

How does a mother's immune system tolerate a semi-foreign fetus?

- Role of Natural Killer cells in protecting the fetus, a semi-allograft with half of its genes paternally derived



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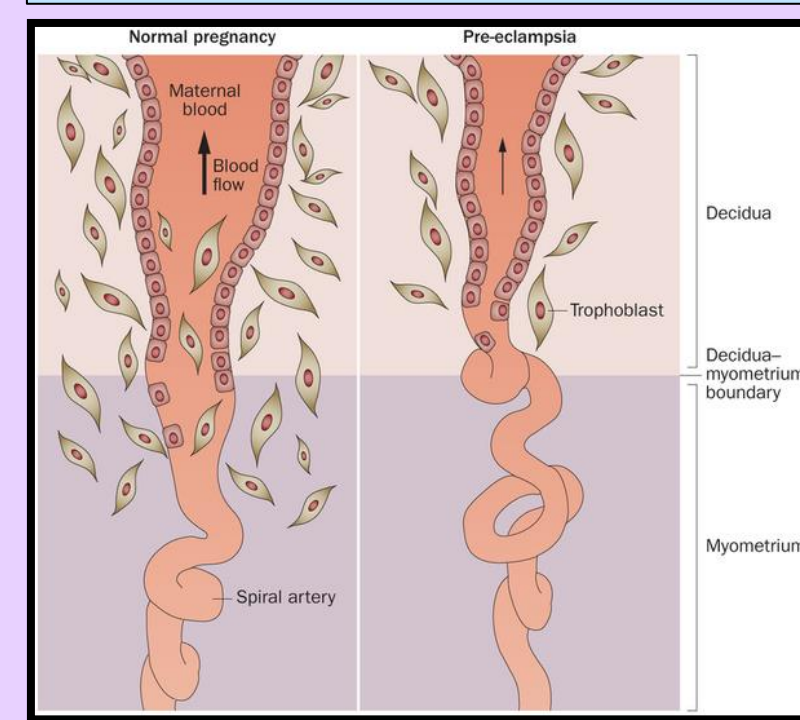
Aim

To determine the distribution of Natural Killer cells 'in space' – location within mouse uterus, and 'in time' – location at different stages of pregnancy (virgin, early gestation and mid gestation)

Introduction

- Natural Killer (NK) cells constitute 70% of leukocytes in the human uterus.
- In pregnancy, a semi-allogeneic fetus is implanted into the mother's uterus. Instead of rejection by the mother's immune system, the fetus is protected by unique interactions between paternally derived MHC on the fetus and maternal NK cell receptors in the uterus. **Certain MHC – NK interactions (e.g. HLA C2 – KIR A) predispose to a greater risk of pregnancy complications like pre-eclampsia, fetal growth restriction and recurrent miscarriages.**
- This is because these interactions inhibit a key NK cell function: remodelling of spiral arterioles in the pregnant uterus. NK cells convert the smooth muscle wall of the arterioles into a fibrinoid epithelium, leading to vasodilation and increased blood supply to the fetus. The remodelling also decreases the pressure of blood flowing to the fetus, thereby preventing fetal damage. **Incomplete spiral arteriole remodelling can lead to pre-eclampsia, fetal growth restriction and stillbirth.**

