Effects of Photoperiod on the Development of Beef Replacement Heifers from Weaning until First Lactation

By

Nanette D. Glover

A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Department of Animal Science

The University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

Effects of Photoperiod on the Development of Beef Replacement Heifers from Weaning until First Lactation

BY

Nanette D. Glover

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

ABSTRACT

Glover, Nanette D. M.Sc., The University of Manitoba, October, 2000. Effects of Photoperiod on the Development of Beef Replacement Heifers from Weaning Until First Lactation. Major Professors; Julie A. Small and Alma D. Kennedy.

The effects of photoperiod on the development of beef replacement heifers from weaning until first lactation were determined by assigning 144 crossbred heifers on the basis of weaning weight (225 ± 23 kg) and sire breed (British/Continental) to two outdoor housing facilities, with different photoperiod treatments, in a completely randomized design. From December 1, 1998 (day 0) until May 20, 1999, heifers in one facility received supplemental light (423 lux, 1 m above ground) to extend the daily photoperiod (natural + supplemental light) to 16 hours (EP). Heifers in the other facility experienced natural photoperiod only (NP). Measurements of body weight gain, backfat, and concentration of prolactin in blood serum were made every 28 d. Observations for estrus behaviour were made twice daily, in the morning and evening, and were confirmed by serum progesterone concentration in blood samples taken 8-12 d after observed estrus. Pre-breeding body weight (388 ± 4 kg), and backfat (3.7 ± 0.1 mm) were similar ($P > 0.05$) between treatments. Prolactin was higher ($P < 0.05$) for the EP than the NP treatment on days 28, 56, but was not different ($P > 0.05$) on days 84, 112 and 140. For EP and NP treatments respectively, 84.7% vs. 69.4% ($P < 0.05$) had one confirmed estrus, and 63.9% vs. 48.6% ($P < 0.05$) had two confirmed estruses before breeding season. The proportion of irregular length pubescent cycles (41.6%) was similar between treatments ($P > 0.05$).

Two methods of estrous synchronization and timed artificial insemination (AI) were used for first service. Progesterone concentration in blood samples taken twice weekly for approximately one month before synchronization, was used to classify heifers as having either regular luteal function (RLF – at least two consecutive progesterone values ≥ 1.0 ng ml^{-1}) or irregular luteal function (ILF - no consecutive progesterone values ≥ 1.0 ng ml^{-1}). Heifers with RLF were assigned to either double prostaglandin ($PGF_{2\alpha}$) method (Lutalyse® 25 mg, 11 d apart, PG-RLF) or gonadotrophin-releasing

hormone (GnRH) method (Factrel® 100 μg followed 7 d later by $PGF_{2\alpha}$, and a second GnRH at AI, GnRH-RLF). All heifers with ILF were assigned to the GnRH method (GnRH-ILF). Response to synchronization was determined by blood progesterone concentration at each injection. Heifers observed in estrus until 36 h post- $PGF_{2\alpha}$ were inseminated 12 h later, and all remaining heifers were inseminated 66 h post- $PGF_{2\alpha}$. Fertile bulls were used 2-45 days after AI. Synchronization response rate (69.8, 65.1, 31.0 ± 4.5%), first service conception rate overall (32.6, 27.9, 13.8 ± 4.3%), and in responders (43.3, 35.7, 16.7 ± 4.2%) were similar ($P > 0.05$) in PG-RLF and GnRH-RLF, but were lower ($P < 0.05$) in GnRH-ILF. Pregnancy rates at 25-d (74.4, 76.7, 74.1 ± 4.3%) and 45-d (79.1, 90.7, 91.4 ± 3.8%) were similar ($P > 0.05$) for PG-RLF, GnRH-RLF, and GnRH-ILF, respectively. Extended photoperiod increased the proportion of heifers with RLF ($P < 0.05$), but had no further effects ($P > 0.05$) on synchronization or breeding.

Maternal performance as two-year olds was evaluated in terms of calving date (n=105), milk production and composition during the period of peak lactation (n=32), and calf weight. Calving distribution, age and body weight were similar between photoperiod treatments ($P > 0.05$). Milk production and composition at 6, 8 and 10 weeks were similar between treatments ($P > 0.05$). Calf weight did not differ from birth until spring turnout to pasture ($P > 0.05$).

The results of this study indicate that exposure to extended photoperiod of 16 h light day^{-1} during the post-weaning period was effective in stimulating the reproductive development of beef heifers, but did not affect response to synchronization and timed AI, or subsequent maternal performance of beef heifers.

ACKNOWLEDMENTS

I would like to express my sincere appreciation to Dr. Julie Small for acting as my advisor during the completion of this project. Thank you Julie for giving me the opportunity to complete my degree, and for your guidance and friendship. I would like to thank Dr. Alma Kennedy for her advice and encouragement, I have really enjoyed her involvement in the project. Thanks also to Dr. Laurie Connor for serving on my advisory committee and for her instruction in reproductive physiology. The financial support of Manitoba Hydro is gratefully acknowledged, as well as the expert advice of Paul Wasney and Ray Borris of Manitoba Hydro in planning the lighting design for this project. The contributions of UpJohn & Pharmacia (Lutalyse®), Ayerst Veterinary Laboratories (Factrel®), American Breeders Service, Canada (semen) and the Agriculture & AgriFood Matching Investment Initiative Fund are appreciated.

I owe a huge thanks to many individuals at the Brandon Research Centre. Thank you to Doug Ward for being one step ahead of me most of the time, for his constant encouragement and for his dedication to this and all projects at the station. Thank you to Bill Sutherland for the many hours spent analyzing samples and for his guidance in the lab. Thanks to Av Singh for his cheery smile and friendship. This project would not have been possible without the support of the BRC Beef Crew. Thanks guys for all the hard work, and for all of the fun and memorable times I have had working with you.

I would like to thank my entire family for all of the support throughout my studies. I am very lucky to have such a caring family. Finally, and most importantly, I would like to thank my husband Dale for travelling this long and at times difficult road with me. I would not have been able to complete this project without the input of my resident computer consultant, statistical advisor, proof-reader, chef, accountant etc. etc. Thank you, Dale, for being the best friend and husband I could ever hope for.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ADG	average daily gain
AI	artificial insemination
BCS	body condition score
cAMP	cyclic adenylate monophosphate
CL	corpus luteum
CRI	colour rendition index
EP	extended photoperiod
ES	estrous synchronization
FSH	follicle stimulating hormone
GnRH	gonadotrophin releasing hormone
HPS	high pressure sodium
ILF	irregular luteal function
LH	luteinizing hormone
MRNA	messenger ribonucleic acid
NP	natural photoperiod
P4	progesterone
PG, $PGF_{2\alpha}$	prostaglandin
RLF	regular luteal function
SE	standard error
TMR	total mixed ration

1 GENERAL INTRODUCTION

"There is considerable flexibility for management in the operation of a cow/calf herd. In addition to pasture management, the rancher chooses breeding and calving dates, length of breeding season, the time to wean and market and a nutritional program. The single area for which there are no choices is conception. The cows must get bred. If not, the banker has his turn at running the ranch. (Diven 1995)."

Proper management of replacement heifers is one very important step in achieving the goal of high conception rates in the beef herd. The replacement heifer represents the future genetic base of the cow herd, therefore selection for desired traits is obviously very important to the long term efficiency and success of the herd. The management tools used to raise replacement heifers also impact lifetime productivity in the herd. Nutritional and environmental factors interact with the heifer's genetic potential to determine her course of reproductive maturation.

The main objective of this thesis is to thoroughly examine a specific element of the environment that may influence the reproductive development of the beef replacement heifer - photoperiod. Presented in this thesis are the description and results of a study examining the effects of providing an extended photoperiod, from just after weaning until breeding, on the development of beef heifers until first lactation.

A second objective is to examine the compatibility of two estrous synchronization methods with timed artificial insemination of beef heifers. A recent survey of Manitoba's cow/calf producers showed that while reproductive performance is a major factor limiting profitability, few producers use estrous synchronization and artificial insemination mainly because of the labour associated with such practices (Small and McCaughey 1999). The development of synchronization procedures that reduce labour inputs may enhance the adoption of artificial insemination.

2 LITERATURE REVIEW

All consumable products of animal origin, including meat, milk, eggs and countless others, are obtained entirely through reproductive processes (King 1993). The importance of this fact is often overlooked or underestimated. Livestock producers generally appreciate the economic importance of the time required for animals to reach market weight, and can usually quantify the feed costs associated with raising an animal from birth (King 1993). Quantifying the economic importance of reproduction seems to be a more elusive task. Melton (1995) compared the relative importance of reproduction with growth and end-product traits. He reported that for the conventional cow-calf operation that markets calves at weaning, reproduction may be as much as four times more economically important than growth and end-product traits. In Manitoba, the majority (68%) of beef producers sell weaned calves (Small and McCaughey 1999).

2.1 Reproductive Efficiency

Given the importance of reproduction, one goal of livestock producers should be to maximize reproductive efficiency. King (1993) defines reproductive efficiency as the number of viable young produced during the lifetime of the female in a herd or flock, and suggests that the intervals from birth to first parturition, and then between births, are the principal components affecting lifetime reproductive performance. Although simplistic, this definition of reproductive efficiency is true in a biological sense and can be expanded upon.

Compared to other livestock species, cattle seem to be at a particular disadvantage from a reproductive standpoint. Generally, beef females experience a prolonged period to reach sexual maturity followed by a long gestation, which usually results in a single offspring. Because of the low reproductive rate of beef cows, a greater proportion of the total energy is required to maintain the breeding herd as compared to other meat producing species (Dickerson 1978). King (1993) suggests that in order for beef

producers to compete with more prolific species, reproductive management should aim to have cows producing a calf annually. King (1993) further states that the reproductive task of a beef cow is not complete until a calf is weaned. Thus, reproductive efficiency in the beef cow is best defined as the ability to wean a calf annually throughout her lifetime in the herd. A more complete definition of efficiency must account for total end product (pounds of calf) versus total input, but first the basic goal of weaning a calf annually for the lifetime of the cow in the herd should be achieved.

2.1.1 Cow Age and Time of Calving

Heifers that first calve as two-year olds have a higher lifetime calf production than those that first calve as three-year olds (Patterson et al. 1992). Lesmeister et al. (1973) reported that heifers first calving as two-year olds calved significantly earlier throughout the remainder of their herd lifetime than did those first calving as three-year olds. Managing heifers to first calve as two-year-olds is currently common practice in the beef industry. While the economic benefits of calving heifers at two are accepted, the importance of time of calving in the first season is perhaps less well appreciated.

Lesmeister et al. (1973) clearly illustrated the importance of date of first calving as a two-year-old on lifetime productivity. Fourteen years of records were collected from 1169 calves born to 291 cows, first bred to calve at two years of age. The breeding season lasted 60 days and all calves were weaned on the same day. Each cow was assigned to an initial calving group based on her time of calving as a two-year old. Each subsequent calving was then assigned to a calving group, thus each cow had one initial calving group and numerous subsequent calving groups. The initial calving season was broken up into three 21-day periods, numbered one through three sequentially. Heifers calving before 283 days were classified as Early, and those calving after the last 21-day period were classified as Late. Initial calving group affected subsequent calving group, with early calvers maintaining a significant ($P < 0.05$) advantage in calving date in subsequent years. Initial calving group also affected first calf performance, as early calving heifers weaned heavier calves ($P < 0.01$) than those calving later in the season. The authors stated that one of the most important findings of the study was that cows

calving early the first time produced more kilograms of calf ($P < 0.01$) in their lifetime than cows calving later the first time. The average lifetime production (weaning weight) for first time early calvers was 185 kg, compared to 179 kg for first time late calvers. Although this study occurred over 25 years ago, the breeding management of the herd was very similar to current practices (restricted breeding season, calving as two-year olds, one weaning date). The findings, therefore, almost certainly apply to current beef production.

In a more recent study, Marshall et al. (1990) reported very similar results to those of Lesmeister et al. (1973). The study by Marshall et al. (1990) built upon earlier studies because it accounted for potential increases in feed costs associated with earlier calving. The first calving season was divided into CG1 (calving before 21 days), CG2 (calving 22-42 days into the season) and CG3 (calving after 42 days). Table 1 illustrates the impact of calving date on various calf production traits. The fact that pre-weaning ADG was similar, while weaning weight differed among groups emphasizes the importance of age on calf uniformity. The increase in weaning weight was only partially offset by increased energy intake by the heifer-calf pair, thus differences existed in the overall production efficiency ratio. Overall, early calving heifers were the most efficient. The authors explained that the increased efficiency is due to the fact that early calvers spend more time in a lactating state, diluting the proportion of total feed energy used for maintenance. CG1 cows tended to have their second calf earlier than CG3 calves, which agrees with findings from the study by Lesmeister et al. (1973). An interesting point that was raised by the authors is that calving interval is not a good measure of reproductive efficiency when a restricted breeding season is used, because open cows are usually culled which would bias the interval in favour of cows that calved late but rebred.

Table 1. First-calf production traits as affected by calving group (adapted from Marshall et al. 1990).

Trait	CG1	CG2	CG3	F-test[a]
Calf Traits				
Birth Date (julian)	78.5 ± .65	94.7 ± .90	116.6 ± 1.41	**
Birth Weight (kg)	34.0 ± .37	34.7 ± .53	34.9 ± .79	NS
Pre-Weaning ADG (g day^{-1})	784 ± 9.2	793 ± 13.1	761 ± 19.4	NS
Weaning Age (days)	220 ± .7	204 ± .9	182 ± 1.4	**
Weaning Weight (kg)	207 ± 2.2	196 ± 3.1	175 ± 4.6	**
Creep Feed ME (Mcal)	461 ± 6.9	402 ± 9.4	335 ± 14.4	**
Cumulative heifer feed ME (Mcal)				
On-test to calving	2677 ± 17	3072 ± 24	3521 ± 32	**
Lactation	6001 ± 30	5475 ± 42	4788 ± 57	**
Total drylot period	8684 ± 36	8554 ± 52	8298 ± 69	**
Production efficiency				
Heifer and calf ME/calf weaning wt (Mcal/kg)	44.6 ± .48	46.6 ± .69	50.9 ± 1.02	**

[a]treatment effect: ** = $P < 0.01$, NS = $P > 0.10$

These studies clearly illustrate the importance of managing beef females to calve early as two-year olds. In order for a heifer to calve early as a two-year old, she must become pregnant at approximately 15 months of age. Given that a calf spends approximately six months nursing its mother, the window becomes quite small for managing the replacement heifer to achieve the goal of pregnancy establishment at 15 months. Genetic, nutritional and environmental factors all may influence the reproductive development of replacement heifers.

2.2 Physiological Control of Puberty

Puberty is defined as the physiological stage of development in which the female first expresses estrus and ovulates (Short 1984). Before discussing the importance of managing pubertal development, a brief review of the physiological mechanisms governing puberty is required. Physiological processes in the body are mediated through the endocrine system. Reproductive processes in the female are governed by the hypothalamic-pituitary-ovarian axis (Figure 1). A series of physiological changes in this axis lead to puberty.

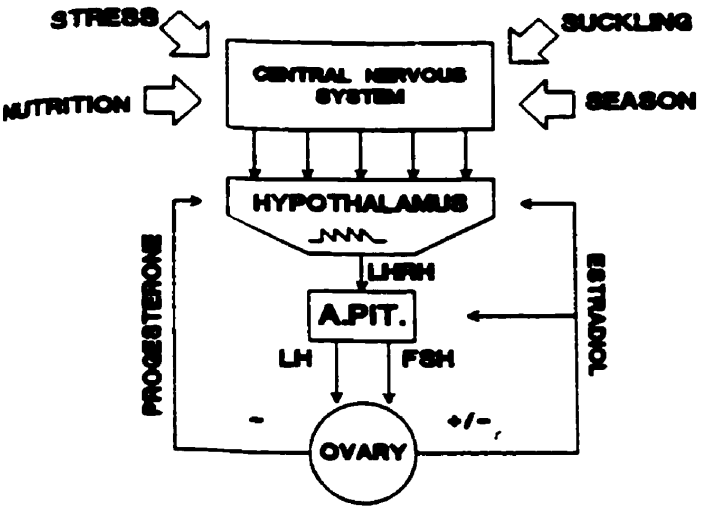

Figure 1. Schematic illustration of the hypothalamic-pituitary-ovarian axis showing neuronal and endocrine inputs that control release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (adapted from Schillo et al. 1992).

Schillo et al. (1992) presented an excellent model of the endocrine events leading to puberty in heifers (Figure 2). One of the curious findings in the study of puberty is that the hypothalamic-pituitary axis is functionally competent long before the onset of puberty, as early as one month of age (Schams et al. 1981). The ovaries of heifer calves are responsive to gonadotropins long before ovulation first occurs (Seidel et al. 1971). Schillo et al. (1992) suggest that changes in the negative feedback effects of estradiol on LH secretion is key in the endocrine control of puberty development. The responsiveness of the axis to the negative feedback of estrogen seems to decrease with age, as estradiol suppressed LH release for a longer duration in 4- month old heifers than in 8- and 12-month old heifers (Schillo et al. 1982). The reduction in responsiveness to estradiol negative feedback allows pulsatile LH secretion to increase to a level that stimulates the development of preovulatory follicles. The prepubertal increase in LH pulse frequency seems to be an important developmental event in the onset of puberty (Schillo et al. 1992). The maturation of ovarian follicles, due to the increase in LH pulse frequency, results in increasing concentrations of estradiol. High levels of circulating estradiol lead to a preovulatory LH surge and estrus.

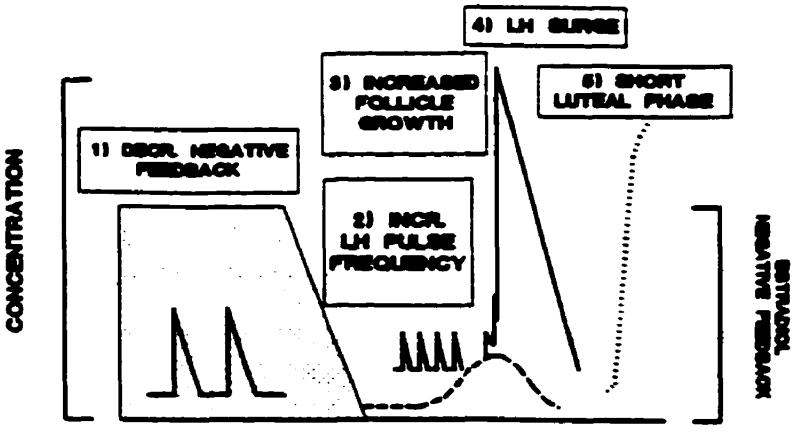

Figure 2. Summary of important endocrine events associated with onset of puberty in the heifer. Patterns of LH, estradiol, and progesterone are represented by the solid, dashed, and dotted lines respectively. The shaded area represents degree of responsiveness of the hypothalamic-pituitary axis to estradiol negative feedback (adapted from Schillo et al. 1992).

2.2.1 Time of Puberty in Relation to Breeding

For heifers to become pregnant, they must have undergone puberty prior to, or at the desired breeding age. The timing of puberty relative to breeding may be an important factor in determining the proportion of heifers that are bred early in the season. Breeding on the pubertal estrus reduced pregnancy rate and number of offspring in sows and ewes (Robertson et al. 1951 and Hare and Bryant 1985 as cited by Byerley et al. 1987). Byerley et al. (1987) examined the pregnancy rates of beef heifers bred either on the pubertal or the third estrus. They found that heifers bred on the third estrus had a higher pregnancy rate ($P < 0.05$) than those bred on the pubertal estrus (78% vs. 57%, respectively). The physiological reason for reduced fertility at puberty was not clear.

A more recent study by Del Vecchio et al. (1992) further examined the dynamic nature of the estrous cycle after puberty. Heifers were observed and daily blood samples taken from the pubertal estrus until the completion of three estrous cycles. The frequency of abnormal length cycles was greater ($P > 0.05$) during the first (40%) and second (35%) cycles than during the third cycle (0%). The causes of the abnormalities are shown in

Table 2. The endocrine data showed that abnormalities in uterine and ovarian endocrine activity occurred during the first and second cycles. Specifically, abnormalities were measured in the serum progesterone, estrogen, and prostaglandin levels and profiles in the first two cycles. The authors concluded that the abnormalities in the endocrine events during the first cycles may have an impact on conception rate and embryo survival, and that by the third cycle the endocrine profiles measured were normal. These findings help to explain the previously discussed reduced conception rates experienced when breeding at pubertal estrus.

Table 2. Specific causes for heifers exhibiting abnormalities during the first and second estrous cycle (adapted from Del Vecchio et al., 1992).

First estrous cycle	No CL developed	Short-lived CL	P4>1ng/ml at estrus	Silent estrus	Total
Short cycle	10 %	0 %	10 %	0 %[b]	20 %
Long cycle	0 %	0 %	0 %	20 %[a]	20 %
Total	10 %	0 %	10 %	20 %[a]	40 %
Second estrous cycle					
Short cycle	5 %	10 %	10 %	0 %[b]	25 %
Long cycle	5 %	0 %	0 %	5 %[b]	10 %
Total	10 %	10 %	10 %	5 %[b]	35 %

[ab] Different superscripts within columns indicate a difference at $P<0.05$.

2.2.2 Factors Affecting Puberty Development

Endocrine control of the development and function of the hypothalamic-pituitary-ovarian axis has been described earlier. In addition to endocrine information, the axis receives inputs from the central nervous system (Figure 1). Inputs that influence reproduction include information about nutritional status, environmental conditions, stress and social interactions (Schillo et al 1992). Genetics also influence puberty and reproduction (Martin et al. 1992).

2.3 Genetics

Great genetic variation exists both within and between breeds of beef cattle for age at puberty. Martin et al. (1992) provide an excellent review of genetic effects on beef heifer puberty and subsequent reproduction. Breeds have diverged in frequency of genes that affect expression of various traits, due to isolation by either geographical or human imposed pedigree barriers (Martin et al. 1992). The divergence of breeds for some characteristics is summarized in Table 3.

Table 3. Breed crosses grouped in biological type on the basis of four major criteria[x] (adapted from Martin et al. 1992).

Breed group	Growth rate and mature size	Lean : fat ratio	Age at puberty	Milk Production
Jersey	X	X	X	XXXXX
Hereford-Angus	XX	XX	XXX	XX
Red Poll	XX	XX	XX	XXX
Devon	XX	XX	XXX	XX
South Devon	XXX	XXX	XX	XXX
Tarentaise	XXX	XXX	XX	XXX
Pinzgauer	XXX	XXX	XX	XXX
Brangus	XXX	XX	XXXX	XX
Santa Gertrudis	XXX	XX	XXXX	XX
Sahiwal	XX	XXX	XXXXX	XXX
Brahman	XXXX	XXX	XXXXX	XXX
Brown Swiss	XXXX	XXXX	XX	XXXX
Gelbvieh	XXXX	XXXX	XX	XXXX
Holstein	XXXX	XXX	XX	XXXXXX
Simmental	XXXXX	XXXX	XXX	XXXX
Maine-Anjou	XXXXX	XXXX	XXX	XXX
Limousin	XXX	XXXXX	XXXX	X
Charolais	XXXXX	XXXXX	XXXX	X
Chianina	XXXXX	XXXXX	XXXX	X

[x] X lowest, XXXXXX highest

Heifers sired by breeds with a large mature size tend to be older and heavier at puberty compared to heifers with a smaller mature size. Also, breeds historically selected for milk production tend to reach puberty earlier than do breeds not selected for milk production. The correlation between mature size and age at puberty is 0.57 and for milk production and age at puberty is -0.87 for *Bos taurus* breeds (Martin et al. 1992). *Bos*

indicus breeds reach puberty at an older age than do *Bos taurus* breeds. Gregory et al. (1991) reported that the correlation between age at puberty and pregnancy rate at 18 months of age was -0.79, for nine purebred breeds which reached puberty at different ages.

The importance of genetic management becomes apparent through the effects of heterosis on age at puberty and subsequent reproduction. Heterosis is defined as the difference between the mean for the reciprocal F1 cross and the mean for the purebred parents (Martin et al. 1992). Heterosis represents the recovery from inbreeding depression that develops within genetically isolated populations (Dickerson 1973). Generally, the percentage of heifers reaching puberty at a certain age is greater for crossbreds than for purebreds (Martin et al. 1992). Utilizing heterosis is one method of managing age at puberty. A number of crossbreeding schemes have been developed to maximize heterosis, including rotational breeding, terminal crosses, and composite breeds (Martin et al. 1992).

Within breeds, selection for age at puberty may be one way to improve fertility, as age at puberty is a moderately heritable trait. The average heritability for this trait was reported to be .40 (Martin et al. 1992). Age at puberty is, however, somewhat difficult to measure directly, and to do so accurately is labour intensive. Selecting for age at puberty in heifers may be more easily achieved by selecting for scrotal circumference in sires (Martin et al. 1992). A high correlation, -0.92, between scrotal circumference in males and age at puberty in heifers was reported by Gregory et al. (1991). Heritability estimates from numerous papers for scrotal circumference averaged 0.45 (Martin et al. 1992). Because scrotal circumference is quite heritable and easily measured, it represents a good selection trait to improve overall fertility by lowering age at puberty.

Most *Bos taurus* heifers have the potential to reach puberty around the yearling age and thus breed at an appropriate age. However, breeds and individuals with an inherent ability to reach puberty and breed early may do so at less cost than their later maturing counterparts (Martin et al. 1992). To fully realize the inherent ability of a heifer to reach puberty and breed early, she must be provided with adequate nutrition and a favourable environment.

2.4 Nutrition

The nutrition of replacement heifers has received much study, thus an abundance of literature exists on the topic. From this abundance of information, general theories on the effects of nutrition on puberty have evolved, yet there still exists much interest in discovering the best strategies for the nutritional management of heifers. Rather than focus on the specifics of past research, the goal of this section is to discuss some of the basic and evolving concepts of heifer nutrition, and to relate these concepts to the physiological mechanisms governing puberty and reproductive development.

The relationship between level of nutrition and age at puberty is well established. Early in this century, reports on the effect of nutrition on reproduction were appearing (Eckles 1915), and subsequent studies have produced similar findings (reviewed by Schillo et al. 1992). The predominant message that resulted from many years of study is that an inverse relationship exists between level of nutrition and age at puberty, or as nutrition increases, age at puberty decreases. Body weight is the major factor affecting age at puberty, and heifers on a higher plane of nutrition achieve the weight gain required for the onset of puberty more quickly than heifers on a lower plane of nutrition, and are thus younger at puberty (Patterson et al. 1992). Realizing the importance of achieving a critical body weight on the onset of puberty allowed for the development of a simple nutritional management strategy called the target weight concept (Goehring 1991). The principle behind this concept is that heifers are fed to achieve 61-65% of mature body weight by the time they are exposed for breeding (Goehring 1991). The mature weights of various frame scores of cattle are shown in Table 4. The target weights are those required for females to be pubertal by 14 months of age, but as previously discussed, heifers should ideally be on the third cycle by breeding, thus these weights should be achieved slightly earlier. Regardless, they illustrate the concept of target feeding heifers in the post-weaning to breeding phase.

Table 4. Weight estimates (kg) for cattle of various ages within a frame score (adapted from Fox et al. 1988).

Frame Score	205-day Weight	14-month (breeding) Weight	Mature Weight
2	170	280	430
3	182	295	465
4	190	313	499
5	200	331	533
6	209	347	567
7	218	363	600
8	227	380	635

While the need for heifers to achieve the target body weight before breeding is well established, the best strategy to achieve the weight is not yet well defined. Whether the post-weaning weight gain should occur uniformly, or can be achieved more economically by feeding a lower cost ration followed by a period of increased nutrient intake remains unclear. Clanton et al. (1983) found that age at puberty was similar between heifers fed at a level that sustained a constant rate of gain post-weaning and those that experienced rapid growth during either the first or second half of the post-weaning period. A study by Yelich et al. (1996) examined the effects of two feeding strategies on puberty and endocrine function in heifers. At nine months of age, heifers were assigned to one of two treatments, HGAIN - fed to gain 1.36 kg/day or LHGAIN - fed to gain 0.23 kg/day for 16 weeks then fed to gain 1.36 kg/day. The HGAIN heifers were younger at puberty than the LHGAIN heifers (369±16 vs. 460±17 days of age, P = 0.001), but body weight (334±18) and condition score (5.6±0.2) were not different. The target gain of 0.23 kg/day in the LHGAIN treatment is a very severe restriction, and one that would not likely be applied in a practical situation. This study did not follow through to measure breeding performance, however a study by Hall et al. (1997) did report the effects of step gain feeding on some reproductive responses. For 122 days before breeding, heifers were fed to either gain continuously at 0.8 kg/day, or in a step-wise fashion of 0.5 kg/day for 84 days then 1.4 kg/day for 38 days. The results of the study indicated that compared to the continuous gain treatment, the step-wise gain treatment impaired both the estrus response to synchronization (90% vs. 35%) and first service conception rate of heifers (90% vs. 6%). However, the step-wise group did not

reach the same breeding weight as the continuous group (358.4 vs. 380.5 kg), because weight gain in the step-wise group was below target.

Feeding represents a large portion of the total cost of raising replacement heifers, thus there is ongoing interest in developing economical feeding strategies. Past research has shown that increased nutrient intake, following a period of nutrient restriction, hastens the onset of puberty (Yelich et al. 1996). More research is required to prove that step-wise gains can produce similar results to continuous gains, and to determine the optimal duration of each stage of intake and the appropriate gains in each stage.

2.4.1 Nutrition and Endocrine Control of Puberty

The relationship between nutrition and puberty is becoming better understood, yet there are still many unanswered questions as to exactly how nutrition influences the endocrine control of puberty. Nutritional status influences pulsatile LH release in heifers (Schillo et al. 1992). Hall et al. (1994) examined the effects of dietary energy intake on LH secretion and puberty in heifers. Heifers were maintained on either a high energy ration (HDE, 14.15 MCal ME/heifer/day) or moderate energy ration (MDE, 10.84 MCal ME/heifer/day) from weaning at 7 months of age until 14.5 months of age. The HDE heifers were younger at puberty than the MDE heifers ($P < 0.001$), but body weight was not different. Pre-pubertal LH pulse frequency increased between 10.5 and 12 months of age in HDE heifers, compared to that in MDE heifers. These findings corroborated earlier findings by Day et al. (1986), that energy restriction suppresses the increase in LH pulse frequency, even at a moderately restricted level that may occur in practical conditions (Hall et al. 1994).

