EFFECT OF AN INJECTION OF $PGF_{2\alpha}$ ADMINISTERED 11 DAYS PRIOR TO DOUBLE-OVSYNCH ON FIRST SERVICE CONCEPTION RISK IN LACTATING DAIRY COWS

by

HILLARY A. MAY

(Under the Direction of Dr. Michael W. Overton)

ABSTRACT

The objective of this study was to determine if the administration of a single injection of $PGF_{2\alpha}$ 11 d prior to the start of Double-Ovsynch would improve first service conception risk and reduce the time from calving to conception. Lactating Holstein cows (n= 1,043) were randomly assigned to one of two groups; the treatment group (n=531) received an injection of $PGF_{2\alpha}$ at 35 ±3 DIM, which corresponded to 11 d prior to starting the Double-Ovsynch protocol. The control group (n=512) received the standard Double-Ovsynch protocol beginning at 46 ±3 DIM without any additional treatments. Results indicate that $PGF_{2\alpha}$ did not increase first service conception risk to Double-Ovsynch; further, first service conception risk was not different for primiparous or multiparous cows. Future studies are needed to assess the potential impact of presynchronization with $PGF_{2\alpha}$ prior to Double-Ovsynch and to develop additional ways of improving first service conception risk and pregnancy rate.

INDEX WORDS: dairy cow, $PGF_{2\alpha}$, reproduction, presynchronization

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DEDICATION

To my family

Thank you for encouraging me to follow my dreams, teaching me the value of hard work, and being my support system throughout this journey.

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CHAPTER 1

INTRODUCTION

Dairy herds often struggle to achieve acceptable levels of reproductive performance and while the exact causes may vary across herds, common issues include poor estrus expression, declining estrus detection efficiency, and low conception risk. Estrus synchronization and ovulation synchronization programs have eliminated or reduced the need for estrus detection in many reproductive management programs and have improved reproductive performance. In an attempt to further increase conception risk, presynchronization programs have been developed to synchronize estrus or ovulation prior to TAI protocols.

Previous studies have looked at presynchronization prior to the start of Ovsynch; however, none have determined the effect of presynchronization or the potential uterine health effects of a dose of $PGF_{2\alpha}$ prior to the start of Double-Ovsynch on first service conception risk. Because exogenous $PGF_{2\alpha}$ has known uterine health benefits, an injection prior to Double-Ovsynch may further increase conception risk by improving uterine health and fertility.
Our hypothesis was that an injection of $PGF_{2\alpha}$ given 11 d prior to starting Double-Ovsynch would improve first service conception risk by improving uterine health and increasing responsiveness to Double-Ovsynch. Cows that have a corpus luteum (CL) present and respond to the initial $PGF_{2\alpha}$ injection should then be in the first 5 to 8 d of their cycle when the first GnRH of Double-Ovsynch is given, thus improving the response to the TAI protocol.

CHAPTER 2

LITERATURE REVIEW

Introduction

Reproductive performance in the United States

United States dairy herds have experienced an apparent decline in reproductive efficiency, which continues to present difficulties for producers. The apparent decline in reproductive efficiency is due in large part to poor estrus expression, declining estrus detection, and low conception risk (Lopez et al., 2004, Washburn et al., 2002, Wiltbank et al., 2006). A major factor affecting reproduction in most U.S. dairy herds is a low rate of estrus detection efficiency (Lopez et al., 2004). Southeastern dairy herds have experienced an apparent decrease in conception risk from about 53% in the 1970s to about 35% in the 1990s based on information gathered from the Dairy Records Management Systems (Raleigh, NC) (Washburn et al., 2002). The decrease in conception risk is likely due to decreased estrus detection efficiency and accuracy and reduced levels of circulating reproductive hormones that have been associated with increased levels of milk production (Wiltbank et al., 2006). High levels of milk production and the associated increase in feed intake lead to an increased rate of clearance of estradiol from circulation which results in a reduction in the duration of standing estrus, prolonged exposure to LH, and a delay in ovulation (Wiltbank et al., 2006). The use of timed-artificial insemination (TAI) protocols has reduced or even eliminated the need for estrus detection when properly implemented and has mitigated many of the impacts of high milk production on reproductive performance.

Multiple factors impact reproductive performance in dairy cows including cow side and sire side parameters. Sire-side factors include reproductive management strategies; semen quality—including motility, morphology, and overall handling; semen placement; heat detection efficiency and accuracy; and timing of semen delivery. Cow-side factors include cyclicity, estrus expression, as well as uterine health. Dystocia, retained placenta, metritis, endometritis, and pyometra are some of the factors that negatively affect uterine health and delay uterine involution. Cyclicity status of the cow impacts reproductive performance because only approximately 46% of cows are expected to be cycling by 40 days-in-milk (DIM); further, only approximately 72% of cows are expected to be cycling by 60 DIM, which indicates that 28% of cows are anestrous and anovulatory at 60 DIM (Staples et al., 1990).

The culmination of these various management and health events is captured as insemination risk and conception risk, the primary drivers of overall reproductive performance (Overton, 2010b). Insemination risk is defined as the number of cows inseminated during a 21-d period divided by the number of cows eligible for breeding (Overton, 2010b). Conception risk (also called conception rate) is defined as the percentage of matings that result in a diagnosed pregnancy (i.e., the number of new pregnancies diagnosed divided by the total number of cows inseminated) (Overton, 2010a). The mean conception risk in the U.S. is approximately 32% (Schefers et al., 2010). Twenty-one d pregnancy rate, or more commonly referred to simply as pregnancy rate, is defined as the total number of cows identified pregnant out of the total number of eligible cow cycles at risk and is commonly examined over 21-d periods of time (Overton, 2006). The average pregnancy rate in the U.S. is approximately 16%, which is also equivalent to 0.16 pregnancies per cow cycle at risk (Overton, 2006).

Economics

A decrease in reproductive efficiency leads to an economic loss through decreased milk per cow per d of life; reduced number of replacement animals produced; increased cost of semen and breeding; losses associated with abortion and embryonic losses; and increased late lactation culling risk with the associated increase in replacement cost. Cows that fail to get pregnant in a timely manner also have an increased risk for becoming over-conditioned, which may lead to dystocia and a greater risk of metabolic disease in her subsequent lactation. The estimated cost of an abortion, which varies based upon level of milk production, milk value, parity, stage of lactation, reproductive efficiency of the herd, replacement costs, and market cow value, ranges from approximately $152 to $1,373 (De Vries, 2006). The average cost estimates per d open (DOPN) range from less than $1 to over $5 (Meadows et al., 2005, Overton, 2006); however, DOPN is not the best measurement of reproductive performance because it is biased and does not take into account the cows that are not currently pregnant, cows that failed to become pregnant, or cows that were culled prior to conceiving (Overton, 2010b). A high producing cow will generally have more opportunities to become pregnant because high producing cows are not likely to be culled early in lactation due to reproductive failure; however, low producing cows are more likely to be culled and have fewer opportunities to become pregnant. In either case, cows are not accounted for in DOPN until a pregnancy is achieved; therefore, a better measure of either individual or herd level reproductive performance is 21-d pregnancy rate.

To estimate the value of changes in reproductive performance, a variety of factors must be considered including heifer replacement cost, milk price, insemination risk, feed cost, conception risk, breeding costs, and level of milk production (Overton, 2009). Synchronization programs are commonly utilized when herds struggle with estrus detection, strive to improve insemination risk, or desire to concentrate labor for breeding management on fewer days. Timed-AI programs are beneficial and cost effective as long as the programs are properly implemented and managed. The cost for a total TAI program was estimated to range from $78 to $86 per cow slot per year; however, the estimated net return for a total TAI program ranged from $13 to $19 per cow slot per year depending upon the reproductive performance of the baseline program used for comparison (Overton, 2006, 2009).

Current Research

Uterine immune function

Once bacteria have gained access to the uterine lumen, neutrophils act as the first line of defense against the development of an infection (Hammon et al., 2006). Polymorphonuclear leukocytes (PMNs), the 'first responders' of the immune response, have been found to aid in the defense mechanism against bacterial contaminants in the uterus (Dhaliwal et al., 2001). The immune defenses of the uterus are suppressed by progesterone, which increases uterine susceptibility to nonspecific infections (Lewis, 2004, Paisley et al., 1986).

Approximately 80 to 95% of cows have bacterial contamination two weeks postpartum (Sheldon et al., 2009). Once in the uterus, bacteria may go on to cause an immediate infection (metritis), a delayed infection (metritis, endometritis or pyometra depending on when the infection occurs and whether or not a corpus luteum is present), or simply result in contamination that is cleared by the cow over a period of days. In some cows, bacteria that are brought into the uterus may colonize and exist without causing an infection until an increase in progesterone concentration causes decreased uterine immune function (Lewis, 2004). The infection will continue until luteolysis occurs and there are decreased concentrations of progesterone, which can no longer diminish uterine immune function and allows the uterus to clear the infection (Jackson, 1977, Lewis, 2004, Ott and Gustafsson, 1981). A decrease in progesterone allows for an increase in estrogen and dilation of the cervix, which results in an increase in blood flow to the uterus and an increase in myometrial contractions, consequently aiding in the expulsion of bacteria.

