—Original Article—

Reproductive Responses to Daily Injections with Porcine Somatotropin Before Mating in Gilts

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Abstract. Litter size and progeny birth weights are lower in gilts than in sows. Somatotropin (ST) is an important regulator of ovulation, fetal growth and survival. We therefore investigated effects of pST treatment of gilts for two to four weeks before mating on ovulation rate, behavioural estrus, fetal growth and survival, litter size and birth weights. In Experiment One, gilts were injected with 0, 30, 60 or 90 µg pST/kg/day for 14 days commencing 7 days after first estrus. Reproductive tracts were collected and corpora lutea and follicle numbers counted 5.5 days after second estrus. Ovulation rate ($P=0.031$) and number of medium-sized follicles ($P=0.059$) correlated positively with pST dose. In Experiment Two, gilts were injected with 0, 12.5, 25 or 50 µg pST/kg/day for 21 days from first estrus, and mated at second estrus. Numbers of corpora lutea, follicles and fetuses were counted at day 31 of pregnancy. Numbers of medium follicles and ovary weights were positively related to pST dose. In Experiment Three, 31 week old (1st replicate) or 27 week old (2nd replicate) gilts were injected daily with 0 or 12.5 µg pST/kg/day until mating 25.9 ± 0.6 days later, and delivered at term. Pre-mating pST increased total litter size in younger gilts in the 2nd replicate only ($P<0.05$). In conclusion, injecting gilts with pST before mating does not consistently alter ovulation rate, increases the number of medium follicles available for recruitment at the second mating after treatment and increases subsequent litter size in younger gilts.

Key words: Embryo growth, Growth hormone, Litter size, Ovulation rate, Pig

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Reproductive performance is poor in primiparous pigs (gilts), compared with multiparous dams (sows). Litter size in the pig is lowest at their first parity, and then increases in either the second or third parity depending on breed [1–3]. The low litter size of gilts appears to be primarily due to low ovulation rates, with small effects of parity on embryo survival, and similar rates of still birth across parities [2, 4–7]. Birth weight is also lower in gilt than sow progeny [1, 3], resulting in decreased survival and postnatal growth. Gilts can comprise 30–50% of modern commercial herds (B Luxford, personal communication), and increasing the litter size and/or birth weight of gilt progeny would therefore have a substantial impact on overall profitability.

Somatotropin (ST) or growth hormone (GH) is an important regulator of ovulation in a range of mammalian species. In transgenic mice and sheep constitutively expressing ST ovulation rate is increased but fertility decreased, which is due to decreased mating rates in mice, and increased fetal loss in sheep [8–10]. Conversely,

resistance to ST reduces litter size in the last week of pregnancy by 35–55% in GH receptor/GH binding protein knockout mice, largely due to reduced ovulation rate [11, 12]. In transgenic pigs continuously expressing ST at relatively high levels, ovulation is completely abolished [13].

Several studies have evaluated pST administration between puberty and second estrus in the gilt. In *ad libitum*-fed gilts, daily administration of 90 µg pST/kg/day from 14 days after first estrus until 24 h after the onset of second estrus increased the ovulation rate of those gilts that cycled from 12.4 to 14.3 (15% increase), but also decreased the proportion of gilts that displayed a second estrus from 100 to 55% [14]. Subsequent studies by this group using the same pST regimen, found similar ovulation rates in pST-treated gilts and control gilts but similar reductions in the proportions of gilts having a second estrus. Interestingly, when pST treatment was started 3 days later, the proportion of anestrous gilts did not increase. This implies that high GH levels must occur for 8–9 days or more to suppress estrus [15, 16]. Longer-term pST treatment before mating (~65 µg pST/kg/day from 125 days of age for ~80 days until puberty did not affect ovulation rate or fetal survival, but decreased fetal length and weight at 47 days after mating [17], highlighting the need to evaluate longer-term outcomes of pre-mating pST treatments.

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Together these results suggest that pST can have beneficial as well as negative effects on reproductive performance, depending on increases in circulating levels and their duration. Moreover, increasing circulating levels of ST temporarily and moderately in the estrous cycle prior to mating might increase ovulation rate without the adverse effects on reproduction of continuous elevated GH. As such, the aim of the present study was to determine a pST dose that increases ovulation rate without decreasing the proportion of gilts ovulating, under commercial conditions (Experiment One). We then evaluated numbers of fetuses and numbers and size of follicles (Experiment Two) and litter size (Experiment Three) in gilts treated with this regimen before their first mating.

