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PHYSIOLOGICAL MECHANISMS MEDIATING NUTRITION-REPRODUCTION INTERACTIONS IN THE LACTATING AND WEANED PRIMIPAROUS SOW

by

LOUISA JANE ZAK 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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PHYSIOLOGICAL MECHANISMS MEDIATING NUTRITION-REPRODUCTION INTERACTIONS IN THE LACTATING AND WEANED, PRIMIPAROUS SOW

submitted by

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in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY in ANIMAL PHYSIOLOGY

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ABSTRACT

The effects of cumulative dietary restriction during lactation have been characterized in the lactating primiparous sow paradigm, however the effects of differential patterns of catabolism during lactation on endocrine and reproductive status had not previously been investigated. The first experiment was designed to address this question. All litters were standardized to 6 piglets, and during a 28 day lactation sows were either fed to appetite (AA; n = 9) throughout or restricted to 50 % from day 1 to 21 (RA; n = 9) or from day 22 to 28 (AR; n = 8). Periods of feed restriction suppressed plasma LH, insulin and IGF-1, when compared feeding to appetite. The time to return to estrus was increased and ovulation rate decreased in AR and RA sows, although embryo survival was lower in AR sows only.

The contribution of follicle and oocyte maturation to embryonic survival was determined in AR (n = 10) and RA (n = 11) sows in a second experiment using in vitro maturation of oocyte/cumulus complexes (OCC) recovered from experimental sows, or incubation of prepubertal OCC with pooled follicular fluid from experimental sows. Sows were slaughtered 38 h before estimated time of estrus. Although plasma estradiol was not different, RA sows had more large preovulatory follicles that AR. More oocytes retrieved from RA sows, or incubated with follicular fluid from RA sows, had matured to metaphase II, although cumulus cell expansion was not different between treatments. Differences in the maturation of the follicle and oocyte in the pre-LH surge period may therefore contribute to nutritional effects on embryonic survival.

The extent to which making sows anabolic during a 28 day lactation could ameliorate catabolic effects on LH secretion and on fertility were investigated by superalimenting sows (SA; n = 8) to 125 % of ad libitum intakes (AL; n = 12) in a third experiment. Although SA

increased and AL decreased body weight during lactation, LH secretion, and plasma insulin or IGF-1 concentrations before or after weaning were not different. Weaning to estrus interval, ovulation rate and embryo survival were also similar among treatments. These results demonstrate that making sows anabolic during lactation did not overcome the inherent suppression of LH by the suckling stimulus nor improve fertility after weaning.

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This document represents not only a number of years of study, but is also a culmination of a lifetime of personal development, and I was fortunate to have been raised in an environment in which inquisitiveness and independence of mind was encouraged, and to Mum and Dad I thank them.

To Mum, Dad and Greg I dedicate this manuscript.

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CHAPTER 1
INTRODUCTION

The success of a population depends on its ability to attain and maintain sexually mature individuals. Puberty and ovulation are readily suppressed by food shortage or increased energy expenditure. The cessation of reproductive processes during metabolically or energetically adverse situations is an evolutionary survival strategy and relates to the females need to reproduce within the context of current environmental conditions (Bronson et al., 1989). For females the energetic priority for reproduction lies below that of maintenance energy requirements. Rodents and other small mammals have evolved a unique reproductive strategy because of their high surface area to volume ratio, relatively small fat stores and relatively large metabolic energy requirements. A female rodent spends the majority of her time in a thermally insulated nest, and thus minimizes the metabolic energy requirements for thermogenesis. She will only leave her nest to forage for food if the nutritional reward for foraging is sufficient, or in excess, of the maintenance energy requirement. However, if her energy requirements for thermogenesis increase, for example when the ambient temperature decreases she may then decide that her best survival strategy is to stay inside the nest. If her energy expenditure for foraging outweighs that of energy intake at a time when she has already committed herself to reproductive activity, she may abort the litter or cannibalize her offspring in order to conserve energy.

The evolutionary strategy employed by large mammals is somewhat different to that observed in rodents. Primarily the differences exist because large mammals have a lower surface area to volume ratio, lower metabolic rate and relatively more nutrient storage capacity. Thus their energetic needs for thermogenesis can be buffered by mobilization of fat. glycogen and protein. Once committed to gestation or lactation it is extremely unlikely that they will terminate the pregnancy or cease to lactate in the face of an energetic challenge. Instead they will mobilize body reserves to supply the gestational or lactational energy requirements.

The overall evolutionary strategies between the opportunistic breeders (e.g. rodents) and of the non-opportunistic breeders (e.g. large mammals) are very different. The daily acute energetic challenges faced by opportunistic breeders has resulted in an evolutionary strategy in which the survival of the dam is of absolute importance. Although she may terminate a pregnancy or leave her litter during metabolically adverse situations, she is still able to reproduce the moment environmental conditions are once again favorable. Non-opportunistic breeders on the other hand, have evolved an evolutionary strategy in which the transfer of genetic material to the next generation is of prime importance. Once committed to reproduction the dam may mobilize vast amounts of body reserves to support the energy requirements of gestation or lactation, to such an extent that she may not be able to rebreed at the next available opportunity.

The energetics of the lactating sow are confounded by the fact that young sows still have a high lean growth potential. Their lean tissue deposition is actually attenuated during lactation when compared to their estimated potential lean body mass, as discussed in a recent review by our research group (Foxcroft et al., 1995). Everts (1994) demonstrated that sows

need to attain an ideal protein mass of 35 kg in order to achieve maximal reproductive success. However, during lactation most primiparous sows mobilize both fat and protein reserves to supply precursors for lactogenesis (Baidoo et al., 1992; Mullan and Williams. 1989) and as such the lean growth potential is greatly attenuated. However, once weaned. the sow will continue to utilize fat as a metabolic fuel which enables protein to be accrued. as demonstrated by elevated glycerol and IGF-1 concentrations after weaning (Clowes et al., 1994).

The energetic demands of lactation are frequently not met in the primiparous sow because feed consumption is insufficient to meet the demands of lactation and she enters a negative energy or nitrogen balance (Aherne and Williams, 1992; Noblet et al., 1990). Hypophagia in early lactation is characterized by a reduced glucose tolerance and insulin resistance (Weldon et al., 1994) which may be a residual effect of preparturient diabetogenesis (Schaeffer et al., 1991). If lactation energetics are further confounded by an imposed restriction in feed intake reproduction can be severely compromised. This may be characterized by an extended weaning to estrus interval (Reese et al., 1982; King and Williams, 1984; King and Dunkin, 1986; Brendemuhl et al., 1987; King and Martin, 1987: Kirkwood et al., 1987; Prunier et al., 1993) and decreased embryo survival after day 25 (King and Williams, 1984; Kirkwood et al., 1987; Baidoo et al., 1992). A decreased ovulation rate associated with restricted feed intake during lactation has been reported by King and Williams (1984). However, the majority of studies have not reported a reduction in ovulation rate. possibly because ovulation rate may be confounded by the extended weaning to estrus interval which is often in excess of 5 to 12 days (Koketsu et al., 1996).

The production of mature, viable gametes is a multifaceted interaction between the central synthesis and release of gonadotropin (luteinizing hormone (LH) and follicle stimulating hormone (FSH)) and the ovarian synthesis of steroidal and non steroidal products. Once mature gametes have been released from the ovarian follicles the fallopian tube now becomes the host for the oocyte and subsequently the conceptus. Finally the embryo will enter into the uterus where it will develop into a viable progeny. It is plausible to imagine therefore, that at any one of these stages changes in the milieu of the conceptus by metabolic status may impact on its developmental competence.

In a series of experiments reported in this thesis the effects of nutritionally induced changes in metabolic state were used to elucidate the mechanisms mediating these effects on reproductive performance. In Chapter 1, literature pertinent to the experimental hypotheses tested in subsequent chapters is reviewed . The first section of the literature review addresses the current literature pertaining to the control of metabolic status and discusses some of the models utilized to determine these factors. In the second part, information on the neuroendocrine regulation of LH secretion and in particular the possible control of LH by factors that may mediate alterations in metabolic state are addressed. The third section reviews the control of folliculogenesis, in particular the non steroidal factors which may be involved in the local regulation of ovarian function in different nutritional states. The fourth section reviews the regulation of oocyte maturation and factors affecting embryo survival, again emphasizing their regulation by non steroidal factors.

Experiment 1 (Chapter 3) addressed the hypothesis that different patterns of feed

intake, and associated differences in metabolic state during the last week of lactation, will affect LH secretion and subsequent reproductive performance. The results demonstrated that inducing sows to become more catabolic during lactation, by feed restriction, increased the weaning to estrus interval and decreased ovulation rate when compared to sows fed to appetite throughout. Interestingly, sows induced to become relatively more catabolic during the last week of lactation also had poor embryo survival. Thus using this experimental paradigm, we were able to differentially affect ovulation rate and embryo survival, and this allowed us to evaluate the potential role of follicle and oocyte maturational state in determining embryo survival in Experiment 2 (Chapter 4).

Experiment 3 (Chapter 5) addressed the hypothesis that inducing sows to become anabolic during lactation, would improve their reproductive performance above that of sows either fed ad libitum or feed restricted, and secondly to observe the degree to which anabolism during lactation could ameliorate the suckling induced suppression of LH.

The experiments presented in Chapter 3, 4, and 5 are all extended versions of papers submitted to peer reviewed journals. A paper based on Chapter 2 has been accepted by 'Journal of Animal Science', a paper based on Chapter 3 has been submitted to 'Journal of Reproduction and Fertility', and a paper based on Chapter 4 has been submitted to 'Journal of Animal Science'.

CHAPTER 2
LITERATURE REVIEW

2.1 Nutritional model development

By decreasing energy availability below that needed for optimal growth rate, puberty can be delayed (Bronson et al., 1987; Foster et al., 1989) or in the mature animal the ensuing negative energy balance will result in cessation of ovarian cyclicity (Armstrong et al., 1986). In both situations reproductive function can be restored by increasing energy intake or reducing energy expenditure. These findings suggest that low energy availability either as a result of increased energy expenditure or reduced energy intake, will lead to a physiological situation that will result in suppression of reproductive function. The mechanisms mediating these changes in reproduction have been studied in conditions of both chronic and acute feed restriction. The chronically feed restricted, ovariectomized, prepubertal lamb (Foster et al., 1989) maintained near weaning weight for several weeks, has been used as a model to study the acute increase in LH secretion in response to feed ingestion. Chronic feed restriction, for 30 to 50 days, in the postpubertal gilt resulted in suppressed LH secretion and a lack ovulatory follicle development (Britt et al., 1988). Interestingly although continued feed restriction plus exogenous GnRH therapy caused a return to ovarian cyclicity within 6 days, it took two weeks of realimentation to increase LH secretion and a further 4 weeks to reinitiate ovarian development. The difference in the follicular response time in the realimented versus GnRH treated gilts indicated that a considerable lag was apparent in the emergence of the metabolic signals controlling the increase in GnRH secretion.

Acute alterations in metabolic state can significantly alter the release of LH. In the male rhesus monkey a 24-hour fast will result in total suppression of LH release, which becomes evident after 4-6 hours, whereas realimentation increased LH secretion within 2 hours (Cameron and Nosbisch, 1991). Thus during the acute nutritional challenge, the metabolic cues which mediate the inhibition of LH secretion are already apparent. It is probable however, that periods of prolonged fasting or feed restriction also involve other more chronic alterations in metabolic status for example IGF-1 or cortisol which may also impact on the control of LH secretion.

Comparable acute nutritional effects have been reported in swine. The prepubertal gilt model described by Booth et al. (1994) was designed to investigate the effects of dietary manipulation in two groups of littermate gilts. All gilts were allowed to gain 10 kg in body weight to achieve a final body weight of 85 kg, however the dynamics of weight gain were quite different between the groups. One group was fed to appetite (85A) until they weighed 85 kg and then fed a maintenance energy ration (85AR) until slaughter. The other group were fed to maintenance (75R) for a period then fed to appetite until they had also achieved 85 kg (85RA) in body weight. Although gilts achieved the same weight, and their body composition was not different at slaughter, short term changes in nutritional status produced very different results between the groups. Periods of maintenance feeding were associated with decreased plasma IGF-1, insulin and glucose, whereas during periods of feeding to appetite they were all elevated.

A modification of this model was used to investigate the mechanisms mediating short

term nutritional effects on follicular development (Booth et al., 1996). Gilts were maintained at 75 kg for 7 days and then either maintained at 75 kg or fed to appetite over the same period. LH increased within 6 hours of feeding to appetite and was associated with increased insulin. IGF-1 was higher in refed gilts. Feeding to appetite for 7 days also resulted in a more latent increase in plasma IGF-1 and follicular development.

Based on the effectiveness of this model for studying nutritionally mediated effects on LH secretion and ovarian development we chose to utilize a comparable model in the lactating/weaned primiparous sow and the results of these studies are presented in Chapters 2 and 3.

2.1.2 Nutritional effects on metabolic state

During periods of realimentation or feed restriction, alterations in plasma concentrations of metabolic hormones and substrates occur (see I'Anson et al., 1991, for review). Alterations in metabolism in these situations ultimately ensure a constant supply of glucose to the brain, through alterations in intermediary metabolism, including glycolysis, lipid beta oxidation, amino acid oxidation, lipolysis and proteolysis (Newsholm and Leech, 1984). The net changes in metabolic substrates during periods of feed restriction versus ad libitum food availability are represented in Figure 2.1.

The experiments described in later chapters specifically focused on nutritionally mediated changes in insulin and the growth hormone / insulin like growth hormone-1 (GH/IGF-1) axis. Primarily this decision was made because nutritionally mediated alterations of these hormones are well documented in the pig and have been correlated with LH secretion in vivo (Booth et al., 1994, Booth et al., 1996; Baidoo et al., 1992; Tokach et al., 1992), and ovarian function in vitro (Hammond et al., 1993).

2.1.3 Insulin

Insulin is involved in the adaptive responses of tissue to changes in glucose concentrations. After the ingestion of a carbohydrate meal, glucose increases and during the postingestive period stimulates the production of insulin from the β-cells of the islets of Langrahans of the pancreas to promote the deposition of metabolites into peripheral tissue (Brockman and Laarveld, 1986). In the liver the principal effects of insulin are to inhibit gluconeogenesis, increase fatty acid synthesis and decrease proteolysis. Whereas, in muscle, insulin increases glucose utilization and decreases protein degradation and in adipose tissue insulin increases the rate of fatty acid synthesis (Newsholm and Leech, 1984).

Centrally administered insulin acts as a satiety signal and reduces feed intake and body weight (Woods et al., 1984). The site of action of insulin appears to be in the region of the ventromedial hypothalamus as administration of insulin into this region decreased feed intake (Leibowitz, 1990) and in the converse situation, administration of insulin antibodies into the ventromedial hypothalamus of the rat increased feed intake (McGowan et al., 1990).

2.1.4 Growth hormone / Insulin-Like-Growth-Factor-I

Growth hormone is essential for somatic growth, promotes protein accretion and decreases fat deposition (Hart and Johnsson ,1986). In lambs (Pell and Bates, 1987) and rats

(Bates et al., 1993) certain muscle types have been characterized as being more responsive to the anabolic actions of growth hormone.

The principle control of growth hormone secretion is accomplished by the interrelationship of two hypothalamic peptides, somatstatin to inhibit its secretion, and growth hormone releasing factor (GRF). GRF receptors are located on the somatotrophs within the pituitary and their activation causes increased growth hormone gene transcription (Theill and Karin for review , 1993). During periods of feed restriction growth hormone is suppressed in the rat. This is partly a result of an increase in somatostatin within the plasma (Tannenbaum et al., 1979). Because injection of antibodies to somatostatin restored plasma growth hormone concentration (Tannenbaum et al., 1979), and also due to a decrease in proGRF mRNA expression in the hypothalamus (Bruno et al., 1990).

Most other large species of mammals differ to rodents in their response to feed restriction, in that growth hormone is actually elevated whereas IGF-I concentration is suppressed. This apparent uncoupling of the growth hormone/IGF-I axis is discussed below.

2.1.5 Insulin-Like-Growth-Factor-I

The primary site of IGF-I synthesis is the liver (Leaman et al., 1990) although IGF-1 gene expression has been identified in a variety of other tissues, such as the granulosa cells of the ovary (Hammond et al., 1993) and uterus (Tavakkol et al., 1988). This reveals a possibility that IGF-I may act in a paracrine or autocrine manner in a variety of tissue. IGF-I is the prime mediator of the anabolic actions of growth hormone during growth (Thissen et al., 1994ab) and elevated IGF-I concentration has been correlated with decreased protein catabolism and excretion of urinary nitrogen (Clemmons et al., 1984). Indeed, passive immunization against IGF-I in the lamb increased protein catabolism (Koea et al., 1992) and acute infusions of IGF-I during a fast in human subjects reduced the degree of proteolysis (Mauras et al., 1992). The relationship between IGF-I and protein metabolism is further exemplified in the study by Clemmons et al. (1985) in which refed rats exhibited greater IGF-I and a more positive nitrogen balance if refed a diet containing essential amino acids as opposed to non essential amino acids. Also in the rat restoration of a growth hormone circadian rhythm after 72 hours of fasting is dependent on the ingestion of protein (Okada et al., 1994). In a recent study of human subjects it was determined that IGF-I acted to increase protein synthesis and insulin acted to reduce proteolysis (Russell-Jones et al., 1994). Thus by acting in synergy these two factors may act to maximize the net deposition of protein during realimentation after a period of feed restriction.

The regulation of IGF-I and IGF-II is further complicated by the fact that 6 binding proteins have been identified; of these, binding protein 3 is the most prevalent and forms a complex with IGF-I and an acid labile unit. The biological activity of IGF-I is modulated according to whether it is complexed or not, and increased biological activity of IGF-I is associated with its free form. Periods of feed restriction may significantly alter the relative ratios of various binding proteins, for example binding protein-3 tends to decreases while binding proteins-2 and 1 increase and may act to alter the bioavailability of IGF-I to tissue (Theissen et al., 1994).

In the well fed animal, plasma concentration of IGF-I is high (Booth et al., 1996), and

in the pig, periods of feed restriction suppress plasma IGF-I concentration (Booth et al., 1996; Charlton et al., 1994) although plasma growth hormone is elevated above basal values (Baidoo et al., 1992, Booth et al., 1994). Thus restricted feed intake results in an uncoupling of the usual growth hormone/IGF-I axis. During periods of feed restriction insulin concentration is low and causes a reduced tissue sensitivity to growth hormone (Clemmons et al., 1994). This physiological adaptation may act as a compensatory function to increase lipolysis and reduce proteolysis (Thissen et al., 1994). The uncoupling of the normal growth hormone/IGF-I ovariectomized axis during a fast or period of feed restriction is due in part to a reduced growth hormone binding sites and growth hormone binding protein in the liver (Maes et al., 1983).

Diabetic rats are characterized as having low IGF-I concentration and decreased hepatic binding for growth hormone, and administration of insulin to diabetic rats restored both IGF-I and growth hormone binding to normal (Maes et al., 1983). These data suggest that suppression of insulin concentration during periods of feed restriction acts to decrease hepatic growth hormone receptor binding. More recent evidence has shown that the IGF-I response to challenge by growth hormone in the protein restricted rat was attenuated although the number of hepatic growth hormone binding sites were similar between the treatment groups (Maes et al., 1988). Thus not only does insulin affect growth hormone receptor binding but also post receptor events may also contribute to the growth hormone/IGF-I uncoupling.

2.2 Mechanism mediating metabolic effects on the reproductive axis

In the studies described in subsequent chapters the characteristics of luteinizing hormone (LH) secretion were measured as a prime indicator of reproductive status at the hypothalamic-pituitary level. The regulation of GnRH secretion and subsequent LH release into the peripheral blood system is essential for the growth of antral ovarian follicles. To better understand the mechanisms whereby metabolic status could potentially influence LH secretion, data pertaining to the control of several neurotransmitter and neuropeptides are presented below.

2.2.1 Neuroendocrine regulation of LH

The central regulation of GnRH activity has been extensively researched and there are three major classes of neurochemicals involved in GnRH regulation: namely, aminergic neurotransmitters, (e.g catecholamine), amino acids (e.g. excitatory amino acids glutamate and aspartate), and thirdly neuropeptides (e.g. neuropeptide Y and the endogenous opioid peptides) (see Kalra, 1993, for review). The net effect of these is also dependent on the steriodogenic environment. Recently evidence has accumulated to suggest that other cell to cell regulation of gonadotropin-releasing-hormone (GnRH) exists which utilizes trophic molecules of glial and neuronal origin. These growth factors arise from glial and astrocyte cells within the central nervous system and probably have indirect, paracrine actions on GnRH neurons. This evidence came from the studies by Ojeda's group in which incubation of the medial basal hypothalamus with transforming-growth-factor-α (TGF-α) enhanced GnRH release, although epidermal growth factor receptors are not found on GnRH neurons (Ma et

al., 1992). In fact epidermal growth factor receptors are actually found on astrocyte cells, and this observation prompted the suggestion that TGF-α stimulates GnRH release via a paracrine effect of the growth factor on glial cells and the release of substances to promote GnRH releasing promoting capabilities from the TGF-α activated astrocytes (Ma et al., 1992). More recently Voigt et al. (1996) demonstrated that fibroblast growth factor may promote the differentiation of GnRH neurons and these neurons develop under a control mechanism mediated by at least two growth factors; one is fibroblast growth factor which directly alters the processing of pro GnRH and the second is transforming growth factor α which acts via astroglial cells. Neuronal networks that impinge on the GnRH neurons arise from caudal regions of the brain, for example catecholaminergic terminals of the hypothalamus arise from cell bodies located within the brain stem and fibers from noradrenergic neurons project to the hypothalamus via the ventral bundle, locus coreleus and from the paraventricular nucleus. Dopaminergic neurons form a main bundle called the 'tuberoinfundibular bundle' whose cell bodies lie within the arcuate nucleus and terminals within the median eminence, and the pituitary (Kalra, 1993).

The neurochemical pathways involved in the regulation of GnRH are the primary triggers for the release of LH from the adenohypophysis, since either passive immunization against GnRH (Esbenshade and Britt, 1985) or hypophyseal stalk transection in the pig (Kraeling et al., 1986) or heifer (Bishop et al., 1996) abolished LH secretion from the adenohypophysis. In the rat, evidence indicates that five structurally distinct regions of the hypothalamus involved in the regulation of LH, these being the supra chiasmatic nucleus, medial preoptic nucleus, medial preoptic area, arcuate nucleus and median eminence.

2.2.2 Catecholaminergic modulation of LH

GnRH neuronal activity is modulated by catecholaminergic neurotransmission and in the rat, a subpopulation of GnRH neurons from the medial preoptic area and diagonal band of Broca and in the arcuate nucleus are innervated by catecholaminergic terminals (Chen et al., 1989). In the ovariectomized monkey electrical activity of presumed GnRH neurons within the medial basal hypothalamus always proceeded an increase in GnRH release from the axonal terminals. In an attempt to identify the neuronal inputs affecting GnRH release (by observing the effects on electrical activity in the medial basal hypothalamus) administered various drugs intracardiacly to monkeys, Kaufman et al. (1985) observed that administration of an α-1 antagonist (phenoxybenzamine or prozosine) or an dopaminergic blocker (metaclopramide) electrical activity decreased, whereas administration of an α-2 (yohimibine) adrenoceptor antagonist had no effect. Thus central norepinepherine and dopaminergic neurotransmitters modulate GnRH activity via α-1 and dopaminergic receptors. In the rat, the progesterone induced LH surge in also blocked by α-1 and α-2 receptor antagonists (prazosin and yohimbine, respectively) (Brann and Mahesh, 1991). Jarry et al. (1990) introduced an α-1 receptor antagonist (Doxazosine) via means of a push pull cannula technique into the preoptic/anterior hypothalamus or medial basal hypothalamus of the ovariectomized rat. These results showed that the α-1 antagonist decreased electrical activity in the preoptic area/anterior hypothalamus only. Thus in this model, norepinepherine secretion from the preoptic area/anterior hypothalamus is a prerequisite for proper function

of the GnRH pulse generator which involves the α-1 receptor subtypes. However within the preoptic area/anterior hypothalamus there is extreme plasticity of the GnRH pulse generator because in vivo studies in the ovariectomized rat in which norepinepherine was deleted by administration of 5-amino-2,4-dihydroxyphenlyethylamine, GnRH activity was inhibited. However within 4 to 6 hours, although norepinepherine within the preoptic area/anterior hypothalamus was still diminished, GnRH activity was restored, via a non α-1 mechanism (Leonhardt et al., 1991). Thus norepinepherine is a permissive neurotransmitter for the GnRH pulse generator rather than the 'pulse generator'.

Generally, norepinepherine is stimulatory to LH in the steroid primed ovariectomized or intact animal, and inhibitory when steroids are absent (Kalra and Kalra, 1983). The study of neuroendocrine regulation of GnRH is further complicated by the fact that neuronal systems do not act in isolation of one another and are probably interdependent on each other. Early reports in the ovariectomized steroid replaced rat that the release of LH associated with electrical stimulation of the ventral noradrenergic tract was potentiated by naloxone (Dyer et al., 1985). This observation lead to a further identification of the interaction between these two systems. In the ovariectomized, progesterone replaced gilt treatment with naloxone, which antagonizes the endogenous opioid receptors, usually causes an increase in LH secretion. However inhibition of norepinepherine synthesis by treatment with N-methyl-[1-methyl-2-proenyl] 1,2 hydrazine-docarbothioamide (AIMAX), blocked the usual increase in LH associated with naloxone treatment (Barb et al., 1993). Similarly in the ovariectomized. steroid replaced rat increased electrical multi unit activity within the medial basal hypothalamus normally associated with naloxone treatment, was abolished after pretreatment with an α-1 (phenoxybenzamine), but not an α-2 (yohimbine) receptor antagonist (Nishihara et al., 1991). Thus from these data, norepinepherine facilitated GnRH neuronal activity through α-1 and α-2 adrenoceptors, whereas the actions of naloxone to increase GnRH activity is probably facilitated by indirect activation of α-1 adrenoceptor on the GnRH neuron.

2.2.3 Excitatory amino acid modulation of LH

Excitatory amino acids (glutamate, aspartate and GABA) exert their effects through at least five receptor subtypes. Glutamate receptor subtypes are categorized into ionotropic. including NMDA, kainic, and DL-α-amino-3-hydroxy-5-methyl-4-isoaxazole propionic acid (AMPA), or metabotropic receptors. NMDA receptors have been identified throughout the hypothalamus and organum ventralis lateralis terminalis OVLT (Brann, 1995, for review) and regional localization of excitatory amino acid receptor subtypes has been recently identified in the rat by Donoso et al. (1990) and Bouguignon et al. (1989).

There is no clear consensus as to the role of NMDA receptor subtype in the activation of LH release in various experimental paradigms. In the male orchidectomized rat (Brann and Mahesh, 1992), ovariectomized lamb (Estienne et al., 1990), and ovariectomized prepubertal gilt (Popwell et al., 1996), NMDA activation is inhibitory to LH secretion. However, in the prepubertal monkey (Gay and Plant, 1987), intact male rat (Donoso et al., 1990) and lactating sow (Sesti and Britt, 1992) administration of NMDA is stimulatory to the release of LH. In gilts that were ovariectomized after puberty, endogenous estradiol replacement (Barb et al.. 1992) resulted in no change in LH secretion, whereas vehicle or progesterone

supplementation actually decreased LH release (Barb et al., 1992; Chang et al: 1993).

Excitatory amino acids are also involved in the induction of the LH surge in the rat because immediately prior to the progesterone induced LH surge aspartate and glutamate concentrations in the preoptic area are elevated (Ping et al., 1994). In the Syrian hamster NMDA receptor types have been identified as having an important role in reversing the suppression of reproductive activity during short daylight periods, because administration of NMDA during this time caused resumption of estrus activity (Urbanski et al., 1990).

The stimulatory actions of excitatory amino acids in GnRH release may also involve catecholinergic neurotransmission. Noradrenergic neurons have been implicated, because pretreatment with an inhibitor of norepinepherine reduced the NMDA stimulated increase in LH secretion in the rat (Kalra and Simpson, 1981), and similarly, pretreatment with the dopamine antagonist, promazine, prevented an increase in LH in response to NMDA (Price et al., 1978). Opioidergic tone may also be a regulatory factor involved in excitatory amino acid induction of LH secretion because pretreatment of rats with morphine abolishes the stimulatory effects of NMDA on LH release (Kalra and Simpson, 1981).

2.2.4 Opioidergic modulation of LH

The endogenous opioid peptides consist of three sub-groups, dynorphins, β-endorphins and enkephalins which have different affinities for 5 receptor subtypes (μ, K, δ, σ, E). Neuroanatomical studies show that β-endorphins are localized in the arcuate nucleus and these cells innervate the median eminence (Meister et al., 1989). β-endorphin immunoreactive fibers innervate the median eminence-arcuate nucleus and synapse with LHRH fibers in the medial preoptic area (Chen et al., 1989). Also dynorphin and enkephalin fibers terminate in the preoptic area and median eminence (Crowley et al., 1985). β-endorphin is also secreted into the hypophyseal portal veins and possibly interacts with GnRH at the level of the gonadotroph (Sarkar and Yen, 1985).

In general opioids exert their influence at the hypothalamic level, because GnRH challenge increased LH to a similar degree in pigs (Barb et al., 1988), rats (Cicero et al., 1977) treated with or without naloxone, and in morphine treated rats (Pang et al., 1977). Conversely, opioidergic tone may also increase the sensitivity of the pituitary to GnRH stimulation, because Barb et al. (1990) noted that naloxone (opioid receptor antagonist) administration enhanced, whereas β-endorphin abolished, the LH response to GnRH challenge (Barb et al., 1990). Also in the steer, in vitro culture of pituitary tissue incubated with naloxone increased the basal release of LH, whereas incubation with physiological levels of enkephalin inhibited LH release (Chao et al., 1986).

Like so many of the other neurotransmitter systems, the steroidal milieu is critical for the net effect of opioidergic modulation of GnRH. In the intact prepubertal or steroid replaced, ovariectomized pig (Barb et al., 1988) or rat (Bhanot and Wilkinson, 1984) treatment with naloxone is stimulatory to LH release, whereas in the steroid deprived environment, naloxone is inhibitory to LH secretion. However in the ovariectomized rhesus monkey, GnRH neuronal activity was reduced with treatment with morphine and reinstated with naloxone injection (Kesner et al., 1986). In the pig progesterone may be a prerequisite for opioid modulation of LH because during the progesterone dominated luteal phase of the

estrous cycle naloxone stimulated the release of LH, whereas during the estrogen dominated. follicular phase naloxone failed to elicit an LH response (Barb et al., 1986). In the intact rat naloxone administration increased LH secretion on all days of the cycle, whereas the LH response was augmented by pre treatment with progesterone (Gabriel et al., 1983; Piva et al., 1985). A reduced opioidergic tone may be facilitatory in the induction of the preovulatory LH surge, as evidenced by the data of Nanda et al. (1990) in which administration of morphine during this time attenuated the LH surge, and this suppressive effect was negated by administration of naloxone.

2.2.5 Neuropeptide Y as a neuromodulator of LH

In the median eminence there is a population of neuropeptide Y immunoreactive fibers in the external zone (Whitkin et al., 1989), which is also the termination site for LHRH neurosecretory axons. Neuropeptide Y is involved in the induction of the GnRH surge in rats (Kalra and Crowley, 1992 for review). However, neuropeptide Y is stimulatory to GnRH only in steroid primed animals, since increased GnRH release from rat median eminence incubated in vitro with neuropeptide Y occurred only when the median eminence had been obtained from steroid primed, ovariectomized animals (Crowley and Kalra, 1987).

Neuropeptide Y has duel sites of action during the proestrous LH surge, one within the median eminence and the other at the gonadotroph to amplify the GnRH induced release of LH (see Kalra and Crowley,1992). Although neuropeptide Y by itself does not stimulate the release of LH from the pituitary, neuropeptide Y does have a facilitatory action on GnRH induced LH release. In agreement with the actions of other neurochemical modulators of LH the steroid milieu is important for its actions on GnRH. Indeed in vitro incubation with neuropeptide Y of adenohypophyseal tissue obtained from rats on the afternoon of proestrus. which are characterized as having high levels of circulating estradiol, increases LH secretion in response to GnRH challenge; however, repeating the experiment with tissue obtained at metestrus did not facilitate the actions of GnRH.

