ANNUAL REPRODUCTIVE CYCLE OF THE MALE FIELD RAT, *RATTUS RATTUS BRUNNEUSCULUS* (HODGSON) IN HILLY TERRAIN OF MIZORAM[1]

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(With a plate)

The males of the field rat, *Rattus rattus brunneusculus* (Hodgson) were collected every month during the period 1976 to 1979 from different areas of Mizoram. Body weight of each rat was noted. The observations were made on the weight and histology of the testes and various accessory sex organs during different months of the year.

The male of this species is a seasonal breeder and shows a single long breeding season from May to October. During this period, the weights of testes and accessory sex organs are high. The testicular histology shows broad seminiferous tubules with sperms and large interstitial cells with vesicular nuclei. The breeding phase is followed and preceded by the short regressive and progressive phases respectively. The non-breeding phase extends from December to February. During these months, there is significant reduction in the testis weight and the seminiferous tubules have germ cells limited to primary spermatocytes. The interstitial cells are inconspicuous and have small often pycnotic nuclei and little cytoplasm. Parallel to the reduction in the testis weight, the accessory sex organs also exhibit a decrease in weight.

INTRODUCTION

The field rat, *Rattus rattus brunneusculus* (Hodgson) is commonly found in Mizoram, a Union Territory of India. It usually inhabits crop fields, forests and tribal settlements. It is observed that the population of this rat increases exponentially at the time of bamboo flowering. It inflicts incalculable loss to paddy (*Oryza sativa*) and vegetable crops as well as stored grains thereby resulting in famine conditions. Therefore, this rat is of great economic importance. An attempt has been made to investigate the physiology of reproduction of this rat. The present study deals with the annual reproductive cycle of the male.