Materials and Methods

Animals

The studies were designed in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* [18] and approved by the Animal Experimentation Ethics Committees of the University of Adelaide and Rivalea Pty (formerly Bunge Meat Industries). All studies were performed in Commercial F1 hybrid (Large White $\times$ Landrace) gilts under commercial management practices at the Research and Innovation Unit of Rivalea Ltd, Corowa.

Experiment 1—dose response study of 14 days pST treatment before the second estrus on behavioural estrus, ovulation and follicle size

Eighty gilts were induced to ovulate by injection with 500 IU of pregnant mare serum gonadotropin and 250 IU human chorionic gonadotropin (as PG600, Intervet, Angers, France) at 23 weeks of age. Gilts were exposed to boars daily to detect estrus. Seventy-three gilts that exhibited a first estrus were weighed and backfat depth measured at the P2 site (65 mm from the midline over the final rib, using linear array 3.5 MHz live ultrasound; Noveko™, Canada), and weight and P2 backfat depth were similarly measured prior to postmortem. Gilts were stratified according to liveweight and then assigned to one of four treatment groups. Gilts were given a daily injection of sterile water (vehicle) containing either 0, 3, 6 or 9 mg of recombinant pST (Reporcin, Southern Cross Biotech Pty, Melbourne, Australia) for 14 days, commencing seven days after their first estrus. These doses delivered pST at initial rates of 0, 29.6 ± 0.5 , 60.3 ± 1.0 and 90.1 ± 1.4 $\mu\text{g/kg/day}$. Data from two gilts was later excluded following post-mortem, due to loss of the reproductive tract at post-mortem for one gilt and because the other gilt had only one ovary. Sixty-six of the gilts, including all gilts that displayed a second estrus, were slaughtered 6 days after the start of their second estrus, on average 6 days after the completion of pST treatment, and their reproductive tracts were collected. The numbers of corpora lutea (CL, for ovulation rate), and the numbers of small (1.0–2.9 mm diameter), medium (3.0–5.9 mm) and large (≥ 6.0 mm) follicles were counted and uterine and ovary weights were measured.

Experiment 2—effects of 21 days pST treatment before the second estrus on behavioural estrus, ovulation and follicle size

Sixty-four gilts were selected at first estrus at ~ 27 weeks of age, and on the following day were allocated to one of four groups, matched by weight, injected daily for the next 21 days with 0, 12.5, 25 or 50 $\mu\text{g pST/kg/day}$, and then mated at their second estrus. Four gilts in the 50 $\mu\text{g pST/kg/day}$ group returned to estrus after mating. The remaining gilts were killed at 31.4 ± 0.4 days after mating, and a further four gilts in the 50 $\mu\text{g pST/kg/day}$ group were found not to be pregnant. In the pregnant animals, ovaries were dissected and weighed, and numbers of small, medium and large follicles, corpora lutea, and embryos were counted.

Experiment 3—effects of 26 days pST treatment before mating on litter size in gilts

Eighty gilts per replicate were selected at ~ 27 weeks of age (replicate one) and ~ 23 weeks of age (replicate two), and induced to ovulate by injection with 500 IU of pregnant mare serum gonadotropin and 250 IU human chorionic gonadotropin (as PG600, Intervet, Angers, France). Gilts were randomly allocated to daily injections with pST at 0 or 12.5 $\mu\text{g pST/kg/day}$ from selection to mating at the second estrus following induction (25.9 ± 0.6 days later), in two replicates (40 gilts per treatment in each replicate). A total of 131 gilts completed the study and delivered at term; 66 gilts in replicate one (34 pST-treated) and 65 gilts in replicate two (33 pST-treated). Gilts were 31.2 ± 0.1 and 26.9 ± 0.1 weeks at mating in replicate one and two respectively. Litter size (total number of piglets, number born alive and number still born) was recorded at farrowing.

Statistical analyses

The effects of pST treatment on the incidences of behavioural estrus and ovulation were analysed by Chi-squared test. Effects of pST treatment on numerical data in Experiments One and Two were analyzed by 1-way ANOVA, and the responses to different pST doses compared using a Bonferroni post-hoc correction. Effects of pST treatment and replicate on responses in Experiment Three were analyzed by 2-way ANOVA. Data are presented as mean $\pm$ SEM unless indicated otherwise.

