REPRODUCTION IN THE MALE STOAT, MUSTELA ERMINEA

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Summary. The male stoat has a well-defined breeding season extending from May until the end of July. The testis then regresses rapidly and remains in quiescence until the end of November when the seminiferous tubules consist of a peripheral layer of supporting cells and occasional spermatogonia. The onset of spermatogenesis in December is marked by the appearance of primary spermatocytes and, by April, most of the tubules contain germinal cells in all stages of spermatogenesis. Spermatozoa are not found in the cauda epididymidis until May. Juvenile males, unlike the females, attain sexual maturity in the spring following the year in which they were born. Testosterone concentrations of plasma samples obtained from adults at intervals throughout the year were determined by gas-liquid chromatography. Levels rose with the onset of spermatogenesis and reached a maximum during March and April. They decreased in May but another smaller peak developed during June and July corresponding to the mating period. This peak may be related to maintenance of the accessory sex organs and libido.

INTRODUCTION

The reproductive organs of male mammals have been investigated in many species and the information available shows interesting differences in the duration of reproductive activity, which allow a differentiation into 'continuous' and 'seasonal' breeders (Asdell, 1964). In the seasonally breeding mammals, a period of sexual quiescence intervenes between the active period and the length of this quiescent period shows species variation; tropical mammals usually have less restricted breeding seasons than those living in temperate regions. Partial or complete cessation of reproductive activity is quite common in the latter, many of which hibernate during the winter months.

The work of Deanesly (1935) and Watzka (1940) has already established that the male stoat, unlike the female, remains sexually immature for about a year and attains sexual maturity only in the spring following the year in which it was born. As in other mammals, the onset of spermatogenesis in the

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juvenile is marked by an increase in the size of the testes which descend into the scrotal sacs and remain there throughout life. The reproductive cycle of certain closely related mustelid carnivores, such as the ferret (Allanson, 1932) and the weasel, Mustela nivalis (Hill, 1939), have also been studied and some remarkable differences in the patterns of breeding are seen.

Androgen determinations in the testis and the peripheral plasma have been carried out in a number of seasonally breeding animals (Short & Mann, 1966; Lincoln, 1971; Racey, 1972) but no comparable studies on carnivores are available. In the present investigation, histological changes in the reproductive organs of juvenile and adult stoats are described. Variations in the plasma testosterone levels throughout the year are discussed in relation to the breeding cycle and also to the changes observed in the reproductive organs.

MATERIALS AND METHODS

Histological material was obtained from twelve 1st year, fifteen 2nd year, and five 3rd year stoats maintained in the laboratory, in which all but three were born. The animals were kept individually in anodized aluminium cages in a well-lit room in normal daylight conditions: the room was unheated to mimic the outside conditions as far as possible. The animals were fed on raw cow or horse flesh (minced) alternated with whole dead mice. They were allowed free access to water and an ‘egg–milk’ mixture was provided every other day. The stoats lived uneventfully under these conditions and never showed any physical abnormalities.

The animals were killed at appropriate intervals during the course of the year. The weights of the paired testes and epididymides were recorded. The tissues were fixed in Bouin's fluid and prepared routinely for light microscopy. Sections (6 μm) were stained with haematoxylin and eosin. Testis sections prepared in this way were subjected to detailed microscopical examination. The seminiferous tubule and the Leydig cell nuclear diameters were measured using an eye-piece micrometer. Twenty measurements were recorded for each specimen. The specimens were arranged in chronological order according to the stage of the reproductive cycle so that corresponding histological changes could be described.

