DNA AND CELL BIOLOGY
Volume 27, Number 11, 2008

Pp. 629–635
DOI: 10.1089/dna.2008.0758

Effects of Plasmid Growth Hormone–Releasing Hormone Treatment during Heat Stress

Patricia A. Brown,[1] Angela M. Bodles-Brakhop,[2] and Ruxandra Draghia-Akli[2]

A gene therapy treatment with plasmid-based growth hormone–releasing hormone (GHRH) delivered by electroporation (EP) was investigated during heat stress; 32 primiparous cows received 2.5 mg of a GHRH-expressing myogenic plasmid (pSP-HV-GHRH), while 20 were designated as controls. Offspring of treated animals showed a reduction in mortality (47%; $p < 0.02$), and survival from birth to 260 days was dramatically improved (0% mortality vs. 21% in controls) along with an increase in weight gain ($p < 0.05$). Milk production was increased compared to controls with an average yield gain of 421 kg/cow ($p = 0.028$). Prolactin (PRL) levels were also significantly increased compared to controls ($p < 0.05$). The second pregnancy rate was improved by GHRH treatment (53.3% vs. 30.8%). This study shows that the use of plasmid-mediated therapy delivered by EP can maintain health status during periods of heat stress, important for both animals and potentially humans in hot, challenging climates.

Introduction

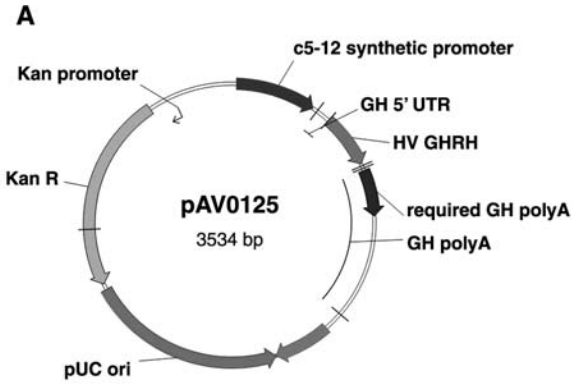
The seasons of the year can have a dramatic impact on humans and animals alike. As a model for heat stress we have chosen dairy cows. Dairy animals' growth, production, fertility, and lactation can be affected by extended periods of high temperature and relative humidity (West *et al.*, 2003). Only environmental solutions, such as cooling systems or misters, have been proposed to date (Collier *et al.*, 2006); nevertheless, this is not a practical solution in most cases, especially for humans (Harlan *et al.*, 2006). Investigation of alternative methods of dealing with heat stress is warranted especially with increasing climatic extremes (Patz *et al.*, 2005).

The administration of recombinant growth hormone (GH) has been used in farm animals for many years to enhance lean tissue deposition and or milk production, while increasing feed efficiency (Etherton *et al.*, 1986; Klindt *et al.*, 1998). Recombinant growth hormone–releasing hormone (GHRH) has also been used as a more physiologic alternative in experimental studies. The use of GHRH in large animal species (e.g., pigs or cattle) not only enhances growth performance and milk production, but also improves production efficiency from both a practical and a metabolic perspective (Dubreuil *et al.*, 1990; Farmer *et al.*, 1992). For example, the use of recombinant GHRH in lactating sows has beneficial effects on the growth of weanling pigs, although optimal nutritional and hormonal conditions are necessary for a dose of GHRH to realize its full potential (Farmer *et al.*, 1996). The administration of bovine somatotropin (bST) is the current method used to improve productivity in domestic animals and has been shown to enhance milk production (Gulay *et al.*, 2003, 2004) even under conditions of heat stress (Johnson *et al.*, 1991; Manalu *et al.*, 1991; Settivari *et al.*, 2007). Nevertheless, the weekly or biweekly administration can be costly, can result in adverse effects due to sudden supraphysiologic increases in GH, and can be time consuming.

Comparisons of GH and GHRH treatments, as peptide formulations, have been conducted in cattle. The two treatments resulted in similar milk composition, body condition score (BCS), and body weight (Binelli *et al.*, 1995). However, the use of the peptide GHRH treatment is impractical in long-term studies due to the short half-life of the hormone in the serum, the need for frequent repeat administrations, and significant costs. Plasmid-based therapy combined with *in vivo* electroporation (EP) is an effective way of administering small plasmid doses to achieve physiologically relevant levels of the desired transgene product (Mir *et al.*, 2005).

We have previously reported that the injection and EP of GHRH in dairy cattle result in improved immune function combined with reduced morbidity and improved BCS (Brown *et al.*, 2004). As an extension of the earlier study (Brown *et al.*, 2004), the objective of the present data analysis was to determine whether a plasmid-mediated GHRH therapy combined with EP would produce long-term beneficial effects in large animals during periods of heat stress when animals are known to not thrive. The results presented herein suggest that

[1]VGX Animal Health, The Woodlands, Texas.
[2]VGX Pharmaceuticals, The Woodlands, Texas.



