

Manipulating the Duration of the VWP to Optimize Herd Performance and Profitability

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- Extending the duration of the voluntary waiting period (VWP) from 50 or 60 DIM to 88 DIM can increase first service pregnancy per AI (P/AI) but delay overall time to pregnancy when either all timed AI (TAI) or combined (AI in estrus and TAI) programs are used for first service.
- The greatest effect of extending the VWP on first service P/AI may be observed in primiparous cows.
- Delaying first service from 60 to 88 DIM may result in greater profitability for primiparous but not for multiparous cows.
- Changes in VWP duration affected profitability primarily through differences in replacement cost and to a lesser extent by income over feed cost.
- In general, the effect of manipulating the duration of the VWP on herd performance and economics depends upon complex interactions between reproductive performance, herd exit dynamics, lactation performance, and economic conditions.

INTRODUCTION

Timing of pregnancy during lactation affects dairy herd profitability by defining the calving interval, milk production efficiency, and herd replacement dynamics. The insemination and conception risk after the end of the voluntary waiting period (**VWP**) are the two major determinants of time to pregnancy during lactation. Nonetheless, the duration of the VWP can also influence timing of pregnancy because it determines when cows become eligible for insemination.

Traditionally, dairy farms in the US began inseminating cows at ~40 to 50 DIM

because sub-optimal estrous detection and fertility to AI required that cows received multiple services to conceive. In recent years, however, better cow health and reproductive management programs that ensure inseminating cows by a set DIM, led to increases in reproductive and productive performance of well-managed dairy herds. Improved detection of estrus and fertility reduces the number of inseminations needed to conceive and the variation in the timing required for cows to become pregnant. Thus, there is an opportunity to better control timing of pregnancy during lactation and thereby maximize profitability. In this regard, extending the VWP by a reasonable amount of time (20 to 30 d) may be a simple and inexpensive change with potential to impact the profitability of dairy herds.

In spite of the potential effect of manipulating the duration of the VWP on herd reproductive performance and profitability, very limited data is available about the reproductive performance and worse yet, on the profitability of dairy cows managed with different VWP duration. Indeed, in recent years many dairy farms have extended the VWP for their cows without a clear understanding of the implications to herd performance and profitability.

Potential Effects of VWP on Physiological Status of Cows before First Service

Extending the duration of the VWP may improve reproductive performance of cows through multiple mechanisms. For example, it may provide more time to recover uterine health (Gilbert et al., 2005; Sheldon et al., 2009) through improved immune status later in lactation, more time to resolve inflammation after calving, or both (LeBlanc et al., 2011;

2014). Likewise, a longer VWP may provide cows more time to resume ovarian cyclicity (Butler, 2003) allowing more estrous cycles before first service which, has been linked to fewer days to first service and greater pregnancies per AI (P/AI; Thatcher and Wilcox, 1993; Butler and Smith, 1989). Delaying first service beyond the period of negative energy balance nadir in early lactation may also improve reproductive performance by avoiding insemination during severe negative energy balance. Optimum body condition score (BCS) at the time of insemination is strongly associated with high probability of pregnancy (Souza et al., 2003; 2008; Carvalho et al., 2014).

Previous Research on VWP Duration and Reproductive Performance

Despite the potential benefits of extended VWP on reproductive performance, the impact of this management strategy on overall herd performance has not been fully elucidated. Few randomized controlled experiments have evaluated the implications of VWP duration on the reproductive performance, herd exit dynamics, and economics of dairy cows. In an experiment with a limited number of second lactation cows (i.e., 54 cows per group), Van Amburgh et al. (1999) found no differences in P/AI at first service, heat detection efficiency, and services per conception when comparing VWPs of 60 versus 150 days. Similarly, Arbel et al. (2001) observed no effect of extending VWP by 60 d (from 90 to 150 in primiparous cows and from 60 to 120 in multiparous cows) on reproductive performance of dairy cows under Israeli conditions. In this experiment, only cows with above-average milk production that did not calve during summer were included. In contrast, in an experiment conducted in Germany using only cows with above- or below-average milk production, an increment of 13 and 20 percentage points in P/AI to first service was observed when VWP was extended from 77 to 98 or from 56 to 77 DIM for high- and low-producing cows respectively (Tenhagen et al.,

2003).

