EFFICACY REVIEW

PRODUCT:	ZonaStat-H
REG. NUMBER:	86833-R
DATE:	March 15, 2011
DP BARCODE:	D370425
DECISION:	420440
GLP:	N/A
CHEMICAL:	Porcine Zona Pellucida (PZP)
CHEMICAL NUMBER:	Porcine Zona Pellucida…176603
PURPOSE:	Review submitted articles to determine if product's efficacy as a contraceptive is supported.
MRID:	**47859801. Grandy, J. (2009) ZonaStat-H (Porcine Zona Pellucida): Product Efficacy: (Wild Horses and Burros). Unpublished study prepared by The Humane Society of the United States. 84 pp.**
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BACKGROUND:

The Humane Society of the United States (HSUS) is applying for Section 3 registration for ZonaStat-H (porcine zona pellucida) to control feral and wild horses and burros. ZonaStat-H will be registered as a Restricted Use product to be used by certified applicators only.

PRODUCT DESCRIPTION:

The active ingredient in ZonaStat-H is porcine zona pellucida (PZP), which is an immunocontraceptive vaccine that is administered to target animals via intramuscular injection in hip or gluteus muscles either by hand delivery (injection), jab-stick delivery, or remote (dart) delivery. ZonaStat-H consists of an emulsion of 2 components: (1) the antigen, a naturally occurring, chemically unmodified glycoprotein, PZP, which is extracted from pig ovaries; and (2) an adjuvant (modified Freund's Complete Adjuvant, mFCA, or Freund's Incomplete Adjuvant, FIA). PZP itself is a composite of four different acidic glycoproteins, ZP1, ZP2, ZP3, and ZP4. Approximately 70-80% of PZP is made of ZP3. mFCA consists of cell wall fragments from a non-pathogenic soil bacterium (*Mycobacterium butyricum*). *M. butyricum* fragments are suspended in a physiologically inert mineral oil and an emulsifier. FIA is identical to mFCA, but does not have the mycobacterial cell wall that is found with mFCA.

Upon injection of ZonaStat-H into a female horse or burro, anti-zona pellucida (ZP) antibodies are produced. The antibodies bind to the ZP glycoproteins that surround the egg of the injected animal, alter the glycoproteins' conformation, and block the attachment of sperm, thus preventing fertilization.

APPLICATION METHOD AND RATE

The application rate is 1.0 cc of PZP (which is already dissolved in 0.5 mL of phosphate buffered saline, PBS) + 0.5 mL adjuvant (mFCA or FIA). The antigen solution (PZP + PBS) is mixed in the field prior to use which is detailed in the Procedures section of the label as noted below:

1. Wear examination gloves while mixing and loading PZP.
2. Attach the Luer-Lok connector to one of the glass syringes (depending on method of application).
3. Place the 1.5 inch needle on the second glass syringe.
4. Draw ont 0.5 cc of adjuvant.
5. Using the same syringe, draw up the 0.5 cc of PZP in the phosphate buffer saline (PBS).
6. While holding the syringe containing the vaccine carefully in order to prevent the plunger from slipping out, take off the needle and attach the syringe to the second syringe using the Luer-Lok connector.
7. Push the PZP-adjuvant mixture back and forth through the two syringes 100 times. The resulting will become thick and white.
8. Make sure that all of the emulsion is in one syringe.
9. Holding the syringe containing the emulsion very carefully, remove the other syringe, leaving the Luer-Lok on the syringe containing the emulsion.

Different materials are needed for mixing depending on the method of application. For hand delivery, the applicator would need a 3cc disposable plastic syringe with Luer-Lok, a 1.5 inch 18 g disposable sterile needle; jab-stick delivery requires a Dan-Inject® Fiskars Combi-Click Jab Stick, a 3cc disposable plastic syringe with Luer-Lok, and a monoject 1.5 inch 14 gauge disposable sterile needle; and the remote (dart) delivery method of application requires the use of a 2.0 inch 18 gauge disposable sterile needle and a 1.0 cc C-type or P-type Pneu-Dart dart with 1.25 inch or 1.5 inch barbless needle.