To correlate with the histological study, blood samples were obtained from twenty adult stoats at different times of the year. When the animals were under fluothane (Halothane: I.C.I.) anaesthesia, about 5 ml blood were usually collected into a heparinized syringe by cardiac puncture. The animal was normally allowed to recover and used again for the same purpose after at least 14 days rest. The plasma was separated by centrifugation and stored at −17°C until the testosterone levels were determined. The volume of the plasma samples was usually 2·6 ml, but volumes of 1·2 to 3·6 ml were also used when the blood sample was smaller or greater than 5 ml. Testosterone in the plasma was determined as the 17β-monochloroacetate derivative. After the addition of 15,000 d/min (38·6 ng) of [4-14C]testosterone as radioactive internal standard, 1 M-NaOH was added to the plasma to a final concentration of 0·15 M-NaOH and the testosterone was extracted four times with 2 vols of
diethyl ether. The ether was pooled and evaporated to dryness under nitrogen and the testosterone was separated from the crude extract by the thin-layer chromatographic (TLC) system, benzene:ethyl acetate (1:1, v/v). The testosterone was chloroacetylated with monochloraacetic anhydride and pyridine (Brownie, van der Molen, Nishizawa & Eik-Nes, 1964), and the chloraacetate was then purified by the TLC system, benzene:ethyl acetate (9:1, v/v). Before final quantification by gas-liquid chromatography (GLC) with electron-capture detection, a known amount of 20β-hydroxy-4-pregnen-3-one mono-chloracetate was added to the sample as GLC internal standard. The steroid determinations were corrected to 100% recovery according to the recoveries of the radioactive and GLC internal standards, and the calculation method was based on that given by van der Molen & Groen (1965). The amount of testosterone associated with the 14C tracer was then subtracted from the final concentration reported here. The conditions of GLC and the purification of chemicals and the [4-14C]testosterone were similar to those reported in an earlier paper (Tam, 1971). Precision tests on the above procedure were performed by using the same technique for the determination of exogenous testosterone added to portions of a pool of male guinea-pig plasma after the endogenous testosterone concentration had been determined. With the addition of 30 ng testosterone to give a final concentration of 5 ng/ml, the results were 30·00±3·00 ng (mean±S.D., coefficient of variation 10·00%, N = 3). After the addition of 70 ng to represent a total concentration of 13·00 ng/ml, the results were 70·30±4·00 ng (coefficient of variation 9·70%, N = 3).

RESULTS

The reproductive organs of the male stoat, like those of many other Carnivora, are comparatively simple. Immediately adjacent to the cauda epididymidis, the ductus deferens is slightly coiled and attains its maximum diameter. Beyond the convoluted portion, it continues as a thin narrowing tube and crosses over the urethra at its junction with the neck of the bladder. The terminal portion of the ductus is enlarged to form an ampulliform swelling. There are no seminal vesicles and no obvious prostate gland.

Histological changes in the reproductive organs

The testes of the immature males showed very little variation in histological appearance in the first few months after birth. Until August, the seminiferous tubules had a diameter of about 60 µm and consisted of a peripheral layer of supporting cells and occasional spermatogonia (Pl. 1, Fig. 1). The tubules had no lumen, and were filled with a cytoplasmic 'syncytium' of the supporting cells. Occasionally, spermatogonia larger than others were seen lying in the 'syncytium'. These enlarged spermatogonia resemble the cells first described in 1896 by van Beneden and later called 'winter spermatogonia' by Courrier (1927). It appeared that the spermatogonia at the periphery of the tubule enlarged and migrated inwards to give rise to 'winter spermatogonia', whose nuclei might measure as much as 10 to 15 µm in diameter. The immature testis contained abundant interstitial tissue. The cells had spherical nuclei 5 to 6 µm
in diameter. Larger interstitial cells were often found next to the tunica (Pl. 1, Fig. 1).

From September until the end of the year, proliferation took place in the seminiferous tubules. Although the tubules were still small and devoid of a lumen, spermatogonia were undergoing mitosis. More spermatogonia were seen in each tubule (Pl. 1, Fig. 2). 'Winter spermatogonia' were still present in the autumn months. Mitoses became more numerous during December when primary spermatocytes first began to appear in the tubules (Pl. 1, Fig. 3). The interstitial tissue underwent no appreciable changes.

