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Fertility Control in Wildlife: The Role of Immunocontraceptive Vaccines

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Abstract

Immunocontraception has gained significant attention as an ethical and effective tool for managing overabundant wildlife populations, particularly in regions experiencing increasing human–wildlife conflicts due to habitat fragmentation, agricultural expansion, and urbanization. Traditional population control methods, such as culling and poisoning, have raised concerns about their environmental impact, non-target species harm and public opposition. Immunocontraceptive vaccines offer a non-lethal alternative by harnessing the body's immune response to suppress fertility, targeting key reproductive hormones and proteins, such as gonadotropin-releasing hormone (GnRH) and zona pellucida (ZP) glycoproteins. This review delves into the mechanisms of action of these vaccines, their applications across various species, and the challenges associated with immunocontraception, including inconsistent immune responses, the need for booster doses, and non-responders in treated populations. Recent advances in vaccine delivery systems, such as oral baits and slow-release implants, offer potential solutions for large-scale wildlife management efforts. Additionally, we explore the long-term ecological impacts of fertility control, its role in reducing disease transmission, and its integration into broader wildlife conservation strategies. Despite its potential, immunocontraception requires further refinement in terms of vaccine efficacy, cost-effectiveness, and practical implementation, particularly for free-ranging and hard-to-reach species. As research continues to evolve, immunocontraception holds promise for achieving sustainable wildlife population control, balancing biodiversity conservation, and mitigating human-wildlife conflicts.

Keywords: Immunocontraception, Wildlife population control, Gonadotropin-releasing hormone (GnRH), Zona pellucida (ZP) vaccines, Human-wildlife conflicts, Fertility control

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Introduction

Overpopulation of humans across the globe, destruction of forests areas and habitat fragmentation lead to overlap of wild animal habitats and land use for human habitation. When resources become scarce and humans and wild herbivores compete for the same resources, whether in the wild or in cultivated fields, the latter become pests or predators. In the past, elephants, wild pigs, bison, monkeys, langurs, bears, bats, porcupines, and various species of seed-eating and omnivorous birds on the Indian subcontinent were not known to raid

crops. However, these animals are currently consuming planted crops, causing enormous losses to subsistence farmers and impacting government measures designed to protect them. As most of these pests are either endangered or threatened, the problem becomes controversial (Sridhara 2006). In such situations, the management of wild and pest-susceptible animals is becoming an issue of concern. Traditionally, wild animals are managed through culling (Rutberg and Naugle 2008; White and Ward 2010; Gionfriddo et al. 2011).

For decades, several vertebrate pest species have been controlled with varied results, primarily through the use of poisons. Several authors have explored the benefits and drawbacks of this traditional population control approach (Bomford 1990). On the one hand, poisoning is a cost-effective, quick, and direct technique to reduce pest species density in certain areas. However, because of rising public concern about animal welfare, the use of poisons creates ethical concerns, because poisons can impact non-target species and remain in the environment, in addition to compound-specific pre-lethal effects on the target species. Furthermore, the development of behavioural avoidance, a common phenomenon observed in rodents in response to rodenticides, can impede the effects of poisons (Drummond and Rennison 1973). These conditions result in antipathy effects for lethal control measures (Beringer et al. 2002; McCann et al. 2008; Sharp and Saunder 2011; Dunn et al. 2018).

For vertebrate pest management, fertility control has emerged as an alternative to lethal control (Bremner and Park 2007; Kirkpatrick et al. 2011; Massei and Cowan 2014). Management through fertility control takes longer than culling to achieve population reductions since infertile animals are not destroyed (Massei and Cowan 2014; Hone 1992). Fertility control, on the other hand, may have advantages over lethal control. Infertile animals in a population, for example, may contribute to density-dependent feedback, which slows population recovery (Zhang 2000). Contraception is especially useful for sustaining lower population numbers when culling has been performed (Merril et al. 2006, Yoder and Miller 2006; Pepin et al. 2017). Fertility control may also reduce disease transmission chances by decreasing both the number of newborn prone individuals (Miller et al. 2004; Killian et al 2007) and animal-to-animal contact during mating (Ramsey 2007).

