Hormonal requirements and the influence of age on the decidual cell reaction in the bank vole (*Clethrionomys glareolus*)

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Summary. Laboratory bred bank voles of different age and reproductive status were bilaterally ovariectomized, treated with exogenous oestrogen or progesterone or both, and given an artificial decidual stimulus (crushing with artery forceps). An hormonal regimen of oestrogen (100 ng) followed by progesterone (1 mg) and small amounts of oestrogen (10 ng) was necessary for maximal development of the decidual cell reaction (DCR). A DCR did not develop if the animals were treated with only oestrogen or progesterone. Middle-aged virgin females 5–7 months old had larger responses than did young virgin females 2–3 months old, and parous females, 15–19 months old and having had at least 10 litters, had a response similar to that of virgin females of about the same age. The size of the DCR in old parous females was inversely related to the time elapsing since the last litter. It is concluded that in its hormonal requirements for a successful DCR the bank vole is similar to the mouse. The results also suggest that the increase in fertility of bank voles up to the age of about 100 days may in part be explained by increased response of the endometrium to blastocysts. The reduction in size of the DCR in old animals is a consequence of ageing rather than of repeated pregnancies.

Introduction

In bank voles from laboratory colonies, vaginal perforation occurs at the age of 30–40 days, but a high proportion of such young females fail to become pregnant at their first mating (Clarke & Clulow, 1973; Westlin & Gustafsson, 1984). It has been suggested that this is due to deficient output of progesterone by the ovaries, attributed to insufficient release of luteotrophin (Westlin, 1982). It has been supposed that either the threshold stimulus arising from mating and required to activate the luteotrophic complex is higher in young females than the satiety point of copulation for males, or that young females do not allow mating to satiety (Westlin & Gustafsson, 1984). However, it also seems likely that the responsiveness of the uterus to blastocysts may alter with age or mating experience, as a consequence of the action on it of ovarian hormones. One manifestation of such a uterine change might be the size of the decidual cell reaction (DCR). The DCR can be evoked artificially in pseudopregnant animals or hormone-treated animals by trauma or intraluminal injection of oil (Loeb, 1908; De Feo, 1967; Finn & Porter, 1975). We report here the results of experiments on the decidual cell reaction in virgin bank voles of various ages and in old parous animals.

Materials and Methods

The bank voles used were from the breeding colony in the Department of Agricultural Science, Oxford University. The husbandry was as described by Baker & Clarke (1986). All animals were kept in the same animal room in a

photoperiod of 16 h light per day (lights on at 08:00 h) at 18–20°C. Females produced by the breeding colony were weaned at the age of 18 days and kept in groups of 4–8 animals until experiments began. They were then housed singly in wire-mesh cages 28 × 18 × 10 cm, and were provided with whole oats *ad libitum*, supplemented with fresh carrots and Dixon's diet 41B. Water was constantly available. Bedding consisted of sawdust, non-absorbent cottonwool and hay.

There were 9 groups of females, all with a perforate vagina, which could be classed by age and reproductive status into 4 types: (1) young virgin animals 2–3 months old; (2) middle-aged virgin females 5–7 months old subdivided into 6 groups; (3) old virgin females aged 12–18 months; and (4) old parous females from the breeding colony, aged 15–19 months, which had had 10 or more litters, the last having been born 2–5 months previously.

Voies in 8 of the groups were bilaterally ovariectomized under Saffan (alphaxalone:alphadolone acetate) (Glaxovet Ltd, Uxbridge, U.K.) anaesthesia (approximately 0.1 ml/10 g body weight). Treatment of ovariectomized animals with hormones or oil began 2 days later. The hormonal doses and regimens for Groups 1, 2a, 3 and 4 had been found by Milligan (1974) to sensitize the uterus of field voles (*Microtus agrestis*) so that a well defined decidual cell reaction followed trauma of the uterus. Oestradiol-17 β and progesterone (Sigma Chemical Company, St Louis, MO, U.S.A.) were given subcutaneously under light ether anaesthesia according to the schedules and doses shown in Table 1. The hormones were dissolved in arachis oil and injected in a volume of 0.05 ml. Daily vaginal smears were taken, examined fresh and scored for the presence of nucleated epithelial cells, cornified epithelial cells and leucocytes. At 7 h after the last progesterone and oestrogen injections (Pe in Table 1) and at corresponding times in Groups 2b to 2e, animals were anaesthetized with Saffan, laparotomized and one uterine horn traumatized by giving 4 transverse crushes with fine artery forceps (see Gosden, 1979). One uterine horn of virgin, intact females (Group 2f) was also traumatized in a similar way.

