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Evaluation of Reproductive Efficiency in Lactating Dairy Cows through Physiologic Evaluation and Synchronization Modification

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To the Graduate Council:

I am submitting herewith a dissertation written by Charles Dexter Young entitled "Evaluation of Reproductive Efficiency in Lactating Dairy Cows through Physiologic Evaluation and Synchronization Modification." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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A Dissertation Presented for the
Doctor of Philosophy
Degree
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Charles Dexter Young

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Dedication

This dissertation is dedicated to my loving wife Miranda and my two sons Luke and Paul. Their unwavering support has driven me to complete this journey and reminded me of the truly important things in life. I look forward to the next chapter in our lives as a family.

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Abstract

Reproductive performance of lactating dairy cows has decreased as milk production has increased as a result of genetic selection. Milk production alone is not the reason for decreased reproductive performance, as fertility issues are multifactorial and collaborative. Research chapters contained within have taken an applied approach focusing in two different areas of fertility. One approach was development of an evaluation system to identify lactating cows with decreased fertility prior to breeding; while another approach was to modify an ovulation synchronization protocol during periods of heat stress in order to improve fertility. The first focus was development and evaluation of a size and position score based on a one to three scale and assigned to lactating cows during a pre-breeding exam. This score was a reflection of the cervical and uterine horn diameter, and of the position of the reproductive tract in relation to the pelvis. Results indicated that cows with the largest reproductive tracts (score 3) had a 15% decrease in conception rates following artificial insemination compared to the smallest reproductive tracts (score 1). Score two reproductive tracts (intermediate) exhibited decreased conception rates compared to score one, but greater than score three. Identification of cows with lower potential fertility will allow producers to make the most economically, efficient decision when artificially inseminating lactating dairy cows. The second research focus modified an ovulation synchronization protocol by including an intravaginal progesterone releasing device (CIDR) nine days prior to artificial insemination during periods of heat stress. Use of a CIDR has shown to be beneficial with modest improvements in fertility in cool seasons, especially in anovular cows. This modification did not prove to be beneficial in improving conception rates during periods of heat stress. Lactating dairy cows with or without a progesterone releasing device had comparable conception rates. This research remains informative since use of a CIDR is expensive. This research exhibits this additional expense

does not lead to positive results, thereby discouraging producers from incurring an unnecessary expense.

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CHAPTER I: Introduction

Pregnancy is the cornerstone of the dairy industry. Effective and efficient reproductive performance is essential if a seemingly endless supply of milk for the domestic and global population is to continue. World populations are expected to increase to 8.9 billion by the year 2050; this 2.6 billion population increase is equivalent to the global population in 1950 (Cohen, 2003). Efficiency of the dairy industry has excelled greatly during this same time period of a 2.6 billion global population increase (1950-2003). In 1944, 25.6 million cows produced 53.0 billion kg of milk annually, compared to 9.2 million cows producing 84.2 billion kg of milk in 2007 (Capper et al., 2009). Furthermore, the dairy cow population required to produce one billion kg of milk in 2007 is 21% of the population needed to produce the same amount in 1944 (Capper et al., 2009). Several factors have contributed to this increase in efficiency including improvements in mechanization of agriculture, nutrition, agronomy, crop and animal genetics, housing facilities, and artificial insemination (Capper et al., 2009). It has been estimated milk production will continue to increase at a rate of 1.3% per year (Santos et al., 2010). This translates to one million fewer cows needed by the year 2050 to produce the same per capita milk available in 2010 in the United States (Santos et al., 2010). This is an alarming trend regarding future milk production, and highlights the urgency placed on the scientific community to accelerate progress related to improving reproductive efficiency.

Causes of reduced reproductive inefficiencies in lactating dairy cows are multifactorial and lead to an estimated 26.5% culling rate each year (USDA, 2002). Decreases in fertility have been attributed to increased milk production consistently throughout the scientific literature over the past decades. While the inefficiencies in fertility of lactating dairy cows have directly corresponded to an increase in milk production, this single factor is not solely responsible. The post-partum period is a critical time that sets the stage for future reproductive success. Uterine bacterial contamination, metabolic disorders, mastitis, body condition losses and negative energy balance in the post-partum period all lead to reproductive inefficiencies during the breeding period later in lactation. Environmental factors can also lead to decreased fertility, and heat stress is one of the greatest environmental factors. Heat stress can result in as many as 20 percentage point's reduction in conception rates, and these effects can be extended through early cooler periods. In the warm temperate climate of the Southeast US, these negative effects have shown to be more detrimental to fertility compared to the cooler Northeast region (Huang et al., 2008); thus allowing heat stress based research to have a significant impact on the Southeast producer.

Research efforts highlighted by this dissertation focus on two different applied approaches aimed at improving reproductive performance in lactating dairy cows. The first research effort focused on identifying those individual animals that may experience decreased fertility prior to insemination. An evaluative system was developed to assess the size and position of the reproductive tract during a pre-breeding exam. We hypothesized that cows possessing larger reproductive tracts would experience decreased fertility due to a larger volume or area for sperm to navigate to the site of fertilization. Identification of animals with lower potential fertility prior to breeding would allow producers to make informed economic decisions regarding insemination, thus allowing for a more economically efficient reproductive program overall. The second research effort focused on improving the efficiency of artificial insemination during periods of heat stress. An approach of incorporating an intravaginal progesterone releasing devise during a fixed timed AI protocol was taken to accomplish this goal. We hypothesized that inclusion of a progesterone releasing device would improve fertility during periods of heat stress. Progesterone is a key steroid hormone involved in the establishment of pregnancy, and evidence exists that circulating progesterone concentrations may be altered during periods of heat stress. If inclusion of this hormone releasing device could result in improved fertility, it would give producers the opportunity to successfully and efficiently inseminate cows during a period that has traditionally been detrimental to fertility.

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CHAPTER II: Literature Review

Introduction

Decreased reproductive success in lactating dairy cows maybe due to many factors which can include physiological differences and environmental challenges. The following review of literature will focus on evaluation of the reproductive tract in heifers and lactating dairy cows; as well as the negative influence of heat stress imposes on fertility and use of supplemental progesterone to potentially negate these influences.

Development and Efficacy of Reproductive Tract Scores for Predicting Fertility in Heifers

Evaluation of the reproductive tract as a means of identifying individuals with potentially improved fertility has shown to be effective in beef and dairy heifers. A reproductive tract scoring (RTS) system was developed and published by Andersen et al. (1991) as a means of determining pubertal status in beef heifers prior to breeding. The scoring system is based on a 1 to 5 scale and evaluates the tone and presence or absence of ovarian structures. An RTS 1 represents an immature or infantile reproductive tract with no uterine tone and no palpable ovarian structures. Heifers assigned a RTS 2 have slightly more tone than an RTS 1 and small follicles ≤ 8 mm in diameter. Heifers given a score of 3 are thought to be on the verge of cycling due to more uterine tone than an RTS 2, and follicles ranging in size of 8 to 10 mm. The RTS 4 and 5 heifers are presumed to be cycling with good or erect uterine tone respectively, and a possible palpable corpus luteum (CL) or palpable CL respectively with follicles greater than 10 mm in size (Andersen et al., 1991). This system proved to be beneficial in identifying beef heifers that were less likely to respond to synchronization and become pregnant in a short breeding season. Synchronized heifers assigned an RTS 1 had a 41 to 58% decrease in pregnancy rates compared to RTS 4 and 5 when multiple studies utilizing this system were evaluated. In addition, heifers having an RTS 1 had 32 to 67% lower pregnancy rates at the conclusion of the breeding season than heifers scoring an RTS 3 (Andersen et al., 1991). This RTS system was later determined to be repeatable and accurate within and between veterinarians (Rosenkrans and Hardin, 2003). Two different veterinarians possessing two different levels of experience performed 174 transrectal palpations on 29 Angus heifers. Substantial agreement was found among veterinarians classifying heifers as prepubertal or pubertal, while moderate agreement was found between veterinarians (Rosenkrans and Hardin, 2003). A later study found this RTS system was a good predictor of fertility when used prior to fixed timed artificial insemination (AI) or natural service (Gutierrez et al., 2014). Beef heifers (n = 4041) were assigned to fixed timed AI followed by exposure to natural service two weeks later (n = 2660), or natural service exposure for the entire 85 day breeding season (n = 1381). Cosynch in combination with a five or seven day CIDR was used for fixed timed AI. Pregnancy rates to AI were increased in heifers having an RTS of 4 (57.6%) or 5 (64.6%) compared to 1 to 3 (40.7% to 48.3%). Only the RTS 5 group exhibited higher pregnancy rates (90.2%) to natural service when compared to RTS 1 or 2 (79.7%). Pregnancy rates following natural service in RTS 3 (84.3%) and 4 (88.4%) were similar to RTS 5 (Gutierrez et al., 2014). A modified version of this scoring system was used to evaluate the efficacy of different synchronization protocols and RTS in dairy heifers (Stevenson et al., 2008a). Heifers were assigned an RTS score of 1 to 3 representing prepubertal, peripubertal and pubertal, respectively. Heifers having toneless uterine horns and follicles < 8 mm in diameter were classified as prepubertal (RTS 1), those with slight uterine tone and follicles 8 to 10 mm in diameter were peripubertal (RTS 2); and heifers with uterine tone, follicles > 10 mm in diameter and a palpable CL were considered pubertal (RTS 3). Heifers were assigned an RTS upon enrollment into the study, not prior to the breeding season as was done previously in beef heifers. Upon enrollment heifers were assigned to one of four treatment groups for AI, which included a combination of estrus detection with or without prostaglandin $F2_{\alpha}$ (PGF) administration and use of a CIDR in combination with TAI or estrus detection. Pregnancies per AI in the 28 day insemination period in this study were not influenced by RTS as they were in previous studies with beef heifers. However, pubertal heifers were inseminated and established pregnancy sooner, and had the least reproductive costs (Stevenson et al., 2008a). These studies demonstrate that some level of reproductive tract evaluation prior to breeding can serve as a good predictor of subsequent fertility in beef and dairy heifers. The size of reproductive tracts is not a major component of this scoring system since reproductive tracts in heifers are usually similar in size. In fact, the presence of various ovarian structures are the major component to evaluating pubertal status in heifers. No known studies have utilized this heifer reproductive tract scoring system as a predictor of fertility in lactating cows. Size and position of the reproductive tract has been used as a means of diagnosis of uterine disorders and assessment of uterine involution, but uncommonly compared to subsequent fertility.

After parturition uterine involution has historically been evaluated by classification of the position of the uterus and is typically thought to be complete if the uterus is positioned within the pelvic cavity (Bosu et al., 1984; Buch et al., 1955; Lindell et al., 1982; Scully et al., 2013). Uterine involution begins with a rapid decrease in uterine size, which occurs within the first three days and is a result of vasoconstriction and peristaltic contractions which occur every three to four minutes (as reviewed by Leslie, 1983). Typically by day-four postpartum, the rate of decreasing uterine size slows, and by day ten the uterus is completely palpable (as reviewed by Leslie, 1983). During the first twelve days postpartum, necrosis of the caruncular stalk takes place and the caruncles are sloughed. The sloughing of the caruncles greatly reduces weight of the uterus and progresses reduction in uterine weight from 10 to 13 kg towards the final goal of 0.8 kg (as reviewed by Sheldon et al., 2008). During this same time period, the lochia discharge is developed which is comprised of sloughed caruncles, remaining fetal fluid and blood from the ruptured umbilicus (as reviewed by Sheldon et al., 2008). Finally, regeneration of the endometrium begins with the intra-caruncular areas and then centripetal growth of cells over the caruncle (as reviewed by Sheldon et al., 2008). Altogether, the involutionary process involves expulsion of the placenta, physical shrinkage of the uterus, necrosis and sloughing of the caruncles, and regeneration of the endometrium. These processes are thought to be complete by 25 days postpartum by some authors (as reviewed by Leslie, 1983; Sheldon et al., 2008), and up to 50 days postpartum by others (Buch et al., 1955; Scully et al., 2013). Uterine involution is also characterized by a massive release of prostaglandin $F2_{\alpha}$ (PGF) that is considered to be the hormonal regulator of uterine involution (as reviewed by Kindahl et al., 1999). Prostaglandin $F2_{\alpha}$ levels parallel the progression of involution and peak by day-four postpartum and remain elevated for two to three weeks. High levels of PGF for a shorter duration are associated with rapid and uncomplicated uterine involution, while decreased levels and extended duration are associated with a complicated involution generally accompanied by bacterial contamination of the uterus (as reviewed by Kindahl et al., 1999). Complications during or following parturition, such as dystocia or retained fetal membranes, nearly always result in uterine contamination and delayed involution. This delayed involution subsequently leads to a delay in first ovulation and decreased fertility (as reviewed by Sheldon et al., 2008).

Evaluation of the size and position of the reproductive tract has been greatly utilized for nearly six decades to evaluate the progression of uterine involution postpartum (Buch et al., 1955; Scully et al., 2013). Complete uterine involution is generally classified as uterine horns being 2 to 5 cm in diameter and relatively symmetrical, and the uterus positioned within the pelvic cavity (Bosu et al., 1984; Scully et al., 2013). However, not all lactating dairy cows have reproductive tracts located within the pelvic cavity. Mid to late lactation cows can have reproductive tracts located outside the pelvic cavity, and these cows are generally thought to have completed uterine involution because they are later in lactation. The final reproductive tract size and position may be influenced by postpartum uterine involution and bacterial contamination. Studies classifying the size and position postpartum as a means of diagnosing uterine disorders (LeBlanc et al., 2002) and involution (Scully et al., 2013) have not evaluated cows beyond 50 days in milk (DIM). If uterine size and position can be associated with uterine disorders and incomplete or delayed involution, it stands to reason that size and position alterations may be permanent and impact fertility throughout lactation. However, it is unknown if larger reproductive tracts located more abdominally result in reduced fertility in lactating dairy cows.

