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EFFECTS OF HORMONE IMPLANTS ON ESTRUS AND OVULATION IN FERAL MARES

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ABSTRACT: Five groups of 30 captive feral mares each were implanted with silastic rods containing estradiol (E) and/or progesterone (P): E only with 8 g, P only with 24 g, P+HE with 8 g P + 8 g E, HP+E with 12 g P + 4 g E, HP+LE with 12 g P + 2 g E. Arbitrary group designations were differentiated by relative high (H) and low (L) amounts of steroid. Thirty mares received silastic rods containing no hormone (CI). Five mares from each group were bled every 2 wk for 4 mo and monthly for another 5 mo. All mares were tested for estrus by allowing them to stand in an alley between two pens of stallions and visually monitoring her response to the stallion. Serum P levels increased from 0.3 ± 0.1 to 1.8 ± 0.1 ng/ml in the P only group during the first 3 wk after implanting. Levels remained stable for the next 2 wk and then began a gradual decline. Serum P levels in the other groups were lower. Serum E levels were slightly increased in the groups receiving 8 g of E (E only and P+HE groups). Significantly fewer animals in the E only and P+HE groups exhibited estrus as compared with control animals (10 of 23 and 13 of 26 versus 22 of 25, respectively, $P \leq 0.003$). However, animals receiving 24 g of P (P only) showed similar occurrences of estrus as controls. All animals detected in estrus ovulated as evidenced by elevations in serum P levels above 5 ng/ml collected 10 days after the mare was detected in heat. In spite of the significant effect of hormone implants on the occurrence of estrus, over 80% of mares bred and conceived when placed with a stallion. Our data suggest that these levels of implanted hormones can raise serum levels of P and E for at least 21 wk. These hormones, when implanted during early anestrus, can alter the occurrence of psychic estrus in captive feral mares without suppressing ovulation and conception.

Key words: Hormone implants, estradiol, progesterone, estrus, ovulation, feral horses, *Equus caballus*, experimental study.

INTRODUCTION

Management of feral horses (*Equus caballus*) to prevent overgrazing on public rangelands in the western United States is a controversial subject. Although designation of rangelands as overgrazed has not been experimentally substantiated (Vale, 1975; Wagner, 1983; Berger, 1986), such categorization is common and has been the subjective opinion of stockmen, wildlife specialists and environmentalists. Livestock interests continue to complain about grazing competition between livestock and feral horses, and management policies of the Bureau of Land Management (BLM) often have favored these interests, primarily because grazing has been the most important commercial activity on public lands in the western United States (Vale, 1975). Language expressing concern for the condition of rangelands and questioning the role of feral equids in degeneration of these ranges was included in the Public Rangeland Improvement Act of 1978 (Public Law 95-514, United States at Large 92[2]: 1803–1810).

Appropriate management levels of horse numbers have been set for rangelands (Boyles, 1986), and since 1978 substantial sums of money have been appropriated to BLM for the removal of horses to achieve these management levels. Horses removed from these rangelands are offered at public auction under the Adopt-a-horse Program (Boyles, 1986). The number of horses removed in recent years has been greater than the number adopted, and the need for permanent confinement has devel-

TABLE 1. Amount of hormone implanted in feral mares in each treatment group.

Group*	Progesterone (g)	Estradiol (g)
E only	0	8
P + HE	8	8
HP + E	12	4
HP + LE	12	2
P only	24	0
CI	0	0

* See text for explanation of abbreviations used to designate groups.

oped. Permanent confinement facilities holding 2,000 to 3,000 horses each were established in Nevada, Texas and Nebraska. Unadoptable horses are maintained at these facilities for the remainder of their lives. To reduce the cost of confining unadoptable horses, the BLM is investigating the potential for reducing the recruitment rate of free-roaming populations of horses by limiting reproduction.

Kirkpatrick et al. (1982) reported successful lowering of reproduction in a population of horses in Idaho with temporary sterilization by the remote injection of testosterone propionate into dominant stallions. However, Wagner et al. (1982) suggested that this technique did not have promise for application throughout the western United States because there often was an exchange of mares between bands and not all foals are sired by the dominant stallion in a band. In this paper we report on our experience with estradiol (E) and/or progesterone (P) impregnated capsules for controlling estrus, ovulation and pregnancy in captive feral mares at one of BLM's permanent confinement facilities.