Animals were killed 3 days after traumatization and the uterine horns were weighed separately. The difference in weight between the horns was taken as the size of the decidual cell response. Uteri were fixed in Bouin's fluid, embedded in paraffin wax, sectioned at 7 μ m, stained with Ehrlich's haematoxylin and eosin, and examined for the occurrence of decidual cells. Since there was no statistically significant correlation between the weight of the untraumatized horn and the size of the decidual response of the contralateral horn ($r = 0.313$ for 26 d.f.), the decidual cell reaction has been expressed as its absolute weight. The effect of the treatments on the size of the response has been examined by analysis of variance (Bailey, 1981).

Results

In all groups there was some enlargement of the traumatized uterine horn, although the size of this differed considerably between the treatments (Table 2). In 5 of the groups (1, 2a, 2b, 3 and 4) the traumatized horn at the sites of crushing had localized swellings which contained decidual cells. The swellings resembled those associated with implanted blastocysts. The much smaller swellings in the other four groups appear to have been inflammatory response to the crushing.

Analysis of variance showed a significant effect of treatments on the size of the DCR. As shown by the mean weights of the DCR, at least two variables affected it. One was the hormonal regimen:

Table 1. Treatment regimen of laboratory bred bank voles for testing the size of the decidual cell reaction

Group	Treatment† on Day:													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) Young virgin	ovx	—	E	E	E	—	—	—	Pe	Pe	Pe*	P	P	K
(2a) Middle-aged virgin	ovx	—	E	E	E	—	—	—	Pe	Pe	Pe*	P	P	K
(2b) Middle-aged virgin	ovx	—	E	E	E	—	—	—	P	P	P*	P	P	K
(2c) Middle-aged virgin	ovx	—	E	E	E	—	—	—	e	e	e*	oil	oil	K
(2d) Middle-aged virgin	ovx	—	—	—	—	—	—	—	P	P	P*	P	P	K
(2e) Middle-aged virgin	ovx	—	oil	oil	oil	—	—	—	oil	oil	oil*	oil	oil	K
(2f) Middle-aged virgin	Intact													
(3) Old virgin	ovx	—	E	E	E	—	—	—	Pe	Pe	Pe*	P	P	K
(4) Old parous	ovx	—	E	E	E	—	—	—	Pe	Pe	Pe*	P	P	K

†ovx = ovariectomy; E = 100 ng oestradiol; e = 10 ng oestradiol; P = 1 mg progesterone; oil = arachis oil;

*traumatization; K = killed.

Table 2. Weight of uterine horns and size of the decidual cell reaction (DCR) in laboratory bred bank voles treated† according to the regimens given in Table 1

Group	Weight (mg) of uterine horn		Wt of DCR* (mg)	No. with decidual cells
	Stimulated	Unstimulated		
(1) Young virgin (EPe)	27.5 ± 3.6	7.3 ± 0.6	20.2 ± 4.0	6/8
(2a) Middle-aged (EPe)	77.4 ± 16.7	12.4 ± 1.4	65.0 ± 16.6	8/8
(2b) Middle-aged (EP)	26.6 ± 8.2	9.4 ± 0.8	16.2 ± 7.4	4/5
(2c) Middle-aged (Ee)	10.2 ± 1.9	8.4 ± 2.1	1.8 ± 0.5	0/7
(2d) Middle-aged (P)	12.8 ± 1.5	6.5 ± 0.7	6.3 ± 0.8	0/5
(2e) Middle-aged (oil)	7.3 ± 2.0	5.1 ± 1.7	2.3 ± 0.6	0/4
(2f) Middle-aged (intact)	13.9 ± 3.0	10.5 ± 2.9	3.4 ± 0.7	0/8
(3) Old virgin (EPe)	44.8 ± 9.1	10.9 ± 2.0	33.9 ± 9.1	8/8
(4) Old parous (EPe)	54.8 ± 8.2	20.3 ± 2.8	34.5 ± 7.5	7/8

Values are mean ± s.e.m.

†E = 100 ng oestradiol; e = 10 ng oestradiol; P = 1 mg progesterone; oil = arachis oil.

