GonaCon™, a Versatile GnRH Contraceptive for a Large Variety of Pest Animal Problems

Lowell A. Miller
USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado
Jack Rhyan
USDA APHIS Veterinary Services, National Wildlife Research Center, Fort Collins, Colorado
Gary Killian
Almquist Research Center, Penn State University, University Park, Pennsylvania

ABSTRACT: As part of the program to develop contraceptive tools to control populations of over-abundant wildlife species, the NWRC has developed a single-injection gonadotropin-releasing hormone (GnRH) immunocontraceptive vaccine, GonaCon™. GonaCon™ has been tested and shown to provide contraceptive effects lasting 1-3 years in many pest species including white-tailed deer, domestic and feral pigs, bison, wild horses, cats, dogs, and California ground squirrels. GonaCon™ contains a GnRH peptide conjugated to keyhole limpet hemocyanin combined with AdjuVac™, an adjuvant also developed at the USDA National Wildlife Research Center. Immunization against GnRH prevents the circulating GnRH from stimulating the release of pituitary luteinizing hormone and follicle-stimulating hormone. This process of immuno-neutralization of GnRH effects a temporary non-surgical castration in both males and females. Contraceptive and behavioral effects of GonaCon™ are discussed for a variety of species.

KEY WORDS: adjuvant, fertility control, GnRH vaccine, GonaCon™, immunocontraception

Proc. 21st Vertebr. Pest Conf. (R. M. Timm and W. P. Gorenzel, Eds.)
Published at Univ. of Calif., Davis. 2004. Pp. 269-273.

INTRODUCTION

A growing interest in nonlethal methods for population control of nuisance or damaging species of wildlife has fostered research in reducing fertility of these pest wildlife species. The most commonly used means of inducing infertility is by immunocontraception, in which the animal is immunized against a protein or hormone needed for reproduction. Wildlife personnel have looked at the values and shortcomings of immunocontraception for several years and all have concluded that further research is warranted (Miller and Fagerstone 2000, Fagerstone et al. 2002).

Traditional immunocontraceptive research in mammals has concentrated on the use of a vaccine made from zona pellucida extracted from the ovaries of pigs (*Sus scrofa*) and therefore called porcine zona pellucida (PZP) (Miller et al. 1999). Animals immunocontracepted with PZP continue to cycle and are bred, but they do not become pregnant due to the PZP antibody coating the egg. In animals such as white-tailed deer (*Odocoileus virginianus*) that continue to cycle throughout the breeding season, the PZP contraceptive technique may introduce physiological stress due to an extended breeding season (Killian and Miller 2000, Miller and Killian 2000). This prolonged estrous cycling results in increased activity during early winter, at a time when conservation of calories is important. Increased activity may also contribute to increased collisions with automobiles in highly populated areas. Because of concerns over prolonged estrous cycling, NWRC developed a second contraceptive (GnRH vaccine) that contracepts by reducing reproductive behavior.

DEVELOPMENT OF THE GnRH VACCINE

GnRH (gonadotropin-releasing hormone) is a small peptide hormone sometimes called the "master hormone" because it is responsible for controlling the reproductive processes in both males and females. The GnRH peptide is identical in all mammals and is not immunogenic both because of its small size and because it is considered "self" to the immune system. However, GnRH can be made immunogenic by coupling it to a carrier such as keyhole limpet hemocyanin (KLH). The coupled GnRH peptide is called a GnRH conjugate, which then is combined with an adjuvant to create a vaccine.

GnRH contraceptive vaccines have been evaluated as immunocastration agents in pets, (cats, *Felis sylvestris catus*; and dogs, *Canis familiaris*), cattle (*Bos taurus*), sheep (*Ovis aries*), swine, and deer (*Odocoileus* spp.) (Adams and Adams 1992, Ladd et al. 1994, Meloen et al. 1994, Oonk et al. 1998, and Schanbacher 1982). All of these GnRH studies have used 2 or more injections, and the duration of the immune responses in many studies has been 6 months or less, which is not practical for wildlife applications.

