ARTIFICIAL INDUCTION OF LACTATION IN NONBREEDER DAIRY COWS

by

Tracy Jewell

Submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

DAIRY SCIENCE (LACTATION)

M. A. Barnes
(Chairman)

R. E. Pearson
R. M. Akers
R. C. Nebel
S. C. Nickerson
Department Head

August 9, 2002

Blacksburg, VA

Keywords: induction of lactation, estrous cycle, dairy cattle

ARTIFICIAL INDUCTION OF LACTATION IN NONBREEDER DAIRY COWS

TRACY JEWELL

M. A. Barnes, Chairman

Department of Dairy Science

ABSTRACT

Thirty-four cows (26 Holsteins and 8 Jerseys) were subjected to an estrous synchronization protocol administering 2 $PGF_{2\alpha}$ injections 11 d apart prior to beginning the lactation-induction protocol. Artificial induction of lactation yielded a 92% success rate for Holstein cows with success defined as achieving >9 kg milk/d, and a 88% success rate for Jersey cows with success defined as achieving > 5 kg milk/d. Mean accumulated milk yield for induced cows at 150 DIM was 65% of mean yield for nontreated cows. Mean peak milk yield for lactation- induced Holsteins and Jerseys was 32 kg/d and 20 kg/d, respectively. Mean serum and milk progesterone concentrations for samples collected during the first 6 d of lactation were not different between lactation-induced and nontreated cows. However, mean serum estradiol concentrations for induced cows were higher ($P < 0.05$) in samples collected 3 and 5 DIM. Lactation-induced cows exhibited an increase in serum alpha-lactalbumin concentrations 2 d prior to initiation of milking, reaching values of ~260 ng/ml. Mean days-to-first service was greatly reduced in cows induced into lactation compared to nontreated cows, while mean services per conception was similar between induced and nontreated cows. Mean days to conception was lower for induced cows than for nontreated cows. By 150 DIM, pregnancy rate of induced cows was 70%, whereas nontreated cows averaged 56% pregnancy rate.

ACKNOWLEDGEMENTS

I have to extend the greatest thanks to my major advisor, Dr. M. A. Barnes, for his patience, advice, guidance, and understanding throughout the past two years. I am also thankful to him for allowing me the opportunity to assist in laboratory and classroom activities and learn the often forgotten other side to education, teaching. I would also like to thank the other members of my committee: Dr. R. E. Pearson for his assistance in the statistical analysis of my data, Dr. R. C. Nebel for his helpful advice in the area of reproduction, and to Dr. R. M. Akers for providing his knowledge in the area of mammary physiology.

I would also like to thank Dr. Thomas Bailey and Monsanto Dairy Business for providing the Posilac which was used throughout the course of this study. Trials and animal care procedures were approved and conducted under Federal Hatch Project #135577, and drug use was approved by FDA under INAD Permit #10876, A-0000.

Special thanks to Cari Culver, Katie Poole, and Dee Guyton for all your help, support, and friendship. To Cari and Katie, thanks for the many wonderful weekends and the many trips to the plantation. To Dee, thanks for being a great roommate and always offering to help even when you were busy.

Lastly, I would like to thank my parents, Bryn and Nancy Jewell, for their love, support, and encouragement. You have consistently reminded me throughout my life to pursue my goals and never let anything discourage me. Without your encouragement and support, I would not have achieved this.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Protocols for the induction of lactation in nonlactating and nonpregnant dairy cows have focused on the practical uses of these procedures on farms (Fulkerson, 1979; Kensinger, 1999). With the use of such protocols, farmers may be able to reduce herd culling losses and replacement costs by retaining animals that would otherwise be culled from the dairy herd. Substantial non-voluntary culling losses and long calving intervals may be attributed to reproductive problems and failure to breed. Increases in calving intervals due to reproductive inefficiency leads to decreases in profit from lost milk sales and increased costs of reproduction. Salvaging cull cows such as problem breeders with the use of artificial induction of lactation offers the producer the opportunity to keep cows that may be genetically superior in potential for milk production and gain additional breeding opportunities.

Many techniques over the past 60 years have utilized the ovarian hormones estrogen and progesterone, alone or in combination, to develop the mammary gland and initiate lactation (Hammond, 1944; Collier, 1976; Chakriyarat, 1978; Peel, 1978). Early attempts to induce lactation used 120-to-180 d injection regimens of estrogen and progesterone (Hancock, 1954; Turner, 1956). These long-term treatments generally resulted in low milk yields and low rates of success (proportion of cows successfully induced into lactation). In 1973, Smith and Schanbacher developed a 7-d estrogen-progesterone protocol and successfully induced lactation in 60% of treated animals. Although the new protocol was successful in 60% of treated animals, there was still substantial variability in milk yields between lactation-induced cows. Since the development of this shorter protocol, many modifications have been employed to improve success rates and reduce the variability in milk yields. The addition of reserpine, a tranquilizer that increases blood prolactin levels for several hours, and dexamethasone, a synthetic glucocorticoid, to induction protocols have increased success rates, but have not reduced the variation in milk yields recorded from induced cows (Collier, 1977; Chakriyarat, 1978; Peel, 1978). Other attempts to improve milk yields have involved administering somatotropin at the start of milking, which increased milk yields (28.3 kg/d) compared with controls (24.1 kg/d) receiving only the estrogen-progesterone injections (Kensinger et al., 1998).

Erb et al. (1976) reported that cows having more successful induced lactations following treatment with 17β-estradiol and progesterone had below average plasma concentrations of estrogen and progesterone before treatment. It was also noted in the same study, that progesterone declined rapidly after the last 17β-estradiol and progesterone injection and estrogen declined 7 d after 17β-estradiol and progesterone treatment. Results obtained from their study indicate that induced lactations would be more successful if cows started the lactation-induction treatment 3 to 8 d after estrus accompanied by ovulation (Erb et al., 1976). The objective of this experiment is to further investigate this hypothesis and to determine the effect of synchronizing estrous cycles with 2 injections of prostaglandin ($PGF_{2\alpha}$) prior to the induction period. We hypothesized that synchronizing estrous cycles of animals prior to the induction period would increase success rates and decrease the variability in response to the lactation induction treatment.

CHAPTER II

LITERATURE REVIEW

Artificial induction of lactation

It has been well established through *in vivo* and *in vitro* studies that hormones play a crucial role in the development and function of the mammary gland (Turner, 1931; Turner, 1956; Williams, 1960; Erb, 1976; Tucker, 2000). Studies examining the role of estrogens in stimulating mammogenesis in dairy cows showed that estrogen stimulates mammary duct growth, and estrogen and progesterone in combination stimulate lobule-alveolar development of the mammary gland (Tucker, 2000). Estrogen is also involved in initiating lactogenesis in cattle at parturition. Estrogen initiates lactation in two ways: 1) it causes release of prolactin from the anterior pituitary gland into blood (Nagasawa, 1969; Tucker, 2000) and 2) estrogen increases the number of prolactin receptors in mammary cells (Sheth, 1978; Tucker, 2000).

Although combinations of exogenous progesterone and estrogen work synergistically to stimulate lobular-alveolar growth, it is the high levels of progesterone during pregnancy that help to regulate lobular-alveolar growth and block lactogenesis. Progesterone blocks lactogenesis in several ways. One example of this is how it blocks glucocorticoid receptors in mammary tissues which would suppress the lactogenic activity of glucocorticoids (Tucker, 2000). The exact mechanism of how progesterone accomplishes this is unclear, but removal of the progesterone block, luteolysis; and a decline in progesterone as parturition approaches, allow the onset of lactogenesis (Fulkerson, 1979; Tucker, 2000).

The ovarian hormones, estrogen and progesterone, are not the only hormones required for lactogenesis, as prolactin is also needed. Blood prolactin levels in cattle surge several hours prior to parturition, (Ingalls, 1973; Tucker, 2000) and this surge in prolactin is apparently necessary for full lactogenesis. The surge in prolactin can be blocked with the use of bromocriptine, reducing milk yield, but this effect can be reversed with administration of prolactin (Akers et al., 1981).

Glucocorticoids, cortisol being the major glucocorticoid in cattle, also play a role in mammary gland development, leading to alveolar cell differentiation of the gland.

Glucocorticoids compete with progesterone for mammary epithelial cell binding sites. Administering glucocorticoids to nonlactating cows with a developed mammary gland aids in the induction of lactation because the increase in glucocorticoids displaces progesterone from mammary cell receptors, thus reducing the progesterone block to prolactin receptor synthesis (Tucker, 1965; Fulkerson, 1975). Milk yields from these induced-lactations can be enhanced with the addition of prolactin, offering additional evidence that the hormones (estrogen, progesterone, prolactin, and glucocorticoids) work synergistically in the onset of lactogenesis (Tucker, 2000; Akers, 2002).

The use of exogenous hormones to mimic mammary development during pregnancy has led to research to answer to questions regarding how the hormones work synergistically to promote lactogenesis. During these attempts to mimic mammary development with varied dosages of exogenous hormones, it was observed that induced-lactations actually occurred in treated animals, and these results have then led to the use of exogenous hormones to induce lactations in nonpregnant cattle.

Techniques to reduce culling rates and replacement costs through the use of hormonal induction of lactation in dairy cattle have been attempted with varied success for more than 60 years (Hancock, 1954; Turner, 1956; Smith 1973). Development of a reliable cost efficient protocol to artificially induce lactation would allow dairy producers to reduce herd culling losses of nonbreeder cows and lower replacement costs by retaining animals that would otherwise be culled from the dairy herd.

Magliaro et al. (1999) compared the profitability of inducing lactation in non-pregnant cows versus purchasing replacement heifers. Healthy, multiparous, nonpregnant cows were induced into lactation using an estrogen/progesterone protocol. Net present value (NPV) was calculated for a 12-month stream of net incomes for induced animals and peer heifers. Magliaro et al. (1999) and Kensinger et al. (2000) calculated NPV for induced cows versus replacement heifers and found that NPV for induced cows was $520 greater than for replacement heifers. These calculations also considered the administration of bovine somatotropin (bST) to lactation induced cows to enhance milk yields, and estimated that the mean annual NPV advantage for using BST was $261/cow. If a protocol similar to this was approved by FDA, then inducing non-pregnant cows into lactation would be a method for dairy producers to increase profitability

(Magliaro et al., 1999). Currently, steroid-based induced-lactation protocols are illegal because of concerns regarding consumer safety and presence of hormones in milk.

Early protocols for induction of lactation consisted of long-term hormone treatment of 120 to 180 d and resulted in low milk yields and low rates of success (Hancock, 1954; Turner, 1956; Williams, 1960). Smith and Schanbacher (1973) utilized a 7-d injection protocol using a combination of the hormones 17β-estradiol (.1 mg/kg BW/d), and progesterone (.25 mg/kg BW/d) and were able to successfully initiate lactation in 60% of treated animals (6 of 9 cows and 1 heifer) after the first series of injections with this shorter protocol. Although Smith and Schanbacher (1973) were successful in inducing lactation in a majority of animals with the shorter protocol, they also experienced a 40% failure rate. This high failure rate left opportunities for other scientists to examine protocols to improve success rate of artificial induction of lactation in nonpregnant and nonlactating dairy cows. Researchers attempted to increase the success of induction of lactation by extending the duration of hormonal treatment (Erb, 1976a; Erb, 1976b; Peel, 1978) or doubling the daily dose of steroids used (DeLouis et al., 1978), and several included use of either exogenous dexamethasone (Collier, 1975; Collier, 1976; Chakriyarat, 1978) and/or reserpine (Collier, 1977; Peel, 1978; Lembowicz, 1982) in the induction schemes. Exogenous reserpine can elicit an increase in blood prolactin concentration that lasts for several hours in cattle (Bauman et al. 1977), thus imitating the dramatic increase in prolactin seen several days prepartum in pregnant cows (Convey et al. 1974). It is also well documented that plasma glucocorticoids increase dramatically at the time of parturition in ruminant species (Convey, 1974; Heald, 1974; Collier, 1975), and administration of the synthetic glucocorticoid, dexamethasone, can mimic increased glucocorticoid levels (Collier et al. 1975).

Long-term treatments using injections of estrogen alone or in combination with progesterone were thought to be important in mimicking pregnancy in the ruminant, and this long-term steroid stimulation was also thought necessary for complete mammary cell differentiation and development (Folley, 1944; Turner, 1956; Meites, 1961; Chakriyarat, 1978). Smith and Schanbacher (1973) were able to prove this theory wrong utilizing higher dosages of estrogen and progesterone injections for 7-d, but other researchers (Erb, 1976b; Peel, 1978) speculated that a longer duration of treatment was needed to improve success rates (the number of animals producing a defined milk yield) and to eliminate the variation in milk yields experienced using the 7-d treatment. Increasing the injection period from 7-d to 11-d, or 12-d, as

performed by Peel et al. (1978) and Erb et al. (1976b) did not improve success rates (success > 1 kg/d within 24 after start of milking) or eliminate the variation in milk yields reported between animals induced into lactation. Lembowicz et al. (1978) were able to successfully reduce the 7-d estrogen and progesterone protocol to 5·5 d, or even 3·5 d, in multiparous Polish Black and White cows, so that its application would be more practical in commercial herds. In that study, doses of 17-β estradiol and progesterone, as outlined by Smith and Schanbacher (1973), were used in addition to eight single injections of reserpine on days 9 to 16 at a dose of 22·5 mg/cow/d. The lower milk yields resulting from this modified procedure were not significantly different from those of cows induced into lactation with the 7-d treatment, but the advantages were its simplicity and reduction of estrus-like excitement (mounting) that would be beneficial to producers (Lembowicz et al., 1978).

