# Contraceptive Vaccines for Wildlife: A Review

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Wildlife, free-ranging and captive, poses and causes serious population problems not unlike those encountered with human overpopulation. Traditional lethal control programs, however, are not always legal, wise, safe, or publicly acceptable; thus, alternative approaches are necessary. Immunocontraception of free-ranging wildlife has reached the management level, with success across a large variety of species. Thus far, the immunocontraceptive research and management applications emphasis have been centered on porcine zona pellucida and gonadotropin-releasing hormone vaccines. Contraceptive success has been achieved in more than 85 different wildlife species, at the level of both the individual animal and the population. At the population management level with free-ranging species, the primary focus has been on wild horses, urban deer, bison, and African elephants. The challenges in the development and application of vaccine-based wildlife contraceptives are diverse and include differences in efficacy across species, safety of vaccines during pregnancy, the development of novel delivery systems for wild and wary free-ranging animals, and the constraints of certain non-contraceptive effects, such as effects on behavior. Beyond the constraints imposed by the public and a host of regulatory concerns, there exists a real limitation for funding of well-designed programs that apply this type of fertility control.

## Introduction

While elective contraception of domestic animals can be traced back hundreds of years, more recent contraceptive research has been almost universally associated with human applications. However, in the past 40 years a new application has arisen, involving certain species of both domestic animals[1] and wildlife.[2] In the past decade, increased public concern over unrestrained dog and cat reproduction has stimulated a great deal of research in this area, and issues such as the prevention of pregnancies in feedlot cattle have even brought the commercial agricultural community into the world of contraceptive research.[3] A few of these activities, however, provide methods that are directly applicable to non-domesticated animals. Not surprisingly, wildlife contraception involves a number of diverse challenges not seen with either human contraception or domestic species.

With regard to wildlife contraception, four commonly referenced case studies include wild horses (*Equus caballus*), urban and park cervids such as white-tailed deer (*Odocoileus virginianus*) and wapiti (*Cervus elphus*), African elephants (*Loxodonta africana*) in game parks, and more recently bison (*Bison bison*). There are other examples but these stand out with regard to free-ranging wildlife. A growing number of non-domestic species housed in zoos and wild animal parks could also be included. With rare exception, all are medium to large mammals.

Efforts at controlling wildlife overpopulation by means of contraception began as early as the 1950s, and most research involved natural and synthetic steroids, or a variety of non-steroidal compounds.[4–6] Virtually all of these early attempts, while

pharmacologically successful, ended in failure, for a variety of reasons including toxicity, passage through the food chain, adverse effects on social behaviors, high cost of application, difficulty in delivering the compounds remotely, health risks in pregnant animals, and a host of regulatory issues. Of course, the constraints within these early approaches varied depending on the species. For example, remote delivery was not a requirement for captive exotic species, and behavioral changes were of less importance in urban deer, which were viewed as 'pests' in some quarters. Conversely, remote delivery is a virtual requirement for free-ranging wildlife, and behavioral changes would not be tolerated in social animals such as wild horses and African elephants. Thus, each species and setting brings with it specific needs and limitations. The cumulative effect of all these issues resulted in the realization that contraception with steroid hormones, the method of choice for humans and dogs, was not acceptable across the broader spectrum of wildlife. Steroids remain useful in some captive wildlife contraception efforts but have been abandoned for use in free-ranging wildlife.

It was only with the advent of practical immunocontraception that significant progress in free-ranging wildlife contraception occurred. Immunological approaches were considered for use in wildlife as early as 1987[7] and the earliest recorded attempts at wildlife immunocontraception included a gonadotropin-releasing hormone (GnRH) vaccine tested in wild horses on Cumberland Island National Seashore in 1986.[8] Shortly thereafter, a porcine zona pellucida (PZP) vaccine was tested in domestic and captive wild horses.[9] The results of the GnRH trial were not promising, while the PZP trial provided clear evidence that an immunocontraceptive could successfully inhibit fertility in equids. In relatively rapid succession, trials with PZP were successful in captive white-tailed deer[10–12] and a variety of zoo animals, including Przewalski's horse (*Equus przewalskii*) and Banteng (*Bos javanicus*)[13] Formosan sika deer (*Cervus nippon taiouanus*), axis deer (*Cervus axis*), Himalayan tahr (*Hemitragus jemlahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), muntjac deer (*Muntiacus reevesi*), and sambar deer (*Cervus unicolor*)[14] and African elephants (*Loxodonta africana*) (J. F. Kirkpatrick, unpublished data).

