

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 Gilts¹

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ABSTRACT: Prepubertal gilts of obese (n = 24) or lean (n = 24) genetic lines were injected (s.c.) daily with 0, 2, or 4 mg of porcine somatotropin (pST) for 6 wk starting at 160 d of age to determine whether pST affects follicular function. Blood and ovaries were collected at slaughter 24 h after the last injection. Surface follicles ≥ 1.0 mm in diameter were counted, and pools of follicular fluid (FFL) and granulosa cells were collected from 1.0- to 3.9-mm (small) and 4.0- to 6.9-mm (medium) follicles. Oocytes were collected from small and medium follicles and evaluated for maturational stage and viability. Porcine somatotropin increased ($P < .08$) the numbers of small but not the numbers of medium follicles per gilt ($P > .10$). Oocyte maturation and viability were not affected by pST or genetic line. Porcine somatotropin increased ($P < .05$) concentrations of insulin-like growth factor I (IGF-I) in serum and FFL of both obese and lean gilts; IGF-I was lower ($P < .01$) in

lean gilts. Treatment with pST decreased ($P < .05$) IGF-II in FFL of lean but not in that of obese gilts. Dose of pST and line had no effect on concentrations of progesterone in FFL of small or medium follicles or on concentrations of estradiol in FFL of small follicles. Concentrations of estradiol in FFL of medium follicles were decreased ($P < .05$) by pST (2 and 4 mg/d) in obese gilts, but estradiol was lower ($P < .01$) and unaffected ($P > .10$) by pST in lean gilts. The numbers of LH/hCG binding sites in granulosa cells were decreased ($P < .05$) by pST (4 mg/d) in medium follicles. The 4 mg/d dose of pST increased ($P < .05$) IGF-I binding protein activity only in serum of obese gilts. We conclude that 2 and/or 4 mg/d of pST can increase concentrations of IGF-I in serum and FFL and stimulate growth of small follicles in two genetically divergent lines of gilts without affecting oocyte viability.

Key Words: Somatotropin, Gilts, IGF-I, Ovaries

J. Anim. Sci. 1992. 70:3149-3157

Introduction

Recent attempts to improve production efficiency in domestic animals have concentrated on the use of exogenously supplied somatotropin (ST; Enright, 1989; Hanrahan, 1989). Long-term (≥ 30 d) porcine somatotropin (pST) treatment can alter body composition in barrows and gilts (Hanrahan, 1989; McLaren et al., 1990; Smith and Kasson, 1991). In addition, pST treatment has been reported to have both stimulatory and inhibitory

¹This work was supported in part by the Oklahoma Agric. Exp. Sta. (Journal article no. 5867). The authors gratefully acknowledge N. R. Mason (Lilly Res. Laboratories) for the generous donation of estradiol antiserum; the National Hormone and Pituitary Program of NIDDK (Univ. of Maryland School of Med., Baltimore) for providing the IGF-I antiserum (UBK-487), hCG (CR-127), and bovine LH (USDA-bLH-B-5); and S. Tinker and T. Boman for expert technical assistance.

²To whom correspondence should be addressed: 114 Anim. Sci. Bldg.

Received September 26, 1991.

Accepted May 22, 1992.

effects on reproductive functions in gilts (Bryan et al., 1989, 1990; Kirkwood et al., 1989). However, little is known about the reproductive responses of genetically obese or lean swine to pST. Although ST can directly stimulate rat (Jia et al., 1986) and porcine (Hsu and Hammond, 1987; Mondschein et al., 1989) granulosa cell functions in vitro, the systemic effect of ST is thought to be mediated, in part, through increased hepatic production of insulin-like growth factor I (IGF-I) (Gluckman et al., 1987; Evoke et al., 1988). Similar to pST, IGF-I (and IGF-II) also has direct effects on growth and differentiated function of ovarian follicle cells in vitro (Adashi et al., 1985; Hammond et al., 1988b; Geisthovel et al., 1990). In female rats, ST is thought to play an important role in the onset of puberty (Ramaley and Phares, 1980) and ovarian function (Advis et al., 1981). Thus, our experiment was designed to determine whether pST affects follicular function in prepubertal gilts obtained from populations selected for either high (obese) or low (lean) backfat.

