

Administration of Porcine Somatotropin by a Sustained-Release Implant: Effects on Follicular Growth, Concentrations of Steroids and Insulin-Like Growth Factor I, and Insulin-Like Growth Factor Binding Protein Activity in Follicular Fluid of Control, Lean, and Obese Gilts¹

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ABSTRACT: Prepubertal gilts of control (n = 30), obese (n = 30), or lean (n = 29) genetic lines were implanted with no, one, or two implants of porcine somatotropin (pST, each delivers 2 mg/d) for 6 wk starting at 160 d of age to determine whether pST affects ovarian function. At 4 mg/d, pST increased ($P < .01$) numbers of 4.0- to 6.9-mm (medium) follicles but not ($P > .10$) numbers of 1.0- to 3.9-mm (small) follicles per gilt. Both doses of pST increased ($P < .01$) serum and follicular fluid (FFL) concentrations of IGF-I and activity of IGF binding protein (IGFBP)-3 and 36-kDa IGFBP in all three lines; IGFBP-3 was the predominant IGFBP. In comparison, binding activity of IGFBP-2 was decreased ($P < .01$) in serum by 4 mg

of pST but increased ($P < .05$) in FFL by 4 mg of pST. Lean gilts had lower ($P < .05$) serum concentrations of IGF-I and less ($P < .05$) total binding activity of IGFBP than control and obese gilts. Concentrations of estradiol in FFL of small and medium follicles tended ($P < .08$) to be increased by 2 mg/d of pST, whereas FFL concentrations of progesterone were unaffected by pST. Obese and control gilts had twofold greater ($P < .05$) FFL progesterone concentrations than lean gilts. We conclude that sustained-release implants of pST can stimulate follicular growth, increase concentrations of IGF-I in serum and FFL, and increase IGFBP activity in serum of genetically divergent lines of gilts without an adverse effect on ovarian function.

Key Words: Somatotropin, Gilts, Insulin-Like Growth Factor, Binding Proteins, Ovaries

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Introduction

Recent attempts to improve production efficiency in domestic pigs has concentrated on the use of exogenous porcine somatotropin (pST) supplied in slow-release implants (Azain et al., 1992; Klindt et al., 1992a; Hacker et al., 1993). Long-term pST treatment supplied in daily injections has stimulatory and inhibitory effects on reproductive functions in gilts

(Bryan et al., 1989; Kirkwood et al., 1989; Spicer et al., 1992). Some of these reproductive responses to pST are affected by genotype of the gilts (Spicer et al., 1992). However, the effect of sustained-release implants of pST on reproductive functions in gilts has not been reported. The ovarian effect of ST is thought to be mediated through direct effects of ST on ovarian follicles (Hsu and Hammond, 1987; Mondschein et al., 1989; Langhout et al., 1991), and, in part, through increased hepatic production of IGF-I (Gluckman et al., 1987; Guidice, 1992). Although ST is thought to play an important role in the onset of puberty in female rats (Ramaley and Phares, 1980), daily injections of pST starting at 3 to 4 mo of age did not alter the age of puberty onset in gilts (Andres et al., 1991; Terlouw et al., 1991). Our experiment was designed to determine whether sustained-release implants of pST affect ovarian follicular function in prepubertal gilts from populations selected for either high (obese) or low (lean) backfat, and a control population.

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Materials and Methods

Animals and Treatments. Gilts from a contemporary line (control, $n = 30$) and from lines selected for high (obese, $n = 30$) or low (lean, $n = 29$) backfat were implanted for 6 wk with no, one, or two implants that deliver 0, 2, or 4 mg of recombinant pST per day, respectively, starting at approximately 160 d of age (59.0 ± 1.4 kg of BW). The control gilts were unselected 1/4 Landrace-1/4 Yorkshire-1/4 Chester White-1/4 Large White gilts. The obese and lean pigs were Duroc obese \times Yorkshire obese and Duroc lean \times Yorkshire lean pigs derived from populations selected solely for high or low backfat thickness over multiple generations (Hetzer and Harvey, 1967). The gilts were allotted into four replicates to be slaughtered on four different days between May 15 and July 2, 1991. Gilts were fed, to appetite, a diet composed of 64.5% corn and 31.6% soybean meal (19.5% CP; as-fed basis), supplemented with dicalcium phosphate, ground limestone, trace mineral premix, and vitamin premix (2.4, .5, .2, and .2% as-fed basis, respectively). The contents of the trace mineral premix and vitamin premix have been described previously (Yen et al., 1990). Calculated lysine concentration of the diet was 1.08% (as-fed basis).

Gilts were slaughtered approximately 6 wk ($41.4 \pm .1$ d) after the implantation of pST. Ovaries and uteri were removed and weighed at slaughter, and the numbers of all follicles ≥ 1 mm on the ovarian surface were recorded. The follicular fluid (FFL) from follicles 1 to 3.9 mm (small) and 4 to 6.9 mm (medium) in diameter was collected and pooled within each size group and ovary. Fluid from large (≥ 7.0 mm) follicles was also collected, but very few of these large follicles were found and therefore this FFL was not analyzed. Blood samples were collected at slaughter for IGF-I analysis. Serum and FFL were stored at -20°C until they were analyzed.

Radioimmunoassays. Immunoreactive IGF-I in FFL and serum was determined with RIA after acid-ethanol extraction as described previously (Hammond et al., 1988; Echternkamp et al., 1990). This procedure resulted in parallelism between human IGF-I standard (Amgen Biologicals, Thousand Oaks, CA) and porcine FFL and serum. Intra- and interassay CV were 12.0 and 14.3%, respectively. Concentrations of progesterone in FFL were quantified with a RIA (Baranao and Hammond, 1985). Intra- and interassay CV were 9.9 and 16.1%, respectively. Concentrations of estradiol in FFL were quantified with a RIA (Cox et al., 1987) as modified by Spicer and Enright (1991). This procedure resulted in parallelism between estradiol standard and porcine FFL. Intra- and interassay CV were 15.4 and 27.9%, respectively.

Insulin-Like Growth Factor I Binding Protein Activity. Total IGFBP activity in serum was determined after incubation with [^{125}I]IGF-I by the method of Moses et al. (1979). Samples of FFL from small or

medium follicles were pooled within gilt before analysis. Briefly, 10- μL aliquots of serum or FFL were incubated overnight at 4°C with 100 μL of [^{125}I]IGF-I (15,000 cpm; counter efficiency was 75%) and 150 μL of assay buffer (PBS containing 2.5 mg of BSA/mL, pH = 7.5). To separate bound from free [^{125}I]IGF-I, activated charcoal (500 μL ; 5% wt/vol in PBS containing 2.5 mg of BSA/mL) was added to each tube, incubated for 30 min at 4°C , and centrifuged at $1,200 \times g$ for 20 min at 4°C . Intra- and interassay CV were 7.9 and 17.4%, respectively.

