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UNIVERSITY OF ALBERTA

METABOLIC INDICATORS IN POST-WEANING SOW FERTILITY

BY

EMMA JAYNE CLOWES

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

IN

ANIMAL NUTRITION
DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING 1993

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled METABOLIC INDICATORS IN POSTWEANING SOW FERTILITY submitted by EMMA JAYNE CLOWES in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

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Date: Jan 13 1993

ABSTRACT

The effect of serving sows at first or second estrus after weaning was determined using 116 Camborough sows (parities 1-8). From day 109 of gestation until farrowing sows were fed their gestation allowance plus an additional 1 kg. Lactating sows were fed according to their metabolic body weight (Wt $kg^{0.75}$) and litter size. Litter size was standardized to 9 to 12 piglets, and sows were weaned at either 21±3 or 28±3 days post-partum.

Eighty sows were paired according to their weaning-to-estrus interval and parity. One sow of each pair was bred at first and the other at second estrus after weaning. The remaining 36 sows were also bred at either first or second estrus, but were catheterized either on day 3 after weaning or day 20 of the first estrous cycle. Sows were blood sampled every 30 mins from 08.00 to 13.30 h on the day after catheterisation, and bred twice by artificial insemination. Sows were bled every 6 h from catheterisation until one day after standing heat, and every 12 h for a further three days.

Litter size was increased ($P<0.002$) by breeding at the second estrus, for parity one and two sows only (10.4 vs. 12.8 total pigs born). These changes were observed in the absence of any significant changes in weight, backfat, fat and protein during lactation and in weight and backfat changes between weaning and breeding. Estrus of breeding did not influence litter size of sows of parity three or more sows.

Similar results were observed for the 36 catheterized sows, but a numerical increase in litter size was only seen in the young sows bred at second rather than first estrus ($P<0.15$). Plasma progesterone measured 50 h after the preovulatory LH surge was significantly higher in sows of parity one and two bred at second rather than first estrus (5.4 ± 0.7 vs. 3.7 ± 0.3). Sows of parity one and two bred at second estrus had significantly higher basal insulin levels ($P<0.09$), and lower IGF-1 ($P<0.05$) and glycerol ($P<0.001$) levels. For sows of parity three or greater, no significant differences were seen in plasma concentrations of basal insulin, insulin-like growth factor-1 and glycerol between first and second estrus, but GH was lower at second estrus ($P<0.03$). Only basal insulin was positively correlated with total litter size ($R=0.70$).

Thus, subsequent litter size was increased, possibly due to an increase in embryo survival, by delaying breeding sows of parity one and two but not of parity three or more until their second estrus after weaning. Furthermore, insulin, IGF-1 and glycerol but not GH are useful measures of metabolic status and likely play a role in sow fertility after weaning.

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INTRODUCTION

Data from a swine software management programme (Swine Graphics, Webster City, Iowa), based on 82,000 sows, indicated that sows which return to estrus within 7 to 12 days after weaning had smaller litter sizes (figure 1a) than sows that return to estrus within six days of weaning (Leman, 1987). This, statistic if expanded over a year, leads to reduced reproductive efficiencies in sows bred more than six days after weaning, as defined by the number of pigs weaned per sow per year (figure 1b). In a smaller trial involving 78 multiparous sows, those that returned to estrus 7 to 13 days after weaning (6 % of the herd) had lower ovulation rates (16.2 ovulations) and embryo numbers (13.3) than sows that returned to estrus within six days (17.5 ova and 14.1 embryos, respectively) or between 14 and 20 days after weaning (18.9 ova and 15.0 embryos, respectively) (Fahmy and Dufour, 1976).

Delaying breeding sows until the second estrus after weaning (skip-a-heat breeding) was widely used in primiparous sow management in large commercial Australian units during the 1980s (King *et al.*, 1982). Skip-a-heat breeding increases the subsequent litter size by 1.0 to 3.4 pigs, compared to sows bred at first estrus (Moody *et al.*, 1969; Pay, 1973; Love, 1979; Fahmy *et al.*, 1979; King *et al.*, 1984; Clark and Leman, 1987; Clark *et al.*, 1988; Morrow *et al.*, 1989; Conner, 1992). In fact, skip-a-heat breeding may increase fertility and improve herd productivity in those sows which return to estrus after more than six days after weaning, especially in primiparous sows.

Obviously, breeding delays are useful only if they are cost efficient. The economics of skip-a-heat breeding can be crudely assessed by estimating the number of piglets per day produced by the sow, and then multiplying this by the extra number of days the sow remains empty (21 days):

piglet days = (#piglets/sow/yr * preweaning piglet mortality) / 365 days

For example, using Alberta averages, each sow produces 18 pigs/sow/yr with a preweaning mortality rate of about 20%, which translates to 0.056 pigs/day. A breeding delay of 21 days (a single estrous cycle) would result in the loss of 1.17 pigs/delayed breeding (0.056 pigs/day * 21 days). However, in higher performing units, a sow would produce 24 pigs/sow/yr giving 0.079 pigs/day. Hence, the production loss arising from breeding delays would be 1.66 pigs. Such figures do not take into account the cost of nonproductive days (NPD), efficient farrowing crate use and other factors (Dial, 1992). Therefore, an increase of 1.5 to 2.0 piglet births may not be a sufficient increase in litter size to cover the cost of maintaining the sow for an additional estrous cycle.

The data of Leman (1987) was collected from sows of different breeds and parities and from various farms under both good and bad management conditions. Therefore the data may be distorted by factors which influence breeding performance. These factors include genetics (Dyck, 1971ab, 1972; Fahmy and Dufour, 1976; Fahmy *et al.*, 1979; O'Grady *et al.*, 1985), parity (Lee and Mitchell, 1989), age (King and Dunkin, 1986a), management practices (Varley, 1982; Britt, 1986), season (Fahmy *et al.*, 1979; Hurtgen *et al.*, 1980; Svarek *et al.*, 1981; Cox *et al.*, 1983; Armstrong *et al.*, 1986), environmental temperature (O'Grady *et al.*, 1985) and boar effects (Britt, 1986). A better understanding of the data of Leman (1987) is required to explain the effects of reduced litter size and pigs/sow/year seen in sows bred between 7 to 14 days after weaning (figures 1ab).

Nutrition is another factor that affects reproduction (Aherne and Kirkwood, 1985; Close and Cole, 1986; King, 1987; Britt *et al.*, 1988; Hughes and Pearce, 1989; Booth, 1990ab; Cole, 1990). Indeed the basis for nutrition-reproduction interactions has been the focus of many recent studies. It has been suggested that the metabolic status of an animal is closely linked to fertility (Booth, 1990b; I'Anson *et al.*, 1991), but the mechanisms by which nutrition affects reproduction are still unclear. Even so, insulin and insulin-like growth factor-1 (IGF-1) have been proposed as key hormones affecting reproduction. The main objective of this thesis was to further investigate the relationship between the sow's metabolic status and her fertility after weaning.

The specific objectives of the research were:

1. To create, under controlled conditions, a group of sows that return to estrus in seven or more days after weaning and determine whether these animals had smaller litters, as previously reported.
2. To Determine whether skip-a-heat breeding increases litter size above that of first estrus sows, especially for those sows that returned to estrus late after weaning.
3. To determine the extent to which metabolic parameters, such as insulin and insulin-like growth factor-1 (IGF-1) might be used as indicators of metabolic status, and thus fertility in the sow after weaning, and therefore help explain the physiological basis for differences in post-weaning sow fertility.

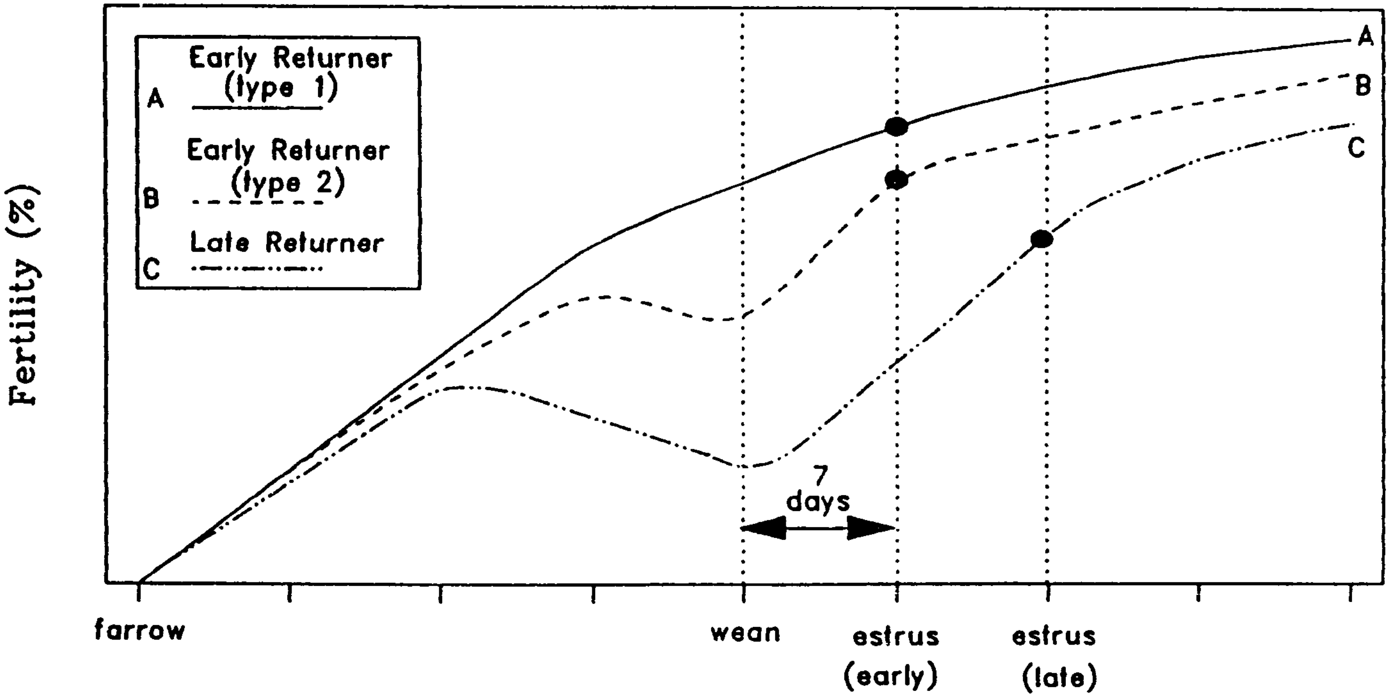
REVIEW OF THE LITERATURE

1.1 INTRODUCTION

A sow must 'escape' from the affects of the suckling stimulus, and the extra nutritional demands placed on her tissue reserves during lactation, in order to return to estrus, ovulate, conceive and successfully deliver a healthy litter. In a recent review, Noblet *et al.* (1990) described these nutritional effects simply as inputs (nutrition) and outputs (maintenance requirements, growth and milk production). This simple method of describing the sows metabolic status will be used to help explain differences in sow fertility after weaning.

Figure 1.1 describes three different types of sow according to their metabolic status and fertility after weaning. Sows may maintain their tissue depots, or even lay down fat and protein, (remain anabolic) for a portion of their lactation, indicated by the positive slope of the line in figure 1.1 (line A). If a sow is in a positive energy and/or protein balance, she will continue to improve her fertility throughout lactation. At weaning, the type 1 early returner (figure 1.1, line A) is anabolic, although the slope of the line decreases in the last 10 to 14 days of lactation, at the time of peak lactation. These sows recover from lactation fairly quickly and return to estrus within three to six days after weaning. However, if a sow's lactational output outweighs her inputs, the inflexion of the line becomes negative (figure 1.1, line B), indicating the sow is in a negative metabolic state and is breaking down

Figure 1.1 Effect of metabolic state on sow fertility between farrowing and subsequent estrus



Early and late returners: sows that return to estrus early and late after weaning: fertility demonstrated in relation to metabolic status.

body tissues to maintain her lactational outputs (becomes catabolic). These sows remain anabolic until the third week of lactation (time of peak lactation) when they become catabolic, as indicated by the negative slope (see figure 1.1 line B). Because these sows lose only moderate amounts of weight during lactation, they recover their metabolic status rapidly after weaning and become anabolic, and return to estrus within six days (type 2 early returner). However, their relative fertility compared to type 1 sows will be lower. The majority of sows (82 %) return to estrus in less than seven days after weaning (Leman, 1987) and would therefore fit into the type 1 and 2 categories described.

Sows and gilts with very large litters and/or low voluntary feed intake or those restricted in feed intake during lactation may have lower lactational inputs than outputs. Such sows (see figure 1.1 line C) draw on their own body tissue reserves to overcome the lactational nutritional deficit and maintain milk yield and thus piglet growth rate (Eastham *et al.*, 1988). If restrict feeding continues over several consecutive lactations, milk yield and piglet weaning weight are reduced, because the sow is unable to draw on sufficient tissue depots to maintain lactation at an adequate level (Lythgoe, 1987). Thus, from a purely metabolic viewpoint, if a sow becomes increasingly catabolic during lactation, she will either remain anestrus or be late in returning to estrus after weaning. Such a sow would have considerably depleted her tissue reserves over lactation, and would take a longer period of time to become anabolic after weaning and increase her fertility enough to ovulate and maintain a pregnancy (late returner). Thus, in this third type of sow, fertility is depressed because of both a delayed return to estrus and a more pronounced reduction in litter size

This idea of an increase in fertility with time from farrowing is consistent with the data showing that breeding sows at the second estrus after weaning (skip-a-heat breeding) increased litter size above that of sows bred at first estrus. This increase in litter size is caused by both an increase in ovulation rate and embryo survival (King *et al.*, 1984). However, the effect of skip-a-heat breeding does not appear to increase the ovulation rate of sows above second estrus (Dyck, 1971b).

1.2 OPTIMIZATION OF THE SOW'S REPRODUCTIVE EFFICIENCY

Understanding how litter size and farrowing interval synergize to optimise a sow's reproductive performance and fertility is very important. Thus, to establish the conditions required for optimal sow fertility after weaning, a number of factors which affect fertility will be discussed.

1.2.1 Farrowing Interval.

The farrowing interval can be reduced to increase the number of farrowings/sow/year by shortening lactation and the weaning-to-estrus interval, because gestation can only be shortened by one or two days before neonatal survival of the offspring is diminished. However, shortening lactation to 10 days tends to extend the weaning-to-estrus interval and decrease the subsequent litter size (Pay, 1973; Aumaitre *et al.*, 1976; Varley and Cole, 1976a; Walker *et al.*, 1979; Kirkwood *et al.*, 1984). Thus an optimum lactation length of 20 to 30 days has been associated with highest reproductive efficiency (review by, Varley, 1982; Foxcroft, 1990).

Reducing lactation length decreases litter size by reducing embryo survival but apparently not ovulation rate (Moody and Speer, 1971; Varley and Cole, 1976b, 1978). Embryo survival may be reduced in early weaned sows because the uterine environment would not have fully recovered from the effects of gestation and parturition: Palmer *et al.* (1965) reported that uterine involution takes 21 to 28 days to complete.

1.2.2 Parity.

Many of a herd's reproductive problems are concentrated in low parity, and especially primiparous sows. Compared to multiparous sows, primiparous sows require additional energy and protein for growth, above those for milk production and maintenance during lactation. Also, the lactational voluntary feed intake of most primiparous sows almost invariably fails to meet their lactational nutrient requirement (O'Grady *et al.*, 1985). This makes the primiparous sow a sensitive but potentially complex model to use when studying nutritional effects on reproduction. Therefore when looking at a herd's performance, parity must always be accounted for, because primiparous sows are more likely to have extended weaning-to-estrus intervals (Aumaitre *et al.*, 1976; Love, 1979; Fahmy *et al.*, 1979; Hurtgen *et al.*, 1980;

Svarek *et al.*, 1981; Esbenshade *et al.*, 1986; Noblet and Etienne, 1987) and decreased subsequent litter sizes (Kirkwood *et al.*, 1988a; Lee and Mitchell, 1989), compared to multiparous sows.

1.2.3 Gestational feed intake.

Reducing lactational feed intake diminishes sow reproductive efficiency and fertility (King and Williams, 1984a; Hughes *et al.*, 1984; King and Dunkin, 1986ab; Kirkwood *et al.*, 1987a, 1990; Baidoo, 1989). Lactational voluntary feed intake is reduced if (1) the plane of nutrition in gestation is too high (O'Grady *et al.*, 1985; Aherne and Kirkwood, 1985; Esbenshade *et al.*, 1986; Lee and Mitchell, 1989; Mullan and Williams, 1989; Yang *et al.*, 1989; Cole, 1990) and (2) the sow is excessively fat at farrowing, which is also a reflection of high gestational feeding (Mullan and Williams, 1989; Yang *et al.*, 1989). In fact, Williams and Mullan (1989) suggested that lactational voluntary feed intake is reduced when a sow's body fat exceeds one third of her body weight. Thus, feeding strategies that consider the entire reproductive lifetime of the sow, rather than a single pregnancy or lactation must be evolved (Cole, 1990).

As many nutritional factors impinge on sow fertility at different times during her reproductive lifetime, the subsequent discussion will attempt to simplify the picture by focusing on three specific periods in the sow's reproductive cycle, lactation, the weaning-to-breeding interval and early gestation. Firstly, changes in body condition and body weight over these periods will be considered. Then, an attempt to further understand the association between metabolism and reproduction will be made by looking at correlations between the weaning-to-estrus interval and other measures of fertility, and fat and protein changes. Finally, certain key metabolic hormones will be discussed as a very probable mechanism linking reproduction and nutrition through the sows metabolic status.

1.3 LACTATION

The nutritional regimen imposed on a sow during lactation profoundly affects her fertility through her metabolic status (see figure 1.1). It is known that sows with an inadequate lactational nutrient intake frequently exhibit diminished conception rates (Hardy and Lodge, 1969; Hughes *et al.*, 1984; Kirkwood *et al.*, 1987a) and reduced subsequent litter size (Kirkwood *et al.*, 1988a). Therefore, it is extremely important to understand the effects of lactational nutrition on subsequent ovulation rate and embryo survival in the sow and, by doing so, develop appropriate nutritional regimens (ARC, 1990; NRC, 1988).

1.3.1 Effect of lactational weight and condition change on sow fertility.

It has long been known that sows that lose the largest amount of weight and fat during lactation, experience the highest incidence of delayed estrus after weaning (Hardy and Lodge, 1969; Reese *et al.*, 1982, 1984; King and Williams, 1984a; King *et al.*, 1984). Researchers have attempted to establish relationships between a sow's reproductive performance and her absolute and dynamic body weight and compositional changes during lactation, but the results are equivocal. Most researchers found no relationship between subsequent ovulation rate and either weight changes or absolute body weight at different stages of a sow's reproductive life (King and Williams, 1984a,b; Hughes *et al.*, 1984; King and Dunkin, 1986a,b). In contrast, others reported an inverse relationship, in multiparous sows, between subsequent ovulation rate and either weight loss during lactation (Hardy and Lodge, 1969), or weight and backfat at weaning (Moody and Speer, 1971). Only weak correlations between the weaning-to-mating interval and a sow's liveweight at weaning (R = -0.35; Mullan and Williams, 1989) and lactational liveweight loss (R = -0.02 to -0.30; King *et al.*, 1984; Reese *et al.*, 1984; Mullan and Williams, 1989; Brooks *et al.*, 1975) have been reported.

Therefore, lactational body weight changes *per se* are not reliable predictors of a sow's reproductive efficiency and fertility, and more predictable indicators were sought. This led to a consideration of the relationship between relative tissue changes and reproductive efficiency. From these data a number of conflicting schools of thought emerged. It was hypothesised, in turn, that either total feed intake (Baidoo, 1989), energy intake (Reese *et al.*, 1982; Nelssen *et al.*, 1985; Johnston *et al.*, 1989), change in body fat content (Whittemore *et al.*, 1980, 1984, 1989; Mullan, 1987; Mullan and Williams, 1989; Yang *et al.*, 1989; Mullan, 1991), protein intake or protein stores (King and Williams, 1984b; King and Dunkin, 1986b; King, 1987) were the most important nutritional factors regulating reproductive function in the sow.

However, in the experiments looking at lactational fat and protein effects on reproduction, the 'thin' sows in the Australian experiments (King, 1987; King and Williams, 1984ab; King *et al.* 1984; King and Dunkin, 1986ab; King and Martin, 1989) were equivalent in backfat thickness (mm) to the 'fat' sows in the Scottish experiments (Whittemore *et al.*, 1984; Eastham *et al.*, 1988; Whittemore and Yang, 1989; Yang *et al.*, 1989). Therefore, the sows used in Australian experiments were by no means short of fat reserves, unlike those in the Scottish experiments. This could bias the results, so care must be taken when interpreting these data.

1.3.2 Effect of energy/protein intake on weaning-to-estrus interval.

i) Fat/energy

More than 20 years ago it was hypothesised that a sow has a 'threshold' level of body fat, below which reproductive failure will occur (Elsley *et al.*, 1968; Maclean, 1969). The hypothesis was supported by research in women, which suggested that a critical ratio of fat to lean body mass is essential for the attainment of puberty and maintenance of reproductive function (for review see Frisch, 1984). However, this concept has been strongly criticised by Bronson and Manning (1991), amongst others, who challenge the hypothesis and suggest that it is 'merely based on correlative evidence and not proven scientific fact'.

Whittemore and Yang (1989) and Yang *et al.* (1989) still maintain that a 'threshold' level of fat of about 10 mm at the P2 site (10th rib and 65 mm from the midline) is required, to prevent breeding problems. Furthermore, they suggested that even if lactational protein loss is zero, lipid loss may still be appreciable, and concluded that the fat content of the body plays an important role in reproduction. However, these researchers continually over-simplify the effects of nutrition on reproduction, and have suggested that the equation [26.6 - 1.28 (backfat (mm) P2) at weaning)] adequately predicts the weaning to estrus interval in primiparous sows. Others suggest that the weaning-to-estrus interval is poorly correlated with backfat at weaning (R=-0.49, Johnston *et al.* 1989; -0.38, Mullan and Williams, 1989) or backfat loss during lactation (R=-0.15 to -0.32; Esbenshade *et al.*, 1986; Mullan and Williams, 1989).

As early as the 1960's, Elsley *et al.* (1968) demonstrated that feeding low energy diets during lactation delayed the return to estrus of sows after weaning. Later, King (1987) proposed that there is a critical lactational energy intake (45 MJ ME/d) below which the weaning-to-estrus interval is adversely affected, even when protein and lysine supplies are adequate. This contradicts the suggestion that critical energy levels vary according to a sow's weight and parity (King and Dunkin, 1986a).

ii) Protein

King and Dunkin (1986b) observed that increasing protein intake during lactation had effects on reproductive efficiency over and above those of energy intake. They also reported that daily lactational nitrogen losses were positively associated with the weaning-to-estrus interval (King and Williams, 1984b; King and Dunkin, 1986ab). Furthermore, sows on a low daily energy intake (33 MJ ME/d or 42 MJ ME/d) during lactation catabolized muscle tissue to a greater extent than sows on a higher energy intake (67, 50 or 59 MJ ME/d) (Reese *et al.*, 1984; Nelssen *et al.*, 1985).

In light of these findings, King (1987), amongst others hypothesised that protein intake and body protein reserves are the primary nutritional factors affecting a sow's reproductive efficiency. Further support for this hypothesis came from the observation that reducing lactational protein intake (414 vs. 746 g CP/d) increased the weaning-to-estrus interval (King and Martin, 1989). Following from this, Williams and Mullan (1989) suggested that a sow has a 'threshold' body weight (150 kg) and protein content above which she exhibits a minimum weaning to estrus interval. However, sows are still very actively growing at 150 kg liveweight. They therefore require more time to recover from the excessive nutrient demands of lactation, and become anabolic after weaning, because they have additional nutrient requirements for growth during lactation often not accounted for in lactational feed requirements.

iii) Protein and Energy.

