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COMPARISON OF EFFECTS OF FOUR IMMUNOCONTRACEPTIVE TREATMENTS ON ESTROUS CYCLE AND RUTTING BEHAVIOR IN CAPTIVE WHITE-TAILED DEER

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Abstract: A behavioral observation protocol was used to detect estrus in white-tailed deer (Odecoileus virginianus) in order to evaluate potential extended estrous cycling effects associated with certain immunocontraceptive vaccines. The study was conducted from 8 November 1994 through 24 February 1995 at the Pennsylvania State University White-Tailed Deer Research Center, where a herd of 100-150 deer is maintained in a 22 acre fence-enclosed area. Estrous-associated behaviors of 24 does were detected by an eight-category rating scale as the animals co-mingled with 4 white-tailed buck deer. Does that were treated with four of the vaccine treatments (PIS, PIK, GnRH, EAP) each showed a mean number of estrus cycles between 1.00 and 1.33 and Control animals (sham-injected with vehicle only) cycled a mean of 1.25 times. In contrast, the PZP-treated does showed a mean number of estrous cycles of 2.89. This effect confirmed previously reported extended estrous cycling for the PZP treatment groups. Only one fawn was born to the nine PZP-treated does. GnRH treatment indicated a possible effect of reducing the birth rate by 50 percent. In a side experiment, four bucks treated with GnRH showed very low levels of interest in pursing estrous does as compared with six non-treated (control) bucks during the rut season. The GnRH treated bucks also lost their antlers much earlier in the season, by 28 October 1994, compared to control animals that lost their antlers between 20 January and 17 February 1995.

Immunocontraceptive vaccines have been receiving increased research and development interest as applied to wildlife damage management problems in recent years (Turner, Liu and Kirkpatric 1992; McShea, Wemmer and Stuwe 1993). One of the primary applications involves controlling the overabundance of white-tailed deer herds, particularly in the Northeastern U.S., in areas where hunting and other management methods have failed to stem the overpopulation levels. Hazards to auto traffic on highways and airplane ground traffic on runways, damage to fruit trees, vegetable gardens and ornamental plantings are some of the problems posed by overabundance. Often, individual land owners may be opposed to hunting as a management method, but they seek other non-reductional control methods such as repellents or habitat alteration which frequently fail to sufficiently reduce damage or safety hazards.

A previous report (Shumake, Wilhelm, Hummel, Miller and Killian - in preparation) indicated that one vaccine, Porcine Zona Pellucida (PZP), is highly effective in preventing

pregnancy in white-tailed deer (O. virginianus). Unfortunately, the PZP vaccine also leads to extended estrous cycling in doe deer with some animals showing 3 to 4 extra cycles over 2 to 3 months beyond the normal rut period.

The objective of the current study was to further evaluate and confirm the PZP vaccine contraceptive efficacy and extended estrus cycling side effects and to compare them with five other vaccine preparations: a Peptide Injected with T-cell Epitope (PIS), a Peptide Conjugated to Keyhole Limpet Hemocyanin (PIK), an Estrus Associated Protein (EAP), a Gonadotropic Releasing Hormone (GnRH), and a vehicle control (physiological saline and an adjuvant). A previously developed deer rut behavioral protocol was used for detection of estrus in the deer under each of the six treatment conditions. In addition and as a secondary objective, four buck white-tailed deer were observed during the rut season after previous injection with the GnRH vaccine. Their behaviors toward each other and the does in the herd was compared with the rut behaviors displayed by six non-injected bucks. This vaccine normally leads to gonadal regression, suppression of sperm production, and loss of sex hormone activity.

STUDY AREA

All observations were made over an 109 day period (8 November 1994 through 24 February 1995) at the Pennsylvania State University Deer Research Facility, University Park, PA. This consists of a 22 acre area enclosed with high chain linked fencing for the captive management and propagation of white-tailed deer. The Facility is divided into 9 paddocks or sub-enclosures (designated A through F), each separated by 8 ft (2.44 m) diamond mesh fence, and topped with 3 strands of barb wire. A centrally-located runway building is used for handling, weighing, blood sampling, treating and examining individual deer.

Vaccine treatment does (n=24) and control males (n=4) were housed together in paddock F. The GnRH treated bucks (n=4) were housed together with does (n=16) in paddock C. Except for blood sampling on 20 February 1995, animals remained in the above areas for the 109 days of observations. Both paddocks contained no underbrush affording unobstructed viewing of the deer herds.

METHODS

Observation Procedures

The observed does were part of a continuing study to evaluate the efficacy of immunocontraceptive vaccines directed at the zona pellucida of the egg, as well as alternate immunocontraceptive vaccines. During the Fall of 1994 and Winter of 1995, the 24 does were assigned to six treatments. Four control does were given sham injections with the vehicle (physiological saline and an adjuvant) per se. Four does that had received PZP injections the previous year were administered "booster" immunizations with PZP, and were designated the PZPB Group. Five does were given their initial PZP injections prior to the observation interval in the Summer of 1994, and these animals were designated as the PZP Group. Another set of four does was administered injections of GnRH. Three does were given EAP injections and two does each received PIK or PIS injections prior to the observation period.