Identifying an effective vaccine was only the initial step in developing a population management for wildlife. Very early it was recognized that the major challenges in wildlife contraception included (i) identifying successful contraceptive agents in captive animals, (ii) documenting safety associated with successful agents, (iii) developing a means of delivering these agents to free-ranging wildlife, and (iv) actually altering populations. With the success of PZP-induced fertility control in captive animals, which addressed the first of the four challenges, the next and more challenging step was the implementation of actual field trials with free-ranging animals. While the strategy remained the same, this represented a major change in tactics and one that is still underappreciated. Fortunately, one of the major advantages of immunocontraception became obvious during captive animal trials, which was the ability to deliver contraceptive doses in very small volumes, which could be delivered remotely. This is now recognized as a key requirement for practical wildlife contraception.

The first field trial of wildlife PZP immunocontraception occurred on Assateague Island National Seashore, a barrier island off the coast of Maryland 23 years ago. This trial proved successful in inhibiting fertility in wild horses.[15] Aside from demonstrating a high degree of contraceptive efficacy, this initial study proved beyond a doubt that the vaccine could be delivered remotely, without any handling of animals. It also demonstrated the safety of the vaccine in pregnant animals[15] and that fertility inhibition could be maintained with annual booster inoculations.[16]

The first field trial with white-tailed deer occurred at the Smithsonian Institute's Conservation and Research Center at Front Royal, VA.[17] Deer were captured, tagged, and given a primer dose of PZP and then released. Subsequent booster inoculations were given remotely via small 1.0 cc darts. As with earlier captive trials, the vaccine proved efficacious (85%) and remote delivery proved successful. In the case of both the wild horse and deer field trials, animals received a 65- to 100-μg primer dose emulsified with Freund's complete adjuvant (FCA) and a booster inoculation of 100-μg PZP emulsified with Freund's incomplete adjuvant (FIA). The first field trial with free-ranging African elephants occurred in 1996, in the Kruger Park, in South Africa. Animals were immobilized from a helicopter, given ultrasound examinations to determine pregnancy status, collared and hand injected with a 600-μg primer dose of PZP + a component of Ribi® adjuvant.[18] Subsequent booster inoculations occurred by remote

treatment. Results were positive but efficacy was lower (75%) than with horses and deer. A change of adjuvants, to Freund's modified adjuvant (FMA), increased efficacy to 100%.[19,20] Since the completion of these trials, management level application of the PZP vaccine has been successful in altering entire populations, stopping population growth and even decreasing populations, of wild horses,[21] urban deer,[22] wapiti,[23] and African elephants.[19,20]

## Safety

At this point, attention turned to safety issues. The questions at hand were (i) did PZP alter ovarian function, (ii) were the contraceptive effects reversible, (iii) were there adverse behavioral effects of PZP immunocontraception, and (iv) were there any short- or long-term adverse physical side effects?

A major advantage of zona proteins as immunogens is the absolute lack of cross-reactivity with other tissues and protein hormones.[24–26] Additionally, the site and mechanism of action (see Clark and Dell[27] for an eloquent discussion of the mechanism of action supporting zona vaccines) is as far 'downstream' for most of the reproductive processes as possible, such that the sequelae of reproductive events that are disrupted are inconsequential. This is essential for wildlife species but may be considered a disadvantage if the target animals are domestic species, such as dogs and cats, where extinguishing undesirable behaviors is as much a goal as contraception.

