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Review Article

Current ZP3-based Immunocontraceptive Vaccine for Free Ranging Wild Pest

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ABSTRACT

There is an overabundance of certain animal species that are considered as destructive and have reproductive control acts as a humane management of pest population. In particular, mammalian zona pellucida 3 (ZP3) has provoked a great interest as a potential antigen for immunocontraception. High levels of long-term infertility have been achieved in many species following ZP3-based immunization. This paper discusses the current updates on the ZP3-based immunocontraception for several major pest species of the world.

Keywords: Immunocontraception, pest control, zona pellucida 3

ABBREVIATIONS

BAC	:	Bacterial Artificial Chromosome
BMP15	:	Bone Morphogenic Protein 15
CHV	:	Canine Herpesvirus
CTL	:	Cytotoxic T Lymphocytes
DES	:	Diethylstilbestrol
FSH	:	Follicle Stimulating Hormone
fZP	:	Fox Zona Pellucida
GnRH	:	Gonadotrophin Releasing Hormone
GMCSF	:	Granulocyte-Macrophage Colony-Stimulating Factor
HCMV	:	Human Cytomegalovirus
ie1	:	Immediate Early 1 Region
ie2	:	Immediate Early 2 Region
IVF	:	In Vitro Fertilisation
IL-4	:	Interleukin 4
KLH	:	Keyhole Limpet Hemocynin
LH	:	Luteinizing Hormone
MBP	:	Maltose Binding Protein
MGA	:	Melengestrol Acetate
mZP	:	Mouse Zona Pellucida
MCMV	:	Murine Cytomegalovirus
NP	:	Nucleoprotein
OGP	:	Oviduct Glycoprotein
PZP	:	Porcine Zona Pellucida
PRL	:	Prolactin
rZP	:	Rat Zona Pellucida
SP56	:	Sperm Protein 56
SLP	:	Synthetic Late Pox Virus Promoter
TK	:	Thymidine Kinase
ZP	:	Zona Pellucida
ZP1	:	Zona Pellucida 1
ZP2	:	Zona Pellucida 2
ZP3	:	Zona Pellucida 3
ZPC	:	Zona pellucida C

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INTRODUCTION

Reproductive control is important for the management of certain overabundant wildlife which has turned into pest species. Three basic available techniques for this purpose include surgical sterilization, hormonal contraception, and immunocontraception (Artois, 1997; Cowan and Tyndale-Biscoe, 1997; Sinclair, 1997). However, it is costly and impractical to conduct surgical sterilization for free ranging wildlife population. It involves invasive procedure, causes discomfort, pain, and poses the risk of infection. Hormonal contraception using highly conserved reproductive steroid sex hormones, such as gonadotropin releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH) to disrupt the oestrus cycle, is not species-specific. It often leads to undesirable side effects that raise ethical issues, such as premature termination of pregnancies or damages to non-reproductive tissues (Talwar, 1997; Delves, 2004; Ferro and Mordini, 2004), alterations in sexual and social behaviour of the target species (Tyndale-Biscoe, 1994; Tuyttens and Macdonald, 1998). Therefore, immunocontraception remains an attractive method to reduce and maintain the populations of these animals. In particular, immunocontraceptive vaccine prevents conception by stimulating the production of antibodies that bioneutralize proteins or hormones essential for reproduction. Meanwhile, the use of immunocontraception has been recognized to provide animal welfare benefits relative to alternative methods (Tyndale-Biscoe, 1994; Oogjes, 1997; Porton, 2005; Rutberg, 2005; Hardy and Braid, 2007). It provides alternatives to the on-farm (e.g. pig production industry) surgical removal of an animal's gonad which is usually undertaken without anaesthetic. For free ranging wildlife, immunocontraceptive control is preferable to most lethal methods of controlling populations by animal welfare proponents (Oogjes, 1997; Porton, 2005; Hardy and Braid, 2007). The application of this method is also suggested in areas where lethal control is restricted, such as at national parks.

