Theriogenology 81 (2014) 170–185

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

40th Anniversary Special Issue

The cow as an induced ovulator: Timed AI after synchronization of ovulation

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ARTICLE INFO

Article history:
Received 10 August 2013
Received in revised form 17 September 2013
Accepted 18 September 2013

Keywords:
Ovsynch
Timed AI
Synchronization of ovulation
GnRH
Prostaglandin $F_{2\alpha}$

ABSTRACT

Timed-AI after synchronization of ovulation has become one of the most used reproductive technologies developed during the past 40 years. Various adaptations of this technology are now extensively used worldwide, in the beef and dairy cattle industry. Our well-cited report, published in Theriogenology in 1995, presented a method termed Ovsynch, that used GnRH and $PGF_{2\alpha}$ to perform synchronization of ovulation and timed AI in lactating dairy cows. This report introduced Ovsynch, more as a concept of induced ovulation, and demonstrated the ovarian dynamics during the protocol. Validation and improvements on this method were subsequently performed in numerous university studies and on commercial dairies, worldwide. This review will provide a brief historical background, some personal recollections, and certain modifications that have been made in synchronization of ovulation protocols. Each section emphasizes the physiology that underlies the most widely-used synchronization of ovulation protocols and key modifications and some practical application of these protocols on commercial operations. Finally, the effect of timed AI in the US dairy industry and in the Brazilian beef cattle industry are compared. Although numerous studies have been done using these protocols, there is still substantial need for research to improve the synchronization, efficacy, simplicity, and practical application of these protocols.

1. Historical introduction

Synchronization has been defined as “the coordination of events to operate a system in unison” (Wikipedia). A long-standing vision of reproductive animal scientists has been the precise coordination of the reproductive hormones with corpus luteum (CL) and follicle development to produce an optimized hormonal environment in which all animals ovulate and become pregnant in unison; perfect synchronization [1–6]. The report published by us in *Theriogenology* in 1995 [7] proposed a novel method, termed Ovsynch, for synchronization of ovulation using two treatments with GnRH and a single treatment with $PGF_{2\alpha}$. Unfortunately, this simple method did not produce a perfect synchronization with all cows ovulating in unison in an ideal hormonal environment. However, a sufficient percentage of cows were synchronized to achieve similar fertility after timed AI after Ovsynch as was achieved after AI to estrus in high-producing lactating dairy cows [8–10]. Since that publication, we have learned a great deal about synchronization of ovulation and timed AI through the research efforts of many different laboratories across the United States and the world. We were invited to write this review to provide our current perspective on Ovsynch and timed AI.

Naturally induced ovulators, such as the rabbit, cat, and llama, will have an LH surge and ovulation induced by the act of coitus. In animals that naturally have spontaneous ovulation, such as the cow, the LH surge and ovulation is induced at a specific, difficult to predict, time of the estrous cycle. The process is initiated by increases in estradiol (E2)

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0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.09.017

to a sufficient concentration for a sufficient duration, in the absence of circulating progesterone (P4), to induce a GnRH surge from the hypothalamus, which induces an almost immediate surge in LH from the pituitary. In response to the LH surge, the original source of the elevated E2, the preovulatory follicle, will begin to undergo changes that result in reduced E2 production, breakdown of the basement membrane, reinitiation of meiosis in the oocyte, and, after a delay of approximately 28 hours in the cow, ovulation. Coitus is linked to ovulation because standing estrus is induced by a similar elevation in E2, in the absence of P4, as is required for induction of the GnRH surge. The objective of turning the cow into an induced ovulator, Ovsynch, was greatly facilitated by three technological advancements that were available at the time we began these studies in 1993. First, transrectal ultrasonography of bovine ovaries allowed visualization and determination of physiological mechanisms regulating the bovine follicular waves [11–15]. Although we had only an old, borrowed ultrasound machine available for these studies, the resolution was sufficiently precise to allow determination of the ovarian dynamics during the Ovsynch procedure. In addition, inducing ovulation in cattle required exogenous GnRH being commercially available for use in dairy cattle. Induction of an LH surge, potentially in the absence of estrus, with exogenous GnRH can induce ovulation, if a responsive dominant follicle is present on the ovary and the GnRH-induced LH surge is of sufficient magnitude. Finally, acceptable fertility during synchronization of ovulation procedures required consistent and synchronous induction of CL regression, which was made possible by the discovery of $PGF_{2\alpha}$ as the luteolysin in ruminants [16,17] and the commercial introduction of $PGF_{2\alpha}$ for induction of CL regression in cattle [1].

Early studies to induce ovulation in cattle used E2, hCG, or pituitary LH [1]. For example, as early as 1960 [18], there were reports that daily P4 injections (25 mg) combined with a subsequent treatment with E2 benzoate, 3 days after the last P4 treatment, could be used to synchronize estrus (97% expression of estrus) with acceptable fertility (38.5%; 35/91 [18]). Similarly, E2 benzoate has been used after progestin treatment of beef heifers [19] or after $PGF_{2\alpha}$ treatment of beef cows [20] to synchronize estrus and the LH surge. However, the reports by the laboratories of Guillemin [21,22] and Schally [23,24] in 1971 showing that GnRH was a small decapeptide changed the synchronization landscape. It was soon demonstrated that native or synthesized GnRH induced a surge in LH and FSH and ovulation in cattle [25,26]. The initial studies primarily focused on ovulation of mature follicles, in the absence of P4, most times after treatment with $PGF_{2\alpha}$ [27,28]. One early study [29] demonstrated that GnRH could induce an LH surge in cows during the luteal phase, although the magnitude of the LH surge was greatly reduced compared with cows with follicular cysts. Practical studies mostly focused on treatment of cystic cows [30,31] or anovular beef cows [32]. Silcox et al., in 1993 demonstrated that 100 μg of GnRH induced either ovulation or luteinization of growing, dominant follicles even during the luteal phase of heifers [33]. Two excellent review articles from the laboratory of Bill Thatcher discussed much of this early work with GnRH and development of a method for synchronization of estrus in heifers [34,35]. These researchers discussed their unpublished results using a GnRH agonist 7 days before a $PGF_{2\alpha}$ treatment to more precisely synchronize the time of estrus in heifers [35]. A subsequent study also demonstrated some changes in follicular dynamics and synchronization of estrus in lactating dairy cows [36]. Near this same time, studies in beef cattle were being performed that also demonstrated improved synchrony of estrus when a GnRH analogue was administered 6 days before a $PGF_{2\alpha}$ treatment [37–41]. Thus, treatments with GnRH before $PGF_{2\alpha}$ had been performed and treatments with GnRH after $PGF_{2\alpha}$ had been performed in different studies but these had not been combined into a complete synchronization of ovulation program.

Along with inducing ovulation in cattle using injection of exogenous GnRH, it was important to induce regression of the CL using treatment with a luteolysin. Identification of the luteolysin in ruminants followed determination that the uterus was the obligatory source of the luteolysin in cattle [42]. In addition, the luteolysin was likely to be a small molecule because it was transported in a local manner between the uterine vein and the ovarian artery [43–45]. The luteolytic effect of $PGF_{2\alpha}$ in rats was demonstrated in 1969 [46]. Abstracts were presented in 1972 at the American Society of Animal Science (ASAS) meetings demonstrating that $PGF_{2\alpha}$ was luteolytic in cattle when delivered using intrauterine, subcutaneous, or intravenous routes [1,47]. Demonstration that $PGF_{2\alpha}$ was the uterine-derived luteolysin has now been shown by many different types of studies, as previously reviewed [17]. The time to estrus and fertility were subsequently demonstrated after $PGF_{2\alpha}$ treatment of cattle [48,49]. Approximately 80% of cows were detected in estrus on Day 2, 3, or 4 after treatment with $PGF_{2\alpha}$ and there was no difference in fertility compared with cows bred to a natural estrus [50]. We particularly used the research by Momont and Sequin [51] in which cows administered $PGF_{2\alpha}$ on Day 7 of the estrous cycle had increased synchrony in time to estrus compared with cows given $PGF_{2\alpha}$ at other times.

2. Recollections on development of Ovsynch

In one sense, our Ovsynch research was a natural consequence of a great deal of previous research on follicular waves, use of GnRH, and use of $PGF_{2\alpha}$ in cattle. When we began this research in 1993, Milo Wiltbank was a new faculty member who had primarily focused his research on understanding the function of the CL and Richard Pursley was a new graduate student who came to University of Wisconsin (UW) with a unique background, having previously owned and operated a dairy farm. Both had a common interest in resolving poor reproductive performance of dairy cows. There were continuous, almost daily, discussions of the best ways to use GnRH for reproductive management. One night, Wiltbank woke at 3 AM with thoughts of giving GnRH after $PGF_{2\alpha}$, potentially producing an optimal time of AI without detection of estrus. More intense discussions ensued, culminating in Pursley initiating the first study using ultrasound on cows in the UW herd to evaluate the ovarian dynamics during the procedure. We hypothesized that: (1) the first GnRH, when administered at a random stage of the estrous cycle, would induce ovulation in cows with a functional dominant follicle; (2) a new follicular wave would be initiated with a dominant follicle selected during the next 7 days; (3) $PGF_{2\alpha}$ would induce luteolysis in a high percentage of cows that had been treated 7 days earlier with GnRH; (4) a dominant follicle would continue to grow, increasing circulating E2, and lactating dairy cows would begin to show estrus at 48 hours after $PGF_{2\alpha}$ treatment; and (5) a final GnRH treatment at 48 hours after $PGF_{2\alpha}$ treatment would induce a surge of LH and synchronized ovulation, allowing for proper timing of AI before ovulation. To our surprise, the first 10 cows that went through the Ovsynch procedure had near perfect synchronization and 70% of the cows became pregnant. At the time we did not realize that this would not be the normal results with this procedure, yet these extraordinary results stimulated us to continue these studies. The subsequent 10 cows brought us back to reality because only three of these cows became pregnant. In addition, evaluations in heifers, with Dr. Michael Mee, a visiting professor from UW-Platteville, demonstrated synchrony was far from perfect with 25% of heifers not synchronized using Ovsynch. However, synchronized cows and heifers were found to ovulate in a tight interval, between 24 and 32 hours after GnRH treatment, based on ultrasound evaluations of the ovaries every 2 hours.

One project that we performed to validate and understand the physiology that underlies Ovsynch used daily ultrasound of a group of lactating dairy cows (N = 66) for 30 days before treatment with Ovsynch. In the experiment, cows were treated with either GnRH (G) or saline (S) was substituted for the two GnRH treatments resulting in four experimental groups: GPG, GPS, SPG, or SPS (Pursley and Wiltbank, unpublished). Anovular cows and cows at random stages of the estrous cycle in the GPS and GPG groups ovulated a dominant follicle to the first GnRH treatment with high frequency (85%). After the $PGF_{2\alpha}$ treatment, a greater ($P < 0.05$) percentage of cows in the GPG group responded to the second GnRH treatment with ovulation of a new dominant follicle compared with the SPS, GPS, and SPG groups; 94% versus 63%, 76%, and 71%, respectively (Fig. 1). In addition, time to ovulation was earlier ($P < 0.05$) and more synchronous for cows in the GPG and SPG groups compared with the GPS and SPS groups (Fig. 1). In the cows that did not receive GnRH at the end of the protocol (SPS and GPS), time to ovulation after $PGF_{2\alpha}$ treatment was dependent on size of the preovulatory follicle at the time of the $PGF_{2\alpha}$ treatment. This is illustrated in Figure 2 with time to ovulation having a high negative association ($R^2 = -0.855$) with follicle diameter at the time of $PGF_{2\alpha}$ treatment. From this study it was clear that ovulation to the first GnRH treatment produced a synchronous follicular wave, a CL that was responsive to $PGF_{2\alpha}$ 7 days later, and increased synchrony of ovulatory follicle size at the time of $PGF_{2\alpha}$ treatment. In addition, the second GnRH treatment induced a synchronized ovulation with all cows that ovulated to GnRH treatment ovulating before the spontaneous ovulations (SPS, GPS). Thus, it was clear that the first and second GnRH treatments of Ovsynch were essential for optimal synchronization of a preovulatory follicular wave and tightly-synchronized ovulation near timed AI in lactating dairy cows.

