



Reproductive biology and reversible male chemical fertility control in feral horses  
by Anne Perkins

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE  
in Animal Science

Montana State University

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Abstract:

In order to function as a management tool for feral horse populations, male fertility control must be reversible and have little or no effect on libido. This thesis project was divided into three separate phases: 1) to determine the breeding season of feral mares, 2) to establish a quantifiable endpoint to evaluate stallion libido, and 3) to field test two reversible chemical fertility drugs on feral stallions. Four mares from the Pryor Mountain National Horse Range were captured and corralled in Billings, Montana. Blood samples were taken every three days for 13 months and assayed for LH, progesterone and total estrogens. A stallion held in an adjacent corral aided in detection of behavioral estrus. The ovulatory period began in April and ceased at the end of August indicating a complete and concise breeding season of five months. Eliminative marking behavior (EMB) was used to evaluate stallion libido. Eliminative marking behavior is the response of a stallion to the urination or defecation by another horse. Stallion response to eliminations by mature mares varied markedly with season, averaging 80% from April through July and 1% from November through February. Thus seasonal changes in eliminative marking behavior correlated with the breeding season. Two separate horse herds were used for testing Quinestrol (Q) and microencapsulated testosterone propionate (mTP). In Challis, Idaho, ten stallions were treated with varying dosages of mTP in December, 1979. The eliminative marking behavior of treated stallions were compared to control (untreated stallions) in Challis during the summer of 1981. There was no discernible difference in eliminative marking behavior. During the summer of 1982 the treated stallions and their bands were relocated and foals were counted. There was a 6.6% successful breeding rate among the treated bands while the control bands had a 37% successful breeding rate. This difference was highly significant ( $P < .01$ ) indicating that use of mTP may be an effective method of controlling or limiting population growth in feral horses. Quinestrol was tested on a smaller herd in Juntura, Oregon. All three stallions in a herd of 21 horses were darted with Q during the winter of 1980. Eighteen months later foals from the Juntura herd were counted. There was a successful breeding rate of 78% among the Q treated herd and 47% among a control herd located 25 kilometers away. The greater percentage of foals to mares in the treated group was insignificant ( $P < .05$ ). Reasons for these results are discussed along with management implications.

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Signature Anne Perkins

Date March 5 1982

In memory of Dr. Edward L. Moody,  
who believed in my ability  
to succeed in graduate school.

REPRODUCTIVE BIOLOGY AND REVERSIBLE MALE  
CHEMICAL FERTILITY CONTROL IN FERAL HORSES

by

ANNE PERKINS

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

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## TABLE OF CONTENTS

	Page
Dedication . . . . .	i
Vita . . . . .	iii
Acknowledgements . . . . .	iv
Table of Content . . . . .	v
List of Tables . . . . .	vii
List of Figures. . . . .	viii
Abstract . . . . .	ix
Introduction . . . . .	1
Chapter I - Literature Review. . . . .	4
A Brief History of Feral Horses in America . . . . .	4
Introduction of Horses to America. . . . .	4
Rise and Fall of Feral Horse Numbers . . . . .	6
Protection for the Feral Horse . . . . .	8
Public Attitudes Toward Feral Horses . . . . .	10
Adopt-A-Horse Program. . . . .	12
Biology of the Feral Horse . . . . .	15
Social Organization of Feral Horses. . . . .	15
Review of Sexual Behavior and Reproduction in Stallions. . . . .	19
Review of Sexual Behavior and Reproduction in the Mare . . . . .	22
Reversible Chemical Fertility Control. . . . .	26
Review of Past Research in Male Contraception. . . . .	26
Screening of Potential Anti-Fertility Compounds. . . . .	30
Chapter II - Investigation into Feasability of Quinestrol (Q) and Microencapsulated Testosterone Propionate (mTP) as a Reversible Chemical Fertility Control in Feral Stallions . . . . .	36
Phase I - Seasonal Estrus Patterns in Feral Mares. . . . .	36
Introduction . . . . .	36
Methods and Materials . . . . .	36
Results . . . . .	41
Discussion. . . . .	47

	Page
Phase II - Evaluation of Behavioral Response to Chemical Fertility Compounds . . . . .	53
Introduction . . . . .	53
Methods and Materials . . . . .	54
Results . . . . .	57
Discussion . . . . .	65
Phase III - Field Test of Reversible Chemical Fertility Control on Feral Stallions . . . . .	67
Introduction . . . . .	67
Methods and Materials . . . . .	68
Challis, Idaho . . . . .	68
Juntura, Oregon. . . . .	70
Results . . . . .	72
Foal Counts - Challis, Idaho . . . . .	77
Foal Counts - Juntura, Oregon. . . . .	81
Discussion . . . . .	87
Challis, Idaho . . . . .	87
Juntura, Oregon. . . . .	88
Chapter III - Discussion and Summary . . . . .	92
Management Implications. . . . .	94
Suggested Future Research. . . . .	98
Appendices . . . . .	99
1. Wild and Free-Roaming Horse and Burro Act. . . . .	100
2. Description of mTP Treated Stallions . . . . .	102
3. Map of Challis Horse Range . . . . .	104
4. Description of Juntura Test Herd . . . . .	105
5. Band Descriptions of Challis Herd, Summer 1981 . . . . .	106
6. Juntura Herd Social Organization . . . . .	112
Literature Cited . . . . .	113

## LIST OF TABLES

Table		Page
1	Frequency of Various Behaviors Exhibited by Feral Stallions . . . . .	58
2	Incidence of Elimination Marking Behavior in Feral Horses. . . . .	58
3	Elimination Marking by Harem Stallions in Response to Urination and (or) Defecation by Other Members of Their Band. . . . .	59
4	Behavior of Harem Stallions in the Challis Herd June 9 - July 15, 1980 . . . . .	75
5	Behavior of Harem Stallions in the Juntura Herd June 28 - July 3, 1980 . . . . .	76
6	Band Composition of mTP Treated and Control Bands. . .	80
7	Foaling Data for mTP Treated and Control Bands . . . .	80
8	Description of Hog Creek Control Herd. . . . .	84
9	Age and Sex of Data on Hog Creek Control Herd. . . . .	85
10	Band Composition of Q Treated and Control Bands. . . .	86
11	Foaling Data for Q Treated and Control Bands . . . . .	86

## LIST OF FIGURES

Figure		Page
1	Progesterone and LH levels for the mare Dahlia. . . . .	42
2	Progesterone and LH levels for the mare Columbine . . . . .	43
3	Progesterone and LH levels for the mare Sunflower . . . . .	44
4	Progesterone and LH levels for the mare Tiger Lily. . . . .	45
5	Frequency of Elimination Marking Behavior by Feral Harem Stallions in Response to Elimination by Harem Mares . . . . .	60
6	Frequency of Occurrence of Each Component of the Elimination Marking Behavior Display by Feral Stallions During the Year . . . . .	62

## ABSTRACT

In order to function as a management tool for feral horse populations, male fertility control must be reversible and have little or no effect on libido. This thesis project was divided into three separate phases: 1) to determine the breeding season of feral mares, 2) to establish a quantifiable endpoint to evaluate stallion libido, and 3) to field test two reversible chemical fertility drugs on feral stallions. Four mares from the Pryor Mountain National Horse Range were captured and corraled in Billings, Montana. Blood samples were taken every three days for 13 months and assayed for LH, progesterone and total estrogens. A stallion held in an adjacent corral aided in detection of behavioral estrus. The ovulatory period began in April and ceased at the end of August indicating a complete and concise breeding season of five months. Eliminative marking behavior (EMB) was used to evaluate stallion libido. Eliminative marking behavior is the response of a stallion to the urination or defecation by another horse. Stallion response to eliminations by mature mares varied markedly with season, averaging 80% from April through July and 1% from November through February. Thus seasonal changes in eliminative marking behavior correlated with the breeding season. Two separate horse herds were used for testing Quinestrol (Q) and microencapsulated testosterone propionate (mTP). In Challis, Idaho, ten stallions were treated with varying dosages of mTP in December, 1979. The eliminative marking behavior of treated stallions were compared to control (untreated stallions) in Challis during the summer of 1981. There was no discernible difference in eliminative marking behavior. During the summer of 1982 the treated stallions and their bands were relocated and foals were counted. There was a 6.6% successful breeding rate among the treated bands while the control bands had a 37% successful breeding rate. This difference was highly significant ( $P < .01$ ) indicating that use of mTP may be an effective method of controlling or limiting population growth in feral horses. Quinestrol was tested on a smaller herd in Juntura, Oregon. All three stallions in a herd of 21 horses were darted with Q during the winter of 1980. Eighteen months later foals from the Juntura herd were counted. There was a successful breeding rate of 78% among the Q treated herd and 47% among a control herd located 25 kilometers away. The greater percentage of foals to mares in the treated group was insignificant ( $P < .05$ ). Reasons for these results are discussed along with management implications.

## INTRODUCTION

Since passage of the 'Wild Free-Roaming Horse and Burro Act' (Public Law 92-195) in 1971, numbers of horses inhabiting public lands have steadily increased. Due to the fixed range size which horses inhabit and the lack of natural predators, these animals are facing a serious overpopulation problem. The need for sound management of horses which is both humane and economical is apparent. A paradox exists because to many Americans wild horses represent a symbol of freedom and spirit and any form of management which destroys or interferes with that symbol would not be acceptable. Unlike livestock which is regulated with annual permits, and game which is regulated through a hunting season, the horse is protected.

The purpose of this project was to investigate the use of a reversible fertility control drug for use on feral stallions. In order to be functional as a management tool, the male fertility control utilized must meet these criteria:

1. It must inhibit spermatogenesis.
2. It must not interfere with the stallion's ability to maintain a harem band. Otherwise, untreated bachelor stallions may take over the band and continue to produce foals.
3. It must be reversible in its action so that the gene pool is not irreversibly altered and the population is capable of regenerating itself whenever necessary.

4. It must be inexpensive and easily administered.

-Because of the social organization of feral horses, a fertility control which focuses on the harem stallion is a logical approach. For each stallion treated there may be one to five mares prevented from foaling the following spring. Another very important advantage for using a fertility control is its reversibility. Feral horse herds have a number of unique individuals. Changes in conformation from the characteristics deemed desirable by domestic breeders (e.g. small heads, small jaws, trim throat latches, large body size, etc) may be the result of natural selection. Feral horses are surviving because of their genetic makeup. The current policy of removing animals eliminates some components of the gene pool. If this continues, we may no longer have a feral horse population but rather a government sponsored horse breeding program. A great deal can be learned about the behavior and physiology of domestic horses from feral horses. To inflict subjective qualities for selection on feral populations contradicts one of the purposes for their existence; PL 92:195 states that feral horses contribute to a diversity of life forms within the nation. A chemical fertility control method would only temporarily interfere with natural selection since it is a reversible process. Therefore, the long term process of natural selection may continue to influence the composition of the population.

Very little is known about the sexual behavior and reproductive

physiology of feral horses. A solid foundation of knowledge is necessary before manipulation of their natural state is practical for management purposes. There are two possible deleterious consequences which could result from the use of a male contraceptive without adequate knowledge. If feral mares are capable of conceiving through the fall, a reversible fertility inhibitor used on stallions may only postpone the reproductive season. High foal mortality would undoubtedly result from such a delayed breeding season because mares would foal in the winter months. Secondly, should the fertility control drug alter the behavior of harem stallions, band stability may be disrupted and bachelor stallions could mate with the mares. A method for determining the effect of the fertility control drug on stallion behavior is necessary. In order to address these problems, this project was divided into three separate phases:

1. To determine the breeding season of feral mares.
2. To establish a quantifiable endpoint for assessing reproductive behavior in feral stallions.
3. To administer a fertility control drug to a selected group of feral stallions and then compare the number of foals in their bands to control bands.

## CHAPTER I

### LITERATURE REVIEW

#### A Brief History of Feral Horses in America

##### The Introduction of Horses to America

The modern horse (Equus caballus) is not a native species to North America (Wyman, 1945; Cunninghaeme, 1949; Dobie, 1952; Rey, 1975; Thomas, 1979). Eohippus, Mesohippus and Merychippus (prehistoric ancestors to Equus) were indigenous to this continent and fossil records of Equus dating back to around 8,000 B.C. have been found in many places in North America (Simpson, 1961; Thomas, 1979). Around 10,000 years ago the horse vanished from North and South America but continued to thrive in Asia. Reasons for the disappearance of Equus in the Americas are unknown, but several theories have been proposed and throughly discussed by Wyman (1945), Simpson (1961), Thomas (1979) and others. From Asia, horses spread through Europe, the Middle East, and Africa.

Lundholm (1949), a Swedish zoologist, has hypothesized that Equus caballus sprang from at least several types of wild horses. By studying fossil remains he divided early wild horses into two major groups: 1) the eastern group which included the Tarpan and Przewalski's horse, and 2) the western group which was subdivided into: a) Germanic type, a large horse which became the forerunner of the heavy draft horses, and b) Microhippus, which became the forerunner of the Arabian horses.

The Tarpan was once thought to be the true progenitor of the modern horse (Wyman, 1945). However, Lundholm (1949) suggested that there were several different types of wild prehistoric horses in Eurasia, and that these different types gave rise to different domestic types in the locations where they existed.

Some of the western group (*Microhippus*) migrated into Asia Minor, eventually into North Africa and the northern shores of the Mediterranean Sea. This group of horses underwent dramatic changes, becoming fine boned, fleeter, and 'hot-blooded' (more red blood cells per cubic centimeter of blood). The changes in these horses were likely due in part to selective breeding for swift war-horses for use in desert terrain. These horses, known as Arabians and Barbs, eventually became the source of breeding for almost all domestic horse breeds except the draft horses (Thomas, 1979).

The first modern horses in America were introduced by the early Spanish explorers (Wyman, 1945; Ryden, 1970; Rey, 1975; Thomas, 1979). Some of these horses escaped or were stolen by Indians and the descendents of these horses became the first free-roaming horses in America (Wyman, 1945; Ryden, 1970; Rey, 1975). From these horses came the Indian ponies and the great herds of horses that roamed the western States (Wyman, 1945).

In addition to the Spanish horses brought to America, northern European breeds were introduced as settlers from other nations arrived

(Wyman, 1945; Ryden, 1970). Escapees from these settlers, miners, ranchers and explorers mingled with the Spanish horses. As a result, from the time of the introduction of northern European breeds until present day, many of the free-roaming horses have a mixture of bloodlines in their lineage including some animals that are only second generation wild (Bureau of Land Management, 1973; Rey, 1975). Regardless of a horse's breeding, if it is roaming free on public lands in the U.S., somewhere in its ancestry it evolved from imported, domesticated stock (Wyman, 1945; Ryden, 1970; Denhardt, 1975; Rey, 1975; Thomas, 1979) and is therefore not truly wild, but rather feral.

#### Rise and Fall of Feral Horse Numbers

The population of feral horses continued to climb from their introduction to America in 1519, until they reached a peak in the early 1800's. Dobie (1952) estimated that two million feral horses existed at that time. These feral horses were competing with a burgeoning cattle industry so ranchers began a concerted effort to eliminate feral horses from the range (Rey, 1975). Prior to 1930, mustangers captured and sold feral horses. The rate of removal generally reflected the market price in the Eastern United States and Europe; and the horses were in no danger of extinction or large scale declines (Wyman, 1945; Ryden, 1970; Rey, 1975).

In 1934 the Taylor Grazing Act was passed which eventually led to

the creation of the Bureau of Land Management (BLM). This federal agency was and still is responsible for managing the majority of federal range lands. The Act was passed in order to regulate grazing in the West. Section nine of the act specifies that forage should be allocated for use of wildlife, but the horse was defined as a feral animal and therefore did not qualify for multiple use consideration under the act (Rey, 1975). The policy of the BLM as quoted by its first director was "...the removal of wild horses from public ranges..." (Wyman, 1945; Rey, 1975).

When the Taylor Grazing Act was passed there were approximately 150,000 feral horses on public lands in 11 western states (Thomas, 1979). Range managers considered the horses a threat to the range and ranchers were reluctant to share their assigned grazing rights with wild horse herds (Ryden, 1970; Rey, 1975). In order to control and reduce horse numbers, cattlemen along with professional mustangers and the BLM cooperated on feral horse roundups (Thomas, 1979).

Although federal policy and cattlemen promoted elimination of horses from public lands, the biggest factor in the reduction of the herds during the late 1940's and early 1950's was the market for horseflesh in chicken feed, canned pet food and for human consumption (Ryden, 1970; Rey, 1975). The demand for pet food boomed as the American population became less rural and more urban. As more people started to buy canned pet food, it became more profitable to capture

and deliver feral horses to the canneries (BLM, 1973; Rey, 1975). With a means of making horse hunting profitable, feral horse populations were steadily reduced from 1930 to 1960 (Rey, 1975).

During the late 1920's and early 1930's, horsemeat was processed for human consumption. Horse slaughtering and processing plants under federal jurisdiction grew in number from ~~1923~~ until 1930. During this period thousands of wild horses were disappearing from the western ranges, and export figures for that period show an increase in pounds of horsemeat being shipped to Europe (Thomas, 1979).

#### Protection for the Feral Horse

Prior to the Wild Free-Roaming Horse and Burro Act, commercial mustangers were interested in capturing as many 'pounds' of horseflesh as possible (Velma Johnston, personal communication). Horses were hazed with airplanes into corrals where they could then be loaded onto stock trucks and delivered to canneries. According to Velma Johnston (Wild Horse Annie), horses suffered a great deal and frequently were loaded on stock trucks bleeding, suffering from broken legs or other wounds.

The public attitude toward these round-ups were not always what it is today. Thomas (1979) thoroughly researched popular magazines during the years following World War II. She found that the feral horse was looked at as a serious competitor for the grass needed to

produce beef and mutton for a hungry world. She said:

"the news media and national magazines carried occasional stories about reductions of the wild horse herds but viewed it as progress or nostalgic regret for the passing of the Old West".

Most people in the United States were not familiar nor knowledgeable about wild horse round-ups. During the 1950's, articles began to appear in popular magazines concerning the inhumane methods of mustanging (Bundy, 1953; O'Brien, 1957; Ryden, 1970). The fight to protect and save feral horses began with a small handful of individuals, but in time gained tremendous strength in numbers. Horse protection organizations were created which are still active today in policing feral horse management.

