



**DEAKIN**  
UNIVERSITY AUSTRALIA

# The extracellular matrix modulates asynchronous concurrent lactation in tamar wallaby (*Macropus eugenii*)

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9th International Symposium



# Wallaby reproductive strategy



**short gestation**

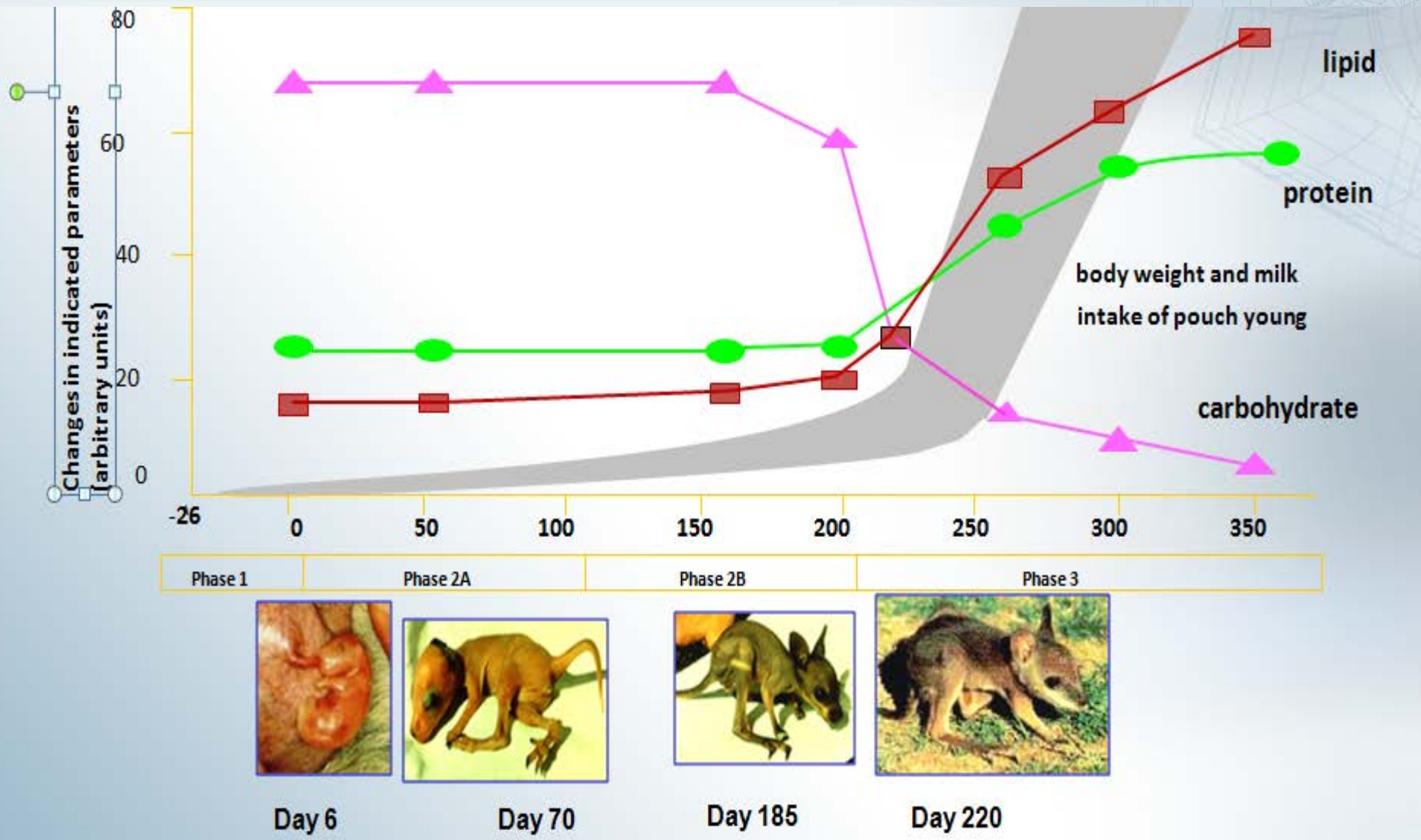
**relatively long lactation**

**greater investment in development during lactation compared to eutherians**

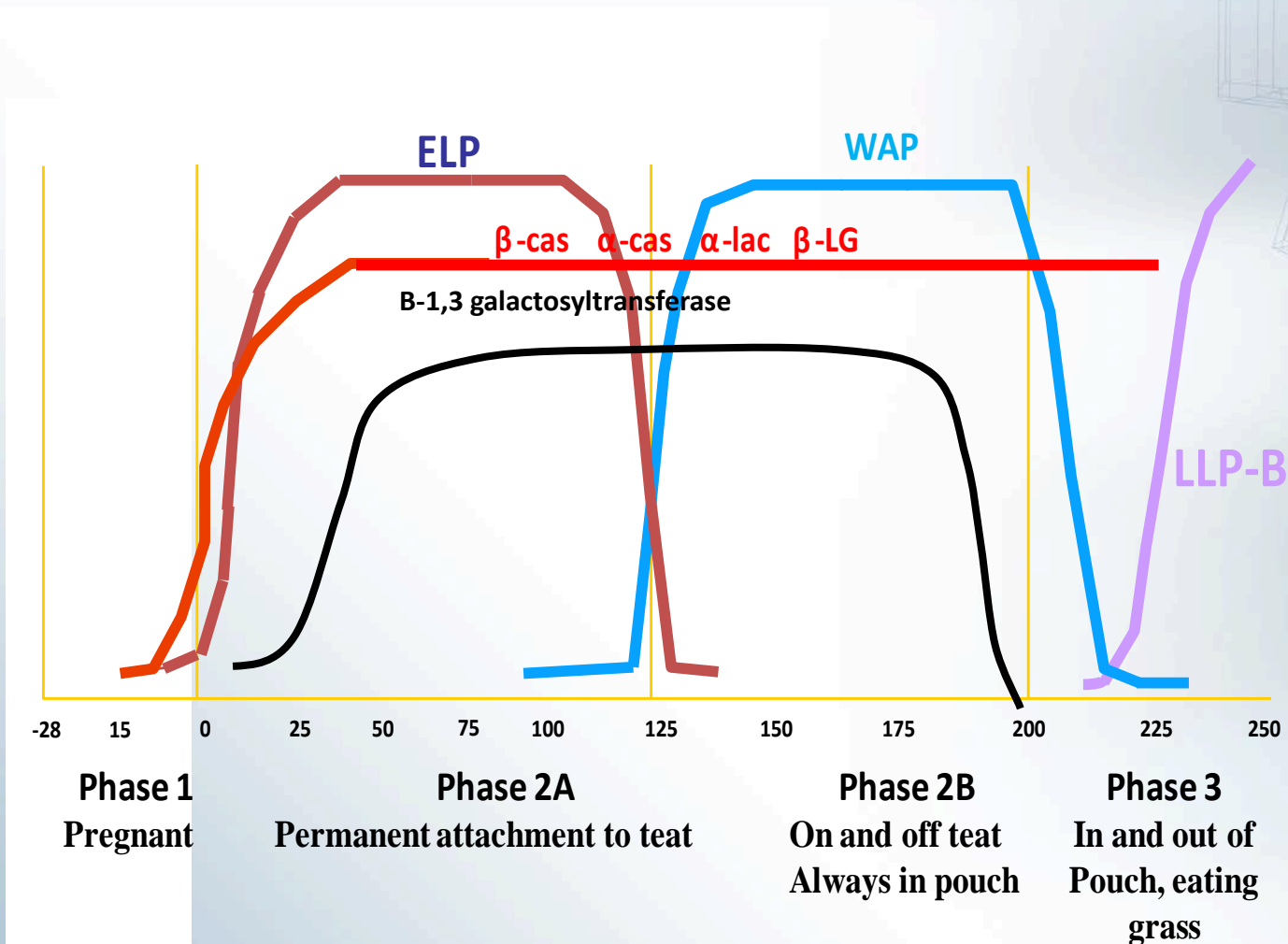
**milk composition and milk production altered significantly during lactation**



