MANAGEMENT STRATEGIES TO MAXIMIZE PREGNANCY RATES IN DAIRY CATTLE

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Introduction

In high producing dairy cows, herd pregnancy rates are reduced due to poor heat expression and/or detection, anestrus, low conception rates and increased embryo mortality. Furthermore, these impediments to optimal reproductive performance are exacerbated under stressful environmental conditions such as heat stress, which is even more detrimental in higher milk producing cows. Reproductive performance has decreased in North America, Europe and Australia. Reasons for the decline are multi-factorial and not entirely associated with an increase in milk production. Epidemiology studies indicate that other factors such as reproductive diseases (i.e., retained placenta, metritis and ovarian cysts) or season of calving were relatively more important than milk yield on reproductive performance. In fact, higher producing herds may have a greater reproductive performance because of better feeding, reproductive management and healthier cows. Nevertheless, the physiological state of lactation is associated with a lower reproductive rate compared to heifers. In lactating dairy cows, the proestrous concentrations of estradiol are lower as are luteal phase concentrations of progesterone compared to non-lactating cows. Altered functional competence of follicles may be due to altered concentrations of LH and IGF-I in lactating cows. Increased liver metabolism of progesterone associated with dry matter intake may limit progesterone availability. These altered endocrine conditions may impede optimal reproductive performance. Major advancements have been made in the regulation of ovarian function such that systems are now available to control and coordinate ovarian follicular, corpus luteum and uterine functions in a manner that is conducive to normal fertility in lactating dairy cows. Objectives of this presentation are to examine various approaches to optimize reproductive efficiency by coordinating and regulating ovarian follicular and CL function in lactating dairy cows.

Development and Optimization of Timed Insemination Programs

Effective estrus synchronization programs provide a number of advantages: cows or heifers are in estrus at a predicted time which facilitates AI, and embryo transfer; time and labor expense for detection of estrus are reduced; AI becomes more practical under extensive conditions; precise control of ovulation permits a timed insemination without the need for detection of estrus; and specific timed treatments to improve embryo survival can be implemented effectively.

The ability to control the time of ovulation precisely following a period in which follicular development and CL regression have been programmed sequentially permits a timed insemination. Such programs are essential in high producing dairy cows that experience a reduction in estrus intensity that contributes to undetected heats, re-occurring luteal phases without estrus expression, or re-occurring waves of follicles that fail to ovulate. Development of timed insemination programs has been based upon a thorough understanding of the factors controlling ovarian follicular growth.

Ovsynch:

One program that has been extremely successful for insemination of cows at a fixed time for first service without the need for detection of estrus is the Ovynch program in which injections of GnRH are given 7 d before and 48 h after an injection of $PGF_{2\alpha}$, and cows are inseminated 16 to 20 h after the second injection of GnRH. This system synchronizes follicle maturation with regression of the corpus luteum before the GnRH-induced ovulation and timed insemination. Numerous studies indicate that pregnancy rates (proportion of all treated cows that were pregnant) to the Ovsynch program were comparable and in some studies greater than the appropriate control group.

There are several stages of the estrous cycle when initiation of the Ovsynch program causes reduced pregnancy rates. Initiation of the program between days 13 to 17 of the cycle is a time in which spontaneous regression of the CL occurs prior to the time that $PGF_{2\alpha}$ is injected at 7 days after the injection of GnRH. These cows will be asynchronized in that they may ovulate prior to the time of insemination and insemination will be to late for the cow to conceive. During the early stages of the cycle (e.g., days 2 to 4), the recruited dominant follicle is not sufficiently developed to ovulate in response to GnRH. As a consequence, the dominant follicle at the second injection of GnRH is considered aged and has expressed dominance for 5 days or longer. Follicles that have periods of dominance longer than 5 days are less fertile and some of these aged follicles fail to ovulate in response to the ovulatory injection of GnRH. An additional point to consider is that GnRH-induced turnover of follicles or induction of a new follicular wave is most efficient if ovulation is induced in response to the first injection of GnRH. Collectively, these findings indicate that presynchronization of cows prior to implementation of the Ovsynch program should improve pregnancy rates if cows enter the Ovsynch program at the most favorable period of the estrous cycle (i.e., days 5 to 12 of the cycle).

