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CHANGES IN THE MALE REPRODUCTIVE ORGANS OF *LORIS TARDIGRADUS LYDEKKERIANUS* (CABRERA)

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Our knowledge of the reproductive phenomena in lower primates, especially the Lemuroidea, is scanty. While extensive work has been done to elucidate the factors regulating reproductive cycles in naturally occurring mammalian populations of rodents, nearly the same attention has not been paid to primate reproduction. It is essential that this gap in our knowledge be filled as a link to the understanding of reproductive processes in higher primates and man. The paucity of information on the reproductive biology of the Lemuroids and Lorisoids is primarily due to the difficulty of obtaining an adequate number of animals throughout the year.

The primates exhibit a variety of reproductive patterns; some show continuous reproductive activity while a few exhibit regular periodicity (ASDELL [1946]). Reports of seasonal reproductive cycles in primates have been based almost entirely on observations of seasonal births and changes in the female genital tract. There are few studies on seasonal changes in the reproductive organs of male primates. SPUHLER [1935] reported that the males of the lesser mouse lemur of southern Madagascar, *Microcebus murinus*, are in "full spermatogenesis during the peak of the dry season, October–November, while during the lesser rainy season, June–July, they are spermato-

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genically inactive". In *Lepilemur mustelinus* and *Cheirogaleus*, the reproductive season extends from the end of May to the beginning of August in Madagascar (PETTER-ROUSSEAUX [1964]); spermatogenesis has not yet been studied in *Lemur*, *Hapalemur* and the Indriidae. SADE [1964] and CONAWAY and SADE [1965] reported that the male rhesus monkeys *(Macaca mulatta)* of the free ranging colony on Cayo Santiago, Puerto Rico, show an annual spermatogenic cycle; spermatogenesis is maximal during the fall breeding season. During the spring, there is extensive regression of the seminiferous tubules and spermatogenesis ceases; redevelopment of the testis begins in mid-summer prior to the breeding season; a similar cycle is present in the epididymis.

HILL [1935; 1953] reported that *Loris tardigradus* experiences two heat periods during which the abdominal testes descend overnight into scrotal sacs which are affected by a reticular pigmentation. RAMASWAMI and ANAND KUMAR [1962] observe that the testes "should descend and become scrotal during March–April and September–October when mating occurs". On the other hand, RAMAKRISHNA and PRASAD [1962] reported that the males of *Loris tardigradus* are spermatogenically active throughout the year with no sign of testicular quiescence during any season of the year.

The present paper records the changes in the reproductive organs of the male slender loris, *Loris tardigradus*, based on collections from the wild state. The report is part of a more extensive study of the physiology of reproduction of this animal, which is in progress.

Material and Methods

One hundred and fifty-one lorises were collected from casuarina forests around Bangalore, India. Live animals were brought to the laboratory and were autopsied within a few hours. Body weight and position of the testes were noted; the genital tract was dissected free of fat and connective tissue and dropped entire into alcoholic Bouin's fixative. The material was transferred to 70% alcohol after 24 h fixation. The testes, epididymes, prostate, bulbourethral glands and seminal vesicles were then separated and weighed. Cross sections of the testis and epididymis, passing through the middle, were cut at 10 μ and stained with iron haematoxylin or haematoxylin and eosin. The diameter of the seminiferous tubules was determined from measurements of width of the tubules along two axes at right angles to each other. Presence of sperms in the testis and epididymis was considered a sign of sexual maturity. The data are presented in Tables I and II.

Observations

Body Weight

Immature animals with body weights ranging from 150–275 g occur in all months of the year except September–October. Sexually mature animals show considerable spread in body weight which therefore appears to bear no relation to spermatogenic activity. There is no reduction in the body weight of the sexually mature animals during any season of the year (Tab. I).

Changes in the Testes

Activity of the males as determined by the presence of spermatozoa in the testis, epididymis and ductus deferens (Tab. I, Fig. 1) extends throughout the year with no period of quiescence during any

Table I. Weights of testes and accessory glands of reproduction in loris.

