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# Does transcriptional activation of STAT3 have a role in ovine mastitis?

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## Background

Mastitis, inflammation of the udder, is a major welfare problem for sheep. It is painful for the animal and also results in significant economic losses. Most mastitis in the UK is caused by bacteria and whilst veterinary interventions focus on prevention through good management, many animals still succumb to infection at which point antibiotics are currently a mainstay of therapy.

The Signal Transducers and Activators of Transcription (or **STAT**) family are molecular factors which coordinate varied cellular processes, including **inflammation**, and **mammary gland development**. Importantly, they are critical in controlling the regression of the mammary gland at the end of lactation (**involution**), a time when dairy animals are particularly prone to mastitis (**dry period mastitis**).

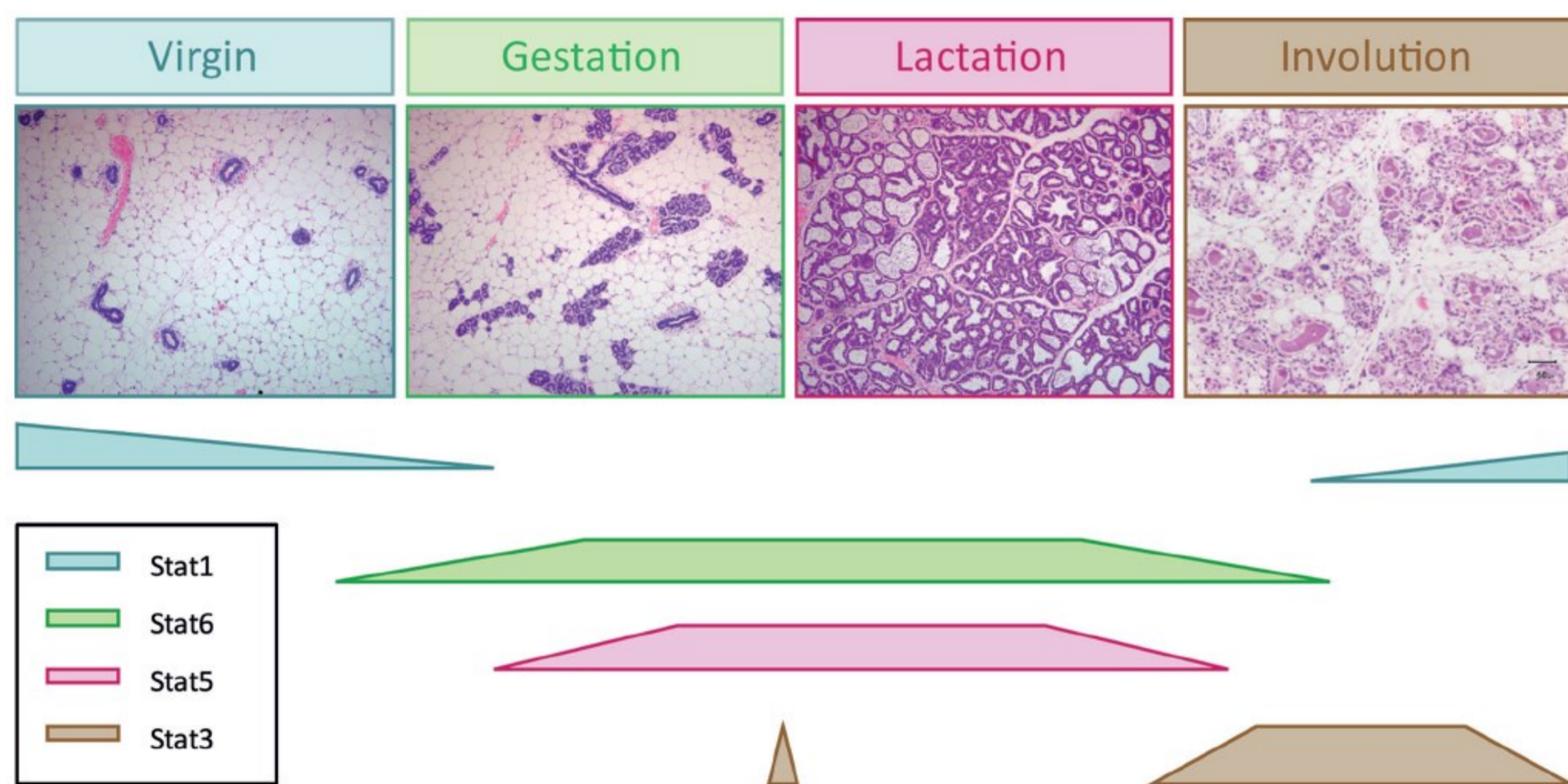
## STAT activity in murine models of mammary gland involution

