

***Immunocontraceptive Reproductive Control
Utilizing Porcine Zona Pellucida (PZP)
in Federal Wild Horse Populations***

(Third Edition)

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Contributors

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**Jay F. Kirkpatrick, Ph.D., Allen T. Rutberg, Ph.D.,
and Linda Coates-Markle**

Compiler and Editor

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Patricia M. Fazio, Ph.D.

June 1, 2010

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Contributors

Jay F. Kirkpatrick, Ph.D. is Director of The Science and Conservation Center, Billings, Montana and holds a Ph.D. in reproductive physiology from the New York State College of Veterinary Medicine at Cornell University, Ithaca, New York.

Allen T. Rutberg, Ph.D. is a Research Assistant Professor, Center for Animals and Public Policy, Cummings School of Veterinary Medicine at Tufts University, North Grafton, Massachusetts; and Consultant and Senior Research Scientist for The Humane Society of the United States. Dr. Rutberg holds a Ph.D. in zoology from the University of Washington (1984) and an A.B. in biological anthropology from Harvard College (1976).

Linda Coates-Markle was, formerly, the Montana/Dakotas Wild Horse and Burro Specialist and the Wild Horse and Burro National Research Coordinator, Bureau of Land Management, Billings Field Office, Billings, Montana. She is now with the BLM's Division of Rangeland Resources as the Society of Range Management Liaison in Wheat Ridge, Colorado.

Compiler and Editor

Patricia M. Fazio, Ph.D. is an environmental writer and editor. Dr. Fazio holds a B.S. in animal husbandry/biology from Cornell University (1964); an M.S. in environmental history from the University of Wyoming, Laramie (1982); and a Ph.D. in environmental history from Texas A&M University, College Station (1995). Her dissertation was an establishment history of the Pryor Mountain Wild Horse Range (1968), in Montana/Wyoming.

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MEMORANDUM OF UNDERSTANDING
between the Humane Society of the United States
and the Bureau of Land Management

After a workshop held in Santa Fe, NM, November 29 and 30, 2005 on wild horse fertility control, the Humane Society of the United States and the Bureau of Land Management have agreed to develop a Memorandum of Understanding to co-operate on: -

- 1) The further development and wider use of contraception in wild horse populations,
- 2) Resolve some of the uncertainties being faced in producing the vaccine and ensuring a continuing supply of a safe and effective vaccine,
- 3) Assist in public outreach on the issues, and
- 4) Maintain healthy and viable herds in the existing BLM wild horse Herd Management Areas.

"The BLM sees this as a way to reduce horse removals, to place fewer horses in short- and long-term holding facilities, and to achieve budgetary savings," said Don Glenn, Acting Group Manager of the BLM Wild Horse and Burro Program, Washington, DC.

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**Source:** HSUS-BLM Press Release on Fertility Control, e-mail from Tom Gorey WO/BLM/DOI, sent 11/30/2005 01:17 PM. Forwarded by Linda Coates-Markle /MTSO/MT/BLM/DOI on 12/05/2005 10:55 AM.

## *Introduction*

*Two fundamental questions exist...*

- 1) Does PZP harm wild horses?**
- 2) Will its use eliminate entire herds?**

The quick answers are that the Food and Drug Administration (FDA), The Humane Society of the United States (HSUS), and animal care committees all carefully review protocols for PZP use, and more than 20 years of data, carried out under these set of rules, clearly show that wild horses are neither injured by this drug, nor do aberrational behaviors occur as a consequence of its application. Too, oversight by The Humane Society of the United States assures that the vaccine is used only to slow reproduction and may not be used for the extermination of entire herds. PZP is designed to bring about short-term infertility and is reversible, if not used beyond five consecutive years. It reduces the need for gathers and preserves the original gene pool in each herd.

Expanding on these central points, the contributors and editor of material presented within this document have aspired to answer, with scientific objectivity, common questions and concerns raised by actual individuals and groups about porcine zona pellucida (PZP) and to provide citations and references that may be accessed through interlibrary loan, or other sources, for further study. Updates and additional questions and answers (Q&A's) will be provided periodically, as research progresses or protocols change.

PZP use in wild horse herds has been studied extensively for more than two decades, with papers published in peer-reviewed scientific journals by experienced reproductive physiologists, equine scientists, wildlife biologists, geneticists, and animal behaviorists, providing a portrayal of safety, high efficacy, and absence of long-term behavioral, physical, or physiological effects from the vaccine. Those involved in the creation of this Q&A have endeavored to produce a factual document of scientific merit, supported by field data, with statistically adequate sample sizes. Data was collected by trained, unbiased individuals, who adhere to established research methodology within his or her respective field.

## *Questions & Answers*

### **THE PZP VACCINE**

***Q.* What is porcine zona pellucida (PZP), and how does it work to block pregnancy?**

A. A non-cellular membrane known as the zona pellucida (ZP) surrounds all mammalian eggs. The ZP consists of several glycoproteins (proteins with some carbohydrate attached), one of which, ZP3, is thought to be the primary component of the sperm receptor (the molecule that permits attachment of the sperm to the egg during the process of fertilization). The PZP vaccine is derived from pig eggs. When this vaccine is injected into the muscle of the target female animal, it stimulates her immune system to produce antibodies against the vaccine. These antibodies also attach to the sperm receptors on the ZP of her own eggs and distort their shape, thereby blocking fertilization. [see Paterson and Aitkin 1990; Miller et al. 2001]

Thus far, PZP has been a promising form of contraception in wild horses and other wildlife for the following reasons:

1. Pregnancy is prevented approximately 90% of the time in treated animals;
2. The vaccine can be delivered remotely by small darts;
3. Contraceptive effects are reversible (up to five years in wild horses);
4. PZP is effective across many species;
5. No debilitating health side effects have been observed, even after long-term use;
6. No effects on social behaviors have been observed;
7. The vaccine cannot pass through the food chain;
8. It is safe to administer the vaccine to pregnant animals.

***Q.* How is it made, and who manufactures it?**

A. The porcine zona pellucida (PZP) vaccine, used on BLM, U.S. Forest Service, and NPS wild horse mares, as well as within several wild horse sanctuaries, is produced by The Science and Conservation Center (SCC) in Billings, Montana. Each batch is subjected to a qualitative and quantitative quality-control program and shipped under the authorization of an Investigational New Animal Drug (INAD) exemption for wild horses (FDA

# 8857-G0002) issued to The Humane Society of the United States (HSUS) by the Center for Veterinary Medicine of the Food and Drug Administration. In collaboration with other investigators, The SCC continues to conduct research with the contraceptive vaccine, focusing on the ability to produce larger quantities, and increasing the efficacy of long-term contraception through a single inoculation (see Turner et al. 2002, 2008).

**Q. How is the PZP vaccine obtained?**

A. Once all necessary authorizations and approvals have been obtained for use of the vaccine, it may be ordered from:

**Kimberly M. Frank**

The Science and Conservation Center (SCC)

ZooMontana

2100 S. Shiloh Road

Billings, MT 59106

(406) 652-9719 (phone)

(406) 652-9281 (fax)

e-mail: [sccpzp@hotmail.com](mailto:sccpzp@hotmail.com)

The vaccine is not commercially available and is provided at 60% of the cost of production, which currently runs about \$24/dose. This is the price of the standard, one-year, 100 microgram dose. The 2-3 year vaccine uses considerably more than 100 micrograms, as well as more adjuvant, and includes the added cost of pelleting. The 2-3 year PZP vaccine costs about \$200 per dose, plus the personnel costs of administration, which are minor, if horses are being gathered anyway. Compared with the \$1,100 - \$1,600 it takes to gather, remove, transport, hold, and adopt a horse (or care for it indefinitely), PZP is a bargain.

**Q. Are any pigs killed, expressly to produce the vaccine?**

A. Pig ovaries are obtained from a slaughterhouse in Iowa, as a by-product of hogs already destined for slaughter. Therefore, no fewer hogs will be killed if the PZP vaccine were no longer made. Major competitors for pig ovaries include Chinese restaurants, and pharmaceutical companies, that use ovarian endocrine components for research and production of products.

**Q. Is this drug FDA approved and patented? If so, who is making all the profit from its use?**

A. In FDA language, “approval” refers to approval for commercial distribution and marketing, and PZP is not a commercial product. No one is profiteering from PZP. The Humane Society of the United States holds the Investigational New Animal Drug exemptions (INAD), which are the oversight process by which FDA compiles data to examine vaccine safety and effectiveness. Basic and applied research that generated most of the knowledge about the vaccine was carried out with public funds (from the National Science Foundation, National Institutes of Health, U.S. Department of Agriculture, Bureau of Land Management, etc.). The research team considers products developed with public monies to be in the domain of the public, and therefore has no intention of commercialization. Therefore, it will always be called “experimental,” under FDA rules, despite the fact that PZP has been studied and field-tested extensively, for safety and efficacy, and is currently being used with more frequency on federal wild horse mares.