Results

Experiment 1—dose response study of 14 days pST treatment before the second estrus on behavioural estrus, ovulation and follicle size in gilts

Gilt liveweight and backfat: Prior to treatment, gilt liveweight ($P > 0.8$, average 100 ± 1 kg) and P2 backfat depth ($P > 0.2$, average 11.7 ± 0.3 mm) did not differ between groups. Gilt liveweight at post-mortem, thirteen days after the end of pST treatment, was altered by treatment ($P = 0.012$; 0 mg pST/day: 117 ± 2 kg, 3 mg pST/day: 128 ± 2 kg, 6 mg pST/day: 126 ± 3 kg, 9 mg pST/day: 125 ± 3 kg), being higher in gilts that had been treated with 3 mg pST/day than in vehicle-injected gilts ($P = 0.009$). Gilts treated with 3 mg pST/day also gained more weight ($+26 \pm 1$ kg) during the study than control gilts ($P = 0.047$, $+19 \pm 2$ kg).

P2 backfat depth at post-mortem and P2 backfat gain did not dif-

Table 1. Effect of pST treatment for 14 days starting 7 days after first estrus on estrus behaviour and ovulation at second estrus in gilts¹

Outcome		Initial pST dose ($\mu\text{g/kg/day}$)				Treatment effect
		0	30	60	90	
Gilts per treatment	(number)	17	20	17	17	—
Gilts that displayed 2 nd estrus	(number)	14	11	15	10	
	(percentage)	82	55	88	59	$\chi^2=0.068$
Cycle length in gilts displaying 2 nd estrus	(days)	21.9 ± 0.3^{ab}	20.5 ± 0.8^a	22.9 ± 0.3^b	21.2 ± 0.2^a	$P=0.002$
Gilts that ovulated at 2 nd estrus	(number)	11	8	9	9	
	(percentage)	65	40	53	53	$\chi^2>0.5$
Cycle length in gilts that ovulated at 2 nd estrus	(days)	21.6 ± 0.3^{ab}	20.3 ± 1.1^a	22.8 ± 0.5^b	20.9 ± 0.1^{ab}	$P=0.033$

¹Data for cycle length are mean $\pm$ SEM. The effects of treatment on the incidence of 2nd estrus and ovulation were assessed by χ^2 analyses.

^{a, b} Means lacking a common superscript differ ($P<0.05$).

fer between treatment groups (each $P>0.3$).

Incidence of estrus and ovulation: Treatment with pST tended to alter the proportion of gilts that displayed behavioural signs of a second estrus in response to boar exposure, but did not alter the proportion of gilts that ovulated within each group (Table 1). However, two of the nine gilts treated with 9 mg pST/day that did ovulate failed to display behavioural estrus, and one of these gilts only had one CL. For gilts that displayed behavioural estrus, treatment altered estrous cycle length ($P=0.002$), which was longer in gilts treated with 6 mg pST/day than in those treated with 3 mg pST/day ($P=0.001$) or 9 mg pST/day ($P<0.05$), but did not differ between vehicle-injected and any pST-injected group (Table 1). For gilts that ovulated at second estrus, treatment also altered estrous cycle length ($P=0.033$), which was longer in gilts treated with 6 mg pST/day than in those treated with 3 mg pST/day ($P=0.033$), but again did not differ between vehicle-injected and any pST-injected group (Table 1).

Reproductive tract, ovulation rate and follicle development: In gilts that displayed behavioural estrus and that ovulated at their second estrus, pST treatment tended to increase uterine weight ($P=0.067$) and increased total ovarian weight ($P=0.043$), although these outcomes did not differ between any pairs of treatment groups ($P>0.1$ for all, Table 2) and were not correlated with the initial rate of pST administration ($P>0.1$ for both). Also in this subset of gilts, pST treatment did not alter ovulation rate ($P=0.154$, Table 2). Ovulation rate did, however, correlate positively with the initial rate of pST administration ($R=0.366$, $P=0.031$, $n=35$; Fig. 1). Of note, all pST-treated gilts that displayed estrus and ovulated had at least twelve corpora lutea, whilst the ovulation rate in control gilts ranged from nine to nineteen (Fig. 1). Treatment of gilts with pST did not affect the numbers of antral follicles in each size range, nor the total number of antral follicles ($P>0.3$ for each, Table 2), although the number of medium-sized follicles tended to correlate positively with the initial pST dose rate ($r=0.322$, $P=0.059$, $n=35$).