In 1951 Velma B. Johnston (Wild Horse Annie) of Reno, Nevada, saw a truckload of horses in transit for slaughter. She was appalled by their poor condition and by the way they were being handled. She decided to do something about it through legislative action. In 1959 Public Law 86-234, better known as the 'Wild Horse Annie Law' was passed. This law made capturing wild horses on public lands using aircraft or motorized vehicles an offence punishable under federal law. There is nothing in the 'Wild Horse Annie Law' to determine whether the horses were the responsibility of the Federal Government or the states, so for many years the status of the feral horse remained uncertain (Rey, 1975). Enforcement of the law was difficult

and round-ups continued to occur through the use of legal loopholes (Ryden, 1970).

Horse enthusiasts became involved and concerned about the fate of feral horses. Letter writing campaigns were initiated by horse protection groups along with a strong lobbying effort (Joan Blue, personal communication; Thomas, 1979). On December 15, 1971, Congress passed Public Law 92-195, the 'Wild Free-Roaming Horse and Burro Act'. This act protected all feral horses and burros from 'capture, branding, harassment, or death', and placed the responsibility for management of these animals with the Bureau of Land Management (BLM) (see appendix 1 for complete law).

#### Public Attitudes Toward Feral Horses

Prior to the efforts of Wild Horse Annie and others like her, very little concern or pressure was placed on management or control of feral horse herds. Horses were rounded up in response to their market value. As their market value increased and round-ups became more frequent, some people felt that feral horses would vanish from the range as did the American bison (Ryden, 1970). Three basic attitudes evolved concerning feral horses and they still exist today.

The first group includes stockmen and some range managers. This group considers the feral horse to be simply a free-roaming domestic horse which in large numbers are a nuisance and a detriment to the

range (Don Gossey, personal communication; Bob Larkin, personal communication).

2 The second group includes native wildlife enthusiasts who are concerned about the encroachment of an introduced and highly competitive species on a delicate habitat (Hansen and Clark, 1977).

3 Finally there are the horse protectionists who view the feral horses as symbols of the Old West with a spirit of freedom and pride. The last group has become the largest, at least in political power, over the past decade. As management decisions are made by the BLM all three groups are heard, but pleasing all three is impossible according to John Boyles, director of free-roaming horses and burros for the BLM.

Joan Blue, president of the American Horse Protection Association (AHPA), believes management of feral horses is unnecessary and that the BLM's approach is a policy of mismanagement (Personal communication, 1981). She states:

"I go along with Hope Ryden, our wild horse advisor, in believing that horse populations will regulate their own numbers if they are left alone."

According to Blue, AHPA has spearheaded efforts to protect and defend the rights of horses. Thousands of dollars have been spent seeking court injunctions to halt BLM round-up efforts.

Adopt-A-Horse Program

The Wild Free-Roaming Horse and Burro Act created a new category of animal (national heritage specie). Since this category was neither livestock nor wildlife, there was no precedent for management. Unfortunately PL 92-195 did not spell out how the BLM was to manage feral horse herds nor did it address the problem of population control.

Forage on the national resource lands was allocated to livestock and wildlife under the Taylor Grazing Act of 1934. Domestic livestock are controlled by an annual license; game animals are controlled by established hunting seasons. Horses and burros have minimal predators and under protective legislation increased in numbers substantially between 1971 and 1976 (Monroe, 1977).

When PL 92-195 was passed, there were 17,300 horses in nine western states (Box, 1975). This number more than tripled in the next six years (Thomas, 1979). Increasing numbers of horses placed increasing demands on forage supply. Loss of vegetation increases erosion which further reduces the productivity of the land (Stoddart, Smith and Box, 1955). In order to minimize deteriorating range conditions, BLM field offices were instructed to initiate plans to reduce horse populations back to the 1971 level (Monroe, 1977). Studies were to continue for determining the optimum number of horses for each area. Due to the time it took for the BLM to initiate action and the difficulties encountered in the rounding up of the horses, they fell

far short of their goal. As of May, 1975, there were 49,000 feral horses on public lands (Thomas, 1979).

In May of 1976, the BLM implemented a nationwide "Adopt-A-Horse" program, appealing to horse enthusiasts to provide "foster" homes for excess horses and burros. The purpose of this program was to round up feral horses and relocate them with people willing to care for and maintain them at their own expense. The animals could not be sold or used for any commercial purpose (Monroe, 1977).

At first the BLM was prohibited from using aircraft to round up the feral horses. Therefore, round-ups were conducted using saddle horses and driving feral horses into traps. This method was not only time consuming and primitive, but costly. The cost of capturing, feeding and finding homes for excess horses averaged \$800.00 per captured horse during the first year of non-motorized round-ups (Rey, 1975).

On October 21, 1976, the use of helicopters for rounding up horses was made legal by the 94th Congress. This effectively reduced the cost of adoption of horses to between \$300 to \$350 per animal (Monroe, 1977). However, problems with the Adopt-A-Horse program have not ended because helicopters are now legal. Reports from various horse ranges have indicated an increase in population ranging from 13% to 28% per year (Englebright, 1975; Feist, 1975). The number of animals rounded up has not kept pace with overall population growth.

The BLM has estimated that approximately 11,000 horses per year should be removed in order to protect forage and maintain the population (BLM report, 1980). By the end of fiscal year 1981, the total number of horses and burros which had been adopted since the beginning of the program totaled 29,000.

The BLM has recently implemented policy changes in the Adopt-A-Horse program in an attempt to place more feral horses and burros in homes. Prior to these recent policy changes, adoptees were merely custodians of federal property. That is, they carried all the expenses of the animals but did not own them. Under the new policy, after caring for a feral horse or burro for one year, the adoptee can apply for a clear title. Also, each individual is now allowed to adopt up to four animals per year. Because of these changes, more people are willing to adopt. In 1981, 10,149 horses were adopted (Fred Batson, personal communication).

In spite of efforts to make the Adopt-A-Horse program an effective method for controlling feral horse populations, numbers continue to increase. There are numerous applicants expressing an interest in adopting feral horses. Currently there are 14,000 applicants requesting 38,000 animals. Most people, however, want only young animals. There are relatively few individuals with the knowledge, expertise, facilities and finances to care for older feral horses (Jerry Wilcox, personal communication).

Under the Reagan Administration, Secretary of the Interior James Watt has announced that the federal government cannot afford to give away horses. As of October 1, 1981, the government will be charging a \$200 adoption fee. This will substantially reduce the number of people willing to adopt. The Department of Interior estimates that 5,000 horses per year will be adopted leaving 6,000 to be destroyed (American Horse Protection Association, 1981).

The Adopt-A-Horse program has been an expensive venture for the BLM. In 1979 the national budget for management of feral horses and burros totalled \$3,391,000. In 1980 and 1981 it grew to \$5,150,000 and \$6,660,000 respectively. The 1982 budget is projected at \$6,826,000. In 1981, 66% of the money budgeted for management of feral horses and burros went to the Adopt-A-Horse program (Fred Batson, personal communication).

### Biology of the Feral Horse

#### Social Organization of Feral Horses

Numerous studies have described the social organization of feral horses (Feist, 1971; Pelligrini, 1971; Hall, 1972; Feist and McCullough, 1975; Hall, unpublished data; Welsh, 1975; Clutton-Brock et al., 1976; Keiper, 1976; Berger, 1977; Green and Green, 1977; Rubenstein, 1978; Salter, 1978; Nelson, 1979; Miller, 1979; Perkins et al., 1979). Free-roaming horses are highly social animals and are

found in three types of family units known as bands. A harem band consists of a single stallion, a lead mare, other mares and their offspring. Multi-male bands have been reported by Keiper (1976); Green and Green (1977); Miller (1979); Boyd (1979); and Denniston (1979). Finally, a less cohesive group consisting of all males is known as a bachelor band (Feist, 1971; Hall, 1972; Perkins et al., 1979). Occasionally older lone stallions are reported (Perkins et al., 1979).

Changes in band composition are usually the result of younger animals leaving their original band (Feist, 1971; Green and Green, 1977). In the Pryor Mountain National Horse Range, multi-male bands have not been reported. It appears that when the testes descend at sexual maturity (approximately 3 years of age), the young males are driven from the band by the harem stallion (Perkins et al., 1979). Feist (1971) found that immature females accounted for most of the changes in band composition he observed in the Pryor Mountains feral horse population.

Two major factors are responsible for maintenance of band structure. They are mare bonding and stallion behavior. Mare bonding has been observed both in feral (Feist, 1971; Kirkpatrick et al., unpublished data) and domestic mares (Imanishi, 1950; Ralston, 1977). Joubert (1972) removed the dominant male from a band and the females appeared to be very closely bonded and actively searched for the

missing male. They rejoined with the male when he was released one month later. Similarly, four feral mares from the Pryor Mountains of Montana held in captivity for one and one-half years were released and remained a cohesive unit which a stallion later joined (Kirkpatrick et al., unpublished data).

In conjunction with mare bonding, stallion behavior is significant in maintaining band cohesion. Feist (1971) studied the behavior of harem stallions in the Pryor Mountains and found that stallions herd the band away from possible outside threat or the closeness of another band. He also observed stallions herding another band or individual not part of his band away from his band. By quantifying initiation of movements and herding behavior, he suggested that stallions are the principal leaders of the band. Inferior stallions (i.e. crippled or old) may lose their mares to another stallion (Feist, 1971).

Not all studies have supported fidelity or band cohesiveness. Nelson (1980) found that prior to the onset of parturition and breeding in late March, bands on the Jicarilla District of the Carlsen National Forest in New Mexico begin to break up, with females and their offspring moving between adjacent bands. Mares were not found with their original primary band, but were either with another band, by themselves, or with all female fractions of a previous band (Nelson, 1980).

Multi-male bands in the Red Desert of Wyoming have been the focus of several studies (Boyd, 1979; Denniston, 1979; and Miller, 1979). Denniston found that in the Red Desert multi-male bands are formed when: bachelor bands acquire females; a bachelor eventually is accepted by an established band; or a colt remains in his original band. Unlike horses on the Pryor Mountain range, if a Red Desert colt remains with his parental band beyond the age of 2 or 3 years, he is not driven off. His presence is largely ignored by the mature stallion(s). If a bachelor attempts to join a well established band, he will be challenged, chased and driven off repeatedly by the mature male(s). Often the bachelor persists in his attempts, trailing the band at some distance, grazing close to them and watering with or immediately after them. They may eventually be tolerated (Denniston, 1979; Miller, 1979). As mares come into estrus, subordinate stallions of a multi-male band may share them with little friction from other band stallions (Boyd, 1979; Denniston, 1979; Miller, 1979).

The kind of social organization consisting of multi-male family units has not been thoroughly researched previous to this study of the Red Desert horses. Klingel (1969, 1974, 1979) describes only two types of social system in wild equids: harems and territorial males. Since feral animals tend to revert toward the wild condition, Denniston (1979) suggests that Red Desert horses are only partly reverted toward a harem system.

Review of Sexual Behavior and Reproduction in Stallions

The physiological mechanism most critically involved in male sexual behavior and reproduction is the hypothalamic-pituitary axis (Dorner et al., 1968). Gonadotropin releasing hormone (GnRH) which is secreted from the hypothalamus releases both follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the adenohypophysis (Sorenson, 1979). In the stallion, LH acts to stimulate testosterone secretion from interstitial cells (cells of Leydig), while FSH is required for the maturation of spermatids into fully developed sperm cells (Hafez, 1974; Sorenson, 1979; Houpt, 1980). More recent research (Davies 1982) has indicated that FSH has a physiological role in stimulating spermatogenesis in immature animals and restoring spermatogenesis in hypophysectomized or estrogen treated adults. However, experiments in normal adults suggest that established spermatogenesis is not FSH dependent. Testosterone then acts on the brain, especially the anterior hypothalamus preoptic area, in conjunction with the appropriate stimuli from a receptive mare to produce male sexual behavior (Houpt, 1980). In addition, testosterone is required for reduction division of the primary spermatocyte to secondary spermatocytes and further division of secondary spermatocytes to spermatids (Hafez, 1974).

Some domestic stallions may show sexual behavior throughout the year. However, stallions are seasonal breeders. Pronounced seasonal

variations have been noted in seminal plasma, total seminal volume and spermatozoa concentrations (Pickett et al., 1970, 1975; Van Der Holst, 1975). Two ejaculates at hourly intervals were collected once weekly from Quarter-horse stallions between May 1969 and May 1970. Sperm concentration/ml in second ejaculates was lowest in December and highest in May (Pickett et al., 1975). Semen composition showed an even greater change in response to season. Van Der Holst (1975) demonstrated that length of photoperiod may be the primary factor influencing stallion semen. Maintenance of normal stallions in continuous light during the winter months appeared to improve spermatogenesis significantly.

Corresponding seasonal changes in plasma androgens and testosterone have been demonstrated by Berndtson et al. (1974), with a nadir in December and peak levels during the spring reproductive season. A study on 34 feral stallions from the Pryor Mountains indicated similar results. Androgen and testosterone concentrations were below 2 ng/ml during the non-breeding months and above 2.38 ng/ml during the breeding months. Field observations indicated that peak breeding activity occurred at the same time as peak androgen/testosterone concentrations (Kirkpatrick et al., 1977b). Behavioral studies of Feist (1971), Feist and McCullough (1975), Pellegrini (1971) and Berger (1975) indicate maximum aggressive and sexual behavior of feral horses occurs during May and June. These behaviors correspond to the

periods of highest androgen and testosterone levels found in both domestic and feral horses and to semen quality in domestic horses.

Diurnal variation of plasma testosterone has been examined both in feral (Kirkpatrick et al., 1976) and domestic (Sharma, 1976) stallions. Both studies revealed stallions possessed a diurnal testosterone rhythm with a peak occurring at 0800 hours. The nadir occurred at 2000 hours in domestic stallions and 2300 hours in feral stallions. Kirkpatrick et al. (1976), noted that one of the feral stallions, an eight year-old bachelor which had never made an effort to acquire a harem since sexual maturity, had a significantly lower mean testosterone concentration than the harem stallions in the study.

Plasma testosterone levels were measured in 21 two year old colts from the Pryor Mountains (Angle et al., 1979). Nineteen of the colts with a mean testosterone concentration of 1.14 ng/ml, had undescended testes. Two of the colts with descended testes had concentrations of 1.9 and 4.8 ng/ml. Field observations of these colts (Perkins et al., 1979) revealed that the 19 colts with undescended testes were permitted to remain in their harem bands, while the two colts with descended testes, were driven from their respective harem bands during their second spring. Angle et al. (1979) suggested that as plasma testosterone levels in colts approach that of a mature stallion, either behavioral changes or testosterone-based pheromonal messages emanating from the colt may trigger their ejection from their harem bands.

In studying reproductive patterns and the regulation of fertility in feral stallions, a method for examining sperm production which was safe and would not cause infection or hematoma formation was needed. A simple testicular biopsy method for use in the field was developed by Turner et al. (1979). The method was not adequate for quantitatively evaluating spermatogenesis. However it proved useful for differentiation of normal sperm production from azoospermia or severe oligospermia.

#### Review of Sexual Behavior and Reproduction in the Mare

Traditionally, the mare as opposed to other domestic livestock has been regarded as having a low reproductive efficiency. This has been attributed to the fact that mares with successful performance records are kept as broodmares without consideration of their existing reproductive problems (Evans et al., 1977). It has also been attributed to the universal birth date which will be discussed later.

In the mare, the estrous cycle is divided into two periods. The follicular phase is the period during which the mare shows behavioral signs of estrus, has rapid follicular growth and finally ovulates (Evans et al., 1977). Most mares in estrus display several characteristic behavioral signs when teased with a stallion. Her stance is characterized by a raised tail without switching, hind legs spread apart, and pelvis flexed. The labia of the vulva contracts and

relaxes, and there is eversion of the clitoris which is commonly referred to as "winking". Mares in estrus usually urinate quite frequently in the presence of a stallion (Hafez, 1974; Evans et al., 1977). The diestrous period of the cycle is the luteal phase which begins with ovulation. During diestrus, the corpus luteum (CL) is formed which secretes progestins. The behavioral response of the diestrous mare to teasing with a stallion varies from a passive, noninterested attitude to complete resentment, often manifested by violent attempts to kick and bite the stallion (Evans et al., 1977).

The change from behavioral estrus to diestrus occurs quite rapidly after ovulation. Hughs et al. (1972b) have reported that approximately 50% of the mares they studied were in diestrus within 24 hours after ovulation and 80% were in diestrus within 48 hours. This finding emphasizes the close relationship between ovulation and the end of sexual receptivity.

The domestic mare is generally considered to be seasonally poly-estrous (Sorensen, 1979) and is regulated by photoperiod (Burkhardt, 1947; Nishikawa, 1959). Sexual activity is usually greatest during the spring. Exposing mares to additional hours of light during winter months will induce estrus and advance the onset of the breeding season (Nishikawa, 1959). However the literature pertaining to the nature of the breeding season in the mare is conflicting and inconsistent. Hafez (1974) suggests this inconsistency results from real differences

among horses studied with regard to both environment and genetics.

Loy (1970) has classified mares into three groups according to seasonal variability. In the first category is the polyestrous mare, which cycles regularly throughout the year even though she has seasonal variations that fall within the normal range. The second group includes the seasonally polyestrous mare which has a definite breeding season and a definite anestrous period. The third classification is the seasonally polyestrous mare which has erratic reproductive patterns.

"Silent heat", the failure to show behavioral estrus prior to ovulation, and estrus behavior without ovulation have been reported (Hughes et al., 1972; Ginther, 1974). In a study at the University of Wisconsin, Ginther (1974) calculated that the mean date for cessation of ovarian activity was mid-October. However, 13 of 14 mares ovulated and demonstrated behavioral estrus from September through January. Nonovulatory estrus is usually observed immediately preceding the onset of winter anestrus (Hughes et al., 1972).

Breed differences in the length of breeding season have not been established but Thoroughbreds tend to have an earlier breeding season. This is probably due to artificial selection. Race horses are aged by the universal birth date of January 1. It has been the practice of race horse breeders to breed mares in order to foal as close to January 1 as possible. This gives their foals maximum physical advan-

tages as two-year old race horses (Hafez, 1974).

Foaling data for the Pryor Mountain and Winnemucca feral horses (Perkins et al., 1979) showed peak foaling activity occurring in May and June. Boyd (1979) also found that most foals in the Red Desert of Wyoming are born from late March through August with a peak in May and June. These foaling data clearly indicates that feral horses are sharply seasonal with a clear and rather complete end to the foaling season.

