Effects of administration of two growth hormone–releasing hormone plasmids to gilts on sow and litter performance for the subsequent three gestations

Patricia A. Brown, PhD, DVM; Amir S. Khan, PhD; Ruxandra Draghia-Akli, MD, PhD; Melissa A. Pope, BS; Angela M. Bodles-Brakhop, PhD; Douglas R. Kern, DVM, MS

Objective—To determine whether a novel optimized plasmid carrying the porcine growth hormone–releasing hormone (GHRH) wild-type cDNA administered at a lower dose was as effective at eliciting physiologic responses as a commercial GHRH plasmid approved for use in Australia.

Animals—134 gilts.

Procedures—Estrus was synchronized and gilts were bred. Pregnant gilts were assigned to 2 treatment groups (40 gilts/group) or 1 untreated control group (24 gilts). Gilts in one of the treatment groups received the commercial GHRH plasmid, whereas gilts in the other treatment group received a novel optimized GHRH plasmid; both plasmids were administered IM in the right hind limb, which was followed by electroporation. Sow and litter performance were monitored for the 3 gestations after treatment.

Results—A significant increase in insulin-like growth factor-I concentrations, decrease in perinatal mortality rate, increase in the number of pigs born alive, and increase in the weight and number of pigs weaned were detected for both groups receiving the GHRH-expressing plasmids, compared with values for the control group. Additionally, there was a significant decrease in sow attrition in GHRH-treated females, compared with attrition in the control group, during the 3 gestations after treatment.

Conclusions and Clinical Relevance—Both of the GHRH plasmids provided significant benefits for sow performance and baby pig survivability for pregnant and lactating sows and their offspring during the 3 gestations after treatment, compared with results for untreated control gilts. Use of a novel optimized plasmid reduced the effective plasmid dose in these large mammals. (*Am J Vet Res* 2012;73:1428–1434)

Many consumers are interested in issues concerning the environment, food safety, animal well-being, and farm structure. Therefore, methods to improve the well-being and general health of production animals, decrease the use of antimicrobials, and reduce offspring morbidity and mortality rates are of particular interest to meet the protein consumption needs of these consumers. Manipulation of the GHRH–GH–IGF-I axis via GHRH[1] or somatotrophin[2] has been used for many years to enhance lean tissue deposition or milk production and increase feed efficiency in farm animals. However, the need for frequent injections is costly and time-consuming. In comparison, the administration of plasmid GHRH via electroporation is a safe, effective alternative that requires only a single injection.

Abbreviations

CpG	Cytosine phosphate guanine
GH	Growth hormone
GHRH	Growth hormone–releasing hormone
IGF	Insulin-like growth factor

Studies conducted over the past 2 decades have indicated that gene therapy is a viable alternative to traditional pharmaceutical or biological treatment in food and companion animals. Studies[3–6] have revealed that administration of GHRH plasmid via IM injection and electroporation and subsequent increases in downstream IGF-I concentrations are associated with improved maternal lactation, increased survival rate of offspring, and a higher rate of success for a second pregnancy. In early 2008, the

Received May 24, 2011.
Accepted September 22, 2011.
From VGX Animal Health Inc (Brown, Kern) and VGX Pharmaceuticals Inc (Khan, Draghia-Akli, Pope, Bodles-Brakhop), 2700 Research Forest Dr, The Woodlands, TX 77381. Dr. Khan's present address is Inovio Pharmaceuticals Inc, 1787 Sentry Pkwy W, Bldg 18, Ste 400, Blue Bell, PA 19422. Dr. Draghia-Akli's present address is European Commission, CDMA 02/169, Brussels, Belgium B-1049.
Supported by VGX Animal Health Inc, a subsidiary of Inovio Pharmaceuticals Inc.
The authors thank Karen S. Rosenbaum and Raymond W. Rosenbaum for assistance with the animals.
Address correspondence to Dr. Kern (dkern@vgxah.com).

Australian Pesticides and Veterinary Medicines Authority approved a plasmid for commercial use in pregnant swine, the first GHRH DNA treatment approved for use in food animals. A single treatment of pregnant gilts or sows reduces the perinatal mortality rate and improves production performance during 3 consecutive gestations.[7] Furthermore, in a study[8] in which baby pigs were cross-fostered, it was found that the improved preweaning growth of offspring born to GHRH plasmid-treated sows was attributable to improved maternal performance.

The commercial plasmid product has been described elsewhere.[7] The purpose of the study reported here was to evaluate the ability of a novel optimized plasmid to achieve a similar response when administered at a lower dose than that of the commercial plasmid. The success of an optimized plasmid could dramatically improve the feasibility for the use of plasmid-based DNA treatments in agricultural animals, companion animals, and humans.