Months		Body weight g	Testis mg	Diameter of seminiferous tubules μ	Weight of epididymis mg	Seminal vesicles mg	Prostate mg	Cowper's glands mg
January	(10)	258±12	1774±183	232	613	1231±219	169±36	437±72
February	(16)	285±16	1674±416	218	685	970±208	94±29	324±90
March	(11)	272±11	1632±146	221	624	1021±107	118±13	303±33
April	(8)	277± 6	1498±122	230	662	1035±130	113±14	320±42
May	(11)	301± 9	1623± 68	229	731	1119± 79	146±16	383±25
June	(11)	299± 6	1736±159	234	612	687± 56	83± 6	221±19
July	(14)	260± 7	1503± 95	211	559	539± 50	79±11	192±27
August	(7)	260± 8	1693±170	237	585	675± 76	82±10	210±19
September	(12)	265± 6	1671±118	237	602	826±228	104±19	259±44
October	(7)	272± 5	2035±229	225	655	1037±317	126±33	318±87
November	(13)	282± 7	2036± 91	247	604	992±187	144±23	390±48
December	(18)	284± 9	1977±115	239	800	1221±166	171±20	447±42

Mean ± standard error of the mean.
Figures in parentheses indicate the number of animals.
Immature animals with testis weights below 750 mg are not included for calculations.

Table II. Weights of testes and accessory glands of reproduction in loris.

Testes weight range mg	Testes weight mg	Seminiferous tubule diameter μ	Epididymis weight mg	Seminal vesicles mg	Prostate mg	Cowper's glands mg
0– 400 (8)	127± 31	94± 6	57± 10	36± 3	10	14± 1
401– 800 (5)	689± 35	120±10	141± 25	482± 82	24	133± 42
801–1200 (15)	1032± 93	202±20	463± 78	663± 90	71±21	217± 65
1201–1600 (34)	1362±223	222±21	557± 92	672± 62	93±18	235± 72
1601–2000 (39)	1811± 85	232±11	666± 85	897± 68	114±25	304±105
2001–2400 (27)	2102± 98	246±15	792±110	1128±109	154±38	407± 92
2401–2800 (10)	2547± 64	249±12	872±101	1765±250	221±19	542±100
2801–3200 (4)	3029± 75	239±11	949± 86	1633±119	229±30	599± 45
3201–3600 (1)	3465	278	1100	1695	—	520

Mean ± standard error of the mean.
Figures in parentheses indicate the number of animals.

season. While there is no periodicity in spermatogenic activity of the sexually mature males, young males with immature testes in different stages of development occur in all months of the year except September–October.

Testes weights of loris included in the data range from 0.13 to 3.465 g. The testes weight/body weight ratio, which is shown in Table III, ranges from 0.4% in the lower body weight groups to 0.96% in the heaviest animals. For convenience of comparison the animals are grouped with an increment of 400 mg of testes weight. In immature animals with testes weight below 400 mg the relative testes weight is 0.4% and gradually increases to 0.96% in the heaviest testes weight group (2801–3200 mg) with an average body weight of 310 g. The ratio is 0.4% until the testes weight reaches 1200 mg after which there is a gradual increase. This probably indicates that during early stages of development the growth of the testes lags behind the growth of the body and increases subsequently as the animals grow. The relation between age and growth of the testis in loris is not clearly understood.

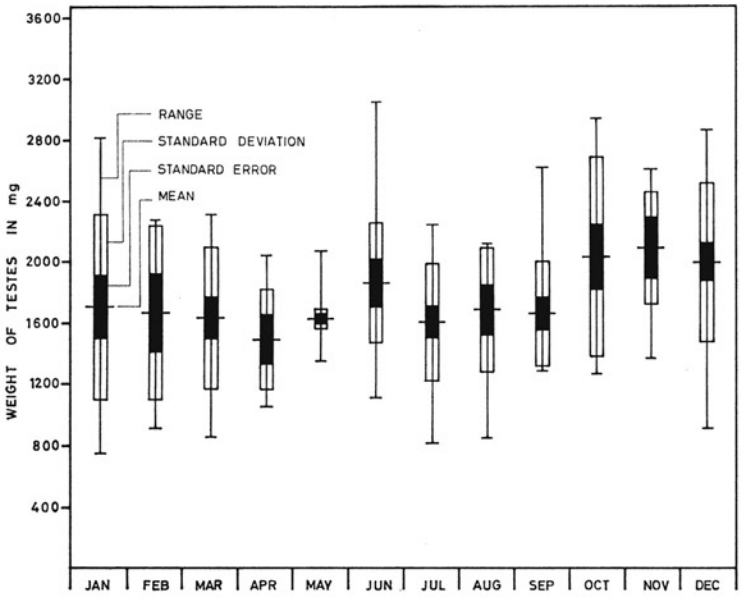

Fig. 1. Diagram showing the variation in the weight of the testes in the different months. Testes weighing over 900 mg are spermatogenically active and sperms are present in the seminiferous tubules and epididymal tubules.

