Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation

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The potential contribution of nutritionally induced differences in follicular and oocyte maturity to embryo survival was addressed in pigs. When primiparous, lactating sows are fed to appetite from farrowing to day 21 of lactation and then with feed intake restricted to 50% from day 22 to 28 (restricted), embryo survival is 64% at day 28 of gestation, compared with 85% in sows fed to 50% from farrowing to day 21 and then fed to appetite from day 22 to 28 (refed). In the present study, 32 sows were equally assigned to these two treatments (restricted or refed) but they were slaughtered 38 h before the estimated time of oestrus. The largest 15 follicles per sow were aspirated and follicular fluid recovered for analysis in vitro. Although plasma oestradiol concentration before slaughter and follicular fluid oestradiol concentration at slaughter were not different (P > 0.05), refed sows had more (P < 0.02) large follicles than did restricted sows. Cumulus expansion scores in vitro were not different between treatments, although more (P < 0.03) oocytes from refed sows had matured to metaphase II than those from restricted sows. Similarly, although cumulus expansion of oocyte–cumulus complexes from prepubertal gilts oocytes incubated with follicular fluid obtained from restricted (n = 1227) or refed (n = 1147) sows was not different (P > 0.05), the rate of oocyte nuclear maturation was greater (P < 0.012) after incubation with follicular fluid from refed than with that from restricted sows. Differences in the maturation of the follicle and oocyte in the period before the LH surge may therefore contribute to the treatment effects on embryo survival.

Introduction

The variance in embryo survival in the pig is large, and on average only 75% of blastocysts recorded on day 9 of pregnancy survive until day 25 (Pope and First, 1985). A high proportion of loss occurs around the time of maternal recognition of pregnancy and implantation (days 12–18) when the more advanced embryos secrete oestradiol, which acts as a signal to prevent luteolysis (Bazer and Thatcher, 1977) and promotes changes in the uterine milieu (Davis and Blair, 1993). The uterine environment per se significantly affects the developmental competence of the blastocyst. At the time of maternal recognition of pregnancy, there is an asynchronous array of developmental stages within the blastocyst population (Pope et al., 1986) and it has been hypothesized that the initiation of changes in the uterine environment by more advanced blastocysts is detrimental to the development of less mature embryos (Pope et al., 1990).

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

It was therefore suggested that heterogeneity within the follicle population is likely to influence oocyte maturation and could have consequences for embryo developmental competence (Hunter and Weisak, 1990). In an experiment in which the distribution of oocyte maturation 13 h before ovulation was compared with zygotic maturation in a comparable set of gilts, Xie et al. (1990a) found that the skewness of oocyte development continued into the zygotic population. Xie et al. (1990b) established that late-ovulating follicles give rise to less well-developed embryos on day 4 of gestation in gilts. Furthermore, the observed diversity of embryo development 160 h after hCG injection (cell cycle number) is not related to the duration of ovulation (Soede, 1992). Together, these data

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suggest that the physiological state of the follicle, and hence the maturational state of the oocyte, give rise to embryos that exhibit different abilities to develop within the same uterine environment.

Experimental paradigms involving manipulation of feed intake around the time of oestrus in gilts (Pharazyn et al., 1991; Jindal et al., 1996) or during lactation in sows (Baidoo et al., 1992; Zak et al., 1997) influence embryo survival. The concept that nutritionally induced manipulation of embryo survival not only involves changes in the uterine environment but also alterations in follicular and oocyte morphology have not previously been addressed in the literature.

Different patterns of feed restriction in primiparous, lactating sows caused differences in ovulation rate and embryo survival compared with sows fed to appetite throughout lactation (Zak et al., 1997). Feed restriction for the last week of a 28 day lactation (restricted) or feed restriction from day 1 to day 21 followed by feeding according to appetite from day 22 to day 28 (refed) was found to decrease the ovulation rate compared with that in sows fed to appetite throughout. However, embryo survival was also reduced in restricted sows compared with that in sows undergoing other treatments. In the present study, we tested the hypotheses that: (1) the observed differences in embryo survival between restricted and refed sows that have reduced but similar ovulation rates is due partly to differences in oocyte quality in the presumptive preovulatory pool (Expt 1); and (2) that such differences in oocyte quality are in turn related to differences in the ability of the follicle to support oocyte maturation (Expt 2).

