Reproductive performance of Holstein cows treated with prostaglandin $F_{2\alpha}$, gonadotropin releasing hormone, and recombinant bovine Somatotropin

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Key words: Timed AI, Presynchronization, bovine somatotropin, ECF

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ABSTRACT

The objective of this study was to examine the effects of presynchronization and recombinant bovine somatotropin (**rbST**) on conception rates following a timed insemination (**TAI**) protocol in lactating dairy cows. A further objective included the evaluation of the efficacy of the Early Conception Factor (**ECF**) test kit. Recombinant bST may offer some benefit when used in conjunction with estrus synchronization and TAI. Presynchronization treatment consisted of two injections of $PGF_{2\alpha}$ given 14 d apart, with the second dose administered 14 d prior to the initiation of a TAI protocol. A total of 216 lactating Holstein cows were presynchronized with $PGF_{2\alpha}$ and then received GnRH (100µg) at 67 ± 7 d post partum (**PP**), administration of $PGF_{2\alpha}$ (25 mg) 7 d later, another GnRH (100µg) administration 2 d after $PGF_{2\alpha}$, and were inseminated 8-18h later (**OvSynch**). First service conception rate (**CR**) was determined by rectal palpation at 42 ± 7 d after artificial insemination (**AI**). Treated cows (n=113) received rbST 67 ± 7 d PP whereas control cows (n = 113) were presynchronized without rbST. The cycling status of all cows was determined by paired milk P_4 levels at 53 and 67 ± 7 d PP. No differences ($P > 0.10$) in conception rate were observed between control and rbST treated cows (44.7 and 40.7% respectively), nor was there any interaction of cyclicity and rbST. Milk samples were collected 7 d following AI for use in ECF test kit evaluation. Samples were stored at -20ºC (n=216) and at 5ºC (n=113) until assayed. Test results for frozen and refrigerated samples were compared to conception rates determined by rectal palpation at 42 ± 7 d after AI. The rate of false positive and negative results for frozen milk samples were 36.1 and 14.8% respectively, and 40.7 and 7.1% for refrigerated milk samples. Treatment with rbST at the time of the first GnRH injection of an OvSynch protocol did not significantly alter first service conception rates. Additionally, an acceptable 92.9% accuracy of the ECF test for the detection of open cows 7 d after AI using milk samples stored at 5ºC was obtained.

Key words: Timed AI, Presynchronization, bovine somatotropin, ECF

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Chapter 1. Introduction

Estrus synchronization protocols have been used to reduce labor and time associated with estrus detection and artificial insemination (AI). Enhanced regulation of estrus depends on controlling the corpus luteum (CL) as well as follicular development (Twagiramungu et al., 1990). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) given in two doses, 14 d apart, has been used as a method of estrus synchronization which has limited time required for detecting estrus. Cows synchronized with two prostaglandin injections, 14 d apart, have produced pregnancy rates of 84% (Folman et al., 1990). Despite successful conception rates, intense estrus detection is necessary for 2 to 5 d following treatment. In order to increase the efficiency of AI in the dairy industry, producers must be presented with an AI program designed to coordinate follicular maturation and luteal regression closely such that ovulation can be predicted. This would eliminate the need for the detection of estrus, permitting insemination at a prescribed time.

Two $PGF_{2\alpha}$ injections, separated by 14 d, would offer partial synchronization of follicular development before luteal regression. If a group of cycling cows is in random stages of the estrous cycle at the time of the first of two $PGF_{2\alpha}$ administrations, luteolysis should be induced in those cows in d 5 to 15 of the estrous cycle, while the rest remain unaffected. At the second $PGF_{2\alpha}$ administration, the initially responsive group should be in the early stages of a new cycle, and the remainder would be in a broader range of the estrous cycle, allowing for a leuteolytic response to $PGF_{2\alpha}$.

This practice would yield a greater number of cows that will have a maturing second wave dominant follicle capable of ovulating in response to a gonadotropin-releasing hormone (GnRH) induced lutenizing hormone (LH) surge than would a GnRH administration given at a random stage of the estrous cycle. A controlled release of GnRH capable of stimulating an LH surge would greatly reduce the time span of ovulations within a group of synchronized cows and greatly benefit reproductive management when breeding at a fixed time (Kraeling et al., 2000). Therefore, following the $PGF_{2\alpha}$ dosage, a timed insemination protocol such as OvSynch could be incorporated. OvSynch consists of a GnRH injection, a $PGF_{2\alpha}$ administration 7 d later followed in 48 h by administration of GnRH (Pursley et al., 1997 a). The combination of the two protocols (two $PGF_{2\alpha}$ injections 14 d apart followed by OvSynch) is known as PreSynch.

This procedure allows for the synchronization of ovulation within an 8 h period, allowing for a timed insemination with greater success, without the use of estrus detection.

Recombinant bovine somatotropin (rbST) has been developed as a commercially available recombinant hormone for use in dairy herds due to its ability to increase milk production. Its effect on reproduction must also be considered. In studies conducted using lactating dairy cows treated with rbST, researchers have reported a decrease in reproductive performance (Collier et al., 1997; Maollem et al., 1997; Kirby et al., 1997 b). Additional experiments have found conception rates to be decreased by as much as 16.9% with rbST treatment (Luna-Dominguez et al., 2000). However, there is evidence that rbST may offer some benefit when used in conjunction with ovulation synchronization. When a reproductive management system that eliminates the need for estrus detection was used in lactating dairy cows, it was observed that administration of rbST increased pregnancy rates following the synchronized service (Moreira et al., 2000). This incongruity suggests that this topic is worthy of additional experimentation. The present study focused on the effects of rbST treatment on the reproduction parameters of lactating Holstein cows in a commercial setting where cows were presynchronized for a TAI.

There are various tools in existence used in the detection of pregnancy in cattle. These include hormonal assays such as milk or blood serum progesterone (P_4) and applied practices such as transrectal ultrasonography and palpation. These means, however effective, are conducted post-implantation and therefore are ineffective in determining conception shortly following insemination. A product that accurately identifies conception pre-implantation would be beneficial in decreasing the interval between inseminations and therefore increasing reproductive efficiency.

Currently, a technology exists that reportedly detects a pregnancy-associated glycoprotein within 48 h of conception. This product is known as the Early Conception Factor (ECF) test (Concepto Diagnostics Inc., Knoxville, TN). The ECF test is marketed as a test to detect non-pregnant or "open" cows 6 to 20 d following insemination. Both milk and blood serum can be used. Only a few studies have compared ECF test predicted conception rates to actual pregnancy diagnosis by palpation or ultrasound, and have reported conflicting results. High rates of false negative and false positive results were

reported when blood serum was used (Cordoba et al., 2001). In another study, blood serum and milk were reported to yield differing results (Gandy et al., 2001). This is an important factor considering a dairy producer can easily obtain a sample of milk as opposed to blood serum.

Chapter 2. Review of Literature

Reproductive Performance and Production

Reproductive performance is a limiting factor in dairy farm profitability (Hamudikauwanda et al., 1987). Within recent years, the dairy industry has experienced a decline in fertility and overall reproductive efficiency. Due to improvements in nutrition, genetics, and management, milk production per cow has increased. This shift toward more productive cows is, however, associated with a decrease in reproductive efficiency. For example, AI conception rates in the 1950's were approximately 55% (Casida, 1961). Present day conception rates are approximately 45% for AIs at spontaneous estrus (Dransfield et al., 1998) and about 35% with timed AI (Pursley et al., 1998). In addition, Stevenson (2000) showed increasing services per conception, days open, and days to first insemination in 143 dairy herds continuously enrolled in the DHIA record system from 1970 to 1999.

Profits from a reproductive program are maximized when most cows exhibit optimal reproductive performance. Inefficient reproductive efficiency decreases profit by reducing efficiency of milk production (Hamudikuwanda et al., 1987). Increased days open or days to first service result in fewer calves per productive life and therefore result in less milk production per day of life. Reproductive inefficiency results in elevated veterinary expenses as well as higher replacement costs due to culling. Calving interval is an important factor within reproductive performance. The cost of a prolonged calving interval is projected to be two to three dollars per day over a 12.5 month period. For dairy cows, a calving interval of 13 months is considered optimal (Nebel, 1998). Calving intervals longer than optimum occur when cows spend more time in the later, less profitable stage of lactation (Call, 1978). Detection of estrus and rate of conception are integral components for achieving this interval. However, detection of estrus efficiency is low. The detection of estrus in Raleigh DRMS (Raleigh, NC) herds is less than 40%.

Efficient and accurate detection of estrus is the key management factor determining the successful use of AI and acceptable reproductive performance in a dairy herd (Heersche and Nebel, 1994). Detection of estrus can be said to be comprised of two factors: efficiency and accuracy. Detection efficiency is usually defined as the percentage of estruses observed that should have occurred within a period of time. Detection accuracy is the percentage of estruses observed that were true estruses. Dransfield et al. (1998) analyzed data generated from a pressure sensitive radio telemetric estrus detection system (Heat Watch System, DDx Inc., Denver, CO) and reported the average dairy cow is in estrus seven hours and stands to be mounted 8.5 times per estrus. Of the tested population (n = 2401), 24.1% of the cows had low intensity of estrus (< 1.5 stands/h) and short duration (< 7 h). This makes detection of estrus difficult under the standard recommendations of 30 min. twice daily. Sturman et al. (2000) found that in a herd of Holsteins (n = 242), 19% of inseminations were performed on cows in the luteal phase or first trimester of pregnancy. Of the pregnant cows inseminated, 17% experienced embryonic loss. Detection of estrus is also affected by the surface on which the cows are housed. Mounting activity is greater on a dirt surface compared to concrete ($P < 0.05$, 3 to 15 fold greater) (Vailes and Britt, 1990). The growing tendency toward larger herd size and increased utilization of confinement housing with concrete floors may lead to a decrease in estrus detection rates. Due to the low rate of estrus detection in most herds, variation in days to first and subsequent AI can be quite large, leading to longer than optimal calving intervals. Studies have linked poor estrus detection to increased calving intervals. Rounsaville et al. (1979) showed that increasing heat detection shortened first service interval, days open and calving interval. A change from 33 to 55% in heat detection resulted in a decrease of almost one day in time to first service and more than one day in time open and calving interval for each percentage that unit change in heat detection. This study supported the contention that heat detection, as compared to conception rate and reproductive culling, most significantly influences days to first insemination, days open, and calving interval.

