

Review Article

Current ZP3-based Immunoconceptive 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	MBP	: Maltose Binding Protein
BMP15	: Bone Morphogenic Protein 15	MGA	: Melengestrol Acetate
CHV	: Canine Herpesvirus	mZP	: Mouse Zona Pellucida
CTL	: Cytotoxic T Lymphocytes	MCMV	: Murine Cytomegalovirus
DES	: Diethylstilbestrol	NP	: Nucleoprotein
FSH	: Follicle Stimulating Hormone	OGP	: Oviduct Glycoprotein
fZP	: Fox Zona Pellucida	PZP	: Porcine Zona Pellucida
GnRH	: Gonadotrophin Releasing Hormone	PRL	: Prolactin
GMCSF	: Granulocyte-Macrophage Colony-Stimulating Factor	rZP	: Rat Zona Pellucida
HCMV	: Human Cytomegalovirus	SP56	: Sperm Protein 56
ie1	: Immediate Early 1 Region	SLP	: Synthetic Late Pox Virus Promoter
ie2	: Immediate Early 2 Region	TK	: Thymidine Kinase
IVF	: In Vitro Fertilisation	ZP	: Zona Pellucida
IL-4	: Interleukin 4	ZP1	: Zona Pellucida 1
KLH	: Keyhole Limpet Hemocynin	ZP2	: Zona Pellucida 2
LH	: Luteinizing Hormone	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; Tuytens 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 bionutralize 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 plague 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 faeces (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 immunoconceptive 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 immunoconceptive 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 immunoconceptive approach. Thus, viral vectors appear to be an ideal delivery system for an immunoconceptive 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 immunoconceptive 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 (GM-CSF) 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 pellucida 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 immunoconceptive success (Chambers *et al.*, 1999; Lloyd *et al.*, 2007). Similarly, a prior exposure to MCMV could limit the immunoconceptive effects of the recombinant MCMV 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 immunoconceptive 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 polypeptide 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 polypeptide antigens by species specific vector, MCMV might improve the safety of viral-vectored immunoconceptive vaccine.

European Red Fox (Vulpes vulpes)

The European red fox is one of the major vertebrate pests in Australia. The overpopulation 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 immunoconceptive 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 decades 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 to incompatible 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 overpopulation in several southern and eastern African countries. The resulting high elephant densities in Southern Africa have led 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 immunoconception 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 immunocontraception 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 oophoritis 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 revaccinated, 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 immunoconceptive

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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REFERENCES

- Andersson, H.A. and Barry, M.A. (2004). Maximizing antigen targeting to the proteasome for gene-based vaccines. *Molecular Therapy*, 10(3), 432-446.
- Anthony, J.D., Kurt, C.V., Paul, D.C.C. and Scott, E. (2000). Managing White-Tailed Deer in Suburban Environments: A Technical Guide. A publication of Cornell Cooperative Extension, the Wildlife Society-Wildlife Damage Management Working Group, and the Northeast Wildlife Damage Research and Outreach Cooperative.
- Artois, M. (1997). Managing a problem of wildlife in the "Old World": A veterinary perspective. *Reproduction, Fertility and Development*, 9, 17-25.
- Bach, J.F., Koutouzov, S. and van Endert, P.M. (1998). Are there unique autoantigens triggering autoimmune diseases? *Immunological Reviews*, 164, 139-155.
- Bell, R.L. and Peterle, T.J. (1975). Hormone implants control reproduction in white-tailed deer. *Wildlife Society Bulletin*, 3, 152-156.
- Bertschinger, H.J., Kirkpatrick, J.F., Fayrer-Hosken, R.A., Grobler, D. and van Altena, J.J. (Undated). Immunocontraception of African elephants using porcine zona pellucida vaccine. Retrieved from <http://elephantpopulationcontrol.library.uu.nl/paginas/txt06.html>.
