

## IMMUNOCONTRACEPTION OF CAPTIVE EXOTIC SPECIES: V. PROLONGED ANTIBODY TITERS IN DALL SHEEP (*OVIS DALLI DALLI*) AND DOMESTIC GOATS (*CAPRA HIRCUS*) IMMUNIZED WITH PORCINE ZONA PELLUCIDA

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**Abstract:** Native porcine zona pellucida (PZP) immunocontraception has been used to inhibit fertility in more than 80 species of ungulates, although the duration of contraception efficacy varies among species in both Perissodactyla and Artiodactyla. This study examined anti-PZP antibody titers in Dall sheep and domestic goats at the Milwaukee County Zoo, and also Himalayan tahr and Armenian Mouflon sheep at the San Diego Zoo Safari Park, and, for comparison, Altai wapiti, lowland wisent, Javan banteng, and southern pudu at the San Diego Zoo Safari Park, all were given a primer dose and booster dose of PZP. Of the San Diego Zoo Safari Park animals, the 4 comparison species demonstrated the typical 1-yr pattern of anti-PZP antibodies, whereas the Armenian sheep and Himalayan tahr showed prolonged (2–3 yr) antibody responses after a single primer and booster dose. The Dall sheep and domestic goats had significantly longer durations of antibody titers (3 yr) from a single year's treatment (primer plus booster). Analysis of the data indicates that Armenian sheep, Himalayan tahr, Dall sheep, and domestic goats have prolonged responses, and are more sensitive to PZP in that they produce a protracted antibody response.

**Key words:** Capridae, contraception, fertility control, immunocontraception, ovidae, porcine zona pellucida, ungulates.

### INTRODUCTION

Native porcine zona pellucida (PZP) vaccine has been used successfully to cause fertility inhibition in more than 85 wildlife species, both free ranging<sup>6</sup> and in captivity.<sup>3,7</sup> However, despite this success across many species, considerable variation exists within the orders Perissodactyla and Artiodactyla with respect to quantitative and qualitative patterns of antibody titers and contraceptive efficacy.<sup>3</sup> The majority of ungulate species follows a pattern of producing high titers 1–3 mo after the initial treatment and then of maintaining these titers at contraceptive levels for approximately 8 mo to 1 yr if a booster inoculation is given 3–6 wk after the initial treatment.<sup>5,8,10</sup> This necessitates an annual booster inoculation to maintain contraceptive antibody titer levels.<sup>3,10</sup> Some species, however, require booster inoculations more frequently than once per year. For example, Muntjac deer (*Muntacus reevesi*) require

booster inoculations at approximately 6-mo intervals, whereas zebras (*Zebra* sp.) require booster inoculations every 9 mo.<sup>3</sup>

Often, contraceptive treatment of captive wildlife species is carried out to delay rather than permanently inhibit fertility, which allows for reproduction at times favored by age, genetics, health, or exhibit space. Significantly prolonged anti-PZP antibody titers might interfere with effective population planning in some species. Thus, this study was undertaken to examine more closely the differences between selected members of Ovidae and Capridae compared with other species of Ungulata with respect to duration of anti-PZP antibodies.

### MATERIALS AND METHODS

#### Study animals

Study animals at the San Diego Zoo Safari Park (SDZSP) in Escondido, California (USA) included 14 Altai wapiti (*Cervus elaphus sibericus*), 10 Himalayan tahr (*Hemitragus jemlahicus*), 9 Javan banteng (*Bos javanicus*), 5 lowland wisent (*Bison bonasus*), 3 southern pudu (*Pudu puda*), and 8 Armenian mouflon (*Ovis musimon*), treated with the PZP vaccine. All the animals were adults, and all except the pudu were housed in large mixed ungulate species outdoor exhibits. The substrate

From the Science and Conservation Center, 2100 South Shiloh Road, Billings, Montana 59106, USA (Kirkpatrick, Lyda, Frank); Milwaukee County Zoo, 100001 West Blue Mound Road, Milwaukee, Wisconsin 53226, USA (Wallace); and San Diego Zoo Safari Park, 15500 San Pasqual Valley Road, Escondido, California 92027, USA (Lamberski). Correspondence should be directed to Dr. Kirkpatrick (jkirkpatrick@Montana.net).

was grass and dirt. The pudu were in an outdoor single-species exhibit with dirt substrate.