On each observation period day, the herd of 24 does was penned in paddock F with four bucks. They were observed for two, one-hour periods each day each starting between 0730 to 0900 EST and between 1600 to 1700 EST. Early in the breeding season in November, a third one-hour observation period was conducted between 1130 and 1230 EST, in order to optimize detection of does in estrus. All regularly scheduled observations were conducted by trained observers: Eric Wilhelm, Heather Trakima, and Bill Taylor. Supplementary observations were made by the Facility employees and herdsman, Robert Mothersbaugh, at various times during each observation day.

Does were identified by their tag color and number. For does with similar colors or numbers, a small patch of hair was shaved on a unique part of the body. A complete list of tag numbers, colors, and shave marks was made available to all observers. The identification cues allowed identification of all does from a minimum of 20 yards with some animals identifiable from any location within the paddock.

Determining Estrus

Observers entered the paddock, located the four bucks, and verified their individual ear tags using binoculars. For the remainder of the time period, the behavior of the bucks and response of individual does were observed from the maximum distance that allowed animal identification. For each behavioral encounter in which the buck(s) showed some interest in a doe, rut behaviors were classified and recorded in one of eight categories as described further in Table 1. All data were recorded manually and documented on videotape. The recorded data sets were later analyzed to extrapolate the time period in which each doe came into estrus. Repeated estrous cycles were a strong indication that animals were not pregnant even though matings may have been repeatedly observed. Typical behavior of the bucks toward a doe in estrus was as follows: (1) subdominant males would actively pursue or accompany the doe as she moved around the paddock; (2) as the doe approached standing estrus, the alpha male would pursue, guard, and attempt to mount the doe; and (3) finally, the subdominant males would be allowed to approach the doe only after the alpha male had copulated with her and was then pursuing a different doe.

The four male white-tailed deer treated with GnRH were similarly observed each day. Records were taken whenever some pursuit or mounting attempts were observed with the herd of 16 does. Dates for loss of each antler were recorded for comparison with control bucks that were not injected.

Data analyses were descriptive and involved tabulation of the number of estrus cycles observed in each doe treatment group. Final fawn birth data were tabulated on 22 August 1995 for a comparison of contraceptive effects among the six treatment groups. Fawn birth data for the 16 does housed with the four GnRH-treated bucks were also tabulated on the above date.

RESULTS

All 24 does were observed to have estrous signs during the observation period. Animals

in the Control, GnRH, and EAP Groups each showed one estrous cycle except for one doe in each respective group that cycled twice. The two animals in each of the PIS and PIK Groups, respectively, both showed one estrous cycle as detected by human observers. PZP animals showed from one to four estrous cycles ($\bar{x} = 2.60$) and PZPB animals showed between two and five cycles ($\bar{x} = 3.25$). Both PZP Groups combined showed a mean estrous cycling count of 2.89. These data are shown in Table 2. Buck number 209, the alpha male, serviced 90 percent (38/42 x 100) of does observed in estrus. The remaining 10 percent (4/42 x 100) of the matings were by subordinate males and they occurred at those times when the alpha male was occupied with interest in pursuing a different doe.

As also indicated in Table 2, both the PZP and PZPB Groups showed an extended period of estrous cycling lasting a combined mean interval of 69.44 ∓ 32.04 days after initiation of observations on 8 November. Compared with Control and all other Immunocontraceptive treatments (GnRH, EAP, PIS, PIK) the two PZP Groups showed a greater number of observed estrous cycles with a combined group mean of 2.89 ∓ 1.19. Only 1 birth occurred amongst the 9 PZP-treated animals. There was a possible partial contraceptive effect as indicated by the 50 percent birth rate among the four GnRH-treated does. None of the other immunocontraceptive treatments effectively reduced pregnancy or birth rates. The PIK treatment resulted in two single births for each of the two treated does; however, although twinning is a very frequent occurrence in white-tailed does, these animal numbers are much too small to infer a potential reduced fertility rate for this vaccine treatment.

With only small sample numbers available for each immunocontraceptive treatment group, a non-parametric statistical analysis was applied to the number of does showing extended cycling beyond the date of 28 December 1994. Fisher's Exact Probability Statistic indicated that the combined PZP Groups ($n = 9$) compared to Control does that survived the observation period ($n = 3$) did not yield a significant increase in animals showing estrus later than the above date ($p = .269$). However, when all animals in non-PZP Groups that survived the observation period ($n = 14$) were compared with the PZP Groups combined ($n = 9$), a significant increase in the late cycling was noted for these latter Groups ($p = .010$).

