 23 d post-AI and were more than 5 days into the new estrous cycle would have responded to the $PGF_{2\alpha}$ by regressing the CL (Howard and Britt, 1990; Pursley et al., 1995; Levy et al., 2000). Sartori et al., (2004) reported that cows with atypical cycles had an average estrous cycle length of 29.3 ± 1.1 d. The $PGF_{2\alpha}$ would have also regressed the CL for any cows that had not yet returned to estrous. The CIDR was used to provide supplemental P_4 to prevent R35 cows from ovulating prematurely by suppressing LH surges (Sirois and Fortune, 1990; Nation et al., 2000; Silvia et al., 2002). The CIDR was also used to potentially benefit PR/AI since cows were set up to have no CL or low circulating P_4 (Bilby et al., 2013). The R42 protocol was initiated 32 d post-AI with GnRH and a CIDR. The initial GnRH would have ovulated a dominant follicle if there was one and started a new follicular wave (Pursley et al., 1995). If there was no dominant follicle present at the time of the GnRH it was likely that a new follicular wave was emerging anyways (Pursley et al., 1995). The CIDR was used to improve synchronization (Lima et al., 2009; Bilby et al., 2013). The R35 protocol did not control follicular development like the R42 protocol did. Instead, the R35 protocol extended follicular development.

Effect of P_4 on the Day of the final $PGF_{2\alpha}$ on Fertility Parameters

R35 had lower circulating concentrations of P_4 on the day of the final $PGF_{2\alpha}$ than R42 despite the use of a CIDR due to the design of the R35 protocol (Table 5.4). R35 had a greater proportion of cows with low P_4 (< 2 ng / mL) than R42 on the day of the $PGF_{2\alpha}$ (Table 5.4). R35 cows classified as having high P_4 (≥ 2 ng / mL) on the day of the $PGF_{2\alpha}$ still had lower average P_4 concentrations (ng / mL) than R42 cows. We did not find a difference in PR/AI between cows with low P_4 (< 2 ng / mL) or high P_4 (≥ 2 ng / mL) on the day of the $PGF_{2\alpha}$ within treatments.

Others have reported that high P_4 on the day of the $PGF_{2\alpha}$ increases the probability of pregnancy (Bello et al., 2006; Martins et al., 2011; Denicol et al., 2012). It is very likely that PR/AI for R35 were hindered by low concentrations (ng / mL) of P_4 on the day of the $PGF_{2\alpha}$.

Cows with high P_4 (≥ 2 ng / mL) on the day of the $PGF_{2\alpha}$ had higher rates of pregnancy loss 24 to 62 d post-AI than cows with low P_4 (< 2 ng / mL) regardless of treatment (12 vs. 0%). This is most likely due to the fact that fewer cows with low P_4 (< 2 ng / mL) on the day of the $PGF_{2\alpha}$ actually became pregnant (27%; n = 62). In a study with a greater number of cows with P_4 data, cows treated to have low P_4 during ovulatory follicle development had a greater rate of pregnancy loss after 35 d post-AI compared to cows treated to have high P_4 (Martins et al., 2018). Similarly, a different study showed that cows with low P_4 during the Ovsynch protocol had greater pregnancy loss 29 to 57 d post-AI compared to cows with high P_4 (Wiltbank et al., 2012). In our study there was no difference in pregnancy loss rates between treatments 34 to 62 d post-AI, but there was 24 to 34 d post-AI (Table 5.2). R35 had greater pregnancy loss than R42 24 to 34 d post-AI (Table 5.2). This was again likely due to lower concentrations of P_4 during ovulatory follicle development in R35.

Effect of Ovulatory Follicle on Fertility Parameters

Savio et al., (1993b) demonstrated that by administering $PGF_{2\alpha}$ and a CIDR simultaneously the growth phase of dominant follicles was extended. The R35 treatment was set up similarly, so it was very likely that the ovulatory follicle in the R35 group was a first wave follicle around 11 d old. First wave dominant follicles have been reported to have reduced fertility compared to second wave dominant follicles (Bisinotto et al., 2010; Denicol et al., 2012). It was not explicated if the reduced fertility of first wave dominant follicles was due to the follicle itself or the hormonal environment during the first follicular wave (Bisinotto et al.,

2010). Progesterone during the first follicular wave is low and increasing (Sartori et al., 2004). We administered $PGF_{2\alpha}$ so the P_4 would have still been low but probably not increasing. Denicol et al., (2012), reported similar PR/AI for cows that ovulated a second wave follicle and cows that ovulated a first wave follicle but were supplemented with P_4 from two unused CIDRs. Using only one unused CIDR in this study probably did not provide enough P_4 to increase the fertility of the first wave follicle.

Lower concentrations of P_4 on the d of $PGF_{2\alpha}$ was related to larger average ovulatory follicle sizes in this study. This has been demonstrated in other studies as well (Vasconcelos et al., 1999; Bisinotto et al., 2010; Martins et al., 2018). Low concentrations of P_4 increases the frequency of LH pulses (Ireland and Roche, 1982; Savio et al., 1993b; Cerri et al., 2011a). This creates larger dominant follicles with reduced fertility (Cerri et al., 2009; Cerri et al., 2011a; Denicol et al., 2012). It is not surprising that R35 had larger average ovulatory follicle sizes than R42. R35 also had greater variation in range of average ovulatory follicle size than R42 (8.85 – 30.45 vs. 10.10 – 24.75 mm) which we attributed to the lack of follicular synchrony in R35.

We did not find an effect of average ovulatory follicle size on PR/AI or pregnancy loss rates 24 to 62 d post-AI. Vasconcelos et al., (1999) reported that groups with larger expected ovulatory follicles had lower PR/AI and greater pregnancy loss. Bello et al., (2006) showed a quadratic relationship between ovulatory follicle size and fertility where ovulatory follicles of approximately 16 mm in diameter resulted in the greatest PR/AI. Ovulatory follicle size may be an indicator of follicle maturity (Bello et al., 2006; Perry et al., 2007). In an *in vivo* study, oocytes that developed under prolonged dominance underwent premature maturation (Revah and Butler, 1996). Cerri et al., (2009) showed that follicles that had extended periods of dominance developed into poorer quality embryos. R35 had older, larger ovulatory follicles than R42 that

grew under lower concentrations of P_4. These suboptimal characteristics may help to explain the reduced PR/AI and increased pregnancy loss 24 to 34 d post-AI observed in the R35 group.

