EFFECTS OF PORCINE ZONA PELLUCIDA IMMUNOCONTRACEPTIVES IN ZOO FELIDS


Abstract: Methods of contraception are necessary for management of zoo felids; however, the most commonly used contraceptive (melengestrol acetate implant) is associated with serious adverse reactions with long-term use. Porcine zona pellucida (pZP) vaccines are promising as contraceptives, but their safety in zoo felids has not been tested. pZP vaccine was administered to 27 female felids representing 10 species, including African lion (Panthera leo), Asian leopard (P. pardus), jaguar (P. onca), tiger (P. tigris), snow leopard (P. uncia), cougar (Felis concolor), Siberian lynx (F. lynx), Canada lynx (F. canadensis), serval (F. serval), and bobcat (F. rufus), in 15 facilities. Over 6 wk, each animal received three i.m. injections of 65 µg pZP with Freund's complete adjuvant (FCA). Freund's incomplete adjuvant, or carbopil as the adjuvant. Behavioral signs of estrus were seen in 14 of the vaccinated felids. An acceptably high incidence of adverse reactions was seen including injection site swelling, lameness, limb swelling, or abscessation (or all) in five felids after injection with FCA as the initial adjuvant. Adverse behavioral signs, including increased irritability and aggression, were seen in four felids. Six of the felids were assayed for antibodies against pZP during the 12 mo after vaccination; all showed antibody production. Antibody levels appeared to peak 1–4 mo after vaccination began, although elevated antibody levels persisted in two animals for >12 mo after the first injection. All vaccinated felids were ovariohysterectomized 3–13 mo after vaccination. Folliculogenesis was present in all treated animals, and there was no histopathologic evidence of inflammatory damage to ovaries. Contraceptive efficacy was not specifically evaluated in this study; however, two of the three felids housed with an intact male became pregnant during the study, one of which gave birth to healthy cubs.

Key words: Panthera sp., Felis sp., contraception, Freund's adjuvant, immunocontraception, zona pellucida.

INTRODUCTION

Contraception is routinely used in zoo felids to prevent overpopulation and allow preservation of genetic variability within captive populations.32 Permanent contraception is safely achieved by surgical sterilization. The genetic priorities of endangered species breeding programs necessitate conservation of maximum genetic resources, so temporary contraceptive methods are preferable. Melengestrol acetate–impregnated silastic implants are currently recommended as the most reliable means of reversible contraception for zoo felids, according to the Contraceptive Advisory Group of the American Zoo and Aquarium Association (AZACAG).7 Long-term or repeated use of these implants has been associated with endometrial hyperplasia, endometrial mineralization, and uterine and mammary cancer.22,46,47 The use of such other progestins as levonorgestrel and medroxyprogesterone acetate, androgens as mibolerone,15 and surgical methods as vas deferens occlusion has been investigated in zoo felids. None has provided reliable contraception, patient safety, ease of administration, and reversibility.

Vaccination using zona pellucida (ZP) antigen shows promise for reversible contraception in many species. Reproductive immunologists continue to attempt to develop a ZP vaccine that could be administered easily to large numbers of women in rapidly growing countries, stemming population growth with minimal ethical concerns and completely reversible contraceptive effects.5,23,34,35,36,37 Porcine-derived ZP (pZP) vaccination has become a valuable population control measure for wild ungulates.23,24,35,36,37,38,39 ZP immunocontraception has been performed effectively in a small number of African lions and a cougar (Kirkpatrick, unpubl. data) but with poor efficacy in domestic cats.17,25 ZP glycoproteins are found in oocytes at all stages of folliculogenesis, even as early as the primordial or primary follicle stage.18 During normal fertilization, sperm attach to receptor glycoproteins in the ZP matrix on the outside of an ovulated ovum,
thus inducing the acrosome reaction in a sperma-
tozoon and allowing the single spermatozoon to
complete fertilization. If ZP antibodies are present
in follicular fluid before ovulation, the resultant anti-
obody-antigen complex prevents sperm penetration
of the ZP matrix primarily by steric hindrance
(physical occlusion of sperm receptor sites) and
thus prevents the cascade of events that lead to fer-
tilization of the oocyte. In some species, the im-
une response to ZP vaccination includes cell-me-
diated as well as humoral components, and T-cell
involvement has caused lymphocytic inflammation
in all stages of follicles in ZP-vaccinated mice.38-49
Previous studies have reported oophoritis, oocyte
depletion, disruption of follicular development, and
ovarian dysfunction in ZP-vaccinated nonhuman
primates, mice, rabbits, and dogs,3-6,20,21,26,30,32,33,35,37,38
although it is not clear whether these effects were
due to the ZP doses used, purity of ZP antigen, or
other factors.

