

# Analysis of Primary Cilia in the Lactating Bovine Mammary Gland

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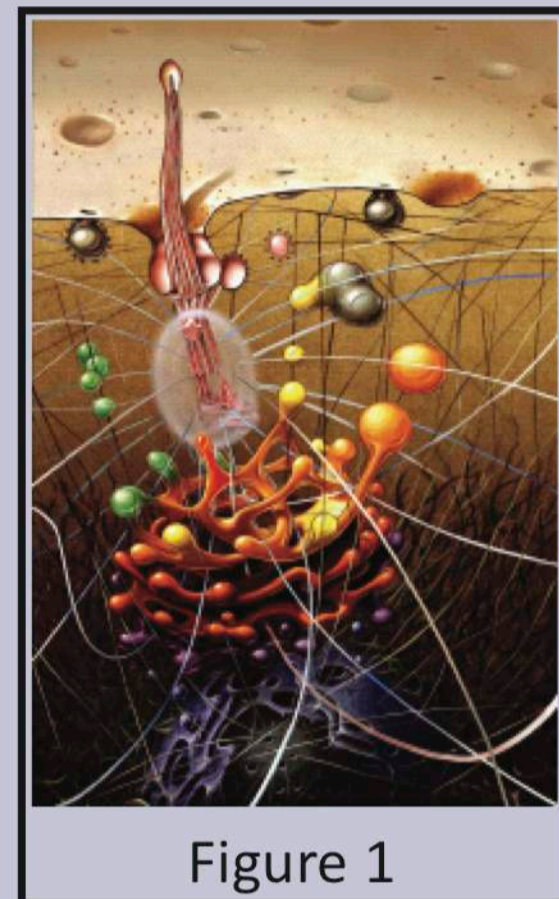
## Introduction

Extended periods of milk accumulation result in reduced milk secretion, increased mammary epithelial cell (MEC) death and eventually, involution of mammary glands. Local intra-mammary signals initiate involution in response to milk stasis (1) although the actual trigger has not yet been identified.

Physical distension of the mammary gland has been postulated as a key trigger of cessation of milk secretion (2) and increased apoptosis, due to a sustained change in shape of MEC from milk accumulation (3). Primary cilia act as mechanosensors in a wide range of cell types (4,5,6), but despite their discovery in mammary tissue from many species (6-10), their role in bovine lactation and involution have not been investigated.

The primary cilium consists of two parts (Fig 1)<sup>4</sup>; a membrane-bound, microtubule-based, 9+0 axoneme that projects into the extracellular microenvironment, and an intracellular basal body that comprises the most mature of the two centrioles located within the centrosome. The centrosome is the microtubule organising centre of the cell, while the integrity of the microtubular cytoskeleton is essential to maintain the trans-Golgi network and thus, protein modification, sorting and secretion.

We believe that understanding the mechanisms initiating involution may ultimately result in selection of dairy animals with persistent lactation and/or tolerant of extended milking intervals.



## Aims

To understand the potential role of primary cilia mechanosensation and/or chemoreception in the milk production process, by analysis of primary cilia distribution and morphology in the bovine mammary gland during active lactation and throughout early involution.

## Methods

### Animal Trial Design

Mammary gland involution was induced by abrupt termination of milking of Friesian, primiparous, non-pregnant, pasture-fed heifers at mid lactation (92 ± 3 DIM), with low SCC (159,000 ± 20,000 cells/ml) and an average milk yield of 14.2 ± 0.4 l/d.

### Alveolar mammary tissue collected at slaughter

6, 12, 18, 24, 36, 72, 192 h post milking

### Histology

Tissue samples were fixed in paraformaldehyde, sectioned at 5-8µm and either, stained with Haematoxylin and Eosin (H and E) for histology and grading for milk stasis (n=6/group), or treated for antigen retrieval in citrate buffer ± pepsin for immunohistochemistry.

### Immunohistochemistry

Immunohistochemistry was used to define primary cilia (anti-acetylated tubulin), centrioles (anti-gamma tubulin), myoepithelial cells (anti-smooth muscle actin, (SMA)) and nuclei (Hoechst) (n=3 per group). Fluorescently labelled sections were examined by confocal microscopy and the images analysed for primary cilia distribution and morphology.