- Booth, T.W., Scalzo, A.A., Carrello, C., Lyons, P.A., Farrell, H.E., Singleton, G.R. and Shellam, G.R. (1993). Molecular and biological characterization of new strains of murine cytomegalovirus isolated from wild mice. *Archives of Virology*, 132, 209-220.
- Bradley, M.P., Hinds, L.A. and Bird, P.H. (1997). A bait-delivered immunocontraceptive vaccine for the European red fox (*Vulpes vulpes*) by the year 2002? *Reproduction, Fertility and Development*, 9(1), 111-116.
- Brochier, B., Kieny, M.P., Costy, F., Coppens, P., Bauduin, B., Lecocq, J.P., Languet, B., Chappuis, G., Desmettre, P. and Afiademanyo, K. *et al.* (1991). Large-scale eradication of rabies using recombinant vaccinia rabies vaccine. *Nature*, 354(6354), 520-522.
- Buller, R.M. and Palumbo, G.J. (1992). Safety and attenuation of vaccinia virus. In M.M. Binns and G.L. Smith (Eds.), *Recombinant poxviruses* (pp. 235-267). Boca Raton: CRC.
- Chambers, L.K., Lawson, M.A. and Hinds, L.A. (1999). Biological control of rodents-the case for fertility control using immunocontraception. In G. Singleton *et al.* (Eds.), *Ecologically-based rodent management* (pp. 215-242). Australian Centre for International Agricultural Research, Canberra.
- Colenbrander, B. (2003a). In B. Colenbrander, J. De Gooijer, R. Paling, S.S. Stout, T. Stout and W.R. Allen (Eds.), *Proceedings of an Expert Consultation on the Control of Wild Elephant Populations* (p. 7). Beekbergen, Netherlands.
- Colenbrander, B., Bertschinger, H.J. and Stout, T.A.E. (2003b). Immunocontraception of elephants

- using porcine zona pellucida vaccination. EU-Asia Link Project Symposium "Managing the Health and Reproduction of Elephant Populations in Asia" (pp. 117-119). Bangkok, Thailand.
- Conner, S.J., Lefievre, L., Hughes, D.C. and Barratt, C.L. (2005). Cracking the egg: Increased complexity in the zona pellucida. *Human Reproduction*, 20, 1148-1152.
- Cowan, P.E. and Tyndale-Biscoe, C.H. (1997). Australia and New Zealand mammal species considered to be pests or problems. *Reproduction, Fertility and Development*, 9, 27-36.
- Curtis, P.D., Richmond, M.E., Miller, L.A. and Quimby, F.W. (2007). Pathophysiology of white-tailed deer vaccinated with porcine zona pellucida immunoconceptive. *Vaccine*, 25, 4623-4630.
- Delsink, A.K. (2003). Contraception of African elephant cows in two private conservancies using porcine zona pellucida vaccine and the control of aggressive behaviour in elephant bulls with a GNRH vaccine. In B. Colenbrander, J. De Gooijer, R. Paling, S.S. Stout, T. Stout and W.R. Allen (Eds.), *Proceedings of an Expert Consultation on the Control of Wild Elephant Populations* (pp. 43-45). Beekbergen, Netherlands.
- Delsink, A.K., Bertschinger, H.J., Kirkpatrick, J.F., Grobler, D., van Altena, J.J. and Slotow, R. (2004). The preliminary behavioural and population dynamic response of African elephants to immunoconception. *Proceedings of the 15th Symposium on Tropical Animal Health and Reproduction: Management of Elephant Reproduction* (pp. 31). Utrecht, Netherlands.
- Delsink, A.K., van Altena, J.J., Grobler, D., Bertschinger, H., Kirkpatrick, J.F. and Slotow, R. (2007). Implementing immunoconception in free-ranging African elephants at Makalali Conservancy. *Journal of the South African Veterinary Association*, 78(1), 25-30.