### Figure 1.

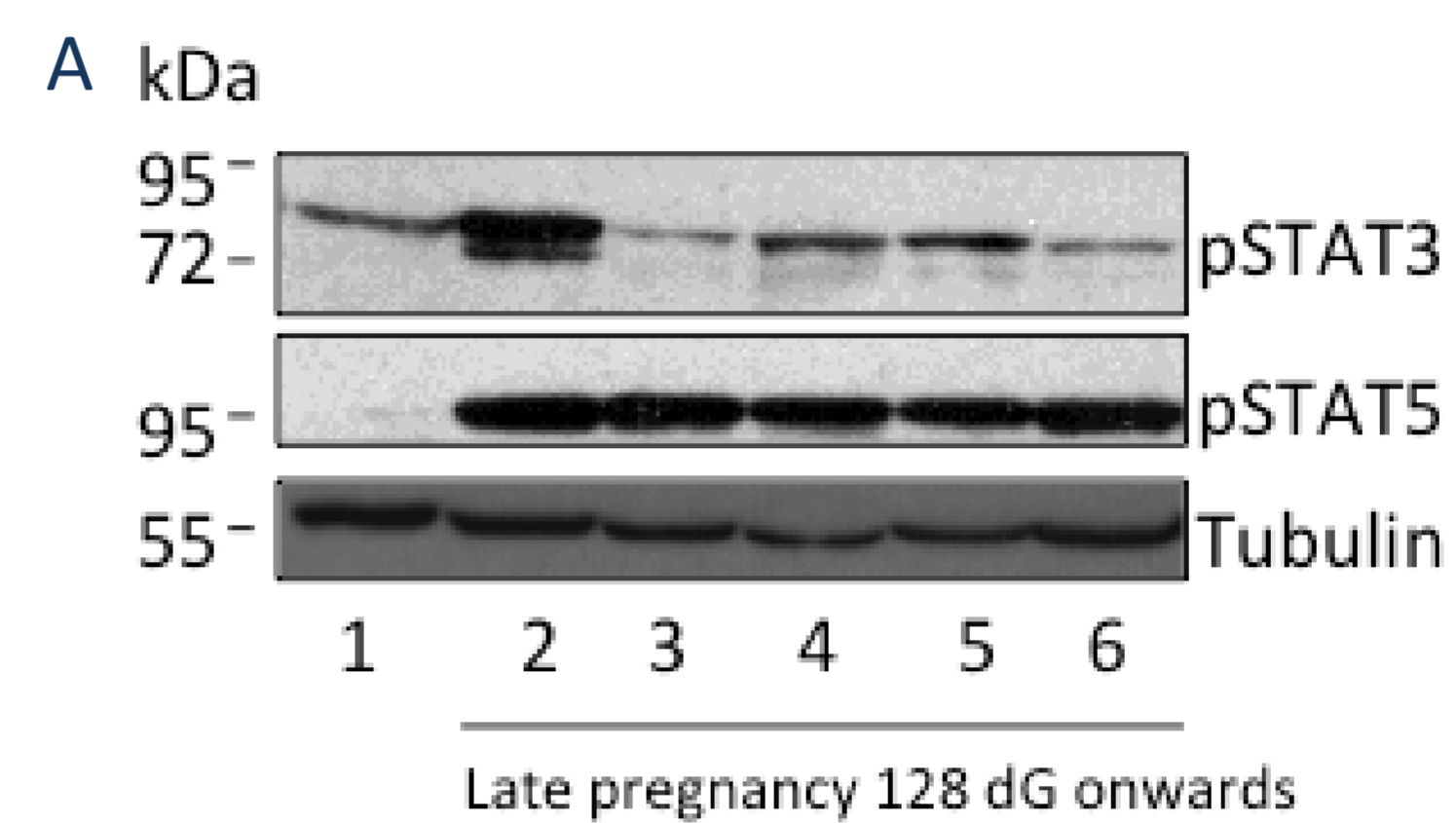
#### STAT activity during the murine mammary gland developmental cycle.

