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Efficacy of Extending the Voluntary Waiting Period in Lactating Dairy Cows to Improve Fertility of Sex-Sorted Semen

Sarah E. Orr
University of Tennessee - Knoxville, sorr6@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Sarah E. Orr entitled "Efficacy of Extending the Voluntary Waiting Period in Lactating Dairy Cows to Improve Fertility of Sex-Sorted Semen." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

J. Lannett Edwards, Major Professor

We have read this thesis and recommend its acceptance:

F. Neal Schrick, Justin D. Rhinehart

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Efficacy of Extending the Voluntary Waiting Period in Lactating Dairy Cows to Improve Fertility of Sex-Sorted Semen

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DEDICATION

I would like to dedicate this thesis to my family and friends. I could not have done this without your love, support, and encouragement!

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ABSTRACT

The objective of this study was to compare fertility of sex-sorted semen in lactating cows using a voluntary waiting period (**VWP**) of approximately 55 days (**VWP55**) or 85 days (**VWP85**). At 21 days in milk (**DIM**), cows were randomly assigned to begin estrus synchronization at 55 or 85 DIM. Only cows confirmed cyclic by 55 DIM were synchronized. At 55 or 85 DIM, cows [VWP55 (n=44); VWP85 (n=45)] having a corpus luteum (**CL**) were administered $PGF_{2\alpha}$ [two alpha]. Estrus was monitored continuously by the HeatWatch® [registered sign] system (**HW**) and by twice daily visual observation seven days following each $PGF_{2\alpha}$. Cows exhibiting estrus were artificially inseminated with sex-sorted Holstein semen using one of two different bulls having similar sire conception rates. After 14 days, $PGF_{2\alpha}$ was administered to any cow that did not exhibit estrus. Cows not responding to either $PGF_{2\alpha}$ administration were administered GnRH and a controlled intravaginal drug releasing device (**CIDR**). Seven days later, CIDR was removed and $PGF_{2\alpha}$ was administered. Pregnancy determination occurred 28 to 35 days after AI and was monitored at 60, 80, and 150 days. No difference in overall percentage of cows expressing estrus (79.55% and 77.78%, P = 0.8393), hours to estrus following either the first or all $PGF_{2\alpha}$ administrations (65.79 h and 72.65 h, P = 0.4908; 62.28 h and 73.00 h, P = 0.2218, respectively), number of mounts (9.05 and 10.54, P = 0.5246), average mount duration (4.59 s and 4.99 s, P = 0.2602), and total estrus length (8.16 h and 9.17 h, P = 0.6389) was observed between VWP55 and VWP85 cows. Conception rates 30 days after AI were significantly higher for VWP85 cows compared to VWP55 (51.43% vs. 22.86%, respectively; P = 0.0181). Conception rates were monitored at 60 (48.57% vs. 20.00%, respectively; P = 0.0166), 80 (42.86% vs. 20.00%, respectively; P = 0.0471), and 150 days after AI (42.86% vs. 20.00%, respectively; P = 0.0471) to monitor embryonic loss. Fertility of sex-

sorted semen in lactating cows can be improved after a VWP of 85 days under specific management conditions.

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CHAPTER 1

INTRODUCTION

Sex-sorted semen is a valuable tool for the dairy industry to increase the number of heifer calves available. Fertility of sex-sorted semen is lower than conventional semen. For instance, in dairy heifers, fertility of sex-sorted semen is on average 30% lower (range: +14% to -77%) than conventional semen (DeJarnette et al., 2008; Seidel and Schenk, 2008; DeJarnette et al., 2009; Schenk et al., 2009; DeJarnette et al., 2010; Norman et al., 2010; DeJarnette et al., 2011; Healy et al., 2013). In lactating dairy cows, fertility of sex-sorted semen is reduced by 26% (range: 0% to -54%) compared to conventional semen (Andersson et al., 2006; DeJarnette et al., 2008; Schenk et al., 2009; DeJarnette et al., 2010; Norman et al., 2010; Karakaya et al., 2014). This is compounded by conventional semen conception rates that are only approximately 32% in lactating dairy cows (Norman et al., 2009; Schefers et al., 2010; Wiltbank and Pursley, 2014), resulting in many recommendations that sex-sorted semen usage be limited to dairy heifers (DeJarnette et al., 2009; Norman et al., 2010; Healy et al., 2013). Multiple studies have evaluated methods to address the fertility deficit associated with sex-sorted semen including altered uterine deposition sites (Seidel and Schenk, 2008), sperm cell concentrations (DeJarnette et al., 2008), sorting and storage processes (Schenk et al., 2009), timing of insemination (Sales et al., 2011), and integration with timed AI (Sá Filho et al., 2013), yielding only marginal, if any, increases in conception rates for lactating cows.

A critical component of dairy breeding strategies that impacts fertility is the length of the voluntary waiting period (**VWP**), which is a defined time period after calving that producers elect to wait before efforts to inseminate those cows begin. Waiting a defined time period after calving is critical to allow a cow adequate time to go through uterine involution, recover from any postpartum health issues, and resume ovarian cyclicity. Many studies have reported benefits of waiting to inseminate cows until later in lactation. Summarizing seven studies, Britt (1975) reported that fertility was 25% for cows inseminated in the first 20 days after calving compared to greater than 60% for cows inseminated after 70 days postpartum. One study demonstrated improved conception rates in cows bred for the first time after 50 days in milk (**DIM**) compared to those bred before 50 DIM (Hillers et al., 1984). Others found that cows inseminated ≥74 DIM had higher fertility than cows inseminated earlier in lactation (Whitmore et al., 1974). Tenhagen et al. (2003) indicated that Holstein cows submitted for timed insemination using conventional semen after an OvSynch protocol had increased first service conception rates as the stage of lactation progressed from 53 to 59 DIM, to 73 to 81 DIM, and 94 to 102 DIM. Stevenson and Phatak (2005) noted improvements in pregnancy rate as DIM increased in cows that were originally synchronized for timed insemination but were inseminated following standing estrus before timed insemination.

Despite obvious improvements of inseminating cows with conventional semen later in lactation (Whitmore et al., 1974; Britt, 1975; Hillers et al., 1984; Weller and Folman, 1990; Tenhagen et al., 2003; Stevenson and Phatak, 2005; Caraviello et al., 2006; Schefers et al., 2010), two producer surveys of 103 and 673 US herds report current practices that employ an average VWP of 52 ± 1.3 and 56 ± 0.6 days, respectively (Caraviello et al., 2006; DeJarnette et al., 2007). Because fertility of conventional semen increases when cows are inseminated later in lactation, we hypothesized that extending the VWP beyond 55 DIM would be beneficial for improving fertility for lactating cows inseminated with sex-sorted semen. In support of this, Schenk et al. (2009) retrospectively found that lactating dairy cows inseminated between 84 to 98 DIM had increased pregnancy rates compared to inseminations earlier in lactation. Thus, the objective of this study was to directly compare the fertility of sex-sorted semen in lactating cows using a VWP of approximately 55 days or a VWP of 85 days.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The following is a review of the pertinent scientific literature describing the factors contributing to the current fertility status of lactating dairy cows. Use of sex-sorted semen in both dairy heifers and lactating cows will be discussed as well as the impact of the voluntary waiting period on fertility. Finally, economic considerations for both sex-sorted semen and extended VWPs will be reviewed.

Increasing World Population and the Need for Improved Dairy Cow Fertility

In 2011, it was estimated that world population would reach 9.15 billion people by the year 2050 (FAO, 2011). With that estimate, production of dairy products needs to increase by more than 121% to accommodate the projected consumption (FAO, 2011). More current estimates by the United Nations suggest that world population will be even greater—9.7 billion people—by 2050, further attesting to the need for more dairy products to feed a growing world (United Nations, 2015).

To this end, US milk production has increased by 1.3% yearly in recent years and is likely to continue at this rate into the future (Santos et al., 2010). More milk produced per cow translates into fewer cows needed to produce an equivalent amount of milk. It has been suggested that 11.1% fewer cows will be needed to meet milk production requirements in 40 years (Santos et al., 2010). Increasing milk production per cow however does not come without a cost. Genetic selection for increased milk production over the past five decades is one of many factors that have been coincident with reduced fertility of lactating dairy cows (reviewed by Lucy, 2001). Because pregnancy and subsequent parturition are required for initiating lactation, efforts to improve fertility of the dairy cow are important to efficiently meet the production needs of the future.

