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Roger K. Rector, Superintendent
Assateague National Seashore
Route 2, Box 294
Berlin, Maryland 21811

Dear Mr. Rector:

Thanks for your letter of November 6, and your interest in our research. I will try to give you a comprehensive picture of the work Dr. Turner and I have done and how it might apply to your ponies.

Our research, funded by Interior and specifically BLM, was intended to find a way to reduce fecundity in feral horses. To make a long story short, we found a drug that, when compounded appropriately, can be delivered to stallions and will reduce sperm counts to a point where fertility is unlikely.

The drug can be delivered to a captured animal by injection, or to free-roaming animals remotely with a dart and a tranquilizer gun. The drug must be delivered a few months in advance of the breeding season and will have its effects only for one breeding season. It is important to understand that we have developed and are endorsing only a reversible approach to fertility control. We do not believe that the gene pool should be unalterably changed.

In a field test in Challis, Idaho we were able to reduce foaling by 83%. The details of that test are given in the two enclosed papers. Since that test we have shown that remote delivery of the drug is quite possible. Upon conclusion of that field test, the project came to its end and we have been unable to carry out a second field-test. Good science requires that we repeat our experiment and duplicate our success before encouraging anyone to utilize the method on a large scale.

Currently the only drawback is the expense of the drug, which is still unavailable commercially and must be compounded by hand in small quantities. Large-scale production is quite feasible however and the cost will eventually be quite reasonable- perhaps \$5.00 per animal.

The advantages of our approach to population increases in feral equids are clear. First, we act humanely. No animals are destroyed and they suffer no more than a dart. Second, the method is flexible in its approach to management. We can treat the

Let's
Discuss

1 Superintendent SR
2 Asst. Superintendent
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Ch. Interpretation
Ch. Ranger
Ch. Maintenance
3 Resource Mgt. Spec. 213K
Dean of Arts and Sciences 406/657-2177
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Supv. Pk Ranger (Ad)
Supr. Pk Ranger (Va)
Park Naturalist (Va)

Roger K. Rector
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animals every year, or every other year, or whenever, or we can treat 25% of the horses, or 50% or 100%. The approach, being reversible can be fitted to specific management needs. Third, the gene pool remains intact. While you can achieve reductions in foaling rates and thereby lower populations, no animals are removed from the gene pool permanently. This is a particularly important consideration when justifying a control program to the public. Fourth, the behavior of individual animals is not changed and the social organization of the population is not disrupted as it often is through programs like Adopt-A-Horse. Fifth, this is a long-range approach which will address the problem for many years to come instead of simply treating the symptoms of overpopulation on an annual basis.

Our approach, despite its documented success, has not been without critics. The two basic criticisms have focused on (1) multiple-stallion bands and (2) exchange of mares between bands. In our own research neither of these conditions have made themselves apparent. In answer to these criticisms however, we see no difficulty in treating all the stallions in a particular band in the case of multiple-male bands. Nor do we see any problem arising from the small number of mares which might move to a new band. If a mare leaves a treated stallion only to join with another treated stallion, the results are predictable.

The situation you describe at Assateague is an ideal setting for a second field test. Dr. Turner and I would be most interested in pursuing the subject if the National Park Service feels this approach is meritorious. Incidentally, I tried to inform the NPS of this work three years ago but the NPS gave no indication of interest.

As a closing remark I might add that there is a second drug which gave good indication of efficacy in laboratory tests but which has not yet been field tested in stallions. Also, we have access to several drugs which can be delivered remotely and which can probably render mares infertile, temporarily, also.

If I can answer any additional questions please don't hesitate to contact me (406) 657-2177. Thanks again for your interest in our work.

Cordially,


Jay F. Kirkpatrick, Ph.D.
Associate Professor of
Physiology
Acting Dean

JFK/bf

xc: Dr. Turner

enclosure

REVERSIBLE CHEMICAL FERTILITY CONTROL IN FERAL HORSES

Jay Kirkpatrick, PhD; John W. Turner, Jr., PhD; and Anne Perkins, B.S.

SUMMARY

A microencapsulated form of testosterone propionate (MTP) was administered to free-roaming feral stallions intramuscularly, in an attempt to suppress spermatogenesis and inhibit fertility. Foal counts for control bands averaged 0.371 foals per mare, whereas for MTP-treated bands the average was 0.066. There were no differences between control and drug-treated groups in any behavior endpoints studied. These results indicate that controlled-release MTP can provide measurable and reasonably effective fertility control in feral horses.

INTRODUCTION

The idea of controlling population increases in wild or feral species with chemical antifertility agents is not new, but the concept is largely untested. A limited amount of research of this nature has been directed toward a variety of species, including the coyote, *Canis latrans* (Balser, 1964), the whitetail deer, *Odocoileus virginianus* (Matschke, 1977), the red fox, *Vulpes vulpes* (Cheatum and Hansel, unpublished), and feral cats, *Felis catus* (McDonald, 1980). The results have not been encouraging and only moderate success has been achieved.

Fertility control in the horse (*Equus caballus*) is an even more limited subject. Historically and in modern times equine fertility control has centered about castration, and most often this procedure is carried out not for the purposes of limiting reproduction, but rather for the purpose of limiting androgen production and modifying behavior. Vasectomy will prevent fertilization without altering stallion behavior but the procedure is time consuming, expensive, irreversible, and has little

practicality with respect to feral horses.

