

Humane Wildlife Solutions

**T H E R O L E O F
I M M U N O C O N T R A C E P T I O N**

Edited by **Allen T. Rutberg**

Humane Society Press

an affiliate of

**THE HUMANE SOCIETY
OF THE UNITED STATES**

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First edition
ISBN 978-0-9748400-3-1

Library of Congress Cataloging-in-Publication Data

Humane wildlife solutions : the role of immunocontraception /
edited by Allen T. Rutberg.

p. cm.

Includes bibliographical references and index.

ISBN 0-9748400-3-3

1. Animal contraception. 2. Contraception, Immunological. I.
Rutberg, Allen Theodore.

SK356.C65H86 2005

639.9'3--dc22

2005002810

Printed in the United States of America



Humane Society Press
An affiliate of The Humane Society of the United States
2100 L Street, NW
Washington, DC 20037

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Foreword

This slim publication reviews some of the considerable progress to date in the field of immunocontraception as a technique to control wildlife fertility and wildlife population growth. While there has been considerable basic research done on immunocontraception using Porcine Zona Pellucida protein (PZP), *Humane Wildlife Solutions: The Role of Immunocontraception* focuses more on the application of the PZP vaccine in the field and its practical impact. The authors of several chapters show that PZP can be effectively applied in field situations to control the population of free-living groups of wild horses, white-tailed deer, and African elephants. In the case of elephants, the field trial is only now, in 2005, at the stage where we can begin to state with confidence that the vaccine controls reproduction while producing few, if any, side effects. Yet, all promise is not borne out in reality: one author describes an inconclusive attempt to use the PZP vaccine to control canine reproduction.

Although *Humane Wildlife Solutions* provides the scientific and technical details of the success of the PZP vaccine, I would like to acknowledge the various individuals who helped to get us to this stage of PZP vaccine development.

Without question, first among these are Jay Kirkpatrick, Ph.D., and John Grandy, Ph.D.. Together with his long-time friend and colleague, John W. Turner, Jr., Ph.D., Jay has been an unflagging promoter of the PZP vaccine. While he would no doubt want to deflect credit to his own mentors and colleagues, for us at The Humane Society of the United States (HSUS), Jay's enthusiasm, meticulous record-keeping, academic integrity and scientific excellence, and willingness to go out into the field to show how the vaccine could be humanely and remotely delivered to the target populations have been the key factors behind the success of the PZP vaccine. However, Jay

needed resources to accomplish what he did: John Grandy, very early on, saw the potential of immunocontraception for humane wildlife population control and argued long and hard that The HSUS should back the technique. He eventually won over HSUS President Emeritus Paul G. Irwin and, as a result, The HSUS has put many millions of dollars over the past fifteen years into proving that the PZP vaccine can work.

Neither Jay nor John would have had the opportunity to pursue the practical application of the PZP vaccine without the help and support of others. Among them are National Park Service (NPS) employees Bruce Rogers, resource management specialist, Assateague Island National Seashore (ASIS, in NPS parlance); Roger Rector (superintendent, ASIS); Larry Points, chief interpretive officer, ASIS; John Karish, Ph.D., chief scientist, NPS Mid-Atlantic Region; and Allison Turner, biological technician, ASIS. Rogers, Rector, Points, and Karish opened the door to wildlife contraception by courageously pushing ahead with an unproved technique in America's most celebrated and protected herd of wild horses. They were risk takers who placed the welfare of the horses before their own career interests. Rogers oversaw the first applications of the vaccine. Rector maintained solid administrative support until we achieved success. Points helped to present the program to the public and pre-empted any problems from that quarter, while Karish supported the project within the NPS Mid-Atlantic Region. Last, but by no means least, much of the success that has been achieved on Assateague in controlling the wild horse population can be ascribed to the dedication, skill, patience, and concern for the animals demonstrated by Allison Turner.

In the West, there was initially relatively little enthusiasm for wild horse contraception. In recent years however, both Linda Coates-Markle, Ph.D., wild horse specialist, Montana-Dakota Region, Bureau of Land Management (BLM), and Ron Hall, program director, BLM National Wild Horse Program, Reno, saw the promise of the technique and helped to open the door, despite significant cultural and political opposition, to large-scale wild horse PZP vaccine trials in the region. In fact, the door has now been jammed wide open by the BLM! In a March 17, 2004, memo, the BLM policy on wild horse gathers was changed. It now states

Fertility control must be considered as an alternative in all Wild Horse Gather Plans/NEPA documents. If the Proposed Action does not include the use of fertility control, rationale should be presented as to why it will not be used. If fertility control is a component of the Proposed Action, EA documents will be submitted to the National Program Office (NPO), Attn.: Ron Hall, for review at least sixty days prior to the planned gather date.

The PZP vaccine is now also an important component of fertility control in zoos, thanks to the efforts and endorsement of Wilbur Amand, V.M.D., executive director of the American Association of Zoo Veterinarians. When others were skeptical, he saw the value of immunocontraception and helped get the technique accepted by some “early adopters” in the zoological park community.

Deer fertility control is probably the most politically contentious area in the field of immunocontraception. State fish and game agencies are very suspicious of any wildlife contraceptive, and at least one pro-hunting organization is attempting to pass laws that would prohibit wildlife contraception. Several individuals deserve special recognition for their contributions in developing and promoting deer immunocontraception. The HSUS team of Allen T. Rutberg, Ph.D., and Rick Naugle have helped plan and implement field trials of the PZP vaccine in two significant suburban deer populations, one of them on the Fire Island National Seashore (FIIS, in NPS parlance). Maria and Sedat Beqaj, two local residents, were the driving force behind the launch of the FIIS project, while Mary Foley, Ph.D., of the NPS (New England Region) helped to overcome internal NPS opposition to the project. Without their activism and support, the FIIS project would most likely have become so mired in New York State fish and game politics that it would never have got off the ground. A parallel role was played at the National Institute of Standards and Technology (NIST) by Guy Chamberlin, Jr., Walter Rabbitt, John Kennedy, Susan Carscadden, and Rhonda Hurt. Only their faith and persistence allowed the NIST study to take wing and soar, despite repeated volleys from the Maryland Department of National Resources and its allies in the Congressional Sportsmen’s Caucus. Finally, one must also mention Priscilla Cohn, Ph.D., of Pennsylvania. She has been a tireless advocate for immunocontraception as a solution to suburban deer population growth. In 1987 she started the international wildlife contraception symposia and helped focus resources and brain power on this field.

Immunocontraception of elephants is relatively new, but 2004–2005 has seen significant movement towards a broader acceptance of the technique for elephant population control as an alternative to culling. There is still some question whether the technique can be applied successfully to large elephant populations (such as the 12,000 strong herd in the Kruger Park in South Africa) but the work of Prof. Henk Bertschinger, University of Pretoria, who has established the first PZP vaccine preparation laboratory in Africa, and Douw Grobler and J.J. van Altena, who played and continue to play an important role in the field trials of the vaccine in Kruger Park and in the neighboring Makalali Conservancy, has helped to demonstrate that the application of the technique is really only limited by the imagination of those who would use it.

There are many others whose roles and influence could also be mentioned here. People such as Bill Lasley, Ph.D., and Susan Shideler, Ph.D., of the University of California, Davis, developed the tools of urinary and fecal hormone metabolite analysis and also helped to influence Jay Kirkpatrick on the road that led to his work on PZP. Bonnie Dunbar, Ph.D., developed techniques to extract and purify PZP that influenced the extraction techniques that Jay now uses in his laboratory.

Because of them and many others, we now stand at the brink of a revolution in wildlife management, where we no longer need to focus on lethal techniques to address situations in which humans and wildlife come into conflict.

Andrew N. Rowan, Ph.D.
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Editor's Note

This book elaborates on the proceedings of a workshop organized by Andrew Rowan, Ph.D., executive vice president, Operations, of The Humane Society of the United States, held in Washington, D.C., June 21–23, 2003.

Although the workshop participants were limited in number, the scope and depth of the research presented was impressive. Many “firsts” were described here: the first dart delivery of immunocontraceptives to wildlife in the field; the first successful attempts to control non-captive wildlife populations with immunocontraceptives (indeed, any kind of contraceptives); the first delivery of immunocontraceptives to elephants. In addition, participants presented data from the most productive, longest-running study of immunocontraception in any wildlife species, the Assateague Island National Seashore wild horse study.

Also evident, and impressive, were the blood, sweat, and tears expended in the effort required to make these studies happen. As Jay Kirkpatrick discusses in “The Elusive Promise of Wildlife Contraception: A Personal Perspective,” these studies demanded much more than scientific and technical skill. Success required diplomacy, media savvy, skill with the public, good political instincts, and patience, patience, patience—even before the first dart flew. These are not necessarily characteristics that scientists are best known for, and I salute my colleagues and collaborators for their versatility as well as their persistence.

Finally, we should not forget what these studies are all about: the animals. Of all the rewards this research has provided us, the greatest is the knowledge that we have lengthened and improved the quality of the lives of the individual animals we have worked with. The studies described here

have prevented the births of “surplus” zoo animals; lengthened the lives of wild horses and kept others on the range and out of stressful adoption programs; and prevented needless killings of deer and elephants. More, we have placed before the world unambiguous evidence that people and wildlife can live together without killing and abuse, and in so doing provided hope to all those who strive for a more humane world.

As this volume goes to press, the need to use this technology to spare animal suffering has become even more urgent. The use of sharpshooters to kill semi-tame deer in suburban communities is rising; so too is pressure to cull elephants in southern Africa. For the first time since 1971, wild horses from western public lands are slated to be sold for slaughter in massive numbers, thanks to the so-called Burns Amendment (named after its author, Montana senator Conrad Burns) to the 2005 Omnibus Appropriations Bill. Intended to reduce the number of wild horses—now approximately 20,000—that the BLM has been keeping in holding facilities after removal from the range, the Burns Amendment requires the BLM to sell at auction any “excess” wild horse more than ten years old, as well as any horse who has been unsuccessfully offered for adoption more than three times. Ironically, almost simultaneously with the passage of the Burns Amendment, the U.S. Geological Survey published an economic analysis (Bartholow 2004) that projected that widespread use of immunocontraception on wild horses could save the BLM \$7.7 million per year—which would earn back, in a matter of months, the agency’s entire investment in immunocontraception research.

It’s time to move along and make this work.

I thank Andrew Rowan and John Grandy, Ph.D., for their organization of and support for the workshop and this volume; Deborah Salem for her heroic editorial oversight; and Kate Natrass, M.S., for her insightful manuscript reviews.

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April 15, 2005

The Elusive Promise of Wildlife Contraception: A Personal Perspective

Jay F. Kirkpatrick

Introduction

During the first half of the 1990s, a great deal of excitement erupted over the promise of managing some wildlife populations with fertility control. Much of that excitement came from the breakthrough with immunocontraception of wild horses on Assateague Island National Seashore in 1988, where twenty-six mares were remotely treated with the porcine zona pellucida (PZP) vaccine and not a single foal was born a year later (Kirkpatrick, Liu, and Turner 1990). Interestingly, the public excitement was directed at the possibility of controlling white-tailed deer rather than wild horses. That is probably reasonable; in areas with large human populations, there are many more troublesome deer populations than there are troublesome horse populations.

In rapid succession it was shown that this vaccine was effective in captive white-tailed deer (Turner, Liu, and Kirkpatrick 1992) and even semi-captive deer (McShea et al. 1997), which only added to the interest among animal protectionists and public officials struggling to defuse deer controversies in their communities. During the early 1990s, requests for the

application of the vaccine to urban and suburban deer multiplied. Although it was not directly related to this expanding interest in deer contraception, the successful application of the vaccine to many different captive wildlife species in zoos (Kirkpatrick et al. 1995, 1996) only fueled the enthusiasm among those who objected to lethal controls.

The first in what would become a series of international conferences addressing this technology was initiated in 1987 in Philadelphia, a year before the breakthrough on Assateague. This was the first time that almost everyone with an interest in the field was brought together to review what was available and what might be developed. It was something of a surprise that so many people attended. It was clear that there was a strong latent interest in a diverse collection of animal welfare and rights groups, government agencies, and wildlife managers. This conference was largely a historic review of what had been accomplished with captive and free-ranging wildlife, but there was a conspicuous absence of successful applications in the field (Cohn et al. 1996). A second conference, in Melbourne, Australia, in 1990, provided little that was new in the way of field experience but focused its attention on the future and what might be possible. By 1993 the success with wild horses on Assateague, the mounting safety and efficacy data, and the captive studies with deer stimulated a third conference, in Denver (Kreeger 1997). There, however, the positive scientific results were greeted by skepticism, and there was open hostility to the very idea of wildlife contraception.

What led to this redirection of what had, until then, appeared to be a monolithic rush toward non-lethal control? The answers can best be understood by carefully studying the issues as they relate to deer.

Deer Contraception

In April 1988 a conference held on the outskirts of Princeton, New Jersey, examined the issues and problems of urban deer (Donald 1993). It barely touched on the possibility of contraception. Nonetheless, it was clear that both the hunting community and the state fish and game agencies perceived non-lethal controls for deer populations as a threat. There was some reasonably civil discourse on the subject but the lines were drawn—lightly, but drawn nevertheless—between opponents and supporters of deer contraception.

A year later, with the as-yet unpublished results of the Assateague horse work starting to make the rounds in wildlife management circles, interest in the possibility of applying the PZP vaccine to deer erupted. A group of citizens in Princeton became intensely interested and lobbied The Humane Society of the United States (HSUS) to sponsor a trial there. Just to the north, an arboretum in Morristown also pleaded to have deer contraception applied on its grounds. Animal welfare and animal rights groups rallied around these initial attempts to initiate deer contraception trials.

Driven by both internal politics and external lobbying from hunters, the New Jersey Fish and Game Department (NJF&G) responded by asserting flatly that deer contraception would not work and that it would never approve such trials. In reaction, several animal rights groups assumed equally radical, but opposite, positions. One group repeatedly warned that contraception was the means by which “all sport hunting would be brought to its knees.” Others joined the battle, and it has not abated in the years since. The hard-nosed stance by NJF&G even led to an effort by some citizens of New Jersey to change the composition of the Fish and Game Commission and thereby to dilute the influence of the hunting community. The PZP wildlife contraceptive research group left the state, for the time being, alone.

A citizen-driven invitation to examine a possible field site in New York was proffered in 1991. Despite its designation, Fire Island National Seashore (FIIS) includes nearly twenty small communities interspersed between the Seashore’s natural areas. Deer populations in these communities had exploded. Many community residents and visitors fed the deer generously, named them, and worried over them. These people were also very wealthy and very influential. They had struggled through a number of legal battles associated with previous attempts to kill what they considered their deer on their island. Ironically, the same conditions that led to the growth of the deer population and the associated controversy made the site an excellent one for a PZP project. Human-habituated deer frequented backyards, boardwalks, and even sundecks, and, rather than fleeing people, approached them for handouts. The PZP research group eagerly agreed to start a project there.

The New York Department of Environmental Conservation (NYDEC), however, was as unenthusiastic about this project as the authorities in New Jersey had been about a project in their state. Because this was federal land, the political and legal issues were more complicated. The Seashore’s enabling legislation permitted hunting, the state managed that hunting, and the NYDEC challenged the National Park Service (NPS) over its right to run a contraceptive project with a species managed by the state. The FIIS staff was ambivalent, caught between a vocal and powerful local constituency and an alarmed state agency. The NPS regional science office, however, was less hesitant and took on the state’s challenge. Ultimately the NYDEC agreed to cooperate. New Jersey officials, stung by this decision, privately urged NYDEC officials to stop the project, but they did not prevail.

Another project, at the Gaithersburg, Maryland, campus of the National Institute of Standards and Technology (NIST, part of the federal Department of Commerce), was even harder to get off the ground. After the Maryland Department of Natural Resources (MDNR) rejected a series of research permit requests from NIST and the research team, irritated NIST officials went to their Solicitor General for legal advice. The Solicitor General issued an opinion that, because NIST was federal land, the managing agency did not need the state’s permission to manage the wildlife there. MDNR counterattacked by soliciting the help of the Congressional Sports-

man's Caucus, several of whose members sat on the committee that authorized NIST's funding. Caucus members wrote NIST to tell it that the campus deer population should be managed with a controlled hunt. After a tour of the urban 570 acre, high-security, twenty-four-hour-a-day research facility staffed by 6,000 federal employees and contractors—the Caucus members and MDNR agreed that a hunt was not feasible and backed off. Contraceptive treatments of NIST deer began in 1996 (Rutberg et al. 2004; Rutberg, this volume).

In the mid-1990s, other small projects began in Ohio, Morristown, New Jersey (following a legislative hearing in which contraception-friendly legislators raked NJF&G officials over the coals), and in Connecticut. But proposed deer contraceptive projects in northern Indiana, Princeton, the Pittsburgh and Philadelphia metropolitan areas, and elsewhere came to naught after acrimonious exchanges between those for and against non-lethal controls. These battles drew in virtually every animal protection group, every sportsmen's group and even a few governors. In the end a combination of red tape and political maneuvering overcame rational discussions and none of these projects was started (Kirkpatrick and Turner 1997). The Indiana Department of Natural Resources (IDNR) finessed a project in Beverly Shores by granting a research permit but imposing conditions that undermined the research objectives and would have introduced unacceptable risks to the health and safety of researchers and animals alike. The state agencies continue to try to thwart projects at this writing; in autumn 2003, Ohio DNR arbitrarily cancelled the research permit for one long-standing study and denied permits for two new deer studies by other contraception research groups.

Ten years after the initiation of the FIIS study, the FIIS and NIST studies have now shown conclusively that certain deer populations can be managed and even reduced by contraception (Naugle et al. 2002; Rutberg et al. 2004). Despite these impressive successes in the field, the battle lines are now clear and well defined. State wildlife agencies and a significant portion of hunters see deer contraception as a threat to hunting. Because of the control these agencies exert over deer research on all but federal lands, the momentum toward establishing contraception as an effective deer management tool in urban and suburban areas has been slowed dramatically.

The Opponents' Arguments

For many years, opponents of deer contraception within state agencies and the wildlife professions pinned their case on scientific issues. The years of contraceptive research with steroid hormones—which do pass through the food chain—allowed some opponents of deer contraception to scare the public by blurring the distinction between steroids and immunocontraceptive vaccines. They argued or implied that contraceptives pass through the food chain and thereby pose a serious risk to wildlife and

people that consume the meat of treated animals (Hanback and Blumig 1993; Guynn 1997). Some even alleged that women who consumed meat from treated animals would become sterile, and men might experience breast enlargement. Gradually the professionals reluctantly accepted that ordinary animal proteins such as PZP cannot pass through the digestive tract and remain biologically active. (Nevertheless, the argument is still posed by hunting groups and other opponents, e.g., Minter 2001.)

A second argument contended that extended breeding seasons, which result from deer contraception, would deplete the energy resources of treated does (Guynn 1997; Nettles 1997). A Front Royal (Virginia) study directly contradicted this claim and showed that by summer treated does who did not fawn were on the average 15 kg heavier than untreated does who got pregnant and fawned (McShea et al. 1997). A more recent study showed no difference between the condition of treated and untreated animals by the subsequent fall (Walter, Kirkpatrick, and Gregonis 2003). A related concern was that, because of the extended breeding season, bucks would exhaust themselves chasing estrous females for two additional months (Hanback and Blumig 1993; Winand 1999). Again, the Front Royal study showed that there was no frenzied chasing or breeding activities associated with the extended breeding season. Older mature bucks did most of the breeding in the early and midseason periods and then stopped. The younger bucks showed some moderate but non-aggressive interest in the treated does. Informal observations at NIST, FIIS, and other sites confirm that there is some elevated winter breeding activity, but it does not seem to harm the animals involved.

A third argument focused on genetics. How would contraception affect the genetic integrity of the deer herd (Nettles 1997)? This is a legitimate question. However, the same people who were expressing concern about the genetic impact of immunocontraception appeared unfazed by the impact of hunting aimed primarily at pursuing the largest bucks in the land. Hunting of trophy males has caused grave harm in other ungulate populations (Coltman et al. 2003; Milner-Gulland et al. 2003). If America's deer herds had survived for a hundred years with such an intense focus on killing the biggest and the best of the animals, it was hard to imagine that inhibiting the fertility of a few hundred or few thousand selected does would do greater harm.

In one approach to the question of genetic impacts, it was argued that only healthy deer would respond immunologically and become infertile, while sick deer would not make antibodies and would therefore get pregnant. This would result in America's deer herd becoming progressively more unhealthy (Nettles 1997). This argument, supported by a single experiment with an inbred strain of chickens (Haddad et al. 1994), was founded on the more-than-dubious assumption that animals too sickly or feeble to mount an immune response to a simple vaccine could successfully assume the heavy physiological and behavioral burdens of birth and lactation. And the evidence didn't support it: the PZP vaccine has been used on

physiologically stressed herds of western wild horses and in zoos all over North America and Europe, sometimes in sick animals whose health might be compromised by another pregnancy. These stressed and sick animals raised normal antibody titers to PZP (Kirkpatrick et al. 1995, 1996).

Critics also challenged the efficacy of immunocontraception. The initial argument was that wildlife contraception wouldn't work at all. The publication of the Assateague wild horse work and the captive deer work (Kirkpatrick, Liu, and Turner 1990; Turner, Liu, and Kirkpatrick 1992) deflated this change. Then the argument became contraception wouldn't work on free-ranging deer. After McShea (1997) and Kirkpatrick et al. (1997), it was that contraception worked on individual free-ranging deer but didn't control populations (Garrott 1995; Grund 1996; Seagle and Close 1996). The Fire Island and NIST studies finished off that argument (Naugle et al. 2002; Rutberg et al. 2004). One by one, the scientific arguments against deer contraception were countered, based on actual research and field application.