Experiment 2—effects of 21 days pST treatment before the second estrus on behavioural estrus, ovulation and follicle size in gilts

The number of antral follicles of 3–6 mm at post-mortem in early pregnancy was positively related to pST dose, with a similar positive trend for ovarian weight (Table 3). Treatment with pST prior to mating did not alter ovulation rate nor the numbers of

Fig. 1. Relationship between pST dose (for 14 days after first estrus to second estrus) and ovulation rate at second estrus. Individual data are included for gilts that showed behavioural estrus and that ovulated in their second estrus.

embryos at ~day 30 of pregnancy (Table 3).

Experiment 3—effects of 26 days pST treatment before mating on litter size in gilts

Gestation length was unaffected by pST treatment or replicate (115.3 ± 0.3 days). The effect of pre-mating pST treatment on litter size varied with replicate (interaction, $P=0.05$). The total number of piglets born was increased by 18% or 1.6 piglets by pST treatment in gilts that were 27 weeks old at mating in replicate two ($P<0.05$), but was not altered by pST treatment in gilts that were 31 weeks old at mating in replicate one (Table 4). Pre-mating pST treatment and replicate did not alter numbers of stillborn or born alive piglets (Table 4).

Discussion

Treatment with pST for 14 days commencing at first estrus had dose-dependent positive effects on ovulation rate, but pST-treated gilts tended to be less likely to display behavioural estrus. When gilts were mated following pre-ovulatory pST treatment, the num-

Table 2. Effect of pST treatment for 14 days starting 7 days after first estrus on ovulation rate and follicle development in gilts that displayed estrus and ovulated at second estrus¹

Outcome	Initial pST dose ($\mu\text{g/kg/day}$)				Treatment effect (P)
	0	30	60	90	
Gilts per treatment (number)	11	8	9	7	—
Uterine weight (g)	442 $\pm$ 24	510 $\pm$ 39	433 $\pm$ 28	550 $\pm$ 47	0.067
Total ovarian weight (g)	11.7 $\pm$ 1.0	15.3 $\pm$ 1.4	12.0 $\pm$ 1.4	16.3 $\pm$ 1.7	0.043
Ovulation rate (number of corpora lutea)	13.1 $\pm$ 1.0	14.1 $\pm$ 0.6	15.2 $\pm$ 0.8	15.6 $\pm$ 0.8	NS
Small follicles, 1.0–2.9 mm (number)	30.7 $\pm$ 6.8	31.6 $\pm$ 7.1	43.4 $\pm$ 6.4	35.0 $\pm$ 4.6	NS
Medium follicles, 3.0–5.9 mm (number)	25.1 $\pm$ 5.0	31.9 $\pm$ 7.0	36.3 $\pm$ 6.4	38.1 $\pm$ 4.9	NS
Large follicles, $\geq$ 6.0 mm (number)	1.1 $\pm$ 0.4	1.9 $\pm$ 0.9	1.2 $\pm$ 0.5	3.0 $\pm$ 1.4	NS
Total follicles (number)	56.9 $\pm$ 9.8	65.4 $\pm$ 10.7	81.0 $\pm$ 11.6	76.1 $\pm$ 4.1	NS

¹Data are mean $\pm$ SEM, NS $P > 0.1$.**Table 3.** Effect of pST treatment for 21 days starting on the day after first estrus on ovulation rate and follicle development in gilts that became pregnant following mating at second estrus¹

