Long-Term Efficacy of Three Contraceptive Approaches for Population Control of Wild Horses

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ABSTRACT: Controlling fertility of feral horses through the use of long-acting contraceptives or sterilization approaches has been championed as a reasonable and humane solution for addressing overpopulation problems in several western states. However, methods to accomplish long-term contraceptive efficacy of horses following a single treatment have been lacking. In fall 2002 and spring 2003, we initiated a study to compare the long-term efficacy of a single-shot contraceptive vaccine directed at gonadotropin releasing hormone (GonaCon™) to that of a single-shot vaccine directed at the zona pellucida (SpayVac) and to the use of intrauterine contraceptive devices (IUD). Both vaccines were administered with AdjuVac™, an adjuvant developed at the National Wildlife Research Center. The objectives of the study were to determine: 1) 3-year efficacy for preventing pregnancy, 2) whether the contraceptive effects are reversible, and 3) whether there are contraindications. The Nevada Department of Agriculture provided the feral mares, which were maintained at the Nevada State Penitentiary, Carson City, facility. Mares were dewormed and given health vaccinations annually. Eight untreated control mares were compared to 12 mares treated with SpayVac, 16 mares treated with GonaCon™, and 15 mares treated with copper-containing IUDs. Only 25% (2/8) of the control mares were not pregnant or infertile in the first year. All mares in the SpayVac group were infertile, and 94% (15/16) were infertile in the GonaCon™ group during the first breeding season. In Year 2, 80% (10/12) of the SpayVac-treated mares and 60% (9/15) of the GonaCon™-treated mares were infertile. In Year 3, 80% of the SpayVac mares and 53% (8/15) of the GonaCon™-treated mares were infertile. For IUD-treated mares, 80% (12/15) were infertile after Year 1, but only 29% (4/14) and 14% (2/14) were infertile after Years 2 and 3, respectively. For IUD mares that were infertile, it was possible to visualize the IUD by ultrasonography, leading us to conclude that mares that became pregnant had lost their IUDs. For mares given SpayVac, uterine edema was commonly observed. In Years 2 and 3, antibody titers for SpayVac were progressively lower compared to titers observed in Year 1.

KEY WORDS: contraception, *Equus caballus*, feral horse, fertility control, GnRH vaccine, intrauterine device, SpayVac-PZP vaccine

Proc. 22nd Vertebr. Pest Conf. (R. M. Timm and J. M. O'Brien, Eds.)
Published at Univ. of Calif., Davis. 2006. Pp. 67-71.

INTRODUCTION

Contraception of wildlife and feral species has been considered a desirable option for addressing concerns of over population for many years. Ideally, the contraceptive approach should be easily administered, be effective for multiple years, and have few if any contraindications in the target species. Immuncontraceptive vaccines are believed to fulfill many of the attributes desired for population control. Data reported previously for several species, including horses (*Equus caballus*), support the notion that immunocontraceptive vaccines are safe and effective in the short term (reviewed in Killian *et al.* 2004). One injectable immunocontraceptive vaccine that has been extensively researched for use in wildlife and feral species is the porcine zona pellucida (PZP) vaccine. This vaccine presumably works by stimulating antibody production against the zona pellucida, the non-cellular outer covering of the egg. Consequently, the normal interaction between sperm and egg is prevented by the presence of antibodies on the surface of the ovum. One of the shortcomings of most formulations of PZP vaccines previously reported, however, is that a single vaccination is effective for only 1-2 years at most (Fagerstone *et al.* 2002). Although less extensively evaluated than the PZP vaccine for wildlife contraception, the gonadotropin releasing hormone (GnRH) vaccine is also of interest as an immunocontraceptive approach, since it provides a means of non-surgical sterilization by compromising reproductive hormone and gamete production of both males and females.

Considering the needs of population control of western, free-ranging mustangs, annual vaccination by injection is not a practical means to maintain infertility. Annual capture is inefficient, costly, and involves added risks of injury to horses and handlers. Although oral forms of contraceptive vaccines may make it practical to annually vaccinate free-roaming species with minimal difficulty, they are not now available and their development will probably take many years. Given the status quo, it was our belief that if the PZP and GnRH vaccines were modified or shown to act longer, they would eliminate the need for annual vaccination and provide a reasonable short-tem solution for controlling fertility in wild mustang populations.

