Exogenous porcine somatotropin stimulates mammary development in late-pregnant gilts

Chantal Farmer^{*,1} and Pieter Langendijk[†]

^{*}Agriculture and Agri-Food Canada, Sherbrooke R & D Centre, 2000 College, Sherbrooke, Québec J1M 0C8, Canada; and [†]School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia 6150, Australia

ABSTRACT: The goal of this project was to determine if increasing insulin-like growth factor-1 (IGF-1) concentrations in late pregnancy can stimulate mammary development in gilts. Yorkshire × Landrace gilts of a similar body weight (BW; 196.2 ± 6.2 kg) on day 89 of gestation were separated in 2 groups, namely, controls (CTL, *n* = 17) that were injected with sterile water, and porcine somatotropin-treated (pST, *n* = 20) that received injections of 5 mg of pST (Reporcin). Injections were given daily from days 90 to 109 of gestation and gilts were slaughtered on day 110 to collect mammary glands for compositional analyses. Blood samples were obtained on days 89, 96, 103, and 109 of gestation to measure IGF-1, free fatty acids (FFA), urea, glucose, and insulin concentrations. Treated gilts gained more BW (22.7 vs. 18.2 kg, *P* < 0.05) and lost more backfat (*P* < 0.05) than CTL gilts during the treatment period. There was a treatment × day effect (*P* < 0.01) on IGF-1, glucose, and urea concentrations. Concentrations of IGF-1 increased 4-fold (*P* < 0.01) in pST compared with CTL gilts

on days 96, 103, and 109 of gestation. Insulin values were also greater on days 96 (*P* < 0.01) and 103 (*P* = 0.01), and tended to be greater (*P* < 0.10) on day 109 of gestation in pST gilts. Glucose was greater in pST than CTL gilts on days 96 (*P* < 0.01), 103 (*P* < 0.01), and 109 (*P* = 0.01). Concentrations of urea were lower (*P* < 0.01) on days 96, 103, and 109 of gestation in gilts receiving pST injections, and FFA was not altered by treatment on any sampling day (*P* > 0.10). Injections of pST did not affect mammary extraparenchymal tissue weight (*P* > 0.10) but increased mammary parenchymal mass (1922 vs. 1576 ± 124 g, *P* < 0.05). The composition of parenchymal tissue was also altered by treatment. Mammary parenchyma from pST gilts contained more (*P* < 0.05) protein, DNA and RNA and less fat (*P* < 0.05) and dry matter (*P* < 0.01) than that from CTL gilts. These findings provide a clear demonstration that increasing circulating IGF-1 in late-pregnant gilts can stimulate mammary development both in terms of total parenchymal mass and of parenchymal tissue composition.

Keywords: exogenous somatotropin, gestation, gilt, IGF-1, mammary development, swine

© Crown copyright 2019.

This article contains public sector information licensed under the Open Government Licence v3.0 (<http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/>).

J. Anim. Sci. 2019.97:2433–2440
doi: 10.1093/jas/skz136

INTRODUCTION

Sows limit growth rate of their piglets because of inadequate milk yield (Harrell et al., 1993). Studies aiming at stimulating mammary

development of growing or pregnant gilts were therefore carried out to increase sow milk yield. The last third of gestation is the period with most rapid mammary accretion before the onset of lactation (Sorensen et al., 2002). The hormone prolactin was found to play an essential role for mammary development in late-pregnant pigs (Farmer et al., 2000; Farmer and Petitclerc, 2003) and increasing its circulating concentrations as of day 90 of gestation

¹Corresponding author: chantal.farmer@canada.ca

Received January 15, 2019.

Accepted April 18, 2019.

led to greater piglet growth rate (VanKlombenberg et al., 2013). The insulin-like growth factor-1 (IGF-1) system was also shown to be important for normal mammary gland development when using transgenic and knockout mice models (review by Hadsell and Bonnette, 2000). Looking at the specific actions of IGF-1 in gestation, Knight et al. (1994) reported increased mammary cell proliferation at parturition in goats that were injected with somatotropin (ST) during the last 6 wk of gestation. An increase in mammary parenchymal cells was also seen when ewes were injected with ST from days 97 to 124 of gestation (Stelwagen et al., 1993), and primiparous cows had greater milk yield following injections with recombinant ST in the last trimester of gestation. An effect via enhanced mammaryogenesis was suggested in that last study (Stelwagen et al., 1992). In swine, IGF-1 was suggested to be a local mediator of mammary development in late pregnancy (Simmen et al., 1988). Yet, transgenic overexpression of IGF-1 did not affect milk yield (Monaco et al., 2005); but, injections with 8 mg/d of porcine ST (pST) from days 75 until 105 of gestation increased parenchymal tissue mass as well as total parenchymal contents of DNA, RNA, and protein (Howard, 1995). However, these last findings were never published and the dose and timing of injections may not have been optimal. Indeed, the most important increase in mammary development occurs as of 90 d of gestation (Sorensen et al., 2002), and doses of 3.5 mg/d (Gatford et al., 2009) or 5 mg/d of pST (Sterle et al., 1995, 2003) brought about significant increases in circulating IGF-1 of pregnant pigs. The goal of the current project was to determine if increasing concentrations of IGF-1 via injections of 5 mg/d of pST from days 90 to 109 of gestation can stimulate mammary development in gilts. The impact of greater circulating IGF-1 concentrations on various metabolic variables was also studied.

