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IMMUNOSTERILIZATION OF FERAL AND CAPTIVE HORSES: A PRELIMINARY REPORT

Robin B. Goodloe, Robert J. Warren and Daniel C. Sharp

Abstract

Preliminary results from the treatment of female feral horses on Cumberland Island, Georgia with freeze-dried GnRH conjugated to ketolymphohemocyanin (KLH) indicated the compound had little effect on animal health, herd stability, or survival of fetuses in utero. The overall impact of the immunosterilizing agent on feral horse productivity cannot be evaluated until the conclusion of the 1988 foaling season. In a controlled experiment with corralled horses, mares treated with GnRH-KLH failed to develop high levels of GnRH antibody, and all but one treated mare produced a conceptus. Emphasis during the remaining 2 years of the study will focus on evaluating other formulations that have greater antibody-stimulating properties than GnRH-KLH conjugate, but that are less easily administered in the field or pose greater health hazards to the animal.

Introduction

Barrier islands along the U.S. Atlantic coast are vulnerable to damage caused by introduced domestic species with limited intrinsic means for population regulation. Feral livestock in island habitats forage extensively on sea oats (*Uniola paniculata*) and other dune and interdune vegetation (Lenarz 1983, Simon et al 1984, Turner 1986). Excessive grazing on this dune-stabilizing vegetation results in poorly developed frontal dunes and produces conditions that permit landward movement of sand from previously stable secondary dunes. Hillestad *et al*, (1975) reported that these changes in dune structure adversely affect nesting habitat of the loggerhead sea turtle (*Caretta caretta*) and deplete inland wetland habitat utilized by wood ducks (*Aix sponsa*), alligators (*Alligator mississippiensis*), river otters (*Lutra canadensis*), wading birds, and other animals.

Feral livestock also feed heavily on smooth cordgrass (*Spartina alterniflora*), the dominant species of the salt marsh (Keiper 1976, Rubenstein 1981, Simon *et al*, 1984). Turner (1985) determined that heavy grazing by horses in the salt marshes of Cumberland Island, in conjunction with horse trampling and compaction of soil, reduced aboveground *Spartina* up to 98% compared to ungrazed areas. Riemold *et al*, (1975) found that ungulates grazing in coastal island marshes reduced primary production, detritus production, and density of fiddler crabs (*Uca pugnax*). Grazing by ungulates also has been related to changes in successional patterns in *Spartina* marshes (Ranwell 1961, Reimold *et*

al, 1975) and to decreased abundance of marsh plants with high forage values (Chabreck 1968).

Feral cattle, sheep, and goats have been removed, to a large extent, from eastern U.S. coastal islands. Free-ranging horses, however, still exist on three eastern barrier islands -- Assateague Island, VA and MD; Shackleford Banks, NC; and Cumberland Island, GA. Only one, privately owned population that exists on the Virginia portion of Assateague Island (Chincoteague Island National Wildlife Refuge) currently is managed. The animals are rounded up annually, and excess animals, usually foals, are sold at auction (Keiper and Houpt 1984).

Horse herds on the other eastern island sites appear to be increasing (Ambrose *et al*, 1983; Lenarz 1983; Keiper and Houpt 1984; Finley 1985; Skip Phrange, pers. comm.). Most of the animals inhabit publicly owned land (Assateague Island National Seashore, Cape Lookout National Seashore, and Cumberland Island National Seashore), and resource managers do not consider roundups and animal removal to be a practical, long-term method to regulate population size. Our study was initiated to evaluate another non-lethal method of horse population control -- sterilization of breeding adults.

Study Sites

Field Trials: Field trials to determine the efficacy and safety of the selected sterilizing agent were conducted on feral horses that inhabit Cumberland Island, the largest and most southern of the barrier islands along the Georgia coast. Cumberland is approximately 28 km long, 0.8-4.8 km wide, and covers an area of 95.5 km. Eighty percent of the island is organized as the Cumberland Island National Seashore and includes an extensive area of federally designated wilderness; the remainder of the land is owned by private, State, and other Federal interests.

