THE EFFECT OF FEED LEVEL DURING THE OESTROUS CYCLE ON OVULATION, EMBRYO SURVIVAL AND ANTerior PITUITARY LH POTENCY IN THE GILT

K. J. COOPER, P. H. BROOKS,* D. J. A. COLE AND N. B. HAYNES

Department of Physiology and Environmental Studies, and Department of Agriculture and Horticulture, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leicestershire

(Received 13th December 1971)

Summary. A series of experiments was conducted to investigate the effect of basal feed level and increased feed intake during oestrus on ovulation rate, early embryo survival, and residual pituitary LH potency in the pig. All the gilts used were fed 1·8 kg food/day from puberty to second heat. Gilts whose food intake was reduced from 1·8 kg to 1·4 kg/day following second heat had more atretic follicles at third heat and had fewer CL and fewer embryos at Day 20 of gestation, than control gilts fed 1·8 kg throughout, but these differences were not significant. Increasing the feed intake of gilts fed only 1·4 kg between second and third heat to 3·6 kg on Day 1 of oestrus only resulted in CL counts intermediate between those of the other two groups. The number of mature and atretic follicles during oestrus was not affected. Anoestrus was more frequent in gilts whose feed intakes were reduced to 1·4 kg/day. Residual pituitary LH potency was higher in gilts receiving only 1·4 kg feed/day than in gilts fed 1·8 kg/day. Increasing feed intake to 3·6 kg on Day 1 of oestrus significantly (P<0·05) reduced residual pituitary LH potency in animals previously fed 1·4 kg/day, but had no significant effect on animals previously fed 1·8 kg/day.

INTRODUCTION

It has been established that the ovulation rate in the pig is influenced by the plane of nutrition received during the oestrous cycle. Gilts on restricted feeding regimens have lower ovulation rates than gilts allowed free access to food (Robertson, Casida, Grummer & Chapman, 1951; Christian & Nofziger, 1952; Self, Grummer & Casida, 1955). The depressant effect on ovulation

* Present address: Scale-Hayne Agricultural College, Newton Abbot TQ12 6NQ, Devon.
rate, and also possibly on litter size, of a reduced feed allowance can be partially rectified by an increase in feed level for 1 day only during oestrus (Lodge & Hardy, 1968). This extra nutrient intake must be supplied early in oestrus, at about the time of the ovulatory release of gonadotrophins in order to affect ovulation rate (Brooks, Cooper, Lamming & Cole, 1972).

The mechanisms by which changes in feed intake modify ovulation rate in the pig are not clear. Follicular development at Day 19 of the oestrous cycle was similar for gilts fed high or low levels of dietary energy (Kirkpatrick, Howland, First & Casida, 1967), but at Day 20, Rigor, Meyer, First & Casida (1963) found that follicular development was greater when energy intake was increased from 5.9 to 10.9 Mcal/day for 12 days before oestrus. Despite the apparent influence of both extended and short periods of increased feed intake on ovulation rate and number of mature follicles, few corresponding changes in pituitary hormone secretions have been demonstrated. Kirkpatrick et al. (1967) measured the pituitary concentration of FSH and LH during the oestrous cycle of gilts fed at two dietary energy levels. They found that residual pituitary FSH and LH concentrations were modified by feed level and postulated that a greater amount of FSH may have been secreted during the proovulatory period when gilts received a higher dietary energy intake for several days before ovulation. No significant changes in residual pituitary LH potency were found by Brooks et al. (1972) as a result of a short term increase in feed level during early oestrus, even though this resulted in a significant increase in ovulation rate. In this study, however, residual LH concentration was measured 48 hr after ovulation, by which time any fluctuation in hormone content resulting from the extra nutrient intake may have disappeared.

The current series of experiments were designed to investigate further the effects of basal feed level and increased feed intake during early oestrus on the ovulation rate, early embryo survival and residual pituitary gonadotrophin levels in the gilt.

MATERIALS AND METHODS

Animals and management

The gilts used in these experiments were by Landrace boars and born to Landrace×Large White sows. The gilts were selected at approximately 170 days of age, and were housed in groups of up to twelve animals in deep-strawed yards. All the gilts were individually fed once daily on a breeding sow ration. The composition of the ration was the same as that used by Brooks et al. (1972). The gilts had unrestricted access to water.

All gilts were tested daily for oestrus using vasectomized boars. Puberty was defined as the day on which intromission by a vasectomized boar was permitted. The first day of oestrus as determined by boar acceptance was designated Day 1 of the cycle or of pregnancy.