Presync-Ovsynch:

A program defined as Presync-Ovsynch was developed in which pre-synchronization is achieved with a standard estrous synchronization protocol ($PGF_{2\alpha}$ given twice at a 14-day interval) with the Ovsynch program initiated 12 days after the second injection of $PGF_{2\alpha}$. A Presynch-Ovsynch program increased pregnancy rates 12 percentage units (i.e., 37% to 49%; [1]) and 18 percentage units (i.e., 25% to 43%; [2]) in lactating cyclic cows. This stimulation in pregnancy rates was attributed to manipulation of the estrous cycle such that the Ovsynch, timed insemination program was initiated at the most favorable stages of the estrous cycle. Future programs for further optimization of fertility likely will consider programs that manipulate ovarian function such that follicular turnover via ovulation or induced follicular atresia occurs in all cows, and luteal phase like progesterone concentrations are sustained until the time of induced CL regression. These future systems in lactating dairy cows may include insertion of intravaginal devices containing progesterone and acute injections of estrogens. However, inclusion of these strategies will require acceptance by regulatory agencies and consumers. For example, the two approved classes of pharmaceuticals for use in lactating dairy cows in the USA are the GnRH and $PGF_{2\alpha}$ drugs.

Success of the Ovsynch program is dependent on whether lactating dairy cows are anestrus or cycling. Pregnancy rates were less in cows that were not cycling at the time the Ovsynch program was initiated (e.g., 22% versus 42%). Overall, the TAI protocol was able to induce cycles in 75% of anestrous cows, based upon the number of anestrous cows which were classified as ovulating to either the first and/or second injection of GnRH. If anestrous cows ovulate to the first and second GnRH injections of the Ovsynch program then pregnancy rates appeared to be normal (e.g., 39%). Thus the Ovsynch protocol was successful in regulating ovarian function in certain anestrous cows such that they conceived. Pregnancy rates to the second service for anestrous cows were similar to those obtained for cyclic cows and constitute further evidence for a beneficial effect of the Ovsynch protocol, when used on anestrous cows in response to two GnRH injections over a 9-day period. Many anestrous cows have re-occurring anovulatory follicles that are not estrogenic and fail to induce a preovulatory surge of LH. The timely injections of GnRH to induce LH and FSH, as part of the Ovsynch protocol, appear to induce functional follicle development and ovulations that permit initiation of a pregnancy in some anestrous cows with anovulatory follicles.

Heatsynch:

An alternative strategy to control the time of ovulation is the ability of exogenous estradiol to induce a LH surge by stimulating hypothalamic secretion of GnRH when given in a low progesterone environment during late diestrus and proestrus. An estradiol induced LH surge lasts for approximately 10 h, which is comparable to a spontaneous LH surge and longer than the LH surge induced by GnRH. Estradiol cypionate (ECP), an esterified form of estradiol-17β that is available commercially for use in cattle, has been used as part of a timed insemination program in lactating dairy cows to replace the second GnRH injection of an Ovsynch program and is called Heatsynch [3].

Pregnancy rates were evaluated in lactating dairy cows when ECP was used to induce ovulation as part of a timed insemination protocol in comparison to Ovsynch for lactating dairy cows in Florida (n=371) and Texas (n=321). In two studies (Florida and Texas), cows were pre-synchronized with two injections of $PGF_{2\alpha}$ given 14 d apart with timed insemination protocols beginning 14 d after the second injection of $PGF_{2\alpha}$. The protocols consisted of an injection of GnRH followed by $PGF_{2\alpha}$ 7d later. Cows were injected either with GnRH (Ovsynch) at 48 h after $PGF_{2\alpha}$ and inseminated 16-24 h later or with ECP (1 mg, i.m.) at 24 h after $PGF_{2\alpha}$, Heatsynch) and inseminated 48 h later. Pregnancy rates did not differ between Heatsynch and Ovsynch programs at either site (Table 1).

Table 1. Pregnancy rates (LSM $\pm$ SE) of lactating dairy cows to a timed insemination following Ovsynch or Heatsynch programs.

Location	Ovsynch Group[a] (n=179)	Heatsynch Group[b] (n=192)	Overall (n=371)
Florida (n=371)	37.1 $\pm$ 5.8	35.1 $\pm$ 5.0	39.6 $\pm$ 2.5
Texas (n=321)	28.2 $\pm$ 3.6	29.0 $\pm$ 3.5	26.8 $\pm$ 2.4

[a] Ovsynch: GnRH (Day -10), $PGF_{2\alpha}$ (Day -3), and GnRH (Day -1) with timed artificial insemination on Day 0.