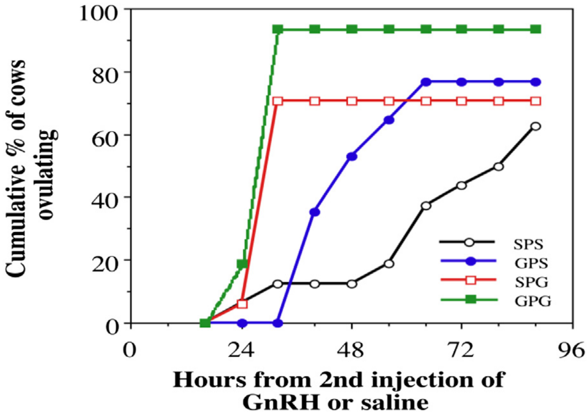

Fig. 1. Cumulative time to ovulation in lactating dairy cows (N = 66) that received saline (S) or GnRH (G) at the start and end of Ovsynch. The cows receiving the complete Ovsynch protocol (GPG) had a greater ($P < 0.05$) percentage of cows that ovulated compared with the other three groups. Cows that received GnRH at the final treatment (GPG or SPG) had earlier and more sychronous ovulation than the other two groups.

Another important analysis that was done during development of Ovsynch [52] was evaluation of timing of AI in relation to the final GnRH treatment. We speculated that time of AI in relation to ovulation was likely to not be optimized in programs using AI after detection of estrus and therefore optimization of the timing of AI, in relation to a synchronized ovulation, might produce a substantial increase in fertility in lactating cows. Cows (N = 732) were bred at the same time as the second GnRH treatment or at 8, 16, 24, or 32 hours after the second GnRH treatment. We found that cows that received AI 16 hours after GnRH treatment had the greatest number of pregnancies per AI (quadratic effect with 16 hours optimal; $P = 0.001$) and 16 hours had a greater number of pregnancies per AI than

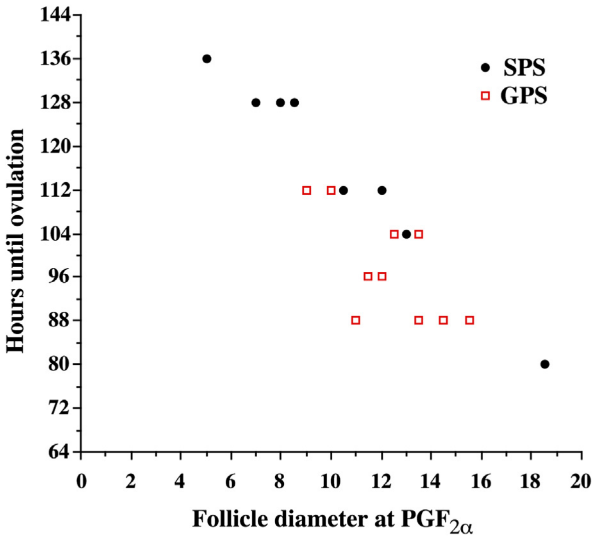

Fig. 2. Time to ovulation compared with follicle diameter of the preovulatory follicle at the time of $PGF_{2\alpha}$ treatment in lactating dairy cows that received either GnRH (G) or saline (S) 7 days before $PGF_{2\alpha}$ and saline treatment 2 days after $PGF_{2\alpha}$ ($R^2 = 0.855$). Only ovulating cows were analyzed.

cows bred 0 or 32 hours after GnRH treatment [52]. The reduced number of pregnancies per AI for 0 hours was likely due to reduced viability of sperm because they remained for an extended period of time in the tract before ovulation (approximately 28 hours). The reduced fertility for the 32-hour group was likely due to reduced viability of the oocyte because AI was done after ovulation and sperm would require time for capacitation and transport to the oviduct before fertilization. Thus, this study somewhat validated our original idea that improved fertility could be obtained by optimizing the timing of AI in relation to a synchronized ovulation.

The name, Ovsynch, was suggested by Pursley after a night of scanning when he was trying to write a manuscript and needed a simpler term for the entire procedure. The suggestion brought a hearty laugh from Wiltbank but the Ovsynch terminology, meaning synchronization of ovulation, was used after that time. In the peer-reviewed literature, there are hundreds of articles that have cited our original Ovsynch article (Google Scholar = 860; Web of Science = 557), and numerous articles use the term Ovsynch in the title (N = 76) or in the abstract (N = 256). A simple search of Ovsynch on Google results in >32,000 results. Obviously, the "Ovsynch" term and technology have become an integral part of bovine research and of the cattle industry during the 18 years since the original publication. In addition, publications have also reported the use of Ovsynch-types of protocols in other domestic animals including: 27 in water buffalo [53], 6 in Yaks [54], and numerous publications on similar protocols in goats [55–57] and sheep [58,59]. We have focused this review primarily on the use of Ovsynch in dairy cattle and will discuss a few selected issues related to this protocol. It is not our intention to provide a comprehensive review of all aspects of the history of synchronization procedures or of all studies that have used synchronization of ovulation and timed AI.

3. Practical implementation of Ovsynch on dairy farms

Whether Ovsynch could become a practical reproductive management tool was a question that we grappled with during the early years. It was unknown if dairy producers would be willing to integrate three hormonal interventions before timed AI into their daily management activities on a commercial dairy farm. We now know that even more complicated presynchronization-Ovsynch-Resynch systems (Fig. 3) are routinely implemented on commercial dairies [60,61]. The practical use of timed AI systems has been facilitated by the adoption of computerized software on many dairy farms which has simplified the production of daily cow lists and improved compliance with these synchronized reproductive management systems. Indeed, today there are numerous combinations of detection of estrus and Ovsynch/timed AI used on commercial dairies. Nevertheless, in the early days of Ovsynch development, a more researchable question related to the effect of Ovsynch on reproductive efficiency on a dairy farm. The question that we believed was most critical was: Does use of Ovsynch alone allow effective reproductive management without the need for detection of estrus?

Two large fertility studies were designed and performed as part of the dissertation of Pursley and to test the efficacy of Ovsynch [8,9]. The first study [8] and arguably the most pivotal study validating the Ovsynch technology, evaluated reproductive efficiency during an entire lactation in cows bred only to Ovsynch/timed AI versus typical reproductive management on three dairy farms (N =333). In this study, the Ovsynch cows received Ovsynch/timed AI for first AI and all subsequent AIs. Cows in the control group received AI after detection of estrus with some selective use of $PGF_{2\alpha}$ and GnRH. As expected, Ovsynch reduced average days to first AI and reduced variability in time to first AI compared with reliance on detection of estrus to perform AI (Fig. 4). Despite the earlier AI and AI of all cows regardless of whether they expressed estrus, there was no difference in percentage pregnancies per AI between the two groups at first or later AIs. In addition, average time, and variation in time to pregnancy were significantly reduced for cows in the Ovsynch group (Fig. 4). Thus, dairy producers could use Ovsynch to reduce "days open", not because of better fertility at the AI but because of better service rates (heat detection rates). Detection of estrus is reduced on many dairy herds because of numerous labor, environmental, milk production, health, genetic, and cow comfort factors [62–64]. The real power of this technology was that dairy

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Week 1				PGF		
Week 2						
Week 3				PGF		
Week 4						
Week 5		GnRH				
Week 6		PGF		GnRH	AI	
Week 7						
Week 8						
Week 9						
Week 10						
Week 11		GnRH				
Week 12		PG+PGF		GnRH	AI	

PGF = prostaglandin $F_{2\alpha}$, GnRH = gonadotropin-releasing hormone, PG = pregnancy diagnosis

Fig. 3. A typical reproductive management program using timed AI. First AI is done after a Presynch-12, note 12 days from final $PGF_{2\alpha}$ treatment to GnRH of Ovsynch, followed by an Ovsynch-56 timed AI protocol. A Resynch-32 procedure with GnRH given on Day 32 after the previous timed AI and the next week cows are evaluated for pregnancy and if not pregnant, cows are given a $PGF_{2\alpha}$ treatment and continue through the Ovsynch protocol and timed AI. PGF, $PGF_{2\alpha}$.

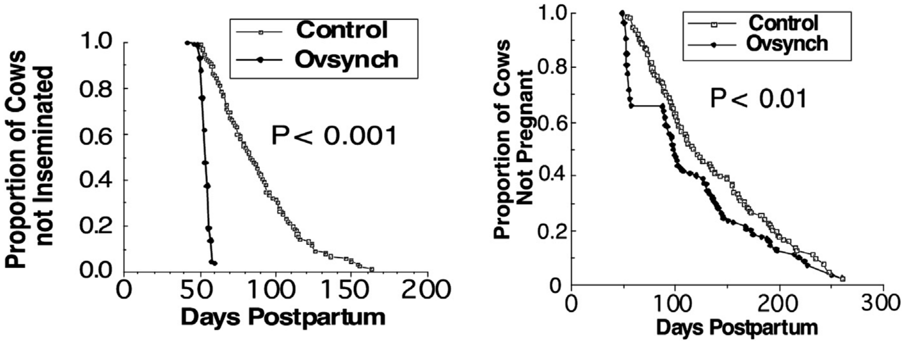

Fig. 4. Survival curves for time to first AI (left) and time to pregnancy (right) for cows bred with typical reproductive management using AI 12 hours after detection of estrus (control) or bred to Ovsynch and timed AI for all breedings (Ovsynch). See text for details. From [8].

producers could choose a precise time for first AI (usually within a 1-week period), compared with previous reproductive management strategies that primarily relied on waiting for cows to cycle, express estrus, and then hopefully detect that estrus to perform first and later AI. In addition, this study introduced the use of Ovsynch as a tool for "resynchronization" (Resynch). Dairy producers could choose their time between AIs (42 days was used in our study) by determining nonpregnant cows (at 32 days) and then initiating the Resynch-Ovsynch protocol. There have now been numerous studies that have evaluated timing and optimization of Resynch strategies [65–71].

The second study to test efficacy of Ovsynch compared Ovsynch with a traditional synchronization program using detection of estrus after $PGF_{2\alpha}$ treatment in lactating dairy cows. This study was a collaborative project with a number of researchers that were part of the NC-113 project [9]. Once again, variation in time to first AI was reduced in the Ovsynch-treated cows. Fertility was similar in dairy cows bred to estrus or Ovsynch (39% vs. 38%); whereas heifers had greater number of pregnancies per AI when bred to estrus than Ovsynch (74% vs. 35%). We concluded, "The ability to achieve acceptable fertility after a timed AI could have a major impact on reproductive management of lactating dairy cows", removing reliance on detection of estrus and allowing more control in AI programs [9]. A study from the laboratory of Dr. William Thatcher near this same time period [72] evaluated Ovsynch-type protocols, using a GnRH agonist or hCG, in dairy heifers. They reported a reduction in pregnancies per AI for timed AI compared with AI after estrus. Another study by Dr. Thatcher's group evaluated an Ovsynch-type of protocol using a GnRH agonist in lactating cows [73]. They reported similar first service conception rate in cows bred to estrus (33.0%) compared with cows bred after timed AI (27.2%). A meta-analysis done in 2005 (71 trials in 53 research publications) reported no difference between fertility in cows bred to Ovsynch compared with other reproductive management strategies [10]. Thus, Ovsynch and timed AI was a reliable, relatively simple way to breed lactating dairy cows and generally produced similar fertility as AI to a detected estrus. Studies showing better fertility in cows bred to AI after detection of estrus than to Ovsynch generally have high fertility in cows bred to estrus (>40) with typical fertility to Ovsynch [10,74,75].

Maintaining reproductive efficiency is a major factor contributing to efficiency, profitability, and sustainability of dairy farms [76–79]. Before development of Ovsynch, reproductive efficiency, in management terms, was dependent on efficient detection of estrus (heat detection rate, also called service rate) and the percentage of cows pregnant per AI (also called conception rate or fertility). Until 2000, reproductive efficiency in the United States was decreasing for more than 40 years probably due to declines in service rate and fertility of dairy cattle. However, there has been a substantial improvement in reproductive efficiency in the past decade, probably partly due to introduction of Ovsynch/timed AI programs in the United States (discussed in section 7). From the early studies on Ovsynch development and validation, it was clear that Ovsynch technology could improve reproductive efficiency in lactating dairy cows by increasing service rate with no change in fertility. The next challenge was to improve fertility to the timed AI. As Ovsynch become a routine part of dairy management systems across the United States, researchers reasoned that more complex Ovsynch strategies that produced enhanced fertility could be efficiently implemented in the industry. The next two sections highlight a few of these Ovsynch modifications and presynchronization strategies that are focused on developing protocols with enhanced fertility.