Prostaglandin $F_{2\alpha}$ is luteolytic and is used to treat uterine infections by reducing the levels of progesterone, thus allowing the infection to clear (Lewis, 1997). Prostaglandin $F_{2\alpha}$ stimulates myometrial contractions, which may help in the expulsion of uterine contents and may increase the phagocytic activity of leukocytes in the uterus (Ott and Gustafsson, 1981, Razin et al., 1978). Prostaglandin $F_{2\alpha}$ and other eicosanoids, such as leukotriene B_4 (LTB_4), are thought to have the ability to improve uterine immune defenses and lessen the effects of progesterone (Lewis, 2004). An injection of $PGF_{2\alpha}$ has been shown to enhance uterine secretion of $PGF_{2\alpha}$, which, in turn, may increase the activity of phospholipase A_2 and cyclooxygenase 2 (COX2) (Lewis and Wulster-Radcliffe, 2006). Phospholipase A_2 potentially increases free arachidonic acid, which can be used to make COX, lipooxygenase, and LTB_4 products (Lewis and Wulster-Radcliffe, 2006). Prostaglandin $F_{2\alpha}$ also allows the display of estrus by eliminating the effects of progesterone.

Uterine health

Uterine health influences reproductive performance primarily via an impact on conception risk. There have been various reports on the prevalence and impact of uterine health problems on reproduction. Uterine health conditions have been described as acute puerperal metritis, clinical metritis, clinical endometritis, subclinical endometritis (SCE), and pyometra

(Sheldon et al., 2006). Following calving, the uterus is often infected with pathogenic bacteria such as *Arcanobacterium pyogenes, Escherichia coli, Fusobacterium spp., Pasteurella multocida, Prevotella spp., Pseudomonas spp., Staphylococcus spp.,* and *Streptococcus spp.* up to two weeks postpartum; however, the bacterial load is drastically decreased by 3 to 4 weeks postpartum in most cows (LeBlanc, 2008).

Metritis

Transition health problems including dystocia, retained placenta, twins, hypocalcaemia, and ketosis are risk factors for the development of metritis in early lactation. Acute puerperal metritis is defined as inflammation of all of the layers of the uterus along with signs of systemic disease, such as a fever greater than 103 degrees Fahrenheit, a foul smelling red-brown watery discharge, and decreased feed intake (Lewis, 1997, Sheldon et al., 2006). Clinical metritis is defined as an enlarged uterus with an abnormal uterine discharge and no signs of systemic illness that is diagnosed within 21 d postpartum (Sheldon et al., 2006). Metritis is associated with an increased risk of culling, decreased milk production, and decreased reproductive efficiency and is thought to predispose cows to the development of endometritis later in lactation.

Endometritis

Endometritis is inflammation of the endometrial lining of the uterus and has negative effects on conception risk, time to pregnancy, culling risk, and profitability (Kasimanickam et al., 2006). Risk factors for endometritis include dystocia, twins, retained placenta, hypocalcaemia, ketosis, puerperal metritis, and clinical metritis (Gautam et al., 2010, LeBlanc, 2008). Due to the lack of a consistent definition of endometritis and the d at diagnosis, the apparent prevalence of endometritis varies widely (Kasimanickam et al., 2004, Kaufmann et al., 2010, LeBlanc et al., 2002a, Sheldon et al., 2006). Pathogenic bacteria commonly associated with endometritis include *Arcanobacterium pyogenes, Fusobacterium necrophorum, Prevotella spp.,* as well as *Escherichia* coli (Sheldon et al., 2006). The mean prevalence of endometritis ranges from 34 to 42% between 21 to 28 DIM (Heuwieser et al., 2000, Kaufmann et al., 2010).

Clinical endometritis

Clinical endometritis has been inconsistently defined and reported in several studies (Correa et al., 1993, Emanuelson et al., 1993, Heuwieser et al., 2000). When defining clinical endometritis, the characteristics of the discharge must be considered as well as the stage of lactation. Clinical endometritis is defined as the presence of a purulent vaginal discharge at greater than 21 d in lactation with no systemic signs of disease; cervical diameter greater than 7.5 cm between 20 and 33 DIM; or mucopurulent discharge after 26 DIM, when examined between 20 and 33 DIM, using vaginoscopy (LeBlanc et al., 2002a, Sheldon et al., 2006). Using the latter definition, the prevalence of clinical endometritis ranged from 16.9% to 18.1% (Kasimanickam et al., 2006, LeBlanc et al., 2002a). Cows with endometritis at 20 to 33 DIM were reported to have a 27% reduction in hazard rate for calving-to-conception interval compared to normal cows; furthermore, cows with clinical endometritis were 1.7 times more likely to be culled due to reproductive failure than normal cows (LeBlanc et al., 2002a).

Subclinical endometritis

Subclinical endometritis is defined as the presence of PMNs in a uterine cytology sample or ultrasonic imaging of fluid in the uterine lumen or both at 21 DIM in the absence of clinical endometritis; as inflammation of the endometrium in the absence of clinical endometritis diagnosed by the presence of greater than 18% neutrophils on cytological evaluation on d 21 to 33; or greater than 10% neutrophils on cytological evaluation at d 34 to 47 postpartum (Kasimanickam et al., 2004, Sheldon et al., 2006). The prevalence of SCE is approximately 37 to 42% at 32 to 49 DIM based on two northeastern studies (Galvão et al., 2009a, Kasimanickam et al., 2006). Cows with endometritis have a longer time to first service and have a decrease in conception risk; furthermore, median DOPN for cows diagnosed with endometritis was reported to be approximately 30 to 60 d longer than normal cows (Borsberry and Dobson, 1989, Gilbert et al., 2005, Kasimanickam et al., 2004).

Treatment with antibiotics

Numerous studies have been conducted to evaluate the effectiveness of treating endometritis using antibiotics, hormones, and disinfectants. Antibiotic treatment has included systemic antibiotics such as ceftiofur or ampicillin and intrauterine (i.u.) antibiotics including ceftiofur, cephapirin, penicillin, and tetracycline; however, antibiotics are not labeled for i.u. use in dairy cows in the U.S. and the research results are often no better than using injections of $PGF_{2\alpha}$. Cows with endometritis at 21 to 27 DIM were treated with either 1.0 mg/kg body weight (BW) of ceftiofur on three consecutive d or 0.5 mg of cloprostenol at 21 to 27 and 35 to 41 DIM (Kaufmann et al., 2010). At 42 to 48 DIM, the proportion of cloprostenol treated cows cured was 80.2% compared to 74.2% in cows treated with ceftiofur (Kaufmann et al., 2010). A single 125 mg i.u. infusion of ceftiofur hydrochloride at 41 to 47 DIM did not successfully mitigate the effects of SCE on fertility of cows compared to untreated cows and, therefore, may not be effective at ameliorating SCE (Galvão et al., 2009b). Another study treated cows with either an i.u. infusion of 100 ml of 3.3% betadine solution, 500 μg i.m. of cloprostenol or 25 mg of dinoprost, or no treatment (Knutti et al., 2000). Conception risks were not different for treatment groups and control (Knutti et al., 2000); however, due to small group sizes, there was insufficient power to detect a reasonable difference.

In a study by LeBlanc et al. (2002b), cows with endometritis were treated with an i.u. infusion of 500 mg of cephapirin benzathine, 500 μg intramuscular (i.m.) of cloprostenol, or received no treatment. Cows treated with cephapirin between 27 and 33 DIM had a higher pregnancy rate compared to controls; however, during the same timeframe, there was no difference in pregnancy rate between cephapirin and $PGF_{2\alpha}$ treated cows or between $PGF_{2\alpha}$ and control cows (LeBlanc et al., 2002b). A numerical increase in first service conception risk was observed for cows treated with 500 μg of cloprostenol i.m. (49%) versus cows treated with 500 mg cephapirin benzathine i.u. (42%) and both groups were statistically higher than controls (27%) (Kasimanickam et al., 2005). However, there may be economic losses that occur due to the use of antibiotics for the treatment of endometritis if there is a milk or meat withdrawal time after treatment.

Treatment with $PGF_{2\alpha}$

Prostaglandin $F_{2\alpha}$, which does not have a milk or meat withdrawal time, has often been reported to be a more beneficial treatment for endometritis than antibiotics (Jackson, 1977, Ott and Gustafsson, 1981, Paisley et al., 1986). Prostaglandin $F_{2\alpha}$ is commercially available as dinoprost tromethamine or as cloprostenol sodium. A single injection of $PGF_{2\alpha}$ resolved chronic endometritis within 7 d in about 85% of cows (Jackson, 1977, Ott and Gustafsson, 1981). Cows were less likely to have *Arcanobacterium pyogenes* isolated from an endometrial biopsy at 40 d after calving when treated with $PGF_{2\alpha}$ at 26 DIM (Bonnett et al., 1990). Although some studies have shown a benefit to $PGF_{2\alpha}$ use prior to 30 DIM (Bonnett et al., 1990, Etherington et al., 1994), many other studies have shown no benefit and indicate that $PGF_{2\alpha}$ should not be used prior to 30 DIM (Galvão et al., 2009a, Hendricks et al., 2006, Pankowski et al., 1995, Sheldon and Noakes, 1998). Studies have also shown $PGF_{2\alpha}$ administration to have favorable effects on fertility (Bonnett et al., 1990, Heuwieser et al., 2000). Heuwieser et al. (2000) observed that treating cows with $PGF_{2\alpha}$ at 43 DIM increased estrus detection efficiency, shortened the time to first service by 12 d, and resulted in fewer DOPN than the other treatments.