Materials and Methods

Animals and Treatments. Gilts from lines selected for high (obese, $n = 24$) or low (lean, $n = 24$) backfat were injected (s.c.) daily with 0, 2, or 4 mg of recombinant pST for 6 wk, starting at 160 d of age (59.0 ± 1.4 kg of BW). 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 equally into four replicates to be slaughtered on four different days between July 7 and August 3, 1989, and averaged 211 ± 5 d of age and 87.4 ± 2.5 kg BW at slaughter. Gilts were fed, to appetite, a diet composed of 64.5% corn and 31.6% soybean meal (19.5% CP; DM basis), supplemented with dicalcium phosphate (2.4% DM basis), ground limestone (.5% DM basis), trace mineral premix (.2% DM basis), and vitamin premix (.2% DM basis). The contents of the trace mineral premix and vitamin premix have been described previously (Yen et al., 1990b). Calculated lysine concentration of the diet was 1.08% (DM basis).

Gilts were slaughtered 24 h after the last injection of pST. Ovaries and uteri were removed and weighed at slaughter. The surface diameter of all follicles ≥ 7 mm was recorded and their follicular fluid (FFL) was collected individually. The 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. Blood samples were collected at slaughter for IGF-I analysis.

Granulosa cells from small and medium follicles were recovered from FFL samples by centrifugation and resuspended in PBS with 20% glycerol for storage at -70°C until they were analyzed. Serum and follicular fluid were stored at -20°C until they were analyzed.

Oocyte Measurements. Oocytes were examined microscopically (phase contrast, 100 \times) for viability (morphologically), nuclear maturation (i.e., germinal vesicles, germinal vesicle breakdown, metaphase I, metaphase II, and so on), and for morphological appearance of the cumulus mass (e.g., tight, loose, or no cumulus; pyknotic nuclei, and so on) surrounding the oocyte (Christenson et al., 1975). Oocytes were categorized by assigning a maturational stage score for subsequent analyses of treatment and line effects on the oocyte-cumulus complex. Maturational stages ranged from 10 to 45; 10 = oocyte degenerate without germinal vesicle, 20 = oocyte degenerate with germinal vesicle, 21 = oocyte intact with germinal vesicle, 30 = oocyte degenerate with germinal vesicle containing fine-threaded chromatin, 31 = oocyte intact with germinal vesicle containing fine-threaded chromatin, 40 = oocyte degenerate with germinal vesicle containing contracted threads of chromatin, 41 = oocyte intact with germinal vesicle containing contracted threads of chromatin, and 45 = oocyte with germinal vesicle breakdown. Oocyte recovery averaged $38.5 \pm 3.2\%$.

Radioimmunoassays. Immunoreactive IGF-I in FFL and serum was determined by RIA after acid-ethanol extraction as described previously (Hammond et al., 1988a; Echterkamp 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 4.6 and 16.3%, respectively. Concentrations of IGF-II in FFL were determined by RIA as described previously (Buonomo et al., 1988). Concentrations of progesterone in FFL were quantified by RIA (Baranao and Hammond, 1985). Intra- and interassay CV were 11.0 and 16.9%, respectively. Concentrations of estradiol in FFL were quantified by 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 10.5 and 22.6%, respectively.

Radioreceptor Assay. Specific binding of LH/hCG to granulosa cells was determined by using a [^{125}I]hCG receptor assay as described previously (Spicer and Ireland, 1986; Spicer et al., 1986). Granulosa cell DNA was quantified by the methods of Burton (1956).

Insulin-Like Growth Factor I Binding Protein Activity. Insulin-like growth factor I binding protein activity in serum and FFL was determined by

Table 1. Effect of porcine somatotropin (pST) on oocyte maturation (OM), granulosa cell (GC) DNA content, insulin-like growth factor II (IGF-II) and progesterone concentrations in follicular fluid (FFL) and ovarian and uterine weights of obese and lean gilts