The need for an effective population control scheme in feral horses is critical, and arises from the protection afforded these animals by the Wild Horse and Burro Act (PL 92-195). In May of 1967 the Bureau of Land Management reported an estimated 17,000 feral horses on public lands in 10 western states. By May of 1975, and after several years of full protection by law, the estimate had increased to 48,000 animals.³ In spite of the increases, population control efforts lagged behind. Adoption programs have proven to be unacceptably costly and the number of animals removed has not kept pace with the population growth.

This study was undertaken to test the effectiveness of using injectable controlled-release testosterone propionate as a reversible antifertility agent in feral horses.

MATERIALS AND METHODS

The feral horse population utilized in this study inhabited the Challis District wild horse range in central Idaho. Ten bands were randomly selected which ranged in size from 5 to 17 horses, with a total of 69 horses. None of the 10 bands possessed more than a single sexually mature stallion (three years or older). Ten control bands, none of which had more than a single sexually mature stallion, and ranging in size from 4 to 14 horses, with a total of 61 horses were also selected. Both the experimental and control bands inhabited an area of 97 sq. km.

Administration of the antifertility drug took place in December of 1979. A Hughes 500C jet helicopter was used to locate the test stallions. The helicopter carried three people in addition to the pilot. They included (1) a BLM employee who was responsible for identifying the stallions, (2) a veterinarian who was responsible for

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immobilizing the stallions, and (3) one of the authors (Perkins) who was responsible for administering the antifertility drug and keeping band identification records.

Previously identified harem stallions were darted from the aircraft with a Palmer Powder Charge Capture gun. Approximately 300 mg of succinyl choline was administered for immobilization, and as soon as each horse went down the helicopter landed. When necessary the stallion was ventilated with the aid of a manually operated raft pump fitted with an endotracheal tube.

The antifertility compound administered was a controlled-release, micro-encapsulated formulation of testosterone propionate (MTP). The MTP^a was homogeneously dispersed in an aqueous solution of polyethylene glycol (PEG), a biodegradable polymer, and the individual microcapsules ranged in size up to 250 microns in diameter and contained approximately 50% steroid by weight. The compound was sterilized by radiation. The MTP was administered intramuscularly in the hip. One stallion received 2.5 g, three received 5.0 g, three received 7.5 g, and three received 10.0 g.

Photographs of all stallions treated were taken and written descriptions of each horse were recorded. Tails were clipped and red flagging was tied in the manes and tails for later identification. Descriptions of band members were also recorded whenever possible.

In June, July, and August of 1980, during and immediately following the period of peak breeding activity, and six months after administration of MTP, the treated and control stallions and their accompanying bands were located. Identification was first made with the aid of a helicopter, and following that, the bands were approached via saddle horse, vehicle, or on foot. Observations were made in daylight with 10X binoculars and a 20-40X zoom spotting scope. Data were recorded on cassette tapes at the time of observation. Band membership for both MTP-treated and control bands was independently established by at least two observers. 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 (stallion, subordinate or immature male, female), 3) coat color, 4) identifying marks (stockings, blaze, paint, etc., and squared tails on the stallions).

Behavioral data were collected at all times of the day throughout the study period. The number of hours

devoted to treated and control bands was similar, and there were never more than two days between observations of control and of treated bands. Since the study included both breeding season and post-breeding season periods, the data are arbitrarily divided as follows: A) 6/9 to 6/28, B) 6/29 to 7/15, C) 7/16 to 8/2, and D) 8/3 to 8/20. Behaviors recorded included 1) elimination marking behavior, 2) mounting, 3) copulation, 4) herding (the stallion rounding the harem group up and moving them out, or rounding up individuals which had strayed), and 5) aggression (the behavioral events associated with confronting and chasing off intruders, which may or may not include fighting). In October of 1980, following the breeding season, treated stallions and control stallions and mares accompanying them were marked with paint balls from a helicopter to aid future identification.

RESULTS

In the foal count study of 1981, it was possible to identify and document 7 of the 10 treated bands and 8 of the 10 control bands. The data for foal counts are presented in Tables 1 and 2, and they reflect foal counts through August 20, 1981. Foal counts for control bands averaged 0.371 foals per mare, whereas for treated bands the average was 0.066; i.e., only 1/6 as many foals were produced in treated bands. Seven of eight control bands had at least one foal, whereas only two of the seven treated bands had a foal. Both foals produced by treated bands were from mares which had been documented in summer, 1980, to be with their respective treated

TABLE 1
BAND COMPOSITION OF
HORMONE-TREATED AND CONTROL BANDS

	Control	Treated
No. of Bands	8	7
No. of Horses	56	50
No. of Mature Mares.....	35	30
No. of Immatures	9	10
No. of Foals	13	2
Avg. Band Size	7.0	7.1

TABLE 2
FOALING DATA FOR
HORMONE-TREATED AND CONTROL BANDS

	Control	Treated
No. of Foals/Mare	0.371	0.066
No. of Foals/Band	0.62	0.28
% of Bands with Foals....	85.5	28.4

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TABLE 3
FREQUENCY OF DISPLAY OF SPECIFIC BEHAVIOR BY
HAREM STALLIONS IN THE CHALLIS HERD, SUMMER 1980

Type of Behavior	Frequency of Behavior*			
	June 9-28		June 29-July 15	
	MTP	Control	MTP	Control
Elimination Marking (EMB)	16.8	16.1	13.9	14.9
Mounting	2.1	1.9	2.0	0.8
Copulation	1.2	1.0	0.9	0.4
Herding	2.3	2.3	1.8	2.1
Aggression	3.0	2.7	2.6	2.3

*Average frequency per 10 hrs. of observation for MTP (n=5) and control (n=5) stallions.

stallions, and each mare with a foal was with the same stallion in 1981 and had foaled prior to June 30. These two stallions had received 5.0 g and 7.5 g of the MTP.