The mechanisms mediating the nutritional effects on pulsatile LH secretion have not yet been fully elucidated (Schillo et al. 1992; Hall et al. 1994). To better understand the relationship between nutrition and LH release, it is necessary to determine the signals that the central nervous system uses to assess nutritional status, and how these signals are translated into a neuroendocrine message (Schillo et al. 1992). Changes in intermediary metabolism that accompany changes in weight and (or) fatness may influence LH

secretion (Figure 3). The changes in nutritional status may be reflected by changes in metabolic hormones or metabolites.

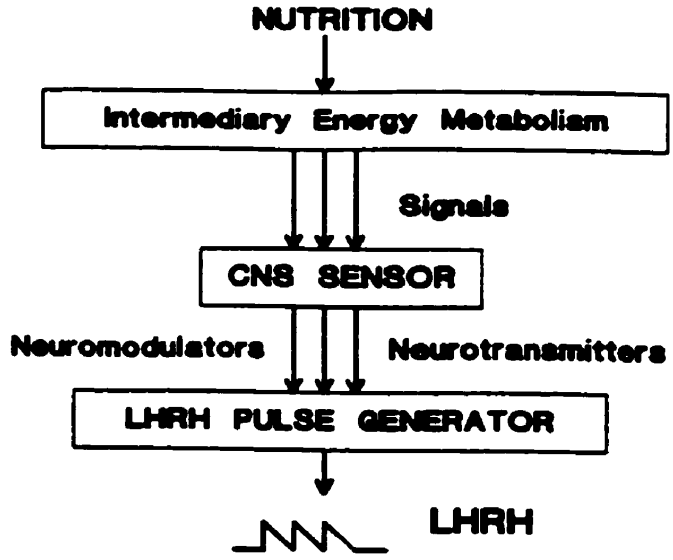

Figure 3. Hypothetical model depicting a possible mechanism whereby nutrition influences pulsatile release of luteinizing hormone-releasing hormone (LHRH) and onset of puberty in the heifer (adapted from Schillo et al. 1992).

Based on a review of the literature, Schillo et al. (1992) suggested that nonesterified fatty acids and growth hormone might inhibit LH release, whereas insulin, IGF-1, and tyrosine may stimulate LH release. Yelich et al. (1996) found that heifers on a high plane of nutrition had greater ($P < 0.05$) concentrations of IGF-1 and insulin than heifers on a step-wise ration. The step-wise heifers had greater ($P < 0.05$) concentrations of growth hormone and nonesterified fatty acids. As previously discussed, the HGAIN heifers had increased LH pulse frequency and reached puberty at a younger age than the LHGAIN heifers, thus these metabolic signals could have acted through the pathway depicted in Figure 3 to affect puberty. These findings provide some insight into the metabolic signals affecting puberty in heifers, and this area will undoubtedly receive much more study.

2.4.2 Other Relationships Between Nutrition and Reproduction

Nutrition affects the development and function of reproductive processes in heifers, in addition to puberty. Both under- and over-feeding heifers have been reported to negatively affect various aspects of reproduction. Low planes of nutrition have been associated with reduced conception rates, later conception dates and increased pregnancy loss (Short and Bellows 1971). Overfeeding has been associated with weak estrous symptoms, reduced conception rates, high embryonic mortality and decreased milk production (Patterson et al. 1992).

The relationship between over-feeding as a heifer, and mammary development and milk production is one of great importance, because the weaning weights of calves of similar age depend mainly on the genetic potential of the calves and the dams' ability to produce milk (McFadden 1991). The mammary gland undergoes two distinct phases of development in preparation for lactation. The first is growth of mammary cells, and the second is differentiation of these mammary cells into secretory cells (McFadden 1991). The growth phase occurs in distinct stages beginning at birth. For the first two to three months, the udder grows isometrically, or at the same rate as the rest of the body (Tucker 1981). Beginning at about three months of age and continuing until about nine months of age, the udder enters a phase of allometric growth in which mammary cell growth proceeds at a much more rapid rate than that of the rest of the body (Tucker 1981). From 9 months until about 15 months, mammary growth returns to isometric rate, and during gestation enters another period of rapid growth. The majority of growth occurs during gestation, but depends upon the foundation of cells provided by the earlier phase of allometric growth (McFadden 1991). Differentiation occurs around the time of parturition, in response to the hormonal cues accompanying pregnancy and parturition (Tucker 1981).

Proper nutrition has been shown to be an important factor influencing mammary growth during the first allometric period from 3 to 9 months of age, with the effects carrying through to subsequent milk production. The mammary gland contains a layer of fatty tissue called the fat pad, into which the secretory cells grow (McFadden 1991). Overfed heifers deposit more fat in the udder (Yelich et al. 1995) which may decrease secretory cell growth (McFadden 1991). The effects of over-feeding may be most critical

during the first allometric growth phase. Johnsson and Obst (1984) studied the effects of level of nutrition before and after eight months of age on milk and calf production in beef heifers. The heifers were reared on either a high or low plane of nutrition until eight months of age, followed by either a high, low, or moderate plane of nutrition from eight to fourteen months of age (Table 5). The results of the study (Table 5) showed that growth rate before eight months had more influence on milking ability than growth rate after eight months of age. Heifers receiving a low plane of nutrition prior to eight months had higher milk production.

Table 5. The average daily gain of heifers fed to grow at either high (H), medium (M) or low (L) rates during two rearing periods and subsequent milk yield, estimated at 30 days of lactation and calf weaning weight (adapted from Johnsson and Obst 1984).

	HM	HL	MM	LH	LM	Significance[a]
Period ADG (kg/day)						
2-8 months of age	0.91 ± 0.02	0.91 ± 0.02	0.67 ± 0.02	0.55 ± 0.02	0.55 ± 0.02	
8-14 months of age	0.57 ± 0.02	0.14 ± 0.02	0.58 ± 0.02	0.97 ± 0.04	0.55 ± 0.02	
30-day milk yield (l/day)						
1st lactation	4.1 ± 0.3	4.9 ± 0.3	4.3 ± 0.3	5.7 ± 0.3	6.3 ± 0.4	**
2nd lactation	5.2 ± 0.4	6.8 ± 0.6	7.0 ± 0.4	6.6 ± 0.5	7.0 ± 0.4	*
3rd lactation	5.1 ± 0.4	6.5 ± 0.6	7.2 ± 0.4	7.4 ± 0.5	7.6 ± 0.5	**
Calf weaning weight (kg)						
1st lactation: 240 days	218 ± 7.3	236 ± 5.5	221 ± 8.1	241 ± 9.6	259 ± 7.9	**
2nd lactation: 200 days	202 ± 6.2	217 ± 8.1	222 ± 5.2	219 ± 7.9	229 ± 8.2	NS
3rd lactation: 200 days	211 ± 4.4	225 ± 7.2	223 ± 5.4	230 ± 5.1	237 ± 4.1	*

[a] treatment effect: **=P < 0.01, *=P < 0.05, NS=P>0.10

From this data, the importance of pre-weaning nutritional management becomes obvious, as under current management practices, the majority of the first allometric growth phase would be spent nursing the cow. Martin et al. (1981) reported the negative effects of creep feeding calves on subsequent calf weaning weight. Heifers that received creep-feed as calves weaned smaller calves than heifers that did not receive creep-feed as calves (417.1 ± 2.2 vs. 425.7 ± 2.0 kg, P < 0.05). McFadden (1991) concluded that the ideal time to increase growth rates is post-weaning, after the first allometric stage of

mammary development and during the period when improved nutritional status may hasten the onset of puberty to allow for timely breeding.

2.5 Environment

2.5.1 Bull Exposure

Stimuli from social interactions may have important modulating effects on reproduction (Patterson et al. 1992). In other species, social interactions with a male may either suppress sexual maturation (primates in a family group) or accelerate sexual maturation (house mice, swine) (Vandenbergh 1989). The effects of exposure to sterilized males on pubertal development in heifers have been inconsistent. Roberson et al. (1987) found that long term exposure (152 days) to sterilized bulls had no effects on the cumulative proportion of heifers reaching puberty by 15 months of age. Previous studies have also indicated that short term exposure to bulls had no effect on age at puberty in heifers (Berardinelli et al. 1978; MacMillan et al. 1979).

Other studies have shown that bull exposure may accelerate puberty in heifers. Izard and Vandenbergh (1982) found that heifers exposed to bull urine reached puberty earlier and calved earlier than non-exposed heifers. Roberson et al. (1991) hypothesized that some of the inconsistencies in research results may be due to an interaction between growth rate and bull exposure. In experiment one, 267 heifers (~345 days of age at beginning of exposure) were used in a four-year study looking at the effects of bull exposure (~75 days), independent of a nutritional treatment. The results of experiment one showed that bull exposure reduced age at puberty in three out of four years. Averaging over all years, approximately twice as many heifers from the bull exposure group (60.3%) had reached puberty by 14 months of age compared to the no bull exposure group (29.8%). Experiment two was a two-year study, in which 159 heifers were assigned to one of four treatments: BE-HG - bull exposure and high growth rate (.8 kg/day), BE-MG - bull exposure and moderate growth rate (.6 kg/day), NE-HG - no bull exposure and high growth rate, and NE-MG - no bull exposure and moderate growth rate. The results of experiment two showed that the effects of bull exposure interacted with

growth rate ($P < 0.05$). The effect of bull exposure was greater in the HG than the MG group, and both bull exposed groups reached puberty before the non-exposed groups. Both bull exposed groups had a higher AI conception rate, which is likely due to a larger percentage reaching puberty before AI.

Growth rate is one factor that influences the effects of bull exposure on puberty. Other factors including heifer breed, duration of bull exposure, differential ability of bulls to elicit a response, location, and season may also explain the contradictory results in the literature (Roberson et al. 1991; Patterson et al. 1992).

The acceleration of puberty is mediated in part, through a pheromone produced by the male and identified by receptors in the vomeronasal organ of the female (Vandenbergh 1989). The neuroendocrine mechanism by which the male pheromone may accelerate puberty development is still unclear. A logical mechanism is that male exposure stimulates the hypothalamic-pituitary axis to secrete the gonadotropin, LH (Patterson et al. 1992). Exposure to males increased LH pulse frequency in anestrous ewes (Martin et al. 1980). Male exposure may also hasten the final stages of ovarian maturation and steroid production (Patterson et al. 1992).

2.5.2 Photoperiod

In some domestic species, reproduction is limited to one season of the year and management techniques are used to manipulate reproduction so that offspring are produced year round. In cattle, reproduction is not limited to one season, perhaps because domestic cattle evolved in situations where natural selection for seasonal breeding was reduced by the provision of feed, shelter and care for the young (Hansen 1985). Although reproduction occurs year round, it does not function in complete independence from seasonal inputs. Research has shown that one of the key inputs responsible for the seasonal influence on reproduction is photoperiod (Hansen 1985).

2.5.2.1 Photoperiod and Puberty Development

Early reports indicated that date of birth influenced age at puberty in heifers (Menge et al. 1960; Roy et al. 1980). Date of birth is obviously confounded with many other factors including seasonal differences in nutrition, temperature and management. Also, sexual development is an ongoing process, occurring over a number of seasons. Schillo et al. (1983) conducted a study to separate seasonal effects during the first six months of life from those in the second six months of life on puberty development. Heifers were born in either the spring or fall and reared outside until six months of age, after which they were housed in environmental chambers that simulated temperature and photoperiod conditions of spring/summer or fall/winter. Regardless of season of birth, heifers exposed after six months to spring/summer conditions were younger at puberty than heifers exposed to autumn/winter conditions. It is not possible to separate the effect of temperature and photoperiod in this study, but other studies have focused specifically on the effects of photoperiod on puberty. Hansen et al. (1983) found in two experiments, that heifers exposed to 18 hours of light per day reached puberty at a younger age than heifers exposed to natural day length from weaning. In experiments one and two respectively, ages at puberty were 318 days and 367 days in the long photoperiod group compared to 367 and 394 days in the natural photoperiod group. Growth rate was not different between the two treatments, therefore the authors suggested that the effects of photoperiod on puberty occurred independently of any nutritional effects, however feed was provided ad libitum and no indication was given that feed intake was measured.

Petitclerc et al. (1983) examined the effects of photoperiod on heifers fed either a high (>1 kg/day ADG) or low (0.7 kg/day ADG) plane of nutrition. Sixty heifers were housed indoors and exposed to either 16 hours light and 8 hours dark (16L:8D) or 8 hours light and 16 hours dark (8L:16D) and high or low plane of nutrition. The results showed that the interval from the start of the experiment to puberty was shorter for heifers exposed to 16 hours of light ($P < 0.07$) or a high plane of nutrition ($P < 0.01$). However, the effects of photoperiod on puberty were accompanied by increased growth rate. In both nutritional treatments, the 16L:8D group had increased growth rate, and improved feed efficiency. Further discussion on the effects of photoperiod on growth will follow, as it is important to be able to distinguish whether the light itself is affecting puberty. In

a more recent study, Ringuet et al. (1994) found that 16L:8D reduced age and body weight at puberty in 24 indoor housed Holstein heifers. Heifers experiencing 16L:8D reached puberty on average 32 days sooner and were 33 kg lighter than heifers experiencing 8L:16D. Although it was reported that feed was provided on an ad libitum basis, no indication was given whether differences in feed intake existed between the two treatments.

2.5.2.2 Photoperiod and Growth and Body Composition

The effects of photoperiod on growth rate remain unclear. Extending photoperiod to 16 hours may stimulate growth rate (Peters et al. 1980; Zinn et al. 1986a), reduce growth rate (Zinn et al. 1986b) or have no effect (Petitclerc et al. 1984; Phillips et al. 1997) compared to animals experiencing natural day length or 8 hours of artificial light. Furthermore, the effects of photoperiod on growth may be confounded with stage of sexual development (Zinn et al. 1986b), level of feed intake (Petitclerc et al. 1984), sex of the animal (Phillips et al. 1997; Tucker et al. 1984) and changes in body composition (Petitclerc et al. 1984). Increased growth rate has occurred independently of increased feed intake (Peters et al. 1978), while other studies have reported increased feed consumption in animals exposed to 16 hours of light (Peters et al. 1980).

The effects of photoperiod on body composition are somewhat less conflicting than those for growth rate. Petitclerc et al. (1984) reported that a photoperiod of 16L:8D enhanced gain in the percentage of protein in the 9th to 11th rib section of ad libitum fed heifers. Zinn et al. (1986b) found that post-pubertal heifers exposed to short days (8 hours of light) had a greater percentage of fat and a reduced percentage of protein in the soft tissue of the 9th to 11th rib section than that in heifers exposed to long days (16 hours of light). Photoperiod did not affect carcass composition in pre-pubertal heifers. In a recent study, Phillips et al. (1997) found that heifers experiencing natural photoperiod (average 9.7 hours/day) deposited more fatty tissue between autumn and winter than heifers receiving 16 hours of light/day. Supplemental light decreased fat and increased lean content of heifers in the winter months.

2.5.2.3 Photoperiod and Milk Production and Mammary Development

Historically, most research on the relationship between season and milk production focused on the effects of ambient temperature with very little attention paid to the potential effects of photoperiod on milk production (Tucker 1988). Early studies on the relationship between photoperiod and milk yield produced conflicting results, with some reporting no effect and others reporting increased milk yield with long photoperiods (Tucker 1988). Peters et al. (1978) reported an increase in milk yield with extended photoperiod. Cows provided with a 16 hour photoperiod produced 10% more milk in 100 days of lactation than cows provided with natural photoperiods of 9-12 hours light. The ability to increase milk production by extending photoperiod is currently becoming well accepted. Lactating cows that are provided with 16 hours of light per day have shown a 5 to 16% increase in milk yield compared to cows receiving 13.5 hours or less of light per day (Chastain and Hiatt 1998). The increase in milk yield has also been accompanied by an increase in feed intake (Peters et al. 1981; Tucker 1988). The dairy industry has embraced the potential of photoperiod to improve milk production, as a manual entitled "Supplemental Lighting for Improved Milk Production" (Chastain and Hiatt 1998) was written to inform dairy producers about the proper design and use of a supplemental lighting program.

There have been few studies examining the effects of photoperiod on the mammary development of heifers. Petitclerc et al. (1984) conducted a study to examine the effects of photoperiod and plane of nutrition on the mammary development of Holstein heifers. As discussed previously, heifers receiving a high plane of nutrition may show reduced milk production because of increased fat deposition in the developing mammary gland. Petitclerc et al. (1984) stated that the improved growth rate sometimes associated with long photoperiod would be beneficial in raising dairy heifers only if the development of mammary parenchymal tissue was not affected. Heifers were fed either a high (1.0 kg/day ADG) or low (0.7 kg/day ADG) plane of nutrition and housed with either a 8L:16D or 16L:8D photoperiod. After the heifers reached puberty, they were slaughtered and the mammary tissue was collected and dissected. The results showed that the amount of mammary parenchymal tissue was lower in heifers receiving a high plane of nutrition. Photoperiod had no effect on either the total amount or composition of

the parenchymal tissue in the mammary gland. In a subsequent study, Petitclerc et al. (1985) found that a 16L:8D photoperiod stimulated the growth of the mammary parenchyma into the fat pad. When reviewing the available literature, no studies were found that measured the effects of extending photoperiod on mammary development of beef heifers. There were also no studies found that followed through to measure milk production from heifers raised with long photoperiods.

2.5.2.4 Photoperiod and Other Reproductive Traits

Most studies on photoperiod focus on specific traits such as age at puberty, growth, or hormone secretion. Very few studies follow through to measure other reproductive traits such as breeding performance (number of services, days open, etc.). Hansen et al. (1983) found that age at puberty was reduced by exposure to 18 hours of light compared to natural photoperiod, but age at conception was not different between the two groups (Table 6). Although not statistically significant, heifers from the long photoperiod treatment conceived on average 16 days earlier. Because heifers were bred on each estrus until conception, the long photoperiod heifers received the first service at a younger age. The authors hypothesized that the younger age at breeding may explain the lower first service conception rate.

Table 6. Effect of photoperiod on onset of reproductive activity (adapted from Hansen et al. 1983).

Trait	Long Photoperiod	Natural Photoperiod
Age at first estrus, d[a]	367 ± 17.2*	394 ± 9.8
Age at first ovulation, d	360 ± 17.9*	389 ± 12.4
Age at conception, d	380 ± 15.4	396 ±10.3
Avg. services/conception	1.4	1.1
Percentage conception, first service	64	91

[a] values are means and standard errors
*P < 0.10

In a recent study, Reksen et al. (1999) reported the effects of photoperiod and photointensity on milk yield and reproductive performance using data collected from 1538 farms in Norway. The objective of the study was to assess whether the findings of previous experiments on photoperiod and sexual development and milk production were

present under field conditions. The traits measured were milk yield at first AI, 305-d milk yield, age at first AI, age at first calving, days open, calving interval, AI per cow, and nonreturn rate at 60 days. Lighting management was classified as either >12 hours or <12 hours of light. In addition, farms were classified as to whether or not dim illumination (average of 36 lux) was used throughout the night. Photoperiods >12 hours (average 14.2 hours) were used by 64% of farms. Farms using photoperiods <12 hours averaged 11.7 hours of light. The findings for milk yield agreed with previous studies, specifically that milk yield increased by 0.5 kg/day with long photoperiods. Age at first AI and first calving decreased 4.8 and 6.6 days respectively, in heifers experiencing long photoperiods. This finding was attributed to long photoperiod stimulation of earlier onset of puberty, or earlier establishment of normal cycles, or both. The use of dim illumination reduced days open, calving interval and AI services per cow and increased the nonreturn rate compared to herds that did not use dim illumination at night. The authors hypothesized that dim illumination at night may have mimicked the natural lighting pattern during summer, which stimulated reproductive traits. An interesting point to note from this study is that differences in various physiological traits were present in conditions less dramatic than those used experimentally. The "short" photoperiod in this study averaged 11.7 hours and the long photoperiod 14.5 hours. Experimentally, the difference is often more pronounced, with 16 hours being a commonly used long photoperiod. Performing a similar study in a large number of barns with a greater difference between long and short photoperiods would be very interesting.

2.5.2.5 Photoperiod and Behaviour

The effect of photoperiod on the behaviour of housed beef cattle has been measured in few studies, therefore preference for illumination level, and the effects of photoperiod on behaviour remain unresolved. Past research has shown that a preference for a lit versus an unlit environment may exist in confined calves (Baldwin and Start 1981 as cited by Weiguo and Phillips 1991). Weiguo and Phillips (1991) designed an experiment to determine whether loose-housed beef calves also exhibit a preference for light. Within a pen, calves were offered a choice between natural (10.5 hour day) or

supplemental lighting (18.5 hour day). Behavioural observations showed that more time was spent in the side with supplemental lighting than in the side with natural lighting. In pens where calves were provided with either supplemental or natural light, the provision of supplemental light reduced the amount of time spent standing and walking in the bedded area and increased the amount of time spent standing in the feed area. The authors concluded that calves may prefer a lit environment, and that supplemental light may decrease activity levels. Phillips et al. (1997) also reported reduced activity levels in heifers provided with 16 hours of light compared to those under natural light (average 9.7 hours light/day). Heifers experiencing long photoperiod spent more time lying awake. Interestingly, the heifers experiencing the long photoperiod exhibited more mounting behaviour in the winter observation period than did the heifers without supplemental light. By the spring observation period, both groups had the same amount of mounting behaviour. Puberty was not measured in this experiment, but it is possible that heifers reached puberty earlier in the long photoperiod treatment, which was reflected by an increase in mounting behaviour. Reksen et al. (1999) state that exposure of animals to inferior light intensity might be an upcoming issue for animal welfare, thus more study is needed to clearly define whether lighting intensities and duration used to achieve improvements in production traits are acceptable in terms of animal behaviour and welfare.

2.5.2.6 Mechanisms Encoding Day Length

2.5.2.6.1 Melatonin

Research in sheep has indicated that the pineal gland is involved with the transfer of information about photoperiod into a neuroendocrine signal (Schillo et al. 1992). Light information is received by the retina and sent to the pineal gland where it is translated into an endocrine message via melatonin secretion (Hansen 1985). Cattle exhibit a circadian pattern of melatonin secretion, which is characteristic of many mammals (Crister et al. 1988). During periods of darkness, or scotophase, melatonin concentrations are high. With the onset of daylight, or photophase, melatonin

concentrations drop very quickly. The pattern of melatonin release encodes information about the duration of both the light and dark phases of each day (Schillo et al. 1992).

2.5.2.6.2 Prolactin

Prolactin is another hormone that is responsive to changes in photoperiod. While melatonin secretion varies daily, prolactin secretion responds to gradual changes in day length (Crister et al. 1988). Twenty-five years ago, Bourne and Tucker (1975) reported that serum prolactin increased approximately fourfold in bull calves when daily light exposure increased from 8 to 16 hours. Because of its responsiveness to changes in day length, prolactin is often measured in photoperiod studies. Increased prolactin was measured in both steers and heifers exposed to 16 hour days (Phillips et al. 1997). Petitclerc et al. (1983) found that 16 hours of light increased prolactin in heifers fed on either a high or moderate plane of nutrition. Zinn et al. (1986b) reported that 16 hours of light increased prolactin secretion in post-pubertal heifers. The magnitude of difference in prolactin levels between heifers exposed to long or natural length days was smaller in this study than in some other studies, which the authors attributed to cold environmental temperatures. Peters and Tucker (1978) found that prolactin levels increased in response to long photoperiod only when ambient temperatures exceeded 0°C. Peters et al. (1980) found no effect of increased day length on prolactin, which they too attributed to cold temperatures. While cold temperatures may inhibit a rise in prolactin, stress has been reported to greatly elevate circulating prolactin levels (Tucker 1971). Hansen et al. (1983) found a prolactin concentration of approximately 100 ng/ml in beef heifers, which is much higher than most of the values reported for cattle in the literature. Generally prolactin concentration with long days is about half or less of what was found by Hansen et al. (1983). Stanisiewski et al. (1984) found that prolactin concentration was higher when blood was collected by jugular venipuncture than when collected via cannula, which they attributed to stress associated with capture and confinement for venipuncture. Realization that other factors may impact prolactin concentration is important in avoiding misinterpretation of the effect of photoperiod on prolactin secretion.

2.5.2.7 Endocrine Mechanisms for the Effects of Photoperiod

2.5.2.7.1 Puberty

The physiological basis for the effects of photoperiod on puberty in cattle is not well understood. In sheep, the onset of the breeding season coincides with a photoperiod mediated reduction in the ability of estrogen to inhibit LH secretion (Webster and Haresign 1981). The effects of photoperiod on the pulsatile release of LH accompanying puberty in heifers have not been thoroughly studied (Schillo et al. 1992). Research in ewes has shown that the pattern of melatonin in the circulation affects the responsiveness of the hypothalamic-pituitary axis to the negative feedback effects of estrogen on LH secretion. Whether the same relationship exists in cattle has not been determined, however it is known that the neural centers involved with LH releasing hormone production contain high affinity melatonin receptors (Cardinalli et al. 1979 as cited by Schillo et al. 1992). Schillo et al. (1992) presented a hypothetical model for the relationship between photoperiod and puberty, suggesting that the pre-pubertal increase in LH pulse frequency may occur through a similar mechanism as in sheep (Figure 4).

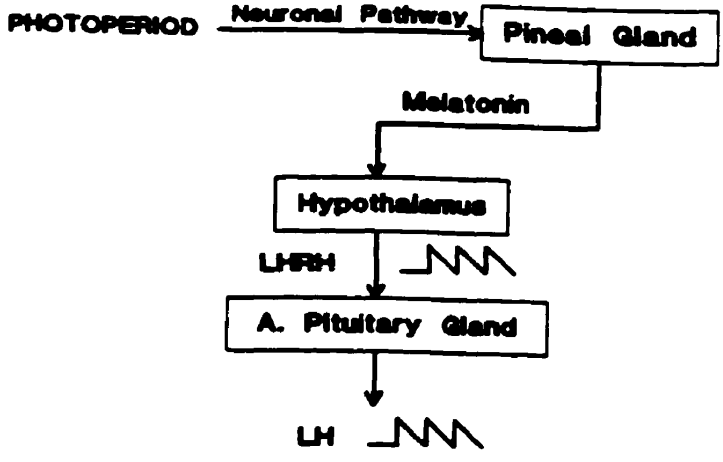

Figure 4. Hypothetical model depicting a possible mechanism whereby photoperiod influences pulsatile LH release and onset of puberty in the heifer (adapted from Schillo et al. 1992).

2.5.2.7.2 Growth

The hormonal coordination of growth and tissue development is a very complex and highly integrated system. Not suprisingly, therefore, the precise mechanisms that are influenced by photoperiod to affect growth are not yet well defined (Phillips et al. 1997). In a review of the influence of photoperiod on growth, body composition and hormone secretion, Tucker et al. (1984) concluded that the hormonal signals associated with photoperiod that mediate changes in growth are probably not associated with changes in insulin, growth hormone, or thyroxin. Glucocorticoids, which are generally catabolic, are reduced by long photoperiods (Tucker et al. 1984). Prolactin may be partially responsible for changes in growth rate, as prolactin has been shown to have an anabolic effect (Nicoll 1980 as cited by Tucker et al. 1984). There is not enough evidence to form conclusive cause and effect relationships between prolactin and changes in growth (Phillips et al. 1997; Tucker et al. 1984).

2.5.2.7.3 Milk Production and Mammary Development

Hormones are the primary physiological factors that stimulate mammary growth and initiate and maintain lactation (Tucker 1981). Ovarian hormones, including progesterone and estrogen, synergize with pituitary hormones, including prolactin and growth hormone, to coordinate mammary growth (Tucker 1981). Prolactin plays a crucial role in lactogenesis and lactation. Other hormones including oxytocin, growth hormone, placental lactogen, thyroid hormone and adrenocorticotropin interact with prolactin to stimulate and maintain lactation (Tucker 1981). Of the myriad of hormones that influence lactation, the relationship between prolactin and photoperiod is probably best understood. Although increased prolactin levels often coincide with improved milk production, a cause and effect relationship has not been firmly established (Tucker 1988). Changes in nutritional status and animal behaviour under different photoperiods cannot be overlooked as factors that may work in conjunction with endocrine factors to mediate the increase in milk production that often occurs with long photoperiods.

2.5.2.8 Lighting Conditions

Many of the discrepancies in the literature with respect to the ability of long photoperiods to elicit consistent physiological responses may be due to different methods of providing the desired photoperiod.

2.5.2.8.1 Duration

A commonly used lighting regime in experiments is 16L:8D for long photoperiod and 8L:16D for short photoperiod. Often 16L:8D is compared to natural, variable length photoperiods. Peters et al. (1980) found that an 8-hour period of darkness was necessary to improve weight gain in Holstein heifers. Chastain and Hiatt (1998) advise dairy producers to use a 16-18 hour photoperiod, as longer photoperiods do not stimulate further milk production and are not economical. Stanisiewski et al. (1988) found that calves receiving 24 hours of light had serum prolactin concentrations that did not differ from those in calves exposed to 8 hours of light and 16 hours of dark.

2.5.2.8.2 Intensity

Natural sunlight may provide an illumination level approaching 80,000 lux on sunny days (Chastain and Hiatt 1998). In a publication produced for the purpose of implementing supplemental lighting programs into the dairy industry, Chastain and Hiatt (1998) recommended that an intensity of 100-200 lux be provided in order to realize an improvement in milk production. An economic analysis showed that at this intensity, the improvement in milk production would quickly pay for the cost of installing the lights. The minimum light intensity required to stimulate milk production is not known (Dahl 1998).