MATERIALS AND METHODS

Animals were cared for according to a recommended code of practice (CCAC, 2009) and procedures were approved by the institutional animal care committee of the Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada.

Animals and Treatments

Thirty-seven Yorkshire $\times$ Landrace gilts were bred via artificial insemination using pools of semen from Duroc boars. During gestation, they were housed in individual pens (1.5 $\times$ 2.4 m) and

were fed 1 daily meal (0800 h) of a conventional corn-based diet (12.85 MJ/kg DE, 14.28% CP, 0.64% lysine). From mating to day 89 of gestation gilts weighing 140, 145, 150, 155, 160, and 165 kg at mating received, respectively, 2.2, 2.1, 2.0, 1.9, 1.9, and 1.8 kg of feed. As of day 90 of gestation all gilts were fed 2.5 kg. On day 89 of gestation, gilts were separated in 2 groups, namely, controls (CTL, $n = 17$) injected with 1 mL of sterile water, and porcine ST-treated (pST, $n = 20$) receiving daily injections of 5 mg of pST (Reporcin, Zamira Life Sciences Pty Ltd, Knoxfield, Australia). The pST was diluted in 1 mL of sterile water and injections were given from days 90 to 109 of gestation. The dose of pST was similar for all treated gilts, with all gilts averaging a body weight (BW) of 196.2 ± 6.2 kg on day 89 of gestation. Gilts were weighed and had their backfat thickness measured ultrasonically (WED-3000, Shenzhen Well D Medical Electronics Co., Guangdong, China) at P2 of the last rib at mating and on days 45, 89, and 109 of gestation. Jugular blood samples were obtained before the morning meal on days 89, 96, 103, and 109 of gestation. Gilts were then slaughtered on day 110 of gestation to obtain mammary glands for compositional analyses. Their uterus was removed and fetuses were counted and weighed; ovaries were also obtained and weighed and the number of corpora lutea was counted.

Blood Handling and Assays

Blood samples were used to measure concentrations of IGF-1, insulin, urea, free fatty acids (FFA), and glucose. Blood sampling was done between 0700 and 0800 h before the morning meal. Blood samples for urea (20 mL) were collected into Vacutainer tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ) and left at room temperature for 3 h, stored overnight at 4 °C, centrifuged for 12 min at $1,800 \times g$ at 4 °C the following day, and serum was then harvested. Blood samples for IGF-1, insulin, and FFA assays (20 mL) were collected in EDTA tubes (Becton Dickinson and Cie, Rutherford, NJ), put on ice and centrifuged within 20 min for 12 min at $1,800 \times g$ at 4 °C, and plasma was immediately recovered. Lastly, blood samples for glucose analyses (7 mL) were collected into tubes containing 10.0 mg of potassium oxalate and 12.5 mg of sodium fluoride to inhibit glycolysis, were put on ice and centrifuged within 20 min at $1,800 \times g$ for 12 min at 4 °C, and plasma was immediately recovered. Serum and plasma samples were

frozen at -20°C until assayed. Concentrations of IGF-1 were measured with a commercial RIA kit for humans (ALPCO Diagnostics, Salem, NH) with small modifications as detailed previously (Plante et al., 2011). Validation for a plasma pool from sows was previously conducted (Plante et al., 2011). Sensitivity of the assay was 0.10 ng/mL . The intra- and interassay CVs were 5.88% and 6.42%, respectively. Insulin was measured with a porcine insulin commercial RIA kit (Millipore, St-Charles, MO) which was previously validated for sow serum (Farmer et al., 2010). Sensitivity of the assay was $1.61\text{ }\mu\text{U/mL}$ and intra- and interassay CVs were 6.93% and 3.13%, respectively. Glucose was measured by an enzymatic colorimetric method with a commercial kit (Wako Chemicals, Richmond, VA). Validation for a plasma pool from gestating sows was demonstrated, parallelism being 100.8% and average mass recovery being 95.5%. Intra- and interassay CVs were 1.83% and 1.67%, respectively. Urea was measured by colorimetric analysis using an autoanalyzer (Auto-Analyser 3; Technicon Instruments Inc., Tarrytown, NY) according to the method of Huntington (1984). Intra- and interassay CVs were 1.68% and 2.24%, respectively. Concentrations of FFA were also measured by colorimetry with a commercial kit (Wako Chemicals, Richmond, VA). Intra- and interassay CVs were 2.46% and 4.97%, respectively.

Mammary Gland Measurements

At slaughter, mammary glands from 1 side of the abdominal wall (aiming for 7 glands) were excised. They were stored at -20°C and once frozen were cut into 2-cm slices and stored again at -20°C . Each slice was later trimmed of skin and teats and mammary parenchymal tissue was dissected from surrounding adipose tissue (i.e., extraparenchymal tissue) at 4°C . Both parenchymal and extraparenchymal tissue weights were recorded. Parenchymal tissue from all dissected and sliced glands was homogenized and a representative sample was used for determination of composition by chemical analysis. The RNA content of parenchymal tissue was measured by ultraviolet (Volkin and Cohn, 1954) and the DNA content of parenchymal tissue was evaluated in all samples using a method based on fluorescence of a DNA stain (Labarca and Paigen, 1980). Both RNA and DNA contents were reported on dry matter. Dry matter (Method 950.46; AOAC, 2005), protein (Method 928.08; AOAC, 2005), and lipid contents (Method 991.39; AOAC, 2005) were also determined in parenchyma.