Interestingly, R35 had a greater proportion of cows with high P_4 ($\geq$ 2 ng / mL) on the day of the final GnRH than R42 (Table 5.4). This was likely due to cows that ovulated early. It is possible that since R35 cows had lower P_4 on the day of the $PGF_{2\alpha}$ that removing the CIDR that day or the day after would create a LH surge before the final GnRH (Ireland and Roche, 1982; Sirois and Fortune, 1990; Savio et al., 1993b). R35 follicles measured on the d of the final GnRH may already have been ovulating. Thus, the time from ovulation to insemination would have been extended and may explain the lower PR/AI and greater pregnancy loss 24 to 34 d post-AI observed in R35 (Pursley et al., 1998). Low circulating concentrations of P_4 has been correlated with a higher incidence of multiple ovulations (Cerri et al., 2011a; Cerri et al., 2011b; Martins et al., 2018) So it was surprising that there was only a 4% difference in multiple ovulation rates between R35 and R42.

R35 had a lower ovulation rate than R42 (91.8 vs. 95.5%). This was a result of RES35 having lower ovulation rates than RES35+1, RES42, and RES42+1 (Table 5.3). Vasconcelos et al., (1999) reported a reduced ovulation rate of 54% when follicles were 10 to 16 d old. If cows were synchronized as expected, R35 would have an 11-d old ovulatory follicle and R42 would have a 9-d old ovulatory follicle on the d of the final GnRH. The absence of a dominant follicle was the main reason for cows not ovulating in this study (Table 5.3). Cows either did not have a follicle $\geq$ 10 mm on the d of the GnRH or cows had a follicle $\geq$ 10 mm that became atretic, characterized by a decrease in size and subordinate follicular growth. Savio et al., (1993a) concluded that low frequency of LH pulses was not sufficient to maintain a functioning dominant

follicle and resulted in atresia. It is possible that instead of ovulating, the dominant follicle in some of the RES35 cows became atretic.

Effects of Parity, Insemination Number, and Month of Insemination on Fertility Parameters

There was an interaction between the effects of treatment and parity on PR/AI (Table 5.1). It appears that this interaction was driven by reduced PR/AI amongst 2nd parity R35 cows. Multiparous cows have reduced fertility compared to primiparous cows (Santos et al., 2009; Carvalho et al., 2014; Middleton et al., 2019), but it is unclear why 2nd parity R35 cows had such fewer PR/AI than 2nd parity R42 cows.

At 2nd AI R42 had greater PR/AI than R35 (Figure 5.3). This relationship was not observed at subsequent inseminations. Second AI would have been 110 – 116 DIM for R35 and 117 – 123 DIM for R42. This difference in PR/AI at 2nd AI was especially important when the goal was to achieve pregnancy by 130 DIM. The range of DIM for subsequent AI would depend on the sequence of treatments for each individual cow.

PR/AI were lower for the months of May, June, July, and August than they were for the months of February, March, April, September, and October. This was likely associated with heat stress during the summer months in Michigan. The effects of heat stress on fertility have been well demonstrated (Putney et al., 1989; Ray et al., 1992; Roth, 2008). PR/AI were not confounded by an interaction between treatment and month of insemination.

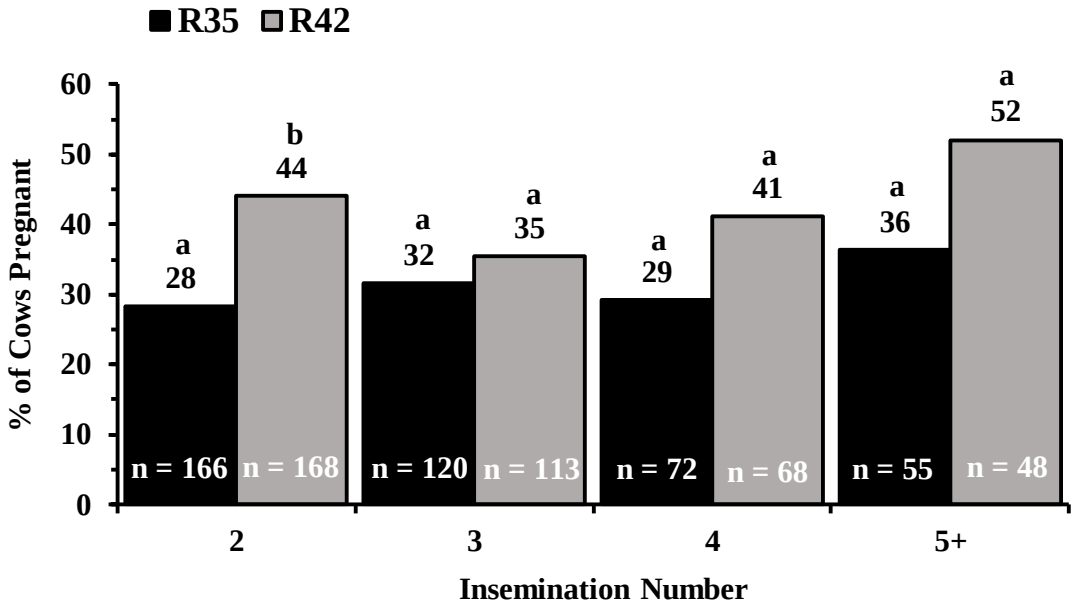

Figure 5.3 Effect of treatment on pregnancy per AI at 24 d post-AI for 2, 3rd, 4th, and 5th + AI. Pregnancy was diagnosed by utilizing the percent change in PSPB levels from d 17 to d 24 post-AI. R35 = cows re-inseminated 35 d post-AI with the progesterone controlled internal drug release device (CIDR) either removed on the d of or d after $PGF_{2\alpha}$ and R42 = cows re-inseminated 42 d post-AI with CIDRs either removed on the d of or d after $PGF_{2\alpha}$. Different superscripts within insemination number represent differences (a, b; $P < 0.05$). Significance between treatment within insemination number for PR/AI are as follows: 2nd AI R35 vs. R42 ($P = 0.003$), 3rd AI R35 vs. R42 ($P = 0.54$), 4th AI R35 vs. R42 ($P = 0.14$), and 5th + AI R35 vs. R42 ($P = 0.10$). R35 cows were 110 – 116 DIM and R42 cows were 117 – 123 DIM at 2nd AI. The range of DIM for subsequent AI depended on the sequence of treatments for each individual cow.

