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Patterns of Dominance and Reproduction in a High Density Herd of White-tailed Deer (*Odocoileus virginianus*)

A Thesis

Presented for the

Master of Science

Degree

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Catherine Elizabeth Kolkmeier

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ABSTRACT

A high density herd of white-tailed deer (Odocoileus virginianus) in Cades Cove, Tennessee, was monitored through both behavioral observation and hormonal analysis to investigate the possibility that recent population trends may be explained in part by the action of an internal regulating mechanism. Behavioral and population census data were collected for two reproductive seasons to provide information on the nature of social interactions among group members and on changes in the structure and stability of social groups. Special attention was given to female dominance hierarchies, within which it was proposed that aggressive social interactions may influence the hormonal state of reproductive adult females.

Census results demonstrated that, during the winter sampling period, there were no significant changes in sex or age composition of family groups. These results were based on the overall proportion of females and of adults observed in daily censuses of family groups for a six-week period.

A procedure was developed and tested which analyzed concentration of progesterone and cortisol in fecal pellets collected from adult females in the Cades Cove deer herd. Behavioral observation and hormonal analyses also were conducted on a captive group of female deer at the University of Georgia. This group served as a control

population for which life history data were available for all females.

Analysis of hormonal data indicated that, among females in the captive herd (n=5, sampled once monthly over a six-month period), there was no significant effect of an individual's social rank or of the month in which an animal was sampled on progesterone concentration in feces. Similar data collected from the Cades Cove deer population were inconclusive for progesterone. Although these effects were found to be statistically nonsignificant, it should be noted that this study was not able to refute its original hypothesis that aggressive social interactions may influence the reproductive success of female white-tailed deer. Finding evidence of an internal density regulating mechanism in this population would require further study.

This study provides preliminary information on both the dynamics of the Cades Cove deer population, as well as on the potentially valuable management tool of non-invasive fecal hormone analysis. Recommendations are made for the continued monitoring of the Cades Cove herd and for the development of sampling techniques which may provide more information on the dynamics of the deer herd and the effects of social activity on the density and reproductive performance of female white-tailed deer in Cades Cove.

TABLE OF CONTENTS

I. INTRODUCTION 1
History of Herd 5
Population Regulation 8

II. METHODS 14
Behavioral Observations: Year I 14
Behavioral Observations: Year II 15
Cades Cove 15
Captive Herd: University of Georgia 17
Laboratory Analysis 19
Validation for Progesterone 20
Validation for Cortisol 24
Statistical Analyses 25

III. RESULTS 26
Behavioral Observation: Cades Cove 26
Males: Descriptive Trends 26
Females: Descriptive and Quantitative Trends 30
Population Parameters 34
Captive Herd: Georgia 36
Laboratory Analysis 37
Progesterone: Cades Cove 37
Progesterone: University of Georgia 38
Cortisol: Cades Cove and University of Georgia 47
Validation for Progesterone 48
Validation for Cortisol 51

IV. DISCUSSION 52
Population Dynamics 52
Behavioral Observations 52
Laboratory Analysis 54
Progesterone: University of Georgia 54
Progesterone: Cades Cove 57
Validation for Progesterone 58
Validation for Cortisol 61
Effects of Density on Population Size 62

V. CONCLUSIONS AND RECOMMENDATIONS 65
Laboratory Analysis 65
Effectiveness of Fecal Sampling. 66
Cades Cove Herd 67

LITERATURE CITED 69
APPENDIX 75
VITA 77

LIST OF FIGURES

FIGURE		PAGE
1.	Topographical representation of Cades Cove and surrounding areas	4
2.	Average number of deer counted in nightly spotlight surveys conducted in Cades Cove, Great Smoky Mountains National Park, TN, 1981-1991.	9
3.	Average size of family groups for white-tailed deer during study period, Cades Cove, 1992.	35
4.	Average progesterone concentration (ng/g feces) for female white-tailed deer in dominant and subordinate rank groups, Cades Cove, 1992.	39
5.	Progesterone concentration (ng/g feces) for captive female R8, Whitehall Experimental Forest, University of Georgia, Athens, GA.	41
6.	Progesterone concentration (ng/g feces) for captive female Y2, Whitehall Experimental Forest	42
7.	Progesterone concentration (ng/g feces) for captive female LR, Whitehall Experimental Forest	43
8.	Progesterone concentration (ng/g feces) for captive female R19, Whitehall Experimental Forest.	44
9.	Progesterone concentration (ng/g feces) for captive female B6, Whitehall Experimental Forest	45
10.	Mean progesterone concentration (ng/g feces) for each of five captive female white-tailed deer, Whitehall Experimental Forest.	46
11.	Progesterone concentration (ng/0.5 g feces) from estrous to three month post-parturition, 1990-1991, Whitehall Experimental Forest	50
12.	Comparison of progesterone concentration (ng/g feces) for five captive female white-tailed deer, Whitehall Experimental Forest.	55
13.	Pathway showing the production of cortisol and progesterone from cholesterol, including several intermediate molecules. Adapted from Norris, 1985.	60

LIST OF TABLES

TABLE PAGE

1. Cross-reactivity of selected ligands related to progesterone. Reprinted from ^{125}I Progesterone Radioimmunoassay, ICN Biomedicals, Costa Mesa CA. 23

2. Interactive behaviors observed occurring among adult females in the Cades Cove herd. Includes both affiliative and aggressive interactions. 31

CHAPTER I

INTRODUCTION

Hypotheses regarding the effects of high density on the reproductive success of ungulate populations have been addressed in numerous studies (McCullough, 1979; Woolf and Harder, 1979; Clutton-Brock et al., 1982; Seal et al., 1983; Teer, 1984; Putman, 1988). In captive populations of white-tailed deer, overcrowding has caused lowered productivity of yearling females (Ozoga et al., 1982), decreased production of eggs and low corpus luteum counts in adult females (Hesselton and Jackson, 1974; Ozoga and Verme, 1986), and increased neonatal mortality due to insufficient maternal care (Langenau and Lerg, 1976; Ozoga et al., 1982). Although the effects of high density are well documented, less is known about the exact mechanism which controls these effects.

The endocrine-behavioral feedback mechanism, as described by Christian and Davis (1964), explains the changes in reproductive success due to high density as a result of alterations in hormonal production. Particularly affected are adrenocortical activity and the secretion of gonadotropins. High density causes an increase in the amount of interactions between individuals, which in turn creates a physiologically stressed environment. Hormonal

response to stress is manifested in part in the form of behavioral changes and alterations in the physiology of the reproductive system (Christian and Davis, 1964; Bronson, 1989). Among ungulates and other mammals, the effects of "endocrine suppression" of breeding females has been described, whereby a dominant female will curtail breeding in other female members of her group by aggression and other threatening behaviors, which in turn produce a stress response in the subordinate females and subsequent difficulty or failure to reproduce (Christian and Davis, 1964); subordinate females have been documented as having lower reproductive success than their dominant counterparts (Ozoga et al., 1982; Bronson, 1989; Moehlman, 1989). The result is a lowering of the birth rate which can be powerful enough to offset the growth of the population, thereby limiting the density.

Low productivity can result from physical and physiological changes occurring at virtually any stage of the reproductive process. Mating may be physically impeded by aggressive dominant females (Clutton-Brock et al., 1982; Marchinton and Hirth, 1984) or by lack of receptivity to a breeding male due to asynchronous ovulation cycles (Ozoga and Verme, 1986). Lack of conception may occur in successfully mated females if they are experiencing stress which has interrupted their reproductive functions (endocrine suppression) (Christian and Davis, 1964; Bronson,

1989). The above also may occur if the females do not come into estrous, the timing of which is regulated by the physiological condition of the female (Christian and Davis, 1964; Teer, 1984; Ozoga and Verme, 1986). Fetuses may be aborted or embryos reabsorbed as a result of physiological stress in the mother, or the female may fail to produce enough milk to support the young after it is born (Clutton-Brock et al., 1982; Verme and Ullrey, 1984; Putman, 1988). Stressed females also have been observed to abandon their newborns (Langenau and Lerg, 1976; Clutton-Brock et al., 1982; Ozoga and Verme, 1982; Verme and Ullrey, 1984; Putman, 1988). The occurrence of any of these conditions in a herd can significantly contribute to a low birth rate and possibly limit the growth of the population.

Exhibiting population dynamics similar to those presented above is the resident herd of white-tailed deer in Cades Cove, Great Smoky Mountains National Park, Tennessee. Cades Cove was established as an historical area in Great Smoky Mountains National Park in the 1930's and consists of 977 ha of rolling hills and meadows; 76.5% of the area is used in local ranching operations, managing a few hundred head of cattle and horses. Some areas are maintained as historical sites, where small log homes, mills and barns are preserved for visitors. The Cove is surrounded by an 18 km paved loop road and two unpaved roads which traverse the Cove, dividing it roughly into thirds (Figure 1). Visitation

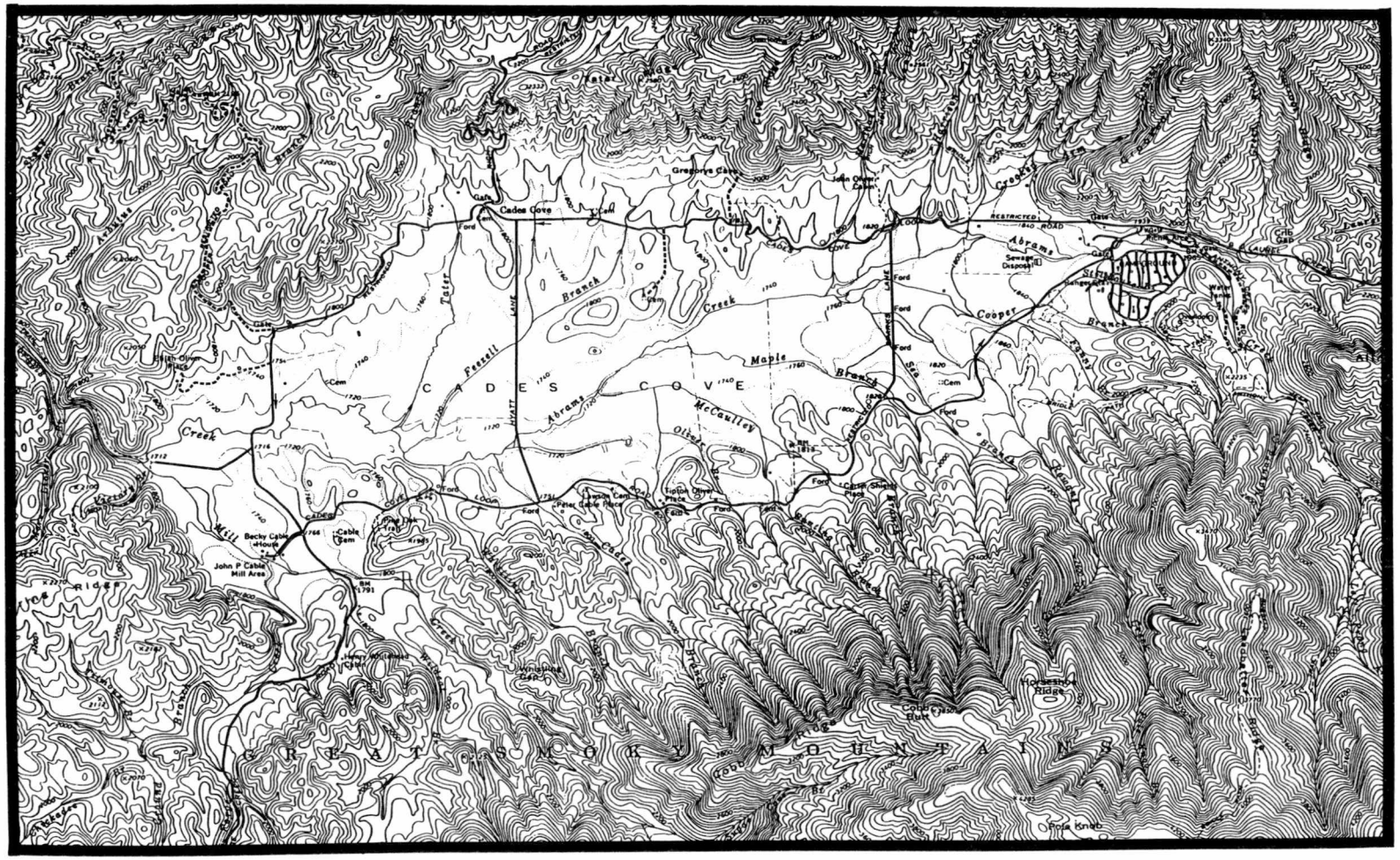


Figure 1. Topographical representation of Cades Cove and surrounding areas, Great Smoky Mountains National Park, TN.

to Cades Cove is among the highest in the Park, which is the most frequently visited national park in the United States. Many species of mammals use the Cades Cove area, including black bears (Ursus americanus), bobcats (Lynx rufus), coyotes (Canis latrans), woodchucks (Marmota monax), raccoons (Procyon lotor), and white-tailed deer (Odocoileus virginianus). The most visible and one of the most popular of these is the resident herd of white-tailed deer.