Declining Fertility of Lactating Dairy Cows

Dairy cow fertility worldwide has declined dramatically since the 1950s (reviewed by Lucy, 2001; Washburn et al., 2002). In 1998, after reviewing the literature, Butler determined that conception rates of lactating dairy cows had decreased from approximately 65% in 1951 to only 40% in 1996 for New York dairy herds (Butler, 1998). From 1975 to 1997, the decline was estimated to be at a rate of 0.5% per year (Beam and Butler, 1999). Increased services per conception and average conception rates of less than 40% have led to a decline in calving rate of approximately one percent per year (Royal et al., 2000b). Reduced fertility results in decreased genetic gain within the herd (Hohenboken, 1999; reviewed by Lucy, 2001; Seidel, 2007; McCullock et al., 2013) and fewer replacement females. These negative consequences result in decreased profitability for the producer (Royal et al., 2000b; McCullock et al., 2013).

Recent evidence from analysis of nearly 20 million inseminations from Dairy Herd Improvement breeding records obtained since 1995 demonstrated that first service conception rates for Holstein cows have begun to improve (Norman et al., 2009). According to Norman et al. (2009), the average first service conception rate for lactating Holsteins rose from 27% to 32% between 2001 and 2007 and has been attributed to the adoption of estrus synchronization or timed artificial insemination protocols. Although fertility in lactating cows is improving, it is still at suboptimal levels, which emphasizes the need for additional work toward improvement.

Milk Production and Calving Related Challenges in Dairy Cows

Numerous reports in the literature have associated decreased fertility with increased milk production. Rolling herd average milk production rose from approximately 6,500 kg per lactation in the 1970s to nearly 9,000 kg per lactation in the late 1990s (reviewed by Lucy, 2001). Corresponding reductions in conception rates, increased calving intervals, and increased services per conception suggest an antagonistic relationship between milk production and fertility (reviewed by Lucy, 2001). In support of this notion, Dhaliwal et al. (1996) determined that high yielding cows required 0.42 more services and needed an additional 25 days to conceive when inseminations began before 80 DIM. Nevertheless, others have reported weak or no impact of milk yield on fertility (Darwash et al., 1997; Loeffler et al., 1999; Gröhn and Rajala-Schultz, 2000).

Many other factors may also negatively influence fertility. Dystocia increased the number of services required for conception, interval to first estrus, interval to first service, days open, and calving interval compared to cows having normal parturitions (Dematawena and Berger, 1997; Gaafar et al., 2011). Furthermore, cows experiencing severe cases of dystocia have reduced conception rates by 200 DIM compared to cows experiencing mild cases of dystocia or having a normal parturition (Tenhagen et al., 2007). Other calving-related issues such as twinning also affect fertility (Roche, 2006). After parturition, uterine involution must occur, whereby the uterus regresses in size and expels fetal membranes and associated tissue attachments. Uterine involution typically occurs by 30 to 40 DIM (Okano and Tomizuka, 1987). However, the uterus may not be capable of supporting a new pregnancy until 60 or more DIM (Roche, 2006). Bacterial contamination of the uterus after calving is a common occurrence. If the immune response is delayed, other postpartum related disorders such as retained placenta, metritis and endometritis may reduce fertility (Thatcher et al., 2006). Furthermore, subclinical and clinical mastitis early in lactation have been reported to negatively impact fertility by increasing services per conception and days open (Barker et al., 1998; Schrick et al., 2001).

Negative Energy Balance

The transition period from gestation to early lactation requires a high level of nutrition to meet increased energy demands for milk production in early lactation. Feed intake must increase to partially accommodate the partitioning of nutrients for lactation as well as maintenance requirements with the remaining energy needs being met by mobilization of fat tissue (Bauman and Currie, 1980; Wathes et al., 2007; Roche et al., 2009). Decreased feed intake and fat mobilization prepartum and early postpartum often cause high producing cows to experience an energy deficit, known as negative energy balance [**NEB;** (Bauman and Currie, 1980)]. Negative energy balance typically extends from approximately two weeks before calving to 8 to 12 weeks postpartum and can lead to negative effects lasting into the breeding period (Butler, 2003, 2005).

Negative energy balance has adverse effects on reproductive functions and fertility (Butler, 2003; Wathes et al., 2007). For instance, cows in NEB may have altered luteinizing hormone pulse frequency and reduced glucose, insulin, and insulin-like growth factor I levels which contributes to increased length of anovulation (Butler, 2005; Wathes et al., 2007). These alterations may lead to reduced estradiol synthesis which slows follicular growth and extends time to first postpartum ovulation (Butler, 2003; Wathes et al., 2007). Negative energy balance also causes the liver of the cow to metabolize steroid hormones at an increased rate, resulting in lower circulating levels of estradiol and progesterone which further reduces the likelihood of ovulation (Butler, 2003; Wiltbank et al., 2006). Furthermore, mobilization of body tissues for energy results in the production of non-esterified fatty acids and beta-hydroxybutyrate acid which negatively impact the follicular environment to impair oocyte quality and developmental competence (Wathes et al., 2007). Subsequent embryo development may be compromised because lower progesterone levels shortly after fertilization may fail to adequately prepare the uterus to support pregnancy (Butler, 2003; Wathes et al., 2007). It is also suggested that NEB impedes uterine repair by hampering the clearance of pathogens from the uterine environment following calving (Wathes et al., 2007).

Negative energy balance can be indirectly monitored through the use of body condition scoring (**BCS**), a subjective method involving assessment of fat stores and assignment of a numerical score, generally using a scale of one (emaciated) to five (obese) (Ferguson et al., 1994). The BCS of the cow at the start of lactation and the amount of body condition lost during early lactation can be important predictors of subsequent fertility (Gillund et al., 2001; Roche et al., 2009). BCS loss of one or more numerical unit(s) after calving resulted in longer calving to conception intervals and required more artificial inseminations for cows to conceive (Gillund et al., 2001). Notably, BCS loss did not affect calving to first service interval, which helps to explain why more services were required for conception (Gillund et al., 2001). Similarly, in a large, U.S. based, commercial dairy herd, when BCS decreased by 0.4 or 0.8 points during the first month of lactation, cows were 1.17 and 1.36 times less likely to conceive after the first AI, respectively (Domecq et al., 1997).

Ovarian Cyclicity

One of the most important factors affecting cow fertility is cyclicity status. Research suggests that atypical cyclicity patterns such as prolonged luteal phase, follicular cysts, and cessation of cyclicity in lactating cows have increased over time (Royal et al., 2000a). Multiple studies reported that approximately 20 to 50% of cows remained anovular by 50 to 60 DIM (Moreira et al., 2001; El-Zarkouny et al., 2004; Santos et al., 2004; Shrestha et al., 2004). Shrestha et al. (2004) classified cows that took more than 45 DIM to return to cyclicity by causative issues including prolonged or shortened luteal phase, delayed first ovulation, or cessation of cyclicity and found that majority of delayed cows had delayed first ovulation or prolonged luteal phase. Evidence from a Japanese study of primiparous and multiparous Holsteins demonstrated that time to first ovulation and first estrus was shorter for first lactation females compared to multiparous cows (Sakaguchi et al., 2004), while others found that first parity cows took longer to return to cyclic status compared to multiparous cows (Tanaka et al., 2008; Chebel and Santos, 2010). Though submitting cows to GnRH-based timed artificial insemination (**TAI**) may induce cyclicity in some cows, pregnancy rates are still reduced compared to cows that regained cyclicity without hormonal induction (Cerri et al., 2004; Santos et al., 2004; Chebel et al., 2010; Chebel and Santos, 2010). The percentage of cows that resumed ovarian cyclicity increased as DIM increased (Shrestha et al., 2004), and correspondingly others have reported that more estrus events before insemination results in increased fertility (Thatcher and Wilcox, 1973; Sakaguchi, 2010).

Managing Fertility of the Lactating Dairy Cow

Voluntary Waiting Period

The voluntary waiting period (**VWP**) is an essential management component in dairy cattle production and is a defined length of time after parturition during which cows are purposely not submitted for insemination (Chebel and Santos, 2010). The VWP allows time for the cow to undergo healing processes including recovering from any postparturient disorders, completing uterine involution, and returning to a cyclic status before to insemination (Stevenson, 2001; Miller et al., 2007; Wathes et al., 2007; Chebel and Santos, 2010). Two surveys of 103 and 673 herds found that the average length of the VWP in American dairy herds is approximately 52 and 56 days, respectively (Caraviello et al., 2006; DeJarnette et al., 2007). In some cases, the range of VWP lengths employed can vary from 40-70 days (Stevenson, 2001).

Though some may vary the length of the VWP for multiple reasons such as post-calving health, parity, and milk yield, waiting at least ~ 55 days before first insemination is imperative. (DeJarnette et al., 2007; Miller et al., 2007). Approximately 20-50% of cows may still be anovular by 50-60 DIM (Linderoth, 2005; Miller et al., 2007; Chebel and Santos, 2010), suggesting that breeding immediately after the traditional VWP of 50-60 DIM may result in fewer successful conceptions.

