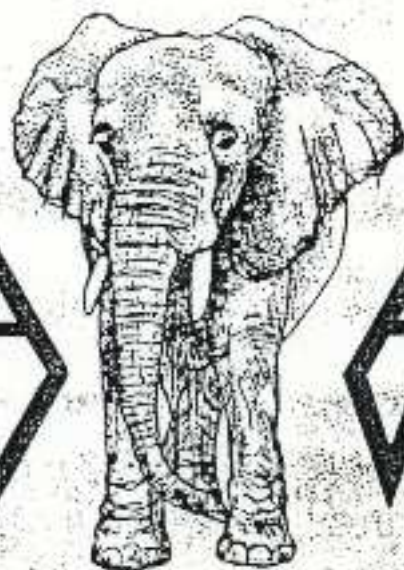


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IMMUNOCONTRACEPTION OF FEMALE CAPTIVE EXOTIC UNGULATES

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In order to reduce surplus or undesirable offspring, 12 species of captive exotic ungulates were immunized with a vaccine containing porcine zonae pellucidae (PZP). The vaccine was prepared by slicing porcine ovaries and recovering oocytes by filtration. The zonae pellucidae were heat solubilized at 70 degrees C and the zona proteins were stored frozen in phosphate buffer until use. The species include three Przewalski horses (*E. Przewalski*), three banteng (*Bos javanicus*), 10 Formosa sika deer (*Cervus nippon*), 11 sambar deer (*Cervus unicolor*), four axis deer (*Axis axis*), three muntjac deer (*Muntiacus reevesi*), 9 Himalayan tahr (*Hemitragus jemlahicus*) and 15 west caucasian tur (*Capra ibex*). Each animal with the exception of the muntjac deer received an initial intramuscular inoculation of 65 ug of PZP (approximately 5000 zonae) in 0.5 cc of PBS + 0.5 cc of adjuvant. The dose for muntjac was approximately 30 ug. Three weeks later each animal received a second inoculation, and some animals received a third six weeks after the initial inoculation. Inoculations were given by hand or remotely. When possible, the first inoculation was given approximately one month prior to the onset of breeding activity. Four different adjuvant protocols were used, including (1) an initial inoculation with Freund's Complete Adjuvant (FCA) + two inoculations with Freund's Incomplete Adjuvant (FIA), (2) three inoculations with FIA, (3) three inoculations with muramyl dipeptide (MDP), or (4) three inoculations with Quil-A adjuvant (QA). FCA was used only with animals at the Cologne Zoo and discontinued after that because of the TB+ reaction which results. Animals which were inoculated for the first time in 1990 received a single booster inoculation a year later. When possible, blood samples were obtained and serum was measured for anti-zonae antibodies. Urine samples were collected from the Przewalski horses and measured for estrone conjugates (E_1C) and progesterone metabolites (iPdG) in order to assess ovarian function.

None of the Przewalski's horses produced foals during the year following inoculation, while all four control mares produced foals. Antibody titers among treated mares exceeded contraceptive levels, and urinary E_1C and iPdG indicated that estrus and ovulation were occurring during the year following inoculation. One of the treated mares was pregnant at

the time of inoculation and produced a healthy foal. No banteng were born from breedings after inoculation; one banteng was pregnant at the time of inoculation and delivered a healthy calf. All three banteng had contraceptive antibody titers approximately one year following inoculation.

Three of 4 PZP-treated axis deer remained infertile through the first year following inoculation. All were inoculated with FIA as the adjuvant. Two of the 11 sambar died from conditions unrelated to this study and six of the surviving nine animals remained infertile through the year following inoculation. Two of the sambar producing fawns were inoculated with MDP as the adjuvant and one with FIA. At the time of inoculation it was noted that many of the sambar did not get complete injections because of the viscosity of the vaccine emulsion. Results from the sika study are inconclusive because males had access to females prior to inoculation; however, antibody titer data suggest contraceptive levels were attained. At 5/92 the incidence of pregnancy for the muntjac and Himalayan tahr is not yet available, but antibody titers are significantly higher in treated animals than in controls and suggest contraception will be effective. At 6/92 only three of 13 PZP-treated tur produced kids (23%). One of those had an antibody titer of only 8% of the positive reference standard, and the other two were animals that received less than complete inoculations because of the viscosity of the vaccine emulsion and the small diameter of the needle. During the three previous years, the pregnancy rates for the same population of animals were 78%, 82% and 69%, indicating a significant contraceptive effect.