Evaluation of Size and Position of Reproductive Tract in Cows

In lactating dairy cows, evaluation of the reproductive tract has predominantly been performed as a means of diagnosing postpartum uterine disorders or evaluating uterine involution postpartum, not necessarily for predicting fertility. LeBlanc (2002) evaluated the size and position of reproductive tracts 20 to 33 DIM in conjunction with other evaluations to diagnose endometritis, and related this information to reproductive performance. Cows (n = 1865) were examined once between 20 and 33 DIM during a biweekly herd visit. Initial examination included the use of a vaginoscope to visualize and classify vaginal discharge. Transrectal palpation followed whereby the location and size of the uterus were classified as entirely within the pelvis, over the pelvic brim but completely palpable following retraction, or over the pelvic brim and not completely palpable (LeBlanc et al., 2002). Cervical diameter was classified as < 5 cm, 5 to 7.5 cm, or > 7.5 cm, and the diameter (cm) of the largest uterine horn was estimated. In 1786 cows, the uterus was located entirely within the pelvis in 45.0% of cows, over the pelvic brim but palpable in 51.9% of cows, and not palpable in 3.1% of cows; however, location of the uterus did not impact pregnancy rate (LeBlanc et al., 2002). Cervical diameter > 7.5 cm found in 6.8% of cows did have a negative impact on pregnancy, along with presence of purulent discharge (LeBlanc et al., 2002). These data support findings that endocervical inflammation found prior to 35 DIM decreases fertility within 300 DIM (Deguillaume et al., 2012). The location of the uterus ultimately did not serve as a good predictor of fertility when evaluated 20 to 33 DIM; however, a large cervix was associated with poor fertility and its effect was greatest if found between 27 and 33 DIM (LeBlanc et al., 2002). Scully et al. (2013)

investigated effects of lactation on uterine involution in primiparous cows by allowing cows to lactate or ceasing lactation immediately postpartum. Beginning one week postpartum, the position of each cow's uterus in relation to the pelvis was evaluated by transrectal palpation twice a week. Position scores were assigned based a 0 to 3 scale with zero being a return to a non-gravid state, and 1 to 3 being varying degrees of the uterine body and horns positioned further over the pelvic brim (Scully et al., 2013). Conclusions of this study were that complete uterine involution was reached by 49 days postpartum in both groups, and uterine position scores were higher in non-lactating cows between 39 and 49 days postpartum. While fertility was not accessed in this study, it does suggest that uterine position can be used as one indicator of uterine involution. Incomplete uterine involution can limit natural defenses and ability to clear bacterial contamination, thereby delaying ovarian cyclicity which has been associated with decreased fertility (as reviewed by Sheldon et al., 2008). Another study used size and position of the uterus to assist in assessing uterine involution in lactating cows with or without uterine disease (Heppelmann et al., 2013). Using a 1 to 6 scale (Grunert, 1979); scores 1 to 3 were if the uterine horns were retractable and palpable and < 2 cm (score 1), 2 to 5 cm (score 2), and > 5 cm (score 3) in diameter. Scores 4 to 6 were assigned if the uterus was not palpable and the greater curvature palpable (score 4), greater curvature incompletely palpable (score 5), or greater curvature poorly outlined (score 6). Higher uterine scores were reported in cows with uterine disease compared to those without up to 18 DIM. Between 35 and 65 DIM mean uterine scores for both groups were reported to be between 1 and 2, and no fertility data were reported with respect to uterine scores (Heppelmann et al., 2013). Classification of size and position of the uterus may be a useful tool to evaluate uterine involution rates; however, no evidence exists as to whether or not this tool can predict potential fertility in lactating cows.

Through the use of ultrasonography, endometrial thickness around the time of ovulation was investigated to determine any effects it may have on fertility (Souza et al., 2011). Two studies were performed, one concentrating around the time of ovulation with few cows, and the other a larger trial evaluating fertility. In both studies, endometrial thickness was obtained by measuring the distance between the endometrial lumen and the visualized interface between the endometrium and myometrium. This measurement was taken on each uterine horn approximately 2 cm beyond the uterine body bifurcation and averaged to obtain an endometrial thickness for an individual. The first trial involved eight lactating cows (4 primiparous, 4 multiparous) that were known to be cycling. Cows underwent the Ovsynch protocol using gonadotropin releasing hormone (GnRH) - 7 days – PGF - 72 hours – GnRH with daily ultrasonography exams beginning at PGF administration and lasting five days. Endometrial thickness increased rapidly from approximately 7 mm to 9.5 mm in 24 hours following PGF administration. This increase in thickness remained for three days then began to decrease following GnRH administration, and returned to approximately 7 mm by two days after GnRH. At GnRH administration, estrogen levels were the highest and subsequently decreased as endometrial thickness decreased. Comparatively, serum progesterone levels were negatively correlated with endometrial thickness and began to rise at the least thickness measured (Souza et al., 2011). In the second study, cows underwent a two injection PGF pre-synchronization followed by the Ovsynch protocol 11 days later. Endometrial thickness was obtained 8 hours prior to the final GnRH (day 0) administration and 8 days later (day 8). All cows underwent fixed timed AI, but estrus expression was documented as well as uterine tone (day 0) and ovulation confirmation via ultrasonography (day 0 and day 8). In addition, blood samples were collected (day 0) to evaluate serum progesterone and estrogen levels. Ultimately, endometrial thickness was classified as ET1 (≤ 8 mm) and ET2 (> 8 mm). Increased endometrial thickness (ET2) was positively associated with a number of parameters including larger ovulatory follicle, increased serum estrogen concentration near AI, increased ovulation to final GnRH, increased percentage of estrus expression, increased uterine tone, and lower percentage of anovular cows.

Conception rates at 35 to 41 days and 58 to 64 days post AI were also improved with ET2 over ET1 (Souza et al., 2011).

Data regarding endometrial thickness around the time of ovulation and AI aid in the identification of cows with potentially higher fertility. This finding is novel in that ultrasonography measurements were used in cows with no uterine disorders; whereas, a previous study used this technology to diagnose endometritis and related that to compromised fertility (Barlund et al., 2008). The disadvantage of this approach in predicting fertility, based on endometrial thickness, is the level of expertise required with ultrasonography. Periodically, authors noted substantial differences in endometrial thickness between two inseminations involving the same cow (Souza et al., 2011), suggesting this tool may not be useful in identifying individual cows with poor fertility except for a specific insemination. This evaluative tool is not practical for all producers to use because of the expertise needed to perform the evaluation; and it cannot be applied to identify potentially poor reproductive performers prior to breeding, as the heifer reproductive tract scoring system can. A system which can be used by individuals of varying palpation skill levels, and be applied to identify potentially poor fertility in mature lactating cows may be useful in improving reproductive efficiency.

In summary, research involving evaluation of the reproductive tract prior to breeding in heifers and cows has generated useful information regarding potential fertility. The heifer reproductive tract scoring system has proven to be effective in identifying pubertal individuals, and resulted in greater success during a breeding season (Andersen et al., 1991; Stevenson et al., 2008a). Evaluation of the size and position of reproductive tracts in lactating cows via transrectal palpation has helped identify animals with uterine disorders (LeBlanc et al., 2002) and classify uterine involution (Scully et al., 2013), both of which can impact subsequent fertility. The use of ultrasonography measurements around the time of ovulation has shown positive results in identifying cows with potentially higher conception rates (Souza et al., 2011); however, this system requires a level of expertise not available to all producers. Ultimately, the size and position of the reproductive tract in lactating dairy cows may impact fertility, but this approach has not been confirmed. Nevertheless, size and position of the reproductive tract is only one factor potentially related to the larger multifactorial problem of fertility in lactating dairy cows. One factor that undeniably contributes to poor fertility is heat stress, and improved fertility during these challenging periods would certainly help achieve the goal of improving fertility in dairy cattle.

Impact of Heat Stress on Fertility in Lactating Cows

It is well documented that heat stress negatively impacts fertility in lactating dairy cows. Decreases in conception rates of 20 to 30 percentage points can be seen during periods of heat stress compared to cooler winter months (as reviewed by Rensis and Scaramuzzi, 2003), and these negative effects usually carry over throughout the autumn period when ambient temperature begins to cool (Badinga et al., 1985). These immediate and delayed effects have been attributed to alterations in follicular dynamics and hormonal profile during and after periods of heat stress (as reviewed by Wolfenson et al., 2000). Reductions in follicular dominance (Badinga et al., 1993; Wolfenson et al., 1995) and delays in luteolysis (Wilson et al., 1998b) are a few key alterations that repeatedly occur during heat stress. Reductions in follicular dominance can lead to decreased inhibin production (Roth et al., 2000; Wolfenson et al., 1995) and allow more large follicles to grow within a single wave (Roth et al., 2000). Additionally, early emergence of subsequent follicular waves (Wolfenson et al., 1995) and delayed luteolysis (Wilson et al., 1998b) can both allow for an extended developmental period for a future ovulatory follicle. These follicles undergoing extended development are considered to be aged or persistent, and have been associated with poor fertility (Mihm et al., 1994) and poor embryonic survival (Ahmad et al., 1995). Impacts of heat stress on follicular dynamics subsequently impact the hormonal profile of the estrous cycle. Several of the studies investigating the follicular dynamics during heat stress have also reported reductions in estradiol at various periods of the estrous cycle (Wilson et al., 1998b; Wolfenson et al., 1995), and an increase in FSH which fuel the increased number of large follicles found (Roth et al., 2000). Alterations in estrus behavior have also been observed with heat stress. Estrus detection still remains the dominant method in the US dairy industry for inseminating lactating cows (USDA, 2009). This method becomes much more challenging during the summer months with decreased estrus expression (Pennington et al., 1985) and a shorter duration of estrus (Gwazdauskas et al., 1981). Fixed timed AI allows opportunities to circumvent this issue by increasing AI submission rates during heat stress. When AI submission rates are increased with fixed timed AI during heat stress, conception rates are comparable to estrus detection along with significant increases in pregnancy rates (Cartmill et al., 2001), and reduced days open (De Rensis et al., 2008).

Heat Stress Effects on Progesterone

Research investigating the influence of heat stress on plasma progesterone concentrations has yielded conflicting results. Plasma progesterone concentrations increase during periods of heat stress applied by various methods (Abilay et al., 1975; Trout et al., 1998; Wilson et al., 1998b). Multiparous cows exposed acute heat stress had increased plasma progesterone concentrations (Trout et al., 1998). Cows were housed in heated (38.3 C) or thermoneutral (18.3 to 24 C) chambers for ten hours each day between days 11 and 21 of their estrous cycles. Plasma progesterone concentrations were greater in heat stressed cows up until day 18 of the estrous cycle (Trout et al., 1998). Rest periods outside of the chambers in this study occurred outdoors in a sand lot adjacent to the chambers. The study was conducted during cool months, and authors even noted that on some cooler days the heating system could not elevate the temperature in the chambers to desired levels (Trout et al., 1998). Cows may have experienced a cooling period during the evening, which is not always the case during periods of chronic heat stress in a commercial production setting. Another study housed nulliparous heifers continuously in a thermoregulated facility and exposed to 18.2 C temperature for two estrous cycles, and 33.5 C for two estrous cycles (Abilay et al., 1975). Plasma progesterone concentrations were greater during the period of mild heat stress up until day 8 of the ensuing second cycle. These results indicate a potential adjustment to the mild heat stress conditions, which better mimicked chronic heat stress by continuous exposure. Wilson et al. (1998) also concluded plasma progesterone concentrations were increased in cows exposed to heat stress. Plasma progesterone concentrations levels in heat stressed primiparous cows (housed at 29 C) was greater after day 16 of the estrous cycle than thermoneutral primiparous cows (housed at 19 C) and the heat stressed cows were noted to have delayed luteolysis (Wilson et al., 1998b), which was not the case in a previous study (Trout et al., 1998).

Differences in these studies make it difficult to discern if plasma progesterone concentrations truly increase during periods of chronic heat stress in a production setting. All heat stressors applied as treatments were artificial and the applied temperatures ranged from 29 to 38.3 C. In addition, general conclusion about increases in plasma progesterone from these studies are difficult considering these studies used multiparous, primiparous or nulliparous animals. Plasma progesterone concentrations between these three groups of animals are difficult to compare because lactation and parity can affect progesterone concentrations. Plasma progesterone is ultimately a balance between luteal and adrenal progesterone production and hepatic metabolism. Stressors can result in significant adrenal contributions of progesterone (Gwazdauskas et al., 1972). High dry matter intakes by multiparous cows are common to support a generally higher level of milk production compared to primiparous cows. These higher levels of feeding increase liver blood flow and increase the metabolic clearance of progesterone, and these increases persist longer with higher intakes (Sangsritavong et al., 2002). Metabolic differences associated with lactation alone make it impossible to compare steroid hormone levels in parous and nulliparous cows. Lactation demands with regards to milk production and dry matter intakes also make it difficult to compare primiparous and multiparous cows.

Plasma progesterone concentrations during periods of heat stress have been found to be decreased specifically during the diestrual period in several studies. In an early study performed by Rosenberg et al. (1977), multiparous and primiparous cows were housed in their normal production environment and all estrous cycles were evaluated until pregnancy or 120 DIM. Progesterone concentrations in the summer were found to be significantly decreased compared to winter 8 to 19 days before estrus. Summer progesterone concentrations were consistently 1.5 ng/mL lower in multiparous cows between days 4 to 15 prior to estrus, and 2.0 ng/mL lower in primiparous cows between days 8 to 15 prior to estrus. During the summer period, cows that reached their peak progesterone concentrations 7 days prior to insemination had greater fertility compared to cows reaching their peak 8 to 11 days prior to insemination (Rosenberg et al., 1977). This early data indicated that progesterone was decreased during the warm months, and an increased progesterone level around the time of insemination was positively related to conception during these months. A later study evaluated the entire estrous cycle daily of lactating cows, housed in a production environment, during spring and summer periods (Howell et al., 1994). Summer progesterone concentrations were decreased between days 6 to 18 of the estrous cycle by an average of 2.5 ng/mL, and the peak magnitude of progesterone tended to be decreased (Howell et al., 1994). These studies performed all in lactating cows under normal environmental conditions paint a more realistic picture of progesterone concentrations during periods of heat stress. If heat stress is in fact decreasing plasma progesterone concentrations, this could contribute to the decreased fertility seen in warm periods.

Progesterone is a key steroid hormone involved in the regulation of the estrous cycle. High or appropriate plasma progesterone concentrations prior to insemination have shown to be positively associated with conception (Fonseca et al., 1983; Martins et al., 2011). During periods of high progesterone, GnRH pulsatility is characterized as high amplitude and low frequency leading to a low pulse frequency of luteinizing hormone (LH) and atresia of dominant follicle. Decline of progesterone following luteolysis leads to high LH pulse frequency and eventual ovulation. Sub-luteal plasma progesterone concentrations (1 to 2 ng/mL) can allow increased pulse frequency of LH, but not to concentrations required for final maturation of the pre-ovulatory follicle and ovulation. These sub-luteal concentrations of progesterone are associated with extended dominance of the follicle leading to the development of persistent follicles and decreased fertility (as reviewed by Lonergan, 2011). Exogenous progesterone regulates release of LH such that a LH surge, required for ovulation, is prevented (Kinder et al., 1996). High progesterone causes a priming effect on the uterine endometrium by facilitating the accumulation of lipids needed for the synthesis of PGF (McCracken et al., 1999), and have an inhibitory effect on the uterine secretion of PGF (as reviewed by Silvia et al., 1991). The inhibitory effect is imposed by inhibiting the synthesis of estrogen receptors. With estrogen receptors inhibited, estrogen cannot induce synthesis of oxytocin receptors on the uterine endometrium; thereby, progesterone indirectly inhibits PGF release (as reviewed by Silvia et al., 1991). The effect of progesterone to inhibit an LH surge, priming of the uterus, and possibly preventing the release of PGF are all key as to why it has been used to assist in manipulation of the estrous cycle during synchronization protocols. Limitation of the LH surge results in a high percentage of animals continuing to develop follicles and not undergoing estrus. At the same time, lipid resources are being accumulated in the uterine endometrium to allow adequate synthesis of PGF, which will contribute towards luteolysis; and premature luteolysis may be prevented to allow greater estrous synchrony. Exogenous progesterone, or progestogens, have historically been utilized in several different fashions to manipulate estrous, including melengestrol acetate (MGA), which is administered orally generally in feed; norgestomet, which is administered as an ear implant; and progesterone which is currently administered via a controlled internal drug release (CIDR) device inserted vaginally. The CIDR is currently the only approved method of progesterone administration to lactating dairy cows in the US; therefore, it is the method of choice for dairy producers.

