Delayed implantation in lactating bank voles, *Clethrionomys glareolus*

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Summary. The time of blastocyst implantation in lactating and non-lactating female bank voles was studied. In primiparous females implantation started 4 days and 9–11 h after mating. A delayed implantation of at least $1\frac{1}{2}$ days was found in lactating, multiparous females when compared to non-lactating, multiparous and primiparous females.

Introduction

The effect of lactation on the gestation period has been investigated in only a few microtine species. In most of these no effect has been found (Hasler, 1975). Manning (1954) showed an extension of the interval between births in lactating collared lemming females (*Dicrostonyx groenlandicus*) and this was confirmed by Hasler & Banks (1975). No effect of lactation on gestation has been found in the lemmings *Lemmus lemmus* (Koponen, 1970) or *L. trimucronatus* (Mullen, 1968). Breed (1969) noted a small increase in the gestation period of lactating field voles (*Microtus agrestis*). In other species of the genus *Microtus* no effect of lactation on gestation has been found (Cowan & Arsenault, 1954; Pinter & Negus, 1965; Richmond & Conaway, 1969; Kirkpatrick & Valentine, 1970; Lee, Horvath, Metcalfe & Inskeep, 1970).

From field data on the bank vole (*Clethrionomys glareolus*) Brambell & Rowlands (1936) reported a lactational delay of implantation which was later supported by Coutts & Rowlands (1969). Buchalczyk (1970) also found a prolonged interval between births in lactating female bank voles. Von Wrangel (1940) reported that one female bank vole, mated *post partum* and lactating, had a prolonged gestation period.

It has been generally assumed that the effect of lactation in lengthening the next gestation period is due to delayed implantation but this has not been shown to occur for any microtine species (Hasler, 1975). The present paper reports studies of the effect of lactation on the time of implantation in bank voles.

Materials and Methods

A total of 99 virgin female bank voles was used. They originated from the laboratory colony maintained at the Department of Zoology, University of Lund. At the beginning of the experiment the animals were between 57 and 390, most of them 60–120, days old. The females were individually housed in wire-topped plastic cages (40 × 20 × 15 cm).

To establish the exact time of mating the following procedure was used. Once a day a fertile male was introduced into the cage. The animals were left together and checked every hour until a copulatory plug was found or mating was observed. The male was left with the female for 3–5 h after mating was confirmed. If no mating occurred, the male was removed and introduced again the following day.

Experiment 1. This experiment was designed to determine the time of implantation in primiparous females. The 52 mated females used (24 excluded, see below) were killed between 4

days 6 h and 4 days 12 h after mating. Uteri were weighed and fixed in a mixture of 96% ethyl alcohol, 40% neutral formalin and glacial acetic acid (15:4:1 by vol.). After embedding in paraffin wax, 7 µm sections were serially cut and stained with Ehrlich's haematoxylin and eosin or according to the KOLW method (see Borg, Andersson & Meurling, 1978) and examined for blastocysts.

Implantation was considered to have started when the uterine epithelium adjacent to a blastocyst appeared to be dissociating. This criterion was chosen because it was readily seen in histological preparations and, in our opinion, provided a safe method for a uniform judgement.

Experiment 2. This experiment was designed to determine whether implantation was delayed during lactation and to compare the time of implantation in primiparous females with that in lactating and non-lactating females pregnant *post partum*. Three groups of females were used. (1) Primiparous females (21 animals, 11 excluded, see below). (2) Lactating, multiparous females, mated *post partum* (14 animals, 2 excluded, see below). (3) Non-lactating, multiparous females, mated *post partum*. The young were removed from the female within 24 h after parturition (12 animals). Parous females had delivered 1–3 litters and lactating females were suckling at least 3 young. Females in Groups 2 and 3 were mated by introducing a male as soon as the young were found and the time of mating was determined in the same way as described for Exp. 1. Females in all groups were killed 6 days after mating and the uteri were examined histologically.

The excluded females were all non-pregnant as judged from the small size of the corpora lutea and/or the absence of blastocysts or embryos. Only a few uteri from females judged non-pregnant from the appearance of the ovaries were sectioned.

Results

Experiment 1

Implantation in primiparous females was found to have started by 4 days 9–11 h after mating (Table 1; Pl. 1, Figs 1–3), but there was some variation between individual females: in 1 female implantation had already started at 4 days 9 h while in 3 others implantation had still not started by 4 days 11 h.