A mosaic of major vegetation communities exists on the island. Bands of primary dunes, interdune meadows, and secondary dunes lie adjacent to the beach and support sparse stands of grasses, forbs, and sedges. Dense shrub thickets composed largely of wax myrtle (*Myrica cerifera*) are located in the interdune meadows and on the rear dunes, where they merge into upland forest habitat. Grasslands occur at the elevated edges of the salt marsh and support a variety of herbs and shrubs, but the majority of the salt marsh is dominated by smooth cordgrass (Hillestad *et al*, 1975, Simon *et al*, 1984).

Lenarz (1983) counted 144 horses on Cumberland Island in July 1981 and estimated that the population would increase between 5% and 21% annually. A minimum of 154 horses were counted in the March 1983 census (Ambrose *et al*, 1983), and 181 horses were recorded in May 1985 (Finley 1985). Results of the 1987 census have not been tabulated, but it is likely that the Cumberland horse herd currently exceeds 200 animals.

Corral Studies: Concurrent studies to determine concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and titers of GnRH antibody in treated and control animals were conducted on captive pony mares maintained at the Horse Research Center, University of Florida.

Methods and Materials

Sterilizing Agent: Our main objective when we evaluated potential sterilizing agents was selection of a compound that would reduce horse productivity but not affect health of the treated animals, herd stability, or survival of fetuses or nursing foals. Emphasis was placed on selecting a cost-efficient compound that could be applied in the field with minimal manpower expenditure or risk to the animal; a remotely delivered compound that did not require capture and handling of animals was preferred. Effective duration and reversibility of the compound were additional considerations.

A number of chemical fertility control agents have been developed and tested on a variety of animals (Dzuik and Cook 1966, Bell and Peterle 1975, Matschke 1977a, 1977b, 1980). Research on chemical fertility control in horses, however, has been limited, since castration effectively regulates reproduction in domestic horses. The only completed field study on chemical control of feral horse reproduction involved injecting free-roaming western stallions with microencapsulated testosterone propionate (MTP) (Kirkpatrick *et al*, 1982). Mares associated with MTP-treated stallions averaged 0.066 foals per mare, compared with 0.371 foals per mare for control bands. Kirkpatrick *et al*, (1982) immobilized the stallions and injected MTP by hand, but recent studies indicate that the compound can be administered remotely via dart gun (Turner and Kirkpatrick 1986).

Fertility control based on administration of MTP or other male-sterilization techniques is effective only if stallions retain herds for relatively long periods and if bachelor males have little access to harem females. Feist and McCullough (1975) reported that 9.1% of the dominant stallions studied on the Pryor Mountain Wild Horse Range, WY and MT, lost dominant status between May 1 and November 1. Nelson (1980) found that approximately 25% of the harem males in the Jircarilla Wild Horse Territory, NM, were replaced annually. Furthermore, he reported that fidelity to a particular band was weak or nonexistent in many mares. Our preliminary observations in 1986 indicated that similar changes in herd structure occurred in the Cumberland Island horse population. Therefore, we limited our search for potential sterilizing agents to those that were effective on female horses.

The reproductive process of most female mammals can be interrupted through treatment with a number of natural or synthetic chemicals. In general, however, oral or intramuscular administration of estrogens or progestins to females

during the breeding season lacks reliability and is impractical as a management tool for large, free-ranging populations. A third method of administering steroids to regulate reproduction, the use of silicone rubber implants, has been used in ewes (Dziuk and Cook 1966), white-tailed deer (*Odocoileus virginianus*) (Bell and Peterle 1975, Matschke 1977b, 1980), and humans (International Committee for Contraception Research 1978, Diaz *et al*, 1982, Sivin *et al*, 1983). Female horses, however, are capable of producing secondary follicles even when progesterone levels are high (Ginther 1979, Neely 1983), and it is unlikely that implants containing progestin would efficiently reduce reproduction rates (J.A. Wesson, U.S. Seal, pers. comm.). The use of estrogen implants currently is under investigation by other researchers (Siniff *et al*, 1985).