Although STATs are expressed throughout the cycle, their **activation by tyrosine phosphorylation** is strictly regulated and occurs at specific stages in the cycle. This pattern of activity reflects the requirement for each STAT as determined using genetic deletion of each individual factor.

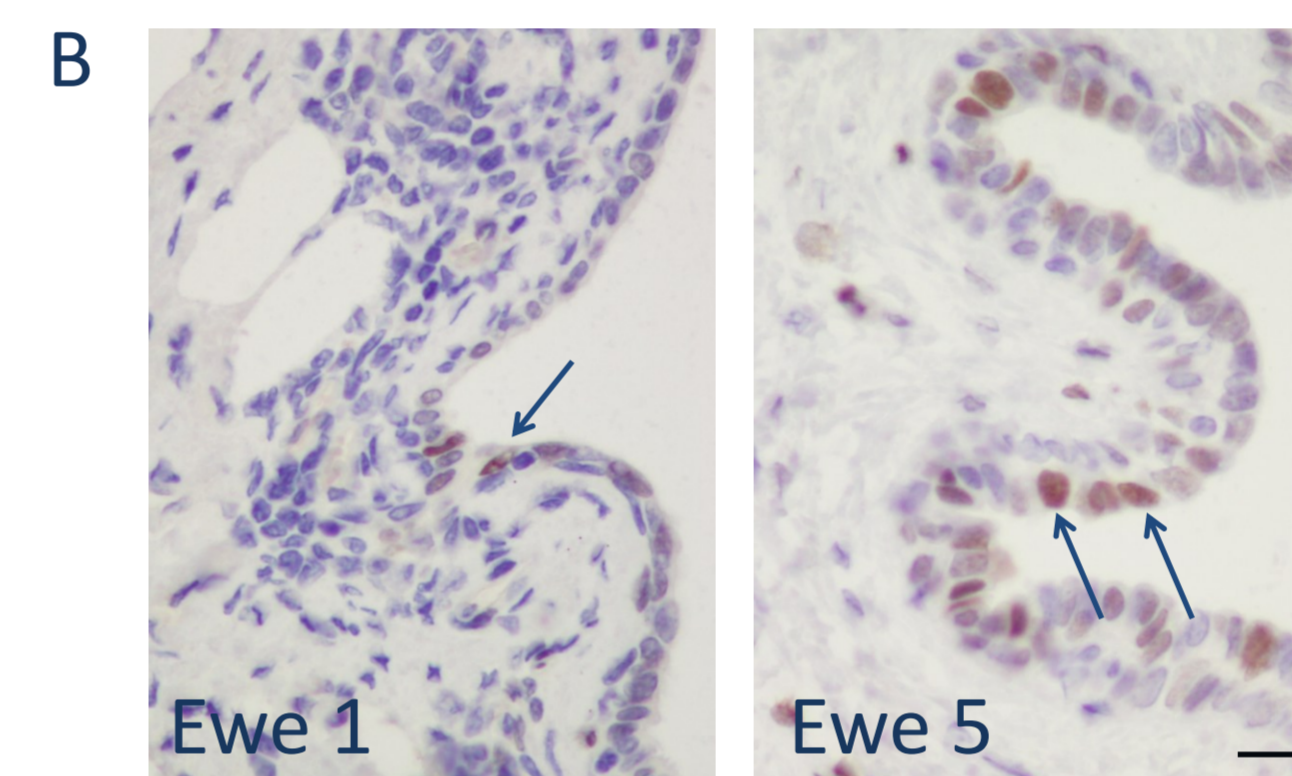
Adapted from Hughes & Watson, 2012.



## Expression of pSTAT3 and pSTAT5 varies with stage of mammary gland developmental cycle



**Figure 3.** (A) Western blot demonstrating basal levels of expression of STAT3 and STAT5 in mammary gland extracts from ewes at various developmental stages. Ewe clinical histories: 1) Mature; not pregnant; previously parous. 2) Approx 135 dG; illness prior to death (but no mastitis). Twins. 3) 130 dG no illness. Single lamb. 4) 130 dG no illness. Triplets. 5) 127 dG no illness. Twins. 6) 128 dG no illness. Single lamb. dG = days gestation.

