

**Effects of Energy Balance on Ovarian Activity and Recovered Oocytes
in Holstein Cows Using
Transvaginal Follicular Aspiration**

by

Kerry W. Kendrick

Thesis submitted to the Faculty of the
Virginia Polytechnic and State University
in partial fulfillment of the requirements for the degree of

Master of Science

in

Dairy Science (Reproduction)

APPROVED:

F.C. Gwazdauskas, Chairman

W.H. Eyestone

T.L. Bailey

W.E. Vinson, Department Head

May, 1997

Blacksburg, VA

Keywords: Transvaginal Follicular Aspiration, Energy Balance, In-Vitro Embryo Culture,
IGF-I, Progesterone, Estradiol, FSH, LH

**Effects of Energy Balance on Ovarian Activity and Recovered Oocytes
in Holstein Cows Using
Transvaginal Follicular Aspiration**

by

Kerry W. Kendrick

F.C. Gwazdauskas, Chairman

Dairy Science (Reproduction)

(ABSTRACT)

The effects of energy balance on hormonal patterns and recovered oocytes were evaluated in 20 lactating Holstein cows during two trial periods (fall/spring). Cows were randomly selected and assigned to one of two dietary treatments formulated so that cows consumed 3.6% BW (HE- 1.78 mcal/kg; n=6 in fall, n=5 in spring) and 3.2% BW (LE- 1.52 mcal/kg; n= 5 in fall, n=4 in spring). Body weight and body condition score (BCS) were recorded prior to parturition and weekly throughout the fall trial. Ultrasound guided transvaginal follicular aspirations were conducted twice weekly between d 30 and 100 of lactation. Follicle size and number were recorded. Follicular fluid (FF) was aspirated from the largest follicle, and serum samples were collected for hormone assay (IGF-1; estradiol (E₂); progesterone (P₄, serum); LH and FSH). Oocytes were collected and graded based upon cumulus density and ooplasm homogeneity, then fertilized and cultured in vitro. Milk yield averaged 41.64 ± .3 kg/d (mean ± SE) for HE and 32.8 ± .3 kg/d for LE. There was a significant cubic day postpartum by treatment interaction for milk yield.

Dry matter intake and BW treatment by week interactions were significant for the cubic and linear components, respectively. Oocyte numbers increased linearly from d 30 to 100 postpartum. HE cows produced more good + oocytes ($1.5 \pm .2$) than LE cows ($1.4 \pm .1$). Follicles ≤ 5 mm predominated throughout the study (6.4 ± 3.0). However, greater numbers of follicles 10 to 14 mm and ≥ 15 mm were found in the fall ($1.98 \pm .08$ and $.50 \pm .06$) than spring ($1.11 \pm .3$ and $.23 \pm .07$). Follicular fluid IGF-1 was higher in HE ($2.3 \pm .2$ ng/ml) than in LE cows ($1.6 \pm .2$ ng/ml). Mean basal serum FSH concentration was lower at 28 d postpartum (173 ± 8 pg/ml) compared to later (521 ± 25 at d 60 and 650 ± 25 pg/ml at d 110). Serum P_4 peaked at 35 d postpartum, with HE cows having 1 ng/ml higher P_4 than LE cows. Low dietary energy reduced milk yield, DMI, BCS, FF IGF-1 and serum P_4 and had a negative impact on oocyte quality.

DEDICATION

This thesis is dedicated to my parents. Their constant support, encouragement and love gave me the strength and ability to believe in myself and believe that anything is possible, including a Masters Degree.

ACKNOWLEDGEMENTS

First I would like to sincerely thank my committee, of Drs. Frank Gwazdauskas, Tom Bailey and Will Eyestone. Their commitment to my research, hours spent at the barn, and guidance made this degree possible. I would especially like to thank Dr. Gwazdauskas for his patience and devotion to this project.

I would also like to thank all the graduate students who made even the hard times bearable with their laughter and words of wisdom. Special recognition goes to Lori Smith, Beth Grove, Sue Pandolfi, Steve Butler, Amy Garst, Steve Ellis, Andy Pryor and Amin Ahmadzadeh whose assistance with this project and unending friendship will never be forgotten.

This projects success is in part do to the dedication and hard work of Megan Irby, Rachel Bethard, Debbie Johnson and Amin Ahmadzadeh. Special thanks to Dr. Vinson, Dr. Herbein and Dr. Pearson.

Last I would to thank my grandparents and nanny, for all their letters that never failed to make my day throughout the years; as well as my sister Kristen, for her friendship and sisterly advice. Finally, I would like to thank John Polson, who was always by my side from beginning to end, for his endless support, encouragement and faith in my ability to succeed in all that I do.

TABLE OF CONTENTS

ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGMENTS.....	v
LIST OF TABLES.....	vii
LIST OF FIGURES.....	ix
PROLOGUE.....	1
INTRODUCTION.....	3
REVIEW OF LITERATURE.....	4
Energy Balance.....	4
Oocyte Classifications.....	7
Follicular Development.....	9
Dominant Follicles.....	10
Reproductive Hormones.....	10
Ultrasound-Guided Transvaginal Follicular Aspiration.....	14
Bovine In-Vitro Maturation, Fertilization and Culture.....	17
MATERIALS AND METHODS.....	19
Treatment Groups.....	19
BCS and BW.....	19
Ultrasound-Guided Transvaginal Follicular Aspiration.....	19
Oocyte Evaluation.....	22
In-Vitro Maturation, Fertilization and Culture.....	24
Radioimmunoassay Validation.....	25
Radioimmunoassay Procedures.....	25
Post-Aspiration Synchronization.....	34
Statistical Analysis.....	34
RESULTS.....	36
DISCUSSION.....	60
IMPLICATIONS.....	71
LITERATURE CITED.....	72
APPENDIX.....	78
VITA.....	90

LIST OF TABLES

Table		Page
1	Dairy cattle ration formula for high and low energy groups.....	20
2	Bovine follicular fluid estradiol parallelism using 1 μ l of sample (pg/ml)	26
3	Recovered estradiol after known amounts of hormone were added to 5 μ l (pg/ml) of bovine follicular fluid	27
4	Progesterone parallelism in 100 μ l (ng/ml) of bovine follicular fluid.....	28
5	Recovered progesterone after known amounts of hormones were added to 1 μ l (ng/ml) of bovine follicular fluid	29
6	Bovine serum progesterone parallelism using .1 ml of sample (ng/ml).....	30
7	Recovered progesterone after known amounts of hormones were added to 50 μ l (ng/ml) of bovine serum.....	31
8	Mean oocyte development scores for high energy and low energy cows, per aspiration	59
9	Analysis of variance for dry matter intake, energy balance and energy intake of lactating dairy cattle from calving to 100 d postpartum	78
10	Analysis of variance for body condition (BC) scores and body weights (BW) in lactating dairy cows from d -8 to 25 wk postpartum.....	79
11	Analysis of variance for milk yield and milk fat percentage in lactating dairy cows from calving to 100 d postpartum	80
12	Analysis of variance for size of largest follicle measured in lactating cows from d 30 to 100 postpartum.....	81
13	Analysis of variance for follicle numbers by size category obtained from lactating dairy cows from d 30 to 100 postpartum.....	82

14	Analysis of variance for total oocyte numbers and quality scores for data obtained from d 30 to 104 postpartum in lactating cows	83
15	Analysis of variance for the number of oocytes by quality score from d 30 to 104 postpartum	84
16	Analysis of variance for follicular fluid hormones from lactating dairy cows from d 30 to 104 postpartum	85
17	Analysis of variance for serum progesterone (ng/ml) obtained from lactating cows from calving to 104 d postpartum	86
18	Analysis of variance for basal FSH and LH (pg/ml) obtained from lactating cows from d 28, 60, and 105 postpartum	87
19	Analysis of variance for development of oocytes collected from lactating dairy cows by ultrasound guided transvaginal follicular aspiration	88
20	Analysis of variance for rate of conception of lactating dairy cows post transvaginal follicular aspiration	89

LIST OF FIGURES

Figure		Page
1	Evaluation of the quality of oocytes derived from transvaginal follicular aspiration.....	23
2	Curvilinear relationships of dry matter intake (DMI) and energy intake (EN INT) for the high energy (HE) and low energy (LE) groups from wk 1 to 16 wk postpartum	37
3	Curvilinear relationship of energy balance (EN BAL) for the high energy (HE)and low energy (LE) groups from 1 wk postpartum to 16 wk	38
4	Linear relationship of body weight (BW) for the high energy (HE) and low energy (LE) groups from 1 to 16 wk postpartum	39
5	Quadratic relationship of body condition score (BCS) for the high energy (HE) and low energy (LE) groups from -8 to 25 wk postpartum.....	40
6	Milk yield (\pm .3) for the high energy (HE)and low energy (LE) groups from 1 to 16 wk postpartum	42
7	Milk fat levels for the high energy (HE) and low energy (LE) groups from 1 to 16 wk postpartum	43
8	Largest follicle sizes recorded for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum, viewed via ultrasound.....	44
9	Number of follicles \leq 5 mm and 6 to 9 mm viewed via ultrasound for high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.....	46

10	Number of follicles 10 to 14 mm and \geq 15 mm viewed via ultrasound for high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.....	47
11	Number of oocytes collected via ultra-sound guided transvaginal follicular aspiration (TVFA) from 30 to 104 d postpartum.....	48
12	Mean quality score of oocytes collected via ultra-sound guided transvaginal follicular aspiration (TVFA) from 30 to 104 d postpartum.....	50
13	Number of oocytes collected via ultra-sound guided transvaginal follicular aspiration per quality group for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.....	51
14	Follicular fluid hormone concentrations (IGF1: Insulin-like growth factor-I; E2: Estradiol; P4: Progesterone) for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.....	53
15	Serum progesterone concentrations for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.....	54
16	Basal LH concentrations for the high energy (HE) and low energy (LE) groups at 28, 60, and 104 d postpartum.....	55
17	Basal FSH concentrations for the high energy (HE) and low energy (LE) groups at 28, 60, and 104 d postpartum.....	57

PROLOGUE

When taking into account the relationship between dietary energy and reproduction in dairy cattle, the most significant time period is that of early and peak lactation when the animals' demand for energy is highest. The general relationship between dietary energy intake and energy utilization is defined as energy balance. Energy balance (NEB) usually reaches its nadir during the first two weeks of lactation and recovers at a variable rate. In dairy cows, NEB is directly related to the amount of time between calving and the cow's first ovulation (postpartum interval). During early lactation the energy required for milk production exceeds that provided by the diet. Ovarian activity is more closely associated to milk production than TDN intake (Whitmore et al., 1974; Butler and Smith, 1987; Wiltbank et al., 1962). A cow producing 35 kg of milk daily requires three times more energy for milk production than for body maintenance.

The energy requirements of a lactating cow are met through a combination of dietary intake and mobilization of body reserves (Butler and Smith, 1987; Stevenson and Britt, 1979; Coppock et al., 1974). Body condition scores may be correlated to the number of small follicles present on an ovary, and follicle numbers are higher in cows with body condition score of 3 to 5 than those with lower body condition. Animals in NEB lose body condition and body weight, and exhibit impaired reproductive performance.

Recovery from NEB after the onset of lactation to a positive state may provide an important signal for initiation of ovarian activity. The reestablishment of ovulatory cycles

soon after parturition assures multiple estrous cycles prior to the recommended breeding period, and can therefore influence conception rates.

Timing and magnitude of NEB interact to determine the extent to which NEB limits luteal function in postpartum estrous cycles and subsequent maintenance of pregnancy. Negative energy balance appears to primarily interfere with the ability of the hypothalamus-hypophyseal axis to develop the pulsating LH pattern necessary for fostering ovarian follicular development and ovulation (Butler and Smith, 1987).

This study will once again resurrect the decades old question of “How is reproduction affected by nutrition?” Using the latest technologies, we employed ultrasound-guided transvaginal follicular aspiration (TVFA) to demonstrate that NEB will reduce milk yield, dry matter intake, body condition score, body weight, and have a negative impact on the quality of oocytes retrieved through TVFA, as well as their ability to develop in vitro.

INTRODUCTION

The effects of prolonged dietary energy restrictions on lactating dairy cows have been shown to be extensive, influencing both ovarian hormones and follicular development. Energy balance (EB) is an important regulator in the resumption of estrous cycling after parturition, with energy balance reaching nadir during the first 2 wk of lactation and recovering at a variable rate. However, it is thought that a delay in the resumption of a positive energy balance state may greatly effect the ability of the animal to produce the quality of oocytes that are essential for conception. Negative energy balance (NEB) has been related to altered hormonal levels that do not support a functional reproductive system during early lactation.

In order to study the immediate and prolonged effects of NEB on the ovarian function of lactating cows, ultrasound-guided transvaginal follicular aspiration (TVFA) can be utilized. TVFA was first introduced in the Netherlands in 1987 and since that time it has been an effective way to obtain oocytes from live cattle. These oocytes can be matured, fertilized and cultured in-vitro. Thus, oocyte development rates can be observed and overall effects of NEB on the oocytes determined.

The purpose of this study was to determine the impact of EB on: 1) twice weekly assessment of the number and size of follicles; 2) quality of oocytes collected; 3) in vitro developmental capacity of oocytes and 4) concentrations of progesterone, estradiol and insulin like growth factor-I (IGF-I) in follicular fluid and progesterone in serum obtained from lactating dairy cattle from d 30 to 100 postpartum.

REVIEW OF LITERATURE

Energy Balance

Nutrition can play important roles in reproduction of cattle (Butler and Smith, 1987; Stevenson and Britt, 1979; Coppock et al., 1974; Dominguez, 1995). However, the precise mechanisms through which nutrition affects reproduction are not completely understood for lactating dairy cows. Dairy cows cannot maintain positive dietary energy balance during early lactation and must mobilize body reserves in order to maintain a supply the combined energy demands of maintenance and lactation require (Nebel et al., 1993; Villa-Godoy et al., 1990). The time taken to re-establish positive energy balance after parturition is affected by the extent of fatty tissue reserves and the efficiency with which these reserves can be mobilized (Grimard et al., 1995).

Energy balance (EB) can be an important regulator in the resumption of estrous cycling after parturition (Dominguez, 1995; Butler and Smith, 1987). Energy balance between days 20 and 60 postpartum is inversely related to days to first postpartum ovulation (Spicer et al., 1990). After estrous cycles have been initiated, predicted EB is not associated with follicular growth or number of follicles within size class (Nebel et al., 1993). Lucy et al. (1991) reported that increasing EB is proportional to the number of large follicles in postpartum dairy cows with significant differences in follicular development related to body condition. They also demonstrated that EB in the early postpartum period was positively correlated with follicular recruitment. Thus, cows with

a higher energy balance had fewer small follicles (1-9 mm) and a greater number of follicles 10-15 mm in diameter.

At least 80% of dairy cows have a NEB during early lactation (Villa-Godoy et al., 1990). Energy balance (EB) usually reaches its nadir during wk 1 to 2 of lactation and recovers at a variable rate (Villa-Godoy et al., 1990; Butler and Smith, 1987). Timing and magnitude of NEB interact to determine the extent to which NEB limits luteal function in postpartum estrous cycles and subsequent maintenance of pregnancy. Nebel et al. (1993) stated that NEB may adversely affect fertility and may be influenced primarily by dry matter intake (DMI). During NEB, homeorhetic events sustain metabolic support of lactation, and apparently mammary function has metabolic priority over ovarian function. According to Butler and Smith (1989), Coppock et al. (1974), and Whitmore et al. (1974) a direct inverse relationship ($r = -.80$) exists between milk yield and energy balance. However in another study, milk yield did not differ among cows with distinctly different energy balances, demonstrating that some cows ingest sufficient nutrients to satisfy requirements for high milk yield and are able to achieve positive energy balance earlier postpartum than others (Villa-Godoy et al., 1988). Grimard et al. (1995) reported that cows in NEB lost weight with low milk production, suggesting they had both low DMI and production requirements. The correlation between milk yield and days to first ovulation became significant only after the period (d 40) when most cows had already ovulated. This suggests that other factors, not just milk yield, are involved in determining the interval to first ovulation (Butler and Smith, 1989).

Body condition (BC) score is a tool that can be used to monitor nutritional status in lactating dairy cows, using a 1 to 5 scale with 1 being an emaciated animal and 5 being an obese animal (Braun et al., 1986; Butler et al., 1981). There may be a relationship between BC and the ovary whereby both follicle number and oocyte quality (proportional of normal oocytes) of follicles may be adversely affected by a low BC, thus playing a role in decreased fertility (Dominguez, 1995). Britt (1991) stated that the loss of BC in early lactation is associated with decreased fertility. An effect of poor BC at calving on the number of follicles was present 5 wk postpartum, but not at 9 wk postpartum (Prado et al., 1990).

Dominguez (1995) found that cows with poor BC scores had fewer normal oocytes than cows with higher scores, leading to a smaller pool of normal follicles for the selection of those that continue developing into the final stages of maturation and reducing the probability of normal fertility. Body condition score is related to the number of small follicles, and follicle numbers are higher in cows with BC score of 3 to 5 than those with lower BC. Additionally, oocytes from small follicles are significantly affected by BC, and the proportion of normal oocytes increases with higher BC. Body condition exerted no influence on the quality of oocytes recovered from medium size follicles. A BC score of 1 yielded approximately 16% normal oocytes, while cows with a BC score of 4 and 5 resulted in 56 and 42% normal oocytes, respectively.

Oocyte Classification

A four level system based on compact multi-layered cumulus investment and homogeneous ooplasm has been used to classify oocytes: 1 - compact multilayered cumulus of greater than three layers with homogeneous ooplasm; 2 - compact cumulus of one to two layers with homogeneous ooplasm having a coarse appearance and a darker zona pellucida; 3 - oocytes with less compact cumulus and irregular ooplasm containing dark clusters; and 4 - nude oocyte or expanded cumulus, irregular ooplasm and a jelly-like matrix (DeLoos et al., 1989). Konishi (1996) found that more than 60% of COCs collected through TVFA classified as grades 2 to 4. Smith et al.(1996) found significantly more Class 1 oocytes cleave (63.7%, compact) than those in class 3 (29.5%, partially nude) and Class 4 (17.7%, nude). It was shown that 57.2% of the oocytes collected from bovine ovaries at abattoirs have compact, thick cumulus cells, 9.6% have thin cumulus layers, and 7.5% are naked (Shioya et al., 1988). They found that about 50% of the naked oocytes undergo maturation division and half of these were fertilized, although 20% of the naked oocytes were abnormal or degenerated when examined microscopically.

