

## Absence of Effects From Immunocontraception on Seasonal Birth Patterns and Foal Survival Among Barrier Island Wild Horses

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Despite a large body of safety data, concern exists that porcine zonae pellucidae (PZP) immunocontraception—used to manage wild horse populations—may cause out-of-season births with resulting foal mortality. Our study at Assateague, Maryland indicated the effects of immunocontraception on season of birth and foal survival between 1990 and 2002 on wild horses from Assateague Island. Among 91 mares never treated, 69 (75.8%) of foals were born in April, May, and June (in season). Among 77 treated mares, 50 (64.9%) were born in season. Of 29 mares foaling within 1 year after treatment (contraceptive failures), 20 (68.9%) were born in season. Of 48 mares treated for greater than 2 years then withdrawn from treatment, 30 (62.5%) of 48 foals were born in season. There were no significant differences ( $p < .05$ ) between either treatment group or untreated mares. Survival did not differ significantly among foals born in or out of season or among foals born to treated or untreated mares. Data indicate a lack of effect of PZP contraception on season of birth or foal survival on barrier island habitats.

The use of porcine zonae pellucidae (PZP) contraceptive vaccine has been widely applied to wild horses in numerous research studies (Kirkpatrick, Turner, Liu, & Fayrer-Hosken, 1996) and also has been applied to several wild horse herds as a management tool (Turner & Kirkpatrick, 2002). Issues of efficacy, reversibility, long-term health (Kirkpatrick & Turner, 2002; Turner & Kirkpatrick, 2002), and behavior (Powell, 1999) have been addressed through past research efforts. Two remaining issues that have not been addressed are the effect of PZP on out-of-season births among treated mares and the effects of PZP on the survival of foals born to treated mares.

The concern with the possibilities of out-of-season births arises from the possibility that anti-PZP antibody titers may, over time, fall below contraceptive levels at times of the year when a pregnancy and the resulting birth would be injurious to the foal. Although no body of data suggests that foals born to treated mares are less healthy or survive at lower rates than foals born to untreated mares, this is a safety issue not addressed since the original study of PZP contraception of wild horses in 1988 (Kirkpatrick, Liu, & Turner, 1990). This retrospective study was conducted to determine if (a) PZP treatment led to significant out-of-season births, (b) PZP treatment led to a significant decrease in foal survival, and (c) foal births out of season led to decreased survival.

#### METHOD

The identities of all 172 wild horses inhabiting Maryland's Assateague Island National Seashore (ASIS) are known by individual markings and band affiliations. Birth dates, by month, exist for 168 foals born on ASIS from 1986 through 2002. Birth dates are acquired by visual sighting of foals during monthly surveys by National Park Service personnel. Foal survival is defined as surviving to age 1 or older.

To insure that very early neonatal losses do not go undetected, mares were tested for pregnancy each year by means of urinary and/or fecal steroid metabolite analysis (Kirkpatrick, Kasman, Lasley, & Turner, 1988; Kirkpatrick, Lasley, et al., 1990; Kirkpatrick, Shideler, Lasley, & Turner, 1991). Specifically, the excreta is analyzed for both estrone conjugates (E1C) or immunoreactive pregnanediol-glucuronide-like progesterone metabolites (iPdG). Both metabolites are significantly higher in pregnant animals. However, although progesterone levels (and therefore iPdG) will remain high for months after a fetal death, E1C concentrations fall within 12 hr of fetal loss (Hyland & Langsford, 1990). Thus, the pregnancy detection methods are virtually 100% accurate. In this way, very early neonatal losses do not go undetected.

The normal season of foaling is defined as April, May, and June (called *in season* hereafter), based on previous studies with wild horses (Hall, 1972; Keiper &

Haupt, 1984; Palmer, 1978; Satoh & Hoshi, 1932). Comparisons were made in the number and percentage of foals born in season and those born outside this seasonal window between mares (a) never treated with PZP, (b) treated with PZP for 1 year before the birth (i.e., contraceptive failures), and (c) those treated with PZP for at least 2 years before the birth of a foal. The group treated with PZP for 1 year before the birth (contraceptive failures) included 9 mares who became pregnant after only a single initial inoculation was given to 41 mares in 1994 as opposed to the standard two-inoculation protocol (Kirkpatrick, Liu, & Turner, 1990). Foal survival also was calculated as a function of whether the foal was born in season or outside this window and whether or when the mother had been treated with PZP prior to the birth. Differences in season of birth, foal survival as a function of birth date, and foal survival as a function of PZP treatment were tested for significance by Fisher's exact test for contingency tables.

