The effects of exogenous pST administration and feeding ractopamine in early pregnancy on the birth weight and growth performance of gilt and sow progeny

Report prepared for the Co-operative Research Centre for an Internationally Competitive Pork Industry

By

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Executive Summary

Piglets of low birth weight have higher pre-weaning and post-weaning mortality, and poorer growth potential after birth, including fewer muscle fibres and hence potential for lean tissue deposition. Previous studies indicated that maternal pST injections through early to mid or late pregnancy and feeding ractopamine through early-mid pregnancy had potential to increase progeny birth weights and/or postnatal performance, but these technologies had not been directly compared, and improvements in litters from gilts and sows had not been compared.

We therefore evaluated effects of maternal pST injections or feeding ractopamine from day 25 to 50 of pregnancy on fetal and placental growth and muscle fibre development in the fetal pig at day 50 of pregnancy, and compared responses in gilts and sows (Study 1). Subsequently, we evaluated effects of maternal pST injections from day 25 to 50 or day 25 to 100 in gilts and sows, on maternal reproductive performance in the treatment pregnancy and lactation, and at the subsequent mating, and on progeny size at birth, postnatal growth, finisher feed consumption and efficiency, and carcass characteristics.

Study 1, fetal responses: Maternal pST injections increased litter average fetal weight by 11% and ractopamine increased this by 9%. Maternal pST had more consistent effects than ractopamine on increasing measures of fetal size, and also increased fetal length, abomdinal circumference and liver weight. Effects of maternal pST, but not ractopamine, on fetal weight remained significant when litters of less than 5 fetuses were excluded, and pST was therefore chosen as the treatment to be evaluated under commercial conditions in Study 2. Maternal pST also increased maternal weight gain in sows, although not gilts. Effects of maternal pST on fetal weight were greatest in the largest fetuses of the litter. Maternal plasma ST and IGF-I were increased, and maternal and fetal plasma urea were decreased, in dams treated with pST, consistent with an anabolic state. Both treatments affected fetal muscle development, with increased primary muscle fibre diameter in heavy fetuses from pST-treated dams and in medium weight fetuses from ractopamine-treated dams, compared to fetuses from control pigs. Based on these results and our previous studies, maternal and progeny responses to maternal pST treatment from day 25 to 50 or 25 to 100 of pregnancy were compared to untreated controls in Study 2.

Study 2, maternal and progeny responses: This research has demonstrated that significant increases in birth weight and postnatal growth can be obtained under commercial conditions when pregnant pigs are injected daily with pST from day 25 to 100 of pregnancy. Increases were greater in sow progeny than in gilt progeny. Carcass weights were increased by 3.3 kg on average in progeny of dams injected with pST from day 25 to 100 of pregnancy. This is currently cost-effective in both parities ($9.85 net benefit/litter in gilts, $50.32 net benefit/litter in sows). Responses are larger in sow litters, but this is partly offset by increased post-weaning cull rates, and strategies to minimize this might be needed (e.g. pST from first litter onwards to maximize foot and leg strength, increased lactation feed intakes or holding over to avoid culling highly productive sows with large litter weight gains in lactation).

Maternal pST injections from day 25 to 75 of pregnancy are financial viable to increase progeny performance. Labour shortages and concerns about consumer perceptions make sustained maternal pST injections unattractive for take-up by the Australian Pork Industry

in its present form, however. Licence approval also needs to be extended to pregnant animals for Reporcin, which is currently only licenced for use in growing pigs. Follow-up studies of the effectiveness of pST injections only during late gestation are underway, and we are also exploring the normal patterns of circulating pST during pregnancy in the pig as a precursor to developing a selection approach. We also suggest that further evaluation of feeding ractopamine and of dietary approaches to increase maternal pST during pregnancy are warranted, as these would be more readily implemented by industry than maternal pST injections.

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1. Introduction

Background: Fetal growth in the pregnant pig is constrained by commercial feed levels, particularly in the gilt, where fetal and maternal growth each compete for nutrients. Increased maternal nutrition improves birth weight as well as feed efficiency and muscle fibre development of progeny (Dwyer, et al. 1994), but decreases lactation feed intake of the sow. Administration of pST to the pregnant pig has shown promise as an alternative strategy to increase fetal growth and progeny performance, and preliminary data also suggests that ractopamine or salbutamol treatment (ß-adrenergic agonists) increase progeny growth rates and muscle development. Studies using nutrition, pST or ractopamine, implicate d25-50 of pregnancy as a critical period for improving later progeny performance (in association with increased fetal growth), but usually without increased birth weight and perinatal survival, which are seen in response to more sustained treatments.

Daily pST injections in pregnancy – effects on fetal growth and survival: Maternal pST treatment during early gestation may improve embryo/fetal growth and survival. In early studies, Kelley et al (1995) found that daily injection of gilts with 30 µg pST/kg/d from d 28 to 41 of gestation increased fetal length (by 20%) and embryo survival (from 77 to 88%) at d 41. Sterle et al (1995) also reported increased embryo survival and fetal weight in response to daily injection with ~35 µg pST/kg/d from d 33 to d 44 of gestation, but gilts in these two studies were fed at higher rates than used commercially. In our earlier studies under commercial conditions, daily injections of ~15 or 30 µg/kg/d in gilts from d 25 to 50 of pregnancy increased fetal weight at d 50, but not at term, suggesting later constraint of fetal growth (Gatford, et al. 2004; Gatford, et al. 2000). In contrast to the studies of Kelley and Sterle (Kelley et al. 1995; Sterle et al. 1995), litter size and embryo survival under commercial conditions were not increased by 15 or 30 µg pST/kg/d, nor in response to daily injection of 12.5 µg pST/kg/d in early gestation (PC Owens, M Nottle, R Smits, APL final report). Importantly, there were no adverse effects of pST on embryo survival under commercial conditions. These previous studies were performed in gilts, but it is possible that increased fetal growth following short-term pST treatment in early gestation may persist to term in older sows, where there is less maternal competition for nutrients. Even in gilts, one study reported small increases in birth weight after pST treatment in early gestation in the smallest piglets of each litter (Rehfeldt, et al. 2001a). Sustained maternal pST treatment appears to be a more effective strategy to increase birthweight, particularly where fetal growth is limited by increased litter size in highly prolific genetic lines, a problem identified already in overseas herds. Under commercial conditions, we have shown that sustained daily pST treatment (2mg/d ≈15µg pST/kg/d) of gilts from d25 to 100 of pregnancy increased birth weight, with the biggest increase in the largest litters, where maternal constraint is greatest (Gatford et al. 2004), providing a potential strategy to decrease losses before weaning and improve progeny growth and performance. The mechanism/s responsible for short and long-term changes in fetal growth in response to pST are unclear. Limited evidence implicates changes in maternal nutrient partitioning, placental growth and placental nutrient transport.

Daily pST injections in pregnancy – effects on progeny performance: Increased maternal nutrition from d 25 to 50 or d 25 to 80 of gestation increases numbers of muscle fibres in progeny of sows (Dwyer et al. 1994). Progeny growth rate and feed efficiency were improved in the latter group but not measured in progeny of sows whose feed intake was increased only from d 25 to 50. This suggests that treatments that increase fetal growth during the period of early muscle fibre development can improve later performance of progeny through

effects on muscle development during fetal life. Therefore, by increasing the supply of nutrients to the fetus at critical periods of development, maternal pST treatment may also improve progeny growth, muscularity and feed conversion efficiency after birth. In our studies, maternal pST treatment from d 25 to 50 of gestation increased semitendinosus cross-sectional area in female progeny which were of median birth weight for their litter, without changing the ratio of secondary to primary muscle fibres (Gatford, et al. 2003). Maternal pST administration from d 25 to 50 or d 25 to 100 of gestation in gilts did not, however, change growth rates or feed efficiency in male or female progeny of median birth weight for the litter (PC Owens, KL Gatford, R Smits, APL final report). We do not currently know whether performance of light or heavy littermates is altered by maternal pST treatment. Kelley et al (1995) also reported increased muscle weights and cross-sectional areas in progeny of gilts treated with pST from d 28 to 40 of gestation, confirming that muscle development can be altered by maternal pST treatment during this period. More recent reports have suggested that increases in muscle fibre numbers and muscle growth after maternal pST treatment in early pregnancy may be greatest in the smallest pigs from each litter (Rehfeldt, et al. 2001b; Rehfeldt, et al. 2004). Progeny across the birth weight range may therefore need to be studied to observe the effects of pST and improvements in overall progeny performance.

Feeding ractopamine in pregnancy – postnatal responses: The evidence for efficacy of dietary ß-adrenergic agonists in pregnancy on progeny performance is much less strong than for pST, but potentially offers a less invasive and less labour-intensive alternative strategy for *in utero* manipulation of progeny performance. Hoshi et al (2005a) reported that feeding sows (2^{nd} to 4^{th} parity) with 20 ppm ractopamine between d 25 and 50 of pregnancy increased the growth rates of their progeny from 20 to 50 kg body weight, and increased carcass weight in progeny killed at 133 d of age, but did not change numbers of muscle fibres in semitendinosus muscle, nor measures of muscle size. Litter size and weights at birth and weaning were not altered by feeding sows ractopamine at this dose from d 25 to 50 of pregnancy, nor after longer treatment from d 25 to 80 of pregnancy (Hoshi, et al. 2005b). In the only other published study to report the effects on progeny of feeding ß-adrenergic agonists during pregnancy in pigs, Kim et al (1994) reported that feeding salbutamol to sows throughout the first third of pregnancy (mating to d 38) altered proportions of fibre types in semitendinosus muscle and increased muscle cross-sectional area in progeny at slaughter (90 kg). This study lacked the power to observe changes in progeny survival or smaller changes in growth rate, however, with only four sows studied per treatment.

Rationale for project strategy:

Increased progeny growth and/or muscle fibre numbers and muscle size have been reported following maternal treatment with increased nutrition (gilts), daily pST injection (gilts), or dietary ractopamine (sows) between d 25 and 50 of pregnancy. For at least nutrition and pST, these later progeny responses are preceded by increased fetal growth during treatment. We will therefore use this treatment window initially to compare the effectiveness of pST and ractopamine, as well as to compare responses to these in gilts AND sows. Our first aim, in Study 1, was to determine which treatment/s or parity/ies give the maximal increase in fetal size and hind-limb muscle fibre number at the end of treatment. Continuation of the study to the commercial trial (Study 2) was subject to a critical review at the end of 2007, and in particular, demonstration of increased fetal growth and/or muscle development in response to pST and/or dietary ractopamine. The treatments and parities chosen for Study 2, run in 2008, were based on outcomes of Study 1.