The wapiti, banteng, wisent, mouflon, and Himalayan tahr were fed ad libitum diets of high-fiber herbivore (ADF-25) pellets (Western Millings, Goshen, California 93227, USA), sudan grass hay, and Bermuda grass hay. The pudu were group-fed low-fiber herbivore (ADF-16) pellets (Western Milling, Goshen, California 93227, USA), leaf eater food (Marion Zoological, Plymouth Minnesota 55441, USA), Bermuda grass hay, and Acacia browse.

Study animals at the Milwaukee County Zoo (MCZ) in Milwaukee, Wisconsin (USA) included 4 female Dall sheep (*Ovis dalli dalli*) and 5 female pygmy goats (*Capra hircus*). Sheep ages ranged from 4 to 11 years. Goat ages ranged from 6 to 8 years. They were housed in a free-stall barn with access to the outside of the exhibit area. The diet consisted of a free-choice mixture of timothy and alfalfa hay, a nonmineralized salt block, and water. For approximately 5 mo of the year, public feeding of the goats occurred (May 1 to September 30), during which the goats were fed pelleted feed (HMS petting zoo treat, HMS Zoo Diets, Bluffton, Indiana 46714, USA) by the public; the amount per goat was undetermined. The Dall sheep received approximately 1 flake of alfalfa hay daily and free-choice ADF-16 (Mazuri, Purina Mills, Richmond, Indiana 47374, USA) year round, supplemented by a variety of fresh browse when available. They had free-choice access to a nonmineralized salt block and water. All of the MCZ animals except 1 goat were multiparous. The goat, a breeding male, was housed separately and introduced to selected females for controlled breeding. At the start of the study, all the subjects at both locations were clinically healthy, and all the subjects were within the normal reproductive life span.

### PZP preparation

The native PZP was prepared at the Science and Conservation Center in Billings, Montana (USA) from porcine ovaries according to the modified method of Dunbar et al.<sup>2</sup> Basically, oocytes were removed from porcine ovaries by using a custom-designed instrument that consisted of ganged razor blades, followed by the manual filtration process to separate the zonae from cellular debris. The zonae were then solubilized in sterile water adjusted to pH 7.2. The antigen was titrated with buffered saline solution, pH 7.0, to doses of 100 µg of PZP protein antigen per 0.5 ml, and stored at -44°C.<sup>2</sup> The PZP was screened for pathogenic

bacteria by using blood agar plates incubated under aerobic conditions. Qualitative analysis of zona proteins was carried out by means of polyacrylamide gel electrophoresis by using the Mini-Protean II Cell System (Bio-Rad Laboratories, Hercules, California 94547, USA), with prestained sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) standard and a low range, of 18–106 Kd, and permanent images of the gels stored on a computer. The application of this vaccine was authorized under the U. S. Food and Drug Administration Investigational New Animal Drug Exemption File 8840-G003-004.