Use of Supplemental Progesterone to Improve Fertility

The use of a CIDR in combination with fixed timed AI has shown improvement in fertility measurements. One study compared three groups including cows inseminated with fixed timed AI utilizing the Ovsynch protocol with or without and CIDR (1.38g progesterone), and estrus detection as a control (El-Zarkouny et al., 2004). Cows were classified as cyclic or anestrous prior to initiation of treatment by evaluating serum progesterone concentrations 20 and 10 days before AI. Induced ovulation in anestrous cows, luteal function, luteolysis and synchronization rate were all similar between the fixed timed AI groups with and without a CIDR; however, these parameters were decreased in the estrus detection group. Indications were that use of the CIDR did not result in improvements in these parameters over and above Ovsynch alone. Pregnancy rates however were increased with the use of a CIDR compared to Ovsynch alone. Pregnancy rates 40 to 56 days post AI in the Ovsynch + CIDR group (45.1%) were comparable to estrus detection (38.8%), but increased over Ovsynch alone (20.9%; $P < 0.05$). In addition, embryo survival rate was increased between 29 to 57 days post insemination with a CIDR (75.9%) compared to Ovsynch alone (54.5%; $P < 0.05$). This study provides evidence that a CIDR used in combination with fixed timed AI can improve pregnancy rates and embryo survival (El-Zarkouny et al., 2004). Similar results of increased pregnancy rates with a CIDR in a fixed timed AI protocol were reported in later studies (Chebel et al., 2010; Melendez et al., 2006; Stevenson et al., 2006; Stevenson et al., 2008d). Simple inclusion or exclusion of a CIDR (1.9g progesterone) at initiation of Ovsynch resulted in increased pregnancy rates overall (Stevenson et al., 2006). Cows not identified in estrus following two injections of PGF 14 days apart were enrolled in an Ovsynch protocol with or without a CIDR containing 1.9g of progesterone (Melendez et al., 2006) or 1.38g of progesterone (Chebel et al., 2010), and pregnancy rates were increased for cows with a CIDR in each study. Cows not possessing a CL at initiation of an Ovsynch protocol were assigned to receive a CIDR or no CIDR, and again cows with a CIDR resulted in improved pregnancy rates 33 and 60 days post AI over those without a CIDR (Stevenson et al., 2008d). Two recent studies have investigated the use of a CIDR in cows with (Bisinotto et al., 2015f) or without a CL (Bisinotto et al., 2015a) at initiation of Ovsynch, a similar approach taken in a previous study (Stevenson et al., 2008d). Cows without a CL at initiation of Ovsynch were assigned to treatment with or without a CIDR for seven days. Cows assigned to CIDR treatment had two CIDRs (1.38g progesterone each) inserted. Use of a new CIDR containing 1.38g of progesterone has shown to only increase plasma progesterone concentrations by 0.78 ± 0.04 ng/mL (Cerri et al., 2009), which are well below plasma progesterone concentrations of 4 to 6 ng/mL observed in cows during diestrus (Bisinotto et al., 2015f; Cerri et al., 2009). Inclusion of two CIDRs in cows without a CL increased plasma progesterone concentrations by 1.85 ng/mL over untreated cows without a CL (Bisinotto et al., 2015a). Average progesterone concentrations of 2.77 ng/mL of cows with two CIDRs still could not match the 4.93 ng/mL progesterone concentrations of diestrus cows (Bisinotto et al., 2015a). Ultimately inclusion of two CIDRs in cows without a CL improved conception rates (42.2%) over those untreated without a CL (31.3%; $P = 0.001$), but were similar to those in diestrus (38.4%; Bisinotto et al., 2015a). These results are in agreement with another study (Stevenson et al., 2008d), which only used one CIDR. Another study evaluated the use of a CIDR (1.38g progesterone) in cows with a CL at initiation of Ovsynch and found the CIDR increased plasma progesterone concentrations significantly only immediately after insertion, and maintained modest improvements until removal (Bisinotto et al., 2015f). No benefits in conception rate at day 32 or 60 post AI were seen with the use of a CIDR in cows with a CL at initiation of Ovsynch (Bisinotto et al., 2015f). These results are in agreement with others, finding the presence of a functional CL at the beginning of Ovsynch in combination with a CIDR did not impact conception rates (Chebel et al., 2010).

The body of literature regarding the use of CIDRs to improve fertility with fixed timed AI is positive as studies have demonstrated benefits of 4.8 to 10% increases in pregnancy rates. In fact, the benefits of a CIDR may only exist under certain conditions since two of the studies only saw improvements in cows without a CL at initiation of the protocol (Bisinotto et al., 2015a; Stevenson et al., 2008d). The CIDR has not only shown benefits of improving pregnancy and conception rate, but also by inducing cyclicity in anestrous cows early in lactation (Cerri et al., 2009). Cows underwent two injections of PGF 14 days apart beginning 31 DIM as a pre-synchronization. Cows were determined to be anestrous if one CL was not detected in two consecutive ultrasonography examinations at 31 and 39 ± 3 DIM (Cerri et al., 2009). Treatments included administration of a new CIDR (1.38g progesterone), a 7-day used CIDR, or no supplemental progesterone. Twelve days after removal of treatment (57 ± 3 DIM) blood was collected for plasma progesterone concentration evaluation and cows were enrolled in Ovsynch. Cyclicity was determined to be resumed if this single plasma progesterone concentration was ≥ 1.0 ng/mL (Cerri et al., 2009). A greater percentage of cows resumed estrous cyclicity with supplemental progesterone, regardless of new or used, compared to untreated cows. Treatment with a CIDR did not demonstrate a benefit in conception rates as all were similar between the two treatments and control. However, cows that initiated estrous cyclicity prior to Ovsynch had greater conception rates compared to anestrual cows (Cerri et al., 2009). While there appears to be no direct effect of CIDR on conception rates, a CIDR can positively affect anestrous cows when administered prior to the breeding period. Overall research supports the use of CIDRs to improve conception rates following fixed timed AI and induction of cyclicity prior to breeding periods. This evidence is key to the logic of using a CIDR during periods of heat stress, a time when fertility is greatly compromised and plasma progesterone concentrations are potentially decreased.

Use of a Supplemental Progesterone to Improve Fertility during Heat Stress

Few studies have tested the use of supplemental progesterone in combination with fixed timed AI during periods of heat stress to improve fertility. Alnimer and Lubbadeh (2003) randomly allocated 60 Friesian cows to three treatment groups. A progesterone releasing intravaginal device (PRID) containing 1.55g of progesterone was used to administer supplemental progesterone, similar to administration with a CIDR. Two of the three treatment groups were administering a PRID at 51 ± 3 DIM concurrently with GnRH. The first treatment group (n = 20) had the PRID removed seven days later with PGF administration, followed by a final GnRH 48 hours later and fixed timed AI 16 to 20 hours after the final GnRH. Second treatment group (n = 20) did not receive a second GnRH, but instead were estrus detected following PRID removal and PGF administration. The third treatment group (n = 20) was based on estrus detection, and cows not detected in estrus by 58 ± 3 DIM were administered PGF and detected for estrus (Alnimer and Lubbadeh, 2003). Cows detected in estrus following insemination were re-inseminated, and those diagnosed not pregnant were administered PGF and re-inseminated upon detected estrus. Blood was collected at each injection to evaluate serum progesterone concentrations, and any sample ≥ 1ng/mL was considered cycling. All procedures were performed in the Dulial area of Jordan during the warm summer months. Conception rates to first inseminations between the three groups did not differ as they reported rates of 30.0, 15.0, and 15.0% for treatments one, two and three respectively. Pregnancy rates for cows at 120 and 150 DIM were greater for cows in treatment one with a PRID and fixed timed AI compared to the other treatments involving estrus detection. These results are not surprising considering Ovsynch has improved pregnancy rates during heat stress mainly due to increased AI submission rates (Cartmill et al., 2001; de la Sota et al., 1998). The experimental design of this study makes it difficult to discern if the benefits in pregnancy rates were due to the PRID or the fixed timed AI. The lack of a direct comparison of fixed timed AI with or without a PRID prevent sound conclusion related to effects of supplemental progesterone during periods of heat stress, and the number of observations in each treatment group were limiting.

Another study performed on lactating Holsteins in northeastern Spain reported positive effects from use of supplemental progesterone during heat stress. This study evaluated 3577 inseminations total, and included multiple inseminations on cows (Garcia-Ispierto et al., 2013), which is different from the previous study evaluating 60 first inseminations (Alnimer and Lubbadeh, 2003). Cows not detected in estrus, based off a pedometer activity system, and inseminated over a 21-day period were randomly allocated to receive a PRID (1.55g progesterone) for five or nine days. Cows inseminated following estrus detection served as controls. Cows were administered PGF and equine chorionic gonadotropin (eCG) upon PRID removal, followed by GnRH 48 hours later and timed AI 12 hours after GnRH. Inseminations were evaluated over the course of 17 months in all seasons; and seasons were divided into two groups, warm and cool. Cows inseminated following estrus (n = 1635) during the cool season served as the reference in odds ratio analysis and resulted in a conception rate of 43.7%. Cows assigned a PRID for nine days and timed AI resulted in decreased conception rates during the warm (22.3%) and cool (32.0%) seasons. In addition, cows inseminated following estrus in the warm season had decreased conception rate of 26.6%. Cows assigned a PRID for five days and timed AI had conception rates in the warm (43.3%, n = 120) and cool (39.7%, n = 600) season, which were comparable to cows inseminated following estrus in the cool season (43.7%, n = 1635). Authors concluded that the use of a PRID for five days in combination with fixed timed AI resulted in similar fertility results observed in estrus detected cows in cool seasons. While these results are positive, they do not allow for clear conclusions regarding progesterone supplementation during heat stress because a direct comparison of cows with no

PRID and timed AI was not used. Limited observations of 120 inseminations during the warm season with a 5-day PRID also raise questions about conclusions drawn by the authors regarding positive effects of progesterone supplementation.

The use of progesterone supplementation during periods of heat stress has been investigated; however, experimental designs in both studies discussed do not allow for a clear comparison (Alnimer and Lubbadeh, 2003; Garcia-Ispierto et al., 2013). Neither study compared cows with or without progesterone supplementation in combination with fixed timed AI during periods of heat stress. Observation limitations also existed in both studies with one having 60 first inseminations (Alnimer and Lubbadeh, 2003) and another having 120 inseminations during heat stress (Garcia-Ispierto et al., 2013). Clear direct comparisons and increased observations are needed in future studies before sound conclusions can be made regarding potential benefits of improving fertility during heat stress with supplemental progesterone.

Taken together, periods of heat stress have a clear negative impact on fertility in lactating dairy cows. Furthermore, this negative impact is immediate during the summer months and delayed in the cooler autumn months (as reviewed by Rensis and Scaramuzzi, 2003). Alterations in follicular dynamics, estrus expression, and steroid hormone concentrations are all key factors contributing to decreased fertility during and following periods of heat stress (as reviewed by Rensis and Scaramuzzi, 2003). Conflicting results exist regarding progesterone concentrations during heat stress; as some authors have reported an increase (Abilay et al., 1975; Trout et al., 1998; Wilson et al., 1998a), while others have reported a decrease (Howell et al., 1994; Rosenberg et al., 1977). Differences in experimental design regarding chronic or acute application of temperature exposure may be a key reason for the discrepancy within the scientific literature. Studies reporting a decrease in progesterone applied a chronic temperature exposure, similar to a production setting. The use of supplemental progesterone with a CIDR has shown positive effects on fertility during unspecified seasons (Chebel et al., 2010; El-Zarkouny et al., 2004; Melendez et al., 2006; Stevenson et al., 2006). Fixed timed AI has been shown to improve some fertility parameters during heat stress by increasing AI submission rates (Cartmill et al., 2001). Few studies have investigated the use of a CIDR in combination with fixed timed AI during periods of heat stress, and those that have lack clear comparisons within the experimental design in order to make sound conclusions (Alnimer and Lubbadeh, 2003; Garcia-Ispierto et al., 2013). Thus, more efforts may be necessary, regarding the use of supplemental progesterone in combination with fixed timed AI during periods of heat stress, before sound recommendations can be made to dairy producers.

Summary

Fertility in lactating dairy cows must be improved if an abundant milk supply for the international and domestic population is to continue. A multitude of factors can have a negative impact on fertility, and many factors work collaboratively to decrease fertility. Size and position of the reproductive tract have been used as an evaluative tool to diagnose postpartum uterine disorders and assess uterine involution. Some cows maintain a larger reproductive tract beyond the postpartum period, and it is unknown if cows with larger reproductive tracts have decreased fertility. If this is the case, an evaluative tool prior to the breeding period could help producers make the most informed decisions regarding their reproductive programs. Heat stress is also a major factor contributing to decreased fertility in lactating cows. Research has reported a decrease in progesterone levels during periods of heat stress, but limited information exists regarding the use of supplemental progesterone to negate these negative effects. One research effort detailed in the following chapters will attempt to answer the question of whether or not cows with larger reproductive tracts experience decreased fertility. A second effort will investigate if supplemental progesterone applied during periods of heat stress, will positively impact reproductive performance. Hypotheses are that cows with larger reproductive tracts will have lower fertility, and supplemental progesterone may improve fertility during periods of heat stress.

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CHAPTER III: Effect of Reproductive Tract Size and Position on Conception Rates in Lactating Dairy Cows

Abstract

Objectives were to develop a reproductive tract size and position scoring system that identify lactating dairy cows with decreased fertility and test the system in a field trial. The reproductive tract size and position scoring system (SPS) utilized only transrectal palpation to evaluate cervical and uterine horn diameter, as well as position of the reproductive tract in relation to the pelvis. Scores were used to identify cows with small (SPS1), medium (SPS2), and large (SPS3) tracts during pre-breeding exams (typically 30 to 60 days in milk). Reproductive tracts determined to be an SPS1 were small and compact with uterine horns resting within the pelvic cavity, SPS2 were intermediate in cervical and uterine horn diameter with longer uterine horns resting partially outside the pelvic cavity, and SPS3 were large diameter, deep reproductive tracts resting mostly outside the pelvic cavity. Cows with SPS1 had a higher conception rate following artificial insemination (43.3 ± 3.7%) compared to SPS 2 (36.9 ± 3.6%, $P = 0.0073$) and SPS3 (27.7 ± 4.3%, $P = 0.0002$). Cows with SPS2 also differed in conception rate compared to SPS3 ($P = 0.0251$). Ultrasound measurements of uterine horn, cervical diameter (TOTAL, $P = 0.01$), and length measurements of uterine horns, cervix, and vagina (TOTAL, $P < 0.003$) confirmed differences among SPS groups. Volume measurements of abattoir reproductive tracts indicated no differences in lumen volume among SPS ($P > 0.20$). In conclusion, cows identified with larger reproductive tracts experienced a 15% decrease in conception rates. These cows can be identified early before entering a breeding program and allow dairy producers to make sound decisions, and allow for more efficient AI programs.