Treatment and line	No. of gilts	No. of follicle samples	OM stage ^a	GC DNA, µg/mg	FFL IGF-II, ng/mL ^b	FFL progesterone, ng/mL ^b	Ovarian wt, g ^c	Uterine wt, g
0 mg of pST								
Obese	6	24	22 (40)	1.58	15.7 ^{de}	322	4.98 ^{de}	156
Lean	7	28	21 (29)	1.96	22.9 ^f	161	4.14 ^d	123
2 mg of pST								
Obese	6	26	25 (53)	1.89	17.3 ^{df}	190	5.54 ^e	120
Lean	8	37	24 (43)	2.01	13.6 ^{de}	163	5.34 ^e	123
4 mg of pST								
Obese	7	37	27 (56)	1.78	14.7 ^{de}	176	6.92 ^f	89
Lean	8	29	22 (41)	2.30	11.0 ^e	497	6.01 ^{ef}	153
Pooled SE	—	—	2	.29	2.2	127	.42	.23

^aNumber in parentheses is average number of oocytes evaluated per gilt.

^bValues are pooled means of both small and medium follicles.

^cSignificant treatment effect ($P < .01$).

^{d,e,f}Means with different superscripts differ ($P < .05$).

incubation with ¹²⁵I-labeled IGF-I by the method of Moses et al. (1979). Follicular fluid samples from 1.0- to 3.9-mm or from 4.0- to 6.9-mm 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 ¹²⁵I-labeled IGF-I (15,000 counts per minute; counter efficiency was 75%) and 140 µL of assay buffer (PBS containing 2.5 mg of BSA/mL, pH = 7.4). To separate bound from free [¹²⁵I]IGF-I, activated charcoal (500 µL; 5% wt/vol in PBS containing 2.0 mg of BSA/mL) was added to each tube, incubated for 30 min at 4°C, and centrifuged at 1,200 × g for 20 min at 4°C. Intra- and interassay CV were 7.9 and 17.4%, respectively.

Statistical Analyses. Hormone data within each treatment were grouped into three follicle-size groups, based on diameter of follicle: 1.0 to 3.9 mm (small), 4.0 to 6.9 mm (medium), and ≥ 7.0 mm (large). Because very few large follicles were found in these prepubertal gilts, only data from small and medium follicles were analyzed. Data were subjected to least squares ANOVA (SAS, 1988) for a 3 × 2 × 2 factorial arrangement in a randomized design; main effects were dose of pST = 0, 2, and 4, line = lean and obese, and follicle size = small and medium. All possible interactions were also evaluated. Only data from prepubertal gilts were analyzed; 2, 2, and 0 gilts had corpora lutea at the time of slaughter in the 0, 2, and 4 mg of pST groups, respectively. Data showing heterogenous variance (FFL progesterone and estradiol, and numbers of LH/hCG binding sites) were analyzed after transformation to ln (x + 1). All means presented in results are least squares means ± SE. Specific differences between means were determined using the PDIF procedure (SAS, 1988)

if significant main effects were observed. Relationships among the variables measured were evaluated by regression and simple correlation analysis (Pearson correlation coefficients; SAS, 1988).

Results

Gross Ovarian and Uterine Morphology. Ovarian weight was increased ($P < .01$) in pST-treated lean and obese gilts. However, ovarian weight was not affected ($P > .10$) by line of gilt (Table 1). Uterine weight was unaffected ($P > .10$) by pST dose, line, or their interaction (Table 1).

The number of 1.0- to 3.9-mm follicles increased ($P < .08$) with dose of pST (Figure 1) but was unaffected ($P > .10$) by line of gilt. Averaged across lines, 4 mg of pST increased the number of 1.0- to 3.9-mm follicles by 52%. The numbers of medium follicles were unaffected ($P > .10$) by pST dose, line, or their interaction (Figure 1).

Oocyte Measurements. Maturation stage of oocytes was not affected by pST dose, line, or their interaction (Table 1). Similarly, oocyte viability averaged 77.2 ± 2.2% and was unaffected ($P > .10$) by pST dose, line, or their interaction.

Insulin-Like Growth Factor I in Serum and Follicular Fluid. Significant main effects on serum IGF-I were dose of pST ($P < .01$) and line ($P < .01$). Both 2 and 4 mg of pST increased ($P < .05$) concentrations of IGF-I in serum at slaughter in lean and obese gilts (Figure 2); 4 mg of pST increased serum IGF-I 3.6- and 3.9-fold above controls in lean and obese gilts, respectively. The largest difference in serum IGF-I between lean and obese gilts were observed in gilts treated with 2 mg of pST (Figure 2). No significant pST dose × line interaction was observed.