Outcome	Initial pST dose ($\mu\text{g/kg/day}$)				Treatment effect (P)
	0	12.5	25	50	
Pregnant gilts per treatment (number)	17	18	14	7	—
Total ovarian weight (g)	12.5 $\pm$ 0.7	13.2 $\pm$ 0.5	13.5 $\pm$ 0.5	14.3 $\pm$ 0.5	0.06
Ovulation rate (number of corpora lutea)	13.6 $\pm$ 0.8	14.9 $\pm$ 0.7	15.1 $\pm$ 1.14	15.3 $\pm$ 1.0	NS
Small follicles, $<$ 3.0 mm (number)	139 $\pm$ 12	150 $\pm$ 9	146 $\pm$ 16	120 $\pm$ 26	NS
Medium follicles, 3.0–6.0 mm (number)	17 $\pm$ 1	22 $\pm$ 2	23 $\pm$ 2	23 $\pm$ 2	0.04
Large follicles, $>$ 6.0 mm (number)	0	0	0	0.1 $\pm$ 0.1	NS
Embryos (number)	11.0 $\pm$ 0.8	11.8 $\pm$ 0.8	11.5 $\pm$ 0.8	11.6 $\pm$ 0.4	NS

¹Data are mean $\pm$ SEM, NS $P > 0.1$.**Table 4.** Effect of pST treatment for 26 days before mating on litter size at term in gilts¹

Outcome	Replicate One (Gilts 31 week old at mating)		Replicate Two (Gilts 27 week old at mating)		Treatment effect (P)
	Initial pST dose ($\mu\text{g/kg/day}$)		Initial pST dose ($\mu\text{g/kg/day}$)		
	0	12.5	0	12.5	
Gilts treated before mating (number)	40	40	40	40	—
Pregnant gilts per treatment (number)	32	34	32	33	—
Litter size—total (number)	10.5 $\pm$ 0.5	10.2 $\pm$ 0.5	9.1 $\pm$ 0.5	10.7 $\pm$ 0.5	0.05 ²
Litter size—still born (% of total)	5.9 $\pm$ 2.1	4.0 $\pm$ 2.1	5.6 $\pm$ 2.1	6.9 $\pm$ 2.1	NS
Litter size—born alive (number)	9.8 $\pm$ 0.5	9.8 $\pm$ 0.5	8.6 $\pm$ 0.5	10.1 $\pm$ 0.5	NS

¹Data are mean $\pm$ SEM, NS $P > 0.1$. ²Pre-mating pST treatment increased total litter size in replicate two ($P < 0.05$) but not in replicate one (NS).

ber of fetuses in early pregnancy was not altered by pST treatment, but litter size at term was increased in younger gilts that were 27 weeks old at mating. Numbers of medium sized follicles in the ovary were also increased in early pregnancy, suggesting that pST treatment might stimulate follicle development and potentially ovulation rate at the *second* ovulation post-pST treatment. A similar promotion of follicle growth in the second follicular wave has been reported in heifers and in superovulated ewes in response to treatment with recombinant bovine GH in the preceding estrous cycle [19, 20].

Our finding that pST treatment at higher rates tended to suppress

expression of behavioural estrus is consistent with previous work using similar doses of pST purified from porcine pituitary glands ($\sim 90 \mu\text{g/kg/day}$), where pST treatment for 8–9 days prior to mating also reduced the proportion of gilts displaying a second estrus [15, 16]. Our results confirm that pST can suppress estrus at high doses in the absence of any potential interference from other pituitary hormones, since we used recombinant pST. The mechanisms by which pST inhibits estrus behaviour and ovulation in some animals are unknown. Porcine somatotropin may be acting centrally at the hypothalamic pituitary axis, as the amplitude of the LH surge was reduced in pre-pubertal gilts injected daily with $90 \mu\text{g/kg/day}$ of

pituitary-derived pST for 6 days after administration of PMSG [21]. In the present series of studies, lower doses of pST, at 25 µg/kg/day did not reduce pregnancy or mating rates in gilts.