Fertilization is a species-specific process (Hoodbhoy and Dean, 2004; Conner *et al*., 2005). Therefore, targeting the gamete proteins should provide an effective contraceptive vaccine with the least risk to non-target species. To date, the most widely tested immunocontraceptive vaccine for wildlife species is on the basis of developing antibodies to zona pellucida (ZP) (Jewgenow, 2009). Other potential immunocontraceptive antigens, particularly protein derived from sperm, appear less efficacious than ZP antigens (Naz *et al*., 2005; Hardy, 2007; Hardy and Braid, 2007). ZP is a unique extracellular glycoprotein matrix that surrounds mammalian oocyte through which the sperms must penetrate during the initial stages of fertilization. It is composed of three major glycoproteins, which are zona pellucida 1 (ZP1), zona pellucida 2 (ZP2) and zona pellucida 3 (ZP3). High levels of long-term infertility in females have been achieved in many species following immunization with native ZP and recombinant ZP3 antigens. In this paper, current updates on the ZP3-based immunocontraceptive vaccine for several world major pest species are discussed.

Rats

Rats have undeniable become major pests. In fact, they are the vectors of many diseases, such as plaque and leptospirosis. They destroy an estimated of 20% of the harvested crops of the world during storage and cause major damages to buildings and equipment. The current control methods used for wild rat population involve trapping, poisoning, and biological means. A biological rodenticide, using *Sarcocystis singaporensis,* has been reported (Wood, 1985; Wood and Chung, 2003). This protozoan alternates as a gut parasite of the reticulate python, passing back to rats which eat the snake feaces (Zaman and Colley, 1975). It causes a debilitating muscle infection, found to be lethal to rat (Wood, 1985). These population control methods are less than satisfactory due to the intrinsic capacity of these species to grow rapidly and replace those that have been killed.

Therefore, curbing the reproductive potential of the pest is a more effective approach in reducing the rat population densities.

In particular, Porcine ZP (PZP) has been extensively used for birth control studies due to its high availability at the abattoirs. Heteroimmunisation of PZP has been found to successfully reduce or inhibit fertility in several species (Wood *et al*., 1981; Mahi-Brown *et al*., 1985; Paterson *et al*., 1992). However, PZP has no fertility control effect on rats (Drell *et al*., 1984). Hence, 'self' ZP is required to suppress the fertility in rats. ZP3-based DNA immunocontraceptive vaccines have been previously developed in which the rat ZP3 glycoprotein (rZP3) is expressed under the control of the human cytomegalovirus (HCMV) immediate early promoter (Lai, 2004). The administration of this DNA vaccines resulted in reduction of an average litter size up to 90%, although the ZP3 antibody titre was significantly low. The ovarian dysfunction was characterized by excessive depletion of follicles and an increase in the number of oocyte-free cell clusters. In order to produce recombinant rZP3 that mimics the native glycoprotein in bulk, rZP3 cDNA was expressed in yeast. Vaccination with recombinant ZP3 protein, expressed by yeast cells stimulated strong antibody response, but no correlation between antibody titres and infertility was observed (Lai, 2004; Lo *et al*., 2009). Meanwhile, rZP3-DNA vaccine was co-administrated with Interleukin 4 (IL-4) to boost up the level of antibody response against ZP3 protein. However, a dramatic increase in the ZP antibody level did not enhance the efficacy but it further weakened its effectiveness in preventing fertilization.

An attempt to improve the potency of the rZP3-DNA vaccine is the incorporation of the Newcastle viral nucleoprotein (NP) to the DNA vaccine (Lai, 2004). The viral nucleoprotein served as an immunologic adjuvant in the form of fusion gene. Being an endogenous antigen, rZP3 is not highly antigenic on its own since it is recognized by the host as "self" molecules. The antigenicity of the rZP3 protein was greatly improved when conjugated with NP because the NP portion is of viral origin, and thereby rendering the entire protein foreign and evoking a stronger cytotoxic T lymphocytes (CTL) response and a high level of antigen specific antibodies, mainly IgG2a subclass via cross priming (Yankauckas *et al*., 1993; Lai, 2004). This may increase the anti-ZP3 antibody production without affecting the cell mediated immune response, unlike the enhancement of antibody response by IL-4 which is sometimes accompanied by a suppression of cell-mediated immune response that is important to ensure permanent infertility. Furthermore, this improved rZP3-DNA vaccine has been found to produce a significant enhancement on the levels of ZP3 antibody. However, despite high ZP3 antibody titres, the vaccinated rats produced normal litter size. This might be due to certain epitopes, specifically the sperm receptor sites or those possessing contraceptive activities might be masked during protein folding when the larger NP protein was expressed as recombinant protein resulting in less capability to prevent pregnancy.