After this string of successful field tests, the opponents of immunocontraception then changed tactics and began to question the cost of immunocontraception. Sportsmen were resolute in the position that no license-generated state fish and game funds should ever be used for this technology. The state agencies agreed. But so, for that matter, did most of the advocates of deer contraception. At potential sites pledges for private money were almost always forthcoming and adequate.

Some hunters and agency staff also suggested that hunters might be deprived of recreation if immunocontraception replaced traditional deer management. However, the shooting of human-habituated deer in city parks has only a minimal connection to sport hunting and the concept of fair chase and, even for many opponents to deer contraception, this was not a legitimate argument.

By this point it was clear that opponents of immunocontraception needed to erect a new set of obstacles, outside of science, and they found them in the issue of regulation.

Regulatory Issues

In 1993 the U.S. Department of Agriculture (USDA) voluntarily bowed out of the regulation of wildlife contraceptives with the rationale that the legislative mandate of the USDA is disease prevention, and that pregnancy is not a disease. The Center for Veterinary Medicine (CVM), of the U.S. Food and Drug Administration (FDA) took over the responsibility for regulation of wildlife contraceptives. The agency issued Investigational New Animal Drug exemptions (INAD) to me for the use of PZP in deer, wild horses, and zoo animals. Lacking the resources required to deal with the ethical and regulatory issues at hand, I soon turned the sponsorship of the INAD over to The HSUS. Unfortunately, assumption of the INAD by The HSUS, which opposes sport hunting, aggravated the suspicions of hunters

and further polarized the entire wildlife contraception issue. In the meantime, the FDA insisted that the development of the contraceptive agent, the PZP vaccine, be moved forward toward testing and licensing through a New Animal Drug Application (NADA). This is the standard process for the development of any commercial drug, human or veterinary. But it posed something of a problem in this case, because neither the scientific team nor The HSUS had intentions of developing the vaccine as a money-making proposition. In fact, by publishing the findings widely, the research team placed the technology in the public domain, thereby preventing the native PZP vaccine from being patented for use in wildlife. Thus, no single party could lock up access to the technology.

In supervising field trials under the INAD, the FDA insisted that animals treated with the PZP vaccine be captured and tagged with a clear warning not to consume the meat of the animal. (Because of its isolation and the absence of hunting on the island, FIIS has remained the lone exception.) Unfortunately, the utility of immunocontraception really lies with remote delivery, and the cost and time involved in capturing urban deer, not to mention the stress and mortality associated with the capture process, may be too great for fertility control to achieve significant and practical results in urban settings unless the tagging requirement is lifted.

The second problem that came along with the FDA regulatory process was the immense cost of testing and clinical trials. By the early 1990s, after the success of PZP with wild horses was understood and accepted, it had become clear that there was no commercial potential for a drug that would only be applied to wildlife. Most pharmaceutical companies think in terms of millions of doses annually, if a profit is to be made, but it is unlikely that more than a few tens of thousands of wild animals, of all species, would ever be treated in a single year. So now, the problem became one of who would invest the millions of dollars necessary to get through the FDA regulatory process when there was no promise of profit at the other end.

By the mid-1990s, some of this pessimism was pushed aside by the prospect of using PZP in companion animals, domestic dogs and cats. This was a potential market that might make the PZP testing and trials cost-effective, and the resulting commercial form of the vaccine would still be available for use in wildlife (although there is precious little money available for the purchase of wildlife contraceptives by the potential users). Unfortunately, the PZP vaccine has not worked well in canids and felids and that hope has receded for now (ACCD 2002; Gorman et al. 2002).

Finally, the prospects of developing an effective inexpensive synthetic or recombinant ZP have eluded just about all scientific efforts. A number of synthetic molecules have been developed that elicit impressive antibody titers but they do not cause significant contraception. The problem is that the carbohydrate groups attached to the ZP protein backbone are essential to the molecule's contraceptive effectiveness, and these have not been effectively mimicked in synthetics (Alexander and Schlaff 1996). That leaves, for the immediate future, native PZP, which will have to surmount

high hurdles to meet the FDA's Good Manufacturing Process (GMP) requirements. Thus, native PZP has moved only slowly toward commercial development despite its success, and its use will remain "experimental" for the time being (even after sixteen years of application to wildlife and another thirty to non-human primates).

This "experimental" label has become a foil for opponents, who argue that we shouldn't be using anything experimental on the public's wildlife. A number of field projects have wilted in the face of agency hostility and associated public nervousness. As the story reached the end of 2003, the U.S. Sportsman's Alliance had selected as one of its two highest priorities putting a stop to deer contraception through federal and state legislation. (The other one is stopping animal rights terrorism.)

Who would have thought that such a simple concept could elicit such strong emotions?

Wild Horses

Wild horse contraception followed a convoluted pathway also, but more progress has been made with this species. The stimulus for wildlife contraception in general was the passage of the Wild, Free-Roaming Horse and Burro Act of 1971 (Public Law 92-195; 16 U.S.C. §133 et seq.), which banned the killing, capturing, and harassing of wild horses on western public lands. While the intent of the law was noble, the new regulations provided for almost no management, and wild horse populations increased dramatically within several years of the passage of the act (Rutberg 2003).

The initial attempts at wild horse contraceptive research, 1971–1976, were carried out with almost no federal support, and there was little interest on the part of the scientific community at large to become involved. In 1977 the Bureau of Land Management (BLM), the federal agency vested by the 1971 Act with the management responsibility for the western wild horses, offered about \$300,000 for contraceptive research. To no one's surprise, several research groups immediately became interested and the competition for that money became intense.

The first attempts at wild horse contraception focused on giving stallions a long-acting form of testosterone, which had the effect of reducing sperm counts to the point of infertility. This research had four outcomes worth noting. First, this approach was pharmacologically successful, reducing foaling rates by 83 percent, but it was cumbersome, expensive, and dangerous to man and beast alike (Kirkpatrick, Turner, and Perkins 1982; Turner and Kirkpatrick 1982). The stallions had to be immobilized from a helicopter and injected by hand with a huge dose of the drug. The second outcome was that public objections to horse contraception increased, because of this stress to animals. The third outcome was that the research experience allowed the scientists to develop, for the first time, a set of

characteristics of the ideal wild horse contraceptive. These included (1) remote delivery of the drug, (2) at least 90 percent efficacy, (3) reversibility in its contraceptive action, (4) safety when administered to pregnant mares, (5) minimal effect on social behaviors, (6) no long-term debilitating health side effects, (7) reasonably low cost, and (8) no passage through the food chain (Kirkpatrick and Turner 1991).

The fourth and final significant outcome was that it signaled to the larger scientific community a willingness of the government to put serious funding behind the effort. Nothing so charges the scientific community with enthusiasm as money. The problem was, only a single research group was conducting any work and somehow a larger segment of the research world had to be permitted to get involved. To solve this problem, the BLM established an advisory committee to review research needs and recommend appropriate research. The National Academy of Sciences (NAS) established the committee, which deliberated for a year or so and recommended that additional money be put into wild horse contraceptive research. Thus in 1985, after a five year hiatus, wild horse contraceptive research was brought back to life (Wagner 1982).

In 1985 BLM allocated \$750,000 for research, and appointed a three-person subcommittee of the NAS committee to screen the proposals and select a research group. There were only three responses to the request for proposals. One was from the stallion contraception group. Another was from the University of Minnesota, proposing to surgically place steroid implants into captured mares. The third, from the University of California, Davis, was for the study of immunocontraception.

With 20–20 hindsight, it is clear that the immunocontraception proposal was the most meritorious, but it was the proposal for the steroid implants that won the day. A large scale research effort was initiated with Nevada horses and steroid implants.

In a totally independent and unrelated event, our research group was contacted by the NPS and requested to produce a proposal (for \$19,000) to develop a contraceptive approach to the wild horses of Assateague Island National Seashore (ASIS), in Maryland. The UC-Davis group promoting immunocontraception was again ignored.

From 1985 to 1990, the Nevada and Assateague studies progressed, with little or no communication between the groups. The steroid implant group in Nevada showed, in rapid order, that the implants could reduce mare fertility, probably for up to five years per treatment (Eagle et al. 1992). On Assateague, we again showed that reproduction could be inhibited with androgens, this time using remote delivery (darting) to treat stallions with long-acting testosterone (Kirkpatrick 1995; chapter 5, this volume). Both groups were successful in inhibiting fertility, but the shortcomings of both approaches were again exposed. The animals given the steroid implants sometimes rejected and expelled the implant. The biggest drawback of the implants, however, was that the mares had to be captured and at least lightly anesthetized before surgery could take place. The cost was high and

the stresses for the animals were significant. At this point, no one had even considered the passage of the drugs through the food chain, which the synthetic steroids used in implants will do. Nor did anyone consider the long-term pathologies associated with these hormones, which had already been shown in other species (Linnehan and Edwards 1991).

The stallion-based research on Assateague followed a similar path. While the approach worked, each stallion had to be darted three separate times just to receive a minimally effective dose—an approach that became increasingly difficult with each successive shot—and the cost of the long-acting steroids was extremely high. In short, it was impractical (Kirkpatrick 1995).

The Nevada project forged ahead despite the obvious impracticalities, but our group shifted gears, throwing out fifteen years of steroid research, and sat down to talk with the previously rejected immunocontraception group at UC-Davis. Fortunately, the NPS persisted, and offered a second chance to the research team. In 1988, after a marriage between the two research groups—our formerly stallion-based group and the immunocontraceptive group—the mares of Assateague were treated with the PZP vaccine.

By 1990 the competition between the two approaches was over. The steroid-implant approach collapsed, not so much because of its impracticality, but because of a disaster with the care and handling of the wild horses recruited for the research. For a variety of reasons, all preventable, nearly 50 of the steroid research group's horses died (Wagner 1992). Public outcry was loud and in little time the research support for this project and group was withdrawn, leaving the BLM with nothing but bad press. On Assateague Island, however, the PZP research was extraordinarily successful and the door was opened to a completely new approach to fertility control in wildlife, including horses (Kirkpatrick, Liu, and Turner 1990).

The Social and Political Response To Wild Horse Contraception

In the 1970s and 1980s, public opinions about wild horse contraception changed in interesting ways in response to the research progress and emerging issues.

When the Wild, Free-Roaming Horse and Burro Act was passed in 1971, there were an estimated 17,000 wild horses on public lands in ten western states. That number has been disputed and is probably, at best, a rough estimate. However by 1980 the estimated number had risen to 80,000 horses. Adopt-A-Horse, which had been the only reduction management tool in the 1970s, was obviously not succeeding.

In 1980 President Reagan appointed James Watt as Secretary of the Interior, which most conservation and animal welfare organizations viewed as hiring the coyote to guard the sheep. As a long-time Wyoming rancher, Watt harbored the time-honored regional dislike of federal government

regulation of public lands in the west, and he sought to change the law to remove much of the protection afforded to wild horses by the 1971 Act. Secretary Watt was naïve about the emotions evoked by wild horses and the reluctance of Congress to get into this battle, and his initiative went nowhere. Nor did Watt last his full term as secretary. With his assault on the public lands in general, and wild horses in particular, Watt single-handedly changed the complexion of the contraceptive research issue.

Interestingly, while the greatest need for an effective management tool belonged to the BLM, the NPS was the agency that took the science to a new level. The news and publication (in 1990) of the NPS success on Assateague Island spread rapidly and gathered interest and (in some cases) support from a collection of diverse groups, including The HSUS, The National Institutes of Health, and the American Zoo and Aquarium Association. Suddenly wildlife contraception was not so bizarre.

In the meantime wild horses were still breeding on the western ranges, and ranching interests were increasing their pressure to do something about them. In June 1991 Senator Harry Reid (D-NV) led a Senate subcommittee hearing to find some way to get a grip on the problem of exploding wild horse populations. This was not a minor problem for Reid, since most of the horses were in his home state. Because the previous contraceptive research with the steroid implants had ended in disaster, there was a deep natural suspicion of any contraceptive approach. The general public and its elected politicians did not and, in reality, could not be expected to distinguish between steroid-based contraception and immunocontraception.

The Senate hearing focused on the research results from Assateague and how these might be applied to western wild horses. Because each mare needed to be darted twice the initial year of treatment and once annually thereafter, this approach would be unworkable on the vast ranges of the west. The proposed solution was to incorporate the PZP vaccine into a single treatment format that would result in at least two years of contraception. With Reid's backing, a large sum of money—perhaps a million dollars—was appropriated to the BLM over the next ten years for the development of a one-inoculation vaccine that would have contraceptive effects for two to three years.

Battling Bureaucracies and Competing for the Research Dollar

When the National Biological Survey (which eventually metamorphosed into the Biological Resources Division [BRD] of the U.S. Geological Survey) was created in 1991, it assumed most of the research functions of the Department of Interior's three major natural resource agencies, the NPS, the BLM, and the U.S. Fish and Wildlife Service. This included responsibility for wild horse contraception research.

Unfortunately, the transition was not smooth. The BRD fell almost two years behind in its payments of research funds, and several times the entire project was shut down for nearly a year at a time. And when experiments were planned, the BLM often was unable to gather a sufficient number of mares to meet the requirements of the research design.

In the meantime at least three other research groups were watching the research dollar figures with increasing interest. The “one-inoculation” PZP research group had such a lead, particularly in the field, that it was going to be difficult for the other groups to catch up. Both political and scientific approaches were taken to slow the momentum of the one-inoculation group. When the BRD created an advisory committee to determine the long-range direction of wild horse contraceptive research, it appointed members who were all linked to competing research groups. The one-inoculation PZP group was not represented. The long-term research plan that emerged recommended continuing the ongoing one-inoculation PZP research but also directing funds towards projects that were related to interests and activities of members of the committee. It was almost a replay of the 1980s era NAS advisory committee that awarded federal funds to the steroid implant group; key members of that committee also had strong ties to the research group that received the funds.

The one-inoculation opponents also attempted to muster scientific arguments against PZP, in particular arguing against the safety of the PZP vaccine. Two U.S. Department of Agriculture/Animal and Plant Health Inspection Service (USDA/APHIS) veterinarians working under contract with the BLM objected to moving ahead with PZP field trials on the grounds that the adjuvant (Freund’s Complete Adjuvant, or FCA) used in previous field trials was dangerous to horse and researcher alike, even claiming (without any supporting data) that an accidental stick would result in the loss of a finger. That argument was debunked by referring them to sixty years of data by researchers using FCA as well as by fifteen years of experience with it on Assateague and elsewhere (Turner and Kirkpatrick 2002). The APHIS veterinarians also raised the possibility that the FCA would cause a false positive tuberculosis (TB) test in treated horses. (This can be a problem in other species, but there is no reliable test for TB in equids and not a single research group in the United States was even working on developing such a test.)

Ultimately, the BLM and the BRD rejected the scientific arguments. Under the revised strategic plan that emerged from the BRD, the one-inoculation group was allowed to go forward with field trials while the other three or four groups would continue using captive horses. As of 2004 no changes in normal behavior had been noted, no abscesses had been formed, and no one had lost any fingers from needle sticks. Other horses are still being actively managed on Assateague, Cape Lookout, Carrot Island, Return-to-Freedom, and Little Cumberland Island (Kirkpatrick, chapter 5, this volume), but the war rages on.

Role of Animal Protection Groups

Horse protection groups, animal welfare groups, and animal rights groups have played shifting and sometimes contradictory roles in the history of horse contraception.

In the 1970s, most wild horse advocacy groups, and even some animal welfare groups, opposed the idea of wild horse contraception. Most of these groups saw only two alternatives during those years: one was to leave the horses alone; the other was to harass the animals, using darts, contraception, capture, and removal. There was almost no recognition that a non-hunted animal that lives twenty years, has (in most locations) no natural enemies, and reproduces impressively fast will ultimately need to be controlled. Driving that failure of recognition was the belief that the BLM was systematically overestimating the size of horse populations, and the groups' deep suspicions of the agency's ultimate motives.

A turning point occurred in the 1980s. In the shadow of James Watt, animal protection groups saw that the choices had been reframed. No longer were the alternatives contraception versus hands-off; now, it was darts versus bullets. In May 1981, in a historic meeting in the HSUS library in Washington, D.C., a coalition of conservation, animal protection, and horse advocacy groups came to the collective realization that wild horse management of some kind was necessary. Only the American Horse Protection Association (AHPA) continued (at that time) to oppose the idea of any management. Overnight, almost all those horse advocacy groups and animal protection groups, which had opposed contraception, took up its banner and promoted the idea.

The involvement of The HSUS proved both helpful and harmful in the initiation of the Nevada one-inoculation studies in the early 1990s. In 1992 The HSUS signed a Memorandum of Agreement (MOA) with the BLM to cosponsor the first western PZP trials. Although the BLM was ambivalent about the MOA due to its history of antagonism with The HSUS, it saw no choice; The HSUS provided it with the political cover it needed to pursue contraception after the humane catastrophe of the 1980s steroid implant studies. On the other hand, HSUS involvement was a mixed blessing. Because Senator Reid was from Nevada, the congressional appropriation was channeled through the University of Nevada at Reno (UNR), even though that institution had never been involved in wildlife contraceptive research. UNR balked, not because it didn't want its share of the money, but because animal researchers at UNR had previously battled with "the humane society." UNR backed down because the BLM refused to proceed without The HSUS, and UNR did not want to explain to Reid why the project had stopped. (Ironically, "the humane society" that had raised UNR's ire was actually a local organization unaffiliated with The HSUS.) Despite a decision to move ahead, the involvement of an animal protection group once again caused discomfort and polarization.

In the post-Watt 1980s, animal protection groups largely supported the concept of horse contraception and enthusiastically urged it upon the BLM at every opportunity. But as the concept has turned to reality, and the BLM has increasingly embraced it, some of these same horse advocacy groups are backing away from the concept. Not trusting the BLM, they do not trust contraception in the BLM's hands. In 2002 The Fund for Animals blocked a wild horse contraception project at the Pryor Mountain Wild Horse Range by filing a formal appeal with the Interior Department. The Fund, which generally supports wildlife contraception, argued that the BLM's novel "humane use" justification for the project—the reduction of suffering and death among the horses by preventing pregnancies in very young mares—had been inadequately debated by the public, and moreover was too intrusive. In the end, The HSUS negotiated an agreement with The Fund and the appeal was dropped.

Zoo Contraception

Application of contraception to zoo animals should have been relatively simple. One of the largest problems zoos have is the production of "surplus animals" from unplanned pregnancies and births, and contraception is an obvious and seemingly easy-to-implement solution. But even this application was not to be simple.

The widespread application of contraception in zoos came into vogue in the 1970s, with the development of the progestin-based melengestrol acetate (MGA) implant. The MGA implants work quite well, but serious pathologies result from their use, and they are used today with a great deal of caution (AZA Contraceptive Advisory Group 2003 Recommendations, St. Louis Zoo; Frank, this volume). Nevertheless, the MGA implants have been among the most commonly used zoo contraceptives for the better part of fifteen years.

In October 1989 the success with PZP was described in a report to the annual meeting of the American Zoo and Aquarium Association (AZA). PZP was intriguing to zoos because, as opposed to the MGA implants, the vaccine could be delivered remotely. At this same meeting, AZA's first advisory committee on zoo contraception was created. The committee was first named the Steroid Contraceptive Advisory Committee, and its role was to advise zoos on contraceptive use. The recent PZP data from Assateague was of interest to some in AZA but the association of the PZP group with The HSUS, which had been at odds with AZA for many years over a variety of issues, cooled enthusiasm for the technology. In any case, there were no inquiries by the AZA committee regarding the use of PZP and there seemed to be little future for immunocontraception in the zoo world at that time.

In 1990, halfway around the world, zoo managers at the Köln Zoo (Cologne) in Germany were looking for a way to control fertility in Przewalski's horses and were considering MGA implants. By coincidence, Ron

Keiper, a long-time wild horse researcher who established his reputation on Assateague Island, was visiting the Köln Zoo. Aware of the success of PZP on Assateague, he passed the information along to the zoo's managers.

The Köln Zoo managers then invited me to a conference in Leipzig about reintroducing Przewalski's horses into Mongolia. The invitation was a pre-text; from Leipzig, I was taken to Köln and initiated a PZP immunocontraception project with Przewalski's horses at the zoo. The zoo added some banteng to the treatment list, and zoo immunocontraception was launched with this modest start. Of course, it worked well (Kirkpatrick et al. 1995).

Word about the trials at Köln filtered their way back to the United States, but interest in the PZP approach was slow to develop. The break came later in 1990. The executive director of the American Association of Zoo Veterinarians (AAZV), who was following the immunocontraception progress with interest, encouraged one of the veterinarians at the Bronx Zoo to give PZP a closer look. In December a number of sika deer, axis deer, Himalayan tahr, elk, and muntjac deer at the Bronx Zoo were treated with PZP and, once again, it worked (Kirkpatrick et al. 1996). It was a modest start in the North American zoo world.

Again, The HSUS played an ambiguous role. The Bronx Zoo trials were to be jointly funded by the zoo and The HSUS. However, when the Bronx Zoo director discovered this arrangement, he balked and insisted that only the zoo pay for the project. As it turned out, both parties paid their share. However, the Bronx Zoo did not continue with PZP contraception after completion of the study.

In 1991 several other zoos started trials with PZP and, in 1992, the AAZV invited the PZP team to speak at its annual meeting in Oakland. Although MGA implants are still the most widely used zoo contraceptive, the PZP vaccine today is used in more than 100 zoos and in more than 100 species (Frank, this volume), and the PZP team is represented on the AZA Contraceptive Advisory Group.

Reliable Field Personnel

If the thirty-one-year experience of wildlife contraception has shown anything, it is that no fertility control project, regardless of the money behind it or the particular technology employed, will be any better than the people in the field delivering the technology. That probably sounds self-evident, but, along with fish and game agency resistance, the scarcity of qualified field personnel is rapidly becoming the most important bottleneck in the entire effort to manage wildlife through contraception. Several case studies make this point very clearly.

