Estrous detection and timed AI protocols: complementary not mutually exclusive

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Introduction

Since the 1990s timed artificial insemination (AI) protocols have been developed to improve the AI submission rate (number of cows that receive AI divided by the number of cows that are eligible to be inseminated over a 21 day interval), also known as heat or estrus detection rate, of lactating dairy cows. More recently, a better understanding of reproductive physiology has resulted in timed AI protocols that may result in pregnancy per AI (P/AI) of up to 45% in high producing lactating dairy cows. (Santos *et al*, 2010; Souza *et al*, 2008). Nonetheless, the greatest benefit of timed AI protocols to reproductive performance of dairy herds is increased AI submission rates. Thus, often the decision of whether or not to use timed AI protocols is based on the AI submission rates achieved when AI occurs based only on estrous detection (ED). Other factors like accuracy of ED and compliance with timed AI protocols chosen are also important to reproductive performance.

Upon the advent of timed AI protocols many suggested that daily ED of lactating dairy cows would no longer be necessary. Timed AI protocols make use of reproductive hormones like GnRH, prostaglandin (PG) $F_{2\alpha}$, and progesterone (P4). The use of these hormones for reproductive management of dairy cows may undergo scrutiny by consumers similar to what has been observed in regards to the use of antimicrobials, possibly limiting their use. The recent growth in number of companies commercializing activity monitors for detection of estrus has resulted in several companies claiming that the implementation of such activity monitors would eliminate the need for timed AI protocols.

The goal of this brief review is to evaluate whether reproductive programs for lactating dairy cows may be solely dependent on timed AI protocols or AI on detected estrus. A few examples of dairies that have attempted to eliminate one or the other will be given, but I caution that some of these examples are merely data extracted from on farm software and not the result of controlled studies.

The Challenges of Estrous Behavior for Lactating Dairy Cows

Unquestionably lactating dairy cows have reduced expression of estrus compared with dairy heifers and beef animals because of physiological characteristics,often because of increased incidence of pathological conditions, and because of management.

Immediately postpartum, cows undergo a physiological anovular condition characterized by the lack of ovulation and formation of a corpus luteum (CL) until approximately 25 to 30 days postpartum (Butler, 2000). However, cows that have postparturient diseases and undergo more severe loss of body condition score (BCS) have more prolonged anovular condition. Cows that had no change in BCS from calving to first postpartum AI (approximately 65 DIM) and cows that lost < 1 unit of BCS from calving to first postpartum AI were 2.0 and 2.4 times more likely, respectively, to be cyclic by 65 DIM than cows that lost > 1 unit of BCS during this period (Santos *et al*, 2009). Furthermore, cows diagnosed with mastitis in early postpartum (mastitis = 39 vs healthy = 32 days; Huszenicza *et al*, 2005) and cows diagnosed as lame within the first 30 DIM (lame =34 vs healthy = 29 days; Garbarino *et al*, 2006) had a prolonged anovular condition than healthy cows. Postponed resumption of ovarian cycles results in delayed establishment of pregnancy because of reduced AI submission rates and reduced P/AI (Chebel *et al*, 2010; Santos *et al*, 2009).

Limited access to open lots/dirt lots also seems to be a limiting factor for AI submission rate among lactating dairy cows. Vallies and Britt (1989) demonstrated that mounting activity was 15-fold greater for lactating dairy cows with access to open lots than cows housed solely on concrete.

Onset of lactation affects expression of estrus by reducing concentrations of estradiol. Lopez *et al* (2004) demonstrated that cows with greater milk yield (102.1 ± 0.9 lb or 46.4 ± 0.41 kg/day) had reduced duration of estrus (6.2 ± 0.5 vs 10.9 ± 0.7 hours) and reduced number of mounts during estrus (6.3 ± 0.4 vs 8.8 ± 0.6 mounts) compared with cows with reduced milk yield (73.7 ± 0.7 lb or 33.5 ± 0.32 kg/day). Furthermore, the same group demonstrated that high producing dairy cows (103 ± 2.2 lb or 46.8 ± 1 kg/day) had reduced estradiol concentration on the day of estrus (6.8 ± 0.5 vs 8.6 ± 0.5 pg/ml) despite having larger follicles (18.6 ± 0.3 vs 17.4 ± 0.2 mm) compared with low producing dairy cows (71.1 ± 1.3 lb or 32.3 ± 0.59 kg/day; Lopez *et al*, 2004). This resulted in reduced length of estrus (7 ± 1.1 vs 11.9 ± 1.4 hours) and number of mounts during estrus (6.5 ± 0.9 vs 9.8 ± 1) for high producing cows compared with low