- Delves, P.J. (2004). How far from a hormone-based contraceptive vaccine? *Journal of Reproductive Immunology*, 62, 69-78.
- Drell, D., Wood, D., Bundman, D. and Dunbar, B.S. (1984). Comparison of the immunological response in rats and rabbits to porcine zona pellucida. *Biology of Reproduction*, 30, 445-457.
- Dunbar, B.S., Lo, C., Powell, J. and Stevens, V.C. (1989). Use of a synthetic peptide adjuvant for the immunization of baboons with denatured and deglycosylated pig zona pellucida glycoproteins. *Fertility and Sterility*, 52(2), 311-318.
- Dunbar, B.S., Avery, S., Lee, V., Prasad, S., Schwahn, D., Schwoebel, E., Skinner, S. and Wilkins, B. (1994). The mammalian zona pellucida: Its biochemistry, immunochemistry, molecular biology, and developmental expression. *Reproduction, Fertility and Development*, 6, 331-347.
- Dunbar, B.S., Prasad, S., Carino, C. and Skinner, S.M. (2001). The ovary as an immune target. *Journal of the Society for Gynecologic Investigation*, 8(suppl. 1), S43-48.
- Fayrer-Hosken, R.A., Brooks, P., Kirkpatrick, J.F., Bertschinger, H.J., Raath, J.P. and Soley, J.M. (1997). Potential of the porcine zona pellucida (PZP) being an immunoconceptive agent for elephants. *Theriogenology*, 47, 397.
- Fayrer-Hosken, R.A., Bertschinger, H.J., Kirkpatrick, J.F., Grobler, D., Lamberski, N., Honnneyman, G. and Ulrich, T. (1999). Contraceptive potential of the porcine zona pellucida vaccine in the African elephant (*Loxodonta africana*). *Theriogenology*, 52, 835-846.
- Fayrer-Hosken, R.A., Grobler, D., Van Altena, J.J., Bertschinger, H.J. and Kirkpatrick, J.F. (2000). Immunoconception of African elephants, a humane method to control animal populations without behavioural side effects. *Nature*, 407, 149.
- Fayrer-Hosken, R.A. (2003). Elephant immunoconception. In B. Colenbrander, J. De Gooijer, R. Paling, S.S. Stout, T. Stout and W.R. Allen (Eds.), *Proceedings of an Expert Consultation on the Control of Wild Elephant Populations* (p. 27). Beekbergen, Netherlands.
- Ferro, V.A. and Mordini, E. (2004). Peptide vaccines in immunoconception. *Current Opinion in Molecular Therapeutics*, 6, 83-89.

- Frayne, J. and Hall, L. (1999). The potential use of sperm antigens as targets for immunocontraception: Past, present and future. *Journal of Reproductive Immunology*, 43, 1-33.
- Gormana, S., Lloyd, M.L., Smith, L.A., McWhorter, A.R., Lawson, M.A., Redwood, A.J. and Shellam, G.R. (2008). Prior infection with murine cytomegalovirus (MCMV) limits the immunocontraceptive effects of an MCMV vector expressing the mouse zona pellucida-3 protein. *Vaccine*, 26, 3860-3869.
- Gu, W., Holland, M., Janssens, P. and Kerr, P. (2003). Antibody response in the female rabbit reproductive tract to influenza haemagglutinin encoded by a recombinant myxoma virus. *Virology*, 313, 286-295.
- Gu, W., Holland, M., Janssens, P., Seamark, R. and Kerr, P. (2004). Immune response in rabbit ovaries following infection of a recombinant myxoma virus expressing rabbit zona pellucida protein B. *Virology*, 318, 516-523.
- Guevara-Patino, J.A., Turk, M.J., Wolchok, J.D. and Houghton, A.N. (2003). Immunity to cancer through immune recognition of altered self: Studies with melanoma. *Advances in Cancer Research*, 90, 157-177.