2.3 Metabolic cues and LH secretion

As discussed earlier, the energetic balance of energy intake versus energy expenditure has a profound impact on reproduction (Bronson, 1987) as does insufficient energy availability to support potential growth rate. as in the chronically growth restricted lamb (Foster., 1988). In other models, acute alterations in nutritional status also have profound effects on reproductive status, for example in the gilt (Booth et al., 1995), lactating sow (Koketsu et al., 1996) or monkey (Cameron, 1991). A common feature of all these studies is that disruption of reproductive processes is associated with the suppression of pulsatile LH. and intermittent infusion of GnRH to feed restricted monkeys (Dubey et al., 1986), or challenge with NMDA in the growth restricted lamb (Landefeld et al., 1989), both restored the pulsatile release of LH. These data suggest that nutritional modulation of LH secretion appears to be affected at the level of the GnRH pulse generator.

2.3.1 Metabolic fuel availability

Reinstatement of LH pulsatility in the fasted monkey is dependent on the size (and

consequently the energy value) of the meal at realimentation (Parfitt et al., 1991). The reinstatement of LH is not due to stomach distension as a result of the mass of feed, since saline infusion into the stomach of fasted monkeys did not increase LH whereas gastric infusion of feed did (Schreihofer et al., 1993). Similarly in the gilt, ingestion of sugar beet fiber, which has low nutritional value, did not increase LH pulsatility (Formigoni et al., 1996). More recently Schreihofer et al, (1996) found that reinstatement of LH pulsatility after a 48-hour fast was not dependent on the source of nutrients ingested, and feeding of macro-nutrient diets containing pure fat, protein or carbohydrate all increased LH secretion. The identification of the metabolic signals which mediate such changes in LH and presumably GnRH neuronal activity are therefore of great interest.

2.3.2 Glucose availability

Wade et al. (1996) have accumulated a vast body of evidence to suggest that the availability of oxidizable metabolic fuel is the prime regulator of central reproductive activity in experimental paradigms in which metabolic status has been manipulated.

The location of glucose sensing sites may lie proximal to the GnRH neuron itself. because peripheral injection of NMDA stimulated the release of LH even after prior administration of 2-deoxyglucose (2DG) (Bucholtz et al., 1996). The location of the glucoprivic center may involve an interaction with the norepinepherine neurons (Nagatani et al., 1996) because in the ovariectomized rat, micro dialysis studies have shown that suppression of LH in response to 2DG is associated with an increase in norepinepherine release from the paraventricular nucleus. It is hypothesized that the area postrema region is a likely integrative site for nutrition and reproduction, because 2DG into the 4th ventricle decreases LH secretion in the orchidectomized rat (Wade et al., 1996).

In the undernourished, gonadectomized, lamb model developed by Foster's group, in which there is little body fat to buffer nutritionally mediated alterations in metabolic status. the GnRH neuronal system is exquisitely sensitive to acute changes in the availability of nutrients. A characteristic of this model is an increase in peripheral glucose associated with feeding, associated with an acute increase in LH pulsatility (Bucholtz et al., 1993). More recently Bucholtz et al. (1996) investigated the effects of glucoprivation on LH secretion by administration of 2DG into the lateral cerebral ventricle which caused a suppression of LH secretion which was not associated with alterations in peripheral glucose concentration. Although parentral administration of 2DG similarly suppressed LH frequency, this time it was in association with reduced plasma glucose concentration. They concluded that availability of glucose to some central brain locus is critical for the regulation of LH release and presumably GnRH activity, and that peripheral glucose concentration is not a good indicator of glucose oxidative availability within the brain. The insulin sensitive glucose receptor, GLUT4, has been identified in the hypothalamus of the rat, and may mediate a functional route by which glucose could modulate the neuronal activity of GnRH either directly or indirectly (Livingstone et al., 1995).

As mentioned earlier, Schreinhofer et al. (1996) realimented monkeys after a 48-hour fast, with intragastric infusion of meals consisting of macronutrients of either fat, protein. carbohydrate or a mixture of the above. Plasma glucose concentration differed amongst

treatments groups, whereas LH secretion was consistently increased in response to realimentation. In lactating sows fed fat versus carbohydrate rich diet, plasma LH was increased to a greater extent after carbohydrate meals, although plasma glucose and insulin were similar between treatment groups (Kemp et al., 1995). Similarly glucose infusion during late lactation did not increase LH pulsatility (Tokach et al., 1992b). All of these studies support the observation of Bucholtz et al. (1996) that peripheral glucose concentration is not a principal regulator of LH secretion. However, the role of oxidative glucose availability within the brain has not been addressed in these models.

2.3.3 Leptin as a neuromodulator of LH

Timing of the first estrous cycle in the rodent may (Frisch, 1988) or may not (Bronson et al., 1987) be correlated to body fat. As such the role of body fat as a regulator of reproductive function was viewed with skepticism (Bronson and Manning, 1991). A principal argument against a role for fat in the regulation of reproduction was that there was no known metabolic/neuroendocrine pathway that provided the linkage between the magnitude of a female's fat stores and the GnRH pulse generator. However recently a likely candidate has been identified.

The obese gene (ob) was initially isolated in the mouse (Zhang et al., 1994) and more recently in the rat (Ogawa et al., 1995). The localization of the ob gene is restricted to the mature adipocyte and its expression is induced during adipose cell differentiation and maturation (Ogawa et al., 1995). The levels of ob gene expression and its protein (leptin) are elevated in obese states and fall in states of starvation (Frederich et al., 1995). The leptin receptor has been isolated from the ovaries of the diabetic mouse (Lee et al., 1996) and the receptor mRNA is isolated in hypothalamus, choroid plexus and cerebellum of the rat (Cheung et al., 1996), which would suggest a probable modulatory role for leptin within the reproductive system. Recent research has used the homozygous recessive ob/ob mouse, which is characterized as being obese and infertile. Administering the ob gene product, leptin, for 14 days into mice that were pair fed with control ob/ob mice, resulted in a significant reduction in body weight, compared to control fed mice; however ovarian and uterine weights were increased and even more surprisingly LH secretion remained unchanged in leptin treated mice (Barash et al., 1996). This study demonstrates that leptin may regulate reproductive activity at both local and central sites because LH remained unchanged during a period of significant body weight loss.

The ob/ob genotype of mouse is also characterized by having elevated plasma levels of neuropeptide Y (Schwartz et al., 1996) which decrease upon leptin administration. This is associated with a reduction of neuropeptide Y gene expression in the arcuate nucleus, which is the location of the principal source of neuropeptide Y cell bodies (Bai et al., 1985). This observation suggests that leptin may function as a negative feedback controller of neuropeptide Y expression, and therefore is a probable modulator of GnRH neuronal activity during differing metabolic states.

Identification of neural pathways will lead to a better understanding of the mechanisms by which leptin can influence feeding and reproduction. To date leptin receptor mRNA has been identified in some key regions in the brain, however to my knowledge no leptin protein

has been identified within the brain, and as such a definitive role for leptin in the regulation of reproductive function cannot be concluded. Leptin is 16 KDa in size and probably cannot cross the blood brain barrier. Schwartz et al. (1996) suggested that leptin may act within the median eminence, which lies outside the blood brain barrier, and effects axons projecting into the arcuate nucleus. Alternatively, a specialized blood brain barrier transport system may facilitate its movement into the brain. In summary therefore, the proposed actions of leptin. in the brain are to reduce hypothalamic neuropeptide Y levels and thus reduce feed intake. body weight and blood glucose levels, and as such may serve as a normal metabolic signal to the reproductive system.

2.3.4 Insulin as a neuromodulator of LH

Alterations in energy balance by feed restriction affect the utilization of metabolic fuels in order to maintain a normal state of homeostasis. Plasma glucose concentration is principally maintained by glucagon and insulin, both of which are released from the pancreas, and changes in tissue sensitivity to insulin are also important for glucose homeostasis. Insulin is a likely candidate for the signaling of metabolic status to the reproductive axis, both at a local level and also centrally. The transport system for insulin into the brain is principally located within the circumventricular organs at the choroid plexus which projects into the ventricles and in the median eminence (Van Houten et al., 1980) and immunoreactive insulin receptors have been identified within the arcuate nucleus (Baskin et al., 1994).

Intracerebroventricular (ICV) administration of insulin (6ng/ml injection) into ovariectomized gilts increased LH frequency (Cox et al., 1989) and in vitro incubation of medial basal hypothalamic fragments with insulin and glucose in the rat potentiated the release of GnRH when compared to glucose incubation alone (Arias et al., 1992). Studies in the mature ram which were feed restricted and then given the equivalent energy supplementation of lupin feeding, in the form of ICV glucose and insulin, or insulin alone for 12 h/day for 4 days, also increased LH pulsatile release (Miller et al., 1995). Similarly in the prepubertal gilt additional energy supplied to maintenance fed gilts in the form of a glucose infusion caused an immediate increase in plasma LH frequency (Booth, 1990). Contrary to this observation. peripheral infusion of glucose for 4 h/d for 10 days, to mimic the energy content of lupin supplemental feeding in the mature ram, did increase peripheral insulin and glucose concentration but did not affect LH secretion (Boukhliq et al., 1996). Furthermore, Hileman et al. (1993) injected insulin (500ug) ICV into growth restricted ovariectomized lambs and did not observe an increase in LH. Recent data from Cameron's laboratory has also shed doubt on the absolute role of insulin in the increase of LH secretion in response to realimentation in the monkey (Williams et al., 1996). After a 24-hour fast, realimentation usually resulted in an increase of LH secretion which is normally associated with increased plasma insulin; if, however, the normal increase in plasma insulin is blocked by administration of diazoxide plasma LH still increased.

In summary the definitive role of any one pathway that is involved in the regulation of metabolic status cannot be determined in an in vivo situation. The net effect of alterations in metabolism are due to a cohort of changes in both the peripheral and central aspects of the organism and as with the neuroendocrine regulation of GnRH, are likely to be dependent on

the actions of one another.

2.3.5 Neuropeptide Y as a neuromodulator of LH

Neuropeptide Y was first isolated from the porcine brain (Tatemoto et al,. 1982) and is a powerful orexigenic agent. Anatomical studies suggest that the principal source of neuropeptide Y containing terminals in the paraventricular nucleus arise from cell bodies originating in the arcuate nucleus (Bia et al., 1985). Additional inputs are likely to be derived from the brain stem afferents in which neuropeptide Y is colocalized with norepinepherine (Sawchenko et al., 1985).

During states of feed deprivation neuropeptide Y increases within the arcuate nucleus and this is a result of increased neuropeptide Y gene expression in the arcuate nucleus as determined by increased mRNA for preproneuropeptide Y within this brain region only (White et al., 1990). Injection of neuropeptide Y antibodies into the paraventricular nucleus or into the third ventricle diminishes hyperphagia in rats associated with refeeding after a period of fasting (Lambert et al., 1993). Conversely, injection of neuropeptide Y into the paraventricular nucleus produced a robust feeding response with a preference for carbohydrates (Stanley et al., 1986). Jhanwar-Uniyal et al. (1993) reported that feeding rats pure macro nutrient diets for 3 weeks resulted in a positive correlation between carbohydrate intake and neuropeptide Y staining in the arcuate and paraventricular nucleus, which was not related to caloric intake. For reviews of neuropeptide Y and feeding behavior interrelationships (Sahu and Kalra, 1993).

The evidence that neuropeptide Y may be involved in the integration of nutritional status and reproduction came from studies by Schwartz et al. (1991) in which central administration of insulin depressed the elevated preproneuropeptide Y mRNA in the fasted rat. Similarly in the streptozotocin induced diabetic rat, insulin administration suppressed neuropeptide Y (McKibbin et al., 1992), which was not associated with changes in plasma glucose concentration. In a subsequent experiment, Sahu et al. (1995) observed an increase in neuropeptide Y in the paraventricular and arcuate nucleus, and in the medial preoptic areas. in rats deprived of food for 3 days, whereas in rats fed ad libitum neuropeptide Y was not elevated. Neuropeptide Y may be involved in inhibition of sexual maturation during adverse metabolic conditions, as determined from the data of Gruaz et al. (1993). Sexual maturation was studied in rats initially subjected to restricted feed intake and then refed for 7 days with or without ICV administration of neuropeptide Y. They found that all refed. vehicle control animals had a vaginal plug, compared to only 1/9 of neuropeptide Y treated animals. Concomitant ad libitum feeding and neuropeptide Y administration also decreased ovarian weight and reduced the number of pituitary GnRH receptors.

2.3.6 Precursor control of neurotransmitters

All neurotransmitters which are dependent on precursor availability are produced from compounds that must be obtained from the diet; for example, serotonin is formed from tryptophan, catecholamine from tyrosine and acetylcholamine from acetylcholine. The ratio of these individual amino acids are correlated with their brain concentrations and with the synthesis rates of individual neurotransmitters themselves (Gibson et al., 1978).

Ingestion of a meal may increase the plasma concentration of these precursors several fold. For example feeding of weanling rats with high protein diets caused an increase in tryptophan to large neutral amino acid ratio (Woodger et al., 1979). The transport of large neutral amino acids (LNAA) and choline is dependent on their concentration gradient across the blood brain barrier, and because the LNAA are competitive for their transporters within the blood brain barrier, their transport into the brain is also dependent on the concentration of other LNAA (Fernstron and Wurtman, 1972).

Brain concentrations of serotonin change acutely after ingestion of a carbohydrate meal. The increased plasma insulin concentration, associated with carbohydrate ingestion, results in an increased serum and brain tryptophan ratio, tryptophan hydroxylase and an increased brain serotonin concentration (Lovenberg et al., 1968). Ordinarily, plasma branched chain amino acids decrease when plasma insulin is elevated, for example after feeding; however, bound tryptophan concentration remains constant, and so the ratio of tryptophan to other LNAA will increase. Unlike the other normal LNAA, tryptophan is normally bound to albumin in the plasma. Insulin facilitates the uptake of free fatty acids (which are normally bound to albumin) into the adipose cells. Serum albumin concentration increases and because tryptophan has a high affinity for it, tryptophan becomes bound (Lipset et al., 1973). Interestingly the ingestion of fat leads to increased plasma concentration of free fatty acids which displace the tryptophan from the binding protein, albumin, and results in increased tryptophan concentrations as well (Curzon and Knott, 1974). Thus the total amount of free and bound tryptophan determined the amount of tryptophan in the brain (See Fernstrom et al., 1981; Wurtman et al., 1981 for reviews).

The concentrations of amino acids have been correlated with LH secretion. In the post partum lactating dairy cow (Zurek et al., 1995) tyrosine concentration was positively correlated to LH pulse frequency, which may be indicative of brain catecholaminergic concentrations, whereas elevated plasma concentration of total LNAA in the wether, was indicative of increased catabolism of protein (Koening et al., 1981). Thus the concentrations of LNAA are responsive to the type of meal being ingested and also to the metabolic state of the animal. As such the concentration of tryptophan to other LNAA may provide a mechanism for both acute and chronic modulation of GnRH.

2.3.7 Opioids as neuromodulators of LH

Opioids are potential mediators of nutritional modulation of GnRH release, because fasting for 120 hours, in the estradiol primed, ovariectomized rat caused a suppression of LH secretion which was reversed by administration of an opioid antagonist, naloxone (Dyer et al., 1985). Cagampang et al. (1990) also found that a fast of 48 hours suppressed LH secretion in the estradiol primed, ovariectomized rat; however in non steroid replaced rats the opioid antagonist, naloxone did not reinstate LH pulsatility. In the male orchidectomized monkey naloxone administration during a 24-hour fast failed to increase LH secretion, however, administration of naloxone during the realimentation period did increase LH secretion above that normally observed (Helmreich and Cameron, 1992). In the 7-day feed restricted prepubertal gilt, Cosgrove et al. (1991) also observed that naloxone did not increase LH during feed restriction, and surprisingly even in the ad libitum fed gilts it suppressed LH

secretion. Thus, in these models opioids do not appear to be a facilitator the suppression of LH during feed restriction or fasting.

More recently, in the ovariectomized, estradiol replaced rat (Cagampang et al., 1992) a 48-hour fast resulted in a suppression of LH secretion which was at least in part mediated by α-2 adrenoceptors. Peripheral administration of piperoxan (an α-2 adrenoceptor antagonist) blocked the usual inhibitory effect of fasting on LH secretion, whereas α-1 adrenoceptors antagonist, prazosin had no effect. Thus in the rat suppression of LH during an acute fast, in part, involves the α-2 adrenoceptors. McCabe et al. (1984) speculated that these receptors are probably located within the paraventricular nucleus, because hyperphagia as a result of administration of an α-2 agonist (clonidine) into the paraventricular nucleus is suppressed when the paraventricular nucleus is lesioned .

2.3.8 Adrenal axis as a neuromodulator of LH

The production of glucocorticosteroids is regulated by adrenocorticotrophic hormone (ACTH) which is released from the adenohypophysis and corticotrophin releasing hormone (CRH) from the hypothalamus. Cortisol stimulates gluconeogenesis in the liver and lipolysis in adipose tissue (Newsholm and Leech, 1984), and during periods of feed restriction in the lactating sow plasma cortisol is elevated (Baidoo, 1989). The role of cortisol in mediating the suppression of LH was suggested by Dubey and Plant (1985). Exogenous administration of hydrocortisone acetate to monkeys diminished the release of LH, and subsequent administration of GnRH restored LH release, thus suggesting a hypothalamic site of action on the control of LH secretion (Dubey and Plant, 1985). CRH can inhibit LH secretion by decreasing GnRH release from hypothalamic tissue in vitro (Gambacciani et al., 1986). This may, in part, involve catecholaminergic neurons because Maeda et al. (1994) found that in the ovariectomized steroid supplemented rat, a 48-hour fast suppressed LH secretion and this was associated with increased activity of the ascending adrenergic projections into the PVN and increased production of CRH.

Despite such evidence for a potential role for the adrenal axis in regulating reproduction in the fasted male monkey extensive evidence has been accumulated by Cameron's group to indicate that activation of the adrenal axis is not a modulator of LH secretion. Helmreich et al. (1993) found no correlation between the rise in cortisol and suppression of LH secretion during a 24-hour fast, and if the increased cortisol was mimicked by administering hydrocortisone acetate into normally fed monkeys LH was not suppressed. To determine whether an increase in hypothalamic CRH activity may act to decrease LH secretion during a 24-hour fast, dexamethasone was administered, and although it reduced plasma cortisol, it did not suppress plasma LH secretion. Finally, to discern between the roles of stress and the metabolic consequences of fasting, Schreihofer et al. (1993) realimented monkeys with either saline or feed through a gastric cannula. Although both groups of animals became agitated during the normal feeding period because they were in visual contact with monkeys fed normally, LH was increased only in those monkeys which were infused with a regular food ration.

In summary, therefore, an increase in plasma cortisol concentration may occur during brief periods of fasting or during more prolonged periods of restricted feed intake during

lactation in the sow (Baidoo, 1989), and this may be a metabolic adaptation to ensure continuous glucose availability. However, cortisol per se is apparently not directly involved as a modulator of LH secretion in these situations.

2.3.9 Secretion of GnRH and LH during lactation

For at least the first 36 hours after farrowing LH secretion is still very pulsatile in the sow, resembling that of follicular phase animals; however, by day 3 of lactation, LH secretion is significantly suppressed by suckling (De Rensis et al., 1993a). Pulsatile LH secretion may increase as lactation progresses (Stevenson et al., 1981) and then in response to weaning LH secretion increases robustly (Shaw et al., 1987).

Suckling per se is the primary inhibitor of LH secretion during lactation and results in a reduced stimulation of the pituitary gland by GnRH (Foxcroft et al., 1992), because challenge with a single dose of GnRH (Rojanasthein et al., 1987) or pulsatile infusion of GnRH for 7 days (Rojanasthein et al., 1988) increased LH secretion. As lactation proceeds more sows can be induced to come into standing heat by pulsatile infusion of GnRH (Rojanasthein et al., 1988; Cox and Britt, 1982). By challenging sows with NMDA, Sesti and Britt (1993a) have demonstrated that the releasable pools of GnRH increased as lactation progresses, and in vitro studies have also shown that the rate of GnRH release increased as lactation proceeds (Sesti and Britt, 1993b).

In the post partum lactating animal opioids play an important role in the suckling induced suppression of LH. For example in the lactating, ovariectomized rat, naloxone infusion increased LH release (Wu et al., 1992), and in the lactating beef cow the opioidergic suppression of LH appears to diminish as lactation proceeds, because more naloxone is required to increase LH (Whisnant et al., 1986). At day 3 in the post partum sow, De Rensis et al. (1993b) failed to increase LH in response to naloxone, whereas naloxone administration at day 10 of lactation caused an increase in LH secretion. Furthermore, the usual increase in LH associated with weaning was blocked by morphine administration (Armstrong et al., (1988). In summary, therefore data from the sow suggests that suckling during established lactation involves an increased opioidergic tone, whereas during the first three days after farrowing, when LH is quite pulsatile and first comes under the suppressive effects of suckling, evidence for an opioidergic involvement is lacking.

2.3.10 Suckling intensity and ovarian function

A variable response to an estradiol benzoate induced LH surge is observed during lactation, possibly as a result of reduced suckling frequency. In early lactation, estradiol benzoate (800 μg) is ineffective at stimulating a LH surge however a partial recovery is observed when sows are challenged later in lactation and in some animals estrus was actually induced (Cox et al., 1988). Similarly Sesti and Britt (1993c) noted that more sows exhibited LH surges in response to estradiol benzoate administered at day 28 of lactation, when compared day 14. Typical bi-phasic LH responses were observed in the weaned sow after administration of estradiol benzoate and resulted in a preovulatory like LH surge 54 hours later (Edwards and Foxcroft, 1983). Interestingly the magnitude of the LH surge was greater in sows weaned after a longer lactation (Edwards and Foxcroft, 1983; Kirkwood et al, 1984).

In the sow, a period of suckling induced suppression of LH after parturition is apparently vital for the production of normal follicles, as De Rensis et al. (1989) observed aberrant follicular development in zero weaned (litters removed 6 h after birth) sows when compared to control suckled sows.

Reducing the intensity of the piglet suckling stimulus, by removal of the heaviest piglets (split weaning) decreases the weaning to estrous interval (Stevenson and Britt, 1981). To determine whether reduced suckling per se or a reduced metabolic demand of milk production, results in enhancement of follicular development after split weaning, Grant (1989) removed the 5 largest piglets from each litter at day 14 of lactation, and allowed the remaining piglets either to suckle all the available teats or only six (covered). Although total litter weights were similar among all treatments, follicular development and aromataze activity was greatest in the covered group. LH increased transiently after split weaning although no consistent relationship between ovarian development and LH were observed. From this experiment it is apparent that the greatest ovarian development was observed in the covered sows, and although the litter weight gains were similar between the covered and split weaned groups, the net suckling intensity in the covered group was reduced. These data demonstrate the complex nature of the interrelationships among LH secretion, follicle development and metabolic status around the time of weaning which culminate in the timing of estrus and ovulation.

2.4 Physiology of follicle maturation in the sow

The ultimate goal of the female reproductive processes is to produce viable oocytes. and in the case of mammalian species, an environment that is conducive to optimal embryo development. At the ovarian level a cohort of events culminate in the synthesis of a unique environment, the follicle.

The initial stages of follicle development (primordial) are independent of gonadotropins. Only when the developing follicle has developed 2 to 3 layers of granulosa cells, and the theca internal and externa begin to differentiate, do FSH receptors appear on the granulosa cells (Nakano et al., 1977) and antrum formation is induced in the follicle. Although LH receptors are located in the thecal cells of follicles greater than 100um in diameter (Channing and Kammerman, 1973; Meduri et al., 1992) it is only during the antral stage of follicle development that the granulosa cells acquire LH receptors (Hseueh et al., 1984). In swine, in contrast to other large mammalian species, both the granulosa and thecal cells of the follicle produce significant amounts of estradiol. The granulosa cells are the main site of estradiol synthesis and these cells express only P_{450} aromatase during the follicular phase; however, although the prime function of the theca interna is to synthesizes androstenedione from cholesterol, it also expresses aromatase activity and also produces estradiol (Conley et al., 1994).

2.4.1 Follicle recruitment

As the pig is a polytocous species, multiple follicles are recruited and selected into the final preovulatory pool. Before day 14 of the estrous cycle as many as 50 small follicles are evident on the ovary at any one time and final follicle recruitment into the preovulatory pool

occurs between days 14 and 16. The selection procedure which determines the final population of preovulatory follicles occurs between days 16 to 20 and involves both atresia and a concomitant block to the recruitment of new follicles into this pool. Only at day 20 of the cycle is a final subset of about 15 preovulatory follicles apparent in the gilt (Hunter et al., 1989).

2.4.2 Follicular development during lactation and after weaning

During lactation, folliculogenesis is regarded as being relatively quiescent (Britt et al., 1985). Generally, although there is a slight increase in the percentage of follicles in the 4 to 5 mm class as lactation progresses, the vast majority of follicles are less than 5 mm (Kungavongkrit et al., 1982). However within 48 hours of weaning large preovulatory type follicles may be present (Foxcroft et al., 1987), which suggests that the robust increase in LH pulsatile secretion once the litter has been weaned is a probable signal for initiation of follicle recruitment and maturation.

If one considers a weaning to estrus interval of 5 days, then the time of weaning would be equivalent to day 16 in cyclic animals, i.e. the time when follicles are being actively recruited into the preovulatory pool. In the pubertal gilt Morbeck et al. (1992) estimated that a follicle took 14 days to grow from antrum formation to 3 mm in diameter and a further 5 days to become preovulatory in size. Thus follicles undergoing antrum formation in late gestation or early lactation could constitute the preovulatory pool from which ovulatory follicles are selected after weaning. If this is true, then it is feasible that nutritional status of the animal during late gestation or lactation may have a profound effect on follicular development and potentially on oocyte maturation (Foxcroft et al., 1995). Indeed, Miller (unpublished observation) observed that follicular development was retarded after a 2 week lactation in sows which were restricted to maintenance energy requirements, compared to sows allowed access to feed on an ad libitum basis. Thus even during lactation, nutritional status may further retard the development of follicles within the proliferating pool.

2.4.3 Local regulation of ovarian function

The normal development of follicles is complex and classical endocrine feedback mechanisms between gonadotropin and ovarian steroids cannot satisfactorily explain all aspects of folliculogenesis, e.g. follicle selection. Recent studies have identified a myriad of potential paracrine and autocrine regulators of ovarian function, some of which have been identified as being involved in the regulation of ovarian function in nutritional models.

2.4.4 Nutritional effects on ovulation rate

Increased feed intake (flush feeding) for at least 6 days before estrus can increase ovulation rate (Dailey et al., 1972; Flowers et al., 1988), likely by improving the metabolic status of the gilt prior to ovulation as evidenced by elevated plasma IGF-I and insulin concentrations, when compared to control gilts (Beltranena et al., 1991). Flush feeding may (Beltranena et al., 1991; Cox et al., 1986) or may not (Cox et al., 1986) be associated with an increase in LH. The increased ovulation rate may be as a result of a decreased rate of atresia because morphometric examination of ovaries from gilts slaughtered at estrus revealed

that the percentage of follicles defined as being atretic was lower in flush fed gilts that in those maintained on a 'normal' feed allowance (Dailey et al., 1975). Similarly, Cox et al. (1990) examined ovaries at day 17 or 19 of the estrous cycle and also found that insulin administered to gilts fed to appetite from day 12 of the cycle resulted in a lower rate of atresia in medium sized follicles, than in saline treated controls. Together these data indicate that increased nutritional intake prior to estrus can significantly increase ovulation rate by improving the metabolic status of the animal prior to ovulation. Follicles which would have otherwise become atretic are then rescued and form part of the preovulatory population.

2.4.5 Gonadotropin-independent changes in follicle characteristics

Nutritional flushing during the follicular phase in gilts increases ovulation rate and as discussed above, this may be independent of changes in gonadotropin secretion. Using a streptozotocin-induced diabetic gilt model, Meurer et al. (1991) found that although LH was unchanged by glycemic state, diabetic gilts had more atretic follicles and the large follicles which were evident had lower IGF-I and estradiol content. In a more recent study Cox et al. (1994) again utilized the streptozotocin-induced diabetic gilt model and found that cessation of insulin therapy at day 12 of the estrous cycle caused the diabetic gilts to have more atretic follicles but similar hCG receptor binding to normoglycemic controls. Together these results indicate that in the diabetic model steroidogenesis during the follicular/late luteal phase was potentially detrimentally affected by alterations in insulin or IGF-I but not due to changes in LH stimulation.

In the ewe, supplemental feeding of lupin during the period of day 2 to 13 of the cycle increased ovulation rate without increasing LH or FSH concentrations, although plasma insulin was increased (Downing et al., 1995). In the prepubertal gilt fed allyl trenbolone to block the usual increase in LH associated with realimentation, 7 days of realimentation still resulted in significant follicular growth which was associated with elevated plasma IGF-I and insulin concentration (Cosgrove et al., 1992). In the superovulated cow (Simpson et al., 1994) concomitant treatment with insulin (.25 iμ/kg) during superovulation therapy resulted in larger follicle diameters and greater IGF-I follicular fluid concentration although plasma glucose and LH were not affected. In conclusion, increased ovulation rates or follicle growth occur in models in which nutrition or insulin status has been changed for some time prior to ovulation, which can be independent of alterations in gonadotropin secretion.

2.4.6 Insulin-like-growth-factor-I

Perhaps the best described growth factor family within the ovary is that of IGF-I (see Hammond et al., 1993 for review) and both IGF-I receptors (Veldhuis et al., 1985) and IGF-I gene expression (Charlton et al., 1992) have been identified in the porcine ovary. IGF-I has been identified as an autocrine amplifier of gonadotrophic actions within the ovary, because it acts to potentiate the actions of FSH induced progesterone synthesis, by increasing the cytochrome P_{450} side chain cleavage enzyme (Urban et al., 1990). Conversely, in vitro incubation of granulosa cells with IGF-I antibody reduced the ability of FSH to induce progesterone synthesis (Mondschein et al., 1989). In human luteal and granulosa cells, IGF-I also potentiates the FSH induction of estradiol synthesis (Erickson et al., 1989) and increases

P_{450} side chain cleavage synthesis (Magofin and Weitsman, 1993). Five IGF binding proteins have been isolated from the porcine ovary (Mondishein et al., 1991)and these act to modulate IGF-I bioavailability in an autocrine/paracrine manner (Hammond et al., 1993).

Surprisingly, during the follicular phase, a negative correlation exists between follicular fluid IGF-I concentration and the diameter of large follicles, and IGF-I concentration is lower in estrogenic than non estrogenic follicles (Khalid and Haresign, 1996). A similar inverse relationship between follicle size and IGF-I concentration was observed in sows after 48 hours of weaning (Howard and Ford, 1992). However within these follicles there are significant alterations in the binding protein profiles. Khalid and Harsign (1996) found that IGFBP-3 increased in large follicles whereas in the weaned sow, IGFBP-2 was lower in large follicles (Howard and Ford, 1992).

Nutritional models utilizing feed restriction or realimentation are very effective in manipulating the hepatic production of IGF-I. Typically during feed restriction IGF-I is decreased whereas during realimentation IGF-I concentration returns to normal concentrations (Booth et al., 1996; Tokach et al., 1993). However, follicular fluid IGF-I concentrations do not necessarily reflect that seen in the peripheral environment. Ten weeks of feed restriction in the heifer did not alter IGF-I concentration in the follicular fluid, although estradiol concentration within the follicles was lower in feed restricted animals (Spicer et al., 1991). Similarly 7 days of realimentation, as compared to continued feed restriction for 7 days in the gilt, did not alter IGF-I gene expression in ovarian tissue, although significant differences in hepatic IGF-I expression were evident (Charlton et al., 1992). Collectively these data indicate that plasma IGF-I concentration may not reflect changes in follicular fluid and that IGF-I concentration alone is not a true indicator of IGF-I's bioavailablility, as alterations in IGF-I binding proteins may be physiologically more important. The control of steroidogenesis and ovarian development is therefore highly complex and is probably a result of temporally controlled expression of paracrine and autocrine factors.

