BLM Research Proposal Format

A. COVER PAGE

US Department of Interior
Bureau of Land Management
Wild Horse and Burro Program

Proposal for Research Effort

(PRIVILEGED COMMUNICATION)

1a. Applicability and efficacy of ZonaStat-H on wild burros in northwestern Arizona.
TITLE OF PROPOSAL (90 Character Maximum)

1b. PI: Boyles Griffin, Stephanie L.; C-I: Henry Jann DVM
INVESTIGATORS (Principal-Investigator LAST NAME, FIRST NAME; Co-Investigators LAST NAME, FIRST NAME)

2a. Stephanie L. Boyles Griffin
NAME OF PRINCIPAL INVESTIGATOR (PI)

2b. (blank)

2c. Senior Director
POSITION TITLE

2d. sboyles@humanesociety.org
EMAIL

2e. The Humane Society of the United States
INSTITUTION AND DEPARTMENT

2f,g. (301) 258-3147 (301) 258-3080
PHONE FAX

2h. ADDRESS: 700 Professional Drive
Gaithersburg MD 20879

3a. THIS PROPOSAL IS A: (Mark one only) X__ NEW APPLICATION ___ CONTINUATION ___ UNPLANNED EXTENSION

3b. FOR COMPLETION, A FUNDING REQUEST IS:

	INCLUDED and REQUIRED	X INCLUDED but NOT REQUIRED	NOT INCLUDED

3c. AMOUNT OF FUNDING REQUESTED:

	FIRST YEAR	SECOND YEAR	THIRD YEAR
	$ 0	$ 18,055	$ 15,640

3d,e DATES OF PROPOSED STUDY:

	START	END
	10/1/15	12/31/2019

AGREEMENT: It is understood and agreed by the undersigned if this proposal / application is approved, whether or not a grant is made, it will be according to the terms of the proposal and the stipulations set forth in the accompanying instructions. In addition, a written agreement appropriate for the nature of the proposed work (e.g., Memorandum of Understanding, Assistance Agreement, Task Order, letter of agreement) will be required to outline the obligations of the researchers and the BLM in the conduct of the study.

PRINCIPAL INVESTIGATOR ASSURANCE: I agree to accept responsibility for the conduct, completion and reporting of the study proposed here and to provide the agreed upon progress and final reports.

4a. SIGNATURE OF PRINCIPAL INVESTIGATOR: _____ DATE: _____

CERTIFICATION AND ACCEPTANCE: I certify that the statements made in this application are true and complete to the best of our knowledge, and I accept the obligation to comply with the above agreement. I understand that the Principal Investigator and his/her department will be responsible for any expenses incurred by this project which exceed the approved funding amount.

4b. OFFICIAL SIGNING FOR ORGANIZATION: _____ DATE: _____

4c. ADDRESS:

4d. EMAIL

4e,f. PHONE FAX

B. RESEARCH OBJECTIVES

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort

Privileged Communication

Name and Address of Applicant or Applicant Organization:

The Humane Society of the United States
700 Professional Drive
Gaithersburg, MD 20879

Title of Project: Effects of ZonaStat-H immunocontraception on free-ranging western wild burros

Use this space for an ABSTRACT of your Proposed Research, Outline Objectives and Methods (250 work maximum).

To help build the scientific foundation for incorporating immunocontraception into the BLM's wild burro management programs on public lands in the West, we propose a four year field study to examine the efficacy of using the immunocontraception vaccine ZonaStat-H (porcine zona pellucida) on a wild burro herd. We propose to study individual effects of ZonaStat-H on 75-125 burros within the Black Mountain Herd Management Area (HMA) in Arizona.

Individual burros will be accessed to apply treatments using bait stations, bait traps, or by random opportunistic darting in the field (where habituated burros can be accessed and easily identified). Jennets will receive an initial ZonaStat-H dose under standard BLM protocols followed by a booster dose two weeks to six months later. It is assumed that some jennets will not be accessed a second time and therefore will not be treated. Individuals will then be observed and documented for approximately three years.