To evaluate the various molecular weight species of the IGFBP, one-dimensional SDS-PAGE was performed on serum and representative follicular fluid samples under nonreducing conditions as described previously for porcine FFL (Howard and Ford, 1992). Briefly, proteins in 1- μL samples (added to 24 μL of buffer) were separated on a 12% polyacrylamide gel, electrophoresed, transferred to nitrocellulose, and ligand-blotted overnight with [^{125}I]IGF-I for IGFBP activity. Band intensity on autoradiographs was characterized with scanning densitometry. Serial dilutions of serum or FFL (i.e., 1:1, 1:3, and 1:5 dilutions of the original 1:25 dilution) produced proportional, quantitative reductions in band intensities (data not shown).

Statistical Analyses. Hormone data within each treatment were grouped into two follicle-size groups, based on diameter of follicle: 1.0 to 3.9 mm (small) and 4.0 to 6.9 mm (medium). Follicle data were analyzed with least squares ANOVA (SAS, 1988) for a $3 \times 3 \times 2$ factorial arrangement in a randomized design. Main effects were dose of pST = 0, 2, or 4; line = control, obese, or lean; follicle size = small or medium. Data on the various molecular weight forms of IGFBP in serum and FFL were analyzed with least squares ANOVA for a $3 \times 3 \times 3$ factorial arrangement in a randomized design; main effects were as follows: dose of pST = 0, 2, or 4; line = control, obese, or lean; source = serum, small FFL, or medium FFL. Serum IGF-I was analyzed as a 3×3 factorial ANOVA. All possible interactions were also evaluated. Data with heterogeneous variances (FFL progesterone and estradiol) were analyzed after transformation to $\ln(x + 1)$. Specific differences between means were determined using the PDIFF procedure (SAS, 1988) if significant main effects were detected. Relationships among selected variables were evaluated with regression and simple correlation analyses (Pearson correlation coefficients; SAS, 1988).

Results

Gross Ovarian and Uterine Morphology. As assessed by the absence of any corpora lutea, all gilts were prepubertal at slaughter. Ovarian weight was influenced by pST ($P < .05$) and line ($P < .001$) but not by the pST \times line interaction ($P > .20$).

Specifically, ovarian weight was greater ($P < .05$) in gilts treated with 4 mg of pST ($6.20 \pm .26$ g) than in gilts treated with 0 mg of pST ($5.22 \pm .25$ g) and intermediate in gilts treated with 2 mg of pST ($5.77 \pm .25$ g). Ovarian weight was greater ($P < .05$) for control gilts than for lean or obese gilts (Table 1). When ovarian weight was expressed as grams/kilogram of BW at slaughter, it was not affected by pST ($P > .10$), because pST increased ($P < .05$) BW proportionately. However, adjusted ovarian weight (grams/kilogram BW) was affected ($P < .01$) by line; control gilts had the greatest ovarian weight ($.084 \pm .003$ g/kg), lean gilts had the least ovarian weight ($.059 \pm .004$ g/kg), and obese gilts had an intermediate ovarian weight ($.068 \pm .003$ g/kg). Uterine weight was unaffected ($P > .10$) by pST dose, line, or their interaction (Table 1).

Numbers of medium follicles were increased ($P < .01$) 2.4-fold by 4 mg of pST (Figure 1), whereas numbers of small follicles were unaffected ($P > .10$) by pST. Numbers of medium, but not small, follicles were also affected ($P < .10$) by line; control gilts had 2.3-fold greater numbers of medium follicles than did lean or obese gilts (Figure 1).

Insulin-Like Growth Factor I in Serum and Follicular Fluid. Dose of pST ($P < .001$) and line ($P < .05$) affected serum IGF-I (Table 1), but there was no pST dose \times line interaction ($P > .20$). Both 2 (227 ± 23 ng/mL) and 4 mg (315 ± 23 ng/mL) of pST increased ($P < .05$) serum concentrations of IGF-I above that of 0 mg of pST (89 ± 23 ng/mL). Four milligrams of pST, relative to 0 mg, increased serum IGF-I 3.6-, 4.6-, and 2.5-fold in control, obese, and lean gilts, respectively. The greatest difference in serum IGF-I

between lean and obese gilts was detected in gilts treated with 4 mg of pST (Table 1).

Porcine somatotropin also increased concentrations of IGF-I in FFL 3.8- and 4.5-fold for 2 and 4 mg of pST, respectively, relative to 0 mg of pST (Figure 2). Significant main effects and interactions were dose of pST ($P < .01$), line ($P < .01$), follicle size ($P < .01$), pST \times line ($P < .01$), pST \times follicle size ($P < .01$), and line \times follicle size ($P < .05$); the three-way interaction was not significant ($P > .10$). In FFL of small, but not medium, follicles (pST \times follicle size, $P < .01$), IGF-I concentrations were further increased by 4 mg of pST (i.e., vs 2 mg of pST, 187 vs 137 ng/mL, SEM = 11, $P < .01$). Pooled across follicle sizes and pST doses, lean gilts had lower concentrations of IGF-I in FFL than control and obese gilts (122 vs 152 and 199 ± 8 ng/mL, respectively). Pooled across lines, medium follicles had 62% greater concentrations of IGF-I than small follicles (195 vs 120 ng/mL, SEM = 7). Treatment ($P < .06$), but not line ($P > .10$), tended to affect the FFL: serum ratio of IGF-I. Ratios for 0, 2, and 4 mg of pST averaged .61, .96, and .88, SEM = .11, respectively.

Follicular Fluid Steroids. Concentrations of progesterone measured in FFL of small and medium follicles were not affected ($P > .10$) by dose of pST. However, line and follicle size affected ($P < .01$) FFL progesterone concentrations (Figure 3). All interactions were nonsignificant ($P > .10$). Concentrations of progesterone were twofold greater in obese and control gilts than in lean gilts, and were 25% greater in small vs medium follicles (Figure 3).

Concentrations of estradiol in FFL were influenced by line ($P < .05$) and follicle size ($P < .001$); none of the interactions influenced ($P > .20$) FFL estradiol concentrations. Obese gilts had 1.9-fold greater FFL estradiol concentrations than control and lean gilts

Table 1. Effect of porcine somatotropin (pST) on serum insulin-like growth factor-I (IGF-I), body weight, ovarian and uterine weights, and serum IGF-I binding protein activity (IGFBP) of control, obese, and lean gilts

Treatment and line	No. of gilts	Serum IGF-I, ng/mL ^a	BW, kg ^a	Ovarian wt, g ^a	Uterine wt, g	Serum IGFBP, % ^{ab}
0 mg of pST						
Control	10	108 ^{cd}	84.1 ^{de}	6.9 ^e	80	6.0 ^{de}
Obese	10	73 ^c	71.4 ^c	5.0 ^{cd}	89	5.2 ^d
Lean	10	87 ^{cd}	80.2 ^d	3.9 ^c	72	4.4 ^d
2 mg of pST						
Control	10	257 ^{ef}	88.0 ^{ef}	7.0 ^{ef}	88	10.0 ^{fgh}
Obese	10	255 ^{ef}	74.7 ^c	5.4 ^d	84	9.9 ^{fg}
Lean	10	168 ^{de}	81.6 ^f	4.9 ^{cd}	87	7.1 ^e
4 mg of pST						
Control	10	389 ^g	89.0 ^f	8.1 ^f	88	11.5 ^{gh}
Obese	10	337 ^{fg}	74.9 ^c	5.1 ^{cd}	81	11.6 ^h
Lean	9	220 ^e	81.2 ^d	5.4 ^d	93	9.7 ^f
SEM	—	40	1.6	.4	12	.6

^aSignificant treatment and line effect ($P < .05$).

