GnRH
Deer Population Control

NATIONAL WILDLIFE RESEARCH CENTER

U.S. Department of Agriculture Animal and Plant Health Inspection Service Wildlife Services

Overabundant deer herds, particularly those in urban or suburban settings, present serious problems for wildlife managers, landowners, and the general public. Problems include increased numbers of deer-vehicle collisions, increased damage to ornamental and native plants, aggressive behavior toward humans by bucks during the rut, greater potential for disease transmission among deer, and reduced nutritional status of deer. Traditional methods of population control, such as hunting, often are impractical or illegal in such settings. The development of safe and effective wildlife contraceptives is needed to control overabundant wildlife populations in situations where traditional management tools cannot be employed.

NWRC Contraceptive Research—The National Wildlife Research Center (NWRC) in Fort Collins, Colorado, has been active in the development and testing of wildlife contraceptives since 1992. To be an effective and useful wildlife contraceptive, a compound should:

- be safe for the target animal and free of undesirable side effects,
- not affect nontarget species adversely,
- not cause treated food animals to become unsafe for human consumption,
- cause little or no negative social effect on target animals, and
- induce complete and long-lasting infertility that, ideally, is reversible.

Though many compounds have been tested at NWRC, including some that were highly effective in sterilizing wild mammals and birds, failure to meet the above criteria precluded their use in many management situations. Additionally, a contraceptive agent may cause undesirable reactions in one target species but not in others. For example, an immunocontraceptive vaccine developed from the zona pellucida of pigs (porcine zona pellucida, or PZP) has been used to temporarily sterilize dogs, coyotes, baboons, burros, wild horses, and white-tailed deer. The PZP vaccine is a highly effective contraceptive, but unfortunately it causes multiple estrous cycles in female deer. These multiple cycles and the recurrent sexual activity (and deer movements) associated with them may increase deer-vehicle collisions and other deer-human conflicts. The PZP vaccine does not seem to cause multiple estrous cycles in other species on which it has been tested, and it may prove to be a highly useful infertility agent for other wildlife.

A Single-shot Vaccine—As part of its program to develop tools for managing populations of overabundant wildlife

species, NWRC scientists have developed a new gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine (named GonaCon™) that shows great promise as a wildlife infertility agent. (For a technical discussion of GnRH immunocontraception, see last page.)

Two major obstacles had to be overcome during the development of this vaccine. First, a new adjuvant had to be developed (an adjuvant is a compound that improves the immune response, causing higher levels of antibodies). U.S. Food and Drug Administration (FDA) concerns about the commonly used Freund's adjuvant prompted the search for a replacement. Accordingly, NWRC scientists developed a new adjuvant, AdjuVac™ (see sidebar) that is more effective than Freund's adjuvant but lacks the negative side effects.

The second major obstacle to the development of a new immunocontraceptive vaccine for wildlife was the need for a single-dose contraceptive, because of the impracticality of capturing free-ranging wild animals twice to vaccinate them. Previous contraceptive vaccines required at least two injections (an initial dose followed by a booster dose). Although it was originally developed as a two-injection contraceptive treatment, NWRC's GnRH vaccine was subsequently tested in a single-injection form that is much more practical as a field delivery system. Development of the single-injection vaccine was possible only because of the creation of AdjuVac™ adjuvant.

The usefulness of a single-shot immunocontraceptive vaccine depends, among other things, on the duration of the contraceptive effect that the vaccine produces. The combination of AdjuVac™ adjuvant and NWRC's GnRH conjugate produces a much longer-lasting contraceptive effect than was produced by earlier efforts that combined Freund's adjuvant with the (same) GnRH conjugate. (See next page for technical discussion of NWRC vaccine conjugate design.)

AdjuVac™ Adjuvant

Although the U.S. Food and Drug Administration has objected to its use on several grounds, including concerns related to target animal safety and human consumption of treated animals, the most popular (and controversial) adjuvant is Freund's Adjuvant. This adjuvant, widely used since 1945, has long remained popular among immunologists because it is so effective with all types of antigens. It is now known that the addition of *Mycobacterium* (as in Freund's complete adjuvant, FCA) provides a critical "danger signal" to the immune system that is the key to Freund's success. Although many other adjuvants have been developed since the initial appearance of Freund's, none has matched the effectiveness of FCA.

A typical disease vaccine primes the immune system to be on the alert for an infection caused by organisms with properties similar to those of the vaccine. Antibodies to the disease may be few in number or absent until the infection occurs. The infection then serves as a booster that stimulates an immediate immune response, protecting the vaccinated animal. For an immunocontraceptive vaccine to be effective, however, it must continually produce a high contraceptive antibody titer, so the booster effect must come from a different, nondisease mechanism—the adjuvant.

