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Growth Hormone–releasing Hormone Plasmid Treatment by Electroporation Decreases Offspring Mortality Over Three Pregnancies

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LifeTideSW5 is a growth hormone–releasing hormone (GHRH)–expressing plasmid delivered by intramuscular (IM) electroporation (EP), and the first therapeutic plasmid delivered by this physical method to be approved for use in food animals. Gestating sows (n = 997) were treated once with a single 5-mg GHRH-plasmid by EP or served as controls. Data on offspring from three parities subsequent to treatment were collected. No adverse effects related to treatment were noted. First parity post-treatment offspring from treated sows displayed a 2.93 kg ($P < 0.0001$) increase in carcass weight (CW), 1.0 mm ($P < 0.0001$) less back-fat (P2), and a 27.0 g CW/day ($P < 0.0001$) increase in rate of gain (ROG) compared with controls. An increase of 21.6% was recorded in the number of offspring surviving. In the second and third parities post-treatment, offspring from treated females displayed higher number of born alive and total born number, and lower stillborn rates. Third parity offspring from treated sows displayed a 1.6 kg advantage in CW ($P < 0.05$), 1.0 mm less P2 ($P< 0.05$), and a 10.0 g CW/day benefit in ROG. Furthermore, offspring from treated females had a 19.04% lower post-wean loss rate. Overall, plasmid GHRH administration decreased morbidity and mortality in treated females and their offspring over three consecutive pregnancies.

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INTRODUCTION

The welfare of farm animals is of paramount importance, particularly in a large industrial setting,[1] while ensuring environmentally friendly and economically efficient protein production for the world where consumption is projected to soar in the next decades.[2] Therefore, methods to improve general animal health and well-being, and decrease the use of antibiotics, while decreasing offspring morbidity and mortality are of particular interest. A novel nonviral gene therapeutic approach, with the LifeTideSW5 licensing studies performed in Australia under the purview of the Australian Pesticides and Veterinary Medicine Authority offers this unique opportunity.

The growth hormone–releasing hormone/growth hormone/insulin-like growth factor-I (GHRH/GH/IGF-I) axis plays an important role in growth and development, declining with age, and being disregulated in pathological circumstances.[3] We have previously shown that plasmid-mediated GHRH supplementation by electroporation (EP) results in enhanced growth[4] with improvements in weight gain and body composition.[5] We have also shown that if GHRH is administered to pregnant rats or pigs, pituitary somatotroph and lactotroph numbers, as well as postnatal growth rate of the offspring are optimized.[6,7] In rats, this was shown to be at least partially due to the hormone crossing the placenta and directly influencing fetal development.[8]

The development of the EP technology is a significant advancement in the field of gene therapy.[9] Plasmid DNA injection alone has had some success, particularly for vaccination purposes;[10] however, the combination of plasmid delivery with EP enables a single, low-dose injection with long-term therapeutic effects in large animal models of disease.[11–14] Nevertheless, the long-term effects of a single GHRH-plasmid administration on the offspring of treated animals have not been examined, in particular over multiple pregnancies. Here, we show that the one-time treatment of female pigs in their late gestation with a porcine-specific GHRH-expressing plasmid followed by EP has the ability to improve the outcome of offspring for at least three parities.

RESULTS

Between October and November of 2003, gestating females (n = 997) located in a single production unit of a large-scale Australian commercial swine production site were entered into a trial to determine the effects of plasmid-mediated GHRH supplementation technology on treated animals and their offspring over three subsequent parities during a 1-year trial.

The period of gestation did not differ between the treated and control groups, averaging 116 ± 0.1 days. The initial treatment parity saw no difference in the number born alive (NBA) of offspring

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between the treated and control dams. However, as the trial females farrowed in their second and third parities, post-treatment, there were differences in the NBA from treated versus control sows (Table 1). In the second parity post-treatment, the resulting 0.4 increase in NBA (10.8 ± 0.12 GHRH-treated versus 10.4 ± 0.14 control females, $P = 0.03$). In the third parity post-treatment, females treated with the GHRH-expressing plasmid farrowed 0.5 NBA more than controls (11.3 ± 0.16 versus 10.8 ± 0.17, $P = 0.066$). Sows that were at their second and third pregnancy during treatment displayed the highest differences in NBA versus controls. There was no difference in the number of total born (TB) or stillborn (SB) in the first parity (Table 2). In the post-treatment parities two and three, there was a 0.1, nonsignificant reduction in the number of SB offspring born to treated females compared with controls.

