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Background:

- Climate change, and resulting increased heat exposure, poses a growing threat to maternal health.
- Pregnancy involves physiological adaptations that increase susceptibility to heat stress
- Exposure to extreme temperatures in pregnancy is associated with:
 - 16% ↑ in preterm labour
 - 46% ↑ in stillbirth
 - 5% ↑ in adverse pregnancy (per 1 degree Fahrenheit temperature increase)¹
- By mid-century, both global temperatures and the number of women of reproductive age will increase²... yet the mechanism remains undefined.

Markers of interest:

- Keratin 18 (KRT18):
 - Pan-epithelial marker
- Insulin-like growth-factor binding protein-1 (IGFBP-1):
 - Endometrial remodelling, foetal development

Aims:

- i) Optimise immunohistochemical staining for KRT18 in porcine endometrium for future analyses.
- ii) Optimise immunohistochemical staining for IGFBP-1 in porcine endometrium to allow comparison between heat stress and thermoneutral treatment groups.

Experimental design:

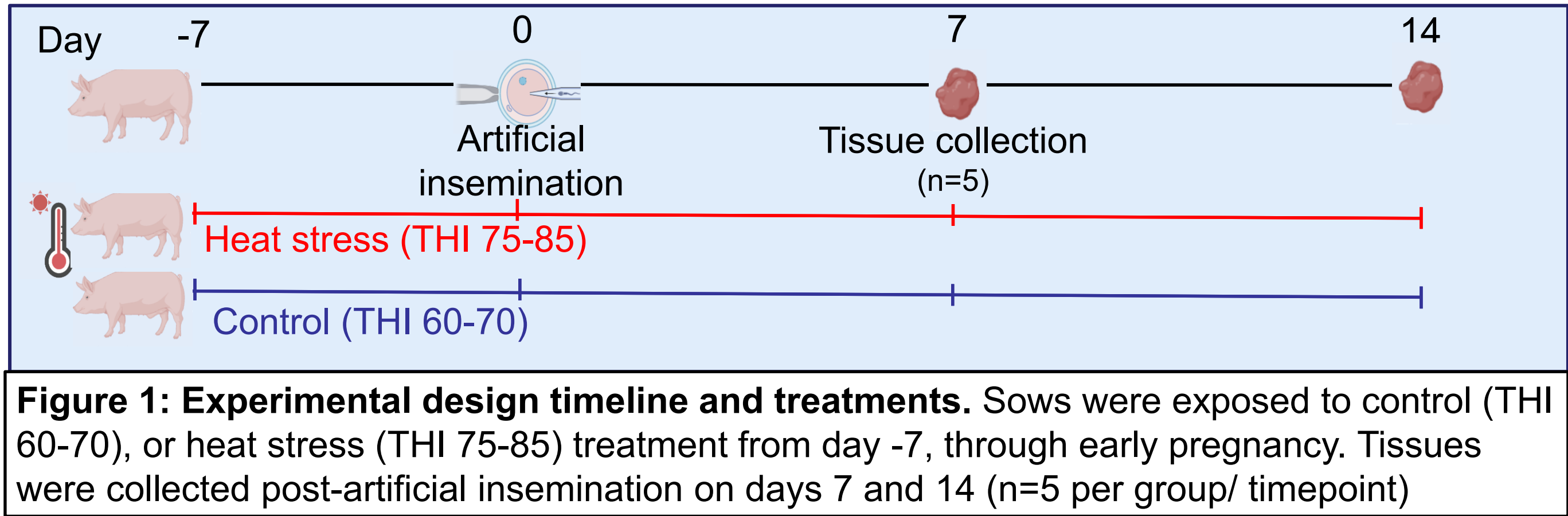


Figure 1: Experimental design timeline and treatments. Sows were exposed to control (THI 60-70), or heat stress (THI 75-85) treatment from day -7, through early pregnancy. Tissues were collected post-artificial insemination on days 7 and 14 (n=5 per group/ timepoint)

Methods:

- Paraffin embedding:** Collected porcine endometrial samples were fixed in wax blocks.
- Tissue sectioning:** Blocks were sliced into 5 μm sections using a microtome.
- Antigen retrieval:** Tris-EDTA, 0.05% Tween-20, pH 9 buffer
- Immunohistochemistry:** Primary anti-KRT18, anti-IGFBP1 and secondary antibodies.
- Staining:** DAB staining of bound targets via HRP-conjugated secondary antibody. Hematoxylin counterstaining for architectural context of tissue.