ZP proteins show similarity between taxonomic
groups,23,42 therefore porcine ovaries (readily avail-
able from abattoirs) have been used as the basis for
most ZP vaccines. However, domestic cat ZP and
pZP share few epitopes, and this has been cited as
the reason for lack of contraceptive efficacy in pZP-
vaccinated domestic cats, despite high levels of
pZP antibodies being present.27 Similar epitope re-
search has not been performed in other felid spe-
cies, and it is uncertain whether pZP antibodies ef-
effectively block sperm binding sites in nondomestic
felids.

High immunogenicity and antibody production
have been achieved with ZP vaccines adjuvanted
with Freund’s complete adjuvant (FCA).4 However,
FCA has been associated with adverse reactions,
including local and systemic granulomatous inflam-
mation, due to mycobacterial cell wall and paraffin
oil components.6,36,62 Adverse reactions have also
been seen in domestic cats (Munson, unpubl. data).
Mycobacterial components in FCA cause tuberculin
test positivity in animals that have had FCA ad-
ministered. Freund’s incomplete adjuvant (FIA)
does not contain mycobacterial components and is
therefore less likely to cause granulomatous injec-
tion site reactions. FIA is also less immunogenic
than FCA. Carbopol is a water-soluble high–molecu-
lar weight carboxomer that has been used as an ad-
juvant in pigs and rabbits without adverse ef-
fects.18,19

Research has recently focused on ways to mini-
mize the adverse effects of ZP vaccination by using
less immunogenic adjuvants and more purified and
specific (recombinant subunit) forms of ZP antigen
protein.1,28,35,37,31,35,37 However, the decrease in
broad-spectrum immunogenicity results in de-
creased contraceptive efficacy, which is problem-
atic for population management of zoo species.

We administered partially purified adjuvanted
pZP to 27 zoo felids representing 10 species, fol-
lowed them clinically, and then evaluated their ova-
ries pathologically up to 14 mo after vaccina-
tion to determine whether ZP immunoc contracep-
tives had adverse effects in these species. Serum
ZP antibodies were also measured in six of the fe-
lids to evaluate the magnitude and duration of their
humoral response.

MATERIALS AND METHODS

Study animals

North American institutions housing nondomest-
cic felids were contacted to recruit permanently sur-
plus (not intended for breeding) females for this
study. Twelve African lions (Panthera leo), six
cougars (Felis concolor), two tigers (P. tigris), one
jaguar (P. onca), one Asian leopard (P. pardus),
one snow leopard (P. uncia), one serval (F. serval),
one Siberian lynx (F. lynx), one Canada lynx (F.
canadensis), and one bobcat (F. rufus) were en-
rolled in the study (Table 1). A control group of
nonvaccinated felids was chosen from the AZA-
CAG’s disease surveillance database to match the
study group animals as closely as possible regard-
ing age, previous contraceptive history, and spe-
cies. All vaccinated felids were sexually mature at
the time of first pZP vaccination, and their ages
ranged from 2 to 18 yr at time of ovariohystere-
tomy. This study was not designed as a contracep-
tive efficacy trial; institutions were advised that
vaccinated felids should not be considered contra-
cepted and should not be allowed to breed during
the study. However, separation from males was not
always feasible, and three felids were housed with
sexually intact males during the study.

Vaccine and adjuvant preparation

pZP were isolated using techniques described
previously.1,12 Frozen, thawed porcine ovaries were
minced in cold phosphate-buffered saline (PBS)
using a ganged razor-blade apparatus. The oocytes
were separated from other tissues, including gran-
ulosa cells, by screen filtration, counted, and ho-
mogenized. The zonae were isolated on a 48-μm
screen, heat-solubilized at 70°C, and frozen in dos-
es of 65 μg protein (approximately 5,000 zonae) in
0.5 ml PBS until shipment and use by participating
institutions.

