EFFECTS OF EXOGENOUS INSULIN AND BODY CONDITION ON METABOLIC HORMONES AND GONADOTROPIN-INDUCED FOLLICULAR DEVELOPMENT IN PREPUBERAL GILTS1,2,3

Isidro A. Matamoros, Nancy M. Cox4 and Alfred B. Moore

Mississippi State University, Mississippi State 39762

ABSTRACT

To determine influences of insulin and body condition on follicular growth, prepuberal gilts (n = 16) treated with pregnant mare's serum gonadotropin (PMSG) were used in a 2×2 factorial experiment with main effects of insulin (0 or .4 IU/kg every 12 h beginning at 1800 on the day before PMSG) and backfat depth (moderate, 25 ± .8; high, 32 ± .7 mm; P < .0001). Body weights were similar. Blood sampling was at 6-h intervals for analyses of LH, FSH, growth hormone (GH), glucagon, cortisol, insulin, insulin-like growth factor-I (IGF-I), plasma urea nitrogen (PUN), nonesterified fatty acids (NEFA), testosterone, estradiol-17β, and progesterone. Ovaries were removed 75 h after PMSG treatment, and visible small (≤ 3 mm), medium (4 to 6 mm), large (≥ 7 mm), and macroscopically atretic follicles were counted. Administration of insulin increased IGF-I in fluid of medium follicles (108.8 vs 60.7 ng/ml; SEM = 13.3; P < .05). Neither insulin nor fatness affected hCG binding by granulosa cells (12.5 ± 1.6 ng/10⁶ cells) or numbers of large (16.7 ± 2.6) and medium (10.4 ± 2.3) follicles. However, insulin increased the number of small follicles (58.9 vs 29.9; SEM = 9.7; P < .05) and reduced the number of atretic follicles (3.8 vs 11.3; SEM = 1.1; P < .05). The predominant effect of insulin on reducing number of atretic follicles was in the small size class (.6 vs 6.9; SEM = .6, P < .01). Follicular fluid estradiol and progesterone were not affected by treatments; however, testosterone concentrations in large follicles were lower in gilts with higher backfat (32.5 vs 59.9 ng/ml; SEM = 4.0; P < .05). Systemic LH, FSH, glucagon, cortisol, PUN, NEFA, estradiol, and testosterone were not affected by insulin or level of feeding. However, GH was lower in gilts that had higher backfat (overall average of 3.2 vs 2.8 ng/ml; SEM = .1; P < .05). Insulin reduced atresia and altered intrafollicular IGF-I independently of body condition and without sustained effects on other hormones.

Key Words: Insulin, Granulosa Cells, Follicle Development, Backfat, Glucose


Introduction

Providing additional dietary energy to swine on restricted feed during the middle and late estrous cycle increased ovulation rate (Anderson and Melampy, 1972; Brooks and Cooper, 1972; Zimmerman, 1972; Dailey et al., 1975; Cole, 1982; Ahern and Kirkwood, 1985; Flowers et al., 1989a). Exogenous insulin and dietary energy acted synergistically to increase ovulation rate (Cox et al., 1987b), and endogenous insulin was elevated during flushing to increase ovulation rate (Flowers et al., 1989a). Insulin may promote the persistence of follicles in preovulatory, rather than degenerative, pathways. Exogenous insulin reduced atresia and correspondingly maintained the proportion of medium-sized follicles...
(4 to 6 mm diameter) during d 17 to 19 of the cycle (Matamoros et al., 1990).

Based on observations of rebounds in glucose concentration after treatment with insulin, Cox et al. (1987b) hypothesized that metabolic hormones that respond to hypoglycemia might be part of the mechanism for increased ovulation rate. Glucagon, growth hormone (GH), and cortisol contribute to regulation of glucose homeostasis (Gerich et al., 1979; Cryer, 1981). In addition, obesity has been associated with elevated endogenous insulin levels (Yalow et al., 1965) accompanied by resistance to glucoregulatory effects of insulin (Friedman and Moon, 1985). One effect of hyperinsulinemia is a reduction in the number of insulin receptors of various tissues (Friedman and Moon, 1985), including the ovaries (Poretsky et al., 1988).