### Treatment protocol

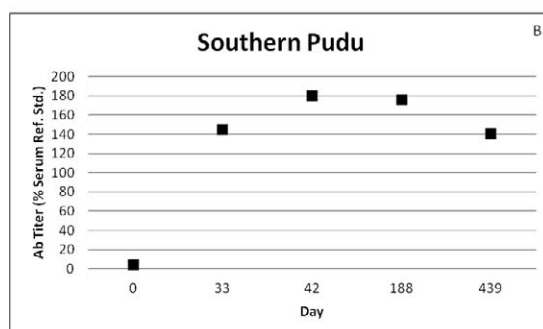
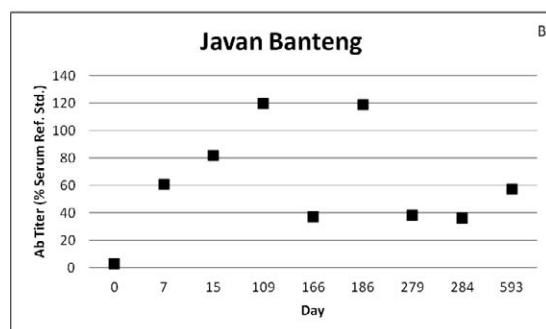
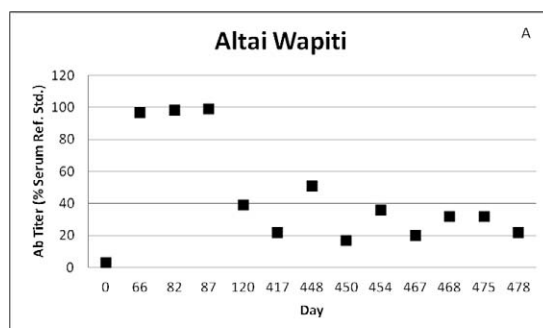
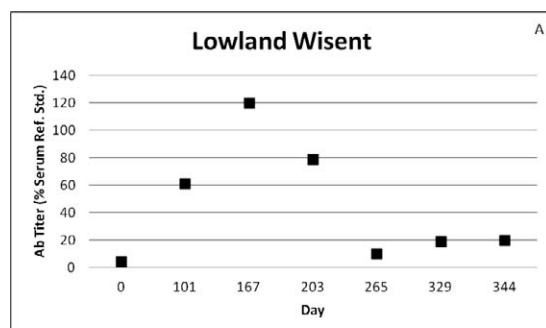
Each animal was given an initial primer dose of 100 µg PZP emulsified with 0.5 ml of Freund's Modified adjuvant (Calbiochem, La Jolla, California 92039, USA) as previously described.<sup>10</sup> Injections were given i.m. in the thigh muscles. Approximately 4 wk later, each animal received a booster inoculation of 100 µg PZP emulsified with 0.5 ml of Freund's Incomplete adjuvant (Sigma, St. Louis, Missouri 63178, USA). The SDZSP animals were treated remotely by means of 1.0-ml darts (Dan-Inject dart guns, Austin, Texas 78754, USA). MCZ animals were restrained manually and injected by hand.

### Blood collection for serum titers

A venous blood sample for antibody titer analysis was collected in calcium ethylenediamine-tetraacetic acid (Ca EDTA) and serum separator tubes at the time of the initial inoculation. Thereafter, blood was collected from the SDZSP animals opportunistically over a 3-yr period by using carfentanil and xylazine anesthetic agents, with no complications. MCZ animals were sampled, without anesthesia, at approximately 2-mo intervals over the first year, at 4-mo intervals over the second year, and twice during the third year. Serum was harvested and stored frozen at -5°C until analysis.

### Antibody titer analysis

Anti-PZP antibodies were quantitatively analyzed as previously described<sup>10</sup> by using immunoglobulin (Ig) G conjugated to alkaline phosphatase (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland 21811, USA). For antibody assays in species of Cervidae, the IgG was anti-white-tailed deer (*Odocoileus virginianus*); for members of Bovidae, the IgG was anti-domestic



**Figure 1.** Anti-porcine zona pellucida antibody titers for lowland wisent (A) ( $n = 5$ ) and Javan banteng (B) ( $n = 9$ ). Data points represent opportunistic blood collections from different animals.

**Figure 2.** Anti-porcine zona pellucida antibody titers from Altai wapiti (A) ( $n = 14$ ) and southern pudu (B) ( $n = 7$ ). Data points represent opportunistic blood collections from different animals.

cow; and, for ovids and caprids, it was anti-domestic sheep. All test sera were assayed in duplicate and expressed as a percentage of the positive reference sera.