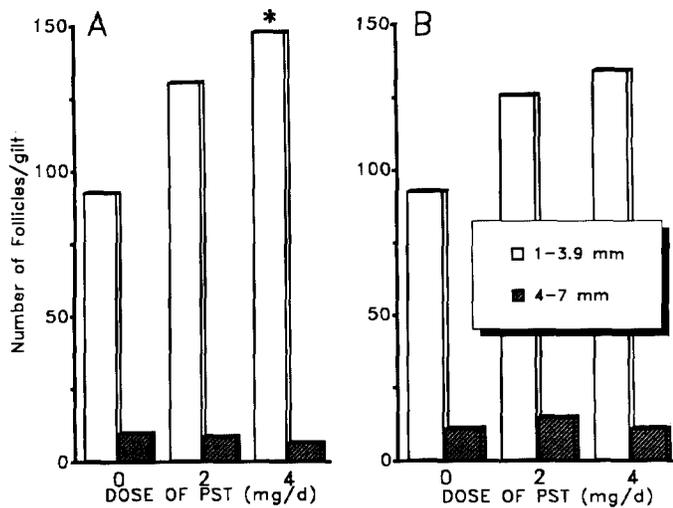


Figure 1. Numbers of small (1.0 to 3.9 mm) and medium (4.0 to 7.0 mm) follicles measured in prepubertal obese (A) and lean (B) gilts. In (A), pooled SE = 21.9 and 3.0 for small and medium follicles, respectively. * $P < .08$ vs 0 mg/d. In (B), pooled SE = 20.4 and 2.8 for small and medium follicles, respectively. PST = porcine somatotropin.

Porcine somatotropin also increased concentrations of IGF-I in FFL (Figure 2). Significant main effects were dose of pST ($P < .01$), line ($P < .01$), and follicle size ($P < .05$), with no significant ($P > .10$) interactions. Obese gilts had 33% greater concentrations of IGF-I in FFL than did lean gilts (1,220 vs 915 ± 69 ng/mL), and medium follicles had 21% greater concentrations of IGF-I than small follicles (1,171 vs 964 ± 78 ng/mL).

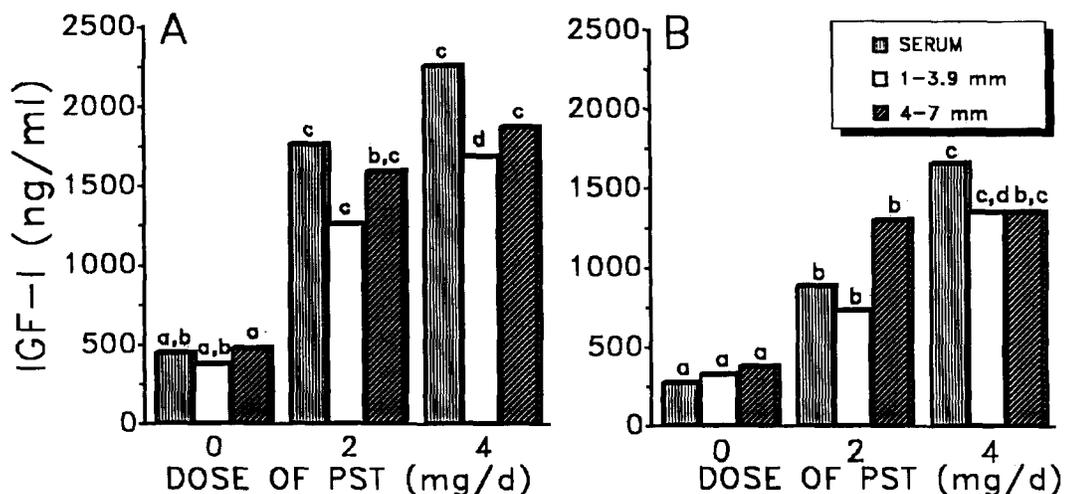


Figure 2. Concentrations of insulin-like growth factor I (IGF-I) in serum and follicular fluid of small (1.0 to 3.9 mm) and medium (4.0 to 7.0 mm) follicles collected in prepubertal obese (A) and lean (B) gilts. For (A) and (B), pooled SE = 195, 138, and 190 ng/mL for serum and small and medium follicles, respectively. ^{a,b,c,d} Within serum and follicle-size categories, but across panels, means with different letters differ ($P < .05$). PST = porcine somatotropin.