In the early 1990s, a member of the research team traveled to Virgin Islands National Park to administer initial PZP treatments to burros (Turner, Liu, and Kirkpatrick 1996). Park personnel were trained to give the booster inoculations at a later date. This project was difficult from the start, burdened with

poor delivery equipment, dense brush, and very smart animals. When the time for the booster inoculations arrived, the assigned personnel were diverted to another project by the park's administration and the animals were not boosted on time. The project ultimately succeeded only because the researcher repeatedly returned to the park to do the work himself.

A similar problem has plagued the deer contraception project on Fire Island National Seashore. After six or seven years of treating the deer there, The HSUS requested that the national seashore take over the responsibility for the project. The NPS has made significant efforts to assist the vaccination; but employee turnover, lack of committed resources, and NPS reluctance to make decisions have meant that, more than ten years after it started, the project is still heavily dependent on HSUS personnel to get the work done. Ironically, the reduction in deer populations brought about by the project has lowered public concern about the deer, which in turn has reduced pressure on the NPS to commit resources to the project.

During the 1990s intense one-week training workshops were organized in Maryland for potential participants in deer contraception projects, and in Montana for BLM personnel assigned to administer the PZP vaccine to wild horses on their own ranges. But, in both cases, unless workshop participants dealt with the vaccine and the delivery systems on at least a weekly basis, the details were quickly forgotten.

Several successful examples of developing field personnel exist. Placing a high priority on field competence, The HSUS employs a person who has little other responsibility than to conduct field work and treat deer, and in some cases wild horses, remotely, with darts. This person is the single largest reason why the Fire Island and NIST deer projects have been so successful. Likewise, Assateague Island National Seashore and Cape Lookout National Seashore each assigns a full-time employee major responsibility for the management of horses. These rangers keep the inventory of horses, monitor band composition, home ranges, and behavior, dart the animals every year, and collect urine and fecal samples for remote pregnancy testing.

In all three success stories, the common thread is that there are personnel dedicated to the field aspect of wildlife contraception. Making this issue even more difficult is the number of other qualities, aside from skill with a dart gun, that field personnel should have. These particular individuals need to be sensitive to the animals they pursue and should not be willing to risk shots that might injure the animal. They need an intimate knowledge of the animals' behaviors and their home ranges. They need to know how to deal tactfully with an inquisitive public. And they need the patience of Job.

Where will these field personnel come from and who will support them? This is a critical question if wildlife contraception is to reach its full potential. At present there are no more than ten people available with the proper training and approach. At least four of them are government employees with little opportunity to work outside their own agencies. Those interested in moving wildlife contraception ahead must assure that this need for trained and committed field personnel is filled.

Conclusion

Beyond the wild horses, urban deer, and zoo animals, PZP has been applied to African elephants, elk in a national park, water buffalo on a military reservation, and a few other species. In every case treated animals showed sharply reduced fertility. Most of these other applications were accompanied by their own strange brand of politics, suggesting that the whole topic of wildlife contraception invites multifaceted controversy. Today wild horse contraception moves steadily along but not nearly so fast as the horses reproduce or the problem requires. Urban deer contraception is progressing sluggishly, and most of the ongoing projects are on federal land where state agencies have limited authority. Only zoo contraception moves ahead, growing annually, and (for now) relatively unscathed by ugly political battles.

Several outstanding factors lie behind the failure of wildlife contraception to advance. By far the largest is the official opposition of fish and game agencies, which see contraception as a threat to hunting. They have done a masterful job of convincing the larger hunting community that several people with dart guns may ultimately destroy public hunting. Few of their scientific arguments hold water and, in many cases, deliberate misinformation has been the primary weapon against contraception.

The most probable explanation, but certainly not the universal answer to this mystery, is the polarization between the animal protection groups, and particularly The HSUS, and the hunting community, official or otherwise. Even when the population of deer at the center of a particular dispute will never, *can never*, be hunted, the deep animosities between hunters and the animal protection groups keep the feud hot and lively.

A second failure of the wildlife contraceptive movement lies with the failure (to date) to have any particular contraceptive agent approved by the FDA for general use. The lack of FDA approval concerns the public, a concern that is fanned into fright by the misinformation circulated by opponents. The extensive published record of safety and efficacy demonstrated for immunocontraceptives suggests that the failure to gain FDA approval is less a scientific issue than a process issue; currently, immense resources are needed to move a new drug through the FDA's formal testing and approval process. Because demand for wildlife immunocontraceptives will be limited, there are no economic incentives to do so. There are ethical, ecological, and social incentives, but no one will make money producing immunocontraceptives for wildlife.

Finally, the challenge of finding, training, and supporting field personnel must be overcome. This is particularly pertinent because it is emblematic of the larger problem of wildlife contraception—that there is no economic incentive to solve it. Personnel must be able to pick up and travel for several weeks at a time, while being able to support themselves in other ways for the better part of each year. They must possess their own delivery equipment, even when they are not in the field, in order to hone skills, and they

must have a sense of caring and ethics that are consistent with those of the animal protection community.

Wildlife contraception has a future, but the emotional, knee-jerk reactions that hunters, fish and game agencies, and animal protection organizations have to one another will have to be overcome. In addition, either some form of concession will be needed from the FDA, or the PZP vaccine and its adjuvants must be taken through the entire FDA New Animal Drug Application process. Some plan for the identification and training—and funding—of field personnel will have to be developed. The development of wildlife contraception technology must be distanced from politics, and decisions about whether (and where) fertility control should be applied to a particular species, and which technology will be used, must be reached not on the basis of political favoritism and factional nonsense, but on proven science.

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Deer Contraception: What We Know And What We Don't

Allen T. Rutberg, Ph.D.

Introduction

Today tens of millions of white-tailed deer (*Odocoileus virginianus*) live in North America. This fact gives us much to celebrate. Deer astonish us with their capacity to thrive in the suburban habitats we have created for ourselves and enchant us with their beauty, grace, speed, and simple wildness. But their success has come at a price that much of the public would prefer not to pay. Millions (one? two? three? it's not clear) of deer collide with cars each year. Their association with the ticks that carry the Lyme disease pathogen and, indeed, the likelihood that they encourage the spread of Lyme disease, tarnishes their public image. They infuriate farmers, gardeners, and nursery owners and worry ecologists, who see a shift in the structure and species composition of deer-browsed forests. They are our beloved pests.

A safe, effective, and humane contraceptive would go far to resolve the deep public ambivalence about deer. Public opinion surveys show significant support for the application of deer contraception, even in the absence of any established technology (Kilpatrick and Walter 1997; Stout, Knuth,

and Curtis 1997; Lauber and Knuth 2000). Every fall the various offices of The Humane Society of the United States (HSUS) field dozens of calls weekly from communities all over the country seeking humane alternatives for deer population control, and many callers are smitten with the possibility of contraception.

In the early 1990s, partly in response to constituency demand, The HSUS joined with Jay Kirkpatrick, John Turner, and Irwin Liu to take the porcine zona pellucida (PZP) immunocontraceptive vaccine to the field and test the potential—and limitations—of the technology as a deer population management tool. The science of deer contraception has been continuously hampered by politics, with most opposition coming from hunting groups and state agencies that (mostly wrongly) view contraception as a threat to hunting and, to a lesser extent, from local political leaders who demand a quick, lethal (but often ineffective) fix to local deer problems (see Kirkpatrick, chapter 1, this volume). In general, the strength (and irrationality) of the opposition has increased in direct proportion to the success of the science; as we have gotten closer to demonstrating the effectiveness of contraception as a deer management tool, the opposition has become more focused and more strident. But we're much too close to fail now.

Background and Goals

With the support of the PNC Foundation, Turner, Liu, and Kirkpatrick (1992) first demonstrated that two to three PZP vaccinations delivered remotely prior to the breeding season could prevent pregnancies in white-tailed deer. PZP looked like it might be a nearly ideal wildlife contraceptive: remotely deliverable, apparently safe, highly effective, reversible, biodegradable, and having minimal effects on behavior (Kirkpatrick and Turner 1991).

Since that time, the HSUS research team and its collaborators have pursued four primary research objectives.

(1) *Demonstrate vaccine effectiveness under field conditions.* Contracepting deer in a pen is one thing; showing that you can efficiently locate and treat deer in the field is quite another. Prior to the PZP studies, there had been a number of physiological successes with administering contraceptives to deer in the field (Bell and Peterle 1975; Matschke 1980; Avazino 1996), but the numbers treated were very small, and the treatment process laborious and expensive.

(2) *Demonstrate that deer populations could be controlled by contraceptives.* From a wildlife conflict resolution viewpoint, if you can't stabilize or reduce a deer population with a contraceptive—no matter how well it works on treated individuals—you don't have a management tool. Deer population size is controlled by birth rates, death rates, and immigration and emigration rates; these rates in turn are influenced by nutrition, population density, predation, hunting, vehicle collisions, habitat disturbance,

social behavior, and other intrinsic and environmental factors. Contraception only influences birth rates, and the strength of that influence depends on the effectiveness of the contraceptive, the proportion of breeding-age females that can be located and treated, and the fertility of the breeding-age females that you can't locate and treat. Thus, population control is the supreme test of contraceptive effectiveness.

(3) *Identify an adjuvant to substitute for Freund's Complete Adjuvant.* Because injections of PZP alone elicit a very weak immune response, all PZP vaccines also include an adjuvant, which stimulates the immune system to produce a stronger response to the vaccine. Most of the initial research with PZP in horses, deer, and zoo animals tested vaccines prepared with Freund's Complete Adjuvant (FCA) in the initial "priming" dose and Freund's Incomplete Adjuvant (FIA) in subsequent doses (Kirkpatrick, Liu, and Turner 1990; Turner, Liu, and Kirkpatrick 1992; Kirkpatrick et al. 1995). FCA, however, has a record of causing serious injection site reactions when given to small research animals such as mice and hamsters (Hanly, Bennett, and Artwohl 1997). Because of this (and other factors not clearly expressed to The HSUS), the Food and Drug Administration informed The HSUS that it would not approve any final vaccine formulation that included FCA, and we were directed to identify another adjuvant that would be effective and safe to use with PZP.

(4) *Design an effective, long-acting, single shot vaccine.* In the initial PZP research with horses and deer, animals were treated with two or three shots prior to the mating season and subsequently with annual boosters (Kirkpatrick, Liu, and Turner 1990; Turner, Liu, and Kirkpatrick 1992, Turner, Kirkpatrick, and Liu 1996). Though it soon became clear that three shots were not necessary, and that there is considerable flexibility in the timing of the first two shots (McShea et al. 1997; Thiele 1999), this condition would clearly limit the usefulness of PZP as a deer contraceptive. In some areas experiencing deer conflicts it is possible to treat the same animal with multiple shots and annual boosters, but it is challenging and labor intensive under the best of circumstances. Consequently, a long-acting, single shot contraceptive is virtually a requirement for widespread management use.

In the past decade, the HSUS research team and its collaborators have accomplished the first three objectives. PZP has been administered to free-ranging deer, often remotely, at a number of sites; we have demonstrated population reductions at Fire Island National Seashore, New York, and the National Institute of Standards and Technology campus in Maryland; and we and other research teams have identified several adjuvants that are highly effective with PZP. The success the research team has achieved with long-acting single shot vaccines for horses (Turner et al. 2002; Kirkpatrick, chapter 5, this volume), however, has not yet been accomplished for deer by our research group.

This paper primarily reviews the research on deer contraception carried out by The HSUS and its collaborators. We also supplement descriptions of HSUS work with discussions of research carried out by other research groups to evaluate the overall status of the field.

Study Sites and Collaborations

The HSUS has sponsored or conducted field studies at numerous sites, with numerous collaborators, including the National Park Service (NPS), the Biological Resources Division of the U.S. Geological Survey, the National Institute of Standards and Technology (NIST), the University of Maryland, the University of New Hampshire, SUNY College of Environmental Science and Forestry at Syracuse (New York), the Connecticut Department of Environmental Protection (CT DEP), and various local governments (Table 1). Several other study sites for which proposals were developed, including Amherst, New York, Beverly Shores, Indiana, Princeton, New Jersey, and Valley Forge, Pennsylvania, were stopped by lawsuits, obstruction by state and federal agencies, or other hostile political actions (Kirkpatrick, chapter 1, this volume). The Mumford Cove/Groton-Long Point (MC/GLP) study was somewhat productive. Unfortunately, the study was shut down prematurely by community hostility that was overtly and covertly encouraged by a CT DEP deeply ambivalent about the research in which it was collaborating.

The studies conducted at Fire Island National Seashore (FIIS) and NIST have been our most productive by far.

Efficacy, Reversibility, and Antibody Titers

Multiple Shot Vaccines Prepared with FCA/FIA

In the initial captive study, Turner et al. (1992) showed that an initial injection of PZP emulsified in FCA followed by one or two booster shots of PZP emulsified in FIA at three and six weeks later prevented pregnancy in all seven treated does, whereas only one of seven control does failed to become pregnant. All shots were delivered remotely via blowgun, with the initial shot being administered in October and the male introduced in December. In follow-up experiments (Turner, Kirkpatrick, and Liu 1996), a single autumn booster shot of PZP/FIA extended infertility an additional year, but fertility was restored to control levels within two years of treatment. No does with anti-PZP titers greater than 50 percent of positive reference serum values produced fawns. Similar efficacy and reversibility results were obtained in semi-captive deer receiving two-shot preparations, except that the contraception threshold for antibody titers was reported to be 80 percent (McShea et al. 1997). In the Mumford Cove, Connecticut, study, Walter et al. (2002) found the contraceptive threshold for PZP/FCA/FIA to be in the 60–70 percent range.

Efficacy of the PZP/FCA/FIA preparation appeared to be lower under field conditions at FIIS (Kirkpatrick et al. 1997; Naugle et al. 2002). From 1993 to 1997, 74–164 individually known (but untagged) does were treated via blowgun in late summer or early autumn with two-shot PZP/FCA/FIA preparations followed by annual boosters of PZP/FIA. Over-

Table 1
A Summary of HSUS Deer Projects, 1993–2003

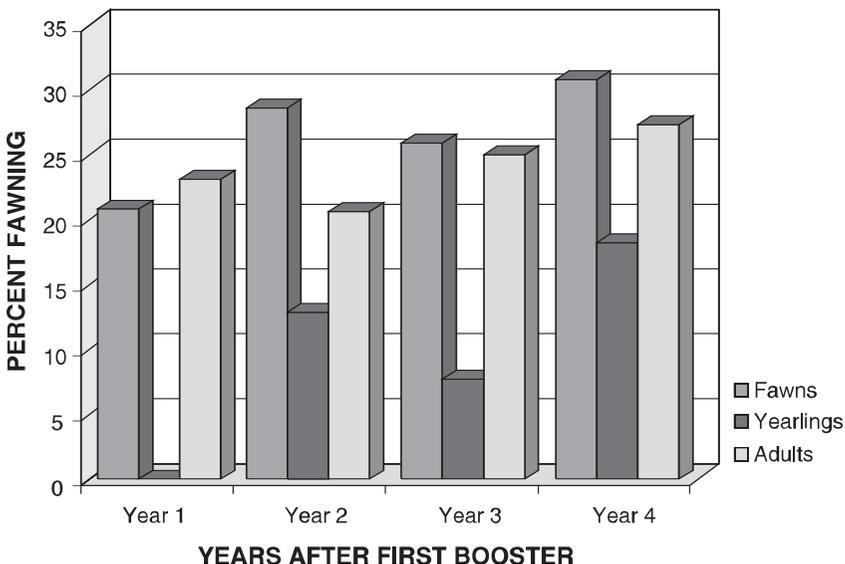
Project	Begun	Status	Results
Fire Island National Seashore, N.Y. (with National Park Service, Biological Resource Division of USGS, SUNY College of Environmental Sciences and Forestry)	1993	Ongoing	<ul style="list-style-type: none"> • Efficacy of PZP (FCA/FIA) reduced under field conditions • Efficacy improves with successive years of treatment • Population fawning rates, density reduced in high treatment areas
National Institute of Standards and Technology, Gaithersburg, Md. (with NIST, University of Maryland)	1995	Ongoing	<ul style="list-style-type: none"> • Efficacy not influenced by seasonal timing of initial treatment • Efficacy in field matches efficacy in captivity when initial treatment delivered by hand • Efficacy when first shot administered to fawns < 1 week old matches efficacy when first shot administered prior to first breeding season (1.5 yrs) • No significant differences in survival of treated vs. untreated fawns • Females treated during second trimester gave birth to healthy fawns at rates comparable to untreated animals • No significant differences in survival of fawns born to treated vs. untreated females • Carbopol®/FIA almost ineffective as an adjuvant • STDCM (RIBI) and Montanide® ISA 50 effective as adjuvants; ISA 50 less effective than FCA/FIA; RIBI as effective or more effective than FCA/FIA • Population reduced by >25% since 1997
Sharon Woods Metro Park, Columbus, Ohio (with Columbus-Franklin County Metro Parks)	1995	Term. 2003	<ul style="list-style-type: none"> • Efficacy of PZP (FCA/FIA) reduced under field conditions • Population stabilized by contraception combined with limited culling
Irondequoit, N.Y. (conducted by SUNY-CESF)	1997	Term. 2001	<ul style="list-style-type: none"> • Effectiveness for population control modeled as a function of effort, deer population attributes
Frelinghuysen Arboretum, Morristown, N.J. (with Morris County Park Commission)	1997	Term. 2000	<ul style="list-style-type: none"> • No useful results; site too small and disturbed, population too transient
Groton Long-Point/Mumford Cove, Conn. (with University of New Hampshire, Conn. Department of Environmental Protection)	1997	Term. 2000	<ul style="list-style-type: none"> • STDCM (RIBI) inconsistent and relatively ineffective as adjuvant (but cf. NIST; methodological concerns) • Costs of contraception for management purposes modeled
Submarine Base Bangor, Silverdale, Wash. (FS 10.1) (with U.S. Navy)	1998	Term. 1999	<ul style="list-style-type: none"> • High, long-lasting antibody titers for PZP/FCA + PZP/QA-21 in lactide-glycolide pellets

all, 17.6 percent of treated does produced fawns, with frequency of pregnancy declining significantly with successive years of treatment (Naugle et al. 2002). Efficacy also improved as delivery techniques improved. Because these animals were not handled, blood was not collected, so antibody titers are not available.

At NIST between 1996 and 2002, 21.4 percent of 295 PZP/FCA/FIA treatments resulted in the production of fawns (Figure 1). There was no trend with respect to number of years of treatment (chi-square = 1.29, $df = 4$, $p = 0.864$). Does first treated as yearlings fawned significantly less frequently (8.5 percent), however, than does first treated as adults (24.0 percent) or as fawns (25.0 percent) (chi-square = 7.32, $df = 2$, $p = 0.026$), and in fact of the sixteen does first treated with FCA as yearlings, only three had had any fawns as of four years post-treatment. Delivering the initial PZP treatment by hand improved efficacy over the first two years of treatment, but the difference disappeared after multiple treatments (Thiele 1999; HSUS, unpublished data). Efficacy rates were not affected by the timing of initial treatments; fawning rates among does receiving their initial injection in winter/spring (5/35, or 12.5 percent) did not differ from those of does receiving their initial injection in fall (2/12, or 14.3 percent) (Thiele 1999).

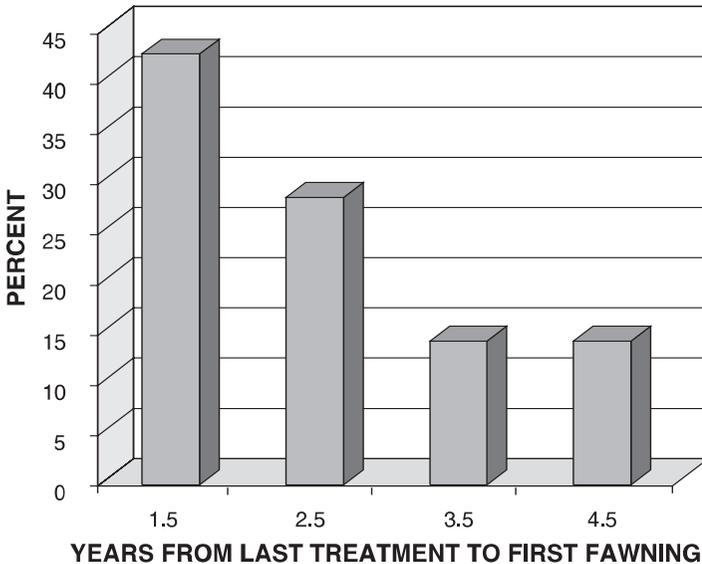
At NIST, annual boosters were stopped in 1999 for twenty-one FCA/FIA deer and reversibility followed for up to four years; 42.9 percent of the does

Figure 1
Fawning Rates of Deer Treated with PZP Plus FCA/FIA at the National Institute of Standards and Technology, 1996–2002



fawned in the spring immediately after boosters ceased, whereas the others remained infertile for up to four years (Figure 2). Sample sizes were too small to determine whether reversibility was influenced by number of treatment years or age at initial treatment.

Figure 2
Reversibility of PZP plus FCA/FIA
Vaccine, National Institute of Standards
and Technology, 1999–2003 (N = 21)



Multiple Shot Vaccines Prepared with Alternative Adjuvants

Adjuvants may boost immune response by several different mechanisms. They may hold the antigen in place for a longer period of time (depot effects); they may process and present the antigen to the surveillance cells of the immune system in a way that amplifies the immune response (presentation effects); or they may include components that are themselves highly immunogenic and that elicit a broad immune response that becomes targeted at the antigen (association effects) (Hanly, Bennet, and Artwohl 1997). Depot and presentation effects are often obtained with adjuvant emulsions; Freund's Adjuvants, for example, are oil-based, and a stable water-in-oil emulsion is created by mixing an aqueous solution of PZP with either FCA or FIA. FCA also contains non-living, non-infective cell wall fragments from the highly immunogenic tuberculosis pathogen, *Mycobacterium tuberculosis*, which adds a powerful association effect. Multiple injections of FIA provide some immune boost, but a much stronger effect is generally found if the priming dose includes FCA.

