SEASONAL CHANGES IN TESTIS MORPHOLOGY AND SPERMATOGENESIS IN ADULT AND YOUNG-OF-THE-YEAR COYOTES (*CANIS LATRANS*)

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SEASONAL CHANGES IN TESTIS MORPHOLOGY AND SPERMATOGENESIS IN ADULT AND YOUNG-OF-THE-YEAR COYOTES (CANIS LATRANS)

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PREFACE

This study is concerned with describing seasonal differences between adult and young-of-the-year coyotes regarding testis morphology and spermatogenesis. Primary objectives are to determine (1) seasonal differences in testis morphology, spermatogenic and indicated reproductive activity, and (2) proportion of males that can produce mature spermatozoa and appear physiologically capable of reproducing during their first year of life.

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CHAPTER I

INTRODUCTION

Historically, management of North American carnivores has primarily involved removal of individuals and populations from areas in which there arise conflict with human interests. During recent years, however, the orientation towards management of communities and ecosystems has increased. Efficient, intensive management of wildlife resources necessitates increased understanding of the ecology of individual species and populations, including larger carnivores. Also important and relevant to investigations of coyote ecology is the emerging demand for the larger carnivores due to their esthetic and recreational values as opposed to indiscriminate killing for alleged economic benefits.

Effective management programs based on sound biological principles represent an imminent need today. Knowledge of reproduction and breeding habits of the male, as well as the female, is of basic importance to management of any given animal species. Effective management of coyote populations requires knowledge of the reproductive biology of this species. Breeding habits and reproductive cycle of the male coyote have been studied in little detail (Gier,

1968). Coyotes are known to be seasonal breeders. Hamlett (1938) found that breeding season of coyotes peak in February, and that males are sterile approximately eight months of the year. However, he made little distinction with respect to seasonal differences in testis morphology and spermatogenic activity that may occur among age groups, particularly between adults and young-of-the-year.

Such factors as population density or changes in habitat conceivably could alter the proportion of young-of-the-year capable of reproducing, thus altering reproductive success. Gier (1968) found that in some years as many as 70 percent of yearling females produced litters, while in other years only 10 percent reproduced. Similar variations are also possible in yearling males. However, factors which influence age of first reproduction and reproductive activity in coyotes are little understood.

Several researchers have studied the effects of age and season on testis morphology and spermatogenic activity of mammals (Kirkpatrick, 1955; Follmann, 1967; Ogle, 1969; Brown, 1969; Chapman and Chapman, 1970; and Christian, et al., 1972). Follmann (1967), in comparing adult and yearling male gray foxes (Urocyon cinereoargenteus) in southern Illinois, found no statistically significant differences in spermatogenic activity with respect to age during the breeding season. Ogle (1969) concluded from his study of coyotes in Washington that puberty may be reached during the first year.

Testis morphology has been used to provide an index to spermatogenic activity in many mammals (Schwartz, 1942; Mossman, 1955). However, gross testis morphology of most seasonal breeding mammals is not necessarily indicative of breeding capabilities (Ecke, 1955; Hoffman and Kirkpatrick, 1956; Dodds, 1965; Bookhout, 1965). Hamlett (1938) indicated that testes of coyotes do not appreciably increase in size during the breeding period, and Ogle (1969) in studying the postreproductive phases of coyotes from March to August, found no correlation between testicular weights and testicular activity. Relationships between seasonal testis morphology and reproductive activity should be studied further to establish whether or not testis morphology varies in relation to season and reproductive activity.

This study was conducted to further observations on the reproductive cycle of the male coyote and to determine (1) seasonal differences in testis morphology, spermatogenic and indicated reproductive activity between adult and young-of-the-year, and (2) proportion of males that produce mature spermatozoa and appear physiologically capable of reproducing during their first breeding season.

CHAPTER II

MATERIALS AND METHODS

A total of 121 coyotes was collected from various areas in eastern and central Oklahoma from December, 1970 until April, 1972. A total of 52 males was obtained, of which 11 were young-of-the-year (Table 1). Animals were collected by trapping with steel traps; by using cyanide "coyote getters", until recently when this technique was outlawed by the federal government; and by "hand calling" and shooting. In the case of trapping, traps were run twice daily and animals caught were shot in the head.