Restriction of both protein (310 vs. 648 g CP/d) (King and Williams, 1984b) and energy (26 to 33 vs. 50 to 67 MJ ME/d) during lactation in primiparous sows (Reese *et al.*, 1982; King and Williams, 1984b) and energy alone in multiparous sows

(Reese *et al.*, 1984) extends the weaning-to-estrus interval. Furthermore, King (1987) in summarising data from a number of experiments on lactational nutrient intake, bodyweight and backfat losses and the weaning-to-estrus interval in primiparous sows, concluded that both protein (R = -0.82; King, 1987) and energy intake (R = -0.70, King, 1987; R = -0.69, Johnston *et al.*, 1989) during lactation are important factors affecting the weaning-to-estrus interval. This suggests that adequate levels of both protein and energy are required during lactation to prevent reproductive inefficiencies.

Thus, even though numerous experiments have been undertaken by many eminent scientists in the field of nutrition-reproduction interactions, the question as to what links nutrition and reproduction still remains unanswered. Therefore, criticism of the once highly accepted hypothesis that a minimum fat:lean ratio is required, in humans, to maintain reproductive function (Frisch, 1984) must be heeded when analyzing the results in sows. The human data (Frisch, 1984) has been strongly criticised by many, and perhaps rightly so, because an energy deficit most likely causes a decrease in sexual development in young females and a cessation of ovulation in adults. But, correlatively, fat storage will also be depressed (Bronson and Manning, 1991).

Research into the link between nutrition and reproduction in the sow has also concentrated on the associations between sow fertility and absolute levels of body fat and protein and their dynamic changes during lactation and the weaning-to-estrus interval. Again, similar to the human work, conclusions from these empirical experiments are tenuous, often producing weak correlations and contradictory results.

For instance, fat loss and tissue changes may correlate very well with fertility (King, 1987), but in other studies no relationship was found (King and Williams, 1984a,b; Hughes *et al.*, 1984; King and Dunkin, 1986a,b, Mullan and Williams, 1989). Therefore it is perhaps surprising that more people have not challenged the hypothesis, accepted by many researchers, that protein and/or energy stores and intake are the key factors affecting reproductive efficiency in animals. Rather the energetic or protein changes occurring at the cellular level reflect the metabolic status of the sow and in turn her endocrine status are more likely to directly cause changes in fertility. The approach of using indicators of an animal's metabolic status is beginning to be taken when looking at fertility in sows.

1.3.3 Effect of lactational feed intake on subsequent ovulation rate.

The majority of researchers observed that lactational nutrient intake had no effect on subsequent ovulation rate (Hughes *et al.*, 1984; King and Williams, 1984ab; King and Dunkin, 1986a; Aherne and Kirkwood, 1985; Kirkwood *et al.*, 1987ab,1990; Baidoo, 1989; Yang *et al.*, 1989) but did reduce embryonic survival (Hardy and Lodge, 1969; Hughes *et al.*, 1984; Baidoo, 1989; Kirkwood *et al.*, 1987ab,1990) (Table 1.1). Therefore, subsequent litter size is smaller in sows fed poorly compared to sows fed well during lactation.

It is difficult to accept that poor nutrition during lactation has no effect on subsequent ovulation rate, because undernutrition during lactation causes a significant depression in metabolic hormones such as insulin and insulin-like growth factor-1 (IGF-1), and these hormones have a profound effect on follicle development (see section 1.6). Furthermore flush feeding a high energy diet for 10 to 12 days to previously restrict fed gilts, increases their ovulation rate to a 'normal' level compared to the ovulation rate of gilts that remain restrict fed (Flowers *et al.*, 1988; Beltranena *et al.*, 1991). This lack of a change in ovulation rate in sows fed different lactational nutrient levels may be explained by the extended weaning-to-estrus interval (see table 1.1) (Fahmy and Dufour, 1976), which would allow a sow time to recover from lactational undernutrition, become anabolic and thus recover fertility (see figure 1.1).

1.3.4 Endocrinology of the lactating sow.

Divergent mechanisms control luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretion during lactation. LH secretion is primarily controlled by hypothalamic gonadotropin-releasing hormone (GnRH) secretion (Kraeling and Barb, 1990), whereas FSH secretion, although dependent on hypothalamic releasing factor(s), is primarily controlled by the ovary and a number of non-steroidal ovarian factors (for review see Tonetta and DiZerega, 1989). Negative feedback of the ovarian protein factor, inhibin, has been suggested as the main factor inhibiting FSH secretion during lactation, but this factor has no effect on LH secretion (Stevenson *et al.*, 1981).

During lactation, mean plasma LH levels (Stevenson, Cox and Britt, 1981; Kirkwood *et al.*, 1984) increase gradually as lactation proceeds, as does the releasable

Table 1.1 Effect of lactational feed intake on the subsequent weaning-to-estrus interval, ovulation rate and embryo survival.

References	Parity	WEI[1] (days)		Ovulation[2] Rate		Embryo[3] Survival	
Lactation FI[4]		High	Low	High	Low	High	Low
Hardy and Lodge (1969)	multi	-	-	15.2	13.8	58.3	53.9
Hughes *et al.* (1984)	multi	-	-	18.6	19.1	68.9	58.4
Kirkwood *et al.* (1987)	2nd	4.3	5.3	18.3	19.2	82.7	68.3
Kirkwood *et al.* (1990)	2nd	6.0	8.9	17.6	17.7	82.6	71.6
Baidoo (1989)	2nd	5.1	9.0	16.4	17.2	81.4	67.2
King and Williams (1984a)	1st	10.8	23.0	14.4	13.6	70.1	71.7
King and Dunkin (1986a)	1st	7.8	29.8	12.0	12.2	-	-
King and Dunkin (1986b)	1st	11.1	12.9	13.3	11.9	-	-

1. Weaning-to-Estrus Interval (days)
2. Mean # corpora lutea
3. Embryo Survival (%) [(# viable embryos / OR)*100]
4. Relative lactational feed intake

pool of pituitary LH (Bevers *et al.*, 1981; Smith *et al.*, 1992). Plasma FSH levels, although not showing as characteristic a pulsatile release as LH, also increase slowly during lactation (Stevenson *et al.*, 1981; Edwards, 1982; Britt *et al.*, 1985; Sesti *et al.*, 1991). Prolactin, on the other hand, is maintained at a elevated level during lactation. Prolactin secretion is induced by suckling in the sow (Mattioli *et al.*, 1986), and by late lactation the secretion of prolactin gradually decreases, as suckling frequency declines (for review Varley and Foxcroft, 1990).

A highly pulsatile pattern of LH secretion prior to weaning has been associated with a shorter weaning-to-estrus interval (Shaw and Foxcroft, 1985; King and Martin, 1989; Mullan and Close, 1989). More recent evidence suggests that even by day 14 of lactation, reduced LH secretion can be associated with a delay in the return to estrus (Tokach *et al.*, 1992a). Furthermore, Shaw and Foxcroft (1985) found a positive correlation (r=0.60) between the weaning-to-estrus interval and the mean FSH:LH ratio just before and after weaning. However, FSH secretion before and after weaning *per se* does not appear to have an effect on sow fertility (Stevenson *et al.*, 1981; Edwards, 1982; Edwards and Foxcroft, 1983a; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987). Therefore the tremendous between-sow variability in gonadotropin secretion seen during and after lactation (Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987) probably indicates different rates of ovarian follicular development, and thus fertility in the sow.

It is generally assumed that both progesterone and estrogen decline shortly after parturition, and are maintained at this low basal level throughout lactation

(Edwards, 1982). However, as reviewed by Varley and Foxcroft (1990), there is evidence in some sows that abnormally high random peaks of estradiol occur during lactation. These peaks appear during peak lactation, at a time when the sow is likely to break down more of her body reserves to maintain milk production. Varley and Foxcroft (1990) suggest that estrogen may be released when fat stores are broken down during lactation, as fat deposits can store 200 times more progesterone than the peripheral circulation (Hillbrand and Elsaesser, 1983). Fat can probably also store estrogen, as estrogen too is a steroid and lipophilic. However, estrogen release from fat stores would probably only occur over a 48 hour period, because fat and peripheral estrogen levels are in dynamic equilibrium with one another (Hillbrand and Elsaesser, 1983). Therefore, release of estrogen from fat stores would only maintain estrogen at slightly elevated levels in the periphery. Another possible explanation for the estrogen peaks is an extra-ovarian estrogen source. The adrenal cortex has been suggested as an estrogen source (Stevenson *et al.*, 1981) because many steroids, including estrogens, are synthesised and released from the adrenal gland during stress (Heap *et al.*, 1966). However, because only a minority of investigators have identified abnormally high estrogen levels during lactation, more research is required to discover whether these lactational estrogen peaks exist, and what their effects may be on the reproductive axis.

1.3.5 Effects of endocrine status on the ovary.

Ovarian antral follicles do not reach ovulatory size during lactation. But, as lactation proceeds, ovarian follicle size increases and the percentage of atretic follicles decreases (for review see Britt *et al.*, 1985). The exact role of the gonadotropins in ovarian follicle maturation have not been fully elucidated. However, evidence suggests that FSH stimulates ovarian follicle development up to a diameter of 5 to 6 mm, whereas LH is necessary for the final follicle maturation (Britt *et al.*, 1985).

The weaning to estrus interval can be reduced by decreasing the suckling stimulus of the sow, by separating litters from sows for 6 to 12 h every day (fractionated weaning) (review by Britt, 1986) or weaning the heaviest piglets 3 to 5 d earlier than the lighter pigs (split-weaning) (Stevenson and Britt, 1981; Cox *et al.*, 1982,1983; Britt, 1986). Grant (1989) established that the neural intensity of the suckling stimulus (number of teats suckled) is the critical factor influencing peripheral prolactin and LH levels and thus ovarian follicular development in the lactating sow. Other researchers also determined that the suckling stimulus alters pre-weaning LH levels independent of plane of nutrition during lactation (Mullan and Close, 1989; Mullan *et al.*, 1991). However, the LH levels after weaning appear to be more related to loss of the bodies tissue reserves during lactation (Mullan *et al.*, 1991), and thus presumably, the sow's metabolic status during and after lactation. Furthermore, the ability of the anterior pituitary to release LH in response to GnRH is not blocked by prolactin or suckling (Bevers *et al.*, 1981) and follicle growth and ovulation can be stimulated by exogenous administration of GnRH pulses during lactation (Cox and Britt, 1982). Therefore, suckling appears to primarily suppress ovarian follicular development during lactation at the level of the hypothalamus and/or higher in the brain, by suppression of GnRH secretion.

An opiate mechanism has been suggested as a mediator of suppressive effects on gonadotropin secretion within the hypothalamus in the lactating sow (Mattioli *et al.*, 1986; Armstrong, Kraeling and Britt, 1988) and rat, but not in the lactating human or seasonally anestrus sheep (for review see McNeilly, 1987). The suckling stimulus may also suppress gonadotropin secretion via neural pathways linked to the hypothalamic GnRH neurones through dopaminergic neurones (Foxcroft, 1992).

Lactation therefore has a tremendous influence on reproductive performance due to the direct neural responses to suckling and indirectly, by the metabolic changes associated with milk production. The mechanisms mediating these effects need to be understood in order to maximize the sow's reproductive efficiency. When considering the nutrient demands of lactation, an evaluation of a sow's metabolic status (which will reflect her energy/protein balance), rather than measures of fat and weight change during and after lactation, will provide the means of adopting a more mechanistic approach to the study of lactational effects on sow fertility. However, a sow's reproductive axis is not only affected by nutrition in lactation. Nutrition in the post-weaning period and in early gestation can also can affect ovulation rate, weaning-to-estrus interval and embryo survival, and this will be described in the following section.

1.4 POST-WEANING

1.4.1 Effects of nutrition on reproductive efficiency

Feeding a high plane of nutrition between weaning and breeding (flushing) had no effect on the weaning-to-estrus interval in multiparous sows (Dyck, 1972; Varley and Cole, 1976b; Tribble and Orr, 1982), but shortened the weaning-to-estrus interval (9.3 vs. 21.6 days) in primiparous sows (Brooks and Cole, 1972). Flush feeding also increased conception rate (100 vs. 58%) (Brooks and Cole, 1972) and subsequent ovulation rate (14.3 vs. 13.2 ova) in primiparous sows (King *et al.*, 1984), but had no effect on ovulation rate in multiparous sows (Brooks *et al.*, 1975; Varley and Cole, 1976b; Baidoo, 1989). Thus, the feeding level between weaning and mating has continually failed to affect reproductive performance in multiparous sows (Dyck, 1972; Brooks *et al.*, 1975).

Variance in the weaning-to-estrus interval was lower in flush fed sows (Dyck, 1972). Therefore, feeding a high plane of nutrition during the weaning-to-estrus period could help synchronise the return to estrus of a group of sows. Furthermore, Baidoo (1989) reported that the weaning-to-estrus interval tended to be shorter and the embryo survival rate higher (70.4 vs. 64.0%) in sows fed poorly during lactation, and then flush fed between weaning and breeding, compared to sows not flush fed. This suggests that flush feeding during the weaning-to-breeding interval can help to partially overcome the affects of poor nutrition during lactation, if the feeding period is over at least six days (Fahmy and Dufour, 1976). As estrus occurs within three to seven days after weaning in most multiparous sows, but not in primiparous sows, multiparous sows are not likely to be maintained on a high energy diet long enough in the postweaning period for changes in the reproductive axis to occur.

1.4.2 Endocrinology of the weaned sow.

Normally, weaning a sow causes an increase in hypothalamic GnRH synthesis and release (Cox and Britt, 1982) and a rapid increase in LH synthesis and pulsatile secretion. This is maintained for a number of days, depending on the weaning-to estrus interval, until the occurrence of a rise in circulating estradiol concentration and ultimately in the preovulatory LH surge (Stevenson *et al.*, 1981; Cox and Britt, 1982; Edwards and Foxcroft, 1983a,b; Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987). Within a few hours of weaning or removal of the suckling stimulus prolactin falls to a basal level (Stevenson *et al.*, 1981; Edwards and Foxcroft, 1983a,b; Shaw and Foxcroft, 1985). Progesterone levels remain low during the early post-weaning period until ovulation.

Follicular growth increases in the weaning to estrus interval, with a corresponding elevation in peripheral estradiol level. Plasma estradiol concentration peaks just before estrus, is immediately followed by the LH surge and then returns to basal levels (Stevenson *et al.*, 1981; Edwards, 1982; Edwards and Foxcroft, 1983a,b). Initially negative estradiol feedback blocks the effect of GnRH for about 60 h; at first at the anterior pituitary level, by blocking the response to GnRH, and then by reducing GnRH secretion from the hypothalamus. But, once estrogen levels exceed a certain level, positive feedback occurs, and increasing GnRH pulse frequency allows the release of the accumulated LH pool causing the LH surge (Britt *et al.*, 1991) and ovulation 30 to 40 h later (Stevenson *et al.*, 1981; Foxcroft *et al.*, 1987).

The preovulatory LH surge is essential for the occurrence of ovulation, and if it does not occur, cystic ovarian follicles form (Armstrong and Britt, 1985). Similarly, if the preovulatory surge is diminished, as seen in early weaned sows (Edwards and Foxcroft, 1983a; Kirkwood *et al.*, 1984), the subsequent ovulation rate may be lower (Archibong *et al.*, 1987). Anestrous sows administered exogenous GnRH 30 days after weaning also produced a greater LH surge and larger subsequent litters (14 vs. 8.5 piglets) compared to sows that do return to estrus following weaning (Armstrong and Britt, 1985). Thus, the magnitude of the LH surge appears to be dependent on the time after parturition and weaning, and should be optimised to ensure high reproductive efficiency in the sow.

Plasma FSH changes are unpredictable immediately following weaning (Foxcroft *et al.*, 1987); a preovulatory FSH surge, coincident with the LH surge, occurs in sows weaned after longer, but not necessarily shorter lactations (Edwards and Foxcroft, 1983a; Kirkwood *et al.*, 1984). It was thought that as long as a 'threshold' level of FSH is achieved after weaning, the sow will be able to return to estrus. However, higher circulating concentrations of inhibin have been associated with similar or higher FSH levels compared to FSH levels seen in non-prolific breeds during the follicular phase in prolific breeds of sheep (Tsonis *et al.*, 1988) and pig

(Foxcroft *et al.*, 1992), compared to animals with 'normal' ovulation rates. Furthermore, immunisation against inhibin increased the ovulation rate in European breeds of pig (Brown *et al.*, 1990). Thus, the FSH/inhibin status of the sow may be important in optimising ovulation rate and, even more so, the pattern of FSH secretion in relation to LH may be a highly important factor affecting fertility.

In sows destined to be anestrus after weaning, steroid and gonadotropin levels remain low, even 45 days after weaning (Almond and Dial, 1990c) and the preovulatory LH surge fails to occur (Armstrong and Britt, 1985). However, the ovarian axis in anestrous sows appears to be functional, as follicular growth, ovulation and fertile estrus can be induced by treatment with exogenous gonadotropins (Dial *et al.*, 1984), estrogen (Dial *et al.*, 1984) and pulsatile GnRH (Armstrong and Britt, 1985). Thus,it would appear that the failure of a sow to resume ovarian activity after weaning is not due to the unresponsiveness of the ovary to gonadotropins, but rather to a dysfunction at the level of the hypothalamus and pituitary. In this context, it is interesting to note that the hypothalamo-hypophyseal axis in anestrous sows is more sensitive to estradiol negative feedback effects (Almond and Dial, 1990b). This increased sensitivity to estradiol may be one mechanism by which LH synthesis and secretion, and thus ovarian development, is suppressed (Almond and Dial, 1990a).

1.5 EARLY GESTATION

1.5.1 Effect of nutrition on reproductive efficiency.

A high level of nutrition from the time of breeding and continuing through early gestation resulted in a reduction in embryo survival in gilts (Dyck *et al.*, 1980) but not in multiparous sows (Moody and Speer, 1971). Increasing feed intake from day three of gestation had no effect on embryo survival in gilts (Kirkwood *et al.*, 1990; Pharazyn *et al.*, 1991a), but similar increases in feed intake, from 1.8 to 2.5 kg/day, from day one after mating, decreased the embryo survival rate (87.7 vs. 70 %, respectively) (Pharazyn, 1992). Thus, the first few days of gestation in the pig are possibly a critical period, during which nutrition affects embryo survival. Possibly, in the earlier experiments in which no change in embryo survival occurred, the gilts feed intake was increased too late after ovulation to have any effect.

1.5.2 Endocrinology of the sow in early gestation.

Progesterone levels begin to rise within hours of ovulation, initially slowly and then more rapidly to reach a plateau level 8 to 14 days after ovulation (Edwards, 1982). The time of the initial progesterone increase can have enormous affects on embryo survival. In fact, lower progesterone levels, in the peri-ovulatory period, have been associated with high perinatal loss in prolific breeds of sheep (Ashworth *et al.*, 1989). Furthermore, variations in embryo survival were greater and the mean embryo survival was lower in gilts (Pharazyn, 1992) and sows (Hughes *et al.*, 1984) with lower peripheral progesterone concentrations.

High levels of nutrition in early pregnancy are associated with low peripheral progesterone levels and decreased embryo survival rates in sheep (Parr *et al.*, 1987) and gilts (Dyck *et al.*, 1980) but not in sows (Prime *et al.*, 1988). Symonds and Prime (1988) proposed that high feed intake increases blood flow rate through the liver, and thereby the metabolic clearance rate of circulating progesterone. To overcome this increase in progesterone clearance in gilts with high ovulation rates due to *ad libitum* feeding prior to mating, Ashworth (1991) supplemented gilts with progesterone after mating and restored embryo survival rates from 66.4% to control levels of 82.8%.

Thus, high progesterone levels in early gestation appear to be important for embryo survival. Several mechanisms may be involved, including, increasing the allantoic fluid volume and glucose content in the pig fetus (Ashworth, 1991), increasing the synchrony between the embryo and uterus (for review see Pope, 1988) and other factors discussed in the comprehensive review of Pharazyn (1992).

Thus, nutrition has profound and complex effects on reproduction, which are by no means fully understood. In general, sows fed a low level of energy/protein during lactation have a reduced reproductive efficiency due to reduced conception rates, extended weaning-to-estrus intervals and reduced litter sizes. The affects of poor lactational feeding can be overcome if a high plane of nutrition is fed for more than six days during the weaning-to-mating interval. However, if this level of feeding is maintained into early gestation in primiparous sows, embryo survival and thus subsequent litter size may be reduced.

Many arguments still centre around the question of whether protein, energy and fat intakes or body stores are the most important factors affecting a sow's reproductive efficiency and fertility. King (1987) failed to explain a physiological role for protein on the reproductive axis, but a number of protein dependent mechanisms may exist (Booth, 1990a). It would appear that tissue measurements alone are not good indicators of fertility, and each new experiment seems to add to the confusion, as most of these experiments report correlative effects rather than 'cause and effect'. To obtain a clearer view of the mechanisms affecting reproductive efficiency, other possibilities need to be explored, and more factorial experiments undertaken. Booth (1990b) suggested that an understanding of the effects of nutrition on reproduction requires study of the metabolic status of the sow, by measurement of some key metabolic hormones and metabolites.

1.6 MECHANISMS MEDIATING NUTRITION-REPRODUCTION INTERACTIONS IN THE SOW

Nutrition can induce acute and chronic changes to the reproductive axis, in the absence of any changes in body composition (for reviews see Britt *et al.*, 1988; Booth, 1990b). Furthermore, sows with similar body composition may differ metabolically and reproductively (Foxcroft, 1990). Much correlative evidence has been collected which relates tissue changes during lactation and following weaning to reproductive performance (see section 1.3.2). However, the physiological basis for differences in fertility still remains elusive.

Factors which modulate metabolic effects on the reproductive axis have been found in the thyroid axis, adrenal axis and the ovary. At the ovarian level, autocrine and paracrine effects due to intra-ovarian regulators such as insulin and peptide growth factors, including insulin-like growth factors (IGFs), epidermal growth factor (EGF), transforming growth factor (TGF)-α and TGF-β, fibroblast growth factors (FGF) (Booth, 1990ab) and the cytokinins (Adashi, 1989) have been demonstrated. Therefore, metabolites and metabolic hormones may act, directly or indirectly, as physiological signals to the hypothalamo-hypophyseal-ovarian axis, and in this way affect a sow's reproductive function.

The effects of an animal's metabolic status on the reproductive axis have been comprehensively reviewed (Booth, 1990ab; I'Anson *et al.*, 1991; Tokach, 1991). This evidence will not be repeated as part of this review. Instead, information on a number of the most likely candidates for the link between nutrition and reproduction, namely insulin and the growth hormone-IGF-1 axis, will be discussed.

1.6.1 The Role of Insulin

Insulin has long been associated with reproduction. For instance, insulin disorders such as diabetes mellitus and eating disorders such as anorexia nervosa are associated with amenorrhoea, anovulation and infertility. But with insulin replacement therapy to patients with diabetes mellitus, normal reproductive function can be restored (for review see Poretsky and Kalin, 1987). Furthermore, Tokach (1992a) found a significant positive correlation between plasma insulin levels during early lactation and the weaning-to-estrus interval in primiparous sows.

i) Central effects of insulin.

