

## EVALUATION OF GnRH CONTRACEPTIVE VACCINE IN CAPTIVE FERAL SWINE IN FLORIDA

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**Abstract:** We evaluated a GnRH contraceptive vaccine in penned feral swine that were captured throughout Florida. In March 2002, pigs (~7-30kg) were segregated by sex and assigned to two GnRH treatments, given as a single IM injection in the rump using AdjuVac adjuvant. Ten males and 9 females received 1000µg GnRH-KLH; 10 males and 9 females received 2000µg GnRH-KLH. Untreated males and females served as controls. In mid-June 2002, blood was taken and males and females were combined into two large breeding pens. Animals were euthanized in December and blood and tissue samples were taken. Reduced testicular and ovarian size, and serum testosterone and progesterone were evident in GnRH-treated animals and corresponded to increased antibody titers to GnRH. The most effective GnRH vaccine dose for females was 2000µg; for males it was 1000µg. The 2000µg GnRH vaccine prevented pregnancy in 100% of the females at slaughter and 90% of the females during the 36-week study. For females receiving the 1000µg GnRH vaccine, 78% were not pregnant at slaughter, but only 44% were not pregnant during the entire study. We conclude that the single-shot GnRH vaccine is highly effective for contraception of feral female swine for up to 36 weeks.

**Key words:** feral swine, GnRH vaccine, immunocontraception

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### INTRODUCTION

Overpopulation of feral swine raises concerns relating to damage of wild ecosystems and agricultural crop damage. Rooting and feeding behaviors can cause considerable damage to native vegetation as well as to forest plantings, row crops and pastures. Feral swine are also recognized as

disease reservoirs for brucellosis and pseudorabies among other diseases, and increase the risk of disease spread to other wildlife, domestic livestock and humans. Population reduction using contra-ceptives has the potential to minimize these concerns providing that their safety and efficacy is demonstrated. Immunocontraceptive

vaccines meeting these criteria, and of interest for wildlife population control, are those that target the zona pellucida of the ovum or the hypothalamic peptide, gonadotropin releasing hormone (GnRH). Although the porcine zona pellucida vaccine has been shown to be effective for contraception of several wildlife species (Fagerstone et al. 2002), its use for swine is problematic because the vaccine is prepared using porcine zona pellucida protein, making it less immunogenic.

The GnRH vaccine has been studied in domestic and farm animals for potential as a non-surgical castrating agent, and in swine to eliminate boar taint (Dunshea et al. 2001). In short duration studies GnRH vaccines have been evaluated as immunocastration agents in cattle (Adams and Adams 1992), horses (Rabb et al. 1990), and swine (Meloan et al. 1994, Oonk et al. 1998, Zeng et al. 2002). Recently, we completed a long-term study using a GnRH vaccine on white-tailed deer establishing its efficacy, safety and reversibility (Miller et al. 2000). The objectives of the present study were to evaluate the effects of a single-shot GnRH vaccine on reproductive physiology and contraception of male and female feral swine (*Sus scrofa*).

## **MATERIALS AND METHODS**

The study was conducted on a farm near Gainesville, FL. The site was a wooded area that included several outdoor pens to confine the feral swine. Wild pigs of unknown history were captured throughout Florida from January-March 2002 and brought to the study site for preliminary testing. Although no body weights were taken, estimates ranged from 7-32 kg. Some pigs were obviously pregnant, most were sexually mature, but some were immature at the start of the study. Initially, pigs were dewormed, tested for brucellosis and

pseudorabies and positive reactors were eliminated.

The GnRH vaccine was developed at the National Wildlife Research Center and has been used previously in white-tailed deer (Miller et al. 2000). A synthetic peptide of GnRH was conjugated to KLH and combined with AdjuVac adjuvant to prepare 1 ml vaccination doses.

Pigs were segregated by sex and randomly assigned to two GnRH treatments, given as a single IM injection in the rump. Ten males and 9 females received 1000 $\mu$ g GnRH-KLH; 10 males and 9 females received 2000 $\mu$ g GnRH-KLH. Five males and 5 females served as untreated controls. On March 21, 2002, blood samples were taken and pigs immunized. Males and females were segregated and also sorted into pens by large and small size. In mid-June 2002, approximately 12 weeks after immunization, blood samples were taken and males and females were combined into two large breeding pens. Observations made throughout the study indicated some mounting and breeding activity. Females having litters during the 36-week study were recorded, as were those females that were pregnant at slaughter.

