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HORMONAL CONTRACEPTION OF FERAL MARES WITH SILASTIC® RODS

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ABSTRACT: Homogeneous Silastic® rods containing ethinylestradiol (EE) (1.5 or 4 g), estradiol-17β (E) (4 g) or progesterone (P) (6 g) were implanted into feral mares (*Equus caballus*) between 4- and 10-yr-old. Six treatment groups ($\geq$10 mares/group) of non-pregnant mares received 36 g P and 12 g E (P+E), 36 g P and 8 g EE (P+HEE), 1.5 g EE (LEE), 3 g EE (MEE), 8 g EE (HEE) or control-implanted mares (CI). CI received implants containing no steroid. Two groups of pregnant mares received P+HEE or HEE. Stallions were placed with the mares 15 to 26 mo after implanting. Blood was collected biweekly for up to 28 mo after implanting and serum analyzed for P by radioimmunoassay. A single *P* value $\geq$2.5 ng/ml indicated ovulation and 2 consecutive values $\geq$2.5 ng/ml indicated pregnancy. Serum from blood collected before and at 4, 12, 24, 50, 64 and 89 wk after implanting was analyzed for EE concentrations. All animals pregnant at the time of contraceptive placement delivered normal foals. Contraceptive efficacy for groups LEE, MEE, HEE and P+HEE were 75, 75, 100, and 100%, respectively after two breeding seasons. Suppression of ovulation appeared to be inversely related to the concentration of EE used in the implant. The percent of animals ovulating after 2 yr of contraception in each group was 100, 100, 88, 62, 20, and 12 for groups CI, P+E, LEE, MEE, HEE and P+HEE, respectively. The pregnancy rate for the same groups was 100, 78, 25, 25, 0 and 0%, respectively. Contraceptive efficacy was followed for 3 yr in one group, P+HEE, and was 88%. Pregnancy rates for groups P+E and CI after 3 yr was 78 and 82%, respectively. Our data demonstrate effective contraception of feral mares for up to 36 mo without compromising a pregnancy in effect at the time of implanting. Calculating the decline in EE concentrations to 150% of pre-implantation concentrations, these data suggest an effective contraceptive life of approximately 16, 26, and 48 to 60 mo for LEE, MEE and HEE implants, respectively. Mechanisms that appear to be involved in contraceptive efficacy include preventing ovulation at higher concentrations of steroids and either suppressing ovulation or implantation at lower concentrations of steroid.

Key words: Ethinylestradiol, horses, hormonal contraception, *Equus caballus*, silastic rods, experimental study.

INTRODUCTION

Management of wild and feral populations of animals to prevent habitat destruction and crop damage in the United States is a controversial subject due to the many different special interest groups involved (Wagner, 1983; Berger, 1986). Livestock interests spur the controversy by complaints about grazing competition between livestock and feral horses (*Equus caballus*). Bureau of Land Management (BLM) management policies often have favored the livestock interests, primarily because grazing has been the most important commercial activity on some public lands in the western United States (Vale, 1975).

Since 1978 substantial sums of money have been appropriated to the BLM for removal of horses to achieve numbers appropriate for management of these rangelands (Boyles, 1986). The number of horses removed in recent years has at times been greater than that adopted, and permanent confinement facilities have been established. To reduce the cost of confining unadoptable horses, the BLM began investigating the potential for reducing the recruitment rate of free-roaming populations by limiting reproduction.

Although, 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, this technique may not be applicable in the western United States

because of exchange of mares between bands and siring of foals by subdominant stallions in a band (Wagner et al., 1982; Stevens, 1990). Kirkpatrick and co-workers have continued to investigate contraceptive methods and recently (Kirkpatrick et al., 1990) presented information suggesting that immunocontraception was effective in controlling the growth of feral horse populations. They reported that multiple injections were required for the method to be effective for 1 yr. Although we (Plotka et al., 1988), recently reported that neither progesterone (P) (24 g), estradiol-17β (E) (8 g), nor combinations of the two (up to 8 g each) impregnated in Silastic® prevented ovulation and/or conception, we now present data demonstrating that ethinylestradiol (EE) with or without P effectively prevents pregnancy for at least 2 yr and possibly up to 4 yr.