The safety of PZP immunocontraception was confirmed for horses, deer, and elephants through a series of follow-up investigations. Ovarian endocrine function was monitored in treated wild mares via urinary steroid metabolites,[28] and it was shown that there were no permanent or significant changes in ovarian endocrine parameters and estrous cyclicity even after long-term treatment.[29–31] This is consistent with the accepted theory of the mechanism of action of zona proteins.[32] Later studies in wild horses confirmed the safety of PZP use in pregnant mares[33,34] and reversibility of contraceptive action, at least through five consecutive years of treatment.[33] demonstrated increased body condition[35] and increased longevity in mares treated chronically,[36] and no significant changes in fundamental social organization or behaviors.[37] Two more recent studies found that PZP treatment resulted in less band fidelity among treated mares outside the breeding season[38] and minor changes in time budgets among treated mares.[39] However, the former study was not controlled for pregnant animals or mares that had foals removed annually. The latter study found that most behavioral changes were associated with changes in body condition or the presence of foals. Considering that untreated mares have more foals than treated mares, and that treated mares develop better body condition than untreated mares, small changes in time budgets would be expected. Ultrasound examinations of ovaries and uteri from PZP-treated elephants also revealed a lack of negative effects.[18,20]

While no long-term negative health effects were detected in PZP-treated wild horses, there was some concern over injection site reactions. Because of the homology of the zona glycoproteins across many mammalian taxon groups, PZP is a poor immunogen. Thus, a powerful adjuvant is necessary and between 1988 and 1998, FCA was the adjuvant of choice for initial inoculations, followed by booster inoculations of PZP + FIA. In reality, as long as inoculations were given in the rump or hip, injection site reactions were uncommon; however, there was concern over causing false tuberculosis-positive tests among some species of animals treated with FCA (there is no TB test for equids; thus, this was not a problem in these species). As a result, for initial inoculations, a new adjuvant was utilized with PZP in wild horses, and later among other species as well. FMA, which utilizes the fractionated cell walls from *M. butyricum*, a common soil bacterium with no known pathologies associated with it was tested and proved to be as efficacious as FCA.[40] Thus, this is the adjuvant of choice since 1998. Rare injection site reactions still occur after inoculation, even with FMA or FIA, but seldom exceed 1%.[40] One study[41] demonstrated a higher incidence of injection site reactions in horses treated remotely, with darts, than those treated by hand-injection. This suggests that injection site reactions may be as much a function of delivery as the actual vaccine. Delivery by dart must result in surface bacteria and debris being pushed into the injection site. In the absence of either short-term or long-term health issues, PZP contraception is now common in free-ranging and sanctuary wild horses, urban deer, zoo animals, and African elephants. Other safety issues have been reviewed in more detail elsewhere.[42]

Other immunogens have been used in horses but thus far none have been applied to free-ranging wild

horses except in small-scale research trials. Several approaches targeted the stallion. GnRH vaccines have demonstrated that testicular function can be suppressed in the stallion.[43,44] However, the application of these vaccines to wild horses is contraindicated because of the suppression of testosterone-mediated reproductive behaviors, which are necessary for holding breeding bands together in the face of competition from other stallions. The entire social structure of wild horses is disrupted if the dominant stallion is chemically castrated. Further, studies have shown that control of dominant males, by means of vasectomy, in wild horses is not effective because this increases the reproductive success of bachelor stallions.[45] A second problem associated with targeting the male of the species, is that, if it is not a harem-based social structure, nearly every male in the population will need to be treated, and this is probably not logistically possible. Thirdly, the removal of functioning males from the breeding population may have far more serious genetic consequences that treating females[46,47] and certainly provides less management flexibility.

Three commercial GnRH vaccines have shown success in suppressing reproduction in mares. Both Equity® (Pfizer Animal Health, Sandton, South Africa) and Improvac® (CSL, West Ryde, NSW, Australia) suppressed cyclic activity in mares.[48,49] GonaCon®, a GnRH vaccine developed by USDA, primarily for use in deer, was tested in captive wild mares and also was successful in blocking cyclic activity and ovulation.[50] A fourth commercial GnRH vaccine, Repro-BLOC® (Amplicon Vaccine, Pullman, WA, USA) has been developed as an alternative to castration in cattle. This latter vaccine utilizes a series of GnRH genes, which were cloned into an ovalbumin carrier via an *Escherichia coli* expression vector. Aside from trials in cattle, Repro-BLOC® has been used to suppress estrus in captive Asian elephants and proposed as a contraceptive for this species;[51] however, five serial inoculations were required to achieve adequate anti-GnRH titers. Despite contraceptive success, and clear potential applications to several other species, the application of GnRH vaccines to wild horses brings to the fore issues that usually are not considered with human contraception. The complex evolutionary social organization of horses and other equids is driven to a large degree by reproductive steroids. In the case of GnRH vaccines, the result is, although temporary, a non-surgical castration. Public opinion, which directs so many aspects of contraception, human, or wildlife, requires in the case of wild horses an intact set of social behaviors. The same is true for elephants and most captive exotic species in zoos.