The number of lactation days was determined by the date from birth to weaning. No difference in the number of lactation days for the treated and control sows was recorded (n = 21.0 ± 4.0 days control versus 20.9 ± 3.5 days treated). Wean fate was also analyzed (Table 3). Overall, there was an increase in the number of animals weaned from post-treatment parity 1 to post-treatment parities 2 and 3. There was no difference in the number of offspring weaned within post-treatment parity 1 when comparing treated with control animals. However,

Table 1 Summary of the number born alive by post-treatment parity and overall parity

	Post-treatment parity 1				Post-treatment parity 2				Post-treatment parity 3			
	Treatment		Control		Treatment		Control		Treatment		Control	
Overall parity	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM
Total	483	8.8 ± 0.15	488	8.9 ± 0.14	352	10.8* ± 0.12	348	10.4 ± 0.14	218	11.3** ± 0.16	239	10.8 ± 0.17
Parity 1	125	8.3 ± 0.30	122	8.6 ± 0.28	—	—	—	—	—	—	—	—
Parity 2	83	8.6 ± 0.30	87	8.5 ± 0.35	83	10.5 ± 0.24	84	10.0 ± 0.31	—	—	—	—
Parity 3	54	9.4 ± 0.38	51	9.4 ± 0.39	62	10.9* ± 0.30	70	10.0 ± 0.26	47	11.1 ± 0.34	60	11.0 ± 0.35
Parity 4	63	8.9 ± 0.44	69	8.9 ± 0.36	47	10.7 ± 0.38	42	10.5 ± 0.43	41	12.0** ± 0.44	46	10.9 ± 0.38
Parity 5	67	9.7 ± 0.42	64	9.7 ± 0.38	54	11.0 ± 0.34	52	10.6 ± 0.41	34	11.2 ± 0.45	29	10.6 ± 0.54
Parity 6	62	8.9 ± 0.39	64	8.5 ± 0.43	51	10.9 ± 0.29	49	11.2 ± 0.30	38	10.8 ± 0.45	42	11.1± 0.37
Parity 7	29	8.3 ± 0.67	31	8.8 ± 0.54	54	10.7 ± 0.33	51	10.3 ± 0.31	30	11.3 ± 0.27	33	10.8 ± 0.44
Parity 8	—	—	—	—	1	12.0 ± (—)	—	—	28	11.4 ± 0.34	29	10.5 ± 0.45

Where *N* is the number of sows with litters.
*Statistically significant *P* value < 0.05, *t*-test; **0.05 < *P* value < 0.10, *t*-test.

Table 2 Summary of the number stillborn by post-treatment parity

	Post-treatment parity 1				Post-treatment parity 2				Post-treatment parity 3			
	Treatment		Control		Treatment		Control		Treatment		Control	
Overall parity	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM
Total	483	1.5 ± 0.09	488	1.5 ± 0.09	352	0.7 ± 0.05	348	0.8 ± 0.06	218	0.8 ± 0.07	239	0.9 ± 0.08

Where *N* is the number of sows with litters.

Table 3 Summary of the number weaned by post-treatment parity and overall parity

	Post-treatment parity 1				Post-treatment parity 2				Post-treatment parity 3			
	Treatment		Control		Treatment		Control		Treatment		Control	
Overall parity	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM
Total	483	6.9 ± 0.13	488	6.9 ± 0.13	340	8.9* ± 0.12	348	8.6 ± 0.12	181	9.7** ± 0.10	185	9.0 ± 0.12
Parity 1	125	5.6 ± 0.28	122	5.5 ± 0.28	—	—	—	—	—	—	—	—
Parity 2	83	7.0 ± 0.31	87	7.3 ± 0.29	79	9.3 ± 0.20	84	8.9 ± 0.26	—	—	—	—
Parity 3	54	7.1 ± 0.39	51	7.5 ± 0.39	61	9.3** ± 0.22	70	8.6 ± 0.26	37	9.8 ± 0.23	47	9.5 ± 0.22
Parity 4	63	7.5 ± 0.29	69	7.3 ± 0.28	45	9.2 ± 0.30	42	9.0 ± 0.23	36	9.6** ± 0.22	38	8.7 ± 0.32
Parity 5	67	7.6 ± 0.23	64	7.3 ± 0.38	54	8.7 ± 0.31	52	8.4 ± 0.35	29	10.1** ± 0.24	23	8.6 ± 0.35
Parity 6	62	7.7 ± 0.39	64	7.3 ± 0.35	48	8.8 ± 0.51	49	8.8 ± 0.30	35	9.5 ± 0.24	34	9.3 ± 0.22
Parity 7	29	6.6 ± 0.52	31	7.2 ± 0.56	52	8.2 ± 0.33	51	8.0 ± 0.32	24	9.3* ± 0.35	24	8.5 ± 0.37
Parity 8	—	—	—	—	1	9.0 ± (—)	—	—	20	9.8** ± 0.20	19	8.8 ± 0.25

Where *N* is the number of sows with litters.
*0.05 < *P* < 0.10, *t*-test; **statistically significant *P* < 0.05, *t*-test.

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treatment differences within post-treatment parities 2 and 3 were significant ($P = 0.06$ and $P < 0.0001$, respectively). At parity 2, this difference was 0.3/litter, and at parity 3 this difference was 0.7/litter. The number of animals weaned at the third post-treatment parity was also significantly increased for sows on their second or third pregnancy during GHRH-plasmid treatment ($P < 0.05$).

The TB, NBA, and number weaned (NW) were analyzed over all parities. Only sows that had three parities recorded during the study were included in this analysis. Because treatment was not given until after the sows were already pregnant, late in the first gestation, no first parity differences were expected, therefore the TB and NBA were only analyzed during parities 2 and 3. The NW was analyzed over all three parities. There was an increase in the TB, NBA, and NW in the treated sows ($P = 0.04$, 0.004, and 0.02, respectively). This difference is near 0.7 NBA/litter and 1.0–1.4 NW/litter.

