Theory and practice of immunocontraception in wild mammals

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Abstract Immunocontraception has been proposed as a technique for managing wildlife populations in urban and suburban settings where traditional, lethal control methods may not be publicly acceptable. Immunocontraception uses an animal's own immune response to disrupt reproductive function. Proteins of eggs, sperm, fertilized eggs, and reproductive hormones have variously been proposed for use in developing a vaccine for fertility control. The most widely tested immunocontraceptive vaccine for wild species is based on developing antibodies to the zona pellucida (ZP), which surrounds the mammalian egg cell. This vaccine has successfully caused infertility in some individual animals, but requires multiple treatments. Enhancement of immune response and efficiency of vaccine delivery will be necessary before this type of management strategy can be applied to wildlife control at the population level. Contraceptive treatment may alter the health and behavior of wildlife populations and therefore must be monitored closely.

Key words contraceptives, ELISA, enzyme-linked immunoabsorbent assay, *Equus caballus*, immuno-contraceptives, immunosterilization, *Odocoileus virginianus*, population management

Increasing attention is being focused on fertility control as a possible technique for controlling wildlife populations in urban and suburban areas. In many of these areas, wildlife populations are becoming overabundant. However, in such settings, traditional hunting or lethal control programs may not be publicly acceptable. A demand has arisen for contraceptive management techniques (e.g., "Battling animal overpopulation—deer herds at heart of park conflict" *USA TODAY*, 25 Mar 1993), but fertility control is not a panacea for population management. First, effective contraception must be achieved, and delivery of the contraceptive must be practical and cost effective. There also are many animal health questions that must be resolved before the use of contraceptives for population control is considered (Nettles 1997). These concerns were also voiced in a proposed resolution on the use of fertility control in free-ranging wildlife by the Wildlife Disease Association (1994) and by the American Association of Wildlife Veterinarians (reviewed by Nettles 1997).

In 1991, Turner and Kirkpatrick reviewed the most recent developments in wildlife immunocontraception. Since that time, a number of advances have taken place in this area of research. It is timely now to present an updated review. Our objectives are to provide a brief review of the immunological theory and biology underlying immunocontraception. Many wildlife managers may not be interested in acquiring a thorough knowledge of immunology; however, there are many important details of reproductive immunology that are directly related to the practical treatment of wildlife species with immunocontraceptives. These details often are not of concern to reproductive specialists that work with either domestic species or human subjects. Therefore, we will review the relevant concepts in both immunology and wildlife management when considering immunology-based fertility control for wildlife management.

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Wildlife biologists in the future may become more involved in the use of contraceptives for population management than they have in the past (Garrott 1995, Warren 1995). Biologists should understand the immunocontraceptive techniques and the advantages or disadvantages of contraceptive fertility control in order to make informed recommendations for wildlife population management.

We review the concepts in reproductive biology and immunology necessary to understand how potential vaccines could cause infertility. Then, we review selected reproductive proteins that have been tested as immunocontraceptives in wildlife. This review is not designed to be exhaustive, but rather, to facilitate understanding of how immunocontraceptives have been applied to wildlife species. Many other proteins are being isolated and tested for use as immunocontraceptive vaccines, all of which rely on an animal's own immune system to disrupt reproductive function. Immunological concepts also are important for understanding vaccine delivery techniques. Finally, we consider selected topics in population health and behavior in regard to contraceptive management techniques.

A brief review of reproductive biology relative to immunocontraception

Many elements combine to bring about successful reproduction in mammals, including precise coordination of hormones, gamete production, fusion of oocyte (egg) with sperm, and maintenance of pregnancy. The hypothalamic–pituitary–gonadal axis controls sperm and egg production. Successful fertilization occurs when the sperm is deposited, travels up the female reproductive tract, and finally fuses with the egg (reviewed in Yanagimachi 1994).

The mammalian egg is surrounded by 3 layers: the vitelline membrane, the ZP (a matrix of protein with attached carbohydrate groups), and a cumulus cell layer (mass of loosely packed cells). Ejaculated sperm, coated with seminal proteins undergo many changes before passing through the layers surrounding the egg. Sperm membrane structure and protein composition change (i.e., capacitation) as they travel through the female's reproductive tract. Seminal proteins are removed as the sperm undergo capacitation. Capacitation prepares the sperm for the acrosome reaction and allows penetration of the cumulus cells (in those species where present). Sperm–egg recognition occurs at the ZP and is followed by the acrosome reaction. This reaction is necessary for sperm penetration through the ZP and then fusion with the egg plasma membrane (reviewed in Yanagimachi 1994).

