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BULLETIN

**The Reproductive Cycle
of the Raccoon in Illinois**

**C. Sanderson
Nalbandov**

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**VOLUME 31, ARTICLE
JULY, 1973**

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VOLUME 31, ARTICLE 2
JULY, 1973

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This report is printed by authority of the State of Illinois, IRS Ch. 127, Par. 58.12. It is a contribution from the Section of Wildlife Research of the Illinois Natural History Survey.

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The Reproductive Cycle of the Raccoon in Illinois

Glen C. Sanderson
A. V. Nalbandov

ALTHOUGH THE RACCOON (*Procyon lotor*) is a commonly recognized, widely distributed, and abundant North American mammal, little has been known about its reproductive cycle except the season of birth, the number of young per litter, and the duration of the gestation period. Basic information on the length of the estrous cycle, whether ovulation is spontaneous or induced, the period of sexual activity in the male, the occurrence of pseudopregnancy, the roles of the various hormones in reproduction, and the anatomy of the reproductive tracts has been either lacking or fragmentary.

The objectives of this study were to gather data on the reproductive cycle and the basic anatomy of the reproductive system of the raccoon and to investigate those aspects of the raccoon's reproductive physiology that gave promise of increasing our knowledge in the general field of mammalian reproductive physiology. This study was part of an effort to obtain a refined understanding of the population dynamics of the species. Other aspects of the study will be published elsewhere.

ACKNOWLEDGMENTS

Prior to 1961 this work was supported by Illinois Federal Aid Project W-56-R, the Illinois Department of Conservation, the U.S. Bureau of Sport Fisheries and Wildlife, and the Illinois Natural History Survey, cooperating. During 1961, 1962, and 1963 partial support for these studies was contributed by the National Institutes of Health under Research Grant

7849. The remainder of the support for this study was provided by the Illinois Natural History Survey.

We thank Dr. T. G. Scott, former Head of the Section of Wildlife Research at the Survey, for his encouragement and advice throughout the study, and Dr. H. W. Norton, Professor of Statistical Design and Analysis in the Department of Animal Science at the University of Illinois College of Agriculture, for his help with the statistical analyses. We also thank Dr. Jean W. Graber, former Research Assistant Professor of Animal Science, University of Illinois, who prepared most of the histological sections and provided other valuable assistance, and the senior author's wife, Beverley C. Sanderson, who drew the sketches of the male and female reproductive tracts and helped in many other ways. W. D. Zehr, Illinois Natural History Survey Technical Photographer, and G. G. Montgomery, former Survey staff member, assisted with the photomicrographs. R. J. Ellis was employed as Research Associate on Project W-56-R from February 1, 1961 through June 30, 1962 and contributed specimens and other assistance to this study. G. G. Montgomery was employed as Research Associate on Research Grant 7849 and made many contributions to the study. C. L. Foley, Illinois Department of Conservation, Paris, Ill., supplied live raccoons for this study and was helpful to the project in other respects. Present and past employees of the Natural History Survey who contributed specimens and information to the study include Dr. B. J. Verts, Dr. G. L. Storm, Dr. R. D. Andrews, Dr.

Frantispiece.—Cages used to hold raccoons in Urbana, Ill. Each double cage held either one pair, one female and her young, or 1-3 adult raccoons in each half. The outside dimensions of the cages were 3 feet (width) X 4 feet (height) X 6 feet (length). Each cage was divided crosswise through the middle with wire and had a nest box on each end. The wire was 1.5-inch mesh, 14-gauge hexagonal netting. The nest boxes had wire bottoms, and wooden bottoms were inserted on top of the wire in winter. A hinged wire top on each nest box fitted under the removable lid.

R. R. Graber, and others. Helen C. Schultz of the Survey staff and Robert M. Zewadski, Associate Technical Editor of the Survey, edited the manuscript.

Dr. H. W. Norton and Dr. A. Sydney Johnson, Associate Director, Institute of Natural Resources at the University of Georgia, Athens, reviewed the manuscript and made many valuable suggestions.

We are especially grateful to Clifford, Albert, and Robert Perardi (Perardi Brothers Fur and Wool Company, Farmington, Ill.) for their active and enthusiastic cooperation with our study.

METHODS

SEASONAL CYCLE OF THE GONADS

Each year from 1955 through 1961 the senior author examined dead raccoons at a number of fur houses in central Illinois. The majority of the raccoons were examined at Farmington in Fulton County and Colchester in McDonough County. Most or all of these animals came from within the range of *Procyon lotor hirtus* (Goldman 1950:24). During the hunting and trapping season, which usually occurred during November through January (but occasionally included late October), large numbers of recently killed raccoons were sold to fur-buying establishments and pelted. Often a majority of the acceptable carcasses were dressed and frozen prior to being sold for human food. Thus, from the large number of raccoons examined, numerous data were recorded and many organs suitable for gross examination were collected as the animals were being skinned.

The present report deals principally with the reproductive organs of the raccoon. Before the animals were skinned, one testis and epididymis were removed from each male, and the condition of the nipples of each female was recorded. All pertinent information was recorded separately for each animal. After the raccoons were pelted, the complete reproductive tracts were removed from fe-

males and were placed separately in 1-pint plastic bags to prevent the tissues from drying. Each plastic bag was placed in a small paper bag on which the data were recorded.

The specimens were usually examined in the laboratory the day after collection but sometimes were examined on the day they were collected. The testes were weighed to the nearest 0.1 gram. A drop of fluid collected from the tail of the epididymis was diluted with a drop of normal saline solution and examined under the microscope for the presence of sperm. Both ovaries were examined visually and weighed to the nearest 0.1 mg.

Raccoons found dead or collected by trapping and shooting specifically for autopsy were processed in the same general manner as those examined in fur houses. A small number of raccoons, obtained from sources other than fur buyers, came from the southern and eastern sections of Illinois within the range of *P. l. lotor* (Goldman 1950:24).

Gonads from both sexes were collected from adult and juvenile raccoons each month. Several gonads were removed immediately after the deaths of the animals and were preserved and prepared for histological study. The average monthly weights of the gonads from all of the raccoons studied, both those freshly killed and those dead for several hours, were used in constructing graphs showing the seasonal gonadal weights for juveniles and adults of both sexes. Histological examinations of the testes, epididymides, ovaries, and uteri contributed information regarding the seasonal sexual cycle.

CAPTIVE RACCOONS

For many phases of the study captive raccoons were kept in outdoor cages in Urbana, Ill. Most of these animals were trapped in the wild, both as adults and juveniles, and some as small young, mostly in Champaign, Piatt, Edgar, and Carroll counties, Ill. We estimated the ages of wild raccoons at the times of their cap-

ture (Sanderson 1961a). Some animals used for the study were born in captivity—some were conceived in captivity and others were born in captivity to females that were pregnant when captured.

Captive raccoons were usually paired and held as one male and one female per cage. Pregnant females were isolated prior to parturition; the males were not returned while the young were with the females. Some females were isolated to determine whether ovulation in the raccoon is induced or spontaneous. In some cases three or more animals—juveniles of both sexes and surplus males—were held in a single cage.

The captives were given fresh food and water daily. The main diet was Dog Chickers or Laboratory Checkers, manufactured by the Ralston Purina Company. Occasionally the diet was supplemented by chickens, fish, eggs, and other available fresh foods.

Captive raccoons that died or were killed were processed as described above, except that all of the gonads, after being weighed, were preserved for histological study. Usually a section of the uterus and occasionally accessory organs of the reproductive tract were also preserved for histological examination.

Males

The annual reproductive cycle in several captive male raccoons was determined by restraining each male in a wire cone at irregular intervals throughout the year and collecting a drop of fluid from the tail of the epididymis. The tail of the epididymis was forced against the skin of the scrotum; then a pointed scalpel was used to prick through the skin, and a drop of fluid was collected on a glass slide. The drop was diluted with normal saline solution and examined under the microscope for the presence of sperm. After the collection of the epididymal fluid, the animal was returned to its cage with no further treatment. No infection or other troubles resulted from this treatment.

Occasionally, a captive male, or a wild

male that had been livetrapped and was to be released at the point of capture for another phase of the study, was unilaterally castrated to obtain a testis and epididymis for study. Captive males that fathered young were assumed to have had sperm in their epididymides at the time that they impregnated the females.

Females

The reproductive cycle of captive female raccoons was studied by examining the ovaries and uteri during laparotomies of anesthetized animals. The anesthetic used was pentobarbital sodium administered at the rate of 1 cc per 4 pounds of body weight. Given intraperitoneally, it usually produced surgical anesthesia in 10–30 minutes; however, individual responses to the anesthetic varied, and animals that required more anesthetic were given larger doses the second time laparotomies were performed.

The raccoon is resistant to infection and withstands surgical incursions well. Instruments were washed in 70-percent alcohol but were not sterilized. As many as 12 laparotomies were performed on one female over a period of several months, sometimes on subsequent days, sometimes two or three times in 1 week, but usually from 2 weeks to several months apart. Animals were usually given penicillin after each operation although no infections developed when it was not used. Surgical silk or cat gut was used to close the peritoneal linings and muscle; these sutures were not removed until a subsequent laparotomy was performed. Wound clips, used to close the skin, were removed approximately 10 days after the operation.

To examine ovaries for evidence of ovulation, it was usually necessary to slit the ovarian capsules. Because the cut edges of the capsules did not always grow together, this procedure was omitted when examining females that were being held to produce young. The uterus was gently withdrawn from the body cavity for examination and gross measurement.

In several cases one or both ovaries were removed for study. Uterine sections were taken from living females for histological study of the development of the endometrium.

MEAN BIRTH DATE OF RACCOON LITTERS

The mean date of birth was determined for 20 litters conceived in the wild in the northern half of Illinois. Of these 20 litters, 7 were born in captivity. The potential birth dates of the others, most of which were examined in female raccoons found dead along roadways, were estimated by measuring the uterine swellings in the manner described by Llewellyn (1953:321). Data obtained during the present investigation were also used in estimating the probable birth dates. Because Llewellyn (1953:321) recorded measurements of only three embryos in one litter at three different stages and at birth, several embryos were measured in captive females during this study. Although the dates of conception were not known, the maximum measurements of the uterine swellings were plotted in relation to the number of days prior to the known birth dates. Many wild females were examined throughout the year for pregnancy, lactation, and the presence of fresh placental scars and corpora lutea. This information helped to determine the limits of the breeding season in wild raccoons.

SECONDARY SEX RATIOS

Secondary sex ratios were obtained by examining 83 embryos and young at birth in 26 litters and by determining the sex of 54 wild raccoons less than 2 months old from 23 litters. Chi-square tests were used to test whether the sex ratio of the wild young less than 2 months of age was different from equality and from the ratio of the embryos and young at birth.

ESTROUS CYCLE AND OVULATION

Estrous Cycle

Estrous cycles were determined for individual captive female raccoons by

examining the ovaries at or near ovulation and then reexamining the ovaries at intervals until the animals ovulated again.

The raccoon's main breeding season was interrupted throughout much of Illinois by colder-than-normal temperatures and deep snows in 1960. Observations of live-trapped raccoons and the body weights of young, wild raccoons weighed during the fall and winter of 1960 indicated that some raccoons were born later than normal during that year. Lenses collected from several young raccoons during the hunting and trapping season of 1960-1961 were used to estimate the months of birth for these juveniles (Sanderson 1961*b*:482-485). The time intervals between the peaks of estimated birth dates were assumed to represent the average interval between ovulations for wild raccoons in central Illinois.

Cotton swabs were used to take daily vaginal smears from several captives in an attempt to delineate the estrous cycle. Observations of vulval swelling, size and pigmentation of the nipples, and general disposition of the animals were made each time the animals were handled. Vaginal tissues were removed from several females for histological study.

Ovulation

Each of two females was placed alone in a small cage in the fall of 1960 to obtain information on the mechanism of ovulation and on pseudopregnancy. These females could see other raccoons but could not come into physical contact with them. Also, one pet female, reported by the owner to have had no contact with other raccoons, was observed. Individual corpora lutea were studied in these females during a series of laparotomies.

Some of the corpora lutea in the ovaries of three females were marked with India ink—and the locations of all corpora lutea were mapped. By following the fate of the marked and mapped corpora until they disappeared, we found that mapping the corpora lutea was as

reliable a method of determining their life-spans as was marking them with ink. Mapping was used in subsequent studies. At each initial observation the ovary was forced through the slit ovarian capsule, the corpora were examined for color and measured grossly, and their locations in the ovary were mapped.

INTERSTITIAL TISSUE

Ovarian interstitial tissue was studied in wild raccoons on which observations as to pregnancy and lactation had been made, in several captive females treated with various hormones prior to the removal of the ovaries, and in untreated captives whose breeding histories were known. A uterine section was usually obtained when ovaries were collected, and the condition of the endometrium was studied in relation to the degree of development of the interstitial tissue. Representative sections selected from each ovary and uterus were photographed by mounting the slide in the carrier of a photographic enlarger and projecting the image directly onto 4- X 5-inch contrast process ortho sheet film. Prints 8 X 10 inches were made on F5 Kodabromide paper. By examining the photographs, we determined the abundance and distribution of cells of each type in the interstitial tissue in relation to the development of the endometrial glands, the time of year, the age of the animal, and the stage of the reproductive cycle.

HISTOLOGY

Tissues were preserved in Bouin's solution or in 10-percent formalin neutralized with either $MgCO_3$ or $CaCO_3$. The organs preserved in Bouin's solution were left for an indefinite period, but those preserved in 10-percent formalin were transferred to 70-percent alcohol after 48-72 hours. With a few exceptions, all tissues prepared for histological examination were stained with hematoxylin and eosin. The ovaries of a few females that had died some time prior to the preservation of the organs

were sectioned at 15-20 microns; the number of corpora lutea was our main interest in these ovaries. In all other cases the sections were cut 6 microns thick. The preserved organs were embedded in paraffin and sectioned and mounted by routine methods.

PLACENTAL SCARS

In dead female raccoons placental scars were counted, using transillumination. The uterus was then slit and the inside surfaces were examined for scars.

In captive pregnant females the uterine swellings were measured and the locations of the embryos were mapped during laparotomies. After parturition the presence and persistence of placental scars at the sites of known placental attachment were studied during a series of laparotomies. The scars were examined in living animals by gently pulling the uterus far enough out of the body cavity to allow it to be transilluminated. Uterine sections containing scars at various stages were removed from living females at intervals for histological study.

MORPHOLOGY OF THE REPRODUCTIVE TRACTS

Males

A few complete male reproductive tracts were removed and preserved for histological study. The entire tract from one male, and individual accessory organs from a few additional males, were sectioned. India ink was injected into one vas deferens of a fresh specimen until the ink ran out the urethral opening of the penis. The tract was then preserved and sectioned for histological study to trace the duct system, containing particles of India ink, through the prostate gland.

A schematic diagram of the male reproductive system was sketched from a fresh specimen that had been partially dissected but was sufficiently undisturbed to show its relationships to adjacent structures. A complete reproductive tract that had been dissected and preserved was used for reference.

Females

A schematic diagram of the reproductive tract (from one female) was prepared from a fresh tract that had been sufficiently dissected to reveal its conformation but that maintained its position relative to adjacent structures. One entire tract that had been removed and preserved was used for reference.

EFFECTS OF CASTRATION

Males

Four captive male raccoons were castrated at ages ranging from 72 days to approximately 9 months to study the effects of castration on the development of the penis bone, the opening of the preputial orifice, and the age at which the epiphyses close in the radius and ulna. These studies were not completed because the four animals died of various causes at different ages; the one that lived the longest attained an age of approximately 22 months.

Females

One female raccoon, born in captivity, was 3 months of age when castrated; the second, born in the wild, was estimated to be 4 months old when castrated. Several adult females were also castrated to study the effects of castration on vaginal smears, the vaginal epithelium, the uterus, and the closure of the epiphyses in the radius and ulna.

Vaginal tissues and uterine sections were taken from castrated females at intervals. These tissues were prepared for histological study and used for comparison with similar tissues from females believed to be anestrus. The females castrated as adults were also used to study the effects of various exogenous hormones on vaginal smears, the vaginal epithelium, and the development of the endometrium.

Two pregnant females were castrated as the first phase of a study of the effect of castration on pregnancy. The first female, with four embryos, was castrated 38 days (estimated time) after conception. The second female, with five em-

bryos, was castrated approximately 11 days after conception. These females were observed daily after castration for signs of abortion. A second laparotomy was performed on the first female 21 days after castration and on the second female 19 days after removal of the ovaries.

EFFECTS OF EXOGENOUS HORMONES

Males

Two captive adult male raccoons were used for preliminary studies of the effects of androgen on spermatogenesis. Beginning in August, near the midpoint of sexual inactivity, injections of testosterone cyclopentylpropionate (Res. No. 8961-1, Upjohn) were administered to both of these males. The first male received seven subcutaneous injections of 30 mg each at 3-day intervals.

Immediately before the first injection of the hormone the left testis and epididymis were removed from each animal. The testis was weighed and a smear from the tail of the epididymis was examined for the presence of sperm. Each testis and epididymis was prepared for histological study.

The first male was killed 21 days after receiving the first androgen injection, and the right testis, right epididymis, and the prostate were removed. The testis was weighed and a smear from the tail of the epididymis was examined for sperm. The second male was similarly treated but received four injections of 12 mg each and was killed 15 days after the first injection was administered.

Females

Several attempts were made to cause the growth and development of Graafian follicles and to cause ovulation by injecting various hormones into female raccoons. The hormones used were pregnant mare's serum (PMS, Upjohn), the pituitary gonadotropins (FSH and LH, Armour), estradiol cyclopentylpropionate (ECP, Upjohn), estradiol valerate (estradiol, Squibb), hydroxyprogesterone caproate (progesterone, Squibb), chorionic gonadotropin (CGH, Up-

john), and human menopausal gonadotropin (HMG-J5, Statens Seruminstitut, Copenhagen). Because these hormones were administered by many different routes and at many different dosage levels and time intervals, the methods used are discussed in connection with the particular animals involved or are given in the tables where the results from the individual animals are summarized.

UTERINE MILK

Studies were made to determine the hormone or hormones responsible for the secretion of uterine milk by the endometrial glands and to learn the nature of this secretory material. Ovaries and uteri were sectioned and stained from 18 raccoons—all were collected during the breeding season and some of them were pregnant—in which corpora lutea were present and from 89 raccoons—collected throughout the year—whose ovaries contained no corpora lutea. None of these 107 raccoons had been injected with hormones. In all cases the endometrial glands were examined for the presence of secretory materials.