MATERIALS AND METHODS

Silastic rods (implants) were prepared in lots of 20 by thoroughly mixing either crystalline P (4 g/implant; Steraloids, Inc., Wilton, Massachusetts 03086, USA) or crystalline E (4 g/implant; Sigma Chemical Co., St. Louis, Missouri 63160, USA) with medical grade silicone rubber (Silastic #382, Dow Corning, Inc., Hemlock, Michigan 48626, USA). Implants were molded in 12 ml syringes and weighed approximately 13.8 g. Each implant therefore had 4 g of steroid and 9.8 g of silastic. The steroid–silastic blend was mixed thoroughly, 1 to 2 drops of stannous octoate catalyst was added and the blend mixed again. Following addition of catalyst, the blend was drawn into 12 cc disposable syringes and allowed to cure for 10 to 24 hr. After curing, the syringes were removed and implants were soaked in sterile saline (0.9%) for a minimum of 24 (usually 72) hr. The saline bath was changed every 24 hr. Six treatment groups were established (Table 1). The range of steroid was chosen based on in vitro release rates of implants incubated at 37 C in saline, a volume of distribution of 50 liters, estimated steroid half-life of less than 20 min, and target serum values of 20 to 40 pg E per ml and 2 to 4 ng P per ml.

Mares used in the study were captured in June 1985 and after initial processing were confined at the BLM's Wild Horse Holding Facility in Lovelock, Nevada (USA; 40°11'N, 118°23'W). Mares were aged according to tooth wear (Ensminger, 1969) by Bureau of Land Management personnel and ranged between 4 and 9 yr of age. They were housed in 30 × 30 m pens at a maximum density of 50 per pen, fed a ration of chopped alfalfa hay and straw twice daily, and allowed water ad libitum.

Mares were palpated to ensure each was not pregnant in September 1985, and implantation of the rods was begun in November 1985. Early anestrus was chosen for two reasons: (1) serum hormone concentrations would reflect the amount released from implants without contribution from endogenous sources, and (2) BLM regulations prohibit handling of free-roaming mares during the months of February through June; thus, if contraception was to be practical, implants would have to be placed during anestrus or pregnancy.

After a mare was restrained in a squeeze chute, a 10 × 10 cm area of hair was clipped from the left side of the neck and the area scrubbed with Betadine solution (The Purdue Frederick Company, Norwalk, Connecticut 06856, USA). Six cc of 2% lidocaine hydrochloride (Vedco Inc., Overland Park, Kansas 66204, USA) was injected intradermally and subcutaneously as a local anesthetic and a 30 to 35 mm incision was made through the skin. A pocket large enough to hold the implants was formed by blunt dissection above the incision, and the implants were inserted. The incision was closed with one mattress suture using two strands of 4-0 stainless steel. All animals received 10.5×10^6 IU penicillin G benzathine and penicillin G procaine in aqueous suspension (Flo-cillin, Bristol Laboratories, Syracuse, New York 13201, USA) intramuscularly following the procedure.

We collected blood from a randomly chosen

TABLE 2. Status of implants 18 wk after implanting in feral mares.

Status of implant	Group[a]					
	E	P + HE	HP + E	HP + LE	P	CI
Good	17	22	13[b]	8	15[c]	18
Encapsulated or infected	7	4	6	3	5	6
Lost	6	4	9	19[d]	9	6

[a] See text for description of groups.
[b] Two mares died.
[c] One mare died.
[d] HP+LE group was deleted from the study.

sample of five mares from each treatment group biweekly through 11 March and monthly thereafter until November 1986. In addition, we collected a sample of blood 10 days after any mare was detected in estrus as described below. The bleeding procedure consisted of restraining the mare in a squeeze chute and puncturing the jugular vein with a 15 ga needle attached to a 35 ml syringe. Two to 5 ml of blood was immediately transferred to a vacuum tube containing Na EDTA for hematological analyses; the remainder was transferred to plain vacuum tubes and allowed to clot for 4 to 12 hr. The clotted blood tubes were then centrifuged, and the serum was decanted and frozen until assayed for levels of P and E.

