SYNCHRONIZATION OF OVULATION IN BOVINES - REVIEW

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INTRODUCTION

Reproductive performance is the pivotal physiological process for a successful dairy farm. Many dairy herds incur a substantial cut in the economy due to the poor conception rate and proper herd replacement. Genetic improvement of bovines for increasing milk yield can be achieved by application of artificial insemination (A.I.). Successful results of A.I. depend on accurate detection of estrus. Development of Timed Artificial Insemination (TAI) programs allows reduced emphasis on detection of oestrus because all cows are inseminated at a specific time in relation to the hormone injection. Estrus detection has been cited as one of the most important factors affecting the reproductive success of artificial insemination programs. Various estrus synchronization protocols have been developed to bring a large percentage of groups of females into estrus at a predetermined time. Earlier protocols have involved controlling estrous cycle length in cattle either by extending the life span of the corpus luteum by the use of progestogens or shortening the life span of the corpus luteum by the use of Prostaglandins. The reduced fertility following the earlier synchronization protocols made it necessary to understand ovarian follicular and corpus luteum dynamics in cattle. An increase in this basic understanding as well as the development of treatment regimens to manipulate ovarian follicular and corpus luteum dynamics over the last decade have resulted in development of better estrus synchronization protocols based on a) elimination of the dominant follicle and initiation of new follicular wave, b) initiation of new follicular wave, synchronization of Ovulation and timed artificial insemination. These protocols are very promising and have the potential to enhance pregnancy rates and the success of artificial insemination programs.

Synchronization of ovulation with GnRH and $PGF_2\alpha$ brought a major impact on managing lactating cows by allowing timed AI and ascertained ovulation time with eliminating oestrus detection (Pursley *et al.,* 1997). Therefore increased rates of estrus detection would ideally increase pregnancy rates within set time limits, thereby leading to shorter calving intervals, increased production, increased economy and improved standard of living of farmers in many developing countries. This review will focus the role of synchronization of ovulation on reproductive performance in the dairy cows.

Induction of ovulation in bovines using GnRH - PGF_2 based protocols

Thatcher *et al.* (1989) found that injec tion of 10 µg buserelin, modulated the ovarian follicular waves and CL function in cows.

Macmillan *et al.* (1985) reported similar results using 5µg buserelin in cows. Thus, the effectiveness of GnRH analogs has been demonstrated for manipulation of reproductive events in bovines. Thatcher *et al.* (1989) also stated that GnRH analogs injected prior to $PGF_2\alpha$ treatment increased the synchrony of estrus response in cows. Twagiramungu *et al.* (1995) reported that pretreatment with burserlin 6 days prior to $PGF_2\alpha$ treatment eliminated the need for estrus detection in cows.

Several synchronization protocols currently recommended for cows used GnRH in combination with PGF_2 for synchronization of estrus and ovulation. Each GnRH based protocol started with the same basic frame work, which involved an injection of GnRH followed with an injection of PGF_2, six (or) seven days later. The inclusion of GnRH analogues with PGF_2 (7 days prior to PGF_2) in estrus synchronization programs not only improved estrus detection rates and synchrony of estrus (Wolfenson *et al.,* 1994 and Twagiramungu *et al.,* 1995), but also induced fertile estrous cycles in both cyclic and anestrus bovines(Thompson *et al.,* 1999 and Stevenson *et al.,* 2000).

The random administration of GnRH during the estrous cycle resulted in LH release(Chenault *et al.,* 1990) which caused ovulation or luteinization of large follicles present in the ovary, synchronized the recruitment of a new follicular wave (Thatcher *et al.,* 1989 and Macmillan and Thatcher, 1991) and equalized the follicular development in wave pattern (Thatcher *et al.,* 1989; Macmillan and Thatcher 1991; Twagiramungu *et al.,* 1992; Wolfenson *et al.,* 1994 and Schmitt *et al.,* 1996). Subsequent administration of $PGF_2\alpha$ induced the regression of an original or GnRH-induced CLand allowed final maturation of the synchronized dominant follicle (Schmitt *et al.,*1996a). Furthermore, there was no apparent detrimental effect of GnRH on the responsiveness of GnRH-induced CL or spontaneous CL to prostaglandin (Twagiramungu *et al.,* 1995).

Select Synch (GnRH - PGF_2 Heat detection - AI)

Select-synch was the first protocol established utilizing GnRH analogs. The select synch cows were injected with GnRH and $PGF_2\alpha$ 7 days apart. Detection of estrus began 24-48 hours before the $PGF_2\alpha$ injection and continued for the next 5-7 days (Dejarnette *et al.*, 2004).

