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Authors

Killian, Gary
Wagner, Donald
Fagerstone, Kathleen
et al.

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Long-Term Efficacy and Reproductive Behavior Associated with GonaCon™ Use in White-Tailed Deer (*Odocoileus virginianus*)

Gary Killian

USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado

Donald Wagner

Deer Research Center, The Pennsylvania State University, University Park, Pennsylvania

Kathleen Fagerstone and Lowell Miller

USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado

ABSTRACT: GonaCon™ is a single-shot immunocontraceptive vaccine that targets the reproductive hormone gonadotropin releasing hormone (GnRH). The GnRH peptide used in the vaccine is secreted by the hypothalamus of the brain and is specifically known as luteinizing hormone releasing hormone. This peptide stimulates the synthesis and secretion of luteinizing hormone and follicle stimulating hormone by the anterior pituitary gland. These hormones in turn stimulate hormone and gamete production by the ovary. In earlier studies with deer, we showed that antibodies produced in response to a single shot of GonaCon™ inactivated endogenous GnRH and greatly reduced fertility, reproductive steroids, and associated behaviors for at least 2 years. In the present study, we report on contraceptive efficacy and behavioral observations up to 6 years after female white-tailed deer were given a single shot of one of several formulations of GonaCon™. We compared the standard GonaCon™ made by conjugating the GnRH peptide to keyhole limpet hemocyanin (GonaCon™-KLH) to one with the GnRH peptide conjugated to a blue mollusk protein (GonaCon™-Blue) and to a 2-shot regimen with GonaCon™-KLH. All GonaCon™ preparations were made into an emulsion with AdjuVac™ and administered to 5 mature female deer per treatment. The results showed that the GonaCon™-Blue preparation significantly out-performed the other single-shot vaccines, as well as the 2-shot regimen of GonaCon™-KLH, with efficacy of 80-100% for each of the 5 years in the study. Interestingly, expression of estrous behavior was minimal in Years 1-2 following treatment for all groups, but expression of estrus increased in later years of the study, even though does remained infertile. This expression of estrus and fertility was reversed with follicle stimulating hormone releasing hormone (FSH-RH) peptide conjugated to a mollusk protein and mixed as an emulsion with AdjuVac™. This suggests that does possess an FSH-RH peptide, secreted by the hypothalamus, which modulates follicle development and resulting estrogen production and reproductive behavior.

KEY WORDS: fertility control, FSH-RH, GnRH vaccine, GonaCon™, immunocontraception, multi-year contraception, *Odocoileus virginianus*, reproductive behavior, white-tailed deer

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INTRODUCTION

The USDA National Wildlife Research Center (NWRC) has spearheaded efforts to develop and test several technologies directed at reproductive management of overabundant wildlife species. Immunocontraceptive vaccines have proven to be the most promising approach based on evaluations in a variety of species (Fagerstone et al. 2002). Immunocontraceptive vaccines work by stimulating antibody production directed at hormones or proteins that are essential for reproduction in the target animal. GonaCon™ is an immunocontraceptive vaccine, developed by the NWRC, which is directed at gonadotropin releasing hormone (GnRH) produced by the hypothalamus. GnRH is a decapeptide that stimulates production and secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary gland, which in turn regulate gamete and steroid hormone production by the ovaries and testes. In the presence of sufficient antibody directed at GnRH, production and secretion of GnRH by the hypothalamus is compromised, and as a result, production of sex steroids and gametes is prevented. Based on behavior studies with white-tailed deer (*Odocoileus virginianus*), treated animals are not only infertile but also show less evidence

of breeding behavior, attributable to the decline in serum sex steroids (Killian and Miller 2001).