Initial tests of alternative adjuvants were not fruitful (see Table 2 for description of adjuvants tested). At NIST, the initial shot of PZP/FCA was replaced with PZP emulsified in FIA plus Carbopol® 934P (B.F. Goodrich, Cleveland, Ohio), a polymer resin widely used in the pharmaceutical industry for producing sustained-release effects. This preparation showed a sharply reduced vaccine efficacy, with 74 percent fawning rates (vs. 84 percent among controls, n.s.).

Table 2
Description and Sources of Adjuvants Tested

Adjuvant	Description	Source
Freund's Complete Adjuvant (FCA)	Non-metabolizable oil, detergent, cell wall fragments of <i>Mycobacterium tuberculosis</i> ; forms water in oil emulsions	Sigma-Aldrich, St. Louis, Mo.
Freund's Incomplete Adjuvant (FIA)	Non-metabolizable oil, detergent; forms water in oil emulsions	Sigma-Aldrich, St. Louis, Mo.
Montanide® ISA 50	Similar to FIA, but emulsion more stable and less viscous	Seppic, Paris, France
Carbopol® 934P	Acrylic acid polymer resin used in cosmetics	B.F. Goodrich, Cleveland, Ohio
QA-21	Saponin extracted from bark of tree, <i>Quillaja saponaria</i> widely used as food additive; water soluble	Aquila/Antigenics Framingham, Mass.
Synthetic trehalose dicorynomycolate (s-TDCM)	Metabolizable oil with s-TDCM, a synthetic bacterial cell wall analog	University of Georgia, Georgia, with components from Ribi Immunochem, Hamilton, Mont.
Ribi Adjuvant System (RAS) R-700	Metabolizable oil (squalene) with combination of s-TDCM and MPL® (Monophosphoryl Lipid A)	Ribi Immunochem/Corixa, Hamilton, Mont.
ImmunEasy® CpG- DNA	Short DNA oligonucleotides (cytosine-guanine pairs) in aqueous suspension	QIAGEN, Hilden, Germany

In a small study on a captive herd in Newton, Massachusetts, in 1996–97, five does received PZP and QA-21 (Cambridge Biotech; later, Aquila Biopharmaceuticals; later, Antigenics; Framingham, Massachusetts) in an aqueous solution. QA-21 is a highly purified extract from the tropical tree, *Quillaja saponaria*, a saponin in the widely used Quil-A family of veterinary adjuvants (Kensil 1996). Unfortunately, four out of five QA-21-treated does produced fawns (vs. 0/4 FCA/FIA does). Consistently, in a 2001 study, cap-

tive deer at the University of New Hampshire receiving 100 μg PZP + QA-21 in a controlled-release pellet showed low anti-PZP titers, with only two of five treated does exceeding 50 percent and the remainder peaking below 20 percent.

Another adjuvant family of interest is that produced by Ribi Immunochem (later, Corixa; Hamilton, Montana). The Ribi adjuvants are oil in water emulsions, some of which contain a variety of immune system stimulators, including s-TDCM (synthetic trehalose dicorynomycolate) and bacterial cell wall skeletons. A two-shot preparation of 400 μg PZP emulsified (at the University of Georgia) with s-TDCM was tested at the Mumford Cove field site. In this study, eight of thirteen (62 percent) PZP/s-TDCM treated females fawned, with seven of nine titer levels from blood sampled in March–April below 50 percent (Walter et al. 2002a).

Subsequent tests at NIST of s-TDCM, the Ribi Adjuvant System R-700, which includes s-TDCM as one component, and Montanide[®] ISA-50 (an FIA-type adjuvant with improved emulsion and flow properties; Seppic, Paris) proved much more encouraging. In 1998 a total of twenty-nine does were hand-injected with 65 μg PZP in FCA (winter), 65 μg PZP in ISA-50 (winter), or 200 μg PZP in s-TDCM (late summer), followed in autumn 1998 by remotely-delivered boosters of 65 μg PZP in FIA, 65 μg PZP in ISA-50, or 100 or 200 μg PZP in R-700, respectively. Annual boosters were administered in autumn of each of the following two years. Across three years, fawning rates for FCA/FIA does were 23.8 percent, for ISA-50 does 22.7 percent, and for s-TDCM/R-700 does 3.7 percent (Table 3). Boosters were not administered to six s-TDCM/R-700 does in autumn 2001; only one of the six fawned in 2002, 1.5 years after the last boosting.

Table 3
Percentage of Females Fawning after Treatments of PZP Mixed with One of Three Adjuvants

Adjuvant	1999	2000	2001	Total
FCA/FIA	12.5	0.0	40.0	16.7 (N = 18)
s-TDCM	0.0	11.1	0.0	3.6 (N = 28)
ISA-50	22.2	22.2	20.0	21.5 (N = 23)

(N = number of doe years)

Titer data are also available from this trial of the NIST deer. For s-TDCM/R-700 deer, titer levels (from blood sampled 63–104 days after boosting) ranged from 44 percent to 151 percent, with all but one value (from the doe that produced a fawn) lying above the 50 percent level (Figure 3). In deer who did not receive boosters in 2001 and did not fawn in

Figure 3
Antibody Titer Levels over Time for Deer Treated with PZP + s-TDCM or RAS R-700, National Institute of Standards and Technology, 1998–2002

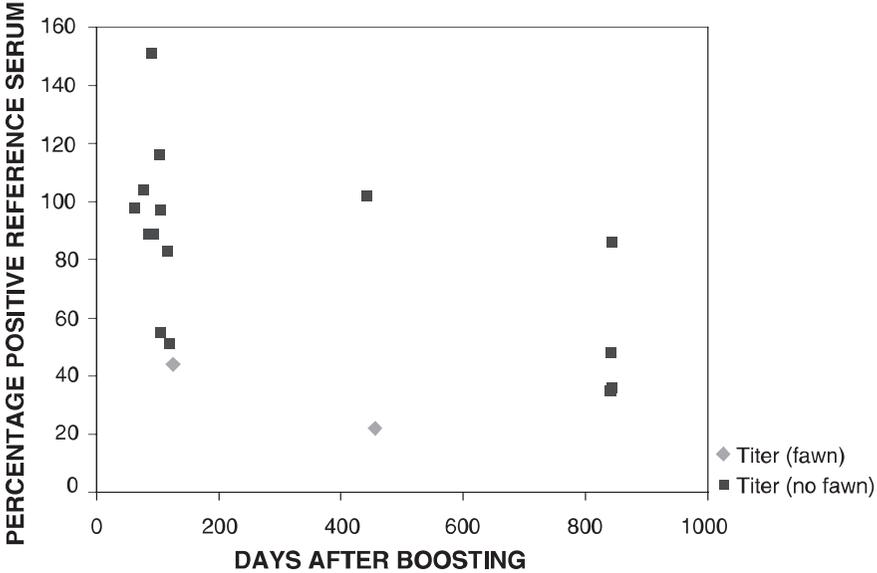
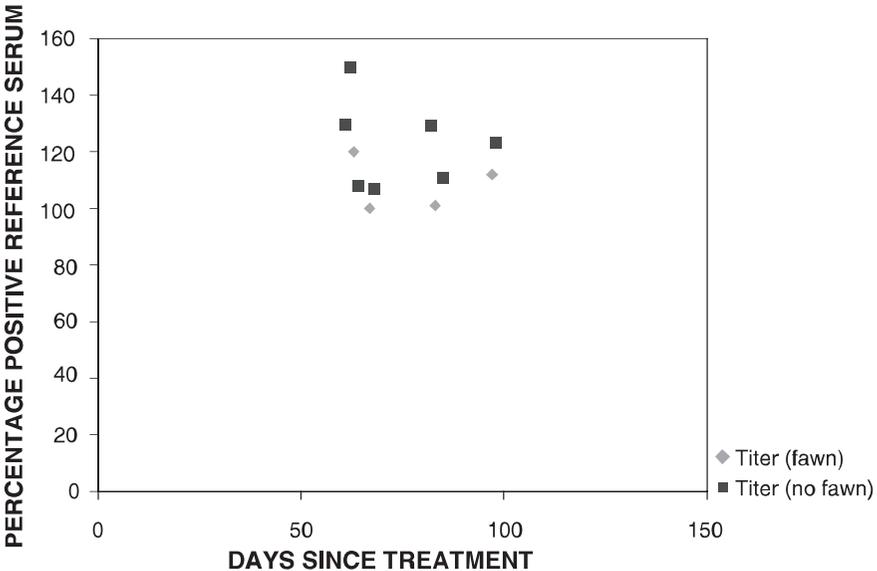


Figure 4
Antibody Titer Levels over Time for Deer Treated with PZP + Montanide® ISA-50, National Institute of Standards and Technology, 1998–2002

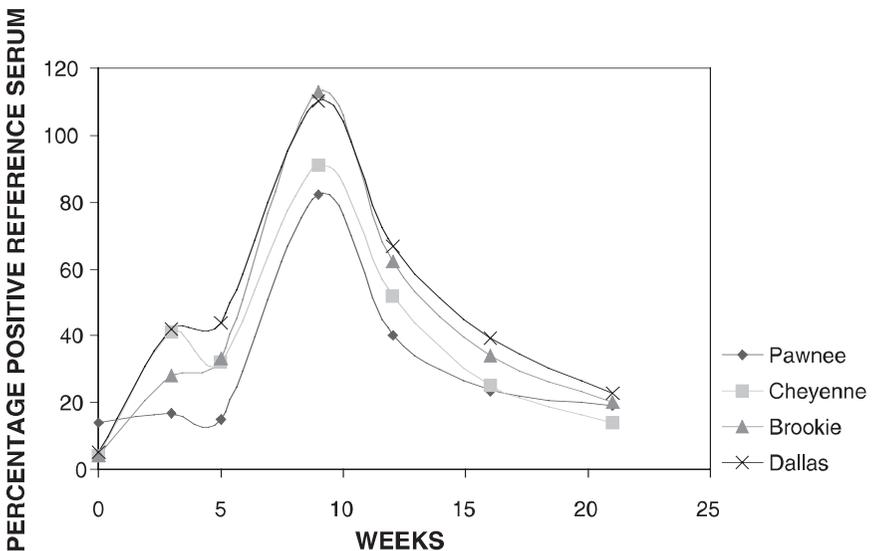


2002, titers obtained in winter 2003 (840–842 days after last boosting) ranged from 35–86. These data, together with those from Mumford Cove, strongly suggest a contraceptive level of approximately 50 percent for Ribí. Failure of the Mumford Cove sTDCM preparation to generate contraceptive levels of titers were very probably due to differences in handling; the preparation of PZP and sTDCM shipped to Mumford Cove from the University of Georgia was stored refrigerated, whereas the PZP sent to NIST was stored frozen until use in the field. Extended refrigeration might have allowed degradation of the Mumford Cove PZP.

Titers for ISA-50-treated does were consistently high between sixty-four and ninety-eight days after treatment, ranging from 101 percent to 151 percent (Figure 4). The fact that four does with titer levels above 100 percent became pregnant, however, suggests that either (1) titer levels declined rapidly for some animals or (2) titer levels are poor predictors of contraceptive efficacy with ISA 50. It has been suggested that, unlike complete adjuvants (such as FCA and R-700), incomplete adjuvants (such as FIA or ISA) act principally on the humoral (Th1) immune system and elicit only weak responses from the cellular (Th2) immune system (Weeratna et al. 2000). This suggests that both immune pathways may be important in achieving contraception.

Preliminary testing has also been carried out with a CpG adjuvant (ImmunEasy™ Plus, QIAGEN, Hilden) on captive deer at the University of New Hampshire (UNH) in collaboration with P.J. Pekins. CpG is an oligonucleotide (short chain of DNA) sequence found naturally in bacterial DNA

Figure 5
Anti-PZP Antibody Titers in Deer Treated
with Two Shots of PZP + CpG (ImmunEasy™)
Adjuvant at the University of New Hampshire



(Measured at the laboratory of J.F. Kirkpatrick, Science and Conservation Center, Billings, Montana)

(but not in mammalian DNA) which was fairly recently shown to have strong immunogenic effects, with minimal side effects (Weeratna et al. 2000). At UNH, five female deer were hand-injected with two shots, five weeks apart, of 65 μg PZP emulsified in 0.5 mL ImmunEasy™ Plus. Titers peaked four weeks after boosting at 82 percent to 113 percent, then declined below 50 percent by sixteen weeks after boosting (Figure 5). The females were not bred, so fawning data is not available.

In summary we have had the best success with two-shot vaccine preparations of PZP in s-TDCM and Ribi Adjuvant System R-700, with high efficacy, sustained high titers, and potential multi-year application. CpG adjuvants may also prove useful; we have not yet tested Modified Freund's Complete Adjuvant (which substitutes the common soil bacterium *Mycobacterium butyricum* for *M. tuberculosis*) in white-tailed deer.

Conjugate Studies

Another way to boost immune response to an antigen is to chemically conjugate that antigen to a more immunogenic molecule. Only one limited study has been carried out with PZP conjugates in deer, in collaboration with Pekins. In 1998–99, five does captive at UNH were treated with one shot of 400 μg PZP conjugated to KLH (keyhole limpet cyanin) emulsified in s-TDCM, plus one shot of 65 μg PZP in FIA. Contraceptive titers were achieved only transiently (one–two months) for the five conjugate does, and all three of the PZP/FCA/FIA positive controls showed consistently and considerably higher titers than all five conjugate does. As in the case of deer treated with s-TDCM-adjuvanted vaccines at Mumford Cove, however, the handling of the conjugated vaccine received from the University of Georgia may have been problematic.

Sustained Release Technologies

One strategy for creating a one-shot vaccine is to use sustained release preparations to mimic a multi-shot antigen exposure. Lactide/glycolide polymers, which are the materials from which dissolving surgical sutures are manufactured, are especially suitable for use as a sustained release vehicle because of their safety record and because they can be engineered to release antigens at different times. This technique has proven very successful in producing a long-term, one shot PZP treatment for horses (Turner et al. 2002).

In the initial trial with deer, only two of seven captive white-tail does (29 percent) treated with a one shot preparation of PZP/FCA + PZP in lactide-glycolide microspheres became pregnant (Turner, Kirkpatrick, and Liu 1996). These results were supported by a small 1993–94 study of captive does at UNH, in which two of three does hand-treated with a single shot of 65 μg PZP in FIA plus 65 μg PZP in lactide-glycolide microspheres produced contraceptive-level titers for nine months or more (while the third doe failed to produce contraceptive titers at all).

Under less controlled conditions, however, seven of nine does (77.8 per-

cent) treated remotely with PZP/FCA + PZP in microspheres became pregnant (McShea et al. 1997). Other attempts to elicit significant titers or prevent fawning using controlled-release pellets have also failed. At NIST, hand injections of 65 μg PZP/ISA-50 + PZP/QS-21 pellets and 65 or 200 μg PZP in Ribi R-700 + 367 μg PZP in nanoparticles did not produce significant titer levels or prevent fawning, and, as noted above, PZP/QS-21 pellets hand injected into captive deer at UNH did not elicit good titer levels.

Turner, Kirkpatrick, and Liu (1996) demonstrated that the one-shot, controlled release technology could work in principle in deer. Consistent success with single shot vaccines has remained elusive, however, in part perhaps because of poor injection quality in remotely-delivered treatments.

Safety and Side Effects

The evidence so far suggests that harmful side effects of PZP vaccines in deer are rare and minor.

At FIIS, approximately 60 percent of deer remotely darted with PZP/FCA developed sterile granulomas 2–8 cm in diameter at the injection site. Two of 353 treated deer developed abscesses that drained and healed in five to fourteen days with no long-term effects (Naugle et al. 2002). No adverse health effects or unusual mortality was reported for treated deer. Anecdotally, no abscesses or granulomas have been observed at NIST among deer treated by hand with any adjuvant or among deer treated with ISA 50 or RAS R-700 adjuvants. One deer with a history of being treated with FIA/Carbopol, FIA, and FCA developed an injection site granuloma and a large, persistent lump following a fourth treatment with FIA, but these reactions disappeared, and no ill-effects were apparent one year later (R.E. Naugle, personal communication).

In the semi-captive Front Royal (Virginia) herd, McShea et al. (1997) observed that PZP-treated females and the males with them extended their mating season as late as March, exhibiting elevated activity levels, but the contracepted-nonpregnant does gained more weight than the control females, suggesting that the metabolic costs of elevated activity were more than compensated for by the elimination of fawning and lactation. Histological examinations yielded no evidence of differences in ovarian structure between treated and untreated females. Walter, Kirkpatrick, and Gregonis (2003) found no differences in weight or body condition between treated and untreated females in the Groton-Long Point study. Underwood, Verrett, and Fischer (1998) observed evidence of a strong secondary birth peak in late summer at FIIS, suggesting an extended breeding season there as well, but this secondary peak has subsequently disappeared (H.B. Underwood, personal communication).

Behavioral observations at NIST have yielded anecdotal evidence for an extended breeding season; the observations are supported by results from the 1997–1998 birth season, which indicated that the birth date of fawns

born to PZP/FCA-treated females was significantly later than those born to untreated females (Thiele 1999). Treatment of pregnant does with PZP/FCA/FIA at NIST did not affect ongoing pregnancies or the survival of offspring, nor did the survival to one year of fawns treated within a week of birth differ from that of untreated fawns (Thiele 1999). Every doe given an initial hand injection of PZP/FCA ($N=8$) or PZP/ISA 50 ($N=14$) in winter 1998 (i.e., during gestation) gave birth to a healthy fawn that spring.

Body Condition at NIST

No systematic study of the effects of PZP treatment on body condition has been carried out at NIST. Body condition scores, however, are available at treatment and at capture one year after treatment for eight does treated with PZP/FCA or PZP/ISA 50 in the winter of 1998. Five of the eight showed increased body condition scores, two stayed the same, and one declined. Moreover, contraception treatment failed for the one who declined in condition and for one of the two who stayed the same; of the six successfully contracepted females, five increased body condition scores and one stayed the same.

Bone-marrow scores at necropsy are available from fifty-five treated and nineteen untreated adult (2+ years) females who died at NIST between 1994 and 2003. Bone-marrow scores of treated adult females were on average slightly (but not significantly) better than those of untreated adult females (treated females, $M = 2.27$, untreated females, $M = 2.89$, $t = 1.317$, $P = 0.192$). When only adults known to have been killed by automobile collisions are included (arguably a more random sample), the difference grows but remains non-significant (treated females, $M = 1.64$, untreated females, $M = 3.67$, $t = 2.207$, $P = 0.087$; only six untreated females remain in the sample). Although sample sizes are small, no difference between adjuvants is apparent.

Thus, the data from NIST, McShea et al. (1997), and Walter, Kirkpatrick, and Gregonis (2003) indicate that PZP-treated female deer are in no worse condition, and in some circumstances may be in better condition, than untreated female deer.

Field Applications

Most data on field applications of immunocontraception in deer have been produced by The HSUS and its collaborators.

Cost and Effort

The cost and effort required to deliver PZP in the field have varied sharply by study site, circumstances, and project objectives (the need for repeated blood draws, for example, increases project costs). So far, the FIIS study

has been the cheapest and most efficient. At FIIS the deer are highly accessible and have been treated without first being captured. Beginning in 1997 we began to use marking darts (Pneu-Dart™, Williamsport, Pennsylvania), which simultaneously vaccinate and place a dye spot on the darted animal, thus making individual recognition unnecessary for darting purposes. Darters at FIIS spent an average of 1.0 hour/treatment and 1.4 hours/deer (Naugle et al. 2002). Treating unidentified deer with marking darts was more efficient than treating individually known deer (0.5 hour/treatment vs. 1.4 hours/treatment). Excluding labor, costs averaged \$64 per treatment. Darting efficiency was slightly lower at NIST; at NIST, contraceptive darts required on average 1.6 hours/deer to deliver. Chemical captures, however, required an average of 12.0 hours/deer, which added considerable time to the field effort (Rutberg et al. 2004).

Costs were considerably higher at the Mumford Cove study site, principally because of reduced darting efficiency, the need to dart different deer with different preparations, the effort needed to capture deer repeatedly for tagging and blood sampling, and the deer's wariness that resulted from repeated captures. Contraceptive darting at Mumford Cove required an average of 5.6–17.5 hours/treatment (varying widely between years), and chemical captures required 7.5–39.0 hours/capture, with capture efficiency generally greater in the spring (13.2–15.9 hours/capture) (Walter et al. 2002b). When costs of equipment, supplies, labor, lodging, and travel were compiled, cost per deer was \$1,128 over two years, with most costs (\$960) incurred in the first year and labor accounting for 63–66 percent of total costs.

In the Irondequoit study, the darting effort averaged 2.5–6.7 hours/treatment (Rudolph, Porter, and Underwood 2000). Capture times ranged from 9.8–21.5 hours/capture. Both capture times and darting times displayed a statistically significant dependence on local deer density (e.g., an estimated 4.4 hours/capture at 50 deer/km²) (Porter and Underwood 2001).

Population Effects

Reduction of fawn production and population size or density have been demonstrated at FIIS and NIST. These sites, along with the Assateague Island wild horse field site (Turner and Kirkpatrick 2002), represent the only studies conducted so far that demonstrate clear effects of contraception on wildlife populations.