B

	Signal peptide	GHRH (1-40)OH
HV GHRH	MVLWVFFFVILTLSNSSHCSPPPPLTLRMRR	**HV**DAIFTNSYRKVL**A**QLSARKLLQDI**LN**RQQGERNQEQGA
Porcine GHRH	MVLWVFFFVILTLSNSSHCSPPPPLTLRMRR	YADAIFTNSYRKVLGQLSARKLLQDI**MS**RQQGERNQEQGA
Bovine GHRH	MVLWVFFLVTLTLSSGSHGSLPS-QPLRIPR	YADAIFTNSYRKVLGQLSARKLLQDI**MN**RQQGERNQEQGA

FIG. 1. Maps and sequences. (**A**) Plasmid map of pAV0125 GHRH. (**B**) GHRH sequences: HV-GHRH analog, as well as bovine and porcine signal peptide, and mature GHRH (1–40)OH sequences are shown with differences in amino acids indicated by bold, underlined type.

plasmid-mediated GHRH therapy with EP can be a viable therapeutic alternative for heat stress.

Materials and Methods

DNA constructs

The expression of the plasmid pSPc5-12-HV-GHRH is driven by a 360 bp SPc5-12 muscle-specific synthetic promoter (Li *et al.*, 1999) (Fig. 1A). The synthetic GHRH cDNA, HV-GHRH was obtained by site-directed mutagenesis of porcine/bovine GHRH cDNA (1–40)OH (Altered Sites II *in vitro* Mutagenesis System; Promega, Madison, WI); the translation of porcine, bovine, and HV-GHRH with signal peptide and mature GHRH(1–40)OH is included (Fig. 1B). The GHRH cDNA is followed by the 3′ untranslated region of GH. Characterization of the vector and the long half-life analog HV-GHRH has been previously described (Draghia-Akli *et al.*, 1999).

Animals

Prior to the start of the experiment (10 days), animals ($n = 52$) were relocated from South Dakota to Texas. All plasmid-treated dairy cows and controls included in this study were heifers at treatment (i.e., at their first pregnancy). Thirty-two primiparous Holstein heifers, 18–20 months of age, were treated with plasmid once during the last trimester of gestation; the heifers were purchased certified pregnant. Similarly, 20 pregnant heifers from same source, breed, and age did not receive plasmid treatment and served as standard-of-care controls. Farm personnel were blinded to the treatment of animals. Animals calved at 23 months $\pm$ 24 days. Cows were housed in a free stall barn fitted with fans and water misters over the feed lane, exposed to natural daylight, and fed a silage-based total mixed ration (TMR) *ad libitum* twice daily. The high-producing ration used during lactation consisted of 18% protein, 18.75% acid detergent fiber (ADF), 32.2% neutral detergent fiber (NDF), and 1.74 mega (M) calories of net energy $(NE)_l$/kg of dry matter (DM). The base ration used during gestation consisted of 16.5% protein, 19.3% ADF, 32.4% NDF, and 1.72 Mcal NE_l/kg of DM. The herd was milked twice per day. Each cow was fitted with a transponder/pedometer that allowed for automatic identification upon entering the stall. Individual milk weights, conductivity, and activity were captured using AfiMilk software (Germania Dairy Automation, Waunakee, WI). One treated and four control animals were culled during the study. All milk and, at the end of the study, the slaughtered animals were treated with lye and buried in an approved location. Animal protocols were approved by VGX Pharmaceuticals, Inc., animal experimentation committee and conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals.

Second breeding and calves

The Ovsynch program (Willard *et al.*, 2003) was used to synchronize the second breeding, which was performed by artificial insemination (AI), of 43 out of the 52 lactating cows (30 treated and 13 controls); the other control cows were culled by the herdsman for genetic pooling reasons or due to poor performance status. A second treatment was not given before or during the second gestation. Approximately 6–8 months after the first calf was born, the cows were synchronized using the Ovsynch protocol and AI for the second time. Pregnancy was determined at 60 days after AI by rectal palpation. The rate of successful insemination that resulted in a second pregnancy was noted.

Calves were not treated with the GHRH-expressing plasmid, only the cows. Calves were separated from dams at day of birth and fed colostrum at a rate of 10% of their body weight in the first 24 h (calves from treated cows received colostrum from treated cows, and calves from controls received colostrum from control cows). The calves were then fed pasteurized milk supplemented with free choice calf starter from 2 days to weaning at 7 to 8 weeks of age. At weaning, the calves were put on pasture supplemented with hay and calf starter to 4 months of age, and changed to ground corn mix after 4 months of age.