Although rZP3-based DNA immuno-contaceptive vaccines has successfully reduced the number of rat populations to a certain extent, the need for booster vaccination raises both the economical and practical issues of using the DNA-based immunocontraceptive approach. Thus, viral vectors appear to be an ideal delivery system for an immunocontraceptive vaccine, especially for those antigens which are highly glycosylated and in which post-translational modification is important for the generation of immune responses to functionally important domains (Lai, 2004). The viral-based delivery system is practical and advantageous due to its long acting persisting nature in the populations even after a single exposure. Extensive *in vitro* studies on adenovirus and retrovirus ZP3 based immunocontraceptive vaccines have been successfully completed (Lo *et al*., 2006a: 2006b). Meanwhile, the experiment to test the effectiveness of these vaccines on reducing the rat population is underway.

Mice

Mice have also caused major damages worldwide. Globally, they have caused damages worth billions of dollars annually. Damages caused by mice in crops, such as rice (specifically in the temperate regions), currently form a major threat to humans. Wild mice in the southeastern grain-belt of Australia have been reported to cause great damages to crops and stored grain and have important social impacts on the rural communities (Redhead, 1988; Singleton *et al*., 1999: 2001; Ylönen, 2001). The current control methods used are apparently less than satisfactory. In more specific, these methods are either lethal, non-humane, and have caused negative side-effects through poisoning of non-target species. Thus, there is a need for continued evaluations and testing of novel approaches aimed at interrupting their fertility.

Mice heteroimmunized with porcine zona pellucida had no significant effect on reducing the mouse fertility (Sacco *et al*., 1981). Meanwhile, the immunization of certain mouse strains with ZP3 peptide (amino acid 330-342) stimulates autoimmune response and oophoritis (Rhim *et al*., 1992). The strain dependent nature reduces the usefulness of this ZP3 peptide in outbred mouse population (Millar *et al*., 1989; Rhim *et al*., 1992). In order to improve this vaccine, a synthetic peptide vaccine contained a 7-mer peptide represents amino acid 336-342 which is immediately adjacent to the most hydrophilic portion of ZP3 and partially overlaps a region that contains a potential amphipathic α helix coupled with a carrier protein, keyhole limpet hemocyanin (KLH) (Millar *et al*., 1989) was studied. Mouse ZP3 (mZP3) is too small to produce antibodies on its own, so it was coupled to a larger protein (KLH) to increase antibody production. This vaccine induces antibody responses to both mZP3 and KLH. It gives long-term contraception without exhibiting ovarian histopathology. Targeting functional regions of the antigens not only reduces the undesirable side-effects, but it also increases the specificity of the vaccine. Contraceptive peptide epitopes with reduced side-effects and increased species specificity have been described. The selected mouse specific immunocontraceptive peptides have been determined to play a key role in reproduction. Mouse specific immunocontraceptive polyepitope vaccines, containing mouse specific epitopes for ZP1, ZP3, sperm protein 56 (SP56) and proliferin fused to maltose binding protein (MBP) produced in bacterial expression system, were found to have caused 40% fertility decrease in inoculated mice (Hardy *et al*., 2002). Meanwhile, conjugated peptides containing SP56, granulocyte-macrophage colony-stimulating factor (GMCSF) and prolactin (PRL) elicited peptide-specific serum antibodies and reduced fertility by 50% (Hardy *et al*., 2004). Multiple peptide antigens could be coupled to immune enhancers to generate stronger immune responses. Furthermore, polyepitope vaccines closely mimic natural autoimmunity more closely, whereby the physiological effects require simultaneous immune responses against a number of different antigenic molecules (Bach *et al*., 1998; Hardy *et al*., 2004).

