Immunocontraception of Florida Feral Swine with a Single-dose GnRH Vaccine

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Problem
Methods to limit fertility of feral swine are needed to reduce transmission of diseases and agricultural and ecosystem damage.

Method of Study
We evaluated a single-shot GnRH immunocontraceptive vaccine in both male and female feral swine for its effect on fertility and functional status of the reproductive tissues. Captive feral pigs were randomly assigned to receive 1000 or 2000 µg GnRH-KLH vaccine treatments or no treatment.

Results
After 36 weeks, none of the 2000-µg-treated females and only 20% of the 1000-µg-treated females were pregnant. This corresponded to reduced serum progesterone, regressed tissues within the reproductive tract and lack of evidence for follicular development leading to ovulation. Males were less responsive to the vaccine than females, but more responsive to the lower dose of the vaccine than the higher dose.

Conclusions
The single-shot GnRH vaccine is effective in controlling fertility of female feral swine and may be useful for population reduction.

Introduction

Populations of feral swine (*Sus scrofa*) have been reported for 23 states with the total population estimated to be 3 million animals.[1,2] Although recreational hunting of feral swine is popular in in some states, overabundance of feral swine is a problem that raises concerns relating to ecosystem and agricultural damage, and of disease threat to wildlife, domestic livestock and humans.

Feral swine are known reservoirs for both brucellosis and pseudorabies. Swine brucellosis is caused by the bacterium *Brucella suis* and is characterized by abortion, infertility, lameness, and vulval discharges in infected animals.[3] The afterbirth of *Brucella*-infected pigs may also transmit the disease to cattle.[4,5] Humans are also susceptible to swine brucellosis, putting persons who come in contact with feral swine at risk. Pseudorabies is a highly contagious herpes viral disease which occurs in swine. The virulence of the feral swine pseudorabies strain is considerably less than that of the strain isolated from domestic pigs.[2] However, once swine are infected with the virus they will remain infected for the rest of their lives, although they may not show clinical signs of the disease. Swine infected with pseudorabies are capable of transmitting the disease to other species including cattle, sheep, goats, horses, dogs, and cats. Pseudorabies infections in these secondary species are usually fatal.

Control of fertility of overabundant wildlife and feral species is a topic that has received considerable attention in recent years.[6] Immunocontraceptive vaccines have been the focus of much infertility research with the goal of population management being a single-dose, long-acting contraceptive that is highly effective with minimal adverse effects. Research on an immunocontraceptive vaccine of particular interest for swine targets gonadotropin-releasing hormone (GnRH). Several short-term investigations have studied GnRH vaccines for commercial swine production with an interest in using the approach as an alternative to surgical castration of boars.[7–9] The GnRH vaccine stimulates antibody production to inactivate endogenous GnRH, and thereby reduces release of gonadotrophic hormones leading to gonadal atrophy.[10] As a result, regression or lack of development of the reproductive organs results in 'immunological castration'. Although evidence from other species suggests that the vaccine is effective in both males and females, and produces both infertility and cessation of sexual activity, studies with swine have primarily focused on evaluating the effects of the vaccine in males. Recently, however, we demonstrated that a single-shot GnRH vaccine developed at the National Wildlife Research Center, USDA-APHIS was 80–90% effective in preventing estrus behavior and pregnancy in domestic pigs.[11]

Evidence suggests that among feral swine, oral contact with reproductive discharges and sexual activity are the primary means of transmission of both brucellosis[4] and pseudorabies.[12] Our interest in evaluation of a GnRH immunocontraceptive vaccine for feral swine is based on recent studies with deer and domestic pigs in which we demonstrated that both fertility and reproductive behavior are greatly diminished.[11,13] We believe that the GnRH vaccine represents a potentially ideal contraceptive vaccine for feral swine because it induces infertility in both sexes and limits the spread of brucellosis and pseudorabies by limiting sexual activity.

Although we have demonstrated the effectiveness of our GnRH vaccine in domestic swine,[11] we were also interested in establishing the efficacy of the vaccine in a mixed feral population of different size males and females, including pregnant, non-pregnant and breeding animals, as this is more typical of what would be encountered in the field. Therefore, the present study was undertaken to evaluate a single-shot GnRH vaccine in both male and female feral swine for its effect on fertility and status of the reproductive tissues. This effort represents the first step in developing a strategy to use the vaccine to limit fertility and reproductive behavior as a means to reducing the transmission of brucellosis and pseudorabies among feral swine.

Materials and Methods

Male and female feral swine of unknown history were captured throughout Florida in January–March of 2002. The animals were brought to a farm near Trenton, Florida where they were tested for pseudorabies and brucellosis and dewormed. Animals which tested positive for brucellosis and pseudorabies were not used in the study. Some of the female pigs were pregnant when they were captured. Although the weights of the animals were not taken, the range was estimated to be 7–32 kg. Pigs were maintained for the entire study in outdoor pens located in a shaded, sandy area. The study was approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University.