Materials and Methods

Experimental design

At farrowing, 32 primiparous Camborough sows (Pig Improvement (Canada) Ltd), randomly stratified according to sow mass at farrowing and the number of piglets born, were allocated to one of two treatments. All sows were fed a wheat–barley–soybean diet formulated to provide 13.4 MJ metabolizable energy kg$^{-1}$ body mass. 15.4% crude protein and 0.74% lysine (Zak et al., 1997) throughout a 28 day (mean of 28.2 ± 0.2) lactation. Sows allocated to the restricted treatment (n = 16) were fed freely from farrowing until day 21; the diet of these sows was then restricted to 50% of the average consumption of the previous 5 day period from day 22 to day 28. Referred (n = 16) sows were restricted to a feed intake of 2.3 kg day$^{-1}$ from farrowing until day 21 and then fed freely from day 22 to day 28. Water was available at all times to the sow and piglets throughout the experimental period. As in our previous study (Zak et al., 1997), all litters were standardized to six piglets within 48 h of farrowing. Creep feed was not available. Sow mass and backfat (65 mm from mid-line at last rib) and litter masses were recorded at farrowing and on day 21 and day 28 of lactation. Sow mass and backfat were again recorded at slaughter. From weaning until slaughter all sows were allowed to consume freely a diet formulated to provide 13.4 MJ metabolizable energy kg$^{-1}$, 13.7% crude protein and 0.56% lysine.

After weaning until the day of slaughter, blood samples (5 ml) were taken via acute venepuncture of an ear vein for the determination of plasma concentrations of oestradiol 24, 48 and 72 h after weaning. Sows were tested twice a day at 07:00 and 19:00 h for the onset of standing oestrus using direct exposure to a vasectomized boar for 15 min. Time of slaughter was determined so that preovulatory oocytes recovered at the same stage of follicular development could be matured in vitro using a standard maturation medium. In this group of animals, the onset of behavioural oestrus occurs 9 h before the LH surge (R. Jindal, unpublished). On the basis of the previously observed time to return to oestrus of 122.3 ± 9.8 h and 134.7 ± 8.7 h in restricted and refed sows, respectively (Zak et al., 1997), animals in replicate I were slaughtered 4.5 days (108 h) after weaning. Although the interval from weaning to oestrus was not different between treatments (P > 0.05), two out of six restricted sows compared with no refed sows had already ovulated by the time of slaughter. To maximize the number of animals contributing to the study, individual treatment means were thereafter used to determine the time of slaughter; in replicates II and III, the time of slaughter after weaning was 83.5 ± 2.4 h for restricted and 97 ± 1.6 h for refed sows. Thus, animals in each group were slaughtered 38 h before the anticipated onset of oestrus (equivalent to day 20 of the cycle).

All procedures carried out in this experiment were approved by the Faculty Animal Policy and Welfare Committee to ensure adherence to the guidelines of the Canadian Council of Animal Care.