Estrus Synchronization

Prostaglandins

Considerable research has been carried out in order to develop technologies to synchronize and efficiently detect estrus. In the past, reproductive management protocols have focused on the synchronization of estrus using $PGF_{2\alpha}$. These were very successful when cows were bred after a detected estrus. Detection of estrus increases and management of AI is more efficient when estrus is synchronized with $PGF_{2\alpha}$ in contrast to daily detection of estrus (Stevenson and Pursley, 1994). Nevertheless, synchronization with $PGF_{2\alpha}$ does not control the time of AI because estrus detection is still required. Lucy et al. (1986) showed that cows receiving a fixed time AI at 72 to 80 h after a second injection of $PGF_{2\alpha}$ resulted in pregnancy rates considerably lower ($P < 0.05$) compared to cows receiving AI at a detected estrus alone. Lack of luteal function prior to the second injection limits the success of the two-$PGF_{2\alpha}$ injection protocol for timed inseminations. Stevenson et al. (1994) showed nearly 15% of $PGF_{2\alpha}$ treated cows (n = 176) displayed a lack of luteal function when P_4 levels were tested. Low pregnancy rates related to timed AI following treatment with $PGF_{2\alpha}$ may be explained by the variation in time of ovulation with respect to time of AI. This variation in time of ovulation is due to the deviation in stage of the pre-ovulatory follicle at the time of $PGF_{2\alpha}$ injection (Pursley et al., 1997 b). If a fully developed and functional dominant follicle is present at $PGF_{2\alpha}$ injection, the time to and variation in time to ovulation, or estrus are significantly less than if the dominant follicle is early in development. Injection of dairy heifers with $PGF_{2\alpha}$ between d 5 and 8 of the estrous cycle resulted in earlier (by 11 h) estrus than did injections of $PGF_{2\alpha}$ given between d 14 and 16 of the cycle (Stevenson et al., 1984). Time of ovulation, however, is apparently not affected by $PGF_{2\alpha}$ treatment. Walker et al. (1996) found the mean time of ovulation in Holstein cows to be 27.6 ± 5.4 h after detected estrus using the HeatWatch system. The time of ovulation did not differ between estruses induced by $PGF_{2\alpha}$ and those occurring spontaneously. Overall, synchronization with $PGF_{2\alpha}$ does not offer desired pregnancy rates with fixed time inseminations.

Gonadotropin-releasing Hormone

Gonadotropin-releasing hormone offers some assistance in a synchronization program. When administered exogenously, GnRH will initiate a surge of LH and therefore lutenize the dominant follicle of an ovary. This, in turn, allows for a surge of follicle stimulating hormone (FSH), initiating recruitment of a new follicular wave. Therefore, GnRH has an impact on follicular dynamics. Second follicular waves emerged earlier in GnRH treated cows than in control cows (9.9 versus 12.8 d; $P < 0.01$) and emergence of a third dominant follicle was shortened by 1.2 d in cows treated with Buserelin (GnRH agonist) (Rajamahendran et al., 1998). Heifers receiving only Buserelin had an increased number of medium-sized follicles compared to controls (MacMillian and Thatcher, 1991). Lucy and Stevenson (1986) showed that the conception rate of cattle receiving GnRH was higher ($P = 0.06$) than that of saline-treated controls. This increase may be attributed to increased plasma P_4 concentrations.

A proportion of infertility in cattle could be attributed to inadequate CL function. Lamming et al. (1989) found lower milk P_4 concentrations between d 10 and 16 following breeding among cows that did not establish pregnancy than for cows establishing and maintaining pregnancy. GnRH treatments stimulated plasma P_4 concentrations above that of the control with the higher response following treatment with 2.1mg of Buserelin (Rajamahendran et al., 1998). Buserelin pretreatment also delayed or prevented complete luteolysis by an injection of $PGF_{2\alpha}$ (MacMillian and Thatcher, 1991). Alteration of ovarian follicular dynamics with a GnRH agonist before induction of CL regression with $PGF_{2\alpha}$ improves precision of estrus response (Thatcher et al., 1989). When a GnRH analogue was injected 6 d prior to an injection of $PGF_{2\alpha}$, there was an increased number of synchronized animals. Variability in the time of estrus in beef cows and heifers was also reduced significantly ($P < 0.01$) (Twagiramungu et al., 1992).

Timed Insemination

It has been determined that GnRH, when administered exogenously, will lutenize an ovarian dominant follicle. A protocol has been devised using this ability of GnRH allowing TAI between 8 and 16 h following treatment (Pursley, 1995). The procedure is not directed a synchronizing estrus, but instead synchronizing the time of ovulation. This greatly impacts the management of reproduction in dairy cows since it eliminated the need for estrus detection. Pursley (1995) described the treatment as involving an initial, intramuscular injection of GnRH at d 0). This will ovulate large, functional, dominant follicles (>10mm) and induce a new follicular wave, increasing the probability of another large follicle to be present at the time of the $PGF_{2\alpha}$ injection 7 d later. A second injection of GnRH is given at d 9 in order to increase the synchrony of ovulation of the second wave dominant follicle. Cows are inseminated 16 to 20 h following the second dose of GnRH. This protocol is termed OvSynch (Pursley et al., 1997 a).

Pursley (1995) found that 20 out of 20 lactating cows and 18 of 24 heifers ovulated a newly formed dominant follicle between 24 and 32 h after the second GnRH injection. This study also showed $PGF_{2\alpha}$ is best given 48 h prior to the second GnRH administration. Two separate studies found pregnancy rates for the first service AI were similar: 37% versus 39% and 38.9% versus 37.8% for treatment and control cows, respectively (Pursley et al., 1997 a; Pursley et al., 1997 b). In addition, median days to first AI (54 versus 83) and days open (99 versus 118) were lower for OvSynch treated cows (Pursley et al., 1997 a). This demonstrates the procedure to be effective for use in place of estrus detection. Additionally, OvSynch has been shown to increase pregnancy rates above those observed after synchronization of estrus alone with $PGF_{2\alpha}$. Specifically, the pregnancy rate of treatment cows (33%) was significantly higher ($P < 0.01$) than that of cows treated with $PGF_{2\alpha}$ 14 d apart (11%) (Momcilovic et al., 1998). However, OvSynch may have less of an effect on conception rates. Two separate studies (Burke et al., 1996; Stevenson et al., 1999) displayed a tendency of conception rates to be higher after a detected estrus than those following OvSynch and one, fixed-time AI, although pregnancy rates were similar or of greater magnitude after fixed-time AI. This may be due to either poor expression or poor detection of estrus associated with OvSynch.

Although OvSynch seems to be an effective management tool for lactating dairy cows, response is dramatically different with regard to heifers. Pregnancy rates were greater (74.4%) for heifers treated with $PGF_{2\alpha}$ and inseminated at a detected estrus than those of heifers treated with OvSynch (35.1%) (Pursley et al., 1997 b). Additionally, significant ($P < 0.05$) differences in pregnancy rates were found by Stevenson et al. (2000) between those heifers treated with OvSynch (48/113; 42.5%) and those treated with the standard $PGF_{2\alpha}$ protocol involving AI after a detected estrus (76/131; 58.0%). Heifers receiving the OvSynch protocol also show a shortened return to estrus following insemination (Schmitt et al., 1996). The stage of the estrous cycle that OvSynch is initiated may affect the competence of the protocol.

Vasconcelos et al. (1999) showed a definitive effect of the initiation of OvSynch relative to the day of the cycle. Cows were scanned via ultrasound to determine day of the estrous cycle. Sixty four percent of cows (n = 156) ovulated in response to the first GnRH administration and varied by stage of cycle ($P < 0.01$). This, in turn, varied the response to the second GnRH dosage (92% if ovulation to first GnRH versus 79% if no ovulation; ($P < 0.05$)). In a similar experiment, heifers were treated with two doses of $PGF_{2\alpha}$ to induce estrus, subsequently scanned to determine the day of ovulation, assigned to begin OvSynch at d 2, 5, 10, 15, or 18 of the cycle, and scanned throughout the cycle to determine ovulation (Moreira et al., 2000 c). During the metestrus phase of the estrous cycle (d 1 to 4), the dominant follicle is too immature and small (<9mm) to respond to GnRH. The second GnRH injection of the OvSynch protocol may result in ovulation but the subsequent CL will produce less P_4 and therefore diminish subsequent pregnancy rates (Moreira et al., 2000). In addition, the follicle may possibly have displayed dominance for an extended period by the time of the second GnRH injection. Follicles that display dominance for too long a period (≥ 5 d) have decreased fertility (Austin et al., 1999). A similar situation exists with initiation of the first GnRH injection in the late luteal phase of the estrous cycle (d 13 to 17). Two-wave cycle cows will have a small, non-GnRH responsive dominant follicle that will not ovulate and form a new CL. Therefore, the cow spontaneously produces endogenous $PGF_{2\alpha}$ from the uterus and regresses the previously existing CL about 4 d later. In this situation, the cow may already have expressed estrus and ovulated prematurely by the time of the exogenous

$PGF_{2\alpha}$ and subsequent GnRHadministration. If the initial GnRH dose is given in the proestrus phase of the estrous cycle, the $PGF_{2\alpha}$ injection may fail to completely regress the CL resulting in lower pregnancy rates following TAI (Moreira et al., 2000). Heifers beginning OvSynch on d 15 of the cycle ovulated prior to insemination. Additionally, failure to ovulate following the second GnRH injection, and incomplete regression or short life span of the induced CL caused a shortened return to estrus (< 16 d).

Prostaglandin $F_{2\alpha}$ offers some natural synchronization of follicular development and estrus. If all cows are cycling, $PGF_{2\alpha}$ injections at an interval of 14 d are expected to place about 90% of the cows in the ideal stage of the cycle to begin OvSynch (d 5 to 10) (Thatcher et al., 2001). With a treatment of two $PGF_{2\alpha}$ doses prior to OvSynch, most cows will have a maturing, second wave dominant follicle that is capable of ovulating in response to a GnRH induced LH surge at an interval after luteal regression is induced by the second $PGF_{2\alpha}$ injection (Stevenson et al., 1999). Therefore, synchronization prior to OvSynch can be considered an effective management tool. Prostaglandin $F_{2\alpha}$ treatment prior to OvSynch is termed presynchronization. Moreria et al., (2001) reported that there was no significant difference in pregnancy rates found between controls and presynchronized cows (36.0% versus 36.9%). In another similar study, however, pregnancy rate for presynchronized cows was 42.6% whereas control cows receiving OvSynch initiated at a random stage of the estrous cycle had a pregnancy rate of only 25.3% (Moreria et al, 2000 a)

Somatotropin

Somatotropin and Milk Production

Somatotropin (ST) is a pituitary hormone that is found to control aspects of both growth and nutrition. Somatotropin was first characterized in 1931 when a crude extract from bovine pituitaries demonstrated growth-promoting effects in rats (Evans and Simpson, 1931). Somatotropin was later isolated from the anterior pituitary (Li et al., 1945). Four decades later, in the early 1980's, ST was produced by recombinant DNA technology which allowed for the first study involving this new recombinant technology

to be administered to lactating dairy cows in 1982 (Bauman et al., 1982). The administration of exogenous ST has been shown to increase lactation performance of a range of species from laboratory animals to humans; however, the majority of research has been done with dairy cows. In fact, recombinantly derived bST has been approved for commercial use in 25 countries (Etherton and Bauman, 1998). This is undoubtedly due to its galactopoetic effects. In fact, since the inception of its use in the United States in 1994, an estimated 2 million dairy cows were receiving bST by three years post-approval (Etherton and Bauman, 1998). Bovine ST alters the lactation curve of dairy cows with an immediate increase in milk production (Galton et al., 1997). Typical milk yield responses are increases of 10 to 15% (4 to 6 kg/d), although good animal management and care further increase response (Bauman, 1992). Generally, however, response is negligible in early lactation, so bST is used in the latter 80% of the lactation cycle. Following treatment with bST, milk yield increases for the first few days and maximizes during the first week. Therefore, greater peak milk yield and increased persistency in yield is reached over the lactation cycle (Bauman et al., 1985). Thus bST shifts the lactation curve and extends the lactation period.