Behavioral data for 1980 and 1981 are presented in Tables 3 and 4, respectively. In 1980, there were no differences between control and MTP-treated groups in any behavioral endpoint in period A. In period B, frequencies of all endpoints were decreased in controls, and in the MTP-treated group similar decreases were observed for all endpoints except mounting and copulation, which remained elevated.

There were no differences between control and drug-treated groups in any behavioral endpoint in any of the three study periods in 1981 (Table 4). In both groups the frequencies of mounting, copulation and elimination marking behavior (EMB) in periods C and D were significantly ($p < .01$, multiple range test) lower than frequencies in period B. Frequencies of both herding and aggressive behavior were progressively, but not significantly, lower in each time period. Although behavior for individual stallions was variable, none of the individual measures were drastically different from the mean. Several stallions (both control and treated) failed to exhibit mounting and/or copulation behavior during the entire observation period.

DISCUSSION

These results strongly suggest that microencapsulated testosterone is an effective antifertility agent in feral horses, and that the reproductive behavior of treated stallions was unaffected by the drug. Previous unpublished studies of the effect of MTP on domestic pony stallions, conducted at the University of Pennsylvania School of Veterinary Medicine under the direction of Dr. Robert Kenney, indicated that 5.0 g of the drug administered intramuscularly caused significant and reversible decreases in sperm counts (36% decrease), total sperm motility (13% decrease), and progressive

motility (29% decrease). In the seven horses recovered in 1981, doses of MTP ranged from 5.0 g to 10.0 g, and there appears to be no difference in the results regardless of the dose. The two treated stallions who each presumably sired a foal received 5.0 g and 7.5 g of MTP, respectively, and these doses were the same and 50% greater, respectively, than the dose given to the stallion of band F, whose band had no foals. The reason for the lack of fertility inhibition in these stallions is unknown. Subjective estimates indicate that the weight of these stallions was similar to the others, and no differences in the measured endpoints were noted among the studs.

The mechanism of action of testosterone appears to be associated with decreased output of the pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH).¹² The fact that sperm motility and sperm counts decreased suggests an impairment of testicular physiology and accounts for the decrease in fertility.

There is no evidence to support the idea that decreased libido interfered with reproduction. The MTP-treated feral stallions were observed mating during the 1980 breeding season and a comparison of the 1981 data with behavioral data for summer 1980 revealed no gross differences in the endpoints studied, with the exception of mounting and copulation frequency. In 1980 mounting and copulation decreased after the peak of the breeding season in control stallions, but not in treated stallions. We suggest the the maintenance of mating behavior in treated stallions reflected continued estrus cycling by their mares which were not yet pregnant due to the stallion's infertility and/or continued high systemic testosterone levels and increased libido in the stallions. Since mating frequency after the breeding peak was decreased in treated stallions as well as in controls in 1981, it appears that the treated stallions were again fertile in 1981. This will be firmly established by foal counts in control and drug-treated bands in summer of 1982.

Recent criticisms of male-focused fertility control in

TABLE 4
FREQUENCY OF DISPLAY OF SPECIFIC BEHAVIOR BY
HAREM STALLIONS IN THE CHALLIS HERD, SUMMER 1981

Type of Behavior	Frequency of Behavior*					
	June 29-July 15		July 16-Aug. 2		Aug. 3-Aug. 20	
	MTP	Control	MTP	Control	MTP	Control
Elimination Marking (EMB)	17.3	15.4	11.2	12.3	10.0	8.5
Mounting	1.1	1.3	0.6	0.8	0.5	0.4
Copulation	0.9	0.8	0.3	0.3	0.2	0.1
Herding	2.0	1.9	1.5	1.3	1.3	1.2
Aggression	2.8	2.3	1.8	1.9	1.6	1.4

*Average frequency per 10 hrs. of observation for MTP (n=7) and for control (n=8) stallions.

feral horses¹¹ directed specifically at the potentially disrupting effects of mare exchange between feral horse bands appear to be unfounded, at least among feral horses in Challis. While others^{10,2} have reported high rates of exchanges of mares between bands on another range (Red Desert, Wyoming) there was little evidence of this in Challis. On the Challis horse range there was ample opportunity for band interaction, with seven treated bands and eight control bands inhabiting an area of 97 sq. km., yet few exchanges were observed during the 1980 breeding season. This corroborates previous findings in the Pryor Mountain horses.⁴ The seasonal rise in androgens and testosterone in breeding stallions⁷ and the accompanying aggressive herding and reproductive behavior^{6,13} indicates that the breeding season is the time when mare exchanges are least likely to occur. In our field test we purposely left a number of untreated bands in the target area in order to test the effect of mare exchange.

