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WILD HORSE POPULATIONS

Field Studies in Genetics and Fertility

National Research Council

WILD HORSE POPULATIONS:
FIELD STUDIES IN GENETICS AND FERTILITY

Report to the
Bureau of Land Management
U.S. Department of the Interior

Committee on Wild Horse and Burro Research
Board on Agriculture
National Research Council

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*Through November 1990.

Preface

When Congress passed the Wild Free-Roaming Horse and Burro Act (P.L. 92-195) in 1971, it decreed that the secretaries of interior and agriculture are to engage in the "protection, management, and control of wild free-roaming horses and burros on public lands . . . [thenceforth] to be considered . . . as an integral part of the natural system" on those lands (U.S. Congress, 1971). The public lands referred to are those under the administration of the Bureau of Land Management (BLM) and the Forest Service. Most horses and burros are on land administered by BLM, and most of the management responsibility rests with this agency.

To casual travelers through the arid and semiarid lands of the western United States, there may seem to be little human land use and ample room for these equine "living symbols of the historic and pioneering spirit of the West." But in fact diverse interest groups demand an array of competing, often conflicting, uses of these lands: mining, timber harvesting, domestic livestock grazing, consumptive and nonconsumptive wildlife uses, recreation, water harvest, and energy production.

Grazing by wild horses and burros is one of these competing uses, and it arouses the animosity of interest groups that feel encroached upon. In particular, livestock groups consider that horses and burros compete with domestic animals for range forage; traditional wildlife advocates have the same concerns for indigenous wildlife; and other environmental groups consider equines to be aliens in ecosystems that have not evolved with, and are damaged by, them. On the other side there are equally strong advocates for wild horses and burros who consider these animals to be part of the western heritage and the aesthetic atmosphere of the region.

Under existing law, it is the responsibility of the BLM and Forest Service to mediate these conflicting demands and to negotiate aggregate uses large numbers of animals across the country to adoption centers. It also places heavy demands on understaffed agencies that have numerous other natural resource management responsibilities across half the area of the western United States. Moreover, when the adoption demand is saturated, the agencies are faced with large and growing numbers of animals that need feed and shelter. This occurred during the mid-1980s when, after unusually large roundups, the BLM maintained several thousand wild horses in pens at Lovelock, Nevada, at an annual cost of several million dollars. In recent years, the BLM budget for wild horse and burro management has ranged between $10 million and $20 million annually.

One alternative herd-control approach to roundup and adoption is reproductive suppression. In 1979, the National Research Council's Committee on Wild and Free-Roaming Horses and Burros was established under mandate of the Public Rangelands Improvement Act (P.L. 95-514) in part to design a wild horse and burro research program and to evaluate research funded by BLM (U.S. Congress, 1978). In 1980 this committee recommended an extensive research program that included studies on reproductive inhibition in mares. Five research projects were initiated in 1980, although none addressed fertility control. In 1982, the committee filed a final report (National Research Council, 1982). In 1983, all of the projects were terminated.

In part because of growing concern with the cost of holding increasing numbers of unadopted horses in the Lovelock corrals, Congress appropriated $1 million in fiscal year 1985 for further research. The report accompanying the appropriations bill for the fiscal year 1985 budget of the U.S. Department of Interior stated (Robert F. Burford, U.S. Department of the Interior, personal communication, November 20, 1984)

> [T]here is still significant disagreement over how many animals are excess, what historic levels were, and what is the current level of reproduction. . . . [Thus we direct] the Bureau, through the National Academy of Sciences, to continue to develop data to answer these and other relevant questions.

The Committee on Wild Horse and Burro Research was constituted in 1985 at the request of BLM and asked once again to consider research needs. BLM officials suggested a need for research on fertility control and computer simulation of alternative population control strategies. Upon committee recommendation, two projects were ultimately initiated and funded by BLM in 1985—one on fertility control and a related one on population genetics.

The committee's final report summarizes its review of the research results. The genetics study has been completed. The fertility control project has one more field season remaining as this report is written, but the data so far accrued have been extensively analyzed and modeled. When the modeling and remaining research have been completed in the fall of 1990, the fertility of the public lands among the interest groups. In areas with wild horses and burros, the compromises include decisions on the number of these animals that should be maintained along with the prescribed number of livestock and indigenous wildlife.

All interest groups agree on one goal: the population of wild horses and burros, in conjunction with other wild and domestic grazing animals, should not be allowed to rise to levels at which the animals degrade the ecological health of rangelands. Hence, there is agreement that the aggregate numbers of wild horses and burros, livestock, and indigenous wildlife, and the specific number decided upon for each class, should be controlled within the carrying capacity of each area. The BLM has adopted a policy stating that the management level—their judgment of the appropriate numbers—for wild horses on the public lands of the western United States should be 31,000. As of early 1990, this limit had not been reached; populations during the 1980s generally numbered between 40,000 and 50,000.

Through yearly reproduction and a relative scarcity of natural population limiting factors in areas with healthy vegetation, wild horse and burro populations increase at annual rates reportedly varying between 6 and 20 percent. Rates in the higher part of this range are more likely at low population densities and in areas where the range vegetation is in good to excellent condition. Thus, western populations of horses and burros increase several thousand each year.

Given the unremitting tendency of these animals to increase, and decisions to hold horses and burros in each area at agreed-upon numbers, the management agencies face a continuing need to control population. P.L. 92-195 (U.S. Congress, 1971) states

> The Secretary may order wild free-roaming horses and burros to be destroyed in the most humane manner possible when he deems such action to be . . . necessary to preserve and maintain the habitat in a suitable condition for continued use . . . or such action is the only practical way to remove excess animals from the area.

But the agencies have rejected mass euthanasia as a socially unacceptable solution and instead have developed the Adopt-a-Horse and Adopt-a-Burro programs. Periodic roundups of animals make those numbers in excess of management levels available for the general public to adopt as pets and work animals.

The adoption program has been generally successful, placing 81,000 animals across the United States from 1972 through 1987 (U.S. Department of the Interior, 1989). However, it requires substantial funds, involving extensive helicopter time for roundups, high-quality pen facilities and feed to hold the animals until adoption, veterinary fees, and transportation costs to move control project will provide not only a technical evaluation of the fertility control methodology used, but also an assessment of its population control efficiency and cost. BLM should then be in a position to compare the new methods with existing ones and to make policy decisions.

Frederic H. Wagner, *Chair*
Committee on Wild Horse and
Burro Research

Contents

1

Introduction

The Committee on Wild Horse and Burro Research was established in 1985 at the request of the Bureau of Land Management (BLM). Its charge was to

- Review research on wild horses and burros completed since 1982;
- Assess the research recommendations of an earlier committee of the National Research Council in light of current issues, and update these recommendations if necessary;
- Develop guidelines to assist the BLM in contracting for additional research studies;
- Monitor the progress of contracted research projects; and
- Evaluate the final reports of the research projects and prepare a final committee report.

Under this arrangement, the BLM expressed its views on the priorities for research to be conducted. The committee then presented its research recommendations and guidelines. Three areas of research were chosen by the BLM: wild horse population genetics, control of fertility in wild horses, and simulation modeling of alternative population-control strategies. The areas chosen for research focused exclusively on wild horses. Wild burros were not studied in any of the research reviewed by this committee.

In 1985, requests for proposals (RFPs) were issued by the BLM. The committee carried out a scientific review of the responses to the RFPs. Subsequently, the BLM awarded a grant to the University of California at Davis for a genetics research project and to the University of Minnesota for a fertility control project. The modeling research was not funded because of data limitations.

This report reviews the design and results of BLM-funded research on wild horse genetics and on fertility control. It is based on meetings with the research groups from the University of California at Davis and the University of Minnesota over a 4-year period as well as numerous other communications. The report also addresses concerns expressed by some individuals and interest groups that injuries to and deaths of horses during the conduct of these research projects have compromised the integrity and usefulness of the research results.

2

Research and Results

The Bureau of Land Management (BLM) awarded research grants in 1985 for the study of wild horse genetics and fertility control. The University of California at Davis conducted its 3-year study on wild horse population genetics and submitted a final report to BLM on January 15, 1988 (Bowling and Touchberry, 1988). The University of Minnesota began its study of wild horse fertility control on October 1, 1985, and will complete it in the fall of 1990. Although the results from one remaining field season in the fertility study are not included in this report, the data gathered to date have been extensively analyzed and permit the conclusions presented in Chapter 5.

GENETICS STUDIES

Research on wild horse parentage and population genetics included a genetic analysis of the inheritance of different proteins present in red blood cells and serum. From December 1985 through October 1986 researchers from the University of California at Davis collected blood samples from nearly 1,000 horses at seven trap sites in the Great Basin area of Oregon and Nevada. The genetics studies on the free-ranging feral horses had the following four objectives:

1. Assess average and individual heterozygosity in the populations to determine if there has been loss of heterozygosity or inbreeding through genetic drift, selection, removals, or management restrictions on animal movement;
2. Estimate the contributions of the original wild mustangs (descendants of animals released by the Spanish) and the current domestic lineages (13 breeds) to the present feral horse populations;

3. Evaluate the several populations for possible divergence in gene frequencies and for the development of population substructure; and

4. Determine parentage and particularly paternity within bands to evaluate the proportion of foals sired by the dominant band stallion.

An important feature of horse social organization and behavior is year-round bands. Bands generally consist of females, their young, and a single stallion. Bands with more than one male do exist, but are generally short lived, lasting from several hours to several months. Bachelor males also form bands, although older horses, more than 14 years old, spend significantly more time alone than younger males, particularly those 2 to 5 years of age. Bands can contain from one to eight (rarely more) females, with an average of between three and four mares per band. Females, however, do not band together for life, and will stray from one band to another. Interestingly, Berger, in his 5-year study of wild horses, reports never seeing a mare driven from a band by a stallion (Berger, 1986).

Methods Used

The assays used included red cell antigens (50 alloantigens at 7 loci), isoenzyme (red cell and serum isozymes), and serum protein electrophoresis (76 alleles at 12 loci) for a total of 19 polymorphic loci. Loci known to be polymorphic in domestic horses were chosen for analysis. Selection was based on the objective of measuring the occurrence and frequencies of alleles for a comparison with the data available on many domestic horse lineages. This approach does not evaluate polymorphisms or rare alleles at other loci. It detects new alleles at the studied loci. Observations on coat color and pattern variants at seven loci were also collected.

Blood samples were collected from 975 horses in five populations. Two sites had two trap locations each, providing paired subpopulations; the other three sites had one trap site each (Flanigan, 175 animals in 30 bands; Wassuks, 119 in 21 bands; Beaty Butte, 112 in 17 bands; Stone Cabin, 239 in 30 bands at site 1 and 127 at site 2; and Clan Alpine, 104 in 17 bands at site 1 and 99 in 12 bands at site 2). Separation of the locations by distance and geography makes it unlikely that genetic exchange occurred between these populations. The band compositions and paternity assignments may have been compromised in two cases: (1) a roundup and removal of some horses was conducted by BLM at the Flanigan study area in September 1985 prior to the sample collection in December; and (2) samples were collected at the Beaty Butte area in February 1986 after a BLM reduction roundup in November 1984. Data on the domestic breeds were drawn from blood samples from a breed registry laboratory; they ranged from 79 to 14,517 samples per breed. Thirteen domestic breeds are thought to be associated with the horses of the Great Basin (Figure 2-1). They include the Arabian, Criollo, and Mangalarga breeds.

FIGURE 2-1 The Great Basin of the western United States.

Results

Data were analyzed by calculating allele frequencies at individual loci, proportion of heterozygous loci in individuals and in populations, Nei's genetic distance, and dendrograms. Parentage was accepted on the basis of congruent allelic specificity at each locus for foal and dam pairs and with the band stallions. The numeric results are summarized in Tables 2-1, 2-2, and 2-3, and in Figure 2-2. They indicate the following:

- There were no significant deviations from Hardy-Weinberg equilibrium proportions indicating that the horses sampled at each site were members of an interbreeding population within the limits of the 19 loci examined.
- The horses from the paired sites within the Stone Cabin and Clan Alpine sites could each have been members of a single randomly mating population.
- The number of effective alleles for wild horses averaged 41.3 ± 2.8 (range 38.8 to 46.3) and for domestic breeds averaged 40.3 ± 4.0 (range 33.7 to 46.8).
- The average heterozygositv was 0.402 ± 0.009 and 0.353 ± 0.011 for wild and domestic horses, respectively.
- The differences between populations of Great Basin horses were less than between breeds of domestic horses, based upon Nei's population measures.
- Unique variants in the wild horses were observed only at the highly polymorphic Pi locus.
- The above data and the dendrograms support the hypothesis that Great Basin horses originated from escaped or released domestic draft, saddle, and cavalry animals.
- Paternity assignments included 121 foals from 69 intact harem bands. The data indicate that about one-third of the foals were not sired by the harem stallions. This exclusion rate did not change when the data from the sites disturbed by roundups were excluded.

TABLE 2-1 Allelic Variation in Horses at 19 Blood Type Loci

Trap Site or Domestic Breed	Number of Alleles			Number of Effective Alleles	Average Heterozygosity (Standard Error)
	Blood Group	Protein	Total		
Trap site, wild and free-roaming horses					
Flanigan	30	41	71	38.3	0.378 (±0.058)
Wassuks	24	33	57	38.8	0.380 (±0.062)
Beaty Butte	25	36	61	39.7	0.442 (±0.045)
Stone Cabin 1	30	48	78	46.3	0.423 (±0.062)
Stone Cabin 2	28	38	66	43.0	0.416 (±0.059)
Clan Alpine 1	31	51	82	42.5	0.404 (±0.058)
Clan Alpine 2	28	45	73	40.5	0.368 (±0.061)
Domestic breed					
Arabian	29	37	68	37.1	0.346 (±0.062)
Thoroughbred	24	34	58	33.7	0.295 (±0.060)
Quarter horse	36	50	86	45.0	0.403 (±0.062)
Standardbred	29	40	69	39.1	0.413 (±0.048)
Morgan horse	34	51	85	42.9	0.410 (±0.056)
American saddlebred	34	47	81	40.7	0.386 (±0.059)
Tennessee walking horse	29	42	71	35.8	0.350 (±0.056)
Belgian	31	42	73	46.8	0.443 (±0.059)
Shire horse	28	43	71	38.3	0.381 (±0.058)
Argentine Criollo	29	46	75	41.1	0.410 (±0.061)
Chilean Criollo	30	44	74	41.8	0.428 (±0.052)
Mangalarga	26	38	64	36.8	0.305 (±0.068)
Mangalarga marchador	30	48	78	45.4	0.412 (±0.066)

SOURCE: Bowling, A. T., and R. W. Touchberry. 1988. Wild horse parentage and population genetics. Final research report to the U.S. Department of Interior, Bureau of Land Management, January 15. University of California at Davis. Photocopy.

TABLE 2-2 Nei's Population Measures for Horses

	Population Cluster[a]		
	Wild	Domestic	Total
Heterozygosity			
Total	0.429	0.443	0.441
Subpopulation	0.402	0.383	0.389
Diversity			
Interpopulational	0.027	0.060	0.052
Subpopulations relative to total population	0.063	0.136	0.117

[a]Population number is 7 wild and 13 domestic horses.

SOURCE: Bowling, A. T., and R. W. Touchberry. 1988. Wild horse parentage and population genetics. Final research report to the U.S. Department of Interior, Bureau of Land Management, January 15. University of California at Davis. Photocopy.

TABLE 2-3 Foal Paternity in Harem Bands

	Trap Site									
Type of Band	F	W	B	SC1	CA1	CA2	Total	Per-cent	Total[a]	Per-cent
Harem bands	12	10	12	20	8	7	69		45	
Bands, 1 stallion	9	4	10	12	6	1	42		23	
Excluded for all foals	3	0	4	4	1	0	12	29	5	22
Sire of all	6	4	3	6	4	0	23	56	14	61
Sire of some, not all	0	0	3	2	1	1	7	26	4	27
Bands, 2 or more stallions	3	6	2	8	2	6	27		22	
Excluded for all foals	2	2	0	0	0	2	6	22	4	18
One is sire of all	1	3	2	4	2	2	14	52	11	50
One is sire of some	0	1	0	4	0	2	7	25	7	32
Foals in band with stallion	18	11	23	40	16	13	121		80	
Without qualifying sire	7	3	9	15	2	4	40	33	24	30

NOTE: Study area designations are F, Flanigan; W, Wassuks; B, Beaty Butte; SC1, Stone Cabin/site 1; CA1, Clan Alpine/site 1; CA2, Clan Alpine/site 2. Stone Cabin/site 1 was located in west central Nevada, Nye County. Clan Alpine/site 1 was located in west central Nevada, Churchill County; site 2 was located in the same area, about 10 miles away.

[a]Considering only W, SC1, CA1, and CA2 sites for which no recent Bureau of Land Management roundups had occurred.

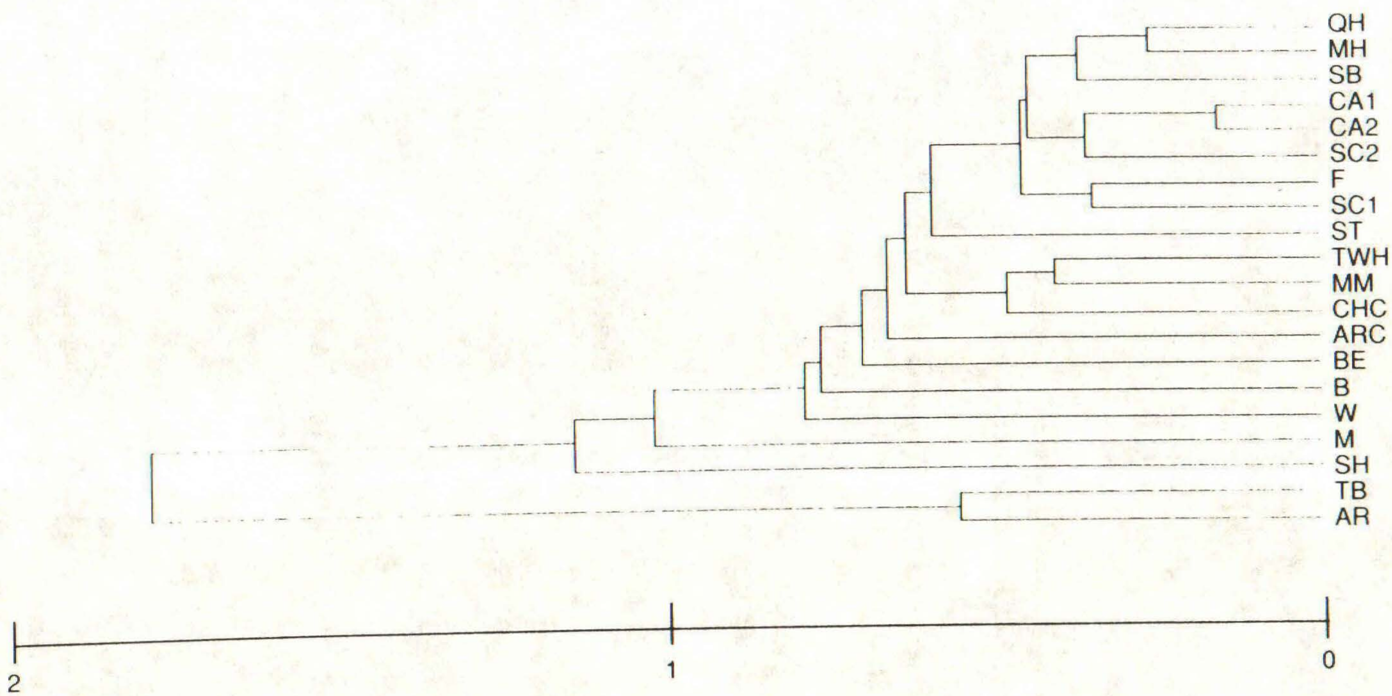

FIGURE 2-2 Nei's genetic distance for 20 horse populations. Source: A. T. Bowling and R. W. Touchberry. 1988. Wild horse parentage and population genetics. Final research report to the U.S. Department of the Interior, Bureau of Land Management, January 15. University of California at Davis. Photocopy.

Note: Designations are QH, quarter horse; MH, Morgan; SB, American saddle horse; CA1, Clan Alpine/site 1; CA2, Clan Alpine/site 2; SC2, Stone Cabin/site 2; F, Flanigan; SC1, Stone Cabin/site 1; ST, standardbred; TWH, Tennessee walking horse; MM, Mangalarga; SH, shire; TB, thoroughbred; and AR, Arabian. Clan Alpine/site 1 was located in west central Nevada, Churchill County; site 2 was located in the same area, about 10 miles away. Stone Cabin/site 1 was located in west central Nevada, Nye County; site 2 was located in the same area, about 10 miles away.

In summary, the genetic data support the domestic origin of these Great Basin horse populations, the lack of differentiation within or between these populations, and their likely origin from a substantial number of founders, with no evidence of a bottleneck or loss of heterozygosity. Because of their substantial sample sizes and high levels of polymorphisms, these studies are very robust and allow substantial confidence in the interpretations. The interpretations, however, apply only to the populations sampled. It is possible that there are other populations that have experienced a long history of isolation, small population sizes, or selective origins. The methodology and data base are now available to allow testing of any such proposed population.

MARE FERTILITY CONTROL STUDIES AT LOVELOCK CORRALS

The overall goal of the fertility control research has been to develop a method to block reproduction in wild and free-roaming horses that would be effective over several years. The research involved vasectomizing dominant band stallions or using steroid hormones to block pregnancy in mares. The research was initiated in the fall of 1985 with a corral study to develop methodology that could be used in the field in 1986 with wild and free-roaming animals. The objectives of the corral studies in Lovelock, Nevada, were to develop dosage levels and techniques for administering steroids to captive wild mares. Such dosages and procedures had not previously been developed in horses, and were needed before the research team could administer steroids to wild and free-roaming mares in the field. These studies at the Lovelock corrals were conducted by researchers from the University of Minnesota.

The relative merits of surgical implants versus remote delivery of steroids were considered. The advantage of remote delivery is that, operationally, the steroids could be administered in the field with darts fired from helicopters without subjecting the mares to capture, anesthesia, and surgical implant. However, the decision to use implants was made for the following reasons:

- The implantation procedure has been used with other animals, including humans, with success in multiyear fertility control.
- At the time research began in the fall of 1985, no studies using remote delivery had achieved more than 1 year of fertility control, nor was any technology known that would allow multiyear dosage.
- No dosage levels of remotely delivered female hormones were known.
- It was necessary to capture the experimental animals in the field in order to determine their ages and to mark them for subsequent observation. Hence, capture and restraint were necessary for either implants or remote-delivery animals.

Methodology

The first phase of the pen studies involved the development of silastic rods—silicone rods impregnated with progesterone or estradiol—to serve as the dosage delivery vehicle. The rate of release of the steroid was measured by testing this release periodically when the silastic rods were immersed in saline solution.

The first set of steroid implants in corraled males was initiated in mid-November 1985 and involved administering six different treatments to 30 mares each. These treatments involved the subcutaneous insertion in the neck of rods containing one of the following dosages:

- 8 g estradiol;
- 24 g progesterone;
- 8 g estradiol and 8 g progesterone;
- 12 g progesterone and 4 g estradiol;
- 12 g progesterone and 2 g estradiol; and
- placebos containing no steroids.

A seventh group of 30 mares was maintained as nonimplanted controls.

A second experimental sequence on corralled horses was initiated in April 1986 involved the seven dosages listed below using one of the following methods: subcutaneous implants in the rump and flank areas, intermuscular implants, and intraperitoneal insertion. Ten mares were used in each of the following treatments:

- 36 g progesterone and 24 g estradiol-17β;
- 36 g progesterone, 24 g estradiol-17β and glycerol;
- 36 g progesterone and 12 g estradiol-17β;
- 36 g progesterone and 12 g estradiol benzoate;
- 36 g progesterone and 8 g ethinylestradiol;
- 36 g progesterone and 8 g ethinylestradiol in methyl ether; and
- 1 g levo norgestrel.

The third series of treatments of corralled horses was run in January 1987 involving 50 mares for the following five treatments:

- 10 pregnant mares given 8 g ethinylestradiol;
- 10 pregnant mares given 3 g ethinylestradiol and 36 g progesterone;
- 10 open mares given 1.5 g ethinylestradiol;
- 10 open mares given 36 g progesterone and 8 g ethinylestradiol; and
- 10 open mares given 8 g ethinylestradiol.

Dr. Gerald M. Peck, the veterinarian under contract to the BLM during the Lovelock studies, was present during the surgical work to oversee the procedures.

Blood samples were taken at bi-weekly intervals from veins of the experimental animals to assay the amount of hormone released by the implants. To indicate the length of time over which fertility was controlled, blood samples were taken from November 1985 through November 1988. Normally, ovulation in horses is followed by an elevation of serum progesterone greater than 1.5 µg/ml. This elevation persists for 10 to 14 days and can be detected through bi-weekly sampling. This correlation of ovulation and elevation of serum progesterone has been validated by rectal palpations in domestic horses (Ginther, 1979) and, to a more limited extent, in feral horses (Wolfe et al., 1989). The failure to observe pregnancy despite elevated progesterone levels in some animals may reflect either the use of too rigorous a criterion to accept ovulation, or the fact that estrogens may exert a contraceptive effect at the level of the fallopian tube (delays migration of ovum or embryo into uterus) and of the uterus in antagonizing the progestational changes necessary for implantation.

Results

The first series of implants proved to be ineffective in preventing pregnancy when the mares were exposed to stallions during their normal estrus periods. In addition, some of the mares rubbed the implants out of their necks.

The second and third series proved more effective.

- Implants containing 36 g of progesterone plus 8 g of ethinylestradiol or implants containing 36 g of progesterone plus 24 g of estradiol-17β with glycerol as a cosolvent effectively blocked ovulation for two breeding seasons in more than 60 percent of the mares.
- Implants containing 8 g of ethinylestradiol without progesterone were capable of blocking ovulation for at least one breeding season.
- Implants containing 8 g of ethinylestradiol with or without 36 g of progesterone when implanted during mid-pregnancy allowed the successful completion of pregnancy and delivery of apparently normal foals.
- Scarring around the implants may be a significant problem that could reduce the longevity and effectiveness of the implants.
- Route of administration had no effect on the level of hormone achieved in the serum and consequently on the effectiveness of the implant.

Pregnancy was blocked through two breeding seasons in more than 90 percent of the animals receiving 36 g progesterone plus 8 g ethinylestradiol, or those animals receiving 8 g ethinylestradiol alone. Pregnancy was blocked through the peak of the breeding season of the third year in more than 70 percent of the animals receiving 36 g progesterone plus 8 g ethinylestradiol or 36 g progesterone plus 24 g estradial-17β and glycerol.

Over 80 percent contraceptive efficiency was achieved through the peak of the second breeding season in open mares that had been treated with 1.5 g ethinylestradiol alone or in pregnant mares that received 3 g ethinylestradiol plus 36 g progesterone. Moreover, the data show that ethinylestradiol at 1.5 g and 3.0 g blocks pregnancy without blocking ovulation, whereas 8.0 g of ethinylestradiol with or without 36 g of progesterone blocks ovulation as well as pregnancy. The data also suggest that 36 g of progesterone plus 24 g of estradiol-17β with glycerol blocks ovulation during the second year, but in the third year the treatment blocks pregnancy while allowing ovulation. More generally, the following conclusions were drawn:

- Effective contraception can be achieved for at least 28 months.
- The length of time that the contraceptive is effective beyond 28 months is not known.
- The mechanism of contraceptive effect is not completely understood. Ovulation could be blocked at the higher concentrations of ethinylestradiol or estradiol with or without progesterone. Or, at lower concentrations of ethinylestradiol, the contraceptive effect might occur at some point after ovulation, such as fertilization or implantation.
- The peritoneal cavity (intraperitoneal) is an efficient and effective location for routine implantation of hormonal contraceptives.

The experimental animals were removed from the Lovelock corrals in 1989 and transported to a wild horse sanctuary in Oklahoma after the steroid levels in their blood had been monitored for about 2 years. They are a valuable resource for establishing the length of time over which the implants are effective. Modeling the cost-to-effectiveness ratio of contraception versus roundup for horse population control depends on the number of years a treatment works. At present, the treatments are contraceptive up to 28 months, but it is not known how much longer they might remain effective.

Hence, the committee recommends that annual monitoring of blood hormonal levels continue until the levels are not significantly higher than the initial control levels. The cost of this monitoring would be low relative to the value of the results in determining the practical utility of contraception.

The committee concludes that the pen studies at the Lovelock corrals achieved their objectives and were handled in a professional manner. These chemosterility studies have led to the following publications in peer-reviewed literature: Plotka et al., 1987; Plotka et al., 1988a,b; and Plotka et al., 1989. At the time of its development, the intraperitoneal approach was on the cutting edge of a rapidly developing technology.

MARE FERTILITY CONTROL STUDIES IN THE FIELD

Steroid implants in the field constitute the second component of the mare fertility control experiments. The objective is to implant mares in selected study areas with steroids and placebos, follow their reproductive histories over 2 to 3 years, and evaluate the effectiveness of the treatments. Research is expected to be completed by the fall of 1990.

Methods

Wassuks Study Area

In January 1986, 41 mares, 3 years old and older, in the Wassuks area of west central Nevada were implanted with silastic placebo rods to serve as undosed comparison animals. These mares and those in the Clan Alpine area (described below) are not formal control animals. The horse populations in these areas are not large enough to support the formation of control groups and treatment groups. Therefore, separate or adjacent areas were used to compare treated and untreated mares.

The 41 placebo-implanted mares in the Wassuks area were equipped with radio collars. An additional 62 mature male and female animals were fitted with marker collars. The marker collar animals at this and other sites were intended to be used to study band stability and structure. This portion of the study was eventually discontinued because of the lack of funds.

Since many of these animals must have been pregnant from the 1985 breeding season, any possible blocking effect on future conception and pregnancy by the placebo would not be observable until the spring or summer of 1987.

Stone Cabin Study Area

In September 1986, 101 mature mares in the Stone Cabin area of south-central Nevada were implanted either with the 8 g ethinylestradiol plus 36 g progesterone (EP) combination (51 mares), or with 8 g ethinylestradiol (EE) only (50 mares). The 101 treated animals were equipped with radio collars. An additional 114 mature male and female animals were fitted with marker collars.

Many of these animals were undoubtedly pregnant from the 1985 breeding season. Hence, any blockage of future pregnancies by the steroids would not occur until the 1987 breeding season and would not be observable until the spring and summer of 1988.

Clan Alpine Study Area

In September 1987, 100 mature mares in the Clan Alpine area of central Nevada were implanted either with the 8 g ethinylestradiol plus 36 g progesterone combination (50 mares), or with 8 g ethinylestradiol only (50 mares). Forty-nine mares were implanted with placebos to serve as untreated comparison animals. All 149 treated animals were equipped with radio collars. An additional 152 adult male and female animals were fitted with marker collars.

Many of these animals were undoubtedly pregnant from the 1987 breeding season. Hence, any blockage of pregnancy as a result of the steroids would not occur until the 1988 breeding season and only be observable by spring and summer 1989.

Aerial Surveys

Four aerial surveys by helicopter were made in each study area each spring between the months of April and June. The purpose of these flights was to observe the foaling rates of treated and placebo-implanted mares.

Results

The results of the 1988 and 1989 aerial surveys are summarized in Tables 2-4, 2-5, and 2-6. Table 2-4 presents the foaling rates in 1988 for each study area and Table 2-5 presents the foaling rates in 1989. Table 2-6 compares the data from each year.

In the Clan Alpine area, where no treatment effects were expected until 1989, 97 of the 100 treated mares were observed in 1988 (Table 2-4). Of the 97 treated mares, 51 (53 percent) were seen with foals. Thirty-one of the 49 placebo implants were observed and 13 (42 percent) were seen with foals. Among an estimated 109 untreated mares with marker collars observed during the aerial observations, 51 (47 percent) were seen with foals.

In the Stone Cabin area where mares had been treated in the fall of 1986 and any treatment effect would have been evident in 1988, 88 of 101 treated mares were observed (Table 2-4). Of the 45 EE-treated mares and 43 EP-treated that were seen during the flights, 5 (11 percent) and 3 (7 percent) were seen with foals, respectively.

In the Wassuks, where 41 mares had received placebo implants, 15 (45 percent) of the 33 mares observed were seen with foals (Table 2-4).

Clan Alpine and Wassuks percentages are remarkably close, and none is statistically different from the others. They indicate that neither the roundup and treatment of the previous September in the Clan Alpine area nor the implants (placebos or steroids) in the Wassuks and Clan Alpine areas caused any statistically detectable signs of abortion or early foal mortality. A proportion of the animals were pregnant at the time of implantation, and the percentages of the four groups seen with foals are approximately what would be expected in a sample of undisturbed 3- to 12-year-old mares.

For 1989 in Clan Alpine, 4 (9 percent) of the observed 45 EE mares and 3 (6 percent) of the observed 50 EP mares were seen with foals. Of the 31 observed placebo mares, 14 (45 percent) were seen with foals. In Stone

TABLE 2-4 Foaling Rates as Determined by Aerial Observation, 1988

Area and Treatment	Number of Mares	Number of Foals	Foaling Rate	95 Percent Confidence Interval[a]
Clan Alpine				
Treatment[b]	97	51	.53	.43–.63
Placebo	31	13	.42	.25–.59
Untreated, marker collared[c]	109	51	.47	.38–.56
Stone Cabin				
Ethinylestradiol	45	5	.11	.02–.20
Ethinylestradiol and progesterone	43	3	.07	.00–.15
Wassuks				
Placebo	33	15	.45	.28–.62

[a]The 95 percent confidence intervals were calculated by the formula $p \pm 1.96(pq/n)^{.5}$, where p is the proportion of mares with foals and q is the proportion of mares without foals.

[b]Treatments in the Clan Alpine mares were not effective until 1989.

[c]This estimate is based on an aerial survey (third census) of the Clan Alpine study area (May 21, 1988). In Clan Alpine, 413 adults (yearlings or older) were captured, 149 of which were females 3 to 12 years old and were implanted with treatment capsules or placebos. The proportion of mares in that sample was 149/413 = .36. On May 21, 1988, 301 adults were counted in bands with no marked horses. Assuming the same distribution of mares as in the collared sample (.36), 109 of the 301 adults were mares in the 3- to 12-year age class.

In Clan Alpine, 66 foals were assigned to collared mares, 56 (.85) to 3- to 12-year-old mares and 10 (.15) to older mares. On May 21, 1988, 60 foals were counted in bands with no marked horses. Assuming the same distribution of foal production as in collared mares, 51 of the 60 foals were attributed to the 109 unmarked 3- to 12-year-old mares (Siniff et al., 1988a,b).

Cabin, 1 (3 percent) of the observed 35 EE mares and 6 (16 percent) of the observed 37 EP mares were seen with foals. In the Wassuks, 21 (70 percent) of the observed 30 placebo-implants were seen with foals (see Table 2-5).

The effects of the Clan Alpine steroid treatment were now evident in 1989 as were the Stone Cabin treatments for the second year. Meanwhile, the placebo implants bore foals in 1989 at about the same rate as in 1988. (In the Wassuks data the 95 percent confidence intervals around the foaling rate of 70 percent in 1989 overlap the 95 percent confidence intervals for

TABLE 2-5 Foaling Rates as Determined by Aerial Observation, 1989

Area and Treatment	Number of Mares	Number of Foals	Foaling Rate	95 Percent Confidence Interval[a]
Clan Alpine				
Ethinylestradiol	45	4	.09	.01–.17
Ethinylestradiol and progesterone	50	3	.06	.00–.12
Placebo	31	14	.45	.27–.63
Stone Cabin				
Ethinylestradiol	35	1	.03	.00–.09
Ethinylestradiol and progesterone	37	6	.16	.04–.28
Wassuks				
Placebo	30	21	.70	.53–.86

[a]The 95 percent confidence intervals were calculated by the formula $p \pm 1.96(pq/n)^{.5}$, where p is the proportion of mares with foals and q is the proportion of mares without foals.

1988, and thus the foaling rates for 1988 and 1989 at Wassuks are not statistically different.)

For the combined areas and years (Table 2-6), the percentage of observed, treated mares seen with foals varied between 6 percent and 11 percent. If the 1988 and 1989 data for each of the two treatments are pooled, the effectiveness in curbing reproduction is essentially the same: 8 percent for 125 EE-treated mares, 9 percent for 130 EP-treated mares. The percentages compare with 49 percent and 57 percent for the 2 years in the placebo implants. Evidently both EE and EP implants effectively reduce pregnancy rates in mares for at least 2 years.

By pooling all of the data, 22 (9 percent) of 255 observed, treated mares were seen with foals, while 114 (51 percent) of 222 observed, placebo-implanted mares were seen with foals.