Methods of Somatotropin Action

Somatotropin affects many physiological processes which lead to an increased amount of nutrients available for milk synthesis during lactation. The biological effects of ST are both metabolic and somatogenic. The direct effect of somatotropins on tissues and changes in tissue metabolism are essential in increasing milk yield. Somatogenic effects are those in which ST stimulates cell proliferation. These effects are mediated by somatotropin-dependent somatomedins such as insulin-like growth factor-I (IGF-I) whose synthesis is increased by bST. Many of the actions of rbST are believed to be mediated by IGF-I and IGF-II which are secreted from various tissues in response to ST and can have paracrine and autocrine actions. The change in lactation curve due to exogenous bST treatment suggests that bST effects involve both an increase in rates of milk component synthesis per mammary epithelial cell as well as improved secretory cell maintenance (Etherton and Bauman, 1998). The somatogenic effects of ST are evident

within mammary tissue. However, close arterial (external pudic) infusion with bST has no effect on milk yield (Prosser et al., 1994). The detection of ST receptors in mammary tissue has been unsuccessful, although IGF receptors are abundant (Akers, 1985). These two facts strongly support the theory that the somatotropin-dependant somatomedin IGF is the mechanism through which bST increases milk production.

Somatotropin and Reproduction

Somatotropin also affects reproduction. This may be due to the fact that somatotropin increases efficiency by reducing the proportion of nutrients used for maintenance. It is understood that ST causes an increase in the synthesis and secretion of IGF-I and IGF Binding Protein (IGFBP). Insulin-like Growth Factor-I is a growth factor that stimulates growth and development within a variety of cell types, and IGFBP is an IGF carrier protein. The somatomedin hypothesis proposes some of the effects of ST on growth and reproduction are mediated by the release of IGF-I from the liver into the blood. Blood IGF-I has been directly correlated with follicular fluid (FF). Leeuwenberg, et al., (1996) found that the majority of FF IGF-I is derived from the blood. Therefore, it can be assumed that endocrine IGF-I under ST control influences ovarian function through its contribution to FF. Insulin-like growth factor-I and gonadotropins are synergistic for growth and differentiation of the follicle (Spicer and Ectherncamp, 1995). In fact, follicular growth and steroidogenesis are correlated with increased LH secretion as well as greater blood concentration of IGF-I (Spicer and Ectherncamp, 1995). Therefore, ovarian function is most likely dependent on both LH pulsatility and IGF-I concentrations.

Work has also been done with respect to specific gene deletion. Somatotropin and ST receptor gene knockout mice have been shown to be fertile, but with decreased efficiency. Zhou et al., (1997) found ST receptor knockout mice had an average litter size of 2.7 compared to 6.9 of the control mice. Insulin-like Growth Factor-I gene knockout mice showed more severe effects. Follicles grew to the preantral stage but failed to ovulate (Baker et al., 1996). The block to ovulation occurred in untreated mice as well as in mice treated with exogenous gonadotropins to induce ovulation. Extreme doses of gonadotropins induced ovulation in the IGF-I knockout mice, but oocytes were

poor in quality and were not fertilized. In this respect, if ovarian IGF physiology is similar in mice and cattle, ovarian IGF-I may be more important than liver IGF-I. It can be assumed that ST and IGF-I facilitate reproductive processes so that reproductive efficiency is improved.

Somatotropin receptor mRNA has been found throughout bovine reproductive tissues. It was initially localized in granulose cells, and later in the theca cells of the CL, both preantral and antral follicles, oviduct, and endometrium (Kolle et al., 1998). Within the ovarian follicles, preantral ST receptor mRNA was greatest in oocytes, and greatest in the cumulus cells of antral follicles. This suggests a direct effect on reproductive tissues possibly through follicular growth and/or oocyte development (Kirby et al., 1996). The IGF system (receptors, ligands, and binding proteins for both IGF-I and IGF-II) is expressed within granulosa and theca cells, and IGF-I and IGF-II are both shown to cause growth differentiation and survival of follicular cells (Adashi et al., 1998). More importantly, IGF acts synergistically with gonadotropins (FSH and LH). This is governed by the ability of IGF to increase gonadotropin receptor numbers and increase the activity of gonadotropin receptor second messenger systems (Adashi et al., 1998). For example, IGF-I knockout mice are characterized by the underexpression of ovarian FSH receptors (Zhou et al., 1997). Improved fertility has been shown for oocytes matured *in vitro* with bST and to be independent of IGF-I. This was shown by the addition of IGF-I neutralizing antibody, which failed to block the ST effect (Izadyar et al., 1997). Somatotropin dependent ovarian IGF-I synthesis has been shown in the rat, rabbit, pig, and sheep, but not in cattle (Kirby et al., 1996). However, IGF-I mRNA was measured from homogenized ovaries. Therefore, subtle changes between cell types were not detectable.

Bovine somatotropin causes a follicular response in cows. The number of recruited follicles (<10 mm) increased in cows and heifers treated with bST (Kirby et al., 1997 a). Tripp et al., (2000) observed more follicles ultrasonically in bST treated cows ($P < 0.01$), and retrieved 50% more follicles per transvaginal ultrasonographic follicular aspiration from rbST treated cows than for control cows. Recombinant bST changed patterns of the ovarian follicular cycle of cattle by causing premature loss of dominance by dominant follicles, as well as increasing the number of recruited follicles (Kirby et al.,

1997 a). One explanation might be that rbST increases follicular growth as well as a synergistic effect of IGF-I and gonadotropin receptors. *In vivo*, however, ST increased FF IGF-I, but not gonadotropin binding sites (Andrade et al., 1996). Another explanation might be that ST decreases atresia of growing follicles. Cushman et al. (1996) showed that rbST decreased follicular atresia following synchronization with estradiol. Bovine ST alters the duration of dominance as well. Kirby et al. (1997 b) demonstrated the dominance phase of the first wave dominant follicle to be shortened by 2 d in rbST treated cows. This leads to earlier emergence of the second wave dominant follicle. The shift in dominance was also associated with an earlier but lesser midcycle follicle FSH peak. Cows treated with rbST had more class 1 (3 to 5 mm) and class 2 (6 to 9 mm) follicles. In a separate study, Kirby et al. (1997 a) showed rbST treatment to increase the number of class 3 (≥10 mm) follicles as well. Jimenez-Krassel et al. (1999) detected that rbST treatment increased FF concentrations of ST and increased the number but decreased the size of both dominant follicles and corpora lutea, and increased the abundance of IGF binding proteins -2, -3, and -4 in the FF of dominant follicles.

Early embryonic death is considered a main cause for pregnancy failure. This is particularly a concern with repeat breeding cows where 50% of embryos die within 16 d of fertilization (Ayalon, 1978). Early embryonic growth and differentiation are highly dependent on uterine secretions, which are in turn dependent on P_4. Repeat breeding cows are found to have lower P_4 levels 7 d after ovulation (Shelton et al., 1990). Bovine ST may reduce embryonic death. Morales-Roura et al. (2001) found increased P_4 levels ($P < 0.05$) at d 18 following insemination as well as increased conception rates ($P < 0.05$) in rbST treated cows. In addition, IGF-I has been found to stimulate *in vitro* CL P_4 secretion (Sauerwein et al., 1992). This suggests a luteotropic effect of bST. Bovine ST may directly act to improve embryo development as well. Increased IGF concentrations within the uterus and oviducts may stimulate embryonic growth and differentiation (Pershing et al., 2001). Insulin-like growth factor is a stimulant for oocyte maturation and subsequent embryo development. Additionally, supplementation of a culture medium with IGF-I had a beneficial effect on preimplantation embryo development by decreasing apoptosis, or programmed cell death, and increasing cell proliferation (Makarevich and Markkula, 2002). Moreover, embryos from cows treated with bST

transferred to untreated recipient cows increased pregnancy rates (56.1%) compared to transfer of control embryos ($P < 0.01$) (Moreira et al., 2001).

Studies conducted on long-term effects of rbST in cows have shown declines in reproductive performance. Additionally, calving intervals are increased with rbST supplementation. The mean interval from calving to conception increased from 128 ± 16 d for control cows to 246 ± 22 d for those cows receiving 86.0 mg rbST per d (Esteban et al., 1994). Luna-Dominguez et al. (2000) demonstrated a decreased pregnancy rate of 48.5% (49/101) for rbST treated cows compared to that of control cows (65.4%; 68/104). Notwithstanding, administration of rbST at estrus improved the conception rate of repeat-breeding Holstein cows in association with an increase in concentration of P_4. The conception rate of rbST treated cows was 29.3% compared to 16.9% for the controls, demonstrating a significant difference ($P < 0.05$) (Morales-Roura et al., 2001). Bovine ST increases number of days between calving and first estrus. Control cows average 42.1 d versus 53.4 d for rbST treated cows ($P < 0.08$) (Maollem et al., 1997). Pregnancy rates were also reduced by rbST treatment ($P < 0.05$) (Maollem et al., 1997). Recombinant bST may extend the negative energy balance (NEB) of a cow. Negative energy balance is a period when cows fail to consume enough feed to meet increased nutrient demands, and mobilize body resources to meet the deficiency. Rebreeding appears to be delayed by NEB. Increasing milk production with the use of rbST increases NEB, which in turn increases the incidence of diseases such as mastitis, retained placenta, cystic ovarian disease, abortion, and laminitis. This all can be held responsible for a decrease in reproductive performance.

Bovine ST has also been documented to lower estrus detection. Kirby et al., (1997 b) after a norgestimate synchronized estrus found 92% of control cows to have an observed estrus, where as only 39% of the rbST treated cows displayed estrus ($P < 0.05$). A possible method to counter this rbST effect would be to eliminate the need for estrus detection. In order to accomplish this, follicular maturation must be coordinated along with luteal regression. This, in turn, will allow for insemination at one prescribed time as with the aforementioned OvSynch and presynchronization programs.

