The testicular and epididymal changes in the soft-furred field rat, *Millardia meltada* (Gray) in relation to some environmental factors

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**Abstract.** The annual reproductive changes in the testes and epididymis of the soft-furred field rat, *Millardia meltada* have been investigated. The males of this species are sexually quiescent between January and April. Two peaks of highest reproductive activity occur annually in October and December. Interstitial cells show seasonal activity which approximately parallels the condition of the testes and epididymis. A correlation of these changes with the environmental conditions during 1977-78 indicates that the lowest temperature, shortest daylength and natural conditions occurring after the rainfall are congenial for reproduction of this species.

**Keywords.** Testicular, epididymal changes; sperm count; environmental factors; *Millardia meltada*.

1. Introduction

Studies on the breeding biology of the wild rodents, are available for voles (Grocock and Clarke 1975), pocket gopher (Brown 1971), field rats and mice (Delany 1971; Jewell 1966; Taylor and Horner 1970; Van de Graaf and Balda 1973; Okia 1976; Mann and Bindra 1977) and hystricomorphs (Weir 1974). Based on the examination of sperm smears, Chandrahas and Krishnaswami (1974) reported no seasonal variation in the sexual activity of the males of soft-furred field rat, *Rattus meltada* in Kolar, Karnataka State. On the other hand, Mock and Frankel (1978) observed that the laboratory rats exhibit seasonal periodicity in several reproductive parameters even under constant laboratory conditions. It may be mentioned here that any result based on a single qualitative parameter may lead to erroneous conclusions. A detailed seasonal study of the testes and epididymis is therefore undertaken. An attempt has also been made to correlate these changes with the environmental temperature, daylength and rainfall.

2. Materials and methods

Adult male soft-furred field rats were collected from fields around Mysore, South India. Animals weighing 47-65 g were killed by decapitation within two days.
after they were brought to the laboratory. Every month 5-8 individuals were sampled. The testes and cauda epididymis were dissected out and were weighed separately. For histological observations pieces of testes and right cauda epididymis were fixed in Bouin’s or Hollande-Bouin fluids, for subsequent paraffin embedding. Five micron thick sections were stained with haematoxylin–eosin or Mallory’s triple stains. The diameter of the testes was measured using the dividers and the graduated scale. The diameter of the seminiferous tubules was measured using the ocular micrometer. An average of two diameters perpendicular to each other was taken as the mean tubule diameter. Twenty measurements from each of the three individuals were recorded in all the monthly samples. Mean value ± S.E. of the testes and the seminiferous tubule diameter were calculated.

The epithelial height of the cauda epididymis was measured with the ocular micrometer. Since there is variation in the cell height, the mean values of twenty measurements were used. Left cauda epididymis was used for sperm counting. The cauda epididymis was macerated in 5 ml of 0.09% physiological saline. The epididymal tissue was separated and a drop of the sample was observed for sperm motility. Sperm count was made using the haemocytometer adapting the technique outlined by Samuell (1977). The sperm count per ml sample was calculated.

3. Observations

3.1. Weight of the testes and epididymis

Seasonal fluctuations are noticed in the weights of testes and epididymis (figure 1). Two peaks in the weights are observed during October and December. Both the testes and epididymis show a sharp decline in their weights by January.

3.2. Diameter of testes and seminiferous tubules

Measurements of testicular and seminiferous tubule diameter reveal essentially a trend similar to that of organ weights with maximum values during October-December and decreases by January (figure 2).

3.3. Histological changes in the testes

Spermatogenesis commences during May at which time the seminiferous tubules enlarge in size, spermatogonia, spermatocytes, early spermatids and a few elongated spermatids are noticed. The lumen of these tubules contain a few mature spermatozoa. This condition prevails till August. During September the spermatozoa increase in their concentration. The seminiferous tubules reveal further increase in their activity as evidenced by enlarged lumen, 7-8 cell layers and large number of spermatozoa during November and December (figures 3 and 4). From the beginning of January the histological picture shows reduction in the number of cell layers, and during February the occurrence of pachytene and diplotene stages of primary spermatocytes is common (figures 5 and 6). Occasionally elongated spermatids are also noticed. Towards the beginning of April the seminiferous tubules contain only pachytene stage of primary spermatocytes (figures 7 and 8). These spermatocytes then undergo degeneration.
3.4. Interstitial cells of the testes

The interstitial cell pattern in *M. meltada* fits into the first category of the classification of the interstitial tissue by Fawcett *et al.* (1973) i.e. species with relatively small volume of Leydig cells and very little connective tissue stroma. The interstitial cells associate with the blood vessels and form a triangle in the interjection of the seminiferous tubules. In *M. meltada*, further, the mean interstitial cell nuclear diameter for monthly samples follows the same seasonal pattern as that of the seminiferous tubule and epididymal epithelium (figure 1). At the beginning of breeding season the nuclei are small but distinct with one or two nucleoli (figure 9). They become voluminous during breeding season (figure 10). From the beginning of January the nuclear size is reduced (figure 11) and the connective tissue then appears prominent. Detectable histological changes are however not observed in the cytoplasm of these cells. Accordingly, maximal and minimal values are recorded in December and February respectively.

