

## **PRESENTATION SUMMARY & POWERPOINT**

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### **Native Porcine Zona Pellucida Vaccine and Lessons Learned Along the Way**

Jay F. Kirkpatrick, Science and Conservation Center, Billings, Montana

The history of native porcine zona pellucida (PZP) is traced from its origins, in the early 1970s, and the work of Alex Shivers and Ralph Gwatkin, through excitement over its possible application to human contraception in the 1980s, its decline in popularity because of the failure to develop a synthetic form of PZP and immense variability in contraceptive reversibility time, its use as a tool for understanding the mechanisms of fertilization, its successful application as a wildlife contraceptive and, most recently, as a possible but failed companion animal contraceptive.

Along the 35-year journey, many lessons learned have accumulated from this endeavor – some scientific, some political, some social, some cultural and some economic. The biological lessons include the significant variability among taxon groups in response to the PZP, the major discrepancies in purity and doses over years of research, a lack of standardization in the purity of even current sources of PZP, a failure to always include contraceptive studies along with antibody studies.

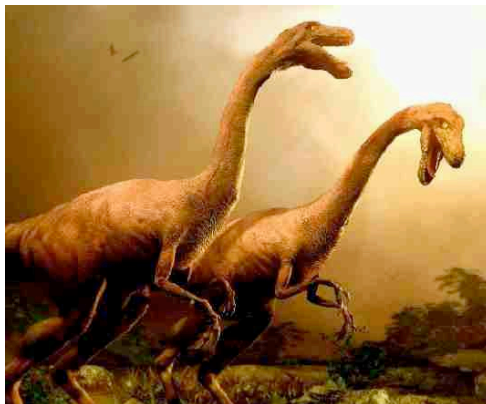
Other lessons learned outside the domain of science include a failure to listen to the needs of practitioners while developing contraceptive vaccines; a failure to understand that there is no such thing as a “magic bullet” that meets all contraceptive needs in all settings; a failure to anticipate and plan for opposition to contraception, whether in humans, wildlife or companion animals; and a failure to understand, or at least acknowledge, that costs will affect the potential application of contraception, that “dead-end” research and products often have second lives with alternative applications, and that the infusion of politics into contraceptive vaccine development, whether in the form of opposition of support, or from within or without the scientific community, can be damaging.

### Session III: What's New in Contraceptive Vaccines?

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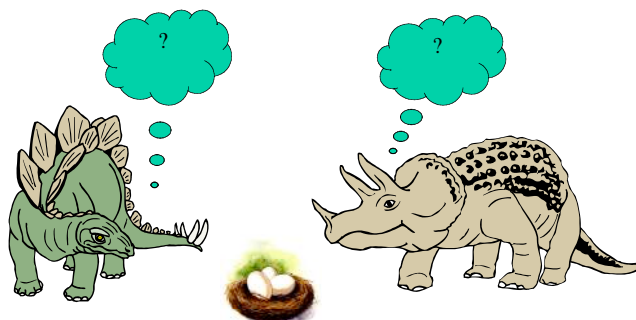
## Evolutionary History of Native Porcine Zona Pellucida and the Lessons Learned



Jay F. Kirkpatrick

The Science and Conservation Center

## The Earliest Beginnings: The Era of Discovery, 1972-1977



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#### THE BEGINNINGS

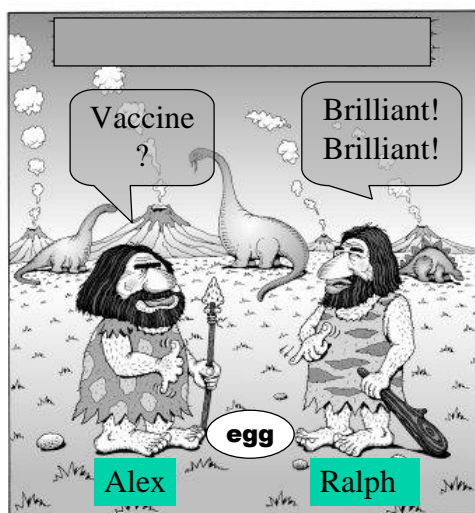
Ownby CL, Shivers CA. 1972. Antigens of the hamster ovary and effects of antiovary serum on eggs. *Bio Reprod.* 6:310-318.

Sacco AG, Shivers CA. 1973. Effects of reproductive tissue-specific antisera on rabbit eggs. *Biol. Reprod.* 8:481-490.

Shivers CA, Dudkiewicz AB, Franklin LE, Fussell EN. 1972. Inhibition of sperm-egg interaction by specific antibody. *Science* 178:1211-1213.