producing dairy cows (Lopez *et al*, 2004). Even though the reasons for the reduced estradiol concentrations of estradiol during estrus in lactating dairy cows are not completely elucidated, the currently most accepted hypothesis is that the elevated dry matter intake of high producing cows,necessary to meet nutritional requirements of lactation, results in greater blood flow through the liver, the most important site of steroidal hormones catabolism. In a series of experiments, Sangsritavong *et al* (2002) demonstrated that onset of feed intake resulted in significant increase in blood flow to the liver, and that the increase in blood flow was dependent on amount of feed consumed (Figure 1). Furthermore, lactating dairy cows fed 7.8 lb (3.5 kg) of dry matter had greater clearance rate of P4 at one and two hours after feeding compared with unfed cows (Sangsritavong *et al*, 2002). Cows fed 23.4 lb (10.6 kg) of dry matter had greater P4 clearance rate from two to four hours after feeding than unfed cows, whereas cows fed 33.4 lb (15.2 kg) of dry matter had greater P4 clearance rate from one to four hours after feeding compared with unfed cows (Sangsritavong *et al*, 2002). Similarly, lactating dairy cows fed ad libitum had greater estradiol clearance rate from 2 to 4.5 hours after onset of feeding compared with unfed cows (Sangsritavong *et al*, 2002).

Clearly, physiological and pathological conditions share the blame for reduced AI submission rates among lactating dairy cows. Because of the great importance of AI submission rate to the overall reproductive efficiency of lactating dairy cows, different ED and/or AI submission strategies (i.e. timed AI protocol) should be implemented in herds where adequate AI submission rates are not achieved.

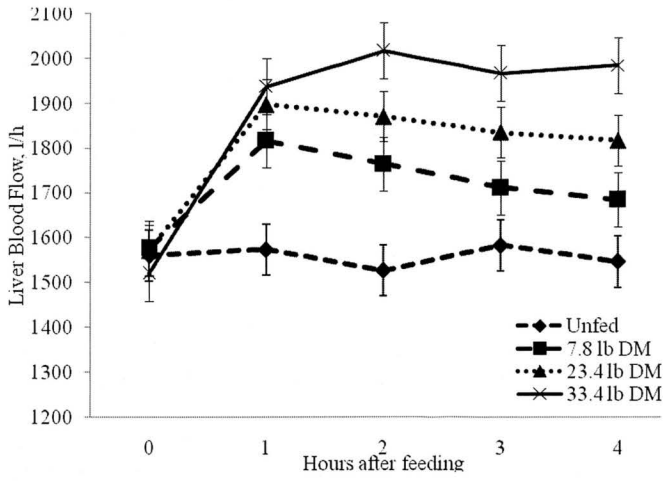

Figure 1. Effect of feed intake on liver blood flow. Adapted from Sangsritavong *et al* (2002).

Estrous Detection and Timed AI protocols: Complementary not Mutually Exclusive

In one comprehensive survey conducted in 103 dairy herds from at least 12 states, 74.8% of the herds indicated that an estrus/ovulation synchronization program for first postpartum AI was implemented (Caraviello *et al*, 2006). However, when data from 33 million inseminations of Holstein and Jersey cows from Dairy Herd Improvement Association herds were analyzed, it was estimated that the percentage of herds that did not use synchronization protocols was 94.8% in 1996 and 72.5% in 2005 (Miller *et al*, 2007). Thus, it is clear that a lot of variability exists in regards to implementation of timed AI protocols, let alone the types of timed AI protocols used.

