COMPARISON OF ENDOCRINE REGULATORS OF METABOLISM AND POSTWEANING REPRODUCTION IN PRIMIPAROUS AND MULTIPAROUS SOWS.

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Kristina J. Orzechowski

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

COMPARISON OF ENDOCRINE REGULATORS OF METABOLISM AND POSTWEANING REPRODUCTION IN PRIMIPAROUS AND MULTIPAROUS SOWS

BY

KRISTINA ORZECHOWSKI

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

MASTER OF SCIENCE

ABSTRACT

Reproductive function in the growing primiparous sow is often compromised. A knowledge of the key processes regulating fertility in the breeding sow is required to limit the negative effects of lactation and early weaning. To that end, comparisons were made to better understand the fundamental differences between mature multiparous and growing first parity Cotswold sows. Equalization of metabolic demand in nine first parity and eight fifth parity sows was made by standardizing litter sizes by day 3 postpartum (pp) to 10 and 12 piglets for first and fifth parity sows, respectively. Weaning of piglets was at 14 days pp. Sows were fitted with venous cannulae at approximately day 107 of gestation to facilitate frequent blood sampling. Blood was collected at 20-minute intervals for 8 hours on days 110 of gestation (g) and 3 pp, for 10 hours on days 13 pp and 4 postweaning (pw), for 5 hours on the day prior to estrus and at estrus, for 3 hours on day 2 postbreeding (pb) and for 5 hours on days 5, 10 and 15 pb. In addition, single daily blood samples were collected from day 10 pp to 14 days pb and on the day prior to slaughter at 27-35 days pb following mating at the first postweaning estrus. Reproductive tracts were recovered after slaughter and ovulation rate and embryo mortality were determined at this time. Number born alive and the percentage of the standardized litter weaned did not differ between the parities ($P>0.054$). Average weaning weight of piglets was greater for first parity sows ($P=0.03$). Fifth parity sows consumed more feed at all times, except in the pb period ($P=0.008$). Although first parity sows lost more backfat throughout lactation and in the 5 days pw ($P=0.01$), weight loss was greater in the first parity sows in the last week of lactation only ($P=0.02$). Average daily weight loss, primarily as total body protein ($P=0.009$), in the 5 days pw ($P=0.015$) was greater in fifth parity sows. The pw interval to

estrus was significantly longer and more variable in first parity sows compared to fifth parity sows (P=0.023). Ovulation rate at 27-35 days pb was greater in fifth parity sows (P=0.0004), but the number of viable embryos and embryo mortality rate did not differ between the parities. Postweaning pregnancy rate was 6/9 and 6/8 for first and fifth parity sows, respectively. Weight and weaning-to-estrus interval (WEI) were negatively correlated across parity and first parity sows with prolonged anestrous periods weighed less in late gestation and lactation than those that returned to estrus by 7 days pw (P=0.04). Mean blood glucose, progesterone, cortisol, LH, T_4, T_3 and the T_4/T_3 ratio did not differ between parity on any of the sampling days. Blood urea nitrogen was greater (P<0.053) in the fifth parity sows during lactation, but was not correlated with total body protein. Estradiol was higher in first parity sows in late gestation (P=0.015). Pulse frequency of LH did not differ between the two groups but baseline concentration was greater on day 13 pp in fifth parity sows (P=0.03). FSH was higher in first parity sows on day 13 pp, estrus and 2 days pb (P<0.053). Insulin was greater in fifth parity sows in lactation and on the day prior to estrus (P<0.02) and IGF-I was greater in first parity sows at estrus (P=0.03). Embryo mortality was not correlated with estradiol at estrus or 2 or 5 days pb or with progesterone at slaughter. Correlation analysis was performed for every hormone on each frequent sampling day. Differences in endocrine correlations between first and fifth parity sows were particularly evident in lactation and the return to estrus period. First and fifth parity sows appear to catabolize body stores differentially in lactation and the immediate postweaning period, with first parity sows sacrificing fat over protein stores. Furthermore, body weight in this study was more related to the duration of the postweaning anestrous period than was backfat. The present data suggests that differences in postweaning reproduction are related to body catabolism resulting in endocrine profiles unique to each parity. And it is hypothesized that postweaning reproduction may be controlled by divergent mechanisms in young and mature sows.

ACKNOWLEDGMENTS

Foremost, I have to extend a great deal of thanks to my advisor, Dr. Laurie Connor who, in the first place, had enough faith to take me aboard. Many thanks go to my advisory committee, Dr. S.K. Baidoo, Dr. G. Crow and Dr. J.E. Eales. Their patience, support and criticism was invaluable.

I have to express my gratitude to Dr. G. Crow and Dr. L. Onischuk for their statistical assistance, availability and especially patience. I thank Ricky Araneda for her immeasurable help and support in the lab. The lab space remained intact and the radiation kept in check.

The help and company from Nadine Funk and Jennifer Dronzek made the job a little lighter during those long and stressful 4 months. I would never have been able to do it without them. Also, thanks go to Jack, Jody, Maurice and Ed for all their help and good nature. As well, I have to thank 5675E, 7563C, 5973E, 10043C, 6032E, 6082E, 10143C, 6140E, 7722C, 9832C, 9595C, 5679E, 5143E, 7180C, 4813E, 7403C and 6068E for greeting me each morning with jubilant snorts.

I thank Manitoba Pork Inc. and Graduate Studies for financial support during the course of this work.

Thanks go to the many smiling faces down the graduate students hallway. The good humour, friendship and laughter was/is unstoppable.

Finally and most importantly, thanks to my family for their never-ending encouragement, love and support. Thank you, thank you, thank you...

TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDIX TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BG	blood glucose
BSA	bovine serum albumin
BUN	blood urea nitrogen
CA	corpora albicans
CH	corpora hemorrhagicum
CL	corpora lutea
cpm	counts per minute
CV	coefficient of variation
DE	digestible energy
dl	decilitre
E2	estradiol 17-ß
eCG	equine chorionic gonadotropin
E	estrus
E-1	day prior to estrus
ELISA	enzyme-linked immunosorbent assay
EOP	endogenous opioid peptides
FSH	follicle-stimulating hormone
g	gestation
GH	growth hormone
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin

IGBP	IGF-binding protein
IGF-I	insulin-like growth factor I
IU	international unit(s)
Kcal	kilocalories
kg	kilogram(s)
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
mg	milligram(s)
ml	millilitre
mm	millimeter(s)
mRNA	messenger RNA
NEFA	nonesterified fatty acids
ng	nonogram
NRC	National Research Council
NRS	normal rabbit serum
pg	picogram
PBS	phosphate buffered saline
PEG	polyethylene glycol
PMSG	pregnant mare's serum gonadotropin
pb	postbreeding
pp	postpartum
pw	postweaning
P4	progesterone
RIA	radioimmunoassay
rpm	revolutions per minute
SAS	Statistical Analysis System

T_3	triiodothyronine
T_4	thyroxine
TRH	thyroid releasing hormone
TSH	thyroid stimulating hormone
USP	United States Pharmacopea
WEI	weaning-to-estrus interval(s)

CHAPTER 1

INTRODUCTION

A primary goal in modern swine production is maximization of annual sow output. Sow reproductive performance, with respect to the number of pigs weaned per sow per year, is composed of two main factors. One of these is the percent weaned, comprised of litter size and preweaning mortality. The other is the number of litters a sow produces per year, which is determined by gestation and lactation length and the number of non-productive days (Foxcroft *et al.*, 1995). Practically, gestation length cannot be altered to increase the farrowing frequency. Therefore, attention is directed towards the other contributing factors. Economically, lactation is often regarded as an unproductive stage in a sow's breeding life. As a result of this, early weaning strategies are currently being employed to increase the number of production cycles per sow per year. As lactation lengths decrease, the time spent in the farrowing facility is shortened and thus, farrowing crate use is maximized. This is of economic importance considering the high capital cost associated with the farrowing facility and the comparably low cost of the nursery phase. Currently, weaning at 10 to 17 days of age is being incorporated into commercial production as a way to combat disease problems and facilitate mixing of multisource piglets, as well as to increase the farrowing frequency. However, the earlier anticipated improvement in annual sow production and economic returns have not been fully realized.

Early weaning strategies are directed towards shortening the length of the postpartum anestrous period. The block to ovarian cyclicity during lactation can be at the neural and/or ovarian level as a result of the suckling action of the piglets. This suckling inhibition is responsible for lowered rate of gonadotropin secretion (Sesti and Britt,

1993a), due primarily to increased levels of prolactin, oxytocin and the endogenous opioids. The depth of anestrus is dependent on the duration and number of piglets suckling. With this in mind, strategies such as partial weaning, split weaning, exogenous hormone therapy and early weaning have been employed with varying degrees of success.

Early weaning is often characterized by a slow return to estrus and increased embryonic mortality (Xue *et al.*, 1993; Foxcroft *et al.*, 1995), with the first parity sow being the most affected. In addition, aberrant gonadotropin profiles are observed following very early weaning and contribute to compromised oocyte development and embryo livability (Varley and Cole, 1976) but, surprisingly not to ovulation rate (Varley, 1982). This altered gonadotropin production may involve a number of factors including the sow's stage of growth, nutrient intake during gestation and lactation, litter size and her metabolic status at farrowing.

Aside from the absence of estrus, lactation also imposes a large metabolic demand on the sow. Sows must be able to meet their own maintenance requirements as well as those of the suckling piglets. It is common for sows to become catabolic during lactation and this problem is often most pronounced in the young first parity sow. The current trend for lean growth rate selection in pigs has provided F1-generation fast growing, lean gilts often with reduced voluntary feed intake (Foxcroft, 1997). This is probably the largest detriment associated with selection pressure for lean growth. Resultant, is a need to reassess the nutritional requirements of the primiparous sow. Often, primiparous sows come out of a lactation period in poor body condition, exhibit a prolonged postweaning return to estrus and subsequent lowered reproductive performance. This often results in an increase in the culling rate (Foxcroft *et al.*, 1995) and these symptoms may also carry through into their second parity. In contrast, mature full-fed multiparous sows tend lose less body condition, promptly return to estrus postweaning and show good reproductive performance. This situation, however, is not usually observed until the third parity or

greater and is believed to be linked to different maternal drives in nutrient partitioning between growing and mature sows.

Changes in live weight and body composition have profound effects on reproductive performance. However, reproductive performance can be affected even in the absence of obvious changes in body condition. Feed restriction results in lowered levels of gonadotropins and metabolic hormones such as insulin, IGF-I and thyroid hormones and increased levels of adrenal steroids. These hormones and other metabolites play a critical role in reproductive function in either direct or permissive roles. Whether a similar situation exists in the lactating primiparous sow who is more likely to be catabolic and unable to consume sufficient dietary energy for maintenance remains, for the most part, unanswered.

In order to limit the negative effects of lactation and early weaning an understanding of the key physiological processes regulating the fertility of the breeding sow is first required. To that end, the main objective of this research was to increase our understanding of the biological differences between mature fifth parity sows and growing first parity sows. By identifying the differences between these animals in terms of endocrine profiles, reproductive function, feed intake and body condition, the mechanisms which impact on reproduction should become clearer and thus, facilitate the development of appropriate management strategies.

CHAPTER 2

REVIEW OF THE LITERATURE

Introduction

From an animal production standpoint, lactation is often considered to be an unproductive time in a sow's reproductive life. The suckling piglets are responsible for an inhibition at the level of the hypothalamus, anterior pituitary gland and/or ovary. Gonadotropin levels are low and prolactin and endogenous opioid peptide levels are elevated, resulting in ovarian quiescence (Varley and Foxcroft, 1990). In order to improve reproductive efficiency of the sow, swine producers are implementing tactics that prevent or 'sidestep' the endocrinological events associated with lactation. These include gonadotropin and/or steroid treatment and a reduction in the lactation length, all of which have debatable benefits.

Reduction of lactation length to less than about 21 days, is a major issue in the modern swine industry. The primary benefits attributed to a reduced lactation length are increased farrowing frequency and facilitation of the mixing of multisource piglets for segregated early weaning. Ideally this should result in an increased number of piglets a sow produces per year. However, the actual outcome is not always this way (Xue *et al.*, 1993; Foxcroft *et al.*, 1995). Reduction of lactation length has inherent problems that often offset or negate any potential gains seen with an increased farrowing frequency. The most common problem associated with shortened lactation periods are longer postweaning intervals to estrus and decreased litter sizes in the subsequent farrowing (Xue *et al.*, 1993; Foxcroft *et al.*, 1995). This is especially true of the first litter sow. This review will focus

on the endocrinology of the lactating sow, principles and problems of early weaning and the role of nutrition and body condition in reproductive status of the sow.

Lactational Anestrus

Gonadotropin suppression

Postpartum anestrus can be due to inhibitory influences at a number of levels: the hypothalamus, the anterior pituitary gland and/or the ovary. Lactational anestrus is characterized by a suppression of the estrogen positive feedback on LH secretion (Mattioli and Seren, 1985). LH suppression during lactation is not ovarian dependent, in that, it is not seen to rise with ovariectomy (Stevenson *et al.*, 1981). During lactation, LH basal levels and pulse frequency are suppressed. In the immediate postpartum period, LH levels are generally slightly higher than in established lactation. However, Varley and Foxcroft (1990) report that LH synthesis and release can be suppressed in the early postpartum period as a direct residual result of the elevated progesterone concentration from the previous gestation. Chronic exposure of the hypothalamic-pituitary axis to progesterone during gestation causes dysfunction of the estrogen-positive feedback mechanism (Varley and Foxcroft, 1990). It was generally thought that in lactation the concentration of LH is low and only begins to rise at approximately day 21 of lactation and/or following weaning (Foxcroft, 1985). However, Sesti and Britt (1993b) found LH to decrease between days 1 and 7 postpartum and then increase to day 21 of lactation. As well, the readily releasable pool of LH increases throughout lactation as shown by GnRH challenge. In agreement with this, Stevenson *et al.* (1981) found the LH response to a GnRH treatment was greater on day 20 of lactation than on day 10. Treatment with *N*-methyl-DL-aspartic acid, a potent neuroexcitatory amino acid analog known to induce GnRH release, elicited increased serum LH concentration as lactation progressed (Sesti and Britt, 1993b). Therefore, these same

authors suggested that hypothalamic stores of GnRH increase throughout lactation and these stores are sufficient to stimulate the synthesis but not secretion of LH. In lactation the pituitary levels of FSH are high but plasma levels are low. This is suggestive of sustained FSH synthesis, but a low rate of release. As well, FSH levels are much higher in late lactation than in early lactation (Stevenson *et al.*, 1981) and similar to those seen in the luteal phase of the estrous cycle (Foxcroft, 1985). FSH release is not as responsive to GnRH treatment as LH and ovariectomy during lactation has been found to increase serum FSH concentration. From this, it is suggested that in lactation, FSH is under the control of nonsteroidal ovarian factor(s), possibly inhibin (Sesti and Britt, 1993b). Thus, the current information points to a clear lactational block to estrus at the neural level.

Inhibitory factors

The low levels of the gonadotropins in lactation are the result of a neuroendocrine mechanism from the suckling action of the piglets. This suckling stimulus is primarily responsible for the lowered rate of secretion of the gonadotropins and the absence of estrus (Sesti and Britt, 1993a). The actual mechanism involved is largely unknown, however. The depth of anestrus is directly related to the number of piglets that the sow is nursing. More than one piglet is required to maintain lactation and prevent cycling. The sensitivity of the ovary, in part, depends upon the duration of lactation and/or the frequency and intensity of suckling (Ellendorff *et al.*, 1985).

Serum prolactin levels are raised with frequent suckling bouts. Some evidence suggests that the levels of prolactin are inversely related to the levels of FSH and LH (van de Wiel *et al.*, 1985) while others have not seen similar results (De Rensis *et al.*, 1993b). Possibly, the block to ovarian function could be due to a lack of an appropriate previous stimulation by LH and/or FSH. *In vitro* studies show that prolactin exerts a direct effect on the granulosa cells by decreasing progesterone synthesis (van de Wiel *et al.*, 1985). As

well, hyperprolactinaemia exerts its effect directly on the hypothalamus modulating the release of LH by activating dopaminergic terminals of the median eminence which are involved in tonic release of LH (van de Wiel *et al,* 1985). However, prolactin does not have a direct effect on the pituitary gland because altering levels of prolactin do not disturb LH responsiveness to GnRH, which increases as lactation progresses (van de Wiel *et al.*, 1985). In order to determine if prolactin acts as the major contributor to LH suppression during suckling periods, physiological levels of prolactin were administered to weaned sows. LH basal concentration and pulse frequency and amplitude were significantly lower than in saline-administered controls, but still higher than in sows which were suckled (van de Wiel *et al.*, 1985). This suggests that prolactin contributes to the effects of the suckling stimulus in conjunction with other factor(s). Bromocriptine, a prolactin antagonist, is effective in blocking the effects of prolactin with a resulting increased LH release. Furthermore, bromocriptine improves the reproductive efficiency of postpartum sows (Mattioli and Seren, 1985). Therefore, prolactin may be the primary inhibitor of gonadotropin secretion during lactation.

Oxytocin inhibits ovarian function (Ellendorff *et al.*, 1985) and its release is highest at the beginning of lactation, even though the suckling periods themselves may be shorter. The piglets stimulate the sow's central nervous system to release oxytocin from the posterior pituitary gland to initiate milk letdown by presuckling massaging. As a result, more oxytocin is released if the frequency of the suckling periods is high (Ellendorff *et al.*, 1985). *In vitro* and *in vivo* studies show oxytocin also acts as a prolactin-stimulating factor. Samson *et al.* (1986) found that suckling periods in rats caused a significant increase in serum oxytocin and a concomitant increase in serum prolactin concentration. When anti-oxytocin was administered following suckling of pups, the normally expected elevation in prolactin was absent. Furthermore, oxytocin perfusion of rat anterior pituitary cells stimulated prolactin release in a dose-dependent manner.

In pigs and sheep, endogenous opioid peptides (EOP) are implicated in the suckling-induced inhibition of GnRH and LH release and they also act to stimulate prolactin secretion (Armstrong *et al.*, 1988; Knight *et al.*, 1986). Suckling bouts increase the ß-endorphin concentration in the hypothalamic-hypophyseal portal blood stream. The EOP do not affect the lactotroph cells directly, rather they act at the neural level either by inhibiting the release of dopamine, and/or by enhancing the release of a prolactin-releasing factor (Knight *et al.*, 1986). As prolactin and EOP levels increase with suckling, the hypothalamic content of met-enkephalin, dopamine and noradrenalin fall. As well, there is an associated increase in hypothalamic serotonin. Morphine acts as an exogenous EOP and acute administration of it blocks the effects of weaning. That is, the rise in LH and decrease in prolactin seen with weaning are not observed. Chronic activation of EOP receptors with morphine delays the onset of estrus following weaning while naloxone, an antagonist of EOP and serotonin, has been shown to prevent the rise in prolactin associated with the suckling stimulus (Armstrong *et al.*, 1988). Indeed, 2 mg kg^{-1} of naloxone administered on day 10 of lactation resulted in increased LH secretion (De Rensis *et al.*, 1993a). Partial consequences of the suckling stimulus therefore, are clearly at the neural level by promoting the production and release of brain opiates which appear to have a suppressive action on gonadotropin release.

Weaning and Estrus Induction

Ovarian response to weaning

As lactation length progresses, the sow overcomes the inhibitory effects of suckling and the number of medium to large-sized follicles gradually increases and the number of atretic follicles decline (Sesti and Britt, 1993b; Foxcroft *et al.*, 1995). This could be due to a decrease in the frequency of suckling and a decrease in milk synthesis, or through some

recovery of the hypothalamic-pituitary-gonadal axis from the suckling inhibition. Partial recovery of the estrogen positive feedback mechanism is seen by day 35 of lactation. Estrogen administration after approximately day 21 postpartum can induce an LH surge, which is seen more quickly in the absence of prolactin (Mattioli and Seren, 1985; van de Wiel *et al.*, 1985). Also, LH responsiveness to GnRH increases as lactation progresses (Varley and Foxcroft, 1990; Sesti and Britt, 1993b).

The gonadotropins are generally the primary stimulus for follicular development and an ovulatory estrus after weaning. In the immediate postweaning period, the pituitary levels of FSH change very little, while LH levels increase quite dramatically (Edwards, 1982; Foxcroft *et al.*, 1987). At weaning, pituitary LH synthesis and release are re-established and are associated with an increased level of GnRH output within 60 hours of weaning (Edwards, 1982). This, in combination with the total removal of the litter, provide the necessary factors for advanced folliculogenesis. A 'productive' sow will usually exhibit a fertile estrus within 7 days of weaning (Foxcroft and van De Wiel, 1982; Killen *et al.*, 1992).

During lactation, there are a number of small, medium and large follicles present on the ovary. Palmer and coworkers (1965a) found the average follicle size at parturition to be 4.6 mm in diameter with a decrease to 2.7 mm after the first week of lactation. The largest of the follicles present on the ovary in lactation are in fact atretic as indicated by chromatolysis in the membrana granulosa cells and pyknosis of the nuclei (Palmer *et al.*, 1965b). Follicle size then gradually increases in lactation and in the postweaning period follicle size increases dramatically (Palmer *et al.*, 1965a). Newton *et al.* (1987) state that it is likely that multiparous sows have a greater proportion of large follicles which are more responsive to gonadotropin stimulation and this is supported by their observation of higher estradiol and lower FSH concentrations in the multiparous sows during lactation. This may be, in part, responsible for their earlier return to estrus postweaning compared to first parity

sows. Foxcroft *et al.* (1987) found that following weaning at 28 days, primiparous sows showed a large degree of between-sow variability in both follicle size and estrogenic activity. After weaning, the number of small follicles decrease and medium and large follicles predominate. More than 70% of the large follicles that are present at 24 and 48 hours after weaning will continue to develop and ovulate normally less than one week later. Unilateral ovariectomy within the first 48 hours after weaning does not affect the weaning-to-estrus interval (WEI), the number of small follicles (Killen *et al.*, 1992) or serum steroid concentrations, although FSH concentration increases (Stevenson *et al.*, 1981). Follicular concentration of progesterone increased six fold within 6 hours of weaning in medium-sized follicles and estradiol and testosterone content of these follicles increased as well. In the small follicles, however, the concentration of testosterone alone increased (Killen *et al.*, 1992) possibly indicating atresia. In large follicles, estradiol concentration increases within 24 hours of weaning while insulin-like growth factor-1 (IGF-I) levels remained static. Killen *et al.* (1992) found that the correlation between IGF-I and follicle diameter is positive at weaning. Also, IGF-I concentration was found to be higher at all times in large follicles compared to small follicles. These results are representative of the situation seen with sows weaned at a time more consistent with the industry standard of 3-4 weeks. However, in the early-weaned and zero-weaned sow, cystic follicles, increased WEI, the absence of estrus and high embryo mortality are more common.

Partial weaning

Partial weaning is a management strategy to promote concurrent estrus and lactation. The piglets are removed from the sow for a period of time each day thus, affording the sow the chance to recover from the suckling inhibition imposed by her litter. The decrease in the frequency of suckling results in rapidly increased levels of LH while oxytocin and prolactin levels decrease. Associated with partial weaning, is an increase in

the number of medium and large-sized follicles as well as an increase in LH release (Foxcroft *et al.*, 1995).

The stage of lactation that the suckling manipulation is imposed appears to have no critically defined time. Some have found the effects beneficial when the piglets were removed from the sow for short periods of time each day before day 21 postpartum, while there are reports that sows separated from their litters for 12 hours per day or for 1 hour twice daily can return to estrus in 1-2 weeks postpartum and still maintain lactation (Stevenson and Britt, 1981; Grinwich and McKay, 1985). Varley and Foxcroft (1990), however, report that only moderate success from this treatment is realized only after the third week of lactation. And in fact, Crighton (1970) was only able to induce lactational estrus when separation of sow and litter, for 12 hours per day, beginning at 21 days postpartum was paired with GnRH treatment.

Although partial weaning may advantageous in inducing estrus during lactation the benefits appear to be primarily seen with multiparous sows (Clark *et al.*, 1986; Newton *et al.*, 1987). Also, partially weaned sows do not appear to have shorter postweaning anestrous periods than normally suckled sows. In fact, Grinwich and McKay (1985) and Newton *et al.* (1987) found no difference between control and restricted suckling groups in the number exhibiting estrus by 7 days postweaning. If used in conjunction with boar exposure and high plane feeding or with the use of exogenous gonadotropins, the responses to partial weaning are improved (Henderson and Hughes, 1984). The lactational estrus that can be induced with partial weaning is of similar duration and the hormonal secretory patterns do not appear to differ from a normal postweaning estrus (Newton *et al.*, 1987). It appears that restricted suckling has no negative effects on conception rates or embryo survival, whereas shortened lactation periods often do (Grinwich and McKay, 1985).

Partial weaning can be useful in increasing the feed consumption of sows, thus minimizing tissue catabolism in the lactating sow. Henderson and Hughes (1984) found a nonsignificant trend towards an increase in feed consumption by sows that are partially weaned and this trend may be attributed to decreased energy requirements as a result of lower milk production. Nevertheless, this potential change in feed intake in combination with reduced milk output due to shorter suckling time, could be advantageous in minimizing lactational weight and backfat losses (Henderson and Hughes, 1984). This could be particularly useful when dealing with the extensive tissue catabolism that is often seen in the primiparous sow. Grinwich and McKay (1985) found that a 22 hours per day suckling restriction was responsible for significantly heavier primiparous sow weights at 35 days postpartum although they did not speculate on the reason.

In addition, the piglets' creep consumption can also be increased significantly (Grinwich and McKay, 1985) with partial weaning although piglet weaning weights are generally not affected (Henderson and Hughes, 1984; Grinwich and McKay, 1985). Piglets that have been partially weaned may be more accustomed to creep feed consumption at weaning and therefore, will not have the same growth lag that is often seen at weaning (Clark *et al.*, 1986).

Split weaning

Split weaning is the removal of some of the piglets from the sow. This allows the sow to nurse a smaller litter. Sows weaned in this manner and still maintaining high feed intake while nursing fewer piglets are more likely to restore maternal tissue losses. This results in a faster return to estrus postweaning and larger subsequent litter size (Mahan, 1993). While decreasing the litter size before weaning is associated with an earlier return to estrus, increasing the litter size from 8 to more than 13 pigs has not been shown to increase the return-to-estrus interval. Sows nursing small litters of 3 pigs, however, had shorter

WEI than did sows nursing medium sized litters of 8 pigs or large litters of 13 or 14 pigs. (Stevenson and Britt, 1981). Despite this, Varley and Foxcroft (1990) in their review, noted that decreasing the litter size is currently not known to proportionately decrease plasma prolactin concentrations. If this is true, split weaning may only be marginally successful and total removal of piglets is therefore required to completely overcome suckling inhibition.

Zero weaning

Zero weaning is accomplished by total removal of the piglets from the sow immediately following parturition. Its function is to remove the suckling inhibition on gonadotropin release. Zero weaning, as a technique to increase the number of litters per sow per year, is problematic. A minimum period of suckling is required for recovery of the hypophyseal-gonadal axis from gestation and total removal of the suckling stimulus at parturition causes acute endocrine dysfunction (Varley and Foxcroft, 1990). The absence of the suckling-induced suppression on LH and FSH release causes rapid growth of follicles that were present on the ovary at parturition. Foxcroft *et al.* (1995) state that large follicles are present at farrowing but one week later only small and medium-sized follicles are present on the ovary of the lactating sow. Varley and Foxcroft (1990) conclude that without lactation, these large follicles can become estrogenically active in the first week following farrowing. This illustrates that the ovary is responsive to gonadotropins in early lactation, and the primary cause of ovarian quiescence in established lactation is lack of an appropriate LH signal.

Estradiol 17-ß is known to be high and variable in the immediate postpartum period in zero-weaned sows, but a clear hormonal pattern is lacking (Varley and Atkinson, 1985). The exceedingly high estrogen concentrations following zero weaning results in cystic follicles (Elliot *et al.*, 1980). There is, however, no clear relationship between the

circulating estradiol concentration and the incidence of anestrus or nymphomaniac behaviour (Varley and Atkinson, 1985; Varley and Foxcroft, 1990). Furthermore, high levels of peripheral estrogens may also be implicated in poor embryo survival which often results from zero weaning. This will be discussed in further detail later.

Zero-weaned sows return to estrus in 2-3 weeks postpartum (Elliot *et al.*, 1980; Varley and Atkinson, 1985). Elliot *et al.* (1980) found that the duration of estrus in zero-weaned sows was significantly longer than a control group weaned at 28 days postpartum. In addition, Varley and Atkinson (1985) found more sows weaned at zero or 1 day postpartum to be anestrous *vs.* sows weaned at 42 days postpartum. As well, the WEI were longer and the subsequent litter sizes were smaller in the zero-weaned sows. There were no differences in the study of Elliot *et al.* (1980), however, in conception rates at first estrus.

Generally, a period of approximately 21-28 days is necessary for the uterus to return to its normal nonpregnant state (Palmer *et al.*, 1965b; Hafez, 1993). In the first 28 days of lactation, uterine weight decreases 11 fold and uterine length decreases by half (Palmer *et al.*, 1965a). Despite this, most uterine rebuilding has occurred by 3 weeks postpartum and at this time the uterus should be fully competent (Varley, 1982). Without lactation, oxytocin release is absent and this may compromise uterine involution and endometrial repair. Perhaps then, incomplete uterine involution is implicated in reduced embryo survival following zero weaning.

Hormone treatment

Other methods for inducing an early ovulation and estrus postpartum are through the use of exogenous gonadotropins, estrogens, and GnRH. Gonadotropin treatment stimulates the follicles to develop and produce estrogens which exert a positive feedback on LH secretion (Sesti and Britt, 1993b). PMSG with hCG treatment is more successful in the

later stages of lactation (Ellendorff *et al.*, 1985; Sesti and Britt, 1993b). In their review, Varley and Foxcroft (1990) report LH and FSH treatment to be far more successful in estrus induction when administered on days 30-36 of lactation compared to days 1-5 of lactation. However, Kirkwood and Thacker (1990) administered PMSG and hCG to lactating sows at 10 days postpartum and found approximately half of the treatment group ovulated between 20 and 28 days of lactation as indicated by elevated progesterone concentrations. In contrast to these findings, Clark *et al.* (1986) found no effect on the WEI with a 3-day administration of 200 μg of GnRH beginning at 3 weeks of lactation.

Estrogen treatment has been used to provide the necessary positive feedback for increasing the frequency of pulsatile LH and inducing ovulation (Foxcroft *et al.*, 1995). Discharge of GnRH and/or sensitivity at the pituitary level is affected by an estrogen surge prior to the preovulatory LH rise (Ellendorff *et al.*, 1985) and exogenous estrogen mimics the naturally occurring estrogen surge and positive feedback on LH secretion. The magnitude of the LH surge is higher if the estrogen treatment is given later in lactation (day 35 *vs.* day 5) and this is probably due to inadequate pituitary pools of LH early in lactation (Varley and Foxcroft, 1990; Sesti and Britt, 1993a). The available stores of LH and GnRH are known to increase significantly as lactation progresses (Sesti and Britt, 1993a; 1993b). Sows that exhibit the exogenous estrogen-elicited LH surge, first show a period of negative feedback that lasts 48-54 hours. This is followed by a period of positive feedback lasting 36-40 hours; similar in duration, but not magnitude to the naturally occurring LH surge. The timing of the estrogen treatment is crucial. The best results are seen when the litter of piglets is weaned at the same time the estrogen is administered. If the litter is allowed to suckle between the time of the estrogen treatment and the LH surge, LH and FSH can be suppressed to a degree sufficient to prevent the development of follicles (Sesti and Britt, 1993a). From the data available, it appears that although concurrent lactation and ovulation can be induced, success of these strategies depends on the time in which they are

implemented. Most beneficial results occur later in lactation when pituitary stores of gonadotropins are sufficient to trigger folliculogenesis and ovulation.

Problems Associated With Early Weaning

Generally, weaning before 21 days is characterized by an increase in the duration and variability of the WEI with a large percentage of sows exhibiting prolonged postweaning anestrus. As well, high embryo mortality in the first three weeks of gestation, high incidence of nymphomania resulting from follicular cysts and possible negative effects on piglet growth and survival are associated with early weaning (Elliot *et al.*, 1980; Varley and Atkinson, 1985; Varley and Foxcroft, 1990; Xue *et al.*, 1993; Foxcroft *et al.*, 1995). Foxcroft *et al.* (1995), noted an increase in the replacement rate of breeding sows, especially primiparous sows, as lactation lengths are shortened. Despite these negative effects, shorter farrowing-to-service intervals are often observed. This would indicate that a sow's productivity could be increased because she is able to have more litters per year (Varley and Foxcroft, 1990; Xue *et al.*, 1993; Foxcroft *et al.*, 1995). Although early weaning (12 *vs.* 28 days postpartum, for example) could lead to an improvement in the number of litters produced per sow per year, the effects do not always point to an actual increase in productivity. It is often seen that subsequent litter size is smaller and the number of piglets the sow produces in the year is not increased (Foxcroft *et al.*, 1995).