Materials and Methods

Animals—Commercial maternal-cross (Large White × Landrace crossbred) gilts[a] (n = 134) were purchased for the study. The gilts used in the present study were genetically similar to pigs used in a previous study[7] in Australia. Gilts were tested prior to entry into the study and routinely during the study for swine influenza virus (H1N1 and H3N2), porcine reproductive and respiratory syndrome virus, and *Mycoplasma hyopneumoniae*. Results of all serologic tests were negative for these pathogens. All procedures conducted were reviewed and completed by or under the direct supervision of the company's licensed veterinary team in compliance with the standards of care set forth in the USDA APHIS regulations as stated in the Animal Welfare Act of 1966 and subsequent amendments.

Gilts were delivered to our research farm in Burton, Tex, via a single shipment from the nucleus herd. Gilts ranged from 60 to 180 days of age, with a 30-day interval between age groups (25 to 29 gilts/group). Each gilt received a double ear tag at the time of arrival at our facility.

Gilts were observed for evidence of their first estrus and placed in groups (ie, farrowing cohort) for subsequent synchronization of estrus and breeding. Within 21 days after the first observed estrus, groups of gilts were synchronized by means of a commercially available 0.22% altrenogest solution[b] in accordance with the manufacturer's recommendations. Gilts were bred by artificial insemination with semen obtained from terminal line boars.

Gilts were assessed for pregnancy via ultrasonographic examination at 28 to 35 days after insemination (day of insemination was designated as day 0 of gestation). Gilts within each farrowing cohort were assigned via a randomization procedure. All eligible pregnant gilts were weighed on the day of treatment. Gilts were then ranked from highest to lowest body weight, and gilts were then allocated into subsets of 3 animals/subset. When gilts reached 84 to 88 days of gestation, each subset of 3 animals was then assigned to 1 of 2 treatment groups or a control group via a random number generator. For each farrowing cohort, 8 gilts were administered the commercial GHRH plasmid,[d] 8 were administered the optimized GHRH plasmid, and the remainder were left as untreated control animals. Thus, there were 40 gilts in each of the plasmid-treated groups and 24 gilts in the control group (6, 5, 4, 4, and 5 for farrowing cohorts 1 through 5, respectively).

DNA constructs—Expression of the commercial plasmid[d] was driven by a muscle-specific synthetic promoter.[9] The plasmid was produced in accordance with current good manufacturing practices[e] and formulated in sterile water and 1% (wt/wt) low–molecular-weight poly-L-glutamate sodium salt. The release criteria for each plasmid were summarized (**Appendix**). The optimized plasmid contained the same porcine wild-type cDNA under the control of the same muscle-specific promoter. To optimize the commercial plasmid, several changes were made. These changes to the optimized GHRH plasmid included a completely synthetic plasmid backbone that was depleted of CpG; deletion of unnecessary plasmid backbone sequences; inclusion of a short, optimized origin of replication; and modifications to the expression cassette (in particular, a shorter, stronger GH polyadenylation signal).[10] The changes resulted in a 1,029-bp difference between the commercial plasmid and optimized plasmid (**Figure 1**).

Treatment—On days 84 to 88 of gestation, 1 mg of the optimized GHRH plasmid or 5 mg of the commercial GHRH plasmid was administered to each pregnant gilt in the respective treatment groups. Ketamine hydrochloride[f] (1.1 mg/kg) and tiletamine-zolazepam[g] (1.85 mg/kg) were injected via an auricular vein to induce short-term anesthesia. Once gilts were anesthetized, the respective plasmid was administered into the semimembranosus muscle of the right hind limb. All plasmid-treated groups received the specified dose in a total volume of 1 mL for the optimized plasmid group and 2 mL for the commercial plasmid group. In all treated gilts, plasmid injection was followed by electroporation[h] (0.5 A, pulse width of 52 milliseconds, 1-second interval between pulses, and 3 pulses/gilt) as described elsewhere.[11] No adverse effects of the treatment were detected.

Data collection—Gilts were housed in groups until shortly before parturition. Approximately 2 to 3 days prior to anticipated farrowing, the gilts were moved into farrowing crates. The number of baby pigs born alive or stillborn and the weight of baby pigs at the time of birth were recorded. Cross-fostering was minimized and, when necessary, was allowed only between sows within the same treatment group. All pigs in a farrowing cohort were weaned at 21 days of age; pigs in a cohort were weaned on the same day, regardless of their actual age. The number of pigs weaned per litter, the weight of individual pigs at weaning, total litter weight, and preweaning pig mortality rate were recorded. Data collected after weaning included the weekly weight of each pen of pigs, daily and weekly feed consumption, and pig mortality rate.