In fall 2002 and spring 2003, we initiated a study to compare the long-term efficacy of a single-shot contraceptive vaccine directed at gonadotropin releasing hormone (GonaCon™) with that of a single-shot vaccine

directed at the zona pellucida of the ovum (SpayVac). Both vaccines were administered with AdjuVac™, an adjuvant developed at the USDA National Wildlife Research Center (NWRC). In addition, we evaluated the use of an intrauterine contraceptive device (IUD), the 380 copper "T", since in humans it has been shown to be effective for maintaining infertility for up to 12 years (Killian *et al.* 2004).

The objectives of the study for these contraceptive approaches were to determine: 1) rates of contraception for up to 3 years, 2) whether the contraceptive effects were reversible, and 3) whether there were contraindications. We now report the results following 3 years of study.

MATERIALS AND METHODS

The details pertaining to the horses used in the study and the treatments they received were previously reported (Killian *et al.* 2004). They are briefly summarized below.

Animals

The Nevada Department of Agriculture provided 53 mares and 3 stallions for use in the project. The mares weighed 225-360 kg and ranged in age from 18 months to 12 years at treatment. The horses were previously gathered from state lands and brought to the Nevada State Penitentiary, Carson City facility, where the study was conducted. All horses were given annual health vaccinations and dewormed routinely.

To handle feral mares for jugular blood sampling and vaccinations, they were run into a hydraulic chute and haltered. Contraceptive vaccines were given intramuscularly in the left lateral neck. For pregnancy evaluations by ultrasound or palpation, or IUD placement, the mares were chemically restrained.

Treatments

The studies were approved by the Institutional Animal Care and Use Committee of Pennsylvania State University. Eight mares were assigned to be untreated controls, 12 mares received a single shot of 400μg SpayVac PZP vaccine, 15 mares received a single shot of 1800μg GonaCon™ vaccine, and 3 mares received a single shot of 2800μg GonaCon™ vaccine. Fifteen mares received copper-containing IUDs placed into the uterus, trans-cervically. Doses of the SpayVac PZP vaccine were kindly provided by the vaccine's developer, Dr. Robert Brown (Brown *et al.* 1997). The single-shot GonaCon™ vaccine was developed at and provided by the NWRC. Both SpayVac and GonaCon™ were used in combination with AdjuVac™ adjuvant (Miller *et al.* 2004). The human copper 380 "T" intrauterine devices were purchased from Family Planning Sales Ltd., Littlemore, Oxford, UK. Mares were penned with a fertile stallion for a breeding trial lasting from June to September of each year. If mares failed to breed or become pregnant during the breeding season, they were considered infertile.

Observations

Nevada mares were routinely observed for breeding activity by staff and prisoner caretakers, and they were checked once or twice annually by ultrasonography for pregnancy, IUD retention, and uterine inflammation. Blood samples were assayed for estradiol, progesterone, and antibody titers to the contraceptive vaccines at the NWRC (Miller *et al.* 2000, 2001). Contraindications evaluated included general health and body condition, and uterine edema, which may be associated with hormonal changes or presence of IUDs. One mare receiving the GonaCon™ vaccine died after the first breeding season of causes not related to the treatment.

RESULTS AND DISCUSSION

Contraceptive Efficacy

All mares in the SpayVac group were infertile and 94% (15 /16) were infertile in the GonaCon™ group during the first breeding season (Figure 1). In Year 2, 80% (10/12) of the SpayVac-treated mares and 60% (9/15) of the GonaCon™-treated mares were infertile. In Year 3, 80% (10/12) of the SpayVac mares and 53% (8/15) of the GonaCon™-treated mares were infertile. For IUD-treated mares, 80% (12/15) were infertile after Year 1, but only 25% and 14% were infertile and after Years 2 and 3.

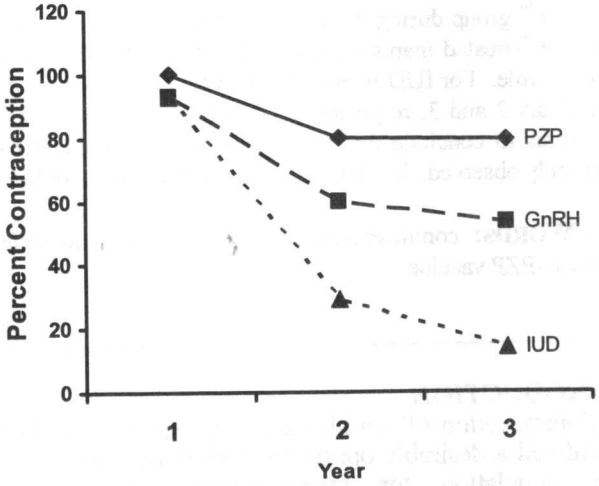

Figure 1. Percent mares contracepted in years following treatment with SpayVac (PZP), GonaCon™ (GnRH), or the 380 copper "T" human intrauterine device (IUD).