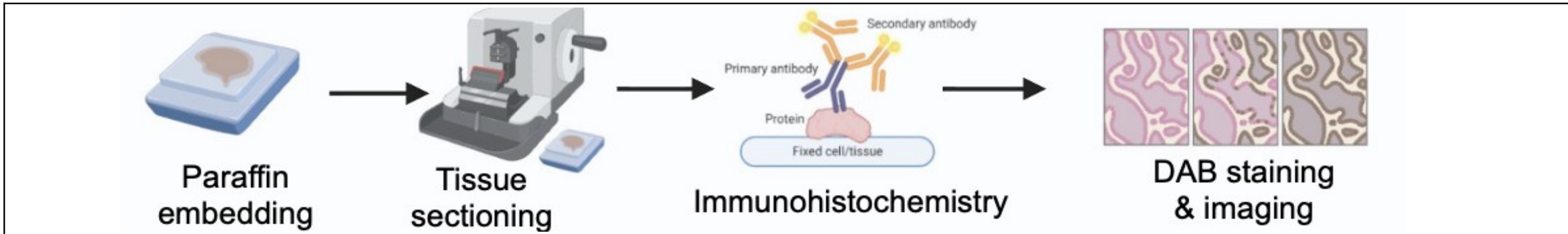


Figure 2: Schematic workflow for IHC protocols for KRT18 and IGFBP1.

Results

i)