Various hormones were administered to castrate females, uterine sections were removed at varying time intervals, and the endometrial glands were examined by histological methods for the presence of secretory material. The hormones used on individual castrate and intact females to study hormonal control of the secretion of uterine milk were progesterone and ECP, ECP alone, and progesterone alone; however, progesterone alone was not given to any castrate animal for a sufficient time to determine whether it would cause the uterine glands to secrete. Also studied were the direct and secondary effects of PMS, FSH, and LH, used primarily in attempts to cause the growth of Graafian follicles and to cause ovulation, and the production of secretory material by endometrial glands in intact females.

Methods described by Pearse (1960: 265-271) and Lillie (1954: 274-299)

were used to demonstrate the nature of the material observed in the lumina of the endometrial glands. Uterine sections from three female raccoons that had material present in the endometrial glands were used. The uterine section from one was fixed in 10-percent formalin neutralized with CaCO_3 . The uterine section from another was fixed in Bouin's solution, and the section from a third was fixed in 10-percent formalin neutralized with MgCO_3 . All of these tissues were imbedded in paraffin for sectioning, and control slides were used in each case.

RESULTS AND DISCUSSION

SEASONAL CYCLE OF THE GONADS

Males

The age at which male raccoons reach sexual maturity may vary from one region to another. In Michigan, on the basis of meager circumstantial evidence, Stuewer (1943*b*: 72) concluded that males "are probably not sexually mature by the first breeding season after their birth." In Illinois Pope (1944: 91) had two captive males—of parent stock supposedly "from northern Illinois or some adjacent region"—that mated successfully before they were 1 year of age.

Stuewer (1943*b*: 63) reported that the testes of juveniles and yearlings were in an abdominal position; those of adults were usually descended during the breeding season and, though variable in position, during the remainder of the year were most often in the coelom. Stuewer's evidence suggested that testis size might reflect the capacity to breed. He measured the lengths of testes in the scrotum with an accuracy of approximately 5 mm. Stuewer (1943*b*: 64) concluded that if "testis size is significant, males are probably capable of breeding at all times of year after reaching maturity." Asdell (1946: 136), on the basis of Stuewer's work but omitting his qualifications, stated that the male raccoon was capable of mating at any time. Nalbandov (1958: 162) cited the male raccoon as

a species in which spermatogenesis is continuous although the breeding season of females is restricted to late winter and early spring.

The data in Table 1 and Fig. 1 show that raccoon testes grew at a rather uniform rate from birth until about 10 months of age (through the February after birth), when the average weight of one testis was 5.6 grams. The testes of juvenile males showed the most rapid gains in weight between December and February. The average weight of a testis from a juvenile male in November was only 30 percent of the average weight in February. The sample sizes for February, March, and April were small, but there was an indication that the weights of testes in juveniles declined after February. After April testicular weights of juveniles were included with those of adults because a majority of the juvenile males were sexually active by April.

In our experience raccoon testes were nearly always found in the scrotum, even at birth, Stuewer's (1943b: 63) statements to the contrary notwithstanding. They were more prominent in adults than in juveniles, and most prominent in adults during the breeding season. Even in immature animals the testes were rarely withdrawn into the body cavity.

In Illinois a majority of the male raccoons reached sexual maturity as yearlings. Although the presence of sperm in the epididymis does not necessarily indicate sexual potency, it does indicate that an animal is in or approaching the period of sexual activity. No juvenile male had sperm in its epididymis prior to October (Table 1). In October the epididymides of about 9 percent of the juveniles contained sperm; by February this figure had increased to 87 percent. An extrusible penis was another indication of a juvenile's stage of sexual development (Sanderson 1961a: 14). Occasionally, a male was found with a non-extrusible penis but with sperm in its epididymides. Among juvenile males, 5 percent had extrusible penes in September. This figure had increased to about 67 percent by February and March but declined slightly in April. These data indicated that, in Illinois, from one-half to two-thirds of the juvenile male raccoons are sexually mature by the time they are 1 year old (Table 1). By sexually mature we mean that the male has an extrusible penis and a relatively high concentration of sperm in the epididymides.

Comparison of data from juvenile and adult male raccoons shows that juveniles became sexually mature 3-4 months later in the year than did adults. Several

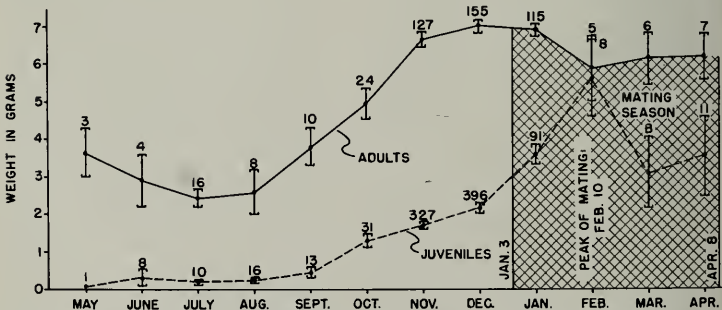


Fig. 1.—Seasonal variations in the average weight of one testis in adult and juvenile raccoons. With each mean are the number of observations and a vertical line representing the mean plus or minus one standard error. All animals were taken in Illinois from November 1955 through April 1961. The data are given in Table 1.

Table 1.—Average monthly testis weights of raccoons and the occurrence of sperm in the epididymis.^a

Time	Juveniles ^b				Adults ^b			
	Average Weight of One Testis in Grams	Standard Error	Range	Percent with Sperm in Epididymis ^c	Average Weight of One Testis in Grams	Standard Error	Range	Percent with Sperm in Epididymis ^c
At birth	0.0042(1) ^e	0 (1)
May ^f	0.01 (1)	0 (1)	3.63 (3)	0.64	2.8-4.9	100 (3)
June	0.34 (8)	0.24	0.1-2.0	0 (13)	2.92 (4)	0.68	2.0-4.9	33 (3)
July	0.21 (10)	0.03	0.1-0.4	0 (10)	2.45 (16)	0.22	1.3-4.4	11 (9)
August	0.24 (16)	0.04	0.1-0.4	0 (16)	2.60 (8)	0.58	0.8-6.0	50 (2)
September	0.46 (13)	0.12	0.2-0.4	0 (13)	3.81 (10)	0.47	1.1-5.9	0 (7)
October	1.26 (31)	0.18	0.3-3.7	9 (32)	4.96 (24)	0.36	1.7-7.6	85 (13)
November	1.68(327)	0.09	0.1-7.3	8(323)	6.65(127)	0.16	2.6-11.3	95(121)
December	2.15(396)	0.10	0.1-7.1	24(395)	6.99(155)	0.15	1.2-13.6	97(153)
January	3.57 (91)	0.25	0.2-9.8	32(118)	6.86(115)	0.16	2.7-11.0	97(113)
February	5.61 (8)	0.99	0.4-9.2	88 (8)	5.84 (5)	0.83	3.3-7.6	100 (6)
March	3.05 (8)	0.86	0.9-5.0	57 (7)	6.13 (6)	0.69	4.6-8.7	100 (7)
April	3.55 (11)	1.03	0.6-8.7	33 (10)	6.14 (7)	0.58	4.5-8.9	100 (7)

^a All raccoons were from Illinois and were collected from November 1955 through April 1961.

^b A juvenile was 0-12 months of age; an adult was more than 1 year old.

^c Determined by microscopic examination of a drop of fluid from the tail of the epididymis.

^d Most adults have easily extrusible penes; rarely is a 13- to 16-month-old male found with a nonextrusible penis.

^e The numbers of observations are in parentheses.

^f Most juveniles are 1 month old in May.

juvenile males had no sperm in their epididymides during the peak of the breeding season but became sexually mature after most of the breeding had been accomplished. At least some adult males were incapable of breeding when the second and third ovulations occurred. Hence, we believe that a majority of the second litters born to raccoons are sired by yearling males.

This study is the first to establish that seasonal variations occur in the testis weights of raccoons (Fig. 1). The average weights of the testes of adult males were minimal in June, July, and August, began increasing during September, and reached their peak in December. Among adults the maximum average weight of one testis was nearly three times the average minimum weight. A decline in testis weight appeared to occur prior to the peak of the breeding season in Feb-

ruary; however, our sample sizes for February, March, and April were small. Testes continued to decline from their peak weight in December to a low point in July. In adults there was a positive correlation between testis weight and the presence of sperm in the epididymis (Fig. 2).

Four males were unilaterally castrated on different dates. The second testis was removed from each at a later date. The weights of these testes are shown in Table 2. In many species the removal of one gonad causes the second one to hypertrophy, but our observations on the effects of unilateral castration in the raccoon on the weight of the remaining testis are inconclusive. The remaining testes in the two adult males castrated unilaterally in July showed greater-than-average increases in weight from July to December. A greater-than-average

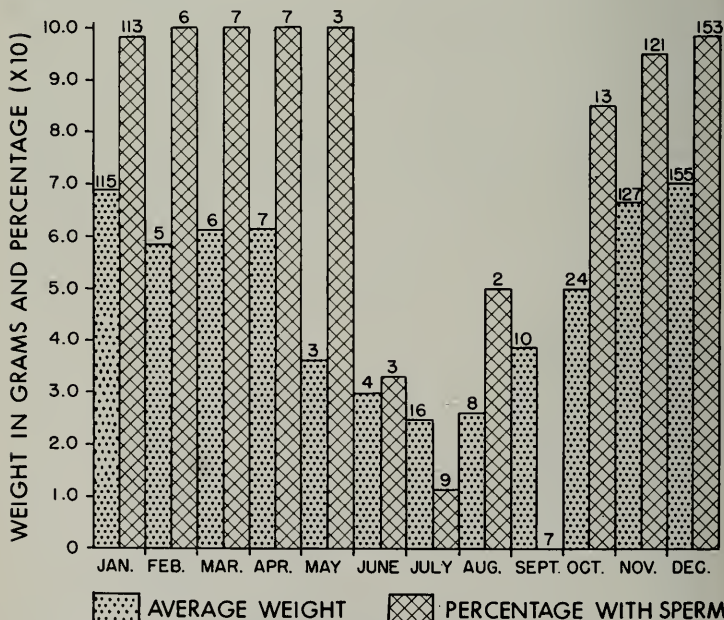


Fig. 2.—Seasonal variations in the average weight of one testis taken from adult raccoons and the percentage of adults with sperm in the epididymis. The numeral at the top of each bar indicates the number of observations.

Table 2.—Testis weights of four raccoons, showing seasonal changes in individual animals.

Raccoon Number and Type	Estimated Age in Months	Date	Weight of One Testis in Grams	Sperm Present or Absent ^a
1771 (wild)	15	7-18-57	1.5	-
	20	12-23-57	9.6	+
1783 (captive)	15	7-19-57	2.6	-
	20	12- 6-57	9.6	+
1803 (captive)	7	11- 6-57	1.5	-
	9	1- 3-58	6.6	+
2121 (captive)	14	5-23-58	3.2	-
	17	9- 3-58	2.7	-

^a The plus sign indicates that sperm was present, and the minus sign that it was absent.

increase in weight occurred from November to January (the period of maximum growth rate in juvenile males) in one juvenile male after the removal of one testis in November. After unilateral castration in May the second testis in the remaining adult showed a decrease in weight from May to September, a period when the average weights of testes from wild adults did not differ significantly.

Several captive raccoons were examined repeatedly for sperm in their epididymides after they became sexually mature (Fig. 3). From July through October the greatest percentage of adult males were sexually inactive. The histories of several captives show that individual males had periods that averaged 3-4 months when they were incapable of breeding although males with

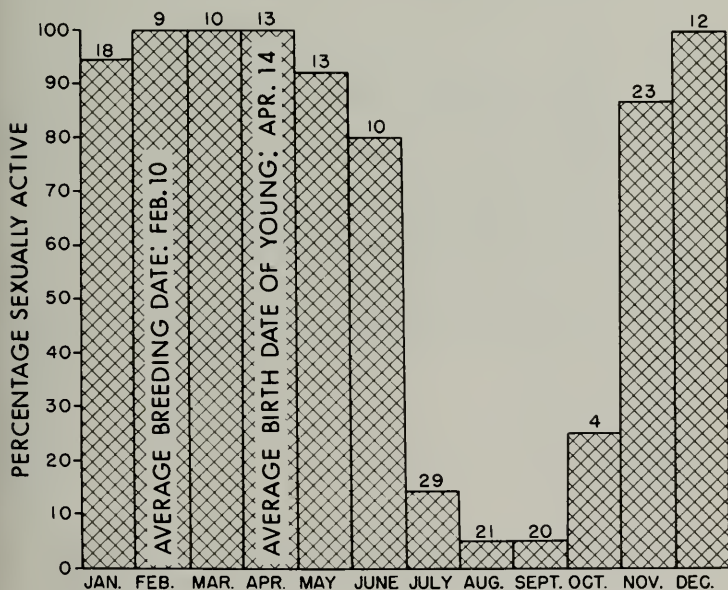


Fig. 3.—Percentage of sexually mature captive male raccoons with sperm in the epididymis. The numeral at the top of each bar indicates the number of observations for each month.

sperm in their epididymides were found in all months (Fig. 3 and 4). Lower concentrations of sperm were found at the beginning and end of the period of sexual activity than were found during

the peak, but how the concentration of sperm is related to a male's fertilizing ability is not known.

Histological examinations of the testes and epididymides of 85 wild and 37 cap-

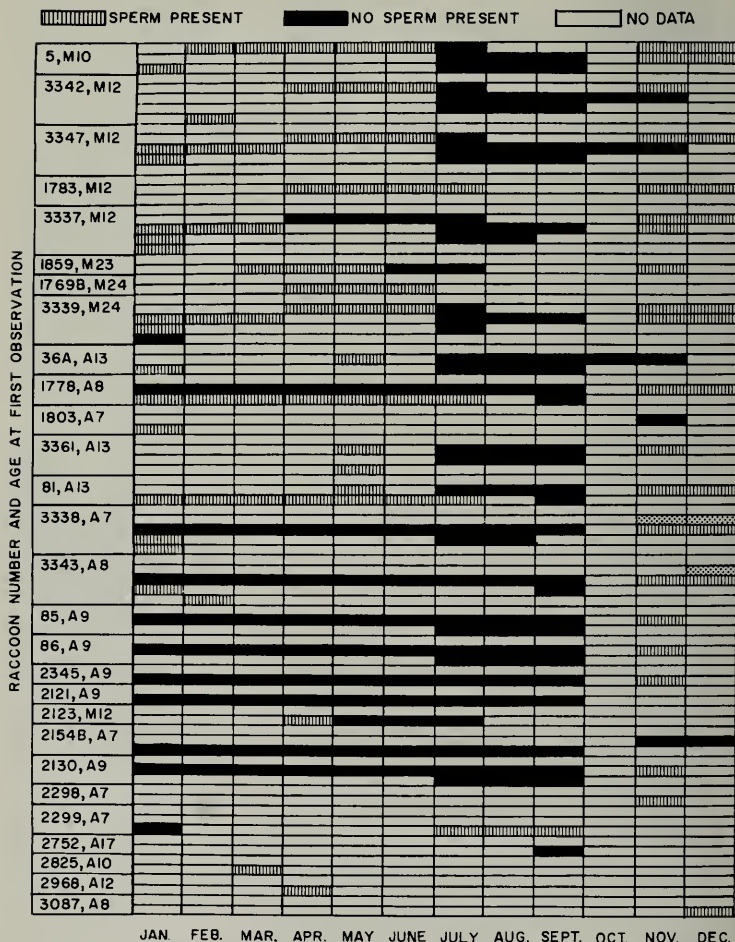


Fig. 4.—Presence or absence of sperm in the epididymides of captive male raccoons. These raccoons were held in outdoor cages throughout the year in Urbana, Ill. The animal's identification number is given first, and the estimated age in months when the first observation was recorded is given next. A = approximate age, M = minimum age. The different lines for each animal indicate different years.

tive male raccoons (Table 3) confirmed the gross observations, reported above, made on captives. In all but five males if sperm were present in the testes, they were also present in the epididymides, and vice versa. Data from these five cases indicate that sperm may be stored in the epididymis for some time after spermatogenesis ceases and that sperm may be found in the testis prior to being stored in the epididymis. Males of some species are able to ejaculate fertile sperm for as long as 4 weeks after castration (Nalbandov 1958: 176). Testes and epididymides removed from two adult raccoons in August (Table 3) are representative of the conditions found. One male had sperm in the seminiferous tubules but none in the epididymides. The second male had low concentrations of sperm in both the testes and the epididymides.

These data show that the male raccoon has a seasonal sexual cycle. The general correlation between the size of the testis and the presence of sperm in the epididymis did not hold in individual cases. Three hundred eighty-four testes with sperm in the corresponding epididymides, taken from adults from October through April, averaged 7.2 grams and ranged from 2.6 to 11.3 grams.

Fifteen testes with no sperm in the corresponding epididymides, taken from adults during the same months, averaged 4.6 grams and ranged from 1.2 to 9.5 grams.

A substantial number of testes were weighed during November, December, and January from the 1950-1951 fur season through the 1960-1961 fur season in Iowa and Illinois. Raccoons in Iowa were examined at a fur house in Bloomfield, Davis County. Most specimens from Illinois were collected at a fur house in Farmington, Fulton County. Farmington is approximately 130 miles, almost due east, from Bloomfield, and the reproductive cycles of the animals collected at these two locations probably were similar. A few Illinois specimens were collected from other fur houses located in the central (north-south) third of Illinois. Each fur buyer bought dead raccoons from hunters and trappers living within a radius of about 100 miles around his location.

These data were collected to study seasonal and annual trends in the weights of testes (Table 4) and the timing of spermatogenesis in relation to age among male raccoons. The testes of juveniles gained weight significantly ($P < 0.02$) from November to January. No signifi-

Table 3.—Occurrence of sperm in the testes and epididymides of adult and juvenile raccoons as determined by histological examination of 85 wild and 37 captive males.^a

Month	Adults ^b		Juveniles ^b	
	Percent with Sperm	Number of Observations	Percent with Sperm	Number of Observations
January	67	3	...	0
February	100	3	0	1
March	100	1	25	4
April	75	4 ^c	57	7
May	25	4	0	2
June	0	1	0	6
July	17	12	0	8
August	25	4 ^c	0	10
September	33	9	0	7
October	80	10	0	8 ^d
November	86	7 ^d	14	7
December	100	1	0	3 ^d

^a All observations were made in Illinois from 1957 through 1960.

^b An adult was more than 12 months of age; a juvenile was 0-12 months of age.

^c One adult male in April and one in August had sperm in the epididymides but not in the testes.

^d One adult male in November and one juvenile male in October and one in December had sperm in the testes but not in the epididymides.

Table 4.—Average testis weights in adult and juvenile raccoons for November, December, and January from the 1950-1951 through the 1960-1961 seasons.

