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THE EFFECT OF ENERGY INTAKE PRIOR TO
OESTRUS AND SUBSEQUENT TO MATING
ON OVULATION RATE AND LITTER
SIZE AT BIRTH IN GILTS

A thesis presented in partial fulfilment of the requirements
for the degree of Master in Animal Science at
Massey University

LE-NGOC CHI.-MINH

1973

A B S T R A C T



The objective of this investigation was to examine the effect of flushing for about eleven days prior to service and plane of nutrition during the first month of pregnancy on the reproductive performance of gilts. Additional observations were also made on plasma progesterone levels during early and late pregnancy and body temperature changes during the oestrous cycle and late pregnancy in gilts.

A flushing ration of 3.6 kg per day representing 10.80 Mcal of digestible energy, given to gilts at about 5th oestrus (270 days old and 115 kg body weight) did not make any significant difference in ovulation rate or litter size at birth from the control gilts under similar physiological conditions, receiving 1.8 kg of the same feed per day. It was thought that the gilts were mated late in their reproductive life and their body conditions might partly explain the discrepancy between the present study and others.

In contrast, high plane of nutrition during the first month of pregnancy significantly increased litter size and weight at birth. Average birth weight of pigs, however, was unaffected. No interaction between pre- and post-mating feeding levels could be found on any of these three characteristics.

Plasma progesterone levels increased significantly from Day 3 to Day 15 and declined gradually from Day 100 through Day 110 to 112 of pregnancy. The effect of feeding treatments (pre- and post-mating) was not significant except at stage 5 (Day 110 of pregnancy). Mummified foetuses were significantly correlated with progesterone levels at the last two stages (Day 110 and 112 of pregnancy).

Rectal temperatures of gilts increased significantly from Day 2 to Day 6 then tailed off until Day 10 of the oestrous cycle. (Day 0: day of oestrus). During late pregnancy, rectal temperatures dropped significantly from Day 6 to Day 3 prior to farrowing day. However, due to big daily variations and the unreliability of the measurement, temperature changes were not suggested as a diagnosis method of ovulation and parturition.

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I N T R O D U C T I O N



Pigs multiply more rapidly, mature more quickly and produce more digestible flesh than other domestic animals (Bostock, 1957). Furthermore, pigs can be successfully bred and reared on smaller areas than other domestic animals. However the pig, unlike the herbivora, cannot be produced and maintained on cheap roughages but subsists for the major part on expensive concentrates; production costs are high and the margin of profit is markedly variable. The profitability of pig production is largely governed by litter size at birth and the number of piglets successfully reared to marketable weight (Burger, 1952; Scofield and Penny, 1969).

Hammond (1921) indicated that fertility in mammals (in particular the pig) is largely controlled by: (1) The number of ova shed; (2) The number of ova fertilized; and (3) The number of embryos which develop normally until birth. Krallinger (1937) proposed a fourth factor, namely the regularity of the oestrous cycle. However, as fertilization percentage (the proportion of cleaved ova to the total number of ova shed) in gilts is very high (Hancock and Hovell, 1962) it may be concluded that for any particular reproductive cycle, fertility in the pig will be determined by ovulation rate and embryonic mortality (Cooper and Scofield, 1969). The heritability

of reproductive traits in the pig is low, and as a consequence, the influence of environment, and in particular nutrition, assumes considerable importance.

The purpose of this investigation was to examine the effect of "flushing" (a sudden increase in feed intake) prior to service and plane of nutrition during early pregnancy on ovulation rate and embryo survival of gilts. One experiment was conducted, but for convenience the thesis is presented in separate chapters under the following main headings:

1. Effect of flushing prior to service on ovulation rate and litter size at birth.
2. Effect of plane of nutrition over the first month of pregnancy on farrowing results.

Additional observations were also made on:

3. Plasma progesterone level during early and late pregnancy in gilts.
4. Body temperature of gilts during the oestrous cycle and late pregnancy.

CHAPTER I

EFFECT OF FLUSHING PRIOR TO SERVICE ON OVULATION RATE AND
LITTER SIZE AT BIRTH

I. Literature Review:

1. Premating flushing and ovulation rate:

Work on ewes (White and Robertson, 1922; Clark, 1934; McKenzie and Terrill, 1937; Howland et al., 1966; Memon et al., 1969) and on rats (Lodge and Cooper, 1967) showed significant increases in ovulation rate in high-fed animals as compared with low-fed ones. Similarly, much work on pigs revealed that an increase in plane of nutrition (Robertson et al., 1951 a,b; Christian and Nofziger, 1952; Self et al., 1955; Lazauskas, 1965; O'Bannon et al., 1966; Schultz et al., 1966; Holmes, 1969; Cooper et al., 1971; Brooks et al., 1972) or in energy intake (Gossett and Sorensen, 1959; Haines et al., 1959; Goode et al., 1960 a,b; Sorensen et al., 1961; Du Mesnil du Buisson et al., 1968 ...) for about three weeks or more resulted in an increase in ovulation rate. However, it is noted that prolonged high level of feeding may also accelerate the growth rate of gilts (Zimmerman et al., 1960 b; Lodge and MacPherson, 1961); and ovulation rate has been shown to be positively correlated with liveweight gain of gilts at oestrus (Squiers et al., 1952; Rathnasabapathy et al., 1956; Zimmerman et al.,

1960a). So, the increase in ovulation rate reported in the above studies may be a response to both increases in feed intake and live weight and/or liveweight gain of gilts at oestrus.

In fact, it has been shown that energy intake by itself can also influence ovulation rate without the secondary effect of live weight and liveweight gain of gilts; since flushing or fasting gilts in a short period of time (from a few days to about two weeks), over which, body weight changes are negligible, can also affect ovulation rate (Zimmerman, 1958, 1959; Zimmerman et al., 1958, 1960 a,b; McGillivray et al., 1962, 1963; Rigor et al., 1963; Kirkpatrick et al., 1967 a; Bazer et al., 1968). A similar trend was noted on ewes (Girou et al., 1971). Accordingly flushed ewes had a significantly higher ovulation rate than the unflushed ones of the same live weight.

In addition Zimmerman (1959) found that although continuously full-fed gilts shed a greater number of ova than continuously limited-fed ones, flushing for two or three weeks prior to breeding produced approximately the same ovulation rates as continuous full-feeding. More recently, Moore et al. (1971) were not able to detect any significant difference in ovulation rate between gilts flushed for fourteen days and those flushed for only one.

Evidence also shows that a few hours of flushing or fasting before ovulation (even after the onset of heat)

suffice to influence ovulation rate in gilts (Ray and McCarty, 1965; Lodge and Hardy, 1968; Bazer et al., 1971) as well as in sows (Heap et al., 1967; Hardy and Lodge, 1969 b). It should be noted that in these experiments flushing was imposed during the first twenty-four hours of heat, ie., before the onset of ovulation (Burger, 1952). In other words the possible time for such an effect of nutrition to be exerted may be limited to the interval between the onset of oestrus and ovulation (Heap and Lodge, 1966; Heap et al., 1967). The effect of short term flushing or fasting after the onset of heat on ovulation rate has also been reported on mice (McClure, 1958, 1959) and rats (Lodge and Cooper, 1967; Cooper et al., 1970).

These results were questioned by Kirkpatrick et al., 1967 b; Frobish, 1970; Brooks and Cole, 1971. The latter could not find significant differences between control gilts and those flushed once on the first day of oestrus. The discrepancy was thought to be due to the difference in time of ovulation between gilts in relation to the time when flushing was imposed. Investigation of the critical time for flushing to be effective (Brooks et al., 1972) showed that flushing for one day of mating, but not on the following day, significantly increased ovulation rate.

It has also been shown that plane of nutrition (or energy intake) does not have any significant effect on

ovulation rate of gilts (Marshall and Potts, 1921; Hart and Miller, 1937; Briggs et al., 1942; Gossett and Sorensen, 1956; Haines et al., 1959; Frobish and Gerrits, 1969; Cumming, 1972). Furthermore Tribble et al. (1956), Goode et al. (1960 a,b); Paquignon et al. (1972) found more corpora lutea in gilts or sows which had been subjected to some degree of feed or energy restriction than those which had not.

The discrepancies between studies remain unexplained since the mechanism through which nutrition affects ovulation rate (if it does) is not known. It has been suggested as involving the regulation of the rate of the release of gonadotrophin. (Samuel, 1948; Ershoff, 1952; Meites, 1953; Follis, 1958; Leathem, 1958, 1959; Allen and Lamming, 1961; Piacsek and Meites, 1967; Howland, 1971, 1972 a).

2. Premating flushing and litter size at birth.

Restricted feeding for four weeks before mating significantly reduces litter size at birth in rats (Lodge and Cooper, 1967). Moore et al. (1971) reported that flushing gilts for one or fourteen days increased live embryos per litter over controls. The increase resulting from the fourteen-day but not the one-day treatment was significant. A similar increase of feed, on the day of mating (Lodge and Hardy, 1968), or on the following day (Moody and Speer, 1971) was found to increase significantly

litter size at birth or the number of embryos at 25 days of gestation. Conversely starving gilts from ten days prior to service resulted in a reduced number of embryos (McGillivray et al., 1962).

In contrast to the above evidence, increasing feed intake of gilts for many days prior to service (Mayrose et al., 1966; Frobish and Gerrits, 1969) or for only one day, on either day one of oestrus (Brooks, 1970; Brooks and Cole, 1971; Brooks et al., 1972; Cooper et al., 1971; te Brake, 1971) or on day two of oestrus (Libal and Wahlstrom, 1970; Brooks et al., 1972) had no significant or consistent effect on either litter size at birth or on the number of embryos present at 20 - 28 days of gestation. This was confirmed by Naber and Zimmerman (1971) stating that gilts flushed for one day or two - three weeks had similar litter size as the control, unflushed.

One reason for the ineffectiveness of flushing on litter size may be that the material limitation of the gilt appears to be approximately eleven pigs, and that under most circumstances it is other material factors and not ovulation rate which limit litter size (Brooks and Cooper, 1972). In a situation where litter size is limited by ovulation rate, increasing feed intake may raise the ovulation rate and consequently the litter size. This theory may explain the significant differences in litter size found by Lodge and Cooper (1967); Lodge and Hardy (1968); Moody and Speer (1971)... . However it cannot

explain the ineffectiveness of flushing on litter size at birth in sows (Nielsen, 1968; Walker, 1972; Brooks and Cole, 1972).

II Methods and Materials:

1. Animals:

Twelve pairs of littermate gilts born from 6.2.71 to 18.3.71 at the Pig Research Centre, Massey University were selected at random from eight families of three breeding groups (Large White (LW) x Landrace (LR)) x LR; LW x LR and LW x Berkshire (B). Four littermate boars born on 19.2.71 of LW x LR breed were also selected. All animals were weaned at about three to four weeks of age when they were about 4.5 kg live weight.

2. Housing:

From about 46 kg body weight (at about 118 days of age) all gilts and boars were kept individually in pens in a totally enclosed building. The layout of this experimental building is illustrated in Figure 1. Attempts were made to maintain the temperature within the building at 18°C, however after the temperature regulator failed to work, it fluctuated considerably from 17°C - 23°C in November, 13°C - 24°C in December 1971 and January 1972, 13°C - 26°C in February 1972. No provision was made for the control of relative humidity.

3. Allocation of pigs to pens:

When the pigs were transferred to the experimental house (11.5.71) they were arranged so that littermates occupied opposite pens. The four boars initially occupied



Plate 1 - Experimental house.



Plate 2 - Inside the experimental house.

FIG.1 -Layout of the Housing Area Used in the Experiment

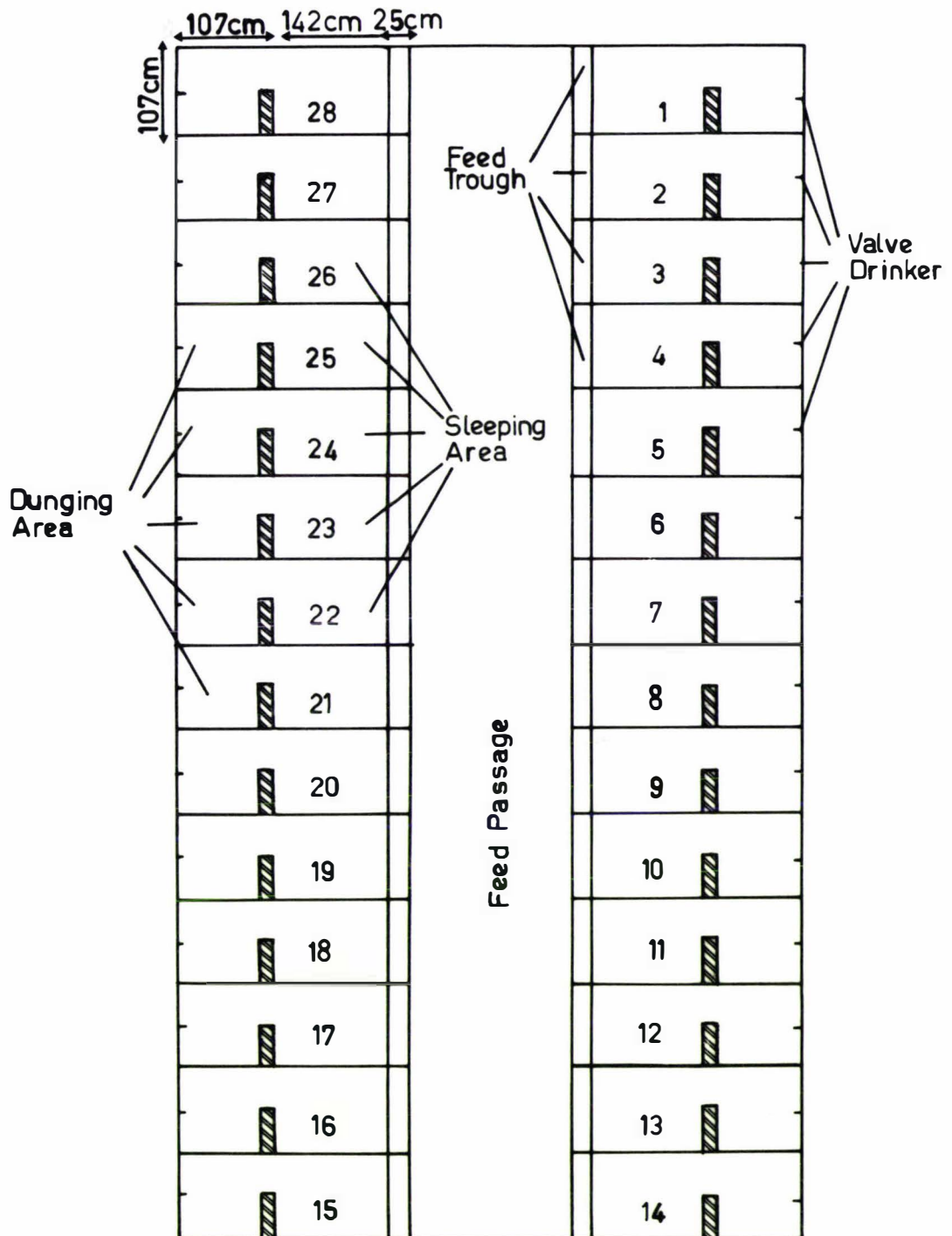




Plate 3 - Individual pen.

pens 4, 8, 21 and 25 (see Figure 1) and were rotated around these pens on a weekly basis. From 21.7.1971 they were moved to pens 1, 2, 27, 28. Later, from 10.8.1971 one boar was removed from the house each week on a rotational basis. Due to an increasing incidence of lameness, which was considered a function of individual penning, the gilts were grouped in batches of six from early November until farrowing and provided with straw bedding. Individual feeding was, however, continued.

4. Experimental Management:

From 22 kg body weight gilts were fed individually, once daily at about 08.00 hr. The gross compositions of the meal fed are shown in Appendix 1. Daily meal allowances were related to body weight (Appendix 3). Water was available to appetite.

5. Experimental Design and Treatment:

The experiment was arranged as a completely randomized block involving two treatments and twelve replicates. Animals were allocated to treatment on a within pair basis. The treatments were:

- (a) Control (unflushed - NF) : Maximum daily meal allowance of 1.8 kg until mating.
- (b) Flushed (F) - As for controls but daily meal allowance increased to 3.6 kg on Day 11 following the oestrus prior to mating.

6. Records:

Animals were weighed at fortnightly intervals, oestrus was checked once daily by observation of the signs of heat and with the aid of boars. A double mating system was used so that two litter sisters were mated by the same pair of boars.

Soon after mating backfat thickness (BFT) of gilts was measured ultrasonically. The measurements were taken at three paired locations (left and right) at about 4 - 5 cm from midline at the shoulder and loin (Rahnefelt, 1964), and at the midpoint of these two locations, ie., midback.

Laparotomies were conducted four to nine days after mating to observe the ovaries and record the number of recently formed corpora lutea assumed to represent recent ovulations. Litter size was determined as the number of fully formed fetuses born.

7. Statistical Analysis:

The number of ovulations and litter size at birth were compared by the analysis of variance in a two-way classification (Snedecor and Cochran, 1969).

Since so little information of litter size at birth was obtained, the results of all gilts were used in the analysis without consideration of the pairing of gilts. The aim of this section is to examine any effect of flushing prior to service on litter size at birth. On the other



Plate 4 - Recently formed corpora lutea of gilts
at laparotomy.

hand the post breeding treatment was imposed equally on two groups (see Chapter 2), so it may be ignored in the analysis for the purpose of this section. Litter size at birth was further adjusted for the difference in age of gilts at mating by an analysis of covariance (Snedecor and Cochran, 1969).