The photointensity required to stimulate earlier onset of puberty is not known. The studies that have examined this relationship generally provide very poor descriptions of the lighting conditions used. Often the duration of light exposure is the only detail given. Studies on the relationship between photoperiod and growth are more abundant, so more information exists on intensity levels required to impact growth. In a recent

study, Phillips et al. (1997) found that an illumination level of approximately 400 lux affected body composition and behaviour of beef heifers. Zinn et al. (1986b) reported similar effects on body composition at about 230 lux. Forbes (1982) concluded that light intensities greater than 100 lux may be required to stimulate body growth. Light intensities between 200 and 600 lux stimulate prolactin secretion (Tucker et al. 1984). Discovering the most economical intensity that is still able to produce the desired response is an important step in transferring photoperiod management to the beef industry.

2.5.2.8.3 Colour/Spectrum

There are a number of lighting products available that are able to provide adequate light intensity. These products, however differ in their spectral properties, which may be perceived by the animals. Commonly used products include fluorescent, high pressure sodium and metal halide lamps. The colour, or degree of whiteness, of a light source is defined by the colour rendition index (CRI) (Chastain and Hiatt 1998). CRI ranges from 0 to 100, with 100 being the greatest degree of whiteness. Incandescent lamps have a CRI of 100, however they are generally not used to light large areas because of the large number required to achieve adequate light intensity. Fluorescent lamps have a CRI of 80 to 85, while metal halide lamps have a CRI of 60 to 80. High pressure sodium lamps provide a CRI of 40 to 60 (Chastain and Hiatt 1998). The spectral properties of high pressure sodium (Figure) give a yellowish-orange tinge to the environment.

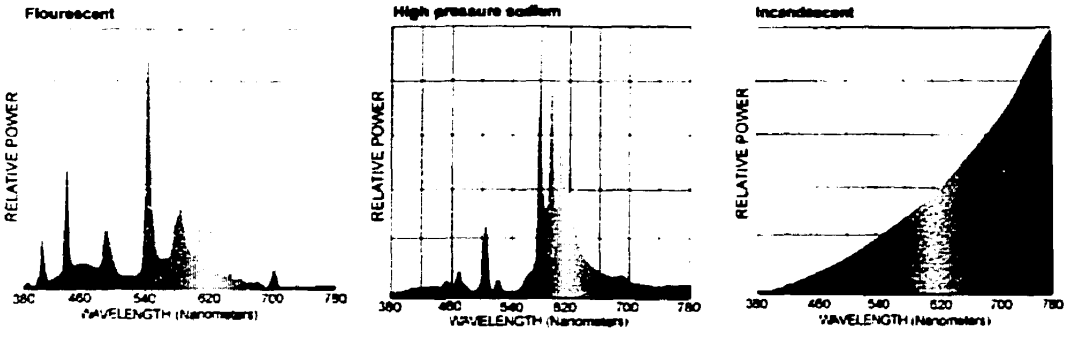

Figure 5. Spectral properties of three lamp types.

Stanisiewski et al. (1984) found that prolactin levels were elevated to the same level when animals were exposed to 16 hours of light of similar intensity from either fluorescent, high pressure sodium, or mercury vapour lamps. Chastain and Hiatt (1998) state that fluorescent, high pressure sodium and metal halide are all acceptable for use in dairy barns. Provided that no negative effects of a certain lamp type emerge, the choice of lamp will likely be based on cost.

2.5.2.8.4 Simulation of Dawn and Dusk

In deer mice, the gradual transition in light intensity that occurs at dawn and dusk provides a more potent cue for photoperiod length than abrupt changes in light intensity (Kavanau 1962 as cited by Zinn et al. 1986a). Zinn et al. (1985) found that Holstein heifers exposed to 16L:8D with gradual transitions in light intensity at dawn and dusk gained 2% more weight than heifers exposed to the same duration of light, but with abrupt transitions. Although the difference was not significant, the authors stated that gradual transitions in light intensity might provide a better cue for day length than abrupt changes. Phillips et al. (1997) produced gradual transitions in intensity in their study on growth, composition and behavioural effects of photoperiod in beef steers and heifers. Tucker (1988) hypothesized that dawn/dusk simulation may be beneficial in increasing milk production, but no studies were found to prove or disprove this theory.

2.6 Artificial Insemination and Estrous Synchronization

Breeding by artificial insemination (AI) accelerates the improvement of heritable traits through the use of genetically superior sires. Adoption of AI has occurred to a much greater extent in the dairy versus the beef sector (Larson et al. 1996) due to the need for the wide scale use of genetically superior dairy sires, and the compatible nature of dairy production with intensive reproductive management (Foote 1996). According to Odde (1990), less than 5% of the U.S. national beef herd is artificially inseminated each year. In a recent survey of Manitoba's beef producers, 20% reported the use of AI although no indication was given of whether this was a routine or occasionally used

management tool (Small and McCaughey 1999). Interestingly, producers identified cost of production, pasture availability, and reproductive success as the three main factors limiting profitability.

Odde (1990) sighted the incompatibility of AI with the extensive range rearing commonly practiced in the U.S. as one of the main barriers to widespread adoption. In Canada, cattle are generally maintained on pasture for the entire breeding season (Small and McCaughey 1999). Additional labour requirements associated with AI are another factor limiting use of the technology. Accurate heat detection is a crucial factor in the success of an AI program and represents a major portion of the labour requirements. 76% of Manitoba's producers do not use AI because of the time needed for heat detection (Small and McCaughey 1999), which if performed properly requires 20-45 minutes of close observation twice daily (Stevenson et al. 1996). This time commitment in combination with pasture rearing presents a formidable deterrent to the use of AI.

Estrous synchronization (ES) may partially remedy this situation. As defined by Odde (1990), estrous synchronization is "the manipulation of the estrous cycle, or induction of estrus, to bring a large percentage of a group of females into estrus at a predetermined time." A closely synchronized group of fertile females would make the labour requirements of AI more compatible with modern cattle production (Larson et al. 1996). ES represents the opportunity to consolidate labour inputs at the beginning of the breeding season, perhaps before cattle are moved from winter housing to pasture. Despite citing time requirements as the major deterrent from AI use, only 12% of producers reported using ES protocols (Small and McCaughey 1999).

In addition to having a compatible relationship with AI, ES offers the additional benefit of allowing for more services, natural or artificial, within a restricted breeding season (Odde 1990; Stephens and Rajamahendren 1998). By experiencing a synchronized estrus at the onset of the breeding season, the animal will have three chances to be bred in a 45-day season. A randomly cycling animal would only have two chances in 45 days, and would require a 63-day season for three opportunities. As discussed previously, a long breeding season perpetuates a long calving season, which is reflected in decreased weaned calf uniformity and failure to rebreed in subsequent years. The current calving season in Manitoba is close to five months (Small and McCaughey

1999). The beneficial effects of ES in giving heifers the opportunity to become pregnant early in the breeding season are obvious.

Given these facts, ES seems to be an underutilized tool by the beef industry. Another explanation for the limited use of ES is the variable nature of current ES protocols in terms of *both* labour requirements *and* fertility. Any ES protocol must be dependable in producing fertile estrus, and be repeatable and practical if it is to receive industry acceptance.

2.6.1 Exogenous Prostaglandin Estrous Synchronization

$PGF_{2\alpha}$ has long been recognized as the predominant luteolytic compound in cattle, although several other hormones also possess luteolytic properties (Pate 1994; Beal 1996). According to Beal (1996) the application of the luteolytic effects of $PGF_{2\alpha}$ and its analogues has been the "most revolutionary advancement in bovine estrous cycle control in the last 50 years". $PGF_{2\alpha}$ synchronization protocols are currently the most commonly used methods in the beef industry (Beal 1996). Ninety percent of Manitoba's producers who practice ES utilize prostaglandins (Small and McCaughey 1999). Commercially available prostaglandin products include Lutalyse ($PGF_{2\alpha}$) and Estrumate (cloprostenol) (Odde 1990).

Prostaglandin ES generally results in conception rates similar to those of heifers bred after a naturally occurring heat (Beal 1996; Odde 1990). This, in combination with the relative ease of prostaglandin use (intramuscular injection), is a likely explanation for the popularity of this system. A shortcoming of prostaglandin use is the variability in the estrus response depending on the stage of the estrous cycle at time of injection. Before day five of the cycle, prostaglandin is unable to initiate luteolysis (Lauderdale 1972). Following day five, the majority of animals respond to prostaglandin injection, although the time of estrus is influenced by the day of the cycle when $PGF_{2\alpha}$ is given (Figure 6).

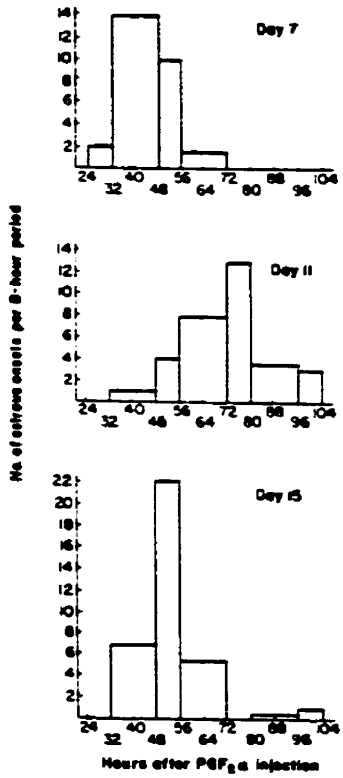

Figure 6. Frequency histograms of estrus onsets per 8-h period after prostaglandin injection at three different stages of the estrous cycle in dairy heifers (adapted from Tanabe and Hann 1984).

The variation in timing of estrus must be realized for insemination to occur at the proper time. If animals are not monitored frequently following injection, early responders may be missed and insemination improperly timed. Provided estrus detection is adequate, there do not seem to be major differences in first service conception rates when prostaglandin is injected at various stages of the cycle (Table 7).

Table 7. Variations in first-service pregnancy rates of heifers injected with $PGF_{2\alpha}$ at various stages of the estrous cycle (adapted from Beal 1996).

	Stage of Cycle[a]		
Reference	**day 5 to 8**	**day 8 to 11**	**day 12 to 15**
King et al. 1982	69%[b]		72%
Stevenson et al. 1984	74%		67%
Tanabe and Hahn et al. 1984	72%	78%	78%
Watts and Fuquay, 1985	57%	62%	78%
Weighted average	68%	68%	75%

[a]Stage of the estrous cycle when $PGF_{2\alpha}$ was injected (Day 0=estrus)
[b]Percentage pregnancy rate after insemination based on detected estrus

2.6.1.1 Physiological Mechanism of Prostaglandin Synchronization

Pate (1994) and Davis et al. (1996) have published excellent reviews of the luteolytic mechanisms of $PGF_{2\alpha}$ in ruminants. $PGF_{2\alpha}$ exerts its luteolytic effect directly at the cellular level of the CL. $PGF_{2\alpha}$ disrupts the normal steroidogenic pathways of luteal cells by interfering with the enzymatic and substrate balances within the cell. The result is a reduction in circulating levels of progesterone. A decreased number of LH receptors follow the reduction in progesterone secretion, resulting in amplification of the drop in progesterone. $PGF_{2\alpha}$ is believed to initiate functional luteolysis through its immediate effects on large luteal cell progesterone production and then subsequently through the chronic effects on LH receptor population. $PGF_{2\alpha}$ may inhibit steroidogenesis at both pre- and post- cAMP sites. An example of a known post-cAMP action is the disruption of the conversion of lipoprotein derived cholesterol to progesterone in the mitochondria of the large luteal cells. Large luteal cells display abundant $PGF_{2\alpha}$ receptor mRNA, while small luteal cells display relatively little receptor mRNA. Large luteal cells are believed to respond initially to the luteolytic signal, followed by intercellular communication between large and small cells. Structural regression follows the initiation of functional luteolysis. Many cellular and hormonal and immune factors contribute to the functional and structural luteolysis initiated by $PGF_{2\alpha}$.

The CL does not respond in the same manner to the luteolytic signal throughout the estrous cycle. The bovine CL has $PGF_{2\alpha}$ receptors whose binding affinity increases 203-fold from days 13-20 of the estrous cycle (Rao et al. 1979). Although $PGF_{2\alpha}$ binding to the CL receptors is low from day 1-4, a lack of receptors is not indicated (Rao et al. 1979; Skarzynski et al. 1997). Rather, Wise et al. (1982) suggest that redirection of blood flow toward the uterus and away from the ovary early in the cycle may result in less $PGF_{2\alpha}$ reaching the CL. There is also a differential luteolytic response following day 5 of the cycle. In a recent study Skarzynski et al. (1997) measured changes in luteal oxytocin secretion as an indication of CL responsiveness to $PGF_{2\alpha}$ treatment during the luteal phase. Oxytocin release is known to occur in response to $PGF_{2\alpha}$, although oxytocin is not believed to be a main factor initiating luteolysis. It is thought to play a supportive role in luteolysis, although the precise mechanism is as of yet unknown. The study showed that

CL sensitivity to $PGF_{2\alpha}$ increased towards the end of the luteal phase of the cycle. The authors suggested that the increased sensitivity might be due to specific changes within the receptors at later stages of the cycle.

2.6.1.2 Current Protocols for $PGF_{2\alpha}$ Synchronization of Estrus

The unresponsiveness of the CL to $PGF_{2\alpha}$ before day five has led to the development of three commonly used $PGF_{2\alpha}$ protocols (Table 8). The *1-shot* method requires that heifers in the follicular phase be bred before ES begins, while the *2-shot* method synchronizes all animals after the second injection. The *varied 2-shot* method is a combination of both, which would reduce the number of animals bred following the second injection. The methods vary both in cost and in total labour inputs; thus the most suitable method for a given situation depends on the relative value placed on these factors by the producer.

Table 8. Commonly used prostaglandin regimes in beef cattle.

Synchronization Day	*1-Shot Method*	*2-Shot Method*	*Varied 2-Shot Method*
1	Check Heat, breed	**1st $PGF_{2\alpha}$ shot**	**1st $PGF_{2\alpha}$ shot**
2	Check Heat, breed		Check Heat, breed
3	Check Heat, breed		Check Heat, breed
4	Check Heat, breed		Check Heat, breed
5	Check Heat, breed		Check Heat, breed
6	Check Heat, breed		Check Heat, breed
7	**$PGF_{2\alpha}$ to unserviced**		Check Heat, breed
8	Check Heat, breed		Check Heat, breed
9	Check Heat, breed		Check Heat, breed
10	Check Heat, breed		Check Heat, breed
11	Check Heat, breed	**2nd $PGF_{2\alpha}$ shot**	**2nd $PGF_{2\alpha}$ to unserviced**
12	Check Heat, breed	Check Heat, breed	Check Heat, breed
13	Check Heat, breed	Check Heat, breed	Check Heat, breed
14	Check Heat, breed	Check Heat, breed	Check Heat, breed
15		Check Heat, breed	Check Heat, breed
16		Check Heat, breed	Check Heat, breed

2.6.2 Control of Ovulation in Estrous Synchronization Programs

Prostaglandin synchronization protocols produce acceptable fertility results, however these regimes are capable of synchronizing estrus only within a 3-5 day period. There has been a recent surge of interest in finding ES methods that facilitate a single

timed insemination. Timed insemination with prostaglandin injection has been attempted, but the general routine is insemination at two times (72 and 96 hours) following the last injection. The expense associated with the use of two straws of semen on each female is likely to exceed the labor costs required for heat detection followed by single insemination. Unless fertility is markedly improved with the double insemination method, visual detection followed by breeding will likely remain the preferred method of prostaglandin ES.

2.6.2.1 Physiological Mechanism of GnRH Synchronization

Asynchrony in follicular wave development accounts for much of the variability in the interval from prostaglandin administration to estrus onset (Bo et al. 1995). A protocol allowing for timed insemination must attempt to control both luteolysis and follicle development, resulting in synchronized estrus and acceptable fertility. Treatment with GnRH alters follicular development so that the estrus response following prostaglandin administration is tightly synchronized. Previous studies have shown that GnRH administration suppresses spontaneous estrus behaviour for a six-day window preceding prostaglandin injection, which is followed by a tightly grouped estrus response following induced luteolysis (Twagiramungu et al. 1992). Twagiramungu et al. (1995) presented an excellent physiological model for the effects of GnRH- $PGF_{2\alpha}$ treatment on follicle development, luteolysis and subsequent expression of estrus (Figure 7).

GnRH exerts its effects on ovarian follicular development and CL function indirectly, via the pituitary gonadotropins, LH and FSH. LH and FSH act directly at the level of the ovary by binding to specific receptors on follicular and luteal cells. When GnRH is administered at a random stage of the cycle, large ovarian follicles respond to increased LH by either ovulating or continuing to undergo atresia, depending on their stage of development. If the dominant follicle is ovulated, a new CL forms. With both ovulation and atresia, circulating estradiol concentrations drop and spontaneous estrus is inhibited. Following GnRH treatment, a new dominant follicle is selected from the upcoming follicular wave, which has been stimulated by increased levels of FSH. When

$PGF_{2\alpha}$ is injected, luteolysis occurs, estradiol and LH pulses increase and the newly selected dominant follicle is ovulated.

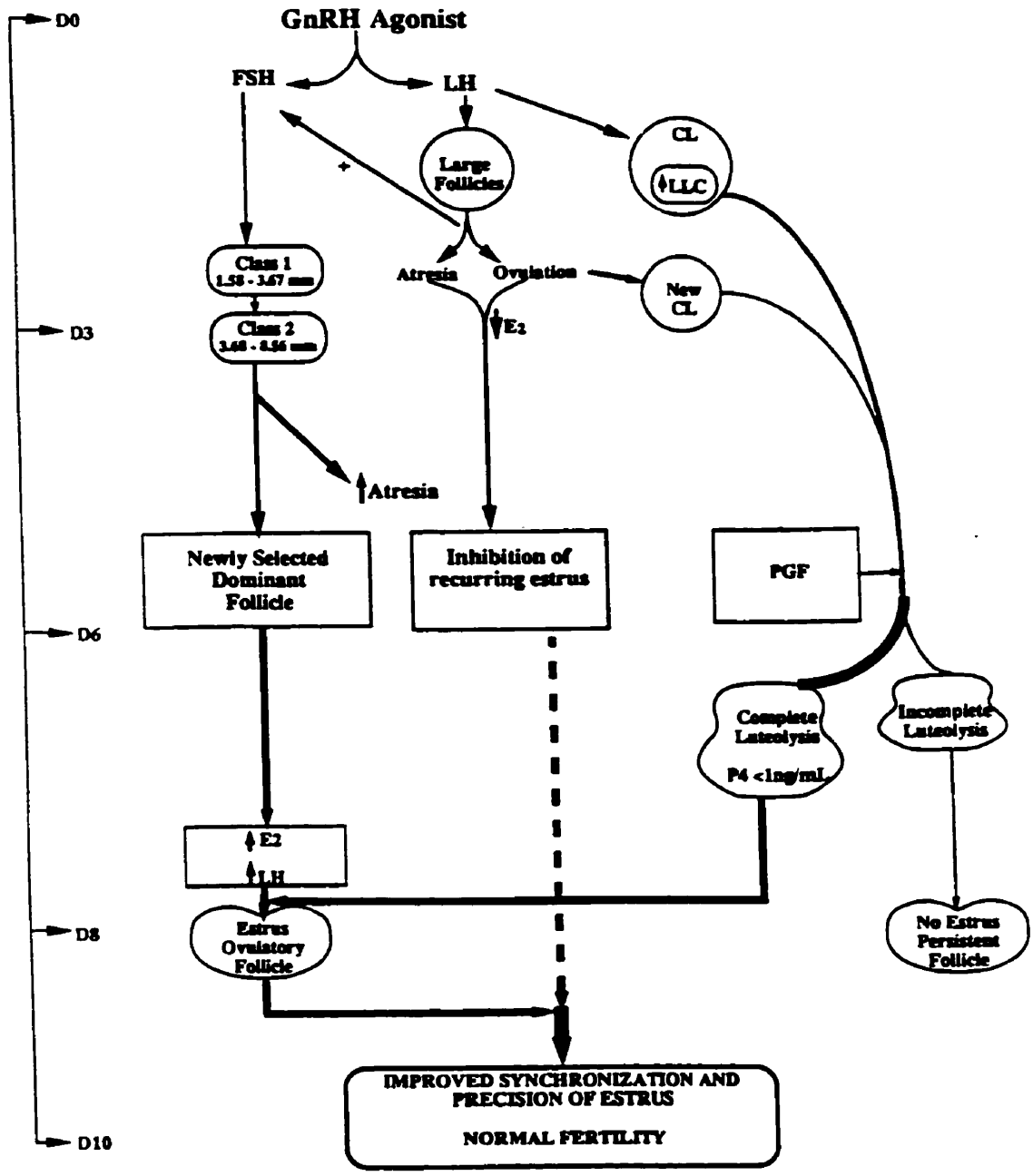

Figure 7. Proposed model for the effect of a GnRH agonist on ovarian follicular dynamics and luteal function to improve the precision of estrus without reducing fertility within a 10-day program for cattle (adapted from Twagiramungu et al. 1995).

2.6.2.2 Developments in GnRH- $PGF_{2\alpha}$ Protocols

The discovery of the ability of GnRH- $PGF_{2\alpha}$ treatment to tightly synchronize estrus seemed very compatible with timed insemination. Through the previously presented mechanism, a new dominant follicle is selected and ovulated, regardless of the stage of the estrous cycle at the initiation of ES (Twagiramungu et al. 1995). In a recent study, timed insemination of beef heifers using a GnRH-$PGF_{2\alpha}$ protocol produced inconclusive results. Stephens and Rajamahendran (1998) compared the double $PGF_{2\alpha}$ method with the GnRH-$PGF_{2\alpha}$ method. The GnRH-$PGF_{2\alpha}$ method consisted of a dose of GnRH followed 7 days later by a dose of $PGF_{2\alpha}$. Both groups were inseminated at 72 and 96 hours following $PGF_{2\alpha}$. Synchronization rate (90% vs. 73%) and conception rate (62% vs. 40%) were similar ($P > 0.05$) for double $PGF_{2\alpha}$ and GnRH-$PGF_{2\alpha}$ respectively; however, the number of animals used was small (n=60). Although the effect was not significant, the double $PGF_{2\alpha}$ method tended to be more successful than the GnRH-$PGF_{2\alpha}$ method. The authors indicated that, based on uterine tone and difficulty of insemination, that inseminations may not have occurred at the appropriate time (i.e. too late) in the GnRH- $PGF_{2\alpha}$ group. They suggested that further study was needed to identify the most appropriate time of insemination, in order to refine the procedure to a single timed insemination.

Another development in this area has been the administration of a second dose of GnRH at, or just before timed insemination, denoted the GnRH- $PGF_{2\alpha}$ -GnRH method (called Ovsynch®). The second GnRH injection is used to control the time that the new dominant follicle is ovulated, by synchronizing the preovulatory LH surge. By exerting control over the actual time of ovulation, the conception rate to timed insemination should improve in comparison to GnRH- $PGF_{2\alpha}$. Twagiramungu et al. (1995) reported similar pregnancy and calving rates in cows bred after GnRH-$PGF_{2\alpha}$, using either visual detection and breeding, or timed insemination with a second GnRH injection 54 hours post-$PGF_{2\alpha}$. There are few reports of the success of this technique in beef heifers. Schmitt et al. (1994) examined the potential of Ovsynch® for use in dairy heifers. In a preliminary study, 28 heifers were given a GnRH agonist on day 0, followed by $PGF_{2\alpha}$ on day 7, and a second dose of GnRH 24 hours later. Breeding took place 15 hours after the

second dose of GnRH (39 hours after $PGF_{2\alpha}$). Pregnancy rate 45 days after AI was 53.6%. A second experiment was conducted under field conditions, using 169 heifers. Half of the heifers received the same treatment as in the preliminary study, while the other half received the first GnRH injection, followed by prostaglandin injection and breeding following visual estrus detection. Pregnancy rate in the heifers bred by visual detection was 50.0%, while that in the timed group was only 25.8%. Many of the heifers from the timed AI protocol exhibited short intervals to the return heat, which the authors said could indicate that the ovulatory dose of GnRH was given too early after prostaglandin for adequate luteinization of the ovulatory follicle.

There were no reports found for heifers where the second injection of GnRH was given at the same time as AI. Results from recent studies conducted at Agriculture & AgriFood Canada, Brandon, Manitoba (Small unpublished data) have shown similar synchronization response and timed AI conception rates in beef cows synchronized with either the OvSynch® method or the 'BRC' method, where the second GnRH injection is given at the same time as AI. Injection of the second GnRH at the same time as AI would reduce the number of handling procedures from four, as required by the OvSynch® method, to three. Development of estrous synchronization protocols requiring minimum labour inputs is desirable from an industry perspective, as the time commitment required for synchronization and AI is currently a major barrier to more widespread use of the technology (Small and McCaughey 1999).

The use of GnRH- $PGF_{2\alpha}$ -GnRH may eliminate the need for estrus detection before and after prostaglandin injection, but will be accepted only if studies confirm that fertility is not compromised. Further research is needed to identify the best time for the second GnRH injection and insemination following prostaglandin injection. Also, studies using GnRH- $PGF_{2\alpha}$ -GnRH are needed in beef heifers, because the insemination time may be different than that used for cows.

3 HYPOTHESIS

There is an abundance of literature on factors affecting the development of reproductive function in heifers. Comprehensive reviews are available on the complex relationships between nutrition, genetics and reproduction (Martin et al. 1992; Schillo et al. 1992). The effects of photoperiod and season on heifer development have also been briefly reviewed (Schillo et al. 1992), but establishing definite relationships in this area is difficult. Some research indicates that photoperiod influences puberty, growth, carcass composition, behaviour, milk production and endocrine function. Many studies on photoperiod utilized intensively housed Holstein heifers, and in some cases the lighting conditions used were incompletely described. The lack of consistency in research conditions and results presents an opportunity for further study on the relationship between photoperiod and beef heifer development. The longer term effects of exposure to different photoperiods during the post-weaning period is an unexplored area.

The experiment described herein was designed to test the hypothesis that exposure to extended photoperiod may affect the development of beef heifers during the period of sexual maturation, with the effects continuing until first lactation. The compatibility of two estrous synchronization procedures with timed insemination is also described.

4 MANUSCRIPT I - EFFECTS OF PHOTOPERIOD ON BEEF HEIFER DEVELOPMENT FROM WEANING UNTIL BREEDING

4.1 Abstract

The effects of photoperiod on the development of beef replacement heifers were determined by assigning 144 crossbred heifers on the basis of weaning weight (225 ± 23 kg) and sire breed (British/Continental) to either of two outdoor housing facilities, with different photoperiod treatments, in a completely randomized design. From December 1 1998 (day 0) until May 20 1999, heifers in one facility received supplemental light (423 lux, 1 m above ground) to extend the daily photoperiod (natural + supplemental light) to 16 hours (EP). Heifers in the other facility experienced natural photoperiod only (NP). Measurements of body weight gain, backfat, and concentration of prolactin in blood serum were made every 28 d. Observations for estrus behaviour were made twice daily, in the morning and evening, and were confirmed by serum progesterone concentration in blood samples taken 8-12 d after observed estrus. Pre-breeding body weight (388 ± 4 kg), and backfat (3.7 ± 0.1 mm) were similar ($P > 0.05$) between treatments. Prolactin was higher ($P < 0.05$) for the EP than the NP treatment on days 28, and 56, but was not different ($P > 0.05$) on days 84, 112 and 140. For EP and NP treatments respectively, 84.7% vs. 69.4% ($P < 0.05$) had one confirmed estrus, and 63.9% vs. 48.6% ($P < 0.05$) had two confirmed estrus before breeding season. The proportion of irregular length pubescent cycles (41.6%) was similar between treatments ($P > 0.05$). Extension of daily photoperiod increased serum prolactin and the proportion of heifers having reached puberty, but did not affect body weight or backfat. Manipulation of photoperiod may be effective in stimulating the reproductive development of beef heifers.

4.2 Introduction

Lifetime productivity is maximized when heifers calve early in the season as two-year olds (Marshall et al. 1990; Lesmeister et al. 1973). In order for heifers to calve at 24 months of age, pregnancy must be established at 15 months of age. The timing of the pubertal estrus in relation to breeding is one factor affecting successful establishment of pregnancy. Byerley et al. (1987) found that heifers bred on the pubertal estrus had 21% lower pregnancy rates than heifers bred on the third estrus. Del Vecchio et al. (1992) examined the dynamic nature of the estrous cycle following puberty and found that abnormalities in uterine and ovarian endocrine activity were linked to a greater frequency of abnormal length cycles following the first and second estrus. By the third cycle, the endocrine profiles reflected those of a regularly cycling animal.

Most management strategies for the development of replacement heifers embrace the relationship between timely onset of puberty and pregnancy establishment. Inputs that influence reproductive development include genetic makeup (Martin et al. 1992), and information about nutritional status, environmental conditions, and social interactions (Schillo et al. 1992). Of these factors, the relationship between reproductive development and the environment (physical and social) has received the least amount of attention.

Photoperiod is one factor in the physical environment that has been linked to changes in the development of heifers. The relationship, however, is far from clearly established. Exposure of heifers to extended photoperiod has been shown to advance puberty (Ringuet et al. 1994; Petitclerc et al. 1983), stimulate growth (Zinn et al. 1986a; Peters et al. 1980) and enhance deposition of lean tissue (Petitclerc et al. 1984), compared to heifers experiencing either natural or artificial short photoperiods. The hormone prolactin has been shown to increase in response to extended photoperiod (Petitclerc et al. 1983; Bourne and Tucker 1975). However, there is also evidence showing that photoperiod may not affect puberty, growth and prolactin (Phillips et al. 1997; Petitclerc et al. 1984; Peters et al 1980). The lighting conditions used in many previous studies were often poorly described, or incompatible with practical implementation into current beef production practices.

The objective of this study, therefore, was to determine the effects of extending photoperiod using a practical and repeatable lighting system, on the development of

outdoor housed beef heifers in the post-weaning period. The effects on growth, backfat, serum prolactin concentration, and pubertal development were of particular interest in this study.

