AN ABSTRACT OF THE THESIS OF

Dennis Marc Bel Isle for the degree of Master of Science

in Animal Science (Physiology) presented on 2 October 1975

Title: INFLUENCE OF MATERNAL INSTINCT STIMULATED BY CALF CONTACT ON THE HORMONAL INDUCTION OF LACTATION IN THE BOVINE

Abstract approved: Redacted for privacy

Lloyd V. Swanson

An experiment was conducted to determine the effect a newborn calf would have on initial success rate, lactation performance, infertility and progesterone levels during mammogenesis and lactogenesis in dairy cows undergoing hormonal inducement to lactate.

Fourteen Holstein, Brown Swiss and Jersey infertile, non-lactating cows, loaned by Pacific Northwest dairymen, were examined to exclude anatomical or pathological causes of infertility. These cows were randomly assigned in equal numbers to two groups. The treatments imposed were as follows: a) The control group received 0.1 mg/kg body weight of 17-beta-estradiol and 0.25 mg/kg body weight of progesterone per day for seven days. The hormones were mixed together in absolute

ethanol at concentrations of 20 mg/ml estradiol and 50 mg/ml progesterone. One-half of the dose was administered subcutaneously at 0800 hr and one-half at 2000 hr each day. b) The experimental group received the same hormone treatment; in addition, a newborn male Holstein calf was placed with each cow at the start of the hormone treatment and remained until the cow began milking.

Blood was collected from all cows ever other day beginning the day prior to initiation of treatment, until day 20 or when milking began. Serum was analyzed for progesterone content by a dextran charcoal radioimmunoassay. Milk production was monitored for the initial 90 days of induced lactation and for 305 days on six of the total cows.

The initial success rate for both groups was 100% based on the criterion of producing in excess of 10 kg milk/day during the first 30 days of milking. The mean day to first secretion was 10.1 days for the control group and 8.9 days for the experimental group. The success rate suggests that immediate calf contact was not an important influence whereas the overall technique used appears to have been a positive influence on increasing the success rate. The technique used included semi-isolation of each cow from the start of the injection series to time of milking and inducement in the calving area of the facility.

Serum progesterone levels averaged 1.5 ± 0.3 ng/ml for both groups on the day preceding the injection series. Progesterone concentration increased to an average maximum level of 3 ng/ml during the injection period for both groups and returned to near normal (1-2 ng/ml) when injections stopped on day 7. Based on a split-plot analysis, the treatment had no significant effect on progesterone levels during the 20 day period measured. The daily hormone level, but not the interaction of days with the treatment, showed significant changes among days. Similarly, a correlation of .38 existed between maximum progesterone level and day of first milk secretion. A negative correlation of -.33 was found between maximum progesterone levels and the day of the estrous cycle treatment began. The correlation was .40 between the day of maximum progesterone level and the day of first secretion.

The control group cows produced an extended 305 day mean of 4292 ± 957 kg of milk compared to 4731 ± 711 kg of milk for the experimental group. Total milk production (extended from 90 days) when compared to the previous lactation, was 52% for the control group and 63% for the experimental group. Average milk production, when again compared to the last lactation but based on six complete lactations (305 days), was approximately 80% for both groups.

There had been no improvement noted in any of the infertility cases studied during the four month treatment period. Each cow that was not disposed of following the experiment was further observed for improvement of her infertility problem. Of the six remaining cows, none were diagnosed as having conceived; the induced lactation had not altered any of the infertility conditions.

Influence of Maternal Instinct Stimulated by Calf Contact on the Hormonal Induction of Lactation in the Bovine

by

Dennis Marc Bel Isle

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed 2 October 1975

Commencement June 1976

APPROVED:

Redacted for privacy

Assistant Professor of Animal Science (major professor)

Redacted for privacy

Head of the Department of Animal Science

Redacted for privacy

Dean of the Graduate School

Date thesis is presented 2 October 1975

ACKNOWLEDGEMENT

I would like to acknowledge my appreciation to Drs. William D. Hohenboken and in particular, to Lloyd V. Swanson, for their suggestions in preparation and review of this thesis; to my major professor Dr. Lloyd V. Swanson for assistance in conducting this research; and to the head of the department, Dr. James E. Oldfield, for the opportunity to study in the department. I am thankful for the advice and for the assistance Dr. J. Ronald Miner has given me over the years in support of patentable research conducted in partial fulfillment of this degree; to Dr. Elisabeth S. Yearick, graduate school representative, for her assistance in correcting this thesis; to K. Bruce Koligian for help and advice in the laboratory; and to the OSU Computer Center for the financial grants received. Perhaps the greatest indebtedness is owed to Carol A. Neuman Bel Isle: assistant, co-sponsor and confidant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INFLUENCE OF MATERNAL INSTINCT STIMULATED BY CALF CONTACT ON THE HORMONAL INDUCTION OF LACTATION IN THE BOVINE

INTRODUCTION

The ability of dairy cattle to produce milk is largely proportional to the number of milk secreting cells present in the mammary gland. Forty years ago very little was known concerning the anatomy of this gland or the hormonal influences which occurred during mammogenesis in preparation for lactogenesis following parturition. Knowledge of the endocrine functions involved in mammary gland growth has been aided by studies involving the use of laboratory animals. Knowledge about the hormones which stimulate mammogenesis in experimental animals was then extended to the dairy cow. Extensive research was conducted on laboratory animals, followed by dairy cows and goats, concerning the role of the two ovarian hormones estrogen and progesterone and their ability to stimulate the growth of the mammary lobule-alveolar system.

Following this, attempts were made to produce a suitable hormone induced lactation that would result in the availability of various new production alternatives such as milking virgin heifers or eliminating reproduction in independent intensified production schemes. Presently, a more feasible situation would be to induce lactation in infertile cows. Many infertile cows are genetically valuable but are disposed of to avoid the financial hardship resulting from loss of production when attempting to resolve the infertility problem.

By developing a hormonally induced lactation similar in respects to that which occurs naturally, valuable infertile cows could be retained. Also, induction of lactation in heifers prior to their first calving could be used as an evaluation tool for predicting production of the heifer and evaluation of the sire at an earlier age.

Current research programs now utilize a very practical method of inducing mammary growth and a subsequent lactation. Nevertheless, these hormone induced lactations are on the average, sub-optimal, resulting in non-competitive production with herdmates. To correct this condition will require a more thorough understanding of mammary physiology. Furthermore, knowledge gained from research on the behavioral-physiological interrelationships of a cow with a calf at the initiation of a lactation may be useful in discovering conditions which cause sub-optimal or low production to exist.

This thesis dealt with the stimulation of maternal instinct by calf contact during the initiation of lactation and attempted to determine its effect on progesterone levels, initial response to the treatment, subsequent production and fertility. This was accomplished by monitoring lactation yield following hormone induction with or without a calf being present, quantification of serum progesterone levels by radioimmunoassay and observing fertility following the treatment to determine the effect on individual infertility problems.

REVIEW OF LITERATURE

This review will discuss hormonally induced lactation in the female with special emphasis given to the bovine. Although the hormonal induction of lactation has been successfully initiated in many species over the years, the methods developed by Smith and Schanbacher (1973) have generated a new potential for induced lactation in dairy cattle. Considerable research in this area has been performed, but the initial response and average production levels have always been below normal.

A. Mammogenesis

As with pregnancy and parturition, normal development of the mammary glands involves several hormones with neuro- or releasing factors (RF) working in concert. Some adenohypophyseal gonadotrophins are indirectly involved in mammary growth since they are essential for the secretion of the ovarian hormones (Turner and Bagnara, 1971; Fournier, Desjardins and Friesen, 1974). In addition to the hypophyseal secretions which are regulated by ovarian or placental secretions during gestation, it has been shown that prolactin (PRL) and growth hormone (GH) can influence mammary development (Grosvenor and Turner, 1958; Meites and Shelesnyak, 1957; Moon, 1961). Normally, progesterone stimulates the development of the lobulo-alveolar system while estrogens stimulate growth of the lobulo-duct system (Cowie, 1972). Adrenocorticotropic hormone (ACTH) and thyroid stimulating hormone (TSH) have been shown to affect mammary tissue by way of their respective target organs (Grosvenor and Turner, 1959; Turner, Yamamoto and Ruppert, 1957).

Falconer (1972), using autoradiographic techniques, observed a direct involvement of PRL in the growth of the mammary glands in the rabbit. However, milk production was not inhibited in dairy cows when serum PRL levels were reduced to approximately 1 ng/ml by ergocryptine administration (Smith et al., 1974). Unfortunately, while there is some information on the hormones required for mammogenesis, the actual mechanisms concerned are not well established (Cowie, 1972).

B. Lactogenesis

Within the cytoplasm of alveolar cells, milk solids are synthesized from blood constituents as the first phase of lactation. The second phase is known as milk removal and is the process whereby the secreted milk, stored in the alveoli and fine ducts, is forced toward the larger ducts, sinuses or cisterns according to the species, by the reflexive releases of oxytocin prior to or during nursing causing the myoepithelial cells surrounding the alveoli to contract (Cowie, 1972).

Research conducted by Cross (1955) suggests that milk removal is a reflexive action mediated through the neurohypophysis and hypothalamus. In addition, the adenohypophysis plays an important role in lactogenesis. The discovery of the lactogenic property of adenohypophyseal extracts led to the discovery of PRL (Turner and Bagnara, 1971). The adrenal cortex also has been found to be of importance in the initiation of lactation in mammals. In the pseudopregnant rat, for example, PRL can induce mammogenesis followed by lactogenesis, but only when accompanied by a cortical hormone (Turner and Bagnara, 1971). Although not of a

necessary for lactogenesis in the rat, it appears that GH, together with TSH, is galactopoietic or stimulating to production (Lyons, Ling and Johnson, 1958; Reece, 1958). Likewise, insulin can improve lactational performance (Growen and Tobey, 1931). Kumaresan and Turner (1965) and Raskin, Raskin and Baldwin (1973) demonstrated increased milk production in rats given exogenous insulin. Also, data from experiments with cows have shown that insulin increased fat and protein content of milk (Baldwin et al., 1972; Kronfeld et al., 1963; and Schmidt, 1966).

C. Hormonally Induced Lactation

Comparing the various hormone levels during gestation with those following parturition, estrogen levels increase as parturition approaches while progesterone levels decrease gradually and continually. Furthermore, progesterone reaches a low titer prior to parturition while estrogen shows a rapid drop immediately postpartum (Cross, 1959). Hormone profiles similar to late pregnancy and early lactogenesis are produced when lactation is induced by injection of estradiol (E_2) and progesterone for seven days (Mollett et al., 1975). Nevertheless, a timed release of these injected hormones apparently does not duplicate this phase of a normal lactation cycle. This may explain why sub-optimal milk production is generally observed when a short injection period is used for inducing lactation (Monk et al., 1973).

Meites (1961) has reviewed many different procedures that have been used in attempts to hormonally induce a lactation in dairy

cattle. Experiments have been performed with laboratory animals using adrenal glucocorticoids during mid-pregnancy to initiate milk secretion; results were similar to those from other methods of inducing lactation (Meites, Hopkins and Talwalker, 1963; Nandi and Bern, 1961; and Talwalker, Nicoll and Meites, 1961). Treatment with PRL failed to initiate milk secretion in pregnant mice (Nandi and Bern 1961) or rats (Talwalker, Nicoll and Meites, 1961) whereas either PRL or cortisol acetate initiated lactation in pregnant rabbits (Meites, Hopkins and Talwalker, 1963). This indicates that there may be a species-specific response to certain treatments.

Estrogen and combinations of estrogen and progesterone have been used for many years in attempts to initiate lactation in cows (Folley and Malpress, 1944; Perrin, 1955; Reece, 1943; Turner, 1959; Turner, Yamamoto and Ruppert, 1956; and Williams et al., 1955). Although prolonged treatment with estrogen has induced lactation in some heifers and cows (Meites, 1961; Hancock, Brumby and Turner, 1954; and Turner Yamamoto and Ruppert, 1956), success rates and lactation milk yields were much improved when various combinations of progesterone and estrogen were used. These experiments involved treatment over an extended period of time with relatively low levels of estrogen and high levels of progesterone. Following the withdrawl of progesterone, additional treatment with estrogen for up to one month was common.

