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Contraceptive Effect of a Recombinant GnRH Vaccine in Adult Female Pigs

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ABSTRACT: GonaCon™ is a gonadotropin releasing hormone (GnRH) contraceptive vaccine developed at the USDA National Wildlife Research Center (NWRC) that contains keyhole limpet hemocyanin (KLH) coupled to GnRH. The vaccine is administered intramuscularly in an emulsion with the NWRC adjuvant, AdjuVac™. This first-generation vaccine, developed in 1998, has been very effective in contracepting every species tested. However, because of the high cost of KLH, NWRC has developed a second generation GnRH vaccine, replacing KLH with a less expensive mollusk protein. This second generation GnRH vaccine, called GonaCon-Blue™, is currently being tested in several species and has proven to be as, or more effective than the original GonaCon™.

Scientists with the Reproductive Control Methods Project at the NWRC are also testing a recombinant GnRH antigen developed by Dr. Talwar of the Talwar Research Institute, India. The recombinant antigen was produced from plasmid gene expression grown in and extracted from *E. coli*. The antigen is combined with the NWRC AdjuVac™ to form an emulsion similar to the GonaCon™ preparation. The recombinant vaccine has been shown to be effective as a single or dual injection for contraception of adult female pigs in a short-term study at the Swine Research Facility at Penn State University. Although the long term effectiveness of the recombinant vaccine remains to be established, this small molecule of approximately 14,000 MW is of considerable interest because it may be effective in oral or transcutaneous applications. It could also be scaled-up for large field applications.

KEY WORDS: AdjuVac™, GnRH vaccine, immunocontraception, recombinant GnRH antigen, RecoVac™, single injection

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INTRODUCTION

The single-injection keyhole limpet hemocyanin-gonadotropin releasing hormone (KLH-GnRH) / AdjuVac (GonaCon™) vaccine developed in the last 10 years at NWRC has been shown to be the most effective GnRH vaccine available, providing multi-year protection with a single injection and even longer with a booster injection. The multi-year contraception by a single injection is thought to be due to antigen retention in the draining lymph node (Burton *et al.* 1994). The use of the contraceptive vaccine in deer has been extensive (Killian and Miller 2000, Miller *et al.* 1999, Miller and Killian 2000, Miller *et al.* 2000). More recently, GonaCon™ has been tested in domestic and feral pigs (Killian *et al.* 2003, Miller *et al.* 2004a). It has been suggested that the vaccine could be used to reduce the spread of Brucellosis disease in bison and feral pigs (Miller *et al.* 2004b, Killian *et al.* 2006). The GnRH vaccine is quite versatile and can be used to reduce fertility in a variety of animals (Miller *et al.* 2004c).

The injectable form of the vaccine labeled GonaCon™ has many applications, although for wide-scale field use a non-injectable delivery system for the GnRH vaccine is needed. Because large-scale use is anticipated for a non-injectable delivery form of the vaccine, recent efforts have been focused on the development and evaluation of two low-cost formulations.

In the search for a less costly formulation of GonaCon™, NWRC scientists discovered and tested a second generation GnRH vaccine designated GonaCon-Blue™. The first-generation GonaCon™ vaccine used KLH as the carrier protein. GonaCon-Blue™ utilizes a hemocyanin as a carrier protein, isolated from the mollusk *Concholepas concholepas*, found off the south shores of Chile. This hemocyanin has a blue color because of its high copper concentration. The copper is essential for oxygen transport in the mollusk. In GonaCon-Blue™, this large protein (approximately 8,000 kDa) is conjugated to many copies of a synthesized GnRH peptide. In addition, the vaccine contains a new adjuvant, AdjuVac™ developed by the NWRC.

The GnRH is a non-immunogenic B cell peptide. All vaccine antigens must contain both T cell and B cell peptides. Without the T cell peptide, the immune system would only make the IgM antibody, which would be short lived and ineffective as a contraceptive. The combination of T and B cell epitopes produce long-lasting IgG antibodies. In the GonaCon™ vaccine, the T cell epitopes are provided by the mollusk proteins, either KLH or blue protein. The large, complex size, as well as the foreign nature of the mollusk protein, make it very immunogenic. Dr. G. P. Talwar, of the Talwar Research Institute in New Delhi, India, has previously used the diphtheria toxoid (DT) or tetanus toxoid (TT) linked to

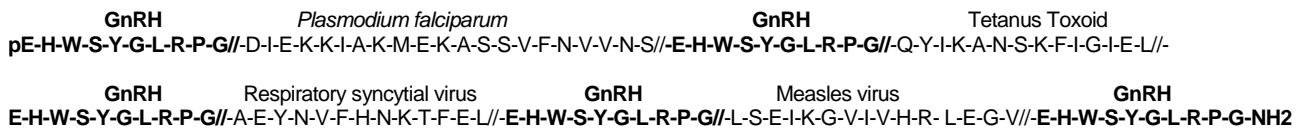


Figure 1. The GnRH-hormone immunogen developed by G. P. Talwar, the “Talwar Recombinant”.

the GnRH as carriers (Shastri *et al.* 1981). Dr. Talwar has also been a leader in defining the GnRH peptide and linkage points for the carriers (Talwar 1985, Talwar *et al.* 1985).