A brief review of immunology relative to immunocontraception

B-cell versus T-cell immune responses

When a foreign protein (i.e., an antigen) enters the body of an animal, the immune system undergoes a complex series of events to protect the animal. The immune response consists of the humoral, or antibody response, and the cell-mediated response. These responses are carried out by lymphocytes. The 2 types of lymphocytes responsible for the division of labor are B cells and T cells. The B cells produce antibodies responsible for the humoral response. The T cells are either involved in target-cell death (cytotoxic T cells) or provide help for both humoral and cell-mediated immune responses (T helper cells). Activated T helper cells release factors that affect lymphocyte growth and differentiation. A subset of T helper cells is involved in delayed-type hypersensitivity reaction, which includes granulomatous inflammation.

Factors affecting the immune response from reproductive proteins

When the body encounters foreign pathogenic proteins (antigens), generally there is a response from the immune system. Most reproductive protein targets for immunocontraception are proteins normally encountered by the immune system; therefore, the immune response against these "self-antigens" may be weak (Alexander and Bialy 1994). The ZP is a normal protein component of the ovary, and therefore is a "self-antigen."

Sperm may introduce foreign proteins in the female. However, the system is adapted to allow sperm entry to the female reproductive tract for fertilization without generating an immune system attack.

Antibody response in the female genital tract may be under hormonal influence (Wira and Sandoe 1977, Murdoch et al. 1982, Wira and Sandoe 1987). A drop in immune response in the cervical mucus at the time of ovulation probably improves conditions for sperm penetration. Additionally, Parr and Parr (1990) found reduced antigen uptake in the vagina at the time of estrus. Reduced immune response in the reproductive tract at ovulation may be a mechanism for ensuring successful fertilization.

Similarly sperm may have evolved mechanisms to reduce their immunogenicity in the female reproduc-

tive tract. Seminal plasma provides most of the volume of a normal ejaculate and is a complex mixture of secretions from the male reproductive tract. Seminal plasma contains factors that suppress immune function (reviewed in James and Skibinski 1995). Also, sperm do not express major histocampatibility complex (MHC) class I molecules (Guillaudeux et al. 1996) which may be a factor in reduced female immune response to sperm.

Modifications of the antigen can be used to overcome the normally weak immune response expected from reproductive proteins (Skinner et al. 1994). When ZP from a different species is injected into the female reproductive tract, it may elicit a stronger immune response than ZP from the same species. Most of the initial work with ZP vaccines has been done with protein isolated from porcine ovaries. Porcine ZP (PZP) has been successful in stimulating the immune response in other species (such as horses and deer), by means of epitopes or antigenic determinants, sites on the antigen that interact with antibodies or T-cell receptors. However, an immune response to PZP also produces antibodies that are directed against common epitopes shared among different species (Skinner et al. 1994). Many of these common epitopes are involved in fertilization or egg cell and follicular development (reviewed in Skinner et al. 1994). Anti-PZP antibodies have been shown to react with the ZP isolated from white-tailed deer (*Odocoileus virginianus*; Miller et al. 1992*a*, Skinner et al. 1994) and horses (*Equus caballus*; Miller et al. 1992*b*).

Immune response of antigens depends on other factors that may be manipulated. Amino acid composition, protein structure, and carbohydrate side chains of target proteins may affect these proteins' ability to stimulate the immune system (Dunbar et al. 1994). Molecular biology techniques have been used to express the complementary DNA (cDNA) of mouse, hamster, and rabbit ZP proteins in various cell lines (Dunbar et al. 1994). In this process DNA is inserted into a foreign cell, which then manufactures the new protein. The protein can be more efficiently produced in large quantities by this process as compared to lengthy purification procedures for ZP isolation from whole ovaries. Although, the protein expressed by the foreign cells duplicate the composition exactly, it may not include modifications found in the naturally produced proteins (Dunbar et al. 1994). These modifications include glycosylation of ZP (addition of carbohydrate groups to the protein), which may be important for optimum immune response (Dunbar et al. 1994, Prasad et al. 1995).