NWRC has modified and tested a USDA-approved Johne's vaccine called Mycopar™ as a replacement for Freund's adjuvant. Mycopar™ has already been approved for use in food animals by APHIS. The new adjuvant, which NWRC scientists have named AdjuVac™, contains a small quantity of *M. avium*, a common, generally nonpathogenic bacterium found in many species of domesticated and wild animals. NWRC scientists are testing AdjuVac™ in numerous wildlife species, and it appears to be an effective replacement for Freund's as an adjuvant for contraceptive vaccines. The GnRH vaccine GonaCon/AdjuVac™, developed by NWRC, has a USDA/APHIS patent-pending status.

Pen and Field Studies of GonaCon™— Recent studies with free-ranging California ground squirrels, captive Norway rats, feral cats and dogs, domestic and feral swine, wild horses, and white-tailed deer have demonstrated the efficacy of the single-shot GnRH vaccine as a contraceptive agent. Infertility among treated female swine and white-tailed deer, for example, lasted up to two years without requiring a booster vaccination.

The NWRC GnRH Vaccine Conjugate Design

The GnRH vaccine generally provides a longer-lasting contraceptive effect in females than in males, probably because the females' demand for GnRH antibody is cyclic, in contrast to the males' constant demand. GonaCon™ contains a GnRH peptide conjugated to KLH combined with adjuvant AdjuVac™.

A single-shot vaccine that provides a multi-year contraceptive effect requires: (1) optimization of the vaccine structural design, (2) optimization of the dose for each target species, (3) use of the best adjuvant available, and (4) development of a delivery system that will protect the injected antigen from rapid destruction by the animal's immune system. Design of multiple-shot vaccines is much less demanding.

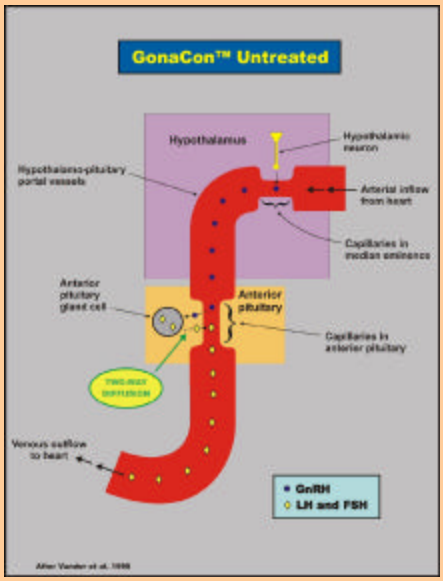
The design of the GnRH vaccine mimics the repetitive epitopes found in many pathogens. Pathogenic viruses and bacteria typically exhibit rigid, highly-organized, highly-repetitive protein epitopes. High epitope density in a highly-organized, repetitive arrangement is important in ß-cell responsiveness. Although ß cells are unresponsive to repetitive epitopes that are poorly organized, repetitive epitopes of proper spacing can stimulate multiple surface receptors of similar spacing. The repetitive epitope pattern permits a cross-linking activation of ß-cell receptors, providing an extremely strong, long-lasting immune response. Mimicry of the repetitive nature of pathogen epitopes is an important aspect of the KLH-GnRH conjugate design. The GnRH peptide, which is analogous to the repetitive epitope, was designed to ensure consistent alignment of the peptide when coupled to the KLH carrier.

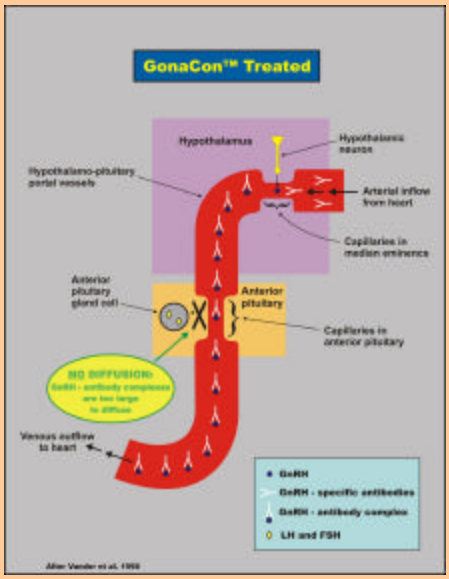
Ongoing studies initiated during July 2004 are examining the practicality of administering GonaCon™ to free-ranging white-tailed deer as well as the efficacy, toxicity, and safety of the vaccine. Near Silver Spring, Maryland, an overabundant herd of white-tailed deer on a completely enclosed site that is owned by the U.S. General Services Administration has provided an excellent opportunity to test the efficacy and practicality of GonaCon™ on a free-ranging deer population. In this field study, 28 adult does were captured, equipped with ear tags and radiotelemetry transmitters, and injected with GonaCon™ immunocontraceptive vaccine. The reproductive behavior and performance of these does will be monitored for two years and compared with those of 15 adult does (unvaccinated, control animals) that inhabit an adjacent, enclosed parcel of similar habitat. NWRC scientists are working closely with Maryland Wildlife Services to complete this field study.