During December, the histological appearances of the testes of both the immature (1st year) and the adult (2nd year +) stoats were essentially similar. The onset of spermatogenesis was marked by the appearance of primary spermatocytes (Pl. 1, Fig. 4). In January, the tubules still contained spermatogonia and primary spermatocytes. The lumen in the tubules first appeared in February. Spermatogenic activity increased greatly with the approach of the spring and, by April, most of the tubules contained all stages of spermatogenesis (Pl. 1, Fig. 5). Active spermatogenesis continued throughout the breeding season which extended from May until the end of July (Pl. 1, Fig. 6 and Pl. 2, Fig. 7).

The testes began to regress towards the end of the breeding season. During August, the regressive changes were quite distinct in the seminiferous tubules. Spermatozoa were still present but the spermatogenic cells, particularly the later stages, appeared to be degenerating. The cells were often clumped together and their degenerating remains were commonly seen in the lumen of the tubule. By September, the tubules contained only a few spermatogonia and degenerating primary spermatocytes (Pl. 2, Fig. 8). The lumen was almost obliterated. All the primary spermatocytes had disappeared by October and the tubules consisted of a peripheral layer of supporting cells and occasional spermatogonia (Pl. 2, Fig. 9).

The testis weights and seminiferous tubule diameters of the animals in the present study are summarized in Text-fig. 1. Little testicular growth took place in the immature (1st year) males in the first few months after birth; enlargement started in January. In adult males, testicular growth was also rapid after December and was maximal by April. After the breeding season, the testicular weight fell sharply and the testes were essentially similar to those of immature males during October and November.

EXPLANATION OF PLATE 1

Fig. 1. Testis of an immature (1st year) 2-month-old stoat during June. The tubules consist mainly of supporting cells and occasional spermatogonia. Note the presence of larger interstitial cells next to the tunica. H & E. ×325.

Fig. 2. Testis of an immature (1st year) 6-month-old stoat during October. More spermatogonia are seen in the tubules at this time. H & E. ×406.

Fig. 3. Testis of an immature (1st year) 8-month-old stoat during December. Primary spermatocytes first begin to appear in the tubules. H & E. ×406.

Fig. 4. Testis of an adult (2nd year) 20-month-old stoat during December. The tubules contain primary spermatocytes. H & E. ×540.

Fig. 5. Testis of an adult (2nd year) 23-month-old stoat during March. Most of the stages of spermatogenesis are represented. H & E. ×325.

Fig. 6. Testis of an adult (3rd year) 25-month-old stoat during the breeding season (May). H & E. ×406.
PLATE 2

(Facing p. 307)
Reproduction in the male stoat

The diameter of the Leydig cell nuclei varied little throughout the year (monthly means: 4·50 to 5·50 µm). The histological appearance of the cell cytoplasm varied considerably between individual animals at any one time during the reproductive cycle and there was no clear indication of seasonal variation in the Leydig tissue.

In the 1st year stoats, the epididymides, like the testes, remained undeveloped and very little growth took place before the following year. Although the testes in the juveniles and the adults exhibited full spermatogenesis in April,

**TEXT-FIG. 1.** Seasonal changes in the weight of the testes (——) and the diameter of the seminiferous tubules (— — —) of immature (○, 1st year) and adult (×, 2nd and 3rd year) stoats. Each mean was determined from two animals.

![Graph showing seasonal changes in the weight of testes and diameter of seminiferous tubules](image)

EXPLANATION OF PLATE 2

**Fig. 7.** Testis of an adult (3rd year) 27-month-old stoat during the breeding season (July). H & E. ×406.

**Fig. 8.** Testis of an adult (3rd year) 29-month-old stoat after the breeding season (September). The tubules contain a few spermatogonia and degenerating primary spermatocytes. H & E. ×406.

**Fig. 9.** Testis of an adult (3rd year) 30-month-old stoat during October. The tubules consist of a peripheral layer of supporting cells and occasional spermatogonia. H & E. ×406.

**Fig. 10.** Cauda epididymidis of an adult (3rd year) 25-month-old stoat during May. Spermatozoa first appear in the epididymal tubules at this time. H & E. ×325.

**Fig. 11.** Cauda epididymidis of an adult (3rd year) 27-month-old stoat during the breeding season. The epididymal tubules are packed with spermatozoa. H & E. ×325.