The present study was designed to determine whether exogenous insulin influenced follicular growth and/or function similarly after feeding management designed to produce similar body weights but different degrees of body fatness in gonadotropin-treated prepuberal gilts. To clarify whether insulin had a direct or indirect effect on follicular growth through glucose counterregulatory mechanisms, other metabolic hormones and metabolites were also measured.

**Materials and Methods**

Twenty crossbred (1/2 Yorkshire, 1/4 Hampshire, 1/4 Duroc), prepuberal gilts were initially divided into two groups (n = 10) based on visual appraisal of body condition (lean or fat) and ultrasonic backfat measurement at the last rib (lean = 9.2 vs fat = 11.5 mm; SEM = 1; P < .05) at 130 d of age and 59 ± 3 kg BW. Then for 40 d the fatter gilts were given ad libitum access to feed and the leaner gilts were fed 2.27 kg/d of a com and soybean meal diet balanced to provide 3,181 kcal ME/kg according to NRC (1987) recommendations. The purpose of the different feeding levels was to enhance the existing differences in body fatness between the two groups yet maintain similar BW. At the end of this feeding period, 16 gilts (n = 8 each) met the criteria for similar BW and different levels of body fat based on backfat probe measurements. Body condition was confirmed at slaughter by measuring carcass backfat at the first rib, last rib, and last lumbar vertebra; backfat is reported as an average of the three carcass measurements. All 16 gilts were given 600 IU of pregnant mare's serum gonadotropin (PMSG)5 s.c. at 170 ± 5 d of age and were used in a 2 x 2 factorial arrangement (n = 4 per treatment combination), in which main effects were body condition (moderate or high) and insulin (Lente pork insulin6; 0 or .4 IU/kg BW s.c. every 12 h beginning 12 h before PMSG injection and continuing until slaughter and ovary removal). During the time of PMSG and insulin treatment all gilts were fed 3.06 kg/d of a diet balanced to provide 9,960 kcal ME/d (Cox et al., 1987b). Elevated dietary energy during insulin administration is necessary to prevent insulin-induced hypoglycemia. In a previous study in which gilts were fed this way, positive effects on ovarian function and a low incidence of insulin-induced hypoglycemia in gilts were observed (Matamoros et al., 1990). Daily diets during the experiment were fed in two equal portions at 0600 and 1800 at the time of insulin or saline injections.

Ovaries were removed at slaughter 75 h after PMSG and immediately placed in .9% saline supplemented with penicillin, streptomycin, and amphotericin-B (10 U/ml, 10 μg/ml, and .25 μg/ml, respectively)7 (May and Schomberg, 1981; Matamoros et al., 1990). The numbers of small (≤3 mm), medium (4 to 6 mm), large (≥7 mm), and atretic follicles were quantified and measured to the nearest millimeter. Macroscopic atretic follicles were classified using the criteria of Moor et al. (1978), based on vascularity of theca and translucency of the follicular fluid. The integrity of granulosa cell membranes during harvesting of granulosa cells and viscosity of follicular fluid were also evidence of atresia (Hunter et al., 1989). Granulosa cells were isolated by mechanical agitation previously described by May and Schomberg (1981) and Matamoros et al. (1990). All granulosa cells of nonatretic medium and large follicles were harvested and resuspended in 1 ml of Ham's F-12 nutrient medium and Dulbecco's modified Eagle medium7 (1:1) supplemented with penicillin (10 U/ml) and streptomycin (10 μg/ml) as described by Matamoros et al. (1990). A total of 1,261 follicles (267 large, 166
medium, 710 small, and 118 atretic) were processed. Follicular fluid from medium and large nonatretic follicles for each ovary was pooled by follicle size class and stored frozen until it was assayed for estradiol, progesterone, testosterone, and insulin-like growth factor I (IGF-I).

