TRANSACTIONS, ILL. STATE ACAD. SCI.
Vol. 71, No. 3 (1978)

ANNUAL REPRODUCTIVE CYCLE OF THE MALE GRAY FOX

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ABSTRACT

One testis and epididymis were examined from each of 130 gray foxes (Urocyon cinereoargenteus) collected throughout the year in five southern Illinois counties. Spermatozoa were found in epididymal ducts October to June, but were present in abundance only December through March. Primary spermatocytes along with spermatogonia were present in testes throughout the year. There was a gradual increase in spermatogenic activity in September and culminating January through March. Rapid regression of seminiferous tubules began in March and was virtually complete by April. A comparison of adults and yearlings during January and February showed no statistically significant difference in spermatogenic activity; however, epididymal analysis during this period suggested yearlings began spermatogenic activity later than adults.

The breeding period for gray foxes in southern Illinois extends from about the last week of January to the end of February (Layne, 1958). Females have only one estrous cycle per year. Males become fertile earlier and remain fertile longer than females. Such an adaptation increases the probability for impregnation of most females in the population from year to year.

Previous studies on the male gray fox have been concerned mainly with ascertaining the fertile period by examination of epididymal smears (Layne, 1958; Wood, 1958) and by examination of testicular and epididymal smears (Sullivan, 1956) for the presence of spermatozoa. Testes and epididymides were examined in this study since examination of both organs is more reliable. Separately, neither is fully representative of reproductive condition.

Most comparisons of sexual activity between adult and yearling (animals in their first breeding season) males in various mammalian

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species were concerned with determining whether yearlings began and ended gonadal activity earlier or later than adults (Rowlands and Parkes, 1935; Ecke, 1955; Wood, 1958) and with comparing testis sizes of adults and yearlings (Stuewer, 1943; Wright, 1947; Hoyte, 1955). My study provides a comparative histological study of testes of the two age groups during the breeding season using statistical analysis of quantitative data. Such analyses indicated whether spermatogenesis was maximal in yearling foxes, and whether testes of adult foxes were more productive due to previous spermatogenic activity.

MATERIAL AND METHODS

A total of 130 testes and epididymides was available from gray foxes collected by shooting or trapping in five southern Illinois counties (Union, Jackson, Williamson, Jefferson, and Saline) during 1955 to 1967. Only adult and yearling age classes were established based on tooth wear (Wood, 1958).

In most cases, animals were frozen before reproductive organs were removed and preserved in 10 percent formalin; in other cases, reproductive organs were fixed in formalin soon after collection. Testes and epididymides were embedded in paraffin using the standard technique, cut at 5μ, and stained with hematoxylin and eosin.

One epididymis from each animal was examined for the presence and relative number of spermatozoa. Only sections of the cauda epididymis were examined for those representing April through September. About 15 percent of the epididymides available October through March had been embedded previously, and no identification could be made as to what portion of the organ was being examined; other samples from these months were from the cauda epididymis.

Relative size of seminiferous tubules and level of spermatogenic activity, as determined by the most advanced stage of spermatogenesis present, were recorded for one testis of each animal. For specimens representing November through April, 10 circular or nearly circular cross-sections of seminiferous tubules from each testis were selected for counts of spermatogenic stages. Counts were made of spermatogonia, primary spermatocytes, spermatids, and spermatozoa. Besides spermatozoa present in the lumina, included in this category were cells still embedded in the Sertoli cells but which had the appearance of spermatozoa. The latter are considered by some authors to be late stages of spermatids (Clermont and Leblond, 1955).

Collection of quantitative data on stages of spermatogenesis before, during, and after the breeding season permitted accurate determination of peak activity of testes. This information was correlated with female reproductive activity as reported by Layne (1958).

RESULTS

Testes

In general, testes of gray foxes undergo the same morphological changes as those of other seasonal breeders. During fall, full reactivation of seminiferous tubules of adult males occurs while those of yearlings begin full activity for the first time. Seminiferous tubules were reduced in size and had thickened basement membranes April through November. In December, there was a generalized increase in size of tubules. Tubules attained greatest diameter and exhibited maximum spermatogenic activity January through March.

Spermatogonia were found in testes throughout the year. There was a slight increase of spermatogonia in one animal during August but a general increase did not begin until September.

Number of spermatogonia increased rapidly from November into January after which there was a gradual decline until April (Table 1). The number in April was slightly higher than in November suggesting that there was a further reduction through summer when testes were less active.

Primary spermatocytes were present in testes throughout the year. Although not abundant during summer, their presence indicated that testes did not become completely quiescent but continued activity at a reduced rate.

Testes of two juvenile foxes collected in July were similar to those of adults, including the presence of primary spermatocytes in seminiferous tubules. Presence of primary spermatocytes in these juveniles indicated that the testes of these foxes were active about four months after birth.

Spermatids did not appear in testes until November. In four of five epididymides examined in October, however, a few spermatozoa were present in the tubules indicating that full spermatogenesis had begun possibly as early as September. No cells beyond the primary spermatocyte stage were observed; perhaps only a few seminiferous tubules were active at this time. Numbers of spermatids increased rapidly from November to a peak in January and declined through February and March (Table 1).