The first application of the NWRC GnRH vaccine was a GnRH/keyhole limpet hemocyanin (KLH)/ Freund's combination, which was tested in the Norway rat (*Rattus norvegicus*). In this study, which used a prime and a boost vaccination (Miller et al. 1997), both male and female rats remained infertile for up to 1 year. The 2-injection GnRH vaccine was then tested in white-tailed deer. Details of this study were reported in Miller et al. (2000). A commercial vaccine (Improvac) that required 2 injections was available for a short time in Australia,

but it was recently taken off the market (Dunshea et al. 2001).

Because GnRH does not stimulate an immune response by itself, the success of the vaccine depends on the design of the conjugation. Each of the previous authors mentioned above have their own GnRH conjugation design. Ferro et al. (2002a, 2002b) have studied several different designs of GnRH conjugation; however, they still required multiple injections.

The design of a single-injection vaccine that will provide multi-year contraceptive effect requires 1) optimizing the vaccine structural design, 2) optimizing the dose for each target species, 3) adding the best adjuvant possible, and 4) designing a delivery system that will protect the injected antigen from rapid destruction by the immune system. The multi-injection vaccine is more forgiving in relation to dose and the optimum vaccine design. Details of the current GnRH vaccine developed by NWRC are presented in a previous article (Miller et al. 2003). However, some general design concepts to improve the vaccine effectiveness are presented here.

ADJUVANT DESIGN (AdjuVac™)

An important part of any vaccine is the adjuvant (a non-specific immune stimulant). The most popular, and most controversial, adjuvant is Freund's Adjuvant, which has been widely used since 1945. This adjuvant has remained popular to immunologists over time because it is so effective with all types of antigens. It is now known that the addition of *Mycobacterium* in Freund's complete adjuvant (FCA) provides a vital "danger signal" to the immune system that is the key to Freund's success. Although many other adjuvants have been developed, none have matched the effectiveness of FCA. However, because of the negative attitude of many animal care and use committees as well as Food and Drug Administration (FDA) concerns on the use of Freund's in any approved product, NWRC began testing a modified USDA-approved Johne's vaccine, Mycopar™, as a replacement for Freund's adjuvant. Mycopar is approved for use in food animals and is therefore not a concern for use in deer, which may be eaten by hunters.

The new adjuvant, which we have named AdjuVac™, contains a small quantity of *M. avium*, a common bacteria found in many wildlife and domestic animals, which provides a similar "danger signal" to the immune system found in Freund's adjuvant. NWRC is currently testing AdjuVac™ in many different wildlife species and it appears to be an effective replacement for Freund's as an adjuvant for immunocontraception.

SINGLE-INJECTION GonaCon™ MIMICS BACTERIA

Many pathogens, including viruses and bacteria, exhibit a rigid, highly organized, highly repetitive pattern of protein epitopes. This repetitive epitope pattern provides a cross-linking activation of B cell receptors, resulting in an extremely strong, long-lasting immune response (Bachmann et al. 1993). This mimic of the repetitive nature of pathogen epitopes is an important part of the KLH-GnRH conjugate design. Banatvala et al. (2001) state that the antigen dose and structure and repetitive eptitope pattern influence the strength of the primary response. He stresses that the greater the primary response, the longer lasting the immune memory.

Bachmann et al. (1993) have shown that in obtaining a high B cell responsiveness to an individual epitope, the design of the immunogen is very important. They found that high epitope density in a highly organized repetitive fashion was important in B cell responsiveness. B cells were unresponsive to poorly-organized but repetitive epitopes. Repetitive epitopes of proper spacing are able to stimulate multiple surface receptors of similar spacing. Long-lasting immune response is thought to be due to a combination of persisting antigen, which is capable of B cell differentiation into plasma cells, and the persistence of long-lasting plasma cells.