However, at the same time, use of public monies for research and development ***does not legally prohibit*** the commercialization of a product. Some researchers are, nonetheless, offended (due to a personal sense of ethics) and will not move forward with commercialization of a product developed with public funds. The rationale is that the public has already paid for the product, and commercialization only allows private companies and individuals to profit from sale without having contributed to the process of research, development, and testing.

Because of this, PZP cannot be used on wildlife without the Investigational New Animal Drug exemption, or INAD. Once the INAD from FDA (one for horses, one for deer and zoo animals) was obtained, the INAD was turned over to The HSUS, leaving that organization to deal with the ethical issues. This means that each project – even at the management level – must have a research question attached to it, and The HSUS must approve the project. An added note is that the Investigational New Animal Drug exemption (INAD) issued to HSUS by the FDA requires a sound safety base before it is issued and would never have been issued were there a significant (or even an insignificant) health or safety concern. Therefore, neither HSUS nor The SCC make money from the vaccine. The SCC provides PZP vaccine at cost of production. Coupled with the paperwork required, The SCC actually

loses money. That is why The SCC is a non-profit. Currently, The SCC's annual budget is about \$120,000, and PZP income results in less than half of that, meaning that a great deal of vaccine is donated.

The patent issue is a different question, not to be confused with the FDA process. Merck patented PZP in the 1970s, but the patent lapsed, and the technology is no longer patentable. Organon International, a large drug company based in the Netherlands, holds the patent for PZP use in humans, but that application may never take place, as scientists have not yet been able to make an effective synthetic form. Also, the variability in time for infertility reversal is significant and could potentially result in litigation.

In recent years, the FDA has transferred authority for wildlife contraceptives (exclusive of captive zoo animals) over to the Environmental Protection Agency (EPA). In 2009, an application is pending for approval of native PZP for use in wild horses by the EPA.

***Q. What groups are on the PZP Contraceptive Research Team?***

A. Today, the team consists of The Science and Conservation Center, Billings; Toledo University Medical College, Ohio; University of California-Davis; Tufts University, Medford, Massachusetts; The Humane Society of the United States, Gaithersburg, Maryland and Washington, DC; and the University of Iowa, Ames. Many other individuals contribute to the effort in one form or another. Governmental agencies that can be considered team members include the National Park Service, U.S. Forest Service, U. S. Department of Commerce, and the Bureau of Land Management.

The entire PZP contraceptive effort involves many people, several institutions, and numerous funding agencies. This team works together, bringing many disparate disciplines and talents in concert to solve the problems at hand.

***Q. Who Funds PZP Contraceptive Research and Applications?***

A. Funding for application of the vaccine to wildlife has been provided by many individual communities, agencies, and organizations, including but not limited to:

- The Humane Society of the United States

- Elinor Patterson Baker Trust
- Geraldine R. Dodge Foundation
- Bernice Barbour Foundation
- Leuthold Family Foundation
- Panaphil Foundation
- Delta-Sonics
- PNC, Inc.
- U.S. Navy
- National Park Service
- Bureau of Land Management
- Rachel Carson National Estuarine Reserve
- U.S. Department of Commerce
- National Institutes of Health
- Fire Island Community Association
- Burkett-Plack Foundation
- Bosack-Kruger Foundation
- Annenberg Foundation
- Cargill Foundation
- THAW Charitable trust
- 112 different zoos in North America, Europe, New Zealand and Australia
- South African National Parks Board
- U.S. Fish and Wildlife Service – African Elephant Conservation Fund
- Fripp Island (SC) Property Owners Association

- Morris County (NJ) Parks Commission
- Franklin County/Columbus (OH) Metro Parks
- and several anonymous donors.

This list is not all-inclusive but provides a picture of the breadth of support for this approach to wildlife management.

***Q.* Who controls vaccine use in wild horse populations?**

A. No agency or organization can use the vaccine without the assent of HSUS, which monitors management plans and the INAD. In fact, the BLM approached the FDA and tried to circumvent HSUS control over vaccine use but was turned down by the FDA. Therefore, oversight and approval by HSUS still exists. No agency or organization will have control of the PZP vaccine in the foreseeable future. Every  $\mu\text{g}$  of vaccine that is produced can only be used in projects where HSUS has reviewed and approved a wild horse herd management plan.

All projects in which the vaccine crosses state lines must be on record with the FDA. As explained previously, the authority to carry out these projects is issued by two separate Investigational New Animal Drug documents (INADs) issued by the FDA to HSUS. As each new project is identified, HSUS reviews the need for the project in the context of scientific, ethical, and moral issues, and, if approved, issues permission to proceed. Notification of each project is accomplished by means of a form, filed with the FDA by The Science and Conservation Center, which specifies how much vaccine is being shipped and what species are to be treated. The INAD also requires that data from each project be gathered in a systematic way and filed, and be made available to the FDA when the need arises. These files are maintained at The Science and Conservation Center. Additionally, the legal managers of the horses (NPS or BLM) or the animal care committee of each zoo must also provide permission to treat animals. This regulatory process is similar for any wildlife species not classified as a food animal by the FDA or as a game animal by a state fish and wildlife agency.

Precisely how procedures will change – or remain the same – after EPA assumes regulatory authority, remains to be seen, but the HSUS will hold the approval; thus, we see no major changes at this time (6/09).

**Q. Does an agency have to do an environmental assessment (EA) or an environmental impact statement (EIS) prior to using PZP on a wild horse herd?**

A. Yes. Environmental Assessments are mandatory for any use by federal agencies. One difference between agencies is that the NPS does a single management EA, that is in force for years, while the BLM does one every five years for application in each herd management area.

**Q. What wild horse populations, within the United States, are presently being managed with PZP?**

A. The vaccine has been used successfully to manage the wild horse population of Assateague Island National Seashore (ASIS), in Maryland, under the sponsorship and authority of the National Park Service (NPS). The population has been treated for 22 years, without health problems, and the population has decreased by almost 40%, since management-level application began in 1995. Wild horses are also being treated on Cape Lookout National Seashore (Shackleford Banks), North Carolina, for the NPS; on Carrot Island, North Carolina; on the Rachel Carson National Estuarine Reserve, North Carolina; and on many areas of Nevada, for the Bureau of Land Management (BLM). Other treated herds include Return To Freedom (American Wild Horse Sanctuary), Lompac, California; Pryor Mountain Wild Horse Range (Montana/Wyoming); Little Book Cliffs National Wild Horse Range, Colorado; McCullough Peaks Horse Management Area, Wyoming; and Little Cumberland Island, Georgia; International Society for the Protection of Mustangs and Burros Wild Horse Sanctuary, Lantry, SD; Black Hills Wild Horse Sanctuary, Hot Springs, SD, Carson National Forest, NM, and the Navaho and Pima/Maricopa Indian reservations. In Nevada and Wyoming, at least 12 different wild horse herds are being treated "experimentally" to evaluate population effects. For Nevada references, see (1) Turner et al. 2001; (2) Turner et al. 1997; (3) Kirkpatrick et al. 1997; and (4) Kirkpatrick et al. 1997. In March, 2009, The Washington Office of the BLM Wild Horse Program issued a memorandum that directs local horse managers to treat all mares returned to the range.

In the case of the four barrier island herds, the Pryor Mountain Wild Horse Range, Little Book Cliffs Wild Horse Range, and Return To Freedom Wild Horse Sanctuary, horses are treated remotely, with dart guns. In Nevada, they are treated in conjunction with gathers, as most of these HMA's are too large, and the horses too wild to dart.

In addition to controlling the horse population on Assateague Island, treatment has extended the lives and improved the health condition of older mares, by removing the stresses of pregnancy and lactation [see Kirkpatrick 1995; Kirkpatrick and Turner 2002, 2003; Kirkpatrick et al. 1990, 1991, 1992, 1995a, 1996a,b, 1997; Liu et al. 1989; Turner and Kirkpatrick 2002, 2008; Turner et al. 1996a]. Horses on Assateague are doing well. About 125 total animals roamed the area in 2009 (27% less than the starting number of 173 in 1995), and their body conditions have improved significantly since 1990 [Turner and Kirkpatrick 2002]. Mortality almost disappeared for the first eight years of management, and the horses are generating new age classes (large numbers between 20-25 years of age, and a growing population between 25-30).