Specimens usually were frozen for later examination, but occasionally freshly killed specimens were necropsied. Frozen specimens were allowed to thaw from 36 to 48 hours, depending on size of animals and length of time frozen.

Body weights were recorded to the nearest pound and later were converted to kilograms (Table 1). Testes and epididymides were removed and preserved in 10 percent neutral buffered formalin. The anterior portion of the lower mandible was removed and frozen so that lower canines could be extracted for aging analysis at a later date. Specimens were aged primarily by examination of dental cementum layers in the canine teeth (Linhart and Knowlton,

Table 1. Mean (± SE) body weight of adult and young-of-the-year coyotes. Number of animals given in parentheses.

Month	Body Weight (kg)	
	Adult	Young-of-the-Year
July	11.8 (1)	- - -
August	- - -	- - -
September	- - -	6.7 (1)
October	11.8 ± 0.0 (2)	10.9 ± 1.5 (3)
November	13.6 ± 0.4 (3)	8.6 (1)
December	15.0 ± 0.5 (10)	14.1 (1)
January	14.1 ± 0.4 (3)	12.3 ± 0.1 (4)
February	13.2 ± 0.5 (8)	13.2 (1)
March	12.7 ± 0.4 (6)	- - -
April	13.2 ± 0.8 (4)	- - -
May	13.2 ± 0.4 (4)	- - -
June	- - -	- - -

1967). Each specimen was aged and a mean age for each month was recorded.

Paired testes volume, to the nearest 0.5 ml. was estimated by displacement of water in a 100 ml cylinder. Smears were obtained from the cauda epididymis (Christian, 1949) and an index to sperm density was recorded using a scale of 0 to 4 (Tiemeier, 1967) (0 = no sperm, 1 = occasional sperm, 2 = frequent sperm, 3 = many sperm, 4 = masses of sperm). This index to density was employed since quantification would yield no better results due to possible factors such as recent ejaculation. Each testis was medially sectioned at right angles to its longitudinal axis with a sharp razor, a thin section was embedded in paraplast, sectioned at 10 microns, and stained with haematoxylin-eosin. Tissue slides were examined microscopically for relative numbers of the different germ cell types and were qualitatively described for each month of the year.

The relative percent area of testis occupied by seminiferous tubules (intratubular area) and/or relative seminiferous tubule diameter have been used by several researchers (Johnson and Buss, 1967; Follmann, 1967; Christian, et al., 1972) as indices of spermatogenic activity. Intratubular area was calculated in the present study using a modification of Chalkley's method (Chalkley, 1943). Tissue sections were projected onto a grid composed of 100 equidistant points and the number of grid points intersecting seminiferous tubules was determined. A total

of 10 fields of each tissue slide was selected randomly and the mean percent of area occupied by tubules was calculated for each testis.

Seminiferous tubule diameter was measured by microscopic examination of the tissue slide at 400X and by use of an ocular micrometer. Ten round, or nearly round, tubules were selected randomly per testis and measured to the nearest micron.

Since age and size of an animal affect organ weight and morphology, significant differences in mean age and body weight among months could account for significant differences in monthly mean organ weights and morphology. Analysis of variance was utilized to test for significant differences among mean age and mean body weights in adults within the breeding season. Student's t-test was utilized to test for significant differences between adults and young-of-the-year concerning testis morphology and spermatogenesis within the breeding season. However, sample size of young-of-the-year was extremely low for several months; therefore, statistical tests could not be used effectively for such times.

CHAPTER III

RESULTS

Age, Weights, and Morphology

Age of coyote specimens ranged from young-of-the-year to seven years. Yearly mean age of adults was three years, and 25 percent of the total sample obtained were young-of-the-year.