For any hog production system to be successful, not only must the sows perform favorably, but so must those pigs destined for market. Early weaning imposes a stress on all domestic animals (Mahan, 1993). Young pigs weaned at lighter weights generally gain less weight throughout the nursery period and often take longer to reach market weight than those that are weaned at heavier weights (Mahan, 1993; Xue *et al.*, 1993). Weaning pigs before two weeks has negative effects on postweaning growth rate and very limited success

(Britt, 1986). Mahan (1993) found that pigs performed better in the first week postweaning if they were weaned at 30 days *vs.* 23 days. Younger and smaller piglets have less developed digestive tracts and lower body fat content, resulting in a poor thermoregulatory system. Furthermore, pigs that are weaned at heavier weights adapt more quickly to their postweaning diet (Mahan, 1993). In contrast, pigs that are split-weaned or partially weaned, do not respond unfavourably in their postweaning growth rate (Stevenson and Britt, 1981). Notwithstanding, new management techniques and specially formulated diets for early weaned pigs are now available allowing optimal performance of these animals in the nursery phase (Beltranena, 1996).

Weaning-to-estrus interval

It is generally agreed that as lactation lengths decrease to less than 21-28 days, the WEI will increase (Varley *et al.*, 1984; Foxcroft *et al.*, 1995). Nevertheless, the findings to date are often conflicting and this is shown clearly by a number of studies in the UK by Varley and associates. No significant differences were found in the WEI for sows weaned at 10, 21 or 42 days of lactation (Varley and Cole, 1976). In another study, multiparous sows that had their piglets weaned at 10 days *vs.* 42 days had significantly longer WEI (Varley *et al.*, 1981). However, in a later study Varley *et al.* (1984) were unable to duplicate these results and found no differences in the WEI between sows weaned at 10, 21 or 42 days of lactation. Nonetheless, the variability was greater for sows weaned at 10 and 21 days postpartum *vs.* sows weaned at 42 days. Likewise, Varley and Atkinson (1985) found that weaning piglets at birth or one day postpartum significantly increased the number of anestrous sows and the postweaning interval to estrus.

In addition to extended postweaning anestrous periods a high degree of variability is evident in the WEI of the very early weaned sow. Elliot *et al.* (1980) found the duration of estrus to be significantly extended in zero-weaned sows and there was increased

incidence of cystic ovaries and these symptoms were similar to sows expressing nymphomania. As well, the farrowing-to-mating interval were greater and more variable for the zero-weaned sows (Elliot *et al.*, 1980). Which, in itself, indicates that the WEI was greatly extended. Despite this, subsequent litter size was not affected by weaning at birth (Elliot *et al.*, 1980). Previously, it was explained that gonadotropin concentrations are detectable at parturition, but fall to nadir levels in lactation and only begin to rise again at about 2 to 3 weeks of lactation (Sesti and Britt, 1993b). Therefore, it can be speculated that if weaning is to occur at a point in lactation when the readily releasable gonadotropin pools are not sufficient to trigger follicular development and ovulation, the period from weaning to ovulatory estrus will necessarily be extended.

Ovulation rate and gonadotropin profile

Shorter suckling periods do not normally affect ovulation or fertilization rates (Varley, 1982). This is intriguing especially considering the differing gonadotropin profiles between conventionally weaned sows (*i.e.* greater than 3 weeks of lactation) and the early weaned sow (Varley and Cole, 1976; Varley, 1982). Early weaning is associated with an LH surge of lower magnitude compared to that seen when the piglets are weaned later. The actual cause of this is unclear but may be related to a decreased responsiveness to GnRH in the early weaned sow (Edwards, 1982). Foxcroft (1985) reported that the LH concentration is significantly higher in sows weaned at 35 days than those weaned at 10 days. Sows that are weaned at three weeks compared to five weeks also have lower serum FSH concentration 2-3 days postweaning (Xue *et al.*, 1993). This relates back to the low availability of releasable LH and FSH stores before day 21 of lactation. A synchronous LH/FSH surge is common in a majority of sows that are weaned at 5 weeks. However, sows that are weaned at 3 weeks or less fail to exhibit any synchrony in the gonadotropin surge. This would indicate that sows that are weaned later in lactation more closely

resemble a normally cyclic sow at estrus in terms of the hypothalamic-pituitary response (Edwards, 1982; Sesti and Britt, 1993b) The reason for this is that the sow is not as influenced by the effects of suckling on gonadotropin secretion in the later stages of lactation possibly as a result of less frequent suckling by the piglets.

Conception rates and embryo mortality

The reduction in litter size resulting from compromised embryo survival, is a major drawback of early weaning. A majority of the losses occur very early in gestation (*i.e.* before day 25 postcoitum) (Varley and Cole, 1976). If conception occurs approximately 3 weeks after parturition, incomplete uterine involution should not be involved with the reduction in embryo survival, although total endometrial repair may take longer. A majority of the losses of fertilized ova falls into two categories; losses of conceptuses prior to elongation and losses at the peri-implantation stage (Varley and Cole, 1976). Most prenatal embryo losses occur before the attachment of the embryos in the uterus (Archibong *et al.*, 1987). Archibong *et al.* (1987) found embryonic loss continues between day 15 and 30 postcoitum in gilts bred at either first or third estrus. In gilts and early weaned sows, the factor influencing these embryonic losses may be an adverse maternal uterine environment and it is likely that inadequate histotroph secretion may be implicated in the poor embryo livability (Varley, 1982; Archibong *et al.*, 1987). A number of uterine proteins exist that are essential to the early stages of embryo development. The production of these proteins is largely controlled by the steroid hormones. The amounts of these proteins change throughout the different stages of gestation and absolute synchrony of the conceptus and these proteins is required to ensure embryonic survival (Varley, 1982).

Early weaning is thought to disturb the delicate balance required within the uterine lumen during early pregnancy (Varley, 1982). The quantity and character of steroid secretions and the progesterone-estrogen ratio play a large role in the control of uterine

secretions. Estrogen concentrations are higher in gilts displaying higher embryo survival on day 30, than gilts with a low embryo survival (i.e. gilts bred at 1st *vs.* 3rd estrus). In addition, serum progesterone on day 15 postbreeding tended to be lower in gilts with a greater degree of embryo wastage (Archibong *et al.*, 1987). The concentration of estradiol 17-ß is higher and more variable between weaning and rebreeding in sows that are weaned early at 10 days compared to those weaned at 21 or 42 days. This was also true of the estradiol concentration in the first ten days following breeding (Varley *et al.*, 1984). Varley *et al.* (1984) found the percentage embryo survival and estradiol concentration in early weaned sows to be significantly correlated, despite a nonsignificant difference in the embryo mortality rate. The source of these high postweaning levels of estrogen is questionable. Considering lactation and early weaning cause acute stress, it is possible that catabolized body fat stores or the adrenal gland are the sources of the elevated estrogen levels and this may carry over into the postweaning period (Varley *et al.*, 1984).

In addition, suboptimal LH surges can result in inadequate luteinization of the corpus luteum and reduced progesterone secretion which may also adversely affect embryo survival (Jindal *et al.*, 1996). However, Varley and associates (1984), found that in the 12 days postbreeding, progesterone concentration was found to rise faster in early weaned sows. This may be due, however, to differences in ovulation rate and are not a sole reflection of weaning age. However, because ovulation rate is not considered to be affected following early weaning, the timing of mating relative to ovulation may be an implicating factor. The effect of lactation length on litter size is more obvious in multiparous sows (Foxcroft *et al.*, 1995) because primiparous sows often already show impaired function in this respect.

High concentrations of estrogen may also adversely affect the success of fertilization, although currently it is not known to be affected by early weaning (Varley, 1982). Despite this, high levels of estrogen do affect the tubal cilia of the oviduct and

subsequent rate of ova transport (Varley *et al.*, 1984). Theoretically, this would lead to the loss of eggs prior to fertilization or implantation (Varley, 1982). Another factor involved may be that the period for egg maturation following early weaning is not sufficient. The magnitude and frequency of the LH surge may be implicated in this. The surge in LH is important for the final stages of oocyte maturation and if altered, the egg destined to be fertilized may not be viable.

Metabolic Influences On Reproduction

Lactation and catabolism

Nutrition plays a large role in the time that is required for the sow to become cyclic after weaning. Indicators of nutritional inadequacy include prolonged WEI (Giesemann *et al.*, 1989), high embryo mortality and decreased subsequent litter size (Brooks and Cole, 1972; Tokach *et al.*, 1992a; Foxcroft *et al.*, 1995). Most mammals partition nutrients towards their nursing young and may have to sacrifice their own requirements to do this. Feed restriction studies have demonstrated the sow's ability to 'milk off her back'. It has been shown that sows which are restricted in their feed intake during lactation and those that are allowed *ad libitum* feed intake during lactation wean litters of similar weight (Zak *et al.*, 1997b; Pluske *et al.*, 1998; Zak *et al.*, 1998). Furthermore, superalimented primiparous sows gain weight and backfat during lactation but do not produce more milk (Pluske *et al.*, 1998). Characteristically, sows that lose more body weight and condition while lactating have a longer postweaning anestrus. This is especially true of the young growing primiparous sow because of the conflict between her own growth and that of the nursing litter. Increasing age seems to be associated with increased resistance to feed deprivation (Prunier *et al.*, 1993). Therefore, in order to improve reproductive performance of first parity sows, feed intake must be optimized. However, evidence suggests that

superalimentation does not ameliorate the ill effects of energy deprivation beyond that of *ad libitum* feeding (Pluske *et al.*, 1998; Zak *et al.*, 1998). In fact, sows fed in excess of their requirements during lactation suppress their postweaning feed intake and lose weight and body condition in the postweaning period (Zak *et al.*, 1998).

Primiparous sows are particularly susceptible to the lactational losses in body condition and impaired reproductive function. First parity sows show longer and more variable postweaning anestrous periods compared to multiparous sows (Clark *et al.*, 1986), and are often removed from the breeding herd because of this increase in non-productive days (Foxcroft *et al.*, 1995). If fed adequately, sows grow and increase their lean body mass at least until parity four. In the feed-restricted gilt model, the metabolic transition from the unfed to the fed state occurs more slowly in older animals. And this may, in part, be due to higher energy requirements of young sows as a result of a higher level of protein accretion (Clowes *et al.*, 1994). The rate of lypolysis after weaning declines more quickly in the multiparous sow compared to the primiparous sow. As a result, mature sows return to their optimal metabolic status more quickly than primiparous sows following weaning. This translates into a situation whereby, at the first postweaning estrus, the multiparous sow is more likely to show better reproductive performance.

Management strategies such as "skip a heat" breeding may prove beneficial in minimizing the ill effects often seen with sows that become highly catabolic during lactation (Clowes *et al.*, 1994). Through this strategy sows have a recovery period of sorts, whereby they can begin to replace lost body stores before they are rebred. The result of which is an increased subsequent litter size, particularly in the first parity sow (Clowes *et al.*, 1994).

Endocrine regulation of postweaning reproduction

A growing body of evidence now suggests that irrespective of body composition, dietary metabolizable energy, and dietary protein fed during lactation exert a direct effect on the postweaning interval to estrus possibly by altering blood metabolites and body metabolism (Johnston *et al.*, 1989; Clowes *et al.*, 1994). Plasma levels of metabolic hormones such as insulin and IGF-I are depressed during lactation, while glycerol and cortisol are elevated pointing to an increased level of catabolism. This is especially true of the primiparous sow postweaning. Clowes *et al.* (1994) suggest that in the postweaning period, young sows actively partition nutrients towards protein accretion at the expense of fat therefore, the concentration of hormones involved with lipogenesis remain low throughout this period. As a result, lipogenic hormones such as insulin, are maintained at low levels and this may exacerbate the effect of losses in body condition.

Gonadotropin level

Nutritional effects on the reproductive axis can occur in the absence of obvious changes in live weight or body composition (Prunier *et al.*, 1993). The consequences of catabolism to reproduction involve both central and local ovarian effects (Zak *et al.*, 1997b). Loss of body fat and protein stores compromise the levels of gonadotropins that build up throughout lactation. The metabolic state of the sow influences the sensitivity of the ovary to gonadotropin stimulation. Foxcroft *et al.* (1995) state that nutritional deprivation in the early stages of lactation not only causes an increase in the postweaning return-to-estrus interval, but also in a reduction of LH secretion (Tokach *et al.*, 1992a), and possibly lasting consequences on the future reproductive performance of the sow. Some evidence suggests that compromising the sow's nutrition in the first few weeks of lactation, is more deleterious than if sows are feed deprived in late lactation (Tokach *et al.*, 1992a). It has also been shown, however, that severe feed restriction in the last week of a 28 day

lactation resulted in the lowest fertility of either group restricted in weeks two or three of lactation (Foxcroft *et al.*, 1995). However, this issue of time frame is equivocal and Zak and associates (1997a) suggest that transient changes in nutritional status at any point in lactation may result in "imprinting" of the follicles and thus, negatively affect subsequent ovulation rate and embryo survival. Also, nutritionally-mediated changes in gonadotropin secretion during the period of follicular recruitment have an effect on subsequent fertility by changing the size of the follicle pool (Foxcroft *et al.*, 1995).

Booth *et al.* (1996), fed nonpregnant gilts either to maintenance or *ad libitum* and found that following dietary repletion, gilts showed significant increases in uterine weight, follicle number and follicular fluid volume. As well, there was a significant increase in the mean concentration of LH, but not FSH, 6 hours following the meal. In agreement with this study, Prunier and coworkers (1993), found mean LH concentration and pulse frequency in gilts to be significantly influenced by feeding level. When both control and restricted-fed gilts were challenged with GnRH, the response to GnRH as measured by LH and FSH release was greater in restricted-fed gilts (Prunier *et al.*, 1993). These researchers concluded that feed restriction affects the GnRH pulse generator and not LH synthesis directly. Similar conclusions have been drawn by others (Armstrong and Britt, 1987; Beltranena *et al.*, 1991). In contrast, Booth *et al.* (1994) found the response to GnRH to be greater in restricted-fed gilts and concluded that there is an increase in the size of the releasable pool of LH as a result of feed restriction which allows for greater gonadotropin release following GnRH treatment. In support of this idea, Zak *et al.* (1997b) found LH pulsatility was completely abolished following feed restriction and immediately restored following realimentation. Serum levels of FSH have been found to remain fairly static regardless of feeding regime (Armstrong and Britt, 1987; Beltranena *et al.*, 1991; Prunier *et al.*, 1993).

The response to a GnRH challenge is also affected by the timing of feed restriction. Gilts fed *ad libitum* until reaching 85 kg body weight and then feed restricted to maintenance, had higher gonadotropin concentration at estrus than gilts fed at a restricted level and then fed *ad libitum* following attainment of 85 kg body weight (Booth *et al.*, 1994). The exhaustive research in this area to date, shows conclusively that feed restriction impairs reproductive function by suppressing gonadotropin synthesis and/or release. Little is currently known, however, regarding what occurs naturally in the growing primiparous sow that becomes catabolic during lactation.

Glucose

Plasma glucose is generally under very strict regulation as it is the primary energy substrate of the brain. Despite this, dietary restriction can cause obvious aberrations in glucose homeostasis. Preprandial concentrations of glucose decreased during a period of feed deprivation in gilts while no effect of feeding regimen was found for postprandial levels (Booth *et al.*, 1994). Plasma glucose was lower at the end of the first week of lactation in restricted-fed sows compared to those fed a high energy diet. At the end of the second and third weeks of lactation, the association between plasma glucose and dietary energy content had decreased but was significant nonetheless (Koketsu *et al.*, 1996). Generally plasma glucose decreases as lactation continues and this may reflect increased glucose uptake by the mammary gland (Armstrong *et al.*, 1986). This may then explain the lower association between energy intake and plasma glucose in later stages of lactation. Plasma glucose concentration does not appear to be related to sow body weight or backfat (Tokach *et al.*, 1992b). Glucose does appear to have a role in gonadotropin synthesis and/or release because glucose infusions in severely diet restricted gilts and ewes restores LH pulsatility (Prunier *et al.*, 1993). In contrast Tokach *et al.* (1992b) were unable to fully restore LH pulsatility in feed-restricted sows with glucose infusions. Maintenance of blood

glucose homeostasis is the most important trigger for insulin secretion which also plays an important role in reproductive competence.

Insulin

Insulin is a lipogenic hormone and its levels are largely regulated by energy status. In normoglycaemic lactating sows increasing insulin concentration hastens the transport rate of plasma glucose into peripheral tissue which in turn leads to increased feed consumption in order to maintain blood glucose homeostasis. When insulin levels are low during lactation, tissue glucose utilization is limited and this may result in more release of nonesterified fatty acids (NEFA) from the adipose tissue (Weldon *et al.*, 1994a; 1994b). Decreased glucose utilization and increased NEFA release increases the amount of substrate available for oxidation and therefore, feed intake is reduced (Weldon *et al.*, 1994b). This is shown by the fact that sows administered exogenous insulin eat more than placebo controls (Weldon *et al.*, 1994b). In steers undergoing compensatory gain following dietary restriction, insulin is positively correlated with body protein and body fat level (Hayden *et al.*, 1993). Losses in body condition, particularly during lactation, are associated with low insulin levels. As well, insulin has been found to be negatively related to urea nitrogen and free fatty acids in the blood (Armstrong *et al.*, 1986). Despite this, Zak *et al.* (1998) were unable to find a similar insulin response in relation to body condition of sows.

Dramatic fluctuations in insulin concentration occur almost immediately following dietary restriction and/or repletion. Both pre and postprandial insulin levels can be affected despite nonsignificant changes in plasma glucose (Beltranena *et al.*, 1991; Booth *et al.*, 1994). Insulin concentration has been found to be lower before and 5 hours after a meal in feed-restricted sows (Prunier *et al.*, 1993), while others report significant changes in insulin only after a meal (Armstrong and Britt, 1987; Zak *et al.*, 1997b). Nonsignificant differences in preprandial insulin concentration between feed-restricted and *ad libitum* fed

sows probably reflects a situation where both groups are in similar absorptive states (Cosgrove *et al.*, 1992). Following realimentation in the prepubertal gilt, the mean insulin concentration, concentration of postprandial peaks and duration of peaks in insulin are increased (Prunier *et al.*, 1993). Other researchers found similar results in the feed restricted-sow and gilt models (Armstrong *et al.*, 1986; Booth *et al.*, 1996; Zak *et al.*, 1998).

The availability of insulin and other metabolic hormones may directly signal the gonads or areas of the brain that are involved with gonadotropin release (Prunier *et al.*, 1993). These hormones may be an important link between nutrition and reproduction where changes in body composition and condition are not obvious. It is well established that insulin and to some degree, IGF-I are responsive to nutritional changes (Beltranena *et al.*, 1991; Buonomo and Baile, 1991; Meurer *et al.*, 1991; Prunier *et al.*, 1993; Booth *et al.*, 1994; Booth *et al.*, 1996). In the pig, insulin and IGF-I act together in ovarian steroidogenesis and together, may have a permissive action with the gonadotropins (Buonomo and Baile, 1991). Cox *et al.* (1987) found that gilts administered either long or short acting insulin and fed a high energy diet had significantly more corpora lutea than control gilts not receiving insulin. Insulin may play a central role in GnRH release, because insulin receptors have been identified in the hypothalamus, arcuate nucleus, median eminence and ventromedial hypothalamus in rats (Booth *et al.*, 1996). Injections of insulin into the cerebroventricular area increased LH secretion in gilts by accelerating GnRH pulses (Tokach *et al.*, 1992a). In humans, diabetes is associated with morphological abnormalities of the LHRH neurons and this is followed by decreased sensitivity of the anterior pituitary gland to LHRH (Booth *et. al.*, 1996). It is well established in the literature that insulin plays an important role in the regulation of LH secretion. Insulin concentration appears to be correlated with the frequency and magnitude of LH peaks (Cosgrove *et al.*, 1992; Tokach *et al.*, 1992a; Foxcroft *et al.*, 1995). The effects of insulin on LH secretion can be

shown by the fact that sows with a delayed return to estrus postweaning have lower serum insulin concentration (Tokach *et al.*, 1992a).

Follicular recruitment and atresia are regulated, in part, by insulin levels as well. Indeed, in gilts, insulin injections increased the LH pulsatility, reduced preovulatory follicular atresia (Cox *et al.*, 1987; Cosgrove *et al.*, 1992; Booth *et. al.*, 1996) and may have participated in follicular recruitment (Zak *et al.*, 1997b). Meurer and coworkers (1991) found diabetic gilts had higher incidence of follicular atresia in small follicles ≤3 mm and other authors have found atresia to be increased in preovulatory follicles of diabetic sows. In the gilt, diabetes results in increased testosterone concentration of medium-sized follicles and lowered estradiol concentration of large follicles. This indicates a level of reduced aromatization in follicles of increasing size, suggesting increased follicular atresia in diabetic sows (Meurer *et al.*, 1991). Insulin administration to prepubertal gilts did not affect the number of medium or large follicles, but, the number of small follicles increased and the rate of atresia decreased (Matamoros *et al.*, 1991). In addition, Matamoros *et al.* (1991) were able to show that exogenous insulin increased follicular fluid IGF-I during eCG stimulated follicular growth. These changes occurred independent of body condition and did not involve alteration to LH, FSH, cortisol, testosterone or blood metabolites. The diabetic sow model also showed intrafollicular IGF-I to be depressed, but not peripheral levels. There was no positive correlation between the follicle diameter or estradiol concentration and the concentration of IGF-I as there is in the non-diabetic control. This data suggests that normal aromatization and follicular development does not occur in the absence of insulin.

In addition to its action at the neural and hepatic levels, insulin may act directly at the ovarian level (Beltranena *et al.*, 1991). As reviewed by Booth (1990), insulin at the ovarian level acts on amino acid transport, stimulates glucose uptake and utilization and low-density lipoprotein metabolism of the granulosa cell. Insulin also augments FSH

stimulated LH receptor induction (Booth, 1990). And this was shown *in vitro* when insulin in conjunction with FSH enhanced LH/hCG receptor binding and progesterone secretion. The level of insulin administration in this study, however, was 1000-fold higher than the physiological level (Maruo *et al.*, 1988). In addition, administration of insulin to cultured porcine granulosa cells *in vitro* enhanced forskolin and dibutyryl adenosine 3', 5'-cyclic monophosphate-stimulated steroidogenesis (Xu *et al.*, 1995). Thus, the intrafollicular effects of insulin can occur independently of LH by affecting ovulation rate and decreasing follicular atresia (Meurer *et al.*, 1991; Booth *et al.*, 1996).

IGF-I

IGF-I or somatomedin-C is involved with gonadotropin receptor induction, granulosa cell proliferation and differentiation (Maruo *et al.*, 1988) and progesterone and estrogen biosynthesis and acts in synergy with FSH-stimulated estradiol production (Veldhuis *et al.*, 1985; Maruo *et al.*, 1988; Cosgrove *et al.*, 1992; Howard and Ford, 1994; Xu *et al.*, 1995). The porcine granulosa cell is known to express the gene encoding IGF-I as well as high affinity receptor sites for IGF-I. Granulosa cells, when cultured *in vitro* with FSH and IGF-I, result in increased release of estradiol (Adashi *et al.*, 1985; Maruo *et al.*, 1988), increased cellular differentiation and LH receptor binding (Maruo *et al.*, 1988). Furthermore, *in vitro* studies with porcine granulosa cells show the effective dose of IGF-I to be at physiological levels (Maruo *et al.*, 1988). In addition, porcine granulosa cells cultured with human IGF-I show a dose-dependent stimulatory effect on progesterone production. It is suggested that this increase in progesterone is attributed to enhanced steroidogenesis by stimulation of the cholesterol side-chain cleavage reaction (Veldhuis *et al.*, 1985). Thus, the current information points to IGF-I having both an autocrine and an endocrine mode of action.

Currently, an active area of research focus is on the IGF-binding proteins (IGFBP) and their potential role in ovarian steroidogenesis. The IGFBP are a group of proteins with varying molecular weights. *In vitro*, granulosa cells can produce IGFBP and influence steroid production which may implicate them as modulators of IGF-I action (Howard and Ford, 1992). The IGFBP profiles in porcine follicular fluid of small and large follicles differ (Howard and Ford, 1992). Howard and Ford (1992), using primiparous sow follicles, found the concentration of IGF-I to be greater in small follicles than in medium and large follicles postweaning and IGFBP-2 to decrease with increasing follicle size. Furthermore, IGFBP-2 had an inverse relationship with follicle steroid production. IGFBP-2, although lowest in medium and large follicles, was more strongly associated with steroid production. The IGFBP-2 band intensity was strong in large follicles with low estradiol production (Howard and Ford, 1992). IGFBP-3, however, did not show a strong relationship with follicle size or steroid production (Howard and Ford, 1992). These same researchers, in a later study, found that IGFBP-3 content (40-44 kDa IGFBP) was similar between loosely associated and tightly bound granulosa cells and was not related to steroidogenesis. Tightly bound granulosa cells that maintain high estradiol production produced less IGFBP-2 and it was suggested that this also implicates IGFBP-2 in the control of follicular steroid production (Howard and Ford, 1994). As well, they were able to show that IGFBP-3 as well as IGFBP-2 (28-30 kDa IGFBP) increased with IGF-I administration. From this, it can be concluded that IGFBP-2 may be implicated in steroid production and possibly follicular atresia because of the inverse association with follicular fluid aromatization. Generally, the binding proteins for IGF-I are associated with a decrease in IGF-I activity (Whitley *et al.*, 1998). Whitley *et al.* (1998) found that insulin administration decreased IGF-I and IGFBP-2, but not IGFBP-3. The level of IGFBP-2 was lower 5 days postweaning compared to 3 days and this is consistent with increasing follicular development.

The effect of feed restriction on IGF-I concentrations is less predictable than for insulin and the results in the literature are conflicting. Rutter and Manns (1991) report that short term changes in feed intake do not usually have a dramatic effect on IGF-I levels. They suggest that, in the cow at least, circulating IGF-I levels are not suppressed further by feed restriction as long as body reserves are being mobilized. However, in the already highly catabolic primiparous sow, feed restriction in the last week of a 28-day lactation causes IGF-I levels to fall and remain low into the postweaning period (Zak *et al.*, 1997b). Feed-restricted prepubertal gilts also have lower mean IGF-I concentration (Booth *et al.*, 1994). In the castrated male pig, a 48 hour fast caused a 58% decrease in plasma IGF-I as a result of decreased peptide synthesis (Buonomo and Baile, 1991). Fasting decreases IGF-I mRNA content in hepatic and extra-hepatic tissue, but, is not considered to alter the half-life (Buonomo and Baile, 1991). Beltranena *et al.* (1991) speculate that during periods of feed restriction, hepatic tissue becomes insensitive to the increased GH concentration explaining the often static IGF-I levels. Whether the intraovarian levels of IGF-I are depressed during feed restriction remains to be determined.

Insulin's effect on the level of circulating IGF-I is equivocal. Normally, in the growing sow, increased insulin in the postweaning period causes up-regulation of GH receptors and a correlated increase in hepatic IGF-I concentration. Matamoros *et al.* (1991) showed that insulin administration with eCG treatment increased follicular IGF-I concentration in prepubertal gilts. However, Whitley *et al.* (1998) found insulin administration decreased intrafollicular IGF-I and suggested that possibly the IGF-I receptor was bound by insulin, although with weak affinity. In the diabetic gilt, intrafollicular IGF-I concentration of large follicles is depressed and there is an absence of a correlation between IGF-I and follicle diameter. The follicular concentration of testosterone was also higher in diabetic gilts indicating a greater incidence of atresia (Meurer *et al.*, 1991). In the normoglycaemic gilt, IGF-I is positively correlated with follicle size and

intrafollicular levels are increased by PMSG and gonadotropin treatment (Meurer *et al.*, 1991). In contrast, Clowes *et al.* (1994) found insulin and IGF-I to be negatively correlated at first estrus in the primiparous sow fed according to their metabolic body size. Booth and associates (1994) found IGF-I and follicular fluid volume in large follicles of gilts to be positively correlated and this was also correlated with live weight. Buonomo and Baile (1991) state that an insulin threshold exists that controls the dissociation of the GH-IGF-I regulatory mechanism. Perhaps then, IGF-I is a long term mediator of the effects of nutrition on reproduction.

Cortisol

Little information exists in the literature on the effects of dietary energy on adrenal steroid production. Feed restriction can be interpreted by an animal as a period of stress and therefore, during periods of feed denial the adrenal axis is activated and plasma glucocorticoid concentrations are increased (Booth, 1990; I'Anson *et al.*, 1994). However, the mechanisms mediating gonadotropin inhibition during periods of restricted feed intake are not fully understood (I'Anson, *et al.*, 1994). Corticosteroids are involved with catabolic processes and the stimulation of gluconeogenesis and are believed to be linked to the thyroid axis (Prunier *et al.*, 1993; van Haasteren *et al.*, 1996). Matamoros *et al.* (1991) found that exogenous insulin treatment had no effect on serum cortisol levels and cortisol was not related to body condition. Despite this, cortisol has been found to be higher in underfed and catabolic animals (Prunier *et al.*, 1993; van Haasteren *et al.*, 1996). Booth *et al.* (1994) found plasma cortisol to be elevated in feed restricted gilts in the postprandial period. However, Prunier and associates (1993) found cortisol concentrations in peripubertal gilts to be higher before a meal than 5 hours following, although this was not affected by feed restriction. Likewise, I'Anson and coworkers (1994) found similar diurnal cortisol rhythms and pulse frequencies in prepubertal female lambs restricted to 25% of

their nutrient requirement and *ad libitum* fed female lambs, despite large differences in LH pulse frequencies. The signal to the hypothalamic-adrenal axis may occur only when starvation is severe and chronic. In fact, the stress response is biphasic with an initial stimulatory period. If the stressor, feed denial in this case, is prolonged, an inhibitory phase follows. The initial effect of stress is at the hypothalamus and if continued, peripheral mechanisms develop and these are characterized by changes in responsiveness of the anterior pituitary gland and the ovary (Rivier and Rivest, 1991). It is established that chronically elevated cortisol levels impair reproductive function by blocking GnRH and LH secretion and thus, preventing ovulation (Rivier *et al.*, 1986; Booth, 1990). Cortisol lowers the responsiveness of the pituitary gland to GnRH (Varley, 1994) and can suppress gonadotropin secretion by pituitary cells *in vitro* (Li, 1989).

Thyroid status

Relatively little is known about the effects of feed restriction on the thyroid axis and the implication to reproduction. In rats, feed deprivation and energy restriction has been shown to affect the hypothalamic-pituitary-axis (Blake *et al.*, 1991; Rondeel *et al.*, 1992; van Haasteren *et al.*, 1996). Feed deprivation significantly decreased pro-TRH mRNA in the paraventricular nucleus and TSHß mRNA in the anterior pituitary gland (Blake *et al.*, 1991). As well, feed restriction in rats lowered plasma thyrotropin-releasing hormone (TRH), thyroxine (T_4), free T_4, triiodothyronine (T_3) and free T_3 (Rondeel *et al.*, 1992; van Haasteren *et al.*, 1996). Giesemann *et al.* (1989) found the level of total T_4 to be unaffected by energy intake during lactation and in the postweaning period. Brendemuhl and coworkers (1987) also found T_4 to be unaffected by absolute energy or protein level in the diet of primiparous sows. However, there was a significant interaction between energy and protein level in the diet, in that, sows fed low protein diets but with high energy had decreased T_4 concentration. When energy was added to the high protein diet, T_4 increased

and the opposite effect occurred when energy was added to a low protein diet (Brendemuhl *et al.*, 1987). The level of T_4 in this study, increased following weaning compared to the levels in lactation (Brendemuhl *et al.*, 1987). In contrast to T_4, changes in T_3 can be elicited by carbohydrates and fat, but not by protein. Buonomo and Baile (1991) found T_4 to decrease by 22% within 48 hours and T_3 to decrease by 51% within 24 hours of feed restriction. In castrated male pigs, T_4 increased within 2 hours and T_3 increased immediately following a meal (Buonomo and Baile, 1991). Booth *et al.* (1994) found both pre and postprandial free T_3 concentration to be lower during feed restriction and to increase following refeeding. Giesemann *et al.* (1989) found that during lactation, plasma levels of total T_3 in primiparous sows fall with energy restriction. Weaning and introducing an isoenergetic feeding level reestablished total and free T_3 levels. In energy-restricted steers, T_4, T_3 and reverse T_3 were decreased, while with energy repletion, they were increased and positively correlated with body protein and body fat (Hayden *et al.*, 1993). In pigs, energy restriction lowers T_3, T_4 remains unchanged and reverse T_3 increases (Eales, 1988). The activity of the deiodinase enzyme which is involved with the conversion of T_4 to T_3 is shown to decrease following feed restriction. Therefore, even with static plasma levels of T_4, the levels of T_3 can be depressed as a result of feed restriction. The fact that T_4 does not appear to change during refeeding or restriction may be related to the fact that the change in thyroid hormone status is more immediate for T_3 than T_4 (Eales, 1988; Buonomo and Baile, 1991).