## RESULTS

Among foals born to mares who never had been treated, 69 of 91 (75.8%) were born in season; among 77 treated mares, 50 of 77 (64.9%) were born in season. The difference was not significant ( $p = .122$ ; standard error (SE) of difference = .085). Among foals born to mares treated with PZP for 1 year before the birth of a foal, 20 of 29 (68.9%) were born in season. The difference was not significant from foals born to untreated mares ( $p = .463$ ; SE of difference = .090). Among foals born to mares who had been withdrawn from treatment or who had experienced a contraceptive failure after at least 2 years of treatment, 30 of 48 (62.5%) were born in season. The difference was not significant from foals born to untreated mares ( $p = .111$ ; SE of the difference = .090). Table 1 shows foal birth by month.

TABLE 1  
Births by Months for PZP-Treated and Untreated Wild Mares

Mare Status	Month of Birth											
	J	F	M	A	May	Je	Jy	Au	S	O	N	D
UT <sup>a</sup>	2	0	3	23	23	23	10	4	2	0	0	1
T1 <sup>b</sup>	1	1	1	12	1	7	1	3	2	0	0	0
T > 2 <sup>c</sup>	1	4	1	12	14	4	4	3	1	1	1	2
Total	4	5	5	47	38	34	15	10	5	1	1	3

Note. J = January; F = February; M = March; A = April; Je = June; Jy = July; Au = August; S = September; O = October; N = November; D = December; UT = untreated; T1 = mares treated the year before the birth of a foal; T > 2 = mares withdrawn from treatment more than 2 years before the birth of a foal.

<sup>a</sup>69 of 91 (75.8%) of foals born in season to UT mares. <sup>b</sup>20 of 29 (68.9%) of foals born in season to T1 mares ( $p = .463$ ). <sup>c</sup>30 of 48 (62.5%) of foals born in season to T > 2 mares ( $p = .111$ ).

Foal survival did not differ significantly ( $p = .471$ ;  $SE$  of the difference = .100) between foals born in season and foals born outside this seasonal window. Among foals born in season, 104 of 119 (92.9%) survived to age 1 or older; of those born outside this window, 42 of 49 (85.7%) survived. Among foals born to mares never treated with PZP, 77 of 91 (84.6%) survived and did not differ significantly ( $p = .825$ ;  $SE$  of difference = .109) from foals born to mares treated at some time before their birth, of whom 67 of 77 (87.0%) survived.

## DISCUSSION

These data indicate there was no significant effect of PZP treatment on out-of-season births, with only 13.3% separating the two treatment groups and untreated mares. This is in contrast to white-tailed deer in which significant extensions of the breeding season and, by implication, ovulation occurred after treatment of does with PZP (McShea et al., 1997), and almost all treated animals extended their breeding season by as much as 2 months. The failure of a larger effect in horses probably is a function of the reproductive biology of this species.

### Photoperiod Influence on Ovulation

Ovulation in the modern horse is seasonal and influenced largely by photoperiod, with peak reproductive activity and foaling correlating with increased photoperiod (Kenney, Ganjam, & Bergmen, 1975). In the domestic mare, ovulation reflects this seasonal pattern, but significant percentages of mares will ovulate well into the fall and winter (Kenney et al., 1975). Photoperiods of 16 to 24 hr induced the onset of breeding in domestic mares, and photoperiods of less than 9 hr caused a cessation in ovulation and breeding (Kooistra & Ginther, 1975).