SpayVac

Mares receiving a single vaccination of SpayVac maintained a high level of contraception throughout the 3-year study. This rate of efficacy exceeds any previously reported rate for use of PZP vaccines in mustang mares or burros (Liu *et al.* 1989, Kirkpatrck *et al.* 1996, Turner *et al.* 2002). In previous studies with mares given a primary and booster vaccination, infertility was achieved in 80-90% of animals for 1 year and further sustained at this level by annual vaccination. Although Kirkpatrick, Turner, and colleagues have used annual vaccination with a PZP vaccine to effectively manage reproduction in some feral horse populations (Turner *et al.* 2002), annual recapture and vaccination of mares is not a practical approach for most field applications.

Various preparations of injectable PZP vaccines have

been used for contraception of a long list of species (Fagerstone *et al.* 2002), including deer (Turner *et al.* 1997, Miller *et al.* 2001, Fraker *et al.* 2002), elephants (Fayer-Hosken *et al.* 1999), dogs (Mahi-Brown *et al.* 1985), baboons (Dunbar 1989), and seals (Brown *et al.* 1997). However, the only formulation of single-shot PZP vaccine that appears to consistently produce a high degree of contraception lasting multiple years is the form known as SpayVac, developed by Dr. Robert Brown. Our findings confirm, in a controlled study, the multi-year efficacy for mares that has been reported for grey seals (*Halichoerus grypus*, Brown *et al.* 1997) and fallow deer (*Dama dama*, Fraker *et al.* 2002) using this formulation of PZP vaccine.

We observed that SpayVac-treated mares were more likely to have elevated serum concentrations of estradiol and show evidence of estrus at sampling than GonaCon™- or IUD-treated mares. These observations were correlated with a high incidence of uterine edema (>80%) in PZP-treated mares (Table 1). Uterine edema associated with estrus would normally be expected to be seen in ~25% of a population of randomly sampled mares during the breeding season. This prediction is based on the assumption that the normal mare estrous cycle is 22-24 days, and for approximately 6 days or 25% of that cycle she will be in estrus. From our observations, it is evident that uterine edema was present in a much higher percentage of PZP-treated mares. Although repeated estrous cycles during the breeding season have been reported for PZP-treated mares (Turner and Kirkpatrick 2002), it was generally concluded that the repeated estrous cycles were of normal length. However, we suggest that the high incidence of uterine edema and behavioral estrus observed for the Nevada mares during sampling in this study indicates abnormal estrous cycles. These abnormal estrous cycles are likely characterized by a prolonged follicular phase and a brief, if not absent, luteal phase. To better characterize the estrous cycle of mares treated with the PZP vaccine, it would be necessary to draw daily blood samples for hormone assay. Unfortunately, mustangs do not lend themselves to frequent handling regimens. Such studies would need to be done on domestic mares.

Table 1. Percentage of reproductive tracts with edema revealed by ultrasonography of mares for each of the treatments compared to the predicted number of mares expected to be in estrus.

	Year 1	Year 2	Year 3
Predicted %	25%	25%	25%
GonCon™ actual %	23% (3/13)	25% (2/8)	25% (2/8)
SpayVac actual %	82% (9/11)	91% (10/11)	100% (10/10)

GonaCon™

The single-shot GonaCon™ vaccine performed quite well during the first year, but the contraception rate decreased to 60% in Year 2 and 53% in Year 3. Although GonaCon™ did not perform as well as SpayVac, the multi-year results achieved with a single vaccination of GonaCon™ far exceed published results for contraception studies with wild horses using PZP vaccines (Turner *et al.* 2002, Kirkpatrick and Turner 2002). In addition, mares did not display evidence of frequent estrus or abnormally high incidence of uterine edema. It is noteworthy that the drop in contraception rate was greatest between Years 1 and 2, with only minimal decrease from Year 2 to Year 3 (Figure 1). A similar trend was seen with SpayVac, although to a lesser degree. This suggests that considering the immunological response, there are two sub-populations of mares: one population responded with antibody titers adequate for contraception that were maintained over several years, while in the other population contraception lasted no more than 1 year. Given the limited data we were able to collect from mustang mares, it is not possible to provide an explanation for the immunological differences between the multi-year and single-year responders.