Thus, at the management level, horses are being treated with PZP for the NPS, Rachel Carson National Estuarine Reserve, the BLM, and at least five private sanctuaries. In addition, new forms of the vaccine are being tested for the BLM in western horses, but not on a management level.

The following HMAs are sites that have been treated with long-acting PZP:

Onaqui Mountain, UT - 56 mares  
 Sand Springs, OR - 31 mares  
 Fox-Hog, NV - 28 mares  
 Green Mountain, WY - 38 mares  
 Monte Cristo, NV - 53 mares  
 Blue Wing, NV - 136 mares  
 Antelope Hills, WY - 28 mares  
 Black Rock East, NV - 19 mares  
 Black Rock West, NV - 19 mares  
 Warm Springs, NV - 27 mares  
 Antelope Complex, NV - 29 mares  
 Calico, NV - 92 mares  
 Groshuite, NV - 44 mares

Granite Range, NV - 79 mares

Nellis Air Force Base Bombing Range, NV (Nevada Wild Horse Range) - 358 mares

McCullough Peaks, WY - 34 mares

Sand Springs, CO - 75 mares

Cedar Mountain, NV - 85 mares

Additionally, another form of the long-acting PZP has been tested in captive mares at Canon City, CO; a reduced dose of PZP was tested successfully in captive mares at Canon City, CO; and a lyophilized form of the vaccine was tested successfully in domestic mares in Clark, WY and Billings, MT.

***Q.* What herds do you propose to treat with contraceptives in the near future? Why did you choose these particular herds? Who decides? What are your long-term goals?**

A. While the PZP vaccine is currently being used on at least 20 horse management areas for the National Park Service or the Bureau of Land Management, its use is appropriate for all free-ranging wild horse herds. Application to particular herds is at the invitation of the managing agency. The long-term goal is to reduce or eliminate the need for gathers and removals.

### ***DELIVERY, APPLICATION, AND BIOLOGICAL EFFECTS OF PZP***

***Q.* How is the vaccine delivered?**

A. The PZP vaccine must be injected into the muscle of the target animal. This can be done by hand if the animal is restrained, or by dart, for remote delivery. There are many commercial dart systems available, but the thick viscosity of the vaccine requires a large needle and a quick injection. Thus far, Pneu-Dart® systems (Williamsport, Pennsylvania) seem to work the best. The Pneu-Dart® 1.0 cc barbless darts can be fired from Pneu-Dart® capture guns or from several other commercial dart guns [Pax-Arms® or Dan-Inject®, for instance]. The darts are disposable, and after hitting the animal in the rump or hip (the only acceptable location for darting), they inject by means of a small powder charge, and then pop out. Because of

their bright colors, the darts are usually retrieved in the field. Darts that have not been discharged cannot be discharged by stepping on them or by any other kind of casual contact. Over a six-year period on Fire Island National Seashore, and with more than 1,000 dartings of deer, only two darts have not been recovered.

Normally, each animal is darted twice the first year, with the first injection being given up to a year before a booster, just preceding the breeding season (March for wild horses or September for deer). Thereafter, a single annual booster inoculation will maintain contraception. The second inoculation of the first year requires that...

- a. you are able to recognize the individual animals; or
- b. you do the first inoculation with a special "marker dart," which leaves a dye mark on the animal at the same time it injects the vaccine; or
- c. selected mares are treated to allow for both genetic diversity within a specific herd and for the promotion of health and improved body condition of an individual animal (through temporary infertility).

An alternative strategy is to give only a single inoculation the first year, from which there will be little contraception, and then a single annual inoculation thereafter, from which there will be significant contraception (see McShea et al. 1997; Turner and Kirkpatrick 2002.)

New approaches using small non-toxic, biodegradable lactide-glycolide pellets, that result in several years of contraception after a single application, are being tested. [Turner et al. 2002, 2008].

***Q.* How would you describe the new pelleted form of PZP-22, and how does it work?**

A. PZP is incorporated into lactide-glycolide pellets, small enough to be injected intramuscularly. After injection, the lactide-glycolide erodes (It is biodegradable and non-toxic) and releases the PZP at varying rates, depending on the ratio of lactide to glycolide. It is an injectable version of a contact cold pill. The pellets are made at the School of Pharmacy, University of Iowa. There have been spotty results in horses. It works in some herds and not in others, and it has worked well in deer, but not in elephants. Not all, but much of the data associated with the pellets has

come from helicopter counts, and it is difficult to match foals with mares from a helicopter. After the summer of 2010, more data will be available. Two herds in the Annenberg Foundation project have been treated with pellets. People are on the ground, who are familiar with the horses; so more solid data should be forthcoming.

**Q. How does the liquid vaccine compare to the pelleted form?**

A. Nothing works as well as a primer dose of native PZP, followed by a booster 2 to 6 weeks later, and then annual boosters after that. Efficacy with the pelleted form, even where it has appeared to work, is not as good. The issue, of course, is having to treat mares every few years versus only once.

**Q. Isn't darting mares painful and potentially harmful or even lethal? Will it result in mares being shot in critical anatomical areas – abdomen or chest, causing inhumane deaths?**

A. As long as only 1.0 cc Pneu-Darts are used, there is almost no risk of injury to the animal. These are very small, light darts. Over a 22-year period, no horse has ever been injured on Assateague Island, the Shackleford Banks, Carrot Island, the Pryor Mountains, or the Little Book Cliffs (translating to well over 1,000 dartings, over the course of 20 years).

On the Pryors, PZP remote-darting operations typically take place in late summer/early fall, and any wild mares receiving the vaccine are individually-identified and tracked regularly with data, non-intrusively gathered on behavior, estrus, fertility, reproduction, survival, and any health concerns. The field studies are conducted by seasonal and term USGS-Biological Resources Division and BLM biological technicians under the supervision of BRD research biologists and the BLM Wild Horse and Burro Specialist.

Several studies of injection site reactions have been carried out. In one study it was shown that of 38 total treated mares, 55% exhibit no reaction to darting, 6% have some level of swelling around the injection site, 22% have a small nodule (granuloma) about the size of a marble, and no animals had abscesses. Only one mare of 38 has ended up with a medium-level swelling, about one year after treatment. In a larger study, data were generated from the records of 329 different horses on Assateague Island, darted over a 19-year period. 1,094 different dartings resulted in a 0.2% abscess rate. The

abscesses healed within two weeks. In another study of captive zoo animals, 1,185 dartings of 25 different species resulted in 16 abscesses (1.3%) (Lyda et al. 2005). In the Pryor Mountains, 151 dartings resulted in 1 abscess (0.7%). At Return To Freedom Wild Horse Sanctuary, in California, 451 dartings of 85 total horses over six years resulted in 15 abscesses (3.0%). At the Black Hills Wild Horse Sanctuary, in 2009, 140 dartings resulted in one abscess (0.7%).

Abscesses typically appear as 25 mm swellings which open and drain within two weeks without complications. More common are granulomas, or subcutaneous nodules, about the size of marbles. These nodules are most notable post-injection but typically disappear over time. Ultimately these nodules are very difficult to discern amongst other natural scars within the coats of these wild mares. Furthermore, there is no indication that the presence of these nodules has compromised the quality of life for these horses. Field technicians have never recorded the mares showing any indication that these nodules are causing any level of discomfort during daily activities and/or interfering with reproductive activities. The granuloma is the counterpart of the smallpox vaccination scar found on humans, but beneath the skin instead of on its surface.

These injection site reactions should be given some context and compared with the same events in domestic livestock. The 1995 Beef Council Quality Audit reported that 11% of cattle inoculated with USDA-approved vaccines produced injection site reactions, including lesions and abscesses. In another study, the Kentucky Beef Council reported that fed cattle had abscess/lesion rates of 3.2-21.6% after vaccinations with USDA-approved vaccines, and in non-fed cattle, the abscess/lesion rate ran from 28.9 to 40.9%.

The most recent study of injection site reactions (Roelle and Ranson 2009) provides some interesting data. In any herd studies, often there are a few horses where abscesses appear more frequently than in other animals. Thus, for some reason, some animals are more prone to abscesses than others, and this often skews the abscess rate within a single herd. For example, in one herd, of a total of six abscesses that appeared after darting, four occurred in the same mare. The other data suggest that vaccinations given by dart produced more abscesses than those given by hand injection. This makes sense, if you consider that hand injections are preceded by antiseptic swabbing of the skin surface, while field darting simply drives dirt and hair

follicles in to the wound, along with the vaccine. Thus, this all suggests that abscesses may not have any relationship to the vaccine or adjuvant, but to the delivery method instead.