MATERIALS AND METHODS

Silastic® rods (implants) were prepared in lots of 20 as described previously (Plotka et al., 1988). Briefly, either crystalline P (4 g/implant) (Steraloids, Inc., Wilton, New Hampshire 03086, USA), crystalline E (4 g/implant) (Sigma Chemical Co., St. Louis, Missouri 63160, USA) or crystalline EE at 1.5 or 4 g/implant (Steraloids, Inc., Wilton, New Hampshire 03086, USA) was thoroughly mixed with silicone rubber (Silastic® #382, Dow Corning, Inc., Hemlock, Michigan 48626, USA). Control implants consisted of silicone rubber without steroid. Following addition of catalyst, the mixture was drawn into either a 3 cc or 12 cc disposable syringe and allowed to cure for 10 to 24 hr. After curing, syringes were removed and implants were soaked in sterile 0.9% saline for a minimum of 24 (usually 72) hr. The saline bath was changed every 24 hr with the final saline bath being saturated with nitrofurazone (Schuyler Laboratories, Rushville, Illinois 62681, USA).

The feral mares used in the study were captured during the first 6 mo of 1985 and after initial processing were confined at the BLM's Wild Horse Holding Facility in Lovelock, Nevada 89419, USA (40°11'N, 118°23'W). Mares were aged according to tooth wear (Ensminger, 1969) by BLM management personnel and ranged between 4 to 9 yr of age. They were housed in 30 × 30 m pens at a maximum density of 50/pen, fed a ration of chopped alfalfa hay and straw twice daily, and allowed water *ad libitum*. Maintenance and veterinary care were provided by personnel at the facility under a contract with BLM.

Mares were palpated to ensure each was open in September 1985. Pregnant mares were palpated to ensure viable pregnancy during September 1986. Control mares (CI) were implanted in November 1985 with the implants placed subcutaneously in the neck as part of a previous study (Plotka et al., 1988). One group of 10 P+HEE (high EE) (Table 1) treated mares was implanted in April 1986. Five of these mares received implants intramuscularly and five received implants subcutaneously. The remainder of the mares were implanted in the peritoneum in January 1987.

The procedure for preparing the site for implant placement was the same for all groups. After a mare was restained, a 10 × 10 cm area of hair was clipped from the left flank and the area scrubbed with iodine solution (Betadine, The Purdue Frederick Co., Norwalk, Connecticut 06856, USA). Six to 15 ml of 2% lidocaine solution (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 stainless steel trochar with stainless steel sleeve large enough to fit over the implants was used to penetrate the muscle and tissue surrounding the peritoneum. After removal of the trochar, the implants were inserted into the peritoneal cavity through the sleeve. The sleeve was removed and the incision closed with one mattress suture using 2-0 chromic cat-gut suture. All animals received 10.5×10^6 IU benzethine penicillin (Flocillin, Bristol Laboratories, Syracuse, New York 13201, USA) intramuscularly following the procedure. Necropsy of mares that died from unrelated causes during the study showed that sometimes the peritoneum was not entered and the implants were in the fat adjacent to the peritoneum wall.

Six groups of non-pregnant mares and two groups of pregnant mares were implanted. The number of mares in each group and the date implanted are shown in Table 1. Low, moderate, and high doses of EE are designated LEE, MEE, and HEE, respectively. The two highest treatments (P+HEE and HEE) were given to both non-pregnant and pregnant mares. The appropriate amount of hormone implanted into each mare was achieved by varying the number of implants. Stallions were placed with the mares in April 1988. Numbers of mares in the control group varied throughout the study because those that became pregnant and delivered were nursing a foal during the next breeding season and not in with the study mares that season.

Blood was collected from all mares biweekly

TABLE 1. Hormone amounts, number of mares and date implanted for each treatment group.