An adjuvant is a substance that enhances the specific immune reaction to an antigen. In early work with remote delivery of ZP vaccines, researchers used Freund's Complete Adjuvant (FCA). This adjuvant is very potent, but often causes severe reactions including granuloma formation and ulceration of overlying skin (Anderson and Alexander 1983, Stills and Bailey 1991). Many synthetic adjuvants are being developed to reduce these side-effects, while promoting an immunological response (Sager 1992).

Quantifying antibody production after immunocontraceptive treatment

A quantitative measure of the humoral immune response (antibody production) is often determined with an enzyme-linked immunosorbent assay (ELISA). This assay measures antibody titers. Several studies evaluating potential wildlife contraceptives have documented the relationship between systemic antibody titer and infertility (Liu et al. 1989, Willis et al. 1994). Although there was apparent individual variation in immune response, mares with higher antibody titers did not conceive (Liu et al. 1989, Willis et al. 1994). As antibody titers of mares treated with PZP declined, fertility resumed (Liu et al. 1989).

Preferentially targeting the cell-mediated immune response with immunocontraceptives

Cell-mediated responses may be stimulated by some epitopes of gamete proteins. Gamete cell destruction may be a target for irreversible contraception or sterilization. This may be especially appropriate in situations where it is infeasible to repeatedly treat free-ranging wildlife with a contraceptive agent. Matschke (1980) argued that efficient and practical management of deer populations in the absence of regulated hunting required a contraceptive capable of lasting the reproductive life of an animal. The preferred mode of action in that case may be cell-mediated tissue destruction causing sterility.

Yewdell and Bennink (1993) reviewed potential antigen processing as a means of specifically targeting humoral or cell-mediated responses. This differential direction of response has been demonstrated with various ZP epitopes (Millar et al. 1989, Tung et al. 1994). Epifano and Dean (1994) reported 1 out of 5 different ZP peptides tested caused oophoritis (a destructive inflammatory disease of the ovary) in an inbred strain of mice. This peptide was 16 amino acids long and caused a cell-mediated response in the mice. Rhim et al. (1992) found a 15-amino-acid ZP peptide caused oophoritis in some but not all strains of mice. In their study, oophoritis probably was mediated via T cells. Upadhyay et al. (1989) found that the adjuvant used with the ZP antigen could have an

effect on how infertility was associated with ovarian morphological changes.

Ovarian dysfunction was demonstrated in dogs treated with a ZP vaccine (Mahi–Brown et al. 1988). However, in this study, ovarian histopathology (defective follicular development and cyst formation) apparently occurred, but a cell-mediated response was not detected. Selection of ZP epitopes that cause tissue destruction or dysfunction may provide a better option for wildlife fertility control than a humoral response (antibody production) that only temporarily interferes with ovarian function.

Peripheral, systemic versus mucosal immune system and the reproductive tract

The immune system can be divided into the peripheral, systemic and separate, mucosal immune system. The lymphoid tissues of the systemic immune system are responsible for antibody types found in the blood circulation. When an antigen challenges the immune system either through intramuscular (IM), intraperitoneal (IP), or intravenous (IV) entry, then the systemic immune system becomes involved in neutralization of the antigen. There is an initial lag in response, followed by production of antibodies by activated B cells. If the system is challenged later with the same antigen, then there is a quicker response. The booster response generally has greater magnitude and affinity for the antigen than the initial response. The antibodies usually are of the immunoglobulin G (IgG) isotype. There are several isotypes or classes of immunoglobulins; each has different molecular weights and biological properties.

The mucosal immune system functions separately as a distinct component of the animal's immune system. The mucosal immune system communicates throughout the body and includes tissues with secretions in the gut; respiratory tract; mammary, salivary and lacrimal glands; as well as the reproductive tract (Kutteh et al. 1988, McGhee et al. 1994). Antibodies found in external secretions from these mucosal immune sites are usually of the IgA isotype (Kutteh et al. 1988, Kagnoff 1993, McGhee et al. 1994). Kutteh et al. (1988) demonstrated that IgA was produced by tissues of the female reproductive tract. Therefore, the mucosal immune system can profoundly affect the function of immunologically based fertility control.

In the mucosal immune system there are discrete, inductive sites where antigen uptake and processing occur (Kagnoff 1993, McGhee et al. 1994). These inductive sites include gut-associated lymphoreticular tissues of the Peyer's patches (PP). Bradley (1994) in-

jected female red foxes (*Vulpes vulpes*) with whole sperm directly into the PP. These females produced anti-sperm antibodies in their sera and vaginal secretions. The anti-sperm antibodies were apparently responsible for embryonic failure.