Although endocrine control of the estrous cycle in the mare is not completely understood, the basic sequence of events have been investigated. The pituitary hormones FSH and LH work in a synergistic manner in the processes of follicular growth, maturation, and ovulation. Follicle stimulating hormone causes ovarian follicles to grow and to produce increasing amounts of estrogenic substances as they grow. The LH causes ovulation and initiates the formation of the corpus luteum. Concentration of LH start to increase in the plasma 2-3 days before ovulation, reaches a peak 1-2 days after ovulation, and declines to diestrus levels 6 days after ovulation (Geschwind et al., 1975). Some authors have questioned whether LH is the causative factor of ovulation in the mare. Unlike other species it peaks after ovulation (Gemmell et al., 1976; Noden et al., 1975; Stabenfelt, 1977; Whitmore et al., 1973).

The ovarian hormones that control the estrous cycle are estrogens

and progestins. The amount of estradiol-17B in the plasma begins to increase at the onset of estrus, reaches a peak 12-27 hours before ovulation, and declines to diestrus levels 5-8 days after ovulation. Because estrogen reaches a peak before LH peaks, Evans (1977) suggests that the estrogen surge may facilitate the ovulatory surge of LH. Within 24 hours after ovulation, the corpus luteum begins to secrete progestins. By the sixth day after ovulation, maximum levels of plasma progestins have been attained. The corpus luteum remains active for approximately 12-14 days, then rapidly undergoes regression.

### Reversible Chemical Fertility Control

#### Review of Past Research in Male Contraception

Today, as in the past, equine fertility control has centered about castration. This procedure eliminates androgen production and in turn reduces aggressive behavior. Normal aggression is necessary for harem stallions to maintain their bands. Vasectomy, on the other hand, is a surgical process which eliminates sperm from being included in the semen. This process does not interfere with the stallion's sexual behavior or sex steroid production. However, the care and time necessary for performing vasectomies make the use of this technique on feral stallions impractical. Rather than borrow previously developed techniques for fertility control of equines, we considered using

chemicals which have been investigated as male contraceptives in laboratory animals and for human use. Such drugs have a more practical application for use on feral stallions.

The problem of controlling fertility in the male by chemical means has not been given much attention compared with the enormous volume of research devoted to the female. Nevertheless, we now know of several compounds which are powerful inhibitors of sperm production or function. A variety of chemicals have been used to inhibit sperm production in laboratory animals, domestic livestock and man. The basic mechanism of action is either attack on spermatogenesis or attack on morphologically mature spermatozoa passing through the epididymis. Production of male reproductive cells is dependent on sustained blood levels of pituitary gonadotrophic hormones. However, the degree of suppression necessary to inhibit spermatogenesis completely is difficult to achieve without also suppressing androgen secretion and hence, libido (Jackson, 1970).

Inhibition of spermatogenesis at different stages has been achieved by a variety of compounds both steroidal and nonsteroidal (Jackson, 1970). In experimental animals antispermatogenic compounds have been discovered during investigations with anti-tumor agents (alkylating chemicals) and from recognition of testicular damage during searches for antibacterial agents (nitrofurazones) and antiprotozoal compounds (nitrofurazones and nitropyroles). Some of these

nonsteroidal chemicals such as triethylenemelamine and alpha-chlorahydrin produce temporary sterility without inhibiting sexual behavior (Bock and Jackson, 1957; Ericsson and Baker, 1970). Steroidal hormones and their synthetic derivatives can inhibit pituitary gonadotrophin secretion via their negative feedback on the hypothalamus which leads to inhibition of spermatogenesis (Jackson, 1970). The inhibitory effects of testosterone on spermatogenesis has been demonstrated both in laboratory animals and in man (Moore and Price, 1937; Hotchkiss, 1934; Heller et al., 1950). Some authors have suggested using testosterone alone as a male contraceptive agent (Mauss et al., 1974; Reddy and Rao, 1972). One of the major drawbacks in utilizing synthetic sex steroids as contraceptive agents has been the fear of inducing undesirable side effects. If testosterone alone was administered, it was feared that it would have to be given at a dose which would elevate circulating testosterone levels above normal, thus exposing the individual to the undesirable effects of high concentration of androgens in the blood.

Steinberger and Smith (1977) demonstrated that testosterone enanthate could induce azoospermia and maintain the azoospermic state without elevation of circulating testosterone levels outside the pretreatment range. Although blood testosterone levels rose approximately 100% over baseline level during the initial phase, it returned to the pretreatment range during the maintenance phase.

Estrogen suppresses the pituitary activity through similar mechanism resulting in regression of both Leydig cells and the seminiferous epithelium. In men treated with high doses of the synthetic estrogen, stilbestrol, the seminiferous tubules were thickened and only spermatogonia and Sertoli cells remained (Jackson and Jones, 1972). Natural and synthetic estrogenic steroids are potent chemicals and cumulative in their action. A few studies have been made in experimental animals on the effects of prolonged exposure to estrogen on male reproductive processes (Jackson and Jones, 1972). Results depend on species, dose and duration of treatment. In mice and rats full fertility was restored 10 to 15 weeks after several months of exposure to estrogen, whereas the recovery process in monkeys progressed more slowly.

The use of natural hormones systems for castration of male sheep is being studied by Dr. R. Garth Sasser at the University of Idaho. According to Sasser the development of antibodies to luteinizing hormones was found to slow down or even prevent the development of testes in male rabbits. Male sheep were given a single injection of the hormone at 30-35 days after birth in combination with a vehicle which stimulates the development of the desired antibodies. When sheep were examined surgically at about 11 months of age, the testes in some, but not all, were found to be between five and 10 percent the size of those in a control group. No untoward effect on other bodily

systems were found (National Wool Growers, May, 1980). However Sasser did not discuss protection of testosterone and subsequent effects on libido. Sasser suggested this method for use in feral horses. However, it is not a reversible process. In light of past research conducted on male fertility control, several chemical compounds showed promise as a reversible antifertility drug for use on feral stallions.

#### Screening of Potential Antifertility Compounds

The screening of potential antifertility compounds for use in equids was carried out at the Georgia and Phillip Hofman Center for Research in Animal Reproduction, University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania (R. M. Kenney, unpublished data). Five treatments were investigated. They include:

1. Silastic implants including 17B Estrodiol and testosterone.
2. Testosterone cypionate
3. Quinestrol (a synthetic estrogen: 17 alpha-ethinylestradiol 3 cyclopentyl ether)
4. Microencapsulated Testosterone Propionate
5. Alpha Chlorhydrin

For each treatment group, pony stallions with two palpably normal testicles and good libido were selected. Ponies chosen weighed approximately 175 kg. as ponies of that size work well in the system at the University of Pennsylvania's Hofman Center for Reproductive

Research. The ponies were trained to ejaculate into a hand-held artificial vagina. Daily sperm output (D.S.O.) was determined by daily semen collection for two weeks. Morphological studies were performed on the sperm from each pony. Blood plasma was taken prior to the initial injection for determination of base-line LH and steroid levels. After the initial injection biweekly plasma samples were taken. In addition, the behavior of the ponies was closely observed and any changes in libido and other "normal" behaviors were recorded.

Of the treatment groups, both Quinestrol and testosterone proved effective in reducing sperm counts and were reversible. That is, the ponies were azoospermia for 3 to 5 months and reestablished normal sperm numbers in 6 to 8 months.

Quinestrol was given to each of three ponies at a dose of 1.3 grams per month once every month until a response was noticed in sperm production. Two of the ponies received three injections. The other pony received a fourth injection because he did not respond as quickly as the first two ponies. There was a decrease ( $P < .05$ ) in total number of sperm per ejaculate (TSN) from November 1978 ( $2.7 \times 10^8$ ) so that by January 1979 TSN was lower ( $0.8 \times 10^8$ ) than for November and December 1978 ( $2.1 \times 10^8$ ). The TSN stayed depressed for 3 months after the last injection of Quinestrol. The apparent increase in TSN started by May ( $.71 \times 10^{10}$ ) and by August ( $2.3 \times 10^8$ ) the TSN was significantly higher than for February and March ( $.3$  &  $.6 \times 10^8$  respectively). The

rate of decline in TSN seems to be similar to the rate of recovery once the trend to increase in TSN began.

The total and progressive motility estimates declined by the third month of treatment, stayed depressed for 3 months (February to April) and abruptly increased within a month (May). Total ejaculate volume and libido estimate did not change significantly for the duration of the experiment.

One objective of this project was to find an agent that would stop spermatogenesis for several months after a single injection. Opportunities for administering a drug to the same feral stallion every month for 3 to 5 months would be rare. A single injection of the drug would be the most practical method for use on free roaming horses. After discovering that multiple doses of Quinestrol did effect spermatogenesis, another experiment was designed to determine the effectiveness of a larger dose in a single injection. Three ponies were given 2.5 grams of Quinestrol in a single injection on May 30, 1979. Although the total sperm number decreased from  $2.73 \times 10^9$  in May to  $1.7 \times 10^9$  in June, it was not as great a decrease as when Quinestrol was given repeatedly. The interaction of season and treatment could not be factored out in this experiment but could have contributed to the apparently lesser response to single Quinestrol administration.

The total and progressive motility estimates showed similar

changes after the treatment as the TSN. Ejaculate volume (minus gel) did not change significantly after treatment except during December when the volume was largest. The reaction time and time to ejaculation did not change significantly post treatment but increased in December, indicating a seasonal influence might be present.

In general the sexual behavior of the second Quinestrol group was not affected. There were slight variations noticed in each pony during the months of December, January, February, and March. It is difficult to attach much significance to these variations in libido considering the effects of seasonality.

The exact site of action of the androgenic and estrogenic steroids used in this study are not known. Aside from the indirect effect on pituitary LH/FSH secretion, a more direct effect on the seminiferous epithelium might also be present. This was suggested by the observation that after allowing for a complete spermatogenic cycle and epidymal transit time, both depression and recovery of spermatogenesis seemed to progress quite rapidly. Monthly treatments of Quinestrol depressed TSN by the third month as did treatments with testosterone Cypionate. Kenney and Garcia (personal communication) suggest that these synthetic steroids could be affecting pituitary LH/FSH release and/or secretion rate. The similarity in the rate of spermatogenic depression and the length of time the ponies stayed oligospermic (approximately 3 months) indicate an involvement of simi-

lar regulatory processes. It seems that once the necessary spermatogenic support (LH & FSH?) is disturbed, the seminiferous epithelial insult occurs rather quickly since TSN is already depressed shortly after the completion of a spermatogenic cycle and transit time of 60-70 days. In addition, recovery occurs rapidly after cessation of treatment (possibly within a month) since increasing TSN were already apparent within the ensuing 60-90 days. In practical terms however, an effective dose of an inhibitory androgenic or estrogenic steroid could be manifested as an oligospermia that would last for 4 to 5 months. In this connection, a single dose of Quinestrol was not as effective as the repeated injections.

A system was developed by Southern Research Institute of Birmingham, Alabama, in which a drug could be incorporated into microcapsules. Drugs are encapsulated in macromolecular coating which are used to produce long-acting drug doses. Microencapsulation provides a protective supply of the drug which is released at specific controllable rates and for a given duration. This is basically accomplished by encapsulating in varying sizes. (L. R. Beck, unpublished data).

✱ Three pony stallions were administered 4.5 grams of microencapsulated testosterone propionate on August 16, 1979. Although azoospermia was not reached, there was a slow decline in total and progressive sperm motility from the start of the experiment to the end

in February, 1980. Total sperm numbers decreased by approximately 36%. Three to five weeks after the initial injection all three ponies showed dramatic increases in libido. A month later they settled down and demonstrated good to excellent libido for the remainder of the test.

## CHAPTER II

### INVESTIGATION INTO FEASIBILITY OF QUINESTROL (Q) AND MICROENCAPSULATED TESTOSTERONE PROPIONATE (TP) AS A REVERSIBLE CHEMICAL FERTILITY CONTROL IN FERAL STALLIONS

#### Phase I - Seasonal Estrus Patterns in Feral Mares

##### Introduction

The research conducted at the University of Pennsylvania Phillip Hofman Center indicated that a reversible male contraceptive was feasible and that its maximum duration would approximate six months. If feral mares were capable of reproduction through the fall and early winter, and a treated stallion managed to generate sufficiently high sperm numbers and motility, the reproductive season might only be postponed. Since the foaling data in the Pryor Mountains of Montana (Perkins et al., 1979) indicated a shorter and abrupt end to the reproductive season, this portion of the study was designed to examine the estrus patterns of feral mares.

##### Methods and Materials

Three mares from the Pryor Mountain Range were captured in May of 1978, transported to Billings and confined in a corral approximately 1000 m<sup>2</sup>. A fourth mare was acquired when she was returned by an Adopt-A-Horse recipient. Three of the mares (Tiger Lily, age 5; Columbine, age 7; Dahlia, age 15) were of proven fertility. The fertility of Sunflower (age 4) was unknown. The mares were fed a

total of 9 kg. of alfalfa hay per day (half in the morning and half in the late afternoon). In addition, they received 500 g. of grain (oats, corn, and barley mixture) every third day. A mineral block was provided which they had free access to along with fresh water whenever snow was not abundant. Under natural conditions, Pryor Mountain horses eat snow during the winter because there are no free water sources at that time. There was no shelter other than the lee side of a small building. A domestic Quarter Horse stallion was kept in a corral adjacent to the feral mares.

Behavioral estrus was observed in the feral mares. A mare coming into estrus would stand close to the stallion and often urinate in his presence. Other signs of estrus included raising the tail, and "winking" (rhythmic eversions of the clitoris) as defined by Ginther (1974).

As most horse breeders know, behavioral estrus is not a reliable method of determining whether ovulation has occurred. Therefore, physiological estrus and ovulation was defined endocrinologically. After the mares were in captivity for one month, we began a two month breaking and training program. The mares were halter broke, taught to stand still and accept a blindfold. Blood collecting began September 1, 1978. Each mare was blindfolded while blood was collected from the unrestrained horse by jugular venipuncture in 10cc heparinized vacutainers. The blood was centrifuged immediately at the corral and

the plasma was frozen. Blood was collected from all four mares every three days through September, 1979.

Evans and Irvine (1975) and Hughs et al. (1975) have demonstrated a clear rise in luteinizing hormone (LH) during the onset of estrus in the mare, with the maximum level shortly after the day of ovulation. Plasma progesterone levels fall rapidly prior to estrus and do not rise significantly until the first day of diestrus (Noden et al., 1975). It is generally agreed that plasma progesterone levels must be less than 0.5 ng/ml for ovulation to occur. Since LH and progesterone paint a fairly accurate picture of ovulation, we measured these two hormones, and total estrogens in laboratories in Toledo, Ohio and at Eastern Montana College respectively. Plasma LH was assayed by the double antibody radio-immunoassay described by Niswender et al. (1968). Total progestin concentrations in peripheral plasma were measured by the competitive-protein-binding assay method of Johnansson (1969). Using the radioimmunoassay of total estrogens were also measured at Eastern Montana College.

Plasma LH was assayed by the double antibody radioimmunoassay described by Niswender et al. (1968). The antibody was GDN15 anti ovine LH antibody, and the equine reference standard used in all assays was LER-1138-1 (potency = 0.27 NIH-LH-S1). The percent equine LH bound was  $29.5\% \pm 2.$ ; the 50% intercept was 17.7 ng ligand/ml; the slope for the standard curve was - 2.11 and the correlation coeffi-

cient of accuracy was  $r = 0.987$ . Since plasma LH has not been previously measured in feral horses, it was necessary to validate the assay in these animals. Domestic equine LH standard curves showed identical slopes to serially diluted plasma from peak estrus feral mares when assayed using the GDN15 anti-LH antibody. PMSG standards were also assayed using GDN15 antibody and showed 35% cross reactivity compared to LH. Measurements of domestic equine LH standards and feral horse plasma described above using (1) GDN15 and (2) an anti-PMSG antibody (supplied by Dr. M. Garcia) validated from LH revealed no difference in slopes between assay (1) and assay (2). These data suggest that the GDN15 double antibody radioimmunoassay measures immunoreactive LH in feral horse plasma which is not assayably different from LH in the domestic horse. Cross reactivity with PMSG in this assay must be considered in data evaluation.

Total plasma progesterin concentrations were measured by the competitive-protein-binding assay of Johansson (1969) with several modifications. Petroleum ether was used to selectively extract progesterins from plasma. Dog plasma was used as a source of binding protein and Florisil (60-100 mesh) was used to separate bound from unbound steroid. Recovery, measured by internal standard, was  $96.3 \pm 5.2\%$ . The coefficient of variation for precision was 8.4% for 12 duplicate determinations assayed on different days. Distilled water blanks, in 12 duplicate assays yielded a value of  $0.13 \pm 0.04$  ng.

Accuracy was determined by measuring known amounts of progesterone in water blanks. Progesterone expected was compared to progesterone measured. The correlation coefficient as determined by linear regression analysis gave a value of  $r = 0.981$ .