Tucker and Meites (1965) used relatively large doses (10 to 15 mg) of ACTH or cortisol for seven to eight days to induce lactation in dairy cattle. Most workers agree that these hormones depress established

lactation in the cow (Cotes et al., 1949; Flux, Folley and Rowland, 1954; Shaw, Chung and Bunding, 1955). But Tucker and Meites (1965) showed that lactations could be initiated in heifers during pregnancy by injecting a synthetic glucocorticoid (9-fluroprednisolone acetate) for a short period of time. Nevertheless, milk yields were low (0.37 kg/day).

In non-lactating, non-pregnant cows, colostrum formation does not occur when levels of E_2 below 0.1 mg/kg body weight/day (estradiol-17B) are given or when progesterone is given simultaneously for up to 14 days (Smith, 1970). Subsequently, Smith et al. (1971) reported that a minimum E_2 dose of 0.1 mg/kg body weight/day was required for 7 days to initiate colostrum formation. The ratio of E_2 to progesterone determined to be optimal for maximum udder development in ovariectomized heifers was 1:25. Smith and Schanbacher (1973) modified earlier induction of lactation procedures by increasing the daily dosage of E_2 and progesterone (using the same ratio of 1:25) and administering the hormones subcutaneously. Estradiol and progesterone were dissolved in absolute ethanol and injected subcutaneously 14 times at 12-hour intervals. An average of 19.1 days elapsed from the first injection of hormones to the occurrence of lactogenesis in an amount sufficient to begin milking. It is interesting to note that during lactation in the ewe, progesterone and E_2 in combination can inhibit an increase in milk yield, and it was suggested that progesterone withdrawal initiated lactogenesis (Hartman, Trevethan and Shelton, 1973).

Subsequently, procedures developed by Smith and Schanbacher (1973) have been refined. Paape, Guidry and April (1973) showed administration

of dexamethasone (DME) at 0.3 mg/kg body weight for 7 days following the final injection of E_2 and progesterone initiated lactogenesis within 24 hours of DME withdrawal. Milk yield response was similar to that observed by Smith and Schanbacher (1973). Also, a lactation can be hormonally induced in dairy cattle within a relatively short period of time utilizing glucocorticoids, provided sufficient numbers of alveolar cells are initially present (Foley et al., 1972).

Most groups of experimental cows have produced approximately 60% of their preceding 305-day milk yield during the induced lactation but substantial individual variability has been observed (Erb et al., 1973). The large variation in hormonally induced lactation production may indicate a relationship between the degree of response and presence of immature ducts and alveoli (Narendran et al., 1974). Heald (1974) reported that differences in lactation response among treated animals may be influenced by two factors: the stage of the cow's estrous cycle when the hormone treatment is begun, and the levels of other endogenous hormones important to lactation. The relatively short period of hormone treatment followed by a rapid lactational response (Narendran and Hacker, 1973; Smith and Schanbacher, 1973), as compared to earlier methods of hormonal induction of lactation (Meites, 1961), may be one of the contributory causes of individual variability. The extended time required for an induced lactation to reach maximum production (Smith and Schanbacher, 1973; Smith and Schanbacher, 1974) may also be explained on the basis of the time taken for a majority of immature

alveoli to mature. Work by Chakriyaret et al. (1975) indicated the importance of the hormone injection scheme when this, along with the hormone sequence, was varied. The resulting milk production was similarly varied but it never exceeded the production results established by the Smith and Schanbacher (1973) technique.

E. Maternal Instinct

Successful production involving large herds of dairy cows relies upon knowledge of the principles of animal behavior (Foley et al., 1972). Although most behavioral studies have been concerned with dominance order in dairy cattle, Schein et al. (1955) failed to show a conclusive relationship between milk production and dominance rank. Selman, McEwan and Fisher (1970a, 1970b) have provided an ethogram (study of behavioral patterns) on dairy cows and their calves during the first five hours postpartum. This is almost the extent of the work that has been done to describe maternal behavior in cattle.

In laboratory animals a virgin mouse, hamster or rat can be induced to exhibit a maternal behavior pattern similar to the post-partum animal in response to the introduction of the young (Rosenblatt, 1967; Terkel and Rosenblatt, 1972; Noirot, 1964a, 1964b, 1969; and Richards, 1966). Several researchers have discussed the possibility that the change in responsiveness of virgin females when exposed to young of their own species is non-hormonally mediated (Bridges et al., 1972; Plume et al., 1968; Rosenblatt, 1967; Terkel and Rosenblatt, 1972).

Nevertheless, this response cannot be designated as being the true maternal response of the primiparous female. The results of Fleming and Rosenblatt (1974b) support the hypothesis that maternal behavior (instinct) exhibited postpartum is hormonally induced.

Prior to the foregoing hypothesis of Fleming and Rosenblatt (1974b) and subsequently in support of it, researchers have investigated concaveation-induced (sensitization) maternal responsiveness in gonadectomized males, females and neonatally androgenized females. No significant differences in maternal responsiveness could be shown between these groups (Quadango et al., 1974). Within a few hours following their first parturition, female rats show entirely adequate maternal behavior toward their young (Rosenblatt and Lehrman, 1963; Weisner and Sheard, 1933). This sudden expression of maternal instinct following parturition seems to be a function of the newly parturient females' unique hormonal condition. Selman, McEwan and Fisher (1970a) observed that following most births in the bovine there appears a fixed-action pattern (instinctual) of maternal behavior, irrespective of the number of previous parturitions experienced by the females.

No reduction in the expression of maternal behavior was reported in multiparous lactating females following enucleation or olfactory bulbectomy (Moltz et al., 1971; Terkel and Rosenblatt, 1968, 1972; Zarrow, Gandelman and Denenberg, 1971; Beach and Jaynes, 1956). In contrast, Roth's (1971) observations of normal maternal behavior in blind, deaf, or anosmic primiparous females points to a multisensory control of maternal response. Furthermore, Roth suggested that the

expression of maternal behavior in virgin females was not dependent on changes in hormone concentration nor was it correlated with them. Rather, this expression appeared to come from continuous sensory input provided by exposure of newborn young to the newly parturient female. Peeters, DeBuysscher and Vandevelde (1973) observed that milk ejection was often accompanied by exhibition of strong maternal behavior (pup retrieval and acceptance of young) in the rat. The results of Fleming and Rosenblatt (1974a) suggested that olfactory bulbs had two functions in relation to maternal behavior: sensory (olfaction) and nonsensory. They observed that the onset of maternal behavior in virgin female rats was delayed when odor from pups was presented to them. Females were more receptive to pups when they were prevented from exposure to their odors. They responded more rapidly to stimuli such as sight, which elicited maternal instinct (Fleming and Rosenblatt, 1974b).

Scott (1966) demonstrated the existence of aggression towards other adults during the lactation period in female mice, but a systematic study of such aggression associated with maternal behavior has not been accomplished (Moyer, 1968). Recent work by Grandelman (1972) documents this phenomenon in mice. The association of aggressive behavior with the lactation period in the female strongly implicates the defense of young as being the primary function of the aggression (St. John and Corning, 1973).

E. Nursing Behavior

Differences between nursing behavior in primiparous and multiparous bovine females were reported by Hafez (1964). He stated that nursing behavior in multiparous animals is facilitated by the reflexes conditioned during previous lactations and, in primiparous animals, is inhibited by the pain and shock of parturition. The establishment of a nursing pattern was discovered to be quite similar in a majority of instances (Selman, 1970b). It has been shown that the presence of a calf near the cow has a favorable influence on milk ejection (Parau, 1968). Conditioning plays an important role in this phenomenon--the sight, sound and odor of a calf probably has become associated with suckling. The discharge of oxytocin in turn is induced by either the suckling or the sensory stimuli provided by the calf. When cows were machine milked 20 to 40 days prior to parturition, milk was secreted only by those quarters stimulated. Prepartum milking results in peak milk production occurring earlier than normal. Prepartum milking increased milk yields approximately 10% during the first 100 days of lactation (Zeliger, Volcani and Sklan, 1972).

The combined activities of the limbic system and hypothalamus probably initiate the ejection phenomena observed in primiparous heifers. The presence of the calf often induces strong or reflexive maternal emotions by the dam (Peeters, Stormorken and Vanschoubroek, 1960). Emotions are assumed to be subjective feelings which are eventually resolved in the limbic system (Grossman, 1967). These emotions, which

are a response to external stimuli, are exhibited by peripheral reaction, for example, in the vascular and respiratory system. The hypothalamus apparently holds a principal role in this motor expression. The anatomical and physiological relationships between the hypothalamus and the limbic system were reviewed by Denamur (1965). The hypothalamus appears to be connected to the limbic system. The paraventricular and supraoptic nuclei are connected to the limbic system by a direct pathway. This pathway is aided by intercalated cells located at the level of the lateral hypothalamus due to very numerous short fibers in the 'limbic system-mid brain circuit' (Nauta, 1958). Electrical stimulation of various parts of the limbic system (septum, hippocampus, cingulate cyrus, etc.) leads to the release of oxytocin as evidenced by milk ejection. Peeters, Debuysscher and Vandevelde (1973) suggested that there needed to be an influence exerted by the hormone combination present at the time of parturition to allow expression of maternal instinct as influenced by the supraoptic and paraventricular nuclei.

In view of the positively correlated change in oxytocin and PRL following nursing (Meites, 1970; Nicoll et al., 1970), it is possible that some of the pathways described for the milk ejection reflex may be the same as those responsible for PRL release (Kordon et al., 1974). There are also indications that the regular occurrence of nursing maintains PRL secretion at a high level (Meites and Turner, 1942). It should be noted, however, that nursing alone can work to a disadvantage in total production due to incomplete withdrawal of milk by the calves (Swanson, 1955).

The neural pathways responsible for hormonal secretion in response to stimulation by the presence of the calf are not well understood. Hayman (1973) hypothesized that separation of the calf from the dam affected the production of lactogenic hormones. Failure of exogenously injected oxytocin to elicit milk ejection indicated that lactation failure was not due to failure of the reflexive functions of milk ejection. Success in lactation could be simply a matter of continuation of the hormonal processes or galactopoiesis in the absense of an offspring.

MATERIALS AND METHODS

A. Experimental Procedure

This experiment was conducted to determine the effect a newborn calf would have on hormonal inducement to establish a lactation in infertile dairy cows. The four categories of study in this project were: 1) initial response to hormonal induction of lactation, 2) determining serum levels of progesterone during and immediately following hormonal inducement, 3) milk yield from the experimental lactation, and 4) the effects of induced lactation on infertility. Milk yield was quantified during the initial three months (90 days) of lactation. Due to injuries, it was necessary in several instances to predict the lactation from a somewhat shorter production period. Only a maximum of two artificial inseminations were possible during the three month period the cow was present in the research herd following the start of the treatment. Therefore, it was often necessary to have the owner diagnose any pregnancy in the cow. Long term results of any change in infertility under category four were obtained from the owner of each cow.

In the course of the experiment a total of 14 non-lactating, infertile cows (five Holstein, five Brown Swiss, four Jersey) were provided by their owners and housed at the Oregon State University Dairy Research Center. Upon arrival the cows were randomly assigned in equal numbers to two groups.

All cows underwent an examination of their reproductive tract by rectal palpation by a veterinarian. Following this, the cows were observed for signs of estrus. The hormone treatment began five to seven days post-estrus, or soon after arrival if there was no evidence or recent history of an estrous cycle. When the udder became distended, or failing this, at 20 days following the start of treatment, the cow was transferred to the milking string. This milking group was kept in a 32 unit free stall area with a concrete surfaced alley. The daily ration consisted of approximately 10 kg of corn silage, 5 kg of alfalfa hay and 5 kg of 16% protein grain per cow. These cows were milked twice each day in a double four De Laval herringbone parlor. Upon completion of the first three months of the lactation the cows were returned to their owners.

B. Determining Sample Size and Type of Analysis Used

A formula was required to determine the number of animals needed to show a significant treatment difference. Methods normally used for determination of sample size were not valid; in measuring milk production between groups of cows it is necessary to take into account the normal variation in production that occurs from lactation to lactation. The repeatability of milk yield for succeeding lactations is approximately 0.52 (Foley et al., 1972). Taking this into account along with the breed phenotypic standard deviation for Holstein, Brown Swiss (1134 kg) and Jersey (907 kg) mature equivalent (ME) milk yield (Norman, 1970), it was decided that the data first be adjusted for breed differences and then be analyzed by covariate analysis (Steel and Torrie, 1960). The covariate

used for adjustment would be the previous Dairy Herd Improvement (DHI) lactation record (2x-305 day-ME) on each cow.