The obvious and consistent difference in the very low fertility of the hormone-treated horses compared with the placebo-treated or untreated horses cannot easily be attributed to any other cause but the hormone treatment. The horses in the Wassuks study area serve as comparisons to the hormone-treated animals in the study. The placebo-treated horses in the Clan Alpine study area, which were originally recruited from the Augusta Mountain area, are also consistent comparisons to the hormone-treated horses. The measure of fertility in the placebo-implanted and untreated horses is consistent

TABLE 2-6 Pooled Foaling Rates

Year and Treatment	Number of Mares	Number of Foals	Foaling Rate	95 Percent Confidence Interval[a]
1988				
Ethinylestradiol (EE)	45	5	.11	.02–.20
Ethinylestradiol and progesterone (EP)	43	3	.07	.00–.15
Placebo[b]	161	79	.49	.41–.57
1989				
Ethinylestradiol (EE)	80	5	.06	.01–.11
Ethinylestradiol and progesterone (EP)	87	9	.10	.04–.16
Placebo	61	35	.57	.45–.69

[a]The 95 percent confidence intervals were calculated by the formula $p \pm 1.96(pq/n)^{.5}$, where p is the proportion of mares with foals and q is the proportion of mares without foals.

[b]Clan Alpine treatments combined with placebo implants in 1988.

with generally observed foaling rates of wild horses, and the difference between them and the hormone-treated mares is clear and consistent in all test areas.

VASECTOMY EXPERIMENTS

The research on reproductive inhibition was designed to experiment with both stallion and mare fertility control. Because the objective is to achieve as many years of inhibition as possible, the decision was made to use vasectomy for stallion fertility control. In order to achieve treatment efficiency, the approach was to vasectomize dominant stallions in bands on the assumption that this would curtail reproduction in the bands.

This assumption carries with it several often-unstated conditions if the procedure is to be effective:

- The dominant stallion must do all or most of the breeding in the band. If a significant amount is done by the subdominant stallions, the effectiveness of sterilizing the dominant is diminished.
- Bands must be relatively stable. If there is a significant exchange of mares or stallions between bands with vasectomized and intact stallions, the technique's effectiveness is reduced.

• Stallions must retain their dominance for several years. If dominance is highly transitory, then one-time sterilization of the dominant stallions of bands can be of only short-term effectiveness.

Methods

The Flanigan and Beaty Butte areas were selected for the vasectomy experiments. In the Flanigan area in northwestern Nevada, dominant stallions from 20 bands were vasectomized and fitted with radio collars; 5 dominant stallions from 5 other bands were radio-collared and left intact as comparison animals; and 139 3-year-old or older animals from these 25 bands were given plastic marker collars. These treatments were administered in December 1985.

In February 1986, 20 dominant stallions were vasectomized in the Beaty Butte area in the southeast corner of Oregon. An additional 67 animals, 3 years old and older, including 5 dominant stallions as comparison animals, were fitted with marker collars.

In the spring of 1988, four aerial surveys were conducted by the research team in the Flanigan area and three in the Beaty Butte area. One survey of the Beaty Butte area was cancelled because of low clouds and high winds. Behavioral observations of horses continued during the spring and summer of 1988. The aerial surveys in the spring of 1988 were the last scheduled surveys for the stallion areas, and aerial data collection for these areas is now complete.

Results

Preliminary results of the stallion study are summarized in Table 2-7. Dominant vasectomized stallions were classified by whether they were (1) in stable bands where they remained dominant, (2) in unstable situations where they were switching bands, or (3) in stud bands. They were further classified as to whether any foals had been born into their bands that year. Examination of these data suggests that sterilizing dominant stallions may have been effective in diminishing reproduction in mountainous habitats, such as the Flanigan area. However, its effectiveness in the flat Beaty Butte area, where bands of horses regularly mingle, is less certain. Final recommendations on the effectiveness of sterilizing dominant stallions will be delayed until thorough analysis of aerial survey data and ground behavioral observations is complete.

In the committee's view, vasectomy is an acceptable and effective method of sterilizing individual wild horses. However, several questions remain regarding its effectiveness as a long-term, population control procedure.

Even though a dominant stallion may be vasectomized and sterile, will

TABLE 2-7 Status of Vasectomized Stallions as Determined by Aerial Observation, 1986 to 1988

Study Area and Stallion Code Number	Year of Observation 1986	1987	1988
Flanigan			
201	Stable, foals	Stable, no foals	Stable, no foals
202	Unstable		Stable, no foals
204	Unstable	Stable, no foals	Stable, no foals
207	Stable, foals		
209	Stable, foals		
211	Unstable		Stable, foals
216	Stable, foals	Stable, no foals	Stable, no foals
219	Stable, foals	Stable, no foals	
523		Stable, no foals	Stable, no foals
529		Stable, no foals	Stable, no foals
Beaty Butte			
520	Stable, foals	Stable, foals	
521	Unstable		
532	Unstable		
537	Unstable	Stable, no foals	Stable, no foals
696	Unstable	Unstable	Stable, foals
697	Unstable	Stud band	Stud band
701	Unstable	Unstable	Unstable
719	Stable, foals	Unstable	
732	Stable, no foals	Unstable	Stud band
734	Unstable	Stable, no foals	Stable, foals
735	Stable, foals		
738	Stable, no foals		
739	Unstable	Stable, no foals	Stable, no foals
744	Stable, foals	Unstable	
747	Stable, foals	Stable, foals	Stable, foals
750	Stable, foals	Stud band	Stud band

SOURCE: Siniff, D. B., J. R. Tester, T. C. Eagle, R. A. Garrott, and E. D. Plotka. 1988a. Fertility control in wild horses. Progress report to the U.S. Department of the Interior, Bureau of Land Management, November 30. Table 2. University of Minnesota and Marshfield Medical Foundation. Photocopy.

subordinate males in the band assume the duties of the dominant male and sire foals? How many foals are actually produced by mares in bands where the dominant stallion has been vasectomized?

Again, the data in Table 2-7 give ambiguous answers to these questions. In the Flanigan area, observations of the bands associated with a vasectomized dominant stallion showed six stable bands with no foals out of the six bands that were observed in 1987 and only one stable band out of the seven observed in 1988 that did have foals.

But in the Beaty Butte area, in 1987 there were two bands with foals out of the five stable bands observed, and in 1988 there were three that contained foals out of the five stable bands that were observed that year. Other bands at Beaty Butte that were observed to include a vasectomized stallion during these 2 years were either unstable or stud bands.

Moreover, in the genetics studies reviewed above, approximately one-third of the foals in the sample bands were not sired by the dominant stallion *of that year*. Either mares were moving between bands or the subdominant males were siring young. Because Berger (1986) has also reported that stallions of differing social status mate with mares, there is reason to withhold judgment on one-time stallion sterilization as a general fertility control procedure.

Vasectomizing the dominant stallion is likely to extend the breeding season. Wild horse mares come into estrus every 21 days, from April through September. Once the mare becomes pregnant this cycle stops, and the stallion does not have to mate with this mare or defend her from other interested stallions. Therefore, another question arises. Will the vasectomized dominant stallion be undernourished as winter arrives because of the additional time and energy required to guard mares during estrus?

The evidence so far indicates that this is not the case. It appears that vasectomized stallions graze sufficiently to maintain body weight and survive the winter satisfactorily.

Does an extended breeding season give the subordinate males more opportunities to breed the recycling females? And if so, do the mares become pregnant and produce foals? Berger (1986) found that stallion dominance was transitory, changing between years. This substantially diminishes the prospect that one-time vasectomy is a population control procedure that would remain effective over a period of years.

These and possibly other factors must be weighed before conclusions can be reached on the efficacy of dominant-stallion vasectomy as a population control technique. On-site behavior observations also need to be integrated into the overall evaluation. At this point its efficacy looks doubtful.

3

Computer Simulation Studies

Although not part of the contract between the Bureau of Land Management (BLM) and the University of Minnesota, the research group engaged in computer modeling to compare the population control effectiveness and costs of various roundup and fertility control options. The group presented its results to the committee at the February 1990 review in Denver.

The researchers modeled the following three, herd management scenarios over a 20-year period:

1. The roundup and adoption procedures used to date. This scenario assumed that a hypothetical herd would need to be rounded up every 4 years. Initial herd size was 600, and at each roundup it would be reduced to 300. The captured horses would then be placed for adoption. Costs were assigned to roundup transportation, holding, and adoption based on BLM's records.
2. Mare contraception. An initial herd of 600 would be reduced to 300 through roundup and adoption. All of the mares that were older than 3 years would be implanted with estrogen. Every 3 years thereafter, enough animals would be rounded up to implant 85 percent of the mares more than 3 years of age.
3. Contraception and selective removal. An initial herd of 600 would be reduced to 300, and the removals placed for adoption. All of the mares older than 3 years would be implanted with estrogen. Every 3 years thereafter, enough animals would be rounded up to implant 85 percent of the mares older than 3 years, and all of the animals 1 to 3 years old would be removed and placed for adoption.

The model predicted that scenarios 2 and 3 would cost 30 to 50 percent less than scenario 1. Furthermore, they would significantly reduce the number of animals needing to be adopted and ameliorate the chronic problem of holding unadoptable animals. The major drawback is that the horses would be captured and handled more frequently in order to implant the mares every 3 years. Hence, the modeling shows that there would be trade-offs, but nonetheless a distinct gain in costs and a reduction in the problems associated with the roundup and adoption program.

As in any modeling exercise, the output is contingent on assumptions made. The Minnesota investigators caution that anyone substituting alternate assumptions, or modifying the simulated protocols, must not expect the same results as those encapsulated above.

4

Research Concerns

Numerous problems confront researchers who study feral horses, and two major logistical problems are inherent in the western United States. First, the rugged, mountainous nature and extensive size of most potential study areas create dilemmas in sampling study animals. It can be difficult to coordinate a large team of biologists to study each of the many populations, although detailed life history and reproductive data can be gathered in this way (Berger, 1986). Depending on available funding, the only feasible method for gathering data over multiple, large study areas is aerial observation. Second, and more important, is the selection of suitable study areas. As pointed out by Berger (1986), several researchers were forced to terminate their projects because study animals were removed by the Bureau of Land Management (BLM) before the studies were completed. The BLM's mandate to manage feral horse herds has made difficult the selection of study areas that fulfill requisites for data collection. In Nevada this problem has been serious, and it has been necessary to select study areas that minimize these conflicts.

Concerns about the conduct of field research activities have been addressed in the following five areas:

- Loss of the Clan Alpine horses;
- Problems and injury with the marker and radio collars;
- Foal orphaning and loss during roundups and aerial surveys;
- Abortion; and
- Disappearance of the penned animals at the Lovelock corrals.

To an extent, these concerns reflect differing views of the ethical acceptability of the methods used to study population control in wild and free-roaming horses. Examination of these ethical concerns, however, are beyond the professional expertise and charge of the committee. The committee can respond to these issues only as they affect the quality of the research results.

LOSS OF THE CLAN ALPINE HORSES

In late August and early September 1987, BLM representatives and the research team began to round up animals from the Clan Alpine area for experiments. However, by September 1 the number of animals rounded up was insufficient for a satisfactory sample size. A decision was made to augment the Clan Alpine numbers with animals from the adjacent Augusta Mountain area.

A fence separates the Clan Alpine and Augusta Mountain areas. On September 2 and 3, 133 animals were rounded up in the Augusta Mountain area and driven to a trap on the Clan Alpine side of the fence. Some horses were driven as far as 15 or 20 miles. The weather was hot and dry, and the horses were in relatively poor physical condition.

Of the 133 captured animals, 42 mares were implanted with placebo capsules and equipped with radio collars, bringing the total number of placebo-implanted mares in the Clan Alpine study area to 49. Another 33 were fitted with marker collars, bringing the total untreated, marker-collared population to 109. The animals were then released on September 2 and 3 in the vicinity of the trap.

Between September 17 and October 30, 48 horses were found dead on the Clan Alpine side of the boundary fence. These included 17 radio-collared animals, 11 animals with marker collars, and 20 unmarked animals. A team composed of a veterinarian and BLM law enforcement and management personnel investigated the situation and concluded the following (U.S. Department of the Interior, 1987):

> The animals were attempting to return North to their home range and were prevented from doing so by the fence. Therefore, not knowing where water sources were located south of the fence, the animals walked the fence in both directions until they died from dehydration.

The committee has received four other versions of the incident, including one account that there were no gaps in the fence and the horses were driven excessive distances around it.

The committee deeply regrets this tragic incident, but it is in no position to impute culpability. It bears, however, the responsibility to assess the effects of this incident on the validity of the research results.

The losses included 17 comparison mares with placebo implants. Another placebo-treated mare died about 12 hours after release, reducing the number of Clan Alpine comparison mares from the original 49 to 31. This reduced the statistical power of the test for the difference in that study area. Nevertheless, the foaling rate of the 31 observed placebo implants in this area in 1989 (Table 2-5) was statistically higher than that of the treated mares.

Moreover, for the study as a whole, there remained 30 comparison mares in the Wassuks in 1989. Thus the loss of the 18 Clan Alpine animals reduced the total number of comparison mares from 90 to 61. Given 2 years of observations on these animals and on the 1988 unmarked mares (Table 2-4), the loss did not render the research invalid or statistically inadequate. As discussed above, the foaling rates of the placebo implants in the different areas and years are consistently similar. Those rates are all statistically higher than the rates of the steroid-treated animals in their second and third years after implantation, and they are in a consistent, low range.

COLLAR PROBLEMS

The use of both radio and marker collars is a widely accepted practice in large-animal field studies. Based on extensive experience with these devices, no adverse effects were anticipated. Between 1987 and 1989, however, a number of horses involved in the study suffered injuries to their necks and ears that were caused by the collars used to locate and identify the experimental animals. Serious questions have been raised concerning the deaths of some of these animals, the nature and extent of the wounds, and possible changes in behavior of the animals as a result of collar problems. Other questions have focused on the design of the collars and the experience of the research team.

Collars were attached to a total of 876 horses. Of these 876 animals, 336 received radio collars (291 mares and 45 stallions); the other 540 adult horses were fitted with marker collars. The radio-collared animals were part of the two fertility control studies. The marker-collared animals were intended to be part of a study of band stability; this study was discontinued due to a lack of funds. The collars will be removed by the BLM at the end of the fertility control study.

Collar Design

The collars are made from an industrial belting material composed of rubber and canvas that is inelastic but somewhat flexible. They are 4 inches wide, with a narrower section underneath the neck where the radios are attached. The collars are adjustable in circumference by means of a system of holes and studs, but fine adjustments are difficult or impossible. Attachment of the radio units stiffens the collars, making them quite inflexible over a span of several inches. All of the collars have 3-inch high numerals to permit identification from the air. These collars are similar, although not identical, to ones employed successfully by members of the research team in an earlier wild horse census study. The belting material used for the collars in the present study appears to be less flexible than the material used previously.

Collar Wounds

Collars were first fitted in the fall of 1986, and problems arose in the spring of 1987. As soon as the problems became evident, the research team began searching for injured horses by helicopter with Dr. Gerald Peck, the on-site consulting veterinarian for the Nevada state office of the BLM. Animals that appeared from the air to have an injury needing treatment were darted and examined. The results are summarized in Table 4-1.

During the years from 1987 to 1989, the research team darted a total of 77 animals. Collars were removed from 54 animals, which constituted 6 percent of the 876 collared animals. Twenty-seven were removed because they were too tight; 27 others were removed because they became too loose and slipped forward over the animals' ears. Six animals were subsequently recollared.

A total of 48 animals had collars permanently removed. Twenty were radio-collared animals that formed part of the experimental population. Three additional radio collars slipped off animals in the field. In all, 23 animals were lost from the experimental population due to collar removal or loss. The other 28 collars that were removed were marker collars with no radio telemetry devices attached. Two other animals died while being captured to have collars adjusted or removed.

The wounds caused by tight collars were unquestionably grim in appearance. In some cases, the horse grew into the collar material, so that the collar became imbedded in the animal's neck. In other cases, the collar abraded the skin under the neck where the radio unit was attached, causing an open sore that subsequently became infected. Loose collars rode up on the animals' necks and over their foreheads, causing sores on the ears.

Primarily the injuries were surface wounds, which responded to treatment and healed. Dr. Peck told the committee that there was no sign of systemic infection, dehydration, or deterioration of body condition in the injured horses. Dr. Peck's diagnoses were communicated to BLM on January 25, 1988, in a report (see Appendix). Six of those animals (designated as recollared in Table 4-1) have subsequently had collars reattached. Dr. Peck, however, questioned the recollaring of horses with neck scar tissue; no animals were recollared after September 1988.

The great majority of the neck wounds were associated with radio collars, rather than marker collars. Most of the wounds occurred under the neck, at

TABLE 4-1 Darting and Collar Summary, 1987 to 1989

Time Period and Study Area	Number of Collared Horses					
	Horses Darted	Collars Removed	Collars Adjusted	Collars Not Adjusted	Horses Died	Horses Recollared[b]
June 1987-September 1988						
Flanigan	11/0[a]	9/0	2/0	0/0	0/0	0/0
Beaty Butte	1/0	1/0	0/0	0/0	0/0	0/0
Wassuks	4/0	4/0	0/0	0/0	0/0	0/0
Stone Cabin	4/31	2/18	2/10	0/2	0/1	0/6
Clan Alpine	4/4	0/2	3/1	0/1	1/0	0/0
Subtotal	24/35	16/20	7/11	0/3	1/1	0/6
October 1988-October 1989						
Flanigan	2/0	2/0	0/0	0/0	0/0	0/0
Wassuks	3/0	3/0	0/0	0/0	0/0	0/0
Stone Cabin	3/6	3/6	0/0	0/0	0/0	0/0
Clan Alpine	3/1	3/1	0/0	0/0	0/0	0/0
Subtotal	11/7	11/7	0/0	0/0	0/0	0/0
Total	35/42	27/27	7/11	0/3	1/1	0/6

[a]Number on left represents animals with collars over their ears; number on the right represents animals with tight collars.
[b]Six animals that had their collars removed were subsequently darted a second time so they could be recollared.

the point where the radio unit was attached. In two cases where the collar had rotated, wounds were observed on the mane under the radio unit. Most observers attributed this effect to the stiffening of the collars at the point where the radios were attached. Dr. Peck also pointed out that the radio collars were put on with the horses lying down, tied up, and anesthetized, while the marker collars were put on with the horses wide awake, and standing in the chute with their neck muscles tensed.

Stone Cabin

Most of the problems with tight collars occurred at Stone Cabin. Collars were placed on 215 animals (101 of which were radio collared) in September 1986. Between June 1987 and September 1988, 31 of those horses were subsequently identified as having collars that were too tight. The research team attributed the problems with tight collars at Stone Cabin to rapid weight gains. They stated that forage conditions were favorable, contraception was effective, and the animals experienced significant increases in body fat. BLM personnel generally agreed, pointing out that the horses at Stone Cabin were collared during the second year of a 2-year drought, which was followed by 2 years of high precipitation and abundant forage production. Both the researchers and BLM personnel stated that the problem of collars becoming imbedded had not happened before and was not anticipated on the basis of previous experience.

Age may also have been a significant factor. According to BLM personnel, many of the animals collared at Stone Cabin were young (2 to 5 years) and may have outgrown their collars in the normal process of maturation. One BLM official told the committee that virtually all of the horses from 2 to 5 years of age experienced collar problems. It is not possible to evaluate the effect of age in the absence of age distribution data. Dr. Peck also suggested that the hormone implants may have caused unusual weight gain in the treated mares.

In 1987, after neck wounds were first observed, seven animals were darted at Stone Cabin to treat collar sores. One collar was removed, and six collars were loosened. One animal was subsequently recollared. In 1988, 28 animals were darted to treat collar problems; 19 collars were removed, 6 were adjusted, 2 horses were released without adjustment, and 1 mare died as a result of recapture. Wounds were cleaned and treated with antibiotics. Collars were removed in cases where it was judged necessary to assist recovery, and the animals were released. Since October 1988, another six tight collars have been removed from the horses at the Stone Cabin study area.

Monitoring

The condition of the study animals and the fit of the collars are monitored from the air during the four census-taking flights scheduled as part of the original study design. In 1988, BLM added an additional flight to check for collar problems in the fall after the animals gained weight over the summer. These measures were applied to all of the study areas and continued in 1989. Dr. Peck recommended increasing the additional collar monitoring to four times a year at regular intervals to detect health problems in the collared horses. In his view, the current schedule of monitoring flights is not sufficient to identify collar problems before they become severe. However, several observers expressed concern that increasing the frequency of the monitoring flights, especially during the spring and summer, could increase the incidence of abortions or orphaning of foals.

Other Study Areas

The collar-related problems experienced at study areas other than Stone Cabin have been less serious (Table 4-1). Five animals were darted to treat complications from tight collars at Clan Alpine; no tight-collared animals were found at Flanigan or Beaty Butte. Of the five at Clan Alpine, two collars were removed, one was adjusted, and one was left in place without adjustment. Three additional collars were adjusted at Clan Alpine, and one animal died during recapture.

The Flanigan and Beaty Butte study areas involved vasectomized stallions only. Collars became loose on several of the experimental animals. In these cases, the collars rode up over the ears of the animals, causing abrasions and concern that they might interfere with vision, hearing, or normal behavior. To correct problems with loose collars, 11 horses were darted at Flanigan through September 1988; nine collars were removed and two adjusted. During the same period of time, one horse was darted at Beaty Butte and the collar was removed. Collars also fell off the vasectomized stallions. By September 1988, 13 of 20 vasectomized males in Flanigan no longer had collars because of removal or loss. In Beaty Butte, 7 of 20 collared animals either lost collars or had them removed. Two additional collared animals died in each area during the course of the study. Since October 1988, one tight collar was removed at Clan Alpine, while a total of eight loose collars were removed at Flanigan, Clan Alpine, and Wassuks. During the fall of 1988 and throughout 1989, the BLM and the research team followed a policy of removing all problem collars, whether or not there was any injury to the animal.

Behavior Effects of Collars

Questions have arisen about the effects of the collars on animal behavior, because the collars may restrict sight or hearing or inhibit normal behaviors such as ear signaling, nuzzling, or neck rubbing. Dr. Cheryl Asa, an animal behavior researcher from the University of Minnesota, told the committee that her field observations of vasectomized and control stallions in the Flanigan and Beaty Butte sites indicated that the collars themselves did not seem to impede normal behavior significantly and did not compromise the animals. In fact, Dr. Asa expressed a concern that loose collars were being adjusted unnecessarily. In her view, the risk from darting the animal to adjust the collar greatly exceeded any risk from the loose collar itself.

Mortality Caused by Collars

The research team has attributed two deaths to collar-related problems. The first was a 25-year-old mare that died at Stone Cabin after being darted to treat a tight collar. The second loss occurred when a stallion fell off a cliff after being darted at Clan Alpine.

Other animals with collars were found dead. One had a collar imbedded in its neck, but no cause of death was determined. Another animal was found dead 12 days after she had been darted but failed to succumb. She was judged to have been dead for 3 to 7 days. The causes of both of these deaths were classified as "other."

The research team classified as natural the deaths of an additional 21 collared horses (4 with marker collars and 17 with radio collars) that were found dead before August 1988. The team found no evidence that these collars were stained from drainage, as were those that had become embedded in the horses' necks before removal. BLM observers believe that collar-related wounds may have caused or contributed to the deaths of at least some of these animals. It is not now possible to determine if the collars played a role in any of these deaths.

Effect on Statistical Adequacy

Questions have arisen regarding whether deaths and collar removals reduced the sample sizes to the degree sufficient to compromise the statistical validity of the study.

A total of 273 mares and 45 stallions in the experimental populations survived roundup and initial collaring. Eighteen placebo-treated mares died at Clan Alpine shortly after being rounded up and collared. The stallions were collared first, and subsequent collar problems with males involved loose collars that slid up over the animals' ears. By the end of the study period, 13 of 20 vasectomized animals in the Flanigan site had lost collars and 2 others had died. In Beaty Butte, 7 of 20 lost collars over the course of the study and 2 other collared animals had died. This gradual loss of collars and a lack of controls throughout the vasectomy study make the results of the vasectomy experiments difficult to interpret.

Tight collars were a problem only with mares. Of the 273 collared mares in the fertility study, 23 or 8.4 percent were lost from the experimental population due to collar removal or loss. An additional 17 horses with radio collars, or 6 percent, died of natural causes during the study. Four other animals died during collaring procedures, and two more with collars were found dead but their deaths were classified as "other." It is not known whether these last six animals were wearing radio or marker collars.

The number of mares from which radio and marker collars were removed and not replaced and the number of collared animals that died total 76, or 9 percent of the number originally collared. As discussed above and summarized in Tables 2-4 and 2-5, the number of animals in each treatment block both in 1988 and 1989 was sufficient to establish statistically significant differences between treatments and placebo implants in all of the cases. Regaining the animals lost to the study would not significantly strengthen the results of the research or the statistical significance of the inhibiting effect of the implants on reproduction.

Summary

To the extent possible, the committee has assessed the problems experienced with the radio and marker collars. There is no doubt that some of the collared animals suffered large and painful wounds, and at least two horses died as a direct result of problems with their collar adjustment. Twenty-three radio-collared horses were lost from the observable population because of collar removal, and two others died while being recaptured to remove their collars. Marker collars were removed from another 28 horses that were not included in the study population. No horses are known for certain to have died from collar wounds.

The collar problems have been attributed to various causes, which include: the design of the collars; the material and construction of the collars; irritation caused by the radio units; tight or loose fitting collars; natural growth of young horses; rapid weight gain because of abundant forage; abnormal weight gain as a result of the hormone implants; and difficulty in making fine adjustments at the first collar fitting. Most observers agree that most of the neck injuries were related to the radio units.

The collars were based on a generally accepted design. Similar collars have been used successfully in field studies of wild and free-roaming horses and many other species of large animals. The research team has had extensive experience with collars, telemetry, and fieldwork of this nature. Based on prior experience, the problems with collars experienced in this study were not anticipated.

Both the research team and the BLM responded as collar problems were observed. Animals with collar wounds were recaptured, and the wounds were treated and the collars removed as necessary. Treated injuries have healed, and some of the collars have been replaced. Additional flights were made to locate horses with collar injuries, and BLM added a fall flight to the monitoring schedule to check the condition of the horses after the period of summer weight gain.

Problems with collar injuries could continue during the remaining months of this study because the construction of the collars, the radio units, and the potential for weight gain remain unchanged. However, because the research team and BLM are now more intensively monitoring the problem, future injuries should be reduced in both incidence and severity. The BLM will round up all collared animals after the 1990 observation season and remove the collars.

FOAL ORPHANING AND LOSS

Questions have arisen over whether the spring and summer monitoring flights, to assess the reproductive status of the mares, separate mares from their foals and cause foal orphaning or death. In the case of collared mares, the numbers on the collars must be read in order to determine whether they are steroid-implanted experimentals, placebo implants, or untreated mares that have been fitted with marker collars. In order to read the numbers, the helicopter must descend to a low altitude over the mares, and they must at times be chased some distance before the numbers can be read. The concern is that, in this monitoring process, mares and foals can be separated and may not be reunited. In such cases, the foals might be permanently separated from the mares at ages too young to survive on their own.

Prior to the committee's 1988 meeting in Reno, Nevada, the committee conveyed concerns to the research team arising from aerial monitoring and the associated risk of foal orphaning and death. At the meeting, the committee examined two sources of evidence provided by the research team to determine whether foal orphaning or loss was occurring.

The first source was the record of observations on collared mares to determine whether mares seen with foals in the initial spring flights were observed without young in subsequent flights. This record proved inconclusive because of ambiguous trends. For example, a mare that may have been seen with her foal in April and sighted twice in May without her foal may have been seen in June with her young. Because of the frequency of these cases, no general pattern of individual mares could be inferred.

The second source of evidence consisted of aggregating all of the flight observations and calculating the percentage of mares with foals seen in each study area. A decline through the four flight periods would suggest foal loss.

Trends were calculated for Wassuks and Stone Cabin in 1987, and Wassuks, Stone Cabin, and Clan Alpine in 1988. Here again, the results were inconclusive. Because the samples were small and individual flight results were therefore variable, trends were difficult to infer. Two areas showed a decline: Wassuks in 1987 between censuses 3 and 4, and Clan Alpine in 1988 between censuses 2 and 4. Stone Cabin in 1988, however, showed a slight, net increase between censuses 1 and 4. Stone Cabin in 1987 and Clan Alpine in 1988, although variable between censuses, showed no net trend between censuses 1 and 4.

In light of available evidence, the committee concluded that the monitoring did not appear to have caused heavy or consistent foal loss. This conclusion did not rule out the possibility that individual foals may have been lost. If such a loss occurred, however, it was not frequent enough to detect through aerial observations. In addition, some natural foal mortality is expected.

Earlier studies have shown that the foal stage is the period of highest natural mortality in the life of a wild horse.

The issue arose again after the 1989 field season, sparked by a memorandum (Sweeney, 1989) that was based on field observations by BLM employees who accompanied the research flights, in a separate helicopter, to identify collared mares and to observe foals. They recorded instances during 1989 when the BLM observers feared that foals may have been separated. After 17 flights over the Wassuks and Clan Alpine areas, one observer wrote, "In my opinion, at least five foals were likely permanently separated from their mares. . . . I believe that the [actual] number of foals [lost] . . . is greater than the five." Sweeney's memorandum concludes "an undetermined number are left behind to become orphaned."

The committee again raised the matter with the research group at the February 1990 review in Denver. In response, the researchers described the flight procedures as follows:

- No band is chased more than 400 to 500 yards. If a foal separates from a band, the research helicopter pulls out of the chase.
- If a foal is separated, the helicopter circles in front of the band and turns it back toward the foal.
- As a precautionary measure, all research flights are accompanied by a second helicopter containing BLM observers who are watching for excessive chases and foal separation. If these occur, they can radio the research helicopter and advise it to end the chase. BLM has never called off the research helicopter.

As in the 1988 review, the research group also reviewed the trends in percentages of observed mares with foals over the four successive flights in the three study areas in 1989. Here again, there were no consistent trends.

A review of available data did not support the assertion that a major, consistent loss of foals occurred. Some small number of foals could have been lost, although even this is not unequivocally shown.

The committee remains concerned about the possibility that the monitoring flights may be causing foal orphaning or loss, and urges that the research team proceed with caution. From the standpoint of the research protocol, both placebo- and steroid-implanted mares are equally at risk. Thus, to the extent there may have been some losses, a comparison of the fecundity of the steroid- and placebo-implanted mares is not invalidated.

ABORTION

Another concern is the possibility that roundup for treatment and subsequent-year censusing flights cause mares to abort. Some observers maintain that (1) abortion may be caused by the stress the animals endure under the research methods of roundup and censusing, and (2) abortion, if it occurs, might have a statistical impact on the research results with regard to the actual effectiveness of the contraceptive implants. Because of observed abortion in corrals following BLM herd-control roundups, an analogy has been drawn between these observations and the possible occurrence of abortion as a result of research-induced stress.

The research team has seen no fetuses or other evidence of abortion. Of course, this does not rule out the possibility of its occurrence. It simply may not have been seen.

However, as discussed above and in Table 2-4, the foaling rate of the Clan Alpine placebo-implanted mares in 1988 (42 percent) and that of the steroid-implanted mares (53 percent) were similar to the foaling rates of the untreated, marker-collared animals that were not approached closely by helicopter (47 percent). The Wassuks placebo-implanted mares also had a similar foaling rate (45 percent). All of these rates fall well within the 95 percent confidence intervals of each other.

These results do not entirely rule out the possibility of abortion. Relatively small samples and year-to-year and site-to-site variation could mask the occurrence of some abortions. However, given the similar foaling rates, if abortion is occurring, it is occurring at too low a rate to be measured statistically.

With regard to the integrity of the research methods and the validity of the results, steroid- and placebo-implanted mares are subject to the same conditions in the conduct of the research. However, because steroid-treated mares are expected to have fewer pregnancies, more abortions would be expected from the placebo-implanted mares. Therefore, abortion, if occurring, would narrow the difference between the foaling rate of the steroid group and that of the placebo group.

Because the foaling rate of the placebo-implanted mares in the Clan Alpine area was within the range of the comparative values of less disturbed, marker-collared mares in 1988, there is no reason to conclude that abortion resulted in a sufficiently narrowed difference between steroid and placebo groups to invalidate the experiment. In the absence of either (1) direct evidence or (2) a statistical indication of abortion resulting from study protocols, the committee concludes that the validity of comparing foaling rates in steroid- and placebo-implanted mares is not compromised by abortion, if any occurs.

DISAPPEARANCE OF PENNED ANIMALS AT LOVELOCK CORRALS

The claim has also been made that large numbers of experimentally treated animals disappeared in the Lovelock corrals. According to the researchers, however, the total number of missing animals was 13. The number of animals in this treatment group was 210 in the first series, 70 in the second, and 50 in the third. These groups were not mutually exclusive; some animals in the first series were used again in the second and third. Hence, the total number of experimental mares was something less than the sum of these three numbers. Nevertheless, the sample sizes were substantial and the disappearance of 13 animals does not pose a serious problem for the integrity of the experiments.

FOOD CHAIN RISKS FROM STEROIDS

Questions have been raised regarding the wisdom of releasing into the wild large numbers of mares with steroid dosages coursing through their tissues. These animals might eventually be rounded up and used for human consumption, or they might die and their flesh eaten by wild carnivores whose reproduction could be impaired.

The dosage trials in the Lovelock corrals were undertaken with estradiol, a natural estrogen that is chemically broken down by the digestive process if consumed by an animal. The first dosages administered were not effective. In the second and third series the estradiol dosages showed some effectiveness, but time was running out and an effective dosage was needed to treat mares in the field by September 1986, according to the schedule of the contract. Therefore, pen trails were begun with ethinylestradiol, a synthetic estrogen that is 30 times as effective as natural estradiol, but withstands digestion if consumed by an animal. This treatment proved effective both in pen- and field-treated animals.

As to the risk of consumption, the possibility of a treated animal being eaten is very small. Moreover, ethinylestradiol is the steroid commonly used in human oral contraceptives. Using liberal calculations, a human must consume 1 pound of horse flesh shortly after implantation (i.e., the peak level of steroid release) in order to ingest the amount of ethinylestradiol contained in a single, low-dose oral contraceptive pill. At some time after implantation, when the steroid release ot the capsule had declined to its longer-term level, the amount ingested would be substantially less.

5

Conclusions and Recommendations

The following four conclusions are based on the data and information available to the committee as of April 1990.

1. The genetics studies show that in any given year about one-third of the foals in bands are not sired by the dominant stallion, and a high degree of heterozygosity exists in wild horses, which originated largely from domestic breeds in the Oregon and Nevada study areas.
2. The Lovelock pen studies have produced steroid dosages, a delivery vehicle, and a surgical procedure that block pregnancy in at least 70 percent of treated mares for at least 28 months. The treatment does not appear to cause abortion in mares that are pregnant when it is administered.
3. The dominant-stallion vasectomy study has had ambiguous results. The data suggest a reduced foal production in the Flanigan area in the first breeding season following surgery. However, the treatment was not effective in Beaty Butte. The research team is analyzing the data in greater depth.
4. The 1988 and 1989 field observations indicate that steroid implantation in mares effectively reduces foaling rates from the 40 percent to 50 percent levels observed in untreated and placebo-implanted mares, to a level of less than 10 percent for at least 2 years in steroid-implanted mares.

This research has been expensive, logistically difficult, and carried out under limiting financial and time constraints. The subjects are powerful, spirited animals that are difficult to handle and risk injury when penned or handled. To a considerable degree, the methodology has been developed anew, under difficult and risky environmental conditions. Fortunately, one helicopter crash during the course of the study did not result in any casualties.

Sadly, there have been injuries to and losses of animals. While regrettable, the numbers lost do not invalidate the results of the research. Overall, the committee feels that the research has been conducted professionally and as effectively as possible under the prevailing constraints and conditions.

The committee recommends continuing observations in the fertility control studies to determine how long the implants effectively control fertility. The implanted mares now held in the Oklahoma horse reserve should be maintained and their blood sampled annually until hormone levels are not significantly different from controls.

To determine the longevity and effects of implants in the field, field monitoring of steroid- and placebo-implanted mares should continue through 1990. Because of objections to helicopter use for this work, attempts should be made to obtain these data from the ground.

The use of chemosterilants in herd management should be evaluated in contrast to other viable control options, and not unduly discounted because of problems that occurred during field research experiments. The loss of 48 animals at the Clan Alpine study area, collar wounds, and possible foal orphaning were products of the research procedures. These problems would not occur normally during the routine application of fertility control for herd management.

The committee believes that the research to date shows some promise for controlling the wild and free-roaming horse population, and at reduced cost and need for adoption. The use of alternative methods is a decision to be made by the Bureau of Land Management (BLM). Clearly, major influences on BLM's decision will be the goals for the program and the resources provided by Congress to administer it.