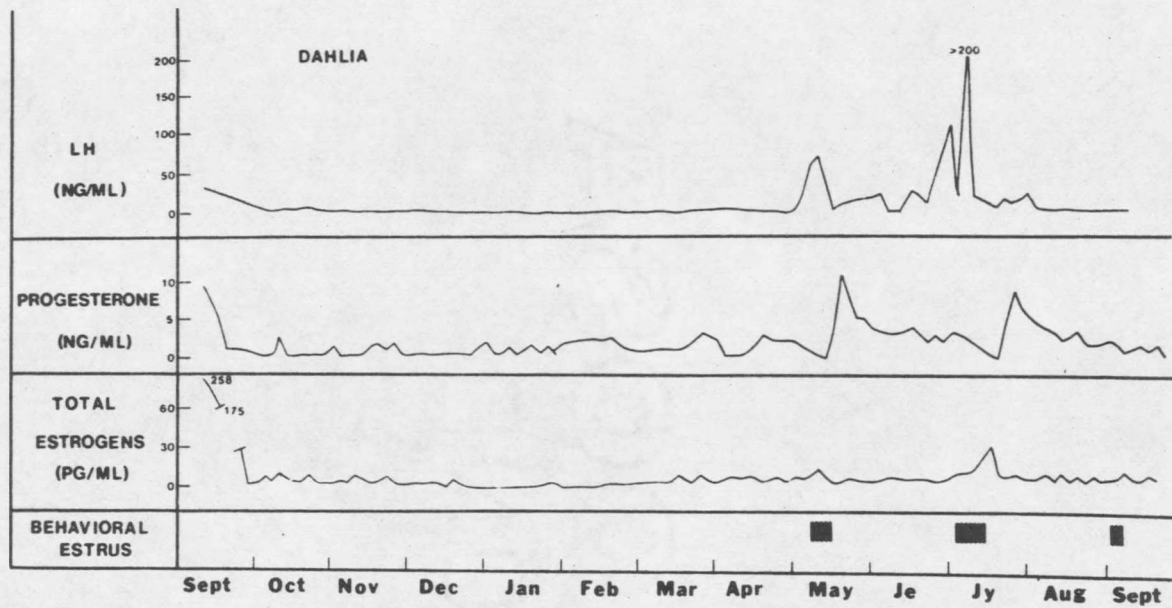
Total estrogens were measured by radioimmunoassay, using ovine antiserum prepared against estradiol - 17 beta-succinyl-bovine serum albumin. The lyophilized antiserum was rehydrated in phosphate buffer (pH=7.0) and labeling was carried out with 6,7 - <sup>3</sup>H estradiol - 17B. The specific activity was 40 - 60 Ci/mM and the total mass of tracer was 35 - 55 pg. The antiserum was diluted to give 50% binding. To aliquots of mare plasma ranging in size from 0.5 to 2.0 ml, 700 - 1000 cpm of 6,7 - <sup>3</sup>H estradiol - 17B was added to determine procedural losses. Estrogens were extracted as described by Nett et al. (1973). To each tube, 0.1 ml of phosphate buffer and 0.05 ml of labelled antiserum were added, and incubation was carried out for 24 hours at 4°C. Separation of free from bound antigen was achieved by selective absorption of the bound with activated dextran-coated charcoal and centrifugation (1200 g x 15 min.) at 4°C. The supernatant was decanted into 5.0 mls of scintillation cocktail (PPO-POPOP) and counted on a Beckman model LS-100C scintillation system. Recovery of estradiol after extraction was  $89.5 \pm 3.5\%$ . The coefficient of variation for precision was 10.8% for 15 duplicate determinations carried out on different days. The interassay and intra-assay variation was

12.7% and 8.6% respectively. Accuracy was determined by measuring known amounts of estradiol-17B. For 15 duplicate samples a linear regression analysis yielded a correlation coefficient of  $r = + 0.99$ . Ten distilled water blanks gave an average value of  $2.7 \pm 2.1$  pg. The lowest value for measurable estradiol standards which differed significantly from the blank value ( $P < 0.05$ ) was 5.0 pg/ml.

### Results

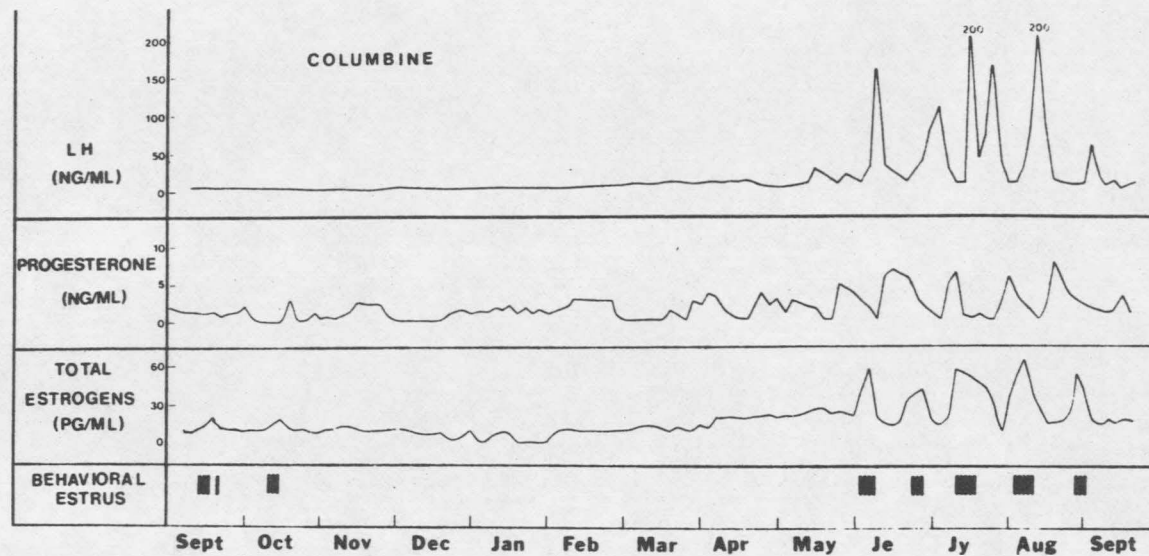
The thirteen-month profiles of plasma LH, progesterone and total estrogen concentrations for the four mares are shown in figures 1 - 4. LH peaks 12 to 25 times greater than the average baseline levels commenced on April 13 and ended August 31. Ovulatory LH peaks were associated with progesterone levels of 0.5 ng/ml or less and with elevations of total estrogens (peak average =  $43.1 \pm 12.1$  pg/ml). Of the 21 LH peaks, 16 were associated with ovulation. LH peaks were followed by increased plasma progesterone concentrations which remained elevated for 6 to 9 days. Average basal (values not involved in a hormone increase or decrease associated with physiological estrus) LH levels were significantly greater ( $P < 0.5$ ) from April through July ( $8.1 \pm 0.5$  ng/ml) than from October through January ( $2.2 \pm .2$  ng/ml). Basal progesterone and estrogen levels did not differ significantly ( $P < 0.05$ ) between those same time periods.

Although behavioral estrus accompanied all ovulations, it was



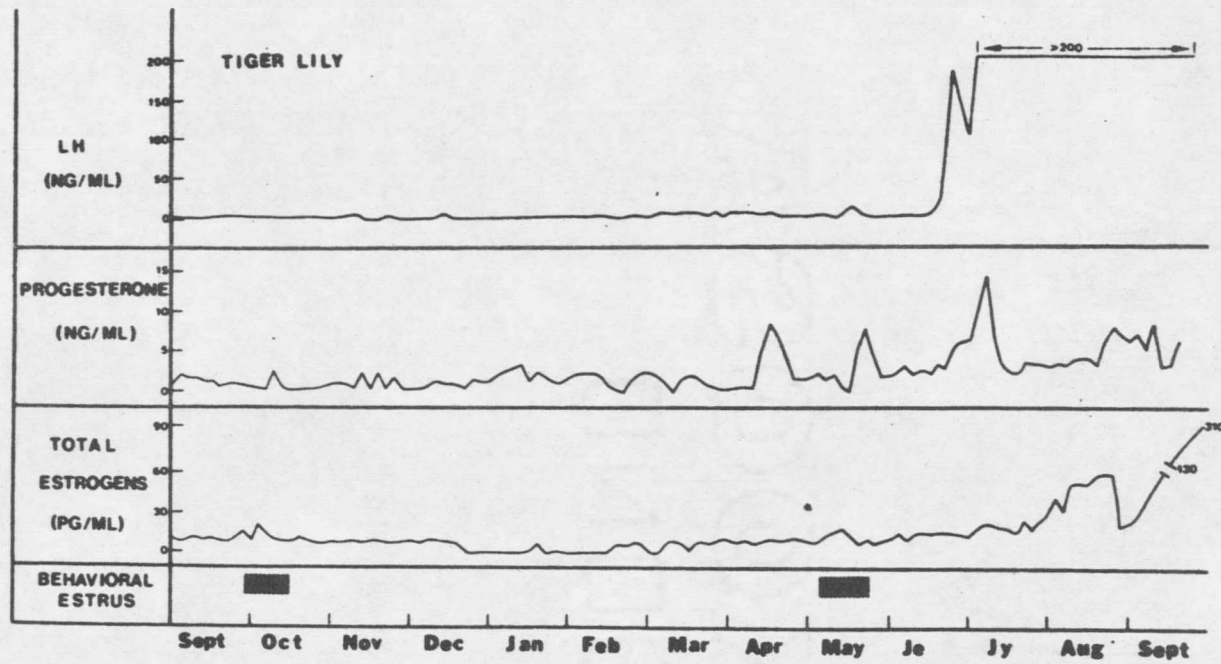
42

Figure 1. Progesterone, LH and total estrogens levels for the mare Dahlia. Bars indicate detection of behavioral estrus.



43

Figure 2. Progesterone, LH and total estrogens levels for the mare Columbine. Bars indicate detection of behavioral estrus.



44

Figure 3. Progesterone, LH and total estrogens levels for the mare Tiger Lily. Bars indicate detection of behavioral estrus.

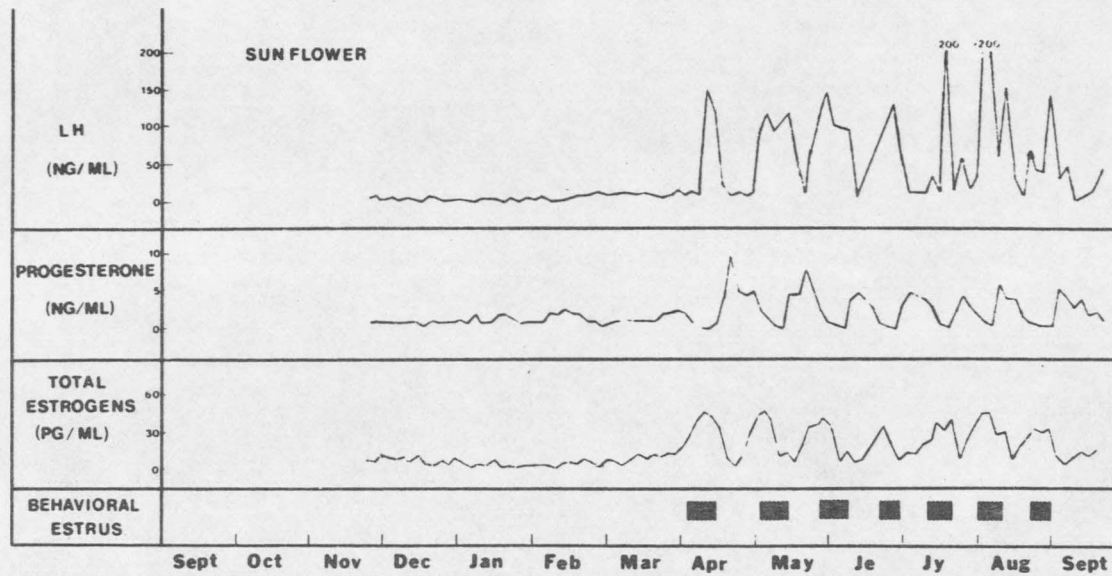


Figure 4. Progesterone, LH and total estrogens levels for the mare Sunflower. Bars indicate detection of behavioral estrus.

\*This mare was not captured until late in the fall and values for September and October are missing.

also observed twice in Columbine, once in Tiger Lily and once in Dahlia during seasonal diestrus in the Fall. Behavioral estrus which accompanied ovulation ranged in length from 6 to 10 days. All behavioral estrus (those associated with ovulation and the four displays not associated with ovulations during the Fall) were accompanied by elevated total plasma estrogens. Diestrus in Columbine and Sunflower ranged from 9 to 15 days. Dahlia demonstrated a 53 day diestrus and Tiger Lily was accidentally bred and became pregnant following her first ovulation.

The precipitous drop of plasma hormones in Dahlia in September, 1978, preceded an aborted foal (September 24). The fetus had a crown-rump length of 41 cm, which aged the fetus at approximately 120 days and would date the conception sometime in mid-May, 1978. The drop in LH probably primarily reflects a decrease in Pregnant Mare Serum (PMS) which cross-reacted with the LH antiserum. There was no behavioral estrus following the abortion and the cause(s) of the abortion is not known. Two domestic brood mares located on the same premises also aborted that Fall.

While Tiger Lily was in physiological estrus during mid-May, 1979, a stallion broke into the mare's corral. The incident occurred at night and the stallion was presumed to have spent 6 hours in the corral. Thirty six to 41 days later, LH levels rose to concentrations greater than 200 ng/ml and remained there until 120 days post

breeding. After that time LH level declined until the end of the study. Progesterone never returned to basal levels and increased slowly but steadily for 120 days. Estrogen concentrations increased sharply at approximately 65 days post breeding. All four mares were returned to the Pryor Mountains immediately following this study. In early June 1980, Tiger Lily was located on the horse range accompanied by a foal.

#### DISCUSSION

It is generally accepted that feral horses in America have lineages that go back to domestication. In fact many free-roaming horses are only a few generations removed from domestication. However, some populations have been free-roaming for over 200 years (Wyman, 1945). Extremely harsh environments and the process of natural selection may have led to physiological adaptive changes which would aid in survival and propagation of the species.

According to Hall (1972) certain anatomical features of the Pryor Mountain, Montana horses, such as their size and build, hoof structure and number of lumbar vertebrae are sufficiently different from domestic breeds to warrant considering the Pryor horses as a distinct variety. In examining diurnal variation of plasma corticosteroids in four Pryor Mountain stallions, Kirkpatrick et al. (1976) found that in general, the diurnal pattern different from the pattern reported in

domestic horses. The wild stallions exhibited a diurnal plasma corticosteroid rhythm, with a nadir at the same time as in domestic horses, but without the marked peak common to domestic horses. In addition, the Pryor Mountain horses appeared to have lower total corticoids and considerably more cortisol when compared to domestic horses. Kirkpatrick et al. (1979) suggested that this may be the means by which feral horses handle more stress than domestic horses, but with lower total corticoid levels. These physiological differences suggest that others may exist.

Whether or not reproductive patterns in feral horses differ from domestic horses was an important consideration of this project. While both feral and domestic horses are clearly seasonal breeders, foaling data from feral horse ranges indicate a sharply defined end to the foaling season (Perkins et al., 1979) with little or no foaling after August. Such data could be the result of foal mortality (inability to survive if born during harsher seasons), or a more sharply defined ovulatory and anovulatory season. The ovulatory season of the captive feral mares in this study (based on endocrine data) corresponds well to the foaling seasons found on the ranges with first ovulations occurring in April and cessation of ovarian activity occurring in August.

Although the ovulatory season for the feral mares begin at roughly the same time of year reported for domestic mares (Kenney et

al., 1975) there is an apparent difference between these feral mares and domestic mares with regard to ovarian activity in the fall and early winter. Although four captive feral mares in this study represent a small sample size, the fact that the three open mares did not ovulate after August 31 strongly indicates an end to the breeding season in these feral mares. This is in contrast to studies reporting significant number of domestic mares ovulating through the fall and into the early winter (Ginther, 1974; Van Niekerk, 1967; Satoh and Hoshi, 1932; and Palmer, 1978). The ovulatory season of ponies ends sooner than in domestic horses and more closely approximates the ovulatory season of the feral mares (Ginther, 1974; Satoh and Hoshi, 1932). Palmer (1978) found no ovarian activity in winter among Welsh ponies compared to 66% in saddle-type mares. Satoh and Hoshi (1932) found in a study of semi-wild Korean ponies, all mares ceased ovulating by October 1. Ginther (1979) speculates that it was the primitive nature of these animals which resulted in a sooner and more abrupt end to the ovulatory season.

Since the Pryor Mountain feral horse population has been reported inhabiting that region for at least 200 years (Wyman, 1945) it is possible that natural selection has created a population of mares with a more sharply defined ovulatory season than found in domestic mares. This shortened breeding season, in contrast to the breeding season

reported for domestic mares, would limit foaling to the period most favorable for survival.

Nutrition did not appear to play a role in determining the onset or length of the ovulatory season in the captive feral mares. Our feeding regime resulted in relatively stable weights over the 13 month study. It was assumed that the captive mares plane of nutrition was equal to or greater than that on the Pryor range for two reasons: 1) in the Pryor Mountains, horses generally lose weight from January through March, a period associated with high mortality, and 2) Hall (1972) has documented the selection of winter forage among the Pryor horses, and found that few vegetation types have substantial nutritive value. In spite of assumed improvement in nutrition in captive mares, their ovulatory season was not hastened nor lengthened.

This apparent lack of influence of nutritional plane on the onset of the ovulatory season is in direct conflict with the results of Bengtsson and Knudsen (1963) Van Niekerk and Van Heerden (1972) and Ginther (1974) which all indicated that there is a correlation between poor nutrition and delayed onset of seasonal ovarian activity. On the other hand, Allen (1978) and Gagnon (personal communication) have suggested that green grass in the spring is superior to dried feeds in stimulating the onset of the ovulatory season. In the Pryor Mountains, the annual green-up occurs in Mid-March, just prior to the start of the ovulatory season. Nelson (1980) found that among horses

in the Carsten National Forest of New Mexico, there were differences in reproductive effort between mares with access to revegetated areas and mares without such access. Females with access to these areas began foaling on April 6 and completed foaling in May. Other females began foaling on May 3 and continued through August. Of the mature females 64% had access to revegetation areas. These females contributed 73% of the foals.

Endocrinologically, ovulation in feral mares was qualitatively similar to ovulation in domestic mares. Since blood samples were taken every three days, the precise daily relationship of LH, progesterone, and total estrogens to one another was impossible to determine, but the general relationships are evident. Quantitatively both estrus and diestrus levels of total estrogens are similar to those reported by Nett et al. (1973) and Plotka et al. (1975). Although seasonal variations for urinary estrogens have been reported previously (Hillman and Loy, 1975), no descriptions on seasonal plasma estrogens are available. Seasonal changes in total estrogens in the captive feral mares appear to result only from estrogen surges during estrus and not from elevated basal levels.

Plasma progesterone concentrations during the ovulatory season were comparable with those reported by Stabenfeldt et al. (1972) for domestic mares and by Sharp and Black (1973) for ponies. However, on a seasonal basis, a distinct difference appears between progestins in

domestic mares and the feral mares. Dohn et al. (1974) measured pregnane-3,20-diols colorimetrically in seasonally anestrus mares and reported consistently high levels ( $76 \pm 20$  micrograms/ml). In the feral mares, plasma progestin concentrations from November through February averaged  $1.8 \pm 0.9$  ng/ml. One possible explanation for these differences could center around the origin of the pregnane-3,20-diols. Dohn et al. (1974) concluded that most of the pregnane-3,20 diols found in their study were of adrenal origin. Differences in adrenal corticosteroid concentrations were demonstrated between feral and domestic horse (Kirkpatrick et al., 1977a). The adrenals of the feral mares might also produce significantly smaller quantities of progestins. Another possible explanation is that feral mare reproductive patterns more closely resemble ponies than domestic horses. Ginther (1979) indicated that persistent corpora lutea, which are so common in horses, rarely occur in ponies. The same may be true of feral mares.

Basal plasma LH levels were similar among the feral mares, and the average basal values were greater from April through July (breeding season) than from November through January. In domestic mares quantitatively similar LH levels and seasonal differences in LH levels have been reported (Garcia et al., 1979).

This phase of the project provided evidence that Pryor Mountain mares have a shortened and well defined breeding season. Consequently, the period of time in which a stallion must remain

infertile was also defined. However, the origin and environment of America's feral horses is so diverse, care must be taken not to attribute any physiological differences noted in this study to all feral horses.

