

論文の内容の要旨

Dissertation title: Establishment of mouse model for analysis of perinatal outcome in Endometriosis and Adenomyosis

(子宮内膜症および腺筋症における周産期転帰の分析のためのマウスモデルの確立)

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Objective

As for endometriosis, there are several studies suggest that endometriosis impairs fertility and causes adverse perinatal outcome. The association between endometriosis and infertility is well known while few studies suggest that endometriosis may cause adverse perinatal outcome. However, the mechanism is still unknown. Our study is aimed to establish endometriosis mouse pregnancy model to elucidate the effect on pregnancy outcome and investigate the underlying etiology.

As for adenomyosis, our study is aimed to establish a novel mouse model of adenomyosis that mimics the human setting and then investigate adenomyosis's effect on the pregnancy outcome.

Methods

As for endometriosis, it was induced by the intraperitoneal injection of homologous minced mouse uteri (ENDO). Vehicle was injected for the control (CONT). Mating of ENDO or CONT mice with fertile male was started 1 or 42 days after the endometriosis induction (Early or Late models, respectively). To evaluate fetal count and weight, mice were sacrificed on 18 dpc. To assess the labor onset, mice were kept until spontaneous

delivery.

As for adenomyosis, the uterine horns of mice were exposed, and the uterine wall was punctured using a 30G needle at a frequency of 100 punctures/1cm. Mice were sacrificed on day14 (D14) or day65 (D65) (n=3). The uterus was stained, lesions were detected and counted, and their volumes were measured. Cell proliferation and fibrosis were assessed by Ki67 and Masson's Trichrome staining, and blood vessels were detected by CD31 immunostaining. Four mice were mated and sacrificed on postpartum day3. Log-rank and Wilcoxon tests were used for statistical analyses.

Results

As for endometriosis, in the early model, ENDO took significantly longer time to conceive than CONT ($p < 0.05$). As for Early model, fetal weight and count were comparable between groups. As for Late model, fetal weight was significantly lower in ENDO (ENDO; 1002.7 ± 7.2 , CONT; 1026.5 ± 7.6 , mean \pm SD, mg/fetus), although fetal counts were equivalent between groups, but resorbed pups were more in ENDO (ENDO; 2.4 ± 0.2 , CONT; 1.7 ± 0.2 , mean \pm SEM, $p = 0.01$). The onset of labor occurs significantly earlier in ENDO than CONT (ENDO; 19.1 ± 0.9 , CONT; 19.8 ± 0.9 , dpc, $p < 0.05$).

As for adenomyosis, the number of lesions was not different between D14 and D65. The entire, glandular and stromal volume of the lesion was larger in D65 ($p < 0.0001$). The proportion of Ki67 positive cells in epithelia was higher in D14 (D14; $15.3 \pm 5.0\%$, D65; $2.6 \pm 1.3\%$, $p < 0.05$), while those in stroma was higher in D65 (D14; $0.5 \pm 0.4\%$, D65; $6.3 \pm 1.6\%$, $p < 0.01$). Blood-vessel density in lesions was higher in D65 ($p < 0.05$). The area of fibrosis in the stroma was higher in D65 ($p < 0.01$). The number and volume of lesions were equivalent between the non-pregnant and the pregnant group.

Conclusion

Our endometriosis pregnancy mouse model demonstrated endometriosis-related infertility, fetal growth restriction, and preterm labor, which may mimic human endometriosis. This model can be applied as a useful model to evaluate the mechanism by which endometriosis affects fertility and perinatal outcome. While the mouse model of adenomyosis established in this study showed similar progression to human lesions and continued after pregnancy. This model is established faster than previously published mice model with less complications. Also, this model is suitable for quantitative longitudinal analysis for lesion's progression. In addition, our model can be used for testing drugs and evaluation of hormonal effect on the lesion. As for pregnancy outcome analysis, this model can evaluate effect of lesion on pregnancy outcome and vice versa.