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Strategies for improving fertility in the modern dairy cow

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Abstract

The high producing dairy cow of the 21st century is subfertile during lactation. Our objectives are to characterize physiological periods limiting reproductive performance and to describe integrated management strategies to improve pregnancy rates. Ovarian recrudescence with normal re-occurring estrous cycles and restoration of fertility to first service are associated with a reduced occurrence of periparturient metabolic and reproductive disorders. Marked negative changes in energy balance and reduced immunocompetence influence gonadotropic and metabolic hormones. Induced ovarian inactivity was associated with enhanced uterine involution. Post-partum health and reproductive performance were improved when by-pass lipids enriched in polyunsaturated fatty acids were fed in the pre- and post-partum periods. Pharmaceutical control of follicle, CL, and uterine function with PGF, GnRH and intravaginal progesterone releasing inserts, has permitted development of more optimal timed-insemination programs for first service. Likewise, resynchronization of nonpregnant cows coupled with the use of ultrasound for early pregnancy diagnosis provides the opportunity for a second timed-insemination within 3 days of a nonpregnant diagnosis. Bovine somatotropin (bST) increases embryo development and embryo survival when coupled with a timed-insemination program or cows detected in estrus. Presence of a conceptus alters endometrial expression of genes and proteins in response to bST and nutraceuticals (i.e., unsaturated fatty acids such as eicosapentaenoic and docosahexaenoic acid in by-pass lipids) to improve pregnancy rates. Postovulatory

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increases in progesterone may enhance pregnancy rates in targeted populations of lactating dairy cows, but timing and magnitude of the progesterone increases are pharmaceutically dependent.

Keywords: Uterus; Insemination; Embryo; Fatty acids; Bovine somatotropin; Progesterone

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1. Introduction

The modern high producing, lactating dairy cow in North America is subfertile as managed under current production systems. Reasons for the decline in fertility are multifactorial and not associated entirely with an increase in milk production [1]. Epidemiological 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 influencing reproductive performance [1,2]. However, it is clear that lactation, as a physiological process, is associated with a lower reproductive rate compared to that of nonlactating heifers [1]. Herds experiencing low pregnancy rates may be encountering an array of possible cow and management inefficiencies such as reduced health and compromised immune function, poor estrus expression and/or detection, extended anovulatory periods, low conception rates, and increased early and late embryonic mortality. Higher producing cows are often the healthier cows because of better feeding and reproductive management, and may actually cycle earlier in the post-partum period [3]. Impediments to optimal reproductive performance are exacerbated under stressful environmental conditions, such as heat stress, which is even more detrimental in higher producing cows. The challenge to improve reproductive performance of lactating dairy cattle involves an understanding of the biochemical and physiological principles controlling reproductive and lactational processes that are then integrated into nutritional management, production medicine, and reproductive management systems to optimize fertility of the herd. Objectives of this presentation are to characterize physiological periods that appear to be limiting reproductive performance and to describe management strategies to improve pregnancy rates.

2. Periparturient/post-partum period

2.1. Uterine–ovarian activities

Slow recovery of reproductive competence during the post-partum period is a major limitation to the success of subsequent reproductive management programs that are implemented for inseminations beginning at the start of the voluntary waiting period. It was proposed initially that early and frequent occurrences of estrus following calving were associated with increased reproductive performance due to a more optimal restoration of the uterine environment [4]. In this study, cows expressing no or only one estrus within 60 days post-partum had reduced fertility resulting in a higher percentage of nonpregnant cows being sold, and more services per pregnancy. These responses decreased linearly as the number of estruses increased, i.e., cows that had two, three or four detected estruses were of greater fertility. The suggestion that spontaneous estruses can enhance uterine involution and subsequent fertility is debatable. Animals with no early estrus periods could represent a greater proportion of cows with periparturient disorders such as dystocia, retained placenta, metabolic disorders, and metritis. Cows expressing one estrus could represent those animals with a prolonged luteal phase (failure to turnover their CL) and/or with possible uterine bacterial contamination such that these cows would have lower fertility. The animals expressing two or more estruses may be representative of reproductively healthy cows. These cows appeared to ovulate once and continued to do so. A certain sequential occurrence of events from the time of parturition to the time of first service is optimal. Therefore, a uterus to ovarian pathway, i.e., completion of physical involution and clearance of the uterus should occur in a short period of time post-partum without the occurrence of ovulations. After completion of uterine involution, sequential ovulations would be a goal towards normal fertility.

