

## **Non-surgical methods of contraception and sterilization in select domestic and wildlife species**

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### **Abstract**

Population control of domestic and wildlife species is an important consideration of veterinarians and wildlife management organizations. Despite decreased rates of euthanasia in United States animal shelters, millions of dogs and cats continue to be euthanized each year. Additionally, almost half of the United States feral horse population is currently housed at government-sponsored facilities and lands, and other populations, such as white-tailed deer, have expanded to be considered pest species in some areas of the United States. Standard surgical sterilization techniques have been used to help regulate small animal populations; however, other, more efficient means of population control are needed, particularly for free-roaming and large animal species. Non-surgical contraception and sterilization methods have the potential of fulfilling this need. Although the ideal method has yet to be developed, vaccines against gonadotropin-releasing hormone and zona pellucida glycoproteins have shown promise as contraceptive agents, and intra-testicular injections have been used to induce sterility in males. This review will focus on methods of non-surgical contraception and sterilization which have been researched in cats, dogs, horses, and select wildlife species, and discuss current or future research which may result in increased efficacy, prolonged immune response, and fewer adverse effects in the target species.

**Keywords:** GnRH vaccine, zona pellucida vaccine, immunocontraceptive, zinc gluconate, infertility

### **Introduction**

Regulation of population size in pet and free-roaming populations is an important consideration for veterinarians and wildlife management organizations. The Humane Society of the United States estimated that although the rate of euthanasia has decreased approximately 77% since the 1970s, 3.4 million dogs and cats were euthanized in United States animal shelters in 2013.<sup>1</sup> Additionally, a number of wildlife populations have exceeded the available resources of their habitat, which can impact natural ecosystems. In these cases, animals may be removed from the population by round-up and adoption (feral horses), increased issuance of hunting tags (white-tailed deer), or euthanasia. In 2007, approximately 46.4% of the United States feral horse population was housed in government-funded facilities at a cost of \$40 million per year.<sup>2</sup> Animal overpopulation is a serious concern, and although standard surgical sterilization methods have shown effect in small animal shelter populations, there remains a need for regulation of population size in free-roaming animal populations. In the past few decades, non-surgical methods of contraception and sterilization have become a focus of research. Currently several products are in use in the United States and other countries, and have shown efficacy where surgical sterilization methods are undesirable or impractical.

Non-surgical contraception or sterilization provides several advantages over the current surgical sterilization methods of castration and ovariectomy or ovariohysterectomy. Administration of an injection to regulate reproductive ability or to induce sterilization is much less invasive compared to reproductive surgery. Pet owners that are averse to anesthesia or surgical sterilization may be more accepting of non-surgical methods. These agents have the potential to greatly increase the number of animals that can be rendered infertile or sterile in population settings, including free-roaming dog populations, feral cat colonies, and wildlife populations. Non-surgical contraception or sterilization requires less surgical expertise, equipment, and time than traditional surgical sterilization.

Several methods of non-surgical contraception or sterilization have been researched, with variable results. Currently, there are two methods of non-surgical contraception and one method of non-surgical sterilization which are used or show promise to successfully inhibit or reduce reproductive ability, or induce sterility. Two methods use the concept of immunocontraception: the gonadotropin-releasing hormone (GnRH) vaccine and the zona pellucida vaccine. The third method uses intratesticular injections

to induce sclerosis and arrest of spermatogenesis. The objective of this paper is to review the methods of non-surgical contraception and sterilization which are currently in use, or which have shown promising results in research trials. These methods will then be discussed in the context of their use and success within select domestic and wildlife species, including cats, dogs, horses, and several wildlife species, with a focus on the control of free-roaming populations.

### **Challenges in development of non-surgical methods of sterilization**

The ideal non-surgical contraceptive or sterilization method would need to meet several criteria, and has yet to be determined. These properties include easy administration, single administration in the animal's reproductive lifetime, 100% efficacy (including both sexes and all ages), and induction of rapid and permanent sterility.<sup>3-5</sup> In addition, pertaining to pet cats and dogs, the ideal method would eliminate sex-related behaviors including estrous cyclicity, roaming, urine spraying, and aggression. In wildlife species, where herd social hierarchy relies on maintenance of sex steroid production (such as in feral horse harems), the ideal contraceptive mechanism must not eliminate production of sex steroids yet prohibit fertility.<sup>6</sup> Finally, the ideal product must be cost-effective, able to be produced in large quantities with little variation in quality control, and result in few side effects.