To describe the effects of ZonaStat-H on foaling rates and foal survival, intensive ground observations will be carried out ten months of the year. Annual booster doses will be attempted on all treated individuals for the length of the study. Ground observations will determine the direct effects of ZonaStat-H on individual jennet reproduction and health and on the timing of foaling. At the conclusion of the study, we will be able to determine how well the Zona Stat-H vaccine, when delivered either remotely and via hand injection, performed as a fertility control agent for wild burros, whether burro populations can be stabilized and reduce over time by treating them with ZonaStat-H, the estimated costs per animal associated with the field application of ZonaStat-H to burros, and the projected cost associated with applying ZonaStat-H to an entire herd of burros in a given HMA.

Name, official title, department, project responsibilities and time commitment (% of annual work effort) of all professional personnel engaged in project:

PI: Stephanie L. Boyles Griffin, Senior Director, Innovative Wildlife Management & Services, The Humane Society of the United States
--20% time commitment

Preparation of BLM proposals, and subsequent reports
Implementation of study
Application of Zona Stat H
Collecting data
Conducting data analysis
Participation in preparation of publications
Oversight of field staff

Hank Jann, DVM
--24 weeks per year

Preparation of BLM and proposals, and subsequent reports
Oversight of study design, preparation of observation protocols
Application of Zona Stat H
Collecting data
Conducting data analysis
Participation in preparation of publications

Field Technician (TBD), The Humane Society of the United States
--50% time commitment

Collecting data
Compiling and organizing data
Application of Zona Stat H

C. RESEARCH PROPOSAL

BLM Wild Horse and Burro Program
Proposal for Collaborative Research Effort

Privileged Communication

All pages must be type written in type font 12, single-spaced with one-inch margins and the pages numbered bottom center. Sections 1-6 cannot exceed a total of 12 pages or individual sections exceed the given page limits. *Guidelines in italics below can be deleted from the proposal prior to submission.*

1. Goals / Objectives / Hypotheses: The field studies described in this proposal will examine the impacts of ZonaStat-H on individual burro health and welfare in the Black Mountain Herd Management Area in northern Arizona. We will evaluate the impacts of ZonaStat-H on 75-150 burros. Jennets will be bait trapped and returned to the range as untreated controls or treated with the standard dose of ZonaStat-H vaccine. Additional habituated jennets in the town of Oatman may be treated with ZonaStat-H via remote opportunistic darting. We will evaluate the effects of ZonaStat-H treatments by comparing foaling data from treated burros obtained during the study to data on untreated burros within the same herd.

Our objectives may be summarized as three questions:

1) *What are the effects of the ZonaStat-H PZP vaccine on individual jennet foaling?*
To answer this question, we will collect data on the number and proportion of treated jennets foaling each year and compare with the same data collected from untreated jennets in the same herd.

2) What are the effects of PZP treatments on the health and social dynamics of treated burros?
To answer this question, we will identify individual jennets, both treated and controls. We will visually examine known animals for injection site abscesses and general health at each encounter, observe new foals, record birth month, and record body condition scores. We will also collect data on group membership, mating, foal survival, and death.

3) Can unhabituated burros be treated initially via bait trapping and then retreated remotely via bait station, bait trapping or opportunistically darting?
To answer this question, we will measure the proportion of originally treated jennets to the retreated jennets and if the retreatment occurred using bait trapping, bait station, or opportunistic darting.

2. Specific Aims: (Sections 1 and 2 are not to exceed 2 pages)
All tasks are to be completed by The Humane Society of the United States' employees, contractors, or volunteers with the exception of BLM participation in bait trapping and freeze marking burros.

2015: Identification of bait trap sites and photograph all habituated burros in the town of Oatman for identification data base.