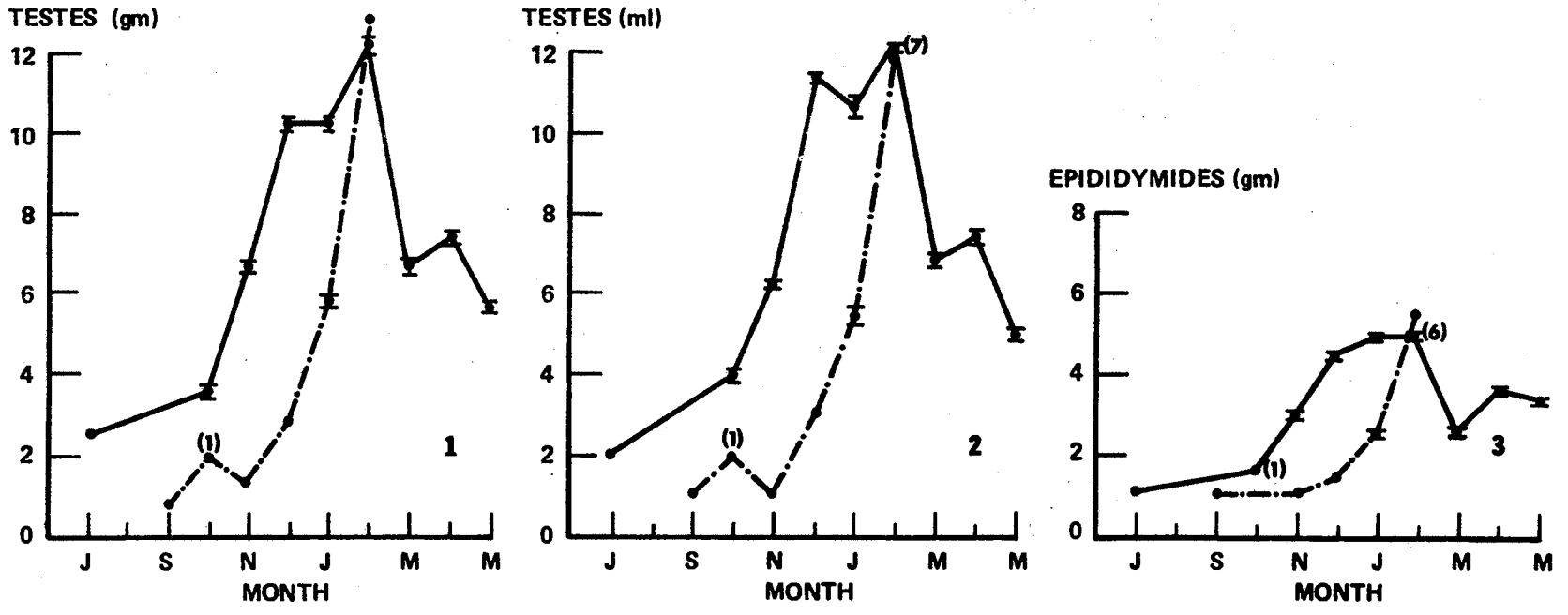
Testes weights of adults increased rapidly from October, peaked in February, and subsequently decreased through May (Figure 1; numbers appearing in parentheses in Figures 1-6 indicate sample sizes that differ from those shown in Table 1). Testes weights of young-of-the-year increased rapidly from November and appeared to also peak in February (Figure 1). However, since no young-of-the-year coyotes were collected during the period of March through August, it can only be assumed that characteristics of reproductive organs peaked in February and did not exceed these values in March. Changes in testes weights of young-of-the-year paralleled but were below those of adults throughout late summer and fall, yet peaked in February at approximately the same weight as those of adults.

Differences in monthly mean testes weights and volumes

Figure 1. Mean (± SE) testes weights of adult (solid line) and young-of-the-year (broken line) coyotes.

Figure 2. Mean (± SE) testes volumes of adult (solid line) and young-of-the-year (broken line) coyotes.

Figure 3. Mean (± SE) epididymal weights of adult (solid line) and young-of-the-year (broken line) coyotes.


TESTES (gm)
12
10
8
6
4
2
0
(1)
1
J S N J M M
MONTH
TESTES (ml)
12
10
8
6
4
2
0
(1)
(7)
2
J S N J M M
MONTH
EPIDIDYMIDES (gm)
8
6
4
2
0
(1)
(6)
3
J S N J M M
MONTH

and monthly mean epididymal weights exist among adults during the period of November through February, and these differences are probably not due to differences in monthly mean age or body weights (Table 1). No significant differences ($P > .05$) in monthly mean age or body weights were found among adults during this period, therefore, observed monthly mean changes in reproductive organ weights and volumes during this period can be considered independent of monthly mean age or monthly mean body weights. Consequently, these changes in monthly mean organ weights and volumes that occur from November through February are assumed to be indicative of spermatogenic activity.