Introduction

Artificial insemination (AI) has been utilized by the dairy industry to increase genetic potential and overall productivity for the last 50 years. Genetic progress in the dairy industry has been made possible, in large part, by advances in semen technology and wide acceptance of artificial insemination (Vishwanath, 2003). However, during this same time AI fertility has continued to decline in the dairy industry (Lucy, 2001). Factors that have been linked to declines in fertility include higher milk production, larger herd size, increase use of confinement housing, and higher levels of inbreeding (Lucy, 2001). Conception rates of 50.8, 48.9, and 48.3% for first, second, and third parity cows respectively were reported in the early 1980s (Gwazdauskas et al., 1981a). More recent research examining records from the Dairy Herd Improvement Association (DHIA) reported conception rates in Holstein cows declined from 33% to 30% from 1996 to 2006 with a low of 27% in 2001 (Norman et al., 2009). Improvement was reported beginning in 2002 and was attributed to increased use of synchronization protocols and better genetic selection (Norman et al., 2009). While improvements in management practices may lead to subtle increases in conception rates, more research is needed to overcome a 20% decrease in the last 25 years (Lucy, 2001; Norman et al., 2009). The growing economic demand on dairy producers also requires AI to become more economically efficient.

General evaluations of the female reproductive tract by transrectal palpation have been used as predictors of fertility. A scoring system was developed to determine pubertal status of beef heifers prior to breeding season (Andersen et al., 1991), and has been effective for identifying individuals with the greatest chance of conceiving in beef (Gutierrez et al., 2014; Holm et al., 2009) and dairy heifers (Stevenson et al., 2008a). This reproductive tract scoring system utilizes a one-five scale and evaluates the diameter and tone of uterine horns, as well as ovarian structures to determine if heifers are prepubertal (1 to 2), peripubertal (3) or pubertal (4 to 5) (Andersen et al., 1991). This reproductive tract scoring system (Andersen et al. 1991) is not applicable in mature cows because of physical differences in reproductive tracts of heifers and mature cows. The focus of the heifer scoring system was to determine pubertal status and identify ovarian structures. Generally heifer reproductive tracts are relatively of similar size and within the pelvic cavity once puberty is reached. Mature reproductive tracts have the potential to change a great deal in size and position following first parturition compared to heifers reproductive tracts, and pubertal status is no longer a concern. Reproductive tract positions in mature dairy cows have been used to assist in determining the existence or severity of uterine infections post-partum (LeBlanc et al., 2002; Scully et al., 2013); and these positions were classified as being entirely within pelvis, over pelvic brim but palpable when retracted, or over pelvic brim and not palpable upon retraction. Studies evaluating the position of the reproductive tract (uterus) 20 to 49 days post-partum have not associated position with conception rate later during the breeding period (LeBlanc et al., 2002; Scully et al., 2013). Uterus positions described by LeBlanc et al. (2002) reflect differences observed in lactating dairy cows beyond the immediate post-partum period.

However, we are not aware of a scoring system accounting for position and size of the reproductive tract to predict fertility in lactating dairy cows. An evaluative tool to predict fertility would allow dairy producers opportunities to make the most informed decisions in order to maximize reproductive and economic efficiencies. Thus, the current study sought to develop and evaluate a reproductive tract size and position scoring (SPS) system in lactating dairy cows that would identify individuals with compromised fertility, not to identify endometritis. The target clientele for this scoring system would be veterinary practitioners and dairy producers. We hypothesized that 1) use of a SPS system based on reproductive tract size and position can be used as a predictor of fertility in lactating dairy cows, and 2) smaller reproductive tracts (lower SPS) will have higher conception rates due to a smaller area for sperm to navigate. Therefore, the objective of the study was to compare conception rates of lactating dairy cows with different SPS.

Materials and Methods

This study was performed in four herds, and all procedures were performed with approval of the University of Tennessee Institutional Animal Care and Use Committee (IACUC).

Development of a Size and Position Scoring System of the Reproductive Tract

Ultrasonography Measurements associated with Size and Position Score

One hundred nonpregnant cows greater than 30 days in milk (DIM) from a single herd were palpated and assigned a SPS. Size and position scores were based on diameter of the cervix and uterine horns; as well as position of the reproductive tract as evaluated by transrectal palpation. Experienced palpators (n = 6) were given the scoring criteria listed in Table 1, and position reference depicted in Figure 1.   Each cow was scored by at least two individuals not informed of other's scores. Following SPS assignment, each animal underwent ultrasonography measurements of the cervix and right and left uterine horns (MyLab 30 Gold, 7.5 MHz transducer). Diameter of the cervix was obtained by placing the ultrasound probe longitudinally along the cervix. Uterine horn diameter measurements were obtained by measuring a cross-section of each uterine horn approximately 2.5 centimeters beyond the external bifurcation. Any cow suspected or confirmed of having a uterine infection, abnormal uterus, or in estrus was not scored or measured.

Length and Volume Measurements associated with Size and Position Score

Length of the reproductive tract as related to SPS was determined using abattoir derived tissues (n = 45 reproductive tracts). Mature Holstein cows were targeted and assigned a SPS by three individuals prior to animal harvest. Following collection of the reproductive tract, length measurements of the cervix, vagina, and uterine horns were obtained with a flexible measuring tape. Cervical and vaginal measurements were taken from caudal to cranial ends of each respectively. Uterine horn measurements were determined from the cranial end of the cervix to the uterotubal junction of each uterine horn.

Luminal volume of the uterus was measured by placing an 18 gauge foley catheter through the cervix into the uterine body. Uterine horns were filled with water using a 60 mL

Table 1: Criteria for Size and Position scores.

SPS	Position of Tract	Size of Tract
1	Cervix and uterine horns located entirely within the pelvis	Small uterine horn diameter (size of 3mL-6mL syringe); Small cervix diameter
2	Cervix within the pelvis and uterine horns at or over the pelvic brim	Small to moderate uterine horn diameter (size of 6mL-12mL syringe); but long normal cervix
3	Majority of cervix and the entire uterine horns beyond the pelvis	Large, thick uterine horns (size of 20mL syringe); Large cervix

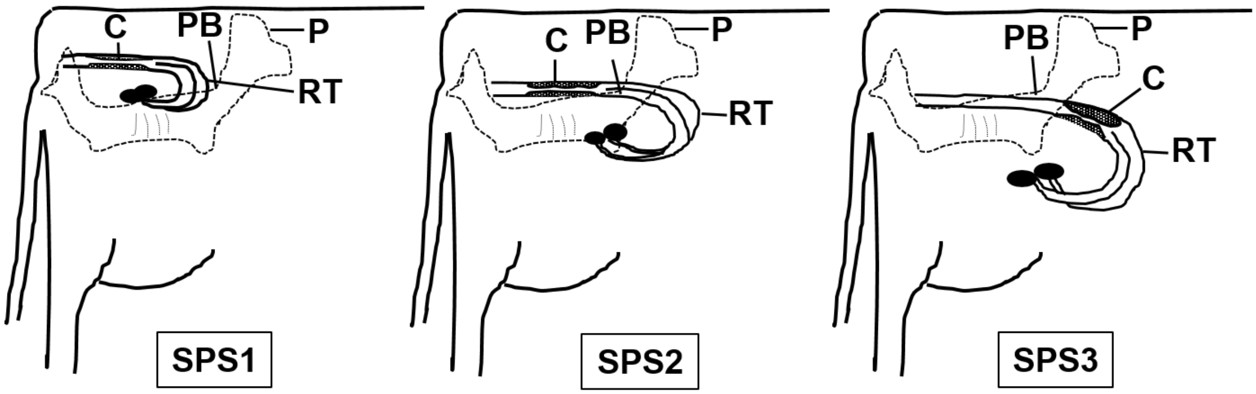

Figure 1: Depiction of reproductive tract position influence on Size and Position Score. Reproductive tracts positioned entirely within the pelvic cavity represent a SPS1. Reproductive tracts in which the cervix is within the pelvic cavity, but uterine horns are outside the pelvic cavity represent a SPS2. Reproductive tracts in which the cervix and uterine horns lie outside the pelvic cavity represent a SPS3. Abbreviations: cervix (C), pelvis (P), reproductive tract (RT), pelvic brim (PB), size and position score (SPS).

syringe and Y-tubing attached to the catheter, until a constant pressure was achieved. Constant pressure was obtained by utilizing gravity, placing the end of the Y-tubing 18.75 centimeters above the reproductive tract. As water entered the uterus, levels in the exit tubing rose and fell as dissipation occurred. Once the water stabilized at the 18.75 centimeters level above the reproductive tract for one minute without expelling more than 2 mL of water, a volume measurement was recorded. Following length and volume measurements, the uterus was dissected to investigate for possible uterine infection or early pregnancy. Eight of the 45 reproductive tracts revealed a uterine infection or early pregnancy upon dissection, resulting in 37 reproductive tracts utilized for volume and length measurements (n = 14, SPS1; n = 11, SPS2; n = 12, SPS3).

Relationship of Size and Position Scores on Fertility in Lactating Dairy Cows

A field trial to test the effects of SPS on fertility in lactating dairy cows was conducted in four herds (University research herd and three privately owned commercial herds). Herd populations ranged from 175 to 700 lactating Holstein cows housed in free stalls or bedded pack, and fed corn silage based total mixed rations. Cows were milked three times a day in three herds, and twice a day in one herd. A total of 1486 cows were enrolled and assigned a SPS at least once (a small subset of cows were enrolled over multiple parities). Nonestrual cows were assigned a SPS before breeding at various stages of lactation. A portion of cows were scored more than once within a single parity due to routine herd checks. In these instances, the most recent score was applied to subsequent insemination(s) (only 3% of scores changed within a single parity). Study leaders participated in SPS assignment in all four herds, while other evaluators were used in each herd following training and review of scoring criteria explained in Figure 1 and Table 1. A total of twelve individuals were used across the four study herds to assign SPS including study leaders, AI technicians, and veterinarians.

Following SPS assignment, cows followed normal herd reproductive management practices for insemination and pregnancy examination. Detection of estrus, estrus synchronization, and ovulation synchronization protocols were used as standard practices and resulted in 1979 inseminations. Inseminators were not informed of the SPS of each cow in all herds. Holstein service sires (n = 55) were selected by commercial mating services, 26 of the 55 service sires had five or more observations. Pregnancy status was determined by ultrasonography 28 to 45 days post insemination, or transrectal palpation 35 to 50 days post insemination. Cows inseminated on return estrus prior to pregnancy evaluation were considered not pregnant for the previous insemination. Cows determined to be not pregnant upon examination were assigned an SPS and subsequent inseminations were recorded and evaluated until pregnancy was established, or individual cows exited a herd's insemination program. Overall study design is depicted in Figure 2.

Freshening date, insemination date, DIM at insemination, parity, service sire, location, milking frequency, milk production at insemination and pregnancy status were recorded for each insemination. Information was retrieved from each herd's respective herd management computer software (Dairy Comp 305; Valley Agricultural Software, Tulare, CA, USA or PC Dart; Dairy Records Management Services, Raleigh, NC, USA). Days in milk at insemination were divided into two groups (DIMGRP) based on values above or below the mean, DIMGRP1 ≤ 116 DIM and DIMGRP2 > 116 DIM. Milk production at insemination was obtained by recording DHIA test day or on farm test day (not DHIA official) weights within 30 days of insemination. Milk production was divided into two groups (MILKGRP) based on values above or below the mean, MILKGRP1 ≤ 39 kg and MILKGRP2 > 39 kg. Parity was divided into three groups including first, second, and three or more.

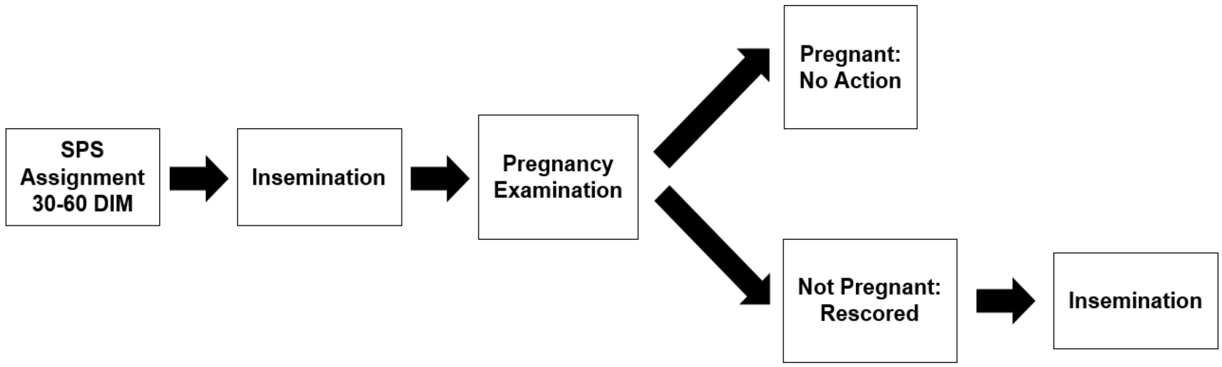

Figure 2: Depiction of Size and Position Score study design. Cows were initially assigned an SPS 30 to 60 DIM, or at various DIM following a non-pregnant diagnosis. Pregnancy evaluation was performed 28 to 50 days post insemination. Cows determined to be not pregnant were assigned a SPS and subsequent inseminations were recorded.

Statistical Analyses

All statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA, 2013). Cows with multiple scores were assigned a final score by averaging the scores and adjusting for palpator differences. Adjustments were performed by testing the correlation between each individual using PROC CORR, and testing for agreement level between individuals using KAPPA test. A mixed model ANOVA was used to produce a least square mean which was used as the final score. The mixed model consisted of score as the dependent variable and cow as the fixed effect, blocked on palpator. Least square means ≤ 1.51 were assigned a final SPS1, 1.52 to 2.51 a final SPS2 and ≥ 2.52 a final SPS3.

Ultrasound diameters (in vivo) and length and volume measurements (ex vivo) were analyzed using a complete randomized design ANOVA. Right uterine horn ultrasound measurements and volume measurement comparisons among SPS were performed with log transformation. Uterus diameter, volume and length means were compared with Fisher's Least Significant Difference mean separation ($P < 0.05$).

Pregnancy data were analyzed using a mixed model ANOVA for binomial data in Glimmix. Basic model consisted of pregnancy as the dependent variable, with SPS the treatment factor; and insemination as the experimental unit. Location, service sire, DIMGRP, MILKGRP, daily milking frequency (MILKX), season or month of insemination, year of insemination, and parity were added individually, and interacted with SPS, as fixed effects to this base model to determine their effect on conception rate. A service sire effect on conception rate was investigated by using sires with 5 or more observations. Milk group ($P = 0.6887$), season ($P = 0.2791$), service sire ($P = 0.4456$), year ($P = 0.8589$) and parity ($P = 0.0844$) did not have a significant effect on conception rate. Location ($P = 0.0076$) and MILKX ($P < 0.0039$) were determined to have a significant effect on conception rate. Days in milk group did not have a significant effect as a single fixed effect ($P = 0.5104$); however, the interaction of

DIMGRP and SPS did have a significant effect (P = 0.0290). Location and service sire were added to the base model as random effects, while DIMGRP and MILKX were included as single fixed effects to comprise the final model. Treatments were compared using Fisher's Least Significant Difference mean separation (P < 0.05).