Complete and Incomplete (e.g. pre-eclampsia) spiral arteriole remodelling

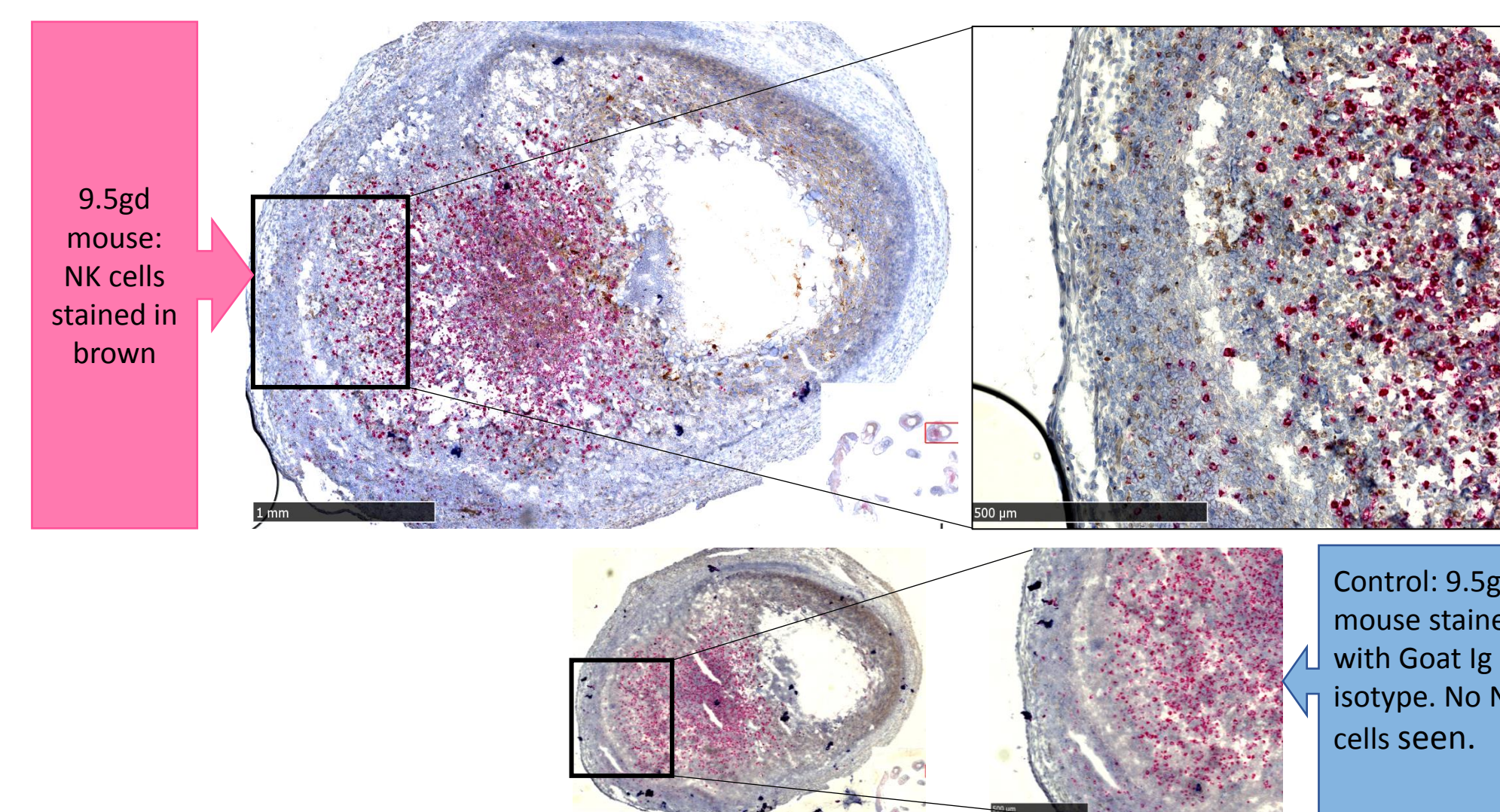
