

博士論文（要約）

**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)

モハメド エルシェルビニ エラザジー エルシェルビニ エルシャル

Mohammed Elsherbini Elazazy Elsherbini Elshal

Objective

As for endometriosis, there have been many studies suggest that endometriosis impairs fertility and causes adverse perinatal outcome: however, the mechanism is still unknown.

My study was aimed to establish endometriosis mouse pregnancy model to elucidate the effect of endometriosis on pregnancy outcome and to investigate the underlying etiology.

As for adenomyosis, my study was aimed to establish a novel mouse model of adenomyosis that mimics the human setting in order to investigate the etiology of adenomyosis, and the association between adenomyosis lesion and pregnancy outcome.

Methods

The endometriosis in mouse was induced by the intraperitoneal injection of homologous minced mouse uteri (ENDO). The 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 study, respectively). To evaluate the number and the weight of the fetus, mice were sacrificed on 18 dpc. To assess the labor onset, mice were left until the spontaneous delivery.

As for adenomyosis mouse model, the uterine horns were exposed, and the uterine wall

was punctured using a 30G needle at a frequency of 100 punctures/1 cm. Mice were sacrificed on day14 (D14) or day65 (D65). The paraffin embedded uterine slices were stained, lesions were detected and counted, and their volumes were measured. The cell proliferation and fibrosis were assessed by Ki67 and Masson's Trichrome staining respectively, and blood vessels were detected by CD31 immunostaining. A part of mice were mated and sacrificed on postpartum day 3. Log-rank and Wilcoxon tests were used for statistical analyses.

Results

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

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; $6.3 \pm 1.6\%$, $p < 0.05$). The blood-vessel density in lesions was higher in D65 (D14; 0.06 ± 0.01 , D65; $0.1 \pm 0.01\%$, $p < 0.05$). The area of fibrosis in the stroma was higher in D65 (D14; $0.007 \pm 0.004\%$, D65; $5.1 \pm 1.01\%$, $p < 0.001$). As for the adenomyosis pregnant model, the number and volume of lesions were equivalent between the non-pregnant and

the pregnant group.

Conclusion

My endometriosis model demonstrated infertility, preterm labor, and fetal growth restriction, which is consistent with previously reported clinical studies of pregnant women with endometriosis. This endometriosis mouse model may therefore be suitable for studies to elucidate the mechanism by which endometriosis adversely affects perinatal outcome.

The currently study also established a novel adenomyosis mouse model that can be analyzed quantitatively in terms of their number and volume. In addition, it was proven that the model was able to conceive, and that the persist in the postpartum period. Therefore, this model can be applied to evaluate the pathogenesis of adenomyosis, to test the efficacy of its therapeutic agents, and the effect of the lesion on pregnancy and vice versa.

It is expected that these models will be used in the future to elucidate the effects of endometriosis and adenomyosis on adverse fertility and perinatal outcome and to establish the therapeutic approach to prevent and treat them.