Tissue and cultures were collected from implant sites from several animals that became infected or showed signs of losing implants. Samples were inoculated to Trypticase Soy Agar with 5% sheep blood, chocolate agar, MacConkey agar and PEA agar, incubated at 35 C with 5% CO_2 and read at 24, 48 and 72 hr. Colonies showing beta hemolysis were selected and confirmed using BBL Strep typing and antibiotic sensitivity was tested. Tissues were prepared for microscopic examination and evidence for active tissue rejection was sought.

The mares were palpated for ovarian activity in April 1986. A stallion was placed in the pens containing hormone treated animals in mid-July and the mares were palpated for pregnancy in September 1986 and again in April 1987. Three control (CI) animals were included to verify that the stallions were fertile in the event none of the treated mares successfully bred.

Most of the P levels were determined by radioimmunoassay as previously described (Plotka et al., 1975). The extraction procedure has a sensitivity of 10 pg and a coefficient of variation of 17% at 10 pg. A few values were determined using a commercial radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California 90045, USA). Confirmation of the kit results were made by comparing 43 samples with duplicate assays by the extraction procedure. Kit values averaged 0.5 ± 0.1 ng/ml less than extraction values but trends over time were similar.

E levels were quantified by the extraction radioimmunoassay previously described by Plotka et al. (1980). This assay is sensitive to 4 pg and has a coefficient of variation of 22% at sensitivity.

Detection of estrus was accomplished by observing mares for behaviors related to estrus (Ginther, 1979), including clitoral winking, frequent urination, posturing, etc. Groups of four to six mares were placed in a runway between two pens of stallions at the holding facility. The mares were allowed to stand for 20 to 30 min, then were observed for a period of 20 to 30 min and the incidence of pertinent behavior was recorded. Status of estrus (in estrus or not in estrus) was determined from frequency of the appropriate behaviors. Ten days following a mare's exhibition of estrous behavior, she was restrained in the squeeze chute, and a sample of blood was collected. Ovulation was considered to have occurred if blood samples had a level of progesterone above 3 ng/ml.

Hormone levels are presented as mean ± standard error throughout. Statistical tests, including analysis of variance for repeated measures, Student's *t*-test, and chi-square analysis were calculated using the Number Cruncher statistical program written by Dr. Jerry Hintze (Kaysville, Utah 84037, USA).

RESULTS

Implants were rejected or lost in varying numbers from all groups (Table 2). Due to the large number of animals losing implants in the HP+LE group, data from this group were not considered representative and the group was dropped from the study. Samples of the exudate were collected from inflamed areas adjacent to