^bIGFBP is expressed as percentage of [¹²⁵I]IGF-I specifically bound per 10- μ L sample.

^{c,d,e,f,g,h}Within column, means with different superscripts differ ($P < .05$).

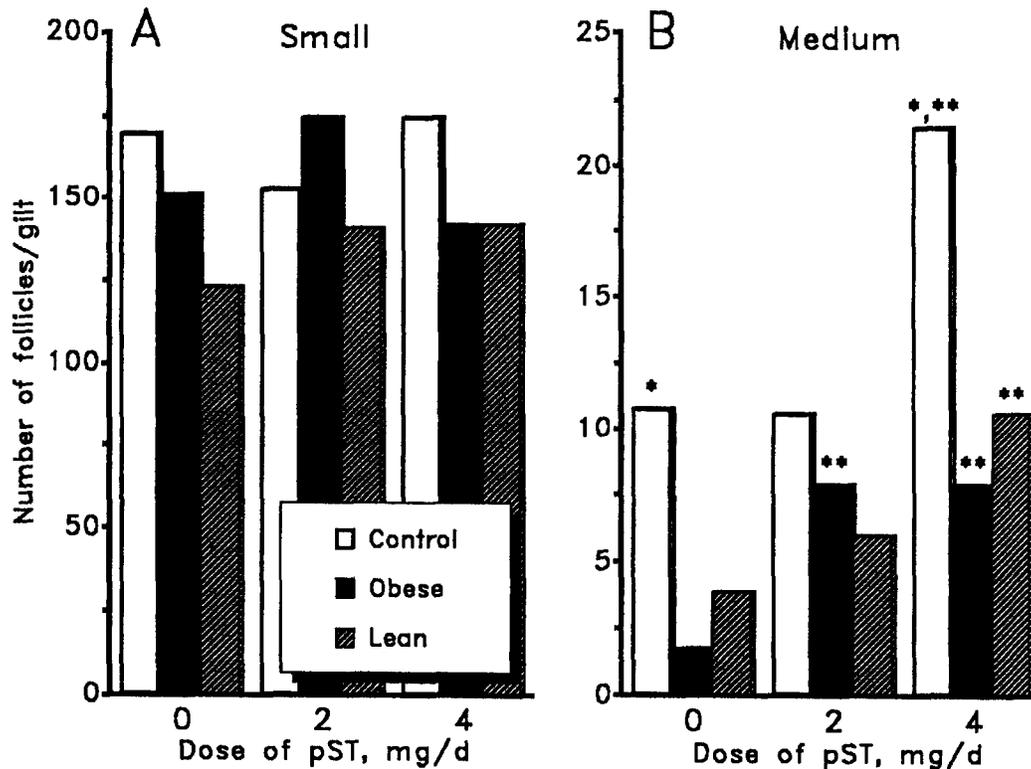


Figure 1. Numbers of small (1.0 to 3.9 mm; Panel A) and medium (4.0 to 7.0 mm; Panel B) follicles measured in prepubertal control, obese, and lean gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) daily for 6 wk. In Panel A, SEM = 21 for small follicles. In Panel B, SEM = 2.1 for medium follicles. * $P < .05$ vs obese gilts within follicle size and pST dose; ** $P < .05$ vs 0 mg/d.

(8.4 vs 4.6 and 4.3 ng/mL, SEM = 1.5, respectively), and medium follicles had 2.3-fold greater concentrations of estradiol than small follicles (8.1 vs 3.5, SEM = 1.2 ng/mL). Dose of pST tended ($P < .08$) to influence FFL estradiol concentrations; estradiol concentrations were increased in medium follicles of control and obese gilts by 2 mg of pST only (Figure 4).

Total Insulin-Like Growth Factor I Binding Protein Activity in Serum. Total IGFBP activity (expressed as percentage of [125 I]IGF-I specifically bound/10 μ L) in serum was influenced ($P < .05$) by pST dose and line but not by pST \times line (Table 1). Treatment with 2 and 4 mg of pST, relative to 0 mg, increased ($P < .05$) IGFBP activity in serum 1.7- and 2.0-fold, respectively. Lean gilts had less ($P < .05$) IGFBP activity in serum ($7.1 \pm .4\%$) than did control ($9.1 \pm .4\%$) or obese ($8.9 \pm .4\%$) gilts. Serum IGFBP activity correlated positively ($P < .05$) with serum IGF-I in control, lean, and obese gilts ($r = .71, .76, \text{ and } .46$, respectively).

Ligand Blot Analysis of Insulin-Like Growth Factor I Binding Protein in Follicular Fluid and Serum. Ligand blots revealed that at least six forms of IGFBP activity existed in porcine FFL and serum (Figure 5). Two major bands of IGFBP (40 and 44 kDa) were identified as doublets of IGFBP-3 and combined for

analysis. The 34-kDa IGFBP was identified as IGFBP-2. The amount of binding activity for IGFBP-2 and for the 36- and 22-kDa IGFBP was affected ($P < .01$) by fluid source (Table 2). Specifically, serum had less IGFBP-2 and 36-kDa IGFBP activity and more 22-kDa IGFBP activity than FFL. Both 2 and 4 mg of pST increased ($P < .01$) binding activity of IGFBP-3 and the 36-kDa IGFBP in serum and FFL; pST tended ($P < .10$) to increase binding activity of the 28-kDa IGFBP. Binding activity of IGFBP-2 was decreased ($P < .01$) in serum by 4 mg of pST, increased ($P < .05$) in FFL of small follicles by 2 and 4 mg of pST, and unchanged ($P > .10$) in FFL of medium follicles by pST (pST \times source, $P < .01$). No significant line or line \times pST effects on IGFBP activity in serum or FFL were evident.

Total binding activity of the IGFBP (i.e., IGFBP-3 + IGFBP-2 + 36-, 28-, and 22-kDa IGFBP) was increased by pST dose (21.2 vs 29.5 vs 33.8 units/ μ L, SEM = .7, for 0 vs 2 vs 4 mg of pST/d, $P < .01$), and was lower ($P < .01$) in serum (26.5 units/ μ L) than in FFL in small (30.3 units/ μ L) or medium (29.8 units/ μ L) follicles. In addition, total serum binding activity of the IGFBP assessed by ligand blot was correlated with total IGFBP activity in serum assessed by charcoal exchange assay ($r = .65$, $P < .01$).