The viral-vectored immunocontraceptive vaccine was first demonstrated in mice using Ectromelia virus. The recombinant ectromelia virus expressed mouse zona pellucia 3 glycoprotein under the direction of a synthetic poxvirus early-late promoter (Jackson *et al*., 1998). A single inoculation with the recombinant virus into the footpad of BALB/c mice caused infertility in 70% of the mice for 5-9 months. Disruption of ovarian follicular development and the depletion of mature follicles without oophoritis were also observed (Jackson *et al*., 1998). Thus, to boost the immunological effect, a gene responsible for activation of interleukin 4 (IL-4) productions was incorporated into the recombinant ectromelia virus (Jackson *et al*., 2001; Ylönen, 2001). All the infected mice died one week after immunization. This recombinant virus was lethal without any contraceptive effect. Meanwhile, the addition of IL-4, which acts as a transmitter substance in inflammations, was aimed at strengthening the immune response. However, it had suppressed the function of the killer T cells to fight the inflammation and

infection of the treated mice and the expression of immune memory response (Ylönen, 2001).

Higher levels of infertility have been achieved by using recombinant murine cytomegalovirus (MCMV) to express the mZP3 glycoprotein at the immediate early 2 (*ie2*) region of the virus under the control of human cytomegalovirus immediate early (*ie1*) promoter (Chambers *et al*., 1999; Lloyd *et al*., 2003; Redwood *et al*., 2005; Hardy *et al*., 2006). MCMV is an attractive vector for fertility associated genes predominantly for its species-specificity and its capacity to infect mice with more than one strain of virus simultaneously (Booth *et al*., 1993; Shellam, 1994). It was also found to establish persistent and latent infection in mice with periodic reactivation (Osborn, 1982). A single inoculation of the recombinant MCMV expressing mZP3 antigen resulted in almost 100% infertility in mice up to 250 days. Persistent anti-ZP3 antibody production and profound changes of ovarian morphology have also been observed in the infected animals. However, in other studies, the innate resistance to MCMV in certain inbred mouse strains was found to significantly reduce the immunocontraceptive success (Chambers *et al*., 1999; Lloyd *et al*., 2007). Similarly, a prior exposure to MCMV could limit the immunocontraceptive effects of the recombinant MCMC vector expressing mZP3 (Gormana *et al*., 2008). The host resistant locus, *Cmv1* which controls the natural killer cell towards the MCMV was the key factor determining the vaccine efficacy. However, specific pathogen free outbred wild mice could be sterilized over 100 days to MCMV infection (Lloyd *et al*., 2007). This finding indicates that wild mice should be susceptible to recombinant MCMV-mZP3 infection. On the contrary, the recombinant MCMV-mZP3 showed no anti-fertility effects on rats. The lack of effect of the immunocontraceptive virus in closely related species indicates the species specificity of this vaccine in the non-host species (Smitha *et al*., 2005).

In a recent study, a range of protein or polyepitope antigens, associated with female reproductive processes expressed by MCMV, were tested for the sterilizing effects in mice (Redwood *et al*., 2007). The antigens tested were bone morphogenic protein 15 (BMP15), oviduct glycoprotein (OGP) and ubiquitin-tagged mZP3. Both BMP15 and OGP proteins were expressed within the reproductive system of female mice. In particular, the mZP3 was N-terminal ubiquitinated to improve CD8+ T cell responses and MHC class I antigen presentation (Rodriguez *et al*., 1997; Anderson and Barry, 2004). The experiment found that only full length mZP3 or ubiquitin-tagged mZP3 induced fertility in mice, but not BMP15 and OGP. Meanwhile, the expression of the mouse-specific polyepitope antigens by species specific vector, MCMV might improve the safety of viral-vectored immunocontraceptive vaccine.

European Red Fox (Vulpes vulpes)

The European red fox is one of the major vertebrate pests in Australia. The over-population of the European red fox has posed a major threat to the survival of endangered native fauna, acted as reservoirs for exotic diseases, and caused a considerable impact on lamb production. Conventional methods for fox population control are by natural predators, commercial trapping, shooting or poisoning with strychnine, phosphorus, arsenic or 1080 (sodium fluoroacetate) (Tyndale-Bisco, 1994). Poisoning with '1080' baits requires repeated applications which need to be delivered into remote and inaccessible regions. This poison is also highly susceptible to non-target animals, such as dogs, and is therefore not suitable to use in the urban areas (Strive *et al*., 2006).