Spermatozoa were found in testes from November through April. From April through July these were present only as debris due to sloughing of the germinal epithelium.

Number of spermatozoa increased rapidly from November to December and continued to increase gradually to a peak in March (Table 1). Because the difference in means between February and March was small (16.6) the period of February-March was considered the peak. The results show, therefore, that maximum production coincided with the peak of the breeding season and extended one month beyond it. From March into April there was a sharp decline in numbers of spermatozoa due to decline in spermatogenic activity.

<u>Epididymides</u>

Although spermatozoa were found in epididymal tubules from October

Table 1. The number of cells representing four stages of spermatogenesis in adult and yearling gray fox testes, November through April. The data indicate the number of cells occurring in 10 5μ-thick cross-sections of seminiferous tubules.

		Spermatogonia	Primary Spermatocytes	Spermatids	Spermatozoa
Month	N	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
November	4	254.8 ± 41.7	174.2 ± 32.9	367.8 ± 93.1	554.8 ± 28.6
December	4	363.0 ± 27.8	119.0 ± 12.0	589.0 ± 72.9	737.8 ± 130.8
January	35	399.2 ± 19.7	268.4 ± 18.7	782.0 ± 50.1	759.6 ± 44.1
February	21	343.0 ± 10.3	288.8 ± 21.4	611.9 ± 47.6	849.8 ± 74.4
March	11	321.2 ± 16.1	152.4 ± 17.1	642.3 ± 80.2	866.4 ± 85.6
April	2	299.5 ± 97.5	153.0 ± 85.0	202.0 ± 77.5	569.0 ± 155.0

through June, they were abundant only during December through March. Only animals from the latter period were considered fertile. With the exception of one fox collected in April, it was doubtful that representatives from October-November and April-June were fertile, because only a few spermatozoa were present in epididymides.

Comparison of Adults and Yearlings

A comparison of number of cells representing stages of spermatogenesis of adults and yearlings for January and February yielded no significant differences in means ($P > 0.05$) using the *t*-test. There was great individual variation among animals; but, nothing could be correlated with age or previous reproductive activity.

A difference between adults and yearlings in the quantity of spermatozoa in the epididymides was evident. Combined data for January and February showed that 12.5 percent of adults and 27.0 percent of yearlings had relatively few spermatozoa in epididymides. This indicated that a greater number of yearlings showed delayed spermatogenic activity than adults as in the absence of frequent copulations, few spermatozoa in the epididymis suggests delayed onset of spermatogenic activity (Martan, personal communication). Also, more yearlings had fewer spermatozoa in epididymides in February (33.3 percent) than in January (22.2 percent), while the number of adults showing similar conditions was the same for both months (12.5 percent).

DISCUSSION

Testes

Spermatogenesis is a lengthy process, requiring 39 and 50 days in the rabbit and ram, respectively (Nalbandov, 1964), and about 74 days in man (Heller and Clermont, 1964). No information is available on the period of spermatogenesis in the gray fox, but it can be assumed to be somewhat similar to the above species. This study showed that for the gray fox spermatogenesis was well under way in November which is approximately 2-1/2 months prior to initiation of the breeding season. Spermatogonia, primary spermatocytes, and spermatids reached their highest numbers just prior to or during the breeding season. Spermatozoa peaked during and immediately after the breeding season. The high numbers of spermatozoa observed during December through March coincided with the male fertile period determined by the large numbers of spermatozoa in epididymides during this period.

Spermatogenesis declined quickly after the breeding season. Although cells could be counted in April, regression of seminiferous tubules and sloughing of germinal epithelium had begun in most cases.

Occurrence of primary spermatocytes in testes throughout the year was unusual. It has been generally accepted that a testis in the quiescent state shows only spermatogonia in seminiferous tubules (Bloom and Fawcett, 1968). Spermatocytes evident throughout the year indicated that gray fox testes continued spermatogenic activity at a reduced state. Though not previously recorded for the gray fox, Rowlands and

Parkes (1935) reported primary spermatocytes in testes of a red fox (Vulpes vulpes) in August.

It has been demonstrated in white-tailed deer (Odocoileus virginianus), another seasonal breeder, that the process of spermatogenesis can be maintained at the primary spermatocyte stage for several months (Robinson et al., 1965), as shown for the gray fox in this study. These authors described it as the "primary developmental state", characterized by presence of spermatogonia and primary spermatocytes but few cells of more advanced stages. This condition in deer is not characteristic of the quiescent state but is intermediate between it and the stage of full spermatozoa production.

Epididymides

Presence of large numbers of spermatozoa in epididymides December through March identified this time as the fertile period for male gray foxes. These 4 months bracketed the fertile period of females identified by Layne (1958) as late January through February. Male fertility during this time would increase the probability that a female could be impregnated should she come into heat prior to or after the usual breeding cycle.