4.3 Materials and Methods

4.3.1 Animals and Management

The experiment described in this chapter was part of an ongoing study at the Agriculture & Agri-Food Canada Research Station, Brandon, Manitoba. The data presented in this chapter was collected between December 1998 and May 1999. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

One hundred and forty four crossbred heifers were used in the study. The heifers were progeny of Continental (Gelbvieh, n=64, Simmental, n=30), British (Red Angus, n=39, Hereford, n=8) and Composite (1/4 Simmental 1/4 Charolais 1/16 Limousin 7/16 British, n=3) sires. All of the dams were either ¼ Simmental ¼ Hereford, or Composite breed. All heifers were born at the research station, between February 26 and May 15, 1998. Weaning occurred on October 5, 1998. Body weight and age (mean ± standard deviation) at weaning were 225±23 kg and 187±14 days, respectively. At weaning, the heifers were assigned to one of two treatment groups applied in two separate facilities (described below): EP = extended photoperiod (n=72) or NP = natural photoperiod (n=72), in a completely randomized design. Each treatment was balanced for sire breed and heifer weaning weight. After weaning, the heifers were housed in their designated facilities until the beginning of photoperiod treatments on December 1, 1998. The age at beginning of treatments was 243 ±14 days in both groups.

Total Mixed Rations (TMR) were formulated using Cowbytes. Until February 15, 1999 (preflush period) the TMR consisted of high quality alfalfa/grass hay and barley silage with free choice mineral and salt (Table 9). The target ADG in the preflush period was 0.6 kg day^{-1}. Between February 15 and May 27,1999 (flush period), barley grain and

a premix pellet consisting of barley grain, minerals, vitamins, salt and limestone were added to the TMR. The target ADG in the flush period was 1.2 kg day^{-1}. During both periods, the amount of barley silage was adjusted as needed to insure animals were on full feed and to achieve the desired weight gains. Adjustments were made equally in both treatments so that both were receiving the same ration at all times. The ration ingredients were weighed, mixed and delivered into the bunk by a feed truck once daily.

The heifers were housed in two similar facilities, with one facility equipped to provide the EP treatment, and the other the NP treatment. The two facilities were separated by about 500 m (Figure 8). Both facilities consisted of a southern exposed, open front pole shed, and a drylot with concrete bunks at the south end (Figure 9). Each facility was divided into two pens to accommodate feeding and handling of the heifers. Each pen had access to a heated water bowl. The recommended space requirement for the shed in a shed/lot situation for yearling animals is 1.86 m^2 per animal, and 15.2 cm of feeder space when animals have continuous access to a total mixed ration (Alberta Agriculture, Food and Rural Development 1995). Both facilities exceeded the minimum requirements. There was an average of 7.6 m^2 of lying space in the EP facility and 5.6 m^2 in the NP facility. Both facilities had 101 cm of bunk space per animal. Fresh straw bedding was provided in the shed twice weekly, and as needed if heavy precipitation was received. The two facilities had similar shelter provided by trees and buildings around the drylot.

4.3.2 Lighting Treatments

4.3.2.1 Equipment & Design

The lighting design for the EP facility (Figure 10) was developed in cooperation with an electrical systems engineer and an agricultural engineer from Manitoba Hydro. In order to achieve the desired light intensity throughout the facility, three types of luminaires were included in the design (Figure 11, Figure 12, Figure 13, Figure 14). All three luminaires used high pressure sodium (HPS) clear lamps.

The floodlights were installed at 5.3 m above ground level, and dusk-to dawns at 4.6 m. The roadway fixtures were mounted 5.5 to 6.1 m above ground level, using a staggered arm arrangement on the pole. Eight roadway luminaires were installed in the shed, at 4.3 m spacings, 4.9 m above ground level.

4.3.2.2 Intensity

The light intensity chosen for the EP facility was 400 lux. The actual light intensity was measured two times during the winter, on November 19, 1998, before the start of the experiment, and on March 3, 1999. The shed lights were installed after the first set of readings were taken, but before the beginning of treatments on December 1, 1998. The illumination levels were measured according to a predetermined grid (Figure 15). At each point on the grid, readings were taken with the light meter in both a horizontal and vertical orientation at 1 m above ground (approximately animal eye level). The NP facility had no light sources within the shed or drylot, and was free from 'spill over light' from surrounding buildings. The absence of intentional or unintentional supplemental light resulted in an environment of complete darkness following natural sunset.

4.3.2.3 Lighting Schedule & Control

The desired photoperiod for the EP treatment was 16 hours of light (natural + supplemental). Sunrise and sunset times for Brandon were obtained from the local weather station, and used to calculate the number of supplemental hours of light required for 16 hours of light each day. To achieve the desired photoperiod, the extra hours of light were added only at the end of the day, therefore both treatments provided a natural sunrise. The lights in the EP facility came on thirty minutes before sunset so that this group did not experience a natural sunset before the beginning of supplemental light. Extension of the photoperiod began on December 1, 1998. The photoperiod in the EP treatment was lengthened once weekly by 2 hours, from 10 hours on December 1, 1998 to 16 hours on December 21, 1998 (winter solstice). Dusk was simulated by staggering shut

down of the lights at 5 minute intervals over a 30 minute period. The lights remained on until May 20, 1999, which was the day the heifers were artificially inseminated.

One timer (EL71/120 Single Channel Controller, Paragon Electric Company, Inc.) controlled the on/off function and another (Logo 230R, Siemens) the dusk simulation. The EL71/120 timer contained an "Astro" feature that adjusted the on time of the lights each day, based on the latitude of Brandon.

4.3.3 Data Collection

4.3.3.1 Measurement of Body Weight, Backfat, Serum Prolactin Concentration and Hair Shedding

Heifers were weighed, had backfat measured and a blood sample taken for serum prolactin analysis, every 28 days throughout the post-weaning period, beginning on December 1, 1998 (day 0). Backfat deposition was measured using real-time ultrasonography (Aloka SSD500, 5.0 Mhz probe). Heifers were restrained in a squeeze chute, and backfat was measured between the 12^{th} and 13^{th} rib. Collection and handling of blood samples for prolactin analysis is described in a subsequent section. Samples for determination of hair shedding were collected from a subset of heifers (20 per treatment) using a curry comb, stencil (16 cm x 7 cm) and collection tray. Hair samples were taken immediately posterior to the ileum with one stroke of the curry comb in the stencil area. Samples were stored in plastic bags prior to drying and weighing.

4.3.3.2 Estrus Detection and Confirmation of Estrus

Heifers were observed twice daily for signs of estrus. Estrus detection was performed in the morning and evening, for a minimum of 20 minutes at each facility, each time. Detection times were 08:30 and 16:00 h in November, December and January, 08:00 and 17:00 h in February and March and 07:00 and 19:00 h in April and May. The times were adjusted to coincide with natural sunrise and sunset. Visual signs used to classify a heifer as exhibiting estrus were standing to be mounted, attempted mounting, restlessness and bawling, matted or roughed hair coat along the back and hip area,

mucous discharge from vulva, and swollen vulva. Estrus with ovulation (puberty) was confirmed by taking a blood sample 8-12 days after the estrus observation, for serum progesterone (P4) analysis. Length of the estrous cycle was calculated as the number of days between confirmed heats.

4.3.3.3 Blood Collection and Analyses

Heifers were restrained in a chute and blood samples were collected by caudal venipuncture. Blood samples were collected into 10 ml non-heparinized, evacuated tubes using 20 gauge, 1" single draw needles. Depending on the time that collection was completed, samples were allowed to either sit at room temperature for 4 hours, or in the refrigerator overnight. Serum was separated from clotted blood components by centrifugation at 1000g (2210 rpm) for 40 minutes at 4°C. After centrifugation, serum was transferred into labeled tubes and frozen (-20°C) until further analysis.

Serum progesterone (P4) concentrations were determined by an established enzyme-immunoassay, which had a detection limit of 0.03 ng ml^{-1} (Del Vecchio et al. 1995). The coefficients of variation within and between assays were 6.71% (n=20) and 11.55% (n=11), respectively. For confirmation of estrus/ovulation and PG response rate during synchronization, P4 concentrations greater than or equal to 1.0 ng ml^{-1} were considered indicative of a functional corpus luteum, and concentrations less than 1.0 ng ml^{-1} were indicative of the absence of a functional corpus luteum.

Frozen serum samples were shipped to the Western College of Veterinary Medicine, Saskatoon, Saskatchewan for prolactin analysis. Serum concentrations of prolactin were measured in a double-antibody radioimmunoassay. The sensitivity of the assay was 0.5 ng ml^{-1}. The range of the standard curve was 0.5 to 32 ng ml^{-1}, and any samples higher than the standard curve were repeated at a dilution. The coefficients of variation within and between assays were 8.6% (n=6) and 23.5% (n=6), respectively.

4.3.4 Statistical Analysis

Data analysis was performed using the Statistical Analysis System v 6.12 (SAS 1996). Data for body weight, backfat, prolactin and hair shedding were analyzed using the general linear models with repeated measures procedures, with type III sums of squares. Sources of variation in the analysis included treatment, day, and the treatment*day interaction, using animal within treatment as the error term for treatment, and residual as the error term for day and treatment*day. The effects of breed and pen were not significant and were therefore excluded from the final model. The average daily gain in body weight was calculated for individual animals by regression analysis. Categorical models (CATMOD) procedures were used to test differences in proportions. The cumulative proportion of heifers from each photoperiod treatment having one, two, or three confirmed estrus observations was compared at ten day intervals beginning 100 days after the onset of treatments.

The standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{error mean square}/n)}$$

n = number of observations / treatment

For proportion data, the standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{sum of variances})}$$

where variance = $(r/n)*(1-(r/n))/n$

n = number of observations/treatment and r = the number responding

4.4 Results

4.4.1 Photoperiod and Weather Conditions

All 144 heifers assigned to the photoperiod treatments completed the post-weaning portion of the experiment. Weather data collected at the location of the experiment showed that the average monthly temperatures were close to the 30-year average temperatures for the same months (Figure 27, Appendix I). With the exception of the adjustment period in the first 28 days, photoperiod remained the same in the EP treatment for the duration of the experiment (Figure 16). Photoperiod gradually lengthened in the NP treatment, approaching a similar duration to the EP treatment by the end of the post-weaning period. The average intensities measured in the drylot on November 19, 1998 were 480 lux with the light meter oriented horizontally and 380 lux with the light meter oriented vertically. On March 3, 1999 the readings averaged 504 lux horizontally and 328 lux vertically. The slight increase in intensity between the two readings can be attributed to: (1) manure build up in the facility which would slightly decrease the distance between the lights and ground level (2) increased reflection of light from snow covered surroundings. In the shed, the average intensity was 90 lux.

The capital costs for the lighting design used in the study were $22,000. The total energy used for extension of photoperiod from December 1, 1998 to May 20, 1999 was 21, 696 kilowatt hours (kWh). The rate used in calculating the cost of operating the lights was $0.055 per kWh. The total cost for operating the lights was $1193.28.

4.4.2 Body Weight & Backfat Deposition

Mean body weights are shown in Figure 17. Body weight did not differ at any point throughout the post-weaning period, although heifers in the EP treatment tended to be slightly heavier (trt*day, $P = 0.12$). The EP and NP treatments achieved a similar body weight (391 vs. 386 ± 3.7 kg; $P = 0.30$) by the end of the post-weaning period (pre-breeding body weight). Average daily gain (ADG) during the pre-flush period was 0.59 kg d^{-1} in the EP treatment and 0.53 kg d^{-1} in the NP treatment ($P = 0.04$, SE = 0.02).

ADG in the flush period was 1.35 kg d^{-1} in the EP treatment and 1.30 kg d^{-1} in the NP treatment (P = 0.10, SE = 0.02).

Backfat thickness (Figure 18) was similar between treatments, although there was a tendency for EP heifers to gain less fat (trt*day, P = 0.11). Pre-breeding backfat thickness was 3.6 vs. 3.8 ± 0.1 mm (P = 0.39) for EP and NP, respectively.

4.4.3 Serum Prolactin Concentration

Serum concentrations of prolactin (Figure 19) increased over the duration of the experiment in both treatments (P = 0.0001), however the increase within treatments differed (trt*day, P=0.02). Prolactin concentration was similar on day 0, at 4.2 vs. 4.7 ± 0.3 ng ml^{-1} for EP and NP respectively. By day 28, however, prolactin was higher (P = 0.05) in the EP treatment, having increased to 10.1 ± 1.6 ng ml^{-1} while concentrations in the NP treatment were approximately half, at 5.6 ± 1.6 ng ml^{-1}. Prolactin remained higher (P = 0.004) in the EP than the NP treatment on day 56, but after day 84 the two treatments were similar (P = 0.50, 0.55 and 0.84 for days 84, 112 and 140 respectively).

4.4.4 Hair Shedding

Hair shedding was negligible from day 0 to day 84 (Figure 28, Appendix II), and increased on day 112 (beginning of April), but there was no effect of photoperiod treatment on (P > 0.05) shedding.

4.4.5 Pubertal Estrous Cycles

Photoperiod affected the proportion of heifers reaching puberty by the end of the post-weaning period (Figure 20), which coincided with the start of estrous synchronization. In the EP treatment, 84.7% of heifers had one confirmed estrus during this period, compared to 69.4% of heifers from the NP group (P = 0.03). The proportion of heifers that had one confirmed estrus was similar (P > 0.05) until 140 days after the photoperiod treatments began, at which time the occurrence of pubertal estruses accelerated in the EP treatment. Heifers from the EP treatment also had an advantage in

the proportion having two confirmed heats by the end of the post-weaning period (63.9% vs. 48.6% for EP and NP respectively, P = 0.06). The proportion of heifers having three confirmed estruses by day 160 was similar between treatments (29.2% vs. 27.8% for EP and NP respectively, P = 0.85). Average age at puberty was similar (P > 0.05) between treatments, at 369 ± 32.9 days for the EP treatment and 365 ± 30.3 days for the NP treatment. However, the proportion of heifers that had reached puberty by 425 days of age was higher for the EP than for the NP treatment. In the EP treatment 85% (61 out of 72 heifers) had reached puberty by 425 days of age, compared to 69% (50 out of 72 heifers) in the NP treatment.

Photoperiod treatments did not affect the cycle length, or the proportion of abnormal length cycles in either the first or second estrous cycles (Table 10). The proportion of abnormal length cycles was similar (P = 0.78) in the first and second estrous cycles.

4.5 Discussion

4.5.1 Lighting Conditions

The results of the light intensity readings showed that the desired intensity of 400 lux was achieved by the design used. The mean intensity was higher than that used in many other studies, but similar to that used in a recent study by Phillips et al. (1997). Tucker et al. (1984) stated that light intensities between 200 and 600 lux increase prolactin secretion. Chastain and Hiatt (1998) recommend that a minimum intensity of 100 lux, but ideally 200 lux, be used to achieve improved milk production of dairy cows. There are currently no established guidelines for optimal light intensity required to elicit changes in reproductive development. Additionally, very little is known about potential differences of light with different spectral properties. Light sources are rated by colour rendition index (CRI), on a scale of 1-100, with 100 being the greatest degree of whiteness (Chastain and Hiatt 1998). The high pressure sodium lamps used in the current study have a CRI of approximately 50, whereas metal halide lamps have a CRI of approximately 70 (Chastain and Hiatt 1998). Stanisiewski et al. (1984) found that the

increase in prolactin concentration with long photoperiod was similar regardless of the spectral properties of the lamps used (incandescent, high pressure sodium, or mercury vapour lamps). There are no known studies available comparing the effects of different lamp types on the development of beef heifers. Further research is required to determine the optimal lighting conditions (intensity and type) for beef heifers.

The capital and operating costs associated with implementing extended photoperiod into a mangement system would vary, depending on many factors including nature of existing facilities, installation costs, duration of photoperiod extension, and type and intensity of lights used in the design. The cost of operating the lights in the present study was very reasonable considering a high light intensity was used for a six month period. Equipment and operation costs will decrease if future studies show that lower light intensities or a shorter treatment period are capable of stimulating reproductive development. Further research is required for determination of a cost to benefit ratio for extended photoperiod.

4.5.2 Growth and Backfat Deposition

The rations for this study were formulated to achieve 0.6 kg d^{-1} gain in the pre-flush period and 1.2 kg d^{-1} gain in the flush period. During the pre-flush period, the EP group was closer to the achieving the target gain than the NP group. The EP treatment gained approximately 60 g d^{-1} more than the NP treatment in the pre-flush period, and this advantage was maintained in the flush period.

In this study, extension of the photoperiod tended to increase ADG in both the pre-flush and flush periods. The differences in ADG appeared early in the study (by day 28) and were maintained throughout. Extended photoperiod appeared to influence the growth rate of both pre- and post- pubertal heifers, as the majority of heifers were prepubertal prior to the flushing period, with an increasing proportion becoming pubertal during the flushing period. There is little consistency in past studies with regard to the effects of photoperiod on growth. Exposure to photoperiods of 16L:8D has been reported to stimulate body weight gain of pre-pubertal heifers (Zinn et al. 1986a, Petitclerc et al. 1983, Peters et al. 1980). Increased growth rate has been associated with an increase in

feed intake (Peters et al. 1980), whereas in the present study, feed intake was held at the same level in both treatments. Zinn et al. (1986b) found that duration of photoperiod did not affect growth rate of pre-pubertal heifers, but that growth rate in post-pubertal heifers was stimulated by short days. More recently, Phillips et al. (1997) found no effects of photoperiod on growth of cold-housed beef heifers.

There was a tendency for backfat to be deposited more slowly in the EP treatment, beginning around day 56 of the study. Zinn et al. (1986b) found that backfat deposition in pre-pubertal heifers was not affected by photoperiod, but that short photoperiod resulted in greater backfat deposition in post-pubertal heifers. Phillips et al. (1997) reported a transient decrease in backfat deposition in post-pubertal heifers exposed to long photoperiod.

The effects of photoperiod on growth and backfat deposition remain unresolved. Tucker et al. (1984) postulated that the effects of extended photoperiod on growth and body composition may depend on the stage of sexual maturity, with the effects of extended photoperiod being related to the presence of functional gonads (post-pubertal animals). While there is evidence to support this theory, data from this and other studies does not support the restriction of effects of photoperiod to post-pubertal animals. Further study is required to determine factors in addition to stage of sexual maturity, such as level of feed intake or environmental temperature, which may also affect the growth and backfat response to photoperiod.

4.5.3 Serum Prolactin Concentration

Heifers experiencing extended photoperiod had higher serum prolactin concentrations for the first 56 days of the present study. Following day 84, however, prolactin concentrations were similar in both treatments. By day 84, the duration of the natural photoperiod was greater than 12 hours per day, and at this time the natural lengthening of photoperiod would have been occurring at an increasing pace. The heifers in the NP treatment could have certainly perceived the natural cues of the approaching spring, which may account for the gradual disappearance of the difference in prolactin between treatments. Peters et al. (1980) suggested that an increase in prolactin may be

dependent upon ambient temperature, with temperatures below 0°C suppressing an increase. The data from the present study does not support this hypothesis, as an increase in prolactin was measured in months where the average temperature was below 0°C. In the study by Peters et al. (1980), the average light intensity used was approximately 100 lux, which may not have been sufficient to stimulate a rise in prolactin. Other studies utilizing higher light intensities (>200 lux) have reported an increase in prolactin under extended photoperiods, even in cold temperatures (Zinn et al. 1986b; Phillips et al. 1997).

The sharp rise in prolactin that occurred on day 140 of this study was an unexpected, but interesting finding. Until this point, prolactin values were well within the range commonly reported in the literature, however on day 140, prolactin measured approximately 130 ng ml^{-1} in both treatments. Stanisiewski et al. (1984) and Hansen et al. (1983) attributed prolactin levels of greater than 100 ng ml^{-1} to a stress response from the animal. Stanisiewski et al. (1984) found that prolactin levels were higher in blood collected by jugular venipuncture than by cannulation, and suggested that the stress associated with capture and confinement for jugular venipuncture may have caused the rise in prolactin.

Stress due to blood sampling is an unlikely cause of elevated prolactin in the current study, as the blood collection routine was similar throughout the experiment and there is no apparent reason for the heifers to have experienced greater stress later in the study. A much more plausible explanation for the increase in prolactin on day 140 is an abrupt increase in environmental temperature leading up to the sampling day. Smith et al. (1977) reported that prolactin increased from 23 ng ml^{-1} to 106 ng ml^{-1} when steers acclimatized to 20 °C for three weeks were abruptly exposed to 40°C. A reduction in metabolic clearance rate and an increase in secretion rate of prolactin with increased environmental temperature accounted for the increase in prolactin concentration. In the present study, there was an abrupt increase in temperature in the days preceding, and on day 140. The mean air temperature in the three weeks proceeding day 140 was approximately 10°C. The temperature on day 140 was 25°C, and in the preceding four days 22°C. This increase in temperature is of approximately the same magnitude as that reported by Smith et al. (1977), as is the increase in prolactin concentration (~20 ng ml^{-1} on day 112 to ~130 ng ml^{-1} on day 140). Curtis (1983) states that the increase in some

hormones in response to heat may be a nonspecific response to stress. While prolactin concentrations are known to increase with ambient temperature (Tucker 1982), values exceeding 100 ng ml^{-1} during extended periods of warm temperature are not commonly reported. Berardinelli et al. (1992) reported prolactin concentrations of approximately 10 ng ml^{-1} in the spring and 5 ng ml^{-1} in the fall. The sharp rise in prolactin measured in the current study is believed to reflect a short term response to a sharp rise in environmental temperature.

4.5.4 Pubertal Estrous Cycles

Previous studies have reported stimulatory effects of long photoperiods on puberty in cattle (Hansen et al. 1983; Petitclerc et al. 1983; Ringuet et al. 1994). This relationship was of particular interest in the present study, as timely onset of puberty is a key goal in the development of beef heifers. The proportion of heifers having reached puberty before the breeding season was increased by extended photoperiod in the present study. Extended photoperiod resulted in a 22% improvement in the number of heifers that had reached puberty, and a 31% improvement in the number having a second confirmed estrus before the breeding season compared to heifers from the NP treatment.

Mean age at puberty was similar between treatments, however a greater proportion of heifers from the EP treatment had reached puberty by 425 days of age. In previous studies that reported a reduction in age at puberty with extended photoperiod, determination of pubertal status continued until all animals had reached puberty (Ringuet et al. 1994; Hansen et al. 1983; Petitclerc et al. 1983). However, in the present study a finite period existed in which heifers had to have one confirmed estrus with ovulation to be deemed pubertal. If confirmation of pubertal status had continued until all heifers were pubertal, average age at puberty would likely have been higher in the NP treatment, as there were twice as many prepubertal heifers in the NP treatment (22) than in the EP treatment (11) prior to the breeding season. From an industry perspective, the proportion of heifers having reached puberty prior to breeding season is a more important measure than age at puberty. Under current cow-calf management systems, the breeding season

typically begins on a given date, therefore the proportion of replacement heifers that are pubertal prior to this date is more important than age at puberty.

The mechanism whereby long photoperiods influence puberty is still unresolved. The findings of this study generate interesting questions regarding the relationship between puberty, growth rate and backfat thickness. Differences in growth rate and backfat deposition rate due to photoperiod arose early in the study, yet the stimulation of puberty by extended photoperiod did not appear until much later in the treatment period. Drawing definitive relationships between these factors is therefore difficult. Schillo et al. (1992) suggested that attainment of a critical body weight may trigger events that induce onset of puberty. Perhaps the increased growth rate of the EP heifers allowed more of them to reach a critical weight earlier, which resulted in more pubertal heifers. The relationship between level of body fatness and puberty is also unclear. Schillo et al. (1992) stated that past research has not demonstrated that the onset puberty is related to a consistent level of body fatness.

Prolactin has been postulated to play a role in sexual maturation (Petitclerc et al. 1983) as hyperprolactinemia leads to precocious puberty in the female rat (Advis and Ojeda 1978). A relationship between prolactin and sexual maturation in cattle has not yet been defined. Schillo et al. (1992) proposed a hypothetical model for the mechanism through which photoperiod affects puberty. Information about the length of the photoperiod is sent to the pineal gland, where the pattern of the photosensitive hormone melatonin acts upon the hypothalamus to influence the secretion of luteinizing hormone releasing hormone, which controls the pulsatile release of luteinizing hormone from the pituitary gland. This model was based upon the findings of studies in the ewe, and remains unproven in cattle.

Following the pubertal estrus, approximately 40% of first estrous cycles, and 35% of second estrous cycles were of abnormal length. The occurrence of short cycles accounted for the majority of the abnormalities in both cycles. These results agree with those of Del Vecchio et al. (1992), who found that improper CL development and asynchrony in the endocrine function of the hypothalamic - pituitary - gonad - uterine pathway largely accounted short cycle length following puberty. Long cycles accounted for a much smaller proportion of abnormalities in cycle length in both inter-estrus

intervals. Del Vecchio et al. (1992) attributed long cycles mainly to the occurrence of silent estrus, or ovulation without the expression of behavioural estrus. In the present study, the proportion of abnormal cycles did not differ between treatments, therefore photoperiod must have influenced the processes leading up to puberty, but the final stages of sexual maturation resulting in regular length estrous cycles must be coordinated through events following puberty, that are possibly unrelated to photoperiod.

4.6 Conclusion

The key finding from this portion of the study was that exposure to extended photoperiod resulted in a greater proportion of heifers having reached puberty prior to the breeding season. Within 28 days of the beginning of the treatments, heifers in the EP group had elevated serum prolactin, which implies that the extended lighting treatment was of sufficient duration and intensity to evoke a physiological response. The mechanism through which photoperiod affects puberty may be related to prolactin, or may function through entirely different pathways, but is definitely an unresolved relationship. The findings of this study suggest that manipulation of photoperiod may stimulate the reproductive development of beef heifers, giving the potential for increasing the proportion of pubertal heifers prior to the breeding season. Past studies have shown that fertility of the pubertal estrus is lower than that of subsequent cycles, therefore increasing the proportion of pubertal heifers before breeding may result in a greater number of fertile heifers earlier in the breeding season.

Table 9. Composition of TMR ingredients[a] from weaning until breeding.

Item	Hay	Barley Silage	Barley Grain	Pellet[b]
Amount in preflush period (% diet as fed)	6.8-50.0	50.0-93.2	0	0
Amount in flush period (% diet as fed)	6.4-9.7	64.5-76.6	8.5-12.9	8.5-12.9
Dry Matter (g kg^{-1})	849	394	885	.
Digestible Energy (MJ kg^{-1})	10.0	12.2	15.4	.
Protein (g kg^{-1})	175	124	125	.
Neutral Detergent Fibre (g kg^{-1})	509	542	230	.
Acid Detergent Fibre (g kg^{-1})	400	346	71	.
Calcium (g kg^{-1})	13.1	4.0	0.7	.
Phosphorous (g kg^{-1})	2.3	3.0	3.8	.
Magnesium (g kg^{-1})	2.3	2.0	1.4	.
Potassium (g kg^{-1})	18.5	18.0	5.4	.

[a] Values for hay and silage are based on laboratory analysis of samples taken at the time of conservation. Values for barley grain are from Cowbytes, as grain was purchased regularly throughout the winter therefore one set of standard values was used for diet formulation.

[b] The pelleted supplement contained per MT crushed barley (941.3 kg), mineral mix (28 kg containing Se .025, Ca 155, P 155, Mg 20, Na .2 I 0.2, Fe 5.0, Cu 4.0, Mn 5.0, Co 0.05, Zn 10, F 2 g kg^{-1}, and Vitamins A 500, D3 55, E 0.5 KIU kg^{-1}), blue salt (10 kg), strong ADE (0.7 kg containing A 10, 000,000, D 1,000,000, E 100,000 IU kg^{-1}) and limestone (20 kg).

Figure 8. Aerial view looking west of extended and natural photoperiod facilities.

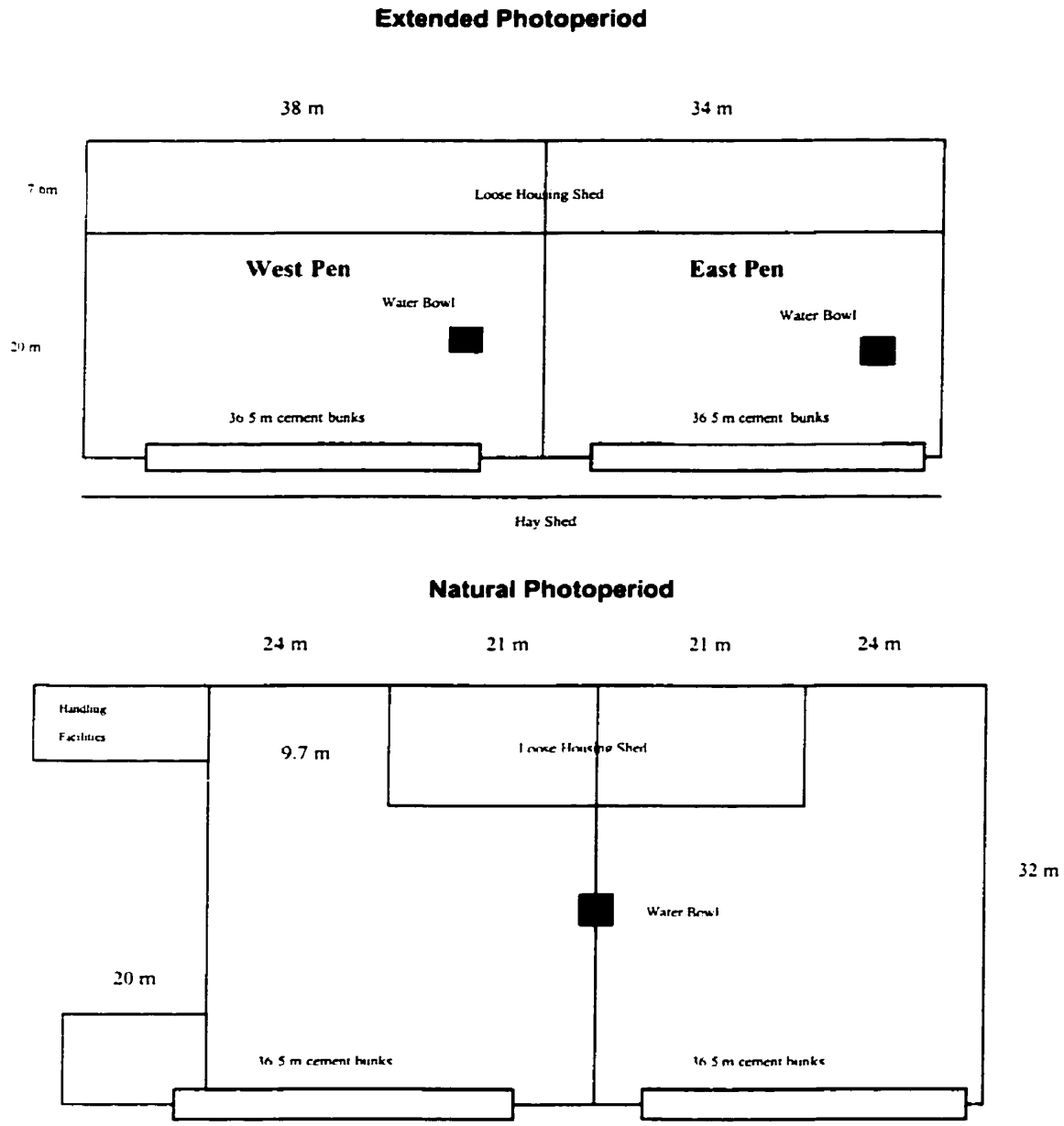

Figure 9. Dimensions of extended and natural photoperiod facilities.