4. Modifications to Ovsynch

4.1. Ovulatory follicle age/size

Excessive duration of follicular dominance can reduce fertility of cattle [4,80–84]. This appears to be due to an effect of prolonged dominance on the oocyte [85] leading to developmental problems in the early embryo [86,87]. During the early studies with Ovsynch, we believed that one of the advantages of Ovsynch could be that the second GnRH treatment would induce ovulation of a follicle with a reduced period of follicular dominance, potentially improving fertility. Therefore, we performed a study to intentionally reduce the size of the ovulatory follicle by aspiration of all follicles at 3 to 4 days after the first GnRH treatment of Ovsynch [88]. This would produce a follicle that was just becoming dominant near the time of $PGF_{2\alpha}$ treatment and therefore would be smaller at the time of the second GnRH treatment. Consistent with our experimental design, cows receiving follicular aspiration had reduced size of the ovulatory follicle compared with control Ovsynch-treated cows (11.5 vs. 14.5 mm). However, contrary to our hypothesis, cows that ovulated smaller follicles had a severe reduction in fertility compared with Ovsynch control cows (14.3% vs. 47.6%). In addition, cows that had follicular aspiration had an increased double ovulation rate (19.1% vs. 9.5%) and increased incidence of short luteal phases (14.3% vs. 0%). The underlying cause of reduced fertility in cows that ovulate a smaller follicle, in addition to the short luteal phase, might be related to reduced preovulatory E2 concentrations and reduced circulating P4 after AI. There are substantial data in beef cattle that cows that are induced to ovulate a smaller follicle during Ovsynch have reduced fertility [89–91]. This appears to be primarily due to reduced circulating E2 in the preovulatory period [91–93]. Thus, in dairy and beef cattle, ovulation of an excessively small follicle at the end of the Ovsynch protocol can result in reduced fertility. In addition, in dairy cattle, ovulation of an older/larger follicle can also result in reduced fertility. We therefore infer that optimization of fertility during Ovsynch necessitates ovulation of a follicle that is neither too old nor too young.

In an attempt to reduce the duration of follicular dominance, a shortened Ovsynch strategy has been developed. The interval between GnRH and $PGF_{2\alpha}$ treatment was reduced from 7 to 5 days along with an increase in the proestrous period from 48 to 56 hours (time of second GnRH treatment) until 72 hours. This strategy, particularly the increased proestrous period, has resulted in improved fertility in beef cattle [94]. A similar strategy in dairy cattle produced encouraging results [95]. However, additional studies are necessary because ideal time of AI, length of proestrus, and number of $PGF_{2\alpha}$ treatments are likely to differ between the two protocols and might not have been optimized in previous studies [96–98].

The optimal size of the ovulatory follicle differs according to type of protocol and type of cattle being synchronized. In a study of 622 lactating dairy cows that had single ovulation after Ovsynch, the effect of follicle size was evaluated [99]. Fertility was greatest (at 60-day pregnancy diagnosis) for cows that ovulated a medium-sized follicle (15–19 mm; 143/302 = 47.4%) compared with cows that ovulated smaller (<14 mm; 63/174 = 36.2%) or larger (>20 mm; 52/136 = 38.2%) follicles. Of particular interest, is the observation that only 48.7% of the ovulating cows ovulated a follicle of optimal size (303/622), whereas many cows ovulated a follicle that was smaller (179/622 = 28.7%) or larger (140/622 = 22.5%) than optimal. Thus, because less than 50% of ovulating cows ovulate an optimal size of follicle, it seems evident that optimization of ovulatory follicle size/age is an area of opportunity for future studies with Ovsynch.

4.2. Supplementation with P4 during Ovsynch

Anovulation can be a major problem in dairy and beef cattle. For example, approximately 25% of dairy cattle are anovular at the end of the voluntary waiting period [100,101]. The physiology that underlies the varied types of anovular conditions has not been fully elucidated [102]. The most common type of anovular lactating dairy cow (approximately 60% of anovular dairy cows) had follicles larger than ovulatory size but smaller than what has classically been defined (≥25 mm) as a follicular cyst [100,102]. Anovular cows of this type probably have hypothalamic resistance to the positive feedback effects of E2 [103,104]. Fertility rate is generally lower in cows that are anovular at the beginning of the Ovsynch protocol [100,105,106]. Interestingly, the reduction in fertility is observed for anovular cows or for cycling cows that are at a stage of the cycle with low P4 at the start of the Ovsynch program [106]. The reduction in fertility is not due to a lack of ovulation to the first GnRH, because ovulatory response is surprisingly high (approximately 80%) in anovular cows [100,107].

The effect of circulating P4 during the week before AI was first reported for lactating dairy cows in 1983 [108]. There have been recent reviews that have discussed the reduction in fertility when the preovulatory follicular wave develops in a low P4 environment [109,110]. Manipulative studies have shown improvements of approximately 5% to 9% in percentage pregnant using a P4 vaginal implant during the Ovsynch program before AI [101,111–115]. In most of these studies, the P4 implant was most effective in cows without a CL at the time of Ovsynch initiation. In seasonally-calving, pasture-based herds, Ovsynch had a lower fertility rate (45%) than AI after a nonsynchronized estrus (53%) and inclusion of a P4 implant during the Ovsynch protocol increased fertility to a value (54%) similar to estrus [74]. Further analyses of these data indicated that the main advantages of inclusion of a P4 implant were in cows that were at an earlier time postpartum at timed AI (<80 days in milk) or anovular at Ovsynch initiation and the reduction in fertility in these cows was associated with reduced synchronization during Ovsynch [75]. Thus, much of the effect of supplementation with a single intravaginal P4 implant might be due to improvements in synchronization rate, in addition to elevating P4 during preovulatory follicle growth in synchronized cows. Supplementation with a single vaginal P4 implant might not elevate circulating P4 sufficiently to optimize fertility and two P4 implants might be needed in dairy cows that lack a CL at the initiation of Ovsynch [114,116]. Studies using presynchronization strategies that result in two CL rather than a single CL during preovulatory follicle growth have also demonstrated a decrease in double ovulation rate and an increase in fertility when P4 is elevated during preovulatory follicle growth [106,109]. Thus, circulating P4 during the Ovsynch protocol might, at times, be insufficient for optimal fertility, particularly in dairy cows that are anovular, near estrus, in the early luteal phase, or in the late luteal phase (CL regression can happen before $PGF_{2\alpha}$ treatment). Supplementation during the synchronized ovulation protocol, with sufficient P4, in these types of dairy cows is likely to improve fertility.

4.3. Dose of GnRH and $PGF_{2\alpha}$

The dose of GnRH that was used in our original studies was 100 μg because this was the labeled dose for treatment of cystic cows in the United States. Due to the high price of

GnRH for dairy producers in Wisconsin (approximately $10 per dose), a lower dose was evaluated [117]. Similar fertility was found in cows treated with 50 μg (46/112 = 41.1%) or 100 μg (48/117 = 41.0%) GnRH at the two treatments [117]. Ovulation to the second GnRH (199/337 = 84.0%) and double ovulation rate (28/199 = 14.1%) were similar for the two treatments; however, ovulation to the first GnRH treatment was not evaluated in the study. Our recent research has shown that the LH surge is greatly reduced when GnRH is given in the presence of elevated P4 compared with GnRH treatment when circulating P4 is low [118]. Therefore, the first GnRH treatment is likely to require a greater dose than the second GnRH to induce an LH surge of adequate magnitude to induce ovulation.

Cows that ovulate to the first GnRH treatment have been found to have greater fertility than cows that do not ovulate [119–121]. There is particularly low fertility in cows that do not ovulate to the first GnRH treatment and have low P4 at the first GnRH, whereas there is little effect of ovulation on fertility in cows that have medium P4 at the first GnRH treatment [68,69,122]. There was increased ovulation to the first GnRH treatment when 200 (217/326 = 66.6%) rather than 100 (187/325 = 57.5%) μg of GnRH was used [122]. Nevertheless, there was no difference in fertility for cows given 200 (263/551 = 47.7%) compared with 100 (246/533 = 46.2%) μg of GnRH [122]. In this study, we observed greater fertility in cows that ovulated (52.2%) compared with cows that did not ovulate (38.5%) to the first GnRH treatment (difference of 13.3%). A simple calculation demonstrates why no improvement in fertility was observed. There was an increase of 9.1% in ovulation to the higher GnRH dose with 13.3% improvement in fertility in cows that ovulated compared with cows that did not ovulate. Thus, only a 1.2% improvement in fertility would be predicted by increasing the GnRH dose (9.1% × 13.3%), very similar to the nonsignificant increase of 1.5% observed in our study. Thus, we believe that increasing the dose of GnRH in Ovsynch is unlikely to produce a substantial improvement in the protocol.

In contrast, increasing the dose or number of treatments with $PGF_{2\alpha}$, might provide important improvements in the Ovsynch protocol. Lack of complete regression of the CL to the $PGF_{2\alpha}$ treatment has been observed in 10% to 25% of cows treated with Ovsynch [68,95,97,99,123]. In these studies, cows that have small elevations in circulating P4 near AI, due to lack of complete CL regression, have greatly reduced fertility. This is particularly important in cows treated with the 5-day Ovsynch protocol [95,124]. Fertility was reduced if only a single $PGF_{2\alpha}$ treatment was given [95] or if two $PGF_{2\alpha}$ treatments were given on the same day (5 days after GnRH) compared with giving one treatment on Day 5 and a second on Day 6 [124]. Increasing the dose of cloprostenol from 500 ug to 750 ug in a 7-day Ovsynch protocol increased CL regression in multiparous (122/154 = 79.2% vs. 135/154 = 87.7%; P = 0.025) but not primiparous (131/146 = 89.7% vs. 129/139 = 92.8%; P = 0.181) cows [122]. An indication of improved fertility (P = 0.054) was observed at the 39-day pregnancy diagnosis with the 750 (247/544 = 45.4%) compared with the 500 (221/540 = 40.9%) μg dose of cloprostenol. In a separate study using the 7-day Ovsynch protocol, cows were given one (Day 7) or two (Days 7 and 8) treatments with 25 mg of dinoprost [97]. An increased percentage of cows with complete CL regression (<0.4 ng/mL 56 hours after $PGF_{2\alpha}$ treatment) was observed after two (326/341 = 95.6%) compared with one (301/356 = 84.6%) $PGF_{2\alpha}$ treatment. No significant improvement in fertility was observed. Nevertheless, an improvement from 0% to 50% fertility in 11% of cows (two vs. one $PGF_{2\alpha}$ treatment) would be expected to result in a 5.5% improvement in fertility, similar to the 5.7% improvement observed in first-service cows in this study (47.0%–52.7%) [97]. Based on all of these results, it seems likely that lack of complete CL regression, particularly in multiparous cows, reduces the effectiveness of the Ovsynch protocol. A second $PGF_{2\alpha}$ treatment is likely to result in a 3% to 5% improvement in fertility, particularly when used in conjunction with presynchronization protocols like Double-Ovsynch, which are discussed in the next section.

4.4. Other modifications of Ovsynch

Numerous other modifications have been made to the original Ovsynch protocol and to other timed AI protocols. One important type of modification is focused on increasing follicle growth during the final stages of Ovsynch using treatment with eCG or FSH. In dairy cows this has generally not improved fertility except in anestrous cows or cows with low body condition score [125–129]. In beef cattle, eCG, FSH, or calf removal have been used to improve follicle growth, ovulation, and fertility near the end of protocols for synchronization of ovulation [130–134]. Treatment with eCG was found to be more effective in suckled, anestrous Nelore beef cows than treatment with a single 10-mg dosage of FSH [134,135]. The improvements in fertility by treatment with eCG, FSH, or by using calf removal seem likely to depend on the physiological status of the cows. Many suckled beef cattle are likely to require a greater number of LH pulses to grow follicles to sufficient size and E2 production to optimize fertility. Most dairy cattle in conventional operations are likely to have sufficient, if not excessive, LH pulses to drive the final stages of follicle growth [136] and are therefore unlikely to require treatments that stimulate follicular growth. In situations with greater negative energy balance, or other physiological situations that would reduce LH pulses, a follicular growth stimulus could improve outcomes with Ovsynch.