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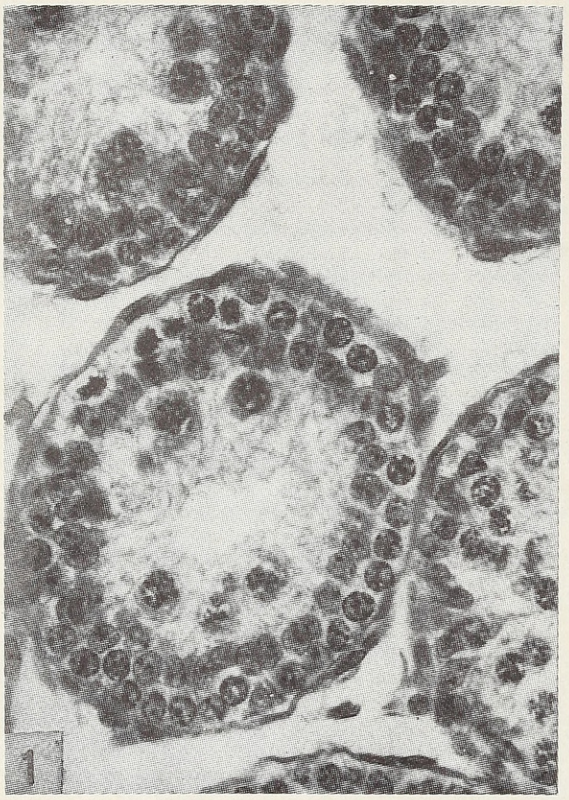
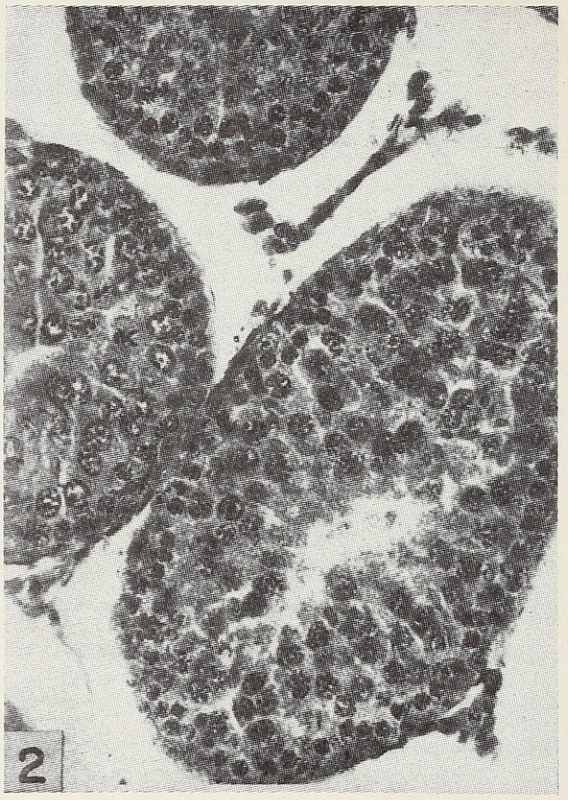
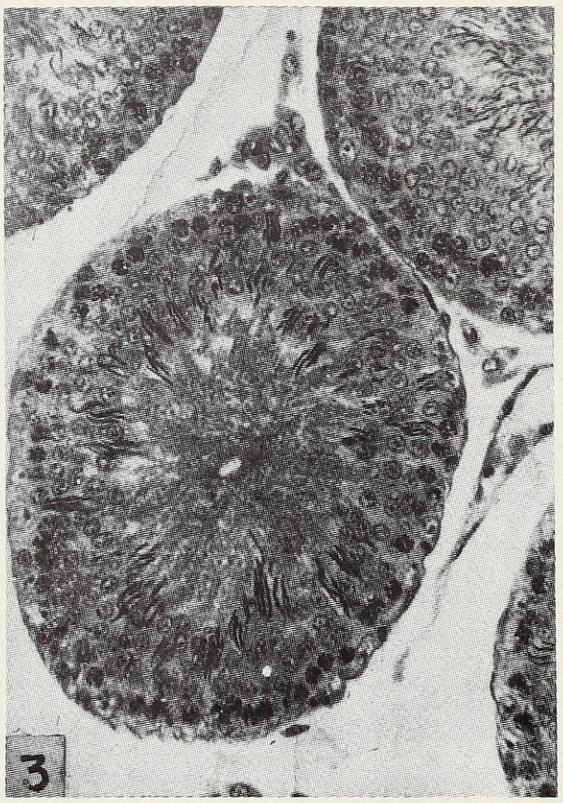
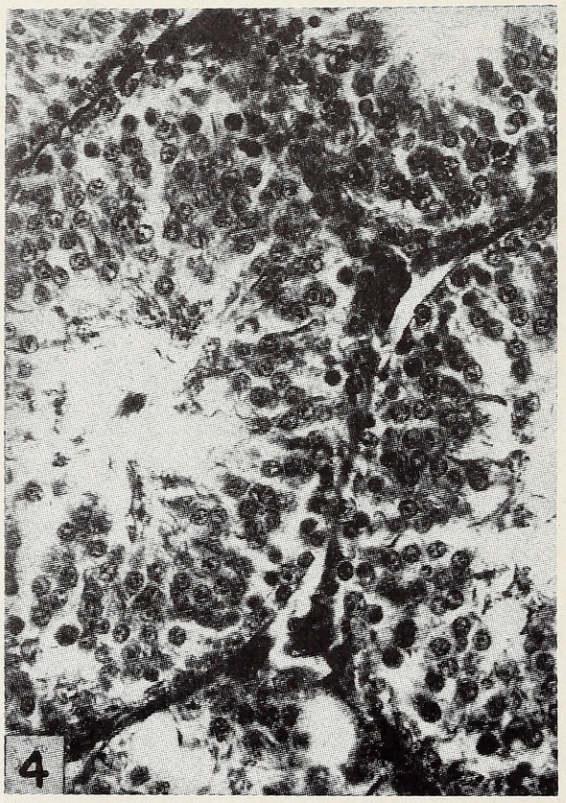
MATERIALS AND METHODS

More than eight hundred adult males of *Rattus rattus brunneusculus* were collected from crop fields and adjacent forests located in different areas of Mizoram from 1976 to 1979. The animals were either caught alive from their burrows or trapped by using 'Sherman' traps. The animals which had minimum body weight of 45 g and length of 28 cm were taken as adults and used in this study. The animals were autopsied within 10-12 hrs of their capture. The body weight was noted prior to autopsy. The testes, epididymides, prostate glands and seminal vesicles were dissected free of fat and connective tissue and weighed on a precision balance to the nearest 0.2 mg. All the weights given in the tables, wherever applicable, are the mean weights of paired organs.