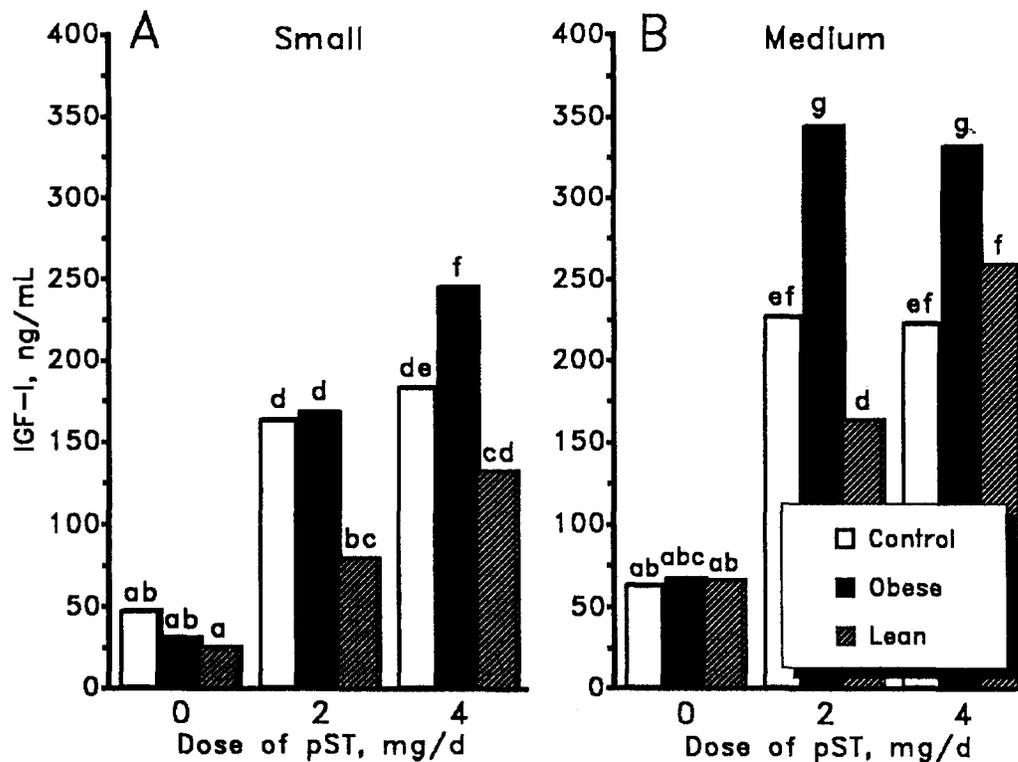


Figure 2. Concentrations of insulin-like growth factor I in follicular fluid of small (1.0 to 3.9 mm; Panel A) and medium (4.0 to 7.0 mm; Panel B) follicles collected from prepubertal control, obese, and lean gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) daily for 6 wk. In Panel A, SEM = 6 ng/mL for small follicles; the number of samples for the control, obese, and lean gilts was 20, 20, and 20 for both 0 and 2 mg of pST and 20, 20, and 18 for 4 mg of pST, respectively. In Panel B, SEM = 8 ng/mL for medium follicles; the number of samples for the control, obese, and lean gilts was 18, 5, and 12 for 0 mg of pST, 18, 17, and 14 for 2 mg of pST, and 20, 18, and 16 for 4 mg of pST, respectively. ^{a,b,c,d,e,f,g}Across panels, means with different letters differ ($P < .05$).

Discussion

Treatment of pigs with daily injections of pST or sustained-release implants of pST has caused 2- to 11-fold increases in serum IGF-I concentrations in previous studies (Evock et al., 1988; Bryan et al., 1989, 1992; Klindt et al., 1992a; Spicer et al., 1992). Similarly, we measured two- to fivefold increases in serum IGF-I in blood of gilts treated with sustained-release implants that delivered 2 or 4 mg of pST daily. This increase in serum IGF-I, and the associated increases in pST, IGF-II, and (or) insulin (Klindt et al., 1992b), may account for the increases in ovarian weight and follicular function (Geisthovel et al., 1990; Guidice, 1992) and for the changes in growth performance and carcass measurements (Buonomo et al., 1992) of pST-treated pigs.

The mechanism by which pST alters follicular growth is unknown. Presumably the increased number of medium follicles in pST-treated gilts resulted from increased IGF-I concentrations in serum and (or) FFL, because IGF-I stimulates granulosa cell mitosis in vitro (Geisthovel et al., 1990; Guidice, 1992). In

support of this suggestion, we found that FFL IGF-I concentrations and number of medium, but not small, follicles were correlated positively ($r = .41$ in obese and $r = .55$ in lean gilts). Previously, a positive association ($r = .3$ to $.5$) between numbers of small ovarian follicles and FFL IGF-I was detected in lean and obese gilts treated with daily injections of pST for 6 wk (Spicer et al., 1992). Similarly, Gong et al. (1991) observed that daily injections of bST for 42 d in postpubertal heifers increased the number of small (2 to 5 mm), but not medium or large, follicles. It is unclear why numbers of medium follicles and not small follicles were affected by pST in the present vs previous study, but it may be due in part to the difference in the delivery system of pST (implant vs injection).

In vitro, IGF-I stimulates estrogen production by granulosa cells from rats (Davoren et al., 1986), pigs (Maruo et al., 1988), humans (Erickson et al., 1990), and cattle (Spicer et al., 1993). Consistent with these in vitro findings, estradiol concentrations in the present study were greater in FFL of control and obese gilts treated with 2 mg of pST daily than in that of