Testes volumes of adults followed a pattern of seasonal change similar to that of testes weights. And testes volumes of young-of-the-year paralleled but were below those of adults, yet peaked in February at a level near that of adults (Figure 2).

There was no significant difference ($P > .05$) between adults and young-of-the-year concerning testes weights or volumes during January. Epididymal weights reached a low during late summer for both adult and young-of-the-year. Weights of this organ began rising in October in adults and November for young-of-the-year (Figure 3). Changes in epididymal weights of young-of-the-year paralleled but were lower than those of adults. Yet, epididymal weights peaked in both age categories at approximately the same time and levels.

Spermatogenesis

July. - Only one adult coyote was collected; its testes were in a quiescent stage (Figure 7). Mature spermatozoa occurred occasionally in the epididymis (Figure 4). Seminiferous tubules contained mostly spermatogonia and only a few primary spermatocytes. Mean percent of intratubular area was still well above the yearly mean of 58 percent (Figure 5), and seminiferous tubule diameter was small (Figure 6).

September. - Only one young-of-the-year was collected during September; its testes contained primarily spermatogonia. Seminiferous tubule diameter, as well as the mean percent of intratubular area, was less than in any other testis examined. No spermatozoa were present in the epididymis.

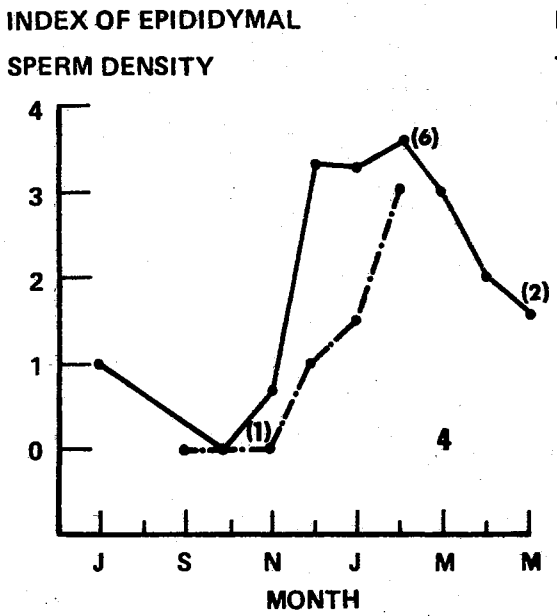
October. - Three coyotes were examined, two adults and one young-of-the-year. The testes of one adult and one young-of-the-year contained principally spermatogonia and primary spermatocytes. The testes of the other adult also contained secondary spermatocytes.

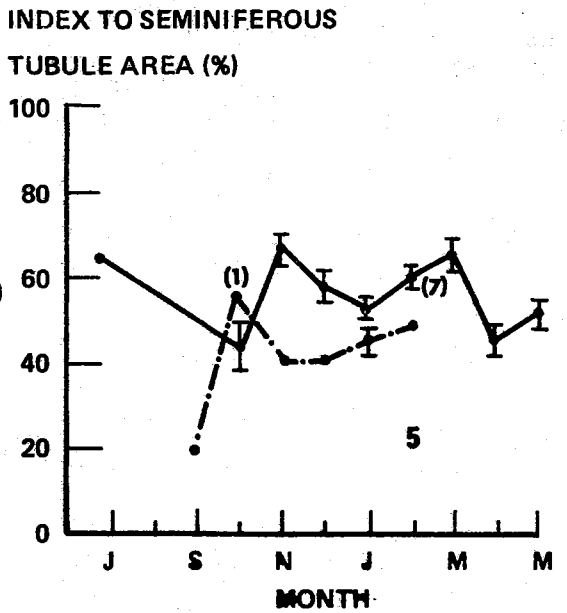
Mean seminiferous tubule diameter (Figure 6), as well as mean percent of intratubular area (Figure 5), of adults reached lowest values during this period. However, mean seminiferous tubule diameter and mean percent of intratubular area of young-of-the-year increased over those occurring in September. No spermatozoa occurred in any of the epididymides.

