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Synchronization of Ovulation in Bovines - A Review

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ABSTRACT

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. Proper oestrus detection coupled with efficient insemination technique using quality semen in a healthy uterine environment critically resulted in high rate of reproduction in bovines (Yildiz, 2005). However, first service conception rate to Artificial Insemination (AI) in cows had fallen away from approximately 65 to 40 per cent or lower (Weigel, 2006). Although, the cause has not yet been fully described, the fertility of dairy cows is declining worldwide (Thatcher *et al.,* 2006). 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 (Yildiz, 2010).
Keywords: GnRH,PGF2α,TAI, ECP, Ovsynch,Heatsynch, Presynch, Select Synch, Co-Synch, Hybrid Synch, Ovsynch plus progesterone

I. INTRODUCTION

Synchronization of oestrus and ovulation protocols currently recommended for cows involve combination of GnRH and $PGF_2\alpha$ (Pursley *et al.,* 1995). Each GnRH based protocol started with the same basic frame work, which involved an injection of GnRH followed with an injection of $PGF_2\alpha$ six (or) seven days later. The inclusion of GnRH analogues with $PGF_2\alpha$ (7 days prior to $PGF_2\alpha$) in oestrus synchronization programmes not only improved oestrus detection rates and synchrony of oestrus (Wolfenson *et al.,* 1994 and Twagiramungu *et al.,* 1995), but also induced fertile oestrous cycles in both cyclic and anoestrus bovines (Thompson *et al.,* 1999 and Stevenson *et al.,* 2000). Not only the degree of synchrony increased, but also the variability in the time to oestrus in beef (Thatcher *et al.,* 1996) and lactating cows (Pursley *et al.,* 1997b and Wolfenson *et al.,* 1994) was decreased with the interval to oestrus after $PGF_2\alpha$ injection averaging three days.

The use of GnRH seven days prior to synchronization of oestrus with $PGF_2\alpha$ altered follicular development and produced preovulatory follicles which were more

II. METHODS AND MATERIAL

GnRH-$PGF_2\alpha$ Based Synchronization Protocols

Numerous new synchronization protocols currently recommended for cows use gonadotropin releasing hormone (GnRH) in conjunction with $PGF_2\alpha$. Each GnRH based protocol uses the same basic framework, which involves an injection of GnRH followed 7 days later with an injection of $PGF_2\alpha$. The way animals are subsequently handled for heat detection and breeding is where the protocols begin to vary. To understand the benefits of GnRH based synchronization protocols and how they work, you must first understand the concept of follicular waves in cattle.

Follicular Waves

Follicles are blister-like structures that grow on the ovaries. Each follicle contains an unfertilized egg that will be released to the oviduct if the follicle ovulates. Research using ultrasound technology has revealed that follicular growth occurs in waves throughout the estrous cycle. Each wave is characterized by rapid growth of numerous small follicles. From this wave of follicles, one follicle is allowed to grow to a much larger size than the others (12 to 15 mm). This large follicle is called the dominant follicle because it has the ability to regulate and restrict the growth of other smaller follicles. A few days after reaching maximum size, the dominant follicle begins to regress. As the dominant follicle regresses, it looses the ability to restrict the growth of other follicles. Thus, a new follicular wave is initiated coinciding with the regression of the previous dominant follicle. From the new follicular wave, another dominant follicle will be selected. Most cows will have two or three follicular waves during an 18 to 24 day cycle.

Follicular Waves and GnRH

An injection of GnRH causes a release of Luteinizing Hormone (LH) from the pituitary gland in the brain. This LH "surge" results in ovulation or luteinization of most large dominant follicles. A new "synchronized" follicular wave is initiated in these animals 2 to 3 days later. Because GnRH stimulates development of luteal tissue in place of the dominant follicle, a higher percentage of cows will possess sufficient luteal tissue to respond to $PGF_2\alpha$ 7 days later. Injecting cows with $PGF_2\alpha$ 7 days after a GnRH injection synchronizes luteal regression in animals with previously synchronized follicular development. The result is a higher estrus response rate and much tighter synchrony of estrus when compared to $PGF_2\alpha$ alone. Although GnRH synchronizes follicular development in most cows, some cows do not respond to the first GnRH injection. If the GnRH injection fails to luteinize a follicle in animals that were due to show heat naturally around the time of the $PGF_2\alpha$ injection.