The bacterial mimic KLH-GnRH conjugate design, combined with the new adjuvant (AdjuVac™), which contains small quantities of *M. avium*, form the basis of the new multi-year single-injection GnRH vaccine GonaCon™.

EFFECT OF GonaCon™ TREATMENT IN FEMALES

Females are treated with GnRH vaccine by intramuscular injection. Effectiveness of the vaccine is tested by measuring the antibody levels to GnRH, and hormonal cyclic activity is measured by periodic sampling of serum concentrations of progesterone and estradiol. Effective contraception in females reduces hormone levels to the point that females do not ovulate or come into estrus. In seasonal breeding animals such as the white-tailed deer, a non-cyclic state is normal 6 months out of the year; GnRH-vaccinated females simply do not come into estrus in the fall. Field testing of the GnRH vaccine includes monitoring the breeding activity, followed by monitoring the reduction of offspring in the treated group as compared to the control group.

GnRH vaccine has been tested in the female white-tailed deer for several years at both Pennsylvania State University and Seneca Army Depot. We have found that GnRH reduces breeding behavior and reduces fawning for up to 4 years (Miller and Killian 2000).

EFFECTS OF GonaCon™ TREATMENT IN MALES

Males are treated with GnRH vaccine by intramuscular injection. Effectiveness of the vaccine is tested by measuring antibody levels to GnRH. Serum testosterone concentrations are also measured. Testicular size is measured, and sperm mobility and number are also measured.

Effective contraception is the male is measured by the drop in testosterone levels, reduction in the size of the testicles, reduction in aggressive behavior, and the lack of interest in females that are in estrus.

The GnRH has been injected in the male white-tailed deer at Pennsylvania State University and at Seneca Army Depot, where we found the annual growth of antlers in male deer is dependant on testosterone, with antler growth increasing with age and increased testosterone concentration. We have found large variation in the size of antlers in GnRH-treated male deer (Curtis et al. 2001). Depending on the timing of the

vaccination, the antlers could fall off early, stay in velvet, or have an abnormally small growth pattern.

DURATION OF CONTRACEPTIVE EFFECT

In animals treated with GnRH vaccine, anti-GnRH in the hypophyseal portal blood complexes to the newly released GnRH from the hypothalamus, preventing GnRH from binding to the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) receptors. GnRH is released from the hypothalamus in a pulse-related fashion; the frequency and high of the pulse is related to seasonal sexual activity or the phase of the estrus cycle. The concentration of newly-released GnRH in the portal blood is picogram/ml (10^{-12} grams/ml), and as it passes into the peripheral blood its concentration drops to femtogram/ml (10^{-15} gram/ml). This concentration is too small to measure even by the most sensitive radioimmunoassay method. GnRH-antibody complexes produced in the portal blood are destroyed by the immune system as they pass through the liver. Continued contraceptive effect is dependent on a continual source of available GnRH antibody. This small quantity of GnRH controls the concentration of the active reproductive hormones, progesterone and testosterone, which circulate at nanograms/ml (10^{-9} grams/ml). It is this small quantity of a master hormone that makes it a good candidate for immunocontraception.

In all the species tested, immunization with resulting antibody titer to GnRH leads to an inhibition of breeding behavior and contraception. Effective contraception continues as long as antibody titers remain sufficiently high.

Effectiveness of the contraceptive response is dose related. Our research has determined that the effective dose can be divided into 3 general categories: small animal = 200 - 400 µg of conjugate, mid-sized animal = 800 - 1,000 µg of conjugate, and large-sized animal = 1,500 - 2,000 µg of conjugate. Intra-muscular injections appear to be the most effective route of delivery. All species show an apparent individual variation in immune response to the vaccine. In species where there was initially 100% contraception, there was always a variation between individuals in the length of the contraceptive response.