Intramuscular injection of plasmid DNA

The endotoxin-free plasmid (Qiagen, Chatsworth, CA) preparation of pSPc5-12-HV-GHRH was diluted in water to 5 mg/mL and formulated with 1% poly-L-glutamate sodium salt (wt/wt). Treated animals were given 2.5 mg pSP-HV-GHRH intramuscularly in the neck using a 21-gauge needle (Becton-Dickinson, Franklin Lacks, NJ). Two min after injection, the muscle was electroporated (five pulses, 1 Amp, 52 ms/pulse), as described in the work by Draghia-Akli *et al.* (2002). Animals were observed immediately after injection and 24 h later for any adverse effects at the EP site, and then returned to pasture.

Prolactin analysis

Prolactin (PRL) levels for GHRH-treated and control heifers were analyzed at a single point on day 90 ($n = 19$ and $n = 4$, respectively) and day 135 ($n = 7$ and $n = 4$, respectively) postcalving. All animals were bled at the same time under the same climatic conditions. This assay was a *post hoc* analysis, and only animals that had sufficient leftover material after all the other tests were performed (CBC, biochemistries, immune markers, etc.) were included. Thus, random animals from both groups were included. Animals were bled from the jugular vein; samples were allowed to clot and then centrifuged immediately on site before being transport on ice to the lab where they were frozen at -80°C until required. Serum PRL was measured using a heterologous ovine immunoradiometric assay (performed in the laboratory of Dr. Spencer, Texas A&M University, College Station, Texas). The limit of sensitivity of the assay was 1 ng/mL. The intraassay variability was 5.6%. The cross-reactivity of ovine PRL antibody for bovine PRL was 100%.

Statistical analysis

Data consisted of repeated measures in different time points with unequal allocation of experimental units to treatment groups (treated $n = 32$; controls $n = 20$). Additional comparisons were performed when a significant ($p < 0.05$) treatment×day interaction was detected. A mixed model using SAS (analysis of simple main effects) was used to examine if there are any significant differences among the groups of each variable at different time points. For growth performance in calves, birth weight was included as a covariant to adjust for weight differences at subsequent time points. Categorical data, such as culling rate and calf mortality rate, were analyzed by ANOVA. Data were coded with numerical values such that ANOVA could be performed. For mortality and fertility rates, we developed an equivalent scoring system: alive = 1, dead = 0, pregnant = 1, and open = 0. Survival was analyzed by the Kaplan–Meier method. PRL was analyzed by ANOVA for repeated measures. The slope of weight gain in calves was calculated by linear regression. Values compared with Student's *t*-test, ANOVA, or linear regressions are presented in the results, with $p < 0.05$ taken as the level of statistical significance.

Results

In this pilot study, we assessed the effects of a plasmid-mediated GHRH therapy on the health status and welfare of a large animal model and their offspring during periods of heat stress. As this was a proof-of-concept study for a gene therapy approach, a relatively small number of animals were used. From June through September 2002 in the vicinity of the dairy farm in Texas, the average monthly temperature ranged from $87.87 \pm 1.01^{\circ}$F to $94.52 \pm 0.40^{\circ}$F with a heat index of $89.98 \pm 1.18^{\circ}$F to $99.77 \pm 0.44^{\circ}$F, indicating a period of severe heat stress for cattle. The animals had been previously acclimatized in South Dakota, where the heat index was significantly less severe and ranged from less than $83.19 \pm 0.99^{\circ}$F to $91.96 \pm 1.45^{\circ}$F, with average monthly temperatures between $74.53 \pm 1.97^{\circ}$F and $88.42 \pm 1.19^{\circ}$F. Periodic measurements at the farm were carried out during the months of July and August ($n = 20$). The average temperature at the farm was the same as at the weather station located 50 miles away ($88.00 \pm 1.04^{\circ}$F vs. $87.90 \pm 1.21^{\circ}$F, respectively; $p = 0.89$); however, the humidity was significantly higher at the farm (68.45 ± 2.79 vs. 60.75 ± 3.38, respectively; $p = 0.04$). Overall, the heat index was significantly higher at the farm than at the weather station (98.57 ± 1.74 vs. 93.54 ± 1.67, respectively; $p < 0.001$).