2.4.7 Transforming growth factor β family

Activin, inhibin and follistatin all belong to the TGF-β family. Inhibins have a common alpha subunit which is covalently linked to either an A or B subunit to give inhibin A or inhibin B. Activins exist in three forms and are covalent dimers of the beta subunits of activin (Ying et al., 1989) and they augment FSH stimulated LH receptor induction in sheep (Hutchinson et al., 1987). A third member of the family called the follistatins. These are weakly inhibitory to pituitary FSH suppression (Ying et al., 1987), however they can bind to activin and neutralize its actions (Xiao et al., 1992). Localization of follistatins and activins within the ovary are suggestive of a role in the paracrine regulation of follicular maturation (Michel et al., 1993 for review). In the development of ovine follicles from preantral to antral and then to atretic, the distribution of follistatin and inhibin gene expression differed in a temporal manner (Braw-Tal, 1994).

Inhibin, which is synthesized within the granulosa cells of the follicle (Shander et al., 1980), is the most clearly identified ovarian factor which acts as the predominant controller of FSH release from the pituitary and inhibin and FSH are normally inversely related. In the

gilt, as follicle size increases during the follicular phase so inhibin increases (Van de Weil et al., 1983) and periods of increased inhibin secretion are associated with a decrease in FSH concentration (Hasegawa et al., 1988). Atretic follicles have lower inhibin concentration than non atretic follicles (Miller et al., 1991).

The plasma concentration of inhibin in the lactating sow has not been determined. however as early as 1981, Stevenson et al. (1981), suggested that FSH was principally controlled by a factor derived from the follicle (inhibin) because ovariectomy during lactation in the sow was associated with an increased plasma concentration of FSH, whilst LH concentration remained unchanged. As lactation progresses and follicular development increases, LH secretion increases (reduced suckling stimulus), whereas FSH concentration tends to decrease in most sows, which is suggested to be due to increased inhibin concentration (Foxroft et al., 1987). A study by Mullan et al. (1989) demonstrated that plasma FSH concentration in sows was greater in sows suckled by 12 piglets. as opposed to 6, and was also lower in sows which were fed ad libitum compared to restrict fed sows. thus demonstrating an inverse relationship between follicle development and FSH concentration. To date, 9 molecular variants of inhibin have been isolated from bovine follicular fluid (Good et al., 1995). Although all forms decrease FSH production from cultured ovine pituitary cells. their ability to potentiate the LH in response to a GnRH challenge, ranged from 40 to 248 %.

Active immunization of gilts (Brown et al., 1990; King et al., 1995), or ewes (Deitrich et al., 1995) against inhibin increased ovulation rate and was often associated with increased FSH concentrations (Kusina et al., 1995). However the advancement of puberty in the lamb by immunoneutralization agonist inhibin is not associated with increased FSH (Anderson et al., 1996).

Work by Scaramuzzi's group, using the autotransplanted ovary model in the ewe, has identified a role for other growth factors as potential regulators of follicular steroidogenesis. Epidermal growth factor perfused across the ovary during the follicular phase reduced estradiol secretion by inhibiting aromataze activity (Murray et al, 1992). Furthermore, TGFα decreased estrogen secretion and enhanced progesterone secretion during the follicular phase and may be indicative of a role in the premature luteinization of granulosa cells (Murray et al., 1994).

2.5 Oocyte maturation

The close association between the oocyte and the surrounding cumulus cells is essential for normal development. Nuclear development of the oocyte is initially arrested at the dictyate stage of meiosis. As primordial oocytes grow they attain the ability to respond to the endogenous preovulatory LH surge and this reinitiates development to metaphase II of meiosis. Only at this stage of development can the oocyte be fertilized (see Thibault. 1977).

2.5.1 Somatic component

During the growth of the follicle granulosa cells differentiate so that the oocyte becomes encompassed by cumulus cells and the mural granulosa cells form the contact between the cumulus cells and the follicle wall. These two cell types respond differently to

the gonadotropin surge, in that the mural granulosa cells become steriodogenic and luteinize, whereas the cumulus cells undergo expansion. The involvement of gap junctions as a means of communication between the somatic component of the follicle and the oocyte (metabolic coupling) is critical for oocyte development. This coupling provides an extensive network of communication between the somatic compartment and oocyte (Anderson and Albertini, 1976) and enables small metabolites such as energy substrates and amino acids to be taken into the oocyte (Colonna and Magnia, 1983). Indeed the rate of follicular growth is related to the extent of metabolic coupling (Brower and Schultz, 1982).

The normal signal for oocyte maturation is the preovulatory LH surge. However, when oocytes are removed from their antral follicles they undergo spontaneous nuclear maturation (Pincus and Enzman, 1935), which suggests that some factors within the somatic compartment of the follicle exert an inhibitory effect on oocyte maturation, since no LH receptors have been located on the oolemma. One hypothesis for the resumption of nuclear maturation is that exposure to the LH surge disrupts the gap junctions between the foot processes of the corona radiata and the oocyte. This will then disrupt the normal pathway by which inhibitory factors can influence the oocyte (Vanderhyden and Armstrong, 1990). One such somatically derived inhibitory factor is cyclic adenosine monophosphate (cAMP) which is primarily located within the mural granulosa cells (Dekel and Beers, 1978). It acts to increase the number and permeability of gap junctions (Loewenstein, 1981). In vivo studies in the pig have shown that cAMP concentration within the oocyte increases after the LH surge and Mattioli et al. (1994) have hypothesized that the high concentration of cAMP maintains meiotic arrest and a transient increase in cAMP in response to the LH surge then facilitates meiosis.

Conversely, not only do somatic cells have inhibitory effects on meiotic maturation (cAMP), but they also produce potential meiotic stimulatory factors, for example maturation promoting factor (MPF). In their review paper, Motlik and Kubelka (1990) have amassed evidence to demonstrate that the acute binding of LH to granulosa cell receptors leads to their final differentiation, which in turn activates 'pre-maturation promoting factor' located within the oocyte cytoplasm. Maturation promoting factor was originally isolated in the oocytes of Xenopus lavea (Dettlaff et al., 1964) and is in fact highly conserved across species. In a recent experiment by Mattioli et al. (1991) injection of nuclei of immature oocytes into the cytoplasm of mature oocytes, which also had high concentrations of maturation promoting factor, induced the initiation of nuclear maturation.

The degree of cumulus cell expansion is related to cytoplasmic maturation and preimplantation embryonic development in cattle (Sirard et al., 1988), and in vitro cumulus cells are necessary at insemination to maximize the occurrence of the acrosome reaction (Fukui, 1990). Similarly in swine, only oocytes functionally connected to the somatic cells of the cumulus could be penetrated by sperm (Mattioli et al., 1988). The persistence of the cumulus cells during maturation is fundamental for the oocyte to maintain its penetrability and the persistence of this interaction stabilizes the distribution of cortical granules in the pig (Galeati et al., 1991). Interruption of the interaction between oocyte and cumulus cells, which occurs naturally at the end of maturation initiates cortical granule migration and prevents polyspermic fertilization. Cumulus-oocyte complex expansion supports the ability

of the oocyte to undergo fertilization (Vanderhyden and Armstrong, 1989). Thus although cumulus cell expansion has not been correlated with oocyte nuclear maturation, the expansion of these cells is essential for the oocyte's release from inhibitory factors that reside within the somatic compartment, and secondly, their presence during and after fertilization optimizes the oocyte's chances of being successfully fertilized.

2.5.2 Role of growth factors in oocyte maturation

It is generally accepted that gonadotropins play a major role in stimulation of oocyte maturation, however it is also possible that other factors influence the maturation of oocytes. Growth factors appear to fulfil this role, as in vitro incubation of mouse oocyte-cumulus complexes with epidermal growth factor (Downs et al., 1988), or transforming growth factor alpha (Brucker et al., 1991), induces germinal vesicle breakdown.

2.5.3 Insulin-like-growth-factor-I

Although most investigations of the biological function of IGF-I have focused on the somatic cell compartment, it seems likely that IGF-I interacts with the oocyte. IGF-I stimulated the meiotic maturation of follicle-enclosed oocytes in the absence of gonadotropins in a dose dependent manner in rabbits, and the maturation was blocked by incubation with an IGF-I type 1 receptor antagonist (Lorenzo et al., 1996). IGF-I type 1 receptors are located in the oocyte of xenopus and induce meiotic maturation by reducing cAMP (Sadler et al., 1987). Exogenous IGF-I added to incubation media of oocytes (porcine) resulted in an increase in the cell numbers within the blastocyst and IGF-I may also affect embryo development by interaction with somatic cells. (Xia et al., 1994).

2.5.4 Epidermal growth factor

Recently several growth factors have been identified as candidates for having a role in the control of oocyte maturation. It is likely that these growth factors interact with the gonadotropins to regulate the maturation of the oocyte. For example, in the pig incubation of oocytes with FSH stimulated germinal vesicle breakdown in vitro, which was potentiated when epidermal growth factor (EGF) was also present (Singh et al., 1993). EGF in combination with gonadotropins increased the rate of nuclear maturation in pig oocytes (Ding and Foxcroft, 1994) and thus it seems likely that EGF may act as an intraovarian regulator of oocyte maturation. In the cow, in vitro incubation of oocytes with EGF or TGF increased the fertilization rate of oocytes and the number of oocytes that developed to the blastocyst stage (Kobayashi et al., 1994).

2.5.5 Follicle size and related oocyte maturation

All oocytes within ovarian follicles are not equally competent to resume meiosis and mature to Metaphase II. This is primarily determined by the developmental stage of the follicle, which is correlated to oocyte size (Sorenson and Wasserman, 1976). Within cattle in vitro maturation /in vitro fertilization (IVM-IVF) systems, follicles greater than 6 mm in diameter yield the greatest percentage of blastocysts (Tan and Lu, 1990). Similarly, incubation of oocytes with follicular fluid retrieved from large follicles is advantageous to

oocyte development (Longeran et al., 1994). Similar observations have been made in the pig. Incubation of oocyte-cumulus complexes with individual follicle shells, obtained at different stages of development and which differed in size within the same preovulatory pool, significantly affected oocyte cytoplasmic maturation (Ding and Foxcroft, 1992). Within polytocous species such as the pig, presumptive preovulatory follicles form a heterologous group in terms of size and biochemical status (Foxcroft et al., 1987). In vitro maturation of oocyte-cumulus-complexes with conditioned media obtained from the largest or smallest follicles at day 20 of the estrous cycle influenced oocyte meiotic maturation, in that media conditioned from large follicles was more able to support oocyte development to metaphase II (Ding and Foxcroft, 1994).

These data support the concept that the maturational stage of the follicle has a profound effect on the cytpolasmic maturation of the oocyte and may have important consequences for early embryo development.

2.6 Embryo survival

Embryo survival is an important component of reproduction. Once a dam has produced one or several oocytes it is important to ensure that the resultant embryo(s) develop in an environment that will optimize their chances of becoming viable offspring. Furthermore, in the polytocous species like the pig it is possible to influence the number of embryos successfully implanting by manipulating the dam's nutritional status.

2.6.1 Nutritional models

Nutritional status of an animal can influence embryo survival. In the lactating sow, lactational and postweaning feed intake interact with one another to influence embryo survival (Baidoo et al., 1992). The patterns of feed intake around the time of ovulation also affect embryo survival. In the Meishan gilt (Ashworth et al., 1995) and European breed of gilts (Pharazyn et al., 1991; Jindal et al., 1996) the highest level of embryo survival was observed in gilts on high feed intakes before ovulation and then feed restricted one day after mating. The timing of changes in feed intake during the periovulatory period appears critical to embryo survival, because Jindal et al.. (1996) found that a reduction in feed intake at day 1 after breeding was associated with the greatest embryo survival, whereas if the feed restriction was delayed until day 3 embryo survival was similar to that observed in gilts which were continued on high feeding levels. In all of these experiments and that of Ashworth. (1991), embryo survival was positively correlated with plasma progesterone concentration during early gestation. The increased embryo survival observed when feed intake was reduced at day 1 after mating in the gilt was associated with a rise in plasma progesterone concentration 10 hours before that observed in 'normally' fed gilts (Jindal, 1996). Thus this relatively small window of time is apparently critical in the determination of embryo survival in the gilt.

2.6.2 Maternal recognition of pregnancy

Swine embryos undergo epitheliochorial placentation and rely on the production of histotroph to provide nutrition for the embryo. A detailed review of embryonic development in the pig was presented by Stroband and Van der Lende (1990). Briefly, the morula is

transported to the uterus as a result of the usual increase in progesterone synthesis from the corpus luteum (Dzuik et al., 1985). Hatching from the zona pellucida occurs at day 6 to 7 and the blastocyst undergoes rapid expansion and retains its ability to move within the uterine lumen up to day 13. Between days 13 and 14 the blastocyst finally becomes attached (Dantzer, 1985).

The uterine environment of a virgin gilt can support the development of a similarly aged blastocyst until about day 9 of pregnancy (Polge, 1982). However after this stage the embryo itself will modify the uterine environment through a cascade of events that are initiated when it signals its presence to the dam, a process that is called 'maternal recognition of pregnancy'. The embryo signals its presence to the dam by producing a pulse of estradiol at day 11 and a second and more prolonged phase of estradiol at day 14 of pregnancy (Geisert et al., 1982), and tissue adjacent to the embryonic disc has the greatest estrogen synthetic ability. The importance of the maternal recognition of pregnancy is that the corpus luteum does not undergo luteolysis and thus continues its synthesis of progesterone. In swine this is achieved by the conceptus estradiol redirecting PGF2α secretion towards the uterine lumen and prevents an effective luteolytic signal from interfering with ovarian progesterone synthesis (Bazer et al., 1977).

2.6.3 Asynchrony between the uterus and embryo

To optimize success rates in embryo transfer experiments, it is essential that the embryo and uterine environment are physiologically synchronized, as indicated by the experiment of Pope et al. (1982). If day 5 embryos were transferred into a day 6 uterus embryo survival was poorer than that observed if embryos of day 7 gilts were to transferred into a day 6 recipient. It has been hypothesized by Geisert et al. (1991) that the asynchrony between the embryos and uterine environment is due to the more advanced embryos initiating changes in the uterine environment to satisfy their physiological needs, by estradiol production. However the less advanced embryos find this changed environment to be hostile and so die.

Roberts et al. (1993) have an alternative hypothesis to explain the death of less advanced embryos at the time of maternal recognition of pregnancy. In response to fetal estradiol production retinol is secreted from the uterine endometrium (Trout et al., 1992), and is important for embryo development. Concomitant with the uterine production of retinol. retinol binding protein produced from the embryos themselves. This binding protein production may not be sufficient in the less advanced embryos to counteract the high concentrations of retinol within the uterus, and so they will perish.

2.6.4 Asynchrony among embryos

There is normally a developmental array of blastocysts within the uterus, ranging from spherical to filamentous forms. When the more advanced blastocysts signal their presence. the uterine changes that are initiated are embryocidal to the smaller less advanced embryos (Pope et al., 1982). However, the death of less advanced embryos is not apparently due to them being less viable than the more advances ones. Wilde et al. (1988) segregated day 7 blastocysts according to size and transferred the small and large blastocysts into individual

ligated horns of the same recipient and found that the small blastocysts were just as viable as the more advanced ones.

The Meishan breed of pig is characterized as being more prolific than the European breeds and in part this may be achieved by the lower estradiol production by embryos. resulting in a more gradual change in the uterine environment (Anderson et al., 1993). Meishan pigs have a slower rate of development from the 4-cell stage to the morula, which may be advantageous to the embryos because it is thought that at this stage they are less sensitive to uterine changes (Youngs et al., 1993).

2.6.5 Within litter diversity: Follicle heterogeneity

Follicle heterogeneity within the preovulatory pool may have implications for oocyte maturation and embryonic development (Hunter and Weisak, 1990). At day 20 of the estrous cycle a subset of 14 to 15 large presumptive preovulatory follicles emerge (Hunter er al., 1989) which exhibit a degree of biochemical diversity. Similarly in the weaned sow. presumptive preovulatory follicles also form a heterologous group and biochemical analysis of these follicles revealed that hCG binding, follicular fluid estradiol and follicle diameter varied greatly (Foxcroft et al., 1987). In the highly prolific Meishan breed of pig more oocytes collected after the endogenous LH surge had matured to metaphase II of meiosis than had comparable oocytes retrieved from Large White animals and this may contribute to the prolifacy of the breed (Faillace and Hunter, 1994).

Within naturally cyclic gilts, approximately 70 % of all ovulations occur within 2 to 4 hours and destruction of unovulated follicles after this time gave rise to a more uniform population of blastocysts at day 11 of gestation (Pope et al., 1986). Further to this observation a comparison of oocyte maturational distribution before ovulation with zygotic maturation in a comparable set of gilts, revealed that the skewedness of oocyte development continued into the zygotic population (Xie et al., 1990a). More recently, Xie et al. (1990b) removed late ovulating follicles by electrocautery and established that late ovulating follicles gave rise to less well developed embryos in early pregnancy. Together these data suggest that the maturational stage of the oocyte at the time of ovulation and the duration of ovulation itself gave rise to a more diverse population of embryos in the pre-implantation period.

If we accept that the maturation of oocytes at ovulation may be variable as a result of follicular heterogeneity, then it is also feasible that endocrine changes around the time of ovulation may also be altered. For pigs with a high embryo survival, subtle differences in endocrine profiles were apparent, in that peak estradiol occurred closer to the onset of estrus and peak LH occurred later after estrus, than in sows exhibiting lower embryo survival (Blair et al., 1994). However, it remains unclear whether these differences are related to follicular heterogeneity and whether they affect the oviductal or uterine environments.

2.6.6 Uterine secretions

The uterus secretes a myriad of factors before and after implantation of the embryo and many of these products have been comprehensively reviewed by Simmen and Simmen (1990). With the advent of molecular biology techniques, other new peptides have been isolated and much current research is aimed at identifying these and determining their temporal relationships to

one another (Roberts et al., 1993).

Uteroferrin is an acid phosphatase that is the source of iron for the developing embryo. It is secreted from the epithelium of the uterus (Roberts and Bazer, 1988). By day 11 of gestation uteroferrin is present in the trophectoderm of embryos, which actively endocytose uteroferrin from the histotroph via their trophoectoderm (Baumbach et al., 1990). Uteroferrin may be necessary for differentiation of embryonic tissue (Thesleff et al., 1985) and also the rapid expansion of trophoblastic tissue at day 11. Interactions among prolactin, estradiol and progesterone modulate uteroferrin gene expression (Fliss et al., 1991) and the production of uteroferrin is closely related to the progesterone: estradiol ratio up to mid pregnancy (Simmen et al., 1988).

The temporal expression of IGF-I and its receptors has been investigated during the preimplantation period. Generally IGF-I stimulates protein synthesis (Harvey and Kaye, 1991) and mitogenesis (Harvey and Kaye 1991b) in mice (Doherty et al., 1994) and pigs (Hofig et al., 1991). In the mouse, genes for IGF-II are expressed at the 2-cell stage, whereas for IGF-I expression occurs at the 8-cell stage of development, which in the mouse is after implantation (Telford et al., 1990). During all stages of preimplantation development (oocyte to blastocyst) IGF binding proteins, 2, 3 and 4 are expressed, whereas IGF binding protein 6 is expressed at the blastocoel stage only (Hahnel and Schultz, 1994). In the pig, peak expression of IGF-I is between days 10 to 12 and coincides with trophoblast elongation (Simmen et al., 1992). Thus similar to the ovarian follicle, the production of IGF binding proteins may prove to be important in the regulation of IGF-I and II bioavailability to the embryo and their temporal relationships to the stage of development may be important for the control of expression of other proteins.

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Figure 2.1 Schematic comparison of the metabolic responses to different nutritional status in swine.

BMR = basic metabolic rate; Cotr's = corticosteroids; Cats = catecholamines; GH = growth hormone; IGF-I = Insulin-like-growth=hormone-I; PRL = prolactin; T4, T3, rT3 = thyroxine, tri-iodothyronine, and reverse tri-iodothyronine; AA = amino acids; FFA = free fatty acids, respectively.

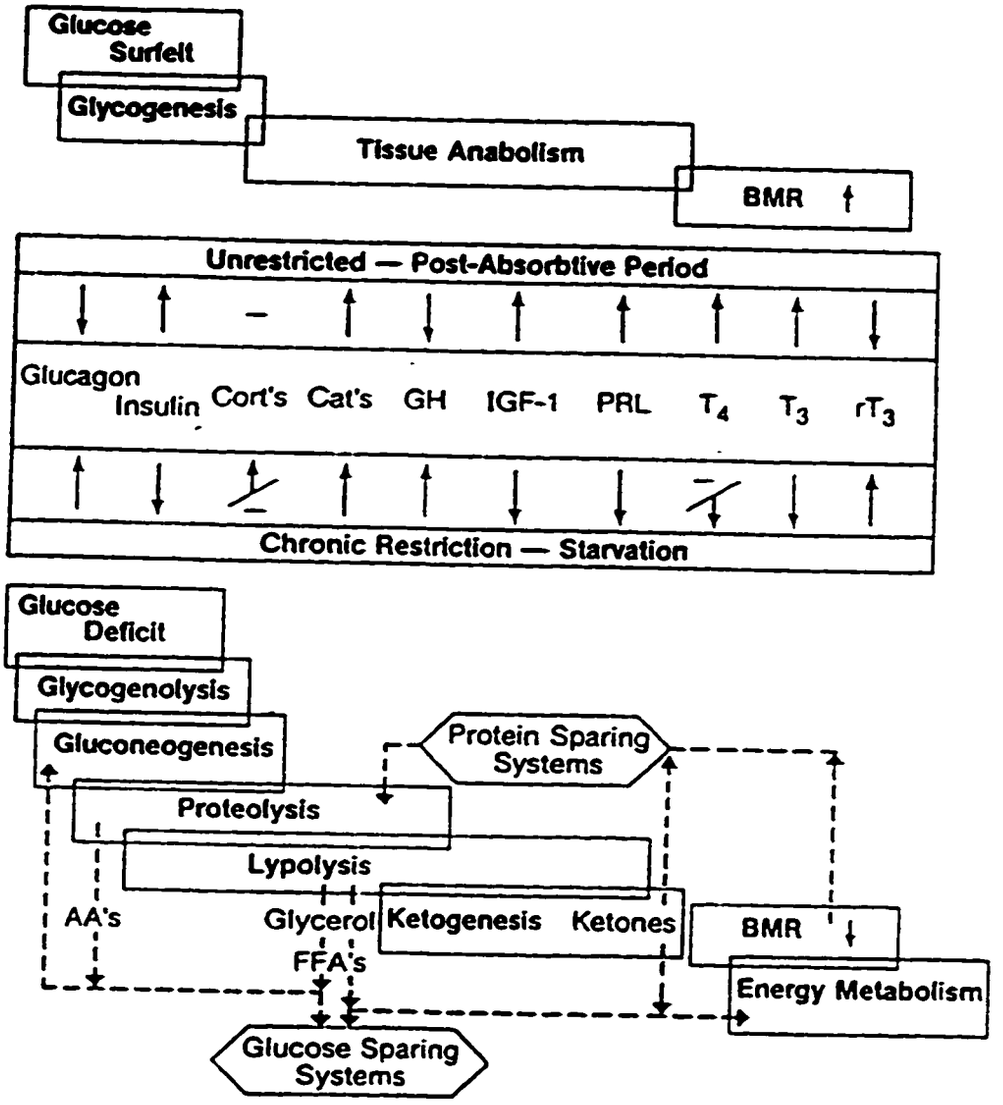
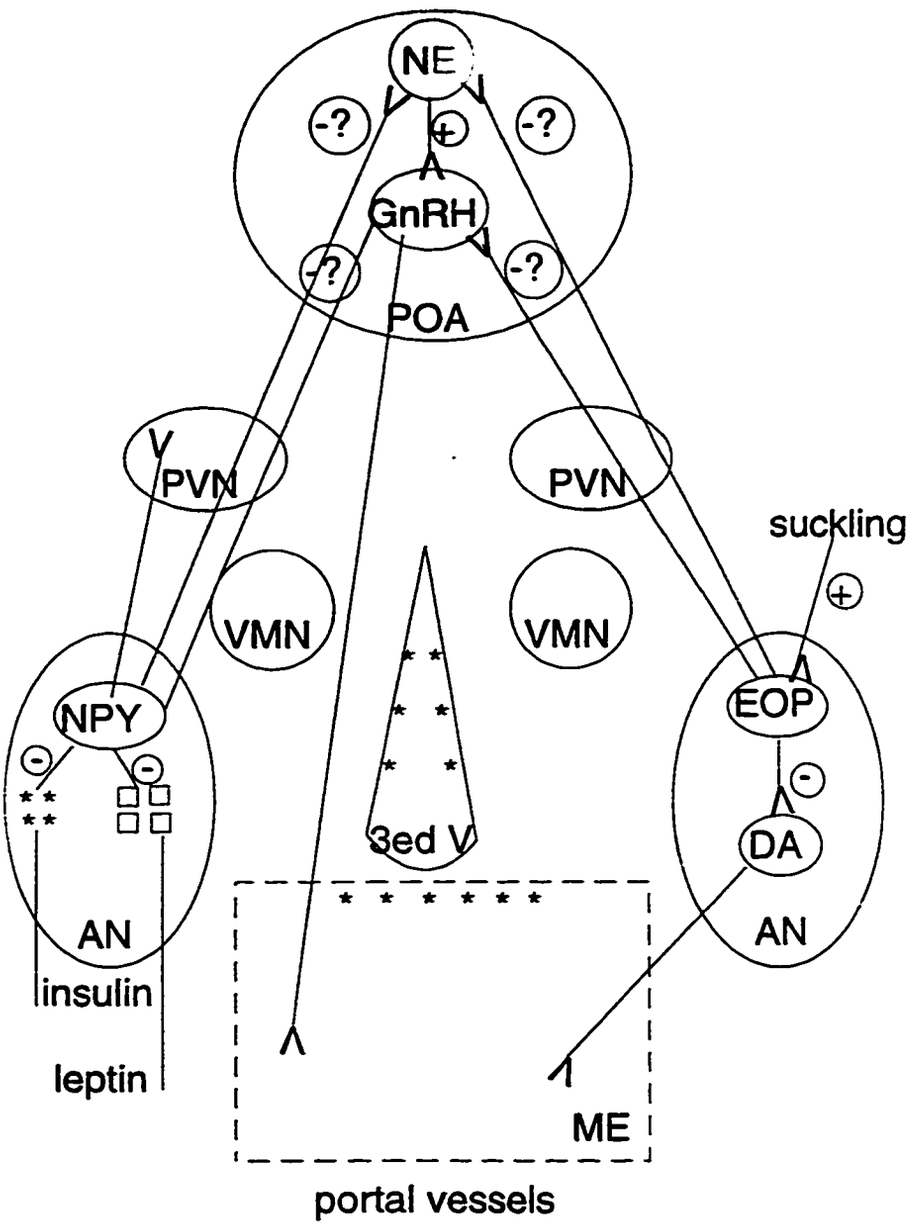

Glucose Surfeit
Glycogenesis
Tissue Anabolism
BMR
Unrestricted — Post-Absorbtive Period
Glucagon
Insulin
Cort's
Cat's
GH
IGF-1
PRL
T_4
T_3
rT_3
Chronic Restriction — Starvation
Glucose Deficit
Glycogenolysis
Gluconeogenesis
Protein Sparing Systems
Proteolysis
Lypolysis
AA's
Glycerol
FFA's
Ketogenesis
Ketones
BMR
Energy Metabolism
Glucose Sparing Systems

Figure 2.2 Diagrammatic representation of the possible neuroendocrine modulation of GnRH by nutritional state and suckling.

Possible mechanisms mediating the suckling induced inhibition of LH is in part achieved by an increased endogenous opioid peptide (EOP) tone, which may act via α-1 adreneoceptors that reside on the GnRH neuron itself, and/or via suppression of norepinepherine induced release of GnRH within the preoptic area. Insulin receptors (*) have been located within the circumvenrticular organs of the 3rd ventricle (3ed) and the median eminence (ME). Leptin (□) receptor mRNA and insulin receptors (*) have been localized in the arcuate nucleus (AN). The proposed mechanism for insulin and leptin induced suppression of GnRH release is via their actions on neuropeptide Y synthesis. During periods of feeding, leptin and insulin plasma concentrations are elevated, whereas neuropeptide Y synthesis in the AN is suppressed and associated with increased GnRH. The mechanisms whereby neuropeptide Y suppresses GnRH may be via α-2 adrenoceptors, because in the rat administration of an α-2 adrenoceptors agonist will prevent the fasting induced suppression of LH.


NE
-?
+
-?
GnRH
-?
-?
POA
PVN
PVN
suckling
+
VMN
VMN
NPY
-
-
EOP
-
DA
3ed V
AN
AN
insulin
leptin
ME
portal vessels

CHAPTER 3
PATTERN OF LACTATIONAL CATABOLISM AND ASSOCIATED ENDOCRINE CHANGES DIFFERENTIALLY AFFECT POST WEANING FERTILITY IN PRIMIPAROUS LACTATING SOWS

INTRODUCTION

Feed restriction during lactation in primiparous sows has detrimental effects on reproductive performance as determined by an increased weaning to estrus interval (Armstrong et al, 1986), a decrease in the percentage of sows in estrus within 14 days (Reese et al, 1984) and increased embryonic mortality (Kirkwood et al, 1987 and Baidoo et al, 1992). A weaning to estrus interval (WEI) of less than 4 days was associated with a high luteinizing hormone (LH) pulse frequency (Shaw and Foxcroft, 1985; Armstrong et al, 1986) or mean LH concentration (King and Martin, 1989) in the 12 hour period before weaning. More recently evidence to suggest that changes in metabolic fuel availability and associated endocrine status are important determinants in the signaling of metabolic status to the reproductive axis (Foxcroft, 1990; Foxcroft, 1992; Quesnel and Prunier, 1995; Wade and Schneider, 1992).

Previous studies have addressed the concept that relative nutritional states are important determinants in reproduction. For example, 'skip a heat' management of weaned primiparous sows has been used to increase the number of piglets born in the second litter. An endocrine study by Clowes et al. (1994) demonstrated that the observed increase in litter size, when young sows were bred at second rather than first post weaning estrus, was associated with the sow approaching a relatively less catabolic state, as indicated by increased mean plasma insulin and decreased glycerol concentration in the proestrus period. Consistent with this concept , the first lactational estrus in dairy cows occurs earlier in those animals most rapidly achieving a less catabolic state (Zurek et al., 1995).

The study reported here was undertaken to test the hypothesis that the pattern of change in metabolic status during lactation would have a critical effect on post weaning fertility and fecundity, and also to identify the endocrine mechanisms mediating these effects.

MATERIALS AND METHODS

During gestation all sows were fed in accordance with the Agricultural Research Council (ARC). At farrowing 25 primiparous Camborough sows (Pig Improvement (Canada) Ltd), randomly stratified according to sow weight at farrowing and the number of piglets born, were allocated to one of three treatments (see Figure 3.1). All sows were fed three times daily at 0800, 1200 and 1500 throughout a 28 day lactation (27.8± 0.5 days) with a wheat-barley-soybean diet formulated to provide 13.4MJ ME/kg, 15.4 % crude protein and 0.74% lysine (see Table 3.1). Sows allocated to group, AA, were fed to appetite throughout lactation. Group AR sows were fed to appetite from farrowing until day 21 and then restricted to 50% of the average per meal consumption of the 'to appetite' fed sows from day 22 to 28. Group RA sows were restricted to 50% of the average per meal consumption of

to appetite fed sows from farrowing until day 21 and then fed to appetite from day 22 to 28. Water was available ad libitum to both the sow and piglets, throughout the experimental period. To optimize the potential for nutritionally induced changes in LH secretion during lactation, all litters were standardized to six piglets within 24 hours of farrowing (Mullan et al., 1991). Creep feed was not available. Sow weight and backfat (Scanoprobe II, Scano, Ithaca, NY) and litter weights were recorded at weekly intervals throughout lactation. From weaning until slaughter all sows were fed ad libitum with a wheat Barley Soybean ration formulated to provide 13.4 MJ ME/kg, 13.7% crude protein and .56% lysine.

After weaning sows were tested twice daily at 0700 h and 1900 h for the onset of standing estrus using direct exposure to a vasectomized boar for 15 minutes. At 12 and 24 hours after the onset of estrus sows were artificially inseminated, by one of two inseminators, using pooled semen ($3x10^9$ spermatozoa /dose) from the same three boars (Alberta Swine Genetics Corp. Box 3310, Leduc, Alberta, Ca). The day of standing heat was designated as 'day 0' and sows were slaughtered on day 28 (28±1.2) of gestation at a local abattoir and their reproductive tracts were recovered. The uterine horns were dissected to determine the number of morphologically viable embryos and ovulation rate was determined by counting the number of corpora lutea on each ovary.