In an ongoing study of captive white-tailed deer at Pennsylvania State University, NWRC researchers are collaborating with university faculty to assess the toxicity and safety of GonaCon™. Responses of treated and control groups of deer will be compared via analyses that will include blood chemistry, hematology, and histopathology, as well as assays of circulating progesterone, luteinizing hormone, and GnRH antibodies.

FDA Registration of GonaCon™—No fertility control agents have been approved for non-investigational use on wildlife populations in the United States. Several materials, however, including GnRH and PZP vaccines, have been classified as investigational drugs that may be used (only) in rigidly controlled research studies. The two GonaCon™ studies underway in Maryland and Pennsylvania are being conducted as pivotal studies that are required as part of the FDA's approval process for a New Animal Drug. The approval process for GonaCon™ vaccine began in 1998 when the FDA established an Investigational New Animal Drug (INAD) exemption for the GnRH vaccine. All research studies of GnRH vaccine have been conducted under this exemption (INAD – 10006).

Advantages of GnRH —NWRC scientists are hopeful that the GnRH vaccine will soon be developed and approved for use for wildlife fertility control. GnRH vaccines have an advantage over PZP because they prevent eggs from being released from the ovaries, thereby eliminating estrus and some undesirable behaviors (e.g., bucks chasing does across roads) associated with it. In addition, GnRH vaccine has promise for reducing or eliminating certain undesirable behaviors in companion animals. For example, fighting, scent-marking, caterwauling, and wandering by cats, and unruly behavior in horses, could be reduced by GnRH vaccine because the vaccine indirectly blocks the production of sex hormones (e.g., estrogen and





Control of Reproduction by GnRH —Gonadotropin-releasing hormone, which is produced in the hypothalamus at the base of the brain, controls the release of the pituitary gonadotropins LH (luteinizing hormone) and FSH (follicle-stimulating hormone). These gonadotropins regulate hormones that drive sperm production in males and follicular development and ovulation in females. Excitation of the GnRH neurons results in the release of stored GnRH peptide from its secretory granules in the hypothalamus. After it diffuses into the surrounding capillary blood, the GnRH travels via the hypophysial portal system to the anterior pituitary, where it diffuses from the capillaries and binds to and activates the LH and FSH gonadotrophs. This activation causes the release of stored gonadotropins, which diffuse back through the capillaries into the bloodstream. The gonadotropins then travel to and activate the reproductive organs, resulting in steroid synthesis and normal sexual activity.

GnRH Immunocontraception—The GnRH vaccine stimulates the production and release of GnRH-specific antibody from the B-cells into the bloodsteam. The antibody circulates throughout the body, and when it reaches the capillary region of the hypothalamus, it comes into contact with GnRH that has diffused into the capillaries after being produced in the hypothalamus. Binding of GnRH to the specific antibody forms large immune-complexes that travel down the hypophysial stalk. Because of their large size, however, the immune-complexes are unable to diffuse out of the blood at the pituitary capillaries. Instead, they remain in the venous blood and leave the pituitary without stimulating the release of LH and FSH. Without the LH and FSH that normally stimulate the synthesis of steroids in the reproductive organs, animals of both sexes remain in an asexual, nonreproductive state. As long as there is sufficient antibody to bind all GnRH circulating in the hypothalamic/pituitary portal system, all sexual activity will be suspended and animals will remain nonreproductive.

testosterone) that contribute to the expression of such behaviors.

The single-shot, multiple-year GonaCon™ vaccine will be a useful tool for the management of enclosed or urban/suburban wildlife populations, such as deer. GonaCon™ still has limitations, however, especially the need to capture and inject each animal. NWRC scientists hope to eventually produce an oral GnRH vaccine that will be attractive only to the target species. For additional information on reproductive control research at NWRC visit the website at:

http://www.aphis.usda.gov/ws/nwrc/research/reproductive_control/index.html

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