**Fig. 12.** Cauda epididymidis of an adult (3rd year) 29-month-old stoat after the breeding season (September). The epididymal tubules are completely devoid of spermatozoa. H & E. ×325.
Text-fig. 2. Seasonal changes in the plasma testosterone of adult stoats. Vertical lines represent the S.E.M. The numbers in parentheses represent the number of samples assayed. The hatched bar indicates the breeding season.

Spermatocytes

Primary

Secondary

Spermatogonia

Spermatocytes

Primary

Secondary

Spermatogonia

Plasma testosterone first appeared in the cauda epididymidis during May (Pl. 2, Fig. 10). The epididymides weighed about 0.30 g in May and this condition persisted until August after which there was a steady decrease in weight as regression set in. Spermatozoa were present in the epididymides throughout the breeding season (Pl. 2, Fig. 11) and they may persist until August. By September, they disappeared completely (Pl. 2, Fig. 12) and were not found in the epididymides until the following May.

**Plasma testosterone concentration**

The hormonal data are shown in Text-fig. 2. The level of testosterone in the plasma began to rise in the winter with the onset of spermatogenesis and reached a maximum during March and April. This peak coincided with the maximum testicular weight attained during this period. During May, testosterone levels decreased. A second, but lower, peak appeared during June and July and covered the major part of the mating period. Testosterone concentration in the plasma began to fall in the autumn and this coincided with the decline in spermatogenic activity of the testes and regression of the accessory sex organs.

**DISCUSSION**

The reproductive patterns in the juvenile male stoat, ferret (Allanson, 1932) and the long-tailed weasel, *Mustela frenata* (Wright, 1947), appear to be similar in
that full spermatogenic activity does not appear until the spring following the animal’s birth, but an interesting difference occurs in male weasels, *Mustela nivalis*. According to Hill (1939), males born early in the breeding season, in April and May, are sexually mature by the age of 4 months (spermatozoa found in the testis and the epididymis) but Heidt (1970) reckons that sexual maturity is not reached until the weasels are 8 months old, the age at which the males first mate. Similar observations have been recorded for the mink (Onstad, 1967). In spite of the difference in opinion on the definition of the term ‘sexual maturity’, it is quite clear that, unlike that of the stoat, the weasel testis is capable of active spermatogenesis by the age of 4 months. Males born later in the breeding season (August and September) remain sexually immature until the following year and then, like the stoat, they attain full spermatogenesis at about the same time as the adult males.

In the stoat, the male breeding season is roughly comparable to that of the ferret. Although male stoats with testes undergoing active spermatogenesis are occasionally found during January and February (Deanesly, 1935), it is unlikely that they would have fertile matings because oestrous females are not found until April and many of these are recently parous (Deanesly, 1943; Gulamhusein, 1973). Since parous females come into heat some time during lactation, it seems that most matings take place after April. It is at this time that spermatozoa first appear in the cauda epididymidis. Fertile matings may take place until August because spermatozoa do not disappear from the epididymis until September. The stoat testis, like that of the ferret, has a definite period of quiescence from September to November and preparation for the next breeding season in both these species begins in December. The quiescent period in the weasel testis is less well marked. At no time does the production of spermatagonia and spermatocytes cease completely (Hill, 1939).

Testis weights and measurements of seminiferous tubules taken from the animals in the present investigation appear to be distributed in accordance with the data given by Deanesly (1935). Unlike the testis, which attains maximum development in April, the epididymis does not reach its maximum weight until May or June (Deanesly, 1935), and in this respect the stoat differs from the weasel and the ferret. In both these species, the testis and the epididymis attain maximum development at about the same time. The usual lag in the development of the accessory sex organs in the stoat resembles the condition in the bat (Courrier, 1927), and is probably well suited to the type of reproductive pattern exhibited by this mustelid. Stoats produce only one litter annually due to the occurrence of delayed implantation which extends from the summer of one year to the spring of the next. Implantation occurs in early March and parturition takes place some 4 weeks later (Deanesly, 1943; Gulamhusein, 1973). It is not clear from the data of Wright (1947) on the reproductive cycle of the male long-tailed weasel whether a condition similar to that in the stoat exists because the weights recorded are those of the combined testes and epididymides. The reproductive patterns exhibited by both the male and the female long-tailed weasel are very similar to those of the stoat.