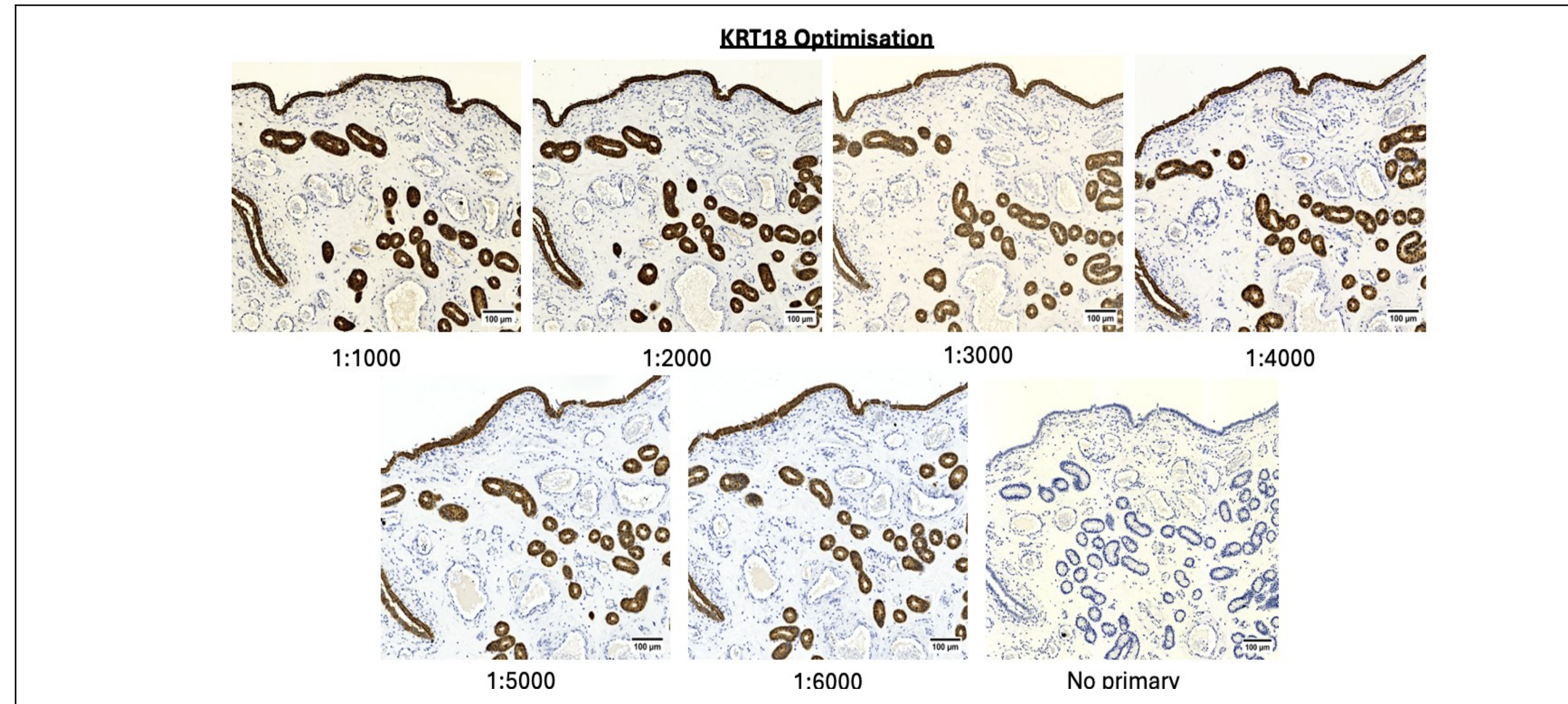


Figure 3: Optimisation of Immunohistochemical Staining for KRT-18. Antigen retrieval with AR9 buffer (Tris-EDTA, 0.05% Tween-20, pH 9) was most effective. Serial dilution of primary antibody revealed highly specific at all dilutions. 1:6000 dilution was selected. No primary control indicated secondary antibody had no non-specific tissue activity.