Our second aim, in Study 2, was to evaluate the effect of the combination of in utero manipulation/s and parity/ies identified in Study 1, on litter size and reproductive performance of gilts and sows, uniformity of birthweight, and the performance through to slaughter of progeny across the range of birthweight under commercial conditions.

2. Methodology

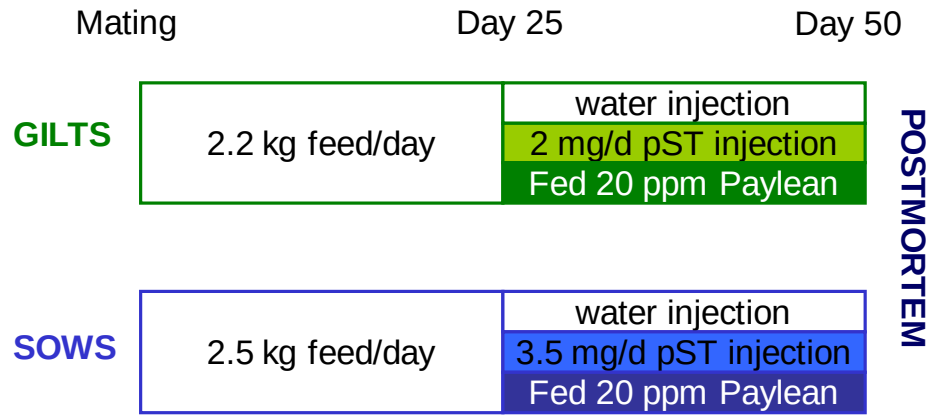
Methodology for Study 1 – fetal growth and muscle fibre responses to ractopamine and pST in early pregnancy in gilts and sows

Gilts and sows (n=8/group) were administered saline (controls) , pST once per day (15 µg pST/kg daily), or fed ractopamine (20 ppm), from d 25 to 50 of pregnancy (Figure 1).

Gilts and sows were euthanased at day 50 of pregnancy and a series of measures taken:

- fetal size and weight
- fetal hindlimb muscle was collected and muscle fibre number and size evaluated in a light, medium and heavy fetus of each litter
- CL numbers were counted to evaluate embryonic survival
- placental weight and area measured
- maternal and fetal blood samples collected to evaluate maternal metabolic responses and placental transport
- placentae collected for morphometric analysis and transporter gene and protein expression studies (to be the subject of a future Honours project)

Figure 1: Experimental design for Study 1, fetal responses to maternal pST injections or dietary ractopamine in early-mid pregnancy



Data analysis Study 1:

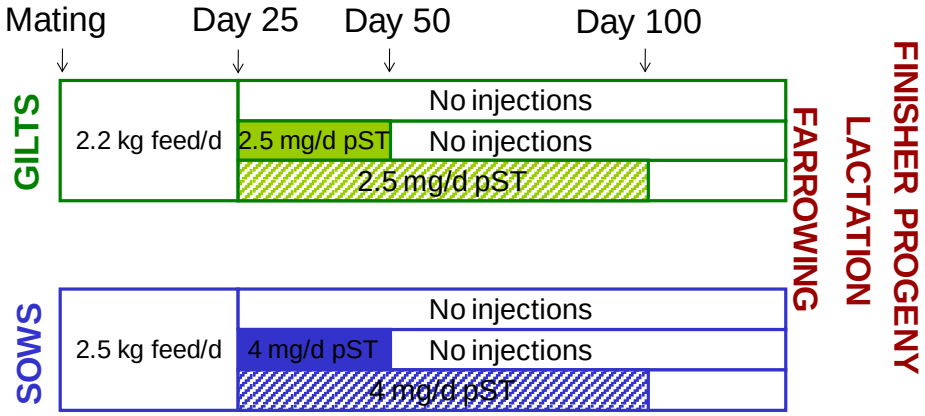
- data from all pregnant dams was included (one pST-treated gilt returned to oestrus ~5 days after pST-injections commenced and was excluded)
- maternal and litter responses (including average fetal and placental size measures) were analysed by 2-way ANOVA for effects of maternal treatment (control/pST-injected/ractopamine-fed) and parity (gilt/sow), with litter size as a covariate

- individual fetal and placental weights were analysed by repeated measures ANOVA for effects of maternal treatment and parity, fetal sex and fetal weight quartile (to determine whether responses were similar in light and heavy fetuses), with litter size as a covariate. Each fetal or placental measure was treated as a measure on the dam, with the dam as the experimental unit.

Methodology for Study 2 – commercial evaluation of progeny performance following maternal treatment

Pregnant animals (n=100/group) were either non-injected (commercial control), or administered pST (gilts 2.5 mg.d^{-1}; sows 4.0 mg.d^{-1}) from d 25 to 50 or d 25 to 100 of pregnancy (Figure 2). Farrowing rate, individual birth weights, and litter size and weight at birth and weaning were measured in all mothers and litters for the treatment pregnancy. Pregnancy losses and dam and piglet removals throughout the study and loss reasons were also recorded. Individual progeny weights at the end of weaner phase (10 weeks), grower (17 weeks), and at slaughter (22 weeks of age) were recorded in heavy, medium and light, male and female piglets from each maternal treatment and parity group (n=approx 240 piglets/week for 10 replicates). Semitendinosus muscles were also collected at birth from male and female piglets of low, medium and high birthweight for their litter, within each treatment (6 progeny/litter x 6 treatments=216). These muscles are currently being analysed and total, primary and secondary muscle fibre numbers counted. Feed:liveweight gain (finisher phase, electronic feeders), and carcass dressing percentage, weight and fatness at slaughter, were measured in male and female progeny from ~980 progeny (~27 individual progeny from each combination of progeny sex, relative birthweight, maternal treatment and maternal parity) under commercial conditions. Subsequent reproductive performance of dams was also followed in the first 10 replicates of the study (replicates 11 and 12 were excluded due to removal of piglets for muscle fibre studies). Time to return to oestrus, conception rate, pregnancy outcome (farrowing and litter size) were recorded in all dams that were weaned following the treatment pregnancy in replicates 1 to 10.

Figure 2: Experimental design for Study 2, maternal and progeny responses to maternal pST injections in early-mid or early-late pregnancy



Data analysis Study 2:

- dam outcomes (e.g. removals from the trial, proportion farrowing, proportions conceiving at subsequent mating) were analysed by Chi-squared analysis for effects of maternal treatment and parity)

- maternal and litter responses (including average fetal and placental size measures) were analysed by 2-way ANOVA for effects of maternal treatment (control/pST-injected) and parity (gilt/sow), with litter size as a covariate
- individual progeny birth and weaning weights were analysed by repeated measures ANOVA for effects of maternal treatment and parity, progeny sex and progeny birth weight tertile (to determine whether responses were similar in light, medium and heavy piglets), with litter size as a covariate. Each progeny measure was treated as a measure on the dam, with the dam as the experimental unit.
- Individual progeny finisher growth and carcass measures were analysed by repeated measures ANOVA for effects of maternal treatment and parity, progeny sex and progeny birth weight tertile (light/medium/heavy birth weight for litter)

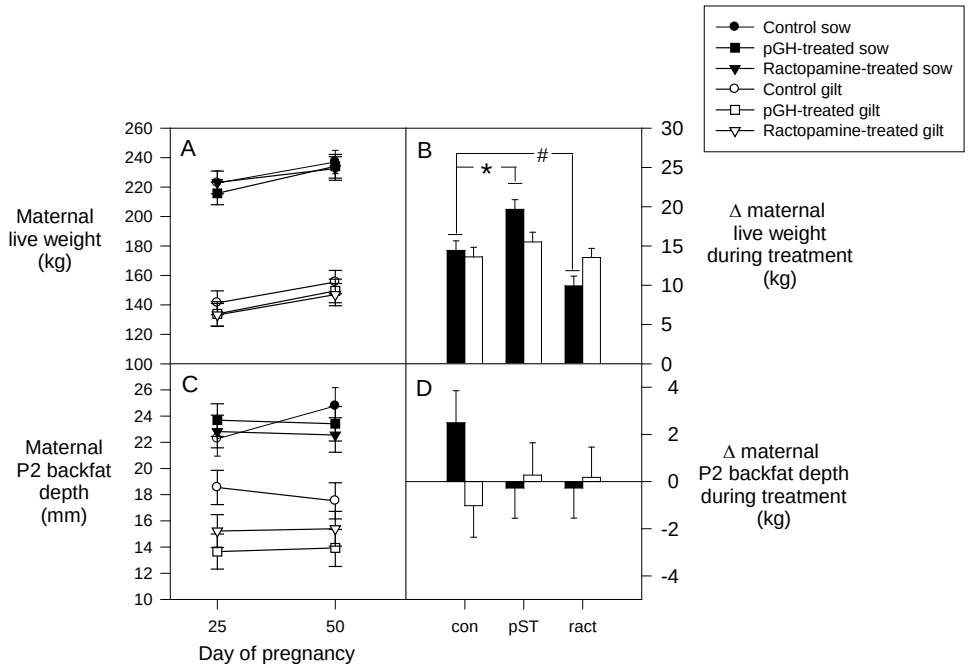
3. Outcomes

Outcomes for Study 1 – fetal growth and muscle fibre responses to ractopamine and pST in early pregnancy in gilts and sows

Maternal weight and backfat depth:

The effect of treatment on change in maternal weight during the treatment period differed between parities (**Figure 1**, P=0.010). In sows, pST-treated dams gained more weight than control dams (P=0.013, controls: 14.4 ± 1.1 kg, pST-treated: 19.7 ± 1.2 kg), and ractopamine-treated dams tended to gain less weight than controls (P=0.070 ractopamine-treated: 9.9 ± 1.2 kg). In gilts, weight change across the treatment period did not differ between groups (P=0.362). Neither maternal treatment (P=0.792) nor parity (P=0.448) affected the change in P2 backfat depth during the treatment period (**Figure 1**, mean 0.23 ± 0.53 mm).

Figure 1 – Effects of maternal treatments and parity on maternal weight (A, B) and P2 backfat depth (C, D). Data are estimated means ± SEM, corrected for the average litter size of 9.87 fetuses. * indicates P<0.05, # indicates P<0.1



Litter size:

Ovulation rate was higher in sows than in gilts (sows: 20.5 ± 0.7, gilts: 13.3 ± 0.6, P<0.001), and did not differ between animals allocated to each treatment group after mating (P=0.299). Litter size (total number of fetuses) tended to be higher in sows than gilts (sows: 10.6 ± 0.8, gilts: 8.8 ± 0.6, P=0.086) and did not vary with treatment (P=0.761).