Figure 4. Mean index of epididymal sperm density of adult (solid line) and young-of-the-year (broken line) coyotes.

Figure 5. Index (mean $\pm$SE) of percent seminiferous tubule area of adult (solid line) and young-of-the-year (broken line) coyotes.

Figure 6. Mean ($\pm$ SE) testis seminiferous tubule diameter of adult (solid line) and young-of-the-year (broken line) coyotes.


INDEX OF EPIDIDYMAL
SPERM DENSITY
4
3
2
1
0
(6)
(2)
(1)
4
J
S
N
J
M
M
MONTH


INDEX TO SEMINIFEROUS
TUBULE AREA (%)
100
80
60
40
20
0
(1)
(7)
5
J
S
N
J
M
M
MONTH

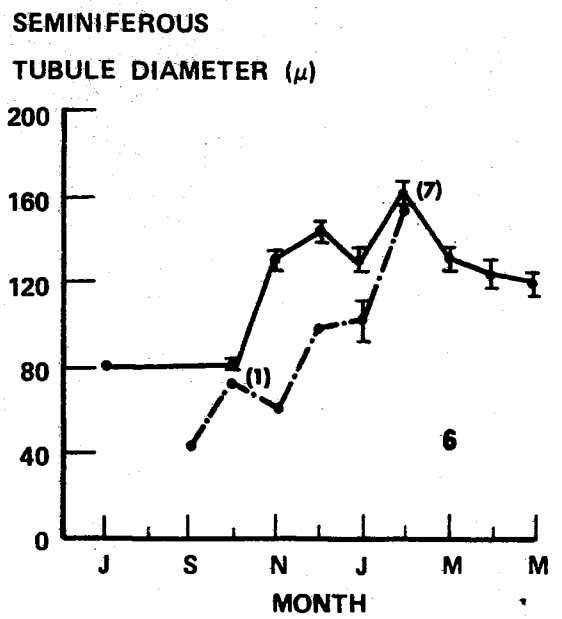
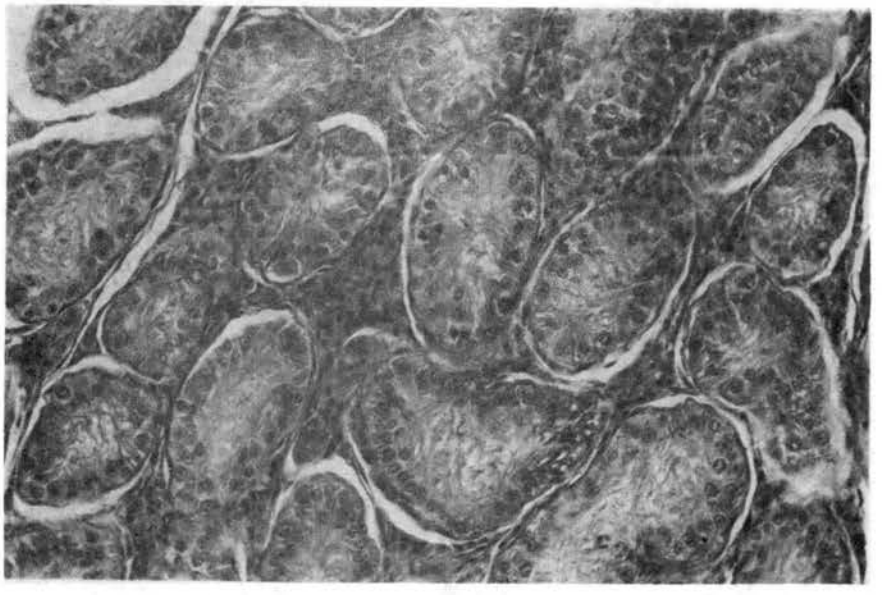
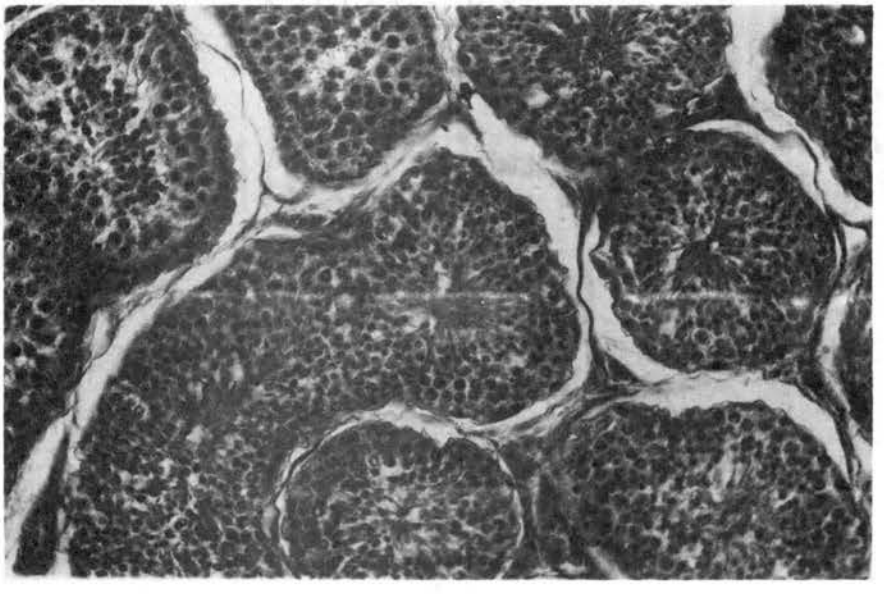