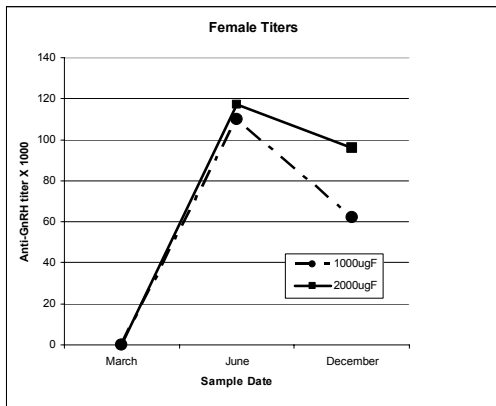
During the second week of December 2002, pigs were euthanized and blood and tissue samples were taken. Reproductive tracts were excised, and testes and ovaries were weighed. Pregnancy status of females was recorded. Blood serum samples were assayed for antibody titers and progesterone or testosterone concentrations as previously described (Miller and Killian 2001).

## **RESULTS**

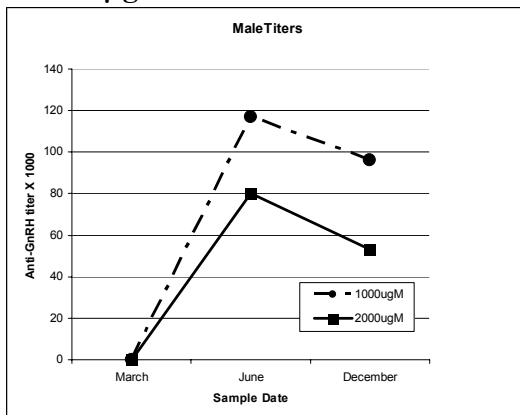
Females given the 2000 $\mu$ g GnRH vaccine produced somewhat higher titers than those given the 1000 $\mu$ g GnRH dose 12 weeks after vaccination (Figure 1a) but the 2000 $\mu$ g dose was clearly more effective in

sustaining the antibody titer 36 weeks post-vaccination than the 1000 $\mu$ g dose. In contrast, the 1000 $\mu$ g GnRH vaccine in males was more effective in producing antibody titers at both 12 and 36 weeks than the 2000 $\mu$ g dose (Figure 1b).

**Figure 1a. Average serum antibody titers (x10000) of female feral swine determined in March at immunization and in June and December, 12 and 36 weeks post-immunization for females receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH-KLH vaccinations.**



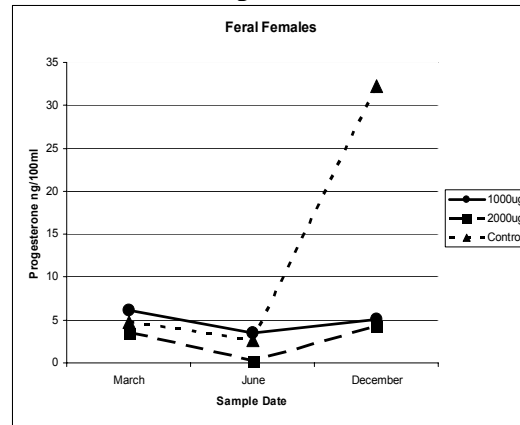
**Figure 1b. Average serum antibody titers (x10000) of male feral swine determined in March at immunization and in June and December, 12 and 36 weeks post-immunization for males receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH-KLH vaccinations.**



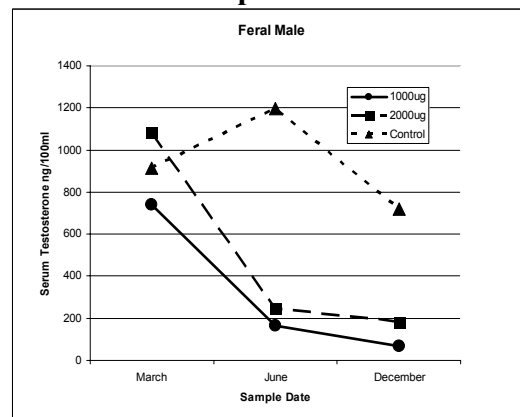
In females, average serum progesterone of treated animals was similar

to controls at 12 weeks post immunization, but was lower than controls at 36 weeks in both 1000 $\mu$ g and 2000 $\mu$ g GnRH vaccine-treated females than in controls (Figure 2a). In males, average serum testosterone was less in treated males than controls at both 12 and 36 weeks (Figure 2b).