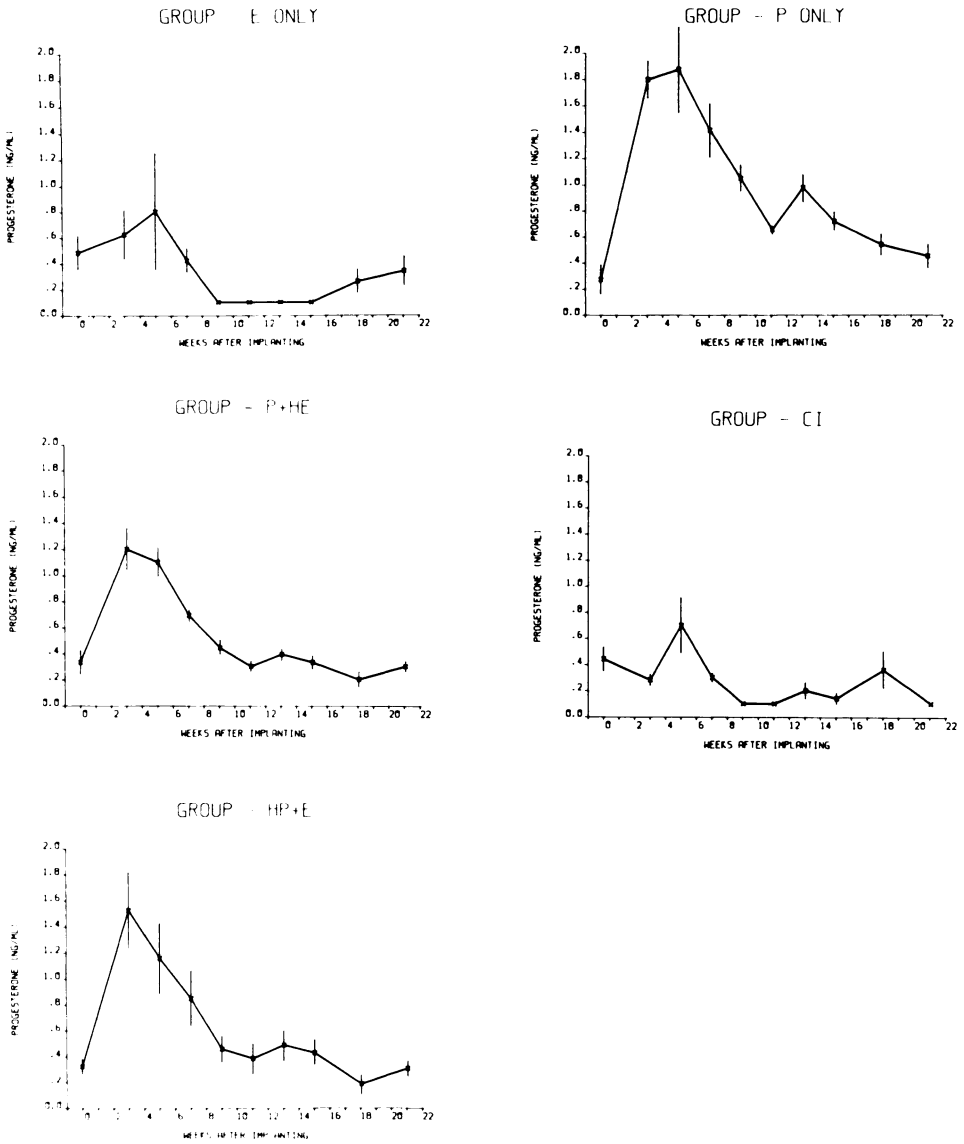

FIGURE 1. Serum progesterone levels for 21 wk following implantation of hormone containing silastic rods. Data are presented as mean ± standard error. In the P+HE and P groups, data from animals ovulating at 21 wk have not been included in the calculation of the mean and standard error.

implants and cultured using sterile techniques. Bacterial cultures revealed a beta-hemolytic streptococcal infection susceptible to penicillin. None of the infected animals had sustained elevated white blood cell counts suggesting that the infections were local. There was no microscopic evidence of an active rejection process. After loss of the implants, all areas healed. Animals being handled for bleeding were treated with 9×10^6 IU penicillin G benzathine and penicillin G procaine in aqueous suspension (Flo-cillin, Bristol Laboratories, Syracuse, New York 13201, USA) when the implant site showed signs of infection. Loss of implants in these groups was much less than in the animals not being handled regularly.