In the GnRH construct developed by Dr. Talwar, the GnRH-hormone immunogen consists of a recombinant multimer of 5 GnRH peptides interspersed with 4 universally immunogenic (“promiscuous”) T cell epitopes of diverse genetic background (Figure 1). The GnRH peptides are positioned at the C-terminal and the N-terminal of the molecule. Dr. Talwar has assembled the gene capable of expressing this recombinant GnRH construct in *E. coli* (Talwar *et al.* 2004). The peptide construct was extracted from the bacteria and checked for proper secondary structure so it would produce antibodies that recognize the native GnRH (Raina *et al.* 2004).

This recombinant vaccine has the advantage of being amenable to large scale production at a reasonable cost. This new Talwar/Miller vaccine has been named RecoVac™. The objective of the current study was to evaluate RecoVac™ when used as a single shot, or a single shot followed by a boost, and to compare those treatments with a single shot of GonaCon-Blue™.

METHODS AND MATERIALS

Contraceptive efficacy was evaluated for two different GnRH contraceptive vaccines on reproductively mature, 5-month-old domestic gilts. Seven pigs received the NWRC GnRH conjugated to Blue protein (800 µg), 5 pigs received 500 µg of the Talwar recombinant GnRH protein, and 5 pigs received 500 µg of the Talwar recombinant GnRH protein, followed in 4 weeks with a 500-µg boost. All GnRH treatment were combined with AdjuVac™, and 6 pigs serving as the negative control received AdjuVac™ alone. At the start of the study, all gilts were bled and received a single IM injection containing their respective treatment vaccine. Approximately 4, 8, and 14 weeks post-immunization, gilts were again bled. Beginning at 14 weeks post-immunization, gilts were evaluated over a 2-month period in a breeding trial for their ability to express estrus, and be bred.

On each day of the breeding trial, each gilt was exposed to a boar and her ability to show “standing heat” recorded. In addition, other behavioral cues relating to readiness to breed and external signs such as swelling of the vulva were noted. Gilts showing standing heat were bred by artificial insemination using semen from proven sires. Pregnancy and farrowing data were recorded. Approximately 6 months after the start of the study, a final blood sample was taken and non-pregnant gilts were euthanized with Euthasol solution. Serum samples from all bleedings were sent to the NWRC on wet ice for titer

and reproductive hormone assays.

The construction of GonaCon-Blue™ was previously described in Miller *et al.* (2004c). The Talwar recombinant GnRH is grown as an expression protein in *E. coli* and purified as described in (Raina *et al.* 2004). Talwar *et al.* (2004) describe the use of this recombinant for treatment of prostate cancer.

RESULTS

All three vaccine treatments produced a reasonable antibody response following a single injection. Five of the 7 gilts treated with GonaCon-Blue™ produced high anti-GnRH titers and were contracepted (Figure 2). Two of the gilts produced litters. The titers resulting from the single GonaCon-Blue™ were higher than those produced from the single Talwar vaccine. However, it is noteworthy that the Talwar recombinant vaccine combined with the NWRC AdjuVac™ produced a significant antibody response with a single vaccination.

This is the first time that the recombinant Talwar GnRH vaccine produced a significant titer with a prime only, resulting in contraception of 3 of 5 gilts. This is a respectable contraceptive response for a small recombinant antigen. The third group, given a boost of the Talwar vaccine 30 days following the prime, was also impressive. The immune response rose rapidly after the boost, and the timing of the peak antibody response corresponded with the breeding period and produced 100% contraception (Figure 3). In contrast, the peak immune response of the single GonaCon-Blue™ antibody occurred about 30 days earlier and started to drop during the breeding period (Figure 2). Although from previous studies with the GonaCon™ we would predict that a boost injection would have also produced a higher percent contracepted, to keep down the cost of the study the GonaCon™ of the Talwar Research Institute in New Delhi, India boost group was not included in the present study.

Because of concerns that GnRH might cause reabsorption of the fetuses, progesterone was used as an early indicator of pregnancy. In Table 1, the first column shows the predicted pregnancy using a progesterone value of over 4 ng/ml on the November 10, 2005 bleed as predictive of pregnancy. The second column indicates the number of pigs in the group that came into heat and were bred. Column three indicates the number of pigs in the group that farrowed. In all treated groups, there was 100% correlation when comparing the progesterone-predicted pregnancy to the actual farrowing. Therefore, it appears that if the GnRH vaccine fails to block pregnancy, the pregnancy will carry through to normal farrowing.