Under fertility control methods, immunocontraception for wildlife management is gaining attraction, and many authorities involved in making such decisions are considering fertility control as a possible humane approach (Cowan and Tyndale-Biscoe 1997). Immunocontraceptive vaccines work by inducing humoral and/or cell-mediated immune responses against hormones/proteins that are important for reproduction and whose biological function is disrupted, resulting in fertility blockage. Contraceptive vaccinations can cause reversible or irreversible reproductive suppression (Gupta and Bansal 2010). To achieve infertility, immune intervention might be addressed at several phases during the reproductive process. These processes divided into three groups: (1) gamete production, (2) gamete function, and (3) gamete outcome. The hypothalamus produces and secretes gonadotropin-releasing hormone (GnRH), which regulates the synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the pituitary. In the testes and ovaries, LH and FSH act and lead to the production of sperm and oocytes respectively. GnRH, LH, and FSH neutralization may interfere with the generation of gametes, reducing fertility. An immune response can be generated against specific and unique antigens present in both male (spermatozoon) and female (egg) gametes, which ultimately results in fertility blockage. After fertilization, human chorionic gonadotropin (hCG) is synthesized and secreted by the embryo, which helps to maintain the corpus luteum and progesterone production, which is essential for the establishment and maintenance of pregnancy. hCG neutralization with the help of antibodies can restrict blastocyst implantation.

1. Contraceptive vaccines designed to target the gamete production inhibition process

For the development of immunocontraceptive vaccines, receptors of GnRH, LH, FSH and gonadotropins have been employed as immunogens to inhibit gamete production. The following is a description of their current situation.

Immunocontraceptive vaccines for neutralization of GnRH

Gonadotropin-releasing hormone, a decapeptide, is present in both males and females. GnRH is responsible for controlling reproduction in males and females by stimulating the production of hormones that lead to ovulation and spermatogenesis. Suppressing GnRH through the generation of anti-GnRH antibodies prevents animals from reproducing (Curtis and Pooler 2002; Miller et al. 2004). Active immunization against GnRH creates an immunological barrier between the hypothalamus and the anterior pituitary gland. Antibodies bind to GnRH in the hypothalamo-hypophyseal portal circulation, which prevents GnRH from binding with receptors on pituitary gonadotrophs. This results in suppression of gonadotropin secretion, the inhibition of follicular development and ovulation, and reproductive behavior (Talwar 1997).

Active immunization with GnRH coupled with various carrier proteins to facilitate the generation of an immune response, has led to blockage of fertility in various animal species. Immunization of female rats with GnRH conjugated to bovine serum albumin (BSA) led to an anestrous state, lower LH levels with unimpaired levels of prolactin and reduced size of ovaries with mostly small- to medium-sized follicles with small antra (Takahashi et al. 1997). The active immunization of white-tailed female deer (*Odocoileus virginianus*) with a priming dose containing 500 mg of keyhole limpet hemocyanin (KLH) -GnRH and, approximately 1 month later, a boost containing 300 mg of KLH-GnRH led to an 88% reduction in fawning rates and failure to conceive; the blockage of fertility was reversible. In the case of captive feral swine, the most effective single shot GnRH vaccine dose for females was 2000 µg; for males it was 1000 µg. The reproductive tracts regressed and was inactive in most of the GnRH vaccinated females. Fully regressed testes were occasionally observed in the treated males, but intermediate stages of regression were the most commonly observed in the treated males (Killian et al. 2003). The major advantages of GnRH are that it can sterilize both sexes and that it has the potential to sterilize rats for at least 1 year. The testes of male rats injected with GnRH began to shrink after 60 days. After 90 days, the testes had regressed completely, and the scrotums disappeared until 0-13 months after treatment. Complete atrophy of internal female reproductive organs was also observed (Lowell et al. 1997).