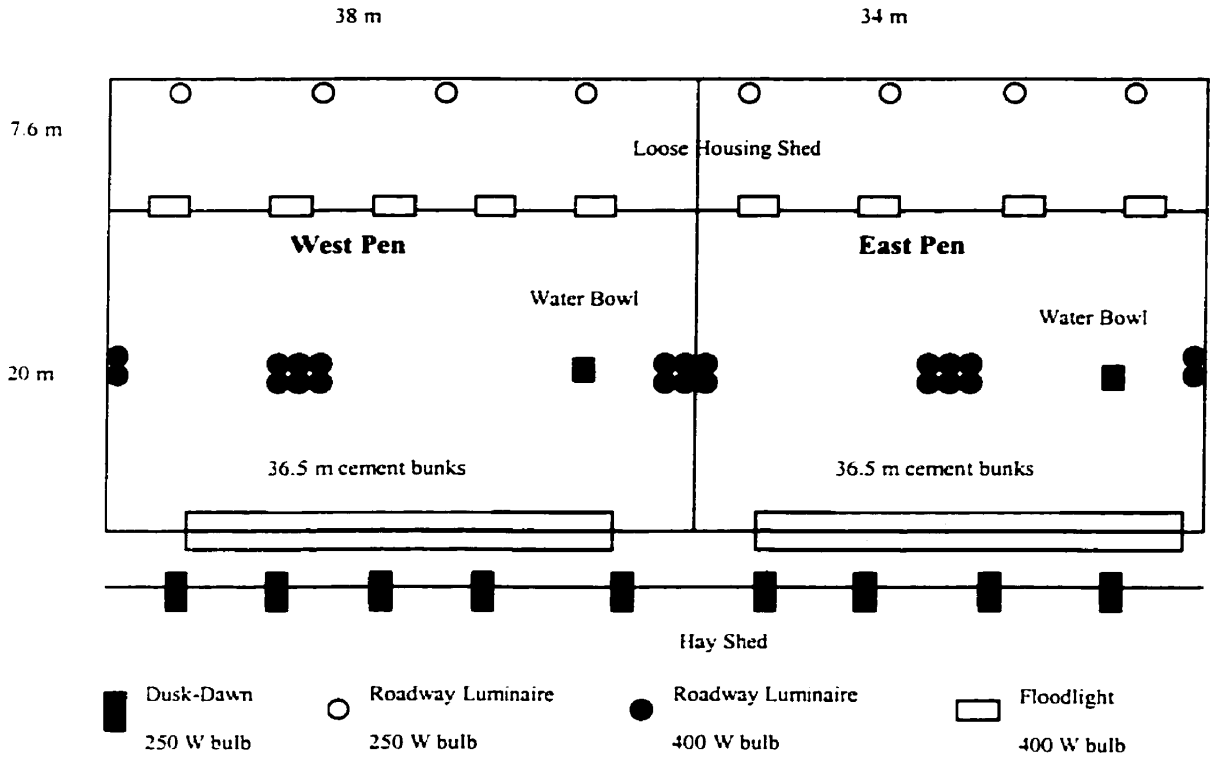

Figure 10. Schematic lighting plan for extended photoperiod facility.

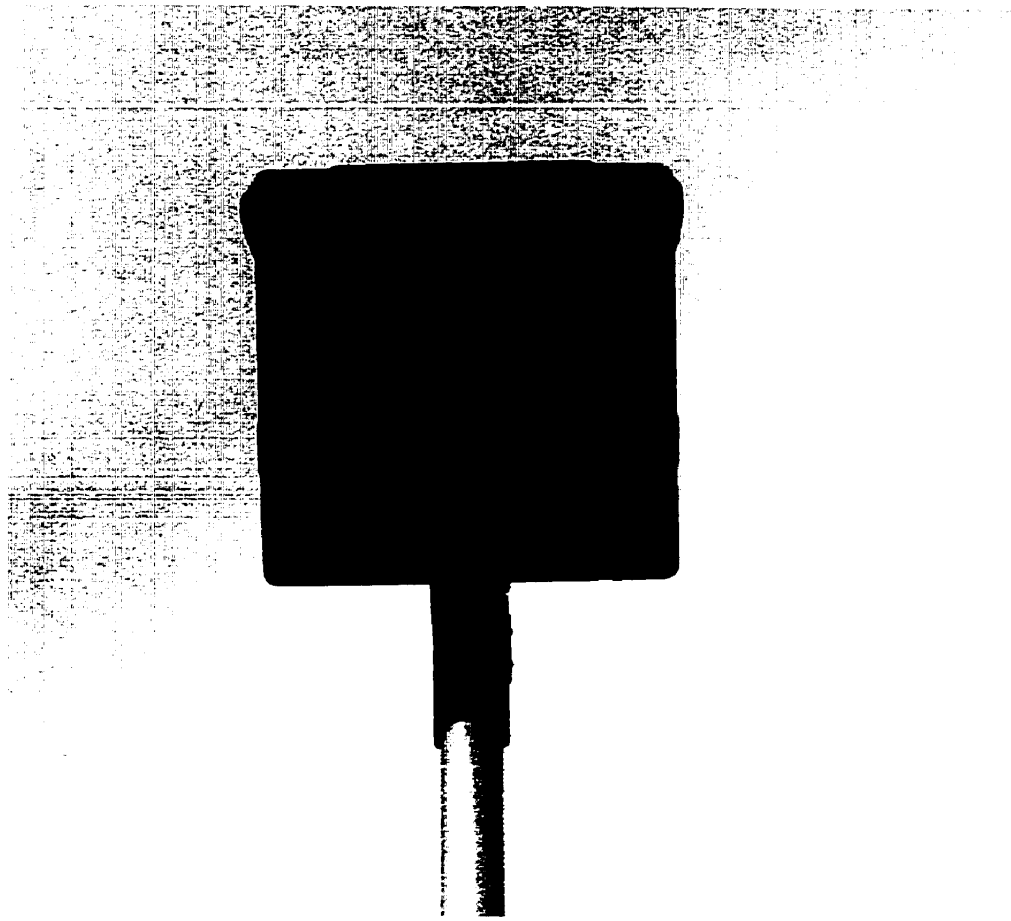

Figure 11. Cast aluminum floodlight, 120V, 400w HPS clear lamp.

Figure 12. Dusk-to-Dawn (sentinel) luminaire, cast aluminum body, 120V, 250w HPS clear lamp

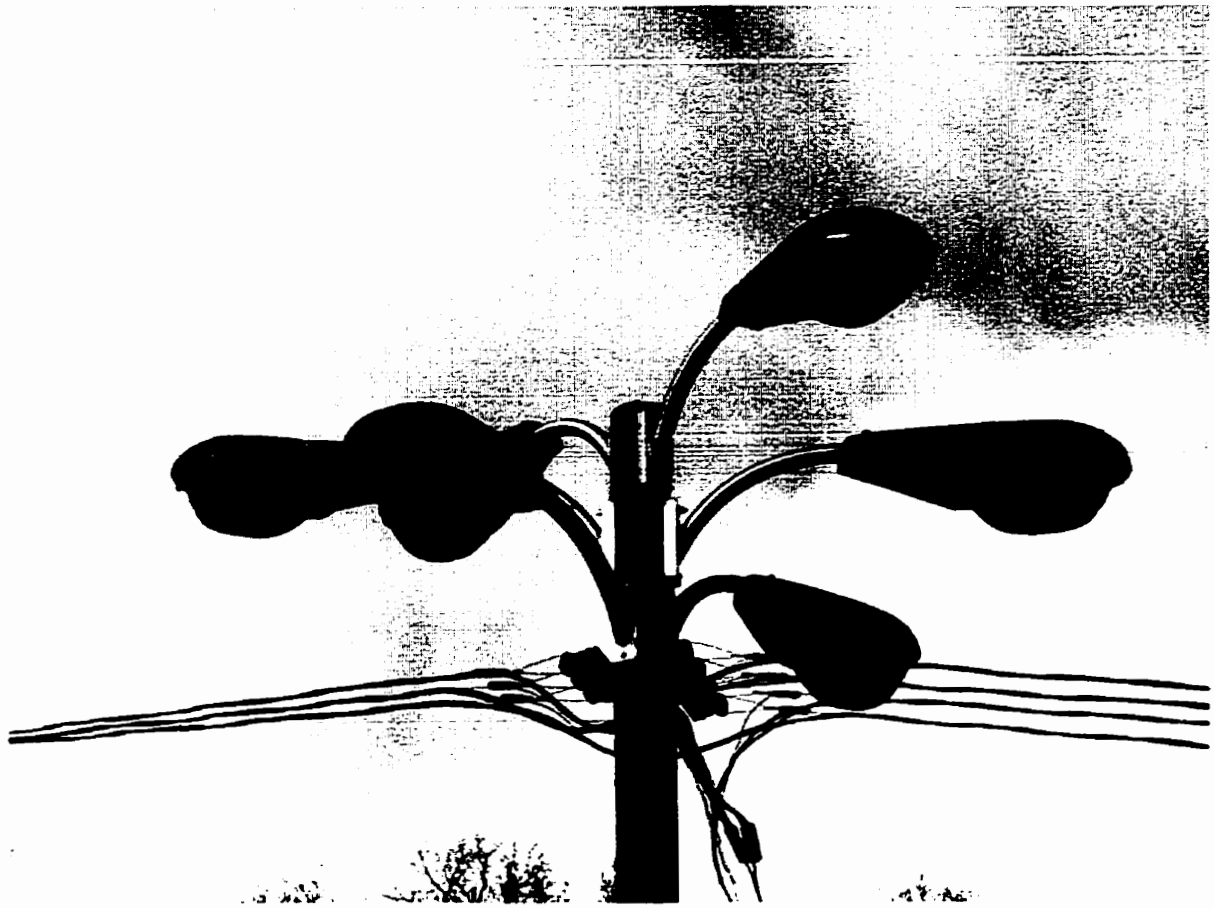

Figure 13. Roadway luminaires (shown in staggered arm arrangement), cast aluminum body, 120V, 400w HPS clear lamp. Roadway luminaires were also installed in the EP shed, however 250W HPS clear lamps were used.

Figure 14. Lighting design used in extended photoperiod facility, showing three fixture types.

	A	B	C	D	E	
1	274	331	658	405	210	
2	353	372	415	287	279	
V	225	505	281	346	250	
3	285	295	194	203	202	
4	276	334	337	329	177	
V	144	384	276	403	309	West Pen
5	318	495	937	641	543	
6	381	571	963	555	398	
V	197	433	448	587	495	
7	361	383	524	371	350	S
8	400	403	505	336	319	H
V	200	488	339	404	358	E
9	374	661	869	760	416	L
10	505	613	929	909	428	T
V	287	396	371	886	717	E
11	491	395	491	443	882	R
12	537	310	609	439	268	
V	287	388	293	392	287	East Pen
13	644	483	788	823	405	
14	668	492	887	734	488	
V	323	394	308	606	504	
15	479	256	264	259	218	
16	396	244	416	225	268	
V	241	308	199	279	234	

	A	B	C	D	E	
1	291	519	384	469	344	
2	297	288	266	463	534	
V	384	384	343	493	418	
3	138	203	289	268	453	
4	405	196	872	394	759	
V	188	147	229	193	217	West Pen
5	537	177	871	413	873	
6	458	180	905	395	990	
V	225	164	492	288	517	
7	264	123	641	296	747	S
8	130	143	494	243	464	H
V	210	89	492	234	535	E
9	689	593	842	411	349	L
10	481	329	555	387	355	T
V	358	375	451	446	356	E
11	690	242	648	244	292	R
12	1046	465	891	294	493	
V	136	142	177	158	118	East Pen
13	890	589	1029	418	563	
14	975	499	1009	404	457	
V	442	277	493	232	221	
15	588	319	725	243	305	
16	395	355	492	288	286	
V	491	276	498	208	238	

V=vertical reading

Figure 15. Light intensity readings (lux) on Nov. 19, 1998 (top) and March 13, 1999 (bottom) in the EP facility. Each number and letter represents a measurement point. 'V' represents the reading at a point with the light meter oriented vertically. The light intensity inside the shed was 90 lux (average of 10 locations).

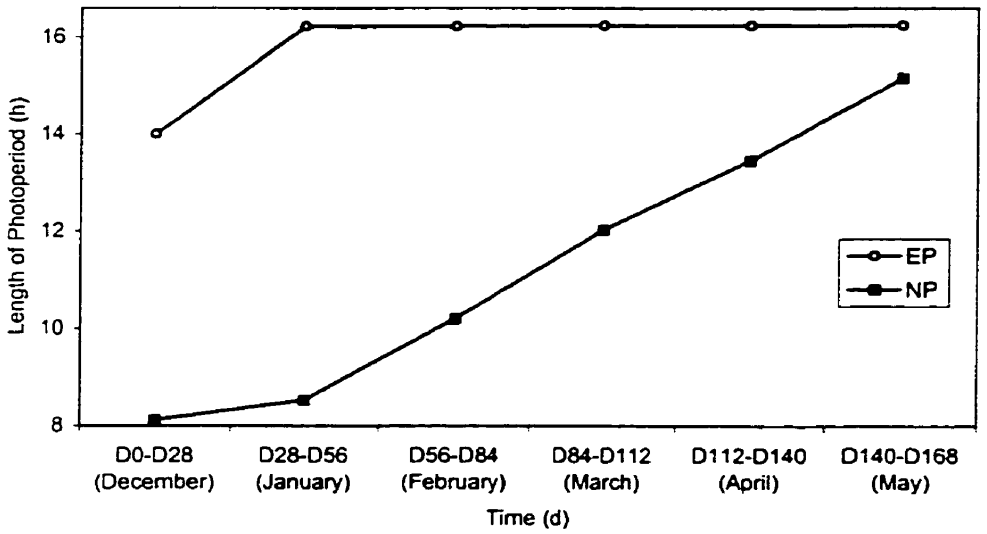

Figure 16. Mean length of photoperiod in extended and natural photoperiod treatments.

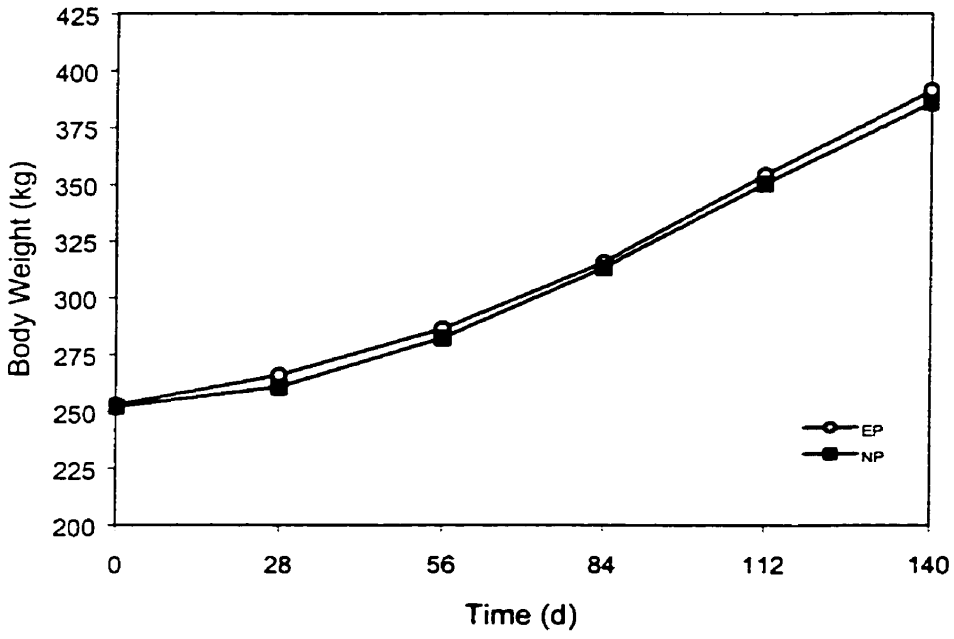

Figure 17. Changes in body weight in response to photoperiod (trt*day P=0.12, SE=0.9).

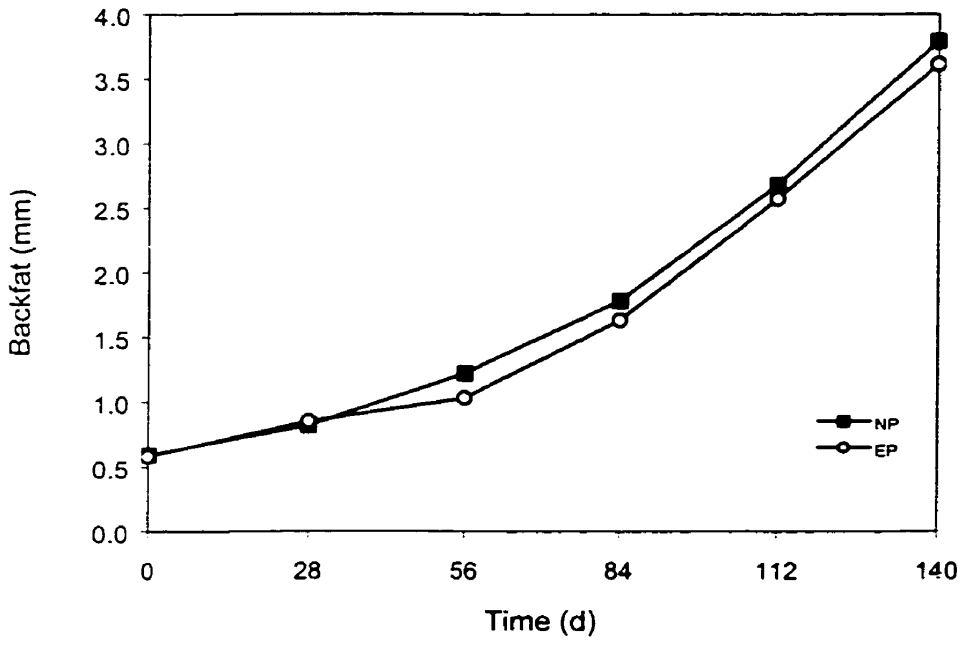

Figure 18. Changes in backfat in response to photoperiod (trt*day P=0.11, SE=0.06).

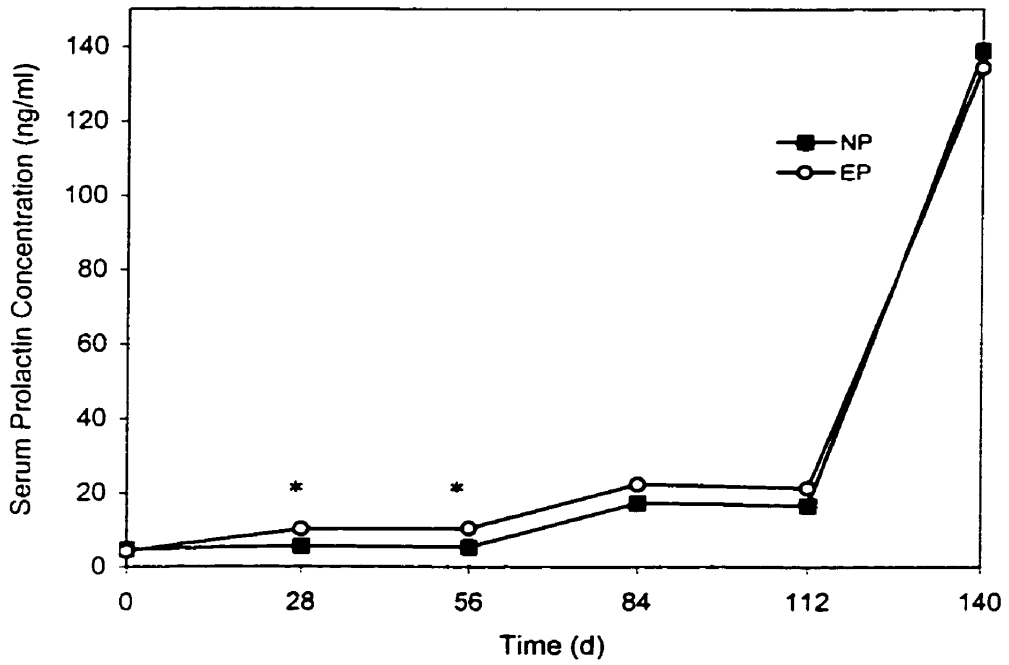

Figure 19. Changes in serum prolactin concentration in response to photoperiod (trt*day P=0.02, SE=3.2).
* Means within a time period were different ($P < 0.05$).

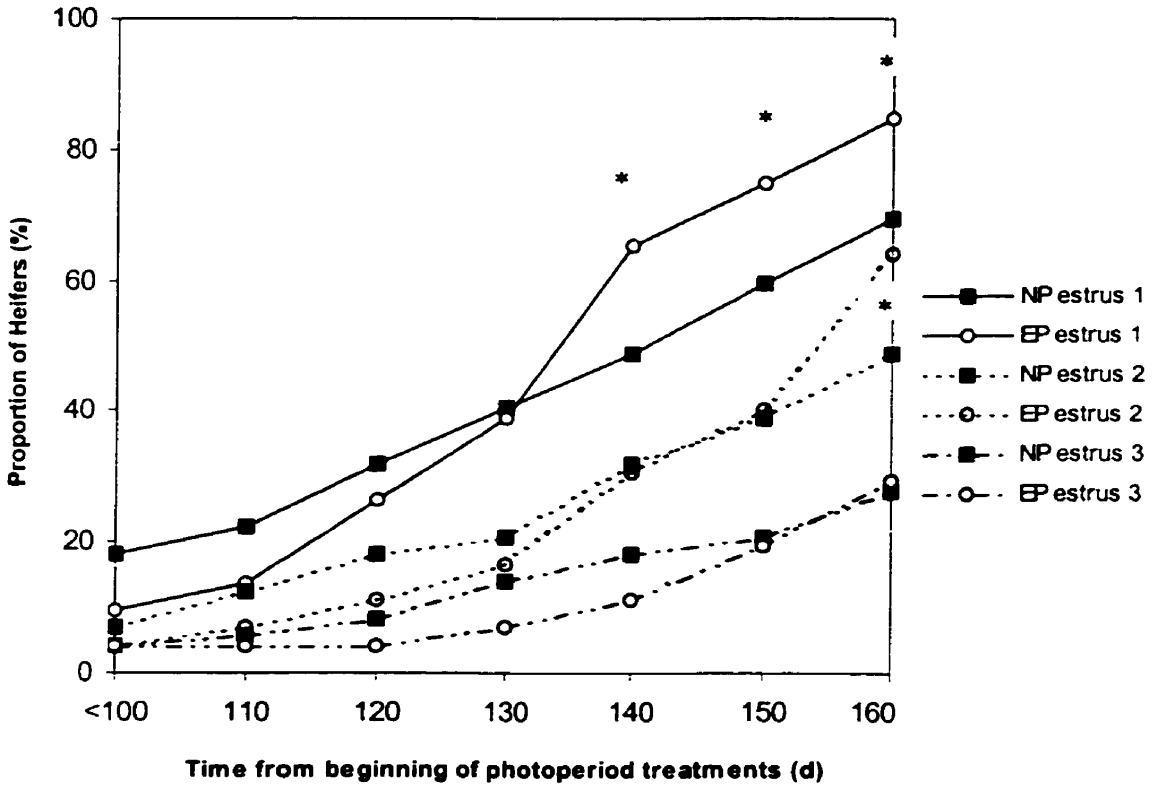

Figure 20. The effects of photoperiod on the cumulative proportion of heifers that exhibited one , two or three confirmed estruses during the post-weaning period, from the introduction of photoperiod treatments on December 1, 1998 to the beginning of synchronization of estrus on May 8, 1999 (day 160). * Means within a time period are different ($P < 0.06$). Means without * were similar ($P > 0.15$). EP increased the proportion of heifers exhibiting one ($P = 0.03$), and two ($P = 0.06$), but not three ($P = 0.85$) estruses by d 160.

Table 10. The proportion of heifers from the EP and NP treatments that exhibited one, two or three confirmed estruses during the post-weaning period, and the proportion of first and second estrous cycles that were of normal, short, or long duration.

Item	Photoperiod Treatment		SE	P-value
	EP	NP		
Number of Heifers	72	72		
One confirmed estrus (%)	84.7	69.4	3.1	0.03
Two confirmed estrus (%)	63.9	48.6	3.4	0.06
Normal 18 to 24 day cycle (%)	58.7	60.0	3.9	0.91
Short cycle (%)	39.1	31.5	3.9	0.48
Long cycle (%)	2.2	8.5	2.6	0.22
Mean first cycle length (d)	17.5	18.3	1.0	0.54
Three confirmed estrus (%)	29.2	27.8	3.3	0.85
Normal 18 to 24 day cycle (%)	61.9	65.0	4.6	0.84
Short cycle (%)	33.3	35.0	4.6	0.91
Long cycle (%)	4.8	0.0	2.2	0.32
Mean second cycle length (d)	18.1	17.5	0.9	0.63
Age at first confirmed estrus (d)	369	365	4.3	0.44

5 MANUSCRIPT II - EFFECTS OF PHOTOPERIOD AND ESTROUS SYNCHRONIZATION METHOD ON BREEDING PERFORMANCE OF BEEF HEIFERS.

5.1 Abstract

A study was conducted to determine the effects of photoperiod and estrous synchronization method on the breeding performance of beef heifers. Crossbred heifers (n=144) were assigned to two photoperiod treatments (extended photoperiod, 16 h light day^{-1}, or natural photoperiod) from December until first service artificial insemination (AI) in May, in a completely randomized design. Progesterone concentration in blood samples taken twice weekly for approximately one month before synchronization, was used to classify heifers as having either regular luteal function (RLF – at least two consecutive progesterone values ≥ 1.0 ng ml^{-1}) or irregular luteal function (ILF - no consecutive progesterone values≥ 1.0 ng ml^{-1}). Heifers with RLF were assigned to either double prostaglandin ($PGF_{2\alpha}$) method (Lutalyse® 25 mg, 11 d apart, PG-RLF) or gonadotrophin-releasing hormone (GnRH) method (Factrel® 100 μg followed 7 d later by $PGF_{2\alpha}$, and a second GnRH at AI, GnRH-RLF). All heifers with ILF were assigned to the GnRH method (GnRH-ILF). Response to synchronization was determined by blood progesterone concentration at each injection. Heifers observed in estrus until 36 h post-$PGF_{2\alpha}$ were inseminated 12 h later, and all remaining heifers were inseminated 66 h post-$PGF_{2\alpha}$. Fertile bulls were used 2-45 days after AI. Synchronization response rate (69.8, 65.1, 31.0 ± 4.5%), first service conception rate overall (32.6, 27.9, 13.8 ± 4.3%), and in responders (43.3, 35.7, 16.7 ± 4.2%) were similar ($P > 0.05$) in PG-RLF and GnRH-RLF, but were lower ($P < 0.05$) in GnRH-ILF. Pregnancy rates at 25-d (74.4, 76.7, 74.1 ± 4.3%) and 45-d (79.1, 90.7, 91.4 ± 3.8%) were similar ($P > 0.05$) for PG-RLF, GnRH-RLF, and GnRH-ILF, respectively. Extended photoperiod increased the proportion of heifers with RLF ($P < 0.05$), but had no further effects ($P > 0.05$) on synchronization or breeding. Results of this study illustrate the importance of RLF on the success of synchronization and AI.

5.2 Introduction

In a recent survey of Manitoba's beef producers, reproductive performance was reported to be one of the top three factors limiting profitability (Small and McCaughey 1999). Many producers reported a prolonged calving season, which is indicative of a long breeding season in the previous year. Routine use of artificial insemination (AI) of beef cattle has received limited acceptance by beef producers, largely due to the extensive nature of beef cattle production, and the time commitment required for accurate heat detection (Odde 1990). The development of estrous synchronization procedures that minimize or eliminate the need for heat detection may increase the use of AI in the beef industry. The combined use of estrous synchronization and AI may aid in shortening the calving season, in addition to facilitating the introduction of superior genetics into beef herds.