Follicular Waves and $PGF_2\alpha$

Any dominant follicle has the capacity to ovulate provided the inhibitory effects of progesterone can be removed at an opportune time. Prostaglandins serve this function by destroying the CL, however, $PGF_2\alpha$ has no direct effect on the normal pattern of follicular waves. Thus, the stage of follicular development at the time of $PGF_2\alpha$ injection will affect the interval from injection to standing estrus. Animals injected when the dominant follicle is in the growing phase will display estrus within 2 to 3 days, whereas animals with aged or regressing dominant follicles may require 4 to 6 days before a new follicle can be recruited for ovulation. Thus, the interval from $PGF_2\alpha$ injection to estrus and ovulation is highly variable due to differences between cows in the stage of follicular development at the time of $PGF_2\alpha$injection.

GnRH-$PGF_2\alpha$ Based Synchronization Options

1) Ovsynch

Ovsynch was introduced by Pursley *et al*. (1995). It is a fixed time AI synchronization protocol that has been developed, tested and used extensively in dairy cattle. It has also proven to be a reliable timed Al (TAI) program for beef cows. The protocol builds on the basic GnRH-$PGF_2\alpha$ format by adding a second GnRH injection 48 hours after the $PGF_2\alpha$ injection (Drost and Thatcher, 1992) has been shown to synchronize oestrus and ovulation effectively. This second GnRH injection induces ovulation of the dominant follicle recruited after the first GnRH injection. All cows are mass inseminated without estrous detection at 8 to 18 hours after the second GnRH injection. Across large numbers of dairy cattle, pregnancy rates to Ovsynch generally average in the 30 to 40% range to a single fixed time Al without heat detection.

In ovsynch technique, a second GnRH injection was administered at 24 hours (Thatcher *et al.*, 1996); 48 hours (Wolfenson *et al.*, 1994 and Pursley *et al.*, 1995) or 54 hours (Twagiramugu *et al.*, 1995) after the $PGF_2\alpha$ injection. This second GnRH injection induced ovulation of the dominant follicle recruited after the first GnRH injection (Pacala *et al.*, 2010).

Timed artificial insemination (TAI) was done without the need for detection of oestrus in dairy cows (Pursley *et al.*, 1997a) at 8-18 hours (Pursley *et al.*, 1995); 10-20 hours (Mee *et al.*, 1990) or 16-24 hours (Geary *et al.*, 1998 and Steveson *et al.*, 1999) after the second GnRH injection in ovsynch programme.

Ovsynch also had the ability to induce fertile ovulation in anoestrus cows due to the GnRH injection (Geary and Whitter, 1998). Large follicles that responded to the GnRH injection ovulated and formed a functional CL (Twagiramungu *et al.*, 1995).

The success of the ovsynch protocol was influenced by the number of follicular waves or length of the follicular waves (Pursley *et al.*, 1997b) as well as the stage of oestrous cycle when the first GnRH dose was administered (Vasconcelos *et al.*, 1997 and Vasconcelos *et al.*, 1999). The early luteal stage of the oestrous cycle (day 5 to 9) was the optimal period for initiating the ovsynch protocol (Vasconcelos *et al.* 1997; Vasconcelos *et al.*, 1999 and Moreira *et al.*, 2000a). A higher pregnancy rate was reported when cows were started on the ovsynch protocol in the early luteal phase compared with the first 3 to 4 days (Vasconcelos *et al.*, 1999) or after day 10 of the oestrous cycle (Moreira *et al.*, 2000).