Insulin-Like Growth Factor II in Follicular Fluid. Concentrations of IGF-II in FFL were affected ($P < .05$) by dose of pST, and there was a pST \times line interaction (Table 1). Porcine somatotropin (2 and 4 mg) treatment decreased ($P < .05$) IGF-II concentrations in FFL of lean gilts but had no effect ($P > .10$) on FFL IGF-II in obese gilts (Table 1). Line, follicle size, or their interaction did not affect ($P > .10$) FFL IGF-II.

Follicular Fluid Steroids. Concentrations of progesterone measured in FFL of small and of medium follicles were not affected ($P > .10$) by dose of pST, line, or follicle size (Table 1).

Concentrations of estradiol in FFL were influenced ($P < .01$) by pST dose, line, and follicle size, and there was a pST \times line interaction. As illustrated in Figure 3, both 2 and 4 mg of pST decreased estradiol in FFL of medium follicles of obese gilts but had no effect on FFL estradiol in medium follicles of lean gilts. Also, dose of pST had no effect on FFL estradiol in small follicles of lean or obese gilts (Figure 3). Averaged across follicle sizes and pST doses, obese gilts had 2.6-fold greater FFL estradiol concentrations than did lean gilts (13.0 vs 4.9 ± 2.2 ng/mL). Averaged across line and pST doses, medium follicles had fourfold greater concentrations of estradiol than did small follicles.

Luteinizing Hormone/Human Chorionic Gonadotropin Receptors. The numbers of granulosa cell LH/hCG binding sites were influenced ($P < .05$) by dose of pST and follicle size. No significant ($P > .10$) interactions were observed. Averaged

across pST dose and line, medium follicles had a 4.1-fold greater ($P < .01$) number of LH/hCG binding sites than small follicles (77 vs 19 ± 17 cpm/ μ g DNA; Figure 4). Porcine somatotropin (2 or 4 mg) had no effect on LH/hCG binding in small follicles, whereas 2 and 4 mg of pST decreased ($P < .05$) LH/hCG binding in medium follicles (Figure 4). Averaged across lines, 4 mg of pST decreased LH/hCG binding in medium follicles by 81%.

Dose of pST, line, or follicle size had no effect ($P > .10$) on granulosa cell DNA content (Table 1).

Binding Protein Activity. The IGF-I binding protein activity (expressed as percentage of [125 I]IGF-I specifically bound/ 10μ L) in serum was influenced ($P < .05$) by pST dose, and there was a pST \times line interaction (Table 2). Porcine somatotropin (4 mg but not 2 mg) increased ($P < .05$) IGF-I binding protein activity in serum of obese gilts but not in serum of lean gilts. Insulin-like growth factor I binding protein activity in FFL of small and medium follicles was not affected ($P > .10$) by pST dose, line, or their interaction (Table 2).

Correlation Coefficients. Data of small and medium follicles were separated within line for correlation analysis (Table 3). In obese gilts, FFL estradiol in small and medium follicles was correlated with FFL IGF-I ($r = -.23$ and $-.36$, respectively), progesterone ($r = .27$ and $.40$, respectively)

Table 2. Effect of porcine somatotropin (pST) on insulin-like growth factor I (IGF-I) binding protein activity (BPA) in serum and follicular fluid (FFL) of obese and lean gilts

Treatment and line	No. of samples ^b	IGF-I BPA, % ^a		
		Serum	FFL	
			Small	Medium
0 mg of pST				
Obese	6	7.9 ^c	10.5	7.8
Lean	5	9.6 ^{de}	9.6	9.2
2 mg of pST				
Obese	5	9.1 ^{cd}	9.8	10.5
Lean	8	9.8 ^{de}	10.9	9.4
4 mg of pST				
Obese	7	10.7 ^e	9.9	9.3
Lean	6	10.1 ^{de}	11.9	11.9
Pooled SE	—	.4	1.0	1.1

^aIGF-I BPA is expressed as the percentage of [125 I]IGF-I specifically bound per $10\text{-}\mu$ L sample.

^bNumber of samples; FFL samples from small and medium follicles were pooled within gilts.