The development of a product with all of these desired properties is challenging. The Michelson Grant and Prize program was developed to provide \$75 million in research monies to develop a one-dose product which would result in permanent sterility in male and female dogs and cats, with a \$25 million prize allocated for the successful research group.<sup>7</sup> Currently \$14 million has been allocated to research the "ideal" product.<sup>7</sup> The major challenge with the use of immunocontraception is that it requires targeting self-antigens – as a result, self-tolerance must be overcome in order to induce an immunologic response.<sup>8</sup> Variable responses exist between individuals, and measuring this response poses a problem because it is unclear if circulating antibodies are representative of efficacy.<sup>9</sup> Long-lasting antibody titers against self-antigens are difficult to achieve, often requiring several boosters for a prolonged response.<sup>10</sup> Side effects due to affected downstream functions of the target hormones, or due to adjuvants required to stimulate a sufficient response, are also a concern.<sup>8</sup>

In addition, delivery of non-surgical methods of contraception to free-roaming feral or wildlife animals presents its own set of challenges. Currently all formulations require injection, which is achieved either by remote darting from vehicles or aircraft or by hand (typically with the animal tranquilized or anesthetized). Access to feral dogs and cats usually requires trapping.<sup>9</sup> If formulations were developed which could be administered orally, baiting would need to be species-specific and have a wide margin of safety.<sup>9</sup> In addition, it would be difficult to identify which animals have already been treated unless animals are permanently identified, which in some small animals has involved tattoo placement in the pinna or inguinal region.<sup>11</sup> For pet dogs, cats, and horses, administration of non-surgical contraceptive vaccines could be easily incorporated into yearly wellness examination visits with the veterinarian.

The goals of a non-surgical contraception or sterilization program vary greatly when considering individual animals versus a population. In companion animals, the induction of complete sterility is vital in each individual animal, as owners are unlikely to consider non-surgical methods acceptable if efficacy is not comparable to surgical methods. In contrast, it is not as vital that every individual animal is rendered infertile in a population setting. For example, studies have shown that only 70-80% of female cats in a feral cat population, or more than 95% of males which breed in polygamous mating systems, must be made infertile in order to control the population size.<sup>12,13</sup> Furthermore, regulation of male fertility is only effective in contained or captive populations. In companion animals, a predictable duration of effect is important, but the need for booster vaccines would be more easily managed than in feral or wildlife settings. It must be recognized, however, that failure of owner compliance with booster vaccines would lead to resumption of fertility in many cases.

In contrast, long duration of infertility must be induced in population settings since frequent boosters would be a severely limiting factor in terms of practicality.<sup>9</sup> In fact, a single dose of an immunocontraceptive vaccine has resulted in infertility of several years duration in female white-tailed deer, feral swine, and horses.<sup>14-18</sup> In feral cat colonies, however, animals tend to have shorter lifespans

and models suggest that a three-year duration of efficacy in females would be sufficient to regulate population size.<sup>12</sup>

With any population, there are concerns about the presence of individuals which are "non-responders" to a vaccine; studies of immunocontraceptives have consistently shown that a small percentage of the population fails to produce an antibody response. This may reflect individual animal genetics, and may lead to a population which, after rendering infertile all susceptible animals, is repopulated by "non-responder" animals, a trait which may be heritable, and which may limit effectiveness of immunocontraceptives in future generations.<sup>5</sup>

### **The concept of immunocontraception**

Reproductive function is regulated via the hypothalamic-pituitary-gonadal axis. Gonadotropin releasing hormone secreted from the hypothalamus stimulates production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland, which act on the ovaries and testes.<sup>19</sup> In females, these hormones regulate follicular activity, ovulation, and the production of estrogen and progesterone. In males, these hormones act on the testes to produce testosterone and support spermatogenesis.

Immunocontraception relies on the stimulation of antibody production against self-antigens which are involved in the regulation of reproductive function in order to inhibit fertility.<sup>9</sup> The goal is to initiate an immune response against a specific hormone or a protein which is integral in the hypothalamic-pituitary-gonadal axis in order to induce infertility. The target antigen can be any hormone or protein that plays a mandatory role in reproductive cyclicity and fertility. Two targets that vaccines have had success at stimulating an effective immune response against to induce infertility are GnRH and zona pellucida glycoproteins. These two methods of immunocontraception will be discussed in further detail.