Energy-restricted animals are hypersensitive to the effects of thyroid hormones on metabolism and thyroid hormones may interact with GH to alter IGF-I levels (Booth *et al.*, 1994). Hypothyroidism is associated with lowered IGF-I concentration and receptor binding (Buonomo and Baile, 1991). However, Giesemann *et al.* (1989) found hyperthyroidism (from feeding thyroprotein) neither enhanced nor diminished the ability of a sow to return to estrus postweaning. During periods of energy or feed restriction,

following a concomitant decrease in thyroid hormone and associated decrease in IGF-I release reproductive function may be impaired.

Follicular development, oocyte maturation and embryo survival

Nutrition and energy intake also affects ovulation rate by changing the character of the follicle pool available for recruitment. There is evidence suggesting that the quantity and the quality of follicles within the presumptive pool are prone to nutritionally-mediated effects (Zak *et al.*, 1997a). As well, nutrient deprivation can have profound effects on normal oocyte maturation and embryonic development (Foxcroft *et al.*, 1995; Jindal *et al.*, 1996; Zak *et al.*, 1997a). Zak *et al.* (1997a) hypothesized that nutritionally mediated changes to embryo survival, in the absence of changes in ovulation rate, are due to differences in oocyte quality in the preovulatory follicle pool and differences in the follicular support of oocyte maturation. These researchers found that restricting feed intake to 50% in the last week of a 28 day lactation period *vs.* refeeding in the last week, showed refed sows characteristically had a greater population of large (≥7 mm) follicles at slaughter 3-4 days following weaning. Restricted fed sows had a significantly larger pool of small (≤5 mm) follicles at slaughter. However, follicular concentrations of estradiol were not different between the two treatment groups at 24, 48 or 72 hours postweaning. In addition, there was no difference in the relationship between follicle size and estrogen concentration for refed or restricted sows. During the first 3 weeks of lactation some follicular recruitment occurs and results suggest that feed restriction at this time hinders folliculogenesis in terms of follicle growth but not steroid production.

Results from *in vitro* oocyte maturation show that although cumulus expansion is not significantly affected by feed restriction and/or refeeding, the proportion of oocytes that advance to metaphase II is larger in refed sows than in feed-restricted sows. Zak *et al.* (1997a) found that the number of oocytes that only reach metaphase I and the germinal

vesicle breakdown phase was greater for the feed-restricted sows. As well, when randomly selected oocytes, from ovaries collected at an abattoir, were cultured in follicular fluid from either refed or restricted-fed sows, the number of those oocytes reaching metaphase II was greater when cultured in follicular fluid collected from refed sows. As before, the greatest number of oocytes that reached only metaphase I were those cultured in follicular fluid collected from feed-restricted sows. There were no treatment differences in the proportion of oocytes having nuclei in the germinal vesicle breakdown or germinal vesicle stage. This study suggests that nutrition influences the follicle and its ability to support the initial stages of oocyte maturation, but, not necessarily its capacity for steroidogenesis. In this way, nutrition may be implicated as a mediator of embryo survival.

The follicles observed in the study of Zak *et al.* (1997a) formed a heterogeneous group. The degree of follicular heterogeneity is important in the preovulatory pool because it is important to oocyte competence (Ding and Foxcroft, 1992) and ability of the follicle to properly undergo luteinization (Zak *et al.*, 1997a). Indeed, follicles in the recruited preovulatory population show a marked size difference and steroid concentration variability. The follicular cells are crucial regulators of oocyte maturation. Follicles of different sizes influence oocyte maturation to different degrees because the follicular cells at various stages produce and secrete maturational factors differentially (Ding and Foxcroft, 1992; 1994). This relationship between the follicle cells and the oocyte is fundamental to oocyte maturation and competence (Ding and Foxcroft, 1992). Large follicles are able to support oocyte maturation to a greater extent than small follicles and this may be a contributing factor to the effects of follicular heterogeneity on early embryonic development. The number of granulosa cells are positively correlated to follicle diameter and the number of granulosa cells may in part, contribute to the amount of oocyte maturation factors that are secreted by the follicle (Ding and Foxcroft, 1992). Ding and Foxcroft (1994) cultured porcine follicle cells from either large or small follicles at day 17

or 20 of the estrous cycle and collected the culturing media for maturing oocytes. Oocyte maturation was greater in media cultured from large follicles. However, fertilization rate of these oocytes showed that sperm penetrability was not different between the oocytes cultured in either media from small or large follicles. The more advanced stage of oocytes cultured in follicular fluid collected from refed sows and the larger follicle size in sows following dietary repletion, suggests that the follicles of these sows are more competent in supporting oocyte nuclear maturation although fertilization per se, may not be compromised. In addition, it may be concluded that perhaps the follicle pool of feed-restricted sows has a greater degree of heterogeneity which may result in poor luteinization of some follicles and thus, compromise the maintenance of pregnancy. Therefore, even in the absence of peripheral or follicular steroidal aberrations between sows on varying nutritional planes, embryo survival may be affected (Zak *et al.*, 1997a).

It may be of importance to note that over-feeding does not necessarily lead to improved reproductive performance (Zak *et al.*, 1998). An interesting example of this is the situation of high energy intake and its relation to embryo survival. Despite the ill effects of feed restriction during lactation on follicular development and oocyte maturation, high energy intake in the very early period following mating can have detrimental effects on embryo survival. High plane feeding postbreeding may alter steroidogenesis and affect embryo survival as assessed by Jindal *et al.* (1996). In this study, gilts were fed 1.5 times maintenance beginning on day 1 (N1) or day 3 (N3) postbreeding or 2 times maintenance on day 1 (H1) postbreeding. These researchers found no differences in ovulation rate although embryo survival in the N1 group was significantly greater than the H1 group. As well, plasma progesterone was significantly higher in the N1 group. Despite these findings, Pharazyn *et al.* (1991) found no differences in plasma progesterone or embryo survival rate between gilts fed diets with two levels of energy and protein from 3 to 15 days postbreeding. Jindal *et al.* (1996) conclude that a high plane of nutrition in the very early

postcoitum period results in increased embryo mortality and they relate this to a much reduced plasma progesterone concentration. They suggest that this may be due to elevated hepatic clearance rate of progesterone as a result of increased blood flow to the liver following alterations in feed intake. There is a "window" of critical time, therefore, that feed level must be carefully monitored in order to minimize early embryonic losses.

Body composition

Cumulative losses in body fat and protein stores during lactation are involved with lowered postweaning reproductive performance. Johnston *et al.* (1989) determined total body fat using D_2O and found a negative relationship between body fat and the postweaning interval to estrus in the primiparous sow. That is, sows with less than 13% body fat had a delayed return to estrus. However, a negative correlation between body weight and WEI in this study was stronger. Other researchers have suggested a "threshold" level of body fat that is required and below which reproductive failure persist. Primiparous sows that are targeted to 20 mm compared to 12 mm of backfat at parturition have shorter WEI (Yang *et al.*, 1989). Notwithstanding, sows with less than 12 mm of P2 backfat at weaning do not necessarily show extended postweaning anestrous periods or compromised litter sizes (Clowes *et al.*, 1994).

Feeding level in lactation also exerts an influence on nitrogen balance. Primiparous sows that are restricted in their nutrient level during lactation excrete less nitrogen in their urine but the nitrogen secreted in the milk is similar to that of *ad libitum* and superalimented-fed sows (Clowes *et al.*, 1998). This suggests that restricted-fed sows partition a majority of their dietary nitrogen towards milk production and consequently lose a larger proportion of body protein in the form of nitrogen. Diets that are deficient in protein but adequate in energy, result in large losses in protein from the muscle and internal organs and extend the WEI (Brendemuhl *et al.*, 1989).

Diet composition

The true nutritional requirements during lactation can be viewed as the sum of the sow's maintenance and production needs and these change with varying milk output over time (Cole, 1982). As lactation progresses, amino acid catabolism increases, but not in sows that are fed high energy-low protein diets. This is related to increasing milk yield through lactation and the need for more glucose and other energy substrates (Brendemuhl *et al.*, 1987). Sow productivity during lactation is dependent on both the levels of protein and energy in the diet and sow diets that are low in both energy and protein increase the duration of the WEI (Brendemuhl *et al.*, 1987; Coffey *et al.*, 1994; Koketsu *et al.*, 1996). Lysine is the first limiting amino acid in swine and inadequate energy intake limits the ability of a sow to utilize dietary lysine. In addition, sows nursing large litters (>10 piglets), require increased dietary lysine to minimize body protein mobilization and maximize milk production (Coma *et al.*, 1996). Brendemuhl *et al.* (1987) report that the level of amino acid catabolism is greater in sows fed high protein diets as well as sows fed low energy diets. Serum urea nitrogen is positively correlated with amino acid metabolism. Coma and associates (1996) increased the lysine content in the diets of adult mixed parity sows from 30 to 64 g day^{-1}, resulting in a quadratic decrease in plasma urea nitrogen. Supporting this, Knabe *et al.* (1996) found that increasing dietary lysine in the diet of primiparous sows nursing ≥9 pigs resulted in a reduction of backfat loss. When the results were pooled across all parities, however, the effects of increased lysine on backfat were not significant. This suggests that primiparous sows may benefit to a greater degree from dietary lysine supplementation, possibly relating to a higher degree of catabolism in these sows. When dietary amino acids levels are below the requirements in lactation, body protein is catabolized to compensate and maintain milk production. Increasing the content

of the first limiting amino acid reduces protein catabolism and the body nitrogen balance is kept positive (Coma *et al.*, 1996).

The ratio of body protein and body fat losses during lactation are affected by dietary energy, protein and levels of lysine (Brendemuhl *et al.*, 1989; Tokach *et al.*, 1992a). If lysine levels are low, protein is compromised, and if the levels are high, fat makes up a larger proportion of the loss. Brendemuhl *et al.* (1987) reported that sows fed diets with high energy and low protein compared to sows fed diets with low energy and high protein had similar weight losses but considerable differences in backfat changes. In a later study, however, it was found that sows fed diets with adequate energy but low protein, lost more weight in the first week of lactation than when sows were fed high protein. However, in the second week of lactation, low energy influenced weight loss to a greater degree and by the fourth week of lactation, there were no energy or protein interactions (Brendemuhl *et al.*, 1989). In addition, supporting their earlier study, diets that were deficient in energy and adequate in protein, caused greater backfat loss than in protein deficient diets. Not surprisingly, sows fed diets high in energy and high in protein lost less weight and backfat than when fed diets low in energy and protein (Brendemuhl *et al.*, 1989). As well, the protein and energy intake affected carcass composition, in that, dietary protein level influenced longissimus muscle area and dietary energy level influenced backfat thickness (Brendemuhl *et al.*, 1989).

Protein level in the diet is correlated with the levels of certain blood metabolites such as free fatty acids, serum urea and glucose. As protein and lipid stores are mobilized for milk synthesis, the levels of insulin and IGF-I fall while glycerol, growth hormone and cortisol increase (Foxcroft *et al.*, 1995). When the total energy consumed is reduced, LH pulsatility and mean concentration are significantly lowered (Koketsu *et al.*, 1996). The influence of lysine on LH secretion increases as the metabolizable energy content in the diet increases. Kemp *et al.* (1995) have shown the energy source in the diet (*i.e.* carbohydrate

vs. fat) influences both insulin and LH secretion in sows. As well, the postprandial insulin response is earlier in isocaloric-starch (carbohydrate) diets than those supplemented with fat. Sows fed diets supplemented with starch also have higher plasma progesterone possibly reflecting increased LH secretion and enhanced granulosa cell luteinization in these sows. In addition, Kemp *et al.* (1995) found that the effects of energy source were not as clear for the lactating sow as those seen in the gilt. These authors attribute this to the confounding effects of a negative energy balance in lactation. Cox *et al.* (1987) enhanced ovulation rate, LH pulsatility and FSH concentration in gilts by feeding high energy diets. They maintain that the effects of nutrition are more obvious in first parity sows. This is primarily due to the fact that primiparous sows have a greater likelihood of being in a negative energy balance and becoming catabolic during lactation and thus, exhibiting chronic anestrus postweaning (Tokach *et al.*, 1992a; Foxcroft *et al.*, 1995). Furthermore, the primiparous sow is usually not able to consume enough energy in her diet to prevent becoming catabolic.

Kirkwood and Thacker (1990), proposed that feed refusal by the sow could be minimized by elevating serum progesterone. Studies with rats has shown that feed intake increases with progestin treatment. Moreover, litter performance can be improved by feeding orally active progestogens to the sow. This treatment appears to be most beneficial, however, when dietary and body conditions are poor (Kirkwood and Thacker, 1990).

Conclusions

Reducing the lactation length of the sow to <20 days is currently a practice being used as a technique aimed at increasing the number of litters she will produce in her breeding life. In lactation there is a suppression of gonadotropin synthesis and/or release primarily as a result of the suckling stimulus from the piglets. This inhibition occurs at all

levels of the hypothalamic-pituitary-gonadal axis and involves increased secretion of prolactin, oxytocin and endogenous opiates. At weaning, there is an almost immediate increase in basal LH concentration, pulse frequency and pulse amplitude and subsequently, follicular development. Theoretically, early weaning should minimize the suppression in gonadotropin levels and the elevated levels of prolactin, oxytocin and endogenous opioid peptides associated with lactation. However, the strategy of early weaning has inherent problems. These problems are not only the potentially ill effects on piglet growth and welfare, but also in terms of the factor that it is trying to improve; the reproductive efficiency of the sow. Early weaning has been shown to be associated with increased WEI, ovarian dysfunction, reduced litter sizes in subsequent pregnancies and possibly negative effects on piglet growth and survival. Because of this, strategies are being employed to overcome these difficulties. Techniques such as partial weaning, split weaning and hormone treatments have been shown to be beneficial in minimizing the ill effects of early weaning.

Confounding the effects of early weaning are the metabolic demands of lactation. Feed restriction has been shown to dramatically hinder reproductive efficiency. Negative effects on reproduction can occur in the absence of obvious changes in body condition, despite the fact that a certain level of body fat and/or protein is required for the resumption of estrous cycles postweaning. The growing first parity sow, who is generally unable to consume enough dietary energy in lactation to prevent a negative energy balance, characteristically exhibits prolonged postweaning anestrous periods. The level of nutrition and nutrient intake affect the buildup and release of gonadotropin stores that are required for ovarian activity postweaning. The levels of certain hormones and metabolites that act as indices of metabolic status are also important regulators of all aspects of reproduction. These include glucose, insulin, intraovarian and hepatic IGF-I, cortisol and the thyroid hormones. Either acting alone or in concert with each other, glucose and the metabolic

hormones are involved with gonadotropin release and thus folliculogenesis. Nutritionally-induced suppression of these result in increased intervals to estrus postweaning and impaired reproductive function.

Therefore, diet is also implicated in reproductive function. Not only are the absolute energy levels which are fed important, but whether dietary energy is in the form of carbohydrate or fat appears to be important. The effects of nutrition and feeding regime are most important in the primiparous sow. The conflict between their own growth and providing for growth of their litter makes these sows particularly problematic to manage. This is especially true in the postpartum and rebreeding period as they approach their second gestation. Although research has indicated the effect and mechanism(s) involved with the aberrations to metabolism resulting from nutritional modification, many questions are still unanswered.

CHAPTER 3

MATERIALS AND METHODS

Animals

At approximately 105 days of gestation, 9 first parity and 8 fifth parity Cotswold sows were placed into individual farrowing pens (1 m high x 2.3 m long x 1.65-1.8 m wide). Some natural light entered through windows at either end of the barn and otherwise, lights remained off unless general or experimental work was being performed. Sow weights and backfat measurements were taken at pen entry, day 112 of gestation (g), days 3, 7, and 14 postpartum (pp), 5 days postweaning (pw), 10 days postbreeding (pb) and on the day prior to slaughter. All sows and piglets in this study were cared for in accordance with the Recommended Code of Practice for Care and Handling of Farm Animals: Pigs (1993) and guidelines of the Canadian Council on Animal Care (1993).

Sow and litter management

From one day prior to farrowing to day 3 pp, sows were confined in a crate (1 m high x 2.3 m long x 0.6 m wide) within their pen. Sows with delays of more than 30 minutes between piglet births were given 2 ml oxytocin (20 IU ml^{-1}; Rhone-Merieux Canada Inc., Victoriaville, PQ, Canada) as long as the birth canal was clear. Sows more than 24 hours late to farrow were induced with 2 ml of prostaglandin (Lutalyse, 5 mg dinoprost ml^{-1}, Upjohn Co., Orangeville, ON, Canada) followed by 2 ml of oxytocin 20 hours later. In order to equalize metabolic demand across parity, litters were standardized to 10 for first parity sows and 12 for fifth parity sows by day 3 pp. These numbers were based on the average litter sizes for parity 1 and 5 sows, respectively, within the university

herd Cotswold sows. Piglets were weighed at birth, days 3 and 7 pp and at weaning on day 14 pp. Piglet deaths occurring during the trial were recorded. Piglets had free access to water, but were not creep fed. All standard practices for piglet processing and sow care were performed.

Feeding schedule

From entry into the farrowing crate to farrowing, sows were fed 3.5 kg day^{-1} of a commercial barley-based lactation diet with 3979 Kcal kg^{-1} DE and 18.2% crude protein. From day 1 pp, sows were brought up to full feed by increasing their feed allowance in 0.5 kg increments daily. Following weaning, sows were fed 3.5 kg day^{-1} of a barley-based dry sow diet containing 3222 Kcal kg^{-1} DE and 15% crude protein. Sows had free access to water at all times via a nipple waterer. Feed offered and orts were recorded daily. Feed allowances met or exceeded the NRC (National Research Council, 1988) recommendations.

Estrus detection and breeding

On the day of weaning and every day thereafter, sows were exposed to a mature boar and allowed fence line contact to aid in estrus detection. On the first day of standing estrus, sows were bred twice at approximately 0900 and 1400 hours and at approximately 0900 hours the following day. All sows were bred by at least two different boars of known fertility. The interval from weaning to estrus was recorded for each sow. Sows were kept in the same pens until 27-35 days pb when they were killed at the abattoir. Reproductive tracts were recovered and returned to the laboratory and examined for ovulation rate and embryo survival.

Ovulation rate and embryo survival

The reproductive tracts were stored in a cooler during transport to a 4°C cold room. All dissections were performed within 36 hours of slaughter. Ovaries were macroscopically inspected for follicular or luteal cysts, corpora albicans, corpora hemorrhagica and corpora lutea. Random slices were made into the ovary to ensure all ovulation sites had been counted. Ovulation rate was defined as the number of corpora lutea or, in sows that returned to estrus postbreeding, the number of corpora albicans.

The uterus was dissected to count the embryos. Uterine horns were carefully cut from the oviduct to the cervix, cutting through the myometrium and endometrium, exposing the lumen. Conceptuses were counted and removed from the uterus. The amniotic and allantoic sacs were removed and the embryos were grossly evaluated for any obvious abnormalities. Crown rump length was measured. Any embryos within a uterus that were less than half the length of all other embryos were classified as abnormal. Any other embryos that were obviously degenerative or deformed were classified as abnormal. Only embryos that were typed as normal were used in the calculation of embryo survival. Assuming fertilization as 100%, embryo survival was determined by calculating the number of normal embryos as a percentage of the ovulation rate for each individual sow.

Catheterization

At approximately 107 days of gestation, each sow was fitted with a venous cannula. For placement of the cannulae, each sow was restrained with a nose snare and anesthetized with intravenous administration of a mixture of Ketamine (100 mg ml^{-1}; M.T.C. Pharmaceuticals, Cambridge, ON, Canada) administered at 5 mg kg^{-1} body weight and Valium (5 mg ml^{-1}; Hoffmann-LaRoche Limited, Etobicoke, ON, Canada) administered at 0.5 mg kg^{-1} body weight. If required, the level of anesthesia was maintained by an additional dose of not more than 700 mg (7 ml) of ketamine.

Sows were positioned laterally on the pen floor and the ear, dorsal region of neck and shoulders were shaved and the ear was cleaned with Hibitane (Wyeth-Ayerst Canada, Inc., Montreal, PQ, Canada) and isopropyl alcohol and swabbed with Betadine solution (Purdue Frederick Inc., Pickering, ON, Canada). A tourniquet was placed at the base of the ear and the ear was covered with a sterile drape. A thin-walled 2-inch 14-gauge needle (CDMV) was inserted into a prominent vein and 1/2 meter of clear vinyl plastic tubing (Dural Plastics & Engineering, Auburn, N.S.W., Australia, Cat. No. SV. 70, Code E20268, I.D. 1 mm, O.D. 1.5 mm) was threaded through the needle. If veins were hard to visualize, a flashlight placed under the ear often helped. Catheters that did not feed easily through the needle and vein, were gently massaged and the catheter tubing coaxed along. Once catheter material was in place, the needle was carefully pulled from the vein while holding the catheter in place, and pulled over the catheter tubing. A catheter adapter, made from 1-inch 18-gauge needle with the tip ground off, was inserted into the catheter and an adapter cap (Becton Dickinson, Sandy, UT, USA) placed on the end.

The catheter was secured at the opening of the vein with a small amount of Vet Bond (3M Animal Care Products, St. Paul, MN, USA) and a small piece of thin white tape was wrapped around the catheter at the point of puncture and secured to the ear. Figure 1 shows the puncture site and cannula entry and attachment of catheter tubing to the animal. Ag Tek skin cement (Kane enterprises, Sioux Falls, SD, USA) was applied along the ear, dorsal region of the neck and shoulders. Three-inch-wide Elastoplast tape (Smith and Nephew; Lachine, PQ, Canada) was secured to the skin over the glue. The remaining 1/2 meter of catheter tubing was laid flat over the tape and 2-inch-wide white fabric tape was secured over the catheter tubing. The catheter was checked for patency with sterile saline and 3 ml of heparinized saline (100 USP ml^{-1}) before it was placed in a whirl pack that was secured dorsally on the shoulders with the skin glue. It was determined that 1 meter of catheter tubing held 2 ml of fluid, therefore 3 ml of heparinized saline was injected into the

catheter. A piece of tight fitting elastic-tube netting (CIDA, Overland Park, KS, USA) was placed over the ear.

Two of the sows were fitted with jugular cannulae as ear vein cannulation proved to be difficult. The procedure was similar for the ear vein cannulation except the sows were placed in dorsal recumbency. A 3-inch 14-gauge thin-walled needle (CDMV) was inserted in the jugular vein with needle entry into the jugular groove directed away from the animal's head. Suction of a saline filled syringe on the needle indicated placement in the jugular vein if it rapidly filled with blood. At this point, the syringe was removed and 1/4 to 1/2 meter of catheter tubing was threaded through the needle. All other procedures for securing the catheter were the same as for the ear vein cannulation, except the tape and tubing were attached laterally and dorsally to the neck and shoulders.

To reduce the incidence of infection, 10-20 ml Penlong (Rogar STB Inc., London, Ont., Canada) was administered intramuscularly to each sow following the cannulation procedure. Aseptic techniques were used throughout the procedure which generally took no longer than 30 minutes.

The catheters were checked for patency every day. At least 3 ml of fluid was drawn off from the catheter and replaced with 3 ml of heparinized saline. Catheters were removed by peeling back the tape on the ear and pulling the catheter out of the vein. A gauze swab was held over the ear to stop any bleeding that may have occurred and the ear was dressed with sterile dressing.

Heparinized saline was prepared with Sigma Sodium Heparin from porcine intestinal mucosa, 179 USP mg^{-1} (Sigma Chemical Corporation, St. Louis, MO., H-9399, Lot # 65H0848). Stock heparin was prepared containing 10000 USP ml^{-1}, weighing 1.397 g, in 25 ml sterile normal physiological saline. Heparin solution for the purpose of flushing catheters was made by the addition of 1 ml stock heparin solution to 100 ml sterile normal

saline and final concentration was 100 USP ml^{-1}. All saline and heparinized saline was kept in a 4°C refrigerator and warmed to room temperature just prior to use.

Blood collection

Samples were collected on days 110 of gestation and 3 pp for 8 hours, days 13 pp and 4 pw for 10 hours, the day before estrus, the day of estrus and 5, 10 and 15 days pb for 5 hours and 3 hours on day 2 pb. All serial blood samples were collected at 20 minute intervals. Eight and 10 hour sampling periods began at 0900 hours and all others at 1100 hours. Single daily samples were collected from day 10 pp through to day 14 pb and on the day before shipping to slaughter (day 27-35 pb).

Serial blood samples were collected from catheters by replacing the injection cap with a 3-way stop cock (Baxter, Deerfield, IL, USA). A syringe was used to draw of 2.5 ml of fluid from the catheter. A clean collection syringe was then used to draw off 10 ml of blood on the hour and 5 ml of blood every 20 minutes between the hour. Following the sample collection, 3 ml of normal saline was injected back into the catheter. At the end of the sampling period, 3 ml of heparinized saline was injected into the catheter and the adapter cap replaced.

All samples were placed into nonheparinized borosilicate glass tubes (16x100 mm) and covered with Parafilm (American National Can., Chicago, IL, USA). Samples were placed into a cooler on ice until transport at the end of the day to a 4°C cold room. Blood samples were centrifuged the following morning at 2200 rpm for 20 minutes at 5°C. Serum was separated, placed into 7 ml plastic scintillation vials and frozen at -20°C until analysis.

Endocrine and metabolite analyses

Progesterone

Serum progesterone (P4) was analyzed by solid phase radioimmunoassay (RIA) with a commercial kit (Coat-A-Count, Diagnostics Products Corporation, Los Angeles, CA, USA). Because of the lack of interspecies difference in the P4 molecule and negligible effects of serum proteins to the assay, as indicated by the manufacturer, this kit was considered appropriate for use with porcine serum. As well, linearity tests showed a high degree of parallelism. The standard curve working range was 0.1 to 40 ng ml^{-1} of P4. ^{125}I P4 was used as the tracer with total counts of approximately 75000 cpm and maximum binding of 40%. In brief, 100 µl of standard and sample serum were aliquoted into tubes pre-coated with anti-P4 and 1 ml of tracer was added to each tube and incubated for 1 hour in a 37°C waterbath. The antibody-bound fraction was isolated by decanting the supernatant and the radioactivity within the tube was read in a gamma counter (LKB Wallac 1282 Compu Gamma Universal Gamma Counter). The nonspecific binding of the assay was ≤1.4%. The sensitivity of the assay at 90% binding was 0.10 ng ml^{-1}. The intraassay and interassay coefficients of variation over 6 assays were ≤15% and ≤12% respectively.

Estradiol 17-ß

Estradiol 17-ß (E2) was assayed by a single antibody RIA as described by Joseph *et al.* (1992). Tritiated E2 (Estradiol [2,4,6,7,16,17-3H(N)], 5.2 TBq $mmol^{-1}$, New England Nuclear (NEN) Life Science Products, Boston, MA, USA, Lot 3248574) was diluted in phosphate buffered saline (PBS) with gelatin and adjusted to 10000-13000 cpm 100 ml^{-1}. NCR Rabbit A11 anti-Estradiol directed against Estradiol 17-ß was supplied by N.C. Rawlings of the Western College of Veterinary Medicine, University of Saskatchewan. Antibody solution was prepared in PBS with gelatin and the final dilution

used was 1:200 100 ml^{-1}. Standards were made up in charcoal stripped pig serum with stock E2 (Estradiol 17-ß, Sigma Chemical Corporation, St. Louis, MO, USA).

For extraction, 4 ml of diethyl ether was added to 500 µl of standards and samples (Fischer Scientific, New Jersey, NY., USA, Lot 970470-36), vortexed 3 times for 1 minute each, allowed to equilibrate for one hour and revortexed. This was followed by freezing of the aqueous phase to approximately -40°C on dry ice for a minimum of 10 minutes, decanting the solvent phase and drying overnight in a fumehood. Following the extraction procedure, 250 µl of antibody solution was added and allowed to incubate for 1 hour. This was followed by the addition of 200 µl tracer which was then incubated overnight on a shaker in a 4°C cold room. To remove any unbound steroids, 500 µl of chilled charcoal suspension containing 200 mg Norit washed charcoal (Aldrich Chemical Company Inc., Milwaukee, WIS, USA) in 100 ml PBS with gelatin was added to all tubes, vortexed, left to sit for 10 minutes at 4°C and then centrifuged. The supernatant was decanted into 7 ml scintillation vials and 3 ml of scintillation fluid (Betamax, ICN Biomedical INC., Costa Mesa, CA., USA) was added to each vial and mixed well.

Standards and unknowns were counted in a beta counter (LKB Wallac) 48 hours after the addition of the scintillation fluid. Total counts were approximately 8500-10500 cpm. Maximum binding was approximately 40-50%. The standard curve working range was 1 to 100 pg ml^{-1}. Nonspecific binding was ≤3%. Sensitivity of the assay at 90% binding was 2.6 pg ml^{-1}. The intraassay and interassay coefficients of variation for the 3 assays were ≤13% and ≤ 20%, respectively.

Cortisol

Serum cortisol was analyzed in 4 assays with a commercial solid phase RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA., USA). For this assay, the tracer was ^{125}I cortisol and the tubes were coated with anti-cortisol. Because the

cortisol molecule remains homologous across species and the effects of serum proteins to the assay are minimal, this kit was considered appropriate for use with porcine serum. As well, tests of linearity showed a high degree of parallelism within each assay. The procedure for this assay was similar to that for P4 except that 25 µl of standard and sample serum was used and incubation time was 45 minutes in a 37°C waterbath. The nonspecific binding was ≤1.6%. The intraassay and interassay coefficients of variation were ≤17% and ≤14%, respectively. The standard curve working range was 5 to 200 ng ml^{-1} of cortisol and the sensitivity of the assay at 95% binding was 2.6 ng ml^{-1}.

Total T_4

Serum T_4 was analyzed in 4 assays with a commercial solid phase RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA., USA). The lack of interspecies differences in thyroxine and absence of serum protein effects on the assay deemed this kit appropriate for use with porcine serum. Tests of linearity gave a high degree of parallelism. For this assay, the tracer was ^{125}I total T_4 and the tubes were coated with anti-thyroxine. The procedure for this assay was similar to that for P4 except that 25 µl of standard and sample serum were used. The standard curve concentration range was 10 to 240 ng ml^{-1}. The nonspecific binding of all assays was ≤1.6%. The sensitivity of the assay at 95% binding was 2.5 ng ml^{-1} thyroxine in the unknowns. The intraassay and interassay coefficients of variation were ≤14% and ≤11%, respectively.

Total T_3

Serum T_3 was analyzed in 4 assays with a commercial RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA., USA). According to the manufacturer, serum proteins have no effect on the assay results and because of the lack of difference across species in the triiodothyronine molecule this kit was considered valid for

use with porcine serum. As well, linearity tests showed a high degree of parallelism. The procedure was similar to that for P4 except for a 120 minute incubation time in a 37°C waterbath. 100 µl of standard and sample serum were aliquoted into tubes pre-coated with anti-triiodothyronine followed by the addition of ^{125}I total T_3 as tracer. The nonspecific binding of all assays was ≤2.3%. The intraassay and interassay coefficients of variation were ≤12% and ≤16%, respectively and the assay was able to detect 5 ng dl^{-1} in the unknowns at 95% binding. The standard curve was T_3 in human serum and the working range was 20-600 ng dl^{-1}.

Insulin

Serum insulin was determined in 8 assays with a commercial RIA kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA., USA) and was validated for use with porcine plasma by Weldon *et al.* (1994b) and Kemp *et al.* (1995). 200 µl of standard and sample serum was aliquoted into pre-coated anti-insulin tubes. One ml of iodinated insulin was added to the tubes and incubated for 18 hours at room temperature. No more than 40 minutes passed between the addition of the first sample and the addition of tracer to all tubes as suggested by the manufacturer. The supernatant was decanted and the radioactivity of the antibody-bound fraction was read in a gamma counter for one minute (LKB Wallac 1282 Compu Gamma Universal Gamma Counter). The nonspecific binding of all assays was ≤3.6%. Intraassay and interassay coefficients of variation were ≤18% and ≤14%, respectively. The standard curve concentration range was from 5 to 400 µIU ml^{-1} and the sensitivity of the assay at 90% binding was 1.5 µIU ml^{-1}. This kit was deemed appropriate for use because of the homology between the human and porcine insulin molecule as well as very negligible effects of serum proteins on the assay results. As well, quality tests of linearity showed a high degree of parallelism within each assay.