In 1999–2000 surveys at FIIS, fawns comprised 13–14 percent of the population in the most heavily treated areas versus 16–33 percent in nearby untreated areas (Naugle et al. 2002). Population density in the most heavily treated area (Kismet-Lonelyville) initially increased by 11 percent per year from the time the surveys started in 1995 to a peak in March 1998 but then decreased at 23 percent per year through October 2000. This decline continued through 2002, though less markedly, and current population

Figure 6
Estimated Deer Population Density,
Kismet-Lonelyville, Fire Island
National Seashore, 1995–2000

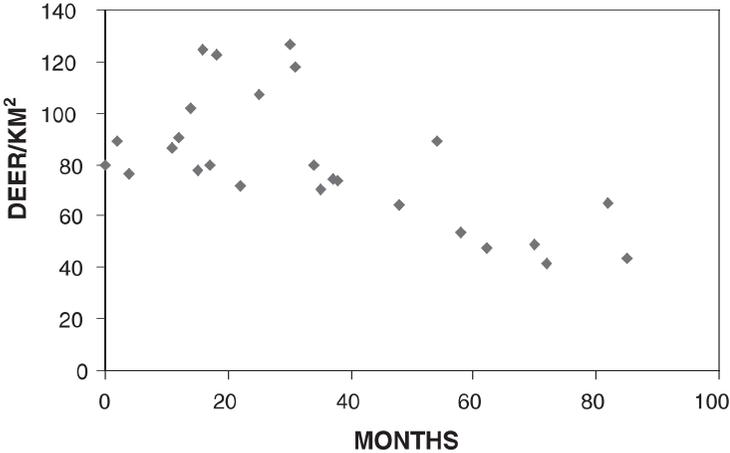
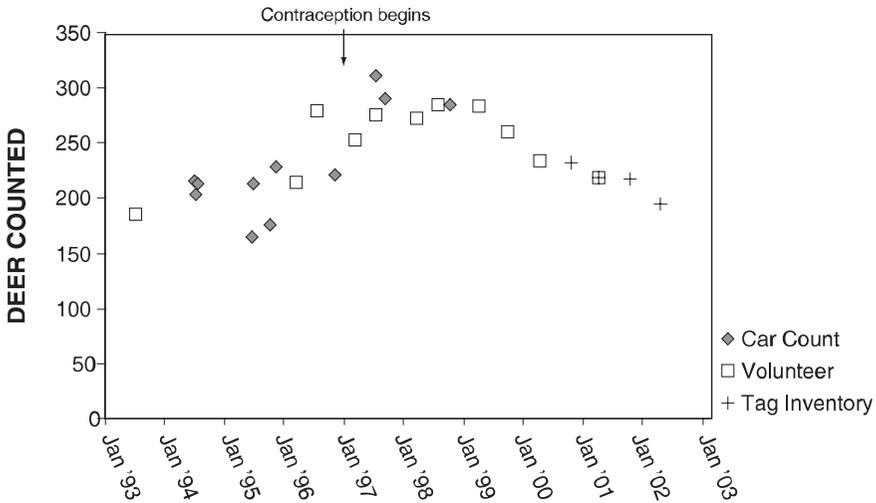


Figure 7
Deer Population Size at the National
Institute of Standards and Technology,
Gaithersburg, Md., 1995–2002



densities in Kismet-Lonelyville are approximately 55 percent of what they were when surveys began in 1995 and approximately 41 percent of what they were at their peak in 1998 (Figure 6). Population densities have not changed consistently in other treatment areas, but changes in deer numbers in some of these areas may be obscured by deer movements in and out of treatment areas and by reduction of available habitat due to construction of deer fencing (H.B. Underwood, unpublished data, personal communication). In other areas, contraception may be failing to affect deer densities because seasonal movements of deer may prevent some females from receiving treatments, an issue that will be addressed in future research.

At NIST, the number of fawns produced in the population declined from approximately 1.08 fawns per female in 1996, the year immediately prior to the onset of contraception, to a low of 0.26 fawns per female in 2001 (Rutberg et al. 2004). Population size increased by approximately 10.6 percent per year between 1993 and 1997 but then declined by approximately 7.9 percent per year between 1997 and 2002. The population size peaked following the initiation of PZP treatments in autumn 1996 and the immigration of 20–45 deer in 1997 from an adjacent parcel of land that had been cleared for development (Figure 7). Changes in population size were significantly (and negatively) correlated with the proportion of females treated, with the regression line indicating that population stabilization is achieved at NIST when approximately 58 percent of the females are treated (Rutberg et al. 2004).

It remains to be seen whether deeper reductions in deer population numbers can be achieved at FIIS and NIST. At both sites, further reductions will probably require an overall increase in the efficacy of vaccines used. Together, however, these studies have firmly refuted the common presumption that contraception alone cannot reduce deer populations.

PZP Studies by Other Investigators

Published literature by other investigators describing tests of PZP on deer is sparse. Miller, Johns, and Killian (2000) confirmed the efficacy of PZP/FCA + PZP/FIA in reducing fertility in captive white-tailed deer, although the workers used 500/300 μg doses injected subcutaneously in multiple sites in the back musculature. The study reported extended mating seasons among treated animals. In the first two years, PZP-treated animals failed to show the elevations of serum progesterone seen in control females, although progesterone increases in treated animals reappeared subsequently. Infertility was seen for as long as four years without boosting. No effects of PZP treatments on blood chemistry, health, body weight, or survival were detected (Miller et al. 2001).

Fraker et al. (2002) hand-injected forty-one free-ranging island fallow deer (*Dama dama*) with a single shot of 100 μg PZP in the form of Spay-Vac® (IVT, Halifax) and FCA. No pregnancies were found among the twenty-

two Spay-Vac[®]-treated does examined over a three-year period (multi-year data were not available for any individual animal). No adverse effects of treatment were observed, although Spay-Vac[®]-treated animals carried significantly less kidney fat than untreated animals. (Spay-Vac[®] effects on kidney fat could not be discriminated from effects of pregnancy.) This represents by far the most encouraging study of a one-shot, multi-year vaccine, although questions of species and adjuvant remain to be resolved. Testing of Spay-Vac[®] on white-tailed deer with Adju-Vac[®] (a diluted Johnes vaccine from Fort Dodge; USDA, Fort Collins, Colorado) on captive and free-ranging deer is underway (M. Fraker, A. DeNicola, personal communication).

Conclusions and Recommendations

The two most important accomplishments of the HSUS deer contraception research effort so far is the identification of the Ribi Adjuvant System as a suitable (and possibly more effective, longer-lasting) replacement for FCA and FIA and the demonstration that PZP can reduce deer population size. From a political and practical viewpoint, the second accomplishment is the more important; the demonstration of a reduction in deer numbers, published in peer-reviewed scientific journals, builds an unassailable base of credibility for deer contraception as a management tool.

In the future, the most valuable role The HSUS can play is to continue to test the limits of field application. How far down can deer populations be pushed and how fast? What kinds of sites are amenable to deer population control through deer contraception? And what are the values of the population parameters—rates of mortality, fertility, immigration, and emigration—that allow a deer population to be controlled by contraception?

The HSUS should continue the quest for more effective, longer-acting vaccines. We have yet to see how long vaccines using the Ribi Adjuvant System may last, or to incorporate components of Ribi Adjuvant System or other adjuvants into controlled-release pellets. At all times The HSUS—and other investigators—should aim for simplicity and practicality of application; these elements will be crucial to successful and widespread distribution and field use.

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The Makalali Elephant Immuno- contraception Program

Audrey Delsink, Douw Grobler, and J.J. van Altena

Introduction

Most biologists agree that some form of intervention is necessary when managing elephant populations due to the destruction of habitat that may arise if elephant numbers are left unchecked. Conservation and management of the African elephant can only be conducted in light of a good understanding of the elephants themselves, based on their distribution, density, movements, and behavior; their impact on the ecosystems; and their response to contact with humans at the human-wildlife interface (Kangwana 1996). Elephants have been studied exhaustively. As a result of this extensive research, perceptions have changed and many biologists and wildlife managers no longer view translocation and culling as acceptable or ethical forms of elephant population control (Whyte, van Aade, and Primm 1998). A new population control method that achieves ecological goals, remains publicly acceptable, and maintains humane standards is necessary. Developing such a method seems impossible, and yet the answer may lie in the form of immunocontraception. For the past twenty years, immunocontraception has been tested extensively

on various species (Kirkpatrick and Turner 1996) and recently has been tested on African elephants (Fayrer-Hosken et al. 2000).

What is Immunocontraception?

Immunocontraception is a non-hormonal, non-steroid method of contraception based on the use of porcine zona pellucida (PZP) proteins. When administered, the PZP vaccine creates an immunological response within the target animal. An antibody layer forms around the egg cell, which binds to and blocks the sperm receptor sites, thus preventing penetration of the sperm cell and successful fertilization. The surface structures of the elephant zona pellucida are very similar to those of the pig zona pellucida, and when female zoo elephants were vaccinated with PZP vaccine and an adjuvant, all developed antibodies that persisted for 12–14 months (Fayrer-Hosken et al. 1997). These results were at a level equivalent to results found in horses treated with the immunocontraceptive (Fayrer-Hosken et al. 2000).

Studies at the Kruger National Park

On the basis of these promising results, field trials were conducted in South Africa's Kruger National Park (KNP) in October 1996. The study was a three-phase trial designed to test the efficacy of PZP as an immunocontraceptive in African elephants and to evaluate dosage and administration regimes. The initial trials included forty-one adult elephant cows: twenty-one treated elephants and twenty untreated control elephants. All the treated cows received an initial vaccination of 600 μg (micrograms) PZP plus s-TDCM adjuvant (synthetic trehalose dicorynomycolate, Ribi Immunochem Research, Montana). Each animal was fitted with a radio collar to allow for monitoring and follow-up treatment, and approximately four weeks later, each animal received a PZP/s-TDCM booster delivered remotely from a helicopter. Twelve months after the initial vaccinations, the elephants were recaptured and scanned for pregnancy through transrectal ultrasonography (except for two elephants whose radio collars had failed). Significantly fewer vaccinated elephants (8/18, or 44 percent) were pregnant than were control females (16/18, or 89 percent; $p = 0.005$; Fayrer-Hosken et al. 2000). One of the treated elephants was in the last trimester of pregnancy and gave birth to a healthy calf, suggesting that the PZP vaccine has no effect on gestation and is safe for the fetus and for parturition.

Subsequently ten elephants were vaccinated with 600 μg PZP/s-TDCM using a revised schedule: an initial vaccination followed by identical boosters at two and four weeks after the initial injection. As with the initial trials, all treated elephants were fitted with radio collars; five of the collars were equipped with global positioning satellite (GPS) transmitters (Lotek,

Newmarket, Canada). Under the revised schedule, only two elephants (20 percent) were pregnant after ten months, which was lower (but not significantly; $p = 0.20$) than the pregnancy rate of the elephants treated under the previous schedule.

These trials also tested the reversibility of the immunocontraceptive and its effectiveness following treatment for a second consecutive year. The ultrasound results showed that all the females left untreated for a second year conceived, compared with none of the treated females (even though the treated females were cycling [Fayrer-Hosken et al. 2000]). This proved that PZP treatment is reversible in elephants; once the immunization is stopped and the blood antibody concentrations have fallen, the cow gradually recovers normal fertility and may once again conceive. Furthermore, treatment using PZP vaccine caused no deleterious effect on the ovary and its cyclicity (Fayrer-Hosken et al. 2000).

Elephants have been referred to as charismatic megafauna, and their highly social natures may be among the most advanced in the animal kingdom (Fayrer-Hosken et al. 1999). Accordingly, field observations to monitor the vaccinated elephants' behavior were initiated. This was facilitated by the presence of radio collars on all treated animals. During the KNP trials, no disruption of social organization or other aberrant behavior in PZP treated elephants was observed (J.v.A., personal observation). This was contrary to the behavior displayed by elephants treated with estradiol implants, who separated from their family groups and, in some cases, even their calves (J.v.A., personal observation).

The Makalali Project

With the KNP trial completed and proven to be highly successful, the next phase of the project was initiated. This involved the development of a strategy to use the vaccine to control free-ranging elephant populations. A discrete population of free-roaming elephants at the Greater Makalali Private Game Reserve (GMPGR), Limpopo Province, South Africa, was identified for this experiment. Due to its manageable size (sixty-six animals in 2004), accessibility (due to the elephants' habituation to vehicles as a result of game-viewing), and the availability of detailed information on individual elephants, the Makalali elephant population was ideal for this next phase (Delsink et al. 2002). The individual records of all the elephants, including photographic identifications, would allow for easy follow-up vaccinations and observations from which extensive records and important data could be gathered.

This study was undertaken to test the following hypotheses (Delsink et al. 2002):

- The porcine zona pellucida vaccine (PZP) could be successfully delivered to free-roaming elephants in a small game park.
- PZP treatment would not alter selected social behaviors.

- Treatment of pregnant females with the PZP vaccine would not harm pregnancies in progress or affect the health of the offspring.
- PZP immunocontraception could reduce the rates of population increase and stabilize elephant population numbers in a small game reserve.

Methods

In May 2000 (Year One) all sexually mature cows (more than twelve years old) were selected for vaccination. Because the KNP trials and work on other species indicated that PZP does not affect gestation, the fetus, or parturition (Fayrer-Hosken et al. 2000, and above), pregnancy status was not used as a criterion for selection in the Makalali study. Following the vaccination schedule used in the second set of KNP trials, eighteen females received an initial booster of 600 μg of PZP and 0.5 ml of Freund's Modified Adjuvant (FMA) followed by the administration of two boosters with the same antigen emulsified in Freund's Incomplete Adjuvant (FIA), 2–3 weeks apart (Delsink et al. 2002). All target animals received boosters in July 2001 (near the anniversary of the date of the second booster), and two additional cows were added to the vaccination regime at that time. In August 2002 the second annual booster vaccinations were administered, and three additional cows were added to the program. Because these cows had recently calved, only a single booster was administered. This brought the number of females contracepted with the PZP vaccine to twenty-three.

For minimal impact and stress on the herds, the target females were darted from the ground (i.e., on foot or from a vehicle) using barbless needles fired from the Dan-Inject® darting system (Dan Inject® International, Denmark). The barbless needles allow the darts to fall out soon after impact due to the movement of the muscle, making it unnecessary to anaesthetize the animal to administer the vaccine or recover the dart. To verify the success of vaccine delivery, we attempted to recover and check all darts. In the case of an incomplete vaccine delivery, the animal was revaccinated (Delsink et al. 2002).

Until 2003 no elephants were fitted with radio collars. As a result, the herds were located using traditional tracking methods of assessing dung and spoor age. Once the herds were located, the target animal was identified according to her unique identifying features. These included ear patterns, such as nicks, tears, and holes, as well as tusk shape, length, and absence. Ear venation patterns were used to identify an individual if no obvious distinguishing ear or tusk features were present (Delsink et al. 2002).

In 2003, due to Makalali's imminent expansion by 8,000 ha, the research team anesthetized and radio-collared four of the target animals to facilitate and maintain the high level of monitoring. During their anesthetization, transrectal ultrasonographs were conducted to determine pregnancy status and reproductive health. A helicopter was used to facilitate these procedures. While the services of the helicopter were available, the

research team administered some of the third annual vaccinations from the air, thereby reducing the time spent darting from a vehicle or on foot.

Behavioral, reproductive, and spatial monitoring of the Makalali elephants has been underway since 1998. As a result, an extensive database exists on the animals' seasonal movements, herd associations, bull/cow interactions, and reproductive behavior. Since the PZP project's inception, the herds have been monitored daily to document any effect of PZP on social behavior and organization. Behavioral observations included focal samples, in which the observer focuses on one animal for a given amount of time (Moss 1988). Ranges of animals from different age groups were monitored, irrespective of whether or not they had been vaccinated. To monitor any fragmentation or change within the family group, among herds, or between bulls and cows, the numbers and identities of all animals present were recorded. Furthermore, the date, time, herd matriarch, mood, weather, and presence of bulls were noted. Sexual behavior (i.e., the presence of musth or signs of estrus and their associated behavior) was carefully monitored and recorded. To monitor change over time, the elephants' home range patterns were determined from systematic sightings of the animals by recording the geographic locations obtained with a GPS receiver (Delsink et al. 2002).

Results

Between 2000 and 2002, twenty-three animals received primary and secondary injections, and annual boosters. Of these, seventeen have calved since the project's inception in May 2000 (Figure 1). Three animals, however, were vaccinated only after the birth of their first calves, and the remaining fourteen were pregnant prior to PZP treatment. Based on the vaccination anniversary of these cows and the known birth date of their calves, the gestational stage at the time of initial vaccine administration ranged from one to twenty-one months. The last calving by a target animal (she was pregnant prior to contraception) was in August 2002. Six target animals had not calved since their initial vaccinations in May 2000, a period of 3.8 years at the time of writing (A.K.D., personal observation).

To date, the only observed effects on behavior have been an increasing wariness toward the vehicle used for darting and a change in the herd's spatial utilization, primarily during the administration of vaccinations. Soon after completion of the initial vaccination program, however, the animals resumed normal movement patterns and were relaxed around the vehicles (Delsink et al. 2002). We saw no separation of vaccinated females from family groups because of bull harassment or any other reason. Apart from one incident in which a newborn calf was killed in an unexplained attack by a young bull elephant, all the new calves remained healthy and displayed no abnormalities, all cow/calf interactions were recorded as normal, and no other unusual or aberrant social behaviors were recorded (A.K.D., per-

Figure 1
Number of Calves Born to Vaccinated Cow Elephants at the Greater Makalali Private Game Reserve, South Africa, 2000–2003

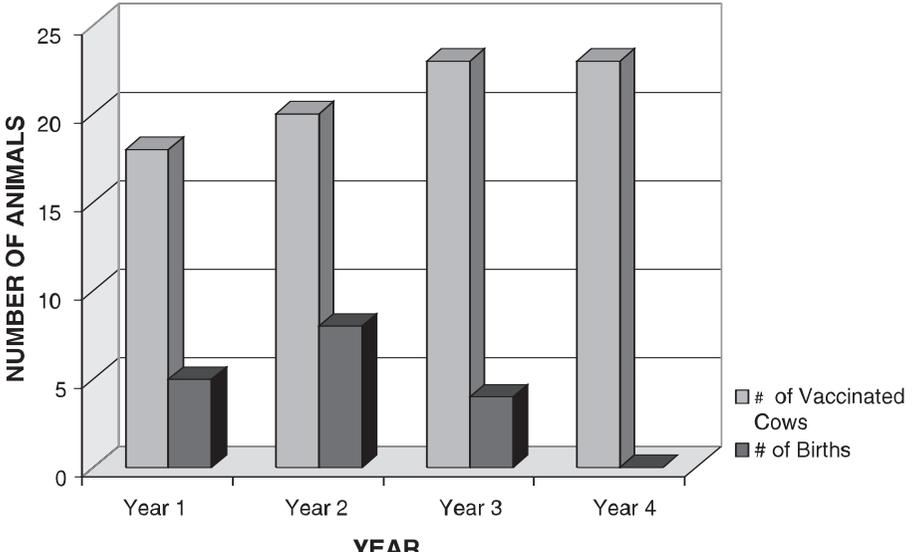
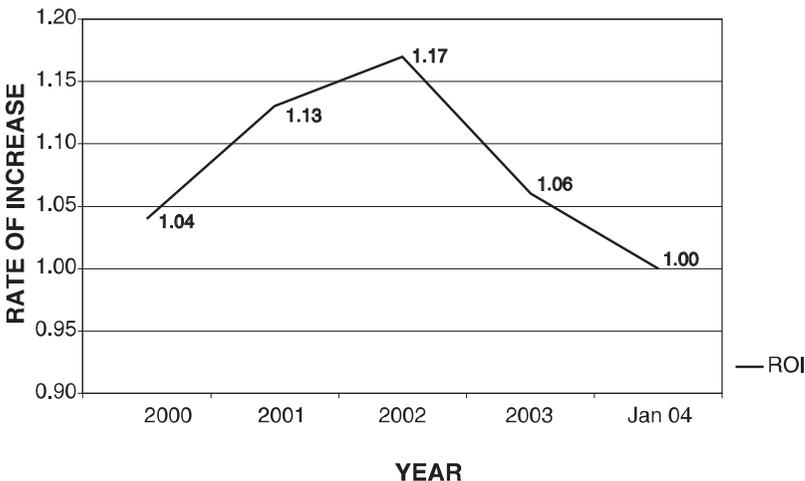


Figure 2
Rate of Increase in the Population of Elephants at the Greater Makalali Private Game Reserve, South Africa, 2000–2003



sonal observation). Estrous behavior has been observed in some of the target animals, and normal bull/cow interactions (including one observed mating) have occurred (A.K.D., personal observation).

As of 2004 82 percent (23/28) of Makalali's breeding population of females had been contracepted. Since the project's inception in 2000, the population's rate of increase (ROI) declined steadily and was at 0 percent for the seventeen-month period between August 2002 to January 2004 (Figure 2).

Discussion and Summary

The KNP and Makalali studies have conclusively demonstrated that, when target females can be individually identified, the PZP vaccine can be successfully and efficiently delivered remotely to free-roaming elephants in both large and small game parks (Fayrer-Hosken et al. 2000; Delsink et al. 2002). As witnessed by the administration of the third annual vaccinations at Makalali, the use of a helicopter dramatically increases the efficacy of immunocontraception by eliminating the time spent tracking animals on foot and allowing a larger number of animals to be vaccinated in a shorter period.

Contraceptive efficacy at Makalali was comparable to or better than that achieved in the second round of studies at KNP, in which 8/10 cows were contracepted for ten months (above).

As in the KNP trials, in which a healthy calf was born from a cow vaccinated in the last trimester of gestation (Fayrer-Hosken et al. 2000), the Makalali calves were born after their dams were vaccinated in various stages of gestation. This provides further evidence that treatment of pregnant females with the PZP vaccine does not harm pregnancies in progress or affect the health of the offspring.

Trials with estradiol implants at KNP resulted in separation of cows from their natal herds and their calves due to excessive bull harassment (J.v.A., personal observation). Preliminary observations at KNP and daily behavior observations over four years at Makalali indicated that no such disruptions accompany PZP vaccinations (Fayrer-Hosken et al. 2000; Delsink et al. 2002; A.K.D., personal observation). Thus, it is absolutely crucial to distinguish between the different contraceptive technologies when evaluating side effects.