Iodination of Human Chorionic Gonadotropin and Receptor Binding. The hormonal material, iodination procedure, activity, and stability characteristics of [125I]iodo-human chorionic gonadotropin (hCG) were as described by Stouffer et al. (1976). Specific activity averaged 37.5 μCi/μg (range 29.7 to 40.3). The active fraction of the preparations used for this study (portion of the [125I]iodo-hCG specifically bound in the presence of excess granulosa cells) ranged from 35 to 40%. The hCG preparation (hCG13VO1) was donated by the National Hormone and Pituitary Program. Binding studies were performed on freshly harvested granulosa cells as described by Matamoros et al. (1990) following procedures of May and Schomberg (1981).

Hormone and Metabolite Assays. Blood samples for serum and plasma were obtained via indwelling catheters every 6 h for 84 h beginning immediately before the first insulin injection (12 h before PMSG). Serum samples were used to measure LH, FSH, GH, insulin, nonesterified fatty acids (NEFA), cortisol, IGF-I, estradiol-17β, progesterone, and testosterone. Plasma samples were used to measure plasma urea nitrogen (PUN) and glucose concentrations. Twelve of the 16 gilts (three per treatment combination) were also sampled for plasma glucagon; individual 3-ml samples of blood were collected in tubes containing 1,000 kIU/ml of aprotinin (proteinase inhibitor, Trasylol®) and 6.7 U/ml of heparin9.

Serum LH concentrations were determined by a double-antibody RIA using a rabbit anti-porcine LH serum (#566) previously validated (Niswender et al., 1970; Cox et al., 1987b). The dilution of the LH antiserum was 1:80,000 in 200 μl, and the second antibody (sheep anti-rabbit gamma globulin, MSU #23, 11-4-83) was diluted 1:10 in 200 μl. The standard and radioiodinated hormone was LER-786-3. Sensitivity of the assay was .17 ng/ml, and intra- and interassay CV were 5.7 and 9.8%, respectively.

Serum concentrations of FSH were determined with a double-antibody RIA using a 1:10,000 dilution of FSH antiserum (USDA 10-1010). The second antibody was the same as described previously for LH. Purified FSH (USDA-FSH-PPI) was used for standards and iodination (according to the procedure of Guthrie and Bolt, 1983, and as validated previously by Cox et al., 1987b). Sensitivity of the assay was .32 ng/ml and intra- and interassay CV were 5.5 and 12.5%, respectively.

Serum GH concentrations were determined by a double-antibody RIA modified from the procedure of Allen and Gerrits (1976a). Purified porcine GH (USDA-pGH-H1) was used for standards and radioiodinated hormone. The first antibody was baboon anti-porcine growth hormone donated by T. Mowles of Hoffman-La Roche, Inc. (1:100,000 in 200 μl of .01 M phosphate-buffered saline, pH 7.5 [PBS], with 1% BSA), and the second antibody was goat anti-monkey (Rhesus) gamma globulin10 (1:10 in 100 μl, P3 Lot 6TA21) and was added 24 h after first antibody and radiolabeled hormone were added to samples and standard. Normal monkey serum11 (1:100 in 100 μl PBS-BSA) was added at this time. Ten minutes later 1 ml of 6% polyethylene glycol (in distilled water) was added, followed by centrifugation to separate antibody-bound hormone from free hormone. The average percentage recovery of 1.25, 2.5, and 5.0 ng of GH added to 100 μl of serum and corrected for endogenous GH was 108%. Increasing amounts of serum (50, 100, 150, 200, and 250 μl) produced a curve parallel to the standard curve. Cross-reactivity with porcine prolactin (USDA-pFRL-B-1) was determined to be less than .001%. The assay sensitivity was .72 ng/ml with intra- and interassay CV of 7.52 and 9.69%, respectively.