LH

LH was analyzed at the University of Saskatchewan in the Department of Veterinary Physiological Sciences at the Western College of Veterinary Medicine. The method used was a heterologous double antibody RIA as described by Kingsbury and Rawlings (1993). The standards for the assay were lyophilized porcine LH (USDA-pLH-B:I) with a working range of 0.0625 to 8.0 ng ml^{-1}. The standards were reconstituted in 5% BSA (Sigma bovine serum albumin A4503) buffered solution (5g BSA in 100 ml PBS with gel). The primary antibody was raised in rabbits against bovine LH with an initial working dilution of 1:40000. This antibody was prepared in 0.2% normal rabbit serum (NRS). The secondary antibody was raised in sheep against rabbit gamma-globulins and diluted in PBS with gel. Iodinated bovine LH was used as the tracer and was diluted in PBS with gel to provide 13000-18000 cpm in 200 µl.

For this assay, aliquots of 200 µl of standard and sample and 200 µl of primary antibody were added to all tubes except the total and nonspecific binding tubes, vortexed and incubated overnight. The following day, 200 µl of tracer was added and left to incubate overnight in a cold room. The next day, 500 µl of secondary antibody and 500 µl of 5% polyethylene glycol (PEG) were added to all tubes except the totals and left in the cold room overnight. Tubes were centrifuged and the supernatant was decanted and the labeled pellets were read on a gamma counter for one minute. Pools and unknowns were analyzed in 5 individual assays and the intraassay and interassay coefficient of variation were ≤16.4% and ≤23.6%, respectively. The non-specific binding was ≤5% and the sensitivity of the assay, defined as the lowest standard concentration different than zero, was 0.06 ng ml^{-1}.

FSH

FSH was analyzed at the University of Saskatchewan in the Department of Veterinary Physiological Sciences at the Western College of Veterinary Medicine. The method used was a homologous double antibody RIA as described by Kingsbury and Rawlings (1993). The standards (USDA-pFSH-1-2) were supplied in lyophilized form and reconstituted in 5% BSA phosphate buffered saline with gel. The standard curve working range was 0.125 to 32 ng ml^{-1} pFSH. The primary antibody (USDA-398-04P, pFSH antiserum, anti-pFSH-ß-subunit) was in a 0.2% NRS solution and had an initial working dilution of 1:5000. Iodinated porcine FSH (USDA-pFSH-I2) was diluted in PBS with gelatin to initially supply 13000-18000 cpm in 100 µl. The secondary antibody was ovine anti-rabbit g-globulin in PBS with gelatin with a dilution of 1:130.

This assay procedure was similar for that of LH (Kingsbury and Rawlings, 1993). The pools and unknowns were analyzed in 2 assays and the intraassay and interassay coefficients of variation were both ≤16.2%. Non-specific binding of the assay was less than 5% and the sensitivity of the assay, defined as the lowest standard concentration not equal to zero, was 0.125 ng ml^{-1}.

IGF-I

IGF-I was analyzed in 10 assays using a commercial ELISA kit (Diagnostic Systems Laboratories, Inc., Webster, TX., USA). The assay required an ethanol-HCL extraction step. The antibody-enzyme conjugate was an anti-IGF-I mouse monoclonal antibody bound to horseradish peroxidase and the substrate for the reaction was tetramethylbenzidine. The standards were supplied in lyophilized form and were reconstituted in deionized water to provide a concentration range of 7.8 to 580 ng ml^{-1}. According to the manufacturer the assay should be able to detect as little as 0.3 ng ml^{-1} of IGF-I in the unknowns. Intraassay and interassay coefficients of variation were ≤26% and

≤20.0%, respectively. Because of the high degree of homology between the human and porcine IGF-I molecule, this kit was considered appropriate for the determination of IGF-I in porcine serum. A high degree of parallelism was observed in the tests for linearity.

Blood glucose

Blood glucose was evaluated on site at the research facilities with the application of a commercial blood glucometer (One Touch® Basic, Lifescan Canada LTD., Burnaby, B.C., Canada) as per the manufacturers directions. Blood samples were analyzed for glucose concentration directly from the blood collection syringe, at room temperature, within 5 minutes of collection.

Serum urea nitrogen

Serum urea nitrogen was measured as an indicator of protein catabolism. Samples were analyzed with a Sigma kit (Sigma Diagnostics, St. Louis, MO, USA, Lot # 106H6096). The methodology followed was without deproteinization and standards, controls and unknowns were read at 540 nm within a 20 minute period. Standards ranged from 15 to 75 mg dl^{-1} urea nitrogen. The intraassay and interassay coefficients of variation were ≤9% and ≤6% respectively.

Statistical analysis

Data were analyzed with primiparous sows (n = 9) and multiparous sows (n = 8) as discrete groups. The two groups were directly compared using analysis of variance and correlations using the General Linear Model functions of the Statistical Analysis System (SAS 6.11, 1986). To test for the effect of parity, endocrine and blood metabolite data were analyzed as a split plot. The model for repeated measures consisted of parity (primiparous *vs.* multiparous), sow within parity, time and the parity-by-time interaction.

The effect of parity was tested using sow within parity as the error term. When a significant parity by time interaction existed, linear contrasts were employed to determine how each parity group differed in this respect. For serial serum sample contrasts, comparisons were made between every hour from the beginning of the sampling period (0900 or 1100) to 1400 hours, between the first sample collected (0900 or 1100) and the last (1600, 1700, or 1900) and between the 1200 sample and the last sample. Contrasts performed for day-to-day comparisons were all days *vs.* day 110 of gestation, day 3 pp *vs.* day 13 pp, day 3 pp *vs.* 4 days pw, day 13 pp *vs.* 4 days pw, 4 days pw *vs.* the day prior to estrus, the day prior to estrus *vs.* estrus, and estrus *vs.* 2 day pb. Bonferroni's test for differences in means was performed when a significant time effect was present to define when those differences existed. As well, a comparison of variance was performed in which the standard deviation in blood parameters for each sow over the course of a sampling period was determined and the mean standard deviation within each parity was compared by a t-test. This analysis was performed on blood parameters for every sampling period. Sows without endocrine data were omitted from these analyses but included in all other parameter analyses.

Litter data, WEI, embryo mortality, and ovulation rate were compared by t-test. Weight, backfat, total body lipid and total body protein were analyzed similarly by comparing the change in either parameter between each of the days of data collection in relation to the number of days between the collection times. Feed intake was directly compared between parity as an average intake in four time periods: the pre-farrowing period, lactation period, return to estrus period and postbreeding period by t-test.

The prediction equations of Whittemore and Yang (1989) for total body lipid (equation 1) and total body protein (equation 2) were used. The r^2 were ≥0.80 and ≥0.90 for fat and protein, respectively.

(1) Lipid (kg) = -20.40(±4.50) + 0.21(±0.02) weight + 1.50(±0.20) P_2 backfat

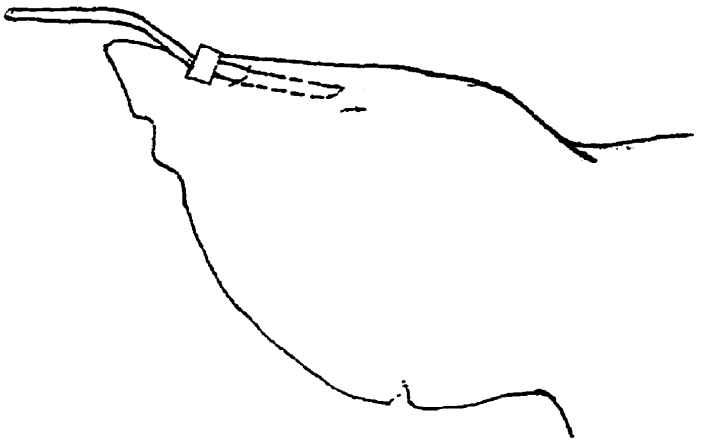
(2) Protein (kg) = -2.30(±1.60) + 0.19(±0.01) weight - 0.22(±0.07) P_2 backfat

LH pulsatility and baseline concentration was determined by the method of Evans *et al.* (1994). In brief, hormone peaks within a profile were defined as 3 standard deviation points above the mean for that animal. To determine the baseline concentration, the peaks were subtracted and the baseline was calculated as the mean concentration without the peaks. Because the half-life for LH is 20 minutes (Hafez, 1993) and samples were collected at twenty-minute intervals, a pulse was only considered a pulse if it passed the previous criteria and there were no more than two peak values in a row. LH baseline concentration and the number of peaks in a day were compared across parity by t-test.

The ∝–level of significance was P<0.054. A trend was defined as P=0.055-0.08.

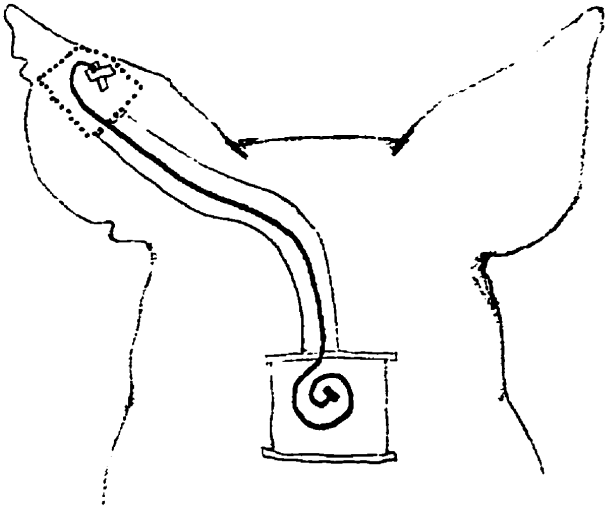
FIGURE 1. Site of puncture and placement of needle and catheter in vein (a), attachment of tape to secure catheter (b) and dorsal view of tape and catheter attachment (c) during ear-vein cannulation in the sow

(a)



(b)

(c)



CHAPTER 4

RESULTS

Sow performance

Sow and litter performance is shown in table 1. There was no significant difference in the number born alive, although fifth parity sows tended to have more piglets born dead in the form of mummies and stillborns (P=0.06). While no difference was found in the percentage of piglets weaned in the standardized litters, first parity sows weaned significantly heavier piglets at day 14 pp (P=0.03).

TABLE 1. Mean pigs born alive, born dead, weaning percentage of standardized litters, average number pigs weaned and mean piglet weaning weight in first and fifth parity sows

	1st Parity		5th Parity		
	LS Mean	SE	LS Mean	SE	P=
Born alive	11.56	0.95	13.5	1.01	0.2
Born dead	0.89	0.46	2.25	0.48	0.06
% of Standardized litter weaned	90	0.06	74	0.06	0.09
Ave. # pigs weaned	9		8.9		
Mean weaning weight (kg)	4.78	0.16	4.15	0.16	0.03

The WEI was significantly longer for the first parity sows (P=0.023) (table 2). The standard deviation in the WEI indicates greater variation for first parity sows in their pw anestrous period (16.67 ± 13.63 days *vs.* 4.25 ± 1.58 days for 1st and 5th parity sows, respectively). All fifth parity and only 3 first parity sows had returned to estrus within 6 days pw. Ovulation rate determined by number of CL or CA, was significantly higher for the fifth parity sows (P=0.0004), although still quite high for the 1st parity. The CL were generally between 6 and 8 mm in diameter. Two fifth parity sows that were subsequently rebred following the first pw estrus, had large blood and fluid filled luteal cysts greater than 15 mm in diameter. Six sows, two of which were rebred, had numerous follicles present on the ovaries at slaughter ranging from 2 to 5 mm in diameter. No differences were found in the number of viable embryos. The average size (crown rump length) of normal, viable embryos for first and fifth parity sows was 18.67 ± 3.83 mm and 21.4 ± 4.98 mm, respectively. Figure 2 shows a gravid sow uterus (a) and pig embryo (b) at 32 days pb. The average percentage of embryo mortality was quite large but not different between the parities. The pw pregnancy rate, determined as the number of sows not returning to estrus pb, was 6/8 for fifth parity sows compared to 6/9 for first parity sows (table 2).

Feed consumption

Average daily feed intake for first and fifth parity sows is shown in table 3. In the prefarrowing period, first parity sows ate almost 1 kg day $^{-1}$ less feed than fifth parity sows (P=0.008). When any daily feed intake less than 2 kg day^{-1} was excluded from the statistical analyses, the results were still significantly different (P<0.05). During lactation, when sows were full fed, fifth parity sows ate almost twice the amount of feed per day as the first parity and this situation remained when feed intake less than 2 kg day^{-1} was excluded from the analyses (P=0.0001). Following weaning, when sows were restricted to 3.5 kg day $^{-1}$ of feed, fifth parity sows continued to eat significantly more feed up until

breeding (P=0.0007). In the pb period, however, there was no difference in the daily feed intake between the two parity groups. In terms of feed consumed per kg bodyweight, first and fifth parity sows did not differ throughout the trial (P>0.40).

TABLE 2. Mean postweaning interval to estrus (WEI), ovulation rate, # viable embryos, percentage embryo mortality and pregnancy rate in first and fifth parity sows

	1st Parity		5th Parity		
	LS Mean	SE	LS Mean	SE	P=
WEI (days)	16.67	3.34	4.25	3.54	0.023
(range)	(5-41)		(1-6)		
Ovulation Rate	18.3	1.27	26.9	1.44	0.0004
# Viable Embryos	11.3	2.97	16.4	3.25	0.30
Embryo Mortality %*	41.1	10.5	45.4	10.5	0.80
Pregnancy Rate	6/9		6/8		

*Embryo mortality % = (1 - # viable embryos/# CL)(100)

TABLE 3. Average daily feed consumption in prefarrowing, lactation, weaning to first estrus and postbreeding periods (kg day $^{-1}$)

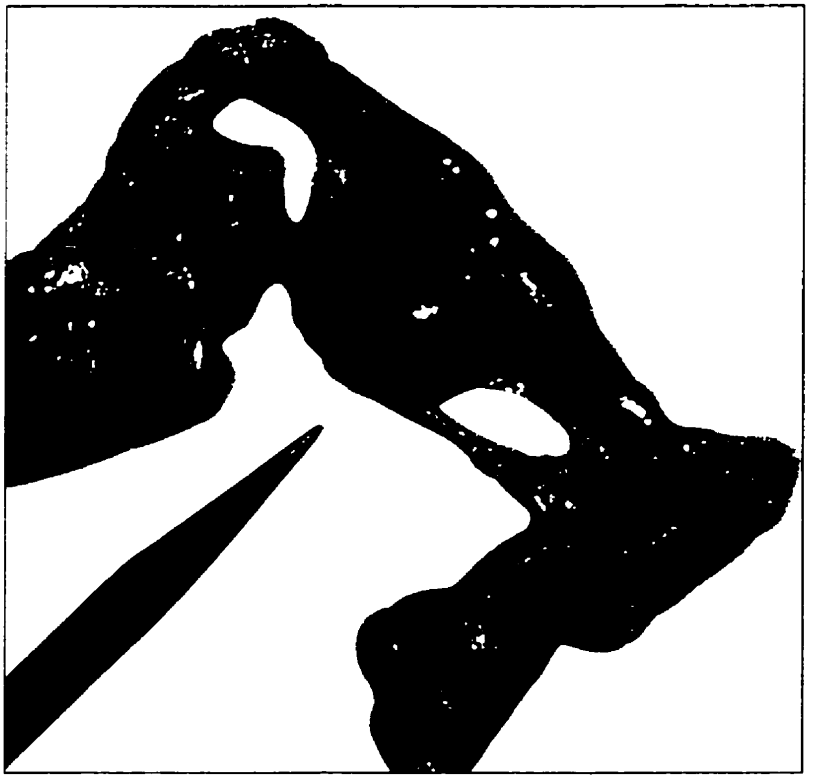
Period	1st Parity	5th Parity	P=
Prefarrowing	2.36 ± 0.20	3.26 ± 0.21	0.008
Day 1 pp to 14 pp	2.91 ± 0.33	5.70 ± 0.35	0.0001
Weaning to first estrus	1.91 ± 0.20	3.13 ± 0.25	0.0007
Estrus to day 27-35 pb	3.08 ± 0.16	3.26 ± 0.18	0.50

values are LS means ± SEM

FIGURE 2. Photograph of gravid sow uterus and ovaries at 32 days postbreeding (a) and viable 32 day old pig embryo. Approximate crown rump length of embryos is 15 mm.

(a)

(b)

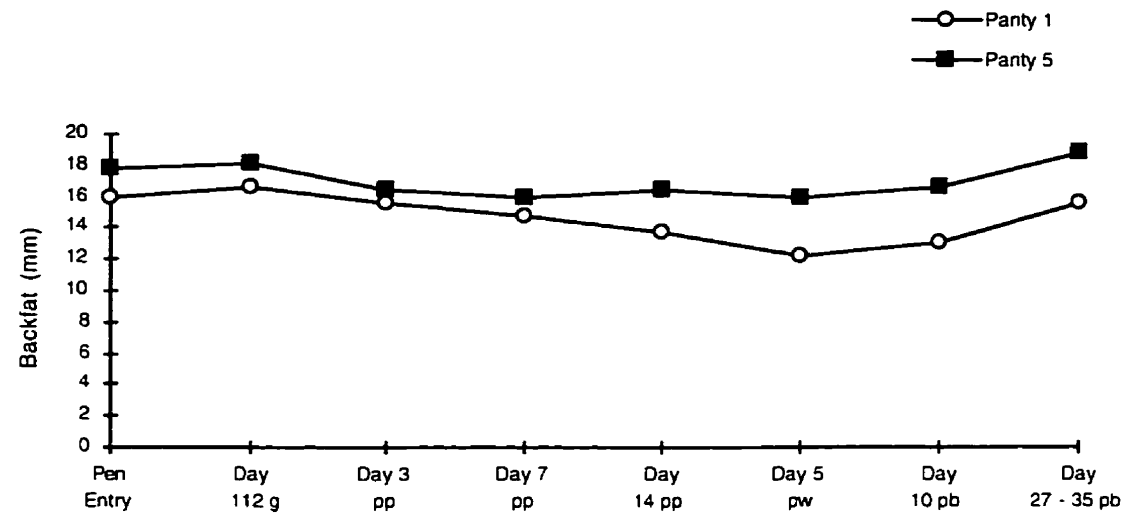


Body composition

No differences were found between parities in backfat thickness (P>0.09) at pen entry (day 105-108 of gestation), day 112 of gestation (g), day 3 pp, day 7 pp, day 14 pp, day 5 pw, day 10 pb or day 27-35 pb. Fifth parity sows, however, were consistently heavier throughout the course of the trial at all weigh periods (P=0.0001). Figure 3 shows the patterns of gain and loss in backfat and weight over the course of the trial. Appendix table A1 gives values for backfat and weight throughout the trial in fifth and first parity sows.

When first parity sows were divided into groups of normal (<7 days, n=3) or prolonged (≥7 days, n=6) pw anestrous periods, there was no significant difference (P=0.11) in their mean weight for normal (188.1 ± 3.4 kg) or prolonged (181.0 ± 2.4 kg) anestrous sows nor in backfat (P=0.60) for normal (15.58 ± 1.92 mm) or prolonged (14.3 ± 1.36 mm) anestrous sows. This was also true for individual days throughout the trial in backfat, total body lipid and total body protein. However, weight for normal and prolonged anestrous sows at day 112 g (218.5 ± 3.9 *vs.* 206.1 ± 2.7), 3 pp (189.3 ± 4.6 *vs.* 177.0 ± 3.2), 7 pp (180.6 ± 3.7 *vs.* 168.1 ± 2.6) and 14 pp (174.9 ± 3.1 *vs.* 163.6 ± 2.2) were significantly different between the groups (P<0.04). The WEI was not significantly correlated with backfat at weaning, backfat at 5 days pw, weight at weaning or weight at 5 days pw for fifth and first parity sows. When pooled across parity, however, the WEI was negatively correlated with weight at weaning (r=-0.51, P=0.05) and 5 days pw (r=-0.52, P=0.047), but not with backfat.

(a)



(b)

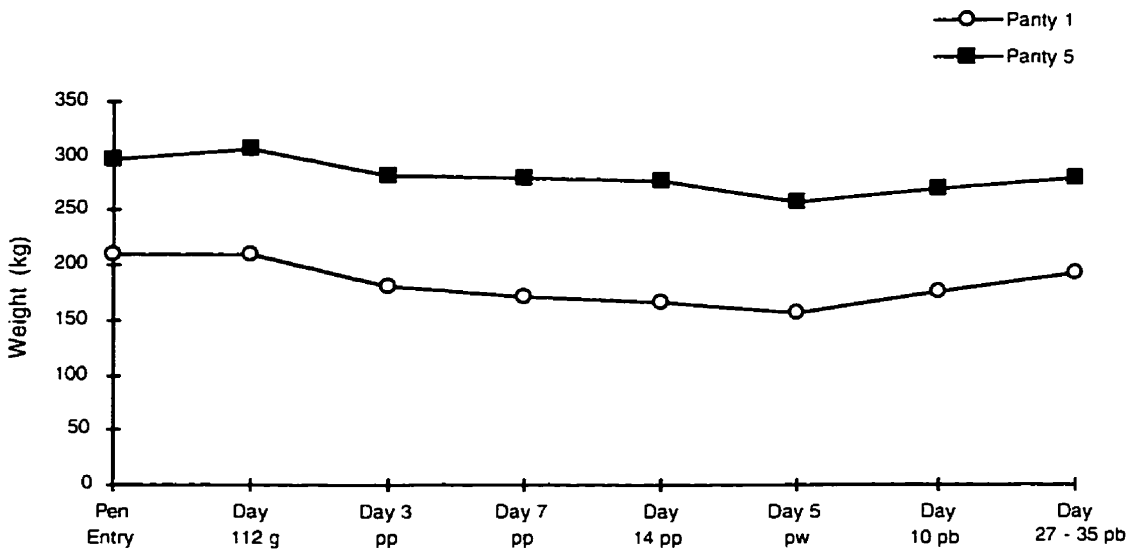


FIGURE 3. Mean sow backfat (a) and sow weights (b) throughout trial. No differences were found in the backfat ($P>0.09$) at any day, however, fifth parity sows were consistently heavier throughout the trial ($P=0.0001$). Refer to text for abbreviations.

When comparing the two parity groups in terms of actual backfat and weight changes over time (table 4), first parity sows lost more backfat per day than fifth parity sows throughout lactation (P=0.03) and through lactation to 5 days pw (P=0.01). There was a trend (P=0.07) for first parity sows to lose more backfat per day in the 2nd week of lactation, where fifth parity sows gained backfat at this time. When comparing the sows from day 3 pp up to termination of the study, fifth parity sows gained significantly more backfat per day than first parity sows (P=0.009).

First parity sows had a weight loss from time of pen entry to day 112 g, while fifth parity sows gained weight and this difference between the two parity groups approached significance (P=0.07). Fifth parity sows lost almost two times more weight per day in the 5 days pw (P=0.015), otherwise the two groups were similar in their weight changes throughout the course of the trial (table 4).

Table 5 shows a comparison of the total changes in backfat and weight over time, not as a daily average. First parity sows lost significantly more backfat during the entire lactation period (P=0.034) and through lactation to 5 days pw (P=0.01). The overall net gain in backfat for first parity sows (0.06 ± 0.62) was much less by 28 days pb than fifth parity sows (2.25 ± 0.66, P=0.03). First parity sows tended to lose more backfat in the last week of lactation (P=0.07) and from 5 days pw to 10 days pb (P=0.072). From the time of pen entry to day 112 g four sows in the first parity group lost weight while the other five sows gained weight in this time period. Since fifth parity sows, on average, gained weight from crate entry to day 112 g and first parity sows averaged a slight loss in weight, the weight change difference between the parities was significant (P=0.05). First parity sows lost significantly more weight in the last week of lactation (P=0.02) compared to the fifth parity sows. From day 10 pb to termination of the study, first parity sows gained more weight than fifth parity sows (P=0.048) and there was a trend (P=0.08) for

fifth parity sows to lose more weight in the 5 days pw. Weight changes were similar for the two groups at all other times.

Appendix table A2 gives prediction values for total body lipid and total body protein for fifth and first parity sows. Fifth parity sows had significantly more body lipid ($P<0.0009$) and body protein ($P<0.0001$) at all times throughout the course of the study compared to first parity sows.

Table 6 shows the daily body lipid and protein changes over time for the fifth and first parity sows. First parity sows compared to fifth parity sows lost significantly more body lipid both in the last week of lactation and throughout the entire period of lactation. As well, there was a trend for the first parity sows to lose more lipid from day 112 g to day 3 pp ($P=0.064$) and in lactation through to 5 days pw ($P=0.066$). No differences in lipid change were evident at any other time. The losses in body lipid are paralleled by the backfat loss in the same time periods (lactation and 5 days pw) for the first parity sows.

The first parity sows tended to lose more body protein from the time they entered their pen to day 112 g ($P=0.078$). Although, first parity sows tended to have a net gain in protein by termination of the study ($P=0.08$), this gain in total body protein occurred after 5 days pw through to termination of the study at 27-35 days pb. Fifth parity sows lost twice the amount of body protein in the 5 days pw compared to first parity sows ($P=0.009$). There were no significant differences in the two groups at any other time (table 6).

TABLE 4. Average daily backfat (mm) and weight (kg) changes in first and fifth parity sows from pen entry to termination of the study at 27-35 days postbreeding

	Average Daily Backfat Changes (mm)			Average Daily Weight Changes (kg)		
Day	**1st Parity**	**5th Parity**	**P<**	**1st Parity**	**5th Parity**	**P<**
Pen entry to 112 g	0.28 ± 0.19	-0.10 ± 0.22	0.22	-0.35 ± 0.54	1.24 ± 0.61	0.07
112 g to 3 pp	-0.14 ± 0.06	-0.14 ± 0.07	1.0	-4.67 ± 0.73	-2.74 ± 0.83	0.11
3 pp to 7 pp	-0.21 ± 0.13	-0.14 ± 0.14	0.73	-2.21 ± 0.77	-0.98 ± 0.81	0.29
7 pp to 14 pp	-0.14 ± 0.07	0.06 ± 0.08	0.07	-0.68 ± 0.23	-0.23 ± 0.26	0.22
14 pp to 5 pw	-0.25 ± 0.07	-0.06 ± 0.08	0.09	-1.82 ± 0.36	-3.33 ± 0.41	0.015
5 pw to 10 pb	0.02 ± 0.05	0.03 ± 0.06	0.88	0.98 ± 0.24	1.33 ± 0.27	0.35
10 pb to 27-35 pb	0.14 ± 0.02	0.12 ± 0.03	0.57	0.91 ± 0.13	0.50 ± 0.14	0.52
3 pp to 14 pp	-0.17 ± 0.04	-0.006 ± 0.05	0.03	-1.24 ± 0.36	-0.47 ± 0.41	0.18
3 pp to 5 pw	-0.2 ± 0.04	-0.03 ± 0.04	0.01	-1.44 ± 0.25	-1.48 ± 0.29	0.92
3 pp to 27-35 pb	0.001 ± 0.01	0.05 ± 0.01	0.009	0.17 ± 0.11	-0.06 ± 0.12	0.17

values are means ± SEM

TABLE 5. Total average backfat (mm) and total average weight (kg) changes in first and fifth parity sows from pen entry to termination of the study at 27-35 days postbreeding

	Total Average Backfat Changes (mm)			Total Average Weight Changes (kg)		
Day	**1st Parity**	**5th Parity**	**P<**	**1st Parity**	**5th Parity**	**P<**
Pen entry to 112 g	0.67 ± 0.48	-0.64 ± 0.55	0.095	-0.09 ± 2.35	7.66 ± 2.66	0.047
112 g to 3 pp	-1.06 ± 0.42	-0.79 ± 0.48	0.69	-29.19 ± 4.05	-18.94 ± 4.60	0.12
3 pp to 7 pp	-0.83 ± 0.52	-0.56 ± 0.55	0.73	-8.83 ± 2.99	-5.49 ± 3.40	0.47
7 pp to 14 pp	-1.0 ± 0.47	0.43 ± 0.54	0.07	-4.89 ± 1.48	1.04 ± 1.67	0.02
14 pp to 5 pw	-1.50 ± 0.40	-0.36 ± 0.46	0.081	-10.64 ± 2.05	-20.34 ± 2.33	0.08
5 pw to 10 pb	0.78 ± 0.48	0.50 ± 0.55	0.72	19.24 ± 4.43	8.49 ± 5.02	0.13
10 pb to 27-35 pb	2.61 ± 0.45	2.06 ± 0.48	0.42	16.71 ± 2.19	9.83 ± 2.32	0.048
3 pp to 14 pp	-1.83 ± 0.49	-0.07 ± 0.56	0.034	-13.72 ± 4.08	-5.32 ± 4.99	0.22
3 pp to 5 pw	-3.33 ± 0.64	-0.43 ± 0.73	0.01	-24.37 ± 4.31	-27.15 ± 5.28	0.69
3 pp to 27-35 pb	0.06 ± 0.62	2.25 ± 0.66	0.03	11.56 ± 5.63	-3.76 ± 6.38	0.093

values are means ± SEM

TABLE 6. Average daily body lipid (kg)[1] and average daily body protein (kg)[2] changes in first and fifth parity sows from pen entry to termination of the study at 27-35 days postbreeding

	Average Daily Body Lipid Changes (kg)			Average Daily Body Protein Changes (kg)		
Day	**1st Parity**	**5th Parity**	**P<**	**1st Parity**	**5th Parity**	**P<**
Pen entry to 112 g	0.402 ± 0.214	0.123 ± 0.243	0.403	-0.128 ± 0.134	0.259 ± 0.152	0.078
112 g to 3 pp	-1.231 ± 0.151	-0.771 ± 0.171	0.064	-0.856 ± 0.144	-0.490 ± 0.163	0.114
3 pp to 7 pp	-0.761 ± 0.271	-0.408 ± 2.88	0.386	-0.374 ± 0.143	-0.154 ± 0.152	0.309
7 pp to 14 pp	-0.345 ± 0.114	0.043 ± 0.130	0.042	-0.099 ± 0.045	-0.057 ± 0.051	0.553
14 pp to 5 pw	-0.758 ± 0.141	-0.772 ± 0.160	0.950	-0.299 ± 0.070	-0.621 ± 0.080	0.009
5 pw to 10 pb	0.231 ± 0.093	0.317 ± 0.106	0.553	0.188 ± 0.050	0.246 ± 0.057	0.453
10 pb to 27-35 pb	0.396 ± 0.048	0.277 ± 0.051	0.111	0.142 ± 0.025	0.069 ± 0.027	0.062
3 pp to 14 pp	-0.498 ± 0.096	-0.106 ± 0.108	0.017	-0.199 ± 0.070	-0.088 ± 0.079	0.305
3 pp to 5 pw	-0.588 ± 0.082	-0.341 ± 0.093	0.066	-0.233 ± 0.049	-0.276 ± 0.056	0.575
3 pp to 27-35 pb	0.037 ± 0.031	0.064 ± 0.033	0.569	0.032 ± 0.020	-0.024 ± 0.022	0.080

values are means ± SEM

[1,2] based on the prediction equations of Whittemore and Yang (1989)

Hormones and blood metabolites

In instances where venous cannulae were not functional, sows were omitted from analyses. Where samples were missing in a collection period, those samples were dealt with as missing data and all other samples analyzed. On day 10 pb only 3 first and 2 fifth parity sows had functional catheters and on day 15 pb only 2 first parity sows and 1 fifth parity sow had functional catheters. Therefore, these days were not included in the results as sample size was very low. Two fifth parity sows were never cannulated and therefore, only their day 27-35 pb single sample from direct veni-puncture were included in the results.

Blood glucose

Figure 4a shows the mean glucose concentrations throughout the trial. On day 3 pp there was a significant hour effect (P=0.0001), shown by the concentration of blood glucose at 0900 < 1100, 1200, 1300, 1400 and 1700 hours. On day 13 pp there was a significant parity by hour effect (P=0.0001) shown by a difference in the blood glucose response from 0900 to 1000, 1000 to 1100, 0900 to 1200 and 0900 to 1900 hours. As well, there was an hour effect (P=0.003) with the concentration at 0900 < 1100, 1300 and 1700 hours. At 4 days pw there was a significant hour effect (P=0.0001) and these differences were 0900<1100, 1000<1800, 1100<1400, 1500, 1600, 1800 and 1900 and 1300<1800 hours. There was also a parity by hour effect (P=0.0013) but the differences in the parity response were not found in the contrasts performed. There was no significant parity by day effect for mean serum glucose over the course of the trial although there was a day effect (P=0.02) shown by serum glucose being lower on the day before estrus compared to day 110 of gestation. Table 7 shows LS mean concentration in blood glucose

± SEM and level of significance between parity. See appendix table A3 for split plot effects in blood glucose.