Effect of Timing of CIDR Removal on Fertility Parameters

We used CIDRs to prevent spontaneous ovulations (Sirois and Fortune, 1990; Chebel et al., 2006; Dewey et al., 2010) and to increase synchrony (Lima et al., 2009; Bilby et al., 2013). Our secondary objective was to determine if it is more optimal to remove the CIDR out on the d of the $PGF_{2\alpha}$ (RES35 and RES42) or the d after the $PGF_{2\alpha}$ (RES35+1 and RES35+2). We hypothesized that RES35+1 and RES42+1 would result in greater PR/AI than RES35 and

RES42, respectively. Although not statistically significant, RES35 had 5-points fewer PR/AI than RES35+1 (Figure 5.2). RES35 also had a lower ovulation rate then RES35+1 (Table 5.3) with a greater proportion of cows not ovulating due to lack of a dominant follicle (Table 5.3). Since R35 is not likely to be implemented as a resynchronization protocol, our data indicates that it makes no difference whether the CIDR is removed on the d of the $PGF_{2\alpha}$ or the d after. The d the CIDR was removed did not affect PR/AI or pregnancy loss in the R42 treatment. From a management perspective, it is more feasible to remove the CIDR the same d $PGF_{2\alpha}$ is administered.

Economic Considerations for R35 and R42

Giordano et al., (2011) concluded that 130 DIM was the optimal time for a cow to become pregnant from a profitability standpoint. The greatest losses in expected monetary value occurred when cows became pregnant after 150 DIM (Giordano et al., 2011). A recent study found that cows that became pregnant by 130 DIM fell into a "high fertility cycle" where they lost less body condition, had fewer health events, greater PR/AI, and less early pregnancy loss in the next lactation (Middleton et al., 2019). This 130 DIM cutoff aligns closely with previous studies that stated 12 to 13- calving intervals were the most profitable (Dijkhuizen et al., 1984; Meadows, 2005). Using 130 DIM as a reference can provide a useful way to evaluate the efficiency of a herd's reproductive program. The difference in PR/AI at 2nd AI between R35 and R42 shows that R42 had a greater proportion of cows achieve pregnancy by 130 DIM (Figure 5.3). R42 appeared to be a more efficient protocol even though cows were inseminated 7 d later then R35.

We used the Wisconsin-Cornell DairyRepro$ (UWCURepro$ v. 1.4) tool to calculate the difference in profit / cow / year for R35 and R42 (Giordano et al., 2012a). Herd size was set to

1,000 cows with G6G as the first AI program and CIDR-Ovsynch for second and subsequent inseminations. We used the farm's 1st AI PR/AI (49%) from the time of the study. Everything was held constant except for the differences in re-insemination intervals, PR/AI and pregnancy loss for R42 (the current program) and R35 (the alternative program). To account for the R35 protocol being initiated with a $PGF_{2\alpha}$ instead of a GnRH, the default GnRH cost per dose for R35 was cut in half and the default $PGF_{2\alpha}$ cost per dose was doubled. R35 was $28.80/cow/year less profitable than R42 in this scenario of a single herd. The value of pregnancy and hence the cost of pregnancy loss was less when cows were given more chances to become pregnant (De Vries, 2006). So arguably, the R35 treatment would have a lower cost of pregnancy and pregnancy loss than the R42 treatment because cows were inseminated 7 d sooner. This gain in 7 DIM, however, was not enough to justify the fewer PR/AI and greater pregnancy loss 24 to 34 d post-AI in the R35 treatment. Giordano et al., (2011) suggested that there may be a tradeoff between DIM and PR/AI where longer programs with higher PR/AI are more economically viable than programs that reduce re-insemination intervals but have lower PR/AI. R42 was more efficient and economically viable than R35.

CONCLUSION

Even though cows in R35 were bred 7 d sooner than cows in R42, the lower PR/AI and higher rate of pregnancy loss 24 to 34 d post-AI made R35 an ineffective resynchronization strategy compared to R42. PR/AI did not differ depending on the d the CIDR was removed. Therefore, it makes the most sense to remove CIDRs on the same d $PGF_{2\alpha}$ is administered in a CIDR-Ovsynch protocol. This was a first attempt to use a non-pregnancy diagnosis at 24 d post-AI to re-inseminate cows as soon as possible. Although unsuccessful we believe that there is

potential for more successful protocols to be developed that will make it possible to achieve pregnancy by 130 DIM.

CHAPTER 6

CONCLUSION

INTRODUCTION

The intent of this thesis is to demonstrate the importance of achieving timely pregnancies in lactating dairy cows and to present some novel reproductive management tools to create timely pregnancies. This final chapter will summarize data in a lay article format that will be re-formatted and submitted for publication in an industry magazine.

THE HIGH FERTILITY CYCLE

The dairy industry has recognized for years that the ideal calving interval from a profitability standpoint is 12 to 13 months. In 2011, a study from the University of Wisconsin concluded that to maximize profitability cows need to become pregnant around 130 DIM (Giordano et al., 2011). Using a 280-d gestation, cows that become pregnant at 130 DIM would have a 13.5 month calving interval. Our research suggests that achieving pregnancy by 130 DIM is also important for fertility and cow health in the subsequent lactation.

All cows were body condition scored within a week of calving on a 1 to 5-point scale with tenth of a point increments and then again at 30 DIM. Cows were synchronized with the G6G/Ovsynch protocol and received 1st timed-AI at 75 to 81 DIM. Pregnancy was diagnosed 35 d later. If diagnosed not-pregnant cows were resynchronized with G6G/Ovsynch and re-inseminated within 56 d. We recorded all health, reproductive, and milk production information for the whole lactation along with reproduction data from the previous lactation.

Cows with longer previous calving intervals had greater body condition at parturition and lost more body condition during the 1st 30 DIM. The incidence of postpartum health events (dystocia, twinning, retained placenta, ketosis, metritis, and displaced abomasum) increased as cows lost more body condition. Interestingly, 20% of the 851 cows in the study maintained or gained body condition during the 1st 30 DIM. Another study from the University of Wisconsin

also found that a portion of cows maintain or gain body condition during the early postpartum period (Carvalho et al., 2014). Multiparous cows that maintained or gained body condition during the 1st 30 DIM had higher PR/AI at 1st AI than cows that had lost body condition (48 vs. 36%). Cows that lost body condition also had a higher rate of pregnancy loss 35 to 60 d after 1st AI than cows that maintained or gained body condition (8 vs. 0%). From our data we determined a cutoff of 130 DIM where cows that became pregnant by 130 DIM had a 75% greater chance of maintaining or gaining body condition during the 1st 30 DIM of the subsequent lactation. This led to fewer health issues, greater PR/AI, and reduced early pregnancy loss which increased the chances of becoming pregnant by 130 DIM once again. We refer to this dynamic relationship as the "high fertility cycle" (Figure 6.1). The incidence of at least one postpartum health event had no effect on PR/AI, but maintaining a "high fertility cycle" may reduce periparturient health disorders.