In other studies, IM injection of sperm plasma membranes did not affect fertility (Willis 1993, White 1995). In these studies the animals developed high titers of anti-sperm antibodies in their sera (antibody titers in the female reproductive tracts were not measured). It is possible that the lack of contraception was due to an immune response to sperm proteins not necessary for fertilization. Alternatively, infertility may have resulted from an inadequate mucosal immune response in the genital tract. Alexander et al. (1992) found uterine wash IgA and IgG concentrations resulting from an injected protein did not mirror serum levels. It is likely the female reproductive tract is protected by both mucosal and systemic immunity (McGhee et al. 1994). However, a stronger antibody response in the reproductive tract may be necessary to neutralize the large number of sperm (Alexander et al. 1992).

Generally only 1 to a few eggs are ovulated per cycle; therefore, only a small number of antibodies may be needed to interfere with ZP function compared to the larger antibody concentrations necessary to neutralize great numbers of sperm (Dunbar et al. 1994). The ZP is exposed to antibodies in the follicular fluid and therefore only a minimal mucosal immune response in the genital tract may be necessary for infertility. Studies that demonstrated infertility using PZP vaccine in horses reported systemic anti-PZP antibodies (Liu et al. 1989, Willis et al. 1994). These investigators did not know what the antibody titers were in the genital tract. However, there must have been sufficient antibody titers around the ovulated eggs to alter ZP function and cause infertility.

A review of potential immunocontraceptive protein targets

Ovarian protein targets

The immunocontraceptive that has been most widely tested in wild species is based on developing antibodies to the ZP. Antibodies to ZP may reduce fertility by inhibiting sperm binding with, or penetration of, the egg (reviewed in Skinner et al. 1990). Additionally, ovarian function may be disrupted by ZP antibodies (Skinner et al. 1984; Fig. 1).

Porcine ZP antigen has been used successfully as an immunocontraceptive in penned white-tailed deer
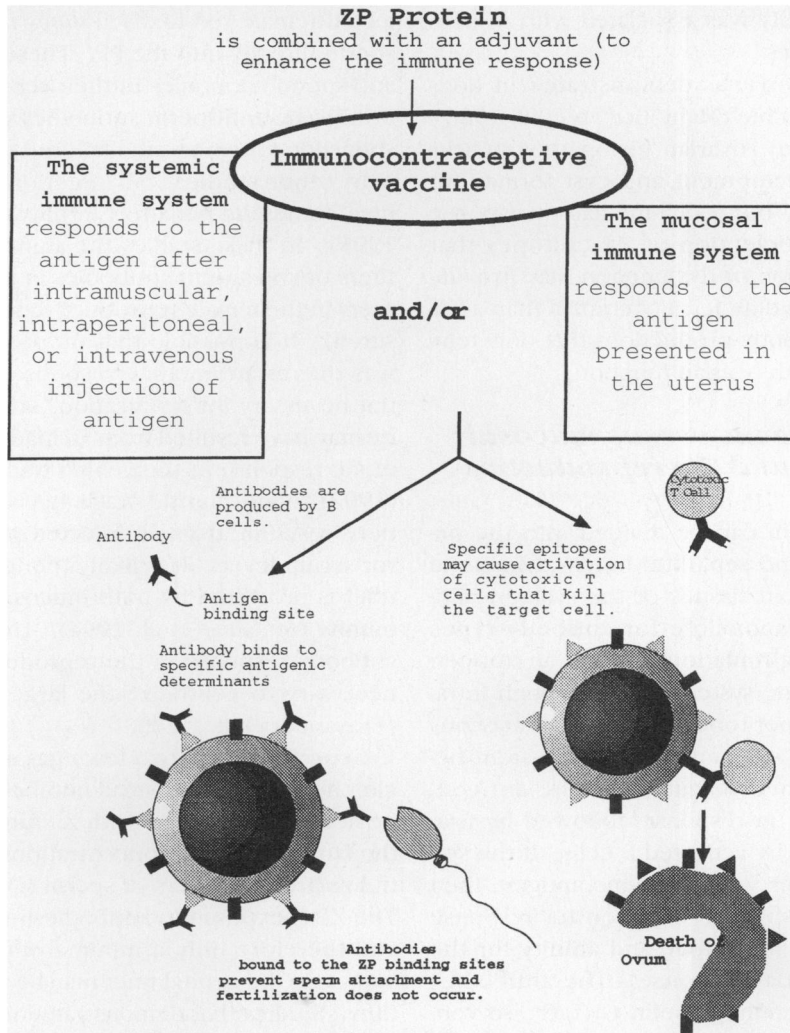
Fig. 1. Immunocontraception using an immune response to zona pellucida (ZP). The ZP is a glycoprotein matrix surrounding the mammalian egg cell. The ZP is isolated, combined with an adjuvant, and injected into an animal. This animal produces an immune response to the ZP antigen in the systemic and mucosal immune system. The immune response to the ZP antigen can inhibit sperm-egg binding or disrupt ovarian function.

