Profitable Reproductive Management of High-Producing Dairy Herds

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▪ Take Home Message

- Infertility remains the leading reason for poor reproductive performance in lactating dairy cows
- Timed AI after the Ovsynch protocol can potentially double the 21-day pregnancy rates from about 12% to 23%, as it increases the insemination rate
- However, the current hormonal injection scheme of Ovsynch does not improve conception rates of lactating dairy cows as it does not effectively control the age and size of the ovulatory follicle
- New data from our laboratory indicates that conception rates could be increased considerably by adopting a pre-ovsynch program of $PGF_{2\alpha}$ and GnRH given 2 days apart, and starting the Ovsynch treatment 6 days later, as the pre-ovsynch program optimises the age, size and function of the ovulatory follicle
- The potential increase in conception rate from this improvement in the function of the ovulatory follicle could improve the profitability on an 800-cow dairy by $120,000/year

▪ Introduction:

Profitable reproduction can likely be defined in a number of different ways. Yet the bottom line for profitable reproduction in most high producing dairy herds is "simply" to achieve a specific number of new lactations each month without purchasing new animals. This is critical from the standpoint of maintaining herd level milk production, but 1) many herds are not achieving their calving goal and are forced to buy replacements, and 2) there are a significant number of efficiencies that can be strengthened, even in the herds that the calving goal is realized. Our work focuses on controlling reproductive processes with Ovsynch in order to control time to 1st and subsequent artificial inseminations, an efficiency tool that controls length of time from calving to conception. This presentation will focus on the development of a new way of thinking related to Ovsynch that can maximize not only numbers of new lactations per month, but also extra numbers of replacement heifers and cows available for sale for dairy purposes.

Infertility remains the leading reason for poor reproductive performance in lactating dairy cows despite efforts to improve accuracy of estrus detection, therapeutic intervention and advancements in synchronization strategies (Lucy, 2001; Washburn et al., 2002). Recent data indicate that conception rates (percent pregnant to a single AI) of cows artificially inseminated **(AI)** in response to a detected estrus are approximately ½ that of virgin heifers (35 vs. 70%) with the gap continuing to widen (Foote, 1952; Herman, 1956; Pursley et al., 1997a; Lopez-Gatius, 2003; Macfarlane, 2003). To improve reproductive performance of dairy cows, many dairy veterinarians prescribe Ovsynch (Figure 1) to their clients. Ovsynch effectively controls time to 1st and subsequent AI in lactating dairy cows (Pursley et al., 1995), thereby, nearly doubling the % of cows that become pregnant in each 21 d period from 12% to 23% (Pursley et al., 1997a; Pursley et al., 1997b). However, the current hormonal injection scheme of Ovsynch does not improve conception rates of lactating dairy cows (Pursley et al., 1997a). Ovsynch does not effectively control age and size of the ovulatory follicle. This leads to asynchrony of ovulation in 10 to 30% of cows treated with Ovsynch (Macfarlane, 2003; Vasconcelos et al., 1999; Peters and Pursley, 2003; DeJarnette et al., 2001; Navanukraw et al., 2004). New data from our laboratory indicates that conception rates of dairy cows treated with Ovsynch can be substantially improved when age, size and function of the ovulatory follicle were optimized (Bello et al., 2006) at the time of the final gonadotrophin releasing hormone **(GnRH)** induced LH surge. These data indicate that circulating concentrations of estradiol **(E_2)** at the final injection of GnRH of Ovsynch, and optimal age and size of the ovulatory follicle were positively correlated with the probability of a pregnancy. From these newly published data, we are confident that hormonal control of follicle and corpora lutea **(CL)** development using GnRH and prostaglandin $F_{2\alpha}$ **($PGF_{2\alpha}$)** prior to Ovsynch will consistently synchronize ovulatory follicles into a prime stage of development at the time of the final GnRH of Ovsynch. The potential increase in conception rate from this improvement in function of the ovulatory follicle could improve farm profit on an 800-cow dairy $120,000/year (Wolf, 1999; Wolf, 2003) and could revolutionize reproductive management of dairy cattle.

