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SELECTED PARAMETERS OF THE REPRODUCTIVE PHYSIOLOGY AND ENDOCRINOLOGY OF COYOTES

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Abstract: The development of the reproductive system and the dynamics of reproductive hormones were studied in captive male and female coyotes (*Canis latrans*). Captive male coyotes exhibited incomplete sexual maturation at the first reproductive season (< 12 months of age). Peak serum testosterone levels in 1-year old males were 50% (300±200 vs. 810±300 pg testosterone/ml) and total sperm production was only 10% (57.4±6.6 vs. 558.8±26 x 10^6 total sperm) of that observed in males older than 1 year. Yearling males were never observed copulating with a female. The sexual maturation of captive female coyotes was less equivocal than their male counterpart's. The pregnancy rate of yearling females was 40% compared to 63% in older females. Average peak serum LH values at the ovulatory surge were 33 ng/ml in yearling females compared to 60 ng/ml in older animals. Serum FSH, estradiol and progesterone levels were similar. There also appears to be an inhibition of fecundity in subordinate females, the mechanism for which is currently unknown. Our long range goal is to capitalize upon this information to develop methodologies for coyote population control

The coyote has been able to adapt and reproduce effectively in a variety of environments from wilderness areas to metropolitan communities. Such success is due in part to its highly flexible social system which is related to its reproductive patterns. In addition to the behavioral patterns necessary for survival, numerous researchers (Bekoff 1976, Bruss et al. 1983, Cary et al. 1982, Hodges 1990, Kennelly 1972, Kennelly 1978, Kennelly and Johns 1976, Stellflug et al. 1981) have described various aspects of coyote reproductive biology.

The coyote is a seasonal breeder, reproductively active between November and June dependent upon geographical location (Gier 1975, Kennelly 1978, Green et al 1984). The female is monestrus The estrous cycle is initiated in December; estrus occurs early in the spring and is distributed over 2 months. Parturition occurs in May or June after a gestation of 60 days (Kennelly and Johns 1976, Bekoff and Diamond 1976, Stellflug et al. 1981). Proestrus lasts 2-3 months and estrus lasts on average of 10.2 days, with ovulation occurring any time between the first and ninth day of estrus (Kennelly and Johns 1976, Cary et al. 1982) Serum estradiol at the pre-ovulation surge averaged 22 8 pg/ml and post-ovulation progesterone levels averaged 15 ng/ml (Stellflug et al 1981).

Electroejaculation of mature males during the height of the reproductive recrudescence yielded total sperm counts of 63 x 10^6 (Bruss, et al., 1983) and 193 x 10^6 (Green et al 1984) Kennelly (1972) reported that the duration of the seminiferous epithelial cycle averaged 13 6 days and epididymal sperm transport was approximately 14 days. The average spermatogenic cycle (the time it takes for a germ cell to develop to a spermatozoa and to be released from the testicle) averaged 54.4 days

The studies summarized here (see Hodges 1990) were undertaken to qualitatively and quantitatively describe the maturation and recrudescence of the reproductive system, and to establish some parameters of behaviorally-associated endocrine responses

Methods

These data were collected over 4 years from wild captive coyotes housed in family units of 1 male and 2 females The foundation animals were trapped, given complete health checks and prophylactic vaccinations, then housed outdoors in 10 X 30-ft enclosures at the Veterinary Medical Research Park at Texas A&M University. All procedures were performed following tranquilization with ace-

promazine maleate (1 1 mg/kg) or with a combination of xylazine (2.2 mg/kg) and ketamine hydrochloride (22 mg/kg); the latter was used at testicular biopsies and electroejaculations.

Blood samples were obtained weekly from the males from November-Apnl and bimonthly thereafter. Testicular biopsies were obtained at monthly intervals. The estrous cycle was identified by vaginal bleeding, vulvar swelling, vaginal smear cytology, increased male interest, and retrospectively, by hormone analysis Blood samples and vaginal smears were collected at 3-day intervals from December-April and bimonthly thereafter. Serum levels of LH, FSH, estradiol, progesterone and testosterone were determined by validated radioimmunoassays (Hodges 1990). Histological sections of formalin-fixed wedge biopsies were evaluated for the presence of spermatogonia, primary and secondary spermatocytes, and spermatids (Clermont 1963).