#### **Zona pellucida vaccine**

Zona pellucid (ZP) vaccination acts by eliciting an immune reaction against the ZP glycoproteins, which form a matrix around the oocyte and which are essential for spermatozoa binding, acrosome reaction, and potentiation of fertilization.<sup>8</sup> The glycoprotein which is targeted by the vaccine is ZP3, which is specifically responsible for spermatozoa binding and induction of the acrosome reaction.<sup>20</sup> The antibodies generated in response to vaccination antagonize the spermatozoa receptor sites, preventing fertilization.<sup>20</sup> It is also hypothesized that cell-mediated immunity may also play a role in the effects of the ZP vaccine by causing atresia of ovarian follicles and ovulation failure.<sup>20,21</sup>

Since the target of the ZP vaccine is far downstream in the hypothalamic-pituitary-gonadal axis, there is little effect on reproductive cyclicity – animals continue to cycle and may be bred.<sup>10</sup> Vaccinated female white-tailed deer and horses were observed to continue normal estrous cyclicity; however, in some cases beyond the normal breeding season.<sup>22,23</sup> Maintenance of sexual behaviors is advantageous in wildlife species, where these behaviors are important in maintaining social interactions and a social hierarchy. However, in females in which antibody titers waned, births were observed later in the year than in non-vaccinated animals, which may put offspring at risk for non-survival in winter months.<sup>24</sup>

In companion animal species, retention of sexual behaviors is not desirable, and can be viewed as a disadvantage of this contraception method. In addition, continued production of sex steroids may result in the development of hormone-dependent uterine or mammary diseases.<sup>12</sup> Furthermore, in contrast to what has been observed in white-tailed deer and horses, it was observed that long-term treatment (i.e. more than five years of annual vaccination) resulted in irreversible changes within the ovary, including cessation of follicular activity, ovulation failure, and undetectable estrogen levels in bitches.<sup>21,25</sup>

Use of ZP glycoproteins as a target for immunocontraception has a number of advantages. Since these proteins are specific only to the ZP, there is no concern of cross-reactivity with other tissues within the body, as demonstrated using porcine zona pellucida (pZP) antibodies and canine and equine somatic tissue samples.<sup>26</sup> Portions of the amino acid structure of the zona pellucida glycoproteins are highly conserved among related species, allowing one product to be cross-reactive in other species.<sup>12</sup> Most ZP vaccines which have been used in wildlife species are porcine in origin; these proteins have shown cross-

reactivity and efficacy in many species, including horses, elephants, and deer.<sup>6,15,18,22,27</sup> Porcine zona pellucida proteins are readily available from slaughterhouses, and therefore inexpensive to obtain. However, pZP proteins have shown a lack of cross-reactivity in more distant species, such as cats and dogs, which will be discussed further in this paper.

There are also a number of disadvantages of the ZP vaccine. Since ZP glycoproteins are only associated with oocytes, this method of contraception can only be used in female animals. Like most self-antigens, ZP proteins are poor immunogens and require a powerful adjuvant, increasing the risk of adverse injection site reactions.<sup>10</sup> Furthermore, studies have shown that initial vaccination has short-term effects, and booster vaccines are required for prolonged antibody production.<sup>10</sup> A booster vaccine two to six weeks after the initial vaccine, followed by an additional booster one to two years later, can induce infertility for several years.<sup>10</sup> A newer vaccine (SpayVac<sup>TM</sup>, Spayvac<sup>TM</sup>-For-Wildlife, Inc., New Jersey) composed of liposome-encapsulated ZP antigens, induced multi-year infertility in white-tailed deer with a single injection.<sup>15</sup> Adverse effects have been studied in research trials involving domestic and captive wildlife species, but would be difficult to monitor in free-roaming populations. Reported side effects of the ZP vaccine in horses included increased intrauterine edema (identified ultrasonographically) and estradiol levels, which suggested ovarian dysfunction such as anovulation. In dogs and cats, injection site reactions included both granuloma and abscess development, and in cats granulomas were also found in the lung and brain, associated to the type of adjuvant used.<sup>28,29</sup> Several cats also developed hypercalcemia and renal dysfunction, and one died of vaccine-induced sarcoma.<sup>29</sup> Some bitches showed prolonged proestrus and estrus with persistently elevated estradiol levels and no rise in progesterone.<sup>28</sup> Histologic examination demonstrated ovarian follicular cysts.<sup>30</sup> Immune-mediated oophoritis has been observed in some treated animals, but is largely attributed to the adjuvant used.<sup>29,31</sup>

A pZP vaccine is commercially available from the Science and Conservation Center at Zoo Montana in Billings, MT. Its effects have been evaluated in wild horses, white-tailed deer, bison, African elephants, seals, and a variety of other species (Table).<sup>32</sup> The cost is \$24 per dose and \$15 for each of two adjuvants.<sup>32</sup> SpayVac<sup>TM</sup> is not yet commercially available. Research has shown contraceptive ability in grey seals, fallow deer, white-tailed deer, and other mammals.<sup>33</sup> Cost of administration of vaccines increases depending on the ability to gather and restrain animals to administer the vaccine.

