APPENDIX 2: 2004 PROTOCOL for the TREATMENT of WILD MARES
PRYOR MOUNTAIN WILD HORSE RANGE
with a
PORCINE ZONA PELLUCIDA CONTRACEPTIVE VACCINE

1. PURPOSE

This is a research field trial designed to delay pregnancy in younger and older wild horse mares within the Pryor Mountain Wild Horse Range (PMWHR) through the use of a native porcine zona pellucida (PZP) contraceptive vaccine delivered remotely, under field conditions. The vaccine would induce one year of infertility, allowing the younger mares to mature in a healthier condition, before becoming pregnant and producing and supporting a foal. The vaccine would also be applied to mares 14 years of age and older. The would allow these mares at least 2 years of recovery post-foaling with an opportunity for improved condition in later life. In 2004, 14 young mares and 7 older mares on the PMWHR, would be treated remotely with the vaccine. The method of delivery would be with 1.0cc Pneu-Darts® and delivery would be by Dan-Inject or PneuDart capture gun.

II. PARTICIPANTS

Project Manager:	Linda Coates-Markle, State Wild Horse and Burro Specialist, BiFO, BLM
Horse Identification:	Field-trained and experienced BLM Seasonal employees and Student Interns.
Vaccine Preparation:	Robin Lyda, The Science and Conservation Center, ZooMontana, 2100 South Shiloh Road, Billings, MT 59106
Designated Darters:	Jay F. Kirkpatrick, Kim Frank and Robin Lyda, The Science and Conservation Center, ZooMontana, Billings, Mt.
Other Certified Darters:	Linda Coates-Markle, BiFO, BLM Melissa Esser, Seasonal Employee, BiFO, BLM Jason Ransom, USGS Wild Horse Crew Leader Susan Hahn, USGS Wild Horse Research Crew
Project Veterinarian:	Lyle Bischoff, DVM, Powell Veterinary Service 522 S. Division, Powell, WY 82435

III. PROCEDURES

A. Vaccine preparation and shipment: Vaccine would be prepared under the supervision of Robin Lyda, Science and Conservation Center (SCC), Billings, MT and transported to the field site in Montana on dry ice, under Food and Drug Administration authority (Investigational New Animal Drug exemption No.8857 G0002 & 0003). FDA form "Notice of Drug Shipment" would be completed for each shipment of the PZP vaccine and filed in the offices of the Science and Conservation Center at ZooMontana, Billings, MT. On the PMWHR, vaccine would be stored frozen in the field.

B. Selection of subject animal: Animals to be treated have all been previously identified by BLM personnel and are identified in EA # MT-010-04-18. The number and identity of animals would be selected on the basis of predetermined animal welfare goals. All animals selected for treatment would be female and at least one year old. If the identification of any horse is questionable, that horse would not be darted, and the ultimate decision rests jointly with the darter and BLM horse identifier.

C. Delivery of contraceptive vaccine: Delivery of vaccine would be by means of 1.0 cc Pneu-Darts®, with 1.5" barbless needles. 0.5 cc of the PZP vaccine (in sterile water) would be emulsified with 0.5 cc of adjuvant and loaded into darts at the time a decision has been made to dart a specific mare. Animals which have never been treated would be treated with PZP + Freund's Complete (or Freund's Modified) adjuvant, while animals, which have been previously treated would be given PZP + Freund's Incomplete adjuvant. Only designated darters would mix the vaccine/adjuvant and prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of a capture gun.

As necessary, the BLM Wild Horse and Burro Specialist would approve those personnel authorized to conduct wild horse darting operations in the PMWHR. At a minimum, authorization would be restricted to those individuals who have appropriate certification, documented and successful experience darting wildlife under field conditions, and are specifically authorized to dart PMWHR horses by the Wild Horse and Burro Specialist. In addition, at least one of the designated personnel in any darting operation must have successfully completed the National Park Service (NPS) or a comparable wildlife immobilization training course.

The decision to dart a horse would ultimately rest with the darter. The accessibility of the horse at a particular point in time and location would trigger the decision-making process. Safety, for both humans and the horse is the foremost consideration in deciding to dart a mare. The Dan Inject gun would not be used at ranges in excess of 30 meters and no attempt would be taken when other persons are within a 30 m radius of the target animal. If a darting attempt is not taken, the gun would be unloaded and the dart stored in a poly-foam container. If a loaded dart is not

used within 2 hours of the time of loading, the contents would be transferred to a new dart before attempting another horse. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart and used the next day. Refrigerated darts would not be used in the field.

Use of the Pneu-Dart® capture gun for dart delivery is often preferred in the field. Safety is again the foremost consideration. Only low velocity (brown) or medium velocity (green) charges would be used in this project. The gun would remain unloaded until the horse has been selected for a darting attempt. No attempts would be taken at ranges greater than 50 yards. No attempts would be taken when other persons are within a 90N angle defined by a line from the darter to the horse.

Only hip or gluteal muscle regions of the horse are acceptable targets. No attempts would be taken in high wind or when the horse is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal angle is when the dart would strike the skin of the horse at a perfect 90N angle. If a horse moves out of firing range after the gun is loaded and it is apparent that another attempt would not be immediately possible, the gun would be unloaded (both cartridge and dart) and stored. Immediately after firing, the empty cartridge would be ejected, and the dart port opened. Every day the capture gun would be used in the field, early morning practice would be required in order to assure that the gun is properly sighted.