Statistical Analyses

The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analyses. The univariate model used for mammary gland composition, ovarian, and fetal data included the effect of treatment, with the residual error being the error term used to test main effects of treatment. Repeated measures ANOVA with the factors treatment (the error term being gilt within treatment) and day of gestation (the residual error being the error term) and the treatment by day interaction were also carried out on BW, backfat, and blood data. Separate analyses of variance for each day were also carried out on these variables. Data in tables are presented as least squares means $\pm$ maximal SEM.

RESULTS

Growth and Metabolic Variables of Gilts

The BW and backfat thickness of gilts are shown in Table 1. Treated gilts tended to weigh more on day 109 of gestation ($P < 0.10$) and gained more BW during the treatment period ($P < 0.05$) than CTL gilts. There were no differences in backfat thickness between treatment groups at any time but the loss in backfat between days 89 and 109 of gestation was greater for pST than CTL gilts ($P < 0.05$). Circulating concentrations of IGF-1, insulin, glucose, FFA, and urea in gilts are shown in Table 2. There were marked differences in all measured variables, except for FFA values that were not altered by treatment on any sampling day ($P > 0.10$). There were treatment $\times$ day effects ($P < 0.01$) on IGF-1, glucose, and urea concentrations, and a day effect ($P < 0.01$) on insulin and FFA. There was a 4-fold increase in circulating IGF-1 in pST gilts compared with CTL gilts on days 96, 103, and 109 of gestation ($P < 0.01$). Insulin values were also greater on days 96 ($P < 0.01$) and 103 ($P = 0.01$), and tended to be greater ($P < 0.10$) on day 109 of gestation for pST gilts compared with CTL gilts. Glucose was also affected by treatment, with values being greater in pST than CTL gilts on days 96 ($P < 0.01$), 103 ($P < 0.01$), and 109 ($P = 0.01$) of gestation. Lastly, urea concentrations were lower ($P < 0.01$) on days 96, 103, and 109 of gestation in gilts receiving pST injections.

Mammary Gland, Ovarian, and Fetal Variables

Mammary gland composition of gilts is shown in Table 3. Injections of pST did not affect the mass of extraparenchymal tissue ($P > 0.10$) but increased parenchymal tissue mass by 22% ($P < 0.05$), hence

Table 1. Body weight and backfat thickness of gilts injected with sterile water (controls, CTL, $n = 17$) or 5 mg of porcine somatotropin (pST, $n = 20$) from days 90 to 109 of gestation

Variable measured	Treatment		SEM ¹
	CTL	pST	
BW ² , kg			
Mating	146.4	148.4	2.2
Day 45 of gestation	172.5	171.4	1.5
Day 89 of gestation	196.3	196.0	1.5
Day 109 of gestation	214.5 ^c	218.7 ^d	1.7
Gain mating to day 89	49.9	47.7	2.2
Gain mating to day 109	68.1	70.3	2.5
Gain days 89 to 109	18.2 ^a	22.7 ^b	1.3
Backfat thickness ³ , mm			
Mating	16.0	16.4	0.3
Day 45 of gestation	17.3	17.5	0.3
Day 89 of gestation	17.5	17.6	0.5
Day 109 of gestation	17.4	17.1	0.4
Gain mating to day 89	1.5	1.1	0.4
Gain mating to day 109	1.4	0.6	0.4
Gain days 89 to 109	-0.1 ^a	-0.5 ^b	0.2

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

^{c,d}Means within a row with different superscripts tend to differ ($P < 0.10$).

¹Maximum value.

²Treatment by day effect ($P < 0.05$).

³Day effect ($P < 0.01$).

decreasing the extraparenchyma/parenchyma ratio ($P < 0.05$). The composition of parenchymal tissue was also altered by treatment. Mammary parenchyma from pST gilts had less percent dry matter ($P < 0.01$) and percent fat ($P < 0.05$), and greater concentrations of protein, DNA, and RNA ($P < 0.05$). Total parenchymal tissue from treated gilts also contained more protein, DNA, and RNA than that of CTL gilts ($P < 0.05$).

Average weight of both ovaries tended to be greater for pST than CTL gilts (11.7 vs. 10.7 ± 0.4 g, $P < 0.10$), but the mean number of corpora lutea was similar across treatments ($P > 0.10$), being 10.4 and 10.2 ± 0.5 for pST and CTL gilts, respectively. Litter size on day 110 of gestation averaged 13.4 and 12.6 ± 1.1 for pST and CTL gilts, respectively, and was not affected by treatment ($P > 0.10$). Average fetal weight (weighted for litter size) was also not affected by treatment ($P > 0.10$), with values of 1.06 and 1.14 ± 0.04 kg for pST and CTL gilts, respectively.