It is believed that the increase in circulating glucocorticoids at parturition is a necessary factor in initiating lactogenesis (Collier et al. 1975), thus leading researchers to examine the addition of exogenous synthetic glucocorticoid, dexamethasone, to induction protocols. Collier and co-workers (1975) modified the 7-d treatment outlined by Smith and Schanbacher (1973), by administering 3 single injections of dexamethasone (20 mg/cow/d) to 6 heifers and 10 cows on d 18, 19, and 20 of the induction protocol. This trial resulted in a 69% success rate (success > 9 kg milk/d at peak yield), but it was concluded that these results were similar to those reported by Smith and Schanbacher (1973) and that dexamethasone did not substantially improve the success rate. Chakriyarat et al. (1978), using 19 dairy cows of varied breed and age, examined the addition of 3 single injections of dexamethasone (.028 mg/kg BW/d) on d 18, 19, and 20 of the 7-d estrogen-progesterone induction protocol. These researchers reported that addition of dexamethasone injections increased the number of cows (9 of 11; 82%) successfully induced into lactation compared with cows induced into lactation without dexamethasone (3 of 11; 27%) (Chakriyarat et al., 1978). Eleven Holstein cows and 9 Guernsey cows were induced into lactation with 17-β estradiol (.10 mg/kg BW/d) and progesterone (.25 mg/kg BW/d) for 21 d, in addition to 3 single injections of dexamethasone (.028 mg/kg BW/d) on days 31 to 34, again improving success rate (success > 5 kg milk/d) but not milk yields compared with cows not receiving dexamethasone injections (Fleming et al., 1986). These results suggest that glucocorticoids may play an important role in initiating lactogenesis in the induced cow but apparently do not enhance milk yields.

Collier and co-workers (1977) continued to pursue modifications to the 7-d estrogen/progesterone protocol with the goal of improving success rates and decreasing the variation in milk yields among animals, by incorporating the addition of reserpine injections. It was hypothesized that prolactin may be a limiting component of the "lactogenic complex" in cows that fail to lactate following the 7-d estrogen and progesterone treatment (Collier et al., 1977). Reserpine injections were administered to nonpregnant cows either on d 13, 14, 15, and 16, or d 8, 10, 12, and 14 of the experiment. Days 13, 14, 15, and 16 were chosen so that prolactin levels in induced cows would mimic the increase in prolactin that occurs in pregnant cows during the period immediately prior to parturition (Convey, 1974; Collier, 1977). Reserpine was administered on d 8, 10, 11, and 12 based on results of an earlier study that indicated that mammary tissue from induced cows was undergoing cellular changes associated with lactogenesis by d 8 and continuing through d 16 of treatment (Collier, 1976; Croom, 1976; Collier, 1977). Use of reserpine to cause prolactin release reduced variation in milk yields between animals and increased the success rate (success > 9 kg milk/d at peak yield) from 40 to 100% in cows administered reserpine on d 13 to 16, and from 75 to 100% in cows receiving reserpine injections on d 8, 10, 12, and 14 (Collier et al., 1977). Peel et al., (1978), utilizing dairy cows of mixed breed, demonstrated that administering reserpine (5 mg/d) on d 1, 6, 11, 16, and 21, in addition to the 7-d protocol described by Smith and Schanbacher (1973), did not increase milk yields, but increased the proportion of cows responding to lactation induction treatment when compared with controls receiving 17-β estradiol and progesterone injections alone.

Several modifications to the 7-d estrogen and progesterone treatment protocol have yielded higher success rates (success defined by the scientist, but usually > 9 kg milk/d peak yield), but little improvement in reducing variation in milk yields among animals have been reported (Collier, 1977; Chakriyarat, 1978; Peel, 1978; Fleming, 1986). Further research into development of new protocols for induction of lactation is required in order to continue improving the success rate, eliminate variability in milk yields between cows, and increase milk yields.

During normal lactogenesis, progesterone concentrations in plasma begin to decrease approximately 1 week before parturition, and estrogen concentrations, already elevated during pregnancy, start to increase dramatically during the final weeks before calving (Fulkerson,

1979). Initiating a lactation induction scheme with all cows in a uniform phase of the estrous cycle may eliminate variation among animals that may be related to differences in concentrations of estrogen, progesterone, glucocorticoids, and prolactin at the time of initiation of lactation. Also, since progesterone competes with glucocorticoids for binding sites in mammary tissue (Fulkerson, 1979), removing the progesterone source (corpus luteum) by administering prostaglandin $F_{2\alpha}$ may allow induced cows to be more responsive to reserpine and dexamethasone treatment following the initial estrogen and progesterone protocol. Induced luteolysis allows glucocorticoids to displace progesterone from binding sites in mammary tissue, thereby removing the progesterone block to lactogenesis (Fulkerson, 1979). Thus, use of $PGF_{2\alpha}$ to induce luteolysis offers the opportunity to initiate a lactation induction protocol during very different stages of the estrous cycle; estrus and metestrus when progesterone is low and estrogen is elevated, or diestrus when progesterone is the dominant steroid hormone. Exogenous $PGF_{2\alpha}$ may then be used following the initial estrogen-progesterone therapy to insure luteolysis and depress circulating progesterone at the time of glucocorticoid and reserpine administration.

Use of prostaglandin $F_{2\alpha}$ for estrous synchronization

Administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been shown to induce luteolysis in the bovine (Lauderdale, 1972; Liehr, 1972; Rowson, 1972). Rowson et al. (1972) injected 0.5 mg/d $PGF_{2\alpha}$ on 2 consecutive days into the uterine horn of cattle ipsilateral to the corpus luteum (CL) and caused regression of the CL between d 5 and 16 of the estrous cycle. Most animals exhibited signs of estrus on the third day after treatment, but administration of $PGF_{2\alpha}$ between d 1 and 4 of the estrous cycle was ineffective in synchronizing estrus among cattle. Lauderdale (1972) administered 30 mg $PGF_{2\alpha}$-tham salt subcutaneously to heifers and observed estrus 2 to 4 days later. Of the five heifers treated on days 2 to 4 of the estrous cycle, none were observed in estrus following treatment. A total of 17 heifers treated on d 6 to 9 of the estrous cycle, and 12 heifers treated on days 13 to 16 of the estrous cycle, expressed estrus. It was noted that blood progesterone levels decreased by 12 hr after $PGF_{2\alpha}$ treatment and the CL decreased in size by 24 hr. In a separate study, Lauderdale et al. (1974) compared the fertility of cattle of varied breed and age following $PGF_{2\alpha}$ injection, and control cows receiving no $PGF_{2\alpha}$. Lauderdale reported that fertility (percent pregnant) was similar between groups ($PGF_{2\alpha}$ injection vs. controls) when cattle were inseminated at synchronized estrus following $PGF_{2\alpha}$, and when cattle were

inseminated at predefined intervals either 12 h or 72 to 90 h after the onset of estrus following $PGF_{2\alpha}$ treatment.

Studies examining the dosage of $PGF_{2\alpha}$ and stage of the estrous cycle when administered, have shown that dose and stage of the estrous cycle affect estrous response. Berardinelli and Adair (1989) reported that low dosages of $PGF_{2\alpha}$ (5 and 10 mg) administered to beef heifers 13 to 14 months of age and of mixed breed, resulted in fewer heifers exhibiting estrus when given $PGF_{2\alpha}$ in the early stage of the estrous cycle, but as the stage of the estrous cycle at which heifers were treated advanced, the number of heifers exhibiting estrus increased. Higher dosages of $PGF_{2\alpha}$ (25 mg or 30 mg) yielded more heifers exhibiting estrus during the early stages of the luteal phase (d 5 to 9 of estrous cycle). In a similar study, Garcia-Winder and Gallegos-Sanchez (1991) used 10, 17.5, or 25 mg $PGF_{2\alpha}$ to synchronize estrus in Holstein cows of different ages and parities between 8 and 12 d of the estrous cycle (10 ± 0.5). Blood progesterone concentration in cows treated with 10 mg $PGF_{2\alpha}$ declined during the first 24 h, but by the end of the experimental period (48 hr following $PGF_{2\alpha}$ treatment), was not different from pretreatment levels, suggesting that the CL underwent partial functional luteolysis, but was able to recover in 24 to 48 hr (Garcia-Winder and Gallegos-Sanchez, 1989). There was no difference in estrous response or pregnancy rate at first service for cattle treated with 17.5 mg or 25 mg $PGF_{2\alpha}$ (Garcia-Winder and Gallegos-Sanchez, 1989). Other researchers (Beal, 1998; King, 1982; Stevenson, 1984; Tanabe, 1984; Watts, 1985) have shown that administering $PGF_{2\alpha}$ to dairy heifers and beef cattle between d 5 and 9 of the estrous cycle results in fewer cows responding to treatment compared with administering $PGF_{2\alpha}$ later in the estrous cycle (d 12 to 18).

The course of events that occur following $PGF_{2\alpha}$ injection, or administration of the $PGF_{2\alpha}$ analog, cloprostenol (CP), between d 5 and 18 of the estrous cycle are: a decrease in blood progesterone concentration within 12 to 24 h, decrease in CL size, gradual increase in estradiol concentration for 48 to 72 h, onset of estrus approximately 72 h after treatment, a surge of lutenizing hormone near onset of estrus, and ovulation 24 to 30 h after onset of estrus (Louis, 1973; Louis, 1974; Seguin, 1980). Only cattle treated during the diestrus phase (d 5 to 18 of the estrous cycle) are responsive to $PGF_{2\alpha}$ injection, with more cattle being responsive to $PGF_{2\alpha}$ during the later stages of the estrous cycle (d 12 to 18) (Berardinelli and Adair, 1989). Cattle

treated with $PGF_{2\alpha}$ during anestrus, proestrus, estrus, and early metestrus are not affected and, except for those in anestrus, continue to cycle (Cooper, 1974; Seguin, 1980).

The ability of $PGF_{2\alpha}$ to induce luteolysis in cattle has led to research and development of protocols that may be used on commercial farms to improve pregnancy rate. One injection of $PGF_{2\alpha}$ causes regression of the CL, but only ~60% of randomly-cycling cows will return to estrus (Seguin, 1980). The animals that respond to $PGF_{2\alpha}$ injection will assume stages of the estrous cycle similar to those of herdmates that did not respond to the first $PGF_{2\alpha}$ injection. After 11 to 12 d, all cows should be in the luteal phase of the estrous cycle and ready to respond to a second $PGF_{2\alpha}$ injection. King and Robertson (1974) administered 2 injections of 30 mg $PGF_{2\alpha}$-tromethamine salt ($PGF_{2\alpha}$ tham salt) 10 d apart to 30 randomly cycling Holstein heifers. Twenty-five of the 30 heifers (83%) treated were in estrus and inseminated 2 to 4 d following the second injection of $PGF_{2\alpha}$ (King and Roberston, 1974). At 60 d postinsemination, 40% of the inseminated heifers were diagnosed pregnant. As reported in similar studies, none of the animals treated with first $PGF_{2\alpha}$ between day 0 and 4 of the estrous cycle exhibited estrus. Burfening et al. (1978) reported similar results with Hereford heifers receiving 2 injections of $PGF_{2\alpha}$-tham salt (33.4 mg) 11 d apart, but only 71% of treated heifers were observed in estrus following the second injection of $PGF_{2\alpha}$.

Use of $PGF_{2\alpha}$ offers an attractive means of regulating the stage of the estrous cycle in order to initiate lactation induction protocols to cows that should be more similar in endocrine status. This may offer the opportunity to reduce variation in number of animals responding to lactation induction and perhaps reduce variation in level of milk yield.

Bovine somatotropin

Asimov and Krouze (1937) were the first researchers to demonstrate that injections of crude pituitary extracts from cattle, increased milk yield in dairy cows. The mean increase in milk yield from four treatment groups (all receiving the same dosage of pituitary extract) was 11 L. Li et al. (1945) isolated somatotropin (ST) from ox anterior pituitary, by treating the fresh anterior pituitary substance or acetone-dried glands with cysteine, which destroys lactogenic, thyrotropic, and gonadotropic activities, but not growth potency. Young (1947) supported the findings of Asmimov and Krouse (1937) and demonstrated in lactating dairy cows that ST was the galactopoietic factor in pituitary extracts that stimulated milk yield in dairy cows. Although

results obtained from studies proved that bovine pituitary extracts could increase milk yields (Asmiov 1937; Young, 1947), the amount of bovine pituitary extract needed to conduct extensive studies was limited because only small amounts of bovine somatotropin (bST) could be purified from each pituitary gland (5 to 15 mg) (Peel and Bauman 1987). Due to limitations in availability of pituitary derived bST, studies could only involve small numbers of cows treated for a few days (Bauman, 1992).