Table III. Testes weight/body weight ratio in loris.

Testes weight range mg	Testes weight body weight ratio %
0– 400 (8)	0.40
401– 800 (0)	—
801–1200 (15)	0.40
1201–1600 (34)	0.52
1601–2000 (39)	0.66
2001–2400 (27)	0.77
2401–2800 (10)	0.82
2801–3200 (4)	0.96

Figures in parentheses indicate the number of animals.

Histology

Testes weighing between 13–62 mg are immature and inactive. The tunica albuginea is thick and a number of closely packed spermatic tubules full of primary spermatogonial cells characterise this stage. The seminiferous tubules are solid and do not show a lumen. In those with testes weights ranging from 72–180 mg, traces of a central lumen are visible and spermatogonial divisions are in evidence. Spermiogenic activity commences when the testes weigh about 760 mg; this stage is characterised by the presence of early spermatids. Testes weighing about 900 mg and over, without exception, are fully functional and their tubules are full of spermatozoa. The testes weight can, therefore, be taken as a fair index of spermatogenic activity. Functional testes may be abdominal, inguinal or scrotal indicating that descent of the testes into scrotal sacs is not a prerequisite for their functional activity in loris.

Diameter of the Seminiferous Tubules

Tables I and II show the variation in the diameter of the seminiferous tubules. In testes weighing below 400 mg the diameter of the tubules is about 80–120 μ. Tubule diameter increases gradually paralleling changes in testes weight to 250 μ (range 230–270 μ) in testes weighing about 2 g. There is no further increase in diameter with the increase in testes weight from 2–3.5 g which is probably more due to relative increase in seminiferous tubule length than to increase in tubule diameter. There is no significant variation either in testicular weight or in the diameter of the seminiferous tubules during different seasons of the year.

Accessory Glands

The weights of the accessory glands of reproduction are closely related to the weight of the testes. This is shown by the gradual increase in the weights of seminal vesicles, prostate and Cowper's glands with the increase in the weight of the testes (Tab. I, II, Fig. 2). The distribution of weights of the accessory glands during different seasons of the year (Tab. I, Fig. 3) exhibits two peaks of activity, one in May and another in December–January. The slight reduction which is noticed in February–March in the weights of the seminal vesicles

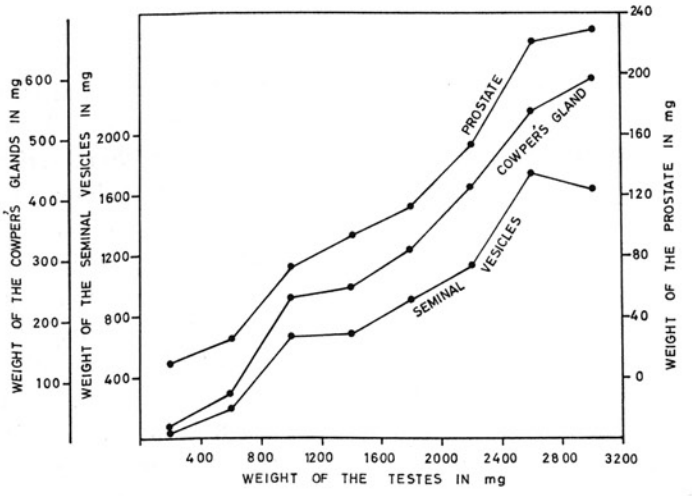

Fig. 2. Diagram showing the relation between the weight of the testes and the weight of the accessory glands.