Phase II - Evaluation of Behavioral Responses  
to Chemical Fertility Compounds

Introduction

The pony stallions at the University of Pennsylvania which were used for testing the fertility control drugs were given "libido" scores. That is, the behavior of the individual ponies were noted and a score was assigned (on a scale of 1-5; 1 = low, 5 = high) for each ejaculation. The personality of each pony could influence such a score. Therefore, subtle changes in each pony were recorded. Such libido scores are helpful in determining whether a drug would drastically affect sexual behavior. However, it is difficult to compare ponies which have been trained to ejaculate into hand held artificial vaginas with free-roaming stallions. An understanding of how anti-fertility control drugs may affect the behavior of harem stallions was necessary before approving its widespread use.

The purpose of this portion of the study was to examine various behavior exhibited by feral stallions including: 1) aggression, 2) herding, 3) copulation, and 4) elimination. A more specific goal

was to develop a reliable behavioral endpoint which would quantitatively express deviation from "normal" behavior.

#### Methods and Materials

Feral horses in the Pryor Mountains National Horse Range were observed with 8X binoculars or 20-40 zoom spotting scope depending on distance and conditions. Observations were made undetected by the horses. Only data collected when horses were unaware of observers were used with the exception of herding in flight. Feral horses tend to move within a given home range and to some extent have habitual diurnal movement patterns.

After spending some time on a horse range (minimum 1 week), it is possible to learn where the various bands may be located at approximate times of the day. The most reliable observation system consisted of locating a band early in the morning and attempting to follow them for as long as possible. Often the horses would vanish over a ridge and it would take several hours of hiking to relocate them. Only direct hours of observation were included. Time of day, weather, location, and horse activity were recorded. All data in this study were collected between December, 1974, and August, 1978.

Stallion elimination marking behavior (EMB) is defined as the behavioral response of a stallion to a urination and/or defecation made by another horse. For the purpose of this study, responses were

recorded only when 1) the observer witnessed the elimination serving as a stimulus, and 2) the marking response was initiated within 1 minute of elimination. Since the source of stimulus could not be determined with certainty for behavior responses to previously deposited urine or feces (including stud dung piles) or other material, these were not included in our calculations. Less than 20% of all elimination marking displays observed were due to undetermined stimuli. Elimination marking behavior was quantified by subdividing the overall response of the stallion into five parts: 1) deliberate movement to the site of elimination, 2) smelling of feces or urine, 3) flehmen (olfaction-associated behavior including extension of head and neck with curling of upper lip), 4) urination on the site, and 5) defecation on the site. Admittedly 'movement to the site' is a prerequisite for the rest of the response. However, it is a distinct part of the response which, unlike the biological necessity of urination, includes coming to attention, turning, and moving deliberately to the site. Urination or defecation on the site was associated with posturing which included arching of the back and a slight reaching and extension of the hind legs. Often urination in response to stimuli occurred in short spurts. Random urination was not associated with this posturing and seldom occurred in spurts, but rather a continuous excretion.

Although some individual variations in the behavior were

observed, the responses were generally very stereotypic. The response of the stallion to a urination by another horse was not different from the response to a defecation, so responses were pooled. Frequency of behavior was determined by dividing the number of responses by the number of eliminations which occurred within approximately 25m of the stallion. This quotient is the elimination marking behavior quotient (EMBQ). Completeness of behavior was measured by recording the number of parts of the sequence which were displayed in a given response; i.e., a stallion displaying all five activities would receive a score of five. Stallions did not always display all five parts of the response. Sometimes flehmen did not occur or stallion urination and defecation did not both occur in a single sequence, although a distinct elimination marking behavior was still apparent in these cases. Therefore, a minimum criteria consisted of 1) deliberate movement to the site, 2) smell with or without flehmen, and 3) urination or defecation. A response was noted only when all three events occurred in a single response.

Herding behavior in harem stallions occurs both actively (driving members of the band) or passively (assuming a rear position as the band leaves an area). This is not to be confused with leadership in regard to escape or simply leaving an area. Lead mares initiate time and direction of movement by simply starting to walk away with band members following. The positioning of band members in a line moving

down a trail is random except for the lead mare in front and the stallion always bringing up the rear.

A stallion will display active herding behavior when members of his herd stray during resting or grazing periods. He will lower his head, lay his ears back and drive the stray horse(s) back toward the other band members. Sometimes stallions display active herding behavior in order to initiate movement from an area. Herding behavior by stallions was noted each time it occurred.

### Results

Preliminary observations of 23 stallions during the spring (April, May and June) indicated that of all the behaviors displayed by feral stallions, the one which occurred with the greatest frequency and was readily observable and quantifiable was EMB (table 1). It was also observed that herding behavior was exhibited only by harem stallions.

Eliminative Marking Behavior was rarely exhibited by mares or immature animals (table 2). Stallions displayed EMB in response to elimination by immature animals in only 3 of 135 observations (table 3). The overall average EMBQ for harem stallion behavior response to urination-defecation by sexually mature mares was 43.4%. However, this response varied markedly with time of year (fig. 5). The EMBQ was highest from April through July (average = 80%) and lowest

TABLE 1. FREQUENCY OF VARIOUS BEHAVIORS EXHIBITED BY FERAL STALLIONS\*

Behavior	Frequency of Display
Mounting	4
Copulation	2
Threat	8
Fight	4
Herding	10
Mutual grooming	8
Submission	6
Elimination marking	44

\*Based on 68 hours of observation of 23 stallions in April, May, and June.

TABLE 2. INCIDENCE OF ELIMINATION MARKING BEHAVIOR IN FERAL HORSES

Band member	No. of animals observed	No. of eliminations observed	Average EMBQ (%)#
Harem stallion	27	598	31.4
Mature mare	68	308	0.5
Immature female	8	70	0
Immature male	9	96	1.0

\*Marking responses to urination and (or) defecation by any band member averaged for the year.

TABLE 3. ELIMINATION MARKING BY HAREM STALLIONS IN RESPONSE TO URINATION AND (OR) DEFECATION BY OTHER MEMBERS OF THEIR BAND

Urination-defecation by:	No. of animals observed	No. of eliminations observed	Overall average EMBQ (%)*
Mature mare	68	610	43.4
Immature female	12	82	2.5
Immature male	14	53	1.8

\*Average of responses over the whole year.

(average = 1% from November through February.) In May and June, the peak of the breeding season, harem stallions responded to 93% and 89% of the eliminations respectively. In contrast, between November and February, only one response was observed in 98 eliminations. Although stallions in bachelor groups displayed EMB, the EMBQ in bachelor groups from April through July was only 37% (51 observations), less than half of the EMBQ for studs with harem bands. A social dominance order existed in bachelor groups, with the more dominant stallions displaying more EMB, more threat and aggressive behavior, and receiving more grooming. Some subordinate stallions showed no EMB. The most dominant stallions displayed EMBQ similar to harem studs.

The completeness of elimination response showed a seasonal pattern somewhat similar to that for average EMBQ, with the most

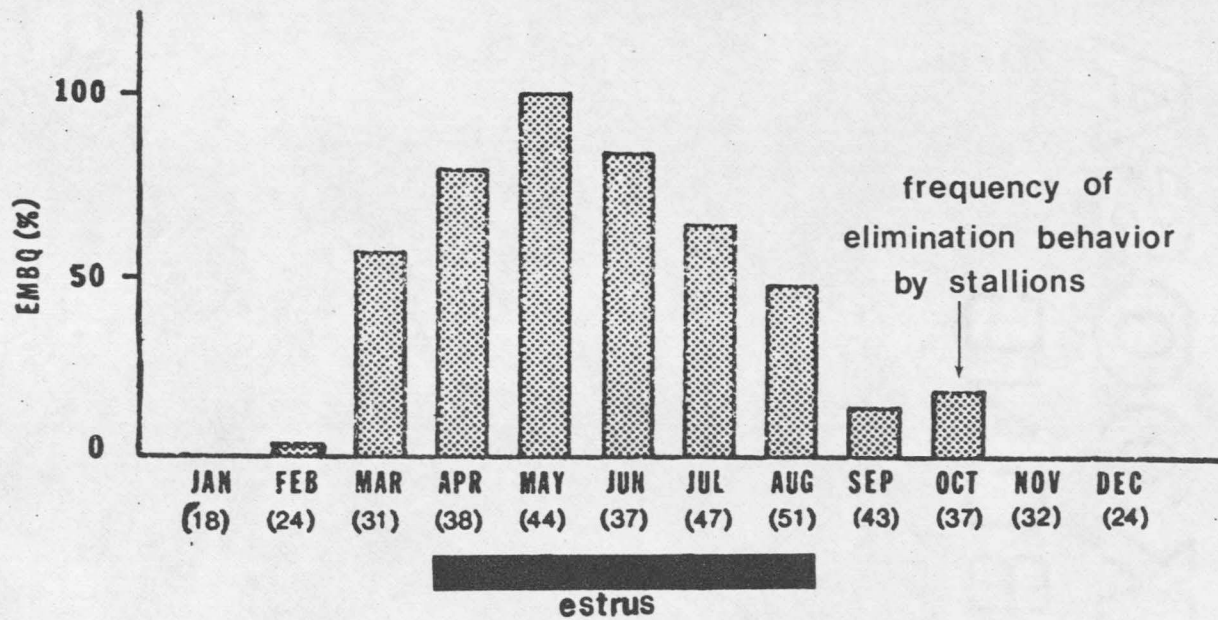


Figure 5. Frequency of elimination marking behavior by feral harem stallions in response to elimination by harem mares. Elimination marking behavior quotient (EMBQ) = number of stallion responses divided by number of mare eliminations within 25 meters of stallion. Values in parentheses are numbers of observations. Estrus bar = the ovulatory period as determined by four mares from the Pryor Mountain Range.

complete response patterns occurring in May ( $4.31 \pm 0.29$  SD) and June ( $4.36 \pm 0.40$  SD) (a complete display = 5). The completeness of the response in these 2 months was greater ( $P < 0.01$ , Multiple Range Test) than in other months. The range for all months was 3.14 - 4.36. Breakdown of the EMB response into its components revealed that the seasonal differences were due primarily to differences in frequency of flehmen and frequency of defecation (fig. 6). Usually when both urination and defecation did not occur in a given marking display, it was defecation which was absent. In fact, defecation was the most frequent omission in the overall marking sequence. Occurrence of defecation in the EMB display ranged from 68% (June) to 25% (September). Occurrence of flehmen in the EMB display ranged from 84% (June) to 30% (October). Most, but not all, stallions displayed flehmen, and flehmen frequency varied among stallions. Flehmen was also occasionally observed in nonmarking-related behavior.

Neither the frequency nor the completeness of the stallions' behavior response appeared to be influenced by the time of day or weather. To determine whether there were seasonal differences in the frequency of eliminations, the number of urinations or defecations per hour was recorded from December through July (except March). Data are from 10 to 20 horses for each month with at least 6 hr. observation per horse. The average number of eliminations per horse per hour ranged from  $0.31 \pm 0.20$  (April) to  $0.58 \pm 0.16$  (June) with no

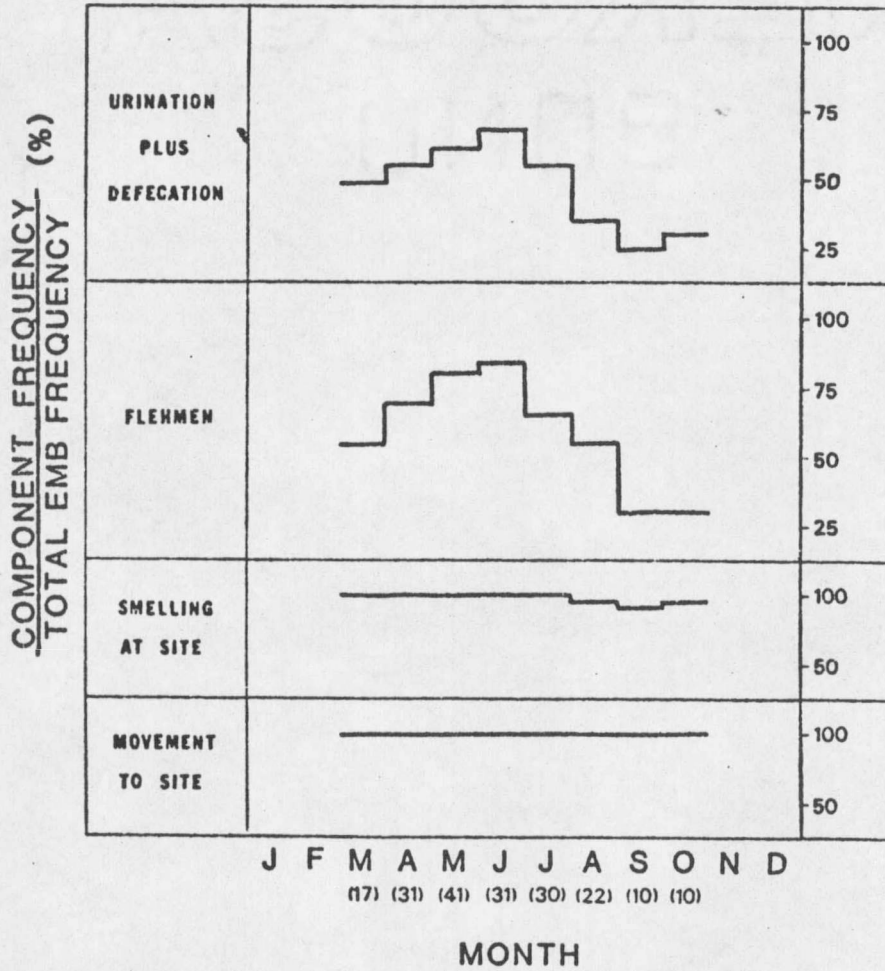


Figure 6. Frequency of occurrence of each component of the elimination marking behavior display by feral harem stallions during the year. Values in parentheses are numbers of observations. No behavior displays occurred November through January. Variation in "urination plus defecation" component was due almost exclusively to omission of defecation.

significant differences (Duncan's multiple range test) among months. The average for all months was  $0.46 \pm 0.12$ .

Herding behavior was unique to harem stallions. Although mares were occasionally seen defending their space (i.e., particular food source, water, or area within 5 m. of their body), they were never observed actively driving other band members or passively bringing up the rear as the band traveled.

Bachelor stallions were not herded by one another. If one bachelor exhibited herding type behavior, other members of the bachelor band responded by walking away, leaving the group entirely or reacting with a threat gesture.

Our observations revealed that EMB can be initiated via olfaction. Mares in many instances urinated or defecated out of sight or hearing (by our judgement), yet after 15-20 seconds, the stallion would raise his head, smell the air, and move directly to the elimination site. In similar situations with the exception that the stallion was up-wind of the elimination site, the stallion did not respond at all.

It is interesting to note that in this study stallions displayed definite seasonality in display of flehmen. Considering the frequent display of flehmen with sexual stimuli (Hafez et al., 1974; Evans et al., 1977) it is not surprising that the greatest frequency of flehmen corresponds to the peak of the breeding season which was determined in

phase I of this study. In addition, it corresponds to periods of peak androgen/testosterone concentrations (Kirkpatrick et al., 1976) found in the Pryor Mountain stallions. However, it has been shown that presumably nonsexual stimuli can also produce the flehmen response (Haupt, personal communication).

The results of this study demonstrate that EMB in feral stallions is most actively displayed during the breeding season (figure 5). It appears to play a role in several social contexts, including the harem, and may be important in harem maintenance during the breeding season. Observing EMB in stallions treated with contraceptive drugs, and comparing them to non-treated stallions is a method for assessing the effect of the drug on a behavioral parameter which may be linked to band cohesion.

Herding behavior and aggression (threat displays toward other horses) are also behaviors which appear to be linked to harem maintenance. However, external factors appear to play a more important role in frequency of aggression and herding behavior as compared to frequency of EMB. Disturbance by an intruder can initiate passive herding behavior and flight. Upon chance meetings of bachelors or another band, harem stallions exhibited aggressive behavior by striking, rearing, fighting or merely vocalizing. Such behaviors of course are dependent on frequency of encounters which in turn is dependent on population density. For this reason EMB and

EMBQ was selected as the primary endpoint for evaluation of the effect of male fertility drugs on stallion behavior. However, aggression and herding are important male associated behaviors, and deviation from what would be considered normal are easily observable. This would also hold true for mounting and copulation.

### Discussion

Male scent marking by urination or defecation has been reported in several species including feral pony (Tyler, 1972), feral horse (Feist, 1971), and plains zebra, mountain zebra and Grevy's zebra (Klingel, 1974). Many pet owners have observed it in their dogs at the proverbial fire hydrant. It has been suggested that marking by elimination may serve as a delineation or claim to territory. Although Pellegrini (1971) suggests that feral horses in the Wassuk Range of western Nevada are territorial (that is, they defend a portion of their home range), no such evidence of this was found in the Pryor Mountains, Vale, Oregon, or Challis, Idaho. Klingel (1974) has reported eliminative marking in both the plains and mountain zebra, which are non-territorial.

In the feral horse, marking by elimination may have a variety of social functions. Stud piles are reported by Feist (1971) in the Pryor Mountains and were observed during this project. They consist of a large pile of dung covering an area 3 meters square or a series

of connected piles ranging up to 25 square meters in size. Schloeth (1958) states that for domestic horses the stud piles and pawing behavior at the piles act as visual markers for territories. Feist (1971) found that feral stallions freely used any pile on a trail to water or on a feeding area. No specific piles belonged exclusively to any one stallion, nor was any plot of ground around the pile defended. An encounter of two harem stallions often resulted in both stallions defecating on a stud pile which Feist (1971) suggested may be a form of threat display. In any case, the eliminative behavior by stallions on stud piles is controlled, and appears to have some social significance even though its relationship to territoriality is unlikely.

Among bachelors, eliminative marking in response to other bachelor stallions generally reflected the social order within the group. That is, the socially subordinate males (as judged by aggressive behavior and grooming) did not display EMB while the apparent leader within the group displayed the greatest EMBQ.

