Morphological Characterizations of the Uterine Lymphatics During Gestation in Mouse

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Abstract

Our study aims to understand the morphology of lymphatic vessels in the uterine tissue, a largely unknown topic. The lymphatic system maintains fluid homeostasis and immune function in the body. If the lymphatics fail to function properly, it can lead to inadequate fluid drainage, often resulting in swelling and discomfort. During pregnancy, this malfunction could potentially interfere with the embryo's development due to fluid imbalances.

Previous research has shown that new lymphatic vessels can form from pre-existing ones in cases such as cancer and embryonic development (Stacker et al., 2014). A viable environment within the uterus is crucial for the embryo to develop immunity and withstand foreign substances. The amniotic fluid surrounding the embryo serves as a protective cushion, facilitating organ development, movement, and overall survival (Modena et al., 2004).

The uterus is a vital female reproductive organ that provides a space for a maturing embryo. Lymphatics might aid in fluid drainage, waste removal, and immune responses. Studying lymphatics in the uterus and the placenta, an organ that forms during pregnancy to support the fetus, may optimize pregnancy outcomes (Red-Horse, 2007). Utilizing a mouse model, we can assess human conditions despite anatomical distinctions.

To investigate the lymphatic system in the uterus during pregnancy, we used the Prox1-tdTomato reporter mouse model that endogenously labels all lymphatic vessels present in the uterus at time point E13.5. Techniques such as whole mount imaging and cryosectioning allowed us to visualize surface lymphatics and internal morphology in cross-sections. Immunofluorescent staining using antibodies against Lyve1, Podoplanin (Pdpn), and Cd31 allowed us to further detect lymphatic vessels and cell markers.

Figure 2. Whole Mount Imaging of Prox1-tdTomato E13.5

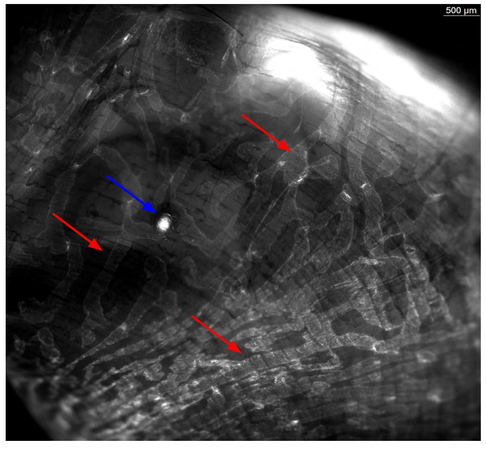
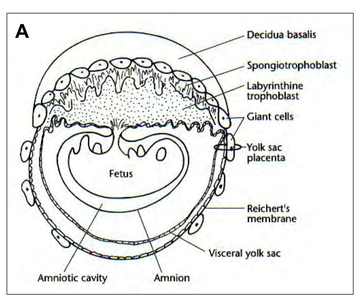


Figure 2. Whole mount imaging of Prox1-tdT E13.5 uterus. Red arrows point to lymphatic vessels on the uterus around the embryo. Blue arrow shows Prox1 expression in the eye lens of the developing embryo.

Figure 1. Uterine Anatomy in Gestation



Zenclussen 2009

Figure 1. Diagram and cross section image of embryo within uterus. A.) The diagram is a representation of the mouse placenta and uterus during pregnancy. B.) Cross section of Prox1-tdT E13.5 uterus. Red arrow points to amniotic membrane, blue arrow to embryo, purple arrow to placenta. Prior to cryosectioning, the uterus was placed into a sucrose solution which drained the excess fluid from the tissue and helped to preserve the sample from freezing damage, explaining the differences in amniotic space and membrane location in the section versus schematic.