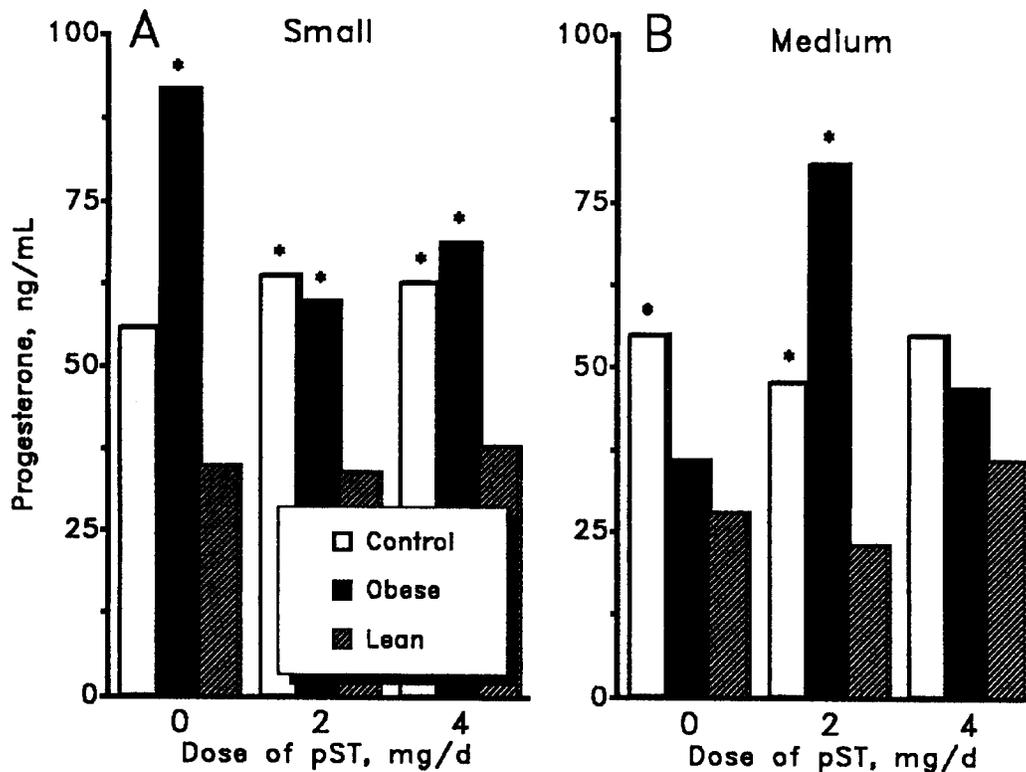


Figure 3. Concentrations of progesterone in follicular fluid of small (1.0 to 3.9 mm; Panel A) and medium (4.0 to 7.0 mm; Panel B) follicles in prepubertal control, obese, and lean gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) daily for 6 wk. In Panel A, SEM = 3 ng/mL for small follicles. In Panel B, SEM = 5 ng/mL for medium follicles. * $P < .05$ vs lean gilts within follicle size and pST dose.

untreated control and obese gilts; these changes in FFL estradiol were coincident with fourfold increases in IGF-I concentrations in serum and FFL. In addition, estradiol and IGF-I concentrations in FFL of control and lean gilts were lower than in FFL of obese gilts. In comparison, Bryan et al. (1989, 1992) found no effect of daily injections of pST on FFL estradiol concentrations in Yorkshire or Duroc gilts, whereas Spicer et al. (1992) found that daily injections of pST had a significant inhibitory effect on FFL estradiol concentrations of obese gilts. Similar to gilts treated with daily pST injections, transgenic prepubertal gilts expressing the bST gene had significantly lower estradiol concentrations in FFL of large, but not medium, follicles than control gilts 3 d after PMSG treatment (Guthrie et al., 1993). The reasons for the discrepancies between the present and prior studies are unknown but may include differences in pST- and/or IGF-I-mediated effects on granulosa cell cytodifferentiation (e.g., expression of gonadotropin receptors). Spicer et al. (1992) reported a decrease in the numbers of LH/hCG binding sites in granulosa cells of medium follicles collected from prepubertal gilts receiving daily injections of pST. In vitro, ST has no effect on FSH-induced estrogen production by rat granulosa cells (Jia et al., 1986) or on basal estradiol

production by porcine granulosa cells (Nitray et al., 1993). Thus, it is unlikely that increased pST directly caused the increase in FFL estradiol concentrations observed in the present study, and that differences in pST concentrations achieved with daily injections vs sustained-release implants of pST account for differences in the effect of pST on FFL estradiol concentrations among studies. Perhaps the greater IGF-I concentrations in transgenic gilts or after daily pST injections vs pST implants caused a down-regulation of the ovarian IGF-I system. Previous research has shown that high IGF-I concentrations can cause post-receptor desensitization (Cascieri et al., 1988). However, further research with pigs will be required to verify these suggestions.

In vitro, ST and IGF-I enhance progesterone production by cultured porcine granulosa cells (Baranao and Hammond, 1984; Veldhuis et al., 1985; Hsu and Hammond, 1987; Kirkwood et al., 1992). These effects seem to be direct because both ST and IGF-I receptors are present in granulosa cells (Carlsson et al., 1992; Guidice, 1992). Also, pST treatment in vivo enhanced subsequent in vitro progesterone production by porcine granulosa cells stimulated by LH but had no effect on basal or FSH-stimulated progesterone production (Bryan et al., 1991). In contrast to in vitro

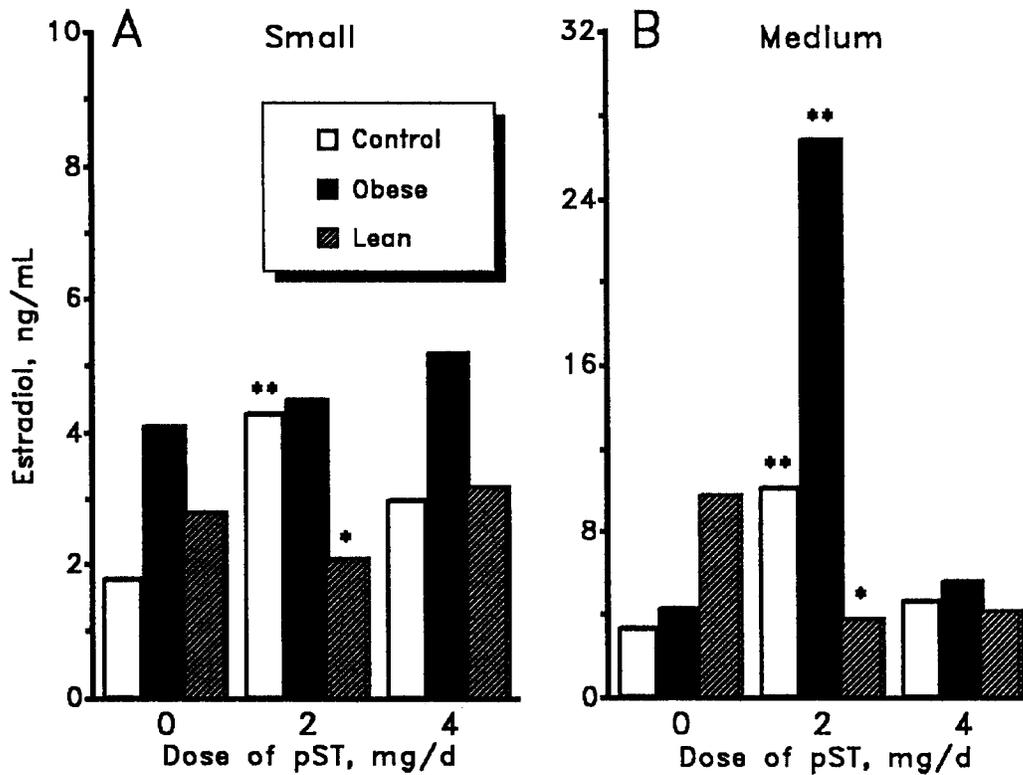


Figure 4. Concentrations of estradiol in follicular fluid of small (1.0 to 3.9 mm; Panel A) and medium (4.0 to 7.0 mm; Panel B) follicles collected from prepubertal control, obese, and lean gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) daily for 6 wk. In Panel A, SEM = 1 ng/mL in small follicles. In Panel B, SEM = 2 ng/mL in medium follicles. **P* < .05 vs obese gilts within follicle size and pST dose; ***P* < .05 vs 0 mg/d.