With the last elephant calf having been born at Makalali in August 2002, the absence of pregnancy among the four cows examined through transrectal ultrasonography, and estrous behavior being observed in some of the targeted cows (who, had they not received the vaccinations, should be pregnant or should have calved again), it appears as though reproductive control has been achieved in the targeted female population. The increase in number of elephants treated with contraceptives and the effectiveness of the treatment are clearly associated with the reduction of the net population ROI, with a 0 percent increase in the last seventeen months of the study.

Thus, the PZP vaccine can reduce population increase rates and stabilize numbers of elephants in small game reserves.

The advisors and participants of this project include the following:

The Humane Society of the United States.

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The Captive Animal Fertility Control Program

Kimberly M. Frank

Introduction

There are several rationales for fertility control in wild animal populations held captive in zoos and aquaria. First, the production of surplus captive wild animals and acceptable management of these animals has become a serious problem for zoos (Lacy 1991). Second, fertility inhibition may be indicated for specific animals because of poor health, undesirable genetic traits, age, or behavioral problems (Kirkpatrick and Turner 1991). Because of the need for contraception in some captive populations, the American Zoo and Aquarium Association (AZA) formed the Contraception Advisory Group (CAG) in 1991. Goals of the CAG include (1) making recommendations regarding what form of contraception to use for specific species, (2) educating the public regarding this issue, and (3) educating the zoo community about the importance of contraception for responsible management of zoo collections (Asa 1993).

In the early years of zoo animal contraception, zoos almost exclusively used progestin-based steroid implants (melengestrol acetate, or MGA). Although these worked well, fifteen years of data demonstrate that serious

pathologies can result from the use of MGA. Kollias, Calderwood-Mays, and Short (1984) demonstrated an association between MGA implants and diabetes mellitus and abdominal adenocarcinoma in big cats. Linnehan and Edwards (1991) also found an association with endometrial adenocarcinoma in tigers implanted with MGA. The implants are still used today, but a great deal more caution is exercised (Asa and Porton 2004). For example, the MGA implant may not be used in felids for more than two consecutive years. Human contraceptives are generally used safely and effectively in captive primates.

Data collected to date suggest that porcine zona pellucida, or PZP, may be a safer alternative for certain species. A noncellular membrane known as the zona pellucida (ZP) surrounds all mammalian ova (eggs). The ZP consists of several glycoproteins, at least one of which (ZP3) is thought to be a primary component of the sperm receptor in most mammalian species (Dunbar et al. 2002). The PZP vaccine is derived from porcine (pig) ovaries; the ova are isolated, the ZP are separated from the ova, and the glycoprotein is solubilized and delivered to target animals intramuscularly. The principle of PZP immunocontraception involves stimulating the animal's immune system to produce antibodies, which will interfere with sperm attachment to the protein receptor on the ovum. When the PZP is injected into the target animal, the animal produces antibodies against the antigen (PZP). These antibodies also attach to the target animal's own ZP3, thus distorting the sperm receptor and blocking the sperm from fertilizing the ovum (Kirkpatrick 1995). Porcine ova are used primarily because pigs are readily available in processing plants across the United States and because pigs produce many ova per ovary.

The application of PZP immunocontraception to captive zoo animals poses a new problem. Because the molecular structure of the PZP molecule is so similar to the endogenous ZP receptor of the target species, the vaccine is a very poor immunogen. Consequently, a powerful adjuvant must be used along with the PZP. In wild horses, the adjuvant of choice is Freund's Complete Adjuvant (FCA). This adjuvant has been recognized as the "gold standard" for vaccine adjuvants (Bennet et al. 1992). FCA, however, contains cell wall fragments from *Mycobacterium tuberculosis*, and for those species for which a reliable tuberculosis (TB) test exists, FCA-treated animals can show false positive tuberculosis tests. Furthermore, data (derived almost exclusively from laboratory animals) indicate that FCA can cause injection-site reactions and even abscesses (Broderson 1989).

The use of FCA in horses is not a problem, because no reliable TB test exists for equids and, as long as the vaccine is given exclusively in the hip or gluteal muscles, injection-site reactions are extremely rare (Frank and Kirkpatrick 2002; Turner and Kirkpatrick 2002).

To overcome this problem with zoo animals, the adjuvant protocol of choice is either three inoculations with Freund's Incomplete Adjuvant (FIA), which is less powerful than FCA but which contains no *M. tuberculosis* components and causes no false positive TB test results, or Freund's Mod-

ified Adjuvant (FMA), which substitutes the innocuous *M. butyricum* for *M. tuberculosis* and appears to be comparable to FCA in effectiveness (Deigert et al. 2003) without the risk of false positive TB tests.

Growth of the Zoo Immunocontraception Program

Of all wildlife contraceptive programs we have attempted, the zoo program has kept The Science and Conservation Center (Billings, Montana) the busiest. Since the onset of the zoo program in 1990, the Center has gathered a large body of information on efficacy and safety across a variety of species.

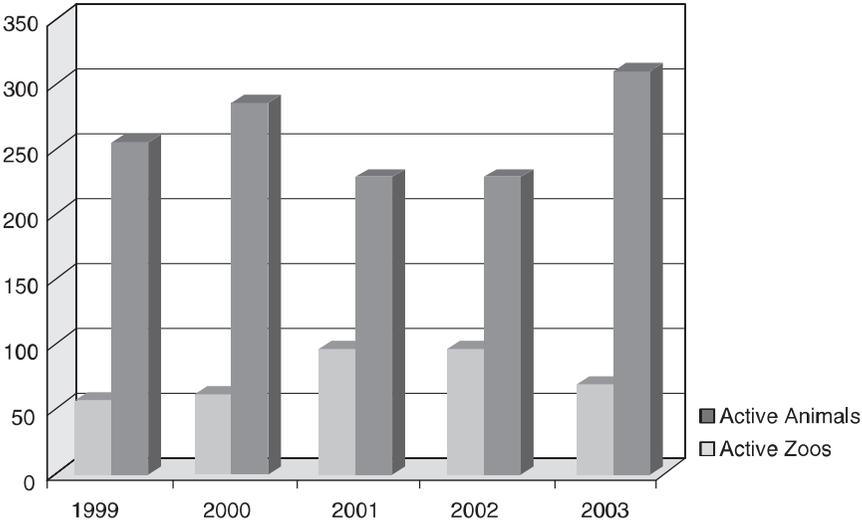
In 1989 J.F. Kirkpatrick reported on the success with PZP in horses at the annual meeting of the AZA. It was those successful trials with wild horses that stimulated the idea to try the PZP vaccine in zoo animals. PZP met the criteria for an ideal contraceptive for wildlife, which are (1) very high contraceptive efficacy, (2) remote delivery, via darts, (3) reversibility after short-term use, (4) effectiveness across many species, (5) no debilitating health side effects, (6) no passage through the food chain, (7) safe to give to pregnant or lactating animals, and (8) no alteration of natural behaviors (Kirkpatrick et al. 1997). Many in the zoo community were particularly enthusiastic because the vaccine could be delivered remotely, eliminating the need to anesthetize and further stress the animals.

The first zoo to use PZP was the Köln (Cologne) Zoo in, Germany, in 1990. Zoo managers were looking for a contraceptive to use on captive Przewalski's horses. The MGA implant was originally recommended, but the PZP vaccine was ultimately chosen because of the success with Assateague Island (Maryland) wild horses. The study included the Przewalski's horses and banteng (a species of Southeastern Asian wild cattle). The PZP worked in both species (Kirkpatrick et al. 1995). Shortly after the start of the Köln study, the research team was contacted by the Bronx Zoo and a study was started, funded jointly by The Humane Society of the United States (HSUS) and the zoo. The species included in this study were sika deer, axis deer, Himalayan tahr, elk, sambar deer, and muntjac deer, forty-five animals in all, and once again the PZP was successfully administered and the animals contracepted (Kirkpatrick et al. 1996).

In 1991, with help from John W. Grandy, Ph.D., and The HSUS, several other zoos began trials with PZP. The following year, the American Association of Zoo Veterinarians (AAZV) invited the PZP team to speak at its annual meeting in Oakland, California. By the end of 1993, the program was growing by leaps and bounds. At the beginning, the records included twelve zoos and approximately 80 animals; by 1995 nearly 300 animals in more than thirty zoos were being managed with PZP contraception. Currently, more than 600 animals of ninety different species have been treat-

ed (Figure 1; Table I) in nearly 100 zoos. Receiving the much-needed data from zoo staff can be a challenge, but with perseverance, we have obtained enough data to evaluate safety and efficacy for many species.

Figure 1
Zoos and Animals Actively Participating in PZP Immunocontraception



Factors Influencing Success and Failure

The majority of the animals we have contracepted have been ungulates (hooved animals), and we have received the most data on zebra and giraffe. Among thirty-eight giraffe for which data have been recovered were ten failures during the first year of treatment and one during the second year of treatment. Most of these could be attributed to various technical failures: incomplete series of inoculations, late administration of boosters, and incorrect use of darts. In addition, some females became pregnant before completion of the vaccine series. There were two apparent contraceptive failures, but excluding the failures attributable to technical problems, the contraceptive efficacy for giraffes was 95 percent. Among the thirty zebra for which complete data have been retrieved, there were two failures in the first year, three in the second year, and one in the third. One of the first year failures was due to the animal getting pregnant before the series of three inoculations was complete (FIA was used). The second was a true biological failure. All three of the second year failures were due to boosters given later than

Table 1
List of Species Treated with PZP
Immunocontraceptives as of December 2003

Addax	Dromedary	Muntjac
Addra Gazelle	Duiker	Nilgiri Tahr
African Lion	Eld's Deer	Northern Fur Seal
Amur Leopard	Elk	Nyala
Angolan Springbok	Fallow Deer	Onager
Anoa	Fennec Fox	Oryx
Arabian Wildcat	Fishing Cat	Owl Monkey
Asian Black Bear	Gemsbok	Pere David's Deer
Axis Deer	Gerenuk	Pronghorn
Babirusa	Giraffe	Przewalski's Horse
Bactrian Camel	Hamadryas Baboon	Reindeer
Banteng	Himalayan Tahr	River Otter
Barasingha	Hippopotamus	Roan Antelope
Bighorn Sheep	Ibex	Rock Hyrax
Binturong	Impala	Sable
Bison	Jaguar	Sambar Deer
Black and White Colobus	Key Deer	Scottish Highland Cow
Black and White Ruffed Lemur	Kirk's Dik Dik	Serow
Black Buck	Kodiak Bear	Sika Deer
Black Lemur	Kudu	Snow Leopard
Bongo	Leopard	Sri Lanka Elephant
Brown Bear	Llama	Tapir
Bush Baby	Lynx	Tiger
California Sea Lion	Malayan Sun Bear	Topi
Chamois	Mandrill	Tur
Coatimundi	Markhor	Water Buffalo
Cuvier Gazelle	Mhorr Gazelle	Waterbuck
Dall Sheep	Moose	White Lipped Deer
Domestic Ass	Mountain Goat	White-tailed Deer
Domestic Goat	Mountain Lion	Wolf
	Mule Deer	Zebra

7–9 months after the initial series of injections, and the third year failure was a contraceptive failure. Excluding the failures attributable to technical problems, the contraceptive efficacy for zebras was 93 percent (Frank and Kirkpatrick 2002).

The majority of contraception failures can be attributed to technical errors. The best way to alleviate the failures may be hands-on training at the facility where the trial is being conducted. The veterinarians and other staff can be trained on PZP protocols, which include mixing the PZP with the adjuvant, proper darting equipment, and proper darting techniques.

In our experience, PZP works inconsistently in most carnivores, although it works well in bears, including black bears, grizzly bears, and sun bears. PZP-treated sun bears, located in Touroparc, a French zoo, had fecal samples tested for hormone metabolites, which showed that the females were still cycling but remained infertile (Schwarzenberger et al. 1998). A Malayan sun bear at the Köln Zoo was also treated successfully for two years, and once taken off the PZP, went on to have healthy cubs. As of 2004 ten black and brown bears at the Rocky Mountain Conservation Center (an animal rescue facility in Colorado) were being treated with PZP, with no pregnancies reported at press time. This facility had received PZP free of charge because of grants received from the Baker Trust and other entities.

Conclusions

Captive breeding is an important goal for most rare and endangered species, but because of limited space and resources, zoo populations of all species need to be managed. PZP is a great management tool for most species because of its many advantages, not the least of which is that it has not been shown to cause any debilitating health problems. Additionally, the PZP vaccine can be delivered remotely, so there is no need to cause additional stress by immobilizing or anesthetizing the animal. Finally, efforts in many zoos have demonstrated that the contraceptive effects of the PZP vaccine are reversible after short-term use (up to three years), providing management flexibility.

An often-overlooked utility for PZP contraception is the compassionate use in animals who are in poor health or old age and, particularly among the ungulates, live in social groups that include males. Such animals, who can no longer bear young because of their health or disabilities, even if there were adequate room for the offspring, can live safely and comfortably within those groups through the use of contraception. Currently, a rhinoceros is being treated in Edinburgh, Scotland. She had experienced a prolapsed uterus several times, and her managers decided it was not healthy for her to continue getting pregnant. Initially they separated her from the male in the collection, but both animals showed distress and were damaging their enclosures. PZP provided a practical solution and, at the same time, improved the welfare of both animals by allowing them to be

housed together without the danger of pregnancy.

PZP immunocontraception has become a common and widely used fertility control approach in North America and Europe, particularly in ungulates. Expanding application of PZP to captive populations will require (1) greater efforts in the area of education, (2) increased opportunities for training practitioners, and (3) greater availability to small non-accredited animal collections and wildlife sanctuaries (few even know about the various wildlife contraceptives for carnivores and other animals). For a variety of biological reasons, PZP does not work well in most carnivores, and providing a safe and effective contraceptive for these animals represents the single greatest technical challenge to captive animal contraception. Funding will drive how quickly solutions can be found to these challenges, and hence how quickly the humane benefits of PZP and other contraceptives will spread through the community of captive wildlife.

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5

CHAPTER

The Wild Horse Fertility Control Program

Jay F. Kirkpatrick

Introduction

The wild horse contraception program may well be the showpiece of the efforts and dreams of the HSUS wildlife fertility control work. The wild horse program, now in its sixteenth year, is the oldest of the organization's five major wildlife fertility control initiatives. The wild horse fertility control program has compiled the single largest body of information on contraception in the field for any technique and any wildlife species and includes information on efficacy, safety, required effort, and population effects. These data, in turn, have proved invaluable in combating the skeptics and educating the larger public about wildlife fertility control.

The driving force behind this program was the passage of the Wild Free-Roaming Horse and Burro Act of 1971 (Public Law 92-195; 16 U.S.C. §133 et seq.). This landmark federal legislation protected wild horses and burros living on Western public lands from being killed, captured for slaughter, or subjected to other forms of abuse and exploitation (Rutberg 2003). The legislation, however, underestimated the reproductive potential of horses and over-estimated the government's ability to manage this species.

The Bureau of Land Management (BLM) was given the authority to manage these horses and burros. Although the numbers are still in dispute, there were an estimated 17,000 horses inhabiting public lands when the Act was passed. In less than ten years, the estimates rose to somewhere between 60,000 and 80,000 animals on these same lands. The federal Adopt-A-Horse program, while providing some relief, has been very expensive (up to \$1,600 per animal removed), and it has fallen far short of reaching its goal of maintaining wild horse populations at desired levels (Biological Resources Division 2001). Perhaps the biggest shortcoming of this program has been its inability to keep track of adopted horses. It is suspected that many of these animals subsequently experience a low quality of life, ranging from neglect as a “backyard horse” to a quick end in the slaughter plants of Texas and Canada. In 2003 excessive numbers of horses were living on marginal rangeland after five continuous years of drought—under conditions that no horse, wild or domestic, should ever have to face (see, for example, Stark 2004).

The earliest attempts at fertility control in wild horses began in 1971 and centered around the application of steroid hormones to stallions. Although typically successful in a pharmacological sense, this approach was impractical. The volume of hormones required was too large (up to 50 cc.) and had to be administered too often to manage large herds (3–4 times over six weeks). The drugs also passed through the food chain, threatening scavengers and altering behaviors. The drugs were expensive (\$100/dose or higher) and probably were not safe to give to pregnant mares. Finally, the drugs could not be delivered remotely, and the stresses placed on animals during capture were not publicly acceptable (Kirkpatrick, Turner, and Perkins 1982; Turner and Kirkpatrick 1983; Kirkpatrick 1995).

A History of Immunocontraception in Horses

These shortcomings led to the abandonment of this approach in 1987. At that time, a new approach, immunocontraception, came into consideration. Irwin Liu, at the University of California-Davis, had demonstrated with captive horses that the porcine zona pellucida, or PZP, vaccine could inhibit fertility in mares if administered three times over six weeks (Liu, Bernoco, and Feldman 1989). Several advantages to this approach became apparent immediately. First, the volume of vaccine necessary for contraception was so small (1.0 cc) that it could be delivered by dart, eliminating the need to capture the animals. At \$10–\$20/dose, it was not unreasonably expensive and, because the vaccine was protein-based, it would not pass through the food chain. PZP’s mechanism of action simply blocks fertilization, and thus there was no reason to believe that it would alter endocrine function or social behaviors or that it would interfere with

pregnancies in progress. These characteristics of the PZP vaccine fit well with the theoretical characteristics of an ideal wildlife contraceptive (Kirkpatrick and Turner 1991).

In what is now recognized as a landmark study, in February, March, and April 1988, twenty-six wild mares on Assateague Island National Seashore (ASIS) were treated with the PZP vaccine. Eighteen were given three inoculations and eight were given two inoculations, all by dart. A year later not a single foal was born to the treated mares, proving the efficacy of the two-inoculation regime (Kirkpatrick, Liu, and Turner 1990). Sixty percent of the mares were pregnant at the time they were treated and these produced healthy foals whose survival was no different from that of untreated mares. The social structure of the herd was unchanged, and individual mare hierarchies were not disturbed. A more recent study (Powell 1999) likewise found no behavioral effects of immunocontraception.

In February 1989 half of the treated mares were given a single booster inoculation and half were left untreated. One year later only one of the booster-treated mares produced a foal, and half of the untreated mares produced foals (a normal reproduction rate), documenting short-term reversibility (Kirkpatrick et al. 1991).

By 1990 interest in this success was rapidly growing in scientific, political, and public domains. In a surprising show of interest, the Reproductive Biology Section of the National Institutes of Health (NIH) funded a three-year project to study the effects of the vaccine on ovarian endocrine function, with the aim of learning about the health of ovaries after repeated treatments. The NIH was interested because, at that time, there was some hope that this vaccine might become the basis for a human contraceptive, and the horse trials were already ahead of the research curve with regard to the many questions posed above.

The outcome of this study was that treatments given for three and four consecutive years caused no significant alteration of ovarian function. In five mares that were treated for seven consecutive years, there was no return to fertility and no ovulation (Kirkpatrick et al. 1992, 1995). These mares resumed ovulating after treatments ended but had not become pregnant as of 2004. It appears that the ovary remains healthy and functional with up to seven years of treatment.

Satisfied by the pilot studies of PZP, the NPS decided in 1994 to consider management-level application to the 166 horses on ASIS. In anticipation of this action, all 74 untreated mares on the island received a single inoculation of PZP in March 1994. The purpose of this initial treatment was not to provide sustained contraception but to ensure that every mare in the herd would require only a single additional treatment for contraception to be effective. In March 1995, after the required Environmental Assessment (EA) was completed (and six foals had been born), a management plan was implemented, and selected mares received additional PZP treatments.

The management plan established by the NPS sought to preserve the genetic integrity of the herd while maximizing the population control

effect. The hallmark of this many-faceted plan, which is too complex to examine in detail here, was that all mares reaching two years of age were immediately treated once, then treated again at three and four years of age. After that the mares were left untreated until they produced one foal, thereby creating an “equal opportunity” foaling plan for the island’s mares. There was an additional benefit to this approach that didn’t become apparent until many years later. By delaying pregnancies in the mares, they were in significantly better condition and health when they finally did become pregnant, at ages six through ten, and foal mortality (as well as mare mortality) decreased. At the other end of the treatment spectrum were those mares who had two or more living generations of offspring; these mares were put on contraception until death. They had made their genetic contribution to the herd and they did not have to endure additional energy-draining pregnancies during later stages of life (Kirkpatrick and Turner 2002; Turner and Kirkpatrick 2002).

It is an interesting footnote that these breakthroughs in wild horse contraception came not from the BLM, the agency which originally stimulated research in this area, but rather at the invitation and sponsorship of the NPS, which falls outside the authority of the Wild Free-Roaming Horse and Burro Act. By 1992, however, the first large-scale tests of PZP on BLM-managed western horses were being conducted in Nevada, with results similar to those seen at ASIS (Turner et al. 1997). In this case, the horses were not darted remotely; they were gathered by helicopter drives and inoculated in chutes.

Today the long-term treatment of the ASIS horses has produced a plethora of valuable data. It is known that efficacy of the two-inoculation protocol is about 90 percent. It is also known that the one-inoculation protocol, if administered in March at the beginning of the breeding season, is about 75 percent effective, and that after an initial inoculation, a single annual booster inoculation will maintain contraception. Injection site reactions are extremely unusual (<1.0 percent) and, when they do occur, are not life-threatening or even serious (Kirkpatrick and Frank 2002). After two years of treatment in March, animals can be successfully treated in August, when they are more easily accessible. Zero population growth can be achieved, while at the same time preserving the gene pool of breeding animals. Body condition scores within the herd also improve significantly after ten years of treatment. This, in turn, has resulted in lower mortality rates and an increase in longevity (up to ten years) for treated mares, improving the quality of life for older animals living under difficult conditions. The data have made it clear there is no danger of foals being born out of season among mares withdrawn from treatment. This and other research also indicate that behaviors are not significantly altered, with the exception of mares being wary of the field personnel wandering about with dart guns. It has been demonstrated that a herd of 170 animals could be managed in this manner for less than \$6,000 per year (including labor) and that, as the program continues, the effort needed to maintain zero population growth decreases. Finally, and significantly, this approach has been accepted and

embraced by the public, when adequate and honest information is provided (Points and Kirkpatrick 1997).