2016: Bait trap and freezebrand 75-125 jennets and treat 70% with ZonaStat-H vaccine. Collect post-treatment baseline data on all captured individuals (foals at side, seasonality, foaling month, body condition score, injection site reaction, age). 50% of all treated jennets will be released and the standard booster dose of ZonaStat-H will be delivered via dart either opportunistically or at a bait trap or bait station. The booster doses will be attempted to be delivered two weeks to six months after the initial treatment. The remaining 50% of treated jennets will be transferred to the BLM Kingman, AZ corrals where they will be held for two to three weeks and then given the standard booster dose of ZonaStat-H. Seventy percent of all habituated burros in the town of Oatman will be vaccinated with ZonaStat-H via remote darting if a positive identification of the individual is well documented.

2017: Collect data on first year of 2016 control and treated jennets. Record individual foaling rates, seasonality, and individual body condition and health. Re-treat all previously treated jennets 8-12 months after initial treatment. Record differences in the ease of access for second and third treatment for each individual.

2018: Collect data on second year of 2016 control and treated jennets. Record individual foaling rates, seasonality, and individual body condition and health. Re-treat all previously treated jennets 8-12 months after initial treatment. Record differences in the ease of access for subsequent treatments for each individual.

2019: Collect data on third year of 2016 control and treated jennets. Record individual foaling rates and seasonality, individual body condition and health. Re-treat all previously treated jennets 8-12 months after initial treatment. Record differences in the ease of access for subsequent treatments for each individual.

3. Background and Significance/Preliminary Studies: (Not to exceed 4 pages)
Briefly discuss the background of the proposal by critical evaluation of existing knowledge and by identification of gaps, which would be addressed by the proposed research. State concisely the importance of the proposed research by relating the objectives of the study to the broad, long-term goals of the BLM Wild Horse and Burro Program in the areas of fertility control and population growth suppression. . Indicate how this effort will advance the fields of equine science, medicine and surgery toward achieving these goals and provide any relevant data (national, regional, local, institutional/ practice) to support the proposed investigation. The rationale for the proposed work must be established by the information presented here. Applicants may also use this section to describe preliminary studies that are pertinent to the application or to provide other information that will help establish the competence of the investigator to undertake the proposed research. Describe the impact of the potential outcome of the investigation to WH&B management, health, and wellbeing specifically regarding population growth suppression.

If not subjected to management, predators, or severe environmental conditions, wild burro populations, like wild horse populations on western public lands, grow rapidly. Published estimates of population growth in western herds range from 11-29% per year, (Morgart, 1978; White, 1980; Ruffner and Carothers, 1982). Wild burros significantly influence the productivity, biomass, and species composition of rangeland plants, utilize water resources, and compact soil. Removal of equid grazing in the Great Basin increases shrub and grass cover and species richness, and excluding equids from water sources results in greater plant species richness,

higher percent plant cover, and greater abundance of grasses and shrubs (Beever and Brussard, 2000; Beever et al., 2007). Wild equids compete with elk, mule deer and wild sheep, and shape wildlife habitat (Marshal et al. 2012; Beever, 2003; Beever and Brussard, 2004, 2000; Hanley and Hanley, 1982; Hansen and Clark, 1977).

Historically, wild equids have been controlled on public lands by gathering and removing them from the range. Between 1971 and 2013, approximately 27,652 burros were removed from the range. In 2004, the BLM was further required by Congress to sell certain horses and burros removed from the range; about 489 burros have been sold under this program.

These management methods are expensive. The cost of a gather typically exceeds $200,000, and the cost of gathering, handling, processing, holding, transporting, and adopting a single wild equid has been estimated to cost more than $9000 (Wild Horse and Burro Advisory Board meeting, April 2014). Moreover, sale and adoption have proven chronically inadequate to dispose of wild burros removed from the range. Following an ambitious program to achieve appropriate management level (AML) on all herd management areas (HMA's), the BLM now finds itself holding more than 900 wild burros in short-term holding facilities and nearly 50,000 wild horses. In FY 2013, BLM spent $46.2 million maintaining these animals, which constituted 46% of the entire budget of the agency's wild horse and burro program. Rational planning of wild horse and burro management on public lands is also hindered by year-to-year fluctuations in budget, court orders, and the vagaries of rainfall, fire, and other environmental factors.