Blood Sampling

At day 17 of lactation an indwelling jugular catheter was surgically implanted, under general anesthesia, via the superficial cephalic vein (Cosgrove at al, 1993). 3 ml blood samples were withdrawn at 10 minute intervals from 0600 to 1800 on day 21, and for 12 hour periods before and after weaning at 1800 h on day 28, for analysis of LH. Additional 15 ml samples were collected hourly for analysis of IGF-I, insulin and follicle stimulating hormone (FSH) by radioimmuno-assay (RIA). Blood samples were collected into heparinized tubes and centrifuged at 1500 x g for 15 minutes. The plasma was poured off and stored at -30^0C until analysis.

Estimation of plasma hormone concentrations

For RIA analysis, all treatment groups were represented in each assay, and all samples from a sow were analyzed in one assay. Plasma LH and FSH concentrations were determined using the homologous double antibody RIA previously described by Cosgrove et al.(1991). For LH intra and interassay coefficient of variance (CV) were 7.8% and 11.2% respectively and average sensitivity, estimated as 85% of total binding was 0.001 ng/tube. For FSH, the intraassay CV for the single assay used was 10.3% and average sensitivity, defined as 88% of total binding was 4.17 ng/tube.

Plasma insulin and IGF-1 concentrations were determined using the homologous double antibody RIA previously described by Cosgrove et al.(1992). For insulin, intra and interassay CV were 6.0% and 6.1% respectively and sensitivity defined as 88% of total binding was 0.007 ng/tube. Plasma IGF-I intra and interassay CV were 4.8% and 5.1% respectively and sensitivity of the assay defined as 92% of total binding was 0.08 ng/tube.

Statistical analysis

All dependent variables were analyzed for normality using the Wilk- Shapiro test (SAS 1990). Data for the dependent variables sow weight, backfat, litter weight, LH, FSH, insulin and IGF-1 across day were analyzed as repeated measures analysis of variance. Analysis were computed using the repeated measures general linear model (GLM) procedure of SAS (1990). For all dependent variables sources of variation were treatment, sows within treatment and the repeated measures of day. Analysis for sow weight, backfat and litter weight also included, as a covariate, sow weight, backfat and litter weight (of the remaining 6 piglets) at farrowing respectively. Significant differences among treatments were determined using sow within treatment as the error term. In the event of a significant day*treatment interaction, differences among days were determined by computing separate analyzes within treatments. Weaning to estrus interval, ovulation rate and embryo survival were analyzed using analysis of variance with sources of variation of treatment and the error term of sow within treatment. Differences among treatment means were determined using Duncan's multiple range test (SAS, 1990).

Comparisons of plasma insulin concentrations amongst treatment groups on day 21 and 28 were made over two time periods: Preprandial (two hours before the morning feed) and postprandial (first to tenth hour after morning feed). After weaning all eleven hourly samples were used in the analysis. Postprandial and post weaning plasma insulin profiles were analyzed by the method of Shaw and Foxcroft (1985) using a sliding windows technique to determine the mean plasma concentration of insulin secretion.

Episodic LH frequency was determined by visual appraisal using the method of McLeod and Craigton (1985): briefly, a pulse is defined as any increase in LH concentration within two successive sampling intervals followed by a decline with at least three sampling intervals between the peak and succeeding base line, occurring at a rate approximating the half life of the hormone.

RESULTS

Sow body weight and backfat did not differ significantly amongst treatment groups at farrowing (Table 3.2). To appetite feed consumption of group AA and AR sows increased from farrowing to day 21 and group AA sows further increased their feed consumption until weaning ($P<.05$). All sows exhibited a loss of body weight and backfat from farrowing to day 21 ($P<.05$). At day 21 body weight and backfat of group RA sows was lower than group AA or AR sows ($P<.001$). Reduction of feed intake for group AR sows from day 22 to 28 did not increase the rate of weight loss, however increased feed consumption of group RA sows resulted in an increase in body weight ($P<.05$). At day 28, weight and backfat in group AR sows was not different from groups AA or RA, however, group RA sow weight and backfat was still lower ($P<.05$) than group AA although not different to group AR. Overall, during lactation, the amount of weight and backfat loss by group AR and RA sows did not differ ($P>.05$) and both were greater than group AA sows ($P<.05$). Overall piglet growth rate did not differ among treatment groups.

Mean plasma insulin concentration revealed a significant day*treatment interaction ($P<.005$). Analysis of treatment by day revealed that post-prandial mean plasma insulin

concentration was not different between day 21 and day 28 for group AA, whereas it was lower (P<.05) at day 28 than day 21 for group AR sows and higher (P<.05, for both) at day 21 than day 28 for group RA sows. In response to weaning, mean plasma insulin concentration decreased (P<.05) for both group AA and RA sows and remained unchanged for AR sows when compared to day 28 of lactation. Analysis of treatment within day, revealed no differences in preprandial mean plasma insulin concentration at day 21 or day 28 (P>.1), as shown in Table 3.3. Postprandial mean plasma insulin concentration at day 21, was lower (P<.05) for group RA than group AA, but neither was different to group AR sows. At day 28, mean plasma insulin concentration did not differ amongst treatment groups. After weaning no differences in mean plasma insulin concentrations were observed amongst treatments. Regression analysis (Figure 3.3) established an association between post-prandial mean plasma insulin concentration at both day 21 and day 28 of lactation and mean feed intake (day 21: r=.62, P<.001, day 28: r=.59, P<.003). As a consequence, feed intake was fitted as a covariate for insulin status in the repeated measures analysis and no effects of treatment, day or day*treatment interaction were then observed. Figure 3.4 and 3.5 demonstrate the temporal change in mean plasma insulin concentration to different feed intakes at day 21 and day 28 of lactation.

There was a significant day*treatment interaction for mean plasma IGF-I (P<.0001). Mean plasma IGF-I concentration was similar at day 21 and day 28 of lactation for group AA sows, whereas mean plasma IGF-I concentration from day 21 to 28 decreased (P<.001) and increased (P<.05) for group AR and RA sows respectively. At weaning mean plasma IGF-I concentration was not different from day 28 values. Analysis of treatment within day revealed that mean plasma IGF-I concentration at day 21 was lower (P<.01) in group RA sows than the remaining groups. At day 28 mean plasma IGF-I concentration was lower (P<.01) in AR sows than groups AA or RA, but still lower (P<.05) in group AR sows than either group AA or RA after weaning (Table 3.3).

There was a day* treatment interaction for LH pulse frequency (P<.002) and mean plasma LH concentration (P<.003) during lactation. Feed restriction of AR sows tended to decreased LH pulse frequency (P<.1) and significantly (P<.01) decreased mean plasma LH concentration. Conversely, refeeding of group RA sows increased (P<.02) episodic LH release frequency and mean plasma LH concentration (P<.03). Continued feeding to appetite in group AA sows had no effect on LH pulse frequency (P<.25) or mean plasma LH concentration (P>.96) (see Table 3.4 and Figure 3.6) . Analysis of treatment within day, revealed that day 21 LH pulse frequency and mean plasma LH concentration were lower (P<.03 and P<.1, respectively) in group RA sows than in AA or AR sows. At day 28 LH pulse frequency was greater (P<.05) in group RA sows than AA sows and neither was different from group AR sows. Mean plasma LH concentration at day 28 was greater (P<.05) in group RA sows than AR sows and neither was different from group AA sows. Irrespective of treatment all sows exhibited an increase in both LH pulse frequency (P<.0001) and mean LH concentration (P<.0002) in response to weaning (Figure 3.7). There were no significant treatment or interaction effects on mean plasma FSH concentration. All treatment groups exhibited an increase (P<.02) in mean plasma FSH concentration after weaning. The time to first post weaning estrus was significantly shorter (P<.05) for group AA sows than for AR

and RA sows. Ovulation rate was greater (P<.05) in AA sows than in group AR or RA sows. Finally, embryo survival was lower (P<.05) in AR sows than group AA or RA sows.

DISCUSSION

Differential sow weight and back fat changes were achieved by the feeding regimen imposed. Mobilization of around 12 kg of body tissue and 3 mm of back fat even in AA sows, is consistent with the losses reported by King and Williams (1984) and Esbenshade et al. (1985). Gross carcass analysis of the primiparous sow after a 31 day lactation demonstrated that both fat and muscle is catabolized (Mullan and Williams, 1990) and plasma glycerol concentrations are greater towards the end of a 28 day lactation than at day 2 (Baidoo, 1989), indicating an increase in lipolysis. Overall group AR and RA lost approximately 22 kg of body weight and 5 mm of backfat over lactation. Even though there were only six piglets suckling per sow the growth rate of each poglet was approximately 25% greater than for piglets in conventional litters of 8-10 piglets in our herd (Clowes et al., 1994). Consistent with the data reported by Prunier et al. (1993), Armstrong et al. (1986) and Baidoo (1989) no treatment differences were observed in litter weight gains despite the differing pattern of weight and backfat losses in AR and RA sows. Thus the partitioning of nutrients during lactation is prioritized towards milk production (Aherne and Kirkwood., 1985) at the expense of catabolizing maternal tissue. The differences we observed in post weaning reproduction are due, therefore, to the extent and timing of catabolic losses during lactation. Indeed data arising from the post partum dairy cow clearly demonstrated that temporal changes in energy balance towards a relatively less catabolic state after the energy balance nadir was positively correlated with the days to first ovulation (Zurek et al., 1995).

Preprandial plasma insulin concentration at day 21 and day 28 was not different amongst treatment groups. Similarly preprandial insulin concentrations, based on a single sample taken at day 6, 12, 20 and 22 of lactation were not different between sows fed ad libitum or restricted to 43.5 MJ ME/d (Armstrong et al., 1986). Again, no differences were found between preprandial insulin in prepubertal gilts (at 75 kg body weight) either fed to appetite or restricted to maintenance feeding for 7 days (Booth et al., 1995). Anderson (1974) has shown that gastrointestinal absorption of nutrients continues for 18 to 20 hours in the gilt. Therefore, the 14 hour duration of the over night fast in the present study, may not have been sufficient for the animals to enter a true fasting state. The postprandial response of insulin is proportional to feed intake in sheep (Bassett et al., 1974); similarly, in the present study feed intake was correlated to mean plasma insulin concentration.

The finding that periods of feed restriction significantly reduced plasma IGF-I concentration is consistent with the results from the study by Tokach et al. (1993) in which primiparous sows exhibiting an increased weaning to estrus interval had lower plasma IGF-I concentration than those returning within 7 days (40 vs 76 ng/ml). Realimentation of prepubertal gilts restricted to maintenance feeding for 7 days was associated with an increase in plasma IGF-I from approximately 58 to 80 ng/ml over a two day period (Cosgrove et al., 1992, Charlton et al., 1993). Indeed using the same experimental model, significant increases

in IGF-I were only observed after 30 hours of refeeding (Booth, 1990). In the lactating sow therefore, periods of feed restriction are characterized by elevated plasma growth hormone concentration (Baidoo, 1989), associated with a change in insulin status and depressed IGF-I concentration (present findings): Thus, as in the feed restricted gilt model (Charlton et al., 1993) the normal GH/IGF-I axis is apparently altered. This may have evolved as a protein sparing mechanism to favor lipolysis and thus make FFA available as a substrate for peripheral tissue (Newsholme and Leech, 1984). A concomitant decrease in IGF-I and LH associated with feed restriction was described in the cow (Richards et al., 1991). There is however, a paucity of data pertaining to the role of IGF-I in the regulation of LH secretion. IGF-I receptors are present in the median eminence (Lesnaik et al., 1988) and pituitary (Rosenfeld, 1984) in rats and incubation of prepubertal rat median eminence with IGF-I elicited a dose dependent increase in GnRH (Hiney et al., 1991).

In the lactating sow LH secretion is predominantly controlled by the suckling stimulus (Foxcroft, 1992) and is inversely related to the intensity of suckling stimulus (Grant, 1987; Varley and Foxcroft, 1990), as concluded from Grants' experiment in which a reduction in the number of piglets in the litters (split weaning) increased mean plasma LH within 48 hours. Overall the LH response was greatest in those sows whose unused teats were covered, as opposed to sows in which the remaining piglets were allowed to access to multiple teats during suckling. Subsequently, work by Mullan et al. (1991) in the lactating sow demonstrated that mean plasma LH concentration before weaning was dependent on the number of piglets suckling (6 versus 12) and not on feed intake (3 kg versus 6 kg/d).

The present experimental model was designed to allow for dynamic changes in metabolic status to be investigated in a situation where the suckling induced suppression of LH was reduced by standardizing litters to 6 piglets. The data presented clearly demonstrates that irrespective of the suckling induced suppression of LH secretion, feed restriction for a period of 7 days (Group AR) reduces plasma LH secretion, and conversely, realimentation for 7 days (Group RA) increases pulsatile LH secretion. These results are comparable with those of Koketsu (1994) and to the results of the study by King and Martin (1989) in which sows fed high protein (175 g/d) compared to low protein (103 g/d) isocaloric diets during a 22 day lactation, exhibited greater mean LH concentration. In the seven day realimentated prepubertal gilt model (Booth et al., 1996) refeeding to appetite after a period of maintenance feeding doubled LH pulsatility within five hours. In the boar (Cosgrove et al., 1993) a 48-hour fast was associated with decreased LH pulsatility. Indeed the rapidity with which LH responds to feed restriction or realimentation has been demonstrated in a number of species, including man (Cameron et al., 1991), rat (Bronson, 1987) and sheep (Foster et al., 1989). Similarly, in the monkey, gastric infusion of nutrients into a previously fasted subject increased LH pulsatility within a few hours (Schreihofer et al., 1993). These widely established effects of feed intake on LH secretion have been reviewed by I'Anson et al. (1991). Effects of feed restriction on LH release have been associated with a decrease in GnRH gene expression in the medial pre optic area of the rat (Grenewald and Matsumoto, 1993) and treatment with the excitatory amino acid, N-methyl D-aspartate (NMDA), was effective in overcoming the block to LH secretion in feed restricted lambs (Ebling et al., 1989). In the present experimental model in which periods of feed restriction reduced LH

pulsatile release when compared to periods of feeding to appetite, it is important to remember that even animals fed to appetite were in varying states of catabolism.

Central insulin has been hypothesized as a regulator of energy balance (Schwartz et al., 1993). In the rat, insulin receptors have been identified in various brain structures, including the hypothalamus, arcuate nucleus (Werther et al., 1987) and median eminence (Van Houten et al., 1979). Oomura and Kita, (1985) have demonstrated that concomitant infusion of insulin and glucose increased the electrical activity of hypothalamic neurons in the rat. Indeed, isolated, perfused rat mediobasal hypothalamic tissue exhibited a synergistic increase in GnRH release only when insulin (10 mU/l) and glucose (10-30 mg/l) are present (Arias et al., 1992).

Intracerebroventricular (ICV) infusion of insulin (0.6 ng/h), or glucose (50 μM/h) plus insulin, for 12 hours per day for 4 days at a rate of 5 ul/min, into maintenance fed mature, intact rams resulted in an increase in LH pulsatility comparable to that observed in animals fed to maintenance and then given a lupin supplement (Downing et al., 1995). However in a previous study by Hileman et al. (1993), ICV injection of 500 ng insulin into ovariectomised lambs which had been realimentated for two weeks after being fed to maintain a body weight of 18.5 kg for 22 weeks, decreased LH pulsatility, associated with a concomitant hypoglycemic state. In the ovariectomized prepubertal gilt (Cox et al., 1989), 6 ng of insulin injected ICV increased LH pulsatility after 3 hours and increased mean plasma LH concentration after 45 minutes, independent of any change in peripheral glucose concentration. A peripheral bolus injection of D-glucose (equivalent to 11.6MJ ME) to maintenance fed, prepubertal gilts doubled the number of LH episodes within 6 hours (Booth , 1990). In contrast, infusion of glucose for a 12 h period into a feed restricted (27.2MJ ME/d) lactating sow (day 18) did not increase LH release although plasma insulin and glucose were concurrently increased (Tokach et al., 1992). Rojkittikhun et al. (1992) reported in the sow, that a 24 hour fast and subsequent refeeding at day 27 of lactation, decreased and then increased plasma insulin and glucose but again LH mean concentration and pulsatility were not affected (.51 vs .53 ng/ml and 1.0 vs 1.3 pulses per 6 hours).

In response to weaning LH increases significantly (Edwards and Foxcroft, 1983) and the weaning to estrus interval has been correlated to LH pulse frequency before weaning (Armstrong et al., 1986; King and Martin, 1989) or after weaning (Shaw and Foxcroft, 1985). However these results have arisen from experimental designs that have effectively observed a cumulative effect of manipulating body condition/nutritional status during lactation. The results presented here and by Koketsu (1994) have clearly demonstrated that LH is affected by dynamic changes in nutritional status during lactation. As a consequence animals which have been exposed to just one week of increased catabolism will experience an extended weaning to estrus interval when compared to those maintained on a high plane of feeding. In contrast to data arising from other laboratories (Koketsu, 1994), the increase in the weaning to estrus interval in our study was very marginal, and this may have important consequences for the effects on ovulation rate. Indeed Tokach et al. (1992) found by modulating the nutritional status of primiparous sows by feeding a matrix of differing lysine and energy concentrations, sows returning early (<day 7) had greater LH as early as day 14 of lactation, and LH was correlated with insulin at day 7. For the studies by Koketsu (1994)

and Tokach et al. (1992) the lactation period was 3 weeks and sows had litter sizes of 8 to 10 piglets whereas in this study lactation length was 4 weeks and sows only had 6 piglets suckling, as such these differences in experimental designs may have contributed to the observed differences in weaning to estrus interval and ovulation rate. The significance of these findings is that changes in nutritional status during lactation may have relatively transient effects on LH episodic release; however, even a relatively short duration of increased catabolism may have lasting detrimental effects on folliculogenesis and as a consequence on the time to return to estrus and ovulation rate. A recent study by Zurek et al. (1995) found that the time to return to estrus after the post partum energy balance nadir in the dairy cow was correlated to the rate at which they were becoming less catabolic. These data support our belief that it is likely that dynamic changes in metabolic status are the essential regulators of the reproductive axis during lactation. The pattern of weight and backfat loss in groups AR and RA sows produced differing effects on the reproductive axis in the present study, although the net losses were similar between treatments. Thus in practice, targeting some particular change in body condition score over the whole of the lactation period may not be very helpful if the pattern of this tissue loss leads to the sow being increasingly catabolic towards the end of lactation.

Folliculogenesis in the lactating sow has not been fully elucidated (Britt et al., 1985), however, based on histological studies in the gilt, the maturation of antral follicles to the preovulatory size is estimated to take 19 days (Morbeck et al., 1992). To our knowledge, the significant decrease in ovulation rate associated with periods of restricted feeding during lactation in the present experiment has not been observed in previous studies in the sow. However a trend for lower (16 vs 20) ovulation rates was noted in sows which lost over 25 kg of body weight compared to those losing less than 10 kg during lactation (Rojkittikhun et al., 1992b). Interestingly in our experiment and that of Rojkittikhun et al. (1992b) the weaning to estrus intervals associated with body weight loss, in absolute terms, were not greatly increased (4.5 vs 5, and 7 vs 4 days respectively), whereas other researchers have typically reported increases in the weaning to estrus interval of 10 days or more (Koketsu, 1994). This probably enabled us to observe the effects of altered nutritional status on ovulation rate, by accelerating the cascade of events leading to recruitment and final selection of preovulatory follicles, with the consequence that final maturation occurred in a relatively adverse metabolic environment. Thus the reported lack of an effect of nutritional status on ovulation rate in many previous studies in the lactating and weaned sow may be analogous to the phenomenon of 'skip a heat breeding', in which there is an increase in litter size when primiparous sows are bred at second as opposed to first post weaning estrus. Alternatively, feeding to appetite during lactation (equivalent to Group AA) may normalize the number of preovulatory follicles. Nutritional flushing of the gilt from day 10 to 15 of the estrus cycle, or from day 15 to estrus caused an increase in the number of large follicles (Dailey et al., 1972). Similarly, flush feeding gilts for at least two estrus cycles increased ovulation rate compared to those which were flushed (46 MJ ME/d) until day 7 of the second cycle and then restricted to 22.6 MJ ME/d until ovulation (Flowers et al., 1988). The underlying endocrinology of flush feeding was investigated by Beltranana et al. (1991), and flush feeding was associated with increased IGF-I and insulin around the peri estrous period and an

increase in LH. Furthermore, follicular development in response to realimentation for a period of 5 days in the prepubertal gilt, as determined by an increase in basal granulosa cell estradiol production, follicular diameter and volume, has been shown to occur even when the rise in LH was abolished by the ingestion of allyl trenbolone (Cosgrove et al., 1992). The concept that aspects of ovarian development is directly affected by nutritional status has been recently reviewed by Giudice et al. (1993) and Ojeda and Dissen (1994). As an extension of earlier studies indicating that insulin could affect ovulation rate by regulating follicular atresia in the follicular phase (Cox et al., 1987), a streptozotocin induced diabetic gilt model was used by Meurer et al. (1991) to investigate the role of insulin in recruitment of preovulatory follicles. Pregnant mare serum gonadotropin (PMSG) was administered at the time that insulin support for the diabetic gilt was withdrawn.. The number of follicles greater than 3 mm, recovered at 75 hours after PMSG, was found to be similar in both diabetic and control animals in which insulin treatment was maintained, however the number of atretic follicles in diabetic gilts was greater than control. These differences in follicular development were not associated with changes in gonadotropin concentrations. Thus, as in the previous studies. insulin administration from day 17 to 19 reduced the number of atretic follicles. In the present study, exposure of the sow to relatively catabolic states during lactation has detrimentally affected the ovarian milieu, as expressed by increased weaning to estrus interval and reduced ovulation rates. Periods of increased catabolism in lactation may therefore result in 'imprinting' of the ovary and effects on both ovulation rate and embryonic survival. Feed restriction in the postpubertal gilt, (Britt et al., 1988), and feed restriction to 50% of ad libitum intake during the first two weeks of lactation in primiparous sows (Miller, unpublished observation), almost abolished the development of follicles greater than 3 mm, demonstrating that maturation of preovulatory follicles is detrimentally affected by an increased catabolic state. Analysis of presumptive preovulatory follicles (as based on expected ovulation rates and weaning to estrus interval) collected 48 hours after weaning in primiparous sows demonstrates there is a large degree of maturational heretogeneity as based on aromatase activity, hCG binding and estradiol concentrations (Foxcroft et al., 1987). Thus prior exposure of the follicle to 'adverse' environment may affect the developmental competency of more immature follicles to be recruited into the ovulatory population immediately after weaning, and may affect the maturational state of the oocyte at the time of ovulation and hence developmental competence.

The significant decrease in embryo survival associated with increased catabolic status prior to weaning is consistent with those data of Kirkwood et al. (1987) and Baidoo, (1989). in which restrict feeding second parity sows to 3 kg per day reduced embryo survival to approximately 64%, compared to 87% for those fed to appetite. The role of progesterone as a mediator of embryo survival has been suggested by Aherne and Kirkwood (1985) and in the gilt progesterone concentration at 72 hours after mating are correlated with embryo survival (Jindal et al., 1996). Evidence for similar associations in the sow in which increased catabolism immediately before weaning has detrimental effects on embryonic survival would be interesting.

In conclusion therefore, the present experimental model appears to have produced an almost unique effect in that the different patterns of lactational catabolism have produced

differential effects on ovulation rate and embryo survival. The collective data clearly confirm that metabolism has both central effects on gonadotropin secretion and potential local effects on the ovary. Collectively, these effects may result in an imprinting effect on the ovary that may affect fertility and fecundity for some time after weaning. In addition, some of the metabolic sequelae of lactational catabolism, such as changes in IGF-I status, persist into the preovulatory period when sows return to estrus relatively soon after weaning and may continue to affect the process of follicle selection and final maturation for ovulation, as well as post ovulatory luteinization.

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Table 3.1 Composition of experimental diets (as fed based)

Item	Lactating sow	Gestating Sow
Ingredients, %		
Barley	40.4	56.4
Wheat	30.0	30.0
Soybean meal, 44% CP[1]	12.0	.7
Tallow	3.0	2.0
Bran	10.0	-
Calcium phosphate	1.7	1.7
Limestone	1.4	1.4
Vitamin-mineral premix[2]	1.0	1.0
Iodized salt	0.5	.5
Chemical Analysis		
Crude protein, %	15.4	13.7
Lysine, %	.74	.56
Metabolizable energy, MJ	13.4	13.4

[1]CP= Crude Protein

[2] Supplied the following per kg of diet: Zinc, 120 mg; Manganese, 12 mg; Iron, 150 mg';Copper, 12 mg; Selenium, 0.1 mg; vitamin A, 5000 IU; Vitamin D, 500 IU; Vitamin E. 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride. 840 mg; Vitamin B_{12}, 30μg; Biotin, 200 μg.

Table 3.2 Mean (± SE) feed intake, sow body weight and backfat and litter weights at weekly intervals during lactation.

	Treatment[a]		
	AA (n = 9)	AR (n = 9)	RA (n = 8)
Feed intake, kg			
day 1 to 7	3.3 ± .7[a]	3.5 ± .9[a]	1.7 ± .3[b]
day 8 to 14	4.6 ± .6[a]	4.7 ± .5[a]	2.3 ± .1[b]
day 15 to 21	4.4 ± .4[a]	4.2 ± .6[a]	2.1 ± .2[b]
day 22 to 28	5.2 ± .1[a]	2.1 ± .2[b]	5.3 ± .2[a]
Body weight, kg			
Farrowing	190 ± 7	193 ± 5	187 ± 7
day 7	188 ± 7	192 ± 6	178 ± 7
day 14	185 ± 7[a]	187 ± 6[a]	168 ± 7[b]
day 21	181 ± 6[a]	179 ± 6[a]	158 ± 8[b]
day 28	179 ± 7[a]	172 ± 4[ab]	163 ± 6[b]
Weight change, kg	- 11 ± 6[a]	- 21 ± 6[b]	- 25 ± 6[b]
Backfat, mm			
Farrowing	20.4 ± 1.5	18.5 ± 1.3	18.8 ± .7
day 7	19.7 ± 1.2[a]	18.5 ± .1[a]	16.3 ± 1.2[b]
day 14	19.6 ± .2[a]	18.0 ± .2[a]	14.4 ± 1.3[b]
day 21	18.2 ±1.3[a]	16.8 ± 1.0[a]	13.0 ± .9[b]
day 28	17.8 ± 1.4[a]	15.1 ± 1.4[ab]	13.4 ± 6.2[b]
Backfat change, mm			
Birth	9.0 ± 1.2	9.7 ± 1.0	10.2 ± .8
day 7	17.2 ± 1.3	18.2 ± 2.5	20.4 ± 2.8
day 14	28.6 ± 3.9	31.1 ± 4.2	31.0 ± 4.0

day 21	41.6 ±4.3	43.6 ±6.1	41.3 ±6.6
day 28	51.9 ±4.8	53.2 ±5.2	52.4 ±4.8
Growth rate, g/d	255 ±89	259 ±101	251 ±80

[a]AA, AR and RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AA = sows receiving feed intake to appetite from d 1 to 28, AR = sows receiving feed intake to appetite from day 1 to 28 then 50% of to appetite feed intake from day 22 to 28, RA= sows receiving 50% to appetite feed intake from day 1 to 21, then feed intake to appetite from day 22 to 28.

[abc]Means within rows lacking a common superscript letter differ (P<.05)

Table 3.3 Mean (± SE) pre and postprandial plasma insulin concentration and, mean IGF-I at day 21 and, before and after weaning at day 28 of lactation.

	Treatment[a]		
Item	AA (n = 8)	AR (n = 9)	RA (n = 8)
Insulin, ng/ml			
Preprandial day 21	1.4 ± .1	1.3 ± .0	1.2 ± .0
Postprandial day 21	4.4 ± .2[a]	3.2 ± .2[ab]	2.1 ± .2[b]
Preprandial day 28	1.3 ± .2	1.4 ± .2	1.7 ± .3
Postprandial day 28	3.4 ± .3	1.7 ± .6	3.5 ± .2
After weaning	2.0 ± .2	1.8 ± .2	2.1 ± .3
IGF-I, ng/ml			
day 21	78 ± 8[a]	60 ± 9[a]	32 ± 8[b]
day 28	74 ± 6[a]	38 ± 7[b]	65 ± 6[a]
After weaning	74 ± 11[a]	36 ± 9[b]	69 ± 10[a]

[a]AA, AR, and RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AA = sows receiving feed intake to appetite from day 1 to 28, AR = sows receiving feed intake to appetite from day 1 to 21 then 50% of to appetite feed intake from day 22 to 28, RA = sows receiving 50% to appetite feed intake from day 1 to 21, then feed intake to appetite from day 22 to 28.

[abc] Means within a row lacking a common superscript letter differ (P<.05)

Table 3.4 Mean (± SE) plasma LH concentration (ng/ml) and LH pulse frequency (per 12 hours) at day 21 and 28, before and after weaning, weaning to estrus interval (h), ovulation rate (OR) and embryo survival (ESR; %)

	Treatment[a]		
Item	AA (n = 9)	AR (n = 9)	RA (n = 8)
Mean LH, ng/ml			
day 21	.12 ± .02	.14 ± .02	.09 ± .02
day 28	.12 ± .02[ab]	.09 ± .02[b]	.18 ± .02[a]
After weaning	.23 ± .06	.23 ± .36	.25 ± .04
LH frequency, pulses/12 h			
day 21	1.77 ± .54[a]	1.92 ± .44[a]	.14 ± .50[b]
day 28	.93 ± .49[a]	1.10 ± .39[ab]	2.30 ± 0.45[b]
After weaning	9.00 ± 2.50	7.08 ± 1.03	9.25 ± 1.26
FSH, ng/ml			
day 28	24.1 ± 4.7	35.8 ± 3.6	37.9 ± 4.0
After weaning	35.4 ± 5.9	40.6 ± 4.6	40.2 ± 5.1
WEI	88.7 ± 11.2[a]	122.3 ± 9.8[b]	134.7 ± 8.7[b]
OR	19.9 ± 1.6[a]	15.4 ± 2.3[b]	15.4 ± 1.9[b]
ESR	87.5 ± 6.4[a]	64.4 ± 6.1[b]	86.5 ± 7.6[a]

[a] AA, AR and, RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AA = sows receiving feed intake to appetite from day 1 to 28; AR = sows receiving feed intake to appetite from day 1 to 21 then 50% of to appetite feed intake from day 22 to 28; RA = sows receiving 50% to appetite feed intake from day 1 to 21, then feed intake to appetite from day 22 to 28.

[abc] Means within rows lacking a common superscript letter differ (P<.05).

Figure 3.1 Schematic diagram of the experimental design. Lactational feeding pattern was imposed from day 0 to 28. Sows were either fed to appetite (100%) or restricted to 50% (50%) of to appetite. At day 19 (D19) surgery was performed for the insertion of a cephalic catheter. At day 21 (D21) a 12 h intensive bleeding window was performed and for 12 hours before and after weaning at day 28 (D28). At 12 and 24 hours after standing heat (SH) sows were artificially inseminated (AI) using mixed semen. At day 28 after standing heat all sows were slaughtered.