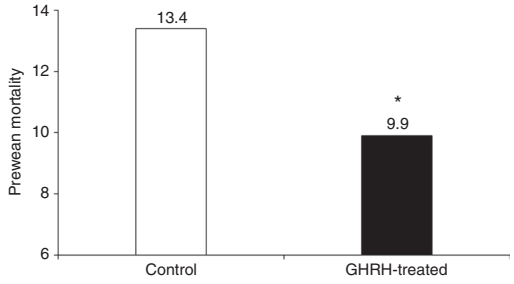

Figure 1 Percentage preweaning mortality in post-treatment parity three. Results are shown as the percentage of number of deaths divided by the total number of animals for treated (n = 203/2,052) and control (n = 288/2,155) animals. *Statistical difference, where $P = 0.0005$ calculated using Fisher's exact test. GHRH, growth hormone–releasing hormone.

Preweaning mortality (PWM) results are shown in **Figure 1**. Offspring PWM rate for the control group was 13.4%, standard number seen in the facility at the time of the trial. However, in the offspring from females treated with the GHRH plasmid, PWM was significantly lower at 9.9% ($P < 0.001$), a 26.1% reduction in PWM. Post-weaning loss rate was also decreased by 19% in offspring from treated sows when compared with offspring from control females (8.76% versus 10.82%, respectively).

Offspring were analyzed when they reached 100 kg for hot standard carcass weight (HSCW), P2, and rate of gain (ROG) data. There were 3,419 (1,911 = treated, 1,508 = control) animals identified by treatment, with 3,279 (1,838 = treated, 1,441 = control) identified by treatment and week born. The GHRH treatment increased offspring survivability by 21.6% compared with untreated control animals. The data from those offspring identified by treatment and week born were analyzed. Offspring from treated females in their first post-treatment parity showed significant changes in their body composition, with higher lean body mass as shown by HSCW, an increase in ROG, and a decrease in P2 back-fat, P <0.0001 (**Table 4**). By the third parity, significant differences were still apparent between offspring from treated females compared with controls for HSCW, P2 back–fat, and ROG, $P < 0.05$. Several other parameters were also analyzed such as the average wean-to-market advantage, estimated live finish weight, number of days to market. All GHRH-treated animals had statistically significant ($P < 0.0001$) improvements in these parameters compared with controls as shown in **Table 5**.

At the end of the trial data collection, there appeared to be a small difference in the number of females removed from the trial (**Figure 2a**), with slightly fewer treated females being removed as a percentage of those females originally placed on trial. **Figure 2b** addresses the reasons for removal.

Table 4 Least squares means for HSCW, P2 and Lifetime Dress Weight ROG variables for data collected from offspring of either treated or non-treated (control) gestating females in their first post-treatment parity and third post-treatment parity

Parity	Treatment group	HSCW (kg)	P2 (mm)	Lifetime dress ROG (kg HSCW/day)
First post-treatment	Control ± SEM*	74.12 ± 0.245	12.6 ± 0.070	0.476 ± 0.0018
	Treated ± SEM*	77.05 ± 0.216	11.6 ± 0.060	0.503 ± 0.0016
Third post-treatment	Control ± SEM**	75.7 ± 1.044	11.1 ± 0.260	0.484 ± 0.0016
	Treated ± SEM**	77.3 ± 1.031	10.1 ± 0.257	0.494 ± 0.0016

Abbreviations: HSCW, hot standard carcass weight; ROG, rate of gain.
All means within a column are significant at *$P < 0.0001$. All means within a column are significant at **$P < 0.05$.

Table 5 Averages of estimated wean-market advantage, estimated live finish weight (kg), estimated days to market post-wean, estimated lifetime ROG – live finish weight (kg) and average of age when reaching ~100 kg (days) for data collected from offspring of either treated or nontreated (control) gestating females in their first post-treatment parity and third post-treatment parity

	GHRH-treated	Control	*P* value	Difference
Average of estimated wean-market (ADG)	0.696 ± 0.001	0.656 ± 0.002	<0.0001	0.040 kg/day
Average of estimated live finish weight (kg)	98.16 ± 0.1	94.13 ± 0.22	<0.0001	4.03 kg
Average of estimated days to market post-wean	132.9 ± 0.1	135.1 ± 0.18	<0.0001	−4.4 days
Average of estimated lifetime ROG – live finish weight (kg)	0.640 ± 0.001	0.606 ± 0.001	<0.0001	+0.034 kg/day
Average age to 100 kg (days)	153.9 ± 0.18	156.1 ± 0.22	<0.0001	−2.2 days

Abbreviations: ADG, average daily gain; GHRH, growth hormone–releasing hormone; ROG, rate of gain.