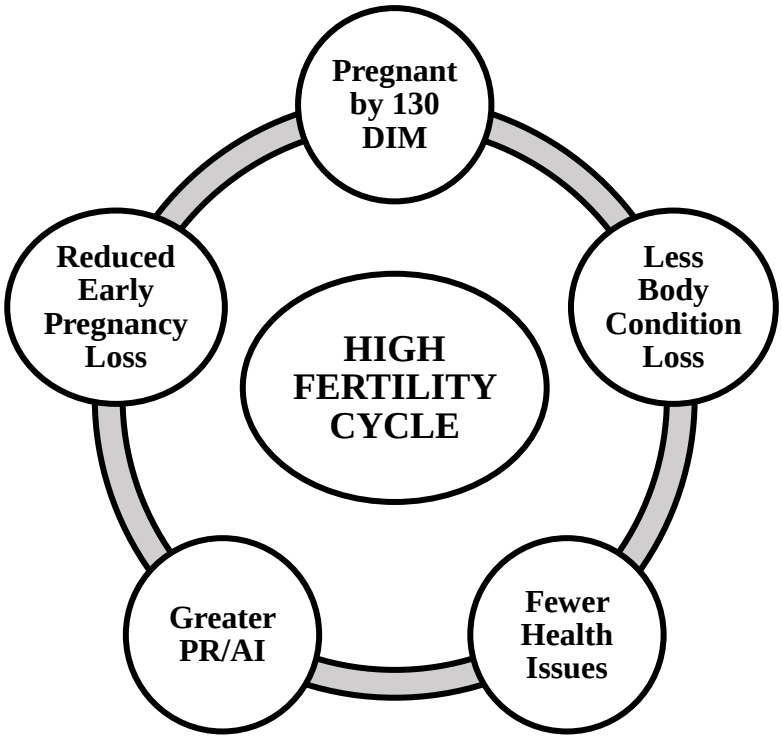

Figure 6.1 A pictorial depiction of the "high fertility cycle".

Cows that maintained or gained body condition had lower milk production at 30 and 60 DIM than cows that lost body condition, so there may be an economic tradeoff. Cows that maintained or gained body condition had lower milk production near peak milk production, but also spent fewer d open and fewer d in late lactation. These cows were also less likely to be treated for a postpartum health issue, which was associated with lower milk production at 30 DIM in our study. Achieving pregnancy by 130 DIM aligns closely with the profitable 12 to 13-month calving interval and can benefit cow health and fertility in the next lactation.

UTILIZING REPRODUCTIVE MANAGEMENT TOOLS TO ENTER A HIGH FERTILITY CYCLE

Reproductive management tools can be used to help cows enter a "high fertility cycle". The first step is to control time to 1st AI while maximizing PR/AI. Synchronization programs allow producers to control time to 1st AI. Fertility programs like G6G, Double-Ovsynch and Presynch-11 have improved PR/AI significantly. It is also critical to diagnose non-pregnant cows as early as possible so cows can be re-inseminated. Thus, giving cows more chances to become pregnant by 130 DIM.

In Chapter 4, we utilized two samples of PSPB taken at d 17 and 24 post-AI to diagnose non-pregnancy with 100% accuracy at 24 d post-AI. Both blood samples were assayed on the same plate utilizing the commercially available BioPRYN assay. This non-pregnancy diagnosis method is incredibly accurate and early; however, we realize the cost of two blood samples and associated labor may not be practical for most dairy farms. Nevertheless, novel early non-pregnancy diagnosis methods like the one we presented will be essential for achieving pregnancy by 130 DIM. It is imperative that pregnant cows are diagnosed pregnant 100% of the time, so they are not given $PGF_{2\alpha}$ for resynchronization. The number of non-pregnant cows diagnosed as pregnant also needs to be kept to a minimum to ensure that non-pregnant cows are re-

inseminated in timely matter. There is a need for further research into developing early and accurate non-pregnancy diagnosis methods. An early non-pregnancy diagnosis may lead to the development of resynchronization protocols that re-inseminate cows sooner.

We tested a short resynchronization protocol in Chapter 5. Cows that were diagnosed non-pregnant at 24 d post-AI were given an injection of $PGF_{2\alpha}$ and a CIDR on d 28 post-AI. Four days later, the cows were given another injection of $PGF_{2\alpha}$ and had their CIDRs removed either that day or the next. Cows then received GnRH 2 d later and timed-AI 35 d post-AI. We compared this protocol (R35) to an Ovsynch with a CIDR that re-inseminated cows 42 d post-AI (R42). R35 cows had lower PR/AI and higher pregnancy loss 24 to 34 d post-AI compared to cows in the R42 treatment. R35 was ineffective as an early resynchronization strategy. The other element to this experiment was to see if it was more optimal to remove CIDRs on the day of the $PGF_{2\alpha}$ or the day after. PR/AI was not affected by the d the CIDR was removed, so the most practical recommendation is to remove CIDRs the same day $PGF_{2\alpha}$ is administered. This study was a first attempt to use an early non-pregnancy diagnosis to develop a resynchronization protocol that re-inseminated all non-pregnant cows as soon as possible. With further research, there is potential for short resynchronization protocols to be developed with higher PR/AI. Using reproductive management tools will make it possible to achieve pregnancy by 130 DIM.

SUMMARY

This research stresses the importance of timely pregnancies and their influence on dairy reproductive efficiency. Cows that became pregnant by 130 DIM had better fertility and fewer periparturient health disorders in the next lactation in this study from a single herd. We proposed a novel method of diagnosing non-pregnancy that was 100% accurate at 24 d post-AI. Diagnosing non-pregnancy as soon as possible can allow for early resynchronization protocols to

be developed that re-inseminate cows quickly. Further research is needed to continue developing early non-pregnancy diagnosis methods and short resynchronization protocols that re-inseminate cows sooner without sacrificing PR/AI. Entering the "high fertility cycle" will depend on utilizing a combination of fertility programs at 1st AI, early non-pregnancy diagnoses and short resynchronization protocols to create pregnancies by 130 DIM.

REFERENCES

REFERENCES

Altman, D. G. and J. M. Bland. 1994. Diagnostic tests 1: sensitivity and specificity. BMJ. 308: 1552.

Arnold, H. J. P. N. Martins, L. Z. Oliveira, R. K. Policelli, K. M. Stomack, G. Sasser, and J. R. Pursley. 2012. Effectiveness of pregnancy-specific protein b in pregnancy diagnosis of dairy cows and heifers. J. Dairy Sci. 95(Suppl. 1): 128.

Bello, N. M., J. P. Steibel, and J. R. Pursley. 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. J. Dairy Sci. 89: 3413-3424.

Berry, D. P., J. M. Lee, K. A. Macdonald, and J. R. Roche. 2007b. Body condition score and body weights on dystocia and stillbirths and consequent effects on post calving performance. J. Dairy Sci. 90: 4201-4211.

