# Immunocontraception in Feral Equids: A possible Approach to Fertility Control of the Przewalski Horse

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## Abstract

The remotely-delivered porcine zonae pellucidae (PZP) vaccine described here is the first successful application of immunocontraception to uncaptured free-roaming equids. The PZP vaccine was administered to free-roaming feral mares on a coastal barrier island by means of darts, and was greater than 93% effective in inhibiting fertility among mares receiving either two or three inoculations. The vaccine's effects are reversible, do not affect pregnancies in progress, and do not affect band integrity. An annual booster inoculation was successful in extending the antifertility effects of the vaccine through a second reproductive season. Results from this study with feral horses suggest that the PZP vaccine may be useful in controlling captive Przewalski Horse populations.

## Introduction

First, I should like to thank Drs. Lydia Kolter and Waltraut Zimmermann for the invitation to speak here today. It is somewhat of a paradox that my topic addresses possible methodologies for interfering with reproduction in such a rare species as the Przewalski horse while much of the focus of this conference will be directed toward increasing reproductive success of this animal, particularly with the current emphasis on reintroduction efforts. However, even with species as rare as the Przewalski horse, genetic anomalies such as the "fox" allele, and the ever present zoo problem of surplus animals provides a sound rationale for careful control of reproduction.

Reducing fertility among free-roaming feral horses (*E. caballus*) has been the goal of numerous studies in the United States over the past two decades. Initial experiments focused on contraceptive androgens which were delivered to stallions as injectable sustained release microencapsulated compounds (Kirkpatrick et al. 1982; Turner and Kirkpatrick 1982). More recently, microencapsulated progestins have been delivered to mares (Kirkpatrick and Turner 1990) and silastic® implants containing ethinylestradiol and progesterone have been placed in mares (Plotka et al. 1989). All of these methods resulted in varying degrees of pharmacological success but the microencapsulated steroids required extremely large doses and the silastic® implants require restraint and surgery. Additionally, there is concern about the alteration of behavior and the passage of steroids through the food chain when they are used to contracept wild free-roaming animals.

An alternative to steroid-induced fertility control in equids is immunocontraception. A conjugated form of gonadotropin-releasing hormone (GnRH) has been used successfully to raise antibodies in captive feral mares (Goodloe et al. 1988) and solubilized porcine zonae pellucidae (PZP) injections inhibited fertility in 13 of 14 captive mares (Liu et al. 1989). The objectives of our study were to (1) test the contraceptive efficacy of a porcine zonae pellucidae vaccine in free-roaming feral mares, under field conditions, (2) determine the effectiveness of remote delivery, (3) determine the contraceptive effectiveness of the vaccine in pregnant and non-pregnant mares, (4) evaluate the safety of the vaccine for use in pregnant mares, and (5) evaluate the contraceptive effectiveness of a single annual booster inoculation.

## Methods and materials

Twenty-six mares of proven fertility were selected from among 100 feral mares inhabiting Assateague Island National Seashore, MD. The ages and fertility records, dating back as far as 1974, were known for almost all the mares. The mares chosen for treatment were purposely selected for high foaling rates, which averaged 10% higher than the overall herd rate for the preceding three years. The PZP vaccine was prepared from porcine ovaries as previously described (Liu et al. 1989) and stored frozen at −5 °C until used.

Between February 29 and March 10, 1988, the 26 mares received an initial inoculation of vaccine. The vaccine consisted of an emulsion of 0.5 cc of phosphate buffer containing approximately 5000 zonae (64.3 µg of protein) and 0.5 cc of Freund's Complete Adjuvant. The mares were darted in the hip region using a Pan-Arms® .527 caliber capture gun and barbless darts. Eight of the mares were acclimated to humans and the initial vaccine delivery was accomplished with a 3 cc syringe and a jabstick and thereafter by dart. Between March 12th and 21st, the 26 mares received a second inoculation. The second inoculation was the same as the first except or the use of Freund's Incomplete Adjuvant instead of the complete adjuvant and the addition of 0.5 cc of phosphate buffer. Between the 16th and 25th of April, 18 of the 26 mares which received the second inoculation also received a third inoculation which was identical to the second inoculation. Six mares received either one or two inoculations of only phosphate buffer and adjuvant. An additional 11 sexually mature untreated mares, which received no sham injections, were selected for a second control group. Identifying markings were recorded for each horse and the animals were observed throughout April for adverse effects and particularly for the presence of abscesses at injection sites.