A chi-squared test investigated an association between parity group and SPS. Repeat breeders were investigated using a chi-squared test, testing whether a higher frequency of repeat breeders was associated with SPS. Repeat breeders were defined as cows having three or more inseminations and not pregnant (Yusuf et al., 2010)

Results

Development of a Size and Position Scoring System of the Reproductive Tract

Ultrasonography Measurements associated with Size and Position Score

Ultrasound measurements of cows assigned an SPS1, SPS2 and SPS3 were increased as SPS increased (P = 0.0020; Table 2). Ultrasound measurements of cows assigned an SPS1 or SPS2 did not differ for the right uterine horn (P = 0.7671), cervix (P = 0.0689), or the collective total (P = 0.1147) of these structures (TOTAL, Table 2). Cows assigned an SPS3 displayed larger left uterine horns (P = 0.0024) and an increase in the collective total of the cervix, right horn, and left horn (TOTAL, P = 0.0049) compared to SPS1 and SPS2 cows. Animals scored an SPS1 had a smaller cervical diameter compared to SPS3 (P = 0.0135). No differences in right horn diameter were observed among all SPS (P = 0.1834).

Length and Volume Measurements associated with Size and Position Score

No length differences were observed between SPS1, SPS2 and SPS3 for right uterine horn (P = 0.0805), cervix (P = 0.0710) and vagina (P = 0.3803; Table 3). Longer left uterine horns were observed for SPS3 compared to SPS1 (P = 0.0053) and SPS2 (P = 0.0347; Table 3). Total (TOTAL) uterine length was greater for SPS3 compared to SPS1 (P = 0.0037) but not

Table 2: Ultrasound diameter measurements across Size and Position Score.

Structure	SPS1 *n=36*	SPS2 *n=46*	SPS3 *n=18*	P value
Left Horn[1]	**14.0 ± 0.6[a]**	**15.5 ± 0.5[b]**	**17.5 ± 0.8[c]**	**0.0020**
Right Horn[1]	**15.4 ± 0.6[a]**	**15.2 ± 0.5[a]**	**17.1 ± 1.0[a]**	**0.1928**
Cervix[1]	**21.3 ± 0.9[a]**	**23.5 ± 0.8[ab]**	**26.0 ± 1.3[b]**	**0.0135**
TOTAL[1,2]	**51.1 ± 1.7[a]**	**54.7 ± 1.5[a]**	**61.1 ± 2.4[b]**	**0.0049**

[1] mm±SEM

[2]TOTAL=Sum of diameter measurements of left horn, right horn, and cervix

[a,b] Means with no common letter within row differ ($P<0.05$)

Table 3: Means for reproductive tract length and volume for Size and Position Scores.

Structure	SPS1[1] (n)	SPS2[1] (n)	SPS3 (n)	P value Overall	P value[2]	P value[3]
Left Horn[4]	34.4 ± 2.1[a] (14)	36.5 ± 2.4[a] (11)	43.8 ± 2.3[b] (12)	0.0151	0.0053	0.0347
Right Horn[4]	33.7 ± 2.1[a] (14)	38.0 ± 2.4[a] (11)	40.9 ± 2.3[a] (12)	0.0805	0.0274	0.3874
Cervix[4]	9.5 ± 0.4[a] (14)	9.4 ± 0.5[a] (11)	10.8 ± 0.5[a] (12)	0.0710	0.0529	0.0395
Vagina[4]	30.6 ± 1.1[a] (13)	32.6 ± 1.1[a] (11)	32.2 ± 1.1[a] (11)	0.3803	0.3085	0.7801
TOTAL[4,5]	104.6 ± 3.8[a] (14)	115.8 ± 4.7[ab] (11)	124.4 ± 4.9[b] (12)	0.0135	0.0037	0.1933
Volume[6]	97.4 ± 16.4[a] (12)	115.4 ± 20.4[a] (11)	126.4 ± 22.3[a] (11)	0.5588	0.2935	0.7173

[1] No differences observed among SPS1 and SPS2

[2] SPS1 and SPS3 comparison

[3] SPS2 and SPS3 comparison

[4]Mean centimeter ± SEM

[5]TOTAL=Sum of left horn, right horn, cervix, and vagina measurements

[6]mL ± SEM

[a,b] Means with no common letter within row differ ($P<0.05$)

different compared to SPS2 (P = 0.1933; Table 3). Uterine lumen volume was not different for all SPS (P = 0.5588; Table 3).

Relationship of Size and Position Scores on Fertility in Lactating Dairy Cows

Frequency and Effect of Parity on SPS

A total of 1490 cows were assigned an SPS within a single parity; however 27 observations had a missing parity, therefore 1463 cows were analyzed. Only 3.1% of the assigned SPS within a single parity changed (n = 46/1463). Cows having an SPS2 had the highest percentage of change within a single parity (5.5%, 32/586), compared to SPS1 (1.6%, 12/747) and SPS3 (1.5%, 2/130). Twenty-seven SPS2 scores that changed, decreased to SPS1 and five increased to SPS3. Evaluation of nine palpator combinations in which 50 common cows were scored revealed the average percent of change from SPS1 to SPS3 or SPS3 to SPS1 was 4.7%. Parity one, two, and three or more had SPS changes of 1.0% (14/606), 0.8% (12/304), and 1.3% (20/553) respectively, with no significant parity effect on SPS change (P = 0.4223). The frequency of SPS1 decreased as parity increased, while SPS2 and SPS3 increased as parity increased (Figure 3, P < 0.0001).

Effect of SPS on Conception Rates

Of the 1490 cows assigned an SPS, some were inseminated with nonconventional semen, semen of a breed other than Holstein, not inseminated, or not evaluated for pregnancy; resulting in 1979 inseminations from 1039 cows utilized for analysis. Cows assigned an SPS1 had a higher conception rate compared to cows assigned an SPS2 (P = 0.0073) and SPS3 (P = 0.0002); furthermore, SPS2 resulted in a higher conception rate compared to SPS3 (P = 0.0251; Figure 4). Milking frequency had a significant effect on conception rate; 2X and 3X milking frequency were 44.1 ± 6.0% and 28.1 ± 3.3% respectively (P = 0.0155). Conception rate for DIMGRP1 (39.2 ± 3.7%) was greater compared to DIMGRP2 (32.3 ± 3.5%, P = 0.0054). Overall, 18.3% (190/1039) of cows were identified as repeat breeders. Percentage of repeat

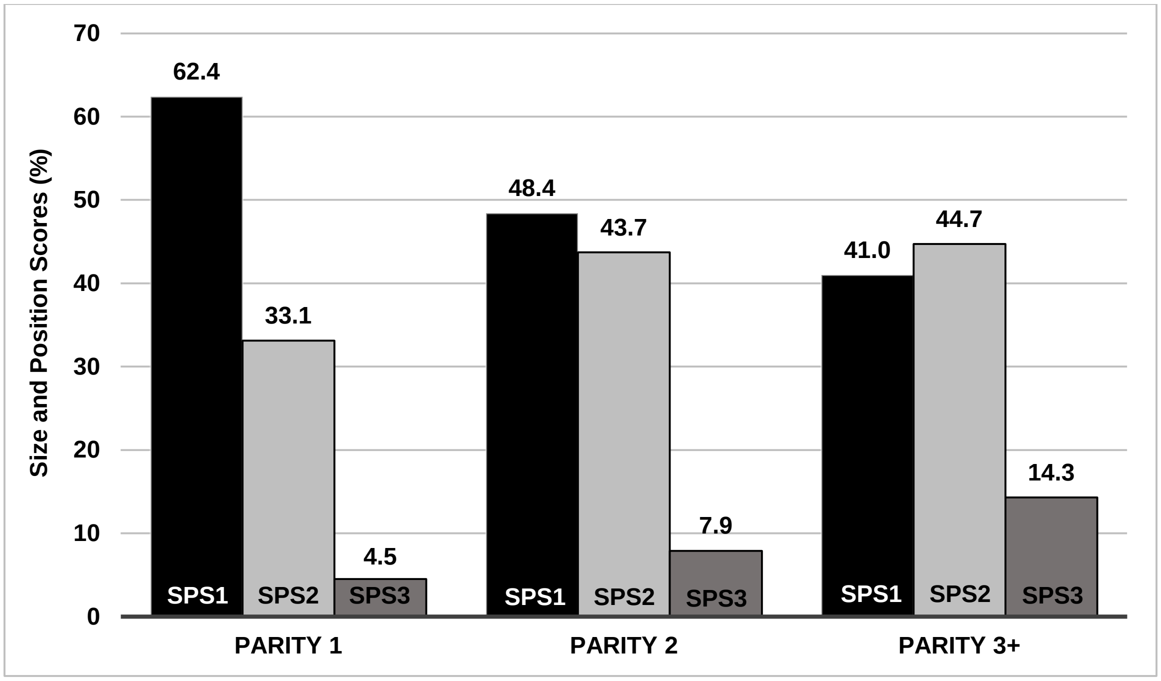

Figure 3: Influence of parity on Size and Position Score. Percentages of SPS1 decreased as parity increased over parity one, two, and three or more (n = 387/620, 153/316, and 235/573, respectively). Percentages of SPS2 increased as parity increased over parity one, two, three or more (n = 205/620, 138/316, 256/573, respectively). Percentages of SPS3 increased as parity increased over parity one, two, and three or more (n = 28/620, 25/316, 82/573, respectively; chi-square $P < 0.0001$).

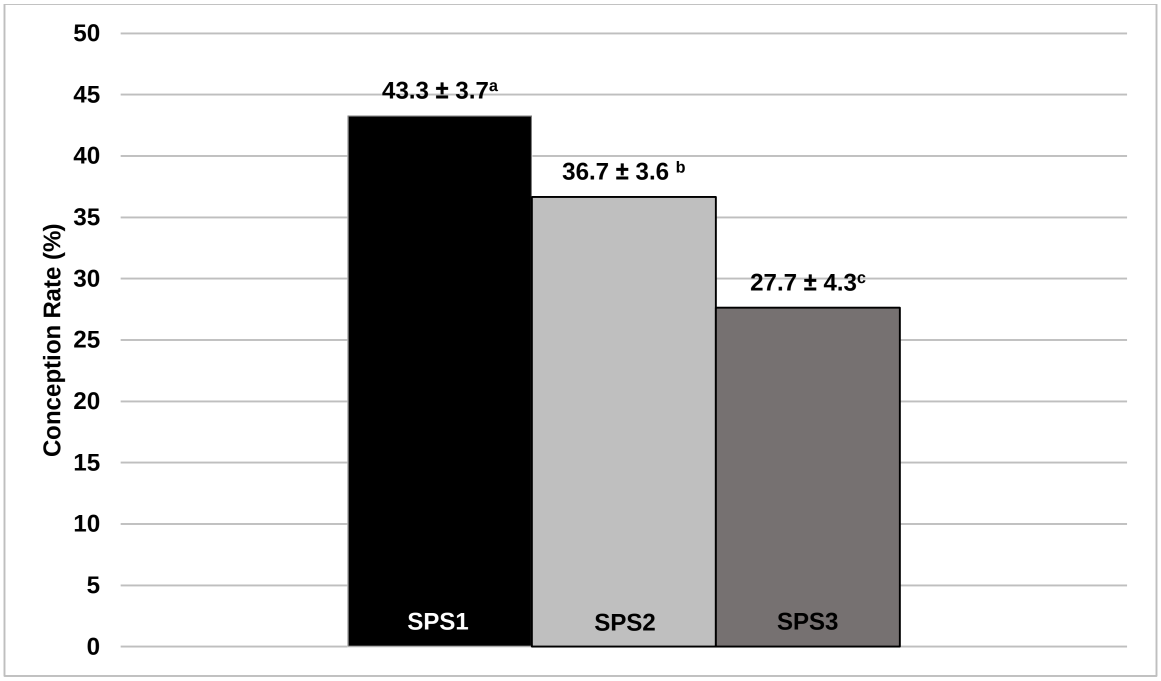

Figure 4: Conception rates of Size and Position Scores. Conception rate for cows with SPS1 (43.3 ± 3.7%, n = 929) were greater than SPS2 (36.7 ± 3.6%, n = 871; P = 0.0055) and SPS3 (27.7 ± 4.3%, n = 179; P = 0.0002). Conception rate for cows with SPS2 was also greater compared to cows with SPS3 (P = 0.0268).

breeders was least for SPS1 (14.5%, n = 76/525) compared to SPS2 (21.5%, n = 92/428) and SPS3 (25.6%, n = 22/86; P = 0.0039).

Discussion

We hypothesized that position and size of the reproductive tract could be used as a predictor of fertility in lactating dairy cows, and to this end we have exhibited that cows with deeper and larger reproductive tracts have decreased conception rates. A primary goal was to develop a system that could be used by veterinarians and producers alike, realizing some may have limited palpation skills beyond AI. Another focus was to classify the position and size of the reproductive tract prior to breeding and not during known estrus, as estrual periods often result in uterine tone and have shown to influence the size of uterine horns (Souza et al., 2011). This is the first known study to classify the collective position and size of the reproductive tract and relate this score classification to fertility. Cows having reproductive tracts positioned more abdominally with thicker uterine horns and cervices exhibited a 15% decrease in conception rates compared to positions in the pelvic region with smaller uterine horns and cervices. Additionally, sums of cervical and uterine horn diameter measurements (TOTAL) confirmed a perceived size difference between SPS1 and SPS3 reproductive tracts. Furthermore, this study revealed the percentage of large reproductive tracts increased as parity increased; and the percentage of repeat breeders increased as reproductive tract size increased. Other studies have used position (LeBlanc et al., 2002; Scully et al., 2013) or size and position (Heppelmann et al., 2013) of the reproductive tract as a means to compliment methods of identifying uterine diseases; however, these classifications were not used in combination to evaluate subsequent fertility.

Definitive influences on reproductive tract position and size following the puerperium period are not clear in the research literature, and influential factors are likely to be complex and interlinked. Parity is one influence on the position and size of the reproductive tract identified during this study. Percentage of deeper and larger reproductive tracts represented as an SPS3 increased along with parity. Initially, this is not surprising considering these cows experienced pregnancy more frequently. Following the physically traumatic process of parturition several events must take place before pregnancy can be reestablished (Sheldon et al., 2008). Uterine involution is the initial step in the progression of the uterus returning to a non-gravid state; followed by regeneration of endometrium and clearance of bacterial contamination (Sheldon et al., 2008). Uterine involution and epithelial regeneration are thought to be completed by 25 days post-partum (Sheldon et al., 2008); however, these events can be negatively impacted by puerperium conditions such as metritis and endometritis (Heppelmann et al., 2013; Sheldon and Dobson, 2004), subclinical hypocalcaemia (Heppelmann et al., 2014; Kamgarpour et al., 1999), and negative energy balance (Swangchan-Uthai et al., 2013). These conditions are also interlinked many times; for example, cows with uterine disease can have a greater degree of negative energy balance (Galvão et al., 2010), and hypocalcaemia can be associated with uterine disease (Martinez et al., 2012).