(Turner et al. 1992). In this study, deer were housed in an 0.5-ha enclosure and treated with PZP vaccine (64.3 µg protein in phosphate buffer) and FCA adjuvant to boost the immune response. The vaccine was administered through the fence with a blow pipe. The deer were vaccinated initially in October and given booster inoculations 3 and 6 weeks later. None of the PZP-treated does produced fawns; however, the necessity of 3 vaccinations in the first year with this method makes it infeasible for treating wild deer populations.

Alternative treatment regimes of PZP have been evaluated (White 1995, Turner et al. 1996). Turner et al. (1996) tested single-injections of PZP using implanted osmotic mini-pumps or microspheres containing PZP. Only 2 of 7 does treated with PZP mi-

crospheres, and none of 3 treated with osmotic mini-pumps, produced fawns. However, some of the infertile PZP-treated does continued breeding into February and March. Control does were not observed breeding after January. Extension of the normal breeding season with contraceptive treatment is an important consideration in this method of population management.

Use of PZP also has been evaluated in horses (Liu et al. 1989; Kirkpatrick et al. 1990, 1991, 1992; Willis et al. 1994). Liu et al. (1989) treated mares with 4 inoculations of PZP. This treatment was effective for contraception and lasted ≥7 months. Using darts for remote delivery of PZP to horses, Kirkpatrick et al. (1990) demonstrated effective fertility control with 2–3 inoculations. After antibody titers in the horses were raised

with the multiple vaccinations, Kirkpatrick et al. (1991) demonstrated that titers could be boosted with an annual inoculation. Kirkpatrick et al. (1992) also found that PZP treatment for 3 years caused altered ovarian function in some mares. Further attempts to make PZP treatment more efficient for horses were evaluated by Willis et al. (1994). In this study, 2 treatments of a higher dose of PZP (remotely delivered with a biobullet) resulted in contraception for 2 breeding seasons in 2 mares. A third mare was given a third treatment after which she became infertile.

Sperm protein targets

Sperm proteins also have received attention as possible protein targets for immunocontraception. Sperm proteins recently have been tested in white-tailed deer (White 1995). This study did not demonstrate effective contraception using whole-sperm plasma-membrane proteins. However, Bradley (1994) showed apparent embryonic failure in red fox females vaccinated with whole sperm. Future research by Bradley (1994) will evaluate specific protein components of fox sperm for potential immunocontraceptives. Several other different spermatozoa antigens also have been considered for use as immunocontraceptives (Anderson et al. 1987, Primakoff et al. 1988, Herr et al. 1990, Naz and Menge 1990, Isahakia and Bambra 1992, O'Hern et al. 1995).

Anti-sperm vaccination may cause infertility in the male or female. In the male, anti-sperm antibodies may cause an autoimmune response (recognition of self proteins as foreign) to the sperm, thus resulting in infertility (Mathur et al. 1988). However, sperm antigens rarely cause an immune response in the male because the blood–testes barrier causes isolation of the testes from the immune system (reviewed in Anderson and Alexander 1983). Naz and Bhargava (1990) found that antibodies to fertilization antigen-1 (FA-1) were directed to the late stages of spermatogenesis in the male. The antibodies gained access to the sperm in the epididymis and vas deferens. These anti-FA-1 antibodies did not react with somatic tissues, such as muscle, heart, blood cell, liver, and kidney.