**Q. How do immune system depression issues affect the formation of abscesses and lameness in horses?**

We have insufficient data regarding how mares will react if they are in poor condition versus good condition, at least not enough for statistical analysis. Then, we just have visual indexes for body condition scores, from which you can simply infer immune competency; thus, we can offer only anecdotal information. Classic immunology says that animals in poor condition (or stressed for any other reason, as well) will not mount as strong an immune response as unstressed animals. Whether or not this effect will cause poorer efficacy, more abscesses, or other issues, is not known. In zoo animals, where we often administer PZP because of health problems, there seems to be no problem. Next, lameness and abscesses may not be at all associated with the vaccine *per se*. Rather, there is some limited data, by the Science and Conservation Center, and by Jason Ransom, U.S. Geological Survey, Ft. Collins, Colorado, that darted horses will produce more abscesses than hand-injected horses. When you hand inject, you wash the surface of the skin with ETOH. However, when you dart, you drive any surface dirt into the dart puncture. The same problem exists when darting wildlife with anesthetics, rather than vaccine. This issue is not any different from differing efficacies among individuals getting flu shots. In general, about all you can say is that animals with compromised immune systems do not do as well as those with competent systems, and there are many variables associated with what causes immune incompetency.

**Q. Will PZP harm mares or foals, physiologically? Have any negative pharmacological side effects been observed? Are any benefits derived from its use?**

A. Safety data has been accumulated over 20 years. It essentially says there are no short- or long-term health problems of any kind, and that the vaccine is reversible, unless the mare is treated for more than five consecutive years (in which case you probably didn't want her to reproduce again anyway). The data make clear that pregnancies in progress are not affected in any way by the vaccine, nor is the health or fertility of the foals compromised, once

they are born. Treating mares carrying female fetuses does not affect the fertility of the offspring.

In fact, as mentioned previously, mares on Assateague Island are living longer than ever, and their mortality has decreased, they are achieving new age classes never before seen on the island, and all of this happened because their body condition scores have increased steadily since 1989, when PZP application started. Historically, a mare never survived to 20 years of age, but now a significant percentage have passed twenty, and about 29 animals (20% of the herd) are between 25 and 30 years old. Removal of the stresses of pregnancy and lactation gives them an immense health advantage, both at the younger and older stages of life.

Foal mortality has dropped significantly. This is probably because their mothers, when they finally do become pregnant, after several years of contraception and then withdrawal of PZP treatments, are much healthier. All of this data (derived not from casual observation) is published.

The other victory for horses is that every mare prevented from being removed, by virtue of contraception, is a mare that will only be delaying her reproduction rather than being eliminated permanently from the range. This preserves herd genetics, while gathers and adoption do not. (Kirkpatrick and Turner 2002, 2008; Turner and Kirkpatrick 2002, 2003; Willis et al. 1994.)

**Q. Does PZP application create late foaling in treated populations?**

A. No. Available data from 20 years of application to wild horses contradicts this claim (Kirkpatrick and Turner 2003). From 1990 to present, Assateague Island has records for 178 horses whose month of birth is known (and in some cases, day of birth known). An examination of the published data, from 1984, of Ron Keiper (retired Distinguished Professor of Biology at Pennsylvania State University and currently Chairman of the Department of Biology at Valencia Community College, Orlando, Florida) in which he looked at eight years of birth dates for the same herd, which at that time was much smaller than we have today (considerably less than 100 horses versus 155) indicates that approximately 85% of the foals were born in April, May and June. Among the 178 horses with known birth dates, 95 were born to mothers who were never treated with PZP, with 70 born in April, May and June (73.6%), and 25 born outside this window. Another 83 foals were born to mares that had at some point been treated with PZP

before their pregnancies, and 65 were born in April, May or June (78.3%), with 18 outside this window. Thus, with a database of 178 horses over an eleven-year period, there is no evidence of late foals being born among treated mothers.

That corroborates published work (Kirkpatrick and Turner 1983), where it was demonstrated that Pryor Mountain wild horses did not extend their season of ovulation even when placed on high planes of nutrition. Mares do not extend their breeding season if they do not get pregnant.

One interesting issue is that the percent of untreated mares giving birth on Assateague Island in the April, May and June window has decreased from 85% down to 74% since 1984. This suggests that as herd size increases, variability in birth dates also increases, but this may simply be a function of larger numbers (in this case a 100% increase in herd size). There is also a moderate pattern among some mares (the N9BF line in particular) with regard to producing foals outside this window. This genetic line was consistently producing foals in March. If that observation is correct (This is only an untested observation.), then it corroborates Eric Palmer's theory that seasonal ovulatory patterns in mares are genetically controlled. In any case, these data, at least, demonstrate that contraception with PZP does not cause early or late births. Once again the Assateague Island (ASIS) horses and the 22-year treatment history produced a wealth of information (Kirkpatrick and Turner 2003).

In the Pryor Mountain Wild Horse Range, the normal foaling period has been well documented (EA #BLM MT010 FY05 -16, figure 10) to primarily take place in May and June, with limited foaling known to happen outside this window, from February to September. Thus, later foaling dates are not considered abnormal.

**Q. For how many years is a mare generally treated with PZP?**

A: This depends on the management plan of the agency, for a particular herd. Perhaps the most effective plan is the one used on Assateague Island, where all two-year-old mares are put on treatment, and then boosted at three and four years of age. After this, they are removed from treatment until they foal, which might occur anywhere from one to five years later. Mares that have already made their genetic contribution to the herd, in the context of the management plan, are treated until extinction.

**Q. How do you determine which mares within a herd will be treated?**

A. The question of which mares to treat with vaccine is an important one, and the answer is embodied in the management plan. The approach used on Assateague Island has proven very effective and safe, i.e., two, three and four year olds are all treated and then withdrawn from treatment until they produce a foal. Older mares with good genetic representation in the herd are treated to extinction. Dr. E. Gus Cothran, an equine geneticist from Texas A&M University, College Station, monitors the plan on Cape Lookout. Immunocontraceptive control is more logical (genetically) than removing animals before they have had a chance to reproduce. Genetic representation is the key element within the management plan.

The most important consideration is to ensure that all genetics are represented, whether or not they reflect "wildness" or band stability, phenotype, or whatever social hierarchy exists. The bottom line is that wild horses are native North American wildlife, and humans should not be selecting for anything other than complete genetic representation and kinship.

**Q. How effective is PZP? Won't some mares still become pregnant after treatment?**

A. PZP treatment in wild horses is about 95+% effective (Turner and Kirkpatrick 2002, 2008). The failure of some horses to respond to the vaccine results from an immune system that either doesn't "recognize" the vaccine's antigen, or from a compromised immune system. This is true for human vaccines as well (consider the less than 100% efficacy with flu vaccines). Regardless, 95% efficacy is enough to manage wild horse populations effectively. In other species, efficacy varies in a species-specific manner (Frank et al. 2005).

**Q. Why can't you block pregnancy with just one inoculation instead of the two shots you use now?**

A. The issue of the "one-shot" is complicated. Currently there are tests with SEVERAL forms of a one-shot vaccine. Despite that, this matter clouds the real issue of putting the vaccine to work NOW.

The initial “primer” dose of PZP causes the immune system to “recognize” and type the antigen, not so much for immediate long-term response, but to prepare the animal’s immune system for future exposures to the vaccine. Thus, a booster inoculation is required the first year, and an annual booster thereafter (Liu et al. 1989).

There is a second reason for the need for booster inoculations. Many human and veterinary vaccines use attenuated (weakened) or killed viruses as the vaccine, and these are powerful stimulators of the immune system. Often a single inoculation lasts for years. The PZP, however, is a relatively small protein that is not especially immunogenic. It is also very close in structure to the native PZP on the target animal’s own ova; thus, the target animal has difficulty in “recognizing” the PZP as foreign to the body. This, in turn, means that multiple inoculations must be given, and with a more immunogenic compound, known as an adjuvant (Lyda et al. 2005).

The subject of the one-shot also clouds the bigger topic of management because it only provides an advantage in the first year. After that, the horses are "one-shot" animals anyway. In 1994, almost every mare on Assateague Island was treated with a single shot. That shot was not meant to cause contraception but to set the herd up as a "one-shot" herd in preparation for management a year later. It was done this way because the National Park Service had to do an environmental assessment (EA) before they were allowed to manage with contraception. Then in 1995, the whole herd was managed with only a single shot per animal. The concept is fairly simple, and a "one-shot" vaccine only aids in the first year. Of course, a single inoculation that lasts multiple years would have more utility. (Turner et al. 2002, 2008)

The present advice given to the BLM is plain. Not a single mare that is gathered, for any purpose, and returned to the range should get back on the range without an inoculation. That makes them "one-shot" animals, and the expense of developing "one-shot" vaccines becomes moot. Once they have had that first shot, they can be re-treated anytime with a single shot.