Gwatkin RBL, William DT, Carlo DJ. 1977. Immunization of mice with heat-solubilized hamster zonae: production of anti-zona antibody and inhibition of fertility. *Fert. Steril.* 28:871-877.

#### Emergence of the Hypothesis That Zona Proteins Could Be Used as a Contraceptive Vaccine



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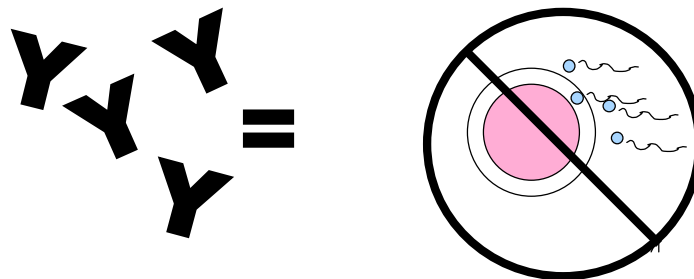
#### PZP PROTEINS AS A CONTRACEPTIVE

Sacco AG, Yurewicz EC. 1987. Use of the zona pellucida as an immunocontraceptive target antigen. In: Dietl J (ed). *The Role of the Zona Pellucida in Mammalian Reproduction*. Berlin. Springer-Verlag

Dunbar BS. 1983. Antibodies to zona pellucida antigens and their role in fertility. In: Wegmann TG, Gill TJ (eds). *Immunology of Reproduction*. New York. Oxford Univ. Press. 507-534.

Sacco AG. 1981. Immunocontraception: consideration of the zona pellucida as a target antigen. In: Wynn RM (ed). *Obstetrics and Gynecology Annual*. Vol. 10. New York, Appleton-Century-Crofts, 1-26.

### Discovery That Antibodies to Zona Proteins Could Block Fertilization: 1974-1978



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#### COULD ZONA PROTEINS BLOCK HUMAN FERTILIZATION?

Shivers CA, Dunbar BS. 1977. Autoantibodies to zona pellucida: a possible cause for infertility in women. *Science* 197:1182-1184.

Sacco AG. 1977. Antigenic cross-reactivity between human and pig zona pellucida. *Biol. Reprod.* 16:164-173.

Between 1974 and 1978 the Task Force (WHO Task Force on Vaccines for Fertility Regulation) conducted experiments that showed that ZP antigens could raise antibodies in marmosets and baboons. Funding restrictions led to the end of vaccine-based experiments by the WHO Task Force.

#### Evidence that Zona Proteins Might Be Used As a Contraceptive Vaccine: 1981-1983

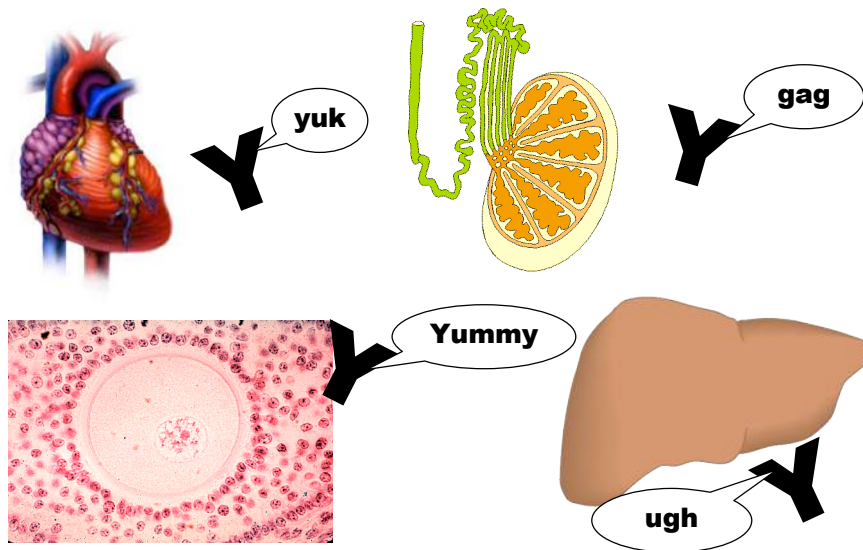


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#### The Discovery That Antibodies Against Zona Proteins Were Tissue-Specific



#### ZONA PROTEIN ANTIBODIES WERE TISSUE-SPECIFIC

Palm VS, Sacco AG, Syner FN, Subramanian MG.  
1979. Tissue specificity of porcine zona pellucida  
antigen(s) tested by radioimmunoassay. *Biol. Reprod.*  
21:709-713.