The most promising technique that we considered for regulating female horse fertility was inhibition of GnRH activity through active immunization. Fraser (1975) and coworkers (Fraser and Gunn 1973, Fraser *et al*, 1975, Fraser and Baker 1978) demonstrated that ovulation was blocked in laboratory rats and rabbits treated with GnRH conjugated to bovine serum albumin and emulsified in Freund's complete adjuvant (FCA). One immunization was sufficient to stimulate production of antibodies to endogenous GnRH, resulting in highly reduced or nondetectable levels of LH and FSH in serum and the anterior pituitary. Other studies indicated that active immunization against GnRH before or at the onset of the breeding season prevented ovulation in sheep and completely suppressed estrous cycles after sufficient antibody titers developed (Clarke *et al*, 1978, Jeffcoate *et al*, 1978). Ovaries and uteri of animals that responded to treatment were in various stages of regression, and normal luteal tissue was not present.

Immunization against endogenous GnRH has been successful in captive horses. Safir *et al*, (1987) reported that 3 of 5 mares immunized with 2 mg GnRh conjugated to human serum albumin and emulsified in FCA failed to ovulate in the 4-month period following an abrupt, artificial increase in day length. Ovulation occurred in the other treated mares, but a booster injection restored acyclicity for all or part of the remainder of the study.

Failure of some animals to respond to treatment is a major drawback of active immunization against GnRH. In horses, Safir *et al*, (1987) found that absence of ovulation in GnRH-immunized animals appeared to be positively correlated to antibody titer levels. Similar variability in immune response among individuals treated with GnRH has been reported for sheep (Foster *et al*, 1977), marmosets (Hodges and Hearn 1977), and gilts (Esbenshade and Britt 1975). Animals that develop high titers of GnRH-antibody, however, are sterilized effectively for relatively long periods. In addition, some formulations of the compound can be administered remotely, thus eliminating the need to handle animals. These characteristics led us to select active immunization against GnRH as the reproductive control method of choice for the horse study.

In most previous immunization research, the GnRH-conjugate complex has been adjuvented to FCA or similar compounds (Fraser and Gunn, 1973, Fraser 1975, Fraser *et al*, 1975, Fraser and Baker 1978) to allow slower uptake of the immunogen and to stimulate greater antibody production. Adjuvented antigens, however, often cause formation of abscesses (immune modules) at or near the injection site. Unadjuvented GnRH-conjugate potentially is less effective at stimulating antibody production than adjuvented formulations. However, it is not known to cause lesion formation and can be freeze-dried for use in biodegradable bullets. Bullet delivery of treatment is preferred over delivery of liquid formulations via syringe darts because bullets are safer for the operator, ensure complete dosage delivery, and are more cost-efficient and practical for field use. Unadjuvented GnRH-conjugate, therefore, was selected for our initial trials of GnRH sterilizing agents on feral and corralled horses. Ketolymphohemocyanin (KLH), a large, water-soluble protein, was selected as the preferred conjugate.

Methods

Field Trials: The feral horses of Cumberland Island were surveyed repeatedly during April and May 1986 to determine herd composition and distribution. Between early December 1986 and mid-February 1987, 29 adult female horses were selected randomly for treatment with biodegradable bullets containing 33 mg of GnRH-KLH conjugate to provide 15 mg of immunogenic activity (Pitman-Moore, Inc., Washington's Crossing, NJ). Each animal received a single 0.25 caliber bullet implanted intramuscularly in the hindquarters using the BallistiVet air-powered gun (BallistiVet, Inc., White Bear Lake, MN). The 31 untreated adult mares known to exist on the island in early 1987 were considered the control group, but none received placebo bullets.