More recently, Gobikrushanth et al. (2014) reported the results of a retrospective cohort study using data from a commercial farm in Florida that extended VWP duration during summer months only. Cows with the extended VWP had improved first service P/AI, more days open, and longer calving intervals. Nonetheless, results from this study might have been confounded by season of AI, as cows with short VWP (5 to 63 d) received first service during summer and fall whereas cows with long VWP (64 to 121) received first service during fall only. Moreover, the reproductive program used to submit cows for first service resulted in overlapped DIM at first service for a substantial proportion of cows.

Collectively, the ambiguous results and multiple exclusion criteria of these previous studies did not allow decisive conclusion that extending the duration of the VWP is beneficial for the reproductive and lactation performance of dairy cows or determination of the potential effects of extending the VWP on the herd exit dynamics and economics of dairy herds.

RECENT RESEARCH ON DURATION OF THE VWP

Extending VWP from 60 to 88 DIM and Using All Timed AI for First Service

We recently conducted an experiment to evaluate the reproductive performance, herd exit dynamics, and economics of dairy cows managed with a VWP of 60 or 88 DIM in commercial dairy farms. We were also interested on evaluating the effect of longer VWP on markers of physiological and energy status before first service. Based on expected physiological benefits of delaying first service (i.e., improved uterine health, reduced rate of anovulation, improved BCS, and reduced systemic inflammation), we hypothesized that extending VWP duration from 60 to 88 DIM would increase P/AI to first service and improve

overall reproductive performance (i.e., reduce overall time to pregnancy after calving).

Cows from three commercial farms in New York State were blocked by parity group (primiparous vs. multiparous) and within the multiparous group cows were stratified by total milk yield recorded for the previous lactation. Thereafter, cows were randomly assigned to a VWP of 60 [**VWP60**; n = 1,265] or 88 [**VWP88**; n = 1,260] DIM. For first service, all cows received the Double-Ovsynch (**DO**) protocol (GnRH-7 d-PGF-3 d-GnRH-7 d-GnRH-7 d-PGF-56 h-GnRH-16 to 20 h-TAI; Souza et al., 2008) for synchronization of ovulation. For second and greater AI services, cows were submitted for insemination after detection of estrus. In the three farms, cows not re-inseminated at detected estrus received TAI after resynchronization of ovulation with the Ovsynch protocol (GnRH-7 d-PGF-56 h-GnRH-16 to 20 h-TAI) initiated 32 ± 3 d after AI (**D32-Resynch**).

All farms housed cows in free-stall barns with four or six rows of stalls, milked cows thrice or twice daily (one farm), and cows were supplemented with recombinant bovine somatotropin (rbST; Sometribove zinc, Posilac, Elanco Animal Health, Indianapolis, IN).

Physiological Parameters before First Service

Our results for multiple markers of physiological status supported the hypothesis that a longer VWP would lead to an improved uterine environment, reduced anovulation, improved BCS, and reduced systemic inflammation before first service. The effect of extending VWP duration on uterine health was evident, as fewer cows were considered to have purulent vaginal discharge (**PVD**) and cytological endometritis (**CYTO**) at the beginning of DO and 10 d before TAI. The longer VWP also resulted in more cows with a BCS ≥ 2.75 , which has been associated with greater first service P/AI. Assuming that most cows lost body reserves after calving, our data

suggested that the longer interval from calving to first service for cows in the VWP88 treatment allowed recovery of more body reserves. Collectively, these observations for physiological markers and overall metabolic status suggested that providing cows more time to recover before first service was a feasible strategy to promote a physiological status more conducive to pregnancy.

A greater proportion of cyclic cows at the beginning of the DO protocol also reflected the effect of additional time for resumption of cyclicity, whereas the similar proportion of cyclic cows observed 10 d before TAI reflected the efficacy of the DO protocol to resolve anovulation. This was expected because previous studies have demonstrated that GnRH-based presynchronization protocols are effective for reducing the proportion of anovular cows before TAI (Souza et al., 2008; Herlihy et al., 2012).

Reproductive Outcomes

In support of our main hypothesis, extending the duration of the VWP from 60 to 88 DIM after synchronization of ovulation with the Double-Ovsynch protocol increased P/AI after first service in lactating dairy cows (Table 1). Nevertheless, most of the observed difference could be attributed to the greater P/AI of primiparous cows in the VWP88 treatment (no statistically significant difference for multiparous cows). The reason for the different response to treatments by parity is unclear at the moment because both groups presented a fairly similar physiological response to the extension of the VWP. Differences between parities in metabolic status, health, or both not captured by the parameters monitored in this experiment may explain such a discrepancy. As expected P/AI was greater ($P < 0.01$) for primiparous than multiparous cows (50.4 vs. 38.0%, respectively) and we also observed that cows with low (45.7%) and medium (46.7%) accumulated milk production up to 30 DIM had greater ($P = 0.02$) P/AI than cows with high

(39.9%) milk yield.