^{c,d,e}Means with different superscripts differ ($P < .05$).

and [125 I]hCG binding ($r = .25$ and $.82$, respectively). Follicular fluid IGF-II was correlated with FFL estradiol ($r = .53$) and progesterone ($r = .23$) in small follicles of obese gilts only. In lean gilts, FFL

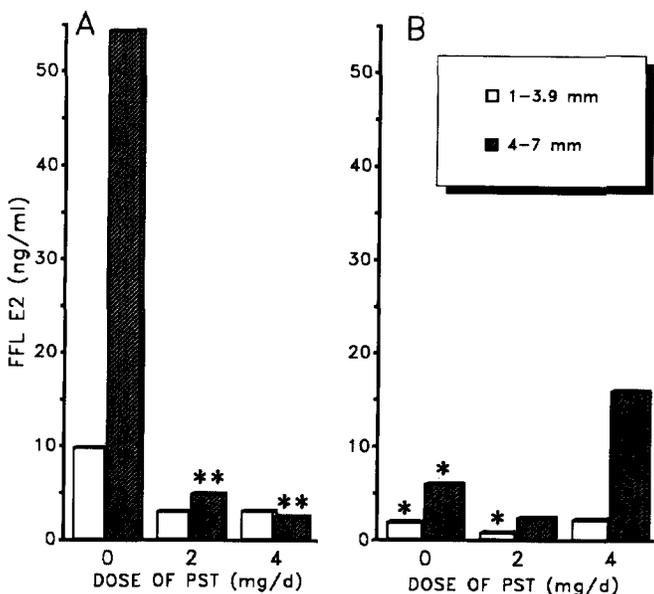


Figure 3. Concentrations of estradiol (E2) in follicular fluid (FFL) in small (1.0 to 3.9 mm) and medium (4.0 to 7.0 mm) follicles collected in prepubertal obese (A) and lean (B) gilts. In (A), pooled SE = 4.3 and 6.7 ng/mL in small and medium follicles, respectively. In (B), pooled SE = 4.4 and 5.7 ng/mL in small and medium follicles, respectively. $**P < .05$ vs 0 mg/d (A). $*P < .05$ vs obese gilts within follicle size and porcine somatotropin (PST) dose.

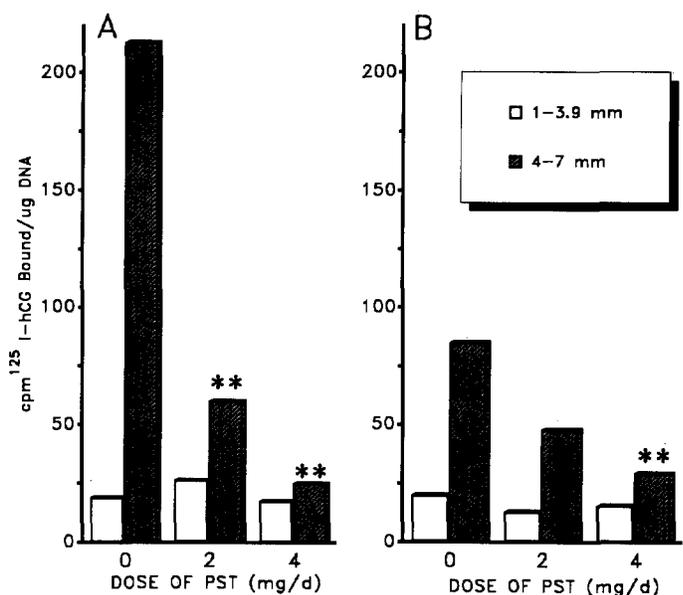


Figure 4. Numbers of luteinizing hormone/human chorionic gonadotropin (hCG) binding sites in granulosa cells of small (1.0 to 3.9 mm) and medium (4.0 to 7.0 mm) follicles in prepubertal obese (A) and lean (B) gilts. In (A), pooled SE = 29.1 and 43.0 cpm/ μ g of DNA for small and medium follicles, respectively. Follicle size effect was significant ($P < .01$). $**P < .05$ vs 0 mg/d within (A) or (B). PST = porcine somatotropin.