Calves born to treated heifers were 25% less likely to die at birth, though this change was not significant (19% vs. 25%) (Fig. 2). Postnatally, causes of death were diarrhea or wasting unresponsive to medical intervention. Overall calf mortality was reduced by 47% ($p < 0.02$) in treated animals compared to controls. During the 260 days' evaluation postnatal, calf mortality rates were 0% versus 21% ($p < 0.03$) for treated and controls, respectively (Fig. 2). We have further examined weight gain and average daily gains in calves born during the period of heat stress to treated heifers compared to those born to control heifers. There was no difference in the proportion of bulls and heifers born to the two groups (57% heifers and 43% bulls), nor in their birth or weaning weights. Growth curves became significantly divergent ($p < 0.05$) approximately 6 months after birth. The weight at 260 days of life was greater ($p < 0.05$) for calves from GHRH-treated heifers compared with calves from nontreated controls (Fig. 3). The slope of the linear regression of body weight on time from birth to 260 days differed ($p < 0.05$) for offspring of GHRH-treated heifers ($b = 0.80$) compared to control heifers ($b = 0.71$). The average

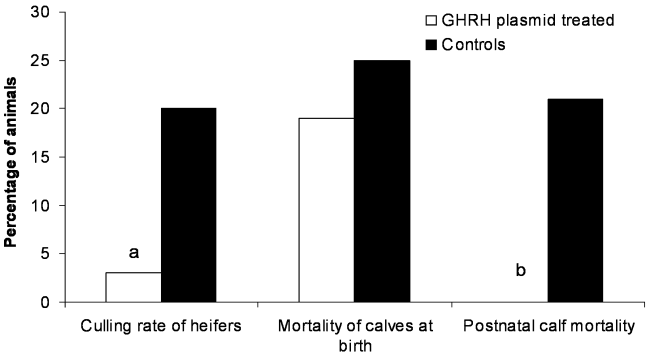


FIG. 2. Percentage of culling rate of heifers ($n = 52$; treated = 32 and controls = 20) and mortality in calves postnatal (treated $n = 24$ and controls $n = 16$). Heifer culling rate, $^{a}p < 0.003$. Overall postnatal calf's mortality, $^{b}p < 0.02$. Mortality of calves at birth ($n = 52$; treated = 32 and controls = 20) was reduced by the GHRH treatment without being statistically significant.

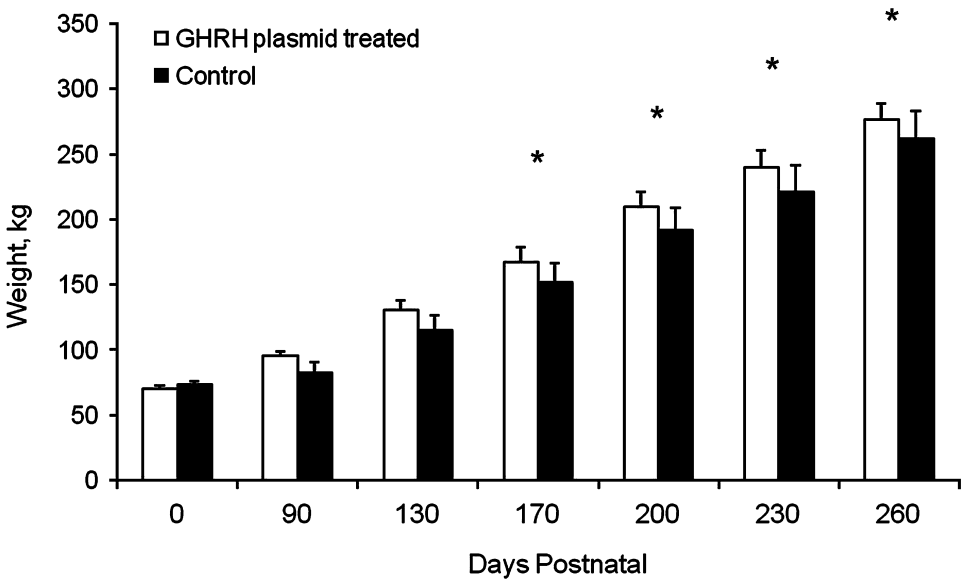


FIG. 3. Body weight of calves born to treated heifers ($n = 19$) versus those born to control heifers ($n = 8$) from birth to 260 days of life. Values are presented as mean ± SEM. *Within day, body weight differed between treatment groups, $p < 0.05$.

daily gain of calves was improved ($p < 0.045$) by the treatment of the dam with GHRH plasmid (1.27 vs. 1.13 kg/day).

The two groups of heifers were further studied by comparing the fertility rates. At the time of synchronization and second breeding, all cows ranged from 156 to 263 DIM with an average 227 DIM. At 60 days after insemination, 53% of the treated and 31% of the control were diagnosed pregnant by rectal palpation. The mean ± SEM was 0.533 ± 0.09 for GHRH-treated cows with a 95% confidence interval range of 0.3439–0.7228, and 0.3077 ± 0.1332 for control cows with a 95% confidence interval range of 0.017–0.598, with a relative risk of 1.73.