## **A One-Inoculation Vaccine**

Because of the need to inoculate animals twice the first year, and the difficulty of doing this with wild species, research is proceeding toward a "one-inoculation" vaccine. Such a vaccine would permit a single darting to

cause one or more years of contraception. The approach under study incorporates the PZP into a non-toxic, biodegradable material, which can be formed into small pellets. The pellets can be designed to release the vaccine at predetermined times after injection (at one and three months, currently), much the same way time-release cold pills work. Initial trials were encouraging, and continued trials are underway (see Eldridge et al., 1989; Turner et al., 2002, 2008). The downside to this approach is the immense amount of vaccine needed for the production of the pellets and the associated cost. While this approach may have some limited use, the difficulty in producing large amounts of vaccine make it unsustainable. What is needed is either a synthetic form of the PZP antigen or a pellet making process that reduces wastage by at least 65%.

**Q. What do you mean by a one-year, two-year, or three-year vaccine?**

Response to the PZP antigen is variable among individual mares. Some mares appear to be naturally poor responders to the vaccine and probably never develop sufficient antibody titer levels to confer infertility (hence the 95% efficacy of the vaccine in horses). Research with the Pryor Mountain wild horse herd indicates that immune response in mares may be correlated with age and fitness. One six-year-old mare, contracepted due to poor physical condition in September 2003, responded poorly to the vaccine, conceived in 2004, and foaled in 2005. Similarly, two 16-year-old mares last boosted in 2003, also produced a foal in 2005. Conversely, younger mares in good condition may have a stronger than expected antibody titer response resulting in a longer period of infertility. This appears to be the case with the first young mares treated with PZP on the Pryor Mountain Wild Horse Range (PMWHR).

Immunocontraception is not an exact science. The vaccines are designed to offer an effective time period based on the average response for many numbers of animals. As the treated number of animals increases, then the known response time for a specific formulation of PZP and adjuvant (carrying-agent) becomes better known. There are many variables to consider, the largest factors being animal condition and related immune response. Young PMWHR mares were healthy when injected (though a small sample size), and it would appear the resulting immune response (to a 90% effective one-year agent) has resulted in 2 years of efficacy. Older Pryor mares are relatively worn-out, and immune response appears limited. As such, the vaccine was not effective in a couple of mares. This is also true

with a younger mare that was treated, due her poor condition, but she still produced a foal.

Perhaps animal condition on Assateague Island (ASIS) is such that one year of efficacy is all that results with the same formulation of PZP and adjuvant that has been used on the Pryors. These are questions that still need to be dealt with, which is why the need exists for continued research. These questions are addressed in individual-based study herds within the Wild Horse & Burro Fertility Control Field Trial program and a project funded by the Annenberg Foundation. This is one of the reasons why the efforts on the Pryors have been critical to knowledge of this vaccine for use on western herds.

**Q. How long does it take for native PZP to stop population growth in a herd?**

A. This depends on the percentage of adult mares treated. On Assateague Island (ASIS), growth was stopped almost immediately. A large number of mares were given the PZP vaccine on ASIS during the first three years (about 87%), and growth stopped quickly. On the Pryor Mountain Wild Horse Range, fewer mares were treated (< 30%), but growth was slowed, as well. So, it depends mostly on the percentage of adult mares treated. However, fertility rates and mortality rates enter into this answer as well. It is precarious to address this any other way than to point out that results are site-specific and cannot be generalized.

**Q. How long does it take for PZP to begin reducing a wild horse population?**

A. On ASIS, the first sign of population reduction took about eight years, but herd reduction has been moving more quickly since then. The following eight years, herd numbers went down from 175 to 116, without any removal of horses, treating anywhere from 48 % to 79% annually. The lag time between initiation of treatment and decline in numbers is largely a function of increased longevity in treated horses, but, as pointed out above, there are other variable factors involved (fertility rates, mortality rates, sex ratios, and so forth). Fertility rates are average on ASIS (45-60%), and mortality is low (<5%).

**Q. How can you tell if a wild mare is pregnant, so you don't treat her with PZP?**

A. In some cases, a fecal or urine sample is collected off the ground, or from yellow snow following urination. Reproductive steroid hormone metabolites are measured that tell us, with almost 100% accuracy, which mares are pregnant and which are not. A pregnancy can be diagnosed from 40 days post-conception until the day of parturition, and the animal does not have to be touched. That said, there is no danger to either the mother or the *in utero* foal, if the mare is treated with PZP during pregnancy (Kirkpatrick and Turner 2003, 2007).

**Q. Is the drug residual in urine or feces or in the dead carcasses of treated mares, where PZP could get into the food chain or cause adverse effects to wildlife, or even contaminate water?**

A. Because PZP is primarily protein, it is readily destroyed in digestion, reduced to amino acids, and therefore cannot pass through the food chain intact and with biological activity (Oser 1965).

**Q. What about compensatory reproduction in PZP-treated herds?**

Thus far, after 22 years of PZP treatment, there is no evidence for compensatory reproduction in a PZP-treated wild horse herd. This might be an issue if a herd is treated for long periods of time and then all treatment withdrawn, but that flies in the face of an effective management plan (Kirkpatrick and Turner 1991b).

## **BEHAVIORAL EFFECTS OF PZP**

**Q. Is wild horse behavior affected by PZP use? Are there any effects on motivation or drive, general contentedness, and the emotional stability of mares treated with PZP? Is band social stability affected negatively? Has any aberrational behavior been seen in PZP-treated mares, band stallions, or bands where mares have been treated with a contraceptive? What behavioral studies have been conducted on wild horses, both in eastern and western wild horse herds? Do you plan additional behavioral studies?**

A. After 22 years of treating the ASIS mares, there is still no evidence of altering behaviors. The baseline behaviors of eastern wild horses were the same as western horses. In order to understand this, a great deal of information must be read, which examines wild horse behavior from a variety of sites around the world. A huge body of literature exists on this subject, and a few of the more salient publications include: Berger 1977; Feist and McCullough 1976; Keiper 1976, 1986; Klingel 1975; McCort 1984; Rubnestein 1981; Rutberg 1990; Rutberg and Greenberg 1990; Salter and Hudson 1982. An independent investigator from the National Zoological Park has confirmed earlier results that show no behavioral changes (Powell 2000). Thirty-years of observing wild horses in North America and Australia and New Zealand have revealed no difference in fundamental behavioral structures. On the other hand, the affects of gathers on social behaviors are obvious (Ashley and Holcombe 2001; Hansen and Mosley 2000).

A good start on this subject can be obtained by reading Powell (1999), which reports on a study done by researchers from the National Zoological Park/Smithsonian. They found no behavioral effects, at that time, after almost eight (8) years of PZP treatment. The same results were reported in several of the Assateague Island papers, including (1) Kirkpatrick 1995, and (2) Kirkpatrick et al. 1995. Also, other studies (Fayrer-Hosken et al. 2000; Delsink et al. 2002) showed a lack of behavioral effects of this same vaccine on free-roaming African elephants, which have an even more complex social order than wild horses.

One critic of PZP claims, "The horses [on Assateague Island] seem more listless than western wild horses..." However, this could only be determined by time budget studies, and, in fact, no evidence for this has been found (referenced above). Casual observation of wild horses proves nothing. For example, casual observation has reported that Pryor horses travel less than ASIS horses, but that has no scientific significance. If one understands the biology of PZP, one would never suggest there is a "psychological" impact of the vaccine.

The research has already shown that band structures do not change and neither do hierarchies. The only major change in hierarchies that occurs is when mares get pregnant, and then they drop down the ladder even more. Most wild horse behavioral researchers still don't know how to measure hierarchy rank.

A more recent study (Nunez et al. 2009) on Cape Lookout National Seashore indicated that treated mares change bands more often outside of the breeding season. However, the control group for this study is a group of mares that become pregnant and have their foals removed from them. One could just as easily conclude that removing foals from untreated mares causes the mares to stay with the band for longer durations.