EFFECTS OF GnRH ON MAMMALS

GnRH Vaccine in California Ground Squirrels

NWRC scientists in collaboration with staff from the Alameda County, California, Vector Control District tested the use of a single-injection GnRH in the California ground squirrel (*Spermophilus beecheyi*). In the 2-year study, 229 male and female squirrels were trapped and injected with a single-injection GnRH preparation. Results suggest a significant reduction in lactation in females through the 2-year period, as well as a reduction in sexually-active males (Nash et al. 2004).

GnRH Vaccine in Feral Dogs and Cats

GnRH vaccine has shown to be effective in reducing the testosterone, sperm count, and sperm motility in a small sample of test male dogs. The vaccine should be very effective in reducing reproductive activity in the female dog. The reproductive inhibition effect lasted 1 year in the male dog with a single injection; however, the contraceptive effect should last longer in the female dog.

NWRC has begun a collaborative study with GnRH in cats. The vaccine has reduced the testosterone and sperm counts in several male cats for up to 1 year with a single injection (Levy et al. 2004). Dr. Julie Levy from the University of Florida has received a Morris Foundation Grant for a collaboration with NWRC to study the reproductive effects of the GnRH in female cats.

Comparing Contraceptives in Wild Horses

In collaboration with Nevada State Veterinarian Dr. David Thain, researchers Dr. Jack Rhyan, Dr. Gary Killian, and Dr. Lowell Miller are comparing the effects of 3 contraceptive Techniques, SpayVac (a PZP formulation), GonaCon™, and insertion of an IUD, in state-owned wild horses in Nevada. The first year's data appear promising for all 3 techniques (Killian et al. 2004a).

GnRH Vaccine Reduces Disease

GnRH Vaccine in Feral Pigs

Sexual transmission of pseudorabies in feral pigs has increased interest in the use of GnRH as a contraceptive, which reduces fertility by reducing breeding activity. The GnRH vaccine has been effective in reducing reproduction in domestic pigs (Miller and Killian 2004a). Feral swine are recognized as disease reservoirs for brucellosis and psuedorabies, among other diseases, and they increase the risk of disease spread to other wildlife, domestic livestock, and humans (Killian et al. 2004b). Although the porcine zona pellucida vaccine has been shown effective for contraception of several wildlife species (Fagerstone et al. 2002), its use in swine is problematic because the vaccine is prepared using porcine zona pellucida protein, which is a "self protein," making it less immunogenic in the pig. Also, as previously mentioned, immunocontraception with the PZP vaccine may cause treated females to continue to cycle, increasing the opportunity to spread pseudorabies.

The GnRH vaccine may have an application in making male pigs more palatable by reducing boar taint. Dunshea et al. (2001) have shown that Improvac (a commercial GnRH vaccine) reduced boar taint in adult male pigs. NWRC has unpublished studies that support these data both in domestic and feral pigs.

GnRH Vaccine in Bison

There are concerns over the spread of disease through calving, such as brucellosis in bison. Bovine brucellosis, a bacterial disease is transmitted between animals including cattle, bison (*Bison bison*), and elk (*Cervus* spp.), primarily through contact with infected aborted fetuses, placentas, parturient fluids, or post-parturient uterine discharge. Following initial infection, a dam often experiences abortion. Subsequent pregnancies may result in abortion or the birth of weak or normal calves and may also result in shedding of the organism. Contraception may therefore reduce the spread of the disease.

PZP is not an optimal contraceptive vaccine, because

prolonging the breeding season of bison in the Greater Yellowstone Area may be deleterious to winter survival of dominant bulls and vaccinated cows, due to increased activity in fall and early winter. Immunocontraception using GnRH vaccine has been shown to reduce reproductive activity in the bison, and it is an alternative to PZP that would not extend the breeding season. Female bison in a zoo setting have been given two GnRH injections, which resulted in 100% reduction in calving for over 2 years. Penned female bison in Idaho, when given a single shot, have been contracepted for 1 year. Four out of 5 bison were in the third trimester of pregnancy at the time of GnRH vaccination and went on to deliver normal calves. The following year, none of the 5 bison calved (Miller et al. 2004).