TABLE 7. Mean blood glucose (mg dl^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	87.86 ± 3.14	85.23 ± 3.59	0.60
3 pp	84.55 ± 2.87	87.74 ± 2.68	0.44
13 pp	84.24 ± 1.57	81.99 ± 1.50	0.33
4 pw	82.87 ± 2.80	74.99 ± 3.27	0.12
E-1	74.15 ± 3.38	78.98 ± 2.85	0.33
E	83.51 ± 2.31	79.20 ± 4.26	0.44
2 pb	77.03 ± 4.51	66.83 ± 4.87	0.19
5 pb	84.75 ± 3.43	75.30 ± 5.31	0.23

values are LS mean ± SEM

BUN

Mean BUN concentrations are shown in figure 4b. On day 110 of gestation there was an hour effect (P=0.003) with BUN concentration at 0900 < 1400 and 1600 hours. Fifth parity sows had significantly higher mean BUN concentration (P=0.037) and variability (P=0.02) and there was an hour effect (P=0.012) on day 3 pp. On day 13 pp, fifth parity sows had significantly higher mean BUN (P=0.053) and an hour effect (P=0.0002) was present. On this day, the BUN concentration at 0900 < 1400, 1500, 1700 and 1800 and 1000 < 1400 and 1500 hours. BUN concentration did not differ by parity on days 10, 11, 12 and 14 pp. In comparing days 110 of gestation, 3 pp and 13 pp, a significant parity effect (P=0.03) was present shown by 5th parity sows having higher BUN throughout, as well as a significant day (P=0.0001) and parity by day (P=0.0001) effect. The difference between the parities in terms of BUN response across days was

found between day 110 g *vs.* day 3 pp and day 110 g *vs.* day 13 pp. The BUN concentration was highest on day 110 g and lowest on day 3 pp with 13 pp being intermediate. BUN on day 3 pp was not significantly correlated with the predicted protein changes on day 3 pp for first (r=-0.51, P=0.38) and fifth (r=-0.84, P=0.07) parity sows. No significant correlation existed between body protein at day 14 pp and BUN on day 13 pp in first (r=-0.15, P=0.81) or fifth parity sows (r=-0.18, P=0.78). Table 8 shows LS mean concentration in BUN ± SEM and level of significance between parity. See appendix table A4 for split plot effects in BUN.

TABLE 8. Mean BUN (mg dl^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	23.57 ± 1.83	22.93 ± 2.06	0.82
3 pp	13.69 ± 1.68	19.19 ± 1.59	0.037
13 pp	15.97 ± 1.66	20.95 ± 1.49	0.053

values are LS mean ± SEM

P4

Figure 5a illustrates mean progesterone (P4) concentration throughout the trial. There were no differences in the mean or the variability in P4 on days 110 g, 3 pp, 13 pp, 4 days pw, day before estrus, estrus or 2 or 5 days pb, nor were there any parity by hour effects on these days. On day 3 pp there was an hour effect (P=0.004) with P4 concentration at 1100 > 1400 and 1700 hours. As well, on day 13 pp, there was a trend for an hour effect (P=0.07) with P4 concentration at 0900 > 1100 hours. There were no differences in concentration in the single samples on days 10 pp, 11 pp, 12 pp or 14 pp although, fifth parity sows had significantly higher P4 concentration 1 day pw (P=0.011), 3 days pw (P=0.037), 5 days pw (P=0.0001) and 1 day pb (P=0.011). There was a

significant day effect (P=0.0001) for mean P4 and this is shown by highest concentration in P4 on day 110 g. As well, the concentration was higher at 5 days pb than all other days except day 110 g. Progesterone was not correlated with embryo mortality at 2 or 5 days pb or 27-35 days pb (r=-0.08; P=0.88 and r=-0.20; P=0.70 for fifth and first parity sows, respectively). Table 9 shows LS mean concentration in P4 ± SEM and level of significance between parity. See appendix table A5 for split plot effects in P4.

TABLE 9. Mean serum P4 (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	18.57 ± 2.50	14.75 ± 3.08	0.36
3 pp	0.54 ± 0.11	0.50 ± 0.10	0.80
13 pp	0.23 ± 0.05	0.26 ± 0.05	0.65
4 pw	0.19 ± 0.06	0.32 ± 0.11	0.34
E-1	0.27 ± 0.09	0.46 ± 0.07	0.18
E	0.22 ± 0.04	0.39 ± 0.12	0.25
2 pb	2.36 ± 1.02	2.03 ± 1.21	0.85
5 pb	8.98 ± 2.07	6.38 ± 2.13	0.42

values are LS mean ± SEM

Estradiol 17-β

Figure 5b shows mean estradiol 17-ß (E2) concentration throughout the trial in first and fifth parity sows. First parity sows had significantly higher mean E2 concentration (P=0.015) on day 110 g and there was an hour effect (P=0.03) but the differences were not detected by comparisons. On day 3 pp, there was an hour effect (P=0.0008) shown by the E2 concentration at 0900 > 1300, 1500, 1600 and 1700 hours. On day 13 pp fifth parity sows had greater variability (P=0.036). At 4 days pw there was an hour effect (P=0.0002) in which the concentration at 0900 > 1500 and 1800 and 1000 > 1800 hours. Also on day

13 pp, there was a significant parity by hour interaction, but the difference between parities were not found in the contrasts performed. Fifth parity sows had higher E2 on day 14 pp (P=0.04), 1 day pw (P=0.01) and 3 days pw (P=0.016) in the single samples. There was a significant day effect for mean E2 (P=0.0001) in the comparison of all days. This was shown by E2 levels being highest at day 110 of gestation. Also, there was a significant parity by day interaction (P=0.05) shown by differing parity responses in E2 concentration on day 110 g *vs.* days 3 pp, 13 pp, 4 pw, day prior to estrus and 2 pb. Estradiol was not correlated with embryo mortality at estrus, 2 or 5 days pb in either parity group. Table 10 shows LS mean concentration in E2 ± SEM and level of significance between parity. See appendix table A6 for split plot effects in E2.

TABLE 10. Mean serum E2 (pg ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	352.47 ± 33.31	210.94 ± 37.13	0.015
3 pp	10.63 ± 0.81	9.58 ± 0.80	0.39
13 pp	8.56 ± 2.03	13.69 ± 1.86	0.13
4 pw	12.21 ± 2.17	18.78 ± 4.47	0.24
E-1	17.76 ± 5.26	33.54 ± 4.82	0.18
E	16.44 ± 3.31	22.21 ± 9.26	0.59
2 pb	6.91 ± 1.74	8.67 ± 1.23	0.47
5 pb	7.37 ± 2.15	6.92 ± 2.23	0.87

values are LS mean ± SEM

LH

In some sampling periods, especially days 3 and 13 pp, many of the serum samples had LH concentration below the detection limit of the assay. Sample size was quite low

when these values were removed from the data set. Therefore, these data points were not discarded and were analyzed with all other values.

Figure 6a shows the mean concentration of LH throughout the trial. There was no parity differences in the baseline concentration except on day 13 pp when fifth parity sows had a higher baseline concentration (P=0.03). Pulse frequency did not differ between the parities on any of the days. Table 12 shows the baseline concentration and pulse frequency for LH. On no day was there a difference in the mean or the variation in LH. From figure 6 it would appear that LH concentration was higher in first (2.96 ± 1.56 ng ml^{-1}) compared to fifth parity sows (0.16 ± 7.90 ng ml^{-1}) at estrus, however, because the variation was so large, no differences were detected (P=0.75). On 2 and 5 days pb there was a significant time effect (P=0.02) but comparisons did not detect the difference. At 5 days pb, there was parity by time interaction (P=0.034). There was no significant parity, day or parity by day interaction in the comparison of all days. See table 11 for LS mean concentration in LH ± SEM and level of significance between parity. Appendix table A7 shows split plot effects in LH.

TABLE 11. Mean serum LH (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	0.14 ± 0.05	0.12 ± 0.05	0.73
3 pp	0.03 ± 0.01	0.06 ± 0.02	0.13
13 pp	0.05 ± 0.27	0.24 ± 0.15	0.55
4 pw	0.19 ± 0.13	0.21 ± 0.38	0.95
E-1	0.70 ± 0.28	0.31 ± 0.24	0.33
E	2.96 ± 1.56	0.16 ± 7.90	0.75
2 pb	0.20 ± 0.08	0.44 ± 0.13	0.18
5 pb	0.24 ± 0.06	0.35 ± 0.07	0.30

values are LS mean ± SEM

TABLE 12. LH baseline concentration and pulse frequency

	Baseline (ng ml $^{-1}$)			Pulse Frequency (pulses sampling period $^{-1}$)		
Day	1st Parity	5th Parity	P=	1st Parity	5th Parity	P=
110 g	0.131 ± 0.041	0.083 ± 0.058	0.51	1.22 ± 0.24	1.20 ± 0.33	0.96
3 pp	0.026 ± 0.011	0.030 ± 0.017	0.86	0.78 ± 0.15	0.75 ± 0.23	0.92
13 pp	0.016 ± 0.008	0.053 ± 0.010	0.03	0.71 ± 0.19	1.25 ± 0.25	0.12
4 pw	0.173 ± 0.051	0.105 ± 0.072	0.49	1.00 ± 0.39	1.33 ± 0.51	0.62
E-1	0.530 ± 0.275	0.325 ± 0.435	0.71	1.00 ± 0.15	0.67 ± 0.19	0.22
E	0.867 ± 0.193	NA		1.75 ± 0.63	0 ± 1.26	0.30
2 pb	0.250 ± 0.090	0.230 ± 0.202	0.93	1.20 ± 0.23	0.50 ± 0.36	0.17
5 pb	0.267 ± 0.038	0.210 ± 0.065	0.53	0.60 ± 0.24	1.00 ± 0.55	0.54

values are LS means ± SEM

FSH

No differences were found in the mean concentration or variability in FSH, nor were there any significant hour or parity by hour effects on days 110 g and 3 pp. On day 13 pp, first parity sows had significantly higher mean concentration (P=0.053) and variability (P=0.016) and there was a trend (P=0.067) for a parity by hour interaction. There was a trend towards an hour effect (P=0.066) on day 4 pw and this was shown by the FSH concentration being higher at 1500 than 0900 hours. Mean FSH concentration was higher at estrus (P=0.03) and 2 days pb (P=0.025) in first parity sows. When comparing all days, there were significant day effect for the mean concentration (P=0.0001), shown by the concentration at day 2 pb being higher than all days except estrus and 5 days pb (figure 6b). As well, there was a significant parity effect (P=0.036), in that, 1st parity sows had higher overall mean FSH throughout the trial. Table 13 shows LS mean concentration in FSH ± SEM and level of significance between parity. See appendix table A8 for split plot effects in FSH.

TABLE 13. Mean serum FSH (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	0.43 ± 0.07	0.40 ± 0.08	0.79
3 pp	0.54 ± 0.13	0.48 ± 0.12	0.76
13 pp	0.87 ± 0.14	0.44 ± 0.13	0.053
4 pw	0.65 ± 0.23	0.21 ± 0.28	0.27
E-1	0.25 ± 0.05	0.21 ± 0.06	0.60
E	0.77 ± 0.34	0.30 ± 0.40	0.03
2 pb	1.54 ± 0.16	0.81 ± 0.17	0.025
5 pb	0.68 ± 0.12	0.51 ± 0.13	0.40

values are LS mean ± SEM

T_4

Figure 7a illustrates the mean T_4 concentrations throughout the trial. No differences were found in the mean T_4 concentration nor were there any significant effects present on any of the sampling days except on day 13 pp, variability was greater for first parity sows (P=0.0008). Also, there was a significant hour effect at 4 days pw (P=0.017). T_4 concentration appeared to be higher in first (19.21 ± 2.50 ng ml^{-1}) compared to fifth parity sows (9.06 ± 7.99 ng ml^{-1}) at estrus, however, because the variation was so large, no differences were detected (P=0.29) (figure 7a). First parity sows had significantly higher T_4 on day 10 pp (P=0.015) but there were no differences in any of the other single samples pw or pb except on the last sample day before slaughter, the first parity sows had higher T_4 concentration (P=0.004). There were no significant day or parity by day effects in mean T_4 in the comparison of all days. Table 14 shows LS mean concentration in T_4 ± SEM and level of significance between parity. Appendix table A9 shows split plot effects in T_4.

TABLE 14. Mean serum T_4 (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	14.74 ± 1.67	13.08 ± 1.89	0.53
3 pp	17.46 ± 1.37	14.51 ± 1.14	0.13
13 pp	14.96 ± 1.65	12.44 ± 1.57	0.30
4 pw	13.99 ± 1.42	13.53 ± 2.67	0.89
E-1	19.16 ± 3.61	16.18 ± 2.73	0.54
E	19.21 ± 2.50	9.06 ± 7.99	0.29
2 pb	19.56 ± 2.49	19.41 ± 2.68	0.97
5 pb	19.67 ± 3.70	21.54 ± 3.79	0.74

values are LS mean ± SEM

T_3

Figure 7b shows the daily mean concentrations in T_3 for first and fifth parity sows throughout the trial. No significant differences in the mean or variation in T_3 concentration were present on any day nor were there any significant effects. However, on day 2 pb, there was a trend for a parity by hour (P=0.064) interaction as noted by a difference in the parity response in T_3 concentration from 1100 to 1200 and between 1100 and 1400 hours. T_3 concentration appeared to be higher in fifth (124.13 ± 43.69 ng dl^{-1}) compared to first parity sows (81.93 ± 46.78 ng dl^{-1}) on day 3 pp, but because the variation was so large, no differences were detected (P=0.53) between each parity (figure 7b). No differences were found between parity in any of the single samples during lactation, pw or in the pb period in T_3 concentration. In the comparison of days, there was a strong tendency for a day effect (P=0.06), and this is shown by the fact that day 3 pp had the highest concentration of any of the sampling periods. Table 15 shows LS mean concentration in T_3 ± SEM and level of significance between parity.

TABLE 15. Mean serum T_3 (ng dl^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	54.58 ± 8.21	31.62 ± 9.40	0.09
3 pp	81.93 ± 46.78	124.13 ± 43.69	0.53
13 pp	28.77 ± 4.17	28.28 ± 3.98	0.94
4 pw	38.58 ± 3.68	34.10 ± 5.55	0.52
E-1	42.04 ± 2.84	38.42 ± 2.12	0.35
E	45.79 ± 4.04	34.93 ± 13.87	0.49
2 pb	36.92 ± 19.38	75.88 ± 21.97	0.24
5 pb	52.64 ± 24.40	31.78 ± 28.18	0.61

values are LS mean ± SEM

T_3/T_4 ratio

There were no differences in mean or variability in the T_3/T_4 ratio. A trend for a parity by hour effect was present on day 2 pb (P=0.07), shown by difference in each parity's response in the T_3/T_4 ratio between 1100 and 1200 and 1100 and 1400 hours. Also on day 2 pb there was a significant hour effect (P=0.05) but comparisons were unable to detect when these existed. When comparing all the days, there were no day or parity by day effects for the mean T_3/T_4 ratio. See appendix table A10 for split plot effects in T_3/T_4 ratio.

IGF-I

Figure 8a shows the mean daily IGF-I concentrations for the fifth and first parity sows. On days 110 g, 3 pp, 13 pp and 4 pw there were no differences in the mean or the variability of IGF-I between the two parities. On day 3 pp there was a significant parity by hour effect (P=0.02) with the differing parity responses in IGF-I concentration occurring

between 0900 and 1000 and 1200 and 1700 hours. Also, on day 3 pp there was an hour effect (P=0.024), with greater concentration of IGF-I at 1600 than at 1300 hours. At estrus, the first parity sows had a higher mean IGF-I concentration (P=0.03). There were no significant differences in the concentration from the single samples. When comparing all days, there was a significant day effect (P=0.0001) and this is shown by the concentration in IGF-I being lowest at day 110 g and days 3 and 13 pp having a lower concentration than on the day before estrus. Table 16 shows LS mean concentration in IGF-I ratio ± SEM and level of significance between parity. See appendix table A11 for split plot effects in IGF-I.

TABLE 16. Mean serum IGF-I (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	76.40 ± 14.12	50.71 ± 16.17	0.26
3 pp	120.49 ± 18.29	156.89 ± 24.38	0.26
13 pp	89.88 ± 22.15	141.25 ± 21.15	0.13
4 pw	142.26 ± 24.26	127.03 ± 45.59	0.78
E-1	208.23 ± 29.73	166.84 ± 23.32	0.32
E	187.36 ± 6.91	110.63 ± 22.06	0.03
2 pb	139.09 ± 27.18	127.11 ± 29.36	0.78
5 pb	135.72 ± 25.39	140.81 ± 29.32	0.90

values are LS mean ± SEM

Insulin

Figure 8b shows the mean insulin concentration for the two parity groups throughout the course of the trial. An hour effect existed on day 110 g (P=0.0001), shown by the insulin concentration being highest at 1000 hours. On day 3 pp, fifth parity sows had a greater mean insulin concentration (P=0.002) and the variation (P=0.004) was greater. As well, mean insulin was higher for fifth parity sows (P=0.02) on day 13 pp

although variation was similar. On day 4 pw, there was an hour effect (P=0.05) but differences between the hours were non detectable. On the day prior to estrus, the mean (P=0.018) and variability (P=0.01) in insulin was higher in fifth parity sows and there was an hour effect (P=0.03). In addition, an hour effect (P=0.04) was present at 5 days pb but again, these differences were non detectable by comparisons. Fifth parity sows had higher insulin on days 12 pp (P=0.048) and 3 pw (P=0.0001) in single samples, but at no time was there a difference in the single samples pb. In the comparison of all days, there was a significant day effect (P=0.0011) and parity by day interaction (P=0.0095). The mean insulin was higher on day 3 pp than on days 13 pp, 4 pw and 2 pb. The difference in the parity by day interaction were present between day 3 pp and day 110 g, day 13 pp and 4 days pw. Table 17 shows LS mean concentration in insulin ± SEM and level of significance between parity. See appendix table A12 for split plot effects in insulin.

TABLE 17. Mean serum insulin ($\mu IU\ ml^{-1}$) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	26.85 ± 6.53	33.08 ± 7.47	0.54
3 pp	16.48 ± 6.19	50.07 ± 5.78	0.002
13 pp	11.41 ± 2.85	22.92 ± 2.72	0.017
4 pw	9.07 ± 3.18	12.63 ± 5.98	0.62
E-1	11.73 ± 4.59	30.05 ± 3.49	0.018
E	14.42 ± 3.60	22.44 ± 11.49	0.55
2 pb	9.42 ± 2.60	10.14 ± 2.80	0.86
5 pb	14.28 ± 4.78	16.40 ± 4.90	0.77

values are LS mean ± SEM

Cortisol

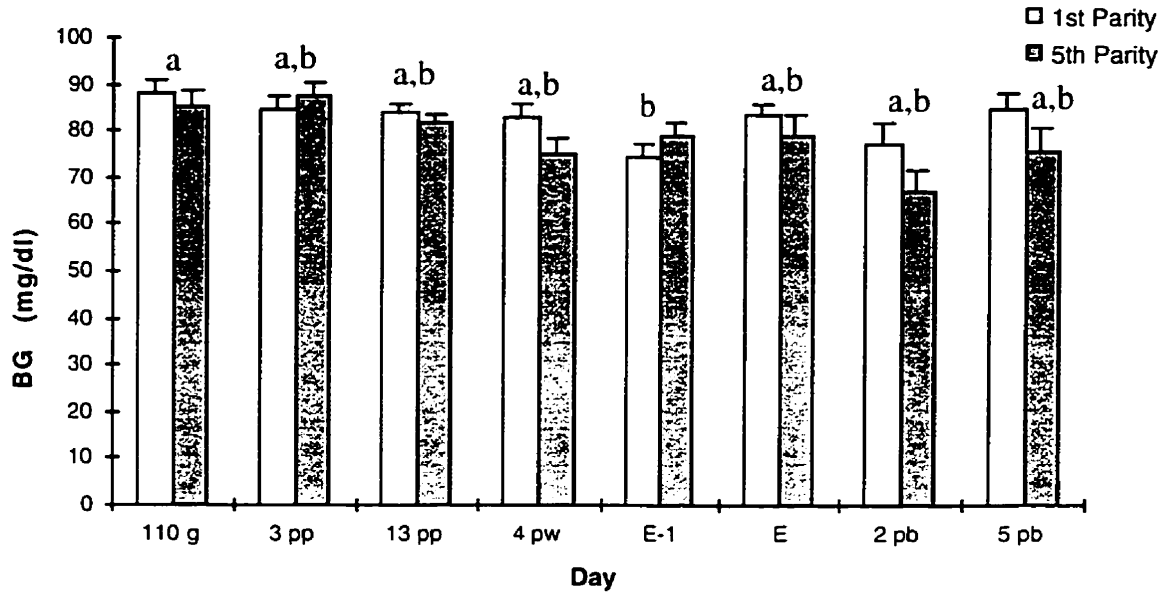
There was an hour effect present on days 110 g (P=0.047) and 13 pp (P=0.05) but comparisons were unable to detect when these differences existed. There was a trend for an hour effect on day 3 pp (P=0.07) and a parity by hour interaction (P=0.06). There were no significant differences or effects on any of the other sampling days. No differences were found in the concentration of the singles samples between the parities except on day 13 pb where fifth parity sow had higher serum cortisol concentration (P=0.034). There was a significant day effect (P=0.01) for mean cortisol in the comparison of days, and this is shown by higher cortisol at 4 days pw and the day before estrus compared to day 13 pp (figure 9). Table 18 shows LS mean concentration in cortisol ± SEM and level of significance between parity. See appendix table A13 for split plot effects in cortisol.

TABLE 18. Mean serum cortisol (ng ml^{-1}) in 1st and 5th parity sows

Day	1 st Parity	5 th Parity	P=
110 g	25.48 ± 2.57	21.16 ± 2.94	0.29
3 pp	19.25 ± 2.76	22.37 ± 2.30	0.40
13 pp	13.69 ± 2.44	14.38 ± 2.62	0.85
4 pw	23.61 ± 5.10	30.30 ± 9.59	0.56
E-1	23.37 ± 5.46	30.08 ± 4.12	0.37
E	17.83 ± 4.11	17.56 ± 7.57	0.98
2 pb	25.06 ± 7.15	24.20 ± 7.72	0.94
5 pb	16.65 ± 11.13	19.82 ± 11.42	0.85

values are LS mean ± SEM

(a)



(b)

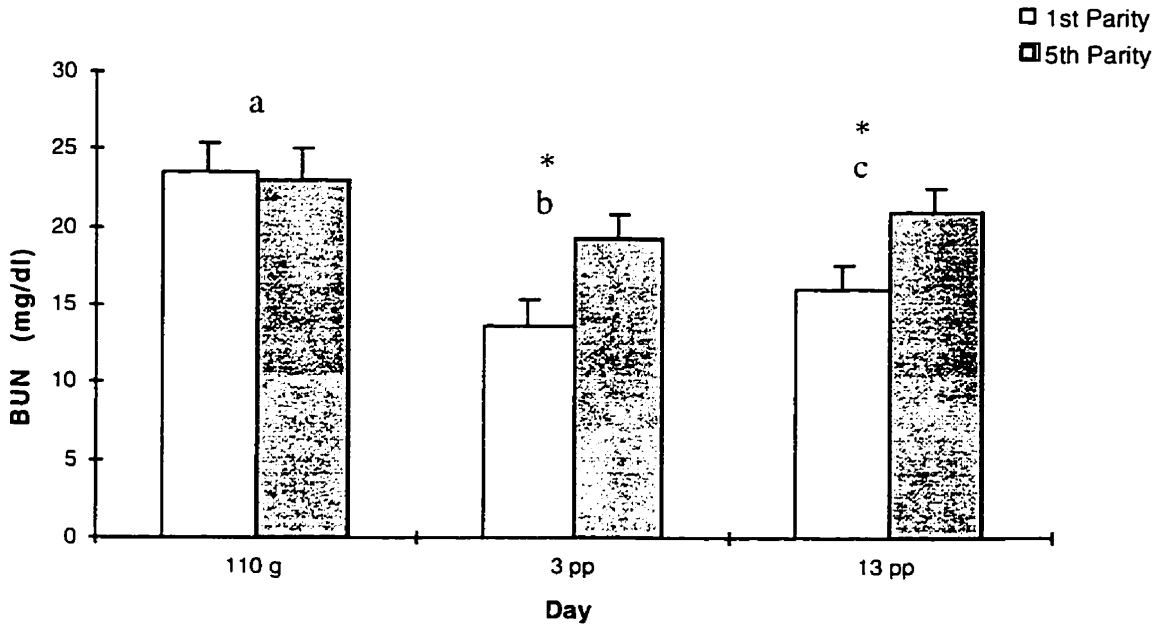


FIGURE 4. Mean blood glucose (BG) (a) and BUN (b) (LS mean ± SEM) values throughout the trial. Different letters denote differences in daily mean concentration across parity for blood glucose (P=0.02) and BUN (P=0.0001). * denotes differences in daily mean concentration between parity for BUN (P<0.053).

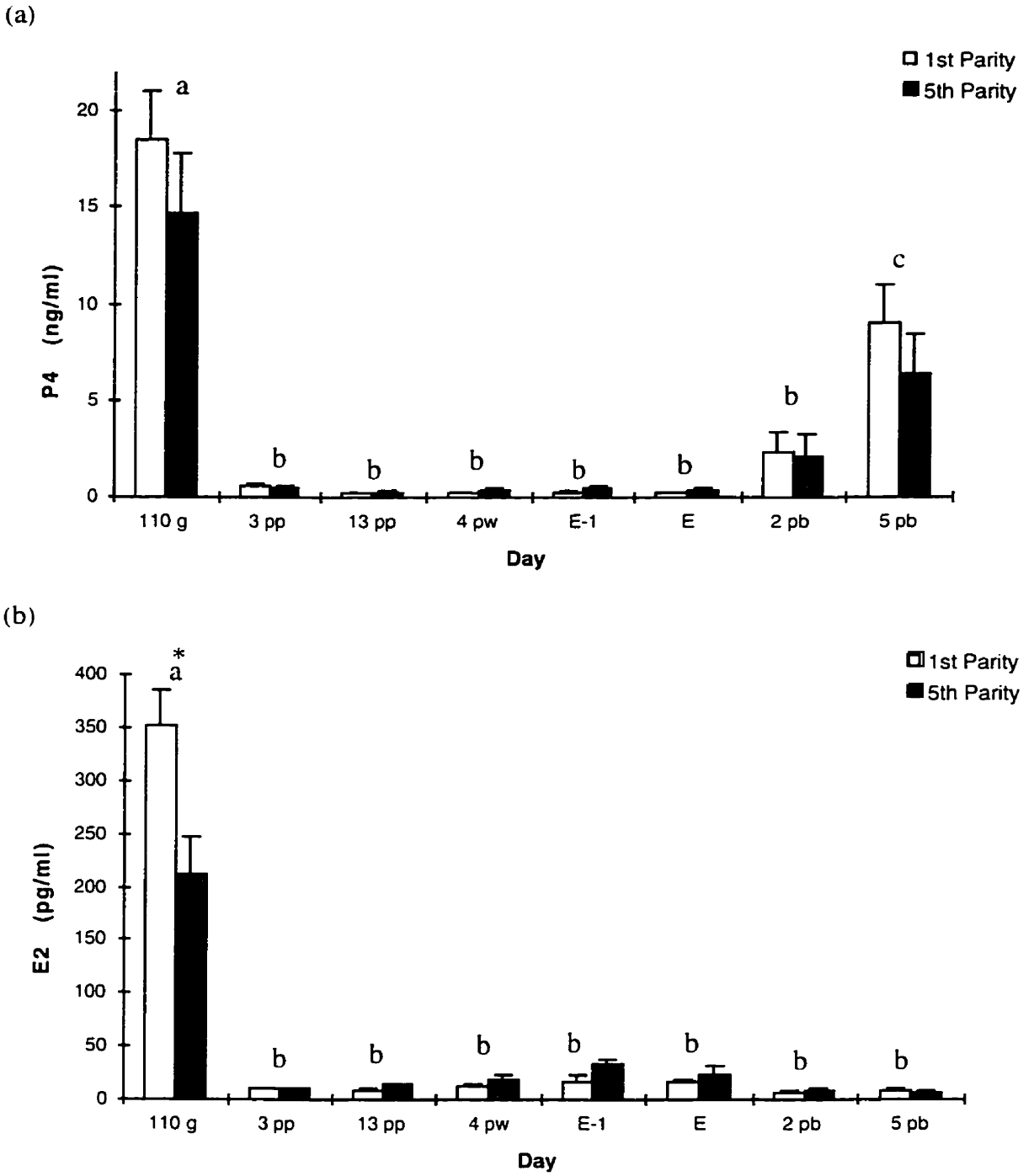


FIGURE 5. Mean serum P4 (a) and E2 (b) (LS means ± SEM) throughout the trial. Different letters denote differences in daily mean concentration across parity for P4 (P=0.0001) and E2 (P=0.0001). * denotes differences in daily mean concentration between parity for E2 (P=0.015).

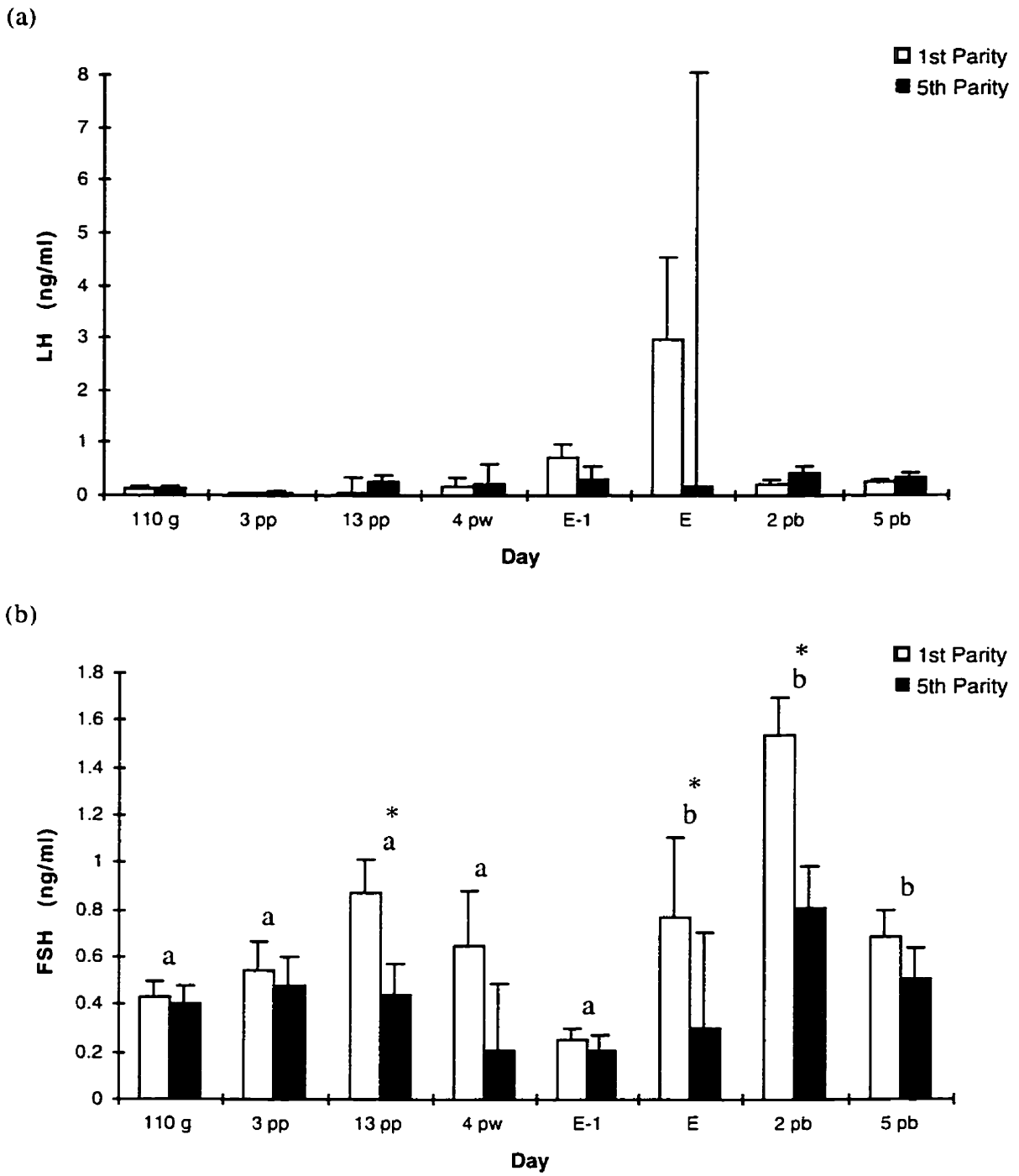
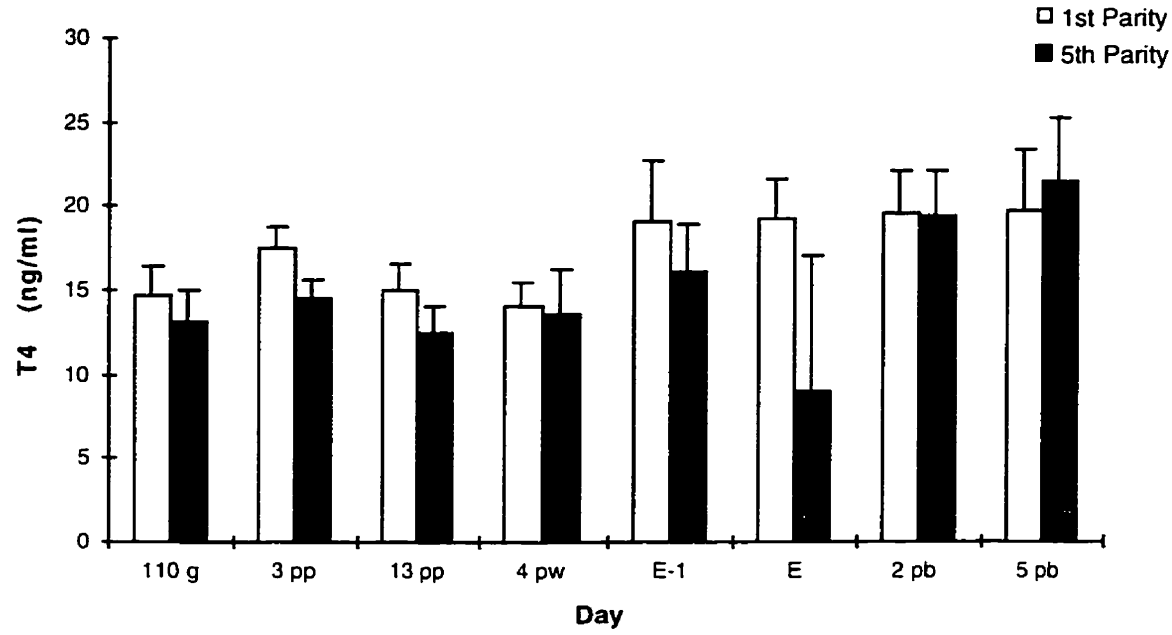


FIGURE 6. Mean serum LH (a) and FSH (b) (LS means ± SEM) throughout the trial. Different letters denote differences in daily mean concentration across parity for FSH ($P=0.0001$). * denotes differences in daily mean concentration between parity for FSH ($P<0.053$). No differences in daily means existed between parity nor was there a day effect present for LH.