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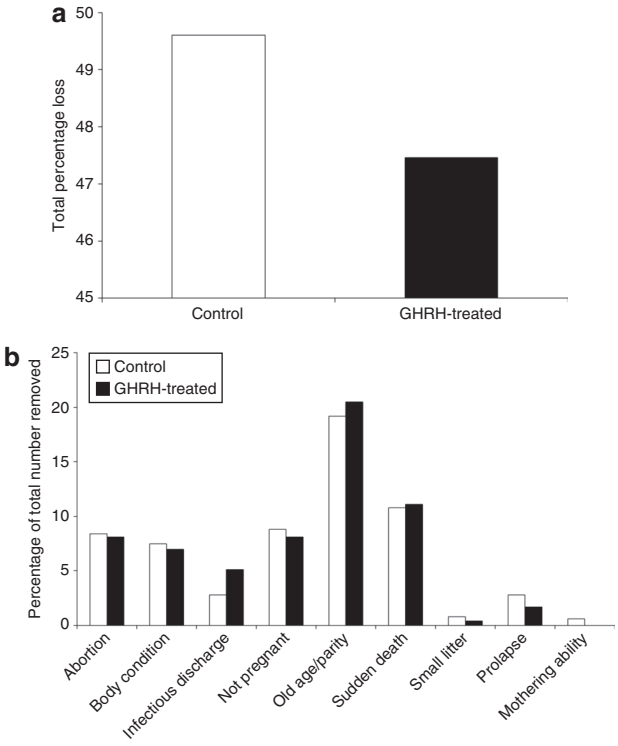

Figure 2 Total loss of females, percentage and causes for death or removal from study.(**a**) Total percentage loss of females. There were 250 control females and 234 treated females removed during the trial period. This equated to a total percentage loss of 49.5% control females and 47.5% treated females. (**b**) Causes of removal of sows during the study. The number of sows removed from the study is shown as percentage of total with the various reasons for the removal indicated. GHRH, growth hormone–releasing hormone.

DISCUSSION

We are presenting herein the first demonstration that a nonviral gene therapy delivered by IM injection followed by EP is safe, efficacious, and feasible in a large animal study under farm conditions, and results in the favorable modulation of health and body composition for three consecutive sets of offspring from treated animals for a year after a single plasmid administration. Control animals were not treated with plasmid or EP but were instead used as a standard of care comparison. We have previously reported[4,7] that neither the injection nor the EP influences the response and the effects are directly due to GHRH expression after plasmid administration. Similar responses have been noted across species (rodents, pigs, and dairy cattle) suggesting that the physiological stimulation of the GHRH axis is a fundamental component of developmental physiology.

Sow health and longevity play a major role in the number of piglets born during their lifetime[15] and the number of high-parity females and lifetime production are linked to financial performance.[16] Currently, there are several interventions used to improve herd productivity and general well-being. Management, such as monitoring and removing at-risk females (females with re-services, lactation length 0–13 days, weaning-to-first-mating interval ≥8 days, and abortion records) increases herd productivity.[17] Genetic differences in reproductive efficiency also exist and need to be considered while choosing a female line.[18] Nutrient intake during gestation also impacts sow health, and can result in increased litter weight, increased pig body weight, and number of marketable pigs at weaning.[19] The use of recombinant somatotropin has also been investigated. One study showed that the daily injection of somatotropin during early gestation (days 10–27) selectively improved the growth conditions for low–birth weight littermates.[20] Another study demonstrated that the daily treatment of sows in gestation with porcine somatotropin for 75 days increased offspring size at birth.[21] Conversely, the administration of recombinant porcine somatotropin to sows in late pregnancy increased blood glucose levels in sows and offspring as well as the number of neonatal deaths.[22]

Gene therapy and EP in the industrial farm setting is relatively novel, yet there are several reports demonstrating the potential of this method. The vaccination of farm animals with plasmid DNA encoding mycobacterial antigens followed by intramuscular (IM) delivery of EP has been shown to improve the primary immune response of goats and cattle.[23] Economically important fish have shown increased growth with GH treatment.[24] The transfer of the tilapia *GH* gene in shrimp embryos resulted in a 32% growth enhancement 3 days after EP of larvae.[25] We have previously shown that EP of GHRH-plasmid results in the improved well-being of horses with laminitis,[12] and cattle treated with GHRH followed by EP during periods of heat stress had reduced calf mortality, increased milk production and weight gain.[26]

Previously, we have shown that the GHRH plasmid–mediated treatment of pregnant rats or pigs in the third part of their gestation results in increased pituitary somatotroph and lactotroph numbers, as well as postnatal growth rate of the offspring.[6,7] In rats, this was shown to be at least partially due to the hormone crossing the placenta and directly influencing fetal development.[8] Nevertheless, these previous studies have not addressed the long-term follow-up after the one-time treatment, and the effects on subsequent parities. Here, we show that when administered to gestating sows, the plasmid GHRH treatment with EP positively affected the treated sow as well as the outcome of three subsequent parities. While the initial treatment parity results for NBA, SB, and TB did not show any difference between the control and treated females, there were significant effects in postnatal performance in the subsequent parities observed in this study. As females were not administered with the plasmid treatment until late gestation, the farrowing results concerning the number of born alive and stillborn found in post-treatment parities 2 and 3 were not expected in the initial treatment parity. While the third parity post-treatment difference in NBA is greater, there is less significance in the results, likely due to the lower number of females remaining in the study. Although not statistically significant, the 0.1 decrease in number of SB offspring in the plasmid-treated females could have significant value over the lifetime of a sow. Coupled with the increase in NBA in parities 2 and 3 post-treatment, the decrease in SB offspring resulted in a greater number of TB offspring for the sows treated with the GHRH-plasmid technology.