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APPENDIX

Report on Collared Horses

The following is an unabridged version of the BLM report, Treatment of Collared Horses at the Stone Cabin Management Area, *written by Gerald R. Peck, D.V.M., on January 25, 1988.*

Horses with tight radio tracking collars were anesthetized by dartgun using M-99 in combination with xylazine and shot from a helicopter. Reversal of this combination was accomplished by intravenous injection of M-5050 and yohimbine. Only horses with collars showing signs of drainage from a wound or excessive tightness were darted. On January 16, 1988, 4 horses were anesthetized and their collars removed, wounds cleaned and debrided, topical antiseptic powder applied, and 70 cc of long acting penicillin administered intramuscularly. On January 21, 1988, 8 horses were anesthetized and collars were removed on 4 horses, readjusted on 2 horses, and no damage found on 2 horses. The same treatment was applied to the 4 horses which had the collars as the horses on 1/16/88.

Summary of injuries is as follows:

- 1 horse with necrosis of skin and subcutaneous tissue about 1 inch deep under the entire collar
- 1 horse with necrosis on top of neck about 1 1/2 inch deep because the radio transmitter had twisted to the top
- 6 horses with necrosis under the neck just caudal to the larynx—ranging from 1 to 2 inches deep

The necrosis on all of these horses was caused by pressure of tight collars. The damage was only to skin and subcutaneous tissues with various amounts of localized infection. No vital structures (i.e., trachea, arteries, or veins) were involved. White blood cell counts on the first 4 horses showed no signs of systemic infection. All horses were in good condition.

One of the horses with no damage from the collar died about 1 hour after being darted. She had recovered from the anesthesia but because of a debilitated condition due to old age (≈30 years) and resulting poor teeth, she could not recover from the stress of the anesthesia.

None of the horses with pressure necrosis from the radio collars appeared to be in imminent danger of dying. As I have stated before, these horses showed no sign of systemic infection and were in good condition. However wounds caused by the tight collars appeared to be quite painful and if the collars were not removed or adjusted more serious damage and the possibility of death could have resulted sometime in the future. There are certain factors that vary with time (i.e., weight gain or loss) that dictate animals in the wild with tracking collars must be monitored on a regular basis to deal with problems that arise before they become serious. Wild horses having problems with tight collars must be identified and treated. They should be observed at regular intervals at least 4 times a year.

Gerald R. Peck, D.V.M.

FINAL REPORT 1992-1996

NEVADA WILD HORSE FERTILITY CONTROL PROJECT

(BLM COOPERATIVE AGREEMENT #1422F950A20002)

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This report summarizes the studies which we performed between October 1992 and July 1996 in order to develop and test a contraceptive vaccine which can humanely regulate feral horse populations. We proposed that such a method could be used in conjunction with or as an alternative to the expensive and over-burdened Round-up/Adoption program which is currently in effect, and we expected that contraception could substantially reduce the cost of wild horse management.

INTRODUCTION

To date, Feral horse management on western public lands has been confined to the removal of excess horses. While we are not convinced that there is an actual overpopulation of horses in many areas, we recognize the need for improved, more effective management of feral horse populations. The removal of horses as the sole management effort, while seemingly effective at the time of removal, does not prevent the subsequent growth of the remaining population and insures that removal must continue year after year. Indeed, there is evidence that, in addition to disruption of family units and skewing of population age structure and sex ratios, the removal of horses actually increases fecundity among those animals remaining behind and accelerates the growth of the population. In other words, removal alone addresses only the symptom of overpopulation (too many horses) and not the cause (reproduction). The result is distortion of population characteristics without solution of the problem.

An alternative approach is to limit reproduction, through some form of fertility control. Toward that goal we have tested a contraceptive vaccine on feral horses which can limit the number of foals born to free-roaming mares. The major characteristics of this vaccine include (1) effectiveness (> 95% effective), (2) remote delivery, which permits humane, non-capture administration of the vaccine, (3) relatively low cost, (4) no effects upon individual or social behavior of the target animals, (5) no effects upon pregnancies already in progress at the time of vaccine delivery, (6) reversible contraceptive action, and (7) no passage of the vaccine through the food chain or into the environment. These characteristics have been previously identified as required for successful feral horse contraception, and the vaccine known as porcine (pig) zona pellucida, or PZP, exhibits all of them.

The zona pellucida is a non-cellular protein membrane which surrounds all mammalian eggs. In order for fertilization to occur, sperm must first bind to this membrane before they can penetrate the egg. The intramuscular injection of PZP into mares causes them to produce antibodies against the pig protein, but these antibodies also bind to the sperm attachment sites on the mares' eggs, thereby preventing sperm attachment and fertilization. Because only fertilization has been blocked, there are no hormonal manipulations which cause behavioral changes. Indeed, immunized mares remain together in their social groups, ovulate regularly during the breeding season, permit mating behavior by the herd stallion, and in general reflect the social behavior of untreated feral horses.

The overall goal of the present project is to develop and test a single-injection contraceptive vaccine for feral horses which will have a 1-3 year period of effectiveness. Specifically, the purpose of these studies, as outlined in the original research plan, was three-fold: (1) development of a functional one-inoculation, one-year PZP contraceptive vaccine which can be delivered remotely to regulate of free-roaming feral horses, (2) extension of the 1-inoculation technology to yield two-years of contraceptive protection, and (3) field test of the vaccine on free-roaming feral horses inhabiting public lands in Nevada.

Before these studies were performed, two separate injections given three weeks apart were necessary for effective contraception in horses: The reason that a one-injection vaccine is needed is that it is neither cost effective nor logistically reasonable for management purposes to maintain horses in captivity for 3 weeks or to mark, relocate and retreat horses that have been released. Thus, the first goal of this research was to develop a method for delivering a single-inoculation PZP vaccine which would provide an immediate exposure of the mare to vaccine and then a second exposure during the next month, without another injection. To achieve the second goal of this research (2-year vaccine), the single-inoculation would also contain a third dose of the vaccine which would be released at about one year, thus resulting in contraceptive protection for two years.

At the time of initiation of these studies there was already a technology which could be applied to the existing PZP vaccine to meet these goals. This technology binds the PZP antigen within an inert non-toxic polymer which, upon injection, will release the antigen continuously but slowly over some period of time. The chemical particles which contain the antigen are referred to as microspheres, and they are composed of poly L-lactide or copolymers of lactide and glycolide. This approach has been used for the intramuscular or subdermal delivery of a large number of agents, including cancer chemotherapeutics and vaccines (Cowsar et al. 1985; Linhardt 1989; Staas et al. 1991).

In summary, the studies which are reported below have focused on (1) the production and assessment of microspheres containing PZP vaccine, (2) the immune response of animals exposed to PZP vaccine released by these microspheres, (3) the ability of selected PZP vaccine preparations to prevent pregnancy in free-roaming feral horses in Nevada.

OBJECTIVES

The specific objectives of this proposed research were directed at the development of a one-inoculation vaccine (in the form of microspheres) for use in feral horses as follows:

1. To determine if the PZP protein (antigen) retains immunological activity during preparation for incorporation into microspheres.
2. To engineer a sustained-release formulation (microspheres) for a one-inoculation PZP vaccine that will impart a full year of contraceptive protection.
3. To test the effectiveness of this one-inoculation, one-year vaccine to produce antibodies in laboratory rabbits (preliminary screen) and in horses (final test).
4. To engineer a controlled-release formulation for a one-inoculation vaccine which will impart two-years of contraception.
5. To test the effectiveness of the one-inoculation, 2-year PZP vaccine to produce antibodies in laboratory rabbits (preliminary screen) and in horses (final test).
6. To assess management-style use of the PZP vaccine by field testing its current 2-injection form in comparison with the single-injection prototypes on free-roaming horses in Nevada.

ACTIVITIES AND RESULTS

The research reported below is presented in approximate chronology and addresses both the laboratory and field studies which were performed.

Preliminary Work

Beginning in 1992, the preparation and lab testing of controlled-release PZP microsphere formulations was initiated, and the BLM and research team organized the first field trial. First, we arranged subcontracts for the study through University of Nevada, Reno, Medical College of Ohio, Deaconess Research Institute, University of California, Davis, and University of Iowa (producer of microspheres). Next, we prepared standard PZP for use in the study, and then we incorporated PZP in to controlled-release microspheres for use in the study we had begun *in vivo* testing in domestic horses of the first PZP microsphere preparation (prior to September 1992). Upon completing the blood collections in this study, we assessed antibody titer data resulting from use of the final PZP microsphere preparation. The initial results indicated up to 7 months of contraceptive effect with single injection. This led us to redesign this PZP microsphere preparation to lengthen the time of contraceptive effectiveness. We then prepared redesigned PZP microspheres in time for use in the initial field trial scheduled for December 1, 1992.

Field Trial 1: Antelope/Antelope Valley HMA (Ely, Nevada), Dec. 1992 - Nov. 1994

After selecting field trial sites (Antelope HMA and Antelope Valley HMA in Nevada) and inspection of the chosen sites and facilities, we met to organize the initial field trial. This involved representatives of the BLM (Nevada State Offices, Ely District and Elko District) and the research team members (Turner, Kirkpatrick, Liu, and Rutberg).

On December 1, 1992, the field trial was begun Implementing the initial field trial required 2-weeks of field work and participation by State and Local BLM, gather contractor (D. Cattoor) and the Research Team. The period of data collection and analysis for the field trial was from December 1992 until November 1994. Regarding this field trial: 1) A summary of the field trial is presented below, 2) a detailed report of this work is currently "in press" for publication in the *Journal of Wildlife Management* (1996), and 3) a copy of this in press paper is included at the end of this final report as Appendix I.

To summarize, we determined contraceptive effectiveness and duration of effect after PZP vaccination in free-roaming feral horses in Nevada. We captured, freeze-branded, treated and subsequently released one hundred thirty-two adult feral mares. Treatment consisted of one or 2 injections (1 month apart) of vaccine emulsion consisting of aqueous PZP and Freund's Complete Adjuvant (FCA, inj. 1) or Freund's Incomplete Adjuvant (FIA, inj. 2). FCA and FIA were obtained from Sigma Co (St. Louis, MO). We gave controls saline and Freund's adjuvant as above. Among 1-injection mares, we gave one group a single dose of raw antigen, and we gave the other group raw antigen plus a second dose of antigen sequestered in controlled-release microspheres. We also studied 63 untreated mares. We administered inoculations in December 1992, and monitored the mares via fecal analysis for pregnancy and via helicopter and ground survey for foal production through September 1994. We determined pregnancy via measurement of estrone sulfate, progesterone metabolites and total estrogens, in fresh feces collected from the ground. Among antigen-treated mares, reproductive success across 1 year was 4.5% (2-injection), 20.0% (1-injection + microspheres) and 28.6% (1-injection, no microspheres). Reproductive success in placebo (55.0%) and untreated (53.9%) mares was significantly greater than in treated groups. The following year, without further treatment, reproductive success was 44.0% (2-injection), and 54.5% (untreated) and these values were not different. Data from other groups were insufficient for comparison. The results of this study confirm the feasibility of capturing large numbers of mares in the field for vaccine treatment and demonstrate: (1) 1-year contraceptive efficacy of several PZP vaccine protocols, (2) greater effectiveness in a 2-injection protocol than in 1-injection protocols, and (3) a return to fertility in the second year after vaccination.

Laboratory Studies: Sept. 1992 - Feb. 1995

While data were being collected for the field trial, laboratory studies aimed at optimizing the controlled-release system for PZP and adjuvant were initiated using rabbits and domestic mares. These studies involved subcontracts with the University of Iowa (Iowa City, IA; Dr. D. Flanagan and Dr. R. Linhardt) and with Medisorb Technologies (A. Stolle-DuPoint Company, Cincinnati, OH; Dr. C. White and Dr. J. Strobel). These sources assisted us in the testing of the PZP protein for its ability to survive the procedure of being incorporated into controlled-release microspheres. They also prepared the microspheres containing the PZP, using PZP which we provided to them. We then tested the ability of these PZP-containing microspheres to stimulate an immune response, by measuring the concentration of anti-PZP antibodies in the blood of rabbits and horses at various times after they had been injected with the PZP microspheres. Appropriate control groups were used to insure that any effects seen were not due to the microsphere material or adjuvant, and to compare the effectiveness of microsphered PZP with ordinary PZP.

The data from the microsphere studies are presented in the next several pages. Before summarizing the data, however, we will briefly describe the course of events associated with the microsphere work. This should provide a clearer picture of the choices we made and why. These studies cover the period from the fall of 1992 through the summer of 1995.

All of the PZP used in the project was prepared under vigorous specifications by M. Bernoco in the laboratory of Co-Investigator, Dr. I. Liu at the University of California, Davis. The viability tests of the PZP antigen (performed at MCO and U. of Iowa) showed that it retained full biological and immunological activity after all steps involved in microsphere preparation (See Appendix IIa, b). The determination that PZP can be lyophilized (freeze-dried) without loss of activity (Appendix IIa) is advantageous for concentrating the PZP and for keeping it in long-term storage.

The first microsphere preparations of the PZP were carried out in 1992 at University of Iowa using lactide and glycolide polymers in a coacervation technique. The lactide: glycolide ratio was 65:35. The loading rate was 1% and the efficiency of the preparation was 75%, i.e., 75% of the starting PZP ended up in beads. The product microspheres were injected into rabbits and into domestic horses and were also incubated *in vitro* to determine PZP-release characteristics. The rabbit, horse and *in vitro* data all suggested that the PZP was being released in a continuous fashion over a 4-5 week period and was effectively stimulating antibody production. Therefore, we decided to use this preparation in one of the treatment groups in the field trial to determine whether it actually effected contraception.

As evidenced in Appendix I, the results of the first field trial showed that the single-injection, controlled-release vaccine was less effective than the standard 2-injection vaccine, which we have used as our reference point for all studies, indicating that more refinement was needed in the single-injection, controlled-release PZP preparation. Thus, late in 1993 a laboratory-based study of improved formulations of controlled-release PZP vaccine was undertaken. This study, utilizing domestic horses and rabbits, was designed to assess temporal release patterns of PZP/adjuvant from microspheres by measuring the time course of plasma levels of anti-PZP antibodies.

The early PZP formulations were prepared as a "best-estimate" for achieving proper delay of PZP/adjuvant release and sufficient anti-PZP antibody titers to insure infertility. The data showing release characteristics of PZP from the Iowa preparation used in the field trial indicated a continuous PZP release across 52 days, but with most release in the second half of the period (Appendix IIc).

The release study was conducted by placing 9.416 mg of PZP-containing microspheres in 1 ml of phosphate buffer (pH 7.4, 0.005M). The PZP microsphere dispersion in buffer was placed in a 1.5 ml plastic capped centrifuge tube which was agitated gently in a 37°C incubator-shaker. Samples (0.75 ml) were removed at various time intervals over 7 weeks. The sample volume was replaced with blank buffer. The samples were frozen at -70°C until analysis. The samples were analyzed by capillary zone electrophoresis (CZE) with UV detection. The accompanying graph (Appendix IIc) gives the cumulative areas for peaks identified as PZP. The areas is proportional to the concentration of PZP which has been released from the microspheres into the medium. There is a progressive increase in the cumulative amount of PZP released over 7 weeks. These results are consistent with other polymer data which have shown that the 65:35 copolymer should not breakdown for at least 6 weeks which correlates with this data.

The results of the rabbit studies using Medisorb preparations indicated that the original formulations were not sufficiently delaying the PZP/adjuvant release to yield maximal antibody response (Appendix III, Fig. 3a). Also, the total amount of PZP incorporated into the microspheres (this is known as "loading") had to be increased. Finally, the data from all of the above studies suggested that adjuvant had to be microsphered along with the PZP, to enhance the immune response to the controlled-release PZP.

The next round of controlled-release preparation was carried out in 1994 and focused on the design and testing of a polymer preparation that would provide delayed PZP/adjuvant release and the amount of PZP/adjuvant release needed to maximize the antibody response. This was carried out by an interactive study involving the Research Team laboratories and Medisorb Technologies. We chose Medisorb for this work, because Medisorb professed to have technology to make both 1-month and 1-year microspheres, while University of Iowa could not make the latter. The antibody titer data obtained by the Research Team from the first Medisorb controlled-release formulation was used to design the next formulation, and this process was carried forward one preparation at a time on a small scale until appropriate release delay and release amount were achieved. Because of the unique conditions which exist in the animal, it was not possible to test the vaccine controlled-release dynamics *in vitro* (test tube), i.e., testing had to be done in the living animal. Since we had good success with rabbits in the early studies, we continued with rabbits. Once a strong candidate preparation was demonstrated in rabbits, we cross tested it in domestic horses.

The studies were arranged to compare different microsphere preparations with each other and with a standard vaccination protocol of 2 separate injections. Titers were monitored weekly to monthly for up to 68 weeks, depending on the treatment and experimental design. In these studies rabbits were vaccinated and blood samples were taken periodically to determine anti-PZP antibody titers. Results of these studies are presented graphically in Appendix III (Fig 3 a-e) and are described below.

We initially tested 3 formulations (I, II, III) of injected microspheres designed to release at about 1 month. Raw PZP was present (Fig. 3b) or absent (Fig. 3a) from these injections. Formulation II yielded the best characteristics (Figs. 3a and 3b), so we arranged for preparation of 3 variations of Formulation II and tested them. Formulation IV utilized regular PZP at regular (65 μg) dose. Formulation V utilized lyophilized PZP at regular dose. Formulation VI utilized lyophilized PZP at 3 times dose. Of these variations (II_b=IV; II_c=V; II_d=VI), the latter two (V and VI) yielded the best titer response (Fig. 3c). The presence of raw (not in microspheres) PZP in the injections which contained microspheres produced highest titers (Fig. 3b), which were sustained many months longer than when raw PZP was absent (Fig. 3a).

We had Medisorb prepare additional controlled-release formulations designed to release PZP at about 12 months. In order to conserve both rabbits and time we treated the same rabbits with both a 1-month and a 12-month controlled-release preparation. We reasoned that the 1-month effect would have worn off long before the 12-month release occurred. Thus, we expected that by the time the 12-month release occurred; titers would be low again, and any booster effect of the 12-month release would be readily apparent. As can be seen in Appendix III, Fig. 3b, there is a titer rebound at 44-48 weeks, suggesting that the microspheres designed to release around 12 months did so around 10-11 months. We were very encouraged by this finding, but we did not have time to pursue the next round of such a long study during the tenure of this project. We planned to continue this work as soon as the 1-injection, 1-year vaccine was sufficiently refined for population-level studies, reasoning that continuation of lab studies could occur simultaneously as we moved ahead with field studies.

In addition to the development of controlled release for PZP antigen, it was necessary to address controlled release of adjuvant. Since adjuvant had been shown to be essential for a good titer response, we had to insure that adjuvant would be present when PZP was present. Although FCA had been quite effective, it was an oil. Controlled-release formulations require that the material to be released is water soluble. Thus, we performed studies to compare the effectiveness of alternative, water-soluble adjuvants against the Freund's adjuvant.

The original vaccination protocol used FCA (injection 1) and FIA (injection 2), and this has been the adjuvant standard against which other adjuvants have been tested. Two water-soluble adjuvants we tested were Carbopol®934 (CAR) and RIBI Adjuvant System (RAS). In the rabbit studies to date, we have tested 1-month microspheres containing PZP + CAR or PZP + RAS in comparison with unsequestered PZP + FCA/FIA sequence or PZP microspheres + FIA injection. Clearly, PZP + FCA/FIA sequence yielded titers which were higher and were sustained longer than any other adjuvant, although the PZP microsphere/FIA injection yielded well elevated titers. In a previous (prior to studies in this final report) study of titer responses in horses, vaccination with unsequestered PZP + CAR was about 80% as effective as PZP + FCA/FIA, while unsequestered PZP + RAS was only about 25% as effective as unsequestered PZP + FCA/FIA. In the rabbit studies, using 1-month microspheres containing PZP + CAR or PZP + RAS, RAS was 63% effective as CAR in elevating antibody titers, but PZP + RAS was nonetheless 2.5 times more effective than PZP without adjuvant. Neither CAR nor RAS was as effective as we needed for contraceptive efficacy under the conditions studied (Appendix III, Fig. 3e).

Regarding timing aspect of the controlled-release of the microspheres containing PZP or adjuvants, it is not clear whether a pulsing of release occurred. Because the release was monitored through a biological response (titers), we did not expect to see sharp peaks but rather blunted hills or gradual shifts over several weeks. This was acceptable, since it is the titer response rather than the release pattern in which we are ultimately interested. Based on this perspective, however, the responses were less vigorous than we expected. Also, it appears that the 1-month release preparation did not release at 1 month. Rather, it appears that there was a release between 9 and 18 weeks (Appendix III, Fig. 3c) or, possibly, soon after injection. An exact timing cannot be clearly determined from the data, but it is unlikely that a response occurred in the 3-6 week period which was desired. We were nonetheless encouraged that the controlled-release approach was viable for both PZP and adjuvant.

The original plan was to test various microsphere formulations and adjuvants in rabbits, and then select the best ones for testing in domestic horses. This was done to limit horse use due to high maintenance cost and difficult handling associated with horses. The domestic horse data, presented in Appendices II and IV, demonstrated that: 1) PZP remained viable during microsphere preparation (Appendix IIa), 2) neither of two different formulations of PZP microspheres (without adjuvant microspheres) augmented the effect of injection of unsequestered PZP + FCA (Appendix IVa), and 3) using the best microsphere formulation from the rabbit studies, microspheres containing PZP and adjuvant (CAR), without injecting unsequestered PZP or unsequestered adjuvant, produced a significant antibody response (Appendix IVb).

These results confirmed in horses the success of microsphered PZP in the rabbit studies in terms of PZP viability. The lack of augmentation of response by PZP microspheres in mares given raw PZP + adjuvant is not readily explained. It may be that the raw PZP/adjuvant maximally stimulated the response so that any microsphere effect was masked. The response obtained with microspheres alone (PZP + adjuvant microspheres) shown that the microspheres do work. However, the release pattern was not a burst at 1 month, since titers were already up at 2 weeks post inoculation. Thus, although the domestic horse data were encouraging in some respects, they left unanswered questions.

One observation which held true for both rabbits and horses is that, in a given sample, some animals respond vigorously and others only moderately to the same dose of vaccine. While it is possible that increasing dosages may yield a higher percentage of vigorous responders, this was not demonstrated in going from 1 X to 3 X PZP dose in a preliminary study in rabbits (Appendix III, Fig. 3d). Another important consideration emerging from comparison of horse and rabbit responses to the same treatment is that the rabbits may not be a reliable model for testing what the vaccine will do in horses. Thus, despite higher costs and greater difficulties, future tests should focus on horses directly.

<u>Conclusions of Studies Performed Through Sept. 1995</u>

The conclusions which we have drawn from the above studies using PZP microspheres and/or adjuvant microspheres are:

1) The microsphere approach will produce well-elevated titers.

2) When PZP-containing microspheres alone were given, titers declined

to probable sub-contraceptive levels after 2-3 months, meaning that the present microspheres given alone will not enable a year of contraception.

3) When raw (unsequestered) PZP + FIA were injected with the microspheres, the titers were highly elevated and in some cases sustained above contraceptive levels for >1 year, indicating that a 1-year, single-injection vaccine should be refineable from this preparation.

4) Increasing the dose of PZP from 1X to 3X did not increase the level or sustainment of titers.

5) The release from the 1-month microspheres did occur, but did not occur near 1 month.

6) The timing and pattern of titers suggested that release from microspheres may have occurred at 9-18 weeks, indicating that controlled-release works for PZP but refinement in technology is needed.

7) The release from the 12-month microspheres occurred at 44-48 weeks and yielded a significant increase in antibody titers, suggesting the possibility of application toward 2-year vaccine.

8) FCA/FIA in sequence was the most effective adjuvant protocol.

9) CAR microspheres provide better adjuventicity than RIBI microspheres, but both were more effective than PZP alone.

<u>Laboratory Studies from Sept. 1995 - Sept. 1996</u>

Although none of the microsphere preparations behaved optimally, high and sustained titers were achieved in some experiments. Thus, we felt that refinement of the microspheres and the protocol could yield the desired vaccine.

Based on the above conclusions, we proposed the following plan of action, which was to:

1) Refine the microsphere formulations to obtain release in the 3-6 week window.

2) Refine microsphere formulations to maximize "pulsing" rather than "gradual" release.

3) Test for the concomitancy of release of PZP and adjuvant from microspheres.

4) Test combination adjuvant preparations, such as FCA/CAR or FIA/CAR to be used as part of the raw-PZP portion of the injection.

Early in 1995 we negotiated with both Medisorb and the University of Iowa regarding plan 1) and 2) above. We concluded that Iowa would provide the most acceptable preparation within the available budget. This work was begun in the summer of 1995. Unfortunately, the funding which was expected to begin October 1995 (fiscal year 1996) did not become available due to failure of Congress to approve the 1996 Federal budget until April 1996. We were forced to drastically limit expenditures of remaining fiscal 1995 funds and to request project extension without additional funds.

It is now August 1996, and we are still without funds. We make this point to place in perspective the project decisions we have made in the past 12 months. First, we were unable to continue the maintenance of previously treated domestic horses kept at the University of California, Davis (UCD). Long-term data on antibody titers from these mares were therefore not available. Second, further planned lab studies on the controlled-release vaccine were prevented due to limited funds. Third, we had to use remaining 1995 (extension) fund to cover the execution of the field study planned for 1996 in the Nevada Wild Horse Range (NWHR, Nellis Air Force Base). Fortunately, we were able to prepare and deliver PZP vaccine to 267 mares in the NWHR with these extension funds, avoiding the loss of an entire year of research. We also were able to perform one lab study comparing combination-adjuvant preparations (see item #4, previous page). The results of this lab study are present below, followed by the description of the NWHR field study, which will be completed in 1997.

Highly elevated and sustained antibody-PZP anti-titers appear to be necessary for extended infertility associated with PZP immunocontraception. Both FCA/FIA and CAR, a water-soluble polymer, have been shown to produce vigorous antibody titer responses when used as individual adjuvants in the PZP vaccine. The specific mechanisms of the adjuvanticity of the adjuvants is not clear, but it appears that there are mechanistic differences, based on water/lipid solubility differences and the presence of carboxymethyl groups (immunostimulatory) only in CAR. Thus, it is possible that additive or synergistic effects could occur if FCA (or FIA) and CAR were used together. FIA used as an adjuvant given as 2 separate injections with PZP and also CAR, given the same way, resulted in moderate antibody titers in a previous study. However, neither adjuvant yielded titers as great or as extended as the FCA (Inj. 1)/FIA (Inj. 2) adjuvant protocol (Appendix V, Fig. 1). We hypothesized that the FIA and CAR adjuvants in combination would yield an even greater response level and duration than if used separately.

FIA was used in the study reported below, since it has less adjuvant effect than FCA, and would better enable observation of a summative response than FCA. We reasoned that observation of an additive adjuvant effect of FIA + CAR could warrant further studies with FCA + CAR.

The effectiveness of PZP immunocontraception using FIA with and without CAR as an adjuvant system was tested in rabbits, beginning in September 1995. Twenty-four rabbits were divided into 4 treatment groups, and each rabbit was given contraceptive vaccine (20 μg PZP + adjuvant) as 2 separate injections, 3 weeks apart. Blood samples (0.5 ml) were taken by ear venipuncture at the time of the first injection and at 13, 27, 42, 69, 91, 125 and 167 days. Anti-PZP antibody titers were measured in these samples (Liu et al., 1989) to assess the antibody response to treatment.

The adjuvant treatment groups were:

Injection 1	Injection 2
FCA	FIA
FIA/CAR	FIA/CAR
FIA/CAR	FIA
FIA	FIA/CAR

The FCA → FIA group served as the positive (active) reference standard for the response.

The results of this study are presented in Appendix IV. As has been observed in previous studies, the FCA/FIA standard protocol produced high antibody titers. All treatment groups showed a similar temporal pattern to the FCA/FIA group across the first 10 weeks, and the response was as vigorous in the other groups as it was in the FCA/FIA group. It appears that beyond 10 weeks the FIA/CAR combinations are all slightly less effective than the FCA/FIA. At 10 weeks, the presence of CAR in both injections yielded a slightly greater response than its presence in only one injection, regardless of whether it was in the first or second injection. However, this condition did not sustain beyond 10 weeks, suggesting that the CAR may not be useful to maintain long-term anti-PZP antibody titers. The FIA/CAR combination was not summative. This is indicated by the fact that CAR alone had yielded titers equivalent to or greater than FIA alone for up to 8 weeks in previous studies (Appendix V, Fig. 1), but in the present study the presence or absence of CAR with FIA made no more than 15% difference in titers (Appendix V, Fig. 2). One difficulty in drawing firm conclusions from Fig. 2 is that in this study the FCA group showed an early, uncharacteristic decline in titers.

We are unable to explain the temporal difference in titer responses between the FCA group in Fig. 1 and the FCA group in Fig. 2. It is possible that this may be due to differences in batches of FCA, since a different batch was used in each study. Unfortunately, the uncharacteristic decline in titers after 70 days in the FCA group precludes a determination of whether FIA/CAR combinations are similar-to or inferior-to the FCA/FIA standard treatment in terms of titer maintenance.

A practical discovery in these experiments was that CAR imparts improved stability to the PZP-adjuvant emulsion. It maintains the integrity of the emulsion through at least one freeze-thaw cycle. The emulsion without CAR breaks down after freezing and thawing, and it must be re-emulsified before use. The presence of 10 mg. CAR prevented emulsion breakdown upon thawing, and it maintained good emulsion quality for several days at 0-4°C. This information is useful in terms of advance preparation, storage and transport of vaccine, especially for field studies.

<u>Field Trial 2: Nevada Wild Horse Range (Nellis Air Force Base, Nevada), Jan. 1996 - Sept. 1996</u>

In January 1996 a field trial of the contraceptive efficacy of a version of a controlled-release PZP vaccine was begun (this trial will not be completed until November 1997). Since CAR appeared to enhance the titer response to FIA, we initiated a test to determine whether CAR would enhance the effectiveness of FCA. Three treatment groups were included in the field study:

	n	Injection 1	Injection 2
Group 1	99	standard PZP-FCA	PZP-FIA
Group 2	99	PZP-FCA/CAR	PZP-FIA
Group 3	69	PZP-FCA plus PZP-CAR microspheres	none

The study area was the Nevada Wild Horse Range (NWHR) in south central Nevada, which is inhabited by approximately 2000 horses with a harem band social structure. The peak breeding period is May and June, and the peak foaling period is April and May. The physical condition of the mares in this study was subjectively estimated to range from poor to very good at the time of capture for treatment.

PZP was prepared from porcine ovaries and the basic vaccine consisted of 0.5 cc. standard phosphate buffer solution containing 65 μg PZP, which was emulsified with 0.5 cc. FCA (Sigma; St. Louis, MO). FIA was used for the second injection. We prepared the emulsion within 24 hours of injection, using 2, 10-cc. glass syringes joined with a plastic connector. After 100 plunger strokes the emulsion was loaded into a 3 cc. plastic syringe for injection via a 18 ga., 3.7 cm needle. The needle and injection site were prewashed with 70% ethanol.

In an attempt to mimic the 2-injection condition with a single injection, one group of mares was given an injection as described above, with an additional component of controlled-release microspheres containing 80 μg PZP and 10 mg CAR adjuvant, given as a separate injection using a 14-gauge, 3.7 cm needle. The microspheres were 10-100 μ in diameter and composed of bioerodable lactide and glycolide polymers. They were prepared by D. Flanagan at the University of Iowa using a coacervation method. Release rates were projected to be continuous over approximately 4 weeks, with greater release in weeks 1 and 4.

Horses were gathered by helicopter for hands-on injection. This access method was chosen because it was used for a scheduled round-up in the NWHR for the BLM adoption program.

Between January 3 and 9, 1996, approximately 800 horses were gathered into portable corrals by helicopter. Stallions and mares were separated and were singly moved through a chute, where they were aged, assessed for physical condition and lactation (yes or no) and given a 1 cc. prophylactic equine flu injection intramuscularly. Healthy mares (n=267) between the ages of 10 and 28 years were permanently marked with consecutive numbers by freeze branding. The brands were located on the upper left hip, were 10 cm. in height and were readable from a helicopter and through a spotting scope at >500 m distance on the ground. A description and data sheet was filled out for each marked mare prior to treatment. It is important to note that in many feral horse herds, reproduction is low among old mares. However, in this study mares >20 years old were included for contraception because data for Nellis horses from prior years had shown incidence of lactation to be similar in mares older or younger than 20, i.e., many old mares were producing foals in the Nellis Herd.

One ml. of freshly prepared emulsion of buffered PZP + adjuvant was hand-injected intramuscularly into the left gluteus muscle. Mares in the microsphere group were also injected at the same location with 2.5 ml. of a 1.0% solution of CAR-in-water containing suspended PZP/CAR microspheres. After one injection, all mares were trucked to a local holding facility and maintained in corrals on grass-hay and water ad lib until release. Mares in the single injection group were released after 4 days into the range area from which they were gathered. Prior to release the mares and their foals were separated, and the foals were retained for eventual adoption. Mares in the 2-injection groups were maintained under observation for 13-17 days on a grass-hay and water (ad lib) diet until the second injection was given. The mares were then returned to their home range area and released. Within 24 hrs of the release the study area was surveyed by helicopter to determine the well-being and dispersal of the mares.

Effectiveness of treatment has not been determined as of August 1996. Effectiveness will be determined by 1) collection of 25-30 fecal samples for selective pregnancy testing in October 1996, and 2) foal counts of all treated animals in June and September 1997.

SUMMARY AND CONCLUSIONS

Entire Project (Sept. 1992 - Sept. 1996)

The conclusions which we have drawn from the studies presented in this final report are presented below. It is clear from these studies that PZP immunocontraception is highly effective in Western feral horses. The PZP antigen of this vaccine and several adjuvants will remain fully biologically active after exposure to several organic solvents, heating to 70° C and freeze-drying; procedures associated with preparation of the vaccine for controlled-release. The controlled-release preparations used in these studies were effective in raising and sustaining elevated anti-PZP antibody titers above control levels for periods >1 year. However, these titers were not as well elevated as when a standard 2-injection protocol was used. The completed field study showed that a 2-injection protocol was also better at reducing fertility than a single-injection, with or without a controlled-released component. A single-injection did decrease fertility by about 50% for 1 year compared to no treatment, but further studies will be necessary to achieve maximal contraceptive effect with a single injection. These studies have been designed and are in the currently submitted proposal to BLM. The preliminary results from the 1-injection, 2-year vaccine preparation encouraged further testing, but this was not feasible prior to this final report. Two-year capability must be a focus of continued controlled-release studies with this PZP vaccine. Of the adjuvants tested, FCA was the most effective. The presence of adjuvant in any future controlled-release preparation will be essential for maximum antibody response and probably for maximum contraceptive effect. The adjuvant combination of FIA and CAR approximated FCA in the initial response, but did not sustain titers as well. In several experiments the results obtained in rabbits were not consistent with later results obtained in horses. This has led us to conclude that the rabbit is not a good titer-response model for the horse, and that further studies should focus directly on horses. Had the rabbit proven a good model, it would make the titer-testing part of these studies far less costly. Unfortunately, horses maintained in captivity are costly, and there are no known alternative, small lab species. Presently the vaccine is well developed enough that much of the remaining research will be at the level of determining its population-control effects in the field. We expect that the limited lab studies outlined in the proposal we have submitted for the 1996 fiscal year will refine the 1-injection vaccine to the point where it can be routinely employed as a 1-injection, 1-year contraceptive. While the proposed 1996 research may yield a 2-year capability, it is likely that this will require further work with controlled-release chemistry, for PZP and water-soluble adjuvants. In the meantime it should be helpful to apply the 1-injection, 1-year capability to population level studies, i.e., considerable field progress in population-control aspects of this contraception field-work progress can be made with what we have now.

Clearly, PZP contraception has a bright future as a feral horse management tool. It is effective, inexpensive, safe and readily applicable within the existing Round-Up/Adoption Program. We feel that the work done to date makes a strong case for continuing the research to optimize PZP contraception. This will begin with the funding of the fiscal 1996 proposal, which will permit completion of the NWHR project, the inhibition of a new field study addressing population-limiting effects of PZP vaccine, the performance of an additional controlled-release study to complete the 1-injection, 1-year technology, and continuation of the 2-year technology.