Somatotropin and Timed Insemination

Synchronization of estrus involving TAI has been used in conjunction with rbST. Specifically, rbST has increased pregnancy rates to a TAI protocol. Moreira et al. (2000 a) treated lactating Holstein cows with rbST at 63±3 d PP ancillary with initiation of OvSynch. Analysis of pregnancy rates to the first synchronized service were diagnosed by ultrasonography at 27 d following insemination and proved a positive main effect of rbST treatment ($P < 0.08$). Additionally, re-examination pregnancy rates at 45 d post insemination were higher for rbST treated cows than that of control cows. In a second experiment conducted by Moreira et al. (2001), rbST was used in conjunction with presynchronization. Treatment cows again received an injection of rbST at 63±3 d PP. An additional treatment group received rbST at 73±3 d PP. OvSynch was started at 63±3 d PP for all groups. Furthermore, all groups were presynchronized with two $PGF_{2\alpha}$ injections 14 d apart, with the second injection administered 12 d prior to the initiation of OvSynch. Pregnancy rates for cows cycling at 63±3 d PP at 32 and 74 d post insemination were increased to show an interrelation of rbST and presynchronization ($P < 0.01$). However, rbST treatment had no effect when cows were not presynchronized.

Early Conception Factor

Identification of non-pregnant dairy cows shortly after insemination is of great importance. Re-breeding an open cow as early as possible can improve reproductive efficiency and pregnancy rate by simply shortening the time between services and thereby increasing the service rate. Available techniques for pregnancy detection in cattle include the hormonal assay for milk P_4, as well as transrectal palpation and ultrasonography. Cows have been reported as non-pregnant with an accuracy of 97.2% using milk progesterone tests between d 18 and 24 following AI (Pennington et al., 1985). Although effective, these methods are performed following implantation. This does not allow for differentiation between fertilization failure and early embryonic loss. Furthermore, early embryonic loss can be exasperated by incorrect diagnosis of the open status, following which $PGF_{2\alpha}$ may be administered to synchronize estrus or ovulation for another AI. On the other hand, incorrect diagnosis of pregnancy or non-open status will

not be effective in presenting non-pregnant cows for re-insemination. A new test for open status has become available commercially for use in cattle. The ECF test kit (Concepto-Diagnostics Inc., Knoxville, TN) reportedly detects the open status of a cow within 6 to 10 d post insemination (Concepto-Diagnostics, 2001).

The ECF test kit reportedly detects a conception or pregnancy-related glycoprotein. This protein is detectable in the maternal host and is known as early pregnancy factor (EPF). Early pregnancy factor is an immunomodulator that may help prevent immunologic rejection of the embryo by the maternal host, and is found to be present as early as 24 h following insemination and persists throughout pregnancy (Morton, 1984). Nancarrow et al. (1981) first detected EPF in both sheep and cattle by using the rosette inhibition assay. Two components comprise EPF: EPF-A and EPF-B. Both components must be present to detect EPF using the rosette inhibition assay. The oviduct produces EPF-A during proestrus and estrus (Koch et al., 1983). Early pregnancy factor-B, otherwise known as zygotin factor, is thought to be released from the oocyte following penetration by spermatozoa as well as from the developing embryo (Threfall, 1994). The detection of EPF may be useful in diagnosing early pregnancy due to the fact that significant differences in rosette inhibition titers were observed between pregnant and non-pregnant cows on d 13 to 16 post AI (Sakonju et al., 1993).

A glycoprotein immunosupressive early pregnancy factor (ISEPF) with a molecular weight of approximately 200,000 g/mol was isolated from the serum of pregnant cows by extraction and purified using chromatography (Threfall, 1994). The purified antigen was administered to two-year-old mixed breed goats for the purpose of polyclonal antibody production. Of the 132 clones produced in the study, two were selected for their specificity to ISEPF. The production of these antibodies led to the development of the ECF lateral flow dipstick test using monoclonal and polyclonal antibodies and antibody-gold conjugate to indicate the presence of EPF (Threfall, 1998). Only a handful of studies have compared ECF test results to pregnancy diagnosis by palpation and ultrasound with conflicting results. Threfall (1994) showed only a 12.5% rate of false negative results for sera collected within 24 h of AI. Threfall (1998) again proved the ECF test to be 94.5% accurate in diagnosing non-pregnancy 24-48 h following breeding. When cows were tested 3 to 7 d post AI, a misdiagnosis by ECF of 48.8% of

cows diagnosed pregnant by rectal palpation was shown (Adams and Jardon, 1999). Cordoba et al. (2001) tested cows for open-status 6 d after AI. A 14% and 96% incidence of false negatives and false positives were found, respectively. Another concern is the effectiveness of the ECF test with the use of milk. When ECF results using both serum and milk were compared, a significant difference was found ($P < 0.05$) (Gandy et al., 2001). False negatives were found to be 10.5% and 31.8% for serum and milk, respectively. Additionally, ECF agreement with confirmed pregnancy rates were only 52.0% and 45.0% for serum and milk, respectively (Gandy et al., 2001).

Chapter 3. Objectives

This experiment focused on the effects of rbST treatment on the reproduction parameters of lactating Holstein cows in a commercial setting. The primary objective of this study was to determine the effects of rbST treatment on first service conception rates for cows treated with a presynchronized TAI protocol. A second objective of this study was to determine the efficacy of the ECF test in a commercial setting using milk stored at either 5 or -25ºC.

Chapter 4. Materials and Methods

Study Population

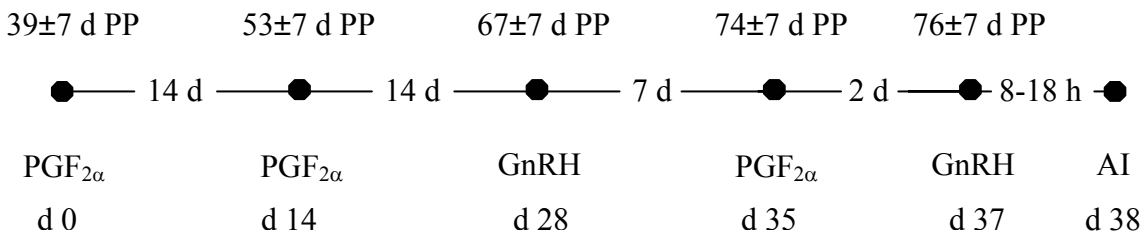
This experiment was conducted from the months of October, 2001 through March 2002 in three commercial dairy herds of lactating Holstein cows. The dairies were Huckleberry Dairy, Floyd, VA (Farm 1), Ingleside Farms, Lexington, VA (Farm 2), and Triple R Farms, Crewe, VA (Farm 3). The selection of cows consisted of 88 from Farm 1, 60 from Farm 2, and 68 from Farm 3 comprising a total of 216 cows. Cows were housed in free stall barns and fed according to the management protocols of each farm. Cows were milked twice daily at each location.

Study Design

Primiparous and multiparous cows were randomly assigned at 39 ± 7 d PP to one of two treatment groups with an equal distribution of parity throughout each group.

Every 14 d a new synchronization cluster was started. Control cows (n = 116) and treatment cows (n = 127) utilized the presynchronization protocol for first service TAI. Cows were administered 25 mg $PGF_{2\alpha}$, (i.m., 5 ml of Lutalyse sterile solution; Pfizer Animal Health, New York, NY) 14 d apart at 39 ± 7 d PP (d 0) and 53 ± 7 d PP (d 14). All cows were then administered 100 μg GnRH, (i.m., 2 ml of Cystorelin; Merial Limited, Iselin, NJ) 14 d following the second $PGF_{2\alpha}$ administration on d 28 (67 ± 7 d PP). On d 35 (74 ± 7 d PP), 25 mg $PGF_{2\alpha}$ was administered followed by 100 μg of GnRH 48 h later on d 37 or 76 ± 7 d PP (Figure 4.1). Randomly, every other cow on d 28 of the protocol received 500 mg rbST, subcutaneously (Posilac; Monsanto Inc., St. Louis, MO).

Figure 4.1. Presynchronization hormone treatment protocol.



Artificial insemination was performed by appointment on d 38 of the protocol, 8 to18 h following the final dose of GnRH. Herd personnel normally in charge of the breeding program performed AI, and proven service sires were chosen by farm management. The corresponding herd veterinarian diagnosed pregnancy between 35 and 49 d following AI by uterine examination via the rectum.

Determination of Cyclicity

Cycling status was determined during the synchronization protocol by milk samples collected for P_4 assay. Specifically, milk samples were collected at the time of the second $PGF_{2\alpha}$ administration (d 14 of PreSynch protocol), at the first GnRH injection (d 28), again on d 35 with the third $PGF_{2\alpha}$ administration, and finally on d 45

corresponding to 7 d post-insemination. Samples were stored at –20° C until assayed for P_4 using a solid-phase RIA (Coat-a-Count Progesterone, DPC, Diagnostic Products Corporation, Los Angeles, CA) with a standard curve, references and duplicates as previously described (Nebel et al., 1988). The sensitivity of the assay was 0.02 ng/ml of P_4 using the standard 3 h incubation period, at room temperature. The antiserum was highly specific for progesterone with a particularly low crossreactivity to other naturally occurring steroids. Intra-assay coefficient of variation was 8.7%. Due to the fact that some milk samples were missing, not all experimental cows were analyzed for cyclicity. A total of, 173 cows (71% of total) were analyzed for cyclicity.

Cows were classified as cyclic or anestrous at initiation of timed AI based on milk P_4 concentrations of samples collected at d 67± 7 and 74 ± 7 PP. Anestrous was classified as a cow with less than 1.0 ng/ml P_4 for both samples. Cows with at least one sample whose P_4 concentration was greater than 1.0 ng/ml were considered cycling.

Early Conception Factor Assay

Milk samples were collected from cows 7 d post-insemination. Milk samples were stored at –20° C (n = 214) or 5° C (n = 113). Samples stored at 5° C had a milk preservative added to the vial before collection (Bronolab-W, D&F Control Systems, CA). Prior to P_4 analysis, all milk samples were ultrasonically homogenized using a cell disruptor tuned to 20 KHz (Sonicator cell disruptor; Heatsystems-Ultrasonics Inc., Plainview, NY) to re-suspend milk fat. Frozen milk was allowed to thaw completely, then vortexed, so that any sediment collected at the bottom of the sample tube was completely resuspended. Samples were then permitted to equilibrate to room temperature (~23ºC).

The ECF test kits were from the same manufacturing lot obtained directly from Concepto-Diagnostics (Knoxville, TN). All ECF test kit components and buffer wash were stored at 5° C until used. Each sample was evaluated by one technician as per the manufacturer's instructions (Concepto-Diagnostics, 2002). Briefly, the ECF test was performed by adding one drop of sample, to the nitrocellulose membrane through the sample cup on the ECF cassette. Immediately following, two drops of buffer solution was added. The cassettes were then incubated at room temperature for 2 h and the results

were recorded. A positive test resulted in two red lines, and a negative test resulted in only one red line.