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### The Non-Human Primate-PZP Era: 1983-1987



#### NON-HUMAN PRIMATE RESEARCH

Gulyas BJ, Gwatkin RBL, Yuan LC. 1983. Active immunization of cynomolgus monkeys (*Macacca fascicularis*) with porcine zona pellucida. *Gamete Res.* 4:299-307.

Aitken RJ, Richardson DW, Hulme M. 1984. Immunological interference with the properties of the zona pellucida. In: Crighton DB (ed). *Immunological Aspects of Reproduction in Mammals*. Boston. Butterworths. 305-326. (Marmosets)

Bamezai AK, Suman, Das C, Talwar GP. 1986. Effect of immunization against porcine zona pellucida (PZP) on steroid hormone profiles and fertility in primates. *J. Reprod. Immunol. (Suppl.* 85) (Bonnet monkeys)

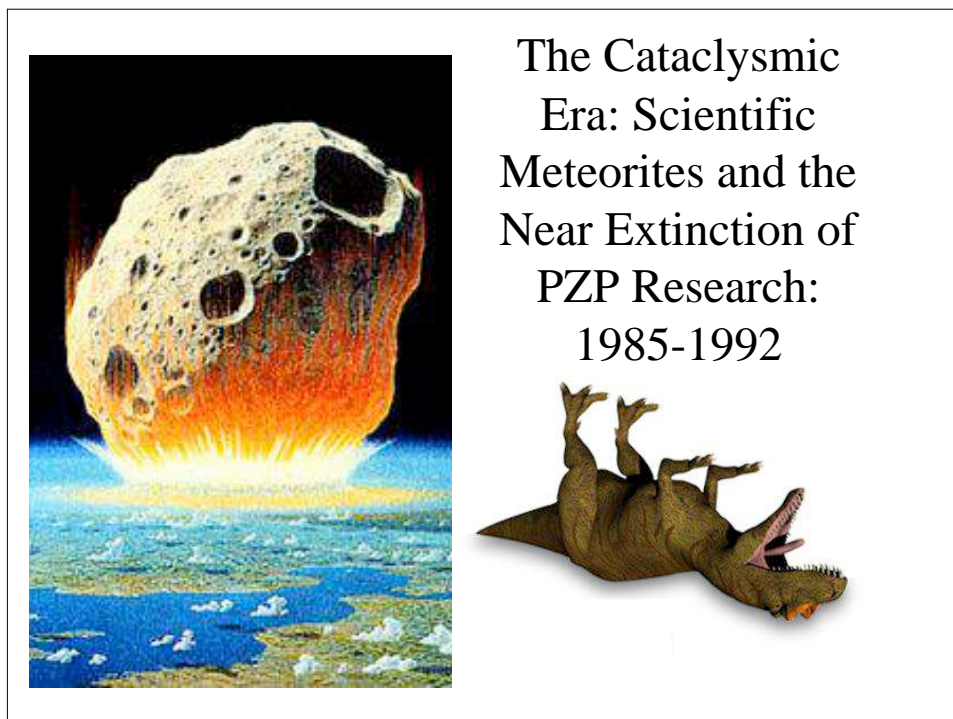
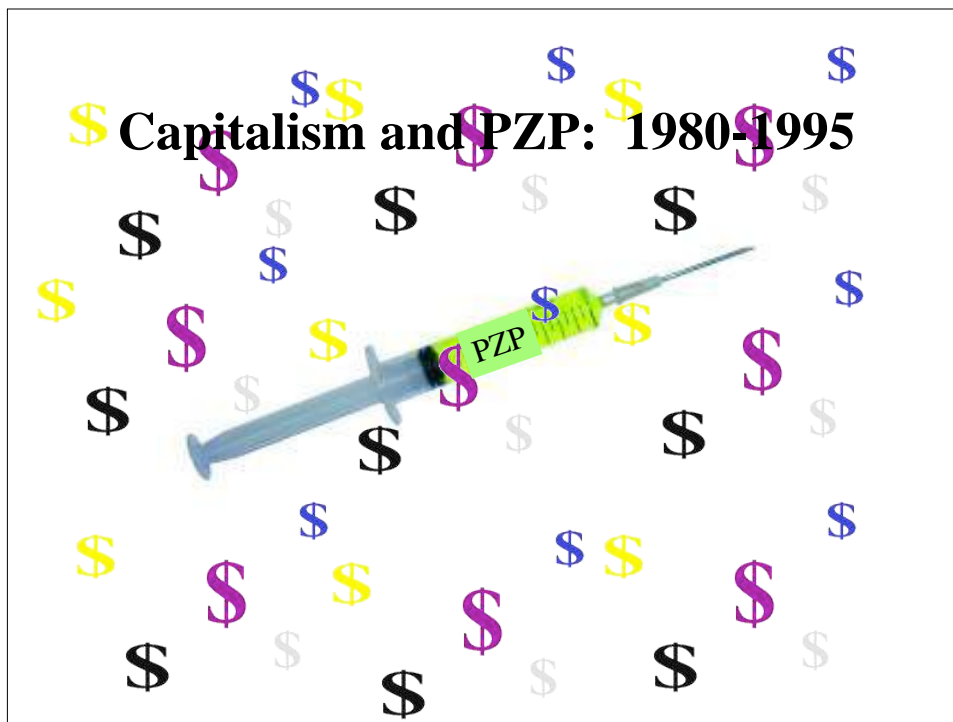
Sacco AG, Subramanian MG, Yurewicz EC, DeMayo FJ, Dukelow WR. 1986. Heteroimmunization of squirrel monkeys (*Saimiri sciureus*) with a purified porcine zonae antigen (PPZA): immune response and biologic activity of antiserum. *Fert. Steril.* 39:350-358.