Berry, D. P., R. F. Veerkamp, and P. Dillon. 2006. Phenotypic profiles for body weight, body condition score, energy intake, and energy balance across different parities and concentrate feeding levels. Livest. Sci. 104: 1-12.

Berry, D.P., F. Buckley, and P. Dillon. 2007a. Body condition score and live-weight effects on milk production in Irish Holstein-Friesian dairy cows. Animal. 1: 1351-1359.

Bilby, T. R., R. G. S. Bruno, K. J. Lager, R. C. Chebel, J. G. N. Moraes, P. M. Fricke, G. Lopes Jr., J. O. Giordano, J. E. P. Santos, F. S. Lima, J. S. Stevenson, and S. L. Pulley. 2013. Supplemental progesterone and timing of resynchronization on pregnancy outcomes in lactating dairy cows. J. Dairy Sci. 96: 7032-7042.

Bisinotto, R. S., R. C. Chebel, and J. E. P. Santos. 2010. Follicular wave of the ovulatory follicle and not cyclic status influenced fertility of dairy cows. J. Dairy Sci. 93: 3578-3587.

Britt, J. H. 1985. Enhanced reproduction and its economic implications. J. Dairy Sci. 68: 1585-1592.

Britt, J. H. 1992. Impacts of early postpartum metabolism on follicular development and fertility. The Bovine Practioner Proceedings. 24: 29-43.

Brusveen, D. J., A. H. Souza, and M. C. Wiltbank. 2009. Effects of additional prostaglandin F2α and estradiol-17β during Ovsynch in lactating dairy cows. J. Dairy Sci. 92: 1412-1289.

Butler, J. E., W. C. Hamilton, R. G. Sasser, C. A. Ruder, G. M. Hass, and R. J. Williams. 1982. Detection and partial characterization of two bovine pregnancy-specific proteins. Bio. Reprod. 26: 925-933.

Butler, W. R. and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy Sci. 72: 767-783.

Butler, W. R., R. W. Everett, and C. E. Coppock. 1981. The relationships between energy balance, milk production and ovulation in postpartum Holstein cows1. J. Anim. Sci. 53: 742-748.

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. L. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. J. Dairy Sci. 89: 4723-4735.

Carvalho, P. D., A. H. Souza, M. C. Amundson, K. S. Hackbart, M. J. Fuenzalida, M. M. Herlihy, H. Aryes, A. R. Dresch, L. M. Vieira, J. N. Guenther, R. R. Grummer, P. M. Fricke, R. D. Shaver, and M. C. Wiltbank. 2014. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. J. Dairy Sci. 97: 3666-3683.

Cerri, R. L. A., H. M. Rutigliano, R. C. Chebel, and J. E. P. Santos. 2009. Period of dominance of the ovulatory follicle influenced embryo quality in lactating dairy cows. Reproduction. 137: 813-823.

Cerri, R. L. A., R. C. Chebel, F. Rivera, C. D. Narciiso, R. A. Oliveira, W. W. Thatcher, and J. E. P. Santos. 2011b. Concentration of progesterone during the development of the ovulatory follicle: I. Ovarian and embryonic responses. J. Dairy Sci. 94: 3342-3351.

Cerri., R. L. A., R. C. Chebel, F. Rivera, C. D. Narciso, R. A. Oliveira, M. Amstalden, G. M. Baez-Sandoval, L. J. Oliveira, W. W. Thatcher, and J. E. P. Santos. 2011a. Concentration of progesterone during the development of the ovulatory follicle: II. Ovarian and uterine responses. J. Dairy Sci. 94: 3352-3365.

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89: 4205-4219.

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, K. N. Galvão, S. O. Juchem, and W. W. Thatcher. 2003. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology. 60: 1389-1399.

Congleton, Jr., W. R. and L. W. King. 1984. Profitability of dairy cow herd life. J. Dairy Sci. 67: 661-674.

Curran, S. 1992. Fetal sex determination in cattle and horses by ultrasonography. Theriogenology 37: 17-21.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle1. J. Dairy Sci. 89: 3876-3885.

De Vries, A., J. D. Olson, and P. J. Pinedo. 2010. Reproductive risk factors for culling and productive life in large dairy herds in the eastern United States between 2001 and 2006. J. Dairy Sci. 93: 613-623.

De Vries, M. J. and R. F. Veerkamp. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci. 83: 62-69.

De Vries, M. J., S. Van Der Beek, L. M. T. E. Kaal-Lansbergen, W. Ouweltjies, and J. B. M. Wilmink. 1999. Modeling of energy balance in early lactation and the effect of energy deficits in early lactation on first detected estrus postpartum in dairy cows. J. Dairy Sci. 82: 1927-1934.

Denicol, A. C., G. Lopes Jr., L. G. D. Mendonça, F. A. Rivera, F. Guagnini, R. V. Perez, J. R. Lima, R. G. S. Bruno, J. E. P. Santos, and R. C. Chebel. 2012. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J. Dairy Sci. 95: 1794-1806.

Dewey, S. T., L. G. D. Mendonça, G. Lopes Jr., F. A. Rivera, F. Guagnini, R. C. Chebel, and T. R. Bilby. 2010. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. J. Dairy Sci. 93: 4086-4095.

Dijkhuizen, A. A., J. Stelwagen, and J. A. Renkema. 1984. Economic aspects of reproductive failure in dairy cattle. I. Financial loss at farm level. Prev. Vet Med. 3: 251-263.

Dohoo, I. R. and S. W. Martin. 1984. Disease, production and culling in Holstein-Friesian cows: III. Disease and production as determinants of disease. Prev. Vet Med. 2: 671-690.

Domecq, J. J., A. L. Skidmore, J. W. Lloyd, and J. B. Kaneene. 1997. Relationships between body condition scores and conception at first artificial insemination in a large dairy herd of high yielding Holstein cows. J. Dairy Sci. 80: 113-120.

Echternkamp, S. E., R. A. Cushman, M. F. Allan, R. M. Thallman, and K. E. Gregory. 2007. Effects of ovulation rate and fetal number on fertility in twin-producing cattle. J. Anim. Sci. 85: 3228-3238.

Edmondson, A.J., I.J. Lean, L.D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72: 68-78.

Engel, C. L., H. H. Patterson, and G. A. Perry. 2008. Effect of dried corn distillers grain plus solubles compared with soybean hulls, in late gestation heifer diets, on animal and reproductive performance. J. Anim. Sci. 86: 1697-1708.

Fricke, P. M. 2002. Scanning the future – Ultrasonography as a reproductive management tool for dairy cattle1. J. Dairy Sci. 85: 1918-1926.

Fricke, P. M., A. Ricci, J. O. Giordano, P. D. Carvalho. 2016. Methods for and implementation of pregnancy diagnosis in dairy cows. Vet. Clin. Food Anim. 32: 165-180.