DISCUSSION

Current results provide a clear demonstration that exogenous pST, likely via increased IGF-1, can stimulate mammary development of gilts in late

Table 2. Circulating concentrations of IGF-1, insulin, glucose, FFA, and urea for gilts injected with sterile water (controls, CTL, $n = 17$) or 5 mg of porcine somatotropin (pST, $n = 20$) from days 90 to 109 of gestation

Variable measured	Treatment		SEM ¹
	CTL	pST	
IGF-1 ² , ng/mL			
Day 89 of gestation	56.3	63.7	6.0
Day 96 of gestation	59.1 ^a	238.4 ^b	14.5
Day 103 of gestation	54.6 ^a	210.3 ^b	13.3
Day 109 of gestation	53.4 ^a	197.5 ^b	17.0
Insulin ³ , μ U/mL			
Day 89 of gestation	8.54	10.45	1.12
Day 96 of gestation	7.20 ^a	11.57 ^b	1.03
Day 103 of gestation	6.81 ^a	10.51 ^b	1.00
Day 109 of gestation	5.61 ^c	8.07 ^d	8.07
Glucose ² , mmol/L			
Day 89 of gestation	3.36	3.43	0.05
Day 96 of gestation	3.30 ^a	3.84 ^b	0.08
Day 103 of gestation	3.30 ^a	3.93 ^b	0.06
Day 109 of gestation	3.50 ^a	3.92 ^b	0.12
FFA ³ , μ Eq/L			
Day 89 of gestation	138.4	109.3	13.5
Day 96 of gestation	75.0	87.5	7.4
Day 103 of gestation	107.8	132.5	12.2
Day 109 of gestation	164.5	169.9	15.7
Urea ² , mmol/L			
Day 89 of gestation	4.67 ^c	4.17 ^d	0.21
Day 96 of gestation	5.36 ^a	2.67 ^b	0.17
Day 103 of gestation	5.29 ^a	2.38 ^b	0.16
Day 109 of gestation	5.64 ^a	2.58 ^b	0.23

^{a,b}Means within a row with different superscripts differ ($P \leq 0.01$).

^{c,d}Means within a row with different superscripts tend to differ ($P < 0.10$).

¹Maximum value.

²Treatment by day effect ($P < 0.01$).

³Day effect ($P < 0.01$).

gestation. The essential role of IGF-1 for normal mammary development has long been recognized in rodents (see reviews by Hadsell and Bonnette, 2000; Kleinberg and Ruan, 2008; Kleinberg and Barcellos-Hoff, 2011) and ruminants (see review by Forsyth, 1996). Hadsell et al. (2002) suggested that the effects of IGF-1 on mammary development depend on the physiological state of the animal. Yet, most studies looking at the impact of IGF-1 on mammary development were performed in the periparturient period. Exogenous ST increased parenchymal mass in ewe lambs (Johnsson et al., 1986; McFadden et al., 1990) and dairy heifers (Sejrsen et al., 1986; Sandles et al., 1987). Knowing that the extent of mammary accretion is greater in the last third of gestation than in prepuberty in pigs (Sorensen

Table 3. Mammary gland composition for gilts injected with sterile water (controls, CTL, $n = 17$) or 5 mg of porcine somatotropin (pST, $n = 20$) from days 90 to 109 of gestation

Variable measured	Treatment		SEM ¹
	CTL	pST	
Extraparenchymal tissue, g	1375	1355	72
Extraparenchyma/parenchyma	0.96 ^c	0.74 ^d	0.07
Parenchymal tissue, g	1576 ^c	1922 ^d	124
Dry matter, %	37.4 ^a	34.2 ^b	0.8
Fat ² , %	62.1 ^c	56.8 ^d	1.7
Fat, g total	361.2	371.9	27.6
Protein ² , %	34.8 ^c	39.5 ^d	1.5
Protein, g total	203.8 ^c	257.9 ^d	18.5
DNA ² , mg/g	9.65 ^c	10.96 ^d	0.43
DNA, g total	5.62 ^c	7.14 ^d	0.49
RNA ² , mg/g	8.63 ^c	9.91 ^d	0.41
RNA, g total	5.03 ^c	6.40 ^d	0.43

^{a,b}Means within a row with different superscripts differ ($P < 0.01$).

^{c,d}Means within a row with different superscripts differ ($P < 0.05$).

¹Maximum value.

²Expressed on a dry matter basis.

et al., 2002), it is of importance to establish whether IGF-1 affects mammogenesis during pregnancy. In dairy heifers, milk yield was increased following injections with recombinant ST in the last trimester of gestation, and enhanced mammogenesis was suggested (Stelwagen et al., 1992). In ewes, the greater milk yield observed after injections with ST from days 97 until 124 of gestation was related to an increase in the number of mammary parenchymal cells (Stelwagen et al., 1993). Knight et al. (1994) also showed that exogenous ST administered during the last 6 wk of gestation in goats increased the number of mammary parenchymal cells at parturition. Furthermore, direct infusion of ST into mammary glands of late-pregnant beef cows increased mammary DNA (Collier et al., 1993). Kann et al. (1999) also suggested that IGF-1 is a mediator for the mammogenic effect of ST during artificial induction of lactation in ewes.