Since it would be necessary to determine minimum sample size in a multiple covariate analysis the following formula was developed by Rowe (1973).

$$n = \frac{2(t^2)(s^2)}{d^2}(1-r^2) + 0.5 = \frac{2(2.69)(6.25\times10^6)}{(4.0\times10^6)}(1-0.27) + 0.5$$

where: n = sample size
t = confidence level (1.64)
s^2 = variance of milk yield (1134 kg^2)
d = measurable difference in milk yield to be detected
r = repeatability of succeeding lactations (0.52)

By using this formula to determine sample size at the 95% confidence level, a total of 12 cows ($\hat{n}$) met the minimum requirements (six per group) to test for significance using a 907 kg difference between groups (d) as the criterion (with the above variance and repeatability factor for milk yield).

C. Experimental Design

1. Experimental Group

The experimental group consisted of seven cows (three Holstein, two Brown Swiss, two Jersey) as illustrated in Table 1. The additional treatment given this group (over controls) was that of exposure to a calf. Individually penned, the cow at initiation of the hormone treatment was separated from a calf by a fence. This fence was removed following the hormone injection series to allow the calf to attempt nursing and to remain with the cow continually. All calves used were Holstein bulls under 14 days of age when they were initially penned with the foster cow. The calf remained with the cow until either the cow was providing more milk than one calf could consume alone

or day 20 from the start of the hormone series, whichever occurred first. No assistance was given the calves to encourage them to nurse or to become accepted by the foster dam.

TABLE 1. EXPERIMENTAL GROUP DATA

Cow	Body Wt. (kg)	Breed[1]	Age (months)	Owner
77	486	Jersey	61	Terhorst[2]
1923	736	Holstein	77	Zylstra [3]
31	744	Brown Swiss	137	Meier[4]
964	631	Holstein	83	OSU[5]
1	562	Jersey	132	Baldwin[6]
13	895	Holstein	106	Barber[7]
Sandra	743	Brown Swiss	63	Weber[8]

[1]all cows were registered

[2]Cornelius, OR

[3]Battleground, WA

[4]Boring, OR

[5]Corvallis, OR

[6]Vancouver, WA

[7]Tillamook, OR

[8]Silverton, OR

2. Control Group

The control group consisted of seven cows also (three Brown Swiss, two Holstein, two Jersey) as shown in Table 2. They were separated from any contact with calves. Except for the first two control cows which were penned together, all other cows were kept in individual pens. The cows were run in pairs, one each from the control and experimental group, and each pair was started at a different time throughout the year as cows became available. This was done to reduce the influence of environmental factors which affect milk production. The dosage, site and time of the hormone injection were similar for both cows in the pair and between all pairs. In this study the first pair began hormone treatment during May 1974 and the last pair completed testing in February 1975.

TABLE 2. CONTROL GROUP DATA

Cow	Body Wt. (kg)	Breed[1]	Age (months)	Owner
80	695	Brown Swiss	89	Meier[2]
5	606	Brown Swiss	120	Meier[2]
33	713	Holstein	49	Straub[3]
40	700	Brown Swiss	89	Meier[2]
Martha	380	Jersey	41	Bielenburg[4]
Surprise	410	Jersey	40	Horning[5]
1040	846	Holstein	66	Boersma[6]

[1]all cows were registered

[2]Boring, OR

[3]Silverton, OR

[4]Scotts Mills, OR

[5]Wilsonville, OR

[6]McMinnville, OR

D. Hormone Injection Schedule

Hormones were injected subcutaneously posterior to the scapula over the dorsal aspect of the rib cage using a 20 ga 2.5 cm needle and a 2.5 cc disposable syringe. The stock solution of hormones per treated pair of cows was prepared as follows:

Stock:

17-beta estradiol (20 mg/ml in absolute ethanol)
progesterone (50 mg/ml in absolute ethanol)
mixed together prior to treatment and stored at
room temperature in the absence of light

Dosage:

17-beta estradiol (0.1 mg/kg body weight daily)
progesterone (0.25 mg/kg body weight daily)
one half this dose was given at 0800 hr and the
other half at 2000 hr

The hormones used in this study were obtained from Sigma Chemical Co., (St. Louis, Missouri): 17-beta estradiol (delta-1,3,4-(10)-estratriene) Lot number 23C-0350 and progesterone (delta-4-pregnen-3,20-dione) Lot number 32C-2310. Hormones were injected twice daily for seven days.

E. Blood Collection

Blood was collected and assayed to establish the circulating systemic progesterone levels during and following the hormone treatment. To reduce the stress caused by collection, blood was drawn by venipuncture from the ventral surface of the tail. Blood was collected the day prior to the

initiation of treatment (day 0) at 2000 hr immediately following the injection, and every other day until day 20 (unless the cow entered the milking string first) following the start of the hormone injection series.

Blood samples were drawn with 10 ml (100x16mm) evacuated glass nonheparinized tubes (Vacutainer) fixed with a 2.5 cm 20 ga needle. The blood was allowed to clot for 10 to 12 hr at room temperature. Using a spatula, the clot was separated from the glass wall of the tube and the samples were centrifuged at 2000 for 10 min at 4° C in a closed refrigerated centrifuge (Ian Sorvall, Inc., Norwlak, Conn.). Following this, the serum was decanted into vials for storage at -4° C until analyzed for progesterone content.

F. Lactation Record

In addition to monthly DHI records, twice daily milk weights were recorded at the Dairy Research Center by use of weigh jars. At the end of the three month milking period the total actual milk production was extended to 305 days by using factors for projecting incomplete lactation records (McDaniel, Miller and Corley, 1965). Subsequently, these records were adjusted to an ME basis (McDaniel et al,. 1967). Also as a result of utilizing different breeds within each group, it was necessary to adjust the data to a common production basis prior to covariate analysis using the adjustments shown in Table 3. Prior records (previous DHI lactation) were similarly adjusted for breed difference and put on a ME basis before use in the comparison analysis.

TABLE 3. ADJUSTMENT FOR THE COVARIATE ANALYSIS

Breed	Breed Average[1] (kg milk)	Standard Deviation[1,2] (kg milk)	Adjustment (kg milk)
Jersey	4016	907	+2309
Brown Swiss	5536	1134	+ 789
Holstein	6325	1134	+ 0

1 twice per day milking; 305 day ME; Foley et al., 1972

2 phenotypic SD assuming normal distributions

G. Radioimmunoassay Procedure

The dextran charcoal single antibody radioimmunoassay (Louis et al., 1973) was used for quantification of serum progesterone.

1. Extraction Procedure

Approximately 4000 to 5000 cpm (10 ul) of 1,2,6,7-^{3}H-progesterone (tracer) was added to a 15x85 mm disposable culture tube and dried under air. Following this 100 ul of serum sample was added to the tube and two additional tubes (without tracer). The labeled progesterone was allowed to equilibrate with the serum at room temperature for 15 minutes. Two ml of benzene:hexane (1:2) was added to all tubes. After rigorous mixing on a vortex mixer for 30 sec, the aqueous phase was allowed to freeze by placing the tubes at -20° C for 24 hours. The organic phase of the serum extract containing labeled progesterone was decanted into a scintillation vial while the solvent from two duplicate

serum extractions were poured off into 12x75 mm assay tubes. The solvent in the scintillation vial was dried under air and the residue dissolved in 10 ml of toluene based scintillation fluid (Appendix 1). This vial was used to determine extraction efficiency.

2. Assay Procedure

The antibody (Ab) was supplied by G.D. Niswender (Colorado State University) and diluted 1:1000 using 0.1% gelatin-phosphate buffered saline (G-PBS; Appendix 2). After the solvent in the 12x75 mm assay tube was dried under air, 100 ul of Ab was added to each extracted sample, to two blank tubes and to two sets of standard tubes. All tubes were mixed for 5 sec and allowed to equilibrate for 30 min at room temperature. One hundred ul of competitor (1,2,6,7 ^{3}H-progesterone; $1x10^{4}$ cpm) diluted in G-PBS was added to all tubes. Following mixing again for 5 sec, the tubes were covered with plastic wrap and incubated for 16 to 20 hr at 4^{o} C.

Subsequently, 1 ml of dextran-coated charcoal (Appendix 3) was added. After mixing for 5 sec and a 15 min incubation period in an ice bath, the samples were centrifuged at 2000xg for 10 min at 4^{o} C. To previously labeled scintillation vials, 0.5 ml aliquots from each assay tube were added along with 7 ml of toluene:triton X-100 (2:1) scintillation fluid (Appendix 4).

Tracer and competitor were added with a Hamilton repeating syringe (Hamilton Co., Reno, Nevada) while Ab and serum were pipetted with an appropriate Eppendorff syringe. The radioactivity (cpm) of each sample was determined by a liquid scintillation counter (Packard 2425 Tri-Carb;

Packard Instrument Co., Inc., Downers Grove, IL). Gain was set at 51% and the discrimination windows were set at 50 and 1000. All samples were counted for 10 min or $1x10^4$ cpm, whichever occurred first. Data from the standard curves were entered into a regression model. Sample data generated from the assay were predicted from the regression coefficients for determination of hormone levels (statistical interactive programming system; CDC 3300; OSU Computer Center). The regression model for these predictions is shown in Apprndix 5. The progesterone data were analyzed using a split-plot analysis of variance as outlined by Kirk (1968).

RESULTS AND DISCUSSION

A. Initial Success Rate

The initial response of the control group to the hormone induction series was 100% when measured as evidence of milk secretion only. The first day the udder began to accumulate milk (as determined visually and by palpation--day for first secretion) and day of first milking are shown in Table 4. Day of first secretion for the control group was 10.1 ± 2.0 and day to first milking was 19.1 ± 0.7.

TABLE 4. DAY OF FIRST SECRETION AND DAY OF FIRST MILKING FOR THE CONTROL GROUP

Cow	Day of First Secretion[1]	Day of First Milking[1]
80	10	21
05	8	18
33	6	21
40	12	17
Martha	5	18
Surprise	9	18
1040	21	21

[1]day 1 = start of hormone injection series

Season of initiation of lactation, reason of infertility, and the day of the estrous cycle on which treatment began are illustrated in

Table 5. For those cows exhibiting estrous cycles, the range of days on which the hormone treatment was initiated was 3 to 10 days postestrus. Effects of these variables cannot be determined from the limited number of occurrences. Since it was not possible to treat all cows identically, some of the variation evident between pairs, such as season of lactation, was assumed to be reduced by the ME and extension factors. Using a criterion of 10 kg/day of milk production by 30 days as a minimal response, six out of six cows (100%) responded.

TABLE 5. REPRODUCTIVE STATUS OF THE CONTROL GROUP AT THE TIME OF INDUCTION OF LACTATION

Cow	Season Induced Lactation was Initiated	Reason for Infertility	Day of Estrous Cycle Injections Began[1]
80	spring	unknown	3
05	spring	cystic ovaries	anestrous
33	summer	abnormal ovaries	7
40	spring	unknown	anestrous
Martha	spring	abortion	6
Surprise	summer	smooth ovaries	10
1040	fall	abnormal ovaries	anestrous

[1]day 1 = estrus

Although a total of 14 cows started, one cow from each group was dropped from the experiment after the first complete week of milking.

Jersey cow Surprise in the control group received a severe injury to her udder. Consequently, she was dried off following a one week production of 17.2 kg. In the experimental group Holstein cow #13 suffered a spinal collapse resulting in her disposal. She had produced 12.9 kg the first week and, estimating from 5 days, 28.9 kg the second week. Neither of these injuries was attributed to increased estrous activity which was noted in earlier trials (Turner, 1959) involving a lengthy induction of lactation procedure.

Of the experimental group, seven of seven cows (100%) responded successfully to the E_2-progesterone treatment as evidenced by milk secretion. This is a significant increase over other reported hormonal induction work done with the bovine using this technique (Smith and Schanbacher, 1973). Nevertheless, this increase cannot be attributed to calf contact since the control group responded similarly. Apparently, the overall technique used was the influencing factor. The technique used included semi-isolation of each cow from the start of the injection series to the time of milking and inducement in the calving area of the facility. Number of days to first secretion and to first milking for each cow are shown in Table 6. Days to first secretion was 8.9 ± 1.1 and number of days to first milking was 19.1 ± 1.0--the same as for the control group. The season lactation began, reason for infertility, and day of the estrous cycle treatment began for each experimental cow are shown in Table 7. Again, using a minimum of 10 kg/day of milk production by 30 days, six of six cows (100%) responded successfully in the experimental group.