In general, the implementation of timed AI protocols results in reduced intervals from parturition to first postpartum AI, reduced variability in interval to first postpartum AI, and may reduce the interval from parturition to establishment of a new pregnancy (Miller *et al*, 2007). These beneficial results, however, are highly dependent on the base line reproductive performance of the herd before adoption of such protocols. Simply put, herds that achieve good AI submission rates and P/AI without timed AI protocols do not necessitate the latter. In the opinion of this author, however, only when P/AI achieved through AI on estrus is extremely poor (poor ED accuracy) should programs based 100% on timed AI protocol be recommended. This is simply a matter of mathematics. Even though a 100% AI submission rate may be achieved in the first 21-day cycle after the end of the voluntary waiting period (VWP), pregnancy diagnosis cannot be conducted until 25 days after AI at the earliest. Thus, re-insemination of nonpregnant cows could only occur as early as 28 days after a previous AI, resulting in the 21-day cycle immediately after AI with AI submission rate of 0% and the following 21-day cycle with a AI submission rate of 100%. Thus, herds with 100% timed AI would struggle to achieve AI submission rate greater than 60%, depending on P/AI.

Therefore, the question that must be answered is, "What are the breakeven points in the decision for 100% timed AI, 100% ED, or both?" To answer that question, published research must be considered and the outcomes obtained with different timed AI protocols and the reported P/AI following AI on estrus. It is important to remind readers that the numbers presented in peer-reviewed manuscripts are often inflated because they result from well controlled studies and often sick cows (i.e. extremely lame, low BCS, etc.) and cows that fail to receive the appropriate treatments are removed from the study.

One of the first experiments to evaluate the economic benefits of reproductive strategy based on ED or

timed AI was conducted in Germany (Tenhagen *et al*, 2004). In this experiment, cows from two herds were either only inseminated based on estrus or were inseminated at fixed time until approximately 200 DIM. In the herd in which the AI submission rate of cows inseminated on estrus was 29%, the timed AI protocol resulted in significant improvements in AI submission rate (65%) and pregnancy rate (14 vs 25%). On the other hand, in the herd where the AI submission rate of cows inseminated on estrus was 55%, the timed AI protocol slightly increased the AI submission rate (70%), but had no significant effect on pregnancy rate (25 vs 29%). Consequently, in the herd with poor AI submission rate when the ED was used, the addition of timed AI to the reproductive management resulted in reduced cost per pregnancy generated (€ 363 vs € 264). On the other hand, the cost per pregnancy was similar among cows submitted to the ED protocol (€ 251) or the timed AI protocol (€ 272) in the herd in which the AI submission rate in the ED treatment was 55%. This was one of the first experiments to suggest that in herds that only inseminate cows in estrus and have AI submission rate greater than 55%, the use of timed AI protocols may not be necessary.

In two recent publications, researchers compared the economic outcomes of reproductive strategies based on ED, timed AI, or a hybrid between ED and timed AI. These experiments used modeling techniques to simulate the economic return of the different reproductive programs.Giordano *et al* (2001) evaluated economic return of reproductive programs for lactating dairy cows based on ED, the double Ovsynch protocol for first AI, and the Ovsynch protocol for resynchronization of cows starting 32 days after the previous AI (DO-Res), and the double Ovsynch program for first AI and resynchronization (DO-DO). The DO-Res ($17 cow/year over the cost of the ED program) and the DO-DO ($21 cow/year over the cost of the ED program) programs were more expensive than the ED protocol. On the other hand, the DO-Res and the DO-DO protocols resulted in income per cow/year $45 and $69 greater, respectively, than the ED protocol. The authors, however, based their calculations of economic return on P/AI results from one study and on farm data for ED cows. As such, P/AI to first AI and resynchronization were 45 and 30%, respectively, for the DO-Res protocol, 45 and 39%, respectively, for the DO-DO protocol, and 33 and 30%, respectively, for the ED protocol. It is not surprising, therefore, that with such differences in fertility, the DO-DO and DO-Res protocols resulted in greater economic return than the ED protocol. Nonetheless, P/AI of cows subjected to timed AI is not significantly greater than P/AI of cows inseminated following synchronized estrus, based on several published papers that did not use the Double-Ovsynch protocol (Chebel and Santos, 2010; Santos *et al*, 2009; Santos *et al*, 2004a; Tenhagen *et al*, 2004).