Genetic engineering advances during the 1970s made it possible to remove DNA from one species (gene splicing) and insert it into another organism. The gene that produces bST in the pituitary gland of the bovine can be spliced into the plasmid (small circular piece of DNA) of an *Escherichia coli* K-12 bacterium. The *E. coli* cells will then make the new protein coded for bST, bacterium is killed, and bST is separated and purified in commercial quantities. These advances in DNA technology in 1982 led to the first study comparing a 6-d administration of recombinantly derived bST (rbST) (25 mg/d) and natural bST (25 mg/d) to dairy cows (Bauman et al., 1982). Milk yields from 12 Holstein cows (92 ± 20 DIM) treated with 25 mg/d of rbST increased by 12.9%, and yields from cows treated with 25 mg/d of natural bST increased by 10.3%, illustrating that recombinantly derived bST was as effective as pituitary derived bST in enhancing milk yield (Bauman, 1982; Peel, 1987). Since the development of rbST, research with this protein has dramatically increased.

Mass production of rbST provided the opportunity to conduct and evaluate long-term studies. Bauman et al. (1985) examined the effect of long-term (188 d) daily administration of either rbST or pituitary derived bST on lactational performance in 30 Holstein cows in their second to fifth lactation, beginning at 84 ± 10 d postpartum. Cows treated with rbST, either 13.5, 27, or 40 to 50 mg/d, had an increase in fat corrected milk yield from 23 to 41% above control cows, while cows treated with pituitary-derived bST (27mg/d) experienced a milk yield increase of 16%. Bauman et al. (1985) also reported that increased milk yield associated with somatotropin treatment caused a decrease in energy balance. Voluntary feed intake increased with increasing dose of rbST to compensate for the decrease in energy balance. Net energy intake of treated cows increased to 35.1 Mcal/d (natural bST 27 mg/d), 36.1 Mcal/d (rbST 13.5 mg/d), 39.2 Mcal/d (rbST 27 mg/d), and 37.5 Mcal/d (rbST 40.5 mg/d) respectively, compared with 34.1 Mcal/d for control cows. By wk 10 of treatment all treated cows were in positive energy balance.

Although Bauman et al. (1985) were successful in demonstrating that rbST was equally as effective as pituitary-derived bST and continued to improve milk yields during long-term treatment (188 d), further exploration was needed to investigate the use of a prolonged-release formulation. It was speculated that a successful prolonged-release formulation would eliminate the need for daily injections of rbST, thus reducing cost and labor. Eighty Holstein cows in first, second, or third lactation were administered (60 ± 3 d postpartum) a prolonged-release formulation containing 500 mg of rbST at 14-d intervals. The prolonged-release formulation was effective in improving fat corrected milk yields by 11.4%, but a cyclic pattern in milk yield was observed within each 14-d injection interval resulting from the formulation providing a diminishing quantity of rbST during the last one-third of the injection interval (Bauman et al. 1989). As previously reported (Bauman et al. 1985), cows receiving rbST increased voluntary feed intake to remain in positive energy balance, with a mean net energy intake of 31.8 Mcal/d for treated cows and 30.2 Mcal/d for control cows. Other researchers using a prolonged-release system of N-Methionyl Bovine Somatotropin (Sometribove, Monsanto Company, St. Louis, MO) with 14-d injection intervals (Hartnell, 1991; Barbano, 1992) have reported similar results.

Administering different dosages of rbST at 96 ± 7 days in milk (DIM) in a sustained-delivery vehicle for intervals of 4 wk has also been attempted. Oldenbroek et al. (1989) administered three different dosages of rbST (320, 640, and 960 mg) in a sustained-release vehicle at 28-d intervals. It was found that the 640 mg dose of bST gave optimum results with a milk energy output increase of 19% in wk 13-36. Another study performed by Leonard et al. (1990) examining a 28-d sustained-release formulation administered to cows in early, mid, and late lactation also incorporated dosages of 320, 640, and 960 mg of rbST and investigated the effect of administering the formulation to cows during two consecutive lactations. Injections of rbST caused an increase in milk yields during two consecutive lactations, but the 28-d slow-release formulation caused an increase in milk yield in cows for only 3 wk, allowing 1 wk for cows to alleviate negative energy balance in early lactation (Leonard et al. 1990). As seen in other studies investigating rbST administration (Bauman, 1985; Bauman, 1989; Hartnell, 1991), all cows receiving somatotropin increased voluntary feed intake to compensate for the extra nutrients required for increased milk yield.

Many studies have investigated effects of rbST use on milk composition. Bauman et al. (1989) reported that fat content of milk increased with rbST treatment (administered 60 ± 3

DIM) if treated cows were in a negative energy balance while receiving bST treatment. When voluntary feed intake increases and a positive energy balance is established, fat content of milk is comparable to that of untreated cows. Barbano et al. (1992) concluded that mean percentages of lactose, fat, total solids, SNF, casein, and true protein during a treatment period (wk 2 to 41 of lactation) were comparable in milk from control and rbST-treated cows. Hartnell et al. (1991) also concluded that administration of rbST did not affect milk composition including milk fat, protein, lactose, ash, Ca, and P concentrations, or somatic cell count (SCC). Other researchers (Dahl, 1991; Kim, 1991; Laurent, 1992) have also reported that use of rbST does not alter milk composition.

With improved milk yield in cows receiving rbST, concerns arise over mastitis, negative effects on reproductive performance (increased days open, lower conception rate, and pregnancy rate), and cases of ketosis, milk fever, and increased susceptibility to diseases. Studies examining effect of rbST on milk composition (Oldenbroek, 1989; Phipps, 1989; Hartnell, 1991; Barbano, 1992) have documented that use of rbST does not increase SCC or increase incidence of ketosis or milk fever. Burvenich et al. (1989) demonstrated that cows receiving rbST treatment recovered more rapidly from experimentally induced *E. coli* mastitis. Bauman et al. (1985) reported no differences in reproductive performance (days from calving to conception, services per conception, and conception rate) in cows treated with rbST compared with nontreated cows. More recent studies (Cole, 1988; Elvinger, 1988; Hard, 1988; Huber, 1988; Burton, 1990; Leonard, 1990) have reported trends for increased number of services per conception, longer calving to conception intervals, and lower conception rate, but effects on reproductive performance were not statistically different when comparing rbST-treated cows to untreated cows.

Studies investigating administration of rbST during early lactation (28 DIM) at dose rates 0, 12.5, 25, and 40 to 50 mg/d indicated that higher doses led to increased days open and a decrease in pregnancy rate (Burton, 1987; Chalupa, 1987; Elvinger, 1988). Eppard et al. (1987) administered rbST beginning 84 DIM at doses of 0, 13.5, 27, and 40.5 mg/d and found days open and services per conception to be comparable to that of controls. Thus, negative effects of rbST on reproductive performance may be attributed to its ability to further depress energy balance of early lactation cows (< 84 DIM) that are already in negative energy balance (Ducker et al., 1985). However, administering rbST at initiation of lactation to nonpregnant, nonlactating cows

induced into lactation should not adversely affect reproductive performance, because these cows are in positive energy balance and normally begin lactation at relatively low levels of milk yield.

CHAPTER III

MATERIALS AND METHODS

Animals

Thirty-four multiparous dairy cows of varied breed (8 Jersey cows; mean age 4.6 ± 1.7 yr, mean BW 397 ± 39.85 kg, and 26 Holstein cows; mean age 3.8 ± 1.10 yr, mean BW 557 ± 60.0 kg) were artificially induced into lactation. Treatment animals were paired with nontreated cows at the Virginia Tech Dairy based on age, calving date, breed, and lactation number. At the start of the experiment, all animals were nonpregnant and nonlactating, and had been dried off for a minimum of 45 d. All animals were subjected to an estrous synchronization protocol prior to the initiation of the lactation induction scheme. Initially, cows were administered two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Lutalyse, Phamacia Animal Health, Kalamazoo, MI) 11 d apart at a dosage of 25 mg/d. Group 1 animals (5 Jersey cows and 13 Holstein cows) received the first $PGF_{2\alpha}$ injection at –16 d and the second injection at –6 d, with d 1 designating the start of the lactation-induction protocol. Group 2 animals (3 Jersey cows, and 13 Holstein cows) received the first $PGF_{2\alpha}$ injection at –10 d and the second $PGF_{2\alpha}$ injection at 0 d, with d 1 designating the start of the lactation-induction protocol (Figure 1). Thus, Group 1 animals were subjected to the lactation-induction protocol beginning 7 d after the last $PGF_{2\alpha}$ injection, while group 2 animals began the lactation induction protocol one day after the last $PGF_{2\alpha}$ injection. One cow from group 1 was culled from the experiment early in her induced-lactation due to injuries acquired during the lactation- induction period. All animals were housed in free-stalls and kept separate from the milking herd during the lactation-induction period. Cows were fed ad libitum a total mixed ration (complete feed) consisting of corn silage, alfalfa haylage, soybean meal, cottonseed meal and high moisture corn (mean DM: 55%, CP: 16.4%, NEL: 0.70 Mcal/lb of DM). After lactation began, milk samples were collected and assayed monthly in the United DHI laboratory, Blacksburg, VA, for protein, fat, and SCC. Estrus activity was monitored utilizing the Heath Watch® System, DDX Inc., Denver, CO. Estrus detection sensors were applied to the tailhead

of all cows 7 d prior to the initiation of the lactation-induction protocol. Reproductive records monitored were days to first estrus, days to first service, days to conception, services/conception, and pregnancy rate, defined as the number of cows pregnant divided by the number of cows inseminated. All lactation-induced cows and some nontreated cows were inseminated on first estrus by the designated AI technician at the Virginia Tech Dairy. The remaining nontreated cows were subjected to the pre-synch program (60 to 150 DIM). Nontreated cows were given two injections of $PGF_{2\alpha}$ (25 mg) 14 d apart followed by GnRH (100 μg) 14 d later and then a third injection of $PGF_{2\alpha}$ (25 mg) was administered 7 d after GnRH. Cows were bred by AI 16 h after the third $PGF_{2\alpha}$ injection.

Lactation-induction protocol

The common stock solution for 17β-estradiol and progesterone contained 20 mg 17β-estradiol and 50 mg progesterone dissolved in absolute ethanol. All cows received twice-daily subcutaneous injections (neck region) of 17β-estradiol (.1mg/kg BW/d) and progesterone (0.25 mg/kg BW/d) for 1 to 7 d, with injections administered 12 h apart alternating from left to right side of the animal (Figure 1). All cows received 1 injection of $PGF_{2\alpha}$ (25mg) on d 13 to initiate luteolysis of existing corpora lutea. Separate stock solutions for the hormones reserpine and dexamethasone contained 5 mg/ml reserpine (Sigma Chemical Co., St. Louis, MO) dissolved in glacial acetic acid, and 20 mg/ml dexamethasone (Sigma Chemical Co.) dissolved in absolute ethanol. Intra-muscular injections of reserpine (5mg/d) and dexamethasone (20mg/d) were administered on d 14 to 17 in the rump region (Figure 1). Stock solutions were prepared prior to the injections and stored at room temperature in the absence of light. All treated cows were injected bi-weekly up to 150 DIM with rbST (Posilac, 500 mg/dose, Monsanto Dairy Business, St. Louis, MO) in the depression on either side of the tailhead beginning at the start of lactation. Twice-daily milking was initiated on d 19 and continued for 150 d into lactation.

Blood and milk sample collection

Beginning on the first day of lactation induction (d 1 ofcollected via tail veni-puncture on alternate days through d 24 (12 samples/cow) (Table 1). Beginning at calving, a total of three blood samples were collected on alternate days from nine nontreated Holstein cows and one nontreated Jersey cow to determine blood hormone content during the first week postpartum.

All samples were refrigerated for a minimum of 18 h and then centrifuged at 3000 rpm for 25 min. Serum was separated and stored at –21 C until assayed to determine concentrations of estradiol-17β, progesterone, and α-lactalbumin. Milk samples were collected from all cows on alternate days for 14 d with the first sample collected at the first am milking (experimental d 20) for a total of 7 samples. Milk samples were also collected on alternate days for the first 14 d of lactation from ten nontreated Holstein cows for later determination of hormone content. Milk samples were aliquated into three 10 ml tubes. One milk sample per cow was sonicated with three 10 second bursts at 20 KHz using a Sonicator Cell Disruptor (Heat Systems-Ultrasonics Inc. Plainview, NY) to break up milk fat globules in order to increase accuracy of progesterone determinations. Milk samples were stored at –21 C until assayed to determine concentrations of progesterone.

Hormone assays

Blood serum samples collected from ten lactation-induced Holstein cows (5 Group 1, and 5 Group 2) on d 1, 19, 21, and 23, and three blood samples, collected on alternate days from day of calving from each of nine nontreated Holstein cows and one nontreated Jersey cow, were analyzed to determine progesterone and estradiol serum concentrations present during the induction protocol and beginning of lactation. Milk samples collected on d 20, 22, 24, and 26 from 10 lactation-induced Holstein cows, and the first four milk samples collected from each of 10 nontreated Holstein cows, were also analyzed to determine progesterone concentrations utilizing purchased radioimmunoassay kits (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA (DPC). The basic radioimmunoassay procedure for detection of progesterone in blood and milk samples required that 100μl of sample and 1.0 ml of ^{125}I Progesterone be incubated for 3 h at room temperature before decanting the supernatant and determining presence of radiolabeled progesterone for 1 min in a gamma counter. The sensitivity of the standard curve ranged from 0.1 to 40 ng/ml. All samples were assayed in duplicate, and mean intra- and inter-assay CV calculated from serum pools were 6.4% and 9.7% respectively, while mean intra- and inter-assay CV calculated from milk pools were 7.5% and 8.2%, respectively.