### GnRH vaccine

The goal of the GnRH vaccine is to stimulate the formation of antibodies against this hormone, and therefore inhibit the downstream release of gonadotropins (LH, FSH) from the pituitary gland. By removing the effects of an upstream hormone in the hypothalamic-pituitary-gonadal axis, suppression of fertility can be achieved. This includes effects such as the suppression of reproductive behaviors, gonadal atrophy, spermatogenic arrest in males, and anestrus in females.<sup>34-37</sup>

Gonadotropin releasing hormone is essential for reproduction in both males and females, making it an effective target for immunocontraception in both sexes. The amino acid structure of GnRH is widely conserved between species, suggesting that one immunocontraceptive product has the potential for use in multiple species. Because inhibition of GnRH has downstream effects, the production of sex hormones is blocked, resulting in the inhibition of sex-related behaviors. For example, a significant decrease in aggression has been noted in GnRH-vaccinated males of various species, including bulls, llamas, alpacas, boars, and elephants.<sup>34,35,38,39</sup> Although studies are lacking, it would be expected that the inhibition of sex hormone production would also limit the development of sex hormone-related diseases, such as mammary gland neoplasia, testicular tumors, pyometra, and prostatic disease.<sup>40</sup> However, other conditions seem to have a higher prevalence in animals spayed or neutered at a young age, suggesting that the persistence of sex hormone production may be beneficial in decreasing the risk of diseases such as prostatic carcinoma, osteosarcoma, cranial cruciate ligament rupture, hemangiosarcoma, and urinary bladder sphincter incompetence in female bitches.<sup>40,41</sup>

Using GnRH as a target for immunocontraception presents several disadvantages. Although the conservation of the GnRH amino acid sequence between species can be advantageous in terms of efficacy across species, this poses a problem if the product is used in bait for wildlife species. For example, an

oral GnRH vaccine for deer is currently being developed, but presents a challenge since strict species specificity is required.<sup>42</sup> Otherwise, wildlife species that are not intentionally targeted may become infertile with exposure to the vaccine. In addition, the advantage of eliminating sex-related behaviors in pets and free-roaming dogs and cats is viewed as a disadvantage in wildlife populations, where the behaviors driven by sex hormones are vital to maintaining social organization.<sup>10</sup> Furthermore, GnRH is a very small peptide, and therefore serves only as a weak immunogen; it must be administered in combination with carrier proteins and adjuvants in order to induce a sufficient and prolonged immune response.<sup>12</sup> Since GnRH is released from the hypothalamus and contained within the blood brain barrier, the only opportunity for antibodies to act on the hormone is during its transport through the hypophyseal portal system before binding to receptors within the pituitary gland. Therefore, these antibodies must be present at high concentrations in order to be effective.<sup>8</sup> Studies have consistently shown that the GnRH vaccine is less effective in males than females.<sup>12</sup> This is thought to be the result of constant pulsatile GnRH secretion in males, which is likely more difficult to counteract than episodic pulsatile secretion that occurs in females. In addition, all studies involving GnRH immunocontraception have shown that a small percentage of the study population fails to respond to the vaccine with significant antibody titers; a cause for this consistent finding is unknown.<sup>12</sup>

GonaCon™ is a GnRH vaccine that was developed by the National Wildlife Research Center. It was approved for use in white-tailed deer in 2009 and feral horses in 2013. The vaccine is listed as a restricted use pesticide and its administration is limited to United States government organizations. It has also shown efficacy in California ground squirrels, prairie dogs, wild horses, elk, and Norway rats.<sup>42</sup> Worldwide, other commercially manufactured GnRH vaccines provide temporary inhibition of reproductive cyclicity and sex hormone-related effects; these vaccines include Equity® (Zoetis, Inc., Australia and Zoetis, Inc., New Zealand), which is registered for use in female horses in Australia and New Zealand, and Improvac® (Zoetis, Inc., Australia), which is approved for use in boars in over 60 countries.<sup>39</sup> A commercially produced GnRH vaccine (Canine Gonadotropin Releasing Factor Immunotherapeutic®, Zoetis, Inc., Florham Park, New Jersey) was licensed in the United States from 2005-2008 for the treatment of benign prostatic hypertrophy in dogs, but is no longer on the market. However, studies have suggested its potential use as an immunocontraceptive. Further research is warranted to evaluate its effects on fertility.<sup>34,43</sup>

### **Intratesticular injections**

Sterilization via intratesticular injections involves injecting chemical irritants into the testes to disrupt spermatogenesis and to initiate a local inflammatory response. This inflammation allows encroachment of the blood-testes barrier and permits the entrance of lymphoid cells, resulting in an immune-mediated reaction against testicular tissues. The outcome of this process is azoospermia and atrophy of the testis and epididymis (due primarily to decreased testosterone and seminiferous tubule diameter).<sup>44,45</sup> Zinc gluconate has been the most extensively studied chemical used as an intratesticular sterilant injection, and will be the topic of this section.