Statistical Analysis

rbST

Data analyses were performed using the logistic regression analysis procedure (Proc Logistic, SAS System). The mathematical model used included effects of rbST treatment, month of first service, parity (i.e. primiparous or multiparous), peak milk yield, cycling status at 67 ± 7 d PP, and all higher order two-way interactions. However, no significant two-way interactions were revealed and were excluded form the model. The effect of P_4 7 d post insemination was not significant and was subsequently removed from the model. It was determined that a sample at this time was not an accurate evaluation of ovulation following synchronization. A 1.0 ng/ml P_4 cutoff for determination of cyclicity may be low with respect to levels of P_4 normally existing 7 d following ovulation. It is possible that a cow ovulated in response to synchronization but the corresponding CL has not yet matured and therefore is producing P_4 at a level lower than 1.0 ng/ml. Further analyses were conducted separating cyclic from anestrous cows.

ECF

Crosstabulation 2X2 tables were constructed for ECF test results and conception rates as determined at 42 ± 7 d after AI (Proc Freq, SAS System) for use in the calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy. Test sensitivity was calculated as the proportion of cows determined to be pregnant at 42 ± 7 d after AI that produced positive ECF test results (number of true positive results / [number of true positive results + number of false negative results]). Test specificity was calculated as the proportion of non-pregnant cows that produced negative ECF test results (number of true negative results / [number of true negative results + number of false positive results]). The PPV of the test was calculated as the probability that a positive ECF test was the result of a pregnant cow (number of true positive results / [number of true positive results + number false positive results]). The NPV of the test was calculated as the probability that a negative ECF test was the result of a non-pregnant cow (number of true negative results / [number of true negative

results + number of false negative results]). The accuracy of the ECF test was calculated as the probability of correctly identifying the conception status of the cow using the ECF test kit (number of true positive and negative results / [number of false positive + false negative results]). The rate of false positive results was calculated as the number of cows predicted positive by the ECF test that were actually open out of the entire population of experimental cows. The rate of false negative results was calculated as the number of cows predicted negative by the ECF test that were actually pregnant out of the entire population of experimental cows. A similar mathematical model to that used to determine the effect of rbST treatment was used to determine factors affecting ECF test results. This model included all the same variables as before with the addition of storage type. No higher order interactions were significant and therefore removed form the model. Differences were considered significant at a probability value of 0.05 or less.

Chapter 5. Results and Discussion

Recombinant bST Supplementation

First service conception rates for rbST treated cows (n = 113) and control cows (n = 103) were not different. Conception rates for treated and control cows were 40.7 and 44.7%, respectively. The main effects of farm, lactation, cyclicity, peak milk yield and month of first service did not affect conception rates (Table 5.1).

Table 5.1. Logistic regression for effects of farm, rbST treatment, lactation, cycling status at 67 ± 7 d PP, ovulation as determined by level of milk P_4 42 ± 7 d after insemination, month of first service, and peak milk yield on conception rates for cows in a presynchronized TAI protocol.

Category		N	Conception Rate (%)	Odds Ratio[1]	95% Confidence Interval[2]
Farm	3	68	35.3	1.00	…..
	1	88	40.9	1.75	(0.66, 4.65)
	2	60	53.3	1.77	(0.63, 4.95)
rbST treatment					
	rbST	113	40.7	1.00	…..
	control	103	44.7	1.02	(0.52, 2.00)
Lactation					
	multiparous	123	45.5	1.00	…..
	primiparous	64	42.2	0.92	(0.41, 2.07)
Cyclicity					
	cyclic	123	44.7	1.00	…..
	anestrous	51	39.2	0.59	(0.28, 1.27)
Month of First Service					
	October-02	34	47.1	1.00	…..
	November-02	46	39.1	0.68	(0.22, 2.11)
	December-02	38	39.5	0.85	(0.23, 3.17)
	January-03	52	40.4	0.41	(0.12, 1.18)
	February-03	26	42.3	0.33	(0.07, 1.52)
	March-03	20	55.0	0.96	(0.28, 3.32)
Peak Milk Yield					
	≤74 lbs	23	47.8	1.00	…..
	75-92 lbs	81	42.0	0.73	(0.25, 2.17)
	93-110 lbs	56	44.6	0.63	(0.19, 2.09)
	≥111 lbs	24	50.0	0.62	(0.15, 2.54)

[1]Odds Ratio is the estimated odds of conceiving for a cow in a particular category relative to the baseline category for that variable. Odds ratio: 1, no effect on conception; >1, increased probability of conception; and <1, decreased probability of conception compared with the baseline category.
[2]When Confidence interval encompasses 1, the odds ratio is not significant.

There was no significant interaction of rbST treatment with peak milk yield. Additionally, there were no further significant higher order interactions. Conception rates for all experimental cows according to farm and treatment group are included in Table 5.2.

Table 5.2. Conception Rates on 42 ± 7d after first service timed insemination for all experimental cows according to farm and treatment group.

	Conception Rates			
	Control		rbST treated	
Farm	**n**	**%**	**n**	**%**
1	**44**	**43.2**	**44**	**38.6**
2	**30**	**53.3**	**30**	**53.3**
3	**29**	**37.9**	**39**	**33.3**
Total	**103**	**44.7**	**113**	**40.7**

Cyclicity was determined from milk P_4 levels on d 14 and 28 of the presynchronization protocol directly corresponding to 53 ± 7 and 67 ± 7 d PP. Due to incomplete sampling and incorrect sampling procedures, only a portion (n = 173) of the original 216 experimental cows was classified as cyclic or anestrus. Cows with P_4 concentrations of <1.0 ng/ml on days 53 ± 7 and 67 ± 7 d PP were considered to be anestrus (quiescent ovaries or anovulatory follicles). Overall, 29.3% of the cows had not started cycling by 67 ± 7 d PP. There was no significant difference among conception rates determined for cyclic and anestrus cows. The conception rate for cyclic cows was 44.7%. Anestrous cows had a 39.2% rate of conception. Frequency of anestrus was greater for multiparous 66.0% than primiparous cows 34.0%. However, there was no significant difference in conception rates by parity, nor was there an interaction between rbST treatment and parity. No significant difference in frequency of anestrus was found among treatment groups. Moreover, there was no interaction between rbST treatment and cycling status. However, it has been previously determined that rbST treatment only

significantly affects conception in those cows that are cycling (Moreira et al., 2001). Therefore, first service conception rates were evaluated for cows sorted by cycling status. A total of 63 of those cows treated with rbST were classified as cyclic. Of the control cows, 60 were determined to be cyclic. When conception rates for cycling cows were determined by uterine examination at 42 ± 7 d post insemination, 46.0% of the rbST treated cows had conceived. Control cows determined as cycling had a 43.3% conception rate. Among cows classified as anestrous, the conception rate for rbST treated cows was 36.0%, and that for control cows was 42.3%. The conception rates analyzed by cycling status are included in Table 5.3.

Table 5.3. Conception rates on 42 ± 7 d after first service timed insemination for those cows determined to be anestrous or cycling at the initiation of the timed insemination protocol (63 ± 7 d PP).

Cycling Status	Treatment Group	n	Conception Rate
Anestrous	Control	26	42.3
	rbST	25	36.0
Cyclic	Control	60	43.3
	rbST	63	46.0

It is improbable that an anestrous cow became pregnant following insemination. Therefore, it must be determined why anestrous cows had a CR not unlike that of cyclic cows. Cows in anestrus that possess a dominant follicle following treatment with two $PGF_{2\alpha}$ administrations will ovulate is response to an injection of GnRH. The administration of GnRH will induce a surge of LH, causing ovulation and subsequent leuteinization of the dominant follicle present at the time of treatment. Ovulation of the dominant follicle would allow for an endogenous surge of FSH to initiate the recruitment of a new follicular wave. Therefore, a second dominant follicle would likely be present

at the time of the second GnRH administration of the OvSynch protocol. This protocol alone may result in the high CR for cows determined to be acyclic before they began the OvSynch protocol.

In a previous study, involving the use of rbST, in accordance with a TAI protocol rbST was shown to increase conception rates (Moreira et al., 2001). However, in their study, rbST treatment only had a significant effect on conception rates of those cows determined to be cycling at the initiation of the TAI protocol. In all, 77.0% of the cows were cycling and 23% were classified as anestrous at the initiation of the first service timed insemination protocol. In fact, Moreira et al. (2001) found that the conception rate for non-cyclic cows was only 24.6%, significantly lower ($P < 0.01$) than the 50.1% conception rate found for cyclic cows. When the investigators considered the effects of rbST treatment among only cyclic cows, presynchronized cows receiving rbST at the initiation of OvSynch had a conception rate of 67.8%. However, presynchronized cows that did not receive rbST had a significantly lower ($P < 0.01$) conception rate (46.9%). In addition, rbST treatment increased ($P < 0.01$) first service conception rates regardless of presynchronization. Cows synchronized with OvSynch alone receiving rbST treatment 63 ± 7 d PP had a conception rate of 39.1% (Moreira et al., 2001).

Similar conception rates to those found for presynchronization cows in the present study have been demonstrated by Moreira et al. (2000 a) and Peters et al. (2002). These conception rates were 42.6 and 41.5% respectively. Moreira et al. (2000 a) reported an increase in conception rates for all rbST treated cows. In their study, cows began the OvSynch TAI protocol at 63 ± 7 d PP, with treated cows receiving an injection of rbST at 63 ± 7 d PP concomitantly with the initiation of OvSynch. Analysis of pregnancy rates to the first synchronized service as diagnosed by ultrasonography at 27 d after AI detected a main effect of rbST treatment ($P < 0.08$) with control cows presenting a conception rate of 29.6% whereas the conception rate of cows treated with rbST was 46.9% (Moreira et al., 2000 a). Similarly, conception rates diagnosed by rectal palpation at 45 d after insemination indicated an effect of rbST treatment ($P < 0.10$) with the conception rate for control cows being 22.1%, and 32.7% for rbST treated cows (Moreira et al., 2000 a).