This study focused on the EMB of harem stallions which is different than elimination on stud piles or by bachelors. In the harem the stallion is responding to the defecation or urination of a female rather than a male. EMB was never followed nor preceded by an act of aggression toward the mare producing the stimuli. Therefore, it is unlikely that EMB is for the purpose of assuring dominance


within the harem. We believe that EMB in the harem stallion may be a claim to the individual producing the stimuli for the following reasons. First, in this study area feral horses are not territorial; therefore, EMB may be marking a harem in lieu of a territory. Secondly, the critical period for maintenance of a harem is the breeding season and EMB is greatest during that period. Finally, Tyler (1972) has suggested that marking by a stallion serves to mask the estrus scent of a mare. Whether the seasonal changes in plasma androgens or the possible changes in the constituency of urine and feces from mares elicit the seasonality of EMB behavior remain unknown. However, both are possible explanations. The fact that urine and feces contain substances that can elicit or modify behavior have been reported by Cheal and Sprott (1971) and Reynolds (1971).

In discussing the evolutionary aspects of Equus, Klinge (personal communication) surmised that territoriality is an older form of social living than non-territorial. Perhaps the original function of eliminative marking by stallions, that of marking a territory, has evolved into a different function, that of marking individuals, which carries the same intent - maintaining a harem.

### Phase III - Field Test of Reversible Chemical Fertility Control on Feral Stallions

#### Introduction

Of all reversible fertility control drugs tested on pony

stallions, the two most promising were microencapsulated testosterone propionate (mTP) and ethinyl estradiol or Quinestrol (Q). The fertility control drug (mTP) was administered to feral horses in the Challis, Idaho district of the BLM on December 17 and 18, 1979. The  intent of this operation was to treat 10 harem stallions occupying a similar geographic area (home range) in order to reduce the number of offspring in that area during the foaling season of 1981. Horses ranging in the area of Spar Mountain, Antelope Flat, and Broken Wagon Road were selected. This range had an approximate population of 800 horses on 66,680 ha. A cooperative effort with the team from Washington State University working on the development of an immobilizing drug for use with feral horses made this part of the operation possible.

Quinestrol (Q) was tested on a range in eastern Oregon. The purpose of this test was to administer all potential sires within an enclosed study area. A control herd of equal number of horses located on a separate range 25 kilometers from the study area was used for comparisons.

#### Methods and Materials

##### Challis, Idaho

A Hughes 500C jet helicopter was used to locate the stallions and facilitate use of the immobilizer. The helicopter carried three

people in addition to the pilot. They were 1) a BLM employee who was responsible for identifying the stallion and keeping the pilot flying within the study area, 2) a veterinarian from the Washington State University team who darted the stallion from the helicopter with an immobilizing drug, and 3) the author who was responsible for administering the fertility drug and keeping records of each band. Within the helicopter, intercom communications were only possible between the BLM employee and the pilot.

Horses were darted from the helicopter with a Palmer Powder Charge Capture Gun. Succinyl Choline was the immobilizing drug. Once the horse dropped to the ground, the helicopter landed. It was everyone's job to reach the horse as quickly as possible in order to restrain and ventilate it. In all cases a go-ahead signal was given by the veterinarian before administration of the anti-fertility drug mTP. Pictures of all horses treated were taken with a 35mm Canon camera, and written descriptions of the animals were recorded. Tails were clipped (squared off) and red flagging was tied in their manes and tails for future identification purposes. Descriptions of band members were also recorded whenever possible.

An aqueous solution of the polyethylene glycol was used as a vehicle for administering the mTP. A vortex mixer was used to keep the drug in suspension. It was necessary to have someone filling syringes and mixing the drug at the helicopter fueling station as the

microencapsulated mTP settled rapidly. Fifty ml syringes and 14 gauge needles were used. The solute and solvent totaled 25 ml per syringe. Each syringe contained 2.5 grams of mTP. Dosage varied from 2.5 to 10 grams per horse (from one to four syringes). Ten horses were treated with mTP. Descriptions of these stallions are found in appendix 2. The location of these horses when they were treated are noted on the map in appendix 3. The data was analyzed by chi square tests.

#### Juntura, Oregon

In February and March, 1980, an attempt was made to administer the drug Quinestrol to feral horses in eastern Oregon. Permission to test the drugs was given by the Oregon State BLM Director and Jerry Wilcox, district wild horse specialist, provided necessary cooperation and support.

A horse range 20 miles south of Juntura, Oregon, was selected. A well defined, enclosed, reasonably small (less than 16,200 ha.) range was needed for this part of the project. According to local BLM personnel, the breeding season of the Juntura horses begins in early April, thus administration of the drug had to be carried out no later than early March.

The Juntura horse range, as it turns out, was almost inaccessible during January and February of 1980. Only on nights when temperatures were below freezing would the mud harden sufficiently to travel with a

vehicle. Travel into Juntura for supplies and showers could only be managed on a few occasions, and care had to be taken not to be gone too long or a quick thaw would prevent a return to the range.

The western-most boundary of the 1,215 ha. range is the Malheur River. Other boundaries include naturally occurring vertical rock walls, and the eastern boundary consists of a BLM fence. The fences are, for the most part, in good repair. There are gates nearly every half-kilometer for cattle. The range itself consists of large steep mountains, canyons and open plateaus. There are occasional juniper trees, but basically the cover is low sage. Water was, if anything, overly abundant. Every draw, coulee, reservoir were filled to capacity and overflowing.

During the two months spent on the Juntura Range all 21 horses were seen at least once. Initially over half of these horses were south of the range. On February 27, these animals were driven back onto the BLM range with a helicopter. The 21 horses consist of a band of 13, a band of seven and a lone mare. The band members are described in appendix 4.

Transportation into the horse range was impossible without saddle horses. Jerry Wilcox provided four saddle horses. Four hours per day were required just to get to and from the range. When the feral horses moved to the northern tip, eight to ten kilometers more were added to the daily travel. Only 40% of the total time spent on the

Juntura range was such that work or travel could be carried out.

Despite innumerable problems with the drug (the cold weather caused viscosity problems), on February 16, the Sunshine Stallion was darted. The drugs were loaded into darts that could be pressurized and fired by a 22 caliber Paxe-Arms tranquilizer gun. The darts were kept in warm water in a thermos bottle until loaded into the gun, in order to keep the drug dissolved. One and one-half hours lapsed between the time of loading into the gun and firing. Whether the drug was successfully delivered is a matter of speculation. On March 2, the subordinate stallion of the Sunshine band may have been darted. No one actually saw whether the dart struck the horse. The dart was recovered, and there was black hair on it. Approximately 2.5 - 3 grams of Q were released. On March 4th the stallion of the Ghost Rider band was darted. Saddle horses aroused his curiosity and he walked to within 10 meters of the author. During pressurization of the dart, it exploded and a new dart had to be prepared. Due to the intense cold only about one half of the required dosage (perhaps 2 grams) could be dissolved. The dart eventually hit the horse and the drug was released.

### Results

#### Effect of Fertility Control Drugs on Behavior

During the summer of 1980 (June 11 through July 15), the drug treated stallions in both the Challis and Juntura herds were

relocated. Detailed description of band members along with behavioral data were recorded. Control bands in Challis were selected during the summer of 1980 and behavioral data from these bands were also noted. Descriptions of the horses were made with the intention of providing distinguishing features to permit recognition the following spring. Since stallions in the Challis herd were treated with the aid of a helicopter, accurate descriptions of band members at that time were not possible. The clipped tails on the treated stallions assisted in relocating them during the summer 6 months later. The Juntura herd had accurate descriptions from the winter. New foals born in the Juntura herd were noted, and stallion behavior was recorded.

In order to evaluate the possible effects of mTP treatment on social organization and behavior of the Challis herd, data were collected on elimination marking, herding, mounting and copulation. Five mTP treated bands and five control bands were selected as the data sources. The size of individual bands varied, but the total number of stallions, mares and immatures for control group and mTP group was similar. The total number of hours devoted to mTP bands and to control bands was similar. Although efforts were made to observe both mTP and control bands on a given day, this was not always possible. However, there were never more than 2 days between observations of controls and mTP treated bands.

Comparison of behaviors in mTP and control bands in the Challis

herd indicated no effect of treatment on EMB or herding behavior (table 4). The treated stallions appeared as capable of maintaining the harem as control stallions as judged by 1) foiling efforts at mare-stealing by bachelors and 2) alertness and responses to intruders or danger and 3) bringing up the rear when fleeing from danger. No difference between mTP treated and control bands in mounting or copulation frequency was noted in June. For July in the control bands, only one mounting display was observed. No copulations were seen. In 4 of the 5 mTP treated bands, however, mounting was observed more than once and in two bands a copulation was observed.

The EMBQ in July was lower than in June in both mTP treated and control bands ( $P < .01$  multiple range test). In addition, the flehmen and defecation components of the EMB display were lower in July than in June in both treated and control bands (table 4). This is similar to results obtained in the Pryor Mountain Range in June and July over several years.

The only two harem stallions in the Juntura herd were treated. Results of observations of their EMB and EMBQ are noted in table 5. There were no untreated stallions in the Juntura herd, so their EMBQ was compared to the EMBQ of the control herd in Challis (table 4). The bands in the Juntura herd remained well separated, never closer than one kilometer. Animals within a band generally remained in a group radius of 25 meters and eliminations by mares were readily

TABLE 4. BEHAVIOR OF HAREM STALLIONS IN THE CHALLIS HERD JUNE 9 THROUGH JULY 15, 1980

No. of displays* Type of behavior	June 9-18		June 19-28		June 5-15	
	mTP	Control	mTP	Control	mTP	Control
Elimination marking (EMB)	60	62	65	59	51	55
Mounting	12	14	9	9	12	1
Copulation	3	2	2	3	2	0
Herding	20	24	20	18	10	14

Aspects of EMB	Percent					
EMBQ**	88	92	94	87	72	77
Movement of site <sup>+</sup>	100	100	100	100	100	100
Smelling <sup>+</sup>	100	100	100	100	100	100
Flehmen <sup>+</sup>	75	82	68	76	55	61
Urination <sup>+</sup>	90	95	96	92	95	95
Defecation	64	58	55	60	44	49

\*No. of displays for all 5 mTP treated or for all control stallions. Number of hours of observation for control and mTP bands is approximately equal.

\*\*EMBQ = number of EMB displays/number of eliminations perceived.

<sup>+</sup>Component displays/EMB displays.

TABLE 5. BEHAVIOR OF HAREM STALLIONS IN THE JUNTURA HERD JUNE 28 THROUGH JULY 3, 1980

Type of behavior	No. of displays
Elimination marking (EMB)	37
Mounting	8
Copulation	2
Herding	6

Aspect of EMB	Percent
EMBQ**	88
Movement to site <sup>+</sup>	100
Smelling <sup>+</sup>	100
Flehmen <sup>+</sup>	80
Urination <sup>+</sup>	95
Defecation <sup>+</sup>	90

\*Harem studs of both Sunshine and Ghost Rider bands.

\*\*EMBQ = number of EMB displays/number of eliminations perceived.

<sup>+</sup>Component display/EMB display.

detectable by the stallion, although occasionally an individual moved as much as 60-80 meters from the group in grazing. Due to the lack of interaction between Q treated harem stallions and other mature males, aggression was not observed.

However, upon accidental disturbance of the bands (horses alerting to an observer), the Q stallions assumed the passive herding position in the rear, and always positioned themselves between the observer and other band members prior to fleeing the area.

Descriptions of the Juntura horses and band affiliations recorded during the summer of 1980 corresponded with the descriptions recorded the previous winter with the exception of foals. This indicates that harem band maintenance was not affected by the drug.

These observations indicated that the treatment of stallions with Q or microencapsulated mTP did not alter male behavior patterns in June and July. In addition, existing social structure within and between bands appeared to be maintained.

#### Foal Counts in Challis, Idaho

The effectiveness of mTP in controlling fertility was determined by counting the number of foals produced in seven bands led by drug-treated studs in the summer of 1981. As a control, foals were also counted in eight bands of similar size led by untreated studs. These bands were the same bands which had been studied for drug effects on

behavior in the summer of 1980 as well.

When the Challis horse range was offered by the BLM for this project, assurances were given that the horses would not be disturbed (rounded-up) during the course of this experiment. In 1971 when the Wild Free-Roaming Horse and Burro Act was passed, there were 150 horses on the Challis range. The herd grew from 150 to 660 by 1978. The BLM had planned to round up some of the horses in the fall of 1976 and give them out for adoption. American Horse Protection Association took the BLM to court in an effort to halt the round-up. This was a test case on BLM management policies. A temporary restraining order against the round-up was made by a federal judge in Washington, D.C. The BLM wanted to appeal the court decision, but didn't expect to have the restraining order lifted.

However, in October of 1980, the restraining order was lifted and three hundred horses (approximately 45% of the herd) were rounded up and removed from the range. The round-up was made band by band so bands in our study were marked from a helicopter with a paint ball gun prior to the roundup. The helicopter pilot made every effort to avoid treated stallions and their bands during the roundup. However, since not all stallions were relocated, it is likely some were captured. In addition, four harem studs escaped during the round-up, and in concert with the usual efforts of bachelor males to obtain harems, some shuffling and loss of mares in treated and control bands resulted.

Furthermore, several attempts at rustling (poaching) were made on the Challis range, and in 1981, CATLIN and members of his band were captured. In this case the rustlers were apprehended and the horses in this band were accounted for. However, it was possible that other horses were poached. The missing mTP treated stallions included MOODY, BURFENING, and GALLAGHER.

Data for each band are based on a minimum of 10 hours of direct observation for each band at distances assuring positive identification. The following sequence and combination of criteria were employed in band identification: 1) number of animals, 2) age (foal, immature, adult) and sex (stud, subordinate or immature male, female), 3) coat color, 4) identifying marks (stockings, blaze, paint, etc., and some treated studs had squared tails from previous cutting).

A helicopter survey taken May 8, 1981, showed the following: 295 adults, 44 yearlings, 33 foals, total 372. Assuming a 5% error in counting the range population maximum at that time was 391. Data presented here are based on more than 360 hours of direct observation between June 29 and August 20, 1981.

The data are presented in Tables 6 and 7, and they reflect foal counts through August 20. It is uncommon for many foals to be born later than mid-July in the Challis area. Foal counts for control bands averaged 0.371 foals per mare, whereas for treated bands the average was 0.066; ( $P < .01$ ) i.e. only 1/6 as many foals were produced

TABLE 6. BAND COMPOSITION OF mTP TREATED AND CONTROL BANDS

	Control	Treated
No. of bands	8	7
No. of horses	65	49
No. of mature mares	35	30
No. of immatures	9	10
No. of foals	13	2
Ave. band size	8.1	7.0

TABLE 7. FOALING DATA FOR mTP TREATED AND CONTROL BANDS

	Control	Treated
No. of foals/mare	0.371	0.066
No. of foals/band	1.63	0.28
% of bands with foals	87.5	28.4

in treated bands. Seven of eight control bands had at least one foal, whereas only two of the seven treated bands had a foal.

Among the treated studs which were not accounted for this year it is possible that foals were produced in their bands. Some shuffling in band membership occurred on the range during the year. There were

two mares missing from the group of treated bands and it is not known whether they had foals.

Both foals produced by treated bands were from mares which had been documented in summer 1980 to be with their respective treated studs (ANSOTEGUI and TURNER). Each mare with foal was still with their stud in 1981 and had foaled prior to June 30. There were no subordinate males in these bands and both ANSOTEGUI and TURNER were observed in 1980 fighting with other stallions on several occasions, suggesting their intolerance of the presence of other males. Whether these stallions sired the foals or the mares wandered away from their bands during the breeding season is unknown. TURNER had received 5.0 g. of the antifertility drug and ANSOTEGUI had received 7.5 g. These doses were the same and 50% greater, respectively, than the dose given to KIRKPATRICK, whose band had no foals. Descriptions of treated and control bands are in appendix 5.

#### Foal Counts in Juntura, Oregon

A four seater fixed wing aircraft was employed on July 9, 1981, to aid in locating the horses and making preliminary foal counts. There were 38 horses spotted from the air, 11 of which appeared to be 1981 foals. The two major bands were still intact. However, two new bands had formed; a bachelor band of five males, and a new band with a stallion, two mares and one foal.

From the air it could be seen that every gate leading into the study area was open. The Sunshine band was first located outside the range just west of the Lavanger well. This band was frequenting this area during the winter of 1980 prior to being driven back into their range with a helicopter. A new power line was being constructed which passed through the southern end of the study area. This construction project accounted for the much improved roads and access to the horse range. It is also the most likely reason for all the open gates. Even though the gates were open and the horses were free to roam over thousands of hectares, they were not observed far from their home range. There are no other feral horses located within 25 kilometers of this range so immigration or migration is unlikely.

Following the aerial survey, attempts were made to locate the horses on foot. After two days of hiking one of the new bands was identified. Using 7X35 binoculars four horses were identified.

With the aid of saddle horses, the majority of the range was traversed. Accurate descriptions of the remaining 34 horses were obtained from no more than 200 meters.

A control herd located in the Hog Creek area approximately 25 kilometers northeast of the Juntura herd was selected for comparing 1981 foal counts. The Hog Creek range is very rugged slate terrain and consists of steep ravines with sage and grasses being the predominant vegetation. It is similar to the Juntura range in that

both are located in Malheur River break country.

The entire range contained approximately 120 horses. Forty-four of these horses were rounded up for adoption and were used as the control group for this study. Individual bands of horses were located and driven into a trap with a helicopter. As the horses were brought in, band composition was recorded. Later these horses were branded and aged by the tooth wear and replacement method.

In the Juntura test herd, eleven of the thirty-eight horses were positively identified as 1981 foals. The control group contained forty-four horses, eight of which were 1981 foals. The information regarding both the Juntura herd and the control herd were compiled in the following figures and tables.

Appendix 6 is a depiction of the herd social organization as it existed during the winter of 1980, the summer of 1980, and the summer of 1981. Table 8 shows the band composition of the control herd. From our hidden vantage point at the roundup, the number of mature horses and foals was all that could be accurately recorded. Table 9 lists all horses in the control herd by sex and age. This information was obtained while the horses were being branded for adoption. Each horse was put down to the ground by heading and heeling in order to positively age and sex each animal. Tables 10 and 11 are a depiction of the band composition and foaling data for the Q treated and control herds.

The ratio of foals to mature mares in the Juntura test herd is 11:14 or 0.78 foals/mares. The ratio of foals to mature mares in the control herd was 8:13 or 0.61 foals/mares. If three year old mares are considered of foal bearing age, the ratio drops to 8:17 or 0.47 foals/mares. The difference between treated and control foals/mare was insignificant ( $P < .05$ ).