Unlike the methods of contraception discussed earlier in this paper, intratesticular injections of zinc gluconate neutralized by arginine resulted in sterility in 99.6% of treated dogs, although long-term follow-up studies have not been performed to substantiate the permanent sterility claim made by the manufacturer.<sup>45</sup> Importantly, its use is limited to males which can impact the ability to regulate population sizes. However, for contained or captive populations, implementation of this method of sterilization can have significant effects and reduce need for repeated trapping and vaccination.<sup>46</sup> Anesthesia is not a requirement for intratesticular injections, although sedation is recommended by the manufacturer to reduce accidental extratesticular injection which can lead to irritation, dermatitis, ulceration, or necrosis.<sup>45,47</sup> Histologically, zinc gluconate injections resulted in testicular degeneration and sloughing of the germinal epithelium which resulted in azoospermia, as well as vacuolization of both Sertoli and Leydig cells.<sup>44</sup> In field trials, a 77% decrease in testicular size, 52% reduction in prostate size, and up to 52% reduction in testosterone levels were observed.<sup>47</sup>

Use of zinc gluconate injections in cats demonstrated suppression of male sex-related behaviors including aggression, roaming, and marking, which was associated to decreased testosterone levels.<sup>48</sup> Furthermore, it is hypothesized that decreased testosterone may decrease the risk of sex hormone-related diseases, including benign prostatic hypertrophy, prostatitis, and testicular neoplasia, although no formal studies have been performed and it remains to be determined if a threshold level of testosterone is required.<sup>45,47</sup> There are no studies which have evaluated the reproductive behavior of animals after treatment; it is not known if males will attempt to breed or can ejaculate.

Zinc gluconate injections may be used for sterilization of male dogs and cats in shelters, or in countries or regions where surgical castration is not culturally acceptable or economically feasible. The manufacturer currently is targeting animal shelters, with an offer to provide the product at one-fifth the cost of a surgical castration.<sup>45</sup> Intratesticular injections reduce the risk of complications associated with anesthesia and surgery but do have known complications. In field trials, the most common side effects were pain upon palpation of the scrotum after injection (6.3%), neutrophilia (6.3%), vomiting (4.4%), and anorexia (4.1%).<sup>47</sup> Twelve of 1738 dogs required veterinary care post-treatment, five of which required surgical castration (0.003%).<sup>47</sup>

It should also be noted that although histologic disruption of spermatogenesis is observed within days of administration, extragonadal sperm reserves may allow the animal to successfully reproduce; the manufacturer recommends to keep treated males away from intact females for 60 days.<sup>45</sup> The use of zinc gluconate as a form of chemical castration in species besides cats and dogs has not yet been described, so this method of non-surgical sterilization is currently limited in its scope of application.

## **Non-surgical contraception and sterilization methods used in cats, dogs, horses, and wildlife**

### Non-surgical contraception and sterilization in cats

As a species that undergoes early sexual maturity, is seasonally polyestrous, and can produce several large litters each year, cats are extremely prolific. In the United States alone, there are estimated to be more than 90 million free-roaming cats.<sup>12</sup> Free-roaming cat populations may be a nuisance, harbor pathogens that can be transmitted to humans and other animals, and compromise many native species due to predation. Trap-neuter-return programs have begun to replace lethal methods of control in order to manage these exponentially growing cat populations. However, the time and resources needed for these programs make them challenging to sustain, making non-surgical contraception or sterilization methods more ideal to control free-roaming cat populations. Gonadotropin releasing hormone vaccination, ZP vaccination, and intra-testicular injections have each been researched in order to determine their efficacy in cats.

The GnRH vaccine is the method that has shown the most promising results during research trials in cats. In a study of GnRH immunocontraception of male cats, six out of nine vaccinated cats developed high GnRH antibody titers, had undetectable serum testosterone, marked testicular atrophy, and the absence of viable sperm.<sup>37</sup> However, the onset of these effects was delayed and varied between individuals, and all but one cat became fertile again by three years after treatment.<sup>37</sup> The remaining three vaccinated cats had an intermediate response in terms of the same variables described above, but breeding trials were not done to determine if they were still fertile.<sup>37</sup> In a study of GnRH immunocontraception of female cats, all 15 vaccinated cats developed a high GnRH antibody titer.<sup>49</sup> Breeding trials showed that 93% of the cats remained infertile for the first year following vaccination, 73% for two years, 53% for three years, 40% for four years, and 27% were still infertile at five years when the study ended.<sup>49</sup> One model found that successful treatment of 60% of the juvenile females in a population with a three-year duration of contraception could be successful in stopping population growth; these results are very encouraging.<sup>49</sup> Although the potential need for booster vaccines could be a limiting factor, the use of a GnRH vaccine in free-roaming cat populations would be ideal for a number of reasons. Gonadotropin releasing hormone vaccines are effective in both males and females, making it a better choice for population control than either of the other methods of non-surgical contraception and sterilization; GnRH vaccination would also result in the elimination of secondary sex behaviors in treated cats.