Nelson¹¹ also posed some questions about the effectiveness of male-focused fertility control because of data which suggest that subordinate stallions participate in breeding activity. In the present study, it is unlikely that foals were sired by stallions other than the harem stallion. Harem stallions of the Challis herd were not observed permitting outside males to mate with their mares and rarely permit mounting of their mares by subordinate males in the band. This is similar to the social pattern in the Pryor Mountain herd^{4,5} but dissimilar to that reported for the Red Desert herd in Wyoming.^{10,2}

Previous data from clinical trials of MTP in domestic pony stallions (Kenney, unpublished) indicate that the drug exerts its antifertility effects for three to six months and that these effects are reversible. Studies of foaling patterns of feral mares in Winnemucca and the Pryor Mountains, and of seasonal ovulatory patterns in Pryor Mountain mares indicate that ovulation rarely begins before April and it ceases by late August. Thus a single MTP treatment can inhibit fertility for an entire breeding season. An important consideration in fertility control

studies of wildlife populations is the integrity of the natural selection process. Since the MTP is reversible in its action, there is no danger of losing a particular segment of the gene pool permanently. Once populations are stable the dominant stallions may continue breeding.

One obvious drawback to the use of MTP is that currently the stallions must be rendered immobile in some manner before the drug can be administered. There were a number of difficulties encountered with the use of succinyl choline, including respiratory arrest. This points to the need for the development of a safer immobilizer for feral horses. There was no difficulty in hitting animals, however, with either darts or paint balls from a helicopter. The MTP must be hand-injected because of the high viscosity of the two available carriers for the drug, polyethylene glycol (PEG) and carboxymethyl cellulose (CMC); however, efforts to develop less viscous carriers are in progress.

The results of the present study strongly indicate that controlled-release microencapsulated testosterone propionate can provide effective reversible fertility control in feral horses. This approach offers a reasonable, cost and time-efficient system for management of these animals.

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Androgens, behaviour and fertility control in feral stallions

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Summary. This field study of feral stallions in Montana and Idaho examines and correlates the seasonal pattern of plasma androgens and specific sociosexual behaviour and reports the effect of a long-acting androgenic steroid on this behaviour and on fertility. Plasma testosterone was measured by competitive protein binding assay in samples obtained by jugular venepuncture from captured animals. In samples taken from 34 sexually mature stallions in 6 different months during the year, a definite seasonal pattern in testosterone was present, with a peak in May (3.04 ± 0.63 ng/ml) and a nadir in December (1.55 ± 0.34 ng/ml). Values were <2.0 ng/ml in non-breeding months and >2.4 ng/ml in breeding months. Behavioural endpoints measured were (1) stallion scent marking in response to elimination by mares (elimination marking), (2) mounting and (3) copulation. The frequencies of each of these endpoints followed closely the seasonal pattern seen for plasma androgens.

In the fertility study microcapsulated testosterone propionate (microTP) was administered i.m. to 10 harem stud stallions 3 months before the 1980 breeding season. In these stallions and in 10 control harem studs, the above behavioural endpoints were examined in the 1980 and 1981 breeding seasons, and foal counts were made in 1981. There were no direct inhibitory or stimulatory effects of microTP treatment on any of the behavioural endpoints in either year. In 1981 foals were produced in 87.5% of the control bands and 28.4% of the microTP-treated bands. These results indicate that microencapsulated testosterone propionate can provide effective fertility control in feral horses without causing significant alterations in sociosexual behaviour.

Introduction

The genus *Equus* is not native to North America. The horse was introduced by exploring Spaniards in 1519. Some horses in the original and ensuing generations were turned out, lost or escaped from domestic control and reverted to the wild state in isolated mountain and desert areas of the western United States. They thrived in these areas for several hundred years in populations estimated at several million, but with the settlement of the West and the human pressure of capture and slaughter their numbers had dwindled to less than 17 000 in 1967. With the passage of the Wild Horse and Burro Act in 1971, which made capture or destruction of these horses illegal, the population again began to expand. Unfortunately, unchecked population growth in the face of limited grazing has led to serious management problems with the current population of nearly 80 000. An Adopt-A-Horse programme, sponsored by the government, has proved unacceptably costly and has been unable to keep pace with population growth.

During the past 7 years (1974–1981) we have studied the reproductive biology of these feral horses and have attempted to develop an endocrine-based method of fertility control for them. Although the idea of controlling wild or feral populations with chemical antifertility agents is not new, implementation of this idea has been limited. Such studies have been performed with several species, including the coyote (Balser, 1964), whitetail deer (Matschke, 1977), red fox (E. Cheatum & W. Hansel, unpublished) and feral cat (McDonald, 1980), but results have not been encouraging.

Most previous studies have been orientated towards controlling female fertility. In the feral horse herds in the present study, dominant stallions usually breed and defend a harem of several (2–11) mares. Thus fertility control of males would be more efficient. Under ideal conditions an antifertility agent would provide reversible infertility without disruption of normal behaviour patterns. The present report examines the effect of a long-acting androgenic steroid on specific behaviour and fertility in feral stallions. Accurate assessment of the effectiveness of this exogenous steroid requires knowledge of the normal seasonal patterns of endogenous androgens and specific behaviour. Therefore, this report incorporates three studies of feral stallions: (1) seasonal patterns of plasma androgens, (2) seasonal patterns of three specific behavioural endpoints, and (3) effect of a long-acting androgenic steroid on those behavioural endpoints and on fertility.