However, an effective wild horse and burro fertility control program offers BLM a potential buffer against environmental, political, and budgetary contingencies and an opportunity to reduce program costs (Bartholow, 2004). The 2013 National Academy of Science reported that PZP was one of the three most promising methods of fertility control for wild horses and burros.

BLM has been supporting research on wild horse fertility control since the 1970's. Early BLM-sponsored research included the use of hand-injected and remotely delivered androgens to stallions in the Challis HMA in Idaho, and estrogen and progestin implants in mares at Clan Alpine HMA in Nevada (Plotka et al., 1992; Turner and Kirkpatrick, 1982). Although some of these methods were pharmacologically successful, i.e., they curtailed reproduction, steroid contraceptives proved difficult to deliver to wild horses in the field. Additionally, they raised concerns about passage through the food chain, behavioral and health side effects, and horse welfare (Turner and Kirkpatrick, 1991).

In 1990, the focus of wild horse contraceptive research shifted to the porcine zona pellucida (PZP) immunocontraceptive vaccine. This shift was spurred by research at Assateague Island National Seashore, Maryland, which demonstrated that a dart-delivered PZP vaccine prevented pregnancies in wild horses with effectiveness of 90% or more (Kirkpatrick et al., 1990). This same formulation was shown to be 100% effective on jennets on the island of St. John while 54% of the untreated control group jennets became pregnant during the same time period. The drug also proved to be safe, effective and reversible (Turner et al., 1996). This study also illustrated that feral burros could be accessed for remotely delivered PZP. Naugle and Grams (2013) also noted that with safer and more sophisticated delivery equipment remote delivery of immunocontraceptive agents have been proven effective. Burro contraceptive research has never

been a focus of the BLM due to the far fewer populations of burros, their slightly slower population growth rate and their historically steady adoption demand. However, burro adoption numbers have decreased since 2002.

In general, the safety of PZP on equids has also been well-established. Even with the use of Freund's Complete Adjuvant in priming doses, draining abscesses at the injection-site are extremely rare, especially when the vaccine is hand-injected (Kirkpatrick et al., 1990; Lyda et al., 2005; Turner et al., 2001). Remotely treated mares in The HSUS's Sand Wash Basin study had a 9% abscess rate that ranged from 3-9 cm in size and dissipated within two months (Rutberg, pers. comm.). ZonaStat-H vaccination does not affect ongoing pregnancies, or survival or fertility of foals of treated mares (Kirkpatrick et al., 1990; Turner et al. 1996; Kirkpatrick and Turner, 2002; Lyda et al., 2005).

The relationship between vaccine reversibility and the number of years treated is fairly well described for the simple PZP/adjuvant Assateague vaccine (Kirkpatrick and Turner, 2002; Turner et al., 1996). Return to fertility is progressively delayed after three consecutive years of treatment, with delays of 4 years or more possible after 4 or more consecutive years of treatment.

Population effects of contraception have been modeled. Garrott developed a stage-structured model using survival and fecundity data from a range of western wild horse populations (Garrott, 1991). These models indicated that high proportions (60-80% or more) of females would need to be treated with contraceptives to achieve stability or herd reduction, with smaller proportions still achieving significant reductions in reproductive rate. However, the model also assumed that mares would be treated at intervals equal to the duration of the contraceptive, which produced the counterintuitive result, that short-acting contraceptives would be more effective at controlling populations than long-acting contraceptives. *WinEquus* (Jenkins, 2002), which is used by the BLM to forecast management outcomes in specific HMA's, is a highly flexible stochastic model that allows the input of site-specific and management-action-specific data, but post-contraception data are yet available for western wild herds, and the outcomes predicted by *WinEquus* for contracepted populations have not been verified. The Assateague and Pryor Mountain wild horse populations have been modeled (and the Assateague model verified using site-specific data) using individual-based models, but such models demand much more information about the life histories of individual horses than is feasible with most western herds (Gross, 2000; Zimmerman et al., 2006).