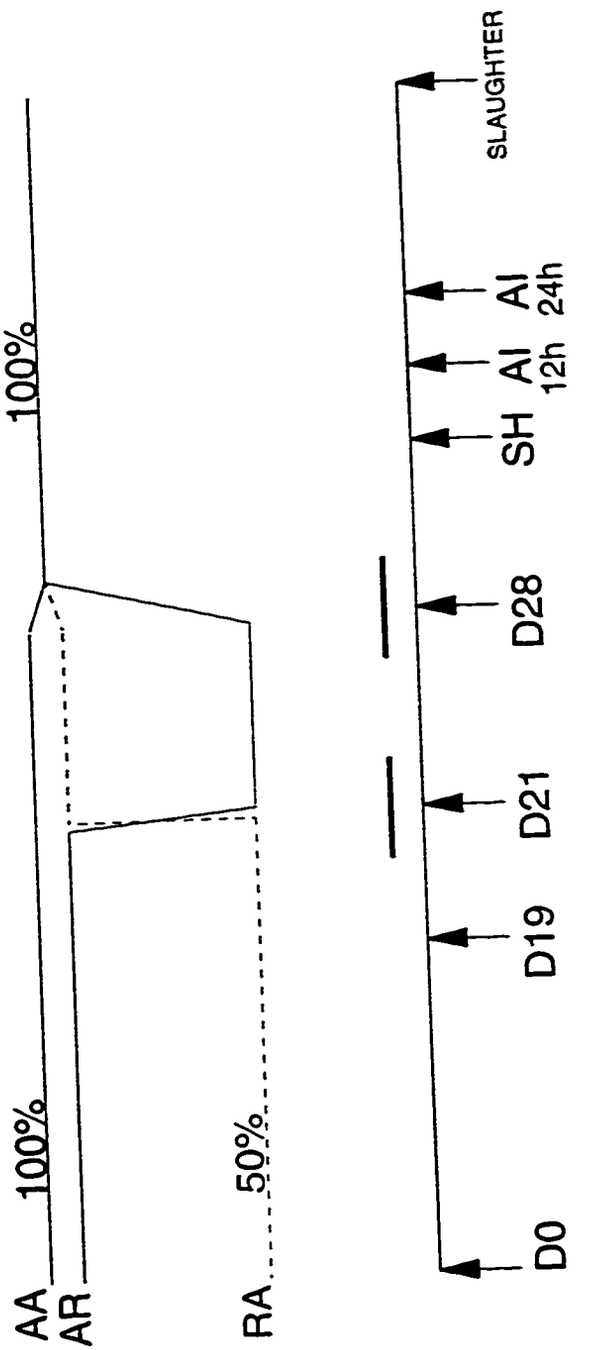

AA
AR
100%
100%
RA
50%
D0
D19
D21
D28
SH
AI 12h
AI 24h
SLAUGHTER

Figure 3.2 Linear regression between mean daily feed intake and mean plasma insulin concentration at both day 21 and 28 of lactation. $Y=1.14 + .58x$, $r=.67$, $P<.01$.

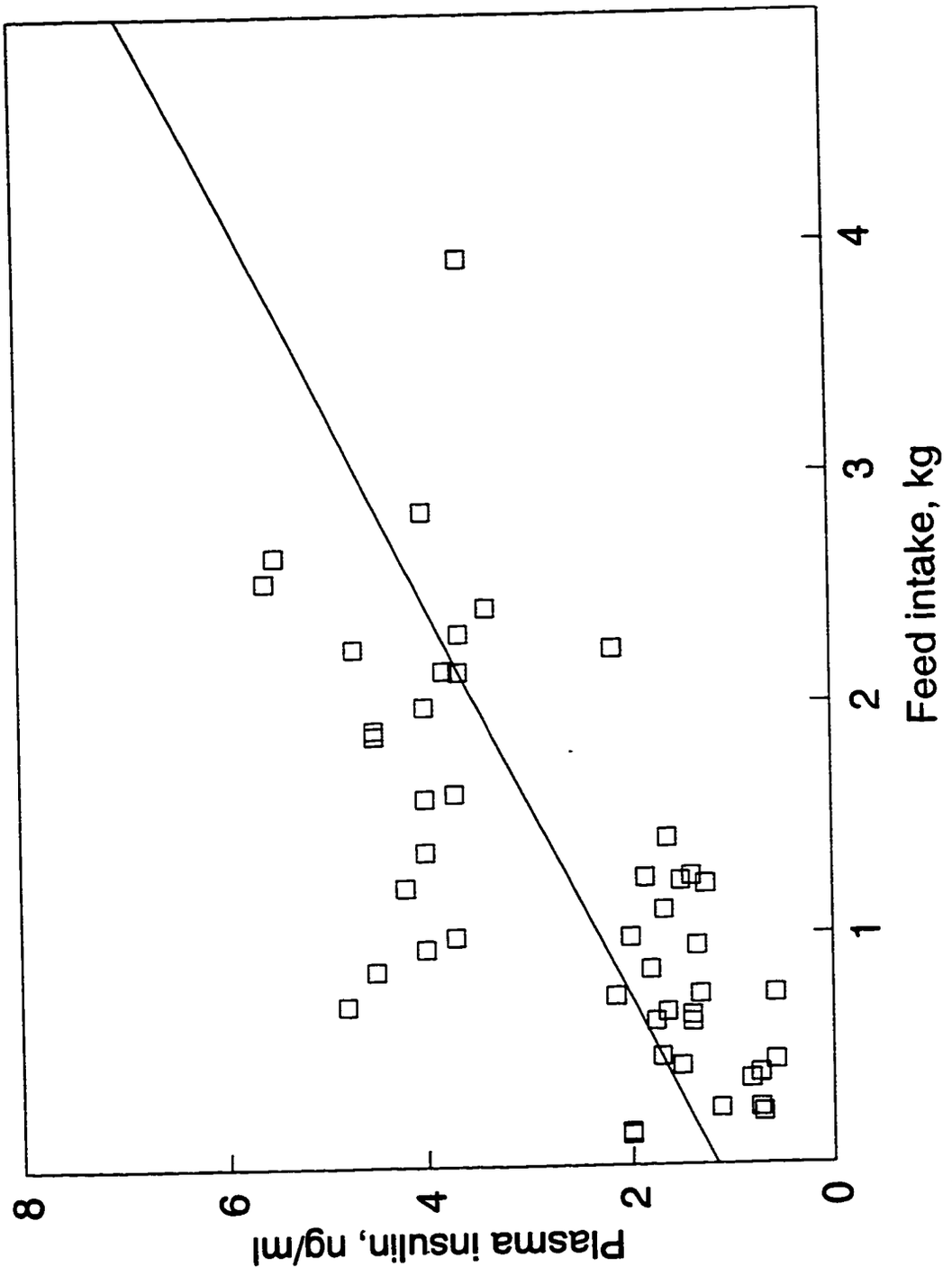

Plasma insulin, ng/ml
0
2
4
6
8
Feed intake, kg
1
2
3
4

Figure 3.3 Mean plasma insulin response to feed intake of sow # 244 (Group AA) at day 28 of lactation to eating 1.7 kg of food at 0800 and 2 kg of food at 1300 h (a) and sow # 233 (Group AA) at day 28 of lactation to eating 1.8 kg of food at 0800 and and 2 kg of food at 1300 (b).

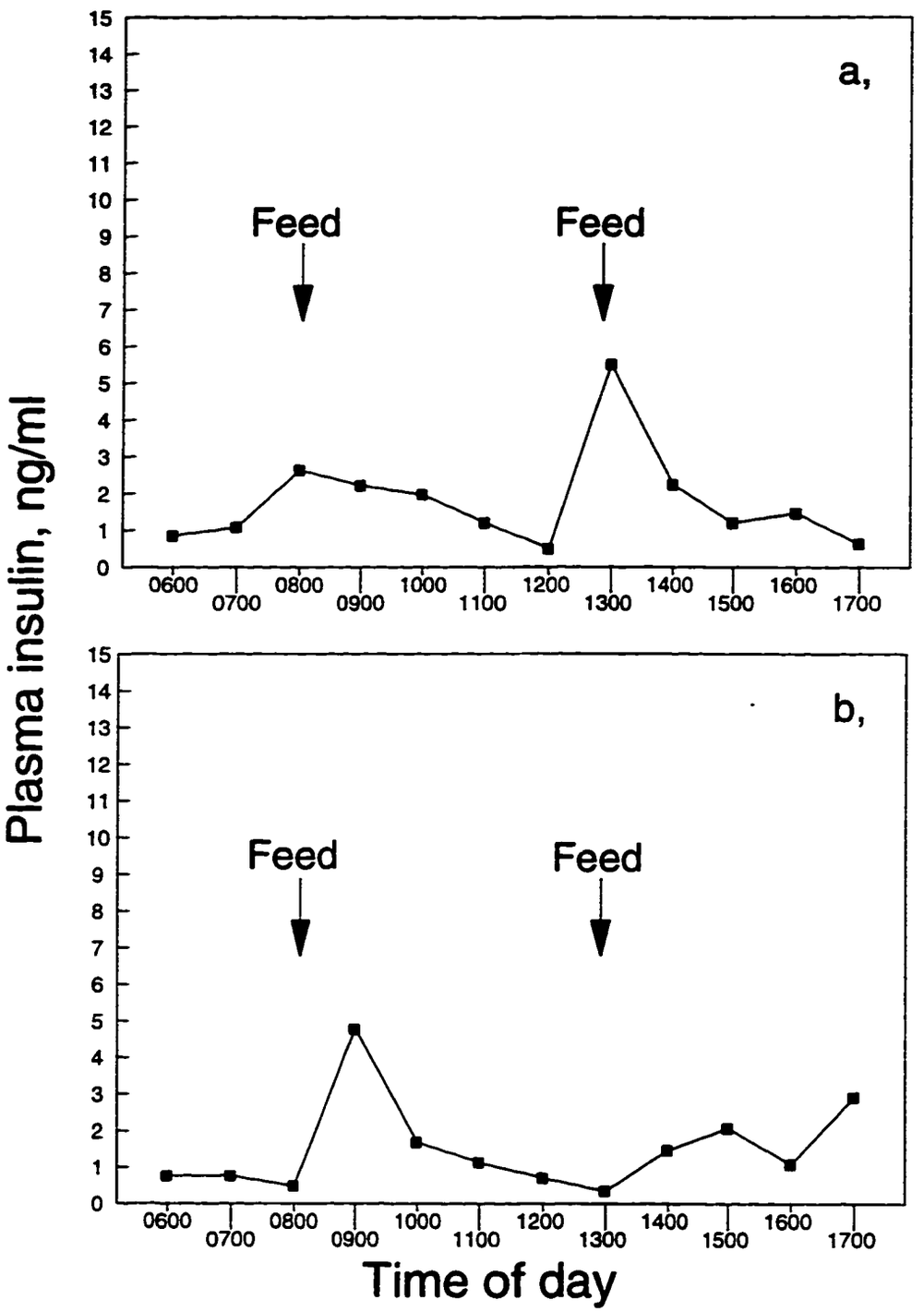

a,
b,
Feed
Feed
Feed
Feed
Plasma insulin, ng/ml
Time of day
0600
0700
0800
0900
1000
1100
1200
1300
1400
1500
1600
1700
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 3.4 Mean plasma insulin response to feed intake of sow #237 (group AA) at day 28 of lactation to eating 3.37 kg of food at 0800 h and 0 kg of food at 1300 (a) and sow # 233 (Group AA) at day 21 of lactation to eating 2.8 kg of food at 0800 and 2.7 kg of food at 1300 (b).

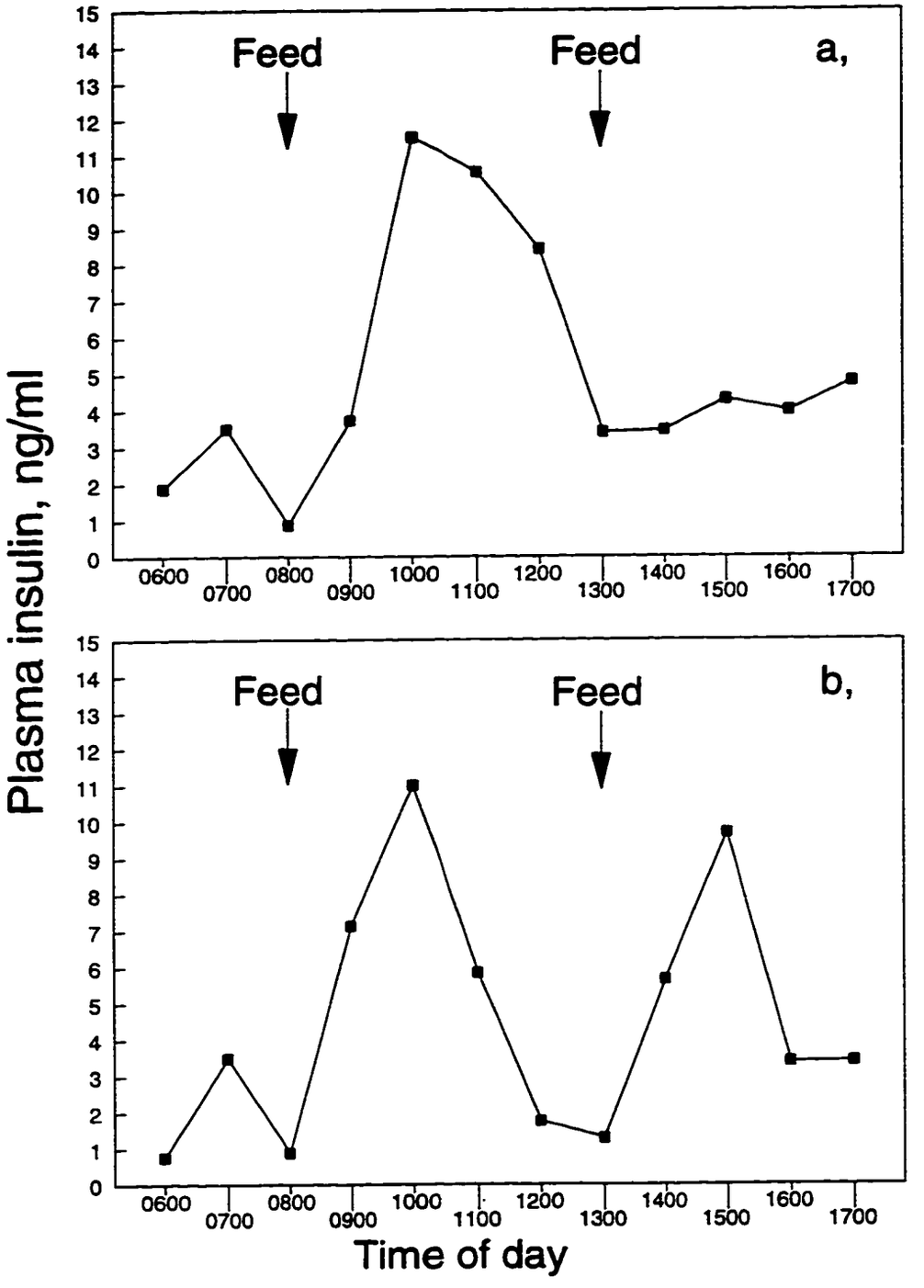

Plasma insulin, ng/ml
Feed
Feed
a,
b,
0600
0700
0800
0900
1000
1100
1200
1300
1400
1500
1600
1700
Time of day

Figure 3.5 Representative plasma LH profiles during a 12-hour bleeding window on day 21 and 28 of lactation, in sow # 247 (Group AA), sow # 32 (Group AR) and sow # 43 (Group RA), respectively.

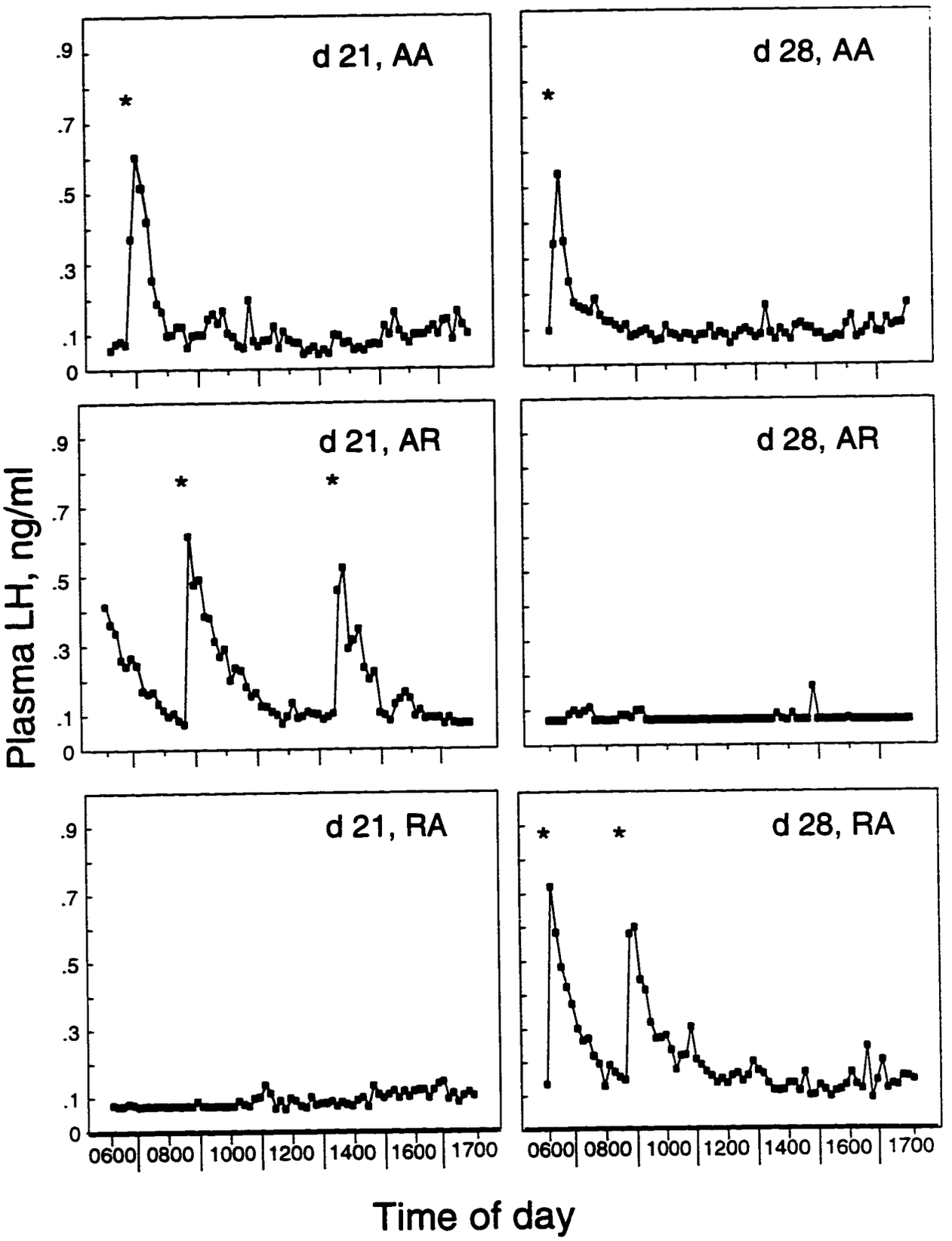
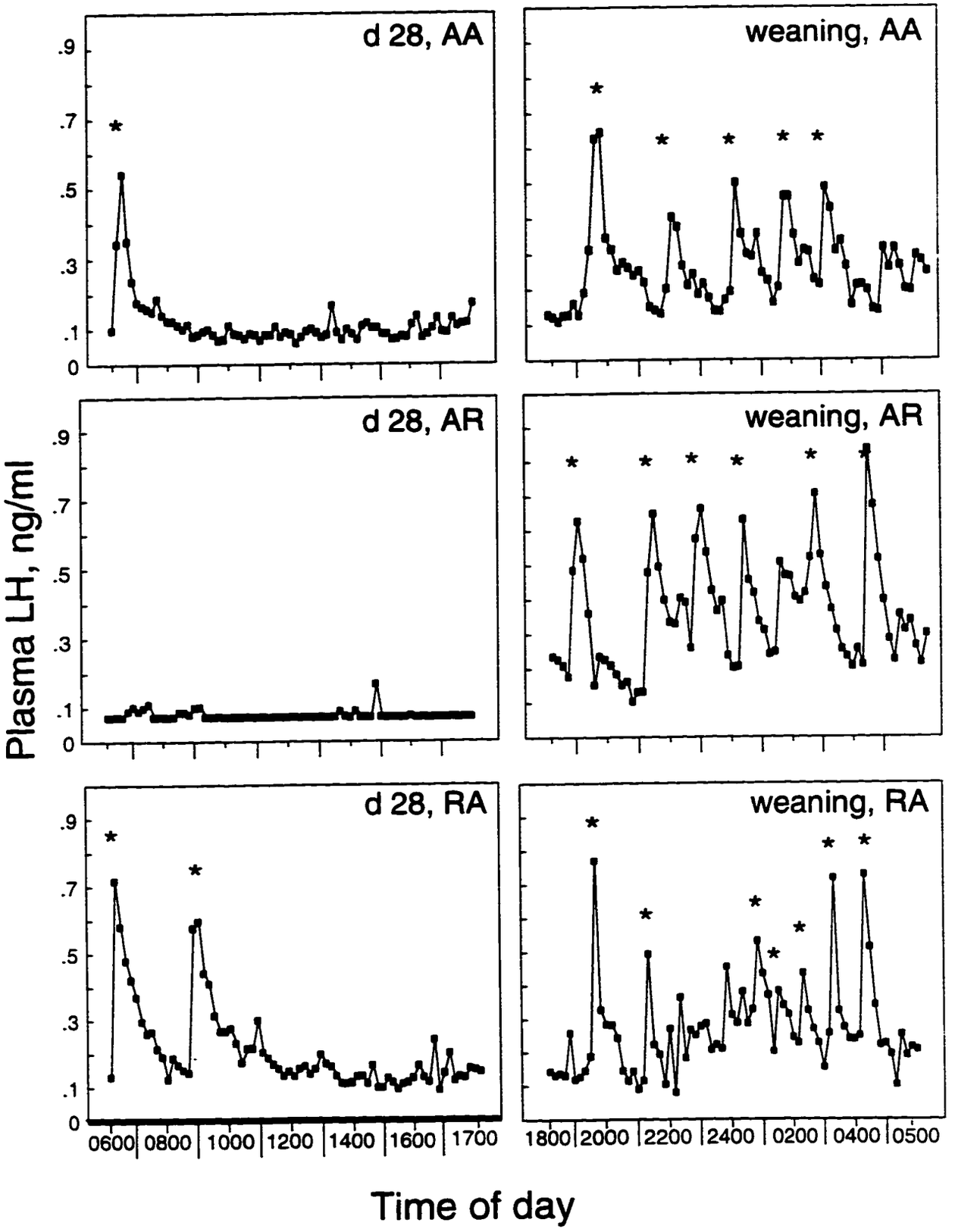

d 21, AA
d 28, AA
d 21, AR
d 28, AR
d 21, RA
d 28, RA
Plasma LH, ng/ml
0
.1
.3
.5
.7
.9
0600
0800
1000
1200
1400
1600
1700
Time of day

Figure 3.6 Representative plasma LH profiles during a 12-hour bleeding window at day 28 before weaning and immediately after weaning in sow # 247 (Group AA), sow # 32 (Group AR) and sow # 43 (Group RA) respectively.


d 28, AA
weaning, AA
d 28, AR
weaning, AR
d 28, RA
weaning, RA
Plasma LH, ng/ml
0
.1
.3
.5
.7
.9
0600
0800
1000
1200
1400
1600
1700
1800
2000
2200
2400
0200
0400
0500
Time of day

CHAPTER 4
IMPACT OF DIFFERENT PATTERNS OF FEED INTAKE DURING LACTATION IN THE PRIMIPAROUS SOW ON FOLLICULAR DEVELOPMENT, AND OOCYTE MATURATION

INTRODUCTION

The variance in embryo survival in the pig is large, and on average only 75% of blastocysts recorded at day 9 of pregnancy survive until day 25 (Pope and First. 1985). A high proportion of loss occurs around the time of maternal recognition of pregnancy and implantation (day 12 to 18) when the more advanced embryos secrete estradiol which acts as a signal to prevent luteolysis (Bazer and Thatcher, 1977) and promotes changes in the uterine milieu (Davis and Blair. 1993). It has been demonstrated that the uterine environment per se significantly affects the developmental competence of the blastocyst. At the time of maternal recognition of pregnancy, an asynchronous array of developmental stages exists within the blastocyst population (Pope, 1988) and it has been hypothesized that the initiation of changes in the uterine environment by more advanced blastocysts may be detrimental to the development of less mature embryos (Pope et al., 1990).

The origins of the asynchronous development of embryos at the time of maternal recognition of pregnancy may, at least in part, be explained by follicular heterogeneity within the preovulatory pool. In the sow, analysis of presumptive preovulatory follicles at 48 h after weaning revealed a range of follicle diameters, estradiol content and hCG-receptor binding (Foxcroft et al., 1987). Similarly in the gilt, Hunter et al. (1989) noted that at d 1 of the estrous cycle, follicles of the presumed periovulatory pool exhibited a range of diameters and steroid concentrations. Furthermore, pig oocytes incubated with media conditioned from large preovulatory-type follicles exhibited a more advanced nuclear maturation than those oocytes incubated with media conditioned with small follicles (Ding and Foxcroft, 1994a).

It was therefore suggested that heterogeneity within the follicle population is likely to influence oocyte maturation and could have consequences for embryo developmental competence (Hunter and Weisak. 1990). In an experiment in which the distribution of oocyte maturation at 13 h before ovulation was compared to zygotic maturation in a comparable set of gilts, Xie et al. (1990a) found that the skewdness of oocyte development continued into the zygotic population. More recently Xie et al. (1990b) established that late ovulating follicles gave rise to less well developed embryos at day 4 of gestation in the gilt. Furthermore, the observed diversity of embryo development at 160 hours after hCG injection (cell cycle number) is not related to duration of ovulation (Soede, 1992). Together these data suggest that the physiological state of the follicle, and hence the maturational state of the oocyte may give rise to embryos which exhibit different abilities to develop within the same uterine environment.

Experimental paradigms involving manipulation of feed intake around the time of estrous in the gilt (Pharazyn et al., 1992; Jindal et al., 1996), or during lactation in the sow (Baidoo et al., 1992; Zak et al., in press) have been shown to influence embryo survival. The concept that nutritionally induced manipulation of embryo survival may not only involve changes in

the uterine environment but also alterations in follicular and oocyte maturity have not previously been addressed in the literature.

The present experiment utilized a model in the primiparous, lactating sow in which differing patterns of feed intake caused differences in embryo survival (Zak et al., in press), to test the hypotheses that the observed differences in embryo survival between may in part, be due to differences in oocyte quality in the presumptive preovulatory pool (part 1) and that such differences in oocyte quality may in turn be related to differences in the ability of the follicle to support oocyte maturation (part 2).

MATERIALS AND METHODS

Experimental design

At farrowing, 32 primiparous Camborough sows (Pig Improvement (Canada) Ltd), randomly stratified according to sow weight at farrowing and the number of piglets born, were allocated to one of two treatments. All sows were fed a wheat-barley-soybean diet formulated to provide 13.4 MJ ME/kg, 15.4% crude protein and .74% lysine (Table 4.1) throughout a 28 day lactation (28.2 ± .2). Sows allocated to group AR (n = 16), were fed on an ad libitum basis from farrowing until day 21 and then restricted to 50 % of the average consumption of the previous 5 day period from day 22 to28. Group RA (n = 16) sows were restricted to 2.3 kg from farrowing until day 21 and then fed on an ad libitum basis from day 22 to28. Water was available at all times to the sow and piglets throughout the experimental period. As in our previous study (Zak et al., 1996) all litters were standardized to six piglets within 48 h of farrowing. Creep feed was not available. Sow weight and backfat (65 mm from mid line at 10th rib) and litter weights were recorded at farrowing, and at day 21 and 28 of lactation, and sow weight and backfat were again recorded at slaughter. From weaning until slaughter all sows were allowed to consume a diet formulated to provide 13.4 MJ ME/kg, 13.7% crude protein and .56% lysine on an ad libitum basis.

After weaning until the day of slaughter, blood samples (5 ml) were taken via acute venepuncture of an ear vein for the determination of plasma estradiol concentration at 24, 48, and 72 hours after weaning. Sows were tested twice daily at 0700 and 1900 for the onset of standing estrus using direct exposure to a vasectomized boar for 15 minutes. Time of slaughter was determined so that preovulatory oocytes recovered at the same stage of follicular development could be matured in vitro utilizing a standard maturation medium (Figure 4.1). In this population of animals (R. Jindal, unpublished observation) the onset of behavioral estrus occurs 9 hours before the LH surge. Based on the previously observed time to return to estrus (Zak et al., in press) of 122.3 ± 9.8 h and 134.7 ± 8.7 h in AR and RA sows respectively, animals in replicate I were slaughtered at 4.5 days (108 h) after weaning. Although weaning to estrus interval between the treatment groups (as based from the data in Zak et al., in press) were not different between treatments (P > .05), 2 out of 6 sows in Group AR, compared to no sows in Group RA had already ovulated by the time of slaughter. To maximize the number of animals completing the study, individual treatment means (Zak et al., in press) were thereafter used to determine the time of slaughter. In replicates II and III the time of slaughter was 83.5 h (83.5 ± 2.4) for AR and 97 h (97 ± 1.6) for Group RA

after weaning, thus animals in each group were slaughtered at 38 hours before the anticipated onset of estrus (equivalent to day 19 to 20 of cycle).

All procedures carried out in this experiment were approved by the University of Alberta Animal Care Committee to ensure adherence to Canadian Council of Animal Care guidelines.

Part one

This experiment was designed to test the hypothesis that nutritionally mediated effects on the pattern of tissue catabolism during lactation may affect the quality of oocyte/cumulus complexes recovered and matured in an in vitro maturation system, as measured by cumulus expansion and nuclear maturation.

Ovaries were obtained from the slaughtered sows, placed in individual plastic bags and transported to the laboratory within 40 minutes in a polystyrene box to prevent major fluctuations in temperature. The following experimental procedures were carried out at a room temperature of 22 to26 °C. Ovaries were washed four times in saline. The external diameter of the largest 15 follicles per sow were measured by taking a mean of two measurements at 90° to one another. These follicles were chosen on the assumption that at least 15 follicles would ovulate (ovulation rates ; AR = 15.4; RA = 15.4; Zak et al., in press) and that the largest follicles present in the late follicular phase represent the presumptive ovulatory population (Foxcroft et al., 1987). The 15 largest follicles were aspirated using a 18 gauge needle and 1 ml syringe. The weight of the needle and syringe before and after aspiration was measured and the difference between the two weights was calculated as the weight of the follicular fluid and assuming a density of 1 g/ml, the volume of follicular fluid was calculated.

Oocytes were removed from the follicular fluid and classed as either denuded or having intact cumulus cells. Detailed techniques for the in vitro culture of cumulus-oocyte complexes were as described by Ding and Foxcroft, (1994b) with some modification. Briefly, oocytes from each sow were incubated in a 35 ml plastic petri dish containing 1.8 ml of Tissue culture medium 199' (TCM 199) supplemented with 200 μl follicular fluid obtained from a pool of randomly selected large, viable follicles present on the ovaries of prepubertal gilts, (Funahashi and Day, 1993) and 100 μl of gonadotropins (2.5 μg/ml NIADDK-oLH-26, AFP-551b; 2.5 μg/ml USDA-pFSH-B-1, AFP-5600) and prolactin [1] (20 ng/ml USDA-pprl-B-1, AFP-5000). Because our objective was to determine nutritionally mediated effects on oocyte development, we excluded the addition of glutamine, L-absorbic acid and insulin. The aspiration procedure was completed within 3 hours. Culture was carried out under an atmosphere of 5 % CO_2 in air at 39 °C for 46 ± 1 hour. The degree of cumulus expansion was then recorded immediately after culture, as described below. The state of nuclear maturation after fixing and staining the oocytes was as described below.

A fraction of follicular fluid from individual follicles was diluted 1 : 500 with TCM 199 and frozen at - 30 °C for analysis of follicular fluid estradiol concentration. The remaining follicular fluid was pooled within sow, ensuring an equal contribution from individual follicles, and filtered using a millipore filter (.2 μm). Pooled follicular fluid was then diluted to 10 % with TCM 199 and frozen (- 30 °C) in 1 ml aliquots for the second part of this study.

Part two

This experiment was designed to address the hypothesis that nutritional status of the sow and related metabolic changes during lactation may affect the composition of the follicular fluid and hence its ability to support oocyte maturation. Randomly allocated oocytes from prepubertal gilts were cultured in pooled follicular fluid obtained from individual sows and assessed for cumulus expansion and nuclear maturation. Briefly, ovaries from slaughtered prepubertal gilts (weighing around 100 kg) were collected from a local abattoir and transported to the laboratory in a polystyrene box to prevent fluctuations in temperature. The ovaries were then washed four times in saline. Follicles with a diameter >3 mm were aspirated using a 18 gauge needle and 10 ml syringe. Oocyte-cumulus complexes were isolated (n = 30 / dish) and incubated in 1.5 ml petri dishes containing 1 ml of diluted (10%) experimental follicular fluid (see part 1) supplemented with 50 μl of hormone stock (2.5 μg / ml NIADDK-oLH-26, AFP-551b; 2.5 μg / ml USDA-pFSH-B-1, AFP-5600) and prolactin (20 ng / ml USDA-pprl-B-1, AFP-5000). Culture was carried out under an atmosphere of 5 % CO_2 in air at 39 ^{0}C for 46 ± 1 h. Cumulus expansion and nuclear maturation were then scored. The incubation of oocytes with individual sow follicular fluid was carried out as a randomized block design involving 3 replicates for each sow and in duplicate (30 oocyte-cumulus complexes per dish on each occasion, providing up to 180 oocytes per sow for determination of treatment effects), during the period from January to April, 1996.