Litter average fetal and placental size:

Litter average fetal weight was increased by maternal pST treatment (11%, P=0.007) and by maternal ractopamine treatment (9%, P=0.018) compared to controls, and did not differ between gilts and sows or with litter size (**Table 2**). Total fetal weight for each litter increased with increasing litter size (P<0.001), was increased by maternal pST-treatment (P=0.014) and tended to be increased by maternal ractopamine treatment (P=0.071), and did not differ between parities (**Table 2**).

Several other measures of fetal size (crown-rump length by 3%, abdominal circumference by 4%, liver weight by 16%) were also increased by maternal pST treatment, but not by ractopamine feeding (**Table 2**). Litter average fetal head width was increased by maternal treatment with pST (4%, P=0.004) or ractopamine (3%, P=0.029) and not altered by litter size or parity (**Table 2**). Fetal liver weight as a percentage of body weight was not altered by maternal treatments (**Table 2**).

Placental weight and area (**Table 2**) were negatively related to litter size, not altered by maternal treatment, and the latter tended to be higher in sows than in gilts (12%, P=0.100). Fetal:placental weight ratios were positively related to litter size and not altered by maternal treatment or parity (**Table 2**).

Table 2 – Effects of maternal treatments and parity on litter average fetal and placental size[1]

Outcome	Gilts			Sows			Significance			
	Control	pST	Ractopamine	Control	pST	Ractopamine	Litter size	Treatment	Parity	T x P
Number of dams	8	7	8	8	8	8				
Number of fetuses	59	73	71	99	78	77				
Fetal weight (g)	37.1 ± 1.5	41.7 ± 1.5	40.6 ± 1.4	36.0 ± 1.5	39.6 ± 1.4	39.4 ± 1.4	NS	0.013[2]	0.288	NS
Fetal crown-rump length (mm)	117 ± 2	121 ± 2	119 ± 1	114 ± 2	118 ± 1	116 ± 1	NS	0.068[3]	0.019	NS
Fetal abdominal circumference (mm)	76.4 ± 1.1	79.9 ± 1.1	78.4 ± 1.0	77.5 ± 1.1	79.5 ± 1.0	78.8 ± 1.0	NS	0.037[4]	NS	NS
Fetal head width (mm)	15.9 ± 0.2	16.8 ± 0.2	16.6 ± 0.2	15.8 ± 0.2	16.3 ± 0.2	16.2 ± 0.2	NS	0.012[5]	NS	NS
Fetal liver weight (g)	2.74 ± 0.14	3.20 ± 0.15	2.94 ± 0.14	2.86 ± 0.14	3.28 ± 0.14	2.95 ± 0.14	0.050	0.010[6]	NS	NS
Fetal liver weight (% of fetal weight)	7.40 ± 0.26	7.65 ± 0.26	7.19 ± 0.25	7.88 ± 0.26	8.29 ± 0.25	7.45 ± 0.25	0.001	0.046[7]	0.034	NS
Placental weight (g)	97 ± 8	116 ± 8	108 ± 8	105 ± 8	115 ± 8	110 ± 8	0.013	NS	NS	NS
Placental area (cm^2)	880 ± 80	875 ± 74	853 ± 70	901 ± 73	994 ± 69	1014 ± 69	0.035	NS	0.100	NS
Fetal:placental weight	0.40 ± 0.03	0.40 ± 0.03	0.41 ± 0.03	0.38 ± 0.03	0.40± 0.03	0.43 ± 0.03	0.002	NS	NS	NS

[1] Fetal data are estimated mean ± SEM of within-litter average, corrected to an average litter size of 9.72. NS indicates P>0.1
[2] Average fetal weight was higher in pST-treated (P=0.007) and in ractopamine-treated than in control dams (P=0.018)
[3] Average fetal crown-rump length was higher in pST-treated than control dams (P=0.022), and did not differ between ractopamine-treated and control dams (P>0.3)
[4] Average fetal abdominal circumference was higher in pST-treated than control dams (P=0.011), and did not differ between ractopamine-treated and control dams (P>0.1)
[5] Average fetal head width was higher in pST-treated (P=0.004) and in ractopamine-treated than in control dams (P=0.029)
[6] Average fetal liver weight (g) was higher in pST-treated than control dams (P=0.003), and did not differ between ractopamine-treated and control dams (P>0.1)
[7] Average fetal liver weight (as a % of fetal body weight) did not differ between pST-treated and control dams (P=0.195), or between ractopamine-treated and control dams (P=203)

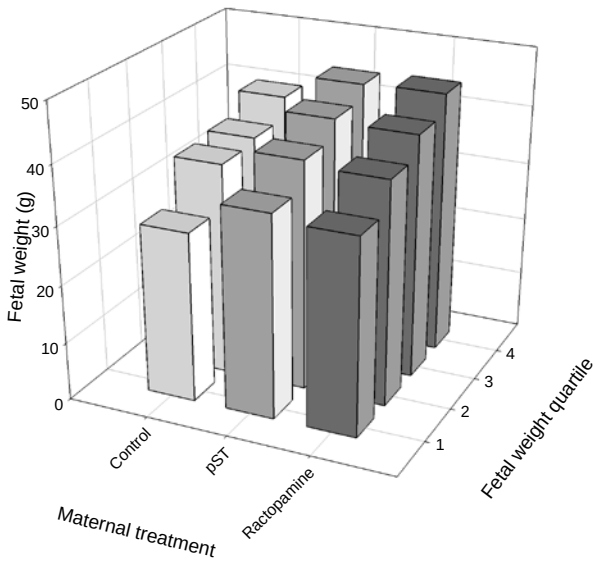
Individual fetal and placental weights:

Weights of individual fetuses did not vary with fetal litter size, tended to vary with maternal treatment overall (P=0.058) and male fetuses were heavier than females overall (P=0.001, males: 39.03 ± 0.62 g, females: 39.00 ± 0.62, estimated means ± SEM). Effects of treatment and fetal sex varied between fetuses of varying weight quartiles within their litters (treatment x quartile interaction: P=0.005, **Figure 2a**; fetal sex x quartile interaction: P<0.001, **Figure 2b**), effect of parity differed between male and female fetuses (P=0.042), and a 3-way interaction between treatment, parity and fetal sex was also evident (P=0.048). Effects of fetal sex, maternal treatment and maternal parity were therefore tested separately for fetuses within each fetal weight quartile, including litter size as a covariate.

Briefly, fetal weight was not affected by maternal treatment or parity in the lightest half of each litter (1st and 2nd quartiles), and was increased in pST-treated dams (P=0.008) and ractopamine-treated dams (P=0.020) compared to controls in the 3rd quartile, and tended to be increased in pST-treated dams (P=0.082) and in ractopamine-treated dams (P=0.070) compared to controls in the heaviest quarter of the litter (4th quartile).

Figure 2 – Effects of (a) maternal treatments and (b) fetal sex on fetal weight within each quartile of fetal weight. Data are estimated means ± SEM. Panel (a): Data for control dams are shown in the lightest bars, for pST-treated dams in mid-gray bars, and for ractopamine-treated dams in dark gray bars. Panel (b): Data for female fetuses are shown in the light gray bars and for male fetuses in dark gray bars.

(a)



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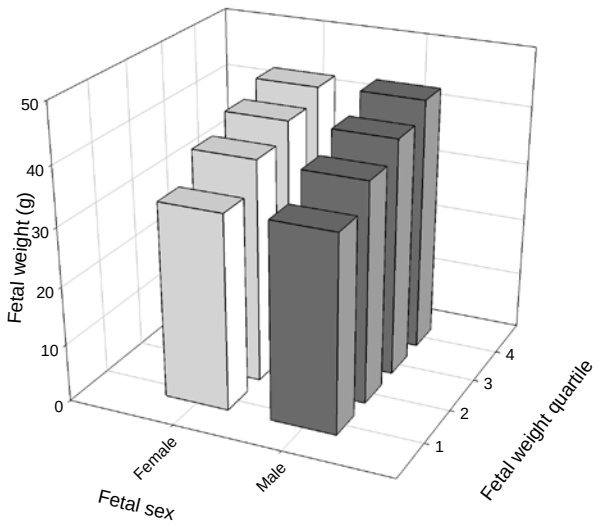
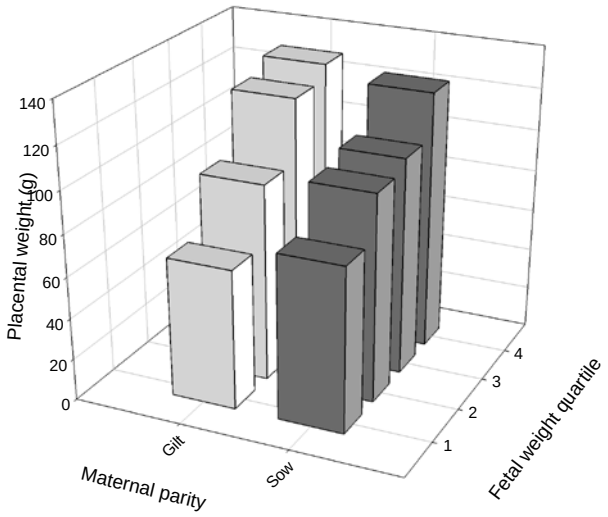


Placental weight was negatively related to litter size (P=0.011), and not altered by maternal treatments. Placental weight varied with fetal weight quartile (P<0.001), being heaviest in placentae from the heaviest fetuses and differing between each quartile of fetal weight (P<0.01 for each). Placentae from female fetuses were heavier than those from males overall (P<0.001, males: 99 ± 4 g, females: 108 ± 4 g). Effects of maternal parity on placental weight varied with fetal weight quartile (P<0.001, **Figure 3**), so that in the 3rd quartile of fetal weight, gilt fetuses had heavier placentae than sow fetuses.

Figure 3 – Effects of maternal parity on placental weight within each quartile of fetal weight. Data are estimated means ± SEM. Data for gilts are shown in the lightest bars and for sows in dark gray bars.