SEMINIFEROUS
TUBULE DIAMETER (μ)
200
160
120
80
40
0
(7)
(1)
6
J
S
N
J
M
M
MONTH

Figure 7. Seminiferous tubules and germinal epithelium in quiescent stage from an adult coyote collected July 29, (X 100).

Figure 8. Seminiferous tubules containing many spermatozoa from an adult coyote collected November 8, (X 100).





November. - Epididymides of two of the three adults examined contained mature spermatozoa. The epididymis of one adult killed November 8, contained spermatozoa, and the seminiferous tubule lumen contained many mature spermatozoa (Figure 8). Mean percent of intratubular area of adults peaked during this period (Figure 5), increasing 52 percent over that present in October, while seminiferous tubule diameter increased 62 percent (Figure 6). However, mean tubule diameter and mean percent of intratubular area of young-of-the-year decreased 20 and 29 percent, respectively, below those of young-of-the-year in October. No spermatozoa were found in the epididymis of the young-of-the-year, and testis development resembled the quiescent stage.

December. - Testes of all adults but one collected during December contained mature spermatozoa in the tubule lumen. Mean seminiferous tubule diameter (Figure 6) increased only nine percent over that of adults in November. Mean percent of intratubular area (Figure 5) decreased 11 percent. Epididymides of all coyotes collected, except one, contained many spermatozoa.

The testes of one young-of-the-year contained only spermatogonia and primary spermatocytes. Mean tubule diameter was 66 percent greater than that of the November young-of-the-year. Mean percent of intratubular area was only slightly greater. Examination of several slides revealed only two mature spermatozoa in the epididymis.

January. - Testes of adults examined during January

Figure 9. Seminiferous tubules from a young-of-the-year coyote collected January 25, (X 100).