Figure 1. Annual changes in the testicular weights, epididymal weights epididymal cell height and interstitial cell nuclear diameter. Vertical bars indicate mean±S.E.
3.5. Histological changes in epididymis

The lining epithelium of the cauda epididymis is constituted of cuboidal principal cells, with basal nuclei. The basal cells are small, pyramidal, alternating with the base of the principal cells. Their nuclei are elongated and lie parallel to the basement membrane. Each tubule is surrounded by muscle layer and intertubular connective tissue.

During the breeding season, the epididymal lumen is seen distended with sperms (figure 12). The cell height of the epithelium appears low. The nuclei are elongated and lie parallel to the basement membrane and the basal cells remain small. A large number of active clear cells are encountered. Large hyalinised areas are seen in between the principal cells. As reported earlier by Guraya and Kaur (1977) for the same species, sperms are commonly seen in the epithelial wall. The active breeding season is further marked by a concomitant decrease in the muscle layer and connective tissue component. From January, the decrease in the size of the epididymis is followed by a parallel decrease in the tubule diameter and a drop in sperm concentration. The epididymal lumen also contains spermatocytes and spermatids (figure 3). During the inactive period the epithelium begins to atrophy and the nuclei infiltrate into the lumen (figure 14). The muscle layer increases its thickness and the connective tissue is seen abundant.
Figures 3–8. Photomicrographs of testis during different seasons of the year. Bouin, 5μ, iron haematoxylin. 3. Seminiferous tubule during December, × 150. 4. A portion of the above enlarged to show spermatogonia, spermatocytes, spermatids, sperms and sertoli cells, × 540. 5–6. Seminiferous tubules during February showing pachytene and deplotene stages of primary spermatocytes and spermatids in a few, × 150; × 540. 7–8. Seminiferous tubule towards the beginning of April with pachytene stages of primary spermatocytes, × 150; × 540.
3.6. Epididymal sperm count

Motile sperms are seen in the cauda epididymis throughout the year except during February and April. The maximum sperm count is recorded during December and January. A sharp fall in the sperm count is noticed in February which is seen prolonged till the end of April (figure 2).

From the above observations it is inferred that the testes and epididymis remain active from May to December. The peak of reproductive activity (October-December) coincides with the winter season. During this period the mean minimum and mean maximum temperatures are at their lowest values (figure 2). The daylength decreased from June and reached minimum in December (11 hr 21.9 min as against 12 hr 52 min in June, figure 2). Rainfall recorded during the period of study is highest in October 1977 and May 1978 (figure 2). From January with the increase in temperature and daylength the testes and epididymal activity regresses. Rains were absent during December 1977, January and March 1978.

4. Discussion

Reproductive status of vertebrates is often implicated with a variety of natural conditions like temperature, daylength and rainfall. Such studies, though extensively undertaken on cattle, swine, sheep and horse, are relatively few on wild rodents. It is understood that the seasonal reproductive periodicity in mammals is common in temperate and sub-arctic regions (Amoroso and Marshall 1960), and to a limited extent in the tropics. Increase in temperature seems to cause cessation of breeding in ground squirrels (McKeever 1966). *M. meltada* lives in burrows. In summer, the temperature inside the burrows will be fairly high and this condition coupled with the scarcity of food during summer, may lead to summer sterility. This view gains support from the observations that during summer, the seminiferous tubules are devoid of lumen and sperms. Only a few spermatids are noticed. Higher reproductive activity is seen in October and December which coincides with low environmental temperature.

Mammals responding to photoperiodism are categorised as short day and long day breeders (Thrope 1967). According to Venge (1968) a small amount of extra light during dark season is known to affect the reproductive status of mink. On the contrary long daylength promotes gonadal activity in vole (Clarke and Kennedy 1967). In *M. meltada* the decrease in daylength is accompanied by the increase in testicular weight. The sperm count and sperm motility are also maximal during shortday periods. From February the increase in daylength is followed by the decline in sperm counts. It appears therefore that the increase in daylength has an inhibitory effect on the testicular activity.

Rainfall seems to have an indirect effect on reproduction. The food of *M. meltada* chiefly consists of swamp grass, various food crops and weed seeds (Walker 1968). These are available in plenty after the rains in the fields around Mysore. The reproductive activity corresponds to the abundance of food available during October and December. In the dry season prevailing after January, the food is scarce and the reproductive potency is decreased. It appears therefore that green vegetation consequent upon the rains and the availability of food crops may serve as yet another factor influencing reproduction indirectly in *M. meltada*. 
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