In swine, no clear correlations were detected between mammary measures and plasma IGF-I concentrations in prepubertal gilts that were either restricted-fed or fed ad libitum (Sorensen et al., 2006). Furthermore, the arteriovenous difference and mammary uptake of plasma IGF-1 in late-pregnant sows did not differ from zero, yet, this does not preclude that increasing IGF-1 concentrations in late gestation could favorably affect mammogenesis (Feyera et al., 2019). When growth hormone-releasing factor was injected from day 100 of gestation until either farrowing or day 29 of

lactation, there was no beneficial effect on mammary development on day 30 of lactation, but the effect on mammogenesis in late gestation was not looked at (Farmer et al., 1997). Only 1 study investigated the effect of exogenous pST on mammary development in late-pregnant gilts. Howard (1995) reported in a PhD thesis that IGF-1 could be involved in mammary development taking place in gestation. Gilts were injected with 8 mg/d of pST from days 75 until 105 of gestation and were then slaughtered to collect mammary glands. Gilts injected with pST had more mammary parenchyma, and more total parenchymal protein, DNA, and RNA than control gilts, whereas extraparenchymal tissue mass was not affected. Current results corroborate those findings, and effects on parenchymal tissue composition were also seen. The decreased percent dry matter and fat, and the increased percent protein and greater concentrations of DNA and RNA in parenchyma are further indicative of a mammogenic effect of IGF-1. These novel effects in the current study are likely due to the timing of the injections. Contrary to the study by Howard (1995), injections started on day 90 of gestation and lasted until day 109 of gestation, being the gestation period with most rapid mammary accretion (Sorensen et al., 2002). DeHoff et al. (1986) previously suggested that ST may not be mammogenic in the pig during pregnancy, but this was solely based on endogenous concentrations. Current results establish that a 4-fold increase in IGF-1 in late pregnancy, via exogenous pST, does stimulate mammary development in swine both in terms of parenchymal mass and parenchymal tissue composition. It is important to mention, however, that the effect of exogenous pST on mammary development may not have been solely due to increased IGF-1 concentrations but also to alterations in metabolism.

In the present study, concentrations of IGF-1 were maximal on day 96, namely 7 d after the onset of pST injections. Injections of 5 mg/d to gilts in early- or mid-gestation led to similar increases in circulating IGF-1 (Sterle et al., 1995, 2003) as seen in the current study. Values reported here remained significantly greater throughout the 3-wk treatment period. Furthermore, the observed changes in concentrations of insulin, glucose, and urea also showed a lasting effect of exogenous pST on the metabolic status of gilts in late pregnancy. These changes are in accordance with reported effects of pST on endocrine and metabolic factors in pregnant pigs (see review by Rehfeldt et al., 2004), and could indicate a homeorhetic shift redirecting nutrients toward the mammary gland. The drastic increase

in IGF-1 was expected (Wray-Cahen et al., 1991). Increases in concentrations of glucose and insulin and a decrease in urea nitrogen were also previously reported in growing pigs injected with pST (Wray-Cahen et al., 1991; Dunshea et al., 1992; Caperna et al., 1993). In pregnant gilts, an increase in serum glucose and a decrease in urea nitrogen were observed when pST injections were given from day 108 of gestation onward (Cromwell et al., 1992) and blood glucose was increased with pST injections from days 80 to 114 of gestation (Trujillo-Ortega et al., 2006). Kveragas et al. (1986) also reported greater glucose and insulin concentrations in late-pregnant sows treated with pST, suggesting a state of insulin resistance. The lower blood urea in pST gilts is indicative of decreased amino acid degradation from breakdown of skeletal muscles for energy use (Nelssen et al., 1985). Injections of growth hormone-releasing factor also led to lower urea concentrations in lactating sows (Farmer et al., 1992). Exogenous pST undoubtedly altered the metabolism of late-pregnant gilts in a way that favored mammary development. Current results also corroborate the effect of pST on improving efficiency of protein metabolism. This is in accordance with the greater BW gain and greater loss of backfat in pST gilts over the treatment period. Exogenous pST is known to increase growth rate and improve feed efficiency in growing pigs (review by Etherton, 1989; Klindt et al., 1998). Treatment with pST increased BW gain and reduced rate of backfat gain in gilts and sows when injections were given from days 25 to 100 of gestation (Gatford et al., 2010).

In previous studies, treatment with pST during pregnancy in pigs was mostly done in early gestation with the goal of increasing fetal and placental growth (Kelley et al., 1995; Sterle et al., 1995, 2003; Rehfeldt et al., 2001). Indeed, increased maternal IGF-1 concentrations may enhance nutrient transfer across the placenta and increase fetal IGF-1, thereby promoting fetal growth (Sterle et al., 1995). Taking into account that most fetal growth takes place in the last third of gestation, one would expect exogenous pST to be most beneficial during that time period. Nevertheless, when injections of pST were given in late gestation, effects on piglet birth weight were contradictory. Trujillo-Ortega et al. (2006) reported greater piglet birth weight when injecting 6 mg/d of pST from days 80 to 114 of gestation. Yet, when Kveragas et al. (1986) injected 10 mg/d of pST during the last 21 d of gestation there was no effect on piglet birth weight. Similarly, in the current trial, fetal weights were not affected by treatment. On the other hand, Gatford