State and Season	Average Weights of One Testis in Grams									
	Juvéniles					Adults				
	November	December	January	Average	November	December	January	Average		
Iowa ^a										
1950-1951	1.05 (28) ^b	0.73 (3)	1.02 (31) ^c	7.99 (23)	6.83 (3)	7.86 (26)		
1951-1952	1.12 (66)	1.37 (87)	1.21 (153) ^d	7.25 (26)	7.72 (56)	7.57 (82)		
1952-1953	1.24 (50)	1.68 (4)	1.29 (54)	7.35 (14)	7.95 (2)	7.42 (68)		
1953-1954	0.86 (32)	1.01 (15)	1.10 (2)	0.92 (49) ^e	6.94 (25)	7.94 (11)	8.70 (1)	7.28 (37)		
1954-1955	2.04 (32)	2.51 (30)	4.65 (2)	2.34 (64)	7.14 (8)	8.03 (16)	7.50 (2)	7.72 (26)		
Illinois ^a										
1955-1956	1.95 (34)	1.59 (19)	2.17 (22)	1.92 (75)	7.13 (3)	5.25 (2)	6.91 (22)	6.81 (27)		
1956-1957	1.67 (47)	2.25 (105)	2.72 (24)	2.16 (176)	7.19 (10)	7.22 (46)	7.37 (31)	7.27 (87)		
1957-1958	1.99 (52)	2.17 (35)	3.29 (23)	2.32 (110)	6.77 (25)	8.43 (12)	6.25 (8)	7.12 (45)		
1958-1959	1.81 (67)	2.41 (62)	3.95 (27)	2.42 (156) ^d	6.93 (27)	6.80 (19)	6.78 (22)	6.84 (68)		
1959-1960	1.26 (32)	2.20 (129)	1.00 (3)	1.99 (164)	7.84 (11)	7.18 (47)	5.80 (6)	7.16 (64)		
1960-1961	1.47 (95) ^f	1.63 (46)	3.70 (19)	1.78 (160)	6.30 (49)	5.96 (29)	4.87 (26)	5.85 (104) ^d		
Monthly average ^g	1.54 (507) ^d	1.96 (560)	2.71 (125)	1.86 (1,192)	6.90 (198)	7.34 (263)	6.89 (121)	7.10 (582)		
Grand average ^g										

^a All raccoons were collected at a fur house in Bloomfield, Davis County.

^b The numbers in parentheses are in parentheses.

^c Significantly different from the grand average ($P < 0.01$).

^d Significantly different from the grand average ($P < 0.05$).

^e Significantly different from the grand average ($P < 0.05$).

^f Most raccoons were collected at a fur house in Farmington, Fulton County.

^g Averages not joined by lines are significantly different from each other ($P < 0.05$).

cant differences in the average weights of adult testes occurred from November to January. This finding was not unexpected, because virtually all adult males were capable of breeding by November but only 8 percent of the juveniles had sperm in the epididymides during November (Table 1). There were some statistically significant annual differences in the weights of testes, but the meanings of these differences were not clear.

Females

The ovaries of raccoons showed a nearly steady rate of growth from birth in April through the following November (Table 5 and Fig. 5). In contrast, the testes of juveniles showed their most rapid increases in weight between December and February (Fig. 1).

The ovaries of juveniles reached their maximum average weight in November, approximately 3 months prior to the peak

of the breeding season. The heaviest normal ovaries encountered were found during November in juveniles; the average weights are shown in Table 5 and Fig. 5 and 6. In October, November, and December the ovaries of juveniles weighed more than the ovaries of parous raccoons. The average weights of ovaries for the two groups of females in January were practically identical (Fig. 5).

The ovaries of juvenile (nulliparous) females showed a significant decline in average weight from November through January, and perhaps through March, but the sample sizes for February, March, and April were too small to be definitive. The small sample of juveniles for these latter 3 months resulted partly from classifying raccoons as nulliparous (juveniles) or as parous or pregnant. During those 3 months many females approximately 1 year of age were either pregnant or parous, and hence their ovaries were placed

Table 5.—Average weights of ovaries by month in the raccoon.^a

Month	Nulliparous			Parous or Pregnant		
	Average Total Weight of Both Ovaries in Milligrams	Standard Error	Range	Average Total Weight of Both Ovaries in Milligrams	Standard Error	Range
April (at birth)	4.2 (2) ^b	0.0	4.2- 4.2	282 (3)	31	249-344
May ^c	10.9 (3)	0.4	10.4- 11.8	217 (4)	50	164-259
June	51 (3)	16	20 - 72	224 (2)	43	181-266
July	121 (6)	24	45 -222	171 (7)	30	93-327
August	118 (7)	14	82 -180	198 (4)	42	76-260
September	184 (14)	23	66 -386	217 (3)	27	164-256
October	253 (14)	25	147 -524	227 (4)	25	163-295
November	312(195)	9	59 -970	271 (80)	12	102-613
December	295(186)	13	78 -699	229 (81)	10	82-555
January	239 (45)	15	100 -452	244 (25)	23	92-655
February	189 (3)	54	112 -294	275 (5)	41	132-381
March	124 (1)	246 (5)	57	74-382
April	260 (1)
May ^d	167 (1)
August	270 (1)
September	149 (2)	70	78 -219
November	367 (2)	70	225 -410
December	233 (12)	27	119 -313
January	261 (3)	43	204 -346
March	136 (1)

^a All raccoons were collected in Illinois, July 18, 1958 through April 18, 1961.

^b The numbers of observations are in parentheses.

^c Most nulliparous juvenile raccoons are approximately 1 month old in May.

^d Most nulliparous adult raccoons are approximately 13 months old in May.

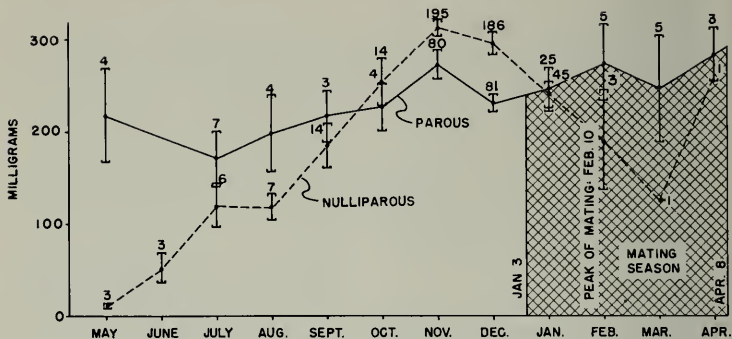


Fig. 5.—Seasonal variations in the average total weight of both ovaries from parous or pregnant and nulliparous raccoons. With each mean are the number of observations and a vertical line representing the mean plus or minus one standard error. All animals were taken in Illinois from July 18, 1958 through May 30, 1961. The data are given in Table 5.

with the adult group. Hence, nulliparous females probably represented only females that did not reach sexual maturity at approximately 1 year of age. All females less than 1 year of age in the January sample were counted as juveniles, as no pregnant female was found during that month.

The seasonal weights of the ovaries in parous raccoons (Table 5) followed a pattern similar to that found for the gonads of adult males (Table 1). The minimum average weight was reached during July, with a slow but consistent increase in weight during August, September, and October. The fall peak

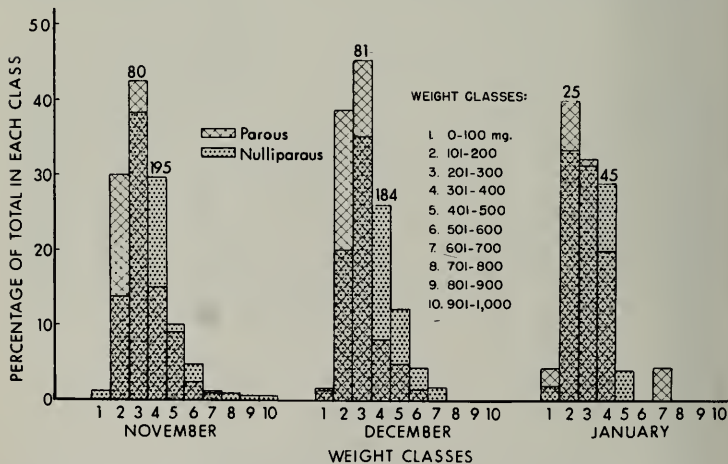


Fig. 6.—The average total weights of both ovaries from parous or pregnant and nulliparous raccoons. All animals were taken in Illinois from November 1958 through January 1961. The numerals at the tops of the bars indicate the numbers of animals in the parous or pregnant and nulliparous groups.

Table 6.—Average total weights of both ovaries in parous and nulliparous raccoons for November, December, and January of the 1958-1959 through the 1960-1961 seasons.*

Season	Average Total Weight of Both Ovaries in Milligrams									
	Parous					Nulliparous				
	November	December	January	Average	November	December	January	Average		
1958-1959	350(15) ^b	270(15)	307(9)	309(39) ^c	355(61) ^d	315(45)	247(20)	324(126)		
1959-1960	219(13)	216(50)	193(2)	216(65) ^c	276(40)	297(110)	246(5)	290(155)		
1960-1961	261(52)	233(16)	211(14)	247(82)	302(99)	252(43)	234(23)	280(165)		
Monthly average ^e	271(80)	229(81)	244(25)		312(200) ^d	295(198)	239(48)			
Grand average ^e				249(186)				296(446)		

* All raccoons were from Illinois.
 b The numbers of observations are in parentheses.
 c Significantly different from the grand average ($P \leq 0.01$).
 d Significantly different from the grand average ($P \leq 0.05$).
 e Averages not joined by lines are significantly different from each other ($P < 0.05$).

weight was reached in November. The ovaries of parous raccoons declined significantly ($P < 0.01$) in average weight from November to December but again increased in weight during January. By April the ovaries of parous raccoons had reached their peak average weight for the year, slightly heavier than in November. The average weight of adults' ovaries in April was a little more than 1.6 times their average weight in July, in contrast to the approximately 2.8-fold increase in average weight reported for the testis in the adult between the low average of July and the high of December.

From the study of ovaries collected during all months and seasons, we gained the impression that differences in weight existed from month to month and year to year. For example, the total weight of both ovaries of parous females averaged nearly 350 mg in November 1958 but only 219 mg in November 1959 (Table 6). Ovaries from nulliparous females killed in November 1958 also weighed considerably more on the average than did ovaries from nulliparous females killed in November 1959. Less striking variations were noted for other months and years. There were also annual differences in the average weights of ovaries from parous females but no significant differences in those from nulliparous females.

MEAN BIRTH DATE OF LITTERS

Wood (1955:409-410) concluded that 7 of the 16 females he examined in Texas had mated by the end of February, but the earliest pregnancy he recorded was March 18. George & Stitt (1951:218) found three litters that were born during March 1950 in Michigan after an unseasonably warm January. Berard (1952:248) observed a lactating female in West Virginia that he estimated had given birth no earlier than August 15, whereas normal births in that area usually occur before May 15. Dorney (1953:123) weighed young raccoons taken in Wisconsin from November 25 through December 22, 1950 and concluded that

Table 7.—Months of birth of raccoons in the northern half of Illinois as determined by actual births or as estimated from examination of embryos.^a

Month	Number of Litters Conceived in Captivity That Were Born in Month Designated	Number of Adult Wild Females Examined for Pregnancy ^b	Number of Litters Conceived in the Wild That Had Actual or Potential Birth Date in Month Designated ^c
January	0	202	0
February	0	6	0
March	2	15	5
April	5	18	12
May	3	9	1
June	1	6	2
July	0	11	0
August	0	4	0

^a All embryos were examined between April 2, 1957 and June 24, 1961.

^b Many nonpregnant, adult females examined from April to August were lactating.

^c Potential birth dates were estimated (Fig. 8).

“a sizable percentage” of the young had been born later than usual in that year. He suggested that the cold spring weather in 1950 had decreased raccoon mobility and thus had decreased the normal number of early conceptions. A similar situation, discussed later, apparently occurred in Illinois during the breeding season in 1960.

The reports cited emphasize the variation in birth dates that is normal in the raccoon. Most raccoons in the northern half of Illinois are born during April (Table 7). The mean date of birth for 20 litters conceived in the wild, 7 of which were born in captivity, was April 18; the earliest date of birth was March 9, and the latest, June 24. The potential birth dates of embryos measured in dead

females were estimated by measuring uterine swellings (Table 8 and Fig. 7).

The mean date of birth for 11 litters conceived and born in captivity in Urbana, Ill., was April 24. The monthly distribution of these births is shown in Table 7. The earliest date of birth for a litter conceived in captivity was March 16; the latest was June 3.

Although 202 wild females were examined for pregnancy during January and 21 were examined during February, July, and August (Table 7), only one pregnant female was observed during these 4 months. When examined on February 25, she appeared to be due to give birth in about 3 weeks.

ESTIMATING BIRTH DATES OF RACCOONS

Table 8.—Estimated number of days prior to birth based on the measurement of uterine swellings in captive raccoons.

Largest Measurement of Uterine Swellings in Millimeters	Days Prior to Birth
8	55
20	39
25	35
30	33
35	33
40	30
45	23
55	17
110	1

Uterine swellings were measured to the nearest millimeter and the dates of birth were recorded for eight litters believed to have been born at full term (Table 8). (One litter was measured on two occasions.) Because it was difficult to measure accurately the crown-rump length of embryos, especially during the early stages of pregnancy, the measurement recorded was the greatest measurement of the external uterine swelling. In the early stages the swellings were essentially round, and the measurement was greater than the crown-rump measurement of

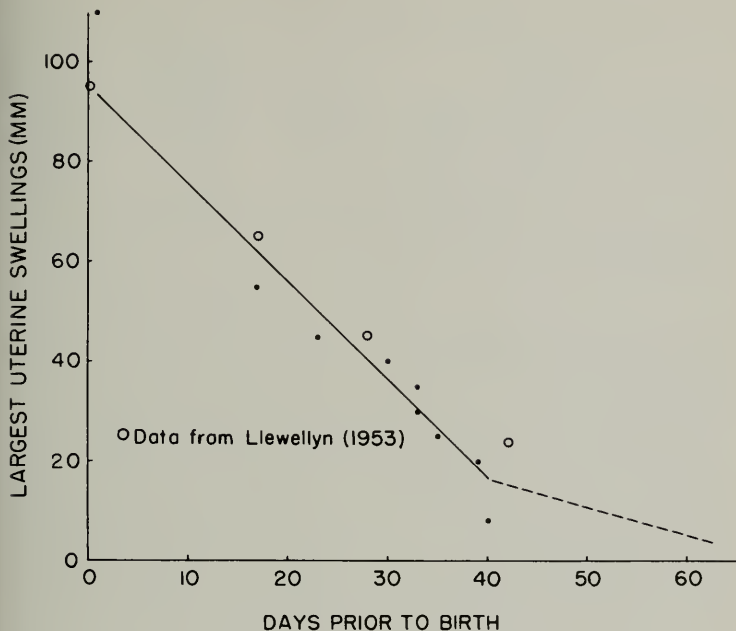


Fig. 7.—Sizes of uterine swellings in raccoons at various numbers of days prepartum. The line was fitted by least squares, not including Llewellyn's data. The dash line, an extension of the line to conception 63 days prepartum, is not based on data. The size used for the uterine swelling at conception was 5 mm, the approximate average diameter of the uterus during estrus. The data are given in Table 8.

the embryo. In the later stages the swellings were elongate and the measurement approximated that of the crown-rump measurement.

The dates of mating were not known, but it was possible to graph the size of the uterine swellings in relation to the number of days prior to parturition (Fig. 7). The line was fitted by least squares and gave a good fit for uterine swellings between 20 and 60 mm in size. When we used this line to estimate the dates of birth for eight litters, the maximum error was 4 days when the uterine swellings were between 20 and 60 mm. In one litter uterine swellings larger than 60 mm were measured and in another litter uterine swellings smaller than 20 mm were measured. Measure-

ments of the swellings in these two litters appear to indicate slower-than-average growth from conception to the 20-mm size and faster-than-average growth from the 60-mm size to birth. Measurements of uterine swellings made during this study were similar to those reported by Llewellyn (1953:321). Our data and Llewellyn's make it possible to estimate the date of birth (Fig. 7). If we assume a gestation period of 63 days, which many authors agree is average for the raccoon, it is possible also to estimate the date of conception.

SECONDARY SEX RATIOS

Incidental information collected during the present study indicated that the sex

Table 9.—Secondary sex ratios in raccoons.^a

Sex of Embryos and of Young at Birth ^b			Sex of Wild Litters Less Than 2 Months of Age		
Females	Males	Percent Males	Females	Males	Percent Males
47	36	43 ^c	21	33	61 ^c
	$\chi^2=1.46$ ($P<0.25$)			$\chi^2=2.67$ ($P<0.10$)	

^a All were from Illinois, taken during the breeding seasons of 1957 through 1961.

^b Includes young conceived in the wild and born in captivity as well as young conceived in captivity.

^c Neither group is significantly different from 50:50 ($P \geq 0.05$); however, the ratios of the two groups may be different from each other ($P < 0.06$).

ratio of raccoon embryos and of young at birth, combined, and the sex ratio of young raccoons less than 2 months old were not significantly different from 50:50 (Table 9). There were more males ($P < 0.06$) among the young less than 2 months old than among the embryos and young at birth, indicating some differential mortality of females between birth and 2 months of age. Other investigators have examined limited numbers of young raccoons at birth or prior to 2 months of age to determine secondary sex ratios. Stains (1956:31) reported that the sex ratio was approximately 1:1 at birth in the raccoon, as shown by counts of litters. Stuewer (1943a:213; 1943b:68) counted all of the young in eight litters ranging in age from 7 to 60 days and found 14 males and 19 females (42 percent males).

ESTROUS CYCLE, OVULATION, AND PSEUDOPREGNANCY

Estrous Cycle

Published reports regarding the estrous cycle in the raccoon do not agree. The raccoon has been reported to have one heat period and one breeding season each year (U.S. Department of Agriculture 1936). Stuewer (1943a:212) found that occasionally an adult female failed to mate successfully in spring and then bred later, but that yearling females either mated during the regular breeding season or did not mate until the next breeding season. Asdell (1946:135) reported: "In New England mating begins in the last week of January and there

may be a later season for young females . . ." Whitney & Underwood (1952:83) stated that, on the basis of actual observations under normal conditions, the period of mating in the raccoon was from January until March; if this period was missed, another normal period of 2 months' duration occurred 4 months later. During his studies of raccoons in Texas, Wood (1955:409) found Graafian follicles in the ovaries of one female examined in April and thus concluded that breeding can occur as late as April. Because ovulation normally occurred early in the year, this finding suggested to him that raccoons were possibly polyestrous.