Insulin may act centrally to affect the reproductive axis. Cerebrospinal fluid insulin is a reliable indicator of an animal's metabolic state, because it is well buffered against transient changes seen in the periphery (Woods and Porte, 1977). In fact, increases in LH pulsatility, possibly due to acceleration of the GnRH pulse generator, have been demonstrated in gilts by injection of insulin into the cerebral ventricles (Cox *et al.*, 1989). This effect is probably mediated by brain and pituitary insulin receptors (Werther *et al.*, 1987), which are in turn influenced by nutrient restriction (Melnyk and Martin, 1984). Furthermore, increases in plasma LH levels and/or pulsatility, together with increases in plasma insulin levels have been observed after refeeding fasted heifers (McCann and Hansel, 1986) and by infusion of glucose into nutritionally restricted gilts (Booth, 1990b). However infusion of glucose into energy restricted lactating sows did not increase LH secretion, even though plasma insulin levels were increased (Tokach, 1992b). This suggests that suckling-induced suppression of the hypothalamo-pituitary axis cannot be overcome simply by increasing insulin levels.

ii) Ovarian effect of insulin.

Ovarian functions of insulin have also been demonstrated. Cox *et al.* (1987) increased the ovulation rate of cyclic gilts (13.4 vs. 15.8 ovulations) by feeding a high energy diet six days before estrus. They further enhanced the ovulation rate (15.8 vs. 19.4 ovulations) by feeding a high energy diet in conjunction with exogenous insulin injections. The increases in ovulation rate were achieved in the absence of any changes in peripheral estradiol and with or without changes in plasma gonadotropins, suggesting that the insulin effect may be directed towards the ovary. Using realimentation of gilts in which the expected increase in LH secretion was suppressed using synthetic progestagins, Cosgrove *et al.* (1992) provided more direct evidence that nutritional effects act directly on the ovary.

The mechanisms by which diet and insulin influence ovulation rate have been partially defined. Increasing dietary energy advanced the increase in large follicle estradiol content in high energy fed gilts (Matamoros *et al.*, 1990) and by doing so may have promoted the growth of other follicles as suggested by Foxcroft and Hunter (1985). This could increase the number of follicles selected into the preovulatory pool, and be associated with follicle recruitment continuing into the follicular phase (Foxcroft *et al.*, 1987; Grant *et al.*, 1989). This mechanism has been proposed as a means of increasing ovulation rate in at least one prolific breed of sheep (Driancourt, 1985). Increasing insulin on the other hand increases ovulation rate by reducing follicular atresia in cyclic (Matamoros *et al.*, 1990) and PMSG treated prepubertal gilts (Matamoros *et al.*, 1991). Thus, increasing insulin and energy levels may work together to increase ovulation rate.

Insulin's ovarian functions are beginning to be understood. Follicular fluid insulin levels are not correlated with plasma levels, which suggests that granulosa cells and/or oocytes play a specific role in accumulating insulin in the follicle. Furthermore, insulin levels are five fold higher in follicles with oocytes that are eventually fertilised (Diamond *et al.*, 1985). This begs the question, "Why is insulin so important to the developing ovarian follicle?".

An explanation for this is in the number of roles insulin plays in follicular maturation. Insulin enhances steroidogenesis (Veldhuis *et al.*, 1985), augments granulosa cell differentiation and estrogen production (Davoren *et al.*, 1986), enhances the differentiated functions of the thecal cell (Cara and Rosefield, 1988) and synergizes with FSH in granulosa cell luteinization (May and Schomberg, 1981). Further effects of insulin on the ovary and granulosa cell are described in section 1.6.2 below.

1.6.2 The Role of Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1)

Growth hormone markedly influences ovarian function. For instance, simultaneous GH and gonadotropin therapy stimulates ovarian function in infertile humans resistant to gonadotropin therapy alone (Homberg *et al.*, 1988). Similarly, administration of exogenous GH to gilts increased their ovulation rate (Kirkwood *et al.*, 1988b; Kirkwood and Thacker, 1991). Some GH effects are probably mediated by IGF-1, because GH promotes granulosa cell immunoreactive (i)IGF-1 content

(Davoren and Hsueh, 1986; Hammond and English, 1987).

Originally it was thought that IGF's were produced as a result of GH action on the liver. However, it has now been established, using *in vitro* techniques, that IGF-1 is synthesised in many tissues (Murphy *et al.*, 1987) including the rat granulosa cell (Oliver *et al.*, 1988, 1989) and the porcine ovary (Hammond *et al.*, 1985; Charlton, 1992). Other factors, including nutrient availability and metabolic hormone status, are important modulators of IGF-1 levels. In fact, gonadal iIGF-1 concentrations are reduced by fasting in the rat (Handelsman *et al.*, 1985). Also, gonadotropins, estradiol and cAMP (the presumed mediator of gonadotropin action) enhance granulosa cell secretion iIGF-1 (Hsu and Hammond, 1987a).

Both IGF-1 and insulin are important modulators of follicle maturation and a number of comprehensive reviews have described their effects (Adashi *et al.*, 1985,1991ab; Baxter, 1988; Eden *et al.*, 1989; Sara and Hall, 1990; Hammond *et al.*, 1991). They help mediate granulosa cell replication and FSH-mediated differentiation by induction of LH receptors (Davoren *et al.*, 1986). Also a special high affinity, low capacity type-1 IGF receptor, identified on the granulosa cells of swine (Veldhuis *et al.*, 1985) and rats (Davoren *et al.*, 1986), to which insulin shows low affinity, is used to mediate the replicative and differentiative effects of IGF. The IGF receptor is inducible by gonadotropins and gonadal steroids and therefore is a potential means of IGF-1 control (Veldhuis *et al.*, 1986).

The dominant actions of IGF-1 and insulin depend largely upon the concommitant influence of other mitogenic and differentiating agents. In the presence of gonadotropins and in the preovulatory follicle milieu, the differentiative effects of IGF-1 are emphasised. For instance, TGF-β in combination with IGF-1, or alone, tends to promote granulosa cell differentiation and inhibit cell proliferation (May *et al.*, 1988). Also, steroidogenesis and aromatase activity is increased in granulosa cells of swine (Veldhuis *et al.*, 1985) and rats (Davoren *et al.*, 1986) exposed to IGF-1. Thus IGF-1, alone, appears more important to granulosa cell differentiation than replication (Mondschein *et al.*, 1989).

The mitogenic effects of IGF-1 become more apparent under the influence of other factors. In fact, the most profound effects of insulin and IGF-1 are seen when these hormones are in combination with other growth factors: IGF-1 potentiates EGF enhancement of granulosa cell DNA synthesis and proliferation (May *et al.*, 1988) and EGF and insulin have additive effects on granulosa cell proliferation (Hammond and English, 1987). Platelet derived growth factor also enhances granulosa cell proliferation (Hammond and English, 1987).

Elevated follicular fluid IGF-1 levels appear to be associated with follicular development. Thus, it makes physiological sense that follicular atresia is lower in gilts with higher follicular fluid IGF-1 levels (Matamoros *et al.*, 1991) and in normal compared to diabetic gilts (Meurer *et al.*, 1991). High levels of peripheral and ovarian IGF-1 are also associated with natural twinning in cows (Echternkamp *et al.*, 1990). This suggests that IGF-1 of ovarian and/or systemic origin plays a role in the regulation of folliculogenesis, and could be selected for like any other genetic trait. Indeed, high and low plasma IGF-1 lines of mice have been genetically selected

(Blair *et al.*, 1988).

Interestingly, Zhou *et al.* (1991) found granulosa cell IGF-1 gene expression to be a function of follicle size, and independent of pituitary hormone effects. Others also found follicular fluid IGF-1 concentrations to be related to follicle diameter and not stage of development in swine (Howard and Ford, 1991) and humans (Eden *et al.*, 1988). Furthermore, dominant follicles contain higher levels of follicular fluid IGF-1 in humans (Eden *et al.*, 1988), pigs (Hammond *et al.*, 1988) and cattle (Echternkamp *et al.*, 1990).

Therefore, high levels of follicular IGF-1, like insulin, appear to be associated with follicles capable of ovulation. In the pig this could influence the recruitment of more follicles into the pre-ovulatory pool and thus increase the ovulation rate and fertility.

1.6.3 IGF Binding Proteins

When considering the effects of the IGFs, one cannot forget the IGF binding proteins (BP). These are capable of modulating the metabolic and mitogenic actions of IGF peptides as a result of high affinity binding (Powell *et al.*, 1991). The IGFBPs represent a family of discrete gene products with variable gene expression and physiological regulation (for reviews see Baxter, 1988; Ooi and Herington, 1988; Holly and Wass, 1989; Sara and Hall, 1990). Five different IGFBPs have been isolated from adult rat serum (Shimasaki *et al.*, 1991a) and identified as IGFBP-1, -2, -3, -4 and -5. Six IGFBPs, including the five identified above and a novel IGFBP-

6, have been isolated and identified from porcine follicular fluid (Shimasaki *et al.*, 1991b), and IGFBP-1 and -2 have been isolated and identified from porcine serum (McClusker *et al.*, 1989). The IGFBPs have many functions: 1) they provide a storage pool for IGFs, 2) they prolong the biological half-life of IGFs in the circulation and 3) they complex with IGFs and may either inhibit or potentiate their actions.

Changes in follicular IGFBPs may have important effects on ovarian function. Granulosa cell steroidogenesis is inhibited by IGFBPs, probably by a combination of sequestration of endogenous IGF-1 and a direct interaction with the cell (Bicsak *et al.*, 1990). Also, IGFBP-2 gene expression is upregulated by estrogen in the rat ovary (Ricciarelli *et al.*, 1991). The increase in IGF-1 and decrease in IGFBPs could also be important in granulosa cell differentiation (Mondschein *et al.*, 1989) because FSH may promote granulosa cell differentiation by stimulating IGF-1 and inhibiting IGFBP-3 (Mondschein *et al.*, 1990).

The binding proteins are also differentially regulated by metabolic signals. Upon fasting, circulating IGFBP-1 levels increase, IGFBP-3 and -4 decrease and IGFBP-2 disappears (McClusker *et al.*, 1991). Furthermore insulin changes the rate of IGFBP-1 gene transcription (Powell *et al.*, 1991), and differentially modulates IGFBP tissue uptake (Bar *et al.*, 1990a). This alteration in IGFBP tissue uptake may alter IGF-1 effects on the target tissue, because IGFBPs transport IGFs to target tissues across endothelial barriers (Bar *et al.*, 1990b). The IGFBPs may also modulate IGF-1 effects on the ovary in a paracrine and/or autocrine fashion, because rat granulosa cell can synthesise and secrete IGFBP-1 (Jalkanen *et al.*, 1989) and porcine granulosa cells can synthesize IGFBP-2 (Mondschein *et al.*, 1990).

The IGFBPs may be a major factor in determining regional levels, bioactivity and metabolic effects of IGF-1 in the ovary. This is because different IGFBPs have different functions and are present at different times during follicle development in the ovary. The mRNAs for IGFBP-2 are localised in interstitial cells, for IGFBP-3 in the corpora lutea, and in IGFBP-4 in atretic rat granulosa cells (Nakatasi *et al.*, 1991). Also, the mRNA for IGFBP-2 decreases and IGFBP-3 increases with maturation and luteinization in porcine granulosa cells (Samaras *et al.*, 1990). Therefore, this evidence, albeit indirect, further adds to the proposal that the IGFBPs contribute to ovarian IGF paracrine/autocrine systems by impacting on follicle regulation.

Research into the IGFBPs and their effects at the ovarian level and in combination with IGF-1 has just begun. But as more evidence is reported, a stronger case for IGFBPs as a modulator of the actions of IGF-1 and ovarian activity unfolds, and further complicates the IGF-1 story.

1.7 CONCLUSION

In conclusion, the sows fertility changes throughout lactation and after weaning (figure 1.1), and these levels of fertility have been linked to the sows metabolic status. Many nutritional factors affect sow fertility. These metabolites and metabolic hormones, for example insulin and IGF-1, and the role they play are finally being understood. Poor fertility after weaning may be due to effects at the level of the brain, for example increased sensitivity to estradiol (Almond and Dial, 1990ab), but effects at the ovarian level cannot be ignored. Insulin and IGF-1 have many and varied effects on the ovary, and different levels of these hormones and factors which control these hormones, such as IGFBPs, are seen at different stages of follicular development (see section 1.6).

A review of the literature reveals very little work on the mechanisms that mediate the nutritional effect on fertility in the sow after weaning. However, from this review it has become clear that changes in metabolic status may be involved in the control of reproduction after weaning. This therefore begs the question as to how the sow's metabolic status varies between first and second estrus after weaning. Furthermore, does metabolic status relate to subsequent ovulation rate, embryo survival and litter size, and at what point can the metabolic status of the sow be altered to improve fertility?

CHAPTER 2

EXPERIMENT 1A: EFFECT ON SOW FERTILITY OF BREEDING AT EITHER FIRST OR SECOND ESTRUS AFTER WEANING

2.1 INTRODUCTION

Sows emerge from lactation at different levels of fertility, often as a result of poor nutrition during this period (Pettigrew and Tokach, 1991). Inadequate nutrition during lactation leads to an extended weaning-to-estrus interval (Fahmy, 1981; King, 1987) a reduced rate of embryo survival (Hughes *et al.*, 1984; Baidoo, 1989; Kirkwood *et al.*, 1987ab, 1990) and a reduced subsequent litter size (Kirkwood *et al.*, 1988a). Furthermore, poor lactational nutrition has been linked to low mean LH levels before and after weaning (King and Martin, 1989; Mullan and Close, 1989), and low LH levels before and after weaning have also been associated with poor fertility after weaning in the sow (Shaw and Foxcroft, 1985; Foxcroft *et al.*, 1987). Lower plasma LH and insulin levels at day 14 of lactation have also been associated with a delay in return to estrus after weaning (Tokach, 1992a). Thus, in the lactating sow, as in other reproductive states, a strong link between nutrition and subsequent fertility is evident.

Leman (1987) proposed that sows that return to estrus 7 to 12 days after weaning (15 % of sows) produce smaller subsequent litters and fewer pigs/sow/year, than sows that return to estrus in less than seven days. Breeding such sows at their second estrus after weaning (skip-a-heat breeding) could help overcome their poor fertility by increasing subsequent litter size (Moody *et al.*, 1969; Pay, 1973; Love, 1979; Fahmy *et al.*, 1979; King *et al.*, 1984; Clark and Leman, 1987; Clark *et al.*, 1988; Morrow *et al.*, 1989; Conner, 1992). Any observed increase would probably be a result of improved nutritional status, rather than by alteration of management *per se*.

The data of Leman (1987) is confounded by many factors including parity and management policy, because they are compiled from a number of different farms under both 'good' and 'bad' management practices. Thus, the objective of this experiment was to establish, under controlled conditions: (1) whether sows that return to estrus greater than six days after weaning are less fertile and produce smaller litters than sows that return to estrus within six days after weaning, and (2) whether a relationship between litter size and weaning-to-estrus interval, as indicators of sow fertility, and body weight and/or fat and protein changes during lactation and after weaning, as indicators of metabolic status, exist. Furthermore, skip-a-heat breeding was evaluated as a method of increasing post-weaning sow fertility.

2.2 MATERIALS AND METHODS

2.2.1 Animals and Treatments

One hundred and sixteen Camborough sows (PIC, Acme, AB., Canada), parities 1-8, at the University of Alberta Swine Research Centre, were fed a conventional dry sow diet (see table 2.1) during gestation, according to their metabolic body weight (Wt kg^{75}; table 2.2). At day 109 of gestation, sows were moved from individual gestation stalls into farrowing crates, and fed an additional 1 kg of feed/day. Upon farrowing, sows were fed a lactation diet (table 2.1), according to their metabolic body weight and number of piglets suckling (equation 2.1). The daily lactation ration was divided into two meals fed at 09.00 and 16.00 h and individual daily feed intakes were recorded.

Equation 2.1:

$$\mathrm{FL}^{a} = [\mathrm{Wt\ kg}^{75\ b} \times 460\ \mathrm{KJ\ DE/kg\ diet}^{c}] + (0.45\ \mathrm{Kg}^{d} \times \mathrm{LS}^{e})$$

[a] Lactational feed level (kg)/day.

[b] Metabolic body weight (kg).

[c] Sows daily maintenance energy requirement is 460 kJ DE/$\mathrm{kg}^{0.75}$

[d] Allowed 450 g of feed/piglet/day.

[e] Number of piglets suckling during lactation.

Sows were weighed at day 109 of gestation, after farrowing and at weaning and breeding. Backfat was measured ultrasonically at the 10th rib and 65mm from the midline (P2) (Scanoprobe II, Scanco, Ithaca, NY) at farrowing, weaning and breeding. Within three days of parturition, litter size was standardized to 9 to 12 piglets/sow by cross-fostering or piglet removal. Piglets were not fed creep feed and were weighed at birth and at weaning.

To increase the number of sows with a weaning-to-estrus interval greater than six days, the sow's lactational feed intake was reduced (King and Williams, 1984ab; King and Dunkin, 1986b; Mullan and Williams, 1989) and number of piglets suckling the sow was increased (Mullan and Close, 1989).

Eighty of the 116 sows were assigned to the breeding trial. Sows were initially weaned on day 21± 3 days post-partum. However, because of management problems, the weaning age was increased to 28± 3 days post-partum. After weaning, sows were moved into individual stalls in the breeding room, within the sight and smell of boars. Sows were grouped into pairs according to length of weaning-to-estrus interval and parity. One sow in each pair was randomly assigned to be bred at the first estrus after weaning (group 1) and the other sow was bred at the second estrus after weaning (group 2). All sows were heat checked daily, using vasectomized boars, from day 3 after weaning until first estrus. Sows in group 2 were again heat checked daily from day 17 of the first estrous cycle until second estrus after weaning. Sows were bred by natural service 12 h after first detection of standing heat and approximately 12 h later by a different boar, to reduce individual boar effects on sow fertility. As far as possible, the same boars were used for the same sow pair.

Sows were fed the dry sow diet (see table 2.1) according to their metabolic body weight (see table 2.2) after weaning, apart from weaning to the first estrus after weaning in group 1 and 2 sows and from day 17 of the first estrous cycle in group 2 sows, when an additional 1 kg/day of the dry sow diet was fed. Subsequent litter size, weaning-to-estrus interval and first estrous cycle length were recorded. Sows showing signs of ill health and/or first signs of estrus more than 19 days after weaning were not included in the trial.

2.2.2 Statistical analysis

Statistical analyses were performed using the General Linear Models procedures of SAS (1990). Preliminary analysis using orthogonal contrasts (SAS, 1990) established that parity one and two sows were not significantly different from one another. Therefore their data were pooled into a single group. Furthermore, this analysis established that sows at parity three or above were not different from one another, but were different from sows of parity one and two. These sows were designated as another group. For statistical analysis, sows which were studied in successive parities (n=18) were considered to be different sows at each parity. Therefore, in the statistical analysis, the measure of experimental variance was sow variance. All quantitative variables, except litter size were analyzed using the linear model below:

$$Y_{ijk} = \mu + E_i + P_j + (EP)_{ij} + e_{ijk}$$

Where Y_{ijk} = dependant variable, μ = overall mean, E_i = treatment effect of breeding at first or second estrus after weaning ($_i$ = 1,2), P_j = parity grouping ($_j$ = 1,2), EP_{ij} = interaction between estrus and parity groups and e_{ijk} = residual error. The linear model above was modified by the addition of the sow's liveweight at weaning as a covariate to account for sow weight differences in the different parity groups. For differences within parity groups, the linear model only included estrus. Associations between variables were explored using Pearson's correlation. Backfat and weight effects were tested on the proportion of sows in estrus after weaning using Chi-square analyses of SAS (1990).

The prediction equations of Whittemore and Yang (1989) were used to estimate the sow's total body lipid (equation 2.2) and protein (equation 2.3). Whittemore's equations for total body protein and fat were chosen over those of King *et al.* (1986), because King's equations (total body lipid (equation 2.4) and protein (equation 2.5)) were derived solely from first parity sows of 120 to 174 kg liveweight and backfat of 18 to 37 mm ((P1 + P2)/2). Sows of parity one to eight were used in this trial, and Whittemore's equations were derived from parity one and four sows. However, care must also be taken in the interpretation of Whittemore's equations because some of the sows of his study were very thin (P2 = 7mm).

Equation 2.2:

$$\text{Lipid (kg)} = -20.4\ (\pm 4.5^{1}) + 0.21\ (\pm 0.02)\text{LW}^{2} + 1.5\ (\pm 0.2)\text{P2}^{3}$$

Equation 2.3:

$$\text{Protein (kg)} = -2.3\ (\pm 1.6) + 0.19\ (\pm 0.01)\text{LW} - 0.22\ (\pm 0.07)\text{P2}$$

Equation 2.4:

$$\text{Lipid (kg)} = 0.362\ \text{LW} + 1.105\ \text{P2} - 33.0$$

(King *et al.*, 1986)

Equation 2.5:

$$\text{Protein (kg)} = 0.146\ \text{LW} + 0.202\ \text{P2} + 5.75$$

(Mullan, 1987; from King personal communication)

[1] Standard error.

[2] Liveweight of the sow (kg).

[3] Backfat of the sow measured ultrasonically at the P2 position (65mm from the midline at the 10th rib)

2.3 RESULTS

Overall, the breeding of sows at their second estrus after weaning increased the total litter size born by 1.2 piglets ($P<0.09$) and the number born alive by 2.0 piglets ($P<0.001$) (table 2.3). These increases in subsequent litter size were achieved in the absence of any between treatment differences in lactation length, lactational feed intake, weaning-to-estrus interval, weight and backfat changes over lactation and the weaning-to-breeding interval (see table 2.3). There was also no difference between treatments in total body fat at farrowing as a proportion of sow liveweight (26.9±0.6 and 26.6±0.6 %) and lactational fat loss as a proportion of fat at farrowing (12.4±1.8 and 13.3±1.8 %) for sows bred at first and second estrus, respectively. Sows lost weight between weaning and mating, but this weight loss did not differ significantly between treatments. Sows maintained body fat depots over this period (table 2.3).

Significantly fewer (4 out of 14) sows with a backfat thickness (P2) of 12 mm or less returned to estrus within 30 days ($X^2 = 14.5$; $P<0.002$), whereas all sows with a backfat thickness (P2) greater than 12 mm returned to estrus. Liveweight had no effect on the length of the weaning-to-estrus interval or on the number of sows failing to return to estrus after weaning, when comparing numbers of sows above and below 150 kg.

Of the 76 sows that returned to estrus within 30 days of weaning, only seven returned to estrus in seven days or greater. One of these sows did not produce a litter, and all the sows apart from this one were first parity. Three of the sows that returned late to estrus after weaning were bred at the first estrus, and the other three sows were bred at the second estrus. Of the three sows that returned to estrus late after weaning, no reduction in either total litter size born (11.3±0.6 vs. 10.1±0.6 pigs) or born alive (10.7±0.7 vs. 9.1±0.5 pigs) was recorded, compared to the first parity sows that returned to estrus in less than seven days and were bred at first estrus (see table 2.4). Furthermore, skip-a-heat breeding resulted in an increase in the total litter size born and born alive in both the late and early returning parity one sows (1.7 vs. 2.6 pigs and 1.6 vs. 2.9 pigs, respectively).

i) Parity Effects

Skip-a-heat breeding significantly increased the total litter size born (2.4 pigs;

$P<0.002$) and born alive (2.4 pigs ; $P<0.001$) in sows of parity one and two. Although a numerical increase in total litter size born (0.2 pigs) and born alive (1.7 pigs) existed for sows of parity three and greater, these increases were not significant (see table 2.5). Skip-a-heat breeding increased total litter size born and born alive, at first estrus; this occurred to a greater extent in first (10.1 vs. 12.7 pigs ($P<0.007$) and 9.1 vs. 12.0 ($P<0.002$), respectively) compared to second parity sows (11.0 vs. 12.8 pigs and 10.4 vs. 12.0 pigs, respectively).