Development of injectable contraceptive vaccines for wildlife poses challenges unlike those encountered with domestic animals or humans. For most wildlife and feral species, administration of an injectable vaccine is limited by the ability to capture or dart a significant portion of the target population in the field. Because the probability of revaccinating the same animal in the future is remote, long-acting single-shot vaccines are considered ideal for field application. For more than 10 years, the NWRC has strived to meet these challenges by developing and evaluating a new adjuvant and formulations of the GnRH vaccine, GonaCon™ (see Miller et al. 2008). The best-performing formulation of GonaCon™ evaluated to date has been named GonaCon™-Blue. Studies with white-tailed deer have demonstrated contraceptive efficacy of 80-100% for up to 5 years following a single vaccination. The purpose of this paper is to present the results of these studies, as well as observations on long-term changes in reproductive behavior for female deer treated with several formulations of GonaCon™.

MATERIALS AND METHODS

Animals

These studies were conducted at the Deer Research Center of the Pennsylvania State University over a 6-year period from 2002-2008. Does were chemically restrained during handling with 2.2-4.4 mg/kg of xylazine administered IM in the rump. Anesthesia was reversed with Tolazine at 4mg/kg given IV or IM.

Treatments

Two GnRH vaccine formulations were evaluated in white-tail does (Miller et al. 2008). One formulation, GonaCon™-KLH, consisted of the GnRH peptide conjugated to keyhole limpet hemocyanin (KLH). GonaCon™-KLH was administered as a single shot given to 5 does in July 2002 followed by a second immunization 4-6 weeks later (Group 1), or as a single shot to 5 does in July 2003 (Group 2). The second formulation, GonaCon™-Blue, consisted of the GnRH peptide conjugated to a blue mollusk protein. GonaCon™-Blue was administered as a single shot to 5 does in July 2002 (Group 3). Both formulations of GonaCon™ contained 1,000 µg of the GnRH peptide and were combined with AdjuVac™ adjuvant (Miller et al. 2008) as a 1-ml dose for IM injection in the rump.

In order to determine whether the expression of reproductive behavior was in some way regulated by the hypothalamic peptide FSH-RH, 7 does previously vaccinated with either GonaCon™-KLH or GonaCon™-Blue and showing evidence of reproductive behavior were given an injection of follicle stimulating hormone releasing hormone (FSH-RH) vaccine consisting of 1,000 µg FSH-RH peptide (Yu et al. 1997) conjugated to the blue mollusk protein (Mollusk-blue-FSH-RH). On August 15, 2006, these does received a single shot of the FSH-RH vaccine prepared as a 1-ml dose with AdjuVac™ and injected IM in the rump.

Blood samples were collected 3-4 times from July through February each year from the jugular vein. After clotting, the serum was harvested by centrifugation and stored frozen at -20°C until assay. Serum was used to determine antibody titers to GnRH and the FSH-RH peptides by ELISA methods previously described (Miller and Killian 2001). Each year pregnancy was evaluated by ultrasonography in early February and confirmed by fawning in the spring.

Behavior Observations

Each year during the months of November through February, reproductive behaviors of treated does were observed by trained personnel. Observations were made 3× daily at 7:30 am, 12:00 pm, and 4:00 pm through mid-January, and thereafter twice daily at 7:30 am and 4:00 pm through the end of February. Observation periods were typically 30 min, but longer periods of observation occurred when breeding activity was observed, to ensure that adequate information was recorded for all active individuals. Criteria used to categorize reproductive behaviors were defined in an earlier publication (Killian and Miller 2001). Behaviors recorded were considered to be indicative of does in estrus or activities surrounding estrus

Table 1. Comparison of the number of reproductive behavior incidents and anti-GnRH titers observed in infertile does receiving different GnRH vaccines during the years of study.

Treatment	Percent Infertile	Incidents / Total Does	Average / Doe
Group 1: GnRH 2-Shot			
2002-03	100	2/5	0.4
2003-04	100	2/5	0.4
2004-05	80	2/5	0.4
2005-06	60	8/4	2.0
Group 2: GnRH-KLH			
2003-04	100	4/4	1.0
2004-05	60	6/3	2.0
Group 3: GnRH-Blue			
2002-03	100	3/5	0.6
2003-04	100	2/5	0.4
2004-05	80	7/5	1.4
2005-06	80	11/5	2.2
2006-07	100	8/5	1.6
2007-08	60	8/3	2.7

and were dependent on the observed interactions between the buck and doe. Each year, reproductive behaviors of individual does were totaled, and then the totals were averaged for all does within the treatment.