The inability of an oocyte to be fertilized may be attributed to incomplete cytoplasmic maturation. Cytoplasmic maturation can be defined as the processes that occur in oocyte that prepare it for fertilization, activation, and preimplantation development (Eppig et al., 1996). The improvement of cytoplasmic maturation in bovine oocytes could be associated with an increased volume of secretions from granulosa and cumulus cells. One possibility is that the secretions of granulosa cells may promote the synthesis of oocyte glutathione. Glutathione has important roles in male pronuclear

formation and early development, and the level within the oocyte increases during maturation and decreases during fertilization and early development (Konishi, 1996). One reason for only a modest IVM/IVF success rate is that the proper conditions to support development in vitro are not well known but may be related to incomplete cytoplasmic maturation.

Early research reported that the size of the follicle from which the oocyte is recovered does not affect IVM of bovine oocytes (Leibfried and First, 1979), suggesting that most oocytes recovered from antral follicles are competent to undergo nuclear maturation. Oocytes recovered from early antral follicles have usually become “competent to resume the first meiotic division” after separation from the follicle. However, small antral follicles contain oocytes which are often only “partially competent” to complete maturation, and meiosis is arrested prior to metaphase II, resulting in an undeveloped oocyte (Eppig et al., 1996).

More recent research by Machatkova (1996), reported significantly higher yield of embryos with more ideal morphological characteristics from follicles with diameters greater than 6 mm than those obtained from follicles less than 6 mm and postulated that larger follicles contained growth factors that enhance the morphological and functional status of the oocytes and the yield of embryos.

Results of IVM/IVF show that only nude oocytes (class 4) had a significantly diminished capacity to mature in vitro and a substantial number of nude oocytes degenerated under in vitro conditions (DeLoos et al., 1989). Konishi (1996) indicated that more than four layers of cumulus cells are optimal for development of embryos to the

blastocyst stage. Naked oocytes, even when fertilized, exhibited a delayed male pronucleus formation and less development to the two-cell stage than higher classified oocytes (Shioya et al., 1988). Looney et al. (1994) reported that out of the 1,970 oocytes they recovered from dairy cows, 722 oocytes cleaved (40.5%), and 813 embryos were transferred to recipient cows resulting in 89 pregnancies. Hasler et al. (1995) reported that there can be a wide variation of the number of oocytes collected from individual cows over the course of an experiment.

A higher rate of development to the blastocyst stage (54% of cleaved embryos) was observed with oocytes never cooled below 35°C, suggesting that careful temperature control over the entire in vitro embryo production process may be necessary to retain the maximum developmental capability of oocytes recovered from ovaries. It may be prudent to maintain bovine ovaries, gametes and embryos at temperatures within the physiological range of 37 to 39°C to maximize both the quantity and quality of embryos obtained by in vitro methods (Pollard et al., 1996). In contrast, Hasler et al. (1994) noted that the percentage of oocytes developing to the blastocyst stage for 38 ± 1 °C was 45.6 %, while the development of oocytes exposed to 25 ± 2 °C only dropped to 43.5%.

Follicular Development

Follicular growth was affected only in cows fed a diet lacking essential nutrients and having a low body condition score at calving (Perry et al., 1991). Differences between underfed and adequately fed cows up to d 50 postpartum were more striking in terms of follicular growth (Grimard et al., 1995). Recruitment and growth of ovarian follicles

increased with time postpartum (Murphy et al., 1990). According to Leibfried and First (1979) the mean relative proportion of non-atretic follicles per pair of ovaries was 23.7% for cycling dairy cows and heifers.

Dominant Follicles

Evidence was presented that the presence of a dominant follicle decreases ovulation rate and the number of transferable embryos in cattle. Therefore, a dominant follicle may indicate a cohort of atretic small follicles, which may not be appropriate for oocyte collection (Smith et al., 1996).

Follicles that start to grow between 10 to 20 d postpartum may be affected by the NEB and carry oocytes with a low developmental capacity. Follicles 1 to 3 mm in diameter tend to have a higher proportion of oocytes possessing a compact, complete investment than those of larger follicles (Leibfried and First, 1979). The average size of large follicles increased in weight gain vs weight loss heifers during the follicular phase of the estrous cycle (Spicer et al., 1991).

Reproductive Hormones

In cattle the concentrations of reproductive hormones in follicular fluid vary considerably with stage of the cycle and with size and physiologic status of the follicle. Follicular fluid is similar to plasma, and many of its components are believed to be derived from plasma. Determination of steroid concentrations in follicular fluid from follicles of known physiologic status is of interest because it may provide information on the synthetic

capabilities of granulosa and theca cells at particular stages of differentiation and nutritional status (Fortune and Hansel, 1985). Healthy large antral follicles in cattle are characterized by high intra-follicular estradiol concentrations and low progesterone, whereas atretic follicles contain higher progesterone or androgen concentrations; atretic follicles have a different and much more variable profile of reproductive hormones in follicular fluid than non-atretic follicles (Smith et al., 1996; Fortune and Hansel, 1985).

One potential hormonal mediator of ovarian function is insulin-like growth factor - I (IGF-I). In vitro studies have implicated IGF-I as a potent stimulator of bovine granulosa and luteal cell steroidogenesis. Specifically, IGF-I increased steroidogenesis of thecal and granulosa cells in vitro, and enhanced granulosa cell mitosis. Ovarian follicular development is controlled by numerous systemic and local intraovarian factors and in vitro studies have established ovarian granulosa and thecal cells as sites of IGF-I action. The available in vivo data suggest that concentrations of IGF-I in follicular fluid increase with increased follicular size in cattle. However, the concentration of IGF-I in small follicles seems to be very low and the levels are comparable to those in blood, and could actually be transferred from the blood. In addition, in vivo data are consistent with the hypothesis that IGF-I concentrations in follicular fluid regulate ovarian follicular progesterone biosynthesis.

IGF-I has been postulated as a potent activator of ovarian follicular growth and for the differentiation of luteal cells. The stimulatory effect of IGF-I on oxytocin and progesterone biosynthesis and release were reported for bovine granulosa and luteal cell-culture. The expression of IGF-I in rat granulosa cells of developing follicles suggests that

IGF-I is produced directly in the follicles. Under in vivo conditions, increased concentrations of progesterone in follicular fluid are associated with increased levels of IGF-I in individual follicles (Einspanier, 1993).

Reduced dietary energy intake had no effect on concentrations of IGF-I in follicular fluid (Spicer et al., 1991). Concentrations of IGF-I were 27% lower in follicular fluid than in plasma. Since IGF-I concentrations in plasma and follicular fluid are not changed significantly with reduced dietary energy intake, it appears that reduced concentrations of progesterone and estradiol in follicular fluid were not mediated by decreased IGF-I. Reduced ovarian follicular function during dietary energy restriction in cyclic heifers was not associated with changes in concentrations of IGF-I in plasma and follicular fluid. Thus, IGF-I of ovarian and systemic origin is unlikely the mediator of dietary induced alterations in ovarian follicular function of heifers (Spicer et al., 1991). Concentrations of IGF-I are low in small follicles, and increase significantly in larger follicles. Concentrations of IGF-I in preovulatory and cystic follicles are higher than in bovine plasma (Einspanier, 1993).

An increase in EB was associated with an increase in concentrations of IGF-I in serum during early lactation. This increase in IGF-I was associated with increased progesterone secretion during diestrus of the first and second postpartum estrous cycles. Also, an inverse relationship between milk production and IGF-I was observed (Spicer et al., 1990).

There was a significant positive correlation between weekly average EB and serum progesterone concentrations ($r = .26$). Spicer et al. (1990) observed that progesterone

concentrations in serum during diestrus of the first and second estrous cycles were greater in positive energy balance (PEB) than NEB cows. Cows losing BW due to restricted diets had decreased plasma progesterone concentrations, which may be due to lower progesterone output by the CL. A reduced level of progesterone caused an increase of LH pulse frequency, which may modify the secretion of other ovarian paracrine and autocrine factors (Dominguez, 1995). Progesterone secretion during diestrus increased only in PEB cows during the second and third (but not first) estrous cycle postpartum (Villa-Godoy et al., 1988). Decreased progesterone secretion occurs with limited luteal function, and insufficient luteal support of the uterus during early pregnancy may be one cause of decreased pregnancy rates (Nebel et al., 1993). Decreased dietary energy intake in cyclic heifers was associated with decreased concentrations of progesterone in follicular fluid of small and medium follicles, and decreased size of large follicles (Spicer et al., 1991). Follicular fluid progesterone levels are correlated ($r=.61$) with the age of the follicle, with levels being higher in regressing than growing follicles. Morphological criteria and follicular fluid progesterone may be able to be used to select those bovine cumulus oocyte complexes (COCs) with higher potential for blastocyst development according to Hazeleger et al. (1993).

Estradiol produced by ovarian follicles is the primary hormone stimulating estrous behavior in cattle. Reduced IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing expression of estrus (Spicer et al., 1990). Estradiol concentrations were higher in large dominant follicles than in small follicles, with levels rising during follicular growth and being higher in preovulatory follicles. Small and

medium follicles contain higher estradiol concentrations in the early days of diestrus (d 1 to 10) when compared with the late (d 11 to 17) luteal phase. These studies imply that small follicles in the midluteal phase are more likely to be atretic than those that are present in the early luteal phase (Spicer and Zinn, 1987). According to Price et al. follicular fluid estradiol levels in cattle are lower in static follicles when compared to growing follicles, with levels being significantly lower ($p < .05$) in follicles that are histologically atretic.

Ultrasound-Guided Transvaginal Follicular Aspiration

Ultrasound-guided transvaginal follicular aspiration (TVFA) was developed as an efficient means of collecting oocytes from live animals for in vitro production of bovine embryos. TVFA is performed by inserting a vaginal probe equipped with an ultrasound transducer into the vagina of a cow. An ovary is then placed adjacent to the ultrasound transducer and a 17 g needle is inserted into one of the present follicles. The oocyte is retrieved through the needle by suction, with little to no damage. The production of embryos following in vitro maturation, fertilization and culture of oocytes aspirated directly from ovarian follicles is performed for therapeutic reasons in humans, for production purposes in domestic animals, and for experimental research in laboratory mammals. In cattle, ovaries obtained from the abattoir constitute a major source of oocytes used in in-vitro fertilization (IVF) procedures, although the quality of these oocytes is highly variable (Smith et al., 1996).

Pieterse et al. (1988) performed 36 transvaginal procedures on dairy cows during which 54 oocytes were recovered from 197 follicles, an overall recovery rate of 27.4% in dairy cows. It was estimated that at that recovery rate the number of oocytes that could be harvested from one animal on a yearly basis (52 wk) would be 135 oocytes. Looney et al. (1994) performed TVFA on 84 dairy problem donor cows with a total of 321 aspirations and 2874 aspirated follicles, a recovery rate of 68.5%.

Because there is an association between the follicle and its oocyte, oocyte quality is affected by the day of the estrous cycle. By collecting all follicles larger than 2 mm in diameter using TVFA, a new follicular wave develops and reaches full numerical development within 3 d. Hence, when TVFA is performed twice weekly the number of follicular waves doubles (6 vs 2 or 3) with respect to a natural estrous cycle. The dynamics of the follicular wave induced by TVFA causes a rapid turnover of the follicular population and decreases the occurrence of atresia (Boni et al., 1996). The mean proportion of normal oocytes decreased with increasing follicular size suggesting a normal process of follicular atresia (Dominguez, 1995).

The ultimate goal of TVFA procedures is to produce more embryos and pregnancies per donor cow than by superovulation. More offspring per cow would also be possible if, during the entire estrous cycle, preovulatory follicles (10 to 15 mm) and smaller follicles could be aspirated and the retrieved oocytes were matured and fertilized (Pieterse, 1988). The combination of TVFA and in vitro embryo production has a higher potential benefit than superovulation for embryo production. In fact, TVFA and in vitro embryo production can be applied regardless of the stage of the estrous cycle of the

donor, in animals with occluded oviducts or with uterine infections, or in animals that do not respond to superovulation treatments (Boni et al., 1996). Follicular aspirations allow for repeated ovum collections without hormonal stimulation of the ovaries or disrupting the animals cyclicity, while avoiding surgical tactics (Pieterse et al., 1991), as well as providing an alternate method for laparoscopy which has been associated with scar tissue and ovarian adhesions (Looney et al., 1994).

Follicle size as well as the location and attachment of the oocyte in cumulus cells can be influenced by the use of hormones, follicles may also play an important role in recovery rates of oocytes. Oocyte recovery rates may be affected by technical aspects of collection procedures such as vacuum pressure, tubing system, needle size, sharpness and bevel, adequate restraint of the animal and methods of manipulating the ovaries.

Oocytes are usually aspirated from follicles which are greater than 2 mm diameter in size without regard to the stage of the estrous cycle. Although the size of oocytes remains unchanged during the growth of follicles between 2 to 7 mm, both nuclear maturation and cleavage development is enhanced in oocytes isolated from larger follicles (Machatkova, 1996). The twice weekly frequency of TVFA has also been documented by Gibbons et al. (1994) in dairy cattle for optimal oocyte retrieval. (Gibbons et al., 1994). Presicce et al. (1997) demonstrated that TVFA can even be utilized for ovum pick-up in prepubertal calves with embryo development being comparable to that of an adult cows.

Oocytes are fixed in place by their cumulus cells or hidden in folds of larger punctured follicles and may remain behind during aspiration. Preovulatory oocytes and oocytes of atretic follicles may be recovered more easily because of loose fixation in the

cumulus cells (Pieterse, 1988). The developmental quality of oocytes used for in vitro fertilization and maturation (IVF/IVM) is dependent upon adequate support of the somatic follicular cells. This cellular support is mediated through an extensive connection between adjacent cumulus and corona cells and between the corona cells and the oocyte through gap junctions (Laurincik et al., 1996). Developmental capacity of oocytes during the postpartum period is low between d 80-120 (Kruip et al., 1996). Oocytes with a dark rim of corona radiata cells display a higher capacity for meiotic maturation and post fertilization development than those without a distinct corona (Laurincik et al., 1996). Revah and Butler (1996) showed that oocytes aspirated from prolonged multiple follicles were characterized by expanded cumulus, condensed chromatin, and possible germinal vesicular breakdown, while oocytes aspirated from growing follicles have compact cumulus with intact germinal vesicles. Bovine follicular oocytes recovered from the follicles void of investment are probably from atretic follicles. Therefore, they are degenerating and losing the ability to mature as judged by nuclear development in culture (Leibfried and First, 1979). A problem with this method is that the length of the needle, tubing and vacuum pressure used for oocyte aspiration tend to remove a portion of the cumulus cells from the cumulus oocyte complex (COC; Konishi, 1996).

Bovine In Vitro Maturation, Fertilization and Culture

In vitro embryo maturation, fertilization and culture (IVM, IVF, IVC) otherwise known as in vitro production, are procedures that give researchers an opportunity to evaluate and understand the concepts behind mammalian reproduction in a laboratory

environment. This is done without having to rely on an animal's estrous cycle or its ability to reproduce naturally.

It has been suggested that the cumulus cells improve the fertilization rate in cattle IVF through specific and non-specific ways. The first, by providing a capacitation-inducing mechanism, and the latter, by facilitating the interaction between capacitated spermatozoa and the zona pellucida surface (Cox et al., 1993). Cumulus cells were shown to increase the sperm penetration of matured oocytes (Park et al., 1989), and stimulate the acrosome reaction during IVF (Fukui et al., 1990). However Cox et al. (1993) found that cumulus cells improved the fertilization rate of matured oocytes, and that the cumulus effect was expressed only when cells were in contact with the zona pellucida. Krisher et al. (1994) reported that embryos derived from oocytes that were matured with intact cumulus cells exhibited improved developments over those derived from denuded oocytes.

Although the presence of cumulus cells is not essential for IVF, cumulus cells are needed for complete oocyte maturation (Shioya et al., 1988). Procedures for the in vitro production of bovine embryos require that oocytes be removed from this highly regulated maternal system to the more variable laboratory environment (Pollard et al., 1996).

MATERIALS AND METHODS

Treatment Groups:

A total of 20 cows were randomly assigned at parturition into one of two dietary treatment groups. Treatment 1 (HE; n=6 in fall, n=5 in spring) was designated as the high energy group and fed 3.6% BW (1.78 mcal/kg). Treatment 2 (LE; n=5 in fall, n=4 in spring) was the low energy group and fed 3.2% BW (1.52 mcal/kg; Cooperative Extension Service, Dept. of DASC, VA Tech). Energy was the only limiting factor in the diets (Table 1). Energy balance was equal to: net energy consumed - net energy required for production and maintenance.

Body Condition Scores and Body Weights:

Body condition scores for each animal were determined prior to calving and through 25 wk postpartum in the fall trial (Braun et al., 1986). Body weights were recorded prior to parturition and weekly throughout the experiment. Orts and milk weights were obtained daily. Milk fat was measured once weekly on pooled daily milk samples and determined by the Virginia Tech DHI lab.

Ultrasound-Guided Transvaginal Follicular Aspiration:

Transvaginal follicular aspirations (TVFA) guided by ultrasound were performed twice weekly (Tuesday and Friday) between 30 and 100 d of lactation on all cows. The TVFA apparatus consisted of an ultrasound transducer (5MHz) and vaginal probe that

Table 1. Dairy cattle ration formula for high and low energy groups

Ingredient	HE (%)	LE (%)
Corn silage	33.0	29.0
Alfalfa silage	18.9	17.7
Orchardgrass hay	6.7	24.4
Corn, Ground	24.4	4.8
Soybean oil meal, 48%	8.1	9.7
Corn distillers grain	6.6	13.3
Megalac ¹	1.8	-
Minerals ²	.5	1.1
Chemical Components		
DM %	66.8	68.5
CP %	17.2	19.2
ADF %	17.2	26.4
NDF %	30.8	45.2
Fat %	6.0	4.1
NE _L Mcal/kg	1.8	1.5

¹ Concentrated form of digestible undegradable energy which does not interfere with rumen function; Church and Dwight

² Contained trace mineral salt (26.8% HE and 22.6% LE), magnesium oxide (12.0% HE and 8.1% LE), monocalcium phosphate (36.6% HE), dicalcium phosphate (29.1% LE), limestone (40.2% LE), and sodium bicarbonate (24.6% HE) per kg of mineral mix, vitamin A (75,000 - HE and 50,000 IU/kg - LE), vitamin D (24,000 - HE and 16,000 IU/kg - LE) and vitamin E (370 - HE and 240 IU/kg - LE)

uses a 17-g, 55 cm needle with an echogenic tip (RAM Consulting, Madison, WI) which was guided by a dorsal mounted needle (16-g). Cows were sedated using acepromazine maleate injected i.v. (44 mg/100 kg BW; Aveco Co., Inc., Fort Dodge, IA). An epidural was administered (6 ml; 2% Lidocaine Hydrochloride; Phoenix Scientific, Inc., St. Joseph, MO) and for the more difficult cows the epidural anesthesia was supplemented with Rompun® (up to .3 ml of 20 mg/ml; Moby Corporation, Shawnee, KS). Both the vulva and anus were cleansed and disinfected (Nolvasan ®; Fort Dodge Laboratories, Fort Dodge, IA). The vagina was infused prior to aspiration with .9% sterile saline to cleanse the vaginal vault. Follicles were observed on an Aloka 500V ultrasound monitor (Corimetrics, Wallingford, CT) and size and number of follicles on each ovary were recorded at each session prior to aspiration (Gibbons et al., 1995). Follicular fluid from the largest follicle (> 10 mm) was aspirated for further hormonal studies and stored at - 20 °C until hormone assays were performed. Jugular blood samples (10 ml) were collected for progesterone analysis beginning at parturition and continued twice weekly throughout the experiment. Blood samples were stored for 24 h at 4⁰ C to allow for clotting, then centrifuged at 2100 g for 30 min for separation of serum. The serum samples were stored at -20⁰C until hormone analysis.