Materials and Methods

The feral horse populations utilized in these studies inhabit (1) the Pryor Mountain National Wild Horse Refuge in south-central Montana and (2) the Challis District wild horse range in central Idaho. The geography, climate and ecology of these ranges are similar. Both the Pryor and Challis ranges were used in behavioural studies. The Pryor Range was the primary site for hormonal studies, while the antifertility field trials were carried out in the Challis range.

Plasma testosterone study

These horses are extremely dangerous to handle and were restrained by the 'heading and heeling' technique of Kirkpatrick *et al.* (1976) who regarded the method to be the only relatively safe way of obtaining blood samples by jugular venepuncture. Kirkpatrick, Baker, Turner, Kenney & Ganjam (1979) observed no difference in stress response as indicated by plasma corticosteroid levels in feral horses sampled while quietly standing and restrained versus feral horses sampled after 'heading and heeling'. All blood samples were taken between 09:00 and 18:00 h which was in the plateau period described by Kirkpatrick *et al.* (1976).

Blood samples were collected in heparinized tubes, centrifuged and frozen on solid CO₂. In the laboratory steroids were ether-extracted from plasma and testosterone was separated from oestrogens and other 17 β -hydroxyandrogens (Kirkpatrick, Wiesner, Kenney, Ganjam & Turner, 1977). Testosterone and fetal 17 β -hydroxyandrogens were measured by competitive protein binding assay as described by Ganjam & Amann (1973). Post-extraction chromatography recovery was $91.2 \pm 3.7\%$. Inter-assay variation was 12.1%, and intra-assay variation was 8.2%. The lower limit of assay sensitivity (differentiable from blank at $P < 0.05$) was 0.28 ng/ml. Data were analysed by ANOVA and Duncan's Multiple Range Test (Alder & Roessler, 1972).

Behaviour study

The primary social unit in feral horses consists of a stud stallion and his harem of mares and their offspring (Plate 1). Bachelor males live singly or in groups until they acquire a harem.



Fig. 1. Adult feral stallion on the range. Photograph taken from seclusion with 500 mm telephoto lens. Note numerous scars on this animal's coat.

Fig. 2. Feral horse band with a foal. Band is alerted to observer. Stallion has protectively moved to right foreground and others are beginning to move off.

(Facing p. 80)

which they maintain and defend. Stallions display several specific behaviour patterns in the harem context, including aggression, grooming, herding, mounting, copulation and elimination (urination-defaecation) marking behaviour. We examined the last three of these patterns, particularly elimination marking, as it was the only readily observable and quantifiable behaviour that occurred with reasonable frequency (Turner, Perkins & Kirkpatrick, 1981).

The data are based on more than 400 h of observation between 1974 and 1978. Horses were observed with $\times 8$ binoculars or a $\times 20$ –40 zoom spotting scope. The system consisted of locating one or two bands of horses in the early morning and observing them throughout the day by following them when they moved to establish reproductive behaviour and daily elimination marking patterns in the context of other daily activities. Only data collected when horses were unaware of observers were used. Conditions existing at the time of observation were recorded, including time, weather, location, and general nature of horse activity i.e. grazing, resting, trailing, watering. A description of each stallion and his location relative to the other horses was also recorded.

Mounting consisted of the stallion approaching the mare from behind, with or without perineal sniffing, rising up over her hind quarters and claspings her sides with his forelegs, with or without pelvic thrusting. Attempted mounts ending prematurely by female resistance were recorded as mounts. Intromission with or without ejaculation was recorded as copulation.

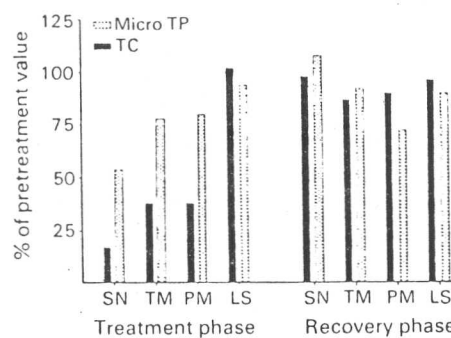
Stallion elimination marking behaviour (EMB) was defined as the immediate behavioural response of a stallion to a urination and/or defaecation made by another horse within 25 m of the stallion. EMB was rarely exhibited by band members other than the harem stallion, and was primarily directed toward eliminations made by mares (see Turner *et al.*, 1981). An EMB response was recorded only when (1) the observer witnessed the elimination serving as a stimulus and (2) the marking response was initiated within 1 min of the elimination. The minimum criterion for an EMB response was (a) deliberate movement to the elimination site, (b) smelling of the faeces or urine with or without Flehmen and (c) urination or defaecation on the site. The frequency of behaviour was determined by dividing the number of responses by the number of eliminations which occurred within about 25 m of the stallion; this is the elimination marking behaviour quotient (EMBQ).

Fertility control study

Ten bands, each of 5–17 animals, of feral horses (totalling 69) from the Challis District of central Idaho were injected with an antifertility compound and another 10 bands (total 61 horses) served as controls. None of the bands contained more than one sexually mature stallion (>3 years of age). The experimental and control herds inhabited an area of 97 km². The compound was timed-release testosterone propionate, given as one i.m. injection of encapsulated microcrystals (microTP) up to 250 μ m in diameter and homogeneously dispersed in an aqueous biodegradable polymer (Southern Research Institute, Birmingham, Alabama). The compound in this form resulted in decreased sperm count and sperm motility for 4–6 months but did not affect libido (Text-fig. 1).

