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Changes in Serum Progesterone and Estrogen of the Nonpregnant Coyote during the Breeding Season[1] (41153)

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Abstract. Vulvar swelling and vaginal bleeding were monitored and blood collected at weekly intervals during proestrus, estrus, and diestrus in 16, captive wild, adult coyotes. Serum progesterone and estrogen were measured by RIA to characterize further the coyote estrous cycle. Coyotes have a prolonged period (2 to 3 months) of intermittent vaginal bleeding generally referred to as proestrus; however, this discharge can occur during estrus and metestrus in some nonpregnant coyotes. Vulvar swelling was extreme for 1 to 2 weeks and the midpoint of extreme swelling occurred about 4 days after the estradiol peak. Progesterone began to increase ($P < 0.01$) concomitantly with the estradiol peak and continued to increase, reaching its peak 3 weeks later. Progesterone then declined gradually toward basal concentrations at 9 to 10 weeks after the extradiol peak and remained at these concentrations through at least the 15th week. Thus, progesterone in nonpregnant coyotes remained above basal concentrations for a period that is about equivalent to that of normal gestation. The lack of progesterone increase from 10 to 15 weeks after the estradiol peak agrees that the coyote is monoestrus. A single estrus is advantageous for controlling reproduction of coyotes, but the distribution of estradiol peaks over a 2-month interval may require prolonged administration of antifertility compounds and thus limit their effectiveness.

The ban on indiscriminate poisoning in 1972 (Executive Order 11643) has led to increased research on nonlethal means for control of predators, particularly the coyote. Antifertility agents may be an effective and environmentally acceptable means for controlling coyotes (2, 3); however, more information about coyote reproductive behavior is needed. Coyote reproductive physiology and endocrinology must be understood to test the effectiveness of antifertility agents and administer them at the appropriate time to interfere with reproduction. In the present study progesterone and estrogen concentrations during the breeding season were measured to characterize further the coyote estrous cycle.

Material and Methods. Sixteen, captive, wild, coyotes were maintained in individual 1.5 × 3.4-m outdoor kennels for about 6 months before the beginning of the experiment. They had access to shade provided by the main kennel building and were given dry dog food and water *ad lib.* They were manually restrained for 5 min each week to monitor vulvar swelling and vaginal bleeding during proestrus, estrus, and diestrus from December 1 to May 1. Vulvar swelling was subjectively evaluated as either none, medium, or extreme. Vaginal bleeding was indicated by the presence of blood in fluid removed from the vagina. During restraint, a jugular blood sample was collected. After centrifugation, serum was decanted and stored 3 to 8 months at −20° until analysis for progesterone and estradiol. Progesterone was measured weekly from December 1 to May 1 and estrogen was measured weekly beginning 3 weeks before until 3 weeks after extreme vulvar swelling. Serum was analyzed for both hormones by specific radioimmunoas-

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says (RIA) and progesterone data were plotted in terms of day of estradiol peak.

The progesterone RIA used rabbit-antiprogesterone 11α-BSA obtained from Miles Laboratories Inc., Elkhart, Indiana.[5] The only appreciable cross-reactivities of the antiserum noted were with 11α-hydroxyprogesterone and 11β-hydroxyprogesterone (44 and 8%, respectively). The antiserum also cross-reacts (3%) with 5α-pregnan-3,20-dione and (2%) with 17α-hydroxyprogesterone. All other possibly interfering steroids cross-reacted less than 0.4%.

Serum assay for progesterone consisted of extraction of samples (50–100 μl) with petroleum ether and reconstitution of evaporated solvent phase in 0.1 *M* phosphate-buffered saline (PBS 0.1% Knox gelatin, pH 7.2).[5] Samples were assayed in duplicate in 10 × 75-mm glass tubes. Each tube received 300 μl of antiserum diluted in PBS to bind 35% of available progesterone and was allowed to equilibrate at room temperature for 20 min. After equilibration, 12,000 cpm tritiated progesterone was added and tubes incubated at 4° overnight. Separation was effected using dextran-coated charcoal at 4°.

Accuracy and precision were tested by addition of several known quantities of progesterone (100, 250, 500, and 1000 pg) to steroid-free plasma. In addition, serum pool samples were included with each assay in duplicate and treated identically to unknowns. Recoveries were corrected for procedural loss, and the results were compared with original quantities; results indicated that the assay measured 95.3 ± 15% ($\bar{X}$ ± SE) of the original quantities added (N = 36). Coefficients of variation for intra-assay and interassay variation averaged 8.5 and 14%, respectively.