Electroejaculations were performed at monthly intervals (Seager 1974). The ejaculate was analyzed for volume, concentration, motility and pH. Vaginal smears were obtained with a vaginal swab or the aspiration of vaginal fluid when present, dried, stained with Diff-Quick R, and evaluated according to Kennelly and Johns (1976) and Cary et al. (1982). Behavior was monitored for 1 hour at dawn each day during proestrus and estrus (December through March) and at biweekly intervals thereafter. Dominance, subordinance, affiliative behaviors and copulatory behaviors were recorded Ethograms of coyotes (Bekoff 1978, Gier 1975) and grey wolves (*C. lupus*) (Packard 1980) were used to categorize these behaviors (see Hodges 1990 for the complete ethograms)

Results

Males. Reproductive system recrudescence appears to be initiated in November as evidenced by the increase in serum levels of testosterone (Fig. 1). Recrudescence was preceded by a rise in LH and the appearance of spermatozoa in testicular biopsies. It was also apparent that full fertility, as predicted by adequate numbers of sperm in the ejaculate (i.e., $>100 \times 10^6$), occurred between January and March (Fig. 2)

Young coyote males entering their first reproductive season exhibited an elevation in serum testosterone and sperm in the ejaculate. However, the increases temporarily lagged behind those of mature males, and the levels were significantly reduced. From behavioral observations made throughout the year, no male coyote less than 1 year of age exhibited any copulatory activity.

Females. The estrous cycle endocrine profile of mature dominant coyotes was unremarkable (Fig. 3). Proestrus was observed as early as late December and estrus (as defined by vaginal cytology) occurred between late February and early March, and lasted 10 days. The ovulatory LH surge was preceded by a rapid rise in estradiol

Although several yearling coyotes exhibited estrus, the entire cycle temporally lagged behind that of mature females by 12-17 days, dependent upon the criterion used Of the 9 trials (defined as 2 females paired together with a male during 1 breeding season) in which behavioral parameters were monitored, only 4 subordinate females exhibited estrus and had an LH surge. Estrus in the subordinate female occurred 11.0±2.7 days after that of her dominant pen mate (Fig. 4). None of the subordinate females gave birth to live young during these trials.

Discussion and Management Implications

These studies substantiated and further delineated the coyote as a seasonal, monestrus canid. The serum endocrine profiles for the estrous cycles of individual animals were qualitatively similar to other mammals However, several issues were raised that may impact on the possibility of exogenously regulating the coyote population by manipulating reproduction.

The yearling male coyote does not enter into the reproductive equation. Neither does he produce enough sperm cells nor attains serum testosterone levels high enough to support copulatory behavior. It has been suggested by others (Bekoff and Wells 1982) that these animals can serve as helpers in obtaining food etc., with no repercussions from the alpha male. It would appear that physiological maturity (sperm in the ejaculate) requires less testosterone than copulation, a behavioral correlate of reproduction.

On a more practical note, it seems counter-productive to attempt to render male coyotes infertile

between April and December. Application of this principle may have some ecological implications on non-coyote species. Agents that induce infertility (as opposed to sterility) in the male coyote should be available from January-March Some non-coyote species would not have access to the agent at critical times in their reproductive cycle.

Our studies (and those of others) on the female coyote indicate that estrus and ovulation occur during a very circumspect time frame, late-February to mid-March. Therefore, to be effective, anti-gametogenic agents should be applied between January and March; antiovulatory compounds in February and March; and abortifacient materials in March and April. Female fecundity appeared to be related to the social hierarchy; however, the effect was not precise nor was it complete. Until the actual mechanism is determined, it is highly unlikely that this characteristic can be exploited.

Summary

Much of this symposium is devoted to discussions on ways to control coyote populations. Previously-used methodologies have been only marginally successful, and a significant portion of those have the potential for producing negative effects on the surrounding ecological systems. We suggest that there may be vulnerable events in the reproductive biology of the coyote that may lend themselves to external manipulation with less damage to the environment, and more precise management of the coyote population. One of our goals should be to identify those vulnerable events in coyote reproduction, then exploit them to our advantage.

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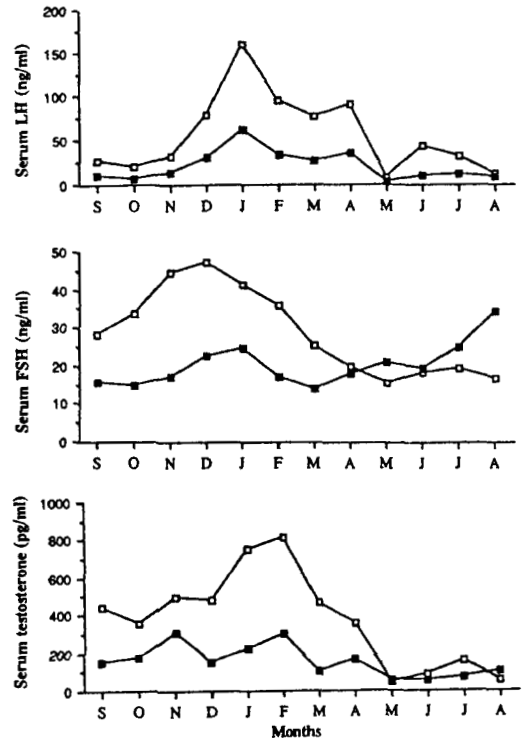