Increased Fertility Coincident with Increased Days in Milk

Fertility in lactating dairy cows may increase as DIM increase (Caraviello et al., 2006; Schefers et al., 2010) as evidenced by improved conception rates when cows were inseminated between 120 and 180 DIM (Niozas et al., 2015). However, majority of these conclusions are based on retrospective analysis of data, and few instances exist where controlled studies examined the effects of altering the VWP, primarily to address summer heat stress (Gobikrushanth et al., 2014) and economic impacts of extending or altering calving intervals (Arbel et al., 2001; Niozas et al., 2015).

Increased Fertility after Estrus Detection at Later Days in Milk

In a 1975 review, sevens studies were summarized and concluded that fertility increased from 25% when artificial inseminations after detected estrus occurred after 20 DIM compared to more than 60% after 70 DIM (Britt, 1975). In a study designed to determine if artificially inseminating cows earlier postpartum would impact future reproductive performance, conception rates to first inseminations after the first postpartum estrus (~39 DIM) were lower compared to inseminations after 74 DIM [67% vs. 37%, respectively; (Whitmore et al., 1974)]. Both Britt (1975) and Whitmore et al. (1974) reported a decrease in services per conception from approximately 2.2 to 1.5 when cows were inseminated at later DIM. Whitmore et al. (1974)

followed cows over three lactations and noted increased fertility of 43, 42, and 22 percentage points higher for cows inseminated at first estrus after 74 DIM for the first, second, and third lactation, respectively. Furthermore, Hillers et al. (1984) reported significantly lower conception rates when cows were inseminated before 50 DIM compared to those that were inseminated between 50-79 DIM (32% vs. 49-54%, respectively). Weller and Folman (1990) concluded that inseminations at 83 DIM achieved desirable fertility without compromising milk production later in lactation.

Increased Fertility after Timed Artificial Insemination at Later Days in Milk

The goal of timed AI (**TAI**) is to utilize different pharmaceuticals to synchronize ovulation to occur at a predicted time, allowing inseminations to be performed at a specific time (Wiltbank and Pursley, 2014). After each $PGF_{2\alpha}$ injection(s) in a given protocol, an opportunity exists for cows to exhibit estrus, and thus insemination may occur if estrus is detected. A common management practice among dairy operations utilizing TAI synchronization practices is to inseminate cows that exhibit estrus before TAI (Stevenson and Phatak, 2005). Holstein cows inseminated with conventional semen after detected estrus during two different TAI protocols had increased pregnancy rates as DIM increased from 54 to 77 DIM [22.6% vs 48.7%, respectively; (Stevenson and Phatak, 2005)]. Inseminations based on estrus during the TAI protocols compared to those occurring only during the $PGF_{2\alpha}$ pre-synchronization demonstrated pregnancy rates that were significantly increased at greater DIM [31.3% at 59 DIM vs. 45.3% at 74 DIM (Stevenson and Phatak, 2005)]. Chebel and Santos (2010) reported numerically higher conception rates for cows inseminated at 72 DIM compared to cows inseminated at 65 DIM (39.6% vs. 33.0%, respectively; $P = 0.14$; $n = 639$). However, it is important to note that cows in the shorter VWP were inseminated after estrus during a pre-synchronization of two $PGF_{2\alpha}$

injections, but if estrus was not expressed, short VWP cows were time inseminated along with the longer VWP cows which were only time inseminated following the OvSynch protocol (Chebel and Santos, 2010). Gobikrushanth et al. (2014) found that extending the VWP from an average of 60 DIM (range: 57 to 63 DIM) to an average of 83 DIM (range: 64 to 121 DIM) resulted in slight improvements (32.1% to 37.7%; $P = 0.02$) in conception rates; however, part of the study was conducted during times of heat stress and could influence observed conception rates.

Modern dairy operations often utilize TAI synchronization to inseminate cows because of the benefits of eliminating estrus detection and having defined time periods for inseminations. Unfortunately, TAI following a traditional VWP of 50 to 60 days often results in decreased conception rates compared to inseminations following estrus detection (Kasimanickam et al., 2005). Tenhagen et al. (2003) reported increased first service conception rates when TAI was performed after at least 73 to 81 DIM among Holstein cows segregated by production level. Using the Ovsynch protocol, cows inseminated at 53 to 59 DIM (low production) and 73 to 81 DIM (average production) had significantly lower conception rates compared to cows inseminated at 94 to 102 DIM [high production; 14.4% vs. 28.7% vs. 41.4%, respectively; (Tenhagen et al., 2003)]. It is noted that milk production level did not have an influence on conception rates when cows were synchronized at similar DIM, thus differences in conception rate could be a result of inseminating cows at differing DIM (Tenhagen et al., 2003).

Fertility of Sex-Sorted Semen

Fertility of a dairy cow is also influenced by the service sire. Currently, two broad categories of semen types exist commercially: conventional and sex-sorted semen. Sex-sorted semen is a valuable reproductive tool for the dairy industry to aid in herd expansion, improve the rate of genetic gain, and increase the number of replacement females available (Hohenboken, 1999; Seidel, 2007; McCullock et al., 2013). A difference in fertility exists between conventional and sex-sorted semen. Moreover, differences also exist when comparing fertility of sex-sorted semen in heifers to that of lactating cows (Bodmer et al., 2005; Andersson et al., 2006; Schenk et al., 2009; DeJarnette et al., 2010; DeJarnette et al., 2011; Healy et al., 2013; Karakaya et al., 2014). In general, fertility of sex-sorted semen is 20% to 30% lower than conventional semen for both heifers and lactating cows (Andersson et al., 2006; DeJarnette et al., 2008; Seidel and Schenk, 2008; DeJarnette et al., 2009; Schenk et al., 2009; DeJarnette et al., 2010; Norman et al., 2010; DeJarnette et al., 2011; Healy et al., 2013; Karakaya et al., 2014). Lower conception rates for sex-sorted semen combined with increased cost prevents its widespread usage (Seidel, 2003).

Dairy Heifers

In four different field trials working with Holstein heifers in multiple locations and under different management conditions, Seidel et al. (1999) found average conception rates for frozen-thawed sex-sorted semen to be 50 to 56% compared to 67% for conventional semen. These rates were not improved by altering sperm concentration, deposition site, or freezing times (Seidel and Schenk, 2008). Schenk et al. (2009) reported sex-sorted semen conception rates that were equivalent to conventional conception rates (49%, 34%, 54% and 43%, 62%, 61%, respectively) following alterations in sorting pressure, storage time, laser sources, and media. Despite these findings, DeJarnette et al. (2011) still questioned the plausibility of ever achieving equivalent conception rates for sex-sorted and conventional semen. Previous efforts were inconclusive about altering semen dosage for improving conception rates for sex-sorted semen. DeJarnette et al. (2008) reported no influence of 2.1, 3.5, or 5.0 x 10^6 sperm per insemination dose on heifer conception rates (46.7%, 51.2%, 52.5%, $P > 0.05$), but DeJarnette et al. (2010) found that differences exist between 2.1 and 3.5 x 10^6 compared to conventional semen (44% and 46% vs. 61%, respectively; $P < 0.05$). Furthermore, Healy et al. (2013) retrospectively analyzed heifer inseminations for sex-sorted and conventional semen found predicted conception rates of 21.3% and 32.1%, respectively, which were noticeably lower than other studies. According to Bodmer et al. (2005), sex-sorted semen pregnancy rates were significantly lower than conventional semen pregnancy rates (33.3% vs. 59.3%; $P = 0.05$). While reductions are noted in dairy heifers for sex-sorted semen, even after multiple approaches to achieve improvements, usage in heifers remains a realistic option for producers due to the inherent increased fertility of nulliparous females (Butler et al., 2014) which may help to justify the added expense to obtain more heifer calves.