The process was repeated at monthly intervals for all 5 farrowing cohorts. Sows were bred and farrowed

a second and third time. Thus, pig growth and survivability endpoints were analyzed and reported for 3 gestations after a single treatment with the plasmid. If ≥ 3 of the 5 cohort groups decreased to < 12 sows/group because of culling or death of sows, the study was terminated after the remainder of the pregnant females farrowed. At the end of the study, all remaining plasmid-treated sows were euthanized under the supervision of a licensed veterinarian. Carcasses of all plasmid-treated sows (died or euthanized) were disposed of via incineration, burial in a landfill, or rendering for nonhuman consumption.

Collection of blood samples—Blood samples (5 to 10 mL) were collected into serum separator tubes from a subgroup of treated sows and offspring. Samples were collected from sows before treatment and at weaning and from offspring 7 and 28 days after farrowing. Samples were allowed to clot for 10 to 15 minutes at approximately 24°C and then centrifuged at 3,000 × *g* for 10 minutes. Serum was harvested and frozen at –80°C. Sera were used for assay of IGF-I concentrations.

Serum IGF-I concentrations—Serum IGF-I concentrations were measured with a heterologous human immunoradiometric assay kit.[i] All samples were assayed in duplicate. The interassay and intra-assay variability for IGF-I were both 4%; cross-reactivity of human IGF-I antibody for porcine IGF-I is 100%.

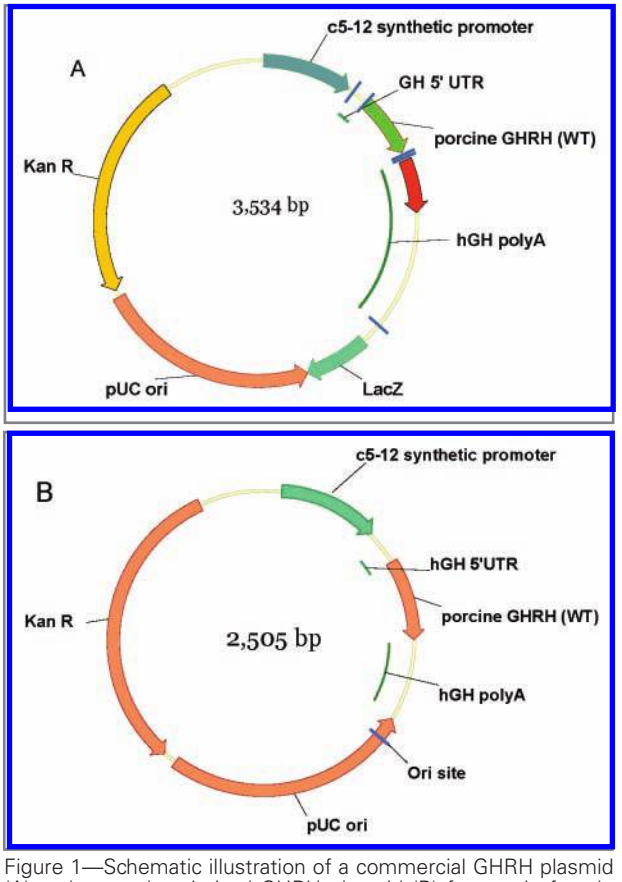

Figure 1—Schematic illustration of a commercial GHRH plasmid (A) and a novel optimized GHRH plasmid (B) for use in female swine. The optimized plasmid contains the same porcine wild-type cDNA under the control of the same muscle-specific promoter. hGH polyA = Human growth hormone polyadenylation site. Kan R = Kanamycine resistance gene. LacZ = β-Galactosidase structural gene. Ori site = Origin of replication. pUC ori = pUC vector origin of replication. UTR = Untranslated region. WT = Wild-type.

Statistical analysis—Results for the plasmid treatment groups were compared with results for the untreated control group. A commercial statistical analysis package[j] was used. Values were reported as mean ± SEM. Comparisons were performed via the Student *t* test. Values of $P < 0.05$ were considered significant.

Results

Treatment with the GHRH-expressing plasmids resulted in significant hormonal changes in the treated females, with significant increases in IGF-I concentrations, compared with the concentrations before treatment. Control females had an increase in IGF-I concentrations of 21% from day 84 of pregnancy to weaning (which was a typical change in IGF-I concentrations during the perinatal period), whereas treated gilts had significant increases in IGF-I concentrations (44% increase [$P = 0.02$] for the optimized plasmid and 46% increase [$P < 0.001$] for the commercial plasmid; **Figure 2**). Gilts treated with the optimized plasmid and the commercial plasmid had an increase in IGF-I concentration of 59% and 63%, respectively, compared with the concentration in control gilts. Offspring from treated and control females did not have significant differences in IGF-I concentrations at days 7 and 28 after farrowing (data not shown).