III Results:

1. General:

Of the twenty-four gilts, one control, although displaying signs of oestrus had apparently not ovulated at subsequent laparotomy. The animal had peritoneal abscesses (post-operative infection) and a second laparotomy was not attempted. Her sistermate had a prolonged oestrus thus she was flushed for twenty-two days. The ovulation rate was surprisingly high (26 ova). Data from both animals was eliminated from the study of ovulation rate.

Of the twenty-four gilts used for the study of litter size at birth, two did not show heat strongly and did not stand for service. Another one failed to conceive and a fourth one died at laparotomy. Of the twenty pregnant gilts, three aborted during the second and third month of pregnancy. The number of gilts providing data for the study of litter size at birth was, therefore, seventeen (six pairs and five individuals). Eight pregnant gilts had peritoneal abscesses for many weeks. During mid pregnancy most gilts were constipated for a few days to two - three weeks. They were off feed, some had a temperature, two had rectal prolapse. Chloramphenicol injection, epsom salts, then bran supplement to feed were given.... The cause of abortion was not determined, although it was once suspected to be leptospirosis (from the results of the blood test).

2. Ovulation rate:

Age, weight and oestrus number of each gilt at mating, length of flushing period, total energy consumed²¹ (by flushed gilts during flushing period and by their sistermate controls during the corresponding period), growth of gilts during the fortnight prior to mating, their ovulation rate, litter size at birth and numbers of mummified fetuses are shown in Appendix 4. The results are summarised in Table 1.

The results of the analysis (Appendix 5) showed that the average ovulation rate of gilts in the flushed group was slightly higher than that of gilts in the control group. However, the difference was not significant.

During the flushing period, most of the control gilts had been mated, consequently the average age, weight of the controls at mating were significantly ($P < .05$) lower than those of the flushed gilts. However neither age nor weight of gilts were significantly correlated with their ovulation rate (Appendix 6). No variables examined (Appendix 6) appeared to be correlated to ovulation rate.

²¹Total energy consumed by the gilts of each group was not the same due to the refusal of feed which became more apparent towards the onset of heat.

Table 1 - Effect of flushing prior to service on
ovulation rate and litter size at birth.

	TREATMENTS		SE of difference between means	Significance test
	NF	F		
Mean age (days) at mating	261.5	278.6	2.52	**
Mean weight (kg) at mating	107.8	123.6	2.49	*
Ave. oestrus number at mating	5.22	5.67	0.376	NS
Ave. length (days) of flushing period	0	11.3	—	—
Total energy consumed (Mcal DE) during flushing period	60.9	115.2	11.25	***
Liveweight gain (kg) during previous fortnight	4.99	7.03	1.279	NS
Weighted mean of BFT ^(a)	3.78	3.97	0.155	NS
Mean ovulation rate	13.9	14.4	0.767	NS
Litter size at birth	5.44	6.87	1.641	NS

*P < .05, **P < .01, ***P < .001

(a) see text

The ultrasonic measurements at three locations (shoulder, midback and loin), average backfat thickness $\left[\frac{1}{3} (\text{shoulder} + \text{midback} + \text{loin}) \right]$ and weighted mean of backfat thickness $\left[\frac{1}{4} (\text{shoulder} + 2 \text{ midback} + \text{loin}) \right]$ of twenty-two gilts are presented in Appendix 7. Since neither backfat measurements, nor ovulation rate were significantly different between the two groups (Table 1), the data were pooled for subsequent analysis. The results are summarized in Appendix 8.

3. Litter Size at Birth:

The results of litter size at birth are presented in Appendix 4 and summarized in Table 1. The results of the analysis (Appendix 5) showed that the average litter size of flushed gilts 6.87, was not significantly different from that of the control, 5.44. Since litter size at birth was slightly correlated to age of gilts at mating (the regression coefficient of litter size on age was -0.112 , $.05 < P < .10$), it was adjusted for the difference of age. The adjusted means of litter size at birth were 7.47 and 5.59 for flushed and control gilts respectively ($.10 < P < .25$). Weight of gilts at mating and their ovulation rate were not significantly correlated to litter size at birth ($r = 0.146$ and 0.010 respectively).

IV Discussion:

1. Ovulation Rate:

The ineffectiveness of flushing in the present experiment does not agree with many results reported in the literature, the exact reasons for this discrepancy are not known, however many factors may partly be responsible.

(a) Length of flushing period -

In work by Robertson et al. (1951 a,b), Self et al. (1955), Schultz et al. (1966), Du Mesnil du Buisson et al. (1968) ... gilts were flushed for a period of about three weeks or more, whereas, those in the present study were flushed for only about eleven days. Wise and Robertson (1953) suggested that the decrease in ovulation rate was correlated with the length of restriction in diet. Conversely there was a tendency for ovulation to increase with greater period of flushing (Zimmerman et al., 1957, 1960a; Zimmerman, 1959; Naber and Zimmerman, 1971; More et al., 1971). The same trend was noted in ewes (Lamond and Bindon, 1959). Kirkpatrick et al. (1967b), in work on gilts, suggested that although the pituitary responded to flushing rapidly follicle growth lagged slightly behind FSH release. In other words, it might take a while for the ovary to respond to the change in the level of FSH... Brooks et al. (1972), Brooks and Cooper (1972) noted that the

number of follicles reaching ovulatory size did not increase in response to short-term flushing. However in these experiments the number of corpora lutea did increase. Furthermore, in the present experiment no significant correlation existed between length of flushing and ovulation rate ($r = 0.04$) thus, the ineffectiveness of flushing on ovulation rate may not be explained on the basis of length of flushing period.

(b) Differences in energy intake between two groups -

Zimmerman et al. (1960a), Kirkpatrick et al. (1967a) reported that within the range of flushing allowance used, ovulation rate increased with the level of energy intake. Schultz et al. (1966) wrote that "to significantly improve ovulation rate, an energy intake of approximately 12.3 Mcal of metabolisable energy (ME) daily was required as compared to a daily energy intake of 6.1 Mcal ME in a three-week-flushing system ...". This confirmed the suggestion by Mayrose et al. (1966) stating that "to show a response in ovulation rate ... it appears that the flushing level must be increased more than 1.82 kg above the control levels of 1.82 or 2.27 kg per day used in these trials" (14-day-flushing system). In the current work flushed gilts previously fed on 1.8 kg feed/day, were given 3.6 kg/day (representing 6.2 Mcal digestible energy (DE) and 12.3 DE respectively, however most of them could not consume all their meals. For the

assessment of the effect of energy intake on ovulation rate, it was decided to examine the variation in ovulation rate of gilts in response to that in total energy intake during flushing period. The results show that energy intake was not significantly correlated with the number of ova shed by gilts.

(c) A possible effect of short-term nutrition

It is well established that the regulation of food intake is controlled by the central hypothalamus mechanism (see Bell, 1970). Two of the biochemical influences which can trigger the hypothalamus are:

- (i) The availability of glucose in body fluid (Liebelt and Perry, 1967)
- (ii) The concentration of fat metabolites in body fluid.

In the present experiment gilts were thought to be too fat by the time flushing was started (will be considered). This might partly explain the increasing refusal of feed from two - three days after being on flushing diet until mating.

On the other hand, cyclical food intake depressions coincident with oestrus have been observed in rats (Slomaker, 1925; Brobeck et al., 1947), heifers (Raun et al., 1967; Carltelin, 1968). The same trend was found in the present experiment.

As previously discussed, feeding from a few hours to one, two days prior to ovulation, suffices to

influence ovulation rate (Lodge and Hardy, 1968; Moore et al., 1971; Cooper et al., 1971; Baker et al., 1968 ...). The reduced feed intake during a few days in flushed gilts before oestrus may be partly responsible for the non-significance of the effect of flushing found in the present study.

(d) Body Condition:

α. - Indices of body fatness used in the analyses.

There appears to be no relevant data bearing directly on the relationship between ovulation rate and BFT of pigs. In the pig, BFT has been shown to be highly correlated with total fat (Manfredini and Palenzona, 1969; Werhahn, 1970; Azzarini et al., 1968). Some studies showed that the measurement at midback location gave the best estimate of the percent fat of the body (Anderson and Wahlstrom, 1969; Aulstad, 1969) whereas some others favoured the measurements taken at the loin (Isler and Swinger, 1968; Manfredini and Polenzona, 1969). Price et al. (1969), Filatov and Sukhorukov (1972) preferred to evaluate fatness and leanness of pigs on the basis of the average BFT. Since different values of correlation coefficients were obtained in different studies, many equations to estimate body fatness of pigs have been suggested. Of course, the indices of fatness obtained from these equations were at least subjected to

certain degrees of error. Therefore in the present work the analysis is based directly on the measurements of BFT instead of those indices. However these indices may be useful in the comparison of the fatness between pigs in this study.

3. Ovulation and BFT measurements.

The results showed that even though the correlation coefficients between backfat traits and ovulation rate did not reach significance, their negative values (except that of the correlation coefficient between shoulder fat thickness and ovulation) showed the trend that the fatter pig had a smaller number of ova shed. Hetzer and Miller (1970) found that there was a tendency for litter size to decrease with fatness of pigs. Accurate measurements of the BFT of pigs were difficult because:

- (i) BFT varies with the locations where the measurements were taken, and it is difficult to determine with accuracy these locations. These problems were faced in the present study when the measurements were taken at the shoulder and loin. Zobriský et al. (1959) reported similar difficulties with the shoulder measurement when using a ruler probe in live animals. Later on, Joblin (1962, 1966) reported that the shoulder measurement repeatability was low, and the poor corre-

lation of this measurement with carcass fatness was not unexpected.

- (ii) Claus (1957) reported that the velocity of ultrasonic waves through fatty tissues was dependent upon the composition and physical characteristics of the tissues. Therefore, variations in fat characteristics other than thickness may be a factor contributing to variations in readings. Thus Price et al. (1960) as well as most workers had to assume that the velocity of ultrasonic waves through fatty tissue was relatively uniform from one pig to another

γ. Fatness and reproductive disorder, ineffectiveness of flushing.

Much work has been done to estimate ultrasonically total or carcass fatness of pigs, so far no standard value of BFT of pigs have been suggested. It is difficult to judge the fatness of pigs. Moreover the values of BFT vary with age, weight and breeds of pigs (Hetzer and Peters, 1965). The gilts in the present study were thought to be too fat by their appearance. Although their daily allowance was lower than that of British recommendation (ARC, 1967), the gilts were kept individually with minimum movement during the pre-experimental period (from about 20 - 25 kg to about 100 kg body weight). They were put in

batches from about a few days to two - three weeks before mating. It is well established that exercise reduces overfat condition in pigs (Andreae, 1968; Zjunkina, 1968; Chernyshev and Karman, 1972), in human beings (Bloom and Eidex, 1967). In the present work, apart from the time of eating, gilts lay down most of the time. Space restriction might be an additional cause to their inherent laziness.

In the human, reproductive disorders have been reported to occur in obese patients of both sexes (Debiase and Cagnazzo, 1970; Cotrazzi, 1970). A dietetic regime improved the condition in respect of restoration of the normal rhythm of menstrual flow, normal cycle of ovulation. The impairment of the oestrous cycle in overfat females might be due to some of the oestrogens secreted by the ovary being dissolved in the fat depots of excessively fat animals and so rendered ineffective in producing oestrus behaviour (Pomeroy, 1960c; Lodge and MacPherson, 1961). This may partly explain the acyclicity in two gilts in the present work following their third and fourth oestrus.

Marshall and Potts (1921), Hart and Miller (1937), Briggs et al. (1942) could not obtain consistent advantage from flushing ewes. Clark (1934) demonstrated that this variability was due to the effect of body condition on the ovarian response to flushing. He showed that if ewes were in good condition, ovulation rates were

high, then there was little response to flushing. However if ewes were in store condition at the commencement of flushing, they showed a marked increase in ovulation rate over unflushed controls. This was confirmed by work on ewes (Hammond 1941; Underwood and Shier, 1941; Wallace, 1953; Allen and Jamming, 1961), mice (Vischer et al., 1952; Lamond and Bindon, 1969), human beings (Suryanarayana et al., 1969), pigs (Pike and Boaz, 1967; Lodge and Hardy, 1968). The latter found that the effect of nutrition on ovulation rate was masked by a "carry over" from previous reproductive cycles. The high plane of nutrition considerably increased ovulation in thin sows but not in fat ones (Pike and Boaz, 1967). This can explain the effectiveness of short-term flushing on ovulation rate in some studies. Brooks (Pig Farming, 1971) wrote "Trials which have appeared to show an increased ovulation rate due to feeding sows more on the day of mating may have been misleading, for the sows in these trials were on a lower feed level throughout previous cycles, so their ovulation may have already been depressed and then simply revived by flushing". This was also supported by work of Cooper et al. (1971) on hormonal changes during flushing.

Gilts in the current work were maintained in high condition during the pre-experimental period, as indicated by their fatness. Therefore, it seems that body condition of gilts prior to flushing may partly

explain the non-significance of the effect of flushing on ovulation rate.

(e) Possible influence of some uncontrolled factors.

Unlike the work by Gossett and Sorensen (1959), Zimmerman et al. (1960a), Rigor et al. (1963), Kirkpatrick et al. (1967a), Schultz et al. (1966) ... the difference in level of feed intake (energy intake) also brought about that in protein intake between treatment groups. Although evidence is not conclusive, it has been suggested that protein intake has some influences on reproductive performance of rats (Guilbert and Goss, 1932; Lyons, 1943; Nelson and Evans, 1953, 1954; Srebnik et al., 1958, 1961; Srebnik and Nelson, 1964), sows (Angelova, 1966).

The number of ova shed by gilts is correlated with age and weight at oestrus (Squiers et al., 1949; 1952; Warnick et al., 1951; Perry, 1954; Rathnasabapathy et al., 1956; Newman, 1963; O'Bannon et al., 1966; Coop, 1966; Fletcher, 1971). Flushed gilts in this experiment were significantly older and heavier than the controls, however the results showed that age and weight of gilts at mating were not significantly correlated with ovulation rate. Squiers et al. (1952), Perry et al. (1954) ... reported that ovulation rate of gilts and sows increased with the order of oestrus. Gilts of

two groups of the current work had experienced a similar number of oestrous cycles. Furthermore oestrus number was shown not significantly correlated with ovulation rate. So these factors might not have any significant effect on the variation in ovulation rate of gilts in response to flushing.

An advantage of the short-term flushing system used in this experiment is that the possible "secondary effect" of growth rate could be eliminated. It has been reported that ovulation rate was associated with growth rate of gilts (Squiers et al., 1952; Rathnasabapathy et al., 1956; O'Bannon et al., 1966). In the present work flushed gilts gained 7.03 kg comparable to 4.99 kg made by the controls over a fortnight prior to breeding. Actually ovulation was not significantly correlated with liveweight gain in this experiment ($r = -0.023$). This agreed with work by Wise and Robertson (1953).

A few other factors reported in the literature which have some relations with ovulation rate of gilts and sows are: genotype (Kappeli, 1908; Robertson et al., 1951a; Boye, 1956; Baker et al., 1968; Warnick et al., 1951; Duncan and Lodge, 1960; Newman, 1963; Kirkpatrick et al., 1967a,b; ... light (Klockova et al., 1961; Gossett and Sorensen, 1959). The latter factor is particularly important in egg production in chickens (Sykes, 1956; McCluskey and Parker, 1963; Martinet, 1963). The present experiment was designed so that two

littermates were on two different treatments, therefore the effects of genotype could be excluded. On the other hand, all gilts were housed in the same building and subjected to the same conditions of light, temperature, humidity... So, it can be assumed that these environmental factors take a very small part, if any at all, in the difference in ovulation rate between the two groups.

(f) The reliability of the method of determination of ovulation rate.

Corner (1923) wrote "... in the examination of many hundreds of pregnant uteri during ten years, only three or four times have specimens been seen in which the number of embryos exceeded the number of corpora lutea ... within a limit of accuracy which is amply close to our purposes, each recent corpus luteum represents one ovum discharged at the latest ovulation". However Brambell (1948) found that in a number of animals the corpora lutea (CL) count was not closely correlated with the number of ova shed. Actually the accuracy of the counting depends upon the stage at which it is done in relation to ovulation time. When fertilization does not occur, the CL regresses and decreases in size, ie., the corpus albicans. In the sow, an abrupt and rapid decrease in size of the CL occurs after the thirteenth day of the cycle, and this regression continues throughout the follicular phase (Hafez, 1968). After a certain stage the corpus albicans

becomes a barely visible scar of connective tissue. In the experiment of Robertson et al. (1951c) and Warnick et al. (1951), ovulations at first oestrus were determined by counting the number of corpora albicantia, while ovulations at second oestrus were determined by counting the number of CL. This was questioned by Dyck (1971b), that some of the corpora albicantia might not be recognizable at the time of counting and this would account for the lower count at first oestrus. On the other hand, within 48 hours of ovulation the ovary underwent a massive structural change, ie., a large increase in size of the CL resulting from a number of factors such as bleeding into the CL, growth of the luteal tissues and perfusion of fluid into the centre... (Cooper and Scofield, 1969). As a result of these changes each CL varied so much in shape and size. Some areas where ovulation had occurred often tended to be hidden or disrupted by other ovulations in close proximity. Consequently it was difficult to obtain an accurate assessment of the number. Perry (1960) found that by counting the CL superficially, he almost invariably was short by one as revealed by dissection of the ovary.