A lack of difference in pregnancy loss, proportion of cows inseminated after a detected estrus, and P/AI for second and greater services suggested that extending the duration of the VWP should not be expected to reduce pregnancy losses for cows pregnant after first service, improve the likelihood of re-insemination to estrus, or the fertility to second and greater AI services. Conversely, extending the VWP from 60 to 88 DIM, affected the total number of AI services up to 350 DIM because cows in the VWP60 treatment had more inseminations ($P = 0.04$) than cows in the VWP88 treatment (2.6 vs 2.4 services, respectively). This was the result of earlier opportunities for re-inseminations in cows not pregnant to previous AI services in the VWP60 group.

Our overall results for P/AI and re-insemination dynamics are in agreement with previous studies, which showed improved P/AI after extending the duration of the VWP (Tenhagen et al., 2003; Gobikrushanth et al., 2014). Nevertheless, direct comparisons between studies are difficult because of differences in experimental design and interactions between treatments and other confounders. Thus, in spite of substantial variation across studies, the collective results of the current experiment and others (Tenhagen et al., 2003; Gobikrushanth et al., 2014) conducted under conditions more similar to ours (i.e., using TAI and less difference in VWP duration) suggest that extending VWP duration increases P/AI to first service. The magnitude of the increment in P/AI, however, may be affected by parity, method of insemination, season, milk yield level, and the magnitude and timing of the extension of the VWP.

Because timing of pregnancy for lactating dairy cows is determined by the combined effect of all AI services rather than first service only, evaluating the pattern of pregnancy creation during the entire lactation is essential to truly determine the effect of VWP duration on reproductive performance. Rather

than focusing on first service outcomes only, dairy managers should consider the potential effect of manipulating the duration of the VWP on pregnancy dynamics during the entire lactation and for all cows. In this regard, cows in the VWP60 treatment in our experiment became pregnant at a faster rate after calving than cows in the VWP88 treatment regardless of parity as evidenced by a greater hazard of pregnancy (i.e., an indication of the different speed at which cows become pregnant in two groups) after calving. As a result, median (days at which 50% of cows were pregnant) and mean days to pregnancy were 102 and 132 d for the VWP60 treatment and 128 and 154 d for the VWP88 treatment. The hazard of pregnancy was also affected by parity ($P < 0.01$) because primiparous cows became pregnant at a faster rate than multiparous cows (HR 1.48, 95% CI 1.36 to 1.62) and milk yield up to 30 DIM ($P < 0.01$) because cows with high milk yield up to 30 DIM became pregnant at slower rate (HR 0.84, 95% CI 0.76 to 0.94) than cows with medium milk yield (no difference between high and low milk yield). Essentially, the reduced P/AI to first service for cows in the VWP60 treatment was fully compensated by the creation of more pregnancies at earlier DIM due to more and earlier opportunities for re-insemination.

Interestingly, the faster rate of pregnancy creation did not result in a reduced proportion of nonpregnant cows at 350 DIM ($P = 0.28$; VWP60 7.3 vs. VWP88 8.6%) for cows that remained in the herd. These results suggest that the greatest consequence of longer VWP is shifting timing of pregnancy towards later lactation rather than generating a different proportion of pregnant cows during lactation. In agreement, two other studies reported the same patterns of pregnancy creation (Tenhagen et al., 2003; Gobikrushanth et al., 2014) than those observed in our experiment.

Herd Exit Dynamics

Cow parity and pregnancy status are

major determinants of the herd exit dynamics in dairy farms. Pregnant cows and younger cows (e.g., primiparous vs. multiparous) have reduced risk of removal from the herd (De Vries et al., 2010; Pinedo et al., 2010). Indeed, in our experiment, a smaller proportion of primiparous than multiparous cows left the herd and primiparous cows had a similar herd exit dynamics regardless of VWP treatment (HR 1.12, 95% CI 0.77 to 1.61). These results reflected the protective effect for culling of early pregnancy and younger age. Conversely, for multiparous cows a greater proportion of cows from the VWP88 treatment exited the herd as lactation progressed ($P = 0.03$; HR 1.21, 95% CI 1.02 to 1.44) reflecting the compounded effect of delayed pregnancy and greater culling pressure in older cows.