Lactating Cows

Conception rates may be considerably lower for sex-sorted semen in lactating cows due to fewer sperm per insemination dose and compromised fertility of the sperm (Seidel and Garner, 2002), the added stress of milk production in cows (reviewed by Lucy, 2001), the timing of the insemination (Sa Filho et al., 2010), or a combination of these or other unknown factor(s). Since the national average conception rate in lactating dairy cows is only 32% (Wiltbank and Pursley, 2014), reductions in fertility of any magnitude negatively affect usage of sex-sorted semen. When conception rates for sex-sorted semen were compared across 211 herds, slightly more than half as many lactating cows conceived compared to dairy heifers [27%, n= 2,298 vs. 45%, n= 48,200; (Schenk et al., 2009)]. In cows selected for absence of uterine and ovarian abnormalities, sex-sorted semen (2 x 10^6 sperm per straw) pregnancy rates were similar to conventional semen [10 x 10^6 sperm per straw; (40.5 ± 6.2% vs. 55.6 ± 6.5%, respectively; n =

115)] when TAI was performed at ~80 DIM (Crichton et al., 2006; Schenk et al., 2009). However, when reproductive status, insemination method, and stage of lactation were not controlled for, pregnancy rates for sex-sorted semen (2 x 10^6 sperm per straw) were significantly lower than unsorted 10 x 10^6 sperm per insemination dose controls [37.7% vs. 25.0%; (Schenk et al., 2009)]. Schenk et al. (2009) retrospectively observed an increase in pregnancy rate of 7.9% when 2 x 10^6 sex-sorted sperm packaged in 0.25 or 0.5 mL straws was used to inseminate non-selected cows after 84 to 98 DIM compared to inseminations earlier in lactation. Turkish Holstein-Friesians inseminated with sex-sorted semen after 140 DIM experienced conceptions rates similar to conventional semen (31.8% vs. 40.9%, respectively) when pregnancy was determined at 31 days post AI (Karakaya et al., 2014). However, Karakaya et al. (2014) also reported high rates of loss (19.1%) by 62 DIM, possibly due to environmental heat stress or lower quality sex-sorted semen. In unsynchronized Holstein-Friesians inseminated with low-dose (2 x 10^6) sex-sorted sperm placed in uterine horns, sex-sorted semen conception rates were significantly lower than conventional semen controls [21% vs. 46%, respectively; (Andersson et al., 2006)]. It is important to note that improper uterine horn inseminations or delaying pregnancy determination to 90 days post AI could be influential factors affecting conception rates in the Andersson et al. (2006) study. A Brazilian study in 2013 evaluated the success of sex-sorted semen when utilized with estrus detection versus timed insemination and found that, while service rates were higher for timed inseminations, pregnancy per AI was higher for cows bred following estrus expression than after two timed insemination protocols [31.7% vs. 19.4% and 23.9%, respectively (Sá Filho et al., 2013)]. Overall, there was a trend for pregnancy rate to be lower for the estrus detection group compared to the two groups of timed insemination cows (14.3% vs. 18.3% and 23.2%), likely due to increased submission rate with timed AI (Sá Filho et al., 2013). Multiple studies evaluating fertility of sex-sorted semen when processed at varying dosages found no appreciable improvement in conception rates (23.0% to 30.3%) when concentration was increased from 2.1 million to 3.5 million or 5 million sperm per straw (DeJarnette et al., 2008; DeJarnette et al., 2010).

Economic Aspects

Sex-sorted semen provides multiple economic benefits for the dairy industry including increased production of heifer calves, allowing producers to be more selective when choosing replacement females, reduced incidence of dystocia at parturition because of smaller calves, and increased genetic progress within the herd (De Vries et al., 2008). Unfortunately, widespread usage of sex-sorted semen has been hampered because of increased cost per straw which has been estimated at $20 to $50 more per insemination dose and because of reduced conception rates compared to conventional semen (Amann, 1999; Weigel, 2004; Norman et al., 2010). For sex-sorted semen to be an economical technology to integrate into reproductive practices, general fertility of a herd must be managed well initially (Seidel, 2003). This is further shown in dairy heifers by Weigel (2004) which reports that the cost of a heifer calf increases from $97 to $106 when conception rates for sex-sorted semen decline from 45% to 35%, assuming semen costs are $50. Thus, in order for sex-sorted semen to be economically beneficial, the value of producing a heifer calf over a bull calf must be twice the cost of production [i.e., if it costs $100 dollars to use sex-sorted semen, the resultant heifer calf must be worth at least $200 more to justify the added expense (Amann, 1999; Seidel, 2003)].

Extending the VWP may help to address some of the fertility issues associated with sex-sorted semen. The increased amounts of milk produced from extending the calving interval may also help to offset the additional cost of sex-sorted semen. Extending the VWP in high producing cows could have economic benefits in terms of milk production (Arbel et al., 2001; Linderoth, 2005; Gobikrushanth et al., 2014). Gobikrushanth et al. (2014) found that extending the VWP from an average of 60 DIM (range: 57 to 63 DIM) to an average of 83 DIM (range: 64 to 121 DIM) improved conception rates by 5% and resulted in approximately 1,600 kg of additional milk yield per lactation in primiparous cows. However, no significant improvements were found for milk production in multiparous cows, which may be attributed to varying levels of production among multiparous cows (Gobikrushanth et al., 2014). When rbST was used to boost persistency of lactation, van Amburgh et al. (1997) showed that cows subjected to extended calving intervals of 16.5 months produced more milk compared to cows having a calving interval of ~13 months. With or without the aid of bST, it seems intuitive that cows which are milked longer would produce increased amounts of milk; the caveat of extended VWPs and subsequent extensions of calving intervals would center on the input cost of maintaining lactating cows for longer periods.

Though Inchaisri et al. (2010) stated that the economically optimal VWP is generally shorter (~60 DIM) for most cows, they also hypothesized that small extensions of the VWP may not negatively impact production from an economic standpoint while allowing producers to achieve increased fertility. Extending the VWP in high producing cows resulted in economic advantages of $0.19 and $0.12 per cow per day of lactation for primiparous and multiparous cows, respectively (Arbel et al., 2001). While this only equates to approximately $11 and $7 of increased revenue, respectively, combined with increased conception rates at later DIM, it demonstrates promise that extending the VWP in lactating cows may be beneficial for fertility and could potentially help to offset the increased cost of sex-sorted semen by increased milk production. Developing a way to improve the fertility of sex-sorted semen in lactating cows that is economically advantageous to the producer could be instrumental for increasing the appeal of this reproductive technology for producers.

CHAPTER 3

MATERIALS AND METHODS

Lactating Holsteins (n = 183), average parity of 1.98 ± 0.97 (range 1 to 5), were housed in a sand-bedded free stall barn, fed *ad libitum* a total mixed ration of corn silage, grass silage, grain and hay (NRC, 2001), provided open access to fresh water, and were milked twice daily. Animal related procedures were approved by the Institutional Animal Care and Use Committee at the University of Tennessee, Knoxville.

Treatment Assignments

Cows calving between August and April over a two year period and having no obvious adverse health or reproductive conditions by 21 DIM (n = 137) were randomly allotted to one of two voluntary waiting periods (**VWP),** either 55 days [**VWP55** (n = 71)] or 85 days [**VWP85** (n = 66); (**Figure 3.1**)]. Reproductive tract scores (**RTS**) were assigned based on the size and length of the reproductive tract with one being the smallest and three being the largest by a single technician as described by Young et al. (2010).

Beginning at approximately 21 DIM, weekly transrectal ultrasound exams were conducted to confirm ovarian cyclicity [i.e. presence of a corpus luteum (**CL**); **Figure 3.1**]. Cyclicity was confirmed by the presence of a CL on either ovary for two of three weeks or visual observation of standing estrus before 55 DIM. Independent of treatment designation, 48 cows (35%) had not resumed cyclicity by 55 DIM and were removed from the trial and returned to normal herd management practices. Additionally, cows that began the synchronization protocol and developed health concerns that could possibly bias treatment results were removed from the study (n = 1), nine cows never expressed estrus during the study, and nine began estrus synchronization but did not express estrus before the study ended.