Evaluation of cumulus expansion

After 46 h of in vitro maturation oocyte-cumulus complexes were evaluated for cumulus expansion (Ding, 1993). Oocytes with a fully expanded cumulus, including the corona radiata were classed as Group 4; those in which the cumulus cells had expanded, but not the corona radiata, were classed as Group 3; those complexes that had only partial expansion of cumulus cells were classed as Group 2; finally, those complexes exhibiting dark looking intact cumulus cells were classed as Group 1. These characteristics were scored independently by two individuals.

Examination of nuclear status

After 46 h of culture oocytes were denuded of cumulus cells (Bavister, 1989), mounted on a slide using the whole mount technique and fixed for 48 h in ethanol/acetic acid (3:1). The nuclear status of oocytes was examined under a phase-contrast microscope after staining with 1 % lacmoid in 45 % acetic acid solution. The nuclear status of oocytes (GV, GVBD, MI, MII) was identified according to the classification of Hunter and Polge (1966).

Steroid analysis

Plasma estradiol was measured by the procedure described by De Rensis et al.(1991) using 1 ml of plasma. All samples were analyzed in one assay with an extraction efficiency of 91 % and intraassay C.V. was 5.6 % and sensitivity defined as 87 % of total binding was 1.91 pg/tube. Follicular fluid estradiol concentration was measured by the direct assay previously described by Ding and Foxcroft (1992) using 100 µl of follicular fluid diluted to 1:6000 in assay buffer. All samples were measured in 3 assays with an intraassay CV of 5.8%

and interassay CV of 9.7%. Sensitivity defined as 85% of total binding was 5.5 pg/tube.

Statistical analysis

Data for the dependent variables sow feed intake, sow body weight, backfat and litter weight were analyzed by repeated measures analysis of variance, using the repeated measures general linear model (GLM) procedure of SAS (SAS, 1990). For all dependent variables sources of variation were treatment, sows within treatment and the repeated measure of day (d 0, d 21, d 28 of lactation). In the event of a significant day*treatment interaction differences among days within each treatment were computed using least squares difference in a split plot analyses of variance (SAS, 1990).

The independence of follicle size and treatment was determined by Chi squared analysis (SAS, 1990). For the dependent variables follicle size, plasma estradiol, and after arcine transformation, the stage of cumulus expansion and nuclear maturation, treatment differences were computed by analysis of variance (ANOVA; SAS, 1990). Sources of variation were block, treatment, block*treatment and sows within block*treatment. The experimental unit, sow within block*treatment was used as an error term.

Linear regression analyses (SAS, 1990) used to determine effects of follicle diameter on cubic root of follicle volume and on follicular fluid estradiol concentration.

RESULTS

Sow responses to treatment

Sow weight and backfat did not differ among treatment groups at farrowing (Table 4.2). There was a significant day*treatment interaction for sow body weight ($P < .01$) and backfat ($P < .01$). At d 21 body weight and backfat for group RA was lower than for Group AR ($P < .01$ for both). However at d 28 no differences in body weight or backfat were observed between treatments. Litter weights at weaning did not differ among treatments ($P > .6$). After weaning the daily feed consumption of AR sows was more ($P < .001$) than during the last week of lactation, whereas Group RA sows ate a similar daily amount after weaning compared to d 22 to 28 of lactation. However, overall, there were no differences from weaning to slaughter in feed intake between the treatment groups.

All of replicate I animals ($n = 9$) were excluded from the following analysis because no oocytes had matured during the in vitro maturation procedure, suggesting that the gonadotropin supplement may have expired. In addition, 3 sows from replicates II and III (2 from Group AR and 1 from Group RA) were also excluded because although large preovulatory size follicles were present, their follicular fluid estradiol concentration was consistently lower than 50 ng/ml, indicating that they had already responded to the preovulatory LH surge before slaughter (Figure 4.4). The following analyzes are therefore based on 10 animals for Group AR and 11 for Group RA .

Follicular status at slaughter

There were no treatment differences in daily mean plasma estradiol concentration from weaning to slaughter (Figure 4.2). Follicular diameter of the 15 largest follicles showed that

Group AR sows had more ($P < .045$) small follicles than RA sows and Group RA sows had more ($P < .02$) large follicles than Group AR (Figure 4.3). Overall, oocytes were retrieved from smaller follicles in Group AR than RA sows ($P < .03$). Regression analysis showed that the diameter of follicles was correlated to cubic root of volume ($P < .001$; $r = .73$; $n = 146$). The diameter of follicles was correlated to follicular fluid estradiol concentration ($P < .01$; $r = .52$; $n = 192$) for estrogenic follicles, whereas the follicles from sows considered to be in the post LH surge period, no correlation between follicle diameter and estradiol concentration ($P > .9$; $r = .003$; $n = 32$) was observed (Figure 4.3). Overall there was no difference in the relationship between follicle diameter and follicle fluid estradiol concentration for Group AR ($r = 68$; $n = 85$) and Group RA ($r = .60$; $n = 107$) (Figure 4.5).

In vitro maturation data

Part 1: Data on the maturation of oocytes retrieved from experimental sows, expressed as the percentage of oocytes with cumulus cells at the start of in vitro maturation, with cumulus cell expansion score 1, 2, 3, and 4 are shown in Table 4.3. No differences in cumulus expansion was observed between treatment groups, however, the number of oocytes reaching metaphase II was greater ($P < .03$) for Group RA than for AR sows. Conversely, Group AR sows tended to have more oocytes at metaphase I ($P < .054$) and the germinal vesicle breakdown ($P < .07$) stage than did Group RA.

Part 2: Incubation of randomly selected oocyte-cumulus complexes obtained from prepubertal gilts and incubated with experimental follicular fluid did not demonstrate any difference in cumulus expansion among treatment groups (Table 4.4). However, the proportion of oocytes reaching the metaphase II stage of nuclear maturation was greater ($P < .012$) for RA than AR sows; conversely, Group AR sows had a greater ($P < .01$) proportion of oocytes at Metaphase I than did RA sows. No differences were observed among treatment groups in the proportion of oocytes with nuclei at the germinal vesicle breakdown (GVBD) ($P > .4$) or germinal vesicle (GV) stage ($P > .3$).

DISCUSSION

Consistent with the data reported by Zak et al. (in press), patterns of feed intake during lactation resulted in differential patterns of body weight and backfat changes in Groups RA and AR. Overall, AR and RA sows mobilized approximately 29 kg of live weight and 2 mm of backfat over lactation. Irrespective of the pattern of weight and backfat loss, sows produced equivalent amount of milk during lactation, as evidenced by similar litter weights at d 21 and d 28.

Central to the interpretation of data presented in this report is the assumption that Group AR and RA sows were at a similar physiological state at the time of slaughter, with respect to time of the LH surge and ovulation. Because the results in replicate I sows indicated that the time of slaughter first chosen was inappropriate, all animals in replicates II and III were slaughtered some 38 hours before the estimated onset of standing heat. This more conservative timing was expected to result in sows being slaughtered in the last 24 to 30 hour

period before the onset of the preovulatory LH surge. Data from only 3 of 24 sows were finally excluded from analysis on the basis that follicular fluid estradiol concentrations in large preovulatory type follicles had already declined. Thus all remaining sows were considered to have been slaughtered at the same relative stage of follicular development. Plasma estradiol concentration of these 21 sows was not different between treatment groups at either 24, 48 or 72 hours after weaning, nor was the mean estimates of plasma estradiol from weaning until slaughter different among treatments.

In agreement with the results of the study reported by Foxcroft et al. (1987) in weaned sows, and of Grant et al. (1989) in cyclic gilts, the follicles examined in the late follicular phase in this experiment (equivalent to d 20 of the estrous cycle) also formed a heterogenous population in terms of follicle diameter and estradiol content. Hunter and Weisak (1990) suggested that the degree of heterogeneity of follicles within the preovulatory pool may have ramifications for the developmental competence of the oocyte once it has been ovulated and for subsequent luteinization of the follicle.

Although there was no difference in plasma estradiol concentration between treatment groups, the follicle size differed. Group AR had a more 'small' follicles, whereas Group RA had more 'large' follicles (Figure 4.3). We suggest that the difference in follicle maturity was not related to animals being slaughtered at different times during the follicular phase, but was a reflection of the sows previous nutritional state. Group RA sows were fed to appetite from day 22 of lactation until slaughter, whereas Group AR sows feed intake was restricted during the last week of lactation and only after weaning were they allowed to feed to satiety. The effects of periods of increased catabolism, due to reduced feed intake on follicle size, has previously been reported in the literature. In the lactating sow, feed restriction to 50% of maintenance requirement for two weeks almost abolished follicular development beyond 3 mm in diameter (Miller, unpublished observation) and periods of increased catabolism during lactation reduced ovulation rate when compared to those sows fed to appetite (Zak et al., in press).

Nutritional status of an animal has profound effects on follicle development. It is common practice to optimize ovulation rate in gilts, by 'flush feeding' animals at a higher level of feeding for at least 6 days before estrus (Dailey et al., 1972) and refeeding a prepubertal gilts after a period of feed restriction increases follicular diameter, volume and estradiol synthesis (Cosgrove et al., 1992). However, the mechanisms whereby follicles are recruited into the preovulatory pool, as opposed to becoming atretic remain unresolved (Foxcroft and Hunter, 1985). Studies by Matamoras et al. (1990) have suggested a role for insulin in preventing follicles from becoming atretic; and in streptozotocin-induced diabetic gilts there was a higher incidence of atretic follicles in all size class than compared to normoglycemic controls (Meurer et al., 1991). Two dimensional electrophoretic analysis of proteins secreted by sheep ovaries determined to be atretic or healthy, or of different sizes, revealed quantitative differences in certain protein spots (Driancourt et al., 1996). Evidence which supports the role of the IGF's and their associated binding proteins (Hammond et al., 1993; Adashi et al., 1991) transforming growth factor alpha (TGF α) and nerve growth factors (Ojeda and Dissen, 1994) in follicular growth and development is accumulating. In particular, IGFBP-2 is positively related to follicle atresia in the gilt (Guthrie et al., 1995) and in the weaned

primiparous sow, Howard and Ford (1992) observed that IGFBP-2 decreased with increasing follicle diameter and estradiol concentration. In vivo infusion of growth factors into an autotransplanted ovary in the ewe has demonstrated functional relationships between growth factors and ovarian function. For example, Murray et al., (1993) demonstrated that EGF decreased estradiol and not androstenedione but increased progesterone secretion, suggesting a possible role in the control of luteinization, and infusion of TGF α (Campbell et al., 1994) decreased the number of large follicles and steroid production, indicative of a role in the regulation of atresia. However, in vitro incubation of porcine oocytes with EGF (Ding and Foxcroft, 1994b) stimulated nuclear maturation. Thus changes in the transcription or translation of proteins and steroids occur as follicles either grow or become atretic, and as such may provide an intrafollicular environment that is conducive to oocyte maturation.

The definitive role of nutritionally induced changes in follicular environment as opposed to nutritionally induced changes in gonadotropin concentrations in models utilizing increased catabolic states has yet to be fully resolved. Receptors for LH on the granulosa, thecal and mural cells of the follicle are essential for follicle persistence and development during the estrous cycle (Esbenshade et al., 1990). During periods of feed restriction in the prepubertal gilt the hypophyseal release of LH decreases within 6 hours but recovers as early as 7 days after refeeding (Booth et al., 1996; Cosgrove et al., 1991). Similarly, LH before weaning in these lactating sows is lower in Group AR following 7 days of feed restriction, compared to RA sows although no differences were evident in the LH response to weaning (Zak et al., in press). The removal of the suckling stimulus at weaning is generally regarded as the signal for resumed follicular growth (Britt et al., 1985) and a lack of LH secretion is the primary causes of retarded follicular growth during lactation in the sow (De Rensis et al., 1991). There is ample evidence that ovarian follicle growth is not absolutely quiescent in lactation (Britt et al., 1985) and in the absence of marked catabolism follicle size does increase during lactation (Kungavonkrit et al., 1982). Thus in the present model it is impossible to delineate between the probable roles of reduced nutritional sequalea and suppressed LH secretion, in terms of their effects on follicular growth, and oocyte maturation.

The observation that more oocytes in Group RA sows developed to metaphase II of meiosis, and that randomly selected oocytes incubated with follicular fluid from RA sows were also more mature than those of Group AR indicates that a factor(s) within the follicular fluid contributed to the observed differences in oocyte nuclear maturation. The ability of the somatic compartment to alter nuclear maturation and cytoplasmic competence of oocytes was demonstrated by Ding and Foxcroft (1994a, b). In the former study randomly selected porcine oocytes were incubated with conditioned media obtained from follicle shells of small or large follicles obtained at day 17 or 20 of the estrous cycle: nuclear maturation was greatest in oocytes matured in conditioned media from large follicles whilst male pronuclear formation was greatest in media conditioned with day 20 as compared to day 17 follicle shells. Collectively these data support the hypothesis that treatment differences observed in the development of the preovulatory pool of follicles may have ultimately been causal in observed treatment differences in oocyte meiotic maturation. Furthermore, associations between genotype effects on follicle oocyte maturation in the immediate preovulatory period in vivo, and the capacity of follicle conditioned media obtained from these animals to support oocyte

maturation in vitro, has been reported (X. Xu, unpublished).

The data reported here demonstrate that the size of follicles in the preovulatory pool and the rate of maturation of oocytes to metaphase II within these follicles can be affected by the nutritional history of the sow. Furthermore, factors of follicular origin appear to mediate these effects. Differences in the maturation of the oocyte (and perhaps the developmental competence of the follicle to luteinize) may potentially contribute to the observed differences in embryo survival in our previous experiments. Thus the management of the lactating sow immediately prior to weaning is important in order to maximize preovulatory follicle development and hence provide an environment which is conducive to optimal oocyte maturation.

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Table 4.1 Composition of experimental diets (as fed basis)

Item	Lactating sow	Gestating Sow
Ingredients, %		
Barley	40.4	56.4
Wheat	30.0	30.0
Soybean meal, 44% CP[1]	12.0	.7
Tallow	3.0	2.0
Bran	10.0	-
Calcium phosphate	1.7	1.7
Limestone	1.4	1.4
Vitamin-mineral premix[2]	1.0	1.0
Iodized salt	.5	.5
Chemical Analysis		
Crude protein, %	15.4	13.7
Lysine, %	.74	.56
Metabolizable energy, MJ	13.4	13.4

1CP= Crude Protein

[2] Supplied the following per kg of diet: Zinc, 120 mg; Manganese 12 mg; Iron, 150 mg; Copper, 12 mg; Selenium, .1 mg; vitamin A, 5000 IU; Vitamin D3, 500 IU; Vitamin E, 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride, 840 mg; Vitamin B_{12}, 30 mg; Biotin, 200 mg.

Table 4.2 Least square means (± SE) feed intake, sow body weight and backfat and litter weights at weekly intervals during lactation.

	Treatment [1]	
	AR (n = 16)	RA (n = 16)
Item		
Feed intake, kg		
day 1 to 21	4.5 ± .7[a]	2.3 ± .1[b]
day 22 to 28	2.8 ± .2[a]	5.3 ± .10[b]
weaning to slaughter	6.2 ± .04	6.0 ± .5
Body weight, kg		
Farrowing	202.4 ± 3.6	196.4 ± 3.7
day 21	190.7 ± 2.3[a]	171.2 ± 1.1[b]
day 28	173.0 ± 3.4	168.2 ± 1.9
Weight change, day 1 to 28	-29.4 ± 2.7	-28.2 ± 1.9
Backfat, mm		
Farrowing	18.6 ± 1.0	17.5 ± .7
day 21	18.0 ± 1.0[a]	12.7 ± 1.2[b]
day 28	15.2 ± 1.2	16.3 ± .3
Backfat change, day 1 to 28	-3.4 ± .4	-1.2 ± .12
Litter weight, kg		
Birth	10.6 ± .3	11.1 ± .3
day 21	44.6 ± 1.4[a]	40.4 ± 1.4[b]
day 28	58.9 ± 1.5	57.8 ± 1.5
Growth rate g/d day 1 to 28	288 ± 12	277 ± 8

[1] AR and RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AR = sows receiving ad libitum access to feed from day 1 to 21 then 50% from day 22 to 28. RA= sows receiving 2.3 kg intake from day 1 to 21, then having ad libitum access to feed from day 22 to 28.

[ab]Means within rows lacking a common superscript letter differ (P<.05)

Table 4.3 Least square means (± SE) of percentage data for cumulus expansion and nuclear maturation after 46 hours of in vitro maturation for oocytes retrieved from experimental sows.

	Treatment [1]	
Item	AR (n = 10)	RA (n = 11)
No oocytes examined	108	107
Cumulus expansion, 4	27.4 ± 5.7	40.4 ± 5.3
Cumulus expansion, 3	36.9 ± 4.1	31.4 ± 3.8
Cumulus expansion, 2	29.7 ± 4.3	17.7 ± 4.0
Cumulus expansion, 1	13.1 ± 4.0	14.6 ± 5.0
No oocytes examined	106	98
Metaphase II	18.4 ± .06[a]	58.2 ± .07[b]
Metaphase I	61.9 ± .08	33.7 ± .08
Germinal vesicle breakdown	24.9 ± .06	9.2 ± .05
Germinal vesicle	11.8 ± .06	12.6 ± .06

[1] AA, AR, and RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AR = sows fed on an ad libitum basis from day 1 to 21 then 50% from day 22 to 28, RA = sows receiving 2.3 kg from day 1 to 21, then fed on an ad libitum basis from day 22 to 28.

[a b] Means within a row lacking a common superscript letter differ (P<.05)

Table 4.4 Least square means (± SE) for percentage data of cumulus expansion and nuclear maturation after 46 hours of in vitro maturation for randomly allocated oocytes incubated in follicular fluid harvested from experimental sows.

	Treatment [1]	
Item	AR (n = 10)	RA (n = 11)
Number of oocytes examined	1227	1147
Cumulus expansion, 4	34.8 ± .03	38.4 ± .1
Cumulus expansion, 3	55.3 ± .03	54.5 ± .1
Cumulus expansion, 2	8.9 ± .02	6.5 ± .02
Cumulus expansion, 1	1.0 ± .01	.6 ± .01
Number of oocytes examined	1218	1135
Metaphase II	43.8 ± .03[a]	54.0 ± .02[b]
Metaphase I	52.3 ± .03[a]	38.6 ± .03[b]
Germinal vesicle breakdown	2.4 ± .01	3.2 ± .01
Germinal vesicle	1.5 ± .01	2.1 ± .01

[1] AA, AR and, RA = sows with different patterns of feed intake during day 1 to 28 of lactation. AR = sows having ad libitum access to feed from day 1 to 21 then 50% from d 22 to 28. RA = sows receiving 2.3 kg/d from day 1 to 21, then ad libitum access to feed from day 22 to 28.

[a b] Means within rows lacking a common superscript letter differ (P<.05).

Figure 4.1 Schematic representation of experimental design. AR and RA denote the experimental groups. Group RA and AR were weaned at 0800 and 2130, respectively. Blood samples were collected at 24-hourly intervals until slaughter.

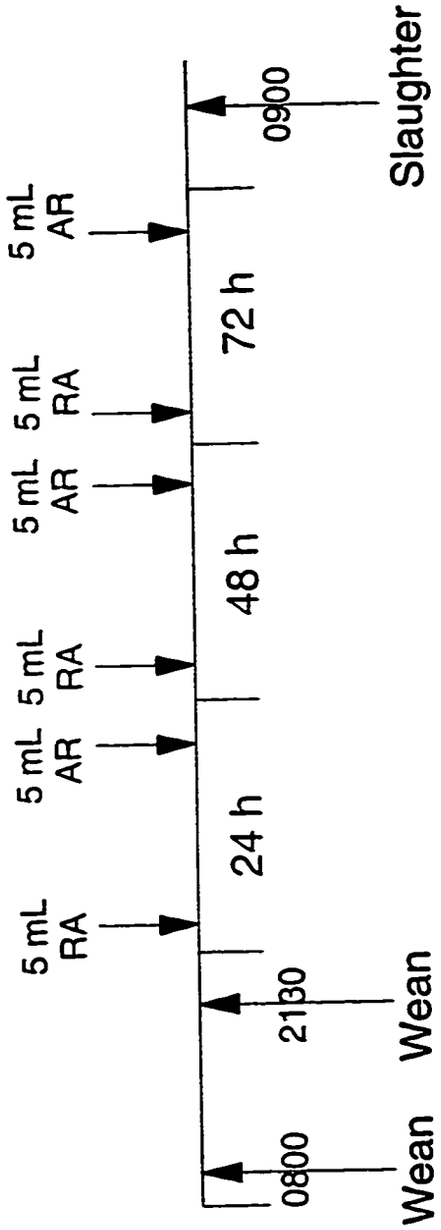

Wean RA
0800
Wean AR
2130
5 mL RA
24 h
5 mL AR
5 mL RA
48 h
5 mL AR
5 mL RA
72 h
5 mL AR
0900
Slaughter

Figure 4.2 Mean (± SE) plasma estradiol concentration at 24, 48 and 72 hours after weaning for treatment groups AR and RA.

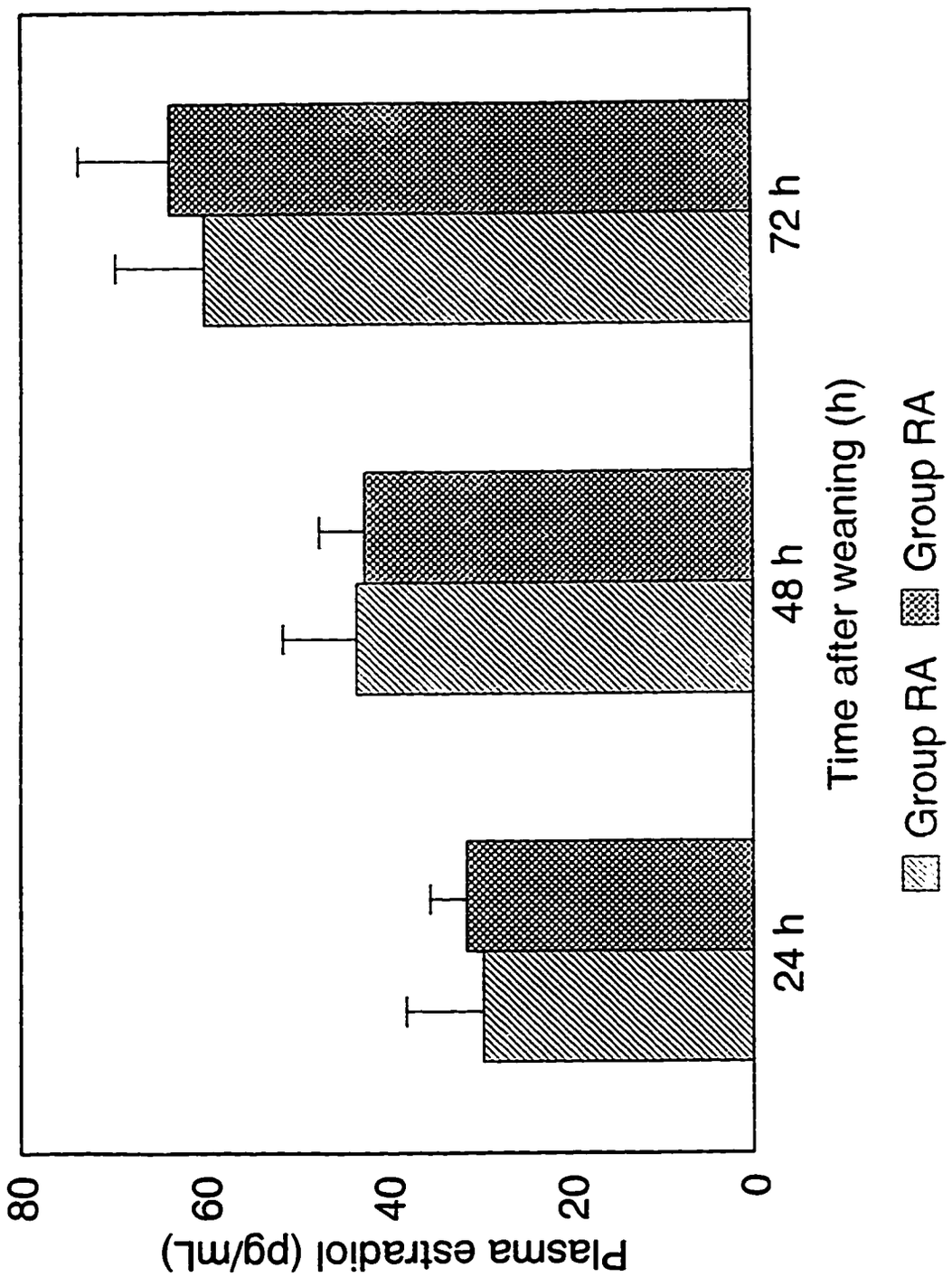

Plasma estradiol (pg/mL)
0
20
40
60
80
24 h
48 h
72 h
Time after weaning (h)
Group RA
Group RA

Figure 4.3 Mean (± SE) for follicle diameter less than or equal to 5 mm (small), greater than 5 mm and less than or equal to 6.5 mm (medium) and, greater than, or equal to 7 mm for treatment groups AR and RA.

Bars with superscripts a and b differ by $P < .05$; and, bars with superscripts a and c differ by $P < .01$, within follicle size class.

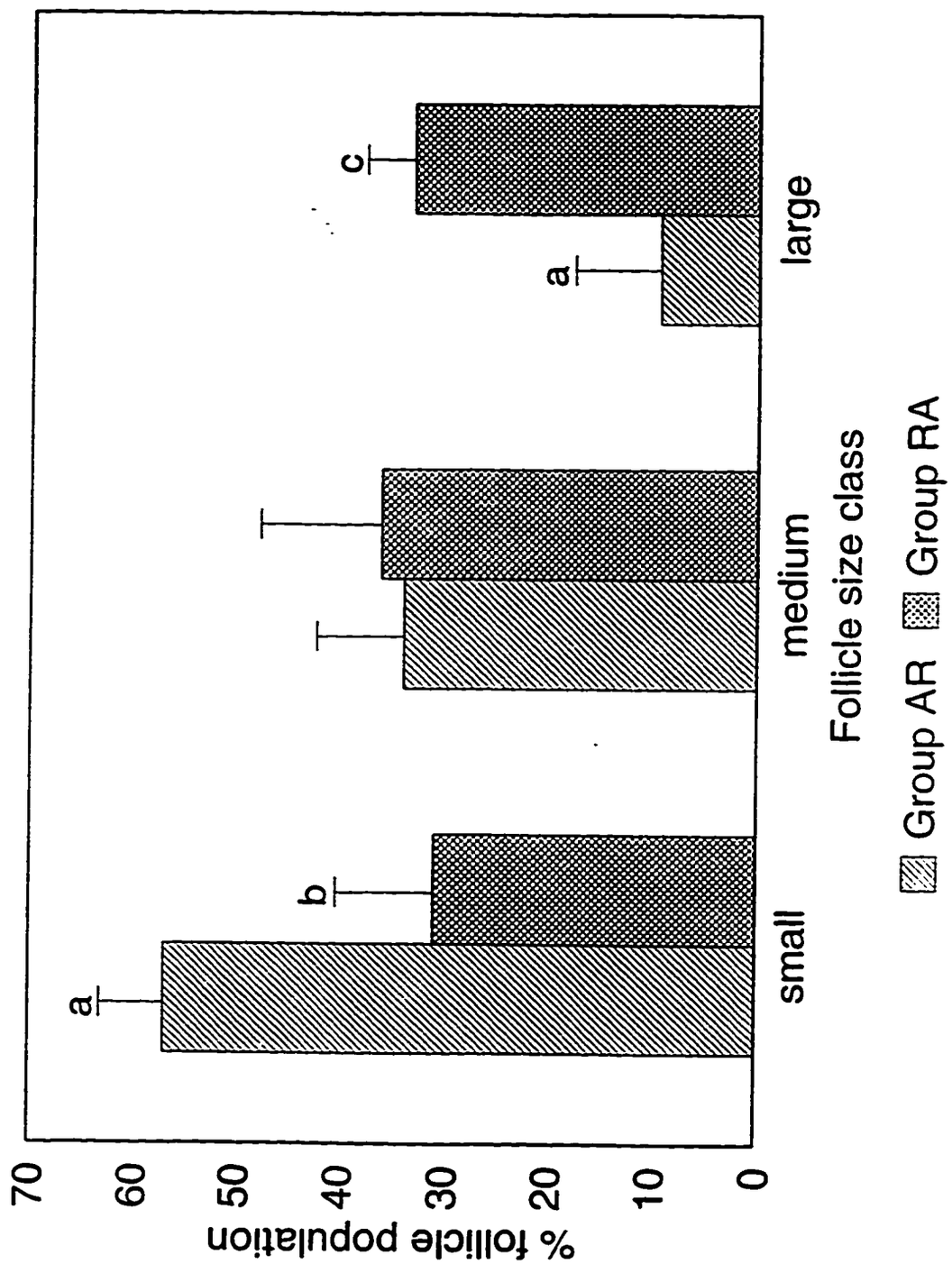

% follicle population
70
60
50
40
30
20
10
0
a
b
c
a
small
medium
large
Follicle size class
Group AR
Group RA

Figure 4.4 Relationships between follicle estradiol concentration and follicle diameter in sows with estrogenic follicles (n = 21) and considered to be in the pre-LH surge period (○) and for sows (n = 3) which were omitted from the data analysis because they had large but non estrogenic follicles and were presumed to be in the post ovulatory LH surge period (●).