Other potential safety issues regarding GnRH vaccines remain. Safety for use in pregnant animals remains a species-specific issue. In those species that rely on pituitary Luteinizing hormone (LH) to maintain the corpus luteum of pregnancy throughout the entire gestation period, GnRH will cause abortion. This is not an issue in a species such as the horse, where placental hormones take over the task of supporting pregnancy, but use in bovids, for example, such as the bison, or during the first half of pregnancy in the goat family would interfere with pregnancies in progress. More troubling, however, are a variety of other issues associated with any vaccine that blocks GnRH. It has now been demonstrated that GnRH receptors exist in a variety of tissues throughout the mammalian body, including the cerebellum,[52] bladder,[53] and cerebrospinal fluid.[54] Now recognized as a form of neurotransmitter, GnRH has physiological effects throughout the central nervous system. GnRH activity in the hippocampus has been implicated in Alzheimers-like syndrome. GnRH activity can alter olfactory function, which is, of course, vital in the reproductive process for so many wildlife species. In the cerebral cortex, GnRH can cause depressed activity, and in the cerebellum GnRH has a correlative link to two genetically based disorders, including Gordon–Holmes Syndrome and Boucher–Neuhauser Syndrome. In the pituitary, it has also been demonstrated that GnRH affects more than just gonadotropic cells.[55] The action of GnRH in cardiac tissue, which is exceptionally rich in GnRH receptors, may also be an important safety issue. At least two studies have demonstrated a serious and negative effect on cardiac function and in men, blocking GnRH can place the patient at greater risk for coronary infarction.[56,57] Whether or not these particular safety issues have any clinical relevance to free-roaming or captive wildlife remains to be seen. However, it becomes intuitive that vaccines that exert their influence further 'upstream' in the reproductive process and which have interactions with non-reproductive tissues will be more problematic than those with target tissue specificity and that exert their effects further 'downstream' in the reproductive process.

In white-tailed deer, safety issues relating to PZP largely paralleled those of wild horses, but with

some exceptions. Reversibility of contraceptive action occurred after 1–3 years in captive PZP-treated deer[11] and after 1–4 years in another study with captive deer.[12] An earlier study demonstrated that there were no changes in ovarian histology, but that the breeding season was extended by one to 2 months in PZP-treated deer.[17] In one study, this resulted in a pulse of late births the first year, but in subsequent years, the late births did not occur.[58] Treated deer were more active than untreated deer but did not spend more time feeding. Despite the increased activity, treated deer gained significantly more weight by the summer following treatment than untreated deer, presumably because of avoidance of lactation, but by the following fall, all weight differences disappeared.[59,60]

Potential changes in ovarian histology were a concern. Prior to application of PZP vaccines to wildlife, some temporal changes in ovarian function were shown to occur in PZP-treated non-human primates[61] and mice,[62] but long-term effects did not occur. Early studies with PZP-treated dogs revealed significant changes in ovarian histology.[63] Another study[64] demonstrated similar effects in PZP-treated rabbits, along with a depletion of primordial follicles. Among PZP-treated sheep, significant changes in the ovarian histology were found.[65] The dog study was probably compromised by a rather impure PZP, and the rabbit and sheep studies revealed species differences in response to the PZP. Collectively, this research raised an issue of concern as wildlife contraception moved forward and into the realm of management.