An alternative approach that has been proposed is to deliver a GnRH agonist (Deslorelin) in a slow-release implant, which leads to a decrease in LH, testosterone, testicular volume, and semen output (Junaidi et al. 2003) and thus has potential as a long-term reversible antifertility agent for male dogs. In the last 20 years, 'single-shot' injectable immunocontraceptive vaccines have been widely tested for use in wildlife management. One such injectable GnRH-based immunocontraceptive vaccine, GonaCon (USDA, Pocatello, ID, USA), has proven effective in many species (Gionfriddo et al, 2011; Miller et al. 2013; Massei et al. 2018), such as white-tailed deer (*Odocoileus virginianus*) (Miller et al. 2000) elk (*Cervus elaphus*) (Killian et al. 2008), wild horses (*Equus caballus*) (Killian et al. 2004; Killian et al. 2006), domestic cats (*Felis catus*) (Levy et al. 2004), California ground squirrels (*Spermophilus beecheyi*) (Nash et al. 2004), and Norway rats (*Rattus norvegicus*) (Miller et al. 1997). One-injection and two-injection formulations of GonaCon™ and GonaCon-B™ produced multiyear contraception in adult female white-tailed deer. GonaCon-B™ provided a longer lasting contraceptive effect (Miller et al. 2008). The single dose immunocontraceptive vaccine gonadotrophin-releasing hormone (GnRH) formulation GonaCon-B™, in a population of free-roaming feral horses significantly reduces fertility for three years without boosters (Gray et al. 2010).

Another commercial GnRH-based contraceptive vaccine, Improvac®, consists of a synthetic incomplete analog of GnRF linked to a carrier protein to make it immunogenic. Immunization of cross-bred Iberian female pigs with Improvac® led to a reduction in the incidence of standing estrous, serum progesterone levels, and the development of the uterus and ovaries (Dalmau et al. 2015). Immunization led to long-lasting immunity of at least 20 months after the third injection (Dalmau et al. 2015). The immunization of male pigs with Improvac® led to a significant reduction in their sexual and aggressive behavior, as observed by a reduction in mounting, fighting, pushing, head butting and tail manipulation (Karaconji et al. 2015). The immunization of a male Asian elephant with a combination of two commercial GnRH-based contraceptive vaccines, i.e., Improvac® and EquityTM, led to a decrease in the serum testosterone concentration, testicle diameter, penile atrophy and weight gain (Lueders et al. 2014). After 1 year of initial treatment, no spermatozoa were observed in the semen, suggesting that the GnRH vaccine may be a useful non-invasive method of contraception for Asian elephants (Lueders et al. 2014).

Bopriva® is one of the commercially available GnRH-based vaccine, developed by Pfizer Animal Health, Parkville, Australia that induces antibodies against GnRH. Active immunization of pubertal bulls with this vaccine led to a decrease in testosterone levels in the blood, testicular development and physical activity, leaving body weight gain unaffected (Janet et al, 2012). Furthermore, two injections of Bopriva® in peripubertal bulls also suppressed testicular growth and blood testosterone concentration for at least 10 weeks after booster injection (Theubet et al. 2010). Bopriva® was also effective at suppressing of testicular functions including sperm production, in boars (Wicks et al. 2013). The immunization of female dairy cattle (cyclic Swiss Fleckvieh cows) with Bopriva® resulted in a decrease in progesterone levels without affecting estrogen levels, the suppression of estrus and impaired folliculogenesis (Balet et al. 2014).

The need to capture animals for treatment limits the field applications of injectable vaccines. The availability of oral immunocontraceptive would increase the scope of fertility control applications in wildlife. The development of oral vaccines is challenging compared with parenteral delivery, as demonstrated by the fact that only a few orally administered vaccines currently exist (Silin et al. 2007). Rapid degradation of compounds in the digestive tract and poor permeation capacity across the intestinal mucosa limit the effectiveness of oral vaccination (Russell-Jones 2000; Antosova et al. 2009; Vela et al. 2017).