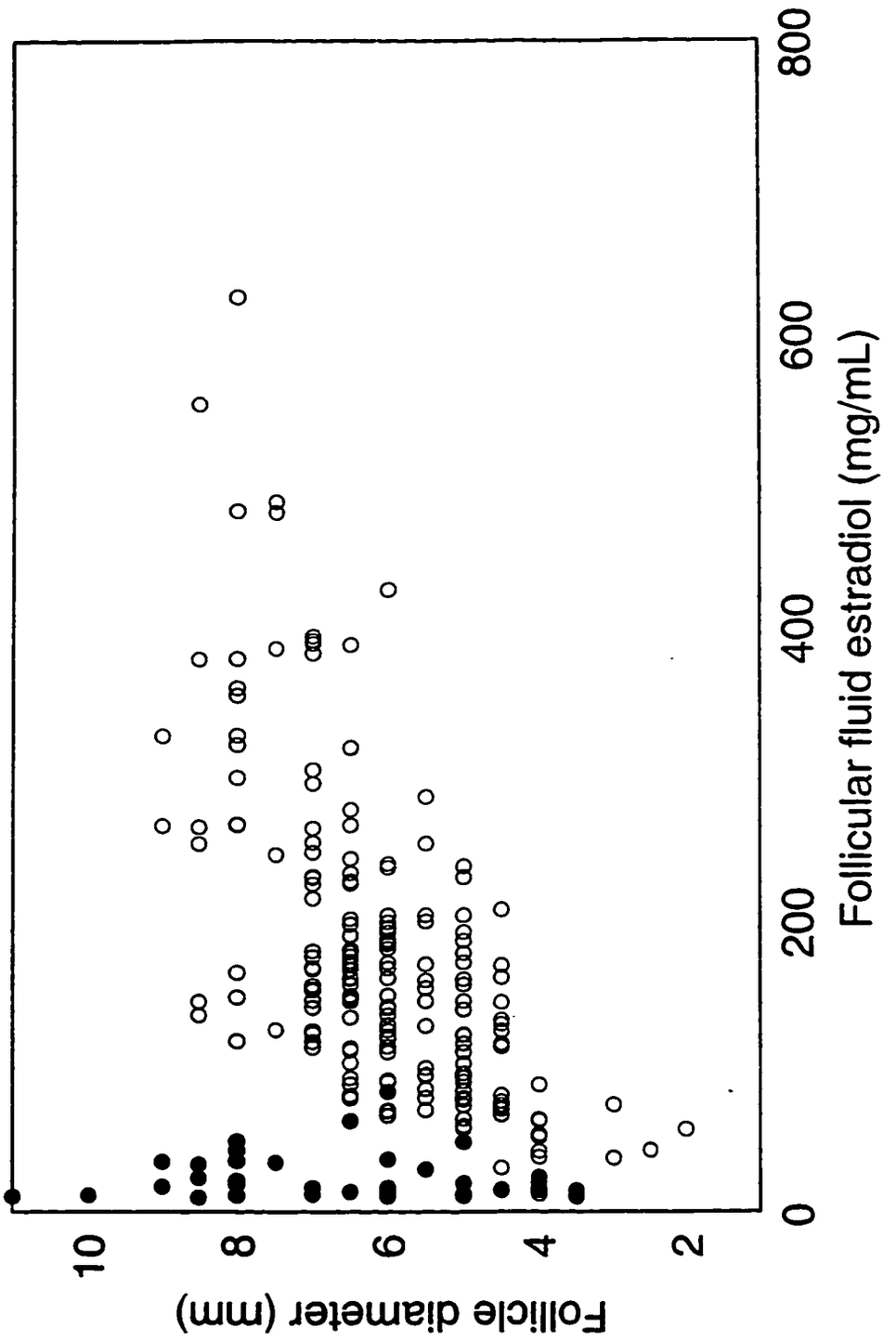
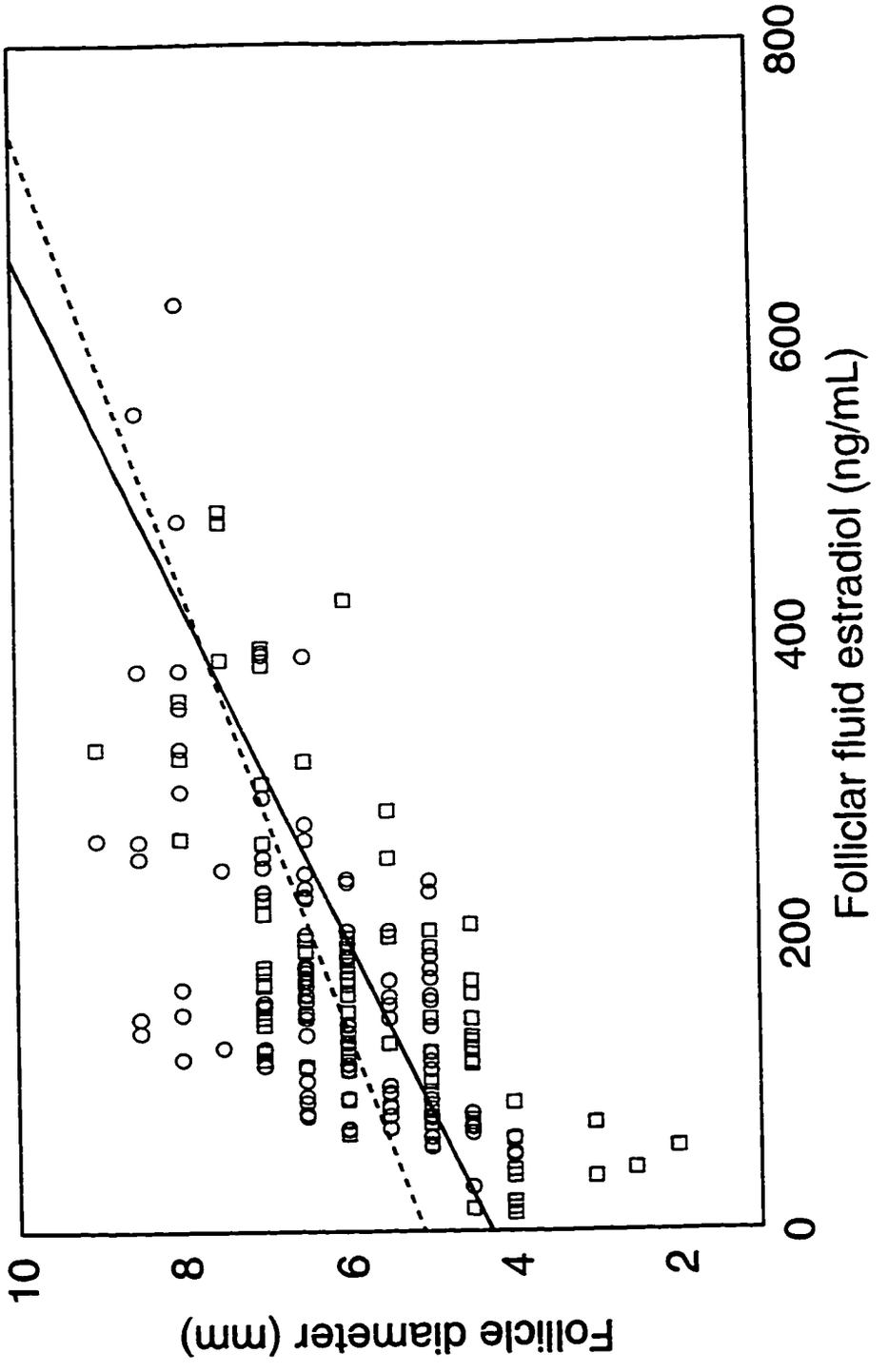

Follicle diameter (mm)
2
4
6
8
10
0
200
400
600
800
Follicular fluid estradiol (mg/mL)
pre LH surge
post LH surge

Figure 4.5 Linear regression of follicular fluid estradiol concentration and follicle diameter for sows bearing estrogenic follicles of treatment groups AR (□) and RA (○) ($P < .01$; for both).


Follicle diameter (mm)
10
8
6
4
2
0
200
400
600
800
Folliclar fluid estradiol (ng/mL)
Group AR
Group RA

CHAPTER 5

DIVERGENT LACTATIONAL METABOLIC STATES, ASSOCIATED ENDOCRINE CHANGES AND SUBSEQUENT REPRODUCTIVE PERFORMANCE IN THE PRIMIPAROUS SOW

INTRODUCTION

A number of experimental paradigms have been developed to investigate the influence of nutrition on reproductive performance. Empirical studies of Reese et al. (1982) and King and Dunkin. (1986) showed that weight loss associated with nutrient restriction during lactation detrimentally affected reproductive efficiency and this effect was correlated with LH immediately prior to weaning (King and Martin, 1989). More recently it has been suggested that metabolic correlates of nutritional state are most probably the functional link with the reproductive axis (Wade and Schneider., 1992; Baidoo et al., 1992; I'Anson et al., 1991).

The concept that dynamic changes in nutrient availability are critically important to reproductive function in swine is reviewed by Booth (1990) and Foxcroft (1992). In the gilt realimentation after 7-day period of feed restriction increased LH pulsatility within 6-hours and follicular development at day 7, which was not associated with changes in body weight or composition (Booth et al., 1994). Initial studies in the primiparous lactating sow by Tokach et al. (1992) established relationships between metabolic state (plasma insulin) and LH secretion as early as day 14 of lactation, and reproductive performance after weaning. Subsequently, Koketsu et al. (1996) reported that a 7-day period of feed restriction in any week of lactation increased the weaning to estrus interval when compared to those maintained on a high feed level throughout lactation. Recent work in our own laboratory (Zak et al., in press) further emphasized the importance of dynamic changes in metabolic state by demonstrating that a move towards an anabolic or catabolic state in the last week of a 28-day lactation in the primiparous sow resulted in differential effects on ovulation rate and embryonic survival.

The primiparous sow fails to consume sufficient feed to maintain a positive energy balance (Aherne et al., 1995) and approaches an increasingly catabolic condition as lactation progresses and mobilizes body tissue to maintain milk production (King and Williams 1984; Zak et al., in press). Although the piglet suckling stimulus provides the primary block to the resumption of estrus during lactation by reducing the pulsatile release of LH (Britt et al., 1985), nutritional restriction can further exaggerate this problem (Foxcroft et al., 1995; Quesnel and Prunier, 1995). Confirmation that a lack of pulsatile LH secretion is the primary cause of lactational anestrus comes from studies in which treatment with exogenous GnRH during lactation resulted in follicular development, behavioral estrus and ovulation (Cox and Britt., 1982; Rojanasthein et al., 1988; De Rensis et al., 1991). Furthermore the data of Sesti and Britt (1991), using the excitatory amino acid NMDA, demonstrated that suckling acts to block the release of GnRH at the hypothalamic level.

Therefore, although there is extensive evidence that increased lactational catabolism

imposes profound inhibitory effects on reproductive function compared to sows fed to appetite, it is not known whether anabolism during lactation could ameliorate the catabolic effects on LH secretion during lactation.

Matzat et al. (1990) have shown that superalimentation of feed through a gastric cannula made sows anabolic during lactation, however little evidence was provided as to the production characteristics of the sows. A comprehensive study was undertaken to compare the impact of different nutritional regiment during lactation on milk production, nitrogen balance and subsequent reproductive performance. In this part of the study our objective was to test the hypothesis that making sows anabolic during lactation would have beneficial effects on postweaning fertility and also to identify the endocrine mechanisms mediating these effects.

MATERIALS AND METHODS

Experimental Design

Primiparous sows were offered a high quality diet during lactation at one of three feeding regimens, ad libitum (AL), restricted (R), or superalimented (SA), designed so that sows were slightly catabolic, grossly catabolic or anabolic. For the AL group, sows were offered food ad libitum. From past experience at the University of Alberta Swine Research Center, gilts fed to ad libitum consume on average 5.0 kg/d and lose .3 kg/d of body weight. Gilts were made grossly catabolic (R) by restricting their feed intake during lactation to about 3 kg/d (fed at 0600, 1330, and 2100), which was calculated to mobilize in excess of 1 kg of body weight per day. Sows were made anabolic by superalimentation (SA) which commenced within 4 days of farrowing. They were offered food ad libitum from a trough and given additional feed by means of a stomach cannula 7 times a day (0600, 0830, 1100, 1330, 1600, 1800 and 2100). The amount of food given via the cannula was adjusted so that the total intake of these sows was 125 % of the estimated food intake of the AL group (Figure 5.1).

Cannulation

Between day 70 to 80 of gestation all experimental primiparous sows (Camborough; Pig Improvement (Canada) Ltd) underwent surgery for the insertion of a stomach cannula (Pluske et al., 1995) to allow for the infusion of additional feed in animals that were allotted to group SA. This experiment and its surgical procedures were approved by the University of Alberta Animal Care Committee to ensure adherence to Canadian Council of Animal Care guidelines.

Diets and Management of Sows

During gestation gilts were offered 2.5 kg/d of a standard diet (Table 5.1). During lactation all sows were fed a wheat-barley-soybean diet formulated to provide 15.4 MJ DE/kg and 18.5% crude protein. All food troughs were thoroughly cleaned once each day and any dry food refusals were weighed. Water was freely available from nipple drinkers at all times.

Within 48 hours of farrowing gilts were randomly allocated to one of three treatments, AL (n = 12), R (n = 9), and SA (n = 8). Within 48 hours of farrowing, litters were standardized to 8 to 10 piglets (8.6 ± 0.9) and creep feed was not available. Within 24-hour of farrowing and at subsequent weekly intervals thereafter, sow weight and backfat depth (65 mm from the midline at the 10th rib; Scanoprobe II, Scano, Ithaca, NY) and litter weights were recorded.

At weaning, superalimentation of SA group ceased and all sows were fed a diet formulated to provide 13.7 MJ DE/kg and 13.7% crude protein at 2.5 times their estimated energy maintenance requirements, based on weaning weight . The morning after standing heat, feed intake was reduced to twice maintenance until slaughter, in accordance with NRC recommendations (1988). Sows were tested twice daily at 0700 and 1900 for the onset of standing heat using fence line contact with a rotation of mature vasectomized boars for 15 minute periods. At 12 and 24 hours after the onset of estrus sows were artificially inseminated, by one of two inseminators, using pooled semen ($3x10^9$ spermatozoa / dose) from the same three boars (Alberta Swine Genetics Corp., PO Box 3310, Leduc, Alberta, Canada). The day of standing heat was designated as 'day 0' and sows were slaughtered on day 28 (27.9 ± 1.2) of gestation at a local abattoir and their reproductive tracts recovered. Ovulation rate was determined by counting the number of corpora lutea on each ovary, and the number of embryos in utero was determined using the method described by Jindal et al. (1996).

Blood Sampling

At day 26 ± 1.3 of lactation an indwelling jugular catheter was surgically implanted, under general anaesthesia via the cephalic vein (Cosgrove et al., 1993). On the day of blood sampling food was withheld during the first 2 hours of sampling to allow for an estimate of preprandial metabolite concentrations. Blood samples (3 ml) were withdrawn at 10 minute intervals for 12 hour periods before and after weaning at 1800 on day 28 (27.9 ± 1.2) for the analysis of plasma LH concentration. Additional 15 ml samples were collected hourly for analysis of plasma IGF-I and insulin concentration. After weaning 5 ml blood samples were collected at 8-h intervals (0700, 1500 and 2300) from 8 hours after weaning until 4 to 5 days after standing heat, for the analysis of plasma LH concentration. Plasma progesterone concentration was analyzed daily from standing heat until 4 days later. Plasma insulin and IGF-I concentrations were analyzed on the morning of standing heat. Blood samples were collected into heparinized tubes and centrifuged at 1500 x *g* for 15 minutes. The plasma was decanted and stored at -30 °C until analysis.

Estimation of Plasma Hormone Concentrations

For RIA. analysis, all treatment groups were represented in every assay and all samples from one sow were analyzed in the same assay. Plasma LH concentrations were determined using the homologous double antibody RIA previously described by Cosgrove et al. (1991). For LH, 200 μL of plasma was assayed, the intra and inter assay coefficient of variance (C.V.) were 7.5% and 8.2%, respectively and average sensitivity, estimated as 85 % of total binding was 0.01 ng/tube. Plasma insulin and IGF-I concentrations were determined in

duplicate using the double antibody RIA's previously described by Cosgrove et al. (1992). For insulin 100 μL of plasma was assayed. the intra and inter assay C.V. were 6.0 % and 6.1 %, respectively, and sensitivity defined as 88% of total binding was .01 ng/tube. For plasma IGF-I 100 μL of plasma was initially extracted, the intra and inter assay C.V. were 4.8 % and 5.1 %, respectively and sensitivity of the assay defined as 92 % of total binding was .08 ng/tube. Extraction efficiencies were routinely high and plasma potencies were not corrected for recovery. Plasma progesterone concentration was determined by the extraction method previously described by Beltranena et al. (1991). Intra and inter C.V. were 4.7 % and 11.2 %, respectively and sensitivity estimated as 85 % of total binding was .05 ng/tube: Extraction efficiencies averaged 82 % and plasma potencies were corrected for recovery.

Statistical Analysis

All dependent variables were analyzed for normality using the Wilk-Shapiro test (SAS. 1990). Data for the dependent variables sow feed intake, body weight, backfat, litter weight at farrowing, day 7, 14, 21 and day 28, and progesterone were analyzed by repeated measures analysis of variance, using the repeated measures general linear model (GLM) procedure of SAS (SAS, 1990). For all dependent variables sources of variation were treatment, sow within treatment, and the repeated measure of day. In the event of a significant day*treatment interaction differences among days were computed using split plot analysis of variance within treatment (SAS., 1990). Energy balance at weaning and standing heat, weaning to estrus interval, ovulation rate and embryo survival (ovulation rate/embryo number) were analyzed using analysis of variance, fitting treatment and the error term of sow within treatment. Differences among treatment means were determined using Duncan's multiple range test (SAS, 1990).

Progesterone data were fitted to the time of peak LH surge. Episodic LH frequency was determined by visual appraisal using the method of McLeod and Craigon, (1985) and the pulse definition used by Cosgrove et al. (1991).

RESULTS

Sows that were superalimented ceased voluntary feeding after about day 2 of lactation. and from then onwards all feed was given via the gastric cannula. Overall, SA sows received more feed ($P < .001$), gained more weight ($P < .001$) and backfat ($P < .001$) than AL sows during lactation. Group R lost more weight ($P < .001$) and backfat ($P < .001$) than Group SA or AL. Litter weights increased linearly during lactation ($P < .001$). Overall, weaning weight and piglet growth rate, adjusted for both birth weight and number of piglets weaned did not differ among treatments ($P > .05$), however litter weight for Group R sows was lower ($P < .05$) than for Group AL and SA at day 28 (Table 5.2).

There were no treatment differences in mean pre or postprandial plasma insulin concentration during the 12-hour period before or after weaning (Table 5.3). A day effect revealed that all treatment groups exhibited a decrease in plasma insulin during the 12-hour

period after weaning, corresponding to an overnight fast. Treatment affected ($P < .001$) mean plasma IGF-I concentration; before weaning, plasma IGF-I in Group R was lower ($P < .001$) than Groups AL or SA and remained so during the 12-hour period after weaning ($P < .001$). There was a significant treatment effect for mean plasma LH concentration ($P < .001$) and LH pulse frequency ($P < .001$). During the 12-hour period before weaning mean LH concentration and pulse frequency were lower ($P < .03$ and $P < .006$, respectively) for R sows than for Group AL or SA and SA, whereas, AL were not different from one another. In response to weaning both mean plasma LH concentration and pulse frequency increased ($P < .001$) independent of treatment. During the 12 hour period after weaning no treatment differences in mean plasma LH concentration or pulse frequency were observed between Group SA or AL sows, although mean plasma LH concentration and pulse frequency were still lower in Group R sows ($P < .01$ for both), see Figures 5.2, 5.3, and 5.4.

There was a significant day*treatment interaction ($P < .05$) for feed intake after weaning. Although all sows were offered 2.5 times maintenance energy requirements during the period from weaning to standing heat, Group SA ate less ($P < .001$) than AL or R sows. During the five days after standing heat SA sows continued to eat less ($P < .01$) than AL or R sows (data not shown). From weaning to standing heat Group R sows gained weight and backfat ($P < .001$ for both). In contrast both AL and SA sows lost weight and backfat and SA sows lost more ($P < .01$) weight and backfat than AL sows. Nevertheless, at standing heat absolute values for body weight and backfat in SA sows were still greater than in R sows ($P < .04$ and $P < .02$, respectively), whereas Group AL body weight was not different from either SA or R sows. Backfat at day 28 for AL sows was different from SA ($P < .03$) and R ($P < .04$) sows. Treatment differences in sow energy balance at day 28 ($P < .03$), standing heat ($P < .02$) revealed that energy balance overall in Group SA was greater than AL and R sows (Table 5.4).

From weaning to standing heat plasma IGF-I concentration in R sows increased ($P < .01$) whereas in AL and SA sows it did not (Table 5.3). From weaning until standing heat mean plasma insulin concentration in Group R and AL increased ($P < .035$) whereas no change was observed in Group SA. At standing heat there was no difference for mean plasma insulin or IGF-I concentration among treatments. There was no effect of treatment but an effect of day ($P < .001$) for plasma progesterone concentration. Regression analysis revealed that embryo survival was correlated with plasma progesterone at 72 hours after the LH surge ($n = 20$; $P < .01$; $r = 0.7$). Weaning to estrus interval was greater ($P < .01$) for Group R than either AL or SA. Ovulation rate and embryo survival did not differ among groups.

DISCUSSION

Differential body weight and backfat changes were achieved by the feeding regimen imposed. Superalimentation of SA group increased body weight and backfat, whereas it was depleted in both AL and R sows. Group SA did not produce more milk than AL sows, hence almost all of the additional nutrients available to the sow were directed to tissue anabolism and could potentially have ameliorated any inhibitory effects of lactational catabolism on

reproductive performance. Restricted feed intake decreased litter weight at weaning (Nelssen et al., 1985; Mullan and Williams, 1989); Koketsu et al., 1996) and increased weaning to estrus interval (Reese et al., 1982; Mullan and Williams, 1989) in R sows, when compared to SA or AL. However, despite the differing patterns of weight loss or gain during lactation, SA fertility was not different to that of AL sows.

Plasma IGF-I concentration was lower in Group R than SA or AL sows, which is consistent with the data reported by Tokach et al. (1993), and pre- or postprandial plasma insulin concentration was not different among treatment groups despite different levels of feed intake. The association of peripheral insulin and feed intake in the lactating sows is equivocal in the sow. Armstrong et al. (1986) and Mullan and Close (1991) did not report an association between the two, whereas Koketsu et al. (1996) and Zak et al.(in press) did report that a period of feed restriction was associated with reduced plasma insulin concentration. Insulin has been suggested as a likely mediator between the metabolic state and the reproductive axis (I'Anson et al., 1991) and insulin status during early lactation has been correlated with luteinizing hormone and subsequent reproductive performance in the sow (Tokach et al., 1992). Intracerebroventricular injection of insulin in the ram (Miller at al.,1995) or gilt (Cox et al., 1989), elicited an acute increase in LH pulsatility, and insulin receptors have been identified within the hypothalamus and OVLT (Werther et al., 1987: VanHouten et al., 1979), thus a role for insulin in the central regulation of LH release is likely. Changes in plasma IGF-I concentration may also be a likely candidate for the metabolic signaling to the reproductive axis, however, because of its latent response to alterations in metabolic state it may be involved in the more long term adaptations of the central reproductive state rather than to acute changes in metabolic status. Relatively severe catabolism experienced by Group R sows caused a suppression in LH secretion during lactation which is similar to the reported data of Baidoo (1989) and Tokach et al. (1992). Despite the fact that SA sows gained body weight and backfat and were anabolic, whereas AL sows were relatively more catabolic during lactation, LH secretion before weaning was similar between these groups. This observation is consistent with the suggestion that the suckling stimulus provided by the piglets is the predominant inhibitor of LH secretion during lactation in the sow (Foxcroft et al., 1992), which cannot be overcome by additional feed intake and associated anabolism. The data reported by Mullan et al. (1991) is in agreement with this conclusion, because they found that LH secretion was consistently lower in sows suckled by 12 as opposed to 6 piglets.

Both Group SA and AL responded to weaning with an increase in LH secretion (Foxcroft et al., 1987), however, Group R sows still exhibited a continued suppression of LH for at least 12 hours after weaning and also exhibited a delay in the weaning to estrus interval. The weaning to estrus interval has been correlated with LH secretion before weaning (Tokach et al., 1992; King and Martin, 1989; Armstrong et al., 1986), and as such the delay in the time to ovulation in Group R may therefore be dependent on the tonic inhibition of LH release during lactation and after weaning, and also a lack of ovarian response to LH stimulation around the time of weaning (Shaw and Foxcroft, 1985). The responsiveness of the ovary to appropriate signals for the initiation of the final maturation of follicles is a prerequisite for ovulation (Britt et al., 1985), and the local regulation of ovarian function by nutritional signals

may be dependent (Cox et al., 1987) or independent (Cosgrove et al., 1992) of central effects.

Data reported in the post partum dairy cow clearly demonstrated a correlation between the timing of the first postpartum estrus with animals approaching a relatively less negative energy balance after the energy balance nadir (Cranfield and Butler. 1990: Senatore et al., 1996; Zurek et al., 1995). Group AL and R sows were also approaching a relatively more positive energy balance between weaning and estrus. Although the weaning to estrus interval was not different between AL and SA sows, estrus occurred in the SA group as they were approaching a relatively less positive energy balance. If the situation between weaning and estrus was analogous to that of lactation, a sow approaching a more catabolic state would be expected to exhibit an extended weaning to estrus interval (Armstrong et al., 1989; Koketsu et al., 1996; Reese et al., 1982). The data arising from King and Williams (1984) and Baidoo et al. (1992) has shown that the weaning to estrus interval cannot be reduced by additional feed consumption after weaning, and conversely if the sows were fed 6 kg during lactation, restricted feed intake after weaning did not extend the weaning to estrus interval (Baidoo et al., 1992). The inability of post weaning feed intake to affect return to estrus interval, combined with the observation that SA sows approached a less positive energy balance after weaning, suggests that the rate limiting events culminating in estrous are dependent on nutritional status and/or LH secretion during lactation, and probably no additional benefit for reduced weaning to estrus interval would have been gained if the sow were to have been superalimented after weaning.

If we accept that metabolic state in lactation exerts the predominant effect on fertility after weaning, then both central and ovarian aspects are potentially involved (Foxcroft et al., 1995). Indeed, LH has the ability to affect ovarian development directly, since frequent injection of GnRH for 3 days caused an increase in follicular development (De Rensis et al., 1991) and pulsatile infusion for 7 days resulted in lactational estrus and ovulation (Rojanasthien et al., 1988; Cox and Britt, 1982). Local effects of insulin (Cox et al., 1987; Meurer et al., 1991) and nutrition on follicle selection (Flowers et al., 1988) and development (Cosgrove et al., 1992) have also been established. The possibility that fecundity is further affected by events after weaning cannot be dismissed, because breeding low parity sows at second rather than first postweaning estrus increased the subsequent litter size, which was associated with sows becoming less catabolic at the time of breeding (Clowes et al., 1994).

In agreement with the data reported by Baidoo et al. (1992) and Kirkwood et al. (1987) lactational feed intake did not affect ovulation rate, which is confounded in all of these studies by treatment induced alterations in the weaning to estrus interval. Interestingly, Group SA sows suckling 8-10 piglets exhibited a similar ovulation rate to 'to appetite' fed sows with only 6 piglets suckling (Zak et al., in press) and other groups in both experiments, which were in relatively more catabolic states also had similar ovulation rates to those observed in this experiment. It is interesting to speculate that minimizing the rate limiting factors affecting folliculogenesis (Fortune, 1994) by maintaining LH pulsatile characteristics (Zak et al., in press) or by optimizing nutrient availability (this experiment) could increase ovulation rate. Indeed, experimental paradigms in the gilt to increase plane of nutritional intake (Flowers et al., 1989; Beltranena et al., 1991; Ashworth et al.,1995), or insulin administration

concomitant with adequate feed intake for several days before estrus all increased ovulation rate (Cox et al., 1987).

In contrast to the data reported by Zak et al.(in press), Baidoo et al. (1992), and Kirkwood et al. (1987) feed restriction during lactation did not affect embryo survival when compared to those maintained on a high feed intake during lactation, although the absolute values reported for high and restrict fed lactational sows were similar in all experimental paradigms. Although SA sows gained body weight and backfat during lactation, and after weaning they 'self restricted' their feed intake, the embryo survival values are not consistent with the data reported by Baidoo et al., (1992), in which embryo survival was maximal for sows allowed a high feed intake during lactation and were then restricted after weaning.

Plasma progesterone is generally considered to have a critical role in mediating embryo survival by affecting the secretion of uterine factors (Roberts and Bazer., 1988). Independent of treatment, a correlation between embryo survival and plasma progesterone was observed at 72 h after the LH surge, this observation is in agreement with the data reported by Pharazyn et al. (1991) and Jindal et al. (1996) in the cyclic gilt and by Foxcroft (unpublished observation) in the weaned primiparous sow.

In conclusion, these data are not consistent with the hypothesis that superalimentation of sows to counteract catabolism due to inadequate feed intake will improve fertility. Collectively these data suggest that the weaning to estrus interval is dependent on metabolic status during lactation rather than changes in metabolic state subsequent to weaning. In part this observation may be dependent on LH secretion at the time of weaning and plasma concentrations of IGF-I may enhance follicular development and maturation via ovarian mechanisms.

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Table 5.1 Composition of experimental diets, % as based on air dry analysis

	Gestation	Lactation
Ingredients, %		
Barley	56.3	24.0
Wheat	30.0	24.0
Soybean meal, 44% CP[1]	7.0	22.0
Fishmeal	-	5.5
Tallow	2.0	-
Sugar	-	16
Canola oil	-	5.5
Dicalcium phosphate	1.7	2.5
Limestone	1.4	-
Vitamin-mineral premix[2]	1.0	1.0
Iodized salt	.5	-
Chemical Analysis		
Crude protein, %	13.7	18.6
Lysine, %	.56	1.05
Digestible energy, MJ DE/kg	13.7	15.4

[1] CP= Crude Protein

[2] Supplied the following per kg of diet: Zinc, 120 mg; Manganese, 12 mg; Iron, 150 mg; Copper, 12 mg; Selenium, 0.1 mg; vitamin A, 5000 IU; Vitamin D_3, 500 IU; Vitamin E, 22 IU; Riboflavin, 12 mg; Niacin, 45 mg; Calcium Pantothenate, 24 mg; Choline Chloride, 840 mg; Vitamin B_{12}, 30 μg; Biotin, 200 μg.

Table 5.2 Least square means (± SE) for feed intake, sow body weight and backfat and litter weights at weekly intervals during lactation, and from weaning to standing heat (SH).

	Treatment[1]		
Item	SA (n = 8)	AL (n = 12)	R (n = 9)
Feed intake (kg)			
Week 1	5.50 ± .60^{x}	4.32 ± .91^{y}	2.67 ± .25^{z}
Week 2	7.54 ± .36^{x}	5.26 ± .86^{y}	3.03 ± .41^{z}
Week 3	8.23 ± .30^{x}	5.70 ± .93^{y}	3.11 ± .43^{z}
Week 4	7.60 ± .45^{x}	4.97 ± 1.83^{y}	2.30 ± .41^{z}
Weaning to estrus (% food refused)	1.36 ± .3^{x} (62 %)	2.30 ± .13^{y} (30 %)	3.2 ± .11^{z} (9 %)
Sow body weight (kg)			
Farrowing	176.5 ± 12.2	178.9 ± 10.0	176.2 ± 10.5
day 7	182.7 ± 5.0^{x}	176.8 ± 3.3^{xy}	164.9 ± 3.8^{y}
day 14	182.3 ± 4.8^{x}	172.5 ± 3.4^{x}	153.9 ± .9^{y}
day 21	185.3 ± .7^{x}	170.0 ± .6^{x}	146.7 ± 1.0^{c}
day 28	181.6 ± .9^{x}	162.6 ± .6^{y}	137.3 ± 1.2^{z}
SH	164.6 ± 5.6^{x}	155.2 ± 4.3^{xy}	147.2 ± 3.4^{y}
Weight change, day 0 to 28	+ 5.1 ± 1.2^{x}	- 16.3 ± .9^{y}	- 38.9 ± 1.1^{z}
Weight change, day 28 to SH	- 17.0 ± 1.8^{x}	- 7.4 ± 2.5^{y}	+ 9.9 ± 3.0^{z}
Backfat (mm)			
Farrowing	17.0 ± 1.4	18.0 ± 1.7	18.3 ± 1.9
day 7	15.6 ± .7^{x}	17.3 ± .5^{x}	16.7 ± .8^{y}
day 14	18.3 ± .6^{x}	16.3 ± .5^{y}	13.5 ± .9^{z}
day 21	18.4 ± .7^{x}	15.6 ± .6^{y}	11.4 ± 1.0^{z}
day 28	18.8 ± .9^{x}	14.3 ± .6^{x}	9.4 ± 1.2^{y}

SH	16.6 ± 1.0^{x}	14.0 ± .9^{y}	10.8 ± 1.3^{z}
Backfat change day 1 to 28	+ 1.8 ± .5^{x}	- 3.7 ± .8^{y}	- 8.9 ± .9^{z}
Backfat change day 28 to SH	- 2.2 ± 1.0^{x}	- .3 ± .5^{y}	+ 1.4 ± .8^{z}
Litter weight (kg)			
piglets weaned	8.8 ± .8	8.5 ± .9	8.4 ± .9
day 1	13.1 ± 1.6	12.4 ± 1.7	12.7 ± 2.5
day 7	23.2 ± 1.6	21.3 ± 1.1	22.1 ± 1.8
day 14	39.1 ± 2.8	36.9± 2.2	36.4 ± 2.9
day 21	57.1 ± 3.5^{x}	53.1 ± 2.5^{xy}	50.9 ± 3.1^{y}
day 28	70.2 ± 3.8^{x}	70.3 ± 2.9^{x}	62.7 ± 3.2^{y}
Piglet growth rate (g / d)	232 ± 50	243 ± 60	213 ± 50

[a]SA, AL and R = sows with different lactational feed intakes. SA = sows receiving feed intake to 125 % of ad libitum, AL = sows receiving ad libitum access to feed, R = sows receiving 50 % of estimated ad libitum feed intake.

^{xyz} Means within a row lacking a common superscript letter differ ($P < .05$).

Table 5.3 Least square means (± SE) for plasma insulin, IGF-I, LH concentration and LH pulse frequency at day 28 of lactation, before and after weaning, and at standing heat (SH). Weaning to estrus interval (WEI), ovulation rate (OR) and embryo survival (ES %).