Maternal and fetal hormones and metabolites:

Maternal plasma ST and IGF-I were each increased in pST-treated dams (~9-fold, P<0.001 and ~2.7-fold, P<0.001, respectively, **Table 3**), and maternal plasma IGF-I was negatively affected by litter size (P=0.004). Maternal plasma insulin tended to be decreased in ractopamine-fed dams (-33%, P=0.053), but not altered by maternal pST-treatment (P=0.513, **Table 3**). Maternal plasma glucose was unaltered by litter size, treatment or parity (**Table 3**). Litter average fetal plasma glucose, however, decreased with increasing litter size (P=0.026), was unaffected by maternal parity (P>0.1), and tended to be decreased in ractopamine-fed dams compared to controls (-23%, P=0.051). Fetal:maternal plasma glucose ratio was negatively related to litter size (P=0.013), tended to be decreased in pST-treated dams (-24%, P=0.059) and was decreased in ractopamine-fed dams (-26%, P=0.034, **Table 3**). Maternal plasma urea was decreased by pST-treatment (-28%, P=0.001) and unaltered by ractopamine-treatment (P>0.7), parity or litter size (**Table 3**). Fetal plasma urea was similarly decreased in pST-treated dams compared to control dams (-21%, P=0.005), but was unaltered in fetuses from ractopamine-treated dams (P>0.3), and not altered by parity or litter size (**Table 3**). Fetal:maternal plasma urea ratios were not altered by litter size or maternal parity, were altered by treatment (P=0.042), but did not differ in pST- or ractopamine-treated dams compared to controls(P>0.1 for each, **Table 3**).

Table 3 – Effects of maternal treatments and parity on maternal and fetal metabolites[8]

Outcome	Gilts			Sows			Significance			
	Control	pST	Ractopamine	Control	pST	Ractopamine	Litter size	Treatment	Parity	T x P
Number of dams	8	7	8	8	6	8				
Maternal plasma GH ($ng.mL^{-1}$)	1.4 ± 3.7	25.5 ± 4.1	1.3 ± 3.5	3.3 ± 3.8	14.7 ± 3.8	1.4 ± 3.8	0.311	<0.001	0.183	0.680
Maternal plasma IGF-I ($ng.mL^{-1}$)	118 ± 15	261 ± 16	95 ± 14	104 ± 15	337 ± 15	94 ± 15	0.004	<0.001	NS	0.027[9]
Maternal plasma insulin ($mU.L^{-1}$)	41.1 ± 6.0	45.4 ± 8.1	24.3 ± 5.7	30.6 ± 6.1	34.8 ± 6.1	23.6 ± 6.6	NS	0.045[10]	NS	NS
Maternal plasma glucose ($mmol.L^{-1}$)	3.26 ± 0.20	3.37 ± 0.22	3.23 ± 0.19	3.10 ± 0.22	3.50 ± 0.20	3.06 ± 0.19	NS	NS	NS	NS
Fetal plasma glucose ($mmol.L^{-1}$)	1.48 ± 0.17	1.26 ± 0.19	1.19 ± 0.16	1.44 ± 0.18	1.26 ± 0.17	1.07 ± 0.16	0.026	NS	NS	NS
Fetal:maternal plasma glucose	0.47 ± 0.06	0.38 ± 0.06	0.37 ± 0.06	0.50 ± 0.07	0.36 ± 0.06	0.35 ± 0.06	0.013	0.067	NS	NS
Maternal plasma urea ($mmol.L^{-1}$)	2.61 ± 0.16	2.00 ± 0.18	2.71 ± 0.15	2.59 ± 0.16	1.73 ± 0.16	2.58 ± 0.15	NS	<0.001[11]	NS	NS
Fetal plasma urea ($mmol.L^{-1}$)	2.88 ± 0.18	2.26 ± 0.20	2.85 ± 0.17	2.97 ± 0.18	2.35 ± 0.18	2.69 ± 0.17	NS	0.005[12]	NS	NS
Fetal:maternal plasma urea	1.11 ± 0.08	1.15 ± 0.09	1.06 ± 0.08	1.16 ± 0.08	1.39 ± 0.08	1.06 ± 0.08	NS	0.042[13]	NS	NS

[8]Data from two dams not treated on the day of post-mortem were excluded from this analysis. Maternal data are estimated mean ± SEM, and fetal data are estimated mean ± SEM of within-litter averages, each corrected to the average litter size of 9.56 in these dams. NS indicates P>0.1

[9] In sows, maternal plasma IGF-I was higher in pST-treated dams than in controls (P<0.001) and not altered by ractopamine treatment (P>0.9). In gilts, maternal plasma IGF-I was increased in pST-treated dams (P<0.001) and decreased ractopamine-treated dams (P=0.046)

[10] Maternal plasma insulin did not differ between controls and pST-treated dams (P>0.5) and tended to be decreased in ractopamine-treated dams (P=0.053 for each)

[11] Maternal plasma urea was lower in pST-treated dams than in control dams (P<0.001) and not altered in ractopamine-treated dams (P<0.7)

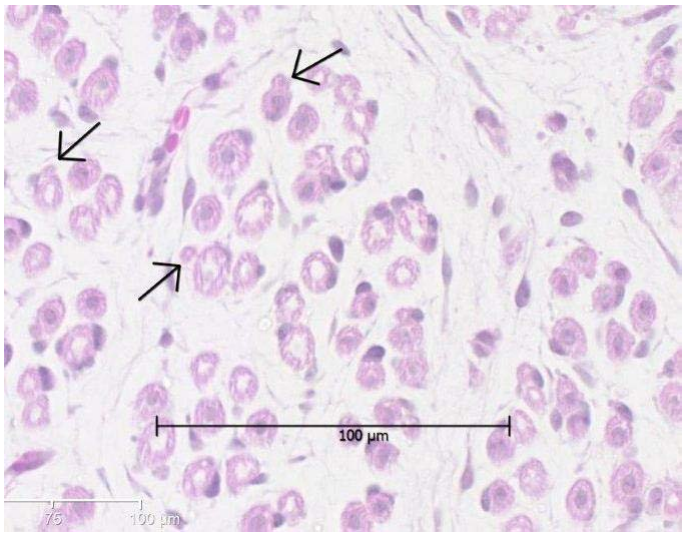
[12] Fetal plasma urea was lower in pST-treated dams than in control dams (P=0.001) and not altered in ractopamine-treated dams (P>0.3)

[13] Fetal:maternal plasma urea was not different in pST-treated dams (P=0.111) or ractopamine-treated dams (P>0.3) than in control dams

Fetal muscle development:

Infrequent secondary fibres (<5% of all fibres) were present in 91% of analysed sections (Figure 4), and the proportion of sections with secondary fibres was similar in fetuses of varying size (smallest non-runts 25/27; median-weight 20/23; largest 22/24). All fibres in a section were counted in order to obtain fibre density, whilst diameter was measured only for primary fibres, since effects on primary muscle fibre size may underlie consequences for later secondary fibre development (Wigmore & Stickland 1983).

Figure 4 – Cross-section of day 50 fetal pig muscle, stained with haemotoxylin-eosin and showing visible secondary fibres (arrows).

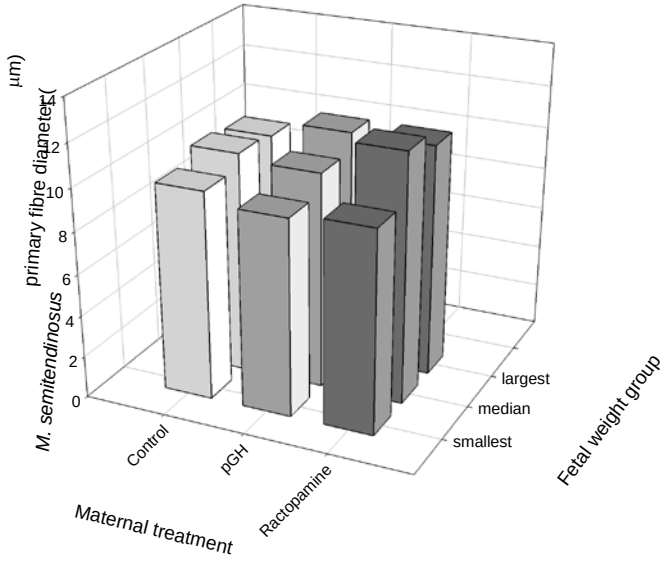


The cross-sectional area of the *M. semitendinosus* tended to differ between fetal size groups, and was not affected by maternal treatment (P=0.109), whilst the effect of maternal parity differed between fetal size groups (P=0.038). Within each fetal size group, maternal parity did not alter *M. semitendinosus* cross-sectional area (P>0.1 for each group), whilst maternal ractopamine tended to increase *M. semitendinosus* cross-sectional area in the heaviest (P=0.078) and lightest (P=0.056) fetuses, but not in median-weight fetuses (P>0.7). Overall, muscle fibre density varied significantly with fetal size group (P<0.001), and tended to differ between parities (gilts > sows, P=0.057), and with maternal treatments (P=0.090). This mainly reflected responses in the heaviest fetuses, where muscle fibre density was higher in fetuses from gilts than those from sows (P=0.015), and was decreased by pST treatment (P=0.018). The total number of muscle fibres in the *M. semitendinosus*, calculated as the product of cross-sectional area and fibre density, did not differ between fetal size groups (P=0.979) or maternal treatments (P=0.219). Effects of parity on total fibre number again differed between fetal size groups (P=0.001). In the heaviest fetuses, total fibre number was greater in gilt than sow fetuses (P=0.017), and ractopamine tended to increase total fibre number (P=0.053). In median and lightest fetuses, total fibre number was unaffected by maternal parity or treatment.

Fetal average *M. semitendinosus* primary fibre diameter (Figure 5) varied between fetal size groups (P<0.001), and was not altered by maternal parity (P=0.134), whilst the effects of maternal treatment differed between fetal size groups (P=0.025). In the largest littermates, fibre diameter tended to be increased by maternal pST (P=0.064), in median-weight littermates, fibre diameter was increased by maternal ractopamine (P=0.025), and in the

lightest littermates, fibre diameter was unaffected by maternal treatment (P=0.895). Across all fetuses, fetal weight correlated positively with *M. semitendinosus* cross-sectional area (r=0.327, P=0.004, n=74) and primary muscle fibre diameter (r=0.285, P=0.014, n=74), and negatively with fibre density (r=-0.483, P<0.001, n=74). Fibre density correlated negatively with primary muscle fibre diameter, across all fetuses (R=-0.624, P<0.001, n=74), as well as within each size group (P<0.011 for each).

Figure 5 – Effects of maternal treatments on fetal *m. semitendinosus* primary fibre diameter within each quartile of fetal weight. Data for control dams are shown in the lightest bars, for pST-treated dams in mid-gray bars, and for ractopamine-treated dams in dark gray bars.