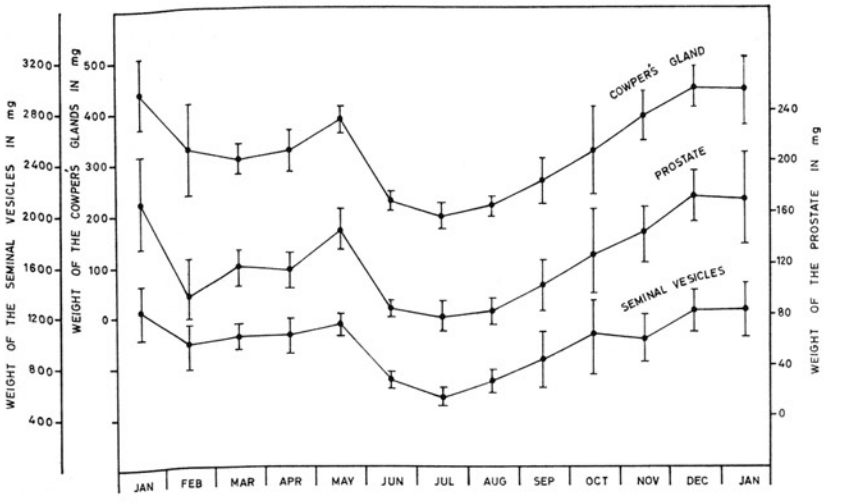

Fig. 3. Diagram showing the seasonal variation in the weight of the accessory glands in the different months.

and prostate and of the seminal vesicles in November is not statistically significant from the levels seen in May and December ($P > 0.05$). There is a decrease in the weights of the accessory glands which

reaches a minimum in July; a gradual increase in weight initiated in August reaches a maximum in December.

Discussion

Breeding Season

There is no unanimity of opinion concerning the breeding season of loris and the season in which the young are born. HILL [1935] states that loris breeds twice a year, with one or occasionally two young being born in late April–May and again in November–December. Based on observations of external genitalia of female loris, MANLEY [1965] observed that loris experienced two periods of activity, each of several successive estrous cycles separated by an anestrous phase of about six months. He further suggests that "the lorisoid primates are fundamentally polyestrous throughout the year, but that in certain forms local conditions may lead to the secondary superimposition of more intensive breeding, a process apparently culminating in loris in which a truer seasonality seems to be the rule".

Growth and Maturity

Figure 4 summarises the cycle of growth, maturity and breeding in the loris. This is based on the observations of HILL [1935; 1953], NICHOLLS [1939], RAMASWAMI and ANAND KUMAR [1962], RAMAKRISHNA and PRASAD [1962], MANLEY [1965] and present data.

The present study shows that immature animals with spermatogenically inactive testes are found in all months of the year except September–October. During this period all male lorises collected are sexually mature and their testes are in full spermatogenic activity. It is reasonable to suggest that young males born during November–December (HILL [1953]) possibly become sexually mature by the beginning of September; similarly young males born in April–May possibly become sexually mature in February–March. The absence of immature males in the collection during September–October may be due to prolonged lactation which is recorded for this species (HILL [1953]). The period of immaturity in male loris possibly lasts 10–11 months. RAMASWAMI and ANAND KUMAR [1962] on the basis of changes in the vaginal smears, record the existence of two estrus periods in loris, one during June–July and another in October–No-

vember. Males which become sexually mature in March may mate with estrus females during June–July. The ensuing pregnancy lasting between 160–171 d (NICHOLLS [1939]; MANLEY [1965]) may result in birth of young in November–December. Similarly young males which become sexually mature in September may mate with estrus females during the second estrus cycle of the year in October–November; the resulting pregnancy leads to birth of young in late April–May. Since the lactation and gestation periods are prolonged each female can breed only once a year. Whether young males which become