Fifteen mares selected for treatment were inoculated with the GnRH-KLH conjugate while they grazed with other herd members. Fourteen other mares were immobilized prior to treatment, using a mixture of etorphine hydrochloride (M 99; Lemmon Co., Sellersville, PA) and xylazine hydrochloride (Rompun; Haver-Lockhart, Shawnee, KN), delivered via dart gun. Entry of the BallistiVet bullet was verified for each immobilized animal. Blood samples were collected from all immobilized animals, and selected mares were fitted with radio transmitters to facilitate animal identification and monitoring of herd movement. Reversal of immobilizing drugs was achieved with intravenous injection of diprenorphine (M 50/50; Lemmon Co., Sellersville, PA).

Treated and control animals were observed frequently in 1987 from mid-March to late August, the period of peak foaling and breeding activity on the island. Information on total annual reproduction was collected through direct counts of live foals and searches for carcasses when foal death was suspected. General health of the treated animals and interactions with their foals, dominant

stallions, and other herd members were recorded. Data also were collected on movement of animals between herds and aggressive encounters between stallions to evaluate the effects of the sterilizing agent on herd stability.

Corral Studies: Five captive pony mares at the University of Florida were treated in December 1986 with GnRH-KLH conjugate, delivered via BallistiVet bullet as described above. Five control mares received a similar, placebo bullet containing only KLH. Eight weeks after initial inoculation, 2 treated and 2 control mares received booster injections of their respective treatments.

Blood samples were collected from all mares by venipuncture at 2-day intervals from 10 May through 1 June 1987, a period that corresponded to the average date of first ovulation in the research herd. Plasma titers of GnRH antibody were determined by indirect ELISA tests using GnRH-coated plates. Plasma concentrations of LH and FSH were quantified by radioimmunoassay using anti-ovine LH antisera and anti-equine FSH antisera, respectively, in conjunction with purified equine LH and FSH for radioiodination and standard preparation. Concentrations of LH and FSH were standardized to date of ovulation and compared by analysis of variance.

Mares were teased daily with a stallion to detect estrus and palpated rectally when estrus was detected to assess ovarian follicular growth and incidence of ovulation. Mares were bred to stallions of known fertility upon display of estrus or detection of ovarian follicles 30 mm in diameter or larger. Daily breeding continued until ovulation occurred, and all mares were checked for pregnancy daily with ultrasound echography beginning on Day 10 after ovulation. Conceptuses were flushed from the uterus on either Day 12 or Day 14. After conceptus removal, mares were administered 5 mg prostaglandin F2a (Lutalyse; Upjohn Co., Kalamazoo, MI) to regress the corpus luteum, then bred during the next estrus.

Results and Discussion

Field Trials: One mare treated with GnRH-KLH conjugate died several hours after immobilization. Etorphine hydrochloride, our primary immobilizing drug, can cause increased arterial blood pressure in horses (Daniel and Ling 1972); death of the mare probably was caused by rupture of pulmonary vessels during the sample collection period.

Two other mares died within 6 weeks of immobilization and administration of the sterilizing agent. Necropsy indicated that one mare had an impacted stomach and possibly died of colic. The second mare had an infected wound on the shoulder and was sacrificed after she collapsed in a high-use visitor area. A fourth treated animal was not located during the 1987 field season and was assumed to be dead or occupying territory not normally traversed by observers.

The other 25 treated mares and 31 control mares appeared healthy when 1987 field studies began, although some mares that still nursed 1986 foals were thin. In mid-March, the animals were organized into 29 herds that contained 1-3 adult females, a dominant adult male (except for one herd with 2 codominant males), and subadult and yearling offspring. Ten herds contained at least 1 adult female from both treatment and control groups. Ten herds contained no treated individuals, and 9 herds contained only adult females that had received the inoculation.