Group	P[a] (g)	E (g)	EE (g)	Date implanted (mo/yr)	Implant site[b]	Number of mares	
						Start	End
P+E	36	12	0	04/86	IP	11	9
P+HEE							
Nonpregnant	36	0	8	04/86	SC, IM	10	8
Pregnant	36	0	8	01/87	IP	10	8
LEE[c]	0	0	1.5	01/87	IP	10	8
MEE	0	0	3	01/87	IP	10	8
HEE							
Nonpregnant	0	0	8	04/86	IP	10	10
Pregnant	0	0	8	01/87	IP	10	10
Control	0	0	0	09/85	SC	30	24

[a] P, progesterone; E, estradiol-17β; EE, ethinylestradiol.
[b] IP, intraperitoneal; SC, subcutaneous; IM, intramuscular.
[c] L, low; M, moderate; H, high.

during the breeding season (April through October) and monthly during the anestrus season (November through March). Mares were restrained in a squeeze chute and the jugular vein punctured 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 analysis; 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 or EE.

Progesterone concentrations were determined by radioimmunoassay using a commercial radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California 90045, USA) as previously described (Plotka et al., 1988).

Serum EE concentrations were determined by radioimmunoassay utilizing a highly specific anti-EE antiserum. The antiserum was raised in rabbits by making an initial intradermal multiple-site injection of 50 μg of 1,3,5(10)-estratrien-17α-ethinyl-3,17β-diol-6-one 6CMO:BSA (Steraloids, Inc., Wilton, New Hampshire 03086, USA) conjugate in 3:1 v/v Freund's complete adjuvant : 0.9% NaCl (w/v in H_2O) containing an additional 20 mg attenuated *Mycobacterium tuberculosis* (Difco Laboratories, Detroit, Michigan 48233, USA). The rabbits also received a second injection of 0.4 ml diphtheria-pertussis-tetanus (DPT) vaccine subcutaneously. Subsequent boosting occurred at 1, 2, 4, and 7 mo with 100–150 μg conjugate in 1:1 Freund's incomplete adjuvant : 0.9% NaCl. One half of this emulsion was injected intramuscularly and the remainder subcutaneously. The rabbits were bled monthly by vacuum suction from a cut in the lateral ear vein. The blood was allowed to clot 2 hr at room temperature and centrifuged. The serum was stored at or below −15 C until tested for immunoreactivity with EE (Steraloids, Inc., Wilton, New Hampshire 03086, USA). The serum aliquot identified as P5-041089 was characterized and utilized in the assay.

One milliliter of horse serum was extracted with methylene chloride and the extract dried and concentrated in the bottom of a 12 × 75 mm glass tube. The dried extract was taken up in 1.0 ml of assay buffer (0.1 M phosphate buffered saline [PBS], 0.1% sodium azide, pH 7.0, with 0.1% w/v gelatin) and incubated with intermittent vortexing for 1 hr at room temperature prior to assay. For the assay, parallel dilutions of samples taken up in buffer were made to 0.5 ml and compared with a standard curve of 250–1.95 pg/tube in 0.5 ml assay buffer. Assay buffer (0.1 ml) containing 4,600 cpm 6,7 ^{3}H-EE2 (NEN Research Products, Boston, Massachusetts 02118, USA) with a specific activity of 59.2 Ci/mmol was added to all tubes. One-tenth milliliter of our rabbit P5-890410 antisera diluted 1:900 in assay buffer was then added to all tubes (except total count and non-specific binding tubes) for a final antisera dilution at incubation volume of 1:3,600. The assay was incubated for 15–20 hr at 4 C and separation of bound from free carried out by addition of 0.4 ml of 0.1 M PBS (no gelatin) containing 0.02% dextran and 0.2% w/v activated charcoal. One-half milliliter of supernatant was counted in a liquid scintillation counter. This assay is specific for EE with <0.15% cross-reaction with known horse estrogens. Sensitivity of the assay

TABLE 2. Ovarian function of captive feral mares during the first year after receiving contraceptive implants.

