CSIRO PUBLISHING
Wildlife Research, 2019, **46**, 713–718
https://doi.org/10.1071/WR18112

Efficacy of dart-delivered PZP-22 immunocontraceptive vaccine in wild horses (*Equus caballus*) in baited traps in New Mexico, USA

Kathleen A. Carey [A], *Alexa Ortiz* [A], *Kayla Grams* [B], *Dan Elkins* [C], *John W. Turner, Jr* [D] and *Allen T. Rutberg* [A,E]

[A]Center for Animals and Public Policy, Cummings School of Veterinary Medicine, Tufts University, 200 Westboro Road, North Grafton, MA 01536, USA.
[B]Humane Society of the United States, 700 Professional Drive, Gaithersburg, MD 20879, USA.
[C]Mt Taylor Mustangs, PO Box 2946, Santa Fe, NM 87504, USA.
[D]Department of Physiology and Pharmacology, University of Toledo College of Medicine, 3035 Arlington Avenue, Toledo, OH 43614, USA.
[E]Corresponding author. Email: allen.rutberg@tufts.edu

Abstract

Context. Federally protected wild horses on public lands are undergoing population growth that overwhelms the historical management strategy of removal and adoption. Porcine zona pellucida (PZP) has been used as an injectable immunocontraceptive vaccine to induce reversible infertility in free-roaming horses. PZP vaccination during February and March, which is the optimal time for administering current vaccines, is not possible for the herd on Jarita Mesa Wild Horse Territory (JM WHT), New Mexico, due to severe weather, terrain and subject wariness.

Aims. The first goal was to assess bait trapping and remote darting as a minimally disruptive alternative to helicopter gathers for treatment. The second goal was to quantify the efficacy over 2 years following spring treatment with a single injection of PZP-22 (a combination of PZP-adjuvant emulsion and controlled-release pellets) by remote dart delivery.

Methods. Bait trapping and dart delivery of PZP-22 was carried out on JM WHT from 4 April to 16 June 2012. The herd was observed in the summers of 2011, 2013 and 2014 to determine the foaling status of the study mares. Outcome (foal or no foal) as a function of treatment was analysed using Fisher's exact test.

Key results. There were 157 individuals, including 66 females >1 year old, documented in 2011. In 2012, 26 females (including three yearlings) identified by colour and markings were bait trapped and darted with PZP-22. The proportion of treated females foaling was lower than that of untreated females in 2013 and 2014, but the difference was only significant in 2013. Of the treated mares observed in 2013, the two that foaled were the last two treated in 2012. Untreated mares >4 years old were significantly more likely to foal than younger mares.

Conclusions. Bait trapping at JM WHT permitted successful delivery of PZP-22 in a previously inaccessible herd. Dart administration of PZP-22 in April–June induced at least 1 year of measurable infertility.

Implications. This is the first demonstration of the efficacy of an initial treatment of PZP-22 delivered by dart instead of hand injection. Considerations for PZP-22 treatment include seasonal timing of treatments and age of treated mares. Treatments need to take place early enough to allow antibody titers to build to contraceptive levels before the breeding season.

Additional keywords: contraception, fertility control, management strategies, natural resource management, population control, population management, reproductive biology, wildlife management.

Received 9 July 2018, accepted 17 August 2019, published online 9 December 2019

Introduction

Management of wild horses on public lands in the US, as required under the 1971 *Wild Free Roaming Horse and Burro Act* (P.L. 92–195, as amended) has proven challenging. Wild horse herds grow at a rate of 15–20% annually through natural reproduction (Garrott 2018). Growing wild horse populations increase pressure on forage that under the Act they share with wildlife and, where permitted, livestock. Since 1975, the US Bureau of Land Management (BLM) and US Forest Service (USFS) have attempted to control wild horse populations by gathering and removing horses from the range and placing them with adopters, privately funded sanctuaries, contract ranches or government-managed long-term holding facilities (Norris 2018). This strategy has not proven sustainable, and the policy of removals may be exacerbating population growth by artificially stimulating reproduction (Kirkpatrick and Turner 1991*a*; Garrott and Oli 2013; National Research Council 2013).