Three adjuvants were used for preparation of
pZP vaccine: FCA (F5506, Sigma-Aldrich Co., St.
Table 1. Descriptive data for 27 female zoo felids vaccinated with porcine zona pellucida, including 12 lions (Panthera leo), six cougar (Felis concolor), two tigers (P. tigris), one jaguar (P. onca), one Asian leopard (P. pardus), one snow leopard (P. uncia), one serval (F. serval), one Siberian lynx (F. lynx), one Canada lynx (F. canadensis), and one bobcat (F. rufus).^c^n

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age (yr)</th>
<th>MGA exposure^b</th>
<th>Recent Adjuvants</th>
<th>Months until OVH</th>
<th>Estrus seen?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lion 1</td>
<td>9</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>6 yes</td>
<td>NAR^c</td>
<td></td>
</tr>
<tr>
<td>Lion 2</td>
<td>9</td>
<td>yes</td>
<td>Carb/Carb/Carb</td>
<td>7 ND</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 3</td>
<td>11</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>13 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 4</td>
<td>11</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>12 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Leopard</td>
<td>14</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>6 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Tiger 1</td>
<td>9</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>12 ND</td>
<td>NAR, pregnancy</td>
<td></td>
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<tr>
<td>Tiger 2</td>
<td>9</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>3 ND</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Jaguar</td>
<td>14</td>
<td>no</td>
<td>Carb/Carb/Carb</td>
<td>7 ND</td>
<td>NAR</td>
<td></td>
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<tr>
<td>Lion 5</td>
<td>15</td>
<td>yes</td>
<td>FCA/FIA/FIA</td>
<td>3 ND</td>
<td>injection site swelling</td>
<td></td>
</tr>
<tr>
<td>Lion 6</td>
<td>6</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>3 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 7</td>
<td>5</td>
<td>yes</td>
<td>FCA/FIA/FIA</td>
<td>6 ND</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 8</td>
<td>8</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>12 yes</td>
<td>behavior change, limb swelling</td>
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<tr>
<td>Lion 9</td>
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<td>FCA/FIA/FIA</td>
<td>12 yes</td>
<td>behavior change, injection site swellings</td>
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<tr>
<td>Lion 10^d</td>
<td>8</td>
<td>no</td>
<td>FCA/FIA/no vaccination</td>
<td>6 yes</td>
<td>behavior change, cellulitis, fistulous tracts</td>
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</tr>
<tr>
<td>Cougar 1</td>
<td>2</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>4 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Cougar 2</td>
<td>3</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>14 no</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Cougar 3</td>
<td>7</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>12 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Cougar 4</td>
<td>7</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>12 no</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Cougar 5</td>
<td>9</td>
<td>yes</td>
<td>FCA/FIA/FIA</td>
<td>12 no</td>
<td>behavior change</td>
<td></td>
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<tr>
<td>Siberian lynx</td>
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<td>no</td>
<td>FCA/FIA/FIA</td>
<td>6 no</td>
<td>lameness</td>
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<tr>
<td>Canada lynx</td>
<td>6</td>
<td>yes</td>
<td>FCA/FIA/FIA</td>
<td>8 no</td>
<td>NAR, pregnancy</td>
<td></td>
</tr>
<tr>
<td>Serval</td>
<td>11</td>
<td>no</td>
<td>FCA/FIA/FIA</td>
<td>12 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Bobcat</td>
<td>6</td>
<td>yes</td>
<td>FCA/FCA/FCA</td>
<td>13 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Cougar 6</td>
<td>10</td>
<td>no</td>
<td>FCA/FCA/FCA</td>
<td>6 yes</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 11</td>
<td>18</td>
<td>yes</td>
<td>FIA/FIA/FIA</td>
<td>6 ND</td>
<td>NAR</td>
<td></td>
</tr>
<tr>
<td>Lion 12</td>
<td>12</td>
<td>yes</td>
<td>FIA/FIA/FIA</td>
<td>6 yes</td>
<td>NAR</td>
<td></td>
</tr>
</tbody>
</table>