The difficulty in counting CL early in pregnancy was also noted by Reitmeyer and Sorensen (1965), Hardy and Scofield (1968), the former found some extra CL at the counting on Day 40 of gestation compared to that on

Day 3. The authors proposed that these accessory CL might be derived from unruptured follicles, which formed luteal tissue later in the cycle and were not observed as early as three days following oestrus. It has been reported that CL atretica or accessory CL are formed from unruptured follicles during pregnancy in several other mammals such as the baboon (Zuckerman and Parkes, 1932), bank voles (Brambell and Rowlands, 1936), mountain viscacha (Pearson, 1949), Canadian porcupines (Mossman and Judas, 1949), rats (Swezy and Evans, 1930), mares (Cole et al., 1931), elephants (Perry, 1953), sheep (Moore et al., 1960). Allen et al. (1947) reported that in rabbits experimental error accounted for 6.8% of the variation in prenatal mortality based on a count of the number of CL.

In a number of experiments on pigs (Gossett and Sorensen, 1959; McGillivray et al., 1963; O'Bannon et al., 1966; Heap et al., 1967 ...), assessment of ovulation rate was based on CL count during 20th - 40th day of pregnancy. On the basis of the above evidence, the value of ovulation rate obtained in these studies might not reflect the true number of ova shed (Hardy and Lodge 1969b). On the other hand, the use of the CL count as an estimate of ovulation rate can theoretically be complicated by the occurrence of polyovulation.

Therefore, the significance of many studies quoted so far is still clouded by the question of the

accuracy of the method of determining ovulation rate. In the present experiment, gilts were laparotomized on about Day 7 after the onset of oestrus. Although difficulties in counting were faced in only a few cases, the accuracy of the results was not known unless a dissection of the ovary was performed. Consequently the non-significance of the difference in ovulation rate between two treatment groups cannot be claimed with certainty.

3. Litter Size at Birth:

The ineffectiveness of flushing prior to service in the present experiment agrees with the results obtained by Brooks (1970), Libal and Wahlstrom (1970), te Brake (1971), Cooper et al. (1971), Brooks et al. (1972), but disagrees with McGillivray et al. (1962), Nielsen (1968), Moore et al. (1971), Brooks and Cole (1972)... In fact in the pig litter size at birth has been shown to be influenced by many factors other than nutrition, and these may at the same time interact with nutritional factors. Age of gilts at mating, for example, has been shown to be positively correlated with litter size at birth (Pomeroy, 1960a; Sherritt, 1962; Omtvedt et al., 1965; Strang, 1970...). In the present study, within the number of animals (seventeen) providing data for litter size at birth, treated gilts were mated at a similar age to the controls. Litter size was found to be slightly correlated with age of gilts at mating. The adjustment for age showed

that litter size of pigs from the flushed group was slightly larger than that from the non-flushed one, (7.47 vs 5.59).

Another factor which has also been reported to have some relations with litter size at birth is weight of gilts at mating (Bowman et al., 1961; Heap et al., 1967...). However there was no correlation between litter size at birth and weight of gilts at mating...

Ovulation rates may be an important factor in determining litter size at birth. High ovulation rates have sometimes been associated with large embryonic losses (Perry, 1954; Rathnasabapathy et al., 1956; King and Young, 1957). However this did not agree with work by Perry (1960), Pomeroy (1960c), Boyd (1965) unless the ovulation rate was exceptionally high. In the latter, e.g., superovulation, the increased embryonic death may be due to uterine capacity (Casida, 1966; Hunter, 1966; Longenecker et al., 1968; Pope et al., 1968).

In the current work, since no real difference in ovulation rate between groups was detected, it was unlikely that this factor biased the result of litter size at birth through its effect on embryonic losses. Moreover the results of the analysis showed that ovulation rate had very little, if any at all, correlation with litter size at birth ($r = 0.01$).

It is noted that litter size at birth in the

present study was determined by both live and dead piglets at birth. The cause of still birth was not determined, but it was thought to be related to the health of gilts during pregnancy rather than to nutritional factors.

V Conclusion:

Most workers agree that flushing prior to service results in an increased ovulation rate in pigs. The present study failed to show this effect of flushing. The reason for this discrepancy is not known with certainty, but a few factors are suggested to be partly responsible for this, viz., body condition of gilts before flushing and/or the accuracy of the method of determining the number of ova shed. Breed difference between the present and other studies may be suspected to take some part in the discrepancy. Some breeds appear to respond to flushing more strongly than others as reported by Self et al. (1955), Kirkpatrick et al. (1972a)... McKenzie (1928) noted an adverse effect of long delay of first breeding. It may be also speculated that the response of the ovary to flushing may decrease with age.

From the present study, it may be concluded that flushing gilts under the condition described is not beneficial. However, since the experiment was designed to examine the effect of flushing, for about eleven days prior to service, on the reproductive performance of gilts, it is difficult to come to any suggestion of an optimum scale of feeding for them. Moreover this scale depends upon the body condition of gilts which in turn is a function of feeding levels and husbandry management

(age, weight of gilts at first mating, housing....) during the growing period. Within the scope of this experiment, it is not known when (at what stage of reproductive life) gilts should be first mated.

Litter size at birth was not affected by flushing. This agreed with many previous experiments. In those studies where litter size at birth did increase with flushing, the increase seemed to reflect that in ovulation rate. Recently Brooks et al. (1972) suggested that there appeared to be a threshold above which it was difficult to increase ovulation rate and litter size.

In fact it is very difficult to judge the effect of feed prior to breeding on litter size at birth without any consideration of the condition of gilts during a long period of pregnancy. Outbreaks of constipation and abortion which occurred in the present work should be taken into account in the final assessment of reproductive performance of the gilts.

CHAPTER 2

EFFECT OF PLANE OF NUTRITION DURING THE FIRST MONTH OF
PREGNANCY ON FARROWING RESULTS

I. Literature Review:

The topic has been studied extensively during the last decade. Many excellent reviews have also been published (Duncan and Lodge, 1960; Casida, 1964; Cassell, 1967; Lucas, 1968; Elsley, 1970, 1971...). The results obtained from studies have been much debated.

1. Plane of nutrition and litter size:

Clawson et al. (1963) found that gilts receiving 3 lb of feed daily during pregnancy farrowed significantly more live pigs than those receiving 6 lb. This is in agreement with work by Self et al. (1955), Gossett and Sorensen (1959), Haines et al. (1959), Goode (1961), Salmon-Legagneur and Jacquot (1961), Pickett and Beeson (1962), Lodge (1969). Recently Frobish and Steel (1970) fed four groups of gilts at four levels of feed intake, representing 3.0, 4.5, 6.0, 7.0, Mcal of metabolisable energy (ME) per day during pregnancy. The average number of pigs farrowed decreased with increasing energy intake, viz., 10.3, 10.1, 9.6 and 8 for the four groups respectively. These authors estimated that each 1.5 Mcal ME increase in daily energy intake accounted for 0.55

less pigs farrowed.

The above findings, however, directly conflict with that of Henson et al. (1964). In the latter study either 3 lb or 5 lb of meal per day were given during three successive pregnancies, the number of piglets born was not affected by levels of feeding. This is in agreement with Eyles (1959). Self et al. (1960), Dean and Eribble (1960, 1961), Salmon-Legagneur (1963), Bowland (1964), Frolish et al. (1966), Elsley (1967, 1968), Elsley et al. (1967, 1968), Hawton and Meade (1969), Anderson and Wahlstrom (1970), Grummer et al. (1971), Jancic (1972). Baker et al. (1969) imposed five planes of feeding, viz. 0.9, 1.4, 1.9, 2.4, and 3.0 kg of feed per day (1.9 kg/day was considered sufficient), on five groups of 55 gilts during pregnancy. They could not detect any significant difference in numbers of pigs born between groups. O'Grady (1962, 1963) and Heap (1966) also failed to find any significant effect of feeding treatments on numbers of pigs born, but high fed sows tended to have slightly larger litters than did low-fed ones.

Furthermore many other studies have shown the beneficial effect of high plane of nutrition on numbers of pigs born by gilts or sows. Vermedahl et al. (1969) found that gilts that were fed 2.27 kg feed per head daily during gestation produced significantly more pigs than those gilts fed 1.3 kg per head daily. This agreed

with the finding of Hanson et al. (1955), Libal and Wahlstrom (1970), Buitrago et al. (1970), Adam and Shearer (1971), Kurbatov (1971) ...

Therefore, so far there are three schools of thought about the effect of plane of nutrition on litter size at birth. They are:

- (a) High feeding decreased litter size at birth.
- (b) High feeding improves litter size at birth.
- (c) Plane of nutrition does not have any influence on litter size at birth.

2. Effect of feed intake during pregnancy on embryo survival:

Studies on the effect of nutrition on embryo survival are also inconclusive. Gilts receiving 2.72 kg feed per day from the time of mating until slaughter at days 25 to 27 had significantly lower embryo survival compared with gilts receiving 1.81 kg feed per day (Schultz et al., 1966). In agreement, many other studies show the trend of a high plane of feeding immediately after mating to adversely affect embryo survival (Robertson et al., 1951; Christian and Nofziger, 1952; Self et al., 1955; Casida, 1956; Gossett and Sorensen, 1959; O'Bannon et al., 1966; Dutt and Chaney, 1968; Holness, 1969 ...). Prenatal mortality for the first 25 days of gestation as well as during the entire gestation period was higher for full-fed gilts compared with limited-fed ones (Haines et al., 1959).

On the other hand Ray and McCarty (1965) removed their gilts from feed 24 to 72 hours after mating and then return them back to feed. Embryo survival was significantly improved in animals exposed to a fasting period.

In contrast, evidence also reveals that the difference in embryo survival between high- and low-fed sows during either the first four weeks (King and Young, 1957; Sorensen et al., 1961; Heap, 1966), the first forty days (Haines et al., 1957) or the first seventy days of pregnancy (Pike and Boaz, 1967) is not significant. Some workers suggested that feed distribution rather than the total amount of feed given during pregnancy affected the reproductive performance of sows. Many recommendations allow extra feed in the first three to four weeks of pregnancy (Pig Feeding and Management, 1963; Vadla et al., 1957), probably as a result of the finding of Ballinger (1940) who reported improved conception rate and number of piglets born from increased feeding after service. In addition, American studies (summarised by Casida, 1964), suggest that high feed intakes in mid-pregnancy increase embryonic mortality. However the results obtained by Lodge (1969) and Pike and Boaz (1969) failed to support this hypothesis. The latter employed three patterns of feeding for their sows during pregnancy, as follows: 3.6 kg then 1.8 kg of feed per day; 2.7 kg/day throughout pregnancy or 1.8 kg then 3.6 kg/day with the changes made from the 49th to 63rd day after service. Sows on the

three patterns had the same total amount of feed during pregnancy. The number of piglets at birth was least for sows given 3.6 kg then 1.8 kg. Furthermore Salmon-Legagneur (1962), Salmon-Legagneur et al. (1960), O'Grady (1971) and Elsley et al., (1969b, 1971) reported that feed distribution during pregnancy does not have any effect upon the number of pigs born ...

3. Effect of feed intake on birth weight of pigs and litter weight at birth:

The birth weight of pigs is generally influenced by the level of nutrition. Many workers agree that the level of feeding in pregnancy has a consistently and highly significant effect on birth weight of piglets. An increase in feed intake in pregnancy results in an increase in the weight of pigs at birth (Hanson et al., 1955; Henson et al., 1955; Henson et al., 1964; Clawson et al., 1963; Tassell, 1967; Elsley et al., 1967, 1969a; Elsley, 1967, 1968; O'Grady, 1967; Vermedahl et al., 1969; Holness, 1970; Buitrago et al., 1970; Jancic, 1972). Elsley (1967) and Frobish and Steel (1970) reported that mean birthweight was increased in a linear manner when the total energy intake of the pregnant sow was increased, with each 1.5 Mcal ME increase in daily energy intake accounting for 121.3 g heavier live pigs farrowed whereas Baker et al. (1969) noted that the increase was quadratic.

The increase in total litter weight at birth in response to increased feed intake during pregnancy has also been observed (Clawson et al., 1963; Lodge et al., 1966; O'Grady, 1967; Adam and Shearer, 1971).

Once again, conflicting evidence to the above finding, available in the literature, is not rare. Accordingly average birth weight of pigs is very little if at all, affected by the variation in feed intake of sows during pregnancy (Eyles, 1959; Self et al., 1960; Dean and Tribble, 1960, 1961; Salmon-Legagneur and Jacquot, 1961; Salmon Legagneur, 1963; Bowland, 1964; Hawton and Meade, 1969; Libal and Wahlstrom, 1970; Panov, 1971). The total litter weight at birth has been also reported to be affected by the level of feed during pregnancy. (Lodge et al., 1966; Elsley et al., 1967; Lodge, 1969).

Furthermore, there is growing evidence that a high plane of feeding during late pregnancy improves the birth weight of piglets (Moustgaard, 1961; Lodge et al., 1966; Elsley, 1970). Thus, the recommendations published by A.R.C. (1967) indicate that feed intake should be increased in the latter part of pregnancy. However Lodge (1969), Elsley et al. (1969b, 1971) failed to demonstrate this beneficial effect of high feeding. The patterns of feed intake (High-Low, Low-High and High-Low-High, all having the same total amount of feed) had no appreciable or significant effect on the birth weight of pigs.

II Methods and Materials:

1. Experimental design and treatments:

After mating (Chapter 1), gilts were allotted to two post-mating treatments, which with the two pre-mating ones formed the basis of a 2 x 2 factorial experiment. Thus one half (five pregnant gilts, which were grouped in one batch) of each pre-mating treatment group was allocated to one of the following post-mating treatments throughout the first month of pregnancy;

(a) High feeding : 2.71 kg meal 2 per day

(b) Low feeding : 1.36 kg meal 2 per day.

From the second month of pregnancy until farrowing all gilts were fed similarly, viz., 1.8 kg meal 2 per day until the last month of pregnancy, when feed allowance was increased to 2 kg meal 2 per day.

The same experimental management described in Chapter 1 was continued. A few days before farrowing gilts were transferred to the farrowing house.

2. Records:

(refer Chapter 1 for the records of litter size at birth)

Litter weight was determined as the total weight of all fully formed piglets at birth in a litter, whereas average birth weight of pigs was the ratio of litter weight to litter size at birth. Days of farrowing were recorded.

3. Statistical analysis:

The data of the three results recorded (litter size, weight at birth and average birth weight of pigs) were analysed according to the method of analysis of variance of a 2 x 2 factorial experiment (Snedecor and Cochran, 1969).

III Results:

1. General

Health (refer Chapter 1).

A summary of results is presented in Table 2 (Interactions between pre-mating and post-mating treatment failed to reach significance for each characteristic and results are shown with reference to post-mating treatment only).

Observation on individual animals for the three characteristics and details of the relevant statistical analysis are given in Appendices 10 and 11.

2. Litter size:

The results show that gilts fed on a high plane of nutrition during the first month of pregnancy had significantly ($P < .025$) larger litters at birth than those on low plane (7.89 vs 4.13).

3. Litter weight:

The high-fed group had significantly ($P < .05$) heavier litters than the low-fed ones (10.65 kg vs 5.88 kg).

4. Average birth weight:

By contrast, the analysis of variance of average birth weight of pigs does not reveal any significant effect of either pre-mating [Flush (F) and Non Flush (NF)] or post-mating [High (H) and Low (L)] treatments.

The average birth weight of the whole experimental herd was 1.34 kg.

Table 2. Litter size, weight (kg) at birth, and average birth weight (kg) of pigs from gilts fed on High and Low plane of nutrition during the first month of pregnancy.

Characteristics	Post-mating treatments		F-test	Significance test
	High	Low		
Litter size at birth	7.89	4.13	7.407	**
Litter weight at birth	10.65	5.88	5.142	*
Average birth weight of pigs	1.36	1.32	0.0002	NS

* $P < .05$ ** $P < .025$ NS. $P > .05$

IV Discussion:

1. Litter size and some possible factors affecting it:

The increase in litter size in response to high plane of feeding during the first month of pregnancy is in agreement with Salmon-Legagneur and Jacquot (1961), Sorensen et al. (1961), Lodge (1969), Frobish and Steel (1970) ... The results also indirectly support the view of increased embryo survival by high feeding sows as reported elsewhere. However, these do not agree with work by Robertson et al. (1951), Eyles (1959), Self et al. (1960), Dean and Tribble (1960, 1961), Mayrose et al. (1966), Lodge (1969) ... The reason for the discrepancy is not known, but some factors other than nutrition might have biased the results.

(a) Physiological factors -

It is known that litter size at birth is influenced by any factor affecting ovulation rate, fertilization rate and embryonic mortality at any stage of pregnancy. In work by Holness (1969), where high plane increased the number of live embryos, ovulation rates were greater in the high-fed group than in the low-fed one (since feeding treatments were imposed long before oestrus). However, this does not explain the situation where plane of nutrition causes significant differences in litter size from gilts of equal ovulation rates as

observed in present work and by Self et al. (1955). The large number of pigs farrowed may be accounted for by either a higher fertilization rate or a lower prenatal death in the high-fed gilts. Alanko (1968) drew attention to the variation of fertilization rate in sows in an extreme case where sperm concentration was too low. It seems probable that a too-low sperm concentration in the oviduct will result, at least for some ova, in a delay of fertilization; these ova being consequently too old at the time of fertilization might result in abnormal development. On the other hand, the ova at a stage of development, not correctly timed to the changes of the uterine mucosa during the pre-implantation time, might be prone to early embryonic death at the onset of nidation. This may be a factor limiting the litter size. However in the present experiment each gilt was double-mated by two different boars for the maximum fertilization. These two boars were chosen randomly among four experimental ones. So it may be assumed that all gilts were similarly treated with respect to semen quality. Moreover semen quality was independent from treatments imposed. In general, fertilization rates in sows are very high and unaffected by plane of nutrition (Robertson et al., 1951; Christian and Nofziger, 1951; Self et al., 1955; Hill et al., 1971). Therefore the increased litter size at birth in the high-fed group as compared with the low-fed one,

obtained in this experiment, may be due to an improvement in embryo survival. However the mechanism is not known.