Incidences of these conditions, whether alone or in combination, can be linked to higher incidence in multiparous cows. Greater incidences of subclinical endometritis and metritis have been associated with increased parity (Bonneville-Hebert et al., 2011). Incidences of hypocalcaemia have been shown to increase in multiparous cows (Sato et al., 2013). In addition Heppelmann et al. (2014) indicated a hypocalcaemic state resulted in lengthened uteri one to three weeks post-partum as a result of reduced contractility. Heppelmann et al. (2013) evaluated uterine involution by measuring uterine blood flow, using Doppler sonography, and evaluating size of the reproductive tract by transrectal palpation in lactating cows until 65 days in milk. A scale of 1 to 6 scale described by Grunert (1979) was used in which: uterus was retractable in scores 1 to 3 and uterine horn diameter were < 2 cm (score 1), 2 to 5 cm (score 2) or > 5 cm (score 3), uterus not retractable in scores 4 to 6 and greater curvature palpable (score

4), greater curvature not completely palpable (score 5) or uterus poorly outlined (score 6) (Heppelmann et al., 2013). Cows with uterine disease had larger uterine size until 18 days postpartum, and a trend reported for these cows to have larger uterine size until 65 days postpartum compared to cows without uterine disease (Heppelmann et al., 2013). Heppelmann et al. (2013) concluded uterine involution had been delayed in cows with uterine disease based on an increase in blood flow volume and decreased pulsatility index in uterine arteries of affected cows. It is not surprising that the current study revealed parity as a contributing factor to size of the reproductive tract considering a number of postpartum conditions can inhibit uterine involution, and increased incidences of these conditions have been linked to increased parity.

Some studies have associated increased parity with decreased fertility (Chebel et al., 2004; Herlihy et al., 2012; Kasimanickam et al., 2006; Souza et al., 2008), while others have observed no parity effect (Pursley et al., 2012; Stevenson and Phatak, 2010), making the relationship between parity and fertility complex and controversial. Potential factors that contribute to the complexity of parity effects on fertility include increased milk production (Lucy, 2001), potential alterations in steroid hormone concentrations due to increased milk production (Sangsritavong et al., 2002), increased risk of puerperium uterine disorders (Bonneville-Hebert et al., 2011; Gröhn and Rajala-Schultz, 2000; LeBlanc et al., 2002), and increased risk of repeat breeder syndrome (Bonneville-Hebert et al., 2011). This study indicates that position and size of the reproductive tract is an additional factor; however, the reason for position and size differences is also complex and not fully understood. Interestingly, cows in this study with increased parity (three or more) and smaller reproductive tracts (SPS1) had similar conception rates (40.6 ± 2.8%) to cows in parity one (41.7 ± 2.5) and two (41.3 ± 3.3%) with an SPS1, indicating size of the reproductive tract may have a greater effect on fertility than parity. This provides evidence that if the reproductive tract remains small and located within the pelvis

(SPS1), regardless of parity; conception rates will not suffer as greatly as cows with larger and more abdominally positioned reproductive tracts. We found only one study linking the size of the reproductive tract to parity (Souza et al., 2011); even though this link is completely logical. Furthermore, no clear link is established between the position and size of reproductive tracts approximately 30 DIM and the uterine environment regarding existing or previous subclinical infection.

It is widely accepted that rectal palpation used to identify uterine disorders alone during the postpartum period is a poor predictor of fertility because it is a subjective evaluation technique (LeBlanc et al., 2002), and tends to underestimate existing disorders (López-Helguera et al., 2012). While rectal palpation alone may not be an accurate predictor of subsequent fertility in the postpartum cow, it has been shown that larger cervices identified by rectal palpation in the postpartum period are related to poor fertility (LeBlanc et al., 2002). Cervices greater than 7.5 cm at 27 to 33 DIM have been associated with decreased fertility as an indication endometritis may exist in those cows (LeBlanc et al., 2002). The current study found significantly larger cervices in SPS3 cows compared to SPS1 using ultrasound diameter measurements. Differences in cervical evaluation techniques in the present study could explain why cervical diameter measurements (ultrasound) of 2.6 cm are much smaller than the > 7.5 cm (transrectal palpation) defined by LeBlanc et al. (2002). These larger cervices may be associated with a previous or existing uterine disorder considering larger cervices and purulent uterine discharge were found to be predictors of decreased fertility (LeBlanc et al., 2002). Cervical inflammation has been shown to reduce pregnancy rates at 300 DIM when identified less than 35 DIM (Deguillaume et al., 2012). Endometrial inflammation also existed in 75% of cows having cervical inflammation (Deguillaume et al., 2012). The works of LeBlanc et al. (2002) and Deguillaume et al. (2012) exhibit that larger or inflamed cervices are associated with endometritis in the puerperium period. Other authors have also found evidence of endometritis by means of endometrial cytology (Gilbert et al., 2005), classification of intrauterine fluid volume (Mateus et al., 2002), and ultrasonography (Mee et al., 2009) in cows 6 to 8 weeks postpartum. Unresolved endometritis at this stage of lactation retards uterine involution (Mateus et al., 2002) and negatively impacts first service pregnancy rates (Mee et al., 2009) and ovarian activity (LeBlanc et al., 2002; Mateus et al., 2002). Uterine disorders, including cervicitis and endometritis, that remained incompletely resolved at the time of a pre-breeding exam could compromise the uterine environment and likely be classified as an enlarged tract. Metritis resulted in enlarged diameter of the previously gravid uterine horn as late as four weeks post-partum (Heppelmann et al., 2014), this period corresponds to the period SPS assignment in the current study. Uterine size was determined to be greater in cows with uterine disease up to 18 days post-partum, and trend was reported for these cows to have enlarged uteri up to 65 days postpartum (Heppelmann et al., 2013). In the present study, cows with previous puerperium uterine disorders were not identified prior to SPS assignment; therefore no conclusions can made be regarding whether previous uterine disorders contributed to an increase in reproductive tract size. Cows thought to have existing uterine disorders at the time of SPS assignment were not scored until disorders were thought to be resolved. Nevertheless, enlarged cervices and uteri have been associated with uterine infections and this could possibly explain why SPS3 cows experienced decreased fertility.

Puerperium uterine disorders have been linked to increased parity (Bonneville-Hebert et al., 2011) and repeat breeder syndrome (Bonneville-Hebert et al., 2011; Salasel et al., 2010), this study provides evidence that percentage of repeat breeders increase as SPS increases. Repeat breeder cows have classically been defined as cows not pregnant following three inseminations, despite no clinically detected reproductive disorders (Yusuf et al., 2010). Cows in this study were not examined for reproductive disorders during transrectal palpation; therefore, cows not pregnant following three inseminations within a single lactation were considered repeat breeders (Bonneville-Hebert et al., 2011). An 18.3% overall occurrence of repeat breeder status in this study population is comparable to other reports ranging from 14 to 24% (Bartlett et al., 1986; Yusuf et al., 2010). Percentage of repeat breeders increased by eleven percent from an SPS1 to SPS3 (14.5% to 25.6% respectively); indicating an increasing likelihood cows with large reproductive tracts could become repeat breeders. Repeat breeder status may also be the cause of negative impacts on conception rates when cows were inseminated with increased DIM. Cows inseminated earlier than the mean DIM (DIMGRP1, mean=116) exhibited greater conception rates compared to cows inseminated later than mean DIM (DIMGRP2). In addition, 7.3% (51/698) of cows inseminated in DIMGRP1 were identified as repeat breeders, while 40.6% (139/342) inseminated in DIMGRP2 were repeat breeders.

Increased milking frequency (3X) had a negative impact on conception rates compared to 2X in the current study. Effects of increased milking frequency on fertility have been investigated extensively over the past three decades; however, a clear positive or negative effect has yet to be established. Negative effects of 3X milking on fertility have been associated with a decreased weight gain throughout a lactation (DePeters et al., 1985), and a hypothesized luteolytic effect of increased oxytocin release as a result of increased udder massage (García-Ispierto et al., 2007). Milking 3X is a management tool utilized by some dairy producers to increase daily milk yield, and has shown to increase production by 6 to 17% over entire lactations (Barnes et al., 1990; DePeters et al., 1985; Gisi et al., 1986; Smith et al., 2002). Questions regarding effects of 3X milking on fertility have been addressed by measuring fertility parameters such as: days to first service, inseminations per conception, days open and calving interval (Allen et al., 1986; Amos et al., 1985; Barnes et al., 1990; DePeters et al., 1985; Gisi et al., 1986; Smith et al., 2002). Studies have shown 3X milking to have no significant effect on inseminations per conception and days open (Amos et al., 1985; Barnes et al., 1990; Gisi et al., 1986), while others have reported increases in these reproductive parameters (DePeters et al., 1985; Smith et al., 2002). Days to first service in 3X cows have been reported to increase (DePeters et al., 1985), have no effect (Smith et al., 2002) and decrease (Allen et al., 1986). Results of some of these studies should be cautiously interpreted since some contained a limited number of cows, using 100 or fewer (Amos et al., 1985; Barnes et al., 1990; DePeters et al., 1985). Few studies have compared effects of milking frequency on conception (DePeters et al., 1985) or pregnancy rates (García-Ispierto et al., 2007), and only one found to examine a great number of inseminations (10,965 inseminations) (García-Ispierto et al., 2007). The current study demonstrates a significant negative effect on conception rates for cows milked 3X by analyzing 1979 inseminations, which agrees with one other large study (García-Ispierto et al., 2007). However, these results should be cautiously interpreted since milking frequency is associated with location in the present study. One of four locations milked 2X and resulted in a 45.4% conception rate, compared to the other three locations milking 3X with conception rates of 33.3%, 39.1%, and 24.3%. Furthermore, 2X milking inseminations only accounted for 14.8% of all inseminations analyzed. García-Ispierto et al. (2007) reported a similar scenario in which four herds were used and 2X inseminations only accounted for 11% of their study population. Without more balanced study populations regarding 2X and 3X milking, it is difficult to make a convincing argument that 3X actually decreases conception rates.

While mechanisms dictating differences in position and size of the reproductive tract remain unclear, collective efforts of this research indicate this SPS system can be used as a predictor of fertility in lactating dairy cows. Cows having reproductive tracts abdominally located outside the pelvic cavity with thicker uterine horns and cervices experienced a 15% decrease in conception rates when compared to cows with smaller reproductive tracts located within the pelvis. These cows can now be identified prior to entering a breeding program and allow producers to make the most informed decisions. Older cows with larger reproductive tracts can establish pregnancy as seen in this field trial (24.3%), but perhaps should not be pursued as aggressively as herd mates with smaller reproductive tracts. These cows may be candidates for fewer inseminations, less expensive semen, higher fertility semen, crossbreeding, or reproductive culling. Similar conception rates for all SPS1 cows regardless of parity also emphasizes the need for early identification and treatment of postpartum uterine disorders. Early identification and treatment could control the size of the reproductive tract, if uterine disorders prove to contribute to uterine position and size during a breeding period. Parity is a contributing factor to position and size of reproductive tracts; however, further investigation is needed in order to gain a better appreciation for other contributing factors. Examination of whether puerperal uterine disease or metabolic conditions affect reproductive tract SPS later in lactation is one step towards understanding potential contributing factors. Previous research has linked increased parity with increased occurrences of uterine disease (Bonneville-Hebert et al., 2011), hypocalcaemia (Sato et al., 2013), and greater negative energy balance (Galvão et al., 2010); however, a clear link of these disorders affecting reproductive tract SPS is not established. Examination of how reproductive tract SPS may change throughout lactation or the estrous cycle may also reveal mechanisms impacting position and size. Evidence exists that measurable differences of endometrial thickness in the time surrounding ovulation occur (Souza et al., 2011), indicating the estrous cycle could influence SPS. Enhanced understanding of underlying causes of reproductive tract position and size differences, as well as how an SPS changes over the course of a lactation can also lead to more efficient use of the SPS system and more efficient AI programs. The cause of why some reproductive tracts are larger than others is likely a complex issue that requires more research efforts to fully understand.

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CHAPTER IV: The efficacy of progesterone releasing intravaginal device prior to insemination during Double-Ovsynch synchronization on conception rates in lactating dairy cows during summer months

Abstract

The objective was to determine if inclusion of a controlled internal releasing device (CIDR) prior to insemination of a fixed timed AI protocol could improve conception rates in lactating dairy cows during periods of heat stress. Cows (n=517) underwent Double Ovsynch and were randomly allotted to treatment of inclusion or exclusion of a CIDR during the insemination Ovsynch portion of the protocol. Treatments were applied at the time of gonadotropin releasing hormone (GnRH) administration, followed by prostaglandin $F2_{\alpha}$ (PGF) 7days later and treatment removal. Final GnRH was administered 56 hours after PGF and cows were inseminated 16 to 20 hours later. The use of a CIDR (38.5 ± 3.6%) did not positively impact conception rates compared to NO CIDR (36.0 ± 3.5%; P = 0.6044). Parity had a significant effect on conception rate with first parity (50.9 ± 4.0%) cows having higher conception rates compared to third (32.9 ± 6.1%) and fourth or greater (25.5 ± 4.5%) parity (P = 0.0014). Temperature and humidity data were collected for each day of insemination, and no weather data impacted conception rates. Calving interval of cows not pregnant to first insemination was not decreased by the use of a CIDR in a previous estrous cycle. Cows receiving CIDR or NO CIDR had a calving intervals of 14.0 and 14.3 months respectively (P = 0.3403). Overall, the use of a CIDR during periods of heat stress did not improve conception rates; however, intriguing numerical results in primiparous cows may justify further investigation of this application.

Introduction

Fertility of lactating dairy cows declines during warm seasons, and these effects have shown to be more detrimental in the warm southeastern climate of the United States compared to the northeastern climate (Huang et al., 2008). Decreased estrus expression (Pennington et al., 1985) coupled with shorter duration of estrus during warm periods (Gwazdauskas et al., 1981) are contributing factors especially since estrus detection remains the predominant method of inseminating lactating cows (USDA, 2009). Use of fixed timed artificial insemination (AI) during heat stress improves in pregnancy rates over estrus detection by increasing submission rates (Cartmill et al., 2001; de la Sota et al., 1998), but is not sufficient at restoring fertility of cows exposed to heat stress. Mechanisms by which heat stress impacts fertility are dependent on severity and duration of exposure, and are multifactorial with effects on maternal environment and oocyte being problematic (as reviewed by Rensis and Scaramuzzi, 2003).

In circumstances of chronic heat stress a reduction in corpus luteum (CL) function may be problematic. Studies have demonstrated a reduction in progesterone concentrations during the luteal phase prior to insemination during periods of heat stress (Howell et al., 1994; Rosenberg et al., 1977). Progesterone concentrations were decreased by an average of 1.5 to 2.0 ng/mL 8 to 19 days prior to estrus (Rosenberg et al., 1977) and 2.5 ng/mL 6 to 18 days (Howell et al., 1994) prior to estrus during chronic heat stress exposure. This is concerning since high progesterone levels in the luteal phase leading up to ovulation are positively associated with conception (Fonseca et al., 1983; Martins et al., 2011). The use of controlled internal releasing device (CIDR) or progesterone releasing intravaginal device (PRID) have shown improvements in conception rates following fixed timed AI (Chebel et al., 2010; El-Zarkouny et al., 2004; Stevenson et al., 2006), since they may increase progesterone concentrations.