# Changes in milk composition during lactation



# Changes in milk protein gene expression during lactation



# Domain-specific bioactives delivered at specific times

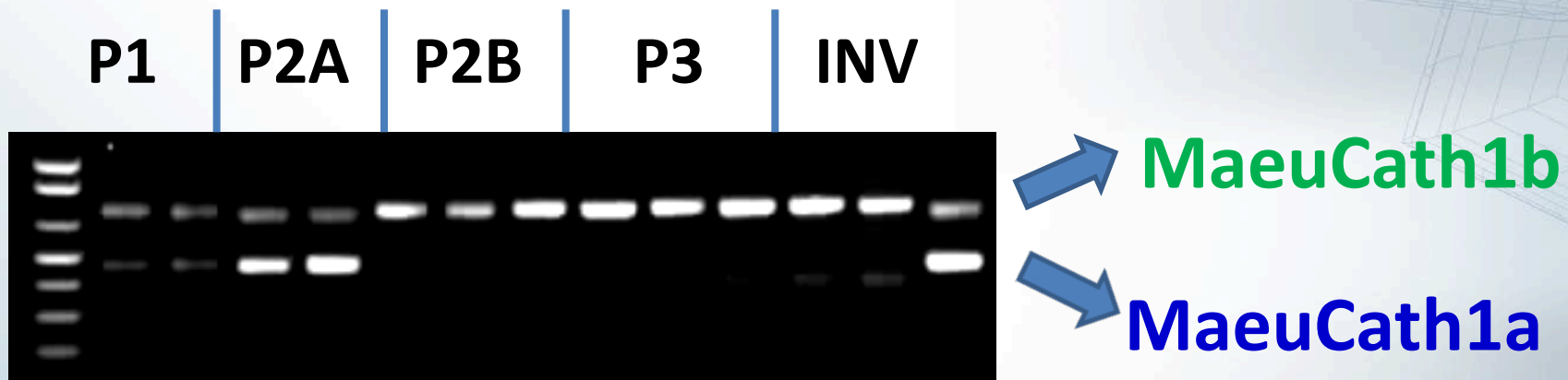
**e.g Cathelicidins**

**Found gastro-intestinal tract, respiratory system and the skin**

- 1. antimicrobial**
- 2. wound healing**

**Role in lactation not clear**

# MaeuCath1 differentially expressed during lactation



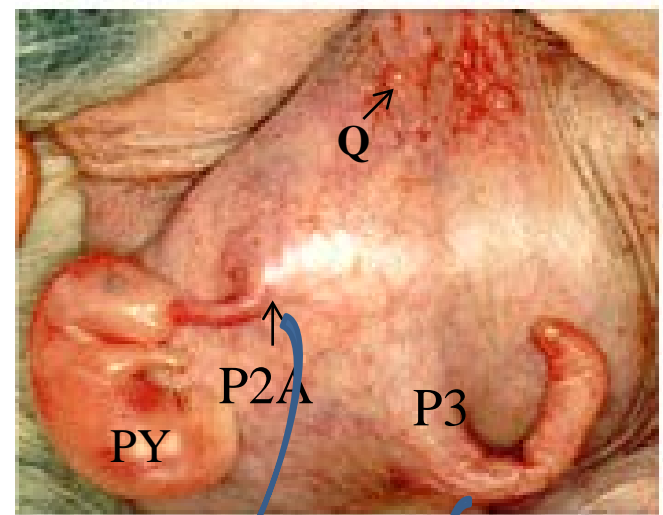
*Wanyonyi. S. et al Comparative Biochemistry and Physiology, 2011*

**Spliced into two variants to**

- Cath 1a may provide antimicrobial defence to the young and mother**
- Cath1b may play a role in mammary growth during peak lactation**

# Asynchronous concurrent lactation (ACL)

The wallaby can  
produce both P2A and  
P3 milk concurrently



tELP

tLLP's

# Possible mechanisms for ACL?

**1**  
**Hormones**

**2**  
**Sucking of  
the young**

**3**  
**The milk?  
The ECM?**



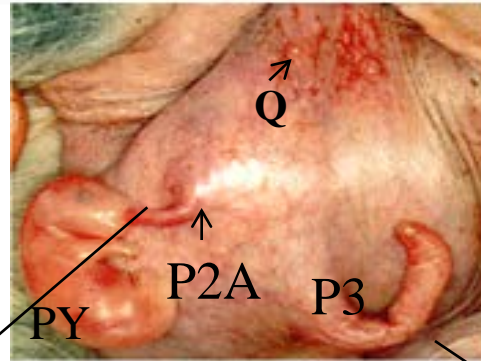
# Possible mechanisms for ACL?