Immunocontraceptives are one possible solution to the control of wild horse populations on public lands. An ideal immunocontraceptive for free-roaming horses is specific, safe for the animal and environment, effective and both cost-effective and practical to administer on a large scale (Kirkpatrick *et al.* 2011; Hobbs and Hinds 2018). First documented to induce infertility in wild and domestic mares by Liu *et al.* (1989), the porcine zona pellucida (PZP) vaccine, tested in a variety of formulations, has been shown to meet many of those requirements (Kirkpatrick *et al.* 1990; Kirkpatrick and Turner 1991*b*; Turner and Kirkpatrick 2002).

Efficacy and cost of administration are key to effective limitation of population size and growth by means of a contraceptive agent. The practicality of delivering PZP to free-roaming mares by hand or dart has been proven in a select group of small, well studied herds (Kirkpatrick *et al.* 1990; Kirkpatrick and Turner 1991*b*; Ransom *et al.* 2011). However, wild horse herds on public lands in the western United States are often challenging to access for vaccine administration because of the rough terrain they inhabit and some horses' avoidance of humans (Turner and Rutberg 2013). In its initial form, consisting of an emulsion of PZP antigen and Freund's Complete Adjuvant, the vaccine required a booster 1 month after the initial injection to reliably decrease fertility for 1 year (Kirkpatrick *et al.* 1990; Turner *et al.* 1997). Extending the period of infertility induced by treatment would eliminate the need for labour-intensive annual boosters and decrease the risk of wary free-roaming horses learning to evade capture and treatment (Naugle and Grams 2013). The goal of extending the longevity of PZP effectiveness has followed two routes. First, PZP has been incorporated into liposomes and delivered with various adjuvants under the trade name SpayVac (IVT, Halifax, NS, Canada). Although SpayVac has shown promise in trials on captive horses (Killian *et al.* 2008; Bechert *et al.* 2013; Roelle *et al.* 2017), it has not yet been field tested on free-roaming wild horses. The second approach has been to add controlled-release components to the PZP emulsion to mimic the effects of boosters. Initial field trials of PZP emulsions combined with PZP embedded in lactide–glycolide polymer microspheres produced 1 year of effectiveness with a single shot, but the microspheres were difficult to handle under field conditions (Turner *et al.* 2001, 2002). Subsequently, a vaccine formulation consisting of the PZP-adjuvant emulsion combined with PZP and QA-21 adjuvant embedded in controlled-release lactide–glycolide polymer pellets ('PZP-22'), delivered by jabstick, induced 2–3 years of substantially reduced fertility with a single injection in January at Clan Alpine HMA, Nevada (Turner *et al.* 2002, 2007). This reduction was followed by a return to a control level of fertility in 4 years (Turner *et al.* 2007). However, attempts to replicate this multi-year effect in mares at three western sites did not meet with the same success (Ransom *et al.* 2011; Rutberg *et al.* 2017). Hypotheses for the observed reduction in effectiveness of PZP-22 primers are currently being tested (Rutberg *et al.* 2017).

Rutberg *et al.* (2017) showed that PZP-22 boosters could be delivered effectively by dart, but remote delivery of PZP-22 priming doses has not been studied. The ability to deliver initial treatments of PZP-22 by dart rifle would permit treatment with less equipment, labour and handling of wild horses.

The aim of the present study was to assess the reduction in fertility of treated mares over 2 years following administration of PZP-22 via dart on unrestrained horses in a corral. Treatments were carried out at Jarita Mesa Wild Horse Territory (JM WHT), Carson National Forest, New Mexico. Access to wild horses for management purposes on JM WHT is challenging. Gathering horses by helicopter is ineffective due to heavily forested terrain and large variations in elevation. JM WHT is generally inaccessible by vehicle in the winter months due to snow cover. Additionally, its resident wild horses avoid direct approaches by people. Thus, access to wild horses on JM WHT requires the development of capture methods other than helicopter gather.

Materials and methods

Study area

The Jarita Mesa Wild Horse Territory (JM WHT) in Carson National Forest, New Mexico, is a 22 200-ha range managed by the U.S. Forest Service (2006; USFS Environmental Assessment). Elevations range from 2300 to 3200 m. Pinon pines (*Pinus* spp.), juniper (*Juniperus* spp.) and sagebrush (*Artemesia tridentata*) dominate the vegetation at lower elevations, and Ponderosa pine (*P. ponderosa*) forest dominates at higher elevations. The JM WHT is home to native mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus*). As in most western landscapes, JM WHT has a long history of human use, including grazing by domestic livestock; in 2011, the US Forest Service permitted private livestock owners to graze 502 cow–calf pairs and 16 bulls. Appropriate Management Level (AML) for wild horses on JM WHT is 20–70 horses.