Sacco AG, Pierce DL, Subramanian MG, Yurewicz EC, Dukelow WR. 1987. Ovaries remain functional in squirrel monkeys (*Saimiri sciureus*) immunized with porcine zona pellucida 55,000 macromolecule. *Biol. Reprod.* 36:481-490.

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## The Second Cataclysm: Ovarian Damage



### OVARIAN DAMAGE

Dunbar BS, Lo C, Stevens V. 1989. Effect of immunization with purified porcine zona pellucida proteins on ovarian function in baboons. *Fert. Steril.* 52:311-318.

Luo Y, Tung KSK. 1993. T cell peptide of a select protein elicits autoantibody to the protein antigen. Implications for specificity and pathogenic role in autoimmunity. *J. Immunol.* 151:5790-5799. (Mice)

Skinner S, Timmons T, Schwoebel E, Dunbar BS. 1984. Immunization with zona pellucida proteins results in abnormal ovarian follicular differentiation and inhibition of gonadotropin-induced steroid secretion. *Endocrinology* 115:2418-2432. (Rabbits)

Wood DM, Liu C, Dunbar BS. 1981. Effect of alloimmunization and heteroimmunization with zonae pellucidae on fertility in rabbits. *Biol. Reprod.* 25:439-450.

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#### OVARIAN DAMAGE (CONT.)

Lee V, Dunbar BS. 1992. Immunization of guinea pigs results in polycystic ovaries. *Biol. Reprod.* (Suppl 46):131 (Abstract).

Mahi-Brown CA, Yanagimachi R, Hoffman J, Huang TTF, 1985. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of different adjuvants and patterns of estradiol and progesterone levels in estrous cycles. *Biol. Reprod.* 32:671-772.

Jones GR, Sacco AG, Subramanian MG, Kruger M, Zhang S, Yurewicz EC, Moghishi KS. 1992. Histology of ovaries of female rabbits immunized with deglycosylated zona pellucida macromolecules of pigs. *J. Reprod. Fert.* 95:513-525.

Paterson M, Thillai Koothan P, Morris KD, O'Bryne KT, Braude P, Williams A, Aitken RJ. 1992. Analysis of the contraceptive potential of antibodies against native and deglycosylated porcine ZP3 in vivo and in vitro. *Biol. Reprod.* 46:523-534. (Marmosets)

## The Third Cataclysm: Variation in Time to Contraceptive Reversibility



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## Remnants of PZP Research Life



### **PZP AS A TOOL FOR UNDERSTANDING THE MECHANISMS OF FERTILIZATION**

Florman PM, Wassarman HM, 1985. O-linked oligosaccharides of mouse egg ZP3 account for its sperm receptor activity. *Cell* 41:313-324.

Saling OM, 1991. How the egg regulates sperm function during gamete interaction: facts and fantasies. *Biol. Reprod.* 44:246-251.

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#### THE SEARCH FOR A USEFUL EPITOPE OF PZP

Dunbar BS, Avery, Lee V, Prasad S, Schwahn D, Schwoebel E, Skinner S, Wilkins B. 1984. The mammalian zona pellucida: its biochemistry, immunochemistry, molecular biology, and developmental expression. *Reprod. Fert. Dev.* 6:331-47.

Dean J. 1991. The zona pellucida genes encode essential proteins for mammalian fertilization and early embryogenesis. *Proc. Soc. Exptl. Biol. Med.* 196:141-146.