In the first trial with PZP and horses,[9] no changes in ovarian histology were found, and other investigators had demonstrated ovarian weights and histology were unchanged after PZP treatment of white-tailed deer.[17] In another study[66] ovarian eosinophilic oophoritis was found in six of eight treated deer, but this rate was not significantly different from controls. This same study revealed that this condition is common in untreated deer at the time of ovulation. Despite the absence of any significant changes in ovarian histology in wildlife studied thus far, it is vital that each species be evaluated for potential changes. Blood chemistry studies in treated white-tailed deer revealed only a few significant changes after PZP treatment, but none was associated with any physiological abnormalities.[66,67] Ongoing studies with Dall sheep (*Ovis dalli*) and domestic goats (*Capra hircus*) reveal the same lack of blood chemistry changes in these two species.[62] Injection site abscesses were found in only 2 of 353 deer (0.5%)[68] and 100% of PZP-treated deer revealed granuloma formation at the injection site[66] but without any complications.

## Efficacy

A major challenge in the development of human immunocontraceptives is the variability in immune responses by individuals, similar to that which is evident in so many prophylactic vaccines, such as influenza. It is unlikely that humans will use contraceptives with efficacy as low as even 95%. There are individual differences within a species in reactions to contraceptive immunogens. For example, examining the anti-PZP antibody titers from several groups of horses,[65] wapiti[69], fallow deer (*Cervus dama*),[70] and white-tailed deer[71] treated with PZP reveals a significant difference in response to the same immunogen, the same dose, and the same adjuvant. However, for wildlife populations, a variety of modeling[71] and results from field trials[21] have demonstrated that contraceptive vaccines with less than 100% effectiveness will suffice.

Clearly there are species differences as well. For wild horses treated with native PZP, efficacy is approximately 95% over 23 years.[21] In the case of white-tailed deer, there is more variability,[11,12] with an efficacy of approximately 75%. Among PZP-treated wapiti, efficacy ranged from a low of 84% to a high of 90%.[23] African elephants in one population have responded with a 100% efficacy over 10 years of management.[20] Among captive exotic species, efficacies ranged from lows of 70% in Sambar deer (*Cervus unicolor*) to highs of 100% in addax (*Addax nasomaculatus*), gerenuk (*Litocranius walleri*) markhor (*Capra falconeri*) bighorn sheep (*Ovis canadensis*), mountain goats (*Oreamnos americanus*), and several other ungulates.[72] Thus, a major challenge to the development of wildlife immunocontraceptives is the ability to inhibit fertility across diverse species. GonaCon-B® and SpayVac® have both been associated with efficacies in the ranges of 39–64% and 27–48%, respectively, in wild horses[73] while SpayVac® was associated with 100% efficacy in fallow deer over 3 years.[74] The GnRH vaccine Improvac®, when given to domestic mares in two inoculations over 35 days provided 100% efficacy with cycling and ovulation as the endpoints.[48]

This latter issue reflects both a strength and weakness of PZP immunoconception, and for that matter

any immunocontraceptive targeting some reproductive tissue (ovum, sperm or pituitary cell) or protein hormone (GnRH). Unlike reproductive steroids, which have absolute homology across species, protein hormones reveal amino acid differences across species. In the case of PZP, there must be an immune response to an epitope of either or both the PZP-1 and the PZP-3 alpha glycoprotein for a successful antibody response and effective contraception. In the case of white-tailed deer, anti-porcine ZP antibodies recognized several epitopes of both the ZP-1 and ZP-3 glycoproteins[75,76] but in domestic cats, the same antibodies do not recognize any segment of the zona glycoproteins.[77,78] While a species-by-species analysis of cross-reactivity with PZP-induced antibodies has not occurred, it is clear that species-specific epitopes vary across taxonomic lines and will affect the contraceptive efficacy of the immunogen. Thus far, PZP-induced contraception has not failed in any of 64 members of Ungulata in which it has been attempted, whether members of Perissodactyla or Artiodactyla,[42] or bears[72,79] or pinnipeds.[72,80] This implies some evolutionary relationships among the epitopes of the zona sperm receptor. Although not directly related to contraceptive research, the application of zona proteins from, and directed toward, a variety of species across broad taxonomic lines will surely reveal interesting evolutionary pathways in the development of the sperm receptor.