The dairy cow is amazing in that ovarian follicular activity resumes early in the post-partum period. With the drop in plasma concentrations of estradiol following delivery of calf and fetal membranes, inhibition of FSH secretion is terminated and early increases in plasma FSH begin to stimulate follicle development as early as 7 days after parturition. A dominant follicle develops in response to FSH and LH. However, the follicle does not always produce estradiol, and this appears to be associated with inadequate amounts of plasma LH and IGF-1 [5,6]. Both LH and IGF-I are necessary for full functional development of an estrogenic follicle.

Concentrations of IGF-I in plasma were related closely to the recrudescence of CL activity [7]. Cows that cycled early in the post-partum period experienced an early increase in plasma concentrations of IGF-I (i.e., 2 weeks post-partum), whereas cows that were anestrous through 8 weeks of lactation did not exhibit a rise in plasma IGF-I concentrations until 5–6 weeks post-partum. It is our contention that these changes in IGF-I were related to metabolic differences among cows that began to cycle at different times and were critical to follicle development and subsequent formation of the CL. The marked deficit in early energy status for anestrous cows exerted a marked carryover effect on conception [3]. Only 33% (5/15) of anestrous cows eventually conceived compared to 84% (21/25) and 93% (13/14) for early and late cycling cows, respectively. Cows that are diagnosed as anestrus or had low body condition scores at the time of fixed-timed insemination had lower pregnancy rates and higher pregnancy losses [8]. Concentration of IGF-I in plasma may be a good candidate to monitor reproductive responsiveness to post-partum dietary treatments in high producing dairy cows.

2.2. Uterine health

During the first 4 weeks post-partum, the cow's immune system is challenged severely [9]. Most cows develop a mild nonpathological endometritis during the early puerperal phase of the post-partum period [10], and uterine fluids (lochia) are usually voided during the first 2 weeks post-partum. The uterine immune system seems to up-regulate during the periparturient period and remains so until progesterone from the first post-partum ovulation down-regulates the uterine immune system. Onset of endometritis has been associated with an increase in progesterone concentrations [11]. Cows that developed endometritis had lower PGFM concentrations during the early post-partum period (i.e., 0–14 days post-partum) perhaps contributing to a reduction in neutrophil function that compromises the ability of the uterus to prevent and/or manage infections.

Administration of a GnRH agonist (deslorelin) implant is able to suppress follicular growth, reduce concentrations of estradiol, and delay both CL development and concentrations of progesterone in post-partum dairy cows [12]. An additional experiment evaluated ovarian follicular activity, presence of a CL and uterine involution in cows treated from Days 1 to 4 post-partum with a non-degradable deslorelin implant (5 mg; $n = 10$) or a control group ($n = 9$) that did not receive an implant [13]. All cows had normal parturitions without dystocia, retained fetal membranes or milk fever. Diameters of uterine horns were monitored by ultrasound. Vaginal endoscopy evaluated the amount, consistency and color of the cervical discharge on Days 14, 21, 28 and 35 after enrollment. Ovarian activity was suppressed by the deslorelin implant due to a reduction in gonadotropin secretion. In deslorelin implant-treated cows, the size of the previously pregnant uterine horn also was reduced, and the frequency of a purulent discharge from the cervical os was lower. Results from this experiment question the merits of stimulating early post-partum ovarian activity and the need for supplemental estrogens during the immediate post-partum period. Absence of ovarian activity during the period of uterine involution appeared to enhance the involutionary process. Perhaps this is the reason that suckled beef cows experience less post-partum problems involving the uterus. Treatments that delay ovarian activity or reduce sustained progesterone exposure warrant further study.

In clinically normal cows, detection of subclinical endometritis, between 34 and 47 days post-partum by endometrial cytology (i.e., $>10\%$ of leucocytes in the endometrial smear) or diagnosis of fluid in the uterus by ultrasonography, was associated with a reduction in subsequent fertility [14]. Thus, the intrauterine environment is critical and predictive of subsequent fertility, and subclinical endometritis is a condition undetected by routine examination via palpation per rectum. It is imperative that the incidence of metritis and subclinical endometritis be reduced so that cows entering the breeding period are most fertile and can respond to the programmed system of reproductive management. A dietary strategy of supplementation, with functional nutrients to increase chronically the availability of prostaglandins as a means to possibly enhance general immunocompetence and neutrophil function, is an attractive means to possibly manage uterine infections [15].