Overall it is not clear how heat stress may compound the challenge of maintaining an appropriate progesterone balance; however, evidence exists it may impact progesterone concentrations (Howell et al., 1994; Rosenberg et al., 1977). The fixed timed AI protocol Double Ovsynch (Souza et al., 2008) has shown to be beneficial during periods of heat stress compared to other protocols (Dirandeh et al., 2015), but its use with a CIDR during heat stress has not been highly explored. We hypothesized that first service conception rates would be improved during these warm periods with the inclusion of a CIDR during the insemination

Ovsynch portion of the Double Ovsynch protocol. Previous work supports this hypothesis as progesterone supplementation in combination with equine chorionic gonadotropin during fixed timed AI has improved conception rates comparable to estrus detection during cool seasons (Garcia-Ispierto et al., 2013). The objectives of this study were to investigate whether Double Ovsynch in combination with a CIDR prior to insemination would improve first service conception rates during periods of heat stress. Experimental design includes a direct comparison of inclusion or exclusion of a CIDR. The direct comparison of CIDR or no CIDR is unique, and this detail has precluded others from making sound conclusions regarding the direct effect of a CIDR during heat stress.

Materials and Methods

Experimental Design

This study was performed in a commercial herd in east Tennessee with all procedures performed with approval of the University of Tennessee IACUC. The study was conducted during the warm months of June through September over the course of two consecutive years in lactating Holstein cows. Cows were milked thrice daily, fed a corn silage based total mixed ration, and housed in a bedded pack barn with fans and sprinklers for heat abatement. This herd also has a history of good reproductive success. A previous study performed by our lab within this herd yielded conception rates of 35.2% over the course of two years during all seasons (Young et al., 2010).

Biweekly, cohorts of cows 39 to 52 days in milk (DIM) began a Double Ovsynch program (Souza et al., 2008). Cows were randomly assigned to one of two treatment groups, CIDR (EAZI-BREED CIDR, 1.38g progesterone; Zoetis, Florham Park, NJ) or NO CIDR. Treatments were applied beginning with the insemination Ovsynch portion of the protocol. During the treatment period, cows were administered gonadotropin releasing hormone (GnRH; 100µg i.m.; 2mL of Cystorelin, Merial Limited, Duluth, GA), prostaglandin $F2_{\alpha}$ (PGF; 25µg i.m.; 5mL of

Lutalyse, Zoetis, Florham Park, NJ) seven days later, and GnRH 56 hours after PGF with timed AI 16 to 20 hours after second GnRH. Cows assigned to the CIDR treatment had a CIDR inserted at the time of first GnRH administration and removed seven days later when PGF was administered. A schematic representation of the synchronization protocol is presented in Figure 5. All inseminations were first inseminations of the respective lactation. Pregnancy diagnosis was performed by herd veterinarians utilizing ultrasonography or transrectal palpation no earlier than 35 days post insemination.

Calving date, insemination date, DIM at insemination, parity, service sire, milk production and pregnancy status were recorded for each insemination. Information was retrieved from herd management computer software (Dairy Comp 305; Valley Agricultural Software, Tulare, CA, USA). Data regarding milk production were obtained within thirty days of insemination. Days in milk at insemination and milk production were divided into three groups (above 75^{th}, intermediate, and below 25^{th} percentile) using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Cary, NC, USA, 2013). Parity was divided into four groups to represent first, second, third and fourth or more parities. Six different Holstein service sires were utilized and balanced relatively equally among treatments. The calving interval of cows not pregnant to first insemination was investigated to reveal if any factors influenced later inseminations.

Weather data were obtained from a regional airport located 49.4 km from study location. Minimum (LOWTEMP) and maximum (HIGHTEMP) temperature and humidity (LOWHUMD, HIGHHUMD) measurements were recorded for each insemination date. The change in temperature (TEMPDIFF) and humidity (HUMDDIFF) for each insemination date was also obtained by calculating the difference between each high and low measurement. Maximum and minimum temperature humidity indices (THI) for each insemination date were calculated using

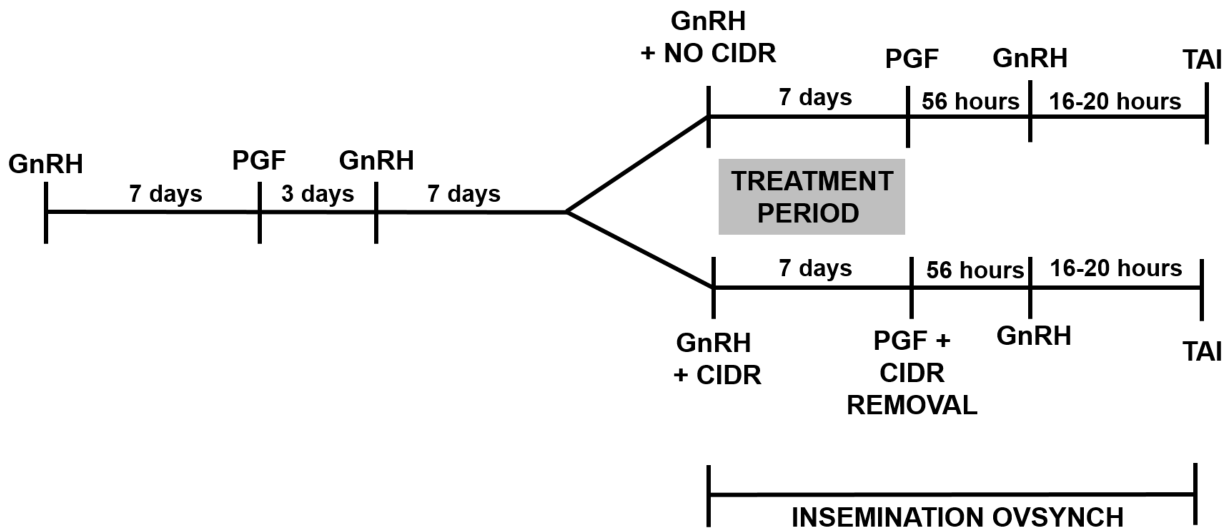

Figure 5: Heat stress synchronization study design and random treatment allotment. GnRH=Gonadotropin-releasing hormone, PGF=Prostaglandin F2α, CIDR=Controlled internal releasing device, TAI=Timed artificial insemination.

the following formula: $THI = T_{db}-[0.55-(0.55*RH)]*32$, where T_{db} is dry bulb temperature in ºF and RH is relative humidity expressed as a decimal (humidity/100) (NOAA, 1976). Maximum THI (MAXTHI) was calculated using maximum temperature (ºF) and minimum humidity, while minimum THI (MINTHI) was calculated using minimum temperature (ºF) and maximum humidity (West et al., 2003). All weather data, within each group, were sub-divided into three groups (above 75th, intermediate, and below 25th percentile) using the UNIVARIATE procedure in SAS 9.4 (SAS Institute, Cary, NC, USA, 2013) and values are represented in Table 4.

Statistical Analyses

Data were analyzed using SAS 9.4 (SAS Institute, Cary, NC, USA, 2013) using a mixed model ANOVA in Glimmix. In all analyses insemination served as the experimental unit, and pregnancy and calving interval were each dependent variables in separate analyses. A binomial distribution option was used for conception rate data, and log transformation was used to normalize calving interval data. Variables collectively included in the model as individual fixed effects to determine their effect on conception rate and calving interval included: service sire, DIM, MILK, YEAR of insemination, PARITY, LOWTEMP group, HIGHTEMP group, TEMPDIFF group, LOWHUMD group, HIGHHUMD group, HUMDDIFF group, MAXTHI group, and MINTHI group. Manual backwards selection was used to eliminate non-significant ($P \geq 0.10$) variables. The final model testing the effects on conception rate included fixed effects of treatment (CIDR/NO CIDR), PARITY, and MILK; with PARITY and treatment interacted and service sire as a random effect. The final model testing the effects on calving interval included fixed effect of MILK. Effects of each variable on each respective dependent variable were measured using Fisher's Least Significant Difference mean separation ($P < 0.05$) in the final model analysis.

Additional analyses were performed with each weather variable's extremes individually interacted with treatment (CIDR), and pregnancy as the dependent variable and service sire as

Table 4: Variable groups representing high, intermediate, and low values.

Variable	Group 1	Group 2	Group 3
DIM (days)	≥ 76	75-70	≤ 69
MILK (kg)	≥ 45.8	45.7-32.3	≤ 32.2
LOW TEMPERATURE (C)	≥ 21.7	21.6-17.3	≤ 17.2
HIGH TEMPERATURE (C)	≥ 33.9	33.8-26.8	≤ 26.7
TEMPERATURE DIFFERENCE (C)	≥ 12.8	12.7-7.9	≤ 7.8
LOW HUMIDITY (%)	≥ 63	62-36	≤ 35
HIGH HUMIDITY (%)	≥ 97	96-94	≤ 93
HUMIDITY DIFFERENCE (%)	≥ 57	56-32	≤ 31
MINIMUM THI	≥ 70.5	70.4-64	≤ 63
MAXIMUM THI	≥ 82	81-77	≤ 76

a random variable, using a mixed model ANOVA in Glimmix to evaluate each variable's individual effect on conception rate. Intermediate groups were eliminated from analyses to test extreme highs and lows effect on conception rate for each variable. Effects were measured using Fisher's Least Significant Difference mean separation ($P < 0.05$).

Results and Discussion

Inclusion of a CIDR before insemination during a Double Ovsynch fixed timed AI protocol (Figure 5) during warm summer months did not improve first service conception rates of 517 inseminations. Cows having a CIDR resulted in 38.5% first service conception rates, while cows without a CIDR resulted in 36.0% (P=0.6044). The present study is unique compared to others that have used progesterone supplementation during periods of heat stress and reported improved pregnancy rates at 120 DIM (Alnimer and Lubbadeh, 2003) and comparable conception rates to cool season inseminations (Garcia-Ispierto et al., 2013). The present study demonstrates a direct comparison of first service conception rates in which all inseminations were fixed timed AI during periods of heat stress, with or without a CIDR.

Alnimer and Lubbadeh (2003) used a PRID which contained 1.55g of progesterone compared to the 1.38g of progesterone in the CIDR used in the current study. Observation limitations existed, having only 20 cows per treatment; and not all cows underwent fixed timed AI (Alnimer and Lubbadeh, 2003). In fact, only one treatment group (n = 20) underwent fixed timed AI while others were inseminated following estrus detection. This experimental design resulted in an increase of in AI submission rate for those that underwent fixed timed AI, and also could have affected the final conclusions (Alnimer and Lubbadeh, 2003). Conclusions were that the use of a PRID and fixed timed AI was beneficial during summer months by increasing pregnancy rates at 120 DIM and decreasing days open (Alnimer and Lubbadeh, 2003). This conclusion is difficult to ascertain considering fixed timed AI alone has increased AI submission rate and pregnancy rate in heat stress cows compared to estrus detection (Cartmill et al., 2001).

Garcia-Ispierto et al. (2013) concluded the use of a five day PRID with fixed timed AI during heat stress achieved similar conception rates to cows inseminated following estrus detection in cool periods; however, no direct comparison was made with or without a PRID during heat stress. This study also had limitations with observations with only 120 cows inseminated during the warm period five day PRID, while a comparison of estrus detected inseminations during cool periods resulted in 1635 observations (Garcia-Ispierto et al., 2013). Cows enrolled for fixed timed AI during this study (Garcia-Ispierto et al., 2013) were those that had not exhibited estrus within a 21 day period, indicating these cows could have been in a state of anestrous or compromised in some way. Inseminations during this study were also performed on cows multiple times, whereas the present study only included first service. A comparison of the current study with previous studies (Alnimer and Lubbadeh, 2003; Garcia-Ispierto et al., 2013) reveals novelty by carrying out a direct comparison of cows (n = 517) under the same environmental conditions, exposed to the same synchronization protocol and all submitted to fixed timed AI for first insemination.

Double Ovsynch was the protocol of choice in the current experimental design because it has shown to be beneficial during heat stress compared to other fixed timed AI protocols (Dirandeh et al., 2015). Greater ovulation rates to the GnRH of the insemination Ovsynch, and increase synchronization and conception rates during heat stress are benefits seen with Double Ovsynch (Dirandeh et al., 2015). This aspect of our experimental design may be a reason we detected no differences with a CIDR. Maximum synchronization and fertility may have been achieved with the use of Double Ovsynch; therefore the CIDR was not able to improve first service conception rates to a statistically significant level. Additionally, induction of an accessory corpus luteum during the final Ovsynch may have increased progesterone levels such that no benefit of a CIDR could be detected (Bello et al., 2006). The use of CIDRs to improve fertility in lactating cows have yielded conditional results. Cows without a CL at initiation of Ovsynch have shown improvement when a CIDR was used (Bisinotto et al., 2015a), as well as cows with low serum progesterone levels prior to PGF administration of an Ovsynch protocol (Stevenson et al., 2006). A large portion of cows fitting these criteria are considered to be anovular, thus the improvement in fertility with a CIDR may be only with anovular cows. Cows having a CL at initiation of Ovsynch and believed to be cyclic have not shown improvements in fertility with the use of a CIDR (Bisinotto et al., 2015b). Heat stress periods can increase incidences of anestrous (as reviewed by Rensis and Scaramuzzi, 2003) and decrease progesterone during diestrus (Howell et al., 1994; Rosenberg et al., 1977); therefore it was reasonable to hypothesize incorporation of a CIDR during periods of heat stress would increase fertility in lactating cows.

Parity effected first service conception rates the greatest in the current study, and conception rates decreased incrementally as parity increased (Table 5). First parity conception rates were greater than third and four or more, and second parity conception rates were greater than four or more. These results are not surprising considering many studies have shown primiparous cows to have better reproductive success (Chebel et al., 2004; Herlihy et al., 2012; Kasimanickam et al., 2006; Souza et al., 2008). Primiparous cows are likely to experience a less severe negative energy balance postpartum due to higher IGF-1 and insulin levels (Wathes et al., 2007); however, energy demands for growth and milk production require an adequate body condition score at parturition for subsequent reproductive success. The energy demands associated with increased milk production seen in multiparous cows may be the reason for decreased fertility in these cows. Cows under greater pressure to achieve high production levels, they have been genetically selected for, are more likely to have a larger negative energy balance. This more severe negative energy state results in decreased reproductive efficiency by delaying first ovulation and extending days open (as reviewed by Butler, 2003). This negative energy state and higher milk production can result in lower progesterone levels near

Table 5: First service conception rates of each parity group and interacted with treatment.

	First Service Conception Rates (%)		
Parity Group	**Overall (n)**	**CIDR (n)**	**NO CIDR (n)**
Parity 1	50.9 ± 4.0 (196) [a]	54.1 ± 5.3 (100)	47.6 ± 5.3 (96)
Parity 2	41.7 ± 4.3 (150) [ab]	42.4 ± 5.9 (76)	41.1 ± 5.9 (74)
Parity 3	32.9 ± 6.1 (66) [bc]	36.0 ± 8.7 (32)	30.0 ± 8.1 (34)
Parity 4+	25.5 ± 4.5 (105) [c]	24.1 ± 6.1 (56)	27.0 ± 6.4 (49)

[a, b, c] Different superscripts within column indicate differences ($P = 0.0014$)

the time of first insemination (Villa-Godoy et al., 1988) and may be caused by a higher hepatic metabolic clearance of steroid hormones (Sangsritavong et al., 2002).