Observations made on captive raccoons held in outdoor cages in Urbana, Ill., confirmed Stuewer's (1943a:212) observations on the absence of delayed breeding in yearling females. General observations made on several captive, yearling females showed that either they became pregnant or pseudopregnant at the time when adults became pregnant, or they did not breed until the next breeding season. These observations were confirmed by examining the ovaries of two yearling females several times from March through August. Their ovaries and uteri remained small and inactive during the entire period. Thus, we believe that delayed breeding in yearling females does not contribute substantially to the number of litters born later in the year than usual.

Dates of ovulation were estimated by observing birth dates and by direct examination of Graafian follicles, corpora lutea, and embryos. Heat and ovulation probably occurred at about the same time.

Table 10.—Approximate number of days between ovulations in five captive raccoons.

Estimated Date of First Ovulation	Estimated Date of Second Ovulation	Days Between Ovulations	Remarks
2-10	5-10	89	During first pregnancy, carried embryos half way or more to term but resorbed them.
Before 3-14	5-26	70 ^a	Pseudopregnant
Before 3-10	5-11	62 ^a	Pseudopregnant
3-2 (± 2)	6-23 (± 2)	84	Pseudopregnant
1-29	6-16	141 ^b	Carried embryos to term each time. Young of first pregnancy all dead 4 days postpartum.

^a Minimum.^b The interval between the births of two litters in one season.

On the basis of our observations of five captive raccoons for which the approximate dates of the first and second ovulations were known (Table 10), we found that the interval between ovulations in captive raccoons in Urbana, Ill., varied approximately from 80 to 140 days—and not invariably 4 months, as reported by Whitney & Underwood (1952:83). The shorter intervals that we observed agree with Millard's (1939:28-29) data. He obtained two litters in one breeding season from 6 of 10 captive raccoons in Wisconsin whose young were removed on the day of birth and whose mates were returned 3 days later. Seven of the females were observed to mate 10-16 days after the young were born. If we assume a gestation period of 63 days and that the female raccoon ovulates on the day of mating, ovulations in Millard's animals occurred 73-79 days apart.

Under normal circumstances wild, adult female raccoons in Illinois rarely skip a breeding season. Special circumstances may interfere with the regular breeding cycle, causing a higher-than-normal percentage of the litters to be born late (Dorney 1953:123). Such interference occurred in some sections during the 1960 breeding season in Illinois. Temperatures at the Urbana and Peoria weather stations (U.S. Weather Bureau 1960) were average for January 1960, but mean temperatures in February were 15.7° F [9.0° C] below normal. Snowfall at Urbana and Peoria for February and March 1960 ranged from 8 to 16 inches [20.3-40.6 cm] per month higher than

the average for the preceding 10 years. On the Allerton Park Study Area (Piatt County, east-central Illinois) young raccoons were caught in live traps beginning in early June of each year from 1957 through 1961, with the exception of 1960. In 1960 the first young were live-trapped after September 1 even though trapping was conducted during the entire summer.

Eyes were collected from 257 juvenile raccoons killed by hunters and trappers over a wide area centered around Farmington in west-central Illinois during the 1960-1961 hunting season. The lens technique (Sanderson 1961b:482-485) was used to estimate birth dates. The lenses indicated that the peak of births in 1960 occurred in mid-April, the usual time, but that a second, smaller peak occurred at the first of July, about 11 weeks later. These two peaks were separated by about the length of one estrous cycle, as it was estimated from our observations of captive raccoons. According to the lens data, approximately 16 percent of the young were born during August, September, and October in 1960—later than the latest date of birth reported in Table 7—indicating that under some circumstances a substantial number of wild raccoons have had more than one estrous cycle in a year.

In view of Millard's (1939:28-29) success in getting two litters in one season from captive raccoons and because the present study demonstrated that some of our captive pseudopregnant and pregnant females had second heat periods in captivity, it was, at first, surprising that so

few second litters were conceived in captivity during our study. Millard's (1939) objective was to rear a large number of young raccoons for restocking purposes, and no doubt he disturbed his animals as little as possible. Our study, on the other hand, required frequent handling of the animals and their subjection to laparotomies. Only two pregnancies are known to have resulted from second ovulations during our study. The first female became pseudopregnant after her first ovulation, and the single embryo from her second ovulation was resorbed; the second female gave birth to her second litter in August, 141 days after the first litter was born. She had killed the last surviving young of her first litter 4 days postpartum.

Female raccoons will not ovulate and come into estrus so long as they are nursing young. Young were removed at birth from four female raccoons and 5 days after birth from one female. All of these females were returned to their mates when the young were removed, but no second matings were observed and no second pregnancies resulted. Young were removed from six females at periods varying from 17 days to 6 weeks after birth, and the males were returned to the females. One female was given a drug that caused her to abort or resorb her young. Her mate remained with her at all times. However, no second pregnancies resulted in any of these animals. In addition to these females several others underwent periods of pseudopregnancy during the normal breeding season while remaining with their mates through the summer. No late pregnancies resulted. Possibly some of the males were no longer capable of fertilization (Fig. 3 and 4) by the time their mates experienced their second estrous cycles.

Our data make it clear that in Illinois it is possible for raccoons to ovulate two times during one season and even to give birth to two litters. However, to give birth to the second litter, the female must lose her first litter on or shortly after the day of birth. We have no evidence that

ovulations occur after lactation ceases in the raccoon. In any case, it appears that raccoons must nurse for 2-3 months in the wild and that probably they usually nurse for 3-5 months (Stuewer 1943a: 213; Montgomery 1969:155-158).

The vaginal smear is frequently used to determine the stage of the reproductive cycle in the laboratory mouse, rat, and guinea pig. Stockard (1932:1612-1627) gives a general review. Although this technique can theoretically be applied to other species, many problems occur with species that have relatively long periods of proestrus and estrus. Nalbandov (1958:103-104) pointed out that all mammalian females show changes in their vaginal histology during the estrous cycle. He further reports:

"The vaginal-smear technique is most useful, however, with animals having short estrous cycles . . . ; in animals with longer cycles . . . vaginal changes lag from one to several days behind ovarian changes, and vaginal smears are therefore less reliable indicators of ovarian events."

Stuewer (1943b:64) observed that from 1 to 2 weeks elapsed from the onset of vaginal swelling in the raccoon until the female would receive the male. After a receptive period of about 3 days, 3 or 4 weeks elapsed before the vulva returned to normal appearance. Whitney & Underwood (1952:83) reported that the onset of the mating cycle could be recognized by a thickening or swelling of the vagina and vulva and traces of bloody fluid (absent in some females), and that the female would accept the male at the onset of the mating cycle and was receptive for a period of 3-6 days.

We obtained estrous-type vaginal smears for a period of several weeks in our raccoons; examples of these smears are shown in Fig. 8. An estrous-type smear was obtained from a castrated female 36 days after the end of treatment with estradiol and progesterone (Fig. 8B). One captive female must have mated during the 7 days between the taking of two vaginal smears; she gave birth

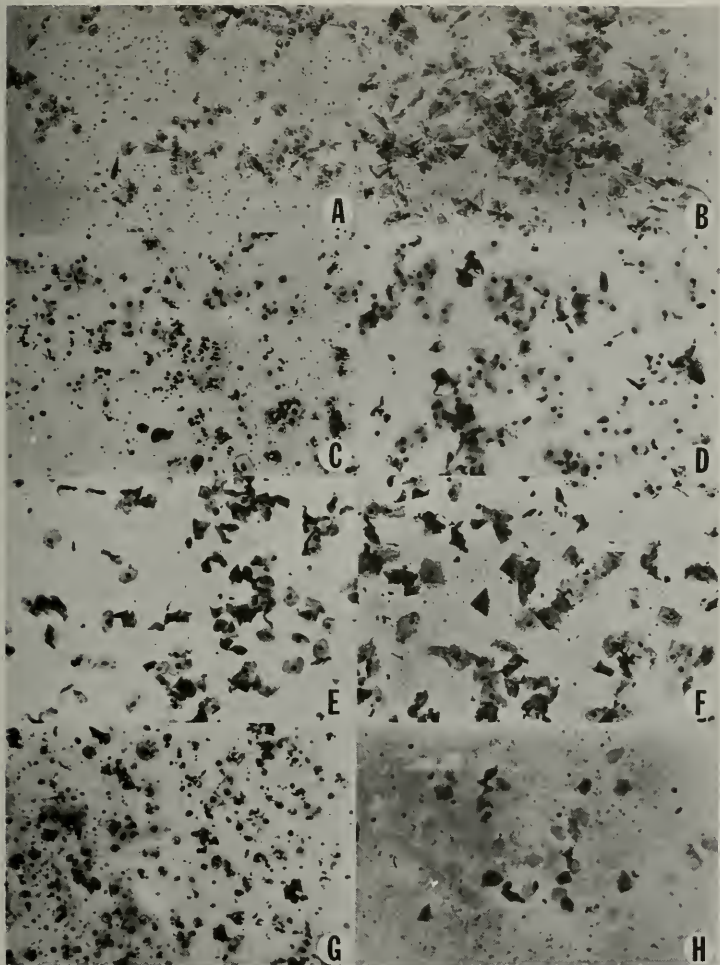
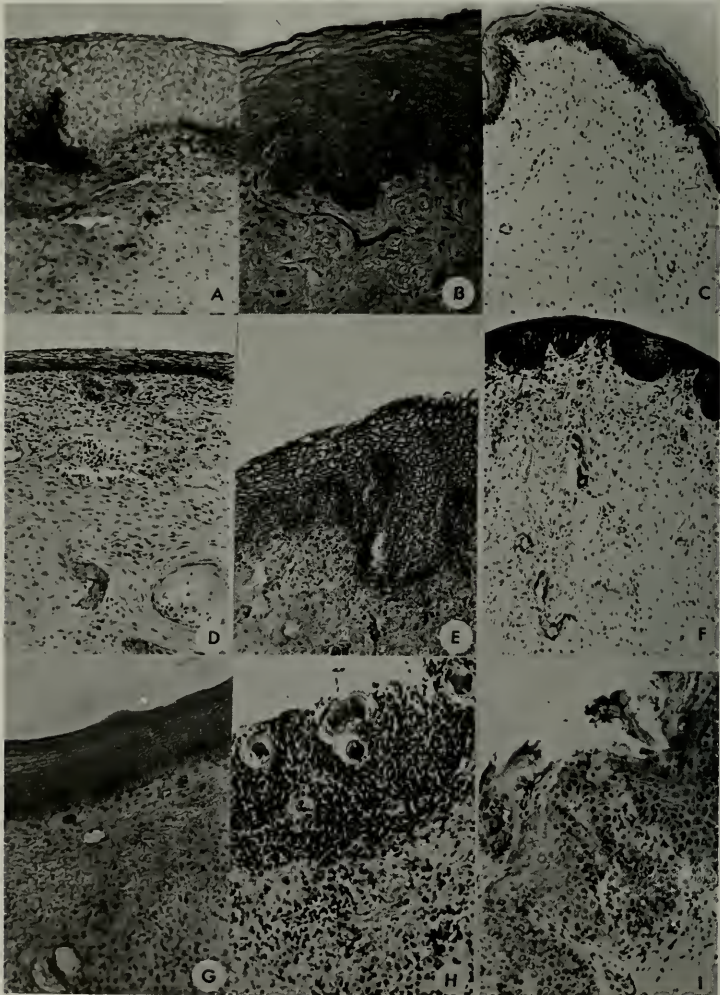


Fig. 8.—Vaginal smears from captive raccoons representing various stages of the estrous cycle. A, female 1292; ovaries removed May 14, 1958; smear taken July 23, 1959. B, castrated female 1297; second ovary removed August 13, 1957; smear taken May 16, 1958, 36 days after treatment with estradiol and progesterone ended. C, nulliparous female 2525; smear taken December 4, 1958. D, female 2959; smear taken January 11, 1958. E, female 2959; smear taken March 7, 1958. F, female 2959; smear taken March 14, 1958; female 2959 must have mated between these two dates, because she gave birth to young on May 13, 1958. G, adult female 1786; smear taken April 12, 1957. H, female 2114; smear taken February 21, 1958; uterine swellings were 8 mm in diameter. The smears were stained with Wright's blood stain and are shown 62 times actual size.

60 days after the second smear was taken (Fig. 8E and F). On the basis of the results obtained from this study, we conclude that the days when a female raccoon will receive a male can not be identified by examination of vaginal

smears. The vaginal smear appeared to be no more specific than gross vulval swelling—which can be observed much more readily. Leucocytes (Fig. 8C and G) were seen in vaginal smears from raccoons only infrequently. The paucity of



vaginal smears containing leucocytes suggested that the raccoon may pass through metestrus in a relatively short time.

Many of the difficulties inherent in using vaginal smears may be avoided by taking vaginal tissue for biopsies—a simple procedure in the raccoon. Samples of vaginal tissues were removed from both anesthetized and unanesthetized animals (Fig. 9). However, vaginal tissues from castrated females (Fig. 9A and B) showed that the histology of the vaginal epithelium is not a reliable indicator of estrus in the raccoon.

Ovulation

Whitney & Underwood (1952:84), without citing evidence, reported that in the raccoon "sufficient stimulation is produced during copulation to insure ovulation." Llewellyn & Enders (1954a: 440) removed one ovary from each of four sexually mature raccoons that had been isolated from males before and during the normal breeding season. They found "well developed follicles" in each ovary but no corpora lutea and, on the basis of this evidence, suggested that ovulation in the raccoon is not spontaneous but is induced by copulation.

In our study one female was approximately 5 months old when captured about 5 months before the breeding season. She was isolated for 3 months prior to the breeding season. Her nipples were moderately stimulated but unpigmented when a laparotomy was first performed on her 3 months after she was isolated. The ovaries were each 8 X 5 mm—small for ovaries with corpora lutea—yet each

ovary had two corpora lutea, each 5 mm in diameter. Thirty-five days later the corpora lutea were essentially unchanged in size and appearance. Sixty days after the first examination the ovaries were 10 X 5 mm, and the corpora lutea were slightly paler and were between 3 and 4 mm in diameter. Ninety-three days after the initial observation no traces of the corpora lutea were visible.

A second female that was isolated was approximately 40 days old when captured. She was isolated 2 months prior to the breeding season, and the first laparotomy was performed on her 2 months after the isolation began. Her nipples were only slightly stimulated, and her ovaries, measuring 8 X 4 mm, contained no corpora lutea. The left ovary had one clear follicle and the right ovary had two, each follicle measuring 2 mm in diameter.

Nine days later the right ovary had four freshly ovulated follicles, each 3 mm in diameter. The left ovary had a single follicle of the same size with a tiny hole in its highest point. It was believed that this female had ovulated no more than 2 days earlier.

Twenty-six days after the freshly ovulated follicles were observed, the right ovary was 11 X 5 mm and had three corpora lutea, each 4 mm in diameter. Either the fourth follicle in the ovary did not form a corpus luteum, or it was obscured by one of the other corpora. The left ovary was 10 X 5 mm and had one corpus luteum of the same size as those in the right ovary.

Eighty-one days after the freshly ovu-

Fig. 9 (Page 52).—Photomicrographs of vaginal biopsies from captive raccoons representing various stages of the estrous cycle. A, female 1292; ovaries removed May 14, 1958; biopsy performed July 23, 1959. B, castrated female 1297; second ovary removed August 13, 1957; biopsy performed May 16, 1958, 36 days after treatment with estradiol and progesterone ended. C, female 2114; biopsy performed February 21, 1958; uterine swellings 8 mm in diameter. D, nulliparous female 2525; biopsy performed December 4, 1958. E, female 1297; biopsy performed July 23, 1959; no treatment with estradiol and progesterone after April 3, 1958. F, female 1298; biopsy performed October 5, 1959; ovulated about September 24, 1959 as a result of injections of pregnant mare's serum. G, female 1782; biopsy performed October 29, 1957; treated with estradiol beginning October 17, 1957; ovaries removed June 10, 1957. H, female 2959; biopsy performed January 27, 1960; ovulated after December 18, 1959 as a result of treatment with follicle-stimulating hormone and luteinizing hormone; corpora lutea present. I, female 2805; biopsy performed February 24, 1960; fresh corpora lutea present. The sections were stained with hematoxylin and eosin and are shown 122 times actual size.

lated follicles were observed, the ovaries were each 8 X 3 mm; the four corpora lutea, each now 3 mm in diameter, were still present. By 102 days after the corpora were first seen, four whitish corpora albicantia (not examined histologically), each measuring 1 mm in diameter, had formed at the sites of the preceding corpora lutea. At this time there were also two follicles, each 2 mm in diameter, in the right ovary and one of similar size in the left ovary. The female was judged ready to ovulate a second time.

Thirty-one days later one corpus luteum was found in the left ovary and five or more were found in the right ovary; each corpus luteum was approximately 5 mm in diameter. The left ovary was removed (133 mg) and sectioned, but no ovum was found in the corpus luteum. Thus, this animal had probably ovulated. Also, the secretory material in the endometrial glands indicated that progesterone had been secreted. Seventy-three days after the follicles were examined, the corpora lutea were still present but measured only 2 mm in diameter.

A female found when approximately 3 weeks old was kept as a house pet until the middle of April, when she was about 1 year of age. At that time she suddenly became vicious, severely biting both owners. She remained the most vicious raccoon we have seen among the many dozens of wild, captive, and pet raccoons that we have handled. According to her owners, she had never come into contact with other raccoons. At the time her behavior changed, her nipples were moderately stimulated and moderately pigmented, indicating that she was either pregnant or pseudopregnant. When first examined, her ovaries were 9 X 6 mm and 7 X 5 mm, respectively, and contained a total of five corpora lutea, each 3 mm in diameter. The size of the corpora indicated that they were regressing when examined, because newly formed corpora lutea in the ovaries of raccoons are approximately 5 mm in diameter. Thirty-five days after the initial examination all five corpora lutea were plainly visible but were regressing and were

slightly smaller than when first examined. Forty-eight days later (83 days after the first examination) no traces of the corpora lutea could be seen by gross examination.

The data on these three isolated females, one of which ovulated twice in one season, show that the raccoon is a spontaneous ovulator, and refute Llewellyn & Enders' (1954a:440) interpretation of their observations. The statement of Whitney & Underwood (1952:84) that ovulation in the raccoon is dependent upon copulation is not true for captive raccoons in Illinois.

In many captive raccoons, especially those reared as pets, the onset of estrus and pseudopregnancy was apparent from changes in behavior. A docile house pet sometimes suddenly became vicious and unmanageable. In all such cases that we examined, corpora lutea were present in the ovaries. The formation of corpora lutea was invariably accompanied by changes in the uteri and nipples whether the animal was pregnant or only pseudopregnant. The nipples always enlarged, and some became heavily pigmented, some became only slightly pigmented, and still others remained unpigmented. With the onset of pseudopregnancy the uteri became turgid and opaque and were considerably enlarged from their size during anestrus; however, they were not fluid filled and somewhat *rubbery*, as they were during estrus.