Fox ZPC (fZPC) antigens have been expressed with baculovirus system due to the lack of variable glycosylation and low yields of recombinant product in both yeast and bacteria expression systems (Bradly *et al*., 1997).

To date, the fZPC-based immuno-contraceptive vaccine has been assessed in two viral vectors, namely the vaccinia virus and canine herpesvirus (CHV) vectors (Reubel *et al*., 2005; Strive *et al*., 2006). Recombinant

vaccinia-based rabies vaccine has been used in the past two decade in Europe and North America to mitigate and prevent the spread of rabies in the European red foxes without any undesirable side-effects (Brochier *et al*., 1991; Masson *et al*., 1996). Therefore, vaccinia virus was studied to evaluate their anti-fertility potential in foxes. The recombinant fZPC vaccinia virus was constructed under the control of a synthetic late poxvirus (SLP) promoter to drive the expression of fZPC which was inserted within thymidine kinase (TK) gene locus of vaccinia virus (Reubel *et al*., 2005). All the recombinant viruses expressed fZPC in the cell culture but were highly attenuated in foxes *in vivo*. None of the treated foxes raised antibodies specific to the transgenes fZPC. Moreover, repeated administration of recombinant fZPC has been found to fail to induce ZPC specific antibodies but it developed a pronounced immune response to vaccinia virus proteins. It has been reported that the inactivation of the vaccinia virus thymidine kinase gene, caused by the transgene insertion into the viral genome, leads to the attenuation of the virus *in vivo* (Buller and Palumbo, 1992). In contrast, self antigens that are derived from homologous tissues can be less immunogenic, specifically when they are linked toincompatible viral carrier or promoter (Dunbar *et al*., 2001; Guevara-Patino *et al*., 2003; Reubel *et al*., 2005). Meanwhile, the absence of infectious virus in foxes indicates abortive infection and probably lack of virally expressed late proteins, including transgene due to the synthetic late promoter which is active only at the late stages of vaccinia virus replication. This may cause insufficient transgene expression to induce adequate immune responses against transgenic proteins. Therefore, a high expression of the transgene is required to overcome the poor immune responses to anti-fertility antigens in foxes.

Canine Herpesvirus is another viral vector that has been assessed for use in foxes (Reubel *et al*., 2001). The bacterial artificial chromosome (BAC) system has been used for CHV to facilitate vaccine development (Strive *et al*., 2006). The BAC-derived recombinant fZP3-CHV vaccine was found to be highly attenuated *in vivo* and failed to induce antibody response against the fZPC protein, although high serum antibody levels against the CHV proteins were detected (Reubel *et al*., 2005; Strive *et al*., 2007). Attenuation was due to the presence of large amounts of additional BAC-related DNA incorporated into the viral genome and to the inactivation of the thymidine kinase gene (Strive *et al*., 2006).

Therefore, in order to improve BAC-derived CHV antifertility vaccines, the development of an improved TK positive, self-excisable CHV-BAC, that enables antigen expression from an intergenic region between the UL21 and UL22 genes within the CHV genome, was reported. In this study, the BAC-derived CHV-PZPC stably expressed the PZPC antigen (Strive *et al*., 2007). However, it was also highly attenuated *in vivo* and failed to induce anti-ZPC antibodies or infertility in foxes (Hardy *et al*., 2006).

European Rabbit (Oryctolagus cuniculus)

The European rabbit (*Oryctolagus cuniculus*) is an introduced species in Australia. High fertility rate and over-abundance of the European rabbit have turned this species into a major vertebrate pest species in Australia and other countries. The species has specifically caused significant losses in agricultural production and severe environmental degradation (Holland and Jackson, 1994; Gu *et al*., 2003: 2004). The current methods used to reduce of rabbit populations are inhumane, non-species specific and met with only partial success (Holland and Jackson, 1994; Tyndale-Biscoe, 1994). Therefore, fertility control was suggested.