^a FCA, Freund's complete adjuvant; FIA, Freund's incomplete adjuvant; Carb, carbopol; MGA, melengestrol acetate; OVH, ovariohysterectomy; ND, not definitely known.
^b MGA impregnated in silastic, placed i.m. or s.c. as a contraceptive implant. Recent MGA exposure was defined as less than 2 yr between MGA implant removal and OVH.
^c NAR, no adverse reactions reported by clinical veterinarian.
^d Lion 10 did not receive a third vaccination.

Louis, Missouri 63178, USA), FIA (F5881, Sigma-Aldrich Co.), and carbopol (Carbopol 934, B. F. Goodrich, Cleveland, Ohio 44141, USA). Individual vaccines were prepared using 0.5 ml pZP and 0.5 ml adjuvant, emulsified together immediately before injection.

Study schedule

pZP vaccine was administered i.m. by hand injection, pole syringe, Capchur® dart (Palmer Chemical and Equipment Co., Douglassville, Georgia 30133, USA), Telinject® dart (Telinject, Saugus, California 91350, USA), or Pneu-dart® dart (Pneudart, Williamsport, Pennsylvania 17703, USA) in a 1-ml volume. Each felid received three inoculations over a 1.5-mo period, except one lion that did not receive a third inoculation because of injection site reactions seen after the first two inoculations (Table 1). Table 1 also shows the order of adjuvants used for the three injections in each individual. One bobcat and one cougar received FCA as the vaccine adjuvant for all three inoculations, instead of receiving FIA in the second and third inoculations as had been intended. One snow leopard and two lions received FIA as the vaccine adjuvant for all three inoculations. In total, 19 felids received FCA or
Antibody analysis

Serum was harvested and frozen until it was shipped to the University of California for antibody measurement. Antizona antibody analysis was accomplished by the enzyme-linked immunosorbent assay (ELISA). Fifty microliters of 5 μg/ml zona antigen solution in 0.1 M glycine buffer (pH 9.5) was placed in each well of a flat-bottom ELISA microplate (“Sumilon” low protein binding, Cat. #MS-3496, E & K Scientific Products, Saratoga, California 95070, USA) and incubated overnight at 4°C. At room temperature (20–22°C), the plate was washed once and incubated with 200 μl PBS-Tween for 30 min to block unspecified binding sites. After two more PBS-Tween washes, the treatment of the plate consisted of subsequent incubations with 50 μl/well of TBS-Tween–diluted reagents used in the following order with three washes each in between: study felid’s serum diluted 1:500, biotinylated goat anti-cat IgG diluted 1:250 (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland 20879, USA), and alkaline phosphatase avidin diluted 1:1,000 (Zymed Laboratories, San Francisco, California 94101, USA). Finally, 50 μl of substrate solution of 1 mg p-nitrophenyl phosphate/ml (5-mg tablets, Sigma-Aldrich Co.) in 10% diethanolamine buffer (pH 9.8) was incubated with prediluted primary monoclonal antibodies against CD3 (T cells, Novocastra Laboratories Ltd., Newcastle on Tyne, U.K.) or CD79a (B cells, Dako Corporation) for 30 min at room temperature. Domestic cat spleen was used as a positive control. Negative control slides were incubated without primary antibodies. Slides were incubated with secondary biotinylated horse antimus IgG (Vector Laboratories, Burlingame, California 94010, USA) for 30 min, followed by streptavidin–horseradish peroxidase (Zymed) for 20 min following the manufacturer’s protocols. Ammonium–ethyl-carbazole (Sigma-Aldrich Co.) was used as the chromagen. Slides were counterstained with hematoxylin (Gill’s formula, Fisher Scientific, Pittsburgh, Pennsylvania 15275, USA).

Statistical analyses

For quantitative ovarian analyses, the mean number of each follicular stage was compared between pZP-treated and control animals by nonparametric tests (Mann–Whitney test). Differences among the two adjuvant groups and controls also were as-
Felids housed with males became pregnant during the study; these felids' sera were not available for antibody measurement.