On day 0, all the cows received an injection of a GnRH analog. Starting 6 days after the injection, and for 6 successive days, the herd was monitored for estrus activity, and bred 8 to 12 hours after estrus detection. On day 7, cows that have not been detected in estrus were given an injection of $PGF_2$to induce luteolysis. The majority of cows exhibited estrus 36 to 72 hours after $PGF_2\alpha$ (Stevenson *et al.,* 2000).This was an effective protocol, but the need for estrus detection was still present (Twagiramungu *et al.,* 1992a) and pregnancy rates ranged from 20to 61per cent and pregnancy rates obtained by different investigators were 20.80 (Lemaster *et al.,* 2001); 40 (Kojima *et al.,* 2000); 41 (Stevenson *et al.,* 2000); 45 (Patterson *et al.,* 2001); 53 (Stevenson *et al.,* 2000); 61 (Dejarnette *et al.,* 2001) and 65 per cent(Constantaras and Kesler, 2004) following select synch.

Co-Synch (GnRH - PGF_2 GnRH + Fixed time AI)

Cosynch was modeled after Select Synch, however, the need for estrus detection was eliminated with a second GnRH injection. On day 0 all the cows received a GnRH analog. Seven days later all cows were injected with $PGF_2\alpha$ to induce luteolysis. Two days after PGF_2injection, all cows received a second GnRH injection followed by immediate insemination (Lamb *et al.,* 2001). Pregnancy rates after Cosynch was 40 to 54 per cent in cows and this system effectively reduced labor costs and higher levels of fertility were achievable in cows (Lamb *et al.,* 2001 and Geary *et al.,* 2001 a).

Hybrid Synch

Hybrid synch was a combination of select synch and co-synch systems (Stevenson *et al.,* 2000). Estrus detection and Al carried out until 72 hours after the $PGF_2\alpha$ injection and then mass-AI along with GnRH injection were done to those cows that did not exhibit estrus until 72 hours (Larson *et al.,* 2004; Dejarnette *et al.,* 2001a and Dejarnette *et al.,* 2004). Pregnancy rates in cows administered with the hybrid synch protocol was 34 (Stevenson *et al.,* 2000); 46 (Dejarnette *et al.,* 2001a); 53 (Larson *et al.,* 2004) and 52 per cent (Dejarnette *et al.,* 2004).

Ovsynch (GnRH - PGF_2- GnRH Fixed time AI)

To synchronize ovulation within a short time period and enable timed insemination in the GnRH-prostaglandin regime, an additional GnRH dose was included at 24 (De Rensis *et al.,* 1999); 48 (Pursley *et al.,* 1995); 54 (Twagiramungu *et al.,*1995) and 60 hours (Peters *et al.,* 1999) after $PGF_2\alpha$ treatment. The effectiveness of the second dose of GnRH 48 hours after prostaglandin treatment in synchronizing the timing of ovulation has been established for dairy and beef cows (Silcox *et al.,* 1995). A second dose of GnRH given at 48 hours after $PGF_2\alpha$ injection improved the precision of ovulation over an 8hours period from 24 to 32 hours after the second GnRH dose. The success of this addition to the standard combined GnRH-$PGF_2\alpha$ regimen in dairy cows gave rise to the recently developed Ovsynch or Timed Artificial Insemination (TAI) protocol, which allowed successful fixed-time AI without the need for estrus detection (Pursley *et al.,* 1995).

In the Ovsynch program, 100 µg of GnRH was given at random during the estrous cycle, followed by 25 mg of $PGF_2\alpha$ and a second dose of 100 µg GnRH (Pursley *et al.,* 1995).Ovulation was synchronized because the preovulatory follicles were at a similar stage in development and was responsive to LH at the time of the second GnRH treatment. This program coordinated follicular recruitment, CL regression and time of ovulation and permitted fixed time AI 16 hours after the second GnRH dose was administered (Burke *et al.,* 1996). Thus by synchronizing ovulation, reproduction in lactating dairy cows could be effectively managed without the need for estrus detection (Pursley *et al.,* 1997).There have also been reports (Geary *et al.,* 1998) of fertile ovulation in anestrus cows after the Ovsynch program attributable to the incorporation of GnRH (Britt *et al.,* 1974). Pursley *et al.*(1998) concluded that AI performed close to 16 hours after the second dose of GnRH in the Ovsynch protocol found to be optimal, though pregnancy rates per AI and calving rates were comparable to rates achieved after AI performed 0 to 24 hours after the second GnRH dose.

