Fertility of the post-partum bank vole (*Clethrionomys glareolus*)

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Summary. Analysis of records of a bank vole breeding colony suggests that fertility is high immediately post partum, declines during established lactation and rises after weaning of young. Mating tests with lactating females and females whose young had been removed at birth showed that receptivity is reduced during lactation, although amongst the females which did mate there was no difference between lactating and non-lactating animals in the proportion which produced litters. However, average size of litters at birth was significantly larger for the lactating than for the non-lactating females. There is some evidence suggesting that this difference may arise after ovulation has occurred. Virgin females were no more receptive or fertile than lactating females.

Introduction

Microtine rodents, of which the bank vole (*Clethrionomys glareolus*) and the short-tailed field vole (*Microtus agrestis*) are examples, exhibit regular alterations in population size (Krebs & Meyers, 1974). Explanations of such population cycles will need to take into account the reproductive biology of a species. Experimental studies have established for field voles and bank voles some of the basic reproductive characteristics which bear upon population growth: breeding seasons appear principally to be regulated by photoperiod (Clarke, 1981; Clarke et al., 1981); ovulation is induced by mating (Breed, 1967; Clarke, Clulow & Greig, 1970); and pregnancy can be blocked by a 'strange' male (Clulow & Clarke, 1968; Clarke & Clulow, 1973; Milligan, 1976a, b). Population growth will also be influenced by post-partum fertility. Breed (1969) found with the field vole that 81% of females mated 2–13 days post partum, and of these 59%, produced litters. Furthermore, field voles were uniformly fertile throughout lactation. We have investigated the fertility of the lactating and post-partum non-lactating bank vole under laboratory conditions to find out whether in this regard too it resembles the field vole.

Materials and Methods

Animals were derived from the breeding colony founded in 1964 from wild-type bank voles trapped in Wytham Biological Reserve, Oxfordshire. The colony is kept on a daily lighting regimen of 16 h light at 18°C. Animals are fed on whole oats and hay, supplemented twice weekly with fresh carrot and rat and mouse pelleted diet. Water is constantly available. Cotton wool and sawdust are

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provided as bedding. Each cage in the breeding colony contains a sexually mature pair kept permanently together. Litters are removed from the parental cage at the age of 18 days.

**Analysis of colony records.** The colony records for each breeding female include the birth date and number of young born for each litter. The spacing of litters and the relationship of this to their size have been examined to gain a general expression of post-partum fertility.

**Experimental study of post-partum sexual receptivity and fertility in lactating and non-lactating females.** This involved 227 sexually mature virgin females, born in the colony, whose initial age was 91·4 ± 2·4 days when they weighed 15·9 ± 0·4 g. Each female was placed in its own cage with a fertile male from the breeding colony. When such females showed a weight increase of 3 or 4 g, signifying that they were probably pregnant, stud males were removed and the cages were checked morning and evening for the birth of first litters. Once this had occurred fertile males were again placed in the cages of the females at 09:00 h on one of the first 20 days post partum. Vaginal smears were taken just before these post-partum pairings, stained with toluidine blue and examined microscopically. Females were inspected every 2–3 h between 09:00 and 18:00 h for vaginal plugs, and again on the following day at 09:00 h when males were removed. Starting 15 days after such 24-h mating tests, females were examined morning and evening for the birth of litters. In this way it was possible to establish the days post partum during which females were receptive and fertile. Some females were again tested for receptivity and fertility in the post-partum period following the birth of the second, and several subsequent litters. The 227 females comprised 103 lactating animals on which 163 tests of receptivity and fertility were carried out, and 124 post-partum non-lactating females, whose young had been removed immediately after birth and on which 146 tests of receptivity and fertility were made. There were no differences in the results of tests for receptivity and fertility limited to the first post-partum period, and those carried out after several subsequent litters.

**Fertility of virgin bank voles, and length of first pregnancy.** An estimate of the length of first pregnancy was necessary to interpret an aspect of colony records. It also seemed desirable to assess the receptivity and fertility of virgin bank voles living with fertile males for the same period as the post-partum females. Accordingly each of 100 sexually mature virgin females, aged 104·8 ± 3·8 days and weighing 17·0 ± 0·3 g, was individually caged for 24 h with a fertile male from the breeding colony. Vaginal smears taken just before pairing were examined as described above. Females were checked for vaginal plugs and for litters in the same way as the post-partum females. The day on which a vaginal plug was found was taken as Day 0 of pregnancy.