Progesterone levels were significantly elevated over preimplantation baseline

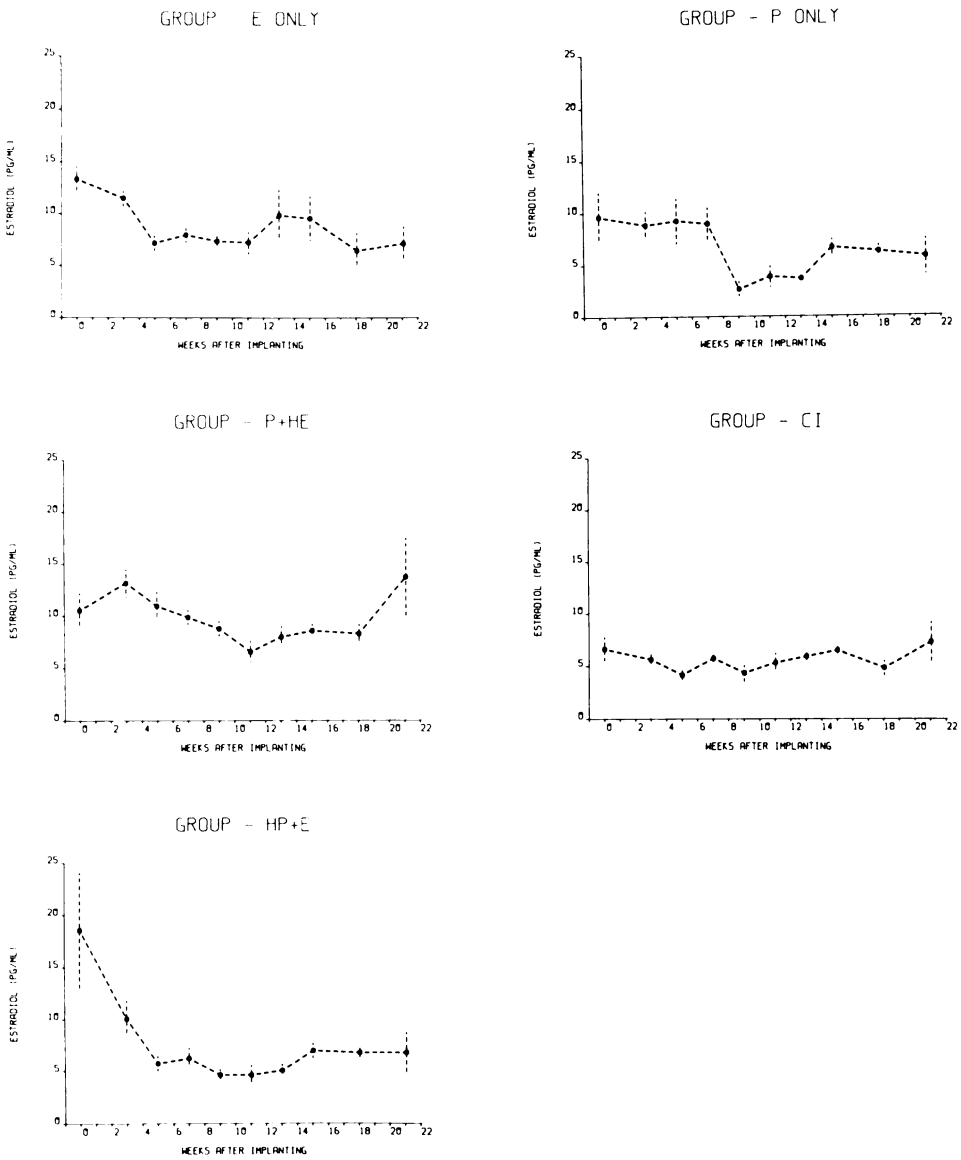

FIGURE 2. Serum estradiol levels for 21 wk following implantation of hormone containing silastic rods. Data are presented as mean ± standard error. Values from animals with aberrantly high E levels (greater than 2 standard deviations from the mean of all animals) have been omitted.

values ($P \leq 0.01$) in all groups receiving P 3 wk postimplantation (Fig. 1). Progesterone levels were stable for 2 wk and then began to decline, reaching pre-implantation levels at 9 wk for groups P+HE and HP+E and by 18 wk for the P only group (Fig. 1). Significant differences among groups were noted through 18 wk. Serum P levels were positively correlated with the amount of P implanted for weeks 3 to 21 with correlation coefficients >0.70 through week 15 ($P \leq 0.01$) and then decreasing to approximately 0.40 for weeks 18 and 21 ($P \leq 0.04$). Progesterone levels in CI and E only groups remained at baseline through 18 wk except for two animals in the E only group that had levels of 1.3 and 2.5 ng/ml 3 and 5 wk, respectively, after initiating treatment.

Three of the five mares in the CI group

TABLE 3. Exhibition of estrus and ovulation in implanted feral mares.