Oocytes were collected in Dulbecco's phosphate buffered saline (DPBS; Gibco, Long Island, NY) enriched with 10% vol/vol Newborn Calf Serum (NCS; Gibco), 1% vol/vol Penicillin/Streptomycin (P/S) and 25 µg/ml Heparin (Sigma Chemical, St. Louis, MO). During follicular aspiration, oocytes were transported through the needle to 2 m silastic tubing (2.0 mm o.d. x .5 mm i.d.; Dow Corning Corporation, Midland, MI) by a

vacuum pressure pump at 75 mmHg and they were collected into an Em-con filter (Professional Embryo Transfer Supply, Canton, TX). The Emcon was drained and rinsed with DPBS to make sure all oocytes were released from the filter. Located oocytes were moved using a 5 μ l Drummond microdispenser pipette (Drummond Scientific Co., Broomall, PA), washed three times in TL Hepes supplemented with BSA (3 g/L; Sigma Chemical), graded and placed into a maturation media containing TCM199 (Gibco), 10% vol/vol Fetal Calf Serum (FCS; Hyclone, Logan, UT), bFSH, bLH (0.01 U/ml each; NOBL Labs, Sioux Center, IA) and 1% vol/vol P/S (Gibco; Gibbons et al., 1994). The two treatment groups were kept separate throughout the entire experiment. Oocytes were placed into 500 μ l of the maturation media with a 1 ml mineral oil overlay, and kept in an incubator, where they remained for 22 to 24 h at 39°C and 5%CO₂.

Oocyte Evaluation:

Oocyte quality scores were based on those of DeLoos et al. (1989). There were four classifications based on ooplasm homogeneity and cumulus investment: 1 - compact multilayered cumulus with greater than three layers and a homogeneous ooplasm; 2 - compact cumulus of one to two layers with homogeneous ooplasm having a coarse appearance and a darker zona pelucida; 3 - oocyte has less compact cumulus with irregular ooplasm containing dark clusters in the ooplasm; and 4 - nude oocyte or expanded cumulus, irregular ooplasm and a jelly-like matrix (Figure 1).

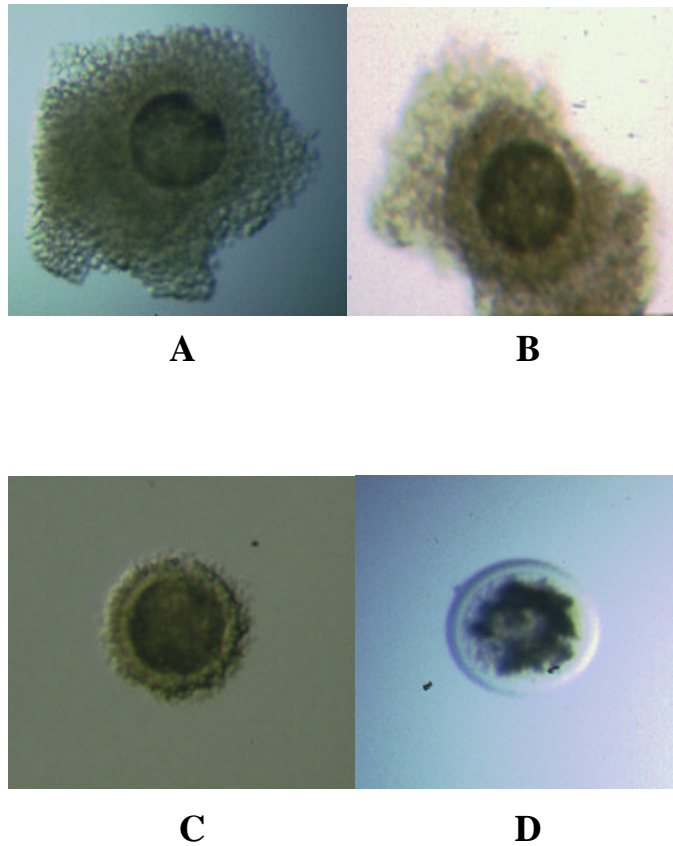


Figure 1. Evaluation of the quality of oocytes derived from transvaginal follicular aspiration.

- A. Good +: compact multi-layered cumulus with greater than three layers and a homogeneous ooplasm
- B. Good 0: compact cumulus of one to two layers with homogeneous ooplasm having a coarse appearance and a darker zona pelucida
- C. Good -: less compact cumulus with irregular ooplasm containing dark clusters
- D. Poor: nude oocyte or expanded cumulus, irregular ooplasm with jelly-like matrix

In-Vitro Maturation, Fertilization and Culture:

After 22 to 24 h of maturation the oocytes were washed three times in TL HEPES supplemented with BSA (3 g/L; Sigma Chemical) and placed into Nunc wells (Thomas Scientific, Swedesboro, NJ) containing fertilization media prepared from a stock media containing 25 mM Pyruvate (Sigma Chemical); 1% vol/vol P/S (Gibco) and supplemented with BSA (.3g/L; Sigma Chemical). Characterized frozen semen from a tested bull was thawed and separated through Percoll separation procedures (Parrish et al., 1995). The final sperm suspension used was 1×10^6 sperm/ml. Heparin and PHE (Penicillamine, 20 mM; Hypotaurine, 10 mM; Epinephrine, 1 mM) were added for increased sperm capacitation and mobility. The final sperm suspension (.5 ml) was added to the Nunc wells with oocytes and incubated for 18 to 22 h.

After IVF (18 to 22 h), the oocytes were vortexed to remove remaining cumulus cells and transferred to a co-culture well containing buffalo rat liver (BRL) mono-layer media (200,000 cells in 500 μ l); no more than 25 oocytes per well. The media consisted of TCM 199, FCS, 1% BSA, 0.1 mM nonessential amino acids (Gibco), 2 mM Glycine, 1 mM Alanine (Sigma), and 1% vol/vol P/S (Gibco). They were placed into the incubator at 39^o C and 5% CO₂, where they remained for 4 d. At the start of the fourth day, the embryos were moved to fresh media until d 7. At this time the oocytes were evaluated under light microscopy and developmental stage for each treatment group was recorded.

Radioimmunoassay Validations:

Prior to using Coat-A-Count assay kits (Diagnostic Products, Los Angeles, CA), the kits were validated for both follicular fluid and serum samples. This was done to determine the optimum volume of sample to be used for each assay. The results of the assay validations for estradiol and progesterone in follicular fluid were assessed in diluted samples and by adding known amounts of hormone to follicular fluid (Tables 3-6). Assay of progesterone in serum was assessed using similar procedures (Tables 7 and 8).

Radioimmunoassay Procedures:

IGF-I concentrations in bovine follicular fluid: Frozen follicular fluid samples were thawed and mixed. An extraction mixture (900 μ l) made up of 87.5% ethanol (100%) and 12.5% 2N HCL and 100 μ l of the follicular fluid sample was added to a microfuge tube, vortexed and incubated for 1 h. The samples were placed in a Hermle Microfuge Z231 and spun for 10 min at 9982 g. Five hundred microliters of the supernatant was added to 200 μ l of 0.855 M/L Tris Base to neutralize the sample. The samples were frozen at -20^o C. After 1 h the samples were removed from the freezer and placed in a Beckman J6-B clinical centrifuge for 30 min at 3935 g. The supernatant was poured off into a 12 x 75 mm plastic tube (VWR, Bridgeport, NJ) and frozen until assayed.

On d 1 of the assay, 100 μ l of the tracer plus 100 μ l of the first antibody (mouse anti-IGF-I) were added to the 40 μ l of the thawed supernatant and stored 4^o C.

Table 2. Bovine follicular fluid estradiol parallelism using 1 μ l of sample (pg/ml)

Dilution	$\bar{x} \pm SE$	(n)	% Undiluted
Undiluted	8.7 \pm 0.2	5	-
1:1.33	9.9 \pm 0.6	4	114
1:2	8.2 \pm 0.8	4	94
1:4	6.5 \pm 0.4	4	75
1:10	10.9 \pm 1.2	4	126
1:20	9.7 \pm 0.3	3	111
1:50	3127 \pm 86		% of 1:50
1:100	3256 \pm 75	4	104
1:200	2968 \pm 40	5	95
1:300	2671 \pm 66	5	85
1:400	2825 \pm 153	5	90

Table 3. Recovered estradiol after known amounts of hormone were added to 5 μ l (pg/ml) of bovine follicular fluid

Standard added	Observed $\bar{x} \pm$ SE	(n)	Expected	% O/E
0	36.00 \pm .04	2		
2	3.38 \pm .14	4	2.36	143
5	5.98 \pm .53	4	5.36	112
15	16.40 \pm .40	4	15.36	107
50	53.55 \pm 1.74	4	50.36	106
180	178.30 \pm 16.79	4	180.36	99
360	358.77 \pm 53.61	4	360.36	94

Table 4. Progesterone parallelism, in 100 μ l (ng/ml) of bovine follicular fluid

Dilution	$\bar{x} \pm SE$	(n)	% 1:100
1:100	399.4	3	-
1:50	355.9	3	89
1:25	571.5	3	143

Table 5. Recovered progesterone of bovine follicular fluid after known amounts of hormones were added to 1 μ l (ng/ml)

Standard added	Observed $\bar{x} \pm$ SE	(n)	Expected	% O/E
0	.95 \pm .07	3		
.1	.92 \pm .09	3	1.05	87
.5	1.33 \pm .13	4	1.45	91
2	3.41 \pm .17	4	20.95	115
10	10.99 \pm .16	4	10.95	100
20	22.02 \pm .86	4	20.95	105
40	41.28 \pm .56	4	40.95	101

Table 6. Bovine serum progesterone parallelism using .1 ml of sample (ng/ml)

Dilution	$\bar{x} \pm SE$	(n)	% Undiluted
Undiluted	.95 \pm .02	4	-
1:1.33	.79 \pm .02	3	83
1:2	.74 \pm .01	4	78
1:4	.86 \pm .03	4	90

Table 7. Recovered progesterone after known amounts of hormones were added to 50 μ l (ng/ml) of bovine serum

Standard added	Observed $\bar{x} \pm$ SE	(n)	Expected	% O/E
0	.88 \pm .09	4		
.1	.79 \pm .03	4	.98	81
.5	1.31 \pm .07	4	1.38	85
2	3.00 \pm .15	4	2.88	104
10	11.39 \pm .47	3	10.88	105
20	22.72 \pm .50	4	20.88	109
40	44.40 \pm 1.60	4	40.88	109

The second antibody (goat anti-mouse) was added on d 2 (100 μ l) to each sample and placed into the 4° C cold room for 3 d. On d 6 1 ml of cold double distilled PBS was added and the samples were centrifuged at 2,200 g for 30 min. The supernatant was poured off and the samples were counted for 1 min in a gamma counter (Akers, personal communication; CV with in assay: 14.9%, between assay:15.8%).

Progesterone and estradiol concentrations in bovine follicular fluid: Samples were removed from the freezer and allowed to thaw and vortexed. Sets of samples were diluted to 1:100 or 1:200 prior to the assay. One hundred microliters of the diluted sample was placed in polypropylene antibody coated tubes (Diagnostic Products, Los Angeles, CA), wherein ¹²⁵I-labeled progesterone/estradiol competed for 3 h with the progesterone/estradiol in the unknown sample for antibody sites. After 3 h the supernatant was removed to terminate the competition and isolate the antibody-bound fraction of the radiolabeled progesterone/ estradiol. These samples were counted using an ¹²⁵I gamma counter (1 min per sample; progesterone CV with in assay: 6.62%, between assay: 11.5%; estradiol CV with in assay: 7.1%, between assay: 10.0%).

Progesterone concentrations in bovine serum samples: Serum samples (.1 ml) were analyzed using the identical kit (Diagnostic Products) and procedure as for the follicular fluid assay without the dilution (CV with in assay: 7.46%, between assay: 8.9%).

Luteinizing hormone concentrations in bovine serum samples: The blood samples used for LH analysis were collected using aseptic techniques. A cannula was placed into the jugular at 0530 and immediately infused with a 3.5% citrate solution, to prevent clotting in the cannulation tubing. Citrate addition was repeated after each sample was

collected. Blood collection began at 0930 and continued to 1300 at 15 min intervals. The blood samples (10 ml) were placed into 13 ml collection tubes and immediately placed on ice. All samples were stored at 4° C for 24 h, then centrifuged for 35 min at 2,200 g. The serum collected was stored at - 20° C until assayed.

The levels of LH present in the collected serum samples were evaluated according to the methods set forth by Bolt and Caldwell (1992). Both serum samples and a purified form of LH (USDA-bLH-B-6) were placed in an assay buffer (500 μ l) and incubated with USDA-309-684p antibody (1:35,000). The samples were allowed to sit for 24 h at room temperature. Then, ¹²⁵I radio-labeled LH was added to each sample and kept at room temperature. Forty-eight hours later, the second antibody (anti-rabbit gamma globulin serum) was added at a 1:8 dilution and stored for 48 h at 4° C. The samples were then centrifuged for 45 min and the supernatant was poured off. The tubes were allowed to drain for 30 min before being placed in a gamma counter for 1 min (CV within assay: 12.0%, between assay: 13.0%).

Serum FSH was quantified by a heterologous double antibody radioimmunoassay in a non-equilibrium assay conditioned as described previously (Bolt and Caldwell, 1992) with the following alterations. The FSH assay utilized rabbit ovine-FSH antiserum (NIDDK-anti-oFSH-1, [1:80,000 dilution]) as the primary antibody and highly purified bovine FSH (USDA-bFSH-I-2) as both iodinated tracer and reference standard. Sheep anti-rabbit gammaglobulin (1:8 dilution) was used as the second antibody. The ovine-FSH antiserum bound 15% of radiolabeled FSH in the absence of the unlabeled hormone with

the sensitivity of the assay being < .1 ng/ml of the reference standard (CV within assay: 7.8%, between assay 11.0%).

Post-Aspiration Synchronization:

Estrus was synchronized in each animal post-aspiration using Synchromate B (Sanofi Animal Health, Overland Park, KS) ear implants. The implants remained in the animal for 9 d. Two injections of GnRH (100 μ g) were administered 5 d after the completion of aspiration and 48 h after the implant was pulled (Pursley et al., 1995). Estrus was monitored using Heatwatch[®], with AI occurring at visual heat.

Statistical Analysis:

The statistical analysis was performed using the General Linear Model procedure in the Statistical Analysis System (SAS, 1985). The basic model included: linear, quadratic, and cubic time by weeks postpartum effects, season, treatment, season by treatment, cow nested in season by treatment, and interactions of treatment by days postpartum (week). Reduced models were based on removal of non-significant terms. Dry matter intake, EB, energy intake, BC, and BW analyses were only conducted on the data from the fall season. This was due to the fact that the Calan doors used for feeding and data collection were only available for the fall trial. Dry matter intake, EB, energy intake, BC and BW were analyzed with a model including treatment (LE or HE), cow nested in treatment, and weeks postpartum by treatment interactions. The second model assessed milk yield, and milk fat, with the dependent variables being season, treatment,

treatment by season, cow nested in treatment by season, and treatment by week interactions. For the analysis of largest follicle size the model included follicles < 5 mm, follicles 6-9 mm, follicles 10-14 mm, and follicles > 15 mm as independent variables, with season, treatment, treatment by season, cow nested in treatment by season, days postpartum by treatment as dependent variables. Oocyte numbers and oocyte quality scores were assessed by the fourth model, with the variables being treatment, season, treatment by season, cow nested in season by treatment, treatment by days postpartum, and aspirator was added to the model. The fifth basic model examined hormone data (follicular fluid: IGF-I, estradiol and progesterone; and serum: progesterone), with the model including treatment, season, season by treatment, and cow nested in season by treatment and days postpartum by treatment. The sixth model assessed both LH and FSH data, with the variables being treatment, cow nested in treatment, with the addition of period, treatment by period, cow by period nested in treatment, and time by treatment by period interactions. The final two analyses were performed on the oocyte culture data and pregnancy models upon the completion of the TVFA experiment. Both model statements included treatment and season in the basic model.

RESULTS

Through the evaluation of dry matter intake, energy intake and energy balance, it was demonstrated that there were cow nested in treatment, and linear, quadratic and cubic week by treatment interactions ($p < .01$ or $p < .05$; Appendix Table 9). Energy intake in HE cows reached 45 kg/d at 7 wk postpartum and remained relatively stable through 14 wk with a slight increase there after. In contrast it took an additional 6 wk for the LE group to maximize EB (Figure 2). Dry matter intake peaked at wk 7 for HE cows, while DMI for LE increased more gradually and peaked at 13 wk postpartum (Figure 2). Energy balance was lowest for the LE group at 3 wk postpartum (-3.25 Mcal/d) and it was -4.25 Mcal/d at wk 1 for HE cows. A positive EB was reached at wk 3 for HE cows, but was delayed to wk 7 for LE cows (Figure 3).