Pyometra

Pyometra is defined as an enlarged uterus with a purulent or mucopurulent material within the uterine lumen in the presence of a CL with a closed cervix post calving (Sheldon et al., 2006). Pyometra can be successfully treated with the use of $PGF_{2\alpha}$, which lyses the CL and causes the cervix to open allowing the release of the mucopurulent material.

Synchronization protocols

Current reproductive performance determines if there is a need to use reproductive management programs such as TAI; additionally, management's consideration of labor organization and needs should factor into the decision as to whether TAI may be beneficial as a supplement to estrus detection or as a replacement for it. A farm that has excellent estrus detection efficiency and accuracy may not derive as much economic benefit from TAI programs since the primary benefit of TAI programs is increasing insemination efficiency. (Overton, 2006). In general, the value of TAI versus estrus detection-based AI depends on the individual farm's compliance to the protocol, the initial level and quality of estrus detection, and other farm level factors (Olynk and Wolf, 2008).

Synchronization programs have decreased or eliminated the need for estrus detection in reproductive management programs and have improved reproductive performance. Programs that synchronize ovulation such as Ovsynch (Pursley et al., 1995), Resynch (Fricke et al., 2003), and Cosynch (Small et al., 2001) have been widely studied. The Ovsynch protocol consists of 3 treatments: GnRH on d 0, $PGF_{2\alpha}$ on d 7, and GnRH 2 d later followed by TAI in 12 to 16 h (Pursley et al., 1995). Conception risk for Ovsynch typically ranges from approximately 26 to 38% (Cartmill et al., 2001, DeJarnette and Marshall, 2003, DeJarnette et al., 2001, LeBlanc and Leslie, 2003, Peters and Pursley, 2002). The Resynch protocol is used in non-pregnant cows 28 or more d after cows have been inseminated. The treatments and schedule are identical to Ovsynch (Fricke et al., 2003). Cosynch-72 is a TAI protocol that was designed to reduce some of the compliance issues of Ovsynch. This program consists of the same 3 treatments as Ovsynch, but with slight variations in the timing: GnRH on d 0, $PGF_{2\alpha}$ on d 7, and GnRH + TAI on d 10 (Portaluppi and Stevenson, 2005). Conception risk for Cosynch is usually slightly lower than Ovsynch and often ranges from approximately 23 to 31% (DeJarnette and Marshall, 2003, Portaluppi and Stevenson, 2005).

Presynchronization protocols

Previous studies have looked at administering $PGF_{2\alpha}$ or a combination of $PGF_{2\alpha}$ and GnRH as a method of presynchronization of estrus prior to starting TAI protocols in an attempt to improve reproductive performance. Prostaglandin $F_{2\alpha}$ has been commonly used in reproductive management programs for presynchronization and synchronization of estrus by causing luteolysis, treating endometritis, and inducing estrus (Jackson, 1977, Moreira et al., 2001, Pursley et al., 1995). Common presynchronization programs include a single injection of $PGF_{2\alpha}$ that is followed by estrus detection; 2 injections of $PGF_{2\alpha}$ administered 14 d apart with estrus detection following the second injection; an injection of $PGF_{2\alpha}$ followed in 2 to 3 d with GnRH; or an injection of GnRH followed in 5 to 7 d with $PGF_{2\alpha}$. Each of these strategies is designed to synchronize estrus or ovulation prior to the initiation of a TAI protocol. Subsequent to a single injection of $PGF_{2\alpha}$, approximately 70% of all cycling cows should come into estrus within a week. Following two injections of $PGF_{2\alpha}$ given 10 to 14 d apart, at least 95% of the cycling cows should be in estrus within 2 to 6 d. Both a single injection and the two injection series of $PGF_{2\alpha}$ have been shown to be effective at synchronizing estrus; however, two injections of $PGF_{2\alpha}$ will synchronize a greater proportion of cows into a smaller timeframe.

Presynch is most commonly used for synchronization of estrus prior to synchronization of ovulation. Presynch consists of two injections of $PGF_{2\alpha}$ 14 d apart with the second given 10 to 14 d prior to the start of Ovsynch (Cordoba and Fricke, 2001, LeBlanc and Leslie, 2003, Moreira et al., 2001, Navanukraw et al., 2004). Numerous studies have examined the interval between the second injection of $PGF2_{\alpha}$ and the initiation of TAI and the best results appear to result from the use of 10 or 11 d (Galvao et al., 2007, Santos et al., 2010). Cows that are cycling usually have improved first service conception risks when enrolled in Presynch due to improved uterine health and an improved response to TAI as a consequence of the staging effect of presynchronization and receiving the GnRH injection at a more optimal time of the estrous cycle (Moreira et al., 2001). Cows that respond to Presynch and start the TAI 10 or 11 d later are likely to be in the first 5 to 8 d of the estrous cycle and are more likely to respond to the first injection of GnRH by luteinizing the dominant follicle. Pregnancy per AI for Presynch-Ovsynch inseminations ranges from 37 to 42% (Silva et al., 2007, Souza et al., 2008). A single injection of $PGF_{2\alpha}$ given 10 to 14 d prior to Ovsynch has also been shown effective and has produced conception risks that range from approximately 33 to 49% (Cartmill et al., 2001, Cordoba and Fricke, 2001, LeBlanc and Leslie, 2003).

An alternative to presynchronization with only $PGF_{2\alpha}$ is the Double-Ovsynch protocol which utilizes 6 treatments: GnRH on d 0, $PGF_{2\alpha}$ on d 7, GnRH on d 10, GnRH on d 17, $PGF_{2\alpha}$ on d 24, and GnRH 56 to 60 hours later followed by TAI in 12 to 16 h (Souza et al., 2008). The Double-Ovsynch protocol has been shown to increase the average pregnancy per AI similarly to Presynch-Ovsynch but first lactation cows respond more favorably than mature cows (Souza et al., 2008). One benefit of Double-Ovsynch as compared to a traditional Presynch-Ovsynch is the potential for improved conception risk in cows that are anestrous and anovulatory just prior to enrollment in the TAI program. The two injections of GnRH in the Double-Ovsynch protocol often facilitate a resumption of cyclicity in some anovulatory cows (Souza et al., 2008).

Another alternative to presynchronization with only $PGF_{2\alpha}$ or Double-Ovsynch is the G6G protocol. The G6G protocol includes 5 treatments: $PGF_{2\alpha}$ on d 0, GnRH on d 2 followed by Ovsynch 6 d later consisting of GnRH, $PGF_{2\alpha}$ 7 d later followed by GnRH 56 to 60 hours later with TAI 12 to 16 hours later (Bello et al., 2006). G6G increased the proportion of cows that ovulated to the initial GnRH of Ovsynch and increased the synchronization rate to Ovsynch compared to the control (Bello et al., 2006).

Conclusion

Reproductive failure or a delay in conception has proven to be costly to dairy producers. In general, improving reproductive efficiency increases profitability by facilitating an earlier return to the next lactation, reducing the risk of over-conditioning, and reducing the risk of culling due to reproductive failure. In addition, cows that avoid over-conditioning are less likely to have transition problems in the subsequent lactation. Common postpartum diseases include acute puerperal metritis, clinical metritis, pyometra, clinical endometritis, and subclinical endometritis and all of these problems have been associated with a decrease in pregnancy rate and decreased profitability.

Prostaglandin $F_{2\alpha}$ is an effective treatment for endometritis and is economically favorable on farms that are currently utilizing $PGF_{2\alpha}$ for presynchronization or TAI. Prostaglandin $F_{2\alpha}$ given prior to starting a synchronization program improves uterine immune function and reduces the negative impact of endometritis by removing the CL and, in turn, eliminating progesterone, which may allow the cow to better respond to an infection. The use of $PGF_{2\alpha}$ as a presynchronization treatment improves first service conception risk through improving uterine health and improving responsiveness to synchronization protocols such as Ovsynch.

Synchronization programs are an effective way to increase reproductive efficiency and profitability, especially on farms that suffer from poor reproductive performance. More research is needed on Double-Ovsynch to confirm present findings; furthermore, research should be done to evaluate the potential benefit from an injection of $PGF_{2\alpha}$ prior to Double-Ovsynch. Because exogenous $PGF_{2\alpha}$ has known uterine health benefits, an injection prior to Double-Ovsynch may further increase conception risk by improving uterine health and fertility.

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CHAPTER 3

EFFECT OF AN INJECTION OF $PGF_{2\alpha}$ ADMINISTERED 11 DAYS PRIOR TO DOUBLE-OVSYNCH ON FIRST SERVICE CONCEPTION RISK IN LACTATING DAIRY COWS[1]

[1] May, H.A., R.D. Berghaus, I.J. Smith, W. Graves, and M.W. Overton. To be submitted to *Journal of Dairy Science*.