**Figure 2a. Average serum progesterone concentrations of female feral swine receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH-KLH vaccinations. Determinations were made for the initial sample in March and for samples obtained in June and December, 12 and 36 weeks post-immunization.**

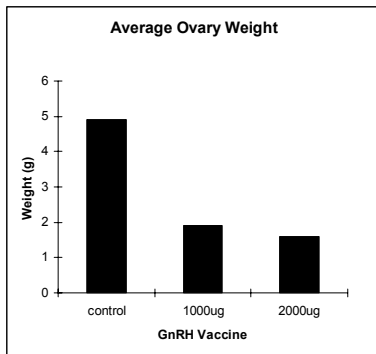


**Figure 2b. Average serum testosterone concentrations of male feral swine receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH-KLH vaccinations. Determinations were made for the initial sample in March and for samples obtained in June and December, 12 and 36 weeks post-immunization.**

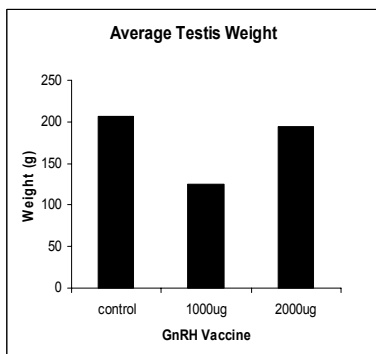


At slaughter, reproductive tracts were regressed and inactive in most of the GnRH vaccinated females, but never in the female controls. Regressed reproductive tracts appeared similar to those of prepubertal animals. Fully regressed testes were occasionally seen in the treated males, but intermediate stages of regression were the most commonly observed in the treated male. Average weight of both ovaries at slaughter was similar for treated females, and less than controls (Figure 3a). Average weight of the testes was only less than the controls in males receiving the 1000 $\mu$ g GnRH vaccine dose (Figure 3b), which corresponded to the higher antibody titers in male seen with this dose (Figure 1b).

**Figure 3a. Average weight of both ovaries recovered from feral swine receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH vaccine treatments or untreated controls.**

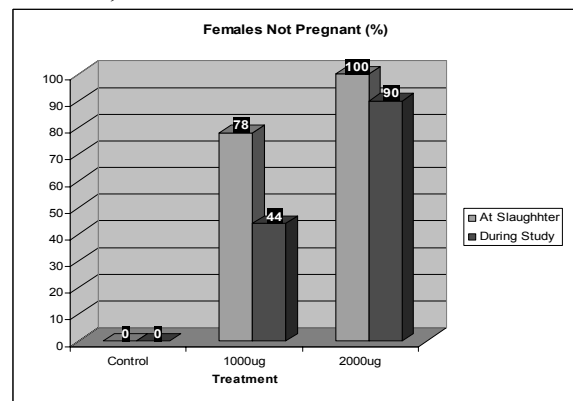


**Figure 3b. Average weight of both testes recovered from feral swine receiving 1000 $\mu$ g or 2000 $\mu$ g GnRH vaccine treatments or untreated controls.**



The ability of the GnRH vaccine to prevent pregnancy and farrowing prior to slaughter was evaluated by recording females who had litters during the 36-week study. Some of these females were pregnant at the start of the study based on their farrowing dates, whereas others became pregnant during the study. For purposes of treatment analysis, these observations are summarized as the percent females not pregnant during the entire study and the percent not pregnant at slaughter (Figure 4). As expected, all of the controls were pregnant during the study and at slaughter. The 2000 $\mu$ g GnRH vaccine was most effective in preventing pregnancy, with 100% of the females not pregnant at slaughter and 90% not pregnant during the 36-week study. Of the females receiving the 1000 $\mu$ g GnRH vaccine, 78% were not pregnant at slaughter, but only 44% were not pregnant during the entire study.

**Figure 4. Percent females not pregnant during the entire study and the percent not pregnant at slaughter for feral swine treated with 1000 $\mu$ g or 2000 $\mu$ g GnRH vaccine, or for treated controls.**



## DISCUSSION

The present study demonstrated that the GnRH vaccine was effective in generating antibody titers in both male and female feral swine that altered several aspects of reproductive physiology. These

effects included reduced ovary and testis weight, reduced plasma testosterone and progesterone concentrations, and reduced pregnancy rates in treated pigs compared to controls. The believed mechanism of action of the anti-GnRH titers produced by the vaccine is to inactivate GnRH from the hypothalamus, and thereby block the normal stimulation of gonadotropic hormones which regulate reproductive steroid and gamete production by the testes and ovaries (Miller et al. 2000). The effects we observed in the treated pigs in the present study are consistent with this mechanism of action.