Plasma glucagon concentrations were determined with a double-antibody RIA modified from the procedure of Allen and McMurry (1984). Glucagon assay buffer was adjusted to pH 8.8 and was used to dilute first and second antisera. First antibody (rabbit anti-glucagon, No. 01-0055-09, lot AGS-R-PLUS12) was diluted 1:5,000 in glucagon assay buffer and
100 µl was added to each assay tube. The second antibody was sheep anti-rabbit gamma globulin diluted 1:10 in glucagon assay buffer and 200 µl was added per assay tube. Standards11 (Sigma G4250) were diluted in assay buffer containing 5% aprotinin and 1% BSA to a volume of 100 µl per tube. Radiolabeled glucagon13 (No. 120) had a specific activity of 300 µCi/µg. Increasing amounts of plasma (50, 100, and 150 µl) produced a curve parallel to the standard curve. The average recovery of .156 and .3125 ng of glucagon added to 50 µl of aprotinin-treated plasma and corrected for endogenous glucagon was 99.2%. The assay sensitivity was .48 ng/ml and intra- and interassay CV were 9.6 and 11.2%, respectively.

Plasma insulin concentrations were determined with a double-antibody RIA procedure validated in our laboratory (Cox et al., 1987b) according to the procedure of Hales and Randle (1963). Sensitivity of the assay was .07 ng/ml and intra- and interassay CV were 9.0 and 11.3%, respectively.

Serum cortisol concentrations were determined using a commercially available RIA kit13. The average recovery when .75, 1.875, and 9.375 ng were added to 12.5 µl of serum was 109.9%. Increasing amounts of serum (12.5, 25.0, and 37.5 µl) produced a curve parallel to the standard curve. The sensitivity of the assay was 1.7 ng/ml and intra- and interassay CV were 6.8 and 6.7%, respectively.

Serum and follicular fluid IGF-I concentrations were assayed using a heterologous double-antibody procedure (Houseknecht et al., 1988). To separate IGF-I from binding proteins, 50 µl of serum or follicular fluid was incubated for 36 and 24 h, respectively. The incubation solution contained 1.0 ml of IGF-I assay buffer and 480 µl of .2 M glycyglycine HCl (pH of solution was between 3.2 and 3.5). The IGF-I assay buffer contained the following: .02% protamine sulfate11 (grade III), 30 mM sodium phosphate (monobasic), .05% Tween-20, .02% Na azide, and .01 M EDTA. The first antibody was anti-somatotropin-C/IGF-I rabbit antiserum (UBK487) diluted at 1:2,400 in 100 µl of assay buffer containing 1:200 normal rabbit serum. The second antibody (sheep anti-rabbit gamma globulin, MSU #24 9-13-83) was diluted 1:10 in 50 µl of assay buffer. The IGF-I standard was lot #1401-3 (Amgen G1410014). The radiolabeled IGF-I (No. IM-17215) was (3-[125I]iodo)[tyrosyl] insulin-like growth factor (Thr59) and had a specific activity of 2,000 Ci/mmol. The average recovery of 50, 100, and 200 pg of IGF-I added to 50 µl of serum and follicular fluid and corrected for endogenous IGF-I was 106%, and increasing amounts of incubation media from serum and follicular fluid (25, 50, and 75 µl) produced a curve parallel to the standard curve. Sensitivity of the assay was 4.7 ng/ml, and intra- and interassay CV were 6.9 and 8.7%, respectively.

Concentrations of estradiol-17β were determined by RIA in serum and follicular fluid (diluted 1:100 in .01 M PBS, pH 7.5) as validated by Cox et al. (1987a) and Matamoros et al. (1990). Intra- and interassay CV were 6.7% and 11.7%, respectively.

Concentrations of testosterone in serum and follicular fluid were determined by RIA as previously described and validated in our laboratory (Matamoros et al., 1990). The follicular fluid was diluted 1:100 in .01 M PBS, pH 7.5. The intra- and interassay CV were 9.7% and 12.6%, respectively.

Progestosterone concentrations in follicular fluid diluted 1:100 in .01 M PBS were determined by RIA methods previously described and validated by Rainey et al. (1990) and Matamoros et al. (1990). The intra- and interassay CV were 11.7% and 14.9%, respectively.