Photomicrographs of T. S. of testes showing:
Fig. 1. Non-breeding conditions. × 400. Fig. 2. Recrudescence. × 250. Fig. 3. Breeding conditions. × 250. Fig. 4. Regressive changes. × 250.

Only the tissues from representative animals of each month collections were fixed in Bouins fluid for histological study. Sections were cut at 5-7 micra and stained with Haematoxylin and Eosin. The diameter of approximately 15-20 seminiferous tubules and nuclei of 25-30 randomly selected interstitial cells from each testis section was measured by means of an ocular micrometer.

Results

Testis

Weight (Table 1)

The testis showed marked variations in the weight during different months of the year. It was lowest in February, started increasing in March and a significant increase was observed in April ($p<0.001$). It further increased in May and this peak value was almost maintained till October. Thereafter, regression sets in and the weight started decreasing gradually and significant reduction was noted in December ($p<0.001$), reaching lowest once again in February. During non-breeding phase (December to February) the testes become abdominal in position while they descend down into the scrotum during breeding phase (May to October).

Histology (Plate 1 — Figs. 1-4, table 1)

From December to February when testes weights were very low, the histology also showed regressed state. It was characterized by the presence of a thick, fibrous and crumpled tunica albuginea. The seminiferous tubules were narrow, the germ cells were restricted to primary spermatocyte stage and lumen was almost clear (Fig. 1). There was an apparent increase in the number of Sertoli cells. The intertubular spaces were enlarged, the interstitial cells were small with oval and small nuclei and appeared non-secretory. In March and April, with the testis recrudescence, concurrent regenerative changes were seen in its histology. It was characterized by an increase in diameter of seminiferous tubules (Table 1) and even the appearance of spermatozoa in a few tubules indicating reinitiation of the germ cell activity. The interstitial cells also became prominent and showed some secretory activity as was evident by their vesicular nuclei (Fig. 2). At the time when testes weights were very high (May to October) the histology showed perfect breeding characteristics. The tunica albuginea was thin and smooth. Spermatozoa were seen in most of the seminiferous tubules which became more compact in arrangement due to increase in their diameters thereby the intertubular spaces were greatly reduced (Fig. 3). The interstitium was less marked. The interstitial cells were large, polyhedral and with spherical vesicular nuclei. In November, when the testes weights were significantly decreased, the seminiferous tubules were reduced in diameter resulting in an increase in the intertubular spaces. Degenerated spermatozoa, spermatids and even secondary spermatocytes in the form of debris were characteristically observed in the lumen of the tubules. At the advance stages of regression, the tubules were almost cleared off their debris. The interstitium became more visible due to narrowing of the tubules and the interstitial cells became less prominent (Fig. 4).

Accessory sex organs

Weight (Table 2)

The changes in the weight and histology of the accessory sex organs were closely parallel to the testicular cycle. From December to February when the testes were regressed, the weights of different accessory sex organs (epididymis, prostate, seminal vesicle) were lowest and the histology showed non-secretory characteristics. While during the breeding phase (May to October) these organs were maximally grown and appeared to be highly secretory. The details of histology of these organs are being presented elsewhere.

TABLE 1

MONTHLY CHANGES IN TESTIS WEIGHTS (MEAN±S.E.) AND DIAMETER OF SEMINIFEROUS TUBULES AND INTERSTITIAL CELLS OF *R. r. brunneusculus*

Month	Testis		Seminiferous tubules		Interstitial cells	
	Number of animals	(mg/100 g body weight)	Number of tubules measured	Diameter (micron)	Number of cells measured	Nuclear diameter (micron)
January	78 'a'	251± 17.3	27	111.5±12.86	38	4.32±0.085
February	75	139± 14.9	18	100.6±12.76	30	4.92±0.360
March	58 'b'	476± 40.5	26	171.2±16.91	41	5.74±0.121
April	37 'c'	1114±126.0	15	275.0±10.00	25	5.73±0.210
May	50 'd'	2105± 46.5	24	257.5± 3.82	37	5.92±0.374
June	54	2111±130.0	30	296.8±11.75	50	5.94±0.234
July	60	1927±100.0	24	289.6±17.63	40	6.22±0.266
August	102	1834± 67.5	30	296.7±18.33	38	5.82±0.158
September	69	2099± 76.5	32	280.8± 6.51	36	5.98±0.104
October	97	1616±113.0	21	297.5± 7.50	39	5.95±0.161
November	84	1417±160.0	26	222.5±13.50	31	5.13±0.251
December	58 'e'	614± 40.4	35	126.7±10.14	35	4.28±0.192
	'p' Values	Testis 'c' vs 'd' < 0.001 'd' vs 'e' < 0.001		Seminiferous tubules 'a' vs 'b' < 0.005 'b' vs 'd' < 0.001 'd' vs 'e' < 0.001		Interstitial cells 'a' vs 'b' < 0.001

