Investigating Autoimmunity in the Etiology of Premature Ovarian Insufficiency in a Mouse Model

Nilay Kuscu*[1], Ruth Appeltant*[1,2], Sergej Petrovic[1], Suzannah A Williams[1]

**equal author contribution*

[1]Nuffield Department of Women's & Reproductive Health, University of Oxford, Oxford, United Kingdom
[2]Department of Veterinary Science, University of Antwerp, Antwerp, Belgium

Introduction

Premature ovarian insufficiency (POI), is a condition that impacts 1-3% of women under 40 years and can be caused by autoimmunity. When POI ovaries contain follicles, this is known as follicular POI and occurs in ~50% of POI cases. Double mutant (DM) mice exhibit follicular POI at 3 months that results from oocyte-specific deletion of *Mgat1* and *C1galt1* alleles (loxP *Cre* technology); these genes generate complex N-glycans and O-glycans respectively (1,2,3).

Objective

This study aimed to determine if the cause of POI in DM mice is autoimmunity.

Methods

Ovaries from DM mice and control siblings (lacking the ZP3:*Cre* transgene), were collected at the day they were born (D0) and transplanted beneath the kidney capsule of immune-deficient mice. After 3-months, the ovaries were collected, fixed, sectioned, H&E stained, and analyzed to assess follicle development. Foxl2 and AMH were detected using immunohistochemistry.

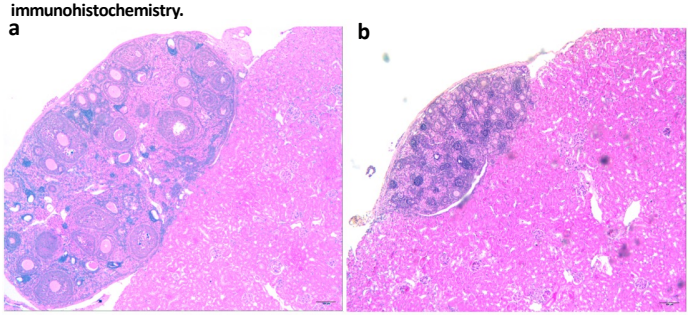

Figure 1. Representative images show ovarian morphology and follicle development in ovarian tissue graft from control (a) and DM mice (b). Magnification: 5X.

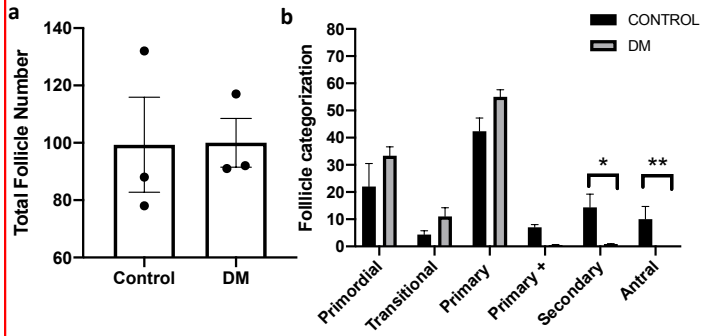

Figure 2. Comparison of follicle numbers and stage in transplanted ovaries from control and DM groups. a: Number of total follicles counted in each ovarian graft by analysis of every 10th section (n=3). **b:** Comparison of the number of follicles at each stage of development in the control and DM ovarian tissue graft. Results are expressed as mean ± SEM. *p < 0.001, **p < 0.0001.

Results

- Transplanted ovaries were successfully retrieved for both control and DM (Figure 1).
- Follicle development was observed in both groups, confirming the functional viability of the transplanted tissue. Follicle numbers did not differ between Control and DM, however, the stage of follicle development did differ (Figure 2a).
- Control ovaries contained primordial follicles as well as healthy primary, secondary and preantral follicles; as expected in a wildtype ovary that has been transplanted to an immunocompromised host. However, in the DM ovary transplants, follicles remained at the primary stage, with a diminished presence of later stages (Figure 2b).
- DM oocytes were also reduced in size compared to Control follicles at the same stage of follicle development (Figure 3).
- Control ovaries contained follicles that generated anti-mullerian hormone (AMH) whereas those in DM ovaries did not, revealing a lack of granulosa cell development.
- Follicles in both Control and DM transplanted ovaries were Foxl2 positive (Figure 4).

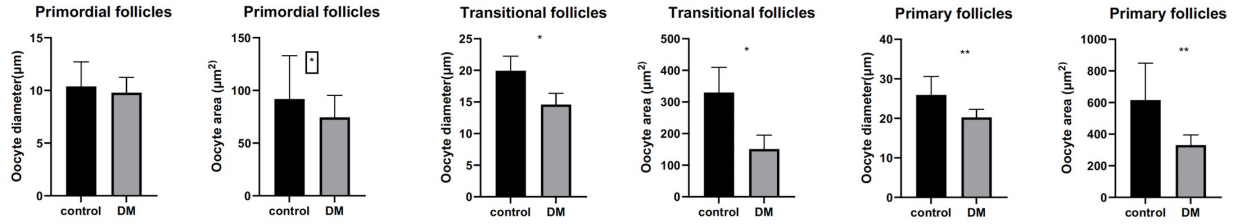

Figure 3. Comparison of oocyte size in primordial, translational and primary follicles between control and DM ovaries. Results are expressed as mean ± SEM. *p < 0.05, **p < 0.01.

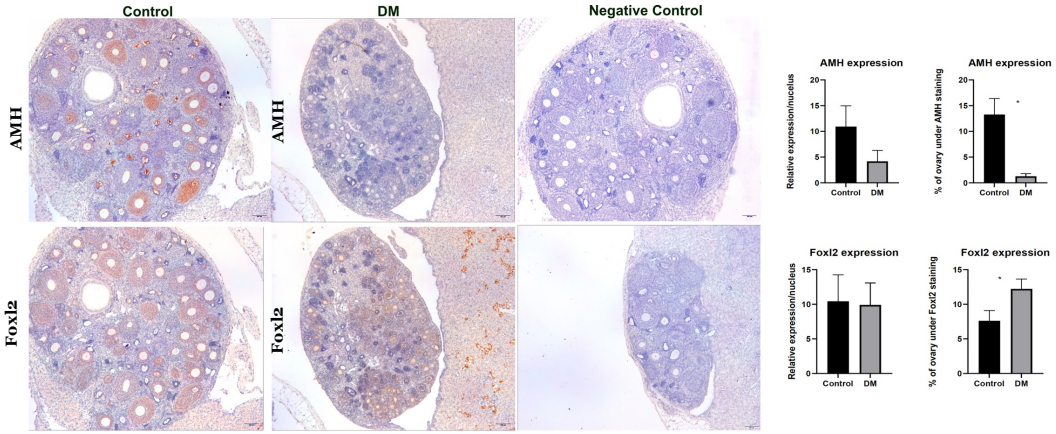

Figure 4. Detection of AMH and Foxl2 in control and DM ovaries. Representative images with AMH and Foxl2 labelling in control and DM ovaries. The intensity was used to assess relative expression of AMH and Foxl2 positive cells in follicles . Results are expressed as mean ± SEM. *p<0.05

Conclusion

These data reveal that autoimmunity is not the cause of POI in DM mice since follicle development did not occur in an immunocompromised environment. Further in-depth investigations into the complex interactions underlying POI are warranted in the DM mice and such investigations hold the potential to comprehensively unravel the condition's underlying causes and potentially identify therapeutic targets.

Funding

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References

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