TABLE 8. DESCRIPTION OF HOG CREEK CONTROL HERD: JULY, 1981

Band #	Stallions	Mares/Immatures	Foals
1.	1	5	1
2.	1	3	2
3.	1	4	1
4.	1	9	2
5.	1	3	1
6.	1	3	1
7.	3 (Bachelor Band)		
Total	9	27	8 (44)

TABLE 9. AGE AND SEX DATA ON HOG CREEK CONTROL HERD: JULY, 1981

Stallions	Mares	Immature Males	Immature Females	Foals Males	Foals Females	
5	4	2	1	1	1	
6	4	2	1	1	1	
7	4	2	2		1	
7	4	3	2		1	
8	5	3	3		1	
11	6	3	3		1	
11	7		3			
11	7		3			
12	9					
	10					
	11					
	12					
	14					
<hr/>	<hr/>	<hr/>	<hr/>	<hr/>	<hr/>	
9	13	6	8	2	6	(44)

TABLE 10. BAND COMPOSITION OF Q-TREATED AND CONTROL BANDS

	Control	Treated
No. of bands	6	3
No. of bachelor bands	1	1
No. of horses	44	38
No. of mature mares	17	14
No. of immatures	7	6
No. of foals	8	11
No. of bachelors	3	4
Ave. harem band size	6.8	11

TABLE 11. FOALING DATA FOR Q TREATED AND CONTROL BANDS

	Control	Treated
No. of foals/mare	0.471	0.786
No. of foals/band	1.33	3.67
% of bands with foals	100	100

DiscussionChallis:

The results in Challis suggest that the concept of fertility control in feral horses is workable and has potential for practical application to some herds. The difference in the number of foals born to mares in treated stud bands compared to control bands was highly significant ( $P < .01$ ). Observations of the test stallions and mares within their bands during the summer of 1980 (six months after drug administration) indicated no effect on their behavior (based on EMB and EMBQ) or herd social structure. The only change in behavior was that treated stallions continued to copulate into late July. This might be a result of open mares cycling later into the summer.

\* A drawback for the use of mTP is that currently the stallions must be restrained before the drug can be administered. The immobilizer used during this project (succinylcholine chloride) precipitated the death of eight out of 25 horses darted and is not acceptable for future use with mTP. Barring the prospect of a safe immobilizer in the near future, the mTP must be administered to restrained horses because of the high viscosity of the drug carrier. Presently, mTP can be administered to captured stallions which in turn can be released back to their home ranges.

Southern Research Institute (SRI) of Birmingham, Alabama is the sole supplier of mTP at present. To date very little research has

been conducted with alternative (less viscous) carriers for mTP. Scientists at SRI have no doubt that new carriers with lower viscosity can be developed and that with some alterations in the delivery design of the darts, remote administration is quite possible. Additionally, SRI has indicated that since field testing at Challis, the compounding process for mTP has been altered significantly to provide longer acting release of the steroid and greater efficacy of release. Such improvements may allow administration of mTP to coincide with regularly scheduled round-ups usually held in October.

The ponies in the clinical trials at the University of Pennsylvania received 4.5 g. doses of mTP. The treated stallions were larger than the ponies (425 kg. vs. 175 kg.). Stallions KIRKPATRICK and TURNER both received 5 g. of mTP; KIRKPATRICK's band had no foals while TURNER's band had one foal. Stallions GAGNON, CATLIN, and ANSOTEGUI received 7.5 g. of mTP; GAGNON and CATLIN had no foals while ANSOTEGUI had one foal. Stallions WAMBOLT and KECK received 10 g. of mTP and there were no foals in either band. These data show no correlation between dose administered and antifertility effects in feral horses; however, further investigations may reveal that the larger dosage provides an explanation for the efficacy of the drug in the feral stallions of the Challis herd.

Juntura:

That Quinestrol has potential as an inhibitor of fertility is

illustrated by the data from clinical trials at the University of Pennsylvania. Our failure to reduce foaling rate in Juntura was most likely due to a combination of problems involving both the weather and the solvent carrier for the steroid. Quinestrol does not remain in liquid suspension when mixed in a vegetable oil base below room temperature. This was not discovered until the first dart was loaded in the field during late January, 1980. That particular winter was cold, often below freezing. When the Q was mixed into a dart, it crystallized within a few minutes. Methods for keeping the darts warm included: carrying them in a warm thermos bottle and keeping the barrel of the dart gun encased in a battery operated electric sock. Although this prolonged the period to crystallization, it was not enough time to locate the target stallion, sneak to within 50 meters and fire the dart. Small aliquots of benzene were added to the steroid to aid in lowering the temperature at which it crystallized. However, the benzene weakened the plastic darts which then cracked and leaked when pressurized.

Scientists at the University of Pennsylvania indicated that a proper dose for a stallion weighing approximately 400 kg. was 4 grams of Q. Although the harem stallions of both the Sunshine and Ghost Rider band were darted, the probability that they received 4 g. of Q is limited. There is no way of knowing how much of the drug had crystallized in the darts prior to striking the stallions.

Although this project failed to reduce the number of foals born in the Juntura herd, data collected over the 18 month period provides valuable information regarding herd social structure. This herd had been reduced from approximately 75 horses to 21 horses by a round-up held in the fall of 1979. When this herd was first identified in the winter of 1980, there were two distinct bands and a single horse (appendix 6). Eighteen months later the two major bands were still intact. No exchange of mature mares occurred during this period. Two mares from the Sunshine band left the band with the subordinate stallion to form a new band. This is contrary to the studies of Nelson (1981) and Miller (1979) who reported unstable band organization in New Mexico and Wyoming respectively.

As the population grew from 21 to 38 horses over two foaling seasons (an increase of 29% and 41% in 1980 and 1981 respectively), the younger males formed a typical bachelor band (appendix 6). The subordinate stallion and two mares from the Sunshine Band formed a new band. These two mares are likely half sisters of the stallion they are with. Although no reliable age data is available on these mares, mare #9 had no foal in 1980 or 1981 and was probably immature. Mare #10 had no foal in 1980 and had a foal at her side in 1981. Judging by her size and length of tail, this is apparently her first foal. This corroborates the findings of Feist (1971) and Hall (1972) that the majority of changes in band structure occurs when immature animals

leave established bands to form new bands.

During the summers of 1980 and 1981 there was no observed interaction among the bands. The development of a bachelor band and a new harem band in Juntura is consistent with what has been reported in the Pryor Mountains (Feist, 1971; Hall, 1972; Perkins et al., 1979). However, it is different from the herd social structure found in the Red Desert of Wyoming which consist of multi-male bands with a great deal of band interaction (Denniston, 1979).

## CHAPTER III

### DISCUSSION AND SUMMARY

The original research proposal and experimental design of this project was approved by the BLM in September of 1978; this proposal designated the Pryor Mountain National Horse Range for field testing of the reversible antifertility drugs. The Pryor Mountain herd was selected for two major reasons: 1) nine years of accumulated behavioral, social structure and fertility data were available on this herd, and 2) the Pryor Mountain Range is only 50 kilometers from the laboratory at Eastern Montana College in Billings. However, citizens of Lovell, Wyoming (closest town to the Pryor Mountain Range) led by Pat Schmidt, editor of the Lovell Chronicle applied pressure on the BLM to halt the project. Hope Ryden, the American Horse Protection Association wild horse advisor said,

"This sort of drugging program cannot work, and may well lead to serious endocrine imbalances that will prevent the males from successfully living through the harsh western winters. The result may be the ultimate destruction of some herds such as the Pryor Mountain Wild Horse Range" (American Horse Protection Association, Inc., Spring, 1980).

This statement led to an organized effort to end the project and the BLM withdrew its approval. Prior to this action, the author spent two months living in the Pryor Mountains gathering base line behavioral data on specific stallions intended for the drug trial.

Results from the experiments with male fertility control on

ponies at the University of Pennsylvania indicated no harmful side effects. A request by the National BLM Wild Horse and Burro Specialist was made asking for a BLM district to volunteer a feral horse herd for continuation of the project. The Salmon, Idaho district offered the Challis herd; and the Vale, Oregon district offered the Juntura herd. The unfamiliarity of these two ranges which were over 1000 km. from our base created the following limitations:

1) Phase I of this project clearly suggested that Pryor Mountain mares have an abrupt end to their breeding season. No concrete evidence on either the Challis or Juntura herd indicated such a breeding season.

2) Every horse on the Pryor Mountain range had been accounted for and accurate band descriptions over several years would have been invaluable for this project. No prior descriptions of horses and band affiliations were available for either the Challis or Juntura herd.

3) Since behaviors of individuals are so variable, before and after drug administration data concerning behavior of test stallions would have been more conclusive than comparison to other individuals. Time did not permit collection of behavior data on test stallions prior to their drug administration.

4) Genetic background of the Challis horses included a considerable influence from draft breeds. Their size was significantly greater than the Pryor Mountain horses which could have

greatly influenced dosage requirements.

5) Technical problems arising with the drugs could not be solved rapidly due to the distance from the laboratory to the horse ranges.

6) Experimental animals were lost and shuffled due to large scale round-ups and rustling.

In spite of these problems, the field testing in Challis and Juntura provided important information regarding the use of fertility inhibitors on stallions in the wild.

#### Management Implications:

Using computer simulated models of population responses to changes in demographic parameters, Nelson (1978) demonstrated that to initiate a population decline in feral horses, the percent of successful breeding females must be reduced to less than 10%. In the Challis test herd, out of 30 potentially reproductive mares, only two had foals or there was a successful breeding rate of 6.6%. Nelson's computer simulated model suggests that the use of mTP over a twenty year period would cause a significant decrease in total population numbers, assuming breeding rates of 6.6%.

Recent criticism concerning male fertility control as a practical means of managing feral horse populations has centered about the lack of fidelity of mares to harem stallions. Nelson (1981) observed females moving between adjacent bands during the breeding season.

Some shuffling and the disappearance of mares from their bands was observed for short durations in and among Challis horses. However, the fact remains that 28 out of 30 mares were not bred by peripheral stallions. This suggests that in feral horse populations under conditions similar to those found in Challis, enough mares remain with their respective harem stallions during the breeding season to enable a male focused fertility control to effectively reduce population growth. Nelson (1981) found that mares in the Carlsen National Forest copulate primarily with dominant stallions which is in agreement with our findings. Therefore treatment of most dominant stallions within a given herd may solve any lack of fidelity problems if they existed. The problem of copulation by subordinate or co-dominant stallions reported by Miller (1979) could also be solved by treating all sexually active males within a band.

Some authors have expressed the opinion that treatment of stallions is an "impractical management approach" (Miller, 1979; Nelson, 1981; Conley, personal communication). Experience with this project proved the contrary. Although it was not possible to administer the fertility control drug remotely, on two separate occasions feral stallions were darted from a helicopter, one with an immobilizer, and the other with a paint ball gun. \*These experiences proved that remote delivery of drugs via helicopter is entirely possible. During the administration of mTP, six horses were darted

with succinylcholine chloride and failed to drop to the ground; one dropped, but recovered and ran away before the team could reach him; and eight horses were darted which subsequently died. Counting these fifteen horses and the ten test stallions that were put down and then administered the test drug, a total of 25 horses were darted in 16 hours of helicopter time. Without having to land and work with the animals on the ground after darting each horse, an estimated 35 to 40 horses could be darted in the same 16 hours of helicopter time. The round-up in Challis during the fall of 1980 required 24 hours of helicopter time. For the same cost of rounding up 300 horses, 60 stallions could have been treated. Once a herd population has been reduced to the established carrying capacity as determined by each BLM district, round-ups may no longer be necessary and the cost of transporting, working, housing and adopting out horses could be eliminated.

\*In addition to potential monetary savings, contraception has another possible advantage over round-ups as a population control method. According to Waring (1979), feral equid management will only be successful if physiological as well as behavioral well-being of the equid populations are maintained. Management based on population disruption will not produce healthy herds and thus will not be successful management. Removal or loss of herd members can create serious disruptions to social units. On Sable Island, for example,

the loss of a herd stallion in winter places the rest of the social unit in jeopardy especially if a new yet inexperienced male herds the band into areas with poor shelter and poor food quality (Welsh, 1973). Culling activities can destroy behavioral stability and lead to herd instability (Waring, 1979). Although administering a contraceptive drug would cause some temporary harassment from the use of a helicopter, there would be no need for loss or disruption of existing individuals within a harem band.

\* Before advocating the use of a fertility control program for managing feral horse populations, a basic knowledge of natural versus artificial selection must be realized. Selective sterilization of harem stallions could eliminate the major contributors of quality genetic material from the gene pool. This is why reversible drugs were chosen. The purpose of reversible chemical fertility control is to reduce the total number of foals sired by an individual, not to permanently sterilize or eliminate an individual's contribution.

The purpose of feral horse management is not to subject free-roaming horses to selective breeding. Attributes of horses described by various breed organizations vary tremendously. It would be impossible to agree on a breed or type of horse that should exist on federal lands (i.e. Quarter Horses and Thoroughbreds are a far cry from Spanish Barb). A great deal can be learned about domestic horses by studying the behavior and physiology of horses that are selected by

nature. Should the results of this project lead to implementation of the use of mTP or Q as a management tool, a statistically valid method for random selection should be designed so that no stallion be treated more than any other stallion.

Suggested Future Research:

Testing of reversible male chemical fertility control drugs on feral stallions should be repeated under more controlled experimental design. A well defined feral horse population not subject to round-ups, poaching, or open gates should be used for a repeat trial. In addition, further development of a safe and effective method for immobilization of free-roaming horses is needed. Problems experienced in the Challis herd with use of succinylcholine chloride have been reviewed in a separate report to the BLM (Borchard, 1980). The longer acting, less viscous mTP should be tested. In addition, the feasibility of administering Q with a dart gun from a helicopter may prove valuable. The results of the Challis field test appear to justify the initiation of a long term management project (e.g. 3-7 years).

APPENDICES

## APPENDIX 1. WILD FREE-ROAMING HORSE AND BURRO ACT.

Public Law 92-195  
92nd Congress, S. 1116  
December 15, 1971

## An Act

To require the protection, management, and control of wild free-roaming horses and burros on public lands.

*Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,* That Congress finds and declares that wild free-roaming horses and burros are living symbols of the historic and pioneer spirit of the West; that they contribute to the diversity of life forms within the Nation and enrich the lives of the American people; and that these horses and burros are fast disappearing from the American scene. It is the policy of Congress that wild free-roaming horses and burros shall be protected from capture, branding, harassment, or death; and to accomplish this they are to be considered in the area where presently found, as an integral part of the natural system of the public lands.

## SEC. 2. As used in this Act—

(a) "Secretary" means the Secretary of the Interior when used in connection with public lands administered by him through the Bureau of Land Management and the Secretary of Agriculture in connection with public lands administered by him through the Forest Service;

(b) "wild free-roaming horses and burros" means all unbranded and unclaimed horses and burros on public lands of the United States;

(c) "range" means the amount of land necessary to sustain an existing herd or herds of wild free-roaming horses and burros, which does not exceed their known territorial limits, and which is devoted principally but not necessarily exclusively to their welfare in keeping with the multiple-use management concept for the public lands;

(d) "herd" means one or more stallions and his mares; and

(e) "public lands" means any lands administered by the Secretary of the Interior through the Bureau of Land Management or by the Secretary of Agriculture through the Forest Service.

SEC. 3. (a) All wild free-roaming horses and burros are hereby declared to be under the jurisdiction of the Secretary for the purpose of management and protection

in accordance with the provisions of this Act. The Secretary is authorized and directed to protect and manage wild free-roaming horses and burros as components of the public lands, and he may designate and maintain specific ranges on public lands as sanctuaries for their protection and preservation, where the Secretary after consultation with the wildlife agency of the State wherein any such range is proposed and with the Advisory Board established in section 7 of this Act deems such action desirable. The Secretary shall manage wild free-roaming horses and burros in a manner that is designed to achieve and maintain a thriving natural ecological balance on the public lands. He shall consider the recommendations of qualified scientists in the field of biology and ecology, some of whom shall be independent of both Federal and State agencies and may include members of the Advisory Board established in section 7 of this Act. All management activities shall be at the minimal feasible level and shall be carried out in consultation with the wildlife agency of the State wherein such lands are located in order to protect the natural ecological balance of all wildlife species which inhabit such lands, particularly endangered wildlife species. Any adjustments in forage allocations on any such lands shall take into consideration the needs of other wildlife species which inhabit such lands.

(b) Where an area is found to be overpopulated, the Secretary, after consulting with the Advisory Board, may order old, sick, or lame animals to be destroyed in the most humane manner possible, and he may cause additional excess wild free-roaming horses and burros to be captured and removed for private maintenance under humane conditions and care.

(c) The Secretary may order wild free-roaming horses or burros to be destroyed in the most humane manner possible when he deems such action to be an act of mercy or when in his judgment such action is necessary to preserve and maintain the habitat in a suitable condition for continued use. No wild free-roaming horse or burro shall be ordered to be destroyed because of overpopulation unless in the judgment of the Secretary such action is the only practical way to remove excess animals from the area.

(d) Nothing in this Act shall preclude the customary disposal of the remains of a deceased wild free-roaming horse or burro, including those in the authorized possession of private parties, but in no event shall such remains, or any part thereof, be sold for any consideration, directly or indirectly.

SEC. 4. 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 marshal or agent of the Secretary, who shall arrange to have the animals removed. In no event shall such wild free-roaming horses and burros be destroyed except by the agents of the Secretary. Nothing in this section shall be construed to prohibit a private landowner from maintaining wild free-roaming horses or burros on his private lands, or lands leased from the Government, if he does so in a manner that protects them from harassment, and if the animals were not willfully removed or enticed from the public lands. Any individuals who maintain such wild free-roaming horses or burros on their private lands or lands leased from the Government shall notify the appropriate agent of the Secretary and supply him with a reasonable approximation of the number of animals so maintained.

SEC. 5. A person claiming ownership of a horse or burro on the public lands shall be entitled to recover it only if recovery is permissible under the branding and estray laws of the State in which the animal is found.

SEC. 6. The Secretary is authorized to enter into cooperative agreements with other landowners and with the State and local governmental agencies and may issue such regulations as he deems necessary for the furtherance of the purposes of this Act.

SEC. 7. The Secretary of the Interior and the Secretary of Agriculture are authorized and directed to appoint a joint advisory board of not more than nine members to advise them on any matter relating to wild free-roaming horses and burros and their management and protection. They shall select as advisers persons who are not employees of the Federal or State Governments and whom they deem to have special knowledge about protection of horses and burros, management of wildlife, animal husbandry, or natural resources management. Members of the board shall not receive reimbursement except for travel and other expenditures necessary in connection with their services.