2.3. *Feeding by-pass fats*

Growing evidence indicates that the design and delivery of supplemental unsaturated fatty acids to the lower gut for absorption (specifically linoleic acid, linolenic acid, eicosapentaenoic acid [C20:5] and docosahexaenoic acid [C22:6]) may target reproductive tissues and alter reproductive function and fertility. We recently examined whether feeding of calcium salts of fatty acids containing 28% linoleic acid would have modulator effects on post-partum dynamics of PGF secretion, health, milk production and reproductive performance [15]. Primiparous ($n = 22$) and multiparous ($n = 25$) Holstein cows were used in a completely randomized block design to determine the effects of timing of the initiation of fat supplementation (Megalac-R®, Church and Dwight Co. Inc., Princeton, NJ) on cow performance the first 14 weeks post-partum. The four dietary treatments were: Group 1 (Control; no supplemental fat source;) and Groups 2–4 supplemented with Megalac-R® (2% of dietary dry matter) beginning 28 days prior to expected calving date (Group 2), at day of parturition (Group 3), or Day 28 in milk (Group 4). Cows fed Megalac-R® received ~227 g per day during the pre-partum period and ~454 g per day in the post-partum periods. Of cows that ovulated to the Ovsynch protocol on ~Day 72 post-partum, cows fed Megalac-R® tended ($P = 0.09$) to experience higher first service conception rates than controls (Group 1: 27.8%, 3/11; Group 2: 40.0%, 4/10; Group 3: 70%, 7/10; Group 4: 63.6%, 7/11).

The profiles of plasma PGFM (Fig. 1) for the first 12 days post-partum differed between the group receiving Megalac-R® beginning pre-partum (Group 2) and those fed Megalac-R® beginning at parturition (Group 3) or not fed Megalac-R® during the first 12 days after parturition (Groups 1 and 4). Our interpretation is that cows fed Megalac-R® pre-partum had their lipid pools enriched in linoleic acid which increased substrate concentrations for arachidonic acid, the precursor for the synthesis of PGF. The increased potential for the uterus and cells of the immune system to secrete prostaglandins because of by-pass feeding of linoleic acid–calcium salts may enhance post-partum uterine health and the immunocompetence of the cow. This is supported by the observation that initiation of feeding of Megalac-R® in the late dry period resulted in fewer health problems (mastitis, retained fetal membranes or metritis) in the first 10 days post-partum compared to cows not fed Megalac-R® pre-partum (1/12 versus 15/35; $P < 0.05$). In a large field experiment [16], lactating Holstein cows ($n = 423$) were fed, beginning 25 days before calving until Day 60 post-partum, either a fat-supplemented diet (1.5% of dietary DM) of a calcium salt blend of primarily C18:2 and *trans* C18:1 (EnerG-1 Transition Formula®; Virtus Nutrition,

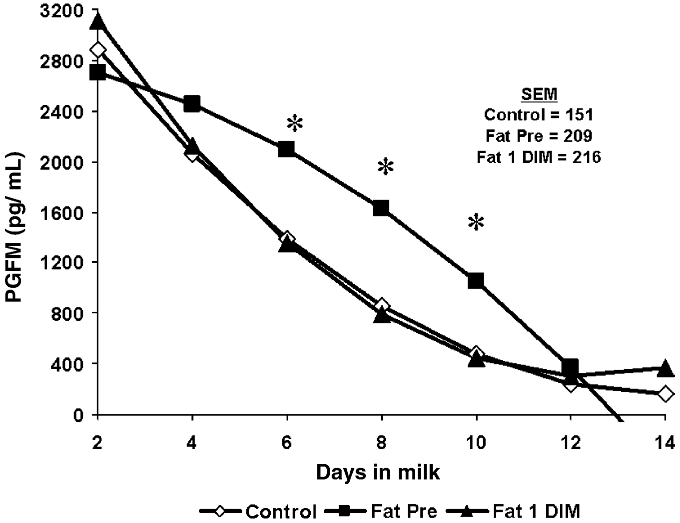

Fig. 1. Effect of Megalac-R® supplementation on plasma 13–14 dihydro, 15 keto-PGF2 (PGFM; ng/mL) between 2 and 14 days in milk (DIM). Lactating dairy cows did not receive Megalac-R® supplementation (control; $n = 23$), received Megalac-R® beginning at 28 days prior to expected parturition (Fat Pre; $n = 12$) or received Megalac-R® at the time of parturition (Fat 1 DIM; $n = 12$).

Fairlawn, OH) or calcium salt of palm oil (EnerG-II®; Virtus Nutrition). First service conception rate to a timed-insemination at 72 ± 3 days post-partum tended ($P < 0.09$) to be improved from 25.9% to 38.9% by supplementation with C18:2 and *trans* C18:1 fatty acids. A sub-sample of these dairy cows ($n = 154$; i.e., fat supplemented from 25 days before calving through 60 days post-partum) were timed-inseminated at Day 47 post-partum and had embryos recovered 5 days later (Day 52 post-partum) [17]. Cows fed EnerG-I Transition Formula® tended to have ($P = 0.11$) a higher fertilization rate (87% versus 73%), more accessory sperm per structure collected (34 versus 21; $P < 0.001$), and tended to have ($P = 0.06$) a higher proportion of embryos classified as high quality (73% versus 51%). Collectively, these studies indicate that feeding fats enriched in selected unsaturated fatty acids, beginning in the dry period and continuing in the post-partum period, improves post-partum health and milk production [15] as well as the development of bovine embryos [17] and subsequent pregnancy rates [15,16]. The beneficial effects on reproductive responses may be due to a hastened restoration of the post-partum reproductive system to support embryo development.