TABLE 6. DAY OF FIRST SECRETION AND DAY TO FIRST MILKING FOR THE EXPERIMENTAL GROUP

Cow	Day of First Secretion[1]	Day of First Milking[1]
77	9	21
1923	13	21
31	6	18
964	12	21
1	8	14
13	9	21
Sandra	5	18

[1]day 1 = start of hormone injection series

TABLE 7. REPRODUCTIVE STATUS OF THE EXPERIMENTAL GROUP AT THE TIME OF INDUCTION OF LACTATION

Cow	Season Induced Lactation was Initiated	Reason for Infertility	Day of Estrous Cycle Injections Began[1]
77	summer	pregnant[2]	anestrous
1923	spring	irregular cycle	33
31	spring	cystic ovaries	anestrous
964	spring	unknown	7
1	spring	chronic uterine infection	3
13	fall	no cycle	anestrous
Sandra	summer	unknown	9

[1]day 1 = estrus

[2]30 to 60 days

In both groups, the mammary gland showed little if any change during the hormone injection series. Regular twice daily milking was begun either when the mammary gland became distended or 20 days from the start of treatment, whichever came first. Table 8 compares the initial performance of both groups. These data showed no significant difference in response times indicating that the calf did not stimulate the initiation of lactogenesis.

TABLE 8. COMPARISON OF THE DAY OF FIRST SECRETION AND THE DAY OF FIRST MILKING FOR THE CONTROL AND EXPERIMENTAL GROUPS

	Day to First Secretion[1]	Day to First Milking[1,2]
Control Group	10.1 ± 2.0	19.1 ± 0.7
Experimental Group	8.9 ± 1.1	19.1 ± 1.0
Overall Mean	9.5 ± 1.1	19.1 ± 0.6

[1]day 1 = start of hormone injection series

[2]mean of day to first milking was biased by our 20 day cut-off time.

The mean day to first milking for both groups compares well with other reported studies (Smith and Schanbacher, 1973). Several cows (3 controls and 4 experimentals) were held for 20 days before milking. Following the start of the hormone injection series, all cows exhibited estrous activity. This activity, except for the length of time, appeared normal in all respects and decreased in all cows during the first weeks of milking. It should be noted that the experimental cows came into

milk (first secretion) at 8.9 ± 1.1 days as compared to 10.1 ± 2.0 days for the controls, but this was not significant. It should also be noted that, of the Jerseys (#77, #1, Martha, Surprise) treated, all reached the first day of secretion (8.5) and first day of milking (17.8) sooner than the larger breeds (10.2 and 19.6, respectively).

B. Lactation Performance

Since three different breeds were used in these trials, it was necessary to adjust production figures to a common breed equivalent (Table 9). Breed averages (DHI averages of cows that calved from 1967 to 1968) and standard deviations (Norman, 1970) on which the breed adjustment was based, were shown in the Materials and Methods section (Table 3). Individual production figures from the previous lactation, adjusted to a 305 day equivalent by region, breed, age, the season that lactation began (McDaniel, Miller and Corley, 1965; McDaniel *et al.*, 1967) and breed adjustment factor, are shown for the control group in Table 9 and for the experimental group in Table 10. For comparisons between groups only six cows from each were included in the analysis. Cow Surprise of the control group and #13 of the experimental group were dropped due to the inability to accurately predict production from the short lactation times. Reasons for the short production were previously discussed.

TABLE 9. ADJUSTMENT OF THE PREVIOUS LACTATION FOR THE CONTROL GROUP

Cow	Breed	Last Actual[1] (kg)	Extension Factor[2,3]	ME Factor[4,5]	Breed Adjustment[6] (kg)	Previous Lactation[7] (kg)
80	BS	5936	1	1	789	6725
5	BS	5903	1	1.01	789	6751
33	H	10290	1	1.08	0	11113
40	BS	7174	1	1	789	7963
Martha	J	3738	1	1.16	2309	6645
1040	H	10593	1	1.02	0	10805

1 last lactation actual kg of milk

2 McDaniel, Miller and Corley, 1965

3 factors for projecting incomplete records of cows according to breed and age

4 McDaniel et al., 1967

5 factors for calculating mature equivalent milk by region, breed, season of calving and age

6 adjustment to equalize breed differences for comparison

7 previous lactation adjusted kg of milk

Comparison of the two groups began with adjustment and extension of the 90 day induced lactation. These data are summarized for the control group in Table 11 and for the experimental group in Table 12. An analysis of this comparison (t-test) showed no significant difference between the groups based on their previous milk production records (Table 13).

TABLE 10. ADJUSTMENT OF THE PREVIOUS LACTATION FOR THE EXPERIMENTAL GROUP

Cow	Breed	Last Actual[1] (kg)	Extension Factor[2,3]	ME Factor[4,5]	Breed Adjustment[6] (kg)	Previous Lactation[7] (kg)
77	J	5144	1	1.05	2309	7710
1923	H	7693	1	1	0	7693
31	BS	7371	1	1.05	789	8529
964	H	2170	3.23	1	0	7009
1	J	5455	1	1.02	2309	7873
Sandra	BS	2298	2.15	1.08	789	6125

[1] last lactation actual kg of milk

[2] McDaniel, Miller and Corley, 1965

[3] factors for projecting incomplete records of cows according to breed and age

[4] McDaniel et al., 1967

[5] factors for calculating mature equivalent milk by region, breed, season of calving and age

[6] adjustment to equalize breed differences for comparison

[7] previous lactation adjusted kg of milk

Table 14 is a comparison of the degree of change of production records in the control group from the last natural lactation to the induced lactation. A significant difference (t-test)[1] between performances was evident at the 95% confidence level. Mean production in the induced lactation was approximately 52% of the last lactation. The SD was 46% of that of the previous records. The decreased variability should be

[1] paired t-test

TABLE 11. ADJUSTMENT OF THE INDUCED LACTATION FOR THE CONTROL GROUP

Cow	Breed	Induced Actual[1] (kg)	Extension Factor[2,3]	ME Factor[4,5]	Breed Adjustment[6] (kg)	Adjusted Production[7] (kg)
80	BS	1533	2.63	1	789	4821
5	BS	1132	2.63	1.01	789	3796
33	H	1234	2.56	1	0	3159
40	BS	1932	2.63	1	789	5870
Martha	J	699	2.50	1.16	2309	4336
1040	H	1443	2.56	1.02	0	3768

1 induced lactation actual kg of milk in 90 days

2 McDaniel, Miller and Corley, 1965

3 factors for projecting incomplete records of cows according to breed and age

4 McDaniel et al., 1967

5 factors for calculating mature equivalent milk by region, breed, season of calving and age

6 adjustment to equalize breed differences for comparison

7 induced lactation adjusted kg of milk

the result of lower production based on the assumption that as production increases variability does also. In comparing the induced production of the experimental group with their last natural production (Table 15) a significant difference is found (t-test)[2] at the 99% confidence level. While the SD was 86% of the previous lactation, production was 63% of that

2 paired t-test

TABLE 12. ADJUSTMENT OF THE INDUCED LACTATION FOR THE EXPERIMENTAL GROUP

Cow	Breed	Induced Actual[1] (kg)	Extension Factor[2,3]	ME Factor[4,5]	Breed Adjustment[6] (kg)	Adjusted Production[7] (kg)
77	J	1077	2.50	1.05	2309	5136
1923	H	1490	2.56	1	0	3814
31	BS	1710	2.56	1.05	789	5386
964	H	1678	2.56	1	0	4296
1	J	1278	2.50	1.01	2309	5536
Sandra	BS	751	4.23	1.08	789	4220

1 induced lactation actual kg of milk in 90 days

2 McDaniel, Miller and Corley, 1965

3 factors for projecting incomplete records according to breed and age

4 McDaniel et al., 1967

5 factors for calculating mature equivalent milk by region, breed, season of calving and age

6 adjustment to equalize breed difference for comparison

7 induced lactation adjusted kg of milk

of the previous natural production as compared to 52% for the control group (Table 16). The reduced SD appears to have been the result of lower production. A 34% difference in SD between groups for the induced lactation indicates that the introduction of the calf stimulus

TABLE 13. COMPARISON OF PREVIOUS MILK PRODUCTION RECORDS BETWEEN GROUPS[1]

	Control Group		Experimental Group
Group size	6		6
Mean (kg)	8334		7490
Standard deviation (kg)	2093		826
Range (kg)	4468		2403
t-value		0.92	
Degrees of freedom		10.00	
t-table value at 95%		2.23	

1 the 305 day records

TABLE 14. COMPARISON OF THE PREVIOUS LACTATION AND THE INDUCED LACTATION FOR THE CONTROL GROUP

	Previous Lactation[1]		Induced Lactation[2]
Group size	6		6
Mean (kg)	8334		4292
Standard deviation (kg)	2093		957
Range (kg)	4468		2711
Mean difference (kg)		4042	
Standard error of difference (kg)		1108	
t-value		3.65	
Degrees of freedom		5.00	
t-table value at 95%		2.57	

1 the 305 day records

2 the 90 day extended records

TABLE 15. COMPARISON OF THE PREVIOUS LACTATION AND THE INDUCED LACTATION FOR THE EXPERIMENTAL GROUP

	Past Lactation[1]		Induced Lactation[2]
Group size	6		6
Mean (kg)	7490		4731
Standard deviation (kg)	826		712
Range (kg)	2403		1722
Mean difference (kg)		2759	
Standard error of difference (kg)		280	
t-value		9.87	
Degrees of freedom		5.00	
t-table value at 99%		4.03	

1 the 305 day records

2 the 90 day extended records

TABLE 16. PERCENTAGE CHANGE OF STANDARD DEVIATION AND PRODUCTION IN BOTH GROUPS

	Previous Lactation (kg)	Induced Lactation (kg)	% of Previous Lactation[1]	SD Previous	SD Induced	% of Previous SD[2]
Control Group	8334	4292	52	2093	957	46
Experimental Group	7490	4731	63	826	711	86

1 the induced lactation shown as a percentage of the last natural (previous) lactation

2 the induced lactation SD shown as a percentage of the last natural (previous) lactation

together with a slightly higher production resulted in little change in the variation among records (when compared to the control group). Comparing this to the 154% difference between SD of both groups during their previous lactation, overall reduction was apparently due to such changes as housing, plane of nutrition, milking practices and handling or care along with the lower production.

When performance of the controls was compared with the experimental group (Table 17) there was no significant difference evident (t-test). The control group produced approximately 91% as much milk as the experimental group but the SD of the experimental group was 25% less than that of the controls. Table 16 showed the amount of change in production and within group variation when induced and previous lactations were compared. These data indicated that the degree of change from normal toward abnormal or low production was more evident in an induced lactation when a calf was not present during the time of induction. In addition, the coefficient of variation for milk production was not substantially changed in either group (Table 18).

The t-test was used for comparison of unadjusted means between change in performance from past or previous to induced production within groups and between either past or induced production records of both groups. Following this, the normal variation inherent from one individual record to a succeeding performance was taken into account in a completely randomized block design analysis of covariance (CRANOCV; Steel and Torrie, 1960) of the production data.

TABLE 17. COMPARISON OF THE CONTROL AND EXPERIMENTAL GROUPS DURING THE INDUCED LACTATION

	Control Group	Experimental Group
Group size	6	6
Mean (kg)	4292	4731
Standard deviation (kg)	957	712
Range (kg)	2711	1722
t-value	-0.90	
Degrees of freedom	10.00	
t-table value at 95%	2.23	

TABLE 18. COEFFICIENT OF VARIATION OF PRODUCTION WITHIN EACH GROUP

	Coefficient
Control - previous records[1]	0.25
Experimental - previous records[1]	0.11
Control - induced lactation records[2]	0.22
Experimental - induced lactation records[2]	0.15

[1] the 305 day records

[2] the 90 day extended records

The CRANOCV was used to measure differences in performance between groups after first adjusting for previous records. The covariate was the previous lactation record on each cow. Table 19 illustrates the degrees of freedom and sums of squares and cross-products used in the analysis. The coefficient used in the adjustment of observed means was:

$$b_{yx} = E_{xy} / E_{xx} = -1.2\text{x}10^{6} / 2.2\text{x}10^{7}$$

$$= -0.06 \text{ kg of milk of the induced lactation for each kg of previous lactation milk}$$

where:

$$E_{xy} = \left(Sx_{ij}\, y_{ij} - \frac{x..y..}{rt}\right) - \left(\frac{Sx_{.j}\, y_{.j}}{t} - \frac{x..y..}{rt}\right) - \left(\frac{Sx_{i.}\, y_{i.}}{r} - \frac{x..y..}{rt}\right)$$

$$E_{xx} = \left(Sx_{ij}^{2} - \frac{x..^{2}}{rt}\right) - \left(\frac{Sx_{.j}^{2}}{t} - \frac{x..^{2}}{rt}\right) - \left(\frac{Sx_{i.}^{2}}{r} - \frac{x..^{2}}{rt}\right)$$

x = previous lactation
y = induced lactation record
r = replications (6)
t = treatments (2)
S = summation

An F value was calculated on the unadjusted means of the induced lactation of each group:

$$F = \frac{T_{yy} / (t-1)}{E_{yy} / (r-1)(t-1)} = \frac{1.0\text{x}10^{6} / (2-1)}{5.0\text{x}10^{6} / (6-1)(2-1)}$$

$$= 1.0 \ (df=1,5)$$

where:

$$T_{yy} = Sy_{i.}^{2} - \frac{y..^{2}}{rt}$$

$$E_{yy} = \left(Sy_{ij}^2 - \frac{y..^2}{rt}\right) - \left(\frac{Sy_{.j}^2}{t} - \frac{y..^2}{rt}\right) - \left(\frac{Sy_{i.}^2}{r} - \frac{y..^2}{rt}\right)$$

There was no significant difference in unadjusted mean milk production during the induced lactation between the two groups. This indicated that along with calf contact, the normal variation that existed from one lactation to the next had not affected the comparison. Any difference that was evident was due to chance.