(b) Differences in feeding levels between treatments:

Hanson (1958), Elsley and MacPherson (1970) reported that extremely low feed intakes during pregnancy reduced both number and the birth weight of pigs, this effect becoming more pronounced with succeeding parity. This is the so-called "Thin Sow Syndrome" resulting from prolonged underfeeding. However this is not the case as reported above ...

In the cases where feeding levels did not have any significant effect on litter size (e.g. Eyles, 1959; Dean and Tribble, 1960, 1961; Lodge, 1969 ...), it is noted that the amount of feed given to the high-fed group was not much bigger than that given to the low-fed one. For example, feed allowance for high- and low-fed sows was respectively 4.6 and 3.1 lb in work by Dean and Tribble (1960, 1961). It might be that these feeding levels were not sufficiently different to cause a significant difference in embryo survival (Bazer et al., 1968).

(c) Stage of pregnancy when nutritional treatments are imposed:

Tassel (1967) reported 25% of sows transferred to the low diet after mating losing their litters. The loss

probably occurred after fertilization. Phillippo (1967) found no significant difference between the percentage mortality at the 13th day compared with that in gilts killed between the 15th and 25th days. This suggests that the loss before the 13th day of pregnancy could account for all the mortality occurring by the 25th day of pregnancy. Furthermore, in sows, the period of elongation of the blastocysts occurs from the 10th to 13th day of pregnancy. This period has been suggested to be very critical for embryonic development, and it is at this time that many of the abnormalities arise (Phillippo, 1967). This was later confirmed by Scofield (1969), Scofield et al. (1971). This stage is also the stage of implantation in the pig (Crombie, 1970). A second, smaller peak of prenatal loss has been also found to occur around the time of organogenesis, i.e., about three weeks of pregnancy (Hafez, 1959).

In sheep either a low plane of nutrition during the first two months of pregnancy (Bennett et al., 1964), or food restriction for seven days during the first twenty days (Edey, 1966), or starvation for four days between the 9th and 15th day (van Niekerk, 1966) resulted in a high percentage of embryo loss; while neither a low plane of nutrition from the 25th day (Foote et al., 1959) nor a submaintenance ration from the 25th day to the 90th day (Wallace, 1948), had a marked effect on foetal mortality. Therefore, a similar conclusion as in the pig

may be drawn, namely, that the most critical period is the time of implantation (van Niekerk et al., 1968). This has also been confirmed by work on rats (McClure, 1959, 1961) and mares (van Niekerk, 1965). The mechanism involved is not known. Some authors hypothesized that a large part of embryonic death during early pregnancy results from mechanism which affects uterine capacity (i.e., the ability of the uterus to support only a limited number of embryos) (Bazer et al., 1969a,b; Fenton et al., 1972).

The embryonic death during mid- and late-pregnancy has not been studied extensively, and it is not known whether plane of nutrition during these periods has any effect. Casida (1964) reported that high feed intake during mid-pregnancy might be harmful to the foetuses. Placental insufficiency (the inability of the placenta to support the foetus) was suggested to be responsible (Scofield, 1972) since at this stage (60 - 70 days of pregnancy) placental growth stops and foetal growth progresses rapidly (Pomeroy, 1960c). That might be why in a number of experiments (Haines et al., 1957; Lodge et al., 1966; Frobish et al., 1966 ...) where feeding treatments were maintained throughout pregnancy, no significant differences in embryo survival could be detected between the groups of gilts.

(d) Body condition of gilts:

With respect to the difference in feeding levels

between high- and low-fed groups and the stage of pregnancy over which feeding treatments are imposed, the studies by King and Young (1957) are similar to the current work. However these authors could not find significant effects of feeding treatments upon embryo survival at 28 days of pregnancy. The only important difference between their work and the present one is that the animals involved in the latter were nulliparous gilts whereas those involved in the former were multiparous sows which had had already 2 - 4 litters. The history of these sows was not mentioned by the authors, it is difficult to come to any explanation as to why the discrepancy existed. Pike and Boaz (1967) noted that the effect of the nutritional regime during pregnancy could be masked by a "carry-over" from previous reproductive cycles. The sow's body reserves would appear to be capable of buffering the effect of nutritional treatments during a particular pregnancy. These authors (Pike and Boaz, 1967) reported that an increase from 4 lb to 8 lb meal daily following service and through pregnancy did not affect fetus numbers in sows in good condition, but it raised to the same numbers of fetuses of thin sows. This may also partly explain the beneficial effect of fasting after breeding reported by Ray and McCarty (1965). In the latter experiment all groups of gilts were self fed during the entire pre-experimental period.

2. Litter weight at birth:

The results obtained herein are in agreement with work by Clawson et al. (1963), Lodge et al. (1966), O'Grady (1967) ... The mechanism involved is not fully understood. It was found that nitrogen retention was significantly higher on the high plane of nutrition during pregnancy (Pike and Boaz, 1967). However Moustgaard (1960) (unpublished data, cited by Elsley, 1967) reported that the increase in the concepta accounted for only a small part of the total liveweight gain for the sow. The main part is the gain of maternal tissue. This was supported by work of Elsley (1968) that the amount of nutrients deposited in the uteri as a result of pregnancy was surprisingly small when compared with the total intake of nutrients by the pregnant sow.

3. Average birth weight of pig:

This characteristic was not significantly affected by feeding treatments. This may be due to the fact that the increase in litter weight at birth was accompanied by that in litter size in response to high feeding. This agrees with work by Eyles (1959), Self et al. (1960), Salmon-Legagneur (1963), Panov (1971) ... but disagrees with Elsley (1967, 1968, 1969), Vermedahl et al. (1969), Holness (1970), Jancic (1972) ... However these latter imposed feeding treatments throughout pregnancy. It has been shown (previously mentioned)

that high levels of feed intake during late pregnancy may enhance the birth weight of pigs. This may partly explain the discrepancies between the results obtained herein and elsewhere.

4. Some factors which might bias the results of three characteristics recorded:

(a) Animal health -
(refer Chapter 1).

Even though the cause of the abortion was not determined, most gilts had a raised temperature (40°C - 41°C) for several days during pregnancy. It was initially thought by pathologists that this abortion was of a non-specific type as a result of the febrile episode of these gilts, since any general infection which raised the temperature of the gilts above 40.6°C for several days will result in prenatal death and abortion (Done, 1968). This was supported by the considerably high incidence of mummification (35.5% of the herd gilts had mummified foetuses. In those gilts the total number of full-term piglets were 28 whereas the number of mummified foetuses were 13). Therefore the parturition results could be biased.

(b) Stresses:

During the critical stage of embryonic development, gilts in the present experiment suffered many stresses, such as blood collections, laparotomy ... Euker et al. (1972) reported that embryonic mortality

was associated with all groups of rats subjected to stresses from Day 1 - 5, 5 - 12 and 12 - 20 of pregnancy.

High ambient temperature (refer Chapter 1) or fighting (as they were put in batches) may also cause embryonic death (Edwards et al., 1968) ...

V Conclusion:

Since the reproductive performance of sows has been shown to be influenced by many factors other than plane of nutrition, caution should be taken in assessing the results obtained from experiments. Under the conditions of the present study, it appears that gilts receiving 2.71 kg of feed per day during the first month of pregnancy had significantly better reproductive performance than those receiving only 1.36 kg per day. The results support some recommendations on sow nutrition. Although, in agreement with most recent work about the advantage of feeding 2.71 kg per day over 1.36 kg per day, the optimum level of feed allowance is not known. However, in determining feeding levels for pregnant sows or gilts, their body condition at mating should be taken into account, since high feeding will not be beneficial in fat sows.

CHAPTER 3

PLASMA PROGESTERONE LEVELS DURING EARLY AND LATE PREGNANCYI Introduction:

Progress in the investigation of the part played by progesterone (P) in reproduction has been hampered for a long time by the lack of a sufficiently sensitive and a specific method of assay. The fact that large doses are usually required to produce a biological response has to some extent invalidated the numerous bioassay techniques that are available. Many techniques for the assay of P have been suggested in the literature. They are either biological (Hooker and Forbes, 1947; Duyvene de Wit, 1938, 1941) or chemical methods (Butt et al., 1951; Edgar, 1953a,b; Zander and Simmer, 1954; Zander, 1955; Raeside and Turner, 1955).

More recently, Murphy (1964) suggested the application of the property of protein binding to the assay of minute quantities of hormones and other substances. Such binding has been shown to exist between certain hormones and globulin. This type of binding had actually been utilized by Berson and Yalow (1957), Unger et al. (1961), Murphy et al. (1963). The modification of the technique by Murphy et al. (1964), Johansson et al. (1968), Johansson (1969a,b) made it possible to study more closely the change in P levels of bovine

jugular venous plasma (Shemesh et al., 1971), in peripheral plasma during the luteal phase of the normal human menstrual cycle (Johansson, 1969a,b), during the oestrous cycle, pregnancy in pigs (Stabenfeldt et al., 1969a,d; Tillson, 1969; Edqvist and Lamn, 1971; Shearer, 1971; Henricks et al., 1972 ...).

II Literature Review:

1. Source and formation of progesterone:

The main source of P is the luteal cells of the corpus luteum. The most typical features of steroid-secreting cells are:

- (a) The abundant, smooth endoplasmic reticulum (s.e.r.) and
- (b) The tubular rather than lamellar cristae of the mitochondria (Abraham and Tait, 1971).

The luteal cells synthesize cholesterol from acetate and store it as fatty acid of the 3-hydroxyl group of cholesterol. This is then converted to 5-pregnenolone ... The biosynthetic pathway of P, based on in vivo and in vitro incubation of ovarian tissue with acetate ^{14}C or some other radioactive intermediate was demonstrated to be : acetate \longrightarrow cholesterol \longrightarrow pregnenolone \longrightarrow progesterone \longrightarrow 17 α -hydroxy progesterone \longrightarrow androstenedione \longrightarrow testosterone \longrightarrow estrone or estradiol (Sweat et al., 1960; Aakvaag and Eik-Nes, 1965a, b; Rice and Savard, 1966; Savard and Telegdy, 1965; Short, 1964 ...). All these reactions take place under the influence of an active series of enzymes (McKerns, 1969). Other pathways may be found because specie differences in the biosynthesis of P are known.

2. Peripheral plasma progesterone changes:

Shemesh et al. (1971) wrote that "the peripheral plasma level of P furnishes a valuable indication of the concentration of the reaching the target organs and the control centres governing the secretion of gonadotrophins at various phases of the reproductive cycle. Alterations in circulating P level also supply information on the functional state of the corpus luteum".

(a) During early pregnancy -

Evidence reveals that P levels in peripheral plasma during luteal phase of non-pregnant sows was similar to those during the corresponding period in pregnant sows (Tillson and Erb, 1967; Tillson et al., 1970; Shearer et al., 1972). Similar results have been reported with cows (Donaldson et al., 1970) and ewes (Bindon, 1971). Although the actual values of P levels were reportedly different between studies (Short, 1957; Tillson and Erb, 1967; Stabenfeldt et al., 1969d ...), in general there was agreement that these levels rose from oestrus to a peak at about Day 10 - 15 after that. The only difference noted between studies is the time when the P levels reach the peak (Table 3).

Table 3 - Progesterone levels in peripheral blood plasma of sows at their minimum and maximum values during early pregnancy or during the oestrous cycle.

Source	Stage of the cycle or pregnancy (days) (Day 0 : onset of oestrus)	Progesterone level ng/ml	
		Pregnant	Non-pregtant
Tillson and Erb (1967)	0 12 - 14	7.5 27.2	8.3 24.5
Tillson (1969)	0 10 - 12	4 32	4 26
Tillson <u>et al.</u> (1970)	0 10 - 12	3-4 26	3-4 25
Edqvist and Lamn (1971)	3 - 4 13 - 14	- -	2.5 26-37
Shearer (1971)	3 14	- -	5-6 45
Henricks <u>et al.</u> (1972)	2 12	- -	5.1 33.2

After reaching the peak, P levels remain high in pregnant sows whereas those in non-pregant ones decrease toward the onset of the next oestrus (Tillson and Erb, 1967; Tillson, 1969; Tillson et al., 1970 ...). This is in agreement with work on cows (Donaldson et al., 1970; Henricks et al.; Shemesh et al., 1971; Christensen et al., 1971; Swanson and Hafs, 1971; Stabenfeldt et al., 1969b) and on ewes (Plotka and Erb, 1967; Stabenfeldt et al., 1969a,c; Gomes et al., 1965; Bindon, 1971; Neher and

Zarrow, 1954).

(b) During late pregnancy -

Results are almost in agreement about the decline in blood plasma P concentration toward parturition (Short, 1960; Masuda et al., 1967; Shearer, 1971). However differences in the timing when P levels start to decline still exist between studies.

3. Supported evidence from work on other source of progesterone:

Formerly, with techniques of poor sensitivity, most workers tended to study the P content in corpora lutea or ovarian venous plasma because of its comparatively high values (Allen, 1930; Elden, 1934; Kimura and Corwell, 1937; Gawienoski, 1956; Loy et al., 1958; Gomes et al., 1965 ...). The results show that during oestrous cycle or early pregnancy the concentration of P in ovarian venous effluent (Gomes et al., 1965; Masuda et al., 1967; Brinkley and Young, 1968) follow the same patten as that observed on peripheral plasma P (Tillson and Erb, 1967; Tillson, 1969; Tillson et al., 1970 ...). This is supported by the positive relationship between P concentration in peripheral and ovarian venous plasma (Moor, 1968; Erb et al., 1971; Sprague et al., 1971). Both of these have been shown to correlate with P content of corpora lutea (Gomes et al., 1963; Stormshak et al., 1963; Masuda et al., 1967;

Rombauts et al., 1965, 1971; Stabenfeldt et al., 1969c; Henrick et al., 1969a; Cavazos et al., 1969c).

In agreement with studies on peripheral plasma, those on ovarian venous plasma (Masuda et al., 1967) and in luteal tissue (Erb et al., 1962; Masuda et al., 1967; Rombauts et al., 1971 ...) show a decline in P concentration during late pregnancy toward parturition.

III Methods and materials:

1. Progesterone assay technique:

(a) The method for P estimation was the competitive protein binding (CFB) assay of Murphy (1967) as described by Johansson (1969a) with the following modifications:

- During the extraction procedure, petroleum ether was mixed with plasma and shaken for five minutes, instead of one minute,
- The mixture was then frozen in a dry-ice bath instead of letting it settle down.
- The temperature of the water bath used in the protein binding assay was 45°C instead of 40°C.

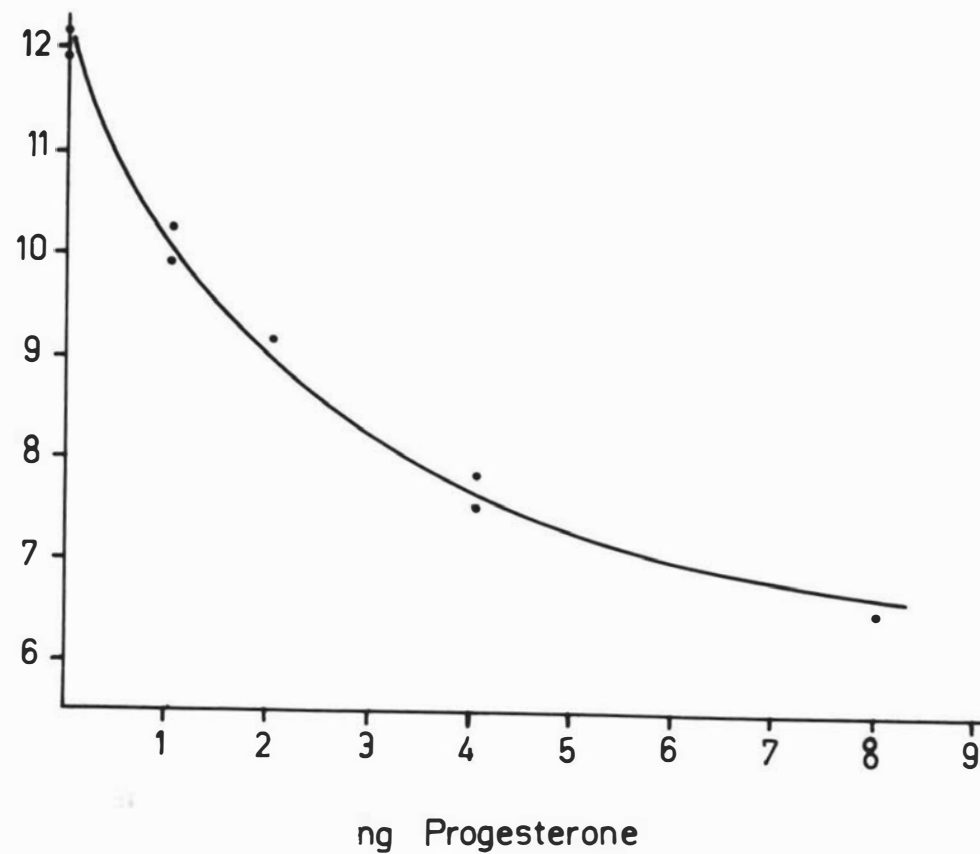
A standard curve was prepared from solutions of known levels of P, viz., 0, 1, 2, 4, 8 mg (Figure 2).