A female raccoon born in April was reared as a pet until the following January, when she became too unruly for the owners to handle and was donated to our project. She is described here as representative of the females, housed with other raccoons, that ovulated but did not become pregnant. According to her owners, she had not come into contact with other raccoons before she was donated to our project. Two days after we received her, she was placed in a cage with four yearling males. Forty-nine days later her nipples were tiny and white, but after 21 more days (March 28) they were elongated and black, indicating that she was either pregnant or pseudopreg-

nant. Five days later her left ovary was removed, and histological examination showed four freshly formed corpora lutea.

Histological examinations of the ovaries from this nonisolated female, from one isolated female, from three nonpregnant wild females, and from two additional nonisolated, nonpregnant, captive females revealed no ova in the corpora lutea. No substantial difference was noted between the corpora lutea of the isolated nonpregnant and of the nonisolated nonpregnant females. Ovaries from several nonpregnant females housed with other females, or with males, were examined during and after the breeding season. In several cases these ovaries had corpora lutea, which were grossly identical to those seen in females isolated prior to the breeding season and to corpora lutea in pregnant females. Thus, we concluded that corpora lutea in both isolated pseudopregnant and nonisolated pseudopregnant females formed from ovulated follicles and not from luteinization of follicles. Normal-appearing corpora lutea were also formed in the ovaries of a female in which ovulation was induced by exogenous hormones.

Data gathered from examination of 13 captive raccoons indicated that corpora lutea persist in pregnant females until parturition. Observations on four of these captives indicated that corpora lutea disappeared 14–16 days after parturition if the young were taken from the mother within 5 days after birth. In one of these four the corpora lutea were not present 16 days after parturition; in another they were present 14 days after parturition.

One female ovulated, apparently for the second time in the season, about May 11. She mated, and one embryo was implanted; approximately 20 days after ovulation the embryo was dead. Traces of one corpus luteum were still present in each ovary approximately 52 days after ovulation, and, on the basis of size and appearance, we concluded that they undoubtedly persisted for a maximum of 60 days.

In five nursing females the corpora lutea disappeared before the ovaries were examined from 11 to 35 days postpartum. A sixth female examined 11 days after parturition had four regressing corpora lutea, each 3 mm in diameter, in her left ovary and none in the right ovary. The corpora were those observed when she was first examined 34 days before the birth of her young. She was examined again 20 days after giving birth, when only four corpora albicantia were present in her left ovary. Thus, in this nursing female, the corpora lutea disappeared between 11 and 20 days after parturition. Corpora lutea were not found in histological preparations of ovaries from two wild, lactating females, nor by gross examination of the ovaries from six other wild, lactating females.

Pseudopregnancy

Our data indicate that corpora lutea persisted for about the same length of time in captive pseudopregnant raccoons as they did in those that give birth to young. Corpora were present 61 days but not 82 days after the estimated date of ovulation in one pseudopregnant captive. Three other pseudopregnant females showed similar periods of pseudopregnancy, although the data for these females were less precise than were the data for the first. The persistence of corpora in females that went at least halfway to term did not appear to differ significantly whether the young were aborted, were resorbed, or were born and were removed at birth or nursed until weaned. In one female, discussed in the preceding section, the young were resorbed at an early stage and the corpora lutea disappeared no more than 60 days after ovulation.

In some species pseudopregnancy may equal normal pregnancy in duration, but in most animals it lasts about half as long (Nalbandov 1958:218). Our observations indicated that all captive raccoons that ovulated, but did not become pregnant, underwent a period of pseudopregnancy much as does the dog. In the raccoon pseudopregnancy lasted ap-

proximately the same length of time as does normal pregnancy and followed ovulation.

Our observations of wild female raccoons during the breeding season indicated the relative incidence of pregnancies and pseudopregnancies, and supplied substantiating evidence that corpora lutea disappear in wild, lactating females, as in captives, shortly after they have given birth. Histological sections were made of ovaries collected from March through June (1957 through 1961) from 15 wild females 2 years of age or older. Six were pregnant, four were pseudopregnant, and five had recently given birth. Corpora albicantia were present in the ovaries of four of the five parous females, and corpora lutea were present in all of the pregnant and pseudopregnant animals. The fifth parous female, collected March 1, had recently given birth or aborted, as indicated by the fresh placental scars in her enlarged uterus and the four corpora lutea in her ovaries; however, she was not lactating. Corpora lutea were not found in histological sections of the ovaries from 39 young-of-the-year, 14 *yearling* (12–20 months old), and 10 adult wild raccoons collected from July through January.

Of 15 wild, parous female raccoons collected from February through September, only 1 had freshly ovulated follicles in February, and another had corpora lutea in March. None of the remaining 13 females, including 6 that were lactating, had corpora lutea. As mentioned earlier, histological examinations of ovaries from lactating, captive females indicated that corpora lutea disappear between 11 and 20 days after parturition, regardless of whether the females nurse their young.

From February through June (1957 through 1961) we made 30 observations on 24 captive female raccoons 2 years of age or older. Five were caught only a few days prior to examination. Of the 30 observations, 18 were of pregnant animals, 8 were of pseudopregnant females, and 4 were of animals neither pregnant nor pseudopregnant when examined. The

one animal that accounted for two of the four latter observations had an abnormally large uterus but inactive ovaries in 1959. Her uterus was enlarged but her ovaries were small when she was examined in May of 1957. Thus, she did not represent the norm. The other two observations of females that were neither pregnant nor pseudopregnant were of adult females that had given birth to litters in previous years; each was examined once during subsequent mating seasons. Because each was examined only once during the breeding season of the year in which corpora lutea were not found, it is conceivable that they had undergone pseudopregnancy but that the corpora had regressed before they were examined. Thus, evidence from both captive and wild females indicated that a majority of the females 2 years of age or older were either pregnant or pseudopregnant each year.

Every year during the hunting and trapping season a small percentage of females, judged to have ovulated on the basis of the stimulated or pigmented nipples, or both, were without uterine placental scars. During the fur seasons in Illinois from 1956–1957 through 1960–1961, uteri were examined from 284 females that appeared, on this basis, to have ovulated, and 7 (2.5 percent) had no placental scars. The evidence indicated that these animals had been only pseudopregnant. Some annual variation occurs in this characteristic. During the 1960–1961 fur season, all 77 females judged, upon examination of their nipples, to have ovulated had placental scars in their uteri.

PERCENTAGE OF YEARLING FEMALES THAT WERE SEXUALLY MATURE

Of 21 captive female raccoons approximately 1 year of age examined from February through June, 11 were either pregnant or pseudopregnant, but 10 were sexually immature. Histological sections of the ovaries from nine wild yearlings collected from February through August showed no corpora lutea in the five non-

pregnant females nor in the two lactating females, but corpora were present in the ovaries of the two pregnant yearlings. Gross examination of the ovaries from five wild, nulliparous yearlings collected from March through August showed that the ovaries of four contained no corpora lutea, but that three corpora lutea were present in one female collected in May. Thus, 10 of 21 captive yearlings and 9 of 14 wild yearlings were sexually immature.

During two fur seasons in Illinois (1959-1960 and 1960-1961) nulliparous adults with tiny unpigmented nipples accounted for 15 of 164 (9.2 percent) adult female raccoons examined. These nulliparous adults, with tiny unpigmented mammae, probably did not ovulate during the first breeding season after their birth.

SECRETION OF PROGESTERONE BY CORPORA LUTEA

The period of the production of progesterone by corpora lutea in the raccoon is unknown, but circumstantial evidence indicates that corpora lutea probably secrete progesterone as long as they are present (discussed later in connection with the production of uterine milk). One female had five corpora lutea, each 5 mm in diameter, when first examined on April 10. At that time we traumatized her left uterine horn by inserting a needle into the uterine lumen two times, each time scratching the entire length of the inside of the uterine horn with the point of the needle as it was withdrawn. Eight days later the ovaries and corpora were unchanged in gross size and appearance. The left uterine horn showed no evidence of trauma, but there is no direct evidence that the uterus of the raccoon will respond to traumatization with a decidual reaction in the presence of progesterone.

PIGMENTATION OF MAMMAE

Several female raccoons were studied to establish a possible physiological cause for the pigmentation or depigmentation

of nipples. Some pseudopregnant yearling females developed heavily pigmented nipples, whereas others did not. The presence or absence of pigment was not correlated with nursing, abortion, resorption of embryos, age at first estrus, or any other factors we could recognize. Unpigmented nipples remained so throughout life, but lightly pigmented nipples sometimes became darker with age. The pigment was not sloughed after nursing as Snyder & Christian (1960:650) found in the woodchuck (*Marmota monax*).

INTERSTITIAL TISSUE

Many studies were conducted before 1920 on the interstitial tissue in mammalian ovaries. Interstitial tissue is present in greater or lesser amounts in the ovaries of some species and is apparently absent in others. Little is known about its function. His (1865) was apparently the first to describe interstitial tissue cells in mammalian ovaries and to discuss their importance. Allen (1904: 120, 141) concluded that interstitial cells were formed from connective tissue during a process of degeneration in both the testis and ovary, and noted many points of similarity between the cells of the interstitial tissue and the lutein cells of corpora lutea. Kingsbury (1914:86) discussed the interstitial cells in the domestic cat (*Felis catus*) and recognized the lipid nature of the granules in these cells, but he found no evidence that the cells constitute morphologically an intra-ovarian gland. He also reported their presence in immature, newly born, and fetal kittens.

Rasmussen (1918:395) believed that in the woodchuck the interstitial cells proliferated from the terminal epithelium during adult life. He found a marked seasonal variation in the number of interstitial cells and in the amount of lipid present in them in the woodchuck. These cells gradually increased in number during hibernation and hypertrophied rapidly immediately after hibernation (Rasmussen 1918:371-372). Maximum numbers were seen in females that did not

become pregnant until late in the breeding season. Retrogression began with pregnancy and the growth of corpora lutea and continued until July. The ovarian interstitial cells were minimal in size in late summer and early autumn but then began to enlarge. After an extensive review of the literature, Rasmussen concluded, in accord with the vast majority of the investigators, that the interstitial cells come either directly from the connective tissue (stroma) of the ovary, or indirectly from the theca interna of atretic follicles.

According to Corner (1932:1597), the stroma of the rabbit ovary consists so largely of epithelioid cells heavily laden with lipid granules that the entire organ is a solid mass of interstitial cells in which the follicles and corpora lutea are embedded. This finding led to the concept that the ovarian stroma in this and similar species was a gland of internal secretion, the so-called interstitial gland. Embryological study showed that interstitial cells were largely derived from the theca interna of atretic follicles and that interstitial cells were found in many species at a very early stage of embryonic differentiation, in which case they seemed to be produced by the modification of the cells of the stroma and of the various epithelial proliferations. The pig ovary (Corner 1932:1597) contains epithelioid cells only in follicles and corpora lutea, the stroma cells being simply fibroblasts. Corner (1932:1597) reported that the cat ovary was between the extremes represented by the rabbit and pig ovaries. In the adult human ovary there appeared to be epithelioid cells only in follicles and corpora lutea.

It is conceivable that interstitial cells, whether found in great numbers in the stroma of the rabbit or in thin layers in atretic follicles in humans, are functionally the same, but proof is lacking (Corner 1932:1597). Corner (1932:1598) further reported that in all of the species he studied the interstitial cells contained granules of neutral fat or, at least, of lipoids, which reduce osmic acid and stain with Sudan III. Some workers are

ready to assume that the lipoids found in the interstitial cells represent a true internal secretion.

Much of the older work, mentioned by Stafford & Mossman (1945:97), showed that in some mammals the development of ovarian interstitial tissue is at its maximum during proestrus and estrus and that all of the animals included in this group, most of which breed annually or semiannually, have long reproductive cycles. The literature reports no evidence of ovarian interstitial tissue in laboratory rodents, which have short estrous cycles, and there is no easily discernible cycle in the amount or state of interstitial tissue that could be correlated with pregnancy in the guinea pig. There is a trend toward a maximum amount of interstitial tissue in the cortex near estrus and into early pregnancy and a minimum in midpregnancy. The high and low in the medulla seemed to occur a week or two later than in the cortex, suggesting that in the guinea pig medullary interstitial tissue originates from that of the cortex.

Patzelt (1955) studied the interstitial tissue in several carnivores and emphasized that age, time of year, and stage of the reproductive cycle greatly affected the interstitial cells. He also pointed out that other investigators considered thecal cells, which are traced back to the particularly active atresia of follicles during pregnancy, to be closely associated with the cells of the corpora lutea. Thus, Altman (1927) thought it conceivable that only a topographical contrast existed between thecal granulosa and lutein cells.

Patzelt (1955) regarded the interstitial tissue cells as producers of hormones and as a storage place for the substance necessary for the formation of new follicles and for propagation in general. The basis for his ideas was the fact that the lipid-containing cells are variously derived within the rudimentary ovary from germ layers, thecal cells, and cells of the surrounding stroma, and that it is not possible to demarcate the source of the interstitial cells. He usually found that ovarian interstitial cells were filled with

stored lipoids after a heat period and during pregnancy. After parturition a decrease in stored lipoids occurred that led to a functional dimorphism simultaneously with the formation and maturation of new follicles.

Hansson (1947) concluded that the abundance of interstitial tissue in the mink (*Mustela vison*) indicated that the tissue performed a special task. Because anestrus in the mink lasts from May to January, when no follicular growth beyond the vesicular stage takes place, the interstitial tissue may serve as a regulator during this time, governing sexual differentiation.

A preliminary study of the abundance of interstitial tissue in histological sections of the ovaries of 119 raccoons taken in all months indicated that interstitial tissue cells were abundant at some stages of the reproductive cycle, often occupying as much as 50-90 percent of the space in the ovary. However, interstitial tissue cells were seldom abundant when corpora lutea were present. Of 21 pairs of ovaries with corpora lutea, only 3 had significant amounts of interstitial tissue. One of these is shown in Fig. 10G.

Females less than about 2 months of age did not have large amounts of interstitial tissue in their ovaries. With this exception the ovaries of females less than 12 months old contained more, both relatively and absolutely, of this tissue, on the average, than did the ovaries of older females.

Seasonal trends in the abundance of interstitial tissue were apparent in ovaries with no corpora lutea. The ovaries removed from 16 adults from January through June contained little interstitial tissue. Ovaries removed from 26 adults killed from July through December contained more interstitial tissue than did those collected earlier in the year. No trend was apparent in the amount of interstitial tissue within the July-December period. During this interval ovaries from adults did not contain as much interstitial tissue as did ovaries from females less than 12 months old.

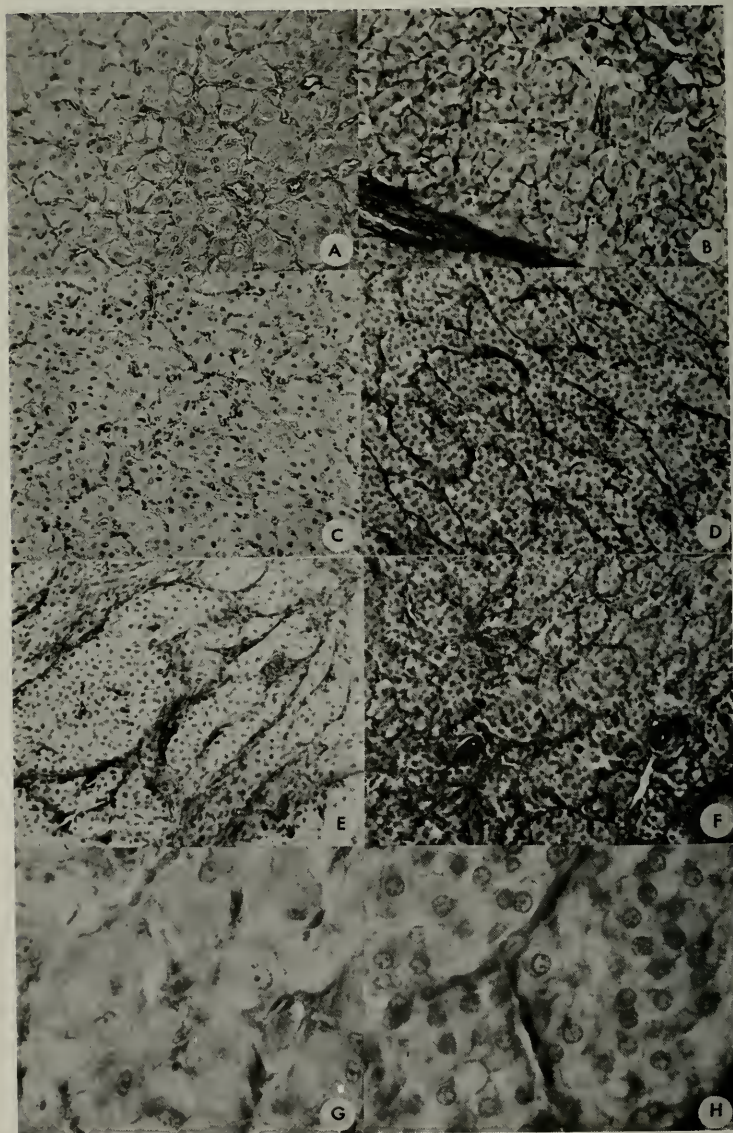
Ovaries from raccoons less than 12

months of age showed less seasonal variation in the abundance of interstitial tissue than did the ovaries from older animals. Small amounts of interstitial tissue were present in ovaries removed from seven juveniles in May and June, when most young were less than 2 months old. The ovaries excised from 24 juveniles in July, August, and September contained more interstitial tissue than did the ovaries examined in May and June, but the differences among the amounts of interstitial tissue found in July, August, and September were slight. The greatest abundance of interstitial tissue was discovered in 16 pairs of ovaries taken from juveniles during October and November. The maximum ovary weights recorded during this study were those of juvenile females in November (Table 5). Nine pairs of ovaries were examined from females not yet 1 year old killed during the period December through April. The abundance of interstitial tissue in these ovaries did not appear to differ from that in the ovaries of juveniles examined from July through September.

In spite of marked seasonal and age differences in the abundance of interstitial tissue, there was no apparent correlation between its abundance and the size or amount of coiling of the uterine glands. The development of the uterine glands and the presence of secretory material in these glands were largely dependent upon the presence of corpora lutea.