Figure 1. Average serum concentrations of LH, FSH and testosterone in immature (<1 year old) and mature (>1 year old) male coyotes Standard deviations are omitted to preserve clarity Open symbols depict immature coyotes(n=3); closed symbols depict mature coyotes (n=4)

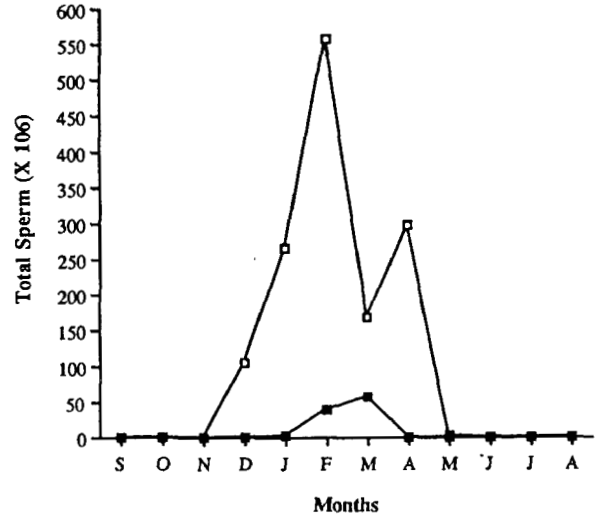

Figure 2. Total sperm in the ejaculate, following electroejaculation, of immature and mature male coyotes. Open circle depicts immature coyotes (n=3); closed diamonds depict mature coyotes (n=4).

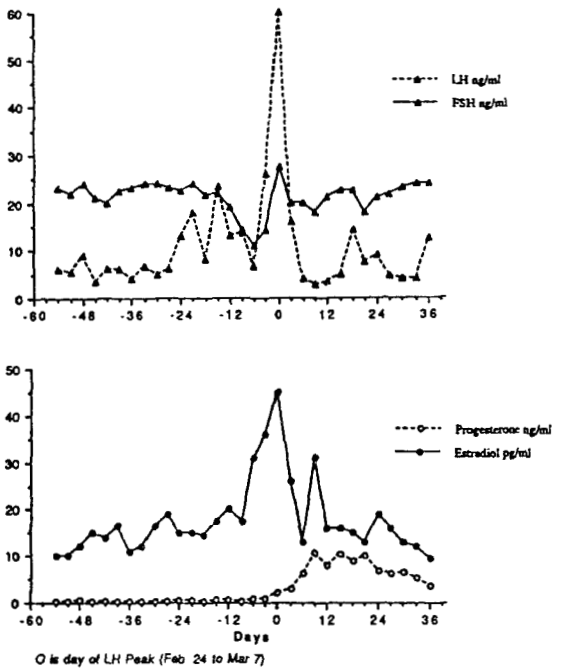

Figure 3 Endocrine profile of reproductive hormones in mature female coyotes (>1 year old) during the estrous cycle (n=8 cycles). Shaded area represents estrus as determined by vaginal cytology. Hormone concentrations of individual animals were initialized to the day of the LH peak. Open triangles depict LH; closed triangles FSH, open circles progesterone; closed circles estradiol.

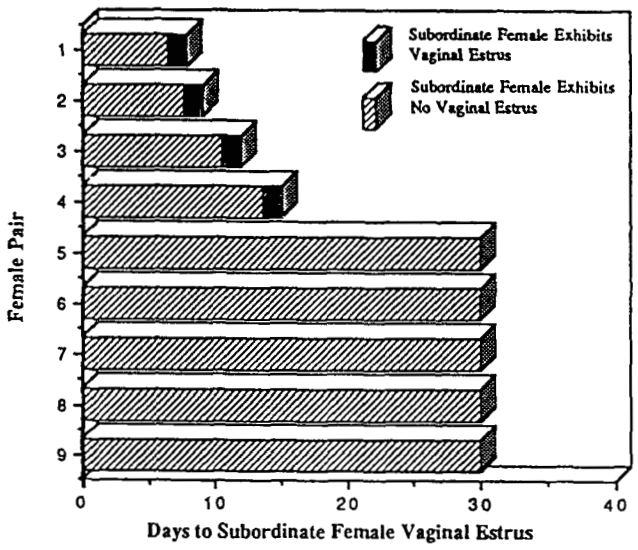

Figure 4. Time (days) to vaginal estrus of subordinate female coyotes Day 0 = first day of vaginal estrus of the dominant female of that pair. Each line represents the response of 1 family unit at 1 breeding season. Mean time to subordinate female estrus was 11.0±2.7 days (range = 8-15 days; n=4 pairs of females). Solid black bar represents estrus. NOTE: In 5 pairs the subordinate female had not exhibited vaginal estrus (or an LH surge) by 30 days following estrus of the dominant female of that pair, these females were not included in the calculation of the mean