The GnRH vaccine and adjuvant used were developed at the National Wildlife Research Center and were previously evaluated in white-tailed deer.[13] The vaccine consisted of the GnRH peptide conjugated to keyhole limpet hemocyanin used in combination with AdjuVac™ adjuvant (National Wildlife Research Center, USDA-APHIS, Ft. Collins, CO, USA). The adjuvant contained a modified Johne's vaccine, Mycopar™ (Ft. Dodge Animal Health, Overland Park, KS, USA), as a replacement for Freund's adjuvant.

Male and female pigs were randomly assigned to 1000- or 2000-μg GnRH-KLH vaccine treatment or no treatment. Eleven females received the 1000-μg dose, nine females received the 2000-μg dose and five females were untreated. Ten males received the 1000-μg dose, 10 males received the 2000-μg dose and four males were untreated.

At the beginning of the study, blood samples were taken and the pigs were immunized with the GnRH vaccine by an intramuscular injection in the rump. Males and females were separated, and large and small pigs of each sex were further sorted into separate pens. Approximately 12 weeks after immunization, blood samples were taken and both treated and untreated males and females were combined together into two large breeding pens where they remained for the duration of the study. Each pen contained both treated and untreated males and female for the remainder of the study. Although

there was no designated observation period, breeding activity was recorded if it was observed. Sows giving birth during the study were noted, as was the pregnancy status of the females at the end of the study. During the study, two females assigned to the 1000-μg treatment and one male assigned to the 2000-μg treatment died. In addition, two females and one male in the control groups died. There was no evidence that the deaths were related to the treatments.

In mid-December, 36 weeks after immunization, pigs were killed, a blood sample was taken and the ovaries and testes were recovered. The gonads were weighed and a sample of tissue was fixed in 10% formalin-Ca^{++} and later prepared for histological evaluation. For a few animals in each treatment, tissue samples of uterus, oviduct, and epididymis were also collected for histological evaluation. Blood samples were used to assay antibody titers for GnRH and testosterone and progesterone.

Histological evaluations were made on coded slides. In males, the testis was evaluated for the presence of cell types associated with spermatogenesis including spermatogonia, primary spermatocytes, round spermatids and elongated spermatids. The functional state of the Leydig cells and the presence of spermatozoa in the epididymis were also considered. For females, the ovaries were examined for stages of follicular development and presence of corpora lutea. Uterus and oviduct samples were examined to determine whether the epithelia lining the uterine and oviduct lumen were active or regressed.

Differences among treated and untreated groups were assessed by least significant difference tests[14] for multiple comparisons at $P < 0.05$.

Results

Antibody titers for GnRH were negative in pre-immunization samples and in all samples taken from untreated pigs. For males, antibody titers were greatest 12 weeks after immunization with either the 1000- or 2000-μg dose vaccine (Fig. 1), although titers for both doses declined after 36 weeks. Values for males receiving the 1000-μg dose were greater than those receiving the 2000-μg dose at both time points, but were only significant at 36 weeks. In contrast, females receiving either the 1000- or 2000-μg dose had similar titers at 12 weeks, but those receiving the 2000-μg dose significantly greater titers at 36 weeks than those receiving 1000 μg of GnRH (Fig. 1).
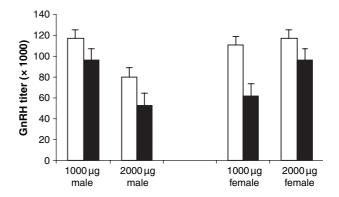

Fig. 1 Serum antibody titers at 12 weeks (open bars) and 36 weeks (solid bars) post-immunization of male ($n = 10$) and female ($n = 9$) feral pigs receiving a single intramuscular injection of either a 1000 or 2000 μg dose of GnRH vaccine. Data are not shown for serum titers of untreated pigs ($n = 5$), which were all negative.

Average serum progesterone concentrations for all females were ≤6 μg/ml at the initial bleed and 12 weeks thereafter. After 36 weeks, average serum progesterone concentrations were also <6 μg/ml for the treated females, and were significantly less than untreated females which averaged 32 μg/ml of progesterone at 36 weeks. Progesterone values for untreated females corresponded with the percentage of females within each treatment group having corpora lutea in ovaries collected at the end of the study (Fig. 2). Treated females had considerably fewer corpora lutea than control females.