	Treatment[1]		
Item	SA (n = 8)	AL (n = 12)	R (n = 9)
Insulin (ng/ml)			
preprandial, day 28	1.42 ± .12	1.35 ± .16	1.22 ± .10
postprandial, day 28	2.65 ± .21	2.57 ± .14	2.20 ± .16
after weaning	1.84 ± .2	2.10 ± .15	1.81 ± .16
standing heat	1.37 ± .3	2.76 ± .93	2.18 ± .43
Change, day 28 to SH	- .47 ± .41^{x}	+ .66 ± .21^{y}	+ .37 ± .31^{y}
IGF-I (ng/ml)			
day 28	51.2 ± 1.8^{x}	53.2 ± 1.9^{x}	25.5 ± 1.0^{y}
after weaning	51.2 ± 1.8^{x}	53.3 ± .1^{x}	25.9 ± .1^{y}
SH	51.6 ± .9	55.1 ± .2	52.1 ± .2
Change, day 28 to SH	+ .4 ± .5^{x}	+ 1.8 ± .2^{x}	+ 26.2 ± .2^{y}
Mean LH (ng/ml)			
day 28	.33 ± .04^{x}	.29 ± .03^{x}	.22 ± .03^{y}
After weaning	.51 ± .04^{x}	.45 ± .03^{x}	.36 ± .03^{y}
LH frequency (pulse/ 12 h)			
day 28	3.3 ± .8^{x}	2.7 ± .6^{x}	0.2 ± .6^{y}
After weaning	7.8 ± .8^{x}	6.5 ± .6^{x}	3.8 ± .6^{y}

WEI	$4.4 \pm .8^{x}$	$4.2 \pm .1^{x}$	$6.3 \pm .1^{y}$
OR*	18.3 ± 2.6	14.4 ± 2.8	15.6 ± 3.14
ES (%)[§]	73.2 ± 15.2	83.3 ± 10.1	72.3 ± 15.8

[a]SA, AL and R = sows with different lactational feed intakes. SA = sows receiving feed intake to 125 % of ad libitum, AL = sows receiving ad libitum access to feed, R = sows receiving 50 % of estimated ad libitum feed intake.

^{xyz} Means within a row lacking a common superscript letter differ ($P < .05$).

* Number of observations: SA = 6, AL = 7, R = 7.

[§] Number of observations: SA = 5, AL = 7, R = 7.

Table 5.4. Least square means (± SE) for energy balance calculations during week 4 of lactation and standing heat (SH).

Item	Treatment[1]		
	SA (n = 8)	AL (n = 12)	R (n = 9)
Sow weight (kg)			
Weaning	181.6 ± .9^{x}	162.6 ± .6^{y}	137.3 ± 1.2^{z}
SH	164.6 ± 5.6^{x}	155.2 ± 4.3^{xy}	147.2 ± 3.4^{y}
Litter weight gain (kg/d) day 21 to 28	1.87 ± .5^{x}	2.45 ± .6^{x}	1.68 ± .5^{y}
Milk production (kg/d)[2]	7.48 ± .8^{x}	9.8 ± 1.1^{x}	6.72 ± .6^{y}
Feed intake (kg)			
day 21 to 28[3]	7.6 ± .5^{x}	4.97 ± 1.8^{y}	2.3 ± .4^{z}
day 28 to SH[4]	1.4 ± .1^{x}	2.3 ± .1^{y}	3.2 ± .1^{z}
Energy balance (MJ DE/d)			
Energy intake (MJ DE) day 21 to 28	117.0 ± 9.2^{x}	76.5 ± 6.3^{y}	35.4 ± 3.2^{z}
Energy intake (MJ DE) day 28 to SH	19.2 ± 6.0^{x}	31.5 ± 1.4^{y}	43.8 ± 1.3^{z}
Maintenance energy[5] (MJ DE/d) day 28	22.8 ± 2.7	21.0 ± 2.7	18.5 ± 3.0
Maintenance energy (MJ DE/d) SH	21.1 ± 1.7	20.2 ± 2.2	19.4 ± 2.2
Energy in milk[6] (MJ DE/kg)	62.8 ± 5.5	82.3 ± 9.8	56.5 ± 7.4
Energy balance[7] (MJ DE/d), day 28	+ 33.1 ± 5.0^{x}	- 26.8 ± 6.9^{y}	- 39.6 ± 5.2^{z}
Energy balance[8] (MJ DE/d), SH	- 1.9 ± .8^{x}	+ 11.3 ± 3.0^{y}	+ 24.4 ± 2.7^{z}

[1] SA, AL and R = sows with different lactational feed intakes: SA = sows receiving feed intake to 125% of ad libitum intake, AL = sows receiving ad libitum access to

feed , R = sows receiving 50% of estimated ad libitum feed intake.

[2] Milk yield of sows is estimated from litter weight gains (4 g milk/ g gain) (ARC, 1981).

[3] Metabolizable energy content of lactational diet = 15.4 MJ DE/kg.

[4] Metabolizable energy content of gestational diet = 13.7 MJ DE/kg.

[5] Maintenance energy requirement = .46 MJ / $Wt^{.75}$ kg /d.

[6] Requirement for milk production = 8.4 MJ DE/kg, based on energy content of milk of 5.2 MJ/kg and efficiency of utilization of 65% (ARC, 1981; Verstegen et al., 1985).

[7] Energy balance at day 28 = (Energy for sow maintenance + energy for milk production) - (Energy in feed consumed).

[8] Energy balance at standing heat = (Energy for sow maintenance) - (Energy in feed consumed).

[xyz] Means within a row lacking a common superscript letter differ ($P < .05$)

Figure 5.1 Diagrammatic representation of events during the experimental trial. Sows were placed on their respective treatments within 3 days of farrowing. At day 26 surgery was performed to insert an indwelling catheter to allow for frequent blood sampling at day 28, for 12 hours before and after weaning. After weaning all sows were allotted 2.5 X maintenance energy until the morning following standing heat. After standing heat they were allowed to eat 2 X maintenance energy requirement. At 12 and 24 hours after standing heat (SH) the sows were artificially inseminated. At 25 days after standing heat they were slaughtered to allow for the recovery of reproductive tracts.

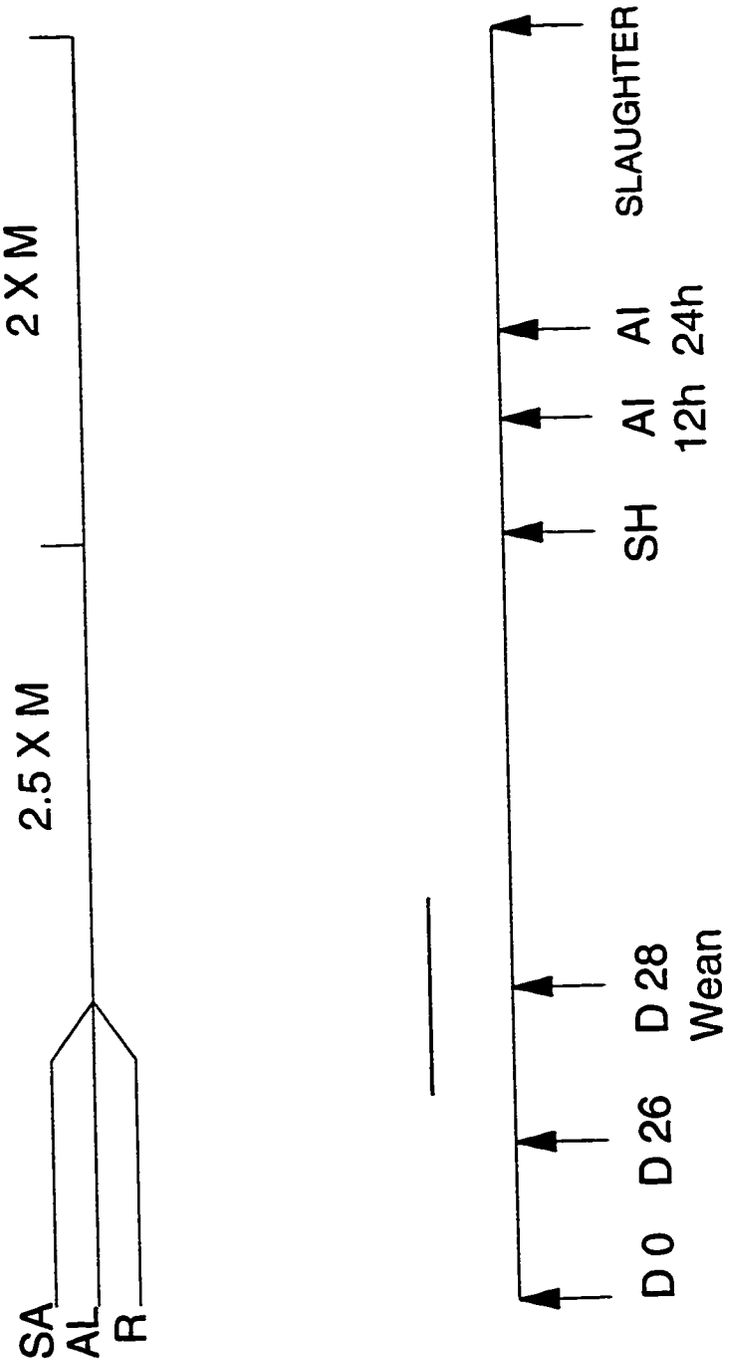

SA
AL
R
2.5 X M
2 X M
D 0
D 26
D 28
Wean
SH
AI
12h
AI
24h
SLAUGHTER

Figure 5.2 Representative profiles of LH secretion, obtained from three superalimented sows (a, b and c) during a continuous 24-hour bleeding window for 12 hours before and after weaning.

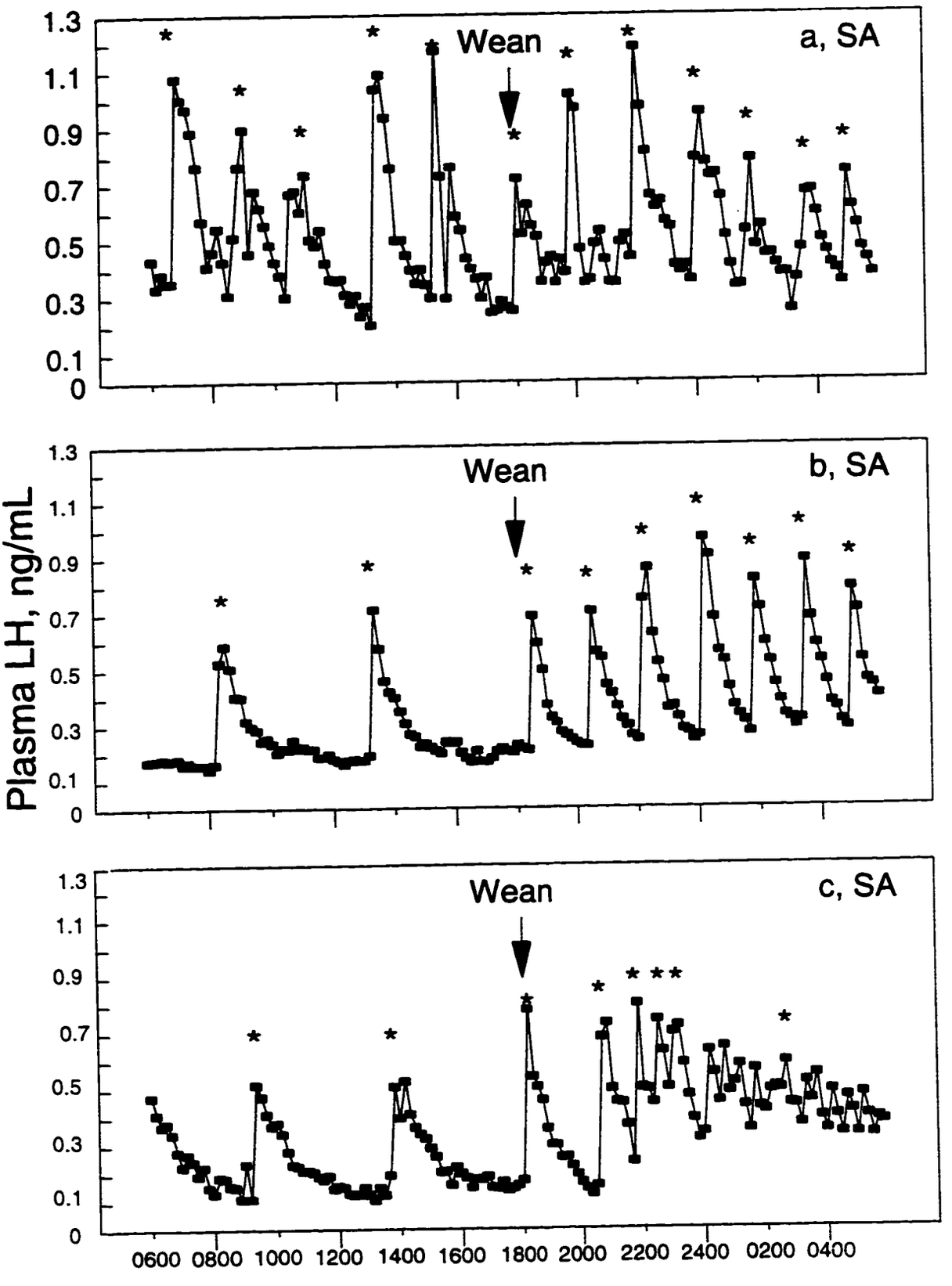

Wean
a, SA
Wean
b, SA
Wean
c, SA
Plasma LH, ng/mL
1.3
1.1
0.9
0.7
0.5
0.3
0.1
0
0600 0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400

Figure 5.3 Representative profiles of LH secretion, obtained from three sows allowed to feed to their ad libitum capacity (a, b and c) during a continuous 24-hour bleeding window for 12 hours before and after weaning.

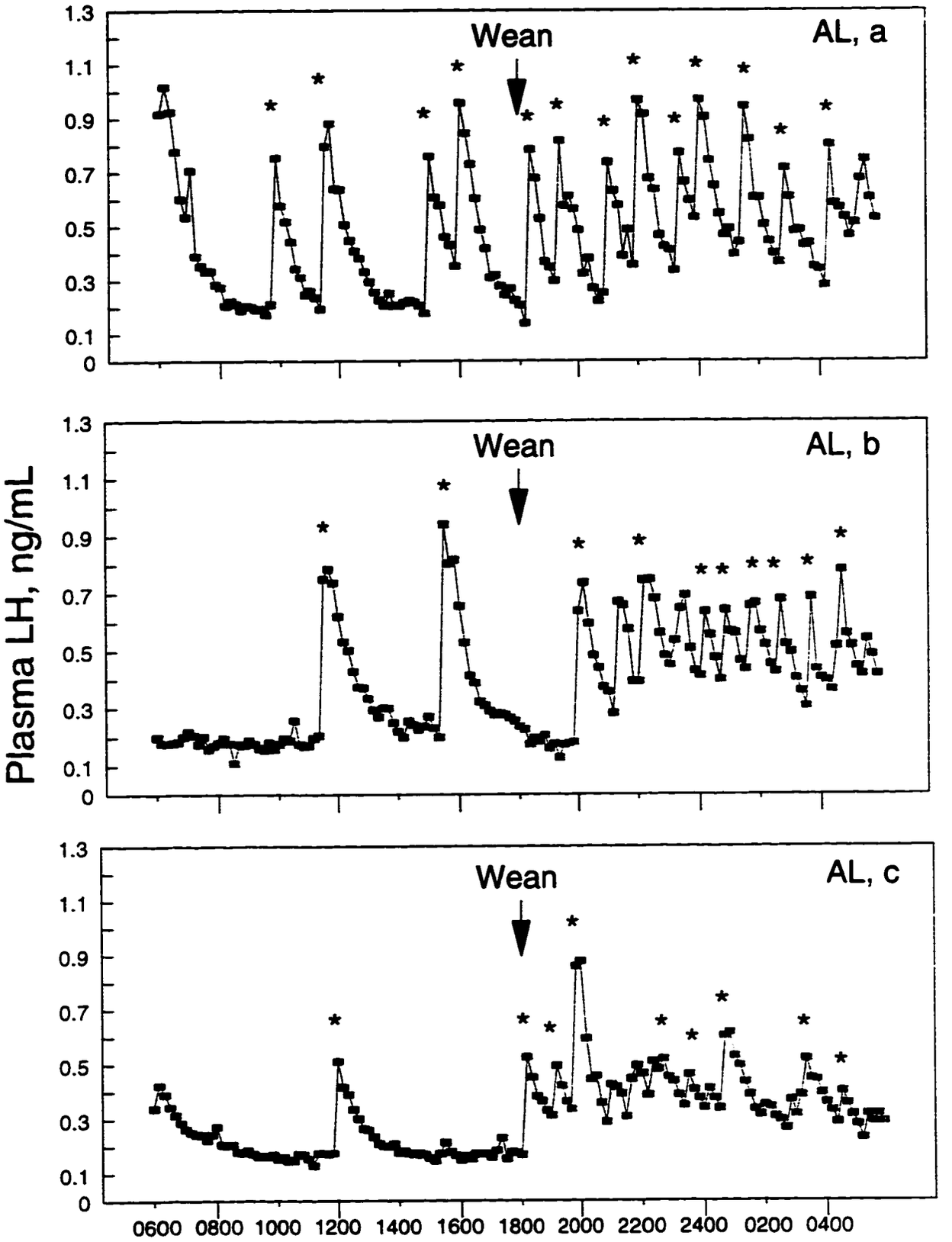
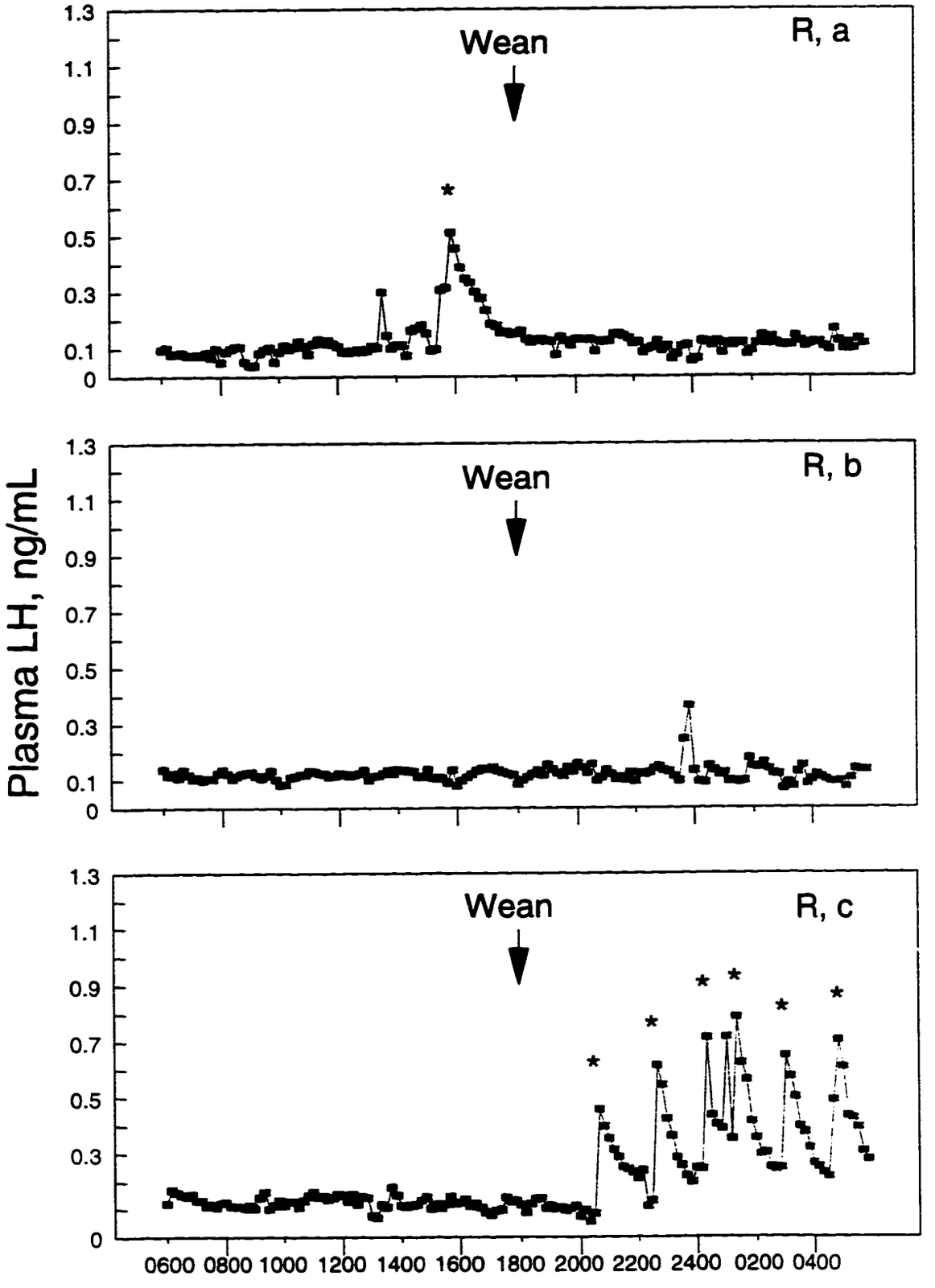

Wean
AL, a
Wean
AL, b
Wean
AL, c
Plasma LH, ng/mL
1.3
1.1
0.9
0.7
0.5
0.3
0.1
0
0600 0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400

Figure 5.4 Representative profiles of LH secretion, obtained from three sows fed to 50 % (a, b and c) during a continuous 24 hour bleeding window for 12-hours before and after weaning.


Plasma LH, ng/mL
Wean
R, a
R, b
R, c
1.3
1.1
0.9
0.7
0.5
0.3
0.1
0
0600 0800 1000 1200 1400 1600 1800 2000 2200 2400 0200 0400

CHAPTER 6
GENERAL CONCLUSIONS

The theme of the experiments described in this thesis was to investigate the effects of different metabolic states during lactation in primiparous sows on subsequent reproductive function. Two models were developed. The first described in Chapters 3 and 4, was developed from a model in the gilt described by Booth et al. (1996), in which altering the pattern of feed intake for only 7 days produced significant changes in metabolic and reproductive status. The main hypothesis tested in Chapter 3 was that similar short term changes in feed intake and associated changes in metabolic status have a critical effect on post weaning fertility . We also sought to identify the endocrine mechanisms mediating these effects. The second model was developed in collaboration with Dr Ian Williams (University of Western Australia). Lactating sows are normally catabolic during lactation and as such the lactating sow is not an easy model which to investigate the potential beneficial effects of an anabolic status on reproductive performance. To test the hypotheses that 1) making sows anabolic during lactation would ameliorate the catabolic effects on LH secretion and 2), would have beneficial effects on postweaning reproductive performance, sows were made anabolic during lactation by infusion of a quantity of feed into the stomach which was calculated to make them gain body weight. As the endocrine and reproductive parameters measured in both experiments were similar, in this chapter the results from all experiments have been integrated in an attempt to better understand the mechanisms controlling reproduction in the lactating and weaned sow.

The principal plasma indicators measured to determine metabolic state were IGF-I and insulin concentration. During periods of feed restriction plasma IGF-I concentration was lower than when compared to sows that were fed ad libitum. These findings are consistent with the data reported by Booth et al. (1996) in which 7 days of feed restriction reduced plasma IGF-I, and in the lactating sow in which decreased body weight was associated with reduced IGF-I (Tokach et al., 1993). Interestingly plasma IGF-I concentration in superalimented sows was not greater than in sows fed ad libitum. There is relatively little comparable data to which this observation can be compared. In obese human subjects plasma IGF-I is highly variable and no consistent relationship exists (see Thissen et al., 1994, for review). In an experiment by Forbes et al. (1989), IGF-I concentration increased by only 19% in human subjects that were over fed for 14 days. The composition of body weight increase was approximately 50% fat and 50% lean mass, which may indicate that the blunted IGF-I response to superalimentation may be in part dependent on the maximal lean growth rate in individuals, above which additional nutrients are deposited as fat.

Both experimental models produced treatment groups that had very different reproductive characteristics and as such the results have allowed us more insight into factors pertaining to the control of reproduction.

LH secretion during lactation is predominantly suppressed by the piglet suckling stimulus (Foxcroft et al., 1995). Moreover, the intensity of the suckling stimulus is

inversely related to LH secretion during lactation because Mullan et al. (1991) found that LH secretion was greater in sows suckled by 6 as opposed to 12 piglets. Furthermore, within a few hours of the piglets being weaned LH pulsatility increased robustly (Shaw and Foxcroft, 1985). Despite the predominant suppressive effect of piglet suckling stimulus, restriction of feed intake during a 28 day lactation can further suppress LH secretion (Baidoo, 1989). The results from Experiment 1 agree with the results in the gilt (Booth et al., 1996), in that periods of altered metabolic status for 7 days, had dramatic effects on LH secretion. Interestingly in Experiment 3, although SA sows were depositing lean body mass (Clowes; unpublished observation) and were anabolic during lactation, this did not ameliorate the suckling induced suppression of LH when compared to sows fed ad libitum. This observation again supports the suggestion that suckling per se is the predominant inhibitor of LH during lactation, or alternatively, by making these sows obese (increased backfat) during lactation may have resulted in a degree of tissue insensitivity to the normal metabolic signals and resulted in blunted insulin, IGF-I and thus, LH responses. The LH response to weaning for all sows in Experiment 1 and the superalimented and ad libitum fed sows in Experiment 3 was robust. Although sows that were feed restricted for the duration of lactation in Experiment 3 still exhibited an increase in LH after weaning the response was lower when compared to other treatment groups. This suggests that the adverse effects of catabolism experienced during lactation is not removed at weaning, and latent effects of metabolic status after weaning , possibly mediated through plasma IGF-I, continue to suppress LH secretion.

Weaning to estrus interval has been correlated with LH secretion immediately prior to weaning (King and Martin, 1989; Baidoo, 1989), and certainly suppression of LH in sows that were restrict fed for the duration of lactation also expressed a delay in the onset of estrous after weaning (Experiment 3). However, as evidenced from Experiment 1, LH immediately prior to weaning was not correlated with the weaning to estrus interval, because sows that were restricted for the first 3 weeks and then fed to appetite for week 4 of lactation (RA), still exhibited a delayed weaning to estrus interval although LH secretion at weaning was similar to that observed in 'to appetite' fed sows. Furthermore, in a recent experiment by Koketsu et al. (1996) restriction of feed intake during any week of lactation suppressed plasma insulin, IGF-I and LH concentrations and extended the weaning to estrus interval. It is interesting to note however, that weaning to estrus interval is not influenced by feed intake after weaning (King and Williams, 1984; Baidoo et al., 1992). In support of this observation, sows that were superalimented during lactation, exhibited estrus at the same time as 'ad libitum' fed sows, even though they lost considerable body weight after weaning. Together these data suggest that the timing of estrus after weaning is dependent on events that occur during lactation, which ultimately affect the responsiveness of the ovary to the robust increase in LH secretion at weaning, which is initiates the final follicular development (Britt et al., 1985).

The events that culminate in altered sensitivity of the ovary to increased LH secretion at weaning may involve both central and local effects (Foxcroft et al., 1995).

During lactation, although LH is suppressed by the suckling stimulus, follicular development can still be triggered by appropriate gonadotropin stimulation, because infusion of pulsatile GnRH for 7 days resulted in lactational estrus and ovulation (Cox and Britt, 1982). Metabolic status during lactation also affects ovarian development. Miller (unpublished observation) observed that after two weeks of lactation, feeding to maintenance energy requirements abolished all follicular development above 3 mm in diameter.

Suppressed plasma concentrations of insulin or IGF-I, often observed during periods of feed restriction, may mediate potential nutritional effects on follicle development because in vitro studies have identified para and autocrine roles for insulin and IGF-I (Hammond et al., 1993). Furthermore in the prepubertal gilt (Cosgrove et al., 1992), realimentation for 5 days after a 7-day period of feed restriction, was associated with increased follicular diameter, volume and estradiol synthesis, although the usual increase in LH associated with realimentation was abolished by feeding of allyl trenbolone.

To date, the results presented in Experiment 1, are the first report that ovulation rate at the first post weaning estrus was significantly suppressed when sows were feed restricted during lactation. Normally feed restriction during lactation is associated with an increased weaning to estrus interval of at least 5 to 6 days (Koketsu et al., 1996). However in Experiment 1, the weaning to estrus interval was extended by only 1.5 to 2 days, which was sufficient to minimize the interaction between elevated ovulation rates and increased weaning to estrus interval. It is well documented in the swine literature that relatively less catabolic states enhance ovarian function. For example, 'flush feeding' of gilts for at least 6 days before onset of estrus normalizes ovulation rate in gilts that were previously feed restricted (Beltranena et al., 1991) and in the lactating sow, 'skip a heat' breeding after weaning in low parity sows is practiced to increase the number of piglets in the subsequent litter. Furthermore, endocrine studies by Clowes et al. (1995) determined that improved litter size was associated with the sows becoming less catabolic at breeding during the second post weaning estrus when compared to the estrus immediately following weaning. There is , however, a disparity between the gilt and lactating sow models. Although RA sows had been effectively flush fed for the last 7 days of lactation, and plasma insulin and IGF-I concentration, and LH secretion were similar between AA and RA sows at weaning, RA sows still exhibited a lower ovulation rate than AA sows which indicates the growth or maturation of follicles during lactation can be affected by metabolic state during lactation.

Embryo survival has previously been shown to be lower in sows fed a restricted amount of feed during lactation (Baidoo et al., 1992), however, no differences in embryo survival were observed in Experiment 3, despite the sows differing metabolic states. In Experiment 1, embryo survival was suppressed in AR sows only, which was quite unexpected. This model has produced a unique effect, in that differential effects on ovulation rate and embryo survival were achieved according to the pattern of lactational catabolism, and increased catabolism during the last week of lactation

severely compromised embryo survival. Thus the duration of ad libitum feeding prior to ovulation may have important consequences for embryo survival. In the gilt the time of follicle development from antrum formation to 3 mm was estimated to take 14 days and a further 5 days to reach preovulatory state (Morbeck et al., 1992). If this data is extrapolated to the lactating sow, follicles undergoing antrum formation in late gestation or early lactation would constitute the pool from which follicles would be selected after weaning. These data combined with the results of Experiment 1 suggest that the follicle or oocyte may be 'imprinted' during lactation by adverse metabolic states which may have important consequences for embryo survival. The concept that nutritionally induced manipulations of embryo survival may not only involve changes in the uterine environment, but also alterations in follicular and oocyte maturation was addressed in Experiment 2.

Follicular diameters and estradiol content of the largest 15 follicle per sow were collected at approximately 38 hours before expected standing heat. Although there was no difference in daily estimates of plasma estradiol concentration, RA sows had more large follicles than AR sows. In vitro maturation of oocytes resulted in a higher rate of nuclear maturation for oocytes retrieved from RA sows than from AR sows, and similarly the nuclear maturation rate of oocytes retrieved from prepubertal gilts was also greater when incubated with the follicular fluid obtained from RA sows. Thus the size of follicles in the preovulatory pool and the nuclear maturational rate of oocytes obtained from these follicles can be affected by the nutritional history of the sow and furthermore, factors of follicular origin appear to mediate these effects. The differences in oocyte maturation that were observed between these treatments is probably as a result of impaired ovarian function. Miller (unpublished observation) found that feed restriction during lactation for two weeks abolished follicle development above 3 mm in diameter. Furthermore restricted feed intake during any week of a 3 week lactation increased the weaning to estrus interval, compared to sows fed to appetite throughout (Koketsu et al., 1996), which is also suggestive of suboptimal ovarian function. The observation that more follicles in Group RA, which were effectively fed ad libitum for 12 days, were larger than in AR sows, which were fed to ad libitum for just 5 days, also supports this suggestion.

Thus, not only does adverse metabolic state during lactation limit follicular development (as determined by ovulation rate and weaning to estrus interval) but the 'metabolic milieu' in which follicular growth occurs significantly influences follicle and oocyte maturation. Cytoplasmic and nuclear maturation of the oocyte is closely regulated by its follicular environment (Tsafriri, 1988; for review) and in turn, this interaction effects the ability of the oocyte to produce a viable blastocyst (Thibault, 1977). Thus if catabolism during lactation results in a less mature follicular environment to which the oocyte must be exposed before ovulation, the ability of the oocyte to respond to the preovulatory LH surge and subsequent cytoplasmic and nuclear maturation may be impeded. Not only could this result in alterations in nuclear and cytoplasmic maturation, but also the ability of the follicle to luteinize may be compromised which would have ramifications for progesterone induced changes

in uterine morphology and glandular secretions in preparation for the developing blastocysts.

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