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IMMUNOCONTRACEPTION LIMITS FOAL PRODUCTION IN FREE-ROAMING FERAL HORSES IN NEVADA

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Abstract: We determined contraceptive effectiveness and duration of effect after porcine zona pellucida (PZP) vaccination in free-roaming feral horses in Nevada. We captured, freeze-branded, treated and subsequently released 132 adult feral mares. Treatment consisted of 1 or 2 injections (1 month apart) of vaccine emulsion consisting of aqueous PZP and Freund's complete adjuvant (FCA; inj. 1) or Freund's incomplete adjuvant (FIA; inj. 2). We gave controls saline and Freund's adjuvant as above. Among 1-injection mares, we gave one group a single dose of raw antigen, and we gave the other group raw antigen plus a second dose of antigen sequestered in controlled-release microspheres. We also studied 63 untreated mares. We administered inoculations in December 1992, and monitored the mares via fecal analysis for pregnancy and via helicopter and ground survey for foal production through September 1994. We determined pregnancy via measurement of estrone sulfate, progesterone metabolites and total estrogens, in fresh feces collected from the ground. Among antigen-treated mares, reproductive success across 1 year was 4.5% (2-inj.), 20.0% (1-inj. + microspheres) and 28.6% (1-inj., no microspheres). Reproductive success in placebo (55.0%) and untreated (53.9%) mares was significantly greater than in treated groups. The following year, without further treatment, reproductive success was 44.0% (2-inj.), and 54.5% (untreated) and these values were not different. Data from other groups were insufficient for comparison. The results of this study confirm the feasibility of capturing large numbers of mares in the field for treatment and demonstrate: (1) 1-year contraceptive efficacy of several PZP vaccine protocols, (2) greater effectiveness in 2-injection protocols than in 1-injection protocols, and (3) a return to fertility in the second year after vaccination.

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Key Words: accessibility, antibody titers, contraceptive vaccine, controlled-release, equids, feral horses, fertility, horse immunocontraception, mares, microspheres, population control, reversibility

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Since the late 1970s, fertility control has been investigated as a potential tool for regulating feral horse (Equus caballus) numbers. In early studies, various steroids were the focal contraceptive agents, with controlled-release androgens used in stallions (Turner and Kirkpatrick 1982, Kirkpatrick et al. 1982) and estrogenic and progestagenic steroids used in mares (Plotka et al. 1988, 1992). Despite contraceptive efficacy obtained with some steroids, interest in these agents waned in the face of undesirable behavioral effects and potential environmental hazards of such steroids (Turner and Kirkpatrick 1991, Kirkpatrick and Turner 1991). In 1987, the antifertility effect of a PZP vaccine was tested successfully in free-roaming feral mares on Assateague Island National Seashore (Kirkpatrick et al. 1990a). Previously this PZP vaccine had prevented pregnancy in 13 of 14 domestic or captive feral mares (Liu et al. 1989). In the Assateague study the dart-delivered vaccine was >95% effective and had no apparent effects on pregnancies in progress, the health of offspring, or the behavior of treated mares.

The effectiveness of the vaccine appears to reside in the antigen-stimulated production of anti-PZP antibodies in the vaccinated animal. These antibodies bind to the zona pellucida surface of the recipients' ovulated egg, presumably preventing sperm attachment (Liu et al. 1989). In ungulates studied to date, 2 separate exposures to PZP in one month have been used to obtain maximal, sustained anti-PZP titers for the first year of contraception (Kirkpatrick et al. 1990a, Turner et al. 1992a). Thereafter, a single annual booster injection has been sufficient to maintain fertility from year to year (Kirkpatrick et al. 1991a, 1992a).

Because it is impractical on a management scale to give a 2-injection sequence to free-roaming wildlife, the potential use of polymers that permit a controlled, gradual, or pulsed release of the vaccine has been investigated. One approach involves forming a homogenous mixture of the drug with a biodegradable nontoxic material, in the form of microspheres (Eldridge et al. 1989, Wang et al. 1990). Upon intramuscular injection and contact with tissue fluids, the biodegradable material erodes and releases the drug over a predetermined period of time (Wang et al. 1991). One goal of the present study was to compare the efficacy of PZP vaccine administered as 2 separate injections with the efficacy of PZP vaccine administered as a single injection with and without a controlled-release PZP component.

Federal agencies have considered contraception for a potential management role in regulating some Western feral horse populations. While barrier-island feral horses and Western feral horses are the same species, there are many regional differences in horse behavior and habitat (Turner and Kirkpatrick 1982, Keiper and Houpt 1984, Berger 1986, Rutberg 1990), as well as agency differences in horse management techniques (T. Pogacnik, Bur. Land Manage., pers. comm.; B. Underwood, Natl. Park Serv., pers. comm.). Techniques for accessing and contracepting barrier-island horses may not be effective with Western horses. Therefore, an additional goal of the present study was to determine field accessibility to Western feral horses for PZP contraception.

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METHODS

Experimental Design

The study examined the antifertility effectiveness and duration of effect of 3 variations of injected PZP contraceptive vaccine in comparison to placebo and untreated control conditions. We (the research team) randomly assigned to groups, adult mares previously identified by freeze-branding. Investigators inoculating the animals were unaware of the content of a given injection. From previously identified individuals, we determined treatment effectiveness and duration of effect by foal counts and pregnancy testing of fecal samples collected upon defecation. Mare groups consisted of: (1) 2 separate injections, 4 weeks apart, of vaccine containing adjuvant and native PZP, (n =

60), (2) 1 injection of vaccine containing adjuvant and native PZP (n = 21), (3) 1 injection of vaccine containing adjuvant and native PZP plus an additional, controlled-release PZP (no controlled-release adjuvant) dose (n = 22), (4) 2-injection placebo (adjuvant and saline, without PZP) (n = 19), and (5) 1-injection placebo (n = 10). In addition to these groups, we tested for pregnancy or observed for presence of a foal 63 unmarked and untreated mares (that had not been captured) during the study. To prevent duplication of data collection among these 63 mares, we identified the mares by coat color, face markings, and by association with other horses in their family band (Turner et al. 1981). Contraceptive treatment was administered in December 1992. We collected fresh feces for pregnancy testing in September-October 1993 and 1994. We counted foals in May, August, September, and October of 1993 and 1994.

Study Area

The study area encompassed about 1,825 km^2 in northeastern Nevada centered at 40°15'N and 113°30'W near the Dolly Varden Mountains. Elevations ranged from 1,600 to 2,400 m. Vegetation on the study area was composed largely of shrub-steppe communities with sagebrush (Artemisia sp.), rabbitbrush (Chrysosamnus sp.), and bitterbrush (Purshia tridentata) dominating. Areas of pinyon pine (Pinus edulis) were present at higher elevations. Average annual precipitation was 25-cm at 1,500 m elevation, with increasing amounts (mostly as snowfall) at higher elevations. Water was available from permanent springs scattered throughout the study area. The northern and southern portions of the study area were administered by the Elko and Ely districts of the Nevada BLM.

Horse Population

The study area was inhabited by about 700 horses with a harem band social structure (Turner et al. 1981, Berger 1986). The peak breeding period was May and June, and the peak foaling period was April and May. We subjectively estimated the physical condition of the mares according to the condition scale (1-10) developed by Henneke et al. (1983). Mare condition ranged from fair (3) to very good (7) during the study. We rated well-fleshed animals with a sleek coat in very good condition. We rated animals with noticeable ribs, mild hip and shoulder bone protrusion and low quality coat in fair condition.

PZP Vaccine Preparation

We prepared PZP from porcine ovaries (Liu et al. 1989). The vaccine consisted of 0.5-cc standard phosphate buffer solution containing 65-μg PZP, which was emulsified with 0.5-cc Freund's complete adjuvant (Sigma, St. Louis, Mo). We emulsified PZP with Freund's incomplete adjuvant for the second injection. We prepared the emulsion within 12 hours of injection, using 2 10-cc glass syringes joined with a plastic connector. After 100 plunger strokes, we loaded the emulsion into a 3-cc plastic syringe for injection via a 19-gauge, 3.7-cm needle. The needle and injection site were prewashed with 70% ethanol.

In an attempt to mimic the 2-injection condition with a single injection, we gave one group of mares an injection, with an additional component of controlled-release microspheres containing 65-μg PZP with no adjuvant. While we recognized that the presence of adjuvant was desirable in the microspheres for maximizing the immune response, the technology for preparing adjuvant-containing microspheres was unavailable. The microspheres were 10-50 μm in diameter and composed of bioerodable lactide and glycolide polymers. They were prepared by D. Flanagan, R. Linhardt and E. Schmitt at University of Iowa using a coacervation method (Wang et al. 1991). We projected release rates to be continuous over about 4 weeks, with greater release in weeks 1 and 4 (Wang et al. 1990, 1991).

Accessing, Handling, and Inoculating of Horses

The BLM gathered horses by helicopter, permitting injection by hand. We chose this method because access to the horses was possible as a part of scheduled roundup for the BLM horse adoption program.

Between 2 and 12 December 1992, the BLM gathered about 500 horses by helicopter into portable corrals. In a cooperative effort, we and the BLM: (1) separated the gathered stallions and mares, (2) moved them singly through a chute, (3) determined aged and physical condition, (4) gave a 1-cc prophylactic Strepguard® (Miles Lab., Shawnee Mission, Ks.) injection intramuscularly, and (5) permanently marked healthy mares (n = 132) between the ages of 5 and 15 (prime reproductive age) with consecutive numbers by freeze-branding. The brands were located on the upper left hip, were 10-cm in height and were readable from a helicopter and through a spotting scope at >500-m distance on the ground.

We hand-injected intramuscularly 1-cc of freshly prepared emulsion of buffered PZP (or buffer alone) and adjuvant into the left gluteus muscle and photographed each marked mare. The BLM maintained mares given a single injection in the portable corrals on grass-hay and water ad libitum for up to 72 hours and then released them into the range area from which they had been gathered. Before release, the BLM separated the mares and their foals and retained the foals for eventual adoption. They separated mares in the 2-injection protocol from the foals and transported them by truck to the Palomino Valley BLM corral facility. The BLM maintained the mares, under observation, for 4 weeks on a grass-hay and water (ad libitum) diet until we gave the second PZP injection. The BLM then returned the mares to their home range area and released them. Members of the same harem band, previously marked with a same-color grease pencil, were released together. Within 24 hours of the release, representatives of the BLM and the research team surveyed the study area by helicopter to determine the well-being and dispersal of the mares.

Antibody Titers

Before release from captivity and after the second inoculation, we examined each mare for possible injection-site abscess, and obtained a 10-cc blood sample by jugular venipuncture for antibody titer analysis from randomly chosen PZP-treated (n = 19) and placebo (n = 8) mares. We harvested the serum and froze it at -20C until assay. We determined anti-PZP antibody titers in the above serum samples by an enzyme-linked immunosorbent assay (ELISA) (Voller et al. 1986), with modification reported previously for horses (Liu et al. 1989). We assayed the sera in duplicate and expressed the results as a percentage of the horse positive reference serum, which consisted of a pool of sera that had demonstrated near-maximal titers, about 4-6 weeks after an initial PZP injection (Liu et al. 1989). Pooled preimmunization sera served as a control (no anti-PZP antibody).

Pregnancy Testing by Fecal Steroid Analysis

Pregnancy testing avoids the potential problem of incorrectly assessing contraceptive effectiveness due to undetected stillbirths or mortality of neonates. In the study area, most foaling occurs in April and May, and breeding is uncommon after mid-June. Pregnancy can be detected reliably with fecal analysis within 80 days of conception (Kirkpatrick et al. 1991b). We sampled mares for pregnancy detection in fall 1993 (to determine treatment efficacy) and fall 1994 (to determine duration of effect). Between mid-September and mid-October of 1993 and 1994, we observed mares for defecation using binoculars and 20-40 x spotting scopes. We insured that each sample collected was from the desired mare by pairing observers such that one person maintained view of the sample through the scope while hand-signalling the other observer to the sample. In any case where the location or specificity of the sample was in questions, none was collected. We collected freshly dropped fecal balls (2-3) in a sealable plastic bag, labelled them, and stored them on ice until they could be placed in a freezer (within 72 hr). We determined pregnancy in each sample via measurement of estrone sulfate (E_1C) and immunoreactive progesterone metabolites (iPdG) by ELISA

(Kirkpatrick et al. 1991b) and total estrogen (TE) by radioimmunoassay (Kirkpatrick et al. 1990b). The combined measures have proven nearly 100% accurate in pregnancy diagnosis in several species (Lasley and Kirkpatrick 1991), including the feral horse. We considered a mare pregnant when values for a given sample were $E_1C > 27.9$, iPdG > 4000.0 and TE > 250.0 ng/g of dry-wt feces.

Foal Counts

We determined the presence of a foal with a given marked mare by helicopter and ground observations in May and September of 1993 and 1994. We verified which foal was with which mare in ground surveys by observing the horses until the foal clearly associated with a given mare by its repeated proximity to her during grazing and travelling and/or by nursing from her. In the helicopter surveys we verified parentage by maintaining sufficient helicopter distance from the family groups of horses to permit foals to associate closely with the mother as they moved away from the helicopter. The latter verification method has correlated well with subsequent ground survey data in previous studies (Turner et al. 1992b). We considered a mare to be successful reproductively if she tested positive for pregnancy and/or was verified to have a foal.

Statistical Analysis

Where appropriate, we have presented data as mean ± SE. We employed Student's *t*-test for statistical analysis of fecal and titer data. We determined possible group differences among reproductive success rates using a Tukey-type multiple comparison test for proportions or a binomial probability distribution (BPD; Zar 1984).

RESULTS

During one month of captivity and at the time of their release in January 1993, none of the 60 2-injection mares showed abscesses. None of the mares observed in the field thereafter showed injection-site marks, suggesting that abscesses had not occurred. Following the treatment of mares in December 1992, severe winter conditions prevailed in northern Nevada through March 1993. However, we observed 93 of the 130 marked mares (71.5%) between May and October 1993. We found an additional 8 marked mares not seen in 1993 between May and October 1994, yielding a total reidentification rate of 77.7% during the study.

In 1993 we collected fecal samples from 51 marked mares. In the 1993 samples obtained from mares that were reidentified and had produced foals in 1994 ($\underline{n}$ = 9) the average values for E_1C, iPdG, and TE were 18.9, 38.7, and 22.2 × greater than the respective average values associated with mares that did not produce foals ($\underline{n}$ = 19) in 1994 (Table 1). For each of the 3 steroids measured, the average value for mares that did produce foals was significantly greater ($\underline{P}$ < 0.01) than for mares that did not produce foals. Among the mares tested, the values for all 3 steroids were consistent, i.e., all were elevated or all were depressed. All mares that were diagnosed as pregnant (by fecal steroids) and were reidentified the following spring were observed to have a foal. We observed that 2 of 19 mares that were diagnosed as not pregnant had a foal the following spring. Overall accuracy was 26/28 (93%).

Of 44 reidentified mares in the 2-injection PZP group, only 2 mares were reproductively successful in the 1993 breeding season. Eleven of 20 mares were reproductively successful in the 2-injection placebo group. In the 1-injection groups reproductive success occurred in 4 of 14 mares (PZP, no microspheres), 3 of 15 mares (PZP plus PZP microspheres), and 2 of 4 mares (placebo). Reproductive success was achieved in 34 of 63 unbranded mares that were studied (Table 2). The proportion of reproductively successful mares in all treatment groups was significantly lower ($\underline{P}$ < 0.05) than in both placebo and untreated control groups, the proportions of which were not different from each other. In addition, the proportion of reproductively successful mares in the 2-injection PZP group was significantly lower ($\underline{P}$ < 0.05) than the proportions in both 1-injection PZP groups. The proportions in the 1-injection PZP groups were not different.

Anti-PZP antibody titers in 2-injection mares at 4 weeks after initial inoculation averaged 86.2% of positive reference control titers in 19 PZP-treated and 0.9% in 8 placebo-treated animals ($\underline{P}$ < 0.01). Control titers were 0-2%. Thirteen of the PZP-treated mares showed titers >90%, and 6 were between 46 and 75%. Sixteen of the titer-tested PZP mares were reidentified for subsequent fertility evaluation. All of these had shown titers >52% at 4 weeks posttreatment, and all were infertile in the first year after treatment.

We determined duration of treatment effect on the basis of 1994 pregnancy testing only. This basis was considered acceptable, because the 1993 correlation between pregnancy-test and foal-count data reported was 0.93 ($\underline{n}$ = 28). In the 2-

injection PZP group (treated Dec 1992) fecal samples from 11 of 25 mares showed pregnancy-positive levels of steroid metabolites in the second year (Sept 1994) after treatment. We obtained a positive pregnancy test in fecal samples from 12 of 22 unbranded mares that we had sampled during the same period as for branded mares. The pregnancy rates were not different ($\underline{P} > 0.05$, BPD) between these groups (Table 3). Among 2-injection placebo mares, samples (Sept 1994) from 2 of 4 indicated pregnancy. In the 1-injection PZP and 1-injection PZP-plus-PZP microspheres groups, samples (Sept 1994) from 1 of 2 and 1 of 2 mares, indicated pregnancy. We did not obtain samples from mares in the 1-injection placebo group. Sample sizes for 1-injection groups were insufficient for statistical analysis.

DISCUSSION

Inoculation of captured and released feral mares with PZP vaccine resulted in a marked suppression of fertility across one breeding season. While a fertility rate of 4.5% occurred with use of the 2-injection protocol, fertility rates in both single-injection groups were higher (20-28%). Nonetheless, single-injection fertility rates were only 1/3 - 1/2 of fertility rates observed in control (placebo and untreated) mares, and this difference was statistically significant. Fertility rates among control mares were similar to those reported for other feral horse populations (Wolfe et al. 1989).

The results indicate that the controlled-release microspheres neither mimicked the condition of 2 separate injections, nor produced significantly greater infertility than a single injection alone. Exposure of the mares to PZP via microspheres differed from a second vaccine injection in that the PZP in the former was not released as a bolus and was not accompanied by adjuvant. In a previous equid study employing FCA (Kirkpatrick et al. 1990a), we observed abscesses in 3 of 26 treated mares. However, abscesses occurred only in mares given 3 injections of vaccine (1=FCA, 2=FIA, 3=FIA), not in mares given 2 injections (1=FCA, 2=FIA). Had there been abscesses in 2-injection mares in the present study, the abscesses should have appeared during the month of captivity. Thus, it appears that, in the 60 mares given 2 injections, abscesses did not occur. Abscesses seemed unlikely in mares given a single injection, since they received less exposure to adjuvant than 2-injection mares.

The presence of well-elevated anti-PZP antibody titers in all of the PZP-treated mares and in none of the placebo-treated mares that were sampled is consistent with previous reports in horses (Liu et al. 1989). Also, these titers were consistent with fertility data, i.e., no mares with elevated titers produced foals in all of the mares (n = 16) for which the assessment could be made.

The presence of anti-PZP antibody titers and infertility are not necessarily related. However, elevated titers consistently have been associated with infertility in the horse. In a previous study, none of 13 mares exhibiting peak (4-6 weeks post-inoculation) titer levels >65% of positive reference serum titers before the breeding season became pregnant (Liu et al. 1989). In the present study, none of 16 mares showing titers >52% at 4 weeks post-inoculation was reproductively successful during the subsequent breeding season.

The effect of PZP treatment in 2-injection mares was sustained through 1, but not 2, breeding seasons, indicating a return to fertility after 1 year. Forty-four percent of 2-injection, PZP-treated mares that were relocated in the fall of 1994 were pregnant. This finding is similar to the pregnancy rates in placebo and untreated control mares, which averaged 56 and 54% in 1993, and 50 and 54% in 1994. We focused the reidentification effort to test duration of infertility on the 2-injection mares, since they were exposed to the greatest stimulation by PZP. We expected that the possibility of extended infertility would be greatest in this group. Also, the 2-injection mares composed the largest group in the study, offering the best chances for reidentifying a large number of these mares. Although the 1-injection data are consistent with the 2-injection data regarding reversibility, the small number of reidentified mares in the 1-injection groups precludes conclusions in these groups.

This study confirms the high accuracy of pregnancy diagnosis via fecal steroid metabolites in feral horses (Kirkpatrick et al. 1991b). The minimum 18-fold difference observed between non-pregnant and pregnant state in the measured metabolite levels favors reliable differentiation of pregnant and non-pregnant state. Although no discrepant values were observed in the present study, an occasional (<2%) discrepant high or low value is possible among the 3 metabolites we have used (Lasley and Kirkpatrick 1991). By measuring all 3 metabolites (E_1C, iPdG and TE), we minimized the chances of mis-diagnosis. Among 28 mares for which we obtained both pregnancy and foaling data, agreement was 93%. Both cases of disagreement were false negatives, i.e., pregnancy diagnosis was negative but a foal was present. We were unable to determine whether the pregnancy test was incorrect or whether we mismatched the foal with the mare or whether conception occurred too late in the year to be detectable at the time of fecal collection.

Management Implications

The study was carried out in Nevada, where more than 60% of Western feral horses reside. Helicopter roundup of horses is a well-established method for accessing feral horses in Nevada and other Western states for roundup/adoption programs, and has been used for other contraception studies (Eagle et al. 1992, Plotka et al. 1992). Incorporation of PZP vaccination into the existing roundup procedures greatly reduces the cost of accessing horses for immunocontraception in years when gathers are performed. In the BLM roundup/adoption program, mares more than 5 years of age are returned to the range. An added advantage of using scheduled periodic roundups for administering immunocontraception is that only mares destined to remain on the range will be treated; i.e., treatment will not be wasted on mares aged 3-5 years, which will be removed and adopted. Options for horses for single-injection immunocontraception in years when adoption roundups are not performed include gathering by helicopter, and remote delivery by dart from helicopter (Turner and Kirkpatrick 1991).

The use of contraception for management purposes has the potential to reduce management costs by reducing the frequency of roundups and eliminating the need to maintain unadoptable horses in captivity. Contraception can also enhance the humaneness of management, since roundups and extended captivity are stressful to the horses. However, Garrott (1991) has concluded from feral horse modelling studies that each population (herd area) is unique and must be considered individually for potential use of contraception. We agree with this point. Also, as suggested by Garrott (1991), the issues of both cost-effectiveness and limitation of population growth must be included in assessing long-term contraceptive management potential.

A 2-injection protocol presents logistical and economic problems for routine use in feral horses, because of the need to keep gathered horses for 3-4 weeks. However, single-injection methodology presently could be used in the field, with the caveat that the first year after treatment the expected fertility rate will be 20-28%. An annual booster in subsequent years should yield >90% efficacy, since Kirkpatrick et al. (1992a) have observed <5% fertility in year 3 among mares given a single injection of PZP vaccine in years 1 and 2. In other words, despite a submaximal response to antigen in year 1, the response to booster antigen in year 2 can be maximal.

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Table 1. Association of fecal steroid metabolite levels in feral mares with subsequent presence or absence of foals[a]

Mare condition	No. of mares	Steroid metabolites concentration[b] (ng/g feces dry wt ± SE)		
		E_1C	iPdG	TE
Without Foal	19	14.6 ± 8.3	378.0 ± 217.4	53.9 ± 21.4
With Foal[c]	9	276.2 ± 69.8	14,637.1 ± 2,250.0	1197.1 ± 214.0

[a] Fecal samples collected in Sept 1993, foal counts performed May-Sept 1994

[b] E_1C = estrone sulfate, iPdG = immunoreactive pregnanediol glucuronide, TE = total estrogens

[c] Values between conditions for all steroids are significantly different; $\underline{P} < 0.01$, Student's $\underline{t}$-test

Table 2. Effect of PZP immunocontraception on fertility of free-roaming feral mares in Nevada

Treatment[a]	No. of mares located[b]	No. of mares reproductively successful[c]	% of mares reproductively successful[c,d]
Untreated	63	34	53.9[q]
Placebo (1- or 2-inj.)	20	11	55.0[q]
PZP (2-inj.)	44	2	4.5[r]
PZP (1-inj. + microspheres)[e]	15	3	20.0[s]
PZP (1-inj.)	14	4	28.6[s]

[a] A given treatment (Dec 1992) consisted of i.m. injection of an emulsion of 0.5-cc Freund's adjuvant and 0.5-cc phosphate buffer solution with or without (placebo) 65-μg PZP

[b] Mares that were located for sample collection and/or observation between May 1993 and Oct 1994

[c] A positive fecal pregnancy test or presence of a foal were criteria for reproductive success

[d] Values with different superscript (q,r,s) are significantly different from each other; $\underline{P} < 0.05$, Tukey-type multiple comparison test for proportions

[e] One dose of microspheres contained 65-μg PZP for controlled release and was incorporated into the injection

Table 3. Reversibility of PZP contraception in free-roaming feral mares

Condition	No. of mares sampled[a]	No. of pregnant mares[b]	% of mares pregnant[c]
PZP (2-inj.)	25	11	44.0
Untreated	22	12	54.5

[a] Treatment in Dec 1992; fecal samples collected in Sept 1994

[b] Based on pregnancy test criteria as presented in Table 1

[c] No differences among groups; ($\underline{P} > 0.05$) binomial probability distribution

APPENDIX IIa

Effect of Freeze-Drying, Heat and Organic Solvent on PZP Induction of Anti-PZP Antibody Titers

		% POSITIVE REFERENCE SERUM				
PREP.#	HORSE I.D.	11/18	12/5	12/18	1/16	2/25
1	Pum	9	38	127	116	101
	Ter	8	15	94	101	82
	Swe	7	21	115	113	98
means				112	110	94
2	Pol	13	23	117	113	109
	Kat	9	53	123	115	93
	Ros	10	36	107	108	102
means				116	112	101
3	Hiv	9	37	121	110	91
	Rai	6	24	102	111	106
	Lah	4	9	99	118	76
means				107	113	91
4	Fox	4	61	122	115	93
	Cor*	92	140	130	125	120
	Mis	3	70	131	123	111
means				127(2)	119(2)	102(2)

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Prep 1: lyophilized with no further treatment
Prep 2: lyophilized, then exposed to heat (65°C for 15 min)
Prep 3: lyophilized, then exposed to CH_2CL_2 vapor for 15 min
Prep 4: untreated (control)

11/18 First vaccination of 50μg prep + FCA
12/5 Second vaccination of 50μg prep + FIA

* It was overlooked that the mare Corky had been vaccinated with PZP 4 years earlier (injections: 12/30/87, 1/13/88, 1/27/88). The still remaining high titer, however, is remarkable.

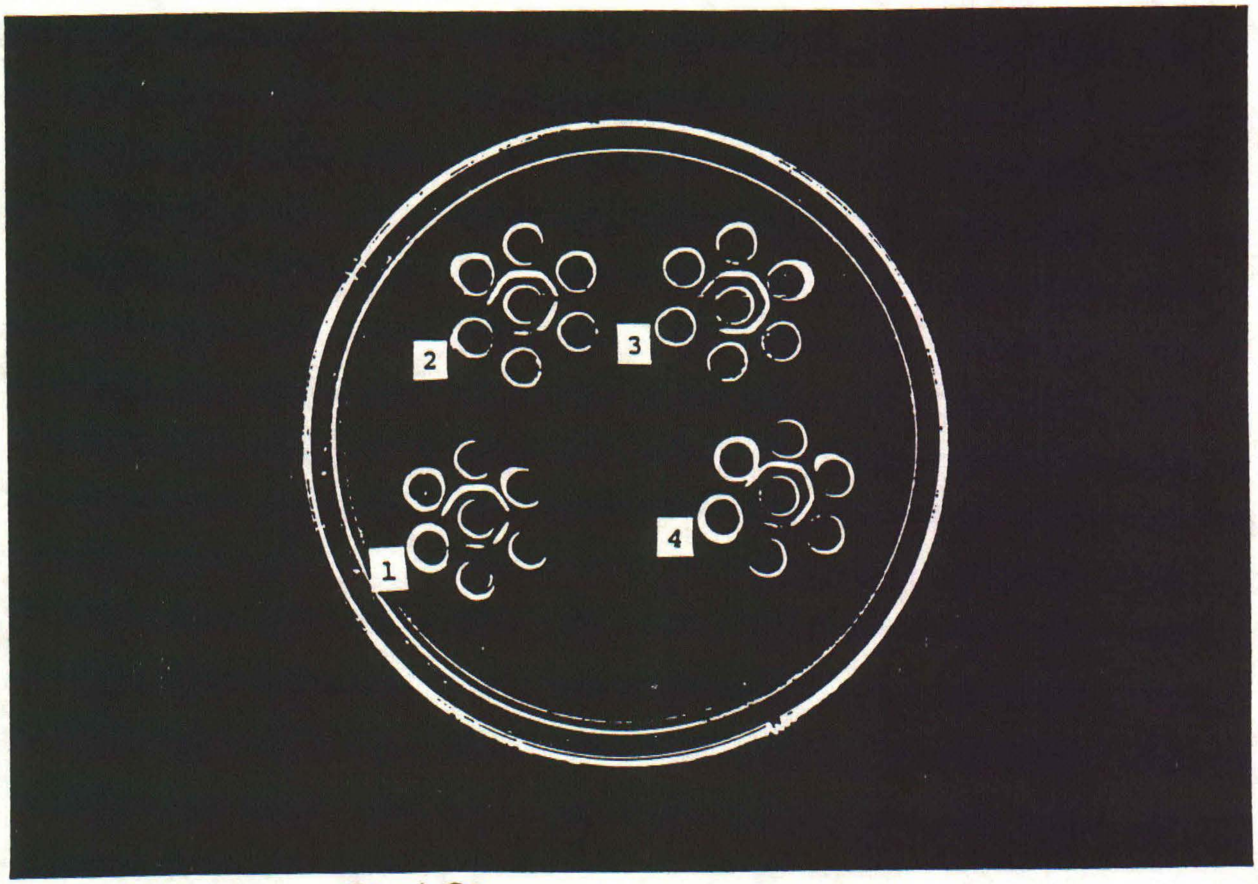
APPENDIX IIb

Conservation of Immunoreactivity of PZP after Incorporation into Lactide/Glycolide Microspheres

Immunodiffusion (Ochterlony) with microsphere preparations #1, 2, 3 and our vaccine as control (#4) at similar concentrations (100μg/ml) in a 0.9% agar gel.

The central well of each cluster of wells contains the preparations as marked #1,2,3,4. The six peripheral wells contain (in clockwise sequence, starting from the well next to the mark) sera from: 1. one control horse with no antibodies to PZP, 2.,3.,4. three horses with antibodies to PZP, 5. one himilayan tahr (goat) with anti-PZP antibodies, and 6. one deer with anti-PZP antibodies.

As you can see, there is no precipitate between the preparations and the antibody-negative serum, but there are precipitates of all the preparations with the antibody-positive sera. The patterns of the 3 microsphere preparations appear similar to the control, espècially when the distance of the precipitate from the central well is considered. The distance of the precipitate from the central well increases with more antigen quantity. The fact that the wells were punched individually and by hand (because we were not equipped with a well pattern template), which results in slightly unequal distances between the central and the peripheral wells, should not matter.



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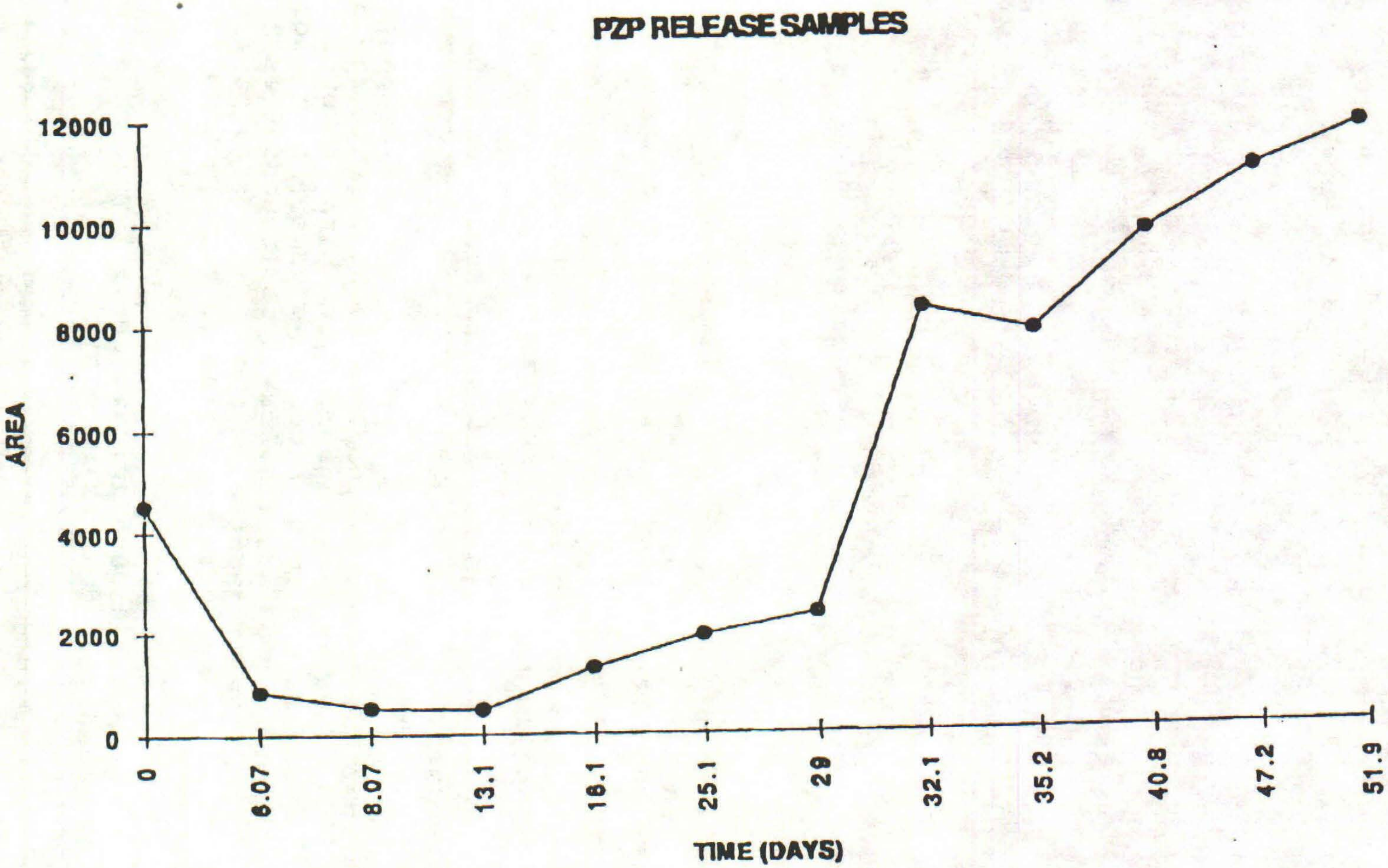
APPENDIX IIc

Release Characteristics of PZP from Lactide/Glycolide Microspheres as Measured by Capillary Zone Electrophoresis



As PZP is released from the microspheres, it becomes detectable in the solution. Area (y axis) is a measure of PZP concentration in the solution,with increasing area reflecting increased PZP.

APPENDIX III

Anti-PZP Antibody Titer Data in Rabbits:

Microsphere Data

Information Regarding Figs. 3 a-e.

The following pages contain figures displaying the results of several microsphere studies performed in rabbits. The microspheres ("beads") contained porcine zona pellucida (PZP) antigen or adjuvant, and they were prepared by Medisorb Technologies (Cincinnati, Ohio) and tested by the research team of J. Turner, J. Kirkpatrick and I. Liu. Testing consisted of injection of microspheres and vehicle with or without additional raw (unsequested; not in microspheres) antigen and adjuvant, followed by periodic blood sampling for up to 68 weeks. The x-axis of each graph is "weeks", and the y-axis is antibody titers as "% of positive reference serum." The positive reference serum is a pool of sera from mares that had demonstrated anti-PZP antibody titers in the high positive range and were subsequently infertile.