5. Presynchronization programs before Ovsynch

Generally when Ovsynch is implemented in a commercial cattle operation, cows will be at a random stage of the estrous cycle. One of our early studies demonstrated that response to Ovsynch differed according to stage of the estrous cycle at which the cow received the first GnRH treatment of Ovsynch [137]. For example, GnRH treatment on Day 1 to 4 of the estrous cycle rarely resulted in ovulation, whereas GnRH treatment on Days 5 to 9 caused ovulation in almost all cows. Thus, cows that were on the Day 1 to 4 schedule had an older and larger follicle that ovulated to the second GnRH treatment (19.2 mm) than cows that began Ovsynch on Days 5 to 9 (16.8 mm). Cows that began Ovsynch on Days 5 to 9 also had greater circulating P4 at the time of the $PGF_{2\alpha}$ treatment (3.6 ng/mL), probably due to the presence of two CL, than cows that initiated Ovsynch on Days 1 to 4 (2.5 ng/mL). In contrast, cows in the later estrous cycle at Ovsynch initiation (Days 12 to 21) also had lower P4 but due to early regression of the CL, before the $PGF_{2\alpha}$ treatment in some cows. Some cows in the later estrous cycle, that did not ovulate to the first GnRH treatment had ovulation prior before the second GnRH treatment. The cows that initiated Ovsynch in the later estrous cycle and did have synchronized ovulation, ovulated a larger follicle than cows on Days 5 to 9. Of particular importance, cows expected to ovulate larger follicles (Ovsynch initiated on Days 1–4 or 14–21) had lower fertility rates (25% at 98-day pregnancy diagnosis) than cows expected to ovulate smaller follicles (36%; Ovsynch initiated on Days 5–13). In another study it was also observed that dairy cows that initiated Ovsynch on Days 5 to 9 had greater fertility than cows that initiated Ovsynch on other days of the cycle [138]. These results and other similar results in heifers [139], provided the physiological basis for subsequent presynchronization methods that attempted to maximize the number of cows at a more optimal stage of the estrous cycle at Ovsynch initiation.

A presynchronization strategy that used two treatments with $PGF_{2\alpha}$ (Presynch-12) was published from the laboratory of Dr. William Thatcher [140]. This study utilized a 2 × 3 experimental design with the first main effect being whether cows received or did not receive the Presynch strategy ($PGF_{2\alpha}$ treatment – 14 d later – $PGF_{2\alpha}$ – 12 d later – Start Ovsynch protocol). The second main effect was initiation of bovine somatotropin (bST) treatment on 63, 73, or 147 day in milk. There was an interaction of bST and Presynch ($P < 0.01$) with bST only showing an effect when cows received Presynch and not in non-Presynch cows. A calculation from the presented results shows that fertility is better in Presynch (113/264 = 42.8% at 74-day pregnancy diagnosis) than cows that were bred to Ovsynch without Presynch (80/272 = 29.4%). Presynch only improved fertility in cows that were cyclic and not in anovular cows, as might be expected with a Presynch strategy that only used $PGF_{2\alpha}$ treatments.

Numerous presynchronization studies have been performed. Three of the most commonly used presynchronization strategies are illustrated in Figure 5 and will be briefly discussed in the next four paragraphs. Subsequent studies confirmed that the Presynch strategy using two $PGF_{2\alpha}$ treatments 10 to 14 days before Ovsynch improved fertility compared with Ovsynch alone [141,142]. A single $PGF_{2\alpha}$ treatment 12 days before Ovsynch and first AI was not effective [143,144], however, a single $PGF_{2\alpha}$ treatment 12 days before Resynch ($PGF_{2\alpha}$ on Day 34 after previous AI and 12 days later, Ovsynch initiation) did increase fertility [145]. A shorter interval from the second $PGF_{2\alpha}$ treatment of Presynch improved fertility compared with a longer interval (11 days = 36.4% vs. 14 days = 30.2% [146]).

One of the most common adaptations of Presynch-Ovsynch is with the use of detection of estrus after the second $PGF_{2\alpha}$ treatment and subsequent enrollment of all cows that were not detected in estrus into the Ovsynch protocol [147]. Use of detection of estrus during Presynch has reduced fertility to the Ovsynch/timed AI and overall

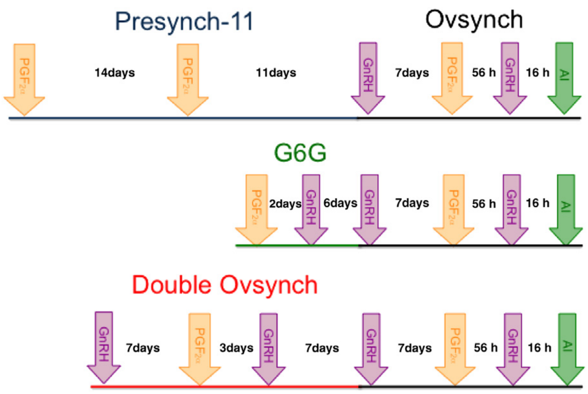

Fig. 5. Hormonal treatments for the three presynchronization programs discussed in the text.

fertility in all enrolled cows in one study (49.2% vs. 33.3%) [148], although the effect was not significant in another study (31.1% vs. 25.3%) [149]. Because Ovsynch produces fertility that is similar to AI after an estrus in high-producing cows, and because Presynch before Ovsynch increases fertility compared with Ovsynch alone, it is logical that Presynch-Ovsynch could be a “fertility program” that could allow increased fertility compared with AI after an estrus. The Pursley laboratory recently tested this idea (Strickland and Pursley, unpublished) and found that fertility was greater with Presynch-Ovsynch (293/651 = 45%) than in cows bred after a standing estrus (219/706 = 31%). Thus, Presynch-Ovsynch, with all cows being bred to a timed AI, might be considered a “fertility program” and not just a program for improving service rate in high-producing, lactating dairy cows.

A presynchronization protocol, now known as G6G or G7G, was developed by the Pursley laboratory using a single $PGF_{2\alpha}$ treatment followed 2 days later with a GnRH treatment with subsequent initiation of Ovsynch 6 or 7 days later [119,150]. This protocol appears to be effective in synchronizing more cows to a stage of the estrous cycle that can respond to the first GnRH treatment of Ovsynch [119,150–152]. The percentage of cows that ovulated to the first GnRH treatment of Ovsynch increased when cows were treated on Day 6 (85%) compared with Day 4 (56%) or Day 5 (67%) after the GnRH treatment of G6G ($PGF_{2\alpha}$ treatment – 2 d later – GnRH – 6 d later – Start Ovsynch) [119]. In a separate study, ovulation rate was greater on Day 6 (94%) than on Day 7 (82%) or Day 8 (73%) (Pursley et al., unpublished).

A third presynchronization strategy, originally developed in the Wiltbank laboratory, is termed Double-Ovsynch [153]. This protocol uses an Ovsynch protocol (GnRH treatment – 7 d later – $PGF_{2\alpha}$ – 3 d later – GnRH) followed 7 days later by Ovsynch. In theory, anovular cows should be induced to cycle and cycling cows should be synchronized so that Ovsynch begins on Day 7 of the estrous cycle. Use of Double-Ovsynch compared with Presynch-12 increased percentage of cows with CL at the time of the first GnRH treatment (343/366 = 93.7% vs. 281/373 = 75.3%) and percentage of cows with elevated

P4 at the time of $PGF_{2\alpha}$ treatment (88% vs. 76%) [154,155]. There was also an increase in fertility with Double-Ovsynch compared with Presynch-12 in the first study [153] and in a more recent study (383/837 = 46.3% vs. 349/850 = 38.2%; P = 0.01) [155]. There was also an increase in fertility with Double-Ovsynch compared with Ovsynch alone as a Resynch procedure [68]. A recent study compared Double-Ovsynch with Presynch-10 before a 5-day Cosynch protocol (GnRH and timed AI given together at either 58 or 72 hours after the $PGF_{2\alpha}$ treatment) [98]. Fertility was very high in all treatments (>50% pregnant per AI) and not affected by presynchronization method. However, the cows treated with Double-Ovsynch had reduced pregnancy losses compared with Presynch-10 cows (38/501 = 7.6% vs. 58/515 = 11.3%; P = 0.03). The lack of an effect of Double-Ovsynch on overall fertility compared with Presynch in this last study could be due to differences in grazing cattle compared with cows fed a total mixed ration (TMR) in free-stall environments or might be due to the use of a shorter, and potentially higher fertility, Presynch program in the grazing study (Presynch-10) compared with our study (Presynch-12). In general, Double-Ovsynch appears to be another "fertility program" with increased fertility compared with traditional Ovsynch and, at times, compared with Presynch-Ovsynch programs.

There are other presynchronization strategies that have been tested including a single GnRH treatment 7 days before Ovsynch [67,156–160] or a P4 intravaginal device before Ovsynch [120,161,162]. Extensive comparisons of the various presychronization strategies are still needed. A recent large study (N = 1870) by the Pursley laboratory (Pursley et al., unpublished) found that ovulation to the first GnRH treatment of Ovsynch was greater for Double-Ovsynch (90%) than for G6G (85%) or Presynch-11 (80%) but that overall fertility was excellent and similar for all three of the programs in lactating dairy cows. Matching the use of presynchronization programs with the facilities, goals, and expertise is probably most critical for success of these programs on specific dairy farms. In particular, producers that use Presynch programs that include GnRH treatments need to consider the potential reduction in expression of estrus [67,159,160] and producers that use Resynch programs, such as Double-Ovsynch or $PGF_{2\alpha}$ treatments before Ovsynch, need to consider the potential increase in interval between breedings [68,69,163]. Before first AI, the length of the presynchronization program might not be a major issue on some dairies and therefore costs, fertility, and farm situation are greater considerations. However, the length of synchronization programs is a major issue during Resynch programs, potentially increasing the interval between breedings. In conclusion, use of timed AI programs can be used not only to increase the service rate, as shown in our original Ovsynch trials, but also to improve fertility, in some herds. Effective use of Ovsynch/timed AI can also allow accurate testing of targeted changes in nutritional strategies, hormonal supplementation, and follicle size/age, to name just a few possibilities, because treatment strategies can be precisely timed to ovarian or embryonic stages.

6. Synchronization of ovulation programs using E2 products

As already discussed, the GnRH-based programs such as Ovsynch use a first GnRH treatment to synchronize the preovulatory follicular wave and to synchronize luteal function by ovulating a follicle. Thus, induction of an LH/FSH surge followed by a prolonged FSH surge leads to a new follicular wave [164,165] and a dominant follicle at the time of the second GnRH treatment, which is used to synchronize the time of ovulation, allowing effective timed AI. An alternative method to remove the dominant follicle is to inhibit gonadotropin secretion using treatment with E2 in the presence of elevated circulating P4. Early studies that combined progestagens with estrogens led to the development of the Syncro-Mate-B/Crestar system for synchronization of ovulation and timed AI [166–170]. These early studies did not have the benefit of ultrasound to determine the follicular wave patterns and used high doses of E2-esters to induce regression of the CL, and regulate follicular waves. Later studies by Gabriel Bo and others in the laboratory of Reuben Mapletoft demonstrated that treatment with E2 could induce regression of follicles with a subsequent synchronized emergence of the dominant follicle 3 to 5 days later [171–174]. Treatment of dairy cows with 2 mg of E2-benzoate (EB) reduced circulating LH and FSH to nadir concentrations approximately 30 minutes after treatment with a subsequent FSH surge at 3 to 4 days after EB [175]. The time from EB treatment until follicular wave emergence was independent of stage of the follicular wave at the time of EB treatment [176]. The dose and type of E2 were critical for timing of follicular wave emergence after E2 treatment [177–179]. The circulating E2 profiles are very different for different types of E2 products [180]. Interestingly, in lactating dairy cows, the time to wave emergence after 2 mg of EB treatment was shorter [125] in cows with high milk production (3.8 days) than in cows with lower milk production (4.5 days) perhaps due the increased E2 metabolism with increasing milk production [181,182].