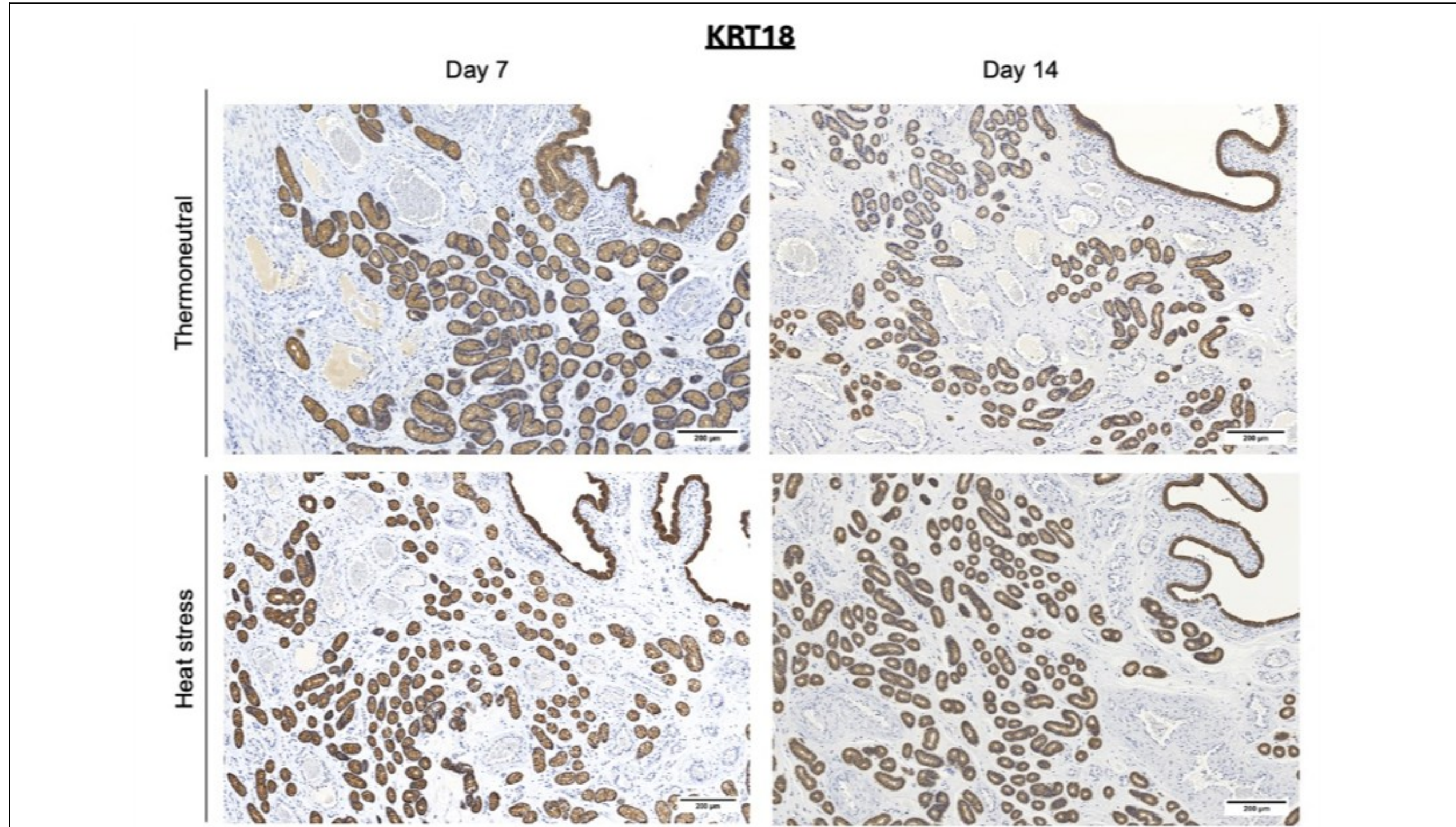


Figure 4: KRT18 experimental sample staining. Representative sections from each treatment group and timepoint. Staining clearly visualised the epithelial and glandular architecture of porcine endometrium in all samples. Images are representative of the samples taken. Staining was broadly consistent across treatment groups.

Results

ii)