**Results**

**Analysis of colony records**

Frequencies of intervals between births of 1491 litters are given in Text-fig. 1. The distribution of litter intervals appears to be bimodal, with a major peak of births on Day 21 and minor peak on Day 38. Out of a total of 1491 litters, 1164 (78·1%) were born at intervals of 17–24 days, and 112 (7·5%) at 37–42 days. The mean length of 74 first pregnancies recorded in various parts of this study was 19·5 ± 0·1 days. Andersson & Gustafsson (1979), studying bank voles from a Swedish laboratory colony, showed that there is a delay in implantation of at least 14 days in mated lactating females suckling 3 or more young. It seems possible that the spread of litter intervals from 17 to 24 days may be partly due to delay in implantation caused by suckling. This is supported by data from 982 litters which had been born at intervals of 17–24 days and which were reared to weaning. Intervals (in days) between litters in relation to the number of young suckled were 20·1 ± 0·1 for 1 or 2 young, 20·8 ± 0·1 for 3 young, 21·1 ± 0·1 for 4 young, and 21·3 ± 0·1 for 5, 6 or 7 young. The change in distribution of litter intervals with increase in number of young suckled was statistically significant ($\chi^2 = 83·7$ $P < 0·001$).
Text-fig. 1. Frequency distribution of litter intervals in the breeding colony of bank voles (Clethrionomys glareolus). Data taken from records of 1491 litters.

Table 1. Sexual receptivity and fertility in parous and virgin bank voles (Clethrionomys glareolus) paired with fertile males for 24 h

<table>
<thead>
<tr>
<th>Days post partum</th>
<th>No. of females</th>
<th>Total no.</th>
<th>Total no. of virgin females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>N-L</td>
<td>L</td>
</tr>
<tr>
<td>Tested</td>
<td>14</td>
<td>7</td>
<td>38</td>
</tr>
<tr>
<td>With vaginal plugs</td>
<td>10</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>Producing litters</td>
<td>7</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Lactating (L) and non-lactating (N-L) females were tested during Days 1–20 post partum; Day 1 was omitted for non-lactating animals because a marked decline in receptivity amongst lactating females did not occur until Day 3 post partum.

Post-partum sexual receptivity and fertility

It is clear from Table 1 that receptivity and fertility of lactating females were high in the first 2 days post partum, but thereafter declined considerably until Days 15–17, when a rise occurred to values resembling those on Days 1–2, and which was maintained until Days 18–20. Overall, lactating animals were receptive in 40.1% of tests and 28.2% of the total and 69.7% of those which mated produced litters. A high proportion of non-lactating females mated and were fertile from Day 2 to Days 18–20 post partum (Table 1): 67.1% were receptive, and 42.5% of the total and 63.3% of those which mated were fertile. Comparing all lactating with all post-partum non-lactating animals, \( \chi^2 \) for the difference in proportion mating was 21.9308 (\( P < 0.001 \)), and for differences in proportion fertile 6.8727 (\( P = 0.01–0.001 \)). However, there was no significant difference in fertility between the lactating and post-partum non-lactating females known to have mated. Receptivity (50%) and overall fertility (28%) of the virgin females were significantly less than those of the combined post-partum non-lactating females (\( \chi^2 = 7.3149, P = 0.01–0.001; \chi^2 = 5.3710, P = 0.05–0.02 \)), but there was no significant difference in fertility between the females known to have...
mated in these two groups. Receptivity and fertility of virgin and lactating females did not differ significantly.

The average size of litters arising from mating of lactating females was $4.2 \pm 0.2$ ($n = 43$), whereas those of the non-lactating post-partum animals was $3.0 \pm 0.1$ ($n = 62$) ($P < 0.001$). There was no relationship between this difference and the parity of the females in the two groups, or between the day post partum on which a female was tested and the size of the subsequent litter.

The occurrence of mating in lactating, post-partum non-lactating and virgin bank voles could not be related to the characteristics of the vaginal smear. Mating occurred after smears comprising only cornified cells, purely nucleated epithelial cells, or mixtures of these two types plus leucocytes. During the first 3 days post partum there were very few cells in the smear. By Day 5 leucocytes were common and there were a few cornified cells. The number of leucocytes declined after Day 7, whereas cornified cells became more abundant.

