Towards novel zona pellucida vaccine formulations for use in wildlife & domestic animals

Martin Schulman  
PhD MMedVet BVSc BSc MRCVS  
Section of Reproduction, Faculty of Veterinary Science, UP, South Africa

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zona pellucida (ZP) vaccines

ZP = zona pellucida

pZP = native porcine zona pellucida vaccine
    = 3 or 4 glycoproteins (spp dependant) = ZP1/2/3/4

reZP = recombinant zp proteins
    cloned porcine ZP3 & ZP4 & expressed in E. coli
zona pellucida (ZP) vaccines

ZP vaccines = subunit vaccines
ZP proteins + adjuvant

immune response

antibodies

infertility

AB-prevented sperm-oocyte binding

effect continues as long as AB titres high enough

an adjuvant will
↑immune response & duration & % responders
↓antigen dose & Nº boosters
zona pellucida (ZP) vaccines

ZP vaccines: the application

- **population management**
  - most notably in
  - **African elephant** *(Loxodonta africana)* in South Africa
  - **feral horse** *(Equus caballus)* in North America
zona pellucida (ZP) vaccines

ZP vaccines: the horse model

= large social herbivore

BUT is domesticated

& can clinically monitor oestrous cycles

⇒ via trans-rectal palpation & U/S

⇒ successive cycles of individual mares
recombinant ZP (reZP) vaccines

reZP vaccines: the advantages

- more efficient & economical production
- no contamination ↔ non-ZP proteins
- eliminate risks ↔ animal-based products
1. investigate reZP-based vaccines
   pZP ↔ commercial & regulatory limitations

2. investigate alternative formulations
   Freund’s adjuvants in ZP-vaccines ↔ SE

3. investigate ovarian effects
   reversiblity & ongoing cyclicity?
STUDY 1: ZP vaccines + Freund’s

21 pony mares (3-14y)

**Group I/pZP‡ (n=7)**
- Initial vaccination = \( pZP + FMCA \) (V1)
- Booster (5wks later) = \( pZP + FIA \) (V2)

**Group II/reZP‡ (n=7)**
- Initial vaccination = \( reZP + FMCA \) (V1)
- Booster (5wks later) = \( reZP + FIA \) (V2)

**Group III/control (n=7)**
- 2x vaccinations 5wks apart = FMCA or FIA only

‡Trumpeter Farms, Winters, CA, USA

‡\( pZP-3 \) & \( pZP-4 \) expressed by E. coli; NII, New Delhi, India
### STUDY 1: OUTCOMES

**ovarian cyclic activity & pregnancy**

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>No. Al’s per group</th>
<th>No. (%) pregnant @14 d post-ovulation</th>
<th>PR per cycle (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (7)</td>
<td>9</td>
<td>7 (100)</td>
<td>78</td>
</tr>
<tr>
<td>pZP (7)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>reZP (7)</td>
<td>11</td>
<td>4 (57)</td>
<td>36</td>
</tr>
</tbody>
</table>

**control**  ➡️  normal cyclic activity throughout  ➡️  7/7 pregnant

**pZP treated**  ➡️  6/7 (86%) extended anestrus  ➡️  0 pregnant

**reZP treated**  ➡️  1/7 (14%) extended anestrus  ➡️  4/7 (57%) pregnant

**note:** all mares resumed cycling by 10m post-treatment
STUDY 1: OUTCOMES

anti-ZP AB response

<table>
<thead>
<tr>
<th>Group</th>
<th>Time Point</th>
<th>Optical Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>pZP</td>
<td>Day 0</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Day 28 post Prim</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>Day 28 post boost</td>
<td>2.000</td>
</tr>
<tr>
<td></td>
<td>End of season</td>
<td>3.000</td>
</tr>
</tbody>
</table>

| reZP  | Day 0      | 0.000          |
|       | Day 28 post Prim | 1.000       |
|       | Day 28 post boost | 2.000       |
|       | End of season  | 3.000         |

injection sites
STUDY 1: OUTCOMES

2015 1st publication of reZP vaccine application in equids

Equine function and pregnancy outcome in pony mares following immunocontraception with native and recombinant porcine zona pellucida vaccines