These protocols generally use an E2 treatment at the end of the protocol to induce ovulation as originally shown to be effective in beef cattle in Syncro-Mate-B protocols [183]. Unfortunately, complete synchronization is a problem in E2-P4 protocols in lactating dairy cows due to lack of regression of the dominant follicle, lack of ovulation to the final estrogen treatment, and lack of complete regression of the CL [125,184,185]. In beef cattle, complete synchronization with E2-P4 protocols seem to be more efficient [185–187]. Thus, there are now two major systems that are used for synchronization of ovulation and timed AI. One system emerged from our research and the research of many others and uses GnRH to synchronize the emergence of the preovulatory follicular wave and to synchronize ovulation. The other system developed from the original work of James Wiltbank, Gabriel Bo, Reuben Mapletoft and many others who evaluated the use of estrogens to synchronize the delayed emergence of a follicular wave and subsequently to induce ovulation of the dominant follicle. Both systems can synchronize follicular wave emergence, luteal function, and final ovulation near timed AI, however, both systems have some deficiencies and some advantages in specific

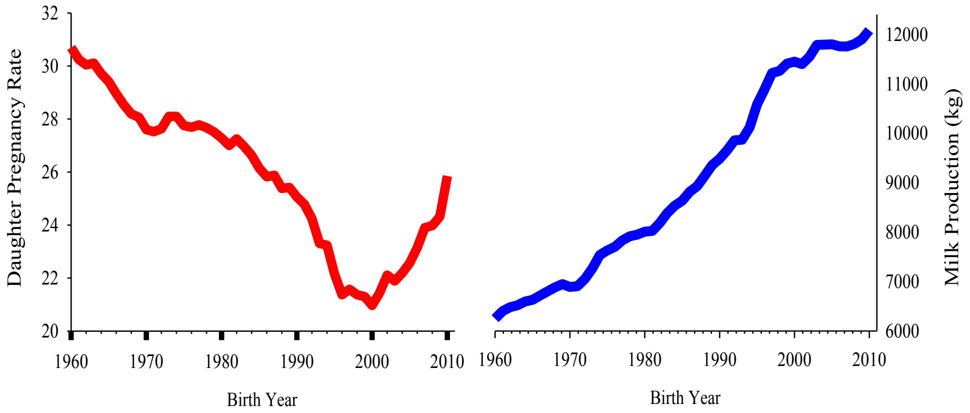

Fig. 6. Phenotypic values for US Holstein heifers (USDA-AIPL) born from 1960 until 2010 for daughter pregnancy rate (left in red) and milk production (right in blue). Data are from USDA-AIPL, United States Department of Agriculture-Animal Improvement Programs Laboratory (http://aipl.arsusda.gov/).

physiological situations. Synchronization success continues to improve compared with the original protocols, as we obtain greater understanding of the limitations of each system.

7. Effect of Timed AI in the US dairy industry and Brazilian beef cattle industry

Use of timed AI technology has taken surprisingly different turns in different countries. This is illustrated by discussion of the use of timed AI in the United States, particularly in the dairy cattle industry, compared with use in Brazil, particularly in the beef cattle industry.

As shown in Figure 6 and Table 1 there have been dramatic changes in reproduction in dairy cattle in the United States based on data collected by the United States Department of Agriculture (USDA) and particularly by Dr. Duane Norman at USDA ([188]; USDA Animal Improvement Programs Laboratory Web site [http://aipl.arsusda.gov/]). The primary measure of reproductive performance tabulated by the USDA is daughter pregnancy rate (DPR), a value derived from days open [189,190]. Until the year 2000, the phenotypic DPR for Holstein cattle declined for many years in dairy herds in the United States probably due to increasing problems with detection of estrus and reductions in fertility to AI. This trend for reduced reproductive efficiency was associated with an increase in milk production (Fig. 6). Since 2000, the increase in milk production has continued but the decline in reproductive performance has been dramatically reversed. During the past decade there has been an increase of approximately 5% in DPR, which translates into a reduction in days open of approximately 20 days. This phenomenal change in reproductive performance could be attributed to improvements in genetic characteristics and/or improvements in management.

Table 1 provides more specific data on some reproductive traits during this time period. Columns 2 and 3 show that the percentage of cows in herds that are using synchronization of ovulation and timed AI has increased from less than 10% in 1998 to 58% by 2008 (Norman et al., 2009 [188]). This increase is likely to have continued in the past 5

Table 1
Evaluation of traits on dairy farms in the United States (data are from [188] and USDA-AIPL web site [http://aipl.arsusda.gov/]).

Year	% Not synchronized	% Likely Synchronized	Mean days to First AI	Mean days calve to last AI	First service pregnant per AI	Number of AI per lactation	Calving interval
1998	91	9	95	**	32	2.2	422
1999	85	15	94	**	30	2.2	428
2000	80	20	93	**	30	2.2	426
2001	75	25	92	**	27	2.3	428
2002	70	30	91	**	30	2.3	426
2003	65	35	90	147	32	2.4	425
2004	58	42	88	143	33	2.5	422
2005	52	48	87	146	31	2.6	423
2006	47	53	86	145	31	2.5	423
2007	45	55	85	141	32	**	423
2008	42	58	83	136	32	**	418
2009	**	**	81	133	32	**	417
2010	**	**	81	133	32	**	412
2011	**	**	80	129	32	**	**

The first two columns represent the percentage of dairy cattle in herds that use synchronized ovulation protocols, showing the increase from 1998 to 2008. The mean days to first AI and last AI (pregnant or do not breed) decreased during this time period. The first service conception rate or percentage pregnant per AI did not change, although number of AIs per lactation was increasing. The calving interval decreased during this time. ** - indicates that data were not available to the authors at the time of writing of this manuscript.

years, although the authors were not able to find a more up-to-date, accurate evaluation of timed AI usage in the US dairy herd population. It should be emphasized that this is not the percentage of cows bred using timed AI but the percentage of cows that are in herds that use timed AI. Some herds might use timed AI as their major breeding technique. However in many dairy herds, timed AI is used as a tool to assure that cows are bred in a timely, efficient manner even though many cows receive AI after detection of estrus. Clearly there has been a reproductive revolution during the early 2000s with synchronization of ovulation and timed AI becoming a major method for reproductive management in dairy herds in the United States.

Columns 4 to 7 in Table 1 help to illustrate the underlying changes in reproduction that account for the dramatic improvement in DPR, shown in Figure 6. Mean days to first AI have been reduced by 15 days from 1998 to 2011. This reduction in days to first AI has occurred almost exclusively in herds that use timed AI and not in herds that are not synchronized [188]. Nevertheless, results of a recent evaluation of dairy herds in Wisconsin indicated that herds that used more timed AI had a longer voluntary waiting period (49.6 vs. 67.5 days [191]), which should, theoretically, make a reduction in days to first AI more difficult. Nevertheless, the use of timed AI provides such control of timing of first AI that greater consistency and reduced days to first AI can be achieved, even while increasing voluntary waiting period. This is illustrated even more dramatically by the reduction of 18 days in US herds of days from calving to last AI observed from 2003 to 2011 (column 4; USDA-AIPL). Surprisingly, there is no change in conception rate (percentage of cows pregnant per AI) during this time period (column 6; 30%–32% from 1998 to 2011). Thus, improvements in fertility in dairy cattle do not underlie this dramatic improvement in DPR. There appears to be primarily improvements in control of reproductive performance with fewer days to first AI, fewer days until the cow becomes pregnant, probably due to reduced time between AIs, and an increase in number of breedings during lactations (column 7). Clearly, within the time period shown, the use of presynchronization “fertility programs” has not yet altered overall fertility to first AI in herds that use timed AI. Nevertheless, more recent data from Wisconsin have shown that herds that primarily use timed AI have greater fertility at first service than herds that primarily use AI after detected estrus (39.9% vs. 34.9%; $P < 0.01$ [191]). In conclusion, improvements in reproductive efficiency in US dairy herds during the past decade appear to be due to improvements in reproductive management, such as improvements in computerized dairy cattle monitoring, improved methods for detection of estrus, and perhaps, most importantly, effective use of synchronized ovulation and timed AI programs.

Table 2
The number of units of semen sold from beef or dairy bulls in the USA (from NAAB website [http://www.naab-css.org/]) and Brazil (from ASBIA website [http://www.asbia.org.br/novo/home/]).

Year	United States			Brazil		
	Total	Beef	Dairy	Total	Beef	Dairy
2008	22.5	1.28	21.2	7.46	3.72	3.75
2009	21.1	1.20	19.9	8.17	4.49	3.68
2010	22.9	1.23	21.7	9.64	5.53	4.11
2011	23.9	1.40	22.5	11.91	7.01	4.90
2012	24.7	1.67	23.0	12.34	7.44	4.90
% Growth per year	2.44%	7.6%	2.12%	16.35%	25.00%	7.67%

Abbreviations: NAAB, National Association of Animal Breeders; ASBIA, Brazilian Association of Artificial Insemination.

In contrast, the Brazilian livestock industry has had an explosion in timed AI usage in beef cattle. The number of units of semen sold in the United States (National Association of Animal Breeders; NAAB) and Brazil (Brazilian Association of Artificial Insemination, ASBIA) for dairy and beef cattle are shown in Table 2. In 2012, semen sales were approximately double in the United States compared with Brazil. More than 90% of semen sales in the United States were for dairy cattle (93.1%), whereas most semen sales in 2012 in Brazil were for beef cattle (60.3%). The most notable difference is the growth rate in beef cattle semen sales in Brazil, averaging a 25% increase in semen sales each year. Almost all AIs in beef cattle in Brazil are done using synchronized ovulation protocols using timed AI [192]. The types of programs that are used are not Ovsynch/GnRH-type programs, but are programs that primarily use E2-benzoate at the beginning of the protocol to synchronize the follicular wave and E2-cypionate at the end of the protocol to synchronize ovulation for the timed AI. These programs primarily require that the herd personnel have to handle the cows only 3 times (3 managements), once at the beginning of the protocol with insertion of a vaginal P4 implant and treatment with E2-benzoate, a second time for removal of the vaginal P4 and treatment with $PGF_{2\alpha}$ and E2-cypionate, and finally cows are handled at the time of AI. These protocols also generally use either calf removal or eCG to stimulate the final stages of follicular growth before timed AI. The overall conception rate for timed AI in Brazilian beef cattle averages approximately 50% with these relatively simple timed AI protocols [134,192]. These protocols are particularly effective in anovular beef cattle, which represent most of the cows that receive timed AI in Brazil [134,192]. In the United States, there seems to be some increase in beef cattle semen usage during the last 2 years (17.9% since 2011), possibly because of increased use of timed AI in the United States. Timed AI protocols in the United States rely on GnRH-type of programs because of lack of approval by the US Food and Drug Administration of any E2 products for use in reproductive management protocols. Whether this trend in the US beef cattle industry continues with the same drive and intensity that has occurred in the Brazilian beef cattle industry remains to be seen. We predict that any dramatic increases in semen sales in the beef cattle industry in any country will hinge on effective development and implementation of synchronized ovulation and timed AI programs in beef cattle herds in that country.

8. Conclusions

This review has highlighted the original development of Ovsych, the major modifications to Ovsynch, and the effect that use of timed AI programs are having in the cattle industry. Surprisingly, despite the numerous studies with these protocols, correct synchronization remains a problem, particularly in dairy cattle protocols, with optimization needed in: hormonal environment before AI, hormonal environment and follicle size near the time of AI, and hormonal environment after AI. It seems clear that a high rate of fertility can be achieved, even in high-producing dairy cattle and in anovular, early postpartum beef cows, if proper synchronization strategies are used. Many physiologic, nutritional, cow comfort, and health issues can also affect the success of these programs. There are also important economic, management, and sociological aspects of use of these synchronization protocols that need to be considered when implementing these programs in commercial dairy and beef cattle operations. Simplification of these protocols could improve practical implementation of these reproductive management strategies on commercial operations. Nevertheless, the reproductive revolution of timed AI has been firmly imprinted in the cattle industry, although it is unclear if we are riding the beginning or end of this synchronized follicular wave. To us, it seems likely that the future of synchronization protocols in cattle will continue to hinge on effectively converting the cow into an induced ovulator to allow timed AI.

Acknowledgments

The authors thank the many colleagues and trainees who performed the research cited in this article and a great deal more research that has not been cited but has added to our understanding of synchronization of ovulation and timed AI, particularly Jerry Guenther, Jose Vasconcelos, Roy Silcox, Paul Fricke, and Michael Mee, who performed many of the early studies discussed in this article.