▪ The Problem:

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 (Washburn et al., 2002; Heersche and Nebel, 1994). Conception rate in cows decreased from approximately 70% (Foote, 1952; Herman, 1956) to 35% (Lopez-Gatius, 2003; Macfarlane, 2003) while conception rate in heifers remained steady at approximately 70% (Herman, 1956; Pursley et al., 1997a). Reproductive efficiency in lactating cows is further compromised due to poor estrus detection rates. Current reports indicate that estrus detection rate is approximately 34% in lactating cows (MI DHIA 2005, yearly summary) and 70% in heifers (Pursley et al., 1997a). The dramatic change in estrus detection rate from the transition of heifer to cow may be attributed to changes in hormonal concentrations (Sartori et al., 2004; Wolfenson et al., 2004) as well as differences in environment, with cows spending more time on concrete (Lucey et al., 1986; Britt, 1985) and being more susceptible to heat stress (Sartori et al., 2004).

The ovulatory follicle plays a key role in reproductive success by coordinating hormonal interactions, final maturation of the oocyte and ovulation. An increasing number of studies reinforce a consistent association between physiological characteristics of the ovulatory follicle and fertility of lactating dairy cows, including follicular age, size, and function (E_2 production capacity) (Macfarlane, 2003; Vasoncelos et al., 1999, 2001; Bello et al., 2006; Sartori et al., 2004; Wolfenson et al., 2004; Bleach et al., 2004; Mihm et al., 1994). Although Ovsynch was developed to synchronize the time of ovulation to allow for timed-AI, few studies have focused on the impact of age, size and function of the ovulatory follicle on the fertility outcomes of this program (Bello et al., 2006; Vasconcelos et al., 2001).

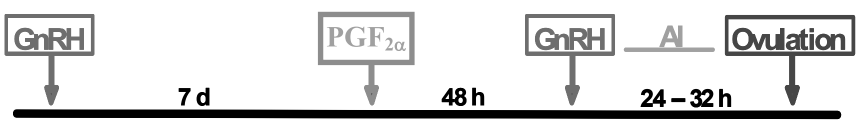

Figure 1: Description of the original Ovsynch program utilizing GnRH and PGF$_{2\alpha}$ to control the time of ovulation in lactating dairy cows.

▪ The Ovsynch Protocol: Strengths and Limitations.

The Ovsynch protocol was developed (Pursley et al., 1995; Pursley et al., 1997b) to control the time to 1st and subsequent AI thus maximizing service rate and improving overall estrus detection rate. Ovsynch is based on three scheduled hormonal injections (Figure 1). The 1st injection of GnRH is intended to cause ovulation of any functional dominant follicle **(DF)** present in the ovary and induce subsequent emergence of a new follicular wave approximately 1.5 d later. Then, a new DF develops and undergoes selection and dominance during the next 7 d. If a follicle does not respond to the 1st GnRH it is likely the cow is in early stages of a follicular wave (1st 3 to 4 d) in which the future DF does not yet have LH receptors. In this case the DF will soon deviate from the subordinate follicle(s) and develop, but this follicle will either be more mature or may reach atresia by the time of the $PGF_{2\alpha}$ injection. On d 7, $PGF_{2\alpha}$ is administered to induce luteolysis, thus allowing for further growth and maturation of the DF, now a pre-ovulatory follicle. Finally, a 2nd GnRH injection is administered 48 hours after $PGF_{2\alpha}$ to induce a pre-ovulatory LH surge that triggers ovulation between 24 and 32 h later (Pursley et al., 1995).