Adverse reactions and behavioral signs

Clinically apparent adverse reactions were seen in several of the felids vaccinated with FCA as the initial adjuvant but not in felids receiving other adjuvants (Table 1). Injection site swellings were seen in four felids for at least 3 mo after their second vaccinations. Injection site reactions did not appear to be associated with a particular injection method or institution because they occurred after pZP had been hand-injected or darted and at more than one facility. Two lions from separate facilities developed firm nodular masses at the site of pZP injection. One of these masses was biopsied within 3 mo of development, and biopsy revealed an extensive pyogranulomatous reaction at the site. Admixed with the abundant epithelioid macrophages and neutrophils were clear vacuoles that are characteristic of paraffin oil from Freund's adjuvant. In another lion, an injection site reaction progressed to cellulitis of both hind limbs with fistulous draining tracts that eventually healed as scars during the subsequent year. In another lion from the same facility, an injection site reaction involved swelling of one entire hind limb after the third vaccination. One lynx showed transient lameness without apparent injection site swelling for 3 days after second vaccination. Abnormal behavior was seen in four felids after vaccination, including increased irritability, "masculine" behavior, and aggression for the entire duration of the study. Behavioral signs of estrus were seen in at least 14 of the vaccinated felids (Table 1), and breeding was often observed in felids housed with intact or vasectomized males.

Ovarian and uterine histopathology

All animals had evidence of ongoing folliculogenesis and many had CL, indicating recent ovulation. Quantitative findings of follicular development are presented in Table 2. Control animals had fewer developing secondary follicles and more atretic secondary follicles than animals treated with pZP-F (FCA and FIA together). Animals vaccinated with pZP-C had significantly more secondary follicles without zonae than either pZP-F or controls. All animals except two controls and one pZP-F had plentiful healthy zonae. Minimal lymphocytic infiltrates composed of small numbers of B- and T cells were present in the stroma and atretic follicles of most animals including 20 of 25 controls, 14 of 19 pZP-F-treated animals, and all pZP-C-treated animals (P = 0.7). There was no qualitative difference in degree or distribution of inflammation.
Table 2. Quantitative and qualitative features in ovaries of 27 zoo felids vaccinated with porcine zona pellucida. 

<table>
<thead>
<tr>
<th>Ovarian feature</th>
<th>PZP-Freund's,  N = 19 (x ± SD)</th>
<th>PZP-Carbopol, N = 8 (x ± SD)</th>
<th>Control, N = 25 (x ± SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial and primary follicles</td>
<td>169.6 ± 141.9</td>
<td>115.6 ± 144.6</td>
<td>165.8 ± 159.6</td>
<td>0.46</td>
</tr>
<tr>
<td>Secondary follicle with no zona</td>
<td>7.7 ± 6.5a</td>
<td>11.3 ± 8.1c</td>
<td>3.3 ± 3.0c</td>
<td>0.001</td>
</tr>
<tr>
<td>Secondary follicle with zona</td>
<td>15.1 ± 12.4p</td>
<td>5.8 ± 4.8</td>
<td>6.7 ± 6.2c</td>
<td>0.01</td>
</tr>
<tr>
<td>Tertiary or preovulatory</td>
<td>13.8 ± 11.7</td>
<td>8.8 ± 7.2</td>
<td>8.9 ± 7.2</td>
<td>0.29</td>
</tr>
<tr>
<td>Corpora lutea</td>
<td>1.1 ± 1.4</td>
<td>1 ± 1.1</td>
<td>0.6 ± 1.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Granulosa cell nests</td>
<td>0.5 ± 1.7</td>
<td>0.5 ± 0.5</td>
<td>1.0 ± 2.0</td>
<td>0.26</td>
</tr>
<tr>
<td>Atretic secondary follicles</td>
<td>1.4 ± 1.3a</td>
<td>1.9 ± 1.6</td>
<td>6.5 ± 6.3a</td>
<td>0.0004</td>
</tr>
<tr>
<td>Atretic tertiary follicles</td>
<td>15.8 ± 7.2</td>
<td>13.8 ± 9.3</td>
<td>17.0 ± 10.6</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* Within rows, values with different superscripts are significantly different (P < 0.05) by Kruskal–Wallis nonparametric tests.

among treatment groups. One pZP-C-treated jaguar with an ovarian papillary cystadenocarcinoma had abundant lymphocytes infiltrating the theca and surrounding zonae. There were no significant differences in parameters among ovaries obtained 3, 6, or 12 mo after pZP-F vaccination. Moderate to severe cystic endometrial hyperplasia was present in 66% of both pZP-vaccinated and control groups (P = 1.24).