Recently, a new approach for developing an oral immunocontraceptive, agents that exploits bioadhesive and immunologically active properties of killed *Mycobacterium avium* cell wall fragments (MAF) was developed. As an adjuvant, *M. avium* is acid resistant is known to be imbedded in the ileal region of the small intestine, associated with the immunologically active area of the Peyer's patch (Bermudez and Sangari 2001). Thus, incorporating antigens into constructs such as *M. avium* may increase mucosal uptake while bypassing the acidic environment of the stomach. The MAF was conjugated to recombinant GnRH protein called IMX294, which is used as a GnRH-specific immunogen. An initial trial using the MAF-IMX294 conjugate provided the first evidence that an orally delivered immunocontraceptive vaccine could generate anti-GnRH antibody titers in laboratory rats. Increasing the dose and frequency of vaccine administered to rats, in a second trial enhanced the immune response, eliciting titers that reduced the proportion of females that gave birth. This provided the first evidence of the contraceptive effect of an oral anti-GnRH vaccine (Massei et al. 2020).

The primary problem with immunization against GnRH is the highly variable response between individual animals. In almost all trials, it is evident that non-responders are present within the population. These are animals that do not have a contraceptive response to the vaccine. In many cases reproductive function is maintained despite an immunological response to the vaccine. When Chinese female pigs (Zeng et al. 2002), feedlot heifers (Adams and Adams 1990) and mares (Dalin et al. 2002) were immunized against GnRH, the number of non-responders mounted immune responses that were significantly lower than those that were successfully immunocontracepted. This suggests that there is a critical immunological threshold that must be attained to adequately neutralize GnRH (Adams and Adams 1990; Zeng et al. 2002).

Another drawback is that most immunization regimens require repeated secondary immunizations to ensure the production and maintenance of high anti-GnRH titers. In some cases three immunizations leads to a significant antibody response compared with that in control animals (e.g., mares; Garza et al. 1986). The need for repeated immunizations can be costly, time consuming and impractical, particularly in large-scale farming operations and free-range wildlife situations. The development of single-injection controlled-release vaccines of 1 year duration could overcome this drawback (Turner et al. 2007), but they are difficult to develop successfully. Mares immunized against GnRH–BSA with Equimune® adjuvant showed a range of reactions to the booster, ranging from fever to edematous swelling and front leg lameness (Dalin et al. 2002). These reactions at the injection site are not uncommon with adjuvants, and animal ethics committees and regulatory bodies throughout the world either do not allow their use or are fazing out the use of some adjuvants, because of animal welfare concerns. These negative effects can be reduced in some species by reducing the amount of adjuvant used (Dalin et al. 2002), altering the route of administration (e.g., subcutaneous instead of intramuscular) and the use of alternative adjuvants (Leenaars et al. 1997).

Immunocontraceptive vaccines for neutralization of LH

The active immunization of female rhesus monkeys with the β-subunit of ovine LH (β-oLH) led to the inhibition of fertility, which was accompanied by reduced progesterone levels during the luteal phase (Thau et al. 1979). In addition to LH, the potential of the LH-receptor has also been studied. Active immunization of prepubertal male mice with baculovirus-expressed recombinant porcine LH receptor proteins corresponding to either 1–297 aa or 1–370 aa resulted in a decrease in testosterone levels and spermatogenesis (Remy et al. 1993). The fertility of the immunized mice was reduced to 75%. The immunization of adult female sheep (ewe) with LH prohibited pregnancy in all the animals during the two breeding seasons. The mode of action was assumed to be prevention of ovulation, presumably by inhibition of the preovulatory surge of LH (Roberts and Reeves 1989). Although immunization with LH has potential application as a contraceptive agent, it is not widely accepted since it potentially affects sex steroids.

Contraceptive vaccines based on immune-mediated neutralization of FSH

The use of FSH appears to be an attractive contraceptive for application in males. The immunization of male bonnet monkeys with ovine FSH resulted in testicular dysfunction, oligozoospermia, and subsequent infertility (Murty et al. 1979). Notably, infertility was not associated with any change in testosterone levels, thus adding an additional advantage to the use of FSH based vaccines.