3a

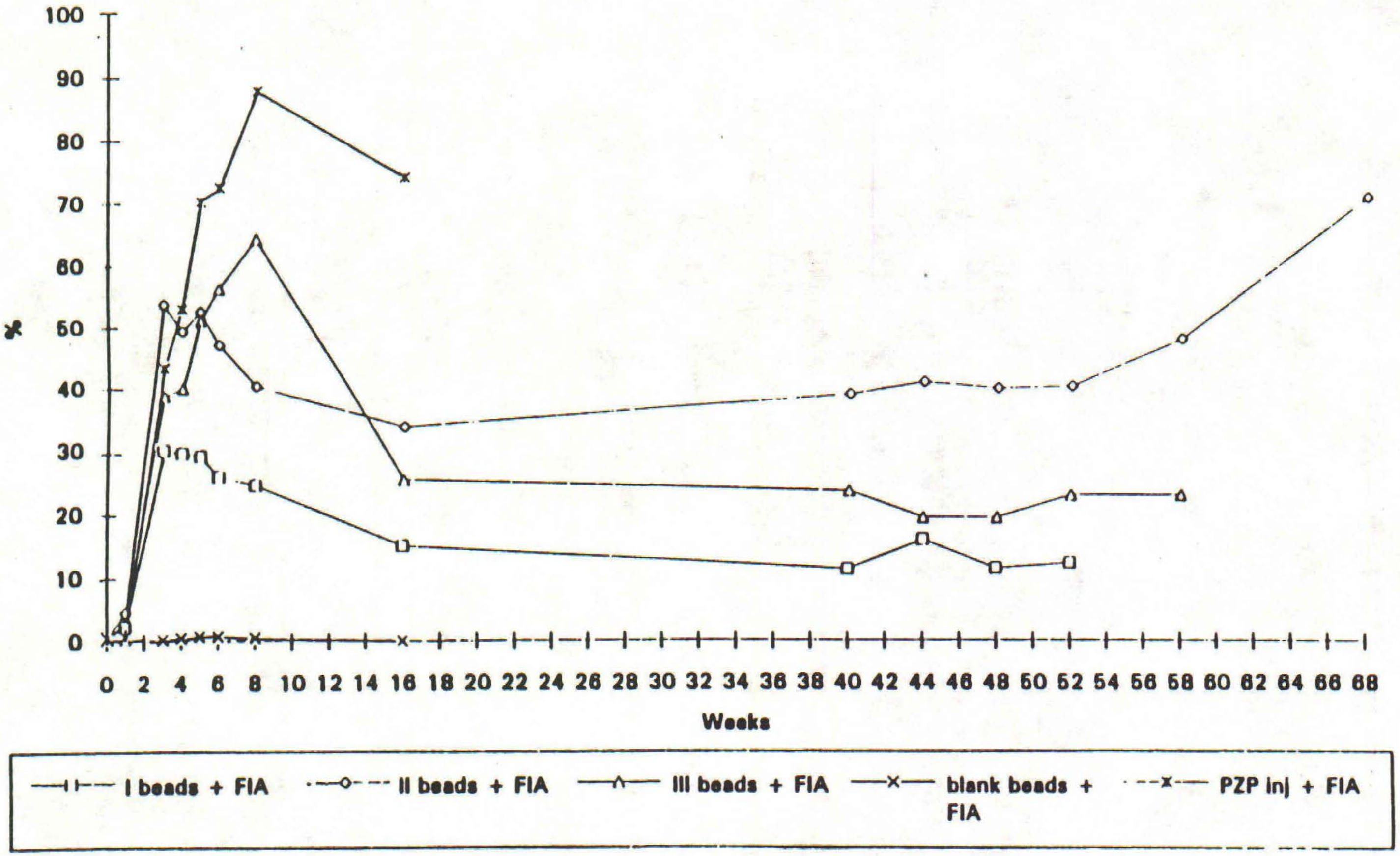


Table 3a. Standard Error Values for Data Presented in Fig. 3a

I beads + FIA	□	.48	.854	4.56	4.66	4.43	5.74	7.12	2.08	2.5	2.4
II beads + FIA	X	.289	1.19	4.09	4.03	5.39	9.12	8.75	10.14	10	19
III beads + FIA	◇	.25	.479	8.31	6.24	10.38	8.75	11.47	7.62	4.16	2.5
Blank beads + FIA	♦	.25	.25	.25	.289	.48	.48	.289	-0-	--	--
PZP Inj. + FIA	▵	.289	.48	9.37	8.53	5.06	3.48	1.70	-0-	--	--
Weeks of treatment		0	1	3	4	5	6	8	16	40	44

3b

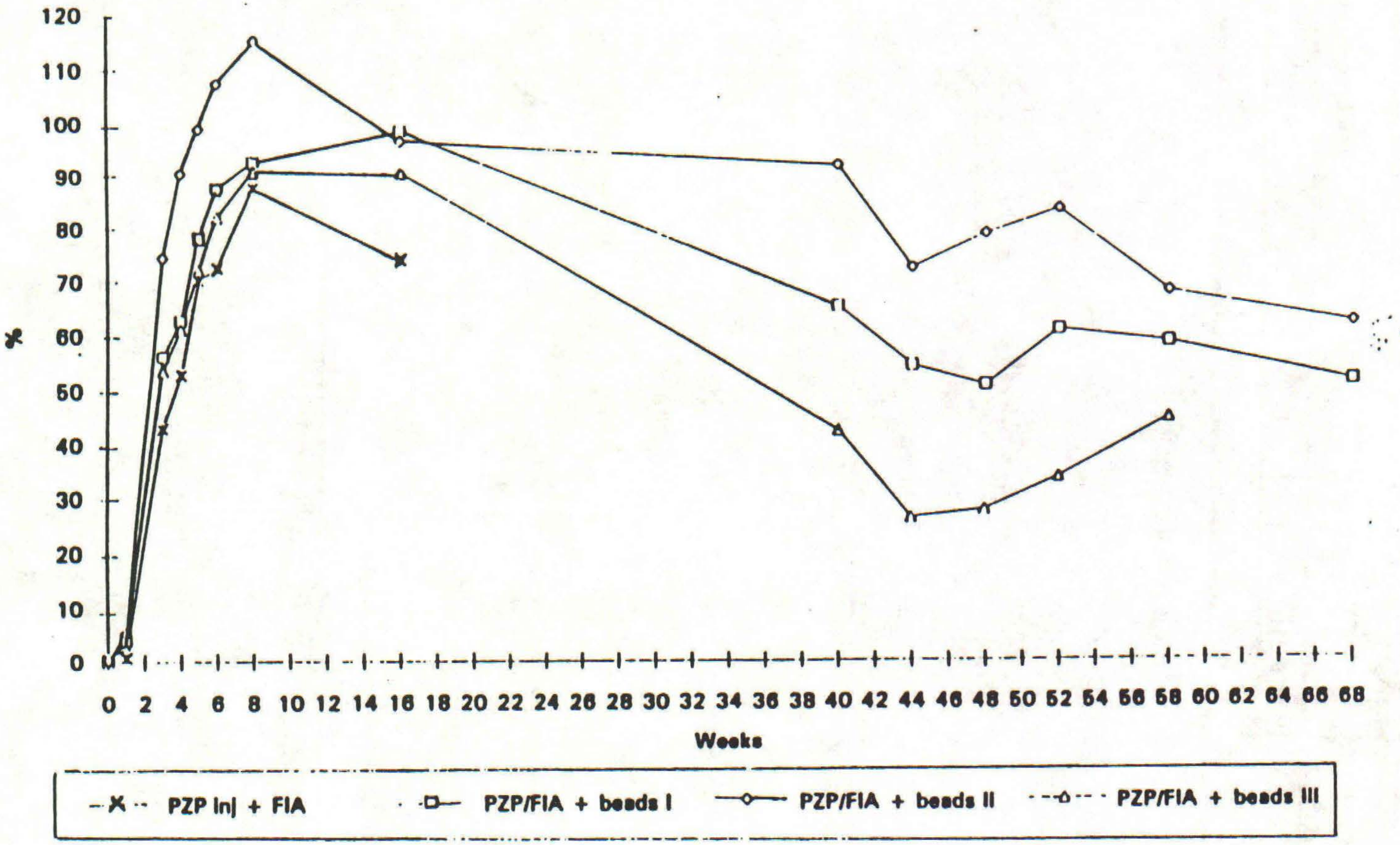


Table 3b. Standard Error Values for Data Presented in Fig. 3b

PZP inj. + FIA	ı	.289	.48	9.37	8.53	5.06	3.48	1.70	0	--	--			
PZP/FIA + beads I	□	0	1.41	3.12	3.07	3.92	4.25	8.04	13.1	15.4	7.5	8	14.5	12 8
PZP/FIA + beads II	◇	.25	.65	10.9	10.9	8.6	8.06	9.6	14.2	10	11.5	5	5 8	5
PZP/FIA + beads III	▵	0	.866	12.3	6.18	6.25	9	10.6	5.8	11.8	11.5	11	10 8	0
Weeks of treatment		0	1	3	4	5	6	8	16	40	44	48	52	58

3c

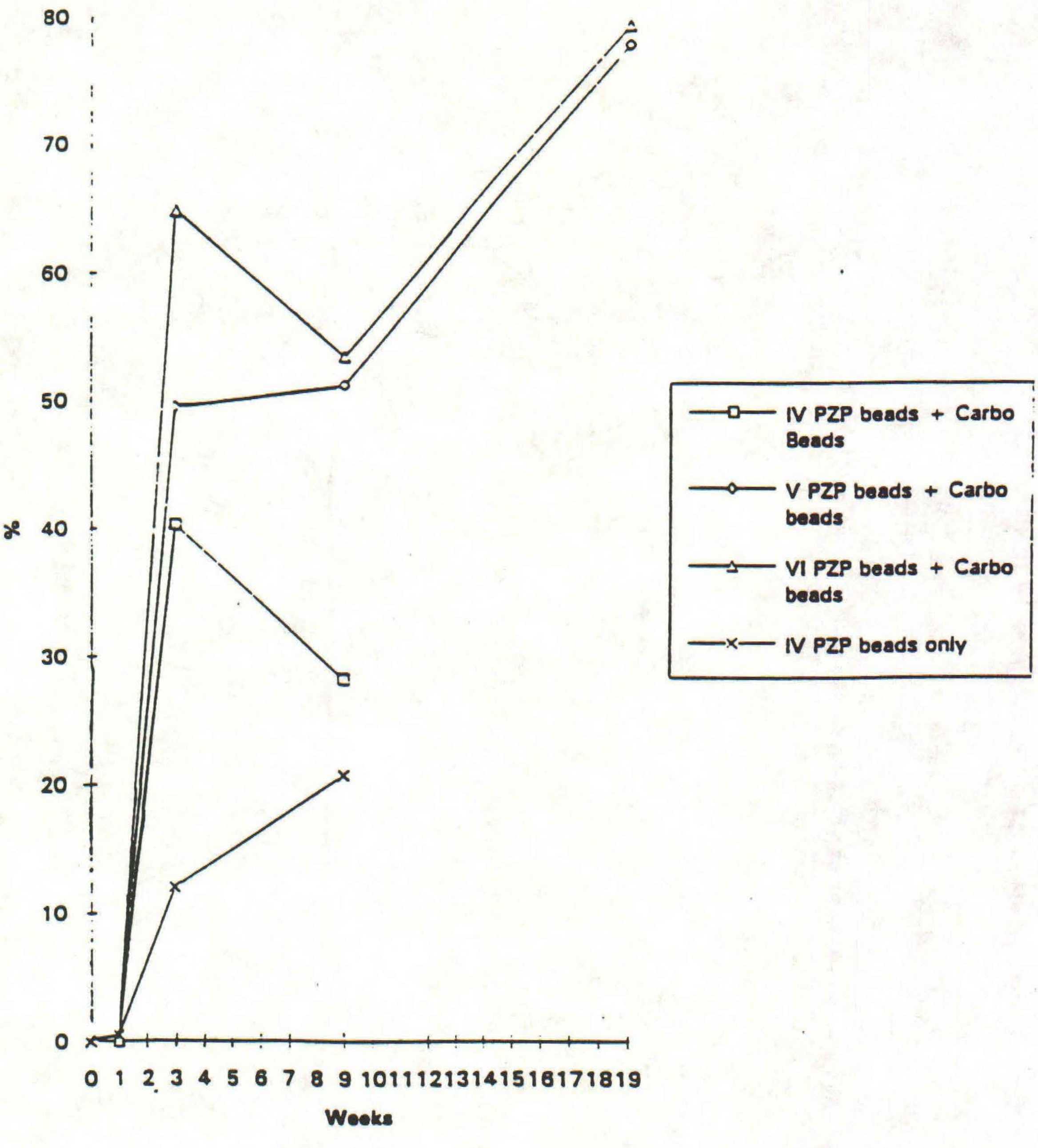


Table 3c. Standard Error Values for Data Presented in Fig. 3c

IV PZP beads + Carbo beads	□	0	0	6.79	3.94	–
V PZP beads + Carbo beads	◇	0	0	6.54	3.92	9.32
VI PZP beads + Carbo beads	▲	0	0	6.02	3.77	9.11
IV PZP beads only	X	0	0	5.12	9.46	–
Weeks of treatment		0	1	3	9	19

3d

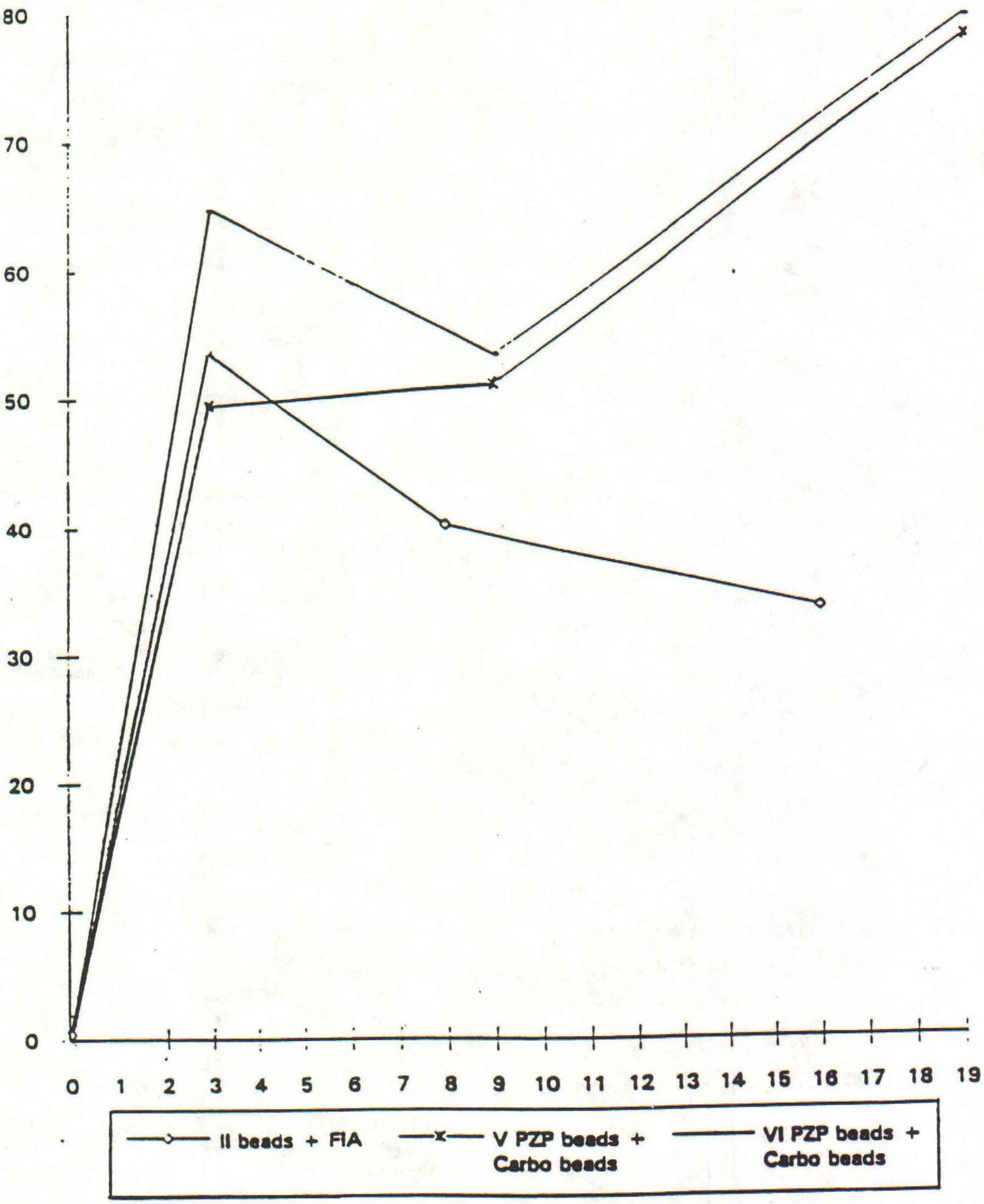


Table 3d. Standard Error Values for Data Presented in Fig. 3d

II beads + FIA	◇	4.09	8.75	–	10.14	–
V PZP beads + Carbo beads	×	6.54	–	3.92	–	9.32
VI PZP beads + Carbo beads	—	6.02	–	3.77	–	9.11
Weeks of treatment		3	8	9	16	19

3e

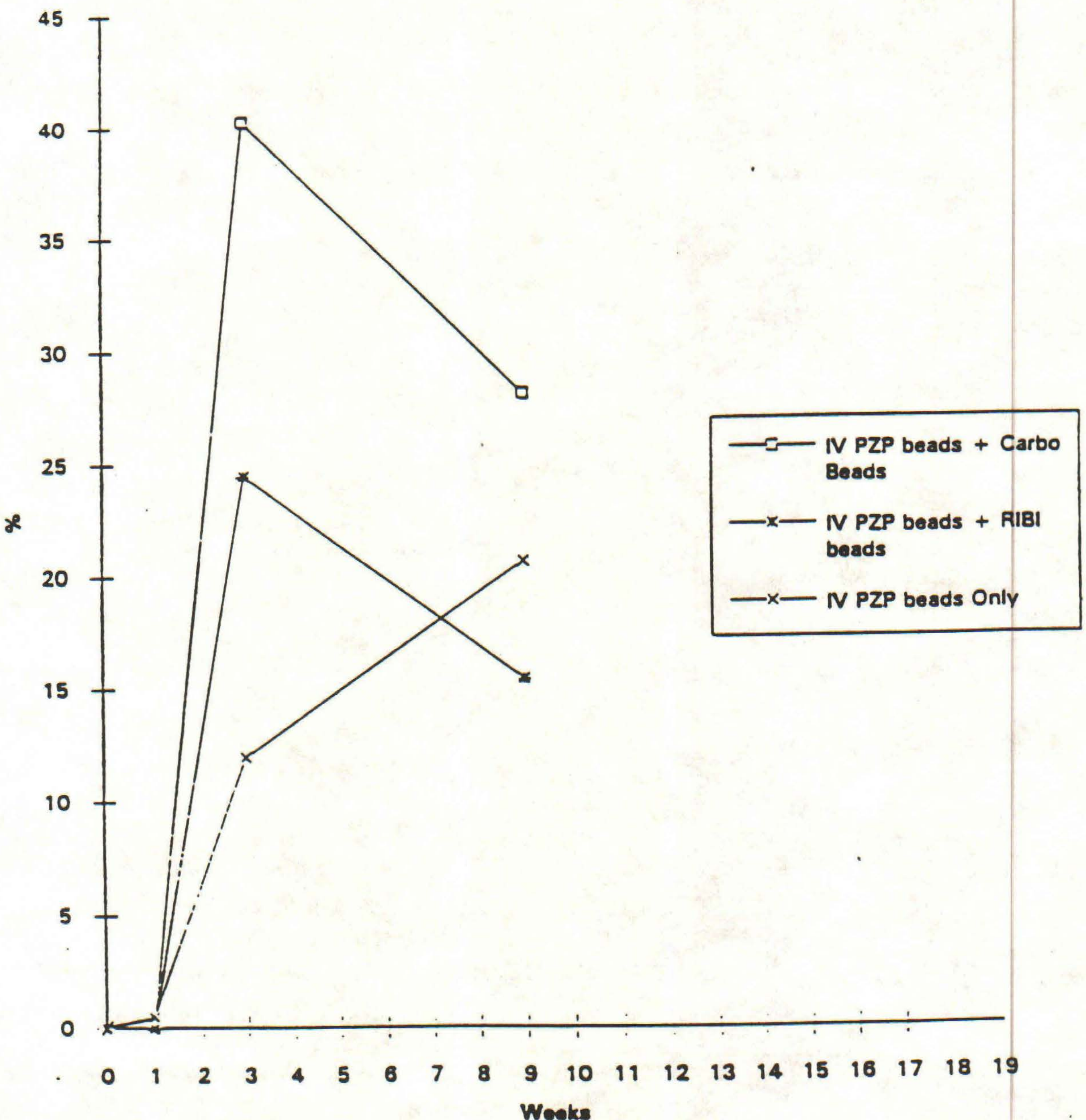


Table 3e. Standard Error Values for Data Presented in Fig. 3e

IV PZP beads + Carbo beads	□	0	0	6.79	3.94
IV PZP beads + RIBI beads	*	0	.25	4.17	3.66
IV PZP beads only	X	0	.5	5.12	9.46
Weeks of treatment		0	1	3	9

APPENDIX IVa

Effect of PZP + Adjuvant + 1-Month Microspheres Containing PZP and Adjuvant on Anti-PZP Antibody Titers in Domestic Horses

Treatment (Dec. 20, 93)
Group 1: PBS-FIA + placebo microspheres in 1 ml vehicle
Group 2: PZP-FIA + placebo microspheres in 1 ml vehicle
Group 3: PZP-FIA + PZP microsph. formulation I in 1 ml vehicle
Group 4: PZP-FIA + PZP microsph. formulation II in 1 ml veh.

ANTIBODY TITERS (% POS. REF. SERUM)
(weeks)

GROUP #	MARE #	0	2	3	4	5	6	7	8	9	10	12	14	16
1	0194	4	4	3	3	3	4	3	3	3	2	3	4	3
	1118	7	8	8	5	8	7	8	4	4	5	4	4	4
	8504	11	10	9	8	8	8	11	9	10	2	2	2	3
	means:	7	7	7	5	6	6	7	5	6	3	3	3	3
2	0122	11	16	44	47	59	91	102	101	106	113	116	104	102
	6523	2	5	46	55	84	107	123	116	116	119	135	109	115
	8546	3	14	41	67	92	96	104	95	92	101	93	110	82
	1513	7	17	43	81	99	101	112	105	101	98	115	97	104
	means:	6	16	44	63	84	99	110	104	104	108	115	105	101
3	1512	3	35	91	96	102	108	123	126	126	124	144	151	155
	9715	1	6	10	23	25	33	37	34	44	42	43	49	38
	9709	4	21	49	52	49	49	63	74	89	88	86	69	66
	0165	5	16	36	36	44	49	53	47	52	55	48	41	35
	means:	3	20	47	52	55	60	69	70	78	77	80	78	74
4	8125	1	13	26	38	35	37	43	62	59	51	35	45	42
	3572	4	46	122	125	123	124	135	128	123	121	148	144	165
	3576	6	18	31	28	29	36	86	82	82	76	80	55	57
	0527*	7	37	50	46	43	56	68	69	-	61	38	45	-
	means:	5	29	57	59	58	63	84	85		77	75	72	

neg. controls: 1-11

* Mare 0527 replaced mare 3575 who died from colic. Mare 0527 was immunized on 1/7/94. All of her serum samples were taken 3 days short of 2, 3, 4 etc. weeks. Because of the resulting lesser sample number in the last 2 columns of group 4, means were not calculated.- Means of groups 2,3,4 peak at 12 weeks. High responders, treated with PZP only, peak at 12 weeks, when treated with PZP + microspheres, increase their titers up to 14 weeks.

APPENDIX IV b

MEDISORB STUDY IN HORSES

Effect of 1-Month Microspheres Containing PZP and Adjuvant on Anti-PZP Antibody Titers in Domestic Horses

Treatment: June 16, 95
PZP microspheres (formulation IId) mixed with Carbopol microspheres

ANTIBODY TITERS (% POSITIVE CONTROL SERUM)

Mare #	weeks 0	1	2	3	4	5	6	7	8	9	10	11	12
2510	9	187	187	175	175	175	174	183	175	172	167	170	168
3538	0	0	40	57	45	56	53	47	64	61	54	54	50
8066	0	0	35	38	33	41	47	34	41	45	47	43	43
9632	4	5	13	13	11	16	20	23	22	20	23	30	23
means of 3:	1	2	29	36	30	38	40	35	42	42	41	42	39

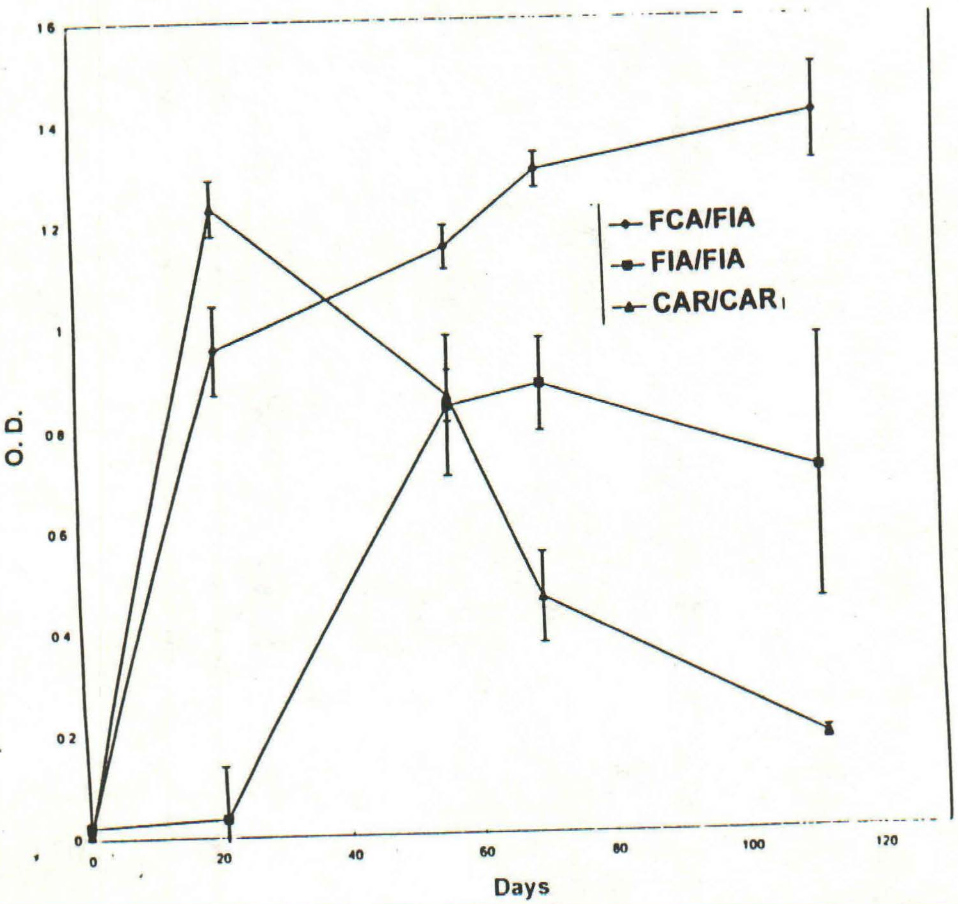
negative control range: 0 - 4

Comment: The "mystery" of mare 2510 has been solved. She had been used in the microsphere - polymer study (Iowa preps) of 1992. We apologize for overlooking that. Her titers were therefore not included in the means.
The mean titers of the remaining 3 mares appear to indicate a continuous small release over the test period, resulting in immune response insufficient for contraception.

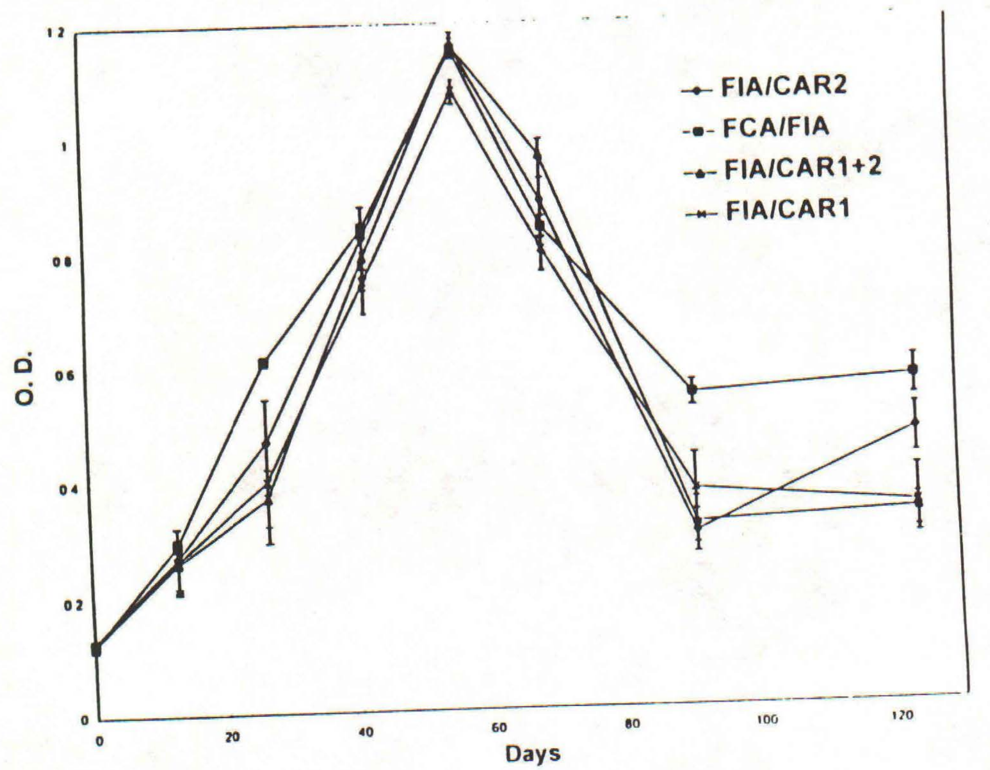
LEGEND FOR FIGURES 1 & 2. O.D. (Y-axis) is plasma optical density, which increases as the antibody concentration increases. Days (X-axis) are days after initial inoculation. A second inoculation of vaccine was given on day 21. Electrophoretic western blots were performed on selected plasma samples, and they demonstrated that the plasma O.D. was well correlated with the anti-PZP antibody protein concentration in the respective western blots. The vertical bars in the graph represent standard error of the mean. FCA = Freund's Complete Adjuvant, FIA = Freund's Incomplete Adjuvant, CAR = Carbopol®. FIA/CAR1+2 = both inoculations contained FIA/CAR. FIA/CAR1 = FIA/CAR in inoculation 1 and FIA only in inoculation 2. FIA/CAR2 = FIA only in inoculation 1 and FIA/CAR in inoculation 2.

APPENDIX V

Effect of 3 Different Adjuvant Protocols on Anti-PZP Antibody Response in Rabbits Given PZP Vaccine



Effect of 4 Different Adjuvant Protocols on Anti-PZP Antibody Response in Rabbits Given PZP Vaccine



RECEIVED
BUR. OF LAND MANAGEMENT
NEVADA STATE OFFICE
98 JAN 26 AM 8:00

MEDICAL COLLEGE OF OHIO

MEMORANDUM

TO: Richard Fayrer-Hosken, U Georgia
Jay Kirkpatrick, ZooMontana
Irwin Liu, UC, Davis
Tom Pogacnik, BLM, Reno
Allen Rutberg, HSUS
Janet Sumner, Research and Grants Administration, MCO
Terry Woosley, BLM, Reno

FROM: John W. Turner

RE: 1997 Progress Report, BLM

DATE: January 13, 1998

Attached is the 1997 Progress Report for BLM Project. This is for your file and information. If you have questions, call me at (419) 383-4146.

JWT:mm

attachment

1997 Progress Report for
BLM Assistance Agreement #1422F915A70001
(Nevada Wild Horse Fertility Control Project)

A final report for the BLM Cooperative Agreement #1422F950A20002, covering the portion of this project from December 1992 through July 1996 was submitted to BLM in August 1996. That report provides detailed background information for the present progress report. In short, the former report describes the development and lab/field testing of a single-injection contraceptive vaccine which endures for one year. The present report describes continued lab/field studies directed at refinement of the 1-year, 1-injection vaccine and development of a 2-year, 1-injection vaccine.

The studies in the present report consist of: 1) completion of a field study begun in January 1996 at Nellis Air Force Base (Nevada Wild Horse Range, NWHR) and 2) laboratory/captive animal studies of different vaccine antigen doses and vaccine adjuvants. Because of funding delays, the project had to operate without additional funding from October 1996 through July 1997. Thus, all studies except the Nellis field trial were severely hampered. Nonetheless, some progress was made in the lab work, and the Nellis field trial was successfully concluded in October 1997. Results are presented below.

NELLIS (NWHR) FIELD TRIAL

In January 1996 a field trial of the contraceptive efficacy of a version of a controlled-release porcine zona pellucida (PZP) vaccine was begun. Since the adjuvant Carbopol 934 (CAR) appeared to enhance the antibody titer response to Freund's Incomplete Adjuvant (FIA) in our previous studies, we initiated a test to determine whether CAR would enhance the effectiveness of Freund's Complete Adjuvant (FCA), which is more potent than FIA. We also wanted to test a new formulation of controlled-release microspheres, which would give the mares a second exposure to both PZP and adjuvant without a second injection. Three treatment groups were included in the field study:

	n	Injection 1	Injection 2
Group 1	99	standard PZP-FCA	PZP-FIA
Group 2	99	PZP-FCA/CAR	PZP-FIA
Group 3	69	PZP-FCA plus PZP-CAR microspheres	none

The study area was the Nevada Wild Horse Range (NWHR) in south central Nevada, which is inhabited by approximately 2000 horses with a harem band social structure. The peak breeding period is May and June, and the peak foaling period is April and May. The physical condition of the mares in this study was subjectively estimated to range from poor to very good at the time of capture for treatment.

PZP was prepared from porcine ovaries and the basic vaccine consisted of 0.5 cc. standard phosphate buffer solution containing 65 µg PZP, which was emulsified with 0.5 cc. FCA (Sigma; St. Louis, MO). FIA was used for the second injection. We prepared the emulsion within 24 hours of injection, using 2, 10-cc. glass syringes joined with a plastic connector. After 100 plunger strokes the emulsion was loaded into a 3 cc. plastic syringe for injection via a 18 ga., 3.7 cm needle. The needle and injection site were prewashed with 70% ethanol.

In an attempt to mimic the 2-injection condition with a single injection, one group of mares was given an injection as described above, with an additional component of controlled-release microspheres containing 80 µg PZP and 10 mg CAR adjuvant, given as a separate injection using a 14-gauge, 3.7 cm needle. The microspheres were 10-100 µ in diameter and composed of bioerodable lactide and glycolide polymers. They were prepared by D. Flanagan at the University of Iowa using a coacervation method. Release rates were projected to be continuous over approximately 4 weeks, with greater release in weeks 1 and 4.

Horses were gathered by helicopter for hands-on injection. This access method was chosen because it was used for a scheduled round-up in the NWHR for the BLM adoption program.

Between January 3 and 9, 1996, approximately 800 horses were gathered into portable corrals by helicopter. Stallions and mares were separated and were singly moved through a chute, where they were aged, assessed for physical condition and lactation (yes or no) and given a 1 cc. prophylactic equine flu injection intramuscularly. Healthy mares (n=267) between the ages of 10 and 28 years were permanently marked with consecutive numbers by freeze branding. The brands were located on the upper left hip, were 10 cm. in height and were readable from a helicopter and through a spotting scope at >500 m distance on the ground. A description and data sheet was filled out for each marked mare prior to treatment. It is important to note that in many feral horse herds, reproduction is low among old mares. However, in this study mares >20 years old were included for contraception because data for Nellis horses from prior years had shown incidence of lactation to be similar in mares older or younger than 20, i.e., many old mares were producing foals in the Nellis Herd.

One ml. of freshly prepared emulsion of buffered PZP + adjuvant was hand-injected intramuscularly into the left gluteus muscle. Mares in the microsphere group were also injected at the same location with 2.5 ml. of a 1.0% solution of CAR-in-water containing suspended PZP/CAR microspheres. After one injection, all mares were trucked to a local holding facility and maintained in corrals on grass-hay and water ad lib until release. Mares in the single injection group were released after 4 days into the range area from which they were gathered. Prior to release the mares and their foals were separated, and the foals were retained for eventual adoption. Mares in the 2-injection groups were maintained under observation for 13-17 days on a grass-hay and water (ad lib) diet until the second injection was given. The mares were then returned to their home range area and released. Within 24 hrs of the release the study area was surveyed by helicopter to determine the well-being and dispersal of the mares.

Treatment success was determined after finding freeze-branded mares on the range and 1) performing fecal analysis for pregnancy in samples collected in October 1996 and 2) counting foals in May and October of 1996 and 1997. Foal production (Table 1) in 1996 (i.e., 1995 pregnancies, before treatment) was 165 foals/250 mares (66.0%). The overall foal production rate in 1997 (representing pregnancy rate during active treatment) was 26 foals/222 mares (11.6%). Regarding comparison of treatments (Table 2), the 1997 foal production was 10/78 (12.8%) in Group 1, 5/47 (10.6%) in Group 2, and 11/97 (11.3%) in Group 3, the one-injection group. The lack of differences in fertility rate among the groups indicates that: 1) the controlled-release component in the 1-injection group in this study provided vaccine exposure equivalent to a second injection of vaccine and 2) in the initial exposure CAR did not enhance the effect of FCA. The greater effectiveness of the standard 2-injection vaccine in the first field study in which it was used (4.5% fertility among 132 mares treated in December 1992 in Antelope/Antelope Valley HMA's) as compared to the present study (NWHR) is unexplained. However, the actual difference in numbers of fertile mares in these two groups is small, and our experience with a variety of species has revealed that a few animals in every population exhibit a poor vaccine response (low antibody titers). It may be that some horse populations show a slightly higher frequency of this characteristic than other horse populations.