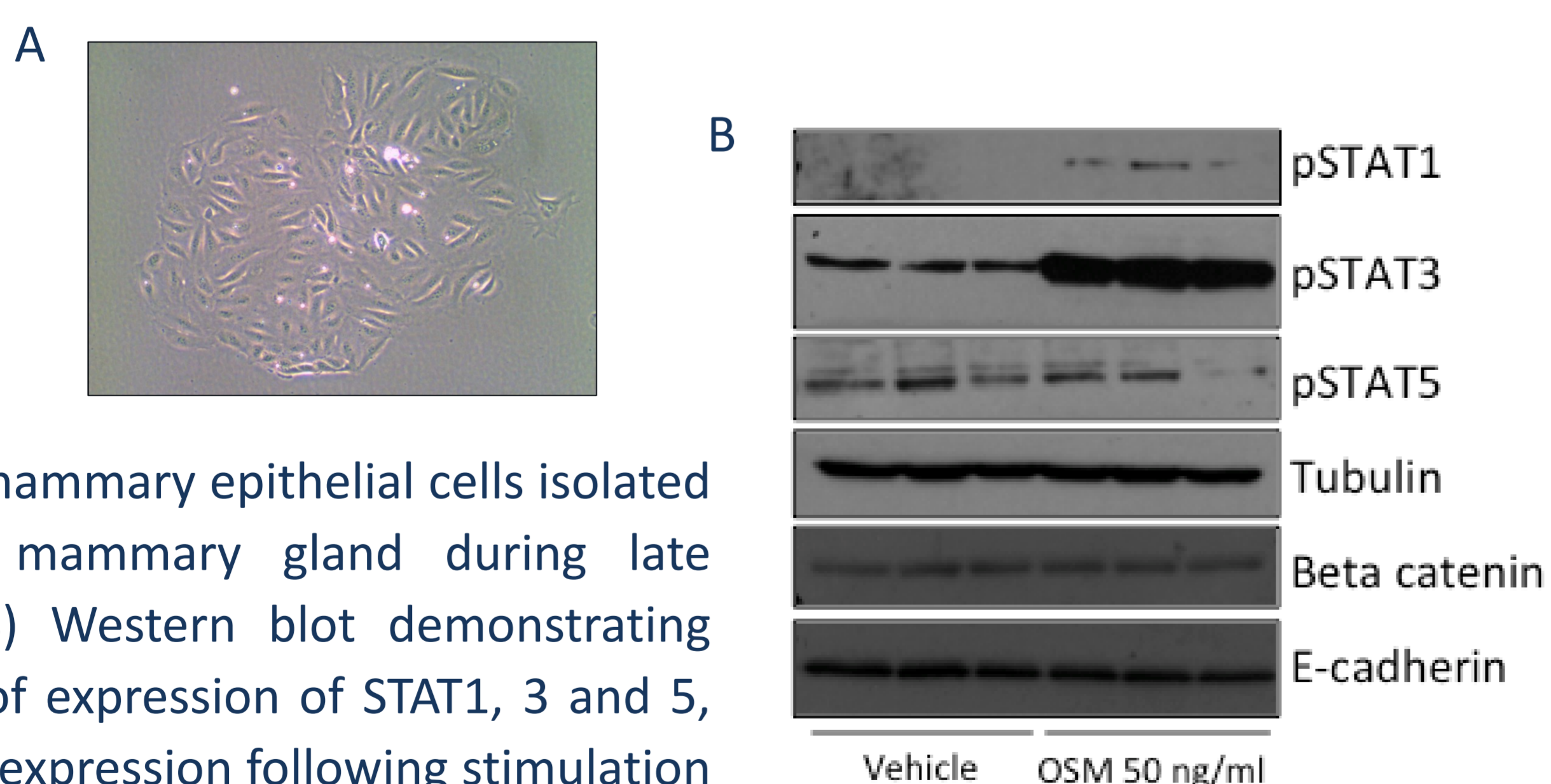


(B) Immunohistochemical staining for pSTAT3 (arrows). Both images show a duct from the left mammary gland. Ewe 5 is pregnant, hence ductular epithelial cells are more prominent. Bar: 30 microns.

## Objectives

This project will examine the expression patterns of three key mammary STATs in sheep mammary epithelial cells and in clinical samples obtained post mortem from sheep at varying times during lactation, natural involution, and during mastitis. We hypothesise that STAT family members may have a role in the pathogenesis of mastitis, given their critical roles in coordinating inflammation and mammary gland development.