It is suggested that no more than two people be present at the time of a darting. The second person should be responsible for locating fired darts. The second person should also be responsible for identifying the horse and keeping onlookers at a safe distance. The safe distance would be determined by the conditions at the time of darting and specifics of the animal involved and darting location.

Fatigue is a concern for darters. Proper treatment of animals requires a clear mind and decisions about veterinary care require careful thought and appropriate responses. Fatigue would not be uncommon because of the hours and habitat associated with horse tracking. It would be the darter's responsibility to determine when work would cease because of fatigue among team members. Weather can also be an important factor and high winds would be a legitimate cause for stopping the operation. The final decision rests with the darter.

To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting. Copies of a one page explanation of the project shall be carried by the participants and given to any non-participants at every opportunity.

D. Recovery of darts: Attempts would be made to recover all darts. If possible, all darts which are discharged and drop from the horse at the darting site would be recovered before another darting occurs. In exceptional situations, with the decision resting with the darter, the site of a lost dart may be noted and marked,

and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine.

E. Record keeping: BLM and SCC personnel would maintain records for the identification of all horses to be darted or used for control purposes. These records would be used to meet FDA regulations for use of the vaccine under the existing INAD. Each horse darted would be permanently identified by the 4-digit BLM identification number. For each horse darted, the following information would be recorded at the time of darting:

1. identification of darter
2. date of inoculation
3. size of PZP dose
4. type of adjuvant
5. type of dart/delivery system
6. precise site of inoculation (right or left side of hip)
7. delivery distance of dart
8. lot number for vaccine

Additionally, other observations regarding estrous behavior, swelling at the site of injection, injection-site reactions, and any other pertinent information collected by researchers or the Wild Horse and Burro Specialist would be maintained by the BLM.

At a minimum, foal counts and birth records shall be carried out annually by BLM personnel. These data shall be recorded by BLM field technicians and transferred to both permanent BLM and SCC records. Other data on mare body condition, fitness and behavior shall be collected under the guidance and research protocol set by the BLM National Wild Horse Fertility Control Field Trial program.

F. Veterinary Emergencies: Personnel conducting darting operations shall be equipped with a two-way radio or cell phone providing a communications link with the Wild Horse and Burro Specialist. In the event of a veterinary emergency, darting personnel would immediately contact the Wild Horse Specialist, providing all available information concerning the nature and location of the incident. As appropriate, the Wild Horse Specialist would contact the Project Veterinarian for advice and/or assistance.

In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected horse until the dart falls out or the horse can no longer be found. The darter would be responsible for daily observation of the horse until the situation is resolved. Possible reasons for a decision to immobilize a horse may include a suspected broken leg, severe lacerations, a dart that has lodged in a bone for more than two weeks, or a severe infection resulting from a dart, which is lodged in a bone or the abdominal cavity. The former are all considered to be rare events in normal field darting practices. Other injuries that may occur as a direct

result of the darting process, such as severe lacerations and infections, may also require the capture and/or immobilization of the horse for evaluation and treatment. Any decision to capture or immobilize would be made in consultation with the Project Veterinarian. Whenever possible, corralling techniques would be used to capture and contain injured horses. If, in consultation with the Project Veterinarian, the use of immobilizing drugs is deemed necessary and appropriate, such agents would be administered exclusively by the Project Veterinarian or by a member of the darting team under the *direct* supervision of the Project Veterinarian.

All injuries would be treated as per the recommendations of the Project Veterinarian, in consultation with the Wild Horse and Burro Specialist. In the event of a broken leg, or other severe injury, where the Project Veterinarian considers the prognosis for full recovery unlikely, the affected horse would be humanely euthanized, after consultation with the Billings Field Manager and the Wild Horse and Burro Specialist.

G. Blood samples/recovery of ovaries: An attempt to recover blood samples for antibody analysis and to recover ovaries for determination of ovarian effects shall be carried out opportunistically. In the unlikely event that a female horse inhabiting the PMWHR must be euthanized for humane reasons, a blood sample would be immediately collected in a red top10 cc tube. The sample would be sent to the Project Veterinarian where the serum would be harvested and stored frozen. If at all possible, at least one and preferably both, ovaries would be excised and placed in 10% buffered formalin, for histological examination.

H. Media relations: All requests by the media (verbal, written or electronic), must ultimately pass through the Wild Horse and Burro Specialist, BiFO, Billings, Mt or their designate, and the decision to release information related to the project shall rest with the BLM. Efforts would be made to inform media and other interested public as to the status of darting efforts on the PMWHR on a regular basis during planned activity.

I. Public Relations: A public communications plan would be prepared soon after the release of the EA addressing the proposed action. Prior to the start of darting activity, the BLM shall distribute to all law enforcement agencies with jurisdiction on the PMWHR, a notice that darting would commence on a particular date and end on a particular date, and that darters may be witnessed by members of the public darting horses with a capture gun. This information would minimize panic calls from a concerned public and provide law enforcement with an opportunity to explain the circumstances and direct the public to BiFO for further details of the operation.

J. Reporting: An annual report would be prepared by the USGS/BRD and provided to the BLM Wild Horse and Burro Specialist. This report would document contraceptive program activities, impacts on the PMWHR herd and program status, successes and/or concerns. At the completion of the research field trial, all results would be analyzed and reported pertaining to guidance and research protocol set by the BLM National Wild Horse Fertility Control Field Trial program.