Yurewicz EC, Zhang S, Sacco AG. 1993. Generation and characterization of site-directed antisera against an amino-terminal segment of a 55 kDa sperm adhesive glycoprotein from zona pellucida of pig oocytes. *J. Reprod. Fert.* 98:147-152.

Mahi-Brown CA, Moran F. 1995. Response of cynomolgus macaques to immunization against a synthetic peptide form the human zona pellucida. *J. Med. Primatol.* 24:258-270.

Hasegawa A, Koyama K, Isojima S. 1988. Immunogenicity of a 92kDa component of porcine zona pellucida isolated using a monoclonal antibody (3A4-2G1) exclusively cross-reactive with porcine and human zonae pellucidae. *J. Reprod. Immunol.* 14:73-82

### The Pleistocene Age of PZP: Wildlife, 1987-Present



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## THE SWITCH FROM HUMANS TO HORSES

Liu IKM, Bernoco M, Feldman M. 1989.

Contraception in mares heteroimmunized with pig  
zonae pellucidae. J. Reprod. Fert. 85:19-29.

## Horses



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#### APPLICATION OF PZP TOP FREE-RANGING WILD HORSES

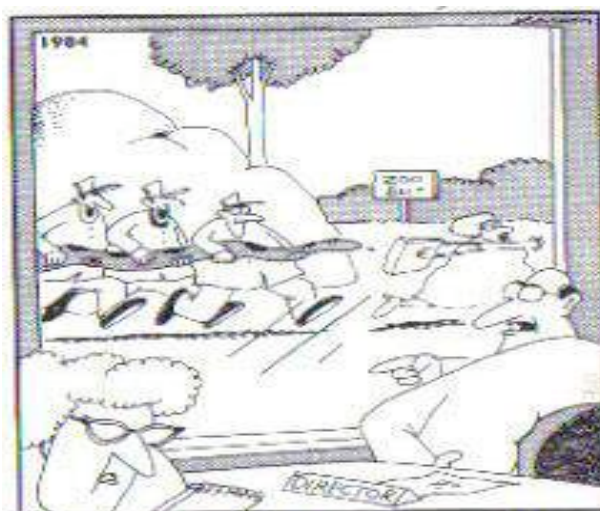
Kirkpatrick JF, Liu IKM, Turner JW. 1990. Remotely-delivered immunocontraception in feral horses. *Wildl. Soc. Bull.* 18:326-330.

Kirkpatrick JF, Liu IKM, Turner JW, Bernoco M. 1991. Antigen recognition in mares previously immunized with porcine zonae pellucidae. *J. Reprod. Fert. (Suppl. 44)*:321-325.

Kirkpatrick JF, Liu IKM, Turner JW, Naugle R, Keiper R. 1992. Long-term effects of porcine zona pellucida immunocontraception on ovarian function of feral horses (*Equus caballus*). *J. Reprod. Fert.* 94:437-444.

Kirkpatrick JF, Naugle R, Liu IKM, Bernoco M, Turner JW. 1995. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. *Biol. Reprod. Monograph Series 1:Equine Reproduction VI.* 411-418.

## Zoo Animals



"Take another memo, Miss Wilkens. ... I want to see all reptile personnel in my office first thing tomorrow morning!"

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#### APPLICATION OF PZP TO CAPTIVE EXOTIC SPECIES (ZOOS)

Kirkpatrick JF, Zimmermann W, Kolter L, Liu IKM, Turner JW. 1995. Immunocontraception of captive exotic species. I. Przewalski's horse (*Equus przewalskii*) and banteng (*Bos javanicus*). *Zoo Biol.* 14:403-416.

Kirkpatrick JF, Calle PP, Kalk P, Liu IKM, Turner JW. 1996. Immunocontraception of captive exotic species. II. Formosa sika deer (*Cervus nippon*), Axis deer (*Cervus axis*), Himalayan tahr (*Hermitragus jemlahicus*), Roosevelt elk (*Cervus elaphus roosevelti*), Reeve's muntjac (*Muntiacus reevesi*) and Sambar deer (*Cervus unicolor*). *J. Zoo Wildl. Med.* 27:482-495.

Deigert FA, Duncan AE, Frank KM, Lyda RO, Kirkpatrick JF. 2003. Immunocontraception of captive exotic species. III. Contraception and population management of fallow deer (*Cervus dama*). *Zoo Biol.* 22:261-268.

Frank KM, Lyda RO, Kirkpatrick JF. 2005. Immunocontraception of captive exotic species. IV. Species differences in response to the porcine zona pellucida vaccine, timing of booster inoculations and procedural failures. *Zoo Biol.* 24:349-358.