Experimental designs

Experiment 1. The design of the experiment was a randomized block. Forty-eight animals were arranged in four blocks of twelve on the basis of age at
selection. Within each block of twelve animals, individuals were randomly allocated to one of three treatment groups at puberty. The treatments were as follows: control animals were fed 1·8 kg food/day, which is similar to that given in commercial practice, from puberty to slaughter. Slaughter procedure has been described by Brooks et al. (1972).

Gilts on the Low Plane diet were fed 1·8 kg food/day from puberty to second heat. From second heat to Day 3 of pregnancy, they were fed 1·4 kg food/day and 1·8 kg food/day from Day 3 to Day 20.

Gilts on low plane/extra nutrient intake (ENI) were fed as the Low Plane group, with the exception of Day 1 of the third oestrous period when their intake was increased to 3·6 kg food. This was achieved by giving the normal morning feed (1·4 kg) and an additional 2·2 kg feed following mating.

All gilts were mated with fertile boars at their third heat period, mating taking place on 2 consecutive days, if the gilts were still in oestrus. Subsequently, they were slaughtered on Day 20 of gestation and the numbers of CL and viable embryos were recorded.

Experiment 2. The experiment was arranged as a completely randomized design. Twenty animals were allocated equally at puberty to one of the two following treatments. Gilts in the Low Plane group were fed 1·8 kg food/day from puberty to second heat. From second heat until slaughter, they were fed 1·4 kg food/day. Gilts in the Low Plane/ENI group were fed as the Low Plane group, with the exception of Day 1 of the third oestrous period when their intake was increased to 3·6 kg food.

The gilts were slaughtered on Day 2 of the third oestrous period and the ovaries were retained for histological examination. The mean concentration of LH in the anterior pituitaries was measured for each group from a pooled pituitary sample.

Experiment 3. The design of the experiment was completely randomized. Fourteen animals were allocated equally at puberty to one of the two following treatments. Control animals were fed 1·8 kg food/day from puberty to slaughter. Gilts in the Control/ENI group were fed as the Control group, with the exception of Day 1 of the third oestrous period when their intake was increased to 3·6 kg food.

The gilts were slaughtered on Day 2 of the third oestrous period and the ovaries were retained for histological examination. The mean pituitary LH concentration for each treatment group was obtained by calculating the geometric mean of the individual pituitary LH concentrations.

Ovarian histology

The ovaries obtained from animals slaughtered at Day 20 of gestation (Exp. 1) were cut into 1-mm sections and the number of CL was recorded. Ovaries from the gilts slaughtered in Exps 2 and 3 were processed routinely, serially sectioned at 7 μm and the numbers of mature follicles above 8 mm and the numbers of atretic follicles above 5 mm diameter were recorded. The atretic follicles were distinguished from mature follicles by the breakdown of the thecal and granulosa-cell layers. The number of corpora albicantia remaining from the previous ovulation (second oestrus) was also determined.
An anterior pituitary assays

The ovarian ascorbic acid depletion assay (Parlow, 1958; Schmidt-Elmendorff & Loraine, 1962) was used with minor modifications as described by Brooks et al. (1972). The doses of standard used were 0.8 and 4.0 mg NIH-LH-S14 and the doses of unknown were either 0.3 and 1.5 or 0.5 and 2.5 mg of dried pituitary tissue.

Statistical analysis

Data from Exp. 1 were analysed using the method of Kempthorne (1952) for a two way classification with unequal numbers. Data from Exps 2 and 3 were analysed using Student's t test. For each assay, the ascorbic acid concentration of the ovary was adjusted by covariance analysis as recommended by Sakiz & Guillemin (1963). The validity, index of precision (λ), relative potency and fiducial limits at P = 0.95 were calculated for each assay using standard methods.

Table 1. Experiment 1. The effect of plane of nutrition and feed level at oestrus on ovulation rate and embryo survival in the gilt

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Low Plane</th>
<th>Low Plane/ENI</th>
<th>Standard error of a difference between two means * †</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of gilts at puberty</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>No. of gilts reaching third heat</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>No. mated</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>No. pregnant at Day 20</td>
<td>13</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Mean pubertal age (days)</td>
<td>196.5</td>
<td>190.2</td>
<td>196.5</td>
<td>4.33</td>
</tr>
<tr>
<td>Mean pubertal weight (kg)</td>
<td>88.0</td>
<td>83.1</td>
<td>89.0</td>
<td>4.00</td>
</tr>
<tr>
<td>Mean weight at third heat (kg)</td>
<td>101.0</td>
<td>95.2</td>
<td>100.6</td>
<td>2.51</td>
</tr>
<tr>
<td>Mean ovulation rate (third heat)</td>
<td>12.5</td>
<td>11.2</td>
<td>11.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Mean no. of viable embryos at Day 20</td>
<td>10.9</td>
<td>10.1</td>
<td>10.3</td>
<td>1.06</td>
</tr>
</tbody>
</table>

* Due to unequal subclass numbers, there are slight variations in the standard errors of differences between means. An average figure is therefore presented.
† None of the differences were statistically significant.