Treatment of female swine with the plasmid GHRH supplementation technology resulted in significant or nearly significant increases in NBA over subsequent parities. Treated females displayed an increase of ~0.7 more pigs born alive over multiple parities. This would equate to a 3.5% increase in the number of animals born alive per year, given at least two parities a year and

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an average of 10 pigs born alive per litter. Offspring from females treated with the GHRH plasmid supplementation technology showed greater survivability rates before weaning. A 21.6% reduction in PWM was observed in the offspring from treated females. The reduced PWM results found in the third parity post-treatment offspring suggest that those same benefits, if not even more substantial results, would have been experienced in the first two post-treatment parities. Death reasons were recorded for piglets; the major reason for death was overlay by sow ($n = 61$), but other reasons included bacterial and viral infections, starvation, or genetic disorders. When coupled together, the results for NBA and PWM found in the third parity post-treatment equate to an increase in pigs weaned per sow when offspring are from females treated with the GHRH plasmid supplementation technology. While in one litter alone these results are quite substantial, the benefits of having these results expressed over multiple parities provide significant advantages. The post-weaning death rate was also decreased in the offspring from treated compared with control sows.

GH and GHRH can influence body composition, in particular lean body mass. Administration of recombinant GH has been shown to have beneficial effects on body composition by reducing fat weight.[27] In genetically lean sheep, the expression of GH is greater than in fat-selection lines of sheep.[28] We have previously shown that the administration of GHRH to young pigs resulted in greater weight gain than controls ($P < 0.01$) and an increase in fat-free mass ($P < 0.05$).[29] Here, we show that the administration of plasmid GHRH can impact the body composition of offspring up to three parities. Body composition parameters such as HSCW, P2, and Lifetime Dress Weight ROG displayed significant increases in offspring from post-treatment parities one and three. As GHRH treatment significantly increased all of these parameters, the resulting increase in leanness could add to the market value of the pig. Although data for post-treatment parity two was not collected, it can be assumed that there would have also been significant advantages in HSCW, P2, and Lifetime Dress Weight ROG further increasing productivity. Furthermore, the increases in other body composition and welfare parameters such as those indicated in **Table 5** demonstrate that treatment with GHRH can improve the health of food animals and decrease their time to market.

Trial female removal rates were also collected and investigated. As previously mentioned, most of the removal reasons were based on an objective decision. However, due to the subjective nature of a number of the removal reasons, these observations could not be fairly analyzed. As sows reach their 7th to 8th litters, they are usually automatically removed from productivity due to old age. However, most sows were at lower parity, and during the trial no adverse effects due to the plasmid GHRH treatment were observed. No females were removed from production as a result of the plasmid GHRH administration or effects. Given the results found in **Table 1**, trends of higher NBA were seen in the older aged (parity 7 and 8) females that had been treated with the GHRH-expressing plasmid. Although the sample numbers were too low to gain significance, it is evident that plasmid GHRH–treated females can maintain superior production efficiency over multiple parities and in older age.

Overall, this study shows that a plasmid and EP gene therapy can be efficiently used in the industrial farm setting. The underlying mechanism by which the long-term effect was observed seems to be multifaceted and has been previously described in our pilot studies: metabolic changes in the mother with better nutrient utilization and higher lactation potential impact both growth pattern and survival of offspring from birth to weaning;[26,29] changes in pituitary lineage in the offspring of treated animals impact the stage of maturation at birth and postnatal growth and development.[7] Also, the better metabolic and health status of the treated sows, as well as improvements in immune responses,[30] may affect reproductive capacity and survival of the offspring. This last finding was described also in plasmid GHRH–treated cows,[26] and is currently addressed in a follow-up study. Regardless, the ability to increase the general well-being in sows, as well as to improve survival and health of offspring, combined with improvements in body composition parameters, are of great value. The approval of the commercial product (LifeTideSW5) in Australia marks the first nonviral gene therapy product delivered by a physical method to be approved by a regulatory agency for use in food animals. This large animal trial provides concrete evidence for the success of EP of plasmid GHRH and will aid in the transition of gene therapy products into day-to-day practice.

MATERIALS AND METHODS

Animals. The trial included 997 females (504 control and 493 treated) that were randomly selected for an even distribution of previous number of pregnancies (*i.e.* parity) (**Table 1**). The sows were located in production units of a large-scale Australian commercial swine production site and followed for three subsequent parities during a 1-year trial. This trial was approved by the Animal Care and Ethics Committee at the sponsor site. Gestating females were kept in individual sow housing crates or multiple female pens (two–three females per pen) until ~110 days of each gestation. Females were then moved to individual crates where they remained through birth (farrowing) and nursing. At ~20 days post-farrowing, offspring were weaned and females were then transferred to individual crates for re-mating via artificial insemination. Offspring were group housed through the weaner (weeks 3/4–9/10), grower (week 9/10–15/16), and finisher (week 15/16–21/22, animals reaching ~100 kg) phases. Although the number of offspring varied from trial location to location, they were evenly distributed by number and assigned randomly per pen (conventional pen or ecosheds). During the trial period, any removal of a female from the production system was recorded per normal production practices.