Fricke, P. M., D. Z. Caraviello, K. A. Weigel, and M. L. Welle. 2003. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J. Dairy Sci. 86: 3941-3950.

Gábor, G. J. P. Kastelic, Z. Abonyi-Tóth, P. Gábor, T. Endrödi, and O. G. Balogh. 2016. Pregnancy loss in dairy cattle: Relationship of ultrasound, blood pregnancy-specific protein B, progesterone production variables. Reprod. Dom. Anim. 51: 467-473.

Galvão, K. N., M. F. Sá Filho, and J. E. P. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. J. Dairy Sci. 90: 4212-4218.

Gearhart, M. A., C. R. Curtis, H. N. Erb, R. D. Smith, C. J. Sniffen, L. E. Chase, and M. D. Cooper. 1990. Relationship of changes in condition score to cow health in Holsteins. J. Dairy Sci. 73: 3132-3140.

Gillund, P., O. Reksen, Y. T. Gröhn, and K. Karlberg. 2001. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. J. Dairy Sci. 84: 1390-1396.

Giordano, J. O., A. S. Kalantari, P. M. Fricke, M. C. Wiltbank, and V. E. Cabrera. 2012a. A daily herd Markov-chain model to study the reproductive and economic impact of reproductive programs combining timed artificial insemination and estrus detection. J. Dairy Sci. 95: 5442-4972.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, R. Pawlisch, S. Bas, A. P. Cunha, and P. M. Fricke. 2012b. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J. Dairy Sci. 95: 639-653.

Giordano, J. O., M. J. Thomas, G. Catucuamba, M. D. Curler, M. Masello, M. L. Stanaferro, and R. Wijma. 2016. Reproductive management strategies to improve the fertility of cows with a suboptimal response to resynchronization of ovulation. J. Dairy Sci. 99: 2967-2978.

Giordano, J. O., P. M. Fricke, M. C. Wiltbank, and V. E. Cabrera. 2011. An economic decision-making support system for selection or reproductive management programs on dairy farms. J. Dairy Sci. 94: 6216-6232.

Giordano, J., P. Fricke, and V. E. Cabrera. 2013. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. J. Dairy Sci. 96: 949-961.

Green, J. A., S. Xie, X. Quan, B. Bao, X. Gan, N. Mathialagan, J. F. Beckers, and R. M. Roberts. 2000. Pregnancy-associated bovine and ovine glycoproteins exhibit spatially and temporally distinct expression patterns during pregnancy. Biol. Reprod. 62: 1624-1631.

Green, J. C., E. M. Newsom, and M. C. Lucy. 2011. Incorporation of a rapid pregnancy-associated glycoprotein ELISA into a CIDR-Ovsynch resynchronization program for a 28-day re-insemination interval. Theriogenology. 75: 320-328.

Groenendaal, H., D. T. Galligan, and H. A. Mulder. 2004. An economic spreadsheet model to determine optimal breeding and replacement decisions for dairy cattle. J. Dairy Sci. 87: 2146-2157.

Gröhn, Y. T., and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim. Reprod. Sci. 60 - 61: 605-614.

Hady, P. J., J. J. Domecq, and J. B. Kaneene. 1994. Frequency and precision of body condition scoring in dairy cattle. J. Dairy Sci. 7: 1543-1547.

Heuer, C., Y. H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial herds. J. Dairy Sci. 82: 295-304.

Howard, H. J. and J. H. Britt. 1990. Prostaglandin F-2α causes regression of an hCG-induced corpus luteum before Day 5 of its lifespan in cattle. J. Reprod. Fertil. 90: 245-253.

Humblot, P. 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology. 56: 1417-1433.

Ireland, J. J. and J. F. Roche. 1982. Effect of progesterone on basal LH and episodic LH and FSG secretion in heifers. J. Reprod. Fertil. 64: 295-302.

Kelley, D. E., L. Ibarbia, R. Daetz, J. H. Bittar, C. A. Risco, J. E. P. Santos, E. S. Ribeiro, and K. N. Galvão. 2016. Combined use of progesterone inserts, ultrasonography, and GnRH to identify and resynchronize nonpregnant cows and heifers 21 days after time artificial insemination. Theriogenology. 85: 230-237.

Kiracofe, G. H., J. M. Wright, R. R. Schalles, C. A. Ruder, S. Parish, and R. G. Sassser. 1993. Pregnancy-specific protein B in serum of postpartum beef cows. J. Anim. Sci. 71: 2199-2205.

Levy, N., S. Kobayashi, Z. Roth, D. Wolfenson, A. Miyamoto, and R. Meidan. 2000. Administration of prostaglandin F2α during the early bovine luteal phase does not alter the expression of ET-1 and of its Type A receptor: A possible cause for corpus luteum refractoriness. Biol. Reprod. 63: 377-382.

Lima, J. R., F. A. Rivera, C. D. Narciso, R. Oliveira, R. C. Chebel, and J. E. P. Santos. 2009. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J. Dairy Sci. 92: 5436-5446.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim. Reprod. Sci. 81: 209-223.

López-Gatius, F., J. M. Garbayo, P. Santolaria, J. Yániz, A. Ayad, N. M. de Sousa, and J. F. Beckers. 2007. Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Domest. Anim. Endocrinol. 32: 29-42.

López-Gatius, F., P. Santolaria, J. Yániz, J. Rutllant, and M. López-Béjar. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Theriogenology 57: 1251-1261.

Louca, A. and J. E. Legates. 1967. Production losses in dairy cattle due to days open1. J. Dairy Sci. 51: 573-583.

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: Where will it end?1 J. Dairy Sci. 84: 1277-1293.

Macmillan, K. L. and A. J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronization, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim. Reprod. Sci. 33: 1-25.

Macmillan, K. L., V. K. Taufa, D. R. Barnes, and A. M. Day. 1991. Plasma progesterone concentrations in heifers and cows treated with a new intravaginal device. Anim. Reprod. Sci. 26: 25-40.

Martins, J. P. N., D. Wang, N. Mu, G. F. Rossi, A. P. Martini, V. R. Martins, and J. R. Pursley. 2018. Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. J. Dairy Sci. 101: 1-21.

Martins, J. P. N., M. J. T. Acevedo, T. O. Cunha, C. Piterini, and J. R. Pursley. 2017. The effect of presynchronization with prostaglandin F 2α and gonadotropin-releasing hormone simultaneously, 7 d before Ovsynch, compared with Presynch-10 / Ovsynch on luteal function and first-service pregnancies per artificial insemination. J. Dairy Sci. 100: 5107-5116.