With a combination of greater milk production and possibly lower serum progesterone levels in multiparous cows, one would expect a benefit from inclusion of a CIDR. In addition, all inseminations analyzed were first inseminations occurring 59 to 84 DIM; a period corresponding to peak milk production; however, we found no significant interaction of CIDR and parity (P = 0.8695). The largest non-significant numerical variation of 6.5% in first service conception rates existed between primiparous cows with those having a CIDR resulting in 54.1% compared to those with NO CIDR resulting in 47.6% (P = 0.3649). A non-significant numerical variation of 6.0% (P = 0.6070) was observed in cows in the third parity; however observation limitations were greatest in this parity group. These non-significant numerical diversities are comparable to benefits seen with a CIDR in a previous study (Chebel et al., 2010). A significant increase of 4.8% conception rate with the use of a CIDR incorporated with fixed timed AI was reported during non-specified seasons (Chebel et al., 2010); while another study reported a non-significant increase in conception rate of 12.4% with a PRID and limited observations during periods of heat stress (Alnimer and Lubbadeh, 2003).

In the present study inclusion of a CIDR during periods of heat stress had no effect on calving interval for those cows not pregnant to first insemination (n = 240). Reports have established that as many as 30 to 41% of cows in a herd can be in a state of anestrous by 65 DIM (Santos et al., 2009; Stevenson et al., 2006), corresponding to the breeding period in this study. Estrous cycles have been induced in previously anestrous cows with a CIDR, with no effect on fertility (Cerri et al., 2009). The cyclic status of the study population used was unknown; therefore, it was logical that the use of a CIDR may influence calving interval. If the CIDR induced cyclicity in anestrous cows, the following cycle could be more fertile. Nevertheless, the calving intervals for cows receiving a CIDR compared to NO CIDR in the current study was 14.0 and 14.3 months, respectively (P = 0.3403), a difference of approximately nine days. Milk production had a tendency (P = 0.0737) to influence conception rates with high producing cows (≥ 45.8 kg; 39.5 ± 4.4%) having similar conception rates with the low producing cows (≤ 32.2 kg; 41.84 ± 5.3%); while the intermediate production group (45.7 to 32.3 kg) suffered the lowest conception rates (30.9 ± 3.1%). Milk production affected calving interval with lower producing cows having an interval of 13.6 months; while high and intermediate producers were 14.5 and 14.3 months respectfully (P = 0.0573). These results did not appear to be influenced by parity as first parity cows were evenly distributed amongst intermediate (n = 50) and low production (n = 44).

Manual backward selection of all variables having an effect on conception rate revealed no weather variables impacted conception rate; therefore, none were included in the final statistical model. Each variable's extreme values were analyzed individually as well, with intermediate groups eliminated, and interacted with treatment (CIDR) to test each variable's extremes influence on conception rate. Cows inseminated on days in which high humidity group was ≥ 97% had lower conception rates compared to when high humidity group measured ≤ 93% with conception rates of 27.9 ± 6.0% (n = 166) and 42.5 ± 6.2% (n = 312), respectively (P = 0.0043). Cows inseminated on days in which low humidity group was ≥ 63% had lower conception rates compared to when low humidity group measured ≤ 35% with conception rates of 31.9 ± 3.6 (n = 166) and 44.8 ± 4.4% (n = 131), respectively (P = 0.0242). Low THI group measurements demonstrated a strong tendency to impact conception rates. Cows inseminated when low THI group measured ≤ 63 (n = 153) had higher conception rates compared to when low THI group measured ≥ 70.5 (n = 110) with conception rates of 43.3 ± 5.9% and 32.0 ± 5.8%, respectively (P = 0.0704). Results of high humidity having a negative effect and low THI and low humidity having a positive effect on conception rates are in agreement with other studies (Ingraham et al., 1974; Rocha et al., 1998). Temperature, high THI and differences in temperature and humidity did not affect conception rates. Treatment with a CIDR did not improve conception rates when humidity was high or with any other weather variable. Service sire exhibited a tendency to effect first service conception rates (P = 0.0689), mainly due to one sire resulting in a conception rate of 14.9% while all others were greater than 37.8%.

Implications

While the current study did not report statistically significant improvement with a CIDR, a noticeable numerical variation of 6.5% was seen in primiparous cows. Multiparous cows did not show similar numerical improvements. The expense of using a CIDR is another matter to consider for producers. This device is likely the most expensive application in any synchronization protocol, and its use has not shown great improvements. If a producer choses to utilize a CIDR improve conception rates it is likely the greatest benefit would be seen in first parity cows, and use of a CIDR in multiparous cows is not likely to generate positive results that overcome the expense of the CIDR. The information presented here encourages further investigation with the application of a CIDR during heat stress in primiparous cows in multiple herds.

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CHAPTER V: Discussion and Implications

The challenges of fertility in lactating dairy cows are great, but continued progress is essential if an abundant milk supply is to continue. High milk production has historically been attributed to decreases in reproductive performance; however, many factors contribute and work collaboratively to decrease fertility. Early lactation factors include the challenges of uterine health postpartum as well as high energy demands for milk production which lead to a negative energy state and loss of body condition. Each of these early lactation challenges can lead to decreased reproductive performance in part by delaying resumption to normal cyclicity (as reviewed by Walsh et al., 2011). Fertility in lactating dairy cows is also seasonal, as dramatic decreases are seen during periods of heat stress. These periods lead to reductions of 20 to 30% compared to cooler periods, and have been attributed to a multitude of factors including alterations in: estrus expression and intensity, hormonal profiles, and follicular dynamics (as reviewed by Rensis and Scaramuzzi, 2003). Fertility improvements have been made in the last decade and have been credited to the adoption of synchronization protocols that allow for a concentrated period for estrus detection or fixed timed artificial insemination (AI) (Norman et al., 2009). Research efforts must continue to focus on all aspects that contribute to fertility so producers can successfully and economically manage their reproductive programs while still maintaining a high level of milk production. Our research efforts focused on two approaches to improve the reproductive efficiency and performance in lactating dairy cows. A scoring system that reflected the size and position of the reproductive tract was developed and field tested in an effort to identify cows with potentially lower fertility. This system proved effective, and found that cows with larger and abdominally positioned reproductive tracts did have lower conception rates compared to smaller reproductive tracts positioned within the pelvis. Next, a modification of the Double Ovsynch protocol to include progesterone supplementation during periods of heat stress was tested to investigate potential benefits on conception rates. Incorporation of supplemental progesterone during the insemination Ovsynch of a Double Ovsynch protocol during periods of heat stress did not significantly improve first service conception rates in lactating dairy cows, but encouraging numerical improvements were noted for first lactation cows. Overall both research efforts have provided information that can be used by producers immediately, and hopefully have a positive economic impact on their reproductive management program.

The size and position score (SPS) system resulted in an evaluative tool that can identify lactating cows with decreased fertility. Our SPS considers the diameter of the cervix and uterine horns, as well as the position of the reproductive tract equally. Abdominally positioned reproductive tracts with large diameter cervices and uterine horns are SPS3, while small diameter uterine horns and positioned within the pelvis are SPS1. Cows assigned a SPS2 have diameters and positions intermediate to SPS1 and SPS3. In our study population, cows with SPS1, SPS2 and SPS3 resulted in conception rates of 43.3 ± 3.7%, 36.7 ± 3.6% and 27.7 ± 4.3% respectively, with 1979 inseminations evaluated over four herds. Each decrease in conception rate for each respective increase in SPS was significantly different. Size and position score also increased as parity increased, which is not surprising considering these cows have undergone parturition more often. Surprisingly we were unable to show a difference in lumen volume between the three different SPS. This leaves questions unanswered as to why differences in fertility exist between the three SPS. One great advantage of this SPS system that we specifically targeted was the lack of expertise required by the evaluator to be beneficial. Dairy producers and practitioners alike with palpation skills required for AI can use this evaluative tool to benefit their reproductive programs.

Use of a CIDR to increase progesterone levels prior to AI during heat stress was a logical application considering progesterone has been shown to be decreased during the diestrual period when natural and chronic heat stressors were applied (Howell et al., 1994; Rosenberg et al., 1977). A CIDR was either included or excluded during the insemination Ovsynch portion of the Double Ovsynch protocol. Overall the inclusion of a CIDR prior to fixed timed AI did not improve first service conception rates with 517 inseminations evaluated. Cows with a CIDR resulted in a conception rate of 38.5%, while those without resulted in 36.0%. In addition, cows not pregnant to first insemination saw no benefit of a CIDR in subsequent inseminations as measured by calving interval. This study was performed in a single herd with historically good reproductive performance, and this fact may be a reason we saw no benefit with a CIDR. Also, previous studies have not found great benefits with the use of CIDR in non-specified seasons (Chebel et al., 2010). Perhaps observation numbers limited our ability to statistically detect improvements.

The current study is the first known study to classify size and position during a pre-breeding exam as a means of identifying cows with decreased fertility, and not for diagnosing uterine disease. Others have associated increased endometrial thickness, based on ultrasonography measurements, around the time of ovulation with improved conception rates (Souza et al., 2011). However, we specifically targeted animals not known to be in estrus for evaluation, and our system does not require ultrasonography expertise to detect millimeter differences in endometrial thickness. Use of the SPS system to identify cows with lower fertility should easily allow a reproductive program to be more economically efficient. Cows assigned a SPS score of 3 should be inseminated with inexpensive semen. Producers may also elect not to expose these cows to as many inseminations. More than half (56%) of producers will artificially inseminate cows five times or more before they take a different approach to establish pregnancy, and artificial insemination is used more than 70% of the time to establish pregnancy (USDA, 2009). A fictional economic scenario helps explain how this SPS system can reduce insemination costs if applied with an approach of using less expensive semen and fewer inseminations. Our population surveyed resulted in 51.1% SPS1, 40% SPS2 and 8.9% SPS3. If we consider 100 cows all inseminated a maximum of five times with semen costs of twenty dollars per unit, we will spend $10,000. If we decrease the number of inseminations of SPS2 cows to four and use semen at fifteen dollars per unit we can save $1,600 on those 40 cows. If we decrease the number of inseminations of SPS3 cows to three and use semen at ten dollars per unit we can save $270 on those nine cows. A total savings of $2,230 on those 100 cows, or 22.3% of our initial cost. This scenario still gives all cows a good opportunity to establish pregnancy, but uses the SPS information to an economic advantage. Another approach to use the SPS information, is identification of cows with higher fertility in which sex sorted semen may work better. Sex sorted semen has been commercially available since 2005, but is currently only recommended in heifers due to the product's decreased fertility capacity. Use of sex sorted semen in lactating cows has been estimated at 3.5% (USDA, 2009) and 0.4% (Norman et al., 2010), indicating some producers wish to use this technology in lactating cows. Application of SPS to identify the best candidates may improve the reported 14% decrease in conception rates with sex sorted semen compared to conventional in greater than half the cows projected to be SPS1 (Karakaya et al., 2014). Future research efforts with the SPS system may include a direct comparison of SPS1 cows with sex sorted or conventional semen. This data could benefit those producers that want to use sex sorted semen in cows and those which are expanding their herd from within. Overall, this research has the immediate potential to allow dairy producers to make more economically sound decisions in their reproductive programs; however, further research with this system is needed to expand on why size and positions are different.

Future research efforts with the SPS system initially should include an agreement and repeatability study. We need to be confident that this system can be easily taught to producers and practitioners, and SPS assignments are repeatable between palpators. The current study was not designed to test agreement and repeatability. One obstacle with this study was the different number of evaluators (n = 12) required to accomplish our goals over four herds. In addition, some herds were evaluated by multiple evaluators; while others only had one evaluator. A similar experimental approach to validate the repeatability and accuracy of the heifer reproductive tract scoring system (Rosenkrans and Hardin, 2003) is an appropriate place to begin. Rosenkrans and Hardin (2003) compared evaluations of two practitioners of different transrectal palpation skill levels. Evaluations were performed on the same 29 animals to test agreement within and between practitioners, and analyzed using a multicategory Kappa test which describes agreement beyond chance. Repeatability validation with our SPS system should involve more cows, multiple herds, and more than two evaluators. The experimental design should include at least two evaluators for each individual to compare their agreement over many observations. Performing this evaluation in multiple herds could also reveal tendencies within herds and may lead to information regarding why reproductive tracts vary in size and position. Validation of this system regarding repeatability is important in order to facilitate it's adoption, considering transrectal palpation evaluation is traditionally viewed as poor predictive tool due to its subjective nature (LeBlanc et al., 2002). We speculate one reason reproductive tracts are different in size and position could be temporary or permanent changes brought on by postpartum uterine disorders. Inflammation during metritis or endometritis could result in irreversible damage to the endometrium allowing for a larger reproductive tract and potentially decreased fertility. The current study did not evaluate postpartum uterine conditions in comparison to SPS later in lactation. Previous studies evaluating the size and position of the reproductive tract as an indicator of endometritis (LeBlanc et al., 2002) or uterine involution (Scully et al., 2013) did not assess cows beyond 50 days in milk. Correlating postpartum disorders such as retained fetal membranes, hypocalcemia and metritis with different SPS during the breeding season could help explain why differences exist. Monitoring SPS throughout lactation to investigate if or when changes occur could also provide information as to why differences exist. Increased parity is clearly a component of differences in SPS, but does not explain everything since parity 3+ cows with an SPS1 had similar conception rates to first and second parity SPS1 cows in the current study.

Incorporation of supplemental progesterone in a Double Ovsynch protocol during periods of heat stress did not prove to improve first service conception rates in lactating dairy cows. This study was novel compared to others including a CIDR during periods heat stress in that only the first service was evaluated and a direct comparison using only fixed timed AI was utilized. Other studies exhibited positive results, but only when fixed timed AI was compared to estrus detection (Alnimer and Lubbadeh, 2003; Garcia-Ispierto et al., 2013). While the current study did not yield the results hypothesized, it is still informative for the producer seeking methods to improve upon poor fertility during warm summer months. Inclusion of a CIDR in any protocol will likely be the most expensive single component, at an approximate rate of ten dollars each. It is estimated that one third of dairy producers have used a CIDR to improve synchrony of cows or heifers (USDA, 2009), indicating producers are willing to incur added expense if they believe it may improve fertility. The CIDR has been shown to induce estrous in anestrous cows (Cerri et al., 2009) and improve their fertility (Bisinotto et al., 2015a); however, similar improvements have not been seen in cycling cows (Bisinotto et al., 2015b). Positive results with a CIDR in lactating cows may mislead producers to utilize this tool to improve fertility during heat stress, a period when fertility is greatly reduced. Our data suggests this practice is not beneficial, and producers would be incurring unnecessary expenses. Use of supplemental progesterone during periods of heat stress may still have promise to improve fertility, but it may require more observations within multiple herds. One disadvantage of the current study was it was performed in a single herd which historically has good reproductive performance. A continuation of this work with multiple herds would allow inclusion of different management practices, which better represents lactating cows in the US population.

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VITA

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