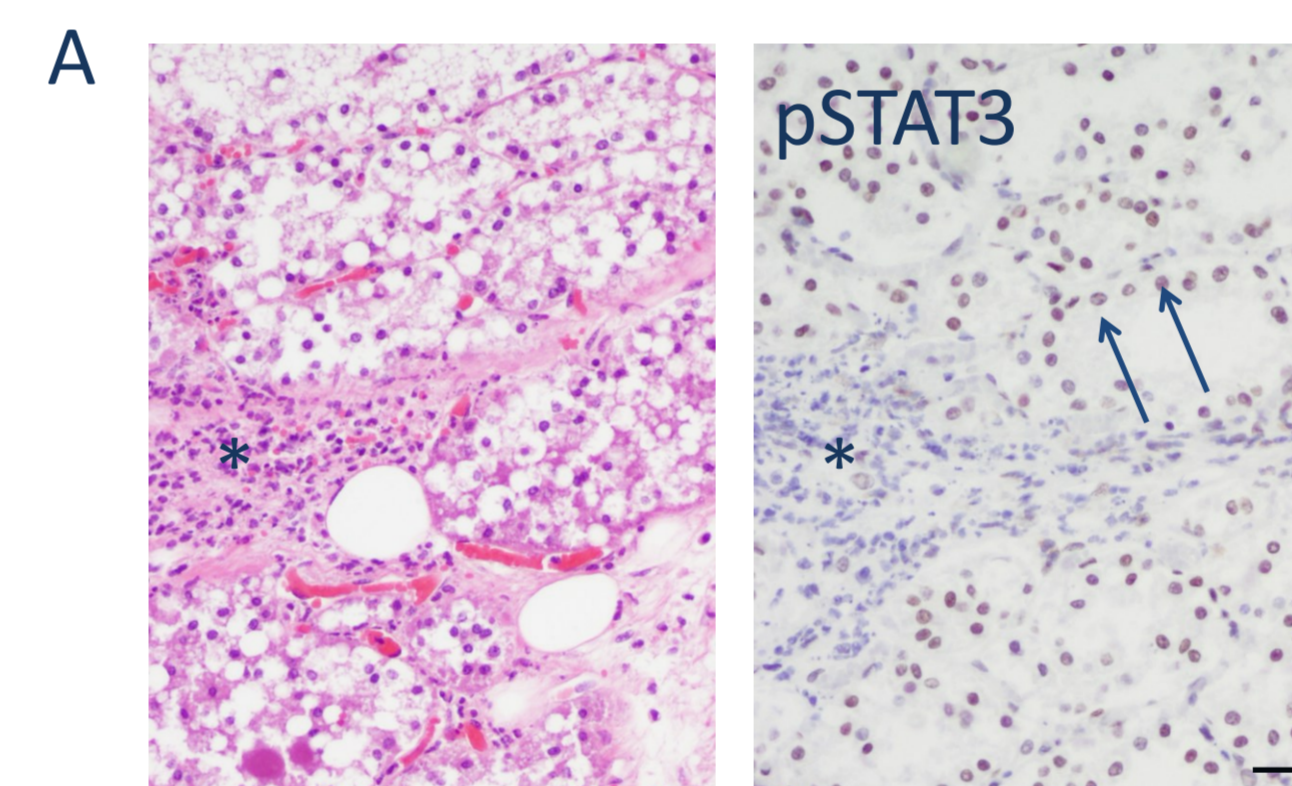
## Primary ovine mammary epithelial cells can be induced to express pSTAT3 by stimulation with oncostatin M (OSM)