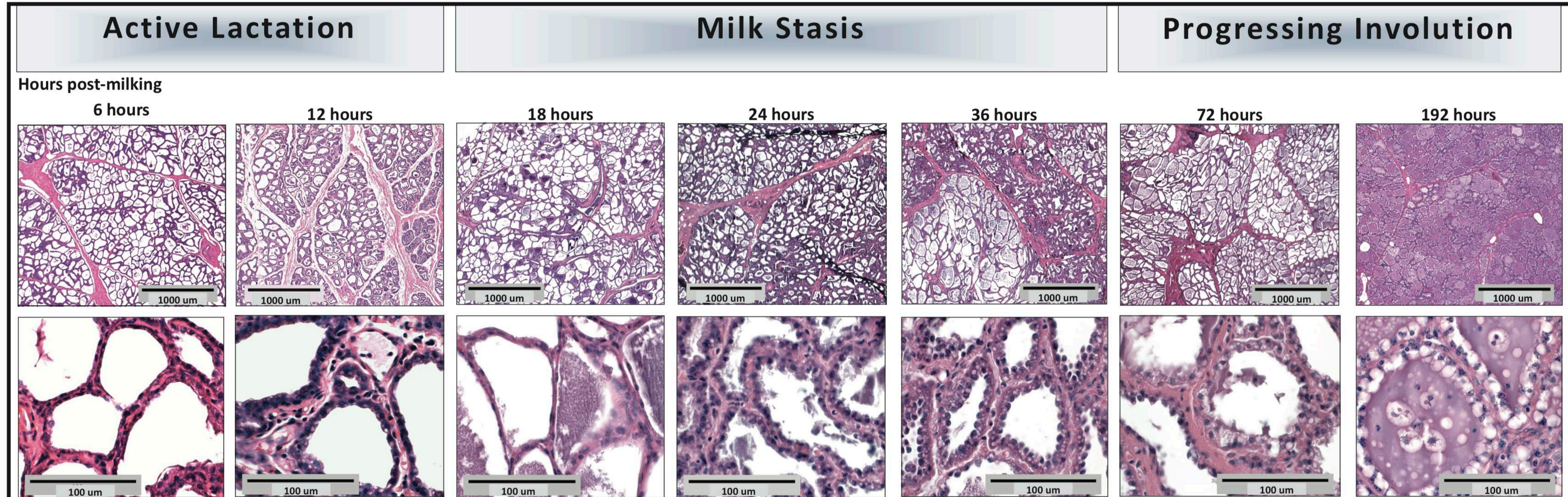
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## Acknowledgements

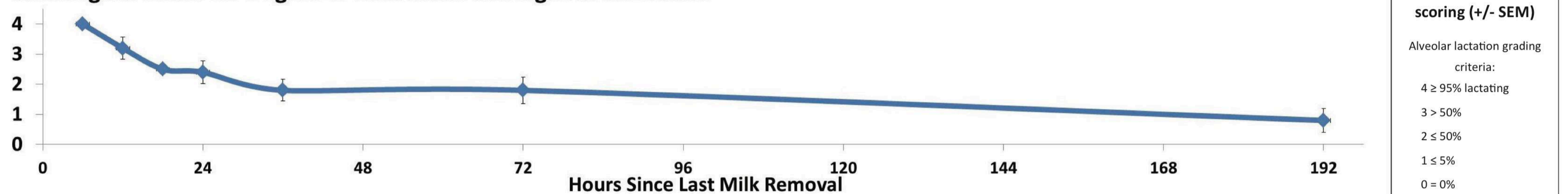
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## Results



H and E staining: Morphological changes in the bovine mammary gland following abrupt cessation of milking in mid-lactation.