Daily sow lactational feed intake increased ($P<0.01$) with parity (see table 2.6). This is not surprising, because the sow's feeding level was based on her maintenance requirements which in turn were based on her metabolic body weight, which increases with parity. The daily feed intake significantly increased between parity one and two sows ($P<0.0001$), but not between parity two and three and greater sows. This is surprising, because first and second parity sows suckled similar litter sizes, and were of similar weights at farrowing and weaning, and therefore they would have been offered similar feed levels. This suggests that first parity sows had a lower voluntary feed intake than sows of second parity and greater.

Sows of parity three and greater contained a significantly larger amount of fat ($P<0.0001$) than sows of parity one and two. When translated into a proportion of liveweight, these differences in total body fat were not significant (see table 2.6). Lactational fat losses did not differ between parity groupings, however lactational fat loss as a proportion of fat at farrowing was significantly less ($P<0.04$) in sows of parity three or more compared to parities one and two. Both the total weight of protein and weight of protein as a proportion of liveweight at farrowing were significantly greater ($P<0.001$) in sows of parity three or more.

Litter size born alive and weaning to estrus interval were highly and negatively correlated with protein loss ($R=-0.86$ and $R=-0.84$; $P<0.0001$, respectively) and fat loss over lactation ($R=-0.87$ and $R=-0.82$; $P<0.0001$, respectively) in sows of parity three or more, however, no such correlation existed for sows of parity one and two. When comparing parity three and greater sows bred at first or second estrus, fat losses (6.0± 1.5 and 7.1± 1.4 kg, respectively) and protein losses (6.3±3.3 and 1.4±3.0 kg, respectively) over lactation were not significantly different. Therefore, the correlations with litter size could not be due to differences in the two treatment groups in sows of parity three or more.

2.4 DISCUSSION

This experiment confirms the results of many other researchers that skip-a-heat breeding increases litter size compared to sows bred at first estrus (Moody *et al.*, 1969; Pay, 1973; Love, 1979; Fahmy *et al.*, 1979; King *et al.*, 1984; Clark and Leman, 1987; Clark *et al.*, 1988; Morrow *et al.*, 1989; Conner, 1992). Interestingly, significant increases in litter size born alive and total born were only seen in parity one and two sows. This is not surprising because these sows were still growing, unlike the majority of sows in parity four and greater. Because sows of parity one and two are still growing, they would have to expend larger amounts of their energy and protein reserves for their growth requirements, because these additional growth requirements were not accounted for in their lactational ration. Thus low parity sows would fall into a 'deeper' catabolic state during lactation than higher parity sows. Furthermore, the voluntary feed intake of first parity sows was lower than that of second parity sows, in agreement with O'Grady *et al.* (1985). Therefore, first parity sows would have become more catabolic during lactation than second parity sows, because their feed intake was less able to compensate for the nutrient drain of lactation and growth. This in turn would account for the larger increase in litter size after skip-a-heat breeding in first parity sows. Such sows would emerge from lactation at a lower metabolic and fertility status than second parity. This would provide these animals with an opportunity to improve their metabolic state and fertility to a greater extent by second estrus after weaning, than second and subsequent parity sows. The increase in total litter size born and born alive with increasing parities after breeding at first estrus is consistent with the above hypothesis and is in agreement with the results of others researcher (Kirkwood *et al.*, 1988a; Lee and Mitchell, 1989).

Sows of parities three or more produced a similar total number of piglets born when bred at first estrus (13.1 pigs) or second estrus (13.3 piglets) after weaning. Furthermore, this number was equivalent to the total number born to sows of parity one and two bred at their second estrus after weaning (12.8 pigs; see figure 2.5), which suggests that skip-a-heat breeding has little effect on sow fertility in older sows (parity three and upwards). Litter size born alive did however increase in skip-a-heat bred compared to first estrus bred sows of parity three or more (11.0 vs. 12.7 pigs, respectively). This suggests that older sows are more fertile at first estrus than younger sows (as described by the number of piglets born alive), but that interpartum piglet survival is lower in sows bred at first estrus. herefore skip-a-heat breeding appeared to increase piglet survival around the time of farrowing, possibly because the sows have more time to recover from parturition and lactation.

Whittemore *et al.* (1984) and Eastham *et al.* (1988) postulated that sows require a critical amount of body fat below which fertility problems occur. In practical terms this amounts to minimum backfat thickness of approximately 10 mm at the P2 site. However, the majority (71%) of 'thin' sows, with a backfat thickness (P2) of 12 mm or less, successfully returned to estrus after weaning (10 out of 14 sows), which adds to the established data which refute the 'threshold' fat theory for fertility maintenance (Lee and Mitchell, 1989). Three of the four sows that remained anestrus were second parity sows that had been in the experiment over two parities. These sows lost weight during their first lactation of restrict feeding, and continued to lose weight during their second lactation, consistent with the results of Lythgoe (1987). Upon commencement of their second lactation they had low fat reserves and therefore probably fell into an increasingly catabolic state throughout their second lactation. Thus these sows were unable to recover their fertility after weaning.

Since sows grow until parity four if fed an adequate diet (Whittemore and Yang, 1989), the increase in fat and protein content of the body across parities was expected (table 2.6). The proportion of fat to liveweight was similar across parities, but the loss of body fat as a proportion of liveweight at farrowing was significantly ($P<0.05$) higher for first and second parity sows. This poses the question whether the relative amount of fat lost is an important signal to the sow; alternatively does a sow read the same message whether she be fat or thin, but loses the same amount of adipose tissue? Whatever the answer, it is essential to elucidate the metabolic signal through which changes in tissue mobilisation mediate effects on the hypothalamo-pituitary-ovarian axis.

It is also interesting to note that litter size born alive was highly and negatively correlated with lactational protein and fat losses in sows of parity three or more. This again points to the sow's metabolic status as being the key indicator of fertility after weaning. According to the hypothesis discussed above, sows with the smallest lactational fat and protein losses would have 'fallen' into a less catabolic state during lactation and so would be more fertile and capable of producing larger litters than sows that lost more tissue over lactation.

Although the time interval between weaning and breeding was short, a matter of four to seven days, animals still appeared to lose substantial and variable amounts of weight at this time, in agreement with the data of O'Grady *et al.* (1975), Zoiopoulos *et al.* (1983) and King and Dove (1987). Brooks (1982) suggested that some gilts that become catabolic during lactation do not immediately revert to an anabolic state after weaning and thus continue to lose weight during the weaning to breeding period. As a result, these sows have reduced ovulation rates. A number of researchers have suggested these weight losses are caused by water loss due to cessation of milk production, mammary tissue involution and also a reduction in gut fill after weaning, rather than actual tissue depletion (Zoiopoulos *et al.* 1983; King and Dove, 1987). These researchers estimated gut fill by fasting sows for 14 to 24 h and water loss by the D_2O method. These research groups observed different results. One established that sows may maintain or even gain body tissue between weaning and breeding (King and Dove, 1987). The other, observed that sows suffer tissue depletion between weaning and breeding (Zoiopoulos *et al.*, 1983), which agreed with the blood parameters they measured. In both cases, gut fill and water loss accounted for a large proportion if not all the weight change between weaning and breeding.

Because estimates of water loss and gut fill are so variable in the literature, no attempt was made to estimate fat, protein and weight changes in sows between weaning and breeding, and therefore no correlates between these measurements were made. Even so, although measured weight losses between weaning and breeding were large and variable in this trial, the sows managed to maintain backfat during this period (see tables 2.3, 2.4 and 2.5), suggesting that the sows did not lose tissue between weaning and breeding.

The increase in number of pigs born alive achieved by delaying breeding must be evaluated to determine whether skip-a-heat breeding is an economically viable proposition to the farmer. For example, a swine unit producing 24 pig/sow/year yields 0.066 pigs/day (24 pigs/ 365 days). If the breeding of the sow is delayed by one estrous cycle (221 days) by skip-a-heat breeding to increase subsequent litter size, she will essentially lose 1.4 pigs (0.066 pigs/day * 21 days). Thus, skip-a-heat breeding would be economically viable only if the increase in litter size achieved was greater than 1.4 piglets, although no cost of extra nonproductive days (Dial, 1992) was taken into account in this calculation. The increase in litter size achieved in the present experiment was 2.4 piglets (both total born and born alive) in first and second parity sows, but only 0.2 total born and 1.7 pigs born alive in sows of parity three or greater. Therefore skip-a-heat breeding would appear to be a useful management tool in parity 1 and 2 sows only. From a practical viewpoint, problems detecting heat at second estrus were encountered and three of the 34 sows (9%) were not bred for this reason. Therefore, unless the increase in litter size is large enough or heat detection is accurate, the widespread use of skip-a-heat breeding is not necessarily an economically beneficial practice.

The sows that returned to estrus late after weaning were from parities one and two and not from higher parity sows, which is in agreement with many other workers (Aumaitre *et al.*, 1976; Love, 1979; Fahmy *et al.*, 1979; Hurtgen *et al.*, 1980; Svarek *et al.*, 1981; Esbenshade *et al.*, 1986; Noblet and Etienne, 1987).

Only nine per cent of sows (4 out of 76 sows) showed a delayed return to estrus after weaning (seven or more days) but only three late returning sows were bred at first estrus and three at second estrus after weaning. Thus, care must be taken in the interpretation of these data. While these limited data are not consistent with the hypothesis of Leman (1987), i.e. that sows that return to estrus between seven and 12 days after weaning are less fertile and produce smaller litters than sows that return to estrus earlier, further evidence is needed to unequivocally disprove it.

2.5 CONCLUSION

In conclusion, skip-a-heat breeding is a management method which improves litter size, regardless of the weaning-to-estrus interval, when compared to breeding of sows at first estrus after weaning. However, this management practice only appears to be an economically viable proposition when used with primiparous or maybe second parity sows. Even then, difficulties in detecting heat at second estrus were encountered which in essence severely limit the advantage of skip-a-heat breeding.

These data also support the hypothesis that differences in fertility in the weaned sow are primarily mediated by changes in metabolic status. However, as only correlative evidence was offered in support of this hypothesis, experiments in which both the sow's fertility after weaning as well as measures of her metabolic status are determined are needed. These would provide direct evidence for such interrelationships. The experiment described in the next chapter in this thesis was designed to meet this objective.

Table 2.1 Composition of diets in Experiment One

	Kg/Tonne	
Ingredients	Dry Sow Diet	Lactation Diet
Wheat	300	300
Barley	564	403.5
Tallow	20	30
Soybean Meal	70	120
Bran	-	100
I. Salt	5	5
Ca Phosphate	17	17
Limestone	14	14
Min-Vit mix[1]	10	10
Lysine HCl	1	1.5
Calculated Analysis (%)		
Crude Protein	13.7	15.4
Lysine	0.56	0.74
Calcium	0.90	0.90
Phosphorus	0.70	0.70
Energy[2] (Mcal DE/kg)	13.4	13.4

[1] Supplied the following per kg of diet: 10000 IU vitamin A, 1000 IU vitamin D, 80 IU vitamin E, 2 mg vitamin K, 30 μg vitamin B_{12}, 12 mg riboflavin, 25 mg niacin, 25 mg calcium pantothenate, 600 mg choline chloride, 200 μg biotin, 200 mg folic acid; 5 mg ethoxyquin, 150 mg iron, 12 mg manganese, 120 mg zinc, 12 mg copper, 0.2 mg iodine, 0.1 mg selenium.

[2] Calculated digestible energy

Table 2.2 Feeding Schedule for Sows in Experiment One

Sow Weight[1] (Kg)	Max. Daily Sow Lactational FL[2] (kg) For Litter Size				Gestational FL[3] (kg)
	9	10	11	12	
140	5.5	5.9	6.4	6.8	2.1
160	5.6	6.1	6.5	7.0	2.2
180	5.7	6.2	6.6	7.1	2.3
200	5.9	6.3	6.8	7.2	2.4
220	6.0	6.5	6.9	7.4	2.5
240	6.2	6.6	7.1	7.5	2.6
260	6.3	6.7	7.2	7.6	2.7
280	6.4	6.9	7.3	7.8	2.8

[1] Liveweight of sows at farrowing (kg).

[2] Feed level offered to the sow (kg).

[3] All sows fed 1 kg additional feed from day 109 of gestation until farrowing.

Table 2.3 Influence of breeding at first or second estrus after weaning on reproductive performance and body composition of sows, irrespective of parity.

Time of Breeding		**First Estrus**	**Second Estrus**	**SEM**[1]
N[2]		42	34	
Lactation:	Length (days)	23.6[3]	22.9	0.53
	Sow feed intake (kg/day)	5.7	5.8	0.11
	Litter weight gain (kg)	50.4	52.9	1.86
Backfat (mm)[4]**:**				
	farrow	22.2	21.6	0.87
	wean	18.8	18.0	0.68
	breed	18.2	17.8	0.74
Change:	lactation	-3.2	-3.3	0.44
	wean-breed	0.2	0.9	0.32
Liveweight (kg):				
	farrow	212.1	212.8	3.75
	wean	201.1	200.8	3.64
	breed	182.6	182.9	3.81
Change:	lactation	-11.0	-11.9	1.72
	wean-breed	-10.0	-8.7	1.70
Wean to Estrus Interval (day)		4.9	5.0	0.17
Litter Size:	Total Born	12.3^{c}	13.5^{d}	0.43
	Alive Born	10.9^{a}	12.9^{b}	0.38

[1] Standard error of the mean

[2] Number of animals in each treatment

[3] Least-square means

[4] Backfat measured ultrasonically at the 10th rib and 65 mm from the midline.

$^{a-b}$ Means within a row lacking a common superscript differ ($P<0.001$)

$^{c-d}$ Means within a row lacking a common superscript differ ($P<0.09$)

Table 2.4. Influence of weaning-to-estrus interval (wean-estrus) on subsequent litter size, in parity one sows bred at first or second estrus.

Wean-Estrus (days)		7			≤ 6		
Estrus of breeding		1	2	SEM[1]	1	2	SEM
N[2]		3	3		32	23	
Litter size:	Total born	11.3[3]	13.0	0.6	10.1^{a}	12.7^{b}	0.7
	Alive born	10.7	12.3	0.7	9.1^{a}	12.0^{b}	0.7

[1] Standard error of the mean
[2] Number of animals in each treatment
[3] Least-square means
[a-b] Means within a row lacking a common superscript differ ($P<0.01$)

Table 2.5. Influence of parity and time of breeding on sow reproductive performance.

Parity		1 and 2			≥ 3		
Estrus of breeding		1	2	SEM[1]	1	2	SEM
N[2]		32	23		10	11	
Backfat (mm)[3]:							
	farrow	21.0[4]	20.3	0.97	23.4	22.9	1.78
Change:	lactation	-3.7	-3.3	0.57	-2.6	-3.2	0.64
	wean-breed	0.2	1.1	0.17	0.1	0.5	0.48
Weight (kg):							
	farrow	183.0	181.6	4.08	241.2	243.9	8.31
Change:	lactation	-11.4	-12.9	2.19	-10.5	-11.0	2.42
	wean-breed	-11.1[c]	-4.9[d]	2.21	-8.9	-12.4	2.28
Litter Size:							
	total	10.4[a]	12.8[b]	0.48	13.1	13.3	1.11
	alive	9.6[a]	12.0[b]	0.45	11.0	12.7	0.98

[1] Standard error of the mean

[2] Number of animals in each treatment

[3] Backfat (mm), measured ultrasonically 65mm from the midline, at 10th rib.

[4] Least-square means

[a-b] Means within a row lacking a common superscript letter differ ($P<0.002$)

[c-d] Means within a row lacking a common superscript letter differ ($P<0.06$)

Table 2.6 Influence of parity on the reproductive and productive performance of sows.

Parity		1 and 2	≥ 3	SEM[1]
N[2]		59	21	
lactation:	Length (days)	23.1[3]	23.1	0.53
	Sow FI (kg/day)	5.5^{a}	6.0^{b}	0.11
	Litter gain (kg/day)	51.9	51.4	1.86
Backfat[4]:	farrow	20.6	23.1	0.87
Change:	Lactation	-3.5	-2.9	0.44
	wean-breed	0.7	0.3	0.32
Fat (kg)[5]:	farrow	48.6^{a}	66.0^{b}	1.96
	(as % farrowing weight)	26.7	26.7	0.59
Change:	Lactation	-7.8	-6.6	0.87
	(% of fat @ farrow)	15.5^{c}	10.2^{d}	1.67
Liveweight (kg):	farrow	182.3^{a}	242.6^{b}	3.75
Change:	Lactation	-12.1	-10.8	1.72
	wean-breed	-8.0	-10.6	1.70
Protein (kg)[6]:	farrow	27.6^{a}	39.4^{b}	0.79
	(as (% kg farrowing weight)	15.2^{a}	16.0^{b}	0.10
Change:	lactation	1.5	1.4	0.33
	(% protein @ farrow)	5.0	3.6	1.09
Wean-estrus interval (days)		5.1	4.9	0.17
Litter size:	Total	10.9^{a}	14.9^{b}	0.43
	Alive	10.1^{a}	13.7^{b}	0.38

[1] Standard error of the mean

[2] Number of animals in each treatment

[3] Least-square means

[4] Backfat (mm): measured ultrasonically 65 mm from midline, at 10th rib.

[5] Total body fat predicted from equation 2.2.

[6] Total body protein predicted from equation 2.3.

[a-b] Means within a row lacking a common superscript differ ($P<0.01$)

[c-d] Means within a row lacking a common superscript differ ($P<0.05$)

CHAPTER 3

EXPERIMENT 1B: EFFECT OF BREEDING SOWS AT EITHER FIRST OR SECOND ESTRUS AFTER WEANING ON SOW FERTILITY, AS INDICATED BY PLASMA INSULIN, IGF-1, GH AND GLYCEROL.

3.1 INTRODUCTION

Inadequate lactational nutrition has been associated with extended weaning-to-estrus intervals (Elsley *et al.*, 1968; Fahmy, 1981; Reese *et al.*, 1982, 1984; King and Williams, 1984ab; King *et al*, 1984; Baidoo, 1989) and reduced embryo survival rates (Hughes *et al.*, 1984; Baidoo, 1989; Kirkwood *et al.*, 1987ab, 1990) resulting in smaller subsequent litter sizes in the sow (Kirkwood *et al.*, 1988a). Thus, sow fertility which varies considerably after weaning has been linked to nutrition (Fahmy, 1981; Clark and Leman, 1987; Leman, 1987; King, 1987).

High plasma insulin levels on day 14 of lactation (Tokach *et al.*, 1992a) and low tissue losses during lactation (Mullan *et al.*, 1991) have been associated with high mean and pulsatile LH levels on day 14 of lactation and after weaning, respectively, and a shorter interval between weaning and estrus. Therefore it is not surprising that delaying the breeding of sows until their second estrus after weaning increases sow fertility (Moody *et al.*, 1969; Fay, 1973; Love, 1979; Fahmy *et al.*, 1979; King *et al.*, This increase in fertility is probably due to recovery of the sow from the energy/protein deficits of lactation, allowing her to move into a more positive metabolic state.

It has been established that nutrition affects reproduction through mechanisms involving metabolic hormones and metabolites (Armstrong *et al.*, 1988; I'Anson *et al*, 1991; Booth, 1990ab; Pettigrew and Tokach, 1991). These factors have been extensively studied over the past five to ten years. Numerous effects of these factors have been discovered at the level of the ovary (Adashi *et al.*, 1985, 1991ab; Hammond *et al.*, 1991) and brain (Cox *et al.*, 1989; Poretsky and Kalin, 1987; Booth, 1990ab; I'Anson *et al.*, 1991). From this research, key factors that have been identified include insulin and IGF-1.

The objective of this study was to establish if differences in peripheral levels of insulin, IGF-1, GH and glycerol exist in sows bred at first or second estrus after weaning. If this is so, it may be possible to use such indicators of a sow's metabolic status to predict her potential fertility after weaning.

3.2 MATERIALS AND METHODS

3.2.1 Animals and Treatments

A subset of 36 sows from the 116 sows used in Experiment 1A were selected for a more detailed experiment, to measure some key metabolic indicators possibly for a more detailed experiment, to measure some key metabolic indicators possibly involved as regulators of sow fertility. Initially, seven sows were blood sampled at first and second estrus, bred at the second estrus after weaning, and slaughtered on day 14 or 28 after breeding. The number of corpora albicantia (CA: a measure of ovulation rate at the first estrus after weaning), corpora lutea (CL: a measure of ovulation rate at the second estrus after weaning) and viable embryo numbers were recorded, together with the weaning to estrus interval and the first estrous cycle length. However, because of management problems and poor patency of the catheters, the experiment was modified, and the remaining sows were bred at either first or second estrus after weaning and also blood sampled at these times. All sows were heat checked twice daily, at 06.00 and 18.00h, from three days after weaning until the first post-weaning estrus was observed. Sows bred at second estrus were also heat checked, twice daily, from day 17 of the first estrous cycle to the second post-weaning estrus. Sows were bred twice by artificial insemination (AI), using mixed semen (Alberta Swine Artificial Insemination Centre, Leduc, Alberta), 12 and 24 h after standing heat was first observed.

3.2.2 Blood Sampling.

Initially, seven sows were twice acutely fitted with an indwelling jugular catheter, via a prominent ear vein, once on the day of weaning and once on day 17 of the first estrous cycle. These sows were blood sampled over four 6 h bleeding windows; the first window occurred on the day after weaning, the next at first standing heat. The remaining 27 sows were fitted with indwelling jugular catheters either on day three or four after weaning if designated to be bred at the first estrus after weaning, or on day 20 of the first estrous cycle, if bred at the second estrus.

At 18.00 h on the day of catheterisation and/or on the evening of observed standing heat, all food was removed from the sows. The following morning, 5 ml blood samples were collected every 30 mins from 08.00 to 13.00 h, and sows were fed at 09.00 h. Each blood sample was divided into two heparinised tubes, and centrifuged at 1510xg for 15 min. The plasma was stored at -20°C. These plasma samples were later analyzed for content of insulin, insulin-like growth factor-1 (IGF-1), growth hormone (GH) and glycerol. From the day of catheterisation until one day after standing heat, 5 ml blood samples were taken every six hours, after which time 12 hourly samples were collected for a further four days, if the catheters were still patent. These samples were analyzed for luteinizing hormone (LH) and progesterone (P_4). The catheter was removed after the final blood sample was taken.

3.2.3. Analysis.

i) Growth Hormone

Plasma growth hormone (GH) concentrations were determined by the double antibody radioimmunoassay (RIA) described by Marple and Aberle (1972). Anti-sera against porcine growth hormone (anti-GH-208) was kindly supplied by D.N. Marple, Auburn University (Auburn, AL). The purified porcine GH of potency 1.5 IU/mg (USDA-pGH-B-1) was kindly provided by the USDA National Hormone and

Pituitary Program, Beltsville, MD and was used for iodination by the Chloramine T method of Greenwood *et al.* (1963) and as standards.