Experiment 1

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

Ovaries were obtained from the slaughtered sows, placed in individual plastic bags and transported to the laboratory within 40 min in a polystyrene box to prevent major fluctuations in temperature. The following experimental procedures were carried out at a room temperature of 22–26°C. Ovaries were washed four times in saline and antibiotic containing kanamycin (10 mg 100 ml$^{-1}$; Sigma). The external diameter of the largest 15 follicles per sow were measured by taking a mean of two measurements at 90° to one another. These follicles were chosen on the assumption that at least 15 follicles would ovulate (ovulation rate for restricted sows is 15.4 ± 2.3 follicles per sow, and for refed sows is 15.4 ± 1.9; Zak et al., 1997) and that the largest follicles present in the late follicular phase represent the presumptive ovulatory population (Foxcroft et al., 1987). The 15 largest follicles were aspirated using an 18-gauge needle and 1 ml syringe. The weight of the needle and syringe before and after aspiration was measured and the difference between the two weights was calculated as the weight of the follicular fluid. Then, assuming a density of 1 g ml$^{-1}$, the volume of follicular fluid was calculated.
Maturation of oocytes in vitro. Oocytes were removed from the follicular fluid and classed as either denuded or having intact cumulus cells. Irrespective of status, all oocyte–cumulus complexes were matured in a standard culture system in vitro, as described by Ding and Foxcroft (1994b), with minor modifications. Briefly, oocytes from each sow were incubated in a 35 ml plastic Petri dish containing 1.8 ml tissue culture medium 199 (TCM 199) supplemented with 200 µl 10% follicular fluid obtained from a pool of randomly selected, large, viable follicles present in the ovaries of prepubertal gilts (Funahashi and Day, 1993) and 100 µl gonadotrophins (2.5 µg NIADDK-oLH-26 ml⁻¹ (AFP-551b) and 2.5 µg USDA-pFSH-B-1 ml⁻¹ (AFP-5600)) and prolactin (20 ng USDA-pPrl-B-1 ml⁻¹ (AFP-5000)). Because the objective was to determine nutritionally mediated effects on oocyte development, we excluded the usual addition of glutamine, L-absorbing acid and insulin (Ding and Foxcroft, 1994b). The procedure was completed within 3 h. Culture was carried out under an atmosphere of 5% CO₂ in air at 39°C for 46 ± 1 h. The degree of cumulus expansion was then recorded immediately and the state of nuclear maturation was assessed after fixing and staining the oocytes as described below.

A fraction of follicular fluid from individual follicles was diluted 1:500 with TCM 199 and frozen at −30°C for the analysis of the concentration of oestradiol in follicular fluid. The remaining follicular fluid was pooled within sow, ensuring an equal contribution from individual follicles, and filtered using a Millipore filter (0.2 µm in diameter). Pooled follicular fluid was then diluted to 10% with TCM 199 and frozen (−30°C) in 1 ml aliquots for the second part of this study.

Experiment 2

This experiment was designed to address the hypothesis that nutritional status of the sow and related metabolic changes during lactation affect the composition of the follicular fluid and hence its ability to support oocyte maturation. Randomly allocated oocytes from prepubertal gilts were cultured in pooled follicular fluid obtained from individual sows and assessed for cumulus expansion and nuclear maturation. Briefly, ovaries from slaughtered prepubertal gilts (weighing around 100 kg) were collected from a local abattoir and transported to the laboratory in a polystyrene box to prevent fluctuations in temperature. Working at a temperature of 22–26°C, the ovaries were then washed four times in saline and antibiotic (kanamycin; Sigma). Follicles with a diameter > 3 mm were aspirated using a 18-gauge needle and 10 ml syringe. Good quality oocyte–cumulus complexes were harvested (n = 30 per dish) and incubated in 1.5 ml Petri dishes containing 1 ml diluted experimental follicular fluid (see Expt 1) supplemented with 50 µl hormone stock (2.5 µg NIADDK-oLH-26 ml⁻¹ (AFP-551b) and 2.5 µg USDA-pFSH-B-1 ml⁻¹ (AFP-5600)) and prolactin (20 ng USDA-pPrl-B-1 ml⁻¹ (AFP-5000)). Culture was carried out under an atmosphere of 5% CO₂ in air at 39°C for 46 ± 1 h. Cumulus expansion and nuclear maturation were then scored as for Expt 1. The incubation of oocytes with individual sow follicular fluid was carried out in duplicate dishes as a randomized block design involving three replicates for each sow, providing up to 180 oocytes per sow for the determination of treatment effects, during the period from January to April, 1996.