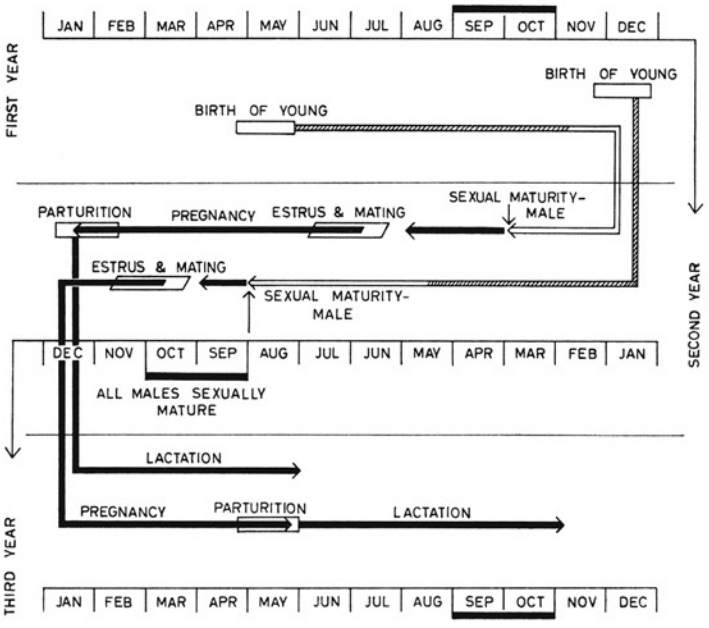

Fig. 4. Cycle of growth, maturity and breeding in the male and female loris. Based on present data and RAMAKRISHNA and PRASAD [1962] for males and observations of HILL [1935; 1953], NICHOLLS [1939], RAMASWAMI and ANAND KUMAR [1962], and MANLEY [1965] for females. – ▯▯▯▯▯▯▯▯▯▯ = period when young are suckling; ═══▷ = period of immaturity; ▬▬▶ = after maturity. The cycle of growth and maturity of young born in April–May is traced from birth, through suckling and growth to maturity, breeding, pregnancy and lactation. A similar cycle is traced for young born in November–December. The age at which female lorises become sexually mature is not clear. On the basis of scanty information available in the literature it is presumed that the period of immaturity in female loris is about 12–15 months.

sexually mature in February–March or in September mate within a few months after the onset of sexual maturity is not clear from our data. The collection of lorises at any given time of the year would consist of a number of generations of animals in various phases of growth and maturity (Fig. 4). The life span and the length of reproductively active life of loris are not known.

Testes Weight/Body Weight Ratio

SCHULTZ [1938] recorded the relation between the body weight and testes weight in a number of simian primates. The relative weight fluctuates between 0.1% and 0.4% among the new world monkeys; in the old world monkeys the testes are proportionately much heavier in the subfamily Cercopithecinae. Among five species of macaques the relative testicular weight varies between 0.46% and 0.92% whereas in the langurs it averages only 0.06% to 0.08%. Among the higher primates the ratio is 0.05% in the orang 0.08% in the gibbon and 0.27% in the chimpanzee. SCHULTZ further pointed out that small testes relative to body weight contain a proportionately much smaller amount of connective tissue than in relatively large testes. HALL-CRAGG [1962] observed that the testes weight/body weight ratio is 0.017% in the adult and 0.01% in the adolescent gorilla *Gorilla gorilla*. The ratio in gorilla is much smaller than that of man or that of the other Pongidae. Comparison of the testes weight ratio of loris which ranges from 0.46% in immature to 0.96% in fully grown adults with those of simian primates shows that loris resembles the Cercopithecinae.

Accessory Glands

The weights of the accessory glands exhibit two peaks of activity in May and December–January (Tab. I, Fig. 3). It should be pointed out, however, that the testes weights do not show any seasonal variation in weight, neither is spermatogenic activity of the seminiferous epithelium arrested during any season of the year (Tab. I, Fig. 1). The decrease in the weights of the accessory glands may indicate fluctuations in the secretory activity of the accessory glands. Whether the two peaks of activity of the accessory glands coincide with the periods when the animals mate cannot be clearly established. HILL [1953] and RAMASWAMI and ANAND KUMAR [1962] pointed out that mating

occurs in June–July and in October–November. The present data on the weights of the accessory glands which show a peak of activity in May suggest that the increase preceeds the mating period in the following two months. Table I shows that accessory gland weights are markedly reduced in June–July, possibly due to discharge of the secretions during copulation. It is, however, difficult to reconcile the occurrence of a peak of activity in the accessory glands in December–January immediately following the period of mating in October–November.

In the loris, an explanation is necessary to account for the occurrence of spermatogenic activity throughout the year while the accessory glands of reproduction exhibit seasonal variations in weight and presumably secretory activity. Gonadotropins and androgens synergise to initiate and maintain spermatogenesis and secretory activity of the accessory glands (PRICE and WILLIAMS-ASHMAN [1961]). It might be speculated that varying levels of androgens are involved in regulating reproduction in loris, a low level of androgen secreted throughout the year which is adequate to maintain spermatogenesis but inadequate to stimulate the sex accessories and a higher level of androgen available during some months of the year for the initiation and maintenance of secretory activity in the accessory glands of reproduction.

