

IMPLICATION OF IMMUNE CELLS IN THE PHYSIOPATHOLOGY OF IMPAIRED FERTILITY IN MICE WITH INDUCED ADENOMYOSIS

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CONTEXT

- Adenomyosis is an enigmatic pathology responsible of impaired fertility in Human, with a still unclear physiopathology.
- One hypothesis is that some changes in immune cells in adenomyosis affected uteri may alter fertility and the environment necessary for a successful implantation and pregnancy.

OBJECTIVE

- To study the involvement of immune cells in the impaired fertility in adenomyosis through a mouse model.

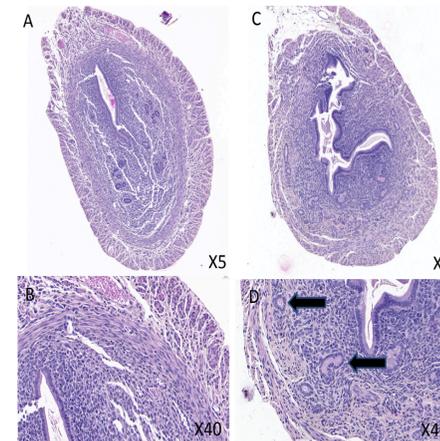
MATERIAL AND METHODS

- Adenomyosis was induced in 36 female CD-1 mice by neonatal oral administration of tamoxifen (TAM group), while 30 neonates received only the vehicle (control group). These mice were put into mating at 3 months.
- To evaluate fertility and pregnancy outcomes, ultrasounds were performed at E7.5 and E12.5 in order to count the number of gestational sacs and the resorption rate in eighteen mice of the TAM group and 15 mice of the control group. Mice were sacrificed at E18.5 and histological, morphometric and functional analysis were performed on the placentas.
- To identify local and/or systemic immune changes during the early implantation period, 18 mice of the TAM group and 15 mice of the control group were sacrificed at E4.5. Uterine horns and spleen were collected for flow cytometry and RT-qPCR analyses in order to analyse immune cells.

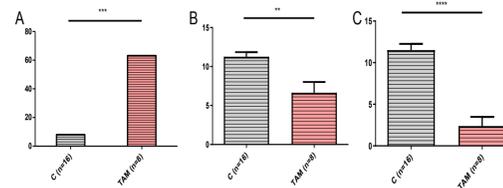
RESULTS

Light microscopy of uteri from the adenomyosis model group and control group stained with H&E

Cross sections of uterine horn in the Control group ((A) x5, (B) x40) show a well-demarcated core of the endometrium surrounded by regular myometrium with concentric layers of smooth muscle and connective tissues. Cross sections of uterine horn in the Tam Group ((C) x5, (D) x40) show smooth muscle of the myometrium penetrated widely by ectopic endometrial glands (Black arrows).



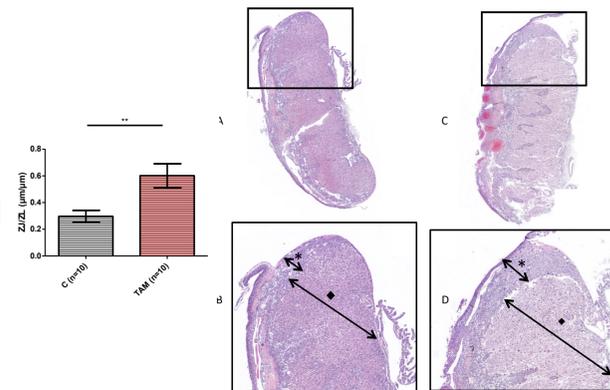
Analysis of implantation and resorption rate



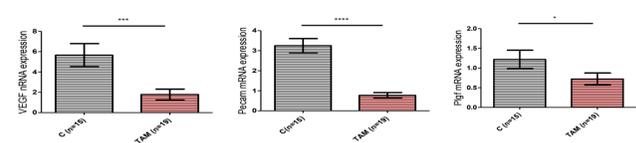
Resorption and implantation rate: «control» group (n=16) versus «TAM» group (n=8)
Number of resorption, (B) number of implantation sites at E7.5, (C) average number of fetuses per litter. Values are means ± SEM.