Evaluation of cumulus expansion. After the oocyte–cumulus complexes had been matured in vitro for 46 h, they were evaluated for cumulus expansion as described by Ding (1993), with slight modifications. Oocytes with a fully expanded cumulus, including the corona radiata, were classed as group 4; those in which the cumulus cells had expanded, but not the corona radiata, were classed as group 3; complexes that had only partial expansion of cumulus cells were classed as group 2; complexes exhibiting dark, intact, cumulus cells were classed as group 1. These characteristics were scored independently by two individuals.

Examination of nuclear status. After oocytes had been cultured for 46 h, they were denuded of cumulus cells (Bavister, 1989), mounted on a slide using the whole-mount technique and fixed for 48 h in ethanol:acetic acid (3:1). The nuclear status of oocytes was examined under a phase-contrast microscope after staining with 1% (w/v) lacoacid in 45% acetic acid solution. The nuclear status of oocytes (germinal vesicle, germinal vesicle breakdown, metaphase I or metaphase II) was identified according to the classification of Hunter and Polge (1966).

Steroid analysis

Plasma concentration of oestradiol was measured by the procedure described by De Rensis et al. (1991) using 1 ml plasma. All samples were analysed in one assay with an extraction efficiency of 91% and an intra-assay coefficient of variation of 5.6%. Sensitivity defined as 87% of total binding was 1.91 pg per tube. The concentration of oestradiol in follicular fluid was measured by the direct assay described by Ding and Foxcroft (1992) using 100 µl follicular fluid diluted to 1:6000 in assay buffer. All samples were measured in three assays with a mean intra-assay coefficient of variation of 5.8% and interassay coefficient of variation of 9.7%. Sensitivity defined as 85% of total binding was 5.5 pg per tube.

Statistical analysis

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

The independence of follicle size and treatment was determined by χ² analysis (SAS, 1990). For the dependent variables of follicle size, plasma concentrations of oestradiol and, after arc sine transformation, the stage of cumulus expansion and nuclear maturation, treatment differences were computed by analysis of variance (ANOVA; SAS, 1990). Sources of variation
Table 1. Least-square means (±SEM) of feed intake, sow body mass and backfat and litter mass at weekly intervals during lactation in primiparous sows given different feed intakes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Restricted (n = 10)</th>
<th>Refed (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–21</td>
<td>4.5 ± 0.7a</td>
<td>2.3 ± 0.1b</td>
</tr>
<tr>
<td>Days 22–28</td>
<td>2.8 ± 0.2a</td>
<td>5.3 ± 0.10b</td>
</tr>
<tr>
<td>Weaning to slaughter</td>
<td>6.2 ± 0.04</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farrowing</td>
<td>202.4 ± 3.6</td>
<td>196.4 ± 3.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>190.7 ± 2.3a</td>
<td>171.2 ± 1.1c</td>
</tr>
<tr>
<td>Day 28</td>
<td>173.0 ± 3.4</td>
<td>168.2 ± 1.9</td>
</tr>
<tr>
<td>Body mass change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–28</td>
<td>-29.4 ± 2.7</td>
<td>-28.2 ± 1.9</td>
</tr>
<tr>
<td>Backfat (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farrowing</td>
<td>18.6 ± 1.0</td>
<td>17.5 ± 0.7</td>
</tr>
<tr>
<td>Day 21</td>
<td>18.0 ± 1.0a</td>
<td>12.7 ± 1.2b</td>
</tr>
<tr>
<td>Day 28</td>
<td>15.2 ± 1.2</td>
<td>16.3 ± 0.3</td>
</tr>
<tr>
<td>Backfat change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–28</td>
<td>-3.4 ± 0.4</td>
<td>-1.2 ± 1.2</td>
</tr>
<tr>
<td>Litter mass (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>10.6 ± 0.3</td>
<td>11.1 ± 0.3</td>
</tr>
<tr>
<td>Day 21</td>
<td>44.6 ± 1.4a</td>
<td>40.4 ± 1.4b</td>
</tr>
<tr>
<td>Day 28</td>
<td>58.9 ± 1.5</td>
<td>57.8 ± 1.5</td>
</tr>
<tr>
<td>Growth rate (g per day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–28</td>
<td>0.288 ± 0.012</td>
<td>0.277 ± 0.080</td>
</tr>
</tbody>
</table>

Restricted and refed refer to sows with different patterns of feed intake during days 1–28 of lactation: restricted sows had free access to feed from day 1 to day 21 and then 50% of this feed intake from day 22 to day 28; refed sows received 2.3 kg intake per day from day 1 to day 21 and then had free access to feed from day 22 to day 28. *Means within rows lacking a common superscript letter are significantly different: **P < 0.05; ***P < 0.01.

were block, treatment, block x treatment and sows within block x treatment. The experimental unit, sow within block x treatment, was used as an error term.