### Histological Grade for Degree of Milk Stasis and Signs of Involution

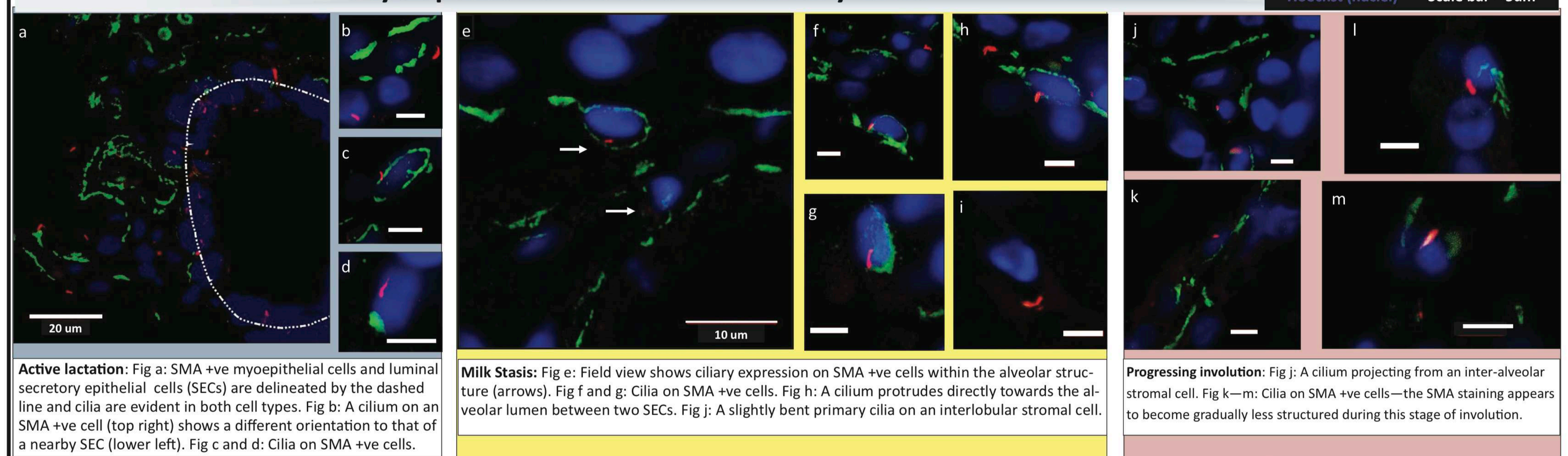


**Average histological scoring (+/- SEM)**

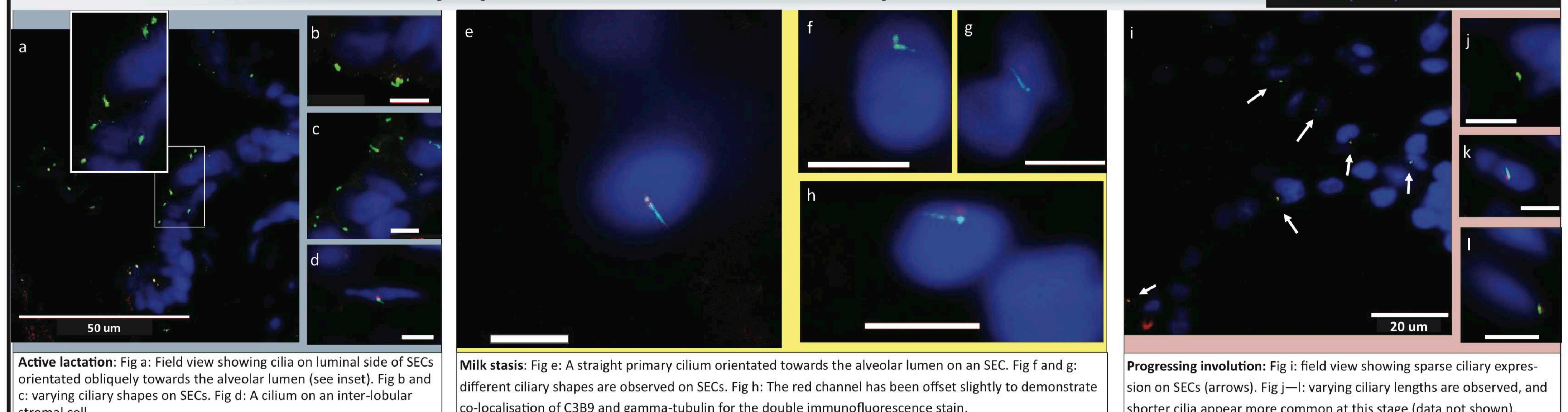
Alveolar lactation grading criteria:

- 4 ≥ 95% lactating
- 3 > 50%
- 2 ≤ 50%
- 1 ≤ 5%
- 0 = 0%

## Myoepithelial Cells and Primary Cilia



## Secretory Epithelial Cells and Primary Cilia



## Conclusions

- All cell types involved with bovine lactation can express a primary cilium.
- Ciliary bending is consistent with mechanotransduction.
- Differences exist in the ciliary orientation of epithelial and myoepithelial cells, suggesting potential differences in ciliary function for these two critical cells.