RESULTS

Compared to the GonaCon™-KLH single- and 2-shot treatments (Groups 1 and 2), the GonaCon™-Blue™ single-shot treatment (Group 3) provided superior contraceptive efficacy for more than 5 years (Table 1). Average anti-GnRH titers were sustained at higher levels for the GonaCon™-Blue for longer periods of time than the other treatments. During the first 2 years of the study, the average number of breeding events for infertile does in the GonaCon™-Blue group was less than the average number of events observed in subsequent years. Similar trends were also evident in the GonaCon™-KLH single- and 2-shot treatments, and all were associated with a general decline in antibody titers (Miller et al. 2008).

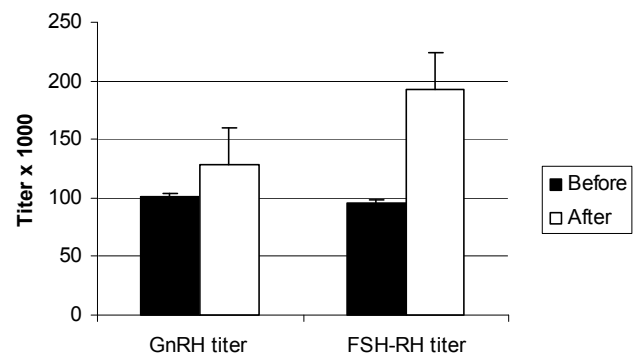


Figure 1. Average serum ELISA antibody titers (±SEM) to the GnRH (LHRH) peptide and the FSH-RH peptide before and after vaccination of does with the FSH-RH vaccine.

Does previously treated with the GnRH-vaccine and subsequently given the vaccine prepared with the FSH-RH peptide produced antibodies to the FSH-RH peptide, but produced antibodies which also cross-reacted with the GnRH peptide (Figure 1). It was also evident that some cross-reaction to the FSH-RH was present from antibodies to the GnRH peptide in the GnRH-treated does, before they were vaccinated with the FSH-RH peptide. The magnitude of the titer response after vaccination with the FSH-RH peptide was greater to the FSH-RH peptide than to the GnRH peptide (Figure 1), indicating that the immune system recognized the FSH-RH peptide as a distinct immunogen.

FSH-RH vaccination of does previously treated with GnRH vaccine was effective in reducing both fertility and reproductive behavior (Table 2). The 3 does in the group that fawned in the 2005-2006 pre-treatment season were infertile in the 2006-2007 breeding season, and 2 of the 3 were also infertile in the 2007-2008 season. Likewise, relative to the pre-treatment season, reproductive behavior was also greatly reduced in the 2 breeding seasons after the FSH-RH vaccination.

DISCUSSION

In earlier studies we conducted with the GnRH vaccine, contraceptive efficacy for the single shot lasted from 1 to 2 years in most does. For the 2-shot regimen, the duration of contraception was extended to 2 to 3 years (Miller et al. 2008). The current results demonstrating 80-100% contraception for 5 years with the single-shot GonaCon™-Blue vaccine exceeds that of any published report involving a GnRH vaccine and is comparable to the multi-year contraception achieved with SpayVac™ in other species (Brown et al. 1997, Fraker et al. 2002, Killian et al. 2008). Return of breeding behavior and fertility in GonaCon™-treated does was associated with a decline in antibody titer to the GnRH peptide. This trend was also apparent with both the single and 2-shot GonaCon™-KLH regimens. It is notable, however, that the return of reproductive behavior occurs in years prior to return of fertility. In years subsequent to receiving the GonaCon™-KLH or GonaCon™-Blue vaccine, does showed a clear trend of increased breeding behavior. The increased expression of reproductive behavior evident with longer periods post-vaccination and multiple years of contraception is a new finding. Although the single-shot does receiving GonaCon™-Blue displayed more breeding behavior in years 3 through 6, they remained infertile. The pattern emerging is that as treated does escape from their immuno-suppression of reproduction with the GnRH vaccine, reproductive behavior returns prior to return of fertility. Reproductive behavior in mammals is driven by the presence of serum estrogen and the absence of progesterone (Killian and Miller 2001, Crowell-Davis 2007). This suggests that follicular development and production of estrogen are sufficient to support reproductive behavior and expression of estrous behavior but inadequate to restore fertility. Under these circumstances, it is difficult to know the mechanism responsible for the infertility. When GnRH-treated does lack behavior to breed, it is the apparent explanation for infertility. How-