References

[1] Lauderdale JW. ASAS Centennial Paper: Contributions in the *Journal of Animal Science* to the development of protocols for breeding management of cattle through synchronization of estrus and ovulation. J Anim Sci 2009;87:801–12.
[2] Odde KG. A review of synchronization of estrus in postpartum cattle. J Anim Sci 1990;68:817–30.
[3] Zimbelman RG, Smith LW. Control of ovulation in cattle with melengestrol acetate. 2. Effects on follicular size and activity. J Reprod Fertil 1966;11:193–201.
[4] Thatcher WW, de la Sota RL, Schmitt EJ, Diaz TC, Badinga L, Simmen FA, et al. Control and management of ovarian follicles in cattle to optimize fertility. Reprod Fertil Dev 1996;8:203–17.
[5] Wiltbank JN, Zimmerman DR, Ingalls JE, Rowden WW. Use of progestational compounds alone or in combination with estrogen for synchronization of estrus. J Anim Sci 1965;24:990–8.
[6] Beal WE. Current estrus synchronization and artificial insemination for cattle. J Anim Sci 1998;76:30–8.
[7] Pursley JR, Mee MO, Wiltbank MC. Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 1995;44:915–23.
[8] Pursley JR, Kosorok MR, Wiltbank MC. Reproductive management of lactating dairy cows using synchronization of ovulation. J Dairy Sci 1997;80:301–6.
[9] Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J Dairy Sci 1997;80:295–300.
[10] Rabiee AR, Lean IJ, Stevenson MA. Efficacy of Ovsynch program on reproductive performance in dairy cattle: a meta-analysis. J Dairy Sci 2005;88:2754–70.
[11] Adams GP, Matteri RI, Kastelic JP, Ko JC, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J Reprod Fertil 1992;94:177–88.
[12] Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. Selection of the dominant follicle in cattle. Biol Reprod 1996;55:1187–94.
[13] Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 1988;39:308–17.
[14] Driancourt MA, Thatcher WW, Terqui M, Andrieu D. Dynamics of ovarian follicular development in cattle during the estrous cycle, early pregnancy, and in response to PMSG. Domest Anim Endocrinol 1991;8:209–21.
[15] Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. J Reprod Fertil 1989;87:223–30.
[16] Baird DT, Land RB, Scaramuzzi RJ, Wheeler AG. Endocrine changes associated with luteal regression in the ewe; the secretion of ovarian oestradiol, progesterone and androstenedione and uterine prostaglandin F2alpha throughout the oestrous cycle. J Endocrinol 1976;69:275–86.
[17] Knickerbocker JJ, Wiltbank MC, Niswender GD. Mechanisms of luteolysis in domestic livestock. Domest Anim Endocrinol 1988;5:91–107.
[18] Ulberg LC, Lindley CE. Use of progesterone and estrogen in the control of reproductive activities in beef cattle. J Anim Sci 1960;19:1132–42.
[19] Hansel W, Schechter RJ, Malven PV, Simmons KR, Black DL, Hackett AJ, et al. Plasma hormone levels in 6-methyl-17acetoxyprogesterone and estradiol benzoate treated heifers. J Anim Sci 1975;40:671–81.
[20] Welch JA, Hackett AJ, Cunningham CJ, Heishman JO, Ford SP, Nadaraja R, et al. Control of estrus in lactating beef cows with prostaglandin F2 alpha and estradiol benzoate. J Anim Sci 1975;41:1686–92.
[21] Burgus R, Butcher M, Ling N, Monahan M, Rivier J, Fellows R, et al. Molecular structure of hypothalamic factor of ovine origin controlling secretion of hypophyseal gonadotropic luteinizing hormone (LH). C R Seances Acad Sci D 1971;273:1611–7 [in French].
[22] Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R. Purification, amino acid composition and N-terminus of hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem Biophys Res Commun 1971;44:205–10.
[23] Schally AV, Arimura A, Kastin AJ, Matsuo H, Baba Y, Redding TW, et al. Gonadotropin releasing hormone - one polypeptide regulates secretion of luteinizing and follicle stimulating hormones. Science 1971;173:1036–42.
[24] Matsuo H, Baba Y, Nair RM, Arimura A, Schally AV. Structure of porcine LH- and FSH-releasing hormone 1. Proposed amino acid sequence. Biochem Biophys Res Commun 1971;43:1334–9.
[25] Fernandez-Limia O, Murphy BD, Manns JG. Synchronization of preovulatory LH surges after PGF2-alpha in beef cattle - effects of D-Ala-GnRH and testosterone benzoate. Theriogenology 1977;7:73–81.
[26] Kaltenbach CC, Dunn TG, Kiser TE, Corah LR, Akbar AM, Niswender GD. Release of FSH and LH in beef heifers by synthetic GnRH. J Anim Sci 1974;38:357–62.
[27] Britt JH, Kittok RJ, Harrison DS. Ovulation, estrus and endocrine response after GnRH in early postpartum cows. J Anim Sci 1974;39:915–9.
[28] Roche JF. Control of time of ovulation in heifers treated with progesterone and gondadotrophin releasing hormone. J Reprod Fertil 1975;43:471–7.
[29] Kittok RJ, Britt JH, Convey EM. Endocrine response after GnRH in luteal phase cows and cows with ovarian follicular cysts. J Anim Sci 1973;37:985–9.
[30] Kittok RJ, Britt JH, Convey EM. Effect of GnRH on LH and progesterone in cystic cows. J Anim Sci 1972;35:1120.
[31] Cantley TC, Garverick HA, Bierschwal CJ, Martin CE, Youngquist RS. Hormonal responses of dairy cows with ovarian cysts to GnRH. J Anim Sci 1975;41:1666–73.
[32] Britt JH, Kiser TE, Seguin BE, Hafs HD, Oxender WD, Ritchie HD. Fertility after GnRH and PGF2 alpha in suckling cows. J Anim Sci 1975;41:345.
[33] Silcox RW, Powell KL, Kiser TE. Ability of dominant follicles (DF) to respond to exogenous GnRH administration is dependent on their stage of development. J Anim Sci 1993;71:513.
[34] Thatcher WW, Drost M, Savio JD, Macmillan KL, Entwistle KW, Schmitt EJ, et al. New clinical uses of GnRH and its analogs in cattle. Anim Reprod Sci 1993;33:27–49.

[35] Thatcher WW, Macmillan KL, Hansen PJ, Drost M. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 1989;31:149–64.
[36] Wolfenson D, Thatcher WW, Savio JD, Badinga L, Lucy MC. The effect of a GnRH analog on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. Theriogenology 1994;42:633–44.
[37] Twagiramungu H, Guilbault LA, Proulx J, Dufour JJ. Synchronization of estrus and fertility in beef cattle with 2 injections of buserelin and prostaglandin. Theriogenology 1992;38:1131–44.
[38] Twagiramungu H, Guilbault LA, Proulx J, Villeneuve P, Dufour JJ. Influence of an agonist of gonadotropin-releasing hormone (buserelin) on estrus synchronization and fertility in beef cows. J Anim Sci 1992;70:1904–10.
[39] Twagiramungu H, Guilbault LA, Proulx JG, Dufour JJ. Influence of corpus luteum and induced ovulation on ovarian follicular dynamics in postpartum cyclic cows treated with buserelin and cloprostenol. J Anim Sci 1994;72:1796–805.
[40] Twagiramungu H, Guilbault LA, Dufour JJ. Synchronization of ovarian follicular waves with a gonadotropin releasing hormone agonist to increase precision of estrus in cattle - a review. J Anim Sci 1995;73:3141–51.
[41] Twagiramungu H, Guilbault LA, Proulx JG, Ramkumar R, Dufour JJ. Buserelin overcomes the effect of the corpus luteum on ovarian follicular development in postpartum cycling cows. Anim Reprod Sci 1995;39:183–92.
[42] Wiltbank JN, Casida LE. Alteration of ovarian activity by hysterectomy. J Anim Sci 1956;15:134–40.
[43] Moor RM, Rowson LE. Local uterine mechanisms affecting luteal function in the sheep. J Reprod Fertil 1966;11:307–10.
[44] Del Campo MR, Ginther OJ. Vascular anatomy of uterus and ovaries and unilateral luteolytic effect of uterus - angioarchitecture in sheep. Am J Vet Res 1973;34:1377–85.
[45] Ginther OJ, Delcampo CH, Rawlings CA. Vascular anatomy of uterus and ovaries and unilateral luteolytic effect of uterus - local venoarterial pathway between uterus and ovaries in sheep. Am J Vet Res 1973;34:723–8.
[46] Pharriss BB, Wyngarde LJ. Effect of prostaglandin F2alpha on progestogen content of ovaries of pseudopregnant rats. Proc Soc Exp Biol Med 1969;130:92.
[47] Rowson LE, Tervit R, Brand A. The use of prostaglandins for synchronization of oestrus in cattle. J Reprod Fertil 1972;29:145.
[48] Louis TM, Hafs HD, Seguin BE. Progesterone, LH, estrus and ovulation after prostaglandin-F2alpha in heifers. Proc Soc Exp Biol Med 1973;143:152–5.
[49] Lauderdale JW, Chenault JR, Sequin BE, Thatcher WW. Fertility of cattle after PGF2alpha treatment. J Anim Sci 1973;37:319.
[50] Lauderdale JW. Estrus detection and synchronisation of dairy cattle in large herds. J Dairy Sci 1974;57:348–54.
[51] Momont HW, Sequin BE. Influence of day of estrous cycle on response to PGF2 alpha products: implication for AI programs for dairy cattle. 10th International Congress on Animal Reproduction and Artificial Insemination. Champaign: University of Illinois at Urbana-Champaign; 1984. p. 336–8.
[52] Pursley JR, Silcox RW, Wiltbank MC. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J Dairy Sci 1998;81:2139–44.
[53] de Araujo Berber RC, Madureira EH, Baruselli PS. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (*Bubalus bubalis*). Theriogenology 2002;57:1421–30.
[54] Sarkar M, Prakash BS. Synchronization of ovulation in yaks (*Poephagus grunniens L.*) using PGF(2alpha) and GnRH. Theriogenology 2005;63:2494–503.
[55] Holtz W, Sohnrey B, Gerland M, Driancourt MA. Ovsynch synchronization and fixed-time insemination in goats. Theriogenology 2008;69:785–92.
[56] Bowdridge EC, Knox WB, Whisnant CS, Farin CE. NCSynch: a novel, progestagen-free protocol for ovulation synchronization and timed artificial insemination in goats. Small Rumin Res 2013;110:42–5.
[57] Nur Z, Nak Y, Nak D, Ustuner B, Tuna B, Simsek G, et al. The use of progesterone-supplemented Co-synch and Ovsynch for estrus synchronization and fixed-time insemination in nulliparous Saanen goat. Turk J Vet Anim Sci 2013;37:183–8.
[58] Amiridis GS, Valasi I, Menegatos I, Rekkas C, Goulas P, Papanikolaou T, et al. Luteal stage dependence of pituitary response to gonadotrophin-releasing hormone in cyclic dairy ewes subjected to synchronisation of ovulation. Reprod Fertil Dev 2005;17:769–74.
[59] Husein MQ, Romano JE, Bailey MT, Ababneh MM, Crabo BG, Godfrey RW, et al. Estrous synchronization and pregnancy rate of transcervically inseminated ewes during the breeding season. Sheep Goat Res J 1998;14:148–52.
[60] Stevenson JS, Phatak AP. Inseminations at estrus induced by presynchronization before application of synchronized estrus and ovulation. J Dairy Sci 2005;88:399–405.
[61] Silva E, Sterry RA, Kolb D, Mathialagan N, McGrath MF, Ballam JM, et al. Effect of interval to resynchronization of ovulation on fertility of lactating Holstein cows when using transrectal ultrasonography or a pregnancy-associated glycoprotein enzyme-linked immunosorbent assay to diagnose pregnancy status. J Dairy Sci 2009;92:3643–50.
[62] Lopez H, Satter LD, Wiltbank MC. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 2004;81:209–23.
[63] Roelofs J, Lopez-Gatius F, Hunter RH, van Eerdenburg FJ, Hanzen C. When is a cow in estrus? Clinical and practical aspects. Theriogenology 2010;74:327–44.
[64] Roelofs JB, van Eerdenburg F, Soede NM, Kemp B. Various behavioral signs of estrous and their relationship with time of ovulation in dairy cattle. Theriogenology 2005;63:1366–77.
[65] Fricke PM, Caraviello DZ, Weigel KA, Welle ML. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J Dairy Sci 2003;86:3941–50.
[66] Stevenson JS, Tiffany SM. Resynchronizing estrus and ovulation after not-pregnant diagnosis and various ovarian states including cysts. J Dairy Sci 2004;87:3658–64.
[67] Lopes Jr G, Giordano JO, Valenza A, Herlihy MM, Guenther JN, Wiltbank MC, et al. Effect of timing of initiation of resynchronization and presynchronization with gonadotropin-releasing hormone on fertility of resynchronized inseminations in lactating dairy cows. J Dairy Sci 2013;96:3788–98.
[68] Giordano JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP, et al. Increased fertility in lactating dairy cows resynchronized with Double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J Dairy Sci 2012;95:639–53.
[69] Giordano JO, Wiltbank MC, Guenther JN, Ares MS, Lopes Jr G, Herlihy MM, et al. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. J Dairy Sci 2012;95:5612–25.
[70] Thompson IM, Cerri RL, Kim IH, Green JA, Santos JE, Thatcher WW. Effects of resynchronization programs on pregnancy per artificial insemination, progesterone, and pregnancy-associated glycoproteins in plasma of lactating dairy cows. J Dairy Sci 2010;93:4006–18.
[71] Bartolome JA, van Leeuwen JJ, Thieme M, Sa Filho OG, Melendez P, Archbald LF, et al. Synchronization and resynchronization of inseminations in lactating dairy cows with the CIDR insert and the Ovsynch protocol. Theriogenology 2009;72:869–78.
[72] Schmitt EJ, Diaz T, Drost M, Thatcher WW. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. J Anim Sci 1996;74:1084–91.
[73] Burke JM, sal Sota RL, de la Risco CA, Staples CR, Schmitt EJP, Thatcher WW. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. J Dairy Sci 1996;79:1385–93.
[74] Herlihy MM, Berry DP, Crowe MA, Diskin MG, Butler ST. Evaluation of protocols to synchronize estrus and ovulation in seasonal calving pasture-based dairy production systems. J Dairy Sci 2011;94:4488–501.
[75] Herlihy MM, Crowe MA, Berry DP, Diskin MG, Butler ST. Factors associated with fertility outcomes in cows treated with protocols to synchronize estrus and ovulation in seasonal-calving, pasture-based dairy production systems. J Dairy Sci 2013;96:1485–98.
[76] Giordano JO, Kalantari AS, Fricke PM, Wiltbank MC, Cabrera VE. A daily herd Markov-chain model to study the reproductive and economic impact of reproductive programs combining timed artificial insemination and estrus detection. J Dairy Sci 2012;95:5442–60.
[77] Giordano JO, Fricke PM, Wiltbank MC, Cabrera VE. An economic decision-making support system for selection of reproductive management programs on dairy farms. J Dairy Sci 2011;94:6216–32.
[78] Galvao KN, Federico P, De Vries A, Schuenemann GM. Economic comparison of reproductive programs for dairy herds using estrus