In December 1979 each of the previously identified harem males was located by helicopter and darted with an immobilizer (~300 mg succinylcholine). The helicopter landed immediately after each horse went down, and the microTP was administered i.m. in the flank in a dose ranging from 2.5 to 10 g. When necessary, stallions were ventilated by a manually operated air pump fitted with an endotracheal tube. Photographs were taken of each stallion and a description of each stallion and his band was recorded. Tails were clipped and red flagging was tied in the manes for later identification.

From May to August, i.e. during and immediately after the peak breeding period, in 1980 and 1981, extensive observations (>440 h) of treated and control bands were made. On these occasions the bands were located by helicopter and subsequently approached via saddle horse,



Text-fig. 1. Effect of a single injection of microTP or 6 monthly injections of testosterone cypionate (TC) on semen parameters and libido in domestic Ponies. SN = total sperm number; TM = total sperm motility; PM = progressive sperm motility, LS = libido score. The treatment was 4 months after initiation of monthly TC or single microTP injection; the recovery phase was 8 months after single microTP injection or 2 months after cessation of monthly TC injection. N = 3 for each group. This study was supervised by Dr R. M. Kenney and Dr V. K. Ganjam at the University of Pennsylvania.

vehicle or on foot. Observation protocol was as described previously. Band membership was independently established by at least 2 observers and data are based on a minimum of 10 h direct observation for each band at distances ensuring positive identification.

The following sequence and combination of criteria were used in band identification: (1) number of animals, (2) age (foal, immature, adult) and sex (stallion, subordinate or immature male, female) (3) coat colour, (4) identifying marks (stockings, blaze, paint, etc, and squared tails on the stallions).

Behavioural data were collected at all times of the day throughout the study. The time given to treated and control bands was similar, and there were never more than 2 days between observations of control and treated bands. Since the study included the breeding season and the period after it, the data were arbitrarily divided as follows: (A) 9–28 June, (B) 29 June–15 July, (C) 16 July–2 August and (D) 3–20 August. Behaviours recorded included (1) EMB, (2) mounting and (3) copulation. Foal counts were initiated in May and continued until 20 August.

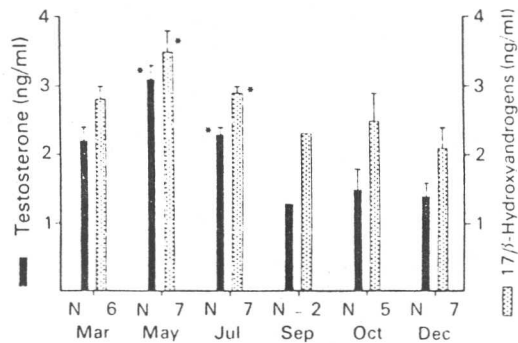
Results

Plasma testosterone

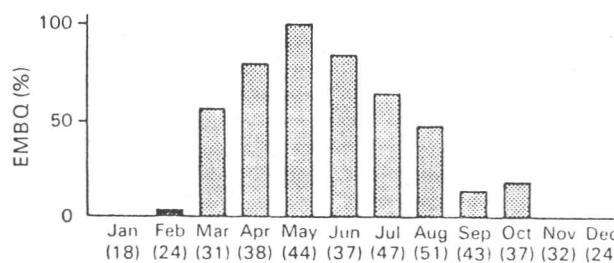
Based on samples taken from 34 stallions in 6 different months during the year, plasma testosterone levels showed a definite seasonal pattern with a peak in May of 3.04 ± 0.63 ng/ml and a nadir in December of 1.55 ± 0.34 ng/ml (Text-fig. 2). Values in all animals were >2.4 ng/ml in breeding months and <2.0 ng/ml in non-breeding months. Total 17β -hydroxy androgens showed a seasonal pattern similar to testosterone (Text-fig. 2). Testosterone as percentage of total androgens increased from 58% in December to 85% in May.

Behaviour

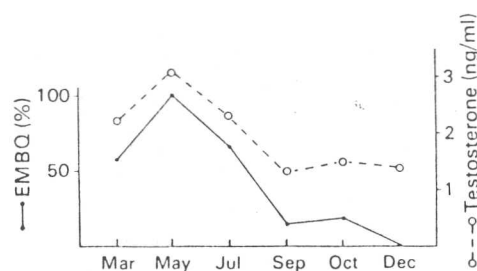
The frequency of EMB responses varied markedly with the time of year, with the EMBQ averaging 80% from April to July (breeding season) and 1% from November to February (Text-fig. 3). During the peak months of the breeding season, May and June, stallions responded to 93 and 89% of eliminations, respectively. In contrast, only one EMB response was observed for 98 eliminations between November and February. Seasonal testosterone and EMB patterns are compared in Text-fig. 4.



Text-fig. 2. Seasonal variation in plasma testosterone and 17β-hydroxyandrogens in feral stallions. Values are mean ± s.d. *Values significantly ($P < 0.01$, multiple range test) greater than respective values in December.