Body weight treatment by week interaction was significant ($p < .05$) for the linear component showing an increased rate of gain for LE cows compared to HE cows (Figure 4; Appendix Table 10). Body weights at parturition were 576 kg for HE cows and 540 kg for LE cows. Body condition score showed a significant quadratic week by treatment interaction ($p < .01$; Appendix Table 10) from -8 to 25 wk postpartum. Body condition scores for the HE cows at parturition averaged 2.9, while the LE cows scored 2.6. Both BC scores were lowest at 12 wk postpartum, however the nadir was lower for LE cows (HE = 2.7, LE = 2.3; Figure 5). Body condition scores did not attain prepartum scores by 25 wk postpartum.

ENERGY INTAKE &DMI

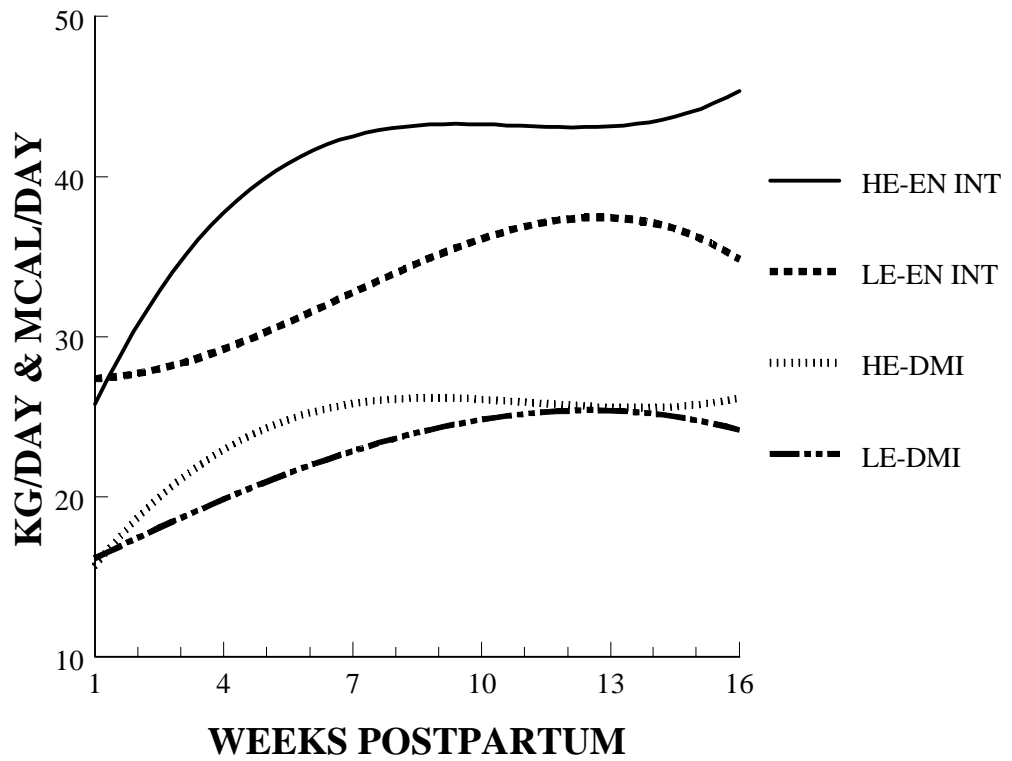


Figure 2. Curvilinear relationships of dry matter intake (DMI) and energy intake (EN INT) for the high energy (HE) and low energy (LE) groups from 1 wk to 16 wk postpartum.

ENERGY BALANCE

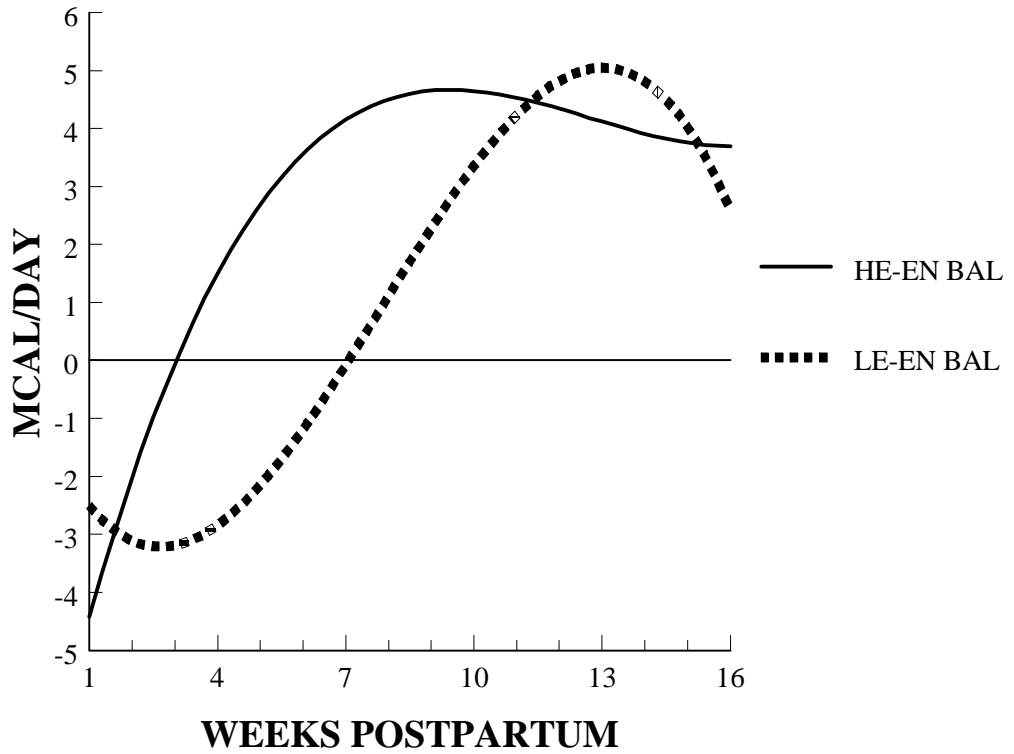


Figure 3. Curvilinear relationship of energy balance (EN BAL) for the high energy (HE) and low energy (LE) groups from 1 wk postpartum to 16 wk.

BODY WEIGHT

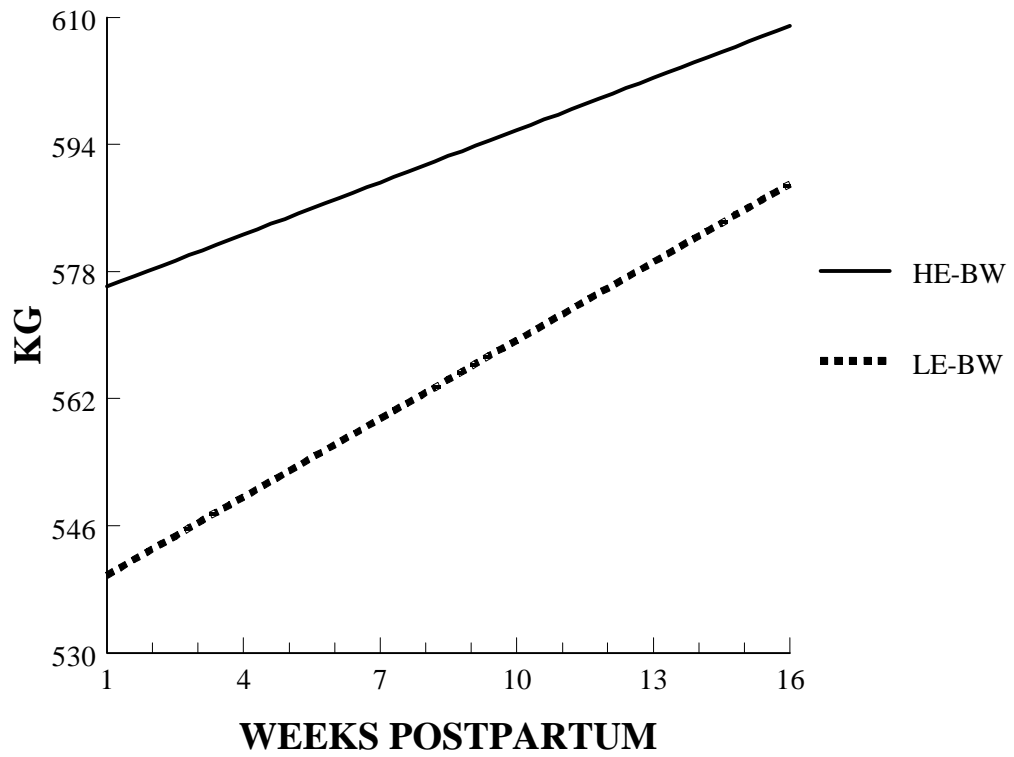


Figure 4. Linear relationship of body weight (BW) for the high energy (HE) and low energy (LE) groups from 1 to 16 wk postpartum.

BODY CONDITION SCORE

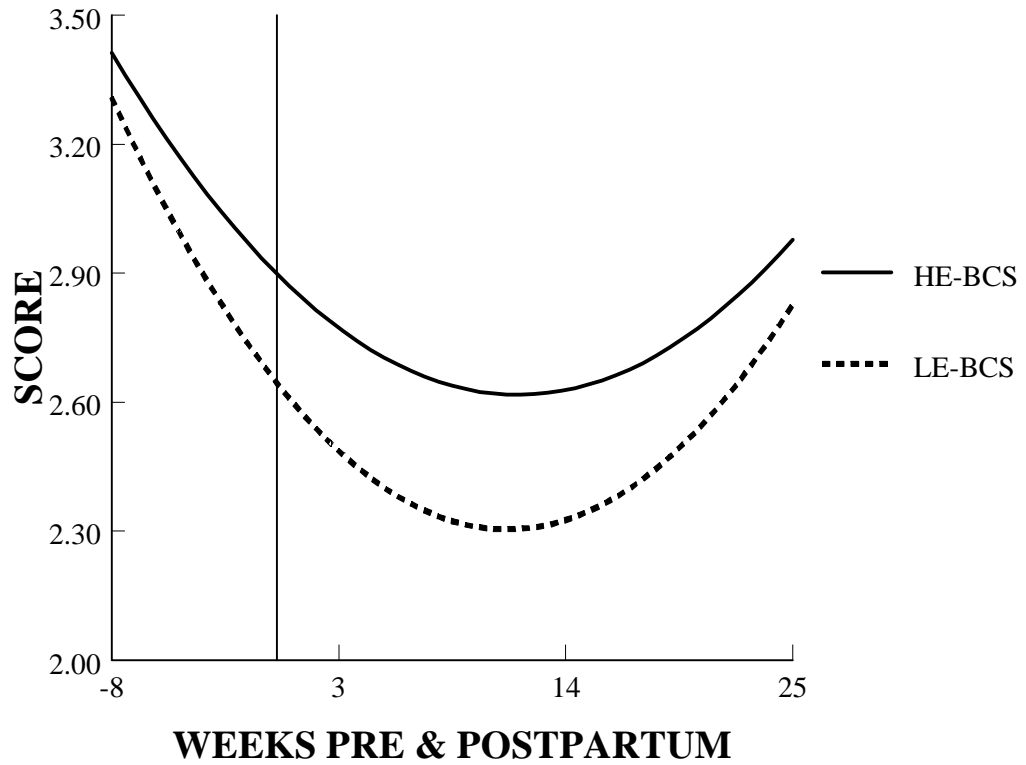


Figure 5. Quadratic relationship of body condition score (BCS) for the high energy (HE) and low energy (LE) groups from -8 to 25 wk postpartum.

Milk yield averaged $41.6 \pm .3$ kg/d (mean \pm SE) for the HE cows and $32.8 \pm .3$ kg/d for LE cows (Figure 6). The significant cubic day postpartum by treatment interaction (Appendix Table 11; $p < .01$) showed a peak in milk yield at wk 7 for HE cows, while the peak for LE cows was between wk 5 and 6 postpartum. Milk fat changed in a quadratic manner over time ($p < .01$; Appendix Table 11). Milk fat was lowest for HE cows at 8 wk postpartum and at 11 wk postpartum for LE cows. At wk 16 the milk fat percentages for both treatments were increasing (HE-5.1%, LE-4.0%; Figure 7).

The sizes of the largest follicle at each aspiration session from d 30 to 100 postpartum were significantly ($p < .05$) affected by the cubic days postpartum by treatment interaction (Appendix Table 12), with LE cows having greater development throughout the aspiration period than HE cows (Figure 8). The average sizes of the largest follicle were greater for LE cows than HE cows. These individual follicles were larger in diameter at the first aspiration session. While the largest follicles in LE cows did not approach the initial aspiration size, the largest follicle in the HE group was approaching initial aspiration size at 90 d postpartum. Additionally, the number of follicles ≤ 5 mm had a negative impact ($b = -.14263$) on size of the largest follicle, while the number of follicles 10 to 14 mm and ≥ 15 mm had a positive association with the size of the largest follicle ($b = .7420$ and 3.8147 , respectively).

The number of follicles ≤ 5 mm was affected by the cubic days postpartum by treatment interaction (Appendix Table 13). Numbers of follicles ≤ 5 mm peaked at 7.2 at 49 d postpartum for HE cows, while in LE follicles ≤ 5 mm peaked at 6.1 at 47 d postpartum and remained relatively constant thereafter (Figure 9). Numbers of follicles 6

MILK YIELD

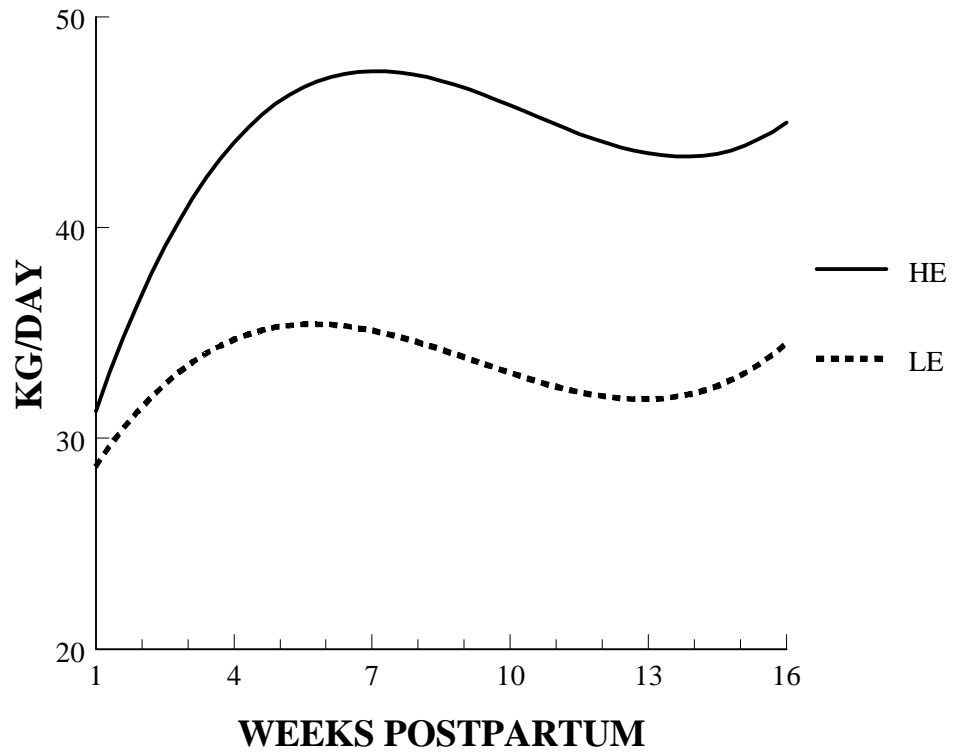


Figure 6. Milk yield ($\pm .3$) for the high energy (HE) and low energy (LE) groups from 1 to 16 wk postpartum.

MILK FAT

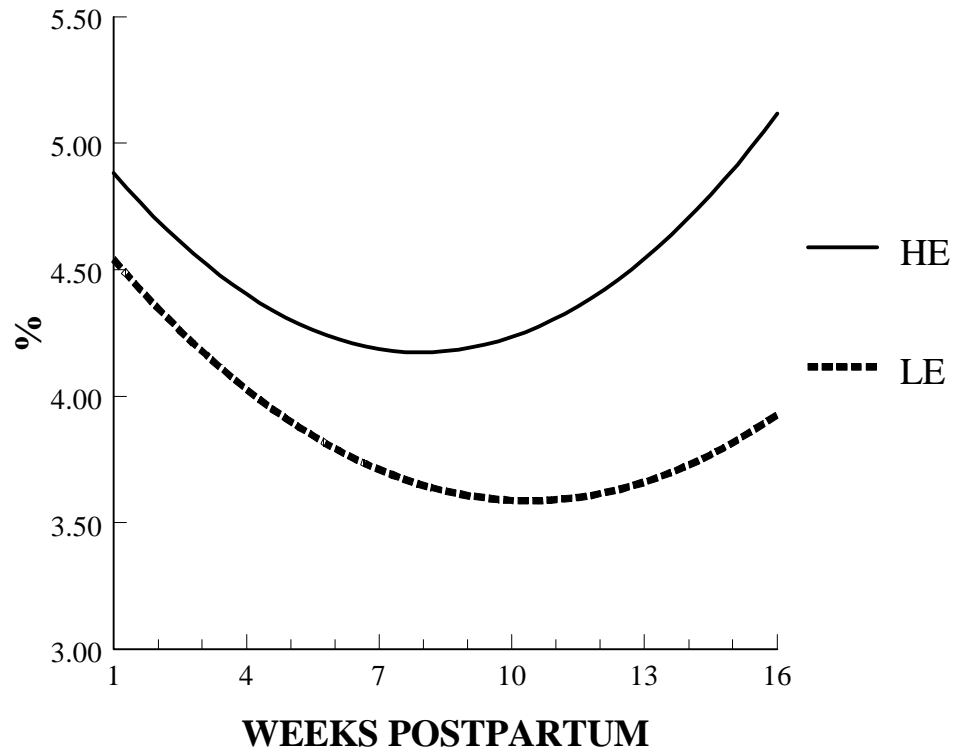


Figure 7. Milk fat levels for the high energy (HE) and low energy (LE) groups from 1 to 16 wk postpartum.