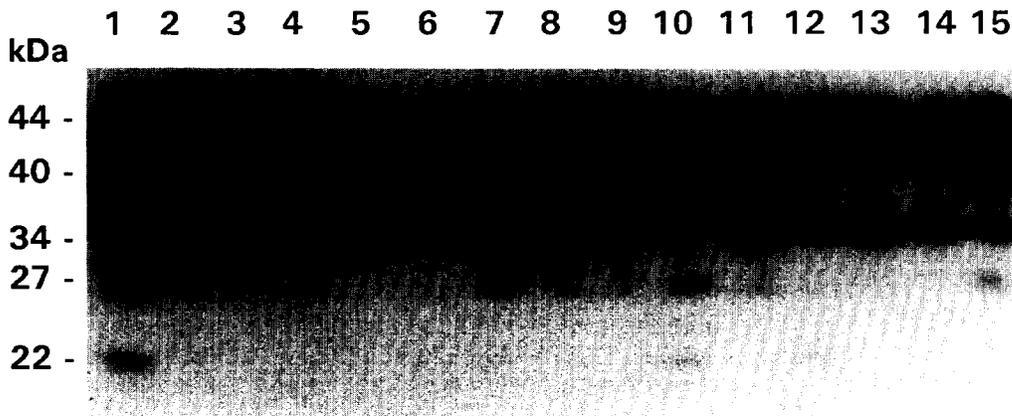


Figure 5. A representative ligand blot analysis of IGFBP in serum and follicular fluid (FFL) of prepubertal gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) daily for 6 wk. Samples (1 μ L) from five individual gilts were analyzed as described in Materials and Methods. Lane 1: pool of bovine FFL; Lanes 2, 3 and 4: FFL sample from a small follicle pool, a medium follicle, and serum, respectively, of a 4 mg/d control-line gilt; Lanes 5 and 6: FFL from a small follicle pool and serum, respectively, of a 0 mg/d lean-line gilt; Lanes 7 to 10: FFL from a small follicle pool, two medium follicles, and serum, respectively, of a 2 mg/d obese-line gilt; Lanes 11 and 12: FFL from a small follicle pool and serum, respectively, of a 2 mg/d control-line gilt; Lanes 13 to 15: FFL from a small follicle pool, medium follicle, and serum, respectively, of a 2 mg/d lean-line gilt.

Table 2. Relative abundance of insulin-like growth factor I binding protein (IGFBP; arbitrary binding activity units/microliter) forms in serum and follicular fluid (FFL) collected from small (1 to 3.9 mm) and medium (4.0 to 6.9 mm) follicles of gilts treated with 0, 2, or 4 mg of porcine somatotropin (pST) per day for 6 weeks^a

Dose of pST and form of IGFBP	Fluid source			SEM
	Serum	Small FFL	Medium FFL	
0 mg				
IGFBP-3 ^b	17.41	17.26	16.53	1.20
IGFBP-2 ^c	2.25	5.03	3.66	.51
36 kDa ^{bc}	.26	.59	.44	.10
28 kDa ^d	.12	.04	.03	.11
22 kDa ^c	.06	.01	.00	.03
No. of samples	24	18	6	—
2 mg				
IGFBP-3	25.53	24.78	22.89	.93
IGFBP-2	1.81	6.17	2.76	.39
36 kDa	.68	1.19	.89	.07
28 kDa	.43	.32	.15	.09
22 kDa	.14	.03	.01	.02
No. of samples	25	20	14	—
4 mg				
IGFBP-3	28.32	26.76	29.76	.93
IGFBP-2	1.11	6.27	4.77	.38
36 kDa	1.04	1.56	1.61	.07
28 kDa	.30	.33	.29	.08
22 kDa	.11	.03	.02	.02
No. of samples	23	19	19	—

^aValues are pooled means of control, lean, and obese lines.

^bEffect of pST ($P < .001$).

^cEffect of fluid source ($P < .001$).

^dEffect of pST ($P < .05$).

studies, pST treatment in vivo has little or no effect on FFL progesterone concentrations (Bryan et al., 1989, 1992; Spicer et al., 1992; present study). The reasons for the discrepancies between in vitro and in vivo studies are unknown.

Similar to previous reports (Buonomo et al., 1987; Walton and Etherton, 1989; Evoke et al., 1990; Owens et al., 1990; Spicer et al., 1992), pST increased total IGFBP and IGFBP-3 activity in serum of gilts in the present study. We also found that pST decreased IGFBP-2 activity in serum. Similarly, Klindt et al. (1992a) showed that serum IGFBP-2 concentrations decreased after 7 to 42 d of 2 or 4 mg of pST/d in pigs. Although total IGFBP activity (measured by charcoal exchange and by ligand blotting) in FFL was increased 1.4- to 2.0-fold by pST, amounts of the various molecular weight species of IGF-binding proteins in FFL were not affected similarly by pST treatment. Specifically, FFL IGFBP-3 was increased by pST, whereas FFL IGFBP-2 was unaffected. Two of the three lower-molecular-weight IGFBP in FFL were also increased by pST. Others have found similar numbers and sizes of IGFBP in FFL of weaned sows (Howard and Ford, 1992) and pools of FFL from abattoir porcine ovaries (Mondschein et al., 1991), except for the 36-kDa IGFBP found in the present study.

Whether this 36-kDa IGFBP is a deglycosylated form of IGFBP-3 remains to be determined. Nonetheless, these IGFBP are thought to sequester IGF-I and inhibit its action on follicular function (Hammond et al., 1991; Guidice, 1992), and thus, local actions of IGF-I may have been attenuated by the twofold increases in IGFBP induced by pST in the present study. However, total IGF-I concentrations in serum and FFL increased by three- to fivefold after pST treatment. Unfortunately, we do not know the effect of pST on biologically available IGF-I. Because the inhibitory effect of IGFBP on granulosa cell estradiol production is blocked in the presence of high (> 5 pM) concentrations of IGF-I (Ui et al., 1989), the greater increase in IGF-I concentrations than in IGFBP activity with pST treatment may have "overridden" the inhibitory effects of IGFBP in the present study. Similarly, we do not know the effect of pST on the number and/or binding affinity of IGF-I receptors on follicular cells. Therefore, whether the IGFBP actually inhibit follicular steroidogenesis may depend on the relative changes in concentrations of both IGF-I, IGF receptors, and IGFBP.