Treatment had no apparent effect on herd stability. Most (82.1%) adult mares remained with the same stallion throughout the 1987 breeding season. A minimum of 3 treated (12.0%) and 3 control mares (9.7%) permanently changed herds during this 6-month period. An additional 2 treated (8.0%) and 2 control (6.5%) mares showed little fidelity to any stallion during parts of the breeding season; they were observed, in the company of their 1986 and 1987 offspring, with different stallions or foraging alone.

Three dominant stallions, including 1 male that died, lost all adult harem females during the period between mid-March and late August 1987. Four new herds were formed when bachelor males or previously subordinate herd stallions acquired adult females. One of the new dominant stallions injured an untreated mare during a copulation attempt, and the mare died in July 1987.

Immobilization and treatment of pregnant mares with GnRH-KLH conjugate several months before parturition did not appear to have great impact on continuance of pregnancy. Seventeen (68.0%) of the 25 treated mares that survived until the beginning of the 1987 breeding season delivered live foals (Fig. 1). This number is not significantly different from the 15 foals produced by the 31 control mares (48.4%) in 1987 or the 27 foals produced islandwide in 1986, before treatment began (50% of known mares; 13 foals by mares treated later in the year and 14 by control mares). One treated mare, however, aborted a near term fetus in February 1987, 2 weeks after immobilization and treatment. No congenital abnormalities were noted in the fetus, but numerous bacterial cocci were found within the small intestine and lungs and on the placenta, indicating that the aborted fetus died of bacterial pneumonia.

Eight additional 1987 foals (25.0%) died within several weeks of birth, including 6 that were born to treated mares. In contrast, in 1986, only 4 foals died within the first few months after birth. Two of these 1986 foals were produced by mares that received treatment the following winter, and parentage of a third foal that was found dead was unknown.

The greater foal mortality observed in 1987 probably is more closely related to sampling chronology than to mare treatment. Field studies in 1986 were conducted only in April, May, and August. June and July, however, appear to be periods of fairly high foal mortality, and some foal deaths in 1986 probably were not documented. In 1987, 5 of the 8 foals that died were born after May 31, a

period during which 55.6% of all newborns died. In contrast, only 13.0% of the animals born on or before May 31 died.

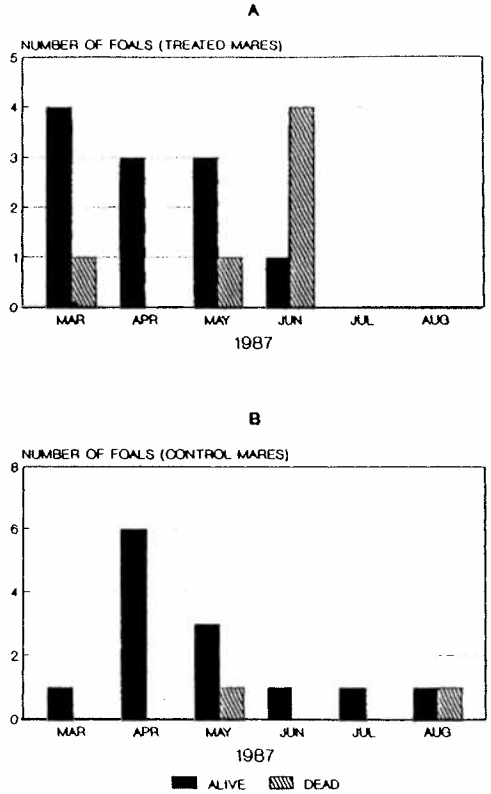

Figure 1. Productivity of free-ranging (a) treated and (b) control mares on Cumberland Island, 1987. Note scale differences for Y axes.

Corral Studies: The average date of first 1987 ovulation for mares in the corral study was May 11 (SE = 4.1, n = 7) compared to May 14 (SE = 2.6, n = 35) for all other mares at the University of Florida's Horse Research Center. Three control mares were excluded from average ovulation date calculations; one mare was pregnant at the time of treatment, and two others ovulated for the first time well beyond the normal time.