Two water blanks were included with each assay to reveal positive or negative interference in the assay. The average blank value was less than 2.5 ± 3 pg.

Estradiol-17β was measured with an antiserum against estradiol 6-BSA obtained from Dr. G. D. Niswender (Colorado State University, Fort Collins, Colo.). The antiserum was diluted 1:60,000 in PBS; its cross-reactivity and specificity have been reported previously (4). Celite column chromatography was used to check the specificity of the antiserum by a method reported previously (5). Estradiol-17β was isolated from 12 samples and compared to the same 12 unchromatographed samples. The correlation coefficient was 0.97, indicating a negligible difference between the chromatographed and unchromatographed samples, so the remaining samples were not chromatographed.

The assay procedure was similar to that for progesterone. Briefly, 1.5 to 2.0 ml of serum was extracted with diethyl ether. Bound and free estradiol were separated by the same method described for progesterone. Accuracy, precision, sensitivity, and blanks were determined by measuring water blanks, standard quantities of estradiol, and pooled serum in duplicate with each assay by the same procedure used to measure experimental samples. Sensitivity was found to be 5.7 pg/ml with a blank of 2–3 pg/ml. Intraassay and interassay coefficients of variation were 6.5 and 8%, respectively. Average recovery after procedural loss was 74.6 ± 0.6% (N = 250).

Data were corrected for extraction losses and analyzed by the split-plot procedure (6) to account for correlation of errors introduced by repetitive measurement on individual animals. Selected contrasts were compared by Scheffé's procedure (7).

Results. Intermittent vaginal bleeding was observed for 2 to 3 months in each of the 16 coyotes. Vulvar swelling was extreme for about 2 weeks. During a 9-week period of weekly sampling, estradiol increased ($P < 0.01$) from 3.7 ± 1.2 to 22.8 ± 4.6 pg/ml and then decreased ($P < 0.01$) to 4.0 ± 1.0 pg/ml (Fig. 1). On the average, estradiol peaked 3.9 ± 0.9 days before the midpoint of extreme vulvar swelling. One estradiol peak was observed in each coyote. For this coyote population estradiol peaks were distributed over a 2-month period.

Progesterone data for each coyote were

[5] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

plotted in relation to time of peak estradiol in each coyote (Fig. 1). Levels remained below 2.5 ng/ml for 6 weeks before time of peak estradiol. At the time of peak estradiol, progesterone increased significantly ($P < 0.01$) to 5.2 ± 0.08 ng/ml; it peaked (15.2 ± 1.8 ng/ml) 3 weeks later. Progesterone again approached basal concentrations (between 2 and 3 ng/ml) 9 to 10 weeks after the time of peak estradiol and remained low throughout the rest of the sampling period.

Discussion. To our knowledge, this is the first report on the changes in estradiol and progesterone during the coyote estrous cycle. Reproductive endocrinology of the dog, however, has been studied extensively. The estrous cycles of the coyote and dog (8) appear somewhat similar, as would be expected since hybridization between the two species is possible (9, 10). Both animals have vaginal bleeding and vulvar swelling during proestrus. Coyotes in this study and others (11, 12) had intermittent vaginal bleeding over a period of 2 to 3 months. Dogs bleed continuously for the average 9-day proestrous period (13), but may also bleed intermittently during anestrus before observed proestrus.

Vaginal bleeding was present 30 days after peak estradiol in 2 of 16 coyotes. This finding suggests that in addition to having vaginal bleeding in proestrus and estrus (12) some coyotes may have vaginal bleeding after ovulation. This finding supports the observation of vaginal bleeding during pregnancy in hand-raised coyotes (14). Weekly observations are not sufficient for an accurate mean time of extreme vulvar swelling but 95% of the animals had extreme swelling for 1 to 2 weeks. If one assumes that estrus begins at or near the time of peak estradiol concentrations, then our midpoint of extreme vulvar swelling, 3.9 ± 0.9 days after peak estradiol, agrees with a previous report that vulvar size was maximal by the fourth day of estrus (12).

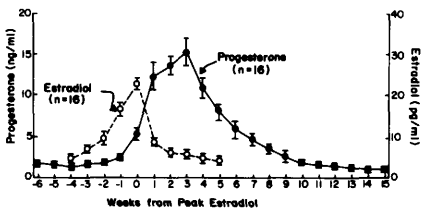

FIG. 1. Serum estradiol and progesterone during the seasonal estrous cycle of the captive wild coyote.