Similar to previous studies examining bovine (Spicer et al., 1988; Echternkamp et al., 1990; Spicer and Enright, 1991) and porcine follicles (Hammond et

al., 1988; Spicer et al., 1992), a positive association between follicle size and IGF-I concentration was detected. Whether this greater concentration of IGF-I in medium vs small follicles is due to increased local biosynthesis or increased diffusion of IGF-I from serum remains to be determined. Concentrations of IGF-I in serum and FFL were positively correlated in the present ($r = .60$ to $.77$, $P < .01$) and a previous ($r = .81$; Spicer et al., 1992) study, supporting the idea that blood serum is a source of intraovarian IGF-I. Regardless of the source of FFL IGF-I, the concentrations measured in FFL in the present study are close to the maximal concentrations that have been tested and shown to stimulate porcine granulosa cell proliferation and steroidogenesis in vitro (Baranao and Hammond, 1984; Veldhuis et al., 1985; Maruo et al., 1988; May et al., 1988).

Implications

Long-term treatment of prepubertal gilts with porcine somatotropin delivered through a sustained-release implant increased numbers of medium ovarian follicles and tended to increase estradiol concentrations in medium follicles, an observation not previously found with daily injections of porcine somatotropin. The mediator(s) of the effects of porcine somatotropin on follicular function is(are) unknown but may be linked to its stimulation of the insulin-like growth factor system. Whether sustained-release implants of porcine somatotropin will increase follicular development and ovulation rate or improve reproductive performance in pubertal gilts and mature sows remains to be determined.

Literature Cited

- Andres, C. J., M. L. Green, J. A. Clapper, T. R. Cline, and M. A. Diekmann. 1991. Influence of daily injections of porcine somatotropin on growth, puberty, and reproduction in gilts. *J. Anim. Sci.* 69:3754.
- Azain, M. J., K. D. Bullock, T. R. Kasser, and J. J. Veenhuizen. 1992. Relationship of mode of porcine somatotropin administration and dietary fat to the growth performance and carcass characteristics of finishing pigs. *J. Anim. Sci.* 70:3086.
- Baranao, J.L.S., and J. M. Hammond. 1984. Comparative effects of insulin and insulin-like growth factors on DNA synthesis and differentiation of porcine granulosa cells. *Biochem. Biophys. Res. Commun.* 124:484.
- Baranao, J.L.S., and J. M. Hammond. 1985. Serum-free medium enhances growth and differentiation of cultured pig granulosa cells. *Endocrinology* 116:51.
- Bryan, K. A., A. M. Clark, J. M. Hammond, and D. R. Hagen. 1991. Effect of constant versus adjusted dose of exogenous porcine growth hormone (pGH) on growth and reproductive characteristics of gilts. *J. Anim. Sci.* 69:2980.
- Bryan, K. A., D. R. Hagen, and J. M. Hammond. 1992. Effect of frequency of administration of exogenous porcine growth hormone on growth and carcass traits and ovarian function of prepubertal gilts. *J. Anim. Sci.* 70:1454.
- Bryan, K. A., J. M. Hammond, S. Canning, J. Mondschein, D. E. Carbaugh, A. M. Clark, and D. R. Hagen. 1989. Reproductive and growth responses of gilts to exogenous porcine pituitary growth hormone. *J. Anim. Sci.* 67:196.
- Buonomo, F. C., J. Klindt, and J. T. Yen. 1992. Endocrine and metabolic responses of contemporary and genetically lean and obese boars and gilts administered porcine somatotropin by sustained release implant (PST-SR). *J. Anim. Sci.* 70(Suppl. 1):209 (Abstr.).
- Buonomo, F. C., T. L. Lauterio, C. A. Baile, and D. R. Campion. 1987. Determination of insulin-like growth factor-I (IGF-I) and IGF binding protein levels in swine. *Domest. Anim. Endocrinol.* 4:23.
- Carlsson, B., C. Bergh, J. Bentham, J.-H. Olsson, M. R. Norman, H. Billig, P. Roos, and T. Hillensjo. 1992. Expression of functional growth hormone receptors in human granulosa cells. *Hum. Reprod.* 7:1205.
- Cascieri, M. M., N. S. Hayes, B. Kelder, J. J. Kopchick, G. G. Chicchi, E. E. Slater, and M. L. Bayne. 1988. Inability of a mouse cell line transformed to produce biologically active recombinant human insulin-like growth factor-I (IGF-I) to respond to exogenously added IGF-I. *Endocrinology* 122:1314.
- Cox, N. M., J. L. Ramirez, I. A. Matamoros, W. A. Bennett, and J. H. Britt. 1987. Influence of season on estrous and luteinizing hormone responses to estradiol benzoate in ovariectomized sows. *Theriogenology* 27:395.
- Davoren, J. B., B. G. Kasson, C. H. Li, and A.J.W. Hsueh. 1986. Specific insulin-like growth factor (IGF)I- and II-binding sites on rat granulosa cells: Relation to IGF action. *Endocrinology* 119:2155.
- Echternkamp, S. E., L. J. Spicer, K. E. Gregory, S. F. Canning, and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid of cattle selected for twins. *Biol. Reprod.* 43:8.
- Erickson, G. F., D. A. Magoffin, J. R. Cragun, and R. J. Chang. 1990. The effects of insulin and insulin-like growth factors-I and -II on estradiol production by granulosa cells of polycystic ovaries. *J. Clin. Endocrinol. & Metab.* 70:894.
- Evock, C. M., T. D. Etherton, C. S. Chung, and R. E. Ivy. 1988. Pituitary porcine growth hormone (pGH) and a recombinant pGH analog stimulate pig growth performance in a similar manner. *J. Anim. Sci.* 66:1928.
- Evock, C. M., P. E. Walton, and T. D. Etherton. 1990. Effect of GH status on IGF-I and IGF-II concentrations and serum IGF binding profiles in pigs. *J. Anim. Sci.* 68:1953.
- Geisthovel, F., I. Moretti-Rojas, F. J. Rojas, and R. H. Asch. 1990. Insulin-like growth factors and thecal-granulosa-cell function. *Hum. Reprod.* 5:785.
- Gluckman, P. D., B. H. Breier, and S. R. Davis. 1987. Physiology of the somatotrophic axis with particular reference to the ruminant. *J. Dairy Sci.* 70:442.
- Gong, J. G., T. Bramley, and R. Webb. 1991. The effects of recombinant bovine somatotropin on ovarian function in heifers: Follicular populations and peripheral hormones. *Biol. Reprod.* 45:941.
- Guidice, L. C. 1992. Insulin-like growth factors and ovarian follicular development. *Endocr. Rev.* 13:641.
- Guthrie, H. D., V. G. Pursel, D. J. Bolt, and B. S. Cooper. 1993. Expression of a bovine growth hormone transgene inhibits pregnant mare's serum gonadotropin-induced follicle maturation in prepubertal gilts. *J. Anim. Sci.* 71:3409.
- Hacker, R. R., A. Deschutter, O. Adeola, and T. R. Kasser. 1993. Evaluation of long-term somatotropin implants in finishing pigs. *J. Anim. Sci.* 71:564.
- Hammond, J. M., C. J. Hsu, J. Klindt, K. B. Tsang, and B. R. Downey. 1988. Gonadotropins increase concentrations of immunoreactive insulin-like growth factor-I in porcine follicular fluid in vivo. *Biol. Reprod.* 38:304.
- Hammond, J. M., J. S. Mondschein, S. E. Samaras, and S. F. Canning. 1991. The ovarian insulin-like growth factors, a local