(a)



(b)

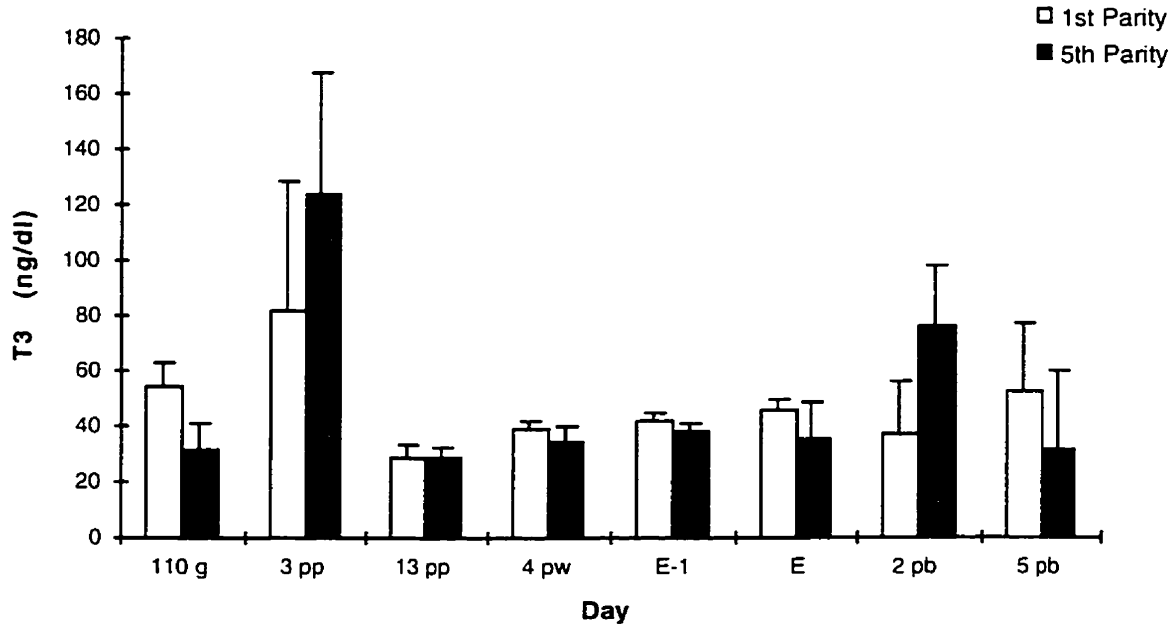


FIGURE 7. Mean serum T_4 (a) and T_3 (b) (LS means ± SEM) throughout the trial. No differences existed in the daily mean concentration between parity nor were there any day effects present in T_4 and T_3.

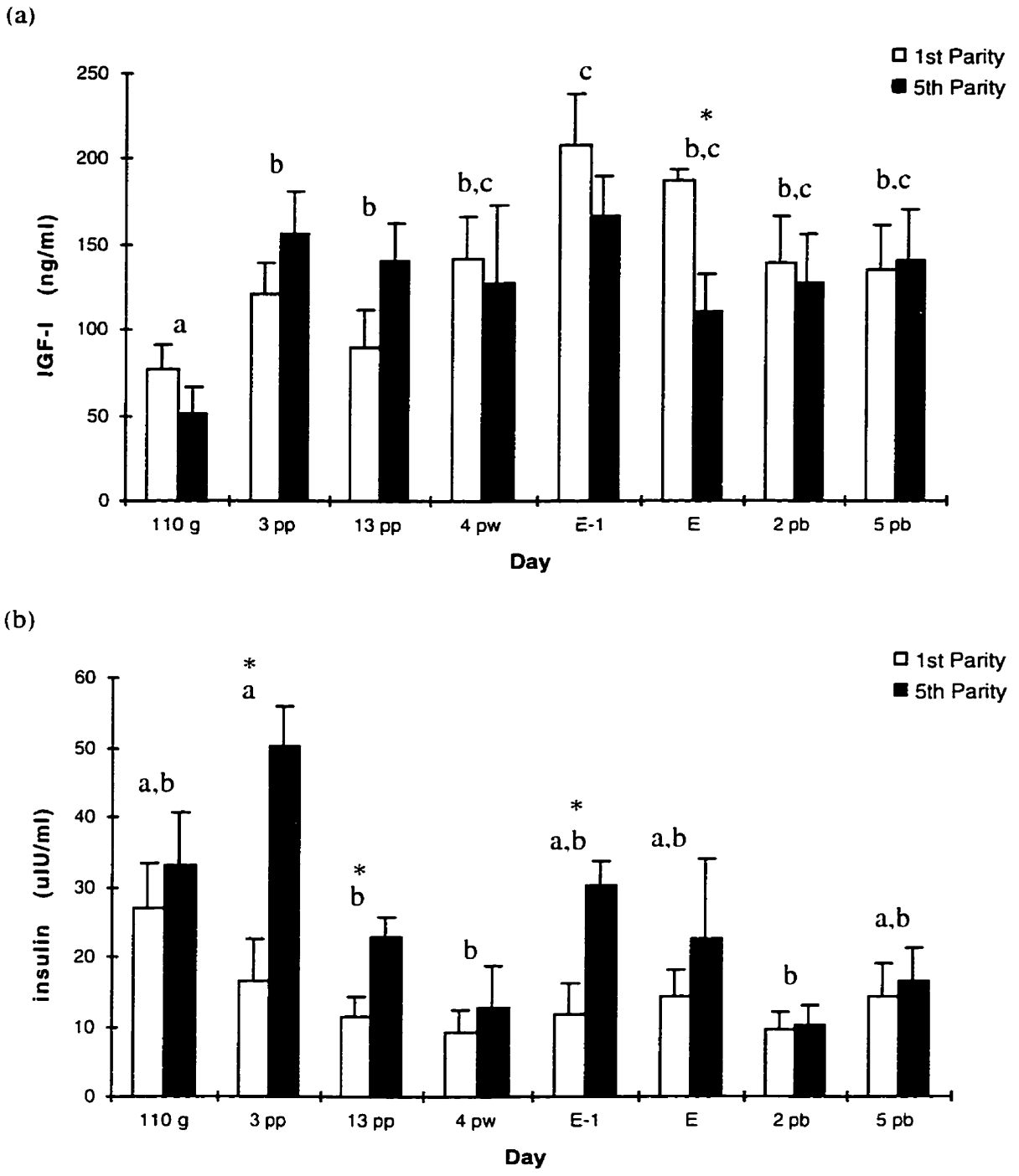


FIGURE 8. Mean serum IGF-I (a) and insulin (b) (LS means ± SEM) throughout the trial. Different letters denote differences in daily mean concentration across parity for IGF-I ($P=0.0001$) and insulin ($P=0.001$). * denotes differences in daily mean concentration between parity for IGF-I ($P=0.03$) and insulin ($P<0.018$).

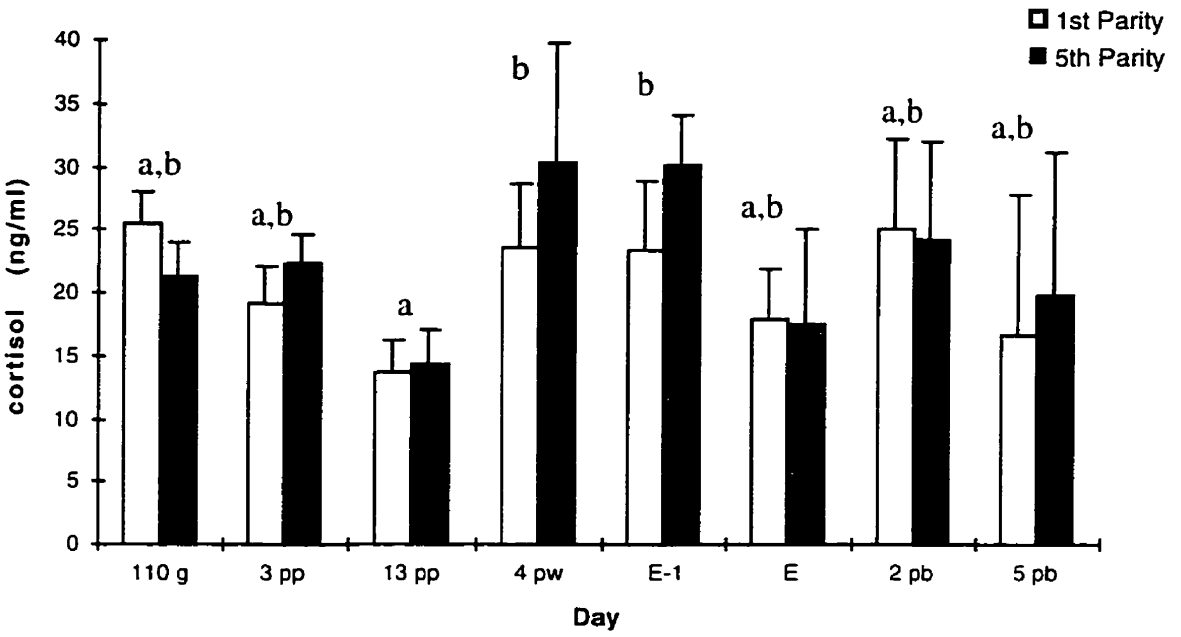


FIGURE 9. Mean serum cortisol (LS means ± SEM) throughout the trial. Different letters denote differences in the daily mean concentration across parity for cortisol (P=0.01). There were no differences in the daily mean concentration between parity for cortisol on any day.

Hormone correlations

(*Nb.* Glucose was only correlated against insulin and IGF-I)

Day 110 g

In fifth parity sows, FSH was negatively correlated with E2 (r=-0.325, P=0.018). IGF-I was positively correlated with LH (r=0.36, P=0.0075), insulin (r=0.287, P=0.036), FSH (r=0.27, P=0.05) and cortisol (r=0.377, P=0.005) but negatively correlated with E2 (r=-0.294, P=0.031) and tended to be positively correlated with T_4 (r=0.251, P=0.067). E2 was also negatively correlated with T_3 (r=-0.441, P=0.0008) and P4 (r=-0.293, P=0.03) and cortisol was positively correlated with LH (r=0.365, P=0.007). There was a trend for insulin to be positively correlated with LH (r=0.262, P=0.056) and cortisol

(r=0.262, P=0.056). As well, there was a strong tendency for T_3 and P4 to be negatively correlated (r=-0.305, P=0.075).

For first parity sows glucose was positively correlated with insulin (r=0.425, P=0.0002) and IGF-I (r=0.324, P=0.006). IGF-I was also positively correlated with FSH (r=0.475, P=0.0001), LH (r=0.348, P=0.001), insulin (r=0.426, P=0.0001) and T_4 (r=0.721, P=0.0001) and negatively correlated with E2 (r=-0.452, P=0.0001) and P4 (r=-0.299, P=0.003). FSH was positively correlated with insulin (r=0.456, P=0.0001) and negatively correlated with P4 (r=-0.294, P=0.004). T_4 was positively correlated with FSH (r=0.588, P=0.0001), LH (r=0.250, P=0.02) and insulin (r=0.543, P=0.0001) but negatively correlated with E2 (r=-0.299, P=0.004). LH was positively correlated with cortisol (r=0.393, P=0.0002) and negatively correlated with E2 (r=-0.248, P=0.024). P4 was positively correlated with E2 (r=0.420, P=0.0001) and negatively correlated with insulin (r=-0.410, P=0.0001). And a trend existed for a positive correlation between insulin and LH (r=0.204, P=0.058).

Day 3 pp

The fifth parity sows had a positive correlation between glucose and insulin (r=0.685, P=0.0001). T_4 was positively correlated with insulin (r=0.302, P=0.052), cortisol (r=0.398, P=0.009) and T_3 (r=0.584, P=0.0001). Cortisol was also positively correlated with P4 (r=0.385, P=0.012) and tended to be positively correlated with E2 (r=0.379, P=0.075). FSH was positively correlated with LH (r=0.373, P=0.015) and T_3 was negatively correlated with IGF-I (r=-0.366, P=0.017).

In first parity sows insulin was positively correlated with glucose (r=0.584, P=0.0001) and negatively with P4 (r=-0.486, P=0.0001). T_4 was positively correlated with IGF-I (r=0.313, P=0.018) and negatively correlated with FSH (r=-0.425, P=0.002). There were positive correlations between E2 and LH (r=0.425, P=0.016) and IGF-I and

cortisol (r=0.415, P=0.0013). As well, there was a trend for positive correlations between T_3 and FSH (r=0.279, P=0.074) and cortisol and P4 (r=0.238, P=0.075).

Day 13 pp

In fifth parity sows, FSH was positively correlated with cortisol (r=0.273, P=0.045) but negatively correlated with E2 (r=-0.512, P=0.0023), IGF-I (r=-0.311, P=0.022) and T_4 (r=-0.285, P=0.037). IGF-I was positively correlated with E2 (r=0.517, P=0.0021), T_3 (r=0.522, P=0.0001), P4 (r=0.488, P=0.0002) and T_4 (r=0.583, P=0.0001). P4 was positively correlated with T_3 (r=0.437, P=0.001), cortisol (r=0.526, P=0.0001) and T_4 (r=0.533, P=0.0001). T_4 was also positively correlated with cortisol (r=0.323, P=0.017) but negatively correlated with LH (r=-0.279, P=0.041). Insulin was positively correlated with glucose (r=0.574, P=0.0001) and tended to be positively correlated with cortisol (r=0.255, P=0.063). As well, E2 was positively correlated with T_3 (r=0.547, P=0.001) and negatively correlated with cortisol (r=-0.431, P=0.012).

The first parity sows had a significant positive correlation between glucose and insulin (r=0.605, P=0.0001). FSH was positively correlated with LH (r=0.355, P=0.043), P4 (r=0.436, P=0.0006) and tended to be positively correlated with IGF-I (r=0.241, P=0.069). However, FSH was negatively correlated with E2 (r=-0.404, P=0.015). T_3 was positively correlated with T_4 (r=0.607, P=0.0001) and insulin (r=0.324, P=0.012) but negatively correlated with E2 (r=-0.513, P=0.0014). T_4 was positively correlated with insulin (r=0.302, P=0.0203) and negatively correlated with P4 (r=-0.395, P=0.002). Cortisol was positively correlated with IGF-I (r=0.560, P=0.0001) and P4 (r=0.393, P=0.003). There was also a trend for a negative correlation between insulin and P4 (r=-0.238, P=0.069).

4 days pw

In fifth parity sows IGF-I was positively correlated with insulin (r=0.482, P=0.008), T_3 (r=0.648, P=0.0002), P4 (r=0.576, P=0.001) and cortisol (r=0.537, P=0.003) but negatively correlated with glucose (r=-0.492, P=0.008) and LH (r=-0.421, P=0.026). LH was positively correlated with FSH (r=0.519, P=0.006) but negatively correlated with T_3 (r=-0.621, P=0.0004) and cortisol (r=-0.406, P=0.032). T_3 was positively correlated with insulin (r=0.452, P=0.016), P4 (r=0.455, P=0.015) and cortisol (r=0.389, P=0.041), however, negatively correlated with FSH (r=-0.467, P=0.014). Also, there were positive correlations between insulin and P4 (r=0.711, P=0.0001) and T_4 and cortisol (r=0.559, P=0.002).

For first parity sows, FSH was positively correlated with LH (r=0.481, P=0.002) and T_3 (r=0.471, P=0.003) but negatively correlated with E2 (r=-0.688, P=0.0001) and IGF-I (r=-0.559, P=0.0003). IGF-I was also positively correlated with E2 (r=0.657, P=0.0001) and T_4 (r=0.312, P=0.042). P4 was positively correlated with E2 (r=0.337, P=0.045), T_4 (r=0.396, P=0.01) and cortisol (r=0.735, P=0.0001). And T_4 was also positively correlated with E2 (r=0.337, P=0.039) and cortisol (r=0.646, P=0.0001).

One day prior to estrus

In fifth parity sows, IGF-I was negatively correlated with FSH (r=-0.733, P=0.007) and E2 (r=-0.659, P=0.02). T_3 was negatively correlated with LH (r=-0.558, P=0.02), E2 (r=-0.645, P=0.023) and cortisol (r=-0.548, P=0.023). And T_4 was positively correlated with P4 (r=0.537, P=0.022) and negatively correlated with FSH (r=-0.677, P=0.016).

The first parity sows showed IGF-I to be positively correlated with LH (r=0.457, P=0.043) and E2 (r=0.567, P=0.043). T_3 was negatively correlated with LH (r=-0.508,

P=0.037), while T_4 was positively correlated with insulin (r=0.585, P=0.017) and negatively correlated with FSH (r=-0.583, P=0.029).

Estrus

For fifth parity sows insulin was positively correlated with E2 (r=0.829, P=0.021), P4 (r=0.786, P=0.036) and T_3 (r=0.814, P=0.026) but negatively correlated with glucose (r=-0.756, P=0.049). E2 was also positively correlated with T_3 (r=0.703, P=0.052) and P4 (r=0.969, P=0.0003). As well, there was a trend for a correlation between cortisol and T_3 (r=0.768, P=0.075).

In first parity sows, E2 was negatively correlated with LH (r=-0.516, P=0.028), FSH (r=-0.673, P=0.002), insulin (r=-0.502, P=0.034) and T_3 (r=-0.510, P=0.031). FSH was also positively correlated with LH (r=0.901, P=0.0001) and insulin (r=0.561, P=0.008). T_4 was positively correlated with T_3 (r=0.436, P=0.05) and negatively correlated with P4 (r=-0.513, P=0.017). As well, there were positive correlations between glucose and insulin (r=0.496, P=0.022) and cortisol and LH (r=0.450, P=0.046). T_3 and LH tended to be negatively correlated (r=-0.411, P=0.072) at estrus.

2 days pb

In the fifth parity sows, insulin was positively correlated with glucose (r=0.777, P=0.005), FSH (r=0.768, P=0.006), E2 (r=0.777, P=0.04) and P4 (r=0.844, P=0.001) but negatively correlated with IGF-I (r=-0.603, P=0.05) and tended to be negatively correlated with T_4 (r=-0.568, P=0.068). IGF-I was also positively correlated with LH (r=0.880, P=0.009), cortisol (r=0.691, P=0.019) and T_4 (r=0.782, P=0.005) although, negatively correlated with P4 (r=-0.669, P=0.024). FSH was positively correlated with E2 (r=0.781, P=0.038) and P4 (r=0.701, P=0.016). P4 was also positively correlated with E2 (r=0.884, P=0.008) and negatively correlated with T_4 (r=-0.821, P=0.002) and cortisol

(r=-0.624, P=0.04). T_4 was positively correlated with cortisol (r=0.782, P=0.005) and negatively correlated with E2 (r=-0.817, P=0.025).

For first parity sows, glucose was positively correlated with insulin (r=0.652, P=0.012) and tended to be positively correlated with IGF-I (r=0.513, P=0.061). As well, T_3 was negatively correlated with P4 (r=-0.856, P=0.0001).

Hormone correlations between LH, FSH, IGF-I, insulin, cortisol, T_4, T_3 and blood glucose for fifth and first parity sows are shown in table 19 and table 20, respectively.

See Appendix for multivariate correlation analysis for the removal of individual sow variation within parity.

TABLE 19. Correlations in LH, FSH, IGF-I, insulin, cortisol, T_4, T_3 and blood glucose (BG) in fifth parity sows

	Day						
	110 g	**3 pp**	**13 pp**	**4 pw**	**E-1**	**E**	**2 pb**
LH-FSH	NS	r=0.37 P=0.015	NS	r=0.52 P=0.006	NS	NS	NS
LH-insulin	NS	NS	NS	NS	NS	NS	NS
LH-IGF-I	r=0.36 P<0.008	NS	NS	r=-0.42 P=0.026	NS	NS	r=0.88 P=0.009
LH-cortisol	r=0.37 P=0.007	NS	NS	r=-0.41 P=0.032	NS	NS	NS
FSH-insulin	NS	NS	NS	NS	NS	NS	r=0.77 P=0.006
FSH-IGF-I	r=0.27 P=0.05	NS	r=-0.31 P=0.022	NS	r=-0.73 P=0.007	NS	NS
T_4-cortisol	NS	r=0.40 P=0.009	r=0.32 P=0.017	r=0.56 P=0.002	NS	NS	r=0.78 P=0.005
T_3-cortisol	NS	NS	NS	r=0.39 P=0.041	r=-0.55 P=0.023	NS	NS
T_4-IGF-I	NS	NS	r=0.58 P<0.001	NS	NS	NS	r=0.78 P=0.005
T_3-IGF-I	NS	r=-0.37 P=0.017	r=0.52 P<0.001	r=0.65 P<0.001	NS	NS	NS
insulin-IGF-I	r=0.29 P=0.036	NS	NS	r=0.48 P=0.008	NS	NS	r=-0.60 P=0.05
insulin-BG	NS	r=0.69 P<0.001	r=0.57 P<0.001	NS	NS	r=-0.76 P=0.049	r=0.78 P=0.005

TABLE 20. Correlations in LH, FSH, IGF-I, insulin, cortisol, T_4, T_3 and blood glucose (BG) in first parity sows

	Day						
	110 g	**3 pp**	**13 pp**	**4 pw**	**E-1**	**E**	**2 pb**
LH-FSH	NS	NS	r=0.36 P=0.043	r=0.48 P=0.002	NS	r=0.90 P<0.001	NS
LH-insulin	NS	NS	NS	NS	NS	NS	NS
LH-IGF-I	r=0.35 P=0.001	NS	NS	NS	r=0.46 P=0.043	NS	NS
LH-cortisol	r=0.39 P<0.001	NS	NS	NS	NS	r=0.45 P=0.046	NS
FSH-insulin	r=0.46 P<0.001	NS	NS	NS	NS	r=0.56 P=0.008	NS
FSH-IGF-I	r=0.48 P<0.001	NS	NS	r=-0.56 P<0.001	NS	NS	NS
T_4-cortisol	NS	NS	NS	r=0.65 P<0.001	NS	NS	NS
T_3-cortisol	NS	NS	NS		NS	NS	NS
T_4-IGF-I	r=0.72 P<0.001	r=0.31 P=0.018	NS	r=0.31 P=0.042	NS	NS	NS
T_3-IGF-I	NS	NS	NS	NS	NS	NS	NS
insulin-IGF-I	r=0.43 P<0.001	NS	NS	NS	NS	NS	NS
insulin-BG	r=0.43 P<0.001	r=0.59 P<0.001	r=0.61 P<0.001	NS	NS	r=0.50 P=0.022	r=0.65 P=0.012

CHAPTER 5

DISCUSSION

Physiological mechanisms controlling postweaning reproduction in the sow are not well defined. Past research indicates that feed denial results in lowered reproductive performance. However, little information exists on the natural situation seen in the primiparous sow which often shows compromised postweaning reproductive performance. Ample evidence indicates that extensive tissue catabolism often results in impaired reproductive performance (Foxcroft *et al.*, 1995). It is not unusual for sows to become catabolic in lactation and growing first parity sows are especially prone to losses in body condition. In our study, comparisons were made of growing primiparous sows with mature fifth parity sows in order to improve our understanding of the main differences between these animals in terms of tissue catabolism, postweaning reproduction and metabolite and endocrine profiles.

Production performance of sows in the present study was good by industry standards and comparable to the university herd averages of 10 and 12 for first and fifth parity Cotswold sows, respectively (Connor *et al.*, 1997) and also is similar to that reported for first parity sows elsewhere (Coffey *et al.*, 1994). These numbers formed the basis for the standardized litter sizes used as a means to equalize the metabolic demand across parity. Although average piglet weaning weight was higher in first parity sows, both parities had 14 day old pigs of similar size to that seen with PIC sows in the study of Pluske *et al.* (1998). The percentage of the standardized litters weaned was similar to those reported by Connor *et al.* (1997) for first and fifth parity sows.

Feed consumption and body composition play an important role in the maintenance of the reproductive cycle. Low dietary energy and protein intake, as a result of feed restriction or low voluntary feed intake, result in extended WEI (Brendemuhl *et al.*, 1987; Coffey *et al.*, 1994; Koketsu *et al.*, 1996). The dietary levels of energy (fat or carbohydrate) and protein are important regulators of endocrine status and may directly or indirectly affect reproductive function. When energy intake is reduced, fat makes up a larger proportion of the loss in body reserves and when protein is deficient, lean body tissue makes up a larger proportion of the loss (Brendemuhl *et al.*, 1987). Extending this, as total feed intake decreases, the absolute levels of energy and protein consumed will be low and the level of lysine consumed will necessarily decrease as well. Knabe *et al.* (1996) found that increasing the level of lysine in the diet of lactating primiparous sows results in reduced backfat catabolism. The larger losses in backfat and weight during lactation in first parity sows in the present study are possibly a reflection of their lower voluntary feed consumption compared to the fifth parity sows. Although feed intake as a proportion of total body weight may be of greater importance. Despite similar feed intake expressed as a percentage of body weight between parity one and parity five sows, the first parity sows had a greater degree of tissue catabolism in lactation. This indicates, therefore, that first parity sows have a greater maintenance requirement.

Brendemuhl *et al.* (1987) found that sows fed diets with various energy and protein combinations did not differ in weight loss but, did differ significantly in backfat changes throughout lactation. A similar situation was seen in this trial between the two parity groups. Although fed the same diet, their absolute energy and protein consumption levels were different, based simply on differences in feed intake during late gestation, lactation and the rebreeding period. First parity sows lost more backfat than protein throughout lactation. A trend for first parity sows to lose more backfat in the last week of lactation is likely a reflection of greater piglet demand and less available body reserves for

mobilization. Although the lean body mass (protein) decreased during lactation, the protein loss did not differ between the first and fifth parities as did body lipid in lactation. This may be an indicator of the metabolic status and growth of primiparous sows and their drive towards protein accretion.

The higher piglet weaning weights for the first parity sows may be a reflection of greater losses in weight and backfat throughout lactation compared to fifth parity sows. Primiparous sows sacrifice and direct their own body reserves towards milk production (Pluske *et al.*, 1998). Despite 'full feed' consumption, the first parity sows mobilized their body stores more readily than fifth parity sows in lactation. This would imply that feed energy was used primarily for milk production and body condition was sacrificed to that end. As well, feed restricted sows produce equivalent milk volume as their *ad libitum* fed counterparts (Zak *et al.*, 1997b; Pluske *et al.*, 1998; Zak *et al.*, 1998) indicating their catabolic drive for milk production. However, there is a biological limit to this drive and sows made anabolic by superalimentation do not wean heavier litters or produce more milk energy than sows fed on an *ad libitum* basis throughout lactation (Pluske *et al.*, 1998). This may be a reflection of the fact that despite greater feed intake, fifth parity sows did not produce more milk than first parity sows as shown by lighter piglet weaning weights.

Sows increase their lean body mass until parity four, however, by parity three protein accretion plateaus (Whittemore and Yang, 1989). Young, growing sows often become somewhat catabolic in late gestation and certainly in lactation (Clowes *et al.*, 1994). The weight loss in some first parity sows in late gestation, but increase in backfat, suggests that in this period, first parity sows lose lean tissue mass. This is supported by the tendency for greater total body protein loss in first parity sows in this time period. Lactational losses in backfat were paralleled by losses in total body lipid and the weight loss seen in the last week of lactation coincided with losses in lipid for first parity sows. For fifth parity sows, weight loss in the 5 days postweaning was paralleled by losses in

total body protein. However, a portion of postweaning weight loss may be attributed to mammary gland metabolism and involution. Weight loss consequent to losses in mammary tissue may be greater in fifth parity sows based on larger mammary glands in older sows. The greater postbreeding weight gain in first parity sows was paralleled by a strong tendency for protein deposition. Thus, results of this study show a trend for increased lean growth in first, compared to fifth, parity sows postweaning at the expense of fat deposition. This is in line with the suggested growth drive of young sows as they approach their second parity compared to the relatively insignificant lean tissue growth seen in mature sows (Whittemore and Yang, 1989; Clowes *et al.*, 1994).

BUN has been used as a rapid response criterion for protein mobilization in the lactating sow (Coma *et al.*, 1996) and BUN levels rise quickly following a meal (Prunier *et al.*, 1993). Similarly, in the present study, BUN increased postprandially throughout the day. The higher BUN levels found in late gestation coincident with lower feed intake are probably a result of higher protein catabolism during gestation and this is consistent with the protein prediction results. However, during lactation, in the absence of a correlation between body protein and BUN and the higher feed intake in fifth parity sows, it is probable that these BUN levels are a reflection of feed intake and not body protein catabolism.

Reproductive function in pigs is influenced by season and often WEI are longer in the summer months (Clark *et al.*, 1986). The major implicating factor is likely photoperiod, however, Simoneau *et al.* (1988) were unable to find a difference in the postweaning anestrus period in sows subjected to short (4 hours) or long (16 hours) light regimens. Despite this, Evans *et al.* (1994) found LH release to be greater in prepubertal gilts during periods of darkness. Temperature may also mediate this effect and barn temperatures throughout the course of the trial were quite high. The average daytime and nighttime temperatures were 26.8°C and 22.2°C, respectively and the maximum temperature in the

barn throughout the trial was 33.9°C. If the barn temperature was high during certain critical times throughout the sows' reproductive cycle it may have had an impact on the performance of sows in this study. Furthermore, high ambient temperatures are known to be a causative factor in reduced voluntary feed intake and increased weight losses in sows (Armstrong *et al.*, 1986; Coffey *et al.*, 1994). Although, in the present study, primiparous sows may have been more influenced by high temperatures compared to the fifth parity sows.

The correlation between body weight and the WEI seen across both parities in this study is in agreement with others (Prunier *et al.*, 1993; Zak *et al.*, 1998). It appears that body weight and not backfat, plays a role in the time required for resumption of estrous cycles postweaning. Although all first parity sows showed a degree of body catabolism during lactation, the relationship between metabolic status and the WEI may not be directly related to body condition. This is in agreement with the findings of Clowes *et al.* (1994) and Johnston *et al.* (1989) who found that primiparous sows with less than 12 mm of backfat can still return to estrus after weaning without delay and subsequent litter size in these sows is not compromised. These findings suggest, as do ours, that backfat and the total amount of body lipid are not the most important predictors of reproductive function and perhaps lean body tissue is a more important factor in the control of reproduction in growing sows. This may not be true of mature multiparous sows, however, considering the large weight and total body protein loss following weaning in the fifth parity group and their prompt return to estrus.