3. Programmed inseminations

The goal for a successful estrus synchronization program in lactating dairy cattle is precise control of estrus, which will allow high fertility to a fixed-timed AI without the need for estrus detection. Strategies for ovulation control have been based on controlling the life-span of the CL with PGF, induction of follicle development and a synchronized ovulation, or prevention of estrus using progestogen treatments. These approaches have been integrated with development of the Ovsynch protocol, i.e., GnRH is given at random stages of the estrous cycle to induce ovulation of the dominant follicle and synchronize follicle wave emergence. Seven days later, PGF is given to regress either the original and/or a newly formed CL, followed by a second GnRH injection 48 h later to induce a synchronous ovulation 24–32 h later. A timed-insemination is carried out at 12–16 h after the second GnRH injection. Fertility with the Ovysnch protocol is highest when cows ovulate to the first GnRH injection. The subsequent ovulatory follicle at the time of the second GnRH injection will have developed with a reduced period of dominance and a responsive CL will be present at the time of the PGF injection. Initiating the Ovsynch protocol prior to Day 12 of the estrous cycle should minimize the number of cows that come into estrus and ovulate prior to the second GnRH injection.

A presynchronization protocol prior to implementation of the Ovsynch protocol was developed by giving two injections of PGF 14 days apart, with the second injection given 12 days prior to the first GnRH of the Ovsynch protocol. The Presynch–Ovsynch protocol increased pregnancy rates 18% (i.e., 25–43%) in lactating, cyclic cows [18]. El-Zarkouny et al. [19] also demonstrated that presynchronization before GnRH-based protocol with a CIDR insert enhanced pregnancy rate compared to that achieved without presynchronization (46.8% > 37.5%; $P < 0.01$), but this effect was not evident in anestrous cows. A modified Presynch–Ovsynch protocol increased the interval from the second PGF to the first GnRH to 14 days [20]. Pregnancy rate with the modified Presynch–Ovsynch protocol was higher than the Ovsynch program alone (49.6% versus 37.3%; $P < 0.05$). This slight modification makes the sequence of injections more producer-friendly relative to implementation of injections on a weekly basis.

An additional modification of the Presynch–Ovsynch protocol in lactating cows is the delay of the second GnRH injection of the Ovsynch protocol until 72 h after the injection of PGF with insemination at the time of the GnRH injection [21]. Delaying the GnRH injection and AI until 72 h after PGF increased pregnancy rate compared to the Presynch–Ovsynch protocol with GnRH injection and AI at 48 h after PGF (31.4% > 22.8%; $P < 0.05$). Although delaying the GnRH injection and AI until 72 h after PGF results in an improvement of pregnancy rate, pre-synchronization is essential. Otherwise some cows will come into estrus early (at 24–48 h after PGF) but will not be inseminated until 72 h.

Presynchronization protocols are only efficacious in cyclic cows. Therefore, successfully optimized presynchronization timed-insemination programs for first service vary from herd to herd, depending on the degree of anestrus and compliance in implementing the sequence of treatments. Additional approaches to optimize controlled breeding include strategies to achieve a high rate of ovulation to the first injection of GnRH of the Ovsynch protocol [22], induction of cyclic activity in anovulatory cows [19,23], and maintenance of a progesterone environment between the first injection of GnRH and PGF of the Ovsynch protocol [19].

4. Programmed reinseminations

Different strategies have been used to resynchronize returns to estrus in order to increase the number of cows reinseminated in a timely manner. Insertion of an intravaginal progesterone releasing insert containing 1.38 g of progesterone (CIDR B, Pfizer Animal Health, Kalamazoo, MI) [24], or in combination with an estrogen injection at CIDR insertion and/or after CIDR removal resynchronizes the return estrus following a prior insemination [25]. However, such programs require detection of estrus, and, as expected, not all nonpregnant cows are detected in estrus.