Minor modifications were made to the radioimmunoassay procedure outlined by DPC protocol for the determination of estradiol-17β. Standards 20 pg/ml and 3600 pg/ml were omitted from this assay and standards 2 pg/ml, 5 pg/ml, and 10 pg/ml were added giving a

standard range of 2 pg/ml to 1,800 pg/ml. Modifications to the standards were made in order to detect lower concentrations of estradiol-17β in serum samples. The basic radioimmunoassay procedure for detection of estradiol in blood samples required that 100µl of sample and 1.0 ml of ^{125}I Estradiol be incubated for 3 hrs at room temperature before decanting the supernatant and determining presence of radiolabeled estradiol-17β for 1 minute in a gamma counter. The sensitivity of the standard curve ranged from 2.0 to 1800 pg/ml. All samples were assayed in duplicate and mean intra- and inter-assay CV calculated from serum pools were 9.3 % and 10.6%, respectively.

Serum concentration of α-lactalbumin in ten lactation-induced Holstein cows was determined by double-antibody RIA by the method of Akers et al. (1986). Purified α-lactalbumin was used for radioiodination and as the reference standard as outlined by Akers et al. (1986). The primary anti-body was raised in rabbits using purified α-lactalbumin (Akers et al. (1986). Sheep anti-rabbit gamma globulin was used as the second antibody (Akers, et al. (1986). The primary antiserum bound ~50% of radiolabled α-lactalbumin in the absence of non-radiolabelled α-lactalbumin. The sensitivity of the standard curve ranged from 0.1 to 25 ng/ml. Serum sample volumes of 40 µl were assayed in duplicate and mean intra- and inter-assay CV calculated from serum pools were 11.4% and 13.7%, respectively. Samples that exceeded the highest standard (25 ng/ml) were assayed a second time at a volume of 10 and 20 µl.

Statistical Analysis

The statistical model consisted of:

$$Y_{ijkl} = \mu + B_i + T_j + L_k + D_l + D*B_{il} + D*T_{jl} + D*L_{kl} + \varepsilon_{ijkl}$$

μ = grand mean

B_i = breed effect

T_j = treatment effect

L_k = lactation effect

D_l = day effect

$D*B_{il}$ = day x breed interaction

$D*T_{jl}$ = day x treatment interaction

$D*L_{kl}$ = day x lactation interaction

ε_{ijkl} = error

Milk yield data and concentrations of serum estradiol-17β, and concentration of progesterone in serum and milk samples were analyzed by least squares analysis of variance by the General Linear Model (GLM) procedure using Statistical Analysis Systems (SAS® Institute, Cary, NC).

Figure 1. Lactation-induction protocol for Groups 1 and 2 with treatment days and hormones administered.[1]

Group 1

Day -16	Day -6	Day 1-7	Day 13	Day 14-17	Day 19
$PGF_{2\alpha}$	$PGF_{2\alpha}$	$E_2\beta$ & P_4	$PGF_{2\alpha}$	Dexamethasone Reserpine	Milk 2 X daily Posilac every 2 weeks

Group 2

Day –10	Day 0	Day 1-7	Day 13	Day 14-17	Day 19
$PGF_{2\alpha}$	$PGF_{2\alpha}$	$E_2\beta$ & P_4	$PGF_{2\alpha}$	Dexamethasone Reserpine	Milk 2 X daily Posilac every 2 weeks

[1] Dosages of hormones administered: 17β-Estradiol 0.1 mg/kg BW/d; Progesterone 0.25 mg/kg BW/d; Reserpine 5 mg/d; Dexamethasone 20 mg/d; $PGF_{2\alpha}$ 25 mg/d; Posilac 500 mg/dose.

Table 1. Day of lactation-induction protocol in Holstein and Jersey cows with corresponding blood or milk sample collected.

Protocol Day[1]	Blood Sample Collected	Milk Sample Collected
1	1	
3	2	
5	3	
7	4	
9	5	
11	6	
13	7	
15	8	
17	9	
19	10	
20		1
21	11	
22		2
23	12	
24		3
26		4
28		5
30		6
32		7

[1] Day 1 = initiation of lactation induction protocol and Day 19 = initiation of lactation.

CHAPTER IV

RESULTS AND DISCUSSION

Milk Yield and Composition

Artificial induction of lactation with the current protocol yielded a 92% success rate for Holstein cows (23 of 25) with success defined as achieving >9 kg milk/d, and a 88% success rate for Jersey cows (7 of 8) with success defined as achieving >5 kg milk/d (Table 2). Eighty-eight percent of induced Holsteins produced >20 kg milk/d and 88% of Jerseys produced > 10 kg milk/d. The success rates achieved in the current study are greater than those of pervious lactation-induction studies which reported success rates of 60-70% with estrogen/progesterone injections only (Smith, 1973; Collier, 1975; Chakriyarat, 1978). Mean Milk yields of induced cows were 65% of yields of untreated control cows for the 150 d of the trial. Milk yield during the last week of the trial was approximately 82% of control Holsteins. As in earlier reports (Smith, 1973; Collier, 1976; Erb, 1976; Peel, 1978; Tervit, 1980; Lembowicz; 1982), lactation yields induced with an estrogen-progesterone protocol were lower ($P<0.05$) than yields of normally calving, untreated control cows (Tables 3 and 4). A breed difference ($P<0.05$) for total milk yields occurred because, as expected, Holstein cows had higher accumulated milk yields than Jersey cows at 30, 60, 90, and 150 DIM for both treatment groups. Mean peak milk yield was 32 kg/d for Holstein cows and 20 kg/d for Jersey cows, with mean yields at first milking of 5.3 kg/d for Holstein cows and 3.7 kg/d for Jersey cows (Figures 2 and 3). As in previous lactation-induction studies, peak milk yields were reached 7 to 8 weeks into lactation (Smith, 1973; Tervit, 1980). Smith et al. (1974) reported a rapid daily increase in milk yield during the first 10 to 12 d following induction of lactation with an estrogen/progesterone protocol. Peak milk yield for cows induced into lactation did not occur until 30 to 60 d after milking was initiated (Smith et al., 1974). Tervit et al. (1980) also reported an increase in milk yield during the first 7 to 8 weeks of lactation with peak yield occurring between 7 and 8 weeks of lactation.

Table 2. Success rate of Jersey[1] and Holstein[2] cows artificially induced into lactation.

Treatment Groups	Number of animals	Percent Success
Jersey	8	88
Holstein	25	92

[1] Milk yields > 5 kg/d were considered successful.
[2] Milk yields > 9 kg/d were considered successful.

Table 3. Milk yields[1] of nontreated and lactation-induced Holstein cows.

		Milk Yields kg ± SE			
Treatment groups[3]	Cows (n)	30DIM[2]	60DIM	90DIM	150DIM
Induced	24	608 ± 62[a]	1596 ± 114[c]	2627 ± 165[e]	4576 ± 268[g]
Nontreated	34	1303 ± 65[b]	2791 ± 120[d]	4167 ± 174[f]	6679 ± 282[h]

[1] Accumulated milk yields least squares means kg ± SE.
[2] Total milk yield for 30 DIM, 60DIM, 90DIM, and 150DIM.
[a, b, c, d, e, f, g, and h] Dissimilar superscripts in columns indicate significant differences ($p < 0.05$).

Table 4. Milk yields[1] of nontreated and lactation-induced Jersey cows.

		Milk Yields kg ± SE			
Treatment groups	Cows (n)	30DIM[2]	60DIM	90DIM	150DIM
Induced	8	415 ± 61[a]	1033 ± 142[c]	1663 ± 221[e]	2892 ± 340[g]
Nontreated	6	765 ± 103[b]	1668 ± 239[d]	2430 ± 373[f]	4156 ± 574[h]

[1] Accumulated milk yields least squares means kg ± SE.
[2] Total milk yield for 30 DIM, 60DIM, 90DIM, and 150DIM.
[a, b, c, d, e, f, g, and h] Dissimilar superscripts in columns indicate significant differences ($p < 0.05$).

Milk composition for Holstein and Jersey cows including total fat (kg), percent fat, percent protein, and somatic cell score (SCS) up to 150 DIM are presented in Tables 5 and 6.

There was a treatment effect for percent protein and total fat yield in Holstein cows whereby lactation-induced Holstein cows had greater percent protein but less total fat in milk by 150 DIM (Table 5). The higher total fat yield observed in nontreated Holstein cows is expected since these animals yielded significantly more milk by 150 DIM and fat percent was similar between the groups. There was also a treatment effect for total fat yield and SCCS in Jersey cows. Again, the higher total fat yield in nontreated Jersey cows is expected, since these animals yielded more milk by 150 DIM and milk fat percent did not differ. Percent milk fat for lactation-induced cows of both breeds was not significantly different from nontreated cows of the same breed. It has been reported in previous induction studies (Tervit, 1980; Sawyer, 1986) in agreement with this study, that percent protein is higher in lactation-induced cows compared with nontreated cows. Sawyer et al. (1986) reported that percentages of protein and fat were higher in milk from lactation-induced heifers than in milk from heifers after normal calving for the first 14 DIM. Somatic cell count score was significantly higher in milk from lactation-induced Jersey cows than from nontreated Jersey cows. This difference was apparently due to the fact that nontreated Jersey cows had abnormally low SCCS (less than 1) up to 150 DIM. There was no difference in SCCS in Holstein cows in the current study. Sawyer et al. (1986) also reported that somatic cell numbers in milk of induced heifers did not differ significantly from that of normally calving heifers.

Tervit et al. (1980) reported higher percent protein in milk of lactation-induced cows compared with controls, but also reported that percent milk fat was similar between controls and induced cows. Narendran et al. (1974) examined percentage of protein and fat in milk of lactation-induced cows and lactations following parturition and found no differences in percentage of fat and protein between the two groups. The increase in percent protein for lactation-induced Holstein cows seen in the current study is in agreement with Tervit et al. (1980). Current results support those of previous studies (Narendran, 1974; Tervit, 1980) where mean fat percentage in milk between lactation-induced cows and nontreated cows was similar. It is apparent from the current results that milk composition is altered in some induced cows (higher percent protein) when compared to nontreated cows, but the composition is still within the range of normal milk. It is also apparent that initiation of lactation-induction protocols in cows following estrous synchronization resulted in a substantial percentage of cows successfully

responding. However, further work is still needed in order to improve milk yields in lactation-induced cattle.

Table 5. Milk composition[1] of nontreated and lactation induced Holstein cows up to 150 DIM.

Treatment Group	Cows (n)	Percent Fat	Total Fat (kg)	Total Protein (kg)	Percent Protein	SCC Score
Induced	24	4.1 ± 0.1^{a}	184 ± 14^{b}	145 ± 8^{d}	3.2 ± 0.1^{f}	2.8 ± 0.5^{h}
Nontreated	34	4.1 ± 0.1^{a}	278 ± 14^{c}	189 ± 8^{e}	2.8 ± 0.1^{g}	2.6 ± 0.5^{h}

[1] Least squares means ± SEM.
[a, b, c, d, e,f, and g] Dissimilar superscripts in columns indicate significant differences ($P < 0.05$).

Table 6. Milk composition[1] of nontreated and lactation induced Jersey cows up to 150 DIM.

Treatment Group	Cows (n)	Percent Fat	Total Fat (kg)	Total Protein (kg)	Percent Protein	SCC Score
Induced	8	4.6 ± 0.2^{a}	137 ± 18^{b}	108 ± 12^{d}	3.8 ± 0.1^{f}	4.0 ± 0.4^{g}
Nontreated	6	5.2 ± 0.3^{a}	212 ± 30^{c}	143 ± 20^{e}	3.3 ± 0.2^{f}	1.5 ± 0.7^{h}

[1] Least squares means ± SEM.
[a, b, c, d, e,f, g, and h] Dissimilar superscripts in columns indicate significant differences ($P < 0.05$).

