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# Reproduction of *Lagurus lagurus* (Pallas, 1773) in the Laboratory

#### [With 5 Figs. & 2 Tables]

In the laboratory Lagurus lagurus reproduced throughout the year. Females matured sexually when approximately 30 days old; males became sexually active a little later, when 45-46 days old. The length of gestation was most frequently from 19 to 23 days and  $35.8^{\circ}/_{\circ}$  of the females mated in post-partum oestrus. Mean number of litters from one female was 4.04 during her entire reproductive life; mean litter size was 3.11. Both number of litters and litter size depend strongly on the age of the female. Females 2 to 6 months old are the best breeders with respect to both the number and the size of young. The mortality of young before weaning was  $36.9^{\circ}/_{\circ}$ . The sex ratio at three weeks of age i. e. at the end of lactation was 1:1 in litters in which none of the young died. In the remaining litters there was a considerable excess of females. In the laboratory *L. lagurus* lives up to 31 months i. e. a year longer than in the field. The only disease observed in our colony was the so called "Lagurus disease" with paresis of the hind limbs, progressive weakening and loss of weight, which always resulted in death of the animal.

# I. INTRODUCTION

The demand for new species of laboratory animals is steadily growing and promotes increasing interest in wild rodents. After adaptation in a controlled environment they can become new laboratory animals. Some of the wild rodents are more suitable for certain types of research than hitherto used »classical« species of laboratory animals.

To adapt new animal species to laboratory conditions it is necessary to provide them with a stable environment, eliminate the influence of external conditions (fluctuations in temperature, illumination and humidity) and the seasonal pattern of reproduction. It is also very important to find a diet suitable for given species and then feed the animals this standard food.

Lagurus lagurus (Pallas, 1773), the so called »steppe lemming« seems to be very promising as a new laboratory animal. This species lives in the steppe (usually clay type soil) of Eastern Europe, namely in the area of the upper and middle

Wolga river, in semi-deserts and deserts of Central Asia (Kazakh SSR and the northen Caucasus) (Ognev, 1950; Ellermann & Morrison-Scott, 1951) and in the north-western part of North America (Burt & Grossenheider, 1964).

Lagurus lagurus is about 1.5 times larger than Mus musculus, cinereous or silver gray with a black stripe along the dorsum from the head to the base of the tail. Adult specimens have a body length of 80.0 to 105.5 mm, tail length of 8.5 to 14.8 mm and body weight of 13 to 20 g. Some individuals may reach 29.0 g.

Breeding of this species in the laboratory was first attempted by Naumov (1948). Beginning in 1956 these experiments were continued in the Department of Vertebrate Zoology, Moscow University (Bashenina, 1957) and at the Institute of Experimental and Clinical Oncology, Medical Academy of USSR in Moscow (Podhossjanc *et al.*, 1959). Freye (1961) is working with *L. lagurus* in Germany. Animals from the Medical Academy in Moscow were twice introduced to the Laboratory Animal Center in England (Cooper, 1964). More recently (since 1963) studies on this species are carried out in three laboratories in the USA: National Institutes of Health, University of California Medical Center and Elmira College (Whitney & Burdick, 1966), all of which received the first animals from England.

At the Mammals Research Institute, Polish Academy of Sciences at Białowieża a colony of L. lagurus was started in summer 1961. The purpose of breeding has been to adapt this species to laboratory conditions and to study the possibility of using it in various experiments.

# II. MATERIALS AND METHODS

#### 1. Conditions of breeding

In August 1961 the Mammals Research Institute, Polish Academy of Sciences received two females and one male *L. lagurus* from the colony of the Department of Vertebrate Zoology, Moscow University. The animals were kept in Białowieża type metal cages  $40 \times 25 \times 15$  cm. These cages have a division with two openings which forms a nesting compartment and a running compartment, the former smaller than the latter. The bottom of the running compartment was covered with turf which was changed every two weeks. The cages were changed and disinfected once a year and more often in case of disease. Sick or crippled animals were eliminated.

Animals were kept one pair to a cage. The last litter often remained with the parents and occasionally, mainly during periods of intensive reproduction, there were two litters with the parents. Some of the young were mated at weaning while the remaining animals were marked and put to stock cages, the males separate from the females. These animals were used to replace dead or eliminated breeders.

The males were left with the females during parturition and lactation as they did not interfere and were not agressive toward the young.