Table 1. Effect of PZP immunocontraception on fertility of free-roaming feral mares in Nevada

YEAR	Treatment	No. of mares located[a]	No. of mares reproductively successful[b]	% of mares reproductively successful[b,c]
1997	PZP	222	26	11.6
1996	NONE	250	165	66.0

[a] Mares that were located for sample collection and/or observation between May 1996 and Oct 1997. Mares sampled in 1996 and 1997 were not necessarily the same individuals.

[b] A positive fecal pregnancy test or presence of a foal were criteria for reproductive success

[c] Historical data indicate similar reproductive rate across years regardless of water/food/climate conditions, suggesting validity of year-to-year comparison for treatment effect.

Table 2. Effect of 3 different versions of PZP contraceptive vaccine on fertility of free-roaming feral mares in Nevada

YEAR	Treatment		No. of mares located[a]	No. of mares reproductively successful[b]	% of mares reproductively successful[b,c]
	Inj. 1	Inj. 2			
1997	PZP/FCA	PZP/FIA	78	10	12.8
	PZP/FCA + CAR	PZP/FIA	47	5	10.6
	PZP/FCA + microspheres	NONE	97	11	11.3

[a] Mares that were located for sample collection and/or observation between May 1996 and Oct 1997

[b] A positive fecal pregnancy test or presence of a foal were criteria for reproductive success

[c] No significant differences among groups for $\underline{P} < 0.05$, Tukey-type multiple comparison test for proportions

DOMESTIC MARE STUDY

In July 1997 a study was begun with 22 domestic mares maintained at the University of California, Davis facility to test the effect of increased priming dose of PZP and the effectiveness of minirods as a controlled-release formulation to replace a second injection of vaccine. The effectiveness of treatment was determined by measuring the serum anti-PZP antibody titer response in these mares in blood samples taken weekly. This study is not yet complete, but treatment groups are presented below.

DOMESTIC MARES STUDY

Treatment		No. of Mares
Priming	Secondary[1]	
65 μg PZP + FCA	65 μg PZP + FIA	2
400 μg PZP + FCA	CAR only	5
400 μg PZP + FCA	200 μg PZP + CAR	5
65 μg PZP + FCA	200 μg PZP + CAR	5
400 μg PZP + FCA	200 μg PZP + QS-21	5

FCA = Freund's Complete Adjuvant, 1 dose = 0.5 ml
FIA = Freund's Incomplete Adjuvant, 1 dose = 0.5 ml
CAR = Carbopol 934 (B.F. Goodrich Corp.), 1 dose = 10 mg
QS-21 = a purified saponin adjuvant (Aquila Pharmaceuticals, Inc.), 1 dose = 200 μg
[1] Secondary dose delivered via controlled-release lactide/glycolide minirods (pellets); 1.5 mm diam. × 3 mm length. One dose of PZP and QS-21 required 1 minirod each; CAR required 6 minirods per dose.

In this study the priming dose was administered by hand injection into the muscle of the upper hip. The minirods were then inserted into the same site using a trocar (a long hollow needle with a plunger to push contents out). The field use of the minirods involves loading the priming dose (liquid) into the chamber of a 1 cc. self-injecting dart and loading the pellets in line in the needle barrel of the dart. The base- and point- ends of the needle barrel are then sealed with sterile petroleum jelly to prevent minirods from slipping out, and the dart is loaded into the gun and fired as in any standard field use.

The study will be completed in April 1998, and the results will be submitted in the next progress report. Preliminary data from the first 3 months of sampling suggest that: 1) the higher PZP dose (400 μg priming and 200 μg secondary, as compared to standard 65 μg/65 μg doses) yields greater serum antibody titers than the standard dose. Whether this difference is significant has not yet been determined. Also, it appears that QS-21 is markedly better than CAR as a controlled-release adjuvant. Again, conclusions must await completion of the study.

RABBIT LABORATORY STUDY

In June 1997 a laboratory study was begun to compare the relative effectiveness of PZP antigen vs. pig sperm antigen vs. rabbit sperm antigen. The reason for this endeavor is that a number of researchers have been studying sperm antigen vaccines as contraceptives, and sperm antigen vaccine has shown effectiveness in preventing fertilization of immunized rabbits. We felt it was important to examine this possibility for the horse. This could be especially useful for avoiding possible permanent infertility from continuous use of the same vaccine across many years. By having an alternate vaccine type, that issue would not arise. Also, sperm antigen vaccines have the potential for use in both males and females, which may offer various management advantages.

Specifically in this study we wanted to determine:

1. The serum antibody response and contraceptive effectiveness of a vaccine derived from porcine and rabbit testis.
2. The effectiveness of the testicular vaccine when compared to an established contraceptive vaccine derived from PZP, in terms of (a) serum antibody response (both strength and duration) and (b) contraceptive effectiveness.

Twenty virgin New Zealand white rabbits were divided into 4 groups depending on the vaccination administered: 1) PZP antigen; 2) pig sperm antigen; 3) rabbit sperm antigen and 4) adjuvant only, as a control. Freund's Complete Adjuvant (FCA) was used for the first inoculation and Freund's Incomplete Adjuvant (FIA) was used for the booster. Antibody titers were quantitatively determined using the Enzyme Linked Immunosorbent Assay (ELISA). A preimmunization blood sample was taken from the rabbits to determine the presence of any PZP/testis antigens and was subsequently used as a negative reference serum in the ELISA. The rabbits were initially infected with 0.3 cc. antigen plus 0.3 cc. FCA with the booster of 0.3 cc. antigen + 0.3 cc. FIA. The injections were administered intramuscularly in the hip to simulate the injection site used in the field.

On day 114, the rabbits were artificially inseminated with 10^7 sperm in a final volume of 0.200 ml per dose consisting of rabbit semen appropriately diluted with Ham's F-10 balanced solution. Each rabbit was injected with 100 I.U. of human chorionic gonadotropin (hCG) at the time of insemination. The rabbits were palpated 25 days later in an attempt to approximate fecundity rates. The results of the fertility trial was summarized in the following table. It should be noted that some rabbits were terminated in an effort to correlate number of ovarian corpora lutea with number of feral implants.

Treatment	Rabbit No.	Viable Offspring	Dead Offspring	Corpora Lutea
PZP	19	0	0	-
PZP	16	0	0	-
PZP	20	0	0	-
PZP	17	0	0	-
PZP	21	0	0	-
PIG	18	0	0	-
PIG	14	5	2	-
PIG	10	9	4	-
PIG	13	1	4	16
PIG	11	5	4	-
RABBIT	15	0	1	-
RABBIT	12	9	0	9
RABBIT	4	0	0	-
RABBIT	5	1	0	10
RABBIT	6	7	2	-
CONTROL	7	10	0	10
CONTROL	8	5	0	13
CONTROL	9	8	0	15
CONTROL	22	9	1	10
CONTROL	23	2	1	3

Although antibody titer analysis and statistical analysis of titers and fecundity have not yet been completed, it is evident that the contraceptive vaccine based on PZP provided dramatic reduction of fecundity. Neither of the testis antigens was close to PZP effectiveness and the pig sperm antigen was little better than no treatment.

RESEARCH TEAM MEETING

The research team gathered for a 2-day assessment and strategy meeting on November 22 and 23, 1997. Emergent from that meeting were several planned studies, which include modifications of studies presented in the funded proposal and several additional studies based on data generated since submission of that funded proposal. The content of the planned studies is summarized in the following sections.

PLANNED CONTROLLED-RELEASE LABORATORY STUDIES

The vaccine literature is extremely difficult to interpret because 1) one antigen may behave differently from another in similar circumstances, 2) antigen and adjuvant effects may vary across species and 3) reports vary on timing and dose of exposures of the recipient to vaccine after the priming dose. However, based on the information currently available and on our own experience, we have decided to pursue a 2-year vaccine with the following characteristics:

1. PZP ligated to an immuno-enhancer such as tetanus toxoid or cholera toxoid. This should maximize the antigenicity of the relatively weak PZP antigen.
2. Controlled release components which co-release antigen and adjuvant, with each in separate microspheres or minirods.
3. Controlled-release timing such that antigen/adjuvant release occurs discreetly 2-3 times after the priming dose and with the final release occurring 6-10 months after injection.
4. Eventual replacement of Freund's Adjuvant with a low toxicity adjuvant in priming and subsequent exposures to vaccine.

1. In Vitro Studies

Since September 1997 we have been developing the capability for monitoring the release of PZP and various adjuvants from controlled-release microspheres and minirods. *In vitro* studies provide an inexpensive reference base for release patterns of various controlled-release formulations. They also offer an alternative to use of live animals in the early stages of assessing controlled-release formulations. Eventually the formulations must be tested in live animals, but *in vitro* tests allow us to generate approximates in the spectrum of possible outcomes, saving both time and money. This is especially important as we develop formulations which release at multiple times after injection and with some long (2-10 months) delays until release occurs.

The first step in the *in vitro* path is development, of means of detecting and quantifying the PZP and adjuvant released. Toward that end, we have modified a highly sensitive protein assay to measure PZP. This assay (PSA) has now been validated and accurately measures PZP in the nanogram range, adequate for detection of the small amounts of PZP released daily from microspheres or minirods. The capability for measurement of adjuvant release is now in development. The results of *in vitro* studies will be provided in the next progress report.

2. ***In Vivo Studies***

These studies, which have been designed but not implemented, are the next step beyond the *in vitro* studies. A statement about them is included here for purposes of continuity. Upon completion of the *in vitro* studies release of PZP/adjuvant from microspheres/minirods will be tested by inserting microspheres/minirods into fiber packets which do not restrict release. The packets will then be implanted into the body cavity of mice. At various times (weeks, months) after placement the packets will be removed and the contents will be assayed to determined amount of PZP/adjuvant remaining in the packeted microspheres/minirods. The *in vitro* database will be used as a guide for projected windows of materials release, and collection of *in vivo* packets will focus around these windows. The best controlled-release preparations evidenced by these studies will then be used to test antibody titer responses in rabbits or horses.

FIELD STUDIES PLANNED FOR 1998-1999

The results of the Nellis field trial indicated that the controlled-release prep used there functioned effectively as a 1-year, 1-injection PZP vaccine. Therefore, the performance of the field trials to assess the population-limiting effect of this vaccine was warranted. Toward that end, 3 sites which had been previously targeted for such studies were designated for treatments in January and February 1998. The planned studies at each site are presented below.

1. *Testing of 2 Different Preparations of Controlled-Release PZP Contraceptive Vaccine*

In January 1998 40-50 mares aged > 9 years will be turned back out on to the range after the KAMA-ANTELOPE area gather. These mares will first be inoculated with PZP contraceptive vaccine to test effectiveness of two different controlled-release formulations of the vaccine. Each formulation will differ in its release pattern. One of the two groups of mares will be marked by squaring the tail hair or roaching the mane. If any untreated mares remain on the range, each treatment group will be marked. The two types of vaccine and their respective delivery methods are presented below:

FORMULATION I. One half of the mares will receive this formulation. It will consist of a liquid dose of the vaccine in which is contained a second and third booster dose sequestered in biodegradable microspheres. Each microsphere is smaller than a grain of salt. The microspheres are suspended in the liquid and are designed to release the PZP hidden in them at several points in time during the first 3 months after injection. This formulation will be delivered as an intramuscular injection by hand-held syringe into mares in the stock chute.

FORMULATION II. One half of the mares will receive this formulation. It will be delivered remotely to the rump from 10-15 ft. away, while the mare is in the stock chute. A CO_2-powered gun will be used. The dart will contain a liquid portion of PZP vaccine in its chamber, and a controlled-release portion in the form of mini-rods (pellets) in the barrel of the dart needle. Upon dart impact the liquid in the chamber will be propelled into the muscle along with the mini-rods. This delivery method has been previously shown to work. The mini-rods will release the PZP hidden in them at several points in time during the first 3 months after injection.

2. *Preliminary Population - Impact Study*

The purpose of this study is to determine maximum contraceptive capability in a given population of feral horses. BLM will gather <u>all</u> horses in a given small/moderate HMA or allotment and we will treat with PZP vaccine all mares scheduled to be turned out (not for adoption). We will determine by foal counts the vaccine effectiveness and will determine by total horse counts the impact on population growth over 2 years. There will be two treated herds (KAMA-ANTELOPE and FISHCREEK) and one control herd (DIAMOND).

This study is preliminary in that one treatment area (KAMA-ANTELOPE) involves a small number of mares (<50), and the other treatment area involves mares of one allotment (FISHCREEK) that mingle with horses of three other allotments. The FISHCREEK mares will be freeze marked for identification, but the site is less than ideal for a definitive total-herd treatment. Such sites were simply not available for study in the present round of gathers. By using the above sites for a preliminary study, we will be optimally prepared for a definitive population-impact study next year.

3. *Computer Population Models and PZP Contraception*

Dr. Steve Jenkins and colleagues at the University of Nevada, Reno have developed computer models for managing feral horse populations. In addition some wild horse managers are using this or a similar model for their specific HMA. Thus, computer modelling is presently in use in planning gathers. However, computer models are only as good as the data upon which they are based. The purpose of our study is twofold: 1) to provide a current contraception data base for use in the computer models, and 2) to test the use of contraception for achieving a specific computer-generated population recruitment goal. Mares in the FISHCREEK and CLAN ALPINE areas will be given PZP vaccine, and the percentage of mares treated in each area will differ according to the prescribed recruitment goal. Foal numbers and base population numbers will be determined in these areas for the next two years. The actual field effectiveness data generated using PZP contraception will permit the desired testing and will provide valuable content for future computer models.

In addition to testing further refinement of the PZP vaccine for applied use, the outcome of these field studies will be of great value toward developing a comprehensive management program for feral horses. These studies will begin the effort to determine whether a BLM horse program which incorporates immunocontraception will enable more humane and cost-effective management.

Program Chart for Planned January - February 1998
Feral Horse Gathers Involving PZP Contraception[1]

CONDITION	FISHCREEK	KAMA- ANTELOPE	CLAN ALPINE
Approximate # of horses to be treated	130-160	40-50	180-220
Approximate gather starting date	January 14-30, 1998	February 1-5, 1998	February 7-28, 1998
Research team arrival date	January 15, 1998 (day after start date)	February 2, 1998 (day after start date)	February 8, 1998 (day after start date)
Untreated Comparator Herd[2]	DIAMOND HMA	DIAMOND HMA	DIAMOND HMA
Type of study	a. Contraception data for computer models[3] b. Preliminary population-impact study[4]	a. Compare two types of controlled-release vaccine b. Preliminary population-impact study[4]	Contraception data for computer models[3]

1. All treatments will be 1-injection, 1-year type.
2. No PZP treatment, but population data will be collected.
3. Percentage of mares to be treated will differ between Fishcreek and Clan Alpine.
4. Data from these preliminary studies will be used to setup definitive population-impact study to be implemented winter 1998-1999.

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16 January 1997

Tom Pogacnik
National Wild Horse and Burro Program
850 Harvard Way
Reno, NV 89502

Dear Tom,

Mike Ashley and I would like to submit this proposal for continuation of our work on wild horses at Garfield Flat and the Granite Range. The proposal covers anticipated expenses for field and laboratory work for 2 ½ years, March 1997 through September 1999. Our total request is for $185,834.

As you know, long-term data on reproduction, mortality, and behavior are critical for managing a variety of species, including native wildlife as well as wild horses. We have established the foundation for two such long-term data sets for wild horses, for populations at Garfield Flat and the Granite Range. As described in the proposal, these data will become increasingly valuable as the studies continue. There are three main reasons for this: (1) Because mortality rates of horses are generally very low, accurate estimation of these rates requires large sample sizes, which can best be obtained by keeping track of known individuals for long periods of time. (2) Because year-to-year variation in survival and reproduction due to stochastic variation in environmental factors is important in population dynamics of wild horses, populations must be studied for fairly long periods of time to assess the full range of such variation. (3) Little is known about density-dependence of birth and death rates of wild horses, and studying populations at a variety of densities is necessary for gaining this important information.

In addition to continuing to collect fundamental demographic data for wild horses at Garfield Flat and the Granite Range, we will complete the genetic analyses of these populations and gather additional data on social behavior. We are particularly interested in how the January 1997 removal of > 50% of the horses at Garfield Flat may affect the behavior of these horses during the spring 1997 breeding season. I will also translate the computer program for modeling population dynamics of wild horses from DOS to UNIX format, and eventually incorporate effects of density dependence into the program in order to make it more realistic. Although not explicitly included in the budget, Mike and I will be pleased to do training sessions on the population model and/or workshops on general aspects of the project for BLM employees, as well as consult with individuals about any aspects of horse demography or population modeling. We also expect to submit several papers for publication based on our work on wild horses within the next two years.

Mike hopes to complete field and lab work for his Ph.D. this year, and write his dissertation and graduate in 1998. I plan to recruit a new graduate student at the M.S. level to work with Mike this summer and take over the field work next year. He or she may develop particular questions to address in a thesis project that aren't included in this proposal, but in any case will continue to monitor the populations and add to the long-term data set.

I hope this proposal for maintaining and extending the long-term data that we have begun to accumulate on wild horses at Garfield Flat and the Granite Range will meet your needs. This project seems to provide a unique opportunity for showing how good science can contribute to more effective management. If you have any questions or suggestions about the proposed work, please contact Mike or me. Thanks very much for your consideration.

Sincerely,

Stephen H. Jenkins
Professor of Biology

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fax: 702-784-1302
e-mail: jenkins@med.unr.edu

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Mary B. Husemoller, *Director*
Sponsored Projects Administration

January 16, 1997

Bureau of Land Management
U.S. Dept. Of the Interior
850 Harvard Way-PO Box 12000
Reno, NV 89520-0006

Re: **Wild Horses in the Desert: A Long Term Study of Life History**

To Whom It May Concern:

Enclosed please find the required number of copies for the above referenced proposal under the direction of Stephen H. Jenkins and Michael C. Ashley. For the period of March 1, 1997 through September 30, 1999, monies in the amount of $185,834.00 are requested.

For questions of a technical or program nature, please contact Dr. Jenkins or Mr. Ashley. For questions of a business nature, please contact this office and refer to **OSPA #1970358 {Jenkins, S.}**

Sincerely,

Mary B. Husemoller

Mary B. Husemoller
Director, Sponsored Projects Administration

MBH:lg
Encl.

WILD HORSES IN THE DESERT: A LONG-TERM STUDY OF LIFE HISTORY, BEHAVIOR, AND GENETICS

A Proposal Submitted to the

Bureau of Land Management

by

Stephen H. Jenkins & Michael C. Ashley

Department of Biology

University of Nevada

Reno, NV 89557

(702) 784-6078

15 January 1997

Time Period of Study: 1 March 1997 - 30 September 1999

Funds Requested: $185,834

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WILD HORSES IN THE DESERT: A LONG TERM STUDY OF LIFE HISTORY, BEHAVIOR, AND GENETICS

INTRODUCTION

Effective management of wild horse populations requires a firm understanding of their demography, behavior, and genetics. Over the past four years, we have been intensively studying the wild horse population at Garfield Flat, Nevada and monitoring a second population in the Granite Range, also in Nevada. We have also produced a computer model of population dynamics that has been distributed to field offices of the Bureau of Land Management responsible for wild horses. This model enables users to project population growth under various management scenarios involving removal and fertility control.

This computer model is potentially a very useful tool for management of wild horses, but requires data on survival rates by age and sex, foaling rates of mares by age, sex ratio at birth, and current age distribution of a population. In addition, a realistic model requires estimates of year-to-year variation in survival rates, foaling rates, and sex ratio at birth due both to stochastic environmental factors such as weather and effects of changing population density. The most difficult set of demographic data to obtain is age-specific survival rates because doing so requires long-term monitoring of known individuals. There are currently only two sources of survival data for wild horses in the western U.S., Garrott and Taylor's (1990) 11-year study of horses in the Pryor Mountains of Montana, and Berger's (1986) 6-year study of horses in the Granite Range of Nevada. These data may not be suitable for widespread use in modeling other populations, for several reasons: (1) Many horse populations occur in lower elevation, more arid environments than the Pryor Mountains or Granite Basin, so may have very different demographic characteristics. (2) Year-to-year variability in survival and reproduction in the Pryor Mountains was strongly influenced by the occurrence of one extremely harsh winter in the 11 years of Garrott and Taylor's study. (3) Estimates of survival and fecundity based on Berger's data are somewhat questionable because of the relatively small sample size and limited duration of his study. (4) Although population densities changed in the Pryor Mountains and Granite Basin during these two studies, there are insufficient data to quantify density-dependence of birth and death rates.

Based on approximately 150 horses that were freeze branded at Garfield Flat in 1993, we have begun to accumulate enough data to estimate age-specific survival and fecundity for this population. Only 12 marked animals have died so far, so survival estimates are still somewhat imprecise. In addition, the length of the study to date is insufficient to estimate the potential range of year-to-year variation in demographic parameters. We have less useful data for the Granite Range at this time because the initial attempt at freeze branding individuals in this population was unsuccessful. However, animals were gathered and branded again in November 1995, and so we will be able to obtain these data for the Granite Range as well in the coming years. Comparison of these two new sets of data with existing data for the Pryor Mountains and Granite Basin will indicate the degree of variation in horse demography and will provide a more realistic range of choices of baseline data for users of the wild horse population model.

Wild horses are gregarious animals that exhibit a variety of social behaviors both within and between populations (Miller 1979, Berger 1986, Rubenstein 1986, Stevens 1990). Horses form associations ranging from year-round harem bands (Berger 1986) to seasonal harem bands (Rubenstein 1986), bachelor bands (Berger 1986), and even loose associations devoid of regular structure (Rubenstein 1986). Harem bands can be strongly territorial (Rubenstein 1986), although most often they are non-territorial (Berger 1986). Dominant stallions within harem bands were once thought to have exclusive access to females (Feist and McCullough 1975, Berger 1986). Subsequent studies have revealed that fertilizations by non-dominant stallions are not uncommon (Bowling and Touchberry 1990, Eagle et al. 1993) and can reach rates as high as 30% (Bowling and Touchberry 1990). Differences in behavior that influence reproductive success and resource use are important parameters for modeling and managing wild populations of horses.

Wild horse populations are frequently reduced to meet management objectives, and sometimes they are severely impacted by environmental fluctuations (Garrott and Taylor 1990). Small populations are more susceptible to genetic disorders than large ones because genetic drift and higher than average levels of inbreeding can increase the expression of deleterious alleles in small populations (Hartl and Clark 1989). Within any population, the sum of breeding males (N_m) and breeding females (N_f) constitutes the effective population size (N_e). Small populations can become genetically smaller as a function of their mating system. For example, the harem band mating system of horses leads to a high degree of variation in male reproductive success (Berger 1986), lowering the number of males that actually breed and reducing effective population size. Combined with behavioral information, knowledge of the genetic characteristics of a population will allow managers to make better decisions regarding reductions to control growth, maintenance of genetic diversity, and introductions of new horses, if necessary, to promote gene flow and reduce inbreeding.

SUMMARY OF RESULTS TO DATE

This summary of results will focus on the Garfield Flat population, because our most intensive work has been done at this site and we have only been able to reliably identify individuals in the Granite Range since November 1995. The Garfield Flat population consists of two sub-populations, which we call the Whiskey Springs herd and the Garfield Springs herd.

The horses at the Garfield Flat HMA behave as two independent units for the majority of the year. During all but the winter months when snow is persistent, there is no overlap between the two herds. The Garfield Springs herd utilizes Garfield Spring, Pepper Spring, and several ephemeral water sources in the vicinity of the playa at Garfield Flat. They forage throughout the Garfield Flat area and often utilize the Pinon/Juniper woodland on the North slope of the Excelsior Mountains. The Whiskey Springs herd utilizes Whiskey Springs exclusively. These horses almost always forage in the Garfield Hills, only occasionally venturing onto Whiskey Flat. When snow is available as a water source, both populations abandon their respective springs and catchments. Many horses from both populations come together in the Garfield Hills, often in areas little used during the summer months (M. Ashley pers. obs.). While there have been no permanent exchanges of animals between herds, aggregations of bands from both herds are common during this period.

The total population has grown considerably over the three years since the 1993 gather, to nearly double its original size (Figure 1), for an average annual growth rate of 21.4% (Table 1). Even though this average growth rate is relatively high compared to those reported for other wild horse populations (Garrott et al. 1991), there was a dramatic decrease to 10.8% in 1996 (Table 1). Although there were differences between the annual growth rates of the two herds (Table 1), both have grown at the same average rate of 21.4% per year over 3 years.

Survival has been quite high for all age classes, especially juveniles. Male survivorship was generally greater than female survivorship at Garfield Flat, but less than female survivorship in the Pryor Mountains (Figure 2). This difference may be due to the short length of our study. Over a longer term, the survivorship curves of males and females at Garfield Flat may converge or, more interestingly and importantly for management, retain their differences. Seasonal variation in weather has been fairly consistent over the past four years at Garfield Flat (M. Ashley pers. obs.). Cold wet spring seasons, reputed to have severe impacts on foal survival (T. Pogacnik, pers. comm.), have not yet occurred. Data for several more years are needed to reveal the range across which survival, especially of juveniles, can vary. These data are important in modeling as the annual growth rate of a feral horse population is most sensitive to changes in age-specific survival rates (Garrott 1991).

The female reproductive rate for horses at Garfield Flat is higher than the rate for the Pryor Mountain population (Garrott and Taylor 1990) and similar to that for the Granite Basin population (Berger 1986) (Table 2). It is important to note that horses in both Nevada populations begin reproduction earlier and reproduce at higher rates overall than those in the Montana population. The data for the Garfield Flat horses are based on only four years of study at stocking densities higher than the AML. Continued study across a wider range of population sizes may reveal substantial differences in the average age-specific rates and their range of variation for improved modeling.

Sex ratio at birth is an important parameter for modeling. Deviation from parity affects the proportion of females in a population which ultimately influences the overall growth rate. We have detected a great deal of variation in the sex ratio of foals across time and between herds (Table 3). Even under the assumption that all undetermined animals are females, the sex ratio at birth remains male biased (Table 3). This trend seems to be consistent with the Trivers-Willard hypothesis (1979) that females in good condition will preferentially produce males while those in poorer condition will produce more females. The rationale is that males born to fit mothers have a competitive advantage from the extra energy invested in them by their mothers, and so will enjoy greater reproductive success than female offspring (Trivers and Willard 1979). Conversely, females in poor condition are better off producing daughters. Given that the majority of the population is still in moderate to moderately thin condition (Heckle et al. 1983), the reduction in

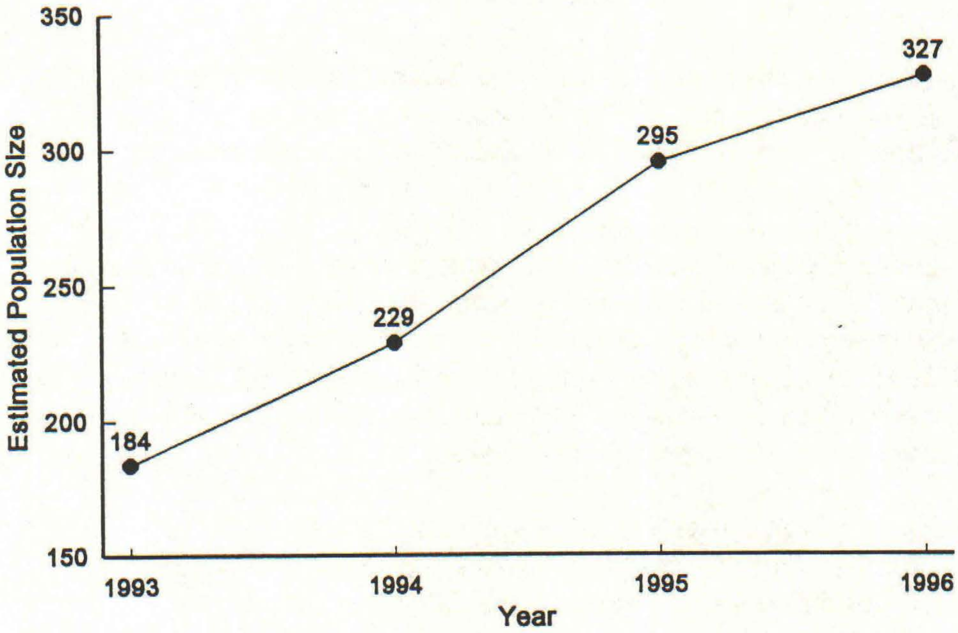
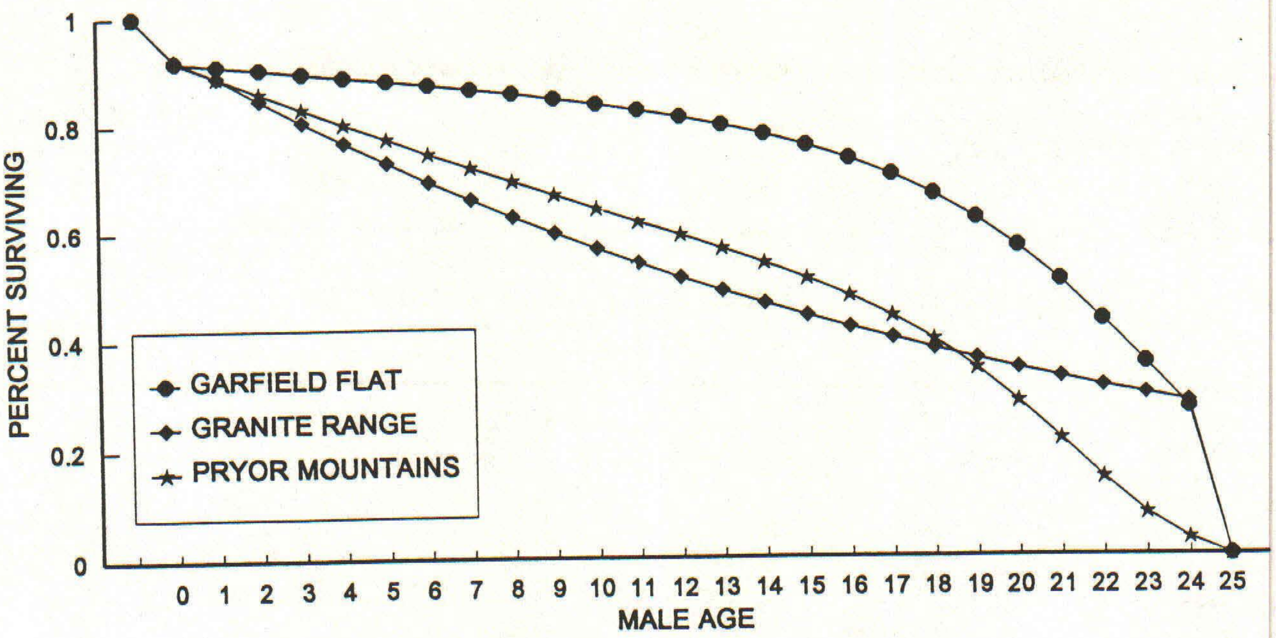


Figure 1. Growth of the Garfield Flat HMA population since the August 1993 gather. The average annual rate of increase is 21.4%.

Table 1.-*Estimated population growth rates for each herd and the combined population of wild horses at the Garfield Flat HMA.*

Year	Garfield Spring	Whiskey Springs	Total Population
1993-1994	27.4	21.3	24.5
1994-1995	24.8	33.3	28.8
1995-1996	11.9	9.7	10.8
Average:	21.4	21.4	21.4



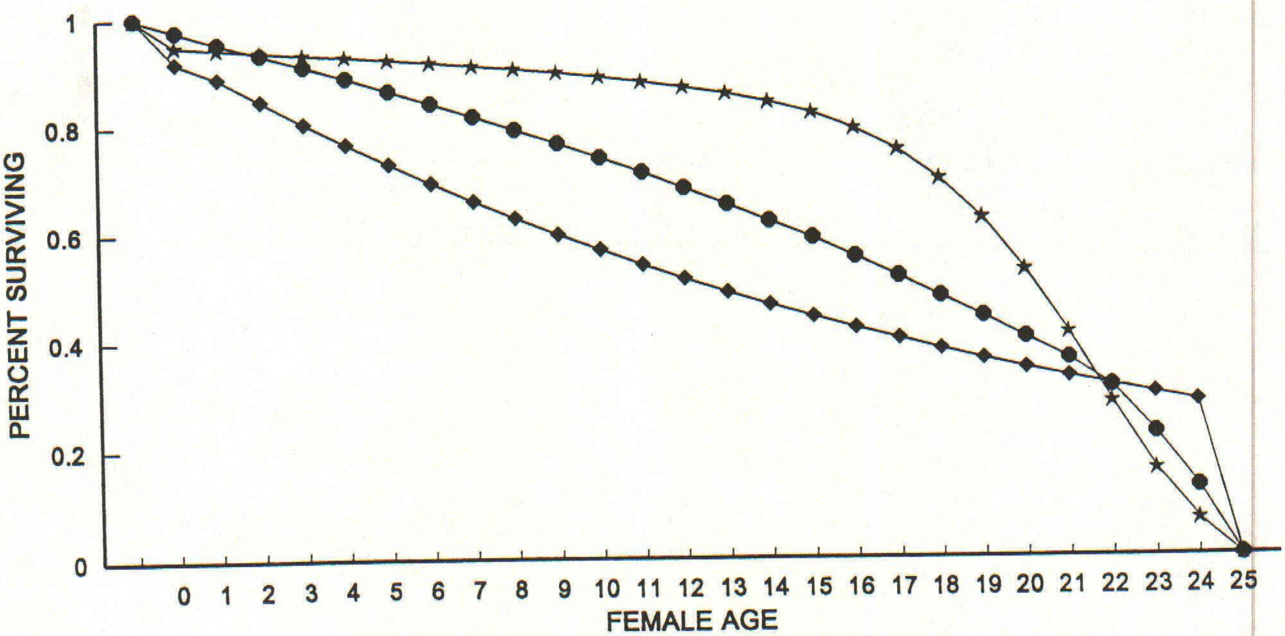


Figure 2. Survival curves for males and females in three wild horse populations: Garfield Flat, the Granite Range, and the Pryor Mountains.

Table 2.-*Foaling probabilities for mares through age 10 for three populations of wild horses.*

Age	Garfield Flat	Granite Range	Pryor Mountains
2	0.310	0.364	0.000
3	0.600	0.440	0.410
4	0.750	0.841	0.410
5	0.600	0.841	0.410
6 - 10	0.860	0.841	0.550

Table 3.-*Sex ratio at birth for each herd and the combined population of wild horses at the Garfield Flat HMA. The ratio is males:females (% identified). The underlying figure is the sex ratio assuming all undetermined animals to be females.*

Year	Garfield Spring	Whiskey Springs	Total Population
1993	0.60 (100)	0.36 (100)	0.48 (100)
	0.60	0.36	0.48
1994	0.90 (73.1)	1.71 (95.0)	1.24 (82.6)
	0.53	1.50	0.84
1995	0.77 (76.6)	1.55 (80.0)	1.13 (78.5)
	0.50	0.94	0.71
1996	0.18 (90.0)	3.17 (75.8)	1.88 (77.8)
	0.76	1.36	1.03
Average:	0.86 (85.0)	1.49 (87.7)	1.14 (84.7)
	0.59	1.02	0.78

female body condition due to increased density does not seem to have been severe enough to effect a shift to a female-biased sex ratio at birth (Trivers and Willard 1979). Continued study of these animals across a longer period of time and a wider range of population densities will help to clarify the nature of this trend and reveal the range of variation for this parameter.

The decrease in population growth rate coincided with an increase in the number of animals in poor body condition, four in 1994 and 1995 compared to 10 in 1996, and an increase in mortality, six marked animals in 1994 and 1995 compared to six in 1996 alone. As stated above, the number of animals in poor condition has been low, but the animals in poor condition were mostly lactating mares (8 of 10). Along with the loss of marked animals, the rate of known foal mortality has doubled from two per year in 1994 and 1995 to four in 1996. Although small, these changes may be indicative of density-dependent impacts on reproduction and survival. Monitoring age-specific survival, age-specific reproductive rate, body condition, and sex ratio over the next few years after the January 1997 removal may show recovery from density-dependent effects. Such indicators would be an increase in the reproductive rate, improved body condition, and increased foal survival.

DNA fingerprinting of the baseline population is proceeding well. A technique has been optimized that allows for identifying alleles that differ by only two base pairs. Several horses have been typed across six microsatellite loci (Marklund et al. 1994) and one case of extra-band paternity has already been found. The majority of the horses have been typed at one locus. The completion of the fingerprinting will identify the instances of extra-band paternity in the 1993 population and provide population level indices of relatedness, gene flow, and heterozygosity. These measures will in turn be used to assess the effective population sizes (N_e) of the herds and the potential for inbreeding effects.