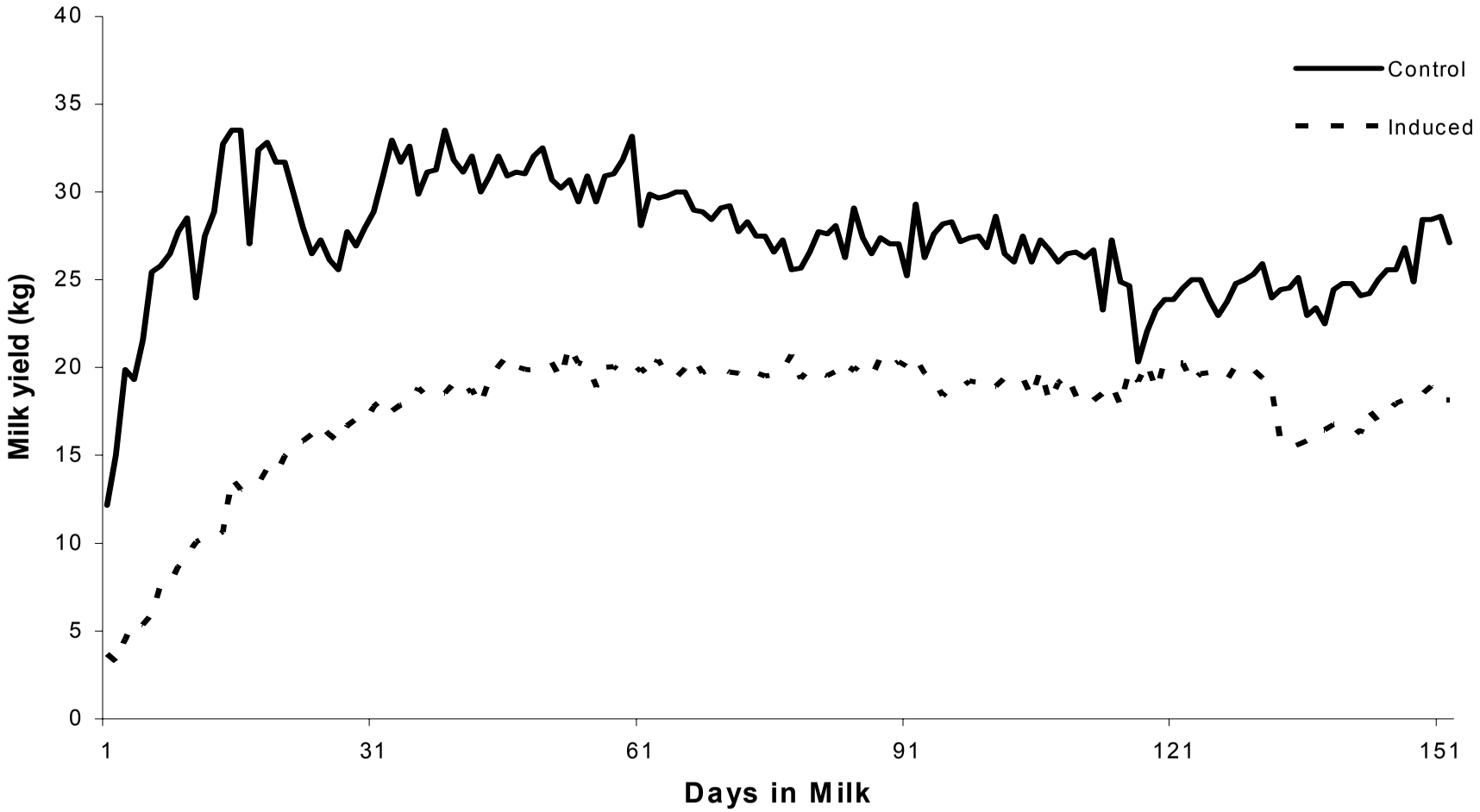

Figure 2: Mean daily milk yield for lactation-induced and nontreated Jersey cows.

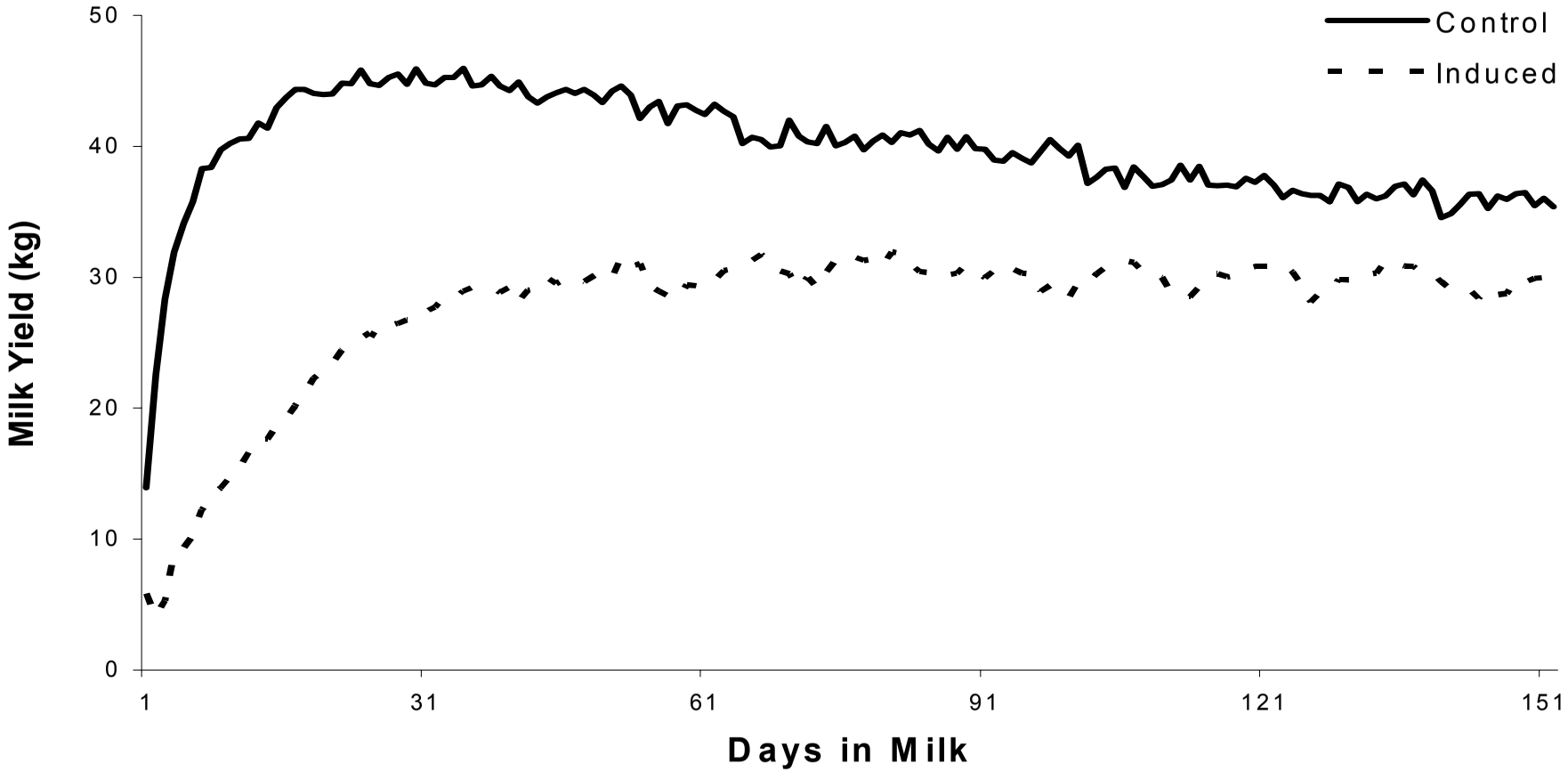

Figure 3: Mean daily milk yield for lactation induced and nontreated Holstein cows.

Serum and milk progesterone concentrations

Least squares means for progesterone (P) concentrations in serum and milk samples from ten lactation-induced cows, and ten nontreated cows are presented in Table 7. Mean concentration of P in serum samples collected at d 1, 3, and 5 of lactation were low (< 0.30 ng/ml) for both treatment groups with no significant difference between induced cows and nontreated cows. These low serum P concentrations are similar to those reported in other lactation-induction studies where samples were collected from 3 d to 24 d after last estrogen-progesterone injection (Erb, 1977; Charkriyarat, 1978; Tervit, 1980; Byatt, 1997). Erb et al. (1977) and Tervit et al. (1980) noted a decline in plasma P concentrations with values < 1 ng/ml 3-7 d after 7-d administration of estradiol-17β and progesterone ceased. Chakriyarat et al. (1978) reported that cows induced to lactate with the 7-d estrogen-progesterone protocol had plasma progesterone concentrations of 0.35 ± 0.17 ng/ml at the start of milking, or 14 d after last estrogen-progesterone injection. The slightly higher plasma P concentrations observed by Erb et al. (1977) and Tervit et al. (1980) may be related to the time samples were collected. In the current study serum samples were collected at 12, 14, and 16 d after last estrogen-progesterone treatment, and Chakriyarat collected samples 14 d after last treatment, with both studies allowing animals more time for serum P concentrations to decline. Low concentrations of P (< 0.1 ng/ml) in cows induced to lactate with the 7-d estradiol-17β and progesterone protocol were also observed by Byatt et al. (1997) in plasma samples collected 20 d after the last estradiol-17β and progesterone injection.

Concentrations of P in milk samples did not exceed 1.5 ng/ml in treated cows, with no significant difference between lactation-induced and nontreated cows. These results are similar to those reported by Sawyer et al. (1986), where milk P concentrations were between 1.0 and 3.0 ng/ml. In the current study, and in the study conducted by Sawyer et al. (1986), P concentrations in milk collected 2 to 8 DIM, and 3 to 28 d into lactation, respectively, after hormonal treatment, were less than that reported in milk collected from dairy cows during the estrous cycle. Tervit et al. (1980) also noted that progesterone concentrations in milk from lactation-induced cows did not at anytime exceed those in milk from control cows during the first 30 d after calving. Erb et al. (1976a) reported higher P during the first 4 DIM, but started to decline by d 7 and by d 14 milk P concentrations were the same as nonpregnant cows. It can be concluded from the current

results and others, that milk progesterone levels decline over time following last estradiol-17β and progesterone injection and at no time exceed those found in milk from nontreated cows during the estrous cycle. Therefore, elevated progesterone concentration in milk from cows artificially induced into lactation should not be a concern.

Table 7. Progesterone (P) concentration[1] in serum and milk of nontreated and lactation-induced Holstein cows during the first week of lactation.

		Serum P (ng/ml ± SE)		Milk P (ng/ml ± SE)			
		Day of Lactation		Day of Lactation			
Treatment Group	Cows (n)	Day 1[2]	Day3	Day2	Day4	Day6	Day8
Induced	10	0.15 ± 0.03	0.12 ± 0.02	0.26 ± 0.13	0.20 ± 0.15	0.14 ± 1.35	0.38 ± 0.43
Nontreated	10	0.15 ± 0.02	0.11 ± 0.01	0.33 ± 0.09	0.19 ± 0.11	1.27 ± 0.90	0.76 ± 0.31

[1] Least squares means ± SEM.
[2] Day represents DIM.
[3] Lactation-induced cows started the experiment during either high or low serum progesterone levels.

Serum estradiol concentrations

Treatment and lactation least squares means for estradiol (E) concentrations in serum are presented in Tables 8 and 9. As expected, concentrations of E in serum samples from lactation-induced and nontreated cows declined 12 to 14 d (d 1 and 3 of lactation) after estrogen-progesterone administration. A rapid decrease in serum E concentrations occurred after calving in nontreated cows, in agreement with previous reports (Dobson, 1974; Kesler, 1976; Erb, 1977; Keller, 1977). There was a significant day effect ($p < 0.05$) for serum E concentrations in samples collected at 1, 3, and 5 DIM. There was no treatment difference in serum E concentrations in samples collected at 1 DIM, but at 3 DIM, lactation-induced cows in Group 1 had higher ($p < 0.05$) serum E concentrations than Group 2 and nontreated cows (Table 8). At 5 DIM, both treatment groups had higher ($p < 0.05$) serum E concentrations than nontreated cows (Table 9). Cows in their third lactation had higher ($p < 0.05$) serum E concentrations at 3 DIM compared to cows in their second lactation (Table 9). A range of 7.6-132 pg/ml for serum E concentration was observed in induced cows, while serum E levels in nontreated cows were 2-

21.5 pg/ml 2 d after calving. Serum E concentrations in lactation-induced cows 16 d after estradiol-17β and progesterone injections (5 d into lactation) were lower than mean serum E (52 ± 3 ng/ml) reported by Erb et al. (1977) in lactation-induced cows 3-25 d in lactation following a 7-d estrogen-progesterone lactation-induction protocol. Data from the present study support findings of Chakriyarat et al. (1978) in that serum E concentrations decline as time from last estradiol-17β and progesterone injection increases, until reaching concentrations similar to those prior to treatment. In the current study, serum E concentrations in lactation-induced cows at last sampling (5 DIM) were similar to those in cows in estrus (31 ± 11 pg/ml) as reported by Monk et al. (1975). Cows induced into lactation using an estradiol-17β and progesterone protocol exhibit an increase in serum E concentrations throughout the induction scheme, but serum E levels decline in a linear fashion within the first 3-4 d of milking as observed in normal calving cows following parturition (Monk et al., 1975).

Milk E concentrations were not determined in the present study, but E values in serum would suggest that elevated E concentrations in milk from cows artificially induced into lactation would not exceed those of normally cycling cows by 5 DIM and would likely not be a cause of concern for consumer health.

Table 8. Estradiol (E) concentration[1] in serum of nontreated and lactation- induced Holstein cows during the first week of lactation.

		Serum E (pg/ml ± SE)		
		Day of Lactation		
Treatment Groups	Cows (n)	Day[2]1	Day3	Day5
Induced	10	45 ± 45[a]	41 ± 10[d]	18 ± 3[e]
Nontreated	10	155 ± 48[b]	6 ± 10[d]	3 ± 3[f]

[1] Treatment Least Squares Means ± SEM.
[2] Day represents DIM.
[a, b, c, d, e] Dissimilar superscripts in columns are significantly different (P <0.05).

Table 9. Estradiol (E) concentration[1] in serum of nontreated and lactation- induced Holstein cows during first and second lactation.