The mean annual temperature of the room was  $18.8^{\circ}$ C (ranging from 16 to  $20^{\circ}$ ) and the mean annual humidity was  $71.0^{\circ}/_{\circ}$ . In late spring, summer and autumn the humidity was increasing to  $83.2^{\circ}/_{\circ}$  due to feeding lots of fresh grass and in winter the humidity was reduced to  $58.4^{\circ}/_{\circ}$  by central heating.

The length of day was fixed at 18 hours using automatic switches and artificial illumination by neon lamps.

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#### 2. Food

In our colony the food of *L. lagurus* was different in different seasons. Throughout the year the animals were given oats with red beets, white beets or carrots. In winter this diet was supplemented with wheat sprouted to 5 cm height and occasionally with fresh apples. In summer the diet was based on grass. Besides, the animals were eating some hay of their nest. Moreover, the animals were given tree twigs during the whole year. Not eaten food was removed on the following day when fresh food was given. Water was available from a rubber bulb with a glass pipe. Both food and water were always given *ad libitum*.

# III. RESULTS AND DISCUSSION

# 1. Sexual maturation

In the laboratory *L. lagurus* females matured sexually when about 30 days old. However, individuals which became pregnant when 21 days old or matured only when 33 to 35 days old were quite common. One female became pregnant when 19 days old.

Males matured sexually slightly later than females, namely when 45 to 46 days old. These observations are in agreement with the data of B a s h e n i n a (1957) concerning the laboratory population of *L. lagurus*. In the field sexual activity of both males and females begins at the age of about 45 days. This however, is only a rough approximation as the sexual maturity of wild living females depends strongly on the date of birth ranging from 26 to 157 days (Pokrovsky, 1962; Schevtschenko, 1963; Krylzov, 1955; 1964). Females born between August and October mature most slowly. Later the rate of maturation increases and is maximal for the females born in spring and early summer. In July the rate of sexualy maturation is nearly two times slower than in June (mean 29.8 days in June and 55.9 days in July) due to the beginning of the dry season.

# 2. Pregnancy length

In *L. lagurus*, similarly to other *Microtidae*, the lapse of time between subsequent parturitions changes slightly depending on pregnancy length. In females nursing the previous litter pregnancy is prolonged.

In our colony the length of gestation defined as the time between two consecutive parturitions, was usually 19-23 days (75.7% of all pregnancies) with a range from 18 to 25 days (Fig. 1). Taking vaginal smears indicated that the pregnancy length in *L. lagurus* is 20-21 days. The same length of gestation (20-21 days) was reported from the colonies of Freye (1961) and Whitney & Burdick (1966). Bashenina

(1957) determined the length of pregnancy from the spacing of litters and reported a range from 18 to 31 days with considerable preponderance of gestations from 19 to 21 days long.

Many females mated in post-partum oestrus; 35.8% of the litters were born between 19 and 23 days after the preceding parturition. However, 27.4% of the litters resulted from matings 6 to 37 days after parturition i.e. in the second oestrus after the parturition or after the end of lactation. The length and the course of the oestrous cycle can not be determined from the data on vaginal smears in our colony. According to Whitney & Burdick (1966) in some females of this species the length of the oestrous cycle was approximately 7 days. Females lactate for 20 days (Freye, 1961). The period of time between subsequent lit-

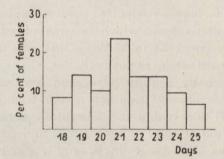


Fig. 1. The length of pregnancy in L. lagurus based on the spacing of litters.

ters does not depend on the size of the nursed litter, thus contrasting with the situation in *Clethrionomys glareolus* (Drożdż, 1963). A considerable proportion of the females (36.5%) had breaks in producing litters lasting up to 4—5 months. These interruptions were most frequent in females which: (a) were mated when female one year old or older, (b) had several (up to 8) litters every 21 days, (c) in spite of early mating started reproduction evry late (for example brother  $\times$  sister matings), (d) in spite of the changing of males several times had only 1 or 2 litters in their life span. This observation could be partially explained by the exhaustion of the female after producing many litters, lack of seasonal breaks in reproduction in the laboratory or inadequate environmental conditions. According to G l a d k i n a *et al.* (1966) *L. lagurus* reproduces much better when the room temperature is 24—26°C.

# 3. Number of litters and litter size

Not all mature animals were mated and only some of the mated animals produced litters. This was especially true in brother  $\times$  sister

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matings (inbred) where frequently there was no progeny or the litter was produced only after changing the male.