TABLE 19. CRANCOV DATA TABLE

Source of Variation	df	Sum of Products				Y Adjusted for X	
		x,x	x,y	y,y	df	SS	MS
Total	11	2.8×10^7	-5.0×10^6	8.0×10^6			
Blocks	5	9.0×10^6	-1.8×10^6	2.0×10^6			
Treatments	1	3.0×10^6	-2.0×10^6	1.0×10^6			
Error	5	2.2×10^7	-1.2×10^6	5.0×10^6	4	4.9×10^6	1.2×10^6
Treatments + error	6	2.5×10^7	-3.2×10^6	6.0×10^6	5	5.6×10^6	
Treatments adjusted					1	7.0×10^7	7.0×10^7

The F value was also determined among unadjusted means for milk produced during the previous lactation:

$$F = \frac{T_{xx} / (t-1)}{E_{xx} / (r-1)(t-1)} = \frac{3.0 \times 10^6 / (2-1)}{2.2 \times 10^7 / (6-1)(2-1)} = 0.68 \quad (df = 1,5)$$

where:

$$T_{xx} = \frac{Sx_{i.}^2}{r} - \frac{x_{..}^2}{rt}$$

There was no significant difference in unadjusted mean milk production between the two groups during the previous lactation. This illustrates that randomization of the cows in both groups was adequate enough so that it was possible to statistically show a difference between groups following induction.

Table 20 shows the covariate adjustment of the observed means following analysis of the data. The difference between the control and experimental groups in adjusted mean milk production during the induced lactation was 489 kg.

TABLE 20. COVARIATE ADJUSTMENT OF THE OBSERVED MEANS OF BOTH GROUPS

Group	Previous Lactation x_i	Deviation $x-\bar{x}$	Adjust $b_{yx}(x-\bar{x})$	Observed Mean	Adjusted Mean
Control	8334	422	-25	4292	4267
Experimental	7490	-422	25	4731	4756

The F value among mean milk production during the hormonally induced lactation between groups with the adjustment was:

$$F = MS \text{ adjustment} / s^2_{y \cdot x} = 7.0 \times 10^7 / 1.2 \times 10^6$$

$$= 0.58 \ (df = 1,4)$$

where:

$$s^2_{y \cdot x} = \frac{S_{yy} - \frac{(S_{xy})^2}{S_{xx}}}{(r-1)(t-1) - 1}$$

There was no significant difference among mean milk production between the two groups although the adjusted means showed a 489 kg advantage for the experimental group. A 907 kg difference[3] was needed to show significance with this number of cows. Therefore, due to the low number of cows in each group, the magnitude of this difference was outside the ability to statistically predict its reocurrence at a 95% confidence level. Apparently, the calf did not influence subsequent production in the hormonally induced lactation. It was also evident from the approximate 40% reduction in the F value between unadjusted and adjusted means of the induced lactation that individual variation of records, not attributed to the treatment, affected the comparison.

---

[3] calculated amount

C. Serum Progesterone Levels

Average serum progesterone level was low (1.5 ± 0.3 ng/ml) and variable for both groups preceding the injection series, and this was unexpected since most of the cows were in estrus five to seven days prior to the start of treatment. Table 21 shows the average progesterone levels for the control group from day 0 through day 16. On day 18 and 20 only two cows which were not milking were sampled. Average serum progesterone increased to near maximum levels in the control group at day 2 (2.8 ± 0.8 ng/ml) and day 6 (2.8 ± 1.0 ng/ml) of the hormone injection series. These levels were somewhat higher than previously reported by Monk et al., (1973). The high SE indicated a high degree of variability in individual response to the injected hormones.

TABLE 21. CONTROL GROUP SERUM PROGESTERONE LEVELS (ng/ml)[1]

Day	0	2	4	6	8	10	12	14	16
N	7	7	7	7	7	7	6	6	6
Mean	1.5	2.8	2.0	2.8	1.7	1.9	1.9	2.1	1.8
SE	0.3	0.8	0.7	1.0	0.3	0.6	0.5	0.3	0.3

[1] hormones were injected from day 1 through day 7

Table 22 illustrates the serum progesterone levels observed in the experimental group. On day 18 and 20 only three cows which were not milking were sampled. Similar to the control group data, maximum progesterone levels were reached by day 2 of the injection series and continued through day 6. Possible causes of the high variability here and in the control group were breed difference, age, season of lactation, weight, prior level of nutrition, assay variability, time of blood sampling and the cause or result of the infertility problem.

TABLE 22. EXPERIMENTAL GROUP SERUM PROGESTERONE LEVELS (ng/ml)[1]

Day	0	2	4	6	8	10	12	14	16
N	7	7	7	7	7	7	6	5	5
Mean	1.5	2.4	2.3	2.2	1.6	1.7	2.0	1.5	1.4
SE	0.3	0.5	0.7	0.5	0.3	0.6	0.3	0.5	0.3

[1] hormones were injected from day 1 through day 7

Table 23 lists the maximum individual progesterone levels and day they occurred during the injection period. Also listed are age, weight, breed, group assigned and percent of previous production produced.

TABLE 23. INDIVIDUAL MAXIMUM SERUM PROGESTERONE LEVELS ACHIEVED AND THE DAY MAXIMUM LEVELS OCCURRED

Cow	Maximum Level (ng/ml)	Day of Maximum Level[1]	Wt. (kg)	Age[2] (mo)	% Previous Production	Breed Group	[3]
1040	6.9	6	864	66	35	H	C
5	6.2	2	606	120	56	BS	C
Sandra	4.8	4	743	63	69	BS	E
964	4.7	4	631	83	61	H	E
Martha	4.5	2	380	41	65	J	C
13	3.9	6	895	106	--[4]	H	E
40	3.2	4	799	89	74	BS	C
77	2.9	6	486	61	67	J	E
31	2.3	7	744	137	63	BS	E
80	2.1	3	695	89	72	BS	C
1923	1.8	7	736	77	50	H	E
1	1.7	1	562	132	70	J	E
Surprise	1.6	1	410	40	--[4]	J	C
33	0.8	1	713	49	28	H	C

1 day 1 = start of injection series

2 age at start of treatment

3 C = control group; E = experimental group

4 lactation not predicted

Maximum progesterone levels ranged from 0.8 to 6.9 ng/ml. These results compare favorably with those reported by Moss et al., (1975) who found a range of 4 to 7 ng/ml between days 3 to 15 of the induction period. Maximum levels were achieved during days 2 to 6 during the injection period.

Correlations between maximum progesterone levels and the factors listed in Table 23 were as follows:

weight	=	.19
age	=	.02
previous production	=	- .10

The correlation between the day that maximum progesterone level occurred and age was low (.21). Nevertheless, a relatively high correlation of .55 ($P < .01$) was found between the day of maximum level and weight. This suggests that body size may negatively influence the rate of either absorption or sensitivity to the hormone.

Comparison of the daily mean progesterone levels of both groups (Fig. 1) revealed similar occurring levels. Progesterone data were analyzed by a split-plot analysis (Kirk, 1968) to evaluate the effect of hormonal induction of lactation treatment (A) at these two criteria:

a_1 = calf influence

a_2 = control (no calf)

Treatment B (days) had six levels corresponding to every other day sampling:

b_0 = day 0, b_2 = day 2, b_4 = day 4, ... b_{10} = day 10

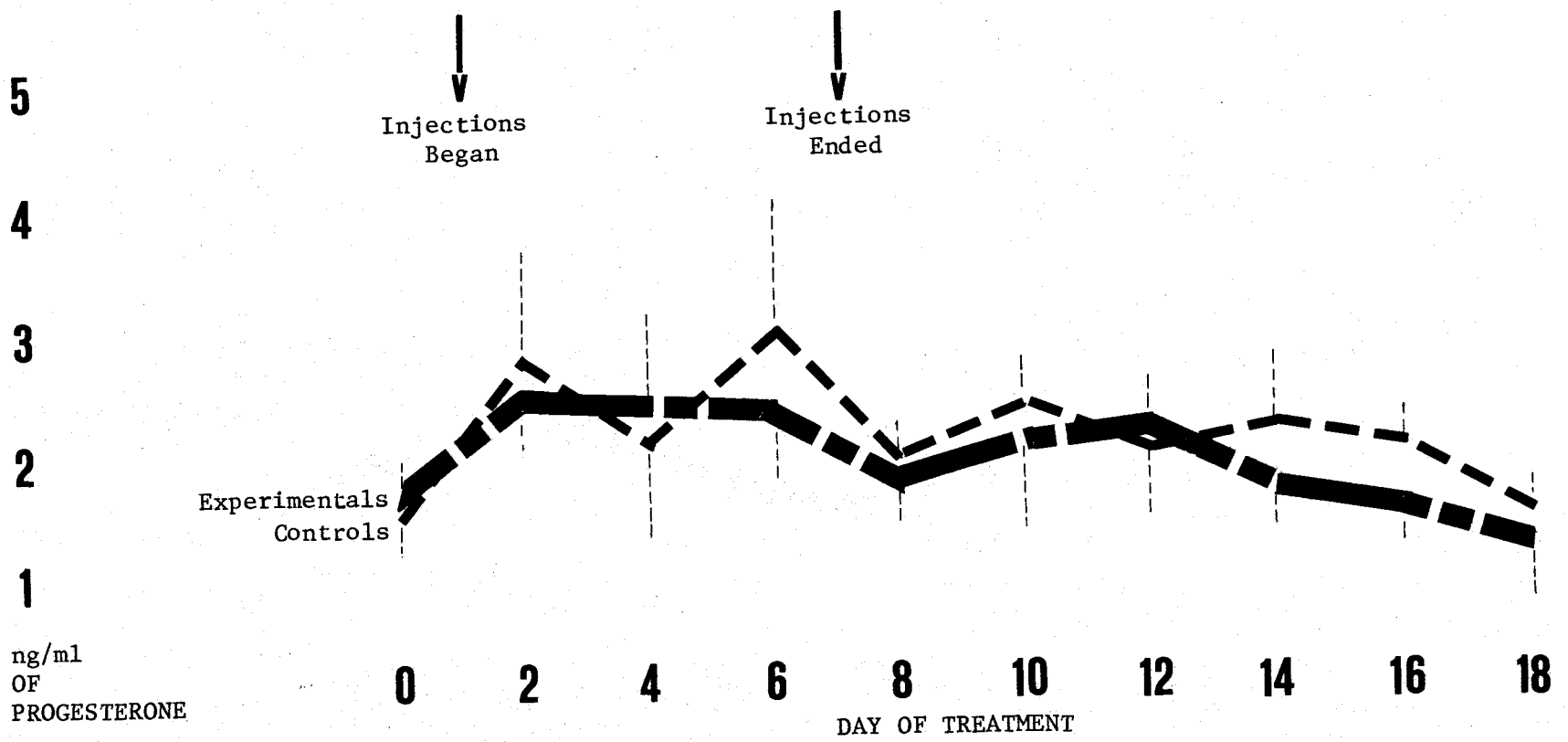

FIGURE 1. Mean Daily Progesterone Levels of the Control and Experimental Groups

A total of 14 cows representing two random samples of seven cows each were assigned to the p=2 levels of A and observed under all q=6 levels of B.