Serum NEFA concentrations were measured by an enzymatic colorimetric method16. Intra- and interassay CV were 4.3 and 5.4% and sensitivity was 10 µeqliter. Parallelism was observed between standard curves and a curve of increasing pool volume from 50 to 200 µl. Recovery of oleic acid averaged 93.1 ± 2.1%.

Plasma glucose concentrations were measured by the glucose-oxidase method (Hill and Kessler, 1961), and PUN concentrations were measured by the glutamate-dehydrogenase method (Talke and Schubert, 1965) in an automated processing system17.

Statistical Analyses. Treatment effects on blood hormones and metabolites were analyzed using a split plot in time design with

13Ventrex Laboratories Inc., Portland, ME.
14Amgen Biological, Thousand Oaks, CA.
15Amersham Corp., Arlington Heights, IL.
16WAKO Chemicals Inc., Dallas, TX.
17Centrisfchem 600, Baker Instruments Co., Allentown, PA.
main effects of insulin, body condition, and time and all interactions. Effects of insulin and body condition were tested with the error term, gilt-within-insulin by body condition (SAS, 1988). Where significant main effects occurred, means were separated by the method of least significant difference (SAS, 1988). Where there was a significant interaction of a main effect (insulin treatment or body condition) with time, a Greenhouse-Geisser Epsilon adjustment (Greenhouse and Geisser, 1959; SAS, 1988) was carried out to determine whether the interaction was a true interaction and not a reflection of correlation present in the data. Where the use of split plot in time was found inappropriate due to such correlation, a multivariate analysis of variance was conducted as described by Allen (1983) and Allen et al. (1983) as modified for use with the GLM procedure (SAS, 1988). This procedure involved fitting an appropriate degree polynomial to the hormonal profile of each experimental unit, thereby reducing the dimension of the hormonal profile. A multivariate analysis was then performed on the regression coefficients of the polynomial fitted to the individual hormonal profiles to determine whether the profiles of the treatment combinations were parallel and/or coincident. If hormonal profiles were found to be parallel and/or coincident, estimation of the proper slopes and intercepts composed of the appropriate treatment combination were made accordingly, using GLM procedures (SAS, 1988).

Follicular fluid hormone concentrations, follicle size distribution, and hCG receptors were analyzed using a completely random design with a $2 \times 2$ factorial arrangement of main effects of insulin treatment and feeding level and their interactions, using the GLM procedures (SAS, 1988). Where significant source effects and/or interactions were detected, mean separations were performed by the method of least significant difference (SAS, 1988). Body weight and carcass backfat depth were analyzed for effects of feeding management using a completely random design, and means of significant effects were separated by the method of least significant difference (SAS, 1988).

Results

Feeding level before the experiment did not affect live BW at slaughter (moderate = 96.0 kg vs high = 98.8 kg; SEM = 2.7; $P > .05$), but animals that had received limited feed had lower backfat depth at slaughter (moderate; 24.6 mm) than those given ad libitum access to feed (high; 32.1 mm; SEM = 1.0; $P < .001$). These analyses confirm that different degrees of body fatness yet similar BW between the two groups were achieved.

Table 1. Least Squares Means for Number of Follicles

<table>
<thead>
<tr>
<th>Follicle classification</th>
<th>Treatment</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large (≥ 7 mm)$^a$</td>
<td>Saline</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>Insulin</td>
<td></td>
</tr>
<tr>
<td>Medium (4–6 mm)$^a$</td>
<td>12.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Small (≤ 3 mm)$^a$</td>
<td>29.9$^b$</td>
<td>9.7</td>
</tr>
<tr>
<td>Macroscopically atretic</td>
<td>11.3$^b$</td>
<td>1.1</td>
</tr>
</tbody>
</table>

$^a$Macroscopically nonatretic follicles only. $^b$Means in a row not followed by the same superscript letter differ ($P < .05$).