One hundred and fifty four pairs were mated of which 78.5% of the females had litters producing a total of 448 litters. One female produced on the average 4.04 litters in her life span. This value is higher than that reported by Bashenina (1957) — 3.2 litters. The number of litters produced by one female during her reproductive life was ranging from 1 to 13 (Fig. 2; females alive and reproducing were not included in these calculations). Relatively many (38.7%) of the females had only

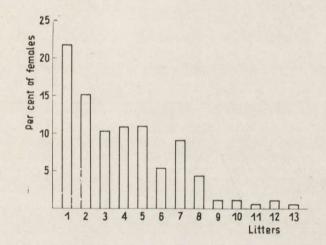


Fig. 2. Number of litters in the life span of a female.

one or two litters although in these cases the males where frequently changed and always present in the females cage. Thirty one and nine tenths per cent of the females had 3 to 5 litters, 18.6% had 6 to 8 litters and the remaining females had from 9 to 13 litters. The maximal number of litters reported by Bashenina (1957) was 10, and in the field it was not more than 5 to 7 litters. This is not a very high fertility as compared to other species of voles reproducing in the laboratory. For example females of *Microtus arvalis* produce on the average 15 to 20 litters in their life cycle (Frank, 1956).

Lagurus lagurus breeds in the laboratory throughout the year (Fig. 3). Between August and January the highest proportion of the females produced litters. Later there is a slight decrease of the per cent of reproducing females which ends only in May. The number of litters and the number of young is slightly higher between August and October and practically the same during the rest of the year. These differences are statistically not significant and could be due to the highest vitamin

content in the food during summer. Similar suggestions were made by Gladkina *et al.* (1963; 1966) and Drożdż (1963).

The total of 1393 young were born in 448 litters; the mean litter size was 3.11. The mean litter size did not change when the females which had only one litter were excluded from the calculations, or when the females mated very late were also omitted. This litter size is in agreement with the data of Cooper (1964) and Gladkina et al. (1966) who reported the mean of 3.0 to 3.2. Bashenina (1957) reported the

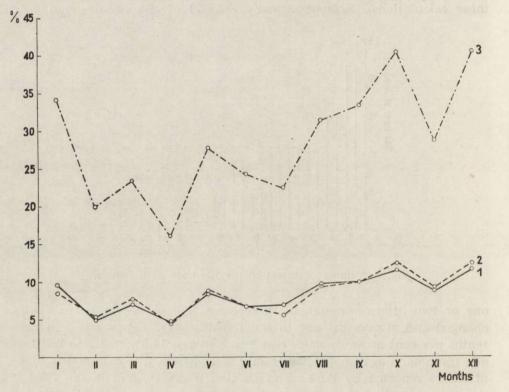


Fig. 3. The distribution of the number of litters, the number of young and per cent of reproducing females in consecutive months.
1 — Per cent of litters; 2 — Per cent of young; 3 — Per cent of reproducing females.

mean of 3.38 young in the litter. Freye (1961) described a still higher litter size, 4.0 and Heptner (1956) reported the mean of 6.0 young in the litter. With the exception of the latter result all these values are much lower than the litter size of free living *L. lagurus* which have on the average 5.5 to 5.9 young in a litter (range from 1 to 11) and in spring even up to 14 young (Schewtschenko, 1963). In the Białowieża animal colony the litter size of *L. lagurus* was ranging from 1 to 8 young,

while in 73.2% of the litters there were 2 to 5 young (Fig. 4). The decreased litter size of *L. lagurus* in the laboratory is most likely caused by food much inferior to that available in the field. In the field the fertility of *L. lagurus* decreases considerably when external conditions and especially food conditions are unfavorable (Krylzov, 1955; Schewtschenko, 1963).

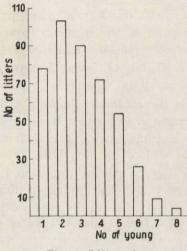


Fig. 4. Litter size.

## Table 1.

The number of litters and the litter size in relation to the age of the females in L. lagurus.

Age in months	2-6	7-12	13 - 18	19 - 24	25 — 31
N	93	89	44	24	8
Per cent of females producing litters	92.4	77.5	53.5	33.3	12.5
Mean litter size	2.43	2.59	1.87	1.75	-
Mean number of young per female	8.27	7.56	4.75	4.62	_

A »statistical« female in our colony produced 12.6 young in her life span (3.11 young  $\times$  4.08 litters). However, some females produced uncomparably more young: female No 29 had 34 young in 7 litters, female No 51 had 46 young in 11 litters and female No 47 had 57 young in 13 litters.