- Hanks, J. (2006). A troubled past. *Africa Geographic*, 14(3), 34-35.
- Harder, J.D. and Peterle T.J. (1974). Effect of diethylstilbestrol on reproductive performance of white tailed deer. *Journal of Wildlife Management*, 38, 183-196.
- Hardy, C.M., Pekin, J. and Have, J. (2002). Mouse-specific immunocontraceptive polyepitope vaccines. *Reproduction Supply*, 60, 19-30.
- Hardy, C.M., Clydesdale, G. and Mobbs, K.J. (2004). Development of mouse-specific contraceptive vaccines: Infertility in mice immunized with peptide and polyepitope antigens. *Reproduction*, 128, 395-407.
- Hardy, C.M., Hinds, L.A., Kerr, P.J., Lloyd, M.L., Redwood, A.J., Shellam, G.R. and Strive, T. (2006). Biological control of vertebrate pests using virally vectored immunocontraception. *Journal of Reproductive Immunology*, 71, 102-111.
- Hardy, C.M. (2007). Current status of virally vectored immunocontraception for biological control of mice. In S.K. Gupta, K. Koyama and J.F. Murray (Eds.), Society of Reproduction and Fertility Supplement Number 63. *Gamete biology: Emerging frontiers in fertility and contraceptive development* (pp. 495-506). UK: Nottingham University Press.
- Hardy, C.M. and Braid, A.L. (2007). Vaccines for immunological control of fertility in animals. *Rev. sci. tech. Off. Epiz.*, 26(2), 461-470.
- Holland, M.K. and Jackson, R.J. (1994). Virus-vectored immunocontraception for control of wild rabbits: identification of target antigens and construction of recombinant viruses. *Reproduction, Fertility and Development*, 6(5), 631-642.
- Hoodbhoy, T. and Dean, J. (2004). Insights into the molecular basis of sperm-egg recognition in mammals. *Reproduction*, 127, 417-422.
- Jackson, R.J., Maguire, D.J., Hinds, L.A. and Ramshaw, I.A. (1998). Infertility in mice induced by a recombinant ectromelia virus expressing mouse zona pellucida glycoprotein 3. *Biology of Reproduction*, 58, 152-159.
- Jackson, R.J., Ramsay, A.J., Christensen, C.D., Beaton, S., Hall, D.F. and Ramshaw, I.A. (2001). Expression of mouse interleukin-4 by a recombinant Ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *Journal of Virology*, 73, 1205-1210.
- Jewgenow, K. (2009). Immunocontraception in wildlife animals. In W.K.H. Krause and R.K. Naz (Eds.), *Immune fertility* (pp. 209-221).
- Kellert, S. (1991). Changes in deer management and public attitudes. In S. Lieberman (Ed.), *Current issues in deer management in urbanizing regions*. U.S. Humane Soc., Washington, D.C., Princeton Univ. Press, Princeton, N.J. In Press.
- Kerr, P.J., Jackson, R.J., Robinson, A.J., Swan, J., Silvers, L., French, N., Clarke, H., Hall, D.F. and Holland, M.K. (1999). Infertility in female rabbits (*Oryctolagus cuniculus*) alloimmunized with the rabbit zona pellucida protein ZPB either as a purified recombinant protein or expressed by recombinant myxoma virus. *Biology of Reproduction*, 61, 606-613.

- Killian, G.J. and Miller, L.A. (2000). Behavioral observations and physiological implications for white-tailed deer treated with two different immunocontraceptives. *Wildlife Damage Management, Internet Center for Wildlife Damage Management Conferences-Proceedings* (pp. 283-291).
- Kirkpatrick, J.F., Turner Jr., J.W., Liu, I.K.M., Fayrer-Hosken, R. and Rutberg, A.T. (1997). Case studies in wildlife immunocontraception: Wild and feral equids and white-tailed deer. *Reproduction, Fertility and Development, 9*, 105-110.