SEC. 8. Any person who—

(1) willfully removes or attempts to remove a wild free-roaming horse or burro from the public lands, without authority from the Secretary, or

(2) converts a wild free-roaming horse or burro to private use, without authority from the Secretary, or

(3) maliciously causes the death or harassment of any wild free-roaming horse or burro, or

(4) processes or permits to be processed into commercial products the remains of a wild free-roaming horse or burro, or

(5) sells, directly or indirectly, a wild free-roaming horse or burro maintained on private or leased land pursuant to section 4 of this Act, or the remains thereof, or

(6) willfully violates a regulation issued pursuant to this Act, shall be subject to a fine of not more than \$2,000, or imprisonment for not more than one year, or both. Any person so charged with such violation by the Secretary may be tried and sentenced by any United States commissioner or magistrate designated for that purpose by the court by which he was appointed, in the same manner and subject to the same conditions as provided for in section 3401, title 18, United States Code.

(b) Any employee designated by the Secretary of the Interior or the Secretary of Agriculture shall have power, without warrant, to arrest any person committing in the presence of such employee a violation of this Act or any regulation made pursuant thereto, and to take such person immediately for examination or trial before an officer or court of competent jurisdiction, and shall have power to execute any warrant or other process issued by an officer or court of competent jurisdiction to enforce the provisions of this Act or regulations made pursuant thereto. Any judge of a court established under the laws of the United States, or any United States magistrate may, within his respective jurisdiction, upon proper oath or affirmation showing probable cause, issue warrants in all such cases.

SEC. 9. Nothing in this Act shall be construed to authorize the Secretary to relocate wild free-roaming horses or burros to areas of the public lands where they do not presently exist.

SEC. 10. After the expiration of thirty calendar months following the date of enactment of this Act, and every twenty-four calendar months thereafter, the Secretaries of the Interior and Agriculture shall submit to Congress a joint report on the administration of this Act, including a summary of enforcement and/or other actions taken thereunder, costs, and such recommendations for legislative or other actions as he might deem appropriate.

The Secretary of the Interior and the Secretary of Agriculture shall consult with respect to the implementation and enforcement of this Act and to the maximum feasible extent coordinate the activities of their respective departments and in the implementation and enforcement of this Act. The Secretaries are authorized and directed to undertake those studies of the habits of wild free-roaming horses and burros that they may deem necessary in order to carry out the provisions of this Act.

Approved December 15, 1971.

## APPENDIX 2. DESCRIPTIONS OF mTP TREATED STALLIONS.

#1 Name: KIRKPATRICK

Markings: Paint. White markings on left mid-side. Tail was cut. 3 socks and bald face.  
Age: 8 years.  
Dosage: 5 g. mTP at 4 injection sites. 12 ml at each site; 2 injections on each hip.

#2 Name: MOODY (lost)

Markings: All black with a small star.  
Age: 7 years.  
Dosage: 2.5 g. mTP at 1 injection site on hip.

#3 Name: BURFENING (lost)

Markings: All brown with light brown muzzle. Red flagging in tail. Left hind sock.  
Age: 15 years.  
Dosage: 5 g. mTP, 25 ml on each hip.

#4 Name: GAGNON

Markings: Nearly white.  
Age: Unknown  
Dosage: 7.5 g. mTP, 25 ml at 3 injection sites - 2 on left hip, 1 on right hip.

#5 Name: WAMBOLT

Markings: Large dark-grey. Flagging in mane.  
Age: 10 years.  
Dosage: 10 g. TF at 4 injection sites. 25 ml at two injection sites on each hip.

## #6 Name: CATLIN (rustled)

Markings: Grey stallion. Clipped tail and flagging in mane.  
(Lone Pine Peak Stud).  
Age: 8 years  
Dosage: 7.5 g. mTP, 2 injection sites on left hip - 25 ml  
each; 25 ml on right hip.  
Band: 17 members (possibly multiple bands as result of  
helicopter disturbance).

## #7 Name: GALLAGHER (lost)

Markings: Bay. Tail clipped and flagging in mane.  
Age: 18+ years. Missing and losing upper teeth.  
Dosage: 10 g. mTP, 25 ml at 4 injection sites - 2 on each hip.  
Band: 6 other members, all bays. 3 mares and 3 foals.

## #8 Name: ANSOTEGUI

Markings: Sorrel. White blaze. 2 stockings. Clipped tail and  
flagging in mane.  
Age: 5 years.  
Dosage: 7.5 g. mTP, 25 ml at each site - 2 on left hip, 1 on  
right hip.  
Band: Unknown. (Was seen fighting with #5 WAMBOLT).

## #9 Name: KECK

Markings: Brown. Small star. Right hind sock. Clipped tail  
and flagging in mane.  
Age: 8 years.  
Dosage: 10 g. mTP, 25 ml in right shoulder; 25 ml in upper  
right hip; 25 ml in lower right hip; 25 ml in left  
hip.  
Band: 7 in band; all brown.

## #10 Name: TURNER

Markings: Bay. Star. 2 hind socks. Clipped tail and flagging  
in mane.  
Age: 7 years.  
Dosage: 5 g. mTP, 25 ml on rear of right hip; 25 ml on upper  
right hip.  
Band: 7 other members. Multicolored band. Foal with star.

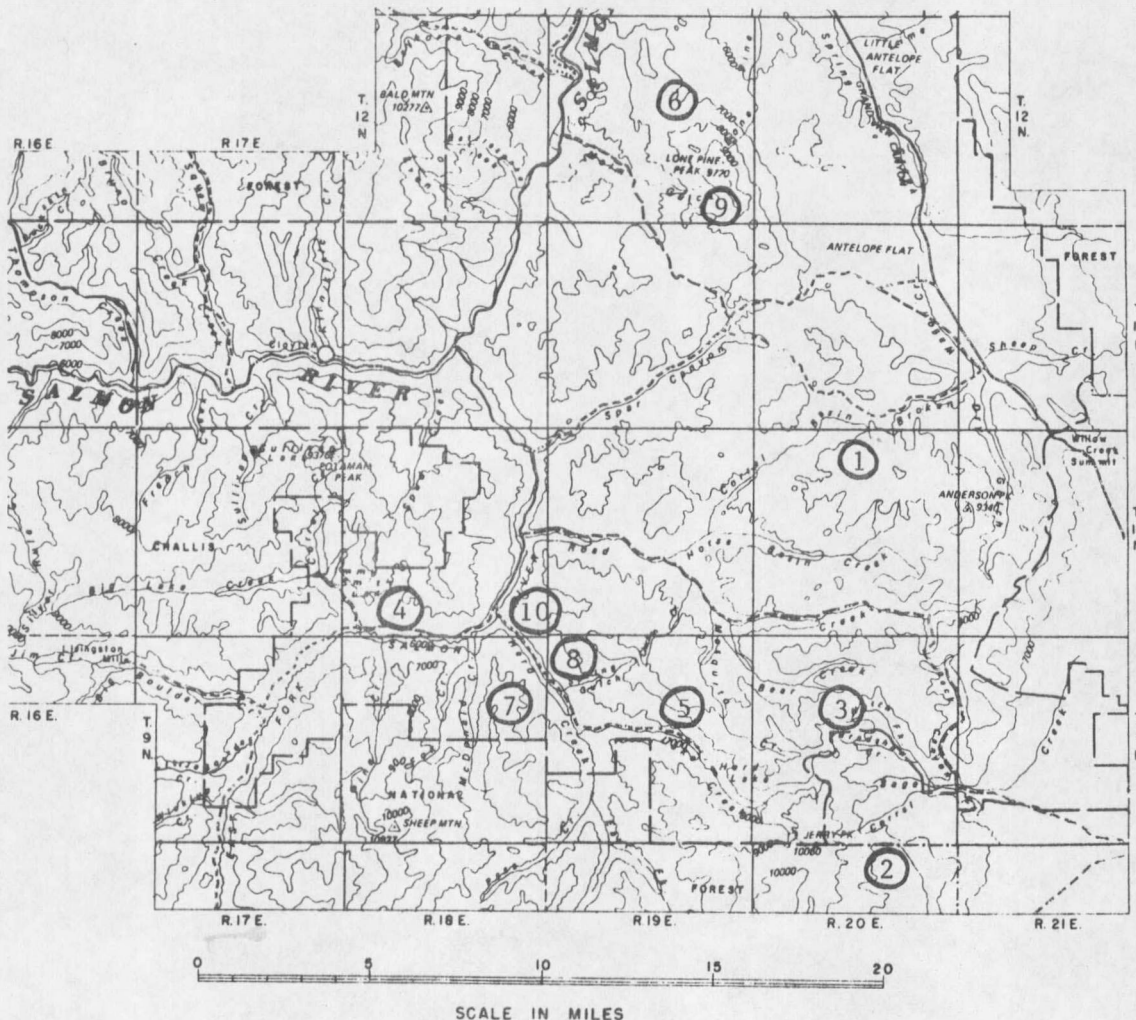
APPENDIX 3. CHALLIS HORSE RANGE

\*Numbers correspond to mTP treated stallions and locations at the time of treatment.

UNITED STATES  
DEPARTMENT OF THE INTERIOR  
BUREAU OF LAND MANAGEMENT

CHALLIS PLANNING UNIT

SALMON DISTRICT  
IDAHO



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## APPENDIX 4. JUNTURA TEST HERD

Sunshine Band

1. Gray Stallion - dark dappled gray with very light colored head; dominant stallion.
2. Black Stallion - bold blaze; no white on legs; subordinate.
3. Bay - immature male; blaze; no white on legs.
4. Bay Mare - small star
5. Palamino Mare
6. Light Grulla - right hind sock
7. Grulla - light colored head; no white on legs (but light colored).
8. Dark Grulla - four creamy stockings and a light head.
9. Dark Grulla - left hind sock
10. Golden Grulla - immature
11. Palamino Filly
12. Palamino Mare
13. Bay - immature male; blaze; left white hind stocking.

(Slides were taken of all horses for positive identification.)

Ghost Rider Band

1. Dappled Grey Stallion - light colored head; wide blaze; hind stocking.
2. Grey Mare - light head
3. Palamino Mare
4. Bay Mare - star
5. Bay Yearling - (belongs to 4 above)
6. Bay immature
7. Dark Brown - nearly black, immature.

## APPENDIX 5. BAND DESCRIPTIONS OF CHALLIS HERD, SUMMER 1981

TREATED BANDS

KIRKPATRICK Sorrel paint stud - large spot on left mid-side, bald face, right forestocking and short hindstockings, squared tail

Bay mare - blaze, hindstockings, small spot lower right side

Brown mare - star

Brown mare - no special marking (NSM)

Bay mare - white nose (snip)

Yearling - dark red-brown paint, wide blaze covers right eye, 3 stockings

Bay Yearling - NSM

No Foals as of August 20

Location: Anywhere on the range south of Corral Basin Creek. Moves a lot.

GAGNON White Stud - dark gray hindstockings, blond mane and tail

White mare - NSM

White mare - NSM

Bay mare - blaze, hindstockings

Bay mare - NSM

Dark gray mare - star, white nose

Dark gray mare - NSM

Black yearling - NSM

Bay immature - blaze, 3 stockings

No foals as of July 25

Location: Plain to S.W. of Spar Mountain.

WAMBOLT  
 Dark gray stud - NSM  
 Bay mare - blaze (white nose)  
 Bay mare - NSM  
 Brown mare - star  
 Gray mare - blond tail  
 Gray mare - dark rump (darker than other gray)

No foals as of August 20

Location: Spar Mountain or hills to east of Spar

CATLIN  
 This band was captured by rustlers in March. All that remains is the stud, two mares and one immature. The rest have been accounted for and are starred (\*) below.

Light gray stud - black muzzle and mane, squared yellow tail  
 Dark gray mare - left hindstocking  
 White mare - black ears and mane, blackish face and forestockings are blackish  
 Brown mare - collar on her, chopped tail

No foals as of August 20

\*Black mare - star  
 Disposition: died, was not pregnant  
 \*Gray mare - bald face, blond tail, black mane, left hindstocking, left frontstocking  
 Disposition: collared and released near Anderson ranch. With a white stud, no foal  
 \*Light gray mare - blackish face, gray mane, brown tail, four white legs  
 Disposition: collared and released in Bear Wallow was not pregnant  
 \*Gray mare - bald face, gray mane, left hindstocking  
 Disposition: died, was not pregnant  
 \*Black yearling - diamond with streak below  
 Disposition: released in Corral Basin with a band of 2 mares with foals and a gray stud

ANSOTEGUI Sorrel stud - bald face, hindstockings and left  
forestocking, squared tail  
Brown mare - blaze (bald)  
Bay mare - NSM  
Bay immature - NSM  
Brown yearling - blaze, hindstocking  
Bay foal - offspring of brown mare with blaze

One foal as of August 20

Location: Top of ridge south of Corral Basin Creek

KECK Brown stud - star, short hindstockings, squared  
tail  
Brown mare - small blaze, hindstockings  
Bay mare - hindstockings  
Black mare - star  
Black mare - NSM  
Brown mare - star  
Bay mare - white snip on nose  
Gray mare - blaze, 4 stockings  
Bay yearling - small blaze  
Sorrel yearling - blaze, hindstockings

No Foals as of August 20

Location: Spar Mountain or Gossey Spring

TURNER Bay stud - small star, hindstockings, squared  
tail  
Bay mare - NSM  
Bay mare - small blaze, hindstockings  
Brown mare - NSM  
Brown mare - NSM  
Brown immature - NSM  
Dark brown foal

One Foal as of August 20

Location: South of Corral Basin Creek; moves a lot



BAND #4

White stud	- blond mane and tail
Bay mare	- blaze, hindstockings
Bay mare	- NSM
Bay mare	- hindstockings, left front stocking
Bay immature	- blond mane and tail, 4 stockings
Bay foal	- blaze, blond mane and tail, 4 stockings

One Foal as of August 20

Location: Spar Mountain, west side

BAND #5

Gray white stud	- NSM
Bay mare	- hindstockings
Black mare	- NSM
Palomino immature	- bald face, hindstockings
Bay foal	- NSM

One Foal as of August 20

Location: Area from Bradshaw Basin east to Lone Pine Peak

BAND #6

Brown stud	- NSM
Bay mare	- star
Dark gray mare	- bald face
Bay mare	- hindstockings
Bay foal	- NSM
Brown foal	- NSM

Two foals as of August 20

Locations: Corral Basin near Spar Mountain

BAND #7

Bay stud	- star
Black mare	- star
Bay mare	- hindstockings
Bay mare	- NSM
Sorrel paint	- white belly, stripe down left rear leg, spot on left rump, blaze
Bay foal	- large star, 4 stockings

One Foal as of August 20

Location: S.W. of Spar Mountain

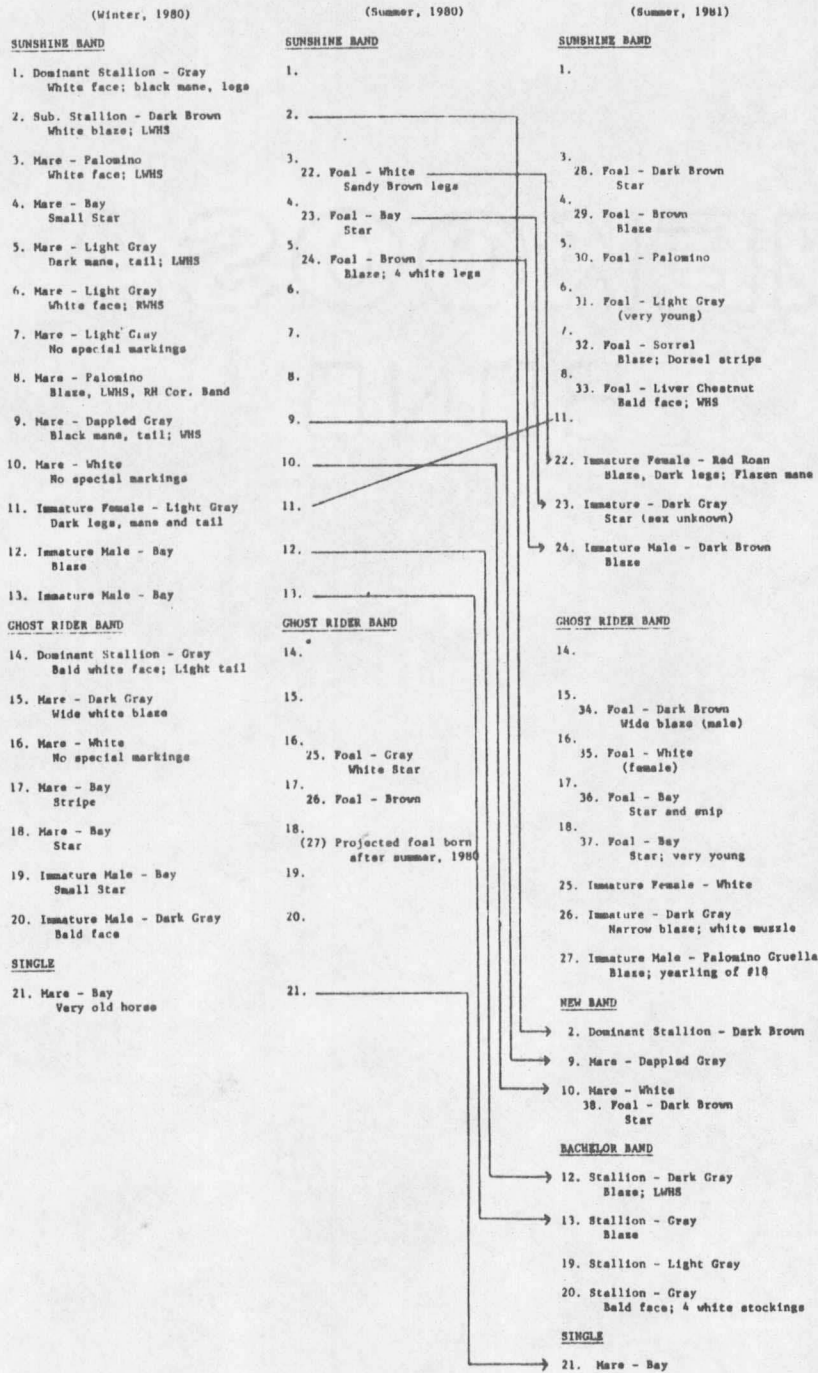
BAND #8

Brown stud - hindstockings  
Black mare - blaze  
Dappled gray mare - black and white tail  
Light gray mare - blond tail, dark points  
Black yearling - hindstockings  
Bay foal - star, hindstockings

One Foal as of August 20

Location: Horse basin area, near horse basin spring

APPENDIX 6. JUNTURA HERD SOCIAL HERD ORGANIZATION



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## LITERATURE CITED

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