Linear regression analyses (SAS, 1990) were used to determine the effects of follicle diameter on the cube root of follicular volume and on the concentration of oestradiol in follicular fluid.

**Results**

**Responses of sows to treatments**

Sow mass and backfat did not differ among treatments at farrowing (Table 1). There was a significant day x treatment interaction for sow body mass (P < 0.001) and backfat (P < 0.01). On day 21, body mass and backfat for refed sows was lower than for restricted sows (P < 0.01 for both). However, on day 28, no differences in body mass or backfat were observed between treatments. Litter masses at weaning did not differ between treatments (P > 0.6). After weaning, the daily consumption of feed by restricted sows was greater (P < 0.001) than during the last week of lactation, whereas refed sows ate a similar daily amount after weaning compared with that eaten on days 22–28 of lactation. Overall, however, there were no differences from weaning to slaughter in feed intake between treatments.

All of replicate I animals (n = 9) were excluded from the following analysis because no oocytes had matured during maturation in vitro, suggesting that the gonadotrophin supplement had expired. In addition, three sows from replicates II and III (two from the restricted group and one from the refed group) were also excluded because although large preovulatory follicles were present, their follicular fluid oestradiol concentration was consistently lower than 30 ng ml⁻¹, indicating that they had already responded to the endogenous preovulatory LH surge before slaughter. The following analyses are therefore based on data from 10 restricted and 11 refed sows.

**Follicular status at slaughter**

The plasma oestradiol concentrations for restricted and refed sows 24 h (34 and 37 pg ml⁻¹, respectively), 48 h (48 and 47 pg ml⁻¹, respectively) and 72 h (63 and 67 pg ml⁻¹, respectively) after weaning were not different (P > 0.05). Considering the distribution of follicles according to the follicular diameter of the 15 largest follicles measured at the time of slaughter, restricted sows had more (P < 0.043) small follicles than refed sows and refed sows had more (P < 0.02) large follicles than restricted animals (Fig. 1). Overall, therefore, more oocytes were retrieved from smaller follicles in restricted than in refed sows (P < 0.03). Regression analysis showed that the diameter of follicles was correlated with the cube root of follicular fluid.
follicular volume ($P < 0.001; r = 0.73; n = 146$). The diameter of follicles was also correlated with follicular fluid oestradiol concentration ($P < 0.01, r = 0.52, n = 192$) for oestrogenic follicles, whereas the follicles from sows considered to be in the post-LH surge period showed no correlation between follicle diameter and oestradiol concentration ($P > 0.9, r = 0.003, n = 32$) (Fig. 2). Overall, there was no difference in the relationship between follicle diameter and follicular fluid oestradiol concentration for restricted ($r = 0.68, n = 85$) and refed sows ($r = 0.60, n = 107$).

Results from maturation data in vitro

Experiment 1. Data on the maturation of oocytes retrieved from experimental sows, expressed as the percentage of oocytes with cumulus cells at the start of in vitro maturation, with a cumulus cell expansion score of 1, 2, 3 or 4, are shown (Table 2). No differences in cumulus expansion were observed between treatments, although the number of oocytes reaching metaphase II was greater ($P < 0.03$) for refed than for restricted sows. Conversely, after culture of oocyte–cumulus complexes in vitro from restricted sows, more oocytes had matured to metaphase I ($P < 0.054$) and the germinal vesicle breakdown stage ($P < 0.07$) than in refed sows.