We did not find consistent effects of pre-mating pST treatment on ovulation rate in the gilt, suggesting that this strategy is unlikely to improve reproductive performance in all gilts within a herd under commercial conditions. However this study provides some evidence that pre-mating pST may improve reproductive outcomes in animals that are younger at mating. Others have also reported variable effects of pST exposure before puberty on follicular development and reproduction. A 6-week exposure to 4 mg/day pST administered via an implant (initial dose ~70 µg/kg/day, decreasing to 50 µg/kg/day) increased the numbers of medium-sized follicles in pre-pubertal gilts [22], whilst immunisation against GHRH at 35 days, but not at 92 days of age, reduced circulating pST and subsequent ovulation rate [23]. Administration of 4 mg/days pST for 10 weeks up to puberty, administered as a daily injection (initial dose ~70 µg/kg/day), did not affect the age of puberty, incidence of ovulation or conception at first estrus, nor litter size and ovulation rate, but reduced fetal weight (–15%) and length (–9%) at 47 days of gestation. In first parity sows, pST treatment (40 µg/kg/day) from day 1 to 5 after weaning did not change follicle size or number, but increased follicular steroidogenesis [24]. Overall, these results suggest that pST might stimulate follicular growth and function in pigs, but that the effects depend on the stage of development and duration of treatment. In the final study reported here, pST treatment prior to mating increased litter size in younger gilts (~27 weeks old at mating) but not in gilts that were more mature when mated (~31 weeks), suggesting that effects of this medium-term pST treatment on follicle development may be greater in the less mature ovary. Ovulation rate naturally increases with age and parity in pigs [4], and pST treatment may therefore accelerate this maturation process. In this herd total litter size is currently ~1.4 piglets higher in mature sows compared to gilts [25], partly reflecting higher ovulation rates in sows.

Consistent with our findings of increased numbers of medium-sized follicles in the ovary following completion of pST-treatment and the subsequent estrous cycle, studies in other species have reported that ST-treatment prior to ovulation increased the size of non-ovulating follicles without increasing ovulation rate. Two studies in pubertal cattle did not find any change in ovulation rate following daily injection of heifers with 25 mg bST for one or two estrous cycles [19, 26], although the numbers of antral follicles were increased after two cycles of bST treatment in one study [26]. In sheep, daily injections of ewes with bST for 7 days increased the numbers of medium-sized follicles during normal or FSH-stimulated cycles, but did not affect ovulation rate following a superovulatory dose of FSH [20, 27]. These increased numbers of medium follicles after ST treatment suggests that ST promotes growth of small follicles and/or promotes the survival of existing medium follicles, either directly or indirectly. Consistent with promotion of follicle survival by ST, seven days of recombinant bovine GH treatment in ewes decreased signs of early atresia in their medium-sized follicles [27], and the proportion of ovarian follicles containing apoptotic cells was reduced in mice transgenic for bovine GH compared to non-transgenic littermates [28].

Effects of pST on ovarian and follicle development may reflect direct and/or indirect effects of pST, including repartitioning of nutrients due to metabolic actions of pST in non-reproductive tissues. Follicles of the porcine ovary bind pST at all stages of follicular development, implying continued presence of pST-receptors [29]. Binding of labelled pST to the granulosa cells of the follicle is higher in preantral than in large follicles [29], which might explain why growth and/or survival of smaller follicles increases and results in greater numbers of medium follicles following pre-ovulatory pST-treatment in gilts, whilst growth and ovulation of larger follicles are not consistently increased. Circulating levels of IGF-I and insulin increase in pST-treated pigs, and might also promote follicular development. IGF-I binds to granulosa cells of healthy follicles, whilst insulin binding can be detected in most cell types within follicles at a range of developmental stages [29]. *In vitro*, IGF-I increased proliferation rates in thecal cells from all follicle size classes, and in granulosa cells from small and medium follicles, but not in granulosa cells from large follicles, suggesting that IGF-I promotes growth to a greater extent in smaller follicle classes [30]. Also in these *in vitro* studies, pST increased proliferation in porcine ovarian granulosa cells from large follicles but not from other size classes, and not in theca cells from follicles of any size [30], implying that effects of pST on follicle growth are at least partially mediated by IGF-I. Conversely, IGF-I administration is not able to rescue infertility of GHR/GHBP knockout mice, implying that effects of pST on ovulation are IGF-I-independent [12]. IGF-I stimulates estradiol secretion more effectively than pST in cultured porcine granulosa and thecal cells [30], and this could be an autocrine/paracrine effect of pST, since it stimulates IGF-I secretion in porcine ovarian granulosa cells [30, 31]. Insulin administration to sows from 1 to 5 days after weaning also increased estradiol and testosterone contents of medium and large follicles to a greater extent than pST administration over the same period [24]. Therefore, increases in circulating insulin may mediate some effects of pST on follicular growth and function.

In conclusion, injecting gilts with pST at doses between 12.5 and 90 µg/kg/day before mating does not consistently alter ovulation rate, but increases the number of medium follicles available for recruitment at the second mating after treatment and increases litter size in younger or less mature gilts.

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