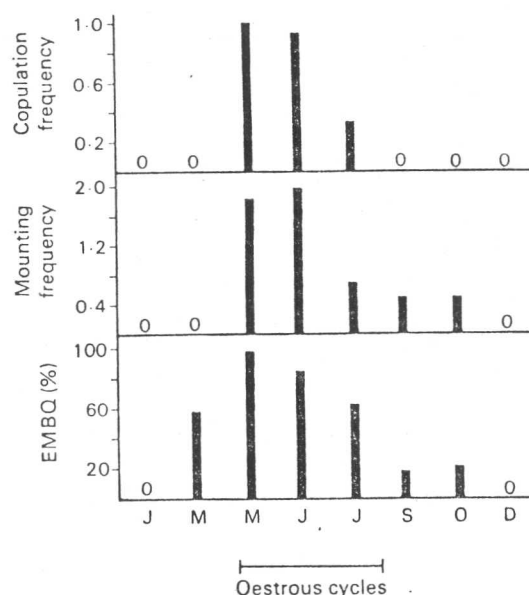


Text-fig. 3. Monthly frequency of elimination marking behaviour by feral stallions in response to elimination by harem mares. Elimination marking behaviour quotient (EMBQ) = number of stallion EMB responses divided by number of mare elimination within 25 m of stallion. No responses occurred in November, December or January. Values in parentheses are number of observations.



Text-fig. 4. Comparison of seasonal pattern of plasma testosterone with pattern of elimination marking behaviour quotient (EMBQ) in feral stallions of the Pryor Mountain Wild Horse Refuge.

Mounting and copulation frequencies showed a seasonal pattern similar to that for EMB (Text-fig. 5). However, mounting and copulation were observed less than 10% as often as EMB, due in part to the fact that mating behaviour is limited to periods of behavioural oestrus (8 out of 21 days) exhibited by harem mares. The ratio of mounting frequency to copulation frequency during the breeding season averaged 2:1, with mounting ceasing slightly later in the season than copulation.



Text-fig. 5. Incidence of mounting and copulation (per 10 h observation) correlated with elimination marking behaviour quotient (EMBQ) for feral stallions in various months during the year. Data base is more than 400 h direct observation of 27 harem stallions and their bands.

Fertility control

The goals of the 1980 and 1981 data collection were (1) to identify accurately treated and control bands, (2) to determine whether treatment had behavioural effects in the field and (3) to determine antifertility effectiveness of the microTP in the field.

Due to changes in band membership, mortality and inaccessibility of some sections of the range, accurate data were obtained from only 7 of the 10 original treated bands and from 8 of the 10 control bands. Behavioural data for the 1980 breeding season were based on more than 180 h of direct observation (Table 1). There was no difference in EMBQ between microTP-treated and control bands at any time although EMBQ in Period A was lower than in Period B in treated and control bands. Mounting and copulation frequencies in microTP-treated bands were not different from frequencies in control bands in Period A. In Period B the frequencies in control bands were lower than in Period A, with mounting and copulation decreased by 56 and 60% respectively. Behavioural data for the 1981 breeding season were based on more than 260 h of direct observation (Table 1). The time of data collection for 1980 and 1981 overlaps only for Period B. There were no marked differences between 1980 and 1981 in any of the 3 behavioural endpoints in Period B. In 1981 there were no differences between treated and control groups in any behavioural endpoint in any of the 3 periods. In both groups in Periods C and D and frequencies of mounting and copulation and the EMBQ were significantly lower ($P < 0.01$, multiple range test) than in Period B. Periods C and D are after the peak of the breeding season. Several stallions (control and treated) failed to exhibit mounting or copulation during the entire observation period.

Foaling data for 1981 are presented in Table 2, reflecting foal counts until 20 August 1981. Foal counts for control bands averaged 0.37 foals per mare, whereas for treated bands the average was 0.07. Seven of 8 control bands had at least one foal, whereas only 2 of the 7 treated bands had a foal. Both the foals in treated bands were from mares which had been noted in summer 1980 to be with their respective treated stallions, and each mare with foal was with the same stallion in 1981 and had foaled before 30 June. One of these stallions had received 5.0 g microTP, the other 7.5 g.

Table 1. Frequency of display of specific behaviour by harem stallions of the Challis herd in summer 1980 and summer 1981

Type of behaviour	1980*				1981†					
	Period A (9–28 June)		Period B (29 June–15 July)		Period B (29 June–15 July)		Period C (16 July–2 Aug.)		Period D (3–20 August)	
	MicroTP	Control	MicroTP	Control	MicroTP	Control	MicroTP	Control	MicroTP	Control
Mounting	2.1	1.9	2.0	0.8	1.1	1.3	0.6	0.8	0.5	0.4
Copulation	1.2	1.0	0.9	0.4	0.9	0.8	0.3	0.3	0.2	0.1
Elimination										
Marking (EMB)	16.8	16.1	13.9	14.9	17.3	15.4	11.2	12.3	10.0	8.5
EMBQ‡	91	87	72	77	85	82	60	64	50	55

* Average frequency per 10 h of observation for microTP (N = 5) and control (N = 5) stallions.

† Average frequency per 10 h of observation for microTP (N = 7) and control (N = 8) stallions.

‡ EMBQ = no. of EMB displays/no. of eliminations by mares within 25 m of stallion.

Table 2. Band composition of microTP-treated and control bands of the Challis herd in 1981

	No. of				
	Bands	Horses	Mature ♀♀	Immature animals	Foals
MicroTP-treated	7	50	30	10	2
Controls	8	56	35	9	13

Discussion

In the present study definite seasonal differences in plasma testosterone of feral horses were observed, with a nadir in September and a peak in May. In domestic horses Berndtson, Pickett & Nett (1974) and Raeside (1978–79) observed seasonal changes in plasma androgen, but Thompson, Pickett, Berndtson, Voss & Nett (1977) reported no seasonal changes in androgen levels. Differences in conditions between the wild and domestic state preclude direct comparison with these studies.