Pursley *et al.* (1995), Dagli *et al.* (2008) and Vijayarajan *et al.* (2009) reported 100 per cent ovulatory response between 24 and 32 hours after the second injection of GnRH in ovsynch treated lactating dairy cows. Vasconcelos *et al.* (1997) demonstrated that 87 to 100 per cent; Keskin *et al.* (2011) recorded 88.4 per cent in Holstein Friesian cows and 88.5 per cent in Swedish Red cattle. Vasconcelos *et al.* (1999) observed 87 per cent in lactating dairy cows. But, Steckler *et al.* (2002) found 88 per cent and Fricke *et al.* (1998) found only 84.90 per cent ovulatory response in ovsynch treated post-partum Holstein dairy cows. Velladurai *et al.* (2015) recorded the overall ovulatory response in retained fetal membrane (RFM) affected and normally calved cows were 75.00 and 81.25 per cent, respectively following Ovsynch treatment.

Pursley *et al.* (1997b) reported similar pregnancy rates for first (37 vs 39 per cent), second (42 vs 45 per cent) and third AI (48 vs 61 per cent) using the ovsynch protocol compared with breeding at a detected oestrus in lactating dairy cows. Stevenson *et al*. (1999) reported 35.60 per cent; Moreira *et al.* (2000) reported 37.5 per cent; 47 to 49 (Fricke and Wiltbank, 1999); 40 to 55 (Pursley *et al.*, 1995; Geary *et al.*, 1998; Sathiyamoorthy and Kathirchelvan, 2010 and Ramakrishnan *et al.* 2012); 59 per cent (Geary *et al*. 1998); 60 to 62 per cent (Cecyre *et al.*, 2002) and 61.00 to 90.00 per cent (Muneer *et al.* 2009) conception rates have been reported following induction of ovulation using ovsynch protocol in lactating dairy cows.

In post-partum lactating cattle varying conception rates of 20 to 27 (Dejarnette *et al.*, 2001a); 30 to 38 (Fricke *et al.*, 1998; Stevenson *et al.*, 1999; Jemmeson 2000; Klindworth *et al.*, 2001 and Punyapornwithaya *et al.*, 2002); 41 (Fricke *et al.*, 1998) and 43.50 (Tenhagen *et al.*, 2001) have been reported following ovsynch treatment. Velladurai *et al.* (2015) recorded the overall conception rate in retained fetal membrane (RFM) affected and normally calved cows were 62.50 and 75.00 per cent, respectively following Ovsynch treatment.

2) Heatsynch

Heatsynch is a newly developed synchronization protocol that used the less-expensive hormone oestradiol cypionate (ECP) in place of the second GnRH injection of the ovsynch protocol (Dejarnette *et al.*, 2001b). ECP is a commercially available form of the natural hormone, estrogen. Estrogen is the hormone that causes cows to show the many signs of heat when they come into estrus, and it creates a surge-type release of gonadotropin-releasing hormone (GnRH) from the brain. GnRH, causes the release of luteinizing hormone (LH), which results in ovulation of the mature follicle. GnRH has a direct and almost immediate effect on the release of LH, while ECP has a delayed effect. A recent study found that cows injected with GnRH have a LH surge within about an hour, while the LH surge of ECP treated cows was not detected for about 41 hours. This difference in time to LH surge means the hormone injection intervals must also be altered when substituting ECP for GnRH (Dejarnette *et al.*, 2004). One milligram of ECP was administered at 24 hours after the $PGF_2\alpha$ injection, while Ovsynch treated cows receive GnRH 48 hours later. Because of the delayed interval to the LH surge, the interval to fixed-time AI is 72 hours after $PGF_2\alpha$ (48 hours after ECP) for Heatsynch, compared with 56-64 hours after $PGF_2\alpha$ (8-16 hours after GnRH) for Ovsynch. Pregnancy rate observed in heatsynch was 30 to 40 per cent (Dejarnette *et al.*, 2001b).