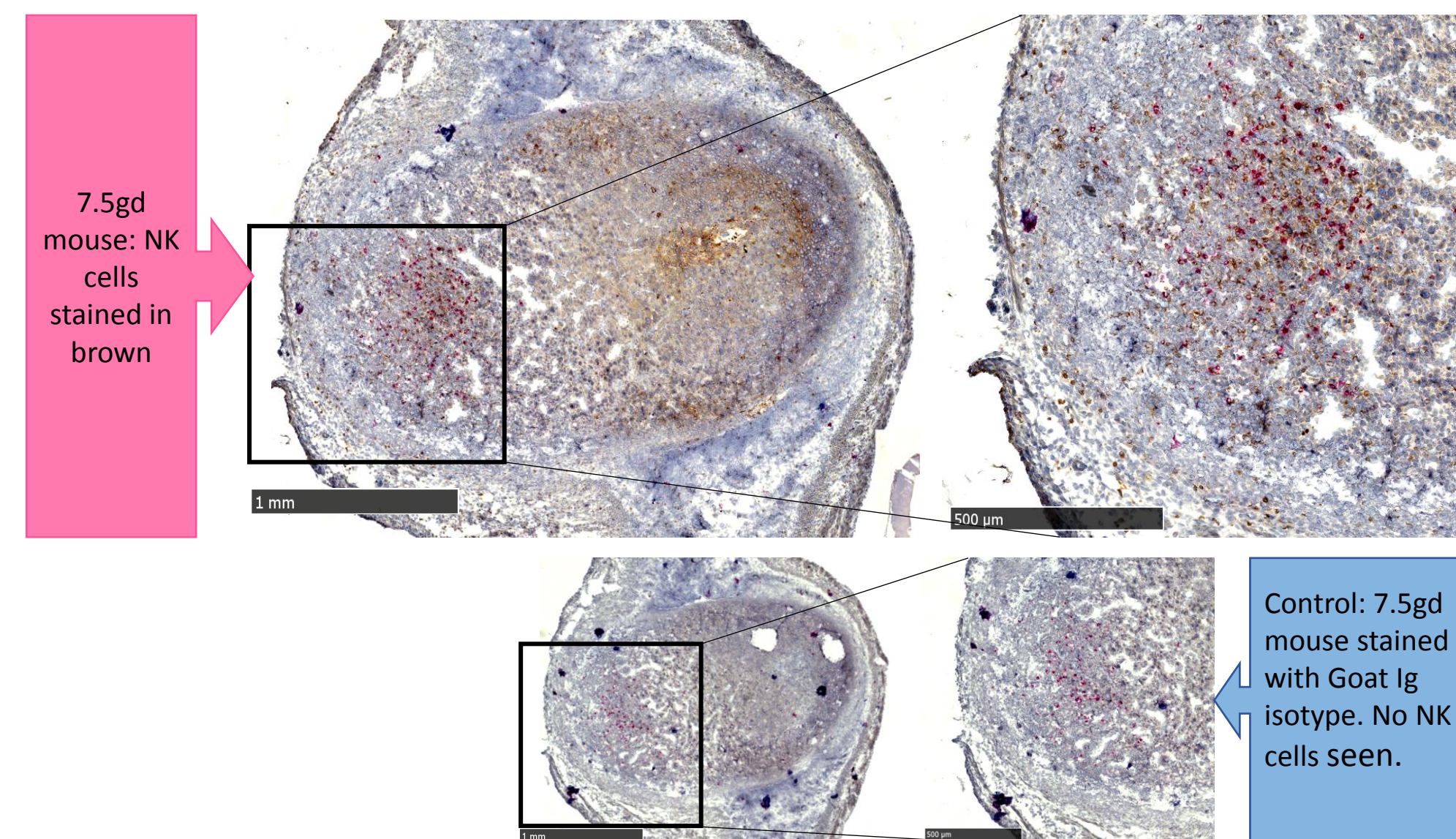
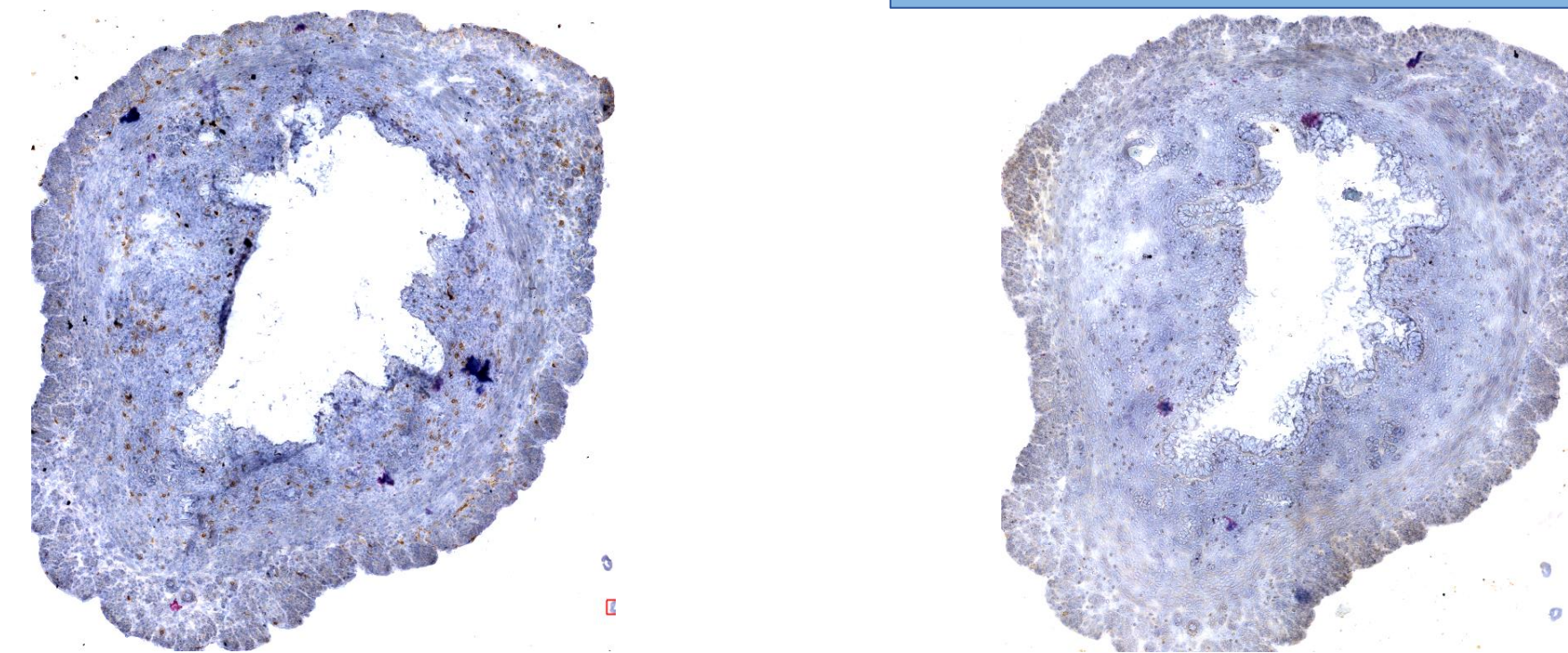