The effects of alloimmunization (rabbit ZP) and heteroimmunization (porcine ZP) on the fertility in rabbits were studied (Wood *et al*., 1981). A rabbit that was heteroimmunized with PZP induces serum antibodies to rabbit ZP antigens and caused infertility with ovarian degeneration and endocrine dysfunction (Wood *et al*., 1981; Skinner *et al*., 1984; Kerr *et al*., 1999). Meanwhile, the rabbit that was alloimmunized with the rabbit ZP did not elicit

a significant immune response and normal offspring were obtained (Wood *et al*., 1981; Skinner, 1987). The fertility levels were also found to be lower than those of the controls, but there was a considerable variability between animals. The fertility reduction rate was also found to be dependent on the state of solubilization of zona prior to immunization (Wood *et al.*, 1981).

In other attempts, an immunization of the female rabbits with bacterially expressed rabbit ZP did not develop detectable antibody level (Dunbar *et al*., 1994; Prasad, 1995). A recombinant myxoma virus expressing rabbit zona pellucida C (ZPC) caused 70% infertility in immunized rabbits for 30-35 days (Mackenzie *et al*., 2006). In a recent study, recombinant myxoma virus expressing rabbit ZPC under the control of a synthetic early/late promoter induced short-term infertility in 90-100% of the treated rabbits (Hardy *et al*., 2006).

African Elephants (Loxodonta africana)

During the twentieth century, the number of African elephants (*Loxodonta africana*) declined dramatically as a result of over-hunting, poaching for ivory, and the loss of habitat through human cultivation and settlement. In 1989, bans were placed on all international trades of elephant products, whereas the protection offered by legislation and the establishment of protected areas has eventually led to a remarkable recovery in the number of elephants (Hanks, 2006). Unfortunately, efforts to nurture the elephant populations have resulted in elephant over-population in several southern and eastern African countries. The resulting high elephant densities in Southern Africa have lead to vegetation and habitat destruction, degradation of the appearance and ecological functioning of the landscape, and thereby reducing biological diversity. Methods such as culling, translocation, range expansion, manipulation of water sources, and contraception are options that have been used to reduce elephant densities (van Aarde and Jackson, 2007). However, culling is greatly opposed due to the ethical and animal rights concerns. Meanwhile, translocation is no longer a viable option due to high cost incurred, cumbersome, lack of suitable wildlife areas available and unrealistic to relocate thousands of animals per year (Colenbrander, 2003a).

With the increasing alarm, the assessment of elephant management in South Africa was conducted. Therefore, PZP immunocontraception for the elephant population control has been reported. The female elephants immunized with the whole porcine ZP developed antibodies that persisted for 12-14 months that were sufficient for an effective contraception in these animals (Fayrer-Hosken *et al*., 1997: 1999: 2000). In the first field trial, 56% of the vaccinated elephants were not pregnant (Bertschinger *et al*., undated). In the second trial, 80% of the inoculated elephants were not pregnant using a revised schedule (Bertschinger *et al*., undated). In more specific, the treated females did not conceive for up to 12 months. When a booster vaccination was given after a year, they remained sterile for up to two years with no deleterious effect on the ovary and cyclicity of the treated elephants (Fayrer-Hosken *et al*., 2000; Fayrer-Hosken, 2003). The trials have also proven that the vaccine is safe to use in pregnant elephants and it is reversible.

A follow-up phase was conducted at the Greater Makalali Private Game Reserve from 2000 and in the span of ~12 years. Forty-three percent of the treated female elephants underwent the 53-month inter-calving period with no early calving indicating 100% reproductive control (Delsink *et al*., 2003: 2004: 2007). This programme seemed to demonstrate that the PZP does not cause herd fragmentation, harassment by bulls, change in rank, and other negative behaviours. Interestingly, it has shown that the elephant anti-PZP antibodies preferentially recognize the theca cells in the primary and secondary follicles, but do not block fertilization in a porcine in vitro fertilization (IVF) system. Therefore, it is likely that the antibodies raised by PZP vaccination of elephant cows are not directed against the molecules involved in either the primary or secondary sperm-oocyte binding, but they exert their effects much earlier, i.e.

during the development of the follicle and zona pellucida (Colenbrander *et al*., 2003b).