Synchronization and AI of beef replacement heifers represents an excellent opportunity to improve the reproductive efficiency of the entire herd. Heifers that calve early as two-year olds have higher lifetime productivity than those that calve late in the season (Lesmeister et al. 1973; Marshall et al. 1990). Replacement heifers are often reared separately from the rest of the herd, providing the opportunity for intensive reproductive management. A commonly used synchronization protocol in heifers is two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), given 11 days apart. This procedure, however, requires heat detection for a five to seven day window, and may result in the development of persistent follicles, which decrease fertility (Stephens and Rajamahendran 1998). As such, synchronization methods that are compatible with timed AI are being investigated. Synchronization protocols for timed AI should attempt to control both luteolysis and follicle development, as asynchrony in waves of follicle growth accounts for much of the variability in the interval from prostaglandin administration to estrus onset (Bo et al. 1995). Gonadotrophin releasing hormone (GnRH) is often used in timed AI protocols, as administration of GnRH results in the selection of a new follicle that is ovulated after $PGF_{2\alpha}$ induced luteolysis (Twagiramungu et al. 1995).

Very little information is available regarding the use of GnRH-$PGF_{2\alpha}$ synchronization protocols for timed AI of beef heifers (Stephens and Rajamahendren 1998). The objective of this study was to compare two methods of estrous

synchronization in heifers reared under two different photoperiods from weaning until breeding. The commonly used double $PGF_{2\alpha}$ method was compared to a method using GnRH and $PGF_{2\alpha}$. The suitability of each method was evaluated in terms of producing acceptable fertility levels, in addition to compatibility with timed AI.

5.3 Materials and Methods

5.3.1 Animals and Management

The experiment described in this chapter was part of an ongoing study at the Agriculture & AgriFood Canada Research Station, Brandon, Manitoba. The data presented in this chapter was collected between April, 1999 and September, 1999. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Chapter 4 contains a complete description of the heifers used in this study, and the management of these heifers during the post-weaning period. All 144 heifers that were initially assigned to the study underwent estrous synchronization and breeding. The heifers received the ration shown in Table 9, (Chapter 4) throughout the post-weaning and breeding phases of the experiment, until they were turned out to alfalfa/grass pasture on June 3, 1999 following AI. Heifers remained on pasture for the entire summer.

5.3.2 Photoperiod Treatments

Chapter 4 contains a complete description of the housing facilities and lighting design used to achieve the two photoperiod treatments; EP = extended photoperiod of 16 hours total light day^{-1} (natural + artificial), artificial light was 423 lux, 1m above ground and NP=natural photoperiod. The photoperiod treatments began on December 1, 1998 and continued until the day of timed AI on May 20, 1999, after which both treatments received natural photoperiod.

5.3.3 Confirmation of Pubertal Status

A bleeding schedule was developed in order to be certain of the pubertal status of all heifers. A single blood sample was collected from each heifer twice weekly from April 8, 1999 until May 3, 1999 for determination of serum progesterone concentration. A total of eight samples were collected from each heifer. The data provided confirmation that heifers that had not been detected in heat throughout the post-weaning period, (via twice daily estrus detection followed by blood sampling 8-12 days later), were truly pre-pubertal. The intensive nature of the program also allowed for characterization of the luteal function of each heifer. Based on the information from the intensive bleed month, heifers were classified as having either regular luteal function (RLF, at least two consecutive progesterone values greater than or equal to 1.0 ng ml^{-1}) or irregular luteal function (ILF, no consecutive progesterone values greater than or equal to 1.0 ng ml^{-1}).

5.3.4 Estrous Synchronization, Artificial Insemination and Natural Breeding

All heifers that were designated as having ILF (n=58) were assigned to the GnRH method (GnRH-ILF). Half of the heifers having RLF were randomly assigned to the GnRH method (GnRH-RLF, n=43) and the other half to the double $PGF_{2\alpha}$ method (PG-RLF, n=43).

The double $PGF_{2\alpha}$ method consisted of two 25 mg doses of the prostaglandin dinoprost tromethamine (5 ml Lutalyse®per dose, Upjohn & Pharmacia) given 11 days apart at 14:00 h. The GnRH method consisted of one 100 μg dose of gonadorelin hydrochloride (2 ml Factrel®, Ayerst Veterinary Laboratories) followed 7 days later with one 25 mg dose of the prostaglandin dinoprost tromethamine (5 ml Lutalyse®, Upjohn & Pharmacia). The protocol was designed so that the second injection of $PGF_{2\alpha}$ in the double $PGF_{2\alpha}$ method, and the single $PGF_{2\alpha}$ injection in the GnRH method, occurred on the same day. Timed artificial insemination was scheduled for 66 hours following this $PGF_{2\alpha}$ injection, at which time a second 100 μg dose of gonadorelin hydrochloride (2 ml Factrel®) was given to heifers assigned to the GnRH method. The timeline for each method is shown in Figure 21.

From initial injections until the $PGF_{2\alpha}$ injection 66 hours before timed AI, estrus detection was performed twice daily at 07:00 and 19:00 h. Following the $PGF_{2\alpha}$ injection prior to AI, estrus detection was performed three times daily at 07:00 h, 13:00 h and 19:00 h. Detection frequency was increased to be certain of the distribution of estrus onset following $PGF_{2\alpha}$ administration.

Heifers that were observed in estrus until 36 h post- $PGF_{2\alpha}$ were inseminated 12 h later. All remaining heifers were bred by AI, 66 hours after the last $PGF_{2\alpha}$. Semen from one proven Gelbvieh sire (ABS Global, DeForest, WI, USA) was used for the first service for all heifers. Fertile bulls were placed with the heifers 48 hours after timed AI, for a 45-day breeding season (bull to heifer ratio 1:36). All bulls used were evaluated for breeding soundness before the breeding season.

Three times daily estrus detection continued for one week after timed AI, after which, detection frequency returned to twice daily at 07:00 h and 19:00 h. To aid in detection, chin ball marking halters were placed on the bulls when they were put in with the heifers after AI. Halters were refilled with paint on a weekly basis, or as needed if a large amount of marking had occurred. Visual estrus detection and use of the marking halters continued until day 25 of the breeding season.

5.3.5 Blood Sampling During Synchronization, AI and Breeding Season

A blood sample for progesterone analysis was taken from all heifers by tail venipuncture at the time of each injection during synchronization, at AI, and 21 and 25 days after AI. For confirmation of the ovarian response to synchronization, progesterone greater than or equal to 1.0 ng ml^{-1} was considered high and indicative of a functional corpus luteum, whereas concentrations less than 1.0 ng ml^{-1} were considered low and indicative of the absence of a functional corpus luteum. Heifers with high progesterone at the time of the final $PGF_{2\alpha}$ and low progesterone at AI were considered responders. Non-responders included asynchronous (high progesterone at GnRH and low progesterone at $PGF_{2\alpha}$, or low progesterone at GnRH, low progesterone at $PGF_{2\alpha}$ and high progesterone at AI), non-cycling (low progesterone throughout synchronization) or having incomplete luteolysis (high progesterone at PGF2α and high progesterone at AI).

All heifers that were observed or suspected to have been in heat the first 14 days after AI were bled within 24 hours of the observation. This blood sample was necessary because ultrasound and rectal palpation cannot accurately differentiate fetal age within 14 days. By measuring progesterone at the time of a suspected heat, it was possible to determine whether a natural service could have resulted in conception, enabling differentiation between an AI conception and a bull-bred conception in the first 14 days of the breeding season.

Serum progesterone concentrations were determined by an established enzyme-immunoassay, which had a detection limit of 0.03 ng ml^{-1} (Del Vecchio et al. 1995). The coefficients of variation within and between assays were 6.71% (n=20) and 11.55% (n=11), respectively.

5.3.6 Pregnancy Diagnosis

Pregnancy diagnosis was performed using real-time ultrasonography (Aloka 560, 5.0MHz probe) 45 days after AI. AI conception rate is defined as the percentage of heifers that were determined, through ultrasonography at 45 days, to have successfully conceived to AI. A veterinarian performed transrectal palpation 60 days after the end of the natural breeding season. AI pregnancy rate is the percentage of heifers that maintained the AI pregnancy throughout the breeding season, as confirmed by rectal palpation (accounts for fetal loss). Cumulative pregnancy rate at day 25 includes heifers that conceived to timed AI, and heifers that became pregnant by natural service between days 2 and 25 after timed AI. Determination of day 25 pregnancy rate was based on the information obtained from ultrasound and rectal pregnancy diagnosis, daily estrus observations and serum progesterone concentration in blood samples taken during synchronization, AI, estrus until day 14 after AI, and on days 21 and 25 of the breeding season. Cumulative pregnancy rate at day 45 is the percentage of heifers that were pregnant at the end of the breeding season, as determined by rectal palpation.

5.3.7 Measurement of Body Weight and Body Condition Score

The heifers were weighed one week after AI, as they were being loaded for transport to pasture. This weight is reported as the weight at breeding. Heifers were weighed on the day of ultrasound for pregnancy diagnosis, and this weight is reported as the weight at the end of breeding season. On both of these weigh days, the heifers were body condition scored by a trained animal herdsperson. The condition score scale ranges from 1-9, with 1 being an emaciated animal and 9 being an extremely obese animal.

5.3.8 Statistical Analysis

Statistical analysis of the data was performed using Statistical Analysis System v 6.12 (SAS 1996). Data for body weight and body condition score were analyzed using the general linear models procedure of SAS with type III sums of squares. Categorical models (CATMOD) procedures were used to test differences in proportions. The standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{error mean square}/n)}$$

$$n = \text{number of observations / treatment}$$

For proportion data, the standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{sum of variances})}$$

$$\text{where variance} = (r/n)*(1-(r/n))/n$$

$$n = \text{number of observations/treatment and } r = \text{the number responding}$$

5.4 Results

5.4.1 Effects of Photoperiod

Photoperiod affected the proportion of heifers classified as having regular or irregular luteal function, and therefore the proportion of heifers assigned to each synchronization method. In the EP treatment, 70.8% of heifers were classified as having RLF, compared to 48.6% of heifers from the NP treatment (P = 0.007, Table 11). Examples of serum progesterone profiles from heifers classified as having RLF or ILF are shown in (Figure 29, Appendix II). All measures of success of synchronization, AI and natural breeding were similar between the two photoperiod treatments (P > 0.05). The interaction between photoperiod and synchronization method was not significant (P > 0.05) for any of the parameters measured during synchronization and breeding.

5.4.2 Ovarian Response to Synchronization

The response to synchronization was lowest in the GnRH-ILF method (P = 0.0002, Table 11). Only 31% of heifers in this treatment responded to the $PGF_{2\alpha}$ injection prior to breeding, compared to 65.1% of GNRH-RLF heifers and 69.8% of PG-RLF heifers. The classification of non-responders into acyclic, asynchronous or incomplete luteolysis differed between synchronization methods (Table 12). Most (52.5%) of the non-responders in the GnRH-ILF group were classified as acyclic, whereas none of the non-respondent heifers in the other two treatments were classified as acyclic (P = 0.001). In the PG-RLF group, the greatest proportion of non-responders were classified as having incomplete luteolysis at the time of AI (69.2%), compared to 26.7% of non-responders in the GnRH-RLF group and 7.5% of non-responders in the GnRH-ILF group (P = 0.001). In the GnRH-RLF group, the greatest proportion of non-responders were classified as asynchronous (73.3%) compared to 30.8% of non-responders in the PG-RLF group and 40% of non-responders from the GnRH-ILF group (P = 0.04).

5.4.3 Pregnancy Establishment at AI and During the Breeding Season

Conception to AI was lower in the GNRH-ILF treatment (13.8%) than in either the PG-RLF treatment (32.6%) or the GnRH-RLF treatment (27.9%) ($P = 0.06$). Success of AI was similar ($P > 0.05$) in the GnRH-RLF and the PG-RLF treatments. AI conception rate in responders also tended to be lower in the GnRH-ILF treatment (16.7%) than in either the PG-RLF treatment (43.3%) or the GnRH-RLF treatment (35.7%). AI conception rate in non-responders averaged 12% for all three groups (8 out of the 68 non-respondent heifers). These heifers may have had 'borderline' P4 values (either slightly below 1.0 ng ml^{-1} at the time of $PGF_{2\alpha}$ or slightly above 1.0 ng ml^{-1} at the time of AI) that resulted in them being classified as non-responders, but still, they conceived to AI. Cumulative pregnancy establishment in the first 25 days of the breeding season was similar in all treatments ($P > 0.05$). Cumulative pregnancy rate after the 45-d breeding season was also similar between treatments, but tended to be lower in the PG-RLF group.

5.4.4 Estrus Distribution Prior to AI

The occurrence of estrus within 7 days of $PGF_{2\alpha}$ differed for heifers that responded to synchronization (Figure 22), compared to heifers that did not respond to synchronization (Figure 23). Over the 7 day window, approximately 65% of respondent heifers showed heat, compared to only 20% of non-responders ($P = 0.001$). Within the classifications of responders or non-responders, method of synchronization had little effect on the distribution of estrus. Fewer respondent heifers from the GnRH-ILF method showed heat within a 7 day period, compared to respondent heifers in the GnRH-RLF and the PG-RLF methods ($P = 0.04$).

5.4.5 Return Estrus Following AI

The pattern of return heats in respondent heifers that did not conceive to timed AI differed between synchronization methods (Figure 24). The proportion of heifers that returned before day 18 was greater ($P = 0.05$) for the GnRH-ILF method, compared to the

other two methods which had virtually identical patterns until this point. By day 20, a greater proportion (P = 0.05) of heifers had returned in both the GnRH-ILF and the GnRH-RLF methods, compared to the PG-RLF. By day 24, all respondent heifers that did not conceive to timed AI from the GnRH-ILF group had returned to heat, compared to approximately 83% of respondent heifers from the GnRH-RLF group and 65% of respondent heifers from the PG-RLF group (P = 0.03).

The pattern of return heats for non-respondent heifers that did not conceive to timed AI was similar between all three synchronization treatments (Figure 25). A comparison of Figure 24 and Figure 25 shows that the occurrence of return heats appeared to occur in a more distinct pattern for respondent heifers than for the non-respondent heifers. In non-respondent heifers, return heats occurred in a gradual pattern for all three synchronization methods.

5.5 Discussion

5.5.1 Effects of Photoperiod

As discussed in chapter 4, one of the major findings of the effects of photoperiod during the post-weaning phase was the stimulation of puberty in heifers housed under extended photoperiod. This stimulation of puberty is almost certainly related to the classification of larger proportion of heifers from the EP as having regular luteal function. The EP treatment resulted in 31% more heifers having undergone two confirmed heats prior to estrus synchronization. Del Vecchio et al. (1992) showed that the frequency of abnormal length cycles dropped dramatically after the second heat, with all heifers experiencing a regular length (18-24 days) cycle between the second and third heat. Progesterone concentrations were higher in the third than in the first cycle. Reports of the occurrence of inadequate corpus luteum function during the pubertal transition phase, as evidenced through serum progesterone concentrations, have ranged from 13% (Nelson et al. 1985) to as high as 63% (Rutter and Randel 1986). Del Vecchio et al. (1992) found that 30% of abnormal length cycles were due to inadequate corpus luteum function.

Classification of luteal function was the only parameter affected by photoperiod in the current study. The high proportion of heifers in both treatments that had not yet undergone three confirmed heats may explain the absence of any effects on synchronization response, and pregnancy establishment. While the heifers from the EP treatment did have an advantage in having regular luteal function, there may be other stages of sexual maturation occurring in the pubertal transition phase that are of equal or greater importance in successful pregnancy establishment. Del Vecchio et al. (1992) suggest the estrogen to progesterone ratio is one factor that may affect conception and embryonic survival, and found that this ratio differed between the first and third cycles. Thus, while the EP treatment advanced puberty and promoted regular luteal function, there may have been some other final stages of maturation that were not advanced by extended photoperiod.

5.5.2 Effects of Synchronization Method on Response to Synchronization

The first dose of GnRH is expected to result in the growth of a new wave of follicles and selection of a new dominant follicle that will ovulate after prostaglandin induced luteolysis (Twagiramungu et al. 1995). Ovulation of the dominant follicle at the time of the first GnRH results in the presence of a CL that is responsive to induced luteolysis (Twagiramungu et al. 1995). The second injection of GnRH is expected to synchronize the LH surge and ovulation of the newly selected dominant follicle (Twagiramungu et al. 1995). The timing of the second GnRH is an important consideration, because the period of increased pulsatile LH frequency between luteolysis and the ovulatory LH surge is essential for the resumption of processes related to meiotic cell division in the oocyte (Greve et al. 1995). If the LH surge is induced prematurely by GnRH, resumption of meiosis may be impaired, resulting in an incompetent oocyte and reduced fertility (Greve et al. 1995).

The GnRH-ILF group showed a poor response to synchronization (31%), compared to the PG-RLF group (69.8%) and the GnRH-RLF group (65.1%). Treatment with GnRH induces the resumption of cyclic ovarian activity in postpartum anestrous cows, and can produce fertility levels similar to that in cyclic cows (Twagiramungu et al.

1995). However, the authors suggest that caution should be used when drawing conclusions on fertility in acyclic cows because only a small number of acyclic cows were used in earlier studies. No studies were found that examined the potential of the GnRH method used in the current study for synchronization of acyclic beef heifers. Prepubertal heifers experience waves of follicular growth similar to those in pubertal heifers (Adams et al. 1994), therefore it is conceivable that administration of GnRH could result in selection of an ovulatory dominant follicle. In this study, only 31% of heifers with ILF responded to GnRH synchronization with high progesterone at the time of prostaglandin and low progesterone at AI. Of the non-respondent heifers, 53% remained acyclic, 40% were asynchronous and 7% had incomplete luteolysis.

Although the overall proportion of non-responders was similar between the PG-RLF and GnRH-RLF treatments, the classification of the non-responders was quite different. In the GnRH-RLF group 73% of non-responders were classified as asynchronous, compared to 31% in the PG-RLF group. Moreira et al. (2000) clearly illustrated the effects that day of the estrous cycle at the initiation of a timed artificial insemination protocol, similar to that used in the current study, has on response to synchronization. Initiation of synchronization on day 2 of the cycle resulted in the highest proportion of heifers failing to ovulate after the first GnRH. When synchronization began on day 15, the incidence of premature CL regression and early ovulation increased. The occurrence of incomplete CL regression was highest when synchronization began on day 18, as the induced CL was less sensitive to prostaglandin. Pursley et al. (1995) found that only 50% heifers ovulated following the initial injection of GnRH, and suggested that failure to respond to the initial GnRH accounted for lack of synchrony in most heifers. In the present study, heifers could have been at any stage of the estrous cycle at the beginning of the synchronization protocol. Thus, failure to respond to synchronization could be attributed to any or all of the factors listed by Moreira et al. (2000).

In the PG-RLF group the highest proportion of non-responders were classified as having incomplete luteolysis at AI (69%). The development of persistent follicles may occur when prostaglandin induced luteolysis is incomplete. In this situation, the dominant follicle becomes non-ovulatory, behavioural estrus is inhibited, and circulating

estrogen increases (Moreira et al. 2000). Sub-luteal concentrations of progesterone for prolonged periods are associated with increased frequency of LH pulses and inhibition of the LH ovulatory surge, resulting in impaired fertility (Moreira et al. 2000). The development of persistent follicles is one problem that has been associated with prostaglandin based synchronization methods (Stephens and Rajamahendren 1998). As previously discussed, the occurrence of incomplete luteolysis was highest when synchronization began late in the estrous cycle (day 18), therefore beginning synchronization at a more appropriate stage is one method of avoiding the problem of incomplete luteolysis and persistent follicles.

5.5.3 Effects of Synchronization Method on Pregnancy Establishment at AI

The success of AI was very poor in the GnRH-ILF treatment (13.8%) and reflects the poor response rate (31%). Furthermore, heifers with ILF were sexually immature at the beginning of synchronization, and fertility at the pubertal estrus is lower than that of subsequent cycles (Byerley et. al. 1987).

Stephens and Rajamahendren (1998) reported AI conception rates in beef heifers of 62% in the double-PG method and 40% in the GnRH method. These results are slightly higher than those obtained in the present study, however the previous study used a smaller number of heifers (n=30) and gave no description of the stage of sexual maturity of the heifers. Schmitt et al. (1994) reported that conception rate at timed AI was 25.4% in beef heifers synchronized with a GnRH-PG protocol. There are relatively few studies in the area of timed AI of beef heifers, as most of the research in the area has been done with dairy heifers. Timed AI conception rates in dairy heifers in the range of 26% (Schmitt et al. 1996) to 35% (Pursley et al. 1997) have been reported.

The reason for the low AI conception rates in both RLF groups is not clear. Prostaglandin ES generally results in conception rates similar to those of heifers bred after a naturally occurring heat (Beal 1996; Odde 1990). In the current study, however, conception rate to timed AI with the double PGF method were lower than that for insemination following detected estrus, which is usually in the range of 60 to 75% (reviewed by Beal 1996). Prostaglandin based synchronization methods are not generally

accepted to be highly compatible with timed AI, as no control is exerted over follicle wave development. Asynchrony between follicle development and timed insemination is a probable cause for the low AI conception rate in the PG-RLF treatment.

As shown above, the low AI conception rate in the GnRH-RLF treatment is not unique to this study. The occurrence of inadequate luteal function, as indicated by short cycles following AI, has been implicated as one factor resulting in low AI pregnancy rates (Schmitt et al. 1996). In the present study, however, the incidence of short cycles following AI was quite low (under 20%, Figure 24) in the GnRH-RLF group. Because most of the heifers from the GnRH-RLF treatment had normal length intervals to return estrus inadequate luteal function is not believed to be the primary cause of failure to conceive to AI. Perhaps the low AI conception rate in this group may be related to failure of the initial GnRH injection to result in selection of a new dominant follicle. Pursley et al. (1995) found that only 50% heifers ovulated following the initial injection of GnRH. Moreira et al. (2000) suggested that failure to ovulate and absence of selection of a new follicle following the initial GnRH injection may result in the continued growth of the first wave dominant follicle. By the time of the second GnRH injection, this follicle may have reached the plateau stage of growth and may be beginning early stages of atresia, which would compromise the subsequent developmental competence of the oocyte (Moreira et al. 2000).

5.5.4 Estrus Distribution Prior to AI

The distribution of estrus in respondent heifers following $PGF_{2\alpha}$ injection indicated that the timing of AI at 66 hours post- $PGF_{2\alpha}$ was appropriate for heifers showing behavioural estrus. Stephens and Rajamahendren (1998) bred by timed double insemination at 72 and 96 hours following $PGF_{2\alpha}$, which they stated may have been too late based on uterine tone and ease of insemination. In the present study, virtually all of the heifers that showed heat did so before 72 hours. These results are similar to the results of Stephens and Rajamahendren (1998), who found that 90% of heifers from the double-PG method and 73% of heifers from the GnRH-PG method showed heat before 72 hours.

Fewer respondent heifers in the GnRH-ILF group showed visible signs of heat following $PGF_{2\alpha}$ injection. Del Vecchio et al. (1992) found that the occurrence of silent estrus, estrous cycles without behavioural estrus, accounted for approximately 25% of abnormalities in the length of pubertal estrous cycles.

5.5.5 Return Estrus Following AI

The pattern of return heats in responders showed that heifers in the GnRH-ILF group returned at a more rapid rate than the other two synchronization groups. Moreira et al. (2000) found that the occurrence of short cycles (<16 days) following GnRH synchronization was due to three conditions: 1) failure of CL to regress following prostaglandin 2) ovulation failure following 2nd GnRH 3) shortened lifespan of the CL induced by GnRH. Because follicular development was not monitored in the present study, it is difficult to speculate on the ability of GnRH to induce ovulation in sexually immature heifers. The occurrence of a short-lived CL following puberty has been previously documented (Del Vecchio et al. 1992).

Most respondent heifers from the GnRH-RLF and PG-RLF groups that returned following AI exhibited a normal length cycle. The occurrence of short cycles was less than 20% in both of these groups. Schmitt et al. (1996) found that giving the second GnRH injection too soon (24 hours) after prostaglandin resulted in a large proportion (35%) of short cycles in dairy heifers. When the second GnRH injection was given 48 hours after prostaglandin, the incidence of short cycles fell to about 15%, which is similar to the occurrence in the present study. These results indicate that the timing of the second GnRH injection did not impair formation of a functional CL as evidenced by a high proportion of normal length cycles following AI.

The PG-RLF group had the lowest return rate following AI. As previously discussed, the development of persistent follicles may inhibit return to estrus, and is one problem that has been associated with prostaglandin based synchronization methods (Stephens and Rajamahendren 1998).

5.5.6 Pregnancy Establishment in the Breeding Season

While heifers having irregular luteal function were at a disadvantage during synchronization and AI, they had a similar rate of pregnancy establishment by day 25 of the breeding season. This data suggests that synchronization with the GnRH method did not impair successful pregnancy establishment. Overall pregnancy rate after the 45-d breeding season was acceptable in all treatments, but appeared to be lower in the PG-RLF treatment. The failure to return to heat due to the presence of persistent follicles may explain the higher proportion of open heifers in the PG-RLF group.

5.6 Conclusion

Exposure to extended photoperiod increased the proportion of heifers with regular luteal function, which is indicative of advanced sexual maturity. The success of estrous synchronization and AI was, however, similar between photoperiod treatments. While conception at AI was not improved in the current study, past research supports the hypothesis that advancement of sexually maturity should provide an advantage in terms of earlier pregnancy establishment. There is no obvious explanation as to why this benefit was not apparent in this study, but further research is required to fully elucidate the relationship between photoperiod, sexual maturity and pregnancy establishment.

The predominant finding that emerged from the present study was the importance of heifers having regular luteal function before estrous synchronization begins. Heifers with irregular luteal function were quite unsuccessfully synchronized and bred by AI. Pregnancy rate at day 25 of the breeding season, however, was similar to the RLF groups, suggesting that synchronization with GnRH did not impair subsequent fertility or pregnancy establishment. AI conception rate for timed AI in the GnRH-RLF group was similar to previous studies, and while not overly desirable from an industry perspective, represents a starting point from which to improve.

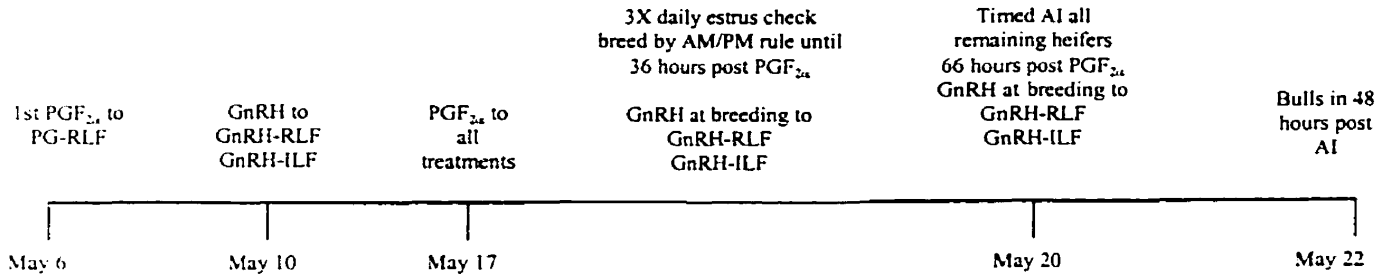

Figure 21. Timeline of procedures used in estrous synchronization treatments.

Table 11. The effects of photoperiod and estrous synchronization method on response to $PGF_{2\alpha}$, pregnancy establishment at AI, and during a 45-day breeding season.

	Photoperiod				Synchronization					
	NP	EP	SE	P-Value	PG-RLF	GnRH-RLF	GnRH-ILF	SE	P-Value	Photoperiod* Synchronization P-Value
Number of heifers	72	72			43	43	58			
Classified as having regular luteal function (%)	48.6	70.8	3.4	**0.007**						
Body weight at AI (kg)	401	407	3.7	0.29	405	403	403	4.5	0.94	0.43
Body condition score at breeding (1-9)	5.5	5.5	0.07	0.46	5.4	5.6	5.6	0.08	0.60	0.74
Body weight, end of breeding season (kg)	417	422	3.5	0.30	420	417	420	4.3	0.75	0.42
Body condition score, end of breeding season (1-9)	4.5	4.5	0.08	0.79	4.4	4.5	4.9	0.09	0.44	0.74
Response rate to $PGF_{2\alpha}$ (%)	50.0	55.6	3.4	0.69	69.8	65.1	31.0	4.5	**0.0002**	0.29
AI conception rate (%)	23.6	23.6	3.2	0.56	32.6	27.9	13.8	4.3	**0.06**	0.44
AI conception rate in responders[a] (%)	33.3	35.0	3.9	0.83	43.3	35.7	16.7	4.2	0.16	0.47
AI pregnancy rate (%)	22.1	22.1	1.7	0.64	29.8	25.5	13.8	4.3	**0.09**	0.44
Cumulative pregnancy rate d 25(%)	77.8	72.2	3.2	0.41	74.4	76.7	74.1	4.3	0.91	0.85
Cumulative pregnancy rate d 45(%)	87.5	87.5	2.8	0.76	79.1	90.7	91.4	3.8	0.14	0.39

[a] number of heifers that conceived to AI as a proportion of responsive heifers, n=40, 36, 30, 28, 18 for EP, NP, PG-RLF, GnRH-RLF, GnRH-ILF respectively.

Table 12. The effects of photoperiod and estrous synchronization method on the classification of heifers that did not respond to synchronization.