For the present assay, 200-300 μl of plasma or 200 μl of standards (0.039 to 10 ng GH/tube) were made up to 400 μl with 1% BSA/PBS buffer. All tubes were vortexed and incubated at 4°C for 24 h with 100μl of the anti-porcine GH, diluted to 1:80000 in 0.05 M EDTA/PBS containing normal guinea-pig serum (NGPS; Calbichem 566400) at a dilution of 1:400, except non-specific binding tubes which received NGPS alone. All tubes were incubated for a further 24 h at 4°C with 100 μl of ^{125}I-labelled pGH (10,000 cpm). Finally, 400 μl of the locally raised horse anti-guinea-pig gamma globulin (HAGPGG) diluted 1:10 in 1% BSA/PBS buffer was added to all tubes, after pre-incubation for 24 h at 4°C with equal volumes of 6% (w/v) polyethylene glycol (PEG 8000; Carbowax, Fisher P-156 #743563). After further incubation at 4°C for 24 h, the bound antigen was precipitated using the second antibody, and bound and free ^{125}I-labelled pGH were separated by centrifugation and aspiration.

Different volumes of a pooled standard control plasma (25-400μl) showed parallelism with the standard curve. The mean intra- and inter-assay coefficients of variation for the series of four assays were 5.9% and 14.6%, respectively. The mean sensitivity of the four assays, defined as (mean Bmax-(2*sd mean Bmax)/mean Bmax)*100, was 0.07 ng/tube.

ii) Insulin Analysis

Plasma samples were assayed for insulin by the double antibody RIA described by de Boer and Kennelly (1989) with modifications as described by de Passillé *et al.* (1992). Assaying 100 to 12.5 μl of a standard plasma pool established parallelism with the standard curve which ranged from 0.003 to 1.6 ng insulin/tube. The assay sensitivity, as defined by (mean Bmax - (2*sd mean Bmax)/ mean Bmax) *100), was 0.008 ng/tube. The mean intra- and inter-assay coefficients of variation for the four assays were 4.2% and 16%, respectively.

iii) Insulin-like Growth factor-1 (IGF-1) Analysis.

Plasma IGF-1 concentrations were determined in two radioimmunoassays after acid-ethanol extraction as described by Booth (1990a), with the following modifications; individual 100μl aliquots of plasma were extracted by vortexing with 3 ml of acid-ethanol (12.5 HCL (2.2 M), 87.5% ethanol(v/v)), and incubated at room temperature for 20 hours before centrifugation at 2090xg for 30 minutes at 4°C. This method of extraction yielded an extraction efficiency of 100%, based on an estimate of cold recovery of IGF-1 added to the standard plasma pool. The double antibody RIA used was that of Glimm *et al.* (1990) with the following modifications. The first antiserum was kindly donated by the USDA National Hormone and Pituitary Program, Baltimore, MA, USA, and was used at a dilution of 1:14,000. This antiserum bound approximately 20% labelled recombinant human IGF-1, obtained from Bachem Fine Chemicals Inc., Torrance, CA, USA (catalogue #DGR012), in the absence of unlabelled antigen. The mean sensitivity of the assay, defined as (me Bmax - (2*mean Bmax sd)/mean Bmax)*100, was 5.0 pg/tube. The standard curve ranged from 0.0039 to 8 ng/tube. Assaying 50, 100, 200 and 400 μl of pooled standard plasma established parallelism with the standard curve. The mean intra- and inter- assay coefficients of variation for the two assays were 8.3% and 12.2%, respectively.

iv) Luteinizing Hormone (LH) Analysis

Plasma LH concentrations were determined by the double antibody RIA, described by Cosgrove *et al.*, (1991) with the following modifications. To enhance precipitation of the antibody-bound hormone, the locally raised second antibody, horse anti-goat gammaglobulin (HAGGG) was diluted 1:40 in BSA/PBS buffer and pre-incubated with an equal volume of 6% (w/v) polyethylene glycol (PEG 8000: Carbowax) for 24 h. Four hundred μl of the HAGGG-PEG solution was added to all assay tubes and incubated for a minimum of 16 hours at 4°C, before centrifugation and aspiration. Reproducible standard curves were obtained using standard potencies from 2.4 to 5000 pg/tube of the pLH preparation SDG-2-65 (0.96-1.18 x NIH oLH-s19). The LH hormone used for iodination was IVO-DHO-pLH, kindly supplied by the Research Institute for Animal Production, The Netherlands.

The mean assay sensitivity, defined as (mean Bmax - (2*mean Bmax sd)/mean Bmax)*100, was 0.01 ng/tube. Assaying 200, 100, 50 and 25 μl of a pooled standard plasma established parallelism with the standard curve. The mean intra- and inter-assay coefficients of variation for the three assays were 9.0% and 20.4%, respectively.

v) Progesterone Analysis

Plasma samples were assayed for progesterone by a double antibody RIA described by Rawlings *et al.* (1977, 1980) with minor modifications as described by Pharazyn *et al.* (1991b). Diluted or undiluted 100 μl aliquots of sample and standard were extracted with 4 ml petroleum ether for 5 min, with a mean extraction efficiency of 73%; unknown potencies were corrected for recovery losses. The standard curve ranged from 0.003125 to 6.4 ng/tube, and no significant deviation from parallelism was evident from assaying a standard plasma pool at different dilutions. The assay sensitivity, defined as (mean Bmax - (2*sd mean Bmax)/ mean Bmax) *100, was 5 pg/tube. The mean intra- and inter-assay coefficients of variation were 7.6% and 12.8%, respectively.

vi) Glycerol Analysis

Plasma samples were assayed for glycerol by gas chromatography (GC) using a capillary column (Fenton and Aherne, 1987). One ml of 95% alcohol was added to deproteinize 0.5 ml plasma samples in micro centrifuge tubes. After mixing, tubes were centrifuged for 5 min in an Eppendorf centrifuge. Duplicate 0.5 ml samples were transferred into 1.8 ml mini-vials with screw caps and PTFE-lined septa and evaporated to dryness at room temperature under vacuum.

A 20 μl volume of the internal standard, 1-dodecanol (3 mg/ml in pyridine), and 100 μl of Sylon TP were added to each vial. Trimethylsilyl (TMS) derivatives were formed by heating at 70°C for 20 minutes, and 1 μl was injected into the GC. Glycerol standards (4 and 8 μg/ml) were subjected to the same procedure as the samples. The relative response factor was calculated as a ratio of internal standard to glycerol peak area, multiplied by the ratio of the amount of glycerol to that of 1-dodecanol in the standard.

3.2.3 Statistical Analysis.

Preliminary analysis, of production and sow composition data, for parity differences, using orthogonal contrasts (SAS, 1990) established that sows of parity one and two were not significantly different from one another. Therefore, as in experiment 1A, data from these sows were pooled into a single group. Further analysis determined that sows of parity three or more were not different from one another, but were different from sows of parity one and two. These sows were therefore designated into a separate group. Two sows from parity one and two were excluded from the analysis because their litter size was three standard deviations away from the mean.

Statistical analyses were performed using the General Linear Models (GLM) procedures of SAS (1990), using the linear model of experiment 1, including parity grouping and estrus of breeding as the class variables. Again, the model was modified when analyzing litter size, by the addition of the sow's live weight at farrowing to account for sow weight differences within the parity groups. However, when analyzing the parity groups individually, the model included only estrus. Associations between variables were explored using Pearson's correlations (SAS, 1990). The endocrine data was analyzed using the GLM procedures of SAS (1990) described above, and progesterone was analyzed using an unequal t-test. The equations of Whittemore and Yang (1989) were used to predict the total lipid content (equation 2.2) and total body protein content (equation 2.3) of the sow.

3.3 RESULTS

In contrast to the results of experiment 1A, the small subset of sows in this experiment initially appeared to have smaller litters when bred at the second compared to the first estrus after weaning. However, analysis of the individual parity groups revealed a numerical increase in total litter size born (11.4 vs. 13.7 pigs ($P<0.15$), respectively) and born alive (10.8 vs. 11.6 pigs ($P<0.52$), respectively) in sows of parity one and two bred at second rather than first estrus after weaning (table 3.1). Skip-a-heat breeding in sows of parity three and greater on the other hand, tended to reduce both total litter size born (10.8 vs. 12.8 pigs ($P<0.27$), respectively) and born alive (8.5 vs. 11.0 pigs ($P<0.25$), respectively) compared to sows bred at first estrus. Consistent with the decrease in litter size in sows of parity three and greater bred at second compared to first estrus, the initial seven sows showed a numerical decrease in ovulation rate in second compared to first estrus (17.7± 1.9 CA and 16.0± 1.9 CL, respectively). However, problems were encountered when counting the number of CAs on the sow's ovaries, because the sows were from high parities, and therefore their ovaries were scarred from previous ovulations. Embryos were unsuccessfully recovered, so unfortunately no data on embryo survival were collected.

3.3.1 Lactation.

Sows of first and second parity, and parity three or more showed no difference in lactation feed intake, backfat, fat, weight and protein levels at farrowing, and losses over lactation between first and second estrus (table 3.2). However, daily litter weight gains were significantly higher in sows of parity three or more bred at second rather than first estrus after weaning (3.3 vs. 2.1 kg/day ($P<0.005$), respectively). These parity three or more sows also lost more backfat and weight during lactation than parity three or greater sows bred at first estrus (4.9 vs. 3.2 mm and 16.1 vs. 14.7 kg, respectively). First and second parity sows bred at second rather than first estrus after weaning, also lost more fat, weight and protein during lactation (10.1 vs. 8.3 kg, 17.0 vs. 5.0 kg ($P<0.06$) and 2.8 vs. -0.7 kg ($P<0.05$), respectively) (table 3.2).

i) Parity effects.

Backfat, total fat, live-weight and total protein at farrowing were significantly higher in sows of parity three and greater, compared to sows of parity one and two (25.9 vs. 20.4 kg ($P<0.05$), 74.5 vs. 49.8 kg ($P<0.001$), 267.1 vs. 188.9 kg ($P<0.001$) and 42.8 vs. 29.1 kg ($P<0.0001$), respectively) (table 3.3). However, differences in total body fatness between parity groups disappeared when total body fat was measured as a proportion of live-weight. Parity three and greater sows lost more weight, protein and protein as a proportion of total body protein over lactation, than sows of parity one and two (15.4 vs. 13.0 kg, 2.0 vs. 1.0 kg and 4.6 vs. 2.8 %, respectively).

3.3.2 Weaning and Breeding.

Both first and second parity and parity three or more sows bred at second rather than first estrus after weaning, lost numerically more weight between weaning and breeding ([illegible].3 vs. [illegible]2 kg and 17.4 vs. 12.4 kg, respectively) (table 3.2). Consistent with the larger weight and tissue losses during lactation and between weaning and breeding seen in sows bred at second rather than first estrus, the weaning-to-estrus interval was slightly extended in parity one and two sows (5.9 vs. 4.6 days ($P<0.09$), respectively) (table 3.1).

3.3.3 Endocrine data

Plasma GH levels decreased over the first four samples in the bleeding window, between 08.00 and 09.30 h, in the five sows represented by figure 3.1a. After this time, plasma GH in individual sows remained stable. Thus, a mean GH value was taken for each sow, for each blood sampling window, from 09.30 h until the end of the window. Plasma IGF-1, unlike GH, did not vary over time as seen in the representative IGF-1 profile from a sow in figure 3.1b. Therefore, a mean

IGF-1 value for each six hour window bleed was taken for each sow. Plasma insulin levels, unlike the other hormones measured was affected by feeding and increased rapidly after feeding at 09.00h, as seen in figure 3.1c. Unfortunately, because the sows were in standing heat, their appetites were very low during the bleed. Therefore, sows did not eat a consistent amount of feed at any given time during the bleed, and many hardly ate at all. Thus, the post-feeding insulin profiles for most sows were quite erratic, and the basal insulin levels were the only consistent portion of the insulin profiles measured. Thus, basal pre-prandial insulin levels, taken after a 16 h overnight fast were used in the analysis of insulin. These insulin values were represented by the mean value of the first three samples in the bleeding window, for each animal. Plasma from the first three samples before feeding were pooled for each animal and bleeding window, for plasma glycerol determination.

i) Hormonal differences between parity groups and time of breeding.

a) Parity one and two

First and second parity sows had higher basal insulin and lower plasma glycerol levels at second compared to first estrus (0.45± 0.15 vs. 0.30± 0.11 ng/ml (P<0.09) and 7.41± 0.47 vs. 4.62± 0.37 ng/ml (P<0.001), respectively) (figure 3.2b). These sows also had significantly lower plasma IGF-1 levels at second compared to first estrus (71.3± 8.2 vs. 101.2± 10.5 ng/ml (P<0.05), respectively), but plasma GH levels were not different between first and second estrus (figure 3.2a).

Total litter size born for both first and second estrus sows was negatively correlated with IGF-1 level (R=-0.77 (P<0.23) and R=-0.84 (P<0.04), respectively) and positively correlated with basal insulin at first estrus (R=0.70 (P<0.30). Basal insulin was highly negatively correlated with IGF-1 at first estrus and with GH at second estrus (R=-0.96 (P<0.08) and R=-0.57 (P<0.14), respectively). Glycerol was highly negatively correlated with GH (R=-0.84 (P<0.08).

b) Parity three and greater

The initial seven sows of parity three or more were sampled for IGF-1, GH, basal insulin and glycerol on the day after weaning, at first estrus, three to four days before second estrus and at second estrus (figure 3.3). Glycerol levels were high just after weaning (8.51±0.70 ng/ml) and then dropped significantly (P<0.003) in the subsequent periods: at first estrus (5.25±0.70 ng/ml), prior to second estrus(5.09±0.76 ng/ml) and at second estrus (4.54±0.76 ng/ml) (figure 3.3b). There was a tendency for IGF-1 levels to fall at second estrus, as seen in first and second parity sows (figure 3.3a). Basal insulin and plasma GH were not different either before or at first and second estrus after weaning, respectively (figure 3.3ab). In parity three or more sows, a significant (P<0.03) decrease in GH was observed in sows bred at second rather than first estrus (2.15±0.13 vs. 1.64±0.18 ng/ml (P<0.03), respectively). No difference between estrus of breeding was seen for the other parameters measured (figure 3.4ab).

Growth hormone levels at first estrus were positively correlated with total litter size born and basal insulin levels at first estrus ($R=0.63$ ($P<0.18$) and $R=0.75$ ($P<0.09$), respectively). On the day after weaning, basal plasma insulin levels were highly and negatively correlated with IGF-1 ($R=-0.71$ ($P<0.07$)) and positively correlated with the number of CLs for the second ovulation ($R=0.97$ ($P<0.001$)). At first standing heat, IGF-1 was positively correlated ($R=0.70$ ($P<0.08$)) and GH was negatively correlated ($R=-0.77$ ($P<0.04$)) with glycerol. Three days before second estrus, glycerol was correlated with basal insulin and subsequent ovulation rate (CL) ($R=0.95$ ($P<0.05$) and $R=0.86$ ($P<0.06$), respectively), and GH was negatively correlated with basal insulin ($R=-0.94$ ($P<0.06$)). Only at the second standing heat was IGF-1 correlated with GH ($R=-0.90$ ($P<0.006$)), but this was a negative relationship, and no correlation was seen between insulin and either GH or IGF-1.

c) Between parities

At first estrus, both plasma IGF-1 and glycerol were significantly higher in first and second parity sows than sows of parity three and greater (96.7 ± 11.1 vs. 69.8 ± 9.3 ng/ml ($P<0.09$) and 7.53 ± 0.62 vs. 5.51 ± 0.52 ng/ml ($P<0.03$), respectively). However, at second estrus glycerol levels were lower in first and second parity sows than sows of parity three and greater (4.54 ± 0.44 vs. 5.67 ± 0.47 ng/ml ($P<0.10$), respectively).

At first estrus, in sows of first or second parity and parity three and greater, IGF-1 was negatively correlated with insulin ($R=-0.96$ ($P<0.08$) and $R=-0.48$

($P < 0.23$), respectively). At second estrus, GH was negatively correlated with insulin in both first and second parity and parity three or more sows ($R = -0.57$ ($P < 0.14$) and $R = -0.74$ ($P < 0.06$), respectively).

ii) Progesterone and luteinizing hormone

In each sow, plasma LH and progesterone were plotted against time to determine the time of standing heat relative to the LH peak (defined as the highest point of the LH surge) (figure 3.5). Standing heat occurred 18 hours before the LH peak, and was highly correlated with the LH peak ($R = 0.97$ ($P < 0.01$)). However, because sampling commenced too late after the LH surge in some sows, the rise in progesterone could only be measured against time of standing heat, and not the time of LH peak in those sows. Heat was only checked every 12 hours, thus a larger error was associated with the time of standing heat than the time of the LH surge.

Fifty hours after standing heat, the variance in progesterone levels was higher across parities in sows bred at second compared to first estrus (figure 3.6a). Mean progesterone levels were also higher in sows bred at second compared to first estrus (3.12 vs. 5.75 ng/ml ($P < 0.09$), respectively). However, no difference was observed between first and second estrus within either parity group (figure 3.6b). When plasma progesterone levels were measured at 30 and 50 hours after the LH peak, first and second parity sows bred at second rather than first estrus showed a significantly higher variance ($P < 0.01$ and $P < 0.02$, respectively) and mean plasma progesterone levels (2.0 vs 1.2 ng/ml ($P < 0.02$) and 5.4 vs. 3.7 ng/ml ($P < 0.04$), respectively) (figure 3.7b). However, no difference in variance and mean progesterone levels at 30 and 50 hours after the LH peak was seen in parity three or more sows bred at first compared to second estrus (2.7 vs 2.6 ng/ml ($P<0.97$) and 4.8 vs 4.8 ($P<0.96$), respectively).

3.4 DISCUSSION

Differences in sow fertility after weaning may be described by differences in their metabolic status before and after weaning (figure 3.8). If a sow is in a positive energy and/or protein balance during lactation, she will continue to improve her fertility throughout lactation. Although the slope of the line decreases in the latter three or four weeks of lactation (time of peak milk production) (figure 3.8), these sows quickly return to estrus after weaning (three to six days), and will be called type 1 early returners. In this experiment, no sows fell into this category. Sows with lower energy/protein intakes during lactation than their maintenance, growth and milk production requirements draw on their own tissue reserves to overcome the lactational energy/protein deficits and maintain their milk yield and piglet growth rate (Eastham *et al.*, 1988). The negative slope of the line indicates catabolism in the sow (figure 3.8 line B). Because these sows only lose moderate amounts of weight, they recover their metabolic status rapidly after weaning to become anabolic, and return to estrus within six days, but at a slightly lower fertility than type 1 early returners (type 2 early returner). The last sow type (figure 3.8 line C) becomes increasingly more catabolic during lactation, as indicated by the decreasing and then negative slope of the line. Such sows take a longer period of time to become anabolic after weaning and would either be late in returning to estrus or, if the sows were in a very 'deep' catabolic state, may remain anestrus after weaning (late returner).

The majority of sows in this experiment were in the early returner (type 2) category. Therefore, delaying breeding these sows until second estrus allowed them to further recover their metabolic status and thus fertility after weaning. This in turn led to an increase in litter size in such sows, due to both an increase in ovulation rate and embryo survival (King *et al.*, 1984).

In agreement with experiment one, first and second parity sows tended to increase both total and alive litter size when bred at second rather than first estrus (13.7 vs. 11.4 pigs and 11.6 vs. 10.8 pigs, respectively). This is not surprising, because these sows would be type 2 early returners (figure 3.8 line B) and therefore continue significantly improving their fertility after first estrus. To obtain an increase in total litter size in first and second parity sows bred at second compared to first estrus, with a probability as high as 0.15, was very encouraging. This is because there were far fewer sows in this trial in parity one and two, and three and greater (13 and 14 sows, respectively) than in experiment 1 (55 and 21 sows, respectively). However, sows of parity three or more showed a numerical decrease in total litter size born and born alive when bred at second rather than first estrus, and a tendency for a lower ovulation rate (16.0 CA vs. 17.7 CL, respectively).

3.4.1. Sows of parity one and two

The relative catabolic status of sows during weaning is affected by the weight, fat and protein losses of sows during lactation. Therefore, the gradient of the negative slope in figure 3.8 (line B) will be greater in sows in a 'deeper' catabolic state during lactation. Sows which are more catabolic during lactation may still return to estrus early (within seven days) but be at a lower relative metabolic status and fertility than sows in a less catabolic state during lactation.

Retrospective analysis indicated that by chance, first and second parity sows allocated to be bred at second estrus lost more liveweight and fat during lactation than sows allocated to be bred at first estrus (17.0 vs. 5.0 kg ($P<0.06$) and 10.1 vs. 8.3 kg, respectively). Furthermore, parity one and two sows bred at first estrus appeared to maintain their body protein content during lactation, whereas sows bred at second estrus lost protein. This further suggests that the sows bred at second estrus were 'less fertile' at first estrus than sows bred at first estrus. Consistent with this, sows of parity one and two bred at second rather than first estrus took longer to return to estrus after weaning (5.9 vs. 4.6 days ($P<0.09$), respectively).

GH has both anabolic and catabolic actions depending on the nutritional and physiological status of the animal. The anabolic actions of GH are mediated by IGF-1 synthesis, predominantly from the liver. However, IGF-1 is also synthesised in a wide variety of other tissues (Murphy *et al.* (1987), including the porcine ovary

(Hammond *et al.*, 1985; Charlton, 1992). Insulin plays a role in liver GH receptor regulation, and in this way modulates the ability of GH to stimulate hepatic IGF production. Thus, it would be expected that when IGF levels are high, peripheral insulin levels would also be high (for review see I'Anson *et al.*, 1991). The insulin-like actions of the IGF's include protein synthesis, glucose transport and oxidation, glycogen synthesis and lipogenesis. During nutritional deprivation, such as the end of lactation in the sow, GH levels are high (Baidoo, 1989) and GH exerts its catabolic effects. This role is most likely enhanced by the low plasma insulin levels seen during lactation, which reduce hepatic IGF production, and therefore permit GH's catabolic actions.

The fact that plasma IGF-1 levels decreased when insulin levels increased in sows bred at second compared to first estrus, initially appears contradictory. However, parity one and two sows were growing, and their growth was halted or at least reduced during lactation due to nutritional inadequacies. So upon weaning, when the nutritional drain of lactation was removed, 'catch-up' growth may occur, and these sows would begin accreting protein. Protein synthesis is one of the physiological roles of IGF, and because other hormones regulate hepatic IGF production, including cortisol, prolactin and thyroid hormones, a combination of these factors could conceivably lead to an increase in IGF-1 levels. Thus, the presence of higher levels of IGF-1 at times of protein accretion after weaning, in first and second parity sows, associated with low insulin levels may not be so illogical. Similarly, in the seasonally malnourished wild boar, higher IGF-1 levels were observed during increased voluntary intake after a long period of undernutrition, and associated with lower insulin levels. Within six weeks of increased feed intake, IGF-1 levels decreased and insulin increased, probably because the boars had completed their 'catch-up' growth, and were in a positive metabolic state and were no longer needing to lay down as much bodily protein (S. Hofäcker, personal communication).

Plasma glycerol concentrations reflect the rate of lipolysis in adipose tissue (Tepperman, 1980), and provide an approximate index of lipid mobilisation. Therefore, the fact that GH was highly and negatively correlated with glycerol at first estrus suggests that GH in these sows was not acting in a catabolic manner, because if GH was high in this case, lipolysis would be occurring. Also, GH and IGF-1 level were not correlated.

The sows in this experiment were bred by AI, using mixed semen, and the first and second parity sows in experiment 1A were bred naturally. In this experiment, the first and second parity sows showed a larger increase in total litter size born and born alive at first estrus compared to sows in experiment 1 (11.4 vs 10.4 pigs and 13.7 vs. 12.8 pigs, respectively). This could be accounted for by the quality of the semen used in the artificially and naturally mated sows, and the fact that the artificial semen was mixed and so could have the advantage of possessing different seminal factors, from the different boars.