[b] Heatsynch: GnRH (Day -10), $PGF_{2\alpha}$ (Day -3), and ECP (Day -2) with timed artificial insemination on Day 0.

In lactating dairy cows, the frequencies of detected estrus and ovulation after ECP were 75.7% and 86.5%, respectively. Estrus occurred at 29.0 $\pm$ 1.8 h (n=28) after ECP. Mean intervals to ovulation were 55.4 $\pm$ 2.7 h after ECP and 27.5 $\pm$ 1.1 h after onset of estrus. Since 75% of the ovulations occurred between $\geq$48 h to $\leq$72 h after ECP, it is recommended that any cow detected in estrus by 24 h after ECP injection be inseminated at 24 h, and all remaining cows be inseminated at 48 h. Based on synchronization of ovulation and pregnancy rates, ECP can be utilized as an alternative to induce ovulation in place of GnRH for a timed insemination. Timing of ECP injection to induce ovulation for a Heatsynch program differs compared to the use of GnRH in an Ovsynch program. The timing differences between ECP injection and insemination (i.e., 24 or 48 h) of the Heatsynch program versus GnRH injection and insemination (i.e., 12 to 16 h) of the Ovsynch program makes the Heat Synch protocol more easily applied relative to the sequence of injections and insemination being made at the same time of day. Greater uterine tone, ease of insemination and occurrence of estrus with the use of the Heatsynch program are well received by inseminators. Alternatively, in facilities with concrete flooring, the reduced estrous expression associated with the Ovsynch program may be preferred. Since fertility between the two programs appears to be comparable, producers have a choice, which also includes relative costs of drugs (i.e., ECP < GnRH).

Alterations in Ovarian Function to Improve Embryo Survival

A major impediment to acceptable herd pregnancy rates in lactating dairy cows is the high rate of embryo mortality (e.g., 44%). The ability to control either the occurrence of estrus or the time of ovulation precisely reduces the problem of poor heat detection considerably and permits investigators to focus on strategies to improve conception rates following insemination.

Bovine Somatotropin

In cycling lactating dairy cows, injection of bovine Somatotropin (500 mg Posilac, Monsanto Co, St. Louis, Missouri) at the time of the first GnRH injection or at insemination in cows of a Presynch-Ovsynch program increased pregnancy rates (57% > 42.6%; [2]). Since bST was effective at insemination, it is likely that bST stimulated embryonic development and survival following insemination in lactating dairy cows. There was no evidence that bST given at the 9th week of lactation is detrimental to fertility when used with a timed breeding protocol such as Ovsynch. A study in Mexico reported, that in cows identified as having three or more prior services, bST given at estrus and again 10 days later stimulated pregnancy rates [4]. The ability to detect this bST beneficial effect likely is attributable to the fact that bST was given at a physiologically important window in which occurrence of estrus and or ovulation was controlled. Several studies showed that bST stimulated bovine in vitro maturation of oocytes and embryonic development. Furthermore, administration of bST at AI to superovulated donor cows decreased the number of unfertilized ova, increased the percentage of transferable embryos, and stimulated embryonic development to the blastocyst stage. Moreover, bST affected both early embryonic development and recipient components to increase pregnancy rates following embryo transfer [30]. Our recent studies indicate that the beneficial effect of bST may be restricted to lactating dairy cows in contrast to non-lactating dairy cows.

HCG induction of Accessory CL:

The opportunity to regulate ovarian function after insemination to improve pregnancy rates is an additional production management strategy. The ability to induce ovulation of the healthy first wave follicle either at day 5 of the cycle or after insemination results in two altered endocrine states. The administration of hCG induces ovulation with the subsequent formation of a functional accessory CL. The majority of the increase in plasma progesterone after hCG was due to the accessory CL. Size of CL, in vitro production of progesterone, and plasma concentrations of progesterone were greater in accessory CL induced by hCG than GnRH. Due to the induction of ovulation at day 5, cows will experience three-follicular wave cycles due to the earlier emergence of the second wave. Furthermore, the third follicular wave is delayed. Development of the conceptus is related to higher concentrations of progesterone and ability of the conceptus to secrete IFN-τ. Therefore, hCG induction of an accessory CL with increased progesterone may enhance embryo survival. Since a greater number of cows conceived that had three follicular waves after insemination compared with cows having two follicular waves, hCG induction of three-wave cycles also may contribute to higher pregnancy rates.