Galvão *et al* (2012) modeled reproductive performance and economics based on the adoption of one of 10 breeding programs. The breeding programs evaluated were based on ED or timed AI and taking into consideration differences in ED efficiency (40 or 60%) and accuracy (85 or 95%), compliance to injections of the synchronization protocols (85 or 95%), and milk price ($0.15 or $0.20/lb; $0.33 or $ 0.44/kg). The reproductive programs evaluated were ED with differing ED efficiency and accuracy, timed AI for all with differing compliance to injections, and timed AI for first AI with differing compliance followed by ED with differing ED efficiency and accuracy. Pregnancy per AI for first AI was assumed to be 33.9% and P/AI of subsequent AI decreasing by 2.6% for every AI, pregnancy loss was assumed to be 11.3%, cows were deemed not eligible for insemination if not pregnant after 366 DIM, and were culled by 450 DIM if not pregnant. All costs associated with the reproductive programs and feeding were taken into consideration. Milk price was set at $0.15 or $0.20/lb ($0.33 or $0.44/kg), cull cows were sold for $0.30/lb ($0.65/kg) of live weight, and calves were sold for $140/calf. Under these assumptions, when the herd used timed AI for first postpartum AI with 95% compliance to injections and ED for subsequent AI with ED with 60% efficiency and 95% accuracy, the greatest 21-day cycle pregnancy rate was achieved (Figure 2A; Galvão *et al*, 2012). Similarly, this reproductive program resulted in the shortest median days to pregnancy (113; Figure 2B) and the greatest profit per cow/year ($375/cow for milk price = $0.15/lb ($0.33/kg); profit of $1,616/cow for milk price = $0.20/lb ($0.44/kg) of milk).

Therefore, postponing re-insemination of cows that return to estrus in order to submit them to timed AI protocols seems illogical because of the consequent increased interval to re-insemination. As mentioned before, the only reason to avoid insemination and, particularly, re-insemination in estrus is poor ED accuracy, which results in reduced P/AI of cows inseminated in estrus. Dairy farms in the United States (US) commonly utilize timed AI protocols in association with insemination on estrus. Approximately 55% of dairy farms rely primarily on detection of estrus as the major method to inseminate cows (NAHMS, 2009). Among the winners of the award for Excellence in Reproductive Management of the Dairy Cattle Reproductive Council between the years of 2009 to 2011, 21 out of 24 used AI on detected estrus associated with timed AI protocol, two out of 24 used only timed AI protocols, and one out of 24 used only AI on detected estrus.

Recently, several companies in the US have started to commercialize activity monitors for detection of estrus. Activity monitors are placed in the collars or on legs of cows, and determine the walking distance and pattern