TABLE 2

MONTHLY CHANGES IN WEIGHT OF EPIDIDYMIS, SEMINAL VESICLE AND PROSTATE OF *R. r. brunneusculus*

Month	Number of animals	Epididymis (mg/100 g body weight)	Seminal vesicle (mg/100 g body weight)	Prostate gland (mg/100 g body weight)
January	78 'a'	28.9± 4.9	12.9± 1.8	30.5± 3.4
February	75	29.1± 4.9	11.9± 2.5	17.2± 6.2
March	58 'b'	166.7± 6.2	32.4± 1.4	122.1± 8.4
April	37	283.1±83.8	134.7±23.1	278.2±57.5
May	50 'c'	404.2±85.9	122.3±25.8	275.4±67.4
June	54	768.8±35.1	188.4±22.9	350.7±41.7
July	60	716.5±34.0	207.4±20.4	409.0±36.7
August	102	662.3±39.3	187.6±23.9	362.3±35.2
September	69	558.6±92.9	188.9±27.1	371.2±62.4
October	97	584.2±66.0	185.5±21.9	318.2±48.8
November	84	436.9±81.8	128.9±17.1	236.8±52.0
December	58 'd'	174.3± 5.2	23.5± 6.6	36.8±16.3
	'p' Values	Epididymis 'a' vs 'b' < 0.001 'b' vs 'c' < 0.001 'c' vs 'd' < 0.001	Seminal vesicle 'b' vs 'c' < 0.001 'c' vs 'd' < 0.001	Prostate gland 'a' vs 'c' < 0.001 'c' vs 'd' < 0.001

Discussion

As revealed by monthly changes in the weight and histology of testes and accessory sex organs, the male of the common field rat, *Rattus rattus brunneusculus* (Hodgson) in Mizoram is a seasonal breeder. The breeding season extends from May to October whereas the non-breeding phase is of a shorter duration (December to February).

Throughout the breeding phase of *R. r. brunneusculus,* the testes remain maximally grown with abundant spermatozoa in their seminiferous tubules. Clusters of interstitial cells with vesicular nuclei are seen in reduced intertubular spaces. Short non-breeding phase (3 months) is characterized by low testis weight, absence of spermatozoa in its seminiferous tubules, germ cells only up to spermatocyte stage and a few small interstitial cells in broadened intertubular spaces. Annual breeding cycle of almost the same pattern was observed in certain other rodents such as *Apodemus sylvaticus* (Asdell 1946) and *Rattus cutchicus cutchicus* (Prakash 1971). Besides these features, the testes also become abdominal during non-breeding phase and descend down into the scrotum during breeding phase. Similar change in testis position has also been reported for *Malacomys longipes* and *Apodemus sylvaticus* (Asdell 1946).

In animals showing regular periodicity in reproductive activity, there exists a close relationship between the absolute number, size and functional activity of the interstitial cells and gametogenic activity. In *Tatera indica cuvierii* (Prasad 1956), *Funambulus pennanti* (Reddy and Prasad 1968) and *Nesokia indica* (Gariyali 1975) the interstitial cells show parallel changes with that of gametogenic activity. Whereas, a reverse condition in which the interstitial cells show increase in size, number and functional activity in regressing or regressed testes, has also been reported in *Myotis griescens* (Miller 1939). In the present rat species, the interstitial cells show changes which run parallel to gametogenic activity. The periodic increase in the number of interstitial cells in sexually active animals may be either due to division of the existing interstitial cells or by transformation of intertubular non-secretory stromal cells into secretory interstitial cells as suggested by Gopalakrishna (1949) and Prasad (1956).