		Serum E (pg/ml ± SE)		
		Day of Lactation		
Lactation Number	Cows (n)	Day[3]1	Day3	Day5
Lactation 2	11	44 ± 55[a]	13 ± 8[b]	11 ± 3[d]
Lactation 3	9	118 ± 51[a]	43 ± 7[c]	14 ± 3[d]

[1] Lactation Least Squares Means ± SEM
[2] Cows in lactation 2 and lactation 3 or greater, including all lactation-induced cows and nontreated cows.
[3] Day represents DIM
[a, b, c, d,] Dissimilar superscripts in columns are significantly different (P <0.05)

Serum α-lactalbumin concentrations

Mean alpha-lactalbumin concentrations in serum samples collected throughout the lactation-induction scheme from ten Holstein cows for the first 5 DIM are presented in Figure 4. The importance of monitoring serum concentrations of α-lactalbumin is that it plays an important role in lactose synthesis (Akers, 1986; Tucker, 2000) and is one of two mammary cell proteins required for lactose synthesis to occur. Monitoring concentration of α-lactalbumin in serum samples of lactation-induced cows provides an indication of the success of the protocol in initiating lactogenesis. Serum concentrations of α-lactalbumin increased in all lactation-induced cows just prior to, or at the initiation of milking on d 19, reaching concentrations as high as 633 ng/ml, which is similar to the time that α-lactalbumin concentrations increased in cows at normal parturition (McFadden et al., 1987). The increase in serum α-lactalbumin concentrations at, or just prior to the initiation of milking, indicates that milk synthesis (lactogeneis) occurred at this time. McFadden et al. (1987) reported that plasma α-lactalbumin concentrations in pregnant Holstein cows increased during the last 50 d of gestation as parturition approached, then reached a maximum of ~900 ng/ml at parturition and declined during the next 3 d to concentrations of

~490 ng/ml. McFadden et al. (1987) hormonally induced lactation (1-7 d estrogen/progesterone protocol, followed immediately with 4 injections of reserpine on alternate days, then concluding with 3 dexamethasone injections) in 12 Holstein cows and measured concentrations of α-lactalbumin throughout the treatment period. These researchers reported that α-lactalbumin concentrations in induced nonpregnant dry cows averaged 15.4 ng/ml during the first 9 d of treatment. Concentrations increased 3 d after the final estrogen-progesterone injection during the time of reserpine administration, and continued to rise over the next 6 d reaching a maximum of ~800 ng/ml, declined over the next 3 d until stabilizing at 185 ng/ml (McFadden et al., 1987). Serum α-lactalbumin concentrations in serum samples in the present study did increase during the treatment period, but not until d 14 of the experiment or 7 d after the last estrogen-progesterone injection, similar to results of McFadden et al. (1987), but there was a difference in the time associated with the increase in α-lactalbumin concentrations. These results likely differ due to differences in times of reserpine and dexamethasone injections between the two induction protocols. McFadden et al. (1987) administered estrogen and progesterone for 7 d followed immediately by 4 injections of reserpine on consecutive days. In the present study we administered estrogen-progesterone for 7 d, then 7 d later administered both reserpine and dexamethasone for 4 d. The difference in time of increase in α-lactalbumin concentrations may be due to differences in time of reserpine injections. Goodman et al. (1983) reported that prolactin induced synthesis and secretion of α-lactalbumin in mammary tissue explants obtained from mulitparous dairy cows. This would suggest that the increase in prolactin levels associated with administration of reserpine likely caused the increase in serum α- lactalbumin concentrations at different times between the current study and that conducted by McFadden et al. (1987). Since serum samples were only collected during the first 5 DIM in the present study, a trend after that time cannot be determined. It can be concluded from the serum α-lactalbumin concentrations that mammary cell differentiation and lactogenesis likely occurred in treated cows prior to the start of milking, but at various times for different cows. There was no apparent relationship between the concentration of α-lactalbumin throughout the lactation- induction protocol and milk yield following treatment.

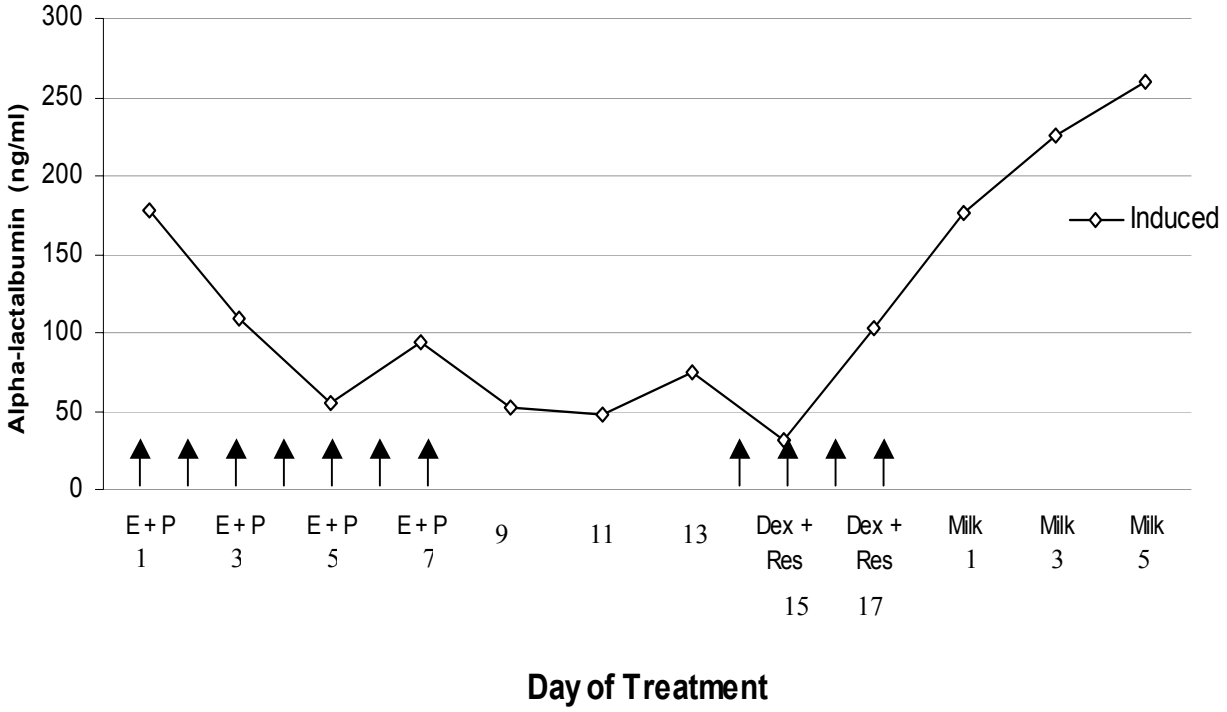

Figure 4: Mean serum α-lactalbumin concentrations in lactation-induced cows,

Reproductive performance

Mean measures of reproductive performance for lactation-induced cows and untreated control cows are presented in Table 10. Reproductive measures were not subjected to statistical analysis because a proportion of nontreated cows were intentionally held open to accommodate a seasonal calving schedule. Thus, days- to-first-service were not statistically analyzed because a majority of nontreated cows were not inseminated on first natural estrus, but were subjected to the Pre-Synch program. Absolute values for days to first service were lower for all treatment groups than those of the control group. Current results support those of Collier et al. (1975), in that lactation-induced cows experienced first service earlier in the lactation period than control cows. Lactation-induced cows were in a more positive energy balance at the start of milking and were inseminated on first estrus, whereas nontreated cows were inseminated at second or third estrus.

It is important to note that control cows were subjected to a voluntary waiting period greater than 45 d after calving, whereas lactation-induced cows were not. It was decided that this voluntary waiting period was not necessary for induced cows since they did not calve and their

reproductive tracts should have been normal. Also, the nonpregnant, lactation-induced cows would be expected to be in a more positive energy balance in early lactation compared to normally calving cows, since milk yields were substantially lower. Mean services per conception (1.25) for all lactation-induced cows were similar to means of control cows (1.32)). Greater than 60% of induced cows were inseminated and conceived without any special practices to improve reproduction in these former "problem breeders". Mean pregnancy rate, defined as the number of cows pregnant divided by the number of cows inseminated, was 66% (21 of 32) for induced Jersey and Holstein cows. The pregnancy rates achieved in the present study are similar to means reported by Collier et al. (1976) (56%) and Peel et al. (1978) (90%). Overall, reproductive performance of lactation-induced cows did not appear to be negatively affected by administering the various steroid hormones during the lactation-induction protocol and was comparable to that of cows with parturient lactation. Once the lactation-induction protocol was completed and the treated cows initiated lactation, many successfully became pregnant. These former nonbreeder cows were able to be salvaged and become pregnant following successful artificial induction of lactation. By using artificial induction of lactation as a management tool to salvage nonbreeder cows a producer may be able to gain an additional, somewhat reduced lactation, and an additional calving to initiate a subsequent normal lactation.

Table 10. Reproductive performance of nontreated and lactation-induced Holstein and Jersey cows.

	Means for Reproductive Performance Measures					
Treatment Groups	Cows (n)	Cows Used	Days to 1st service	Days to conception	Services/ conception	Percent Pregnant[1]
Nontreated Jersey	6	4	86	92.17	1.16	67
Induced Jersey	8	6	46	46	1.0	75
Nontreated Holstein	30	13	95	101	1.47	44
Induced Holstein	25	18	53	76	1.5	64

[1] Percent pregnant is defined as the number of cows pregnant divided by the number of cows inseminated x 100

Financial comparison of induced-lactation vs. replacement heifer

The use of artificial induction of lactation as a management tool on farms may allow dairy producers two options for open cows at the end of their lactation. Producers could cull open cows and replace them, or attempt to artificially induce lactation in nonbreeder cows to salvage them. To determine which option to follow, a comparison of the costs required to induce a cow into lactation (setup costs) versus replacing her with a heifer (high replacement costs or low replacement costs) must be made. Percent lactation-induction success (Table 11) and mean milk yields (Table 4) from the current study have been used for this comparison (costs for Holstein cows and heifers were used for all calculations). The comparisons were made at four combinations of net replacement heifer prices and net milk prices H/HM, H/LM, L/HM, L/LM.

Any open cow that is sold and replaced with a heifer results in a net cost to the producer of $1300 ($1800 replacement cost - $500 salvage value) for high replacement costs and $750 ($1200 replacement cost - $450 salvage value) for low replacement costs. If an open cow is kept and placed on a lactation-induction protocol, there are 3 possible outcomes for this animal: 1) the cow is induced and is not successful in producing > 9 kg milk/d by 30 DIM, 2) the cow is successfully induced into lactation, but does not conceive, 3) the cow is successfully induced into lactation and conceives. The current study had animals represented in each of the three possible categories, with 10% unsuccessful (outcome 1), 25% successful, but not pregnant at 150 DIM (outcome 2), and 65% successful and pregnant at 150 DIM (outcome 3).

A partial budget that accounts for additional costs for the induced cow will be used to determine the economic feasibility of inducing lactation to salvage nonbreeders. For a complete cost comparison, total costs for the dry period required before lactation induction are determined along with costs associated with the loss of a calf, lactation induction costs (hormones), replacement costs at the end of lactation, and loss in milk yield (Table 11). The feed cost for 60 d dry period was determined to be $1.50/d. The cost of lactation induction varies depending on the success of the protocol. Cows that are not producing > 9 kg milk/d at the end of 30 DIM are considered unsuccessful and will be culled and replaced. Thus, these animals do not receive the full bST regimen that is calculated in the cost of lactation induction. The setup costs for animals in this group are: dry period ($90), induction cost ($50), and loss of calf ($250). The cost of loss

in milk yield for 30 DIM is (600kg))*($17.96/100kg milk at the low milk prices(LM) and $31.19/100kg milk at the high milk prices (HM)) and the cost of a replacement animal ($1300 for high replacement costs (H) and $750 for low replacement costs (L)). These combined costs yielded a total of $1877 using H/HM, $1798 H/LM, $1327 L/HM, and $1248 L/LM (see Table 11). Cows that are successfully induced into lactation but do not conceive by 150 DIM cost $1620 H/HM, $1401 H/LM, $1345 L/HM, and $1126 L/LM. The replacement costs ($650) represented for animals in outcome two (successfully induced into lactation but do not conceive) considers that approximately 12.5 % of the replacement heifers will not normally conceive following a first lactation. Cows that are successfully induced into lactation and conceive, have lower costs compared to the other two possible lactation induction outcomes. Total cost at 150 DIM for these cows is $970 H/HM, $751 H/LM, $970 L/HM, and $751 L/LM. Weighting the net costs for the three possible outcomes by the percentage occurrence from the present study (outcome 1 = 10%, 2 = 25% and 3 = 65%) incurs an average net cost of $1223 H/HM, $1018 H/LM, $1099 L/HM, and $895 L/LM. From these calculations, it seems evident that the use of artificial induction of lactation on farms could be beneficial for producers who want to salvage superior cows when replacement costs are high, but older cows of less than average ability and/or with more health problems should not be induced. Any improvements in lactation-induction protocols that result in greater success rates, greater milk yields, or improvements in rebreeding success will make this approach more attractive to producers attempting to reduce culling losses.

Magliaro et al. (1999) and Kensinger et al. (2000) compared the profitability of inducing lactation in order to salvage nonpregnant cows versus purchasing replacement heifers. Both scientists found it more profitable ($520) to induce lactation in nonbreeder cows compared to purchasing replacement heifers. The calculated net present value of a nonpregnant cow induced into lactation in these two studies was greater than that calculated in the current study. The current study includes in the calculation the increased value of replacement heifers and a greater net loss for the loss of a calf prior to the initiation of induced lactation. These two financial differences in the current calculations offer some insight in the difference in total net loss between the two profitable comparisons made by Magliaro et al. (1999) and Kensinger et al. (2000) and
the current trial.