**Q. What about behavioral changes, recently observed in mares, attributed to native PZP?**

A. After 23 years of experience in the field, using native PZP, researchers observing wild horse mares feel that fundamental wild horse social behavior is not changed by the vaccine. However, other researchers are looking at issues such as "time budgets" to find any potential changes. It is the opinion of long-term PZP researchers, however, that these changes are neither of significance, nor are these studies controlled properly. For example, do time budgets differ for horses on good range as opposed to poor range? No one has looked at this, but if there are differences (and logic tells you there should be differences in time budgets between horses living in knee-deep alfalfa and those scratching out a living in a place like the Pryor Mountain Wild Horse Range), then we might expect the time budget of a chronically treated horse to change from untreated horses because of the better body condition scores of the former. These studies have not been controlled for mares with foals (usually untreated) and those without (usually treated), but the important point is that if the fundamental social structure and behavior do not change, it is of no consequence if time budgets change.

**Q. Won't mares just keep coming back into estrus (heat) if they don't get pregnant? Won't prolonged estrus cycling make stallions "edgy" and aggressive, creating continuous "unrest?" In the chaos, won't foals be harmed or even killed?**

A. At the heart of this issue is the subjective nature of casual observation. Science is based on data, not informal surveillance. Systematically collected data, reviewed by other scientists, accepted as legitimate, treated appropriately statistically, and published in a recognized journal is the only acceptable means for arriving at generalizable, accurate behavioral information. A good start for understanding the rudiments of behavioral research in social animals can be found in Craig (1986).

In 1983, it was shown (Kirkpatrick and Turner 1983; 1986 a,b) that wild horses do not have the same ovulatory patterns as domestic horses, and that wild horses have well-defined breeding seasons (usually from about late March until July, but this will vary somewhat from herd to herd). Wild mares do not extend their breeding season if they do not become pregnant. Keiper and Houpt (1984) also showed this for Assateague horses. This DOES happen, however, in deer (McShea et al. 1997) but not with wild horses. In 27 years of data collection on Assateague Island, only a single documented incident of a stallion killing another stallion, because of fighting, has been observed. This occurred in the middle of breeding season (late May).

The PZP vaccine does not prevent ovulation in horses and there is some limited data that it might interfere with ovulation in deer, at least after the initial treatments. Wild horses do not come into estrus every month, whether or not they have been treated with a contraceptive. They have a breeding season that barely makes it from April through July. Many wild mares have but a single estrous cycle and some have none in a given year, but they are highly seasonal and do not ovulate year-round, or even half the year.

The implication of the question is that by treating wild horse mares with PZP, they will continue to cycle throughout the year, and that this will cause stallion "unrest," aggression, and potential injury. By contrast, documented evidence shows that mares will NOT continue to cycle if they do not conceive, at least beyond the normal 3-4 month breeding season.

If critics were correct, and PZP did cause "unrest" and continuous cycling, which, in turn, led to worked-up stallions and foals dying as a result of this behavioral turmoil, then any area using PZP would have greater foal mortality. However, after 15 years of management-level treatment on ASIS, foal mortality has decreased. That is a data-driven fact.

Granted... wild horse behaviors are subtle, and individual horses will show a wide range of variability in behavioral patterns. It takes a great deal of observational experience to pick them up. We do know that hierarchies and band fidelity, are not affected by PZP application. We also know that aggressiveness and aberrational behaviors are not caused by PZP use. Perhaps, however, there may be a subtle change in daily routine. However, the larger question is, even if subtle alterations in behavior may occur, this

is still far better than the alternative of wild horses being rounded up, bands broken apart, and all of the other negatives that go with traditional management. These issues need to be put into the perspective of risk-benefit by PZP opponents.

There may be some minor time budget changes, but this was addressed several questions above. In any case they are not significant in the world of wild horse behavior.

**Q. I “live” with wild horses and feel that some of your behavioral studies (measuring PZP effects) are wrong or incomplete.**

A. Living with wild horses and seeing them every day is one thing, but while of interest, this doesn't necessarily hold noteworthy meaning, unless a parameter for study is identified, a hypothesis established, and a means of testing that hypothesis is conducted. Additionally, this still does not indicate a significant behavioral or other casually observed pattern for an individual animal or for a band or herd unless the data is analyzed properly.

**Q. I wish the BLM had used a bit of its research money or allocated new money to do a study regarding the effects of PZP on the social structure and health of wild horses in the west.**

A. The effects of PZP on social structure and herd health have been studied on herds within the Pryor Mountain Wild Horse Range and Little Book Cliffs Wild Horse Range, and no behavioral changes have been noted, nor have any behavioral changes been noted on wild horses at Return To Freedom (American Wild Horse Sanctuary) in California, where animals are observed daily.

**Q. Isn't the use of PZP “against nature?” Why can't you just leave these animals alone?**

A. Except in a small number of wild horse herds (such as in the Montgomery Pass herd (Turner et al. 1992), Bordo Atravassado in New Mexico, and a few others), mountain lions cannot predictably predate a sufficient number of wild horses to keep herd levels at population numbers in balance with the carrying capacity of their ecosystem. The potential for both wolf and grizzly bear predation of wild horses is in question, as well,

especially if grizzlies and wolves are delisted from the Endangered Species Act, making grizzlies, wolves, and mountain lions all open to hunting pressures.

## **OPPOSITION TO PZP**

***Q.*** Why are some wild horse advocacy groups so vehemently opposed to PZP? It seems like the perfect solution.

A. Opposition to the contraception arises for several reasons. Wild horse advocates do not trust the BLM with the PZP vaccine, despite assurances that the agency cannot use it without approval from The Humane Society of the United States (HSUS). BLM has inflamed the issue by asking FDA for their own INAD, so they could bypass The HSUS. FDA told the BLM that no more "use" INADs would be issued; so, currently, the agency has no choice but to work with HSUS, unless they are willing to change horses in the middle of the race. The HSUS will permit the use of PZP to manage, even reduce, but not to eliminate wild horses.

This has now led the BLM to seek as many other forms of wild horse contraception as possible, from other groups, but it will take significant time to match the safety data studies that have been generated on PZP over many years.

All wild horse advocates want horses to have a better life, but if this entails a choice between having 130 healthy horses versus 200 living on the nutritional edge (in the event of a drought or a severe winter), some would choose the 130 healthy-horse option, out of (what they see as) concern for the well being of the horses. The irony is that those who seek to control wild horse populations through immunocontraceptive measures often spar with other horse advocates who (in the opinion of PZP supporters) object to what pro-PZP factions perceive as the humane treatment of wild horses. The anti-PZP community, who often question factual information and historic success, distrust the morals of those who strongly profess they care (about the well being of the herds). Pro-PZP individuals and groups believe, with conviction, that the primary motivation behind wild horse contraception is keeping healthy wild horses in the wild, on the land forever, in as natural a state as possible, with minimal interference from humans.

Many of these opponents dislike PZP because they fear it will reduce the herd to lower numbers than they want. That has been the major contention with most gathers. After 22 years of contraception on ASIS, and 15 at the management level, contraception has only been able to reduce that herd from 173 to 116. Zero population growth was achieved, and, to date, there has been no need for gathers, but reduction has been slow. So, there is little danger of massive reductions happening anywhere. Even the event of a catastrophic winter has less danger inherent than most advocates might think. The Pryor Mountain herd in Montana/Wyoming went from about 140 horses to 70 in a tragic winter die-off of 1977-1978, but the population had recovered within three years. The only thing that would have changed, had there been 200 animals instead of 140 is that more animals would have had less to eat, and therefore more would have died. The severity of the winter determined that 70 horses would survive, and not the starting population number.

***Q. Aren't you trying to bring wild horses to extinction (using PZP)?***

**A.** The concerns of some anti-PZP wild horse groups over BLM use of the vaccine are justifiable, and the BLM's proposal to use PZP to cause an Oregon herd to approach extinction, and other proposals that have surfaced to use PZP to manipulate herd composition, prove this. These apprehensions are legitimate and acknowledged by PZP researchers. However, Assateague Island data have proven the safety and efficacy of the vaccine, and it is obvious that the BLM is not going to be able to treat 30,000 wild horses to extinction with contraception, especially with continued involvement from The HSUS. Vigilance is important in the continued utilization of the PZP vaccine and in monitoring questionable research studies by state and federal agencies not sanctioned by The HSUS or the BLM, some involving the use of contraceptives that may potentially cause complete sterilization or deleterious effects in mares. Despite the utter rejection of scientific data as a legitimate form of persuasion by some anti-PZP individuals and groups, their hearts are in the right place. They are not the natural enemies of those promoting PZP as a humane management tool. Affable cooperation is needed to resolve issues of wild horse over-population, when and where it legitimately exists, once other means of population reduction have failed or are found to be undesirable.