Abstract

The objective of this study was to determine if the administration of a single injection of $PGF_{2\alpha}$ 11 d prior to the start of Double-Ovsynch would improve first service conception risk and reduce the time from calving to conception. Lactating Holstein cows (n = 1,043) were randomly assigned to receive either a presynchronization injection of $PGF_{2\alpha}$ (Treatment; n = 531) at 35 ± 3 d in milk (DIM) prior to starting Double-Ovsynch or no presynchronization treatment prior to Double-Ovsynch (Control; n = 512). The treatment group received a single injection of $PGF_{2\alpha}$ 11 d prior to the Double-Ovsynch protocol. Both the treatment and control groups received the standard Double-Ovsynch protocol beginning at 46 ± 3 DIM (d-0 GnRH, d-7 $PGF_{2\alpha}$, d-10 GnRH, d-17 GnRH, d-24 $PGF_{2\alpha}$, 56-hr GnRH, 12-hr TAI). Results indicate that the presynchronization injection of $PGF_{2\alpha}$ did not increase first service conception risk to Double-Ovsynch; further, first service conception risk was not different for primiparous cows or multiparous cows. Cows that became eligible for their first service between mid-May and mid-September, however, had 41% lower odds of first service conception than did those that became eligible during the mild months of the year, after adjusting for treatment group and lactation. Neither treatment with $PGF_{2\alpha}$ nor lactation was associated with time to conception. Milk yield was associated with time to conception; for every 1,000 kg increase in 305 d mature equivalent milk yield, the hazard rate for conception increased by an average of 3% with the other variables held constant. Cows that became eligible for their first service during the hottest months of the year had a 32% lower hazard rate for conception during the first 77 DIM; however, there was no difference in the hazard rate for conception after 77 DIM. Future studies are needed to assess the effects of presynchronization $PGF_{2\alpha}$ prior to Double-Ovsynch and to further improve first service conception risk and pregnancy rate.

Introduction

Dairy herds often struggle to achieve acceptable levels of reproductive performance and while the exact causes may vary across herds, poor estrus expression by the cow, declining estrus detection efficiency, and low conception risk are issues commonly cited (Lopez et al., 2004, Washburn et al., 2002, Wiltbank et al., 2006). The decrease in estrus expression, estrus detection efficiency, and accuracy of estrus detection have been blamed on reduced levels of circulating reproductive hormones which are thought to be a consequence of higher milk production and feed intake (Wiltbank et al., 2006). High levels of milk production and the associated increase in feed intake lead to an increased rate of clearance of estradiol from circulation which may result in a delayed onset of estrus, prolonged exposure to LH pulsatility, a delay in ovulation, and a reduction in the duration of standing estrus leading to compromised conception risk (Wiltbank et al., 2006). The use of timed-artificial insemination (TAI) protocols has reduced or even eliminated the need for estrus detection when properly implemented and has mitigated many of the impacts of high milk production on reproductive performance.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been commonly used in reproductive management programs for presynchronization and synchronization of estrus and for the management of endometritis (Jackson, 1977, Moreira et al., 2001, Pursley et al., 1995). Estrus synchronization programs are effective at inducing estrus and consolidating the onset of estrus within a smaller window of time and often result in improved insemination efficiency. However, because these programs do not precisely control ovulation, timed breeding using only $PGF_{2\alpha}$ programs is not very successful. Ovulation synchronization programs add additional components such as GnRH to facilitate improved control over folliculogenesis and timing of ovulation. Together, these programs have eliminated or reduced the need for estrus detection in many reproductive management programs and have improved reproductive performance. Pursley et al. (1995) developed the Ovsynch protocol (d-0 GnRH, d-7 $PGF_{2\alpha}$, d-9 GnRH, 12-hr TAI), which has allowed producers to synchronize ovulation and utilize TAI. Conception risk for Ovsynch typically ranges from approximately 26 to 38% (Cartmill et al., 2001, DeJarnette and Marshall, 2003, DeJarnette et al., 2001, LeBlanc and Leslie, 2003, Peters and Pursley, 2002).

In an attempt to further increase conception risk, presynchronization programs have been developed to synchronize estrus or ovulation prior to TAI protocols. Common presynchronization programs include a single injection of $PGF_{2\alpha}$ that is followed by estrus detection; two injections of $PGF_{2\alpha}$ administered 14 d apart with estrus detection following the second injection; an injection of $PGF_{2\alpha}$ followed in two to three d with GnRH; or an injection of GnRH followed in five to seven d with $PGF_{2\alpha}$. Approximately 70% of all cycling cows should come into estrus within a week after a single injection of $PGF_{2\alpha}$; furthermore, at least 95% of the cycling cows should be in estrus within 2 to 6 d following two injections of $PGF_{2\alpha}$ administered 10 to 14 d apart. Both a single injection and the two injection series of $PGF_{2\alpha}$ have been shown to be effective at synchronizing estrus; however, two injections of $PGF_{2\alpha}$ will synchronize a greater proportion of cows into a smaller timeframe.

Presynch is most commonly used for synchronization of estrus prior to synchronization of ovulation. Presynch consists of two injections of $PGF_{2\alpha}$ 14 d apart with the second given 10 to 14 d prior to the start of Ovsynch (Cordoba and Fricke, 2001, LeBlanc and Leslie, 2003, Moreira et al., 2001, Navanukraw et al., 2004). Cows that are cycling usually have improved first service conception risks when enrolled in Presynch due to improved uterine health and an improved response to TAI as a consequence of the staging effect of presynchronization and receiving the GnRH injection at a more optimal time of the estrous cycle (Moreira et al., 2001). Pregnancy per AI for Presynch-Ovsynch inseminations ranges from 37 to 42% (Silva et al., 2007, Souza et al., 2008). A single injection of $PGF_{2\alpha}$ given 10 to 14 d prior to Ovsynch has also been shown effective and has produced conception risks that range from approximately 33 to 49% (Cartmill et al., 2001, Cordoba and Fricke, 2001, LeBlanc and Leslie, 2003)

Recently, Souza et al. (2008) developed an alternative to presynchronization with only $PGF_{2\alpha}$ known as the Double-Ovsynch protocol (d-0 GnRH, d-7 $PGF_{2\alpha}$, d-10 GnRH, d-17 GnRH, d-24 $PGF_{2\alpha}$, 56-hr GnRH, 12-hr TAI). The Double-Ovsynch protocol has been shown to increase the average pregnancy per AI similarly to Presynch-Ovsynch; however, first lactation cows respond more favorably than mature cows (Souza et al., 2008). One benefit of Double-Ovsynch as compared to a traditional Presynch-Ovsynch is the potential for improved conception risk in cows that are anestrous and anovulatory when enrolled into the TAI program. The two injections of GnRH in the Double-Ovsynch protocol facilitates a resumption of cyclicity in some anovulatory cows (Souza et al., 2008).

Previous studies have looked at presynchronization prior to the start of Ovsynch; however, none have determined the effect of presynchronization or the potential uterine health effects of a dose of $PGF_{2\alpha}$ prior to the start of Double-Ovsynch on first service conception risk. Because exogenous $PGF_{2\alpha}$ has known uterine health benefits, an injection prior to Double-Ovsynch may further increase conception risk by improving uterine health and fertility.

Our hypothesis was that an injection of $PGF_{2\alpha}$ given 11 d prior to starting Double-Ovsynch would improve first service conception risk by improving uterine health and increasing responsiveness to Double-Ovsynch. Cows that have a CL present and respond to the initial $PGF_{2\alpha}$ injection should then be in the first 5 to 8 d of their cycle when the first GnRH of Double-

Ovsynch is given, thus improving the response to the TAI protocol. The objective of this study was to determine if the administration of a single injection of $PGF_{2\alpha}$ 11 d prior to the start of Double-Ovsynch would improve first service conception risk and reduce the time from calving to conception.

Materials and Methods

Farm and Animals

The experiment was conducted in southeastern Georgia at a dairy herd consisting of 1,900 lactating Holstein dairy cows housed in free-stall, tunnel-ventilated barns. Barns were equipped with fans and sprinklers to cool the cows during periods of heat stress. Study design and all procedures were approved by the Animal Care and Use Committee of the University of Georgia, College of Veterinary Medicine. The enrollment period for the study was from January to October 2010. The herd utilized the Double-Ovsynch protocol prior to the start of the study, and the owners agreed to participate in the study. Individual data were recorded on farm by using the radio frequency identification system (RFID) and DairyCOMP 305® Software (Valley Agricultural Software, Tulare, CA), and the dairy is already visited by the investigators on a weekly basis for the delivery of routine herd health and reproductive services. All cows were fed a total mixed ration (TMR) twice daily that met or exceeded NRC (2001) requirements and were milked three times daily. Based on detecting a treatment effect of a 6% point improvement in first service conception risk (38% to 44%) and allowing for a 12% loss to follow-up we estimated that 930 cows would be required to detect a significant difference using an alpha of 5% and a power of 80%; however, due to a higher than expected number of animals calving during our planned period of study, we were able to enroll more cows than were in our sample size calculation.