Milk production was also analyzed. Milk yield from day 30 to day 300 revealed up to an increase of 10–22% (27.45 ± 0.89 kg/day vs. 23.2 ± 1.0 kg/day) for GHRH-treated compared to nontreated controls. The total average increase in milk production between the GHRH-treated and nontreated controls was 421 kg ($p = 0.028$) (Fig. 4). No differences were detected in milk protein content.

Detection of GH was not practical in this study, given the variable GH levels during the day, and the fact that the cows were not continuously housed in the barn but were also turned out to pasture. PRL was significantly elevated at day 90 in GHRH-treated animals compared to controls (324.95 ± 37.66 vs. 166.20 ± 88.59, respectively; $p = 0.044$), and although the levels decreased with time, they were still significantly higher in the GHRH-treated animals at day 135 (120.27 ± 43.48 vs. 1.00 ± 0.00, respectively; $p = 0.034$) (Fig. 5).

The mortality of the heifers (involuntary cull rate) differed between the GHRH-treated and control animals. Twenty percent of the controls animals had to be culled. The causes of death were one John's disease, one systemic infection from hoof conditions and an infected cut, one animal with severe hoof problems complicated by rear leg paralysis, and one severe mastitis case. One treated animal was culled due to an accidental ingestion of a nail that perforated its stomach. The decision to remove an animal from the study was that of the attending herdsman.

Discussion

In this study 52 pregnant Holstein cows were relocated from South Dakota to Texas just before calving, which occurred between June and September. The heat index ranged from 97°F to 106°F, and the temperature humidity index (THI) ranged from 79°F to 83°F. A heat index of 71°F or below indicates a thermal neutral zone, and values ranging from 72°F to 79°F indicate mild stress, 80–89°F indicate moderate stress, and above 90°F indicate severe stress, while any THI over 77°F is considered severe (West *et al.*, 2003). Thus, the animals included in this study were under severe heat stress. While coolers, fans, and misters can be used to decrease heat stress, this is not a practical solution when animals and humans are in the field. Here we show that the physiologic stimulation of the GHRH axis by a plasmid-mediated therapy could alleviate some of the negative consequences of heat stress.

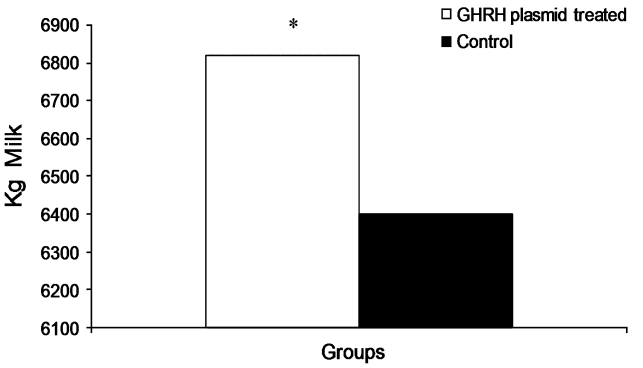


FIG. 4. Milk production increased in GHRH-treated animals compared to nontreated controls over 300 DIM. The total average milk yield per cow at 300 DIM resulted in an increase in total average milk yield/cow of 421 kg (*$p = 0.028$) for GHRH-treated animals ($n = 19$ at 300 DIM, GHRH-treated animals; $n = 13$ at 300 DIM, nontreated controls).

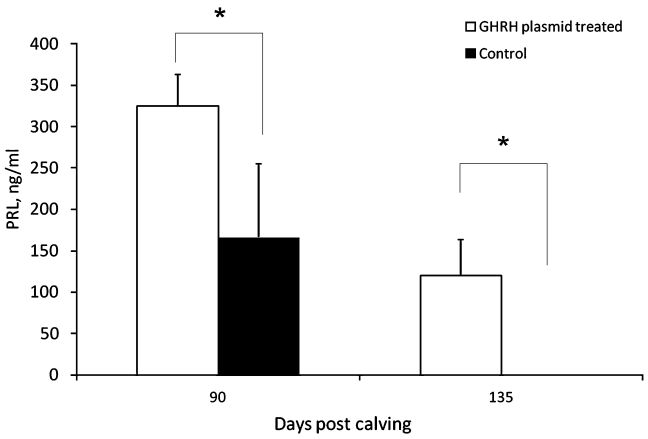


FIG. 5. PRL levels for GHRH-treated and control heifers at day 90 ($n = 19$ and $n = 4$, respectively) and day 135 ($n = 7$ and $n = 4$, respectively) postcalving. At day 135 the control PRL levels were too low to be detected. Asterisk (*) indicates significant difference where $p < 0.05$ between treated and control groups at the time points indicated.