Table 1. Least Squares Means for Number of Follicles

Follicular fluid IGF-I and Steroid Concentrations. Follicular fluid IGF-I in large follicles was not affected by exogenous insulin (115 and 125 ng/ml for saline and insulin treatments, respectively; SEM = 8.2). However, for medium follicles concentration of IGF-I was almost doubled by exogenous insulin treatment. Least squares means were 60.7 and 108.8 ng/ml for saline and insulin treatments,
respectively (SEM = 13.3). The IGF-I concentration for medium follicles of insulin-treated animals was similar to that for large follicles. There was no effect of body condition on follicular fluid IGF-I concentrations.

Higher backfat was associated with decreased follicular fluid concentration of testosterone in medium and large follicles (Table 2). Follicular fluid estradiol was greater in large follicles but was not affected by insulin treatment or body condition (Table 2). Follicular fluid progesterone concentrations were not affected (P > .05) by insulin but were decreased in large follicles of fatter gilts and were greater in large than in medium follicles (Table 2). Ratios of estradiol to progesterone (3.8 ± 1.4) and estradiol to testosterone (7.2 ± 5.4) were not affected by insulin treatment, body condition, or follicle size.

Other Peripheral Hormones and Metabolites. Serum concentrations of NEFA were not affected by any main effect (overall mean = 58.2 μg/liter; SEM = 4.56). Peripheral IGF-I concentrations were not affected by exogenous insulin (overall average for saline = 80.3 ng/ml; SEM = 4.7) or body condition (moderate = 77.8 vs high = 87.5 ng/ml; SEM = 4.7).

For serum LH, a significant insulin treatment × body condition × time interaction (P < .01) was found to be unrealistic after adjustment for correlation. Polynomial regression equations describing LH were similar for all treatment combinations. Average LH was less than 1 ng/ml for all gilts until 84 h of sampling, which was 72 h after PMSG. The beginning of the preovulatory surge in LH was evident in some gilts at 84 h. There was no effect of treatment on the proportion of gilts with LH > 1 ng/ml at 84 h; 2/4 gilts each in the treatment combinations saline-moderate and insulin-high, 1/4 gilts in the saline-high, and 3/4 gilts in the insulin-moderate groups had LH > 1 ng/ml at 84 h.

Serum FSH was not affected by insulin (overall average for saline = .79 vs insulin = .85 ng/ml; SEM = .16; P > .05) or body condition (overall average for moderate = .85 vs high = .74 ng/ml; SEM = .16; P > .05). Growth hormone was reduced (P < .05) in gilts fed to reach higher backfat (overall average for moderate = 3.2 vs high = 2.8 ng/ml; SEM = .11) and was not affected by exogenous insulin.

A significant body condition × time interaction for plasma glucagon (P < .05) was found to be unreal after adjustment for correlation, and upon multivariate analysis of variance the prediction equations were found to be similar among treatment combinations. Overall average for glucagon was .3 ± .01 ng/ml.

Serum cortisol was not affected by insulin treatment (overall average for saline = 34.1 vs insulin = 25.0 ng/ml; SEM = .56; P > .05) or body condition (overall average for moderate = 27.6 vs high = 32.3 ng/ml; SEM = .56; P > .05).

As expected, serum insulin concentrations were increased by insulin treatment (saline = 1.2 vs insulin = 1.6 ng/ml; SEM = .12; P < .06) but were not influenced by body condition. The greatest difference in insulin concentration was in samples taken at 6 h after each injection, when least squares means were 1.90 and 1.36 ng/ml for insulin and saline treatments, respectively (SEM = .09; P < .001). Glucose was decreased over time by insulin treatment (treatment × time interaction; P <

### Table 2. Follicular Fluid Concentrations of Steroids in Pooled Follicular Fluid of Large and Medium Follicles

<table>
<thead>
<tr>
<th>Item</th>
<th>Size</th>
<th>Moderate</th>
<th>High</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone, ng/ml</td>
<td>Large</td>
<td>39.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>35.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.2</td>
</tr>
<tr>
<td>Estradiol&lt;sup&gt;c&lt;/sup&gt;, ng/ml</td>
<td>Large</td>
<td>289.4</td>
<td>206.3</td>
<td>34.9</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>83.7</td>
<td>96.9</td>
<td>27.8</td>
</tr>
<tr>
<td>Progesterone&lt;sup&gt;c&lt;/sup&gt;, ng/ml</td>
<td>Large</td>
<td>131.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.2</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>34.2</td>
<td>42.5</td>
<td>9.4</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means in a row not followed by the same superscript letter differ (P < .05).