History of Herd

The dynamics of the Cades Cove deer population are of interest because of the circumstances surrounding recent changes in the density of the herd. In 1971, the herd experienced a die-off due to an outbreak of hemorrhagic disease in an epidemic which affected many deer populations in the Southeast, though the Cades Cove herd was the only reported population affected in the state of Tennessee (Prestwood et al., 1974). In white-tailed deer, hemorrhagic disease interferes with blood clotting functions and causes a loss of vascular integrity, resulting in fever, severe shock, and death within 2-6 days (Karstad et al., 1961); it is caused by both the bluetongue and epizootic hemorrhagic disease viruses, both of which also occur in cattle (Thomas et al., 1974). Effects on the Cades Cove herd could be seen in spotlight surveys conducted prior to and during the epidemic; these counts revealed an 84% decrease in the

utilization of fields and pastures for feeding, dropping from 723 to 196 animals utilizing pastures in early 1971 (Fox and Pelton, 1973). Fifty-two deer carcasses were found in Cades Cove and surrounding areas. Additional surveys conducted in the year following the die-off, however, showed the density of the herd approaching levels similar to those seen prior to the die-off, probably due in part to an influx of animals from surrounding wooded areas (Fox and Pelton, 1973).

By 1977, the size of the population had returned to a high level of an estimated 519 deer, causing concern that the herd was increasing in density at such a rate as to become overpopulated (Burst and Pelton, 1977). Density, estimated from direct sightings of deer in both day counts and nightly spotlight counts, was estimated a 0.52 deer per hectare. Since dense deer populations are vulnerable to outbreaks of hemorrhagic disease (Nettles, 1982), as was true of all populations suffering high mortality in the 1971 epidemic (Prestwood et al., 1974), it was possible that Cades Cove could again be affected by the disease. Scattered outbreaks of hemorrhagic disease have continued in the Southeast since 1971 (Nettles, 1982). In 1980, the Cades Cove population was among the densest in the Southeast region, appearing to have stabilized at a high density of 0.29-0.58 deer per hectare (Kiningham, 1980). A later study (Wathen and New, 1986) supported this finding, with an

estimated density of 0.38 deer per hectare. Both studies determined density from nightly roadside spotlight counts. Other studies conducted in Cades Cove during the 1980's concluded that, not only was the herd maintaining a high density, but that it also was in good physical condition, was supported by a reliable year-round food source (Dlutkowski, 1985), had a low parasite load (National Park Service records), and exhibited a healthy, high level of genetic heterozygosity with minimal inbreeding (Major et al., 1984).

In Cades Cove, food supplies are high. Because of federal protection by the National Park Service, hunting is not allowed and predation pressure is uncharacteristically low. This creates ideal conditions for rapid population growth, which can lead to overpopulation and an eventual depletion of food resources (Marburger and Thomas, 1965). Concern for the growth potential of the herd led to recommendations that deer be removed from the area and relocated (Burst and Pelton, 1977) that prompted a series of doe removals beginning in February 1981. Contrary to the expectation that the herd would be maintained at a stable level, population counts conducted before and during the removal period showed an increase in field usage. This led to additional removals over the next two years, none of which were successful in reducing the size of the herd (National Park Service Records). In subsequent years,

however, nightly spotlight counts have shown a decline in the number of deer utilizing fields, reaching a low yearly average of 87 in 1989 (National Park Service Deer Counts; Figure 2). Data from spotlight counts were collected periodically by various visitor and student groups. Counts were not conducted on a regular schedule and were not available for 1984-1986.

Population Regulation

The steady drop in density of the Cades Cove herd in an environment favorable to population growth may suggest the action of a self-regulating mechanism which limits the growth of the herd by way of the dynamics of the population. Before the existence of such a mechanism can be sought, however, all extrinsic population regulators must be dismissed as major contributors to the regulation or decrease of this herd. Historically, one of the most visible limiting factors in Cades Cove has been disease. Wathen and New (1986) conducted a survey of the deer population, focusing on diseases present among deer and cattle herds in Cades Cove. Their conclusions maintain that, although disease has the potential of exerting a force on the health of the herd, disease alone is not enough to limit overall growth of the population in the long term. In additional studies, abomasal parasite counts performed from

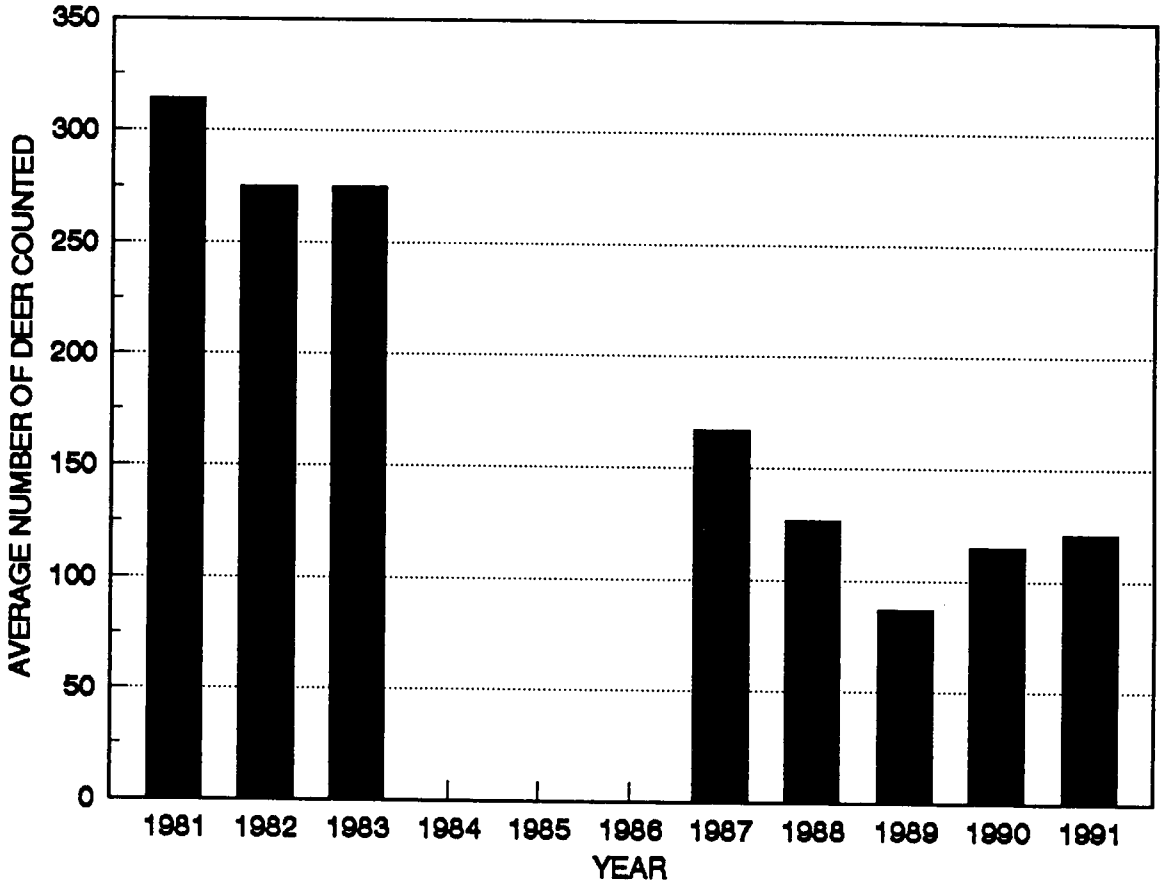


Figure 2. Average number of deer counted in nightly spotlight surveys conducted in Cades Cove, Great Smoky Mountains National Park, TN, 1981-1991. Data were not available for 1984-1986.

1980 to 1989 show a parasite load well below levels considered unhealthy. Abomasal parasite levels measured by the Tennessee Wildlife Resources Agency (TWRA) in 1989 found an average parasite count of 888 ± 326.6 (TWRA Abomasal Parasite Count Report to the National Park Service, 1989). These levels are considered average parasite loads for a dense herd and are most commonly associated with herds of good overall health (Davidson et al., 1982).

The effects of hunting and predation are reduced in Great Smoky Mountains National Park, as hunting is illegal within the park boundaries, and most of the large predator population, which once consisted of cougars (Felis concolor) and possibly red wolves (Canis rufus), were extirpated from the area. Only coyotes and bobcats remain, though their effects on the herd are not yet fully assessed. Although prohibited in the Park, both poaching and predation by domestic dogs (Canis familiaris) do occur, though their effects are difficult to measure. Wathen and New (1986) reported mortalities occurring during their study period (n=31), attributing 25% to predation; this also included predation by black bears.

Genetically, the population exhibits a high level of heterozygosity and a low degree of inbreeding, both of which are indicative of a genetically healthy population (Major et al., 1984). Behavioral regulators, such as the dispersal of juvenile males and recruitment of individuals from the

surrounding Cherokee National Forest, have not been extensively studied, although it is speculated that deer move between the Cove area and the surrounding Cherokee National Forest.

The most frequently cited regulator of deer populations is food availability (McCullough, 1979; Matschke et al., 1984; Teer, 1984; Putman, 1988). Limited food supply results in differential survival of members of the population, limiting the number of individuals contributing to the next generation. Primarily a browsing species, white-tailed deer remain in forested areas, feeding on twigs and buds, moving into grasslands for grazing after depleting the browse resources (Verme and Ullrey, 1984). The impact of deer browsing in Cades Cove is heavy, and a distinct browse line can be seen in the wooded areas throughout the year, whereas grass is readily available in cattle and horse pastures planted in fescue (Festuca spp.), timothy (Phleum spp.), and other grasses. While grazing pressure is heavy, the population is not limited by its availability (Wathen and New, 1986). In a survey of deer hematology, serum biochemistry, and physical parameters, Dlutkowski (1985) concluded that the herd was in good physical condition, with no overall health problems resulting from malnutrition.

The conditions surrounding the Cades Cove deer population provide a suitable backdrop against which the existence of a self-regulating behavioral population

regulator may be tested. The question of how this mechanism may act on the Cades Cove herd is answered in behavior patterns common to white-tailed deer, which live according to a highly structured social system of dominance and subordination. The social system of white-tailed deer is polygynous, with a core group consisting of one or more adult females and their recent offspring (Marchinton and Hirth, 1984; Putman, 1988). Males congregate in the non-breeding season in bachelor herds, which break up in the fall and travel among the female groups, where males compete for access to estrous females. Strong dominance hierarchies exist both in male and female groups within a herd. The male hierarchy is based on size and strength; the female system is based on age (Townsend and Bailey, 1981). The female hierarchy is expressed in the behavioral interactions between females, that range from benign to aggressive.

It is suggested by this study that the stress experienced by subordinate females as a result of their rank in the hierarchy is strong enough to be detrimental to their potential to successfully produce viable offspring. To test this hypothesis, the Cades Cove herd was monitored for the presence of a dominance hierarchy as seen through aggressive interactions among adult females. Individual females were also tested for production of both reproductive and stress hormones so that females of differing ranks could be compared by their exposure to stress and their ability to

reproduce successfully under potentially stressful conditions.

CHAPTER II

METHODS

Behavioral Observations: Year I

The purpose of the first year of behavioral observations was to collect descriptive information on both changes in the general behavioral repertoire of deer in Cades Cove as well as on the timing of major reproductive and physiological events. Observations were conducted for approximately three to four hours, one from pre-dawn to mid-morning, one from early evening to dark. In colder months when the days were shorter, and the herd became active earlier in the day, evening watches began in the early afternoon. Observations were taken from the Cades Cove Loop Road or from adjacent wooded areas using 7x25 binoculars or a 20x spotting scope, and occurred from September, 1990, to June, 1991.

Initially, data were collected for both sexes, describing and categorizing foraging behaviors, movements, and social interactions such as fighting and grooming. Beginning in January, 1991, an ethogram of interactive behaviors of females was created, and observations began to focus more specifically on social interactions among females of differing rank. Rank was determined for a female by subtracting the number of times she was physically displaced

from an area from the number of times she actively displaced another female. This method of rank determination was adapted from Clutton-Brock et al. (1987). Only interactions with other adults were included in determining relative rank. Individual females were placed into two age categories, adult and young, based on body size and the length and shape of the head. Physical displacement was considered to have occurred when one female moved due to the threatening posture, expression, or action of another female.

Behavioral Observations: Year II

Cades Cove

The second year of observations resumed in December, 1991. Observations focused more specifically on interactions occurring within female family groups. Population parameters such as group size and composition also were noted in censuses conducted twice daily, at dawn and dusk. Information collected during censuses included the number of animals in each group, which were then categorized according to age and gender. Only two age groups could be determined with any certainty, adults and young under one year of age. Attempts to determine the sex of individuals were abandoned in mid-February, when males began losing their antlers. As in the previous year, data also were collected on the relative ranks of females that

were involved in interactions with other females in the group. Data collected during censuses were used to track the patterns of movement of family groups through the fields throughout the day and to monitor changes in the size of individual groups throughout the reproductive season.

In order to measure the effects of rank on hormone production in females, fecal pellet samples were collected and analyzed for concentrations of progesterone and cortisol. Progesterone is a steroid hormone produced by both the ovaries and the adrenal glands; it is responsible for the maintenance of pregnancy in mammals. Progesterone levels were measured in fecal pellets collected from females in one of two rank groups, dominant and subordinate, to determine if there were differences in the degree to which members of different rank groups maintained their pregnancies. Cortisol was measured in fecal pellets collected in Cades Cove as well. Cortisol is the primary glucocorticoid for ungulates; it is produced as a response to stressful conditions and affects the production of other hormones and bodily functions to maintain constant physiological conditions within the body. Differences in cortisol levels for the two rank groups would provide information about the level of stress adult females were experiencing. If correlations could be drawn between the degree of stress experienced by a female of known rank and her ability to maintain a concentration of progesterone

sufficient to sustain her pregnancy, there may be grounds to suggest the existence of a behavioral regulating mechanism acting upon females in the two rank groups. Since rank is a relative term, pellets were only collected from females holding the highest or lowest rank, based upon female interactions, for any observation period.