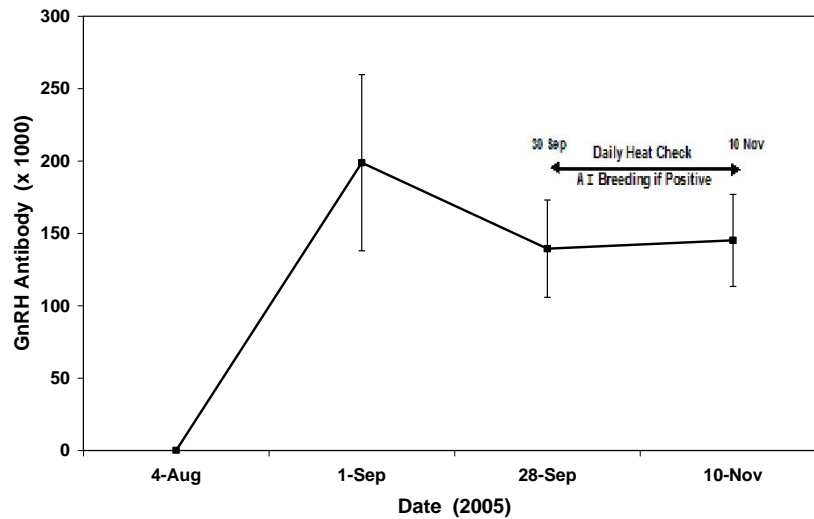


Figure 2. Antibody response in 7 gilts to a single injection of GonaCon-Blue™ and the titer level during the breeding period. Prime injection was given 4 Aug.

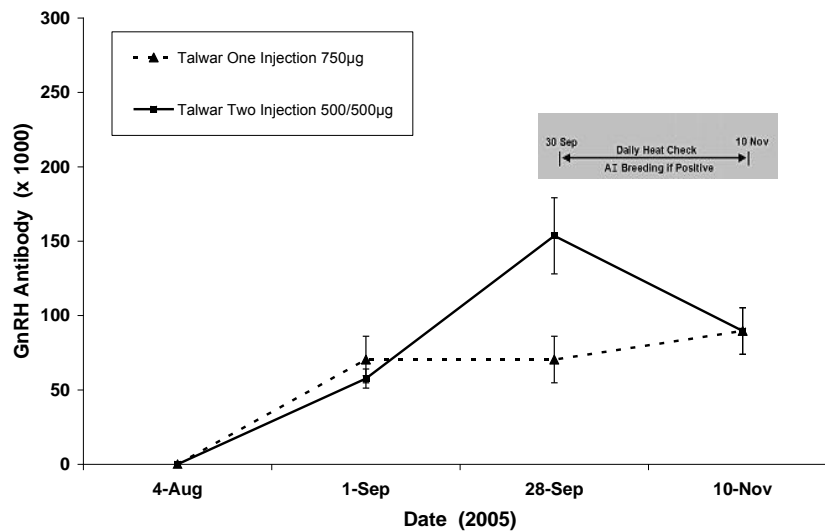


Figure 3. Comparison of antibody titers (mean ± SE) to Talwar single and dual injection. The boost given on September 1 provided high antibody titer through the breeding period.

Table 1. Comparison of the contraceptive results of a single injection of GnRH Blue™ to the single and dual injections of the Talwar recombinant vaccine.

Treatment	n	"Progesterone Positive" (≥4 ng/ml)	Bred	Farrowed
Control	6	4/6	6/6	3/6
GnRH Blue one injection (1000µg)	7	2/7	3/7	2/7
Talwar one injection (750µg)	5	2/5	3/5	2/5
Talwar two injections (500/500µg)	5	0/5	0/5	0/5

DISCUSSION

In the design of his recombinant vaccine, Dr. Talwar chose a number of universally immunogenic (promiscuous) T cell epitopes from many pathogens that have been previously identified (Panina-Bordignon *et al.* 1989). From the many possible, 4 disease T cell epitopes were chosen: *P. falciparum*, tetanus toxin, respiratory syncytial virus (RSV), and measles virus. This variety of disease epitopes provides a diverse genetic background and assures a broad spectrum of species response. Inter-dispersement of the disease epitopes with the GnRH peptide is apparently very effective for producing an immune response to the GnRH peptide. The small size of the recombinant should allow the vaccine to be used in micro-encapsulation technology that we are developing for an oral vaccine delivery system. The GonaCon-Blue™ and the RecoVac™ GnRH contraceptive vaccines are

relatively cheap, which will allow us to test both vaccines in an oral delivery system.

CONCLUSIONS

The small molecule of approximately 14,000 MW included in RecoVac™ has a unique design that makes it very effective as a contraceptive. Most antigens of this size are not very immunogenic and therefore would need to be coupled to a carrier to be effective as a contraceptive vaccine. However, the unique design allows the Talwar vaccine to be effective without the carrier, although the long-term effectiveness of the recombinant vaccine remains to be established. This recombinant vaccine is of considerable interest because it may be adaptable for oral or transcutaneous applications which could be scaled-up economically for large field applications.

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