<sup>c,d</sup>Means in a row not followed by the same superscript letter differ (P < .07).

<sup>e</sup>Difference due to follicle size (P < .05).
INSULIN AND BODY CONDITION AFFECT FOLLICLES

![Graph showing glucose concentration over time for insulin- and saline-treated gilts.](image-url)

**Figure 1.** Least squares means for glucose concentration for insulin- and saline-treated gilts (SEM = 4.0). The prediction equations for the insulin-treated glucose profile \( Y = 77.42 - 6 \times \text{time} + 0.333 \times \text{time}^2 - 0.0083 \times \text{time}^3 + 0.0000534 \times \text{time}^4 - 0.000000939 \times \text{time}^5 \) and saline-treated glucose profile \( Y = 90.5 + 0.43 \times \text{time} - 0.1055 \times \text{time}^2 + 0.0041 \times \text{time}^3 - 0.000057 \times \text{time}^4 + 0.0000027 \times \text{time}^5 \) are indicated.

Body condition did not affect plasma glucose.

The PUN concentration was associated with a three-way interaction (insulin treatment \times body condition \times time; \( P < 0.05 \)), which was found to be unreal because of correlation in the repeated measurements. The prediction equations for PUN were similar among treatment combinations, and the overall average PUN was 11.7 \( \pm \) 0.3 mg/dl.

Serum testosterone was not affected by insulin treatment or body condition (overall mean = 21 \( \pm \) 0.3 ng/ml; \( P > 0.05 \)). A body condition \times time interaction for estradiol (\( P < 0.05 \)) was found to be unreal after adjustments for correlations in the repeated measurements. The prediction equations were similar for all treatment combinations, but there was a tendency (\( P < 0.12 \)) for elevation in the animals with moderate backfat (Figure 2).

**Discussion**

Number of small follicles was doubled and atresia in small follicles was dramatically reduced by exogenous insulin administration. These findings are supported by the study of Meurer et al. (1991), who reported the converse of these results in diabetic animals. In that study, the absence of insulin in diabetic gilts was accompanied by increased atresia and reduced number of small follicles. These data confirm the effect of insulin on reduction of follicular atresia. The distribution of atretic follicles by size class in both studies indicates that the main effect on prevention of atresia by insulin in PMSG-treated gilts is directed toward small follicles.

In cyclic gilts we observed that reduction of atresia by insulin was not confined to small follicles (Matamoros et al., 1990). Instead, in that study incidence of atresia in the medium follicle population between d 17 and d 19 of the cycle was reduced by exogenous insulin. Accordingly, Meurer et al. (1990) observed that atresia in medium and large follicles was increased by the removal of insulin therapy in cyclic diabetic gilts. Thus, in cyclic gilts, effects of insulin on atresia seem to be concentrated in medium-sized follicles, which are likely to become ovulatory follicles by d 21 of the estrous cycle. In the present study, follicular stimulation by PMSG may have been sufficient to prevent an effect of insulin on atresia of larger follicles. Reduction of atresia by PMSG has been demonstrated in sheep (Dott et al., 1979).

In vitro studies have demonstrated that insulin synergizes with gonadotropins to differentiate and facilitate morphological develop-
Figure 2. Least squares means for serum estradiol-17β for gilts with moderate (SEM = 4.3) and high backfat depth (SEM = 4.3). The solid line describes the overall estradiol profile \( Y = 2.84 + .351 \text{ (time)} + .214 \text{ (time}^2) - .0068 \text{ (time}^3) \).
GH has been negatively associated with mediated by an increase in feed restriction (Buonomo and Baile, 1987). Exogenous insulin or feeding, obesity (Althen and Gemts, 1976b) suggested that the effects of GH may be detected. In the present study, only plasma circulating concentrations would have been.(Kirkwood 

Strated to increase ovulation rate during the stage of follicular development, diet could play a complementary role to insulin in advancing follicular development. However, interactions of insulin treatment and body fat were not observed for any single variable in the present study. Furthermore, there was no evidence for a differential sensitivity to insulin in leaner and fatter animals.