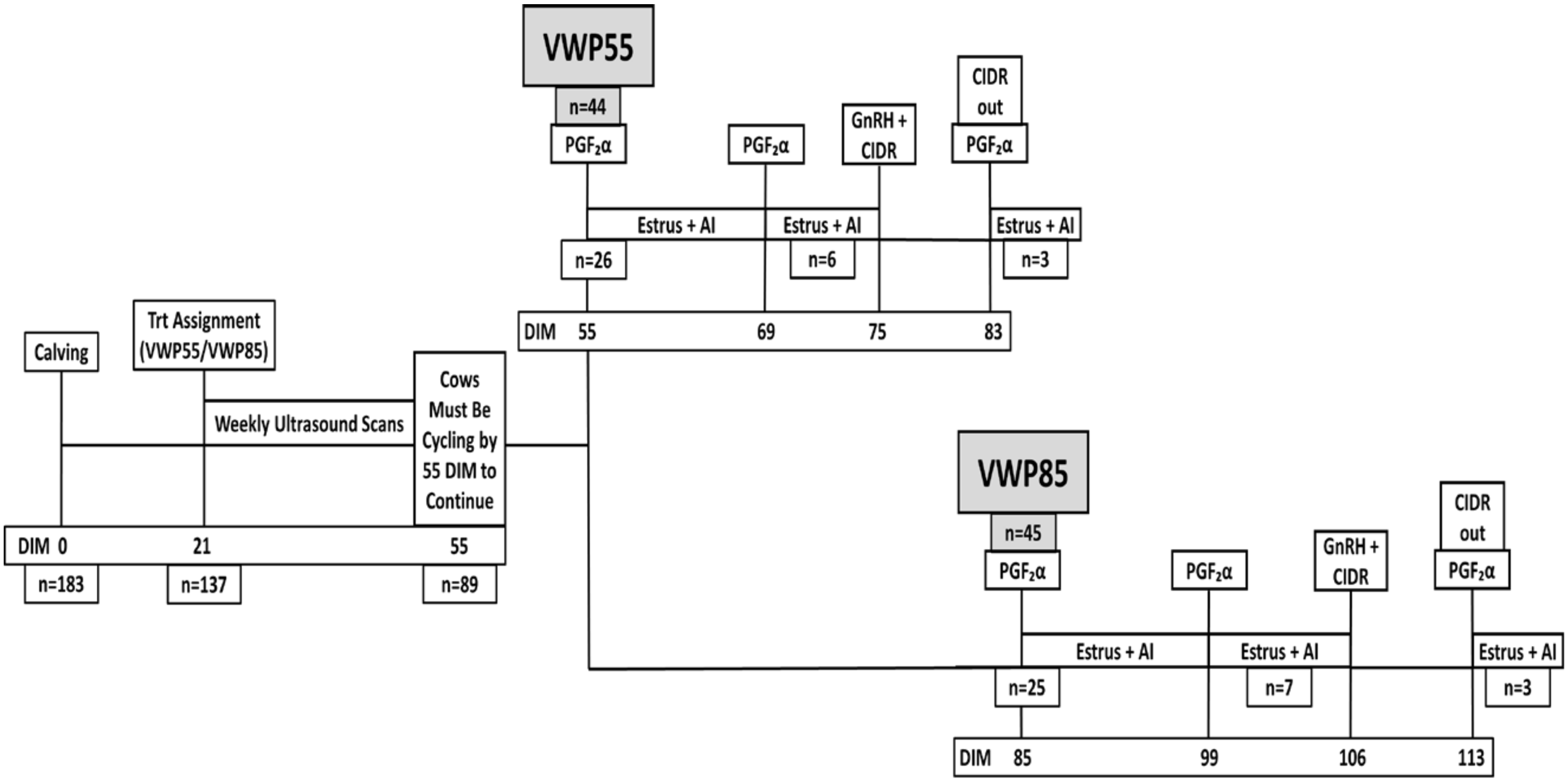

Figure 3.1. Experimental schematic and estrus synchronization protocol for VWP55 and VWP85 cows. Following calving, cows were assigned to VWP55 or VWP85 treatment groups at 21 DIM. From 21 DIM to 55 DIM, resumption of cyclicity was monitored by weekly ultrasound scans such that all cows utilized were cyclic before 55 DIM. Relevant for cyclic cows and initial treatment designation, estrus synchronization began at ~55 or ~85 DIM. Cows were administered $PGF_{2\alpha}$ at appropriate times if a CL was present. Following two $PGF_2\alpha$ administrations (14 days apart) without estrus expression, cows were administered GnRH and CIDR. Seven days later, CIDRs were removed and $PGF_{2\alpha}$ was administered a final time. Any cow exhibiting estrus was submitted for AI with sex-sorted semen from one of two different Holstein bulls.

Estrus Synchronization and Detection

On a weekly basis, cows that were confirmed cyclic by 55 DIM were body condition scored (**BCS**) by a single evaluator and reported based on the standard, dairy industry accepted scale of 1 to 5, with 1 being emaciated and 5 being obese (Ferguson et al., 1994) at either 55 to 65 DIM (**VWP55**; n = 44) or 85 to 95 DIM (**VWP85**; n = 45). HeatWatch® (**HW**; Cow Chips, LLC, Manalapan, NJ) patches were attached to the sacral portion of the spine of each cow with adhesive. Each patch contained a radiotelemetric device that recorded the number and duration of each mount for cows exhibiting estrus. Mount information was sent to a computer database where estrus intensity and duration information was compiled and stored for later analysis.

Cows detected to have a CL present by ultrasound were administered $PGF_{2\alpha}$ (25 mg/mL im; Lutalyse®, *dinoprost tromethamine*, Zoetis, USA; **Figure 3.1**). If no CL was present, cows were monitored for spontaneous estrus until the next stage of the protocol (n = 25). Estrus detection occurred continuously via the HW system and by visual observation twice daily (0600 and 1900) in a deep sand bedded concrete alley way for seven consecutive days after each $PGF_{2\alpha}$ administration (**Figure 3.1**). Estrus was defined as at least two mounts of two seconds or greater within six consecutive hours if HW was the only detection source and/or the observation of a cow standing to be mounted by another. Cows detected in estrus were artificially inseminated ten to eleven hours before predicted ovulation, which was assumed to occur approximately 28 h (Hockett et al., 2005) after first recorded HW mount. After 14 days, cows not detected in estrus were examined by ultrasound, and if a CL was present, a second injection of $PGF_{2\alpha}$ was administered (**Figure 3.1**). Cows not detected in estrus and thus not artificially inseminated in the 7 days following the second prostaglandin administration received an injection of GnRH (50 µg/mL im; Cystorelin®, *gonadorelin diacetate tetrahydrate*, Merial Limited, Duluth, GA) and a controlled intravaginal drug releasing device (CIDR; Eazi-Breed™ CIDR® Cattle Insert, Zoetis, USA) impregnated with 1.38 g of progesterone was placed intravaginally for 7 days (**Figure 3.1**). Following removal of the CIDR, a final dose of $PGF_{2\alpha}$ was administered if a CL was present (**Figure 3.1**).

Artificial Insemination & Pregnancy Determination

Cows exhibiting estrus were submitted for AI by one technician with sex-sorted semen using one of two different Holstein bulls. Sire conception rates for the two different bulls were +1.4 and +1.5 at the beginning and +0.7 and +0.9 at the end of the study, respectively. At the time of insemination, an AI score (**AIS)** relating the general difficulty of insemination was assessed on a subset of cows [score of 1 (reproductive tract easily traversed) to 4 (semen deposited in cervix) and modeled after an embryo transfer score described by Scenna et al. (2005)]. In addition, a cervix score (**CS)** was also recorded relating the overall size of the cervix as discerned via transrectal palpation [(score of 1 to 3 as described by Young et al. (2010)]. Pregnancy status was determined between 28 to 35 days after AI using transrectal ultrasonography. Maintenance of pregnancy was assessed at 60, 80 and 150 days after AI.

Statistical Analyses

In general, data were analyzed as a completely randomized design using PROC GLIMMIX (SAS, Version 9.4, SAS, Inc., Cary, NC, USA) with a binomial or normal distribution. In instances where an effect of year was significant, some estrus characteristics (i.e., mount duration) were analyzed as a randomized block design, blocking on year. All relevant variables were included in initial analyses and manual backwards selection was used to remove non-significant ($P > 0.05$) variables one-by-one from the model until only significant variables remained. Treatment differences from all analyses were determined using F-protected least significant differences and reported as least squares means ± standard error of the mean (**SEM**).

All milk production values were segregated into three groups using the PROC UNIVARIATE procedure such that 25% of cows were classified as low production, 50% as medium production, and 25% as high production for each analysis. Body condition score was separated into two groups such that cows were evenly divided above and below the average score of 2.5. Additionally, parity was grouped as first, second, and third or greater due to limited observations of parities greater than three. All models included treatment, parity, and year as fixed effects. The statistical model for reproductive tract scores included milk production as a fixed effect; whereas, cyclicity analyses included treatment, parity, year, milk production, and RTS. Estrus characteristics models included treatment, parity, milk production (3 d average), and year. Additionally, the PROC LIFETEST procedure was used to compare percentage of estrus expression and time to estrus for treatments. Finally, conception rate models used the following variables in the analysis: treatment, RTS, parity, sire, BCS, milk production (day of AI), and year.

CHAPTER 4

RESULTS AND DISCUSSION

Milk production was similar for VWP55 and VWP85 cows when treatments were initially assigned at 21 DIM (41.33 ± 0.85 and 39.47 ± 0.82 kg, respectively; P = 0.1165), when cyclicity was confirmed at 55 DIM (43.57 ± 0.86 and 44.48 ± 0.80 kg, respectively; P = 0.4413), when estrus synchronization efforts began (43.28 ± 0.84 and 42.51 ± 0.79 kg, respectively; P = 0.5082), and at the time of AI (40.16 ± 1.46 and 40.89 ± 1.46 kg, respectively; P = 0.7250). Reproductive tract scores were similar for cows assigned to either a 55 day VWP or 85 day VWP when evaluated at 21 DIM (1.99 ± 0.07 and 1.94 ± 0.07, respectively; P = 0.6941), at 55 DIM when cyclicity was confirmed (1.64 ± 0.08 and 1.80 ± 0.08, respectively; P = 0.1647), at the time of estrus synchronization (1.73 ± 0.09 and 1.66 ± 0.09, respectively; P = 0.5406), and at the time of AI (1.57 ± 0.10 and 1.66 ± 0.10, respectively; P = 0.5549). The percentage of VWP55 and VWP85 cows cycling by 55 DIM was similar (77.29% ± 5.90 and 79.03% ± 5.29, respectively; P = 0.8158). Additionally, parity for cows assigned to a 55 versus 85 day VWP was similar for all cows beginning estrus synchronization (1.82 ± 0.14 vs. 2.13 ± 0.14; P = 0.1200, respectively). Body condition score was not different for VWP55 and VWP85 cows at onset of estrus synchronization efforts (2.31 ± 0.06 and 2.45 ± 0.06; P = 0.0805). Average rectal temperature at AI for VWP55 cows was similar to those of VWP85 cows (38.41°C ± 0.08 vs. 38.41°C ± 0.08; P = 0.9428). In summary, random treatment allocation at 21 DIM resulted in two groups of cows there were essentially equivalent in milk production, reproductive tract scores, ovarian cyclicity, parity, and body condition score. Hence, any inferences about estrus activity or conception rates were not influenced by these factors and were the result of our imposed treatment groups of either a 55 or 85 day VWP.