Both the number of litters and the litter size depend strongly on the age of the female (C h itty, 1957; G l a d k in a *et al.*, 1966). According to these investigators females 5 to 7 months old had the largest litters. In our colony 92.4% of the females 2 to 6 months old were reproducing while only 28.1% of the older (19 to 31 months old) females were still producing litters (Table 1). In the age group 7—12 months there was the highest number of litters per female, but the litter size was higher in younger females. The mean number of young produced by one female also decreased with the age of the mother; from 8.27 in the youngest group to 4.62 in two year old females. Females of *L. lagurus* retain their fertility for a long time (Table 1). Some 2 year old and older females produced litters and raised them successfully to weaning.

Of 1393 young born in the Białowieża colony 43 (3.08%) were stillborn or died within a few hours after birth. Of these only 6 were killed by the female, the rest did not have any external damages. During the first week after birth 148 (10.9%) of the live born animals died of which 15 were killed by their mothers. Some killing of young by the female was also observed by Cooper (1964) although feeding was the only interference by man. The mortality during the first week of life is not limited to any season (Fig. 5). The lowest mortality was observed in February

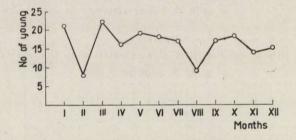


Fig. 5. Mortality of young during the first week of life in different seasons.

and August, while in the remaining months there were only insignificant fluctuations.

Before weaning another 323 young died. However, the young were weaned at different age (when 1—3 month old). Between birth and separation from the mother the total of 514 animals died (36.9%). During the first two weeks after weaning 51 more animals succumbed. Consequently, only 59.4% of the young survived and could have been used for further experiments. This high mortality of young can certainly be reduced in future breeding. The young should be separated when lactation stops i. e. when 20—30 days old.

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#### 4. Sex ratio

Sex ratio at birth is unknown. In litters in which all young survived for three weeks the sex ratio was 1:1. In litters in which there was some mortality the sex ratio was significantly ( $\chi^2$  test) different from 1:1. Assuming an even number of males and females at birth, half of the males and one fourth of the females died. This would indicate that the mortality of males is higher. A similar observation was reported by Whitney & Burdick (1966) who observed considerable excess of females in litters in which some animals were missing. However, Freye (1961) reports a sex ratio 1:1 with a slight and not significant excess of males.

# 5. Life span

Both males and females of L. lagurus lived in laboratory conditions up to 31 months (Table 2). However, only 3.6% of the animals lived longer than two years. The largest number of animals, namely 46.6%died between the second and fifth month of their life. These were mostly

 Age in months
 2-6
 6-12
 13-18
 19-24
 25-31

 Mortality in per cent
 46.6
 32.1
 11.2
 6.6
 3.5

 Table 2.

 Mortality of L. lagurus in the laboratory.

animals kept in stock cages which either were killed soon after being placed with other individuals or died and then were eaten by others. The cause of fighting is not entirely clear. All animals placed in a stock cage were of the same age but animals from different litters might have been quite different with respect to strength and endurance. The difficulty of strange individuals to adapt to living crowded in a relatively small cage might have been another cause of mortality especially at the beginning of our experience. Besides, the fights might have been provoked by especially agressive animals which were later almost completely eliminated from our colony. Persistent fights and eating of dead animals were also observed by Schewtschenko (1962).

In the Białowieża animal colony L. lagurus were usually healthy. The only sickness which occured sporadically was the so called "Lagurus disease" (Cooper, 1964). Sick animals had paresis of the hind limbs, were getting progressively weak and skinny and eventually died. This disease was observed in animals of different age.

# IV. CONCLUSION

The above characteristics, considerable disease resistance and docility of presently bred generations fully qualify *L. lagurus* as a new laboratory animal. Actually this species has already been successfully used for cancer research (Olshevskaja, 1957; Poghossjanc *et al.*, 1959). The reduction of both the number of litters and the litter size in the laboratory is a rather serious difficulty. This can be prevented by feeding a more complete diet, employing stronger selection and higher turnover rate of generations. Only females which reproduce well should be used and they should be discarded when 6–7 months old. The relatively high mortality of young can be reduced by weaning right after the end of lactation.

Further work in the Białowieża animal colony is directed toward obtaining inbred lines. This project is already considerably advanced. Recently (1966) the degree of inbreeding was tested at the Immunological Laboratory, Department of Histology and Embryology, Medical Academy, Kraków. In the siblings skin grafts from the dorsum were rejected only after 32 days. This indicates a high degree of inbreeding in our laboratory population of *L. lagurus*.