The frequency of peak estradiol was distributed biphasically over the second weeks of February and March, with a mean date of February 27th for the estradiol peak. The mean basal (3.7 pg/ml) and peak (22.8 pg/ml) estradiol concentrations were of similar magnitude to the respective concentrations of estradiol (15–17) and total unconjugated estrogen (18) in the dog. Although the behavior of coyotes was not observed in this study, estradiol probably peaks just before or at the onset of estrus, as has been reported for dogs (18).

The time of peak progesterone and its magnitude were similar to values reported for the dog (18) and for the red fox (19). Daily or biweekly rather than weekly samples are required to more accurately show the sequential relationship of progesterone and estrogen concentrations in coyotes. A recent study provides good evidence that progesterone was produced when luteinization of the follicle began before ovulation in the dog (20). The same sequence of events is expected in the coyote. However, confirmation of this hypothesis must await further studies of the temporal relationship between LH, progesterone, and estradiol in coyotes.

Progesterone declined slowly toward the basal value and reached it 9 to 10 weeks after peak estradiol. This phenomenon in which progesterone in the nonpregnant coyote remains elevated above basal concentrations for approximately the length of gestation is similar to that reported for the dog (17, 18) and dissimilar to that reported for the red fox (19). Progesterone and the corpora lutea persist for up to 5 months after whelping in the pregnant red fox or for 5 months after an interval of 52 days (normal gestation) in the nonpregnant red fox (19). The lack of progesterone increase from 10 to 15 weeks after the estradiol peak agreed with observations that the coyote is monoestrus (12). This conclusion was further documented by reports for males that the testes start regressing by early to

middle March (21) and that sperm production has ceased and epididymal sperm are few or absent by June (22).

Monoestrus is advantageous for controlling reproduction of coyotes, but the distribution of the estradiol peaks of the test population over a 2-month interval may require prolonged administration of antifertility compounds and thus limit their effectiveness.

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1. Stellflug, J. N., Muse, P. D., Gates, N. L., and Louis, T. M., J. Anim. Sci. **47**, (Suppl. 1) 392 (1978) (Abstract).
2. Balser, D. C., J. Wildl. Manage. **28**, 352 (1964).
3. Stellflug, J. N., Gates, N. L., and Leathers, C. W., *in* "Proceedings of Eighth Vertebrate Pest Conference," p. 185 (1978).
4. England, B. G., Niswender, G. D., and Midgley, A. R., Jr., J. Clin. Endocrinol. Metab. **38**, 1 (1974).
5. Tulchinsky, D., and Abraham, G. E., J. Clin. Endocrinol. Metab. **33**, 775 (1971).
6. Gill, J. L., and Hafs, H. D., J. Animal Sci. **33**, 331 (1971).
7. Scheffe, H., Biometrika **40**, 87 (1953).
8. Evans, H. M., and Cole, H. H., Memoirs of the University of California, Vol. 9, p. 65. Univ. of Calif. Press, Berkeley (1931).
9. Silver H., and Silver, W. T., Wild. Monogr. **17**, 41 (1969).
10. Mengel, R. M., J. Mammal. **52**, 316 (1971).
11. Whiteman, E. E., J. Mammal. **21**, 435 (1940).
12. Kennelly, J. J., and Johns, B. E., J. Wildl. Manage. **40**, 272 (1976).
13. Smith, M. S., and McDonald, L. E., Endocrinology **94**, 404 (1974).
14. Barnum, D., and Flinders, J., "Final Report of Investigation Concerning Biological Management of Coyote Populations," USDA-WR-SEA (R. D. Plowman Area Director, and J. S. Green, Director of Predator Research) 1980.
15. Jones, G. E., Boyns, A. R., Cameron, E. H. D., Bell, E. T., Christie, D. W., and Parkes, M. F., J. Endocrinol. **57**, 331 (1973).
16. Edgvist, L. E., Johansson, E. D. B., Kasstrom, H., Olsson, S. E., and Richkind, M., Acta Endocrinol. **78**, 554 (1975).
17. Hadley, J. C., J. Reprod. Fert. **44**, 445 (1975).
18. Concannon, P. W., Hansel, W., and Visek, W. J., Biol. Reprod. **13**, 112 (1975).
19. Bonnin, M., Mondain-Monval, M., and Dutourne, B., J. Reprod. Fert. **54**, 37 (1978).
20. Concannon, P. W., Hansel, W., and McEntee, K., Biol. Reprod. **17**, 604 (1977).
21. Gier, H. T., Kans. Agr. Exp. Stn. Bull. **393**, 118 (1968).
22. Kennelly, J. J., *in* "Coyotes Biology, Behavior and Management" (M. Berkoff, ed.), p. 73. Academic Press, New York (1978).

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