Field observations and individual identification

Wild horses were surveyed by vehicle and on foot from 26 May to 17 December 2011, 14 June to 7 August 2013 and 6 June to 7 August 2014. High horse-traffic areas were identified by the presence of tracks and faeces. Two Bushnell (Overland Park, KS, USA) wildlife cameras with 24-h motion activated sensors were placed at heavily used water holes and high traffic areas to document movement of the wild horse groups. When observed directly, wild horses were photographed with a Rebel EOS T3i camera (Canon, Tokyo, Japan) and telephoto (120–400 mm) lens (Sigma, St Louis, MO, USA) and further observed with a Prostaff (20–60 × 82 mm) spotting scope (Nikon, Tokyo, Japan). Horses were photographed for positive identification based on colour, unique face and leg markings, scars and sex. Band composition was recorded, including associations among mares, immatures and stallions. Foals were associated with the dam using affiliative behaviours such as nursing or, occasionally, repeated proximity while grazing or traveling. A few mares were associated with foals by ruling out all other mares present in the group as the possible dam, because they closely associated with other foals present. Foals were aged at first observation to approximately the nearest 2 weeks based on size, tail length and behaviour. Disturbance of the horses' natural activities by the observer was avoided whenever possible.

The Wild Horse Identification Management System (WHIMS; Wildwise Ecological Solutions, http://wildwisesolutions.com/Whims.aspx) is application-based software used to visually catalogue all the horses identified in a study herd. Each horse is assigned a unique ID number. Information associated with each entry includes sex, age, colour, markings, current and past band associations, dam and photos from each year observed (U.S. Geological Survey 2013). New entries were generated when sufficient photographic evidence was collected during the 2013 and 2014 surveys to prove the animal was not previously identified. New entries were made for all foals of the year.

Capture methods

Groups observed during 2011 field observations were chosen for the PZP-22 treatment regime based on ease of identification, access and regular sightings. As part of a management action that also included capture and removal from the range, eight sites were identified for bait placement based on locations where horses had been observed in the past, road access, horse trails and water sources. Only two sites were set up at a time. Wild horse bands were attracted to gather sites using a proprietary bait formula provided by Mt Taylor Mustangs (Santa Fe, NM), which was hired by USFS under independent contract to treat the JM WHT horses. Timing of bait set-up depended on seasonal horse movements, road condition, snow cover and presence or absence of livestock, which may also be drawn to bait. When a band had acclimated to the bait at a gather site, a 10–13-m diameter catch corral made of portable metal pipe panels was set up at the bait site and the gate left open for several days (typically 3–7), so that horses could come and go freely from the catch corral. Cameras were used to monitor band activity with observers out of the horses' sight. After acclimatisation, when most horses in a group were in the corral, the gate was shut and secured by remote control, with the operator in a nearby blind. All mares trapped in April through mid-June were vaccinated; all mares trapped after mid-June were removed.

Porcine zona pellucida treatment

Females were injected with PZP-22 between 4 April and 16 June 2012 in catch corrals during daylight hours; horses corralled after sunset were held until daylight for darting. All females $\geq$1 year old that were not removed were treated. The PZP-22 consisted of 100 µg PZP (Science and Conservation Center, Billings, MT, USA) dissolved in 0.5 mL phosphate-buffered saline and emulsified in 0.5 mL modified Freund's Complete Adjuvant (mFCA, Calbiochem, LaJolla, CA, USA) plus three heat-extruded lactide–glycolide polymer pellets containing a total of 450 µg PZP and 625 µg QA-21 (Antigenics, Lexington, MA, USA) (Turner *et al.* 2007). The vaccine was loaded into a 1.0-mL Pneu-Dart (Williamsburg, PA, USA) dart designed with a rod within the 13-gauge, 1.5-inch needle to simultaneously inject the emulsion and the pellets. Darts were delivered into the gluteal musculature via Pneu-Dart X-Caliber rifle at a range of 10–15 m. The corral was videotaped during darting to confirm identification of treated horses and successful dart discharge. Once all mares in a captured group had been treated, the gate was opened and the group was released.

Statistical analysis

For purposes of statistical testing, foaling by PZP-treated and untreated mares was determined using only positively identified females 2 years and older (determined by size and, where known, individual histories) with a definitive foaling status. Mares that were positively identified but had an unclear foaling status were excluded from calculations relating to the outcome variable of foal or no foal.