The success of the Ovsynch program has been proven to be influenced by the number of follicular waves or length of the follicular wave (Pursley *et al.,* 1997) as well as the stage of estrous cycle when the first GnRH dose was administered (Vasconcelos *et al.,* 1997; Vasconcelos *et al.,* 1999 and Moreira *et al.,* 2000b). Moreira *et al.* (2000b) concluded that the early luteal stage of the estrous cycle (day 5 to 12) was the optimal period for initiating the Ovsynch program. Vasconcelos *et al.*(1997) also recorded a higher pregnancy rate when cows were started on the Ovsynch protocol in the early luteal phase compared with the first 3 days or after day 13 of the estrous cycle. These findings were inconsistent with those of Keister *et al.* (1999) who noted similar reproductive performance in dairy cows whether Ovsynch treatment was initiated at random or on day 7 of the estrous cycle.

Pregnancy rates of 6 (Momcilovic *et al.,* 1998), 20 to 28 (Thompson *et al.,* 1999 and Oliveira *et al.,* 2002), 30 to 40 (Pursley *et al.,* 1997a; Mialot *et al.,* 1999; Pantoja *et al.,* 2002; Punyapornwithaya *et al.,* 2002; Gabor *et al.,* 2002 and Steckler *et al.,* 2002); 45 to 60 (Britt and Gaska, 1998; Geary *et al.,* 1998 and Mialot *et al.,* 2003) and 75 to 79 per cent (Keister *et al.,* 1999 and Jemmeson, 2000) have been reported in postpartum lactating cows following treatment with Ovsynch.

Ovsynch with Presynch

The stage of the estrous cycle at which the synchronization protocol was initiated affected ovarian responses to hormonal treatments, synchrony of ovulation and consequently pregnancy rates (Vasconcelos *et al.,* 1999). Initiation of the Ovsynch protocol in various stages of the estrous cycle showed that the interval between days 5 and 9 resulted in greatest fertility (Vasconcelos *et al.,* 1999). When the protocol was initiated after day 10 of the estrous cycle, a large proportion of cows had spontaneously regressed CL before the injection of $PGF_2\alpha$, which has been associated with a decline in fertility (Chebel *et al.,* 2006). Furthermore, the ability of the initial GnRH to induce ovulation of the dominant follicle and synchronize the emergence of a new follicular wave was largely reduced when the injection was administered between days 1 and 4 of the estrous cycle (Vasconcelos *et al.,*1999 and Cerri *et al.,* 2009).

Failure to ovulate in response to the initial GnRH was associated with the presence of a larger follicle at the end of the protocol (Vasconcelos *et al.,* 1999 and Cerri *et al.,* 2009). When given at random stages of the estrous cycle, only 50 to 60 per cent of the treated cows ovulated in response to GnRH, although only 41.10 per cent ovulated when this concept was tested in postpartum dairy cows (Navanukraw *et al.,* 2004). Therefore, presynchronization protocols have been developed to optimize fertility in response to timed AI programs to assure that the first GnRH was administered at the exact stages of the estrous cycle with greatest ovulatory response. Presynch, as the name implied, was a protocol that "pre-synchronizes" cows to the early stage of the estrous cycle for optimum response to GnRH, and thereby improved pregnancy rates to Ovsynch (Moreria *et al.,* 2000).

Presynch consisted of two injections of $PGF_2\alpha$ 14 days apart with the second $PGF_2\alpha$ given 10 to 14 days prior to the start of Ovsynch (Cordoba and Fricke, 2001; LeBlanc and Leslie, 2003; Moreira *et al.,* 2001 and Navanukraw *et al.,* 2004). Numerous studies have examined the interval between the second injection of $PGF_2\alpha$ and the initiation of TAI and the best results appeared to be from the use of 10 or 11 days (Galvao *et al.,* 2007 and Santos *et al.,*2002). Cows that responded to Presynch and started the TAI 10 or 11 days later were likely to be in the first 5 to 8 days of the estrous cycle and were more likely to respond to the first injection of GnRH by luteinizing the dominant follicle. Pregnancy per AI for Presynch-Ovsynch inseminations ranged from 37 to 42 per cent (Silva *et al.,* 2007 and Souza *et al.,* 2008). A single injection of $PGF_2\alpha$ given 10 to 14 days prior to Ovsynch has also been shown to be effective and has produced conception rates that ranged from 33 to 49 per cent (Cartmill *et al.,* 2001; Cordoba and Fricke, 2001 and LeBlanc and Leslie, 2003).

Presynchronization with Ovsynch protocol (Double-Ovsynch protocol) which utilized 6 treatments: GnRH on day 0, $PGF_2\alpha$ on day 7, GnRH on day 10, GnRH on day 17, $PGF_2\alpha$ on day 24, and GnRH 56 to 60 hours later followed by TAI in 12 to 16 hours (Souza *et al.,* 2008). The Double-Ovsynch protocol has been shown to increase the average pregnancy per AI similar to Presynch-Ovsynch but first lactation cows responded more favorably than pluriparous cows (Souza *et al.,* 2008). One benefit of Double-Ovsynch as compared to a traditional Presynch-Ovsynchwas the potential for improved conception rates in cows that were anestrous and anovulatory just prior to enrollment in the TAI program. The two injections of GnRH in the Double-Ovsynch protocol often facilitated a resumption of cyclicity in some anovulatory cows (Souza *et al.,* 2008).