In this connection it is of interest to consider two suggestions which are relevant to the problem. WOODS and SIMPSON [1961], in discussing the hormonal control of spermatogenesis, observed that higher doses of testosterone, given systemically, were required for spermatogenic than for androgenic action; in contrast ICSH had demonstrable spermatogenic action in doses inadequate to stimulate male accessory glands. On the basis of these results they suggest that the Leydig cells, in response to ICSH, may secrete an additional not yet identified hormone, more efficient in its "gametokinetic" action than in eliciting other androgen responses. An alternative explanation based on the observations of LINDNER [1963] obviates the need to postulate a new testicular hormone. He observed that testosterone concentration in the testicular lymph of rams was two to eight times higher than that in systemic blood plasma and suggests that the androgen release is attended by escape of testosterone into the tissue fluid of the testes, and hence, the seminiferous tubules may be exposed to a much higher concentration of hormone than are androgen target organs remote from the testis. He postulated that "...low grade of stimulation of the Leydig cells by a small dose of ICSH could

give rise to a low concentration of testosterone in the testis adequate to support spermatogenesis, without achieving an effective androgen level in systemic blood". This explanation may account for the activity of the testis and accessory glands in loris. Biochemical work on the levels of testosterone in the testis and plasma during different months of the year, the factors regulating the synthesis and/or release of androgens, and quantitative fluctuations, if any, in the biochemical composition of the secretions of the accessory glands of loris needs to be completed before this problem is clearly understood. These studies are now in progress.

In a study of the reproductive cycles of naturally occurring mammalian populations the primary criterion for determining reproductive activity is the spermatogenic activity in the testis and to a lesser degree the weight of the accessory glands of reproduction. In species which occur in the subarctic, temperate and subtemperate regions seasonal variations in the activity of the testes and accessory glands of reproduction are generally synchronous. The species occurring in the tropical and subtropical regions offer an entirely different problem; in many species testicular activity is continuous throughout the year while it is distinctly seasonal in others (ASDELL [1946]). An analysis of reproduction in loris from the subtropical region around Bangalore, 77° E and 12° N, situated at a height of about 3000 ft above the mean sea level, demonstrates seasonal variations in the activity in the accessory glands while the testes are spermatogenically active throughout the year. Continuation of spermatogenic activity throughout the year need not necessarily indicate the existence of continuous breeding activity in the male. In spite of the production of spermatozoa throughout the year, the breeding season for the male may be restricted to specific delimited periods of the year to coincide with the secretory activity of the accessory glands of reproduction. In such cases it would be necessary to consider the activity of the testes and accessory glands separately on the basis of biochemical studies indicated earlier.

Summary

Changes in the reproductive organs of the male slender loris, *Loris tardigradus lydekkerianus* (CABRERA), have been studied by observations on 151 male lorises collected from forests near Bangalore, South India, over a period of over three years.

The males are spermatogenically active throughout the year with no period of quiescence during any season. The weights of the seminal vesicles, prostate and Cowper's glands show an increase parallel with increase in testicular weight. However, there is a seasonal variation in the weight of the accessory glands; they reach a peak of activity in May prior to the period of mating in June–July.

The cycle of growth, maturity and breeding of loris based on present data and observations of other workers is presented. The period of immaturity in male loris is about 10–11 months; the first set of young are born when the females are about two years old.

The accessory glands show seasonal fluctuations in weight though the testes are spermatogenically active throughout the year.

ADDENDUM

RAMASWAMI and ANAND KUMAR [1965] observe that female lorises are in estrus during June–July and September–October–November. Pregnancy occurs during one of these estrus cycles. Females conceive only once a year. RAMASWAMI and ANAND KUMAR [1962], commenting on the observations of HILL [1953] point out that if there are two heat periods in the female and there is a descent of the testes twice, the testes should descend and become scrotal during March–April and September–October. On the basis of their extensive observations subsequently on loris maintained in captivity, RAMASWAMI and ANAND KUMAR [1965] point out that in the slender loris the testes never descend into the scrotum at any time of the year.

MANLEY [1966] observed changes in the external genitalia of *Loris tardigradus* maintained in captivity and pointed out that *Loris* experiences more than one estrus state in a season with a prolonged anestrum in between. Post-partum estrus and mating does not occur in loris when the young is being suckled. Death of the young during lactation results in the onset of estrus and mating within 5 days thereafter.

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