In the present study, ratios of estradiol to other steroids were not influenced by body condition or exogenous insulin administration. Matamoros et al. (1990) observed that intrafollicular ratios of estradiol to progesterone were increased on d 17 of the estrous cycle by exogenous insulin, implying an enhancement of steroidogenic activity by insulin. A possible explanation may be that the stage of follicular development examined in this study is more similar, with respect to predicted time of ovulation, to d 19 of the estrous cycle, on which there were no effects of insulin on steroid ratios (Matamoros et al., 1990).

The present study does not support the supposition of Cox et al. (1987b) that other metabolic hormones and metabolites that respond to sustained low levels of blood glucose may signal ovarian function. The sampling frequency of every 6 h prevented identification of acute responses of metabolic hormones to insulin or feeding, so only sustained changes in circulating concentrations would have been detected. In the present study, only plasma glucose exhibited a sustained lowering by exogenous insulin. The only metabolic hormone affected by body condition was GH, which was elevated in leaner gilts. Circulating GH has been negatively associated with obesity (Althen and Gerrits, 1976b) as well as feed restriction (Buonomo and Baile, 1987). Exogenous growth hormone has been demonstrated to increase ovulation rate during the follicular phase of the estrous cycle in gilts (Kirkwood et al., 1988a). The same authors suggested that the effects of GH may be mediated by an increase in serum IGF-I. However, these positive effects of GH have been accompanied by anestrus of some cycling gilts (Kirkwood et al., 1988a), cystic-like conditions associated with PMSG/hCG-induced follicular development, and reduced pituitary responsiveness to estradiol in prepuberal gilts (Kirkwood et al., 1988b).

In dogs with concomitant brain and peripheral hypoglycemia, glucagon was the most important factor in restoration of normal glycemia (Biggers et al., 1989). However, in the present study, metabolic hormones and metabolites other than growth hormone were not affected sufficiently to confirm involvement in ovarian follicular development. The failure of restoration of normal levels of glucose suggests that actions of insulin were not fully counterbalanced by these hormones. Results of the present study are consistent with a direct effect of insulin rather than mediation by other metabolic hormones.

It should be noted that our experimental model does not rule out the interactions of the hypothalamo-hypophyseal-ovarian axis in controlling follicular development. In this study, basal levels of gonadotropic hormones were unchanged by insulin and/or body condition, but exogenous gonadotropic support by PMSG may have obviated the need for endogenous gonadotropic support. Pregnant mare’s serum gonadotropin stimulated follicular growth without appreciable changes in gonadotropins (Flowers et al., 1989b). Furthermore, this experiment does not distinguish between endocrine or paracrine actions of IGF-I on ovarian functions affected by insulin. However, it should be noted that although insulin administration did not affect peripheral IGF-I, intrafollicular IGF-I was indeed increased. We conclude that insulin affects follicular dynamics by reducing atresia and increasing the number of potential ovulatory follicles. The role of insulin on ovarian function seems to involve enhancement of intrafollicular production of IGF-I.

**Implications**

During follicular growth induced by an exogenous gonadotropin, exogenous insulin altered follicular growth patterns. As we observed earlier, the most striking effect of insulin is in reduction of atresia. Reduction of atresia during follicular growth is one mechanism by which more follicles could enter ovulatory, rather than degenerative, pathways.
Effects of dietary energy on enhancing ovulation rate are not as clear, but they may involve alterations in steroid production by follicles. The other major finding of this study is that in medium-sized follicles, exogenous insulin increased concentrations of insulin-like growth factor I. There is strong evidence based on in vitro observations that this substance controls follicular development and differentiation in the ovary.

Literature Cited


INSULIN AND BODY CONDITION AFFECT FOLLICLES