Diet. The gestation diet was 13 mega joules (MJ) of digestible energy and maintained at a minimum of 13% protein with an available lysine-to-digestible energy ratio of 0.4 g/MJ of digestible energy. All amino acids were maintained to at least the ideal amino acid ratio in reference to available lysine. Calcium and total phosphorous levels were maintained at 0.9% and 0.63%, respectively. Vitamins and minerals were added at commercial levels with some minerals provided in organic form.

The lactation diet was predominately a wheat-based ration providing 14 MJ of digestible energy and 1% total lysine. All other amino acids were balanced to available lysine levels in an ideal amino acid ratio. The diet contained 2% added fat in the form of animal tallow. The calcium and total phosphorous levels were maintained at 0.93 and 0.65% of the total diet. Vitamins and minerals were added at commercial levels with some minerals provided in organic form.

GHRH-plasmid treatment

Treatment allocation: Females were randomly assigned to a treatment group using a separate permuted blocks randomization list for each

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treatment week. An even number of females per trial group and per parity was attempted (**Table 1**). Approximately 100 females/group/week were entered into the trial over a 5-week period. All females were held to normal prefarrowing-handling practices. Before treatment and farrowing, 25 females (16 control and 9 treated) were removed from the herd due to various management reasons (*i.e.* abortion, not pregnant (also called "not in pig") sudden death, and structural failure), and subsequently removed from the trial. One treated female was not in pig at the time of the first farrowing parity, however she was left in the herd and subsequent data (second parity post-treatment) was collected. Therefore, at the time of first farrowing, 971 females remained on trial (488 control and 483 treated).

DNA construct: Plasmid expression was driven from a muscle-specific SPc5-12 synthetic promoter.[31] Wild-type porcine GHRH complementary DNA was cloned into the *Bam*HI/*Hin* dIII sites of pSPc5-12, to generate pSP-GHRH.[4] The 3′-untranslated region of GH was cloned downstream of GHRH complementary DNA. The plasmid was produced under Good Manufacturing Practice (VGX Pharmaceuticals, The Woodlands, TX) and formulated in sterile water for injection + 1% high-performance liquid chromatography-purified low-molecular weight poly-L-glutamate sodium salt.

Plasmid treatment: Females between 79 and 89 days of gestation were treated with 5 mg of the myogenic GHRH plasmid; controls were not treated, but maintained at standard of care. Each animal received a pre-test physical examination by a veterinarian. Females were moved to a treatment facility using a separate pen per female, and then were anesthetized before treatment using an IM or intravenous injection of Zoletil (Ketamine and Telazol). For IM anesthesia, 1 ml (50 mg Ketamine, 83.33 mg Telazol)/45 kg BW was used. For intravenous anesthesia, 0.1 ml (5 mg Ketamine, 8.333 mg Telazol)/10 kg BW was used. Plasmid solution (5 mg in a 2 ml volume) was injected into the semimembranous muscle using a 3-ml syringe and a 21-gauge, 1.25″ needle. The plasmid injection was followed by EP using a constant current EP machine (CELLECTRA constant current EP device, VGX Pharmaceuticals as previously described[32]) at 0.5 Amps, 5 pulses, 52 millisecond in duration with 1-second interval between pulses. Once fully recovered, females were returned to their pens of origin. Only those females designated for plasmid treatment were handled.

Farrowing: At ~110 days of gestation, trial females were moved into a farrowing shed and crate with other females due to farrow around the same time. Farrowing crate assignment was done randomly, and due to even treatment and control numbers in a given week, trial females were evenly distributed throughout the farrowing sheds. Data were collected for NBA, SB, mummified piglets, and TB. Immediately after farrowing, offspring were identified using an ear tattooing system to denote the week born and trial group. This allowed for the offspring to be tracked throughout their lifetimes. Female identification was arranged in a manner that allowed for farrowing house personnel to be blinded to treatment groups.

Minimal cross-fostering was attempted, however, due to the number of females used as both treatment and controls, versus the total number of females in a production week in the facility, and the random selection of trial females, it was difficult to eliminate cross-fostering between treatments or to "off trial" females. Furthermore, extreme perinatal death rates were experienced throughout the entire facility during the first parity and cross-fostering was inevitable.

Post-treatment parity 2. Farrowing data were collected for the second litter post-treatment. Data were recorded for NBA, SB, mummified piglets, and TB and were analyzed. Before the second parity post-treatment farrowing, 137 control and 132 treated females were removed from the production herd per criteria previously described and subsequently removed from the trial. Three control females were not pregnant and did not farrow during the second period post-treatment parity data collection; these females remained in the herd and enrolled in the trial. Therefore, at the time of second parity post-treatment farrowings there were a total of 700 trial females (348 control and 352 treated) evaluated for perinatal effects.

Post-treatment parity 3

Animals: Before the third parity post-treatment farrowing, 83 control and 89 treated females were removed from the production herd (per criteria described above), and subsequently removed from the trial. There were 531 (268 control and 263 treated) trial females remaining in the study. Of these females, it was then determined that those that farrowed between production weeks 32 and 39 would be monitored for third parity farrowing effects. These dates were set due to logistical requirements of the trial. Therefore, 457 (239 control and 218 treated) trial females were eligible for trial data collection on the third parity farrowing effects. Seventy-three (29 control and 44 treated) trial females were not eligible for further trial data collection due to production system removal. Trial females were randomly entered into various farrowing sheds. Females were identified by an **R** (control) or **L** (treatment) on their farrowing card, at farrowing shed entry, to ensure farrowing shed operators would correctly notch/identify trial offspring.