- amplification mechanism for steroidogenesis and hormone action. *J. Steroid Biochem. Mol. Biol.* 40:411.
- Hetzer, H. O., and W. R. Harvey. 1967. Selection for high and low fatness in swine. *J. Anim. Sci.* 26:1244.
- Howard, H. J., and J. J. Ford. 1992. Relationships among concentrations of steroids, inhibin, insulin-like growth factor-I (IGF-I), and IGF-binding proteins during follicular development in weaned sows. *Biol. Reprod.* 47:193.
- Hsu, C.-J., and J. M. Hammond. 1987. Concomitant effects of growth hormone on secretion of insulin-like growth factor I and progesterone in cultured porcine granulosa cells. *Endocrinology* 121:1343.
- Jia, X.-C., J. Kalmijn, and A.J.W. Hsueh. 1986. Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. *Endocrinology* 118:1401.
- Kirkwood, R. N., P. A. Thacker, G. D. Guedo, and B. Laarveld. 1989. The effect of exogenous growth hormone on the endocrine status and the occurrence of estrus in gilts. *Can. J. Anim. Sci.* 69:931.
- Kirkwood, R. N., P. A. Thacker, and K. Rajkumar. 1992. Effects of growth hormone and triiodothyronine on insulin-induced progesterone production by granulosa cells from prepubertal gilts. *Can. J. Anim. Sci.* 72:589.
- Klindt, J., F. C. Buonomo, and J. T. Yen. 1992a. Administration of porcine somatotropin by sustained-release implant: Growth and endocrine responses in genetically lean and obese barrows and gilts. *J. Anim. Sci.* 70:3721.
- Klindt, J., F. C. Buonomo, and J. T. Yen. 1992b. Growth performance of contemporary and genetically lean and obese boars and gilts administered porcine somatotropin by sustained release implant (PST-SR). *J. Anim. Sci.* 70(Suppl. 1):206 (Abstr.).
- Langhout, D. J., L. J. Spicer, and R. D. Geisert. 1991. Development of a culture system for bovine granulosa cells: Effects of growth hormone, estradiol, and gonadotropins on cell proliferation, steroidogenesis, and protein synthesis. *J. Anim. Sci.* 69:3321.
- Maruo, T., M. Hayashi, H. Matsuo, Y. Ueda, H. Morikawa, and M. Mochizuki. 1988. Comparison of the facilitative roles of insulin and insulin-like growth factor I in the functional differentiation of granulosa cells: In vitro studies with the porcine model. *Acta Endocrinol.* 117:230.
- May, J. V., J. P. Frost, and D. W. Schomberg. 1988. Differential effects of epidermal growth factor, somatomedin-C/insulin-like growth factor-I, and transforming growth factor- β on porcine granulosa cell deoxyribonucleic acid synthesis and cell proliferation. *Endocrinology* 123:168.
- Mondschein, J. S., S. F. Canning, D. Q. Miller, and J. M. Hammond. 1989. Insulin-like growth factors (IGFs) as autocrine/paracrine regulators of granulosa cell differentiation and growth: Studies with a neutralizing monoclonal antibody to IGF-I. *Biol. Reprod.* 41:79.
- Mondschein, J. S., T. D. Etherton, and J. M. Hammond. 1991. Characterization of insulin-like growth factor-binding proteins of porcine ovarian follicular fluid. *Biol. Reprod.* 44:315.
- Moses, A. C., S. P. Nissley, J. Passamani, and R. M. White. 1979. Further characterization of growth hormone-dependent somatomedin-binding proteins in rat serum and demonstration of somatomedin-binding proteins produced by rat liver cells in culture. *Endocrinology* 104:536.
- Nitray, J., A. V. Sirotkin, J. Poltarsky, and J. Bulla. 1993. The effect of recombinant somatotrophic hormone on secretion of ovarian hormones of sexually mature gilts in vivo and in vitro. *Vet. Med. (Prague)* 38:53.
- Owens, P. C., R. J. Johnson, R. G. Campbell, and F. J. Ballard. 1990. Growth hormone increases insulin-like growth factor-I (IGF-I) and decreases IGF-II in plasma of growing pigs. *J. Endocrinol.* 124:269.
- Ramaley, J. A., and C. K. Phares. 1980. Delay of puberty onset in females due to suppression of growth hormone. *Endocrinology* 106:1989.
- SAS. 1988. SAS/STAT[®] User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.
- Spicer, L. J., E. Alpizar, and S. E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and (or) insulin-like growth factor I production in vitro. *J. Anim. Sci.* 71:1232.
- Spicer, L. J., S. E. Echternkamp, S. F. Canning, and J. M. Hammond. 1988. Relationship between concentrations of immunoreactive insulin-like growth factor-I in follicular fluid and various biochemical markers of differentiation in bovine antral follicles. *Biol. Reprod.* 39:573.
- Spicer, L. J., and W. J. Enright. 1991. Concentrations of insulin-like growth factor I and steroids in follicular fluid of preovulatory bovine ovarian follicles: Effect of daily injections of a growth hormone-releasing factor analog and (or) thyrotropin-releasing hormone. *J. Anim. Sci.* 69:1133.
- Spicer, L. J., J. Klindt, F. C. Buonomo, R. Maurer, J. T. Yen, and S. E. Echternkamp. 1992. Effect of porcine somatotropin on number of granulosa cell luteinizing hormone/human chorionic gonadotropin receptors, oocyte viability, and concentrations of steroids and insulin-like growth factors I and II in follicular fluid of lean and obese gilts. *J. Anim. Sci.* 70:3149.
- Terlouw, S. L., A. R. Rieke, T. C. Cantley, L. F. Miller, and B. N. Day. 1991. The effects of recombinant porcine somatotropin on reproductive function in gilts treated during the finishing phase. *J. Anim. Sci.* 69:4294.
- Ui, M., M. Shimonaka, S. Shimasaki, and N. Ling. 1989. An insulin-like growth factor-binding protein in ovarian follicular fluid blocks follicle-stimulating hormone-stimulated steroid production by ovarian granulosa cells. *Endocrinology* 125:912.
- Veldhuis, J. D., R. W. Furlanetto, D. Junchter, J. Garmey, and P. Veldhuis. 1985. Trophic actions of human somatomedin C/insulin-like growth factor-I on ovarian cells: in vitro studies with swine granulosa cells. *Endocrinology* 116:1235.
- Walton, P. E., and T. D. Etherton. 1989. Effects of porcine growth hormone and insulin-like growth factor-I (IGF-I) on immunoreactive IGF-binding protein concentration in pigs. *J. Endocrinol.* 120:153.
- Yen, J. T., H. J. Mersmann, J. A. Nienaber, D. A. Hill, and W. G. Pond. 1990. Responses to cimaterol in genetically obese and lean pigs. *J. Anim. Sci.* 68:2698.