Treatment Groups and Pregnancy Diagnosis

Lactating Holstein dairy cows were enrolled in the experiment at 35 ± 3 DIM regardless of parity and calving history. Any cow that was in the hospital pen or was not in the fresh pen at the time of enrollment was excluded from the study. Cows were randomly allocated to control group (Double-Ovsynch) or treatment group ($PGF_{2\alpha}$ administered 11 d prior to Double-Ovsynch) using a computerized random number generator on a weekly basis and then completed the Double-Ovsynch protocol (Figure 1). The standard dose of 2 ml of synthetic prostaglandin analogue, cloprostenol sodium (Estrumate®, Intervet/Schering-Plough Animal Health; Millsboro, DE; 500 μg, i.m.) was used as the treatment drug. Intramuscular injections of $PGF_{2\alpha}$ and GnRH (Fertagyl®, Intervet/Schering-Plough Animal Health; Millsboro, DE; 100 μg, i.m.) were given as outlined in figure 1 for the Double-Ovsynch protocol (d-0 GnRH, d-7 $PGF_{2\alpha}$, d-10 GnRH, d-17 GnRH, d-24 $PGF_{2\alpha}$, 56-hr GnRH, 12-hr TAI) starting at 46 ± 3 DIM (Souza et al., 2008). All cows were bred by 1 of 2 AI technicians at the farm at 73 ± 3 DIM. Pregnancy diagnosis was done by 1 of 2 herd veterinarians at 111 ± 3 DIM which corresponds to 38 d post insemination (± 3 d). Pregnancy was diagnosed via trans-rectal palpation of the amniotic vesicle.

Statistical Analysis

A total of 1,071 cows (541 treatment cows and 530 control cows) were enrolled between January and October of 2010. Cows that started the lactation as an abortion (4); were a reproductive cull (RC is defined as a voluntary decision made by the producer to not breed and cull the cow; however, the decision was made after enrollment in the study, but prior to first service.) (6); were purchased and had incomplete cow information (1); or were in noncompliance with the research protocol (17) were excluded, resulting in a total of 1,043 cows (531 treatment cows and 512 control cows) that were available for analysis.

Cows that were bred and remained in the herd until they were evaluated from pregnancy via palpation per rectum at 38 ± 3 d post-AI were eligible for inclusion in the analysis of first service conception risk. Univariate analysis of first service conception risk was performed by using Fisher's exact test and the multivariable analysis was performed using logistic regression. Variables associated with conception ($P < 0.2$) in the univariate analysis were considered for inclusion in the multivariable model. Multivariable model selection was performed using a manual backward elimination procedure. Variables with $P > 0.1$ were removed from the multivariable model if their removal did not change the estimated treatment coefficient by more than 10%. Treatment and lactation were retained in all models regardless of their significance. After reaching a preliminary main effects model, all possible two-way interactions with the treatment variable were evaluated. The final logistic regression model was evaluated using the Hosmer-Lemeshow goodness of fit test.

Survival analysis was used to evaluate the effect of variables on time to conception during the first 160 DIM. Univariate analysis of time to conception was performed using Kaplan-Meier survival analysis and multivariable analysis was performed using Cox regression. Cows that were culled or that had not conceived by 160 DIM were censored at the time when they left the herd or at 160 DIM, respectively. Multivariable model selection proceeded as described for the logistic regression analysis. The proportional hazards assumption for each variable was evaluated based on the Schoenfeld residuals.

In addition to the assessment of treatment, potential confounding variables that were evaluated in the analysis included early mastitis (defined as having a first occurrence of clinical mastitis between calving and first service), mid-lactation mastitis (defined as having a first occurrence of clinical mastitis between first service and 160 DIM), lactation (primiparous or multiparous), 305 d mature equivalent milk yield (305 ME), and breeding season (defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise). Mastitis was defined on the farm by the workers via visual inspection of the milk. Somatic cell counts were not evaluated because the herd does not record monthly somatic cell counts on individual cows.

The analysis was performed using commercially available statistical software (Stata version 11.1, StataCorp LP, College Station, TX). All statistical testing was performed assuming a two-sided alternative hypothesis and $P \leq 0.05$ was considered statistically significant.

Results

The distribution of cow characteristics by treatment group is summarized in Table 1. Cows ranged from 1 to 10 lactations with a median of 2 lactations and had an average 305 ME of 12,000 kg. The two groups did not differ with respect to lactation ($P = 0.10$), 305 ME ($P = 0.38$), the occurrence of mastitis in early ($P = 0.36$) or mid-lactation ($P = 0.41$), or the percentage of twin births ($P = 0.22$). The $PGF_{2\alpha}$ treatment group did, however, have a greater proportion of cows that were eligible for their first service during the hot time of the year than did the control group (51.0% vs. 44.5%; $P = 0.04$).

First Service Conception Risk

Results of the univariate analysis for first service conception risk are summarized in Table 2. There were no differences between treatment and control groups in the overall analysis ($P = 0.56$), and no difference was observed between treatment and control groups when cows were limited to those in their first lactation (39.4% and 39.0%; respectively; $P = 0.94$) or to those in their second lactation or higher (40.2% and 37.5%; respectively; $P = 0.54$). Only breeding season was associated with first service conception risk in the univariate analysis, with cows that became eligible for their first service between mid-May and mid-September having a lower conception risk (32.7%) than cows that became eligible for their first service during the cooler months of the year (44.6%; $P < 0.001$).

Results of the final multivariable logistic regression model are summarized in Table 3. Neither treatment with $PGF_{2\alpha}$ nor lactation was associated with first service conception risk in the multivariable analysis. Cows that became eligible for their first service between mid-May and mid-September, however, had 41% lower odds of first service conception than did those that became eligible during the cooler months of the year, after adjusting for treatment group and lactation. The two-way interactions between treatment and lactation ($P = 0.89$) and between treatment and breeding season ($P = 0.67$) were both nonsignificant and were excluded from the final model. Results of the Hosmer-Lemeshow test indicated that the final logistic regression model provided a good fit to the data ($P = 0.94$).

Time to Conception

Results of the univariate analysis for time to conception are summarized in Table 4. Neither treatment nor lactation was associated with time to conception in the univariate analysis

(Figures 2-3). Having early mastitis, a 305 ME below the median of 11,800 kg, and becoming eligible for first service during the hotter months of the year were all associated with longer median times to conception (Figures 4-6).

Results of the final multivariable Cox regression model are summarized in Table 5. Neither treatment with $PGF_{2\alpha}$ nor lactation was significantly associated with time to conception in the multivariable analysis. Having mastitis prior to the first service was not associated with time to conception ($P = 0.10$); however, the point estimate suggested that early mastitis corresponded to a mean reduction of 23% in the hazard rate after adjusting for the other variables in the model. Milk yield was associated with time to conception, although the magnitude of the effect was small; for every 1,000 kg increase in 305 ME, the hazard rate for conception increased by an average of 3% with the other variables held constant. The effect of breeding season was time-dependent. Cows that became eligible for their first service during mid-May to mid-September had a 32% lower hazard rate for conception during the first 77 DIM [hazard ratio (95% CI): 0.68 (0.55, 0.83)], but there was no difference in the hazard rate for conception after 77 DIM [hazard ratio (95% CI): 1.0 (0.81, 1.3)]. The two way interactions between treatment and early mastitis ($P = 0.79$), between treatment and lactation ($P = 0.48$), between treatment and 305 ME ($P = 0.47$), and between treatment and breeding season ($P = 0.28$) were all nonsignificant and were excluded from the final model.

Discussion

Our results indicate that the presynchronization injection of $PGF_{2\alpha}$ did not increase first service conception risk to Double-Ovsynch. When all cows were included in the analysis, first service conception risk was not different for primiparous (39.2%) cows as compared with that observed for multiparous (38.8%) cows. The results of our study are not consistent with previous findings, which indicated higher pregnancy per AI in primiparous cows (65.2%) than in multiparous cows (37.5%; $P = 0.02$) (Souza et al., 2008). The results of our study are comparable to previous findings where a single injection of $PGF_{2\alpha}$ 10 d prior to Ovsynch did not increase conception risk compared to Ovsynch (LeBlanc and Leslie, 2003, Peters and Pursley, 2002). Likewise, a single injection of $PGF_{2\alpha}$ 12 d before Ovsynch failed to increase conception risk compared to Ovsynch (Cordoba and Fricke, 2001); however, Cartmill (2001) reported a numerical increase in first service conception risk in multiparous cows that received a single injection of $PGF_{2\alpha}$ 12 d before Ovsynch but not in primiparous cows (42% and 40%, respectively). These results are similar to our study, where multiparous cows that received the injection of $PGF_{2\alpha}$ for presynchronization had a numerically higher first service conception risk than control cows. On the other hand, Galvao (2007) administered 2 injections of $PGF_{2\alpha}$ 14 d apart and 11 d before a modified Ovsynch and observed an increase in pregnancy per AI compared to control; however, the second injection of $PGF_{2\alpha}$ was later in lactation than in the current study.