Martins, J. P. N., R. K. Policelli, L. M. Neuder, W. Raphael, and J. R. Pursley. 2011. Effects of cloprostenol sodium at final prostaglandin F2α of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J. Dairy Sci. 94: 2815-2824.

McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: A neuroendocrine-mediated event. Physiol. Rev. 79: 263-324.

Meadows, C. 2005. Reproductive record analysis. Vet. Clin. Food Anim. 21: 305-323.

Middleton, E. L. and J. R. Pursley. 2019. Short Communication: Blood samples before and after embryonic attachment accurately determines non-pregnant lactating dairy cows at 24 d post-AI

using a commercially available assay for PSPB. J. Dairy Sci. *Accepted for Publication 28 March 2019.*

Middleton, E. L., T. Minela, and J. R. Pursley. 2019. The high-fertility cycle: How timely pregnancies in one lactation may lead to less body condition loss, fewer health issues, greater fertility, and reduced early pregnancy losses in the next lactation. J. Dairy Sci. *In Press, Corrected Proof, Available online 21 March 2019.* DOI: https://doi.org/10.3168/jds.2018-15828.

Momont, H. W. 1990. Rectal palpation: safety issues. Bovine Pract. 25: 122-123.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84: 1646-1659.

Moreira, F., C. Risco, M. F. A. Pires, J. D. Ambrose, M. Drost, M. DeLorenzo, and W. W. Thatcher. 2000. Effect of body condition on reproductive efficiency of lactating dairy cows receiving a timed insemination. Theriogenology. 53: 1305-1319.
Morris, R.S. 1971. Economic aspects of disease control programmes for dairy cattle. Aust. Vet. J 47: 358-363.

Nation, D. P., C. R. Burke, G. Parton, R. Stevenson, and K. L. Macmillan. 2000. Hormonal and ovarian responses to a 5-day progesterone treatment in anoestrous dairy cows in the third week post-partum. Anim. Reprod. Sci. 63: 13-25.

Nielen, M. Y. H. Schukken, D. T. Scholl, H. J. Wilbrink and A. Brand. 1989. Twinning in dairy cattle: a study of risk factors and effects. Theriogenology. 32: 845-862.

Northey, D. L. and L. R. French. 1980. Effect of embryo removal and intrauterine infusion of embryonic homogenates on the lifespan of the bovine corpus luteum. J. Anim. Sci. 50: 298-302.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Otto, K. L., J. D. Ferguson, D. G. Fox, and C. J. Sniffen. 1991. Relationship between body condition score and composition of ninth to eleventh rib tissue in Holstein dairy cows. J. Dairy Sci. 74: 852-859.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85: 684-689.

Piechotta, M., J. Bollwein, M. Friedrich, T. Heilkenbrinker, C. Passavant, J. Branen, G. Sasser, M. Hoedemaker, and H. Bollwein. 2011. Comparison of commercial ELISA blood tests for early pregnancy detection in dairy cows. J. Reprod. Dev. 57: 72-75.

Pieterse, M. C., O. Szenci, A. H. Willemse, C. S. A. Bajcsy, S. J. Dieleman, and M. A. M. Taverne. 1990. Early pregnancy diagnosis in cattle by means of linear-array real time ultrasound scanning of the uterus and a qualitative and quantitative milk progesterone test. Theriogenology. 33: 697-707.

Plazier, J. C. B., G. J. King, J. C. M. Dekkers, and K. Lissemore. 1997. Estimation of economic values for indices for reproductive performance in dairy herds using computer simulation. J. Dairy Sci. 80: 2775-2783.

Prakash, B., V. L. Telegu, A. M. Walker, and J. A. Green. 2009. Characterization of the bovine pregnancy-associated glycoprotein gene family - analysis of gene sequences, regulatory regions within the promoter and expression of selected genes. BMC Genomics. 10: 185

Pryce, J. E., M. P. Coffey, and G. Simm. 2001. The relationship between body condition score and reproductive performance. J. Dairy Sci. 84: 1508-1515.

Pryce, J.E., M.P. Coffey, and S. Brotherstone. 2000. The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. J. Dairy Sci. 83: 2664-2671.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology. 44: 915-923.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80: 301-306.

Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81: 2139-2144.

Putney, D. J., M. Drost, and W. W. Thatcher. 1989. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. Theriogenology. 31: 765-778.

Rabiee, A. R., I. J. Lean, and M. A. Stevenson. 2005. Efficacy of Ovsynch program on reproductive performance in dairy cattle: A meta-analysis. J. Dairy Sci. 88: 2754-2770.

Ray, D. E., T. J. Halbach, and D. V. Armstrong. 1992. Season and lactation number effects on milk production and reproduction of dairy cattle in Arizona. J. Dairy Sci. 75: 2976-2983.

Remnant, J. G., M. J. Green, J. N. Huxley, and C. D. Hudson. 2014. Variation in the interservice intervals of dairy cows in the United Kingdom. 98: 889-897.

Revah, I. and W. R. Butler. 1996. Prolonged dominance and reduced viability of bovine oocytes. J. Reprod. Fertil. 106: 39-47.

Ribeiro, E. S., F. S. Lima, F. Greco, R. S. Bisinotto, A. P. A. Monteiro, M. Favoreto, H. Ayres, R. S. Marsola, N. Martinez, W. W, Thatcher, and J. E. P. Santos. 2013. Prevalence of periparturient diseases and effects on fertility and seasonally calving grazing dairy cows supplemented with concentrates. J. Dairy Sci. 96: 5682-5697.

Ricci, A., P. D. Carvalho, M. C. Amundoson, R. H. Fourdraine, L. Vincenti, and P. M. Fricke. 2015. Factors associated with pregnancy-associated glycoprotein (PAG) levels in plasma and milk of Holstein cows during early pregnancy and their effect on the accuracy of pregnancy diagnosis. J. Dairy Sci, 98: 2502-2514.

Roberts, R. M., S. Xie, and N. Mathialagan. 1996. Maternal recognition of pregnancy. Biol. Reprod. 54: 294-302.

Roche, J. R., D. P. Berry, J. M. Lee, K. A. Macdonald, and R. C. Boston. 2007. Describing the body condition score change between successive calvings: A novel strategy generalizable to diverse cohorts. J. Dairy Sci. 90: 4378-4396.

Roche, J. R., J. M. Lee, and D. P. Berry. 2006. Pre-conception energy balance and secondary sex ratio – partial support for the Trivers-Willard hypothesis in dairy cows. J. Dairy Sci. 89: 2119-2125.

Romano, J. E., J. A. Thompson, D. W. Forrest, M. E. Westhusin, M. A. Tomaszweski, and D. C. Kraemer. 2006. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology. 66: 1034-1041.