DISCUSSION

Immunocontraception through the use of GnRH vaccine has intrigued reproductive physiologists for 20 years. The vaccine has been used to immunocastrate a variety of animals with mixed success. Because of the seasonal pulsating picogram quantity of GnRH, it becomes the most logical hormone to bioneutralize, and yet there is no GnRH vaccine on the market. Because GnRH must be linked to a large protein to make it immunogenic, the conjugate design has presented a limiting factor in developing a successful long-acting GnRH vaccine. The product development program of NWRC has spent more than 10 years testing multiple vaccine designs in white-tailed deer. As a result of these studies, the current combination of the KLH-GnRH conjugate design, combined with a newly-developed adjuvant AdjuVac™, appears to provide an effective, long-lasting vaccine, which we have named GonaCon™.

GnRH vaccines have been criticized because of lack of species specificity. Because GnRH has a similar peptide sequence in all mammals, species specificity must come with the injection delivery method. The positive side of the lack of species specificity is that the same vaccine can be used for multiple species with slight variations in dosage.

GnRH has also been criticized because it reduces the normal breeding behavior of the target animal. However, seasonal breeders exhibit breeding behavior for 6 months out of the year, so GnRH vaccine merely prolongs the lack of sexual interest throughout the year. At this point in time, there is no reproductive control method that does not in some way interfere with reproductive behavior. As GonaCon™ becomes available as a contraceptive vaccine, we should consider it a tool available for certain situations, while we continue to research new and improved control methods for future use.

The GnRH vaccine GonaCon™ has been shown to be an effective single-injection immunocontraceptive vaccine. GonaCon™ is useful for many applications, one of which is reducing breeding behavior of a particular animal. Another application is to reduce aggression in males. For example, male deer in parks become quite used to people and can be fed in the non-breeding season; however during the breeding season, these male deer can become quite dangerous. A GnRH vaccination could reduce this aggressive behavior. The vaccine can be used as a single or multiple injections, and response is generally reversible in 1 to 4 years, depending on the dose given. The GnRH/AdjuVac™ vaccine, called GonaCon™, has an APHIS USDA patent-pending status.

LITERATURE CITED

ADAMS, T. E., AND B. M. ADAMS. 1992. Feedlot performance of steers and bulls actively immunized against gonadotropin-releasing hormone. J. Anim. Sci. 70:691-698.

BACHMANN, M. F., U. H. ROHRER, T. M. KUNDIG, K. BURKI, H. HENGARTNER, AND R. M. ZINKERNAGEL. 1993. The influence of antigen organization on B cell responsiveness. Science 262:1448-1451.

BANATVALA, J., P. VAN DAMME, AND S. OEHEN. 2001. Lifelong protection against hepatitis B: therole of vaccine immunogenicity in immune memory. Vaccine 19:877-885.

CURTIS, P. D, R. L. POLLER, M. E. RICHMOND, L. A. MILLER, G. F. MATTFELD, AND F. W. QUIMBY. 2001. Comparative effects of gonadotrophin-releasing hormone and porcine zona pellucida immunocontraceptive vaccines for controlling reproduction in white-tailed deer. Reprod. Suppl. 60:131-141.

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. An. Sci. 79:2524-2535.

FAGERSTONE, K. A., M. A. COFFEY, P. B. CURTIS, R. A. DOLBEER, G. J. KILLIAN, L. A. MILLER, AND L. M. WILMOT. 2002. Wildlife contraception. Tech. Review 02-2, The Wildlife Society, Bethesda, MD. 29 pp.