(b) Specificity -

Of the present CFB methods in the literature, there are two basically different methods of attaining specificity. One approach used chromatographic fractionation (Neill et al., 1967; Yoshimi and Lipsett, 1969 ...) and the other employs selective extraction with a solvent (Hagerman and Williams, 1969; Martin et al., 1970) to exclude interfering compounds. While the former method was used to eliminate the interference of a large number of steroids, these have not yet been exhausted.

Figure 2- Standard Curve Prepared from CBG-H³B with Human Plasma. (12.9.72)

- A Standard Curve was Processed Simultaneously for Each Set of Samples Analysed.



Furthermore, as indicated by Rosenthal et al. (1969) and Reeves et al. (1970), the association constants of various steroids seem to vary with different concentrations of P in plasma ... Because of these reasons and the cumbersome thin layer chromatography technique, it was not used in the present experiment. By excluding the thin layer step, the specificity of the method depends on the selectivity of the petroleum ether used for extraction as well as on the degree of displacement of corticosterone-1,2-T (H^3B) from the protein binding system.

Evidence shows that among several interfering steroids present in blood plasma, only two are of importance, viz., 17α -hydroxy progesterone (high ability to replace H^3B) and 20α -hydroxy progesterone (high recovery rate). The influence of the former is limited by its low extraction and that of the latter is also limited by its affinity for CBG as compared to P. Furthermore, according to Rombauts et al., (1965, 1971), the principle steroid of luteal tissues of sows was P. Very few derivatives of P such as 17α -hydroxy progesterone, 20α - or 20β -hydroxy progesterone could be detected. This was confirmed by Cook et al. (1967) and Henricks et al. (1972). So it may be reasonable to assume that the results of the CPB assay presented herein reflect truly the P levels in blood plasma in gilts.

(c) Reasons for using CPB technique -

The technique has many advantages:

- (i) Among the three techniques for P assay described in the literature, viz., CPB; Double isotope derivative (Riondel et al., 1965; Tillson and Erb, 1967; Shearer (1971) and gas liquid chromatography (Wyman and Sommerville, 1968; Stabenfeldt et al., 1969d). The CPB appeared to be the best one from the standpoint of sensitivity, ease of performance and expense. (Demetriou and Austin, 1970; Edqvist and Lamn, 1971).
- (ii) Very small amounts of plasma are required for the assay (only 0.25 ml in the study of Johansson (1969a) and only 0.2 ml was needed in the present work.
- (iii) The method is very rapid and easy to handle. At least twenty samples can be assayed in one day by one technician with good precision and accuracy.

2. Materials:

(a) Equipment -

- Scintillation counter : Liquid scintillation system (Beckman Instrument Inc., California).
- Vortex mixer : "Super Mixer" Cat. No. 1291 - Lab-line Instruments, Inc., Melrose Park, Ill., U.S.A.
- Microlitre pipets 200, 500 ml and pipet tips were available from Eppendorf Gerarebau

Netheler, Hinz GmbH 2000 Hamburg 53
(Hummelsbuttel).

(b) Reagents used were similar to those described by Shearer (1971) with some differences:

- To prepare liquid scintillation solution, additional Triton x-100 (Rombauts and Haas) was mixed in the solution at the ratio of one in three;
- Plasma containing the binding protein was obtained from pregnant women, during the third trimester (since corticosterone binding globulin concentration increases with stages of pregnancy).

(c) All glassware was prepared following the procedure described by Shearer (1971).

(d) Collection of blood samples:

Samples of blood were collected from the ear veins of pregnant gilts at 3, 15, 100, 110, 112 days after mating (Day 0 : oestrus) were named stages 1, 3, 4, 5, 6 respectively. Samples at stage 2 were collected at laparotomy from the vena cava of gilts. The blood was drawn into test tubes containing heparin (except the samples taken at laparotomy, these being drawn into heparinized containers). The blood was then centrifuged as soon as possible after collection at 5000 r.p.m.

for about 30 minutes. The plasma obtained was then stored at -20°C until required for analysis.

The number of gilts contributing samples analysed were: stages 1,2,3 - 20 gilts; stage 4 - 17 gilts; and stages 5 and 6 - 15 gilts; as three sows aborted at mid-pregnancy while two farrowed on day 109.

3. Statistical analysis:

Plasma progesterone levels of subgroup gilts were compared by split-plot technique (stages 1 - 3) (Snedecor and Cochran, 1969). In the case of missing plots (stages 4 - 6) the formulae for inserting estimates of missing values developed by Anderson (1946) were used. The missing plot technique (Cochran and Cox, 1950) of analysis of variance was adopted to compare P levels at these stages.

Analysis of variance - samples within samples (Snedecor and Cochran, 1969) were used to test for differences in P levels between gilts of breeding groups.

IV Results:

1. Plasma P changes during pregnancy:

The values of P concentration in blood plasma of individual pigs at each stage are given in Appendix 12. The results are summarized in Table 4. The overall mean of P levels increased significantly ($P < .001$) from 10.74 ± 3.78 ng/ml on Day 3 to 20.84 ± 5.35 ng/ml on Day 15 of pregnancy. The level at laparotomy (28.55 ± 6.03 ng/ml) was surprisingly higher than that at any stage recorded. (Laparotomy was performed on average at about Day 7 of pregnancy). The levels of P decreased gradually from 10.71 ± 2.72 ng/ml on Day 100 to 9.27 ± 3.79 ng/ml on Day 110 and continued to 7.54 ± 3.53 ng/ml on Day 112 of pregnancy.

2. Effect of feeding on plasma P levels:

In general, feed treatments, either pre- or post-breeding did not have any significant effect on P levels of gilts during pregnancy with an exception at stage 5. At stage 5, low-group gilts had significantly ($P < .05$) higher levels of P than the high-group ones (Appendix 13). Since interaction between main factors were not significant, the results are presented in relation to main effects only (Table 5).

Table 4: Plasma progesterone levels (ng/ml) at six stages of blood collection.

Stage	Mean	S.E. of mean difference	Comparison of P levels between stages (a) (b)
1	10.74	0.798	
2	28.55	1.729	
3	20.84	1.174	1 ***
4	10.71	0.814	3 ***
5	9.27	0.984	3 ***
6	7.54	0.733	1 * 3 *** 4 **

(a) Attempt was not given to compare plasma P levels at stage 2 with those at other stages.

(b) Code numbers indicate the stages that are significantly different from the stage in the respective rows shown in the first column from the left side of the table.

Non-significant: $P > .05$, (*): $P < .05$, (**):

$P < .01$, (***) : $P < .001$.

3. Correlations between plasma progesterone levels and other reproductive traits:

The correlations between P levels at first three stages and ovulation rate were not significant ($r = 0.234$, 0.137 and 0.278 for stage 1,2,3 respectively). For the last three stages, the only two significant correlations noted were those between P levels and:

(a) Numbers of mummified fetuses at stage 5

($r = 0.576$, $P < .05$).

(b) Numbers of mummified fetuses at stage 6

($r = 0.644$, $P < .01$).

The test for differences in P levels between gilts of different breeding groups showed that at no stage of blood collection did breeding have a significant effect on P levels in peripheral blood plasma.

Since, in general, there are no significant differences between breeding groups, feeding groups (except stage 5), the data were pooled for the preparation of figure 3. At stage 2, blood samples were not peripheral. In addition they were collected when gilts were not under normal physiological conditions (anaesthetized), so the values of P levels are not included in figure 3.

Figure 3- Peripheral Blood Plasma Progesterone Concentration
in Gilts During Early and Late Pregnancy

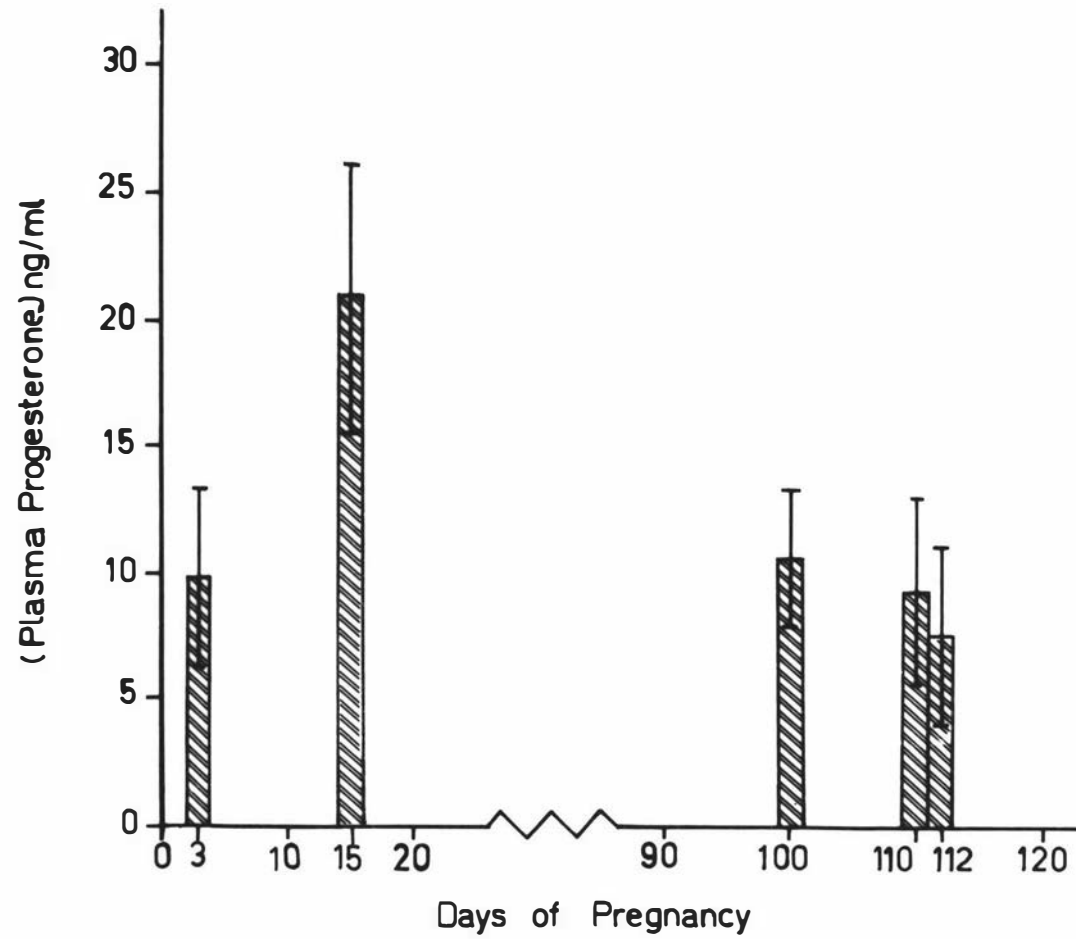


Table 5: Mean values of progesterone levels (ng/ml) from gilts of each main groups of six stages of blood collections.

Stage	Pre-breeding treatment			Post-breeding treatment		
	Flush (F)	Non-Flush (NF)	SE of mean difference	High (H)	Low (L)	SE of mean difference
1	9.82	11.67	2.107	8.39	13.10	3.719
2	29.49	27.62	4.174	29.06	28.05	2.893
3	20.53	21.15	2.309	21.12	20.56	2.734
4	11.0	10.42	0.970	10.64	10.78	0.949
5	8.08	10.46	1.218	7.29	11.74	1.168
6	7.59	7.50	1.154	6.30	9.100	1.054

V. Discussion:

1. Pattern of plasma progesterone levels during pregnancy:

In the current work, although it is not known what the values of P levels between Day 3 and Day 15 are, there appear to be an increase in P levels from about 10 ng/ml on Day 3 up to about 20 ng/ml on Day 15. This is in agreement with most previous studies on sows during early stage of pregnancy (Tillson and Erb, 1967; Tillson, 1969; Shearer, 1971) and on non-pregnant sows during the luteal phase (Tillson and Erb, 1957; Stabenfeldt et al., 1969a,d; Edqvist and Lamn, 1971; Henricks et al., 1972). This confirmed the trend noted by Stabenfeldt et al. (1968) (unpublished data, cited by Akins and Morrisette, 1968) that peripheral blood plasma level of P changed with the morphological change of corpora lutea in sows, since it is well established that during oestrus the mitochondria of the theca lutein cells do not contain many tubulae cristae. From Day 8 to Day 12 after oestrus these cells are packed with s.e.r. and there are large numbers of mitochondria with abundant tubulae cristae creating a membranous internum (Corner, 1956; Cavazos et al., 1969). Moreover the results obtained by Jacoby (1962) showed that states of luteal activity are associated with the changes in the levels

and distribution of ovarian cholesterol.

The increase in P concentration in peripheral blood reported therein also agreed well with that noted by Jones et al. (1965), Masuda et al. (1967), Brinkley and Young (1970) ... in P concentration in ovarian venous plasma and with that noted by Du Mesnil du Buisson et al. (1964); Rombauts et al. (1965); Cavazos et al. (1969); Stabenfeldt et al. (1969c) ... in P concentration in luteal tissue.

The decline in P concentration in peripheral blood plasma toward parturition reported herein agreed well with that by Masuda et al. (1967), Shearer (1971) ... This also supported the trend of decreasing P concentration in ovarian venous blood (Masuda et al., 1971) and in luteal tissue (Kimura and Cornwell, 1938; Duncan et al., 1960; Erb et al., 1962; Rombauts et al., 1971). This phenomenon has also been reported to occur in many other species such as cows (Short, 1960; Stabenfeldt et al., 1970), dogs (Smith and McDonald, 1972), rabbits (Mikhail et al., 1961; Hilliard et al., 1968; Polidoro and Black, 1970), goats (Blom and Lyngset, 1971) and ewes (Bassett et al., 1969; Challis et al., 1971 ...). The decline in plasma P toward parturition as observed in the present experiment indirectly supports the theory of P block to myometrial activity (Courrier, 1941; Courrier and Colonge, 1950; Csapo, 1961, 1969; Schams et al., 1972). In other

words the reduced circulating P in late pregnancy would be a trigger of parturition (Bedford et al., 1972). Conversely the high P level during early pregnancy may be beneficial for implantation because of the inhibitory effect of P on uterine contraction (Hammond, 1957; Bindon, 1971).

It is noteworthy that measurements of peripheral concentrations of hormones are static values that give little indication of the dynamics of metabolism and utilization. For example, a 100-fold increase in plasma P concentrations is caused by a decrease in metabolic clearance rate of approximately 90% and an increase in production rate of only about 3-fold (Illingworth et al., 1970; Challis et al., 1971b). However in the case of the declined P concentration in peripheral blood plasma prior to parturition, it has been shown to be due to both the failure of corpora lutea (Stabenfeldt et al., 1970) and an increase in metabolic clearance rate (Bedford et al., 1972).

2. Magnitude of plasma progesterone concentration:

The concentrations of plasma P recorded in the present work were quite different from those reported in the literature. Tillson and Erb (1967) found that P concentration increased from 8.4 ng/ml on Day 2 to 27.2 ng/ml on Day 12 - 14 after breeding, whereas the values

by Tillson (1969) were 14.2 and 29.6 on Day 2 - 4 and 14 - 16 after breeding respectively. It should be made clear first that the time when the P levels start rising and the time when the maximum P level occurs vary between studies. It may be that the values obtained at stages 1 and 3 in the present experiment are not the minimum and the maximum ones during early pregnancy. Therefore the author does not try to compare these values with the minimum and the maximum ones reported in the literature. Attempt will be given at the comparison of P levels on the day-of-pregnancy basis. During late pregnancy, the values of 10.71, 9.27 and 7.54 ng/ml for P concentration taken on day 100, 110 and 112 respectively, obtained here were markedly smaller than those reported by Shearer (1971), viz., 15, 35-40 and 35-40 ng/ml recorded at these stages respectively. Although the difference in the technique used to determine P levels in blood plasma has shown to have very little effect on the results obtained, it might also be a possible factor contributing to the discrepancy between studies (Neill et al., 1967; Stamenovic et al., 1970; Edqvist and Lamn, 1971). Other factors likely to contribute to the discrepancy between studies include:

(a) Factors involving CPB technique:

(i) Florisil - The absorbent used in the present work to separate the protein-bound from the unbound H^3B was florisil. As pointed out by Demetriou and Austin

(1970) one problem area in P assays is the quality of the florisil. Strott and Lipsett (1968) have indicated a variable response of H^3B to different batches of florisil. Mill (1968) has reported that the sodium sulphate content is the principal cause of variation of adsorbancy between batches of florisil.

(ii) Mixer - The mixing procedure for separation of the free H^3B and CBG- H^3B may also be a source of error. The introduction of a rotary mixer by Demetriou and Austin (1970) has eliminated certain problems and provided other advantages over the individual mixer. With the vortex mixer used in the present work, variability was encountered due to the individual technician, a fatigue factor when large numbers of samples were processed. Another problem was the generation of florisil fines by this vigorous agitation device.

(iii) Standard protein solution (CBG- H^3B) - The quality of CBG- H^3B may also affect the result of the assay. Many of the reports related difficulties with the stability of the CBG- H^3B (De Souza et al., 1970; Johansson, 1970). A possible explanation for this instability may be the adsorption of P to glass surfaces (Demetriou and Austin, 1970).