As stated above, there have been no transfers of horses from one herd to the other. There has also been no detectable immigration from areas outside the Herd Management Area. This is important in that it suggests that there is no gene flow between or into the herds, making them genetically distinct and having different effective population sizes. The only potential for gene flow between these populations exists during the winter months when the two herds are in contact. A few foals are born during this period every year giving rise to post-partum estrous cycles in their mothers. Some of the other mares as well may come into early season estrous. Opportunistic copulations between mares of one herd and males from another facilitate gene flow without immigration. The results of the DNA fingerprinting will reveal if this is indeed the case. It will be interesting to see if levels of gene flow respond to changes in population size across time.

Our data on social structure are extensive for the Garfield Flat population. The freeze branding was very effective, only a few brands showing poorly. Because of this, eleven harem bands have been monitored nearly continuously since the August 1993 gather. These data are expected to yield information regarding the stability of bands as a function of overall size, adult composition, and age of dominant stallion. This is important in assessing the relative impact of gathers and removals on band structure. Changes in band structure can affect reproduction through stress-induced abortions (Berger 1986). A majority of the harem bands in the entire population have been identified each year during the foaling season yielding a set of putative sires for paternity testing with molecular data (Ellegren et al. 1992, Marklund et al. 1994). The January 1997 gather is expected to provide blood samples from the majority of horses born during the last three years for this analysis. An additional benefit will be the collection of blood from those animals captured in 1997 that eluded capture in 1993. These samples are important as some of these animals have been part of harem bands as dominant stallions, subdominant stallions, or reproducing mares.

RESEARCH OBJECTIVES

(1) Continue to gain information on life history and demography to improve understanding of the system and better estimate variation in these parameters.

Long-term demographic data from Pryor Mountain, Montana showed how much annual variation in survival and reproduction could exist for wild horses in a relatively mesic, forested environment. We have the opportunity to produce an equally extensive and valuable data set for a much more arid environment at Garfield Flat, Nevada. The Garfield Flat population may be more representative of other populations in Nevada and elsewhere than the Pryor Mountain population, and so demographic data collected at Garfield Flat may be more useful for projecting the growth of these other populations and assessing the effects of various management plans. Our knowledge of habitat use and movement patterns of horses at Garfield Flat gained over the last four years, together with the fact that most horses were marked in 1993 and the population will be gathered and marked again in 1997, provide the foundation for significantly broadening our understanding of the effects of variation in habitat, population density, and stochastic environmental factors on population growth of wild horses. Such understanding, in turn, is critical for effective and economical management.

(2) Test the hypothesis that wild horse populations experience density-dependent control of population growth through increased mortality and/or decreased fecundity.

While the current version of the population model applies stochastic variation to modify growth rate and survival to mimic natural variation in the environment, there are no functions that reflect the effects of population density (e.g., decreased per-capita availability of forage). This does not pose any real problems at low horse densities; however, at increasing densities both survival and reproduction may be overestimated, making the model less realistic. The proper addition of this functionality to the model depends on reliable data from empirical studies.

Reproduction in 1996 was the lowest in the three years since the gather. Continued monitoring of the study population in the years following the January 1997 gather may reveal a rebound in reproduction, indicative of density-dependent effects at higher stocking levels. Intensive monitoring of this study population across several cycles of growth and removal may stand alone as a detailed study of these effects and also will add a well documented case study to our meta-analysis of this phenomenon from extensive BLM records of population censuses, gathers, and removals. An assessment of whether or not feral horses are limited by density-dependent effects and a measure of their magnitude can add an important dimension to the understanding of population growth in general and greatly improve population modeling.

(3) Continue the study of population structure and social organization in Garfield Flat horses and compare these data to similar data for populations in other environments.

Study of population structure and social organization of horses in several different locales has revealed that there are nearly as many differences as there are similarities (Miller 1979, Berger 1986, Keiper and Sambraus 1986, Rubenstein 1986). While these studies are valuable and informative, it is important to consider that all of the major differences may not yet have been described. Increased knowledge of the way different populations are structured and how their members interact will lead to a better understanding of the species as a whole.

Although there are no significant differences in harem band size and composition between the horses at Garfield Flat and those in the Granite Range, the formation of large temporary aggregations of bands (>40 animals) has been seen in both herds at Garfield Flat. Although this behavior was also described by Miller (1979), its ecological significance has yet to be determined. The long-term monitoring of the horses at Garfield Flat across a number of growth/removal cycles may reveal if this is a persistent trait even at low numbers or if there is a threshold of population size at which it reappears.

Following the January 1997 gather and reduction of the wild horse population at Garfield Flat, we will have the opportunity to assess how the remaining population responds as follows: a) effects of the stress of the gather on reproduction in horses returned to the wild, b) effects of the disruption of band structure that occurs in the course of the gather, c) the effect of reduction in population density on reproductive rate, and d) the effect of reduced density on survival. These data will be useful in determining the range of variation for these parameters, information that can be incorporated in future versions of the population growth model.

Pregnancy testing will also be carried out using blood samples collected at the upcoming gather. This will serve as a replication of the testing done in 1993 to assess effects of stress induced by gathers on reproduction (Berger 1986). Arrangements have been made to track foaling by horses removed from the study population for adoption. These data will augment follow-up field data on age-specific fecundity. Continued study of these animals in light of removal and regrowth of the population will greatly enhance the quality of the age-specific fecundity estimates and their range of variation.

In summary, more long-term studies of wild horse populations are needed. This type of knowledge is important for revealing the range of variability in survival, reproduction, and behavior of horses. A suite of long term data on wild horses from a broad range of habitats will be of great value to wild horse managers. For example. if managers responsible for a particular HMA don't have long-term data of their own, they may be able to select an appropriate set of data from such a suite of long-term studies based on similarities in habitat and other environmental conditions between their site and the site at which long-term data were collected. At present, the only choices for such data are for relatively mesic sites (the Pryor Mountains in Montana or Granite Basin in Nevada), so adding a much more arid site to the collection of data sets available (Garfield Flat) will be invaluable.

STUDY SITES

The extensive work done at the Garfield Flat HMA warrants its continued use as a primary study site. The horses to be returned after the January 1997 gather will be freeze branded if not already marked. This is an opportunity to study these populations at stocking levels far below those of 1993 as well as during a period of regrowth to a level similar to that at the start of the original study. Knowledge of population dynamics and behavior across a continuum of densities will enhance our understanding of the system in general.

The unmarked or poorly marked horses in the Granite Range HMA that were gathered in 1995 were returned with new or improved freeze brands. Subsequent surveys have shown that this recent effort was very successful. Surveys to be carried out in the Granite Range will provide additional comparative data on age-specific fecundity and survival as well as general population characteristics.

Although no direct evidence of mountain lion predation has been found, scat and tracks indicate that these animals are occasionally present in the Garfield Springs area. Continued study may reveal evidence of predation on horses. At a reduced population level, predation, if significant, may become a limiting factor.

DEMOGRAPHIC DATA

Two fundamental parameters of population modeling are age-specific survival probabilities and fecundities. Even though the number of animals to be returned to the HMA is relatively small ($\approx$84), the continued study of these animals will allow for improved estimation of stochastic variation in survival and foaling rate. Continued monitoring will enhance the database we have already developed, from which more realistic modeling may be implemented.

DENSITY DEPENDENCE AND POPULATION REGULATION

We have done extensive analyses of gather, removal, and census data from many BLM district offices to test for density-dependent effects in wild horse populations. We predicted that, if these effects were present, a rebound in population growth rate would occur in response to a reduction in density through removal. Meta-analyses, long used in the social sciences and gaining more widespread use in other disciplines, are valuable in detecting significant effects by pooling the results of often individually non-significant studies (Gurevitch and Hedges 1993). We carried out a meta-analysis to test for a density-dependent decrease in population growth rate at higher densities and an increased growth rate following reduction. We found a significant effect, indicating that wild horse population growth is sensitive to increased density (in preparation).

Additional long-term data sets such as the one proposed here will tend to make the conclusions of such analyses more robust.

Preliminary observations at the study site have revealed no qualitative difference in the forage available to the two herds. Because of the size and heterogeneity of the study site, a prohibitive number of random transects would be necessary to quantify any differences between the two. We will investigate use of remote sensing data as well as vegetation coverages in the form of a GIS database being developed by land use agencies in Nevada as sources of quantitative data for such a comparison. Forage quality and quantity are important factors affecting survival rates (Choquenot 1991) and juvenile condition (Berger 1986, Choquenot 1991). These data will be used to evaluate past population trends and make predictions about future reproduction and survival.

The persistence and amount of water has been reasonably constant across the four years of the current study. The flow at Pepper Spring, the most abundant source of water for the Garfield Springs herd, is approximately twice that of Whiskey Springs. At lower stocking levels, each population has a greater per capita water availability. If water is limiting, this may mark a temporary equality of this parameter for the two populations. Differences between the herds in reproduction and survival at low densities, if found, might then be attributable to factors other than water availability. We will continue to monitor the persistence and accessibility of water to insure that this critical factor does not get overlooked should conditions change.

BEHAVIOR AND SOCIAL ORGANIZATION

Rubenstein (1986) reported substantial variation in the social organization of a population of horses on an Atlantic barrier island. It will be of interest to see if the Garfield Flat study population maintains the same social structure at low density as it did at a higher density. Band size, band composition, age at dispersal, age of first reproduction, and interband interactions will be tracked intensively across the foaling season and periodically monitored throughout the year. Changes in social structure can affect reproductive rate by altering the sex ratio of breeding animals, increasing or decreasing effective population size (N_e). Changes in habitat use and movement patterns as a function of social organization may affect body condition and improve survival independently of a simple decrease in density. Conversely, documenting no substantial difference in social structure and behavior at low densities is also valuable information. Knowledge of how these parameters react to changes in density is important to the long-term management and modeling of wild horse populations.

The next three years at Garfield Flat represent a valuable opportunity. The dramatic reduction in population size resulting from the gather in 1997 will provide a more rigorous test of density-dependent population regulation in wild horses than simply observing how survival and foaling rates change as population density increases naturally. As the population will likely return to the 1993 stocking level, we will be able to follow changes in age-specific survival and fecundity to supplement our observations at higher densities. We will also be able to document any changes in social organization that may result from the population reduction and subsequent growth.

Ultimately, we will have more years of data, necessary for more reliable measures of variation in the aspects of life history, behavior, and genetics that we have been studying. This data set will be valuable to many managers in and of itself, and, together with other long-term data from the Granite Range and the Pryor Mountains, serve managers of almost any herd of wild horses.

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Keiper, R.R., and H.H. Sambraus. 1986. The stability of equine dominance hierarchies and the effects of kinship, proximity and foaling status on hierarchy rank. Applied Animal Behaviour Science 16:121-130.

Marklund, S., H. Ellegren, S. Eriksson, K. Sandberg, and L. Andersson. 1994. Parentage testing and linkage analysis in the horse using a set of highly polymorphic microsatellites. Animal Genetics 25:19-23.

Miller, R. 1979. Band organization and stability in Red Desert feral horses. Pages 113-123 *in* Symposium on the ecology and behavior of wild and feral equids. Laramie, University of Wyoming.

Rubenstein, D. I. 1986 Ecology and sociality in horses and zebras. Pages 282-302 *in* D.I. Rubenstein and R.W. Wrangham, editors. Ecological aspects of social evolution. Princeton University Press, Princeton, New Jersey.

Stevens, E.F. 1990. Contests between bands of feral horses for access to fresh water: the resident wins. Animal Behaviour 36:1851-1853.

Trivers, R.L., and D.E. Willard. 1979. Natural selection of parental ability to vary the sex ratio of offspring. Science 179:90-92.

CURRICULUM VITAE

for

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PRESENT POSITION

Professor of Biology, University of Nevada, Reno.

EDUCATION

1968	A.B., Dartmouth College, Biology (Magna cum Laude, with Highest Distinction in major subject).
1975	Ph.D., Harvard University, Biology.

EMPLOYMENT

1974-Present	Lecturer (1974-1975), Assistant Professor (1975-1980), Associate Professor (1980-1990), Professor (1990-present) of Biology, University of Nevada, Reno (UNR).
1990-1991	Instructional Computing Coordinator, UNR.
1978-1988 (summers)	Scientist-in-Residence, Whittell Forest and Wildlife Area, UNR.
1977 (summer)	Biologist (GS-11), Bureau of Land Management, Nevada State Office.
1973-1974	Tutor in Biology, Harvard University.
1970-1973	Teaching Fellow in Biology, Harvard.
1968-1970	Science and Math Teacher, Roeper City and Country School, Bloomfield Hills, Michigan.

HONORS AND AWARDS

1964-1968	National Merit Scholarship at Dartmouth College.
1967	Elected to Phi Beta Kappa.
1970-1971	National Science Foundation Traineeship, Harvard University.
1971-1974	Richmond Scholarship, Harvard.
1972 (summer)	NSF Evolutionary Biology Training Grant, Harvard.
1978	First-place award for innovative course development (for summer field ecology course), Honors Study Board, UNR.
1983-1984, 1992-1993	Sabbatical Leave from UNR.

PROFESSIONAL SERVICE

Subject-Matter Editor for **Ecology** and **Ecological Monographs** (1995-1998)

Associate Editor for Animal Ecology of the **American Midland Naturalist** (1990-1995).

Member of National Research Council biological sciences panel for NSF Minority Graduate Fellowship Program (1987-1989).

Reviewer for numerous journals and several granting agencies and of student papers and proposals for the American Society of Mammalogists, Animal Behavior Society, and Ecological Society of America.

PROFESSIONAL SOCIETIES

American Society of Mammalogists (chair of Merriam Award Committee), American Society of Naturalists, Animal Behavior Society, Ecological Society of America, Sigma Xi.

PUBLICATIONS

1975 Jenkins, S. H. Food selection by beavers: a multidimensional contingency table analysis. **Oecologia** 21:157-173.

1978 Jenkins, S. H. Food selection by beavers: sampling behavior. **Breviora** 447:1-6.

1979 Jenkins, S. H. Ecology and management of ground squirrels in north-central Nevada. Pp. 79-91 *in* D. L. Koch et al., editors, Cal-Neva Wildlife 1978.

1979 Jenkins, S. H., and P. E. Busher. *Castor canadensis*. Mammalian Species No. 120:1-8 (a special publication of the American Society of Mammalogists).

1979 Anderson, M. M., and S. H. Jenkins. Gene frequencies in the domestic cats of Reno, Nevada: confirmation of a recent hypothesis. **Journal of Heredity** 70:267-269.

1979 Jenkins, S. H. Seasonal and year-to-year differences in food selection by beavers. **Oecologia** 44:112-116.

1980 Jenkins, S. H. A size-distance relation in food selection by beavers. **Ecology** 61:740-746.

1981 Jenkins, S. H., and J. B. Llewellyn. Multiple captures of *Peromyscus maniculatus* and *P. truei*: age, sex, and species differences. **Journal of Mammalogy** 62:639-641.

1981 Jenkins, S. H. Common patterns in home range - body size relationships of birds and mammals. **The American Naturalist** 118:126-128.

1981 Jenkins, S. H. Problems, progress, and prospects in studies of food selection by beavers. Pp. 559-579 *in* J. A. Chapman and D. Pursley, editors, Worldwide Furbearer Conference Proceedings, Volume I.

1982 Jenkins, S. H. Management implications of optimal foraging theory: a critique. **Journal of Wildlife Management** 46:255-257.

1983 Busher, P. E., R. J. Warner, and S. H. Jenkins. Population density, colony composition, and local movements in two Sierra Nevada beaver populations. **Journal of Mammalogy** 64:314-318.

1984 Jenkins, S. H., and B. D. Eshelman. *Spermophilus beldingi*. Mammalian Species No. 221:1-8 (a special publication of the American Society of Mammalogists).

1984 Vig, B. K., M. L. Figueroa, M. N. Cornforth, and S. H. Jenkins. Chromosome studies in human subjects chronically exposed to arsenic in drinking water. **American Journal of Industrial Medicine** 6:325-338.

1985 Busher, P. E., and S. H. Jenkins. Behavioral patterns of a beaver family in California. **Biology of Behavior** 10:41-54.

1986 Mewaldt, W. T., and S. H. Jenkins. Genetic variation of woodrats (*Neotoma cinerea*) and deer mice (*Peromyscus maniculatus*) on montane habitat islands in the Great Basin. **Great Basin Naturalist** 46:577-580.

1986 Price, M. V., and S. H. Jenkins. Rodents as seed consumers and dispersers. Pp. 191-235 *in* D. R. Murray, ed., Seed dispersal. Academic Press Inc., Orlando, FL, xiv + 322 pp.

1987 Llewellyn, J. B., and S. H. Jenkins. Mechanisms of niche shift in mice: seasonal changes in microhabitat breadth and overlap. **The American Naturalist** 129:365-381.

1987 Longland, W. S., and S. H. Jenkins. Sex and age affect vulnerability of desert rodents to owl predation. **Journal of Mammalogy** 68:746-754.

1988 Jenkins, S. H. Comments on relationships between native seed preferences of shrub-steppe granivores and seed nutritional characteristics. **Oecologia** 75:481-482.

1988 Basey, J. M., S. H. Jenkins, and P. E. Busher. Optimal central-place foraging by beavers: tree-size selection in relation to defensive chemicals of quaking aspen. **Oecologia** 76:278-282.

1988 Peacock, M. M., and S. H. Jenkins. Development of food preferences: social learning by Belding's ground squirrels *Spermophilus beldingi*. **Behavioral Ecology and Sociobiology** 22:393-399.

1988 Jenkins, S. H. Use and abuse of demographic models of population growth. **Bulletin of the Ecological Society of America** 69:201-207.

1989 Jenkins, S. H., and P. W. Bollinger. An experimental test of diet selection by the pocket gopher *Thomomys monticola*. **Journal of Mammalogy** 70:406-412.

1989 Jenkins, S. H. Comments on an inappropriate population model for feral burros. **Journal of Mammalogy** 70:667-670.

1989 Eshelman, B. D., and S. H. Jenkins. Food selection by Belding's ground squirrels in relation to plant nutritional features. **Journal of Mammalogy** 70:846-852.

1990 Basey, J. M., S. H. Jenkins, and G. C. Miller. Food selection by beavers in relation to inducible defenses of *Populus tremuloides*. **Oikos** 59:57-62.

1992 Jenkins, S. H., and R. A. Peters. Spatial patterns of food storage by Merriam's kangaroo rats. **Behavioral Ecology** 3:60-65.

1992 Pierce, B. M., W. S. Longland, and S. H. Jenkins. Rattlesnake predation on desert rodents: microhabitat and species-specific effects on risk. **Journal of Mammalogy** 73:859-865.

1993 Jenkins, S. H., and R. Ascanio. A potential nutritional basis for resource partitioning by desert rodents. **American Midland Naturalist** 130:164-172.

1993 Basey, J. M., and S. H. Jenkins. Production of chemical defenses in relation to plant growth rate. **Oikos** 68:323-328.

1994 Jenkins, S. H. RAMAS Population Modeling Software. Pp 68-74 *in* Valuable viable software in education: cases and analysis (P. Morris, S. Ehrmann, R.Goldsmith, K. Howat, and V. Kumar, eds.) McGraw-Hill, Inc., New York, New York, USA.

1995 Jenkins, S. H. Carfentanil, bison, and statistics: the last word? **Journal of Wildlife Diseases** 31:104-105.

1995 Jenkins, S. H., A. Rothstein, and W. C. H. Green. Food hoarding by Merriam's kangaroo rats: a test of alternative hypotheses. **Ecology** 76:2470-2481.

1995 Basey, J. M., and S. H. Jenkins. Influence of predation risk and energy maximization on food selection by beavers (*Castor canadensis*). **Canadian Journal of Zoology** 73:2197-2208.

1997 Breck, S. W., and S. H. Jenkins. Use of an ecotone to test the effects of soil and desert rodents on the distribution of Indian ricegrass. **Ecography**, *in press.*

1997 Jenkins, S. H. Perspectives on individual variation in mammals. **Journal of Mammalogy**, *in press.*

1997 Hayes, J. P., and S. H. Jenkins. Individual variation in mammals. **Journal of Mammalogy**, *in press.*

1997 Sweitzer, R. A., S. H. Jenkins, and J. Berger. The near-extinction of porcupines by mountain lions and consequences of ecosystem change in the Great Basin desert. **Conservation Biology**, *in press.*

1997 Smith, D. W., and S. H. Jenkins. Seasonal change in body mass and size of tail in northern beavers. **Journal of Mammalogy**, *in press.*

SOFTWARE

1993 Wild Horse Population Model, version 2.1. This is a stochastic model which simulates growth of wild horse populations under various management options. It is programmed in C++ and is accompanied by a 37 page User's Guide.

1996 Wild Horse Population Model, version 3.2. This is a completely new version of the above program, written in True BASIC. It is user friendly and has extensive on-line help files.

REVIEWS

1981 Jenkins, S. H. Deer population ecology and management (review of The George Reserve Deer Herd, by D. R. McCullough). **Ecology** 62:285.

1988 Jenkins, S. H. Review of Foraging Theory, by D. W. Stephens and J. R. Krebs. **Journal of Mammalogy** 69:877.

1993 Jenkins, S. H. RAMAS/age. **Quarterly Review of Biology** 68:159-160.

PAPERS SUBMITTED FOR PUBLICATION

1996 Ritorto, J. E., and S. H. Jenkins. Intraspecific and interspecific agonistic behaviour of Merriam's and Panamint kangaroo rats. Submitted to **Animal Behaviour**.

1996 McMurray, M. H., S. H. Jenkins, and W. S. Longland. Effects of seed density on germination and establishment of a native and an introduced grass species dispersed by granivorous rodents. Submitted to **American Midland Naturalist**.

1996 Jenkins, S. H., and R. D. Duncan. Randomization methods for analysing behavioural data: unused or under-appreciated? Submitted to **Animal Behaviour**.

GRANTS AND CONTRACTS

1975,1977, 1979, 1989 Research Advisory Board grants from UNR for studies of food selection by woodrats, social organization of beavers, population genetics of woodrats and deer mice, and food hoarding by kangaroo rats.

1987 Instructional Enhancement Grant for software for teaching computer programming to life science students, UNR.

1992-1996 Behavioral adaptation to a desert environment: an experimental comparative study of food hoarding. **National Science Foundation**, $92,904.

1992-1996 Population dynamics of wild horses in Nevada. **Bureau of Land Management**, $210,948.

1994-1997 Impacts of granivory on Indian ricegrass populations. **USDA Competitive Grants Program**, $199,962 (with W. S. Longland and S. B. Vander Wall).

TEACHING EXPERIENCE

I have taught the following courses in my 22 years at the University of Nevada, Reno (courses highlighted in bold have been taught regularly in recent years):

lower division	Biology: Principles and Applications (aka "Biology for Non-Majors") Animal Biology
upper division	**Ecology and Population Biology** (core course for undergraduates) **Mammalogy** **Population and Community Ecology** Laboratory in Ecology and Population Biology Animal Ecology Principles of Animal Behavior Experimental Field Ecology Computer Acquaintance for Biological Sciences
graduate	**Research Design in Ecology** (core course for graduate students) Mathematical Modeling in Ecology Advanced Population Ecology Advanced Wildlife Ecology Topics in Ecology

GRADUATE STUDENTS

Four Ph.D. students and 13 M.S. students have completed their degrees under my supervision since 1974. The Ph.D. students were J. Basey, P. Busher, J. Llewellyn, and W. Mewaldt. The M.S. students were J. Basey, S. Benner, P. Bollinger, S. Breck, S. Cox, B. Eshelman, R. Haley, T. Kepler, W. Longland, M. Peacock, B. Pierce, R. Peters, and R. Warner. I have also advised 2 postdoctoral fellows, W. Green and A. Rothstein. I currently supervise 1 M.S. and 5 Ph.D. students.

UNIVERSITY SERVICE

I have served on numerous University, College of Arts and Science, and Departmental committees. Among the most important have been University Tenure and Promotion Committee, Sabbatical Leave Committee (chair one year), Whittell Forest and Wildlife Area Board of Control (chair several years), College Personnel Committee, College Courses and Curricula Committee, College Apportionment Committee, Ecology, Evolution, and Conservation Biology Admissions and Examination Committees, and search committees for Director of the Library, Chair of the Biology Department, and several new faculty members.

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PRESENT POSITION Graduate Teaching Assistant, Biology University of Nevada, Reno

EDUCATION

1974	A.A., Northeastern Oklahoma A&M, Social Sciences
1976	B.S.E., Missouri Southern State College, Social Science Education, American History Minor.
1993	B.S., University of Nevada, Reno, Biology (Zoology Emphasis).

EMPLOYMENT

1993-Present	Teaching Assistant/Research Assistant, University of Nevada, Reno.
1976-1991	Keno writer/game supervisor, Harrah's, Reno.

HONORS AND AWARDS

1993	Undergraduate Italian Studies Award, University of Nevada, Reno.
1991	Isola Buck Scholarship for Italian Studies

PROFESSIONAL SOCIETIES

American Association for the Advancement of Science, American Society of Mammalogists, Animal Behavior Society, Society for Conservation Biology.

PUBLICATIONS

1993 Ashley, M. Desertification and wild horses in the northern Great Basin. Journal of Undergraduate Research (Electronic) Volume 1. University of Colorado.

1997 Ashley, M. C. Observations of black-billed magpies (*Pica pica*) grooming feral horses (*Equus caballus*). In preparation for **The Condor**.

1997 Ashley, M. C., J. A. Robinson, L. W. Oring., and G. A. Vinyard. Estimation of macroinvertebrate production and effects of shorebird predation at a constructed wetland. In preparation for **Wetlands**.

VIDEO CREDIT

1995 Ashley, M. Life Springs Eternal. Wild Horse Research, Scientific Consultant. Essence of Life Series, The Esse Group.

PRESENTATIONS

1994 Jenkins, S. H., and M. C. Ashley. Population dynamics of feral horses: a meta-analysis of messy data. American Society of Mammalogists 75th annual meeting.

1996 Ashley, M. C. A non-radioactive, high resolution microsatellite DNA fingerprinting technique applied to paternity assessment of feral horses (*Equus caballus*). American Society of Mammalogists 1996 annual meeting.

Proposed Budget: March 1997- September 1997

Salaries and Wages	
Principal Investigator, 1 month summer salary	$6,500
Ph.D. Student Research Assistant (Mike Ashley), 4 months	$4,632
M.S. Student Research Assistant (new student), 4 months	$3,600
Undergraduate Wages (730 hrs @ $7.00/hr)	$5,110
(field and lab assistance)	
Fringe Benefits (3% of P.I. salary + 1.2% of grad. asst. salary +	$746
8.85% of undergraduate wages)	
Total salaries, wages, and fringe benefits	**$20,588**
Travel	
To field sites	
vehicle rental + mileage (90 days)	$4,636
per diem (2 people x 90 days x $30/day)	$5,400
To scientific meeting for graduate student	$600
Total travel	**$10,636**
Materials and supplies	
film, video tapes,software, miscellaneous field supplies	$1,500
chemicals for DNA fingerprinting	$3,300
Total materials and supplies	**$4,800**
Other expenses	
fees for 2 graduate students (20 credits x $87/credit)	$1,740
fees for use of molecular biology lab	$600
computer programming services (150 hrs x $20/hr)	$3,000
publication costs	$600
Total other expenses	**$5,940**
Total direct costs	**$41,964**
Indirect costs (44.3% of direct costs - fees for graduate student	**$17,819**
Total Budget for March 1997 - September 1997	**$59,783**

Proposed Budget: October 1997 - September 1998

Salaries and Wages	
Principal Investigator, 1 month summer salary	$6,825
Ph.D. Student Research Assistant (Mike Ashley), 12 months	$14,591
M.S. Student Research Assistant, 12 months	$11,340
Undergraduate Wages (900 hrs @ $7.35/hr)	$6,615
(field and lab assistance)	
Fringe Benefits (3% of P.I. salary + 1.2% of grad. asst. salary +	$1,101
8.85% of undergraduate wages)	
Total salaries, wages, and fringe benefits	**$40,472**
Travel	
To field sites	
vehicle rental + mileage (101 days)	$5,463
per diem (2 people x 101 days x $30/day)	$6,060
To scientific meeting for graduate students	$1,200
Total travel	**$12,723**
Materials and supplies	
film, video tapes,software, miscellaneous supplies	$2,500
Total materials and supplies	**$2,500**
Other expenses	
fees for 2 graduate students (30 credits x $87/credit)	$2,610
publication costs	$800
Total other expenses	**$3,410**
Total direct costs	**$59,105**
Indirect costs (44.3% of direct costs - fees for graduate student	**$25,027**
Total Budget for October 1997 - September 1998	**$84,132**

Proposed Budget: October 1998 - September 1999

Salaries and Wages	
Principal Investigator	$0
Ph.D. Student Research Assistant (Mike Ashley), 3 months	$3,830
M.S. Student Research Assistant, 12 months	$11,907
Undergraduate Wages (500 hrs @ $7.72/hr)	$3,860
(field and lab assistance)	
Fringe Benefits (3% of P.I. salary + 1.2% of grad. asst. salary +	$530
8.85% of undergraduate wages)	
Total salaries, wages, and fringe benefits	**$20,128**
Travel	
To field sites	
vehicle rental + mileage (50 days)	$2,840
per diem (2 people x 50 days x $30/day)	$3,000
To scientific meeting for graduate student	$600
Total travel	**$6,440**
Materials and supplies	
miscellaneous supplies	$1,000
Total materials and supplies	**$1,000**
Other expenses	
fees for 2 graduate students (8 credits x $87/credit)	$696
publication costs	$1,000
Total other expenses	**$1,696**
Total direct costs	**$29,263**
Indirect costs (44.3% of direct costs - fees for graduate student	**$12,655**
Total Budget for October 1998 - September 1999	**$41,918**

Materials and Supplies

This budget item includes expenses for DNA fingerprinting, analysis of blood samples for pregnancy testing, and miscellaneous field and laboratory supplies.

Other Expenses

(1) We will cover fees for graduate student course work, as this is a necessary component of their degree programs.

(2) A modest fee is assessed for use of the molecular biology core lab of the Center for Environmental Sciences and Engineering where Michael Ashley will continue to do the DNA fingerprinting work. Money is requested in the first year for computer-programming assistance; this will be used to hire someone to help convert the Wild Horse Population Model from DOS to UNIX format.

(3) We have budgeted a small amount each year of the study for publication costs.

Wild Horse and Burro Advisory Board Report to the Secretaries of the Interior and Agriculture

December 1986

December 5, 1986

Honorable Donald Paul Hodel
Secretary of the Interior
Washington, D.C. 20240

Honorable Richard E. Lyng
Secretary of Agriculture
Washington, D.C. 20250

Dear Mr. Secretaries:

The Wild Horse and Burro Advisory Board is pleased to submit this report containing recommendations concerning issues in the administration of Public Law 92-195, as amended, commonly referred to as the Wild Free-Roaming Horse and Burro Act.

In accordance with its charter, the Board has gathered and analyzed information and heard public testimony in order to offer advice and develop recommendations from a national, public-interest perspective on matters pertaining to management and protection of wild free-roaming horses and burros. To accomplish these objectives, the Board held meetings in Washington, D.C., Reno, Nevada, and Ontario, California.

We wish to assure you that this report is the result of a careful and thorough review of the issues and presents a balanced perspective of the varied interests concerned about the protection and management of wild horses and burros. It is our hope that the recommendations in this report will provide a reasonable basis for more efficient management of the wild horse and burro program. Implementation should be monitored carefully by this or a similar board to ensure compliance with the spirit of the report.

Thank you for giving us the opportunity to serve on this Board.

Sincerely yours,

Norman B. Livermore
Chairman, Wild Horse and
Burro Advisory Board

Laurence R. Jahn, PhD | Brad Little | James H. Magagna

Michael J. Pontrelli, PhD | Helen A. Reilly | Terry D. Swanson, DVM

William E. Towell | Dennis J. White

Report

Submitted to

the Secretary of the Interior and the Secretary of Agriculture

by the

Wild Horse and Burro Advisory Board

December 5, 1986

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Introduction

In 1971, Congress unanimously approved the Wild Free-Roaming Horse and Burro Act to "require the protection, management, and control of wild free-roaming horses and burros on public lands." This legislation reflected concern that these animals were "fast disappearing from the American scene." At the time the law was passed, it was estimated that about 17,000 wild horses and burros inhabited public lands administered by the Secretary of the Interior through the Bureau of Land Management (BLM) and the Secretary of Agriculture through the Forest Service.

The law does not apply to horses and burros on lands administered by other agencies, such as the National Park Service, the U.S. Fish and Wildlife Service, or the Department of Defense.

Under Federal protection and with no natural predators, wild horse and burro herds increased steadily, creating unforeseen conflicts and pressures on certain areas of the public lands. The controversial nature of many of the issues involved in the program made efficient administration more and more difficult. By 1976, improved census techniques indicated wild horse and burro populations of about 63,000, and the need to amend the Act became clear. In that year, an amendment to the Act authorized the use of aircraft and motor vehicles in the management of wild horses and burros on those public lands administered by BLM and the Forest Service.

By 1978, wild horse and burro populations were estimated to be nearly 67,000, and Congress amended the Act again, providing for the following:

1. Transfer of title to as many as 4 animals per year to an adopter who maintained the animals humanely for 1 year. The purpose of this change was to encourage adoptions.

2. An order and priority for removal and disposition of excess wild horses and burros. This change recognized the increasing need for control of wild horse and burro numbers.

3. A research study to be conducted through the National Academy of Sciences.

The 1976 and 1978 amendments sought to increase the efficiency of program administration in light of changing conditions. However, controversy over issues such as appropriate herd management levels and disposition of excess animals continued.

For several years, the number of excess animals removed from the range has exceeded the number of animals for which an adoption demand exists. The 1978 amendment providing an order and priority for removal and disposition of excess animals directs that healthy excess animals for which there is no adoption demand be humanely destroyed. However, the agencies have maintained a moratorium* on destruction of healthy animals since 1982 while seeking an alternative to destroying large numbers of healthy animals. More than 10,000 unadopted horses are now being maintained in BLM corrals and contract facilities at a cost to the public of approximately $25,000 per day, or more than $9 million a year. Meanwhile, as many as 20,000 wild horses above the estimated appropriate herd management levels remain on the public lands.

In February 1986, in an effort to find solutions to these problems and attempt to develop a consensus among affected interests, the Secretaries of the Interior and Agriculture chartered the Wild Horse and Burro Advisory Board for a period of 2 years. The nine members appointed to the Board and the interests they represent are listed below.

Norman B. Livermore, San Rafael, California, Chairman, Public-at-Large
Helen A. Reilly, Reno, Nevada, Wild Horse and Burro Management
Laurence R. Jahn, Washington, D.C., Wildlife Management
William E. Towell, Southern Pines, North Carolina, Conservation
Dennis J. White, Denver, Colorado, Humane Organizations
James H. Magagna, Rock Springs, Wyoming, Livestock Management
Brad Little, Emmett, Idaho, Rangeland Management
Michael J. Pontrelli, Reno, Nevada, Research
Terry D. Swanson, Littleton, Colorado, Veterinary Medicine

In the summer and early fall of 1986, the Board heard public testimony at meetings in Washington, D.C., Reno, Nevada, and Ontario, California. After a review of the issues, the Board focused on two major problems:

1. Large numbers of unadopted horses being maintained in corrals.

2. The presence on public lands of an estimated 15,000-20,000 wild horses and burros in excess of appropriate herd management levels.

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*In 1982, BLM and the Forest Service established uniform adoption fees of $200 per horse and $75 per burro. This action responded to recommendations by a congressional subcommittee and the Office of Management and Budget that the agencies should recover a greater part of the cost of adoptions. Some horse interest groups charged that the fees were excessive and would create the need to destroy animals by decreasing adoption demand. Because of these concerns, the agencies placed a moratorium on destruction of healthy animals until the effect of the fees could be evaluated. The adoption fee for a horse was eventually lowered to $125, but by then unadopted horses had begun to accumulate in Government corrals and the moratorium was continued.

In this report to the Secretaries, the Board makes several recommendations related to these two major problems. In addition, the Board offers recommendations to improve the effectiveness of the program in the areas of research and legislation. The recommendations provide an approach to solving the major problems in the wild horse and burro program and to achieving reasonable goals in keeping with the intent of the Act. The Board sees these goals as:

- Preservation on the public lands of healthy herds of wild free-roaming horses and burros at appropriate herd management levels determined through the resource planning process in accordance with the principles of multiple-use land management.
- Maintenance of the habitat on which these animals depend.
- Humane disposition of excess animals removed from the land.
- Reduction of program costs to the taxpayer.

Summary of Recommendations

The Wild Horse and Burro Advisory Board unanimously agrees that the fundamental concerns of the wild horse and burro program must continue to be the protection and management of wild horse and burro populations on public lands administered by the BLM and the Forest Service and the humane treatment and disposition of excess wild horses and burros removed from the range. Additionally, the Board affirms that protection of the land and its resources is the underlying principle that must guide the land management agencies in all decisions affecting wild horses and burros, as well as other uses of the public lands.

In the course of its meetings, the Board explored many elements of wild horse and burro program administration related to these major problems. As a result, the Board is making specific recommendations on 21 issues in 4 categories, as summarized below.