Once a female could be ranked, she was continuously watched until she defecated. The location of the pellets was determined using a compass line, and distance was estimated by sight. Pellets were only collected after the group had left the area to avoid stressing the animals. Only fresh pellets were taken, and no pellets were collected if there was any doubt as to which was most recent. Pellets and dominance information were not collected for groups with fewer than five adult females present. Pellet sampling was restricted to adults until late February, when it became difficult to distinguish between young and adult females.

Captive Herd: University of Georgia

Since individual females in Cades Cove were not tagged, it was impossible to distinguish individuals from one observation to the next. Because of this, the rank of a particular female was only valid for that observation, making it difficult to detect long-term changes in the dominance hierarchy. The above also eliminated the

possibility of measuring changes in hormone concentration over the gestation period for any single individual.

To provide a control group in which individuals could be distinguished, a captive group of white-tailed deer was utilized at the Whitehall Experimental Forest of the University of Georgia through the cooperation of the School of Forest Resources in Athens, GA. The group consisted of five adult females of three and a half years of age, one eighteen-month-old male, and young from previous years. The group was housed in a large wooded pen, divided into three sections, and was supplementally fed daily and provided with water ad libitum. All females were clearly tagged and were allowed to mate with the male. The female group was observed monthly for 1-2 days until an established dominance hierarchy could be recognized and pellets could be collected from all females. Interactions within the female group as well as interactions between females and the rest of the group were observed and recorded. For grooming and fighting sessions, both the initiator and recipient were recorded, and sleeping locations were noted for each female. Pellets were analyzed for progesterone and cortisol concentrations along with pellets collected from Cades Cove using a radioimmunoassay technique. Radioimmunoassay is a procedure by which an unknown amount of hormone is allowed to compete for binding sites, provided by a hormone-specific antibody, with a known quantity of hormone labeled with radioactive

iodine. After the competitive binding has reached equilibrium, the ratio of labeled to unlabeled hormone is used to calculate the amount of hormone present in the original sample.

Laboratory Analysis

Pellets were kept in plastic zipper bags, frozen within two hours of collection, dried in a 50°C oven for 2-3 days, and ground in a grinding mill using a 30 grade screen. Fecal powder was weighed, placed in a citrate buffer solution, and rotated to suspend the steroid. After the suspension was centrifuged to remove solids, a small aliquot of supernatant was taken for assay.

Ether was used for all extractions, as it effectively strips the steroid from its bound protein (Desaulniers et al., 1989; Messier et al. 1990). Extraction consists of adding ether to the aliquot, centrifuging the solution to separate and suspend the steroid molecules in the ether, and snap-freezing the solution in an ultracold freezer; this freezes the aqueous portion of the solution to the tube. The ether solution containing the steroid is then poured off into an assay tube. The ether is evaporated from the tube, leaving the steroid molecules adhered to the sides of the tube. The above procedure was performed twice for each sample to allow for maximum steroid extraction.

The extract was reconstituted using a small amount of ether to clean the sides of the tube and to concentrate the extract at the bottom. The ether was aspirated and the extract was mixed with a solution of phosphate-buffered saline and assayed using a prepared radioimmunoassay kit (ICN Biomedicals, Inc., Costa Mesa CA; Table A-1).

Validation for Progesterone

The following procedure was developed using seven samples collected at random from a group of seven female deer in Cades Cove. These samples were pooled and used in validation tests performed at the Henry Doorly Zoo, Omaha NE. The extraction procedure was initially tested for its ability to recover progesterone. Two hundred μl of progesterone labeled with beta particle-emitting tritium were added to 0.5 g aliquots of the pooled sample. Half of the aliquots were extracted with 5 ml ether, and tritium β-particle emissions were measured for both sets using a liquid scintillation counter. The tritium-labeled sample also was tested for the necessity of using an enzyme to isolate steroids by adding 100 μl glucuronidase enzyme.

The pooled sample, treated with tritium-labeled progesterone, was tested in different amounts ranging from 100 μl to 2 ml to determine an appropriate quantity to be used with the assay. All samples were extracted with 5 ml ether and counted in a scintillation counter for measuring

tritium emissions. Based on the results of this count, the same procedure was used to test a smaller range of sample quantities (50 µl-200 µl). Samples were extracted with ether and counted using an ^{125}I radioimmunoassay kit (ICN Biomedicals, Costa Mesa CA). Results determined which quantity provided the best recovery for this assay kit.

To test for the interference of other compounds present in the fecal pellets, an internal standard, consisting of a 0 ng/ml to 50 ng/ml range of progesterone concentrations, was run within the assay. Any compounds present which may interfere with the accurate measure of steroid would distort the readings from the internal standard.

Following the validation procedure, 62 samples collected from individual deer at Cades Cove were analyzed using the above protocol. An internal standard, as well as the pooled validation sample, was run with these samples to test for further interference.

In September, 1991, the above procedure was revalidated for progesterone to account for differences in equipment and chemicals. The same pooled samples were used early in the revalidation, though in later stages of the procedure, new samples were included that were taken from three captive adult females resident at the University of Georgia Whitehall Experimental Forest.

Recovery of progesterone was tested using two 100 µl samples from one captive female to which was added 15 µl

tritiated progesterone. The samples were extracted with 5 ml ether and reconstituted with 2 ml isooctane instead of phosphate-buffered saline. A second recovery test was performed to test for the most appropriate sample quantity. The pooled Cades Cove sample was tested in quantities of 100 µl, 500 µl, and 1 ml. Recovery of tritium-labeled progesterone was measured by a beta counter, and all samples were assayed using an ^{125}I assay kit.

One ml aliquots of the pooled Cades Cove sample were mixed with six known concentrations of unlabeled progesterone ranging from 0 ng/ml to 25 ng/ml. This procedure was designed to test the effectiveness of the assay in detecting known amounts of progesterone. These samples also were treated with 15 µl tritiated progesterone to test again for extraction recovery. Also included in this assay were pellet samples from the three captive females, collected from October, 1990, to September, 1991. All samples were assayed in an ^{125}I radioimmunoassay.

Although the prepared assay kit reports cross-reactivity percentages for related ligands, three of these, corticosterone, cortisol, and 17α-hydroxyprogesterone, were tested in an ^{125}I radioimmunoassay to determine if they interfered with progesterone readings, since all three compounds are products resulting from the synthesis of progesterone (Table 1). Each of the compounds was measured in three concentrations, with the lowest concentration

Table 1. Cross-reactivity of selected ligands related to progesterone. Reprinted from ^{125}I Progesterone Radioimmunoassay, ICN Biomedicals, Costa Mesa CA.

LIGAND	% Cross Reaction
20α-Dihydroprogesterone	5.41
Deoxycorticosterone	3.80
Corticosterone	0.70
17α-Hydroxyprogesterone	0.67
Pregnenelone	0.41
Androstenedione	0.23
Testosterone	0.16

typical to that measured in assays designed for human blood. The higher two concentrations were added to pooled deer pellet samples and extracted to measure the effects of extraction on the ability of the assay to detect known quantities of related ligands.

Validation for Cortisol

Cortisol levels follow no specific seasonal pattern (Bubenik et al., 1975), as they are dependent on the presence and intensity of stressors in the environment of an animal. For this reason, the validation procedure focused on retrieval of cortisol in measurable quantities, rather than comparing the levels found to known patterns. The laboratory procedure used to detect cortisol was identical to that for progesterone, with the exception that higher quantities of sample (0.5 g) were used to detect the small concentration of cortisol present in fecal material.

To determine the appropriate amount of sample to be used, five pellet samples, two from Cades Cove and three from Georgia, were chosen at random and extracted with 5 ml ether. After being reconstituted with 4 ml ether, the extract was divided into three aliquots of varying quantity, 0.5 ml, 1.0 ml, and 1.5 ml.

The same five samples were extracted again, in duplicate to avoid dividing the extract, and reconstituted with 3 ml ether. To increase the concentration, the initial

amount of dry fecal material was doubled to 1.0 g. Samples were extracted in duplicate and no aliquots were taken to prevent dilution of cortisol.

Statistical Analyses

Numerical data that were analyzed statistically were tested by the Kruskal-Wallis one-way Analysis of Variance. This nonparametric test is used to test the null hypothesis that sets of samples are from the same population or identical populations with the same medians (Siegel and Castellan, 1988).

CHAPTER III

RESULTS

Behavioral Observation: Cades Cove

Observations were conducted in Cades Cove for 20 weeks from 16 December 1991 to 29 May 1992. Four observation periods were conducted each week for three consecutive days. They lasted for three hours in the mornings, beginning at sunrise, and lasted for four hours in the evenings, ending one half hour after sundown. A total of 93 observations were conducted during the 1991-1992 field season for a total of 326 field observation hours. When possible, behavioral data were analyzed by statistical tests; however, most behavioral data collected were of a descriptive nature and were used to describe and categorize observed behavior patterns. Unless otherwise noted, statements regarding activity levels and behavioral trends occurring over time are meant to be descriptive estimates and are not based on quantitative measures or statistical comparisons.

Males: Descriptive Trends

Within the two bachelor herds resident in Cades Cove, there was little interaction among males. Although activity levels were not measured quantitatively, visual observations show that most of male activity was confined to such

solitary behaviors as feeding, sleeping, and walking. Distances of 3-6 meters were maintained between individuals during the weeks prior to the beginning of the November rut, when males dispersed from their herding areas and began moving through the female family groups. Bachelor herds became more concentrated in February, keeping distances of less than 3 meters between nearest neighbors. In the spring of 1992, there were two bachelor herds in Cades Cove, numbering approximately 20 and 35 individuals each. Males remained in their respective fields for the duration of the study, moving on only one occasion, when cattle were temporarily transferred to their resident field. On three occasions, young females were seen in small groups on the periphery of bachelor herds.

Bachelor herds began to break up in mid-October when the first evidence of male fighting was observed. A typical fight sequence began with two males engaged in a parallel walk, followed by one male lowering his head in invitation to his opponent. Confrontations can escalate to an antler lock with or without pushing, with the more aggressive fights lasting for over thirty minutes. Fights can be accompanied by grunting and snorting, although this was only witnessed twice. Males also were seen to smell the hind feet or tails of their opponents prior to a fight or between bouts, and would mount one another. In groups containing

younger males, young tried to interfere with adult confrontations.

In November and early December, sparring increased in frequency, occupying an estimated 50% of an adult male's active time during observation periods. Males began to wander among the female groups until early December, when male courting of females began to be evident. Fighting began to taper off in February, ending in March when adult males were no longer seen traveling through female groups. Less aggressive head-butting behaviors occurred year-round, especially among younger males when their antlers were beginning to grow back. By mid-February, injuries and large scars were observed on adult males.

Courting behavior consisted of a male following a female, head lowered and ears flat. Courting was rejected by females in all observations, approximately 40% of which progressed to a chase. During the rutting season, females stayed together, feeding in the center of the group, paying little or no attention to the fighting males; although, during courting, females in the group became alert, scattering at the slightest movement from the pursuing male. While in pursuit of a female, a male would stop and smell any urine or feces left by the female, showing flehmen afterwards. Flehmen consists of raising the head and curling back the upper lip (Clutton-Brock et al., 1982); its function is not known.

By early January, the boundaries of the small mixed groups were well defined, with as many as four family groups visible within a single large field. While in the family groups, males remained on the periphery of the group, except during courting. They did not defend the group against other males, as in a harem system, nor did they travel with the group from field to field. When family groups retreated into the woods to avoid humans or other interference, larger adult males were the last to remain in the open. Courting by males continued into February, when groups began to congregate in the larger fields, especially in the early morning hours. Mating was not observed.

In March, males began to leave the female groups and return to their bachelor herds. Within the herd, male grooming became more frequent than in the fall, when males had kept larger distances between them. Younger, male offspring of adult females remained with their natal groups. Although during the rut and mating season, males were aggressive and socially dominant to adult females, they quickly lost this high status beginning in mid-February, when they started to shed their antlers. Without their antlers, males were subordinate to adult females in the group, receiving the brunt of female aggression until April, when antlers began to grow back. Within family groups, young males were active and playful, initiating play-fights and chases, and engaging in solitary play. Observations of

aggression among older juveniles were directed toward younger members of the group.

Females: Descriptive and Quantitative Trends

Family groups, consisting of adult females and their recent offspring, remained constant in age composition in observations in which age groups could be defined. The proportion of adults present in family groups was measured daily for six weeks from February to April, 1992, after which age distinctions could not be made. Statistical analyses failed to detect significant changes in the percentage of adults in daily censuses over the six week period ($X^2 = 9.98$; $p = .07$). Deer were active in the fields during the early morning and late afternoon, spending the midday sleeping far out in the pastures. At first light, and continuing until just past dawn, small groups of 5-12 deer entered the fields from wooded sections of the Cove. Their entry was rapid, running across roads and jumping fences. It was during such traveling periods that an estimated 40% of female interactions were observed; the remaining 60% were seen during feeding bouts. Observed interactions between females ranged from affiliative grooming to aggressive fighting (Table 2). Grooming was observed between adult does and their young, as well as within age classes. Grooming consisted of licking the head

Table 2. Interactive behaviors observed occurring among adult females in the Cades Cove herd. Includes both affiliative and aggressive interactions.