3.4.2 Sows of parity three or more

Mullan *et al.* (1991) observed that sows that are more catabolic during lactation, lose more tissue during lactation and are less fertile after weaning, as demonstrated by lower mean plasma LH and pulsatile LH activity after weaning, than sows that lose little body tissue during lactation. In this experiment, sows bred at second rather than first estrus tended to lose more fat and weight during lactation and more weight between weaning and breeding than sows bred at first estrus (table 3.1 and 3.2).

Therefore, even though parity three or more sows selected to be bred at second rather than first estrus tended to have a higher feed intake during lactation, this larger energy/protein intake was still insufficient to compensate for the larger ($P<0.0005$) daily litter weight gains. Thus, in retrospect, parity three or more sows allocated to be bred at second rather than first estrus were more catabolic during lactation. Because of this, they were not able to recover their metabolic status sufficiently by the second estrus after weaning to improve their fertility and thus subsequent litter size above sows bred at first estrus.

Both IGF-1 and basal insulin failed to increase between first and second estrus in sows of parity three or more (figure 3.4ab). Furthermore, basal insulin at second estrus was negatively correlated with GH ($R=-0.74$ ($P<0.06$) and IGF-1 ($R=-0.51$ ($P<0.24$). Whereas, GH at first estrus was positively correlated with basal insulin at first estrus (0.75 ($P<0.09$) and negatively correlated with glycerol and IGF-1 at first estrus ($R=-0.52$ ($P<0.23$) and $R=-0.48$ ($P<0.33$)). Therefore, high GH was associated with low plasma glycerol and IGF-1 levels with high basal insulin, suggesting that GH was undergoing its anabolic functions. Thus, first estrus sows may have already recovered their metabolic status after lactation and become anabolic. This accounts for their increased fertility, as noted by their total litter size born (12.8 pigs) which is comparable to the total litter size in first and second parity sows bred at second estrus (13.7 pigs). Furthermore, GH at first estrus was positively correlated with total subsequent litter size ($R=0.63$ ($P<0.18$)).

The higher plasma glycerol levels in first and second parity than parity three or more sows at first estrus, reflect a higher rate of lipolysis in first and second parity sows. Thus sows of parity three or more, bred at first estrus, recovered from their lactational nutritional deficit quicker than first estrus sows of parity one and two. Glycerol levels fell significantly between the day after weaning and the day of first standing heat (a matter of two to four days) (figure 3.3), showing how quickly older sows recover their metabolic status after weaning compared to first and second parity sows. Plasma glycerol levels were higher in parity three or more sows compared to parity one and two sows bred at second estrus, thus, the parity three or more sows were possibly still undergoing lipolysis at second estrus.

A number of researchers have demonstrated a possible diurnal GH rhythm, with higher GH levels at night (Kirkwood *et al.*, 1987c; Charlton, 1992). The gradual decline in GH levels seen in five sows in this study between 08.00 and 09.30 h at both first and second estrus is in agreement with this diurnal GH rhythm.

3.4.3 Progesterone Effect

First and second parity sows bred at second estrus showed an increase in litter size, presumably due to an increase in ovulation rate and/or embryo survival. No such effect was seen in parity three or more sows bred at second compared to first estrus. High progesterone levels in early gestation appear to be important for embryo survival in sheep (Parr *et al.*, 1987), gilts (Pharazyn 1992) and sows (Hughes *et al.*, 1984). Furthermore, increased plasma progesterone variances and mean levels, three days after standing heat, in gilts were related to increased embryo survival (Pharazyn *et al.*, 1991a). First and second parity sows bred at second rather than first estrus, had greater variance in progesterone concentrations and higher mean progesterone levels measured 50 hours after the LH peak. These sows also had a larger total litter size, which suggests that high progesterone could indeed be necessary for high embryo survival in sows.

Correlative data suggests that insulin is related to sow fertility, because in parity one and two sows at first estrus, and in the initial seven parity three or more sows at first and second estrus, insulin was highly and positively correlated with total litter size and number of CL's (R=0.70, 0.97 and 0.84). This could be because high insulin levels are seen when the sow is in a higher metabolic status. Increasing peripheral insulin levels in conjunction with high energy levels, increased the ovulation rate in cyclic gilts (Cox *et al.*, 1987) by reducing ovarian follicle atresia (Matamoros *et al.*, 1990, 1991). This lower ovarian follicular atresia is associated with higher follicular fluid IGF-1 levels (Matamoras *et al.*, 1991) and is seen in normal compared to diabetic gilts (Meurer *et al.*, 1991). Thus, high IGF-1 levels in human (Eden *et al.*, 1988) and swine follicular fluid (Hammond *et al.*, 1988; Echternkamp *et al.*, 1990), and high insulin follicular fluid levels in human follicles (Diamond *et al.*, 1985) are associated with follicles capable of ovulation. However, in this study, total litter size was negatively correlated with plasma IGF-1 levels in sows of parity one and two at first and second estrus (R=-0.77 and -0.84). This was unexpected, but it is not totally clear what the function of IGF-1 at first and second estrus was in first and second parity. Thus it appears both that insulin and IGF-1 may be important metabolic indicators signalling sow fertility.

Does basal plasma insulin reflect the level seen by the reproductive axis? This hormone acts at the level of the brain and the ovary. At the level of the brain, cerebrospinal fluid (CSF) insulin appears to affect the reproductive axis (Cox *et al.*, 1989) via brain and pituitary insulin receptors (Werther *et al.*, 1987), which are also influenced by nutrient restriction (Melnyk and Martin, 1984). Cerebrospinal fluid insulin is buffered against the transient changes due to feeding, seen in the periphery (Woods and Porte, 1977). Thus, basal insulin levels have the greatest effect on this insulin pool, because transient daily variations in peripheral insulin would be smoothed out in CSF.

Both IGF-1 and insulin also have effects on the ovary. The rat and porcine ovary synthesize IGF-1 (Oliver *et al.*, 1988, 1989; Hammond *et al.*, 1985; Charlton 1992) and insulin levels are higher in follicles containing oocytes (Diamond *et al.*, 1985). In these cases, measuring peripheral hormones appear to say nothing about the effect at the ovarian level. But, gonadal IGF-1 concentrations are reduced by fasting (Handelsman *et al.*, 1985), thus peripheral levels of IGF-1 could very well reflect the level at the ovary, and high peripheral IGF-1 levels are associated with high fertility in cattle (Echternkamp *et al.*, 1990). Thus, relative peripheral hormone levels could give an indication of the fertility of the animal. But to be more specific, follicular fluid insulin and IGF-1 levels should be measured.

3.5 CONCLUSIONS

In conclusion, the relationship between metabolic indicators of fertility, insulin and IGF-1 are very complex. However, the correlative data does suggest that insulin plays a role in sow fertility after weaning. The role of IGF-1 on fertility could not be properly evaluated in this experiment because of differences in the metabolic status of sows bred at first and second estrus, at weaning. In order to investigate the effects of metabolic state on fertility, the initial metabolic status of sows immediately after lactation must be similar between groups bred at first and second estrus after weaning.

Table 3.1. Influence of parity and time of breeding on sow reproductive performance.

Parity		1 and 2			≥ 3		
Estrus of Breeding		1	2	SEM[1]	1	2	SEM
N[2]		5	8		7	7	
Wean-estrus interval (days)		4.6[3]	5.9	0.47	4.4	4.9	0.23
Litter size:	total	11.4	13.7	0.85	12.8	10.8	1.22
	alive	10.8	11.6	0.69	11.0	8.5	1.46

[1] Standard error of the mean
[2] Number of animals in each treatment
[3] Least-square means

Table 3.2. Production performance of parity 1 and 2 sows and parity 3 and greater sows bred at either first or second estrus.

Parity		**1 and 2**			**≥ 3**		
Estrus of Breeding		**1**	**2**	**SEM**[1]	**1**	**2**	**SEM**
N[2]		5	8		7	7	
Lactation:	length (days)	22.0^c	26.1^d	1.15	23.3^a	19.7^b	0.79
	Feed Intake (kg/day)	5.7^b	5.8	0.16	5.9	6.8	0.50
	Litter gain (kg/lactn)	2.3	2.1	0.20	2.1^a	3.3^b	0.25
Backfat (mm)[4]	farrow	20.5	20.2	1.03	25.7	26.0	2.50
Change:	lactation	-5.0	-4.0	[illegible]	-3.2	-4.9	1.02
	wean-breed	0.5	0.4	0.88	-0.7	-0.1	0.74
Fat[5] **(kg):**	farrow	47.6	52.1	4.05	73.6	75.4	6.02
	(as % farrowing wt[6])	26.6	26.1	1.38	27.8	27.9	1.22
	lactation	-8.3	-10.1	2.6	-8.4	-10 9	2.00
Weight (kg):	farrow	179.8	198.0	9.80	263.7	270.4	15.46
Change:	lactation	-5.0	-17.0	3.97	-14.7	-16.1	3.42
	wean-breed	-6.2	-10.3	2.23	-12.4	-17.4	2.89
Protein[7] **(kg):**	farrow	26.9	31.4	1.92	42.1	43.4	2.72
	(as % farrow wt)	15.1	15.6	0.24	16.0	16.1	0.21
	lactation	0.7^c	-2.8^d	0.84	-2.0	-2.0	0.57

[1] Standard error of the mean

[2] Number of animals in each treatment

[3] Least-square means

[4] Backfat (mm), measure ultrasonically 65mm from the midline, at the 10th rib.

[5] Total body fat predicted from equation 2.2.

[6] As a percentage of the liveweight of the sow at farrowing

[7] Total body protein predicted from equation 2.3.

[a-b] Means within a row lacking a common superscript differ ($P<0.01$)

[c-d] Means within a row lacking a common superscript differ ($P<0.05$)

Table 3.3 Influence of parity on the reproductive and productive performance of sows.

Parity		**1 and 2**	**≥ 3**	**SEM**[1]
N[2]		13	14	
Lactation:	Length (days)	24.1^c	21.5^d	0.69
	Sow FI (kg/day)	5.7^3	6.3	0.28
	Litter gain (kg/day)	2.2	2.7	0.16
Backfat (mm)[4]**:**	farrow	20.4^c	25.9^d	1.72
Change:	Lactation	-4.5	-4.2	0.53
	wean-breed	0.4	-0.4	0.57
Fat (kg)[5]**:**	farrow	49.8^a	74.5^b	4.04
	(as % of kg farrow)	26.3	27.8	0.85
Change:	Lactation	-9.2	-9.7	1.59
	(as % of fat farrow)	18.2	13.3	2.36
Liveweight (kg):	farrow	188.9^a	267.1^b	9.31
Change:	Lactation	-11.0	-15.4	2.61
	wean-breed	-8.2^c	-14.8^d	1.84
Protein (kg)[6]**:**	farrow	29.1^a	42.8^b	1.83
	(as % of kg farrow)	15.4^c	16.0^d	0.16
Change:	lactation	-1.0	-2.0	0.49
	(as % protein farrow)	2.8	4.6	1.22
Wean-estrus interval (days)		5.2	4.6	0.26
Litter size:	Total	12.7	11.8	0.79
	Alive	11.3	9.8	0.85

[1] Standard error of the mean

[2] Number of animals in each treatment

[3] Least square mean

[4] Backfat (mm): measured ultrasonically 65 mm from midline, at 10th rib.

[5] Total body fat predicted from equation 2.2.

[6] Total body protein predicted from equation 2.3.

[a-b] Means within a row lacking a common superscript differ ($P<0.001$)

[c-d] Means within a row lacking a common superscript differ ($P<0.05$)

Figure 3.1 Representative pre- and post-prandial plasma a) GH, b) IGF-1 and c) insulin profiles in sows after weaning

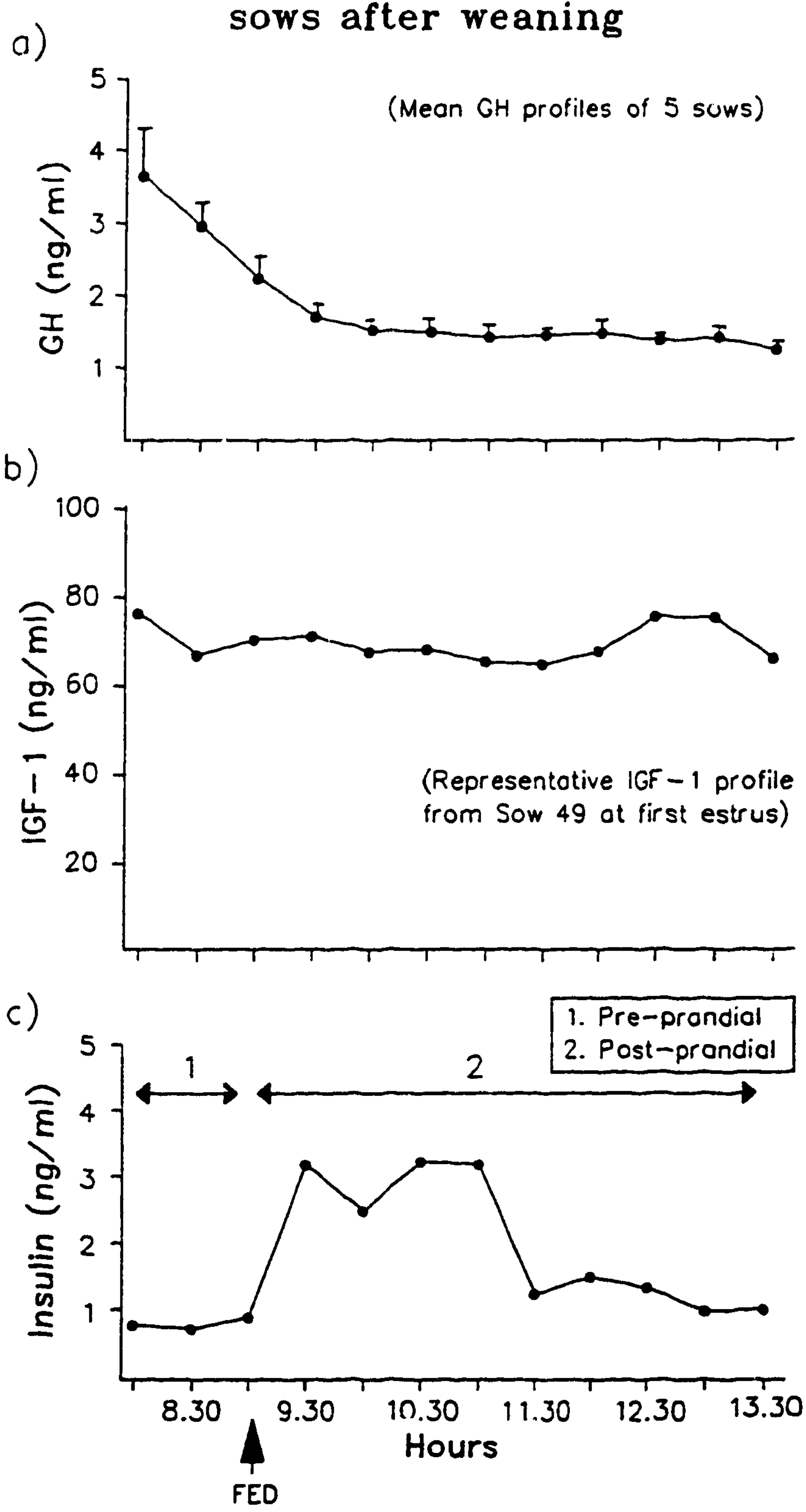
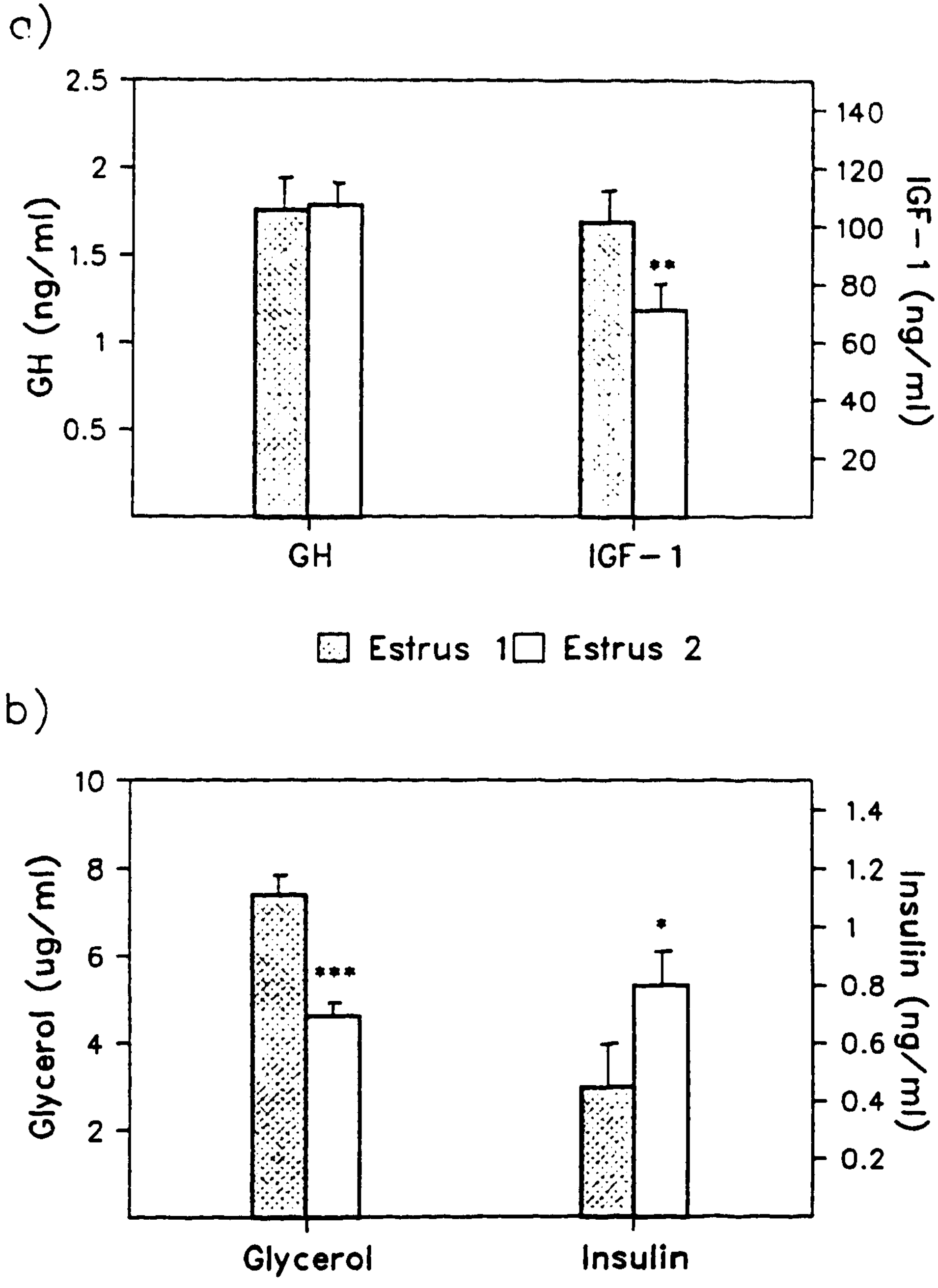
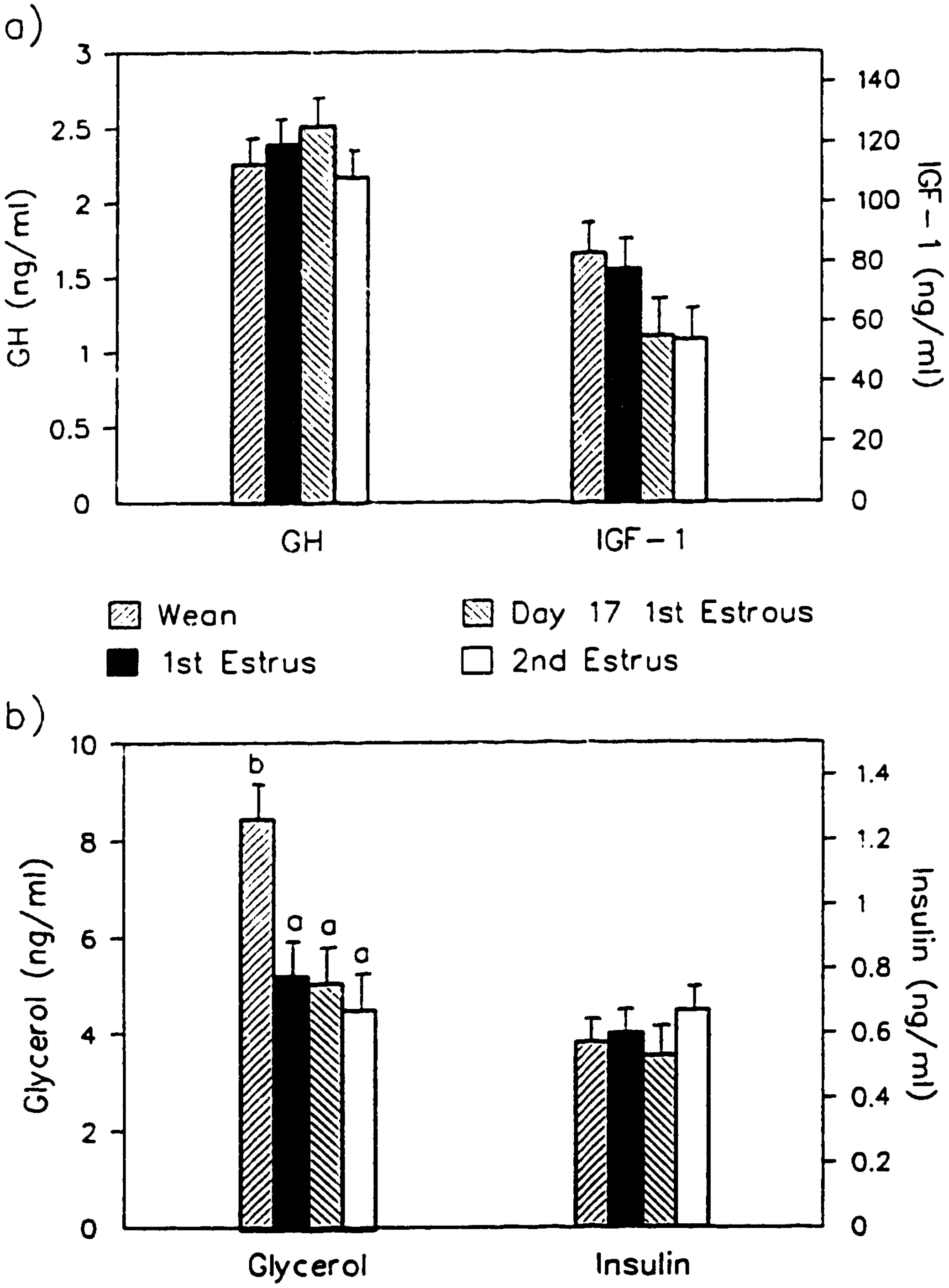


Figure 3.2. Plasma (a) growth hormone and IGF-1 and (b) glycerol and basal insulin, at first and second estrus, in first and second parity sows.