As noted, these models have not yet been tested with data from western wild horses or burros. Field data on the population effects of PZP have been reported for Assateague horses, Pryor Mountain horses, Little Book Cliffs horses and for white-tailed deer, all of these studies have used vaccines that were administered annually (Naugle et al., 2002; Ransom et, al., 2011; Rutberg et al., 2004; Turner and Kirkpatrick, 2002).

4. Experimental Approach: (Not to exceed 5 pages)

The experimental design and procedures should be described in detail. Include the process by which data will be collected. Discuss potential difficulties and limitations of the proposed procedures and alternative approaches to achieve the stated aims (Section 2 above). Provide a tentative sequence or timetable for the investigation (e.g., a Gantt chart).

Study Herd Characteristics and General Design
The Black Mountain HMA in Arizona has been identified for this adaptive management study. This herd should allow for at least 20 habituated jennets and over 75 unhabituated jennets for study. To facilitate observation, it is desirable that this study herd be accessible by vehicle.

Once approved, monitoring of this population would begin immediately to establish identification logs and pretreatment reproduction. Approximately two-thirds of the habituated jennets will be treated with ZonaStat-H and then boostered two-to-six weeks later. 35-50 unhabituated jennets will be bait trapped, treated with the initial dose of ZonaStat-H and uniquely freezebranded and then held for 2 weeks at the Kingman, AZ corrals and then will be given the standard booster dose of ZonaStat-H and then returned to the range. An additional 35-50 jennets will be bait trapped, treated, and uniquely freezemarked and immediately returned to the range where they will be accessed via remote darting at a bait trap or bait station or opportunistically for their standard booster dose of ZonaStat-H. Thirty control jennets will be captured and freeze branded and immediately returned to the range. Animals will be randomly assigned to treatment and control groups. ZonaStat-H treatments will be given following BLM's Standard Operating Procedures for Fertility Control Treatment. These contraceptive treatments will be delivered by dart rifle in the corral or in the field, and consist of one priming injection of 100 µg PZP in 0.5 mL phosphate buffer solution (PBS) emulsified in 0.5 mL Freund's Modified adjuvant (FMA) and a booster dose of 100µg PZP in 0.5 mL PBS emulsified in 0.5mL Freund's Incomplete Adjuvant. Handling of burros and the vaccination procedures will follow BLM's standard protocol, and all treated jennets will be photographed and cataloged for later identification.

Annual booster treatments will be attempted via remote darting at bait traps, bait stations or opportunistically 8-12 months after the initial booster vaccination. We propose here to treat these jennets with 100µg PZP in 0.5 mL PBS emulsified in 0.5mL Freund's Incomplete adjuvant, delivered in 1 cc Pneu-dart® darts with 1.5" needles fired on the ground from Pneu-dart .22 cartridge-powered dart rifles, or equivalent.

We will collect observational data from all treated and control jennets to examine:

1. Direct effects of PZP vaccination on treated animals, including the proportion of treated jennets foaling relative to controls, timing of foaling, body condition, injection site reactions, and general health; and seasonality of breeding;

Data Collection

One half-time researcher, one quarter-time researcher and possibly a third half-time researcher plus several interns and volunteers will be available for collecting data, as well as some additional support from the local BLM field staff as management allows. Data will be collected from both populations using ground observations. Aerial surveys will not be conducted because of the difficulties in locating burros from the air. In the initial months of the study, bait trapping efforts will be the focus of our efforts. Ideal bait trapping months have been identified as November-December and June-August (depending on the monsoon season). Once individuals have been treated, ground observations will be conducted on two different schedules. From

November to January, we will conduct monthly (as resources allow) surveys that focus on identifying individual animals and their group associations so as to assess body condition, injection site reactions, general health, group stability, and foal survival. From February through October, we will attempt to contact and observe every study animal approximately twice per month.