Use of hCG to increase pregnancy rate:

An experiment was designed to examine the potential of hCG to increase conception rates [5]. Objectives were to evaluate the effect of injecting 3,300 IU of hCG (Chorulon, Intervet Inc., Millsboro, DE) on day 5 after insemination on CL number and plasma progesterone concentration. In addition, effects of hCG on conception rates at days 28, 45, and 90 days after insemination were examined. The experiment was conducted on a commercial dairy farm in the south central valley of California. A total of 406 high producing dairy cows (43.0 ± 0.45 kg/day, mean milk ± SE at the time of AI) were housed in a free-stall barn and milked twice daily. Multiparous and primiparous cows were housed separately.

The study was divided into two periods: period 1 of May 14 to September 16, 1999 when daily maximum temperatures ranged from 22 to 38 °C (warm period), and period 2 from October 5, 1999 to March 02, 2000, when daily maximum temperatures ranged from 9 to 29 °C (cool period). One hundred and eighty-two cows were enrolled during the warm period and 224 cows during the cool period. Once a week, a group of 30 to 60 cows between 40 and 145 days in milk had their estrous cycle synchronized with an i.m. injection of 100 µg of GnRH (Gonadorelin; Factrel®, Fort Dodge Animal Health Inc., Fort Dodge, IA) followed 7 days later by an i.m. injection of 25 mg of $PGF_{2\alpha}$ (Lutalyse®, Pharmacia Upjohn Company, Kalamazoo, MI). Estrus was detected once daily, from 2 to 5 days after the $PGF_{2\alpha}$ injection, both visually and by tail chalking . Cows in estrus were assigned to initiate the experiment based on lactation number and days in milk. Seventy two % of the treated cows were detected in estrus. All cows were artificially inseminated once daily, in the morning, by the same technician throughout the experimental period. Semen from five different proven sires was used. Cows found open at day 28 received an injection of 25 mg of $PGF_{2\alpha}$ and were artificially inseminated as detected in estrus within 5 days of the prostaglandin treatment. For analyses of the second service conception rates, only cows that were inseminated in the subsequent estrus at 18 to 24 days after the initial AI and those that came in estrus after the $PGF_{2\alpha}$ injection at day 28 were evaluated.

On day 5 after AI, cows received an i.m. injection in the neck area of either 3,300 IU (hCG group) of hCG (Chorulon®, INTERVET, Inc., Millsboro, DE) or 3 ml of saline solution (control group). One blood sample was collected from all cows between days 11 and 16 after insemination and subsequently analyzed for concentrations of plasma progesterone. At the same time of blood sampling, the ovaries of all cows were scanned by ultrasound to evaluate presence, location (ovary), size (area and volume), and number of CL. Diagnosis of pregnancy was performed by ultrasonography on day 28±1 after AI. During ultrasonography, a cow was determined pregnant when an embryonic vesicle with a viable embryo (presence of heart beat) was detected. At the same time, ovaries of pregnant cows were scanned to determine the presence and location of the ovulatory and accessory CL. Pregnant cows at day 28 were reconfirmed by rectal palpation on day 45±1, and again on day 90±2 after AI. At the time of AI and on day's 28±1, 45±1, and 90±2 after insemination, cows were body condition scored by the same person. To evaluate changes in BCS, cows were divided into three groups in which cows lost, had no change, or gained BCS.

Administration of 3,300 IU of hCG on day 5 after AI successfully induced the formation of one or more accessory CL in 175 of the 203 treated cows. More cows in the hCG-treated group had multiples CL (86.2% vs 23.2%; $P < 0.001$). The increase in number of CL for cows treated with hCG compared with control cows was greater during the cool than during the warm period ($P <$

0.02). Number of CL on day 14 was not influenced by BCS at breeding. However, the change in BCS between AI and day 28 after breeding affected CL number ($P < 0.01$), with cows losing BCS having the highest mean number of CL, followed by those that did not change or gained BCS in the same period.

Plasma progesterone concentrations during the mid luteal phase were increased in the hCG-treated cows compared with the control cows ($P < 0.0001$). Differences in progesterone concentrations between hCG and control cows were +10.6 ng/ml for primiparous cows and +7.5 ng/ml for multiparous cows ($P < 0.02$). Cows with more observable CL during ultrasonography had higher progesterone concentrations ($P < 0.0001$), and an interaction between CL number and treatment on plasma progesterone was observed ($P < 0.0001$). Within the hCG group, cows with more than 1 CL had higher plasma progesterone than those with only 1 CL, and it was increased by 9.8 ng/ml. Such an effect of accessory CL on progesterone concentrations was not observed for cows in the control group, in which progesterone levels were similar.