Estrus Activity

The percentage of cows confirmed to be cyclic by 55 DIM that exhibited natural or pharmacologically induced estrus was similar for cows after a VWP of 55 or 85 days (**Table 4.1**). An exhaustive review of the literature determined no previous studies examining estrus activity at these specific times in lactation; however, this finding is similar to another report that examined estrus expression between 70 and 100 DIM using only a radiotelemetry system in Holstein cows (Lopez et al., 2004). Furthermore, the responsiveness of cows having a CL was also similar for VWP55 and VWP85 cows when evaluation of estrus expression within seven days from the first $PGF_{2\alpha}$ administration (**Table 4.1**). Response rates for both VWP55 and VWP85 cows are similar to those reported by Momcilovic et al. (1998), Walton et al. (1987), and Simmons et al. (1979), which evaluated $PGF_{2\alpha}$ responsiveness between 40 and 60 DIM. While some report higher response rates (81.2%) to $PGF_{2\alpha}$ when cows were inseminated at an average of 83 DIM in both visual observation and HW herds (Jobst et al., 2000), our finding is consistent with studies not specifying DIM which reported $PGF_{2\alpha}$ response rates of 58% to 72% (Lauderdale et al., 1974; Archbald et al., 1993).

The time from $PGF_{2\alpha}$ administration to estrus was measured on a subset of cows (n = 38) using HW system. The time to estrus from the initial $PGF_{2\alpha}$ administration was similar for cows assigned to a VWP of 55 days and 85 days (**Table 4.1**). When all $PGF_{2\alpha}$ administrations from the estrus synchronization protocol were considered, time to estrus was still similar for both VWP55 and VWP85 cows (**Table 4.1**). Our fining is consistent with Macmillan et al. (1980) which reported that stage of lactation [(early or peak lactation versus late lactation (> 240 DIM)] did not impact interval to estrus after $PGF_{2\alpha}$ administration. Times to estrus reported herein are similar to other works reporting approximately

Table 4.1. Estrus response and activity for VWP55 and VWP85 cows[1]

	Treatment[2]		
	VWP55	VWP85	P-Value
Overall percentage that exhibited estrus[3]	$79.55^{a} \pm 6.08$	$77.78^{a} \pm 6.20$	0.8393
Percentage exhibiting estrus within 7 d of $PGF_{2\alpha}$ [4]	$64.37^{a} \pm 11.36$	$57.14^{a} \pm 9.57$	0.6275
Time to estrus for first $PGF_{2\alpha}$ administration (h)[5]	$65.79^{a} \pm 7.58$	$72.65^{a} \pm 6.27$	0.4908
Time to estrus for all $PGF_{2\alpha}$ administrations (h)[5]	$62.28^{a} \pm 6.68$	$73.00^{a} \pm 5.46$	0.2218
Number of mounts	$9.05^{a} \pm 1.75$	$10.54^{a} \pm 1.52$	0.5246
Mount duration (s)	$4.59^{a} \pm 0.43$	$4.99^{a} \pm 0.41$	0.2602
Estrus length (h)[6]	$8.16^{a} \pm 1.64$	$9.17^{a} \pm 1.39$	0.6389

[1]The total number of cows that exhibited estrus, hours to estrus from $PGF_{2\alpha}$, and estrus characteristics as detected by HW were recorded for VWP55 and VWP85 cows.
[2]Least squares means ± SEM reported. Treatment means within a row having unlike superscripts differ ($P < 0.05$)
[3]Includes all cows that exhibited natural or $PGF_{2\alpha}$ induced estrus detected by visual observation and/or HW
[4]Only includes cows exhibiting estrus in response to any $PGF_{2\alpha}$ administration within seven days
[5] As detected by HW

[6]Length of time between the first and last HW recorded mount

73 h to estrus in lactating cows (Renegar et al., 1978; Archbald et al., 1993; Walker et al., 1996). Survival curve analysis indicates that cows exhibited estrus at similar rates after a VWP of 55 day and 85 days for the initial $PGF_{2\alpha}$ administration and when all $PGF_{2\alpha}$ administrations were considered ($P = 0.3848$ and $P = 0.2480$, respectively; **Figure 4.2**). Similar to other reports, the largest increase in cows expressing estrus occurred between 40 and 80 hours following both administrations of $PGF_{2\alpha}$ [**Figure 4.2**; (Lauderdale et al., 1974; Walton et al., 1987)].

Number of mounts per estrus event, duration of each mount, and the total length of time that estrus was exhibited were similar when efforts to synchronize estrus began after a VWP of 55 or 85 days (**Table 4.1**). Independent of DIM when estrus was exhibited in cyclic cows, the number of mounts for both VWP55 and VWP85 cows is consistent with other reports in various settings and situations (Britt et al., 1986; Walker et al., 1996; Dransfield et al., 1998; Xu and Burton, 1999; Lopez et al., 2004). Our result for number of mounts contrasts with Peralta et al. (2005), who found that average number of mounts were higher for cows that were less than 80 DIM compared to cows ranging from 80 to more than 140 DIM. Average mount duration in our study was one to two seconds longer than reported by Lopez et al. (2004), Walker et al. (1996), or Xu and Burton (1999). Mounts of longer duration may have been detected due to facilities (deep sand bedded alley way) and because cows prefer more secure footing to exhibit estrual signs (Britt et al., 1986). Mounts of longer duration may be more easily observed when utilizing only visual estrus detection. Consistent with our findings, most studies evaluating the length of time cows exhibited estrus, independent of DIM, ranged from seven to nine hours (Walker et al., 1996; Dransfield et al., 1998; Xu and Burton, 1999; Lopez et al., 2004), though Britt et al. (1986) reported longer estrus lengths (~13 h). Similarities in estrus activity of cows when the VWP was either 55 days or 85 days is likely due