Vaccine Antibody Titers

Contraceptive efficacy of both SpayVac and GonaCon™ was clearly related to antibody titer (Figures 2 and 3). Average titers for contracepted mares in each of the 3 years of study were considerably greater than those mares that became pregnant. The average titer for contracepted mares receiving SpayVac progressively declined during each year of study. However, the average titer in Year 3 for contracepted SpayVac mares was still nearly 8-fold greater than the average "breakthrough" titer for all SpayVac-treated mares that became pregnant. There was a 37% decline in titer between Years 1 and 2 and a 33% decline between Years 2 and 3. If we assume an average annual rate of decline in titer of 35%, this suggests that, on average, the majority of SpayVac-treated mares will remain contracepted for 4 additional years before the breakthrough titer is reached. This projection of a total of 7 years of contraception for SpayVac-treated mares is supported by the literature report of long-term efficacy of SpayVac use in grey seals (Brown *et al.* 1997).

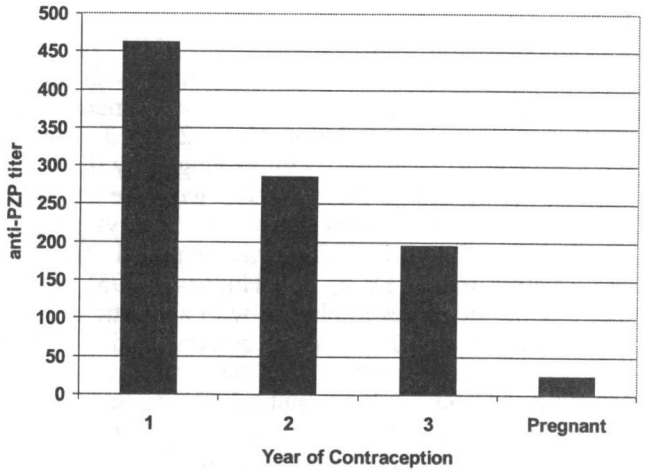

Figure 2. Average antibody titers for contracepted mares treated with SpayVac in years following treatment compared to the average titer of all pregnant mares.

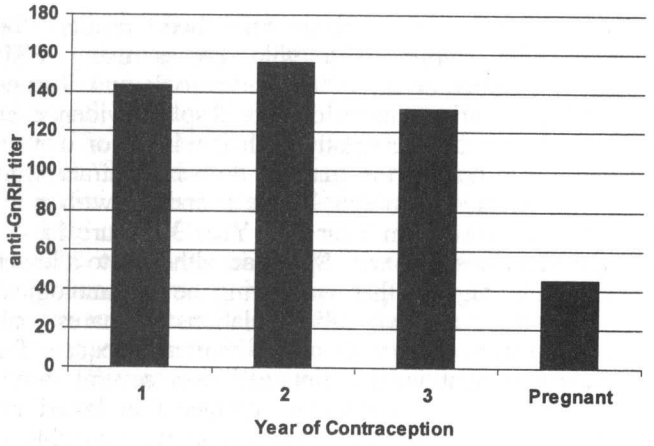

Figure 3. Average antibody titers for contracepted mares treated with GonaCon™ in years following treatment compared to the average titer of all pregnant mares.

Average titers for contracepted GonaCon™-treated mares did not show the same rate of decline in annual titer as seen with SpayVac. However, this observation is somewhat misleading, since in Year 2, 40% of the GonaCon™-treated mares became pregnant. The lower titers for these mares were averaged in the pregnant group, favoring a higher average titer for the mares in the GonaCon™-contracepted group. The fact that pregnant mares with lower titers were no longer included in the contracepted group explains the small artifactual rise in titer in Year 2. The 15% decline in GonaCon™ titer for the contracepted mares between Years 2 and 3 may more accurately reflect the annual rate of titer decline to expect for GonaCon™-treated mares following a single injection. The average titer for the 8 remaining GonaCon™-treated mares contracepted in Year 3 was 132×10^3, or nearly 3-fold greater than the 45×10^3 titer for the 5 mares that became pregnant. However, one of the mares that became pregnant had a titer of 128×10^3, while the average for the other 4 was only 24×10^3. This emphasizes the individual variability that can occur relative to titers and efficacy of a contraceptive vaccine.