In the context of contraception of feral swine, the most impressive results were obtained with the single-shot 2000 $\mu$ g GnRH vaccine given to the females. None of the females in this group were pregnant at slaughter and only one of the females farrowed during the study. Because she farrowed in late November, we estimate she conceived in early August. Most of the females in the 2000 $\mu$ g group had reproductive tracts that were clearly regressed and ovaries that were inactive. Almost 80% of the females receiving the 1000 $\mu$ g GnRH vaccine were infertile at slaughter, whereas only about 45% remained infertile for the entire study. The differences observed between the two treatments are likely related to the greater anti-GnRH titers present at slaughter in the females receiving the 2000 $\mu$ g vaccine. It is noteworthy that while titers were similar between both treatments 12 weeks after, the titer was better sustained after 36 weeks in females receiving the 2000 $\mu$ g dose.

The physiological responses of males to the vaccine were generally less definitive than that observed for the females. The experimental design did not enable us to specifically test the fertility of individual males, because control and treated males were commingled with females. However, based on testicular weights taken at

slaughter and serum testosterone values, we are able to make some inferences about the treatment effects on the males. Serum testosterone was clearly less in both groups of treated males than in controls. Interestingly, reduction in testicular weight compared to the controls was greatest in males receiving the 1000 $\mu$ g treatment. This is likely associated with higher anti-GnRH titers in the 1000 $\mu$ g vaccine-treated males throughout the study than those receiving the 2000 $\mu$ g dose.

An unexpected finding was that the optimal dose for the maximal negative effect on reproductive physiology was the 1000 $\mu$ g dose for males and 2000 $\mu$ g for females. It is difficult to know why these gender differences exist, but it may be related to cyclic hormone secretion patterns associated with females, but not males. Furthermore, without histochemical analyses of the male reproductive tissues we do not know the extent to which spermatogenesis may have been disrupted as a result of the treatment.

In conclusion, these studies have demonstrated that the single-shot GnRH vaccine was highly effective in reducing fertility of females during the 36-week study. The vaccine also negatively impacted testis weight and serum testosterone. These observations are significant in that most prior studies using GnRH vaccines require booster vaccinations to produce antibody titers sufficient to impair reproductive physiology. Remaining questions to be addressed in future studies are the duration of infertility, the reversibility of the effects, and whether an oral form of this vaccine can be developed for field applications.

#### LITERATURE CITED

- ADAMS, T.E. AND B.M. ADAMS. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. *Journal of Animal Science* 70:691-698.
- DUNSHEA, F.R., C. COLANTONI, K. HOWARD, I. MCCAULEY, P. JACKSON, K.A. LONG, S.

- LOPATICKI, E.A. NUGENT, J.A. SIMONS, J. WALKER, AND D.P. HENNESSY. 2001. Vaccination of boars with a GnRH vaccine (Improvac) eliminates boar taint and increases growth performance. *Journal of Animal Science* 79:2524-2535.
- FAGERSTONE, K.A., M.A. COFFEY, P.D. CURTIS, R.A. DOLBEER, G.J. KILLIAN, L.A. MILLER, AND L.M. WILMOT. 2002. Wildlife Fertility Control. Wildlife Society Technical Review 02-2, Washington, D.C., USA.
- MILLER, L.A., B.E. JOHNS, AND G.J. KILLIAN. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. *American Journal of Reproductive Immunology* 44:266-274.
- \_\_\_\_\_, AND G.J. KILLIAN. 2001. Seven years of white-tailed deer immunocontraceptive research at Penn State University: A comparison of two vaccines. *Proceedings of the Wildlife Damage Management Conference* 9:60-69.
- MELEON, R.H., J.A. TURKSTRA, H. LANKHOF, W.C. PUIJK, W.M.M. SCHAAAPER, G. DIJKSTRA, C.J.G. WENSING, AND R.B. OONK. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. *Vaccine* 12:741-6.
- OONK H.B., J.A. TURKSTRA, W. SCHAAAPER, M.M. ERKENS, M.H. SCHUITEMAKER-DEWEERD, A. VAN NES, J.H.M. VERHEIJDEN, AND R.H. MELOEN. 1998. New GnRH-like peptide construct to optimize efficient immunocastration of male pigs by immunoneutralization of GnRH. *Vaccine* 16:1074-1082.
- RABB M.H., D.L. THOMPSON, JR., B.E. BARRY, D.R. COLBORN, K.E. HEHNKE, F. GARZA, JR. 1990. Effects of active immunization against GnRH on LH, FSH and Prolactin storage, secretion and response to their secretagogues in pony geldings. *Journal of Animal Science* 68:3322-3329.
- ZENG, X.Y., J.A. TURKSTRA, R.H. MELOEN, X.Y. LIU, F.Q. CHEN, W.M.M. SCHAAAPER, H.B. OONK, D.Z. GUO, AND D.F.M. VAN DE WIEL. 2002. Active immunization against gonadotrophin-releasing hormone in Chinese male pig: effects of dose on antibody titer, hormone levels and sexual development. *Animal Reproductive Science* 70:223-233.