*Difference between stimulated horn and unstimulated horn.

a sequence involving oestrogen followed by progesterone was necessary for an appreciable response, and this was boosted by the addition of very small amounts of oestrogen at the time of the progesterone treatment. A DCR did not develop if the animals were treated with only oestrogen, progesterone, or oil, nor did it occur in intact females untreated with oestrogen, progesterone or oil. However, the uterine stroma of animals treated only with progesterone was extremely oedematous.

The other factor was the age of the animal: middle-aged virgin females, 5–7 months old, had larger responses than did young virgin females 2–3 months old. Parous females, 15–19 months old and having had at least 10 litters, had a response similar to that of old virgin females. The size of the DCR in the old parous females decreased significantly with time elapsing since the birth of the last litter ($P < 0.01$). The regression of size of the DCR (y) on time in days since the last litter (x) is given by $y = 86.59 - 0.51(\pm 0.11)x$. There was no significant regression of the size of the reaction on the age of these old females.

After ovariectomy, vaginal smears contained abundant leucocytes exclusively. By the 3rd day of oestrogen treatment, smears consisted of numerous cornified cells only. During treatment with progesterone and oestrogen, smears were again leucocytic, but by the time of traumatization there were very few cells of any sort. These types of vaginal smear occurring during hormone treatment are very similar to those found in untreated female field voles (*Microtus agrestis*) before mating and during early pregnancy (Breed, 1968).

Discussion

It is clear from these experimental results that the development of the DCR in the bank vole in response to traumatization of the uterus is most satisfactorily brought about through the priming of the uterus with oestrogen and the subsequent action on it of progesterone in conjunction with very small amounts of oestrogen. Priming with oestrogen followed by treatment with progesterone alone, produced a weak response. For present purposes it was required to establish a simple regimen of hormone treatment which would reliably allow the DCR to develop. With the omission of oestrogen or progesterone or both, the DCR failed to develop, nor did it occur in intact virgin females untreated with exogenous hormones and which, of course, lacked corpora lutea (see Clarke *et al.*, 1970). The regimen which has proved satisfactory for the bank vole is one that had been

developed for the laboratory mouse (Finn & Martin, 1972) and successfully applied to the field vole (Milligan, 1974). The need for priming with oestrogen followed after 3 days by treatment with progesterone and very small amounts of oestrogen is an approximation to the likely endogenous endocrine changes which occur before and after mating. There is circumstantial evidence that the prior occurrence of such a sequence of hormonal stimuli greatly increases the fertility of bank voles: although the first pregnancy in sexually mature females is achieved with difficulty, the second and subsequent ones follow promptly and with great regularity (Clarke & Clulow, 1973). Previous sexual experience in the form of sterile mating also improves fertility (Westlin & Gustafsson, 1983). There is evidence from wild bank voles that such priming of the reproductive tract takes place in natural conditions (Brambell & Rowlands, 1936; Westlin & Nyholm, 1982).

The size of the response changed with age, being weaker in young and old, than in middle-aged virgin females. The DCR in old parous females was the same size as in old virgin females so that the decline in old animals is an effect of age rather than of use of the uterus (Finn, 1963). Artificial decidualizing stimuli have been employed in mice (Finn, 1966; Shapiro & Talbert, 1969; Holinka & Finch, 1977; Gosden, 1979) and hamsters (Blaha, 1967) to show age-related changes in uterine response. These studies also indicated a reduction in the ability to develop deciduomata with increasing age of the females. Since the DCR plays a crucial role in implantation in rodents it has been suggested that this decrease in responsiveness may be a significant factor in senile decline of reproductive capacity (Finn, 1970). There is also evidence from the present study that in old parous bank voles the size of the DCR decreases with time elapsing since the last litter. This may reflect a reduction in the concentration or availability of the steroid receptors necessary for expression of the full progestational response (Janne, 1981).

The increase in the size of the DCR from young to middle-aged bank voles correlates with the increase in fertility at first mating from 20–25% in 30–50-day-old bank voles to 80% in females more than 100 days old (Westlin & Gustafsson, 1984). The present results would suggest that one contribution to the rise in fertility might be an increased sensitivity of the endometrium to the presence of blastocysts. However, this does not rule out the possibility that there is a change with age in the luteotrophic response to copulation (Westlin, 1982; Westlin & Gustafsson, 1984), although this has not been investigated experimentally.

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