When Ovsynch for resynchronization was initiated on Day 21 or 28 after a prior insemination [26], similar pregnancy rates to the prior insemination were detected by ultrasound on Day 28 (33.1% versus 33.6%) and Day 42 (27.0% versus 26.8%). Furthermore, pregnancy rate to the second (resynchronized) insemination on Day 31 or 38 after prior service did not differ between the two treatments (28.3% versus 31.4%). Therefore, it would appear to be safe to initiate an Ovsynch protocol for resynchronization as early as on Day 21 after breeding, before pregnancy determination by ultrasonography on Day 28. In contrast, pregnancy rates to the resynchronized insemination were lower when the Ovsynch protocol was initiated on Day 19 (23%) compared to Day 26 (34%) or Day 33 (38%) after a prior insemination [27]. Perhaps the difference between a detrimental effect on Day 19 [27] but not on Day 21 [26] reflects differences in follicle turnover induced by GnRH. It is possible that a greater portion of cows treated on Day 21 would have had an induced ovulation of a potential preovulatory follicle such that a newly recruited follicle and a CL would be present at the time of PGF injection in nonpregnant cows.

Resynchronization with GnRH given on Day 23, with PGF following a diagnosis of nonpregnancy on Day 30 and timed inseminations following an injection of either GnRH (Ovsynch) or ECP (Heatsynch) resulted in 30-day pregnancy rates of 28.4% and 28.6%, respectively [28]. In addition, pregnancy losses between Days 30 and 55 were 9.4% and 14.3% for Ovsynch and Heatsynch protocols, respectively.

An experiment was designed to integrate the reproductive management of the first and second inseminations in lactating dairy cows and to evaluate the use of the CIDR insert [29]. Cows ($n = 718$) were randomized into a 2 × 2 factorial design to receive PGF on Days 39 and 53 post-partum, GnRH on Day 67, PGF on Day 74, and GnRH and timed AI on Day 77 (experimental Day 0). Cows in estrus between Days 74 and 77 were inseminated according to estrous behaviour. Between Days 67 and 74, cows received either a CIDR insert (Presynch–Ovsynch–CIDR group) or were used as nontreated controls (Presynch–Ovsynch–Control group). Between experimental Days 14 and 23 after timed AI, cows received a CIDR (Resynch–CIDR group) or were used as nontreated controls (Resynch–Control group). After first service, cows detected in estrus were inseminated (AIDE) and did not continue with either resynchronization protocol. On Day 23 cows received GnRH and on Day 30 examined for pregnancy by ultrasonography. Nonpregnant cows received PGF on Day 30 and GnRH and timed AI on Day 33. Pregnancy rates to all first and second inseminations were determined at 30 days after insemination using ultrasonography and at 55 days using per rectum palpation of the genital tract. Blood samples were collected on Day 53 (second PGF treatment) and Day 67 post-partum (first GnRH treatment), and cows were classified as having either low ($\leq$1 ng/mL) or high ($>$1 ng/mL) progesterone. First service pregnancy rates (i.e., cows diagnosed pregnant/cows inseminated at detected estrus or timed inseminated) were determined for all cows that had blood samples collected on both Days 53 and 67 ($n = 585$).

First service pregnancy rates were higher in cows with low progesterone on Day 53 (second PGF) and high progesterone on Day 67 (first GnRH) in the Presynch–Ovsynch–CIDR group than in the Presynch–Ovsynch–Control group (44.4% versus 17.4%; Table 1). Cows in this low-high progesterone category would have been in late diestrus at the time of initiation of the Ovsynch protocol on Day 67 post-partum. The presence of a CIDR insert

Table 1
First service pregnancy rates on experimental Days 30 and 55 for cows in the Presynch–Ovsynch–Control and Presynch–Ovsynch–CIDR groups with different categories of plasma progesterone measured on Days 53 and 67 post-partum

Progesterone category	Treatment group	Pregnancy rate	
		30 days[a]	55 days[a]
High–high	Presynch–Ovsynch–Control	37.23 (35/94)	30.85 (29/94)
	Presynch–Ovsynch–CIDR	39.81 (41/103)	39.81 (41/103)
High–low	Presynch–Ovsynch–Control	33.33 (17/51)	27.45 (14/51)
	Presynch–Ovsynch–CIDR	34.21 (13/38)	31.58 (12/38)
Low–high	Presynch–Ovsynch–Control	17.39 (8/46)	13.04 (6/46)
	Presynch–Ovsynch–CIDR	44.44 (28/63)	38.10 (24/63)
Low–low	Presynch–Ovsynch–Control	31.52 (29/92)	29.35 (27/92)
	Presynch–Ovsynch–CIDR	30.30 (30/99)	27.27 (27/99)

[a] $P < 0.05$ synchronization protocol × progesterone category for pregnancy rates on experimental Days 30 and 55.

would have allowed for a higher frequency of synchronized ovulations resulting in a higher pregnancy rate to the timed insemination.