FERRO, V. A., M. A. H. KHAN, E. R. EARL, M. J. A. HARVEY, A. COLSTON, AND H. STIMSON. 2002a. Influence of carrier protein conjugation site and terminal modification of a GnRH-I peptide sequence in the development of a highly specific anti-fertility vaccine. Part 1. Am. J. Repro. Immun. 48:361-371.

FERRO, A. V., M. J. A. HARVEY, A. COLSTON, AND W. H. STIMSON. 2002b. Part II: Influence of dimerization of a modified GnRH-I peptide sequence on a male antifertility vaccine. Am. J. Repro. Immun. 48:372-380.

KILLIAN, G. J., AND L. A. MILLER. 2000. Behavioral observation and physiological implications for white-tailed deer treated with two different immunocontraceptives. Proc. Wild. Dam. Manage. Conf. 9:283-291.

KILLIAN, G., L. MILLER, N. DIEHL, J. RHYAN, AND D. THAIN. 2004a. Evaluation of three contraceptive approaches for population control of wild horses. Proc. Vertebr. Pest Conf. 21:263-268.

KILLIAN, G. J., L. A. MILLER, J. RHYAN, T. DEES, AND H. DOTEN. 2004b. Evaluation of GnRH contraceptive vaccine in captive feral swine in Florida. Proc. Wildl. Damage Manage. Conf. 10:128-133.

LADD, A., Y. Y. TSONG, A. M. WALFIELD, AND R. THAU. 1994. Development of an antifertility vaccine for pets based on active immunization against luteinizing hormone-releasing hormone. Biol. Repro. 51:1076-1083.

LEVY, J. K., L. A. MILLER, P. C. CRAWFORD, J. W. RITCHEY, M. K. ROSS, AND K. A. FAGERSTONE. 2004. GnRH immunocontraception of male cats. Theriogenology 62(6): 1116-1130.

MELOEN, R. H., J. A. TURKSTRA, W. C. LANKHOF, H. PUIJK, W. C. SCHAAPER, W. M. M. DIJKSTRA, G. WENSING, AND R. B. OONK. 1994. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine 12:741-746.

MILLER, L. A., AND K. A. FAGERSTONE. 2000. Induced infertility as a wildlife management tool. Proc. Vertebr. Pest. Conf. 19:160-168.

MILLER, L. A., B. E. JOHNS, D. J. ELIAS, AND K. A. CRANE. 1997. Comparative efficacy of two immunocontraceptive vaccines. Vaccine 15:1858-1862.

MILLER, L. A., B. E. JOHNS, AND G. J. KILLIAN. 1999. Long-term effects of PZP immunization on reproduction in white-tailed deer. Vaccine 18:568-574.

MILLER, L. A., B. E. JOHNS, AND G. J. KILLIAN. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am. J. Reprod. Immun. 44:266-274.

MILLER, L. A., AND G. J. KILLIAN. 2000. Seven years of white-tailed deer immunocontraception research at Penn State University: a comparison of two vaccines. Proc. Wildl. Damage Manage. Conf. 9:60-69.

MILLER, L. A., J. C. RHYAN, AND M. DREW. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. J. Wild. Dis: 40(4):724-729.

MILLER, L. A., J. C. RHYAN, AND G. J. KILLIAN. 2003. GnRH contraceptive vaccine in domestic pigs: a model for feral pig control. Wildl. Damage Manage. Conf. 10:120-127.

NASH, P. B., D. K. JAMES, L. T. HUI, AND L. A. MILLER. 2004. Fertility control of California ground squirrels using GnRH immunocontraception. Proc. Vertebr. Pest. Conf. 21:274-278.

OONK, H. B., J. A. TURKSTRA, W. SCHAAPER, M. M. ERKENS, M. H. SCHUITEMAKER-DEWEERD, J. H. M. VAN NES, A. 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.

SCHANBACHER, B. D. 1982. Responses of ram lambs to active immunization against testosterone and luteinizing hormone-releasing hormone. Am. J. Physiol. 242:E201-205.