Patton ML, Jochle W, Penfold LM. 2005. Contraception in ungulates. In: *Wildlife Contraception: Issues, Methods and Applications*. Asa CS, Porton IJ (eds). Baltimore. Johns Hopkins University Press. Pp. 149-167.

## Urban Deer



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#### **APPLICATION OF PZP TO CAPTIVE WHITE-TAILED DEER**

Turner JW, Liu IKM, Kirkpatrick JF. 1992. Remotely-delivered immunocontraception in captive white-tailed deer. *J. Wildl. Manage.* 56:154-157.

Turner JW, Kirkpatrick JF, Liu IKM. 1996. Effectiveness, reversibility and serum antibody titers associated with immunocontraception in captive white-tailed deer. *J. Wildl. Manage.* 60:45-51.

#### **APPLICATION OF PZP TO FREE-RANGING DEER**

McShea WJ, Monfort SL, Hakim S, Kirkpatrick JF, Liu IKM, Turner JW, Chassy L, Munson L. 1997. The effect of immunocontraception on the behavior and reproduction of white-tailed deer. *J. Wildl. Manage.* 61:560-569.

Walter DW, Kilpatrick HJ, Gregonis MA. 2003. Does immunocontraception improve condition of free-ranging female white-tailed deer? *J. Wildl. Manage.* 67:762-766.

Miller LA, Crane K, Gaddis S, Killian GJ. 2001. Porcine zona pellucida immunocontraception: Long-term health effects on white-tailed deer. *J. Wildl. Manage.* 65:941-945.



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## Elephants



"My God, Carlson! After years of searching, this is an emotional moment for me! ... Voila! I give you the Secret Elephant Breeding Grounds!"

### APPLICATION OF PZP TO AFRICAN ELEPHANTS AND WAPITI

Fayrer-Hosken RA, Grobler D, Van Altena JJ, Bertschinger HJ, Kirkpatrick JF. 2000. Immunocontraception of African elephants. *Nature (Lond)* 407-149.

Delsink A, Van Altena JJ, Kirkpatrick JF, Grobler D, Fayere-Hosken RA. 2002. Field application of immunocontraception in African elephants (*Loxodonta Africana*). *Reproduction (Suppl. 60)*:117-124.

Shideler SE, Stoops MA, Gee NA, Howell JA, Lasley BL. 2002. Use of porcine zona pellucida vaccine as a contraceptive agent in free-ranging tule elk (*Cervus elaphus nannodes*) at Tomales Point, Point Reyes National Seashore. *Reproduction (Suppl. 60)*:169-176.

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## Era of Population Studies with PZP and Wildlife: 1995-Present



### POPULATION MANAGEMENT BY MEANS OF PZP CONTRACEPTION

Turner A, Kirkpatrick JF. 2002. Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). *Reproduction (Suppl. 60)*:187-195.

Rutberg AT, Naugle RE, Thiele LA, Liu IKM. 2004. Effects of immunocontraception on a suburban population of white-tailed deer (*Odocoileus virginianus*). *Biol. Cons.* 116:243-150.

Naugle RE, Rutberg AT, Underwood HB, Turner JW, Liu IKM. 2002. Field testing of immunocontraception on white-tailed deer (*Odocoileus virginianus*) on Fire Island National Seashore, New York, USA. *Reproduction (Suppl. 60)*:143-153.

Delsink AK, Bertschinger HJ, Kirkpatrick JF, Grobler D, Van Altena JJ, Slotow R. 2004. The preliminary behavioral and population dynamic response of African elephants to immunocontraception. *Proc. 15<sup>th</sup> Symposium on Tropical Animal Health and Production*. Utrecht University, Utrecht, Netherlands. Pg. 19-22.

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#### LONG-ACTING FORMS OF PZP CONTRACEPTION

Turner JW, Liu IKM, Flanagan DR, Rutberg AT, Kirkpatrick JF. 2001. Immunocontraception of feral horses: One inoculation provides one year of infertility. *J. Wildl. Manage.* 65:235-241.

Turner JW, Liu IKM, Flanagan DR, Bynum KS, Rutberg AT. 2002. Porcine zona pellucida (PZP) immunocontraception of wild horses (*Equus caballus*) in Nevada: A 10-year study. *Reproduction (Suppl. 60)*: 177-186.

Fraker MA, Brown RG, Gaunt GE, Kerr JA, Pohajdak B. 2002. Long-lasting, single dose immunocontraception of feral fallow deer in British Columbia. *J. Wildl. Manage.* 66:1141-1147.

Brown RG, Bowen WD, Eddington JD, Kimmins WC, Mezei M, Parsons JL, Pohajdak B. 1997. Evidence for a long-lasting single administration contraceptive vaccine in wild grey seals. *J. Reprod. Immunol.* 35:43-51.