As expected, we also observed that cows in the low milk-yield group had a greater hazard of culling ($P < 0.01$) than cows in the medium (HR 1.72, 95% CI 1.43 to 2.07) and high (HR 2.04, 95% CI 1.69 to 2.47) milk-yield groups. Therefore, milk yield level also played a role because non-pregnant cows with medium and high milk yield within each parity group had lower culling pressure than cows with low milk yield. These data suggest that through its effect on timing of pregnancy during lactation, manipulating the duration of the VWP can also affect the herd exit dynamics which may have important economic implications. As pregnancy is delayed cows are more likely to leave the herd, in particular multiparous cows.

Economic Outcomes

We monitored cows enrolled in our experiment for a total of 18 mo after calving to determine individual cow profitability based on income over feed cost (IOFC), replacement costs, reproductive programs costs, rbST supplementation cost, operating expenses, and value of calves born. Cash flow for an 18-mo period after calving for each cow enrolled in the experiment was calculated by addition of all these expenses and revenues. In order to better represent the reality of a dairy farm, we

considered that every cow enrolled in the trial filled up a slot at the dairy and the slot had to remain occupied for the entire 18-mo period to maintain herd size constant. Therefore, every cow that left the herd due to sale or death was replaced by a randomly selected first lactation cow from the same experimental treatment. This cow contributed with expenses and revenues up to the end of the 18-mo period (i.e., filled up the original slot occupied by the cow it replaced). If the replacement cow left the herd before the end of the 18-mo period it was also replaced by another randomly choose first lactation. The method used for our economic analysis (i.e., fixed period of time including a significant portion of the lactation following application of the experimental treatments), was meant to better represent the effect of reproductive performance on herd profitability. Otherwise, the effect of timing of pregnancy on the current and subsequent lactation is not captured. It also important to note that due to substantial differences in performance and profitability between primiparous and multiparous cows, data was analyzed separately by parity group. Data for profitability by parity group is presented in Table 2.

For primiparous cows, cash flow per slot per 18-mo or per day was similar ($P = 0.32$) for the VWP60 and VWP88 treatment despite a \$68 numerical difference in favor of the VWP88 group. Interestingly, most of the difference between treatments was due to greater replacement cost for the VWP60 treatment because the small differences observed for the rest of the parameters offset each other. The difference in replacement cost was primarily due to greater cost in the subsequent lactation because a slightly greater percentage of second lactation cows (i.e., first lactation during experimental lactation) left the herd before the end of the 18-mo period. Thus, when attempting to extrapolate the results of our experiment to other farms, it is important to recognize the dominance of replacement cost over total profitability because a different replacement cost dynamics (i.e., different

culling pressure and different cash cost of culling) may be observed across herds and changing market conditions. Indeed, when we simulated the potential effect of changes in economic conditions (i.e., milk prices, reproductive cost, heifer replacement costs, calf values), replacement costs accounted for up to ~80% of the total variation in profitability.

Interestingly, the results for multiparous cows were opposite to those of primiparous cows. Although a statistically significant difference between treatments was not observed, cows in the VWP60 were more profitable by \$85 per slot per 18-mo than cows in the VWP88 treatment. In this case, however, replacement cost was greater for the VWP88 reflecting increased culling pressure in non-pregnant cows in the VWP88 treatment in later lactation. Such contrast in results for overall cash flow likely reflected differences in milk production persistency (i.e., lactation curves are less persistent for multiparous than primiparous) and the interaction between parity and risk of leaving the herd as lactation progressed (i.e., only multiparous but not primiparous cows in the VWP88 were more likely to leave the herd). Although to a lesser extent than for primiparous cows, replacement cost explained a substantial proportion of the numerical economic differences for the results with fixed economics values or when we simulated varying economic conditions.

In summary, the economic outcomes for our experiment suggest that extending the VWP from 60 to 88 DIM when using all TAI to submit cows for first service may result in greater (numerical) profitability for primiparous cows, primarily through a reduction in replacement costs. Conversely, the same extension of the VWP duration for multiparous cows may lead to economic losses (numerical) primarily due to greater replacement cost and reduced IOFC which, cannot be offset by reduced reproductive program costs.

Results from our experiment should be interpreted with caution because in spite of the large number of cows in our experiment we did not detect statistically significant differences for overall cash flow, the fact that all cows received rbST, and the particular replacement dynamics of the herds involved in our research which, may have been affected by individual farm management decisions and the economic conditions during the trial. Of note, the method used to calculate cow profitability can also vary depending on whether profitability per unit of time and slot or, per cow regardless of time and herd size constraints are calculated.