For hand delivery (injection) the applicator attaches a 2.0cc or 3.0cc plastic syringe to the glass syringe via the Luer-Lok, and injects the emulsion into the plastic syringe. The applicator then disconnects the glass syringe and connects an 18 gauge 1.5 in. needle to the syringe containing the emulsion.

For jab-stick delivery, the applicator injects the emulsion from the glass syringe into the plastic syringe after attaching the plastic syringe tightly into the Luer-Lok. Then the Monoject 14 gauge 1.5 in. needle is attached to the plastic syringe containing the emulsion after the glass syringe is removed. Then the plastic syringe is placed into the jab-stick.

For remote delivery (via dart), the applicator first attaches the 18 gauge, 2 in. needle to the glass syringe containing the emulsion, then injects the emulsion into the dart. Following emulsification in the field of antigen solution and adjuvant, the applicator remotely injects ZonaStat-H in the hip, gluteus, or hamstring muscles with a syringe dart fired from a CO_2 or cartridge-powered projection system.

EFFICACY OF ZONASTAT-H

As ZonaStat-H does not bear claims to control pests that may pose a threat to human health, pursuant to OPPTS 810.1000(b)(2), the requirement for demonstration of efficacy is waived. In lieu of efficacy studies, the registrant provided various peer-reviewed published articles demonstrating ZonaStat's efficacy as a contraceptive for wild horses and burros.

Reduction of free-roaming feral horse populations via contraception has been a goal of researchers since the early 1970s (Kirkpatrick et al., 1990). Various methods have been attempted leading up to the use of PZP. Initially, fertility reduction was demonstrated by using an injectable microencapsulated testosterone propionate (mTP) in stallions which resulted in an 83% decrease in foaling by mares (Kirkpatrick, et al. 1990). Delivery of mTP was done by first immobilizing the stallions and then injecting them. This method of delivery incurred high costs and stress to the animal, resulting in a remote method of delivery. Though mTP was effective in stallions, remote delivery made it difficult to deliver enough steroid to make it effective (Kirkpatrick, et al. 1990).

Another option was tried which also utilized steroid-induced fertility control, but this time the mares were the target animal. The use of ethinylestradiol-progesterone Silastic® implants showed effectiveness, but once again much stress was placed on the target animal because the method of delivery required the mare to be captured, restrained, then undergo field surgery to place the implants peritoneally (Kirkpatrick, et al. 1990). Focus then turned to immunocontraception as an alternative to steroid-induced fertility control. Efficacy had already been demonstrated for PZP by Liu et al. in 1989 by inhibiting fertility in 13 of 14 domestic and captive feral mares.

The principle of efficacy of PZP in horses was first demonstrated by Liu et al. (1989) by inhibiting fertility in 12 of 14 captive fertile domestic and wild mares (*Eqqus caballus*), which persisted for 7 months. The researchers inoculated the mares with 4 hand injections of PZP with aluminum hydroxide gel. As the aluminum hydroxide gel was found to be only moderately effective in most of the horses, it was therefore substituted by FCA and FIA at 2-4 week intervals. A fifth booster injection was administered 6-9 months after the fourth injection. This study also demonstrated that anti-PZP antibody titers of 64% or greater were associated with effective contraception, and that a decline in contraceptive effect correlated with a decline in antibody titers.

Kirkpatrick et al. (1990) demonstrated PZP effectiveness in a study conducted at Assateague Island National Seashore (ASIS), MD in which 26 mares were remotely injected with a priming dose of 65-100 µg PZP in FCA and either one or two boosters of PZP in FIA at three-week intervals based on the determination by Liu et al. (1989) that at least two inoculations are required in horses so antibody titers are raised high enough for a minimum of 6 months. Upon the first inoculation, antigen recognition is initiated which increases antibody titers temporarily. Then, the second inoculation causes increased titers that last for several months, with each follow-up inoculation prolonging the duration of high titers (Kirkpatrick, et al. 1990).