During October, 1988, five months after the last inoculation (two months after the end of the breeding season), the mares were located and identified, and the presence or absence of foals was recorded. Urine samples were collected from the 26 treated and six control mares, without capture, by extracting urine from the soil immediately after urination. The urine samples were assayed for estrone conjugates (E$_1$C) and indexed to creatinine (Cr) concentrations (KIRKPATRICK et al. 1988). Pregnancy determinations were made on the basis of the urinary E$_1$C concentrations in excess of 1.0 µg/mg Cr.

In February, 1989, 14 of the mares which had received inoculations the previous year and were not pregnant received a single booster inoculation of PZP and Freund's Complete Adjuvant. In August, 1989 the mares were again located and identified and foals counted. In August and October of 1989, urine samples were collected from all treated and control animals. In addition to analysis of urinary E$_1$C, a non-specific urinary progesterone metabolite (iPdG) which provides an accurate reflection of plasma progesterone (KIRKPATRICK et al. 1990), was also measured. The 1989 foal production for these mares was compared to foal production in (1) the same group of mares for 1988 and 1987, (2) the six control mares, and (3) the 11 untreated mares for 1989. Pregnancy tests from the August/October urine collections were used to determine the effectiveness of the booster inoculation and the reversibility of the vaccine's effects. Differences in foaling rates between treated and control groups were tested for significance by means of binomial probability distribution.

## Results

Of the 26 treated mares, 15 were pregnant (57.6%) at the time of inoculation and all 15 produced foals in the spring of 1988. Of the six control mares three (50%) produced three foals. Thirteen of the 15 foals born to treated mares survived their first year and were in good health as yearlings, in August of 1989. None of the 18 mares receiving three inoculations, one of the eight mares receiving two inoculations, three of the six control mares, and five of the 11 untreated mares were pregnant and produced foals. Foaling data and pregnancy data based on the urinary E$_1$C measurements showed a 100% correlation. Of the 26 mares receiving two or three inoculations, the overall fertility rate was 3.8%. In contrast these mares had a 53.8% fertility rate for each of the two pre-treatment years 1987 and 1988. The fertility rates for the six control mares and for the 11 untreated sexually mature mares in the study area were 50% and 45.4% respectively, in 1989. The differences in foaling rates between the treated group



for the post-treatment yea
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jected control group and
presented in Table 1.

Table 1. Foaling r

| Group | In H |
|---|---|
| Treated (0.00) | 3 |
| Treated (12.4) | 2 |
| Control (50.0) | 0 |
| Untreated (45.4) | 0 |

Of the 14 mares given a
oculated in 1988 but given
mares receiving sham inocu
7(43.7%), respectively, are
six sham injected mares an
fidence intervals, respective

Table 2. Pregnancy

| Treatment Group | Nu Ma |
|---|---|
| Booster | 14 |
| Control | 6 |
| Untreated | 16 |

A total of three abscesse
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## Discussion

The success of the PZP
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for the post-treatment year 1989 and the two pre-treatment years was significant at the $P < 0.002$ confidence interval. Differences were significant at the $P < 0.0019$ confidence interval for both the sham-injected control group and the 11 untreated mares. Foaling data for treated and untreated mares are presented in Table 1.

Table 1. Foaling rates for treated and untreated mares for pre-treatment and post-treatment years

| Group | Inoculations/ Horse | No. Horses | No. of Foals Produced/group (%) Pre-treatment 1987 | 1988 | Post-treatment 1989 |
|---|---|---|---|---|---|
| Treated (0.00) | 3 | 18 | 9(50.0) | 11(51.1) | 0 |
| Treated (12.4) | 2 | 8 | 5(62.4) | 3(37.4) | 1 |
| Control (50.0) | 0 | 6 | 2(33.3) | 2(33.3) | 3 |
| Untreated (45.4) | 0 | 11 | unknown | unknown | 5 |

Of the 14 mares given a booster inoculation, one is pregnant (7.1%). Of the 12 mares originally inoculated in 1988 but given no booster inoculation in 1989, four (33%) are pregnant. Of the six control mares receiving sham inoculations and an additional 16 sexually mature untreated mares, 3(50%) and 7(43.7%), respectively, are pregnant. The differences between the foaling rate for the treated mares, the six sham injected mares and the 16 untreated mares are significant at the $P < 0.01$ and $P < 0.018$ confidence intervals, respectively. Data for the booster inoculation experiment are presented in Table 2.