Evaluation of the efficacy of pZP vaccination in cats demonstrated no effects on ovulation, fertilization, conception, or litter sizes.<sup>11</sup> Vaccinated cats developed antibody titers; however, antibodies did not have cross-reactivity with feline ZP. The ZP glycoproteins from other closely-related species (i.e. cows, minks, ferrets, and dogs) were also evaluated as components of a ZP vaccine for cats, but each of these vaccines elicited the formation of antibodies that were much more reactive against the ZP glycoproteins of the species of origin than against feline ZP proteins, and pregnancy rates were unaffected.<sup>50</sup> Development of feline zona pellucida-derived (fZP) antigenic epitopes resulted in inhibition of both spermatozoa binding (57.3%) and fertilization (41.5%) of feline oocytes in vitro.<sup>51</sup> Additionally, use of DNA vaccines using fZP proteins in plasmid vectors was examined. Twelve cats were treated with one of two vaccines (against different zona pellucida epitopes), and of nine which mated, two became pregnant and each delivered two kittens.<sup>52</sup> One vaccinee sustained fetal resorption, and three developed pseudopregnancy. All ovaries demonstrated normal histology and follicular dynamics after ovariectomy.<sup>52</sup>

Although newer vaccine formulations using feline-specific antigens appear to have promise for contraceptive use, ZP vaccines may be less than ideal for controlling free-roaming cat populations. The trapping of feral cats is indiscriminate, and therefore, much time would be wasted trapping male cats. Requirement for booster vaccines remains a concern. As mentioned previously, the ZP vaccine would not suppress the estrous cycle, and sexual behaviors would continue even if the female was rendered infertile.

Current research on immunocontraceptive vaccines in cats is focused on the use of recombinant technology using viral or bacteriophage vectors.<sup>8,53,54</sup> Researchers are exploring the use of feline herpesvirus-1 (FHV-1) as a vector, with the potential that if an animal's infection recrudesces, the vaccine would "self-boost."<sup>8</sup> Furthermore, FHV-1 is not transmissible to dogs, which would limit potential spread of vaccine virus.

The efficacy of intratesticular injections has recently been evaluated in cats. A study using intratesticular zinc gluconate in male cats showed that 83% became azoospermic, and the remaining cats had decreased sperm concentration and motility.<sup>48</sup> Although there were no significant changes in testosterone levels of these cats, testicular atrophy, absence of penile spines, and a decrease in sexual behaviors were observed.<sup>48</sup> Use of intratesticular injections in cat populations will likely have a limited effect on controlling population sizes since only males would be infertile, whereas targeting females for contraception has a much greater overall effect.<sup>12</sup> Furthermore, ability to copulate or ejaculate has not been examined after intratesticular injections of male cats. Although intratesticular injections would require sedation and more handling than administration of GnRH vaccination, it holds potential for permanent sterility in treated animals. Further research is warranted.

#### Non-surgical contraception and sterilization in dogs

Approximately 75% of the world's total dog population consists of homeless stray and feral dogs.<sup>55</sup> Many of these animals, especially in countries with large free-roaming dog populations, are responsible for fatal dog attacks and human rabies deaths, in addition to other zoonotic infections.<sup>56,57</sup> Dog removal campaigns and euthanasia in shelters provide only short-term relief of these problems, while large-scale surgical sterilization campaigns have shown to dramatically improve these issues.<sup>55,56</sup> However, much like trap-neuter-release programs for cats, these campaigns are greatly limited by cost and availability. Non-surgical methods of contraception or sterilization could provide similar benefits as surgical sterilization, but in a more efficient and inexpensive manner.