Biting: Directed toward opponent's body or near the tail

Charging: Running directly toward an opponent with head down and ears back against head

Chasing: Performed on four legs or with body raised on two legs. Two legged chases are short-lived (< 5 seconds) and are often accompanied by flailing.

Displacement: Movement of a female from an area as a result of an aggressive encounter with another

Flailing: Consists of thrashing forelegs at opponent's face or forelegs, while the body is raised up on hind legs

Kicking: Performed with one foreleg; directed toward opponent's back or chest region

Licking: Affiliative or submissive action; directed toward face, body, or neck of another

Lunging: Quick, sudden jump directed toward a passing female

Parallel walk: Follows posturing in the escalation of an aggressive encounter; involves two opponents facing one another, bodies parallel, with both animals walking sideways

Rearing: Precursor to flailing or chasing; involves raising the body up onto two legs

Posturing: Ears flattened against head with head raised high accompanied by staring at opponent

Pushing: Can be performed with head or chest region; directed toward opponent's head or side of body

Smelling: Directed toward nose, underside of tail, or tarsal gland of another female

Snorting: Sudden force of air through the nostrils; directed toward intruding humans, coyotes, dogs, and other deer

and neck region or the back, and smelling under each others' tails. Both adult and young females were observed mounting one another (n=5). Females fed and traveled in small subgroups of approximately 5-12 animals ($\bar{x}$ = 7.86 ± 2.39), remaining within 3-6 meters of other subgroups. In dense groups, where individual deer were within one meter of one another, or in situations where a group was trapped by fences or traffic, aggressive interactions increased by as much as 100%. Aggressive behaviors ranged from slight changes in posture in which a female would raise her head high with her ears back, to violent fights where females raised up on their hind legs, flailing and snorting (Table 2). Between these extremes lay gentle nosing, pushing, chasing, biting, jumping, and a variety of kicking actions. Females kicked with one foreleg or two, raised on their hind legs or from a standing position, and targeted their opponents' faces, legs, or backs.

Fights were preceded by the attacking female walking sideways toward her opponent with ears back, although some aggressive behaviors, such as kicking, were observed to occur without visible warning. Females were noted running on their hind legs, flailing their forelegs in front of them, during intense fighting. Chasing could be accompanied by hissing, grunting, or headshaking. Approximately 90% of all observed fights lasted less than 10 seconds, consisting of a single attack and resulting in the displacement of the

lower-ranking female. However, resistance from an opponent lead to prolonged fights in a few cases, and in two observations, aggressive counterattacks occurred which resulted in the displacement of the initial attacker.

Family groups were concentrated during the December courting period, with nearest neighbors at distances of less than 3 m, and remained so until the spring. During colder days, groups remained in fields longer than on warmer days, foraging in the mornings as late as 11:00 am and resuming feeding as early as 2:00 pm. Family groups entered fields in the late afternoon from a nearby forested area, running cautiously across the Loop Road. Females proceeded single file into fields, and remained in those fields until dusk. At dusk, small groups of 5-20 animals in adjoining fields converged onto one central field, forming larger groups that numbered as many as 52 animals. Large groups of deer spent the night together either in fields or in neighboring woods. At dawn, the large groups split into smaller groups, which spent the morning feeding until about 10:00 or 10:30 am, when they retreated to the woods or to larger fields for the afternoon.

Movements in fields were led by a single female or a pair. One female would begin walking, followed by a second. This caused a subgroup to break off and follow. Eventually, all females in the group moved to the next feeding area. During movements to other fields, females at the front of

the line were seen to urinate along the trail, their urine being smelled by each member of the line, followed by their urinating on a nearby spot on the trail.

Population Parameters

The overall size of the group changed depending on the time of day, as small groups (5-12 individuals) on the periphery of a wooded area in the early evening, for instance, would congregate with many other groups of similar size in more centrally located fields by nightfall, forming a group of 50 or more. Generally, groups would remain loyal to one field or general area from day to day, moving among fields according to a predictable schedule, and were easy to identify in those fields. Average group size, as determined from data collected while small groups had not yet congregated in the larger fields, varied with the seasons. Average group size ranged from a low of 5.71 ± 3.89 in May to a high of 12.42 ± 9.79 individuals in March (Figure 3). Collection of sex ratio data could not be taken accurately following the antler drop, which began in late February, thus the exact change in group composition during the spring months could not be determined. However, for observations in which sex determinations could be made accurately (n = 94), nonparametric statistical analyses failed to show a significant difference over a six week period in either the

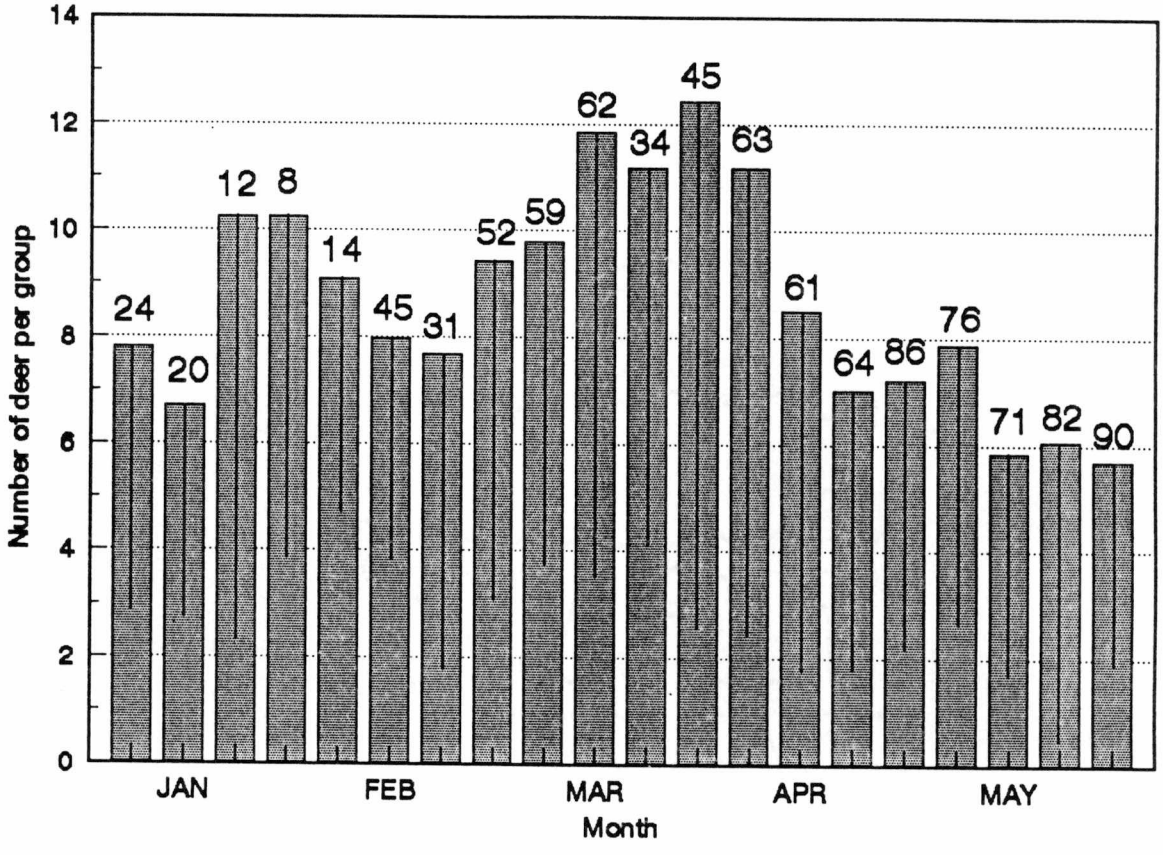


Figure 3. Average size of family groups for white-tailed deer during study period, Cades Cove, Great Smoky Mountains National Park, TN, 1992. Error bars represent one standard deviation. Numbers represent sample sizes.

proportion of males ($X^2 = 4.95$; $p = 0.422$) or of females ($X^2 = 4.61$; $p = 0.466$) present in family groups.

Captive Herd: University of Georgia

Deer were observed in the captive holding pens once monthly for a total of 85 observation hours between 18 January 1992 and 7 June 1992. Observations were conducted for 1-2 days each month until fecal samples could be successfully collected from each female. As in Cades Cove observations, except where otherwise noted, descriptions of general behavioral trends are not based on quantitative data and are not results of statistical tests.

Determining if a dominance hierarchy existed in the captive herd was difficult, since there was little aggression expressed among the adult females. Females acted aggressively toward both the male and the young, although interactions among the adult females were restricted to grooming. Throughout the study period, the hierarchy remained weakly recognizable, with little change in aggression levels even near parturition. Dominance was determined primarily on behaviors seen in movements, grooming, and feeding.

R8 appeared to hold the dominant position, followed by Y2 and LR, who were very aggressive to the young. R19 and B6, both of whom had injuries, seemed to hold the subordinate positions, yielding to the other females during

movements and feeding periods. Y2 was the first to enter the feeding area in all observations and was the most solitary of the group. She was followed in movements by R8 and then by B6 in all feeding observations (n=12).

R8 received grooming from all other females, always keeping her head higher than the other females, but only groomed R19, with whom she also shared her sleeping area. When they were not feeding alone, R19 and B6 fed together in all observations (n=8) and were more interactive with the young than other adults. LR was the most timid of the adults, remaining in the back of the pen during all times of observation and staying close to R8 when R8 was near her. Despite her reluctance to enter the feeding are, LR was aggressive toward fawns and the adult male during movements to other areas of the pen. When in the primary feeding area, LR showed the most aggression toward other members of the group, but showed no aggression to other adult females.

Laboratory Analysis

Progesterone: Cades Cove

Successful sample collection was contingent upon a number of conditions. Interactions must have been observed, individuals must have been recognized and followed through subsequent interactions, and defecation had to be witnessed. Pellet samples must have been located and accurately identified before they could be collected. Following the

determination of the dominance rank of a female, about 80% of all attempts (n≈150) at pellet collection failed due to movements of groups, interference from visitors, or low visibility due to fog, rain, or snow. A total of 25 pellet samples were successfully collected from both dominant and subordinate females between 16 December 1991 and 29 May 1992.

Radioimmunoassay procedures proved to be effective in measuring small quantities of progesterone in fecal samples collected from the Cades Cove population. All progesterone dosages were corrected for initial differences in fecal quantity and are given in ng progesterone per gram fecal powder. Progesterone concentration ranged from 0.24 to 52.8 ng per 0.25 gram of dried fecal material ($\bar{x}$=9.31 ± 14.85 ng/0.25 g). Because of the low number of samples available for analysis, distinct patterns for the two rank groups were not established (Figure 4).

Progesterone: University of Georgia

Progesterone levels during the six-month sampling period were successfully determined for all but four of 26 samples collected from the five females at the University of Georgia facility. Each female was sampled once monthly from January to June, with two exceptions. In March and May, samples could not be collected from female LR, who was reluctant to enter easily viewable areas of the pen. Two

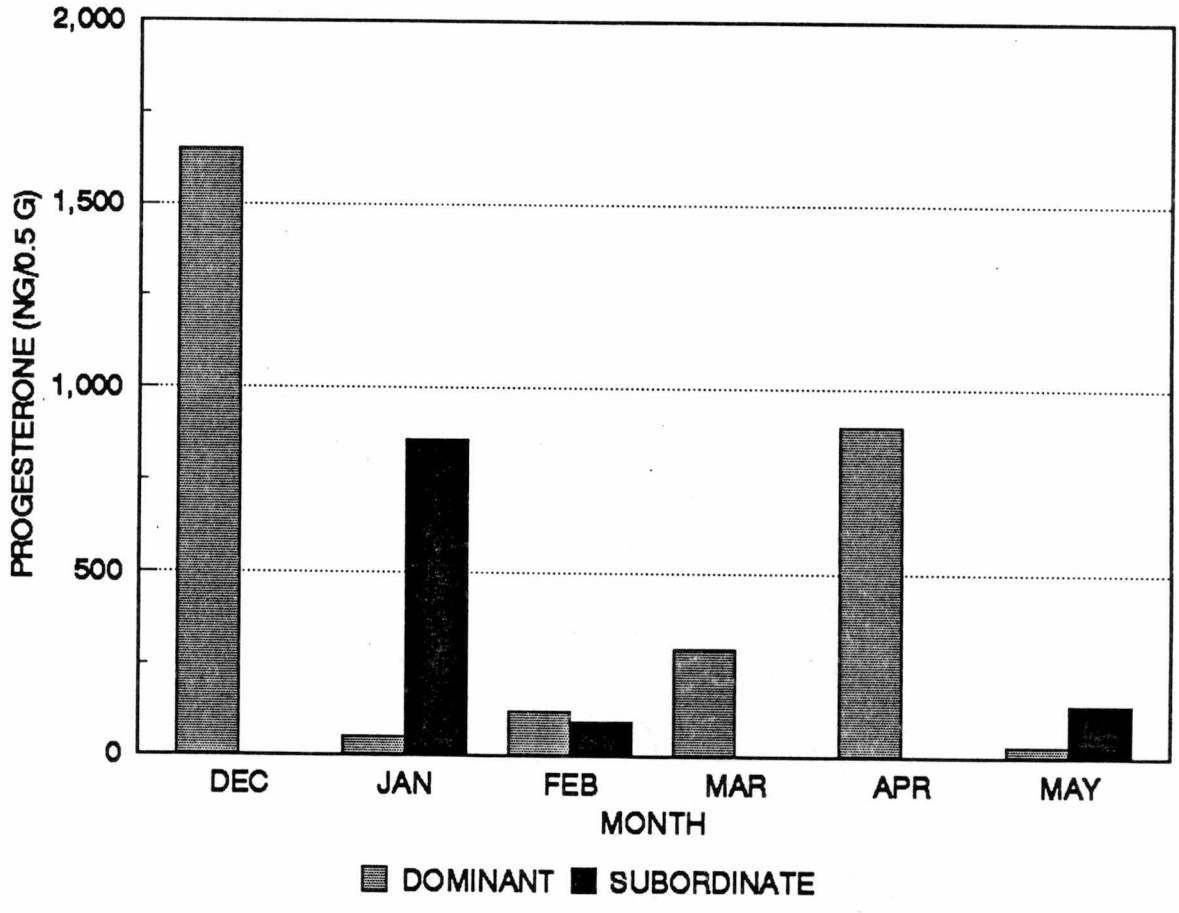


Figure 4. Average progesterone concentration (ng/g feces) for female white-tailed deer in dominant and subordinate rank groups, Cades Cove, Great Smoky Mountains National Park, TN, 1992.