Fisher's Exact tests (FET) were used to compare outcome (foal or no foal) among treatment groups and years. Because previous studies have not shown a difference in fertility between placebo-treated females and untreated females (Turner *et al.* 1997), the fertility of PZP-22-treated females was compared within a given year with that of individually identified females not captured for treatment. The proportion of females foaling in 2011 (before PZP treatment) also served as a comparison to the post-treatment proportions in 2013 and 2014. Alpha was set at $P = 0.05$ for all tests.

Results

Between 4 April and 16 June 2012, 26 females (three yearlings and 23 mares $\geq$2 years of age) were darted with PZP-22 in catch corrals and released back to the range. In addition, 78 wild horses were captured and removed; 60 of these (including 15 adult females, and three 2-year old females) had been previously identified in 2011. Horses did not avoid bait areas after corral panels were put in place. Reactions to injection were moderate and non-violent. We saw no abnormalities at or near the injection site on any of the treated mares at any time during the study, and no mares sustained injuries during capture or darting.

Among known mares observed from June to August 2013 (Year 1), the proportion foaling was 79% lower in PZP-treated mares (2/17; 12%) than in untreated mares (15/27; 56%) (FET, $P = 0.0046$). Among known mares observed from June to August 2014 (Year 2), the proportion foaling was 36% lower in PZP-treated mares (6/17; 35%) than untreated mares (14/26; 54%); this difference was not statistically significant (FET, $P = 0.349$). The pooled proportion of all treated and control mares in the sample foaling did not differ among the 3 years of the study (2×3 FET, $P = 0.736$).

Except for the decline associated with removals during trapping activity in 2012, the sample of individually recognised animals continued to grow throughout the study (Fig. 1). Part of that growth was due to identification of new adult animals not observed on the study site in previous years, and part due to birth and recruitment of new foals. The number of females $\geq$2 years old included in the individually recognised sample was 69 in 2011, 60 (post-removal) in 2012, 78 in 2013 and 86 in 2014. Thus the number of females treated with PZP-22 in 2012 represented only a small proportion of the sample of individually recognised mares on JM WHT (43.3% in 2012, 33.3% in 2013 and 30.2% in 2014).

With respect to the sample of all identified mares, 27 (40.3%) of 66 mares observed had a foal present in 2011 (pretreatment), 16 (37.2%) of 43 mares observed had a foal present in 2013, and

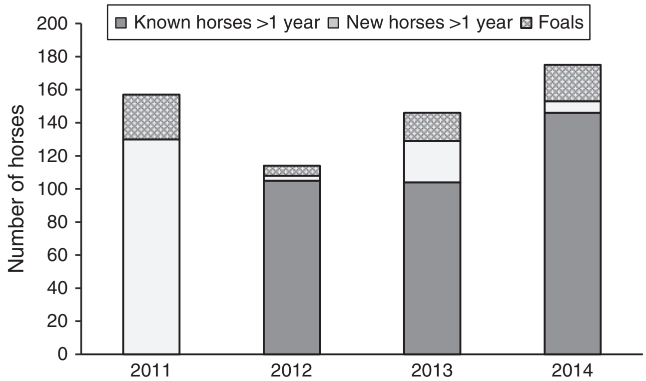

Fig. 1. Total sample of wild horses individually identified on the Jarita Mesa Wild Horse Territory, NM, USA in 2011–14. Numbers of known horses declined in 2011–12 because of removals conducted in 2012, then increased with the addition of newly identified individuals and new foals of the year. Clear, new horses >1 year old; solid grey, horses >1 year old documented in previous years; stippled, foals of the year.

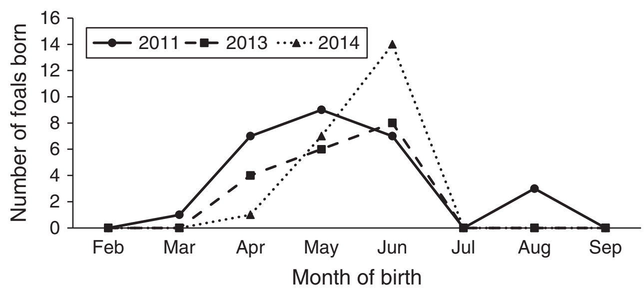

Fig. 2. Dates of foaling for individually identified mares on Jarita Mesa Wild Horse Territory, NM, USA in 2011–14. Dates estimated to within 2 weeks based on foal size, tail length and behaviour at first observation.