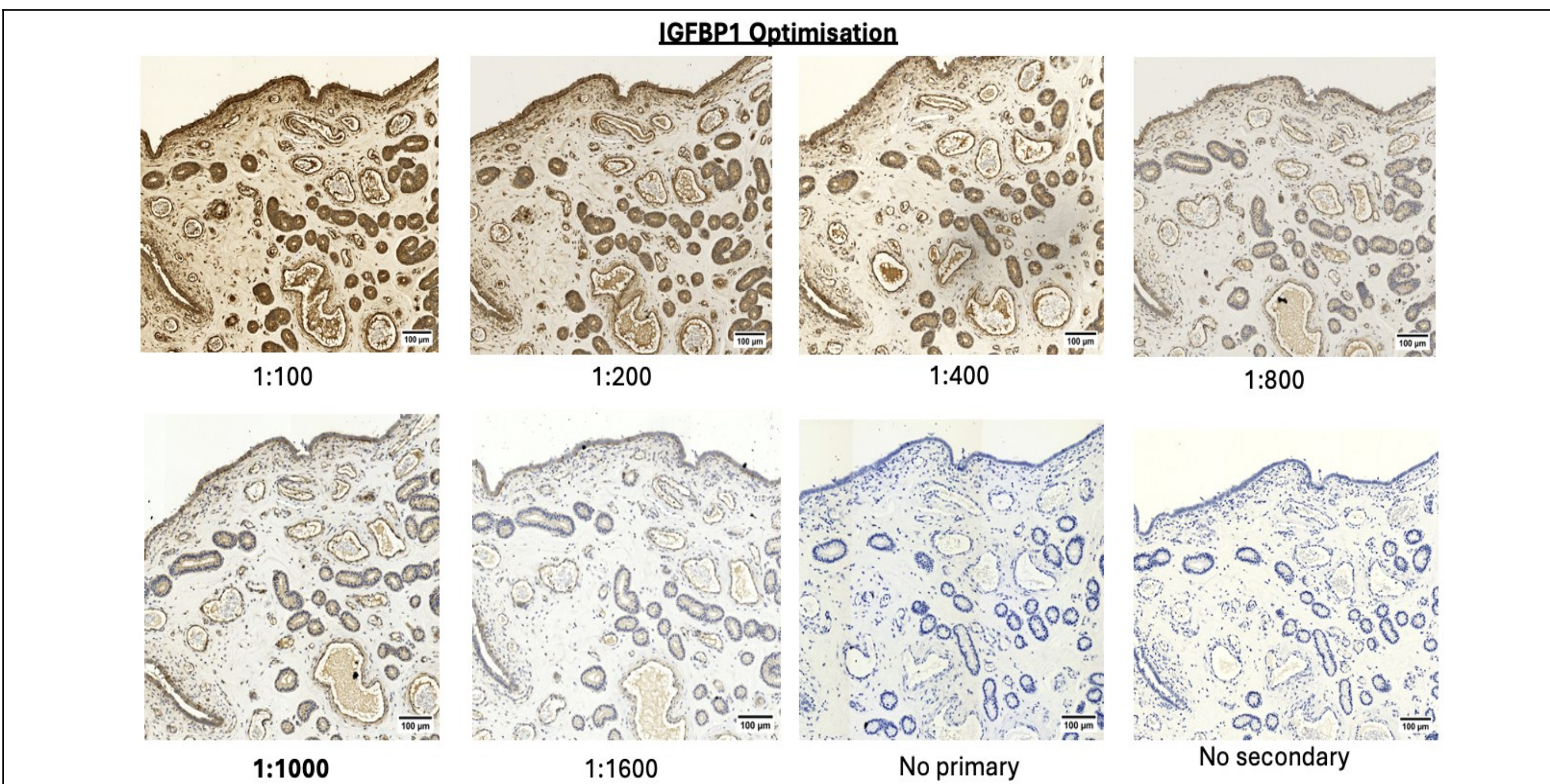


Figure 5: Optimisation of Immunohistochemical Staining for IGFBP-1. Antigen retrieval with AR9 buffer (Tris-EDTA, 0.05% Tween-20, pH 9) was most effective. Serial dilution of primary antigen showed 1:1000 dilution to be optimal. No primary and no secondary confirm true target binding.