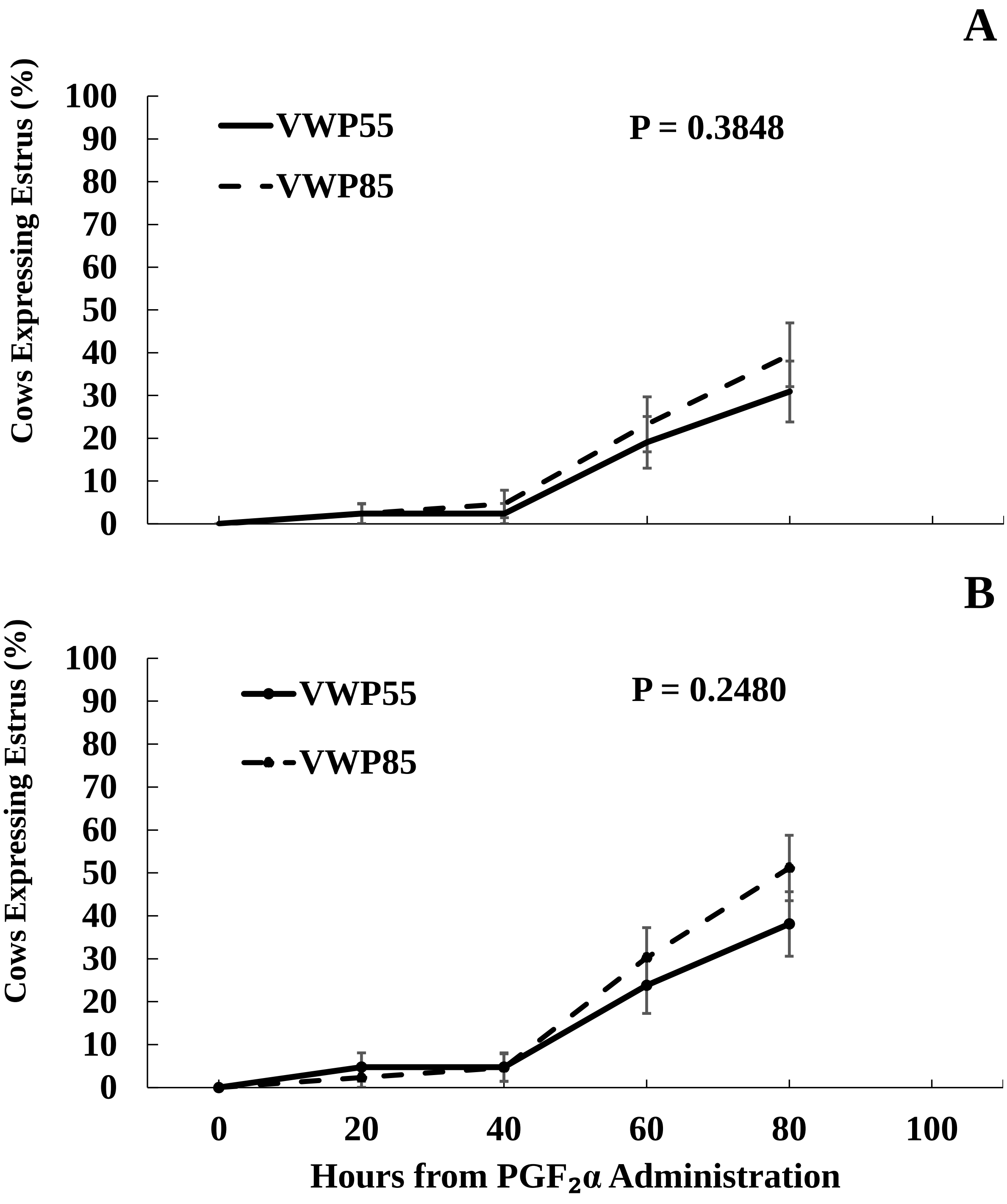

Figure 4.2. Percentage of estrus expression over time after $PGF_2\alpha$ administration
Depiction of survival curve for proportion of VWP55 and VWP85 cows expressing estrus by a given number of hours from the first $PGF_{2\alpha}$ administration (Panel A) or all $PGF_{2\alpha}$ administrations (Panel B) as recorded using the HW system (i.e., 30% of VWP85 cows had exhibited estrus by 60 hours after $PGF_{2\alpha}$ when all administrations were considered).

to the inherent similarities of our treatment groups for general production, cyclicity status, and overall management.

In our study, estrus activity was more influenced by average milk production in the three days around estrus (i.e., one day before, day of estrus, and one day after). Specifically, when estrus activity within seven days of the first $PGF_{2\alpha}$ was considered, larger percentages of cows with milk production ranging from 33.48 to 46.95 kg (medium) and from 46.95 to 62.78 kg (high) exhibited estrus compared to the cows producing 24.09 to 33.48 kg (low; 66.75% ± 11.13 and 85.03% ± 8.22 vs. 24.68% ± 8.81, respectively; P = 0.0014). Increased rates of estrus expression by higher producing cows were not necessarily expected because previous research has shown that higher producing cows have increased negative energy balance (Harrison et al., 1990; Butler, 2003; Wathes et al., 2007; Ospina et al., 2010) and higher rates of $PGF_{2\alpha}$ clearance (Davis et al., 1980; Shrestha et al., 2012), which may lead to reduction of estrus expression.

Artificial Insemination and Conception Rates

Artificial insemination score was not different among treatments (1.43 ± 0.13 vs. 1.68 ± 0.14; P = 0.2024), and neither was cervix score (1.30 ± 0.12 vs. 1.50 ± 0.12; P = 0.2520). No differences in sire conception rates were observed (35.29% ± 8.20 vs. 38.89% ± 8.13; P = 0.7567). Conception rates at approximately 30 days after AI were higher for VWP85 cows compared to VWP55 cows (P = 0.0181; **Figure 4.3, Panel A**), and were higher than reported conventional semen conception rates for lactating cows (Andersson et al., 2006; Norman et al., 2009; Chebel and Santos, 2010; DeJarnette et al., 2010; Karakaya et al., 2014). Benefits of extending the VWP by 30 days (VWP85) were still observed at 60 (P = 0.0166; **Figure 4.3, Panel B**), 80 (P = 0.0471; **Figure 4.3, Panel C**), and 150 days after AI (P = 0.0471; **Figure 4.3, Panel D**). Herein, pregnancy loss between 30 and 60 days after AI (11.5%; 3/26) was similar to

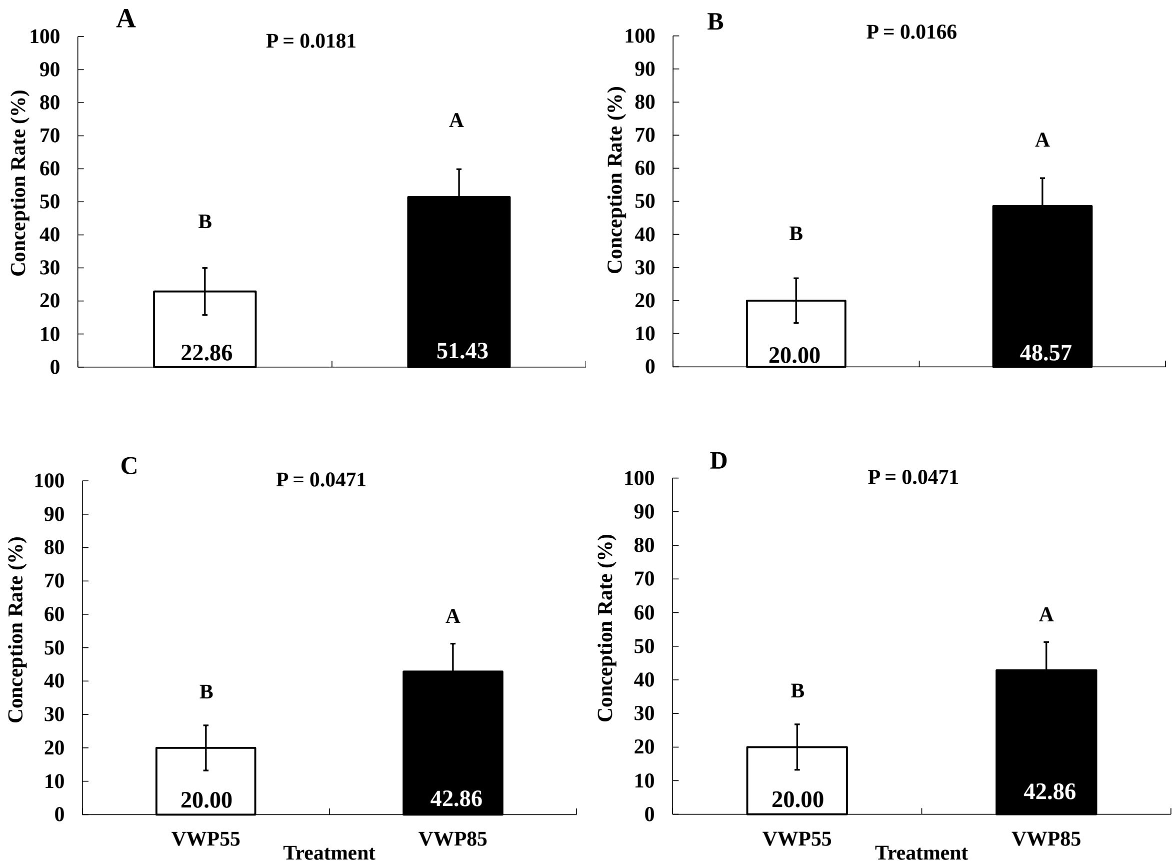

Figure 4.3. Conception rates for VWP55 and VWP85 cows

Conception rates at 30 d (Panel A), 60 d (Panel B), 80 d (Panel C), and 150 d (Panel D) after AI for lactating cows bred with sex-sorted semen after a 55 d or 85d VWP. Least squares means are reported, and bars with different letters were significantly different ($P \leq 0.05$).

reports by Schenk et al. (2009), but less than the 19.1% (9/47) reported in Holsteins inseminated with sex-sorted semen in Turkey (Karakaya et al., 2014). Cows that were not confirmed pregnant to first AI with sex-sorted semen after a 55 or 85 day VWP required a total of 2.5 and 1.5 services per conception, respectively ($P = 0.0010$).