detection, timed artificial insemination, or a combination. J Dairy Sci 2013;96:2681–93.
[79] Ribeiro ES, Galvao KN, Thatcher WW, Santos JEP. Economic aspects of applying reproductive technologies to dairy herds. Anim Reprod 2012;9:370–87.
[80] Austin EJ, Mihm M, Ryan MP, Williams DH, Roche JF. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. J Anim Sci 1999;77:2219–26.
[81] Roche JF, Austin EJ, Ryan M, O'Rourke M, Mihm M, Diskin MG. Regulation of follicle waves to maximize fertility in cattle. J Reprod Fertil Suppl 1999;54:61–71.
[82] Wiltbank MC, Sartori R, Vasconcelos JL, Nascimento AB, Souza AH, Cunha AP, et al. Managing the dominant follicle in high-producing dairy cows. Soc Reprod Fertil Suppl 2010;67:231–45.
[83] Bleach EC, Glencross RG, Knight PG. Association between ovarian follicle development and pregnancy rates in dairy cows undergoing spontaneous oestrous cycles. Reproduction 2004; 127:621–9.
[84] Ahmad N, Beam SW, Butler WR, Deaver DR, Duby RT, Elder DR, et al. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. J Anim Sci 1996;74:1943–52.
[85] Revah I, Butler WR. Prolonged dominance of follicles and reduced viability of bovine oocytes. J Reprod Fertil 1996;106:39–47.
[86] Ahmad N, Schrick FN, Butcher RL, Inskeep EK. Effect of persistent follicles on early embryonic losses in beef cows. Biol Reprod 1995; 52:1129–35.
[87] Cerri RL, Rutigliano HM, Chebel RC, Santos JE. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reproduction 2009;137:813–23.
[88] Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 2001;56:307–14.
[89] Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, et al. Relationship between follicle size at insemination and pregnancy success. Proc Natl Acad Sci U S A 2005;102:5268–73.
[90] Geary TW, Smith MF, Macneil MD, Day ML, Bridges GA, Perry GA, et al. Influence of follicular characteristics at ovulation on early embryonic survival. J Anim Sci 2013;91:3014–21.
[91] Atkins JA, Smith MF, MacNeil MD, Jinks EM, Abreu FM, Alexander LJ, et al. Pregnancy establishment and maintenance in cattle. J Anim Sci 2013;91:722–33.
[92] Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, et al. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. J Anim Sci 2013;91: 1176–85.
[93] Pohler KG, Geary TW, Atkins JA, Perry GA, Jinks EM, Smith MF. Follicular determinants of pregnancy establishment and maintenance. Cell Tissue Res 2012;349:649–64.
[94] Bridges GA, Helser LA, Grum DE, Mussard ML, Gasser CL, Day ML. Decreasing the interval between GnRH and PGF(2 alpha) from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. Theriogenology 2008;69:843–51.
[95] Santos JE, Narciso CD, Rivera F, Thatcher WW, Chebel RC. Effect of reducing the period of follicle dominance in a timed artificial insemination protocol on reproduction of dairy cows. J Dairy Sci 2010;93:2976–88.
[96] Brusveen DJ, Cunha AP, Silva CD, Cunha PM, Sterry RA, Silva EPB, et al. Altering the time of the second gonadotropin-releasing hormone injection and artificial insemination (AI) during ovsynch affects pregnancies per AI in lactating dairy cows. J Dairy Sci 2008;91:1044–52.
[97] Brusveen DJ, Souza AH, Wiltbank MC. Effects of additional prostaglandin F-2 alpha and estradiol-17 beta during Ovsynch in lactating dairy cows. J Dairy Sci 2009;92:1412–22.
[98] Ribeiro ES, Monteiro AP, Lima FS, Ayres H, Bisinotto RS, Favoreto M, et al. Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. J Dairy Sci 2012;95:2513–22.
[99] Souza AH, Gumen A, Silva EP, Cunha AP, Guenther JN, Peto CM, et al. Supplementation with estradiol-17 beta before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. J Dairy Sci 2007;90:4623–34.
[100] Gumen A, Guenther JN, Wiltbank MC. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J Dairy Sci 2003;86:3184–94.
[101] Chebel RC, Al-Hassan MJ, Fricke PM, Santos JE, Lima JR, Martel CA, et al. Supplementation of progesterone via controlled internal drug release inserts during ovulation synchronization protocols in lactating dairy cows. J Dairy Sci 2010;93:922–31.
[102] Wiltbank MC, Gumen A, Sartori R. Physiological classification of anovulatory conditions in cattle. Theriogenology 2002;57:21–52.
[103] Gumen A, Wiltbank MC. Follicular cysts occur after a normal estradiol-induced GnRH/LH surge if the corpus hemorrhagicum is removed. Reproduction 2005;129:737–45.
[104] Gumen A, Wiltbank MC. An alteration in the hypothalamic action of estradiol due to lack of progesterone exposure can cause follicular cysts in cattle. Biol Reprod 2002;66:1689–95.
[105] Wiltbank MC, Sartori R, Herlihy MM, Vasconcelos JL, Nascimento AB, Souza AH, et al. Managing the dominant follicle in lactating dairy cows. Theriogenology 2011;76:1568–82.
[106] Bisinotto RS, Chebel RC, Santos JE. Follicular wave of the ovulatory follicle and not cyclic status influences fertility of dairy cows. J Dairy Sci 2010;93:3578–87.
[107] Wiltbank MC, Gumen A, Lopez H, Sartori R. Management and treatment of dairy cows that are not cycling or have follicular cysts. Cattle Pract 2008;16:14–9.
[108] Fonseca FA, Britt JH, McDaniel BT, Wilk JC, Rakes AH. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J Dairy Sci 1983;66:1128–47.
[109] Wiltbank MC, Souza AH, Giordano JO, Nascimento AB, Vasconcelos JM, Pereira MH, et al. Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. Anim Reprod 2012;9:231–41.
[110] Pursley JR, Martins JP. Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. Reprod Fertil Dev 2012;24: 267–71.
[111] Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS, et al. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J Dairy Sci 2006;89: 2567–78.
[112] Stevenson JS, Tenhouse DE, Krisher RL, Lamb GC, Larson JE, Dahlen CR, et al. Detection of anovulation by heatmount detectors and transrectal ultrasonography before treatment with progesterone in a timed insemination protocol. J Dairy Sci 2008;91:2901–15.
[113] Dadarwal D, Mapletoft RJ, Adams GP, Pfeifer LF, Creelman C, Singh J. Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. Theriogenology 2013;79:859–66.
[114] Bisinotto RS, Ribeiro ES, Lima FS, Martinez N, Greco LF, Barbosa LF, et al. Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. J Dairy Sci 2013;96: 2214–25.
[115] Melendez P, Gonzalez G, Aguilar E, Loera O, Risco C, Archbald LF. Comparison of two estrus-synchronization protocols and timed artificial insemination in dairy cattle. J Dairy Sci 2006;89:4567–72.
[116] Lima JR, Rivera FA, Narciso CD, Oliveira R, Chebel RC, Santos JE. Effect of increasing amounts of supplemental progesterone in a timed artificial insemination protocol on fertility of lactating dairy cows. J Dairy Sci 2009;92:5436–46.
[117] Fricke PM, Guenther JN, Wiltbank MC. Efficacy of decreasing the dose of GnRH used in a protocol for synchronization of ovulation and timed AI in lactating dairy cows. Theriogenology 1998;50: 1275–84.
[118] Giordano JO, Fricke PM, Guenther JN, Lopes Jr G, Herlihy MM, Nascimento AB, et al. Effect of progesterone on magnitude of the luteinizing hormone surge induced by two different doses of gonadotropin-releasing hormone in lactating dairy cows. J Dairy Sci 2012;95:3781–93.
[119] Bello NM, Steibel JP, Pursley JR. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. J Dairy Sci 2006;89:3413–24.
[120] Chebel RC, Santos JE, Cerri RL, Rutigliano HM, Bruno RG. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J Dairy Sci 2006;89: 4205–19.
[121] Keskin A, Yilmazbas-Mecitoglu G, Gumen A, Karakaya E, Darici R, Okut H. Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology 2010;74:602–7.
[122] Giordano JO, Wiltbank MC, Fricke PM, Bas S, Pawlisch RA, Guenther JN, et al. Effect of increasing GnRH and PGF2α dose

during Double-Ovsynch on ovulatory response, luteal regression, and fertility of lactating dairy cows. Theriogenology 2013;80:773–83.

[123] Martins JP, Policelli RK, Neuder LM, Raphael W, Pursley JR. Effects of cloprostenol sodium at final prostaglandin F2alpha of Ovsynch on complete luteolysis and pregnancy per artificial insemination in lactating dairy cows. J Dairy Sci 2011;94:2815–24.

[124] Ribeiro ES, Bisinotto RS, Favoreto MG, Martins LT, Cerri RL, Silvestre FT, et al. Fertility in daily cows following presynchronization and administering twice the luteolytic dose of prostaglandin F-2 alpha as one or two injections in the 5-day timed artificial insemination protocol. Theriogenology 2012;78:273–84.

[125] Souza AH, Viechnieski S, Lima FA, Silva FF, Araujo R, Bo GA, et al. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology 2009;72:10–21.

[126] Ayres H, Ferreira RM, Cunha AP, Araujo RR, Wiltbank MC. Effect of supplemental FSH during Ovsynch in high producing holstein cows during summer. Reprod Domest Anim 2008;43:30–1.

[127] Ayres H, Ferreira RM, Wiltbank MC, Baruselli PS. Effect of supplemental FSH or eCG during a protocol for timed AI in high-producing Holstein cows. Reprod Fertil Dev 2010;22:203.

[128] Bryan MA, Bo G, Mapletoft RJ, Emslie FR. The use of equine chorionic gonadotropin in the treatment of anestrous dairy cows in gonadotropin-releasing hormone/progesterone protocols of 6 or 7 days. J Dairy Sci 2013;96:122–31.

[129] Pulley SL, Wallace LD, Mellieon Jr HI, Stevenson JS. Ovarian characteristics, serum concentrations of progesterone and estradiol, and fertility in lactating dairy cows in response to equine chorionic gonadotropin. Theriogenology 2013;79:127–34.

[130] Bo GA, Baruselli PS, Martinez MF. Pattern and manipulation of follicular development in *Bos indicus* cattle. Anim Reprod Sci 2003; 78:307–26.

[131] Sa Filho MF, Ayres H, Ferreira RM, Marques MO, Reis EL, Silva RC, et al. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (*Bos indicus*) cows. Theriogenology 2010;73:651–8.

[132] Pitaluga PC, Sa Filho MF, Sales JN, Baruselli PS, Vincenti L. Manipulation of the proestrous by exogenous gonadotropin and estradiol during a timed artificial insemination protocol in suckled *Bos indicus* beef cows. Livest Sci 2013;154:229–34.

[133] Small JA, Colazo MG, Kastelic JP, Erickson NE, Mapletoft RJ. Effects of presynchronization and eCG on pregnancy rates to GnRH-based, fixed-time artificial insemination in beef heifers. Can J Anim Sci 2010;90:23–34.

