New Ovsynch Strategies to Manage Reproduction of Dairy Cows

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1. Introduction

Efficient reproduction is a cornerstone to profitable dairy production. Long-term sustainability of dairy farms depends largely on the timeliness of parturitions and the benefit from the physiological consequences that follow. The objective of this presentation is to examine new findings in ovarian physiology that relate to reproductive performance of dairy cows and apply them to the development of new methods to control follicular and luteal function that may optimizing reproductive efficiency of lactating dairy cows.

During the past 50 years, reproductive efficiency of lactating dairy cows progressively decreased due primarily to low and steadily decreasing conception rate and estrus detection rates (Heersche and Nebel, 1994; Washburn et al., 2002). Conception rate in cows decreased from approximately 70% (Foote, 1952; Herman, 1956) to 35% (Lopez-Gatius, 2003; Macfarlane and Pursley, 2003) during this period. Reproductive efficiency in lactating cows is further compromised due to poor estrus detection rates. Current reports indicate that estrus detection rate is approximately 32% in lactating cows (MI DHIA December 2005, monthly summary report).

The nature of poor reproductive performance of dairy cows appears to be multifactorial. Potential contributing factors include: genetics and breed (Fonseca et al., 1983), age and parity (Pursley et al., 1997), resumption of ovarian cyclicity after parturition (Thatcher and Wilcox, 1973), nutrition (Butler, 2000), level of milk production (Butler and Smith, 1989; Peters and Pursley, 2002), use of bovine somatotropin (Moreira et al., 2001; Santos et al., 2004), uterine health (LeBlanc et al., 2002; Gilbert et al., 2005), environment conditions (Rensis and Scaramuzzi, 2003) and management practices (Jordan and Fourdraine, 1993; Nebel et al., 1994), among others. These factors either have a direct effect on, or are indirectly associated with, ovarian physiology at the level of follicular and luteal development and function (Burke et al., 1995; Butler, 2000; Lucy, 2000; Sartori et al., 2004; Sheldon and Dobson, 2004). Altered ovarian physiology may be a common pathway for disrupted reproductive performance in lactating dairy cows.

2. Estrus detection rate and synchronization of ovulation

Approximately 2 out of every 3 dairy cows in estrus go unidentified (MI DHIA December 2005, monthly summary report). Until a decade ago, observation of standing estrus was the only appropriate indicator for breeding. Thus, low service rate was the first limitation to reproductive efficiency in dairy cows.

2.1. Control of service rate with Ovsynch

In 1995, Pursley et al. developed the hormonal program Ovsynch to synchronize ovulation in lactating dairy cows (Ovsynch; Pursley 1995). Ovsynch allowed the insemination of cows by appointment independently of detected estrus. As a consequence, it became possible to control time to first and subsequent AI, thus maximizing service rate and improving overall estrus detection rate. Since 1995, Ovsynch has been widely accepted as a reproductive management tool in dairy farms.

Ovsynch is based on three scheduled hormonal injections. A first injection of gonadotropin-releasing hormone (GnRH) is intended to cause ovulation/luteinization of any functional dominant follicle present in the ovary and induce the subsequent emergence of a new follicular wave approximately 1.5 to 2 d later (Pursley et al., 1995). If no dominant follicle is present at the time of first GnRH, spontaneous emergence of a new follicular wave is assumed to be synchronous within the previous 3 d. The newly emerged follicular wave is allowed to develop and undergo selection and dominance during the following 7 d. On d 7, prostaglandin $F_{2?}$ ($PGF_{2?}$) is administered to induce luteolysis, thus allowing for further growth and maturation of the dominant follicle. Finally, a 2nd GnRH injection is administered 48 hours after $PGF_{2?}$ to induce a pre-ovulatory LH surge that triggers ovulation within an 8 h period, beginning approximately 24 h after the injection (Pursley et al., 1995).

2.2. 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., 1997). However, a major limitation to Ovsynch is the wide variability of synchronization rates (defined as having a regressed CL and ovulation following the final GnRH) in Ovsynch-treated cows. Up to 30% of cows may not synchronize (Vasconcelos et al., 1999; DeJarnette et al., 2001; Macfarlane and Pursley, 2003; Peters and Pursley, 2003). It is unlikely for non-synchronized cows to be inseminated at an appropriate time relative to ovulation, thereby decreasing their chances of becoming pregnant.

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 therefore have a greater chance of conception, than cows at any other stage of the estrous cycle (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 DF capable of ovulating to first GnRH of Ovsynch, and 2) presence of a CL that remained functional during the 7-d period between first GnRH and $PGF_{2??}$ 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 $PGF_{2??}$ of Ovsynch reduces the occurrence of spontaneous ovulation prior to final GnRH of Ovsynch.