Ground observations. The first priority will be to identify individuals in the study population. Ground observations will be carried out by one or two observers. Observations will be made through binoculars, 20-45X spotting scope, or remote triggered camera data. For each group encountered, observers will identify all known burros present, describe any new burros in the group that had not previously been described, tabulate the age-sex composition of the group, match jennets and their foals (by persistent proximity and nursing), and, using GPS, maps, and field notes, note the location of the band. Within limits of visibility and accessibility, the observer will also estimate and record body condition scores (using the 1-9 Henneke scale) and note any apparent injuries, lameness, abscesses, or other signs of ill-health or each group member. Ground observations will be carried out at two different levels of intensity, depending on season.

From November through January, ground observations will be conducted on a monthly schedule (depending on resource availability and feasibility) with the objective that every study animal is observed once during this time period. Ground observations will also track changes in group composition and membership, record foaling, and track health and condition of individual treated and untreated jennets. Remote triggered camera data may be used to supplement ground observations.

From February through October, we will conduct intensive ground observations with the objective of observing each individual twice monthly. This period is chosen for intensive observations because it should bracket the majority of foaling and mating seasons, when the direct and indirect effects of PZP (including those on foaling and courtship and mating behavior) are likely to be most evident.

Animal data collected will include: animal id, capture date, age, capture method, location, dose date and batch number, delivery method, number of animals in group, date when each new foal is observed for the first time, body condition, and observations of overall health.

Data associated with hours required to complete tasks associated with bait trapping, re-treating, darting, and costs associated with these efforts will be collected to assist with future management decisions.

5. Statistical Methods: (Not to exceed 1 page)

Describe what type of data will be collected and how the data will be analyzed, interpreted, and what assumptions will be made during the analysis and interpretation. State the statistical methods to be used. Specify the number of observations required to yield statistically significant results at a particular confidence level (e.g., 95%) or with sufficient power as stated.

Individual parameters including proportion of jennets with foals, and seasonality of foaling will be summarized descriptively across time and displayed graphically as appropriate. The

proportion of treated jennets that foal will be compared to the proportion of untreated jennets foaling.

Effectiveness and longevity of ZonaStat-H boosters following treatment with the initial two shot vaccine will be examined by comparing treated and control jennets for the presence or absence of associated foals in subsequent years using contingency table analysis.

For body condition scores and other health measures, the sampling unit will be the individual animal (N~130).

6. Anticipated effects (Not to exceed 5 pages)
Describe the anticipated effects of any treatments on individual animals and populations. To the extent possible, quantify the rates of those outcomes, preferably providing a range of rates for the specified outcomes, based on cited published literature or other prior studies. It is also essential to present here what effects are not known, but which will be clarified as a result of this study. If your study will be following a set of standard operating procedures (SOPs), those should be included as appendices. This section and any related appendices should provide justification and clarification of anticipated effects, which may be necessary if BLM will need to draft NEPA-related documentation in support of the proposed project.

This adaptive management project will help illustrate the solutions and challenges associated with bait trapping and treating wild burros with ZonaStat-H. ZonaStat-H has been proven to be a safe and effective form of fertility control on equids (Kirkpatrick et al., 1990; Turner et al., 1996; Ransom et al., 2011).