A potential explanation for the lack of significant effect in the current study to the single $PGF_{2\alpha}$ presynchronization injection is the stage of lactation when it was given. At 35 ± 3 DIM, a large proportion of cows are not yet expected to be cycling (Cartmill et al., 2001, Moreira et al., 2001). Staples et al. (1990) reported that only 46% of cows were cycling at 40 DIM, which indicates that less than 46% of the cows in our study were capable of responding to the first injection of $PGF_{2\alpha}$. This injection date was chosen to coincide with the farm's management plan for first service. Delaying the injection may have improved the response, but may have been offset in benefit by the additional delay for achieving first service. Future work in examining the impact of presynchronization prior to Double-Ovsynch should consider the impact of cyclicity. In the present study, we were unable to measure progesterone concentrations due to budgetary limitations, but knowing the cyclicity status of the cows at presynchronization may have improved our understanding of the potential impact of the additional $PGF_{2\alpha}$ injection.

The $PGF_{2\alpha}$ treated cows had a greater proportion of cows that were eligible for first service during the hot season than control cows (51% vs. 44.5%; $P = 0.04$). Cows that were eligible for first service during the hot season had lower conception risk (32.7%) than cows that became eligible during the mild season (44.6%; $P < 0.001$); however, the impact of heat stress only affected cows during the period up to first service because the subsequent breeding was in the mild season (Figure 5). Heat stress has been reported to negatively impact reproduction in dairy cows. Cows exposed to heat stress (≥ 29°C) from 20 to 50 d before AI were reported to have a lower conception risk compared to cows not exposed to heat stress (< 29°C) (23% vs. 31.3% respectively; $P < 0.001$) (Chebel et al., 2004). Heat stress has been reported to have its greatest impact on reproduction early in development via a reduction in fertilization rate and greater embryonic loss during the first six d of development, resulting in large impacts on apparent conception risk (Hansen and Arechiga, 1999).

Although some studies report negative impacts of high milk yield on reproduction (Lucy, 2001, Nebel and McGilliard, 1993), the results from our study indicate that milk yield was positively associated with a shorter time to conception (HR = 1.03 per 1,000 kg; $P = 0.049$). Consistent with our report, Canadian researchers reported an increase in 305 ME was significantly associated with shorter time to pregnancy (HR = 1.07 per 1,000 kg; $P < 0.001$) (Leblanc, 2010). Similarly, Chebel and coworkers (2004) reported the odds ratio (OR) for conception increased as milk yield increased. Further, cows that had milk yield above the mean had greater fertility than cows below the mean (Peters and Pursley, 2002). The studies that report negative impacts of high milk yield may also be influenced by other factors such as increased periparturient disease, increased incidence of retained placenta, heat stress, or poor nutrition, which have all been reported to have detrimental effects on milk yield and reproduction as well as overall cow health. In addition, many studies that report a negative association between fertility and milk production base their results on a comparison of average days open which is a biased outcome. One explanation for the positive association between higher producing cows and shorter time to pregnancy is higher producing cows are more likely to have few health problems, consume more feed, and have overall better health status; further, excellent overall management of the transition dairy cow is crucial for the cow to reach her potential for milk production and thus subsequently allowing for higher reproductive performance.

Mastitis occurring before first service was associated with longer median times to conception in the univariate analysis (Figure 4; $P = 0.050$), but due to a small subset of cows with mastitis occurring before first service, was not significant when other variables were accounted for in the multivariable model. Consistent with our findings, Santos et al. (2004) reported that first service conception risk was decreased when cows had their first case of clinical mastitis prior to first AI (22.1%) or between first AI and pregnancy diagnosis (10.2%) compared to control cows (28.7%; $P < 0.01$). Cows were 2.4 times more likely to experience pregnancy loss from d 28 to 42 when the cow had a high linear somatic cell count (LSCC > 4.5) before AI compared to cows with a lower (≤ 4.5) LSCC (Moore et al., 2005). Conversely, mastitis between AI and pregnancy diagnosis was reported to have no effect on conception risk; however, mastitis was associated with an increased pregnancy loss (Chebel et al., 2004).

In the present study, it is possible that we were unable to detect any impact of presynchronization with $PGF_{2\alpha}$ prior to Double-Ovsynch due to the stage of lactation when the $PGF_{2\alpha}$ injection was administered and that perhaps we may have been able to measure a significant impact by delaying the injection. However, the potential improvement in fertility would have to be significant to overcome the additional delay in achieving first service. Future studies utilizing progesterone sampling and additional study sites are needed to investigate the potential impact of presynchronization with $PGF_{2\alpha}$ prior to Double-Ovsynch; further, studies are warranted to investigate additional approaches for improving first service conception risk and pregnancy rate.

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CHAPTER 4

CONCLUSION

Our results indicate that the presynchronization injection of $PGF_{2\alpha}$ did not significantly increase first service conception risk to Double-Ovsynch and was not significantly different for primiparous cows or multiparous cows. The data also suggest a significant effect of heat stress on conception risk, because cows that became eligible for their first service between mid-May and mid-September had 41% lower odds of first service conception success than those that became eligible during the cooler months of the year. Although a greater proportion of treated cows than control cows were exposed to heat stress at the time of their first service, the effect of treatment was still nonsignificant after adjusting for breeding season in the multivariable analysis. Neither treatment with $PGF_{2\alpha}$ nor lactation was associated with time to conception. Milk yield was associated with time to conception; for every 1,000 kg increase in 305 ME, the hazard rate for conception increased by an average of 3%, which is likely a reflection of better overall health status of the higher producing cows. Our study found a positive association between milk yield and reproductive performance that often differs with many commonly held beliefs regarding the impact of milk yield on reproduction. Well managed herds can utilize tools such as TAI to mitigate some of the negative physiological impacts of high milk yield and additional research is necessary in both genetic and management approaches to improving reproductive performance in high producing dairy cows. Cows that became eligible for their first service during the hottest months of the year had a 32% lower hazard rate for conception during the first 77 DIM, but there was no difference in the hazard rate for conception after 77

DIM. Mastitis occurring before first service had a large numerical impact on time to conception and may have been statistically significant if the incidence of early mastitis had been larger or if the study population had been increased in size.

Tables

Table 1. Distribution of 1,043 cows enrolled in a randomized trial by cow characteristics and treatment assignment.

	Treatment Group		
Variable	Control[1] No. Cows (%)	$PGF_{2\alpha}$[2] No. Cows (%)	†*P*-value
Lactation			
1	149 (29.1)	180 (33.9)	0.10
2+	363 (70.9)	351 (66.1)	
305 ME[3]			
< 11,800 kg	247 (48.6)	271 (51.5)	0.38
≥ 11,800 kg	261 (51.4)	255 (48.5)	
Early Mastitis[4]			
No	468 (91.4)	494 (93.0)	0.36
Yes	44 (8.6)	37 (7.0)	
Mid-lactation Mastitis[5]			
No	465 (90.8)	474 (89.3)	0.41
Yes	47 (9.2)	57 (10.7)	
Breeding Season[6]			
Mild	284 (55.5)	260 (49.0)	0.04
Hot	228 (44.5)	271 (51.0)	
Twins			
No	511 (99.8)	526 (99.1)	0.22
Yes	1 (0.2)	5 (0.9)	
Total	512 (100)	531 (100)	

†Fisher's exact test

[1]Double-Ovsynch

[2]$PGF_{2\alpha}$ administered 11 d prior to the start of Double-Ovsynch

[3]305 d mature equivalent milk yield. The numbers within this category do not equal the total sample size by group due to missing test-day results.

[4]Defined as having a first occurrence of clinical mastitis between calving and first service

[5]Defined as having a first occurrence of clinical mastitis between first service and 160 DIM

[6]Defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise

Table 2. Univariate analysis of first service conception risk in 978 cows that either did or did not receive an injection of $PGF_{2\alpha}$ 11 d prior to the initiation of the Double-Ovsynch protocol and that were bred and retained in the herd long enough to be palpated for pregnancy diagnosis.

Variable	n	No. Conceiving at First Service (%)	†*P*
Treatment			
No[1]	480	182 (37.9)	0.56
Yes[2]	498	199 (40.0)	
Lactation			
1	311	122 (39.2)	0.94
2+	667	259 (38.8)	
305 ME[3]			
< 11,800 kg	473	179 (37.8)	0.51
≥ 11,800 kg	505	202 (40.0)	
Early Mastitis[4]			
No	907	359 (39.6)	0.17
Yes	71	22 (31.0)	
Mid-lactation Mastitis[5]			
No	877	343 (39.1)	0.83
Yes	101	38 (37.6)	
Breeding Season[6]			
Mild	513	229 (44.6)	< 0.001
Hot	465	152 (32.7)	

†Fisher's exact test

[1]Double-Ovsynch

[2]$PGF_{2\alpha}$ administered 11 d prior to the start of Double-Ovsynch

[3]305 d mature equivalent milk yield

[4]Defined as having a first occurrence of clinical mastitis between calving and first service

[5]Defined as having a first occurrence of clinical mastitis between first service and 160 DIM

[6]Defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise

Table 3. Multivariable logistic regression model for the prediction of first service conception risk in 978 cows that did or did not receive an injection of $PGF_{2\alpha}$ 11 d prior to the initiation of the Double-Ovsynch protocol and that were bred and retained in the herd long enough to be palpated for pregnancy diagnosis.