(iv) Temperature - During the procedure of protein

binding assay, at the stage when 0.1 ml of CBG-H³B was added to the tubes containing extracted unknown plasma, the tubes were placed in a 45°C water bath to bring the P into solution (Murphy et al., 1963)... However Robertson and Sarde (1971) noted that satisfactory results can be obtained at 25°C rather than by using a water bath at 40°C. On the other hand it should be noted that the adsorption of corticoids decreases at higher temperature from 10°C to 45°C (Murphy, 1967).

(v) Recovery rate - The effectiveness of the technique for P assays can be judged by the recovery rates of P added to plasma. The spectrophotometry technique described by Short (1958) and used by Short (1960) gave a rather poor recovery rate, 40 - 43%. The method of Zander (1962) used by Brinkley and Young (1968) had a recovery rate of 73%. The value reported by Abraham et al. (1971) in using radioimmunoassay was 84.2%. The difference in the recovery rate may be a source of contradiction between studies. With the value of recovery rate (92%) obtained, the present CPB assay appeared superior to most of those reported in the literature, e.g. Donaldson et al. (1970): 80.8%; Demetriou and Austin (1970): 81% ...

(vi) Reading method - Many investigators have been content to fit the standard curve "by eye", and to read the sample concentrations without any attempt to

provide confidence limits for the results obtained. Burger et al. (1971) suggested a statistical technique for obtaining the best fit of the standard points, with subsequent calculation of unknown hormone concentrations and their errors. These authors also suggested the model for the standard curve. However they did not mention the use of the model is the case of the CPB assay. In the present work, the standard curves were fitted "by eye" and the values of P concentrations in unknown plasma were also read very approximately. This procedure was likely to involve some degree of error and this in turn contributes to the discrepancies between the values of P concentration reported in previous studies.

(b) Other factors affecting P levels in peripheral plasma in sows:

(i) Number of corpora lutea -

In most species, including the pig, during early pregnancy, corpora lutea are the most important source of P. It may be expected that the production of P is proportional to the number of corpora lutea. Stormshak et al. (1963), Short (1964), Rombauts et al. (1965, 1971), have proved this proposition. Brinkley and Young (1970) also found that numbers of corpora lutea were linearly and positively correlated to ovarian P secretion rate (mg P secreted per minute). It may be then speculated that

the P content in peripheral plasma varies with numbers of corpora lutea or ovulation rate. However, this was not proved by work of Niswender et al. (1970) on gilts and Polidoro and Black (1970) on rabbits. The results reported herein too, did not show any significant correlation between ovulation rate and P level in peripheral blood plasma taken at the first three stages. Polidoro and Black (1970) suggested that ovarian P secretion might be greater in females with higher ovulation rates, but these females might also have higher metabolic clearance rates. This hypothesis remains to be proven. In summary, even though evidence is not conclusive on the relationship between numbers of corpora lutea and P levels during early pregnancy in sows, it is not unreasonable to exclude this possibility, ie., higher ovulation rates may result in higher rates of P production and higher levels of P in peripheral plasma. Consequently, the difference in ovulations rates between sows of different studies may result in the difference in P levels of peripheral blood plasma ...

(ii) Numbers of foetuses or embryos -

During the later stage of pregnancy, it was reported that the peripheral levels were significantly higher in ewes (Bassett et al., 1969), women (Johansson, 1969b) with twins than those with single foetuses. However, in these species the placenta has been shown

to be an important source of P (Zander and von Munstermann, 1956; Maire et al., 1968; Gadsby et al., 1972). In the pig, on the other hand the placenta does not produce P (Rombauts et al., 1971), so the situation may not be the same. Erb et al. (1962) reported that correlations between numbers of implants and P contents and concentration in luteal tissue were not significant. Tillson and Erb (1970) also reported that levels of LH in blood plasma showed no variation associated with numbers of embryos. So it appears that the production rate of P by corpora lutea is not influenced by the number of fetuses (or embryos). In agreement with this trend, the present results did not show a relationship between litter size at birth and P levels in peripheral plasma at either of the last three stages. The correlations between numbers of corpora lutea and the P levels at these stages were not significant.

It is noteworthy that in the present experiment the numbers of mummified fetuses were significantly correlated to the P levels at stage 5 ($r = 0.576$, $P < .05$) and stage 6 ($r = 0.644$, $P < .01$). Pomeroy (1955) has theorized that excess P may account for relatively greater embryo death in some sows. Administration of P at levels of 150-200 mg/day commencing 4 - 8 days after breeding markedly increased embryo death as observed at Day 25 of pregnancy (Sammelwitz et al., 1956; Spies et al., 1957). There does not appear to be any clear

cut hypothesis concerning why this phenomenon occurs. It is not known also with any certainty whether the embryo death in the present study is the result or cause of high levels of circulating P. It has been shown that embryos additively influence ovarian steroid metabolism (increase) (Edgerton et al., 1971). Edgerton and Erb (1971a) found that gilts having more fetuses excreted more total pregnanes (metabolites of P in sow urine) throughout the experimental period from Day 28 to 75 of pregnancy.

In summary, an increase in the numbers of fetuses was not followed by an increase in production rate of P in corpora lutea, but in the rate of metabolic clearance. It may be speculated that P levels in peripheral plasma decrease in sows with larger litter size. Moreover in the present work the results did not show any significant correlation between numbers of viable piglets and P levels.

(iii) Nutrition --

Nutrition has been shown to be an important factor contributing to embryonic loss in the sow ... As P is essential for the maintenance of pregnancy in this species nutrition is expected to have some effects on circulating P. Cumming et al. (1971) reported that during early pregnancy P levels were greatly elevated in low-fed ewes compared to high-fed ones. The same trend was found by Lamond et al. (1972) on ewes, Donaldson

et al. (1970) on cows, but no work on sows has been reported. The results obtained in the current experiment also revealed this adverse effect of nutrition on P levels. The high-fed group had significantly lower levels of P than the low-fed one ($P < .025$). The mechanism involved is not understood, particularly as this effect of nutrition was found only at stage 5. The increased P levels in the low-fed group may not be due to an increased rate of P secretion (Cumming et al., 1971).

(iv) Stress -

Stress has been shown to affect P production. Blood samples collected at slaughter had higher levels of P in peripheral plasma than those collected under normal physiological conditions (unpublished observations, cited by Stabenfeldt et al., 1969d). The stress of killing may have caused a release of P by the adrenal cortex. Harrisson and Heap (1968) showed that transplanted adrenals secreted P throughout gestation in the ewe. Thus the adrenals may not only release large amounts of P during stress, but also may serve as a source of P in normal physiological conditions (Stabenfeldt et al., 1969d). The adrenal gland has been also shown to be a source of P in sows, especially under stressed conditions (Balfour et al., 1957; Erb et al., 1962; Heap et al., 1966), in cows (Shemesh et al., 1968;

Donaldson et al., 1970), rats (Feder et al., 1971), sheep (Harrisson and Heap, 1968). The stress caused by blood collection may bias the results of P outlined in some studies. Gilts in this experiment were restrained during collection and the process was rather long; whereas in some other experiments (Shearer, 1971) blood samples were taken through a catheter which was inserted into the jugular vein permanently. So blood would be collected easily without causing much stress on sows.

This is also supported by the fact that blood samples collected at laparotomy in the present work had considerably higher levels of P as compared with those at stages 1 and 3. Therefore it is legitimate to conclude that the difference in stressed condition of pigs at blood collection may contribute to the difference in P levels between studies. It should be pointed out that, at laparotomy (stage 2) blood samples were collected from the vena cava, instead of ear veins as were collections at the other five stages. A comparison of 142 pairs of plasma samples (one was taken from jugular vein, the other was from vena cava) showed a significantly higher P concentration in the vena cava (Davis et al., 1970).

The time in the day, when blood samples are collected, may be of importance. In women, Lurie and Willee (1966) suggested that plasma P concentration

during pregnancy might show a diurnal rhythm. Johansson (1969b) also found that morning samples of blood had higher levels of P than those taken during the remainder of the day. There is no evidence whether this situation exists in the pig (and other animals). Blood samples in the current work were not collected at any particular time in the day, but most of them were collected in the morning.

In addition to the above factors, P levels have also been shown to vary with breeds (Stabenfeldt et al., 1969c), age (Das and Magilton, 1971) of animals. They are also different between individuals (Bindon, 1971) and seasons (Lamond et al., 1972).

It is noted that all gilts in the present experiment were sick during pregnancy. As previously presented, three gilts aborted during the second and last month. Leptospirosis was suspected to be the cause, but this was not confirmed. Small numbers of E.coli and Streptococci were cultured from a swab from the allantoic fluid. Johansson (1969b) reported a lowered P concentration in peripheral plasma of two women aborted by severe toxemia. Osburn et al. (1969) inoculated viable cultures of vibrio fetus into the uteri of cows in the second and third month of pregnancy. This resulted in abortion, and peripheral plasma P levels declining at the time of foetal death. Similar results were obtained by Wiest et al. (1970); Shemesh et al. (1968); Wood et al. (1971). So far, no

explanations for this phenomenon have been suggested.

VI Conclusion:

It is difficult to determine with any certainty a detailed pattern of P levels in peripheral plasma during pregnancy, unless blood samples are collected on a daily basis. From the results obtained herein, it may be concluded that during early pregnancy P levels in peripheral plasma rise considerably from Day 3 to Day 15. This coincides with the stage of development of corpora lutea reported elsewhere. The P levels after Day 15 are not known from this work. No comment could be given on the method of pregnancy diagnosis based on the maintenance of P levels toward the onset of the oestrus following breeding (Robertson and Sarda, 1971; Walker, 1972 - in sows; Shemesh et al., 1968; Pope et al., 1969; Robertson et al., 1971 - in cows.)

Within a limited number of peripherally blood collections, it is not known when (stages of pregnancy) plasma P levels start rising and when they reach maximum values during early pregnancy. The minimum and maximum values of P are not known.

The gradual drop in plasma P levels during late pregnancy might also be used as an indication of farrowing. However it is not known from this experiment at what stage (in relation to farrowing day) P level starts declining, and what the standard levels of P during large pregnancy are ... Actually the values of plasma P levels obtained from each experiment may vary with the technique of assay

used, ovulation rate, number of embryos or foetuses, breeds, ages ... of pigs and plane of nutrition employed.

Low-fed gilts had higher levels of plasma P than the high-fed ones. The results obtained did not reveal any explanation or mechanism for this. The situation became more difficult because this effect of nutrition was found only at stage 5, eighty days after feeding treatments terminated.

The mummified foetuses were significantly correlated with P levels at the last two stages of blood collection. No reasonable explanation for this has been suggested.

CHAPTER 4

RECTAL TEMPERATURE DURING OESTROUS CYCLES AND LATE
PREGNANCY IN GILTSI Literature review:1. During oestrous cycle:

The effect of estrual activity on body temperature has not been studied extensively in the pig. In women, during the follicular phase, the basal temperature remains low until at approximately mid-cycle the temperature rises shortly over a period of normally two days often by as much as 1°F (Marshall, 1965; Brown, 1965). This higher level is generally maintained until the onset of menstruation. The temperature rise, when it is greater than 0.4°F , is considered to be indicative of the occurrence of ovulation. A biphasic temperature curve during oestrous cycle has also been reported in cows (Vollman and Vollman, 1942; Wrenn et al., 1958; Fallon, 1959; King, 1964; Valdivia and Vallenias, 1966; Kumaran and Iya, 1966), in ewes (Cockrem and McDonald, 1969), in mares (Chieffi et al., 1964), in goats (Parer, 1963) and in rabbits (Behren et al., 1970). Accordingly, the daily variation in body temperature was random in nature throughout the estrual cycle except a significant rise during oestrus. The only report on pigs, which could be

found in the literature (Sanders et al., 1964) also showed a cyclic trend in body temperature with temperature elevations occurring near the time of ovulation.

However this biphasic pattern of body temperature is not in agreement with those reported by Du Bois (1948) on human beings, by Christie and Bell (1970) on beagle bitch with respect to the timing of the rise of temperature in relation to oestrous cycle. The authors noted a drop in body temperature occurring at ovulation, followed by a rise to a level higher than the previous one. Furthermore, data obtained by Brown et al. (1962), Christian (1966) and Kumaran and Iya (1966) indicate that irrespective of ovulation occurring, there is pre-estruual drop followed by a rise in temperature during oestrus. Many other authors also agree that there is generally a poor correlation between fluctuations in temperature and the oestrous cycle (Brown et al., 1958; Greenstein et al., 1958; Tsutsumi et al., 1969).

In summary, evidence so far is not conclusive about the changes in body temperature during oestrous (or menstrual) cycles. Some authors reported the random fluctuation of temperature during oestrous cycles, whereas others noted that body temperature changes followed a biphasic pattern. The timing of the rising portion of temperature curve in relation to oestrous cycle was also not in agreement between studies.

2. During late pregnancy:

Several domestic animals show a slight rise in body temperature in late pregnancy and a decline in the period just before parturition (see Weisz, 1943). In cattle (Ewbank, 1963) this drop measures some 0.6°C and commences, on average, 54 hours before calving. The healthy cow, even when exhibiting external signs of imminent parturition, such as mammary distension, relaxation of the sacrosciatic ligaments and vulva enlargement, is unlikely to calve within the succeeding 12 hours if its rectal temperature is above 38.8°C , the normal temperature being 38.5°C . A similar drop in rectal temperature has also been reported in pregnant ewes (Ewbank, 1969) and rabbits (Tsutsumi et al., 1969). However, Hafez et al. (1956) found that pregnancy has no effect upon the body temperatures of Egyptian fat-tailed sheep; and Roberts (1964) briefly reported that the deep vaginal temperature has little practical value in predicting the time of the onset of parturition.

II Methods and Materials:

1. Animals:

Based on regularity of heat, sixteen gilts were chosen for the study of body temperature during oestrous cycles. Due to many reasons mentioned in Chapters 1, 2 (failure to come into heat, abortion ...) six of these sixteen gilts were replaced by another six pregnant ones for the study during late pregnancy.

2. Housing the experimental management:

Refer Chapter 1, 2 -

A few days prior to parturition, the gilts were transferred to farrowing house, a "semi-opened" building.

3. Measurement:

For fifty consecutive days (from 23.9.1971 to 12.11.1971), rectal temperatures of gilts were recorded once daily between 09:00 and 10:00 hr, with an electronic thermometer, accurate to $\pm 0.1^{\circ}\text{C}$, by inserting an all-purpose probe 10 to 12 cm into the rectum. The thermometer used was frequently checked for accuracy. The first week of this period was used to condition the gilts to the experimental procedures; hence, the test period consisted of about two cycles.

Rectal temperatures were also recorded daily during the last fifteen days of pregnancy (this period extended from 19.2.1972 to 8.4.1972). All readings were

recorded to the nearest 0.1°C . Difficulties in measurement faced were a great variation in the readings of rectal temperatures with the condition of the rectum of gilts, ie., constricted or relaxed. The error of measurement might be as great as $\pm 1.0^{\circ}\text{C}$.

4. Statistical analysis:

The results were subjected to analyses of variance of a two-way classification (Snedecor and Cochran, 1969). The rectal temperatures were tested against days of oestrous cycles or pregnancy. The comparison of rectal temperatures between days were done by the "least significant difference" method (Clarke, 1969).

III Results:

Daily rectal temperatures of each gilt during oestrous cycle and late pregnancy are presented in Appendices 14 and 15. The results are summarized in Table 6. The results of the analysis (Table 7) shows that rectal temperatures of gilts vary significantly between days recorded (during oestrous cycles and late pregnancy).

Table 6: Mean of rectal temperature ($^{\circ}\text{C}$) during oestrous cycle and last 15 days of pregnancy.

(n = 16)

Oestrous Cycle		Pregnancy	
Days (a)	Mean	Days (b)	Mean
-10	38.84	-15	39.53
- 9	38.77	-14	39.55
- 8	38.88	-13	39.56
- 7	38.74	-12	39.51
- 6	38.83	-11	39.49
- 5	38.75	-10	39.52
- 4	38.82	- 9	39.49
- 3	38.75	- 8	39.55
- 2	38.84	- 7	39.51
- 1	38.83	- 6	39.58
0	38.81	- 5	39.45
1	38.75	- 4	39.28
2	38.73	- 3	39.14
3	38.88	- 2	39.23
4	38.91	- 1	39.36
5	38.94		
6	38.96		
7	38.90		
8	38.90		
9	38.93		
10	38.87		
SE of mean difference ± 0.07		SE of mean difference ± 0.09	

1. During oestrous cycle:

From Day -10 until Day 2 of the oestrous cycle, rectal temperatures fluctuated slightly around 38.79°C . Then they rose significantly ($P < .01$) from 38.73°C on Day 2 to 38.96°C on Day 6 and tailed off until Day 10. The results are illustrated in Figure 4.

2. During late pregnancy:

From Day 15 to Day 6 prior to farrowing, gilts showed relatively constant temperatures. Rectal temperatures dropped significantly ($P < .001$) from 39.58°C on Day 6 to 39.14°C on Day 3 and then rose slightly to 39.36°C on Day 1 before farrowing (Figure 5). Records on farrowing days were not available, since most of the gilts had farrowed early in the morning before temperatures were taken.

Table 7: Analysis of variance of rectal temperatures during oestrous cycle and last 15 days of pregnancy.

Source of Variation	Oestrous Cycle			Last 15 days of pregnancy		
	df	MS	F.test	df	MS	F.test
Days	20	0.080	1.901 ^{**}	14	0.281	4.257 ^{***}
Gilts	15	0.531	12.360 ^{***}	15	0.490	7.405 ^{***}
Error	300	0.042		210	0.066	
Total	335			239		

^{**} $P < .01$, ^{***} $P < .001$

Figure 4 - Average Daily Rectal Temperatures of 16 gilts During Oestrous Cycle.