Most epididymides from March contained great numbers of spermatozoa while in April only one animal had a sufficient number to be considered fertile. Layne's (1958) data showed that no matings occurred after February. If this is generally true, the drastic reduction in numbers of epididymal spermatozoa from March to April cannot be explained by repeated copulations. Reduction of spermatozoa could have resulted from spontaneous ejaculations or voidance with urine. Dissolution of spermatozoa in the epididymis was not a probable explanation as in the laboratory rat 4 months were required for this to occur (MacMillan, 1954). Occurrence of spermatozoa in urine of male arctic foxes during the breeding season in northern Alaska was noted by the author when urine was collected with a catheter.

Comparison of Adults and Yearlings

Statistical comparison of numbers of cells representing the four stages of spermatogenesis yielded no differences between adult and yearling foxes. Ecke (1955) found similar results in the cottontail (Sylvilagus floridanus mearnsi). Level of spermatogenesis during the breeding season of the gray fox, therefore, was not influenced by age or previous spermatogenic activity. Testes of adult males were not more prolific than those of animals entering their first reproductive season.

Fewer spermatozoa in epididymides of yearling males than in adult males during January and February, strongly suggested that yearlings began spermatogenesis later than adults or that testes of yearlings were slower to reach full activity during the months preceding the breeding season. Either occurrence could reduce the number of spermatozoa available to fill the epididymis even though the rate of spermatogenesis during January and February was equivalent to that of adult males. Lack of sufficient samples of the two age groups during the fall precluded verification of either possibility. Starkov (cited by Asdell, 1964, p. 438) reported that testicular development of yearling red foxes lagged that of mature males.

Various studies (Richards and Hine, 1953; Layne, 1958; Wood, 1958; Lord, 1961) showed that 48-69 percent of gray fox populations consisted of subadult or yearling animals. Wood (1958) also found that 92.3 percent of yearling females produced litters or showed definite signs of sexual maturation. Based on the generally accepted monogamous behavior of gray foxes and evidence that sex ratios do not vary significantly from 50:50 (Richards and Hine, 1953; Layne, 1958; Wood, 1958), it appears that yearling male gray foxes are necessary to mate with the available females in the population. It is biologically sound, therefore, that the majority of yearling males be as fecund as adults during the breeding season as found in this study.

ACKNOWLEDGEMENTS

I thank J. Martan for assistance and guidance during the course of this study; W. D. Klimstra for supplying animals and facilities and for reviewing the manuscript; R. E. Hawkins for review of the manuscript; and W. A. Krumrey for assistance with statistical analyses. This work is a contribution from Project 46, Cooperative Wildlife Research Laboratory, Southern Illinois University and represents a portion of a thesis presented as partial fulfillment of the requirements for the Master of Arts Degree in Zoology.

LITERATURE CITED

ASDELL, S. A. 1964. Patterns of mammalian reproduction. Cornell University Press, Ithaca. 670pp.

BLOOM, W. and D. W. Fawcett. 1968. A textbook of histology. W. B. Saunders Co., Philadelphia. 858pp.

CLERMONT, Y. and C. P. Leblond., 1955. Spermiogenesis of man, monkey, ram and other mammals as shown by the "periodic acid-schiff" technique. Amer. J. Anat., 96:229-254.

ECKE, D. H., 1955. The reproductive cycle of the Mearns cottontail in Illinois. Am. Midl. Nat., 53:294-311.

HELLER, C. G. and Y. Clermont., 1964. Kinetics of the germinal epithelium in man. Recent Progress Hormone Research, 20:545-575.

HOYTE, H. M. D., 1955. Observations on reproduction in some small mammals of arctic Norway. J. Anim. Ecol., 24:412-425.

LAYNE, J. N., 1958. Reproductive characteristics of the gray fox in southern Illinois. J. Wildl. Mgmt., 22:157-163.

LORD, R. D., Jr., 1961. A population study of the gray fox. Am. Midl. Nat., 66:87-109.

MACMILLAN, E. W., 1954. Observations on the isolated vaso-epididymal loop and on the effects of experimental subcapital epididymal obstructions. Studies on Fertility, 7:57-64.

NALBANDOV, A. V. 1964. Reproductive physiology. W. H. Freeman and Co., San Francisco. 316pp.

RICHARDS, S. H. and Ruth L. Hine. 1953. Wisconsin fox populations. Wis. Cons. Dept., Tech. Wildl. Bull. No. 6. 78pp.

ROBINSON, R. M., J. W. Thomas and R. G. Marburger., 1965. The reproductive cycle of male white-tailed deer in central Texas. J. Wildl. Mgmt., 29:53-59.

ROWLANDS, I. W. and A. S. Parkes., 1935. The reproductive processes of certain mammals. VIII. Reproduction in foxes (*Vulpes* spp.). Proc. Zool. Soc. Lon., B105:823-841.

STUEWER, F. W., 1943. Reproduction of raccoons in Michigan. J. Wildl. Mgmt., 7:60-73.

SULLIVAN, E. G., 1956. Gray fox reproduction, denning, range, and weights in Alabama. J. Mammal., 37:346-351.

WOOD, J. E., 1958. Age structure and productivity of a gray fox population. J. Mammal., 39:74-86.

WRIGHT, P. L., 1947. The sexual cycle of the male long-tailed weasel (*Mustela frenata*). J. Mammal., 28:343-352.