Cows treated with Ovsynch yield overall conception rate similar to those obtained after breeding to detected estrus (37 versus 39%, respectively; P > 0.05; Pursley et al., 1997a). 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 (Vasconelos et al., 1999). It is likely that non-synchronized cows will not be inseminated at an appropriate time relative to ovulation, thereby decreasing their chances of becoming pregnant. Vasconcelos et al. (1999), attributed most of the variability in synchronization rate in cows to the stage of the estrous cycle in which Ovsynch was initiated. Cows started on Ovsynch at mid-cycle (d 5-9 of cycle) had a greater probability of synchronizing and therefore had a greater chance of conception. The key reasons for increased synchronization rate in mid-cycle are the presence of a functional DF from the first follicular wave that is capable of ovulating and a CL that remains functional during the 7 d period between GnRH and $PGF_{2\alpha}$. Early in the estrous cycle (d 1 to 4) synchronization is impaired by the presence of newly emerging follicles incapable of ovulating in response to the 1st GnRH. Later in the estrous cycle (≥ d 10) the presence of a functional DF at the time of 1st GnRH varies depending on follicular wave pattern of the cow and time of follicular emergence. Moreover, it is possible to encounter spontaneous luteolysis triggered by endometrial $PGF_{2\alpha}$ during this period. Characterization of the ovarian events that take place when starting Ovsynch at different stages of the estrous cycle indicate that the main causes of synchronization failure are: 1) lack of ovulatory response to the first GnRH, 2) atresia of the DF prior to $PGF_{2\alpha}$, and 3) spontaneous and premature luteolysis between the 1st GnRH and $PGF_{2\alpha}$ (Vasconelos et al., 1999). Early atresia of the DF prior to $PGF_{2\alpha}$ is primarily a function of lack of ovulatory response to the 1st GnRH. For example, it would be unlikely for a follicle 3 to 4 d post-emergence at the time of 1st GnRH to have LH receptors and respond by ovulating. This follicle would be 10-d old at the time of $PGF_{2\alpha}$ and may already be undergoing atresia. Therefore, by the time of the 2nd GnRH, another follicular wave would be emerging spontaneously and the follicle destined to be dominant would be too young to respond to the final GnRH with ovulation. Cows in the latter situation would likely be detected in estrus 3 to 5 d after the 2nd GnRH. In addition, cows with spontaneous luteolysis during the time from the 1st GnRH to $PGF_{2\alpha}$ would likely be in late stages of the estrous cycle and undergoing natural luteolysis before $PGF_{2\alpha}$. Such cows would likely be in estrus from 1 d prior to 2 d after $PGF_{2\alpha}$. In either case, cows would not have a synchronized ovulation in response to the 2nd GnRH, and would likely not conceive to the timed-AI. Improving synchronization rate of Ovsynch increases the percentage of cows with a chance to conceive.

Since synchronization to Ovsynch primarily depends upon the ovulatory response to the 1st GnRH followed by emergence of a new follicular wave (Vasconelos et al., 1999), several studies have tried to develop pre-synchronization strategies to assure presence of a functional mature DF at the beginning of the Ovsynch program (Navanukraw et al., 2004). The most popular ones were based on the use of $PGF_{2\alpha}$ at specific times prior to starting Ovsynch. The absence of consistent synchronization results is likely due to $PGF_{2\alpha}$ only controlling the lifespan of the CL and not follicles. Thus, stage of follicular development at the beginning of Ovsynch would likely have too much variation if pre-synchronized only with $PGF_{2\alpha}$-based programs. Moreover, none of these studies provided data on follicular events throughout pre-synchronization and Ovsynch to assess if pregnancy outcome was actually related to increased synchronization of follicular development.

To summarize this section, an insufficient percentage of cows have an ovulatory response to the 1st GnRH of Ovsynch, subsequent luteal regression to the $PGF_{2\alpha}$, and ovulation and final GnRH of Ovsynch. Only 70 to 90% of cows treated with Ovsynch have successful luteal regression and ovulation, and only 60% of cows have an ovulatory follicle that both emerges following the 1st GnRH and goes on to ovulate following CL regression. Thus, higher rates of synchronization of the development of ovulatory follicles, and in turn enhanced conception rates following fixed-time AI would be expected, provided the 1st GnRH injection of Ovsynch consistently initiates a new follicular wave and the DF from that wave goes on to ovulate.