Three sources of interstitial tissue have been suggested, germ layers, thecal cells, and cells of the surrounding stroma, and all three may be present in the raccoon. Small amounts of interstitial tissue were present in some raccoon ovaries at birth. Judging from appearance alone, we believe it probable that some interstitial tissue in the raccoon is formed from degenerating follicles. Several cases similar to the one shown in Fig. 10A were seen during this study. In other ovaries there appeared to be a *streaming* of the cells as the interstitial tissue formed, presumably from the germinal epithelium

One striking feature of interstitial cells, although the luteal cells were generally larger (Fig. 10). Under the microscope



these two kinds of cells appeared more alike than the photographs in Fig. 10 indicate.

PLACENTAL SCARS

Deanesly (1935:464) first reported that she could recognize parous uteri in the stoat (*Mustela erminea*) by the presence of pigment granules that later workers called *placental scars*. Deno (1937: 433, 445) found that placental scars were produced in the mouse by accumulations of hemosiderin in the cells of the reticulo-endothelial system and that the placental scars were associated with the involuting metrial gland. Deno (1941) later reported that placental scars were visible in both the rat and mouse for a year or longer. Conaway (1955:516-517) stated:

"The placental scars of the rat appear as yellow to black pigmented areas along the utero-mesometrial border. Their origin seems identical with that of the scars in the mouse In both the rat and mouse, the metrial gland is a prominent structure at the base of the placenta Presumably it is formed by an extension of the decidual response into the connective tissue of the myometrium. The pigment-laden cells are concentrated in this area between the longitudinal and circular muscle layers although some are found in the deeper stroma of the endometrium. As the age of the scar increases the pigmented area may decrease in size and appear darker in color."

Sooter (1946:69-70) counted placental scars to determine the numbers of young produced by muskrats (*Ondatra*

zibethicus) although no critical work has been done to determine whether the number of placental scars corresponds to the number of young born. Elder (1952) reported the failure of placental scars to reveal breeding history in captive mink. Brambell & Mills (1948:241), working with the European rabbit (*Oryctolagus cuniculus*), again pointed out

"that although there is little likelihood of failure to detect implantation sites containing living embryos the possibility remains of the disappearance before full term of sites in which the embryos had died and were reabsorbed soon after implantation or, more probably, that such sites might be overlooked, through becoming less conspicuous, and hence omitted from the counts."

In laboratory rats and wild brown rats placental scars were only a crude indication of the number of young produced (Davis & Emlen 1948: 166), with errors as high as 100 percent in either direction. Conaway (1955:531) found that placental scars in the laboratory rat were always formed if all embryos were resorbed after the 11th day of pregnancy, whereas total resorption prior to this time never caused the formation of scars. If some of the embryos were resorbed, death on the seventh day or later resulted in scar formation at all resorption and term sites. If some embryos were resorbed between the 8th and 11th days and the remainder after that, scars were formed at all sites. The size and appearance of resorption scars were similar to those of term scars. Momberg & Conaway (1956: 379) found that 32 of 312 placental scars from previous pregnancies were overlapped by scars of second pregnan-

Fig. 10 (Page 60).—Photomicrographs of luteal and interstitial cells of raccoons, showing similarities in the two. A, female 2137; luteal cells (X 94); ovary removed March 19, 1958; pregnant. B, female 1292; luteal cells (X 94); ovary removed May 14, 1958; ovulation caused by injections of pregnant mare's serum. C, female 2805; luteal cells (X 94); ovary removed February 24, 1960; fresh corpora lutea resulted from natural ovulations; pseudopregnant. D, female 2232; interstitial cells (X 94); ovary removed August 7, 1958; wild animal approximately 3 months old. E, female 2403; interstitial cells (X 94); ovary removed November 6, 1958; wild animal 7 months old. F, female 2234; interstitial cells (X 94); ovary removed August 8, 1958; wild animal 3 months old. G, female 1292; luteal cells (X 375); ovary removed May 14, 1958; ovulation caused by injections of pregnant mare's serum. H, female 2242; interstitial cells (X 375); ovary removed August 27, 1958; wild animal 4 months old. The sections were stained with hematoxylin and eosin.

cies in the white rat. They could not always recognize the superposed scars by gross examination, but microscopic recognition was possible.

The placenta of the raccoon was first described by Watson (1881:280-296). The zonary placenta of the raccoon is similar to that of other carnivorous mammals. Watson (1881:279) noted:

"The placenta formed a complete ring, but at the centre of its widest part, *i.e.*, opposite the back of the foetus, there was a spot similar to that figured by Daubenton in the placenta of *Martes domestica*, and described by Bischoff in that of *Lutra vulgaris*, *Mustela foinea* and *Mustela martes*, where the substance of the placenta was deficient. This deficiency involved the entire thickness of the placenta, so that a probe could be passed from the uterine to the chorionic surface of the organ without injury to its substance."

The placenta of *Procyon* is truly deciduous in character, as it is in the dog, cat, fox, and seal. According to the classification of Mossman (1937:224), the raccoon placenta is endotheliochorial. Placental scars in the raccoon were apparently first noted by Stuewer (1943b:68), who autopsied a female raccoon in May and found four placental scars in the uterus; he believed they indicated that four young had been born. Sanderson (1950:399) examined uteri from six captive females and concluded that "placental scars may be an accurate measure of litter size in raccoons."

If placental scars are to be useful in estimating the reproductive performance of a species, several facts about them must first be known. Pertinent questions are: (1) Is one placental scar formed for each implantation site regardless of the fate of the developing embryo? (2) If the answer to the first question is no, then what stages of embryonic development result in the formation of placental scars? (3) Is it possible to differentiate placental scars formed from embryos that go to term from those formed from embryos that are aborted or resorbed? (4)

How long do the placental scars persist, and is the length of time they persist affected by the female's subsequent breeding history? (5) Are the placental scars recognizable at all seasons of the year? (6) If the placental scars persist beyond a subsequent pregnancy, is it possible to recognize scars representing litters from different years? Some preliminary information on all of these questions has been obtained.

In only 2 of 27 litters with a total of 98 embryos in 2 of 20 captive female raccoons that we examined did we find discrepancies between the number of embryos observed and the number of placental scars identified later. One female (No. 2960) had four embryos, estimated to be 30 days of age when examined on May 26, but five grossly identical placental scars when the uterus was removed 6 months later. The additional scar may have represented a litter of one from a previous year. If so, the scar was overlooked when this same uterus was examined during the fall before the four embryos were observed. The extra scar may have also represented an additional embryo that was aborted or resorbed prior to the time the four embryos were examined. The second female (No. 4022) had two live and one dead embryo when first examined on February 19. She gave birth to two live young 29 days later, but when her uterus was examined 11 days after parturition, there were two grossly identical scars in each horn. There were four corpora lutea in her left ovary and none in the right. Thus, the additional scar observed at the second laparotomy was probably from an embryo that was aborted or resorbed prior to the first examination.

A captive female raccoon (No. 2960) had four embryos, estimated to be 30 days of age, when examined. She was given a drug, Malucidin, that caused either abortion or resorption. The embryos were gone 13 days later, and the sites of attachment were indicated by large bumps. When the uterus was removed 6 months later, five placental scars were identified by slight bumps. We

split the uterine horns and identified the five placental scars as typical for captive females. (Possible differences in placental scars of captive and wild animals are discussed below.) Thus, in this female, one placental scar was formed for each of the four embryos even though all four embryos were either aborted or resorbed at midterm. As has been discussed, the fifth scar either persisted from the previous year or resulted from an embryo resorbed prior to the first examination when the four embryos were about 30 days of age.

Another captive female raccoon (No. 3333) had four live embryos and one that was being resorbed in her uterus on March 21. It was estimated that the embryo being resorbed had died 30 days after conception. The young were born 33 days after the initial examination. Sixteen days after the birth of the litter the placental scar representing the resorbed embryo was smaller than the others, but 47 days after parturition no gross difference could be detected among the five scars.

One pregnant captive female raccoon (No. 2824) was castrated approximately 50 days prepartum, but her embryos continued to grow for about 20 days before they were aborted and resorbed. A second captive pregnant female (No. 2151) was castrated approximately 30 days prepartum, and her young were aborted about 1 week prepartum. Two months after abortion or resorption the placental scars in these females could not be differentiated grossly from those formed by normal embryos born at term. Female No. 2824 was killed 4.5 months after she was castrated. When she was killed, only one placental scar was found, both before and after the uterus was split, even though the exact locations of the embryos were known. The one scar was dark and broad, and appeared to be typical of those formed from young born during the current breeding season. The scar was formed at the site of one of three embryos present 19 days after castration. All three of the embryos were aborted prior to 26 days after castration.

The data from the two castrated females (No. 2824 and 2151) that lost their young and from six intact captive females that resorbed or aborted some or all of their embryos indicated that one placental scar was formed for each embryo that existed for approximately 30 days, whether or not any embryo went to term.

We have made some observations on the persistence of placental scars in the raccoon (Table 11). Placental scars were present, although indistinct, in one female (No. 2959) when her uterus was removed nearly 19 months after her young were born. Scars were visible 12 and 17 months after parturition in another female (No. 1786), but could not be seen in her enlarged uterus stimulated by hormones 14 and 24 months after parturition. Her ovaries were removed approximately 1 year after the birth of her young, and, after castration, she was treated with estradiol and progesterone at various intervals. These treatments may have affected the rate of disappearance of her scars. A third female (No. 2779) had placental scars for 14 months, but not 23 months, after

Table 11.—Persistence of placental scars in captive raccoons.

Raccoon Number	Number of Months Scars Persisted After Parturition	
	Minimum	Maximum
19	4.0	...
1782	5.0	...
1786	17.0 ^a	30.0
2120	6.0	...
2124	4.5	18.5
2124	8.0	...
2125	16.5 ^b	...
2151	3.0	...
2184B	15.0	27.0
2230	6.0	...
2779	14.0	23.0
2959	18.5	...
2960	19.0	30.0
3333	14.0	...
3350	3.5	...

^a Not visible macroscopically — approximately 14 months after parturition — in the uterus stimulated by hormones.

^b Not visible macroscopically during the subsequent estrus, approximately 10 months after parturition, but the same scars were again visible macroscopically 16.5 months after parturition.

parturition, and a fourth female (No. 2125) retained placental scars nearly 17 months after parturition. None of these females gave birth in the second year.

There was no macroscopic evidence of placental scars from a 1958 litter (Female 2124) 18.5 months postpartum, but histological examination revealed a few scattered pigment granules, and scars from a 1959 litter were prominent. Thus, the 1958 scars disappeared, for practical purposes, prior to 18.5 months after parturition, when she had a litter the following year. All female raccoons had placental scars when examined from 2 to 10 months after the birth of their young. The evidence indicated that if a female failed to give birth to a litter in the next year, placental scars persisted for approximately 19 months in captives, but not as long as 24 months. If a captive gave birth to a litter the next year, scars from the first litter persisted for 10 or more months but not as long as 19 months.

One captive raccoon became pregnant at the second ovulation during one season. The single embryo, in the process of being resorbed when it was first observed, was estimated to be 20 days old. Twenty-one days later the site of placental attachment was readily identified as a bump 8 X 7 mm in size; 61 days after the initial observation no trace of the scar could be seen. Thus, this scar disappeared between 21 and 61 days after the resorbing, 20-day embryo was observed. The absence of living embryos in this captive may have been an important factor in the rapid disappearance of the scar.

The variability in the length of time that placental scars were visible in the raccoon after parturition is shown in Table 11. In two females scars were not grossly visible in their stimulated uteri during or near estrous cycles of the ensuing years, because their enlarged uteri caused a diffusion of the pigment granules of the scars, making them invisible. Scars in these females were again visible macroscopically when the uteri regressed.

Scars from a litter born in May 1958 (discussed above) could not be seen (No. 2124, Table 11) 18.5 months later (December 1959) even though their exact locations were known and the uterus was removed and split. After we sectioned the site of one scar, we were able to identify a few scattered pigment granules in the endometrium. Two scars from a litter born in April 1959 were easily identified macroscopically in this same uterus 8 months (December 1959) after the birth. In a second female (No. 2959, Table 11) scars from young born in May were not visible with translucent light after the uterus, which was stimulated, was removed 18.5 months later. All four scars from this litter were located and were identified by the slight bumps visible at the placental sites. After the uterus was opened, all four scars were visible as pale, brownish areas, but they might have been overlooked had not their exact locations been known. When one of these scars was examined histologically, moderate numbers of pigment granules were seen in clumps and scattered in the endometrium and in the adjacent myometrium.

The distribution of pigment granules in the uteri of two wild females was studied in histological sections. Each of these females had four scars at autopsy. Pigment granules in the uterus of one female were somewhat scattered but seemed to concentrate in a ring deep in the endometrium near the myometrium. Many pigment granules were scattered throughout the endometrium of the uterus of the other female.

Pale placental scars were often difficult to see *in situ* in a live animal, and early in the study some scars may have been overlooked. We believe that, after we became experienced in looking for scars, no visible placental scar was overlooked, but they could not be seen in pregnant females and females at or near estrus. When the scars had practically disappeared, they could be observed only by splitting the uterus. Thus, these pale scars would be overlooked when examining live females by laparotomy. If the

uterus of a live female was stimulated, many of the placental sites could best be identified by slight, opaque bumps rather than by the pigmentation. Identification of the location of scars by the presence of bumps was possible for several weeks after parturition, when the uterus was still stimulated, as well as in the stimulated uterus at or near estrus. After the uterus regressed, scars were usually readily visible as bumps or could be identified by using translucent light to observe the pigmented areas. The pigmented areas could also be located when the uterus was opened or by histological examination.

Placental scars seem to persist longer in wild raccoons than they do in captives. The placental scars of captives that we examined from October through January after the births of their litters were generally pale brown, small, and slightly opaque. A majority of the wild females examined during these same months had larger, more opaque scars, often black. Many (55.2 percent in 1959 and 41.0 percent in 1960) uteri of wild, parous females had more than one group of scars, which differed in size and density (Fig. 11). Presumably these scars were from different years; however, some might have been from different litters born in 1 year. There was no evidence that as many as 40 or 50 percent of the wild females gave birth to second litters during a single season. Thus, placental scars probably persist for 20 months or

longer in many — perhaps in all — wild females. In the few wild females with three groups of scars, the first group may have persisted for as long as 32 months.

The placental scars of raccoons are useful for estimating litter size and rate of productivity. However, these scars must be used with caution, and care must be taken to separate properly the groups of scars. We do not know for certain the significance of multiple groups of scars. We can say with reasonable confidence that each embryo that reaches 1 month of age is represented by one scar for 10 or more months. Scars in wild females with only one group of scars probably reflect implantation rates for the preceding breeding season. Most single groups of placental scars occur in females that have mated successfully only once.

MORPHOLOGY OF THE REPRODUCTIVE TRACTS

Males

The duct system and accessory glands in the reproductive system of the male raccoon (Fig. 12) are similar to those found in the dog, as described and shown by Nalbandov (1958: 42-44). Seminal vesicles are lacking, as they are in the dog, fox (*Vulpes fulva*), and wolf (*Canis lupus*). The Cowper's glands (bulbo-urethral glands) are also absent. The walls of the vasa deferentia thicken prior to entering the prostate and form the ampullae. The ampullae and the urethra



Fig. 11.—Raccoon uterus (X 0.75) split to show two groups of placental scars. This female was killed on January 23. Two light scars were only barely visible in the photograph but were readily visible in the fresh specimen. Their locations and densities relative to the three dark scars are indicated by the light stippling (arrows).

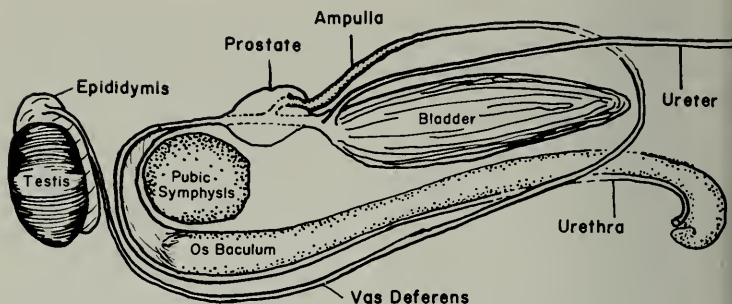


Fig. 12.—Schematic drawing (side view) of the reproductive system (X 0.85) of an adult male raccoon.

unite inside the prostate to form a common duct. The many compartments of the prostate gland open into this duct system.

The *os penis* or *os baculum* (bone of the penis) is well developed in the raccoon. Its stage of development has been used to separate males into two age groups (Sanderson 1950: 395-396; 1961a: 11-14). The os baculum was once used by tailors as a ripping tool for taking out basting threads (Jaeger 1947: 297).

We found several raccoon bacula that had been broken and then healed. Sanderson (1950: Plate 11) showed a photograph of some of these bones. Our data from wild males shed some light on possible causes for these broken bones. During four hunting and trapping seasons in Illinois (1957-1958 through 1960-1961), 7,233 bacula from juvenile raccoons were examined. Forty-three (0.6 percent) of these had been broken but were healed or healing, and 238 (3.3 percent) were freshly broken. At the same time, 4,152 bacula from adults were examined. Eighty-six (2.1 percent) of these had been broken but were healed, whereas 41 (1.0 percent) were freshly broken.

These data indicate that most of the breaks in the os baculum of the raccoon occur in juveniles. The bacula of juveniles are much softer and more easily broken than are those of adults. Hunters often shake a raccoon out of a tree and

let their dogs fight it. Fighting with dogs could account for the freshly broken bones found in both adults and juveniles, and the more durable bones of adults would explain the smaller percentage of freshly broken bacula found in older raccoons.

Females

The raccoon uterus (Fig. 13) is somewhat intermediate between the bicornuate uterus found in the pig and insectivores, and the bipartite uterus found in the cat and dog. There is a single cervix and the horns are distinct, but after the horns join externally to form the single, small uterine body, the uterine lumina remain separate — even though this separation is not apparent from the outside — to a point near the cervix.