In the female, anti-sperm antibodies may cause clumping of sperm (reviewed in Shulman 1986), or reduced penetration of sperm through the cervical mucus (Clarke 1988). Sperm proteins also may be important for penetration of the cumulus-cell layer around the egg (Lin et al. 1994); antibodies that interfere with these proteins may prevent sperm from reaching the egg. Anti-sperm antibodies also may prevent sperm attachment and penetration (Gocial et al. 1988) or alter sperm binding to the ZP (Bronson et al. 1982, Coddington et al. 1992, Naz et al. 1992).

Anti-sperm antibodies may be involved in postfertilization loss of the embryo (Menge and Naz 1988, Bradley 1994). This may indicate that some sperm-surface antigens become incorporated into embryos and are involved in normal embryonic development (Menge and Naz 1988). Anti-sperm antibodies may disrupt embryo development resulting in postfertilization reproductive failure (Menge and Naz 1988).

When determining potential targets for sperm-based immunocontraception in the female, the target protein must be accessible to the anti-sperm antibodies (i.e., ejaculated vs. capacitated sperm proteins). Okabe et al. (1986) demonstrated an inconsistent response of an anti-sperm monoclonal antibody binding to sperm. A monoclonal antibody is a product of a cloned hybridoma cell (derived from biotechnological fusion of an antibody-producing white blood cell with a malignant tumor cell) that is specific for only 1 part of the antigenic protein. The anti-sperm antibody appeared to react with this antigenic component that was only available on the surface of the sperm after capacitation.

A successful sperm-based contraceptive vaccine must be directed against sperm antigens that target specific tissues, affect fertilization, and raise antibody titers in genital tracts (Naz and Menge 1990). In the future, there may be more effective target proteins identified as researchers refine and address these specific concerns for a successful sperm-based immunocontraceptive.

Other reproductive proteins

Other potential protein targets for immunocontraception interfere with the reproductive endocrine system. Disruption of the hypothalamic–pituitary–gonadal axis can occur when antibodies to gonadotrophin-releasing hormone (GnRH; released from the hypothalamus) or follicle-stimulating hormone (FSH; released from the pituitary) are produced (reviewed in Alexander and Bialy 1994). An anti-LHRH (type of GnRH) vaccine was used unsuccessfully in feral horses (Goodloe 1991). Other studies have achieved infertility with this type of approach (Fraser 1983), but there is a potential for altered health and behavior with immunocontraceptives that cause endocrine dysfunction.

Proteins involved in maintenance of pregnancy also have been considered for immunocontraception. Antibodies to β-human chorionic gonadotrophin (βhCG) have been targeted (reviewed in Alexander and Bialy 1994). Additional proteins associated with pregnancy may provide immunocontraception. All of these methods would interfere with pregnancy and cause abortion in the treated animal.

A review of delivery techniques for immunologically based fertility control

Intramuscular vaccine injection by remote delivery

Vaccine administration is also an important consideration for immunocontraceptive management of wildlife. Many techniques have been developed and already evaluated for administration of vaccines in wild populations. Remote delivery is a more efficient method for delivering contraceptive vaccines than methods requiring trapping and immobilization of animals. Porcine ZP antigen has been used successfully as a remotely delivered immunocontraceptive in free-ranging white-tailed deer (Turner et al. 1992) and horses (Kirkpatrick et al. 1990, 1991, 1992). In these applications, dart rifles, FCA, and multiple booster injections were used, all of which are limiting when applied to free-ranging populations. Darts are difficult to deliver and unretrieved darts may pose a threat as an environmental hazard.

A remote biobullet delivery system has been developed (DeNicola et al. 1996). This system uses an air gun to project a 0.25-caliber biodegradable bullet filled with vaccine. The bullets are accurate for ≤25 m, and lost bullets degrade quickly in the environment. Biobullets have been used to remotely treat free-ranging feral horses with an anti-LHRH vaccine (Goodloe 1991), as well as captive horses (Willis et al. 1994) and white-tailed deer (White 1995) with anti-PZP vaccine.

The use of darts or biobullets to remotely inject a vaccine IM is difficult and may require multiple boosters. Booster vaccines often are necessary to maintain the immune response at a sufficient level to prevent fertility. The use of microspheres containing the antigen and adjuvant are being evaluated as a replacement for multiple boosters (Turner et al. 1996). The microspheres contain inert polymers that allow for slow, controlled release of antigen (Shalaby 1995). Presumably these microspheres could be delivered remotely by dart or biobullet to provide a "1-shot" vaccine. In addition to controlling the rate of antigen release, microspheres may function as an adjuvant (Eldridge et al. 1993).