IGF-II and IGF-I were negatively correlated ($r = -.31$) in medium (but not in small) follicles. Progesterone in FFL was correlated with FFL IGF-I in small (but not in medium) follicles of lean ($r = .61$) and obese ($r = .27$) gilts. Mean FFL IGF-I was positively correlated ($r = .81$) with serum IGF-I in each line of gilts (Table 3). In addition, serum IGF-I and mean FFL IGF-I were positively correlated with numbers of small follicles in lean ($r = .48$ and $.50$, respectively; $P < .05$) but nonsignificantly in obese gilts ($r = .29$ and $.32$, respectively; $P > .10$). Numbers of medium follicles were not correlated ($P > .10$) with serum IGF-I or mean FFL IGF-I in either line of gilts.

Discussion

Treatment of pigs with pST has caused 2- to 11-fold increases in serum IGF-I concentrations in previous studies (Sillence and Etherton, 1987; Evock et al., 1988; Bryan et al., 1989). Similarly, we observed three- to fourfold increases in serum IGF-I in blood of gilts treated with 2 or 4 mg of pST daily. This increase in serum IGF-I, and the associated increases in pST, IGF-II, and insulin (Buonomo et al., 1990) (either alone or in

combination), may account for the increases in ovarian weight and follicular function (Hsueh et al., 1984; Geisthovel et al., 1990) and for the changes in growth performance and carcass measurements (Yen et al., 1990a). However, pST treatment did not affect oocyte maturation in gilts of the present study, an observation not previously reported. Studies using *Xenopus* oocytes (Hainaut et al., 1991) and rat cumulus-oocyte complexes (Feng et al., 1988) have shown direct stimulatory effects of low doses of IGF-I (10 to 50 ng/mL) on oocyte maturation in vitro. The cause(s) for the differences between in vivo and in vitro effects of IGF-I on oocyte maturation remain to be determined.

Also, our study is the first to report effects of exogenous pST treatment on the number of LH/hCG binding sites in granulosa cells. This may explain why Bryan et al. (1989) observed a significant reduction in LH responsiveness of porcine granulosa cells cultured in vitro from pST-treated gilts. Based on in vivo (Advis et al., 1981) and in vitro (Jia et al., 1986) work in rats, increased pST should increase the number of LH receptors in granulosa cells. Similarly, Kirkwood et al. (1990) have shown a significant increase in LH/hCG binding in corpora lutea of gilts treated with pST

Table 3. Correlation coefficients between concentrations of insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), progesterone (P_4), and estradiol (E_2) in follicular fluid (FFL) and granulosa cell luteinizing hormone (LH)/human chorionic gonadotropin (hCG) binding in small (S) and medium (M) follicles of obese and lean gilts

Line and hormone	n ^a	IGF-II	E_2	P_4	LH/hCG binding, cpm/ μ g of DNA
Obese-S					
IGF-I	58	-.09	-.23*	.25*	-.20
IGF-II	59		.53**	.23*	.20
E_2	61			.27**	.25*
P_4	61				.30**
Obese-M					
IGF-I	24	-.07	-.36*	-.30	-.14
IGF-II	23		-.23	-.15	-.32
E_2	24			.40**	.82**
P_4	28				.29
Lean-S					
IGF-I	56	-.22	.01	.61**	-.21
IGF-II	54		-.04	-.09	-.09
E_2	54			-.07	.17
P_4	57				-.23
Lean-M					
IGF-I	35	-.31*	.26	.02	-.10
IGF-II	33		-.21	-.16	-.02
E_2	32			.19	-.14
P_4	35				-.28

^aNumber of samples.

* $P < .10$; ** $P < .05$; Mean FFL IGF-I correlated ($P < .001$) with serum IGF-I in obese and lean gilts ($r = .81$ and $.81$, respectively).

for 8 d. Moreover, because IGF-I enhanced FSH-induced increases in numbers of LH receptors in rat (Adashi et al., 1985) and porcine (Maruo et al., 1988) granulosa cells, it was anticipated that the increased IGF-I (or ST) would increase rather than decrease LH receptors in medium follicles in pST-treated gilts of the present study. However, the high concentrations of IGF-I may have caused a down-regulation in the ovarian IGF-I response system, as has been shown in other systems (Cascieri et al., 1988). Because estradiol increases the numbers of LH receptors in cultured porcine (May et al., 1980) and rat (Hsueh et al., 1984) granulosa cells, perhaps the decreased concentrations of estradiol were, in part, responsible for the decreased number of LH/hCG binding sites observed in the present study, at least in obese gilts. In support of this suggestion, FFL estradiol and numbers of LH/hCG binding sites in medium follicles were positively correlated in obese ($r = .82$) gilts but not in lean ($r = -.14$) gilts. Greater LH binding to granulosa cells from medium than from small porcine follicles agrees with previous studies (Nakano et al., 1977).