Bello *et al.* (2006) used a Pre-Ovsynch program using $PGF_2\alpha$ and GnRH prior to Ovsynch in which cows were treated with 25 mg $PGF_2\alpha$ followed by 100 µg GnRH 2 days later. Then, either 4 (G4G), 5 (G5G) or 6(G6G) days later, cows received the 1st GnRH of Ovsynch, and continued the Ovsynch program. Controls received only the Ovsynch treatment with no presynchronization. Compared to Ovsynch alone, G6G dramatically improved the percentage of cows ovulating to 1st GnRH, percentage of cows responding to $PGF_2\alpha$ by luteolysis and ovulation to the final GnRH of Ovsynch and also found doubling of percentage of cows pregnant to G6G compared to Ovsynch alone.

Ovsynch Plus Progestogen / Progesterone

An alternative to improve synchronization without lengthening timed AI programs is progesterone (P_4) supplementation during the protocol. The use of intra vaginal devices for controlled release of progesterone from the GnRH to the PGF_2 injections maintains blood progesterone concentrations that prevent premature estrus behavior, LH surge, and ovulation. These devices have been used during timed AI protocols to improve fertility of dairy cows (El-Zarkouny *et al.,* 2004 and McDougall, 2010).

Intra vaginal inserts for controlled release of progesterone have been used to improve synchrony of ovulation and pregnancy rate in response to timed AI protocols (Lima *et al.,* 2009 and Chebel *et al.,* 2010). Progesterone released by the CIDR insert acted to reduce LH pulsatility and blocked the pre-ovulatory LH surge (Rathbone *et al.,* 2001); therefore, it potentially minimized the negative effects of spontaneous luteolysis before the injection of $PGF_2\alpha$ and reduced the occurrence of premature ovulations during a timed AI protocol. When cows had their estrous cycles presynchronized before a timed AI program, incorporation of a CIDR insert to the synchronization protocol was not beneficial to fertility (El-Zarkouny *et al.,* 2004 and Galvão *et al.,* 2004). Conversely, when cows were not presynchronized, the incorporation of a CIDR insert to the Ovsynch protocol improved pregnancy rate from 40 to 50 per cent on day 28 after AI, and from 33 to 38 per cent on day 56 after AI (Stevenson *et al.,* 2006). However, no benefit of incorporation of a CIDR insert to the Ovsynch protocol fornon-presynchronized cows when compared with those subjected to a Presynch program was also reported by El-Zarkouny *et al.* (2004).

Conclusions and future research

Significant developments have taken place in the past decade leading to a better understanding of ovarian physiology in cattle. The use of ultrasound technology has contributed immensely to bridge major knowledge-gaps in ovarian follicle dynamics. This new knowledge has led to the development of improved protocols for manipulation and control of the bovine estrous cycle. The use of GnRH to synchronize follicle growth and Ovulation is the most recent of these developments. Though $PGF_2\alpha$ is still the most widely used hormone for induction and synchronization of estrus, the Ovsynch/TAI protocol, which strategically uses GnRH and $PGF_2\alpha$to synchronize Ovulation offers potential "freedom" to dairy farmers from their daily time-consumingchore of estrus detection. Pregnancy outcome with Ovsynch/TAI is poor in dairy heifers and cattle. However, the use of CIDR-B device in combination with the

Ovsynch/TAI protocol has the potential to improve pregnancy rates (Ravikumar, 2014). Cows with high concentration of progesterone at the initiation of the Ovsynch protocol have a greater chance for conception (Ambrose *et al.,* 2000; Moreira *et al.,* 2000; Stevenson *et al.,* 1997). Therefore, pre-synchronization of cows by giving two injections of $PGF_2\alpha$ 14 d apart, followed by initiation of Ovsynch/Timed AI protocol12 d after the second of the two $PGF_2\alpha$ injections and Pre-Ovsynch program using $PGF_2\alpha$ and GnRH prior to Ovsynch are another ways of improving pregnancy rates in bovines. Further research is needed to determine if this approach would consistently increase pregnancy rates following Ovsynch/TimedAI in dairy heifers and cattle. Alternative products such as hCG, porcine LH or ECP are being evaluated for their potential benefits in new follicular wave emergence, Ovulation synchronization, formation of robust CL, increase P4 concentrations and enhance pregnancy rates.

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