IUDs

Although the 380 copper "T" IUD performed respectably during the first year of study with an 80% contraception rate (Figure 1), its performance as a contraceptive was poor during Years 2 and 3. Because we were able to visualize by ultrasonography the IUD in infertile mares, but in Years 2 and 3 we were not able to visualize the IUD in mares that were <50 days pregnant, we believe that the IUD was expelled from the reproductive tract. Daels and Hughes (1995) reported that 6 domestic mares implanted with a silastic ring IUD were infertile for 1 breeding season, and all mares returned to fertility after IUD removal the following breeding season. In unpublished studies we are conducting using the 380 copper "T" in domestic pony mares, 4 out of 5 mares have remained contracepted for 3-4 years. Given the difference in uterine size between the pony and mustang mare, it is likely that the human 380 cooper "T" is too small to be consistently retained in the uterus of the mustang mare. Retention of foreign objects in the uterus of the mare is clearly related to the size of the object. In studies using glass balls inserted into the uterus of mares to suppress estrous behavior, it was indicated that the size of the glass ball influenced its ability to be retained by the mare (Nie *et al.* 2001, Thomas 2002). Although the human 380 copper "T" did not perform for multiple years as we expected, based on studies with humans, further research to develop an IUD better suited to mustang mares could produce an effective means of long-term contraception.

Contraindications

We found no significant contraindications that would affect the well-being of mares given any of the treatments. All mares were in good body condition throughout the study. The presence of the copper-containing human IUD in the uterine lumen did not appear to cause a uterine inflammatory response, as reported for domestic mares equipped with silastic IUDs (Daels and Hughes 1995).

While a high incidence of uterine edema was observed in SpayVac-treated mares, which may have been due to irregularities in the estrous cycle, there was no indication that this naturally-occurring response to estrogen adversely affected the mares. There was no pattern of elevated estradiol or reduced progesterone in SpayVac-treated mares compared to GonaCon™- or IUD- treated mares. However, given the limited blood sampling we were able to do, it is not surprising. Frequent blood sampling is necessary to accurately characterize hormonal changes during the estrous cycle, a study better pursued with domestic mares.

Our results with the PZP vaccine agree with the report and general conclusion that mares treated with the PZP vaccine do not suffer from ill effects (Kirkpatrick *et al.* 1995, Turner and Kirkpatrick 2002). This conclusion is also supported by our study in white-tailed deer (*Odocoileus virginianus*) treated with PZP vaccine, which showed no significant adverse health effects (Miller *et al.* 2001). We also came to the same conclusion for white-tailed deer treated with GonaCon™, evaluated in a target safety study that is reported in a companion paper at this conference (Killian *et al.* 2006).

CONCLUSIONS

Three years of study on mustang mares treated with immunocontraceptive vaccines and an IUD suggest that they are safe and effective. Multiyear contraceptive efficacy was greatest for SpayVac, followed by GonaCon™ and the IUD. Given the number of mares becoming pregnant in Years 2 and 3, we conclude that the IUD and GonaCon™ approaches are reversible. Further study is needed to establish the rate of reversibility for the SpayVac vaccine, although the reversibility of other PZP immunocontraceptive vaccines has been reported (Kirkpatrick and Turner 2002).

LITERATURE CITED

BROWN, R. G., W. D. BOWEN, J. D. EDDINGTON, W. C. KIMMINS, M. MEZEI, J. L. PARSONS, AND B. POHAJDAK. 1997. Evidence for a long-lasting single administration

vaccine in wild grey seals. J. Reprod. Immunol. 35:43-51.

DAELS, P. F., AND J. P. HUGHES. 1995. Fertility control using intrauterine devices: an alternative for population control in wild horses. Theriogenology 44:629-639.

DUNBAR, B. S. 1989. Use of a synthetic peptide adjuvant for the immunization of baboons with denatured and deglycosylated pig zona pellucida glycoproteins. Fert. Steril. 52:311-318.

FAGERSTONE, K. A., M. A. COFFEY, P. D. CURTIS, R. A. DOLBEER, G. J. KILLIAN, L. A. MILLER, AND L. M. WILMOT. 2002. Wildlife fertility control. Technical Review, The Wildlife Society, Bethesda, MD. 29 pp.