Pregnancy loss between Days 30 and 55 of pregnancy was reduced in the Presynch–Ovsynch–CIDR group (7.0%) compared to cows in the Presynch–Ovsynch–Control group (15.6%). In cows classified as noncyclic, based on blood samples collected on Days 53 and 67 post-partum, the Presynch–Ovsynch–CIDR group had a reduced ($P < 0.05$) pregnancy rate for first insemination (18.8%) that was restored if cows received a CIDR for resynchronization (41.2%).

Pregnancy rates 30 and 55 days after second service were lower ($P < 0.05$) for cows categorized as an AIDE in the Resynch–CIDR group (28.6% and 26.8%, respectively) compared to AIDE in the Resynch–Control group (38.8% and 36.2%, respectively). Pregnancy rates 30 and 55 days after timed inseminated in cows with a CL at ultrasonography were higher ($P < 0.05$) in the Resynch–CIDR group (29.7% and 27.5%, respectively) than in the Resynch–Control group (19.4% and 13.4%, respectively). Cows without a CL in both groups had low pregnancy rates 30 and 55 days after time-insemination (Resynch–CIDR: 10.0% and 7.5%; Resynch–Control: 15.4% and 15.4%). There was no difference in pregnancy losses between treatment groups following second service.

In lactating dairy cows, the CIDR insert results in subluteal concentrations of progesterone in the circulation (i.e., ~1.1 ng/mL) [30] that will block ovulation and permit management of follicle dynamics. For example, in cows diagnosed with cystic ovaries, insertion of a CIDR between the administration of GnRH and PGF of the Ovsynch protocol increased pregnancy rates to the timed-insemination [28]. The subluteal concentrations of progesterone associated with use of the CIDR likely would be insufficient to directly increase progesterone concentrations sufficiently for a sustained period to enhance embryo survival after Day 6 of pregnancy.

5. Bovine somatotropin (bST) and by-pass fats

Bovine somatotropin is used widely in the dairy industry to promote increases in yields of milk and milk components. Treatment with bST also improves fertilization rate, accelerates embryo development, and improves embryo quality [31,32]. Indeed, timely treatment with bST increased pregnancy rates when given in concert with the Ovsynch protocol [33,34] or in cows detected in estrus [35,36]. Improvements in conception rates were also associated with a reduction in embryonic death between Days 31 and 45 [35].

A study was designed to examine the effects of bST, pregnancy and dietary fatty acids on reproductive responses in lactating dairy cows [37,38]. Two diets were fed, starting approximately 17 days in milk (DIM), in which oil of whole cottonseed (control diet) was compared with a calcium salt of fish oil-enriched lipid (FO). Ovulation was synchronized with a Presynch–Ovsynch protocol, and cows were time-inseminated or not inseminated at the time of the synchronized ovulation (Day 0; 77 ± 12 DIM). On Days 0 and 11, cows received bST (500 mg) or no bST. All cows were sacrificed on Day 17. The number of cows in each group was as follows: the control diet had 5 bST-treated cyclic (bST-C), 5 non-bST-treated cyclic (C), 4 bST-treated pregnant (bST-P), and 5 non-bST-treated pregnant (P) cows; fish oil-enriched lipid diet had 4 bST-treated (bST-FO) and 5 non-bST-treated cyclic (FO) cows.

Feeding fish oil-enriched lipid increased milk production ($P < 0.01$), the number of follicles 2–5 mm ($P < 0.05$), and decreased plasma insulin concentrations ($P < 0.01$) during the period before Day 0 compared with control-fed cows. The bST increased milk production ($P < 0.01$), pregnancy rate (83%, 5/6 versus 40%, 4/10; $P < 0.09$), conceptus length (45 cm versus 34 cm; $P < 0.09$) and interferon-τ in the uterine luminal flushings (9.4 μg versus 5.3 μg; $P < 0.05$) with no effect on interferon-τ mRNA concentration in the conceptus. Treatment with bST increased plasma growth hormone and IGF-I. Among control-fed cows (cyclic and pregnant), bST decreased ($P < 0.05$) progesterone concentrations in plasma, perhaps associated with a greater rate of metabolism induced by bST. The cows fed fish oil-enriched lipid had lower plasma insulin ($P < 0.01$) than control-fed cyclic cows, and feeding fish oil-enriched lipid altered the plasma growth hormone (bST-FO > bST-C; $P < 0.05$) and IGF-I (bST-FO < bST-C; $P < 0.05$) responses to bST injections. Endometrial IGF-I mRNA was reduced in pregnant cows ($P < 0.01$) and tended to be reduced ($P < 0.06$) in those fed fish oil. The IGF-II mRNA was increased in the endometrium of pregnant cows ($P < 0.01$) and bST-treated cows fed the control diet ($P < 0.01$). Cows fed fish oil-enriched lipid had increased ($P < 0.05$) concentrations of IGF-II mRNA when bST was not administered.