Average serum testosterone concentrations were similar for all groups of males at the start of the study. However, serum testosterone concentrations were significantly less than those of untreated males
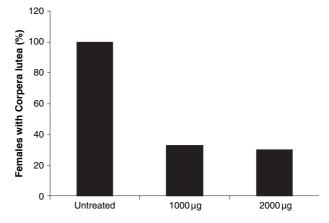

Fig. 2 Percentage of females with ovaries containing corpora lutea in the untreated ($n = 3$), 1000 μg ($n = 9$) and 2000 μg ($n = 8$) treatment groups.

at both 12 and 36 weeks for males receiving either 1000 or 2000 µg of GnRH (Fig. 3). However, testis weight at 36 weeks was only significantly lower in males receiving the 1000-µg dose (Fig. 4).

Testis tissue from untreated males contained all cell types associated with normal spermatogenesis. In the 1000-µg group, Leydig cells were regressed in four of the 10 males examined, while only two of eight males examined in the 2000-µg group had regressed Leydig cells. No males in the 2000-µg group and only three of 10 males (30%) in the 1000-µg showed a negative effect on primary sper-
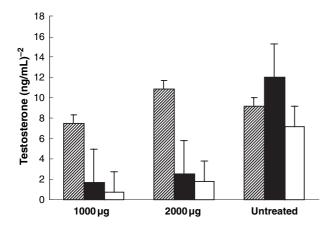

Fig. 3 Plasma testosterone concentrations at the start of the study (striped bars) and at 12 weeks (solid bars) and 36 weeks (open bars) post-immunization. For the 1000 µg, $n = 10$ for each time point. For the 2000-µg group $n = 8$ at the initial and 12 week samples and 7 at the 36-week sampling. For the untreated males, $n = 4$ for the initial sample and $n = 3$ for the 12- and 36-week samplings. Error bars are mean ± standard error.
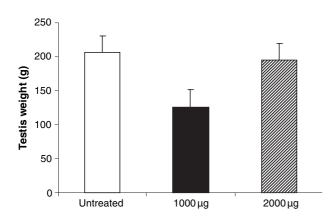

Fig. 4 Testis weights at 36 weeks post-immunization for untreated ($n = 3$), 1000 µg ($n = 10$) and 2000 µg ($n = 7$) treated boars. Error bars are mean ± standard error.

matocytes. Four of 10 males (40%) in the 1000-µg treatment had evidence of a negative effect on round and elongated spermatids, and reduced sperm in the epididymis. However, in the 2000-µg group, no males showed a negative impact on round spermatids or epididymal sperm, and only two of seven males (29%) had a negative effect on elongated spermatids. These observations are consistent with the conclusion that the lower vaccine dose was more effective in interfering with spermatogenesis than the higher vaccine dose.

Ovarian tissue from untreated females contained primary and tertiary follicles and evidence of recent corpora lutea. Although all females in both treated groups showed evidence of follicular development through the tertiary stage, the majority of these females in both treated groups (70% and 67%, respectively) lacked evidence of recent corpora lutea, indicating an absence of recent ovulations (Fig. 2). Although uterine tissue was not recovered from all treated animals, three of five samples recovered from the 1000-µg-treated females (60%) and seven of nine samples from the 2000-µg-treated females (78%) had evidence of regressed uterine epithelia. These observations are consistent with the reduced fertility and the regressed reproductive tracts of the treated females.

Pregnancy status was noted during the study and at slaughter (Fig. 5). All untreated females gave birth
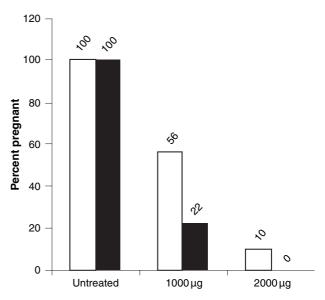

Fig. 5 Percentage females pregnant in untreated ($n = 3$) and 1000 µg ($n = 9$) and 2000 µg ($n = 9$) treated groups during the study (open bars), and at the conclusion of the study (closed bars).

sometime during the course of the study ($n = 5$), and all surviving swine were pregnant at slaughter ($n = 3$). In contrast, only 56% and 10% of the females gave birth during the study in the 1000-μg ($n = 9$) and 2000-μg ($n = 9$) treated groups, respectively. Moreover, at slaughter, only 11% of the females receiving the 1000-μg dose ($n = 9$) were pregnant and none of the females receiving the 2000-μg dose ($n = 9$) were pregnant.

Discussion

GnRH vaccines have been under development and of primary interest for use in several domestic species, including cattle, horses, sheep, as well as swine.[15] Males have been the primary focus of these studies with an interest in developing a vaccine capable of immunological castration. GnRH vaccines that produce adequate antibody titers are believed to act on the hypothalamus and inhibit synthesis and secretion of GnRH which normally stimulates production of follicle stimulating hormone and luteinizing hormone by the anterior pituitary gland. In the absence of these hormones, normal stimulation of the testes and ovaries is compromised and gonadal regression occurs. In the present study, a single-injection GnRH-KLH vaccine combined with AdjuVac adjuvant was effective in producing antibody titers that were sufficient to impact several aspects of reproduction in both male and female feral pigs.