### Patterns of Foaling

Among other horses, the patterns of foaling differ. On Assateague Island, Keiper and Houpt (1984) reported that 88% of foals were born in season. Feist and McCullough (1975) reported that no foals were born in the Pryor Mountains, MT outside season, and Tyler (1972) reported that 96% of births among New Forest ponies occurred in this same period. On Sable Island, Nova Scotia, Canada, foals were born year round, but 77% were born in season (Welsh, 1975). Similar patterns have been reported for wild horses of Wyoming's Red Desert (Boyd, 1979) and the Great Basin (Berger, 1986).

### Foaling and Ovulatory Activity

Foaling patterns however, do not necessarily reflect ovulatory activity. Among domestic mares, the stallion does not always have access to the mare, and the timing of reproduction most often will reflect management of the stallion rather than environmental, nutritional, or genetic influences.

Palmer (1978) found that Welsh ponies had no ovarian activity during the winter months, whereas 66% of saddle breeds did. Satoh and Hoshi (1932) found that semiwild Korean ponies ceased ovulating by October. Kirkpatrick and Turner (1982) demonstrated that ovulation, determined endocrinologically, among Pryor Mountain wild mares was confined to April to July—even when the plane of nutrition was increased—whereas estrous behavior was observed occasionally well into October. Ginther (1979) speculated that wild horses, as they adapt to a free-ranging life and most often a harsh environment, develop a breeding pattern that begins and ends more abruptly and during a period when survival of the foal would be most favorable. This, in turn, implies a genetic basis for the control of ovulation.

Liu, Bernoco, and Feldman (1989) demonstrated that antibody titers to PZP treatment fall over time and that fertility will be restored when they reach minimal levels (below 64% of positive reference standards). If ovulatory activity is confined more or less to a highly seasonal pattern, successful reproduction out of season would not be significant. In any case, the more restricted breeding/ovulatory season in wild horses is the most plausible explanation for the lack of effect seen in PZP-treated wild horses.

### Two Phenomena

This study draws attention to two additional phenomena that must be taken into consideration when examining the effects of PZP contraception in wild horses. First, there is a considerable spread in the percentage of foals born in season among the eight different populations referenced previously. This in turn requires, to assess any possible effects, that careful examination of foaling patterns be conducted prior to PZP treatment. If 23% of foals normally are born out of season among Sable Island horses, and only 4% are born out of season among New Forest ponies, these figures must be incorporated into any assessment of PZP treatment effects on a site-specific basis. Put another way, individual herds may be predisposed more or less to out-of-season births among PZP-treated animals, depending on their normal foaling patterns.

The second interesting aspect of this study is that out-of-season births in untreated mares have been increasing on ASIS since 1984, from 12% to 26% in 2001. The causes for this phenomenon are unknown but merely may reflect increased

variation coincident with increased population size. There were 80 horses on ASIS in 1984. Currently, there are 173.

### A Surprising Discovery

The discovery that month of birth has no effect on survival on Assateague Island is surprising considering the harsh winter ocean-side weather patterns and storms. This suggests that horses living in milder climates such as the Shackleford Banks of Cape Lookout, NC, where horses also are being managed with PZP, will not suffer from out-of-season births whether they are associated with PZP treatment. In harsher climates, however, such as mountainous country where significant snow accumulates, the survival data derived here may not be valid or predictive.

The lack of any effect on survival as a function of PZP treatment in this retrospective study was expected. In the original study of PZP contraception in wild horses (Kirkpatrick, Liu, et al., 1990), 60% of treated animals were pregnant at the time of PZP treatment; survival of the foals was no different from that of foals born to untreated mares. This is consistent with the mechanism of the PZP vaccine: fertilization block. There is no known physiological link between an immunological block to fertilization and postfertilization gestational physiology.

### SUMMARY

The use of PZP immunocontraception among selected wild horse populations can be carried out without fear of causing out-of-season births. Any population control measures applied to wild horses on public lands will come under severe scrutiny. The possibility of causing the death of foals, even indirectly, will be unacceptable to the public. The data in this retrospective study indicate that PZP immunocontraception is not implicated in out-of-season births and that this characteristic is driven by the unique reproductive physiology of equids. The difference in the percentage of out-of-season births between different wild horse ranges, and even the changing figures for the same herds over time point to the need to understand the reproductive biology of each individual herd before population models are constructed or contraceptive management plans are developed.

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