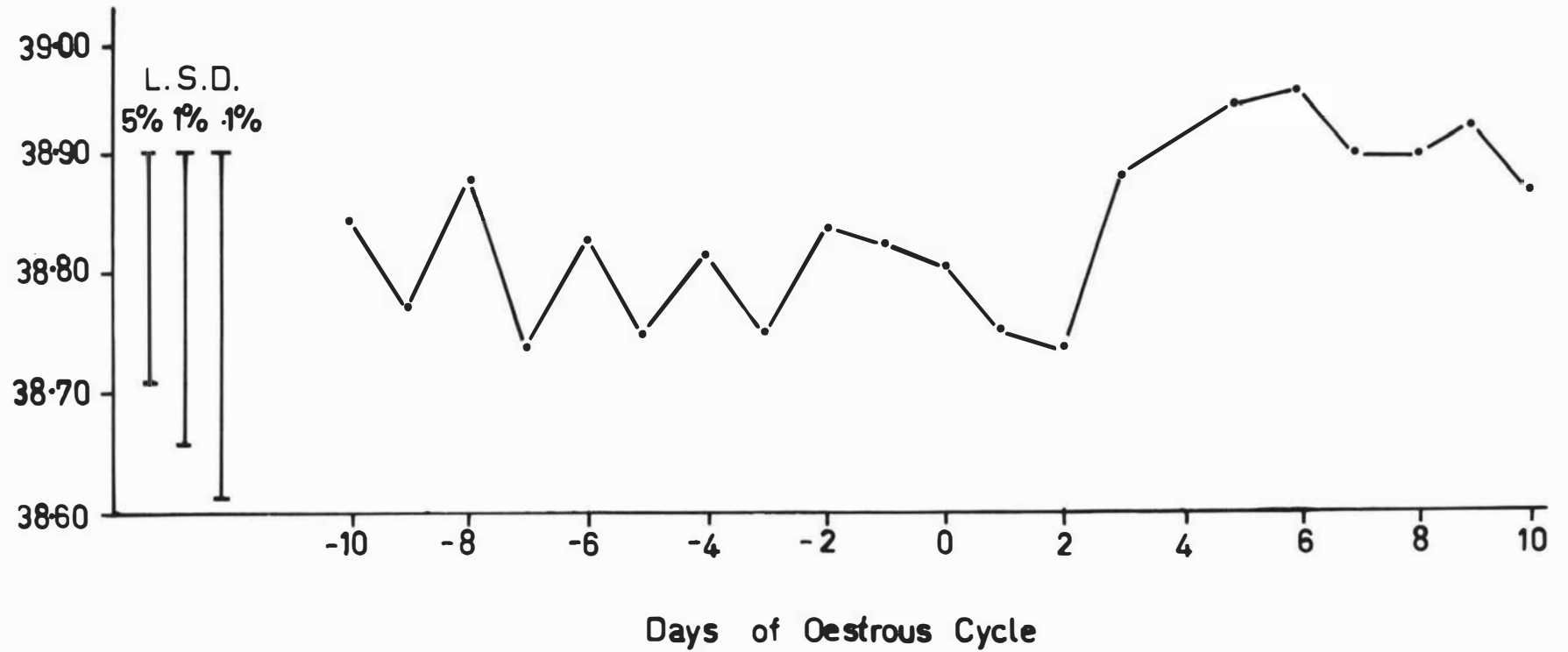
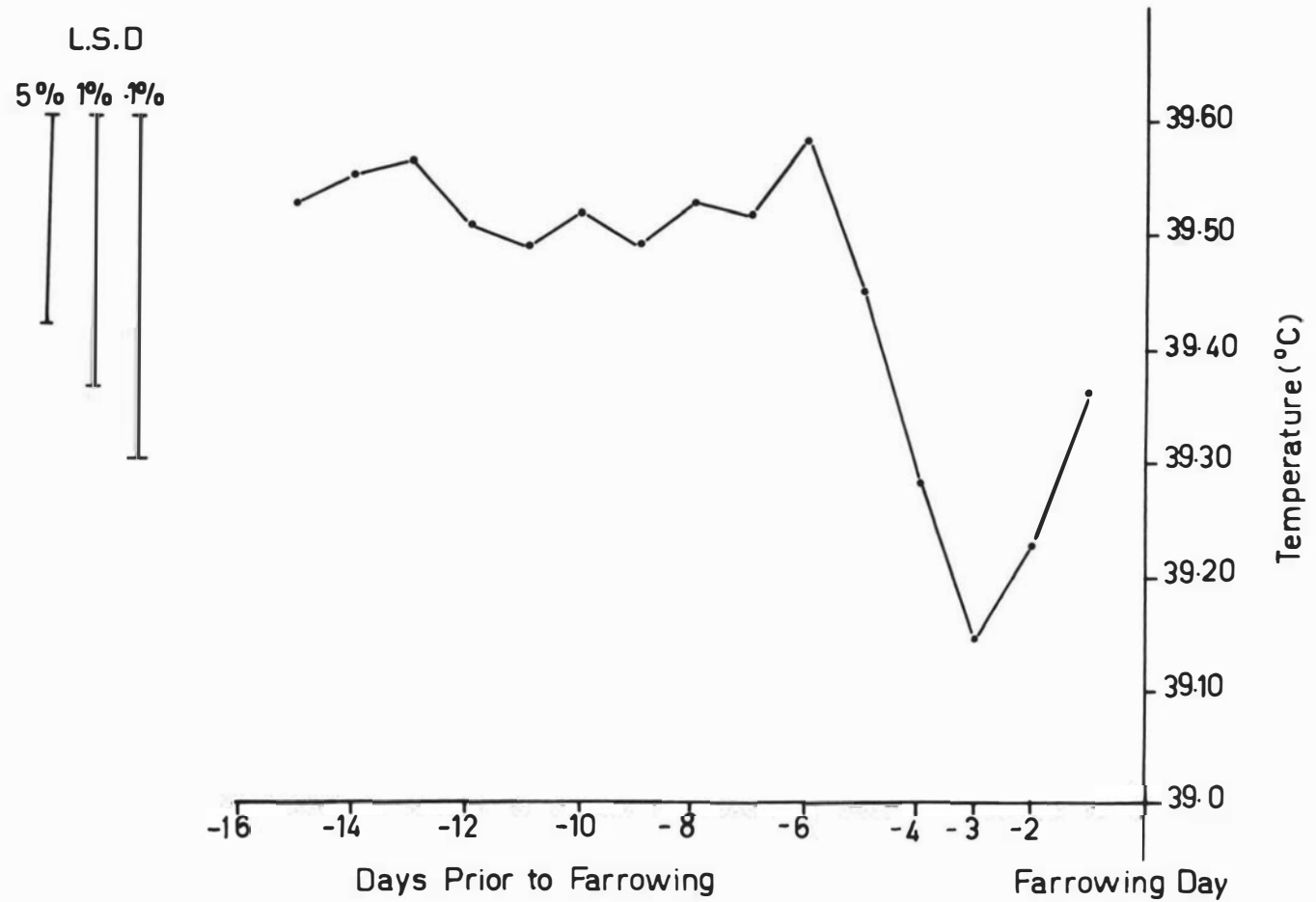


Figure 5 - Average Daily Temperatures of 16 Gilts During the Last 15 Days of Pregnancy



IV Discussion:

In the present study, no attempt was made to regulate the humidity of the experimental house. Behren et al. (1970) noted that humidity significantly affected rectal temperatures of rabbits. Therefore this should be borne in mind in comparing the results obtained herein and those reported elsewhere.

1. During oestrous cycle:

The values of rectal temperature during Day -10 to Day 2 of the oestrous cycle obtained in this experiment were similar to those reported by Sanders et al. (1964). The rise in temperature from Day 3 to Day 6 was in agreement with work by Du Bois (1948), Christie and Bell (1970). The latter found that most of their experimental beagle bitch showed a thermal nadir during oestrus followed by a period of higher temperature values. However many authors found the rise in temperature one, two days prior to oestrus or during oestrous period (Marshall, 1965; Brown, 1965; Cockrem and McDonald, 1969). It is difficult to find an adequate explanation for this difference when the mechanisms involved in this temperature rise (if any) are not understood. Sanders et al. (1964) suggested that the increased muscular activity associated with oestrus contributed to increased temperature, since it is well known that the body temperature rises during exercise (Nielson, 1938; Benzinger, 1959; Bradbury et al., 1964).

Furthermore, small animal studies (Lee and Van Buskirk, 1928) suggest that basal metabolism increases at oestrus independent of activity trends. However this explanation does not fit the situation where temperature rises a few days after heat as observed in the present experiment and elsewhere. Brown (1965) noted that in some women the temperature rise may extend over a number of days and occasionally show a step-rise upward progression. It is possible that this extended rise is associated with the retarded growth of the corpus luteum due to the unduly prolonged period of escape of follicular fluid after rupture (Decker, 1951). In the present work, it was not known exactly when ovulation occurred in relation to the time of temperature rise. Burger (1952) found sows with all follicles intact up to 54 hours after the onset of oestrus. It might be that the rise in rectal temperature reported herein was about the time ovulation ... On this basis the increased muscular activity and basal metabolism may be the causes of the temperature rise during oestrus. However this cannot explain the temperature rise in ewes whose oestrus was suppressed and ovulation did not occur (Christian, 1966; Brown et al. 1962). So far no explanations have been suggested.

2. During late pregnancy:

Decreased rectal temperature on Day 3 prior to farrowing is in accordance with the results obtained by

Ewbank (1963, 1969), Tsutsumi et al. (1969), but not with those by Hafez (1956), Roberts (1964) ... The mechanisms involved have not been investigated. One factor affecting body temperature is the rate of heat loss and it is of interest that Brockway et al. (1963) reported an increase in heat loss to the environment for sheep during the last 24 hours of pregnancy. However this does not explain the drop of temperature at as early as 3 days in the present work or 54 hours (Ewbank, 1963) before parturition. It should be noted that gilts in the present work were transferred to farrowing house which was not a totally enclosed building as the experimental one. Ingram (1964), Rawson et al. (1965), Spies et al. (1965), Holmes (1970) and Ingram and Legge (1970) noted that body temperature was significantly influenced by environmental temperature. The variation in environmental temperature explained 35% of the daily variation in body temperatures (Christian, 1966). So the results of the rectal temperature during the time in farrowing house might be biased.

3. Some possible factors which might have some influence on the results:

In general, discrepancies still exist between the present results and those reported elsewhere. No explanation can be suggested since the mechanism involved is not known. However, in judging the results obtained,

in addition to some factors mentioned above (eg., time of ovulation, ambient temperature, humidity ...), the following factors should be taken into account:

- Body temperature may vary with age of animals (Cockrem and McDonald, 1969).
- Meat-type pigs have been shown to have significantly higher temperature than fat-type ones (Ilancic et al., 1965).
- There are differences in body temperature recorded at different time of the day (Fallon, 1959; Parer, 1963; Mendel and Raghaven, 1964; Chieffi et al., 1964; Taneja, 1966; Behren et al., 1970).

4. Validity of body temperature measurement:

From the above results it appears that the increase in rectal temperature during oestrous cycle as well as their drop during late pregnancy cannot be used as a reliable prediction of the time of ovulation and parturition. This was formerly suggested by Fallon (1959), Schilling and Rostell (1965), Ewbank (1963, 1969), Christian and Bell (1970). Moreover, it is well established that body temperature is maintained by a theremo-regulatory system. A comprehensive text on the environmental physiology in relation to pig production has been published by Mount (1972). Accordingly the rate of heat retention was calculated as in the following equation:

$$ME = HL + ER$$

where ME : intake of metabolisable energy

HL : rate of heat loss

ER : rate of retention of energy.

The ER, on a given feed intake, varies with the rate of heat loss. Panting (Ingram, 1964; Ingram and Legge, 1972) or increased pulse rate (Ingram, 1964) are some usual ways to dissipate heat (vaporisation) ... Therefore, if body temperatures do change during oestrus or late pregnancy, the amplitude will not be very big. For examples, the increase in body temperatures during oestrus period was recorded as 0.11°C in mares (Chieffi et al., 1964), about 0.3°C in one gilt (Sanders et al., 1964) ... So, with only one observation, it is very difficult to detect this variation in body temperature.

V Conclusion:

Under the conditions of the present experiment, gilts showed a rise in rectal temperature at about a few days after the onset of heat and a drop at about a few days prior to parturition. However a big variation in the readings due to measurement technique, environmental factors including feeding and activity, makes it unreliable for use to predict the time of ovulation and parturition.

GENERAL CONCLUSION

As body condition at mating has been suggested to be partly responsible for the ineffectiveness of flushing on ovulation rate; and as continuously full-fed gilts produced the same ovulation rate as gilts full-fed for only two - three weeks (Zimmerman, 1959), it is suggested that gilts can be kept on a low plane of feeding until two or three weeks prior to service, when they are flushed. However, within the scope of the present experiment an accurate scale of feeding for gilts is not known. In other words, the question of at what stage of growth gilts should be kept on a low plane of feeding; and what are the daily allowances for flushed gilts or for those on low plane, still remain to be answered.

It has been suggested that high feeding of a thin sow or gilt may improve her litter performance but high feeding a fat sow or gilt may not be advantageous. However although gilts in this study were thought to be fat, a high level of feed intake during the first month of pregnancy was shown to increase litter size at birth, the results were still questionable because of the outbreak of abortion, ~~unknown-cause~~ constipation ...

Though the results do not give any information about progesterone (P) level during oestrous cycle, the high level of plasma P partly supports the suggestion

of using P measurement as a tool to diagnose pregnancy in gilts or sows. The drop in P prior to parturition might also be useful as a sign of parturition. However since P levels declined gradually toward parturition, it is difficult to predict with accuracy when parturition occurs.

Body temperature does not appear to be a reliable measurement in predicting ovulation and parturition time in this experiment because of its great daily variation.

It is well established that corpora lutea (CL) are the main source of P. Flushing did not have any effect on either ovulation rate or P level.

Both endogenous and exogenous P have been shown to improve embryo survival (Glassgow et al., 1951; Davis and Sorensen, 1959). High plane of nutrition during the first month of pregnancy was shown in the current work to enhance embryo survival. Therefore high plane of nutrition was expected to be positively correlated with P levels. However this was not the case. On the other hand, high-fed gilts tended to have lower P levels than low-fed ones at four stages (1, 4, 5 and 6, the difference was significant at stage 5). Actually results about the effect of P on embryo survival are not conclusive. Furthermore information about litter size at birth and P measurements might be biased by the incidence of disease during pregnancy.

Both P level and body temperature of gilts

decreased prior to farrowing. Whether or not there is any relation between these two measurements is not known. However it was noted that body temperature of gilts tended to increase around ovulation time whereas P levels remained low during this period.

APPENDICES

APPENDIX 1 - Composition (%) and calculated nutrient contents (% dry matter) of meal mixtures.

Meal	1	2
Body weight range (kg)	22-53	54- farrowing
Barley Meal	85.40	87.18
Meat Meal	6.83	10.03
Fish Meal	6.83	1.92
Bone Flour	0.43	0.44
Salt	0.21	0.22
Trace element/vitamin supplement	0.21 (a) [‡]	0.22 (b) [‡]
Digestible energy (Mcal/kg DM)	3.47	3.44
Crude Protein	19.2	18.2
Lysine	0.95	0.72
Methionine + cystine	0.63	0.55
Ca	0.93	1.02
P.	0.86	0.90

(a)[‡] (b)[‡] See Appendix 2 for the composition of trace element/vitamin supplement

APPENDIX 2 - Reported composition of one kg Mineral
and vitamins mixture.

Mixture	(a)	(b)
Vit A	4400 IU	7997 IU
Vit D ₃	660 IU	799.7 IU
Vit E	19.8 IU	28.6 IU
Vit B ₁	1.375 mg	15.4 mg
Vit B ₂	3.509 mg	4.4 mg
Vit B ₆	4.004 mg	2.64 mg
Vit B ₁₂	0.011 mg	0.011 mg
Pentothenate acid	11.99 mg	13.2 mg
Nicotinic acid	13.904 mg	17.6 mg
Choline	220 mg	220 mg
Iron	69.96 mg	124.96 mg
Zinc	150.04 mg	130.02 mg
Manganese	44.99 mg	44.99 mg
Copper	179.94 mg	10.0 mg
Potassium	2.101 mg	2.101 mg
Cobalt	0.33 mg	0.33 mg
Selenium	0.154 mg	0.154 mg
3- nitro 4 hydroxy phenyl arsonic acid	25.5 mg	—

APPENDIX 3 - Scale of feeding.

Live weight of pigs (kg)	Daily meal allowance (kg)
20	1.04
25	1.22
30	1.40
35	1.55
40	1.71
45	1.87
50	1.99
55 and over	3% of body weight to a maximum of 2.3 kg [‡]

[‡]Due to general refusals the maximum daily allowance was reduced to 1.8 kg when animals weighed about 120 kg.

APPENDIX 4 - Age, weight, oestrus number of gilts at mating, length of flushing period, total energy consumed during flushing period, growth during previous fortnight, ovulation rate, litter size at birth and number of mummified foetuses.