Analysis of the placenta

Light microscopy of placenta at E18.5 from the adenomyosis model group and control group stained with H&E, A higher junctional (ZJ*)/labyrinthine (ZL*) area ratio was observed in the TAM group (C,D) compared to the control group (A,B)



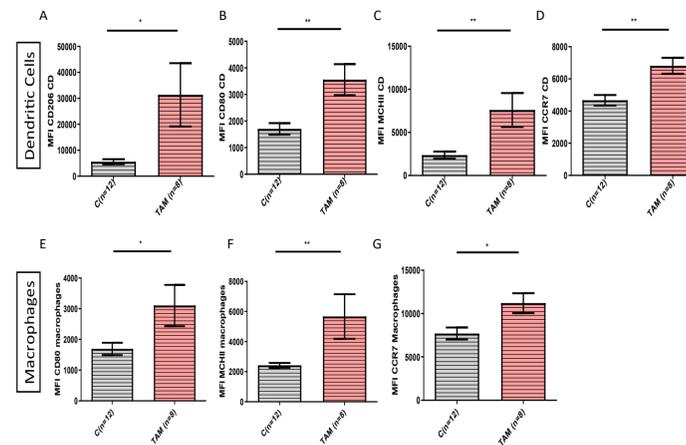
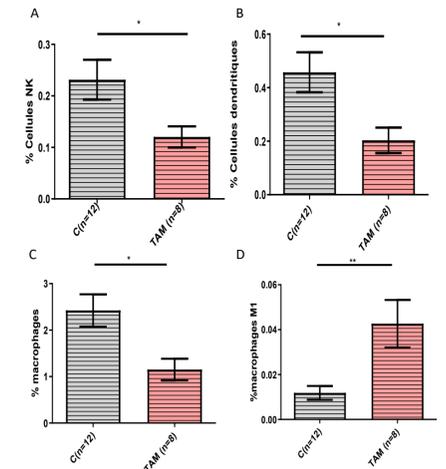
Vegf (A), Pecam (B), Plgf (C) (RT-qPCR gene expressions) in placenta at E 18.5 of Tam-induced adenomyosis and control uteri.



Analysis of the immune cells in uteri during early implantation

Flow cytometry analysis in uteri at E4.5 of TAM group (n=8) and control group (n=12)

(A) number of NK cells; (B) Number of dendritic cells; (C) Number of macrophages; (D) Number of macrophages M1.

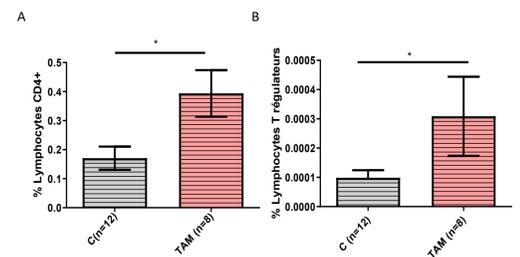


Flow cytometry analysis in uteri at E4.5 of TAM group (n=8) and control group (n=12)

(A) MFI CD206 CD; (B) MFI CD80 CD; (C) MFI MCHII CD; (D) MFI CCR7 CD; (E) MFI CD80 macrophages; (F) MFI MCHII macrophages; (G) MFI CCR7 macrophages.

Flow cytometry analysis in uteri at E4.5 of TAM group (n=8) and control group (n=12)

(A) Number of CD4+ lymphocytes; (B) Number of Regulatory T cells.



Statistical significance: p < 0.05*, p < 0.01**, p < 0.001***

KEY MESSAGES - CONCLUSION

We confirmed the presence of **fertility disorders** in mice with induced adenomyosis, notably :

- the increase in the number of resorption,
- the reduce number of implantation site,
- the presence of vascular placental abnormalities.

This could be link to **local and systemic immune changes** observed in the uteri and spleen during the **early period of implantation**.