Anticipated effects on the use of ZonaStat-H on individual wild jennets include a significantly decreased foaling rate similar to what was found on St John, Virgin Island of 6% (Turner et al., 1996). This decrease in foaling rate is expected to be significantly different from untreated jennets where a foaling rate of 40-73% is expected (Wolfe, et al., 1989; Turner et al., 1996)

Some noted side effects of ZonaStat-H treatments on equids that could be expected in this adaptive management project are minor injection site reactions that are not detrimental to normal behavior (Roelle and Ransom 2009) and decreased ovarian function when treatments are applied for >3 years (Kirkpatrick et al. 1995). However, ovarian function returned in 10-50% of wild horses after >3 years of consecutive treatments. There were no differences noted in pregnancy rates of ZonaStat-H treated and control burros 11 to 24 months after an initial primer and booster dose followed by a single annual booster, indicating that the drug was completely reversible after two consecutive treatment (Turner et al., 1996). This study will be the first to collect data on the reversibility of ZonaStat-H after three consecutive years of treatment on wild burros.

There is a large amount of literature discussing the behavioral effects of immunocontraception on wild horses (Powell 1999; Nunez et al., 2009; Ransom et al., 2010) but no literature is available on the use of ZonaStat-H and its behavioral effects on wild burros.

Concerns regarding any social and behavioral impacts from the use of ZonaStat-H on wild burros can be easily addressed in the NEPA process with literature relating to the impacts of PZP use on both wild horse and wild burro behavior. Shackleford Banks, North Carolina wild horse mares receiving PZP were found to change harem groups more often and initiate and receive more

reproductive behaviors than do untreated mares (Nunez et al. 2009). For a gregarious species like the horse, such changes may have social and demographic effects. Burros do not form long lasting family bands like wild horses do (Moehlman 1974). Permanent bonds do not seem to exist, groupings break up and reform on an almost daily basis and territoriality is rare (Nowak 1997; Walker and Ohmart 1978). No measured differences were noted in reproductive behavior between PZP treated and untreated burros were noted in a 3 year study of feral burros on the U.S. Virgin Islands (Turner et al. 1996). Wild burros also exhibit a larger breeding and foaling window, in some areas year round reproductive behavior occurs (Walker and Ohmart 1978, Turner et al. 1996) thus eliminating the concern that the use of ZonaStat-H may affect the timing of foaling. These preliminary findings indicate that there are no extreme deleterious effects expected with the use of ZonaStat-H on a western U.S. wild burro herd.

Gaining access to burros via initial bait trapping is expected to occur within a six month period (personal conversation BLM, C. Benson). Retreating 50% of the treated individuals using bait trapping, bait stations or opportunistically will be a test of patience and ingenuity. Animals treated repeatedly will most likely become more wary and challenging to retreat with each successive treatment (Naugle and Grams 2013). However, this challenge can be overcome with patience, focus, persistence and resourcefulness. Using varied approaches to treatments (bait stations, concealment in blinds, water trapping) will assist in successive treatments (Naugle and Grams 2013).

Increased longevity in wild equids has been a noted effect of long term Zona stat H use (Kirkpatrick and Turner 2007). This will not be measured in this work due to the shorter duration of this project.

7. Pitfalls and Limitations: (Not to exceed 1 page)
List and describe any potential pitfalls or limitations to successful completion of the study and address how these will be addressed.

Environmental and ecological changes (such as appearance or disappearance of predators, or changes in rainfall, with associated changes in water availability and forage quality) could act to produce differences over time that would mask behavioral and demographic effects of contraception as well as decrease the success of bait trapping. All data will have to be interpreted with a careful regard for environmental, biological, management, and behavioral context.

The ability to gain access to individual treated animals for subsequent treatments will be a challenge and if access becomes difficult will be a pitfall in this adaptive management study. However, treating 50% with initial and booster doses in a corral will allow for the collection of individual reproductive data at a smaller scale if retreatment of the remaining 50% proves to be difficult.

On occasion, contraception studies have been undermined or stopped by failures to gather the required number of animals, or by sightability of individual animals within the study. There also exist extrinsic management contingencies such as court orders or the need for emergency gathers. Such risks will be minimized by choice of study area and cooperation with local BLM.