Fertility of sex-sorted semen in our study using two different Holstein bulls having sire conception rates of +0.7 and +0.9 when the VWP was extended to 85 DIM in confirmed cyclic cows was comparable to studies using conventional semen at later DIM reporting conception rates (Hillers et al., 1984; Tenhagen et al., 2003; Niozas et al., 2015) and overall fertility (Whitmore et al., 1974; Britt, 1975; Stevenson and Phatak, 2005). Our results suggest that current practices for VWP (Caraviello et al., 2006; DeJarnette et al., 2007) may contribute to poor success rates in studies using sex-sorted semen after a VWP of 55 days, which is more consistent with industry standards. Though some sex-sorted semen studies reported inseminations at average DIM similar to our treatment groups, a VWP longer than the industry standard of ~55 DIM was not specifically controlled for because sperm dosages and comparison of estrus detection versus timed AI were the original endpoints, resulting in reduced conception rates by comparison [29.4% and 31.7%, respectively; (DeJarnette et al., 2008; Sá Filho et al., 2013)]. There are instances where extended VWPs or increased DIM at time of insemination did not impact pregnancy rates for conventional or sex-sorted semen, respectively (Arbel et al., 2001; Schenk et al., 2009). Interestingly in one trial, Schenk et al. (2009) selected cows for absence of uterine or ovarian abnormalities before starting the study, similar to our experimental design, though did not explicitly monitor cyclicity and still achieved similar fertility to our results after timed inseminations (average 80 DIM) following OvSynch synchronization. In a separate trial, Schenk et al. (2009) reported pregnancy rates of approximately 25% when cows not selected for reproductive status were inseminated with sex-sorted semen, but upon retrospective analysis cows inseminated between 84 to 98 DIM had pregnancy rates that were 7.9 percentage points higher compared to those inseminated earlier in lactation.

After statistical analysis, only VWP length significantly influenced conception rates in our study. Although DeJarnette et al. (2008) reported that parity tended to impact conception rates of lactating cows inseminated with sex-sorted semen, we did not observe that effect (first: 36.67% ± 8.80, second: 36.00% ± 9.60, third or greater: 40.00% ± 12.65; $P = 0.9660$). This is not surprising since the majority of cows used in our study were first and second lactation due to recent repopulation of our dairy research herd. Additionally, conception rates were similar among reproductive tract scores of 1, 2, and 3 (40.63% ± 8.68, 33.33% ± 8.2, and 40.00% ± 21.91, respectively; $P = 0.8242$) and numerically higher than those reported by Young et al. (2010).

Independent of VWP55 and VWP85 treatment, milk production increased significantly as parity increased from first to third or greater at all stages of the study ($P < 0.0001$; **Table 4.2**), which is generally consistent with Tanaka et al. (2008) and Lee and Kim (2006). Moreover, parity impacted reproductive tract score ($P \leq 0.08$) throughout the entire study (**Table 4.3**). First and second parity cows had smaller numerical reproductive tract scores compared to third and greater parity cows, similar to reports from Young et al. (2010).

Resumption of cyclicity was affected by parity and milk production at 55 DIM, independent of VWP55 or VWP85 treatment. The percentage of first parity cows that were cyclic by 55 DIM was significantly lower than second and third or greater parity cows ($P = 0.0093$; **Table 4.4**) and generally similar to other reports in the literature (Bulman and Lamming, 1978; Tanaka et al., 2008; Chebel and Santos, 2010). In our study, cows ranging in milk

Table 4.2. Influence of parity on milk production[1]

	Parity[2]			
	First	Second	Third or greater	P-Value
Treatment assignment[3]	$31.65^{c} \pm 0.90$	$42.75^{b} \pm 0.96$	$46.80^{a} \pm 1.22$	<0.0001
Cyclicity confirmation[4]	$33.34^{c} \pm 0.95$	$46.97^{b} \pm 0.92$	$51.76^{a} \pm 1.18$	<0.0001
Estrus synchronization[5]	$33.18^{c} \pm 0.94$	$45.97^{b} \pm 0.90$	$49.54^{a} \pm 1.16$	<0.0001
Time of AI	$32.99^{c} \pm 0.96$	$43.79^{b} \pm 1.06$	$50.16^{a} \pm 1.39$	<0.0001

[1]The influence of parity on milk production (kg), independent of VWP55/VWP85 treatment
[2]Least squares means ± SEM reported. Means within a row having unlike superscripts differ ($P < 0.0001$)
[3]At 21 DIM
[4]At 55 DIM
[5]Either 55 DIM or 85 DIM, depending on treatment designation

Table 4.3. Influence of parity on reproductive tract scores[1]

	Parity[2]			
	First	Second	Third or greater	P-Value
Treatment assignment[3]	$1.80^{b} \pm 0.08$	$1.96^{ab} \pm 0.08$	$2.14^{a} \pm 0.11$	0.0460
Cyclicity confirmation[4]	$1.54^{b} \pm 0.08$	$1.72^{b} \pm 0.09$	$2.05^{a} \pm 0.12$	0.0021
Estrus synchronization[5]	$1.56^{b} \pm 0.10$	$1.51^{b} \pm 0.10$	$2.02^{a} \pm 0.13$	0.0063
Time of AI	$1.53^{b} \pm 0.11$	$1.52^{b} \pm 0.12$	$1.93^{a} \pm 0.16$	0.0829

[1]The influence of parity on reproductive tract scores, independent of VWP55/VWP85 treatment
[2]Least squares means ± SEM reported. Means within a row having unlike superscripts differ ($P < 0.10$)
[3]At 21 DIM
[4]At 55 DIM
[5]Either 55 DIM or 85 DIM, depending on treatment designation

production from 24.95 to 35.90 kg (low) were more likely to be cyclic by 55 DIM than cows ranging from 35.90 to 51.10 kg (medium) or from 51.10 to 62.32 kg (high; P = 0.0006; **Table 4.4**).

Conclusions

Though other studies report information about VWPs and fertility increases at later DIM, few were controlled for this specific hypothesis, and to our knowledge, none were controlled experiments using sex-sorted semen (Whitmore et al., 1974; Britt, 1975; Hillers et al., 1984; Weller and Folman, 1990; Tenhagen et al., 2003; Stevenson and Phatak, 2005; Niozas et al., 2015). In circumstances where factors such as milk production, reproductive tract scores, ovarian cyclicity, parity, and BCS are similar, our study is the first that we are aware of to indicate improvements in fertility of sex-sorted semen after extending the VWP to 85 days. While the underlying mechanisms of these benefits are unclear, differences in conception rates were not a result of differing ovarian cyclicity, estrus response to $PGF_{2\alpha}$, or estrus characteristics. While difficult to explain, improved fertility of cows after extending the VWP to 85 days may be attributed to important cellular changes occurring in the uterus after the time of involution that may result in enhanced ability of the uterus to support a pregnancy (Sheldon et al., 2008). It is also possible for benefits of extending the VWP to be related to increased exposure to hormones produced by another 1 to 1.5 estrous cycles before insemination (Thatcher and Wilcox, 1973; Sakaguchi, 2010). The possibility also exists that cows may have returned to positive energy balance (Butler, 2003, 2005) before inseminations begin could be an influential factor.

Table 4.4. Influence of parity and milk production on resumption of ovarian cyclicity[1]

	Cycling by 55 DIM (%)	P-Value
Parity		0.0093
First	$52.89^{b} \pm 9.91$	
Second	$86.39^{a} \pm 4.65$	
Third or greater	$86.56^{a} \pm 6.17$	
Milk Production		0.0006
Low (24.95 – 35.90 kg)	$94.61^{A} \pm 3.15$	
Medium (35.90 – 51.10 kg)	$56.82^{B} \pm 7.08$	
High (51.10 – 62.32 kg)	$66.51^{B} \pm 10.67$	

[1]The influence of parity and milk production resumption of ovarian cyclicity by 55 DIM, independent of VWP55/VWP85 treatment

[2]Least squares means ± SEM reported. Means within a column having unlike superscripts of the same case differ ($P < 0.05$)

Implementing the use of sex-sorted semen for lactating cow inseminations may be possible under specific management conditions, specifically, when cows are managed to be healthy and cyclic, estrus detection is employed, and the VWP is extended to 85 DIM.

Implications

Extension of the VWP from 55 days to 85 days improved conception rates for sex-sorted semen in lactating Holstein cows that were previously determined cyclic and inseminated following detection of standing estrus. $PGF_{2\alpha}$ responsiveness and estrus characteristics were similar between VWP55 and VWP85 cows suggesting that cyclic cows under similar management are capable of exhibiting estrus at varying stages of lactation. Preliminary economic analysis suggests that extending the VWP to 85 days when using sex-sorted semen in lactating cows can be beneficial.

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VITA

Sarah Elizabeth Orr was born on December 13, 1990 in Greeneville, Tennessee. She attended West Greene High School and graduated in the spring of 2009. She furthered her education by attending the University of Tennessee, Knoxville, where she earned a Bachelor's of Science in Animal Science in 2013, and continued on to graduate education under the guidance of Drs. J. Lannett Edwards and F. Neal Schrick. In December of 2015, Sarah graduated with a Master's of Science degree in Animal Science with a concentration in reproductive physiology.