2. Contraceptive Vaccines Based on Sperm and Egg Specific Proteins

Spermatozoa- and egg-specific proteins that are involved in their development and functions leading to successful fertilization, also provide an exciting option for developing contraceptive vaccines.

Contraceptive vaccines based on Spermatozoa-associated proteins

Immunization of female mice or humans with either sperm or their extracts led to the production of anti-sperm antibodies and infertility (Edwards 1964). Owing to the target sperm antigen being used to propose a

contraceptive vaccine, both zona-denuded and zona-encased oocytes can be used. A variety of sperm-specific proteins have been identified, and characterized, and their potential to inhibit fertility has been evaluated in suitable animal models**.** To date, no contraceptive vaccine based on sperm-specific proteins has undergone pre-clinical safety evaluation in animal models and thus has not entered phase-I clinical trials in humans.

Contraceptive vaccines based on zona pellucida glycoproteins

ZP glycoproteins have emerged as potential candidates for immunocontraception because of their essential role in fertilization and tissue specificity. Immunization against the ZP might result in the generation of antibodies that bind to the ZP. Thus, when a sperm encounters the ovum in immunized animals, binding is inhibited because the ZP is already occupied by the antibodies. Owing to their critical role in reproduction, ZP glycoproteins have been used as candidate antigens for contraception via immunological intervention.

The contraceptive potential of either native porcine SIZP (PZP) or its purified component (ZP3) has been demonstrated in female rabbits (Skinner et al. 1984), bitches (Mahi-Brown et al. 1985), non-human primates (Sacco et al. 1987; Bagavant et al. 1994), domestic and captive wild horses (Liu et al. 1989), captive white-tailed deer (Miller et al. 2000) and a variety of zoo animals (Kirkpatrick et al. 1996). The contraceptive efficacy of the PZP vaccine was also demonstrated in free ranging African elephants (*Loxodonta africana*) in the Kruger Park, South Africa (Fayrer-Hosken et al. 2000). Thus, PZP-based contraceptive vaccines have been successfully used for the management of populations of wild horses (Kirkpatrick and Turne 2008; Kirkpatrick et al. 2012), urban deer (Curtis et al. 2002), wapti (Shideler et al. 2002), and African elephants (Delink et al. 2007). In view of the above, three commercial vaccines based on PZP currently available:

1. Spayvac®: Spayvac® showed 100% contraceptive efficacy in the first year and 83% efficacy in the next 3 years in wild horses. Moreover, 85–90% infertility has been reported in female deer (Rutberg et al. 2013).
2. ZonaStat-H: Only the PZP vaccine was approved by the Environmental Protection Agency (EPA) for use in female wild horses and burros. It increased fertility by 86% in domestic and wild horses (Rutberg et al. 2012).
3. PZP-22: Provides two years of infertility in feral horses (Turner et al. 2007; Kirkpatrick et al. 2012).

The ovarian specific expression of zona proteins and lack of cross-reactivity of antibodies generated against zona proteins with other tissues and protein hormones (Palm et al. 1979; Barber et al. 2000) are advantage of PZP-based vaccines compared with GnRH-based contraceptive vaccines.

VACCINE DELIVERY

Remote vaccine delivery by either a dart gun or oral bait

Immunization of free-ranging large animals such as horses and white-tailed deer by remote delivery of a contraceptive vaccine via a dart gun has been very useful (Kirkpatric et al. 1990; Naugle et al. 2002). However, vaccine delivery via dart guns may not be feasible in small free-ranging animals such as monkeys, as vaccine dart may affect some sensitive parts of the body and thereby leads to injury. Another strategy to deliver vaccines may be to use oral baits. The oral baits were dropped by helicopters in the forests inhabited by wild foxes. Within the past 30 years, the overall incidence of rabies in Europe has decreased by approximately 80%, and it has been completely eliminated from Western and Central Europe (www.who-rabies-bulletin.org). However, the delivery of contraceptive vaccines as oral baits for the management of free-ranging wildlife populations may be difficult as it has adverse consequences for the fertility of other animal species, which may also consume these baits in addition to the target species.