### Biological Warfare and PZP



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#### **PZP AND BIOLOGICALLY MODIFIED ORGANISMS AS VECTORS**

Tyndale-Biscoe CH. 1994. Virus-vectored immuno-contraception of feral mammals. *Reprod. Fertil. Dev.* 6:281-187.

Martinez ML, Harris JD. 2000. Effectiveness of zona pellucida protein ZPB as an immunocontraceptive antigen. *Reproduction* 120:19-32.

#### **PZP Moves from Wildlife to Companion Animals: 2000-?**



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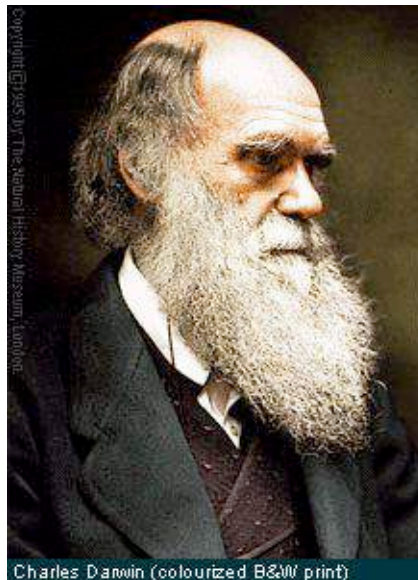
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#### REDISCOVERY OF MAHI-BROWN'S DOG RESEARCH

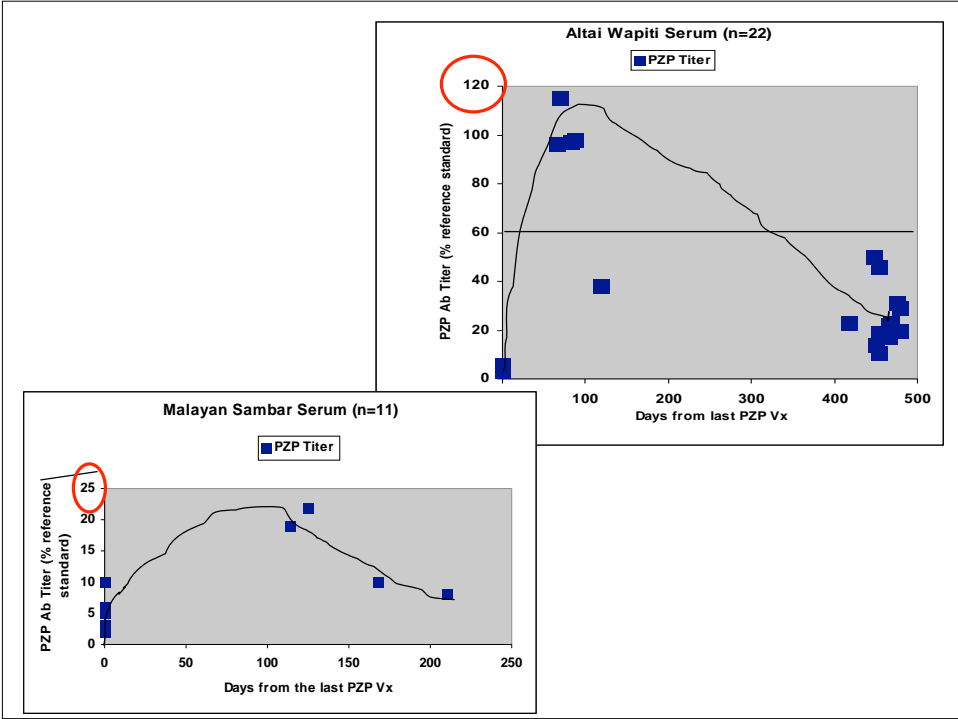
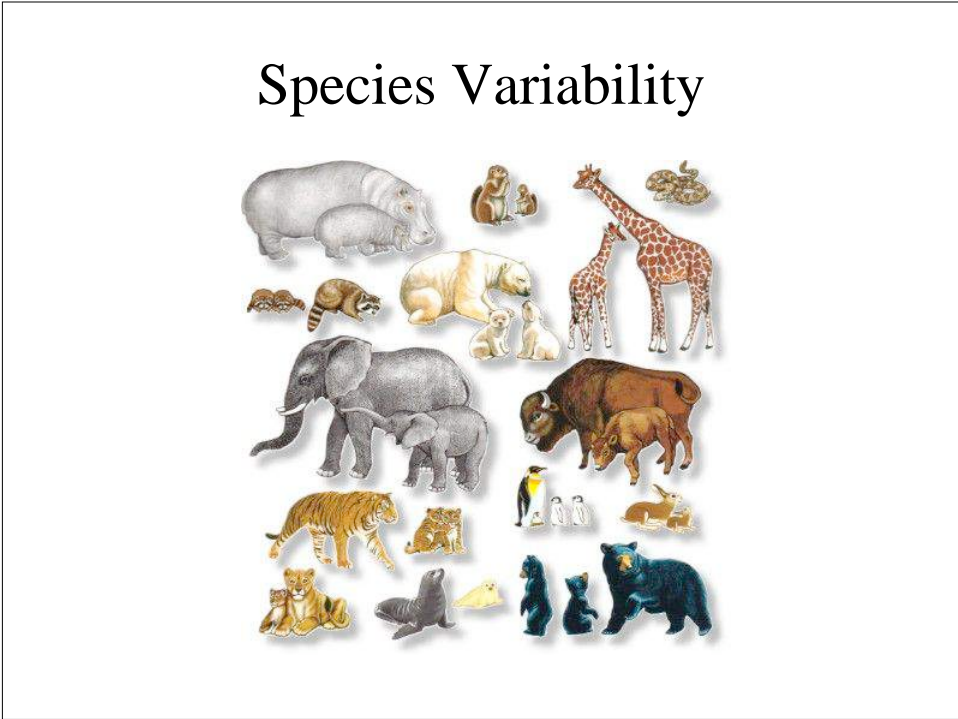
Dunbar BS. 2005. Immunosterilization in dogs using zona pellucida (ZP)-based vaccine. In: Humane Wildlife Solutions: The Role of Immunocontraception. Rutberg AT (ed). Washington, Humane Society Press. Pg. 77-92.