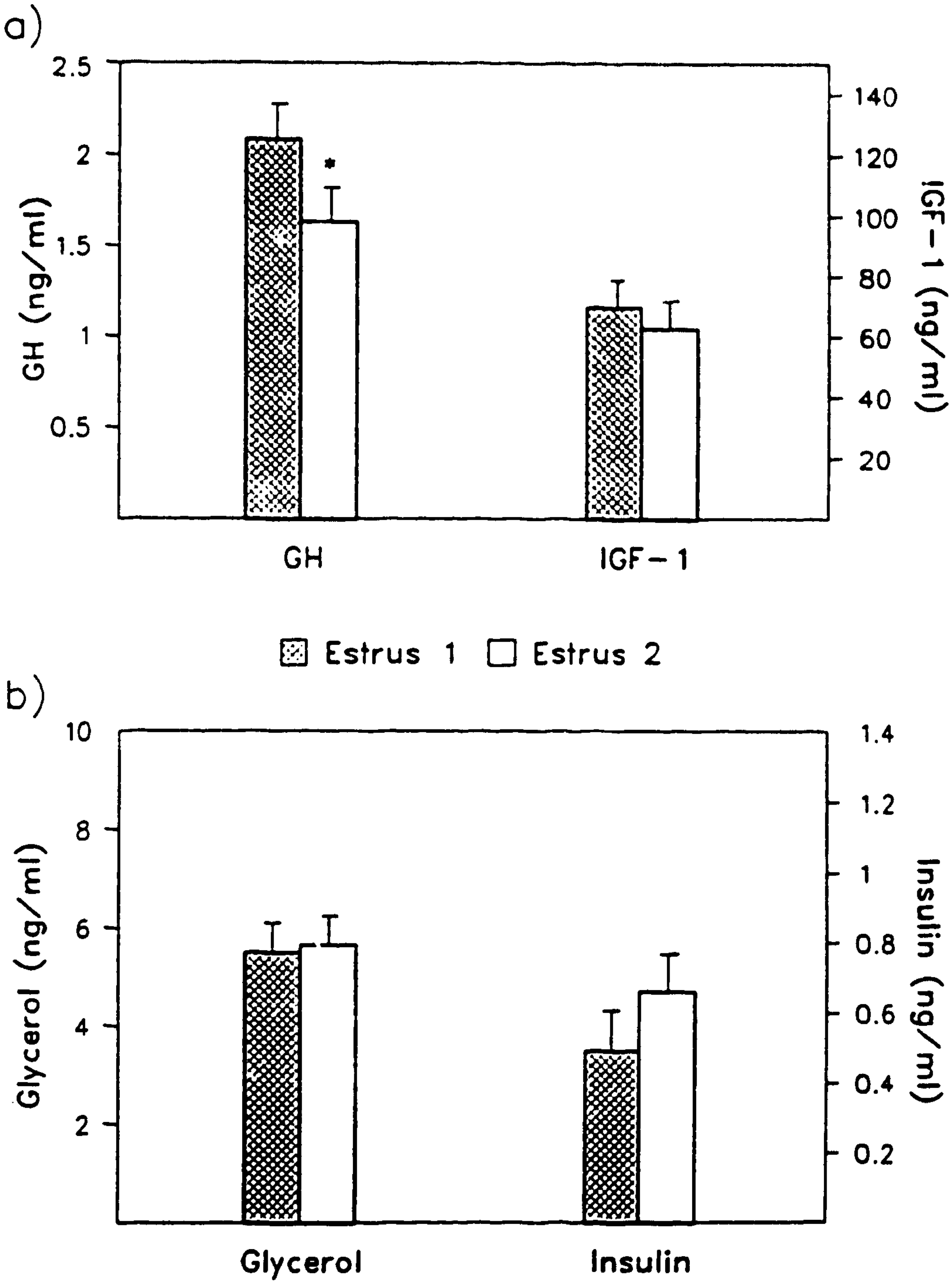
* P<0.09; ** P<0.05; *** P<0.001

Figure 3.3. Plasma a) growth hormone and IGF−1 and b) glycerol and basal insulin, before and after first and second estrus.



a−b: P<0.01

Figure 3.4. Plasma (a) growth hormone and IGF-1 and (b) glycerol and basal insulin, at first and second estrus, in third and greater parity sows.



* $P<0.05$

Figure 3.5. Plasma levels of luteinizing hormone (LH) and progesterone (P4) at around standing heat in sow 49.

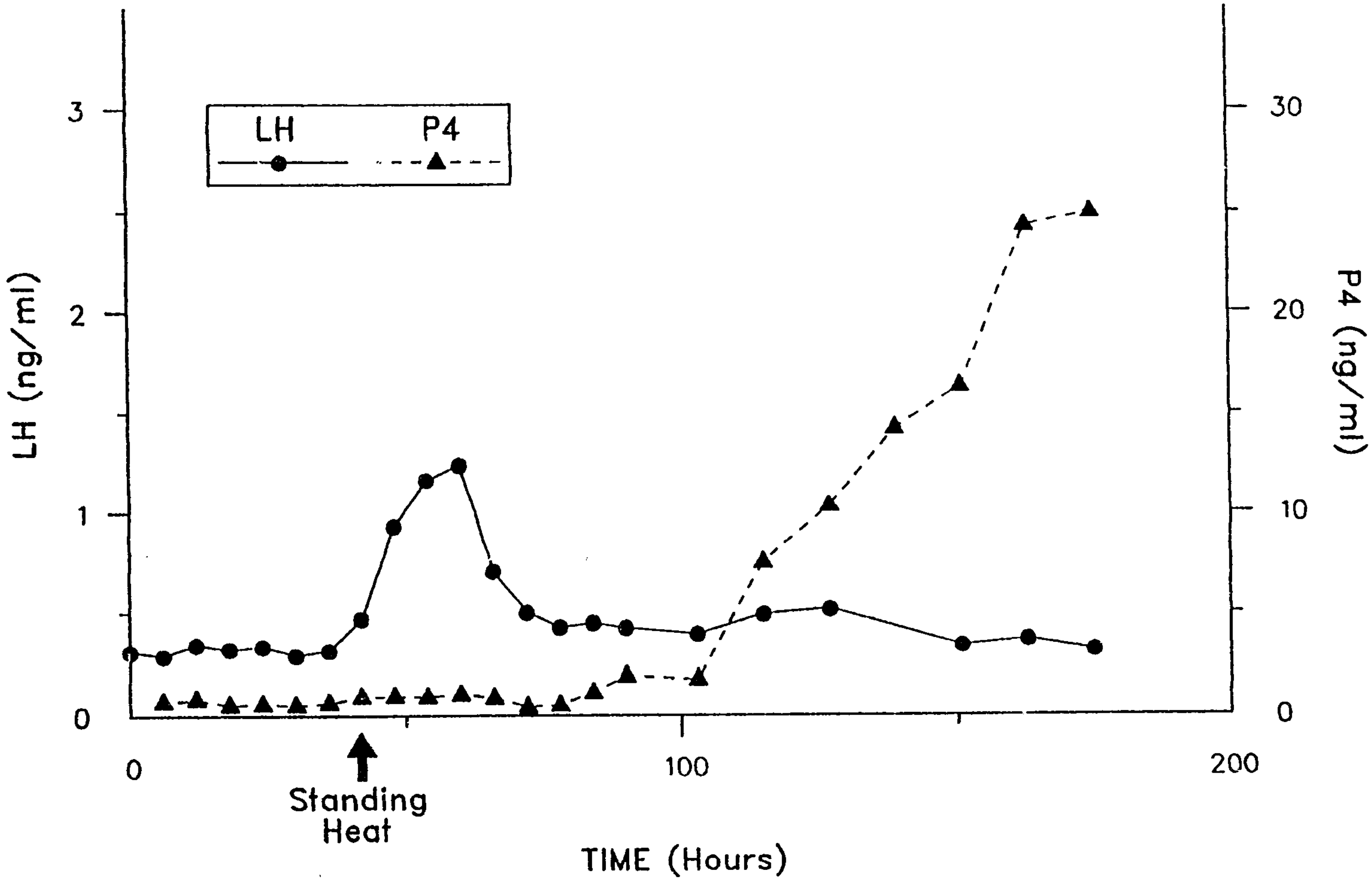


Figure 3.6. Plasma progesterone levels (ng/ml) at 50 hours after standing heat in (a) estrus one and two and (b) in parity 1 and 2 and 3 or more in sows at estrus 1 and 2.

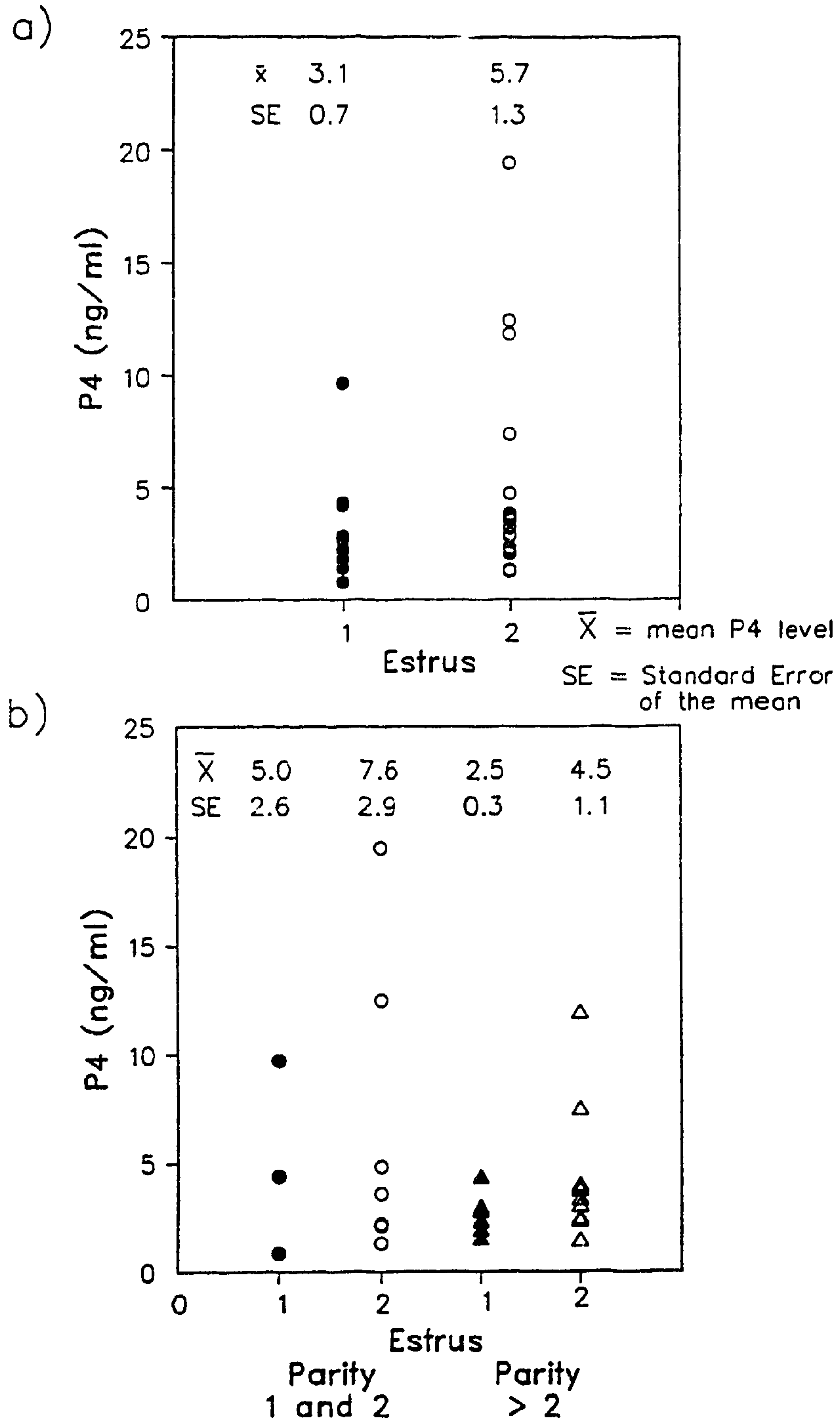


Figure 3.7. Plasma progesterone (P4) (ng/ml) 50 hours after LH peak in a) estrus one and two and b) in parity 1 and 2 and 3 or more at first and second estrus.

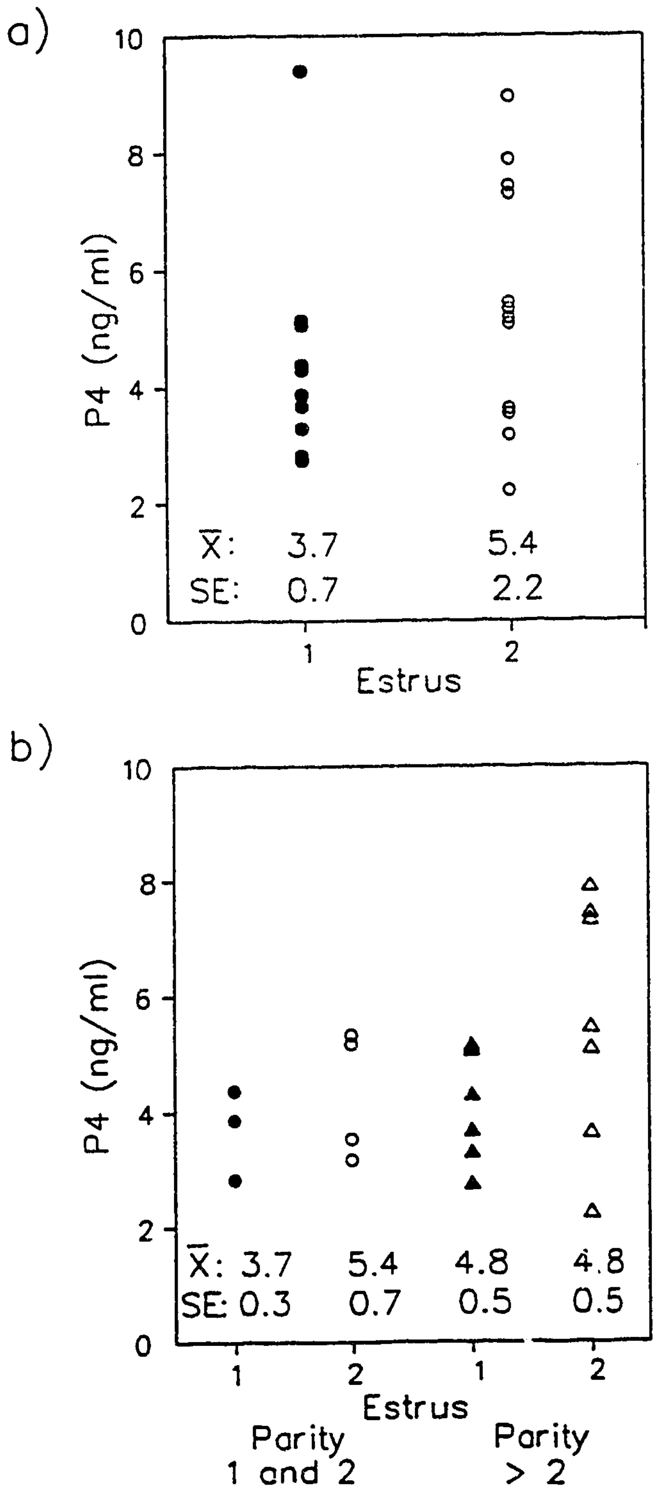
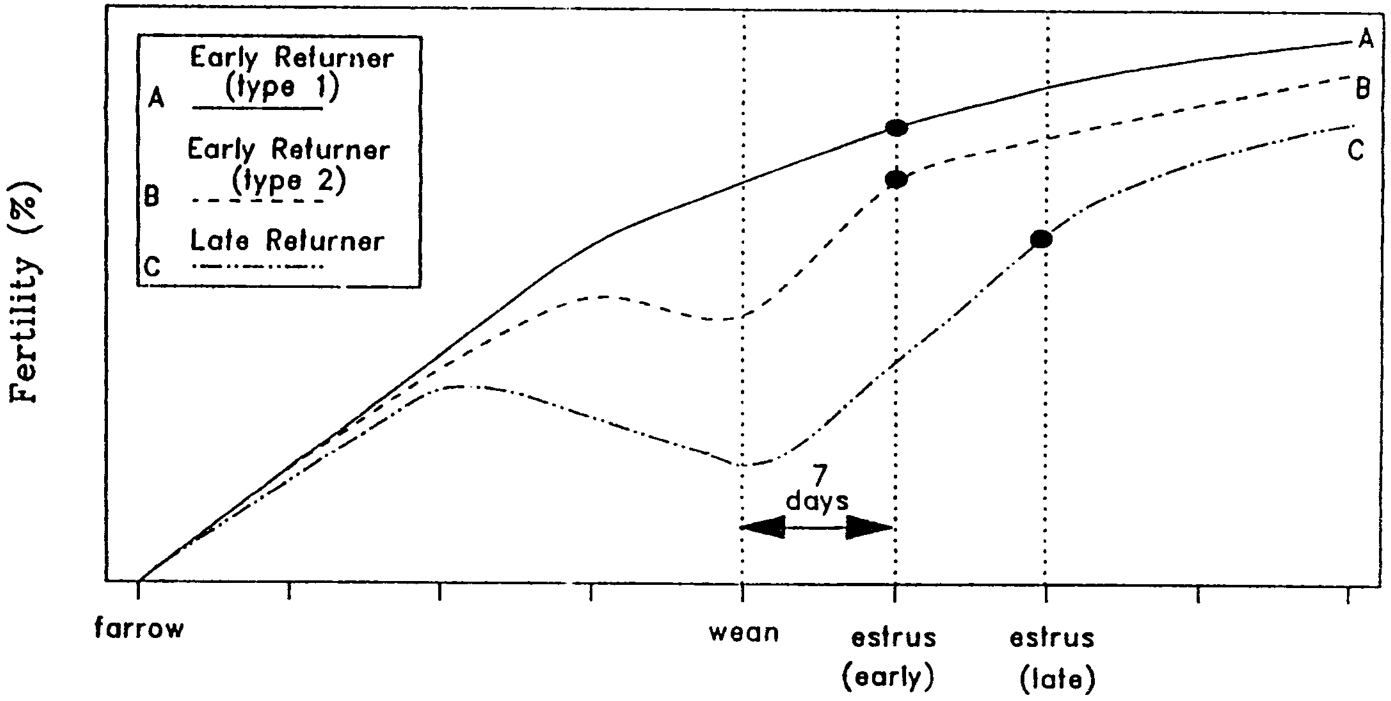


Figure 3.8. Effect of metabolic state on sow fertility between farrowing and subsequent estrus



Early and late returners: sows that return to estrus early and late after weaning: fertility demonstrated in relation to metabolic status.

CHAPTER 4

EXPERIMENT 2: EFFECT OF BREEDING SOWS AT EITHER FIRST OR SECOND ESTRUS AFTER WEANING ON FERTILITY.

4.1 INTRODUCTION

It has been suggested that sows that return to estrus in 7 to 12 days after weaning produce smaller subsequent litters and fewer pigs/sow/year, than sows that return to estrus in less than seven days (Leman, 1987; figure 4.1). Breeding sows that return to estrus late after weaning at their second estrus (skip-a-heat breeding) could overcome their poor fertility (Moody *et al.*, 1969; Pay, 1973; Love, 1979; Fahmy *et al.*, 1979; King *et al.*, 1984; Clark and Leman, 1987; Clark *et al.*, 1988; Morrow *et al.*, 1989; Conner, 1992).

Inadequate nutrition during lactation has been linked to poor fertility after weaning as indicated by 1) lower mean plasma LH levels before and after weaning (King and Martin, 1989; Mullan and Close, 1989, Mullan *et al.*, 1991), 2) extended weaning-to-estrus intervals (Fahmy, 1981; King, 1987) and 3) reduced embryo survival rates (Hughes *et al.*, 1984; Baidoo, 1989; Kirkwood *et al.*, 1987ab, 1990) leading to 4) a smaller subsequent litter size born (Kirkwood *et al.*, 1988a). The increase in litter size seen when breeding was delayed, is probably a result of improved nutritional status.

The data of Leman (1987) was confounded by many factors including parity and management practices, because it was compiled from a number of different farms under both 'good' and 'bad' management practices. Thus, the objective of this experiment was to (1) establish whether sows that return to estrus in greater than six days after weaning are less fertile and produce smaller litters than sows that return to estrus within six days after weaning, and (2) to determine whether the fertility of sows with prolonged weaning-to-estrus intervals can be increased by breeding at the second post-weaning estrus rather than the first.

4.2 MATERIALS AND METHODS

4.2.1 Animals and Treatments

Eighty-one first and/or second parity (Lacombe or Yorkshire) sows, at the Lacombe Federal Research Station, were fed conventional lactation and gestation diets of 16% and 14% crude protein, respectively (table 4.1). During gestation, the sows were housed outdoors and fed *ad libitum* every second day. On day 109 of gestation, sows were brought into the barn and placed into individual farrowing crates. Upon farrowing, the sows were fed up to 8 kg of the lactation diet daily (table 4.1), in two meals. Sows raised their own litters, unless litters were greater than 13 piglets in which case cross-fostering was practised. Sows were weaned at 28±3 days post-partum and were selected at random to be bred at either first (group 1) or second estrus (group 2) and then moved into the breeding area where they were penned in groups of 10. They were then fed 2 kg/sow per day of the gestation diet (table 4.1) on a per pen basis.

Group 1 sows were heat checked daily by running a boar in with the group, and they were then bred at least twice to the same boar at their first estrus after weaning. These sows remained in their pens for a further 21 days after breeding and were pregnancy checked at the time of predicted second estrus. When confirmed pregnant the sows were moved outdoors, and fed *ad libitum* every second day.

Group 2 sows were mixed in the same pen as group 1 sows, in their weaning groups, and were heat checked daily by back pressure to detect first estrus. Second estrus was detected by running a boar in with the group, and the sows were bred in the same way as group 1 sows, at least twice to the same boar. The sows remained in their pens until the time of their predicted third estrus, when they were again heat checked by back pressure, to confirm pregnancy. Then, as in group 1 sows, they were moved outdoors and penned in groups and fed *ad libitum* every second day.

Sows were weighed on day 109 of gestation and at weaning. Piglets were weighed at birth, three weeks of age and at weaning. The day of farrowing and weaning, the weaning-to-estrus interval, the first estrus cycle length and the subsequent litter size were recorded.

4.2.2. Statistical Analysis

Statistical analyses were performed using the General Linear Models (GLM) procedures of SAS (1990), including parity and estrus of breeding as the class variable. Associations between variables were explored using Pearson's correlations (SAS, 1990).

4.3 RESULTS

A decrease in both total litter size and pigs born alive was seen in sows bred at second rather than first estrus after weaning (10.2 vs. 11.6 pigs ($P<0.08$) and 9.5 vs. 10.6 pigs ($P<0.08$), respectively) (table 4.2). Furthermore, sows bred at second rather than first estrus had significantly longer weaning to first estrus intervals (7.1 vs. 5.4 days ($P<0.02$), respectively).

No difference between first and second parity sows was seen between total and alive litter size born, lactation length or weaning-to-estrus interval (table 4.3). The weaning to first estrus interval tended to be longer in sows bred at the second rather than the first estrus after weaning, in sows of both parity 1 and 2 (7.3 vs. 5.6 days ($P<0.06$) and 7.0 vs. 5.2 days ($P<0.08$), respectively). The total litter size born and born alive in the second estrus bred sows tended to decrease in both parity 1 (9.8 vs. 10.9 pigs ($P<0.28$) and 9.4 vs. 10.1 ($P<0.43$), respectively) and parity 2 sows (10.2 vs. 12.4 pigs ($P<0.17$) and 8.8 vs. 11.1 pigs ($P<0.12$), respectively) (table 4.4).

Furthermore, sows bred after seven days tended to have a smaller litter size compared to sows bred before seven days, when bred at first estrus (10.8 ± 0.83 vs. 11.8 ± 0.53, respectively). Litter size was not increased by delaying breeding in late returning (10.4 ± 0.9 vs. 11.8 ± 0.5 sows, respectively) or early returning sows (10.0 ± 0.6

vs. 10.8± 0.83 sows).