Mean ± SEM duration of gestation did not differ among groups (commercial plasmid, 114.5 ± 1.4 days; optimized plasmid, 114.3 ± 1.3 days; untreated control group, 114.2 ± 1.4 days). The number of days of lactation was the interval from parturition to weaning; there was no difference in the mean ± SEM number of days of lactation for the commercial plasmid (22.3 ± 2.1 days), optimized plasmid (22.5 ± 2.3 days), and untreated control (22.9 ± 2.4 days) groups.

Mean number of pigs born alive and mean number of pigs weaned during all 3 gestations were significantly increased for both plasmid-treated groups, compared with values for the untreated control group (**Figure 3**). For all 3 gestations after treatment, gilts treated with the optimized plasmid and commercial plasmid gave birth to significantly ($P = 0.04$) more live pigs per litter (mean, 1.0 and 1.4 more pigs born alive/litter, respectively) and had a significantly ($P = 0.029$) higher number of pigs weaned per litter (mean, 1.0 and 1.5 more pigs/litter, respectively), compared with results for the untreated control gilts (**Table 1**). For all 3 gestations after treatment, females treated with the optimized plasmid and commercial plasmid had a mean increase of 0.6 ($P = 0.033$) and 0.7 pigs/litter ($P = 0.038$) born alive and weaned a mean of 0.8 ($P = 0.038$) and 1.0 more pigs/litter ($P = 0.021$), respectively, compared with results for the untreated control females. Perinatal mortality rate was reduced by 10% and 14% and litter weaning weights increased by 13% and 7% for litters of females treated with the optimized plasmid and the commercial plasmid, respectively, compared with results for the untreated control females. There was no significant difference in the number of stillborn or mummified pigs during the 3 gestations among the 3 groups (**Table 2**).

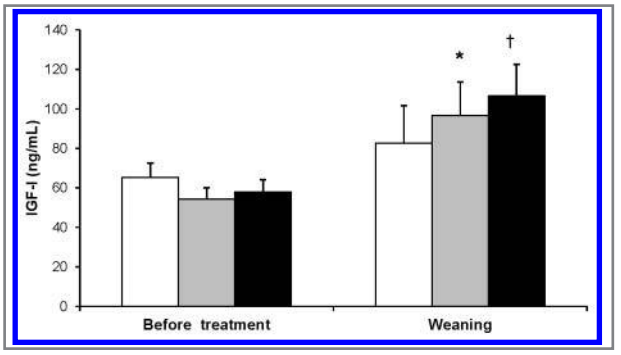

Figure 2—Mean ± SEM serum IGF-I concentrations in samples obtained from 13 gilts administered an optimized GHRH plasmid (gray bars), 15 gilts administered a commercial GHRH plasmid (black bars), and 8 untreated control gilts (white bars). Plasmids were injected between days 84 and 88 of gestation (day of insemination = day 0). All litters were weaned 21 days after farrowing. All samples were assayed in duplicate. *†Within a treatment group, value at weaning differs significantly (*$P = 0.02$; †$P < 0.001$) from the value before treatment.

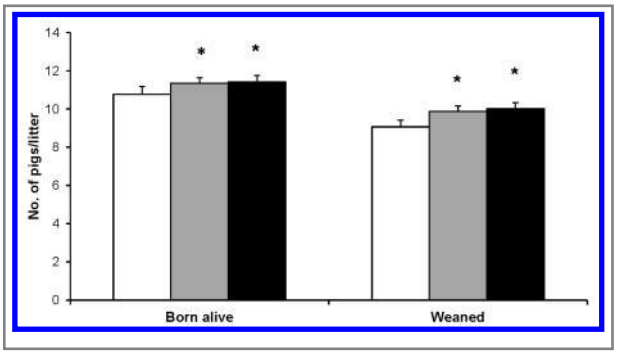

Figure 3—Mean ± SEM number of pigs born alive and number of pigs weaned for untreated control females (53 litters; white bars) and females treated with an optimized GHRH plasmid (93 litters; gray bars) or a commercial GHRH plasmid (114 litters; black bars) during the 3 gestations after treatment. *Within a variable, value differs significantly ($P < 0.05$) from the value for the control group.