et al. (2010) injected 2.5 mg/d of pST to gilts and 4.0 mg/d to sows from days 25 to 100 of pregnancy and saw an increase in piglet birth weight. However, when 2.5 mg/d of pST was injected for a shorter period of time later in gestation (days 75 to 100), birth weight of piglets was increased in sows but not in gilts (Gatford et al., 2012). It is therefore apparent that discrepancies in results are linked to dosage, duration of treatment, and parity. The absence of effect in the current study is likely linked to the short period of treatment in late gestation and to the use of gilts. It would be of interest to compare concentrations of IGF-1, yet, in none of these trials were concentrations of IGF-1 measured. In conclusion, using daily injections of 5 mg of pST to increase IGF-1 concentrations in gilts from days 90 until 109 of gestation stimulates mammary development both in terms of amount of parenchymal tissue and composition of parenchymal tissue but has no effect on piglet birth weight.

ACKNOWLEDGMENTS

The authors wish to thank A. Bernier, É. Fortin, L. Marier, and C. Paradis for their invaluable technical assistance, the staff of the Swine Complex, especially É. Bérubé, J. Boudreau, and J.-P. Brousseau, for care and slaughter of the animals, and S. Méthot for statistical analyses.

LITERATURE CITED

- AOAC. 2005. Official methods of analysis international. 18th ed. Assoc. Off. Anal. Chem., Arlington, VA.
- Caperna, T. J., N. C. Steele, C. M. Evock-Clover, D. Brocht, J. P. McMurtry, and R. W. Rosebrough. 1993. Acute effects of administration of porcine growth hormone on circulating levels of hormones and metabolites in 20-, 40-, and 60-kilogram gilts. *J. Anim. Sci.* 71:897–905.
- CCAC. 2009. Canadian council on animal care guidelines on: the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care in Science, Ottawa, Ontario, Canada.
- Collier, R. J., M. F. McGrath, J. C. Byatt, and L. L. Zurfluh. 1993. Regulation of bovine mammary growth by peptide hormones: involvement of receptors, growth factors and binding proteins. *Livest. Prod. Sci.* 35:21–22.
- Cromwell, G. L., T. S. Stahly, L. A. Edgerton, H. J. Monegue, T. W. Burnell, B. C. Schenck, and B. R. Schrick. 1992. Recombinant porcine somatotropin for sows during late gestation and throughout lactation. *J. Anim. Sci.* 70:1404–1416.
- DeHoff, M. A., C. S. Stoner, F. W. Bazer, R. J. Collier, R. R. Kraeling, and F. C. Buonomo. 1986. Temporal changes in steroids, prolactin and growth hormone in pregnant and pseudopregnant gilts during mammary development and lactogenesis. *Domest. Anim. Endocrinol.* 3:95–105.
- Dunshea, F. R., D. E. Bauman, R. D. Boyd, and A. W. Bell. 1992. Temporal response of circulating metabolites and