	Photoperiod				Synchronization					
	NP	EP	SE	P-Value	PG-RLF	GnRH-RLF	GnRH-ILF	SE	P-Value	Photoperiod* Synchronization P-Value
Number of heifers	72	72			43	43	58			
Non-response rate to $PGF_{2\alpha}$ (%)	50.0	55.4	3.4	0.69	30.2	34.9	69.0	4.5	0.0002	0.29
Classified as acyclic[a] (%)	41.7	18.7	3.8	0.04	0	0	52.5	2.8	0.001	0.44
Classified as asynchronous[b] (%)	44.4	46.9	4.1	0.84	30.8	73.3	40.0	5.6	0.04	0.47
Classified as having incomplete luteolysis[c] (%)	13.9	34.4	3.8	0.04	69.2	26.7	7.5	5.3	0.001	0.44

[a] heifers with low progesterone values at all synchonizing injections were classified as acyclic

[b] heifers not having high progesterone at $PGF_{2\alpha}$ and low progesterone at breeding (but not acyclic) were classified as asynchronous

[c] heifers with high progesterone at $PGF_{2\alpha}$ and high progesterone at breeding were classified as having incomplete luteolysis

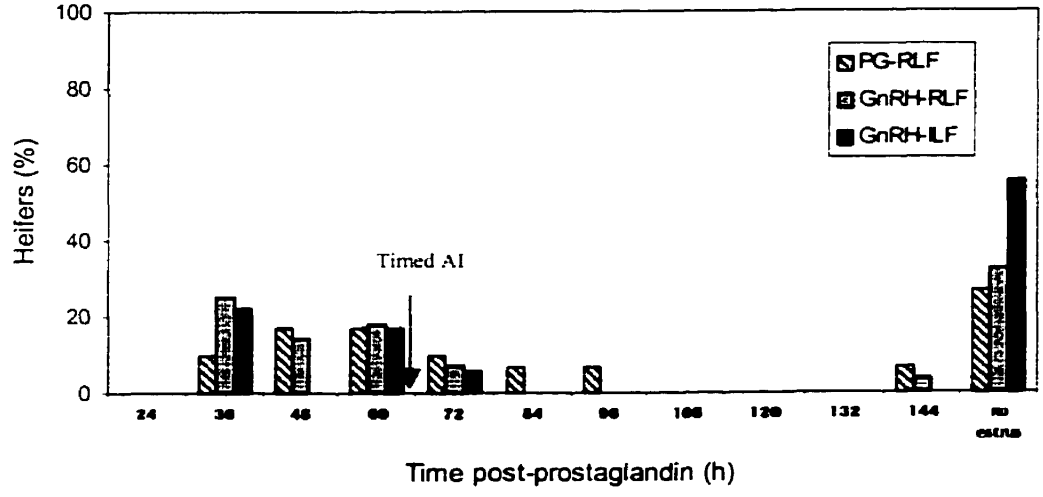

Figure 22. The distribution of estrus in heifers that responded to $PGF_{2\alpha}$ administration before AI. Fewer heifers from the GnRH-ILF method showed heat within a 7 day period, compared to heifers in the GnRH-RLF and the PG-RLF methods (P=0.04, pooled SE=9.6).

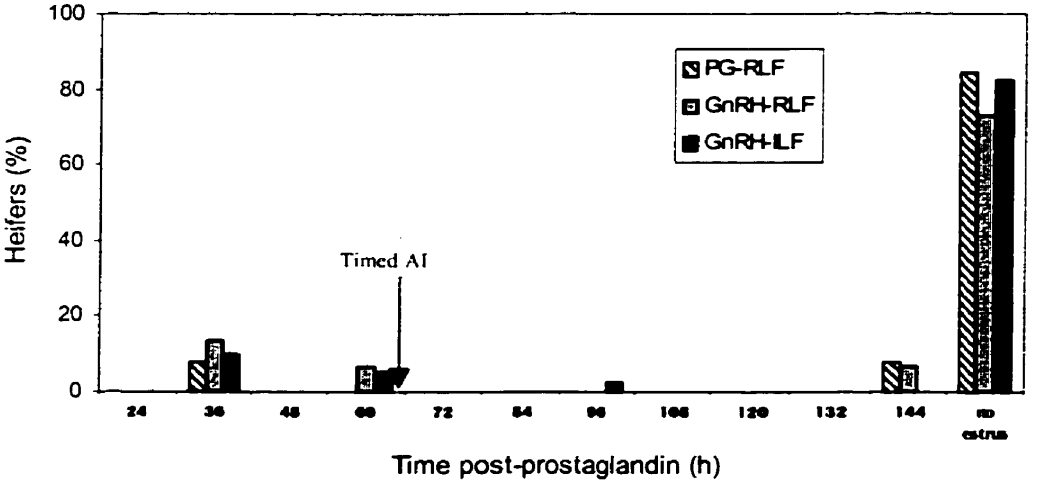

Figure 23. The distribution of estrus in heifers that did not respond to $PGF_{2\alpha}$ administration before AI. The proportion of heifers showing no signs of estrus was similar between synchronization methods (P = 0.69, pooled SE= 6.9)

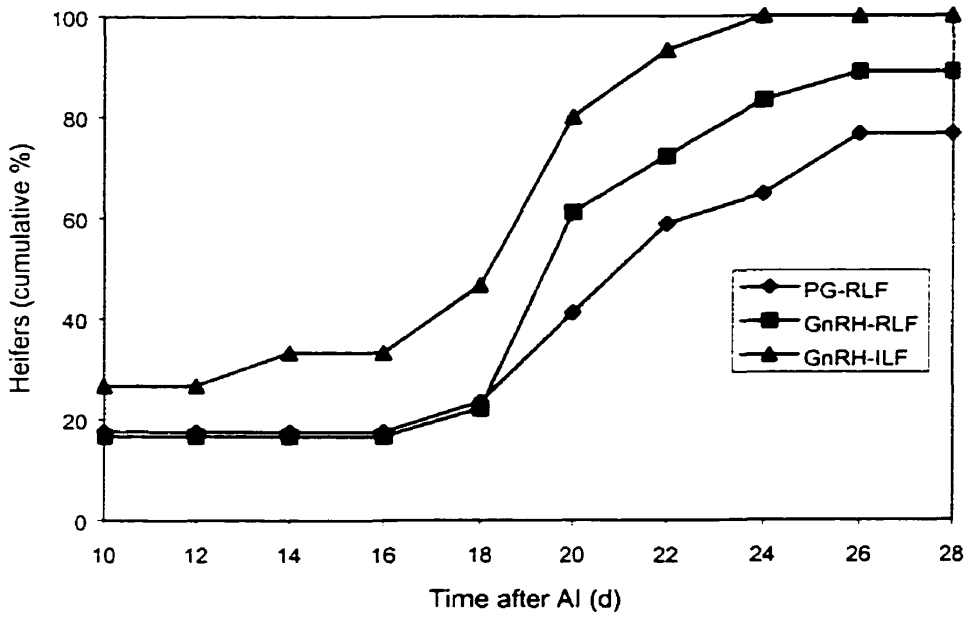

Figure 24. The cumulative percentage of return heats in respondent heifers that did not conceive to timed AI. The proportion of heifers that returned before day 18 was greater (P = 0.05, SE = 5.7) for the GnRH-ILF method, and by day 24 all three synchronization treatments differed (P = 0.03, SE = 4.5).

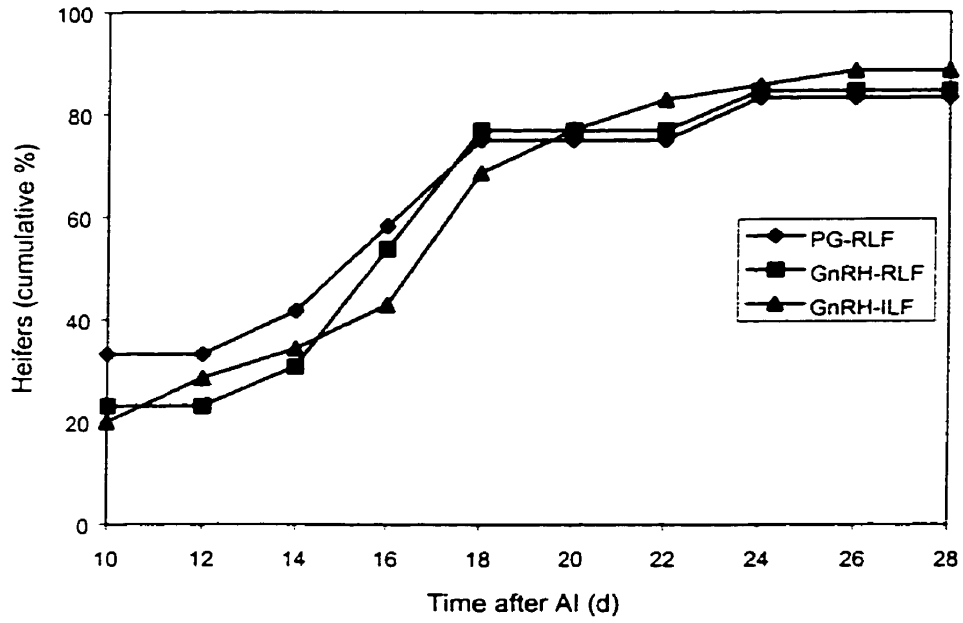

Figure 25. The cumulative percentage of return heats in non-respondent heifers that did not conceive to timed AI. The pattern of return heats was similar at all points ($P > 0.05$, SE = 5.5).

6 MANUSCRIPT III - EFFECTS OF PHOTOPERIOD FROM WEANING UNTIL FIRST BREEDING ON MATERNAL PERFORMANCE OF BEEF HEIFERS

6.1 Abstract

A study was conducted to determine the effects of photoperiod during the post-weaning period (~9-14 months of age) on the subsequent maternal performance of beef heifers. Crossbred heifers (n=144) were assigned to two photoperiod treatments (EP = extended photoperiod, 16 h light day^{-1}, or NP = natural photoperiod) from December until first service artificial insemination (AI) in May, in a completely randomized design. Maternal performance as two-year olds was evaluated in terms of calving date (n=105), milk production and composition during the period of peak lactation (n=32), and calf weight. Calving distribution, age and body weight were similar between photoperiod treatments ($P > 0.05$). Milk production at 6, 8 and 10 weeks was similar between treatments ($P > 0.05$). Milk fat was higher in the NP treatment than in the EP treatment at 10 weeks ($P < 0.05$), and milk protein was similar ($P > 0.05$) between treatments at all milkings. Calf weight did not differ from birth until spring turnout to pasture ($P > 0.05$). The results of this study indicate that exposure to extended photoperiod of 16 h light day^{-1} during the post-weaning period did not affect subsequent maternal performance of beef heifers as compared to heifers reared under natural photoperiod.

6.2 Introduction

The reproductive development of replacement beef heifers is determined by genetic, nutritional and environmental factors. Ideally, management schemes for heifer development attempt to provide the optimum combination of these factors, with the goal of achieving timely onset of puberty in relation to the breeding season. Heifers that calve early as two-year olds generally have higher lifetime productivity than those that calve late (Lesmeister et al. 1973; Marshall et al. 1990). Timely onset of puberty before the breeding season may promote early pregnancy establishment, as fertility was 21% lower in heifers bred on the first estrus than that in heifers bred on the third estrus (Byerley et al. 1987). Photoperiod is one factor in the environment that has been related to the reproductive development of heifers. Past studies have shown that long photoperiods, of approximately 16 hours, may hasten the onset of puberty in comparison to heifers housed under artificial or natural short photoperiods (Hansen et al. 1983; Petitclerc et al. 1984; Ringuet et al. 1994). Very few studies, however, have followed through to measure the effects of photoperiod during rearing on subsequent maternal performance.

One important measure of maternal performance is milk production. The weaning weight of calves of similar age depends mainly on the genetic potential of the calves and the dam's ability to produce milk (McFadden 1991). Exposure to long photoperiods has been shown to increase milk production by 5 to 16% in dairy cows (Chastain and Hiatt 1998). There have been no studies examining the effects of photoperiod on milk production in beef cows. Furthermore, the effects of exposing heifers to long photoperiods on subsequent milk production have not been studied. Petitclerc et al. (1985) found that a 16 hour photoperiod stimulated the growth of mammary parenchyma into the fat pad. Feeding melatonin in the middle of a 16 hour photoperiod to mimic nocturnal melatonin elevation increased the weight of extraparenchymal fat and reduced total mammary parenchymal DNA compared to heifers under 16 hours of light without melatonin feeding (Sanchez–Barcelo et al. 1991).

The objective of this study was to determine the effects of photoperiod, from weaning until first breeding of beef heifers, on subsequent maternal performance as two-year olds. Measures of maternal performance included date and age at calving, milk production at the time of peak lactation and calf performance.

6.3 Materials and Methods

6.3.1 Animals and Treatments

The experiment described in this chapter was part of an ongoing study at the Agriculture & AgriFood Canada Research Station, Brandon, Manitoba. The data presented in this chapter was collected between September, 1999 and May, 2000. All animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Chapter 4 contains a complete description of the housing facilities and lighting design used to achieve the two photoperiod treatments; EP = extended photoperiod of 16 hours total light day^{-1} (natural + artificial), artificial light was 423 lux, 1m above ground and NP=natural photoperiod. The photoperiod treatments began on December 1, 1998 and continued until the day of timed AI on May 20, 1999, after which both treatments received natural photoperiod. The breeding management of the heifers is described in Chapter 5.

Although desirable, due to limited resources it was not possible to keep all bred heifers. Therefore, the number of heifers that were kept and calved out was 105 (52 from EP treatment and 53 from NP treatment).

6.3.2 Pre- and Post-Calving Management

The heifers were brought in from pasture in the fall (November 1999) and wintered in a southern exposed shed and drylot, divided into two pens, with bunk feeders in each pen. Heifers were divided into two pens according to body condition at the time of entering the wintering facility (BCS >=6 'fatter' vs. BCS <6 'thinner') and received two mid-gestation diets, in order to achieve uniform body condition scores at calving. The gestation and post-calving diets are shown in Table 13. During the calving season, heifers were housed outdoors with access to a loose housing shelter, well bedded with straw. Experienced herdspersons monitored calving 24 hours per day. After parturition, the heifer and calf were moved to indoor clean, straw-bedded pens for one to three days

to ensure bonding before turning the pair out to a post-calving shed and drylot. Within 24 hours of birth, calves were weighed, ear-tagged, injected with vitamin E and selenium, and elastrators placed on male calves for castration.

Heifer body weight was determined at the beginning, and middle of the overwintering period, and within 24 h of calving. Calves were weighed within 24 h of birth, at the time of each milk collection, and at turnout to pasture (June 1, 2000).

6.3.3 Measurement of Milk Production

A representative subsample of 32 heifers, 16 from each photoperiod treatment, was selected for determination of milk production. Heifers were selected based on calving date, as it was desirable to have all heifers at the same stage of lactation (i.e. similar number of days post-calving). Milk production was determined at six, eight and ten weeks from the mean calving date of February 25, 2000.

In the morning of each milk collection, calves were weighed and separated from their mothers. Heifers were milked in pairs, one from each photoperiod treatment, using intramuscular injection of 3 cc oxytocin (MTC Pharmaceuticals) to stimulate milk let down. Milk collection was performed using an automated milking claw and pressurized container. After a separation time from their calves of 8 h, heifers were milked a second time, again using intramuscular injection of 3 cc oxytocin (MTC Pharmaceuticals) to stimulate milk let down. The milk from the second collection was weighed, and a subsample taken from each heifer for composition analysis. Samples were sent to MFC Testing and Research Inc, Winnipeg, Manitoba for determination of fat, protein and non-fat solids. Milk weight was corrected to a theoretical 24-h milk production for each heifer.

6.3.4 Statistical Analysis

Statistical analysis of the data was performed using Statistical Analysis System v 6.12 (SAS 1996). Data for body weight, age and date of calving, and milk yield and composition were analyzed using the general linear models procedure of SAS with

type III sums of squares. Categorical models (CATMOD) procedures were used to test differences in proportions.

The standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{error mean square}/n)}$$

n = number of observations / treatment

For proportion data, the standard error of the difference between means was calculated using the formula (Snedecor and Cochran 1980):

$$SE = \sqrt{(\text{sum of variances})}$$

where variance = $(r/n)*(1-(r/n))/n$

n = number of observations/treatment and r = the number responding

6.4 Results

The distribution of calving was similar in the EP and NP treatments throughout the calving season ($P > 0.05$, Figure 26). There were no effects of photoperiod on day, age or weight at calving ($P > 0.05$, Table 14). Milk production was similar ($P > 0.05$) between treatments at 6, 8 and 10 weeks (Table 14). Milk protein content was similar ($P > 0.05$) at all three milk collection periods. Milk fat content was higher ($P = 0.02$) in the NP than the EP treatment at 10 weeks. Calf weight was similar ($P > 0.05$) at each milk collection and at turnout to pasture in the spring.

6.5 Discussion

The similarity in the distribution of calving was not unexpected, as the establishment of pregnancy during the breeding season was very uniform between photoperiod treatments (Chapter 5). The average age at calving in both treatments was

approximately 711 days, which is a desirable age in terms of maximizing lifetime productivity. The similarity in body weight at calving was also not surprising, as both treatments maintained similar body weights throughout the post-weaning and breeding periods (Chapters 4 and 5).

The daily milk production did not differ at 6, 8 or 10 weeks post-calving. This time frame represents peak lactation, thus it was postulated that differences in milking ability should be evident at this time. The peak milk production of lactating cows is quite variable, but typically ranges from 5 to 14 kg day^{-1} for cows typical of beef production enterprises (National Research Council 1996). The peak milk production of two-year olds is generally 25% lower than that of mature cows (National Research Council 1996), therefore the range is about 3.75 to 10.5 kg day^{-1}. The milk production of the heifers in the current study fell well within this range, at about 8 kg day^{-1}. Buskirk et al. (1996) reported similar peak values, of 7 to 8 kg day^{-1} for crossbred beef heifers reared on a moderate plane of nutrition. Milk composition in the present study was also well within the expected range reported by the National Research Council (1996). In 18 studies, milk fat averaged 4.03 ± 1.24% and in 10 studies, milk protein averaged 3.38 ± 0.27 (National Research Council 1996). In the present study, milk fat averaged 4.33% and protein 3.65%.

Provision of long photoperiods during lactation has been shown to increase milk production by 5 to 16% in dairy cattle (Chastain and Hiatt 1998), however there have been no studies that examined the effects of exposure to long photoperiods as calves, on subsequent milking ability. Extended photoperiod has been reported to stimulate growth of the mammary parenchyma into the fat pad in Holstein heifers (Petitclerc et al. 1985). Sanchez-Barcelo et al. (1991) examined mammary development in prepubertal Holstein heifers exposed either to 16 hour photoperiod, or the combination of 16 hour photoperiod with melatonin feeding to simulate the nocturnal increase in melatonin secretion. Extraparenchymal fat pad weight was 43% higher, and parenchymal DNA content 24% lower in melatonin fed heifers. Serum prolactin concentration was lower in melatonin fed heifers. Sanchez-Barcelo et al. (1991) postulated that melatonin may be the mediator of the inhibitory effect of short day length on mammary growth, with the melatonin-induced suppression of mammary parenchymal development being mediated by increased lipid

accumulation. Prolactin may also mediate the effects of photoperiod on mammary development, as prolactin stimulates parenchymal growth either directly or in cooperation with steroid hormones (Topper et al. 1980 and Muldoon 1987, as cited by Sanchez-Barcelo et al. 1991).

The similar milking ability may be attributed to the timing of photoperiod treatments in relation to the development of the mammary gland in heifers. The mammary gland undergoes two distinct phases of development in preparation for lactation. The first is growth of mammary cells, and the second is differentiation of these mammary cells into secretory cells (McFadden 1991). The growth phase occurs in two distinct stages, beginning at birth. For the first two to three months after birth, the udder grows isometrically, or at the same rate as the rest of the body (Tucker 1981). Beginning at about three months of age and continuing until about nine months of age, the udder enters a phase of allometric growth, in which mammary cell growth proceeds at a much more rapid rate than that of the rest of the body (Tucker 1981). From nine until fifteen months, mammary growth returns to isometric rate, until gestation when another allometric phase begins (Tucker 1981). The majority of growth occurs during gestation, but depends upon the foundation of cells provided by the earlier phase of allometric growth (McFadden 1991). Mammary cell differentiation occurs around the time of parturition, in response to hormonal cues accompanying pregnancy and parturition (Tucker 1981).

In the present study, photoperiod treatments began when heifers were approaching nine months of age and continued until approximately fifteen months of age. This time period coincides with the previously described isometric growth phase of the mammary gland. Petitclerc et al. (1984) reported that long photoperiod (16 hours) had no effect on the development and composition of mammary tissue of Holstein heifers. The authors did not provide a complete description of the age of the heifers during photoperiod treatments, but stated that heifers were slaughtered for mammary dissection after two estrous cycles. Thus, it is logical to assume that heifers were likely in the phase of isometric growth during photoperiod treatments. The negative effects of overfeeding on mammary development have been reported to be most critical during the first phase of allometric growth (Johnsson and Obst 1984). Creep feeding as calves has been shown to

decrease subsequent calf production of beef heifers (Martin et al. 1981). As previously discussed, the effects of photoperiod on mammary development may be mediated through both melatonin-induced increase in mammary fat deposition and prolactin mediated stimulation of parenchymal development (Sanchez-Barcelo et al. 1991). Perhaps, the mammary gland is most sensitive to the effects of photoperiod during the allometric growth phase, when the effects of over-feeding are also most critical.

Milk composition was similar between photoperiod treatments, with the exception of higher milk fat content at 10 weeks in the NP compared to the EP treatment ($P = 0.02$). The higher milk fat was not related to an improvement in calf performance at the time of milking, or at turn out to pasture. Rahnefeld et al. (1990) reported that milk yield was the most important predictor of calf growth, and that variation in milk constituents (fat, protein, non-fat solids) accounted for a very small proportion (less than 1.8%) of calf growth rate. Gleddie and Berg (1968), Butson et al. (1980) and Mondragon et al. (1983) also reported a negligible influence of differences in milk components on calf growth. Milk production, therefore, appears to be the main predictor of calf performance, but the differences in milk fat synthesis may represent differences in mammary function and should not be overlooked in future studies.

6.6 Conclusion

Exposure of beef heifers to extended or natural photoperiod from weaning until first breeding did not result in differences in maternal ability as two-year olds. Both groups of heifers calved at a desirable age in terms of lifetime productivity. Milk production during peak lactation was similar between treatments, and this was reflected in similar calf performance. To abandon future study of the effects of photoperiod on milking ability would be premature, as in the present study the photoperiod treatments may not have been appropriately timed to influence the development and subsequent function of the mammary gland. Previous research has demonstrated that photoperiod does indeed have the potential to affect mammary development. Additionally, milk production is increased in dairy cows exposed to extended photoperiods during lactation.

Thus it seems that the absence of photoperiod induced changes in milking ability in the present study may be attributed to improper timing of treatments, rather than the absence of a relationship between photoperiod and mammary development and function. An interesting avenue for future research would be one in which the effects of timing of photoperiod treatments, such as before 9 months of age, or at the time of calving, are related to milk production of beef females.

Table 13. Composition[a] of diets fed to bred heifers.

	Mid-Gestation		Pre-Calving	Post-Calving
Item	**Fats**	**Thins**		
Barley Silage	20	30	15.0	16.0
Reed Canary Grass Hay	Free Choice	0	0	0
Alfalfa/Grass Hay	0	Free Choice	7.0	6.0
Barley Grain	0	0	0	2.0
Pre-Calving Pellet	0	0	2.0	0
Post-Calving Pellet	0	0	0	2.0

[a] Barley silage (35.82% DM) contained on a g kg^{-1} DM basis: crude protein 120, ADF 298, P 3.4, Ca 3.4, K 14.8, Mg 1.7 and 5.8 MJ kg^{-1} DE. Reed Canary Grass hay (84% DM) contained on a g kg^{-1} DM basis: crude protein 51, ADF 454, P 16, Ca 2.4, K 15.4, Mg 1.2 and 9.24 MJ kg^{-1} DE. Alfalfa/grass hay (87.4% DM) contained on a g kg^{-1} DM basis: crude protein 93, ADF 434, P 2.3, Ca 7.5, K 16.5, Mg 1.6 and 9.5 MJ kg^{-1} DE. The pre-calving pelleted supplement contained per MT crushed barley (954.5 kg), custom vitamin mineral mix (28 kg containing, Ca 155, P 155, Mg 120, S 100, Cl 100, I 0.2, Cu 4.0, Mn 5.0, Co 0.05, Zn 8, Se .035 g kg^{-1}, white salt 325 g kg^{-1}, Rumensin 28 kg MT^{-1} containing 200 g Monensin kg^{-1}, and Vitamins A 1,000,000, E 6000 IU kg^{-1}), white salt (7.5 kg), cane molasses (5.0 kg) and vegetable oil (5.0 kg). The post-calving pelleted supplement contained per MT crushed barley (947 kg), custom vitamin mineral mix (28 kg containing, Ca 400, Mg 120, S 100, Na 100, Cl 100, I 0.2, Cu 4.0, Mn 5.0, Co 0.05, Zn 8, Se .035 g kg^{-1}, white salt 135 g kg^{-1}, Rumensin 28 kg MT^{-1} containing 200 g Monensin kg^{-1}, and Vitamins A 1,300,000, E 6000 IU kg^{-1}), white salt (15 kg), cane molasses (5.0 kg) and vegetable oil (5.0 kg).

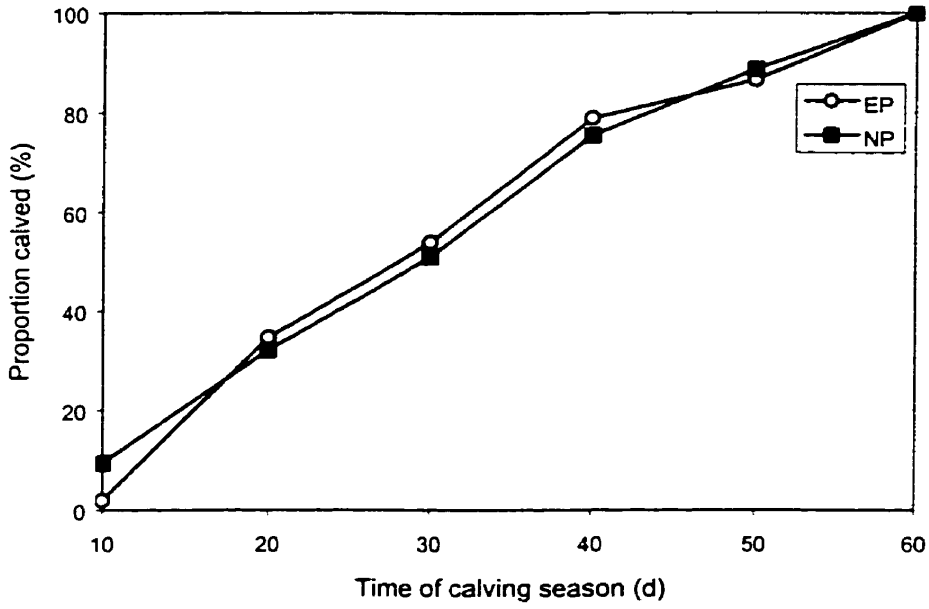

Figure 26. The effects of photoperiod from weaning until first breeding, on the cumulative proportion of heifers calved during the two-year old calving season. Day 0 is 15 days before the predicted 285 d AI calving date of February 27, 2000. The cumulative proportion calved was similar between treatments at all time points ($P > 0.05$, pooled SE=7.3).

Table 14. The effects of photoperiod from weaning until first breeding of beef heifers, on maternal performance as two-year olds.

	Photoperiod Treatment			
	EP	NP	SE	P-value
Age at Calving (d)	711.8	711.0	2.9	0.85
Calving Date (d) [a]	28.7	28.9	2.0	0.95
Calving Weight (kg) [b]	533.7	537.3	3.2	0.44
6 Week Milk Production				
Milk Yield (kg d^{-1})	8.03	7.96	0.59	0.93
Fat Content (%)	4.62	4.64	0.22	0.95
Protein Content (%)	3.79	3.65	0.14	0.48
Calf Weight (kg)[c]	73.4	74.7	2.0	0.65
8 Week Milk Production				
Milk Yield (kg d^{-1})	7.49	7.34	0.41	0.80
Fat Content (%)	4.01	4.29	0.15	0.21
Protein Content (%)	3.78	3.76	0.17	0.93
Calf Weight (kg)	82.4	82.8	2.0	0.89
10 Week Milk Production				
Milk Yield (kg d^{-1})	7.88	8.00	0.37	0.81
Fat Content (%)	3.93	4.46	0.15	0.02
Protein Content (%)	3.52	3.39	0.11	0.40
Calf Weight (kg)	98.4	97.2	2.1	0.70
Calf on Pasture Weight (kg)	118.9	116.4	2.5	0.48

[a]calving date is the number days from February 12, 2000, which is 15 days before the predicted 285 d AI calving date of February 27, 2000.
[b]calving weight adjusted for body weight of the heifer at the beginning of the wintering period.
[c]calf weight adjusted for birth weight for all milking days and for on pasture weight.

7 GENERAL DISCUSSION AND CONCLUSIONS

This study is unique in that it is one of the first to examine the effects of photoperiod on not only pubertal development, but also subsequent reproductive and maternal performance of beef heifers. Although the advancement of sexual maturity with extended photoperiod did not translate into robust differences in reproductive or maternal performance, there are certainly many avenues for future research that may lead to improvements in these areas. Improved understanding of the mechanism whereby photoperiod influences puberty is necessary. Also, further research is needed in the area of estrous synchronization for timed artificial insemination of beef heifers. Better coordinating the exposure to extended photoperiod with the critical period of mammary development may affect milk production ability. Because the results of this study indicate that manipulation of photoperiod may be effective in stimulating reproductive development, further study is required to determine the most appropriate and economical lighting conditions for practical implementation.

Under current beef production systems, most replacement heifers experience the shortest photoperiods of the year in the months immediately preceding puberty. Results of this, and past research indicates that these conditions are not ideal for reproductive development, as in this study 22% more heifers exposed to extended photoperiod reached puberty before the breeding season than did heifers exposed to winter conditions typical of most of Canada. Obviously raising heifers in conditions that undermine the provision of optimum nutrition and selection of desirable genetics is undesirable, and represents an area where progress can be made. Expanding upon existing knowledge of the relationship between environment, nutrition, genetics and reproductive development is one important step in reaching the goal of improved reproductive efficiency of the beef cow.

8 LITERATURE CITED

Adams, G.P., Evans, A.C.O. and Rawlings, N.C. 1994. Follicular waves and circulating gonadotrophins in 8-month-old prepubertal heifers. J. Reprod. Fertil. **100**: 27 – 33.

Advis, J.P. and Ojeda, S.R. 1978. Hyperprolactinemia – induced precocious puberty in the female rat: Ovarian site of action. Endocrinology **103**: 924-935.