Summary of findings, Study 1:

We have shown for the first time, that treating the mother with pST in early-mid pregnancy, increases fetal growth in mature multiparous sows as well as in gilts in their first pregnancy. Maternal treatment with ractopamine also increased fetal weight, and this response did not differ between dams of different parities, contrary to our initial hypothesis. This suggests that these maternal treatments increase nutrient availability to the fetus to a similar extent in both parities, and that the faster relative growth rates of gilts, and hence likely nutrient demand for maternal growth, do not limit the actions of these hormones, at least at this stage of pregnancy. The lack of difference in fetal weight between control gilts and sows at day 50 of gestation also suggests that primiparity and adolescent pregnancy may not restrict fetal growth until later in pregnancy in the pig, resulting in the lower birth weight of gilt compared to sow progeny (Ritter, et al. 1984). Fetal growth was increased by 14% and 11% respectively in pST-treated gilts and sows, compared to the control dams in each parity, with no significant parity x treatment interactions. This is consistent with previous reports of increased fetal growth in pST-treated gilts (Gatford et al. 2000; Kelley et al. 1995; Sterle et al. 1995). In contrast with previous reports of greater progeny muscle development and placental length responses to pST in small than in large littermates (Rehfeldt & Kuhn 2006; Rehfeldt et al. 2001a; Rehfeldt et al. 2001b; Sterle et al. 1995), we found greater fetal growth responses to pST in heavier littermates than in lighter littermates. This difference in relative responses may reflect a dose effect (2 mg/d in our gilts cf. 5-6 mg/d in previous studies), or greater maternal constraint of large fetuses in the present study due to more

restricted maternal nutrition, reflecting current commercial practice, such that large fetuses had a greater potential to respond to increased nutrient availability than small fetuses.

In the present study, maternal treatment with the □$_2$-adrenoreceptor agonist ractopamine in early-mid pregnancy increased fetal growth at the end of treatment but was less effective than maternal pST. Although maternal pST and ractopamine increased fetal weight, only pST increased fetal length, abdominal circumference or liver weight, suggesting that the two hormones may differentially affect growth of particular fetal tissues. Nevertheless, a 9% increase in average fetal weight in response to maternal ractopamine may have practical significance if maintained to term. This is the first report of fetal growth responses to maternal ß-agonists in the pig. Similar to effects of maternal pST, previous studies of maternal ß$_2$-adrenergic agonist administration in early-mid pregnancy in the pig have reported changes in progeny postnatal development without changes in birth weight (Hoshi et al. 2005a; Hoshi et al. 2005b; Kim et al. 1994). Taken together, the smaller fetal weight responses to maternal ß$_2$-adrenergic agonist in early-mid pregnancy compared to maternal pST in the present study in pigs, and lack of increase in fetal or neonatal weights in previous studies of sustained fetal exposure in rat (Downie, et al. 2008; Maltin, et al. 1990) and sheep (Shackelford, et al. 1995), imply that ß$_2$-adrenergic agonists do not induce large and sustained changes in fetal growth.

Although placental weight and size were not altered by maternal treatments in the present study, evidence from other studies and in other species does show persistent effects of altered maternal GH-IGF-I axis on placental growth and function, and implicate changes in placental function in GH-induced increases in fetal growth and development. We are now seeking to recruit an Honours student to explore differences in placental structure and function in placentae collected from this study.

Effects of maternal treatments and parity on fetal muscle development in fetal pigs differed between small, median and large littermates in the present study. Maternal treatments did not affect total fibre number at day 50 gestation (predominantly primary fibres in the present study), with the exception of a trend to increase in large fetuses from ractopamine-treated dams, which suggests that changes in secondary fibre numbers account for the majority of increases in progeny muscle fibre numbers seen at birth in response to maternal treatments including increased nutrition and pST treatment (Dwyer et al. 1994; Rehfeldt, et al. 1993). Interestingly, secondary fibres were apparent, although comprising <5% of all fibres, in the majority of sections in the present study and the proportion of sections in which secondary fibres could be distinguished was consistent between fetal size groups. Previous studies had suggested that secondary fibre hyperplasia did not begin until day 50 of gestation in the pig (Wigmore & Stickland 1983), and that maternal pST or nutritional treatment between days 25 and 50 of pregnancy acted on primary fetal muscle fibres, which begin to develop in fetal pigs by day 35 of gestation (Ashmore, et al. 1973). Increases in secondary fibre numbers at birth were proposed to occur via maternal treatments increasing the size and hence surface area of primary fibres to form the scaffold for later secondary fibre development (Wigmore & Stickland 1983), and/or by increasing proliferation of myoblasts that subsequently fuse to form secondary muscle fibres (Rehfeldt et al. 2001b). The presence of secondary fibres in fetal muscle sections at day 50 of gestation suggest that maternal treatments between days 25 and 50 of pregnancy may also act directly on secondary fibres during their early development. In the present study, maternal pST increased *M. semitendinosus* fibre diameter in the largest littermates, suggesting that this second mechanism may contribute to the increased muscle fibre numbers in progeny of pST-treated dams.

Our results suggest that maternal treatments with hormones that repartition nutrients in the dam and increase fetal growth in gilts will also increase fetal growth in sows, despite the lower growth rates and hence nutrient demands for maternal growth in the former. Fetal growth increases were larger in response to maternal pST than ractopamine, suggesting that it is likely to have greater effects on fetal development and lead to greater improvements in postnatal performance than ractopamine. Indeed, when data were restricted to litters of more than 5 fetuses, reflecting normal litter sizes in commercial herds, the effect of ractopamine become non-significant, but maternal pST still increased fetal weight and size (we have previously reported findings for these larger litters in progress reports). We therefore chose maternal pST-treatment as the intervention to test in the large commercial study of progeny responses in sow and gilt progeny in Study 2.

We have previously shown that maternal pST treatment from d 25 to 50 of gestation did not increase birth weight of gilt progeny, but continuing treatment to day 100 did increase progeny birth weight. The shorter treatment protocol did, however, affect progeny development, including altered hormone secretion and increased *M. semitendinosus* size in female progeny of median birth weight for their litters {Ekert, 2000 #562; Gatford, 2003 #734}. In Study 2, we also therefore compared progeny responses to pST injections from d 25 to 50 or from d 25 to 100 of gestation and untreated controls, between parities, between progeny sexes and between light, median and heavy littermates.

Presentation of results – Study 1:

Parts of the findings of Study 1 were reported in preliminary form at the Australasian Pig Science Association meeting in Brisbane November 2007, and at the Perinatal Society of Australia and New Zealand meeting at the Gold Coast in April 2008. The complete findings of Study 1 were initially submitted to the *Journal of Animal Science* in January 2009, and subsequently to the *Journal of Endocrinology* in March 2009. We have just completed analyses of circulating growth hormone and insulin-like growth factor-I in maternal blood and reanalyzed the data to include responses for all litters, in response to referee requests, and resubmitted the manuscript, and expect that this manuscript will be accepted within the next month.

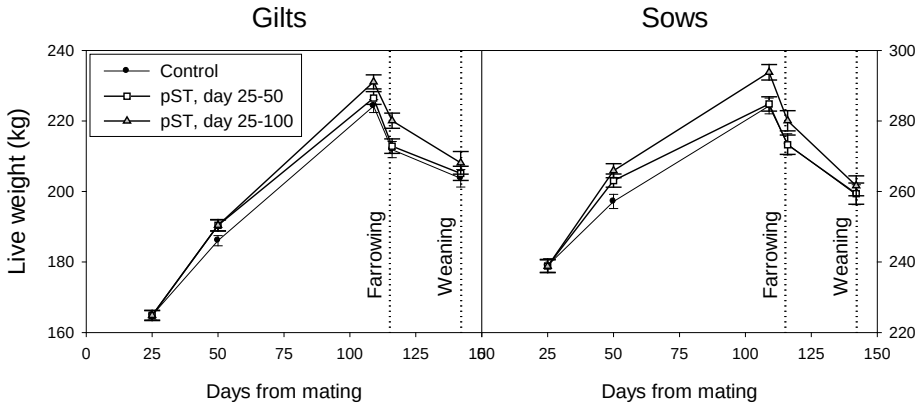
Outcomes for Study 2 – commercial evaluation of progeny performance following maternal treatment

Maternal weight and backfat depth:

Maternal weight prior to injections was higher in sows than gilts ($P<0.001$, Gilts: 164.9 ± 0.8 kg; Sows: 238.9 ± 1.1 kg), and did not differ between treatments ($P=0.997$). Maternal pST treatment increased maternal weight at the end of treatment, at day 50 in all pST-treated dams ($P<0.003$, **Figure 6**) and at day 109 in dams treated with pST from day 25 to 100 ($P<0.001$), regardless of parity. In those dams treated with pST until day 50 of pregnancy, maternal weight returned to similar levels as controls after the end of treatment and these animals were of similar weight as controls at day 109 of pregnancy ($P>0.4$, **Figure 6**). Maternal weight on the day after farrowing was increased in dams treated with pST from day 25 to 100 of pregnancy ($P=0.004$), but not in dams treated with pST from day 25 to 50 of pregnancy ($P>0.8$), compared to controls. Maternal weight loss during lactation varied between treatments, so that weight at weaning did not differ between treatment groups ($P=0.429$, **Figure 6**). Daily maternal weight loss during lactation tended to be faster in dams treated with pST from day 25 to 50 of pregnancy ($P=0.068$, 477 ± 44 $g.day^{-1}$), and was increased in dams treated with pST from day 25 to 100 of pregnancy ($P=0.011$, 643 ± 54 $g.day^{-}$

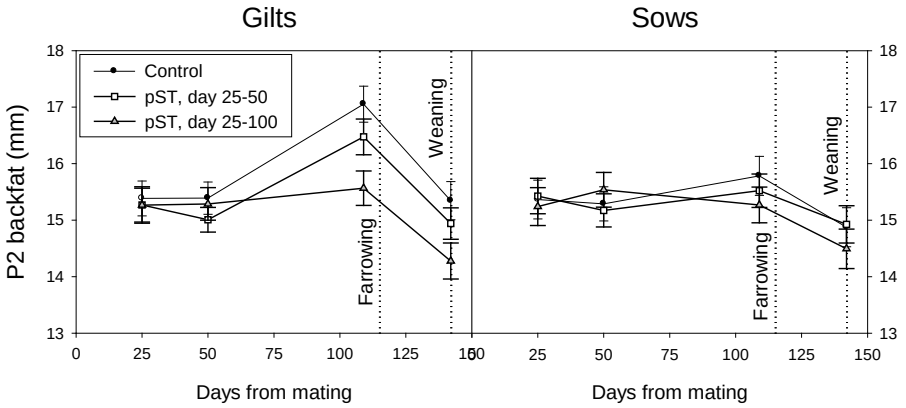
^{1}), compared to controls (465 ± 59 g.day^{-1}). Sows also lost weight more rapidly than gilts during lactation (P<0.001, sows: 722 ± 46 g.day^{-1}; gilts: 333 ± 34 g.day^{-1}).