[134] Sa Filho OG, Meneghetti M, Peres RF, Lamb GC, Vasconcelos JL. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: strategies and factors affecting fertility. Theriogenology 2009;72:210–8.

[135] Sales JN, Crepaldi GA, Girotto RW, Souza AH, Baruselli PS. Fixed-time AI protocols replacing eCG with a single dose of FSH were less effective in stimulating follicular growth, ovulation, and fertility in suckled-anestrus Nelore beef cows. Anim Reprod Sci 2011;124:12–8.

[136] Vasconcelos JL, Sangsritavong S, Tsai SJ, Wiltbank MC. Acute reduction in serum progesterone concentrations after feed intake in dairy cows. Theriogenology 2003;60:795–807.

[137] Vasconcelos JL, Silcox RW, Rosa GJ, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 1999;52:1067–78.

[138] Keith BR, Leslie KE, Johnson WH, Walton JS. Effect of presynchronization using prostaglandin F2alpha and a milk-ejection test on pregnancy rate after the timed artificial insemination protocol, Ovsynch. Theriogenology 2005;63:722–38.

[139] Moreira F, de la Sota RL, Diaz T, Thatcher WW. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J Anim Sci 2000;78:1568–76.

[140] Moreira F, Orlandi C, Risco CA, Mattos R, Lopes F, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci 2001;84:1646–59.

[141] Ei-Zarkouny SZ, Cartmill JA, Hensley BA, Stevenson JS. Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. J Dairy Sci 2004;87: 1024–37.

[142] Navanukraw C, Redmer DA, Reynolds LP, Kirsch JD, Grazul-Bilska AT, Fricke PM. A modified presynchronization protocol improves fertility to timed artificial insemination in lactating dairy cows. J Dairy Sci 2004;87:1551–7.

[143] LeBlanc SJ, Leslie KE. Short communication: presynchronization using a single injection of PGF2alpha before synchronized ovulation and first timed artificial insemination in dairy cows. J Dairy Sci 2003;86:3215–7.

[144] Cordoba MC, Fricke PM. Evaluation of two hormonal protocols for synchronization of ovulation and timed artificial insemination in dairy cows managed in grazing-based dairies. J Dairy Sci 2001;84: 2700–8.

[145] Silva E, Sterry RA, Kolb D, Wiltbank MC, Fricke PM. Effect of pretreatment with prostaglandin F-2 alpha before resynchronization of ovulation on fertility of lactating dairy cows. J Dairy Sci 2007;90: 5509–17.

[146] Galvao KN, Sa Filho MF, Santos JE. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. J Dairy Sci 2007;90:4212–8.

[147] Fricke PM. Preparing cows for the early postpartum artificial insemination. Large Anim Rev 2013;19:95–9.

[148] Gumen A, Keskin A, Yilmazbas-Mecitoglu G, Karakaya E, Alkan A, Okut H, et al. Effect of presynchronization strategy before Ovsynch on fertility at first service in lactating dairy cows. Theriogenology 2012;78:1830–8.

[149] Chebel RC, Santos JE. Effect of inseminating cows in estrus following a presynchronization protocol on reproductive and lactation performances. J Dairy Sci 2010;93:4632–43.

[150] Peters MW, Pursley JR. Fertility of lactating dairy cows treated with Ovsynch after presynchronization injections of PGF(2 alpha) and GnRH. J Dairy Sci 2002;85:2403–6.

[151] Bello NM, Pursley JR. Strategies to maximize ovulation to first GnRH of Ovsynch in lactating dairy cows. J Dairy Sci 2007;90: 327–8.

[152] Yilmaz C, Yilmaz O, Ucar M. Effect of PGF2 alpha and GnRH injections applied before Ovsynch on pregnancy rates in cows and heifers. Kafkas Univ Vet Fak Derg 2011;17:641–4.

[153] Souza AH, Ayres H, Ferreira RM, Wiltbank MC. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 2008; 70:208–15.

[154] Ayres H, Ferreira RM, Cunha AP, Araujo RR, Wiltbank MC. Double-Ovsynch in high-producing dairy cows: effects on progesterone concentrations and ovulation to GnRH treatments. Theriogenology 2013;79:159–64.

[155] Herlihy MM, Giordano JO, Souza AH, Ayres H, Ferreira RM, Keskin A, et al. Presynchronization with Double-Ovsynch improves fertility at first postpartum artificial insemination in lactating dairy cows. J Dairy Sci 2012;95:7003–14.

[156] Dewey ST, Mendonca LG, Lopes G, Rivera FA, Guagnini F, Chebel RC, et al. Resynchronization strategies to improve fertility in lactating dairy cows utilizing a presynchronization injection of GnRH or supplemental progesterone: I. Pregnancy rates and ovarian responses. J Dairy Sci 2010;93:4086–95.

[157] Alkar A, Tibary A, Wenz JR, Nebel RL, Kasimanickam R. Presynchronization with GnRH 7 days prior to resynchronization with CO-Synch did not improve pregnancy rate in lactating dairy cows. Theriogenology 2011;76:1036–41.

[158] Bruno RG, Farias AM, Hernandez-Rivera JA, Navarrette AE, Hawkins DE, Bilby TR. Effect of gonadotropin-releasing hormone or prostaglandin F(2alpha)-based estrus synchronization programs for first or subsequent artificial insemination in lactating dairy cows. J Dairy Sci 2013;96:1556–67.

[159] Chebel RC, Scanavez AA, Silva PRB, Moraes JGN, Mendonca LG, Lopes G. Evaluation of presynchronized resynchronization protocols for lactating dairy cows. J Dairy Sci 2013;96:1009–20.

[160] Mendonca LG, Dewey ST, Lopes G, Rivera FA, Guagnini FS, Fetrow JP, et al. Effects of resynchronization strategies for lactating Holstein cows on pattern of reinsemination, fertility, and economic outcome. Theriogenology 2012;77:1151–8.

[161] Rutigliano HM, Lima FS, Cerri RL, Greco LF, Vilela JM, Magalhaes V, et al. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J Dairy Sci 2008;91:3323–36.

[162] Bicalho RC, Cheong SH, Warnick LD, Guard CL. Evaluation of progesterone supplementation in a prostaglandin F2alpha-based presynchronization protocol before timed insemination. J Dairy Sci 2007;90:1193–200.

[163] Giordano JO, Fricke PM, Cabrera VE. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. J Dairy Sci 2013;96:949–61.

[164] Haughian JM, Ginther OJ, Diaz FJ, Wiltbank MC. Gonadotropin-releasing hormone, estradiol, and inhibin regulation of follicle-stimulating hormone and luteinizing hormone surges: implications for follicle emergence and selection in heifers. Biol Reprod 2013;88: 165–77.

[165] Bodensteiner KJ, Kot K, Wiltbank MC, Ginther OJ. Synchronization of emergence of follicular waves in cattle. Theriogenology 1996; 45:1115–28.

[166] Miksch ED, Lefever DG, Mukembo G, Spitzer JC, Wiltbank JN. Synchronization of estrus in beef cattle 2. Effect of an injection of norgestomet and an estrogen in conjunction with a norgestomet implant in heifers and cows. Theriogenology 1978;10:201–21.

[167] Spitzer JC, Burrell WC, Lefever DG, Whitman RW, Wiltbank JN. Synchronization of estrus in beef cattle 1. Utilization of a norgestomet implant and injection of estradiol valerate. Theriogenology 1978;10:181–200.

[168] Spitzer JC, Jones DL, Miksch ED, Wiltbank JN. Synchronization of estrus in beef cattle 3. Field trials in heifers using a norgestomet implant and injections of norgestomet and estradiol valerate. Theriogenology 1978;10:223–9.

[169] Smith MF, Burrell WC, Broadway J, Wiltbank JN. Estrus and pregnancy in beef heifers following use of the Syncro-Mate-B treatment (SMB)12. Theriogenology 1979;12:183–95.

[170] Smith MF, Burrell WC, Shipp LD, Sprott LR, Songster WN, Wiltbank JN. Hormone treatments and use of calf removal in postpartum beef cows. J Anim Sci 1979;48:1285–94.

[171] Bo GA, Adams GP, Caccia M, Martinez M, Pierson RA, Mapletoft RJ. Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Anim Reprod Sci 1995;39:193–204.

[172] Bo GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. Theriogenology 1995;43: 31–40.

[173] Bo GA, Adams GP, Nasser LF, Pierson RA, Mapletoft RJ. Effect of estradiol valerate on ovarian follicles, emergence of follicular waves and circulating gonadotropins in heifers. Theriogenology 1993;40:225–39.

[174] Bo GA, Adams GP, Pierson RA, Tribulo HE, Caccia M, Mapletoft RJ. Follicular wave dynamics after estradiol 17-Beta treatment of heifers with or without a progestogen implant. Theriogenology 1994;41:1555–69.

[175] Cavalieri J, Hepworth G, Parker KI, Wright PJ, Macmillan KL. Effect of treatment with progesterone and oestradiol when starting treatment with an intravaginal progesterone releasing insert on ovarian follicular development and hormonal concentrations in Holstein cows. Anim Reprod Sci 2003;76:177–93.

[176] Kim UH, Suh GH, Hur TY, Kang SJ, Kang HG, Park SB, et al. The effects of administering estradiol benzoate plus progesterone during the growth or static phases of the dominant follicle in CIDR-treated lactating dairy cows. J Reprod Dev 2007;53:591–6.

[177] Burke CR, Mussard ML, Gasser CL, Grum DE, Day ML. Estradiol benzoate delays new follicular wave emergence in a dose-dependent manner after ablation of the dominant ovarian follicle in cattle. Theriogenology 2003;60:647–58.

[178] Martinez MF, Kastelic JP, Bo GA, Caccia M, Mapletoft RJ. Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. Anim Reprod Sci 2005;86:37–52.

[179] Colazo MG, Martinez MF, Small JA, Kastelic JP, Burnley CA, Ward DR, et al. Effect of estradiol valerate on ovarian follicle dynamics and superovulatory response in progestin-treated cattle. Theriogenology 2005;63:1454–68.

[180] Souza AH, Cunha AP, Caraviello DZ, Wiltbank MC. Profiles of circulating estradiol-17ß after different estrogen treatments in lactating dairy cows. Anim Reprod 2005;2:224–32.

[181] Sangsritavong S, Combs DK, Sartori R, Armentano LE, Wiltbank MC. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17 beta in dairy cattle. J Dairy Sci 2002; 85:2831–42.

[182] Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gumen A. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 2006;65:17–29.

[183] Wiltbank JN, Sturges JC, Wideman D, Lefever DG, Faulkner LC. Control of estrus and ovulation using subcutaneous implants and estrogens in beef cattle. J Anim Sci 1971;33:600–6.

[184] Pereira MH, Sanches CP, Guida TG, Rodrigues AD, Aragon FL, Veras MB, et al. Timing of prostaglandin F-2 alpha treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. J Dairy Sci 2013;96: 2837–46.

[185] Diskin MG, Austin EJ, Roche JF. Exogenous hormonal manipulation of ovarian activity in cattle. Domest Anim Endocrinol 2002;23: 211–28.

[186] Sales JN, Carvalho JB, Crepaldi GA, Cipriano RS, Jacomini JO, Maio JR, et al. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in *Bos indicus* cows submitted to a timed artificial insemination protocol. Theriogenology 2012;78:510–6.

[187] Baruselli PS, Sa Filho MF, Ferreira RM, Sales JN, Gimenes LU, Vieira LM, et al. Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. Reprod Domest Anim 2012;47:134–41.

[188] Norman HD, Wright JR, Hubbard SM, Miller RH, Hutchison JL. Reproductive status of Holstein and Jersey cows in the United States. J Dairy Sci 2009;92:3517–28.

[189] VanRaden PM, Sanders AH, Tooker ME, Miller RH, Norman HD, Kuhn MT, et al. Development of a National Genetic Evaluation for cow fertility. J Dairy Sci 2004;87:2285–92.

[190] Kuhn MT, Hutchison JL, Wiggans GR. Characterization of Holstein heifer fertility in the United States. J Dairy Sci 2006;89: 4907–20.

[191] Souza AH, Carvahlo PA, Shaver RD, Wiltbank MC, Cabrera V. Effect of timed AI use on reproductive performance and culling rate in Wisconsin dairy herds. J Dairy Sci 2013;96:390.

[192] Baruselli PS, Sales JN, Sala RV, Viera LM, Sa Filho MF. History, evolution and perspectives of timed artificial insemination programs in Brazil. Anim Reprod 2012;9:139–52.