females, B6 and R19, both of whom were injured, were taken from the pen in late April to be used in another study. B6 was put in an indoor stall by herself until May. R19 was placed in an outdoor pen with six other individuals, where she aborted at least one fetus prior to 9 May 1992. Results were highly variable among the females, with each female following an independent pattern of change in progesterone concentration over time (See Figures 5-9). Patterns also varied with respect to the range of concentrations covered, with the samples collected from R19 measuring within a range of concentrations one order of magnitude lower than the other four females. Statistical analyses of progesterone concentration patterns (ng/g feces) of the five females based on six monthly collections failed to show a significant effect of either the month in which a sample was collected ($X^2 = 4.86$; $p = 0.302$) or the rank of individual ($X^2 = 6.74$; $p = 0.150$). The effect of the individual on progesterone was also non-significant, although it was relatively strong ($X^2 = 8.80$; $p = 0.066$). In a test for differences among the five females, mean progesterone concentrations were compared for each female (Figure 10), and no significant difference was found ($X^2 = 6.57$; $p = 0.255$). On 17 May 1992, both females B6 and R19 were euthanized as part of another study, after having been placed on a corn diet for three weeks. At time of death, B6 weighed 44 kg and was pregnant with one fetus of

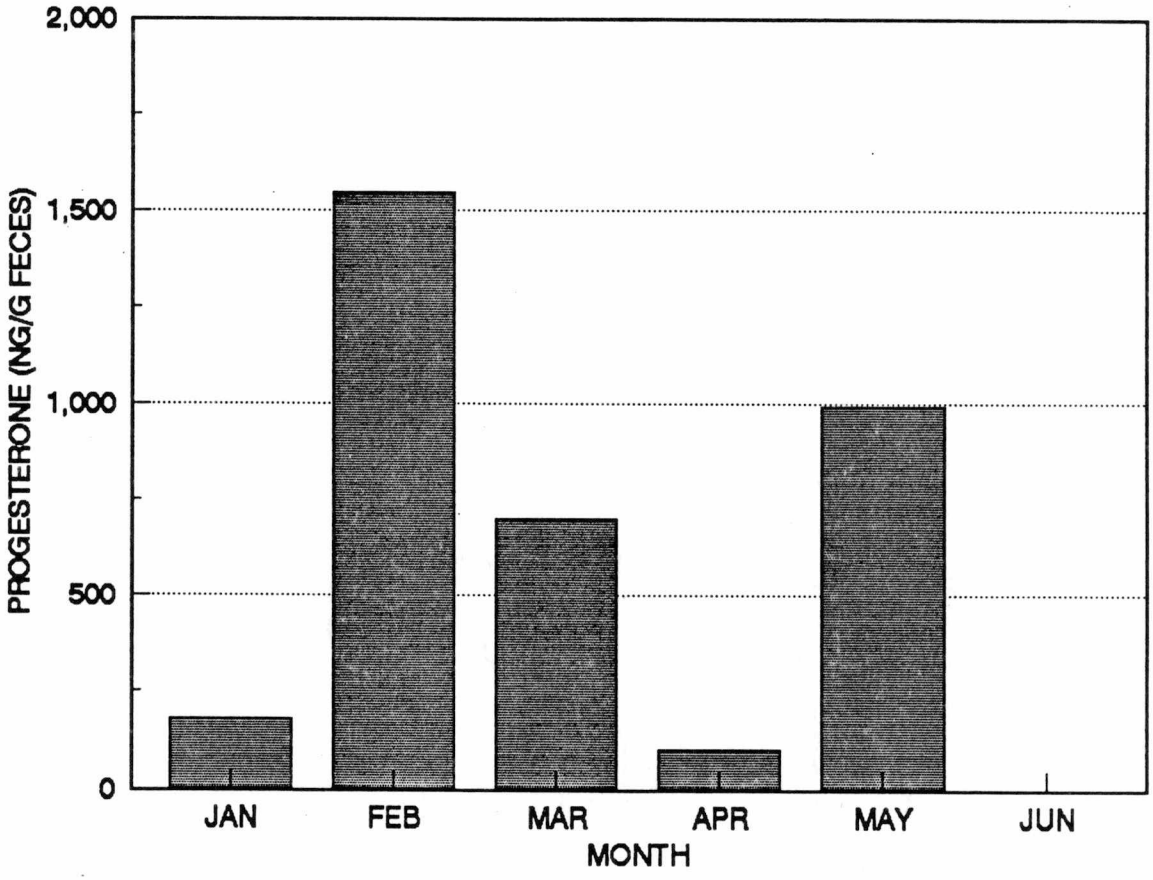


Figure 5. Progesterone concentration (ng/g feces) for captive female R8, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected from January-June, 1992.

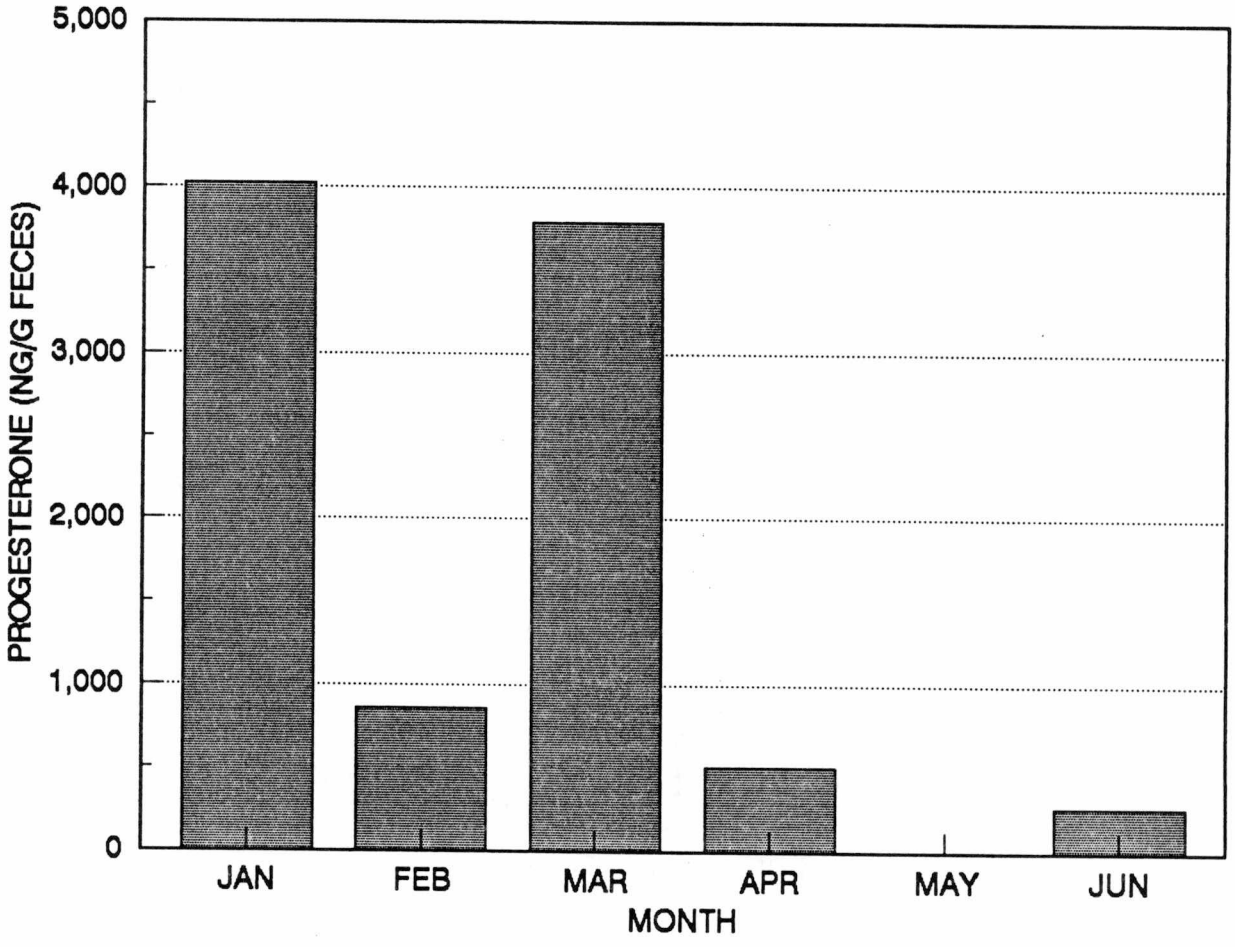


Figure 6. Progesterone concentration (ng/g feces) for captive female Y2, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected from January-June, 1992.

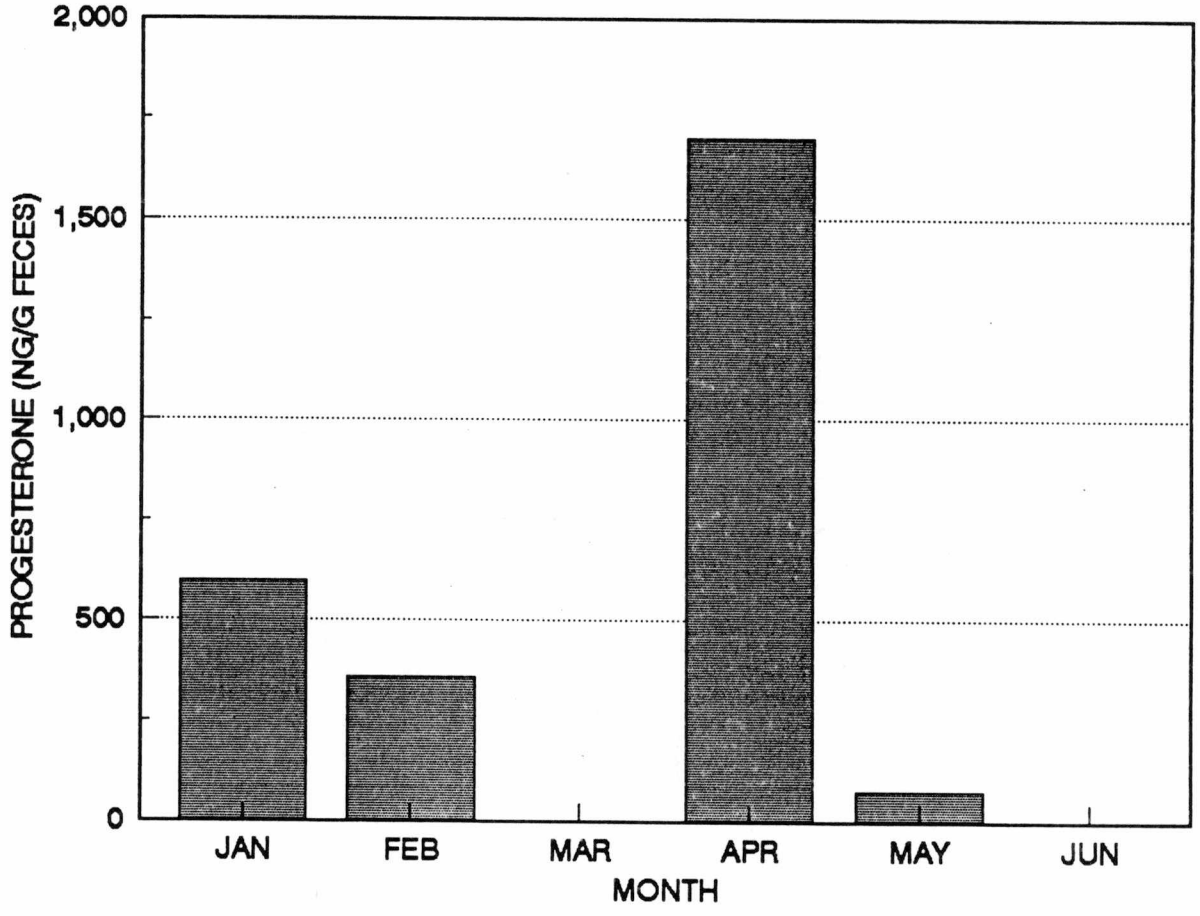


Figure 7. Progesterone concentration (ng/g feces) for captive female LR, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected from January-May, 1992. Data missing for March and June, 1992.