Farrowing–weaning: Data were recorded for NBA, SB, mummified piglets, and TB on 457 (239 control and 218 treated) trial females at farrowing. Offspring from those females that remained at the original production unit and farrowed within the given production week frame (weeks 32–39) were evaluated. Offspring from 381 trial females (200 control and 181 treated) were notched in either the right or left ear according to trial group as previously described. All offspring were notched before any cross-fostering. Minimal cross-fostering was attempted. In all circumstances, an attempt was made to ensure that offspring from control animals were cross-fostered to control sows, while offspring from treated animals were cross-fostered to treated females. Due to the necessary event of cross-fostering, all offspring from trial females were evaluated as individual sample units. All offspring deaths were recorded by trial group. At the time of weaning, all trial offspring were counted by ear notch, and final wean numbers were also used to validate prewean death records. After weaning, trial offspring remained with their production week groups and were sent to wean-to-market grower/finisher sheds. Carcass weight was collected using a certified online scale and is reported as HSCW (AUSMEAT Trim 1). Fat depth is reported as P2 fat depth using a calibrated Hennessey Grading Probe (back fat is measured at the P2 position which is 65 mm down the left side from the midline, at the level of the head of the last rib).

Statistical evaluation. The statistical analyses summarized in this report for all farrowing parities, as well as third parity post-treatment pre-wean mortality data were conducted by inVentiv Clinical Solutions LLC (formerly Synergos, Consultants in Biomedical Research) of The Woodlands, Texas. Data for HSCW were analyzed using SPSS, Univariate Analysis of Variance, in a corrected model with Age at Harvest as the covariate. Data for P2 were analyzed in a corrected model with HSCW as the covariate. Data for Lifetime Dressed ROG were analyzed using simple one-way analysis of variance.

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REFERENCES

1. Reeves, DE (2006). The application of biotechnical and epidemiologic tools for pig health. *Anim Biotechnol* **17**: 177–187.
2. Plain, RL and Lawrence, JD (2003). Swine production. *Vet Clin North Am Food Anim Pract* **19**: 319–337.
3. Frutos, MG, Cacicedo, L, Fernandez, C, Vicent, D, Velasco, B, Zapatero, H *et al.* (2007). Insights into a role of GH secretagogues in reversing the age-related decline in the GH/IGF-I axis. *Am J Physiol Endocrinol Metab* **293**: E1140–E1152.