The close correlation between seasonal changes in plasma testosterone and seasonal frequencies of mounting, copulation and EMB (annual testosterone and EMBQ curves were almost superimposable), suggest that these behaviour displays in feral horses may be androgen dependent. The androgen dependence of mounting and copulation is well known for many species. In addition to EMB, other feral stallion behaviour associated with harem maintenance has shown a correlation with plasma testosterone (Angle *et al.*, 1980). Since EMB (1) can be correlated with plasma testosterone, (2) is related to the integrity and activity of the harem, (3) occurs frequently and (4) is readily quantifiable (Turner *et al.*, 1981), it appears to be a useful endpoint for monitoring possible behavioural effects of antifertility agents in feral horses.

Based on comparison of the behavioural data for control and microTP-treated stallions in 1980, when the drug was active, it appears that the treatment had no significant effect on EMB. This is further supported by the similarity of the 1980 and 1981 data, i.e. a drug-active year versus a non-drug year. There is no evidence in the mounting and copulation data that microTP

treatment interfered with libido in 1980 or that it had a delayed inhibitory effect carried into 1981.

One puzzling observation was that mounting frequency in 1980 decreased after the peak of the breeding season in controls, but persisted at Period A (peak breeding season) levels in the drug-treated bands during Period B (after peak breeding season). It was initially considered that microTP may increase libido above normal. However, while mounting frequency in treated stallions persisted during Period B, it was not increased, and was never above control levels, in Periods A or B. It is unlikely that the stallions were impotent, since mounts were almost always associated with penile erection. An alternative possibility is that persistence of mounting behaviour in treated stallions reflected continued oestrous cyclicity by mares that were not yet pregnant due to the stallion's infertility. Since few copulations were observed it was not possible to document mare receptivity, although, judged subjectively, resistant behaviour by mares was not greater in Period B than in Period A. Previous studies (Turner & Kirkpatrick, 1980) have demonstrated consistent oestrous cyclicity in feral mares from 15 April until 30 August.

The behavioural data in conjunction with the 1981 foal count for control and treated bands strongly suggest that microTP is an effective antifertility agent that does not inhibit libido or interfere with maintenance of the harem. In the 7 treated bands for which foal counts were reported in 1981, the dose of microTP ranged from 5 to 10 g. Two of the treated stallions, one receiving 5 g and the other 7.5 g, presumably sired a foal, while another stallion given 5 g had no foals in his band. The reason for lack of fertility inhibition in the two stallions is unknown. Subjective estimates indicate that all the stallions were of similar weight, and there were no notable difference in behavioural endpoints amongst these 7 stallions. The fertile stallion receiving 5 g microTP had 2 mature mares in his band, while the stallion receiving 7.5 g microTP had 4 mature mares. The foaling rate of 0.25 foals per mare for the latter group was less than the rate of 0.37 foals per mare observed for control bands in this study. However, it is not possible to confirm decreased fertility in this stallion, since a control band with 4 mares also had only 1 foal.

The mechanism of microTP action in producing infertility is unknown. Direct and indirect effects (by inhibition of gonadotrophin release) on spermatozoa and spermatogenesis have been reported in several species (Steinberger, 1980). A study of microTP treatment of domestic Ponies demonstrated decreased sperm count and decreased motility and progressive motility at a dose of 5.0 g. Although the sperm count did not decrease to severe oligospermic levels, the combined effect on count and motility may have been sufficient to produce infertility.

In the present study it is unlikely that foals were sired by other than the harem stallion, because these stallions of the Challis herd do not permit outside males to mate with their mares and rarely permit mounting of their mares by subordinate males in the band. Furthermore, a number of attempts by other stallions to challenge or steal mares from microTP-treated males in 1980 and 1981 were unsuccessful. This is similar to the social pattern in the Pryor Mountain herd (Hall, 1972; Feist & McCullough, 1976), but dissimilar to that reported for the Red Desert herd in Wyoming (Miller & Denniston, 1979), in which some stallions permit outside and subordinate males to mate with their mares. In this latter situation effective fertility control would require treatment of a considerably larger number of stallions, although it would still be more efficient than fertility control of mares. Three important considerations in fertility control studies of wild life populations are: (1) ecological safety, (2) efficiency/economy and (3) reversibility of treatment. In the present study the treatment was ecologically safe since the drug was injected directly into the animal and was degraded before excretion. The treatment was efficient and economical since a single injection inhibited fertility for an entire breeding season and infertility in one stallion prevented pregnancy in a number of mares. In the domestic Pony the microTP effect was completely reversible. In the 1981 field study, non-pregnant mares would still have been cyclic in July and August. Since mounting and copulation frequencies were low in July and August, mares were probably pregnant and not cyclic, suggesting that treated stallions

were again fertile (this will be documented in 1982 foal counts). Therefore, microTP appears to be reversible in its action, permitting the integrity of the natural selection process and minimizing the possibility of losing a segment of the gene pool forever.

The results of the present study indicate that controlled-release microencapsulated testosterone propionate can provide effective, reversible fertility control in feral horses. This approach may offer a reasonable and efficient system for management of these animals.

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