Roth, Z. 2008. Heat stress, the follicle, and its enclosed oocyte: Mechanisms and potential strategies to improve fertility in dairy cows. Reprod. Dom. Anim. 43: 238 – 244.

Ruegg, P. L. and R. L. Milton. 1995. Body condition scores of Holstein cows on Prince Edward Island, Canada: Relationships with yield, reproductive performance, and disease1. J. Dairy Sci. 78: 552-564.

Ryan, D. P. and M. P. Boland. 1991. Frequency of twin births among Holstein-Friesian cows in a warm dry climate. Theriogenology. 36: 1–10.

Santos, J. E. P., H. M. Rutigliano, and M. F. Sá Filho. 2009. Risk factors for resumption of herd-level risk factors for on-farm mortality in Midwest U.S. dairy herds. J. Dairy Sci. 98: 4401-4413.
Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82-83: 513-535.

Santos, J.E.P., H.M. Rutigliano, and M.F. Sá Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110: 207-221.

Sartori, R., J. M. Haughian, R. D. Shaver, G. J. M. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy Sci. 87: 905-920.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler, and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concentrations during gestation. Biol. Reprod. 35: 936-942.

Savio, J. D., W. W. Thatcher, G. R. Morris, L. Entwistle, M. Drost, and M. R. Mattiacci. 1993b. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. J. Reprod. Fertil. 98: 77-84.

Savio, J. D., W. W. Thatcher, L. Badinga, R. L. de la Sota, and D. Wolfenson. 1993a. Regulation of the dominant follicle turnover during the oestrus cycle in cows. J. Reprod. Fertil. 97: 19-203.

Senger, P. L. 1994. The estrus detection problem: New concepts, technologies, and possibilities. J. Dairy Sci. 77: 2745-2753.

Shahid, M.Q., J.K. Reneau, H. Chester-Jones, R.C. Chebel and M.I. Endres. 2015. Cow- and herd-level risk factors for on-farm mortality in Midwest US dairy herds. J. Dairy Sci. 98: 4401-4413.

Silva, E., R. A. Sterry, D. Kolb, M. C. Wiltbank, and P. M. Fricke. 2007. Effect of pretreatment with prostaglandin F2α before resynchronization of ovulation on fertility of lactating dairy cows. J. Dairy Sci. 90: 5509-5517.

Silvia, W. J., T. B. Hatler, A. M. Nugent, and L. F. Laranja da Fonesca. 2002. Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. Domest. Anim. Endocrinol. 23: 167-177.
Sirois, J., and J. E. Fortune. 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology. 127: 916-925.

Souza, A. H, A. Gümen, E. P. B. Silva, A. P. Cunha, J. N. Guenther, C. M. Peto, D. Z. Caraviello, and M. C. Wiltbank. 2007. Supplementation with estradiol-17β before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. J. Dairy Sci. 90: 4623-4634.

Souza, A. H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum times AI in lactating dairy cows. Theriogenology. 70: 208-215.

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Effect of interval from timed artificial insemination to initiation of resynchronization of ovulation on fertility of lactating dairy cows. J. Dairy Sci. 89: 2099-2109.

Strickland, J. M., Martins, J. P. N., Neuder, L. M., and Pursley, J. R. 2010. Effect of 14/11 Presynch/Ovsynch on 1st service conception rates of lactating dairy cows compared to AI following a detected estrus. In: '2010 American Association of Bovine Practitioners Meeting', 2010, Albuquerque, New Mexico, EUA.

Szelényi, Z., O. G. Balogh, F. López-Gatius, I. Garcia-Ispierto, and G. Gábor. 2018. Is twin pregnancy, calving and pregnancy loss predictable by serum pregnancy-specific protein B (PSPB) concentration 28 – 35 days after AI in dairy cows? Acta. Vet Hun. 66: 451-461.

Trivers, R. L., and D. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science 179: 90-92.

Van Cleeff, J., M. Drost, and W. W. Thatcher. 1991. Effects of post insemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. Theriogenology. 36: 795-807.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrus cycle in lactating dairy cows. J. Dairy Sci. 52: 1067-1078.

Vasconcelos, J. L. M., R. W., Silcox, J. A. Lacerda, J. R. Pursley, and M. C. Wiltbank. 1997. Pregnancy rate, pregnancy loss, and response to heat stress after AI at 2 different times from ovulation in dairy cows. Biol. Reprod. Abstract. Madison: Soc. Study Reproduction. 56: 230-230.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. J. Dairy Sci. 71: 1063-1072.

Washburn, S. P., W. J. Silvia, C. H. Brown, B. T. McDaniel, and A. J. McAllister. 2002. Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. 85: 244-251.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Bolman, H. F. Troutt, and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65: 495-501.

Wiltbank, M. C. and J. R. Pursley. 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology. 81: 170-185.

Wiltbank, M. C., A. H. Souza, J. O. Giordano, A. B. Nascimento, J. M. Vasconcelos, M. H. C. Pereira, P. M. Fricke, R. S. Surjus, F. C. S. Zinsly, P. D. Carvalho, R. W. Bender and R. Sartori. 2012. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. Anim. Reprod. 9: 231-241.

Wiltbank, M. C., G. M. Baez, F. Cochrane, R. V. Barletta, C. R. Trayford, and R. T. Joseph. 2015. Effect of a second treatment with prostaglandin F2α during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. J. Dairy Sci. 98: 8644-8654.

Wooding, F. B. P. 1992. Current Topic: The synepitheliochorial placenta of ruminants: Binucleate cell fusions and hormone production. Placenta. 13: 101-113.

Xie, S., B. G. Low, R. J. Nagel, K. K. Kramer, R. V. Anthony, A. P. Zoli, J. F. Beckers, and R. M. Roberts. 1991. Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. Proc. Natl. Acad. Sci. USA. Biochemistry. 88: 10247-10251.

Xu, Z.Z. and L. J. Burton. 2000. Estrus synchronization of lactating dairy cows with GnRH, progesterone, and prostaglandin F2α. J. Dairy Sci. 83: 471-476.

Youngquist, R. S. 2006. Pregnancy Diagnosis. Current Therapy in Large Animal Theriogenology. WB Saunders Co. Philadelphia, PA. pp, 295-303.

Yousef, M. R., J. P. N. Martins, N. Ahmad, K. Nobis, and J. R. Pursley. 2016. Presynchronization of lactating dairy cows with PGF2α and GnRH simultaneously, 7 days before Ovsynch have similar outcomes compared to G6G. Theriogenology. 86: 1607-1614.

Zoli, A. P., L. A. Guilbault, P. Delhaut, W. B. Oritz, and J. F. Beckers. 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its application for pregnancy diagnosis. Biol. Reprod. 46: 83-92.