Prior to attainment of perfect breeding or non-breeding characteristics, testes show gradual but marked changes in weight and spermatogenesis. After termination of breeding activity, a decrease in testis weight and degeneration of spermatozoa along with some other types of germ cells occurs. The deposition of degenerated components as debris within the lumen of tubules marks the regressive phase (November). Subsequent clearance of the debris and presence of germ cells only up to spermatocytes leads the animals to non-breeding phase. After non-breeding phase the recrudescence of testes, as marked by increase in their weights and onset of spermatogenesis, begins and culminates into perfect breeding condition. This growth period is called as progressive phase (March-April). Similar progressive and regressive phases have also been identified in *Funambulus pennanti* (Reddy and Prasad 1968). Thus, on the basis of the changes occurring in testes, the annual reproductive cycle of the male of this rat can be divided into breeding (May to October), regressive (November), non-breeding (December to February) and progressive (March-April) phases.

These cyclical changes in the testis may be possibly due to variations in the levels of pituitary gonadotropins. In certain seasonally breeding mammals like ram (Ortavant *et al.* 1964, Pelletier 1973, Katongole *et al.* 1974,

Sanford *et al.* 1974a, b, Gomes and Joyce 1975), snow shoe hare (Davis and Meyer 1973a, b) and white tailed deer (Mirarchi *et al.* 1978) a definite relationship has been shown between testis activity and pituitary gonadotropins. During the breeding phase, the level of pituitary gonadotropins was found to be highest while it was lowest during the non-breeding phase. Recently, Lincoln and Kay (1979) observed increasing circulating levels of LH during growth phase of the testis and consequently testosterone peak was observed coinciding with the active spermatogenesis in the red deer stag. All these observations lead us to speculate that in this rat also, the seasonal increase and decrease in the levels of pituitary gonadotropins may be the primary factor controlling the cyclic changes in the gonadal activity. Besides, various other factors, both intrinsic and extrinsic either independently or jointly, may also be responsible for the regulation of the reproductive cycle.

It may be possible that the regression occurs due to cumulative effect of negative feedback by high levels of sex hormones secreted during the breeding phase because of which the circulating levels of gonadotropins decrease and consequently gradual regression sets in which leads to non-breeding phase. This phase is maintained for a considerable duration either because of non-stimulatory levels of gonadotropins or occurrence of a refractory period following the breeding phase as reported in most of the seasonally breeding animals (Reiter 1972, Turek *et al.* 1975, Sansum and King 1975, 1976, Murton and Westwood 1977, Grocock 1980, Zucker *et al.* 1980, Soares and Hoffmann 1982). It is during this period that hypothalamo-hypophyseal-gonadal axis or any one of its components becomes unresponsive to a stimulus. However, when the levels of gonadotropins start increasing possibly due to positive feedback effect of low levels of sex hormones from regressed testes or when the refractoriness is over, the gonads once again show recrudescence and the animals get into progressive phase.

The increase and decrease in the weight and functional activity, as judged by histological studies, of various accessory sex organs are seen to be closely related with the testicular cycle. The interstitial cells are known to be the principal source of androgens which control the growth and functional activity of the accessory organs. Seasonal variations in the androgen synthesis and release by these cells, associated with the testis cycle are reflected in a series of changes in the accessory organs. During the breeding phase when the testes show maximum gametogenic activity and the interstitial cells are conspicuous, large and active, the accessory sex organs show maximum weights and functional activity. At the termination of breeding phase, a gradual regression of the accessory sex organs occurs following regression of the testes which ultimately leads to regressed state of these organs in December, the beginning of non-breeding phase. The recrudescence of testis is accompanied by increase in weight and reinitiation of functional activity of the accessory sex organs. Such cyclical changes in the accessory sex organs related with testicular cycle are also reported in other seasonally breeding mammals (Wislocki 1949, Mossman *et al.* 1955, Short and Mann 1965, Reddi and Prasad 1968, Ellis and Balph 1976, Lincoln and Kay 1979). The high levels of androgens during the breeding phase seem to be responsible for the maximum growth and functional activity of the accessory sex organs. Whereas, the reverse may be for the non-breeding phase. Our results can be explained on the basis of the work of Lindner (1963) which shows differences in the concentration of androgen between the lymph and blood of the testis in