- hormones during somatotropin treatment of growing pigs. *J. Anim. Sci.* 70:123–131.
- Etherton, T. D. 1989. The mechanisms by which porcine growth hormone improves pig growth performance. In: R. B. Heap, C. G. Prosser, and G. E. Lamming, editors, *Biotechnology in growth regulation*. Butterworths, London. 97–105.
- Farmer, C., M. F. Palin, and R. Hovey. 2010. Greater milk yield is related to increased DNA and RNA content but not to mRNA abundance of select genes in sow mammary tissue. *Can. J. Anim. Sci.* 90:379–388.
- Farmer, C., G. Pelletier, P. Brazeau, and D. Petitclerc. 1997. Mammary gland development of sows injected with growth hormone-releasing factor during gestation and/or lactation. *Can. J. Anim. Sci.* 77:335–338.
- Farmer, C., and D. Petitclerc. 2003. Specific window of prolactin inhibition in late gestation decreases mammary parenchymal tissue development in gilts. *J. Anim. Sci.* 81:1823–1829. doi:10.2527/2003.8171823x
- Farmer, C., D. Petitclerc, G. Pelletier, and P. Brazeau. 1992. Lactation performance of sows injected with growth hormone-releasing factor during gestation and/or lactation. *J. Anim. Sci.* 70:2636–2642.
- Farmer, C., M. T. Sorensen, and D. Petitclerc. 2000. Inhibition of prolactin in the last trimester of gestation decreases mammary gland development in gilts. *J. Anim. Sci.* 78:1303–1309.
- Feyera, T., P. Zhou, M. Nuntapaitoon, K. U. Sørensen, U. Krogh, T. S. Bruun, S. Purup, H. Jørgensen, H. D. Poulsen, and P. K. Theil. 2019. Mammary metabolism and colostragenesis in sows during late gestation and the colostrual period. *J. Anim. Sci.* 97:231–245. doi:10.1093/jas/sky395
- Forsyth, I. A. 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminants: action and interaction with hormones. *J. Dairy Sci.* 79:1085–1096. doi:10.3168/jds.S0022-0302(96)76462-7
- Gatford, K. L., M. J. De Blasio, C. T. Roberts, M. B. Nottle, K. L. Kind, W. H. van Wetters, R. J. Smits, and J. A. Owens. 2009. Responses to maternal GH or ractopamine during early-mid pregnancy are similar in primiparous and multiparous pregnant pigs. *J. Endocrinol.* 203:143–154. doi:10.1677/JOE-09-0131
- Gatford, K. L., R. J. Smits, C. L. Collins, C. Argent, M. J. De Blasio, C. T. Roberts, M. B. Nottle, K. L. Kind, and J. A. Owens. 2010. Maternal responses to daily maternal porcine somatotropin injections during early-mid pregnancy or early-late pregnancy in sows and gilts. *J. Anim. Sci.* 88:1365–1378. doi:10.2527/jas.2009-2265
- Gatford, K. L., R. J. Smits, C. L. Collins, M. J. De Blasio, C. T. Roberts, M. B. Nottle, W. H. van Wetters, K. L. Kind, and J. A. Owens. 2012. Maternal low-dose porcine somatotropin treatment in late gestation increases progeny weight at birth and weaning in sows but not in gilts. *J. Anim. Sci.* 90:1428–1435. doi:10.2527/jas.2011-4227
- Hadsell, D. L., and S. G. Bonnette. 2000. IGF and insulin action in the mammary gland: lessons from transgenic and knockout models. *J. Mammary Gland Biol. Neoplasia* 5:19–30.
- Hadsell, D. L., S. G. Bonnette, and A. V. Lee. 2002. Genetic manipulation of the IGF-I axis to regulate mammary gland development and function. *J. Dairy Sci.* 85:365–377. doi:10.3168/jds.S0022-0302(02)74083-6
- Harrell, R. J., M. J. Thomas, and R. D. Boyd. 1993. Limitations of sow milk yield on baby pig growth. In: *Proc. Cornell Nutr. Conf. Rochester, NY*; p. 156.
- Howard, K. A. 1995. Effect of porcine somatotropin, soybean oil and energy intake on mammary development in gestating gilts. PhD Thesis presented to the Michigan State University, Dept. of Anim. Sci, East Lansing, MI.
- Huntington, G. B. 1984. Net absorption of glucose and nitrogenous compounds by lactating Holstein cows. *J. Dairy Sci.* 67:1919–1927. doi:10.3168/jds.S0022-0302(84)81525-8
- Johnsson, I. D., I. C. Hart, and A. Turvey. 1986. Pre-pubertal mammary development in the sheep. 3. The effects of restricted feeding or daily administration of bovine growth hormone and bromocriptine on mammary growth and morphology. *Anim. Prod.* 45:53–63.
- Kann, G., A. Delobelle-Deroide, L. Belair, A. Gertler, and J. Djiane. 1999. Demonstration of in vivo mammary and lactogenic effects of recombinant ovine placental lactogen and mammary effect of recombinant ovine GH in ewes during artificial induction of lactation. *J. Endocrinol.* 160:365–377.
- Kelley, R. L., S. B. Jungst, T. E. Spencer, W. F. Owsley, C. H. Rahe, and D. R. Mulvaney. 1995. Maternal treatment with somatotropin alters embryonic development and early postnatal growth of pigs. *Domest. Anim. Endocrinol.* 12:83–94.
- Kleinberg, D. L., and M. H. Barcellos-Hoff. 2011. The pivotal role of insulin-like growth factor I in normal mammary development. *Endocrinol. Metab. Clin. North Am.* 40:461–471. doi:10.1016/j.ecl.2011.06.001
- Kleinberg, D. L., and W. Ruan. 2008. IGF-I, GH, and sex steroid effects in normal mammary gland development. *J. Mammary Gland Biol. Neoplasia* 13:353–360. doi:10.1007/s10911-008-9103-7
- Klindt, J., J. T. Yen, F. C. Buonomo, A. J. Roberts, and T. Wise. 1998. Growth, body composition, and endocrine responses to chronic administration of insulin-like growth factor I and/or porcine growth hormone in pigs. *J. Anim. Sci.* 76:2368–2381.
- Knight, C. H., J. R. Brown, and K. A. Sejrnsen. 1994. A comparison of growth hormone-induced mammary development in pregnant and lactating goats. *Endocrinol. Metab.* 1(Suppl. B):52.
- Kveragas, C. L., R. W. Seerley, R. J. Martin, and W. L. Vandergrift. 1986. Influence of exogenous growth hormone and gestational diet on sow blood and milk characteristics and on baby pig blood, body composition and performance. *J. Anim. Sci.* 63:1877–1887.
- Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* 102:344–352.
- McFadden, T. B., T. E. Daniel, and R. M. Akers. 1990. Effects of plane of nutrition, growth hormone and unsaturated fat on mammary growth in prepubertal lambs. *J. Anim. Sci.* 68:3171–3179.
- Monaco, M. H., D. E. Gronlund, G. T. Bleck, W. L. Hurley, M. B. Wheeler, and S. M. Donovan. 2005. Mammary specific transgenic over-expression of insulin-like growth factor-I (IGF-I) increases pig milk IGF-I and IGF binding proteins, with no effect on milk composition or yield. *Transgenic Res.* 14:761–773. doi:10.1007/s11248-005-7219-8
- Nelssen, J. L., A. J. Lewis, E. R. Peo, Jr., and B. D. Moser. 1985. Effect of source of dietary energy and energy restriction