Group	Number tested	Number ovulating	Percent ovulating	P*
P+E	11	2	18	<0.0001
P+HEE	19	0	0	<0.0001
LEE	8	4	50	<0.01
MEE	8	4	50	<0.01
HEE	20	1	5	<0.0001
Control	24	23	96	

* All comparisons made against the control group.

TABLE 3. Ovarian function of captive feral mares during the second year after receiving contraceptive implants.

Group	Number tested	Number ovulating	Percent ovulating	P*
P+E	11	11	100	NS
P+HEE	16	2	12	<0.0001
LEE	8	7	88	NS
MEE	8	5	62	0.02
HEE	20	4	20	<0.0001
Control	18	18	100	

* All comparisons made against the control group.

is 10 pg with a coefficient of variation of 18% at sensitivity.

Data are presented as means ± standard error throughout. Differences between each treatment group and controls were determined by Fishers exact test utilizing the Number Cruncher Statistical Program written by Jerry Hintze (Kaysville, Utah 84307, USA).

RESULTS

A total of 101 captive feral mares received hormone-containing or control implants in 1985, 1986 or 1987 (Table 1). Although some mares from each group died due to problems related to handling during the course of the study, each group had a minimum of eight mares at the end of the experimental period.

Serum P concentrations were similar among mares receiving subcutaneous, intramuscular and intraperitoneal implants ($P > 0.1$) at 3, 6 and 9 mo after treatment (data not shown).

During the first year after treatment all mares in the P+HEE group were anovulatory based on having serum P concentrations <2.5 ng/ml. Some mares in all other groups appeared to have ovulated, i.e., had serum P concentrations ≥2.5 ng/ml (Table 2). During the second breeding season after treatment, 12–100% of all groups ovulated (Table 3). However, only two of eight mares in each of the LEE and MEE groups and seven of nine mares in the P+E group became pregnant (Table 4). None of the mares in the HEE or P+HEE groups became pregnant (Table 4). Differences in ovulation and pregnancy rates between treated and CI mares were highly significant ($P < 0.001$).

Serum EE concentrations were highest 1 mo after implanting and exhibited a log-linear decline over time (Fig. 1). Although not directly proportional, serum concentrations reflected the amount of EE implanted. Mean serum EE concentrations increased from 11 ± 2 pg/ml before implanting to 46 ± 7, 79 ± 7, and 417 ± 64 pg/ml in non-pregnant mares receiving LEE, MEE, or HEE, respectively and to 291 ± 29 pg/ml in pregnant mares receiving HEE. No difference was observed between peak EE values in non-pregnant or pregnant mares receiving HEE ($P > 0.10$). Non-pregnant and pregnant mares receiving P+HEE achieved serum concentrations of EE of 320 ± 68 and 277 ± 17 pg/ml, respectively ($P > 0.10$). Serum EE concentrations at 15 mo (89 wk) after

TABLE 4. Pregnancy rate of captive feral mares 2 years after receiving contraceptive implants.

Group	Number tested	Number pregnant	Percent pregnant	P*
P+E	9	7	78	NS
P+HEE	16	0	0	0.001
LEE	8	2	25	0.06
MEE	8	2	25	0.06
HEE	20	0	0	<0.001
Control	3	3	100	

* All comparisons made against the control group.

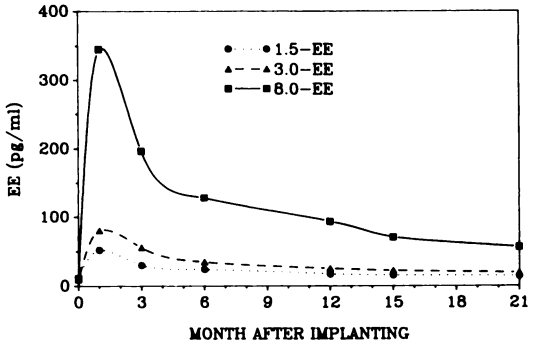

FIGURE 1. Concentrations of serum ethinylestradiol (EE) in mares over 21 mo after being implanted with Silastic® rods containing 1.5, 3.0, or 8.0 g of EE. All implants were placed into the peritoneal cavity.