The extended postpartum anestrus period seen in the first parity group is characteristic of primiparous sows (Clark *et al.*, 1986; Foxcroft *et al.*, 1995). All fifth parity sows returned to estrus within 6 days postweaning and in fact, one sow returned to estrus the day following weaning. In the current study, three first parity sows had normal WEI but, the variability in the WEI was very high in the first parity sows. Although the

WEI may not always be a major problem following early weaning (Varley, 1976), others have found the duration and variability to increase as lactation lengths decrease (Varley *et al.*, 1985; Foxcroft *et al.*, 1995). Therefore, the long WEI in the first parity group may not be the result of the short lactation length imposed in this study.

The management strategy of "skip a heat" breeding is aimed at improving reproductive performance by allowing lost body condition from lactation to be regained before breeding. This strategy is most beneficial in primiparous sows who lose extensive body reserves during lactation (Clowes *et al.*, 1994). In the present study, primiparous sows who returned to estrus within 7 days were significantly heavier in late gestation and lactation than those sows that returned to estrus in ≥7 days although there was no difference in backfat, total body lipid or protein. Postweaning, however, there was no difference in weight between the two groups. All sows with WEI of >7 days were pregnant at the termination of the study. However, this was not the case for the sows that returned to heat within 7 days of weaning. Possibly then, reproductive performance was improved when weight was gained after weaning, indicated by their ability to maintain their pregnancies compared to the sows that returned to estrus in <7 days. Similarly "skip a heat" breeding allows sows to be potentially anabolic prior to breeding and this may improve reproductive performance (Clowes *et al.*, 1994). Furthermore, Jindal *et al.* (1996) found a strong relationship between embryo mortality and feed intake and the level of body catabolism. Despite this, Zak *et al.* (1998) found that superalimentation to make sows anabolic during lactation does not improve fertility after weaning beyond that of *ad libitum* fed sows.

Litter size has been found to be compromised subsequent to early weaning (Varley, 1982). Two major factors influencing litter size are ovulation rate and embryo survival. In the current study, ovulation rate for first parity sows after a 14 day lactation period was higher than reported elsewhere (Jindal *et al.*, 1996) and not highly variable. Because

ovulation rate and fertilization are generally not considered to be affected following early weaning (Varley, 1982), lowered embryo survival may be implicated in decreased litter size. Most prenatal embryo losses occur before attachment of the embryos in the uterus (Archibong *et al.*, 1987) and these losses can be upwards of 40%. It is normal to expect a 10% embryo loss beyond the implantation or placentation period (Archibong *et al.*, 1987). Under normal circumstances, it is generally observed that litter size increases until parity three and plateaus thereafter (Connor *et al.*, 1997). In this experiment, the embryo mortality rate was high leaving the number of viable embryos at day 27-35 postbreeding close to the expected litter size for parity five sows. In first parity sows, however, the number of viable embryos present was less than the actual number of piglets born alive in the previous farrowing. Therefore, the subsequent litter size would be less than what is normally expected for a parity two sow. This decrease in litter size is consistent with early weaning but generally not with conventional weaning systems of 28 days.

A majority of uterine involution is largely complete by 3 weeks postpartum (Palmer *et al.*, 1965a). Following early weaning, however, the uterus may not be fully competent and able to maintain a pregnancy to term and this may be related to the pregnancy rates seen in this trial. In the present study, all fifth and first parity sows were bred at an average of 17.5 and 30.7 days postpartum, respectively. However, all sows which were unable to maintain a pregnancy had been bred at ≤20 days postpartum. First parity sows who maintained their pregnancy at their first postweaning service all had anestrous periods ≥7 days. This may have allowed them time to restore lost body tissue and possibly allow for complete uterine involution which may not have been the case for the sows that returned to estrus in <7 days postweaning. In contrast, all fifth parity sows were bred within 7 days of weaning. Therefore perhaps uterine involution takes longer in first parity sows.

Maintenance of pregnancy is also dependent on a number of uterine proteins (histotroph) which are secreted in varying amounts throughout gestation. The production of

these proteins within the uterus is dependent on the steroidal milieu. Because early weaning is thought to disturb steroid secretion, it is likely that the balance and/or quantity of these pregnancy specific proteins may be disturbed (Varley, 1982). Low levels of estradiol seen in the present study possibly resulted in suboptimal histotroph content within the uterine lumen to support the developing embryos, resulting in a considerable amount of embryo wastage. Therefore, the short lactation length imposed in this study may have been a contributing factor to the high embryo mortality.

An extended WEI may also be implicated in reduced litter sizes (Kemp and Soede, 1996). This, however, is often more a reflection of improper timing of insemination relative to ovulation and not reduced sow productivity per se. In the present study, the extended WEI seen in the first parity sows did not correspond to compromised embryo numbers in relation to those sows with short WEI. And, in fact, only the sows with extended WEI maintained pregnancies to term.

The source of P4 during pregnancy in the pig is the corpus luteum and to a much lesser degree, the placenta (Ash and Heap, 1975). Consistent with others (Ash and Heap, 1975; Parvizi *et al.*, 1976; Foxcroft *et al.*, 1982), P4 was highest in late gestation, low in lactation and began to rise again in the postbreeding period. In our findings, P4 was higher on day 5 postbreeding compared to all other days except late gestation.

The developing fetus produces high levels of estrogen and in the current study, E2 was highest on day 110 of gestation compared to all other days for both first and fifth parity sows. These findings are in agreement with Ash and Heap (1975) who report estrogen concentrations approximately 30-fold higher in late pregnancy compared to at estrus. In our study, the concentration of E2 was higher in the first parity sows on day 110 of gestation and this contrasts with Ash and Heap (1975) who found estrogen levels to increase with increasing parity in late gestation. This was likely due to the greater number of fetuses in the later parity sows in their study, and therefore higher estrogen output from

the fetal-placental unit. However, in our study, there was no significant difference in the number of piglets born alive between the two parities and one sow in the first parity group had exceedingly high E2 levels (>500 pg ml^{-1}) in late gestation.

Similar to the current study, Newton *et al.* (1987) found the levels of FSH and the FSH/LH ratio to be higher in primiparous sows than in later parity sows. FSH is known to build up in the pituitary during lactation and the blood concentrations to increase following weaning. Normally FSH decreases following estrus and then rises again approximately 2-3 days following the ovulatory LH surge (Foxcroft, 1982). This postovulatory increase in FSH is believed to be involved with follicle recruitment and may occur as a result of low estrogen levels and possibly inhibin (Foxcroft, 1982; Fortune, 1994). A similar rise in FSH following breeding was seen in our study and appeared to last for approximately 3 days.

In the present study, the overall hormone levels around estrus tended to be lower than others for E2 (Foxcroft, 1982; Soede *et al.*, 1994), LH (Foxcroft, 1982; Varley and Foxcroft, 1990; Sesti and Britt, 1993b; Koketsu *et al.*, 1996) and FSH in lactating sows (De Rensis *et al.*, 1993a; Sesti and Britt, 1993b), weaned sows (Foxcroft *et al.*, 1987) and restricted fed sows (Zak *et al.*, 1997b). However, information regarding estrogen and gonadotropin concentrations in Cotswold sows is lacking in the literature.

Inadequate LH surges may result in poor luteinization of the corpus luteum which affects P4 secretion (Jindal *et al.*, 1996). In our results P4 secretion did not appear to be affected except that the post estrus rise appeared to take 1 or 2 days longer than expected for sows (Foxcroft, 1982). Furthermore, the eggs destined for ovulation may not be viable if the LH surge is not optimal as it is required for the final stages of oocyte maturation (Jindal *et al.*, 1996). Therefore, this could be implicated in the high embryo mortality seen in this study.

Jindal *et al.* (1996) found P4 around mating to be related to embryo survival and influenced by energy intake level but similar results were not obtained by Pharazyn *et al.* (1991). The lack of a correlation between P4 concentration on days 2 and 27-35 postbreeding and embryo mortality may have been because the number of sows pregnant at these times were low. Therefore, it is possible that any relationship between the P4 concentration and embryo survival was not detected. Also, no significant relationship was found between the E2 concentrations at estrus or 2 or 5 days postbreeding and the embryo mortality rate. Generally, early weaning results in high and variable steroid concentrations that affect the production of pregnancy dependent proteins (Varley, 1982; Varley *et al.*, 1984) as mentioned earlier. This was not the case in our study and in fact E2 production was low which may have resulted in impaired LH release (Foxcroft, 1982; Conley *et al.*, 1994) and possibly in impaired folliculogenesis. However, ovulation rate in our study was high. Despite an apparently high ovulation rate, the percentage retained as viable embryos postbreeding was relatively low. Possibly many of the oocytes which were shed were not fully viable and therefore, any resulting conceptuses were also not viable. Also with the low levels of LH seen in the current study, follicular luteinization may have occurred in the absence of ovulation in some instances. It would appear, therefore, that ovulation occurred on the ovary when in fact only few oocytes may have been released and subsequently fertilized thus, giving the impression of low embryo survival.

The low levels of LH may be associated with the low estrogen levels in this study because the positive feedback required for the LH surge may not have been adequate. Estrogen concentration is negatively correlated with the WEI (Soede *et al.*, 1994). Therefore, the prolonged WEI in our first parity sows may also have reflected lower E2 concentrations. The higher baseline levels of LH on day 13 postpartum for the fifth parity sows is not unexpected for this group considering they had significantly shorter WEI, which indicates advanced folliculogenesis compared to first parity sows. Because LH

concentration is known to increase as lactation progresses (Sesti and Britt, 1993b) a quick postweaning return to estrus would be expected if the levels of LH were sufficiently high towards the end of lactation. Normally we would expect to see LH levels increase quickly and dramatically following weaning (Edwards, 1982; Armstrong *et al.*, 1986; Foxcroft *et al.*, 1987) at least in those sows exhibiting a reasonable WEI. This is primarily a result of the release of pituitary gonadotropin stores following weaning. If however, the levels of the gonadotropins are not sufficient following weaning, the WEI will necessarily be delayed. In the present study, although not statistically significant, LH did begin to rise around day 13 pp and reach maximal levels at estrus. In fifth parity sows, the WEI was not delayed and it is possible that the fifth parity sows had a greater proportion of large follicles at weaning which responded to the gonadotropins, even at low levels. This would translate into a more prompt return to estrus postweaning in the fifth parity group compared to the first parity.

In the current study, positive correlations existed between LH and E2 in first parity sows on days 110 of gestation and 3 postpartum and a negative correlation existed at estrus. The negative correlation at this time is likely a result of E2 beginning to fall in the postovulatory period while LH was high compared to other days for first parity sows. LH and FSH should be positively correlated with each other just prior to estrus and negatively correlated 2-3 days post estrus (Foxcroft, 1982). Positive correlations between LH and FSH were found at 4 days postweaning but not on the day prior to estrus or at estrus. Asynchrony of the periovulatory FSH/LH is common to early weaning (Edwards, 1982; Sesti and Britt, 1993b). It is possible that the lack of a correlation between FSH and LH at this time resulted from the short lactation length imposed in the study. No correlation was found between LH and FSH at 2 days postbreeding indicating that the concomitant rise in FSH and fall in LH did not occur at this time.

In the present study, similar levels of blood glucose in non-restricted fed sows (Tokach *et al.*, 1992; Booth *et al.*, 1994; Koketsu *et al.*, 1996) and insulin in multiparous (Kemp *et al.*, 1995) and primiparous sows during lactation (Tokach *et al.*, 1992a; Koketsu *et al.*, 1996) were seen. The levels of IGF-I in this study were slightly higher than those seen in other studies with *ad libitum* fed lactating primiparous sows (Zak *et al.*, 1998), prepubertal gilts (Booth *et al.*, 1994; Booth *et al.*, 1996) and normoglycaemic gilts (Meurer *et al.*, 1991), but similar to primiparous sows administered exogenous insulin (Whitley *et al.*, 1998).

Similar to other findings (Prunier *et al.*, 1993; Booth *et al.*, 1996), in the present study, glucose was found to rise in the postprandial period in both parities. The differing response between the parities over the course of the day in their blood glucose response probably results from sows consuming their feed at different times. A general observation in this study was that fifth parity sows usually ate all of their feed within minutes of it being placed in the hopper. This was not the case for first parity sows who often ate small portions throughout the day. Because blood glucose rises quickly following a meal this situation would necessarily result in a differential response in blood glucose throughout the day between the two parity groups. Circulating concentrations of glucose have been found to decrease with the progression of lactation and increased milk production (Armstrong *et al.*, 1986) reflecting increased glucose uptake by the mammary gland. Contrasting with this, the present study and others have found little or no change in blood glucose levels throughout lactation (Tokach *et al.*, 1992; Koketsu *et al.*, 1996).

Developing preimplantation pig embryos require glucose as an energy source and this may result in lowered levels of circulating glucose in early gestation (Flood and Wiebold, 1988). Nichol *et al.* (1992) found that in the early preovulatory period, glucose is lower than post ovulation in the oviduct in the pig. Despite these findings, changes in

peripheral blood glucose concentration possibly resulting from glucose uptake by developing embryos were not evident in our results.

The normally expected postprandial increases in insulin concentration were not found throughout lactation in the present study. This is possibly a result of milk production and increased need for glucose by the mammary gland (Armstrong *et al.*, 1986) which resulted in erratic changes in insulin throughout the day in order to maintain glucose homeostasis. As mentioned, feed intake for first parity sows was not generally confined to one meal, rather these sows ate smaller portions throughout the day. The elevated insulin level across parity on day 3 pp most likely is a result of increased feed intake in the absence of a high degree of body catabolism early in lactation. The parity by day interaction illustrates a situation where first parity sows' insulin levels dropped dramatically in early lactation and did not change postweaning, whereas the levels remained the same from late gestation until early lactation in fifth parity sows but fell dramatically postweaning. This is probably related to the large weight loss and total body protein loss in the 5 days postweaning in the fifth parity group as insulin is related to protein catabolism (Armstrong *et al.*, 1986).

Feed restriction results in lowered insulin levels in both the pre and postprandial periods (Prunier *et al.*, 1993; Zak *et al.*, 1997b). In the present study, insulin and feed intake were significantly higher in lactation and prior to estrus in fifth parity sows. Because insulin is involved in lipogenesis (Armstrong *et al.*, 1986; Weldon *et al.*, 1994a; 1994b) the losses in backfat in first parity sows throughout lactation may be related to the low levels of insulin. Furthermore, insulin has been found to be negatively correlated with BUN (Armstrong *et al.*, 1986) and therefore, possibly lean tissue mass. However, in the present study, both insulin and urea nitrogen were depressed in the primiparous sows during lactation. Although as mentioned before, urea nitrogen in this study is probably more a reflection of feed intake and not body catabolism.

Contrasting with others (Buonomo and Baile, 1991; Booth *et al.*, 1994; Zak *et al.*, 1997b), IGF-I levels, in the current study, remained fairly static throughout the day and following meals. Booth *et al.* (1994) found that refed gilts actually have a rebound effect, in that IGF-I levels following refeeding remain higher than control levels. It does, appear somewhat that each parity may respond differently in their IGF-I levels throughout the day shown by a significant parity by time response early in lactation.

First parity sows in the present study, had higher IGF-I at estrus although feed intake was similar to fifth parity sows. Perhaps the rebound effect mentioned is unique to growing animals due in part to IGF-I and its positive correlation with anabolism (Booth *et al.*, 1994; Zak *et al.*, 1997b). Indeed Booth *et al.* (1994) found a positive correlation with IGF-I and body and carcass weight and to longissimus muscle area but not to backfat. Perhaps the absence of a difference in IGF-I during lactation is related to similar changes in body protein between the two parities, despite differing feed consumption and backfat losses. Furthermore, protein deposition in the rebreeding period was higher for first parity sows than for fifth parity sows possibly reflecting the higher IGF-I levels at estrus in the first parity sows. Because IGF-I is regulated in part by growth hormone (Matamoros *et al.*, 1991) it may be that the growing first parity sows if not in a highly catabolic state, have higher IGF-I concentrations because of their growth status. Therefore, when feed intake is decreased or body stores are mobilized, the IGF-I levels may not necessarily fall below that seen in older sows consuming more feed and in a less catabolic state.

A positive correlation between blood glucose and insulin is expected because of the rapid insulin response to changes in blood glucose in order to maintain blood glucose homeostasis (Booth *et al.*, 1996). In the present study, this situation is seen at day 110 of gestation in first parity sows, lactation, estrus and 2 days postbreeding. By extension, glucose may be correlated with IGF-I based on the strong relationship between glucose and insulin and the fact that insulin exerts a direct effect on IGF-I. Insulin administration in the

postweaning period causes GH receptor induction and a correlated increase in IGF-I (Matamoros *et al.*, 1991). In this study, however, a positive correlation existed between glucose and IGF-I in fifth parity sows only in late gestation and 4 days postweaning. Also it is expected, that insulin and IGF-I would be positively correlated in the sow. Indeed, positive correlations existed in both parities in late gestation, but not at other times. In lactation, insulin concentrations in this study were low, especially in the primiparous sow, but not IGF-I levels possibly explaining the lack of correlation between the two at this time. As well, IGF-I and insulin have a permissive action with the gonadotropins on steroidogenesis by ovarian follicles (Buonomo and Baile, 1991; Booth *et al.*, 1996). It could be that these hormones would be positively correlated with each other especially in the early preovulatory period and at estrus. However, in this study clear relationships were not observed.

Because of the positive association with LH, insulin concentration should be related to the WEI (Tokach *et al.*, 1992a). In support of this idea, first parity sows overall had a prolonged postweaning return to estrus and depressed insulin levels in lactation and just prior to estrus. As well, insulin appears to play a role in follicular recruitment (Meurer *et al.*, 1991) as shown by the higher postweaning ovulation rate in fifth parity sows in the current study. However, ovulation rate for first parity sows was quite high, and therefore, it is difficult to conclude if low insulin concentrations in first parity sows had an effect on ovulation rate. IGF-I is also correlated with gonadotropin level and follicle size (Meurer *et al.*, 1991). This likely causes periovulatory IGF-I levels to be increased, and this is noted by elevated levels just prior to estrus. Therefore, the low IGF-I levels in lactation compared to the day prior to estrus are likely a reflection of increased periovulatory IGF-I levels despite similar degrees of body catabolism.

Thyroid hormones are known to decrease following periods of feed restriction in the rat (Rondeel *et al.*, 1992; van Haasteren *et al.*, 1996) and cattle (Hayden *et al.*, 1993)

but T_4 especially, does not appear to be affected to a large degree in pigs following energy or protein intake (Brendemuhl *et al.*, 1987; Giesemann *et al.*, 1989). In contrast, following feed restriction, T_4 and T_3 have been found to fall and are increased following realimentation in pigs (Buonomo and Baile, 1991; Booth *et al.*, 1994). In the present study, the levels of both T_4 and T_3 as well as the T_3/T_4 ratio were similar between first and fifth parity groups despite differences in feed intake. Furthermore, within parity and across parity there were no differences in either T_4 or T_3 throughout the trial on different days despite differing feed intake. A strong tendency for elevated T_3 on day 3 pp in relation to other days of the cycle may be due to increased feed intake in relation to prefarrowing and postweaning but a lower level of catabolism compared to day 13 pp as indicated by less weight and backfat losses.

Cortisol is known to be elevated during periods of stress and feed restriction (Booth, 1990; I'Anson *et al.*, 1994). Because the first parity sows in our study were significantly more catabolic in lactation, elevated cortisol concentrations in these animals could have been expected. However, cortisol, remained similar between first and fifth parity sows throughout the trial. Nonetheless, the levels of cortisol found in this study are consistent with the findings of Ash and Heap (1975) in nonstressed sows through pregnancy, lactation and postweaning. It is probable then, that the period of increased losses in weight and body condition were not perceived as a chronic stress situation by sows of either parity. Cortisol secretion often follows a diurnal rhythm (I'Anson *et al.*, 1994) however, visual inspection of our results did not show any obvious daily rhythm. Perhaps the time and/or duration of the sampling period was insufficient to pickup diurnal changes. During periods of acute stress or low feed intake, cortisol would likely be positively correlated with LH however if the period of stress is chronic, a negative correlation would be present (Rivier and Rivest, 1991). A similar situation was not observed, however, in the present results.

The results of the current study indicate that first parity sows can perform comparably to mature sows in terms of milk production and mothering capabilities. However, the young growing sows mobilize more body reserves for their milk production and this appears to affect their postweaning reproductive performance in relation to fifth parity sows. A notable difference between first and fifth parity sows was the differential mobilization of body stores. Young lactating sows partition nutrients towards milk production and any excess energy is directed towards lean tissue deposition. If the energy intake is below maintenance, body reserves are mobilized. In primiparous sows, body protein stores are maintained at the expense of fat stores. Loses in body condition in the primiparous sow affects the levels of certain hormones, especially insulin, and this has the potential to hinder resumption of estrous cycles postweaning. Differences in endocrine profiles in first and fifth parity sows were primarily seen in lactation and the postweaning periods and this is possibly related to differences in feed consumption and body catabolism.

Postweaning, first and fifth parity sows differed in their reproductive performance. A major problem seen in the first parity sows in the current study, was in the extended duration and variability of the WEI. This is often a primary reason for high culling rates in primiparous sows (Foxcroft *et al.*, 1995). Although ovulation rate appeared to be high, embryo survival was low and this would result in decreased litter size in the first parity sows. Fifth parity sows, even with high embryo mortality rates, had extremely high ovulation rates likely giving good litter sizes at term. Therefore, it appears that differences in reproductive performance in young and mature sows is primarily the result of differing degrees of body catabolism which result in endocrine profiles unique to each parity. It is hypothesized that reproduction is controlled by divergent mechanisms in first and fifth parity sows.

CHAPTER 6

CONCLUSIONS

In this study, the primiparous sows performed well when compared to fifth parity sows in terms of reproductive performance, although the time required for the resumption of estrous cycles postweaning was significantly longer and more variable in the first parity. Throughout this study, body catabolism and the composition of change in body stores differed between parity. Young growing sows appear to mobilize body fat stores more readily in order to keep protein catabolism low. Nutrient partitioning during lactation, as indicated by the high growth rate of piglets, seemed to be directed towards milk production at the expense of maternal body stores. *Ad libitum* feed consumption in fifth parity sows appears to be sufficient during lactation to prevent large losses in weight and body stores. In the early postweaning period, however, with reduced energy intake and the continuation of some milk production, fifth parity sows lost more weight. This was primarily a result of protein mobilization, while first parity sows tended to lose backfat. Mammary gland catabolism may also contribute to this large weight loss postweaning. In the postbreeding period, first parity sows replaced lost body stores from lactation as shown by increased levels of fat and protein anabolism. The duration of the WEI was negatively correlated with body weight across parity. Furthermore, first parity sows with prolonged WEI weighed significantly less than in late gestation and lactation than those with a prompt return to estrus. Therefore, we conclude that the duration of the postweaning anestrous period is likely influenced by changes in body condition, despite an absence of a correlation between the WEI and the backfat or weight of first parity sows. This situation is largely controlled by metabolic hormones and in the present study, insulin appears to play a central role. In

addition, it is hypothesized that postweaning reproduction in first and fifth parity sows is controlled differentially as indicated by differences in endocrine correlations particularly in lactation and the postweaning period.

Further research is recommended to increase the number of experimental animals and to compare primiparous sows weaned at different times to examine the extent of body catabolism occurring throughout different stages of lactation. As well, it is suggested to further examine nutrient partitioning in these animals and its link with metabolic and reproductive hormones and other indices of body metabolism such as serum free fatty acids and creatinine. In addition, a comparison of reproductive function in primiparous and fifth parity sows fed diets differing in energy composition would be recommended and to relate this to body metabolism and endocrine profiles.

REFERENCES

Adashi, E.Y., Resnick, C.E., D'Ercole, A.J., Svoboda, M.E. and Van Wyk, J.J. 1985. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr. Rev. **6**: 400-420.

Archibong, A.E., England, D.C. and Stormshank, F. 1987. Factors contributing to early embryonic mortality in gilts bred at first estrus. J. Anim. Sci. **64:** 474-478.

Armstrong, J.D. and Britt, J.H. 1987. Nutritionally-induced anestrus in gilts: Metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. **65:** 508-523.

Armstrong, J.D., Britt, J.H. and Kraeling, R.R. 1986. Effect of restriction of energy during lactation on body condition, energy metabolism, endocrine changes and reproductive performance in primiparous sows. J. Anim. Sci. **63:** 1915-1925.

Armstrong, J.D., Kraeling, R.R. and Britt, J.H. 1988. Morphine suppresses luteinizing hormone concentrations in transiently weaned sows and delays onset of estrus after weaning. J. Anim. Sci. **66**: 2216-2223.

Ash, R.W. and Heap, R.B. 1975. Oestrogen, progesterone and corticosteroid concentrations in peripheral plasma of sows during pregnancy, parturition, lactation and after weaning. J. Endocrinology. **64:** 141-154.

Beltranena, E. 1996. Contemplating 7 to 10 day weaning? Some essential feeding recommendations. Manitoba Swine Seminar Committee. Volume 10. G. Wruck, ed. Pages 173-178.

Beltranena, E., Foxcroft, G.R., Aherne, F.X. and Kirkwood, R.N. 1991. Endocrinology of nutritional flushing in gilts. Can. J. Anim. Sci. **71**: 1063-1071.

Blake, N.G., Eckland, D.J.A., Foster, O.J.F. and Lightman, S.L. 1991. Inhibition of hypothalamic thyrotropin-releasing hormone messenger ribonucleic acid during food deprivation. Endocrinology. **129:** 2714-2718.

Booth, P.J. 1990. Metabolic influences on hypothalamic-pituitary-ovarian function in the pig. J. Reprod. Fert., *Suppl.* **40**: 89-100.

Booth, P.J., Cosgrove, J.R. and Foxcroft, G.R. 1996. Endocrine and metabolic responses to realimentation in feed-restricted prepubertal gilts: Associations among gonadotropins, metabolic hormones, glucose, and uteroovarian development. J. Anim. Sci. **74:** 840-848.

Booth, P.J., Craigon, J. and Foxcroft, G.R. 1994. Nutritional manipulation of growth and metabolic and reproductive status in prepubertal gilts. J. Anim. Sci. **72**: 2415-2424.

Brendemuhl, J.H., Lewis, A.J. and Peo, Jr., E.R. 1987. Effect of protein and energy intake by primiparous sows during lactation on sow and litter performance and sow serum thyroxine and urea concentrations. J. Anim. Sci. **64:** 1060-1069.

Brendemuhl, J.H., Lewis, A.J. and Peo, Jr., E.R. 1989. Influence of energy and protein intake during lactation on body composition of primiparous sows. J. Anim. Sci. **67**: 1478-1488.

Britt, J.H. 1986. Improving sow productivity through management during gestation, lactation and after weaning. J. Anim. Sci. **63:** 1288-1296.

Brooks, P.H and Cole, D.J.A. 1972. Studies in sow reproduction. 1. The effect of nutrition between weaning and remating on the reproductive performance of primiparous sows. Anim. Prod. **15:** 259-264.

Buonomo, F.C. and Baile, C.A. 1991. Influence of nutritional deprivation on insulin-like growth factor 1, somatotropin, and metabolic hormones in swine. J. Anim. Sci. **69**: 755-760.

Canadian Council on Animal Care. 1993. Guide to the care and use of experimental animals. Olfert, E.D., Cross, B.M. and McWilliam, A.A., eds. Vol. 1, 2nd Edition.

Clark, J.R., Komkov, A. and Tribble, L.F. 1986. Effects of parity, season, gonadotropin releasing hormone and altered suckling intensity on the interval to rebreeding in sows. Theriogenology. **26:** 299-308.

Clowes, E.J., Aherne, F.X. and Foxcroft, G.R. 1994. Effect of delayed breeding on the endocrinology and fecundity of sows. J. Anim. Sci. **72:** 283-291.

Clowes, E.J., Williams, I.H., Baracos, V.E., Pluske, J.R., Cegielski, A.C., Zak, L.J. and Aherne, F.X. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: II. Effect on nitrogen partitioning and skeletal muscle composition. J. Anim. Sci. **76:** 1154-1164.

Coffey, M.T., Diggs, B.G., Handlin, D.L., Knabe, D.A., Maxwell, Jr., C.V., Noland, P.R., Prince, T.J. and Gromwell, G.L. 1994. Effects of dietary energy during gestation and lactation on reproductive performance of sows: A cooperative study. J. Anim. Sci. **72**: 4-9.

Cole, D.J.H. 1982. Nutrition and reproduction. In: Control of Pig Reproduction. D.J.A. Cole and G.R. Foxcroft eds. Butterworth Society, London. Pages 602-619.

Coma, J., Zimmerman, D.R. and Carrion, D. 1996. Lysine requirement of the lactating sow determined by using plasma urea nitrogen as a rapid response criterion. J. Anim. Sci. **74:** 1056-1062.

Conley, A.J., Howard, H.J., Slanger, W.D. and Ford, J.J. 1994. Steroidogensis in the preovulatory porcine follicle. Biol. Reprod. **51:** 655-661.

Connor, M.L., Fulawka, D.L. and Onischuk, L. 1997. Alternative low-cost group housing for pregnant sows. Livestock Environment V, Volume I. Proceedings of the Fifth International Symposium. Bottcher, R.W. and Hoff, S.J. eds. American Society of Agricultural Engineers, St. Joseph, Michigan.

Cosgrove, J.R., Tilton, J.E., Hunter, M.G. and Foxcroft, G.R. 1992. Gonadotropin-independent mechanisms participate in ovarian responses to realimentation in feed-restricted prepubertal gilts. Biol. Reprod. **47**: 736-745.

Cox, N.M., Stuart, M.J., Althen, T.G., Bennet, W.A. and Miller, H.W. 1987. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J. Anim. Sci. **64**: 507-516.

Crighton, D.E. 1970. Induction of pregnancy during lactation in the sow. J. Reprod. Fert. **22:** 223-231.

De Rensis, F., Cosgrove, J.R. and Foxcroft, G.R. 1993a. Luteinizing hormone and prolactin responses to naloxone vary with stage of lactation in the sow. Biol. Reprod. **48:** 970-976.

De Rensis, F., Hunter, M.G. and Foxcroft, G.R. 1993b. Suckling-induced inhibition of luteinizing hormone secretion and follicular development in the early postpartum sow. Biol. Reprod. **48:** 964-969.

Ding, J. and Foxcroft, G.R. 1992. Follicular heterogeneity and oocyte maturation *in vitro* in pigs. Biol. Reprod. **47:** 648-655.

Ding, J. and Foxcroft, G.R. 1994. Conditioned media produced by follicular shells of different maturity affect maturation of pig oocytes. Biol. Reprod. **50:** 1377-1384.

Eales, J.G. 1988. The influence of nutritional state on thyroid function in various vertebrates. Amer. Zool. **28:** 351-361.

Edwards, S. 1982. The endocrinology of the post-partum sow. In: Control of Pig Reproduction. D.J.A. Cole and G.R. Foxcroft eds. Butterworth Society, London. Pages 439-458.

Ellendorff, F., Elsaesser, F., Parvizi, N. and Smidt, D. 1985. Endocrine interactions during lactational anestrus in sows. Curr. Top. Vet. Med. Anim. Sci. **31**: 145-153.

Elliot, J.I, King, G.J. and Robertson, H.A. 1980. Reproductive performance of the sow subsequent to weaning piglets at birth. Can. J. Anim. Sci. **60:** 65-71.

Evans, N.M., Evans, F.D., Maher, A., Friendship, R. and Hacker, R. R. 1994. Influence of light dark cycles on estradiol-17ß induced luteinizing hormone patterns of the prepubertal gilt. J. Anim. Sci. **72:** 1995-2000.

Flood, M.R. and Wiebold, J.L. 1988. Glucose metabolism by preimplantation pig embryos. J. Reprod. Fert. **84:** 7-12.

Fortune, J.E. 1994. Ovarian follicular growth and development in mammals. Biol. Reprod. **50:** 225-232.

Foxcroft, G.R. 1985. Hypothalamic-pituitary-ovarian responses to weaning. Retrospective evidence for the cause of lactational anestrus? Curr. Top. Vet. Med. Anim. Sci. **31:** 133-144.

Foxcroft, G.R. 1997. The challenge of breeding herd management: Making the best of a bad job. Proceedings. Manitoba Swine Seminar Committee. Volume 11. G. Wruck, ed. Pages 27-38.