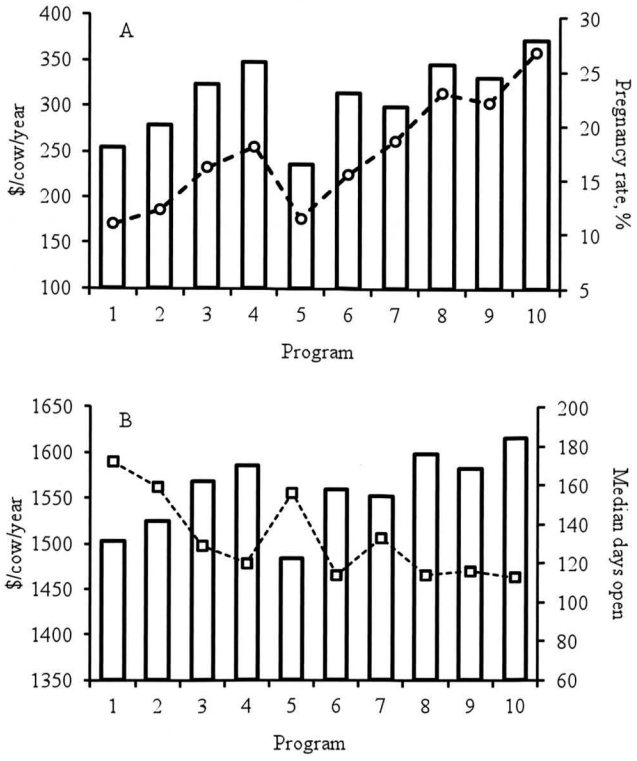

Figure 2. Profits per cow per year ($/cow/year) of cows subjected to 1 of 10 breeding programs: 1) ED at 40% efficiency and 85% accuracy; 2) ED at 40% efficiency and 95% accuracy; 3) ED at 60% efficiency and 85% accuracy; 4) ED at 60% efficiency and 95% accuracy; 5) timed AI for all AI (85% compliance); 6) timed AI for all AI (95% compliance); 7) timed AI for first AI (85% compliance) followed by ED at 40% efficiency and 85% accuracy; 8) timed AI for first AI (95% compliance) followed by ED at 40% efficiency and 85% accuracy; 9) timed AI for first AI (85% compliance) followed by ED at 60% efficiency with 85% accuracy; and 10) timed AI for first AI (95% compliance) followed by EDat 60% efficiency with 95% accuracy. In panel A, bars represent the profit per cow per year calculated using milk price at $ 0.33/kg and dashed lines represent the 21-day cycle pregnancy rate. In Panel B, bars represent the profit per cow per year using milk price at $ 0.44/kg (panel B). Dashed lines represent either the 21-day cycle pregnancy rate (panel A) or median days open (panel B). Courtesy of Ribeiro *et al* (2012): Adapted from Galvão *et al* (2012).

of cows. Once a cow presents an excessively elevated walking pattern, the system flags the cow as suspect for estrus. These systems have been used in other countries (i.e. Israel) for several years and have presented very good results. Interestingly, however, it has been proposed that implementation of electronic methods for detection of estrus would eliminate the need of any timed AI protocol because of its efficiency and accuracy. This is a somewhat ambitious claim, particularly considering the physiological and pathological challenges that affect onset of estrus and estrous behavior of high-producing lactating dairy cows. Anovulation, low estrous expression associated with high-production, and other less prevalent abnormalities such as persistent corpora lutea or pregnancy loss after day 21 of the preceding AI would all reduce the efficacy of estrous detection and result in more nonpregnant cows being diagnosed at the day of pregnancy diagnosis. Some have claimed that electronic monitoring systems can detect 99% of the cows that display estrus. This high sensitivity should not imply that electronic monitoring systems will result in 99% AI submission rate. The key issues here are cows that remain anovular after the end of the VWP (10 to 50% of cows depending on interval from calving, herd, parity, etc.) and cows not pregnant from previous inseminations that will not return estrus within 21 days after a previous AI. Therefore, anovular cows and cows with an abnormal interestrus interval would not be detected in estrus and AI submission rates, which are calculated using 21-days cycles, would likely be approximately 50 to 60%, and not any higher.

Nonetheless, until recently there were no controlled experiments to determine whether activity monitors could eliminate the need for time AI protocols completely. Two recent experiments, however, indicated that activity monitors are not able achieve AI submission rates of 90 to 95% as some companies were claiming because some cows will not display estrus. Valenza *et al* (2011) fitted 42 cows with an activity monitor system (collar) and a mounting detection system (Kamar). The cows were synchronized and allowed to come in estrus. Cows were then examined by ultrasound to determine ovarian activity and occurrence of ovulation. In this small experiment, according to activity monitor and mounting detector, 67 and 62%, respectively, of cows were observed in heat and ovulated; seven and 12%, respectively, of cows were not observed in heat and ovulated; 5% of cows were observed in heat and did not ovulate; and, 21% of cows were not observed in heat and did not ovulate. Therefore, based on an activity monitor system and a mounting detection system, 28 to 33% of cows, respectively, were not observed in estrus. Furthermore, considering ovulation as the 'gold standard', cows that ovulated and were in estrus were +/+, cows that did not ovulate and were in estrus were -/+, cows that ovulated and were not in estrus were +/-, and cows that did not ovulate and were not in estrus were -/-. Thus, the activity monitor system and the heat detection system resulted in sensitivity of 91 and 84%, respectively, specificity of 81%, positive predictive value of 93%, and negative predictive value of 75 and 64%, respectively. Therefore, based on this small experiment the activity monitor and mounting detection system had similar performance.