Beginning in the 1990s, we sought an effective and reasonably inexpensive one-inoculation form of the vaccine that provides a full year, or perhaps two years, of contraception. In the first trials of a one-inoculation PZP vaccine, mares inhabiting the Nevada Wild Horse Range at Nellis Air Force Base were treated with the PZP antigen encapsulated in lactide-glycolide microspheres. The results indicated that a single inoculation of the slow-releasing microspheres provided the same efficacy as the standard two-inoculation protocol (Turner et al. 2001). This approach, however, had serious flaws: the microspheres, which are suspended and not dissolved, occlude the syringe needles and prevent their delivery via dart. The next obvious step was to find a way to incorporate the PZP into the lactide-glycolide material in such a manner that it could be delivered by dart. This is being accomplished through the use of lactide-glycolide pellets that release the vaccine slowly, in pulses, and that can be administered by dart or jab-stick (Turner et al. 2002). Field trials of these pellets on wild horses in the Clan Alpine Herd Management Area of Nevada have yielded a 90 percent rate of efficacy after one year and an 83 percent efficacy after two years. The present line of research now aims to replicate these results and reduce the cost of these long-acting forms of the vaccine.

The success of the wild horse program has promoted the spread of fertility control to other wild horse herds throughout the United States (Table 1). In 1999 the Rachel Carson Estuarine Reserve began administering PZP contraception to the wild horses of Carrot Island, North Carolina, and one year later, Cape Lookout National Seashore (known as CALO) initiated PZP treatment of the famed Shakleford Banks horses, the oldest known population of wild horses in the United States.

On Carrot Island nearly every mare is treated annually, and in 2002 not a single foal was born (P. Deal, personal communication). On CALO, similar success has been demonstrated among treated mares, although the incorporation of an adoption program into the CALO management plan prevents a higher percentage of adult mares from being treated (69 percent of mares were treated in 2003). Efficacy of treated mares on CALO stood at 97.5 percent in 2003 (S. Stuska, CALO, personal communication).

One year later the privately owned wild horses of Little Cumberland Island were treated, as were the wild horses of the Return-to-Freedom Wild Horse Sanctuary in Lompoc, California. In 2002 the wild horses of the Little Bookcliff National Wild Horse Range in Colorado and the famed horses of the Pryor Mountain (Montana) National Wild Horse Range were treated with PZP. All these applications are designed to control populations, yet they all serve as useful research projects. In its sixteen-year lifespan, the wild horse PZP immunocontraception program has generated more than twenty published book chapters and scientific papers in respected peer-reviewed journals and has involved more than twenty researchers.

Table 1
A Summary of Major HSUS/Research Team Wild Horse Immunocontraception Projects, 1988–2003

Project	Dates	Purpose
Assateague Island National Seashore, Md. (NPS)	1988–present	Feasibility of field delivery, population management
Antelope/Antelope Valley HMAs, Nev. (BLM)	1992–1994, 1999–2002	Feasibility of field delivery, efficacy of one-shot vaccine
Nevada Wild Horse range (Nellis Air Force Base), Nev. (BLM)	1996–1998, 2003–present	Feasibility of field delivery, efficacy of long-term vaccine, population management
Fish Creek HMA, Nev. (BLM)	1998–2001	Efficacy of one-shot vaccine, population modelling
Kamma Mountains HMA, Nev. (BLM)	1998–2001	Efficacy of one-shot vaccine, population modelling
Dolly Varden HMA, Nev. (BLM)	1999–2002	Efficacy of one-shot vaccine
Rachel Carson Estuarine Reserve, N.C.	1999–present	Population management
Monte Cristo HMA, Nev. (BLM)	1999–2002	Efficacy of one-shot vaccine
Cape Lookout National Seashore, N.C. (NPS)	2000–present	Population management
Clan Alpine HMA, Nev. (BLM)	2000–2003	Efficacy of long-term vaccine, population modelling
Little Bookcliffs Wild Horse Range, Colo. (BLM/BRD)	2002–present	Intensive study of behavior, genetic, and demographic effects
Pryor Mountain Wild Horse Range, Mont. (BLM/BRD)	2002–present	Intensive study of behavior, genetic, and demographic effects
Buckhorn/Coppersmith HMAs, Calif. (BLM)	2003–present	Efficacy of long-term vaccine
Lower Snake River HMAs, Id. (BLM)	2003–present	Efficacy of long-term vaccine

Key:

BLM = Bureau of Land Management;
 BRD = Biological Resource Division, U.S. Geological Survey;
 HMA = Herd Management Area;
 NPS = National Park Service

The Economics of Wild Horse Contraception

The economics of wild horse contraception are interesting if not exact. Often economic considerations must be viewed in the context of politics and public opinion, and contraception must be evaluated against available alternative management methods, especially with wild horse management.

In 2003 96 person-hours of work were required to dart twenty-one mares on ASIS (about 4.5 hours per horse). These particular mares are very difficult to approach and dart because of the difficulty of the terrain and their wariness toward darters. With an employee earning \$25/hour to conduct this work, labor cost of the project was about \$112/horse; the total cost remains below \$150/horse when the cost of vaccine, supplies, and equipment are included.

Typically, only half of untreated ASIS mares produce foals each year, so the number of horses darted does not strictly equate to the number of births prevented. Using conservative historic data on reproduction, however, we estimate that the contraceptive program on ASIS has prevented a minimum of 180 births over a fifteen-year period. At an estimated cost of contraceptive treatment of approximately \$5,500 per year, this totals \$82,500 over the fifteen-year period, or at most \$458 per birth prevented.

The BLM's costs to conduct the Adopt-a-Horse program for wild horses removed from Western public land are instructive, although not strictly comparable to those for ASIS. The BLM presently manages its horse populations primarily through gathers, or roundups. Depending on management needs, a substantial fraction of all the horses on a herd management area are gathered, usually driven into temporary corrals by helicopter. The number of horses involved ranges from a few dozen to several hundred, with the cost of the gather generally exceeding \$100,000. The gathered horses are then sorted. Some, especially the more appealing younger animals, are designated for adoption; the less adoptable animals (older animals, and especially older stallions) may be sent to long-term holding facilities. The remaining horses are returned to the range to breed again. The BLM has estimated the cost of gathering, preparing, transporting, and adopting a wild horse at between \$800 and \$1,600 per horse; feeding a horse in a long-term holding facilities costs several hundred dollars per year, potentially for the remainder of the life of the horse. These cost estimates dwarf those of even the most generous estimates of the cost of contraception at ASIS.

Considering the expense of conducting gathers on Western public lands, significant economic efficiency would be gained by immunizing every mare captured and returned to the wild. Although this type of immunization would only provide contraception for a limited time, it would cause antigen recognition and create a "one-shot" mare for subsequent management.

Although this discussion is based on estimates, it becomes clear that even less conservative figures would demonstrate a clear economic basis for fertility control.

The Genetics of Wild Horse Contraception

The genetic integrity of free-ranging wild horses is an important consideration. This issue, however, also must be viewed in the context of alternative management approaches. As an example let us examine the Assateague horses once more. Contraceptive management of this herd has achieved zero population growth (Turner and Kirkpatrick 2002). To achieve this same level of population management without contraception, about 180 foals or young horses would have to have been removed, particularly if adoption was the goal. In the context of genetics, that means that 180 genetic contributions to the herd would have been removed forever. Using contraception to delay reproduction, rather than eliminate it, permits every mare the opportunity to make a genetic contribution, although not all mares will actually make a genetic contribution (i.e., have a foal that survives to breed during his or her life).

The ongoing removal of horses that have never bred from small herds, although probably not catastrophic, is genetically unsound. At the very least, it is a more significant alteration of the herd's genetic profile than delaying reproduction through fertility control.

The Current Status of Wild Horse Contraception

In 2003 wild horse contraception stood at a critical juncture. The NPS and the Rachel Carson National Estuarine Reserve continued to use PZP contraception to manage wild horses. The BLM, through the Biological Resources Division of the U.S. Geological Survey, had initiated limited management-level application in the Pryor Mountain and Little Bookcliff herds. At the same time, the national wild horse program was entering a period of crisis, more for the horses than the agency. The number of wild horses on BLM range lands across the west exceeded mandated—and wise—population levels in many areas, yet the BLM had very limited funding even for emergency gathers. Even if adequate funds were available for gathers, holding facilities for removed horses were full. This problem stimulated a movement within the agency and advisory groups to seek one-time Congressional authorization to sell wild horses at public auction, which will lead to the very abuses the Wild Free-Roaming Horse and Burro Act sought to end more than thirty years ago.

At the same time, the agency has resisted applying contraception on a large scale. Some of the resistance originates with agencies that are advising the BLM, such as the USDA/APHIS, which refuses to acknowledge the published safety and efficacy data from the work described above. It is probable that other agendas are also being advanced through this particular group.

A second source of resistance comes from a limited number of wild horse advocacy groups that do not trust management-level contraception in the hands of the BLM, which they view with suspicion. This is a particularly troublesome source of opposition and though well-intentioned, it is ill-advised. Several of these groups are suspicious that the BLM's ultimate goal is to eliminate horses rather than to maintain sustainable populations. Some argue that a larger population of wild horses would be sustainable if all domestic livestock were removed from public lands, and some have even suggested that wolf and mountain lion populations should be reestablished to control the horses. Although these ideas are not without merit, they are unlikely to be implemented widely or soon, or possibly ever, and in the meantime, horses on the range continue to reproduce. The fear of eliminating wild horses with available contraceptive technology is unreasonable and ignores the Assateague experience. Although we can achieve zero-population growth with this technology, we have been unable to reduce a population through contraception in less than ten years. The increased longevity of the treated animals prevents this. Like the USDA/APHIS veterinarians advising the BLM, the opposing horse advocacy groups ignore sound data from previous studies.

Many opposing advocacy groups also misunderstand or ignore the oversight and control of the BLM contraception program by The Humane Society of the United States (HSUS), which holds an Investigational New Animal Drug exemption (INAD) from the FDA for the use of PZP in wild horses. All PZP field studies conducted to date by the NPS and the BLM have been conducted and administered under the auspices of the INAD held by The HSUS. The HSUS, which is a nationally known, moderate animal protection organization, provides oversight to each project. Before any contraceptive project can proceed, the BLM must produce a management plan that ensures responsible management of the herd, or the project will not be approved by The HSUS.

Finally, there is some opposition from other scientific groups that would like to assume the role of leadership in wild horse contraception, which in turn is probably driven by the promise of increased research funding. Collectively, these political, public, and internal forces have prevented wild horse contraception from growing in the West as rapidly as it might have.

The Future of Wild Horse Contraception

We have seven goals for the next five years.

First, we must strive to help the BLM to understand the need to immunize all mares who are gathered and returned to the range. It is counter-intuitive that the agency should spend up to \$1,600 per horse to gather the animals and then release mares back onto the range without immunizing them with PZP.

Second, more than 75 percent of BLM horse herds are comprised of fewer than 150 horses, and these herds are especially suited for remote-delivery contraception. It is our goal to add at least one new BLM herd management area per year to the list of those using PZP contraception.

Third, we must find the opportunity to test Freund's Modified Adjuvant (FMA) in horses. We routinely use Freund's Complete Adjuvant (FCA) in equids (including Przewalski's horses and zebras) without complications. A large body of data collected from hundreds of zoo animals as well as hundreds of wild horses shows that injection site reactions are not a problem, despite unsupported claims by the opposition. There is also the concern that the FCA will cause the recipient animal to test positive for tuberculosis (TB), but no reliable test for TB exists for equids. Although opponents have offered no scientific data to support their concerns regarding injection site reactions in horses, these concerns may be alleviated through the use of FMA. FMA does not cause injection site reactions in horses, nor can it cause a positive TB test even in species for which a reliable test exists. At the time of this writing, PZP with FMA is being tested on captive mares in collaboration with the BLM.

Fourth, we must encourage the BLM to put the long-acting contraceptive pellets to work on larger ranges, in concert with scheduled gathers. The early and promising results in wild horses in Nevada must be replicated in large scale and highly controlled studies.

Fifth, a great deal more effort must be put into educational endeavors to increase the public's understanding of this technology and how it benefits horses. We know that well-designed educational efforts garner support for wild horse contraception from the general public, which, in turn, often drives federal agency actions.

Sixth, we must ensure that a constant and high-quality supply of native PZP, supported by a strong quality-control program, be available for the potentially large demand prompted by the impending wild horse crisis (as well as the application to animals in zoos and deer). The BLM has already gone on record to insist that reasonable quality control procedures be applied to the native PZP used in BLM horses. At a minimum this would include accurate quantitative analyses for dose determination, qualitative analysis by way of polyacrylamide gel electrophoresis, which can be stored, and screening for pathogenic bacteria and selected viruses that might affect horses. The mechanism for this achievable goal is in place, but it requires a sustainable source of funding.

Seventh, and as important as any initiative listed above, is the need to find the means to recruit and train field personnel to deliver the contraceptives. Time is no longer on the side of those who developed this technology and brought it to where it stands today. A new cadre of people who care passionately about wild horses must be found.

America's wild horses face an impending crisis provoked by agency budget problems, a history of mismanagement, and the worst drought in sixty-five years, compounded by indecision in the managing agency. Unless contraception, as well as other tools, is put to work very soon, we will witness deteriorating health and large die-offs of animals on our public lands. The research has been done, the safety has been documented, the efficacy is understood, and the technology is available to help these animals. The time for talking is over.

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Immuno- Sterilization In Dogs Using Zona Pellucida (Zp)-Based Vaccine

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Introduction

As the global human population exploded in the past century, so, too, did the populations of man's best friends. According to statistics from the American Veterinary Medical Association (2003), there were approximately 130 million cats and dogs in the United States in 2002 (although the methods used in this survey may overestimate the population; Patronek and Rowan 1995). The European Pet Food Industry Federation (2004) estimates dog and cat populations in the European Union (EU) for the same year to be approximately 86 million.

Pet overpopulation was first recognized as a serious global problem in the mid-1980s. By the 1990s estimates of the total number of cats and dogs euthanized by U.S. shelters ranged widely, but the number was probably close to 6 million (Arkow 1994; Patronek and Rowan 1995). In the early 1990s, a substantial effort began to try to reduce the overpopulation of pets by conducting widespread surgical spay-neuter clinics. Despite fifteen years of aggressive surgical spay-neuter campaigns, humane education, and legislative efforts, only modest progress has been made in reduc-

ing the staggering numbers of “surplus pets.” In 2002 6–7 million dogs and cats were still being euthanized in U.S. shelters, at an estimated cost of \$2 billion (A. Rowan, personal communication), although in some individual states, such as New Jersey, the trend was clearly downward (Clancy and Rowan 2003).

In addition to the burgeoning population of owned animals is the enormous number of stray, feral, and community-fed dogs and cats, especially in developing countries. Statistics for Latin America, Africa, and Asia are not available or are unreliable, but the numbers of these animals are believed to be exceptionally high, especially in and around cities where garbage provides a reliable food source (Matter and Daniels 2002). Density estimates of stray dogs in Chihuahua, Mexico, and Kathmandu, Nepal, for example, were 735 per km² and 3,000 per km², respectively (Daniels and Bekoff 1989; Kato et al. 2003). Dog bites, rabies, and other zoonotic diseases are rampant in areas where stray dogs are abundant (Wandeler and Bingham 2000; Kato et al. 2003).

By the late 1990s, it became clear to many animal experts that an alternative to lethal and surgical population control methods was needed to offer a real solution for this large and pervasive problem. The goal became to develop a safe and effective alternative: a one-shot, low-cost, mass-produced chemical or immunosterilant that causes permanent sterility without causing any harmful side effects. Such a “spay/neuter shot,” which would be cheaper, faster, and more convenient than surgical sterilization, would hold the promise of greatly reducing the horrendous annual killing of tens of millions of dogs and cats across the world.

Developing a “spay/neuter shot” will not be easy and will take several years, as well as a substantial amount of funding and research. The religious, ethical, and cultural undercurrent of birth control is tricky territory, as many involved in the early surgical spay/neuter efforts can testify. But many of these issues and roadblocks have been addressed via the surgical system that was developed over the past fifteen years, and it can generally be said that now there is widespread support for humane animal population control. Currently, many national and international groups are working on the development of alternatives to surgical spaying and neutering. We are in the very initial stages of developing these new alternatives, and the pace of development is rapidly accelerating.

The Zona Pellucida

The zona pellucida (ZP) is the unique extracellular matrix (egg coat) produced by both the oocyte (egg) and the surrounding granulosa cells (egg nurse cells) during the development of the oocyte in the ovary. This structure is important because it is the matrix to which the sperm binds during fertilization. With the isolation and sequencing of a number of the DNA segments that code for ZP in different species, it has

been possible to classify these proteins into three major glycoprotein families on the basis of their amino acid sequence similarities: ZP1, ZP2, and ZP3 (mouse ZP nomenclature; see reviews by McLeskey et al. 1998; Prasad, Kaul, and Dunbar 2002). The proteins of each of these three families share 40 to 90 percent of their amino acid sequences, suggesting a common evolutionary origin, as well as a functional relationship.

It has long been established that immunization with zona pellucida (ZP) proteins can elicit an immune response that alters normal development of ovarian follicles and can result in either temporary or permanent infertility (Dunbar et al. 2001). Immunization with zona pellucida proteins has distinct advantages over other proposed immunological contraceptive methods: (1) it inhibits fertilization but does not cause abortions; (2) the animal's ZP is directly exposed to antibodies in the follicular fluid of the antral follicle for long periods of time, which promotes opportunities for interaction with anti-ZP antibodies; (3) binding of antibodies to newly formed ZP antigens disrupts junctional complexes between the oocyte and the granulosa cells, which leads to oocyte degeneration and infertility; (4) the zona antigens studied to date are tissue specific, which reduces the likelihood of immune-mediated side effects; and (5) the zona of a variety of animal species are immunologically cross-reactive, so ZP vaccines may potentially be effective in many species (Table 1).

Table 1
Conservation of ZP Proteins Across Species

IDENTITY TO HUMAN ZP PROTEINS, BY PERCENTAGE			
Species	ZP1	ZP2	ZP3
Mice	39	60	67
Rabbit	71	72	69
Pig	68	64	74
Dog	Not sequenced	71	70
Monkey	92	94	94

Many of the studies on immunocontraception have used native PZP as an immunogen. PZP is relatively easy to obtain. It also has a high degree of sequence homology with ZP proteins of other species, increasing the likelihood that antibodies raised in response to PZP injections will cross-react with the ZP of the treated animal. Females of many species have been immunized with PZP or its three major glycoproteins (ZP1, ZP2, and ZP3). In some species, including dogs, rabbits, and marmosets, immunization with pig ZP proteins elicits an immune response that is associated with ovarian dysgenesis (see reviews by Fayerer-Hosken et al. 2000; Patterson et

al. 2000; Dunbar et al. 2002) (Table 2). The animals fail to ovulate, and eventually all their follicles and oocytes are destroyed, leading to permanent sterility. The destruction of the follicles and subsequent absence of corpora lutea also ends the secretion of progesterone and estradiol, the hormones that regulate the estrus cycle and stimulate estrus behavior. In other species, such as horses and deer, the effects of PZP vaccination can be reversible; once treatment is discontinued, declining antibody titers lead to a return to normal fertility (Kirkpatrick et al. 1990; Turner et al. 1996; and chapters in this volume).

Table 2
Effect of Pig ZP Immunization on Ovarian Function and Fertility in Various Species

Species Immunized	Immunogen (Mouse ZP Nomenclature)	Ovarian Pathology/Fertility Status
Rabbit	Native Pig ZP (ZP1, 2, 3)	Infertile/ovarian dysfunction
	Native Pig ZP3 (ZP1, 3)	Infertile/ovarian dysfunction
	Deglycosylated Pig ZP3 ^α (ZP1)	Normal ovarian follicular development
	Deglycosylated Pig ZP3 ^β (ZP3)	Infertile/ovarian pathology
Dog	Native Pig ZP (ZP1, 2, 3)	Infertile/ovarian dysgenesis, polycystic ovaries
Marmoset	Native Pig ZP3 (ZP1, 3)	Infertile/ovarian dysfunction
	Deglycosylated Pig ZP3 ^β (ZP3)	Infertile/ovarian pathology
	Recombinant human ZP3 (ZP3)	Infertile/ovarian pathology
Squirrel monkey	Native Pig ZP3 (ZP1, 3)	Infertile/functional ovaries
	Deglycosylated Pig ZP3 (ZP1, 3)	Infertile/functional ovaries
Baboon	Deglycosylated Pig ZP3 (ZP1, 3)	Infertile/ovarian dysfunction
Mice	Native PZP (ZP1, 2, 3)	Fertile

A crucial factor in the development of effective vaccines has been the identification of adjuvants, which enhance the immune system response. The use of adjuvants has become common, especially with antigens (such as ZP) that do not themselves elicit a strong immune response.

The most commonly used adjuvant, Freund's Complete Adjuvant (FCA), comprises mineral oil, cell wall fragments from *Mycobacterium tuberculosis*, and mannide monosleate (a detergent). Although a potent immunogen, FCA has been known to have side effects such as production of abscesses, granulomas, and tissue necrosis at the site of injection. Modified Freund's

Complete Adjuvant (mFCA) is similar in composition to FCA but substitutes fragments of *M. butyricum* (a benign soil bacteria) for those of *M. tuberculosis* and would be expected to have less severe side effects.