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References below are a sampling of the only known published reports, to date, on the subject of PZP use in wild or domestic horses, or in zebras, wild burros or Przewalski's (Mongolian) horses. However, additional peer-reviewed papers, not listed below, have been published on these topics. Those selected were chosen to support material included in answers to the questions above.

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146:59-66. *Describes the fundamental technology used for developing a one-shot, multi-year vaccine.*

Feist, J.D., and D.R. McCullough (1976). Behavioral patterns and communication in feral horses. *Z. Tierpsychol.* 41:337-371.

Frank, K.M., and J.F. Kirkpatrick (2002). Porcine zona pellucida immunocontraception in captive exotic species: Species differences, adjuvant protocols and technical errors. *Proc. Amer. Assoc. Zoo Veterinarians*, October 2002, Milwaukee, WI, pp. 221-223. *This reports on the effectiveness and safety of PZP in 29 zebras and is about the same as in horses except for the need to give the booster inoculation sooner. No deleterious effects of any kind were found.*

Frank, K.M., R.O. Lyda, and J.F. Kirkpatrick (2005). Immunocontraception of captive exotic species. IV. Species differences in response to the porcine zona pellucida vaccine and the timing of booster inoculations. *Zoo Biology* 24:349-358.

Frisbie, K.M., and J.F. Kirkpatrick (1998). Immunocontraception of captive exotic species: A new approach to population management. *Animal Keeper's Forum* 25:346-351. *This is the first report on the use of PZP in zebras. About the only difference from horses is the need to apply booster inoculations at 9 months rather than a year.*

Hansen, K.V., and J.C. Mosley (2000). Effects of roundups on behavior and reproduction of feral horses. *Journal of Range Management* 53:479-482.

Kaul, R., A. Afzalpurkar, and S.K. Gupta (1996). Strategies for designing an immunocontraceptive vaccine based on zona pellucida synthetic peptides and recombinant antigens. *Journal of Reproductive Fertility (Supplement 50)*:127-134. *This paper also describes the properties and efficacy of the zona vaccine.*

Keiper, R.R., and K. Houpt (1984). Reproduction in feral horses: An eight-year study. *American Journal of Veterinary Research* 45:991-995.

Keiper R.R. (1976). Social organization of feral ponies. *Proc. Pennsylvania Acad. Sci.* 50:69-70.

Keiper R.R. (1986). Social behavior. *Veterinary Clinics of North America.* 2:465-484.

Kirkpatrick, J.F. (1995). Management of Wild Horses by Fertility Control: The Assateague Experience. National Park Service Scientific Monograph, National Park Service, Denver, CO. (60 pp.). *This scientific monograph describes the early experiments on Assateague Island that led to the successful application of PZP to wild horses.*

Kirkpatrick, J.F. (1996). Some recent developments in immunocontraception. In: Living with Wildlife, A. Rowen, and J.C. Weers (eds.). Tuft's Center for Animal and Public Policy, North Grafton, MA, pp. 87-98. *This is a general review of PZP immunocontraception through the mid-1990s.*

Kirkpatrick, J.F. (2005). The wild horse fertility control program. In: Wildlife Contraception. A.T. Rutberg (ed.) Humane Society Press, Washington, DC., pp. 63-76.

Kirkpatrick, J.F., and J.W. Turner, Jr. (1983). Seasonal patterns of LH, progestins and estrogens in feral mares. *Journal of Equine Veterinary Science* 3:113-118.

Kirkpatrick, J.F., and J.W. Turner, Jr. (1986a). Hormones and reproduction in feral horses. *Journal of Equine Veterinary Science* 6:250-258.

Kirkpatrick J.F., and J.W. Turner, Jr. (1986b). Comparative reproductive biology of feral horses. *Journal of Equine Veterinary Science* 6:224-230.

Kirkpatrick, J.F., and J.W. Turner, Jr. (1985). Chemical fertility control and wildlife management. *BioScience* 35:485-491. *This paper is the earliest review of the topic of wildlife contraception, using all methods and all species.*

Kirkpatrick, J.F., and J.W. Turner, Jr. (1991a). Reversible fertility control in nondomestic animals. *J. Zoo Wildlife Medicine* 22: 392-408. *An updated version of the BioScience paper above.*

Kirkpatrick, J.F., and J.W. Turner, Jr. (1991b). Compensatory reproduction in feral horses. *J. Wildlife Management* 55(4):649-652.

Kirkpatrick, J.F., and K.M. Frank (2005). Fertility control in free-ranging wildlife. In: *Contraception in Captive and Free-Ranging Wildlife*. Asa C. and I. Porton (eds.), John Hopkins Press, Baltimore, MD, pp. 195-221.

Kirkpatrick J.F., and J.W. Turner, Jr. (1991). Reversible fertility control in non-domestic animals. *J. Zoo Wildlife Medicine* 22:392-408.

Kirkpatrick, J.F., and A. Turner (2002). Reversibility of action and safety during pregnancy of immunization against porcine zona pellucida in wild mares. *Reproduction (Supplement 60)*: 197-202. *This paper reported on 12 years of research and 7 of management with regard to safety in pregnant mares, the lack of injection site reactions, reversibility (which was almost total after 5 consecutive years of treatment but not after 7 consecutive years of treatment).*

Kirkpatrick, J.F., and A. Turner (2003). Absence of effects from immunocontraception on seasonal birth patterns and foal survival among barrier island horses. *Journal of Applied Animal Welfare Science* 6: 301-308. *As the title implies, this paper showed that treated mares taken off contraception do not produce subsequent foals out of season, and that survival of foals born to previously treated mares is actually better than that of foals born to untreated mares.*

Kirkpatrick, J.F., and A. Turner (2007). Immunocontraception and increased longevity in Equids. *Zoo Biology* 25:1-8. *This paper describes the significantly greater longevity resulting from PZP treatment in wild horses.*

Kirkpatrick, J.F. and A. Turner (2008). Achieving population goals in a long-lived wildlife species (*Equus caballus*) with contraception. *Wildl. Res.* 35:513-519. *This paper describes the significant population changes achieved on Assateague Island using PZP contraception.*

Kirkpatrick, J.F., I.K.M. Liu, and J.W. Turner, Jr. (1990). Remotely-delivered immunocontraception in feral horses. *Wildlife Society Bulletin* 18:326-330. *This records the first use in wild horses and showed (1) high efficacy; (2) no effect on pregnant mares; (3) no short-term effects on behavior; (4) ability to deliver PZP remotely; and (5) no short-term debilitating effects.*

Kirkpatrick, J.F., I.K.M. Liu, J.W. Turner, Jr., and M. Bernoco (1991). Antigen recognition in mares previously immunized with porcine zonae pellucidae. *Journal of Reproductive Fertility (Supplement 44)*:321-325. *This was the second paper on PZP use in wild horses and demonstrated (1) reversibility; (2) the ability to maintain contraception with single annual*

*treatments; (3) no effects on behavior; (4) no short-term effects on behavior.*

Kirkpatrick, J.F., I.K.M. Liu, J.W. Turner, Jr., R. Naugle, and R. Keiper. (1992). Long-term effects of porcine zona pellucida contraception on ovarian function in feral mares. *Journal of Reproductive Fertility* 94:437-444. *This paper reported on four years of treatment and showed (1) no debilitating effects; (2) absence of injection site reactions; (3) no changes in behavior; and (4) efficacy approaching 90%.*

Kirkpatrick, J.F., R. Naugle, I.K.M. Liu, M. Bernoco, and J.W. Turner, Jr. (1995a). Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Biology of Reproduction, Monograph Series 1: Equine Reproduction*: 411-413. *This showed essentially the same things as the paper immediately above, but after 7 consecutive years. At this point, the PZP appeared to be effective, causing no injection site reactions, was reversible, didn't change social behaviors, had no long-term (7-year) debilitating health effects, etc.*

Kirkpatrick, J.F., W. Zimmermann, L. Kolter, I.K.M. Liu, and J.W. Turner, Jr. (1995b). Immunocontraception of captive exotic species. I.: Przewalski's horse and Banteng. *Zoo Biology* 14: 403-413. *This reports the use of PZP in Przewalski's horses in the Koln Zoo (Germany). It was effective, and there were no other problems.*

Kirkpatrick, J.F., I.K.M. Liu, and J.W. Turner, Jr. (1996a). Contraception of wild and feral equids. In: *Contraception in Wildlife Management*. T.J. Kreeger (ed). U.S. Government Printing Office, Washington, DC. pp. 161-169. *This paper was given at the Third International Symposium on Wildlife fertility Control, in Denver, Co, in 1993, and reports on all equid immunocontraception up to that point in time.*