At day 28 after AI, CR was increased from 38.7% for control cows to 45.8% for the hCG-treated cows ($P < 0.01$; Table 2). Similar CR was observed during the warm and the cool periods, but a tendency for an interaction between treatment and period was detected ($P < 0.09$). Control and hCG-treated cows had similar CR during the warm period, but hCG increased CR compared with control during the cool period (47.8 vs 34.2%). Cows with 0, 1 or more than 1 CL on d 14 had CR of 0, 31.7, and 53.6%, respectively ($P < 0.0001$). Plasma progesterone in pregnant cows was 3 ng/ml higher than in cows that were open at day 28 (18.2 vs 15.2 ng/ml; $P < 0.001$). Similar effects of progesterone were observed for CR at 45 and 90 days post AI.

Table 2. Effect of treatment on CR at days 28, 45 and 90 post AI.

	Treatment[1]		
Conception rate, %	Control	hCG	P‹
Day 28	38.7	45.8	0.01
Day 45	36.3	40.4	0.005
Day 90	31.9	38.4	0.008

[1] hCG = human chorionic gonadotropin (3,300 IU)

Cows with moderate BCS at the time of AI had higher CR than cows with low BCS ($P < 0.001$). Conception rates on day 28 were 34.4 and 48.7% for cows with low and moderate BCS, and these effects were similar for control and hCG-treated cows. Similar to the results observed for BCS at the day of AI, changes in body score from AI to day 28 were also associated with changes in CR. Cows that gained BCS from AI to day 28 had higher pregnancy than those that lost or maintained BCS (47.0% vs 42.7% vs 37.4%; $P < 0.03$). Interestingly, an interaction between treatment and BCS change was determined for pregnancy at day 28. Cows that lost BCS when treated with hCG had a CR of 57.1% compared with only 24.2% for those in the control group ($P < 0.05$). When pregnancy diagnosis was considered at day 45 and day 90 after AI, the effects of treatment, number of CL, plasma progesterone concentration, BCS at the day of AI, and BCS change were similar to those observed for CR at day 28 after insemination.

Pregnancy loss was determined from days 28 to 45, 45 to 90 and the overall loss from 28 to 90 days. Only cows that were initially found pregnant and subsequently found open were included in these analyses. For pregnancy losses between 28 and 45 days after insemination, the data from 172 cows were utilized, 79 in the control group and 93 in the hCG-treated group. Cows in the hCG-treated group had a slightly higher pregnancy loss during this period, but no statistical difference was detected (11.8 vs 6.3; $P < 0.21$). Number of CL on day 14 influenced pregnancy loss from 28 to 45 days. Increasing the number of CL from 1 to more than 1 decreased pregnancy loss from 13.2% to 7.6% ($P < 0.007$). Overall pregnancy loss from day 28 to day 90 was similar for hCG and control cows, and averaged 17.4%.

This study supports the concept that increased progesterone during the luteal phase increases embryo survival. However, this effect was not evident in the heat stress period where early embryo losses probably precluded any subsequent increases in embryo survival.

Implications

A vast array of options has been recently developed for the reproductive management of lactating dairy cows. Such management systems have been fine-tuned to result in maximum pregnancy rates and increase the overall reproductive efficiency of lactating dairy herds. As it can be observed in the experiments described above, pregnancy rates as great as 50% to a single service were achieved. It is important to emphasize, that as reproductive systems become more efficient and incorporate several levels for the control of reproductive processes, a thorough understanding of the technology is needed.

It is important for producers to realize that such reproductive management systems cannot solve all reproductive problems per se. For instance, incidence of cows in anestrus greatly reduces the reproductive efficiency of dairy herds and such a problem may not be solved by synchronization systems. Providing optimal nutritional management, maximizing cow comfort, and maintaining a good herd health program are all pre-requisites for the success of any reproductive program.

To date, management of dairy cows has been driven by the necessity to maximize milk production with a high overall success. With the advent of new technologies to precisely manipulate reproductive function in lactating dairy cows, dairy producers are presented with a new opportunity. Further research is necessary to fine tune management systems that are both practical and able to fulfill this objective. Producers can look forward to new strategies to reduce anestrus, synchronize return services and enhance embryo survival.

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