8. References: (No limit)

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Beever, E. A., and P. F. Brussard. 2004. Community- and landscape-level responses of reptiles and small mammals to feral-horse grazing in the great basin. Journal of Arid Environments 59: 271-297.

Beever, E. A., R. J. Tausch, and W. E. Thogmartin. 2007. Multi-scale responses of vegetation to removal of horse grazing from great basin (USA) mountain ranges. Plant Ecology.

Garrott, R. A. 1991. Feral horse fertility control - potential and limitations. Wildlife Society Bulletin 19: 52-58.

Gross, J. E. 2000. A dynamic simulation model for evaluating effects of removal and contraception on genetic variation and demography of pryer mountain wild horses. Biological Conservation 96: 319-330.

Hanley, T. A., and K. A. Hanley. 1982. Food resource partitioning by sympatric ungulates on great-basin rangeland. Journal of Range Management 35: 152-158.

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9. Appendices: (No limit)
Proposed standard operating procedures (SOPs) or other supporting information may be included as appendices.

Appendix I

BLM Standard Operating Procedures for Population-level Fertility Control Treatments
One-year liquid vaccine:

1. PZP vaccine would be administered through darting by trained HSUS or BLM personnel or collaborating research partners only. For any darting operation, the designated personnel must have successfully completed a nationally recognized wildlife darting course and have documented and successful experience darting wildlife under field conditions.

2. Jennets that have never been treated would receive 0.5 cc of PZP vaccine emulsified with 0.5 cc of Freund's Modified Adjuvant (FMA) and loaded into darts at the time a decision has been made to dart a specific jennet. Jennets identified for re-treatment receive 0.5 cc of the PZP vaccine emulsified with 0.5 cc of Freund's Incomplete Adjuvant (FIA).

3. The liquid dose of PZP vaccine is administered using 1.0 cc Pneu-Darts with 1.5" barbless needles fired from appropriate projectors designed for the dart or delivered by hand with a standard 3.0cc syringe and 1.5" 18 gauge needle.

4. 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.

5. Delivery of the vaccine would be by intramuscular injection into the left or right hip/gluteal muscles while the jennet is standing still.

6. Safety for both humans and the burro is the foremost consideration in deciding to dart a jennet. Any smooth bore gun (projector) would not be used at ranges in excess of 30 m while rifled gun (projector) would not be used over 50 m, and no attempt would be taken when other non-darting persons are within a 30-m radius of the target animal.

7. No attempts would be taken in high wind or when the burro is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal is when the dart would strike the skin of the burro at a perfect 90° angle.

8. If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another burro. 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 the next day. Refrigerated darts would not be used in the field.

9. No more than three people should be present at the time of a darting. A non-darter is responsible for locating fired darts. The second person should also be responsible for identifying the burro and keeping onlookers at a safe distance.

10. 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.

11. Attempts will be made to recover all darts. To the extent possible, all darts which are discharged and drop from the burro at the darting site would be recovered before another darting occurs. In exceptional situations, 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.

12. All jennets targeted for treatment will be clearly identifiable through individual unique freeze brand or by photograph (for the habituated Oatman burros) to enable researchers and HMA managers to positively identify the animals during the research project and at the time of removal during subsequent gathers.

13. Personnel conducting darting operations should be equipped with a two-way radio or cell phone to provide a communications link with a veterinarian for advice and/or assistance. In the event of a veterinary emergency, darting personnel would immediately contact a veterinarian, providing all available information concerning the nature and location of the incident.

14. In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected burro until the dart falls out or the burro can no longer be found. The darter would be responsible for daily observation of the burro until the situation is resolved.

Appendix II

Standard Protocol for jennets receiving ZonaStat-H

All jennets treated with ZonaStat-H will be:

- One year old or older
- Have a body condition score of 2 or greater
- Receive a freeze brand on both hips with a unique number or letter combination that is approved by the Arizona Department of Agriculture and the BLM (with the exception of the habituated burros within the town of Oatman that can be identified easily)
- Are visibly in good health