It is theorized that rbST treatment increases conception rates through several mechanisms. Recombinant bST increases production of IGF-I in the liver and therefore,

levels in the peripheral blood. Increased IGF-I has also been detected within the ovarian follicle where IGF-I receptors are present (Spicer and Echternkamp, 1995). Therefore, rbST may affect follicular growth and development. In fact rbST treatment has altered the follicular dynamics of cattle, increasing the number of class 1 (3 to 5 mm) and class 2 (6 to 9 mm) follicles (Kirby et al., 1997 b). In addition, the administration of rbST also accelerated the emergence of a second follicular wave by 48 h (Kirby et al., 1997 a). These effects suggest an effect before the time of AI. However, Moreira et al. (2001) determined the effects of rbST must occur after AI by showing that conception rates did not differ between cows receiving rbST at the initiation of a TAI protocol and those receiving treatment at AI. Administration of rbST may enhance CL development and P_4 production post insemination. Levels of P_4 as well as conception rates have been increased in rbST treated cows. Morales-Roura et al. (2001) found P_4 levels increased at d 18 following insemination with rbST treatment in addition to increased conception rates. Treatment with rbST may also decrease early embryonic death and improve embryo development following insemination. Pershing et al. (2001) detected increased IGF concentrations within the uterus and oviducts following treatment with rbST. Specifically, exogenous rbST induces oviductal IGF-II mRNA as early as d 3 of a synchronized estrous cycle. This supports a role of IGF-II in oviductal differentiation and early embryonic development. At d 7 of the synchronized estrous cycle, rbST down regulated IGF-II transcripts in the endometrium when IGF-I mRNA increased (Pershing et al., 2002). This suggests distinct or overlapping roles in the control of oocyte cleavage and subsequent blastocyst development. Moreover, rbST and IGF-I receptors have been identified at different stages of embryonic development, and IGF-I stimulated blastocyst development *in vitro* (Palma et al., 1997). Thus increased plasma concentrations of IGF-I may enhance embryonic growth and development. It has been observed that the addition of growth hormone (i.e. rbST) during maturation of cultured cumulus oocyte complexes accelerates nuclear maturation, induces cumulus expansion, and results in a subsequent increase in embryonic development (Izadyar et al., 1996). Further research determined that growth hormone enhanced developmental competence of the oocyte leading to a higher fertilization rate (Izadyer et al., 1997).

Previous studies involving the use of rbST in conjunction with a TAI protocol have evaluated treatment effects with continued rbST treatment every 14 d following its initiation and shown rbST to increase CR (Moreira et al., 2001, Moreira et al., 2000 a). In the present study, rbST treated cows only received one dose of the drug at the initiation of the TAI protocol (67 ± 7 d PP). This may account for the deviation in results of the present study to those of previous experiments. Continued treatment with rbST may allow for systemically increased levels of IGF and ST required to increase CR as well as early embryonic growth and development. One dose may be sufficient to increase conception, but may not be adequate for further support of embryonic growth and development required for a viable pregnancy.

Conclusion

First service conception rates for cows presynchronized with two administrations of $PGF_{2\alpha}$ prior to OvSynch were not affected by treatment with rbST. Conception rates for treated and control cows were 40.7 and 44.7% respectively. Incidence of anestrous did not significantly influence overall conception rates. However, considering all cyclic cows, conception rate was 44.7% where as conception rate for anestrous cows was 39.2%. When only cyclic cows were considered, rbST treatment resulted in a conception rate of 46.0%. Cycling control cows had a conception rate of 43.3%. Overall, one administration of rbST at 67 ± 7 d PP in coordination with a TAI within this study, did not significantly affect first service conception rate.

Early Conception Factor

The efficacy of the ECF test kit was analyzed using milk samples from cows used in the rbST study. Each sample used in an ECF test kit was collected 7 d post insemination and stored either at -20^{o}C or at 5^{o}C. The total number of samples for each storage method does not coincide due to a change in experimentation that required samples to be refrigerated in addition to frozen. It was not the original objective of the experiment to compare ECF test results between frozen and refrigerated milk samples. Storage of milk samples at 5^{o}C did not occur until after sampling started. Milk (~5ml) from 216 cows was frozen for ECF analysis. Duplicate samples were collected from 113

cows and were refrigerated in addition to being frozen. The validity of each ECF test kit was based on the presence of a reddish colored line located in the control region of each test cassette as determined by the reader by the end of a two hour incubation period.

The comparison of uterine examination by rectal palpation and ECF test results are shown in Table 5.4. The effects of farm, rbST treatment, lactation, cyclicity at 67 ± 7 d PP, and peak milk yield did not significantly influence ECF assay results (Table 5.5).

Table 5.4. ECF test results for frozen and refrigerated samples collected on d 7 following first service insemination versus pregnancy status determined by uterine examination by rectal palpation (42 ± 7 d post AI).

Storage Type	ECF Test Result	Pregnancy Status by Return to Estrus or Palpation		Total
		Pregnant	Non-Pregnant	
Frozen	**ECF Positive**	**60**	**78**	**138**
	ECF Negative (open)	**32**	**46**	**78**
	Total	**92**	**124**	**216**
Refrigerated	**ECF Positive**	**43**	**46**	**89**
	ECF Negative (open)	**8**	**16**	**24**
	Total	**51**	**62**	**113**

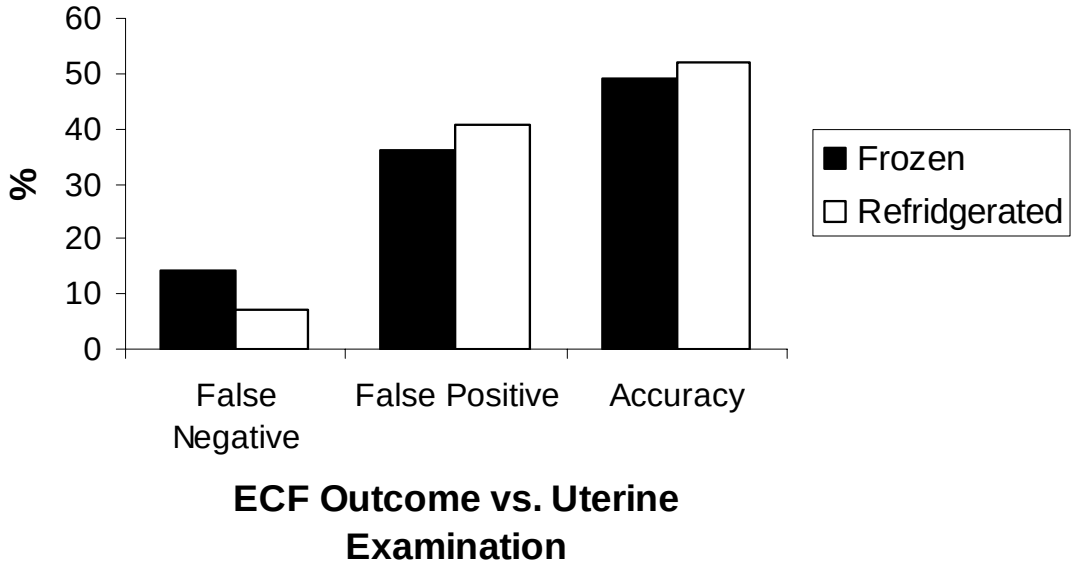
Table 5.5. Logistic regression for effects of farm, rbST treatment, lactation, cyclicling status at 67 ± 7 d PP, ovulation as determined by level of milk P_4 42 ± 7 d after insemination, storage, and peak milk yield on ECF "open" results for cows in a presynchronized TAI protocol.

Category		N	Open ECF Results (%)	Odds Ratio[1]	95% Confidence Interval[2]
Farm	3	101	34.7	1.00	…..
	1	130	43.1	0.63	(0.31, 1.30)
	2	98	11.2	0.17[3]	(0.07, 0.41)
rbST treatment					
	rbST	176	30.1	1.00	…..
	control	153	32	1.38	(0.76, 2.49)
Lactation					
	multiparous	184	31.5	1.00	…..
	primiparous	107	34.6	1.07	(0.55, 2.09)
Cyclicity					
	cyclic	183	29.0	1.00	…..
	anestrous	87	32.2	1.13	(0.60, 2.12)
Storage					
	5°C	216	36.1	1.00	…..
	-25°C	113	21.2	0.53[3]	(0.28, 0.99)
Peak Milk Yield					
	≤74 lbs	37	16.0	1.00	…..
	75-92 lbs	122	44.7	0.71	(0.28, 1.75)
	93-110 lbs	86	29.8	0.58	(0.21, 1.61)
	≥111 lbs	40	9.6	0.59	(0.17, 1.97)

[1]Odds Ratio is the estimated odds of a open result of an ECF assay in a particular category relative to the baseline category for that variable. Odds ratio: 1, no effect on ECF open result; >1, increased probability of ECF open result; and <1. decreased probability of ECF open result compared with the baseline category.
[2]When Confidence interval encompasses 1, the odds ratio is not significant.
[3]$P < 0.05$

A novel aspect of the present study is that frozen milk samples were used in the ECF assay. It was thought that frozen milk samples could be beneficial if retesting was required at a later date, or if the samples were required to be shipped for diagnostic testing elsewhere. Using frozen milk samples from a total of 216 cows, the ECF assay predicted conception for 63.9% of the cows. The ECF assay classified 32 of the cows diagnosed pregnant by palpation to be "open" or non-pregnant, translating to a 14.8% rate of false negative results. In contrast, 124 cows were diagnosed open by uterine examination. The ECF assay classified 78 of the total 216 cows to have conceived. This translates to a 36.1% rate of false positive ECF test results using frozen samples. The rates of false positive and false negative results are displayed in Figure 5.1.

Figure 5.1. Rates of false negative, false positive and accuracy of ECF tests compared to uterine evaluation by rectal palpation at 42 ± 7 d post insemination. (Frozen: n = 216; and refrigerated: n = 113).



The ECF predicted conception rate differed from that determined by palpation by 34.8%. The sensitivity, specificity, PPV, NPV and accuracy of the test for frozen milk samples are included in Table 5.6.

Table 5.6. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for Early Conception Factor (ECF) tests results.

Storage	N	Sensitivity[1]	Specificity[2]	PPV[3]	NPV[4]	Accuracy[5]
Frozen	**216**	**65.2 (60/92)**	**37.1 (46/124)**	**43.4 (60/138)**	**59.0 (46/78)**	**49.1 (106/216)**
Refrigerated	**113**	**84.4 (43/51)**	**25.8 (16/62)**	**48.3 (43/89)**	**66.7 (16/24)**	**52.2 (59/113)**

[1]Proportion of milk samples from pregnant cows with a positive ECF result.
[2]Proportion of milk samples from non-pregnant cows with a negative ECF result.
[3]Probabality that a positive test is the result of a pregnant cow.
[4]Probability that a negative test is the result of a non-pregnant cow.
[5]Probability of correctly identifying pregnancy status.

The test sensitivity, which measures the ability of the test to correctly identify pregnant animals, was 65.2%. By contrast, the specificity of the test, which measures the test's ability to correctly identify open animals, was 37.1%. Herein lies the true ability of the ECF test. The PPV, which determines the probability that a positive ECF test result is from a cow that conceived was 43.4%. The probability that a negative ECF test was the result of an open cow, known as the NPV, was 59.0%. The accuracy, or probability of the test to correctly identify conception status, was 49.1%.

The ECF assay using refrigerated samples predicted conception to be 78.8% when compared to uterine examination at 35 to 49 d post AI. A total of 51 of the 113 total cows whose milk was refrigerated were diagnosed as pregnant by rectal palpation. However, the ECF assay determined 8 to be open resulting in a 7.1% incidence of false negative results. In contrast, 46 of the 62 cows diagnosed open by return to estrus or palpation were classified as having conceived by the ECF test. This resulted in a 40.7% incidence of false positive results. The ECF predicted conception rate determined using refrigerated milk samples differed from the palpation conception rate by 15.7%. The sensitivity, specificity, PPV, NPV, and accuracy of the refrigerated milk ECF test was 84.3, 25.8, 48.3, 66.7, and 52.2% respectively (Table 5.6). Overall, frozen and

refrigerated milk samples used in the ECF assay differed by 14.9% ($P < 0.05$) when determining conception.