Experiment 2. Incubation of randomly selected oocyte–cumulus complexes obtained from prepubertal gilts with experimental follicular fluid did not reveal any differences in cumulus cell expansion among treatments (Table 3). However, the proportion of oocytes reaching the metaphase II stage of nuclear maturation was greater ($P < 0.012$) with follicular fluid from refed than from restricted sows. Conversely, oocytes incubated with follicular fluid from restricted sows had a greater ($P < 0.01$) proportion of oocytes at metaphase I than did those incubated with follicular fluid from refed sows. No differences were observed between treatments in the proportion of oocytes with nuclei at the germinal vesicle breakdown ($P > 0.4$) or germinal vesicle stage ($P > 0.3$).
Discussion

Consistent with the data reported by Zak et al. (1997), the patterns of feed restriction imposed during lactation resulted in differential patterns of body mass and backfat changes in refeed and restricted sows. Overall, restricted and refeed sows mobilized approximately 29 kg of livemass and 2 mm of backfat during lactation. Irrespective of the pattern of mass and backfat loss, sows produced equivalent amounts of milk during lactation, as shown by similar litter masses at day 21 and day 28.

Central to the interpretation of data presented in this report is the assumption that the ovarian follicles of restricted and refeed sows were at a similar physiological state at the time of slaughter, with respect to time of the LH surge and ovulation. Because the results from sows in replicate I indicated that the time of slaughter first chosen was inappropriate, all animals in replicates II and III were slaughtered 38 h before the anticipated onset of standing oestrus. This more conservative timing was expected to result in sows being slaughtered in the last 24–30 h before the onset of the preovulatory LH surge. Data from only three of 24 sows were finally excluded from analysis on the basis that the oestradiol concentration in follicular fluid in large preovulatory-type follicles had already declined. Thus, all remaining sows were considered to have been slaughtered at the same relative stage of follicular development. The plasma oestradiol concentration of these 21 sows was not different between treatments 24, 48 or 72 h after weaning, nor was the mean estimate of the concentration of plasma oestradiol from weaning until the time of slaughter different among treatments. Overall, for a given size of follicle there was no difference in the effect of treatment on the concentration of oestradiol in follicular fluid.

In agreement with the results of the studies reported by Foxcroft et al. (1987) in weaned sows and of Grant et al. (1989) in cyclic gilts, the follicles examined in the late follicular phase in this experiment (equivalent to days 19–20 of the oestrous cycle) also formed a heterogeneous population in terms of follicle diameter and oestradiol content. Hunter and Weisak (1990) suggested that the degree of heterogeneity of follicles within the preovulatory pool has ramifications for the developmental competence of the oocyte once it has been ovulated and for subsequent luteinization of the follicle.

Although there was no difference in plasma oestradiol concentration between treatments, during the phase of follicular growth after weaning follicle size and follicular fluid oestradiol concentration at slaughter differed. Restricted sows had more ‘small’ follicles whereas refeed sows had more ‘large’ follicles. Because of the design of the experiment, we suggest that the difference in follicular maturity was not related to animals being slaughtered at different times during the follicular phase but was a true reflection of the sows’ previous nutritional state. The effects of periods of increased catabolism, due to the effects of reduced feed intake on follicle size, have previously been reported. In the lactating sow, feed restriction to total energy requirement for 2 weeks almost abolished follicular development beyond a diameter of 3 mm (Miller, 1996); in the gilt, follicle diameter and oestradiol content was increased after refeeding for 5 days (Cosgrove et al., 1992).