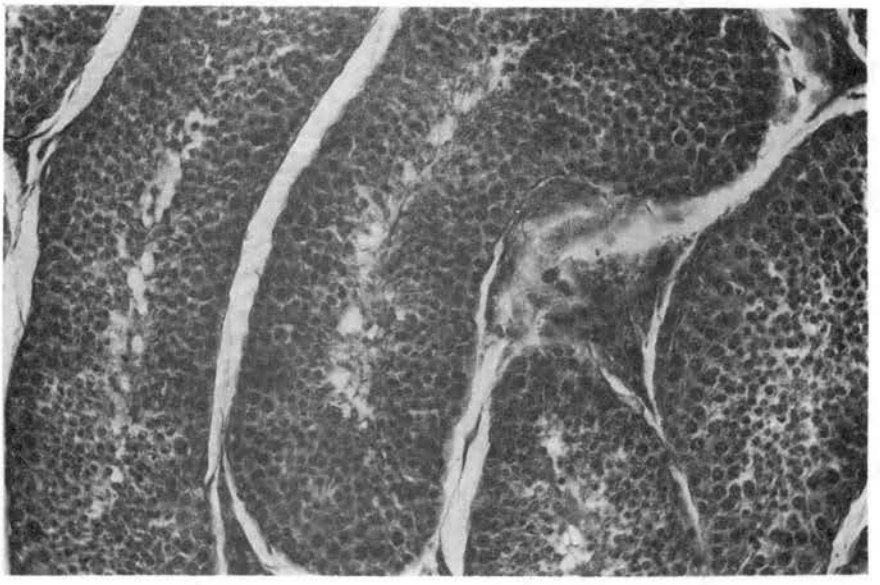
Figure 10. Seminiferous tubules showing germinal epithelial degeneration from an adult coyote collected March 6, (X 100).

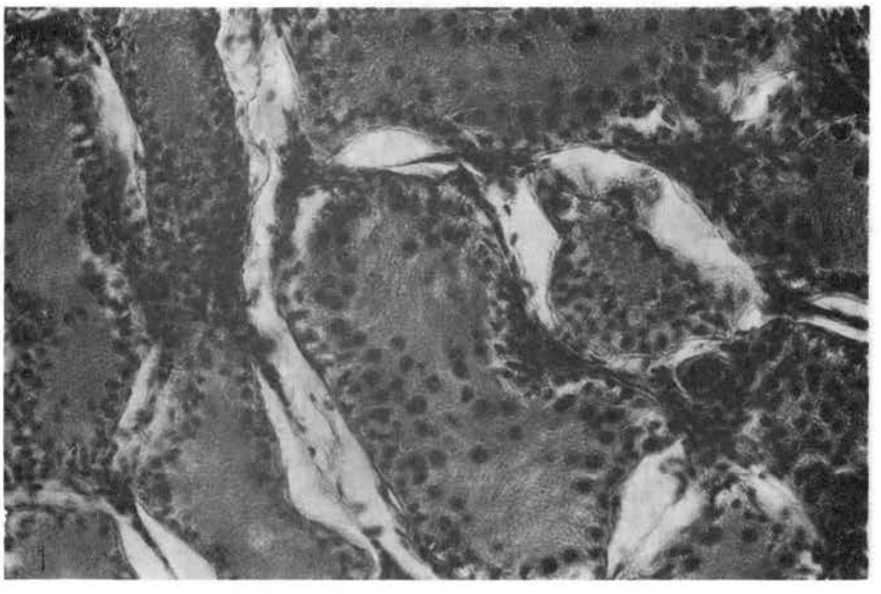
contained mature spermatozoa in the tubule lumen and were in peak spermatozoa production. Epididymides of all adults contained mature spermatozoa (Figure 4). Mean seminiferous tubule diameter and mean percent of intratubular area decreased 10 percent below those of December adults.

Mean seminiferous tubule diameter (Figure 6) and mean percent of intratubular area (Figure 5) of young-of-the-year were four and nine percent greater, respectively, than those of the December young-of-the-year. Mean seminiferous tubule diameter and mean percent of intratubular area of young-of-the-year were not significantly different ($P > .05$) from those of adults during January. Testes and epididymides of all young-of-the-year coyotes contained mature spermatozoa (Figure 9).

February. - Mean seminiferous tubule diameter (Figure 6) of adults peaked during February, increasing 23 percent over those of January; mean percent of intratubular area (Figure 5) increased 15 percent. Testes of all adult and young-of-the-year contained masses of spermatozoa in the tubule lumen and in the epididymides. However, signs of degeneration of the germinal epithelium were evident in some tubules of a few adults, especially by late February.

March. - Mean percent of intratubular area (Figure 5) of adults collected during March showed an increase of eight percent, while mean seminiferous tubule diameter (Figure 6) decreased 18 percent below those of February adults. Testis germinal epithelium showed marked signs of





degeneration (Figure 10), although most testes still contained mature spermatozoa. With the exception of two epididymides, the index value to epididymal sperm density remained high.