Kirkpatrick, J.F., J.W. Turner, Jr., I.K.M. Liu, R.A. Fayer-Hosken (1996b). Applications of pig zona pellucida immunocontraception to wildlife fertility control. *Journal of Reproductive Fertility (Supplement 51)*:183-189. *The only new information here was that the antibodies raised by PZP treatment did not cross-react with any other somatic tissues in the horse, or even with basic reproductive hormones. Translated, this meant the vaccine was even safer than previously thought.*

- Kirkpatrick, J.F., J.W. Turner, Jr., I.K.M. Liu, R.A. Fayrer-Hosken, and A.T. Rutberg (1997). Case studies in wildlife immunocontraception: Wild and feral equids and white-tailed deer. *Reproduction, Fertility and Development* 9:105-110.
- Klingel, H. (1975). Social organization and reproduction in equids. *Journal of Reproductive Fertility (Supplement 23)*:7-11.
- Liu, I.K.M., and A. Shivers (1982). Antibodies to the zona pellucida in mares. *Journal of Reproductive Fertility (Supplement 32)*:309-313.
- Liu, I.K.M., M. Bernoco, and M. Feldman (1989). Contraception in mares heteroimmunized with porcine zonae pellucidae. *Journal of Reproductive Fertility* 89:19-29. *This paper reports on the first application of the PZP vaccine to horses.*
- Lyda, R.O., R. Hall, and J. F. Kirkpatrick (2005). A comparison of Freund's Complete and Freund's Modified adjuvants used with a contraceptive vaccine in wild horses. *J. Zoo Wildl. Med.* 36:610-616. *This paper describes the issues of adjuvants and injection-site reactions in horses after treatment with PZP.*
- McCort, W.D. (1984). Behavior of feral horses and ponies. *Journal of Animal Science* 58:493-499.
- Miller et al. (2001). Characterization of equine zona pellucida glycoproteins by polyacrylamide gel electrophoresis and immunological techniques. *Journal of Reproductive Fertility* 96: 815-825. *This was in vitro work that explained the molecular structure of the equine zona pellucida and speculated on how fertilization is blocked by PZP.*
- Oser, B.L. (1965). Chapter 24. Protein metabolism. In: Hawke's *Physiological Chemistry*, McGraw-Hill, NY, p. 810.
- Paterson, M., and R. Aitkin (1990). Development of vaccines targeting the zona pellucida. *Current Opinion in Immunology* 2:743-747. *Reports on the actual mechanisms behind PZP contraception.*
- Powell, D. (1999). Preliminary evaluation of porcine zona pellucida immunocontraception for behavioral effects in feral horses. *Journal of Applied Animal Welfare Science* 2:321-335. *Working with the behaviorist from the National Zoological Garden, Powell showed no behavioral effects after years of use.*

Roelle, J. E. and J. Ransom (2009). Injection-site reactions in wild horses (*Equus caballus*) receiving an immunocontraceptive vaccine. Scientific Investigations Report 2009-5038, USGS, Fort Collins, CO. 15 pp. *This report describes injection-site reactions among western wild horses treated with PZP.*

Rubenstein, D.I. (1981). Behavioral ecology of island feral horses. *Equine Veterinary Journal* 13:27-34.

Rutberg, A.T. (1990). Inter-group transfer in Assateague pony mares. *Animal Behaviour* 40:945-952.

Rutberg, A.T., and S.A. Greenberg (1990). Dominance, aggression frequencies and modes of aggressive competition in feral pony mares. *Animal Behavior* 40:322-3331.

Salter, R.E., and R.J. Hudson (1982). Social organization of feral horses in western Canada. *Applied Animal Ethology* 8:207-223.

Seal, U.S. (1991). Fertility control as a tool for regulating captive and free-ranging wildlife populations. *J. Zoo Wildlife Medicine* 22:1-5. *This paper is another review of the broader subject of wildlife contraception.*

Shivers, A., and I.K.M. Liu (1982). Inhibition of sperm binding to porcine ova by antibodies to equine zona pellucida. *Journal of Reproductive Fertility (Supplement 32):315-318. In this paper, we see the first investigation of how antibodies against PZP might interfere with fertilization. This work was all in vitro.*

Stafford, K.J., E.O. Minot, W.L. Linklater, E.Z. Cameron, and S.E. Todd (2001). Use of an immunocontraceptive vaccine in feral Kaimanawa mares. *Conservation Advisory Science Notes* 330, Department of Conservation, Wellington, New Zealand. *This paper reports on the failure of an attempt to inhibit fertility in New Zealand wild mares. They used the wrong dose, the wrong adjuvant, and the wrong delivery system; so it is not surprising that it didn't work. Other than that, there were no harmful effects.*

Turner, A., and J.F. Kirkpatrick (2002). Effects of immunocontraception on population, longevity and body condition in wild mares. *Reproduction (Supplement 60):187-195. This paper reported on 12 years of research and seven of management of wild horses with PZP. It showed that (1) a*

*significant population effect can be achieved; (2) that horses thus treated achieved better body condition and lived significantly longer than before treatment and still no behavioral effects had occurred.*

Turner, Jr., J.W., M.L.Wolfe, and J.F. Kirkpatrick (1992). Seasonal mountain lion predation on a free-roaming feral horse population. *Canadian Journal of Zoology* 70:929-934.

Turner, Jr., J.W., I.K.M. Liu, A.T. Rutberg, and J.F. Kirkpatrick (1996a). Immunocontraception limits foal production in free-roaming feral horses in Nevada. *Journal of Wildlife Management* 61:873-880. *This report examines the large-scale application of PZP to western wild horses. It is significant because it presents solid data for lack of injection site reactions.*

Turner, Jr., J.W., I.K.M. Liu, J.F. Kirkpatrick (1996b). Remotely-delivered immunocontraception in free-roaming feral burros. *Journal of Reproductive Fertility* 107:31-35. *This reports the first use of PZP in burros, and other than efficacy and reversibility, no other data were collected. It was a short-term study, but there were no negative facets to the work, physically or behaviorally.*

Turner, Jr., J.W. et al. (1997). Immunocontraception limits foal production in free-roaming feral horses. *Journal of Wildlife Management* 61:873-880. *This is the first report of large-scale use of PZP (in more than 100 animals at a time) in Nevada horses. Other than the efficacy of nearly 90%, the primary noteworthy data included the complete absence of any injection site reactions. In this case, the animals were inoculated and kept in corrals for three weeks and examined daily for problems. There were none.*

Turner, Jr., J.W. et al. (2001). Immunocontraception in feral horses: A single inoculation vaccine providing one-year of infertility. *Journal of Wildlife Management* 65:235-241. *This paper showed that the effectiveness of a prototype one-shot form of the vaccine was as good as the standard two-shot treatment. More than 200 horses were treated, and none showed any deleterious effects.*

Turner, Jr., J.W., I.K.M. Liu, D.R. Flanagan, K.S. Bynum, and A.T. Rutberg (2002). Porcine zona pellucida (PZP) immunocontraception of wild horses in Nevada: A 10-year study. *Reproduction (Supplement 60):177-186. As the title indicates, this is a summary of 10 years of research with PZP, in*

*several forms and with several adjuvants. The paper reports on effectiveness and showed no deleterious effects of any kind.*

Turner, Jr., J.W., A.T. Rutberg, R.E. Naugle, M.A. Kaur, D.R. Flanagan, H. J. Bertschinger, I.K.M. Liu (2008). Controlled release components of PZP contraceptive vaccine extend duration of infertility. *Wildl. Res.* 35:555-562. *This paper is an update of the Turner et al. 2002 paper (above).*

Willis, P., G.L. Heusner, R.J. Warren, D. Kessler, and R.A. Fayrer-Hosken (1994). Equine immunocontraception using porcine zona pellucida: A new method for remote delivery and characterization of the immune response. *Journal of Equine Veterinary Science* 14:364-370. *This reports on a study with domestic horses at the University of Georgia. Aside from efficacy, there were no abscesses, no cross reactivity with somatic tissues, and no clinical problems. This study was carried out in a veterinary school.*

### ***FOR ADDITIONAL INFORMATION***

[http://www.zoomontana.org/conservation\\_center/index.php](http://www.zoomontana.org/conservation_center/index.php)

(The Science and Conservation Center, ZooMontana, Billings)

[http://www.zoomontana.org/conservation\\_center/pzp\\_q\\_and\\_a.pdf](http://www.zoomontana.org/conservation_center/pzp_q_and_a.pdf)

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