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# ROZRÓD LAGURUS LAGURUS (PALLAS, 1773) W WARUNKACH LABORATORYJNYCH

#### Streszczenie

Od 1961 r. prowadzona jest w Zakładzie Badania Ssaków PAN w Białowieży, hodowla *L. lagurus*, której celem jest przystosowanie tego gatunku do warunków laboratoryjnych.

Stwierdzono, że samice dojrzewają płciowo około 30 dnia życia (w nielicznych wypadkach wcześniej). Samce natomiast dochodzą do dojrzałości płciowej około 45—46 dnia życia.

Długość ciąży, obliczona na podstawie odstępów między kolejnymi miotami, trwa najczęściej 19–23 dni (75,7%), przy rozpiętości 18–25 dni (Ryc. 1); natomiast według danych z rozmazów pochwowych 20–21 dni. Stosunkowo dużo samic krytych jest w rui poporodowej (35,8%), natomiast 27,4% miotów zostało urodzonych przez samice kryte w okresie 6–37 dni po ostatnim wykocie. Odstępy między kolejnymi miotami *L. lagurus* nie były uzależnione od wielkości poprzedniego miotu, co zdarza się u innych gatunków gryzoni. Duży procent samic (36,5%) miał przerwy w rozrodzie trwające do 4–5 miesięcy. Występowały one u samic, które łączone były z samcem w wieku 1 roku i starszym; kilkakrotnie (do 2 razy pod rząd) miały mioty co 21 dni; mimo wczesnego połączenia przystępowały do rozrodu późno (np. przy chowie wsobnym); mimo kilkakrotnej zmiany samca, miały tylko 1 lub 2 mioty w życiu.

Na jedną samicę w ciągu życia wypadało średnio 4,04 miota, a ilości skrajne wynosiły od 1 do 13 (Ryc. 2). *L. lagurus* rozmnaża się w hodowli przez cały rok (Ryc. 3). Średnia ilość młodych w miocie wynosi 3,11 sztuk, przy czym ilości młodych w poszczególnych miotach wahają się od 1 do 8 sztuk (Ryc. 4).

Tak ilość jak i wielkość miotu związana jest ściśle z wiekiem samicy. W grupie wiekowej 2–6 miesięcy rozmnażało się  $92,4^{0/0}$  samic, natomiast wśród starszych w wieku 19–31 miesięcy, tylko  $28,1^{0/0}$  (Tabela 1). Średnia ilość młodych uzyskanych od samicy również znacznie maleje z wiekiem matki z 8,27 w grupie najmłodszej, do 4,62 w grupie samic dwuletnich.

Z 1393 młodych urodzonych w białowieskiej hodowli, 3,08% było martwo urodzonych lub padłych w ciągu kilku godzin po urodzeniu. W ciągu najbliższego tygodnia po urodzeniu padło 10,9% zwierząt. Śmiertelność ta nie jest związana z jakąś określoną porą roku (Ryc. 5). Do momentu odjęcia od matki (co następowało w różnym wieku młodych) padły jeszcze 323 osobniki. W ciągu 2 tygodni po odłączeniu padło jeszcze 3,7%. Tak więc z początkowej ilości przeżyło i zostało użyte do dalszych doświadczeń 59,4% młodych.

Stosunek plci w miotach, które w całości dożyły do wieku 3 tygodni wynosi 1:1. Natomiast w miotach z których tylko część młodych osiągnęła ten wiek, zarówno ilość samców jak i samic, różni się istotnie od teoretycznego stosunku 1:1. Samców jest o połowę mniej niż wynosi wartość teoretyczna, a samic mniej o <sup>1</sup>/<sub>4</sub>, co wskazuje na fakt, że wśród młodych większa jest śmiertelność samców.

*L. lagurus* w hodowli dożywał do wieku 31 miesięcy, przy czym w jednakowym stopniu osiągały ten wiek samice i samce (Tabela 2). Jednakże tylko 3,6% początkowej ilości zwierząt przeżyło ponad 2 lata.

Osobniki tego gatunku w hodowli białowieskiej na ogół mało chorowały. Jedyną chorobą, kończącą się zresztą śmiercią zwierzęcia, była tzw. "choroba lagurusów". Objawiała się ona porażeniem tylnych kończyn oraz postępującym osłabieniem i wychudzeniem zwierząt; notowana była u osobników w różnym wieku.