Correlative analysis revealed that the length of the weaning-to-estrus interval was negatively correlated with total and alive litter size born in parity 2 sows at both first (R=-0.29 (0.12) and R=-0.21 (P<0.27), respectively) and second estrus after weaning (R=-0.68 (P<0.21) and R=-0.33 (P<0.59), respectively), but not with parity 1 sows. The length of the weaning-to-estrus interval was also negatively correlated with lactation length in parity 1 and 2 sows bred at first (R=-0.23 (P<0.23) and R=-0.36 (P<0.17)) and second estrus after weaning (R=-0.57 (P<0.001) and R=-0.89 (P<0.11), respectively). In first parity sows bred at first estrus after weaning only, the length of lactation was correlated with total and alive litter size born (R=0.42 (P<0.02) and R=-0.45 (P<0.01), respectively).

4.4 DISCUSSION

A decrease in litter size (both total born and born alive) was seen in sows bred at the second rather than first estrus after weaning. However, the weaning the estrus interval was longer in second compared to first estrus bred sows, and in both parity 1 (7.3 vs. 5.6 sows) and parity 2 sows (7.0 vs. 5.2 days). These results were the reverse of those observed in experiment 1. However, sow management in this experiment was different to that in experiment 1, and may have influenced the results.

The sows in this experiment were housed outdoors, except between farrowing and breeding, and sows were only individually fed during lactation. Thus, between weaning and breeding and during gestation, sows were fed on a pen basis. Feed intake in the post-weaning period and during early gestation can have a significant effect on embryo survival (Pharazyn, 1992). High feed intake between weaning and breeding has been shown to increase conception rates (Brooks and Cole, 1972) and subsequent ovulation rates (King *et al.*, 1984) in primiparous sows. These feed effects, however, only take effect if the period of high feeding is at least five days (Fahmy and Dufour, 1976), which was the case for many of the sows in this experiment. Furthermore, embryo survival was reduced in gilts fed a high level of nutrition from time of breeding or the day after breeding, into early gestation (Dyck *et al.*, 1980; Pharazyn, 1992). High levels of nutrition in early gestation are associated with low peripheral progesterone levels and decreased embryo survival rates in sheep (Parr *et al.*, 1987) and gilts (Dyck *et al.*, 1980). Symonds and Prime (1988) proposed that the high feed intake increases the liver blood flow rate, and thereby increased the clearance rate of circulating progesterone.

The nutrition of sows during the critical period after weaning was not controlled in this experiment. There was competition within the pen groups for food and feeder space. In the weaning-to-breeding period for sows bred at first and second estrus, there was similar competition for food, thus no effect of feeding on ovulation rate would have occurred. However, those sows selected to be bred at second estrus had a longer weaning-to-estrus interval than sows bred at first estrus (7.1 vs. 5.4 days $P<0.02$), respectively). Therefore, from just before the detection of their second estrus, the numbers of animals in a pen would have been reduced to at most five sows, because by this time the sow [illegible] at first estrus would have been moved outside. Thus, competition between sows for food and feeder space in the pens may have been lower. Thus sows bred at second estrus may very well have eaten more feed in very early gestation than sows bred at first estrus. Pharazyn (1992) observed that increasing feed intake from 1.8 to 2.5 kg/day from day one after mating in gilts, decreased the embryo survival rates 20 % (87.7 vs. 70 %, respectively). Thus, the reduced litter size in those sows bred at second rather than first estrus after weaning may be a result of reduced embryo survival, even though ovulation rate may have been increased in these sows, because of recovery of the sows metabolic state after weaning.

Fewer sows were also selected to be bred at second estrus compared to first estrus after weaning in parity 1 and 2 sows (n=17 vs 31 sows and 5 vs. 30 sows, respectively). Therefore, finding significant differences between sows bred at first or second estrus may have been difficult.

Unlike experiment 1, sows that returned to estrus in seven or more days after weaning rather than before seven days, tended to have a decreased litter size (10.8 vs. 11.8 pigs, respectively). This is in agreement with the work of Leman (1987), which suggests that sows that show a delayed return to estrus after weaning are less fertile. The correlative data further confirms Leman's hypothesis, because parity 2 sows with longer weaning-to-estrus intervals tended to have smaller subsequent litter sizes at both first and second estrus.

4.5. CONCLUSION

A reduction in litter size in sows bred at second rather than first estrus was seen in this experiment. This reduction in litter size due to delayed breeding is opposite to the result observed in experiment 1. However, the two experiments were run under very different management systems. Furthermore, as in experiment 1, problems of non-detection of second estrus were encountered. Therefore the practice of skip-a-heat breeding to improve herd fertility should be carefully evaluated, dependant on the management of the herd, before being put into practice.

Table 4.1 Composition of diets in experiment Two

Ingredients	Diet (kg/tonne) Dry Sow	Lactating Sow
Wheat (13.5% CP[1])	200	250
Barley (11.5% CP)	632	500
Tallow	1	25
Soybean Meal (46.5% CP)	45	105
Alfalfa meal (17.5% CP)	50	50
Canola meal (36.0% CP)	30	30
I Salt	5	5
Ca Phosphate	22	19
Limestone	12	13
Min-Vit mix[2]	2.5	2.5
Methionine (98%)	0.1	0.05
Pelletaid	0.5	0.5
Analysis (%)		
Crude Protein (CP)	14	16
Lysine	0.62	0.75
Methionine + Cystine	0.54	0.56
Calcium	1.00	1.00
Phosphorus	0.85	0.80
Salt	0.50	0.50
Energy[3] (Mcal ME.kg^{1})	2.7	3.0

1 CP = Crude Protein

[2] Supplied the following per kg of diet: 4000 IU vitamin A, 600 IU vitamin D_3, 25 IU vitamin E, 27 μg vitamin B_{12}, 4.4 mg riboflavin, 28 mg pantothenic acid, 82 mg niacin, 0.35 mg biotin, 1.6 g choline, 170 mg zinc, 64 mg manganese, 225 mg iron, 35 mg copper, 0.14 mg iodine, 0.15 mg selenium.

[3] Metabolisable energy of the diet.

Table 4.2 **Effect of breeding at first or second estrus after weaning on subsequent litter size.**

Estrus of breeding		1	2	SEM[1]
N[2]		48	35	
Litter size:	Total born	11.6[3]	10.2	0.50
	Alive born	10.6	9.5	0.46
Lactation length (days)		27.7	26.6	0.29
Wean-estrus (days)		5.4[a]	7.1[b]	0.39

[1] Standard error of the mean
[2] Number of animals in each parity
[3] Least-square means
[a-b] Means within a row lacking a common superscript differ ($P<0.05$)

Table 4.3 **Effect of parity of the sow on subsequent litter size.**

Parity		1	2	SEM[1]
N[2]		48	35	
Litter size:	Total born	10.3[3]	11.6	0.50
	Alive born	9.7	10.4	0.46
Lactation length (days)		26.9	27.4	0.29
Wean-estrus (days)		6.4	6.1	0.39

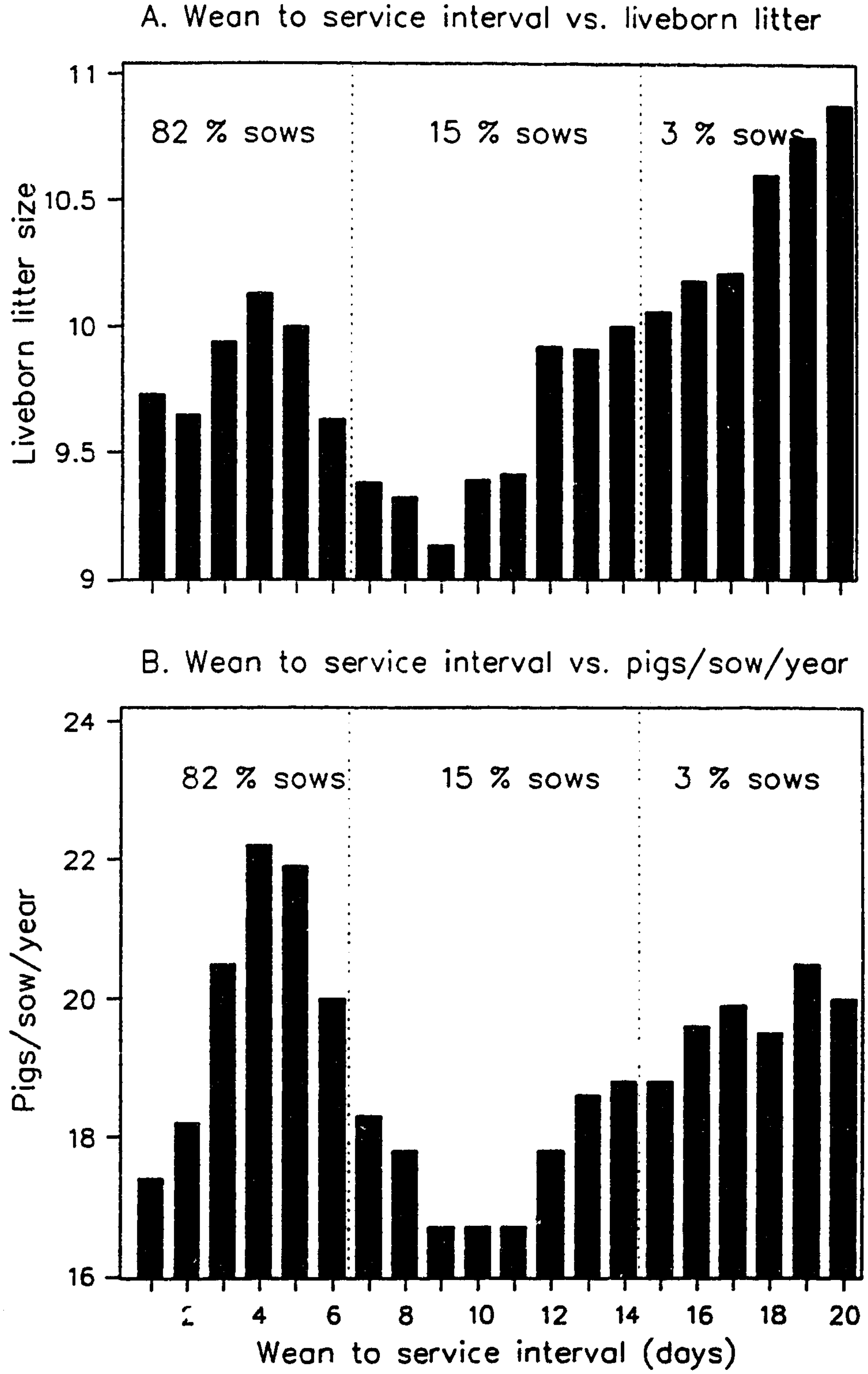
[1] Standard error of the mean
[2] Number of animals in each parity
[3] Least-square means

Table 4.4. Effect of parity of the sow and estrus bred on subsequent litter size.

Parity	1			2		
Estrus	**1**	**2**	**SEM[1]**	**1**	**2**	**SEM**
N[2]	31	17		30	5	
Litter size: Total born	10.9[3]	9.8	0.65	12.4	10.2	0.76
Alive born	10.1	9.4	0.59	11.1	8.8	0.72
Lactation length (days)	27.7[a]	26.0[b]	0.57	27.7	28.3	0.48
Wean-estrus (days)	5.6	7.3	0.48	5.2	7.0	0.54

[1] Standard error of the mean
[2] Number of animals in each treatment
[3] Least-square means
[a-b] Means within a row lacking a common superscript differ ($P<0.05$)

Figure 4.1. Wean to service interval as a predictor of sow fertility



n = 82000 matings
(Leman, 1987)

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

It has been well established that a strong relationship exists between nutrition and reproduction (Aherne and Kirkwood, 1985; King, 1987; Cole, 1990) and in order to achieve efficient reproduction in a swine herd this relationship must be optimised. The majority of work on nutrition-reproduction interactions in sows has been concentrated in the lactational period and not between weaning and breeding. However, selection and recruitment of the ovarian follicle occurs 4 to 5 days before ovulation i.e in the weaning and breeding interval. Therefore, this period could be very important in helping to determine the number of follicles that ovulate, and thus the subsequent litter size born.

It is well known that sows fed inadequate nutrient levels during lactation frequently exhibit an extended weaning-to-estrus interval (Baidoo, 1989) often associated with large body weight and fat losses (Hardy and Lodge, 1969; Reese *et al.*, 1982, 1984; King and Williams, 1984a) and reduced conception rates (Hardy and Lodge, 1969; Hughes *et al.*, 1984). This appeared to link large fat, and to a lesser extent, body weight loss during lactation to poor fertility, and led to the hypothesis of a 'threshold' level of body fat in the sow below which the possibility of reproductive failure increased (Elsley *et al.*, 1968; Maclean, 1969). Even now, Whittemore and Yang (1989) maintain that 10mm of backfat at the P2 site is required to prevent breeding problems. This idea is consistent with the "fat" hypothesis in women which suggested that a critical ratio of fat to lean mass was essential for the attainment of puberty and maintenance of reproductive function (Frisch, 1984). However, even though a "fat" hypothesis was well accepted by many pig researchers of the time, it has also been refuted (Lee and Mitchell, 1989) and fatness *per se* does not appear to be the cause of poor fertility, although in some cases the amount of body fat may correlate well.

The search therefore continued for nutritional factor(s) which affect the reproductive axis, and conclusions from many experiments suggested that inadequate protein and/or energy intake were important in eliciting components of the nutritional effects on the reproductive axis, but were not the direct cause of these effects (Reese *et al.*, 1982; Whittemore *et al.*, 1984, 1989; Mullan, 1987, 1991; King and Williams, 1984b; King and Dunkin, 1986b; King and Martin, 1989). So another candidate for the main nutritional factor modulating fertility in the sow emerged, the animal's metabolic status, and especially energy balance.

Much evidence has arisen to support the arguments that a consideration of the animals metabolic status is more universally applicable than say the threshold fat theory to the development of a hypothesis on how nutritional factor(s) affect the reproductive axis. Bronson and Manning (1991) present state arguments for and against both sides and argue convincingly for the animal's energy balance as a key link between nutrition and reproduction. This argument can also be used in swine, for example given inadequate energy and/or protein intake, as is often the case during lactation, a sow moves towards a more negative energy balance and correlatively may use her own body fat and protein reserves to sustain lactational and maintenance requirements. Thus, even though the sow may lose body fat during lactation, this is not the cause for the change in fertility. Rather, complex biochemical modulations at the cellular level change the metabolic status and energy balance of the animal and affect the CNS-hypothalamo-pituitary-ovarian axis, which may change sow fertility (for review see I'Anson *et al.*, 1991).

A few key factors central to energy balance have emerged as possibilities for the main regulation of reproduction and include insulin, IGF-1 and measures of fat metabolism i.e glycerol. Peripheral levels of these metabolic hormones and metabolites vary during lactation and between weaning and breeding and potentially effect the differences in fertility seen in sows (figure 5.1). However, other factors that affect the reproductive axis have also been identified. These include transforming growth factors (TGF's), epidermal growth factors (EGF), fibroblast growth factors (FGF) and the ratio of the large neutral amino acids, tryptophan and tyrosine, which are precursors for serotonin and the catecholamines, respectively (I'Anson *et al.*, 1991; Booth, 1990ab). Thus these other factors should be taken into account when designing future studies to elucidate the metabolic signals regulating reproductive function.

To begin to comprehend how these nutritional effects may work, it is essential to understand how the metabolic status of a sow varies between farrowing and breeding. This I have attempted to do in the hypothetical model based on experimental work in this thesis and earlier work described in this thesis (figure 5.1).

Figure 5.1. Diagrammatic representation of the metabolic and endocrine status of a sow at parities 1 and 3 during lactation and until first estrus after weaning, based on data from this thesis.

	Parity 1			Parity 3		
	Lactation	Wean	Estrus	Lactation	Wean	Estrus
Protein	↓↓↓	↑↑↑	↑	↓↓	↑	—
Fat	↓↓↓↓	↓(↓)	↓	↓↓↓	↑↑	-
GH	↑	↓	-	↑	↓	-
Cortisol	↑	↓	-	↑	↓	-
Glycerol	↑	-	↓	↑	↑	↓
Insulin	↓	↑	↑	↓	↑	-
IGF-1	↓	↑	↓	↓	↑	-
Glucose	↓	↑	↑	↓	↑	-

The upward facing arrow (↑) represents the animal depositing body tissue reserves (protein and fat in this diagram) and the downwards facing arrow (↓) represents the animal using up body tissue reserves for lactation, maintenance and growth requirements. For the metabolic hormones and factors, (↑) represents an increase in the peripheral levels, the (↓) represents a decrease in peripheral levels and (-) represents no change in peripheral levels comparing gestational plasma metabolic hormone and metabolite concentrations to concentrations in lactation, lactational concentrations to concentrations at weaning, and weaning concentrations to concentrations at first estrus.

This model is based on experimental work in this thesis and earlier research work described in this thesis.

Often the sow's voluntary feed intake during lactation is inadequate to meet the demands of lactation and maintenance (O'Grady *et al.*, 1985). In order to compensate for these inadequacies, the sow must break down her own body tissue reserves to meet these demands. In turn she moves into a more negative energy balance with concomitant changes in her metabolic hormones and metabolites (figure 5.1).

Younger sows (parities 1 and 2) tend to become catabolic during lactation sooner than older sows (parity 3 or more), because the younger sows are still growing. The young sows, even in late pregnancy and, certainly during lactation, progressively move towards a more negative energy balance. The negative energy balance is associated with decreased peripheral plasma insulin, IGF-1 and glucose concentrations and increased peripheral glycerol, cortisol and growth hormone (figure 5.1). Furthermore, either the increase in the sow's lean body mass (growth) stops or the sow may even break down existing body protein, as well as fat reserves, to maintain the nutrient demands during lactation. After weaning, the sow's metabolic demands are reduced and the animal actively partitions nutrients and energy into renewed growth, preferentially laying down protein at the expense of fat, until she has achieved the lean body mass associated with her particular age.

The rapid catch-up growth that occurs in the first parity sows after weaning, should be associated with appropriate changes in the sow's endocrine system and the hormones encouraging protein accretion increase at the expense of those for lipogenesis. Thus plasma IGF-1 levels increase because they aid protein accretion in association with a lowering of peripheral insulin levels at the first estrus after weaning. At the same time, glycerol levels remain high because fat reserves are still being broken down to aid protein accretion (figure 5.1). At the time of second estrus catch-up growth has occurred and the drive towards protein accretion drops to the level seen prior to farrowing and the sow reaches a stable metabolic or predictably an anabolic state.

During lactation under most practical systems of nutritional management, older sows also break down their body tissue reserves to meet the metabolic requirements of lactation and maintenance and move towards a negative energy balance with concomitant changes in the peripheral metabolic hormones, as described for the younger sows above. However, upon weaning, these sows recover more quickly from the lactational nutrient drain because they are either no longer growing, or are growing at a much slower rate than younger sows. Therefore these sows do not have as strong a drive for protein accretion after weaning as younger sows so they do not have to undergo catch-up growth. The rate of lipolysis, which is still high at weaning, as indicated by high plasma glycerol levels, declines rapidly after weaning and may even switch to lipogenesis by first estrus as the older sow quickly recovers her energy balance and metabolic status.

In turn these differences in metabolic status would explain the difference in response of younger and older sows to delayed breeding. Younger sows show an increase in subsequent litter size if bred at the second rather than the first estrus after weaning because at first estrus they have not recovered their metabolic status and have not achieved a positive energy balance. In contrast, delaying breeding older sows has no effect on litter size because by first estrus these sows have already recovered their metabolic status and moved into a more positive energy balance.

The increases in subsequent litter size seen in younger sows bred at second rather than first estrus may partially be caused by an increase in embryo survival. Higher and more variable progesterone levels just after ovulation have been associated with increased embryo survival in gilts (Pharazyn *et al.*, 1991a). Similarly lower progesterone levels in the peri-ovulatory period have been associated with high perinatal loss in prolific breeds of sheep (Ashworth *et al.*, 1989). The younger sows bred at second rather than first estrus after weaning had higher and more variable plasma progesterone levels 10 to 20 hours after ovulation (5.4 vs. 3.7 ng P_4/ml). This suggests that a progesterone related decrease in embryo survival could have been involved in the observed reduction in litter size of sows bred at first rather than second estrus. In the older sows no difference in progesterone levels was seen between first and second estrus (4.8 vs. 4.8 ng P_4/ml), and presumably there would also be no difference in embryo survival in older sows bred at first or second estrus.

After measuring the different metabolic hormones and metabolites the question still remains to be asked, how useful are peripheral measures of the different factors. Insulin-like growth factor-1 is one of the proposed metabolic factors affecting the nutrition-reproductive axis. However, peripheral IGF-1 concentrations may not be the ultimate determinants of ovarian function. At the ovarian level, IGF-1 potentiates the action of FSH on the differentiation of granulosa cells and IGF-1 also potentiates the proliferative effects of EGF on granulosa cells (May *et al.*, 1988). Further, many other tissues synthesise IGF-1, including the ovary (Hammond *et al.*, 1985; Charlton, 1992).

Furthermore IGF binding proteins (IGFBPs) regulate the bioactivity of the IGF's. These binding proteins are differentially regulated by metabolic signals (McClusker *et al*, 1991; Powell *et al.*, 1991), their tissue uptake varies (Bar *et al.*, 1990a) and the number and type of IGFBP in the ovarian follicle varies with maturation. Also, granulosa cells can synthesise and secrete IGFBPs suggesting paracrine and autocrine actions of IGFBP, together with IGF-1, on the ovary (Jalkanen *et al.*, 1989; Mondoschein *et al.*, 1990). Thus, direct actions of IGF-1 at specific areas of the reproductive axis such as the ovary cannot be assessed by peripheral measures. However, the metabolic status and energy balance of the sow can be assessed by measuring peripheral metabolites and metabolic hormones and this is essential for a complete understanding of nutrition-reproduction interaction. Even so, direct evidence for the factors causing these interactions and effects cannot be completely uncovered at the peripheral level.

Studies following up from this series of experiments should use first and maybe second parity sows only as the experimental model, because the nutrition-reproduction interactions are more easily measured in such sows, because of their greater nutrient demand. A more detailed blood sampling schedule should be put into place, with a blood sampling window prior to weaning and more blood sampling windows between weaning and breeding. Protein turnover should be estimated both during and after lactation to give a fuller picture of the depletion of body protein reserves. Better measures of body fat changes during and after lactation should also be made, possibly by measuring total body water, thus allowing a better estimate of true body weight change after weaning. The sows could be challenged with glucose infusions to obtain a measure of energy/insulin status, as an alternative to measuring responses to feed intake. This approach would overcome the feed intake problems found in the experimental work for this thesis. Also ovulation rate and embryonic survival should be measured.

Future research could use the very fat, highly fertile, precociously pubertal and possibly early maturing breeds of Chinese pig to study the effect of metabolic status on fertility (Foxcroft *et al.*, 1992). These sows produce very large litter sizes which far surpass those of the European breeds, due to higher embryonic survival rates at higher ovulation rates.

As the Chinese pig appears to increase litter size by increasing embryonic survival rates in association with increased ovulation rates, these animals could be used as a model to study effects of nutrition on reproduction to be related to European breeds, which also increase litter size by increasing embryonic survival. In such a study, the metabolic status and energy balance of the first parity Chinese pig would be challenged during lactation by reducing the lactational feed intake of these sows. Then, in comparison with a similar study in first parity European breed sows, the recovery from lactation would be monitored. This would demonstrate if first parity Chinese pigs are more mature than first parity European breed pigs. In this way, physiological and metabolic factors in the Chinese pig that effect fertility, could be isolated and related to affects seen in European breeds of pig.

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