Alberta Agriculture, Food and Rural Development. 1995. Feeding and handling facilities. pp. 880-1 – 811-2 *in* Beef Herd Management Reference Binder and Study Guide. Alberta Agriculture, Food and Rural Development. Edmonton, Alberta, Canada.

Baldwin, B.A. and Start, I.B. 1981. Sensory reinforcement and illumination preference in sheep and calves. Proc. R. Soc. London, Ser. B **211**: 513-526.

Beal, W.E. 1996. Application of knowledge about corpus luteum function in control of estrus and ovulation in cattle. Theriogenology **45**:1399-1411.

Berardinelli, J.G., Godfrey, R.W., Adair, R., Lunstra, D.D., Byerley, D.J., Cardenas, H. and Randel, R.D. 1992. Cortisol and prolactin concentrations during three different seasons in relocated brahman and hereford bulls. Theriogenology. **37**: 641 – 654.

Berardinelli, J.G., Fogwell, R.L. and Inskeep, E.K., 1978. Effect of electrical stimulation or presence of a bull on puberty in beef heifers. Theriogenology. **9**: 133-141.

Bo, G.A., Adams, G.P., Person, R.A. and Mapletoft, R.J. 1995. Exogenous control of follicular wave emergence in cattle. Theriogenology. **43**:31-40.

Bourne, R.A. and Tucker, H.A. 1975. Serum prolactin and LH responses to photoperiod in bull calves. Endocrinology **97**: 473-475.

Buskirk, D.D., Faulkner, D.B., Hurley, W.L., Kesler, D.J., Ireland, F.A., Nash, T.G., Castree, J.C. and Vicini, J.L. 1996. Growth, reproductive performance, mammary development, and milk production of beef heifers as influenced by prepubertal dietary energy and administration of bovine somatotropin. J. Anim. Sci. **74**: 2649 – 2662.

Byerley, D.J., Staigmiller, R.B., Berardinelli, J.G. and Short, R.E. 1987. Pregnancy rates of beef heifers bred either on puberal or third estrus. J. Anim. Sci. **65**: 645-650.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Volume 1, Second Edition. Canadian Council on Animal Care, Ottawa, Ont.

Cardinalli, D.P., Vacas, M.I. and Boyer, E.E. 1979. Specific binding of melatonin in bovine brain. Endocrinology **105**: 437-441.

Chastain, J.P and Hiatt, R.S. 1998. Supplemental lighting for improved milk production. Electric Power Research Institute, Inc. National Food and Energy Council. Columbia, MO, USA.

Clanton, D.C., Jones, L.E. and England, M.E. 1983. Effect of rate and time of gain after weaning on the development of replacement beef heifers. J. Anim. Sci. **56**: 280-285.

Crister, J.K., Block., T.M., Kirkpactrick, B.W., Lindstrom, M.J. and Hauser, E.R. 1988. The effect of photoperiod on diurnal rhythms of serum gonadotrophins, prolactin and melatonin in ovariectomized heifers. Domes. Anim. Endocrin. **5**: 23-34.

Curtis, S.E. 1983. Control and integration of thermoregulatory processes. pp 6.1 – 6.7 in Environmental Management in Animal Agriculture. The Iowa State University Press. Ames, Iowa, USA.

Dahl, G.E. 1998. Controlling barn light makes more milk. Hoard's Dairyman. W.D. Knoz (ed). W.D. Hoard's and Sons Co., Fort Atkinson, Wis. Vol 143, No. 17, p. 683.

Davis, J.S., May, J.V., and Keel, B.A. 1996. Mechanisms of hormone and growth factor action in the bovine corpus luteum. Theriogenology **45**:1351-1380.

Day, M.L., Imakawa, K., Zalesky, D.D., Kittok, R.J. and Kinder, J.E. 1986. Effects of restriction of dietary energy intake during the peripubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J. Anim. Sci. **62**: 1641-1648.

Del Vecchio, Sutherland, W.D. and Conner, L.M. 1995. A solid phase enzyme-immunoassay for progesterone determination in plasma. Can. J. Anim. Sci. **75**: 525-529.

Del Vecchio, R.P., Neuendorff, D.A., Stahringer, R.C. and Randel, R.D. 1992. Concentrations of 13.14-dihydro-15-deto-prostoglandin $F_2\alpha$, estradiol-17β and progesterone during the peripubertal period in heifers. Theriogenology. **38**: 419-429.

Dickerson, G.E. 1973. Inbreeding and heterosis in animals. pp. 54-77. *in*: Proc. Anim. Breed. and Genet. Symp. in Honor of Dr. jay L. Lush. Am. Soc. of Anim. Sci., Champaign, Ill. U.S.A.

Dickerson, G.E. 1978. Animal size and efficiency: Basic concepts. Anim. Prod. **27**:367-368.

Diven, D. 1995. Allow nature to supply nutrition. pp. 28-32 *in* Proc. Total Ranch Management in the Northern Great Plains. Swift Current, Canada.

Eckles, C.H. 1915. The ration and age of calving as factors influencing the growth and dairy qualities of cows. Mo. Agr. Exp. Sta. Bull. **135**.

Foote, R.H. 1996. Review: Dairy cattle reproduction physiology research and management-Past progress and future prospects. J. Dairy. Sci. **79**:980-990.

Forbes, J.M. 1982. Effects of lighting pattern on growth, lactation and food intake of sheep, cattle and deer. Livest. Prod. Sci. **9**: 361-374.

Fox, D.G., Sniffen, C.J. and O'Conner, J.D. 1988. Adjusting nutrient requirements of beef cattle for animal and environmental variations. J. Anim. Sci. **66**: 1475-1495.

Gleddie, V.M. and Berg, R.T. 1968. Milk production in range beef cows and its relationship to calf gains. Can. J. Anim. Sci. **48**: 323-333.

Goehring, T. 1991. Hitting the target with heifer development. pp 50-59 *in* Investing in the Future, Replacement Heifer Management. Elanco Animal Health, Indianapolis, Indiana.

Gregory, K.E., Lunstra, D.D., Cundiff, L.V. and Koch, R.M. 1991. Breed effects and heterosis in advanced generations of composite populations for puberty and scrotal traits of beef cattle. J. Anim. Sci. **69**: 2795-2807.

Greve, T., Callesen, H., Hytell, P., Hoier, R. and Assey, R. 1995. The effect of exogenous gonadotropins on oocyte and embryo quality in cattle. Theriogenology. **43**: 41-50.

Hall, J.B., DiCostanzo, A., Woodward, B. and Brown, D. 1997. Body weight and reproductive response to step gain feeding for replacement beef heifers. J. Anim. Sci. **75**(suppl.): 41.

Hall, J.B., Schillo, K.K., Fitsgerald, B.P. and Bradley, N.W. 1994. Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development, and onset of puberty in beef heifers. J. Anim. Sci. **72**: 709-718.

Hansen, P.J. 1985. Seasonal modulation of puberty and the postpartum anestrus in cattle: a review. Livest. Prod. Sci. **12**: 309-327.

Hansen, P.J., Kamwanja, L.A. and Hauser, E.R. 1983. Photoperiod influences age at puberty of heifers. J. Anim. Sci. **57**: 985-992.

Hare, I. and Bryant, M.J. 1985. Ovulation rate and embryo survival in young ewes mated at either puberty or at the second or third oestrus. Anim. Reprod. Sci. **8**: 41.

Izard, M.K. and Vandenbergh, J.G. 1982. The effects of bull urine on puberty and calving date in crossbred beef heifers. J. Anim. Sci. **55**: 1160-1168.

Johnsson, I.D. and Obst, J.M. 1984. The effects of level of nutrition before and after 8 months of age on subsequent milk and calf production of beef heifers over three lactations. Anim. Prod. **38**: 57-68.

Kavanau, J.L. 1962. Twilight transitions and biological rhythmicity. Nature **194**: 1293.

King, G. 1993. Reproductive Efficiency. pp 239-253 *in* Martin, J., Hudson, R.J. and Young, B.A. Animal Production in Canada. University of Alberta. Edmonton, Alberta, Canada.

Larson, R.L., Corah, L.R. and Peters, C.W. 1996. Synchronization of estrus in yearling beef heifers with the melengesterol acetate/ prostaglandin $F_{2\alpha}$.system: Efficiency of timed insemination 72 hours after prostaglandin treatment. Theriogenology. **45**:851-863.

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

Lesmeister, J.L., Burfening, P.J. and Blackwell, R.L. 1973. Date of first calving in beef cows and subsequent calf reproduction. J. Anim. Sci. **36**: 1-6.

MacMillan, K.L., Allison, A.J. and Struthers, G.A. 1979. Some effects of running bulls with suckling cows or heifers during the premating period. N.Z. J. Exp. Agric. **7**: 121.

Marshall, D.M., Mingiang, W. and Freking, B.A. 1990. Relative calving date of first-calf heifers as related to production efficiency and subsequent reproductive performance. J. Anim. Sci. **68**: 1812-1817.

Martin, G.B., Oldham, C.M. and Lindsay, D.R. 1980. Increased plasma LH levels in seasonally anovular Merino ewes following the introduction of rams. Anim. Reprod. Sci. **3**: 125-132.

Martin, L.C., Brinks, J.S., Bourdon, R.M. and Cundiff, L.V. 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J. Anim. Sci. **70**: 4006-4017.

Martin, T.G., Lemenager, R.P., Srinivasan, G. and Alenda, R. 1981. Creep feed as a factor influencing performance of cows and calves. J. Anim. Sci. **53**: 33-39.

McFadden, T.B. 1991. Effects of growth rate on mammary development and milk production of replacement beef heifers. pp 43-49 *in* Investing in the Future, Replacement Heifer Management. Elanco Animal Health, Indianapolis, Indiana.

Melton, B.E. 1995. Conception to consumption: The economics of genetic improvement. pp 40-71. Proc. Beef Improvement Federation 27th Research Symposium and Annual Meeting, Sheridan, WY.

Menge, A.C., Mares, S.E., Tyler, W.J. and Casida, L.E. 1960. Some factors affecting age at first puberty and the first 90 days of lactation in Holstein heifers. J. Dairy Sci. **43**: 1099-1107.

Mondragon, I., Wilton, J.W., Allen, O.B. and Song, H. 1983. Stage of lactation effects, repeatabilities and influences on weaning weights of yield and composition of milk in beef cattle. Can. J. Anim. Sci. **63:** 751-761.

Moreira, F., de la Sota, R.L., Diaz, T. and Thatcher, W.W. 2000. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive response in dairy heifers. J. Anim. Sci. **78**: 1568 – 1576.

Muldoon, T.G. 1987. Prolactin mediation of estrogen-induced changes in mammary tissue estrogen and progesterone receptors. Endocrinolgy **121**: 141 – 149.

National Research Council. 1996. Nutrient requirements of beef cattle – seventh revised edition. National Academy Press. Washington, D.C.

Nelson, T.C., Short, R.E., Phelps, D.A. and Stagmiller, R.B. 1985. Nonpubertal estrus and mature cow influences on growth and puberty in heifers. J. Anim. Sci. **61**: 470 – 473.

Nicoll, C.S. 1980. Ontogeny and evolution of prolactin's function. Fed. Proc. **39**: 2563.

Odde, K.G. 1990. A review of synchronization of estrus in postpartum cattle. J. Anim. Sci. **68**:817-830.

Pate, J.L. 1994. Cellular components involved in luteolysis. J. Anim. Sci. **72**:1884-1890.

Patterson, D.J., Perry, R.C., Kiracofe, G.H., Bellows, R.A., Staigmiller, R.B. and Corah, L.R. 1992. Management considerations in heifer development and puberty. J. Anim. Sci. **70**: 4018-4035.

Peters, R.R, Chapin, L.T, Emery, R.S. and Tucker, H.A. 1980. Growth and hormonal response of heifers to various photoperiods. J. Anim. Sci. **51**: 1148-1153.

Peters, R.R. and Tucker, H.A. 1978. Prolactin and growth hormone responses to photoperiod in heifers. Endocrinology **103**: 229-234.

Peters, R.R., Chapin, L.T., Emery, R.S. and Tucker, H.A. 1981. Milk yield, feed intake, prolactin, growth hormone, and glucocorticoid response of cows to supplemental light. J. Dairy Sci. **64**: 1671-1678.

Peters, R.R., Chapin, L.T., Leining, K.B. and Tucker, H.A. 1978. Supplemental lighting stimulates growth and lactation in dairy cattle. Science **199**: 911-912.

Petitclerc, D., Chapin, L.T. and Tucker, H.A. 1984. Carcass composition and mammary development responses to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. **58**: 913-919.

Petitclerc, D., Chapin, L.T., Emery, R.S. and Tucker, H.A. 1983. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in Holstein heifers. J. Anim. Sci. **57**: 892-898.

Petitclerc, D., Kineman, R.D., Zinn, S.A. and Tucker, H.A. 1985. Mammary growth response of Holstein heifers to photoperiod. J. Dairy Sci. **68**: 86-90.

Phillips, C.J.C., Johnson, P.N. and Arab, T.M. 1997. The effect of supplementary light during winter on the growth, body composition and behaviour of steers and heifers. Anim. Sci. **65**: 173-181.

Pursley, J.R., Mee, M.O., and Wiltbank, M.C. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology. 44: 915 – 923.

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

Rahnefeld, G.W., Weiss, G.M. and Fredeen, H.T. 1990. Milk yield and composition in beef cows and their effect on cow and calf performance in two environments. Can. J. Anim. Sci. **70**: 409-423.

Rao, C.V., Estergreen, V.L., Carman, F.R. Jr., and Moss, G.E. 1979. Receptors for gonadotrophin and prostaglandin $F_{2\alpha}$ in bovine corpora lutea of early, mid and late luteal phase. Acta. Endocrinol. **91**:529.

Reksen, O., Tverdal, A., Landsverk, K., Kommisrud, E., Boe, K.E. and Ropstad, E. 1999. Effects of photointensity an photoperiod on milk yield and reproductive performance of Norweigan Red cattle. J. Dairy Sci. **82**: 810-816.

Ringuet, H., Pelletier, G., Brazeau, P., Gaudreau, P., Guilbault, L.A., Morisset, J., Couture, Y. and Petitclerc., D. 1994. Long-term effects of human growth hormone-releasing hormone and photoperiod on hormone release and puberty in dairy heifers. J. Anim. Sci. **72**: 2709-2717.

Roberson, M.S., Ansotequi, R.P., Berardinelli, J.G., Whitman, R.W. and McInerney, M.J. 1987. Influence of biostimulation by mature bulls on occurrence of puberty in beef heifers. J. Anim. Sci. **64**: 1601-1605.

Roberson, M.S., Wolfe, M.W., Stumpf, T.T., Werth, L.A., Cupp, A.S., Kojima, N., Wolfe, P.L., Kittok, R.J. and Kinder, J.E. 1991. Influence of growth rate and exposure to bulls on age at puberty in beef heifers. J. Anim. Sci. **69**: 2092-2098.

Robertson, G.L., Grummer, R.H., Casida, L.E. and Chapman, A.B. 1951. Age at puberty and related phenomena in outbred Chester White and Poland China gilts. J. Anim. Sci. **10**: 647.

Roy, J.H.B., Gillies, C.M., Pefitt, M.W. and Stobo, I.J.F. 1980. Effect of season of year and phase of the moon on puberty and the occurrence of oestrus and conception in dairy heifers reared on high planes of nutrition. Anim. Prod. **31**: 13-26.

Rutter, L.M. and Randel, R.D. 1986. Nonpubertal estrus in beef heifers. J. Anim. Sci. **63**: 1049 – 1053.

Sanchez-Barcelo, E.J., Mediavilla, M.D., Zinn, S.A., Buchanan, B.A., Chapin, I.T. and Tucker, H.A. 1991. Melatonin suppression of mammary growth in heifers. Biol. Reprod. **44**: 875 – 879.

SAS, Inc. 1996. SAS/STAT user's guide (Release 6.12). SAS Institute, Inc., Carry, NC.

Schams, D., Shallenberger, E., Gombe, S. and Karg, H. 1981. Endocrine patterns associated with puberty in male and female cattle. J. Reprod. Fertil. **30**: 103-110.

Schillo, K.K., Dierschke, D.J. and Hauser, E.R. 1982. Regulation of luteinizing hormone secretion in prepubertal heifers: Increased threshold to negative feedback action of estradiol. J. Anim. Sci. **54**: 325-336.

Schillo, K.K., Hall, J.B. and Hileman, S.M. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Anim. Sci. **70**: 3994-4005.

Schillo, K.K., Hansen, P.J., Kamwanja, L.A., Dierschke, D.J. and Hauser, E.R. 1983. Influence of season sexual development in heifers: age at puberty as related to growth and serum concentrations of gonadotropins, prolactin, thyroxine, and progesterone. Biol. Reprod. **28**: 329-341.

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

Schmitt, E.J.P., Diaz, T.C., Drost, M. and Thatcher, W.W. 1994. Use of a GnRH agonist for a timed insemination protocol in cattle. J. Anim. Sci. **72**: 292 (Suppl.).

Seidel, G.E. Jr., Larson, L.L. and Foote, R.H. 1971. Effects of age and gonadotropin treatment on superovulation in the calf. J. Anim. Sci. **33**: 617-622.

Short, R.E. and Bellows, R.A. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J. Anim. Sci. **32**: 127-131.

Short, R.V. 1984. Estrous and menstrual cycles. pp. 137-141. *in*: Austin, C.R. and Short, R.V. (eds.). Hormonal control of reproduction.. Cambridge University Press, Cambridge, U.K.

Skarzynski, D.J., Bogacki, M., and Kotwica, J. 1997. Changes in ovarian oxytocin secretion as an indicator of corpus luteum response to prostaglandin $F_{2\alpha}$ treatment in cattle. Theriogenology **48**:733-742.

Small, J.A. and McCaughey, W.P. 1999. Beef cattle management in Manitoba. Can. J. Anim. Sci. **79**: 539-544.

Smith, V.G., Hacker, R.R. and Brown, R.G. 1977. Effect of alterations in ambient temperature on serum prolactin concentration in steers. J. Anim. Sci. **44**: 645 – 649.

Snedecor, G. W. and Cochran, W.G. 1980. Statistical methods – seventh edition. The Iowa State University Press. Ames. Iowa, USA.

Stanisiewski, E.P., Chapin, L.T. and Tucker, H.A. 1984. The effect of lamps with different spectral properties on prolactin release in prepubertal bulls. Proc. Soc. Exp. Biol. Med. **175**: 226-232.

Stanisiewski, E.P., Chapin, L.T. Ames, N.K., Zinn, S.A. and Tucker, H.A. 1988. Melatonin and prolactin concentrations in blood of cattle exposed to 8, 16 or 24 hours of daily light. J. Anim. Sci. **66**: 727 – 734.

Stephens, L.A. and Rajamahendran, R. 1998. A comparison of two estrus synchronization methods in beef heifers. Can. J. Anim. Sci. **78**:437-439.

Stevenson, J.S., Smith, M.W., Jaeger, J.R., Corah, L.R. and LeFever, D.G. 1996. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. J. Anim. Sci. **74**:729-735.

Tanabe, T.Y. and Hann, R.C. 1984. Synchronized estrus and subsequent conception in dairy heifers treated with prostaglandin $F_{2\alpha}$. 1. Influence of stage of cycle at treatment. J. Anim. Sci. **58**:804-811.

Topper, Y.J. and Freeman, C.S. 1980. Multiple hormone interactions in the developmental biology of the mammary gland. Physiol. Rev. **60**: 1049-1106.

Tucker, H.A. 1971. Hormonal response to milking. J. Anim. Sci. **32**: 137-141.

Tucker, H.A. 1981. Physiological control of mammary growth, lactogenesis, and lactation. J. Dairy Sci. **64**: 1403-1421.

Tucker, H.A. 1982. Seasonality in cattle. Theriogenology. **17**: 53-59.

Tucker, H.A. 1988. Photoperiodic influences on milk production in dairy cows. Pages 46-56 *in* W. Haresign and D.J.A Cole, eds. Recent developments in ruminant nutrition-II. Butterworths, Toronto, Ontario.

Tucker, H.A., Petitclerc, D. and Zinn, S.A. 1984. The influence of photoperiod on body weight gain, body composition, nutrient intake and hormone secretion. J. Anim. Sci. **59**: 1610-1620.

Twagiramungu, H., Guilbault, L.A., and Dufour, J.J. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A Review. J. Anim. Sci. **73**:3141-3151.

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

Vandenbergh, J.G. 1989. Coordination of social signals and ovarian function during sexual development. J. Anim. Sci. **67**: 1841-1847.

Webster, G.M. and Haresign, W. 1981. The hormonal control of seasonality of breeding in the ewe. Anim. Prod. **32**: 327 (Abstr.).

Weiguo, L. and Phillips, C.J.C. 1991. The effects of supplementary light on the behaviour and performance of calves. Appl. Anim. Behav. Sci. **30**: 27-34.

Wise, T.H., Caton, D., Thatcher, W.W., Barron, D.H. and Fields, M.J. 1982. Ovarian function during the estrous cycle of the cow: Ovarian blood flow and progesterone release rate. J. Anim. Sci. 55:627-637.

Yelich, J.V., Wettemann, R.P., Dolezal, H.G., Lusby, K.S., Bishop, D.K. and Spicer, L.J. 1995. Effects of growth rate on carcass composition and lipid partitioning at puberty and growth hormone, insulin-like growth factor I, insulin, and metabolites before puberty in beef heifers. J. Anim. Sci. **73**: 2390

Yelich, J.V., Wettermann, R.P., Marston, T.T. and Spicer, L.J. 1996. Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed gain at two rates. Dom. Anim. Endocrin. **13**: 325-338.

Zinn, S.A., Chapin, L.T. and Tucker, H.A. 1985. Previous photoperiod exposure and gradual transition in light intensity at dawn and dusk influence the growth response to photoperiod in Holstein heifers. J. Anim. Sci. **61**(Suppl 1): 266.

Zinn, S.A., Chapin, L.T. and Tucker, H.A. 1986a. Response of body weight and clearance and secretion rates of growth hormone to photoperiod in Holstein heifers. J. Anim. Sci. **62**: 1273-1278.

Zinn, S.A., Purchas, R.W., Chapin, L.T., Petitclerc, D., Merkel, R.A., Bergen, W.G. and Tucker., H.A. 1986b. Effects of photoperiod on growth, carcass composition, prolactin, growth hormone and cortisol in prepubertal and postpubertal Holstein heifers. J. Anim. Sci. **63**: 1804-1815.

9 APPENDICES

APPENDIX I

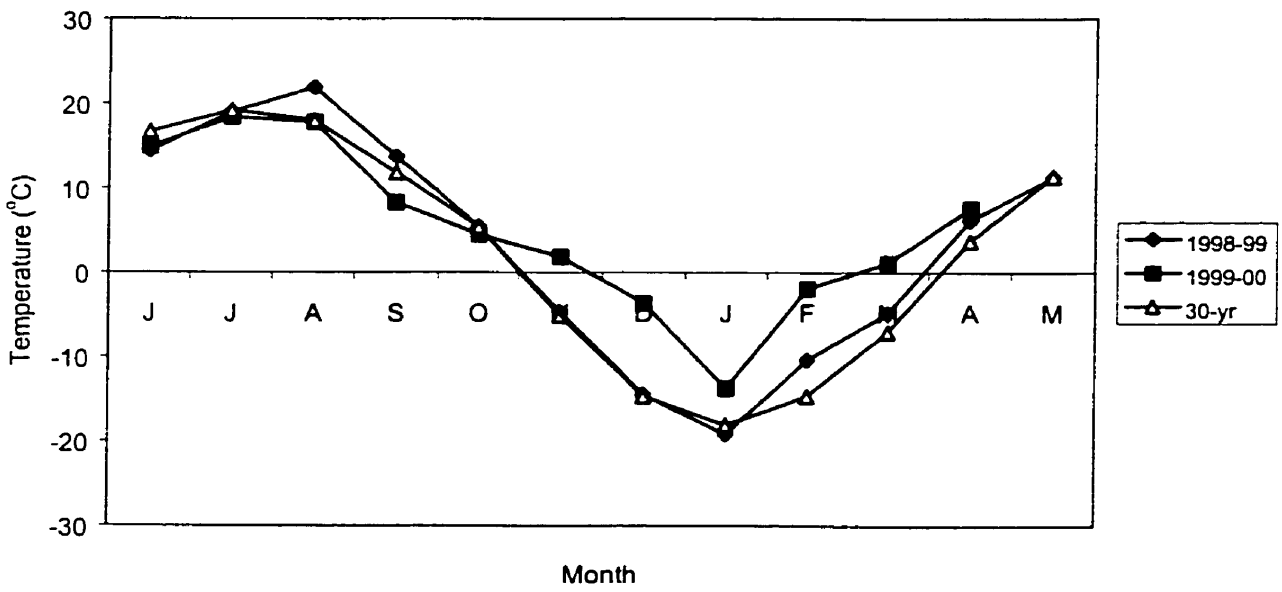

Figure 27. Temperature data collected at the Brandon Research Centre during the experiment, in comparison to the 30-year average temperatures.

APPENDIX II

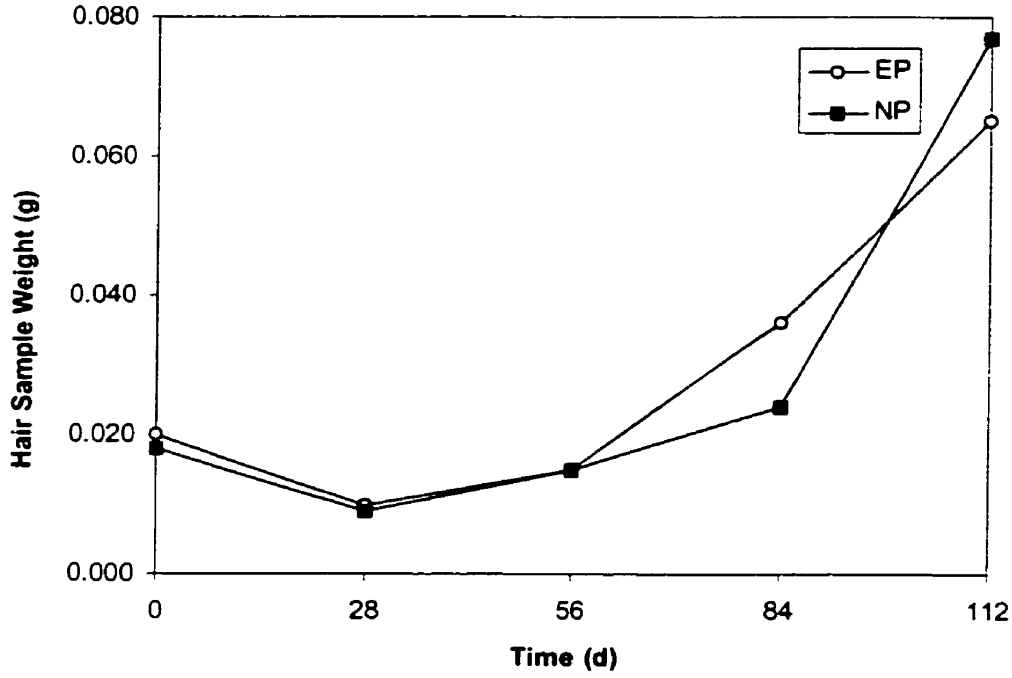

Figure 28. Effects of photoperiod on hair shedding (trt*day P = 0.24, SE = 0.006).

APPENDIX III

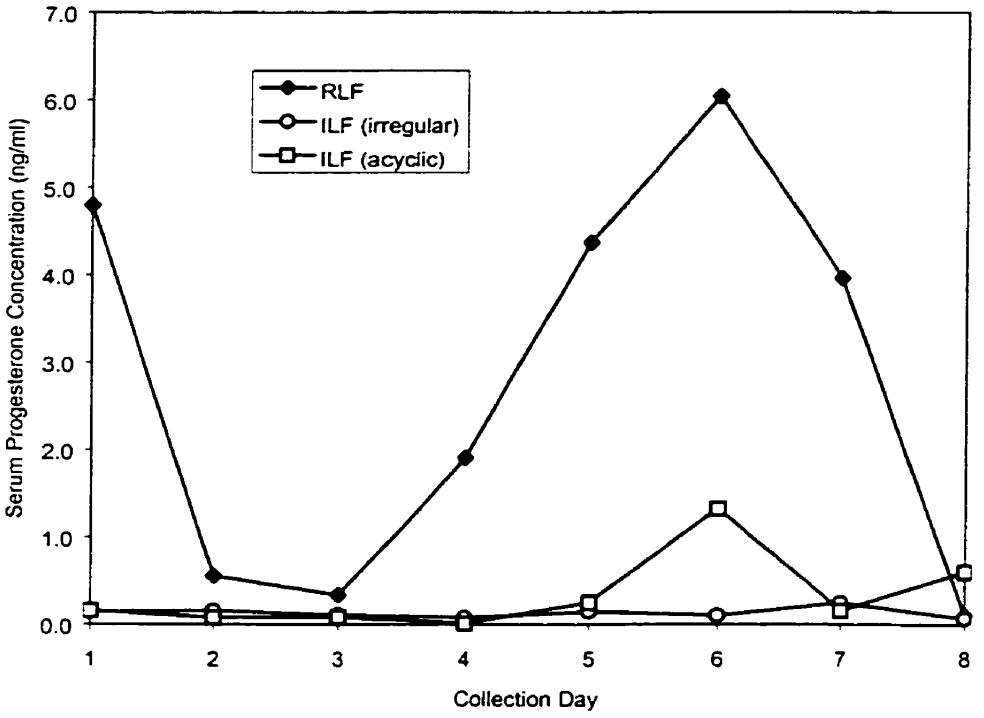

Figure 29. Examples of serum progesterone profiles for individual heifers classified as having regular luteal function (RLF - 2 consecutive progesterone values ≥ 1.0 ng ml^{-1}) or irregular luteal function (ILF - no consecutive progesterone values ≥ 1.0 ng ml^{-1}) based on blood samples taken twice weekly for approximately one month before estrous synchronization.