In a previous study, Cordoba et al. (2001) precluded the possibility of false positive results by using non-inseminated cows and heifers as an unequivocal source of open cows. The researchers also used cows from which at least one embryo, of transferable quality, was retrieved as an unequivocal source of pregnant cows. Blood sera were collected 6 d after estrus from the inseminated cows. An incidence of 14% false negative results and a 4% incidence of false positive results were reported (Cordoba et al., 2001). Gandy et al. (2001) used cows synchronized with a modified OvSynch protocol. Milk samples were taken between 3 and 30 d post AI. The investigators determined that milk taken at d 9 post AI predicted an 82.5% conception rate, which agreed with the actual pregnancy rate by only 52.5%. The same study also determined rates of false negative and false positive results to be 15.8 and 84.2% respectively. The ability of the ECF assay to accurately predict nonpregnant cows between 3 and 30 d post AI varied greatly from 20 to 70% (Gandy et al., 2001).

High rates of false positive results for both milk storage temperatures and those found by previous investigators may be attributed to early embryonic death. Early embryonic loss is associated with the time between milk sample collection and uterine examination. Ayalon (1978) determined that 50% of embryos die within 16 d of fertilization. This is well after samples for the ECF assay were collected (7 d post AI). Adams and Jardon (1999) reported that 49% of rectally diagnosed pregnant cows were misdiagnosed as open by the ECF test. The positive predicted value of the test was also low at 37% (Adams and Jardon, 1999). Therlfall (1998) sampled blood sera of 436 cows between 24 and 48 h after insemination. All cows were examined for pregnancy status 32 to 45 d following breeding. A low incidence of false negatives (5.5%) was reported, although there was a high incidence of false positives among open cows (53.6%).

When conception rate as determined by palpation at 42 ± 7 d following first service insemination was included in the mathematical model, no significant difference was seen between it and the ECF predicted conception rate. Furthermore, month of first service, parity, peak milk yield, and cycling status did not have a significant effect on ECF assay prediction of open cows. However, farm did have a significant effect ($P <$

0.05) on ECF assay results. The conception rates predicted by ECF test were 56.3 (Farm 1), 88.8 (Farm 2), and 66.0% (Farm 3). This may be explained by differing management procedures among herds; specifically, the time at which cows were inseminated following synchronization. Dalton et al. (2001) demonstrated that variation in the time of AI between 0 and 12 h following estrus held a significant effect on fertilization rates and accessory sperm numbers.

The method of storage for the milk samples had a significant ($P < 0.05$) effect on ECF predicted conception rate. This difference may be attributed to the effects of freezing on the EPF protein. The protein may be sheared or degraded, its bonds changed or broken causing the structure to be altered. Following freezing and thawing, the protein may also clump together with other proteins, rendering them indiscernible by the ECF assay.

Conclusion

The specificity of the ECF assay differed between 37.1 and 25.8% for frozen and refrigerated samples, respectively. This difference among specificity indicates that some variability exists in the ECF assay when using frozen versus refrigerated milk. Additionally, the low specificity observed indicates the failure of the test to accurately identify non-pregnant cows. A false positive result of the ECF test rules out the possibility of recycling a cow. In essence, high rates of false positive results correlate to the number of open cows missed by the ECF assay that could be recycled and rebred. The commercially available ECF test kit is marketed as a test for use in the identification of the open cow between 6 to 20 d post breeding. This would allow for a non-pregnant cow to be recycled with administration of $PGF_{2\alpha}$. However, a false negative ECF test suggests non-pregnancy when in actuality, conception had occurred. Therefore such a cow would receive $PGF_{2\alpha}$ causing the abortion of the developing embryo. False negative ECF test results for frozen and refrigerated samples were (14.8 and 7.1% respectively). Thus, using refrigerated samples for ECF analysis of open status was 92.9% accurate. This is close to the advertised 94 to 95% rates of early detection of open cows (Progressive Dairyman, 2004). This accuracy was acceptable for the commercially developed test for the open status of dairy cows.

Reference List

Adams, C.S., and P.W. Jardon. 1999. Evaluation of the early conception factor (ECF™) test in cows 3-7 days post-breeding. Proc. Am. Assoc. Bov. Pract. 32: 240-241.

Adashi, E.Y. 1998. The IGF family and folliculogenisis. J. Reprod. Immunol. 39:13-19.

Akers, RM. 1985. Lactogenic hormones: binding sites, mammary growth, secretory cell differentiation, and milk biosynthesis in ruminants. J. Dairy Sci. 68:501-19.

Andrade, E.Y., S.M. Rhind, I.A. Wright, S.R. McMillen, P.J. Goddard, and T.A. Bramley. 1996. Effects of bovine somatotropin (bST) on ovarian function in postpartum beef cows. Reprod. Fertil. Devel. 8:951-960.

Austin, E.J., M. Mihm, M.P. Ryan, D.H. Williams, and J.F. Roche. 1999. Effect of duration of dominance on the ovulatory follicle on onset of estrus and fertility in heifers. J. Anim. Sci. 77:2219-2226.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fertil. 54:483-493.

Bauman, D.E., J.H. Eisemann, and W.B.Currie 1982. Hormonal effects on partitioning of nutrients for tissue growth: role of growth hormone and prolactin. Fed Proc. 41:2538-44.

Bauman, D.E., M.J. Degeeter, C.J. Peel, G.M. Lanza, R.C. Gorewit, and R.W. Hammond. 1982. Effect of recombinantly derived bovine growth hormone (bGH) on lactational performance of high yielding dairy cows. J. Dairy Sci. 65:Suppl. 1: 21.

Bauman, D.E., P.J. Eppard, M.J. DeGeeter, and G.M. Lanza. 1985. Responses of high-producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci. 68:1352-62.

Bauman, DE. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432-51.

Burke, J.M., R.L. de La Sorta, C.A. Risco, C.R. Staples, E.J-P. Schmitt, and W.W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. J. Dairy Sci. 79:1385-1393.

Call, E.P. 1978. Economics associated with calving intervals. Large Dairy Herd Mgt. C.J. Wilcox and H.H. Van Horn, ed. Univ. Presses Florida, Gainesville. 190-201.

Casida, L.E. 1961. Present status of Repeat breeder cow problem. J. Dairy Sci. 44:2323-2329.

Collier, R.J., J.C. Byatt, T. Curran, P.J. Eppard, A.C. Fabelar, R.L.Hintz, R. Hoffman, M.M. McCrate, C.L. McLlaughlin, R.H. Sorbet, and J.L. Vicini. 1997. Post-approval evaluation of Posilac bovine Somatotropin in 28 commercial dairy herds. J. Dairy Sci. 80 (Suppl. 1):169.

Concepto Diagnostics. 2001. Early conception factor (ECF) assay kit for cattle, product insert. Concepto Diagnostics, Inc., Knoxville, TN.

Cushman, R.A., J.C. DeSouza, V.S. Hedgpeth, and J.H. Britt. 1996. Alteration of bovine folliculogenesis by long term treatment with estradiol (E2) and bovine somatotropin (bST). J. Anim. Sci. 74 (Suppl. 1):220(Abstr.).

Dalton, J.C., S. Nadir, J. H. Bame, M. Noftsinger, R. L. Nebel, and R. G. Saacke. 2001. Effect of Time of Insemination on Number of Accessory Sperm, Fertilization Rate, and Embryo Quality in Nonlactating Dairy Cattle. J. Dairy Sci. 84:2413–2418.

Dransfield, M.B., R.L. Nebel, R.E. Pearson, and L.D. Warnick. 1998. Timing of insemination for dairy cows identified by a radiotelemetric estrus detection system. J. Dairy Sci. 81: 1874-1882.

Esteban, E., P.H. Kass, L.D Weaver., J.D. Rowe, C.A. Holmberg, C.E. Franti and H.F. Troutt. 1994. Reproductive performance in high producing dairy cows treated with recombinant bovine somatotropin. J. Dairy Sci. 77:3371-81.

Etherton, T.D., and D.E. Bauman. 1998. Biology of somatotropin n growth and lactation of domestic animals. Physiol. Rev. 78:745-761.

Evans, H.M., and M.E. Simpson. 1931. Hormones of the anterior hypophysis. Am. J. Physiol. 98:511-546.

Galton, D.M., R.W. Everett, M.E. Van Amburg, D.L. Bauman, and W.A Knoblauch. 1997. Extended calving intervals with the use of bST. Pages 116-121 in Proc. Western Large Herd Dairy Manage. Conf., Las Vegas, NV.

Gandy, B., W. Tucker, P. Ryan, A. Tucker, A. Moore, R. Godfrey, and S> Williard. 2001. Evaluation of the early conception factor (ECF) test for the detection of nonpregnancy in dairy cattle. Theriogenology 56:637-647.

Hamudikuwanda, H., H.N. Erb, and R.D. Smith. 1987. Effects of sixty-day milk yield on postpartum breeding performance in Holstein cows. J. Dairy Sci. 70:2355-2365.

Heersche, G. Jr,and R.L. Nebel. 1994. Measuring efficiency and accuracy of detection of estrus. J. Dairy Sci. 77:2754-2761.

Izadyar, F., B Colenbrander, and M.M. Bevers. 1997. Growth hormone enhances fertilizability of in vitro matured bovine oocytes. Theriogenology 47:23-32.

Judge, L.J., P.C. Bartlett, J.W. Lloyd, and R.J. Erskine. 1999. Recombinant bovine somatotropin: association with reproductive performance in dairy cows. Theriogenology 52:481-496.

Kirby, C.J., M.F. Smith, D.H. Keisler, and M.C. Lucy. 1997 a. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. J. Dairy Sci. 80:273-285.

Kirby, C.J., S.T. Wilson, and M.C. Lucy. 1997 b. Response of dairy cows treated with bovine somatotropin to a luteolytic dose of prostaglandin $F_{2\alpha}$. J. Dairy Sci. 80:286-294.

Kirby, C.J., W.W. Thatcher, R.J. Collier, F.A. Simmen, and M.C. Lucy. 1996. Effects of growth hormone and pregnancy on expression of growth hormone receptor, insulin-like growth factor-I, and insulin-like growth factor binding protein-2 and-3 genes in bovine uterus, ovary, and oviduct. Biol. Reprod. 55:996.

Koch, E., H. Morton, and F. Ellendorff. 1983. Early pregnancy factor: biology and practical application. Br. Vet. J. 139:52-58.

Kolle, S., F. Sinowatz, G. Boie, and D. Lincoln. 1998. Developmental changes in the expression of the growth hormone receptor messenger ribonucleic acid and protein in the bovine ovary. Biol. Reprod. 59:836-842.