Variable	Coefficient (SE)	Odds Ratio (95% CI)	*P*
Treatment			
No[1]	Referent	Referent	0.38
Yes[2]	0.116 (0.133)	1.1 (0.87, 1.5)	
Lactation			
1	Referent	Referent	0.41
2+	-0.119 (0.145)	0.89 (0.67, 1.2)	
Breeding Season[3]			
Mild	Referent	Referent	< 0.001
Hot	-0.536 (0.136)	0.59 (0.45, 0.76)	
Constant	-0.180 (0.157)	NA	0.251

[1]Double-Ovsynch

[2]$PGF_{2\alpha}$ administered 11 d prior to the start of Double-Ovsynch

[3]Defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise

Table 4. Univariate analysis of time to conception for 1,043 cows that either did or did not receive an injection of $PGF_{2\alpha}$ 11 d prior to the initiation of the Double-Ovsynch protocol.

Variable	n	Median Days to Conception (95% CI)	†*P*
Treatment			
No[1]	512	114 (100, 117)	0.73
Yes[2]	531	114 (103, 117)	
Lactation			
1	329	115 (99, 130)	0.54
2+	714	114 (105, 116)	
305 ME[3]			
< 11,800 kg	518	116 (113, 123)	0.027
≥ 11,800 kg	516	112 (98, 115)	
Early Mastitis[4]			
No	962	114 (103, 116)	0.050
Yes	81	135 (113, 159)	
Mid-lactation Mastitis[5]			
No	939	114 (104, 116)	0.69
Yes	104	116 (103, 149)	
Breeding Season[6]			
Mild	544	98 (93, 113)	< 0.001
Hot	499	118 (115, 134)	

†Log rank test

[1]Double-Ovsynch

[2]$PGF_{2\alpha}$ administered 11 d prior to the start of Double-Ovsynch

[3]305 d mature equivalent milk yield

[4]Defined as having a first occurrence of clinical mastitis between calving and first service

[5]Defined as having a first occurrence of clinical mastitis between first service and 160 DIM

[6] Defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise

Table 5. Multivariable Cox regression model for time to conception in 1,034 cows with complete information that did or did not receive an injection of $PGF_{2\alpha}$ 11 d prior to the initiation of the Double-Ovsynch protocol.

Variable	Coefficient (SE)	Hazard Ratio (95% CI)	*P*
Treatment			
No[1]	Referent	Referent	0.86
Yes[2]	-0.014 (0.077)	0.99 (0.85, 1.15)	
Lactation			
1	Referent	Referent	0.71
2+	0.031 (0.085)	1.03 (0.87, 1.22)	
305 ME[3] (1,000 kg)	0.030 (0.015)	1.03 (1.00, 1.06)	0.049
Early Mastitis[4]			
No	Referent	Referent	0.10
Yes	-0.264 (0.160)	0.77 (0.56, 1.05)	
Breeding Season[5]			
Mild	Referent	Referent	< 0.001
Hot	-0.379 (0.106)	†NC	
Breeding Season x DIM			
DIM ≤ 77	Referent	Referent	0.008
DIM > 77	0.414 (0.156)	†NC	

†Not calculated because there is a significant interaction between breeding season and DIM. (See text)

[1]Double-Ovsynch

[2]$PGF_{2\alpha}$ administered 11 d prior to the start of Double-Ovsynch

[3]305 d mature equivalent milk yield

[4]Defined as having a first occurrence of clinical mastitis between calving and first service

[5]Defined as hot if breeding occurred between mid-May and mid-September, or mild otherwise

Figures

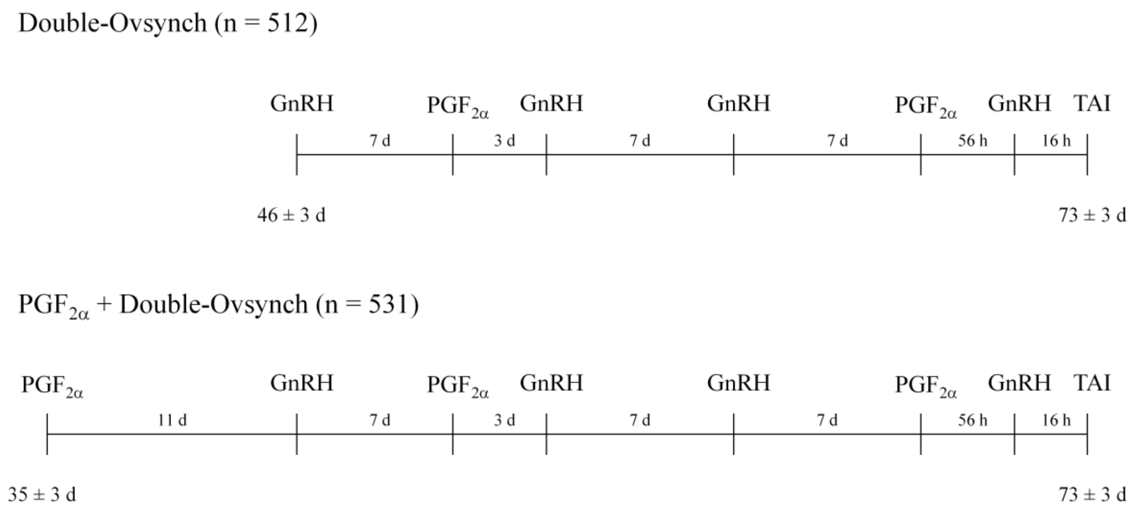

Figure 1. Schematic diagram of hormonal treatments in lactating dairy cows (mean ± range of DIM).

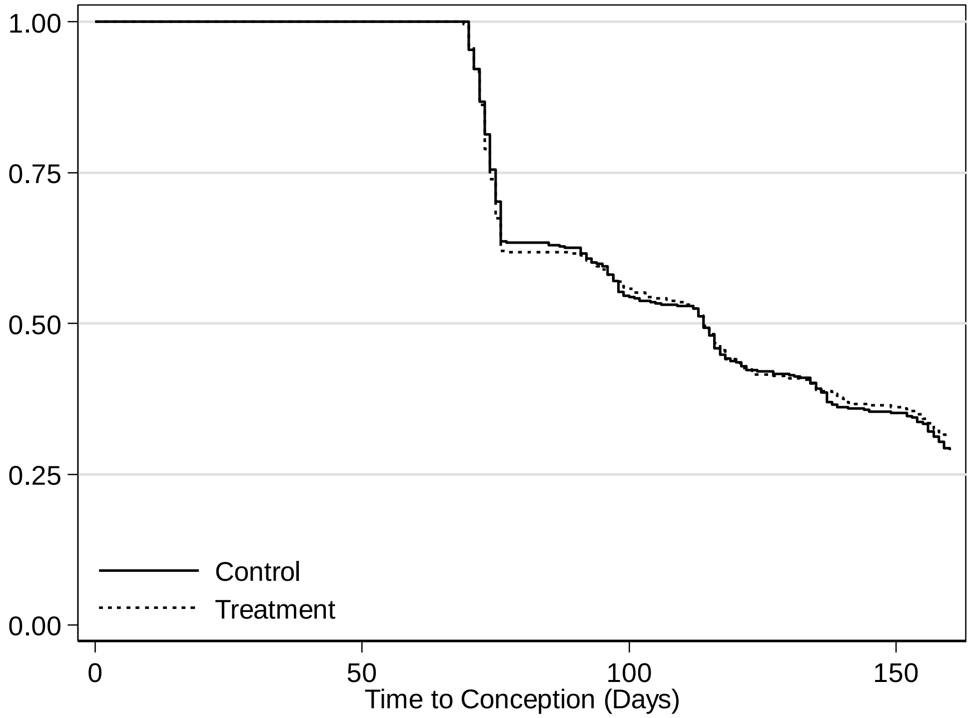

Figure 2. Survival curve for time to conception up to 160 DIM for control cows (solid line: —) and $PGF_{2\alpha}$ treated cows (dashed line: - -). Effect of treatment on conception according to the unadjusted univariate analysis: $P = 0.73$.