Table 11. Financial comparison of inducing nonbreeder cows into lactation vs. purchasing replacement heifers.

				I N C R E A S E D C O S T[1]			
Lactation Induction (Outcome)	Setup costs[2]	Loss in Milk Yield (kg)	Replacement Fraction	H[3]/HM[4]	H/LM[4]	L[3]/HM	L/LM
Unsuccessful lactation (10%)	$390	600	1	$1877	$1798	$1327	$1248
Successful lactation, No conception (25%)	$455	1650	0.5	$1620	$1401	$1345	$1126
Successful lactation, Conceived (65%)	$455	1650	0	$970	$751	$970	$751
Weighted Average[5]				$1223	$1018	$1099	$895
Net Cost of a Replacement Heifer				$1300	$1300	$750	$750
Difference[6]				-$77	-$282	$349	$145

[1] [Increased cost = setup costs + (Loss in milk yield (kg)/100)* (High (HM) or Low (LM) net milk price per 100kg) + Replacement Fraction * (High replacement net cost (H) or Low replacement net cost (L)]

[2] Set up costs ($390 or $455) included loss of a calf ($250), feed cost $1.50/d for 60 d dry period ($90), lactation induction hormones ($50),.for unsuccessful lactation or those cost plus bi-weekly bST (Posilac), and supplies ($65 for 150 DIM).

[3] High net replacement costs (H) = $1800 – cull cow value (500) = $1300
Low net replacement costs (L) = $1200 – cull cow value (450) = $750

[4] HM= High net milk price = High milk price/100kg ($39.68) – feed cost for added milk ($8.49) = $31.19/100kg.
LM =Low net milk price = Low milk price/100kg ($26.44) – feed cost for added milk ($8.49) = $17.96./100kg

[5] Weighted average increased cost over the three possible outcomes for each combination of prices.

[6] Difference between the induction alternative and purchasing a replacement straight away. Positive values indicate that induction was more expensive, negative values indicate that induction was less expensive than replacement.

CHAPTER V

CONCLUSIONS

In this experiment and others (Collier, 1975; Erb, 1976; Chakriyarat, 1978; Peel, 1978; Lembowicz, 1982), modifications to the 7-d estrogen/progesterone induction protocol developed by Smith and Schanbacher (1973) have been employed with the goal of improving success rates (proportion of animals responding to the induction treatment) and reducing the variability in milk yields among lactation-induced cows. In an attempt to improve success rates and reduce variability in milk yields, cows were subjected to estrous synchronization prior to the lactation-induction protocol.

Mean success rates of 92% and 88% were achieved for Holstein and Jersey cows, respectively. Smith and Schanbacher (1973) achieved success rates of 60% following a 7-d treatment with estradiol-17β and progesterone alone. The proportion of cows responding in this experiment was also higher than those of previous induction studies (Collier, 1975; Chakriyarat, 1978), which achieved success rates between 60 and 70% with the 7-d estrogen/progesterone treatment. Peak milk yields from induced cows were reached 7-8 weeks into lactation, with a mean peak yield of 32 kg/d for Holstein cows and 20 kg/d for Jersey cows.

Milk from lactation-induced cows was similar in composition to milk from nontreated cows in percent fat, but differed significantly in somatic cell count score (SCCS) and total fat yield for Jersey cows, and percent protein and total fat yield for Holstein cows. The increase in percent protein reported in this study is in agreement with other lactation-induction studies (Tervit, 1980; Sawyer, 1986). Somatic cell count score was significantly higher in lactation-induced Jersey cows, but this probably resulted from nontreated cows having abnormally low scores for the 150 DIM because there was no difference in SCCS in Holstein cows. A significant difference in total fat yield was expected between nontreated cows and lactation-induced cows because nontreated cows produced more milk by 150 DIM with similar milk fat percent, and therefore had significantly higher total fat by 150 DIM. Although SCCS and percent protein in

milk from lactation-induced cows differed significantly from nontreated cows, these values were still within range of normal milk.

Concentrations of P, E, and α-lactalbumin in serum, and milk P concentration were similar to those results obtained in previous lactation-induction studies (Erb, 1977; Charkriyarat, 1978; Tervit, 1980; Byatt, 1997). Progesterone concentrations in serum and milk during the first 8 DIM did not exceed those of nontreated cows. Concentrations of E in serum samples collected at 3 and 5 DIM were significantly higher in lactation-induced cows than nontreated cows. Nontreated cows had higher (not significant) serum estradiol concentrations at the start of lactation than lactation-induced cows, but they declined to values significantly lower than lactation-induced cows for samples collected at 5 DIM. By 5 DIM serum E concentrations in cows in the present study were similar to those of cows in estrus (Monk et al., 1975). Elevated P and E concentrations in milk from cows artificially induced into lactation should probably not be a concern, since serum concentrations of both these hormones at 5 DIM in the present study are similar to those of nontreated cows during the estrous cycle.

Serum α-lactalbumin concentrations were measured during the lactation-induction treatment and the first 5 DIM. As expected, concentrations of α-lactalbumin in serum samples increased during the time of reserpine and dexamethasone injection in lactation-induced cows. This observation was also reported by McFadden et al. (1987) when lactation-induced cows were administered reserpine alone. There was no apparent relationship between the concentration of α-lactalbumin in serum samples collected throughout the lactation-induction protocol and milk yield following treatment.

Artificially inducing lactation in nonpregnant Holstein and Jersey cows improved reproductive performance after lactation was established. Pregnancy rate was 70% across both breeds by 150 DIM. Cows induced into lactation had lower days to first service than nontreated cows, because they can be bred earlier after initiation of lactation , and nontreated cows may often have to be grouped and bred utilizing a programmed estrous and ovulation control regimen. The number of services per conception was similar among lactation induced and nontreated cows.

In conclusion, this study confirms earlier reports that reproductive performance (Collier, 1975; Peel, 1978) and milk composition (Narendran, 1974; Tervit, 1980; Sawyer, 1986) are not adversely affected by the artificial induction of lactation. Although the use of estrous

synchronization prior to the lactation induction protocol was successful in increasing the proportion of animals responding to the induction treatment compared to other studies, milk yields were not increased. Lactation induced cows produced 65% of milk yields achieved by 150 DIM compared to nontreated cows.

Current results suggest that synchronizing estrous cycles of cows prior to initiation of lactation-induction protocol with two injections of $PGF_{2\alpha}$ 11 d apart, improves success rates of induced lactations, but this method of induction of lactation was not successful in increasing milk yields. Further research in the area of artificial induction of lactation with various programs for estrous synchronization and/or using other modifications to this protocol may increase milk yields of cows induced into lactation.

LITERATURE CITED

Akers, R. M. 2002. Lactation and the mammary Gland. Iowa State Press, Iowa.

Akers, R. M., D. E. Bauman, A. V. Capuco, G. T. Goodman, and H. A. Tucker. 1981. prolactin regulation of milk secretion and biochemical differentiation of mammary epithelial cells in periparturient cows. Endocrinology 109:23.

Akers, R. M., T. B. McFadden, W. E. Beal, A. J. Guidry, and H. M. Farrell. 1986. radioimmunoassay for measurement of bovine α-lactalbumin in serum, milk, and tissue culture media. J. Dairy Res. 53:419.

Asmiov, G. J. and N. K. Krouse. 1937. The lactogenic preparations from the anterior pituitary and the increase in milk yield in cows. J. Dairy Sci. 20:289.

Barbano, D. M., J. M. Lynch, and D. E. Bauman. 1992. Effect of a prolonged-release formulation of n-methionyl bovine somatotropin (Sometribove) on milk composition. J. Dairy Sci. 75:1775.

Bauman, D. E. 1992. Bovine somatotropin: review of an emerging animal technology. J. Dairy Sci. 75:3432.

Bauman, D. E., R. J. Collier, and H. A. Tucker. 1977. Effect of reserpine on serum prolactin, growth hormone, and glucocorticoids in dairy cows. Proc. Soc. Exp. Biol. Med. 155:189.

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):121 (Abstr.).

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.

Bauman, D. E., D. L. Hard, B. A. Crooker, M. S. Partridge, K. Garrick, L. D. Sandles, H. N. Erb, S.E. Franson, G. F. Hartnell, and R. L. Hintz. 1989. Long-term evaluation of a prolonged-release formulation of n-methionyl bovine somatotropin in lactating diary cows. J. Dairy Sci. 72:642.

Beal, W.E. 1998. Current estrus synchronization and artificial insemination programs for cattle. J. Anim. Sci. 76 (Suppl. 3):30.

Berardinelli, J. G. and R. Adair. 1989. Effect of prostaglandin $F_{2\alpha}$ dosage and stage of estrous cycle on the estrous response and corpus luteum function in beef heifers. Theriogenology 32:301.

Burfening, P. J., D. C. Anderson, R. A. Kinkie, J. Williams, and R. L. Friedrich. 1978. synchronization of estrus with $PGF_{2\alpha}$ in beef cattle. J. Anim. Sci. 47:999.

Burton, J. H., B. W. McBride, K. Bateman, G. K. MacLeod, R. G. Eggert. 1987. recombinant bovine somatotropin: effects on production and reproduction in lactating cows. J. Dairy Sci. 70 (Suppl. 1):175 (Abstr.).

Burton, J. L., B. W. McBride, J. H. Burton, and R. J. Eggert. 1990. Health and reproductive performance of dairy cows treated for up to two consecutive lactations with bovine somatotropin. J. Dairy Sci. 73:3258.

Burvenich, C., G. Vandeputte-Van Messom, E. Roets, J. Fabry, and A-M. Massart-Leen. 1989. Effect of bovine somatotropin on milk yield and milk composotion in periparturient cows experimentally injected with *Escherichia coli*. Page 177 *in* Use of somatotropin in livestock production. K Sejersen, M. Vestergarrd, and A. Neimann-Sorensen, ed. Elsevier Appl. Sci., New York, NY.

Byatt, J. C., R. H. Sorbet, P. J. Eppard, T. L. Curran, D. F. Curran, and R. J. Collier. 1997. The effect of recombinant bovine placental lactogen on induced lactation in dairy heifers. J. Dairy Sci. 80:496.

Chakriyarat, S., H.H. Head, W.W. Thatcher, F.C. Neal, and C.J. Wilcox. 1978. Induction of lactation: Lactational, Physiological, and Hormonal Responses in the bovine. J. Dairy Sci. 61:1715.

Chalupa, W., D. T. Galligan, and W. E. March, 1987. Single lactation responses of cows supplemented with somatotropin daily for 266 days. Proceedings of the National Invitation Workshop on bovine somatotropin. St. Louis, MO. Page 34.

Cole, W. J., P. J. Eppard, E. M. Lanza, R. L. Hintz, S. E. Madsen, S. E. Franson,, T. C. White, W. E. Ribelin, B. G. Hammond, S. C. Bussen, R. K. Ceak, and L. E. Metzger. 1988. Response of lactating dairy cows to multiple injections of Sometribove, USAN (recombinant methionyl bovine somatotropin) in a prolonged release system. Part II. Health and reproduction. J. Dairy Sci. 71 (Suppl. 1): 184 (Abstr).

Collier, R.J., D.E. Bauman, and R.L. Hays. 1975. Milk production and reproductive performance of cows hormonally induced into lactation. J. Dairy Sci. 58:1524.

Collier, R.J., D.E. Bauman, and R.L. Hays. 1977. Effects of reserpine on milk production and serum prolactin of cows hormonally induced into lactation. J. Dairy Sci. 60:896.

Collier, R.J., W.J. Croom, D.E. Bauman, R.L. Hays, and D.R. Nelson. 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: lactose and fatty acid synthesis. J. Dairy Sci. 59:1226.

Convey, E.M. 1974. Serum hormone concentrations in ruminants during mammary growth, lactogenesis, and lactation. A review. J. Dairy Sci. 57:905.

Cooper, M. J. 1974. Control of the oestrous cycles of heifers with a synthetic prostaglandin Analogue. Vet. Rec. 95:200.

Croom, W. J., R. J. Collier, D. E. Bauman, and R. L. Hays. 1976. Cellular studies of mammary tissue from cows hormonally induced into lactation: Histology and ultrastructure. J. Dairy Sci. 59:1232.

Dahl, G. E., L. T. Chapin, M. S. Allen, W. M. Moseley, and H. A. Tucker, 1991. Comparison of somatotropin and growth hormone-releasing factor on milk yield, serum hormones, and energy status. J. Dairy Sci. 74:3421.

DeLouis, C., J. Djiane, G. Kann, M. Tergui, and H.H. Head. 1978. Induced lactation in cows and heifers by short-term treatment with steroid hormones. Ann. Biol. Anim. Bioch. Biophys. 18:721

Djiane, J., and P. Duran. 1977. Prolactin-progesterone antagonism in self regulation of prolactin receptors in the mammary gland. Nature. 266:641.

Dobson, H., and P. D. G. Dean. 1974. Radioimmunoassay of oestrone, estradiol-17α and -17β in bovine plasma during the oestrous cycle and last stage of pregnancy. J. Endocrinol. 61:479.