## Duration of contraceptive action

Vaccines generally, but not universally, employ killed or attenuated virulent organisms and the immune response by humans and domestic animals is very powerful, with lasting effects even after a single inoculation. In contrast, contraceptive vaccines generally target reproductive hormones or tissue receptors, and the ability to cause long-lasting elevations in antibodies, individual differences notwithstanding, is far more challenging, because of the relative homology of these protein molecules between species. It is intuitive that a single inoculation, long-lasting contraceptive vaccine is valuable and a worthy goal for free-ranging wildlife.

The current major shortcoming of the most widely used wildlife contraceptive, PZP, is that contraceptive duration is short-lived, at least during the first few years of treatment. An initial inoculation followed by a booster inoculation 2–6 weeks later generally provides a year of contraception. But then, an annual booster is required for a second year of contraception. At least for the first 2–3 years, this is a requirement for successful contraception. Following 3 years of treatment, the duration of high antibody titers is sufficient for successful contraception over several years and eliminates the need for annual booster inoculations. For example, in the wild mare, 3 years of treatment, from age 2 through 4, result in contraception that lasts for a mean of 3.7 years. However, the range is 1–8 years.[53]

This shortcoming of native PZP has stimulated a search for more lasting contraceptive vaccines. Several approaches to this challenge have occurred. The first approach has attempted to incorporate the PZP glycoprotein in biodegradable, non-toxic matrices, which can be injected and which then release the PZP molecule slowly in a sustained release or in pulsed releases. The matrix used thus far has incorporated various ratios of lactide-glycolide, either in the form of microcapsules[81] or in the form of small pellets that can be injected either by hand or by dart.[82–84] Results thus far have been encouraging, but not definitive, and a great deal of variability in both the manufacturing process and the results has resulted. Another difficulty with this approach is that it has not been possible to incorporate powerful oil-based adjuvants in the microcapsules and pellets.

A second approach has incorporated a novel 'packaging' of the PZP. In the case of a proprietary product, SpayVac®, native PZP is incorporated into a multilamellar liposome. This platform appears to provide a much longer duration of high antibody titers and contraception in seals,[80] fallow deer,[74] white-tailed deer[85,86] and horses.[71] Contraceptive duration appears to last for at least 3 years following a single treatment. The primary drawbacks to this approach appear to be the high viscosity of the preparation and the inability to deliver it remotely, and possible side effects on reproductive tissues,[50] at least in horses. The former is a technical problem and may be overcome, and the latter requires substantially more safety testing before this approach becomes practical. Nevertheless, an important point here is that native PZP vaccine must not be confused with more complex vaccines containing PZP.

A third approach that has shown promise is the GnRH vaccine GonaCon®. This vaccine targets the GnRH molecule and blocks ovulation and estrus by preventing endogenous GnRH from stimulating FSH and LH producing cells of the pituitary. In turn, the lack of gonadotrophs inhibits ovarian and testicular

function and gamete production. As with PZP, there are structural differences in the GnRH of various species, with varying degrees of homology. This means that the vaccine will work in some species but not as well or at all in others. GonaCon™ was developed primarily for application to white-tailed deer,[12,87–90] but also works in wapiti,[91] swine,[92] and horses.[50] In the case of GonaCon™ the longer lasting duration of antibodies has been accomplished by the addition of aromatic amino acids to the structure, which enhances immunogenicity but without interfering with binding to pituitary receptors. Puzzling, however, trials with free-ranging deer resulted in significantly lower efficacy than those with captive animals.