Foxcroft, G.R., Aherne, F.X., Clowes, E.C., Miller, H., and Zak, L. 1995. Sow fertility: The role of suckling inhibition and metabolic status. Animal Science Research and Development: Moving Toward a New Century. M. Ivan, ed. Pages 377-393.

Foxcroft, G.R., Shaw, H.J., Hunter, M.G., Booth, P.J. and Lancaster, R.T. 1987. Relationships between luteinizing hormone, follicle-stimulating hormone and prolactin secretion and ovarian follicular development in the weaned sow. Biol. Reprod. **36:** 175-191.

Foxcroft, G.R. and van de Wiel, D.F.M. 1982. Endocrine control of the oestrous cycle. In: Control of Pig Reproduction. D.J.A. Cole and G.R. Foxcroft, eds. Butterworth Society, London. Pages 161-177

Giesemann, M.A., Peo, Jr., E.R., Lewis, A.J. and Hancock, J.D. 1989. Effect of exogenous thyroxine on the interval from weaning to estrus of the primiparous sow. J. Anim. Sci. **67**: 157-167.

Grinwich D.L., and McKay, R.M. 1985. Effects of reduced suckling on days to estrus, conception during lactation and embryo survival in sows. Theriogenology. **23:** 449-459.

Hafez, E.S.E. 1993. Reproduction in Farm Animals. 6th Ed. E.S.E. Hafez, ed. Lea and Febiger, Philadelphia.

Hayden, J.M., Williams, J.E. and Collier, R.J. 1993. Plasma growth hormone, insulin-like growth factor, insulin, and thyroid hormone association with body protein and fat accretion in steers undergoing compensatory gain after dietary energy restriction. J. Anim. Sci. **71:** 3327-3338.

Henderson, R. and Hughes, P.E. 1984. The effects of partial weaning, movement and boar contact on the subsequent reproductive performance of lactating sows. Anim. Prod. **39**: 131-135.

Howard, H.J. and Ford, J.J. 1992. Relationships among concentrations of steroids, inhibin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins during follicular development in weaned sows. Biol. Reprod. **47:** 193-201.

Howard, H.J. and Ford, J.J. 1994. Differential steroidal response of subpopulations of porcine granulosa cells to insulin-like growth factor-I (IGF-I) or IGF-I analogs. Biol. Reprod. **51:** 108-115.

I'Anson, H., Quint, E.H., Wood, R.I., England, B.G. and Foster, D.L. 1994. Adrenal axis and hypogonadotropism in the growth-restricted female lamb. Biol. Reprod. **50:** 137-143.

Jindal, R., Cosgrove, J.R., Aherne, F.X. and Foxcroft, G.R. 1996. Effect of nutrition on embryonal mortality in gilts: Association with progesterone. J. Anim. Sci. **74:** 620-624.

Johnston, L.J., Fogwell, R.L., Weldon, W.C., Ames, N.K., Ullrey, D.E. and Miller, E.R. 1989. Relatioship between body fat and postweaning interval to estrus in primiparous sows. J. Anim. Sci. **67:** 943-950.

Joseph, I. B. J. K., Currie, W. D. and Rawlings, N. C. 1992. Effects of time after ovariectomy, season and oestradiol on luteinizing hormone and follicle-stimulating hormone secretion in ovariectomized ewes. J. Reprod. Fert. **94:** 511-523.

Kemp, B. and Soede, N.M. 1986. Relationship of weaning-to-estrus interval to timing of ovulation and fertilization in sows. J. Anim. Sci. **74:** 944-949.

Kemp, B., Soede, N.M., Helmond, F.A. and Bosch, M.W. 1995. Effects of energy source in the diet on reproductive hormones and insulin during lactation and subsequent estrus in multiparous sows. J. Anim. Sci. **73**: 3022-3029.

Killen, J.H., Christenson, R.K. and Ford, J.J. 1992. Ovarian follicular changes after weaning in sows. J. Anim. Sci. **70:** 2801-2808.

Kingsbury, D. L. and Rawlings, N. C. 1993. Effect of exposure to a boar on the concentrations of LH, FSH, cortisol and oestradiol in prepubertal gilts. J. Reprod. Fert. **98:** 245-250.

Kirkwood, R.N., and Thacker, P.A. 1990. Failure of an induced ovulation during lactation to improve sow or litter performance. Can. J. Anim. Sci. **70**: 135-138.

Knabe, D.A., Brendemuhl, J.H., Chiba, L.I. and Dove, C.R. 1996. Supplemental lysine for sows nursing large litters. J. Anim. Sci. **74:** 1635-1640.

Knight, P.G., Howles, C.M. and Cunningham, F.J. 1986. Evidence that opioid peptides and dopamine participate in the suckling-induced release of prolactin in the ewe. Neuroendocrinology **44:** 29-35.

Koketsu, Y., Dial, G.D., Pettigrew, J.E., Marsh, W.E. and King, V.L. 1996. Influence of imposed feed intake patterns during lactation on reproductive performance and on circulating levels of glucose, insulin, and luteinizing hormone in primiparous sows. J. Anim. Sci. **74:** 1036-1046.

Li, P.S. 1989. Catecholamine inhibition of luteinizing hormone secretion in isolated pig pituitary cells. Biol. Reprod. **40:** 914-919.

Mahan, D.C. 1993. Effect of weight, split-weaning, and nursery feeding programs on performance responses of pigs to 105 kilograms body weight and subsequent effects on sow rebreeding interval. J. Anim. Sci. **71:** 1991-1995.

Maruo, T., Hayashi, M., Matsuo, H., Ueda, Y., Morikawa, H. and Mochizuki, M. 1988. Comparison of the facilitative roles of insulin and insulin-like growth factor I in the functional differentiation of granulosa cells: *In vitro* studies with the porcine model. Acta Endocrinological (Copenh). **117:** 230-240.

Matamoros, I.A., Cox, N.M. and Moore, A.B. 1991. Effects of exogenous insulin and body condition on metabolic hormones and gonadotropin-induced follicular development in prepubertal gilts. J. Anim. Sci. **69:** 2081-2091.

Mattioli, M., and Seren, E. 1985. Effects of bromocriptine treatment during lactational anestrus in pigs. Curr. Top. Vet. Med. Anim. Sci. **31:** 165-178

Meurer, K.A., Cox, N. M., Matamoros, I.A. and Tubbs, R.C. 1991. Decreased follicular steroids and insulin-like growth factor-1 and increased atresia in diabetic gilts during follicular growth stimulated with PMSG. J. Reprod. Fert. **91**: 187-196.

National Research Council. 1988. Nutrient requirements of swine. 9th rev. ed. National Academy Press, Washington, D. C.

Newton, E.A., Stevenson, J.S., Minton, J.E. and Davis, D.L. 1987. Endocrine changes before and after weaning in response to boar exposure and altered suckling in sows. J. Reprod. Fert. **81:** 599-609.

Nichol, R., Hunter, R.H.F., Gardner, D.K., Leese, H.J. and Cooke, G.M. 1992. Concentrations of energy substrates in oviductal fluid and blood plasma of pigs during the peri-ovulatory period. J. Reprod. Fert. **96:** 699-707.

Palmer, W.M., Teague, H.S. and Venzke, W.G. 1965a. Macroscopic observations on the reproductive tract of the sow during lactation and early postweaning. J. Anim. Sci. **24:** 541-545.

Palmer, W.M., Teague, H.S. and Venzke, W.G. 1965b. Histological changes in the reproductive tract of the sow during lactation and early postweaning. J. Anim. Sci. **24:** 1117-1125.

Parvizi, N., Elsaesser, F., Smidt, D. and Ellendorff, F. 1976. Plasma luteinizing hormone and progesterone in the adult female pig during the oestrous cycle, late pregnancy and lactation, and after ovariectomy and pentobarbitone treatment. J. Endocrinology. **69:** 193-203.

Pharazyn, A., Den Hartog, L.A., Foxcroft, G.R. and Aherne, F.X. 1991. Dietary energy and protein intake, plasma progesterone and embryo survival in early pregnancy in the gilt. Can. J. Anim. Sci. **71:** 949-952.

Pluske, J.R., Williams, I.H., Zak, L.J., Clowes, E.J., Cegielski, A.C. and Aherne, F.X. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: III. Milk production and pig growth. J. Anim. Sci. **76:** 1165-1171.

Prunier, A., Martin, C., Mounier, A.M. and Bonneau, M. 1993. Metabolic and endocrine changes associated with undernutrition in the peripubertal gilt. J. Anim. Sci. **71:** 1887-1894.

Recommended Code of Practice For the Care and Handling of Farm Animals: Pigs. 1993. M.L. Connor, ed. Agriculture and Agri-Food Canada Publication.

Rivier, C. and Rivest, S. 1991. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. Biol. Reprod. **45:** 523-532.

Rivier, C., Rivier, J. and Vale, W. 1986. Stress-induced inhibition of reproductive functions: Role of endogenous corticotropin-releasing factor. Science. **321:** 607-609.

Rondeel, J.M.M., Heide, R., de Greef, W.J., van Toor, H., van Haasteren, G.A.C., Klootwijk, W. and Visser, T.J. 1992. Effect of starvation and subsequent refeeding on thyroid function and release of hypothalamic thyrotropin-releasing hormone. Neuroendocrinology. **56:** 348-353.

Rutter, L.M. and Manns, J.G. 1991. Insulin-like growth factor I in follicular development and function in postpartum beef cows. J. Anim. Sci. **69:** 1140-1146

Samson, W.K., Lumpkin, M.D. and McCann, S.M. 1986. Evidence for a physiological role for oxytocin in the control of prolactin secretion. Endocrinology. **119:** 554-560.

Sesti, L.A.C. and Britt, J.H. 1993a. Influence of stage of lactation, exogenous luteinizing hormone releasing hormone, and suckling on estrus, positive feedback of luteinizing hormone and ovulation in sows treated with estrogen. J. Anim. Sci. **71**: 989-998.

Sesti, L.A.C. and Britt, J.H. 1993b. Agonist-induced release of gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone and their associations with basal secretion of luteinizing hormone and follicle-stimulating hormone throughout lactation in sows. Biol. Reprod. **49:** 332-339.

Simoneau, C., Estrada, R., Matton, P., Roy, P.E. and Dufour, J.J. 1988. Effects of litter size and light regimen imposed during lactation on ovarian follicular population at weaning in third-parity sows. J. Anim. Sci. **66:** 2606-2613.

Soede, N.M., Helmond, F.A. and Kemp, B. 1994. Periovulatory profiles of oestradiol, LH and progesterone in relation to oestrus and embryo mortality in multiparous sows using transrectal ultrasonography to detect ovulation. J. Reprod. Fert. **101:** 633-641.

Statistical Analysis System Institute Inc. 1986. SAS User's Guide. Statistics 1986 ed. SAS Institute Inc., Cary, NC.

Stevenson, J.S. and Britt J.H. 1981. Interval to estrus in sows and performance of pigs after alteration of litter size during late lactation. J. Anim. Sci. **53**: 177-181.

Stevenson, J.S., Cox, N.M. and Britt, J.H. 1981. Role of the ovary in controlling luteinizing hormone, follicle-stimulating hormone, and prolactin secretion during and after lactation in pigs. Biol. Reprod. **24:** 341-353.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A. and Johnston, L.J. 1992a. Characterization of luteinizing hormone secretion in the primiparous, lactating sow: Relationship to blood metabolites and return-to-estrus interval. J. Anim. Sci. **70**: 2195-2201.

Tokach, M.D., Pettigrew, J.E., Dial, G.D., Wheaton, J.E., Crooker, B.A. and Koketsu, Y. 1992b. Influence of glucose infusions on luteinizing hormone secretion in the energy-restricted, primiparous, lactating sow. J. Anim. Sci. **70:** 2202-2206.

van de Wiel, D.F.M., Booman, P., Willemse, A.H. and Bevers, M.M. 1985. Relevance of prolactin to lactational and post-weaning anestrus in the pig. Curr. Top. Vet. Med. Anim. Sci. **31:** 155-163.

van Haasteren, G.A.C., Linkels, E., van Toor, H., Klootwijk, W., Kaptein, E., de Jong, F.H., Reymond, M.J., Visser, T.J. and de Greef, W.J. 1996. Effects of long term food reduction on the hypothalamus-pituitary-thyroid axis in male and female rats. J. Endocrinology. **150:** 169-178.

Varley, M.A. 1982. The time of weaning and its effects on reproductive function. In: Control of Reproduction, D.J.A. Cole and G.R. Foxcroft, eds. Butterworth Society, London. Pages 459-478.

Varley, M.A. 1994. Stress and reproduction in the pig. In: Livestock Production in the 21st Century. Priorities and Research Needs, P.A. Thacker, ed. University of Saskatchewan, Saskatoon, Saskatchewan, Canada. Pages 147-157

Varley, M.A. and Atkinson, T. 1985. Weaning at birth: The effect on the reproduction of the sow. Anim. Prod. **41:** 375-382.

Varley, M.A., Atkinson, T. and Ross, L.N. 1981. The effect of lactation length on circulating concentrations of progesterone and oestradiol in the early weaned sow. Theriogenology. **16:** 179-184.

Varley, M.A. and Cole, D.J.A. 1976. Studies in sow reproduction. The effect of lactation length of the sow on the subsequent embryonic development. Anim. Prod. **22:** 79-85.

Varley, M.A. and Foxcroft, G.R. 1990. Endocrinology of the lactating and weaned sow. J. Reprod. Fert. *Suppl.* **40**: 47-61.

Varley, M.A., Peaker, R.E. and Atkinson, T. 1984. Effect of lactation length of the sow on plasma progesterone, oestradiol 17-ß and embryonic survival. Anim. Prod. **38:** 113-119.

Veldhuis, J.D., Furlanetto, R.W. with the technical assistance of **Juchter, D, Garmey, J. and Veldhuis, P. 1985.** Trophic actions of human somatomedin C/insulin-like growth factor I on ovarian cells: *In vitro* studies with swine granulosa cells. Endocrinology. **116:** 1235-1242.

Weldon, W.C., Lewis, A.J., Louis, G.F., Kovar, J.L., Giesemann, M.A. and Miller, P.S. 1994a Postpartum hypophagia in primiparous sows: I. Effects of gestation feeding level on feed intake, feeding behaviour, and plasma metabolite concentrations during lactation. J. Anim. Sci. **72:** 387-394.

Weldon, W.C., Lewis, A.J., Louis, G.F., Kovar, J.L. and Miller, P.S. 1994b. Postpartum hypophagia in primiparous sows: II. Effects of feeding level during gestation and exogenous insulin on lactation feed intake, glucose tolerance, epinephrine-stimulated release of nonesterified fatty acids and glucose. J. Anim. Sci. **72:** 395-403.

Whitley, N.C., Quirk-Thomas, M.N., Skeleton, J.O., Moore, A.B., Purvis, J., Qui, Y. and Cox, N.M. 1998. Influence of insulin on follicular development and the intrafollicular insulin-like growth factor I (IGF-I) system in sows after weaning. J. Reprod. Fert. **112:** 175-184.

Whittemore, C. T. and Yang, H. 1989. Physical and chemical composition of the body of breeding sows with differing body subcutaneous fat depth at parturition, differing nutrition during lactation and differing litter size. Anim. Prod. **48:** 203-212.

Xu, Y.X., Kirkwood, R.N., Rajkumar, K. and Thacker, P.A. 1995. Effects of metabolic hormones and growth factors on forskolin and dibutyryl adenosine 3', 5'-cyclic monophosphate induced steroidogenic responses by porcine granulosa cells *in vitro*. Can. J. Anim. Sci. **75:** 85-91.

Xue, J.L., Dial, G.D., Marsh, W.E., Davies, P.R. and Momont, H.W. 1993. Influence of lactation length on sow productivity. Livestock Prod. Sci. **34**: 253-263.

Yang, H., Eastham, P.R., Phillips, P. and Whittemore, C.T. 1989. Reproductive performance, body weight and body condition of breeding sows with differing body fatness at parturition, differing nutrition during lactation, and differing litter size. Anim. Prod. **48:** 181-201.

Zak, L.J., Cosgrove, J.R., Aherne, F.X. and Foxcroft, G.R. 1997b. Pattern of feed intake and associated metabolic and endocrine changes differentially affect postweaning fertility in primiparous lactating sows. J. Anim. Sci. **75**: 208-216.

Zak, L.J., Williams, I.H., Foxcroft, G.R., Pluske, J.R., Cegielski, A.C., Clowes, E.J. and Aherne, F.X. 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: I. Associated endocrine changes and postweaning reproductive performance. J. Anim. Sci. **76:** 1145-1153.

Zak, L.J., Xu, X., Hardin, R.T. and Foxcroft, G.R. 1997a. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J. Reprod. Fert. **110**: 99-106

APPENDIX

TABLE A1. Mean backfat (mm) and weight (kg) in first and fifth parity sows from pen entry to termination of the study at 27-35 days postbreeding

	Mean backfat (mm)			Mean weight (kg)		
Day	**1st Parity**	**5th Parity**	**P=**	**1st Parity**	**5th Parity**	**P=**
Pen entry	16.00 ± 1.61	17.88 ± 1.71	0.44	210.34 ± 5.53	297.19 ± 5.87	0.0001
112 g	16.67 ± 1.59	18.21 ± 1.80	0.53	210.26 ± 5.90	306.70 ± 6.69	0.0001
3 pp	15.61 ± 1.52	16.50 ± 1.61	0.70	181.07 ± 6.47	282.95 ± 6.86	0.0001
7 pp	14.78 ± 1.37	15.94 ± 1.45	0.57	172.23 ± 5.33	279.05 ± 5.65	0.0001
14 pp	13.78 ± 1.45	16.43 ± 1.65	0.25	167.34 ± 5.64	279.10 ± 6.39	0.0001
5 pw	12.28 ± 1.43	16.07 ± 1.62	0.11	156.34 ± 5.05	259.09 ± 5.73	0.0001
10 pb	13.06 ± 1.38	16.69 ± 1.46	0.09	175.94 ± 5.75	271.20 ± 6.10	0.0001
27-35 pb	15.67 ± 1.62	18.75 ± 1.72	0.22	192.66 ± 5.55	279.61 ± 5.89	0.0001

values are LS means ± SEM

TABLE A2. Mean total body lipid (kg)[1] and body protein (kg)[2] in first and fifth parity sows from pen entry to termination of the study at 27-35 days postbreeding

	Mean body lipid (kg)			Mean body protein (kg)		
Day	**1st Parity**	**5th Parity**	**P=**	**1st Parity**	**5th Parity**	**P=**
Pen entry	46.40 ± 3.22	66.98 ± 3.41	0.0005	34.15 ± 0.87	50.24 ± 0.93	0.0001
112 g	47.37 ± 3.26	69.44 ± 3.72	0.0006	33.98 ± 0.93	51.97 ± 1.05	0.0001
3 pp	39.83 ± 3.27	62.03 ± 3.47	0.0003	28.67 ± 1.04	47.83 ± 1.11	0.0001
7 pp	36.78 ± 2.94	60.39 ± 3.12	0.0001	27.17 ± 0.81	47.22 ± 0.86	0.0001
14 pp	34.30 ± 3.09	61.13 ± 3.50	0.0001	26.47 ± 0.87	47.12 ± 0.99	0.0001
5 pw	29.82 ± 2.94	56.50 ± 3.33	0.0001	24.71 ± 0.77	43.39 ± 0.87	0.0001
10 pb	34.99 ± 3.20	59.60 ± 3.63	0.0002	28.26 ± 0.88	45.47 ± 1.00	0.0001
27-35 pb	42.28 ± 3.44	64.25 ± 3.90	0.0009	30.86 ± 0.85	46.59 ± 0.96	0.0001

values are LS means ± SEM

[1,2] based on the prediction equations of Whittemore and Yang (1989)

TABLE A3. Degree of significance for split plot effects in blood glucose

Day	Parity	Hour	Parity*Time
110 g	NS	NS	NS
3 pp	NS	***	NS
13 pp	NS	**	***
4 pw	NS	***	**
E-1	NS	NS	NS
E	NS	NS	NS
2 pb	NS	NS	NS
5 pb	NS	NS	NS

** 0.001 P< 0.01
*** P <0.001

TABLE A4. Degree of significance for split plot effects in BUN

Day	Parity	Hour	Parity*Time
110 g	NS	**	NS
3 pp	*	*	NS
13 pp	*	***	NS

* 0.01 P< 0.05
** 0.001 P< 0.01
*** P <0.001

TABLE A5. Degree of significance for split plot effects in P4

Day	Parity	Hour	Parity*Time
110 g	NS	NS	NS
3 pp	NS	**	NS
13 pp	NS	NS	NS
4 pw	NS	NS	NS
E-1	NS	NS	NS
E	NS	NS	NS
2 pb	NS	NS	NS
5 pb	NS	NS	NS

** 0.001 P< 0.01

TABLE A6. Degree of significance for split plot effects in E2

Day	Parity	Hour	Parity*Time
110 g	*	*	NS
3 pp	NS	***	NS
13 pp	NS	NS	NS
4 pw	NS	***	*
E-1	NS	NS	NS
E	NS	NS	NS
2 pb	NS	NS	NS
5 pb	NS	NS	NS

* 0.01 P< 0.05
*** P <0.001

TABLE A7. Degree of significance for split plot effects in LH

Day	Parity	Time	Parity*Time
110 g	NS	NS	NS
3 pp	NS	NS	NS
13 pp	NS	NS	NS
4 pw	NS	NS	NS
E-1	NS	NS	NS
E	NS	NS	NS
2 pb	NS	*	NS
5 pb	NS	*	NS

* 0.01 P< 0.05

TABLE A8. Degree of significance for split plot effects in FSH

Day	Parity	Hour	Parity*Time
Day 110 g	NS	NS	NS
Day 3 pp	NS	NS	NS
Day 13 pp	*	NS	NS
Day 4 pw	NS	NS	NS
E-1	NS	NS	NS
E	*	NS	NS
Day 2 pb	*	NS	NS
Day 5 pb	NS	NS	NS

* 0.01 P< 0.05

TABLE A9. Degree of significance for split plot effects in T_4

Day	Parity	Hour	Parity*Time
Day 110 g	NS	NS	NS
Day 3 pp	NS	NS	NS
Day 13 pp	NS	NS	NS
Day 4 pw	NS	*	NS
E-1	NS	NS	NS
E	NS	NS	NS
Day 2 pb	NS	NS	NS
Day 5 pb	NS	NS	NS

* 0.01 P< 0.05

TABLE A10. Degree of significance for split plot effects in T_3/T_4 ratio

Day	Parity	Hour	Parity*Time
Day 110 g	NS	NS	NS
Day 3 pp	NS	NS	NS
Day 13 pp	NS	NS	NS
Day 4 pw	NS	NS	NS
E-1	NS	NS	NS
E	NS	NS	NS
Day 2 pb	NS	*	NS
Day 5 pb	NS	NS	NS

* 0.01 P< 0.05

TABLE A11. Degree of significance for split plot effects in IGF-I

Day	Parity	Hour	Parity*Time
Day 110 g	NS	NS	NS
Day 3 pp	NS	*	*
Day 13 pp	NS	NS	NS
Day 4 pw	NS	NS	NS
E-1	NS	NS	NS
E	*	NS	NS
Day 2 pb	NS	NS	NS
Day 5 pb	NS	NS	NS

* 0.01 P< 0.05

TABLE A12. Degree of significance for split plot effects in insulin

Day	Parity	Hour	Parity*Time
Day 110 g	NS	***	NS
Day 3 pp	**	NS	NS
Day 13 pp	*	NS	NS
Day 4 pw	NS	*	NS
E-1	*	*	NS
E	NS	NS	NS
Day 2 pb	NS	NS	NS
Day 5 pb	NS	*	NS

* 0.01 P< 0.05
** 0.001 P< 0.01
*** P <0.001

TABLE A13. Degree of significance for split plot effects in cortisol

Day	Parity	Hour	Parity*Time
110 g	NS	*	NS
3 pp	NS	NS	NS
13 pp	NS	*	NS
4 pw	NS	NS	NS
E-1	NS	NS	NS
E	NS	NS	NS
2 pb	NS	NS	NS
5 pb	NS	NS	NS

* 0.01 P< 0.05

Multivariate Correlation Analysis

Day 110 g

5th Parity

IGF-I and T_3 (r=-0.423, P=0.003)

T_4 and IGF-I (r=0.292, P=0.044)

cortisol and IGF-I (r=0.283, P=0.05)

1st Parity

LH and insulin (r=0.332, P=0.003)

insulin and IGF-I (r=-0.266, P=0.02)

T_4 and T_3 (r=0.327, P=0.004)

P4 and T_3 (r=0.246, P=0.032)

cortisol and P4 (r=0.372, P=0.0009)

Trend: P4 and LH (r=0.209, P=0.07)

Day 3 PP

5th Parity

FSH and E2 (r=-0.503, P=0.02)

T_4 and LH (r=0.433, P=0.05)

T_4 and T_3 (r=0.909, P=0.0001)

FSH and P4 (r=-0.459, P=0.037)

T_4 and cortisol (r=0.485, P=0.026)

cortisol and P4 (r=0.54, P=0.012)

Trend: cortisol and LH (r=0.411, P=0.064)

cortisol and E2 (r=0.404, P=0.07)

1st Parity

LH and T_3 (r=-0.556, P=0.003)

E2 and T_3 (r=-0.541, P=0.004)

cortisol and IGF-I (r=0.537, P=0.005)

Day 13 PP

5th Parity

LH and FSH (r=0.616, P=0.0002)

E2 and IGF-I (r=0.366, P=0.043)

P4 and LH (r=0.354, P=0.05)

cortisol and T_4 (r=0.357, P=0.049)

cortisol and P4 (r=0.591, P=0.0005)

Trend: cortisol and LH (r=0.326, P=0.074)

1st Parity

LH and FSH (r=0.592, P=0.006)

E2 and IGF-I (r=-0.468, P=0.038)

insulin and T_3 (r=0.496, P=0.026)

T_4 and T_3 (r=0.496, P=0.026)

cortisol and IGF-I (r=0.731, P=0.0003)

IGF-I and P4 (r=0.628, P=0.003)

T_4 and P4 (r=-0.559, P=0.01)

cortisol and P4 (r=0.639, P=0.002)

4 days pw

5th Parity

T_4 and LH (r=0.498, P=0.022)

T_4 and IGF-I (r=0.549, P=0.01)

P4 and E2 (r=0.676, P=0.0008)

P4 and insulin (r=0.568, P=0.007)

P4 and IGF-I (r=0.479, P=0.03)

cortisol and IGF-I (r=0.487, P=0.03)

T_4 and cortisol (r=0.427, P=0.053)

T_4 and P4 (r=0.451, P=0.04)

cortisol and P4 (r=0.43, P=0.052)

1st Parity

T_3 and E2 (r=0.387, P=0.038)

cortisol and IGF-I (r=0.374, P=0.046)

cortisol and T_3 (r=0.395, P=0.034)

cortisol and T_4 (r=0.699, P=0.0001)

cortisol and P4 (r=0.901, P=0.0001)

P4 and T_4 (r=0.501, P=0.006)

P4 and T_3 (r=0.433, P=0.019)

IGF-I and T_4 (r=0.579, P=0.001)

Trend: E2 and cortisol (r=0.353, P=0.06)

Day before estrus

5th Parity

FSH and IGF-I (r=-0.797, P=0.003)

T_4 and FSH (r=-0.672, P=0.024)

T_4 and IGF-I (r=0.599, P=0.052)

cortisol and insulin (r=-0.709, P=0.015)

Trend: T_3 and E2 (r=0.591, P=0.056)

1st Parity

IGF-I and T_3 (r=-0.745, P=0.055)

Trend: T_4 and P4 (r=0.718, P=0.07)

Estrus

5th Parity

No correlations existed.

1st Parity

FSH and insulin (r=0.531, P=0.042)

T_4 and cortisol (r=0.642, P=0.01)

Trend: cortisol and LH (r=0.477, P=0.072)

2 days pb

5th Parity

LH and IGF-I (r=0.99, P=0.01)

FSH and insulin (r=0.974, P=0.03)

T_4 and LH (r=0.954, P=0.05)

T_4 and IGF-I (r=0.95, P=0.05)

cortisol and LH (r=0.973, P=0.027)

cortisol and IGF-I (r=0.979, P=0.021)

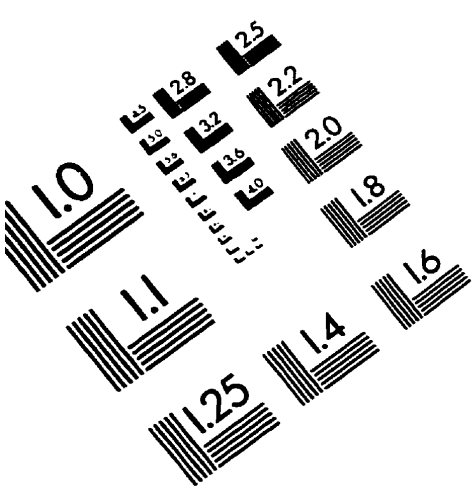
1st Parity

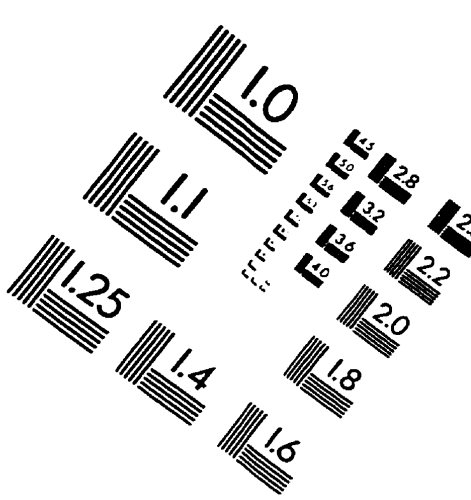
E2 and insulin (r=-0.885, P=0.046)

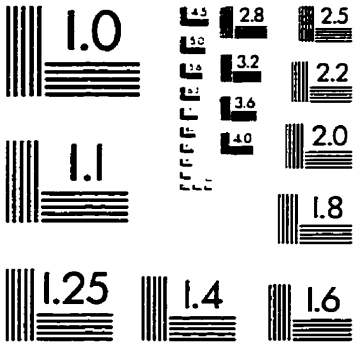
cortisol and LH (r=0.971, P=0.006)

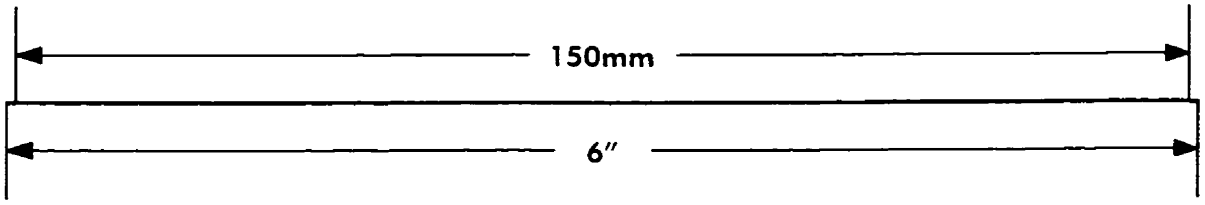
P4 and LH (r=0.93, P=0.022)

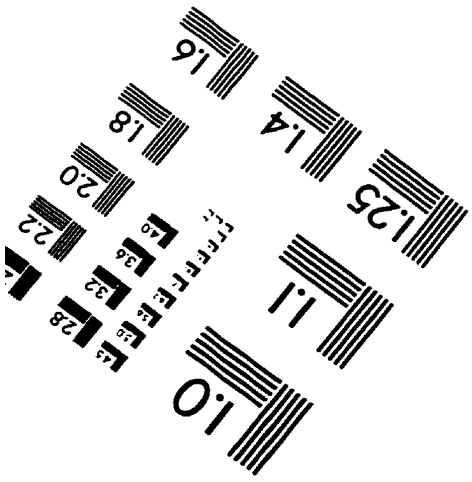
IMAGE EVALUATION
TEST TARGET (QA-3)

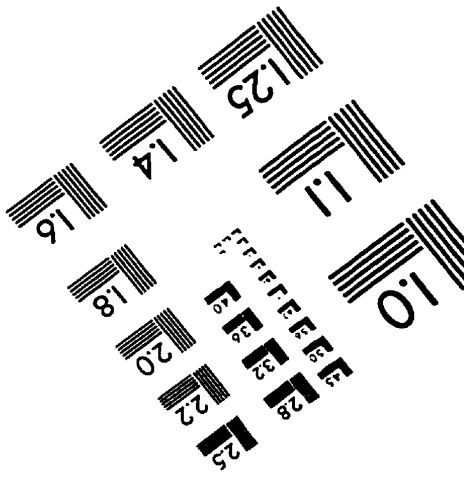












APPLIED IMAGE . Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989