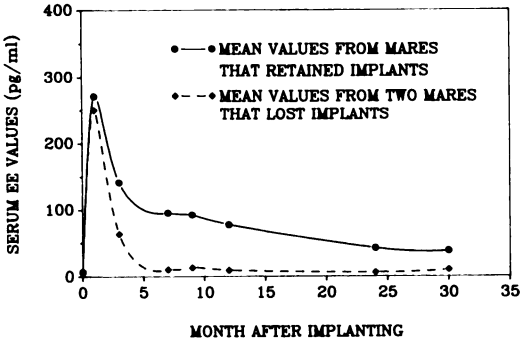

FIGURE 2. Serum concentrations of ethinylestradiol (EE) in mares retaining implants and two mares suggested, by lack of palpation, to have lost implants over 30 mo after receiving 8.0 g EE.

implanting averaged 14 ± 2, 18 ± 2, 56 ± 5 and 60 ± 8 pg/ml for the LEE, MEE, HEE, and P+HEE groups, respectively. Efficacy of contraception (percent not pregnant) through 15 mo (two breeding seasons) was 75, 75, 100, and 100% for the four groups, respectively. All of the mares that were pregnant when the implants were placed (Table 1) produced live, normal offspring during the next foaling season.

The P+HEE group implanted in April 1986 was followed through a third breeding season. One of the eight mares remaining in that group became pregnant during that breeding season (Table 5). Serum EE concentrations dropped from 80 pg/ml to <10 pg/ml during the 1986 breeding season (Fig. 2). Since the implants were placed intramuscularly in this animal, the rapid drop in serum EE suggested that the implants had been lost. We were also unable to palpate any implants at the implant site at that time. A second mare also had a precipitous drop in serum EE concentrations at the same time but did not become pregnant. Serum EE concentrations in the six remaining mares in that group averaged 35 ± 4 pg/ml 30 mo after implanting (Fig. 2).

To predict the contraceptive life of the treatments, we calculated the time for serum EE concentrations to decline to 150% of pre-implantation concentrations. Based on a regression calculated from the serum EE values obtained more than 6 mo after implanting, we predict that effective contraception would be realized for 16, 26, and 48–60 mo for the 1.5 g, 3.0 g, and 8.0 g EE implants, respectively. The addition of P did not lenghten the effective life of the HEE implants.

TABLE 5. Pregnancy rate of captive feral mares 3 years after receiving contraceptive implants.

Group	Number tested	Number pregnant	Percent pregnant	P*
P+E	9	7	78	NS
P+HEE	8	1	12	0.002
Control	17	14	82	

* All comparisons made against the control group.

DISCUSSION

Implantable contraceptive preparations have been used in captive, wild species for several years. The first attempts at implantable contraception of non-domestic species came concurrently from independent studies of Bell and Peterle (1975) with white-tailed deer (*Odocoileus virginianus*) and Seal et al. (1975) with lions (*Panthera leo*), tigers (*Panthera tigris*), jaguars (*Panthera pardus*), and leopards (*Panthera onca*). Since then, only a few have been published. Most of these were with captive animals and for only short

periods. Seal, however, has used melengestrol acetate in Silastic® implants for several years in various species of primates and felids (U.S. Seal, pers. comm.). In addition, Silastic® capsules of levonorgestrel have successfully prevented reproduction for up to 5 yr in humans (Holma, 1985).

Kirkpatrick and Turner (1985) reported that a microencapsulated form of testosterone propionate administered to the only sexually mature stallion in a band of free-roaming feral horses successfully suppressed reproduction without altering behavior. Although this study reported efficacy, the fact that there was only a single mature stallion in the treated bands leaves one with the question of how effective this treatment would be in bands where multiple sexually mature stallions were present and whether all stallions would have to be treated. Nelson (1980) challenged the efficacy of sterilization of dominant stallions as a method of limiting feral horse population growth.