I. Disposition of Excess Wild Horses and Burros

A. That the Secretary implement the five-step process (see pp. 7-8), which provides several opportunities for disposition of the excess animals, and subsequently evaluate it.

B. That the agencies continue the current adoption fees of $75 per burro and $125 per horse, with variances to be made administratively to resolve specific placement problems. The agencies should also periodically compile a study of the grade horse market in relation to adoption fee levels.

C. That the concept of private sanctuaries for wild horses and burros be explored further and encouraged by and receive assistance (but not funds) from BLM and the Forest Service to monitor the efforts and establish a system of accountability for the animals.

D. That old, sick, or lame wild horses and burros be disposed of humanely in the field whenever possible, with a veterinarian determining the need for destruction.

E. That BLM review the existing policy and establish specifications to ensure that equipment used to transport wild horses minimizes the risk of injury to the animals, yet is reasonably cost effective.

F. That BLM require and constantly urge its employees, as well as contractors, to carry out quality control to maintain equipment to prevent and minimize risks of injury to horses and burros processed for adoption. BLM should also review veterinary contracts to assure they require appropriate techniques and medications.

G. That BLM seek the assistance of a marketing expert to review the strategy and publicity efforts to place excess animals through adoption. The BLM also should develop an aggressive adoption policy and seek approval for use of paid advertising when free advertising cannot be obtained.

H. That the adopter be given certificate of title 1 year after the adoption date, thus automatically conveying title unless complaints of inhumane care or conditions are registered to BLM or humane officials.

II. Management

A. That BLM and the Forest Service continue to establish appropriate herd management levels through their existing resource management planning processes. The agencies should increase their effectiveness in using the planning process.

B. That fertility control, if available and practicable, be used on a temporary basis where deemed necessary in specific herd management areas.

C. That excess animals be removed in the following priority:

1. from private lands when requested by the landowner, and
2. from the public lands where resource degradation is the most significant.

D. That the agencies endeavor to develop and utilize more reliable inventory methods for wild horses and burros, recognizing habitat differentials.

E. That the rate of herd increase be established for each herd area through repeated censusing of the herd, undertaken at the same time of year and using the same technique each time.

F. That the agencies (1) emphasize continued monitoring of habitat condition, trend, and utilization within herd management areas, and (2) develop and publish a policy statement that outlines the criteria that will be used and clearly articulates the basis for adjustments in wild horse and burro numbers.

G. That management for particular herd characteristics continue to be determined for each herd area through the planning process.

III. Research

A. That all research on wild horse and burro herds and habitats be coordinated by BLM at the national level; that BLM reevaluate long-term research needs when the results of research in progress are available; and that BLM establish and maintain a list of short-term, site-specific projects to be considered for a share of any future wild horse and burro research funding.

IV. Legislation

A. That sale authority is not recommended at this time. The five-step process should be given priority for disposition of excess animals. If the five-step process does not prove effective, consideration should be given to this and other alternatives for disposition of excess wild horses and burros.

B. That Public Law 86-234 be amended to allow Federal agencies besides BLM and the Forest Service to use helicopters and motor vehicles to remove horses and burros from lands under their jurisdiction.

C. That the Wild Free-Roaming Horse and Burro Act be amended to replace the requirement for public hearings before the use of helicopters and motor vehicles with a requirement for public notification of such use.

D. That Section 1 of the Act be amended to delete the language indicating the animals are in danger of disappearing. The section should be further amended to establish, as a policy of Congress, that wild horses and burros are to be managed under the principles of multiple-use land management.

E. That Section 11 of the Act be amended to eliminate the joint biennial report requirement and replace it with an annual reporting requirement that can be incorporated in each agency's primary annual report to Congress.

Conclusion

As this report indicates, the Board believes that changes are needed in many areas to improve the administration of the Wild Free-Roaming Horse and Burro Act. However, the Board wishes to affirm its conviction that the Forest Service and the Bureau of Land Management are commited to the protection and management of wild horses and burros on the public lands, based upon the principles of multiple-use land management.

For BLM, which is responsible for more than 95 percent of all wild horses and burros, current policy has resulted in a program where increasingly larger portions of funds are spent to maintain thousands of excess wild horses for an indeterminate period of time, rather than to manage and protect animals in their natural habitat.

Without passing judgment on past decisions in an extremely complicated and difficult program, the Board finds that the present situation fulfills neither the spirit nor the letter of the law. The Board therefore strongly urges the Secretaries to give careful consideration to the recommendations in this report and to develop a clear policy statement that will guide them in administering the Act in the future. Implementing actions should be based on factual information rather than solely on emotional reaction.

Issues and Recommendations

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE A: Five-Step Process for Disposition of Excess Wild Horses and Burros

FINDING: In Fiscal Years 1984, 1985, and 1986, more excess animals were removed from the public lands than the number for which the agencies could find qualified individuals willing to provide private maintenance. As a result, the number of animals being maintained in holding facilities has grown each year. More than 10,000 excess wild horses are being maintained at a cost of approximately $25,000 per day in BLM's corrals and contract facilities. Many of the animals in these facilities have little chance of adoption by qualified individuals and under current policy will be maintained there at great cost to taxpayers until they die. Humane organizations, as well as others, believe the horses should be removed from the corrals promptly. Truly a moral and financial crisis exists now. With more horses from roundups anticipated in 1987 and 1988, even more horses will be placed in corrals.

BACKGROUND: The Wild Free-Roaming Horse and Burro Act directs the Secretary to destroy in the most humane and cost efficient manner possible excess wild horses and burros for which no adoption demand exists. However, the agencies placed an administrative moratorium on destruction of healthy excess animals in 1982 when uniform adoption fees were established. In response to recommendations from both a congressional subcommittee and the Office of Management and Budget to recover from the benefiting public a greater part of the Government's adoption-related costs, the agencies imposed fees of $200 per horse and $75 per burro. Some interest groups believed that the fees were too high and would reduce adoption demand, leading to a surplus of unadopted animals that would be destroyed. The fee for a wild horse was eventually lowered to $125, but the agencies have maintained the moratorium. During this time, large numbers of unadopted excess animals have accumulated in BLM's corrals and contract facilities.

RECOMMENDATION: The Secretary should implement the five-step process below, which provides several opportunities for disposition of the excess animals, and subsequently evaluate it.

1. Regular adoption program at full fees.
2. Special adoptions at altered fees.
3. Training of horses at prisons by inmates, with the trained animals to be made available for adoption. Animals not adopted within 30 days after training should be handled through steps 2 and 4, and if not adopted within 30 days, destroyed in accordance with step 5.
4. Placement of horses on private sanctuaries, with the animals maintained with non-Federal funds.
5. Euthanasia for any animal not disposed of within 90 days following BLM's certification of its availability for adoption.

To solve the immediate problem of the large number of animals on hand, some of which have been held for more than a year, the Board recommends that:

- All horses present in contract facilities should be removed before September 30, 1987, using the five-step process. The first four steps are to be used concurrently, with the more desirable animals placed under the first step and animals with poor conformation or 4 years of age or older placed through steps 2, 3, or 4.

- Because of the large number of animals on hand, humane destruction under step 5 will be accomplished gradually during the remainder of Fiscal Year 1987.

To address the problem of unadopted animals after the five-step process has been phased in during Fiscal Year 1987 (October 1, 1986, to September 30, 1987), all excess horses and burros removed from the public lands after September 30, 1987, shall be subject to the five-step process, without the special extension of time described in the above paragraph.

RATIONALE: All interested parties need to be assured that healthy animals destroyed pursuant to the Act are ones for which no adoption demand exists. More experience is needed in distributing horses, especially "unadoptables," through experimental placements (Step 2), training at prisons (Step 3), and private sanctuaries at nongovernment expense (Step 4). Although the numbers of horses that can be handled using each procedure are uncertain, there is hope that sizable numbers can be processed. For example, it is suggested that up to six candidate prisons each may take 600 horses, or up to 3,600 horses, in the next few months. Animals that cannot be placed through one of the first four steps are determined to be unadoptable and thus subject to destruction.

There is a surplus not only of wild horses and burros in the United States, but also dogs and cats. Shelters for homeless dogs and cats are operated by humane organizations. If they are unable to locate a suitable benefactor for an animal within a prescribed period of time (usually no longer than 30 days and frequently less), the animal is euthanized. Some 13 million dogs and cats are destroyed annually by humane organizations in this country. It is reasonable for the Federal Government to implement the legal provision for humane destruction when faced with a surplus of wild horses or burros.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE B: Adoption Fees

FINDING: The current levels of adoption fees are reasonable.

BACKGROUND: BLM and Forest Service regulations set adoption fees at $125 per horse and $75 per burro regardless of age, sex, conformation, or color of the animal. In addition, the Director may adjust or waive the adoption fee upon determining that the animals are unadoptable when the full adoption fee is required and that it is in the public interest to do so.

Based on previous experience, BLM has found that adopters prefer female animals of less than 4 years of age. Several members of the Board, as well as some individuals testifying before the Board, feel a more flexible approach to adoption fees may stimulate adoption of older animals.

RECOMMENDATION: The agencies should continue the current adoption fees of $75 per burro and $125 per horse, with variances to be made administratively to resolve specific placement problems. The agencies should also periodically compile a study of the grade horse market in relation to adoption fee levels.

RATIONALE: The present adoption fees help to ensure that individuals applying to adopt horses or burros are truly interested individuals who will care for the animals properly. Further, these fees discourage adoption for commercial gain or adoption by individuals for whom proper maintenance of the animals would be a financial burden, in which case the risk of inhumane treatment through neglect is increased.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE C: Sanctuaries Financed by Private Donations

FINDING: While documented experience is limited in holding wild horses or burros on private lands with private financial support, reports show some potential for accommodating more horses using this procedure. The potential number of private cooperators, as well as the potential number of horses, that could be involved remain to be identified.

BACKGROUND: The Board heard testimony in regard to the possibility of offering unadopted wild horses and burros to private sanctuaries, where they would be adopted and maintained on private lands through private donations. At least one such sanctuary exists at the present time in California.

RECOMMENDATION: The Board recommends that this option for removing horses from corrals be explored further and encouraged by and receive assistance (but not funds) from BLM and the Forest Service to monitor the efforts and establish a system of accountability for the animals. Information also should be assembled by these two agencies on the key question: what volume of horses potentially can be handled through this option?

RATIONALE: Efforts are needed immediately to help remove horses from corrals. This approach should help meet that goal. Private sanctuaries would serve two important purposes: they would relieve the Government of the financial burden of maintaining these animals indefinitely, and they would allow the animals to exist in a state approximating the wild free-roaming condition in which they lived on the public lands.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE D: Destruction of Old, Sick, and Lame Animals

FINDING: Transporting animals that are to be humanely destroyed to processing facilities that are somewhat distant from the capture site adds additional stress to the animals and incurs an unnecessary cost.

BACKGROUND: The Wild Free-Roaming Horse and Burro Act requires the humane destruction of old, sick, or lame animals as the first priority in removing excess animals. BLM currently captures all excess animals and usually transports them to the nearest BLM facility before destroying the old, sick, or lame. Approved methods for destruction are based on techniques approved by the American Veterinary Medical Association and include chemical injection or gunshot to the brain.

RECOMMENDATION: Wild horses and burros that are classified as old, sick, or lame, or that are severely injured, should be disposed of humanely in the field whenever possible and not be transported to facilities for destruction. In all cases, decisions on which animals are to be destroyed should be made by a veterinarian. Remains should be disposed of consistent with prevailing State health laws, e.g., burying, burning, rendering.

RATIONALE: Definitions of old, sick, and lame contained in BLM's regulations (43 CFR 4700.0-5) are reasonable. Where feasible, destroying old, sick, and lame animals on the ground is both more humane and more cost effective than transporting them to a processing center before destruction.

The legislative history of the Public Rangelands Improvement Act (PRIA), which established the order and priority for removal of excess wild horses and burros, indicates a strong preference for field destruction. In the legislative report accompanying the House version of PRIA, the Committee on Interior and Insular Affairs noted that selective culling of wild animal populations is a worldwide accepted method of controlling animal populations. The report continued: "For wild horses and burros, culling by Federal officials on the range appears to be far preferable [emphasis added] from a humane standpoint than rounding up animals and disposing of them."

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE E: Transportation of Captured Animals

FINDING: Head and other injuries can occur when pot-bellied, two-decked trucks are used to transport horses. Model national legislation that deals with equipment and procedures used to transport horses is expected to be introduced in the near future.

BACKGROUND: All captured wild horses and burros are transported by truck from the capture site to the BLM facility or from one adoption center to another. In most cases, BLM uses double-decker trailers to significantly reduce transportation costs. Care is taken to ensure that each animal has adequate head room; however, some groups have asserted that such transportation is inhumane to horses.

RECOMMENDATION: BLM should review the existing policy and establish specifications to ensure that equipment used to transport wild horses minimizes the risk of injury to the animals, yet is reasonably cost effective.

RATIONALE: Actions called for above would help ensure that horses and burros are transported in a safe manner, with risks for injury minimized. The Board recognizes that implementation of the recommendation may increase transportation costs but believes that ensuring the safety of the animals during transport should be the primary concern of the agencies.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE F: Preparation of Animals for Adoption

FINDING: Procedures used to prepare excess horses and burros for adoption are adequate and humane, but must be subject to frequent reviews to make sure they are executed in a continuing sensitive manner for each animal handled.

BACKGROUND: Board members visited two facilities (Palomino Valley and Lovelock) where animals are prepared for adoption. All animals receive necessary medical treatment, vaccinations, a worming compound, and blood testing. They also are aged and freeze marked. Animals maintained in corrals for some time also must have their hooves trimmed. To perform this work, each animal is either put through a squeeze chute, tranquilized, or secured by roping.

RECOMMENDATION: BLM should require and constantly urge its employees, as well as contractors, to carry out quality control to maintain equipment to prevent and minimize risks of injury to horses and burros processed for adoption. BLM should review the contracts used to obtain veterinary services and make sure stipulations are included that require use of appropriate techniques and medications in treating horses and burros, as well as conducting quality control on a frequent basis.

RATIONALE: The volume of animals being processed increases the risk of laxity in carrying out all steps necessary to ensure humane treatment of all the animals. Actions called for above will help meet the overall goal of preparing horses and burros for adoption using accepted safe and humane procedures and equipment. A "common sense principle" should be used to do whatever is necessary to prevent injuries to horses and burros. Some items warranting special attention include: (1) methods to restrain horses, (2) padded sides on squeeze chutes, (3) chute floors that are dirt-covered or of wood to avoid noise and stumbling encountered with metal floors, (4) medications that require refrigeration, and (5) sterile needles for each horse and burro.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE G: Adopt-A-Horse Program Marketing

FINDING: The BLM and Forest Service have tried numerous and creative methods for locating qualified individuals willing to provide feed and humane care for excess animals removed from the public lands. In Fiscal Year 1985, the cost of publicity efforts alone for the Adopt-A-Horse Program amounted to more than $490,000. Despite this investment, there is criticism that the agencies are not doing enough to locate qualified adopters for excess animals and that more funds and personnel should be directed to the effort.

The current expenditure by BLM to publicize the Adopt-A-Horse or Burro Program is inadequate to accelerate the rate and volume of adoptions. More publicity and other marketing efforts are needed to identify opportunities to adopt horses and burros, especially horses. The Board recognizes that BLM might benefit from assistance, such as that available through a marketing expert, to strengthen its adoption efforts.

BACKGROUND: On the generic level, such products as information packets, public service announcements, displays, publications, videotapes, and slide programs are developed. Public affairs expenditures for satellite adoption centers are primarily personnel related, although site-specific posters, flyers, etc., also may be developed.

RECOMMENDATION: The Board encourages BLM to seek assistance of a marketing expert to review the marketing strategy and publicity efforts to place excess animals through adoption. In addition, BLM should help citizens understand why surplus horses and burros must be removed periodically to protect and perpetuate desired vegetation in each herd management area.

The agencies should develop a written policy that reflects an aggressive attitude toward locating qualified adopters and identify those measures that the agencies could reasonably be expected to undertake to assure wide distribution of information about the availability of excess wild horses and burros.

BLM also should seek approval from the Department to use paid advertising in support of adoption efforts. Although every attempt should be made to obtain public service advertising first, in those areas where free advertising cannot be obtained, BLM should seek low-cost, effective alternatives to ensure the success of the adoption effort.

RATIONALE: Increasing adoptions is in large part a marketing function. Since BLM and the Forest Service are resource oriented agencies, they have limited marketing expertise. Agency publicity efforts should be supplemented with coordinated communications promoted and/or carried out by others, including previous adopters, humane organizations, etc.

DISPOSITION OF EXCESS WILD HORSES AND BURROS

ISSUE H: Titling Procedures

FINDING: At the beginning of this fiscal year, more than 50,000 animals had been maintained in private care for a year or more, and thus were eligible for transfer of title from the Government to their adopters. However, only about half of these animals had been titled. Responsibility for untitled animals remains with the Federal Government.

BACKGROUND: The Act presently provides that an adopter may be granted title to the adopted animal(s) upon application* and a determination by the Secretary that he/she has provided humane conditions, treatment, and care for the animal(s) for a period of at least 1 year. It has been suggested that the Act be amended to delete the requirements for both the application and the determination. The Secretary would be authorized to grant title to individuals who have maintained the animals for a period of 1 year where there is no evidence of inhumane care or treatment.

RECOMMENDATION: The adopter should be given certificate of title 1 year after the adoption date, thus automatically conveying title unless complaints of inhumane care or conditions are registered to BLM or humane officials.

RATIONALE: Streamlined procedures would eliminate Government responsibility and expense after 1 year; minimize violations of law; and improve compliance.

*A recent revision of BLM's regulations requires the adopter to apply for title at the time of adoption.

MANAGEMENT

ISSUE A: Appropriate Management Levels

FINDING: The population of wild horses and burros to be maintained, protected, and managed on the public lands continues to be a significant source of conflict among interest groups. In its simplest terms, the conflict pits local needs and priorities against the desires of those who would like to see substantial numbers of wild horses and burros maintained on the public lands. The appropriate management level for wild horses and burros is best arrived at as part of an allocative process that considers all uses of the area. The resource management planning process is a satisfactory vehicle for establishing appropriate herd management levels; however, evidence exists that the agencies have not always been fully effective in complying with the process.

BACKGROUND: BLM and the Forest Service determine appropriate management levels for wild horses and burros through similar resource management planning processes. The processes take into account all of the uses of the resources available that are recognized in the Federal Land Policy and Management Act and attempt to strike a reasonable balance among these uses based on the needs and desires of the participating public. Resource management plans are flexible and may be amended as conditions change.

The Forest Service has completed planning on its lands in areas where wild horses and burros will be maintained and estimates appropriate management levels at 1,225 wild horses and 350 wild burros. Although its planning is not yet complete for all herd areas, BLM estimates that appropriate management levels on its lands will total approximately 25,000-30,000 wild horses and burros. All BLM resource management plans are scheduled to be completed by September 30, 1988.

RECOMMENDATION: BLM and the Forest Service should continue to establish appropriate herd management levels through their existing resource management planning processes. The agencies should increase their effectiveness in using the planning process.

RATIONALE: The agencies are required by law to implement a planning process to provide for the uses of the public land resource. The processes being used by the agencies provide the flexibility to assure that resource conditions are maintained, local needs are met, a reasonable number of wild horses and burros are maintained on the public lands, and opportunities for public participation exist throughout the process. In addition, the planning process provides for periodic review of all areas where the animals existed in 1971 to determine their suitability for current wild horse and burro management.

MANAGEMENT

ISSUE B: Fertility Control

FINDING: Fertility control may be a possible alternative to, or adjunct of, removals to control population size.

BACKGROUND: Currently the agencies manage wild horse and burro populations by physical removal of excess animals from the public lands. It appears technically possible to implement fertility control, either in stallions or mares, thereby preventing unwanted reproduction. However, the cost of such a program is unknown at the present time. Implementing such a program would reduce the need for periodic roundups.

RECOMMENDATION: Fertility control, if available and practicable, should be used on a temporary basis where deemed necessary in specific herd management areas.

RATIONALE: Once appropriate herd management levels are attained, population control can be achieved effectively and cost-efficiently by periodic removals. However, it is imperative that the possibility of fertility control as an alternative to removals be thoroughly studied and evaluated. When sufficient data are available, decisions as to the practicality and timing of fertility control can be made.

MANAGEMENT

ISSUE C: Priority for Private Land Removals

FINDING: At times, private citizens have had to tolerate the continued presence of wild horses and burros on privately owned land, even after requesting removal of the animals, until BLM was able to schedule the removal.

BACKGROUND: The Wild Free-Roaming Horse and Burro Act states:

> "If wild free-roaming horses or burros stray from public lands onto privately owned land, the owners of such land may inform the nearest Federal marshall or agent of the Secretary, who shall arrange to have the animals removed."

BLM has been removing wild horses and burros from private lands, but has not necessarily given such removals a higher priority than those from public lands. BLM normally determines its removal schedule from public lands based on the animals' impact on the available resources and identification of appropriate management levels through resource management plans. Since the Act was passed, BLM has been involved in eight court actions filed by private landowners seeking to force BLM to remove animals in a more timely manner.

The issue of removals from private lands is particularly difficult in areas of checkerboard land patterns. In these areas, public and private land holdings are intermingled in alternating sections. Wild horses removed from private land in checkerboard areas almost invariably move back onto that land from adjacent public lands unless the herd is totally removed.

RECOMMENDATION: Excess animals should be removed in the following priority:

1. from private lands when requested by the landowner, and
2. from the public lands where resource degradation is the most significant.

RATIONALE: Although the language of the law requires immediate removal of excess animals from public lands, no timeframe is given in the Act for removal of wild horses or burros from private lands. The Board feels that private individuals should not be required to allow wild horses and burros to use their private lands and resources; therefore, it is recommended that requests from private landowners take first priority.

MANAGEMENT

ISSUE D: Inventory of Herds

FINDING: In spite of improvements in censusing techniques, considerable doubt remains in the minds of the public about the accuracy of wild horse and burro population estimates published by the agencies.

BACKGROUND: In 1983, BLM issued an Instruction Memorandum outlining appropriate wild horse and burro census techniques based on research conducted by the University of Minnesota under the auspices of the National Academy of Sciences. The two techniques most often used are the total count and mark-resight methods.

Total Count Method

Due to time and funding constraints, most censuses involve an attempt to count all animals on an area. These are always less than total counts, but by an unknown factor (error). The data are useful, however, in determining population trends. This method has its limitations in areas of high vegetation (e.g., trees) and rough terrain.

Mark-Resight Method

The mark-resight method involves marking (by paint, colored collar, etc.) a known number of animals in a herd area and then counting all animals observed. Using the number of resighted marked animals, the specialist projects an estimated population in the herd area.

RECOMMENDATION: The agencies should endeavor to develop and utilize more reliable inventory methods for wild horses and burros, recognizing habitat differentials.

RATIONALE: Consistent use of proven censusing techniques, which are made available for public review, should in time instill confidence in the agencies' population estimates. Increased credibility for these estimates will decrease the controversy surrounding management actions that are based on population estimates.

MANAGEMENT

ISSUE E: Rate of Increase of Herds

FINDING: The lack of quantitative data on individual herd areas regarding the rate of herd increase contributes to skepticism about the usefulness of the agencies' estimate of an average rate of population increase of 16 percent.

BACKGROUND: Several individuals testifying before the Board have expressed concern over BLM's use of an average estimated rate of increase in wild horse and burro populations. The National Academy of Sciences has looked at rates of increase and determined that they vary by herd, ranging from 3 to 20 percent. Rates also will vary within each herd from one year to another, often because of weather-related conditions or resource conditions.

RECOMMENDATION: The rate of herd increase should be established for each herd area through repeated censusing of the herd, undertaken at the same time of year and using the same technique each time.

RATIONALE: Like population estimates, the rate of increase used by the agencies has been questioned, mostly as being too high. Establishment of more accurate rates for individual herds and herd management areas will allow more credible projections for budget and planning purposes.

MANAGEMENT

ISSUE F: Adjustments to Appropriate Management Levels

FINDING: One basis for increasing or decreasing the number of wild horses and burros on public lands should be the condition of their habitat, but this concept is not widely accepted by parties interested in the protection and control of wild horses and burros nor adequately addressed by the agencies in the planning documents. In some quarters, the perception exists that wild horses and burros bear a disproportionate share of reductions to protect the public land resource. Much of the testimony heard by the Board reflected the viewpoint that the agencies place more emphasis on maintaining the levels of other users than on maintaining wild horses or burros.

BACKGROUND: An important consideration used by BLM and the Forest Service in determining the need for adjustments to wild horse and burro herd size is range trend: the change or lack of change in the condition of the habitat. However, changes in vegetative and other conditions occur very slowly in arid and semi-arid regions and are detectable only in the long term or with highly intensive monitoring systems. To enable evaluation of wild horse and burro impact on their habitat in the short term, study of the extent of utilization of major forage species is most often the only available monitoring method.

RECOMMENDATION: The agencies should (1) emphasize continued monitoring of habitat condition, trend, and utilization within herd management areas, and (2) develop and publish a policy statement that outlines the criteria that will be used and clearly articulates the basis for adjustments in wild horse and burro numbers.

RATIONALE: The factor common to all species of animals found on the public lands is the habitat (soil, vegetation, water, terrain, and climate) in which they live. Proper management of these lands should provide for the optimum number and mix of animals, including wild horses and burros, while improving the ecological site. The monitoring process provides a satisfactory mechanism for identifying needed changes in resource use levels by domestic livestock, wildlife, and wild horses and burros.

MANAGEMENT

ISSUE G: Intensive Management

FINDING: In some areas, management to perpetuate certain herd characteristics responds to public interest in preserving wild horses typical of those that historically inhabited the area.

BACKGROUND: In some locations, herd management area plans require that certain characteristics (such as color, conformation, size, markings, etc.) be maintained in the herd. When excess animals are to be removed from these areas, those animals displaying the desirable characteristics are returned to the area, while those without such characteristics are removed.

Some groups and individuals charge that this exceeds the "minimal feasible level" of management specified in the Wild Free-Roaming Horse and Burro Act.

RECOMMENDATION: Management for particular herd characteristics should continue to be determined for each herd area through the planning process.

RATIONALE: Management to perpetuate traditional characteristics of a herd is in keeping with the concept of preservation of wild horses and burros as "symbols of the historic and pioneer spirit of the West."

RESEARCH

ISSUE: Research

FINDING: The BLM works in conjunction with the National Academy of Sciences (NAS) to identify research needs, develop requests for proposals, and award research contracts through the competitive bidding process. Current research policy has rigidly defined scopes of work and contracts resulting in long-term projects that do not allow response to local conditions and management needs of the BLM. In the course of carrying out their studies, researchers sometimes identify additional areas of investigation outside the scope of work that would provide useful information. Some research must be specific to individual herd management units because of the uniqueness of some of those units as to types of horses and habitat differences.

BACKGROUND: Prior to 1978, nine separate and unrelated studies on wild horses and burros had been initiated by the agencies. In the Public Rangelands Improvement Act of 1978 Congress directed that:

> ". . . the Secretary shall contract for a research study of such animals with such individuals independent of Federal and State government as may be recommended by the National Academy of Sciences for having scientific expertise and special knowledge of wild horse and burro protection, wildlife management and animal husbandry as related to rangeland management. The terms and outline of such research study shall be determined by a research design panel to be appointed by the President of the National Academy of Sciences."

From this mandate came the NAS 1980 Phase I Report, a comprehensive review of current knowledge of wild horses and burros and recommendations for additional research. The Phase I Report was followed in 1982 by the NAS Final Report on Wild and Free-Roaming Horses and Burros. The Final Report cited the need for a firm base of scientific information for sound and effective management and called for completion of the long-term equid research program recommended in the Phase I Report. Five of these recommended research projects were funded by BLM and the Forest Service.

In 1985, Congress again provided funds for research to be conducted through the NAS. A $150,000 contract was negotiated with the NAS for its involvement in analyzing research needs, designing the solicitation, evaluating proposals, and interpreting the results of contracted research. After an analysis of field office research needs and recommendations, BLM consulted with the NAS about appropriate research topics. The BLM recommended that a higher priority be placed on methodologies, strategies and costs for population management, including herd manipulation and fertility control. The NAS concurred, and a solicitation was issued for research on three topics:

Population and Cost Projection Study
Wild Horse Parentage and Population Genetics Study
Fertility Control in Wild Horse Populations

The NAS also recommended additional analysis of wild horse blood samples previously collected in demography research. Based on the proposals received, no contract was awarded for the Population and Cost Projection Study. Three contracts are currently in progress on the following topics: fertility control in wild horses, blood analyses for condition evaluation in feral horses, and wild horse parentage and population genetics.

RECOMMENDATION: All research on wild horse and burro herds and habitats should be coordinated by BLM at the national level; BLM should reevaluate long-term research needs when the results of research in progress are available; and BLM should establish and maintain a list of short-term, site-specific projects to be considered for a share of any future wild horse and burro research funding.

RATIONALE: Future research needs should be determined in light of all information available from past studies. The Board believes that the existing research effort lacks adequate coordination within the agencies, and that opportunities for applied research have not been adequately identified.

LEGISLATION

ISSUE A: Sale Authority

FINDING: Sale authority is a controversial concept. In the past, bills introduced in Congress to provide sale authority have been supported by most livestock organizations and many wildlife and conservation groups. On the other hand, most humane and wild horse interest groups, many members of Congress, and many individuals are unalterably opposed to sale authority. Experience indicates that a bill to provide sale authority would be vigorously opposed.

BACKGROUND: In the past, legislation has been introduced in Congress that would have authorized the agencies to sell unadoptable animals at public auction. The Administration supported such legislation as offering another opportunity for the animals to be placed in private maintenance rather than being destroyed, as is currently required by the Act. Although one bill was approved by the Senate Committee on Energy and Natural Resources during the 98th Congress, no action was taken by the full Senate. No such legislation is now pending before Congress.

RECOMMENDATION: Sale authority is not recommended at this time. The five-step process should be given priority for disposition of excess animals. If the five-step process does not prove effective, consideration should be given to this and other alternatives for disposition of excess wild horses and burros.

RATIONALE: The Board believes that the five-step process should be given an opportunity to dispose of excess animals before other options are considered. However, large numbers of excess animals must not be allowed to remain in corrals for long periods of time at Government expense.

LEGISLATION

ISSUE 8: Extension of Helicopter and Motor Vehicle Use Authority

FINDING: The properly supervised use of helicopters and motor vehicles to gather wild horses and burros is safer and more efficient than other methods of removal.

BACKGROUND: The Federal Land Policy and Management Act of 1976 authorizes the Secretaries of Agriculture and the Interior to use or contract for the use of helicopters and motor vehicles in administering the Wild Free-Roaming Horse and Burro Act. Since the Act only applies to public lands under the jurisdiction of BLM and the Forest Service, other Federal land-managing agencies (such as the National Park Service, U.S. Fish and Wildlife Service, and Department of Defense agencies) must gather horses and burros under their jurisdiction without benefit of helicopters and motor vehicles.

RECOMMENDATION: Public Law 86-234 (passed in 1959 and sometimes called the Wild Horse Annie Act) should be amended to allow Federal agencies besides BLM and the Forest Service to use helicopters and motor vehicles to remove horses and burros from lands under their jurisdiction. To accomplish this, section 47 of chapter 3 of title 18 of the U.S. Code should be amended as follows:

Change the period at the end of section 47(a) to a comma and add:

. . . except that the Secretary of Agriculture, the Secretary of the Interior, and the Secretary of Defense may authorize the use of helicopters or, for the purpose of transporting captured animals, motor vehicles, in order to capture, remove, or relocate unbranded and unclaimed horses or burros on lands under their respective jurisdictions. Such use shall be undertaken only after public notification and, in order to ensure humane treatment of the animals, only under the direct supervision of the Secretary or a duly authorized representative of the Department involved.

RATIONALE: The 1959 law (PL 86-234) prohibiting the use of aircraft and motor vehicles was designed to reduce inhumane treatment of wild horses and burros. BLM and Forest Service accumulated experience demonstrates that the use of aircraft and motor vehicles for rounding up wild horses and burros is effective and should be made available to other Federal agencies.

LEGISLATION

ISSUE C: Public Hearings on the Use of Helicopters and Motor Vehicles

FINDING: The use of helicopters and motor vehicles in roundups is now generally accepted as routine, provided appropriate standards are met.

BACKGROUND: The Wild Free-Roaming Horse and Burro Act requires that the agencies hold public hearings prior to using helicopters and motor vehicles in the management of wild horses and burros. These meetings are generally poorly attended, with discussion centering on planning decisions rather than on the use of helicopters. In recent years, each BLM State Office has held one hearing to cover helicopter and motor vehicle use for the entire year. Previously introduced legislation would have modified this requirement to allow for public notification rather than public hearings.

RECOMMENDATION: Amend the Act to replace the requirement for public hearings before the use of helicopters and motor vehicles with a requirement for public notification of such use.

RATIONALE: The hearing requirement has become superfluous over time. Public notification would meet the public's need to know.

LEGISLATION

ISSUE D: Wild Free-Roaming Horse and Burro Act Preamble

FINDING: Healthy herds of wild horses and burros, which have no natural predators, are thriving on many areas of the public lands in the 10 Western States.

BACKGROUND: Congress found in Section 1 of the Wild Free-Roaming Horse and Burro Act that ". . . these horses and burros are fast disappearing from the American scene." Since passage of the Act in 1971, wild horse and burro populations on public lands administered by BLM and the Forest Service increased fairly steadily to more than 67,000 animals in FY 1980. Removal efforts in the last 2 years have resulted in total wild horse and burro populations estimated at 48,000 at the beginning of FY 1987.

RECOMMENDATION: Section 1 of the Wild Free-Roaming Horse and Burro Act should be amended to delete the language indicating the animals are in danger of disappearing. The section should be further amended to establish, as a policy of Congress, that wild horses and burros are to be managed under the principles of multiple-use land management.

RATIONALE: This change in the Act would reflect the change in the status of the wild horse and burro since the Wild Free-Roaming Horse and Burro Act was passed in 1971 and would recognize the multiple-use mandate contained in the Federal Land Policy and Management Act of 1976.

LEGISLATION

ISSUE E: Biennial Program Reporting Requirement

FINDING: Six reports to Congress on the administration of the Wild Free-Roaming Horse and Burro Act have been prepared by BLM and the Forest Service. Such reports are costly and duplicate other agency reports to Congress.

BACKGROUND: Section 11 of the Wild Free-Roaming Horse and Burro Act requires joint biennial reports to Congress on the administration of the Act.

RECOMMENDATION: Section 11 of the Wild Free-Roaming Horse and Burro Act should be amended to eliminate the joint biennial report requirement and replace it with an annual reporting requirement that can be incorporated in each agency's primary annual report to Congress.

RATIONALE: BLM and the Forest Service can provide similar information to Congress on a more timely (yearly rather than biennially) basis within other existing reports, such as required by the Federal Land Policy and Management Act of 1976.

APPENDIX 1: WILD HORSE AND BURRO ADVISORY BOARD CHARTER

1. Official Designation: Wild Horse and Burro Advisory Board.

2. Board Objectives and Scope: Assist and advise the Secretary of the Interior, through the Director, Bureau of Land Management, and the Secretary of Agriculture, through the Chief, Forest Service, on policy formulation and matters pertaining to the provisions of the Wild Free-Roaming Horse and Burro Act (Public Law 92-195).

3. Period of Time Necessary for the Board's Activities: Since its functions are related principally to application of Public Law 92-195 and associated policies, programs, and regulations, its need is expected to continue indefinitely. However, Board continuation will be subject to biennial review and renewal as required by Section 14(a)(2) of the Federal Advisory Committee Act (Public Law 92-463).

4. Official to Whom the Board Reports: Secretary of the Interior (Secretary), through the Director, Bureau of Land Management (Director).

5. Administrative Support: Administrative support for activities of the Board will be provided by the Bureau of Land Management.

6. Duties of the Board: At the request of the Director or his authorized representative, and in an advisory capacity only, the Board will gather and analyze information, make studies, and hear public testimony in order to offer advice and develop recommendations from a national, public-interest perspective for the Director, the Secretary, the Chief of the Forest Service, and the Secretary of Agriculture, on matters pertaining to management and protection of wild free-roaming horses and burros and such other related matters as the Secretaries may from time to time prescribe.

Specifically, the Board may be directed to:

a. Review current and proposed management policies on protection, management, and control of wild horses and burros and disposition of excess wild horses and burros and recommend appropriate changes within the constraints of Public Law 92-195 that will facilitate these actions.

b. Conduct public hearings or conferences to ascertain the views of the scientific community, humane groups, the conservation community, ranchers, and other affected interests.

c. Stimulate public and private participation to expedite the adoption process and to provide for private care and maintenance of animals determined to be unadoptable.