A major enzyme essential for the biosynthesis of prostaglandins is prostaglandin H synthase-2 (PGHS-2), also known as cycloxygenase-2. The regulation of this enzyme is important to prevent luteolytic peaks of PGF in pregnant cows. The amount of PGHS-2 protein in the endometrium was elevated ($P < 0.01$) and localized to the luminal epithelium of pregnant cows. In contrast, pregnancy was associated with decreased relative concentrations of estrogen receptor alpha (ERα) mRNA ($P < 0.01$), ERα protein ($P < 0.05$) and oxytocin receptor (OTR) mRNA ($P < 0.01$) in endometrial tissue compared to cyclic control cows. Although, PGHS-2 protein was high in pregnancy, the initial regulatory components to activate the system (i.e., ERα protein and OTR) were reduced in the endometrium of pregnancy due to interferon-τ secretion from the conceptus. Consequently, luteolytic peaks of PGF do not occur in early pregnancy, but the PGHS-2 protein is there for regulation of embryo development, implantation, angiogenesis and localized immune responses in early pregnancy. Both fish oil-enriched lipid and bST treatments reduced immunocytochemical PGHS-2 protein expression in the luminal epithelial cells of the endometrium of cyclic ($P < 0.05$) and pregnant ($P < 0.01$) cows. This may assist in attenuating the potential luteolytic system and partially account for the ability of these treatments to improve pregnancy rates. Prostaglandin E synthase mRNA levels were elevated ($P < 0.01$) in cyclic cows and pregnant cows treated with bST, whereas prostaglandin F synthase mRNA levels decreased ($P < 0.01$) in bST-P cows but not in bST-C cows. These changes in gene expression could potentially favor an increase in the PGE/PGF ratio within the endometrium that would support ultimate maintenance of the CL. Feeding fish oil-enriched lipid modulated immunocytochemical expressions of progesterone receptor, ERα, and PGHS-2 within particular endometrial cell types. Collectively, this array of endometrial responses indicates that bST and fish oil-enriched lipids appeared to modulate endometrial expression of certain genes, and that responses varied depending upon exposure to the conceptus (i.e., cyclic versus pregnant effects).

These responses induced by bST and fish oil-enriched lipids appear to act in a manner that may be beneficial for establishment and maintenance of pregnancy.

Responses of plasma IGF-1 among the groups treated with bST are interesting. Pregnant-control cows treated with bST had higher concentrations of IGF-1 (261 ± 18 ng/mL) than bST-treated-cyclic control cows (211 ± 17 ng/mL). This difference may have contributed to the greater degree of conceptus development in bST-treated cows. Although growth hormone in the plasma was markedly elevated in cyclic fish oil-enriched lipid-fed cows that received bST, their concentrations of IGF-1 (180 ± 20 ng/mL) remained relatively low and stable throughout the sampling period. This reflects the potential dietary interactions of fat feeding on endocrine and metabolic responses in lactating dairy cows.

Our first study to examine the effects of bST on endocrine and uterine gene expression on Day 17 after timed AI was with nonlactating dairy cows using a similar protocol for timed AI and bST injections [39]. The pregnancy rate in nonlactating dairy cows was less ($P < 0.01$) following bST treatment (27.2%; 9/33) than in Controls (63.6%; 14/22), emphasizing the importance of utilizing lactating dairy cows as the experimental model in a subsequent study [37,38] in which pregnancy rate was higher ($P < 0.09$) for bST-treated cows (83%; 5/6) than for control cows (40%; 4/10). Although these were two separate experiments conducted in comparable months, but different years, the radioimmunoassay of plasma samples for IGF-1 and growth hormone were done at the same time. The bST-induced increase in plasma growth hormone was substantially lower in nonlactating pregnant cows (10 ng/mL) compared to lactating pregnant cows (25 ng/mL). This may reflect a greater utilization by the liver that accounted for a hyperstimulation in IGF-1 concentrations in bST-treated non-lactating pregnant cows (Fig. 2). It is clear that basal concentrations of IGF-1 are higher in more fertile, nonlactating, pregnant cows, than in