FAYER-HOSKEN, R. A., H. J. BERTSCHINGER, J. F. KIRKPATRICK, D. GROBLER, N. LAMBERSKI, G. HONNEYMAN, AND T. ULRICH. 1999. Contraceptive potential of the porcine zona pellucida vaccine in the African elephant (*Loxodonta africana*). Theriogenology 52: 835-846.

FRAKER, M. A., R. G. BROWN, G. E. GAUNT, J. A. KERR, AND B. POHAJDAK. 2002. Long-lasting, single-dose immunocontraception of feral fallow deer in British Columbia. J. Wildl. Manage. 66:1141-1147.

KILLIAN, G., J. EISEMANN, D. WAGNER, J. WERNER, D. SHAW, R. ENGEMAN, AND L. MILLER. 2006. Safety and toxicity evaluation of GonaCon™ immunocontraceptive vaccine in white-tailed deer. Proc. Vertebr. Pest Conf. 22:82-87.

KILLIAN, G., L. A. MILLER, N. K. DIEHL, J. RHYAN, AND D. THAIN. 2004. Evaluation of three contraceptive approaches for population control of wild horses. Proc. Vertebr. Pest Conf. 21:263-268.

KIRKPATRICK, J. F., R. NUAGLE, I. K. M. LIU, J. W. TURNER, AND M. BERNOCO. 1995. Effects of seven consecutive years of porcine zona pellucida contraception on ovarian function in feral mares. Biol. Reprod. Monogr. Series 1: Equine Reproduction VI, pp. 411-418.

KIRKPATRICK, J. F., AND A. TURNER. 2002. Reversibility of action and safety during pregnancy of immunization against porcine zona pellucida in wild mares (*Equus caballus*). Reprod Suppl. 60:197-202.

KIRKPATRICK, J. F., J. W. TURNER JR., I. K. LIU, AND R. FAYRER-HOSKEN. 1996. Applications of pig zona pellucida immunocontraception to wildlife fertility control. J. Reprod. Fertil. Suppl. 50:183-189.

LIU, I. K. M., M. BERNOCO, AND M. FELDMAN. 1989. Contraception in mares hetero-immunized with porcine zona pellucida. J. Reprod. Fert. 85:19-29.

MAHI-BROWN, C. A., R. YANAGIMACHI, J. C. HOFFMAN, AND T. T. F. HUANG JR. 1985. Fertility control in the bitch by active immunization with porcine zonae pellucidae: use of different adjuvants and pattern of estradiol and progesterone levels in estrous cycles. Biol. Reprod. 32:761-772.

MILLER, L. A., K. CRANE, S. GADDIS, AND G. J. KILLIAN. 2001. Porcine zona pellucida immunocontraception: long-term health effects on white-tailed deer. J. Wildl. Manage. 65: 941-945.

MILLER, L. A., B. E. JOHNS, AND G. J. KILLIAN. 2000. Immunocontraception of white-tailed deer with GnRH vaccine. Am. J. of Reprod. Immun. 44:266-274.

MILLER, L. A., J. RHYAN, AND G. J. KILLIAN. 2004. GonaCon™, a versatile GnRH contraceptive for a large variety of pest animal problems. Proc. Vertebr. Pest Conf. 21:269-273.

NIE, G. J., C. E. JOHNSON, AND J. G. W. WENZEL. 2001. Use of a glass ball to suppress behavioral estrus in mares. Amer. Assoc. Equine Pract. Proc. 47:246-248.

THOMAS, H. S. 2002. Marbles for mares: an alternative for preventing estrus in mares. Amer. Quart. Horse J. 55(5):84-87.

TURNER, A., AND J. F. KIRKPATRICK. 2002. Effects of immunocontraception on population longevity and body condition in wild mares (*Equus caballus*). Reproduction (Suppl. 60):187-195.

TURNER, J. W. JR., I. K. LIU, D. R. FLANAGAN, K. S. BYNUM, AND A. T. RUTBERG. 2002. Porcine zona pellucida (PZP) immunocontraception of wild horses (*Equus caballus*) in Nevada: a ten year study. Reproduction (Suppl. 60):177-186

TURNER, J. W. JR., I. K. M. LIU, A. T. RUTBERG, AND J. F. KIRKPATRICK. 1997. Immuno-contraception limits foal production in free-roaming feral horses in Nevada. J. Wildl. Manage. 61:873-880.