There was no difference in mean FSH or LH concentration between control and treated mares (Table 1), and all but one mare produced at least one conceptus during the breeding season. Antibody titers were significantly less than the 1:33,000 (maximum titer) obtained by Safir *et al*, (1987) and did not greatly

exceed the 1:50 dilution for a known negative horse against which the plasma samples were tested.

Table 1. Mean LH and FSH concentrations (ng/ml) and maximum GnRH antibody titer obtained in corralled mares treated with a KLH placebo or GnRH-KLH conjugate. Blood samples were taken on 19 January 1987 and every 2 days from 10 May - 2 June 1987.

Mare No.	Treatment Group	LH X	LH SE	FSH X	FSH SE	Maximum[a] Antibody Titer	Date of Maximum Titer
167	Control	7.87	0.83	15.85	1.86	< 1:50	N.A.[b]
180[c,d]	Control	3.76	1.25	25.18	6.00	< 1:50	N.A.
488	Control	5.58	0.49	14.77	1.11	> 1:100	1/19
491[d]	Control	N.A.	--	18.66	1.38	1:200	1/19
507[c,e]	Control	18.49	2.45	15.10	2.55	< 1:100	1/19
	Group Mean	6.23	0.51	16.84	1.00		
133[c]	GnRH-KLH	4.18	0.36	17.78	0.94	1:400	5/13
159	GnRH-KLH	7.87	0.83	20.06	1.68	1:100	1/19
176[c]	GnRH-KLH	7.69	1.56	15.89	3.03	1:50	5/11
289	GnRH-KLH	12.10	1.50	19.13	1.55	1:400	1/19
480	GnRH-KLH	5.67	0.83	18.76	2.39	1:400	1/19
	Group Mean	8.66	0.71	18.25	0.82		

[a]Antibody titers determined based on a 1:50 dilution of a known negative horse.
[b]N.A. = not applicable.
[c]Animals received booster inoculations in January 1987.
[d]Animal was not cycling during sampling period but cycled later in the breeding season.
[e]Animal was pregnant at the time of sampling.

Conclusion and Future Plans

The initial phase of this study was designed to evaluate the sterilizing efficacy of freeze-dried GnRH-KLH conjugate, a cost-efficient, easily administered compound with potential for a wide range of management applications. Preliminary results indicate that treatment has little impact on horse health, herd stability, or survival of fetuses in utero. The effect of the compound on reproduction of feral mares on Cumberland Island cannot be determined until the 1988 foaling season; however, results from the corralled horses treated with GnRH-KLH conjugate at the University of Florida indicate that GnRH-KLH conjugate is not an adequate sterilizing agent for horses.

Emphasis during the remaining 2 years of the study will focus on evaluating adjuvented GnRH compounds that have greater antibody-stimulating

properties than the unadjuvented compound used in 1986-1987 but that are less easily administered or pose greater health hazards to the animal. In January 1988, 16 mares at the University of Florida (none of which were treated with the GnRH-KLH conjugate in 1986) were divided randomly into 2 treatment and 2 control groups. Animals in each treatment group were inoculated with either triple-adjuvented GnRh-KLH or alum-precipitated GnRH-KLH. Control mares received placebos consisting of the vaccine without GnRH.

Each treatment was microencapsulated to provide 15 mg of GnRH immunogenic activity delivered at the time of initial treatment, 1-3 months after initial treatment, and 6-9 months after initial treatment. The mass of the microencapsulated treatments 1-2.0 g) exceeded the capacity of the BallistiVet bullet, therefore treatments were delivered in liquid form via syringe in the hindquarters.

Preliminary results from the 1988 corral study suggest that triple-adjuvented GnRH-KLH is an efficient immunosterilizing agent (Goodloe *et al*, 1988). Injection-site lesions were observed in mares administered this treatment, but no lesions posed major health hazards to the animals. Based on these results, triple-adjuvented GnRH-KLH was selected as the treatment method to be evaluated during the 1988-1989 study of immunosterilization of the Cumberland Island mares.

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