Gorman SP, Levy J, Hampton AL, Collante WR, Harris AI, Brown RG. 2002. Evaluation of a porcine zona pellucida vaccine for the immunocontraception of domestic kittens (*Felis catus*). *Theriogenology* 58:135-149.

#### The Lessons of History



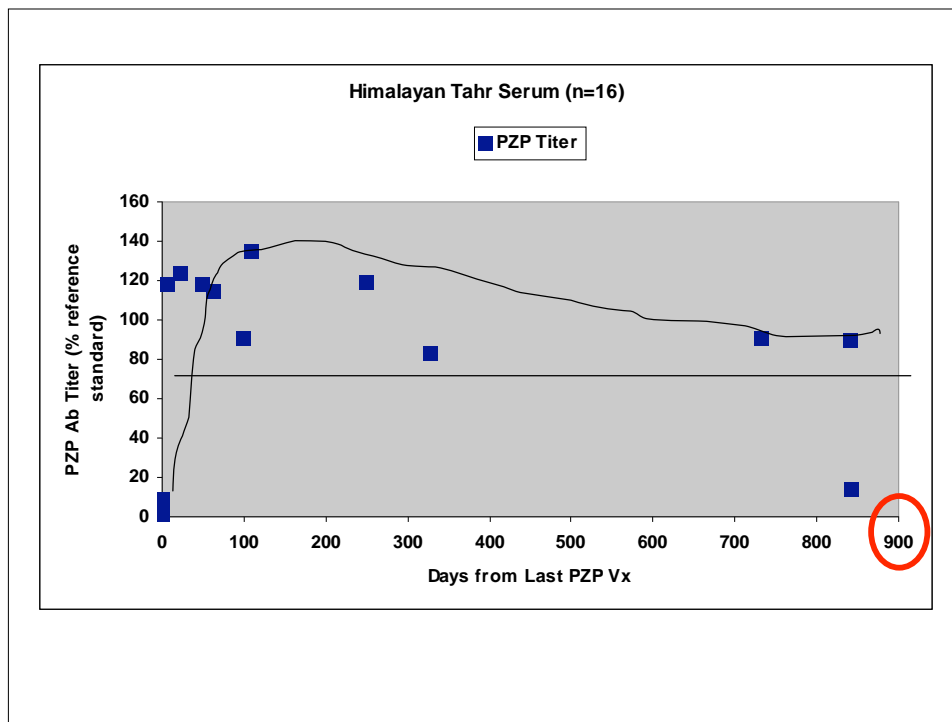
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## Purity of PZP and Consequences

- Cannot Extrapolate from Study to Study
- No Standardization of Product Purity

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Never Look at  
Antibody Titers in  
New Species  
Without Looking at  
Contraception Too!

No Magic Bullet





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Low Cost



Profitability??



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## Politics



## Evolutionary History...The End