LARGEST FOLLICLE SIZE

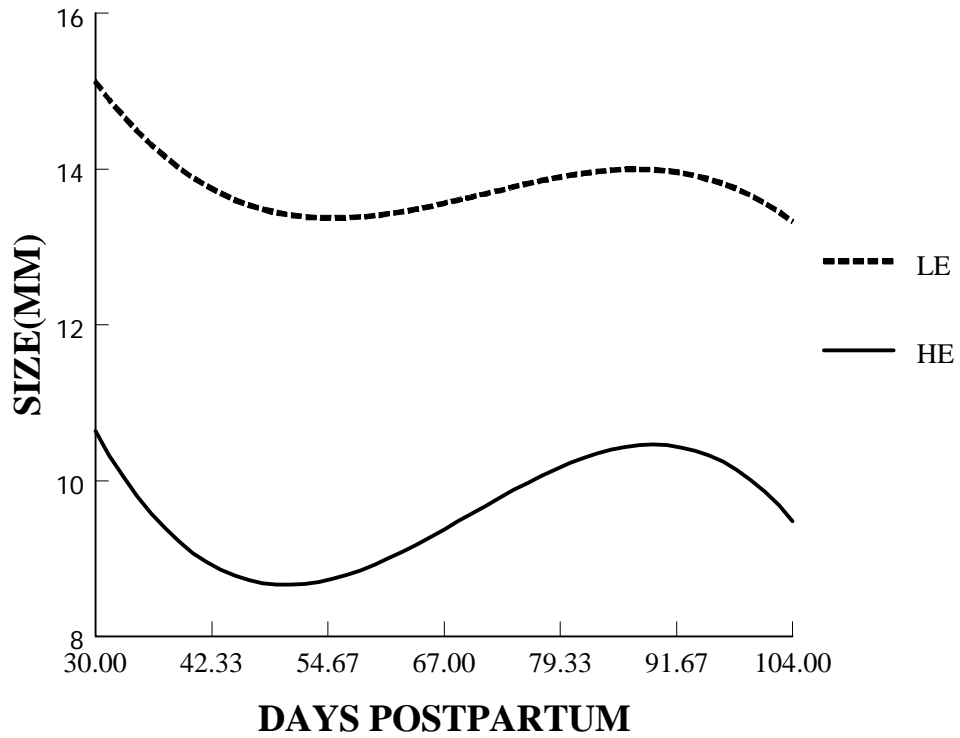


Figure 8. Largest follicle sizes recorded for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum, viewed via ultrasound.

to 9 mm in HE cows were slightly above one per aspiration at 30 d postpartum (Figure 9) and increased linearly (Appendix Table 13) to almost 3 by 104 d postpartum, while in LE cows they were 2.0 and 2.4, respectively. Numbers of follicles 10 to 14 mm in diameter changed in a curvilinear manner for HE and LE cows over the aspiration period (Appendix Table 13). Number of 10 to 14 mm follicles changed in a curvilinear manner (Appendix Table 13) and were at a nadir for HE cows at 45 d postpartum and peaked at 93 d postpartum, whereas the nadir and peak for LE cows was d 40 and d 88, respectively (Figure 10). However, greater numbers of follicles 10 to 14 mm ($p < .01$) and ≥ 15 mm ($p < .05$) were found in the fall than in the spring ($1.98 \pm .08$ and $.50 \pm .06$ vs $1.11 \pm .3$ and $.23 \pm .07$, respectively). Number of follicles ≥ 15 mm changed in a curvilinear manner (Appendix Table 13) and were lowest around 45 d postpartum and peaked about 90 d postpartum with LE cows averaging slightly more than HE cows throughout (Figure 10). Cow (treatment by repetition) was significant ($p < .01$) for both follicles ≤ 5 mm and 6 to 9 mm (cow ranges for ≤ 5 mm follicles was $3.89 \pm .65$ to $10.06 \pm .67$, and for 6 to 9 mm follicles was $.56 \pm .55$ to $3.96 \pm .48$; Table 13).

The overall oocyte numbers per aspiration increased ($p < .05$) linearly from d 30 to 104 postpartum (Appendix Table 14; Figure 11) with the rate of increase greater in LE than HE cows. The mean ova quality scores was weighted by the number of oocytes for each cow, and was determined by assigning each oocyte graded a specific value (1-4) and adding these scores for each cow for each aspiration. This number was divided by the total number of oocytes at that aspiration session. High energy cow mean oocyte quality scores at d 30 postpartum peaked at 2.1 and declined linearly from that point to 1.7 at d

FOLLICE NUMBERS BY SIZE

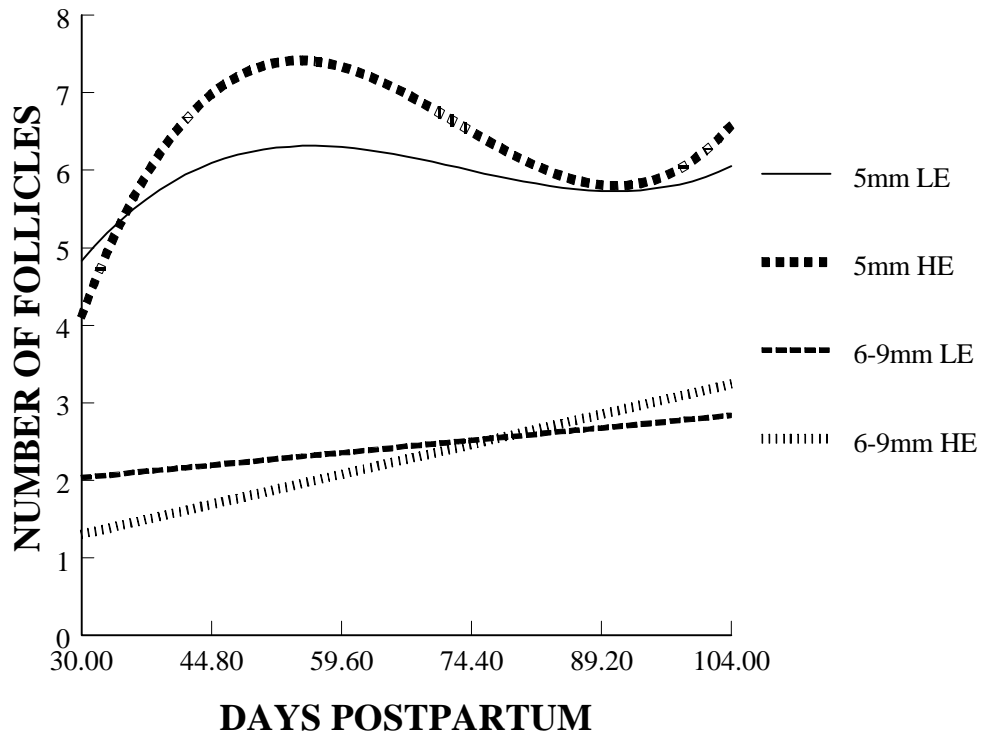


Figure 9. Number of follicles ≤ 5 mm and 6 to 9 mm viewed via ultrasound for High Energy (HE) and Low Energy (LE).

FOLLICLE NUMBERS BY SIZE

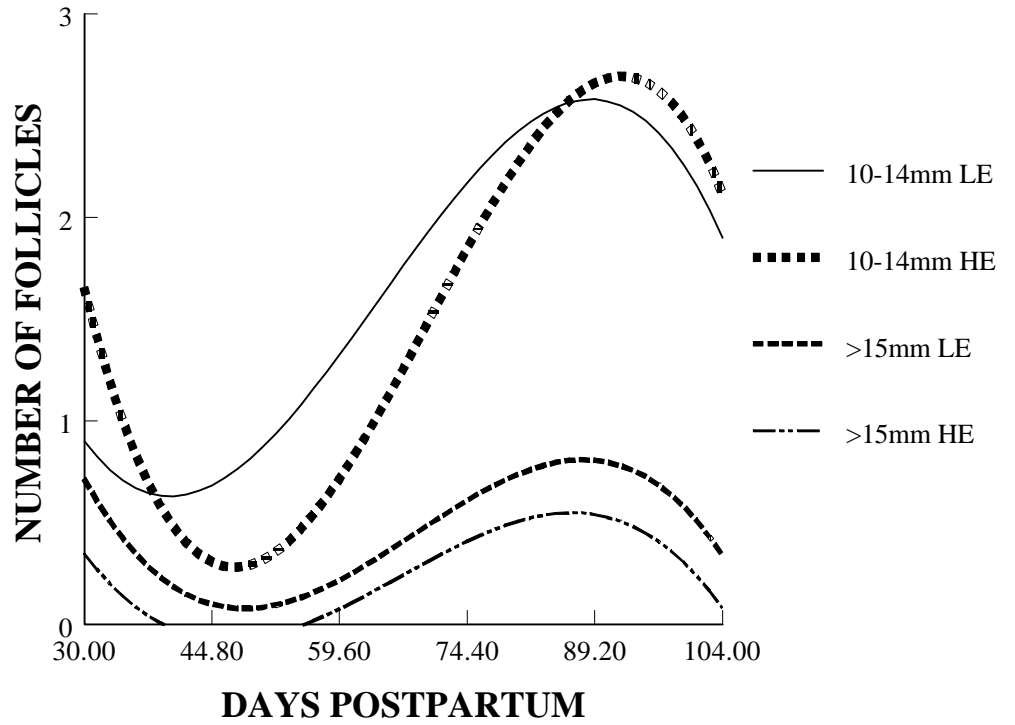


Figure 10. Number of follicles 10 to 14 mm and ≥ 15 mm viewed via ultrasound for high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.

OOCYTE NUMBERS

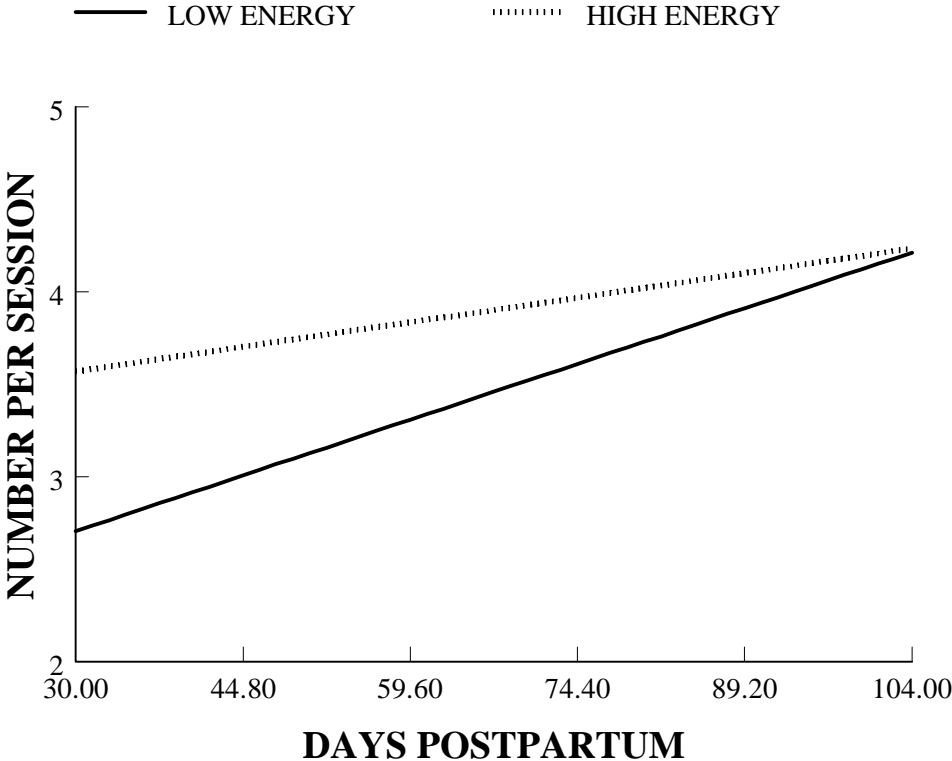


Figure 11. Number of oocytes collected via ultra-sound guided transvaginal follicular aspiration (TVFA) from 30 to 104 d postpartum.

104 postpartum. The mean quality scores for LE cows also declined throughout the experiment. However, the LE cow scores only dropped from 2.05 to 2.0 (Figure. 12). Total oocyte number, mean ova quality score and total ova quality score were significantly different ($p < .01$) for the cow nested in treatment by repetition interaction (cow range for total oocyte numbers: $.90 \pm .68$ to $7.34 \pm .58$; cow range for total oocyte quality: 3.05 ± 1.78 to 13.64 ± 1.21 ; cow range for mean quality score: $1.44 \pm .14$ to $2.51 \pm .14$; Appendix Table 14). Total oocyte quality scores were determined by assigning each oocyte a separate grade from initial quality, of 1 to 4 with 1 being poor and 4 good +. The total figure derived was the sum of all oocytes quality scores at that aspiration session. Both total oocyte number and mean ova quality score were significantly affected by linear days postpartum by treatment interaction and aspirator effect ($p < .01$ or $p < .05$; Figure 11 and 12). Both total oocyte number and mean oocyte quality scores had an aspirator effect ($p < .05$), with total numbers ranging from $3.8 \pm .4$ to $4.6 \pm .2$ and mean oocyte quality scores from $1.7 \pm .05$ to $1.95 \pm .10$.

Analyses of variance for individual oocyte quality scores are in Appendix Table 15. The good quality oocytes were not effected by any of the variables tested. The HE group produced more good + oocytes ($1.5 \pm .2$ vs $1.4 \pm .1$) than the LE cows ($p < .05$). The numbers of good - oocytes were significantly affected by the linear days postpartum by treatment interaction ($p < .01$; Appendix Table 15). The HE cows had 1.3 good - oocytes at d 30 postpartum and about 1.5 at d 104 postpartum, while the LE cows had 1.1 at d 30 postpartum and the numbers increased to around 2.1 by d 104 postpartum (Figure 13). Poor oocyte quality scores were significantly affected by ($p < .01$) cow nested in treatment

OOCYTE QUALITY SCORES

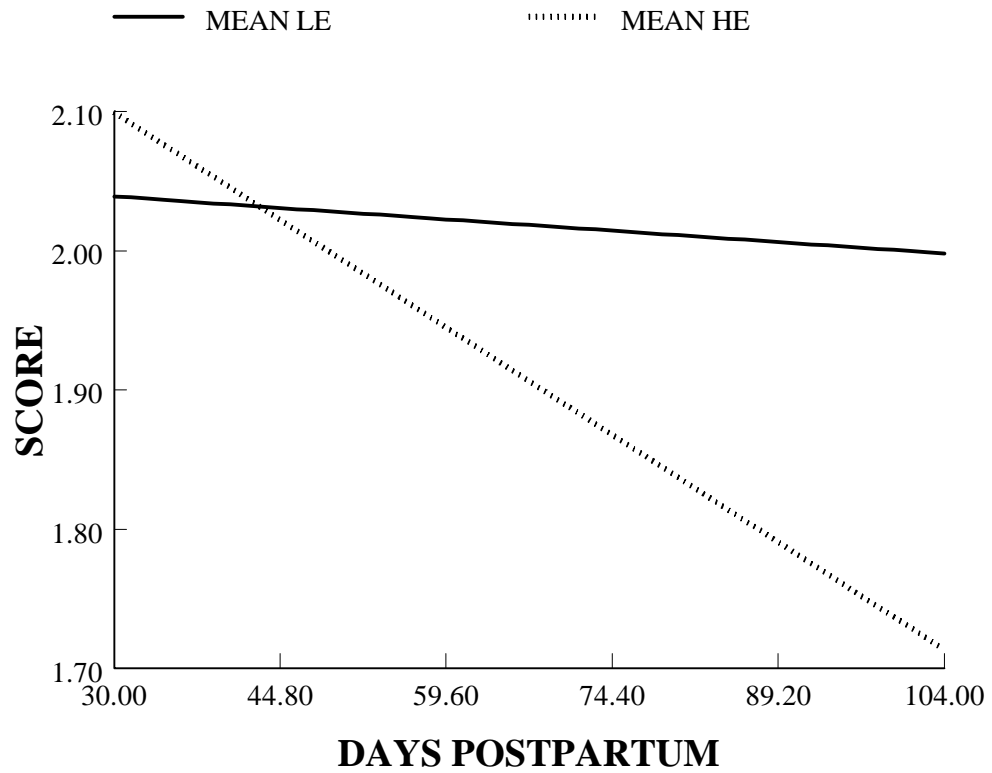


Figure 12. Mean quality score of oocytes collected via ultra-sound guided transvaginal follicular aspiration (TVFA) for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.

OOCYTE NUMBERS BY QUALITY

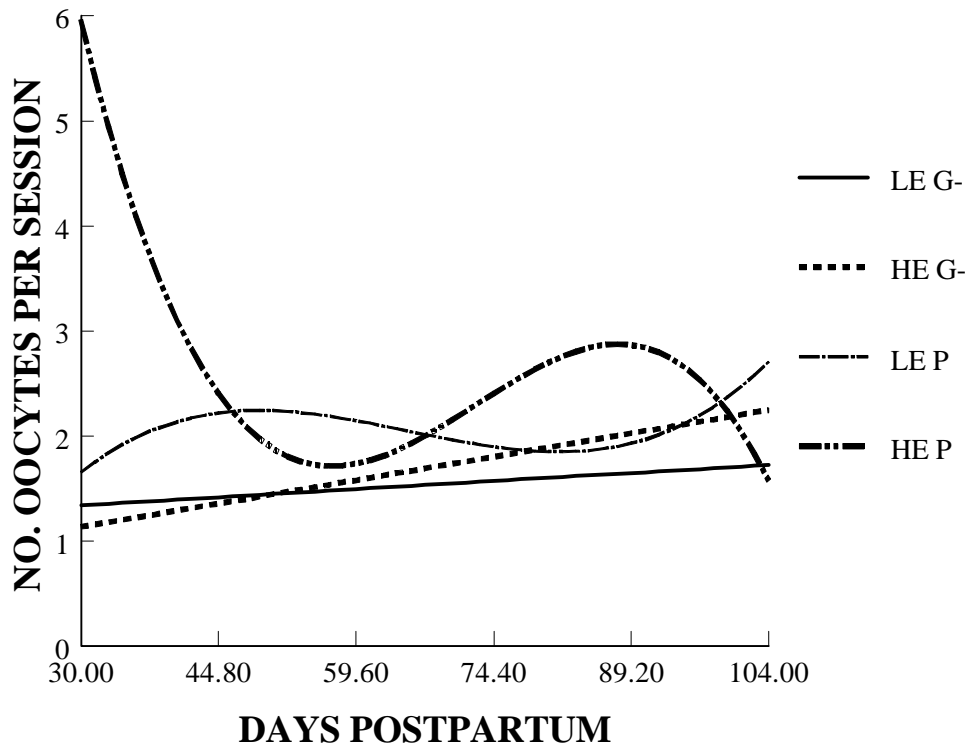


Figure 13. Number of oocytes collected via ultra-sound guided transvaginal follicular aspiration per quality group for the high energy (HE) and low energy (LE) group from 30 to 104 d postpartum (G-: good minus; P: poor)

by repetition (cow ranges for poor oocytes: $.87 \pm .54$ to $4.88 \pm .41$) interactions, as well as for the cubic components of the days postpartum by treatment interaction (Appendix Table 15). Cows in HE had more poor quality oocytes during the first 2 wk of aspiration and between 70 to 90 d postpartum than LE cows (Figure 13). Poor quality oocyte numbers were relatively stable for LE cows during the aspiration period. The aspirator effect was most evident in the poor quality oocytes, with the numbers ranging from $2.37 \pm .17$ to $3.00 \pm .15$.