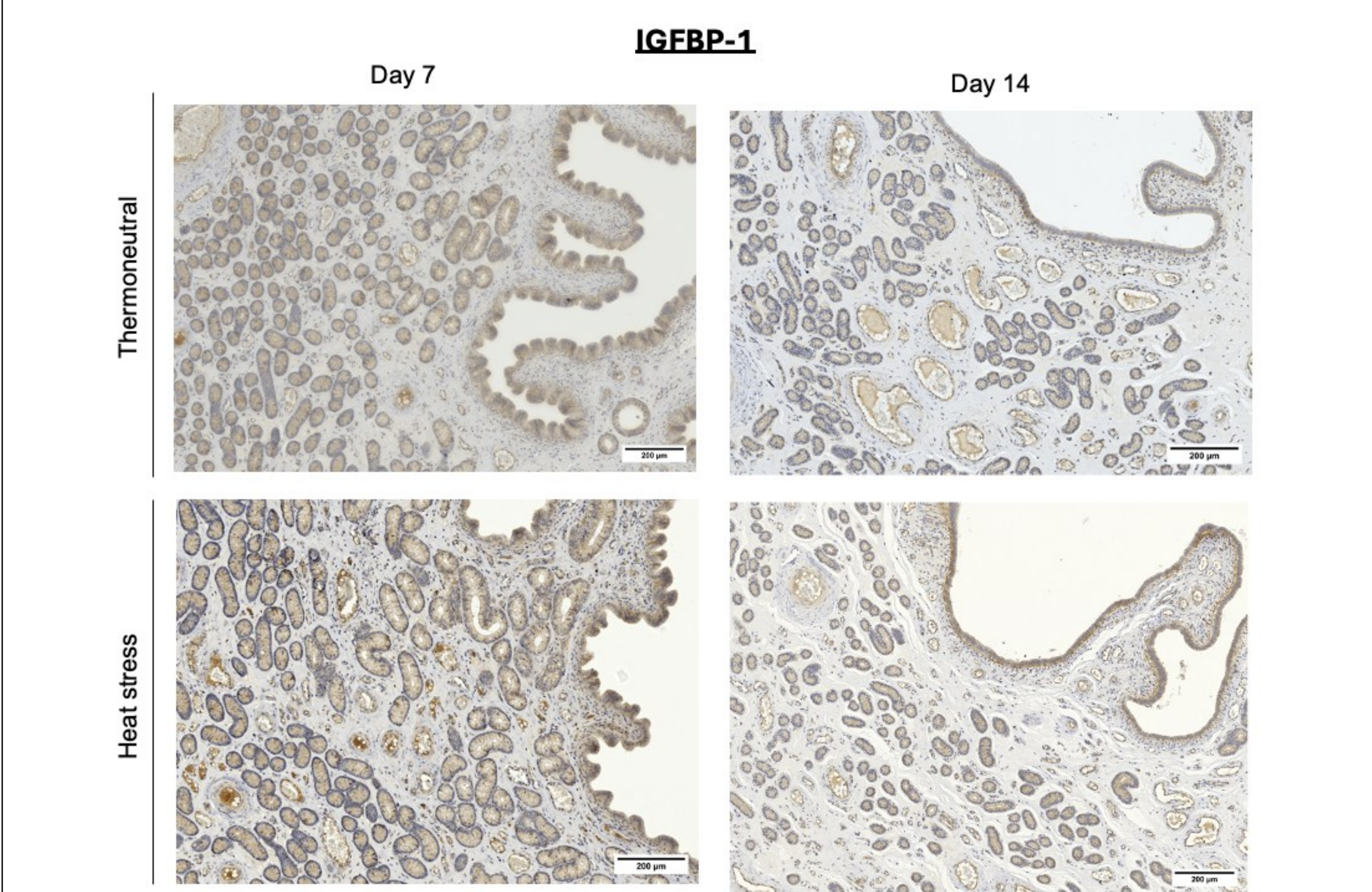


Figure 6: IGFBP1 Experimental Sample Staining. Representative sections from each treatment group and time point. Heat stressed endometrium appeared apparently stronger and more heterogenous immunoreactivity than thermoneutral tissue at both time points, particularly in the glandular epithelium.

Conclusions:

- KRT-18 IHC protocol was successfully optimised in porcine endometrium,:**
 - allowing clear visualisation of epithelial and glandular structures, and for use in future studies.
- IGFBP-1 IHC protocol was successfully optimised in porcine endometrium:**
 - visualising potentially heterogeneous localisation of expression within endometrial glands.

Next steps:

- Investigate IGFBP-1 mRNA expression to assess the impact of heat on protein transcription levels.
- Validate observations with larger sample numbers.

References

1) Chersich, M.F., Pham, M.D., Areal, A., Haghighi, M.M., Manyuchi, A., Swift, C.P., Wernecke, B., Robinson, M., Hetem, R., Boeckmann, M. and Hajat, S. (2020). Associations between high temperatures in pregnancy and risk of preterm birth, low birth weight, and stillbirths: systematic review and meta-analysis. *BMJ*, 371, p.m3811. doi: <https://doi.org/10.1136/bmj.m3811>

2) United Nations (2024). World Population Prospects 2024 Summary of Results. [online] Available at: https://population.un.org/wpp/assets/Files/WPP2024_Summary-of-Results.pdf.