Inflammation in Progesterone Receptor B Knockout (PRBKO) Mice Alters Aldo-Ketoreductase Family 1 Member C18 (Akr1c18) Expression in the Ovary

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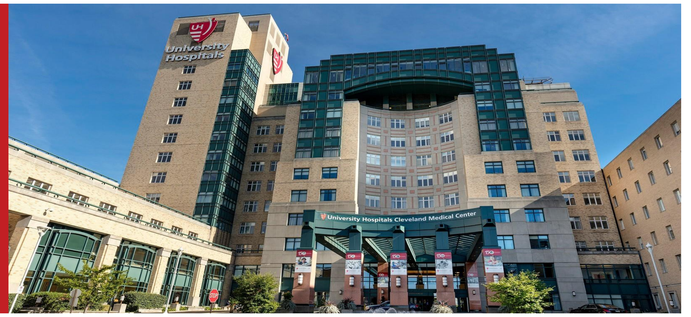


Background

- Progesterone signaling is essential for decidualization and successful embryo implantation.
- The Primary Decidual Zone (PDZ), Secondary Decidual Zone (SDZ), and Mesometrial Zone (MZ) have distinct roles in implantation, immune regulation, and vascular remodeling.
- Inflammation impairs decidualization and is associated with reduced progesterone signaling.
- Progesterone receptor B knockout (PRBKO) mice are subfertile, suggesting PR-B is critical for early pregnancy.
- Akr1c18, an enzyme involved in progesterone metabolism, may further regulate progesterone activity during implantation.[1]

Study Design

- *Tissues and Slides:*

Female CD-1 wild-type (WT) and progesterone receptor B knockout (PRBKO) mice were mated with proven-fertile B6D2F1/J males. On embryonic day (ED) 0.5, mice received an intraperitoneal injection of lipopolysaccharide (LPS; 5 µg in 100 µL PBS) or vehicle control (100 µL PBS). Mice were euthanized on ED 5.5 or ED 7.5. Uteri and ovaries were harvested, fixed, paraffin embedded, sectioned, and prepared for immunohistochemical analysis.

- *Image Analysis:*

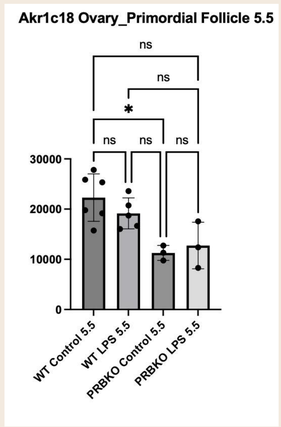
Slides were digitized and analyzed using QuPath v0.6.0. Akr1c18 expression was quantified using the Positive Cell Detection tool with a threshold of 0.15. Staining intensity was assessed using cytoplasmic and nuclear DAB optical density (OD). Total analyzed area, number of detections, positive and negative cell counts, percentage of positive cells, and positive cell density (cells/mm^2) were quantified within the uterine primary decidual zone (PDZ), secondary decidual zone (SDZ), and mesometrial zone (MZ), as well as ovarian primordial follicles, advanced follicles, and corpus luteum.

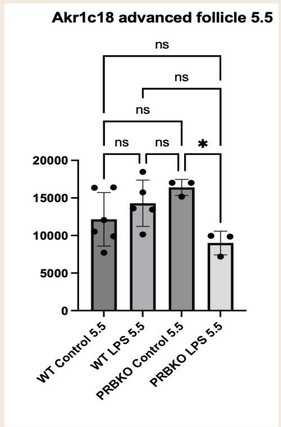
- *Statistical Analysis:*

Positive cell density (cells/mm^2) was compared among WT control, WT + LPS, PRBKO control, and PRBKO + LPS groups at ED 5.5 and ED 7.5 using one-way ANOVA or the Kruskal–Wallis test, as appropriate. Statistical significance was defined as $p < 0.05$.

Results

- In the WT groups, there were 6 mice per group at ED 5.5 and 5 control and 7 LPS mice at ED 7.5. In the PRBKO groups, there were 3 mice per group at both ED 5.5 and ED 7.5. No significant differences in uterine Akr1c18 expression were observed among WT control, WT LPS, PRBKO control, and PRBKO LPS groups in the PDZ, SDZ, or MZ at either time point.
- In the ovary, PRBKO primordial follicles at ED 5.5 expressed less Akr1c18 than WT (3.3 vs. 12.8, p = 0.04). LPS exposure decreased Akr1c18 expression in PRBKO advanced follicles at ED 5.5 (14 vs. 3, p = 0.045). Additionally, at ED 5.5, LPS-treated PRBKO corpus luteum expressed less Akr1c18 than LPS-treated WT mice (2 vs. 13, p = 0.017).
- No other ovarian comparisons were statistically significant.





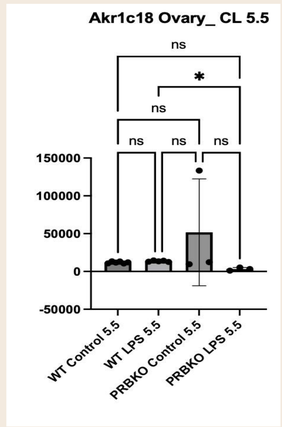


Figure 1. (A) Graph showing reduced Akr1c18 expression in primordial follicles of PRBKO vs. WT mice at ED 5.5. (B) Graph showing reduced Akr1c18 expression in advanced follicles of PRBKO vs. WT mice following LPS exposure. (C) Graph showing reduced Akr1c18 expression in corpus luteum of PRBKO vs. WT mice following LPS exposure. No other ovarian differences reached statistical significance. Data are presented as mean positive cells/mm^2 (or mean staining intensity, whichever metric you used). $p < 0.05$ indicates statistical significance. Error bars represent SD (or SEM, as appropriate).

[Figure 2. (A) Sample IHC of uterine tissue showing staining and cell count method, with left most box representing MZ, middle box representing PDZ, and right-most box representing SDZ. (B) Sample IHC showing ovarian tissue staining with advanced follicle surrounded by stroma and mix of primary and smaller advanced follicles.

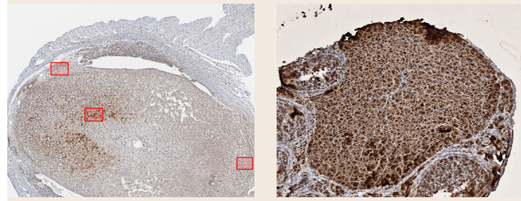


Figure 2A and 2B

Conclusions

- In PRBKO mice, Akr1c1 expression in the ovary was decreased in response to an inflammatory challenge with LPS compared to control mice. This demonstrates that progesterone metabolism in response to inflammation is altered in the absence of the PRB receptor.
- Our findings suggest that PRB is not only essential for progesterone responsiveness but may also play a role in progesterone regulation via expression of the progesterone-metabolizing Akr1c18, particularly in response to an inflammatory challenge.
- These findings, if confirmed by human studies, may help elucidate another mechanism for progesterone resistance in conditions where PRB expression is reduced, such as endometriosis.

Acknowledgements

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References

1. Hevir, N., Vouk, K., Sinkovec, J., Ribič-Pucelj, M., & Rižner, T. L. (2011). Aldo-keto reductases AKR1C1, AKR1C2 and AKR1C3 may enhance progesterone metabolism in ovarian endometriosis. Chemico-biological interactions, 191(1-3), 217-226.

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