The definitive role of nutritionally induced changes in the local follicular environment, as opposed to indirect nutritionally induced effects on gonadotrophin secretion, has yet to be fully resolved in models using increased catabolic states. Receptors for LH on the granulosa, thecal and mural cells of the follicle are essential for follicle persistence and development during the oestrous cycle (Esbenshade et al., 1990). During periods of feed restriction in the prepubertal gilt (Booth et al., 1996) or lactating sow (Tokach et al., 1992; Zak et al., 1997), the hypophysial release of LH is almost totally suppressed but recovers as early as 6 h after feeding to appetite (Cosgrove et al., 1991; Booth et al., 1996). Similarly, in the experimental model used in the present experiment, episodic LH secretion was virtually absent during feed restriction in the last week of a 28 day lactation compared with refeed sows, although no differences were evident in the response of LH to weaning (Zak et al., 1997). The removal of the suckling stimulus at weaning is generally regarded as the signal for resumed follicular growth (Britt et al., 1985; Foxcroft et al., 1995) and a lack of LH secretion is the primary cause of retarded follicular growth during lactation in sows (Cox and Britt, 1982; De Rensis et al., 1991). There is ample evidence that ovarian follicular growth is not absolutely quiescent in lactation (Britt et al., 1985). In the absence of marked catabolism, follicle size does increase during lactation (Kungavonkrit et al., 1982). Thus, in the present model, it is impossible to delineate between the effects of nutrition and metabolic sequelae on the ovary and the indirect effects of suppressed LH secretion in late lactation on follicular growth and oocyte maturation.

Compared with the data from restricted sows, the observations that more oocytes in refeed sows developed to metaphase II of meiosis and that randomly selected oocytes incubated with follicular fluid from the refeed group were also more mature, indicates that a factor(s) within the follicular fluid contributed to the observed differences in oocyte nuclear maturation. Close examination of the data for Expts 1 and 2 indicates that fewer oocytes in Expt 1 that is, oocytes retrieved from experimental sows) were able to undergo cumulus expansion and more remained at the germinal vesicle stage of nuclear maturation when compared with oocytes retrieved from prepubertal gilts obtained from an abattoir. These observations may partly represent differences in the quality of cumulus–oocyte complexes, since all oocytes from experimental sows were matured in vitro, independent of whether they had intact cumulus cells, whereas in Expt 2 oocytes retrieved from prepubertal gilts from an abattoir were carefully selected to ensure that they had a full complement of cumulus cells (Ding, 1993). In addition, although the conditions for maturation in vitro were similar for treatment groups in Expts 1 and 2, culture conditions were not identical. The number of oocytes per well within the maturation medium in Expt 1 was lower than in Expt 2, which may also have consequences for the rate of oocyte maturation. Although a comparison of absolute rates of maturation between Expts 1 and 2 is not possible, oocytes obtained from refeed sows or oocytes retrieved from prepubertal gilts and incubated with follicular fluid from refeed sows exhibited a greater rate of nuclear maturation.

The ability of the somatic compartment to alter nuclear maturation and cytoplasmic competence of oocytes has been
demonstrated by Ding and Foxcroft (1994a, b). In their first study, randomly selected pig oocytes were incubated with conditioned media obtained from follicle shells of small or large follicles obtained on day 17 or 20 of the oestrous cycle. Nuclear maturation was greatest in oocytes matured in conditioned media from large follicles, while male pronuclear formation was greater in media conditioned with follicle shells from day 20 compared with those from day 17. Collectively, these data support the hypothesis that treatment differences observed in the development of the preovulatory pool of follicles in the present experiment are related to observed treatment differences in oocyte meiotic maturation. Furthermore, associations between genotypic effects on follicle and oocyte maturation during the immediate preovulatory period in vitro (Faillace and Hunter, 1994) and differences in the capacity of follicle-conditioned media obtained from these genotypes to support oocyte maturation in vitro have been observed (X. Xu, M. G. Hunter, L. Faillace and G. R. Foxcroft, unpublished).

The data reported here demonstrate that the size of follicles in the preovulatory pool and the rate of maturation of oocytes obtained from these follicles can be affected by the nutritional history of the lactating sow. Furthermore, factors of follicular origin appear to mediate these effects. Differences in the maturation of the oocyte (and perhaps the developmental competence of the follicle to luteinize) may contribute to the observed differences in embryo survival in our previous experiment (Zak et al., 1997). Thus, the management of the lactating sow immediately before weaning is critical in order to maximize preovulatory follicle development and hence provide an environment that is conducive to optimal oocyte maturation.

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