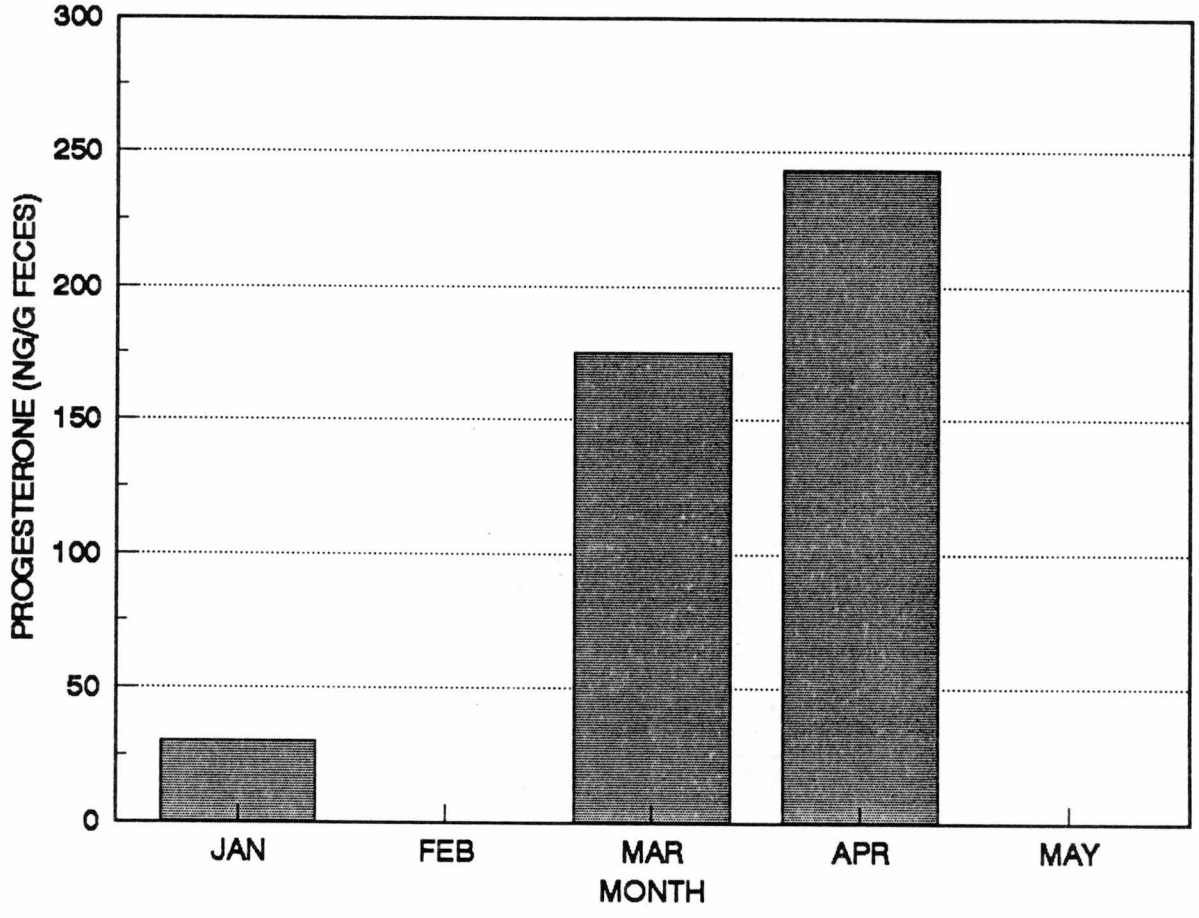


Figure 8. Progesterone concentration (ng/g feces) for captive female R19, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected from January-May, 1992.

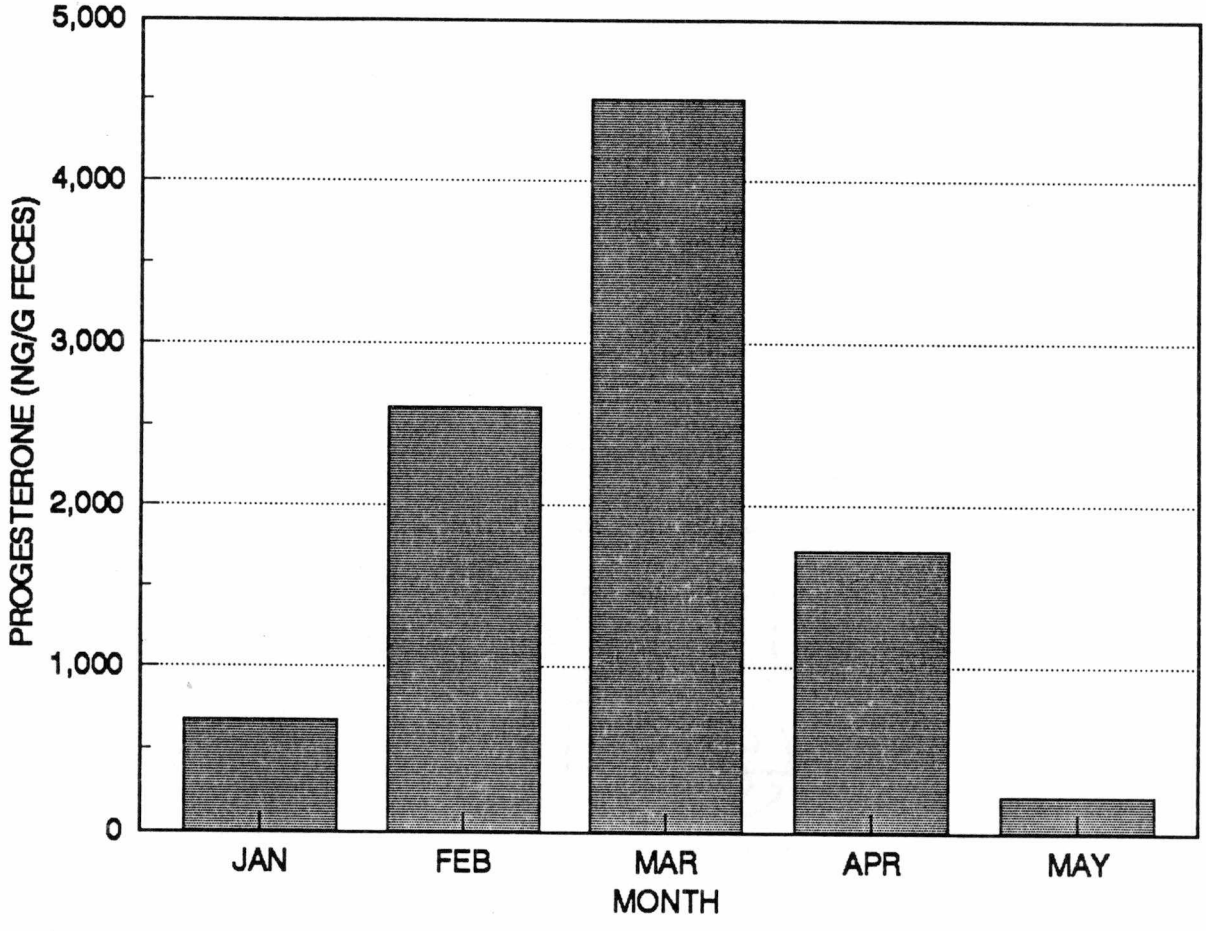


Figure 9. Progesterone concentration (ng/g feces) for captive female B6, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected from January-May, 1992.

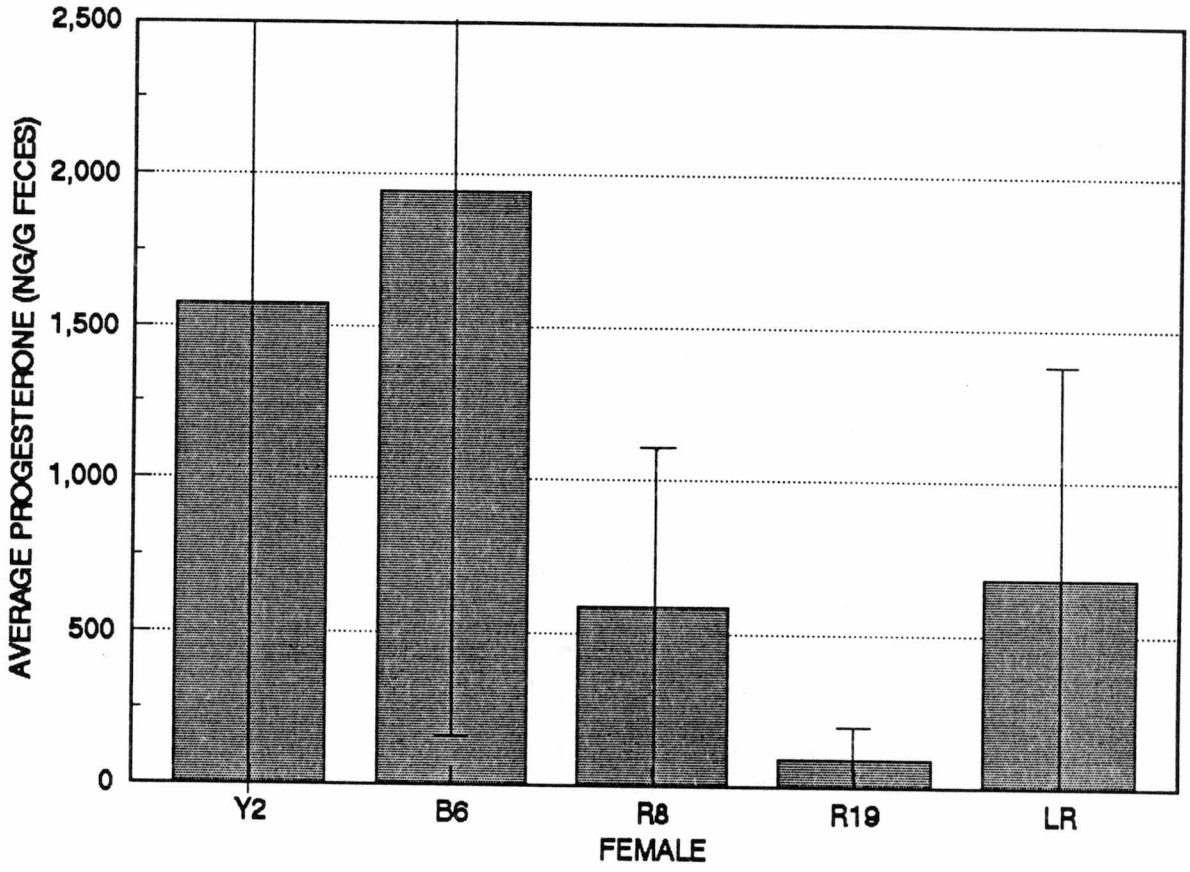


Figure 10. Mean progesterone concentration (ng/g feces) for each of five captive female white-tailed deer, Whitehall Experimental Forest, University of Georgia, Athens, GA. Data collected January-June, 1992. Error bars represent one standard deviation.

approximately 145 days. R19 also weighed 44 kg, and had completely recovered from her abortion; it was difficult, if not impossible, to tell by her regressed uterus that she had been pregnant (L. White, pers. comm.). R8 succeeded in giving birth to one living young, although there may have been others. It is not known whether Y2 or LR gave birth to live young, as their births occurred after the conclusion of the sampling period. Both were pregnant at the time of the June sampling.

Cortisol: Cades Cove and University of Georgia

In an assay of all samples collected in Cades Cove and at the University of Georgia, only ten of 48 samples registered within the range of the standard curve. Binding percentages of these ten samples averaged 79.9% ± 6.3. Measurements of progesterone concentration at this high binding percentage would be inaccurate. Binding percentage (%B, or "percent bound") is a measure of the proportion of radiolabeled progesterone that is bound to anti-progesterone antibodies added in the assay procedure. The relationship between %B and the amount of unlabeled progesterone, present in the initial sample, is inverse and logarithmic; i.e. as %B increases arithmetically, the amount of progesterone in the sample decreases logarithmically. For this reason, measurements made of the amount of progesterone present in samples with a high %B are inaccurate.

Validation for Progesterone

Initial testing of the ability of the extraction procedure to isolate steroids proved that the use of ether was an effective extractant and that the addition of a glucuronidase enzyme did not significantly change the recovery of progesterone. Enzymes were not used in further extractions.

After testing first a broad range and then a more constricted range of quantities, 100 μl of suspended sample yielded the most consistent results and was used for the remainder of the initial stage of the validation. In the first assay for progesterone, steroid concentrations fell well within the limits of the standard curve, and the internal standard showed no interference from related compounds.

In the second assay, containing 62 samples from individual females plus an internal standard, results indicated that samples contained concentrations of progesterone considerably lower than the initial pool of samples and that some were too small to detect with this assay. An attempt to increase the amount of sample used to 200 μl distorted the precision of measurements made by the assay. Although the amount of sample was doubled, the concentration of progesterone measured was increased by 0.02% to 7.15% in twenty randomly selected samples.

When ether was used for both extraction and reconstitution, tritium recoveries averaged 95.1% ± 7.14. Of the assayed samples, only those of either 0.5 ml or 1 ml quantities were able to be measured accurately, and 1.0 ml was chosen for all remaining assays, to be run at a 1:10 sample to ether extraction ratio.