Ducker, M. J., R. A. Haggett, W. J. Fisher, S. V. Morant, and G. A. Bloomfield. 1985. Nutrition and reproductive performance of dairy cattle. Animal Prod. 41:1.

Edgerton, L. E. and H. D. Hafs. 1973. Serum luteinizing hormone, prolactin, glucocorticoid and progestin in dairy cows from calving to gestation. J. Dairy Sci. 56:451.

Elvinger, F., H. H. Head, C. J. Wilcox, R. P. Natzke, and R. G. Eggert. 1988. Effects of administration of bovine somatotropin on milk yield and composition. J. Dairy Sci. 71:1515.

Eppard, P. J., D. E. Bauman, C. R. Curtis, H. N. Erb, G. M. Lanza, and M. J. DeGeeter. Effect of 188-day treatment with somatotropin on health and reproductive performance of lactating cows. J. Dairy Sci. 70:582.

Erb, R.E., E.L. Monk, T.A. Mollett, P.V. Malven, and C.J. Callahan. 1976 a. Estrogen, progesterone, prolactin, and other changes associated with bovine lactation induced with estradiol-17β and progesterone. J. Anim. Sci. 42:644.

Erb, R.E., P.V. Malven, E.L. Monk, T.A. Mollett, K.L. Smith, F.L. Schanbacher, and L.B. Willett. 1976 b. Hormone induced lactation in the cow. IV. Relationships between lactational performance and hormone concentrations on blood plasma. J. Dairy Sci. 59:1420.

Fleming, J. R., H. H. Head, K.C. Bachman, H.N. Becker, and C.J. Wilcox. 1986. Induction of lactation: histological and biochemical development of mammary tissue and milk yields of cows injected with estradiol-17β and progesterone for 21 days. J Dairy Sci. 69:3008.

Folley, S. J. and F. H. Malpress. 1944. The chemical composition of bovine mammary secretions induced by the subcutaneous implantation or oral administration of synthetic estrogens. J. Endocrinol. 4:37.

Fulkerson, W.J. 1979. *Hormonal control of lactation*, *Volume* 1. Annual Research Reviews, ed DF Horrobin. Eden Press, Montreal Canada.

Fulkerson, W. J. and G. H. McDowell. 1975. Artificial induction of lactation in cattle by use of dexamethasone trimethylacetate. Aust. J. Biol. Sci. 28:183.

Garcia-Winder, M. J. and J. Gallegos-Sanchez. 1991. Estrus synchronization in Holstein cows using reduced doses of prostaglandin $F_{2\alpha}$. Theriogenology 36:191.

Goodman, G. T., R. M. Akers, K. H. Friderici, and H. A. Tucker. 1983. Hormonal regulation of α-lactalbumin secretion from bovine mammary tissue cultured *In vitro*. Endocrinology 112:1324.

Goral, H. E., R. W. Turnell, and J. L. Wittliff. Properties of progesterone-binding proteins in mammary tissue. Proc. Am. Assoc. Cancer. Res. 16:154(Abstract).

Hammond, J. and F.T. Day. 1944. Oestrogen treatment of cattle: induced lactation and other effects. J Endocrinol. 4:53.

Hancock, J., P. J. Brumby, and C. W. Turner. 1954. Hormonal induction of lactation in identical twin dairy cattle. NZ J. Sci. Technol. 36:111.

Hard, D. L., W. J. Cole, S. E. Franson, W. A. Samuels, D. E. Bauman, J. T. Huber, and R. C. Lamb. 1988. Effect of long term sometribove, USAN (recombinant methionyl bovine somatotropin), treatment in a prolong release system on milk yield, animal health, and reproductive performance-pooled across four sites. J. Dairy Sci. 71 (Suppl. 1):210.

Hartnell, G. F., S. E. Franson, D. E. Bauman, H. H. Head, J. T. Huber, R. C. Lamb, K. S. Madsen, W. J. Cole, and R. L. Hintz. 1991. Evaluation of sometribove in a prolonged-release system in lactating dairy cows-production responses. J. Dairy Sci. 74:2645.

Heald, C.W. 1974. Hormonal effects on mammary cytology. J. Dairy Sci. 57:917

Heap, R. B., M. Gwyn, J. A. Laing, and D. E. Walters. 1973. Pregnancy diagnosis in cows; changes in milk progesterone concentration during the oestrous cycle and pregnancy measured by a rapid radioimmunoassay. J. Agr. Sci. Camb. 81:151.

Huber, J. T., S. Wilman, K. Marcus, C. B. Theurer, D. Hard, and L. Kung, Jr. 1988. Effect of sometribove (SB), USAN (recombinant methionyl bovine somatotropin) injected in lactating cows at 14-d intervals on milk yields, milk composition and health. J. Dairy Sci. 71 (Suppl. 1): 207.

Ingalls, W. G., E. M. Convey, and H. D. Hafs. 1973. Bovine serum LH, GH, and prolactin during late pregnancy, parturition and early lactation. Proc. Soc. Exp. Biol. Med. 143:161.

Keller, H. F., B. P. Chew, R. E. Erb, and P. V. Malven. 1977. Mammary transfer of hormones and major constituents when cows are milked or secretions are sampled prepartum. J. Dairy Sci. 60:1000.

Kensinger, R.S. 2000. Induced lactation physiology, perception, profitability and propriety. J Dairy Sci. 83 (Suppl. 1): 23.

Kensinger, R.S., R. Graboske, and A.L. Magliaro. 1998. Somatotropin augments milk yields of cows induced into lactation. J.Anim. Sci. 76 (Suppl. 1): 210.

Kesler, D. J., R. C. Peterson, R. E. Erb, and C. J. Callahan. 1976. Concentrations of hormones in blood and milk during and after induction of parturition in beef cattle with dexamethasone and estradiol-17β. J. Anim. Sci. 42:918.

Kim, J., R. C. Campling, and J. I. D. Wilkinson. 1991. Evaluation of a slow-release form of recombinantly derived bovine somatotropin in dairy cattle. Anim. Prod. 52:49.

King, M. E., G. H. Kiracofe, J. S. Stevenson, and R. R. Schalles. 1982. Effect of stage of the estrous cycle on interval to estrus after $PGF_{2\alpha}$ in beef cattle. Theriogenology 42:79.

King, G. J. and H. A. Roberston. 1974. A two injection schedule with prostaglandin $F_{2\alpha}$ for the regulation of the ovulatory cycle of cattle. Theriogenology 1:123.

Lauderdale, J. W. 1972. Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246 (Abstr.).

Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C.K. Vincent, and F. Loyancano. 1974. Fertility of cattle following $PGF_{2\alpha}$ injection. J. Anim. Sci. 38:964.

Lembowicz, K., A. Rabek, and L. Skrezeczkowski. 1982. Hormonal induction of lactation in the cow. Br. Vet. J. 138:203.

Leonard, M., M. Gallo, G. Gallo, and E. Block. 1990. Effects of a 28-day sustained release formulation of recombinant bovine somatotropin (rbST) administered to cows over two consecutive lactations. Can. J. Animal Sci. 70:795.

Li, C. H., H. M. Evans, and M. E. Simpson. 1945. Isolation and properties of the anterior hypophyseal growth hormone. J Biol. Chem. 159:353.

Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrous cycles. J. Anim. Sci. 35:247 (Abstr.).

Louis, T. M. H. D. Hafs, and D. A. Morrow. 1974. Intrauterine administration of prostaglandin $F_{2\alpha}$ in cows: Progesterone, estrogen, LH, estrus and ovulation. J. Anim. Sci. 38:347.

Louis, T. M., H. D. Hafs, and B. E. Seguin. 1973. Progesterone, LH, estrus and ovulation after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 143: 152.

Magliaro, A.L., S.A. Ford, L. O'Connor, L.D. Muller, R.Graboski, and R.S. Kensinger. 1999. Induced lactation of nonpregnant cows or use of replacement heifers: a profitability comparison. J Dairy Sci. 82 (Suppl. 1):19.

McFadden, T. B., R. M. Akers, and G. W. Kazmer. 1987. Alpha-lactalbumin in bovine Serum: relationships with udder development and function. J. Dairy Sci. 70:259.

Meites, J. 1961. Hormonal induction of lactation and galactopoiesis. Page 321 *in* Milk: the mammary gland and its secretions. Vol. 1. S. K. Kon and A. T. Cowie, eds. Academic Press, New York and London.

Monk, E.L., R.E. Erb, and T.A. Mollett. 1975. Relationships between immunoreactive estrone and estradiol in milk, blood, and urine of dairy cows. J. Dairy Sci. 58:34.

Nagasawa, H., C. L. Chen, and J. Meites. 1969. Effects of estrogen implant in median eminence on serum and pituitary prolactin levels in the rat. Proc. Soc. Exp. Biol. Med. 132:859.

Narendran, R., R.R. Hacker, T. R. Batra, and E.B. Burnside. 1974 Hormonal induction of lactation in the bovine: mammary gland histology and milk composition. J Dairy Sci. 71:1334.

Oldenbroek, J. K., G. J. Garssen, A. B. Forbes, and L. J. Jonker. 1989. The effect of treatment of dairy cows of different breeds with recombinantly derived bovine somatotropin in a sustained-delivery vehicle. Livest. Prod. .Sci. 21:13.

Peel, C. J. and D. E. Bauman. 1987. Somatotropin and lactation. J. Dairy Sci. 70:474.

Peel, C.J., J.W. Taylor, I.B. Robinson, A.A. McGowan, R.D. Hooley, and J.K. Findlay. 1978. The importance of prolactin and the milking stimulus in the artificial induction of lactation in cows. Australian J. Biol. Sci. 31:187

Phipps, R. H. 1989. A review of the influence of somatotropin on health, reproduction and welfare in lactating dairy cows. Page 88 *In* Use of somatotropin in livestock production. K Sejersen, M. Vestergarrd, and A. Neimann-Sorensen, ed. Elsevier Appl. Sci., New York, NY.

Rowson, L. E., A. R. Trevit, and A. Brand. 1972. The use of prostaglandins for synchronization of estrus in cattle. J. Reprod. Fertil. 29:145.

Sawyer, G.J., W.J. Fulkerson, G.B. Martin, and C. Gow. 1986. Artificial induction of lactation in cattle: initiation of lactation and estrogen and progesterone concentrations in milk. J. Dairy Sci. 69:1536.

Seguin, B. E. 1980. Role of Prostaglandins in bovine reproduction. JAVMA 176:1178.

Sheth, N. A., S. S. Tikekar, K. J. ranadive, and A. R. Sheth. 1978. Influence of bromoergocryptine on estrogen-modulated prolactin receptors of mouse mammary gland. Mol. Cell. Endocrinol. 12:167.

Smith, K.L., and F.L. Schanbacher. 1973. Hormone induced lactation in the bovine. I. lacatational performance following injections of 17β-estradiol and progesterone J Dairy Sci. 56:738.

Smith, K.L., and F.L. Schanbacher. 1974. Hormone induced lactation in the bovine II. response of Nulligravida heifers to modified estrogen-progesterone treatment. J Dairy Sci. 57:296.

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 $F_{2\alpha}$. J Dairy Sci. 67:1798.

Swanson, L. V., H. D. Hafs, and D. A. Marrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoids from first estrus to breeding size in Holstein heifers. J. Anim. Sci. 34:284.

Tanabe, T. Y. and R. C. Hahn. 1984. Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin $F_{2\alpha}$. I. Influence of stage of cycle at treatment. J. Anim. Sci. 58:805.

Tervit, H.R., R.J. Fairclough, L.T. McGowen, D.D.S. MacKenzie, K.L. Macmillan, and A.J. Peterson. 1980. Induction of lactation in dry dairy cattle. N.Z. vet. J. 28:15.

Tucker, H. A. 2000. Hormones, mammary growth, and lactation: a 41-year perspective. J. Dairy Sci. 83:874.

Tucker, H. A. and J. Meites. 1965. Induction of lactation in pregnant heifers with 9-fluoroprednisolone acetate. J Dairy Sci. 48:403.

Turner, C. W. and W. U. Gardner. 1931. The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agr. Exp. Sta. Res. Bull. 158.

Turner, C.W., H. Yamamoto, and H.L. Ruppert, JR. 1956. The experimental induction of growth of the cow's udder and the initiation of milk secretion. J Diary Sci. 39:1717.

Watts, T. L. and J. W. Fuquay. 1985. Response and fertility of dairy heifers following injections with prostaglandin $F_{2\alpha}$ During early, middle, or late diestrus. Theriogenology 23:655.

Williams, R. and C. W. Turner. 1960. Effect of increased levels of ovarian hormones and duration of treatment on the experimental induction of growth of the cow's udder. J. Dairy Sci. 44:524.

Young, F. G. 1947. Experimental stimulation (galactopoiesis) of lactation. Br. Med. Bull. 5:155.

VITA

Tracy Jewell, the daughter of Bryn and Nancy Jewell, was born on December 24, 1976, in Richmond, VA. She graduated from Northumberland High School, in 1995. She completed her Bachelor of Science degree in Animal and Poultry Sciences from Virginia Tech, in spring 2000. In the fall of 2000, she began her Master of Science degree in Dairy Science in the area of Lactation Physiology. The author will be attending the Atlantic Veterinary College in the fall of 2002 to begin working towards her doctorate degree in Veterinary Medicine.