RESULTS

Experiment 1

The numbers of animals providing data at the various stages are shown in Table 1. Three gilts receiving only 1.4 kg/day became acyclic following their second heat. The results obtained are summarized in Table 1. There was no significant difference in the mean age and weight of the three treatment groups at puberty or at third oestrus. Although a decrease in feed level to 1.4 kg/day for one oestrous cycle reduced ovulation rate, the difference did not reach significance. An increase in feed level to 3.6 kg for 1 day during early oestrus in animals fed 1.4 kg/day resulted in an ovulation rate intermediate
between that of the Control and Low Plane groups. Differences in numbers of embryos recorded at Day 20 of pregnancy were not significant.

Experiment 2

Two animals from the Low Plane group and three animals from the Low Plane/ENI group became acyclic following their second oestrus and were therefore excluded from the data. The results obtained are summarized in Table 2. There were no significant differences in the mean age and weight of the two treatment groups at puberty or at third oestrus. Histological examination of the ovaries following slaughter at Day 2 of oestrus showed that none of the animals had completed ovulation or were in the process of ovulating. There was no significant difference between the number of corpora albicantia remaining from the second oestrous period, or the number of mature follicles at third oestrus. The mean number of follicles exhibiting atresia before ovulation was approximately 8% of the number of mature follicles. Although a transient increase in food allowance to 3-6 kg, for 1 day in early oestrus, did not significantly increase the number of mature follicles before ovulation, there was a significant \((P<0.05)\) difference in the amount of LH retained in the anterior pituitary gland. The reduction in feed level to 1-4 kg/day resulted in a greater retention of hormone than that found by Brooks et al. (1972) when gilts were allowed a higher feed level of 1-8 kg/day. The pituitary concentration of LH returned to a value similar to that found by Brooks et al. (1972) following the increase in food allowance during early oestrus.

Experiment 3

One animal from each treatment group became acyclic between puberty and third oestrus and these were excluded from the data.

Table 2. Experiment 2. The effect of plane of nutrition and feed level during oestrus on the number and type of follicles and pituitary LH potency in the gilt

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Low Plane</th>
<th>Low Plane/ENI</th>
<th>Standard error of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>8</td>
<td>7</td>
<td>—</td>
</tr>
<tr>
<td>Mean pubertal age (days)</td>
<td>197.0</td>
<td>198.0</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean pubertal weight (kg)</td>
<td>97.5</td>
<td>96.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean weight at third heat (kg)</td>
<td>106.0</td>
<td>104.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Mean no. of mature follicles at third heat</td>
<td>11.3</td>
<td>11.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Percentage of atretic follicles</td>
<td>9.6</td>
<td>7.4</td>
<td>—</td>
</tr>
<tr>
<td>Mean no. of corpora albicantia</td>
<td>11.0</td>
<td>11.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean anterior pituitary dry wt (mg)</td>
<td>44.8</td>
<td>44.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Mean LH concentration/mg dried anterior pituitary tissue ((\mu g \text{ NIH-LH-S14})^*)</td>
<td>(3.9 to 11.1)</td>
<td>(0.89 to 3.07)</td>
<td>—</td>
</tr>
<tr>
<td>Index of precision ((\lambda))</td>
<td>0.20</td>
<td>0.29</td>
<td>—</td>
</tr>
</tbody>
</table>

* Values in parentheses are fiducial limits at \(P = 0.95\).
The results are summarized in Table 3. There were no significant differences in the mean age and weight of the two treatment groups at puberty or at third oestrus. Examination of the ovaries following slaughter on Day 2 of oestrus revealed that a number of animals from both groups were in the process of ovulating. The ovaries of these gilts contained both ruptured follicles and mature uniovulated follicles. In all the animals, there was a tendency for ovulation rate to increase with increase in sexual age from the second to the third oestrus, with the difference in the Control group reaching significance \((P<0.05)\). There was no effect of the additional feed intake in early oestrus on the number of mature follicles and ovulated follicles. The proportion of atretic follicles in each treatment was 1.4% of the number of ruptured follicles and mature uniovulated follicles. Residual LH level in the pituitary was not affected by treatment. There was considerable variation in the LH potency between individual animals within each treatment group. The LH concentration varied from 0.35 to 3.2 \(\mu g/mg\) pituitary tissue in the Control group and from 1.6 to 8.0 \(\mu g/mg\) pituitary tissue in the Control/ENI group.