Although recombinant GHRH protein therapy entrains and stimulates normal cyclical GH secretion with virtually no adverse effects, the short half-life of GHRH *in vivo* requires frequent administration (Evans *et al.*, 1985; Thorner *et al.*, 1986), and is therefore not practical as a therapy or as an experimental tool to study the long-term effects of GHRH administration. A plasmid-mediated supplementation could overcome this limitation to GHRH use. For instance, gilts treated in the last part of gestation with a HV-GHRH–expressing plasmid had heavier piglets, and the offspring's postnatal growth rate and welfare was enhanced. Changes in pituitary cell lineage in the offspring of treated pregnant sows and rats have been shown, with higher numbers of GH and PRL-producing cells, which can then directly impact their growth and welfare once the postnatal growth comes under the control of GH and IGF-I (Khan *et al.*, 2003; Fiorotto *et al.*, 2006). The increased growth in offspring is not due to maternal pregnancy diabetes (glucose and insulin were normal in all studies, including this one).

Intramuscular injection of plasmid followed by EP has been used in many models, including humans (Prud'homme *et al.*, 2007), for vaccination or therapeutic applications. Previous studies using GHRH showed that plasmid therapy with EP represents a promising approach to induce regulated production and secretion of proteins in large animal models (reviewed in Prud'homme *et al.*, 2006). Here, we have compared GHRH treatment by EP with the standard of care for dairy cattle. Previously, we have discussed (Brown *et al.*, 2004) and experimentally shown (Khan *et al.*, 2003) that it is not the injection and/or the EP that determines these responses (i.e., CpG sequences in the plasmid backbone that may be immunomodulatory, as our plasmids contain a similar number of immunostimulatory and immunodepressant CpGs), but the effects of GHRH expression after plasmid administration. The similarity of the response across species (rats, pigs, and dairy cattle) suggests that the physiologic stimulation of the GHRH axis is a fundamental component of developmental physiology. Notably, in this study we have shown that physiologic changes can be obtained by using the IM plasmid injection and EP in a large farm animal by using a very small plasmid quantity: 2.5 mg of plasmid in a 530 kg animal, that is, 4.7 μg/kg, rendering this technique a valuable alternative to repeat peptide administration for experimental or therapeutic purposes.

Culling is a major economic problem in the farm animal industry (Radke and Shook, 2001). Methods that could decrease the rate of both voluntary and involuntary culling, improve BCS, reduce pathology in the herd, and improve reproductive parameters especially under conditions of heat stress are highly desirable. We have previously shown that the overall welfare of the GHRH-treated dairy cattle improved, with decreased mortality and morbidity rates (3% vs. 20% in controls, $p < 0.003$). IGF-I levels were analyzed, and physiologic increases correlated with improved BCS (Brown *et al.*, 2004). Increases in immune responses were observed in GHRH-treated animals. As a consequence, animals treated with GHRH displayed an improvement in their hoof pathology compared to control animals (7 of 32 GHRH-treated animals vs. 7 of 20 controls) (Brown *et al.*, 2004). Other studies report the association of hoof pathology with decreased milk yield (Hernandez *et al.*, 2002, 2005; Sogstad *et al.*, 2007). However, in this follow-up report, we show up to a 22% increase in milk production over 300 DIM for GHRH-treated compared to control. Thus, while a small percentage of the milk production increase can be directly attributed to be the resolution of hoof problems, the majority is not. We also report that offspring calves did not experience any adverse effects from the therapy, such as associated pathology or death. Mortality rates were reduced in the calves, and their growth rates enhanced. It is also known that calves born to 2-year-old cows are more susceptible to severe weather conditions than calves born to older cows (Azzam *et al.*, 1993). The GHRH treatment seems to substantially decrease this risk.

Previous studies showed that first-service pregnancy rates at day 45 are increased in cows not resynchronized that initiated bST treatment at 63 days postpartum, compared with cows initiating bST treatment at 105 days postpartum ($37.7 \pm 5.8\%$ and $22.1 \pm 4.2\%$, respectively), but the effect of bST treatment is not observed when cows were resynchronized ($25.6 \pm 4.3\%$ and $25.8 \pm 5.5\%$, respectively) (Moreira *et al.*, 2000). Ovsynch was evaluated for its practical use in multiple herds; the first-service conception rates continued to average 35% (Cartmill *et al.*, 2001; Pancarci *et al.*, 2002), but are lower in herds located in Texas at around 28% (Pancarci *et al.*, 2002). It is well known that maternal heat stress reduces oocyte competence for fertilization and postfertilization development, but the mechanism is unknown (de Castro e Paula *et al.*, 2008). In the current study, treated heifers received pSP-HV-GHRH in the third part of their first gestation. No subsequent treatment was administered, but GHRH-treated cows displayed a 53% rate of first insemination compared to 31% of the control, a 67.7% increase.