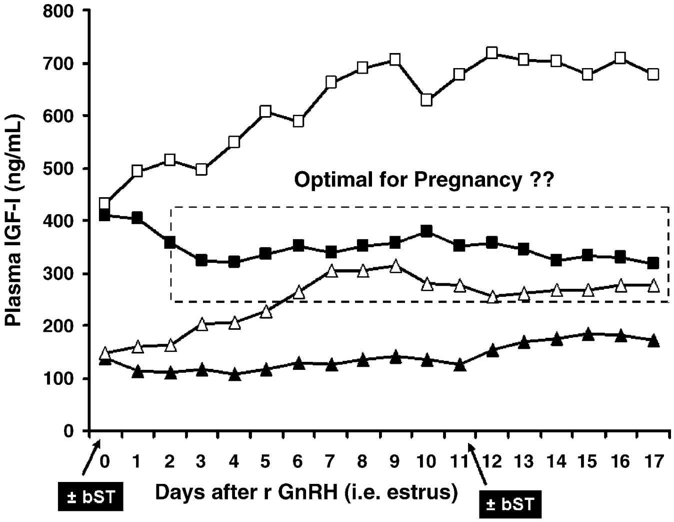

Fig. 2. Plasma IGF-1 (ng/mL) of pregnant cows that were nonlactating or lactating. Cows received injections of bST (±; 500 mg) on days 0 (i.e., day of GnRH) and 11 after a synchronized insemination (Days 0–17). Nonlactating pregnant no bST (■; $n = 14$); nonlactating pregnant bST (□; $n = 9$); Lactating pregnant no bST (▲; $n = 4$); lactating pregnant bST (△; $n = 5$).

lactating pregnant cows. However, when nonlactating dairy cows were treated with bST, there was a hypersecretion of IGF-1 which may have accounted for the bST-induced decrease in pregnancy rate. In contrast, lactating pregnant cows that received bST had an increase in plasma IGF-1 concentrations that approximated the range in basal concentrations of pregnant, non-lactating cows that did not receive bST and were more fertile (Fig. 2). Perhaps there is an optimal level of plasma IGF-1 concentrations for pregnancy that can be achieved in lactating dairy cows with bST injections (Fig. 2). An increase in concentrations beyond this range may lead to a decrease in pregnancy rate.

It is clear that bST injections in cows experiencing their first service at the voluntary waiting period of ~70 days post-partum have an increase in pregnancy rate. This was detected utilizing, for the most part, timed AI protocols or evaluating the effect of bST in cows that were detected in estrus and inseminated. Indeed protocols that synchronize the time of ovulation, like the Presynch–Ovsynch protocol, allow for a test of potential fertility products like bST. The beneficial effect of bST is not easily detected in commercial dairy herds that do not adapt a rigorous timed AI protocol, but are dependent upon estrus detection. Treatment with bST has been shown to reduce expression of estrus [35] and, as a consequence, delays interval to first AI [40].

6. Induction of accessory CL with hCG

On Day 5 of the estrous cycle, granulosa cells of the dominant follicle contain LH receptors such that hCG will induce ovulation and formation of an accessory CL [41,42]. Therefore, administration of hCG 5 days after AI has the potential to increase progesterone secretion during early pregnancy, and to alter ovarian follicular dynamics so that cows have three follicular waves within the period approaching the time of CL maintenance [42]. Injection of 3300 IU hCG in lactating cows on Day 5 after AI increased the number of CL and substantially elevated plasma progesterone concentrations [43]. Conception rates on Days 28, 42, and 90 were increased by hCG treatment, but late embryonic and fetal losses remained unaltered. Therefore, the positive effect of hCG on conception rates was mediated by reducing early embryonic losses. In addition, most of the benefit of hCG treatment was observed in lactating dairy cows that were losing body condition during the breeding period. A recent study with embryo transfer recipients detected an increase in pregnancy rate of recipients treated with hCG [44]. Pregnancy rate in cows receiving hCG on Day 6 was higher (67.5%) than in control cows (45.0%) or cows receiving hCG on Day 1 (42.5%) after estrus. This reinforces that induction of an accessory CL and increased progesterone concentrations reduce early embryonic mortality in cattle. A within herd assessment indicated that cows with an additional CL were eight times less likely to experience fetal loss than those with a single CL [45]. The effect of a spontaneous double ovulation on pregnancy maintenance was not related to twin pregnancies because cows with twins in the same study were 3.1 times more likely to experience pregnancy loss. Since high producing dairy cows have a greater metabolism of progesterone, they are more likely to be responsive to hCG treatment. The literature is replete with studies showing no benefit of GnRH treatment to increase pregnancy rates when used to induce an accessory CL. It is important to recognize that use of GnRH, as opposed to hCG, is associated with a shorter duration of LH exposure, with the induction of an accessory CL that is less responsive to LH in vitro, and a substantially lower increase in plasma progesterone concentrations during the subsequent luteal phase [41]. Furthermore, use of hCG should be targeted to populations of cows that are subfertile such as the high producing, lactating cow that is losing body condition.

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