Analysis of follicular fluid hormones showed that follicular fluid IGF-I was higher ($p < .05$) in HE ($2.3 \pm .2$ ng/ml) than in LE ($1.6 \pm .2$ ng/ml) cows (Appendix Table 16). Follicular fluid estradiol was higher in HE (302.0 ± 59.3 ng/ml) than in LE cows (148.7 ± 53.3 ng/ml; $p < .09$). The days postpartum by treatment interaction ($p < .09$) for follicular fluid estradiol is in Figure 14. Follicular fluid estradiol increased in HE cows but remained relatively stable for LE cows from d 30 to 104 postpartum. There were significant cows nested in treatment by repetition interactions ($p < .01$ for FF IGF-I and FF E₂; Appendix Table 16). The cow ranges for IGF-I were $.91 \pm .2$ to $3.86 \pm .24$ ng/ml and for follicular fluid estradiol ranges were 20.1 ± 104 to 756 ± 120 ng/ml. Mean follicular fluid progesterone was 3.54 ± 6.15 μ g/ml. The follicular fluid estradiol and progesterone ratio was not significantly affected by any variables in the model (Appendix Table 16).

Serum progesterone was affected by the cubic days postpartum by treatment interaction ($p < .01$) and by cow nested in treatment by repetition ($p < .01$; Appendix Table 17). Serum progesterone peaked at 2.5 ng/ml serum at d 39 for the HE group and 1.5 ng/ml serum at d 35 postpartum for LE (Figure 15). Following the beginning of

FOLLICULAR FLUID HORMONES

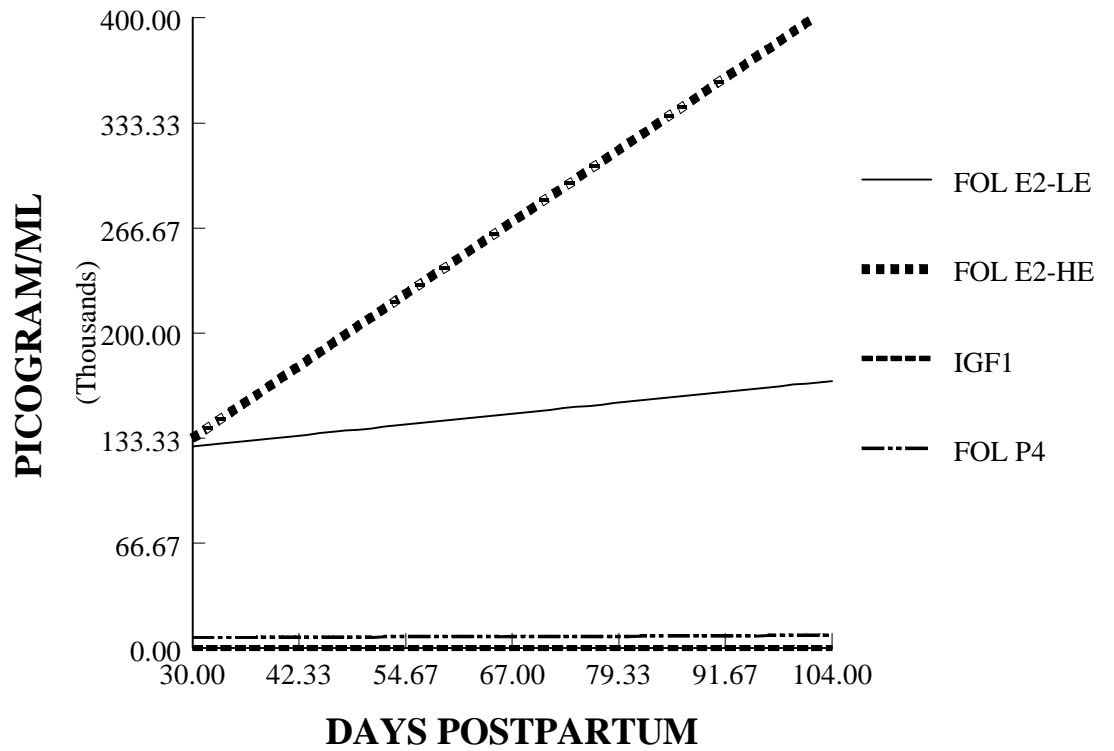


Figure 14. Follicular fluid hormone concentrations (IGF1: Insulin-like growth factor-I; E2: Estradiol; P4: Progesterone) for the high energy (HE) and low energy (LE) groups from 30 to 104 d postpartum.

SERUM PROGESTERONE

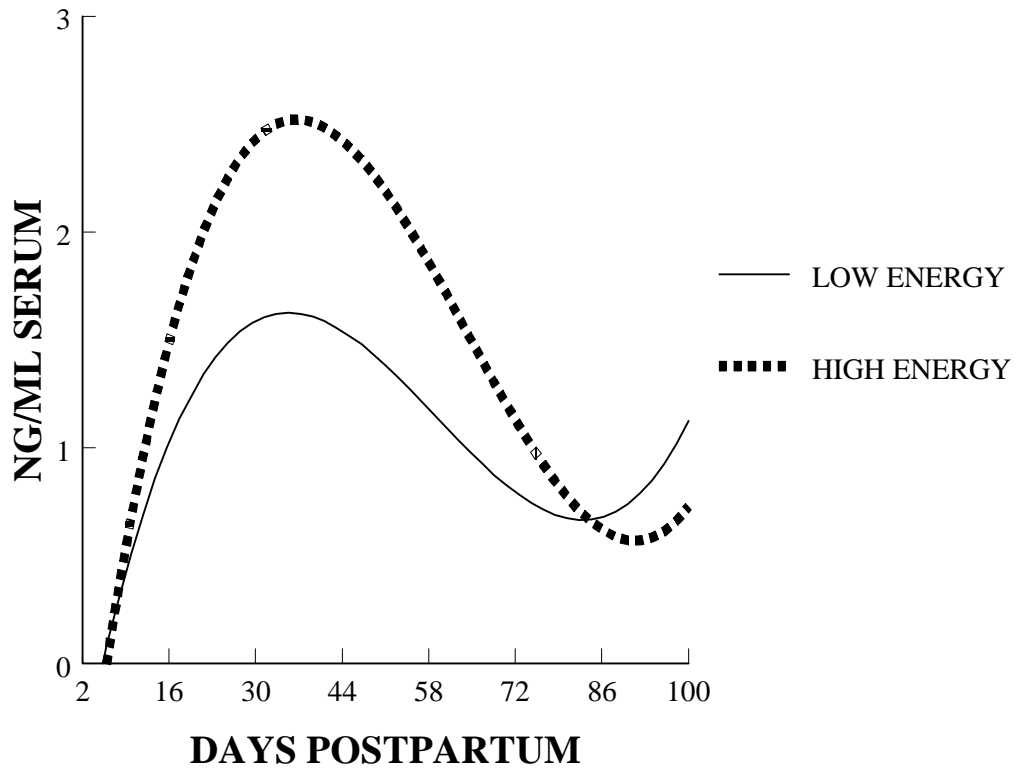


Figure 15. Serum progesterone concentrations for the high energy and low energy groups from 30 to 104 d postpartum.

BASAL LH

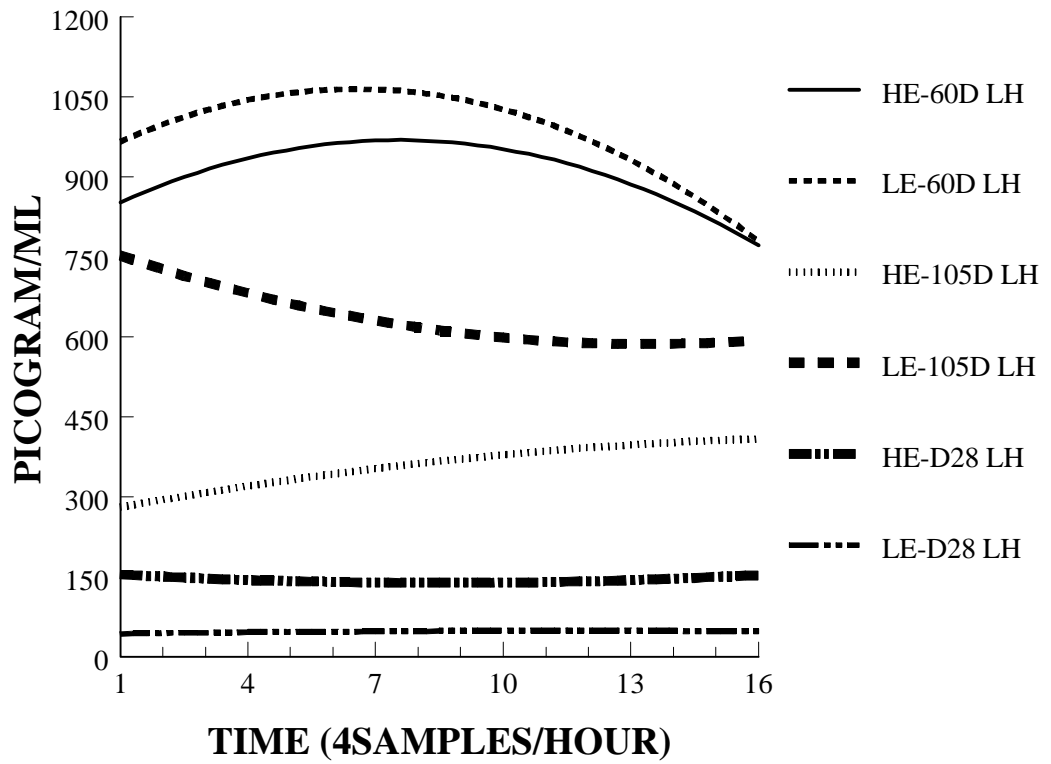


Figure 16. Basal LH concentrations for the high energy (HE) and low energy (LE) groups at 28, 60 and 104 d postpartum.

aspirations at d 30 postpartum, and after the CL had regressed serum progesterone generally was below 1 ng/ml. There was a significant cow by period nested in treatment interaction ($p < .01$) and a significant treatment by period by time (quadratic) interaction ($p < .01$) for both LH and FSH (Appendix Table 18). Basal LH patterns were highest for both treatments at d 60 (HE-1050 pg/ml, LE-925 pg/ml) and lowest at d 28 (HE-150 pg/ml, LE-50 pg/ml; Figure 16). However, basal changes in LH were intermediate at d 105 postpartum and patterns were opposite over the sampling time. Basal LH was affected by cow nested in treatment ($p < .01$).

Basal FSH was affected also by period ($p < .05$; Appendix Table 18). Mean basal FSH was lowest at d 28 (172.7 ± 7.8 ng/ml) and increased to 521 ± 24.8 at d 60 and 650.1 ± 24.1 pg/ml at d 105. At d 60 basal FSH in the HE group peaked at 400 pg/ml, while the LE group had a peak of 680 pg/ml (Figure 17). At d 28 both the HE and LE group basal FSH concentrations were low and similar. At d 28, at d 105 basal FSH concentrations were highest and patterns were similar.

The oocytes collected by TVFA were matured, fertilized and cultured in vitro. The overall development was poor for both treatments in both sessions with no development beyond the early morula stage. The total number of oocytes cultured for the LE and HE treatment groups were 717 and 962 oocytes, respectively, with the development being 14.1 ± 1.2 for the LE group and 20.3 ± 1.2 for the HE group. Slight seasonal differences were observed, with 1002 oocytes collected in the fall session ($91.1/\text{cow}$) and decreasing to 677 in the spring ($75.2/\text{cow}$). The average number of oocytes that cleaved per aspiration in the fall was 1.7 for HE and 2.6 for LE, while the

BASAL FSH

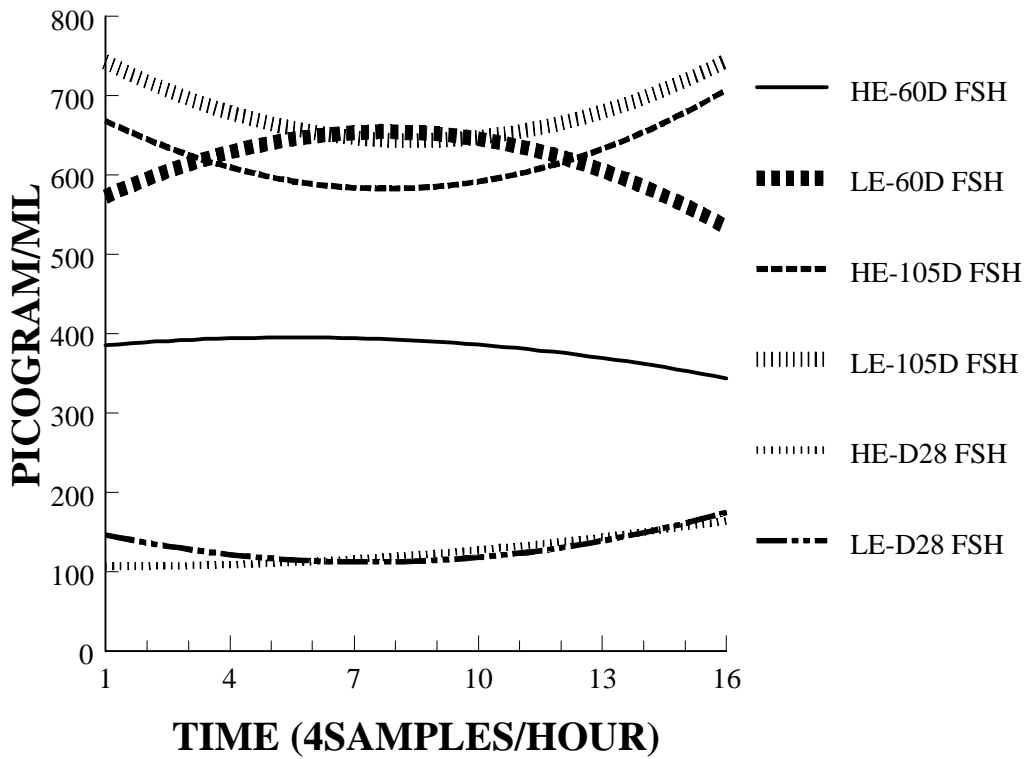


Figure 17. Basal FSH concentrations for the high energy (HE) and low energy (LE) groups at 28, 60 and 104 d postpartum.

spring values declined to 1.2 and 1.7, respectively (fall development: 16.0 ± 1.3 ; spring development: 18.5 ± 1.2). The average mean development scores for both treatments are listed in Table 8.

At the conclusion of the experiment, the cows in both treatment groups were observed for standing heat and serviced by artificial insemination. The number of mounts recorded for each animal in estrus prior to breeding differed between seasons (Fall: 6 and 4.1; Spring: .75 and 0; HE and LE, respectively). The pregnancy rate for the fall cows, for both groups was 91%, with an average of 1.25 services per cow (67.8 ± 11.5). Only one cow (LE) was culled. However, the spring cows had a zero percent pregnancy rate, no reported standing heats and only 1 service was performed on each of two cows out of nine (0 ± 32.4). The final conception rates were 50.7 ± 20.7 and 14.9 ± 20.0 , for the high and low energy groups, respectively.

Table 8. Mean oocyte development scores for high energy and low energy cows, per aspiration

Development	HE	LE
2 cell	1.5	2.1
4 cell	1.4	1.5
8 cell	1.4	1.1
10 cell	1.5	0.5
16 cell	0	0.5
morula	0.5	0
blastocyst	0	0

Average number of oocytes that cleaved for both the high energy (HE) and low energy (LE) groups per aspiration session.

DISCUSSION

After parturition, the ability of a lactating dairy cow to resume estrus is dependent upon the EB of the animal. Infertility is brought on primarily by low DMI which keeps the cow in NEB (Nebel et al., 1993). Both the energy intake and DMI in this study peaked at 16 wk postpartum for the HE cows and at wk 13 for the LE group (40 and 35 kg/d; 24 and 26 kg/d, respectively). After wk 13, energy intake and DMI began to decline for the LE cows, while HE cows continued to increase. Energy balance usually reaches its nadir during wk 1 to 2 of lactation (Villa-Godoy et al., 1990). However, the energy balance for LE cows did not reach its lowest point until wk 3 postpartum before beginning to recover, this agrees with the work of Villa-Godoy et al. (1990). These results demonstrate that the two imposed diets achieved the effects desired in carrying out this experiment. The success of obtaining differences between treatments assured the experiment two distinct groups for evaluation of the effects of EB on bovine reproductive functions.

Body weights for both groups increased in a linear fashion with LE animals having the greater rate of weight gain. This contradicts the findings of Yung et al. (1996), whose work with heifers indicated that only HE animals gained BW, while LE heifers lost .410 kg/d over a 10 wk trial period, and Rhodes et al. (1996) who reported a loss of .8 kg/d in heifers on “restricted” diets. The increasing BW of the LE cows on the “restricted” diet may have been due to a shift in energy utilization away from milk production, as milk yields were lower than in the HE cows. In contrast to the heifers (Yung et al., 1996; Rhodes et al., 1996), the change in metabolic activities lead to the increase in BW.

Another possible theory could be that the LE animals consumed more feed, therefore giving them a greater gut fill than the HE cows. Moreover, the final BC scores were higher in the HE group, with both groups at their lowest at 12 wk postpartum. The HE group lost less BC over the course of the experiment. In both groups BC scores were similar at 8 wk prior to calving, however at the lowest BCS the HE group was approximately .3 above the LE cows.

Milk yield averaged 8.8 kg/d higher for HE than LE cows suggesting a direct relationship between EB and milk production. These results agree with the findings of Grimard et al. (1995), who reported that cows in NEB had lower milk production than those in a positive energy balance. The LE cows in this study peaked in milk production (34 kg/d) at 5 wk postpartum and then steadily declined until 15 wk where milk production began to increase. Although the treatments differed by 8.8 kg/d, the two groups followed the same milk yield pattern over the course of the experiment. In a previous study, Butler and Smith (1989) reported a direct relationship ($r = -.80$) between milk yield and EB, suggesting that an animal must first be in a positive energy balance prior to producing substantial milk yields. The results of this research agree, with the HE cows producing greater amounts of milk than the LE cows throughout the course of the study.