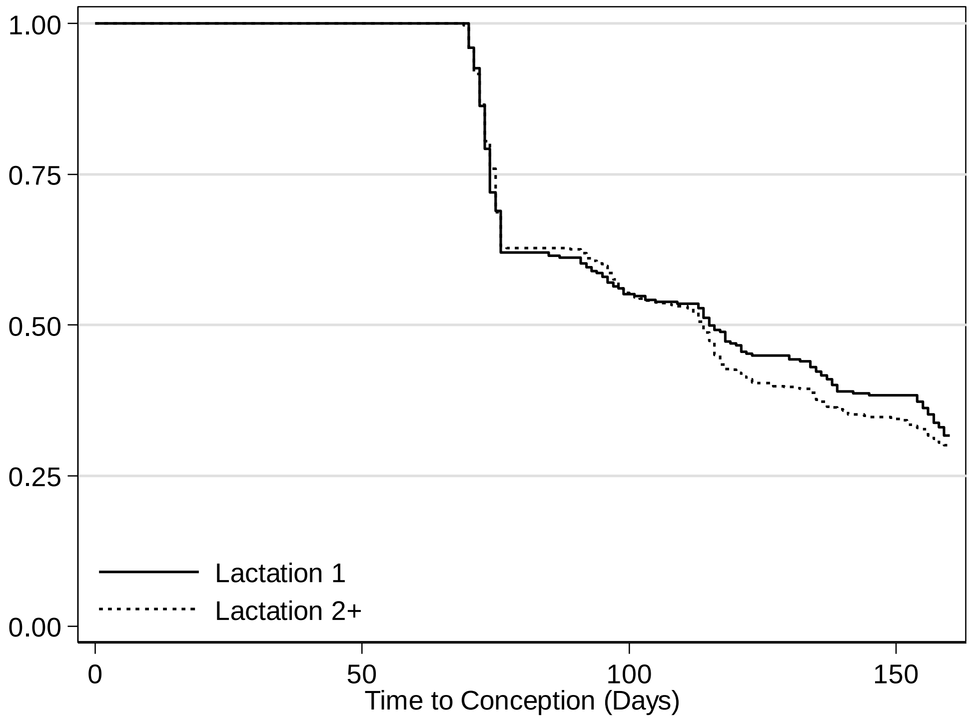

Figure 3. Survival curve for time to conception up to 160 DIM for primiparous cows (solid line: —) and multiparous cows (dashed line: - -). Effect of lactation on conception according to the unadjusted univariate analysis: $P = 0.54$.

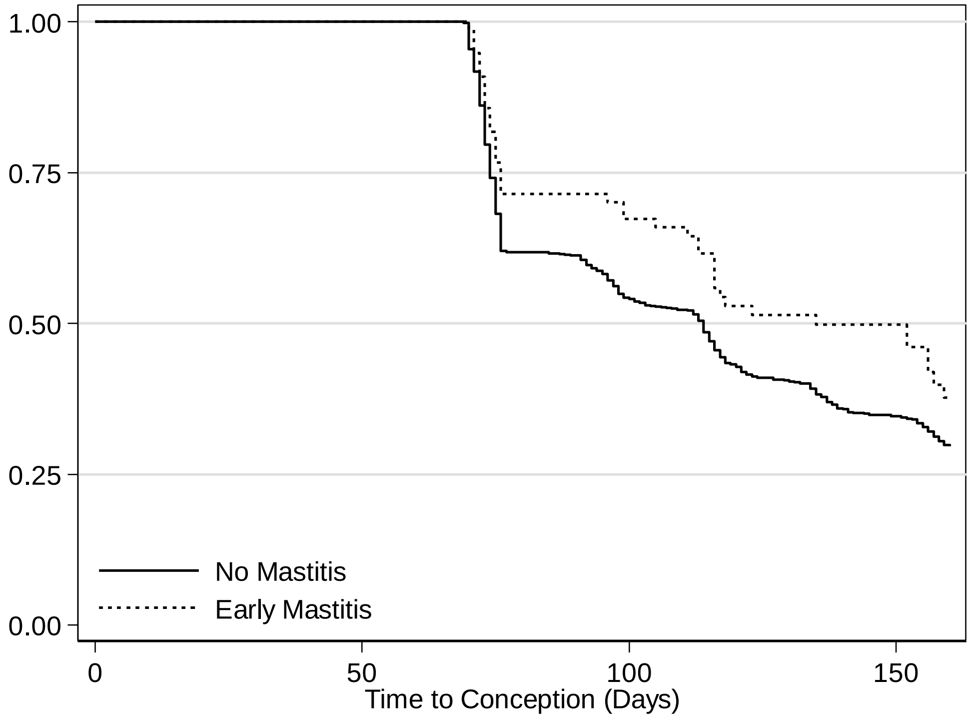

Figure 4. Survival curve for time to conception up to 160 DIM for cows with no mastitis occurring prior to first service (solid line: —) and for cows with mastitis occurring prior to first service (dashed line: - -). Effect of early mastitis on conception according to the unadjusted univariate analysis: $P = 0.050$.

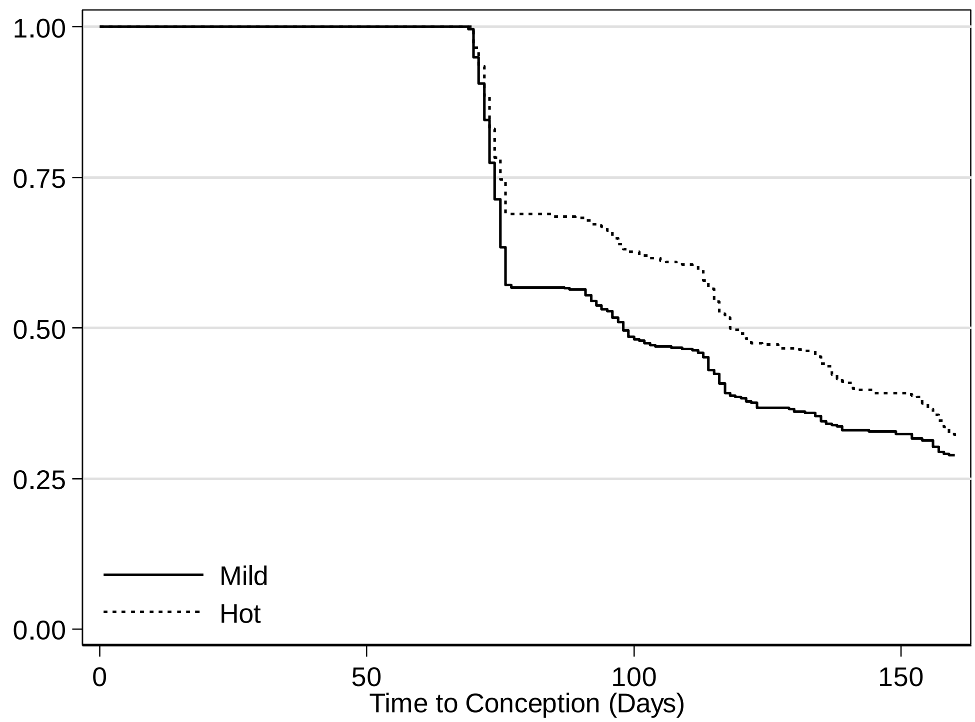

Figure 5. Survival curve for time to conception up to 160 DIM for cows eligible for first service during the mild season (solid line: —) and for cows eligible for first service during the hot season (dashed line: - -). Effect of breeding season on conception according to the unadjusted univariate analysis: $P < 0.001$.

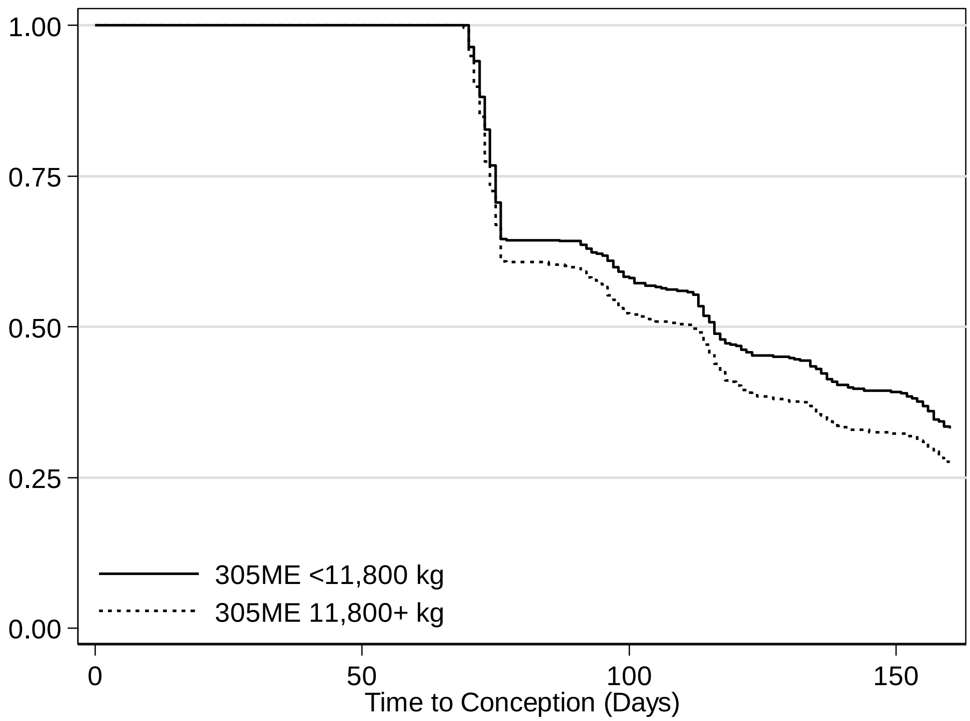

Figure 6. Survival curve for time to conception up to 160 DIM for cows that have a 305 ME less than 11,800 kg (solid line: —) and for cows that have a 305 ME greater than 11,800 kg (dashed line: - -). Effect of milk production on conception according to the unadjusted univariate analysis: $P = 0.027$.