20 (46.5%) of 43 mares observed had a foal present in 2014. Untreated mares 4 years old or more had significantly higher foaling rates than mares less than 4 years of age in both 2013 (15/26, 58% for mares ≥4 years of age *v.* 0/5 for females <4 years of age, FET, $P = 0.0098$) and 2014 (13/20; 65% for mares ≥4 years of age *v.* 1/6, 17%, for mares <4 years of age, FET, $P = 0.0192$).

Of 79 foaling dates estimated from 2011 to 2014, 94.5% occurred in April–June (Fig. 2). Estimates of birth dates for the eight known births to treated mares in 2013 and 2014 fell within that window.

Discussion

Single injections of PZP-22 by dart delivery to mares accessed using bait trapping in late spring 2012 reduced foaling rates in the treated mares in the JM WHT for at least 1 year. Although fertility rates for treated mares were lower than those of untreated mares in 2014 (the second year after treatment), this reduction in fertility was not statistically significant. The efficacy data reported here is more similar to those shown in recent work (Ransom *et al.* 2011; Rutberg *et al.* 2017) than those shown in initial tests in the Clan Alpine, Nevada, study (Turner *et al.* 2007). This diminished initial efficacy of PZP-22 could be due to a variety of factors, including variation in the composition and manufacturing of vaccine or the other components of PZP-22, including the adjuvant and polymer used to make the controlled-release pellets (Ransom *et al.* 2011; Rutberg *et al.* 2017).

Timing of delivery also may play some role. Previous field testing of hand-injected PZP-22 at Cedar Mountains Herd Management Area (HMA), UT, USA, and Sand Wash Basin HMA, CO, USA, suggests a relationship between timing of delivery and effectiveness. Hand-injected PZP-22 primers in these studies showed highest effectiveness when delivered in February, moderate effectiveness when delivered in December and lowest effectiveness when delivered in October–November (Rutberg *et al.* 2017). Depending on the timing of the breeding season at a given site, however, there is likely to be a drop-off for the efficacy of late-spring delivery. At JM WHT, where most foaling occurs between April and June, one would expect diminishing first-year efficacy in late May through June, because more treated mares will already be pregnant (given a roughly 11-month gestation period for horses and a 2–3-week time lag in elevation of antibody titres to contraceptive levels; Liu *et al.* 1989, 2005). Anecdotally, the two PZP-22-treated mares observed to have produced offspring in 2013 were treated on 1 and 2 June 2012, which were the latest treatment dates for females observed in 2013 (one gave birth in April and one in June; unfortunately, three mares treated as late as or later than those two in 2012 were not located in 2013). It is also not known whether delivering a PZP-emulsion primer (ZonaStat-H) alone this late in the season would produce a comparable reduction in fertility, i.e. whether the pellet components of PZP-22 enhance the effectiveness of the priming vaccine.

Because this was a pilot study of the efficacy of PZP-22 remote delivery, we did not examine nor expect population-level effects. Fewer than 50% of individually recognised sample mares were treated in 2012, and in a challenging site like JM WHT we are confident that the sample significantly undercounted the population. Either way, treating a larger proportion of the fertile mares in a herd will be necessary to create a substantial and biologically important reduction in overall population fertility.

Another factor affecting the proportion of females foaling in the total sample might have been the preferential removal of younger horses in 2011 and 2012. Mares ≥4 years old were observed to be more fertile; therefore, preferential removal of younger mares could also have driven the sample foaling rates higher post-treatment and post-removal. Similar effects of age on foaling rate have been documented in other wild horse herds on public lands (Ransom *et al.* 2011; Rutberg *et al.* 2017) and, depending on vaccine costs and ease of access to the horses, female age might be a consideration in deciding which mares to treat.

Conclusions and implications

It was shown here for the first time that initial treatments of PZP-22 are effective for at least 1 year when delivered by dart, as long as vaccination occurs early enough for antibodies to reach contraceptive levels before the onset of the local breeding season. We also showed that corral trapping with bait can be used to access very wary wild horses in remote and challenging terrain, allowing administration of contraception or removal of surplus wild horses, or both.