In a study presented at the 2012 American Dairy Science Association, researchers evaluated the insemination pattern and P/AI of cows that were fitted with activity monitors and were submitted to the Ovsynch protocol with ED (Ovs), to the Presynch/Ovsynch with ED (PresOvs), and to the Presynch/Ovsynch protocol without ED (100%TAI; Fricke *et al*, 2012). In this study, 70% of cows that received the two $PGF_{2\alpha}$ presynchronizing injections were observed in estrus, whereas approximately 57% of cows that were not presynchronized with $PGF_{2\alpha}$ were observed in estrus. The P/AI of cows inseminated in estrus was 30% and the P/AI of cows inseminated at fixed time was 36%. These numbers are very similar to those reported by Stevenson and Phatak (2005), Chebel *et al* (2006), Lima *et al* (2009), and Chebel *et al* (2010). In these studies, the percentage of cows that were inseminated in estrus after two presynchronizing injections of $PGF_{2\alpha}$ ranged from 50 to 62%. On the other hand, P/AI of cows inseminated in estrus ranged from 27 to 44%, and P/AI of cows inseminated at fixed time ranged from 21 to 41%. The results from these studies suggest that activity monitors may perform just as well as detection of estrus based on tail paint removal, and that P/AI of cows inseminated in estrus based on activity or tail paint removal may be similar, these being extremely dependent on farm and personnel.

Field observations of two herds that adopted the activity monitor systems for estrus detection and abolished the use of fixed time AI for first postpartum AI demonstrate that there is a significant risk of significantly increasing the variability in interval to first AI, increasing interval to first postpartum AI, and reducing AI submission rate and pregnancy rate. In Figure 3A and 3B, the patterns of first postpartum AI of herds that started using timed AI protocols for first postpartum AI are depicted. In Figure 3C and 3D, the patterns of first postpartum AI of herds that stopped using timed AI protocols once they implemented activity monitoring systems are depicted. Although this is not data from controlled studies, it is possible to observe that once timed AI protocols stopped being used in the herds that adopted the activity monitoring system, their pattern of first postpartum AI started to resemble the pattern of first postpartum AI before timed AI protocols were widely adopted.

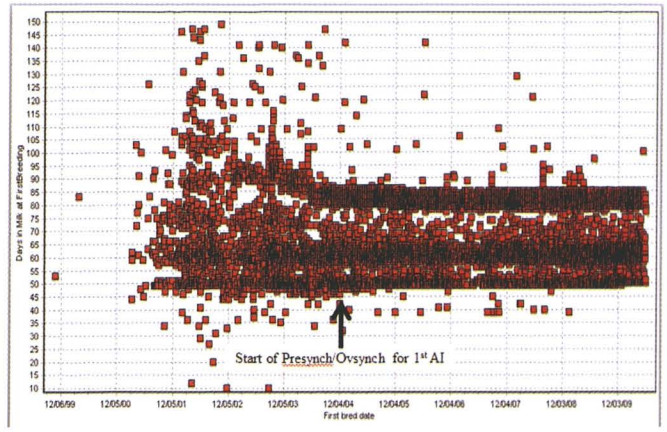

Figure 3A. Pattern of first postpartum AI of a dairy herd in CA (1,600 lactating cows) that implemented timed AI starting December of 2003.