One of the obstacles for a large population control is the need to administer multiple boosters to individual animals (Kirkpatrick *et al*., 1997; Turner *et al*., 1997; Kirkpatrick and Rutberg, 2001; Perdok *et al*., 2007). In order to overcome this hurdle, a single administration (one-shot), with multiple slow releases PZP pellet vaccine, has recently been developed (Frayne and Hall 1999; Kirkpatrick and Rutberg 2001; Kirkpatrick, 2003). All the captive elephants immunized with the one-shot vaccine were found to develop antibody titres that were better than with the conventional vaccine. In fact, this one-shot vaccine can greatly save the cost of contraception (single darting instead of triple darting) and provide a more feasible contraceptive treatment for larger populations of elephants.

White-tailed Deer (Odocoileus virginianus)

The over-population of the white-tailed deer (*Odocoileus virginianus*) has become a serious problem in many areas of the United States, particularly in the northern and eastern parts of the United States. Their adaptability, acute senses, and other physical attributes have enabled them to boom in the wilderness and metropolitan suburbs. Meanwhile, problems associated with over-abundance of white-tailed deer pose adverse effects to ecological communities, economic losses from crop damage, damages to ornamental plantings, damages to automobile, and injuries due to deer and vehicle collisions, as well as spread of Lyme disease from deer ticks (Anthony *et al*., 2000). Methods such as sport hunting, sharpshooting, and trap-and-kill have traditionally been used to maintain white-tailed deer populations. However, public opposition and municipal ordinances make these lethal methods illegal in some locations (Kellert, 1991). Surgical sterilization not only prolongs the breeding season of the female animals, it also requires licensed veterinarians and thus increases the cost incurred for this method. Thus, the fertility control seems to be a potential resolution to control the population of deer. Early studies of deer fertility control used synthetic steroid hormones, primarily melengestrol acetate (MGA), diethylstilbestrol (DES), administered either orally, through implant or infection (Harder and Peterle, 1974; Bell and Peterle, 1975; Matschke, 1977a, b: 1980; Roughton, 1979). However, this vaccine requires capture, immobilization and daily exposure of the agent to the animals. Deer are prey for several species, including human, and therefore, synthetic steroid contraceptives vaccine which resist biodegradation are unfavourable as a deer contraceptives agent.

Immunocontraception is a potential possibility for a more permanent solution. The first successful, remotely delivered immuno-contraception in captive, unrestrained white-tailed deer using porcine zona pellucida antigen was reported (Turner *et al*., 1992). All the PZP treated did not produce fawn but showed extended estrous cycle. The PZP protein was metabolized prior to ingestion or excretion; therefore, it could not be passed through food chain to the predators. In the first field test, single PZP inoculation provided limited contraceptive effect ($\leq$ 20%) and it was also found to yield full contraceptive effect (70-100%) with two inoculations. Meanwhile, a single annual booster inoculation in the second year reduced fertility to 20% thereafter. The second large scale field trial, with free-roaming deer that were acclimatized to human presence, revealed that only 28% of the does which had received the initial two inoculations produced fawns (Kirkpatrick *et al*., 1997).

The possibility for the use of one inoculation PZP vaccine in a two injection protocol was examined (Kirkpatrick *et al*., 1997). Does were given an initial inoculation of native PZP and an osmotic mini-pump that continuously released PZP for 28 days implanted subcutaneously in the neck. The continuous release of PZP elevated the antibody titres to contraceptive levels (Turner *et al*., 1996; Kirkpatrick *et al*., 1997).