Group[a]	Mean date of first estrus[b]	Estrus: Number showing behavioral estrus/ number tested (%)	Estrus: P[c]	Ovulation: Number ovulating/ number tested (%)	Ovulation: P
CI	April 22	22/25 (88)	—	20/25 (80)	—
E	April 11	10/23 (43)	0.001	21/22 (95)	>0.05
P	April 20	15/20 (75)	>0.05	14/20 (70)	>0.05
HP + E	April 17	9/18 (50)	0.007	9/18 (50)	0.03
P + HE	April 22	13/26 (50)	0.003	20/26 (77)	>0.05

[a] See text for description of groups.
[b] Calculated from those animals within the group that exhibited estrus.
[c] Significance of chi-square test versus CI.

exhibited P levels indicative of ovulation by 10 April. Also, one of the five mares in group HP+E and three of the nine mares in group P+HE appeared to have ovulated. These values were not included in the analyses.

Estradiol levels were higher at implanting than at any other time measured (Fig. 2). Significant differences were noted among groups in the preimplanting sample with CI mares having significantly lower E levels than the other groups (Fig. 2). Although serum E levels were slightly higher in the E only and P+HE groups 9 to 13 wk after implanting, the difference in preimplantation levels confounded interpretation of long term differences.

The number of mares exhibiting estrus and/or ovulation is depicted in Table 3. Significantly fewer mares receiving E, regardless of dose, exhibited estrus ($P \leq 0.007$). Alternatively, P appeared not to effect the incidence of estrus. The incidence of ovulation through mid-July appeared to be suppressed only in the HP+E group ($P = 0.03$).

Some animals in all groups became pregnant after a stallion was placed in the pen. Significant differences were not noted between treatment groups in animals identified as pregnant based on two consecutive P levels above 3 ng/ml 2 wk apart or on rectal palpation. Pregnancy rates in the groups were: E only, 91%; P+HE, 81%; HP+E, 94%; and P only, 94%. Only three control mares were bred and these became pregnant.

DISCUSSION

Our objective in this study was to determine if mare reproduction could be controlled utilizing silastic rods containing naturally occurring steroid hormones that would be gradually released into the circulation. In the present study, estrus behavior was suppressed by low levels of E, but not by P. However, ovulation was not suppressed significantly as demonstrated by the elevated P levels in sequentially collected sera in July (Table 3). Because most of the mares successfully bred, none of the treatments were effective as contraceptives.

The loss of implants from the horses was significant in this study. Two mechanisms for this loss were investigated. Cultures and skin and tissue biopsies were collected during the active phase of infection. The histological results were not consistent with an active rejection of the implants and a beta-hemolytic *Streptococcus equi* was

grown in culture. Because beta-hemolytic streptococcus is a common skin organism of horses and the horses were housed in large groups, we concluded that the infection was a result of the bacteria entering through the incision or through an opening caused by drainage of a serum pocket. In addition, the horses were fed in troughs and had to put their heads through bars to get to the feed. Since the implants were placed in the neck, the horses rubbing their necks on the bars made the implants extremely vulnerable to irritation and infection.

The most significant observations of this study are the amount of hormone necessary to raise serum hormone levels and the fact that continuous release of the hormones could not maintain serum levels. It appears that E and P have extremely short half-lives in the horse. Ganjam et al. (1975) reported a triphasic half-life of P with the first component being 2.5 min and the second component being 20 min. The third component was not measured.

It is possible that the reason for the decline in P levels was that release of the hormones from the implants was not sustained at a consistent level. Ganjam et al. (1982) reported that injections of 2 mg E cypionate given every 4 days for 5 wk maintained serum total estrogen levels at 18 to 35 pg/ml. Intramuscular injections of 500 mg P given on the same schedule maintained serum P at 7.3 to 11.9 ng/ml (Ganjam et al., 1982). Although the duration of that study was much shorter, the authors did not mention that serum levels showed any evidence of declining despite the continued injections of hormone. However, Weithenauer et al. (1986) reported that in spite of repeated injections of 150 mg P, serum P levels declined over time. In addition, these workers reported lower levels of serum P after daily injections of 150 mg P + 10 mg E as compared with 150 mg P alone. Their data are consistent with the data presented in this study and suggest that the horse may have some mechanism for disposing of excess hormone, possibly by increasing the level of metabolic enzymes.

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