Table 2. Pregnancy Rates for Booster Inoculated, Control, and Untreated Mares

| Treatment Group | No. Mares | Pregnancy Rates (based on urinary E1C and iPdG) |
|---|---|---|
| Booster | 14 | 1(7.20%) |
| Control | 6 | 3(50.0%) |
| Untreated | 16 | 7(43.7%) |

A total of three abscesses were observed among the 26 treated horses. The abscesses appeared at the site of injection about two days after the third treatment, were about 10–25 mm in diameter, and drained from six to nine days after treatment. Complete healing had occurred within 14 days following treatment.

## Discussion

The success of the PZP vaccine in suppressing fertility among the Assateague mares is based on its ability to inhibit fertilization or possibly implantation (Sacco et al. 1984). The PZP consists of three glycoproteins. One of these, ZP3, is the receptor molecule for sperm surface molecules (Florman and Wassermas 1985). Antibodies raised against PZP are thought to block the sperm receptor sites on the equine zona pellucida, probably by steric hindrance of sperm-receptor attachment (Skinner et al. 1984), thereby preventing fertilization. The results of pregnancy testing suggest, but do not prove, that PZP immunization of mares results in a failure to conceive. Urinary E.C concentrations are not diagnostically useful until day 35 of pregnancy and earlier pregnancies cannot be detected in this non-invasive manner, therefore the effects of PZP immunization upon very early pregnancies cannot be evaluated.

Two major questions to be answered by this study were whether the immunosuppression which normally accompanies pregnancy would interfere with the effectiveness of the antifertility effects of the vaccine, and whether the pregnancies underway at the time of immunization would be successful and the foals healthy. These are important considerations since the use of this vaccine for the management of exotic equids such as the Przewalski Horse will often include pregnant mares among the treated animals. Pregnancy did not alter antifertility efficacy of the vaccine nor was the normal progress of pregnancies or the health of the foals, based on observation, affected by the vaccine.

A minimum of two inoculations is required in horses in order to raise antibody titers sufficiently high to provide contraception for six to nine months. The schedule of inoculations in this study was based on the spacing of inoculations in a previous study with horses (Liu et al. 1989) and the breeding and foaling activity patterns for the Assateague horses, which peak in April, May and June (Keiper and Houpt 1984). The first inoculation causes antigen recognition and temporary increases in antibody titers. The second inoculation causes increased titers which last nine months to a year and each subsequent inoculation increases the duration of elevated titers. The choice of Freund's Complete Adjuvant for the first inoculation and Freund's Incomplete Adjuvant for the second and third inoculation was also based on the work of Liu et al. (1989). While only three abscesses were noted in this study, the evaluation of other adjuvants which are less likely to cause abscesses is an important direction for future research and is already under investigation in a number of other laboratories.

A major advantage of the PZP vaccine is the small aqueous volume which facilitates administration by dart. Remote-delivery eliminates the need to capture the mare and the consequent possibility of injury. Another advantage is the reversibility of the vaccine's contraceptive effects. Earlier studies with captive horses (Liu et al. 1989) demonstrated that treated mares which failed to conceive after PZP treatment became pregnant the following year. This result is corroborated in the present study by the return to normal pregnancy rates among PZP-treated mares.

Despite the return of normal fertility observed to date, the long-term effects of chronic PZP immunocontraception are not defined at present. In some species, such as the rabbit (Wood et al. 1981), dog (Mahi-Brown et al. 1985), and the baboon (Dunbar et al. 1989) some data suggest that the antibody response of the treated animal includes not only effects on the mature ovum but also on oocytes and other ovarian tissues, with resulting changes in estradiol and progesterone secretion. These effects have not been demonstrated in horses thus far or most of the non-human primates which have been studied. Histological studies of ovaries from captive PZP-treated horses revealed no changes three years after treatment, and plasma progesterone values during treatment were consistent with normal cyclicity (Liu et al. 1989). We are currently conducting a study of the long-term effects of this vaccine on ovarian function among Assateague Island mares which are now in their third year of treatment.

Behavioral integrity of treated animals is important, particularly in the case of the Przewalski horse, where animals housed in zoos are often the focus of behavioral study. In the Assateague study, bands with treated mares remained intact during the 18 months following vaccine administration with only three mares moving to new bands. This incidence of exchange of mares between bands is within normal limits for the Assateague herd. Since PZP contraception is directed at fertilization and is non-hormonal, behavioral side-effects are unlikely.

These results suggest that PZP immunocontraception may be useful in the management of the Przewalski Horse. In cooperation with Dr. Waltraut Zimmermann, of the Cologne Zoo, we have initiated a small study of the efficacy of this vaccine in two Przewalski mares bearing the "fox" allele. In addition to evaluating the efficacy of the contraceptive effects, we are also monitoring ovarian function in these mares by means of urinary steroid metabolites.

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