Litters of both groups treated with GHRH plasmids outperformed litters of the control group during all 3 gestations (**Figure 4**). Litters of the females treated with the optimized plasmid had significantly higher birth weight ($P = 0.031$), weaning weight ($P = 0.006$), and total weight gain per litter ($P = 0.006$), compared with results for the litters of the control females. Within posttreatment gestations 1, 2, and 3, values for litters of the females treated with the commercial plasmid did not differ significantly from values for litters of the untreated control females with regard to birth weight, weaning weight, and total weight gain per litter. However, treatment with the optimized plasmid significantly increased birth weight, weaning weight, and total weight gain per litter, compared with values for the control treatment, within posttreatment gestations 1 ($P = 0.052$, 0.025, and 0.025, respectively) and 2 ($P = 0.025$, 0.030, and 0.046, respectively). For posttreatment gestation 3, there were no significant differences between the optimized plasmid groups and untreated control group with regard to birth weight, weaning weight, and total weight gain per litter.

Sow attrition was reduced for both plasmid treatments. For all 3 gestations after treatment, the attrition rate was 43.80%, 24.32%, and 23.68%, respectively, for the untreated control females and females treated with the optimized plasmid and commercial plasmid. Thus, there was a reduction of 44.5% and 45.9% for females treated with the optimized plasmid and commercial plasmid respectively, compared with results for the untreated control females. For the females treated with the commercial plasmid, there were initially 38 gilts successfully bred for gestation 1, which decreased to 34 sows for gestation 2 (attrition rate, 10.53%), with a further decrease to 28 sows for gestation 3 (attrition rate, 23.68%). For the females treated with the optimized plasmid, there were initially 37 gilts successfully bred for gestation 1, which decreased to 31 sows for gesta-

Table 1—Mean ± SEM number of pigs born alive and number of pigs weaned for each of 3 gestations after treatment in untreated control females and females injected on day 84 to 88 of gestation with a commercial GHRH plasmid or a novel optimized GHRH plasmid.

	Gestation 1			Gestation 2			Gestation 3		
Group	No. of litters farrowed	No. of pigs born alive	No. of pigs weaned	No. of litters farrowed	No. of pigs born alive	No. of pigs weaned	No. of litters farrowed	No. of pigs born alive	No. of pigs weaned
Commercial plasmid	38	11.53 ± 0.44*	10.08 ± 0.45*	34	10.44 ± 0.64	9.35 ± 0.63	28	12.50 ± 0.59	10.79 ± 0.51
Optimized plasmid	37	11.11 ± 0.41	9.54 ± 0.49	31	12.06 ± 0.33*	10.42 ± 0.38*	28	10.89 ± 0.63	9.75 ± 0.59
Control	23	10.09 ± 0.74	8.57 ± 0.67	19	10.32 ± 0.63	8.897 ± 0.51	14	12.46 ± 0.38	10.14 ± 0.41

*Within a column, value differs significantly ($P < 0.05$; Student *t* test) from the value for the control group.

Table 2—Mean ± SEM number of stillborn pigs and number of mummified pigs for each of 3 gestations after treatment in untreated control females and females injected on day 84 to 88 of gestation with a commercial GHRH plasmid or a novel optimized GHRH plasmid.

		Gestation 1		Gestation 2		Gestation 3		Total	
Variable	Group	No. of litters	Mean ± SEM	No. of litters	Mean ± SEM	No. of litters	Mean ± SEM	No. of litters	Mean ± SEM
Stillborn	Commercial plasmid	38	0.6 ± 0.14	34	0.4 ± 0.15	28	0.8 ± 0.22	100	0.6 ± 0.10
	Optimized plasmid	37	0.4 ± 0.13	31	0.8 ± 0.21	28	0.5 ± 0.17	96	0.6 ± 0.10
	Control	23	0.4 ± 0.12	19	0.5 ± 0.24	14	0.7 ± 0.32	56	0.5 ± 0.13
Mummified	Commercial plasmid	38	0.3 ± 0.11	34	0.4 ± 0.14	28	0.1 ± 0.05	100	0.3 ± 0.07
	Optimized plasmid	37	0.3 ± 0.09	31	0.6 ± 0.18	28	0.4 ± 0.17	96	0.4 ± 0.09
	Control	23	0.3 ± 0.16	19	0.5 ± 0.17	14	0.4 ± 0.15	56	0.4 ± 0.10

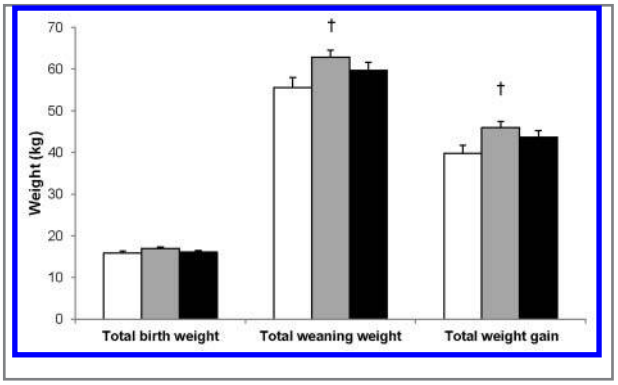

Figure 4—Mean ± SEM total birth weight, total weaning weight, and total weight gain per litter for untreated control females (56 litters; white bars) and females treated with an optimized GHRH plasmid (95 litters; gray bars) or a commercial GHRH plasmid (101 litters; black bars) during the 3 gestations after treatment. *†Within a variable, value differs significantly (*$P < 0.05$; †$P = 0.01$) from the value for the control group.