April. - Mean percent of intratubular area (Figure 5) decreased 30 percent and seminiferous tubule diameter (Figure 6) decreased 16 percent below those of adults collected during March. Gross disorganization of germ cells occurred in the seminiferous tubules of all testes examined, whereas spermatozoa still were present in the tubule lumen and were associated with much cellular debris. Spermatozoa were present in the epididymides of all coyotes examined.

May. - A 13 percent increase in mean percent of intratubular area (Figure 5) and a four percent decrease in seminiferous tubule diameter (Figure 6) occurred among adults as compared to values of these characteristics in April. Testes of only one coyote contained spermatozoa in the tubule lumen. Most testes contained spermatogonia and primary spermatocytes mainly, some contained a few spermatids. Only two epididymides were examined, and both contained spermatozoa.

CHAPTER IV

DISCUSSION

Males constituted 43 percent of the total sample obtained; male young-of-the-year comprised 25 percent of these. Consequently, this allows only small numbers of young-of-the-year to be distributed among the various months. However, seasonal changes in testis morphology and spermatogenic activity of adult coyotes observed in this study agree essentially with those reported by Hamlett (1938), Gier (1968), and Ogle (1969). Spermatogenic activity is greatest during December through February, reaching a peak during the latter month, and decreasing thereafter.

According to Hamlett (1938) and Gier (1968), mature spermatozoa do not reach the epididymis until mid-December, and male coyotes are not capable of breeding until sometime during January. In male coyotes from Oklahoma, however, mature spermatozoa were present in both the testes and epididymides during November. Consequently, some males may possibly be capable of breeding by early December (Kennelly, 1972). This agrees essentially with findings of Gipson (1972) concerning coyotes in Arkansas. Gipson (1972) observed mature spermatozoa in the testis and epididymis of an adult coyote collected November 22.

The epididymides of six young-of-the-year collected from December through February all contained mature spermatozoa. The epididymis of one young-of-the-year collected in December contained only a few spermatozoa, but, since spermatogenic activity had apparently only begun recently, probably would have contained many spermatozoa at a later date. The other five young-of-the-year were probably physiologically capable of breeding or would have been during February when spermatogenic activity would have been at peak level. Thus, it appears that 80 to 100 percent of the young-of-the-year coyotes are physiologically capable of breeding. This agrees with the findings of Gier (1968), Ogle (1969), and Gipson (1972). Gier (1968) and Gipson (1972) concluded that probably all male coyotes are capable of reproducing, and Ogle (1969) found that puberty in coyotes is reached during their first breeding season.

Changes in gross testis morphology appeared to parallel changes in spermatogenic activity. Testis weight and volume of adults began to increase during October, increased rapidly during March. Likewise, evidence of spermatogenic activity in testes of adults appeared during November, reached peak levels during February, and ceased during March. However, this correlation is in contrast to findings of Hamlett (1938) and Ogle (1969). Hamlett (1938) found that testis weight did not appreciably increase during the breeding season. Ogle (1969), in studying the

postreproductive phases of the coyote, March through August, found no correlation between testicular weights and spermatogenic activity. During this period, however, testes weights were probably reduced drastically. In the present study, mean testis weight was reduced 44 percent from February to March, subsequently becoming more variable during the later months.

Also, seminiferous tubule diameter and percent of intratubular area seemed indicative of spermatogenic activity. Values of these characteristics paralleled values of gross testis morphology and spermatogenic activity throughout the year.

Seasonal changes in testis morphology of young-of-the-year closely paralleled those of adults between October and February. Mean testis weight and volume remained below that of adults until February, at which time testis weight and volume essentially equalled those of adults. It appears that by late February, young-of-the-year are practically indiscernable from adults. Body weight (Table 1), testes weights (Figure 1) and volumes (Figure 2), epididymal weights (Figure 3), and spermatogenic activity are essentially the same for these two age classes.

Effective management of coyote populations could be enhanced with such information as dates of initiation and duration of the breeding season, and proportion of males that appear physiologically capable of reproducing, which have been given in this study. However, additional

information concerning the breeding biology of the coyote is essential for implementation of effective, intensive management plans.

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