Milk fat fell to its lowest at wk 8 and 11 for HE and LE, respectively. When cows are in NEB, their body fat and protein stores are mobilized to meet the nutritional demands they face. Barbano and Lynch (1997) found these changes to occur when the animal's milk yields begin to increase, resulting in an increase in milk fat concentrations.

Increasing EB was demonstrated to be proportional to the number of large follicles in postpartum dairy cows with significant differences in follicular development related to BC (Lucy et al., 1991). However, LE cows had greater follicular development throughout the aspiration period for the largest follicle sizes from d 30 to 100 postpartum. Boni et al. (1996) found that the dynamics of the follicular wave induced by TVFA caused a rapid turnover of the follicular population and decreased the occurrence of atresia. In the current study follicular aspiration occurred every third and fourth day, ridding the ovary of follicles ≥ 3 mm. Furthermore, the number of ≤ 5 mm follicles had a negative impact ($b = -.14263$) on size of the largest follicle and the number of follicles 10 to 14 mm and ≥ 15 mm had a positive association with the size of the largest follicle ($b = .74$ and 3.81 , respectively), suggesting that as a greater number of follicles mature there is an inhibitory effect on the new follicular wave. This was the case for the LE cows (Figure 8) that had a maximum of 15 mm size follicles compared to 11 mm size follicles for the HE cows. Differences among small and intermediate follicle numbers (Figure 9 and 10) may indicate a possible treatment effect on ≤ 5 mm, 6 to 9 mm and 10 to 14 mm follicles.

Body condition scores exert an effect on the number of small follicles, with their numbers being higher in cows with BC scores of 3 to 5 (Dominguez, 1995). Follicular growth was affected in cows with a low BC fed diets that did not meet essential requirements (Perry et al., 1991). The numbers of small follicles (≤ 5 mm) peaked at 49 d at 7.2 and 47 d at 6.2 for ≤ 5 mm for HE and LE cows, respectively, and may be related to differences in BW and BC scores during the early postpartum period. The numbers of 6 to 9 mm follicles increased in a linear manner over the aspiration period, starting with 1

per aspiration at 30 d postpartum and increasing to almost 3 by 104 d postpartum for HE cows, while the LE cows only had a slight increase from 2 to 2.4 in 6 to 9 mm follicles per aspiration session. Numbers of 10 to 14 mm follicles were at a nadir at 45 d and 40 d postpartum and peaked at 93 d and 88 d postpartum for HE and LE cows, respectively. These changes in follicular population support the observations of Murphy et al. (1990), who found that recruitment and growth of ovarian follicles increases with time postpartum and is in accordance with Grimard et al. (1995), who reported striking differences in follicular growth between underfed and adequately fed cows up to 50 d postpartum. Theoretically, greater numbers of large follicles should be observed in adequately fed cows when compared to cows in NEB, suggesting that physiological mechanisms responsible for ovarian follicular growth and dominance may become functional at an earlier postpartum stage than in underfed cows. Burns et al. (1997) found that dietary restriction decreased the follicular growth of the largest follicle in contrast to this study. Interestingly, there were greater numbers of 10 to 14 mm and ≥ 15 mm follicles found in the fall season when compared to the spring aspiration trials. The difference between seasons could be due to weather or season of calving (Ray et al., 1992). Total follicle numbers increased with respect to days postpartum, while the number of CL decreased as days postpartum increased. After 3 wk of aspiration CL were not detected by ultrasound and serum progesterone remained < 1 ng/ml until the end of the study.

The total oocyte numbers recorded per aspiration increased linearly from 30 d to 100 postpartum for both treatments, with a greater increase in oocyte numbers for LE cows. At d 30 postpartum the difference between the two groups was about one oocyte

per session. This gap declined and closed as days postpartum increased to 104, and resulted in an average of 4 oocytes per session for both treatments. This trend corresponds to that of BW and BC scores for the two groups, both following the same pattern with the LE cows having the greater rate of increase in weight gain and BC overall. Kruip et al. (1996) found no difference among the number of oocytes collected via aspiration, between animals on either high or low energy diets.

Mean oocyte quality scores for LE cows were stable throughout the experiment. However, in HE cows quality scores decreased over time. Both treatments at 30 d postpartum were at their peak for mean quality. The oocyte quality scores were lower (good -) than anticipated for both treatments when compared to the work of Gibbons et al. (1995) who recovered 52.5% good quality oocytes from twice weekly TVFA. However, the trends over the course of the experiment were comparable to those found by Carlin (1995) with levels of good - oocytes rising by 104 d postpartum (Figure 13).

The HE group produced a greater number of good + oocytes throughout the experiment (70 to 90 d postpartum), but had more poor quality oocytes. However, this was only for the first 2 wk of aspiration. Konishi (1996) using a comparable grading scale found that more than 60% of COCs collected through TVFA were classified as good 0 to poor. Leibfried and First (1979) found that bovine oocytes obtained from follicles varied in the appearance of ooplasm and chromatin configuration. These physical differences were present in a larger proportion of follicles > 3 mm than smaller size follicles. Follicles > 3 mm contain a greater number of degenerating oocytes. Britt (1991) found that preantral follicles exposed to deleterious environmental conditions had altered

gene expression leading to impaired and altered development ending in dysfunctional follicles, CL and low quality oocytes around 80 to 100 d postpartum. There are no reliable methods for predicting the quality of an oocyte that is obtained from a given size follicle. Dominguez (1995) reported that a mean proportion of normal oocytes decreases with increasing follicular size suggesting a normal process of follicular atresia. Factors that may have led to greater numbers of good - and poor quality oocytes obtained from both treatments, when compared to higher quality oocytes numbers, could have been due to varying TVFA techniques performed by the different aspirators.

Available in vivo data on follicular fluid IGF-I concentrations show an increase in IGF-I with an increase in follicular size in both pigs and cattle (Einspanier, 1993). The expression of IGF-I in smaller follicles seems to be very low and comparable to blood levels (Einspanier, 1993). There was a higher follicular fluid concentration of IGF-I in HE ($2.3 \pm .2$ ng/ml) than LE cows ($1.6 \pm .2$ ng/ml) and no difference over the time course of the aspirations. This result conflicts with data presented by Spicer et al. (1991), who reported that reduced dietary energy intake had no effect on IGF-I concentrations in follicular fluid. Reduced IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus. Echtenkamp et al. (1994) reported that follicular fluid IGF-I levels increased as follicular fluid estradiol and follicle size increased. Although we report dietary energy differences in follicular fluid IGF-I, the absolute values were lower than those of de la Sota et al. (1996). However, our aspirations were at 3 to 4 day intervals and the size of the largest follicle at each aspiration

only ranged from 13 to 15 mm for LE cows and 8.5 to 11 mm for HE cows. Thus, it may be unrealistic to expect dramatic changes in follicular fluid IGF-I.

In the present study, follicular fluid estradiol increased in HE cows but remained stable for LE cows during the course of aspirations (Figure 14). Estradiol levels were higher in large dominant follicles, with rising concentrations during follicular growth (Spicer and Zinn, 1987). Spicer et al. (1991) reported higher estradiol concentrations in estrogen active (healthy) follicles in heifers, as well as Smith et al. (1996) who reported healthy large antral follicles in cattle are characterized by high intra-follicular estradiol concentrations. Ginther et al. (1997) reported that follicular fluid estradiol increases in growing follicles, with the concentration of estradiol being approximately 100 ng ml^{-1} in the dominant follicle ($> 8\text{mm}$) and increasing curvilinearly. The production of estradiol by the maturing ovarian follicle seems to be crucial for the growth of granulosa, theca cells and development of the mature follicle (Rhodes et al., 1996). The greater IGF-I concentrations in follicular fluid of HE cows and the increases in follicular fluid estradiol as lactation advanced support the data of Echtenkamp et al. (1994), except that the largest follicle was smaller in HE cows than LE cows. This would suggest that the higher energy diet may provide an element of follicular development not dependent on size alone.

Serum progesterone levels peaked at 2.5 ng/ml at 39 d for HE and 1.5 ng/ml at 35 d postpartum for LE cows. This supports Spicer et al. (1990), who observed that progesterone concentrations in serum during diestrus of the first and second estrous cycles were greater in PEB than NEB cows, suggesting that luteal function is adversely affected by NEB. Villa-Godoy et al. (1990) also observed an increase in progesterone secretion

during the second and third estrous cycle postpartum. Rhodes et al. (1996) reported lower peripheral serum concentrations of progesterone in animals fed a restricted diet. Cows with restricted diets may have lower progesterone levels due to lower progesterone output by the CL. A reduced level of serum progesterone causes an increase of LH pulse frequency, possibly modifying the secretion of other paracrine and autocrine functions (Dominguez, 1995). Britt (1991) conducted hormonal studies for five estrous cycles, and noted that progesterone levels decreased over time. Burns et al. (1997) found that serum progesterone concentrations did not decline until the cycle before anestrus in beef cattle fed diets limited in energy. Differences in serum progesterone were not reflected in follicular fluid progesterone, and associations with follicular fluid IGF-I and follicular fluid estradiol remain to be established. The role of progesterone as a precursor to estradiol in the follicle needs further evaluation (Giudice, 1992).

Basal FSH was highest for both treatments at 105 d postpartum and at its lowest at 28 d postpartum. Similarly, basal changes in LH were lowest at d 28 postpartum but highest at d 60 postpartum, agreeing with Grimard et al. (1995) who noticed that with increasing time postpartum, LH pulse frequency increases. Ahmad et al. (1996) also reported an increased pulse frequency of LH in animals that had greater numbers of primordial follicles. However, Burns et al. (1997) reported cows fed restricted diets had a lower LH pulse frequency when compared to control animals. The low LH base lines occurring at the onset of NEB in LE cows may be because after calving the milk yield needs more energy than is supplied by the diet. This lack of energy leads to a lower glucose concentration, inducing lipolysis and resulting in a lower LH release (Kruip et al.,

1996). At 60 d FSH in HE peaked at 400 pg/ml and LE peaked at 750 pg/ml, the greatest differences in concentrations between treatments in a sampling period. At 28 d basal FSH was low and similar in both treatments. Reduced secretion of basal LH is believed to be due to a reduction in LHRH release from the hypothalamus, not by an impaired pituitary gland. This reduction in LHRH due to restricted diets is thought to be the main cause of altered reproduction functions, possibly resulting in a cessation of the estrous cycle (Rhodes et al., 1996). Basal concentrations of both LH and FSH had differences due to treatment effects: d 28, both basal LH and FSH concentrations were low; at d 60, HE cows had intermediate FSH concentrations and high LH concentrations, LE cows had high concentrations of both LH and FSH; at d 105 HE cows had high concentrations of FSH, with low LH levels, while LE cows also had high concentrations of FSH, with intermediate LH levels.

In vitro oocyte development was poor when compared to similar studies (Konishi, 1996; Smith et al., 1996; Shioya et al., 1988), with development never surpassing the early morula stage. The reasons for these results could be numerous and more likely a combination of factors preventing blastocyst formation in either treatments. One theory is that the environmental conditions in which the oocytes were collected and subsequently transported was detrimental. Oocytes exposed to ambient temperatures below 35° C during oocyte recovery may be adversely affected, therefore affecting the overall quality of oocytes available for in vitro production. Pollard et al. (1996) showed that chromosomal abnormalities can be brought on through exposure of oocytes to room temperature, leading to a reduction in development in vitro. The group noted that it would be prudent

to maintain oocytes at the physiological range of 37° to 39° C, to insure maximum quantity and quality of recovered oocytes. Since TVFA in this experiment was conducted in an enclosed, yet non-temperature regulated barn, aspirated oocytes were exposed to a wide range of temperatures, sometimes for a period of 30 min.

The second theory is related to the abundant number of poor quality oocytes obtained from TVFA in both treatments. De Loos et al. (1989) reported that oocytes of poor quality have a significantly diminished ability to mature and develop under in vitro conditions. This could be due to oocytes lacking cumulus cells that are needed for proper nuclear and cytoplasmic maturation, leading to the formation of the male pronuclei, essential for further embryonic development. It is thought that the secretions from both the granulosa and cumulus cells promote the synthesis of oocyte glutathione, which is a promoter of male pronuclear formation (Konishi et al., 1995). A further possibility is that nude or corona enclosed oocytes have a reduced penetration rate when exposed to capacitated spermatozoa, therefore reducing normal fertilization rates and ability of the oocyte to develop (Laurincik et al., 1995).

At the completion of the experiment, all cows were synchronized and heat checked. The fall trial resulted in 91% pregnancy rates, with only one culled cow from the LE group. This cow was removed from the herd due to failure to conceive. In contrast, the spring trial resulted in no pregnancies. Only one of the nine cows (LE) was known to have adhesions and was culled for this reason, seven other failed to show a standing heat and were not bred. Only two were bred by AI (1-HE, 1-LE) and neither conceived to a

single service. These results are difficult to explain, since both replicates followed the exact same format.

IMPLICATIONS

This study demonstrated the importance of energy balance as it relates to reproduction in the dairy cow. The low energy diet reduced milk yield, DMI, BCS, FF IGF-I, serum progesterone, and had an impact on the quality of oocytes retrieved through TVFA. After calving, a cow needs to be placed on a diet to maximize DMI. Dairy cattle need adequate nutrients for milk production and must be able to recover from the NEB brought on by calving. Only when the cow is able to function in a positive energy state can she successfully be a high producing, reproductively functioning animal.

LITERATURE CITED

- Ahmad, N., S. W. Beam, W. R. Butler, D. R. Deaver, R.T. Duby, D. R. Elder, J. E. Fortune, L. C. Jr. Griel, L. S. Jones, and R. A. Milvae. 1996. Relationship of fertility patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J. Anim. Sci.* 74(8):1943-1952.
- Barbano, D. M., and J. M. Lynch. 1997. Composition and manufacturing properties of milk. [Http://www.nalusda.gov/bic/BST/ndd...ufacturing_properties_of_milk.html](http://www.nalusda.gov/bic/BST/ndd...ufacturing_properties_of_milk.html).
- Bergfeld, E. G. M., F. N. Kojima, A. S. Cupp, M. E. Wehrman, K. E. Peters, V. Mariscal, T. Sanchez, and J. E. Kinder. 1996. Changing dose of progesterone results in sudden changes in frequency of LH pulses and secretion of 17β estradiol in bovine females. *Biol. Reprod.* 54:546-553.
- Bolt, D. J., and D. W. Caldwell. 1992. Inhibin-like activity in plasma from ovariectomized ewes and serum albumin: pitfalls for radioimmunoassay. *Domest. Anim. Endocrinol.* 9:57-69.
- Boni, R., S. Roviello, and L. Zicarelli 1996. Repeated ovum pick-up in Italian Mediterranean buffalo cows. *Theriogenology.* 46:899-909.
- Braun, R. K., G. A. Donovan, T. Q. Tran, J. K. Shearer, E. L. Bliss, D. W. Webb, D. K. Beede, and B. Harris. 1986. Body condition scoring dairy cows as a herd management tool. *Comp. Contin. Educ. Pract. Vet.* 10:F62-F67.
- Britt, J. H. 1992. Impacts of early postpartum metabolism on follicular development and fertility. *The Bovine Practitioner Proc.* 24:39.
- Burns, P. D., J. C. Spitzer, and D. M. Henricks. 1997. Effect of energy restriction on follicular development and luteal function in nonlactating beef cows. *J. Anim. Sci.* 75:1078-1086.
- Carlin, S. K. 1995. Effects of transvaginal follicular aspiration on oocyte recovery, hormonal profiles before and after GnRH and growth factor influence on embryo development.
- Cox, J. F., J. Hormazabal, and A. Santa Maria. 1993. Effect of the cumulus on in vitro fertilization of bovine matured oocytes. *Theriogenology.* 40:1259-1267.

- De la Sota, R. L., F. A. Simmen, T. Diaz, and W. W. Thatcher. 1996. Insulin-like growth factor system in bovine first wave dominant and subordinate follicles. *Biol. Reprod.* 55:803-812.
- DeLoos, F., C. Van Vliet, P. Van Maurik, and Th.A.M. Kruip. 1989. Morphology of immature bovine oocytes. *Gamete Research.* 24:197-204.
- Dominguez, M. M. 1995. Effects of body condition, reproductive status and breed on follicular population and oocyte quality in cows. *Theriogenology.* 43:1405-1418.
- Echternkamp, S. E., u Usda, ARS, U.S. Meat Animal Research Center, Clay Center, H. J. Howard, A. J. Roberts, J. Grizzle, and T. Wise. 1994. Relationships among concentrations of steroids, insulin-like growth factor-I and insulin-like growth factor binding proteins in ovarian follicular fluid of beef cattle. *Biol. Reprod.* 51(5):971-981.
- Einspanier, R., H. Schuster and, D. Schams. 1993. A comparison of hormone levels in follicle-lutein-cysts and in normal bovine ovarian follicles. *Theriogenology.* 40:181-188.
- Eppig, J. J., M. O'Brien, and K. Wigglesworth. 1996. Mammalian oocyte growth and development in vitro. *Molec Reprod Develop.* 44:260-273.
- Fortune, J.E., and W. Hansel. 1985. Concentrations of steroids and gonadotropins in follicular fluid from normal heifers and heifers primed for superovulation. *Biol. Reprod.* 32:1069-1079.
- Fukui, Y. 1990. Effect of follicle cells on the acrosome reaction, fertilization and developmental competence of bovine oocytes matured in vitro. *Molec Reprod Develop.* 26:40-46.
- Gibbons, J. R., R. L. Krisher, S. K. Carlin, R. E. Pearson,, and F. C. Gwazdauskas. 1995. In vitro embryo production after microinjection and ovarian dynamics following transvaginal follicular oocyte aspiration. *Theriogenology.* 43:1129-1139.
- Gibbons, J. R., W. E. Beal, R. L. Krisher, E. G. Faber, R. E. Pearson, and F. C. Gwazdauskas. 1994. Effects of once- versus twice-weekly transvaginal follicular aspiration on bovine oocyte recovery and embryo development. *Theriogenology.* 42:405-419.
- Ginther, O. J., K. Kot, L. J. Kulick, and M. C. Wiltbank. 1997. Sampling follicular fluid without altering follicular status in cattle: oestradiol concentrations early in a follicular wave. *J Reprod. Fertil.* 109:181-186.