tion 2 (attrition rate, 16.22%), with a further decrease to 28 sows for gestation 3 (attrition rate, 24.32%). For the untreated control females, there were initially 23 females successfully bred for gestation 1, which decreased to 19 sows for gestation 2 (attrition rate of 17.39%), with a further decrease to 14 sows for gestation 3 (attrition rate, 43.48%).

Discussion

In the study reported here, optimization of a nonviral gene therapy delivered via IM injection and followed by electroporation was as safe and effective as the commercial plasmid at improving production performance and reducing morbidity and mortality rates for 3 consecutive sets of offspring of treated females after a single administration of the plasmid. Control gilts were not treated with a plasmid and did not receive electroporation; instead, they were used as a standard of care comparison. In another study[7] conducted by our research group in a farm setting, a single administration of the commercial plasmid significantly improved the general well-being of sows and offspring, increased production variables, and decreased morbidity and mortality rates. In the present study, our objective was to test the efficacy of an optimized GHRH plasmid administered at a dose lower than that of the commercial plasmid.

Administration of plasmid DNA alone has yielded some success, although it has been limited by inefficient amounts of expression. Delivery methods such as electroporation enhance plasmid DNA transfer to muscle, which increases long-term expression and has led to numerous ongoing clinical trials.[12] The combination of injection of DNA plasmid with electroporation is more effective than injection of DNA plasmid alone in large animals[13–15] and is becoming one of the most important means for delivery of nonviral gene treatments.[16] Administration of a GHRH plasmid followed by electroporation to 10-day-old pigs is effective at promoting growth, thus avoiding the need for daily injections associated with its peptide-hormone counterpart.[8] In the present study, a single administration of an optimized plasmid or commercial plasmid followed by electroporation improved the performance of offspring from 3 subsequent gestations, which indicated its long-term potential.

In the study reported here, optimization of the plasmid included a completely synthetic plasmid backbone that was depleted of CpG. The presence of CpG dinucleotides can induce an inflammatory response[17] and negatively affect the long-term expression of the desired protein[18,19] because of recognition by the immune system, in particular by the Toll-like receptor,[10] which elicits immune responses against transfected cells.[20] Therefore, CpG motifs were eliminated to increase the duration of expression. Optimization of transcriptional regulatory elements can also have substantial effects on expression.[21] Deletion of unnecessary plasmid backbone sequences; inclusion of a short, optimized origin of replication; and modifications to the expression cassette[22] (in particular, a shorter, stronger GH polyadenylation signal) were included to stabilize the mRNA and consequently improve expression and favor secretion of the newly produced protein from the cells.[10] Changes made to the novel optimized plasmid resulted in a 1,029-bp difference from that of the commercial plasmid. This smaller plasmid size means there are more plasmid copies per milligram. As a result, the optimized GHRH plasmid can be administered at one-fifth the dose of the commercial plasmid and still maintain similar physiologic effects. Although there was a difference in the volume used to administer the 2 plasmids (the optimized plasmid was administered at 1 mg in a 1-mL dose, compared with the commercial plasmid administered at 5 mg in a 2-mL dose), this variable alone appeared unlikely to substantially affect the results. In fact, concentrated formulations result in better expression.[23] Changes in IGF-I concentrations indicated that treatment with the optimized plasmid or commercial plasmid induced similar significant beneficial increases, compared with concentrations for untreated control females, which further supported our contention that the optimized plasmid at the lower dose was as effective as the commercial plasmid at the higher dose for optimizing the GHRH–GH–IGF-I axis.

Treatment with GHRH gene therapy followed by electroporation results in metabolic changes in the dam with improved maternal performance,[3,8] coupled with improvements in immune responses,[24] which impact the growth pattern and survival rate of offspring from birth to weaning as well as the overall health of the dam. Furthermore, GHRH-treated sows maintained better (although not significantly different) production efficiency during multiple gestations and in older age, compared with results for untreated control sows.[7] Hence, we suggest that a 1-time treatment with plasmid GHRH followed by electroporation is a safe and effective alternative to currently available treatments and protocols for maintaining and enhancing the longevity, productivity, and general well-being of an adult sow in a modern farm setting.