The reference sera consisted of a pool of sera from PZP-treated domestic cows (provided by the Potawatami Zoo, South Bend, Indiana, USA), big horn sheep, and white-tailed deer that had demonstrated anti-PZP titers in the high positive range (mean of experimental serum absorbance per mean of reference serum absorbance) and that had not become pregnant after treatment. The dilutions used in these determinations corresponded to the dilution of the reference serum, which gave a 50% maximum binding.

## RESULTS

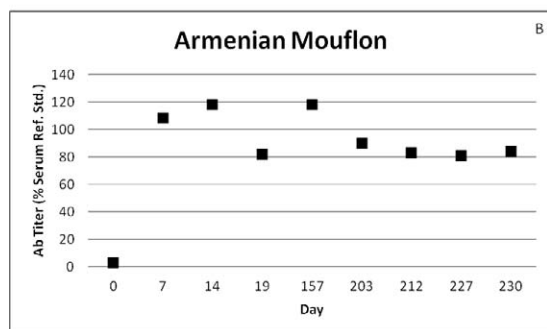
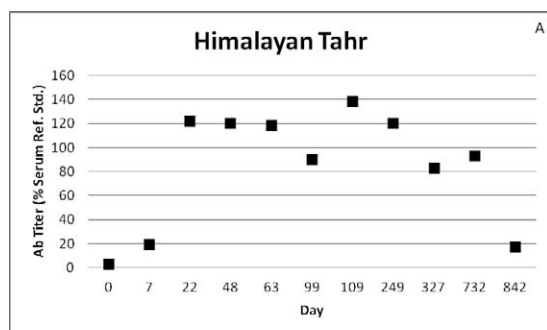
### Animal health

One of the Dall sheep died before the conclusion of the study (age 13 years). Results of the necropsy indicated chronic renal failure and cardiac valve degeneration. One of the goats was euthanized before completion of the study (age 10 years). Results of the necropsy indicated the cause of death as metastatic pulmonary and lymph

carcinoma that originated from a transitional cell carcinoma. No health problems occurred with any SDZSP animals.

### Antibody titers

The data points for anti-PZP antibody titers for all the SDZSP animals represent single samples collected opportunistically from different animals, thus, there are no standard deviations or error bars. Anti-PZP antibody titers are given for the 2 species of Bovidae, the wisent and the banteng, in Figure 1a and b. Basically, the pattern of titers represents a rapid rise and a decline to low levels within a year. Anti-PZP antibody titers for the 2 members of Cervidae, Altai wapiti and pudu, are shown in Figure 2a and b. The titers for the wapiti also revealed a common pattern of rapid rise and a decrease within a year. The pattern for the pudu, however, represents a much slower decline up through day 439. In contrast, anti-PZP antibody titers for the tahr and the Mouflon sheep, in Figure 3a and b, remain relatively high. The tahr titers do not show any appreciable decline until after day 700, and, although samples were not available for the same length of time for the sheep, there was no sign of a



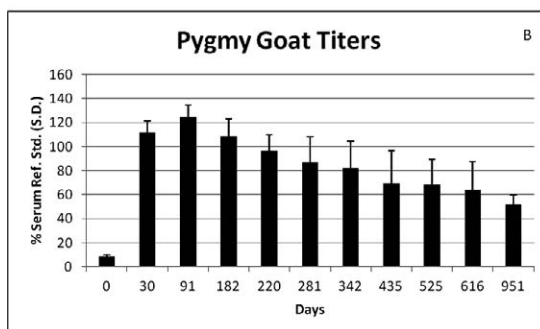
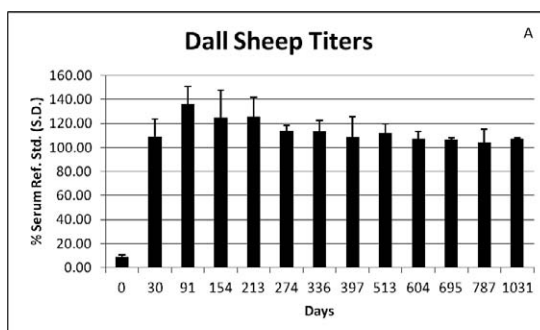
**Figure 3.** Anti-porcine zona pellucida antibody titers from Himalayan tahr (A) ( $n = 10$ ) and Armenian mouflon (B) ( $n = 8$ ). Data points represent opportunistic blood collection from different animals.