▪ Evidence That Age of the DF Compromises Fertility

Several lines of evidence demonstrate an important role for age of the ovulatory follicle.

First

Studies indicate that extending the lifespan of the ovulatory follicle reduces fertility. Treatments based on maintenance of sub-luteal levels of P_4 (1-2 ng/ml; P_4) for an extended period are known to cause prolonged growth and dominance of the DF. This follicle is described as a persistent DF (Mihm et al., 1994). When the persistent DF is allowed to ovulate, fertility is decreased when compared with younger ovulatory follicles. 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 preventing a preovulatory LH surge from occurring. 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 these persistent DF indicate that the oocyte undergoes early germinal vesicle breakdown and continues a progression through the cell cycle towards metaphase I or II. By the time of ovulation of the persistent follicle, the oocyte has already matured and aged resulting in lower fertility, which may be explained either by low fertilization rates, high early embryonic mortality 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, thus compromising sperm and/or oocyte transport and embryonic development.

Second

Follicles that are induced to ovulate before reaching a maturity level that would induce an endogenous LH surge are less fertile. Several studies have shown the relationship between smaller follicular size at the time of induced ovulation with GnRH and lower conception rate. In a recent study from our laboratory, cows treated with Ovsynch that responded to a GnRH-induced LH surge with ovulatory follicles < 12 mm in diameter had lower conception rate compared with ovulatory follicles > 12 mm in diameter (36% for ovulatory follicles > 12 mm vs. 27% for ovulatory follicles < 12 mm; $P<0.01$) (Macfarlane, 2003). They also comprised nearly 34% of the cows in the study (n = 1424). Perry showed a quadratic relationship between follicular size and fertility in beef heifers, with a maximum conception rate of 71% at a follicular size of ~13 mm at the time of the LH surge. Follicles that ovulated at less than 11.5 mm or greater than 16 mm were less likely to support pregnancy than those that ovulated at an optimum size. It is possible that the larger follicles in this study could be persistent follicles. Compromised oocyte quality and subsequent luteal function are reasons proposed why reduced age, size and/or physiological status of follicles reduces conception rate. Lower circulating P_4 concentrations or early induction of luteolysis can impair embryo recognition, development and/or implantation. Another possibility could be related to a non-synchronized, and therefore incorrect, timing of endocrine signals controlling final follicle and oocyte maturation, leading to ovulation of an immature oocyte. Reduced estradiol concentrations due to a younger DF may also alter uterine and oviductal environment, affecting either gamete or embryo survivability.

Third

Cows with three follicular waves per cycle appear to have greater conception rates than cows with two follicular waves. It is widely accepted that ovarian follicles primarily develop in two- or three-wave patterns throughout the estrous cycle of cattle. Lactating dairy cows generally have two waves of follicular growth per cycle. In either wave pattern, the DF of the final wave is the one intended to ovulate. The mean inter-ovulatory interval is shorter for 2-wave vs. 3-wave cows (approximately 20 d vs. 22-23 d, respectively) and the emergence of the ovulatory wave occurs earlier for 2- vs. 3-wave cows (approximately d 10 vs. d 16 of the cycle) leading to different life spans of the ovulatory follicles for each wave pattern. So, at the time of ovulation, a difference in age can be observed between the pre-ovulatory follicle coming from a two-wave (10 to 11 d) vs. a three-wave (6 to 7 d) pattern of follicle development (Bleach et al., 2004). Therefore, the two-wave pattern that predominates in lactating dairy cows is generally characterized by a larger DF reaching ovulation compared with three-wave patterns (Bleach et al., 2004). Moreover, second-wave ovulatory follicles were found to be approximately 3 d older than those of a third wave when the interval from emergence to estrus was measured (Bleach et al., 2004). This difference in lifespan between second and third wave ovulatory follicles was explained by a longer period of follicular dominance rather than by the interval of follicle growth from emergence to dominance, which remained the same in both cases (Bleach et al., 2004). As previously stated, the ovulatory follicle of a two-wave cycle is older than that of a three-wave cycle. By analogy with the prolonged dominance of a persistent follicle, the older age of a second-wave ovulatory follicle could be expected to have a negative impact on fertility. If most dairy cows have two waves, then a high proportion of the population could be expected to be ovulating an older DF, thus providing a possible explanation of the low conception rate problem in lactating dairy cows. Only a few studies compared fertility between three- vs. two-wave cows (Bleach et al., 2004). There were significant trends towards greater conception rate in three- vs. two-wave cows. Unfortunately, sufficient numbers of 3-wave cows are difficult to procure due the fact that most lactating dairy cows have 2 follicular waves during a normal estrous cycle.