Results

Below are the immunohistochemistry stains of mouse uteri sections for NKp46+ NK cells.

Virgin mouse; NK cells stained in brown.

Control: Virgin mouse stained with Goat Ig isotype. No NK cells seen.



Conclusions

- From this project, I built a timeline of the distribution of NK cells within the mouse uterus at different gestation ages (virgin, 6.5 days, 7.5 days, 9.5 days). After mid-gestation (9.5 days), NK cells do not seem to have a functional role in pregnancy.
- We discovered the close proximity of NKp46+ NK cells to the implanting blastocyst as well as to the spiral arterioles in the mouse implantation site from early in gestation. This provides further evidence of NK cell role in interactions with trophoblast cells as well as spiral arterioles as soon as the blastocyst implants. Thus, NK cells are important in establishing as well as maintaining healthy pregnancy.
- We also saw NKp46+ NK cells in the muscular wall of the uterus from as early as 6.5 gestation days, which could potentially hint us to the origin of NK cells in the uterus (although they could be entering from maternal arteries as well).
- Previously it was thought that NK cells are absent in the virgin mouse uterus but my staining on cryosections using anti-NKp46 antibody has shown their presence in the virgin uterus as well.

Implications of this project

My qualitative data can be combined with quantitative data from flow-cytometry experiments on numbers of NK cells in different uterine compartments to understand fully how NK cells are distributed within the mouse uterus throughout pregnancy.

This will help understand the pathophysiological processes behind pre-eclampsia, fetal growth restriction and recurrent miscarriages. Hopefully in the future we can develop therapeutics to prevent or reverse mismatched MHC-NK cell interactions to avoid these complications altogether for the betterment of Maternal and Child Health.

Methods

Implantation sites from mouse uteri were frozen into cryo-blocks and then cut and placed onto glass slides by the lab technician. I researched on Virgin, early gestation (6.5days), 7.5days and mid-gestation (9.5days) sections.

I then used immunohistochemistry to stain for NK cells. All NK cells have a receptor called NKp46, so antibodies that bind to this receptor were introduced onto the sections. The signal was further amplified using an avidin-biotin complex. A chromogen (3,3'-Diaminobenzidine) was used to visualize the stained NK cells under the microscope.

Immunohistochemistry had to be troubleshooted to get a signal specific for NK cells without background staining or tissue damage.

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