- during lactation on sow and litter performance. *J. Anim. Sci.* 60:171–178.
- Plante, P.-A., J.-P. Laforest, and C. Farmer. 2011. Effect of supplementing the diet of lactating sows with NuPro® on their performances and that of their piglets. *Can. J. Anim. Sci.* 91:295–300.
- Rehfeldt, C., G. Kuhn, G. Nürnberg, E. Kanitz, F. Schneider, M. Beyer, K. Nürnberg, and K. Ender. 2001. Effects of exogenous somatotropin during early gestation on maternal performance, fetal growth, and compositional traits in pigs. *J. Anim. Sci.* 79:1789–1799.
- Rehfeldt, C., P. M. Nissen, G. Kuhn, M. Vestergaard, K. Ender, and N. Oksbjerg. 2004. Effects of maternal nutrition and porcine growth hormone (pGH) treatment during gestation on endocrine and metabolic factors in sows, fetuses and pigs, skeletal muscle development, and postnatal growth. *Domest. Anim. Endocrinol.* 27:267–285. doi:10.1016/j.domaniend.2004.06.005
- Sandles, L. D., C. J. Peel, and P. D. Temple-Smith. 1987. Mammogenesis and first lactation milk yields of identical-twin heifers following pre-pubertal administration of bovine growth hormone. *Anim. Prod.* 45:349–357.
- Sejrsen, K., J. Foldager, M. T. Sorensen, R. M. Akers, and D. E. Bauman. 1986. Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. *J. Dairy Sci.* 69:1528–1535. doi:10.3168/jds.S0022-0302(86)80569-0
- Simmen, F. A., R. C. Simmen, and G. Reinhart. 1988. Maternal and neonatal somatomedin C/insulin-like growth factor-I (IGF-I) and IGF binding proteins during early lactation in the pig. *Dev. Biol.* 130:16–27.
- Sorensen, M. T., C. Farmer, M. Vestergaard, S. Purup, and K. Sejrsen. 2006. Mammary development in prepubertal gilts fed restrictively or ad libitum in two sub-periods between weaning and puberty. *Livest. Sci.* 99:249–255.
- Sorensen, M. T., K. Sejrsen, and S. Purup. 2002. Mammary gland development in gilts. *Livest. Prod. Sci.* 75:143–148.
- Stelwagen, K., D. G. Grieve, B. W. McBride, and J. D. Rehman. 1992. Growth and subsequent lactation in primigravid Holstein heifers after prepartum bovine somatotropin treatment. *J. Dairy Sci.* 75:463–471. doi:10.3168/jds.S0022-0302(92)77782-0
- Stelwagen, K., D. G. Grieve, J. S. Walton, J. L. Ball, and B. W. McBride. 1993. Effect of prepartum bovine somatotropin in primigravid ewes on mammogenesis, milk production, and hormone concentrations. *J. Dairy Sci.* 76:992–1001. doi:10.3168/jds.S0022-0302(93)77427-5
- Sterle, J. A., T. C. Cantley, W. R. Lamberson, M. C. Lucy, D. E. Gerrard, R. L. Matteri, and B. N. Day. 1995. Effects of recombinant porcine somatotropin on placental size, fetal growth, and IGF-I and IGF-II concentrations in pigs. *J. Anim. Sci.* 73:2980–2985.
- Sterle, J. A., T. C. Cantley, R. L. Matteri, J. A. Carroll, M. C. Lucy, and W. R. Lamberson. 2003. Effect of recombinant porcine somatotropin on fetal and placental growth in gilts with reduced uterine capacity. *J. Anim. Sci.* 81:765–771. doi:10.2527/2003.813765x
- Trujillo-Ortega, M. E., D. Mota-Rojas, R. Hernández-González, E. Y. Velázquez-Armenta, A. A. Nava-Ocampo, R. Ramírez-Necoechea, M. Becerril-Herrera, and M. Alonso-Spilsbury. 2006. Obstetric and neonatal outcomes to recombinant porcine somatotropin administered in the last third of pregnancy to primiparous sows. *J. Endocrinol.* 189:575–582. doi:10.1677/joe.1.06748
- Vanklompenberg, M. K., R. Manjarin, J. F. Trott, H. F. McMicking, and R. C. Hovey. 2013. Late gestational hyperprolactinemia accelerates mammary epithelial cell differentiation that leads to increased milk yield. *J. Anim. Sci.* 91:1102–1111. doi:10.2527/jas.2012-5903
- Volkin, E., and W. E. Cohn. 1954. Estimation of nucleic acids. *Methods Biochem. Anal.* 1:287–305.
- Wray-Cahen, D., D. A. Ross, D. E. Bauman, and R. D. Boyd. 1991. Metabolic effects of porcine somatotropin: nitrogen and energy balance and characterization of the temporal pattern of blood metabolites and hormones. *J. Anim. Sci.* 69:1503–1514.