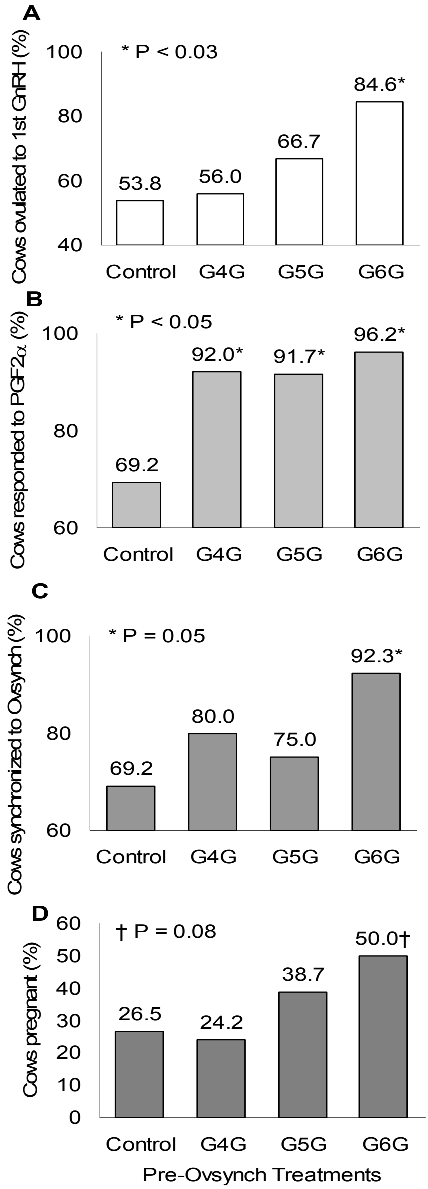

Figure 2. Effect of Pre-Ovsynch treatment prior to Ovsynch on ovulation response to 1st GnRH of Ovsynch, CL regression, synchronization and pregnancy rates in lactating dairy cows (n=130). Cows were given PGF2∀ and GnRH 2 d apart; then Ovsynch treatment began at 4 (G4G), 5 (G5G), or 6 (G6G) days later. Control cows did not receive Pre-Ovsynch treatment.

Fourth

Length of time from emergence and deviation to ovulation of the ovulatory follicle appears to affect fertility. The interval from emergence of the ovulatory follicle to estrus was approximately 1 d shorter in cows diagnosed pregnant compared to cows diagnosed not pregnant (7.8 ± 0.2 d vs. 8.6 ± 0.2 d, respectively; $P < 0.01$) regardless if they had two or three follicular waves (Bleach et al., 2004). A key result from the same study was a significant inverse relationship between follicle lifespan and dominance duration, and CR. The longer the ovulatory follicle remained in the ovary waiting for ovulation, the lower the probability of establishing a pregnancy. Furthermore, a recent study found differences in follicular development in this regard between lactating cows and nulliparous heifers (Sartori et al., 2002). Heifers had a significant trend towards a 1.2 d shorter duration of dominance of the ovulatory follicle when compared to cows. The relevance of this issue becomes clear when considered together with the current conception rate for both groups: 35% for lactating dairy cows vs. 70% for dairy heifers.