Llewellyn & Enders (1954b: 439) removed one ovary, ovarian capsule, oviduct, and proximal end of the uterine horn in each of two raccoons. After closing the cut ends of the uteri with sutures, they released the females. When retrapped the next year, each female was carrying three embryos, two each in the normal horns and one each in the ovariectomized horns. Thus, even though the internal separation of the uterus extends nearly to the cervix, ova can pass from one uterine horn to the other. In our study some indirect evidence of transuterine migration of ova was noted. In a few cases more embryos were found

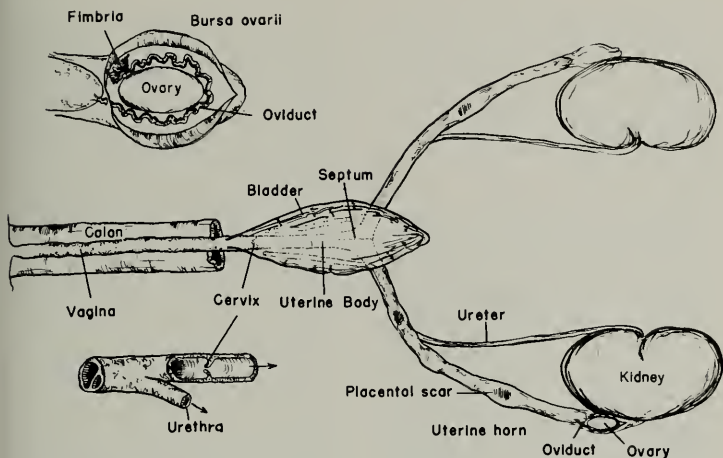


Fig. 13.—Schematic drawing (ventral view) of the reproductive tract (X 0.5) of a parous female raccoon.

in a uterine horn than there were corpora lutea in the corresponding ovary, but the total number of corpora lutea present in both ovaries was usually the same as the number of embryos or placental scars present in both uterine horns.

The ovary in the raccoon, ovoid in shape, is completely surrounded by the bursa ovarii (Fig. 13). This sac is intact except for a small slit on one side, not large enough to permit passage of the ovary as in the mink (*Mustela vison*), dog, and fox. One of our captive females had a congenital deficiency of the bursa that was large enough to permit passage of the right ovary. This opening was slightly dorsal to the normal slit in the bursa but was not connected with it. The left ovarian bursa was normal. This captive was the only such animal among several hundred examined. Watson (1881: 273-274) observed one raccoon and reported that the ovary was destitute of any peritoneal pouch or pavilion such as formed an almost complete sac in many animals.

The fimbria is extensive, and in the estrous female the edge of the fimbria is bright red and protrudes through the

slit in the capsule. This bit of fimbria grossly resembles the gills of a fish. The fimbria joins with the end of the oviduct. The oviduct is highly convoluted and makes an almost complete circle around the ovary before entering the uterus (Fig. 13).

Two, three, and sometimes four ova were observed in a single follicle. When an ovary contained one follicle with multiple ova, several other follicles with multiple ova were usually present.

Approximately 25 female raccoons from Iowa and approximately 25 from Illinois were examined for the presence of the *os clitoridis*. A bone — 11 mm in length — was found in only one clitoris. Rinker (1944: 91) found four ossa clitoridae in four female raccoons examined in Kansas, but found no bones in the clitoria of four other females from a "distant locality," apparently in Kansas. Burt (1960: 8) used Rinker's observation as the basis for stating that the *os clitoridis* is present in the raccoon. Sanderson (1950: 398) found only one *os clitoridis* among 100 female raccoons in Missouri. Because only a small percentage of females examined from Mis-

souri, Iowa, and Illinois had ossa clitoridæ, there may be geographic variation in the presence of this bone. Its presence is not of general occurrence in raccoons in all localities.

EFFECTS OF CASTRATION

Males

Some effects of castration on the development of the os baculum in the raccoon have been discussed by Sanderson (1961a: 13-14). The information in that report, with additional observations, is presented here. The lack of sex hormones in males was reflected by the much shorter and thinner bacula in castrated animals in comparison with bacula from intact animals of similar ages (Sanderson 1961a: Fig. 4). The lack of sex hormones became apparent at 8-11 months of age in castrated males. Sanderson (1950: 396) showed that in intact males the penis normally became extrusible at about 10 months of age, but a castrate male (Sanderson 1961a: Fig. 6, No. 59) had a nonextrusible penis and a small baculum at 22 months of age. This baculum was only slightly longer and heavier than one from a castrate raccoon only 10 months of age (Sanderson 1961a: Fig. 5, No. 209), but both were much shorter and thinner than were the bacula from intact males 18-23 months of age (Sanderson 1961a: Fig. 6). The baculum from the castrated raccoon 22 months of age was dense like an adult bone and not spongy at the base as were bacula of similar size from raccoons 12 months of age and younger.

Thus, we concluded that the level of sex hormones affected the enlargement of the preputial orifice and maturation of the penis bone but had little or no effect on the development of the baculum prior to 7 months of age.

Castration in males also apparently caused a slight delay in the closure of the epiphyseal cartilage in the radius and ulna, but because most of the castrated males in this study died of disease at early ages, not enough information was available to demonstrate this relationship conclusively.

Epiphyseal plates were classified as closed (without cartilage), thin (intermediate condition), or broad (with a thick plate of cartilage) (Sanderson 1961a: 7). One castrated male had broad epiphyses at 17 months of age and thin epiphyses at 20 months of age. His epiphyses were still thin when he died at 22 months of age. When examined, 35 intact males with broad epiphyses were 15 months of age or less, whereas 14 of 17 males (82 percent) with thin epiphyses were 13-19 months of age. Epiphyseal plates in 11 of 13 intact males closed between 16 and 21 months of age (Sanderson 1961a: 16). The effects of castration on epiphyseal closure merit further study.

Females

The time of closure of epiphyses in females was much like that in males, but the greater variation in the upper ages of females with thin epiphyses indicated that epiphyseal closure was delayed in some females or occurred later in some than in others.

One factor that perhaps influences age at epiphyseal closure is the level of circulating hormones. Two females were castrated to study the effects of the absence of ovarian hormones on epiphyseal closure. One female, born in the wild, was castrated at an estimated age of 4 months, and one, born in captivity, was 3 months old when castrated. The first had broad epiphyses at 14 months of age, thin epiphyses at 20 months of age, and thin epiphyses when she died at 23 months of age. The second had broad epiphyses at 22 months of age, thin epiphyses at 25 months, and nearly closed epiphyses at 27 months of age. Epiphyseal development and closure in these two castrated females were delayed in comparison with the rate of development and closure found in the average intact female. Four additional females were castrated at estimated ages ranging from 13 to 24 months. Our observations suggested that the removal of the ovaries, even after raccoons had reached sexual maturity but before the epiphyses closed, delayed the rate of epiphyseal closure.

Major factors that may have contributed to the variations we observed in age at epiphyseal closure in female raccoons were (1) age at first mating, (2) hormone secretion level, and (3) quality and quantity of nutrition. Factors influencing age at epiphyseal closure should be studied further because the available data are somewhat contradictory.

Thus, our data and those of Sanderson (1961a: 10-11) suggest that epiphyseal closure in the castrated female raccoon is delayed in comparison with that found in the average intact female but falls within the limits of variability for intact females.

In mammals castration after implantation and during the first third or first half of pregnancy usually leads to abortion or resorption of the fetuses (Nalbandov 1958:221). In some mammals the ovaries are required throughout gestation, but other mammals do not lose their young after castration, once the crucial period is past.

Two pregnant females were castrated to learn whether the raccoon is a species in which pregnancy is maintained after castration. To establish limits after which castration is tolerated, these two animals were castrated approximately 11 and 38 days after conception, respectively. (It had been established earlier that performing laparotomies on pregnant raccoons did not interfere with pregnancy.)

The first of these two pregnant female raccoons that we castrated was born in 1959 and reared as a pet. She gave birth to a litter in 1960. On February 5, 1961 she forcibly repelled the approaches of her mate. Twenty-five days later she had three embryos in her left uterine horn and two in the right but only four corpora lutea. Each uterine swelling was 10 mm in diameter. We estimated the embryos to be 11 days old, suggesting that mating had occurred about February 17. Both ovaries were removed on March 2, and 19 days later two embryos in the left uterine horn were being resorbed. These two swellings were almost as large as the other three, but the surfaces were collapsed and flaccid, not turgid like those of a normal swelling. The embryos

were still present at both sites. The remaining three embryos, 45 X 20 mm, looked almost normal, except that the swellings appeared less round and turgid than normal swellings are. We could not discern whether the embryos were alive or dead. We estimated that, if they were alive, they would be born in 25 days (Fig. 7). Thus, these embryos had a normal rate of growth for 19 days after the castration of the female. No embryo was found 26 days after castration. From gross appearances we concluded that the last three embryos were aborted and the first two were resorbed.

Twenty-eight days after this female raccoon was castrated, her mate was returned to her cage. The next day, only 3-10 days after her young were aborted, this pair was observed in copulation. This activity suggests the possibility that postpartum heats, which occur in several species such as the sow and mare, may not be dependent upon the presence of the ovaries.

This female escaped 3 months after she was castrated and was taken in a steel trap 52 days later. After she was killed, it was discovered that she was lactating profusely. When the mammary gland was sliced with a scalpel, the entire cut area immediately filled with milk. She was lactating more than 4.5 months after her ovaries had been removed and 4 months after her young had been resorbed and aborted. However, the second pregnant female that was castrated showed no indication of lactation 5 months after removal of her ovaries.

No traces of ovarian tissue were found during the autopsy performed on the first of these females. The uterus, measuring 7 X 4 mm, was turgid and appeared similar to uteri of animals at estrus, but sectioning showed the endometrium to be devoid of even traces of glands. Other female raccoons, months after being castrated, had thick epithelia lining their vaginas (Fig. 9A), suggesting the possibility of an extraovarian source of estrogen in the castrated female.

The second of the pregnant raccoons that was castrated was placed in captivity in 1958 when she was about 2 months

old. She had two embryos in each uterine horn on February 28, 1961, when both ovaries were removed (approximately 38 days after conception and 25 days before parturition), but the left ovary had three corpora lutea and the right ovary only one corpus luteum. Nineteen days after castration and 6 days prior to expected parturition, one dead embryo weighing 43 grams was found in her nest box. The average birth weight of eight newly born raccoons that we weighed was 61.8 grams. The embryo was well developed but did not have much hair, and its hair was shorter than in most young at birth.

Twenty-one days after castration the uterus contained enlarged areas where the young had been attached. One of these sites was opened and examined. Detritus was present, but there was no other evidence of resorption, which was occasionally seen in both wild and captive females. Thus, all four embryos were probably aborted about 1 week prepartum.

Although the two females were castrated at different stages of pregnancy, the embryos apparently persisted for about the same length of time in each—19 days after castration—33 days short of term for the first female and approximately 7 days short of term for the second.

EFFECTS OF EXOGENOUS HORMONES

Males

Two male raccoons were studied to learn whether injections of androgen would initiate or prolong spermatogenesis during the male's period of summer sterility. The first animal chosen was an adult male at least 20 months old when he was captured. In August (during the period of sexual inactivity) the left testis was removed, weighed (1.6 grams), and preserved for histological study. The average weight of one testis from an adult in August was 2.6 grams (Table 1). Sperm could not be found in the epididymis, but spermatogenesis was occurring in a few seminiferous tubules. This male was given six testosterone doses of 30

mg each subcutaneously over a period of 18 days. He was killed 21 days after the removal of his left testis and the first injection of testosterone. The right testis weighed 1.6 grams, and no sperm were present in either the seminiferous tubules or epididymis.

Histological comparison of the two testes and the epididymides showed slight changes that we attributed to the testosterone injections. Both before and after the hormone treatment most spermatogenic cells were approximately 8 microns, and the nuclei 3 microns, in diameter. However, after testosterone injections a few of the cells were as large as 11 microns in diameter. The lumina of the seminiferous tubules remained about the same size after the treatment as they were before, but after the injections the cells of the seminiferous tubules were more scattered than they were before treatment. Sperm were present in the seminiferous tubules prior to treatment, but not afterwards. Sperm could not be found in either epididymis, one of which was removed and examined before and the other after the hormone treatment. The epithelial lining of the tubules was 46 microns tall prior to treatment and 30 microns after treatment (each height is an average of five measurements), indicating degenerative changes, perhaps caused by the hormone. The average outside diameter of the tubules was 140 microns prior to treatment and 65 microns afterwards.

A second male raccoon, captured when approximately 3 months of age, was reared as a pet. He was approximately 16 months old when his left testis, weighing 3.3 grams, was removed in August. The epididymis contained many motile sperm.

He was treated with four doses of 12 mg each of testosterone over a 13-day period. He was killed 15 days after the removal of the left testis and the first injection of the hormone. At that time his right testis weighed 2.5 grams, and many motile sperm were in the epididymis. Histological examination revealed few changes in the cells of the seminiferous tubules.

A few sperm were present in the seminiferous tubules both before and after treatment. After treatment sperm were not found in a section of the epididymis, but a few were observed in a drop of fluid collected from the tail of the epididymis.

Females

Ovulation can be induced during anestrus in several species of domestic and laboratory animals by the injection of gonadotrophic hormones. Hammond (1952:218) used pregnant mare's serum (PMS) as a follicle-stimulating agent and chorionic gonadotropin to cause ovulation in ranch mink.

We made several attempts, using 19 individuals, to cause the growth and development of follicles and to cause ovulation in the raccoon by injecting hormones. Only four individuals ovulated, and three of these cases involved the use of PMS (Table 12). Only the four cases in which ovulation occurred are discussed.

In one series of experiments various dosages of follicle-stimulating hormone (FSH) given subcutaneously were followed by luteinizing hormone (LH) given intravenously. Later FSH and LH were mixed and given subcutaneously, followed by intravenous injection of LH. With one exception all attempts using FSH and LH were unsuccessful in causing ovulation. In some cases normal-appearing follicles were numerous in the ovaries after injections of FSH and mixtures of FSH and LH, but attempts with LH and with a mixture of LH and FSH to cause the follicles to ovulate were unsuccessful. The ovaries generally were overstimulated; that is, they were larger than normal and contained more follicles than normal.

The successful ovulation that did not involve injections of PMS occurred in a female raccoon (the first female in Table 12) approximately 44 months old, weighing 6.7 kg. Each ovary was 11 X 6 mm, with no follicles or corpora lutea approximately 2 months prior to the breeding season. A section of her uterus was removed when the ovaries were measured (approximately 2 months prior to the

breeding season) so that we could study the pigment granules. The next day subcutaneous injections of a mixture of 10 Armour units (AU) each of FSH and LH were begun. These injections were given for 10 days, and on the 12th day a mixture of 80 units each of FSH and LH was injected intravenously. At that time each ovary was 12 X 8 mm and contained 10-20 clear follicles, each about 1 mm in diameter. On the 13th day 100 units each of FSH and LH were injected intraperitoneally as a mixture. On the 16th day the left ovary was 18 X 9 mm and contained approximately 20 follicles, each about 2 mm in diameter, but ovulation had not occurred. The left ovary weighed 760 mg, compared with an average weight of about 137 mg for one ovary of parous or pregnant females during the mating season (Table 5). When the raccoon was killed 45 days after the first injection, her right ovary weighed 290 mg and contained 11 corpora lutea.

In a second series of experiments PMS was injected into eight females in attempts to cause the development of follicles and to cause ovulation. Three of these attempts were successful. The first female was approximately 2 years of age and had been in captivity for more than a year when hormone treatments were begun. We injected 100 international units (IU) of PMS subcutaneously each day for 12 days and 500 IU each on the 13th and 16th days. Thirteen days later, 28 days after the treatment was begun, the uterus and both ovaries were removed. The contents of the oviducts and uterine horns were flushed out, but no ova or blastocysts were found. Each ovary contained approximately 30 corpora lutea. Even though no ovum was recovered, the abnormally large number of corpora lutea containing no ova indicates that this female probably ovulated. The secretory material found in the lumina of the uterine glands indicated that progesterone had probably been secreted.

The second female was captured when she was at least 18 months of age; however, she was not injected with hormones until she was about 53 months old. Sub-

Table 12.—Attempts to cause ovulation in raccoons by injections of exogenous hormones.

Estimated Age of the Raccoon in Months ^a	Weight in Kilograms	Injection Date ^b	Dose (each) ^c	Hormone	Laparotomy or Autopsy Date and Results
21	5.80	1-17-58	2.5 mg	Estradiol ^d	5-6-58: 4 embryos in uterus. 5-13-58: Young born.
		4-16-58-4-28-58	200 IU	PMS ^e	
		5-1-58	500 IU	PMS	
24	3.18	12-3-59-12-12-59	10 AU ^f	FSH ^g , LH ^{h,i}	12-14-59: Ovaries 12 x 8 mm; 10-20 follicles 1 mm diam in each ovary. 12-18-59: Left ovary 18 x 9 mm, 760 mg; 20 follicles 2 mm diam, not ovulated. 1-17-60: Right ovary 290 mg; 11 corpora lutea.
		12-14-59	80 AU ^j	FSH, LH	
		12-15-59	100 AU ^k	FSH, LH	
53	4.76	4-16-58-4-27-58	100 IU	PMS	5-14-58: 60 corpora lutea. 9-21-59: Many unovulated follicles. 9-24-59: Approx. 12 <i>blast punkte</i> in each ovary. Left ovary 826 mg; 26 early-stage corpora lutea. 10-5-59: Right ovary 2,147 mg; 29 early-stage corpora lutea.
		4-28-58, 5-1-58	500 IU	PMS	
		9-9-59-9-20-59	100 IU	PMS	
		9-21-59	550 IU ^j	PMS	

Estimated Age of the Raccoon in Months ^a	Weight in Kilograms	Injection Date ^b	Dose (each) ^c	Hormone	Laparotomy or Autopsy Date and Results
53	5.35	12-2-59—12-13-59 12-14-59	50 IU 200 IU ^j	PMS PMS	12-1-59: Ovaries 10 x 16 mm. 12-14-59: Ovaries 12 x 6 mm; 9 follicles 2 mm diam in each ovary. 12-16-59: Left ovary 12 x 6 mm; follicles 2 mm, ready to ovulate. 12-17-59: Blood oozed from follicles. Left ovary 242 mg, 10 x 6 mm; 6 early-stage corpora lutea 2,000 microns diam. 12-19-59: Right ovary, 8 early-stage corpora lutea 2,400 microns diam; 3-4 corpora lutea with ova.
14	4.54	6-25-57—6-29-57 6-30-57	Unsuccessful Attempts 100 IU 500 IU	PMS PMS	7-1-57: Right ovary, 3 follicles 1,075 x 1,400 microns; no corpora lutea.
		8-3-57—8-7-57 8-9-57—8-11-57	200 IU 500 IU	PMS PMS	8-13-57: Left ovary, follicles to 480 microns; no corpora lutea.
4	4.44	8-24-59 8-24-59—9-2-59 9-3-59 9-4-59	12.5 mg 50 IU ^m 1,000 IU ^j 2,000 IU ^j	P ^t PMS CGH ⁿ CGH	9-4-59: Large follicles, none ovulated. 9-7-59: Ovaries 1,277 and 1,784 mg; 20 follicles, 2,544 x 3,265 microns, in each ovary; no ovulations.