**Discussion**

The frequency distribution of litter intervals for the breeding colony of bank voles, showing a major peak at 17–24 days (mode 21) joined to a minor one at 36–42 days, can be interpreted in the following way: the majority of females in the permanently mated pairs conceive immediately after parturition, but a small proportion are not fertilized at this time and become pregnant during lactation, fertility apparently rising after the weaning of litters about 16 days post partum. Gustafsson, Andersson & Westlin (1980) found essentially the same distribution of litter intervals for a laboratory bank vole colony in Sweden, and similar observations and interpretation have been made on the prairie vole, *Microtus ochrogaster* (Richmond & Conaway, 1969). Delayed implantation of blastocysts during lactation is known to occur in wild and laboratory bank voles and in the Skomer vole (*Clethrionomys glareolus skomerensis*) (Brambell & Rowlands, 1936; Coutts & Rowlands, 1969; Andersson & Gustafsson, 1979), and may have contributed to the spread within the major peak of the frequency distribution of litter intervals seen in the present study.

We have demonstrated experimentally that lactation reduces fertility through a decrease in the sexual receptivity of females. Mating occurred in only 40.1% of the tests upon lactating females, contrasting with 67.1% in non-lactating post-partum animals. However, receptivity, and with it fertility, changes during lactation, both being high immediately post partum, then declining considerably and rising again at about the time of weaning. Such a decline in fertility associated with suckling of young and the release of prolactin is well known in sheep, cows, rabbits, rats, the white-toothed shrew, the Mongolian gerbil and women (Hunter & Lishman, 1967; Beyer & Rivaud, 1969; Harned & Casida, 1969; Land, 1971; Short, Bellows, Moody & Howland, 1972; England, Hauser & Casida, 1973; Fletcher, 1973; Foxcroft & Hasnain, 1973; Shevah, Black, Carr & Land, 1974; Hellwing, 1975; Flint & Ensor, 1980; McNeilly, 1980; Norris & Adams, 1981). In spontaneously ovulating animals there is a suppression of oestrous or menstrual cycles during lactation. By contrast, receptivity and fertility are uniformly high throughout lactation in the field vole (Breed, 1969). Similarly in the rabbit receptivity is high throughout lactation, but the occurrence of ovulation in Dutch Belted rabbits is low early in lactation and then rises. On the other hand Large Albino rabbits show no change during lactation in the proportion of animals ovulating. However, fewer suckling than post-partum non-suckling does of this strain ovulate after mating (Foxcroft & Hasnain, 1973).

The proportion of receptive lactating bank voles producing litters (69-7%) was not significantly different from the figure for receptive non-lactating females (63-3%), but the average size of litters conceived by lactating females was appreciably greater than that of the post-partum non-lactating animals. Lactating females injected with 200 ng LH-RH form about the same number of corpora lutea per female ($4.5 \pm 0.5, N = 4$) as post-partum non-lactating animals ($4.0 \pm 0.0, N = 3$) (J. R. Clarke, unpublished observations). Therefore the difference in size of litters produced by lactating and post-partum non-lactating females may be through effects upon fertility after ovulation has
occurred. On the other hand, amongst those post-partum Large Albino rabbits which do ovulate, suckling females have a significantly higher ovulation rate than do non-suckling animals (Foxcroft & Hasnain, 1973).

The greater sexual receptivity of post-partum non-lactating bank voles, translated to natural populations, would serve as some compensation for loss of sucking litters, but it is difficult to see the advantage of parous non-lactating bank voles producing smaller litters than lactating animals. The low receptivity and fertility of virgin bank voles recorded in the present study has been noticed in previous investigations. It appears that for a high proportion of such females it is necessary for stud males to be present for more than 24 h to evoke adequate sexual behaviour or to bring about some priming of the reproductive tract (Clarke & Clulow, 1973; Westlin, 1982; Berger & Negus, 1982). Such laboratory findings presumably reflect aspects of the social organization within natural populations of bank voles.

We thank Judith Baker and Valerie Petts for expert assistance.

References


Received 20 December 1982