The long-term effects of the PZP vaccine on the white-tailed deer were also studied. In particular, the PZP treated does have a 76%

reduction of fawning rate in 6 years. The deer remained infertile for 1-4 years after the booster. Infertility is directly related to the antibody titres to the PZP. In addition, an increase in estrous cycles also leads to prolonged breeding seasons in the treated does. The ovarian follicular cycle continued to produce ovulatory follicles in the PZP treated does but conception failed to occur. This suggests that the oocytes exposed to immune serum from PZP treated does is less capable of binding spermatozoa (Way *et al*., 1999; Killian and Miller, 2000). It is also possible that infertility results from the failure of a normal corpus luteum to develop sustain pregnancy due to the lower level of serum progesterone concentrations during the luteal phase (Miller *et al*., 1999).

In another study, the peptides that reacted with sera from infertile deer, including six peptides from ZP1 and six peptides from ZP3, were selected and produced to test the immunogenicity and immunocontraception in deer. The ZP3 peptides pin 46-54 (ZP3 279-332) induced high antibody titres towards PZP but does were found to have remained fertile. Meanwhile, ZP1 peptides pin 10–16 (ZP1 79–130) induced lower titres, but deer exhibited multiple estrus cycle and infertility. A less consistent response to PZP peptides as compared to whole molecules of native PZP was possibly due to the smaller peptides which did not contain sufficient T cell epitopes to ensure a sufficient binding by MHC and presentation to T cells to stimulate immunocontraception response (Miller and Killian, 2002). It is important to note that peptides will need to be coupled with a larger protein carrier or included sufficient T cells epitopes to provide sufficient T cells stimulation. Moreover, the three dimensional confirmation and glycosylation of the synthesized peptides may have been altered (Miller and Killian, 2002).

Although several previous studies reported the success of the PZP immunocontraception in white-tailed deer with minimal health side-effect but Curtis *et al*. (2007) identified pathophysiology resulting from the PZP treatment. Most of the does were detected with granulomas at injection sites, even after 2 years of the treatment. The majority of the treated does developed eosinophilic oophorities in ovaries. The ovarian ZP antigens presented to the immune system stimulated release of chemokines which attracted eosinophils. Then, the eosinophils stimulated inflammatory response in the ovary (Curtis *et al*., 2007). It was found that the does without oophoritis revaccinates, with the last booster, had the greatest reduction in normal secondary follicles. This condition has been related to inhibited ovarian function, abnormal cycling, and suppressed progesterone levels (Skinner *et al*., 1984; Mahi-Brown *et al*., 1988; Dunbar *et al*., 1989; Sacco *et al*., 1991; Dunbar *et al*., 2001; Stoops *et al*., 2006; Curtis *et al*., 2007).

Efforts to control overabundant of the white-tailed deer with a long lasting single dose contraceptive vaccine, SpayVac®, have been carried out. SpayVac® consist liposome with encapsulated intact PZP. A single dose of SpayVac® was highly effective for more than 2 years but the practical application is limited to small enclosed areas due to high labour cost (Locke *et al*., 2007).

CONCLUSIONS

It can therefore be concluded that immunocontraception is an attractive and ethically supported alternative for animal control as it is more humane and less invasive. The effectiveness of this particular method has been studied in captive and small population of free-ranging wildlife animals. Nonetheless, further research is needed to safely apply immunocontraception to over-abundant free ranging wildlife populations. Parameters that require crucial considerations are needed with regard to the distribution of the species under study, whether large scale or localised control is desired, and the issue of directed specificity with regard to other species. Antigens that are highly conserved between the species should be avoided. Besides, specificity can also be built within the vaccine which might include the target antigen or epitopes, microbial or other delivery vector. Ideally, a potential immunocontraceptive

vaccine that induces infertility should not cause ovarian dysfunction and significant health side-effects. Different vaccine delivery systems, such as dart delivery, disseminating viral vectors and oral bait systems, have been assessed to ensure that the immunocontraceptive vaccine is capable of inducing a long-lasting immune response to a high percentage of the target wildlife population. Moreover, it is vital that all public concerns and legal requirements in relation to the risks of disseminating genetically modified organism are adequately addressed during the development phase and prior to any environmental release. If issues such as long-term efficacy and safety, the requirement for booster vaccinations, the use of adjuvants and high production cost can be resolved, immunocontraceptive vaccines will then be able to provide consistent, long-term infertility after a single inoculation, and offer realistic alternatives to free ranging wildlife population control in the near future.

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