Since the female ferret and weasel do not exhibit delayed implantation, they become anoestrous soon after the breeding season and remain in quiescence.
until the following spring. As both the sexes attain maximum sexual development at about the same time, the synchronous development of the primary and secondary sex organs is essential for successful breeding in these two mustelids.

Testosterone concentrations in the peripheral plasma are closely correlated with the development of the primary and secondary sex organs of the male stoat. The onset of the first peak in December corresponds with the initiation of spermatogenesis, a stage which may be dependent on testosterone (Steinburger, 1971). This correlation has also been established in the red deer stag (Lincoln, 1971) and studies involving the production of testicular androgens *in vitro* by the cobra, *Naja naja* (Tam, Phillips & Lofts, 1969). Testosterone concentration reached a maximum during March and April, which corresponded to the time of attainment of maximum testicular weight. This probably reflects the requirement of androgens for spermatogenesis and the anabolic effect of testosterone on testicular growth.

Low testosterone levels during May suggest a reduction in the secretion of pituitary gonadotrophin and it seems that after an initial demand, spermatogenesis in the stoat testis is maintained with lower levels of androgen production. The appearance of the second, but lower, peak indicates resurgence in pituitary activity and coincides with the maximum development of the accessory sex organs as reported by Deanesly (1935). Since this peak also covers the major part of the mating period, it appears that testosterone may also be required to maintain libido over a relatively long mating period extending from May to July, or even until August. Lincoln, Youngson & Short (1970) have reported that red deer stags exhibited libido for up to 4 to 5 months after the rut when the concentration of testosterone in the testis had fallen to a low level, and the observations by Rosenblatt & Aronson (1958) on the sexual behaviour of castrated cats suggest that libido is not always associated with high levels of testosterone. The reduction in the concentration of testosterone in the plasma after the breeding season coincides with the decline in spermatogenic activity and the regression of the accessory sex organs.

Although testosterone determinations were not made during the period of reproductive quiescence, it is unlikely that the testis stops secreting androgens over this period. The activity of the steroid enzyme, $\Delta^4$-3$\beta$-hydroxysteroid dehydrogenase (using DHA as the substrate), which is believed to hold a key position in the biosynthesis of many steroid hormones (Talalay, 1965), remained unaltered during October, December, March and May (A. P. Gulamhusein, unpublished observation). This pattern of activity is identical to that observed in the Leydig tissue of many continuously breeding mammals (Baillie, Ferguson & Hart, 1966; Hay & Deane, 1966). Since the activity of this enzyme is reduced following hypophysectomy (Blackshaw, 1970), it appears that, in species such as the stoat, the pituitary secretes some gonadotrophin throughout the year. In our study, the histological appearance of the Leydig cell cytoplasm varied very little throughout the year and the diameter of the nuclei remained unaltered. These observations are at variance with those reported by Deanesly (1935) that Leydig cell cytoplasm and nuclei attained their maximum size in March. Johnsen (1962) considers that androgen production by the Leydig cells cannot be correlated with their histological appearance.
although Muschke (1953) reported that Leydig cell nuclear diameter, which decreases after hypophysectomy, is restored after HCG treatment.

Steroidogenesis in the Leydig tissue is thought to be under the control of LH (Eik-Nes, 1964) and the fact that the testis recommences activity during December indicates that the secretion of gonadotrophins from the pituitary is presumably increased at this time. Since many members of the family Mustelidae breed in the spring when the daylength is increasing, it is interesting to note that resumption of activity in the testis of the stoat probably takes place after the winter solstice. The observation that testosterone production is lowered by September, when the daylength is decreasing, also suggests that light may influence the pattern of gonadotrophin release in this species.

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