F L U S H E D									
Pig No.	Age (Days)	Weight (kg)	Oestrus No.	Length of Flushing Period (Days)	Total Energy Consumed (Mcal DE)	Growth during previous fort- night(kg)	Ovul- ation Rate	Litter Size at Birth	No. of Mummi- fied Foetuses
21/68	280	130.5	6	11	118.8	9.31	16	10	0
20/78	264	122.7	3	9	97.2	6.69	14	10	0
17/67	284	117.3	7	10	88.5	7.01	15	NA	NA
16/9	281	124.5	6	11	115.0	10.00	13	NA	NA
14/5	277	128.2	-	15	152.9	4.48	15	9	3
14/68	275	123.6	-	12	129.6	6.10	14	8	0
12/8	279	112.3	6	10	94.5	4.09	16	NA	NA
17/9	285	122.3	7	11	117.5	6.11	15	1	0
16/6	275	134.1	5	11	108.6	10.45	11	NA	NA
11/6	283	131.8	5	12	126.1	10.00	15	3	0
25/68	282	112.7	6	12	118.1	3.09	14	12	0
10/68*	314	140.0	8	22	217.7	8.18	NA	2	3
Mean	278.6	123.6	5.67	11.3	115.2	7.03	14.4	6.87	0.75

NA: data not available.

*Data of these gilts were not included in all analyses of ovulation rate.

APPENDIX 4 - continued

NON FLUSHED								
Pig No.	Age (Days)	Weight (kg)	Oestrus No.	Total Energy Consumed (Mcal DE)	Growth during previous fort- night(kg)	Ovul- ation Rate	Litter Size at Birth	No. of Mummi- fied Foetuses
21/56	262	126.8	5	59.4	8.18	13	NA	NA
20/7	259	116.8	4	48.6	7.86	15	7	0
17/6	276	108.6	5	54.0	5.91	13	5	0
16/68	262	114.1	6	59.4	5.15	18	5	0
14/567	274	129.1	-	81.0	6.40	16	9	0
14/7	274	117.7	-	64.8	6.45	11	8	1
12/5	266	112.3	6	54.0	0.91	13	5	1
17/57	287	124.1	6	59.4	5.0	15	2	2
16/56	261	120.5	6	59.4	1.82	13	NA	NA
11/7	271	108.6	3	64.8	4.85	13	NA	NA
25/7	284	119.1	6	64.8	2.36	13	2	3
10/678 ^x	327	118.0	6	89.1	2.00	NA	6	0
Mean	270.5	107.8	5.22	60.9	4.99	13.9	5.44	0.78

APPENDIX 5 - Analyses of variance of ovulation rate
and litter size at birth.

Source of variation	Ovulation rate		Litter size at Birth	
	df	MS	df	MF
Treatments	1	1.136	1	8.668
Pairs	10	2.509	-	
Error	10	3.236	15	11.984

No treatment effects were significant ($P > .05$)

APPENDIX 6 - Correlation coefficients between ovulation
rate and some uncontrolled variables.

	Correlation Coefficient	R.S.D.	Sign. Test
Age of gilts at mating	0.058	1.709	NS
Weight of gilts at mating	0.116	1.563	NS
Liveweight gain during 14 days prior to mating	-0.008	1.763	NS
Oestrus number at mating	0.279	1.604	NS
Total energy consumed	0.138	1.692	NS
Length of flushing period	0.041	1.510	NS

NS: $P > .05$

APPENDIX 7 - Measurements of Backfat thickness (BFT) (in cm) of gilts.

NON FLUSHED						FLUSHED					
Gilts	Shou.	Mid	Loin	Ave. BFT	Weighted Mean	Gilts	Shou.	Mid	Loin	Ave. BFT	Weighted Mean
21/56	5.1	3.0	3.9	4.0	3.7	21/68	5.6	3.7	3.9	4.4	4.2
20/7	4.7	2.8	3.3	3.6	3.4	20/78	5.0	3.3	3.8	4.0	3.8
17/6	4.8	3.1	4.4	4.1	3.8	17/67	4.8	3.0	3.8	3.8	3.6
16/68	5.7	3.2	4.4	4.4	4.1	16/9	5.4	4.2	4.9	4.8	4.6
14/567	5.5	3.9	4.5	4.6	4.4	14/5	4.6	3.3	4.0	3.9	3.8
14/7	4.7	3.1	3.7	3.8	3.6	14/68	4.9	3.1	3.3	3.7	3.6
12/5	5.1	3.1	3.8	4.0	3.7	12/8	4.5	2.9	4.0	3.7	3.6
17/57	4.3	3.4	4.3	4.0	3.8	17/9	5.0	3.2	4.3	4.2	3.9
16/56	5.3	2.9	4.7	4.3	3.9	16/6	6.5	4.6	6.0	5.7	5.4
11/7	4.9	2.2	3.0	3.4	3.0	11/6	4.2	2.9	3.5	3.5	3.4
25/7	5.6	3.5	4.4	4.5	4.2	25/68	4.3	3.4	4.0	3.9	3.8

APPENDIX 8 - Correlations between live-animal ultrasonic measurements and ovulation rate.

Measurements	Correlation coefficient	R.S.D.	Significance Test
Shoulder	0.170	1.687	NS
Midback	-0.141	1.694	NS
Loin	-0.218	1.671	NS
Midback + Loin	-0.193	1.679	NS
Ave. BFT	-0.105	1.702	NS
Weighted means	-0.189	1.681	NS

NS: $P > .05$

APPENDIX 9 - Analysis of covariance and test of significance of adjusted treatment means - Litter size at birth adjusted.

Source of Variation	df	S_x^2	S_{xy}	S_y^2	Deviation from regression			F. Test
					df	SS	MS	
Treatment	1	167.0	634.7	8.54				
Error	15	2996	-336.4	171.23	14	133.46	9.53	
Total	16	3163	298.3	179.77	15	151.63		
Between adjusted means					1	18.17	18.17	1.907 ^(a)

(a) NS: $P > .05$

Adjusted treatment group means:

$$NF \quad 5.444 + 0.05 (276.2 - 279.17) = 5.592$$

$$F \quad 6.875 + 0.18 (282.5 - 279.17) = 7.474$$

APPENDIX 10 - Litter size, weight at birth and
average birth weight of pigs

Over- all	Pre- mating Groups	Post- Mating Groups	Pig No.	Litter Size	Litter Weight (kg)	Average Birth Weight (kg)	
	Non Flu- shed		17/57	2	2.14	1.07	
			Low	25/7	2	3.24	1.62
			12/5	5	7.13	1.43	
			10/678	6	9.07	1.51	
		\bar{x}_1'	3.75		5.40	1.41	
		High	14/567	9	14.04	1.56	
			14/7	8	9.43	1.18	
			20/7	7	10.83	1.55	
			16/68	5	6.44	1.29	
			17/6	5	7.18	1.44	
		\bar{x}_1''	6.80		9.58	1.40	
	\bar{x}_1	5.44		7.72	1.41		
	Flu- shed	Low	17/9	1	0.68	0.68	
			25/68	12	17.48	1.46	
			11/6	3	5.03	1.68	
			10/68	2	2.25	1.13	
		\bar{x}_2'	4.5		6.36	1.24	
		High	14/5	9	11.57	1.29	
			14/68	8	11.82	1.48	
			20/78	10	12.34	1.23	
			21/68	10	12.19	1.22	
		\bar{x}_2''	9.25		11.98	1.31	
	\bar{x}_2	6.88		9.17	1.27		
\bar{x}	6.12		8.40	1.34			

APPENDIX 11 - Summary of analyses of variance for
litter size, weight at birth and
average birth weight of pigs

Source	df	Litter Size (MS)	Litter Weight (MS)	Ave. Birth Weight(MS)
Pre-mating treat- ment	1	8.67	8.87	0.076
Post-mating treat- ment	1	60.0 ^{***}	96.42 [*]	0.006
Interaction	1	5.79	5.76	0.003
Error	13	8.10	18.75	0.069

^{*}P < .05, ^{***}P < .025

APPENDIX 12 - Progesterone levels in blood plasma (ng/ml) of gilts during early and late pregnancy.

	Pig No.	Stage 1			Stage 2			Stage 3			Stage 4			Stage 5			Stage 6		
		F	NF	\bar{x}	F	NF	\bar{x}	F	NF	\bar{x}	F	NF	\bar{x}	F	NF	\bar{x}	F	NF	\bar{x}
	12/8 - 12/5	18.7	18.1		30.6	17.1		19.1	20.3		12.5*	11.8							
	17/9 - 17/57	8.4	5.1		29.7	29.1		22.1	13.3		7.9	6.4		5.9	17.2*		5	10.7	
I	11/6 - 11/7	10.8	13.6		23.7	40.6		15.6	30.5		10.1	9.3*		8.3	10.5*		7.9	6.6*	
	10/68 - 10/678	13	5.8		35.5	8		18.2	20.7		17.1	9.7		12.8	11.1		13.6	3.8	
	25/68 - 25/7	7.3	12.5		30.5	30.7		23.1	22.7		8.2	14.8		15.5	12.6		12.5	12.7	
	\bar{x}	9.82	11.67	10.74	29.49	27.62	28.55	20.53	21.15	20.84	11.0	10.42	10.71	8.08	10.46	9.27	7.59	7.50	7.54
	20/73 - 20/7	8.8	11.2		19.9	27.3		21.4	25.5		11.1	12.5		5.6	3.2		5.3	4.5	
	17/67 - 17/6	6	6.7		22.8	34.4		13.9	16.9		13.9*	13.5		6.7*	3.2		8.3*	9.2	
II	14/5 - 14/567	9	7.6		29.3	39.9		27.9	25.7		8.6	8.7		10.3	8.6		6.4	8.3	
	14/68 - 14/7	9.3	10.5		35.1	22.7		30.3	20.1		12.1	3.1		7.1	9.6*		5.7	6.6*	
	21/68 - 16/68	6.4	7.9		32.8	25.9		13.7	15.8		8.5	9.4		6.5	13.1		3.6	5.1	
	\bar{x}	8.39	13.10		29.06	28.05		21.12	20.56		10.64	10.78		7.29	11.74		6.30	9.10	

*estimates of missing plots

APPENDIX 13 - Summary of analyses of variance of plasma progesterone levels at six stages of blood collection (a)

Source	df	Stage 1 MS	Stage 2 MS	Stage 3 MS	df	Stage 4 MS	df	Stage 5 MS	Stage 6 MS
Level (H vs L)	1	110.921	5.101	1.568	1	0.098	1	87.912 [*]	34.844
Litter within level	8	69.171	41.343	37.365	8	10.355	7	14.757	8.653
Treatment (F vs NF)	1	17.113	17.485	1.922	1	1.682	1	25.442	0.036
Interaction	1	5.725	81.205	7.938	1	0.162	1	0.084	5.280
Error	8	22.206	87.115	26.668	5	11.695	4	19.811	15.968

(a) All differences are not significant ($P > .05$) except where asterisks are shown.

^{*} $P < .05$

APPENDIX 14 - Rectal temperature ($^{\circ}\text{C}$) during oestrous cycle

Gilts	16/6	16/9	16/68	16/56	21/56	21/68	17/57	17/6	17/67	17/9	12/8	12/5	25/7	25/68	10/68	10/678	\bar{x}
Days of the Oestrus cycle (a)																	
- 10	39.0	38.8	38.7	38.6	39.0	38.9	38.8	39.2	38.6	38.8	38.7	39.1	39.0	38.6	38.9	38.8	38.844
- 9	38.9	38.6	38.5	38.9	38.7	39.2	38.7	38.3	38.9	38.9	38.7	38.8	38.8	38.7	38.9	38.3	38.769
- 8	39.1	38.6	38.8	39.0	39.3	39.0	39.0	38.6	38.4	38.8	38.7	39.0	39.2	38.3	39.0	38.8	38.882
- 7	39.0	38.9	38.8	38.5	38.9	39.1	38.7	37.5	38.3	38.5	38.9	39.0	38.7	38.8	38.8	38.9	38.738
- 6	39.3	39.3	38.9	38.5	39.1	39.1	38.6	37.9	38.7	38.8	39.0	39.0	38.9	39.1	38.6	38.5	38.832
- 5	38.8	38.5	38.6	38.6	39.1	39.3	38.5	38.1	38.9	38.6	38.7	38.7	39.1	39.2	38.7	38.6	38.750
- 4	39.1	38.3	38.5	38.6	38.8	39.3	38.6	38.6	38.9	38.6	38.7	38.8	39.3	39.0	39.0	38.5	38.819
- 3	38.5	38.5	38.9	38.4	39.2	39.1	38.7	38.6	38.9	38.6	38.7	38.7	39.3	38.3	38.5	38.6	38.750
- 2	38.9	38.9	38.7	38.7	38.9	39.4	38.7	38.7	38.9	38.6	38.9	38.9	38.9	38.7	39.0	38.6	38.838
- 1	39.2	39.1	38.8	38.3	39.0	39.2	38.8	39.0	38.9	38.5	38.8	38.3	38.7	38.9	38.9	38.8	38.825
0	39.0	39.1	38.8	38.6	39.0	39.2	38.8	38.7	38.7	38.6	38.6	38.5	38.8	38.7	39.0	38.8	38.807
1	39.1	39.2	38.3	38.3	38.9	39.0	38.4	38.7	38.6	38.9	38.5	38.4	38.3	38.8	38.7	38.9	38.750
2	38.9	38.8	38.5	38.4	39.0	39.2	38.6	38.8	38.9	38.8	38.6	38.5	38.7	38.5	38.7	38.9	38.738
3	39.3	39.1	38.9	38.5	39.2	39.3	38.5	38.6	38.9	38.8	38.6	38.9	38.9	38.7	38.9	39.0	38.882
4	38.8	39.1	38.6	38.3	39.4	39.5	38.8	38.8	39.1	38.7	38.7	38.7	39.2	38.6	39.1	38.7	38.913
5	39.3	39.3	38.7	38.9	39.6	39.0	38.3	38.8	39.2	38.8	38.6	38.7	39.0	38.8	39.0	38.6	38.944
6	39.4	39.0	38.9	38.9	39.3	39.0	38.9	38.9	39.2	39.0	38.7	38.7	39.0	38.5	39.1	38.8	38.957
7	39.4	38.9	38.5	39.1	39.2	39.3	38.7	38.8	38.6	38.8	38.8	38.8	39.1	38.9	38.9	38.6	38.900
8	39.1	39.0	38.7	38.9	39.0	39.3	38.8	38.3	38.9	39.2	39.0	38.6	38.7	38.7	39.0	38.7	38.900
9	39.3	38.8	39.1	39.1	39.2	39.3	38.8	38.9	38.8	38.6	38.8	39.1	38.7	38.7	38.9	38.7	38.925
10	38.9	39.1	38.9	38.6	39.1	38.9	38.8	38.4	39.0	38.6	38.7	39.1	39.0	39.0	38.7	39.1	38.869

(a) Day 0 : onset of oestrus

APPENDIX 15 - Rectal temperature ($^{\circ}\text{C}$) 15 days prior to farrowing

Gilts Day prior to farrowing ^(a)	17/57	17/6	17/9	20/7	11/6	21/68	16/68	12/5	25/7	25/68	14/5	14/68	14/567	14/7	10/68	10/678	\bar{x}
- 15	39.6	39.5	39.8	39.5	39.4	40.1	39.0	39.5	39.6	39.6	39.3	39.7	39.7	39.2	39.6	39.3	39.525
- 14	39.6	39.6	39.8	39.6	39.3	40.1	39.2	39.4	39.6	39.6	39.6	39.6	39.8	39.1	39.6	39.3	39.550
- 13	39.4	39.2	39.7	39.6	39.8	40.1	38.8	39.5	39.6	40.0	39.6	39.8	39.6	39.3	39.5	39.5	39.563
- 12	39.0	39.5	39.8	38.8	39.5	39.9	39.2	39.7	39.7	40.1	39.7	39.7	39.6	39.3	39.4	39.2	39.507
- 11	39.4	39.4	39.4	38.8	39.3	40.0	39.0	39.6	39.5	39.6	39.7	39.8	39.8	39.5	39.2	39.8	39.488
- 10	39.5	39.7	39.0	39.1	39.6	40.0	39.0	39.5	40.0	39.6	39.4	39.9	39.9	39.4	39.2	39.5	39.519
- 9	39.4	39.7	39.5	39.0	39.3	39.9	38.9	39.6	39.6	39.6	40.0	39.4	39.8	39.5	39.3	39.3	39.488
- 8	39.4	39.3	39.6	39.6	39.6	39.7	39.2	39.8	39.7	39.5	39.8	39.7	39.8	39.2	39.3	39.2	39.525
- 7	39.4	39.4	39.4	39.9	39.5	39.5	39.1	39.4	39.5	39.7	39.7	39.9	39.7	39.8	39.3	39.0	39.513
- 6	39.3	39.5	39.6	39.7	39.3	39.7	39.5	39.6	39.7	40.0	40.1	39.6	39.5	39.2	39.7	39.2	39.582
- 5	39.2	39.6	38.7	39.6	39.5	39.8	39.5	38.9	39.3	39.9	39.8	39.6	39.6	39.6	39.5	39.1	39.450
- 4	39.1	39.0	38.7	39.5	38.9	40.0	39.4	39.3	39.6	39.3	39.4	39.5	39.7	39.2	39.4	38.5	39.282
- 3	39.2	39.0	38.7	39.6	39.2	39.8	39.3	39.2	38.3	39.3	38.6	39.2	39.6	39.3	39.5	38.5	39.144
- 2	39.2	39.7	39.0	39.1	39.1	39.4	39.2	39.2	38.9	39.3	39.3	39.5	39.3	39.5	38.9	39.0	39.225
- 1	39.2	39.3	39.4	40.0	38.6	39.0	39.2	39.8	39.1	40.1	39.5	39.1	39.8	39.7	39.0	39.0	39.363

(a) Day 0 : farrowing day

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