Figure 3. Immunostaining for Endothelial Markers

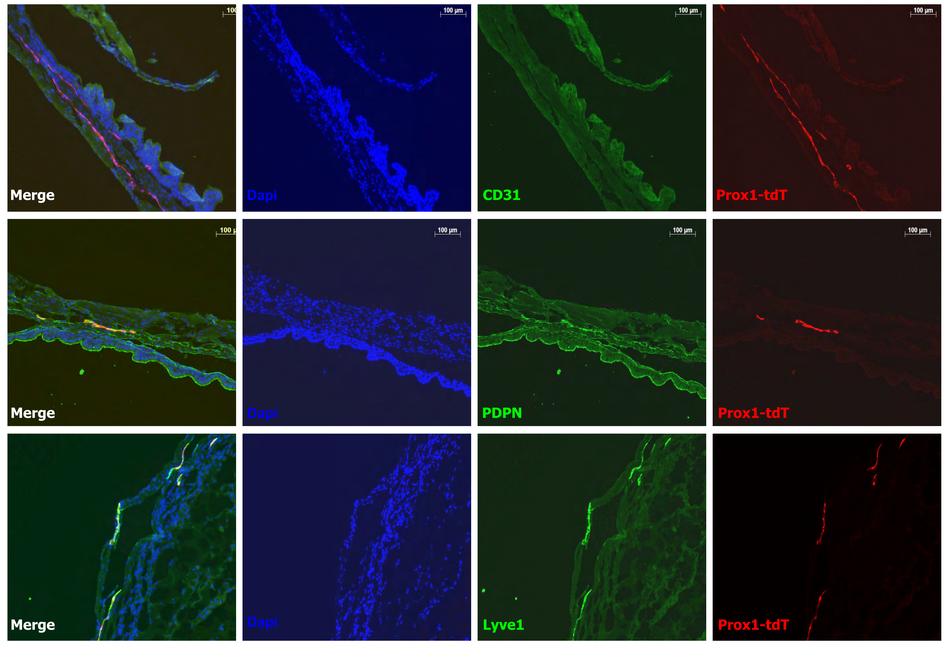


Figure 3. Images of immunofluorescent staining depicting Prox1-tdT E13.5 samples of stained tissue sections. The distinct colors indicate the presence of specific markers such as Cd31, Pdpn, and Lyve1. Pdpn labels lymphatic endothelial cells, while Cd31 labels all endothelial cells. Lyve1 is expressed in capillary lymphatics, but not in collecting lymphatics.

Figure 4. Distribution of Lymphatics in E13.5 Uterus

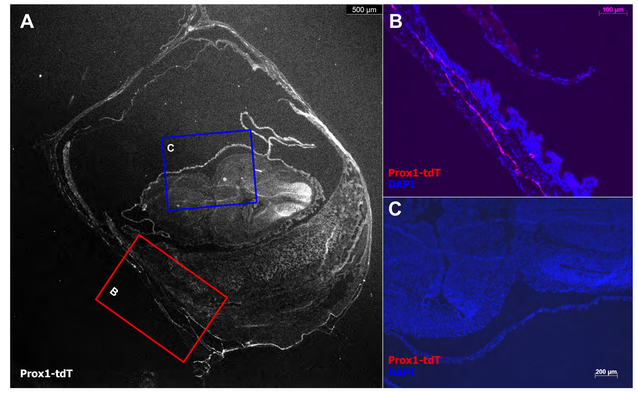


Figure 4. Cross sections of Prox1-tdT E13.5 embryo in uterus showing distribution of lymphatic vessels in fetal and maternal tissues. A.) Cross section of Prox1-tdT E13.5 uterus B.) Close up showing Prox1+ lymphatic vessels along the section perimeter showing location within maternal uterus tissue. C.) Close up showing lack of Prox1+ lymphatic vessels in the fetal amniotic membranes. The image suggests the absence of lymphatic vessels within the amniotic space and membranes.

Summary

Our data suggest that lymphatics are highly present in the uterus during pregnancy.

- Whole mount imaging shows coverage over the surface.
- Our cryosectioning images suggest that lymphatic vessels are present within the uterine tissue, yet do not appear in the amniotic membranes and placenta.
- Immunostaining suggests that Prox1+ vessels express Pdpn and Lyve1, and weakly express Cd31, suggesting that some uterine lymphatics in gestation may be capillary lymphatics.

Future studies should focus on clarifying the specific functions of lymphatics in the uterus during pregnancy and their potential implications in certain diseases such as polyhydramnios, as they may maintain fluid balance and protect a growing fetus against infections and harmful substances.

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