Comparison of the untreated control group with the treated groups revealed that both the optimized plasmid and commercial plasmid groups had improved longevity and performance during the lifetime of the sows. Offspring birth weight, weaning weight, and total weight gain per litter were improved for both treatment

groups and were significantly better for the optimized plasmid group, compared with the untreated control group, during 3 gestations. However, there was no significant difference between the 2 treatment groups, which indicated that treatment with the optimized plasmid was as effective as treatment with the commercial plasmid. There were significant differences in the number of pigs born alive and number of pigs weaned for the 2 treatment groups, compared with results for the untreated control group. In another study[7] conducted by our research group, treatment with the commercial plasmid resulted in a mean increase of 0.7 pigs born alive/litter during multiple gestations. In the present study, we detected the same mean increase of 0.7 pigs born alive/litter during multiple gestations after treatment with the commercial plasmid, whereas treatment with the optimized plasmid resulted in a mean increase of 0.6 pigs born alive/litter. Treatment with the commercial plasmid in a large study[7] in Australia resulted in a mean increase of 1.0 weaned/litter. In the present study, we detected the same mean increase of 1.0 pig weaned/litter after treatment with the commercial plasmid, whereas treatment with the optimized plasmid resulted in a mean increase of 0.8 pigs weaned/litter. Importantly, there was no significant difference in the number of pigs born alive or the number of pigs weaned between the 2 treatment groups. In the present study, we did not detect any significant differences in the number of stillborn or mummified offspring between treatment groups or when treatment groups were compared with the untreated control group. In that previous study[7] by our research group, there was a nonsignificant mean reduction of 0.1 pigs/litter in the number of stillborn pigs for commercial plasmid–treated litters, compared with the number in untreated control litters; similar results were obtained in the present study. The nonsignificant differences in the present study may have been attributable to low sample numbers, although small changes in the number of stillborn and mummified fetuses can have considerable value during the lifetime of a sow, especially when coupled with increases in the number of pigs born alive, resulting in a greater number of total pigs born to treated sows.

Treatment with the commercial GHRH plasmid or the optimized GHRH plasmid resulted in significant improvements in reproduction capacity and offspring survival rate for 3 gestations after a single treatment. Both treatments resulted in significant improvements in the number of baby pigs produced and lactation performance, compared with results for untreated control litters. These findings indicate that treatment with the optimized GHRH plasmid at a lower dose results in similar economic benefits as treatment with the commercial GHRH plasmid. Overall, plasmid optimization can be effective and, when used in combination with electroporation, will lead to further improvements for use of gene therapy in food animals.

a. SuperMom Parent Gilts, Newsham Choice Genetics, West Des Moines, Iowa.
b. Matrix, Intervet/Schering-Plough Animal Health, Summit, NJ.
c. XM boar line, Newsham Choice Genetics, West Des Moines, Iowa.
d. LifeTide SW5, VGX Animal Health Inc, The Woodlands, Tex.
e. VGXI Inc, The Woodlands, Tex.
f. Ketamine hydrochloride, Fort Dodge Animal Health, Fort Dodge, Iowa.
g. Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa.
h. Cellectra, Inovio Pharmaceuticals Inc, Blue Bell, Pa.
i. Diagnostic System Laboratories Inc, Webster, Tex.
j. Excel Spreadsheet, Microsoft Corp, Redmond, Wash.