Some species may be more suited to IM remote delivery of vaccines than others. For example, forest dwelling white-tailed deer would be more difficult to dart than relatively tame horses in a field. Garrott et al. (1992) found that certain situations affected the ease of darting. Darting semi-tame feral horses on is-land settings was cheaper and more efficient than darting wary horses on western rangelands.

Oral delivery of immunocontraceptives

Another proposed method for immunocontraceptive vaccine delivery is through oral dosing. Antigen uptake and processing following oral immunocontraceptive vaccine delivery can target discrete inductive sites of the gut and generate a mucosal immune response (Kagnoff 1993). This response is reflected in the reproductive tract because there appears to be a common mucosal immune system (Kagnoff 1993, McGhee et al. 1994).

Enzymatic degradation and conformation changes of proteins in the gut present obstacles to effective oral delivery of vaccines (Shalaby 1995). Combining the antigen with polymers in microspheres may protect the protein from enzymatic digestion. Also, the vaccine could be administered using recombinant-DNA technology. Viruses or bacterial genes could be altered to express specific immunocontraceptive protein targets as live vectors (Tyndale–Biscoe 1994, Srinivasan et al. 1995). Some viruses and bacteria have great affinity for PP (Shalaby 1995). These microspheres and live vectors would escape degradation in the gut and possibly promote preferential mucosal-immune responses (Hruby 1993, Bradley 1994).

Microspheres may be safer for oral vaccine delivery than using a live vector because of the risks associated with releasing living, replicating organisms that promote infertility (Tyndale–Biscoe 1994). Researchers are aware of the risks associated with these live vectors and are targeting species-specific vectors. The myxoma virus appears to only affect rabbits and therefore is being examined for potential as a recombinant carrier for immunocontraception (reviewed in Tyndale–Biscoe 1994, Robinson et al. 1997). Perhaps an ideal vector to maintain species specificity would be based on a sexually transmitted herpes-type vector (Barlow 1994).

Research has shown that the strongest mucosal immune response may be in response to an initial priming of the systemic immune system through IM or IP injection, followed by mucosal booster (Pierce and Gowans 1975, Alexander et al. 1992, Eldridge et al. 1993). Pierce and Gowans (1975) used an initial IP injection with either an oral or intraintestinal booster to generate a high IgA response. If the booster was delivered IP, then there was only a weak mucosal response.

Immunocontraceptives administered initially by remotely delivered IM injection (e.g., via biobullet) followed by oral boosters (microencapsulated so that the antigen is exposed to the mucosal immune sys-

tem in the intestines) may provide an effective and easily administered method for some wildlife populations. Remotely treating wild animals with contraceptives is labor intensive. However, biobullet delivery of booster injections would be even more time and cost prohibitive compared to oral boosters. Oral administration of these boosters would probably be more efficient. Furthermore, nontarget species would not be affected by eating the oral bait because they would not have been exposed to the initial biobullet (i.e., IM) treatment. Administration of a single oral vaccination to nontarget species should be ineffective in generating a mucosal immune response (Pierce and Gowans 1975, Parr et al. 1988).

Use of biological markers to monitor immunocontraceptive treatment

The efficiency of contraceptive treatments will need to be monitored very closely. Biomarkers may be used to evaluate the affects of contraception on population growth. Also biomarkers may be useful to evaluate exposure of nontarget animals to vaccine treatments. Savarie et al. (1992) reviewed biomarkers used to monitor animal movements and to determine bait acceptance by target and nontarget animals. Tetracycline, rhodamine B, and iophenoxic acid have been evaluated as biomarkers for potential oral vaccine programs (Fletcher et al. 1990, Hable et al. 1992)

A brief review of population health and behavior concerns relative to immunocontraception

There may be broad ecological effects where wildlife contraception is used as a management tool. It has been proposed that only those animals with the "best" immune response to an immunocontraceptive would become infertile (Mitchison 1995, Nettles 1997). Those animals with poor immune responses would be affected less. Thereby, immunocontraception could artificially select for those individuals that were immunodeficient and therefore more susceptible to pathogenic organisms. This concern warrants close attention; there is evidence that vaccine-induced antibody responses may be controlled genetically (Newman et al. 1996). However, not all individuals respond equally to all antigenic determinants. Rhim et al. (1992) found that only certain strains of mice were susceptible to oophoritis from injection of a 15-amino-acid ZP peptide combined with adjuvant. The strains of mice that did not respond to the ZP peptide were not immunocompromised. If an animal

responded weakly to all antigenic challenges, then it probably would have an increased risk of mortality from environmental pathogens and also would be selected against.