Effect of Method of Submission for First Service and VWP Duration on Reproductive Performance

Dairy managers need to determine not only the duration of the VWP for their cows but also, the type of management strategy to submit cows for first service. In this regard, the effect of extending the VWP from 60 to 88 DIM on first service P/AI and subsequent reproductive performance in the experiment described above may have been specific to the use of all TAI with a GnRH-based fertility protocol (i.e., Double-Ovsynch). For example, using all TAI results in a narrow range of DIM to first service regardless of the ability of cows to display estrus behavior. This reduces variation of not only DIM to first service but also for second and greater AI services. By resolving anovulation, proper synchronization of ovulation, and optimization of the endocrine environment before insemination in a majority of cows (Souza et al., 2008; Herlihy et al., 2012; Giordano et al., 2013); GnRH-based protocols may also offset the detriment of shorter VWP on P/AI to a greater extent than programs not including synchronization of ovulation or synchronization of ovulation with PGF-based protocols. Thus, the method of submission to first service and the type of synchronization of ovulation protocol, if any is used, are important considerations at the time of defining the

duration of the VWP. In this regard, many dairy farms continue to submit cows for first service through a combination of detection of estrus and TAI after the Presynch-Ovsynch protocol. Cows detected in estrus after Presynch (two PGF treatments 14 d apart) are inseminated, whereas the rest of the cows receive TAI after completion of the protocol. In this case many farms use a VWP of approximately 50 to 60 DIM. Therefore, a reasonable question is how programs that combine AI at detected estrus and TAI compare to all TAI programs at different VWP durations.

Effect of a Combined Approach versus All TAI and Different VWP on Reproductive Performance of Dairy Cows

As part of the experiment described above, in one of the participating farms we also included an additional treatment that consisted of a typical combined program with the Presynch-Ovsynch (**PSOv**) protocol. Cows in this treatment were allowed to be inseminated at detected estrus any time after 50 ± 3 DIM coincident with the second PGF treatment of Presynch. The other two treatments consisted of all TAI after the Double-Ovsynch protocol at 60 ± 3 (**DO60**) or 88 ± 3 (**DO88**) DIM, as described. Cows in the three treatments were managed equally for second and greater AI services.

Our most relevant findings were that cows managed for first service with the combined approach and VWP of 50 DIM had similar time to pregnancy during lactation than cows managed with all TAI and VWP of 60 DIM. In addition, both treatments with a shorter VWP had reduced time to pregnancy than the DO88 treatment group (Figure 2). As a result, median and mean days to pregnancy were 90 and 123 for DO60, 96 and 126 for PSOv, and 116 and 150 for DO88.

Although the overall effect of VWP duration on P/AI at first service followed the same trends (not the same differences were detected due to lack of statistical power) as for

the larger experiment (presented above), the positive effect of longer VWP on this farm was not as dramatic favoring the groups with shorter VWP. This was particularly important for multiparous cows which had the exact same P/AI after all TAI at 60 or 88 DIM, and only ~4 percentage points lower overall P/AI for cows in the Presynch-Ovsynch treatment. Thus, we concluded that first service management programs that result in a similar range of DIM to first service regardless of being a combined approach or all TAI (e.g., AI at detected estrus and TAI with Presynch-Ovsynch with 50 d VWP and all TAI with Double-Ovsynch and 60 d VWP) can lead to similar time to pregnancy after calving. In addition, these programs with shorter VWP and similar range of DIM at first service can reduce time to pregnancy when compared to an all TAI program with an extended VWP (i.e., 88 DIM) which does not result in a substantial increment in first service P/AI. Our observations, are particularly important for herds that extend the duration of the VWP and do not observe a substantial increment in first service P/AI. Indeed, in our trial we estimated that to have the same proportion of pregnant cows at approximately 90 DIM, P/AI at first service for the program with extended VWP should have been 10 to 11 percentage points greater for primiparous and 7 to 12 percentage points greater for multiparous cows.

Because economic differences between reproductive management programs depend on multiple factors beyond timing of pregnancy during lactation, as clearly seen in our comparison of all TAI at 60 vs 88 DIM, it remains to be determined which one of the strategies was the most profitable in our experiment for programs using a combination of AI at detected estrus and TAI vs all TAI.