**DISCUSSION**

This study showed that pZP vaccination induces an immunologic response in zoo felids, but that vaccination is not necessarily efficacious. Folliculogenesis and ovulation continued in the felids of this study, despite the presence of ZP antibodies. These data are consistent with findings of a previous pZP clinical trial in African lions (Kirkpatrick, unpubl. data), in which pseudopregnancies were seen.

No ovarian damage was noted in any of the study animals. Differences among treatment groups for quantitative ovarian parameters were not considered biologically relevant because all stages of folliculogenesis were plentiful. Unlike reports of immune-mediated damage to ovaries in ZP-vaccinated dogs, rodents, rabbits, and primates, no significant inflammation was observed in the ovaries of study felids. The prominent lymphocytic infiltrates in the pZP-C–treated jaguar with ovarian cancer were likely in response to the tumor. It is possible that the inflammatory lesions in other studies were due to adjuvant effects or lack of purity of the ZP antigen.

Although previous studies involving wildlife species have suggested correlation between pZP antibody levels and contraceptive efficacy, these findings may not be applicable to felids. In our study, neither pZP-F nor pZP-C vaccines were 100% effective in preventing conception and pregnancy. The two felids that became pregnant in our study did not have sera collected for antibody measurement, so it was not possible to correlate antibody levels to contraception. These two animals may have had low antibody levels, so that ova were not effectively coated with antibody and therefore not protected from fertilization. Alternatively, the contraceptive effects of ZP vaccination may be due in part to cell-mediated immunity. However, the ovaries of the ZP-vaccinated animals of our study did not contain significantly more lymphocytes than control animals and those lymphocytes present were randomly distributed (not spatially associated with follicles). These findings suggest that cell-mediated immune damage did not occur in ZP-vaccinated zoo felids. It is possible that zoo felids’ ZP epitopes differ significantly from pZP epitopes, therefore making pZP antibodies ineffective in blocking ZF sites on the zonae of zoo felids, as has been described in domestic cats. Further research regarding epitope characteristics of porcine and zoo felid ZP and antibody cross-reactivity will be necessary to understand the contraceptive potential of pZP antibodies in zoo felids.

The Freund’s–adjuvanted pZP vaccine cannot be recommended for zoo felids because six of the 16 felids given FCA as one of the vaccine adjuvants experienced significant adverse reactions. Tissue changes were characterized by a marked granulomatous reaction, which was likely due to both the *Mycobacterium tuberculosis* antigens and the paraffin lipid in FCA. Behavioral reactions including irritability and dominant behavior may have been due to pain associated with vaccination site reactions and resultant defensive responses toward caretakers. The mycobacterial antigens and paraffin lip-
id of FCA induce a marked immunologic response and make FCA an effective adjuvant. It is not known whether Freund’s modified adjuvant containing M. butyricum would induce similar reactions in zoo felids or be equally immunogenic. Carboxipol-adjuvanted pZP vaccination did not induce adverse reactions in this study.

CONCLUSIONS

Freund’s-adjuvanted pZP contraceptive vaccines used in this study were associated with an acceptably high incidence of adverse vaccine reactions in zoo felids, and the cause of these reactions was likely FCA. pZP vaccination regardless of the adjuvant was not associated with ovarian lesions in this study. Pregnancy and successful parturition occurred despite pZP vaccination in some felids. Until safer, effective adjuvants are available and pZP epitopes can be designed that incite an effective immune response, pZP vaccination should not be used for contraception in zoo felids.

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LITERATURE CITED

18. Groothuis, A. J., H. L. A. Philipsen, J. T. M. de Breet-Grijisch, and M. van Duin. 1996. Immunocyto-


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