Other population-health considerations relate to altered reproductive behavior and population dynamics. Health and fitness of a population may be affected if all females continue estrous cycling beyond the normal breeding season. This problem may have serious implications for a seasonally breeding species such as white-tailed deer in which does may continue estrous cycling for ≤ 7 months (Knox et al. 1988). Rutting bucks reduce food intake, resulting in significant body weight losses (Warren et al. 1981), during the breeding season. Bucks may continue breeding infertile females beyond the normal breeding season, and thereby expend more of their body reserves. Therefore, extending the rutting period by treating a population with contraceptives could increase over-winter mortality.

Increased over-winter mortality of bucks resulting from immunocontraceptive treatment of does in a population should be closely monitored. However, a study by McShea et al. (1997) indicated that buck behavior may shift during the breeding season. In a population in which ZP-treated does continued cycling beyond the usual breeding season, the researchers found that larger bucks ceased breeding as the winter progressed. The ZP-treated does that continued cycling until March were bred by smaller bucks. Long-term effects of contraception on deer social behavior are not known.

In addition to potential effects of contraceptives on male breeding physiology, there may be important changes in female reproductive behavior. Knox et al. (1988) reported that captive, unbred, female white-tailed deer exhibited 2–7 recurrent estrous cycles. The last estrus was detected on 7 April 1986. At the same facility in 1990–1991, White et al. (1995) evaluated the potential effects of breeding stimulus on estrus duration. They monitored recurrent estrous cycles of 9 does. Each estrous cycle was assigned randomly to: (1) no stimulus, (2) copulation with a vasectomized male, or (3) injection of gonadotrophin releasing hormone (GnRH). The does underwent 3–9 estrous cycles with the last estrus detected on 6 June 1992. Although there were many confounding factors involved, the stimulus of breeding without conception may have lengthened the breeding season. This potential extension of recurrent estrous cycles from a repeated breeding stimulus (as would occur with ZP immunocontraception) should be evaluated carefully. Unpredictable health effects could result if a population treated with contraceptives became aseasonal in its reproductive chronology.

Conclusions

Immunological methods of contraception or sterilization show promise for effective and efficient fertility control of individual animals. However, many factors must be considered before implementing this type of control on wild animal populations. Knowledge of immune function may aid in an effective choice of antigen and delivery method. Such knowledge should be combined with an understanding of the normal behavior and the reproductive biology of an animal. With these tools, population-management alternatives may be evaluated where traditional methods are not possible.

Further research should concentrate on the applicability of immunocontraceptive techniques to wild animal populations. Successful immunocontraceptives are available and new ones are likely to be developed soon. Success of wildlife contraceptives at the individual-animal level may not translate into effective fertility control at the population level (Warren 1995). Delivery techniques must become more practical and efficient to treat populations. Targeting both the systemic and mucosal immune system appears to be a promising method for efficient vaccine delivery. Systemic priming with an immunocontraceptive vaccine delivered IM followed by oral boosters needs further evaluation.

Irreversible immunological fertility control or immunosterility also deserves further attention. Immunosterility may be achieved by means of specific epitopes that stimulate cell-mediated or delayed-type hypersensitivity reactions resulting in gamete dysfunction. Immunosterility is not being examined for human immunocontraception because for a successful protein target in humans, reversibility is considered a necessary prerequisite. Immunosterility may facilitate contraceptive treatment of wildlife at the population level and lead to more effective wildlife management.

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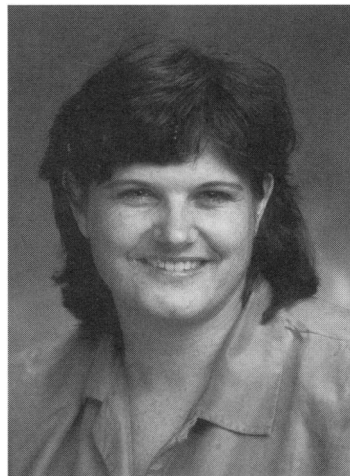
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