It has already been shown that PZP boosters (either PZP-22 or simple PZP emulsion formulations) administered by dart to mares primed with PZP-22 provide at least three additional years of contraception (Rutberg *et al.* 2017). The present study implies that two dart-delivered treatments of PZP – an initial PZP-22 primer followed by a PZP booster delivered 2–3 years later – should produce 4–5 years of contraception. This protocol significantly reduces the level of effort relative to the annual darting required by simple PZP emulsions and should extend the range of management applications of dart-delivered PZP. Additional field-testing of PZP-based SpayVac and the gonadotropin-releasing-hormone-based vaccine GonaCon (National Wildlife Research Center, Fort Collins, CO, USA) could also provide foundations for long-term wild horse contraception and management applications (Baker *et al.* 2018; Bechert and Fraker 2018). Ultimately, the use of fertility control for populations to treat and prevent overabundance could decrease the financial burden of humanely protecting wild horses and ease tension among diverse stakeholders seeking to use resources on multiple-use public lands (Bartholow 2007; NRC 2013; Garrott 2018).

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

Special thanks are extended to: Diana Trujillo, Estevan Gallegos, Ezekiel Rael and Kristen Linner, U.S. Forest Service; Jody Elkins, Mt Taylor Mustangs; and Karen Herman, Sky Mountain Wild Horse Sanctuary. K. A. Carey and A. Ortiz were funded by the National Institutes of Health through the Summer Research program of the Cummings School of Veterinary Medicine at Tufts University. K. Grams was funded in part through a gift from the Annenberg Foundation to The Humane Society of the United States. The authors gratefully acknowledge the efforts of the late Dan Elkins, founder of Mt Taylor Mustangs, whose commitment and no-chase gather methods are foundational to this research and without whom this project, and hopefully more to come, would not have been possible.

References

Baker, D. L., Powers, J. G., Ransom, J. I., McCann, B. E., Oehler, M. W., Bruemmer, J. E., Galloway, N. L., Eckery, D. C., and Nett, T. M. (2018). Reimmunization increases contraceptive effectiveness of gonadotropin-releasing hormone vaccine (GonaCon–Equine) in free-ranging horses (*Equus caballus*): limitations and side effects. *PLoS One* **13**, e0201570. doi:10.1371/journal.pone.0201570

Bartholow, J. (2007). Economic benefit of fertility control in wild horse populations. *The Journal of Wildlife Management* **71**, 2811–2819. doi:10.2193/2007-064

Bechert, U. A., and Fraker, M. A. (2018). Twenty years of SpayVac® research: potential implications for regulating feral horse and burro populations in the United States. *Human–Wildlife Interactions* **12**, 117–130.

Bechert, U., Bartell, J., Kutzler, M., Menino, A., Jr, Bildfell, R., Anderson, M., and Fraker, M. (2013). Effects of two porcine zona pellucida immunocontraceptive vaccines on ovarian activity in horses. *The Journal of Wildlife Management* **77**, 1386–1400. doi:10.1002/jwmg.600

Garrott, R. A. (2018). Wild horse demography: implications for sustainable management within economic constraints. *Human–Wildlife Interactions* **12**, 46–57.

Garrott, R. A., and Oli, M. K. (2013). A critical crossroad for BLM's wild horse program. *Science* **341**, 847–848. doi:10.1126/science.1240280

Hobbs, R. J., and Hinds, L. A. (2018). Could current fertility control methods be effective for landscape-scale management of populations of wild horses (*Equus caballus*) in Australia? *Wildlife Research* **45**, 195–207. doi:10.1071/WR17136

Killian, G., Thain, D., Diehl, N. K., Rhyan, J., and Miller, L. (2008). Four-year contraception rates of mares treated with single-injection porcine zona pellucida and GnRH vaccines and intrauterine devices. *Wildlife Research* **35**, 531–539. doi:10.1071/WR07134

Kirkpatrick, J. F., and Turner, J. W., Jr (1991*a*). Compensatory reproduction in feral horses. *The Journal of Wildlife Management* **55**, 649–652. doi:10.2307/3809514

Kirkpatrick, J. F., and Turner, J. W., Jr (1991*b*). Reversible contraception in nondomestic animals. *Journal of Zoo and Wildlife Medicine* **22**, 392–408.

Kirkpatrick, J. F., Liu, I. K. M., and Turner, J. W., Jr (1990). Remotely-delivered immunocontraception in feral horses. *Wildlife Society Bulletin* **18**, 326–330.