■ New Data

New published data from our laboratory (Bello et al., 2006) indicate that when lactating dairy cows are given pre-synchronization treatments of $PGF_{2\alpha}$ and GnRH, injected 2 d apart, prior to the initiation of an Ovsynch protocol 6 d later, more cows have an ovulatory response to the 1st GnRH injection of the Ovsynch protocol. Cows that ovulate to the 1st GnRH likely initiate emergence of a new follicular wave. Thus, these cows have a greater % of follicles in an ideal stage of development at the time of the final injection of GnRH with regard to the relationship between age, size, and E_2 concentrations and the increased probability of a pregnancy. Here forward, I will describe how these outcomes are strategically important to the reconstruction of the Ovsynch program and its potential to dramatically change the way we think about designing synchronization strategies for cattle.

Optimizing Ovsynch - Step 1: Control over ovulation to 1st GnRH of Ovsynch

Attaining consistent ovulation in response to 1st GnRH of Ovsynch constitutes the first key step to optimizing synchronization of ovulation to Ovsynch in lactating dairy cows (Vasconelos et al., 1999; Bello et al., 2006). Ovulation to 1st GnRH of Ovsynch is followed by emergence of a new follicular wave, from which the ovulatory follicle of Ovsynch develops (Pursley et al., 1995). Thus, variation in response to 1st GnRH leads to extreme variation in the timing of emergence of the ovulatory follicle. This, in turn, translates into substantial variation in age and size of ovulatory follicles at the time of the final GnRH of Ovsynch (Bello et al., 2006). This variation may lead to reduced conception.

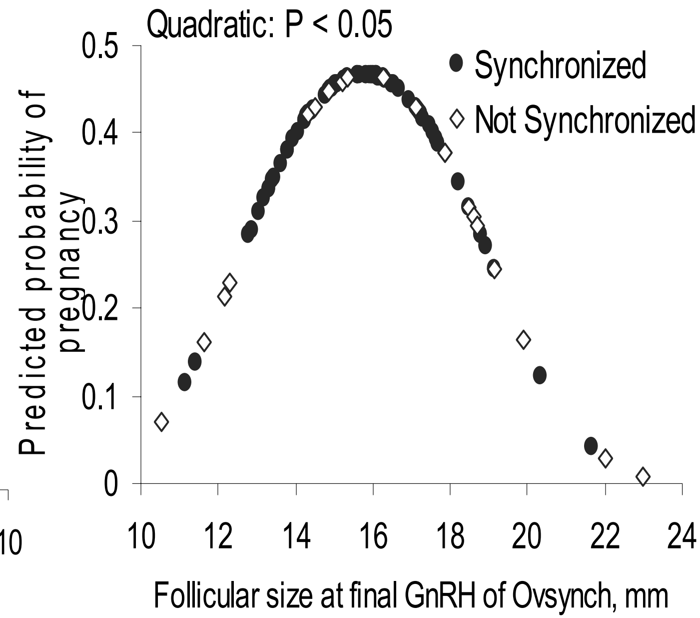

Figure 3: Association between follicle size at final GnRH of Ovsynch and predicted probability of pregnancy 35 d after AI in lactating Holstein dairy cows. Synchronized cows were cows that responded to both $PGF_{2\alpha}$ (by regressing a functional CL) and final GnRH of Ovsynch (by ovulating).