Overall, treatment with the plasmid GHRH resulted in an increase in the rate of pregnancy after first insemination, decreased morbidity and culling rates, increased body scores, and increased milk production, as well as improved welfare in a herd animal and their offspring during a period of heat stress. This gene therapy proof-of-concept study demonstrates the effectiveness of GHRH in a large animal model that will pave the way for future studies in humans.

Acknowledgments

The authors would like to thank Dr. Robert H. Carpenter for his contribution and members of our research team for their assistance, Melissa A. Pope and Kathleen K. Cummings. Our special acknowledgments go to the Chaloupka family for their excellent animal husbandry. We acknowledge support for this study from VGX Pharmaceuticals, Inc. (formally ADViSYS, Inc.), The Woodlands, Texas.

References

Azzam,S.M.,Kinder,J.E.,Nielsen,M.K.,Werth,L.A.,Gregory,K.E., Cundiff, L.V., and Koch, R.M. (1993). Environmental effects on neonatal mortality of beef calves. J Anim Sci **71,** 282–290.

Binelli, M., Vanderkooi, W.K., Chapin, L.T., Vandehaar, M.J., Turner, J.D., Moseley, W.M., and Tucker, H.A. (1995). Comparison of growth hormone-releasing factor and somatotropin: body growth and lactation of primiparous cows. J Dairy Sci **78,** 2129–2139.

Brown, P.A., Davis, W.C., and Draghia-Akli, R. (2004). Immune enhancing effects of growth hormone releasing hormone delivered by plasmid injection and electroporation. Mol Ther **10,** 644–651.

Cartmill, J.A., El Zarkouny, S.Z., Hensley, B.A., Lamb, G.C., and Stevenson, J.S. (2001). Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J Dairy Sci **84,** 1051–1059.

Collier, R.J., Dahl, G.E., and VanBaale, M.J. (2006). Major advances associated with environmental effects on dairy cattle. J Dairy Sci **89,** 1244–1253.

de Castro e Paula, L., Andrzejewski, J., Julian, D., Spicer, L.J., and Hansen, P.J. (2008). Oxygen and steroid concentrations in preovulatory follicles of lactating dairy cows exposed to acute heat stress. Theriogenology **69,** 805–813.

Draghia-Akli, R., Fiorotto, M.L., Hill, L.A., Malone, P.B., Deaver, D.R., and Schwartz, R.J. (1999). Myogenic expression of an injectable protease-resistant growth hormone-releasing hormone augments long-term growth in pigs. Nat Biotechnol **17,** 1179–1183.

Draghia-Akli, R., Khan, A.S., Cummings, K.K., Parghi, D., Carpenter, R.H., and Brown, P.A. (2002). Electrical enhancement of formulated plasmid delivery in animals. Technol Cancer Res Treat **1,** 365–371.

Dubreuil, P., Petitclerc, D., Pelletier, G., Gaudreau, P., Farmer, C., Mowles, T.F., and Brazeau, P. (1990). Effect of dose and frequency of administration of a potent analog of human growth hormone-releasing factor on hormone secretion and growth in pigs. J Anim Sci **68,** 1254–1268.

Etherton, T.D., Wiggins, J.P., Chung, C.S., Evock, C.M., Rebhun, J.F., and Walton, P.E. (1986). Stimulation of pig growth performance by porcine growth hormone and growth hormone-releasing factor. J Anim Sci **63,** 1389–1399.

Evans, W.S., Vance, M.L., Kaiser, D.L., Sellers, R.P., Borges, J.L., Downs, T.R., Frohman, L.A., Rivier, J., Vale, W., and Thorner, M.O. (1985). Effects of intravenous, subcutaneous, and intranasal administration of growth hormone (GH)-releasing hormone-40 on serum GH concentrations in normal men. J Clin Endocrinol Metab **61,** 846–850.

Farmer, C., Petitclerc, D., Pelletier, G., and Brazeau, P. (1992). Lactation performance of sows injected with growth hormone-releasing factor during gestation and(or) lactation. J Anim Sci **70,** 2636–2642.

Farmer, C., Robert, S., and Matte, J.J. (1996). Lactation performance of sows fed a bulky diet during gestation and receiving growth hormone-releasing factor during lactation. J Anim Sci **74,** 1298–1306.

Fiorotto, M.L., Lopez, R., Oliver, W.T., Khan, A.S., 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.

Gulay, M.S., Hayen, M.J., Liboni, M., Belloso, T.I., Wilcox, C.J., and Head, H.H. (2004). 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 **87,** 948–960.

Gulay, M.S., Hayen, M.J., Teixeira, L.C., Wilcox, C.J., and Head, H.H. (2003). Responses of Holstein cows to a low dose of somatotropin (bST) prepartum and postpartum. J Dairy Sci **86,** 3195–3205.