PLATE 1

Transverse sections of uteri to illustrate different states of implantation and embryonic development in lactating and non-lactating bank voles. Figs 1–4 are at the same magnification: the scale line in Fig. 1 is 50 µm; Figs 5–6 are at a lower magnification: the scale line in Fig. 5 is 250 µm. All sections are stained with haematoxylin and eosin.

Fig. 1. Primiparous female 4 days 7 h after mating. Implantation has not started. The uterine epithelium is still intact.

Fig. 2. Primiparous female 4 days 10 h after mating. Implantation has started. The uterine epithelium has become dissociated and the blastocyst is in close contact with the endometrium.

Fig. 3. Primiparous female 4 days 12 h after mating. The blastocyst has penetrated deeper into the endometrium.

Fig. 4. Multiparous and lactating female 6 days after post-partum mating. Implantation has not started (see Fig. 5).

Fig. 5. Multiparous and non-lactating female 6 days after post-partum mating. The embryo has reached a developmental stage similar to that seen in primiparous females 6 days after mating (see Fig. 6).

Fig. 6. Primiparous female 6 days after mating, showing a well developed implanted embryo.

PLATE 1

(Facing p. 350)

Table 1. Occurrence of implantation in primiparous female bank voles at various times after mating

	Time after mating, 4 days and:						
	6 h	7 h	8 h	9 h	10 h	11 h	12 h
Implantation started	0	0	0	1	5	4	4
Implantation not started	3	3	3	2	0	3	0

Experiment 2

All of the primiparous females in Group 1 ($N = 10$) had implanted by 6 days after mating, as had all the lactating ones in Group 3 ($N = 12$), but none of the lactating females in Group 2 ($N = 12$) had done so (Pl. 1, Figs 4–6). The embryos in the females in Group 3 had reached a developmental stage similar to that seen in Group 1 females (Pl. 1, Figs 5 and 6).

Discussion

The time of implantation in primiparous bank voles found in this study, 4 days 9–11 h after mating, appears to be in conflict with that determined by Ozdzenski & Mystkowska (1976b) who stated that implantation took place in the afternoon of the 5th day after mating, but they did not give any criterion for the start of implantation. Furthermore, they only checked their females for evidence of mating once a day (09:00–11:00 h) and therefore there was a potential error in the time of mating of up to 24 h. In the present study the females were checked every hour or the mating was actually observed. If the matings in the study of Ozdzenski & Mystkowska (1976b) took place during the night before a copulatory plug was found, the actual time of implantation in their study was in very good agreement with that observed by us.

In Exp. 2 the females of Group 2 (lactating, multiparous) and Group 3 (non-lactating, multiparous) were killed 6 days after mating, i.e. about $1\frac{1}{2}$ days after the time of implantation in primiparous females (Exp. 1), and there was a clear difference in the occurrence of implantation in the two groups. Since the developmental stage of the embryos in Group 3 was comparable to that seen in Group 1, parity did not appear to be a factor influencing the preimplantation period. The delay of at least $1\frac{1}{2}$ days in Group 2 females must therefore have been related to the lactational state of those females.

Coutts & Rowlands (1969) reported an average pregnancy extension of 3 days in lactating bank voles. Trying to show a delay of implantation of 2 or 3 days instead of $1\frac{1}{2}$ could have given questionable results, because small but implanted embryos in a lactating female could have resulted from normal implantation but repressed post-implantation development, instead of delayed implantation. We therefore decided only to demonstrate the existence of delayed implantation in lactating females, rather than to determine its exact duration. Two casual observations of lactating females that had not implanted 7 days after mating show, however, that the delay of implantation may be longer than the $1\frac{1}{2}$ days shown here.

The possibility that a combination of delayed implantation and repressed post-implantation development caused the longer pregnancy during concurrent lactation could not be ruled out. The developmental rate of bank vole embryos during non-lactational conditions is known from the work of Ozdzenski & Mystkowska (1976a), but whether this rate changes in lactating females has still not been investigated.

The need to exclude 37 females from this study because they were not pregnant at the time of autopsy was of interest because most of them were primiparous females. The reason for this low fertility (35 out of 73 females becoming pregnant) of the first matings in bank voles is now being investigated.

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