- Kirkpatrick, J.F. and Rutberg, A.T. (2001). Fertility control in animals, from mortality control to fertility control (chapter 12). In D. Salem and A. Rowan (Eds.), *The state of the animals* (pp. 183-198). Humane Society Press.
- Kirkpatrick, J.F. (2003). Elephant contraception: Looking beyond the pharmacology. In B. Colenbrander, J. De Gooijer, R. Paling, S.S. Stout, T. Stout and W.R. Allen (Eds.), *Managing African elephant populations: Act or let die? Proceedings of an Expert Consultation on the Control of Wild Elephant Populations* (pp. 43-44). Beekbergen, Netherlands.
- Lai, K.Y. (2004). Development of an immunocontraceptive vaccine for biocontrol of rats. PhD. Thesis, Universiti Putra Malaysia.
- Lloyd, M.L., Shellam, G.R., Papadimitriou, J.M. and Lawson, M.A. (2003). Immunocontraception is induced in BALB/c mice inoculated with murine cytomegalovirus expressing mouse zona pellucida 3. *Biology of Reproduction, 68*, 2024-2032.
- Lloyd, M.L., Nikolovski, S., Lawson, M.A. and Shellam, G.R. (2007). Innate antiviral resistance influences the efficacy of a recombinant murine cytomegalovirus immunocontraceptive vaccine. *Vaccine, 25*, 679-690.
- Lo, S.C., Lai, K.Y., Zeenathul, N.A. and Mohd. Azmi, M.L. (2006a). Retroviral-based approach for pest control. *9th A-IMBN Conference 16th MSMBB Scientific Meeting*. From genomics and proteomics to translational initiatives (pp. 49). Kuala Lumpur, Malaysia.
- Lo, S.C., Lai, K.Y., Zeenathul, N.A. and Mohd. Azmi, M.L. (2006b). An approach towards viral-vectored immunocontraception for rat models. *18th Veterinary Association in Malaysia Scientific Congress 2006*. Enhancing knowledge for advancement in animal health and production. (pp. 201-202). Kuala Lumpur, Malaysia.
- Lo, S.C., Zeenathul, N.A., Lai, K.Y., Sheikh Omar, A.R. and Mohd. Azmi, M.L. (2009). Production of recombinant rat ZP3 immunocontraceptive vaccine in yeast, *Pichia pastoris* for fertility control. *18th Scientific Meeting*. "Reflecting 25 years of molecular biology and biotechnology in Malaysia: from small steps to giant leap. (pp. 42). Kuala Lumpur, Malaysia.
- Locke, S.L., Cook, M.W., Harveson, L.A., Davis, D.S., Lopez, R.R., Silvy, N.J. and Fraker, M.A. (2007). Effectiveness of Spayvac® for reducing white-tailed deer fertility. *Journal of Wildlife Diseases, 43*(4), 726-730.
- Mackenzie, S.M., McLaughlin, E.A., Perkins, H.D., French, N., Sutherland, T., Jackson, R.J., Inglis, B., Muller, W.J., van Leeuwen, B.H., Robinson, A.J. and Kerr, P.J. (2006). Immunocontraceptive effects on female rabbits infected with recombinant myxoma virus expressing rabbit ZP2 or ZP3. *Biology of Reproduction, 74*, 511-521.
- Mahi-Brown, C.A., Yanagimachi, R., Hoffman, J.C. and Jr. Huang, T.F. (1985). Fertility control in the bitch by active immunization with porcine zonae pellucidae, use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. *Biology of Reproduction, 32*, 761-772.
- Mahi-Brown, C.A., Yanagimachi, R., Nelson, M.L., Yanagimachi, H. and Palumbo, N. (1988). Ovarian histopathology of bitches immunized with porcine zonae pellucidae. *American Journal of Reproductive Immunology, 18*(3), 94-103.