Kirkpatrick, J. F., Lyda, R. O., and Frank, K. M. (2011). Contraceptive vaccines for wildlife: a review. *American Journal of Reproductive Immunology* **66**, 40–50. doi:10.1111/j.1600-0897.2011.01003.x

Liu, I. K. M., Bernoco, M., and Feldman, M. (1989). Contraception in mares heteroimmunized with porcine zonae pellucidae. *Journal of Reproduction and Fertility* **85**, 19–29. doi:10.1530/jrf.0.0850019

Liu, I. K. M., Turner, J. W., Jr, Van Leeuwen, E. M. G., Flanagan, D. R., Hedrick, J. L., Murata, K., Lane, V. M., and Morales-Levy, M. P. (2005). Persistence of anti-zonae pellucidae antibodies following a single inoculation of porcine zonae pellucidae in the domestic equine. *Reproduction* **129**, 181–190. doi:10.1530/rep.1.00168

National Research Council (2013). 'Using Science to Improve the BLM Wild Horse and Burro Program: A Way Forward.' (The National Academies Press: Washington, DC.)

Naugle, R., and Grams, K. (2013). Long-term methods and effects of remotely treating wildlife with immunocontraception. *Journal of Zoo and Wildlife Medicine* **44**, S138–S140. doi:10.1638/1042-7260-44.4S.S138

Norris, K. A. (2018). A review of contemporary U.S. wild horse and burro management policies relative to desired management outcomes. *Human–Wildlife Interactions* **12**, 18–30.

Ransom, J. I., Roelle, J. E., Cade, B. S., Coates-Markle, L., and Kane, A. J. (2011). Foaling rates in feral horses treated with the immunocontraceptive porcine zona pellucida. *Wildlife Society Bulletin* **35**, 343–352. doi:10.1002/wsb.66

Roelle, J. E., Germane, S. S., Kane, A. J., and Cade, B. S. (2017). Efficacy of SpayVac® as a contraceptive in feral horses. *Wildlife Society Bulletin* **41**, 107–115. doi:10.1002/wsb.729

Rutberg, A. T., Grams, K., Turner, J. W., Jr, and Hopkins, H. (2017). Contraceptive efficacy of priming and boosting doses of controlled-release PZP in wild horses. *Wildlife Research* **44**, 174–181. doi:10.1071/WR16123

Turner, A., and Kirkpatrick, J. F. (2002). Effects of immunocontraception on population, longevity and body condition in wild mares (*Equus caballus*). *Reproduction Supplement* **60**, 187–195.

Turner, J. W., Jr, and Rutberg, A. T. (2013). From the pens to the field: real-world wildlife contraception. *Journal of Zoo and Wildlife Medicine* **44**, S102–S110. doi:10.1638/1042-7260-44.4S.S102

Turner, J. W., Jr, Liu, I. K. M., Rutberg, A. T., and Kirkpatrick, J. F. (1997). Immunocontraception limits foal production in free-roaming feral horses in Nevada. *The Journal of Wildlife Management* **61**, 873–880. doi:10.2307/3802196

Turner, J. W., Jr, Liu, I. K. M., Flanagan, D. R., Rutberg, A. T., and Kirkpatrick, J. F. (2001). Immunocontraception in feral horses: one inoculation provides one year of infertility. *The Journal of Wildlife Management* **65**, 235–241. doi:10.2307/3802902

Turner, J. W., Jr, Liu, I. K. M., Flanagan, D. R., Bynum, K. S., and Rutberg, A. T. (2002). Porcine zona pellucida (PZP) immunocontraception of wild horses (*Equus caballus*) in Nevada: a 10 year study. *Reproduction Supplement* **60**, 177–186.

Turner, J. W., Jr, Liu, I. K. M., Flanagan, D. R., Rutberg, A. T., and Kirkpatrick, J. F. (2007). Immunocontraception in wild horses: one inoculation provides two years of infertility. *The Journal of Wildlife Management* **71**, 662–667. doi:10.2193/2005-779

U.S. Forest Service (2006). Environmental Assessment for wild free-roaming horse management on the El Rito Ranger District. USDA Carson National Forest, Albuquerque, NM.

U.S. Geological Survey (2013). About WHIMS. USGS, Fort Collins Science Center, Fort Collins, CO. Available at http://www.fort.usgs.gov/resources/spotlight/horse/whims.asp [verified 16 September 2013].