3) Presynch

Thatcher reported the Presynch system in 1998. The Presynch uses 2 injections of $PGF_2\alpha$ at 14 days intervals to pre-synchronize most of the cycling animals. 14 days after the 2nd $PGF_2\alpha$ injection, these cows will be in the proper stage of the estrous cycle to respond to the first GnRH injection in any of the GnRH-$PGF_2\alpha$ (Ovsynch, Heatsynch or Select Synch) based breeding protocol (Thatcher, 1998). Preliminary results using Presynch in front of Ovsynch suggests pregnancy rates were improved by 10-20 percent (Moreria *et al.*, 2000 and EI-Zarkouny *et al.*, 2004). In presynch protocol, the pregnancy rate was 40 to 50 per cent (Thatcher *et al.*, 1998).

4) Select Synch

In select synch system, the cows were injected with GnRH and $PGF_2\alpha$ 7 days apart. Detection of oestrus began 24-48 hours before the $PGF_2\alpha$ injection and continued for the next 5-7 days (Dejarnette *et al.*, 2004).

The majority of cows exhibited oestrus 36 to 72 hours after $PGF_2\alpha$ injection (Stevenson *et al.,* 2000). Animals were inseminated 8 to 12 hours after being observed in standing oestrus (Geary *et al.,* 2001; Dejarnette *et al.,* 2001a and Dejarnette *et al.,* 2004). Overall, estrus response rates in well-managed herds average approximately 70 to 75% with no adverse effect on conception rates (60 to 70%), resulting in synchronized pregnancy rates that average between 45 and 50%. Pregnancy rate was 41 (Stevenson *et al.,* 2000); 45 (Patterson *et al.,* 2001); 47 (Kojima *et al.,* 2000); 61 (Dejarnette *et al.,* 2001a) and 65 (Constantaras and Kesler, 2004) per cent following select synch system.

5) Co-Synch

Co-Synch was an alternative to ovsynch in which, second GnRH injection was given at the time of AI (Geary et al., 2001). Larson et al. (2004) reported that the cows were bred at 54 hours after the injection of PGF2α following co-synch protocol. However, small reduction in conception rate was observed in cows following co-synch when compared to ovsynch (Pursley et al., 1998; Geary et al., 2001 and Dejarnette and Marshall, 2003). Overall, pregnancy rates have averaged 48%. The protocol is quite simple to employ as all injections and timed AI can be done at the same time of the day. In co-synch, the pregnancy rates were between 40 and 50 per cent (Dejarnette et al., 2004).

6) Hybrid Synch

Hybrid synch was a combination of select synch and co-synch systems (Stevenson *et al.,* 2000). Oestrus 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 exhibited oestrus until 72 hours (Larson *et al.,* 2004; Dejarnette *et al.,* 2001b and Dejarnette *et al.,* 2004). Pregnancy rates in cows administered the hybrid synch protocol was 34 (Stevenson *et al.,* 2000); 46 (Dejarnette *et al.,* 2001b); 53 (Larson *et al.*, 2004) and 52 per cent (Dejarnette *et al.,* 2004).

6) Ovsynch plus progesterone

An alternative to improve synchronization and conception without lengthening timed AI programs is progesterone (P_4) supplementation during the protocol. The use of intra vaginal devices (CIDR) for controlled release of progesterone from the GnRH to the $PGF_2\alpha$ injections maintains blood progesterone concentrations that prevent premature estrous behavior, LH surge and ovulation. These devices have been used during timed AI protocols to improve fertility of dairy cows (EI-Zarkouny *et al.,* 2004 and McDoughall, 2010). Steckler *et al.* (2002) reported the conception rates of 49 and 72 per cent respectively at 28 days post timed artificial insemination in ovsynch plus CIDR treated post-partum dairy cows. He also found 55.20 per cent conception rate at 28 days post timed artifical insemination in ovsynch plus CIDR treated anovular lactating dairy cows.