- Masson, E., Cliquet, F., Aubert, M., Barrat, J., Aubert, A., Artois, M. and Schumacher, C.L. (1996). Safety study of the SAG2 rabies virus mutant in several non-target species with a view to its future use for the immunization of foxes in Europe. *Vaccine, 14*(16), 1506-10.
- Matschke, G.H. (1977a). Microencapsulated diethylstilbestrol as an oral contraceptive in white-tailed deer. *Journal of Wildlife Management, 41*, 87-91.

- Matschke, G.H. (1977b). Fertility control in white-tailed deer by steroid implants. *Journal of Wildlife Management*, 41, 731-735.
- Matschke, G.H. (1980). Efficacy of steroid implants in preventing pregnancy in white-tailed deer. *Journal of Wildlife Management*, 44, 756-758.
- Millar, S.E., Chamow, S.M., Baur, A.W., Oliver, C., Robey, F. and Dean, J. (1989). Vaccination with a synthetic zona pellucida peptide produces long-term contraception in female mice. *Science*, 246, 935-938.
- Miller, L.A., Johns, B.E. and Killian, G.J. (1999). Long term effects of PZP immunization on reproduction in white-tailed deer. *Vaccine*, 18, 568-574.
- Miller, L.A. and Killian, G.J. (2002). In search of the active PZP epitope in white tailed deer immunocontraception. *Vaccine*, 20, 2735-2742.
- Naz, R.K., Gupta, S.K., Gupta, J.C., Vyas, H.K. and Talwar, G.P. (2005). Recent advances in contraceptive vaccine development. *Human Reproduction*, 20, 3271-3283.
- Oogjes, G. (1997). Ethical aspects and dilemmas of fertility control of unwanted wildlife: An animal welfarist's perspective. *Reproduction, Fertility and Development*, 9, 163-167.
- Osborn, J.L. (1982). Cytomegalovirus and other herpesviruses. In H.L. Foster, J. D. Small and J. G. Fox (Eds.), *The mouse in biomedical research*, 2, 267-92. Academic Press: New York.
- Paterson, M., Thillai Koothan, P., Morris, K.D., O'Byrne, K.T., Braude, P., Williams, A. and Aitken, R.K. (1992). Analysis of the contraceptive potential of antibodies against native and deglycosylated porcine ZP3 in vivo and in vitro. *Biology Reproduction*, 46, 523-534.
- Perdok, A.A., de Boer, W.F. and Stout, T.A.E. (2007). Prospects for managing African elephant population growth by immunocontraception: A review. *Pachyderm*, 42, 97-107.
- Porton, I.J. (2005). The ethics of wildlife contraception. In C.S. Asa and I.J. Porton (Eds.), *Wildlife contraception: Issues, methods, and applications*. Baltimore, Maryland: The Johns Hopkins University Press.
- Prasad, S.V., Mujtaba, S., Lee, V.H. and Dunbar, B.S. (1995). Immunogenicity enhancement of recombinant rabbit 55-kilodalton zona pellucida protein expressed using the baculovirus expression system. *Biology Reproduction*, 52, 1167-1178.
- Redhead, T.D. (1988). Prevention of plagues of house mice in rural Australia. In I. Prakash (Ed.), *Rodent pest management* (pp. 191-206). Boca Raton: CRC Press.
- Redwood, A.J., Messerle, M., Harvey, N.L., Hardy, C.M., Koszinowski, U.H., Lawson, M.A. and Shellam, G.R. (2005). Use of a murine cytomegalovirus K181-derived bacterial artificial chromosome as a vaccine vector for immunocontraception. *Journal of Virology*, 79, 2998-3008.
- Redwood, A.J., Harvey, N.L., Lloyd, M.L., Lawson, M.A., Hardy, C.M. and Shellam, G.R. (2007). Viral vectored immunocontraception: Screening of multiple fertility antigens using murine cytomegalovirus as a vaccine vector. *Vaccine*, 25, 698-708.