Alternatives to longer acting vaccines have examined delivery systems. As early as 1993, the idea was put forward to engineer the genes for immunogenic proteins into bacterial or viral vectors.[93–95] Known collectively as genetically modified organisms (GMO), the theory was that a gene for an immunogenic molecule that causes fertility inhibition, such as PZP, or GnRH, might be engineered in a non-pathogenic virus, which could then be used to infect the target species and express the gene. There are multiple questions associated with this technology. Can the vector persist for a long enough time to express the immunogen? Could the vector express the immunogen in sufficiently large quantities to elicit adequate antibodies? Will the vector reach a sufficiently large portion of the population to alter density?[96] Research followed that examined a number of possibilities. Cloned genes for PH-20, LDH-C4, and ZP3, all of which encode for gamete proteins were proposed for incorporation into GMO vectors,[97,98] but none ever reached the field test stage. Significant issues of regulation were raised for a contraceptive method that, once released into the environment, could never be called back.[99] The issue of mutation rates in viruses, and the potential for the vector to infect non-target species, was also an issue. For example, a vector that could successfully infect and cause contraception in non-native foxes in Australia would probably also bring about the same result with domestic dogs. One study concluded that the effort to assess risks of contraceptive GMOs would be greater than the effort to develop the technology.[99] These and many other concerns have led to a cessation of this line of research for wildlife immunocontraceptives and have been summarized elsewhere.[100–102]

**Table 1** Comparison of Zona Protein and Anti-GnRH Contraceptive Vaccines

| Parameter | ZP | Anti-GnRH |
|---|---|---|
| Efficacy | 80–100%[a] | 39–100%[a] |
| Male | – | + |
| Female | + | + |
| Reversibility | +[b] | +[c] |
| Remote delivery | + | + |
| Safety in pregnant animals | + | Species Dependent[d] |
| Passage through food chain | – | – |
| Target tissue specificity | + | – |
| Debilitating side effects | – | ??[e] |
| Behavioral effects | Minor | Major |
| Available as a synthetic | – | + |

[a]Values are for horses and deer; beyond those, efficacy is variable depending on the species.
[b]Reversible through five consecutive years of treatment; not reversible after 7 years of consecutive treatment.
[c]Long-term data not available.
[d]Species in which the corpus luteum of pregnancy is supported by pituitary LH will abort if treated with an anti-GnRH agent.
[e]Long-term data not available.
GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

One exception to this trend is a research effort in New Zealand.[103,104] Here, the vector is a nematode parasite *Parastrongyloides trichosuri* and the target species is the invasive brushtail possum (*Trichosurus vulpecula*). The parasite is species-specific and mutation into some form that might infect other species is unlikely. If a gene for a contraceptive protein can be engineered into this parasite, and can be expressed effectively, it may open the door to similar approaches in other species. Of course, there is no guarantee that this particular parasite could not find its way to Australia and infect native possums.

One final approach to the problem of long-term immunocontraception of wildlife remains. Oral delivery of a contraceptive vaccine would overcome the need for laborious delivery systems currently necessary for sustained antibody titers in target animals.[105] Currently, this approach is not a practical reality, but the challenges have been clearly delineated. First, either the contraceptive vaccine itself has to be species-specific or the oral delivery system must be species-specific. Approaches under consideration include transgenic plants[103] and bacterial ghosts,[106] both carrying genetic information for a contraceptive immunogen. The research challenges

are immense and the regulatory hurdles daunting, and it is unlikely that this approach will bear fruit for many years to come.

## Conclusions

Immunocontraception for wildlife management has become a reality since its first application in 1988. Today, animals treated annually number in the thousands, and controlled alterations in population size are occurring in various settings at ever-increasing frequency. The most widely used wildlife contraceptive is native porcine zona protein, largely because of its ability to inhibit fertility in such a large breadth of species, its tissue-specific action, and because of its impressive safety record. Currently six different free-ranging wildlife species are being managed by means of PZP immunocontraception on 52 sites and 76 captive exotic species are being treated in 67 zoological gardens around the world. Several GnRH vaccines show promise, but widespread application will have to wait for more extensive safety testing. Table I summarizes and compares the major characteristics of PZP and anti-GnRH vaccines. The extraordinary success with actual field applications of wildlife immunocontraception over the past two decades has created a new paradigm. Only 23 years ago, the primary question was whether or not we could economically deliver, safely treat, and effectively regulate wildlife populations with vaccine-based contraception. Today, the new question is how we can achieve this goal easier, with less effort and cost and gain public approval at all levels. The remaining challenges now center on improving delivery systems, modifications of the vaccine components to enhance the duration of effectiveness, assuring comprehensive safety, developing a sustainable funding base for research, and achieving general acceptance.

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