- Granger, A. L., W. E. Wyatt, W. M. Craig, D. L. Thompson, and F. G. Hembry . 1989. Effects of breed and wintering diet on growth, puberty and plasma concentrations of growth hormone and insulin-like growth factor-I in heifers. *Domest. Anim. Endocrinol.* 6:253-262.
- Grimard, B., P. Humblot, A. A. Ponter, J. P. Mialot, Sauvant, and M. Thibier. 1995. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. *J Reprod. Fertil.* 104:173-179.
- Giudice, L C. 1992. Insulin-like growth factors and ovarian follicular development. *Endocr. Rev.* 13:641-669.
- Hasler, J. F., W. B. Henderson, P. J. Hurtgen, Z. Q. Jin., A. D. McCauley, S. A. Mower, B. Neely, L. S. Shuey, J. E. Stokes, and S. A. Trimmer. 1995. Production, freezing and transfer of bovine IVF embryos and subsequent calving results. *Theriogenology.* 43:141-152.
- Hazeleger, N. L., D. J. Hill, J. S. Walton, and R. B. Stubbings. 1993. The interrelationship between the development of bovine oocytes in vitro and their follicular fluid environment. *Theriogenology.* 39:231.
- Konishi, M., Y. Aoyagi, T. Takedomi, H. Itakura, T. Itoh, and S. Yazawa. 1996. Presence of granulosa cells during oocyte maturation improved in vitro development of IVM-IVF bovine oocytes that were collected by ultrasound-guided transvaginal aspiration. *Theriogenology.* 45:573-581.
- Krisher, R. L., J. R. Gibbons, R. E. Pearson, and F. C. Gwazdauskas. 1994. Gene microinjection into the germinal vesicle of bovine oocytes. *Transgenics.* 1:395-398.
- Kruip, Th. A. M., H. Van Beek, A. DeWit, and A. Postma. 1996. Influence of food intake ante partum on the quality of oocytes postpartum in dairy cows. *Reproduction and Animal Breeding Advances and Strategy.* 327-331.
- Laurincik, J., P. Hyttel, W. Baran, F. Schmoll, H. Niemann, G. Brem, and K. Schellander. 1996. Corona radiata density as a non-invasive marker of bovine cumulus-corona-oocyte complexes selected for in vitro embryo production. *Theriogenology.* 46:369-377.
- Leibfried, L., and N. L. First. 1979. Characterization of bovine follicular oocytes and their ability to mature in vitro. *J. Anim. Sci.* 48(1):76-86.
- Looney, C. R., B. R. Lindsey, C. L. Gonseth, and D. L. Johnson. 1994. Commercial Aspects of oocyte retrieval and in vitro fertilization (IVF) for embryo production in problem cows. *Theriogenology.* 41:67-72.

- Lucy, M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. *J. Dairy Sci.* 74:473-482.
- Machatkova, M., E. Jokesova, J. Petelikova, and Dvoracek. 1996. Developmental competence of bovine embryos derived from oocytes collected at various stages of the estrous cycle. *Theriogenology.* 45:801-810.
- Murphy, M. G., M. P. Boland, and J. F. Roche. 1990. Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows. *J. Anim. Sci.* 90:523-533.
- Murphy, M. G., W. J. Enright, M. A. Crowe, K. McConnell, L. J. Spicer, M. P. Boland, and J. F. Roche. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrous cycle in beef heifers. *J. Reprod. Fertil.* 92:333-338.
- Nebel, R. L., and M. L. McGilliard. 1993. Interactions of high milk yield and reproductive performance in dairy cows. *J. Dairy Sci.* 76:3257-3268.
- Park, C. K., O. Oghoda, and K. Niwa. 1989. Penetration of bovine follicular oocytes by frozen-thawed spermatozoa in the presence of caffeine and heparin. *J. Reprod. Fertil.* 86:577-582.
- Parrish, J. J., A. Krogenaes, and J. L. Susko-Parrish. 1995. Effect of bovine sperm separation by either swim-up or percoll method on success of in vitro fertilization and early embryonic development. *Theriogenology.* 44:859-869.
- Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991. Influence of dietary on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. *J. Anim. Sci.* 69:3762-3773.
- Pieterse, M. C., K. A. Kappen, Th. A. M. Kruip, and M. A. M. Taverene. 1988. Aspiration of bovine oocytes during transvaginal ultrasound scanning the ovaries. *Theriogenology.* 30(4):751-762.
- Pieterse, M. C., P. L. A. M. Vos, Th. A. M. Kruip, Y. A. Wurth, Th. H. van Beneden, A. H. Willemse, and M. A. M. Taverne. 1991. Transvaginal ultrasound guided follicular aspiration of bovine oocytes. *Theriogenology.* 35(4):857-861.
- Pollard, J. W., A. Martino, N. D. Rumph, N. Songsasen, C. Plante, and S. P. Leibo. 1996. Effect of ambient temperatures during oocyte recovery on in vitro production of bovine embryos. *Theriogenology.* 46:849-858.

- Prado, R., S. M. Rhind, I. A. Wright, A. J. F. Rusel, S. R. McMillen, A. J. Smith, and A. S. McNeilly. 1990. Ovarian follicle population, steroidogenicity and micromorphology at 5 and 9 weeks post-partum in beef cows in two levels of body condition. *Anim. Prod.* 51:103-108.
- Presicce, G. A., S. Jiang, M. Simkin, L. Zhang, C. R. Looney, R. A. Godke, and X. Yang. 1997. Age and hormonal dependence of acquisition of oocyte competence for embryogenesis in prepubertal calves. *Biol. Reprod.* 56:386-392.
- Price, C. A., P. D. Carriere, B. Bhatia, and N. P. Groome. 1995. Comparison of hormonal and histological changes during follicular growth, as measured by ultrasonography, in cattle. *J. Reprod. Fertil.* 103:63-68.
- Pursley, J. R., M. O. Mee., and M. C. Wiltbank. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *Theriogenology.* 44:915-923.
- Ray, D. E., A. H. Jassim, D. V. Armstrong, F. Wiersma, and J. D. Schuh. 1992. Influence of season and microclimate on fertility of dairy cows in a hot-arid environment. *International Journal of Biometeorology.* 36(3):141-145.
- Revah, I., and W. R. Butler. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. *J. Reprod. Fertil.* 106:39-47.
- Ronge, H., J. Blum, C. Clement, F. Jans, H. Leuenberger, and H. Binder. 1988. Somatomedin C in dairy cows related to energy and protein supply and to milk production. *Anim. Prod.* 47:165.
- SAS[®] Institute, INC. 1985. SAS User's Guide: Statistics. Cary, NC, USA.
- Shioya, Y., M. Kuwayama, M. Fukushima, S. Iwassaki, and A. Hanada. 1988. In vitro fertilization and cleavage capability of bovine follicular oocytes classified by cumulus cells and matured in vitro. *Theriogenology.* 30(3):488-499.
- Smith, L. C., M. Olivera-Angel, N. P. Groome, B. Bhatia, C. A. Price. 1996. Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle. *J. Reprod. Fertil.* 106:193-199.
- Spicer, L. J., W. J. Enright, M. G. Murphy, and J. F. Roche. 1991. Effect of dietary intake on concentrations of insulin-like growth factor-I in plasma and follicular fluid, and ovarian function in heifers. *Domest. Anim. Endocrinol.* 8(3):431-437.

Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-I in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrous behavior. *J Dairy Sci.* 73:929-937.

Spicer, L. J., and S. A. Zinn. 1987. Relationship between concentrations of cortisol in ovarian follicular fluid and various biochemical markers of follicular differentiation in cyclic and anovulatory cattle. *J. Reprod. Fertil.* 81:221-226.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, E. P. Stanisiewski, and R. L. Fogwell. 1990. Influence of energy balance and body condition on estrus and estrous cycles in holstein heifers. *J. Dairy Sci.* 73:2759-2765.

Villa-Godoy, A., T. L. Hughes, R. S. Emery, L. T. Chapin, and R. L. Fogwell. 1988. Association between energy balance and luteal function in lactating dairy cows. *J. Dairy Sci.* 71:1063.

Yung, M. C., M. J. Vandehaar, R. L. Fogwell, and B. K. Sharme. 1996. Effect of energy balance and somatotropin on insulin-like growth factor I in serum and on weight and progesterone of corpus luteum in heifers. *J. Anim. Sci.* 74:2239-2244.

APPENDIX

Table 9. Analysis of variance for dry matter intake, energy balance and energy intake of lactating dairy cattle from calving to 100 d postpartum

Source	df	Mean Squares	df	Means Squares	df	Mean Squares
		Dry Matter Intake		Energy Balance		Energy Intake
Treatment ^a	1	7.9	1	35	1	58
Cow (Treatment)	9	36.9 **	9	46 **	9	89 **
Treatment x Week	2	86.2 **	2	56 **	2	214 **
Treatment x Week ^{2b}	2	37.9 **	2	43 **	2	115 **
Treatment x Week ^{3c}	2	22.0 **	2	37 *	2	80 **
Residual	142	3.8	139	9	141	10

^a Treatment tested by Cow(Treatment)

^b Quadratic effect

^c Cubic effect

* = $p < .05$

** = $p < .01$

Table 10. Analysis of variance for body condition (BC) scores and body weights (BW) in lactating dairy cows from -8 to 25 wk postpartum

Source	df	Mean Squares	df	Mean Squares
		Body Condition		Body Weight
Treatment ^a	1	1.14	1	11,658
Cow (Treatment)	9	.48 **	9	17,090 **
Treatment x				
Week	2	2.65 **	2	10,493 **
Treatment x				
Week ^{2b}	2	1.78 **	-	-
Residual	97	.05 **	146	1,004

^a Treatment is tested by
Cow(Treatment)

^b Quadratic effect

** = < .01

Table 11. Analysis of variance for milk yield and milk fat percentage in lactating dairy cows from calving to 100 d postpartum

Source	df	Mean Squares	df	Mean Squares
		Milk Yield		Milk Fat
Season ^a	1	663.6	1	3.5
Treatment ^a	1	11.9	1	.4
Treatment x Season ^a	1	.4	1	1.7
Cow (Treatment x Season)	15	251.0 **	15	1.2 **
Treatment x Week ^b	2	732.4 **	2	4.7 **
Treatment x Week ^{2c}	2	462.1 **	2	4.5 **
Treatment x Week ^{3d}	2	304.1 **	-	-
Residual	249	14.1	244	.4

^a Treatment, Season, and Treatment x Season are tested by Cow (Treatment x Season)

^b Linear effect

^c Quadratic effect

^d Cubic effect

** = $p < .01$

Table 12. Analysis of variance for size of largest follicle measured in lactating cows from d 30 to 100 postpartum

Source	df	Mean Squares
Season ^a	1	11.7
Treatment ^a	1	1.2
Treatment x Season ^a	1	1.9
Cow(Treatment x Season)	15	4.6
Treatment ^b x Days Postpartum	2	28.4 **
Treatment ^c x Days Postpartum ²	2	27.3 **
Treatment ^d x Days Postpartum ³	2	25.2 *
Follicles < 5 mm	1	22.6 *
Follicles 6-9 mm	1	1.7
Follicles 10-14 mm	1	154.8 **
Follicles > 15 mm	1	1507.7 **
Residual	345	5.5

^a Treatment, Season, and Treatment x Season are tested by Cow (Treatment x Season)

^b Linear effect

^c Quadratic effect

^d Cubic effect

* = $p < .05$

** = $p < .01$

Table 13. Analysis of variance for follicle numbers by size category obtained from lactating dairy cows from d 30 to 100 postpartum

Source	Follicles < 5 mm		Follicles 6-9 mm		Follicles 10-14 mm		Follicles > 15 mm	
	df	Mean Squares	df	Mean Squares	df	Mean Squares	df	Mean Squares
Season ^a	1	19.5	1	30.0	1	66.1 **	1	6.6 *
Treatment ^a	1	10.4	1	8.9	1	4.1	1	.2
Treatment x Season ^a	1	31.8	1	2.7	1	.1	1	.1
Cow(Treatment x Season)	15	84.8 **	15	12.9 **	15	2.6	15	.8
Treatment ^a x Days Postpartum	2	39.1 *	2	32.8 **	2	19.5 **	2	4.7 **
Treatment x Days Postpartum ^{2b}	2	34.3 *	-	-	2	21.6 **	2	5.0 **
Treatment x Days Postpartum ^{3c}	2	29.2 *	-	-	2	20.8 **	2	5.0 **
Residual	349	8.9	353	4.6	349	2.3	349	0.6

^a Treatment, Season, and Treatment x Season are tested by Cow (Treatment x Season)

^b Linear effect

^c Quadratic effect

^d Cubic effect

* = p < .05

** = p < .01

Table 14. Analysis of variance for total oocyte numbers and quality scores for data obtained from d 30 to 104 postpartum in lactating dairy cows

Source	df	Mean Squares	df	Mean Squares	df	Mean Squares
		Total Number		Mean Ova Quality Score ^b		Total Ova Quality Score ^c
Treatment ^a	1	13.72	1	.32	1	61
Season ^a	1	.52	1	5.99 *	1	150
Treatment x Season ^a	1	.02	1	1.38	1	32
Cow(Treatment x Season)	15	62.88 **	15	.96 **	15	167 **
Treatment ^d x Days						
Postpartum	2	17.79 ^	2	1.09 †	2	22
Aspirator	2	19.63 †	2	1.26 *	2	7
Residual	356	6.81	331	.37	331	28

^a Treatment, Season, and Treatment x Season were tested by Cow (Treatment x Season)

^b Derived from averaging all quality scores for each individual treatment

^c Derived from averaging scores for each individual quality grade

^d Linear effect of days postpartum

^ = p < .08

† = p < .06

* = p < .05

** = p < .01

Table 15. Analysis of variance for the number of oocytes by quality score from d 30 to 104 postpartum

Source	df	Mean	df	Mean	df	Mean	df	Mean
		Squares		Squares		Squares		Squares
		Good +		Good		Good -		Poor
Treatment ^a	1	1.26 *	1	.03	1	1.04	1	52.6
Season ^a	1	.73	1	.21	1	.97	1	4.5
Treatment x Season ^a	1	.04	1	.24	1	.18	1	2.5
Cow(Treatment x Season)	13	.24	15	.59	15	1.56	15	18.4 **
Treatment ^b x Days postpartum	2	.74	2	.09	2	6.61 **	2	35.8 **
Treatment ^c x Days Postpartum ²	2	.74	-	-	-	-	2	31.1 **
Treatment ^d x Days Postpartum ³	2	.76	-	-	-	-	2	27.1 **
Aspirator	2	.69	2	.34	2	.51	2	12.1 *
Residual	83	.57	131	.55	212	1.13	266	2.9

^aTreatment, Season, and Treatment x Season were tested by Cow (Treatment x Season)

^bLinear effect

^cQuadratic effect

^dCubic effect

* = $p < .05$

** = $p < .01$

Table 16. Analysis of variance for follicular fluid hormones from lactating dairy cows from d 30 to 104 postpartum

Source	df	Mean	df	Mean	df	Mean	df	Means
		Squares		Squares		Squares		Squares
		FF IGF-I ^b		FFE2 ^c		FFP4 ^d		E:P ^e
Season ^a	1	3.3	1	1.3E + 11	1	2,997,624	1	7,483,520
Treatment ^a	1	33.8 *	1	7.2E + 11 [^]	1	9,582,388	1	12,379,452
Treatment x Season ^a	1	9.7	1	4.9E + 11	1	5,965,805	1	28,840,504
Cow(Treatment x Season)	15	6.4 **	15	2.0E + 11**	15	63,693,777 [†]	15	11,896,264
Treatment x Days Postpartum	1	0.4	1	2.5E + 11 [^]	1	27,522,774	1	14,538,695
Residual	266	0.7	144	8.5E + 10	201	37,799,131	130	10,515,358

^a Treatment, Season, and Treatment x Season are tested by Cow (Treatment x Season)

^b IGF-I concentrations in follicular fluid

^c Estradiol concentrations in follicular fluid

^d Progesterone concentrations in follicular fluid

^e Ratio between follicular fluid estradiol and progesterone concentrations

[^] = p < .09

[†] = p < .06

* = p < .05

** = p < .01

Table 17. Analysis of variance for serum progesterone (ng/ml) obtained from lactating dairy cows from calving to 104 d postpartum

Source	df	Mean Squares
Season ^a	1	15.6
Treatment ^a	1	2.3
Treatment x Season ^a	1	.2
Cow(Treatment x Season)	15	11.7 **
Treatment x Days Postpartum ^b	2	57.1 **
Treatment x Days Postpartum ^{2c}	2	49.4 **
Treatment x Days Postpartum ^{3d} x	2	37.9 **
Residual	450	1.9

^a Treatment, Season, and Treatment x Season are tested by Cow (Treatment x Season)

^b Linear effect

^c Quadratic effect

^d Cubic effect

** = $p < .01$

Table 18. Analysis of variance for basal FSH and LH (pg/ml) obtained from lactating dairy cows from 28, 60, and 105 days postpartum

Source	df	FSH	LH
		Mean Squares	Mean Squares
Treatment ^a	1	104,435	218,836
Cow (Treatment)	5	1,218,538 *	231,298 **
Period ^b	2	807,776 *	1,061,536
Treatment x Period ^b	2	26,740	146,175
Cow x Period (Treatment)	7	106,251**	1,265,141 **
Treatment x Period ^c x Time	6	25,174 **	42,463 *
Treatment x Period x Time ²	6	30,744 **	54,545 **
Residual	258	8,635	17,561

^a Treatment tested by Cow (Treatment)

^b Period and Treatment x Period tested by Cow x Period (Treatment)

^c Quadratic effect

* = $p < .05$

** = $p < .01$

Table 19. Analysis of variance for development of oocytes collected from lactating dairy cows by ultrasound guided transvaginal follicular aspiration

Source	df	Mean Squares
Season	1	140.8
Treatment	1	901.6*
Residual	89	67.1

* = $p < .05$

Table 20. Analysis of variance for rate of conception of lactating dairy cows post transvaginal follicular aspiration

Source	df	Mean Squares
Treatment	1	.75
Season	1	.60
Residual	17	.21

VITA

Kerry Wyn Kendrick was born in Alexandria, VA on August 14, 1973. She completed high school at Paul VI High School in Fairfax, VA and in 1991 came to Virginia Polytechnic Institute and State University. In May 1995, Kerry received her Bachelor of Science degree in Animal Science, and subsequently began her Masters research in Dairy Science at Virginia Polytechnic Institute and State University. In the spring of 1997, Kerry received her Masters degree and began her career as a research associate at PPL Therapeutics, INC., in Blacksburg, VA.