According to the split-plot analysis (Table 24), the treatment (A) had no significant effect on progesterone levels during the 10 day period of measurement. The possibility still exists that the calf influenced endogenous progesterone levels, but that the influence was overshadowed by the exogenous administration of the hormone. Treatment B (days), but not interaction of day with treatment, was significant. Therefore, Table 24 suggests that the progesterone level on various days of the treatment differed but that groups did not. Apparently the variation evident was due to the individual response to the hormone treatment rather than calf contact.

Figure 1 shows an increase in progesterone levels during the injection period until day 7. Following this, the progesterone levels again rose (2.0 ± 0.3 ng/ml) to those observed during the injection series. Toward the end of the blood sampling (day 12 to 20 depending on the time to first milking) progesterone returned to a level slightly above that at the start (1.5 ± 0.3 ng/ml). This was similar to the results reported by Mollett et al., (1975). It is interesting to note that during days 8 through 18 when the absorption factor (injection site) was eliminated, the variation was reduced.

TABLE 24. SPLIT-PLOT ANOV DATA

Sources	df	SS	MS	F
Blocks (cow)	27	64.6	2.4	
A (treatment)	1	1.0	1.0	0.40
Error	26	63.6	2.4	
Within blocks (cows)	140	197.4	1.4	
B (days)	5	14.8	3.0	3.28[1]
Interaction (AB)	5	1.2	0.2	0.26
Error	130	181.4	1.4	
Total	167	262.0		

[1] $P < .01$

Individual observations varied considerably about the overall mean. In Figure 2 the progesterone levels of the control group are shown individually from day 2 through day 19 (when sampled) inclusively. Most cows showed an apparent rise in serum progesterone concentration following the end of treatment (day 7). This was possibly due to an increase in corpus luteum activity resulting from the hormones given. Such cows as #5, Martha and #1040 had a high spike release followed by a return to previous levels during the injection period. Cow #80 showed a similar response but at a much lower level. Both cows #33 and Surprise had experienced a marked decrease in progesterone levels to undetectable amounts by day 10 and day 2 respectively. The data

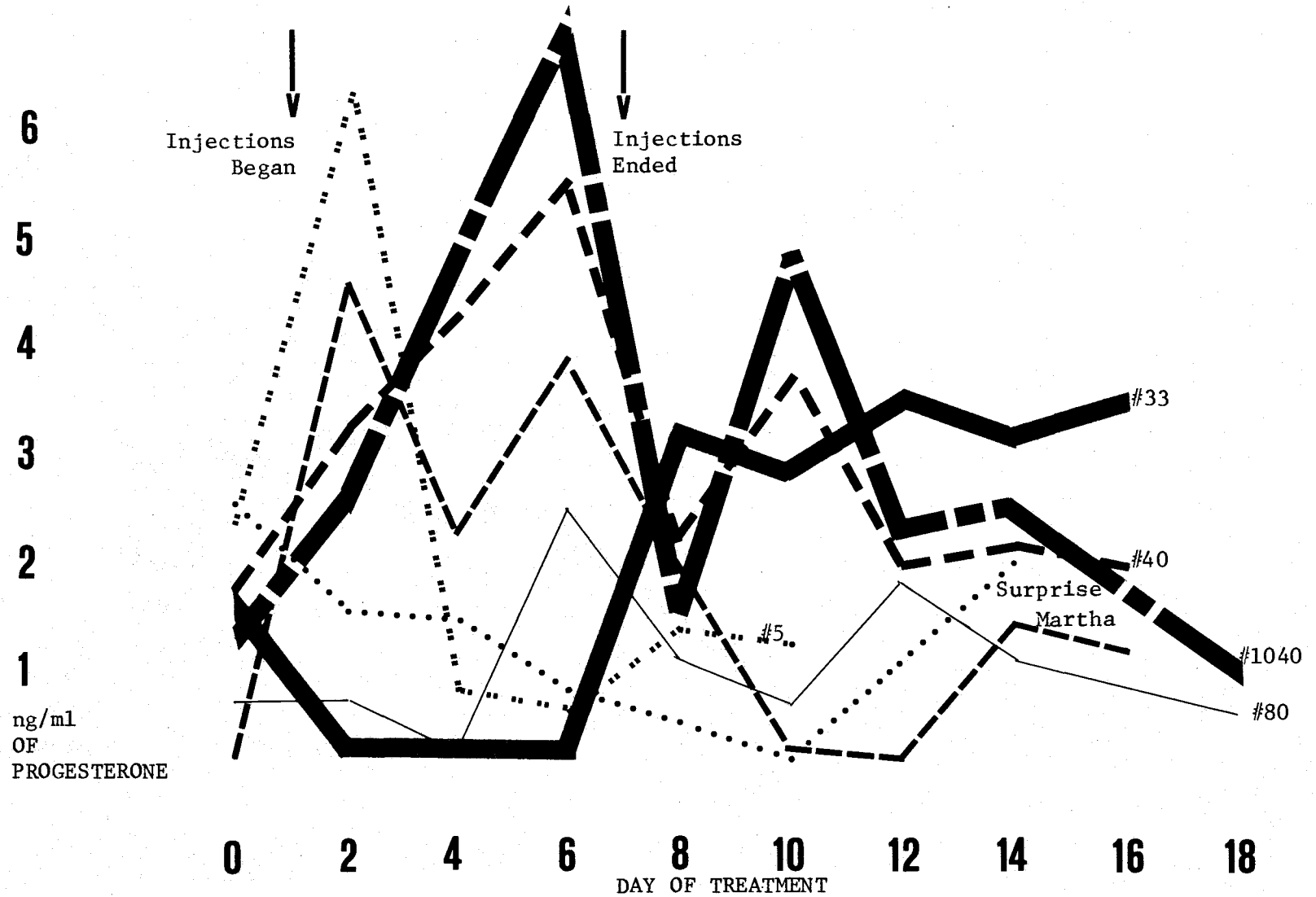

FIGURE 2. Individual Daily Serum Progesterone Levels of the Control Group

may indicate a sensitivity of each cow to the hormones given or their affect at a particular time of the estrous cycle.

The correlations between maximum progesterone level and day of first secretion, day of first milking or the time treatment started were as follows:

day of first secretion	= .38	($P < .05$)
day of first milking	= .10	
day of the estrous cycle treatment began	= - .33	

Similar correlations were determined between the day of maximum progesterone levels and the following:

day of first secretion	= .40	($P < .05$)
day of first milking	= .46	($P < .05$)
day of the estrous cycle treatment began	= .14	

These results showed a correlation existing between the day of first secretion and both the progesterone levels (maximum) and the day of maximum level. A correlation of .46 between the day of first milking and the day of maximum progesterone level suggests that the time to first milking was dependent on individual response time to the hormone.

Similarly illustrated in Figure 3 are the individual progesterone levels from day 2 through day 19 for the experimental group. As with the control group, several of the cows reached high progesterone levels during the time when the hormones were given. Only the progesterone levels of one cow (#1) declined appreciably during that period. It should be noted that, as with the previous group data, not only did the

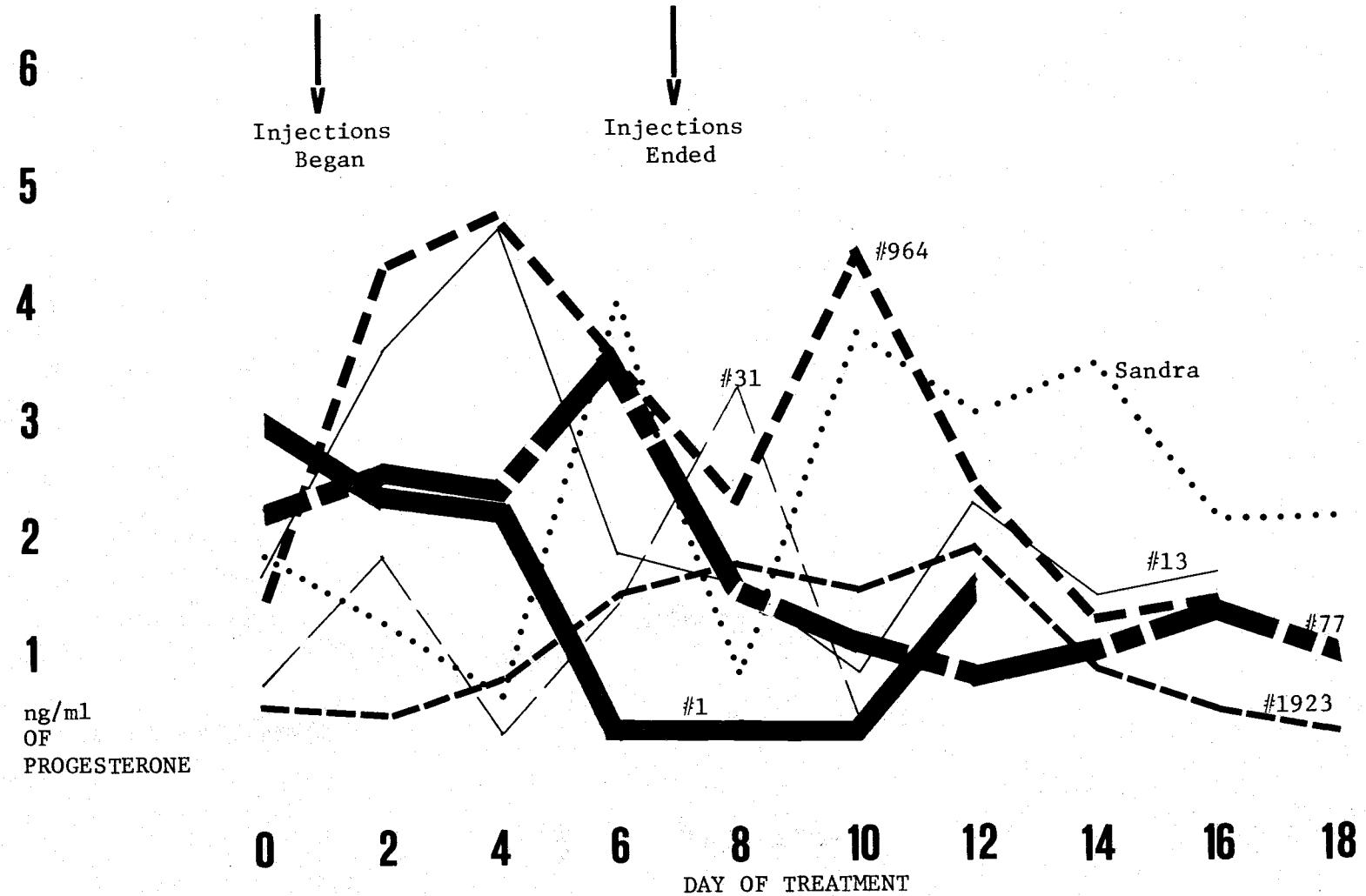

FIGURE 3. Individual Daily Serum Progesterone Levels of the Experimental Group

maximum levels and durations of progesterone levels differ but also, the time to regeneration of higher levels following the withdrawal of treatment varied.

Although established procedure[4] is for day 1 (start of the hormone treatment) to occur between the fifth and seventh day of the estrous cycle, some cows from both groups began the induction series between the third and tenth day of the cycle. Also, many of the cows were not cycling or had irregular cycles during the time prior to treatment (approximately one month). Those cows which did start the induction treatment while cycling are compared to the maximum level of progesterone responses in Figures 2 and 3 and these data are listed in Table 25. The correlation is - .33 between the time treatment began during the cycle (cows which were cycling) and the maximum progesterone level response during the hormone injection series treatment period.

Some cows reached a high maximum progesterone level but began treatment during anestrous (#5) while others had a low peak or response and either started on the seventh day of the estrous cycle (#33) or third day. There was a slight correlation between the day of the estrous cycle treatment began and progesterone level response (.33). In contrast, the declining progesterone response of several cows such as #1 (Fig. 3) was not evident of her induced lactation performance which was 70% of previous production (Table 23).

---

[4] Smith and Schanbacher 1973; 1974

TABLE 25. COMPARISON OF THE STARTING TIMES DURING THE ESTROUS CYCLE WITH MAXIMUM PROGESTERONE LEVEL AND THE DAY MAXIMUM PROGESTERONE LEVEL OCCURRED IN BOTH GROUPS

Cow	Day of Estrous Cycle[1]	Maximum Level (ng/ml)	Day of Maximum Level[2]
1	3	1.7	1
80	3	2.1	3
Martha	6	4.5	2
964	7	4.7	4
33	7	0.8	1
Sandra	9	4.8	4
Surprise	10	1.6	1

[1] treatment started on this day of the cycle

[2] day 1 = start of treatment

D. Extended Effects

1. Milk Production

Of the original 14 cows that were returned to their owners following the first 90 days of milking, two were withdrawn from the experiment and further study as previously mentioned. In the control group #1923, #13 and Sandra were either sold or their records were similarly lost. Table 26 shows the remaining cows of each group

and their final records. Again, breed adjustments were necessary to compare production of both groups.