Edible vaccines

Plants can be engineered to produce large amount of foreign proteins, which can be fed to animals, thereby eliciting an appropriate immune response. As a proof-of-principle, the feeding of genetically modified potatoes (generated by the insertion of a gene encoding the heat labile enterotoxin unit B from enterotoxigenic *E. coli* bacteria) into both mice and humans led to the generation of an immune response against the heat labile enterotoxin unit B antigen (Mason et al. 1998; Tacket et al. 1998). In an effort to control the population of possum in New Zealand, attempts are being made to develop genetically modified carrots expressing possum ZP3 (Polkinghorne et al. 2005). However, the outcome of these efforts has not as yet published.

Virus-like particles and bacterial ghosts

Virus-like particles (VLPs) are essentially non-infective viruses, that are composed of self-assembled viral envelope proteins without genetic material. VLPs have the unique ability to display multiple copies of small

foreign peptides on their surface without losing the self-assembly property of the envelope protein, thereby leading to a potent immune response against the foreign peptide (Grgacic and Anderson. 2006). Their size typically falls within the nanoparticles range and is stable and versatile in nature. VLPs offer excellent adjuvant characteristics that can induce innate and adaptive immune responses. The immunization of mice with Johnson grass mosaic virus (JGMV) with protein-based VPLs expressing a fusion peptide comprising mouse ZP3 led to subfertility in the immunized animals (Choudhury et al. 2009).

Bacterial ghosts which are made-up of non-living bacterial cells without genetic components, have also been effectively used as platform to deliver antigens to elicit immune responses. The immunization of female brushtails possum with bacterial ghosts expressing either N-terminal (41–316 amino acid residues) or C-terminal (308–636 amino acid residues) fragments of possum ZP2 fused to maltose-binding protein in the *E. coli* NM522 strain led to the generation of both humoral and cell-mediated immune responses (Walcher et al. 2008).

Live-vector based contraceptive vaccines

In a reverse genetic approach, attenuated Salmonella typhimurium-expressing mouse ZP3 was developed and delivered to mice via the oral route. Immunized animals exhibit a reduction in fertility (Zhang et al. 1997). Host-specific live vectors expressing various zona proteins have also been evaluated for their contraceptive potential in mice (Jackson et al. 1998), and female rabbits (Gu et al. 2004; Mackenzie et al. 2006). The basic goal of developing host-specific live vector-based contraceptive vaccines is to release them in the environment so that the recombinant virus is transmitted from one animal to another, thereby leading to effective management of pests such as those in rats and rabbits. This approach saves the task of individually capturing the animal, followed by vaccination and the release of immunized animal in its natural habitat. The overall concept was good; however, recombinant viruses have lower infectivity than does with the wild type virus, which will eventually reduce the contraceptive efficacy of live vector-based contraceptive vaccines (Hardy et al. 2006).

Microparticles for contraceptive vaccine delivery

One of the exciting options for delivering a contraceptive vaccine is the use of inert nanoparticles or microparticles, which themselves may not be immunogenic but provide a sustained immune response against an entrapped antigen for a long period. This is primarily due to the slow release of the antigen from such particles. Gray seals (*Halichoerus grypus*) immunized with porcine ZP entrapped in liposomes, decreased fertility in 90% of the immunized animals over a 5-year period (Brown et al. 1997). In general, nanoparticles favor the generation of a cell mediated immune response whereas microparticles favor a humoral immune response (Kanchan et al. 2007). It has been shown that polymer particle-entrapped vaccines can elicit a memory antibody response from single point immunization (Kanchan et al. 2009). Therefore, particles-based formulation supplemented with suitable adjuvant(s) is expected to reduce the number of doses of the vaccine required to achieve the desired immune response and efficacy.

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