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4. Draghia-Akli, R, Fiorotto, ML, Hill, LA, Malone, PB, Deaver, DR and Schwartz, RJ (1999). Myogenic expression of an injectable protease-resistant growth hormone-releasing hormone augments long-term growth in pigs. *Nat Biotechnol* **17**: 1179–1183.
5. Draghia-Akli, R, Ellis, KM, Hill, LA, Malone, PB and Fiorotto, ML (2003). High-efficiency growth hormone releasing hormone plasmid vector administration into skeletal muscle mediated by electroporation in pigs. *FASEB J* **17**: 526–528.
6. Khan, AS, Fiorotto, ML, Hill, LA, Malone, PB, Cummings, KK, Parghi, D *et al.* (2002). Nonhereditary enhancement of progeny growth. *Endocrinology* **143**: 3561–3567.
7. Khan, AS, Fiorotto, ML, Cummings, KK, Pope, MA, Brown, PA and Draghia-Akli, R (2003). Maternal GHRH plasmid administration changes pituitary cell lineage and improves progeny growth of pigs. *Am J Physiol Endocrinol Metab* **285**: E224–E231.
8. Fiorotto, ML, Lopez, R, Oliver, WT, Khan, AS and Draghia-Akli, R (2006). Transplacental transfer of a growth hormone-releasing hormone peptide from mother to fetus in the rat. *DNA Cell Biol* **25**: 429–437.
9. Prud'homme, GJ, Glinka, Y, Khan, AS and Draghia-Akli, R (2006). Electroporation-enhanced nonviral gene transfer for the prevention or treatment of immunological, endocrine and neoplastic diseases. *Curr Gene Ther* **6**: 243–273.
10. Cheung, YK, Cheng, SC, Sin, FW and Xie, Y (2004). Plasmid encoding papillomavirus Type 16 (HPV16) DNA constructed with codon optimization improved the immunogenicity against HPV infection. *Vaccine* **23**: 629–638.
11. Bodles-Brakhop, AM, Brown, PA, Pope, MA and Draghia-Akli, R (2008). Double-blinded, placebo-controlled plasmid GHRH trial for cancer-associated anemia in dogs. *Mol Ther* **16**: 862–870.
12. Brown, PA, Bodles-Brakhop, AM and Draghia-Akli, R (2008). Plasmid growth hormone releasing hormone therapy in healthy and laminitis-afflicted horses-evaluation and pilot study. *J Gene Med* **10**: 564–574.
13. Pavlin, D, Tozon, N, Sersa, G, Pogacnik, A and Cemazar, M (2008). Efficient electrotransfection into canine muscle. *Technol Cancer Res Treat* **7**: 45–54.
14. Luckay, A, Sidhu, MK, Kjeken, R, Megati, S, Chong, SY, Roopchand, V *et al.* (2007). Effect of plasmid DNA vaccine design and *in vivo* electroporation on the resulting vaccine-specific immune responses in rhesus macaques. *J Virol* **81**: 5257–5269.
15. Serenius, T and Stalder, KJ (2006). Selection for sow longevity. *J Anim Sci* (84 suppl): E166–E171.
16. Lucia, T Jr.,Dial, GD and Marsh, WE (2000). Lifetime reproductive and financial performance of female swine. *J Am Vet Med Assoc* **216**: 1802–1809.
17. Takai, Y and Koketsu, Y (2007). Identification of a female-pig profile associated with lower productivity on commercial farms. *Theriogenology* **68**: 87–92.
18. Moeller, SJ, Goodwin, RN, Johnson, RK, Mabry, JW, Baas, TJ and Robison, OW (2004). The National Pork Producers Council Maternal Line National Genetic Evaluation Program: a comparison of six maternal genetic lines for female productivity measures over four parities. *J Anim Sci* **82**: 41–53.
19. Crenshaw, JD, Boyd, RD, Campbell, JM, Russell, LE, Moser, RL and Wilson, ME (2007). Lactation feed disappearance and weaning to estrus interval for sows fed spray-dried plasma. *J Anim Sci* **85**: 3442–3453.
20. Rehfeldt, C, Kuhn, G, Nurnberg, G, Kanitz, E, Schneider, F, Beyer, M *et al.* (2001). Effects of exogenous somatotropin during early gestation on maternal performance, fetal growth, and compositional traits in pigs. *J Anim Sci* **79**: 1789–1799.
21. Gatford, KL, Boyce, JM, Blackmore, K, Smits, RJ, Campbell, RG and Owens, PC (2004). Long-term, but not short-term, treatment with somatotropin during pregnancy in underfed pigs increases the body size of progeny at birth. *J Anim Sci* **82**: 93–101.
22. Trujillo-Ortega, ME, Mota-Rojas, D, Hernandez-Gonzalez, R, Velazquez-Armenta, EY, Nava-Ocampo, AA, Ramirez-Necoechea, R *et al.* (2006). Obstetric and neonatal outcomes to recombinant porcine somatotropin administered in the last third of pregnancy to primiparous sows. *J Endocrinol* **189**: 575–582.
23. Tollefsen, S, Vordermeier, M, Olsen, I, Storset, AK, Reitan, LJ, Clifford, D *et al.* (2003). DNA injection in combination with electroporation: a novel method for vaccination of farmed ruminants. *Scand J Immunol* **57**: 229–238.
24. Martinez, R, Estrada, MP, Berlanga, J, Guillen, I, Hernandez, O, Cabrera, E *et al.* (1996). Growth enhancement in transgenic tilapia by ectopic expression of tilapia growth hormone. *Mol Mar Biol Biotechnol* **5**: 62–70.
25. Arenal, A, Pimentel, R, Pimentel, E, Martin, L, Santiesteban, D, Franco, R *et al.* (2008). Growth enhancement of shrimp (*Litopenaeus schmitti*) after transfer of tilapia growth hormone gene. *Biotechnol Lett* **30**: 845–851.
26. Brown, PA, Bodles-Brakhop, AM and Draghia-Akli, R (2008). Effects of plasmid growth hormone releasing hormone treatment during heat stress. *DNA Cell Biol* (in press).
27. Castillo, C, Cruzado, M, Ariznavarreta, C, Gil-Loyzaga, P, Lahera, V, Cachofeiro, V *et al.* (2005). Effect of recombinant human growth hormone administration on body composition and vascular function and structure in old male Wistar rats. *Biogerontology* **6**: 303–312.
28. French, MC, Littlejohn, RP, Greer, GJ, Bain, WE, Mcewan, JC and Tisdall, DJ (2006). Growth hormone and ghrelin receptor genes are differentially expressed between genetically lean and fat selection lines of sheep. *J Anim Sci* **84**: 324–331.
29. Draghia-Akli, R and Fiorotto, ML (2004). A new plasmid-mediated approach to supplement somatotropin production in pigs. *J Anim Sci* **82** (E suppl.): E264–E269.
30. Thacker, EL, Holtkamp, DJ, Khan, AS, Brown, PA and Draghia-Akli, R (2006). Plasmid-mediated growth hormone-releasing hormone efficacy in reducing disease associated with *Mycoplasma hyopneumoniae* and porcine reproductive and respiratory syndrome virus infection. *J Anim Sci* **84**: 733–742.
31. Li, X, Eastman, EM, Schwartz, RJ and Draghia-Akli, R (1999). Synthetic muscle promoters: activities exceeding naturally occurring regulatory sequences. *Nat Biotechnol* **17**: 241–245.
32. Khan, AS, Smith, LC, Abruzzese, RV, Cummings, KK, Pope, MA, Brown, PA *et al.* (2003). Optimization of electroporation parameters for the intramuscular delivery of plasmids in pigs. *DNA Cell Biol* **22**: 807–814.