Figure 6. Effects of maternal treatment and parity on maternal live weight.



Maternal P2 backfat depth (**Figure 7**) prior to injections did not differ between parities (P=0.885) nor treatment groups (P=0.924). Maternal P2 backfat depth at day 50 of pregnancy was also unaffected by parity or treatment (P>0.4 for each). Maternal P2 backfat depth prior to farrowing was higher in gilts than sows (P=0.001), and reduced in dams treated with pST from day 25 to 100 of pregnancy (P=0.002), but not in those treated from day 25 to 50 only (P=0.183). Rate of maternal P2 backfat loss during lactation did not differ between treatments (P=0.571) or parities (P=0.357). Consequently, maternal P2 backfat depth at weaning (**Figure 7**) was lower in dams treated with pST from day 25-100 than controls (P=0.028), but not reduced in dams whose treatment finished at day 50 (P=0.585).

Figure 7. Effects of maternal treatment and parity on maternal P2 back fat depth.



<u>Litter outcomes for treatment pregnancy:</u>

Gestation length was longer in gilts than sows, and in sows but not gilts, gestation length was reduced following pST treatment from day 25 to 100 of pregnancy (**Table 4**). Numbers of total and live born piglets (each P<0.001), mummified piglets (P=0.062) and still born piglets (P=0.010) were higher in sows than in gilts (**Table 4**). The numbers of live born piglets in each litter were reduced by ~0.6 piglets/litter following pST treatment from day 25 to 50 of pregnancy (P=0.018) or from day 25 to 100 of pregnancy (P=0.024), compared to control litters (**Table 4**). This was due to increased numbers of mummified piglets (P=0.014) and a

tendency for increased numbers of stillborn piglets (P=0.079) in litters from dams treated with pST from day 25 to 50 of pregnancy and lower total numbers of piglets in litters from dams treated with pST from day 25 to 100 of pregnancy (P=0.038), relative to controls.

When corrected for litter size, average birth weight was negatively related to litter size, was higher in sows than gilts (P<0.001), and effects of maternal treatment differed between parities (P=0.010, **Table 4**). In gilts, average birth weight corrected for an average gilt litter size of 12.02 piglets, was altered by maternal treatment (P=0.039), such that average birth weight was increased in litters from gilts treated with pST from day 25 to 100 of pregnancy (P=0.014, 1.49 ± 0.02 kg) and tended to be increased in litters from gilts treated with pST from day 25 to 50 of pregnancy (P=0.071, 1.47 ± 0.02 kg), relative to control litters (1.42 ± 0.02 kg). In sows, average birth weight corrected for an average sow litter size of 13.03 piglets, was altered by maternal treatment (P<0.001), such that average birth weight was increased in litters from sows treated with pST from day 25 to 100 of pregnancy (P<0.001, 1.72 ± 0.02 kg), but not increased in litters from sows treated with pST from day 25 to 50 of pregnancy (P>0.5, 1.59 ± 0.02 kg), relative to control litters (1.57 ± 0.02 kg).

Birth weight was increased to a greater extent by maternal pST from day 25 to 100 in sow litters (9.5% when corrected for litter size) compared to gilt litters (5.0% when corrected for litter size).

Table 4 – Effects of maternal treatment and parity on treatment pregnancy outcomes

Outcome	Gilts			Sows			Significance		
	Control	pST day 25 to 50	pST day 25 to 100	Control	pST day 25 to 50	pST day 25 to 100	Treatment	Parity	Treatment*parity
Gestation length (days)	116.4 ± 0.1	116.4 ± 0.1	116.1 ± 0.1	116.3 ± 0.1	116.4 ± 0.1	115.5 ± 0.1	<0.001	0.016	0.033[14]
Litter size at birth									
No of litters	114	116	107	114	109	113			
Live born piglets	11.5 ± 0.2	11.3 ± 0.3	11.0 ± 0.3	12.7 ± 0.3	11.6 ± 0.3	12.0 ± 0.3	0.028	<0.001	0.247
Still born piglets	0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	0.9 ± 0.1	0.136	0.010	0.826
Total piglets	12.1 ± 0.2	12.1 ± 0.2	11.7 ± 0.3	13.5 ± 0.3	12.7 ± 0.3	12.8 ± 0.3	0.107	<0.001	0.433
Mummies	0.11 ± 0.03	0.32 ± 0.09	0.28 ± 0.06	0.29 ± 0.05	0.41 ± 0.08	0.32 ± 0.07	0.048	0.062	0.589
Progeny size at birth									
Average birth weight (kg)	1.42 ± 0.02	1.47 ± 0.02	1.50 ± 0.03	1.55 ± 0.02	1.60 ± 0.02	1.73 ± 0.02	<0.001	<0.001	0.051[15]
Average birth weight (kg) [16]	1.40 ± 0.02	1.45 ± 0.02	1.47 ± 0.02	1.59 ± 0.02	1.61 ± 0.02	1.75 ± 0.02	<0.001	<0.001	0.010[17]

[14] Gestation length was unaffected by maternal treatment in gilts (P=0.260), and varied between treatments in sows (P<0.001). In sows, gestation length was reduced following pST treatment from day 25 to 100 (P<0.001), but not following pST treatment from day 25 to 50 (P=1.0), compared to controls

[15] Average birth weight was altered by maternal treatment in gilts (P=0.029) and in sows (P<0.001). Maternal pST treatment from day 25 to 100 of pregnancy increased average birth weight relative to control litters, in gilts (P=0.008) and in sows (P<0.001), and maternal pST treatment from day 25 to 50 of pregnancy did not increase average birth weight in either parity (P>0.1 for each)

[16] Corrected to an average total litter size of 12.53 piglets overall and within each litter (gilts: 12.02 piglets born, sows: 13.03 piglets born) for analyses of the treatment *parity interaction

[17] Average birth weight, corrected for litter size, was altered by maternal treatment in gilts (P=0.039) and in sows (P<0.001). Maternal pST treatment from day 25 to 100 of pregnancy increased corrected average birth weight relative to control litters, in gilts (P=0.014) and in sows (P<0.001), and maternal pST treatment from day 25 to 50 of pregnancy tended to increase corrected average birth weight relative to control litters in gilts (p=0.071), but not in sows (P=0.501)

Lactation feed intake, litter survival and growth:

Litter size after fostering was higher in sows than gilts and did not differ between treatments (**Table 5**). Average litter size in gilts was greater than the target of 10 piglets as insufficient dams were available to reduce litter size to 10 in all gilt litters at fostering. Litter sizes remained higher in sows than gilts throughout lactation, and the numbers of piglets that were removed or died during lactation tended to be higher gilt litters than in sow litters (**Table 5**). Maternal treatments did not affect litter size after fostering or piglet losses during lactation, and piglet losses during lactation were negatively correlated with average piglet weight after fostering, overall ($R=-0.277$, $P<0.001$, $n=506$), and within each parity ($P<0.001$ for each). Litter size at weaning was not different between maternal treatment groups (**Table 5**). Nevertheless, increased average birth weight of the litter at birth was associated with decreased pre-weaning losses in the litter (**Figure 8)**, and with increased average weight at weaning (**Figure 9**). A 1 kg increase in litter average birth weight decreased piglet losses by 2.6 pigs/litter and increased litter average weaning weight by 1.42 kg.

Figure 8. Increased birth weight decreases pre-weaning losses in sow and gilt litters.

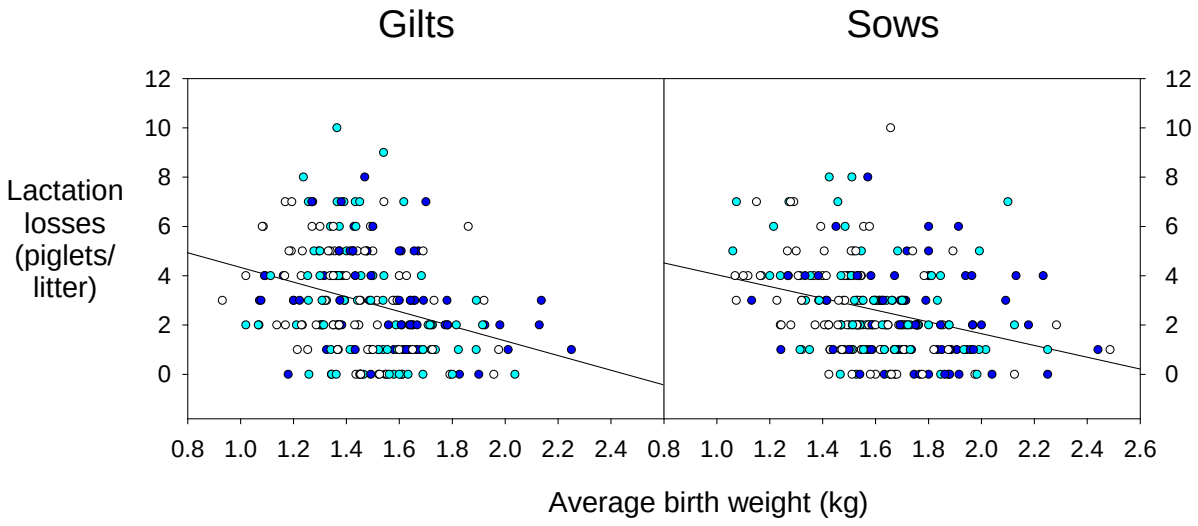
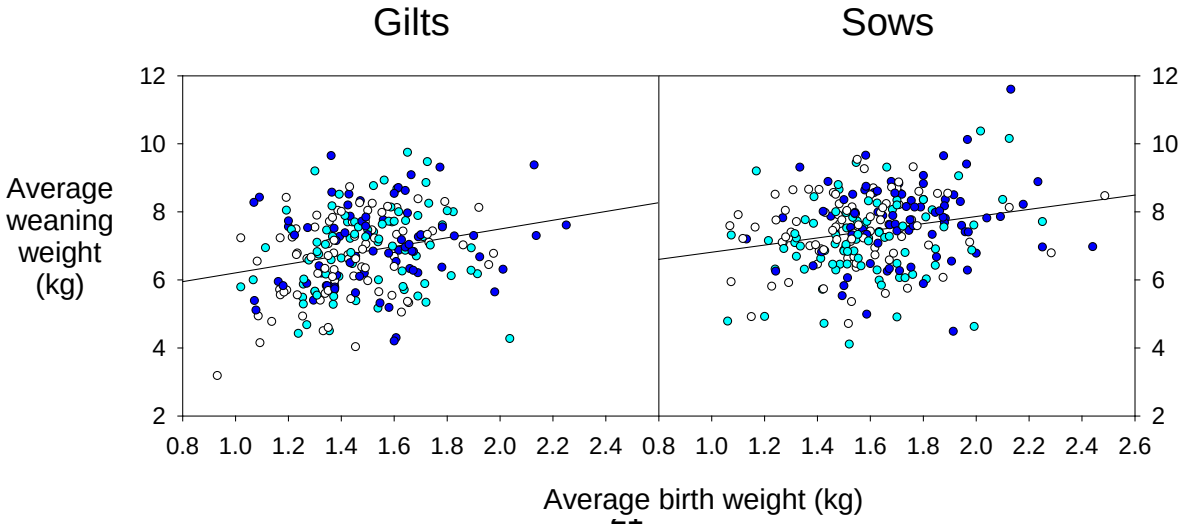


Figure 9. Increased birth weight increases weaning weights in sow and gilt litters.