TABLE 26. COMPLETED 305 DAY RECORDS OF INDUCED LACTATION FOR BOTH GROUPS[1]

Cow	Actual Lactation (kg)	Days of Lactation	Adjusted Lactation (kg)		% of Previous Production
Control group					
5	4220	305	5051		75
80	4558	305	5347		80
40	5853	305	6642		83
Average				5680 ± 489	80 ± 2
Experimental group					
1	4543	305	6897		88
31	5385	305	6443		76
77	2171	146	6116		74
Average				6485 ± 226	80 ± 4

[1] average of both groups was 67% of previous production when extended from 90 days

The final results indicate no real difference existing between groups in complete induced lactations. Table 26 illustrates the fact that when the induced production was extended from 90 to 305 days, the actual production was underestimated. It appears that the lactation curve of hormonally induced cows differs to a point where

conventional extension of records and subsequent adjustment tend to underestimate actual production. From the data presented, all six cows completing a lactation produced approximately 80% of their previous record. This is almost 33% higher than data reported earlier for completed induced lactations by Smith and Schanbacher (1972).

The resulting production for both groups, when compared to the day of the estrous cycle treatment began (Table 27), contravenes work done by Smith and Schanbacher (1974). In their studies treatments initiated between the fifth and seventh day of the estrous cycle produced the best results. Table 27 shows that as the day of the estrous cycle advances (from the third to seventh day) production appeared to decline. Correlation between the day of the cycle treatment began and induced production yield was - .70 ($P < .01$).

TABLE 27. COMPARISON OF THE DAY OF THE ESTROUS CYCLE TREATMENT BEGAN TO THE INDUCED LACTATION YIELD ON SIX COWS COMPLETING A 305 DAY LACTATION

Cow	Day of the Estrous Cycle Treatment Began	Induced Production (kg)
1	3	5536
80	3	4821
Martha	6	4336
964	7	4296
33	7	3159
Sandra	9	4220

The SE shown in Table 26 with the average milk produced indicates a higher degree of variation between cows in the control group than in the experimental group. This variability is related to the initial analysis done with past records to indicate the homogeneity of production in both groups. The control group variation was higher in that initial comparison, as it is here, when compared to the experimental group.

2. Fertility

Finally, the concern with infertility and the beneficial effect a hormonally induced lactation might have on it was certainly not obvious here. None of the remaining six cows conceived and none of the infertility problems previously noted in all 14 cows were altered. Those cows who were exhibiting anestrous prior to the induced lactation also exhibited anestrous following treatment. And, of those six cows remaining after 305 days, the following conditions existed:

#5 = no change in anestrous condition
#80 = no change in infertility status
#40 = no change in anestrous condition
#1 = no change in infertility status
#31 = no change in anestrous condition
#77 = pregnancy terminated

Jersey cow #77 aborted her calf at approximately 50 days of gestation. This occurred 22 days following the hormone injection series, five days following a difficult palpation exam of her reproductive tract and two days following the confirmation of two cases of leptospirosis in other

cows in the herd. These variables do not allow speculation on whether or not the aborted calf could have survived the hormone injection series of the amount and technique used.

Most of the cows used in this experiment should be considered to be severe infertility cases, judged by their breeding history and estrous cycle activity (Tables 5 and 7). Perhaps when cows with an infertility problem, which could be resolved by known treatment -- particularly hormone therapy -- are induced to lactate, their improvement is mistakenly attributed to the initiation of lactation rather than to the hormones administered.

SUMMARY AND CONCLUSIONS

Maternal instinct in cows hormonally induced to lactate was assumed to be enhanced by the presence of a newborn calf in addition to the influence of circulating systemic hormones. It was hoped that by providing a cow with a calf during the hormonal initiation of a lactation, stimulation of maternal instinct would in turn result in reduced variation in response and increased milk production. Initial response to the induced lactation was 100% in this study, whether or not a calf was present during the induction period. It appeared as if the overall technique used to induce lactation was responsible for the high success rate (higher than any previous work shows). The unusual steps taken in the technique used were semi-isolation of the cow prior to, during and following the injection series, inducement in the calving area and movement of the cows from the home facility.

Time to the first noticed secretion in both groups occurred on the average of nine days from the beginning of treatment. Similarly, the first day of milking occurred at an average of 19 days in both groups and was not influenced by the immediate presence of the calf. These figures were close to the expected results for hormone induced lactations under this procedure except for the initial success rate which normally ranges from 60 to 65% (Smith and Schanbacker, 1973). The first day to milking figure was apparently biased by the cutoff time used rather than it being a function of the cow.

Milk production for the two groups was compared not only between the hormonally induced lactations but also between and with the last previous records. Although there was a slight tendency for the influence of the calf to result in increased production (4756 kg vs. 4267 kg) and reduced variation, the small number of cows and amount of difference prevented statistically significant change in production from being detected. When the initial milk production (90 days) was extended to 305 days and compared to the last previous lactation, the control group averaged 52% and the experimental group 63%. When data were eventually collected from 305 day records rather than extended from 90 days, it was found that both groups (three cows remaining in each group) averaged approximately 80% of their previous natural lactations.[5] This and the previous data suggests that when hormone induced lactation is used it can equal initial success rates found under natural conditions as well as producing a substantial lactation. Similarly, it was demonstrated that it is possible to induce a lactation in infertile cows which previously were liabilities when kept for treatment of their problem.

Serum progesterone levels of 1.5 ± 0.3 ng/ml in both groups, prior to treatment, were correlated (.33) with length of time from previous estrus to start of treatment as would be expected. Progesterone levels increased to an average of 3 ng/ml for both groups during the hormone injection period. At the completion of hormone injections, serum progesterone temporarily returned to levels found at the start of the

[5] average of both groups was 67% of previous production when extended from 90 days

treatment. There was a similar increase again following the injection period. Individually, each cow responded somewhat differently, suggesting that sensitivity to the hormones may be associated with differences involving the endogenous circulating hormone levels. No significant correlation was found between progesterone levels or its response and production. Apparently, the circulating titers of the progesterone hormone measured are not directly influencing production during this period.

No improvement in fertility was noted in any of the cows one month following the induction of lactation or during the following observation period (three months). Six of the cows were available for further analysis one year following treatment. None of these cows had responded to this or any treatment for their infertility problem. The small success other researchers (Smith and Schanbacker, 1973) have had in eliminating reproductive problems following an induced lactation were possibly due to other improved or changed conditions rather than the initiation of a lactation.

BIBLIOGRAPHY

Baldwin, R.L., E. Osborne, J. Reichl, N.E. Smith, and Y.T. Yang. 1972. Effect on chronic insulin administration and a high concentrate diet on cow mammary glucose oxidation and lipogenesis. Fed. Proc. 31:675.

Beach, F., and J. Jaynes. 1956. Studies of maternal retrieving in rats. III. Sensory cues involved in the lactating females response to her young. Behav. 10:104.

Bridges, R., M.S. Zarrow, R. Gandelman, and V.H. Deneberg. 1972. Differences in maternal responsiveness between lactating and sensitized rats. Devel. Psycho. 5:123.

Chakriyarat, S., H.H. Head, W.W. Thatcher, K.C. Bachman, and C.J. Wilcox. 1975. Lactational responses of ovariectomized cows to two hormonal induction schemes. J. Dairy Sci. 58:140 (Abstr).

Cotes, P.M., J.A. Crichton, S.J. Folley, and F.G. Young. 1949. Galactopoietic activity or purified anterior pituitary growth hormone. Nature 164:992.

Cowie, A.T. 1972. Lactation and its hormonal control. In: C.R. Austin and R.V. Short (eds.), Reproduction in Mammals: Hormones in Reproduction. Cambridge University Press.

Cross, B.A. 1955. The posterior pituitary gland in relation to reproduction and lactation. British Med. Bull. 11:151.

Cross, B.A. 1959. Neurohypophyseal control of parturition. In: C.W. Lloyd (eds.), Endocrinology of Reproduction, New York, Academic Press.

Denamur, R. 1965. The hypothalmo-neurophysiological system and the milk ejection reflex. Dairy Sci. Abst. 27:193.

Erb, R.E., E.L. Monk, C.J. Callahan, and T. A. Mollett. 1973. Milk yield and reproductive traits subsequent to treatment of cows with progesterone--estrogen. J. Dairy Sci. 56:565. (Abstr).

Falconer, I.R. 1972. Hormone metabolism in the mammary gland of the rabbit. Biochem. J. 126:8.

Fleming, A.S., and J.S. Rosenblatt. 1974a. Olfactory regulation of maternal behavior in rats: I. Effects of olfactory bulb removal in experienced and inexperienced lactating and cycling females. J. Comp. Physiol. Psycho. 86:221.

Fleming, A.S., and J.S. Rosenblatt. 1974b. Olfactory regulation of maternal behavior in rats: II. Effects of peripherally induced anosmia and lesions of the lateral olfactory tract in pup-induced virgins. J. Comp. Physiol. Psycho. 86:233.

Fleming, A.S, and J.S. Rosenblatt. 1974. Maternal behavior in the virgin and lactating rat. J. Comp. Physiol. Psycho. 5:957.

Flux, D.S., S.J. Folley, and S.J. Rowland. 1954. The effect of adrenocorticotropic hormone on the yield and composition of the milk of the cow. J. Endocrinol. 10:333.

Foley, R.C., D.L. Bath, F.N. Dickinson, and H.A. Tucker. 1972. Hormonal control of lactation. In: Dairy Cattle: Principles, Practices, Problems, Profits. Lea and Febiger.

Folley, S.J. and F.H. Molpress. 1944. Artificial induction of lactation in bovines by oral administration of synthetic oestrogens. J. Endocrinol. 4:23.

Gandelman, R. 1972. Mice: Postpartum aggression elicited by the presence of an intruder. Horm. Behav. 3:23.

Grossman, S.P. 1967. Emotional behavior. In: Textbook of Physiological Psychology. J. Wiley and Sons, Inc.

Grosvenor, C.F. and C.W. Turner. 1958. Pituitary lactogenic hormone concentration and milk secretion in lactating rats. Endocrinol. 63:535.

Grosvenor, C.F. and C.W. Turner. 1959. Thyroid hormone and lactation in the rat. Proc. Soc. Expt. Biol. Med. 100:162.

Growen, J.W., and E.R. Tobey. 1931. The influence of insulin and phloridzin. J. Gen. Physiol. 15:67.

Hafez, E.S.E. 1964. Nursing behavior in primiparous and multiparous females. Cornell Vet. 59:549.

Hancock, J., P.J. Brumby, and C.W. Turner. 1954. Hormonal induction of lactation in identical twin cattle. N. Zealand J. Sci. Tech. 36:111.

Hayman, R.H. 1973. Bos Indicus and Bos Taurus crossbred dairy cattle in Australia. II. Effect of calf removal and prolactin treatment on lactation in crossbred Bos Indicus x Bos Taurus females. Aust. J. Agr. Res. 24:449.

Kirk, R.E. 1968. Split-Plot Design. In: Experimental Design: Procedures for the Behavioral Sciences. Wadsworth.

Kordon, C., C.A. Blake, J. Terkel, and C.W. Sawyer. 1974. Participation of serotonin containing neurons in the suckling induced rise in plasma prolactin levels in the lactating rats. Neuroendocrinol. 13:213.

Kronfeld, D.S., G.P. Mayer, J.McD. Robertson, and F. Raggi. 1963. Depression of milk secretion during insulin administration. J. Dairy Sci. 46:449.

Kumaresan, P., and C.W. Turner. 1965. Effect of graded levels of insulin on lactation in the rat. Proc. Soc. Expt. Biol. Med. 119:415.

Louis, T.M., H.D. Hafs, and B.E. Seguin. 1973. Progesterone, LH, estrus and ovulation after $PgF_{2\ alpha}$ in heifers. J. Anim. Sci. 35:1121.

Lyons, W.R., C.H. Ling, and R.E. Johnson. 1958. The hormonal control of mammary growth and lactation. Rec. Prog. Hormone Res. 14:219.

McDaniel, B.T., R.H. Miller, and E.L. Corley. 1965. DHIA factors for projecting incomplete records to 305 days. DHI Letter, ARS-44-164.