decline, and, in fact, a plateau occurred at close to the 100% level after 230 days, which suggests a prolonged immune response.

Anti-PZP antibody titers in the MCZ Dall sheep, shown in Figure 4a, were in excess of 100% of the positive reference standard for most of the 3 yr after treatment, and the decline was exceptionally slow and moderate compared with the other species. The goat anti-PZP antibody titers remained above 100% of positive reference standards for the first 6 mo of the study and declined more rapidly than those of the sheep (Figure 4b). Despite the decline, the titers were still well above 50% after 2 yr. The larger standard deviations in the goat titers were the result of one poor responder among the 5 animals. Qualitatively, the decline over 3 years was more reminiscent of the 1-yr declines seen in other ungulate species.

## DISCUSSION

The issue of species differences in the response to an immunocontraceptive is important if populations of captive exotic species are to be managed effectively and safely. The primary consideration when managing reproduction with immunocontraceptives is the target animals' immune responses



**Figure 4.** Mean (standard deviation) anti-porcine zona pellucida antibody titers for Dall sheep (A) ( $n = 4$ ) and domestic goats (B) ( $n = 5$ ).

to the vaccine, and these responses differ both within species and across species. If booster inoculations are given too infrequently, then efficacy decreases and pregnancies occur; if booster inoculations are given too frequently, then antibody titers can remain high for extended periods of time and interfere with planned breeding.<sup>9,11</sup> Thus, it is important to understand the nature of species differences.

The focus of research and management with the PZP vaccine has been ungulates. To date, none of the 80+ species of the Order Perissodactyla or Artiodactyla treated with PZP, with the exception of the Family Suidae (which does not recognize the PZP antigen as foreign), has failed to respond to the vaccine with regard to fertility inhibition. Most species demonstrate a typical pattern of antibody increase and decrease over the course of a year, after a standard primer and booster treatment a month later, which leads to the practice of an 8 mo to annual booster inoculation to maintain contraceptive antibody titers, as exemplified by white-tailed deer (*O. virginianus*),<sup>12</sup> Przewalski's horses (*Equus przewalskii*),<sup>8</sup> the horse (*Equus caballus*),<sup>9,10</sup> North American wapiti (*Cervus elaphus*),<sup>4</sup> and Fallow deer (*Cervus dama*).<sup>1</sup>



The opposite phenomenon occurs in members of the Family Capridae. A single year's treatment led to extended anti-PZP antibodies in Himalayan tahr.<sup>5</sup> In another trial, big horn sheep treated for a single year with a primer and booster inoculation remained infertile for 4 yr (Frank, unpubl. data) before reversing and producing lambs, which suggested a more prolonged response in at least some Ovidae and Capridae. The data generated in the current study confirm that the species we evaluated do in fact maintain contraceptive antibody titer longer than other species that have been evaluated over time.

The results of this study strongly suggest very conservative PZP treatment among at least some members of Ovidae and Capridae if a rapid return to fertility is the desired outcome. Conservative treatment might be defined as a single year's treatment or possibly even a single primer inoculation. Conversely, if permanent infertility is the desired outcome, because of space limitations or genetic or health issues or if the vaccine is intended to limit populations of feral sheep or goats, then less conservative PZP treatment is indicated.

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