C. J. JOONÈ*, H. J. BERTSCHINGER, S. K. GUPTA†, G. T. FOSGATE‡, A. P. ARUKHA†, V. MINHAS†, E. DIETERMAN§ and M. L. SCHULMAN
STUDY 2: ZP vaccines + (Pet Gel A™ & Poly I:C™)

antigens

**porcine ZP proteins** (100µg pZP)$^1$

**novel reZP = ZP3 + ZP4 proteins** (250µg ZP3 + TT & 250µg ZP4 + bovRNase)$^2$

*note: ZP3 with tetanus toxin & ZP4 with promiscuous T-cell epitope bovine RNAse*

combined **pZP + novel reZP**

adjuvants

combination formulation

**6% polymer adjuvant**$^3$ + **500µg polyinosinic-polycytidylic acid-TLR3-agonist**$^4$

$^1$Trumpeter Farms; Winters, CA, USA
$^2$CSIR Biosciences, Pta, RSA
$^3$Montanide™ Pet Gel A; Seppic, France
$^4$HMW VacciGrade™, Invivogen, USA
STUDY 2: ZP vaccines + (Pet Gel A™ & Poly I:C™)

31 cyclic horse mares (2-10y) living on extensive area (4000ha)

- pZP⁺ (n=7) 2x ▼ pZP + combination adjuvant
- reZP⁺ (n=8) 3x ▼ reZP + combination adjuvant
- pZP & reZP (n=8) 2x ▼ pZP & reZP + combination adjuvant
- control (n=8) 2x ▼ combination adjuvant
STUDY 2: OUTCOMES

Serum antibody immunoreactivity and safety of native porcine and recombinant zona pellucida vaccines formulated with a non-Freund’s adjuvant in horses

Margaret B. Nolan\textsuperscript{a,b,\*}, Martin L. Schulman\textsuperscript{a,b}, Alma E. Botha\textsuperscript{a}, Anne-Marie Human\textsuperscript{b}, Robyn Roth\textsuperscript{c}, Michael C. Crampton\textsuperscript{c}, Henk J. Bertschinger\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>injection site score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>T° ≥39°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tx +</td>
<td>1 day</td>
<td>7 days</td>
<td>1 day</td>
<td>7 days</td>
</tr>
<tr>
<td>Primary</td>
<td>7/31</td>
<td>31/31</td>
<td>23/31</td>
<td>0/31</td>
</tr>
</tbody>
</table>
Ovarian function following immunocontraceptive vaccination of mares using native porcine and recombinant zona pellucida vaccines formulated with a non-Freund’s adjuvant and anti-GnRH vaccines

Margaret B. Nolan a, b,⁎, Henk J. Bertschinger a, b, Robyn Roth c, Michael Crampton c, Isabela S. Martins a, Geoffrey T. Fosgate a, Tom A. Stout a, d, Martin L. Schulman a, b

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Treatment</th>
<th>Control (n = 8)</th>
<th>pZP-only (n = 7)</th>
<th>reZP-only (n = 8)</th>
<th>pZP and reZP (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Active Inactive</td>
<td>Active Inactive</td>
<td>Active Inactive</td>
<td>Active Inactive</td>
</tr>
<tr>
<td>d = 0</td>
<td></td>
<td>8 0</td>
<td>7 0</td>
<td>8 0</td>
<td>8 0</td>
</tr>
<tr>
<td>d = 35</td>
<td></td>
<td>8 0</td>
<td>7 0</td>
<td>8 0</td>
<td>8 0</td>
</tr>
<tr>
<td>d = 70</td>
<td></td>
<td>8 0</td>
<td>6 1</td>
<td>3 5</td>
<td>5 3</td>
</tr>
<tr>
<td>d = 105</td>
<td></td>
<td>5 3</td>
<td>4 3</td>
<td>1 7</td>
<td>3 5</td>
</tr>
<tr>
<td>d = 175</td>
<td></td>
<td>3 5</td>
<td>3 4</td>
<td>0 8</td>
<td>0 8</td>
</tr>
</tbody>
</table>

pZP, native porcine zona pellucida vaccine; reZP, recombinant porcine zona pellucida vaccine; GnRH, anti-GnRH vaccine (Improvac®).
DONKEYS: ZP vaccines + Freund’s adjuvants