Sows ate more feed each day than gilts throughout lactation (P<0.001), and effects of maternal treatments on feed intake differed between parities (**Table 5**). Daily feed intakes during lactation did not differ between treatments in sows (P=0.240). In gilts, lactation feed intake was increased in dams treated with pST from d 25 to 100 of pregnancy (+450 g/d, P=0.001), and tended to be increased in dams treated with pST from d 25 to 50 of pregnancy (P=0.073), relative to control gilts (**Table 5**).

Average piglet weights after fostering (**Figure 10**) were increased in litters from dams treated with pST from d 25 to 50 of pregnancy (P=0.011), and in litters from dams treated with pST from d 25 to 100 of pregnancy (P<0.001). Average piglet weights at d 14 of lactation (**Figure 3**) tended to be increased following maternal pST treatment from d 25 to 100 of pregnancy (P=0.053), and were not increased following maternal pST treatment from d 25 to 50 of pregnancy (P>0.4). Average piglet weights at weaning (**Figure 3**) were increased following maternal pST treatment from d 25 to 100 of pregnancy (P=0.018), and were not increased following maternal pST treatment from d 25 to 50 of pregnancy (P>0.8).

Figure 10. Effects of maternal treatment and parity on litter average piglet weights, after fostering, at day 14 of lactation, and at weaning.

Maternal removals during treatment pregnancy and lactation:

The numbers of dams removed in early pregnancy (d 25 to 50 of pregnancy), from mid-pregnancy to entry to the farrowing house (d 50 to 109), or from late pregnancy through lactation to weaning following the treatment pregnancy (d 109 of pregnancy to weaning), were not different between parities, nor between maternal treatment groups (P>0.16 for all).

Table 5 – Effects of maternal treatment and parity on lactation outcomes

Outcome	Gilts			Sows			Significance		
	Control	pST day 25 to 50	pST day 25 to 100	Control	pST day 25 to 50	pST day 25 to 100	Treatment	Parity	Treatment*parity
Litter size									
After fostering	10.4 ± 0.1	10.4 ± 0.1	10.4 ± 0.1	11.9 ± 0.2	12.0 ± 0.2	12.0 ± 0.1	0.735	<0.001	0.782
Day 14 of lactation	8.2 ± 0.2	8.3 ± 0.2	8.3 ± 0.4	10.0 ± 0.2	9.9 ± 0.2	10.2 ± 0.2	0.599	<0.001	0.757
Weaning	7.4 ± 0.2	7.7 ± 0.2	7.5 ± 0.2	9.3 ± 0.2	9.3 ± 0.2	9.8 ± 0.2	0.424	<0.001	0.256
Lactation piglet losses	3.0 ± 0.2	2.8 ± 0.2	2.9 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	2.3 ± 0.2	0.512	0.089	0.407
Maternal feed intake									
Fostering – weaning ($kg.day^{-1}$)	6.34 ± 0.08	6.57 ± 0.11	6.79 ± 0.08	7.51 ± 0.11	7.33 ± 0.12	7.57 ± 0.09	0.022	<0.001	0.065[18]
Litter lactation weight gain									
Fostering - weaning (kg)	34.5 ± 1.8	37.9 ± 2.0	36.1 ± 1.7	51.2 ± 1.6	47.5 ± 1.8	54.4 ± 1.7	0.276	<0.001	0.036[19]

[18] Daily feed intakes during lactation were affected by treatment in gilts (P=0.003), but not in sows (P=0.240). In gilts, lactation feed intake was increased in dams treated with pST from day 25 to 100 of pregnancy (P=0.001), and tended to be increased in dams treated with pST from day 25 to 50 of pregnancy (P=0.073), relative to control gilts

[19] Litter weight gain throughout lactation was not affected by maternal treatments in gilts (P=0.427), was affected by maternal treatment in sows (P=0.019), but did not differ from control litters in sow treated with pST from day 25 to 100 of pregnancy (P=0.129) nor in sows treated with pST from day 25 to 100 of pregnancy (P=0.176)

Subsequent maternal reproductive performance:

A total of 500 dams remained on-trial at weaning from the lactation immediately following the treatment pregnancy (242 gilts and 258 sows). Removals of dams prior to mating were higher in sows than gilts (□2 P<0.001), and in gilts were not affected by maternal treatment (3% of each group in gilts was removed prior to re-mating). In sows, removals prior to mating were highest in sows treated with pST from days 25-100 of the preceding pregnancy (□2 P=0.037; 9% of controls, 8% of sows treated with pST from d 25 to 50, 21% of sows treated with pST from d25 to 100). The majority of the 18 sows (21% of weaned sows) culled prior to mating in this group were removed either due to foot and leg problems (9 of 18) or due to poor body condition at weaning (6 of 18). Sows that were removed from the herd prior to re-mating (33 of 258 weaned) were thinner (P=0.001) at day 25 of pregnancy before commencing treatment, were thinner (P=0.003) and tended to be lighter (P=0.068) on the day after farrowing, lost more weight (P=0.003) and backfat depth (P=0.023) during lactation, weaned heavier litters (P=0.024) with a greater litter weight gain during lactation (P=0.008), and ate less during lactation (P=0.005) than sows that remained in the herd and were remated following the treatment pregnancy and lactation.

A total of 456 dams were mated at the first post-weaning oestrus. Weaning-remating interval did not differ between sows and gilts from the treatment pregnancy (P=0.135), nor between maternal treatment groups in the treatment pregnancy (P=0.535). The proportion of dams that farrowed following the mating after the treatment pregnancy and lactation, tended to be higher in gilts than sows (gilts: 80.68%, sows: 73.6%), largely due to parity differences in conception rates. Subsequent litter size (numbers of live born piglets) was similar in dams who were gilts or sows during the treatment pregnancy, with greater numbers of stillborn piglets (P=0.024) in the higher parity sows, and no effects of treatment in the previous pregnancy on litter sizes of live born, stillborn or mummified piglets (P>0.16 for each).

Post-weaning progeny performance:

Weaner shed: Approximately 600 progeny per maternal group were followed through the weaner shed. Weaner shed losses were higher (P=0.002) in gilt progeny (112 of 1180) than sow progeny (73 of 1192), but not altered by maternal treatments (P=0.856). Progeny weight at the end of the weaner (**Table 6**) was increased after maternal pST treatment from day 25 to 50 of pregnancy in gilts only (P=0.017) and increased after maternal pST treatment from day 25 to 100 of pregnancy in either parity (P<0.001). Sow progeny were heavier than gilt progeny at the end of the weaner shed (P<0.001, **Table 6**). At the end of the weaner phase, male progeny were heavier than female progeny (P=0.014, males: 24.1 ± 0.2 kg, females: 23.5 ± 0.2 kg), and live weight varied with birth weight tertile (P<0.001). Live weight at the end of the weaner phase increased from light to medium birth weight, and from medium to heavy birth weight progeny (P<0.001 for each comparison; light birth weight progeny: 60.9 ± 0.6 kg, medium birth weight progeny: 63.9 ± 0.5 kg, heavy birth weight progeny: 67.7 ± 0.5 kg).

Grower shed: Approximately 150 progeny per maternal group were studied as growers and finishers. Maternal pST from day 25 to 100 increased progeny weight at the end of the grower phase by 3.5 kg across all progeny (**Table 6**). Sow progeny were heavier than gilt progeny at the end of the grower shed (P<0.001, **Table 6**). At the end of the grower phase, male progeny were heavier than female progeny (P=0.003, males: 64.9 ± 0.5 kg, females: 63.4 ± 0.5 kg), and live weight varied with birth weight tertile (P<0.001). Live weight at the end of the grower phase increased from light to medium birth weight, and from medium to heavy birth weight progeny (P<0.001 for each comparison; light birth weight progeny: 21.8 ± 0.3 kg, medium birth weight progeny: 23.7 ± 0.3 kg, heavy birth weight progeny: 25.9 ± 0.2 kg).

Finisher shed: Progeny were followed through from the grower to finisher phase. Maternal pST from day 25 to 100 increased progeny weight at the end of the finisher phase by 3.1 kg across all progeny (**Table 6**). Sow progeny were heavier than gilt progeny at the end of the finisher shed (P<0.001). Final live weight did not differ between male and female progeny (P=0.247), and was altered by birth weight tertile (P<0.001). Final live weight increased from light to medium birth weight, and from medium to heavy birth weight progeny (P<0.001 for each comparison; light birth weight progeny: 82.6 ± 0.7 kg, medium birth weight progeny: 86.1 ± 0.6 kg, heavy birth weight progeny: 90.5 ± 0.6 kg). Maternal treatments did not alter progeny growth rates, feed consumption or feed:gain during a 4-week measurement period in the finisher shed using electronic feeders in group pens (**Table 6**). Male progeny grew faster as finishers than females (P=0.008, males: 882 ± 12 $g.day^{-1}$, females: 851 ± 12 $g.day^{-1}$). Litter mates in the top 1/3 of birth weights for their litter (heavy: 900 ± 12 $g.day^{-1}$) grew faster as finishers than medium (P=0.010, 862 ± 13 $g.day^{-1}$) or light birth weight progeny (P<0.001, 838 ± 15 $g.day^{-1}$). Average daily feed intake was lower in male than female progeny (P<0.001, males: 1.91± 0.02 $kg.day^{-1}$, females: 2.05 ± 0.02 $kg.day^{-1}$), and also differed between progey of different birth weight tertiles (P=0.007). Average daily feed intake was higher in heavy birth weight progeny (2.03 ± 0.2 $kg.day^{-1}$), than in light birth weight progeny (P=0.006, 1.93 ± 0.3 $kg.day^{-1}$), but not different from either group in medium birth weight progeny (1.98 ± 0.2 $kg.day^{-1}$). Feed:gain was lower in male than female progeny (P<0.001, males: 2.17 ± 0.05, females: 2.43 ± 0.05). Feed:gain tended to be lower in light birth weight (2.22 ± 0.06) than in medium birth weight progeny (P=0.066, 2.36 ± 0.05) and did not differ from either group in heavy birth weight progeny (2.32 ± 0.05).