Unlike the trials performed in cats, GnRH has shown limited efficacy in dogs. A GnRH vaccine (Canine Gonadotropin Releasing Factor Immunotherapeutic®) was previously available in the United States for the treatment of benign prostatic hyperplasia in dogs. Studies of this vaccine demonstrated a decrease in testicular and prostatic volumes, as well as a decrease in testosterone production in treated dogs.<sup>43</sup> However, the study was only short-term, and fertility was not directly evaluated. A GnRH vaccine developed by the USDA for wildlife species (GonaCon™), showed unsatisfactory results in a pilot study in male dogs, with inconsistent effects on fertility and severe injection site reactions.<sup>55</sup> Another study in male dogs showed that, although GnRH antibodies were produced, the antibodies failed

to have an effect on spermatogenesis and rapidly declined despite booster immunizations.<sup>58</sup> In contrast, a study evaluating vaccination with GnRH conjugated to a canine distemper protein in male dogs showed encouraging results – the vaccine was highly immunogenic, resulting in testicular degeneration, Sertoli and Leydig cell atrophy, and spermatogenic arrest.<sup>59</sup> However, the treatment group contained only two dogs and the duration of the vaccine's effects were not evaluated.<sup>59</sup>

In female dogs, GonaCon™ was administered in combination with rabies vaccination; development of antibodies to both immunogens was observed.<sup>56</sup> However, no reproductive evaluations or breeding trials were performed.<sup>56</sup> In another study, female dogs were administered GonaCon™ with or without concurrent rabies vaccination.<sup>60</sup> All animals developed titers to both immunogens. Granulomatous myositis at the injection site developed in seven of 14 animals. Two animals were pregnant at the time of vaccine administration and delivered normal litters. Animals were euthanized and necropsy demonstrated pituitary congestion, edema, and coagulative necrosis.<sup>60</sup> Ovaries demonstrated follicular atresia. Further investigation of the contraceptive effects of GnRH vaccines on female dogs is needed.

One reason which dog owners may elect to delay or elect against surgical sterilization of female dogs is the risk of development of urinary bladder sphincter dysfunction, a disorder with incidence of 4.9% to 20% and higher incidence in bitches spayed at less than three months of age.<sup>40</sup> It has been suggested that elevated LH levels after ovariohysterectomy have effects on development of bladder sphincter dysfunction. Recently, it was demonstrated that treatment of affected spayed bitches with GnRH vaccination resulted in resolution of incontinence in four of nine dogs (44.4%), potentially due to a decrease in LH levels.<sup>61</sup> More research in this area is warranted.

Unlike the reversible effects on fertility observed in horses and white-tailed deer, administration of ZP vaccines to dogs not only has been shown to provide contraception, but in some cases irreversible effects on ovarian function. Effective contraception was achieved after a series of booster doses in bitches. The pZP proteins were effective at eliciting a cross-reactive antibody response, and treated female dogs failed to conceive in breeding trials.<sup>21</sup> However, treated bitches also demonstrated prolonged proestrus and estrus cycles, failure of progesterone to rise during estrus, and development of follicular ovarian cysts, which may represent anovulation.<sup>28,30</sup> Immune mediated oophoritis was observed in some dogs.<sup>31</sup> Findings suggested that contraception may be due to not only inhibition of spermatozoa binding, but also to alteration of follicular dynamics and ovulation. Most side effects of the ZP vaccines were attributed to the adjuvant used. No long-term studies have evaluated the duration of contraception. Due to the observed side effects and requirement for booster doses, ZP vaccination does not appear to be the best option for control of population sizes of free-roaming dogs.

The results of these studies demonstrate that current GnRH and ZP vaccine formulations do not provide consistent contraceptive effects in dogs. The most promising research models appear to be recombinant vaccine vectors including canine distemper virus or canine herpesvirus.<sup>8,59</sup> Continuing research in these areas may lead to development of more effective, longer acting vaccines for control of canine reproductive ability.

The use of intratesticular injections of zinc gluconate neutralized by arginine for sterilization of male dogs has been approved for use in the United States and several other countries. From 2003-2005 a product called Neutersol™ was available in the United States, which now is manufactured by a different company under the name Zeuterin™ (Ark Sciences, Irvington, New York). Zeuterin™ was approved for use in 2014 for use in male dogs aged three to ten months, with normal, descended testes. Injection of zinc gluconate results in sclerosis, arrest of spermatogenesis, and testicular degeneration. Testosterone levels of treated dogs decreased 41-52%.<sup>45</sup> The effects are thought to be permanent; however, no long-term studies have evaluated testicular function in treated animals. Side effects and recommendations for use were discussed earlier in this manuscript. Use of intratesticular injections may be beneficial to regulate male reproductive function in captive or contained populations; however, it has been estimated that at least 95% of males in a population must be infertile to affect population growth.<sup>13</sup>

### Non-surgical contraception in horses

In the past few decades, the population of feral horses within the United States has increased drastically from approximately 25,000 to 69,000. Approximately half of these horses range freely on public land, while the other half are maintained in government facilities at the cost of millions of dollars per year.<sup>2</sup> The large number of feral horses compromises the survival of native species and causes damage to natural ecosystems.<sup>2,18</sup>