During this study, 14 of the 26 treated mares were already pregnant upon inoculation and gave birth to healthy foals approximately 1 – 3 months after the last inoculation. By October 1998, there was only one pregnancy out of the 26 treated mares, as indicated by analysis of urinary steroids, with zero pregnancies among the 18 receiving 3 inoculations, and one pregnancy out of the 8 receiving two inoculations. The following spring, August 1989, only one of the 26 treated mares produced foals. (Kirkpatrick, et al. 1990). Of the 26 treated mares, 14 were boosted again a year later with a single remotely delivered dart containiug PZP in FIA. Only 1 of the 14 boosted mares was pregnant and produce a foal the following year, compared to 10 of 22 "sham-treated and untreated mares (45.5%) (Kirkpatrick, et al. 1991). Additional studies were carried out during the next 6 years which demonstrated foaling rates of 3.8% (4 foals in 105 mare-years) among PZP-treated mares compared to 46.2% in untreated mares (Kirkpatrick, et al. 1991). Zero population growth was achieved in 2 years, with an initial decline in the population becoming apparent in 8 years of inoculations and by year 11, the population declined from 175 to 135 horses, a decrease of 22.8% (Kirkpatrick and Turner 2008).

Turner et al. (1996) conducted a study at Virgin Islands National Park, St. Johns, VI (VINP) on free-roaming feral burros (*Eqqus asinus*) to assess the effectiveness of PZP as a contraceptive with results comparable to those seen in the Assateague Island studies. In this study, 16 female burros were treated with PZP contraceptive. Burros were given an initial one- or two-injection PZP treatment and, after 10 – 12 months,

were given a one-injection PZP booster treatment. Initial treatment consisted of: (1) two separate injections (3 weeks apart) of a 1.0 mL emulsion, containing 65 µg PZP plus FCA (first injection) followed by a booster of FIA (n = 13); or (2) a single injection containing 130 µg PZP emulsified in FCA (n = 3). The single injection was a time-released method with release rates projected to be continuous across 4 weeks, with greatest release in weeks 1 and 4 followed by a booster shot at the end of the 4 weeks (Turner et al., 1996).

Zero of 13 females darted with a priming dose of 65 – 100 µg in FCA and a booster of 65 – 100 µg PZP in FIA produced foals in the period 12 – 24 months after treatment, while 1 of the 3 females receiving the single dose produced foals. Furthermore, 6 of 11 control females gave birth in that time period. Unlike wild and feral horses, feral burros are not seasonal breeders, and some of the burros were pregnant at the time of treatment. The results of this study indicate the two-injection protocol was more effective than the single-injection in preventing pregnancies.

The effectiveness of the adjuvant used is an important factor in how efficacious the PZP epitope is as an immunocontraceptive (Lyda, et al. 2005). Since 1998, PZP has been used in captive free-ranging wild horses with a high degree of efficacy, utilizing Freund's Complete Adjuvant (FCA) as the adjuvant of choice for the initial inoculation and Freund's Incomplete Adjuvant (FIA) for booster inoculations. The use of FCA has resulted in 90% or greater efficacy, however two side effects can occur from its use: 1) Injection site reactions, including open abscesses and 2) false-positive tuberculosis (TB) tests in treated animals. The primary ingredient in the FCA is *Mycobacterium tuberculosis* which can cause antibodies against the TB organism. As a result of these side effects, the United States Department of Agriculture (USDA) has voiced opposition to the use of FCA.

Therefore, modified Freund's Complete Adjuvant (mFCA) has been substituted for FCA in titer trials of captive mares. These trials demonstrated no significant difference between mares hand-injected with 65-100 µg PZP in mFCA followed by a booster shot of 65-100 µg in FIA and mares treated with 65-100 µg PZP in FCA followed by a booster of 65-100 µg in FIA. Lyda et al. (2005) reported that 7 of 8 (87.5%) of mares treated with PZP and mFCA remained above the contraceptive titer threshold 10 months after treatment. The effectiveness of mFCA as an adjuvant was verified with these studies.

CONCLUSIONS

The articles submitted by the HSUS assigned MRID Number 47859801 are acceptable in that they support the efficacy of ZonaStat-H as a contraceptive for the control of wild and feral horses and burros.

Literature Cited

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