Carcass characteristics: Carcass weight was increased by 3.3 kg in progeny of dams treated with pST from day 25 to 100 of pregnancy, and not altered by pST treatment from day 25 to 50 of pregnancy, compared to progeny of control dams (**Table 6**). Carcasses from sow progeny were heavier than those from gilts (P<0.001, **Table 6**). Female progeny carcasses were heavier than males (P=0.003, males: 64.5 ± 0.5 kg, females: 65.9 ± 0.5 kg), and carcass weight also varied with birth weight tertile (P<0.001). Carcass weights increased from light to medium, and from medium to heavy littermates (P<0.001 for each comparison; light birth weight progeny: 62.3 ± 0.6 kg, medium birth weight progeny: 64.8 ± 0.5 kg, heavy birth weight progeny: 68.6 ± 0.5 kg hot carcass weight). Maternal treatments did not alter carcass P2, in absolute terms or corrected for carcass weight, nor dressing percentage (**Table 6**). Carcass P2, corrected for carcass weight, was lower in male than in female progeny (P<0.001, males: 7.27 ± 0.09 mm, females: 8.19 ± 0.09 mm, evaluated for a hot carcass weight of 66.0 kg). Corrected carcass P2 also varied with birth weight tertile (P=0.010), being lower in heavy birth weight progeny (7.51 ± 0.10 mm) than in either medium birth weight (P=0.038, 7.81 ± 0.11 mm) or low birth weight progeny (P=0.026, 7.87 ± 0.12 mm). Dressing percentage was higher in female than male progeny (P<0.001, males: 74.6 ± 0.2 %, females: 76.4 ± 0.2 %), and did not differ between progeny of light, medium or heavy birth weight for their litter (P=0.255).

Table 6 – Effects of maternal treatment and parity on post-weaning progeny growth and carcass measures. Data are estimated means ± SEM corrected for effects of progeny gender and relative size at birth.

Outcome	Gilts			Sows			Significance		
	Control	pST day 25 to 50	pST day 25 to 100	Control	pST day 25 to 50	pST day 25 to 100	Treatment	Parity	Treatment*parity
Progeny weights (for progeny followed through to finisher)									
End of weaner shed	21.7 ± 0.5	23.2 ± 0.5	23.0 ± 0.6	24.4 ± 0.5	24.0 ± 0.5	26.4 ± 0.5	0.036	<0.001	0.195
End of grower shed	60.7 ± 1.0	62.6 ± 1.0	63.6 ± 1.1	64.1 ± 0.9	64.9 ± 1.0	69.3 ± 1.0	<0.001	<0.001	0.207
End of finisher shed	83.4 ± 1.2	84.7 ± 1.1	85.6 ± 1.3	86.4 ± 1.1	87.1 ± 1.1	91.1 ± 1.2	0.011	<0.001	0.377
Finisher performance									
Growth rate ($g.day^{-1}$)	864 ± 27	888 ± 26	841 ± 28	875 ± 25	877 ± 25	854 ± 26	0.429	0.837	0.882
Feed consumption ($kg.day^{-1}$)	1.97 ± 0.04	1.97 ± 0.04	1.97 ± 0.04	1.97 ± 0.04	1.99 ± 0.04	1.99 ± 0.04	0.922	0.562	0.970
Feed:gain	2.23 ± 0.11	2.29 ± 0.10	2.25 ± 0.12	2.35 ± 0.10	2.34 ± 0.10	2.35 ± 0.11	0.970	0.328	0.942
Carcass characteristics									
Hot carcass weight (kg)	62.7 ± 1.1	63.6 ± 1.0	64.9 ± 1.1	65.1 ± 0.8	65.5 ± 1.0	69.5 ± 1.1	0.006	0.001	0.439
Carcass P2 fat depth (mm)	7.51 ± 0.25	7.61 ± 0.24	7.56 ± 0.27	7.38 ± 0.23	7.90 ± 0.23	7.94 ± 0.24	0.342	0.364	0.538
Carcass P2 fat depth (mm)[20]	7.87 ± 0.19	7.85 ± 0.18	7.69 ± 0.21	7.48 ± 0.18	7.93 ± 0.18	7.58 ± 0.18	0.321	0.358	0.419
Dressing %	75.4 ± 0.3	75.4 ± 0.3	75.3 ± 0.3	75.6 ± 0.3	75.4 ± 0.3	76.0 ± 0.3	0.674	0.173	0.523

[20] Corrected to an average hot carcass weight of 66.02 kg

Presentation of results – Study 2:

Abstracts describing maternal and progeny responses to maternal pST during pregnancy have been submitted for presentation at the Australasian Pig Science Association meeting in Cairns in November 2009. A manuscript describing maternal responses to maternal pST treatment from day 25-50 or day 25-100 of gestation and effects of maternal parity is currently in preparation for submission to *Journal of Animal Science* in July 2009. A second manuscript describing progeny responses to these factors is in preparation for submission to *Journal of Animal Science* in September 2009.

4. Application of Research

Maternal pST injections from day 25 to 100 of pregnancy are financially viable under commercial conditions, particularly in sows. Limited labour availability and ethical/consumer acceptance concerns might, however, make development of a non-injection approach more attractive, and we have made several suggestions for potential future research under section 7, Recommendations. To apply the technology in the present form, APVMA license approval would also need to be widened from the current approval for growing pigs to also include pregnant animals. We therefore suggest that the Pork CRC pursue this option with OzBiopharm.

Cost-benefit analyses of sustained maternal pST in pregnancy (per litter basis):

GILTS

Costs (total $47.90/litter):
pST cost = 2.5 mg/d in gilts = 187.5 mg/75 d, $11.90/gilt litter
Labour = $30/h, 2h/500 pigs/day, 75 days = 0.3 h/pig= $9/gilt litter
0.6 pigs/litter fewer born alive ($45/piglet) = $27.00/gilt litter
No change in gilt cull rates after weaning
Benefits (total $57.75/litter):
2.2 kg increase carcass weight per gilt progeny (7.5 pigs weaned/litter in gilts) = average $3.50/kg x 2.2 kg x 7.5 progeny = $57.75/litter
Net benefit in gilts: $9.85/litter

SOWS

Costs (total $100.60/litter):
pST cost = 4 mg/d in sows = 300 mg/75 d, $19/sow litter
Labour = $30/h, 2h/500 pigs/day, 75 days = 0.3 h/pig= $9/sow litter
0.6 pigs/litter fewer born alive ($45/piglet) = -$27.00/sow litter
*12% more sows culled after lactation $380/replacement sow) *12% cull rate = - $45.60/sow litter*
Benefits (total $150.92/litter):
4.4 kg increase carcass weight per gilt progeny (9.8 pigs weaned/litter in sows) = average $3.50/kg x 4.4 kg x 9.8 progeny = $150.92/litter
Net benefit in sows: $50.32/litter

5. Conclusion

The present study clearly shows that sustained maternal pST increases progeny weight at birth, and throughout postnatal life, resulting in a significant increase in carcass weight. Progeny feed:gain and growth rates as finishers were not affected, suggesting that most of the benefit derives from faster growth rates in earlier postnatal life. Further studies are warranted to improve the treatment protocol and develop non-injection approaches to increase maternal pST during pregnancy, and these are detailed under section 7, Recommendations. A pilot trial to investigate whether ractopamine also increases progeny size at birth may also be warranted.

6. Limitations/Risks

Benefits in sows are partly offset by increased cull rates after weaning, due mostly to foot and leg problems and poor condition. This might be able to be reduced by:

- Treating dams with pST from their first pregnancy onwards, so that pST is able to increase skeletal strength (foot and leg problems were not evident in pST-treaetd gilts, although they were also heavier than controls)
- Strategies to increase feed or nutrient intake in highly productive sows. Feed intake increased in pST-treated gilts but not in the sows, suggesting that a limitation on sow feed intake exists

Lack of labour availability and concerns with potential adverse consumer perception might mean that the technology is not taken up by the Australian Pork Industry (labour costs for 75 days of pST injections are approximately $9/dam). Non-injection approaches to increase maternal pST (or other approaches to increase fetal growth) would therefore be desirable.

7. Recommendations

This study has established that maternal pST increases progeny weights from birth to slaughter and increases carcass weights in pigs.

- Pork CRC on behalf of the Australian Pork Industry should open discussions with OzBiopharm regarding applying for licensing of Reporcin for use in pregnant pigs, as the current APVMA license only covers growing pigs.
- Subject to licensing, sows should be injected with pST (4 mg/day) from day 25 to 100 of pregnancy to increase progeny birth, weaning and carcass weights

To reduce potential issues with labour availability and consumer concerns, we also recommend further development of non-injection approaches to increase progeny growth in utero, birth weight and postnatal growth:

- Further evaluation of ractopamine in pregnancy, which can be given in feed, although fetal growth responses were not as large as in pST-treated pigs
- Evaluation of piglet birth weight responses to feeding cysteamine and medium-chain fatty acids in pregnant pigs in a pilot study. These approaches both increase endogenous circulating pST in growing pigs (Previous Pork CRC-supported studies by Prof Frank Dunshea and Dr David Miller), and although growth rate is not increased by fatty acids in growing pigs the increase in circulating levels would be sufficient to increase fetal growth based on the doses we have used in pregnant pigs. Ideally, circulating pST profiles should also be measured in a subset of pigs to demonstrate that these are increased. We propose this as a PhD project in conjunction with a new project
- Preliminary studies investigating relationships between endogenous pST and IGF-I levels during pregnancy and litter birth weights, to establish whether a selection approach to increase pregnancy pST is viable

In addition, effects of maternal pST treatment for more than a single pregnancy have not been evaluated, and this is needed before repeated treatments can be recommended. We therefore suggest:

- Further evaluation of sustained maternal pST responses over multiple pregnancy-lactation cycles, including effects on dam retention in the herd.

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