Harlan, S.L., Brazel, A.J., Prashad, L., Stefanov, W.L., and Larsen, L. (2006). Neighborhood microclimates and vulnerability to heat stress. Soc Sci Med **63,** 2847–2863.

Hernandez, J., Shearer, J.K., and Webb, D.W. (2002). Effect of lameness on milk yield in dairy cows. J Am Vet Med Assoc **220,** 640–644.

Hernandez, J.A., Garbarino, E.J., Shearer, J.K., Risco, C.A., and Thatcher, W.W. (2005). Comparison of milk yield in dairy cows with different degrees of lameness. J Am Vet Med Assoc **227,** 1292–1296.

Johnson, H.D., Li, R., Manalu, W., Spencer-Johnson, K.J., Becker, B.A., Collier, R.J., and C.A Baile, C.A. (1991). Effects of somatotropin on milk yield and physiological responses during summer farm and hot laboratory conditions. J Dairy Sci **74,** 1250–1262.

Khan, A.S., Fiorotto, M.L., Cummings, K.K., Pope, M.A., Brown, P.A., 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.

Klindt, J., Yen, J.T., Buonomo, F.C., Roberts, A.J., and Wise, T. (1998). Growth, body composition, and endocrine responses to chronic administration of insulin-like growth factor I and(or) porcine growth hormone in pigs. J Anim Sci **76,** 2368–2381.

Li, X., Eastman, E.M., Schwartz, R.J., and Draghia-Akli, R. (1999). Synthetic muscle promoters: activities exceeding naturally occurring regulatory sequences. Nat Biotechnol **17,** 241–245.

Manalu, W., Johnson, H.D., Li, R.Z., Becker, B.A., and Collier, R.J. (1991). Assessment of thermal status of somatotropin-injected lactating Holstein cows maintained under controlled-laboratory thermoneutral, hot and cold environments. J Nutr **121,** 2006–2019.

Mir, L.M., Moller, P.H., Andre, F., and Gehl, J. (2005). Electric pulse-mediated gene delivery to various animal tissues. Adv Genet **54,** 83–114.

Moreira, F., Risco, C.A., Pires, M.F., Ambrose, J.D., Drost, M., and Thatcher, W.W. (2000). Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. J Dairy Sci **83,** 1237–1247.

Pancarci, S.M., Jordan, E.R., Risco, C.A., Schouten, M.J., Lopes, F.L., Moreira, F., and Thatcher, W.W. (2002). Use of estradiol cypionate in a presynchronized timed artificial insemination program for lactating dairy cattle. J Dairy Sci **85,** 122–131.

Patz, J.A., Campbell-Lendrum, D., Holloway, T., and Foley, J.A. (2005). Impact of regional climate change on human health. Nature **438,** 310–317.

Prud'homme, G.J., Draghia-Akli, R., and Wang, Q. (2007). Plasmid-based gene therapy of diabetes mellitus. Gene Ther **14,** 553–564.

Prud'homme, G.J., Glinka, Y., Khan, A.S., 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.

Radke, B., and Shook, G. (2001). Culling and genetic improvement programs for dairy herds. In *Food Animal Production Medicine*. O.M. Radostits, ed. (W.B. Saunders Company, Philadelphia, PA), p. 291.

Settivari, R.S., Spain, J.N., Ellersieck, M R., Byatt, J.C., Collier, R.J., and Spiers, D.E. (2007). Relationship of thermal status to productivity in heat-stressed dairy cows given recombinant bovine somatotropin. J Dairy Sci **90,** 1265–1280.

Sogstad, A.M., Osteras, O., Fjeldaas, T., and Refsdal, A.O. (2007). Bovine claw and limb disorders at claw trimming related to milk yield. J Dairy Sci **90,** 749–759.

Thorner, M.O., Vance, M.L., Evans, W.S., Rogol, A.D., Rivier, J., Vale, W., and Blizzard, R.M. (1986). Clinical studies with GHRH in man. Horm Res **24,** 91–98.

West, J.W., Mullinix, B.G., and Bernard, J.K. (2003). Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J Dairy Sci **86,** 232–242.

Willard, S., Gandy, S., Bowers, S., Graves, K., Elias, A., and Whisnant, C. (2003). The effects of GnRH administration postinsemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. Theriogenology **59,** 1799–1810.

Address reprint requests to:
Ruxandra Draghia-Akli, M.D., Ph.D.
VGX Pharmaceuticals
2700 Research Forest Drive
The Woodlands, TX 77381

E-mail: rdraghia@vgxp.com

Received for publication April 21, 2008; received in revised form June 3, 2008; accepted July 7, 2008.