- Reubel, G.H., Pekin, J., Venables, D., Wright, J., Zabar, S., Leslie, K., Rothwell, T.L., Hinds, L.A. and Braid, A. (2001). Experimental infection of European red foxes (*Vulpes vulpes*) with canine herpesvirus. *Veterinary Microbiology*, 83, 217-233.
- Reubel, G.H., Beaton, S., Venables, D., Pekin, J., Wright, J., French, N. and Hardy, C.M. (2005). Experimental inoculation of European red foxes with recombinant vaccinia virus expressing zona pellucida C proteins. *Vaccine*, 23(35), 4417-4426.
- Rhim, S.H., Millar, S.E., Robey, F., Luo, A.M., Lou, Y.H., Yule, T., Allen, P., Dean, J. and Tung, K.S. (1992). Autoimmune disease of the ovary induced by a ZP3 peptide from the mouse zona pellucida. *Journal of Clinical Investigation*, 89, 28-35.
- Rodriguez, F., Zhang, J. and Whitton, J.L. (1997). DNA immunization: Ubiquitination of a viral protein enhances cytotoxic T-lymphocyte induction and antiviral protection but abrogates antibody induction. *Journal of Virology*, 71(11), 8497-503.

- Roughton, R.D. (1979). Effects of oral melengestrol acetate on reproduction in captive white-tailed deer. *Journal of Wildlife Management*, 43, 428-436.
- Rutberg, A.T. (2005). *Humane Wildlife Solutions: The Role of Immunocontraception*. Washington, DC: Humane Society Press.
- Sacco, A.G., Subramanian, M.G. and Yurewicz, E.C. (1981). Active immunization of mice with porcine zona pellucidae: Immune response and effect on fertility. *Journal of Experimental Zoology*, 218, 405-418.
- Sacco, A.G., Yurewicz, E.C., Subramanian, M.G., Lian, Y. and Dukelow, W.R. (1991). Immunological response and ovarian histology of squirrel monkeys (*Saimiri sciureus*) immunized with porcine zona pellucida ZP3 (Mr = 55,000) macromolecules. *American Journal of Primatology*, 24(1), 15-28.
- Shellam, G.R. (1994). The potential of murine cytomegalovirus as a viral vector for immunocontraception. *Reproduction, Fertility and Development*, 6, 401-409.
- Sinclair, A.R.E. (1997). Fertility control of mammal pests and the conservation of endangered marsupials. *Reproduction, Fertility and Development*, 9, 1-16.
- Singleton, G., Hinds, L., Leirs, H. and Zhang, Z. (Eds.). (1999). Ecologically Based Management of Rodent Pests, ACIAR Monograph No. 59.
- Singleton, G., Krebs, C.J., Davis, S., Chambers, L. and Brown, P. (2001). Reproductive changes in fluctuating house mouse populations southeastern Australia. *Proceedings of Royal Society London B*, 268, 1741-1748.
- Skinner, S.M., Mills, T., Kirchick, H.J. and Dunbar, B.S. (1984). Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. *Endocrinology*, 115(6), 2418-32.
- Skinner, S.M., Niu, E.M., Bundman, D.S., Lo, C. and Dunbar, B.S. (1987). Use of immunoaffinity purified antibodies to zona pellucida to compare alloimmunization of male and female rabbits. *Journal of Reproductive Immunology*, 12, 81-92.
- Smitha, L.M., Lloyd, M.L., Harvey, N.L., Redwood, A.J., Lawson, L.M. and Shellam, G.R. (2005). Species-specificity of a murine immunoconceptive utilizing murine cytomegalovirus as a gene delivery vector. *Vaccine*, 23, 2959-2969.