Recovery of progesterone in the second assay of collected pellet samples averaged 107.8% ± 9.5. Typical progesterone patterns, consisting of a small peak in early winter (estrous) and a large peak at parturition, could be detected for two of the three females from the University of Georgia facility, although few samples were available during mid-pregnancy (Figure 11). Progesterone levels were high for all samples, ranging from 6.42 ng to 44.77 ng per 0.5 g sample.

In a test for cross-reactivity of ligands similar to progesterone, though very high concentrations of these compounds were added, the effect on progesterone readings was negligible. In future assays, cross-reactivity reported by the RIA kit was accepted as correct.

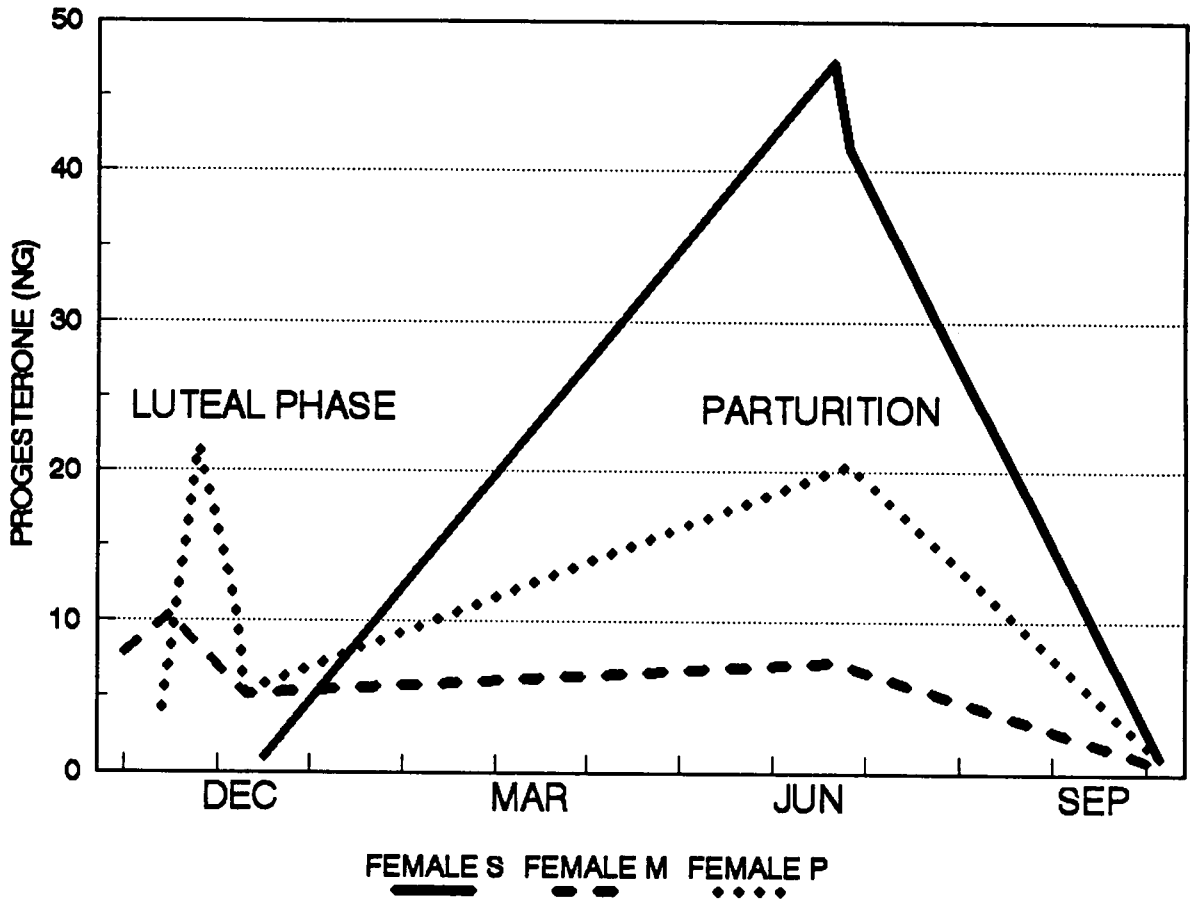


Figure 11. Progesterone concentration (ng/0.5 g feces) from estrous to three month post-parturition, 1990-1991. Data collected from three captive female white-tailed deer, Whitehall Experimental Forest, University of Georgia, Athens, GA.

Validation for Cortisol

In three attempts to increase the concentration of cortisol in samples, assay results showed that cortisol levels consistently fell outside the accurate detection range of the assay. Concentrations that were detected by the assay had binding percentages of 70-95%, indicating cortisol measurements were not accurate.

CHAPTER IV

DISCUSSION

Population Dynamics

Because of the timing of the study period, limited data were collected on changes in the sex ratio and age composition of groups in the Cades Cove population. However, for the period in which sex and age distinctions could be made, there were no detectable patterns in sex or age structure of family group. Because these data were collected during a time of year when single sex groups are beginning to form, it is assumed that movements of the last few breeding males through female groups are responsible for variations in sex composition during the early part of the sampling period. Movements of males also may have affected the age composition of groups by adding to the proportion of adults present in some groups.

Behavioral Observations

General behavioral observations supported information found in the literature on basic patterns and timing of deer behaviors seen in the wild (Marchinton and Hirth, 1984; Putman, 1988). Unlike in woodland areas, where it is common for a family group to consist of only one or two adult females, Cades Cove female groups consisted of many adult

females, that were tolerant of one another except during movements and feeding. This tolerance may be because the females are sisters, or because it is generally safer among prey species to congregate in larger groups when feeding in the open (Marchinton and Hirth, 1984). The formation of large sleeping groups in the late evening may exist as a defense against predation. Group structure was not measured in wooded areas, but may be different from groups in the fields.

Aggressive behaviors were consistent with those reported in other studies, although discrepancies are found with regard to social ranking. Marchinton and Hirth (1984) report that adult males are dominant to adult females, though females in Cades Cove were seen to dominate males of all ages after their antlers had been dropped, even excluding them from a group's feeding area. Males found in female groups in mid-spring were probably previous years' offspring of the females, and they were being encouraged to disperse by their mothers.

In late spring and early summer, young females which have been chased from their natal groups are often seen associating with large bachelor herds, where they are tolerated until mid-summer (Marchinton and Hirth, 1984). In Cades Cove, however, young females were seen in bachelor groups as early as December, prior to the mid-February antler drop. It is likely that these females may have been

small family groups passing through areas inhabited by males and had not been expelled from their own groups. Because bachelor herds occupied large tracts of land, while females in family groups remained more closely associated with one another, small, traveling, family groups may be unable to avoid passing through areas occupied by males in many cases.

Laboratory Analysis

Progesterone: University of Georgia

Plotka et al. (1977) report that all female deer follow the same general pattern of progesterone activity through the gestation period, but it is possible that the ranges of different females will not overlap. For this reason, it is misleading to attempt to detect pregnancy from a single sample, although some serum assay techniques have been shown to provide fairly accurate results (Wood et al., 1986) in detecting progestins. Samples from the five Georgia females show not only different ranges of progesterone levels, but ranges different in scale, by an order of magnitude in the case of female R19 (Figure 12). Superficially, it appears that patterns of progesterone concentration over time are inconsistent with one another, although an analysis of the average concentration of progesterone for each female showed no significant differences. Effects of time on progesterone

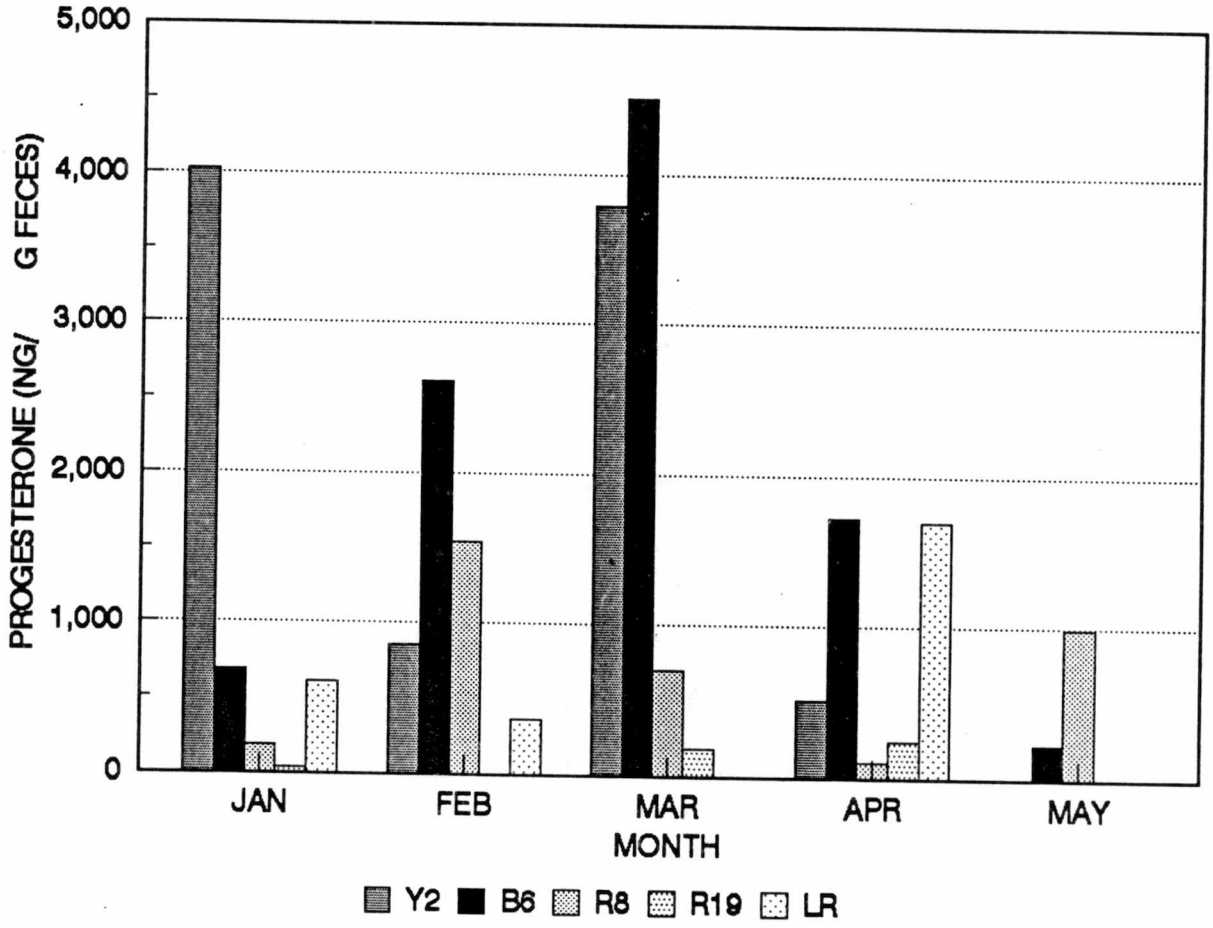


Figure 12. Comparison of progesterone concentration (ng/g feces) for five captive female white-tailed deer, Whitehall Experimental Forest, University of Georgia, Athens, GA. Figure represents data collected post-breeding to parturition, 1992.

concentration also were shown to be non-significant, indicating that the patterns found did not reflect blood levels of progesterone that follow a distinct pattern. However, the analysis did show that there was a low, though non-significant, probability that differences based on the individuals were due to chance. It is speculated that more stringent sampling in this group may have shown more significant differences based on individuals.

The dominance hierarchy appeared stable throughout the study, although a lack of expression of dominance-related behaviors, such as fighting and aggressive posturing, casts some doubt on the ability to accurately detect a hierarchy. Effects of rank on progesterone concentration also were found to non-significant. This might provide further evidence that the hierarchy was not measured accurately; however, this is not a strong argument because individual effects also were found to be non-significant. It is possible also that the ranking order was somewhat artificial as a result of the injuries sustained by two members of the group. Their injuries may have been responsible for their subordinate behaviors.

Conditions surrounding the pregnancies of the five females are not clear. All five females were of the same age, and each had given birth twice before. All females were maintained on the same diet and were housed under the same conditions until April 1992, when R19 and B6 were

removed from the group. It is assumed that all five deer were bred within a few weeks of one another, judging by proximity to the male and stage of pregnancy, although there does not appear to be a consistent response to birth or to the stages of pregnancy in the progesterone levels detected for each of the females. The abortion of R19's young is thought to have been caused by peritonitis, resulting from complications surrounding rumen sampling after her relocation to the new pen (L. White, pers. comm.). The rapid recovery of her uterus remains unexplained.

Progesterone: Cades Cove

Although it was possible to detect varying levels of progesterone for animals in the two rank groups (dominant and subordinate) over a period of five months, the sample size was too low at 25 samples to draw statistically testable conclusions about the reproductive conditions of the animals sampled; additional field collection could have provided the necessary sample size, but the high visitation to the Cades Cove area during some parts of the week limited the time in which sampling could occur. December and January were represented by one sample each. It would have been preferable to have had at least five samples per month from each rank group.

Validation for Progesterone

Henry Doorly Zoo, Omaha, Nebraska. Results of the first validation demonstrated that the assay was effective in detecting some quantities of progesterone in deer feces, though the samples assayed were collected when no deer were present, and were therefore from animals of unknown sex, age or health. The inability of the assay to detect progesterone in some of these samples indicated that further validation was needed, since many of the samples in which no progesterone was detected may have come from males or nonreproductive females.

