

# Gelatinase B deficiency impairs reproduction

Letter  
TO THE EDITOR

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Gelatinase B has been suggested to intervene at different stages of the cyclical changes in female reproduction (1–3): in the menstrual cycle, ovulation, implantation, parturition, and involution of the mammary glands after lactation. Gelatinase B has also been implicated in the process of growth and development of the embryo (4, 5). So far, the only reported spontaneous phenotype of gelatinase B deficiency is a delayed ossification of the growth plate in long bones (6). Most other phenotypes of gelatinase B are induced tissue reactions of the skin (7), the lung (8), the central nervous system or bone (9), and the aortic wall (10). Is there any spontaneous effect of gelatinase B ablation on reproductive capacity?

Gelatinase B-deficient mice were generated by a functional knockout of the active and zinc-binding domain of MMP-9 as previously described (9). In the three reports published to date (6, 9, 11), which described structurally distinct null alleles in the gene, gelatinase B-deficient mice were found to reproduce. However, in the course of studying the induction of autoimmune diseases in these animals, we noted a significantly diminished breeding efficiency of the gelatinase B-deficient mice as compared with wild-type controls.

During a test period of 3 months, the number of mice born per breeding pair is significantly lower in the gelatinase B-deficient than in the wild-type mice. In addition, the individual litters in knockout mice are smaller, and the percentage of infertile breeding pairs is elevated in the gelatinase B-deficient mice, whereas maximal litter numbers were observed in the wild-type mice (Table 1). The percentages of breeding pairs with 0, 1, 2, or 3 litters were

respectively 18, 5, 18, and 59 for the wild-type mice ( $n = 39$ ), versus 36, 20, 17, and 27 for the gelatinase B-deficient mice ( $n = 89$ ) ( $P = 0.007$ ,  $\chi^2$  with Yates's correction = 12.10,  $4 \times 2$  contingency table, 3 degrees of freedom). Control and knockout mice were bred on C57BL/6 background. We observed a quantitatively similar suppression of fertility in gelatinase B-deficient mating pairs whether we compared *MMP-9*<sup>-/-</sup> animals with their wild-type littermates or with wild-type breeding pairs that carried the pure C57BL/6 background genotype. Hence, the differences seen in fertility are not caused by genetic background effects but, indeed, by gelatinase B deficiency. Further studies are required to elucidate whether this spontaneous phenotype may be attributed to male or to female fertility.

Matings of heterozygous mice resulted in the expected mendelian frequency of *MMP-9*<sup>+/+</sup> (101/359, 28.1%), *MMP-9*<sup>-/-</sup> (81/359, 22.6%) and *MMP-9*<sup>+/-</sup> (177/359, 49.3%) mice (9), suggesting that embryonic and fetal development of homozygous mutant mice were not impaired, as also observed by Vu et al. (6).

Based on the combination of a reduced breeding efficiency of homozygous gelatinase B-deficient

mice and the occurrence of the expected mendelian frequency of *MMP-9*<sup>+/+</sup>, *MMP-9*<sup>-/-</sup>, and *MMP-9*<sup>+/-</sup> mice in heterozygous matings, we conclude that decreased fertility is a spontaneous phenotype of gelatinase B deficiency. This finding reinforces the role of gelatinase B in the establishment and maintenance of a normal pregnancy and suggests that influencing gelatinase B activity may be a target in birth control. In addition, decreased fertility is anticipated in the treatment with nonselective and selective MMP-inhibitory drugs that are currently used in clinical trials for inflammatory and neoplastic diseases.

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**Table 1**  
Gelatinase B deficiency impairs fertility in mice

	KO	WT	P
Mean number of newborn mice per breeding pair	8.18 ( $n = 89$ )	14.05 ( $n = 39$ )	< 0.001
Mean number of mice per litter	6.03 ( $n = 120$ )	7.64 ( $n = 72$ )	< 0.001
Percent of breeding pairs without litters	36 (32/89)	18 (7/39)	0.064
Percent of breeding pairs with three litters	27 (24/89)	59 (23/39)	0.0013

Pairs of gelatinase B-deficient (KO) or wild-type controls (WT) of C57BL/6 background were left together during an observation period of 3 months, and the mean number of newborns as well as the numbers of mice per litter were calculated. Statistical analysis was performed using the Wilcoxon and the  $\chi^2$  method with Yates's correction.

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