CONCLUSION

In conclusion, manipulating the duration of the VWP affects herd reproductive

performance, exit dynamics (i.e., cow sales), and profitability. From a reproductive performance perspective the greatest effect of delaying the end of the VWP is greater P/AI to first service (in particular for primiparous cows) and an overall delay in time to pregnancy which, may increase the risk of leaving the herd (in particular for multiparous cows).

Economically, extending the duration of the VWP as in our experiments may increase profitability of primiparous cows and reduce profitability of multiparous cows. Such effect would depend mostly on the herd replacement dynamics and milk production efficiency.

First-service management strategies that combine insemination of cows at detected estrus and TAI for first service (e.g., Presynch-Ovsynch) may result in similar days to pregnancy during lactation provided that average DIM at first service is similar than for all TAI programs and first service P/AI for the combined program is reasonable. Management programs that reduce DIM at first service through AI at detected estrus and TAI or all TAI can reduce time to pregnancy when compared with all TAI programs with longer VWP. In particular, when the extension of the VWP does not substantially increase first service P/AI.

Collectively, data from our recent research suggest that the effect of VWP duration and first service management strategies on dairy herd performance depends upon complex interactions between the pattern of insemination for first service, pregnancy per AI, and herd exit dynamics, all of which may vary for primiparous and multiparous cows. As a result, dairy managers should consider these complex interactions when defining VWP duration for their lactating dairy cows.

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REFERENCES

- Arbel, R., Y. Bigun, E. Ezra, H. Sturman, and D. Hojman. 2001. The effect of extended calving intervals in high lactating cows on milk production and profitability. *J. Dairy Sci.* 84:600-608.
- Butler, W. and R. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767-783.
- Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211-218.
- Carvalho, P., A. Souza, M. Amundson, K. Hackbart, M.Fuenzalida, M. Herlihy, H. Ayres, A. Dresch, L. Vieira, and J. Guenther. 2014. Relationships between fertility and postpartum changes in body condition and body weight in lactating dairy cows. *J. Dairy Sci.* 97:3666-3683.
- De Vries, A. 2006. Economic value of pregnancy in dairy cattle. *J. Dairy Sci.* 89:3876-3885.
- Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology* 64:1879-1888.
- Giordano, J. O., M. C. Wiltbank, P. M. Fricke, S. Bas, R.Pawlisch, J. N. Guenther, and A. B. Nascimento. 2013. Effect of increasing GnRH and PGF 2 α dose during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. *Theriogenology* 80:773-783.
- Gobikrushanth, M., A. De Vries, J. Santos, C. Risco, and K. Galvão. 2014. Effect of delayed breeding during the summer on profitability of dairy cows. *J. Dairy Sci.* 97:4236-4246.
- Herlihy, M., J. Giordano, A. Souza, H. Ayres, R. Ferreira, A. Keskin, A. Nascimento, J. Guenther, J. Gaska, and S. Kacuba. 2012. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. *J. Dairy Sci.* 95:7003-7014.

LeBlanc, S. 2014. Reproductive tract inflammatory disease in postpartum dairy cows. *Animal* 8:54-63.

LeBlanc, S. J., T. Osawa, and J. Dubuc. 2011. Reproductive tract defense and disease in postpartum dairy cows. *Theriogenology* 76:1610-1618.

Pinedo, P., A. De Vries, and D. Webb. 2010. Dynamics of culling risk with disposal codes reported by Dairy Herd Improvement dairy herds. *J. Dairy Sci.* 93:2250-2261.

Sheldon, I. M., J. Cronin, L. Goetze, G. Donofrio, and H.-J. Schuberth. 2009. Defining Postpartum Uterine Disease and the Mechanisms of Infection and Immunity in the Female Reproductive Tract in Cattle. *Biol. Reprod.* 81:1025-1032

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

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

Tenhagen, B.-A., C. Vogel, M. Drillich, G. Thiele, and W. Heuwieser. 2003. Influence of stage of lactation and milk production on conception rates after timed artificial insemination following Ovsynch. *Theriogenology* 60:1527-1537

Thatcher, W. and C. Wilcox. 1973. Postpartum Estrus as an Indicator of Reproductive Status in the Dairy Cow. *J. Dairy Sci.* 56:608-610.

Van Amburgh, M.E., D.M. Galton, D.E. Bauman, and R.W. Everett. 1997. Management and economics of extended calving intervals with use of bovine somatotropin. *Livest. Prod. Sci.* 50:15-28.