In males, the effects included reduced testis weight, reduced plasma testosterone and some histological evidence for effects on spermatogenesis and Leydig cell regression. However, the histochemical analyses suggested that spermatogenesis was disrupted in only a few males as a result of the treatment. A limitation of this study was that it was not possible to actually measure the fertility of individual males, making it difficult to assess the true effect of the vaccine with respect to contraception. In addition, the 36-week study may not have been adequate to assess the full effect of antibody titers on spermatogenesis.

An unexpected result was that the negative effect of the vaccine appeared to be greater in males given the lower 1000-μg dose than that of the 2000-μg dose during the entire 36-week study. Although difficult to explain, this observation was consistent for all end points measured and was clearly associated with greater antibody titers for males receiving the 1000-μg dose. Higher titers were associated with greater physiological effects, but not with greater vaccine dose. Under the conditions of this study, it does not appear that either of the GnRH vaccine doses had a clear negative effect on the reproductive physiology of male feral swine. These observations may justify evaluation of additional vaccine doses for males below 1000 μg, or between 1000 and 2000 μg to determine if greater antibody titers and physiological effects can be achieved than were observed in this study.

In contrast to the observations made for males, the GnRH vaccine was highly effective for contraception of females. Contraceptive effects of the single-shot vaccine prevented pregnancy after 36 weeks in all the females receiving the 2000-μg dose, and in 80% of the females receiving the 1000-μg dose. During the study, only one female receiving the higher-dose vaccine produced offspring, while 55% of the females receiving the lower dose had piglets during the 36-week study. Differences observed between the two treatments appear to be related to anti-GnRH titers present at slaughter. It is noteworthy that while titers were similar between both treatments 12 weeks after immunization, the higher titer was better sustained after 36 weeks in females receiving the 2000-μg dose.

Because the pregnancy status of the females during the course of the study could only be determined for those that farrowed or were obviously pregnant, it is not possible to know with certainty the mechanism of the anti-fertility effect. Relative to controls, the percentage of females with corpora lutea was considerably less in the treated groups suggesting that fewer ovulations occurred in the treated females leading to a negative effect on fertility. However, as approximately 30% of the treated females had evidence of recent ovulations at slaughter, and only 22% (1000 μg) and 0% (2000 μg) of the treated females were pregnant at slaughter it is possible that the infertility effect was the result of early embryonic mortality or abortion.

An intriguing observation related to the vaccine doses was that males responded better to the lower dose than the higher vaccine dose, contrary to what was observed for females. Gender differences do exist in the pattern of GnRH and gonadotropic hormone secretion. Non-pregnant females typically have a cyclic pattern of hormone secretion related to the estrous cycle in contrast to males that secrete GnRH at a relatively constant rate. Although the absolute titers of males and females were similar at

12 weeks, titers of males appeared to decline at 36 weeks while those of females appeared to increase. It is possible that the cyclic pattern of hormone secretion in females served as a periodic 'self-boosting' immunization, resulting in a greater ability to sustain an immune response over time. In addition, with a steady pattern of GnRH secretion in males, it is possible that circulating levels of GnRH antibodies could have been reduced through the formation of antibody-GnRH complexes. Another difference between male and female gamete physiology is that over the period of the female reproductive cycle, 10–20 oocytes are ovulated, whereas in males, literally billions of sperm are produced. These relative differences in the number of gametes produced clearly suggest that the challenge to eliminate or interfere with sperm production is far greater than that for oocyte production.

Evaluation of the injectable single-shot GnRH vaccine in feral swine has shown it to be highly effective in reducing fertility of females for the 36-week study. These observations agree with results obtained with the single-shot GnRH vaccine used on domestic gilts.[11] In contrast to the study with domestic gilts, however, the feral females treated were comprised of a mixed population of sows and gilts of different sizes and pregnancy status. These findings indicate that the anti-fertility effects of the GnRH vaccine would be broadly effective for treating diverse populations of feral females encountered in the field. Although the vaccine gave less impressive results in males, in practical terms these finding are encouraging because females are the likely target of contraceptive use in the field. An additional beneficial effect of GnRH vaccine use in the field would be the reduction of mating behavior, which would serve to reduce venereal transmission of pseudorabies and brucellosis among feral swine. Because an injectable form of a contraceptive vaccine will have only limited field application for feral swine, emphasis and resources in future research will be directed toward further development of this GnRH vaccine into a form for oral delivery.

Conclusions

The single-shot GnRH immunocontraceptive vaccine was effective in reducing fertility of a mixed population of male and female feral swine and may be useful for contraception and disease control of feral populations.

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