24 feral pregnant jennies from Nevis transport → St Kitts post-foaling → treatment groups allocation

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SCHOOL OF VETERINARY MEDICINE
STUDY 1: ZP vaccines + Freund’s

reZP‡ (n=9) reZP3 200ug (0.5ml) + reZP4 200ug (0.5ml) + FMCA (0.5ml) (V1)

reZP3 200ug (0.5ml) + reZP4 200ug (0.5ml) + FIA (0.5ml) (V2)

reZP3 200ug (0.5ml) + reZP4 200ug (0.5ml) + saline (0.5ml) (V3)

pZP‡ (n=8) pZP 100ug (0.5ml) + FMCA (0.5ml) (V1)

pZP 100ug (0.5ml) + FIA (0.5ml) (V2)

control (n=8) FMCA (0.5ml) + saline (0.5ml) (V1)

FIA (0.5ml) + saline (0.5ml) (V2)

‡pZP-3 & -4 expressed by E. coli; NII, New Delhi, India

†Trumpeter Farms, Winters, CA, USA
## DONKEY STUDY 1 OUTCOMES

<table>
<thead>
<tr>
<th>group</th>
<th>estrous cycles</th>
<th>pregnant (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>reZP (n=9)</td>
<td>9/9 anestrus</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10wks post final booster</td>
<td>1 @23wks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 @28wks</td>
</tr>
<tr>
<td>pZP (n=8)</td>
<td>7/8 anestrus</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10wks post final booster</td>
<td>1 @23wks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 @28wks</td>
</tr>
<tr>
<td>control (n=8)</td>
<td>N/A</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>inside 15wks</td>
</tr>
</tbody>
</table>
DONKEY STUDY 2: ZP vaccines & Pet Gel A™ + Poly (I:C)™

25 feral pregnant (>6m) Nevisian jennies (3-14y), BCS = 3-6

↑reZP (n=8) 3x ▼ reZP (250µg ZP3 & 250µg ZP4) + Pet Gel A + Poly (I:C)

↑pZP (n=9) 2x ▼ pZP (100 µg) + Pet Gel A + Poly (I:C)

control (n=8) 2x ▼ Pet Gel A + Poly (I:C)

combination adjuvant = 6% Pet Gel A + 500 µg Poly (I:C)

‡pZP-3 & -4 expressed by E. coli, CSIR Biosciences, Pta, RSA

↑Trumpeter Farms; Winters, CA, USA
## DONKEY STUDY 2 OUTCOMES

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal Cyclic (n)</th>
<th>Non-Cyclic (n)</th>
<th>Pregnant (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>intervals (average &amp; range)</td>
<td>= post-boost to shutdown &amp; resume</td>
<td>intervals (average &amp; range)</td>
</tr>
<tr>
<td>control</td>
<td>8</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>(8)</td>
<td></td>
<td></td>
<td>6 (2-15) weeks</td>
</tr>
<tr>
<td>pZP</td>
<td>5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>(9)</td>
<td>all cycled until pregnant</td>
<td>13 (11-17) weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = resume (13 &amp; 21 weeks)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = no resumption</td>
<td></td>
</tr>
<tr>
<td>reZP</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(8)</td>
<td>all cycled until pregnant</td>
<td>18.5 (11-23) weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>length shutdown = 8 (1-23) weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 = resume (23 &amp; 28 weeks)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 = no resumption</td>
<td></td>
</tr>
</tbody>
</table>

Data summary at week 44
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Veterinary Population Management Laboratory
ROSS UNIVERSITY SCHOOL OF VETERINARY MEDICINE
CSIR
HUMANE SOCIETY INTERNATIONAL AFRICA