**DISCUSSION**

It was postulated by Lodge & Hardy (1968) that the lowered reproductive performance of pigs, which results from feed restriction, establishes a potential within the animal to respond to short-term increases in feed level during oestrus, the ovulation rate returning to a level similar to that of animals fed at a higher level throughout the oestrous cycle. They considered that this enhancement of ovulation rate might be expected to result in an improved litter size. Supporting evidence for such a response in ovulation rate was found by...
Brooks et al. (1972) but this did not support the hypothesis that this would result in increased litter size. In their experiments, increasing the feed level from 1·8 to 3·6 kg food on Day 1 of oestrus only, significantly increased the ovulation rate. It was considered likely that a more severe dietary restriction would create a greater potential and, hence, a greater response to increased feed intake during oestrus. The results obtained in the current experiments do not confirm this hypothesis. In Exp. 1, reducing food intake from 1·8 to 1·4 kg/day tended to depress the ovulation rate and increase the number of gilts becoming anoestrous. Increasing the feed intake to 3·6 kg on Day 1 of oestrus did not significantly affect the ovulation rate.

Reduction of the feed intake appears to suppress the increase in ovulation rate normally associated with an increase in sexual age (Robertson et al., 1951; Warnick, Wiggins, Casida, Grummer & Chapman, 1951). When gilts were fed 1·8 kg/day (Exp. 3), the increase in ovulation rate between second and third oestrus was 1·5 ova. When the feed level was reduced from 1·8 to 1·4 kg/day (Exp. 2), however, the increase in ovulation rate was only 0·3 ova. This effect was accompanied by a corresponding increase in the percentage of atretic follicles.

From the present data, it is difficult to reach a definite conclusion as to the origin of any additional ovulations resulting from an increased nutrient intake during oestrus. In Exps 2 and 3, it was not possible to demonstrate any change in the number of mature follicles shortly before or during oestrus which was associated with a change of nutrition during that period. This may indicate that the additional CL reported by Lodge & Hardy (1968) and Brooks et al. (1972) were formed following the rupture of immature or regressing follicles. If the extra nutrient intake stimulated the rupture of such follicles, these additional ovulations would not be detected in gilts slaughtered on Day 2 of oestrus, as in Exps 2 and 3. The additional ovulations would, however, be detected as additional CL in animals slaughtered at Day 20, as in the experiments of Lodge & Hardy (1968) and Brooks et al. (1972).

The results reported here indicated that raising feed intake for one feed during oestrus, significantly reduced residual LH in the anterior pituitary glands of gilts previously fed 1·4 kg/day, but had no effect on the residual pituitary LH concentration of gilts which were fed 1·8 kg/day.

The variability of individual pituitary LH concentrations found in Exp. 3 indicates that, at this level of feed intake, some animals may show a greater response to the extra nutrient intake than others. However, the mean LH concentrations recorded were similar to those determined from a pooled pituitary sample (Brooks et al., 1972).

These results would indicate that a measurable effect of plane of nutrition on pituitary hormone concentration is unlikely to be observed except at a relatively severe degree of dietary restriction. It remains possible that the rate of synthesis and release of gonadotrophic hormones may be influenced by a short-term alteration in feed level during oestrus while the residual pituitary hormone potency remains unchanged.

Although it is not possible to relate the pituitary and ovarian responses with certainty, it seems likely that these two areas concerned with the reproductive
process in the female pig can be affected by the nutrient supply during oestrus. It would appear that a reduction in feed intake may induce a pituitary block to gonadotrophin secretion, which may then be alleviated by an increased nutrient intake at about the time of the normal ovulatory gonadotrophin release. Niswender, Reichert & Zimmerman (1970) have shown that such a release occurs at the onset of behavioural oestrus.

ACKNOWLEDGMENTS

This work was conducted while two of us (P.H.B. and K.J.C.) were receiving postgraduate scholarships from the Meat and Livestock Commission. The authors would like to express their thanks to Mr H. L. Back for help with statistical analysis of the results and to Mr J. Corbett for technical assistance.

REFERENCES