The pZP vaccine has been widely used in free-ranging horses in order to control population sizes. Studies have shown that a single injection of pZP can provide multiple years of infertility in an individual mare, but the efficacy gradually declines each year.<sup>17</sup> Application of the pZP vaccine in feral horse population control programs typically includes annual vaccination, with ovulation failure in individuals and 100% population efficacy occurring after five consecutive injections.<sup>23</sup> However, 100% efficacy is unnecessary in controlling populations. A study in a herd of wild horses demonstrated that the pZP vaccine could successfully stop population growth within two years and noticeably decrease the herd size within eight years.<sup>62</sup> Although the target of the pZP vaccine is far downstream in the hypothalamic-pituitary-gonadal axis and should have no effect on reproductive cyclicity or behavior, some studies have shown vaccinated mares may display increased and prolonged reproductive behaviors.<sup>23</sup> Given the social nature of horses, such changes may alter the interactions and structure of the population as a whole.

Gonadotropin releasing hormone vaccination has been shown to be successful in inhibiting reproduction in horses in several studies, but with a variable duration of induced infertility.<sup>6,63</sup> A series of two vaccinations a few weeks apart was sufficient at rendering most individuals infertile for one breeding season.<sup>10,64</sup> In one study, a primary injection and booster several weeks later resulted in anestrus in all mares.<sup>63</sup> All but four of the mares returned to cyclicity at a mean time of 418 days (range 232–488 days); older mares showed a trend towards earlier return to cyclicity.<sup>63</sup> However, it can be hypothesized that anestrus would be maintained with implementation of annual booster vaccination. One potential benefit of GnRH vaccination is its efficacy in both sexes. In addition to inhibiting cyclicity in females, GnRH vaccines have been shown to temporarily suppress testicular function, greatly affecting semen quality and reducing testosterone levels in stallions.<sup>36,65</sup> However, since reproductive behaviors are vital to maintaining the complex social structure of feral horse herds, the inhibitory effects of GnRH vaccination on reproductive hormones and behaviors would have a significant detrimental effect on harem and bachelor herd function when used in free-ranging populations.<sup>10</sup>

### Non-surgical contraception in wildlife species

The use of immunocontraceptives to control the population sizes of wildlife species has gained popularity over the past few decades. The table summarizes published studies on immunocontraception in several wildlife species. All of these studies focused on the efficacy of immunocontraceptive vaccines in females, which showed high success rates. Vaccines were administered using sedation and injection by an operator, or remote darting. Antibody titers, hormone levels, ultrasonographic examinations, and birthing rates were all used to determine effectiveness of the vaccines. It is important to keep in mind that most of these studies suffer from small sample sizes and were limited to captive or isolated herds. Although this type of setting allows tracking of the animals over time in order to determine effects on fertility and adverse effects, it may not be representative of the true efficacy seen in large-scale use within free-roaming populations. In addition, many studies have conflicting results. These differences may be attributed to variation in vaccination components, such as dose, antigen structure, and adjuvant used.

### Conclusion

A non-surgical sterilization method that would fit all of the criteria for the “ideal” technique has not yet been developed. However, even an immunocontraceptive that fails to have 100% efficacy may be sufficient for population control, as long as an adequate number of females within the population are rendered infertile.<sup>12</sup> One of the main limiting factors of the techniques that have been developed is the duration of efficacy. The need for booster vaccinations limits the practical use in population management, and methods that induce long-term infertility or permanent sterility are needed. In order to

address this, more recent research approaches have focused on developing an immunocontraceptive with a stronger and more prolonged immune response. This may be achieved in the near future by the use of more effective adjuvants, incorporation of antigens into matrices that allow for slow-release, and development of recombinant vaccines with viral or bacteriophage vectors.<sup>8,10</sup> Non-surgical contraception and sterilization methods may be used in individual companion animals, but would be especially useful in large-scale settings for population control. These techniques may provide a more efficient, less costly, and less invasive method of rendering animals infertile compared to surgical sterilization.

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**Table.** Reported immunocontraception efficacy using zona pellucida (ZP) and gonadotropin-releasing hormone (GnRH) vaccination in females of selected wildlife species

Species	Method Used	Efficacy	Duration	References
White-Tailed Deer	ZP	76%	multi-year with boosters	22
	GnRH	100%	24 months	14
African Elephants	ZP	100%	multi-year with boosters	10, 27
Various Bear Species	ZP	100%	30 months	66
Zebra	ZP	96.5%	56 months	66
Bighorn Sheep	ZP	100%	14 months	66
Elk	ZP	91%	unknown duration	5
	GnRH	90-100%	3 years	67
Grey Seal	ZP	92%	unknown duration	5
Feral Swine	GnRH	100%	36 weeks	16
Bison	GnRH	100%	1 year	68