- Stoops, M.A., Liu, I.K.M., Shideler, S.E., Lasley, B.L., Fayrer-Hosken, R.A., Benirschke, K., Murata, K., van Leeuwen, E.M.G. and Anderson, G.B. (2006). Effect of porcine zona pellucida immunization on ovarian follicular development and endocrine function in domestic ewes (*Ovis aries*). *Reproduction, Fertility and Development*, 18, 667-76.
- Strive, T., Hardy, C.M., French, N., Wright, J.D., Nagaraja, N. and Reubel, G.H. (2006). Development of canine herpesvirus based antifertility vaccines for foxes using bacterial artificial chromosomes. *Vaccine*, 24, 980-988.
- Strive, T., Hardy, C.M., Wright, J. and Reubel, G.H. (2007). A virus vector based on Canine Herpesvirus for vaccine applications in canids. *Veterinary Microbiology*, 119, 173-183.
- Talwar, G.P. (1997). Vaccines for control of fertility and hormone-dependent cancers. *Immunology and Cell Biology*, 75, 184-189.
- Turner Jr., J.W., Liu, I.K.M. and Kirkpatrick, J.F. (1992). Remotely delivered immunocontraception in captive white tailed deer. *Journal of Wildlife Management*, 56(1), 154-157.
- Turner Jr., J.W., Liu, I.K.M. and Kirkpatrick, J.F. (1996). Effectiveness, reversibility and serum antibody titers associated with immunocontraception in white-tailed deer. *Journal of Wildlife Management*, 60, 45-51.
- Turner Jr., J.W., Liu, I.K.M., Rutberg, A.T. and Kirkpatrick, J.F. (1997). Immunocontraception limits foal production in free roaming feral horses in Nevada. *Journal of Wildlife Management*, 61, 873-880.
- Tuytens, F.A.M. and Macdonald, D.W. (1998). Fertility control: An option for non-lethal control of wild carnivores? *Animal Welfare*, 7, 339-364.
- Tyndale-Biscoe, C.H. (1994). Virus-vectored immunocontraception of feral mammals. *Reproduction, Fertility and Development*, 6, 281-287.

- van Aarde, R.J. and Jackson, T.P. (2007). Megaparks for metapopulations: Addressing the causes of locally high elephant numbers in southern Africa. *Biological Conservation*, 134(3), 289-297.
- Way, A.L., Miller, L.A., Dunbar, B.S., Hagen, D.R. and Killian, G.J. (1999). In vitro sperm-egg binding for use in evaluating efficacy of different zona pellucida (ZP) immunogens for contraception in white-tailed deer (*Odocoileus virginianus*). *Biology of Reproduction*, (Supplement 1) 60, 432.
- Wood, B.J. (1985). Biological control of vertebrates—a review and an assessment of prospects for Malaysia. *Journal of Plant Protection in the Tropics*, 2(2), 67-79.
- Wood, B.J. and Chung, G.F. (2003). A critical review of the development of rat control in Malaysian agriculture since the 1960s. *Crop Protection*, 22, 445-461.
- Wood, D.M., Liu, C. and Dunbar, B.S. (1981). Effect of alloimmunization and heteroimmunization with zonae pellucidae on fertility in rabbits. *Biology of Reproduction*, 25, 439-450.
- Yankauckas, M.A., Morrow, J.E., Parker, S.E., Abai, A., Rhodes, G.H., Dwarki, V.J. and Gromkowski, S.H. (1993). Long-term anti-nucleoprotein cellular and humoral immunity is induced by intramuscular injection of plasmid DNA containing NP gene. *DNA and Cell Biology*, 12, 771-776.
- Ylönen, H. (2001). Rodent plagues, immunocontraception and the mousepox virus. *Trends in Ecology and Evolution*, 16(8), 418-420.
- Zaman, V. and Colley, F.C. (1975). Light and electron microscopic observations of the life cycle of *Sarcocystis orientalis* sp. n. in the rat (*Rattus norvegicus*) and the Malaysian reticulated python (*Python reticulatus*). *Z. Parasitenk*, 47, 169-185.