More recently, Kirkpatrick et al. (1990) reported successful contraception of feral horses after immunization of the mares with porcine zona pellucida. Their protocol required three injections over a 3 to 4 mo period before contraception was achieved. In addition, the adjuvant that was added to the vaccine to enhance the immune response generated an undesirable reaction at the injection site of some animals. A major disadvantage of that procedure for field use is the need to identify and reinject individuals at least three times.

The primary objective of this study was to develop an effective method for preventing reproduction in wild and free-roaming horses for a period of >2 yr. Availability of reproductively prime age mares (4- to 9-yr-old) for use in the study was limited. This constraint caused us to utilize control mares from a previous study which had been implanted 6 mo earlier. These control mares received implants in the neck rather than in the peritoneum. However, since serum P concentrations were similar in mares receiving subcutaneous, intramuscular and intraperitoneal implants, we concluded that release of hormone from the implant was independent of implantation site. The fact that some mares which received intraperitoneal implants ovulated during the first year suggested that the physical placement and/or presence of the implant did not suppress ovulation (Table 2). Intraperitoneal implantation was chosen to reduce the likelihood of mares loosing implants.

Our data demonstrate that Silastic® implants containing 8.0 g of EE effectively accomplished this goal with a single treatment in captive animals. Furthermore, the hormone treatments used did not cause abortion of pregnancies existing at the time of implantation. High survival of treated mares 30 days following treatment indicated that the implantation procedure caused few deletrious effects. As far as we are aware, this is the first demonstration of long-term contraception of an equine species.

The type of hormone release curves exhibited by Silastic® rods and the ability to make implants that contain enough hormone to inhibit reproduction for periods of >2 yr will allow use of these implants in free-roaming animals. A small sample (n = 8) of mares treated with P+HEE suggest that the treatment effect lasted through the third breeding season following implantation. Hormone implants appear to be the only method presently available for effecting contraception for that amount of time without repeated treatment.

In order to determine the length of time the contraceptives would be effective, we set up an assay for EE. This allowed us to determine the amount of hormone in animals sequentially over time. Serum EE reflected a rapid release of hormone from the implant to peak serum concentrations by 60 days after implanting. Release dropped off quickly until reaching equilibrium by approximately 6 wk. Release then slowed and was linear over the rest of the study (Fig. 1). Utilizing the fact that

the LEE group had a 75% efficacy of contraception after two breeding seasons with serum EE concentrations averaging 14 ± 2 pg/ml, this level could be considered the minimal serum concentrations of EE for effective contraception. Calculating the length of time for serum EE concentrations in the HEE groups to decline to 15 pg/ml (150% of background), the effective life of contraception in these groups should be between 48 and 60 mo.

One of the disadvantages of any contraceptive procedure for free-roaming species is the cost and effort involved in effectively administering the contraceptive agent. The cheapest and least labor-intensive method of delivering a contraceptive is to place it in a palatable feed that could be put out for the animals. However, past attempts at oral administration of contraceptives to several species have met with only partial success (Kirkpatrick and Turner, 1985). A significant shortcoming of oral administration is that it results in immediate high blood levels that decrease with time, and repetitive doses must be given at frequent intervals to keep the blood levels in an effective range. Injectable contraceptives are longer acting and would be more applicable for free-roaming animals. However, as mentioned above, currently available injectable contraceptives require reinjection of the animals at frequent (monthly or quarterly) intervals. The studies reported here have demonstrated that implants containing EE are effective for at least 3 yr and suggest efficacy for up to 5 yr. The cost of materials and labor for constructing the implants was between $75.00 and $150.00 (U.S.), depending on the treatment. The major disadvantage of our procedure is the need for catching the mares for delivery of the implants. The cost of rounding up and handling the large numbers of animals necessary to effect population control significantly increases the cost of implementation of a contraception program. However, as Garrot (1990) has shown, the cost would be significantly less than the BLM's current program of periodic removal and sale of excess horses. Therefore, we conclude that hormonal contraception of feral horses can be accomplished safely, effectively, and relatively economically using Silastic® implants.

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