These data suggest that (1) PZP immunocontraception of exotic hoofstock is effective, (2) there are differences in the effectiveness of the vaccine depending upon the adjuvant used, (3) no significant health problems result from PZP immunocontraception of exotic hoofstock, (4) the vaccine is safe to give to pregnant animals, and (5) the vaccine can be administered remotely if necessary. Future trials will use larger bore needles to administer the vaccine. Additional trials are underway with hippopotamus (Amsterdam and Calgary Zoos), llama (Virginia Zoo), giraffe (Milwaukee Zoo) and addax (Calgary Zoo).

CHEMICAL AND IMMUNOLOGICAL FERTILITY CONTROL IN WILDLIFE

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Reversible fertility control is a potentially valuable management tool for wildlife populations where traditional mortality control methods are not legal, wise, safe or publicly acceptable. Immunological inhibition of fertility in wildlife was first accomplished in free-roaming feral horses. A porcine zona pellucida (PZP) vaccine was administered remotely to 26 free-roaming feral mares on Assateague Island National Seashore, using barbless darts fired from a capture gun. This vaccine raises antibodies in the mare which bind to sperm receptor sites on her own zona pellucida, thereby blocking fertilization. In the initial study, mares received either 2 or 3 intramuscular inoculations of 65 ug of zona antigen over a 6-week period. After one year there were no pregnancies among the 26 PZP-treated mares compared to 3 pregnancies among 6 control animals, which had received only phosphate buffer and adjuvant, and 5 pregnancies among 11 untreated mares. During the second year of that study, 14 of the original 26 mares were given a single booster inoculation, and 12 were left untreated. One of the 14 boosted mares produced a foal compared to 5 foals produced by the 12 mares which received no booster inoculation. Ten of the 14 boosted mares received a single booster inoculation in years 3, 4 and 5 of the study; and in 70 mare years only 2 foals have been produced, while 40-50% of all untreated mares on the island produced foals during the same period of time. The two foals produced among treated animals during those 4 years were born to the same mare. The results of that study indicate that PZP immunization of feral horses (1) can be carried out remotely, (2) can be applied to pregnant mares without interfering with pregnancies already in progress or with the health of the foals, (3) will not affect social behaviors, and (4) is reversible, at least for up to 2 consecutive years of treatment. The results from booster inoculations indicate that once antigen recognition has occurred, a single annual inoculation can raise antibody titers to contraceptive levels. This same PZP vaccine has also proven effective in inhibiting fertility in white-tailed deer. Five immunized deer, over a 2-year period, have produced no fawns compared to an 83% fawning rate among controls. Trials are also underway with free-roaming feral donkeys in Virgin Islands National Park. Trials with captive exotic ungulates, including sika, axis, sambar, and muntjac deer, ibex, Himalyan tahr, llama, hippopotamus, banteng and giraffe, indicate that this vaccine will be an effective fertility inhibitor across a wide variety of ungulates.

Use of the vaccine in its original form requires a minimum of 2 inoculations during the first year of treatment. This limits its usefulness with secretive species such as deer. Experiments with implantable osmotic pumps in horses and deer indicate that sustained continuous release of the PZP antigen will also cause contraceptive concentrations of antibodies. This has led to the development of nontoxic biodegradable microspheres which contain the PZP antigen and which can be delivered remotely. Preliminary experiments in captive and free-roaming feral horses with this prototype one-inoculation vaccine suggest that it will be effective in free-roaming wildlife after only a single administration.

The control of reproduction in small mammals may be important in controlling predation or diseases such as rabies or Lyme disease. In a somewhat different approach to reproductive control, skunks were live-trapped, lightly anesthetized with a pole syringe and a single Norplant contraceptive implant placed subdermally. The single implant was placed without surgery through a 10 gauge trocar. Over a 3-year period 20 treated skunks have failed to produce a single litter. Previous research has demonstrated that the active ingredient, levonorgestrel, is effective in rodents, and trials are underway in Denver with urban beaver.