McDaniel, B.T., R.H. Miller, E.L. Corley, and R.D. Plowman. 1967. DHIA age adjustment factors for standardizing lactations to a mature basis. DHI Letter, ARS-44-188.

Meites, J. 1961. Farm Animals: Hormonal induction of lactation and galactopoiesis. In: S. Kon and A.T. Cowie (eds.), Milk: The Mammary Gland and Its Secretion, Vol. II, Academic Press.

Meites, J. 1970. Direct studies of the secretion of the hypothalamic hypophysiotropic hormones (HHA). In: J. Meites (ed.), Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry. Williams and Wilkins.

Meites, J., T.F. Hopkins, and P.K. Talwalker. 1963. Induction of lactation in pregnant rabbits with prolactin, cortisol acetate, or both. Endocrinol. 73:261.

Meites, J. and M.C. Shelesnyak. 1957. Effects of prolactin on duration of pregnancy, viability of young, and lactation in rats. Proc. Soc. Expt. Biol. Med. 94:746.

Meites, J. and C.W. Turner. 1942. Influence of suckling on lactogen content or pituitary of postpartum rabbits. Endocrinol. 31:340.

Mollett, T.A., R.E. Erb, E.L. Monk, and P.V. Malven. 1975. Estradiol-17-beta and progesterone effects on lactation. J. Anim. Sci. 41:369. (Abstr).

Moltz, H., M. Lubin, M. Leon, and M. Numan 1971. Hormonal induction of maternal behavior in the ovariectomized nulliparous rat. Physiol. & Behav. 5:1373.

Monk, E.L., T.A. Mollett, R.E. Erb, and C.J. Callahan. 1973. Hormone changes associated with lactation induced with progesterone-estrogen. J. Dairy Sci. 56:656. (Abstr).

Moon, R.C. 1961. Growth hormone and mammary gland lobule--alveolar development. Amer. J. Physiol. 201:259.

Moss, G.E., R.G. Sasser, U.L. Estergreen, S.A. Becker, and S.L. Davis. 1975. Effect of TRH on induced Lactation. J. Anim. Sci. 41:370. (Abstr).

Moyer, K.E. 1968. Kinds of agression and their physiological basis. Commun. Behav. Biol. 2.65.

Nandj, S., and H.A. Bern. 1961. The hormones responsible for lactogenesis in BALB/cCrgl mice. Gen. Comp. Endocrinol. 1:195.

Narendran, R., and R.R. Hacker. 1973. Hormone induction of lactation in heifers and cows: Procedures and problems. Canadian J. Anim. Sci. 53:772. (Abstr).

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. 57:1334.

Nauta, W.J.H. 1958. Hippocampal projections and related neural pathways to the midbrain in the cat. Brain 81:319.

Nicoll, C.S., R.P. Fiorinou, C.T. McKerinse, and J.A. Parsons. 1970. Assay of hypothalamic factors which regulate prolactin secretion. In: J. Meites (ed.), Hypophysiotropic Hormones of the Hypothalamus Assay and Chemistry. Williams and Wilkins.

Noirot, E. 1964a. Changes in responsiveness to young in the adult mouse: The effects of external stimuli. J. Comp. Physiol. Psycho. 57:97.

Noirot, E. 1964b. Changes in responsiveness to young in the adult mouse: I. The problematical effect of hormones. Anim. Behav. 12:52.

Noirot, E. 1969. Serial order of maternal responses in mice. Anim. Behav. 17:547.

Norman, H.D. 1970. Dairy cattle type appraisal: Sources of variation and relationships to producing ability. Ph.D. thesis. Cornell University, New York.

Paape, M.J., A.J. Guidry, and M. April. 1973. Preliminary observations following hormonally induced lactation. J. Dairy Sci. 56:567. (Abstr).

Parau, D. 1968. Altertumliches melken in licht neuerer physiologischer erkenntnisse. Milchwissenschatt. 23:399.

Peeters, G., H. Stormorken, and F. Vaschoubroek. 1960. The effect of different stimuli on milk ejection and diuresis in the lactating cow. J. Endocrinol. 20:163.

Peeters, G., E. DeBuysscher, and M. Vandevelde. 1973. Milk ejection in primiparous heifers in the presence of their calves. Zbg. Vet. Med. A. 20:531.

Perrin, D.R. 1955. Milk composition studies in the hormonal induction of lactation using identical twin dairy cattle. New Zealand J. Sci. Tech. 37:88.

Plume, S., C. Fogarty, L.J. Grota, and R. Ader. 1968. Is retrieving a measure of maternal behavior in the rat? Psycho. Reports 23:627.

Quadango, D.M., J.F. DeRold, B.B. Gorzalka, and R.E. Whalen. 1974. Maternal behavior in the rat: Aspects of concaveation and neonatal androgen treatment. Physiol. Behav. 12:1071.

Raskin, R.L., M.Raskin, and R.L. Baldwin. 1973. Effects of chronic insulin and cortisol administration on lactational performance and mammary metabolism in rats. J. Dairy Sci. 56:1033.

Reece, R.P. 1943. The hormonal preparation of dairy cows for lactation. J. Dairy Sci. 26:746.

Reece, R.P. 1958. Mammary gland development and function. In: J.T. Velardo (ed.), The Endocrinology of Reproduction. Oxford University Press.

Richards, M.P. 1966. Maternal behavior in the golden hamster: Responsiveness to young in virgin, pregnant, and lactating females. Anim. Behav. 14:310.

Rosenblatt, J.S., and D.S. Lehrman. 1963. Maternal Behavior of the laboratory rat. In: H.L. Rheingold (ed.) Maternal Behavior in Mammals. Rheingold.

Rosenblatt, J.S. 1967. Nonhormonal basis of maternal behavior in the rat. Science 156:1512.

Roth, L.R. 1971. Sensory regulation of maternal behavior in rats. Paper presented to Expt. Psycho. Assoc., 1971.

Rowe, K.E. 1973. Determining sample size in covariate analysis. Personal communication. Oregon State University.

Saake, R.G. 1974. Freshening without calving is here. Hoard's Dairyman. July 10. pp 818.

St. John, R.D., and P.A. Corning. 1973. Maternal agression in mice. Behav. Biol. 9:635.

Scott, J.P. 1966. Agnostic behavior of mice and rats: A review. Amer. Zool. 6:683.

Schmidt, G.H. 1966. Effect of insulin on yield and composition of milk of dairy cows. J. Dairy Sci. 49:381.

Schein, M.W., and Fohrman, Mi II. 1955. Social dominance relationships in a herd of dairy cattle. British J. Anim. Behav. 3:45.

Selmen, I.B., A.D. McEwan, and E.W. Fisher. 1970a. Studies on natural suckling in cattle during the first 5 hours post partum. I. Behavioral studies (dams) Anim. Behav. 18:276.

Selmen, I.B., A.D. McEwan, and E.W. Fisher. 1970b. Studies on natural suckling in cattle during the first 5 hours post partum. II. Behavioral studies (calves) Anim. Behav. 18:284.

Shaw, J.C., A.C. Chung, and I. Bunding. 1955. The effect of pituitary growth hormone and adrenocorticotropic hormone on established lactation. Endocrinol. 56:327.

Smith, K.L. 1970. The IgG immunoglobulin of bovine lacteal secretion and a possible role of estrogen on IgG concentration. Ph.D. thesis. Ohio State University.

Smith, K.L., L.A. Muir, L.C. Ferguson, and H.R. Conrad. 1971. Selective transport of IgG into the mammary gland: Role of estrogen and progesterone. J. Dairy Sci. 54:1886.

Smith, K.L. and F.L. Schanbacher. 1973. Hormone induced lactation in the bovine. I. Lactational performance following injections of 17-beta-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.

Smith, O.W., K. Mongkonpunya, H.D. Hafs, E.M. Convey, and W.D. Oxender. 1973. Blood serum testosterone after sexual preparation or ejaculation, or after injections of LH or prolactin in bulls. J. Anim. Sci. 37:979.

Smith, V.G., T.W. Beck, E.M. Convey, and H.A. Tucker. 1974. Bovine serum prolactin and milk yields after ergocryptine. Neuroendocrinol. 15:172.

Swanson, E.W. 1955. The effect of nursing calves on milk production of identical twin heifers. J. Dairy Sci. 38:615.

Steel, R.G., and J.H. Torrie. 1960. Covariance in the randomized complete block design. In: Principles and Procedures of Statistics. McGraw-Hill, Inc.

Talwalker, P.K., C.S. Nicoll, and J. Meites. 1961. Induction of mammary secretion in pregnant rats and rabbits by hydrocortisone acetate. Endocrinol. 69:802.

Terkel, J. and J. Rosenblatt. 1968. Maternal behavior induced by maternal blood plasma injected into virgin rats. J. Comp. Physiol. Psycho. 65:479.

Terkel, J. and J.S. Rosenblatt. 1972. Humoral factors underlying maternal behavior at parturition: Cross transfusion between freely moving rats. J. Comp. Physiol. Psycho. 80:365.

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

Turner, C.W. 1959. The experimental induction of growth of the cow's udder and the initiation of milk secretion. Res. Bull. 697. University of Missouri Agr. Expt. Station.

Turner, C.D. and J.T. Bagnara. 1971. The hormones of pregnancy and lactation. In: General Endocrinology. W.B. Saunders. Co.

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

Turner, C.W., H. Yamamoto, and H.L. Ruppert. 1957. Endocrine factors influencing the intensity of milk secretion: estrogen, thyroxine, and growth hormone. J. Dairy Sci. 40:37.

Williams, R., O.A. Childs, D. Smith, and C.W. Turner. 1955. The effects of the hormones progesterone and estrogen in initiating lactation in dairy cows. J. Dairy Sci. 38:609.

Wiesner, B.P. and N.M. Sheard. 1933. Maternal behavior in the rat. In: B.P. Wiesner and N.M. Sheard (eds.), Maternal Behavior in the Rat. Oliver and Boyd.

Zarrow, M., R. Gandelman, and V.H. Denenberg. 1971. Prolactin: Is it an essential hormone for maternal behavior in the mammal? Hormone Behav. 2:34.

Zeliger, Y., R. Volcani, and D. Sklan. 1972. Yield and protein composition in cows milked prepartum. J. Dairy Sci. 56:869.

APPENDICES

APPENDIX 1

COMPOSITION OF TOLUENE-BASE SCINTILLATION FLUID

5.0 g 2,5-Diphenyloxazone (PPO) / liter

Dissolve fluor in technical grade toluene.

APPENDIX 2

COMPOSITION OF GELATIN-PBS (G-PBS)

0.01 M Phosphate buffered saline, pH 7.0 (PBS)

a) NaCl 8.183 g/liter

b) monobasic phosphate (NaH_2PO_4) 1.38 g/liter (0.012 M)

c) sodium azide 1 g/liter

d) bring to partial volume with distilled H_2O

e) adjust pH to 7.0 with NaOH (Approx. 1.43 ml 5N NaOH/liter)

f) store at 4° C

Gelatin-PBS

a) 0.1% G-PBS is made by dissolving 0.5 g of Knox Gelatin in 400 ml PBS at 37° to 40° C and then bringing it up to final volume (500 ml) with PBS on ice.

b) Store at 4° C.

APPENDIX 3

DEXTRAN-COATED CHARCOAL

a) 0.25 g Dextran T-70 (Pharmacia) / liter

b) 2.50 g Neutralized Norit charcoal (Sigma) / liter

c) Bring to volume (1 liter) with cold PBS.

d) Mix for 5 min and store in refrigerator.

e) Keep on ice and stir when being used.

APPENDIX 4

COMPOSITION OF

TOLUENE-TRITON X-100 SCINTILLATION FLUID

a) 7.0 g 2,5-Diphenyloxazone (PPO) / liter

b) 333.3 ml Triton X-100

c) 666.6 ml Toluene

APPENDIX 5

REGRESSION MODEL FOR ASSAY DATA

Linear Regression Model

$$Y_1 = B_0 + B_1 X_1 + B_2 X_1^2 + B_3 X_1^3 + e$$

Third order model

$$Y_2 = b_0 + b_1 X_2 + b_2 X_2^2 + b_3 X_2^3 + e$$

Predicting third order model

Where:

X_1 = independent variable

Y_1 = random variable

B = parameters / regression coefficients (based on $X_1 Y_1$)

X_2 = independent variable = time (cpm)

Y_2 = random variable = predicted ng/ml

b = based on the standard curve (known data)

$e = N(0, \sigma^2)$ (distribution, mean, variance)