### Figure 2.

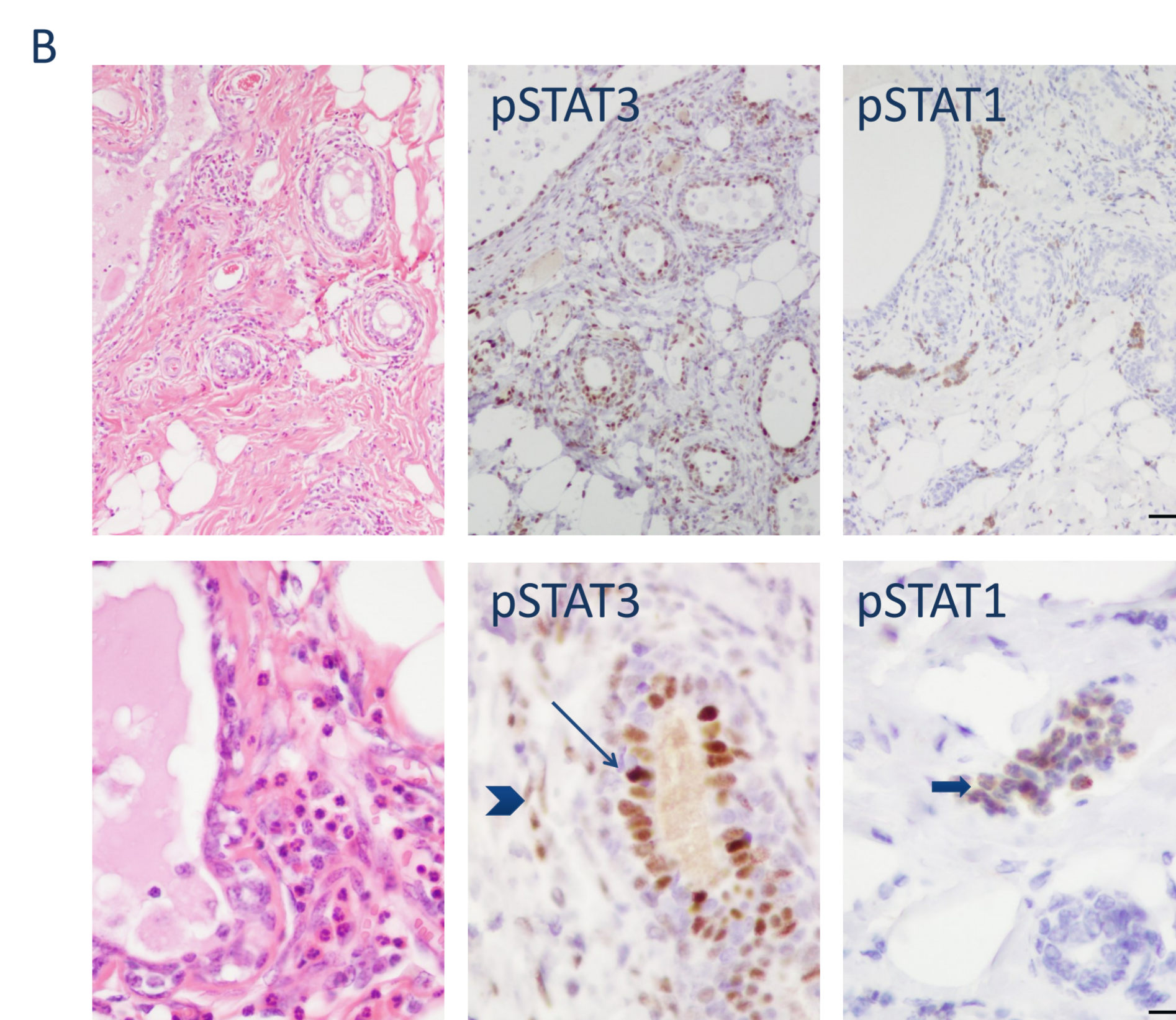
(A) Primary mammary epithelial cells isolated from ovine mammary gland during late gestation. (B) Western blot demonstrating basal levels of expression of STAT1, 3 and 5, and levels of expression following stimulation with OSM.

## Preliminary data suggests phosphorylated STAT1 and STAT3 (pSTAT1 and pSTAT3) may be upregulated in ovine mastitis



### Figure 4.

(A) 4 year-old mule ewe with mastitis during lactation. \* indicates immune cells. Arrows indicate upregulation of pSTAT3 in numerous mammary alveolar luminal epithelial cells. Bar: 50 microns.



(B) 9 year-old dry, nulliparous, Zwartbles ewe with mastitis. pSTAT3 is upregulated in epithelial cells (arrow), stroma (chevron) and immune cells, whereas pSTAT1 is predominantly expressed in immune cells, including lymphocytes, macrophages and neutrophils (short arrow). Upper row bar: 50 microns. Lower row bar: 30 microns.

## Preliminary conclusions

As in other species, pSTAT3 and pSTAT5 are most likely critical to ovine postnatal mammary development. Cases of mastitis may be associated with upregulation of pSTAT1 and pSTAT3; the cellular compartments in which these STATs are expressed during mastitis requires further investigation. During mastitis, STAT1 and STAT3 activity may modulate the gland's inflammatory phenotype, similar to that described in association with Stat3 activity in mouse models of involution.

## Acknowledgements

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If you are interested in collecting tissue samples to contribute to this project, please contact Kate Hughes: kh387@cam.ac.uk.

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