In contrast, during early (d 1 to 4) or late (d >10) estrous cycle, the physiological follicular and luteal scenarios are not conducive to synchronization to Ovsynch. 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 $PGF_{2?}$ 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. Similarly, when cows are started on Ovsynch later in the estrous cycle (? d 10), the presence of a functional DF at the time of first GnRH varies depending on follicular wave pattern of the cow and time of follicular wave emergence (Vasconcelos et al., 1999). Moreover, if cows are started on Ovsynch in late estrous cycle, spontaneous luteolysis is likely to occur before $PGF_{2??}$ of Ovsynch (Vasconcelos et al., 1999; Moreira et al., 2000). In cows with spontaneous luteolysis prior to PGF, the dominant follicle is likely to trigger spontaneous ovulation prior to final GnRH of Ovsynch. In summary, 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.

Ovulatory response to first GnRH of Ovsynch and prevention of premature luteolysis are critical for successful synchronization of ovulation in lactating dairy cows. Control over follicular development prior to initiation of Ovsynch can set up favorable conditions for ovulatory response to first GnRH of Ovsynch. In addition, control over luteal function can help prevent the occurrence of premature luteolysis during Ovsynch. As a result, greater synchronization rates to Ovsynch could be expected.

2.3. Previous approaches to improve outcome to Ovsynch: Limitations

Several pre-synchronization strategies were developed based on the idea that controlling ovarian function prior to initiation of Ovsynch could improve synchronization rates to Ovsynch (Peters and Pursley, 2002; DeJarnette and Marshall, 2003; LeBlanc and Leslie, 2003; El-Zarkouny et al., 2004; Navanukraw et al., 2004). The most popular pre-synchronization strategies were based on the use of $PGF_{2?}$ at specific times prior to initiation of Ovsynch. Conclusions on the effectiveness of $PGF_{2?}$-based pre-synchronization strategies to improve Ovsynch were based only on pregnancy outcomes and were not consistent across studies. Unfortunately, most of these studies did not provide data on the effect of $PGF_{2?}$-based pre-synchronization strategies on ovulatory response to first GnRH of Ovsynch or on overall synchronization rate to Ovsynch. Since the effect of $PGF_{2?}$ is limited to controlling luteal lifespan, we can speculate that pre-synchronization with $PGF_{2?}$ did not consistently synchronize follicular development at first GnRH of Ovsynch. Further research is needed to develop and validate strategies to optimize synchronization rate to Ovsynch by increasing ovulatory response to first GnRH and by preventing occurrence of spontaneous luteolysis prior to the PGF.

It should be noted that the overall positive impact of Ovsynch on reproductive performance of dairy herds is primarily mediated through maximization of service rate. Ovsynch's current hormonal injection scheme does not increase conception rate compared to insemination following detected estrus (Pursley et al., 1997).

3. Factors that impact conception rates in cattle.

Regardless of the insemination trigger (estrus or timed AI to synchronized ovulation), approximately 2 out of every 3 dairy cows fail to conceive and establish a pregnancy after insemination. Given the decreasing trend of conception rates during the past 50 years, it can be anticipated that fertility of lactating dairy cows will continue to be a paramount challenge to profitable dairy herd management. The exact physiological reasons for poor fertility of lactating dairy cows are unknown. As previously mentioned in this review, the problem appears to be multifactorial. Evidence from several studies support an association between functional and morphological features of the ovulatory follicle and fertility.

3.1. Evidence for an effect of lifespan and duration of dominance of the ovulatory follicle on fertility.

Several lines of evidence indicate that an extended follicular lifespan and duration of dominance is detrimental to fertility. The persistent follicle model provides direct evidence for this. A persistent follicle can be created by maintaining sub-luteal levels of progesterone (1-2 ng/ml; P_4) for an extended period (Sirois and Fortune, 1990; Adams et al., 1992; Savio et al., 1993; Mihm et al., 1994; Bridges and Fortune, 2003). Under these conditions, the dominant follicle is induced to grow persistently and remain dominant for longer than normal physiological intervals by temporarily being prevented from ovulating (Sirois and Fortune, 1990; Adams et al., 1992; Savio et al., 1993; Mihm et al., 1994; Bridges and Fortune, 2003). When persistent follicles are finally allowed to ovulate, fertility is decreased when compared with younger ovulatory follicles (Savio et al., 1993; Stock and Fortune, 1993; Mihm et al., 1994; 1996; Revah and Butler, 1996). It was proposed that the negative effect of prolonged growth and dominance of the ovulatory follicle on fertility is associated with the hormonal environment in which the follicle is induced to persist. Sub-luteal levels of P_4 during a prolonged period cause increased frequency of LH pulsatility (Roberson et al., 1989; Kojima et al., 1992) and prevents a preovulatory LH surge from occurring (Rahe et al., 1980; Cupp et al., 1992; Savio et al., 1993; Stock and Fortune, 1993). As a result, the oocyte resumes meiosis while still contained in the follicle and starts undergoing premature nuclear maturation dissociated from follicular maturation and ovulation. Histological characteristics of a persistent follicle indicate that the oocyte undergoes early germinal vesicle breakdown and continues to progress through the cell cycle towards metaphase I or II (Revah and Butler, 1996; Mihm et al., 1999). By the time ovulation is permitted, the oocyte contained in the persistent follicle has already matured and aged, thus yielding lower fertility (Fugo and Butcher, 1966; Butcher and Fugo, 1967). Potential reasons to explain low fertility of persistent follicles include low fertilization rates (Fugo and Butcher, 1966; Butcher and Fugo, 1967), high early embryonic mortality (Ahmad et al., 1995), or both. Also, persistent follicles are known to maintain a high and sustained production of estradiol, which may alter intrafollicular, oviductal and/or uterine environments. This may compromise sperm and/or oocyte transport and embryonic development (Savio et al., 1993; Stock and Fortune, 1993; Mihm et al., 1994; Revah and Butler, 1996; Mihm et al., 1999; Bridges and Fortune, 2003).