Table 2. Observed estrous behavior events and fawning data for GnRH-treated does in the year before and 2 years after treatment with FSH-RH vaccine.

Doe #	Pre-Treatment 2005-2006		Post FSH-RH Vaccine Treatment			
	Behavior	Fawn	2006-2007		2007-2008	
	Behavior	Fawn	Behavior	Fawn	Behavior	Fawn
113	2	0	1	0	2	1
142	3	1	1	0	2	0
2064	1	0	0	0	0	0
2103	2	1	0	0	1	0
3057	3	0	0	0	0	0
4009	1	0	0	0	2	0
4042	4	2	0	0	2	1
Total	16 / 7	4	2 / 7	0	9 / 7	2
Average	2.28	0.57	0.29	0	1.29	0.29

ever, when infertility occurs with breeding behavior, alternative explanations are needed, such as ovulation failure, fertilization failure, or early embryonic mortality. Without detailed study, it is impossible to know the precise mechanism for infertility, but at least two possibilities are suggested. GonaCon™, prepared with the LHRH peptide (Levy et al. 2004), is intended to block the release of luteinizing hormone for the anterior pituitary gland and thereby block follicle development and ovulation. However, evidence also suggests that does possess a follicle stimulating hormone releasing hormone (FSH-RH). FSH-RH has been suggested to stimulate follicle development and estrogen production in the ovary but is not known to stimulate ovulation (McCann et al. 1993, Yu et al. 1997, McCann et al. 1998, Padmanabhan and McNeilly 2001, McNeilly et al. 2003). Because the peptide sequences of the FSH-RH and LHRH are similar, we suspect that during the first year or two following immunization with GonaCon™ there was sufficient cross-reaction between the anti-LHRH antibodies and the FSH-RH peptide to inactivate it. However, as the anti-LHRH titer declined in subsequent years, the cross-reactivity of GonaCon™ and FSH-RH also declined, allowing the stimulation of FSH secretion by the anterior pituitary. Consequently, follicle development and estrogen production would occur, allowing for the expression of reproductive behavior, but not restoration of ovulation and fertility.

The results of the experiment we conducted to determine whether breeding behavior and fertility of does previously immunized with the GnRH vaccine could be shut down by vaccination with the FSH-RH peptide provides some support for the notion that does have a functional FSH-RH peptide. The results suggest that the FSH-RH peptide was inactivated by antibodies produced by the FSH-RH vaccine, resulting in reduction of follicle development. In the absence of normal follicle development, estrogen production would be compromised along with reproductive behavior. In addition, without follicular development, ovulation would not occur and infertility would result. As titers to FSH-RH dropped in the future,

one would predict that cross-reaction to the LHRH peptide would also decline, resulting in restoration of reproductive behavior and fertility.

CONCLUSIONS

These findings lead us to conclude that long-term contraceptive efficacy is possible in female white-tailed deer using the single-shot, GonaCon™-Blue formulation of the GnRH vaccine. During the period of contraception, reproductive behavior may occur without restoration of fertility. Evidence suggests that follicle development in the does may be stimulated by the FSH-RH peptide, which may restore reproductive behavior without fertility in GonaCon™-treated animals.

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