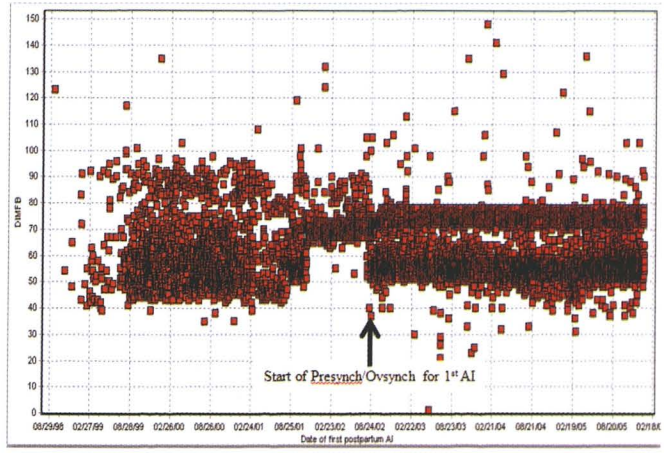

Figure 3B. Pattern of first postpartum AI of a dairy herd in CA (2,300 lactating cows) that implemented timed AI starting August of 2002.

Conclusions

It is widely known that estrous expression and estrous detection of lactating dairy cows are compromised by several physiological, pathological, and managerial factors. The advent of timed AI protocols has resulted in significant improvements in AI submission rates, a very important component of reproductive efficiency and perhaps the easiest parameter to manipulate with different managerial strategies. Activity monitoring systems are also an exciting tool for the reproductive management of dairy cows that has significant value. Nonetheless, the selection of reproductive strategies should be made in light of estrous detection efficiency and accuracy, and in consideration of availability of facilities and personnel.

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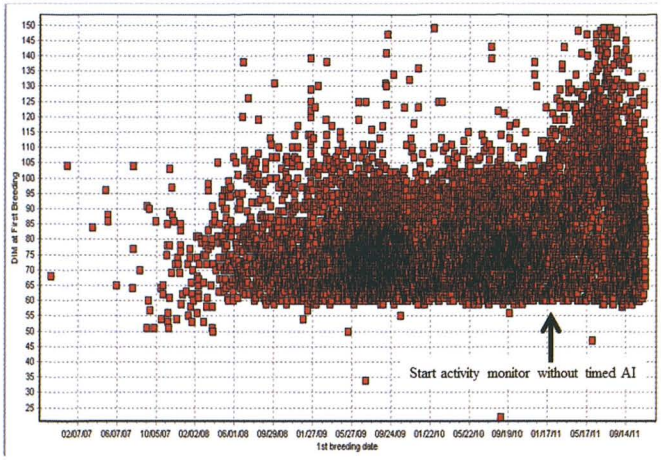

Figure 3C. Pattern of first postpartum AI of a dairy herd in MN (3,100 lactating cows) that implemented the activity monitoring system without timed AI starting January of 2011.

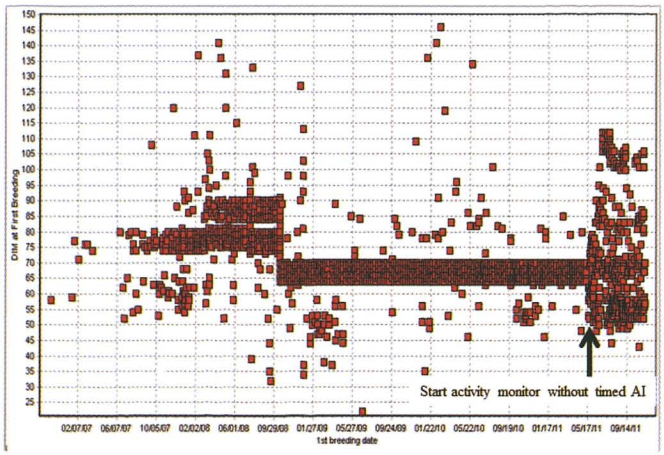

Figure 3D. Pattern of first postpartum AI of a dairy herd in MN (800 lactating cows) that implemented the activity monitoring system without timed AI starting May of 2011.

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