In vitro, IGF-I stimulates estrogen production by granulosa cells from rats (Adashi et al., 1985) and pigs (Maruo et al., 1988). However, in the present study estradiol concentrations were lower in FFL of pST-treated obese gilts than in that of untreated obese gilts in spite of fourfold increases in serum and FFL IGF-I concentrations. Estradiol concentrations in FFL of lean gilts were lower than those in FFL of obese gilts and were unaffected or slightly increased by pST, thus explaining the significant pST dose \times line interaction. Because IGF-II concentrations decreased in FFL of lean but not of obese gilts, it is unlikely that changes in IGF-II are responsible for the decrease in FFL estradiol concentrations or numbers of LH/hCG binding sites observed in medium follicles of obese gilts. In comparison, Bryan et al. (1989) found no effect of pST on FFL estradiol concentrations in Yorkshire or Duroc gilts. In vitro, ST has no effect on FSH-induced estrogen production (Jia et al., 1986), and thus it is unlikely that increased pST caused the decrease in estradiol concentrations in FFL of the present study. Reasons for the discrepancies between in vitro and in vivo studies are unknown.

In vitro, ST and IGF-I enhance progesterone production by cultured granulosa cells (Adashi et al., 1985; Veldhuis et al., 1985; Jia et al., 1986; Hsu and Hammond, 1987). Although pST treatment had no effect on FFL progesterone, FFL IGF-I was positively correlated with FFL progesterone in small (but not in medium) follicles of obese and lean gilts. Similarly, a positive correlation between FFL progesterone and IGF-I has been reported for bovine follicles (Spicer et al., 1988; Spicer and

Enright, 1991). In a previous report (Bryan et al., 1989), a significant increase in FFL progesterone was observed in only one of two experiments in which gilts were treated with pST for 30 to 65 d.

The mechanism by which pST alters follicular growth is unknown. Presumably the increased number of small follicles in pST-treated gilts resulted from increased IGF-I concentrations in serum and/or FFL because the in vitro stimulatory effect of IGF-I on follicular granulosa cell mitosis is well known (Adashi et al., 1985; Hammond et al., 1988b). In support of this suggestion, we observed that serum and FFL IGF-I and numbers of small (but not of medium) follicles were positively correlated ($r = .29$ to $.32$ in obese and $r = .48$ to $.50$ in lean gilts). Because the positive association between numbers of ovarian follicles and serum and FFL IGF-I was limited to small follicles, perhaps IGF-I is involved with follicular recruitment and not with follicular selection in the gilt.

As previously reported (Walton and Etherton, 1989; Owens et al., 1990), pST increased IGF-I binding protein activity in serum of the present study. Although total IGF-I binding protein activity in FFL was not altered by pST, it is possible that the amounts of the various molecular weight species of IGF-binding proteins in FFL may have been altered by pST treatment, thus affecting local actions of IGF-I (Mondschein et al., 1991).

Similar to previous studies examining bovine (Spicer et al. 1988; Echternkamp et al., 1990; Spicer and Enright, 1991) and porcine follicles (Hammond et al., 1988a), a positive association between follicular size and IGF-I concentration was observed. Whether this increased concentration of IGF-I in medium 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 ($r = .81$), 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 at least 10-fold greater than 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 porcine somatotropin treatment tended to increase the numbers of small follicles and significantly decrease estradiol concentrations and numbers of granulosa cell luteinizing hormone/human chorionic gonadotropin binding

sites in medium follicles of prepubertal gilts. The hormonal mediator(s) of the effects of porcine somatotropin on follicular function are unknown but may include insulin-like growth factor I and/or porcine somatotropin directly. Additional studies will be required to determine whether exogenous porcine somatotropin can alter reproductive performance.

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