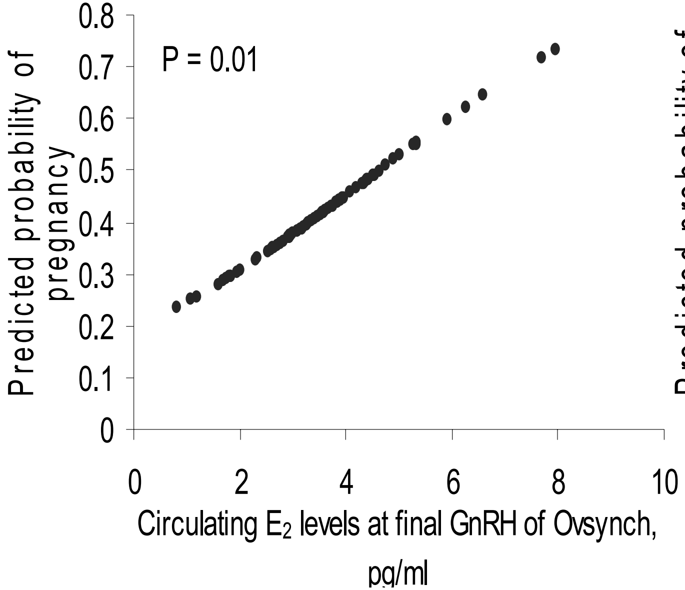

Figure 4: Association between circulating concentrations of E_2 at final GnRH of Ovsynch and predicted probability of pregnancy 35 d after AI in lactating Holstein dairy cows.

Our laboratory tested a Pre-Ovsynch program that maximizes ovulation to 1st GnRH of Ovsynch, which decreased the variability in size of the preovulatory follicle and increased synchronization rate to Ovsynch (Bello et al., 2006). Based on previous data, we designed an injection scheme 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 d later. Then, either 4 (G4G), 5 (G5G) or 6 (G6G) d later, cows received the 1st GnRH of Ovsynch, and continued the Ovsynch program. Controls received only the Ovsynch treatment with no presynchronization. Figure 2 describes the primary outcomes of these data (Bello et al., 2006). Compared to Ovsynch alone, G6G dramatically improved % of cows ovulating to 1st GnRH (A), % of cows responding to $PGF_{2\alpha}$ by luteolysis (B), and % of cows with both a luteolytic response to $PGF_{2\alpha}$ and ovulation to the final GnRH of Ovsynch (C). In these newly published data, we also show nearly a doubling of percent cows pregnant to G6G compared to Ovsynch alone. While additional numbers will be required, data clearly support the concept that enhanced percent cows ovulating to 1st GnRH of Ovsynch holds potential to increase percent cows pregnant to Ovsynch.

Additional data reveal that cows ovulating in response to the 1st GnRH of Ovsynch, regardless of treatment, yielded significantly less variability in pre-ovulatory follicle size at the final injection of GnRH (Figure 3), a greater chance of luteolysis in response to the $PGF_{2\alpha}$ of Ovsynch, and a greater chance of ovulating to the final GnRH (Bello et al., 2006). Also from this study (Bello et al., 2006), a positive linear relationship was detected between concentrations of E_2 at the final GnRH of Ovsynch and the probability of a pregnancy (Figure 4). In addition, a quadratic relationship was also detected between ovulatory follicle size at final GnRH and the probability of a pregnancy. Thus, it is of critical importance to optimize the age and size of the ovulatory follicle to allow these follicles to secrete as much E_2 as possible at the time of the final injection of GnRH of Ovsynch.

▪ Next Steps

We just completed a follow-up to the above study re-testing 6 d between Pre-synchrony and Ovsynch vs. 7 and 8 d and once again using no pre-synchronization as the control. Preliminary analyses reveal that 6 d from pre-synchrony to Ovsynch had greatest synchronization outcomes numerically but was not different from 7 d statistically. Analysis of these data will be completed and published soon.

Also, we are in the process of testing various ways to reduce the age of the ovulatory follicle from emergence to time of ovulation. If we are successful, conception rate studies will be performed to test the effect of ovulation of a younger follicle on fertility of the lactating dairy cow.

■ Summary

It appears that a pre-synchrony program that enhances the % of cows that ovulate to the 1st GnRH of Ovsynch increases synchronization rate to Ovsynch and optimizes ovulatory follicle size and function. Alone, increased synchronization will give more cows a greater chance to become pregnant. I recommend utilizing this technology to improve reproductive performance of dairy cattle. Finally, in Table 1, I have outlined my suggested schedule for a synchronization program in lactating dairy cows.

Table 1: Daily injection schedule for utilizing a PGF/GnRH pre-synchronization program with Ovsynch.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	PGF AM		GnRH AM			
		GnRH AM				
		PGF AM		GnRH P.M.	AI A.M.	

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