Table 12.—Continued

Estimated Age of the Raccoon in Months ^a	Weight in Kilograms	Injection Date ^b	Dose (each) ^c	Hormone	Laparotomy or Autopsy Date and Results
28	4.40	8-24-59—8-30-59	100 IU	PMS	8-31-59: Died; follicles to 1,030 microns.
5	4.35	9-30-59—10-4-59	100 IU	PMS	10-5-59: Died; ovaries 252 mg; follicles to 160 microns. 4-2-58: Left ovary removed; 4 corpora lutea.
12	4.94	6-18-58—6-22-58	10 AU	FSH	6-23-58: Right ovary, 25 or more follicles to 875 microns.
2	1.50	7-16-58—7-20-58 7-21-58	10 AU 500 AU ^d	FSH LH	7-24-58: Right ovary removed; packed with follicles to 140 microns.
39	3.95	9-8-58—9-12-58 9-15-58—9-23-58 9-22-58—9-23-58	15.6 mg 5 AU 50 AU ^d	P FSH, LH LH	9-24-58: Left ovary, follicles to 219 microns.
19	5.72	7-16-58—7-20-58 7-21-58 12-4-58—12-8-58 12-9-58 12-10-58—12-14-58 12-16-58 12-17-58	20 AU 50 AU ^d 10 AU 5 AU 200 IU ^d 10 AU 5 AU 200 IU ^d 15 AU 10 AU	FSH LH FSH, LH FSH, LH PMS FSH, LH	7-24-58: Ovaries removed; 17-20 follicles to 875 microns; ova in follicles. 12-10-58: Ovaries 7 x 5 mm. 12-17-58: Ovaries 10 x 7 mm; small follicles.

Estimated Age of the Raccoon in Months ^a	Injection Date ^b	Dose (each) ^c	Hormone	Laparotomy or Autopsy Date and Results
19 (cont.)	12-18-58-12-21-58 12-22-58	10 AU 100 IU ⁱ	FSH, LH LH	12-23-58: Left ovary removed, 282 mg, 11 x 6 mm; 25 follicles to 1,115 microns. 5-5-59: Right ovary 11 x 7 mm.
	5-7-59-5-11-59 5-13-59	10 AU 200 AU ^j	FSH, LH LH	5-14-59: Right ovary removed; 3 old corpora lutea. 4-2-58: Right ovary removed; 3 corpora lutea.
23	9-15-58-9-19-58 9-22-58-9-23-58	10 AU 50 AU ^j	FSH, LH LH	9-25-58: Left ovary 1,180 mg; many follicles 850 x 2,545 microns; ova in 3.
	6-22-59-6-23-59 6-25-59-6-26-59 6-28-59 6-30-59	10 AU 10 AU 200 AU ^j 500 U	FSH, LH FSH, LH LH CGH	6-30-59: Ovaries 12 x 6 mm; 12 follicles in each. 7-2-59: Ovaries 17 x 7 mm; follicles twice as large as on 6-30-59.
14	7-2-59	500 U ⁱ	CGH	7-6-59: Left ovary removed; follicles 1,475 microns. 7-13-59: Right ovary removed; several luteinized follicles 825 microns and smaller.
	8-24-59-9-5-59 9-7-59	10 AU 200 AU ^j	FSH, LH FSH, LH	9-7-59: Ovaries 11 x 7 mm. 9-8-59: Ovaries removed; 11-15 follicles 1,150 x 1,300 microns in each.
17				

Table 12.—Continued

Estimated Age of the Raccoon in Months ^a	Weight in Kilograms	Injection Date ^b	Dose (each) ^c	Hormone	Laparotomy or Autopsy Date and Results
2	1.04	7-7-59—7-13-59 7-14-59 7-16-59 7-17-59	2 AU 10 AU 150 U 200 U ^j	FSH, LH FSH, LH CGH CGH	7-20-59: Ovaries 7 x 5 mm, total weight of both ovaries 104 mg; a few follicles to 100 microns.
4	1.54	7-22-59—7-29-59 7-30-59 7-31-59	50 IU 100 IU 250 IU	PMS PMS PMS	8-3-59: Right ovary removed, 9 x 4 mm, 28 mg; follicles to 105 microns.
3	2.13	7-22-59—7-30-59 7-31-59	10 AU 25 AU	LH LH	8-3-59: Right ovary removed, 7 x 4 mm, 39 mg; follicles to 200 microns.
2	0.91	7-22-59—7-23-59 7-24-59—7-30-59	408 mg 425 mg ^m	HMG-J5 ^o HMG-J5	8-3-59: Right ovary 7 x 3 mm, 34 mg; few follicles to 109 microns.

^a Estimated age as of the first injection date shown in the table.

^b One injection was given on each date listed except as otherwise noted.

^c All injections were subcutaneous unless otherwise noted.

^d The injection was intramuscular.

^e Pregnant mare's serum.

^f AU = Armour Units.

^g Follicle-stimulating hormone.

^h Luteinizing hormone.

ⁱ FSH, LH indicates that the hormones were mixed and injected; equal quantities of each were given except as noted.

^j The injection was intravenous or via cardiac puncture.

^k The injection was intraperitoneal.

^l Progesterone.

^m Two injections were given daily.

ⁿ Chorionic gonadotropin hormone.

^o Human menopausal gonadotropin.

cutaneous injections of PMS at the rate of 100 IU daily for 12 days were begun in September. Many large follicles were found on the 13th day of treatment. This female was given 550 IU of PMS intravenously on the 13th day. Seventy-two hours later many *blut punkte* were observed in each ovary. The left ovary (826 mg) and a piece of the uterus were removed. Histological examination revealed 26 corpora lutea in early stages. Most had blood in the lumina and appeared to be freshly ovulated. The uterus showed a fairly typical effect of estrogen, and no material was present in the uterine glands, indicating the near absence of progesterone. Eleven days later (26 days after the first injection) the female was killed and the right ovary was removed (2,147 mg). There were 29 early-stage corpora lutea, most of them packed with luteal cells, but lumina were present in 2-4 corpora. The cytoplasm and nuclei of these luteal cells were more darkly stained and the nuclei were smaller than usual. The intracellular space exceeded the norm. Secretory material was present in the uterine glands.

The third female was about 22 months old when caught, but was 53 months of age when these experiments were begun. Subcutaneous injections of PMS were begun 2 months before the breeding season, at the rate of 50 IU per day, and were continued for a total of 12 injections. On the 13th day 200 IU were injected intravenously. At that time each ovary measured 12 X 6 mm and contained approximately nine follicles, each about 2 mm in diameter. Two days later the ovaries and follicles had not changed in size, but one follicle was hemorrhagic and one had a thin red line across the surface at its highest point. Twenty-four hours later when the ovaries were examined, blood oozed from most or all of 11 or 12 follicles in each. There were tiny holes in the highest points of most, and perhaps in all, of them. The ovulated follicles were partly hollow and partly filled with fluid and stringy material. The left ovary, measuring 10 X 6

mm, was removed (242 mg). When examined histologically, it was found to contain six or more blood-filled, early-stage corpora lutea. Among 16 wild raccoons the average number of corpora lutea per ovary, determined by histological examination, was 2.1. Thus, in this most nearly normal ovulation induced by exogenous hormones, the ovaries were somewhat less than twice normal weight, but the ovulation rate was approximately 5.7 times normal.

The female just discussed weighed 5.35 kg and received a total of 800 IU of PMS, a dosage of about 150 IU per kg, a rate similar to that used successfully to cause ovulation in ranch mink (Hammond 1952:219).

In a third series of experiments four different hormones were used on four sexually immature female raccoons from 2 to 4 months old (the last four animals in Table 12) in an attempt to learn how immature ovaries respond to hormones and to study differential responses to the several hormones. The injection of human menopausal gonadotropin (HMG-J5, largely FSH) subcutaneously twice a day for 7 days immediately after 2 days of single injections resulted in little stimulation of either the ovary or the uterus in one immature female. In the second young raccoon 50 international units (IU) of PMS daily for 8 days, followed by 100 IU and 250 IU on the 9th and 10th days, respectively, resulted in a slightly more stimulated uterus than did the HMG-J5 injected into the animal just discussed. In the third animal in this age group 10 Armour units of LH injected subcutaneously daily for 9 days, followed by 25 units on the 10th day, resulted in larger follicles than did either of the two previous treatments.

In the raccoon that received only LH this hormone caused more development of the follicles than did either PMS or HMG-J5 in the other young females. PMS and HMG-J5 contain both FSH and LH and might be expected to cause greater stimulation than LH alone. The ovaries stimulated by HMG-J5 contained

more interstitial tissue than did the ovaries of the females that received the other hormones, and the ovaries of the raccoon that received PMS had less interstitial tissue than those of the female that received LH. The uterus of the female injected with HMG-J5 was somewhat less stimulated (endometrium 650 microns) than that (endometrium 820 microns) of the female that was given PMS although the differences in these uteri were slight. The uterus of the female that received LH was more stimulated (endometrium 1,275 microns) than was either of the other two.

On the basis of the information obtained from our experiments with four female raccoons, it appears that 35-50 IU of PMS given subcutaneously each day for 12 days caused the development of follicles at any time of year in adult females. A dose of 200 IU given on the 13th day might be expected to cause ovulation 48-60 hours later.

UTERINE MILK

Uteri, and ovaries containing corpora lutea, were sectioned from 18 raccoons that had not been treated with hormones. In 17 of the 18 secretory material (uterine *milk*) was present in the lumina of most, but not all, of the uterine glands although it may have been present in all 18 uteri but overlooked in some of the sections.

Histological sections of ovaries containing no corpora lutea and the corresponding uteri were examined from 89 raccoons collected throughout the year. February and March were each represented by a single animal, but each other month was represented by three or more animals. The uterine sections from these animals, with two exceptions, contained no secretory material in the endometrial glands. Small amounts of secretory material were present in the uterine glands of one nulliparous adult killed in September and in another, approximately 7 months of age, collected in November. Secretory material was not abundant in either one, but was definitely present.

These data indicate that, in the raccoon, secretory material (presumably uterine milk) is present when corpora lutea are present. In one female, judged to have been only 10 days prepartum, secretory material was present.

Progesterone alone or in combination with estrogen was probably responsible for the secretion of uterine milk (Table 13). Progesterone alone was given for an insufficient length of time to determine whether it alone can cause the uterine glands to secrete. Two castrated females (No. 1297 and 1786) received a combination of progesterone and estrogen for several days, and the endometrial glands of both contained uterine milk (Table 13). Any combination of gonadotrophic hormones that resulted in the formation of corpora lutea caused secretion by the uterine glands. Five treatments of 2.5 mg each of estradiol over periods of 10 and 20 days, respectively, did not cause secretion by the uterine glands in one castrated female. One intact female (No. 2184B) received five daily injections of 20 units each of FSH, followed on the 6th day by 50 units of LH. Three days later, when she was killed, each ovary contained approximately 20-30 follicles measuring up to 750 X 1,250 microns, but no corpora lutea. The lumina of a few endometrial glands contained small bits of secretory material. Several hormones, including various combinations of FSH, LH, CGH, PMS, and HMG-J5, were given to intact females. Except possibly in the female just discussed, none of these hormones caused the uterine glands to secrete except indirectly by causing the formation of corpora lutea.

Methods described by Pearse (1960: 265-271) and by Lillie (1954:274-299) were used in an attempt to demonstrate the nature of the secretory material. In no case did digestion with either ptyalin or diastase remove the secretory material from the endometrial glands. This finding was taken as evidence that it was not glycogen. According to the information on the identification of carbohydrate-containing materials given by

Table 13.—Presence of secretory material in the uterine glands of captive raccoons as related to injections of exogenous hormones.

Raccoon Number	Estimated Age in Months	Hormone	Number of Days After First Treatment	Corpora Lutea	Uterine Milk
1292	24	PMS ^a	28	+ ^b	+
1297	14	PMS	49	—	—
	22	Progesterone, estrogen	70	Castrated	+
1298	53	PMS	15	+ ^c	—
			27	+	+
1782	14	Estradiol	10	Castrated	—
		Estradiol	20	Castrated	—
1786	24	Progesterone, estradiol	28	Castrated	+
2184B	38	FSH ^d , LH ^e	8	—	T ^f
2276	14	FSH, LH, CGH ^g	14	—	T
			21	+	T
2525	19	FSH, LH, PMS	19	—	—
2805	3	LH	12	—	—

^a Pregnant mare's serum.

^b The plus symbol indicates the presence of corpora lutea or uterine milk, and the minus symbol indicates their absence.

^c Early stage.

^d Follicle-stimulating hormone.

^e Luteinizing hormone.

^f T = traces.

^g Chorionic gonadotropin hormone.

Pearse (1960:236-237), it was either a mucoprotein or a glycoprotein.

SUMMARY

1.—The testes of raccoons in Illinois grew at a uniform rate from birth until about 10 months of age; at that time the average weight of one testis was 5.6 grams. Most male raccoons reached sexual maturity as yearlings, but juvenile males became sexually potent 3-4 months later in the year than did adult males. Seasonal variations occurred in testis weights; the average weights were minimal in June, July, and August, and were highest in December. The average maximum weight of one testis was 2.8 times the average minimum. There was a positive correlation between testis weight and the presence of sperm in the epididymis, but the weight of the testis did not infallibly indicate whether sperm was present in the epididymis. In a large group of raccoons sperm may be found in some animals at any given time, but individual males had periods averaging

3-4 months when they were incapable of breeding.

2.—Ovaries of raccoons showed a nearly steady rate of growth from birth in April through the following November. The heaviest normal ovaries found were in juveniles during November, approximately 3 months prior to the peak of the breeding season. The ovaries of juveniles declined in weight from November through January, and perhaps through March. Seasonal weights of ovaries in parous raccoons followed a pattern similar to that found in the gonads of adult males. The minimum average weight was reached in July, with a slow but consistent increase in weight occurring from then until November. The weights of ovaries of parous raccoons declined from November to December but increased during January and reached their peak average in April, when they were slightly heavier than they were in November. The average peak weight of ovaries of adults in April was slightly more than 1.6 times their average weight in July.

3.—The mean birth date for 20 litters

conceived in the wild was April 18 (range, March 9–June 24) and for 11 litters conceived and born in captivity it was April 24 (range, March 16–June 3).

4.—The measurement of the largest external uterine swelling enabled us to estimate birth dates with a maximum error of 4 days.

5.—The sex ratios of young raccoons less than 2 months of age and of embryos and young at birth were not significantly different from 50:50, but there were more males among the young less than 2 months old than among the other group, possibly indicating some differential mortality of females between birth and 2 months of age.

6.—Yearling females either bred when adults bred or did not breed until they were almost 2 years of age. If female raccoons ovulated but did not become pregnant, if they aborted or resorbed their young, or if they lost their young at or near birth, they sometimes ovulated a second time in one season. The interval between ovulations in five captive raccoons held in Urbana, Ill., varied approximately from 80 to 140 days. Severe weather conditions (extreme cold or deep snow) interfered with the normal breeding cycle and resulted in an unusually large number of late litters. Female raccoons sometimes gave birth to two litters in one season, but they did not rear more than one litter in one season. The vaginal smear was no more specific for indicating estrus than was gross vulval swelling.

7.—Contrary to published reports, the raccoon is a spontaneous ovulator. Ovulation was followed by the formation of corpora lutea whether the animal became pregnant or pseudopregnant. The formation of corpora lutea always resulted in changes in the uteri and nipples. The nipples always enlarged; some became heavily pigmented, some became slightly pigmented, and others remained unpigmented. Thus, it was possible to determine whether a female raccoon had ovulated by examining her nipples. Corpora lutea in both isolated and non-isolated pseudopregnant females formed

from ovulated Graafian follicles and not from luteinization of follicles. Corpora lutea persisted in pregnant females until parturition and apparently disappeared 14–16 days after parturition.

8.—Raccoons that ovulate become either pregnant or pseudopregnant, and the corpora lutea persist for about the same time in pseudopregnant raccoons as they do in those that give birth to young. Corpora in females that went at least halfway to term persisted about the same length of time whether the young were aborted, were resorbed, or were born and were removed at birth or nursed until weaned. Field evidence indicated that in Illinois about 2.5 percent of the adult females were pseudopregnant each year.

9.—Ten of 21 captive yearling females and 9 of 14 wild yearling females were sexually immature.

10.—Interstitial tissue occurred in the ovaries at some stage of the reproductive cycle, often occupying as much as 50–90 percent of the space in the ovary, but seldom occurred when corpora lutea were present. Ovaries of females less than 2 months old did not contain large amounts of interstitial tissue, but with this exception the ovaries of females less than 12 months of age contained, on the average, more interstitial tissue—both relatively and absolutely—than did those of older females. From January through June, ovaries of adults contained little interstitial tissue even when corpora lutea were not present; from July through December, ovaries from adults contained more interstitial tissue than did those collected earlier in the year. The greatest abundance of interstitial tissue was in ovaries taken from juveniles during October and November, and the maximum ovarian weights recorded during this study were those of juvenile females in November.

11.—The placenta of *Procyon* is deciduous, as in the dog, cat, fox, and seal, and is endotheliochorial. If used with caution, placental scars in raccoons are useful for estimating litter size and rate of productivity. The significance of multiple groups of scars is not clear, but

it appears that each embryo that reaches 1 month of age is represented by one scar that persists for 10 or more months. Scars in wild females with only one group of scars probably reflect implantation rates for the preceding breeding season. Placental scars apparently persist longer in wild females than in captives.

12.—The reproductive system of the male raccoon is similar to that of the dog; seminal vesicles and Cowper's glands are lacking.

13.—The uterus of the raccoon is intermediate between the bicornuate and the bipartite uterus. There is a single cervix and the horns are distinct, but after they join externally to form the single uterine body, the uterine lumina remain separate to a point near the cervix. The ovoid ovary is completely surrounded by the bursa ovarii. The sac is intact except for a small slit on one side, not large enough to permit passage of the ovary, as in the mink, dog, and fox.

14.—The level of sex hormones in the male affected the enlargement of the preputial orifice and the maturation of

the penis bone but had little or no effect prior to 7 months of age. Castration in the male also apparently caused a slight delay in the closure of the epiphyseal cartilage in the radius and ulna. Removal of the ovaries, even after raccoons had reached sexual maturity but before the epiphyses had closed, delayed the rate of epiphyseal closure.

15.—Embryos persisted for about 19 days after castration in each of two raccoons—to 33 days short of term in one female and 7 days short of term in the other.

16.—Limited studies indicated that injections of androgen did not initiate nor prolong spermatogenesis and apparently did not influence the size of the testes.

17.—A dose of 35–50 IU of pregnant mare's serum given subcutaneously each day for 12 days caused development of Graafian follicles in adult females at any time of the year. A dose of 200 IU given on the 13th day caused ovulation 48–60 hours later; however, in all cases of successful ovulation, the ovaries were much larger—and the rates of ovulation much higher—than normal.

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