GnRH Immunocontraception: A Possible Control for Populations of Feral Cats

*Kathleen A. Fagerstone and Lowell A. Miller
USDA/APHIS/WS, National Wildlife Research Center
4101 LaPorte Ave.
Fort Collins, CO 80521-2154
(970) 266-6161
FAX (970) 266-6157
E-mail kathleen.a.fagerstone@aphis.usda.gov

Introduction

A growing interest in non-lethal methods for population control of nuisance or damaging species of wildlife has fostered research in reducing fertility of these pest wildlife species. Immunocontraceptive vaccines have been among the most successful of the many infertility agents tested in wildlife species. Two immunocontraceptive vaccines that have been shown to be effective anti-fertility agents in several wildlife species include zona pellucida (PZP) and gonadotropin-releasing hormone (GnRH). These contraceptive strategies utilize the immune system of the animal to produce antibodies against naturally occurring proteins that are essential in the chain of events for successful reproduction. In the case of zona pellucida vaccine, porcine zona pellucida proteins (PZP) harvested from porcine ova are used as an immunogen to stimulate antibody production against the eggs of the target animal. The resulting antibodies prevent normal sperm-egg interaction and conception. For the GnRH vaccine, a modified molecule is prepared which has the capacity, when used as an immunogen, to stimulate antibodies against native GnRH secreted from the hypothalamus and prevent the normal cascade of hormone secretion that is essential for gonad regulation and gamete production.

PZP and GnRH Tests in Deer

The National Wildlife Research Center (NWRC) has tested these two immunocontraceptive vaccines in white-tailed deer in a cooperative agreement with Pennsylvania State University. All vaccinations used two or more injections of the respective vaccines. PZP produced reversible infertility lasting 1 to 4 years. The first two years of active immunization resulted in an 89% reduction in fawning. Reduction in fawning for the entire 7-year study (4 years of no boosting) was 72%. PZP immunization resulted in multi-estrus behavior, with contracepted deer returning to estrus up to 7 times. The average number of sexually active days for the control group was 45 whereas the PZP treated does remained sexually active for 98 days; therefore, there was an extended breeding season. The PZP vaccine is effective in the female only. The PZP vaccine has been found to be an effective contraceptive in most mammals with the exception of rodents and cats. Anti-PZP titers can be developed in the rodent and cat, but reproductive inhibition has been limited.

A five year study of GnRH immunization was conducted in both male and female deer at Pennsylvania State University. Treatment of does led to reduced fawning rates, reduced estrus behavior and reduced concentrations of progesterone. During active

immunization, GnRH does bred to untreated bucks had an 88% reduction in fawning caused by either immunocontraception or immunocontragestion. Reduction in fawning for the 5-year study (2 years of no boosting) was 74%. The vaccine effect was reversible and directly related to the antibody titer. Infertility lasted up to two years without boosting. GnRH immunization induced a decrease in fawning rate, while the average estrous cycles observed per doe were comparable to averages observed for control animals. The GnRH treatment did not cause repeated estrous cycling. GnRH-immunized bucks had no interest in sexual activity when paired with control females. Depending on the immunization schedule, antlers either dropped early or remained in velvet. The GnRH molecule is common in all mammals; therefore the GnRH contraceptive vaccine should be effective in all mammals.

Development of a new Immunocontraceptive Adjuvant

Previously, the only adjuvant that provided effective immunocontraceptive results was Freund's adjuvant. However, as the NWRC began to work with the FDA to develop a commercial immunocontraceptive product, we were told that Freund's adjuvant would not be allowed in a final product because it has been reported to cause granulomas at the site of injection. This meant that we needed to develop a new adjuvant; many different adjuvants were tested in rabbits but all produced low unacceptable titers. A decision was made to try (Mycopar), a licensed Johne's vaccine. FDA verbally told us that they would have not concerns with this vaccine because it was licensed for use in food animals (bovine). We have developed a new adjuvant using a modification of the currently licensed Johne's disease vaccine which contains *M. avium.* We are proposing to name the new adjuvant AdjuVac. In experimental studies with PZP and GnRH immunocontraceptive vaccines, AdjuVac provided immune responses in rabbits and deer exceeding those found using Freund's adjuvant. This encouraged us to develop a single shot immunocontraceptive vaccine.

Developing the Single Shot Immunocontraceptive technology

In most wildlife situations an opportunity to capture an animal is very difficult and expensive so providing a prime and a booster dose would be infeasible. Therefore NWRC has spent the last four years developing single shot vaccines for both PZP and GnRH in in deer at Pennsylvania State University.

Immunologists have always been trained that long lasting immune responses from a vaccine require a prime and a boost. The first shot prepares the body for a quick response to a disease, which acts as a boost dose. Therefore, achieving a good response with a single dose is difficult. Because a few literature articles indicated that a single dose might be effective if given sufficient time to develop the immune response, and because we had a new effective adjuvant, we began development of a single-shot vaccine with rabbits at NWRC. When given a higher dose than normal, both the PZP and GnRH vaccines with AdjuVac give high immunity titers for up to 2 years using a single dose.

The single shot PZP design was tested at PSU in white-tailed deer in 1999 and the vaccines have been effective in deer for 2 years. Both the timing for when the single dose was given and the quantity of vaccine were changed from the 2 shot technique. This single shot PZP immunocontraceptive vaccine has been chosen for use in a white-tailed deer population control feasibility and efficacy trial in Cleveland, Ohio which began in

March 2001. This study is conducted under the FDA-issued INAD 9958 experimental research permit. The park where the study hasl been undertaken is suffering from the effects of deer overpopulation and immunocontraception is being tested as a method of population management.

Developing the GnRH vaccine was a greater challenge than developing the PZP vaccine. Although there were examples of GnRH vaccines in the literature when we started this project in 1994, those vaccines did not produce long lasting titers, probably because of poor conjugate designs. To produce high antibody titers to a small "self" peptide such as GnRH, one must make it appear foreign to the target animal by coupling it to a large foreign protein; we are using a mollusk protein called keyhole limpet hemocyanin (KLH) for this purpose. NWRC's unique conjugate produces antibody to the small peptide GnRH as well as to the foreign KLH. This new GnRH conjugate combined with the new adjuvant us promise for a single shot GnRH vaccine.

A single-shot vaccine is being tested on female deer at Penn State University and all were contracepted the first year using a single shot. We also tested the GnRH vaccine on male deer, with all effective contracepted (very low testosterone values) during the first year. Field research of the GnRH vaccine is being conducted under an INAD 10006.

Summary

Two immunocontraceptive vaccines are available as a single-shot. In species where multi-cycling induced by PZP immunocontraception is a problem, GnRH may be a better vaccine choice. The single shot PZP vaccine is presently being tested in the coyote, a mon-estrus animal.

The GnRH conjugate, combined with the new AdjuVac adjuvant, provides consistently high titers of anti-GnRH antibody in all animals tested by the NWRC: rabbits, rodents, deer, coyotes, horses, bison and pigs. A large pig study, funded by APHIS/Veterinary Services, is underway at Pennsylvania State University. This study, using the domestic pig as a model for the feral pig, compares the efficacy of 2 dose levels of the single shot to a two shot paradigm. Initial estrus data indicate that all 3 treatment shut down estrus in the 5 month old domestic pig. This vaccine is also being tested in a field trial with the California ground squirrel. It appears that this vaccine provides a longer lasting immunocontraceptive response than others reported in literature. It has application in immunocontraception of companion animals, domestic livestock (immunocastration of males raised for meat), and immunocontraception of wildlife. We have applied for and received a provisional government patent for the GnRH conjugation design along with the new adjuvant.