References

1. Farmer C, Petitclerc D, Pelletier G, et al. Lactation performance of sows injected with growth hormone-releasing factor during gestation and(or) lactation. *J Anim Sci* 1992;70:2636–2642.
2. Gulay MS, Hayen MJ, Liboni M, et al. Low doses of bovine somatotropin during the transition period and early lactation improves milk yield, efficiency of production, and other physiological responses of Holstein cows. *J Dairy Sci* 2004;87:948–960.
3. Brown PA, Bodles-Brakhop AM, Draghia-Akli R. Effects of plasmid growth hormone releasing hormone treatment during heat stress. *DNA Cell Biol* 2008;27:629–635.
4. Draghia-Akli R, Fiorotto ML, Hill LA, et al. Myogenic expression of an injectable protease-resistant growth hormone-releasing hormone augments long-term growth in pigs. *Nat Biotechnol* 1999;17:1179–1183.
5. Draghia-Akli R, Ellis KM, Hill LA et al. High-efficiency growth hormone releasing hormone plasmid vector administration into skeletal muscle mediated by electroporation in pigs. *FASEB J* 2003;17:526–528.
6. Khan AS, Fiorotto ML, Cummings KK, et al. Maternal GHRH plasmid administration changes pituitary cell lineage and improves progeny growth of pigs. *Am J Physiol Endocrinol Metab* 2003;285:E224–E231.
7. Person R, Bodles-Brakhop AM, Pope MA, et al. Growth hormone-releasing hormone plasmid treatment by electroporation decreases offspring mortality over three pregnancies. *Mol Ther* 2008;16:1891–1897.
8. Khan AS, Draghia-Akli R, Shypailo RJ, et al. A comparison of the growth responses following intramuscular ghrh plasmid administration versus daily growth hormone injections in young pigs. *Mol Ther* 2009;18:327–333.
9. Li X, Eastman EM, Schwartz RJ, et al. Synthetic muscle promoters: activities exceeding naturally occurring regulatory sequences. *Nat Biotechnol* 1999;17:241–245.
10. Rabinovsky ED, Draghia-Akli R, Insulin-like growth factor I plasmid therapy promotes in vivo angiogenesis. *Mol Ther* 2004;9:46–54.
11. Khan AS, Smith LC, Abruzzese RV, et al. Optimization of electroporation parameters for the intramuscular delivery of plasmids in pigs. *DNA Cell Biol* 2003;22:807–814.
12. Hojman P, Gissel H, Gehl J, Sensitive and precise regulation of haemoglobin after gene transfer of erythropoietin to muscle tissue using electroporation. *Gene Ther* 2007;14:950–959.
13. Bodles-Brakhop AM, Brown PA, Pope MA, et al. Double-blinded, placebo-controlled plasmid ghrh trial for cancer-associated anemia in dogs. *Mol Ther* 2008;16:862–870.
14. Brown PA, Bodles-Brakhop A, Draghia-Akli R. Plasmid growth hormone releasing hormone therapy in healthy and laminitis-afflicted horses-evaluation and pilot study. *J Gene Med* 2008;10:564–574.
15. Pavlin D, Tozon N, Sersa G, et al. Efficient electrotransfection into canine muscle. *Technol Cancer Res Treat* 2008;7:45–54.
16. Reed SD, Li S. Electroporation advances in large animals. *Curr Gene Ther* 2009;9:316–326.
17. Hyde SC, Pringle IA, Abdullah S, et al. CpG-free plasmids confer reduced inflammation and sustained pulmonary gene expression. *Nat Biotechnol* 2008;26:549–551.
18. Hodges BL, Taylor KM, Joseph MF, et al. Long-term transgene expression from plasmid DNA gene therapy vectors is negatively affected by CpG dinucleotides. *Mol Ther* 2004;10:269–278.
19. Mitsui M, Nishikawa M, Zang L, et al. Effect of the content of

unmethylated CpG dinucleotides in plasmid DNA on the sustainability of transgene expression. *J Gene Med* 2009;11:435–443.
20. Kumagai Y, Takeuchi O, Akira S. TLR9 as a key receptor for the recognition of DNA. *Adv Drug Deliv Rev* 2008;60:795–804.
21. Xu ZL, Mizuguchi H, Ishii-Watabe A, et al. Strength evaluation of transcriptional regulatory elements for transgene expression by adenovirus vector. *J Control Release* 2002;81:155–163.
22. Papadakis ED, Nicklin SA, Baker AH, et al. Promoters and control elements: designing expression cassettes for gene therapy. *Curr Gene Ther* 2004;4:89–113.
23. Draghia-Akli R, Khan AS, Brown PA, et al. Parameters for DNA vaccination using adaptive constant-current electroporation in mouse and pig models. *Vaccine* 2008;26:5230–5237.
24. Thacker EL, Holtkamp DJ, Khan AS, et al. 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* 2006;84:733–742.

Appendix

Release criteria for a commercial GHRH plasmid and a novel optimized GHRH plasmid injected into gilts.

Release test	Specification	Commercial plasmid	Optimized plasmid
Concentration by A260 (mg/mL)	≥ 2.5	3.0	4.1
Purity by A260:A280	2.0	NA	2.0
Identity by restriction analysis	Conforms to standard	Conforms to standard	Conforms to standard
Circular forms (%)	≥ 80	95	94
Host-cell DNA (%)	≤ 5	≤ 0.000002	NA
Host-cell RNA (%)	≤ 5	0.6	≤ 0.2
Host cell protein (%)	≤ 5	≤ 0.1	≤ 0.1
Endotoxin (U/mg)	≤ 100	1	0.3
Osmolality (mOsm/kg of H_2O)	≤ 50	15	10
pH	5.0–10.0	6.0	NA
Appearance	Clear, colorless solution with no visible particles	Clear, colorless solution with no visible particles	Clear, colorless solution with no visible particles

A260 = Absorbance measured at 260 nm. A280 = Absorbance measured at 280 nm. NA = Not applicable.