Other adjuvants are also commercially available. One, which is currently being used in human clinical trials, is CpG-DNA. CpG-DNA is an unmethylated dinucleotide common in bacterial DNA but virtually nonexistent in mammalian DNA; therefore, it is apparently used by mammalian immune systems as a signal of bacterial invasion. CpG-DNA induces proliferation of most B cells and triggers immunoglobulin secretion, with fewer and less severe side effects than FCA and other adjuvants (McCluskie et al. 2000; Weeratna et al. 2000).

Currently, the most common source of native PZP for this vaccine is pig ovaries obtained from slaughterhouses. Although this source of ZP protein has provided adequate material for many studies testing the efficacy of ZP proteins as immunocontraceptive or immunosterilant vaccines, this source is not adequate for large-scale animal immunization programs. Many recent studies have reported developing recombinant ZP vaccines that would not require harvesting raw or native material from slaughterhouse products, and some of the studies using recombinant ZP proteins have demonstrated the generation of antibodies that inhibit fertility (e.g., Martinez and Harris 2000; Prasad et al. 2002; Srivastava et al. 2002; Ringleb, Rohleder, and Jewgenow 2004).

The present studies were designed to develop a humane and non-surgical method to control dog populations by developing an immunosterilization vaccine for female dogs that could be produced on a large scale. To evaluate the efficacy of native and recombinant ZP proteins in inducing permanent sterility in female dogs, we have compared different ZP protein preparations (native vs. recombinant proteins), using different adjuvants. We looked at safety, effectiveness as immunocontraceptives or immunosterilants, and other features that would encourage acceptance by veterinarians.

Experimental Design

The animal studies were carried out at the Johannesburg Society for the Prevention of Cruelty to Animals (SPCA), an animal shelter recognized by the National Council of SPCAs, Republic of South Africa. The facilities were newly constructed to provide state-of-the art housing for the dogs in this study.

Dogs of different sizes and breeds were housed for several months prior to administration of the vaccines. Dogs were exercised twice daily and fed Eukanuba-brand dog food. All the dogs were vaccinated and boosted for rabies and the standard DHLPP vaccine, and implanted with AVID identification chips. Caretakers and animal handlers provided daily care, exercise, and evaluation of the dogs' health status. All immunizations and blood collections were carried out by veterinarians. The dogs were allowed to adjust

to their surroundings, their health status was monitored, and their reactions to routine vaccines were evaluated.

Six females ages 4–6 months were selected for each treatment group. This age group was selected because the status of ovarian follicular development can be more accurately evaluated in young animals than in older animals, who have ovaries that are “scarred” from old corpora lutea and an excess of fibrous connective tissue. All the dogs were placed in adoptive homes at the conclusion of the study, except for two dogs who were euthanized due to extreme aggressiveness.

We tested three antigens: PZP, native rabbit zona pellucida (RZP), and a recombinant human ZP3 (rhZP3). Each antigen was tested with mFCA. In addition PZP was tested with CpG-DNA. Thus, there were six treatment groups:

Group 1. PZP + mFCA. Each dog was vaccinated intramuscularly (i.m.) at two sites (0.5 ml subscapular and 0.5 ml lumbar) with 300 μg PZP in 0.5 ml phosphate-buffered saline (PBS) + 0.5 ml mFCA ($N = 6$).

Group 2. PZP + RZP + mFCA. Each dog was vaccinated i.m. at three sites (0.5 ml subscapular, 0.4 ml right lumbar, and 0.4 ml left lumbar) with 300 μg PZP + 100 μg RZP in 0.65 ml PBS + 0.65 ml mFCA ($N = 6$).

Group 3. PZP + CpG-DNA. Each dog was vaccinated i.m. at two sites (0.5 ml subscapular and 0.5 ml right lumbar) with 200 μg PZP + 60 μg CpG-DNA + Rehydrogel (Alum) + sterile saline ($N = 6$).

Group 4. rhZP3 + mFCA. Each dog was vaccinated i.m. at two sites (0.5 ml subscapular and 0.5 ml right lumbar) with 20x solutions rhZP3 + 0.5 ml mFCA ($N = 6$).

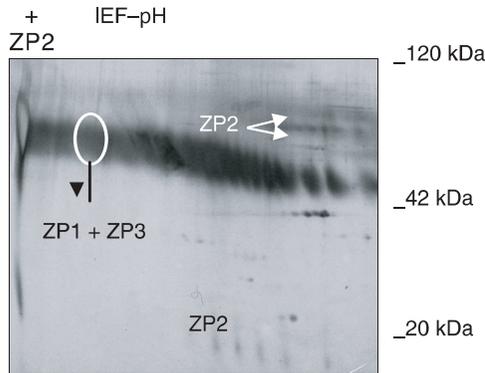
Group 5a. mFCA control. Each dog was vaccinated i.m. at two sites (0.5 ml subscapular and 0.5 ml lumbar) with 0.5 ml phosphate-buffered saline (PBS) + 0.5 ml mFCA ($N = 3$).

Group 5b. CpG-DNA control. Each dog was vaccinated i.m. at two sites (0.5 ml subscapular and 0.5 ml right lumbar) with 60 μg CpG-DNA + Rehydrogel (Alum) + sterile saline ($N = 3$).

Thirty days after the initial injection, dogs in Groups 3 and 5b received booster shots identical to the first shot (except the left lumbar region was used instead of the right). For reasons described later in this chapter, none of the other dogs (those receiving mFCA) was boosted.

Vaccine Preparation. Heat-solubilized porcine ZP (PZP) and rabbit ZP (RZP) protein were obtained using the method described by Wood et al. (1981). The PZP was prepared in the laboratory of Jay Kirkpatrick (Billings, Montana), and analyses of the PZP were carried out in the Dunbar laboratory at the Texas Medical Center in Houston, Texas. The purity of the ZP preparation was evaluated by high-resolution two-dimensional polyacrylamide gel electrophoresis, and the proteins were detected by silver stain (Figure 1). The ZP protein mixture was diluted in sterile physiological saline.

Figure 1
Analysis of Porcine ZP by High-Resolution Two-Dimensional Polyacrylamide Gel Electrophoresis (Isoelectric Focusing in First Dimension and SDS-PAGE Separation by Molecular Weight in the Second Dimension) and Protein Detection by the Color Based Silver Stain



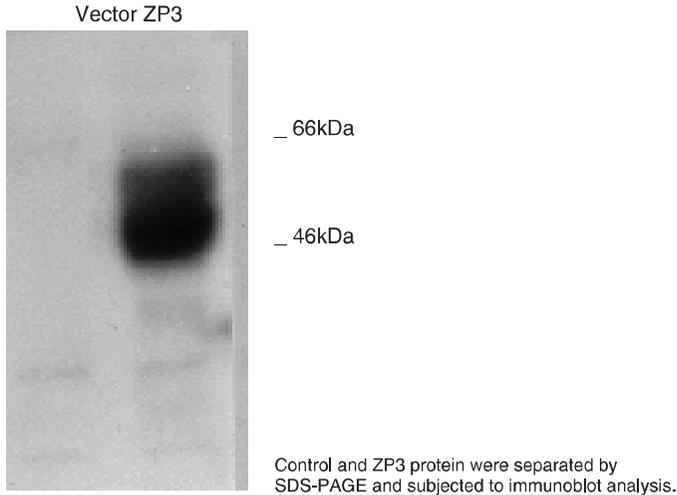
The mFCA (Calbiochem) was vortexed and emulsified with 300 μ g PZP in a 1:1 ratio using 18-gauge needles with connecting syringes. 60 μ g CpG-ODNA (Oligonucleotides, Inc.) and 25 μ Rehydrogel alum (Reheis) were emulsified with 200 μ g PZP using procedures outlined by Davis et al. (1998), Jones et al. (1999); and Segal, Chang, and Shevach (2000).

Human ZP3 cDNA was expressed in wild type CHO cells using the mammalian expression vector pcDNA3.1/Zeo (Invitrogen, California). ZP3 expression was evaluated by SDS-PAGE and immunoblot analysis using antibodies to native pig ZP (Figure 2; Prasad et al. 1995). 1 ml of 20x concentrated ZP3 protein solution was emulsified with 2 ml of saline and 3 ml of MFA.

Antibody Titers. Serum samples were collected from dogs at three weeks post-immunization and monthly thereafter. Sera from the immunized animals were evaluated by Enzyme Linked Immuno Assay (ELISA) for anti-PZP antibody titer levels.

Immunoblot Analysis. To determine the relative strength of response to different ZP antigens, and therefore the potential for effectiveness in different species, antisera from animals with the highest antibody titer were analyzed by immunoblot against deglycosolated rabbit and pig ZP proteins and native PZP and RZP.

Figure 2 Analysis of Recombinant ZP3 Protein Expression Using Antibody to Pig ZP Proteins



Tissue Sampling, Fixation, and Histochemical Analysis. Animals were spayed seven months following initial ZP immunization, at approximately 11–13 months of age. The removed ovaries were fixed in Bouin's Fixative or Buffered Formalin. The ovaries were sectioned, and the ovarian follicles were evaluated by counting the number of follicles from five different sections (300 micron-thick paraffin sections) from each of two ovaries per animal.

Results

Immunization Reactions and Animal Health. All the dogs who received mFCA (controls as well as ZP-immunized) developed severe injection-site lesions. Although all the animals were treated and recovered completely, no additional boosters were given to these animals because of the potential for more severe reactions following a booster immunization. One animal in each of Groups 4 and 5 had to be removed from this study due to factors unrelated to this study (aggression).

Antibody Titer Evaluation by ELISA. Antibody titers peaked at three months (Table 3). All the animals immunized with mFCA and native ZP proteins (Groups 1 and 2) had significant antibody levels even after a single immunization with no boosters. Likewise, all six PZP-CpG-DNA-treated animals showed significant antibody titers. Two of the five animals immunized with the recombinant ZP3 protein had significant antibody titers after a single shot.

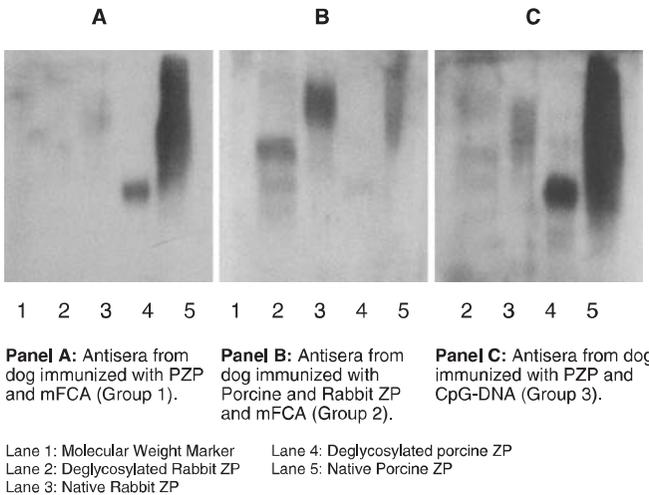
Table 3
ELISA of Sera from Immunized Animals
on PZP to Determine Antibody Titers at
Three Months Following First Immunization

Groups	Animal #	Titer (x 1000)
Group 1 PZP/MFA	1	80
	2	20
	3	40
	4	80
	5	10
	6	10
Group 2 PZP/RZP/MFA	7	40
	8	20
	9	20
	10	20
	11	10
	12	40
Group 3 PZP/CpGDNA	13	40
	14	40
	15	40
	16	80
	17	40
	18	40
Group 4 ZP3/MFA	19	1
	20	1
	21	20
	22	20
	23	1
Group 5 Adjuvant control	24	0
	25	0
	27	0
	28	0
	26	40

(Titers are given as dilution of sera giving detectable antibody)

Immunoblot Analysis of Antibodies Against ZP Proteins. Antisera from animals with the highest antibody titer were analyzed by immunoblot analysis (Figure 3). In dogs immunized with PZP plus mFCA (Group 1), antibodies predominantly recognized PZP (Panel A, lanes 4 and 5); there was very little recognition of RZP (Panel A, lanes 2 and 3). Antibodies primarily recognized the major ZP protein ZP3 (35Kd) (Panel A, Lane 4). The antisera representative of dogs immunized with both rabbit ZP and porcine ZP using the mFCA (Group 2) contained antibodies that recognized both rabbit and porcine ZP (Panel B). Antisera from dogs immunized with PZP and CpGDNA adjuvant also recognized both rabbit and pig ZP proteins (Panel C).

Figure 3 Immunoblot Analysis of Dog Antisera Demonstrating Recognition of Different Epitopes



Histochemical Analysis of Ovarian Follicular Development. All the treatment groups showed reduced numbers of primary, secondary, and antral follicles relative to controls (Table 4; Figure 4). The lowest numbers of primary and secondary follicles were seen in the PZP and PZP-RZP-mFCA groups (Groups 1 and 2). Likewise, all the treatment groups showed elevated levels of atretic cell clusters relative to controls, with the highest levels shown by the PZP-CpG-DNA and PZP-RZP-mFCA groups (Groups 2 and 3). Generally, dogs who showed positive antibody titers showed a higher ratio of cell clusters to follicles than those who showed no measurable titers (Figure 5).

Table 4 Summary of Ovarian Histology

Each follicle value is an average of the number of follicles from five different microscopic views (300 microns section) from different ovarian sections fixed with either Bouin's fixative or Formalin fixative.

Groups	Animal #	Primary Follicles	Secondary/ Tertiary Follicles	Antral Follicles (including atretic)	"Atretic" Cell Clusters
PZP/MFA	1	1	6	0.3	11
	2	2	3.3	1.5	6
	3	1.5	2.8	1.3	13
	4	1.4	1.3	0.5	19
	5	0	0.5	1.5	14
	6	1.8	1.5	1.7	8
		$1.3 \pm .7$	2.6 ± 2	$1.1 \pm .6$	12 ± 4.8
PZP/RZP/MFA	7	3.7	2.5	0	14
	8	1	0	0.25	21
	9	3.3	6	0	12
	10	3.2	1.5	0	20
	11	2.8	1.2	1.8	14.8
	12	0.8	3.3	0.25	29
		2.5 ± 1.3	2.4 ± 2.0	0.1 ± 0.14	19 ± 5.4
PZP/ CpGDNA	13	2.4	2.4	1.5	18.2
	14	2.3	2	1.5	14.5
	15	2.5	3.8	0	22
	16	5.5	2.8	0	56
	17	4	1.3	0.75	27
	18	3	5.8	0	12.3
		3.3 ± 1.3	3.0 ± 1.6	$0.63 \pm .7$	25 ± 16
ZP3/MFA	19	5	6.7	1.5	4.3
	20	2.8	4	0.8	5.3
	21	2.8	4.6	0.3	14.5
	22	2.6	0	0.3	10
	23	2	1.3	1	12.3
		3.0 ± 1.1	3.3 ± 2.7	$0.78 \pm .5$	9.6 ± 4.0
Adjuvant	24	3	3.5	3.3	2.5
	25	7.5	16	3.3	4
	27	6.5	10	0.8	4
	28	6.3	3.8	0.3	5.3
		5.8 ± 1.9	8.3 ± 5.9	2.0 ± 1.4	4.0 ± 1.1

Figure 4
Effects of Antibodies against ZP Immunogens on Ovarian Follicular Development

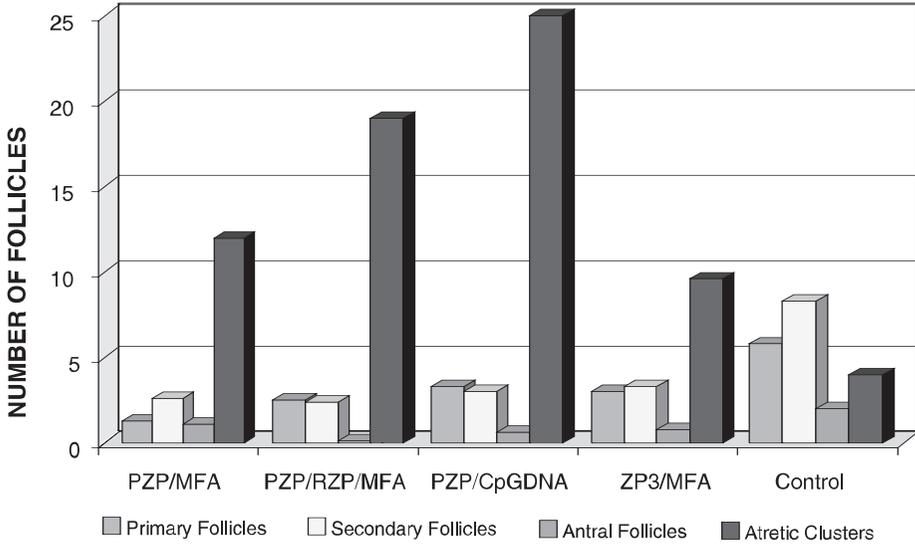
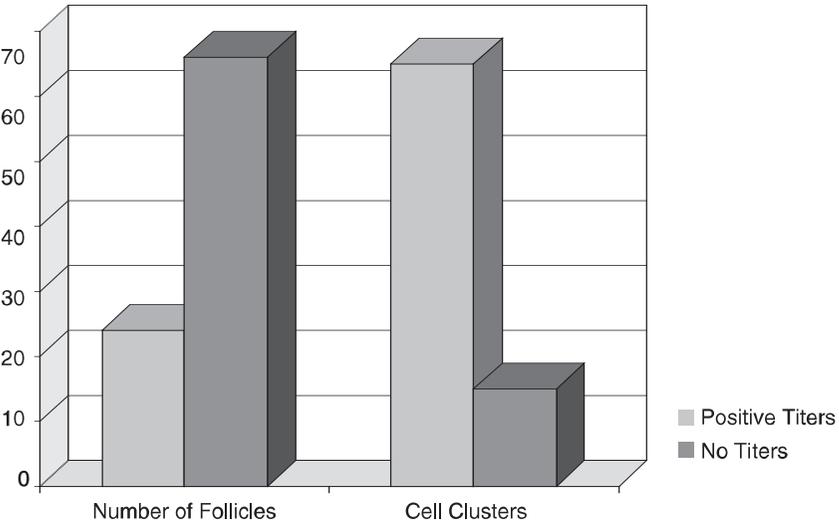


Figure 5
Effects of Antibodies against ZP Immunogens on Follicle Number and Cell Clusters



Follow-Up Studies. Because the recombinant ZP3 group received MFA and could not be boosted, the recombinant ZP3 antigen did not receive a fair test in the first round. Consequently, we performed a follow-up trial. Six female dogs received four injections at four week intervals of recombinant human ZP3 with CpG-DNA, while six (control) dogs received CpG-DNA alone on the same injection schedule. Blood was collected from these animals two weeks after each injection and the sera were analyzed for the presence of PZP antibodies by ELISA. In addition, as a more definitive test of effectiveness, the six treated dogs were bred twice on consecutive heats. Titers remained low in the treated group, and four of the six treatment dogs conceived. It appears that the immunogenicity of the recombinant protein needs to be enhanced to achieve a higher level of antibodies and to elevate contraceptive effectiveness.

Summary and Discussion: Further Prospects

Because of the serious injection-site lesions experienced by all the dogs who received mFCA, it should not be used in dogs. CpG-DNA, however, was not associated with any adverse reactions and should receive further investigation.

All the animals immunized with PZP and PZP-RZP showed elevated titers. Antibody titers were highest in the CpG-DNA immunized animals, and immunoblot analyses showed that the animals immunized with the CpG-DNA showed a broader spectrum of cross-species antigen recognition. The comparison between adjuvants is not straightforward, however, because the CpG-DNA-treated animals received boosters, whereas boosters were withheld from mFCA-treated animals because of the injection-site lesions observed after initial injections.

The animals from the three treatment groups that showed significant antibody titers (Groups 1, 2, and 3) all had significantly reduced levels of developing follicles and increased atretic granulosa cell clusters. These studies imply that, with time, the ovaries would be depleted of developing follicles. Further studies have to be carried out for longer periods of time to evaluate fertility and time until full ovarian depletion and sterility are accomplished. Although the animals in the ZP3 recombinant group (Group 4) had very low titers, a decrease in follicles and an increase in atretic GC cell clusters were apparent and might be enhanced over the long term with boosters.

Strengthening the immunogenicity of the recombinant ZP might be achieved in at least four ways. These include (1) chemically conjugating the CpG adjuvant to the ZP antigen; (2) chemically conjugating the ZP antigen to a more immunogenic protein (Srivistava et al. 2002); (3) immunizing with CpG adjuvant and antigen bound to polylactide-co-glycolide microparticles; or (4) using chimeric recombinant ZP protein containing

immunodominant regions of all three ZP proteins. Short-term (three month) dog studies would be needed to determine the best combination to enhance immunogenicity of recombinant ZP proteins (both ZP3 and chimeric ZP proteins containing antigenic determinants of all three ZP proteins). Only if antibody titers are significant would further studies or fertility trials be warranted.

It would also be desirable to carry out studies on ZP-DNA vaccines. DNA vaccine systems are now being developed for humans, and the FDA has already approved use of DNA vaccines for various human trials. Consequently, a future animal DNA vaccine might pass the FDA approval process more easily. For a DNA immunocontraceptive vaccine, ZP cDNA could be cloned into a mammalian expression vector, and the combined DNA product would be injected into the animal subcutaneously or intramuscularly. Ideally, the expression vector carrying the ZP cDNA would be taken up by the host's own cells, which would then express the ZP antigen on a sustained basis. These antigens would then stimulate the immune response.

DNA vaccines have been shown to elicit high levels of antibody production in numerous animals, and they offer many potential advantages: a large number of epitopes can be included in one construct, and DNA vaccines are very pure, extremely safe, easily mass-produced, and able to be customized to any species.

Although developing effective immunocontraceptives with recombinant ZP proteins has proven to be very challenging, the payoff to animal welfare would be enormous. Continued pursuit of this research path is crucial.

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