In the side experiment, bucks treated with GnRH displayed only passive interest towards

does in estrus, and on four occasions attempted to mount individual does. It was originally hypothesized that no GnRH male could have successfully mated with any doe based on observational data. However, three of the 16 does had fawns by 22 August 1995, indicating a possible incomplete contraceptive effect on the order of 80 percent efficacy. Does housed in the paddock with the four GnRH-treated bucks appeared to be cycling normally as detected by behavioral responsiveness of males contained in adjacent paddocks. Three animals died during the course of the study: a doe in the control group (518 on Dec. 2) and one buck each in the GnRH (561) and control groups (558).

DISCUSSION

Only 24 of 39 does (sixty-two percent) had estrus cycles detected by human observers in a previous study (Shumake et. al., in preparation). In contrast, in the current study, 23 of the 24 does were detected in estrus at some points during the 109-day observation period. The added one-hour observation period in November 1994, along with the relatively mild Fall and Winter weather conditions, probably contributed significantly to boosting the detection rate to this high degree. The additional experience of the observers on this second year of evaluating estrus in does may have also increased their sensitivity to estrous signs. It is still possible and probably quite likely, however, that some cycles also remained undetected in the current study.

Estrus dates for each doe as detected by the behavioral observation protocol, fell mainly in the month of November. PZP treated does, however, continued to cycle on the average of every 24 days, for up to 5 estrus cycles. This same effect had been reported previously (Shumake et al., in preparation) with one animal cycling as late as 22 February 1994. Our data on extended estrous cycling with PZP vaccine are also in agreement with other authors affiliated with other research facilities (Turner et al. 1992; Mc Shea et al. 1993). This may be an inevitable result of effective contraception with certain ZP vaccines that prevent sperm penetration into ova in white-tailed deer. GnRH treatment produced partial contraceptive effects in the does, but in bucks, the vaccine also can produce a side-effect of early antler loss which could be viewed as undesireable from the hunting perspective.

Substitute

Table 1. Eight categories of rutting behavior in white-tailed deer used to detect estrus in does.

Behavioral Category	Brief Description
No Interest	No male displayed any interest in activities of a given doe. The doe was assumed to be in anestrus.
Following/ Standing/Lying within 8 yards	The buck and doe were observed together, either walking or bedding down, for a period of at least 15 minutes. ⊛ add
Short Pursuit ✓	The buck follows the doe wherever she moves in the paddock, for a period of less than 2 minutes. While exhibiting this activity, the couple is frequently observed standing within 8 yards of one another.
Extended Pursuit ✓	This is the same description as listed above for short pursuit, but the activity is continued over at least a 5 minute interval.
Aggressive Guarding/Pursuit ✓	Highly aggressive behavior is shown by the buck toward any other male that approaches a doe in estrus. If the doe attempts to escape from this buck, the alpha male, he continually pursues her.
Female Stands for Male ✓	This activity involves the buck licking the urogenital area of a receptive, estrous doe, with a mounting attempt. This is often accompanied with the buck resting his chin on the back of the doe. At this time, the alpha male will aggressively defend the doe from pursuits of other bucks.
Mounting and Copulation ✓	The doe allows mounting and copulation. Often, the doe will step out from under the buck on his first few mounting attempts. Actual mating lasts only 8-15 seconds and is therefore rarely observed.
Post Copulatory Posture ✓	These postures exhibited by does typically follow a successful copulation, where ejaculation has occurred. Posture of the doe consists of a urination stance (legs spread, tail raised, and a hunched back). The buck may also urinate at this time.

⊛ The buck shows visual orientation in the direction of the doe and may exhibit urine sniffing and Flehmen responses.

Table 2. Summary of outcomes of the 109-day Immunocontraceptive treatment and behavioral observation study on 24 white-tailed doe deer.

Treatment Group (n)	Number of Days before Cycling Ceased $\bar{x} \mp$ S.D.	Range in Days before Cycling Ceased	Number of Whole or Partial Cycles Observed $\bar{x} \mp$ S.D.	Proportion of Does Pregnant by Ultra-sound Detection	Percent Pregnancy Based on Fawns born by 22 Aug.	Mean Number of Fawns Born per Pregnant Doe
Control (4)	25.25 ∓ 13.50	12-46	1.25 ∓ 0.43	.67	100%	1.67
GnRH (4)	12.75 ∓ 7.98	4-23	1.25 ∓ 0.43	.50	50%	1.50
PZP (5)	70.20 ∓ 34.46	3-98	2.60 ∓ 1.02	.00	0%	----
PZPB (4)	68.50 ∓ 28.71	32-107	3.25 ∓ 1.30	.00	25%	1.00
EAP (3)	22.00 ∓ 13.44	12-41	1.33 ∓ 0.09	.33	100%	1.67
PIS (2)	32.50 ∓ 3.50	29-36	1.00 ∓ 0.00	.50	100%	2.50
PIK (2)	33.00 ∓ 11.00	22-44	1.00 ∓ 0.00	.50	100%	1.00
Combined PZP + PZPB (9)	69.44 ∓ 32.04	3-107	2.89 ∓ 1.19	.00	11%	1.00

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