Among those GnRH-$PGF_2\alpha$ based synchronization protocols, the Ovsynch protocol is the best method for Synchronization of ovulation but some limitation is present in this method.

III. RESULT AND DISCUSSION

Ovsynch: Limitations

Cows treated with Ovsynch yield overall conception rates similar to those obtained after breeding to detected estrus (37 versus 39 %, respectively; $P > 0.05$) (Pursley et al., 1997a). However, a major limitation to Ovsynch is the wide variability of synchronization rates. Up to 30% of cows may not synchronize (Vasconcelos *et al.,* 1999; DeJarnette *et al.,* 2001a and Peters and Pursley, 2002). Variation among dairy cows in their synchronization rate to Ovsynch was attributed primarily to the stage of the estrous cycle in which Ovsynch is initiated (Vasconcelos *et al.*, 1999).

Cows started on Ovsynch at mid-cycle (d 5-9 of cycle) have a greater probability of synchronizing to Ovsynch, and have a greater chance of conception (Vasconcelos *et al.,* 1999; Moreira *et al.,* 2000). The key physiological reasons for increased synchronization rate in mid-cycle are: (1) presence of a functional Dominant follicle (DF) capable of ovulating to first GnRH of Ovsynch (2) presence of a CL that remained functional during the 7-d period between first GnRH and $PGF_2\alpha$ of Ovsynch. Ovulation to first GnRH of Ovsynch is followed by emergence of a new follicular wave. The dominant follicle from this new wave generally develops to become the ovulatory follicle of Ovsynch. Presence of a functional CL at PGF2α of Ovsynch reduces the occurrence of spontaneous ovulation prior to final GnRH of Ovsynch.

In contrast, if cows are started on Ovsynch early in the estrous cycle (d 1 to 4), ovulation to first GnRH of Ovsynch is impaired by the presence of an emerging follicular wave, (Vasconcelos *et al.,* 1999; Moreira *et al.,* 2000). It is unlikely for a follicle 3 d post-emergence to have LH receptors and respond to first GnRH of Ovsynch with ovulation (Sartori *et al.,* 2001). Thus, at the time of $PGF_2\alpha$ of Ovsynch, this follicle would be 10-d old and likely already undergoing atresia. By the time of final GnRH of Ovsynch, another follicular wave would be emerging and the follicle destined to be dominant would be too young to ovulate in response to final GnRH of Ovsynch. In these cases, ovulation is likely to occur 3 to 5 d after finalization of Ovsynch.

If cows are started on Ovsynch in late estrous cycle, spontaneous luteolysis is likely to occur before $PGF_2\alpha$ of Ovsynch (Vasconcelos *et al.,* 1999; Moreira *et al.,* 2000). In cows with spontaneous luteolysis prior to $PGF_2\alpha$, the dominant follicle is likely to trigger spontaneous ovulation prior to final GnRH of Ovsynch.

IV. CONCLUSION

In conclusion, successful synchronization of ovulation is less likely if cows are started on Ovsynch during early or late estrous cycle compared to mid-cycle. Since insemination occurs at a fixed time after final GnRH of Ovsynch, chances of conception to timed AI are lower in non-synchronized cows. Cows started on Ovsynch at mid-cycle (d 5-9 of cycle) have a greater probability of synchronizing to Ovsynch, and have a greater chance of conception (Vasconcelos *et al.,* 1999; Moreira *et al.,* 2000). The use of GnRH to synchronize follicle growth and Ovulation is the most recent of these developments. $PGF_2\alpha$ 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-consuming 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 Ovsyncy/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 (Stevenson *et al.,* 1999; Ambrose *et al.,* 2000; Moreira *et al.,* 2000). Pre-Ovsynch program using $PGF_2\alpha$ 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/Timed AI in dairy heifers and cattle.

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