Indirect evidence further supports an association between fertility and length of follicular lifespan and/or duration of dominance. A recent study indicated that the interval from emergence of the ovulatory follicle to estrus was shorter in cows subsequently diagnosed pregnant compared to cows diagnosed not pregnant (7.8 ? 0.2 d versus 8.6 ? 0.2 d, respectively; $P < 0.01$) (Bleach et al., 2004). A key result from the same study was a significant inverse relationship between duration of follicular dominance and conception rates. The longer the ovulatory follicle remained in the ovary waiting for the ovulation trigger, the lower the probability of establishing a pregnancy.

Additional indirect evidence for an effect of follicular lifespan on fertility is based on comparing follicular development between lactating cows and nulliparous heifers. The ovulatory follicle in heifers undergoes a shorter period of dominance compared to that of cows (Sartori et al., 2004; Wolfenson et al., 2004; Burns et al., 2005). In parallel, it is well established that heifers are consistently more fertile than dairy cows (Pursley et al., 1997).

*3.2. **Evidence for an association between morphology of the ovulatory follicle and fertility**.*

Several studies have repeatedly shown the relationship between size of the ovulatory follicle and fertility. Ovulation of small follicles yields lower conception rates (Lane et al., 2001; Vasconcelos et al., 2001; Macfarlane and Pursley, 2003; Mussard et al., 2003). In a recent study from our laboratory, Ovsynch-treated cows that ovulated follicles < 12 mm in diameter had significant lower conception rates compared to ovulatory follicles > 12 mm in diameter (Macfarlane and Pursley, 2003). In this study, cows ovulating a <12-mm follicle comprised 34% of the study. In contrast, ovulatory follicles >18 mm in diameter were also associated with lower fertility (Vasconcelos et al., 1999). More recently, a quadratic relationship was identified between follicular size and fertility: conception rates were impaired after ovulation of follicles below or above a threshold size (Perry et al., 2005). Taken together, these studies indicate that fertility is compromised if a preovulatory follicle is larger or smaller than a corresponding minimal or maximal size threshold. Thus, there appears to be a defined window of ovulatory follicle size in which fertility could be optimized.

Follicular size is related to lifespan of a follicle (Ginther et al., 1989): The longer a dominant follicle grows, the larger it becomes. Thus, dominant follicles larger than a certain threshold are likely follicles with extended lifespan and duration of dominance. Thus, the association between follicular size and fertility indirectly supports an effect of follicular lifespan and duration of dominance and fertility.

3.3. Evidence for an association between ovarian function and fertility

Estradiol produced by the dominant follicle is key to the normal functioning of the reproductive axis. Estradiol is required for follicular growth and development, manifestation of estrus behavior, triggering of ovulation and final maturation of the oocyte (Greenwald and Roy, 1994). Decreased circulating concentrations of estradiol at induction of ovulation were associated with impaired fertility (Perry et al., 2005). Reduced estradiol concentrations due to a younger and smaller ovulatory follicle can also alter uterine and oviductal environment, affecting either gamete or embryo survivability (King et al., 1994; DeSouza and Murray, 1995; Murdoch and Van Kirk, 1998).

Finally, turning once again to the cow-heifer model for differential fertility, greater circulating concentrations of estradiol were observed in heifers compared to cows (Sartori et al., 2004; Wolfenson et al., 2004). It should be acknowledged, however, that physiological differences between cows and heifers expand beyond follicular function and to include age, parity, milk production and uterine health, among others.

4. Summary

Poor reproductive performance of lactating dairy cows is a function of low estrous detection rate/service rate and low conception rate. Service rate can be maximized by synchronizing ovulation with Ovsynch and inseminating cows by appointment. The efficiency of Ovsynch for effectively synchronizing ovulation in dairy cows can still be improved. A greater proportion of cows with a synchronized ovulation will probably result in a greater proportion of cows pregnant to timed AI. On the other hand, functional and morphological features of the ovulatory follicle are associated with fertility. Control of follicular function and morphology could help improve conception rate of dairy cows.

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