University of Tennessee. Tests for the ability of the extraction process to isolate progesterone proved that ether was effective both as an extracting agent as well as a reconstitute. Since recoveries ranged 100%, it was unnecessary to run a recovery test with each assay set.

High progesterone levels detected in the final validation assay may stem from a variety of causes. Pipetting error could cause a shift in the range of readings. The use of an incorrect quantity of radiolabel would upset the balance between labeled and unlabeled progesterone in the assayed solution, causing the interpretation that less radiolabeled steroid is present, which in turn indicates an increase in the proportion of unlabeled steroid detected.

An alternative, and more probable, explanation for high progesterone readings would be an additional source of progesterone, which may be inflating the levels of the steroid in the deer sampled. While the assay was designed to detect ovarian progesterone in humans, it is possible that, in ungulates, adrenal progesterone may exist in high quantities. The adrenal gland produces progesterone as an intermediate step in the production of cortisol (Figure 13). In a stressed animal, large quantities of cortisol would be produced. Under extreme stress, the production of cortisol may be overloaded in a sense, creating a surplus of intermediate compounds, including progesterone (Plotka et al., 1983).

Since the assay used is not capable of distinguishing between progesterone produced in different locations, adrenal progesterone may be the cause of inflated readings. The animals sampled for this assay were both held in captive conditions, being placed in small pens during the time of sampling. Although they were born and raised in captive conditions, and were accustomed to the pen surroundings, inferences about their baseline level of stress should be directed toward the concentration of cortisol as well as progesterone.

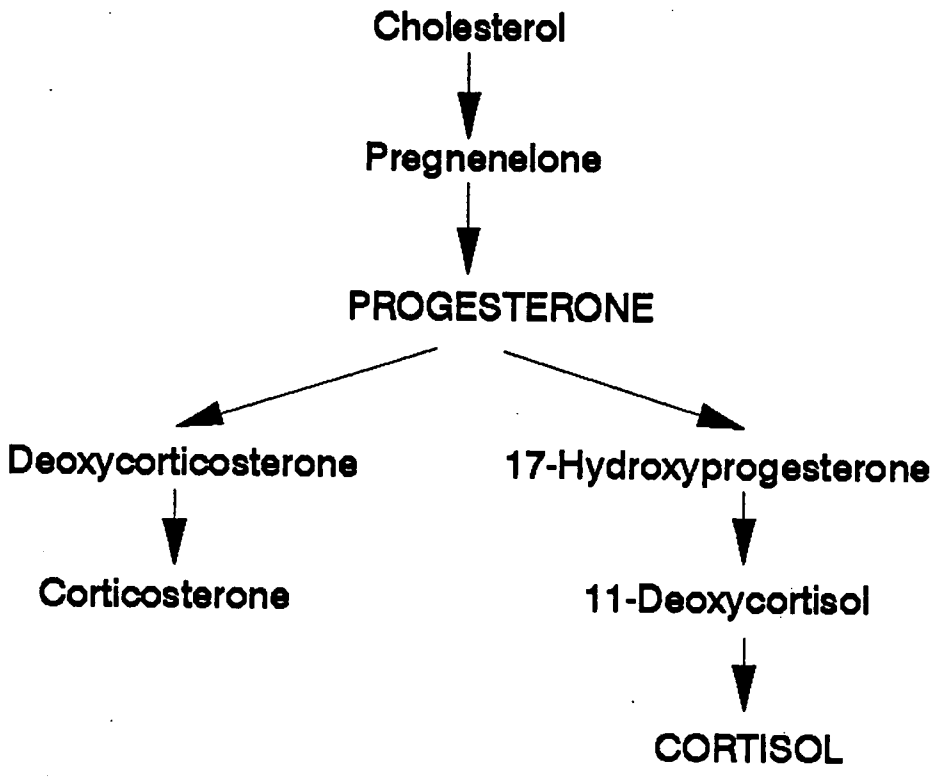


Figure 13. Pathway showing the production of cortisol and progesterone from cholesterol, including several intermediate molecules. Adapted from Norris, 1985.

Validation for Cortisol

Attempts to detect measurable quantities of cortisol in the deer feces were unsuccessful, indicating that quantities of cortisol in feces are lower than the sensitivity of the assay. Binding percentages ranged from 75-90%, which indicates that low levels of steroid are present. High binding percentages are found near the end of the standard curve, where detection of steroid begins to be inaccurate, dramatically distorted by error. Since the assay kit is designed for use with humans, which have cortisol levels ranging from 40-180 ng/ml (Kaplan, 1988), this would explain the inability to detect small concentrations of cortisol. Cortisol levels in deer are an order of magnitude lower than in humans, on average (Bubenik et al., 1975; Seal et al., 1983).

Another factor is the means by which cortisol is processed and excreted in white-tailed deer. Cortisol is produced by the adrenal gland as controlled by the hypothalamus and the anterior pituitary (Kaplan, 1988). Steroid hormones, such as cortisol, are processed differently among animals, entering the blood stream, the digestive system, and eventually being excreted through both the urine and the feces. Non-invasive studies of hormone activity measure quantities of steroid present in the urine, since, among the herbivores, most of the steroids present in the blood stream are excreted through urination (Kirkpatrick

et al., 1988, 1990; Delgiudice et al., 1989; Lasley and Kirkpatrick, 1991). Among carnivores, however, large amounts of steroid are present in the feces, and many studies on both carnivores and herbivores have succeeded in monitoring physiological events through fecal steroid analysis (Kirkpatrick et al., in press; Safar-Hermann et al., 1987; Desaulniers et al., 1989; Messier et al., 1990; Lasley and Kirkpatrick, 1991). The results of cortisol analyses in this study may indicate that cortisol is not present in high enough quantities to be detected in the feces and that much of the cortisol excreted is lost in the urine.

Effects of Density on Population Size

Several opinions exist on whether social stress can be powerful enough to limit the growth of a population. Although results of progesterone and cortisol analyses were inconclusive, this study uncovered a number of new hypotheses both independently and in the literature, which warrant further study.

The same conditions under which the Cades Cove herd exists has been documented in a number of cases, in which a wild, high-density deer population experienced low productivity and population decline in an apparent absence of external regulators (Christian et al., 1960; Welch, 1962 (both quoted in Welch, 1966); Woolf and Harder, 1979; Teer, 1984). These and other studies, including a study of

captive deer (Seal et al., 1983), have postulated that social stress may have influenced the reproductive ability of the females by way of endocrine abnormalities. This suggestion is a point of some debate.

It was the hypothesis of this study that increased stress may cause an elevation of cortisol levels and a subsequent decrease in progesterone levels, which would result in pregnancy dysfunction. Seal et al. (1983), in a study on captive deer, maintained that there is no effect of increasing density on adrenal corticoid activity. This conclusion is similar to that of Woolf and Harder (1979), who found no endocrine abnormalities with increased density, though cortisol activity was not measured. Supporting both these conclusions without contradicting the hypothesis of this study, is a theory suggested by Plotka et al. (1983). In times of extreme stress, a pregnant female will produce high levels of cortisol. In the production of cortisol, several precursors, including progesterone, also are produced (Figure 13). As cortisol production increases, so does that of adrenal progesterone. While increased cortisol can effectively terminate a pregnancy when present in high concentrations, a surplus of adrenal progesterone may supplement the decrease in ovarian progesterone, thus neutralizing the effect of the cortisol (Plotka et al., 1983). If this were the situation in Cades Cove, detection of abnormalities in ovarian progesterone levels could be

clouded by the presence of adrenal progesterone. This could explain the abnormally high levels of progesterone found in the Cades Cove samples.

At the beginning of May, when pregnancy became easily detectable among the females, it appeared that nearly all adult females were pregnant, having carried their fetuses almost to term. The above indicates that, if stress is affecting the productivity of the herd, it is acting late in pregnancy or post-parturition. Data on the parturition and maternal activities of females would provide information on the ability of females in this herd to successfully produce offspring. If stress is acting post-delivery, females may be abandoning their young, or may not be producing sufficient quantities of milk (Clutton-Brock et al., 1982; Verme and Ullrey, 1984; Putman, 1988).

Conclusions drawn from Seal et al. (1983), must be viewed carefully when comparing to the Cades Cove situation, since their study was on captive, hand-reared deer. Deer that are born and raised in captivity may experience a more constant, low-level stress caused by captive surroundings and possible human handling not found in the wild. In this study, average progesterone levels between captive (10.63 ± 14.44 ng/0.25g feces) and wild (9.31 ± 14.85 ng/0.25g feces) deer did not differ significantly, though only five females were sampled in captivity.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Laboratory Analysis

The use of radioimmunoassay procedures to measure fecal steroids provided mixed results. Methods of extraction and assay proved to be sufficient to detect the concentrations of progesterone found in ungulate feces. These methods should be further tested for use in ungulates, particularly in comparison to quantities of progesterone found in the blood of white-tailed deer. Because a single sample of blood or feces is not sufficient to diagnose pregnancy (Plotka et al., 1977), future studies would benefit from marking individual deer and sampling known individuals over time to determine if known patterns of progesterone concentration can be detected accurately. Blood and urine samples need to be collected concomitantly with fecal samples to test for the accuracy of assay readings, as little is reported in the literature as to how steroids are processed and excreted in this species.

The inability of this study to detect cortisol concentration with any accuracy indicated that human-based assay kits should be avoided for use with ungulates. White-tailed deer have cortisol levels far below the range of humans, ranging from less than 10 to 40 ng/ml of serum in

one study (Bubenik et al., 1975) and 40-108 ng/ml serum in another (Seal et al., 1983). Human cortisol levels range on the order of 100-150 ng/ml serum (Kaplan, 1988).

Effectiveness of Fecal Sampling

In a wild herd of white-tailed deer, fecal sampling can be an effective and non-invasive method of collecting physiological data. It does not require anesthesia or trapping, and is essentially cost-free. Implementation of a sampling regime in the field, however, must be cautious and thorough. Obstacles such as human interference, non-visible feeding locations, and lack of cover for viewers must be considered. This method has the potential to be successful, provided ample time and assistance are available. Statistically useful sample sizes cannot be collected by one person, if a large population is to be sampled.

The use of fecal sampling with wild, captive and zoo species has many possibilities. It has been successfully used to study both pregnancy diagnosis and ovarian function in a number of ungulate species (Safar-Hermann et al., 1987; Desaulniers et al., 1989; Messier et al., 1990). It could also be used to monitor the activity of other steroids such as testosterone and estrogen, provided information was available on their excretion rates.

Cades Cove Herd

Past population trends of the white-tailed deer herd in Cades Cove may be a result of natural causes, artificial influences, or a combination of both. It is possible that the herd has reached its carrying capacity and is experiencing natural fluctuations in density as it settles at a stable level. Little studied effects on population density and size such as predation pressure, and dispersal may exert an influence on this population that has not yet been determined. Poaching and predation by domestic dogs may also have an influence on the size of the herd. Density measures should be continually monitored if this phenomenon of density fluctuation is to be understood.

Behavioral studies analyzing patterns and frequencies of specific behaviors could provide valuable information on the population dynamics of the herd. Marking individuals and monitoring changes in rank, behavior, and group affiliation may provide insight on the social structure and stability of dominance hierarchies. Reproductive behaviors, including post-parturition behavior of females must be determined before any conclusions can be drawn regarding the reproductive condition of the herd. With the reintroduction of red wolves to the area, the effects of predation should be monitored, especially with regard to its effect on the changing density of the herd.

Future studies in Cades Cove or other wild herds would benefit by incorporating a control population into their monitoring. A wild herd of lower density would allow for comparisons of both behavioral and endocrinological data as they relate to density. Ideally, this study should be conducted over the course of several seasons to incorporate changes in the dominance hierarchy, and the social organization of the herd.

With respect to the hypothesis set forth by this study, the full effects of density on aggression, and of aggression on stress levels of individuals has not been determined. However, lack of evidence to support this hypothesis in hormonal data does not dismiss the potential for there to be effects of stress on the reproduction of this herd. With further refining of the assay procedure and sampling methods, it will be possible to test this hypothesis more rigorously in Cades Cove and in other populations as well.

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APPENDIX

Table A-1. Assay Procedure (Adapted from ^{125}I Progesterone RIA kit, ICN Biomedicals, Inc., Costa Mesa, CA)

1. Bring samples, antibody, label, and precipitant to room temperature.

2. Add 500 µl phosphate buffered saline and 100 µl 0 ng/ml standard to tubes 1 and 2 to measure non-specific binding.

3. Add 100 µl 0 ng/ml standard to tubes 3 and 4. Continue adding 100 µl of each standard to their respective paired tubes.

4. Add 100 µl sample to remaining paired tubes.

5. Add 200 µl ^{125}I-progesterone to all tubes plus one pair of empty tubes to measure total gamma counts.

6. Add 500 µl anti-progesterone to all tubes except total count tubes and non-specific binding tubes.

7. Vortex all tubes and incubate at room temperature for 60 minutes.

8. Incubate all tubes at 4°C overnight.

9. Add 500 µl precipitant solution to all tubes except total counts.

10. Vortex all tubes and centrifuge at 2000 rpm for 15 minutes.

11. Decant all tubes except total counts and blot tubes before turning upright.

12. Count precipitate in a gamma scintillation counter calibrated for ^{125}I.

13. Compare dosages measured to standard curve determined from tubes containing standards.

VITA

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