Lamming, G.E., A.O. Darwash, and H.L. Black. 1989. Corpus luteum function in dairy cows and embryo mortality. J. Repro. Fertil. 37:245-252.

Lauderdale, J.W., B.E. Seguin, J.N. Stellflug, J.R. Chenult, W.W. Thatcher, C.K. Vincent, and A.F. Loyancano. 1974. Fertility of cattle following PGF2α injection. J Anim. Sci. 38:964-967.

Leeuwenberg, B.R., N.L. Hudson, L.G. Moore, P.R. Hurst, and K.P. McNatty. 1996. Periferial and ovarian IGF-I concentrations during the ovine oestrous cycle. J. Endocrinol. 148:281- 289.

Lucy, M.C., and J.S. Stevenson. 1986. Gonadotropin releasing hormone at estrus: lutenizing hormone, estradiol, and progesterone during the periestrual and postinsemination periods indairy cattle. Biol. Reprod. 35:300-311.

Lucy, M.C., J.S. Stevenson, and E.P. Call. 1986. Controlling first service and calving interval by prostaglandin F2 alpha, gonadotropin-releasing hormone, and timed insemination. J. Dairy Sci. 69:2186-2194.

Luna-Dominguez, J.E., R.M. Enns, D.V. Armstrong, and R.L. Ax. 2000. Reproductive performance of Holstein cows receiving Somatotropin. J. Dairy Sci. 83:1451-1455.

Macmillian, K.L., and W.W. Thatcher. 1991. Effects of agonist of gonadotropins-releasing hormone on ovarian follicles in cattle. Biol. Reprod. 45:883-889.

Makarevich, A.V. and M. Markkula. 2002. Apoptosis and cell proliferation potential of bovine embryos stimulated with insulin-like growth factor-I during in vitro maturation and culture. Biol. Of Reprod. 66:386-392.

Moallem, U., M. Kaim, Y. Folman, and D. Sklan. 1997. Effect of calcium soaps of fatty acids and administration of somatotropin in early lactation on productive and reproductive performance of high producing dairy cows. J. Dairy Sci. 80:2127-2136.

Momcilovic, D., L.F. Archbald, w. Walters, T. Tran, D. Kelbert, C. Risco, and W.W. Thatcher. 1998. Reproductive performance of lactating dairy cows treated with gonadotropin-releasing hormone and/or prostaglandin F2α for synchronization of estrus and ovulation. Theriogenology. 50:1131-1139.

Morales-Roura, J.S., L. Zarco, J. Hernandez-Ceron, and G. Rodriguez. 2001. Effect of short term treatment with bovine somatotropin at estrus on conception rate and luteal function of repeat breeding dairy cows. Theriogenology 55:1831-1841.

Moreira, F., C. Orlandi, C.A. Risco, R. Mattos, F. Lopes, and W.W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J Dairy Sci. 84:1646-1659.

Moreira, F., C.A. Risco, M.F.A. Pires, J.D. Ambrose, M. Drost, and W.W. Thatcher. 2000 a. Use of bovine somatotropin in lactating dairy cows receiving timed insemination. J. Dairy Sci. 83:1237-1247.

Moreira, F., L. Badinga, C. Burnley, and W.W. Thatcher. 2002. Bovine Somatotropin increases embryonic development in superovulated cows and improves post transfer pregnancy rates to lactating recipient cows. Theriogenology 57:1371-1387.

Moreira, F., R.L. de La Sorta, T. Diaz, and W.W. Thatcher. 2000 b. Effect of day of estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78:1568-1576.

Morton, H. 1984. Early pregnancy factor (EPF): A link between fertilization and immunomodulation. Aust. J Biol. Sci. 37:393-407

Nancarrow, C.D., A.L. Wallace, and A.S. Grewal. 1981. The early pregnancy factor of sheep and cattle. J. Reprod. Fertil. 30:191-199.

Nebel, R.L. 1998. Benchmarks for evaluating reproductive performance of the milking herd. Dairy Pipeline. Vol. 19, No. 4.

Nebel, R.L., D.L. Altemose, T.W. Munkittrick, D.J. Sprecher, and M.L. McGilliard. 1988. Comparisons of eight commercial on-farm milk progesterone tests. Theriogenology 31:753-764.

Palma, G.A., M. Muller, and G. Brem. 1997. Effect of insulin like growth factor I (IGF-I) at high concentrations on blastocyst development of bovine embryos produced in vitro. J Reprod Fertil. 110:347-333.

Pennington, J.A., L.H. Shultz, and W.F. Hoffman. 1995. Comparison of pregnancy diagnosis by milk progesterone on day 24 post breeding :field study in dairy cattle. J. Dairy Sci. 68:2740-2745.

Pershing, R.A., W.W. Thatcher, and L. Badinga. 2002. Effects of bovine somatotropin on oviductal and uterine genes encoding components of the IGF system in lactating dairy cows. J. Dairy Sci. J Dairy Sci. 85:3260-3267.

Peters, M.W., and J.R. Pursley. 2002. Fertility of lactating dairy cows treated with OvSynch after presynchronization injections of PGF2α and GnRH. J. Dairy Sci. 89. 2403-2406.

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Prosser, C.G., S.R Davis., V.C. Farr, L.G. Moore and P.D. Gluckman. 1994. Effects of close-arterial (external pudic) infusion of insulin-like growth factor-II on milk yield and mammary blood flow in lactating goats. J Endocrinol. 142:93-9.

Pursley, J.R., M.C. Wiltbank, J.S. Stevenson, J.S. Ottobre, H.A. Garverick, and L.L. Anderson. 1997 b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295-300.

Pursley, J.R., M.O. Mee, and M.C. Wiltbank. 1995. Snchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44:915.

Pursley, J.R., M.R. Kosorok, and M.C. Wiltbank. 1997 a. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.

Pursley, J.R., R.W. Silcox, and M.C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81:2139-2144.

Rajamahendran, R., J.D. Ambrose, E. J-P Schmitt, M.J. Thatcher, and W.W. Thatcher. 1998. Effects of burserlin injection and deslorelin (GnRH-agonist) implants on plasma progesterone, LH, accessory CL formation, follicle and corpus luteum dynamics in Holstein cows. Theriogenology 50:1141-1155.

Sakonju, S., S. Enomoto, S. Kaminura, and K. Amana. 1993. Monitoring bovine embryo viability with early pregnancy factor. J. Vet. Med. Sci. 55:271-274.

SAS. 1999. SAS system for windows. Version 8.2. SAS Institute, INC., Cary NC.

Sauerwein, H., A. Miyamoto, J. Gunter, H.H.D. Meyer, and D. Schams. 1992. Binding and action of insulin-like growth factors and insulin in bovine luteal tissue during the estrus cycle. J. Reprod. Fertil. 96:103-115.

Schmitt, E. J-P., T. Diaz, M. Drost, and W.W. Thatcher. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. J. Anim. Sci. 74:1084-1091.

Shelton, K., M.F. Gayerie De Abreu, T.J. Parkinson, G.E. Lamming. 1990. Luteal inadequacy during the early luteal phase of subfertile cows. J. Reprod. Fertil. 90:1-10.

Spicer, L.J., and R.E. Stewart. 1996. Interaction among bovine somatotropin, insulin, and gonadotropins on steroid production by bovine granulosa and theca cells. J. Dairy Sci. 79:813-821.

Spicer, L.J., and S.E. Echternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Domest. Anim. Endocrinol. 12:223-245.

Stevenson J.S., and J.R. Pursley. 1994. Use of milk progesterone and prostaglandin F2 alpha in a scheduled artificial insemination program. J. Dairy Sci. 77:1755-1760.

Stevenson, J.S., J.F. Smith, and D.E. Hawkins. 2000. Reproductive outcomes for dairy heifers treated with combinations of prostaglandin F2alpha, norgestomet, and gonadotropin-releasing hormone. J. Dairy Sci. 83:2008-2015.

Stevenson, J.S., M.K. Schmidt, and E.P. Call. 1984. Stage of estrous cycle, time of insemination, and seasonal effects on estrus and fertility of Holstein heifers after prostaglandin F2 alpha. J. Dairy Sci. 67:1798-1805.

Stevenson, J.S., Y. Kobayashi, and K.E. Thompson. 1999. Reproductive performance of dairy cows in various programmed breeding systems including OvSynch and combinations of gonadotropin-releasing hormone and prostaglandin F2 alpha. J. Dairy Sci. 82: 506-515.

Sturman, H., E.A. Ohenaco, and R.H. Foote. 2000. Importance of inseminating only cows in estrus. Theriogenology 53:1657-1667.

Thatcher, W.W., F. Moreira, J.E.P. Santos, R.C. Mattos, F.L. Lopes, S.M. Pancarci, and C.A. Risco. 2001. Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology 55:75-89.

Thatcher, W.W., K.L. Macmillian, P.J. Hansen, and M. Drost. 1989. Concepts of regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. Theriogenology 31:149

Thatcher, W.W., R.L. De La Sorta, E.J. Schmitt, T.C. Diaz, L. Badinga, F.A. Simmen, C.R. Staples. And M. Drost. 1996. Control and management of ovarian follicles in cattle to optimize fertility. Reprod. Fert. Devel. 8:203-217.

Therlfall, W.R., and G.M. Bilderbeck. 1998. Early conception factor (ECF) assay for non-conception determination in cattle. Proc. Soc. Theriogenology 157.

Threlfall, W.R. 1994. Immunosuppressive early pregnancy factor (ISEPF) determination for pregnancy diagnosis in dairy cows. Theriogenology 41:317.

Tripp, M.W., J.C. Ju, T.A. Hoagland, J.W. Reisen, X. Yang, and S.A. Zinn. 2000. Influence of somatotropin and nutrition on bovine oocytes retrieval and in vitro development. Theriogenology 53:1581-1590.

Twagiramungu, H., L.A. Guilbault, J. Proulx, P. Villeneuve, and J.J. Dufour. 1992. Influence of an agonist of gonadotropin-releasing hormone (buserelin) on estrus synchronization and fertility in beef cows. J. Anim. Sci. 70:1904-1910.

Vailes, L.D., and J.H. Britt. 1990. Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows. J. Anim. Sci. 68:2333-2339.

Vasconcelos, J.L.M., R.W. Silcox, G.J.M. Rosa, J.R. Pursley, and M.C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067-1078.

Yakar, S., J.L. Liu, B. Stannard, A. Butler, D. Accili, B. Sauer, and D. LeRoith. 1999. Normal growth and development in the absence of hepatic insulin-like growth factor-I. Proc. Natl. Acad. Sci. USA. 96:7324-7329.

Zhou, Y., B.C. Xu, H.G. Maheshwari, L. He, M. Reed, M. Lozykowksi, S. Okada, L. Cataldo, K. Coschigamo, T.E. Wagner, G. Bauman, and J.J. Kopchick. 1997. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron Mouse). Proc. Natl. Acad. Sci. USA 94:13215-13220.