1

## Hormones

Changing hormone concentrations does not significantly affect milk protein gene expression

# Mechanism 2: Sucking by the young



**Highly sensitive to oxytocin**



**Gentle but continuous sucking by the newborn causes sustained release of small amounts of milk**

**Low sensitivity to oxytocin**



**Only aggressive sucking by older sibling stimulates milk secretion**

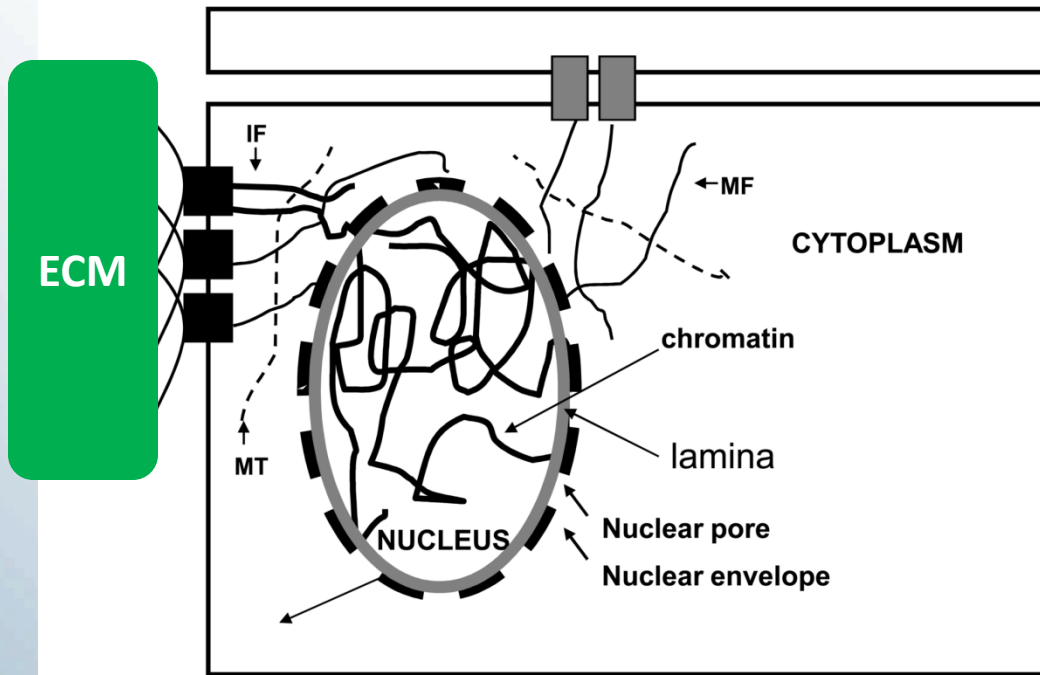
*D. W. Lincoln and Marilyn B. Renfree, 1981*

# Mechanism 3: The Extracellular matrix (ECM)

## Rationale

**Stromal/ epithelial interactions regulate mammary differentiation**

# ECM-Cell interaction



**Cell comes in contact with ECM**

**Mechanical and biochemical signals transduced to the nucleus**

**Change in gene expression profile**

**New proteins secreted into ECM**

# Our hypothesis

P2A cells  P2B ECM = P2B phenotype

P2B cells  P3 ECM = P3 phenotype

P2A phenotype = P2A ECM  P2B cells

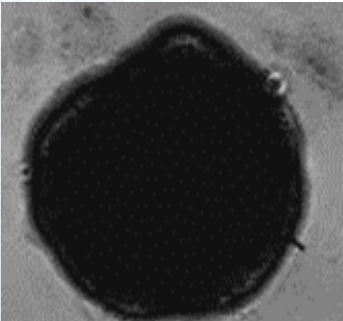
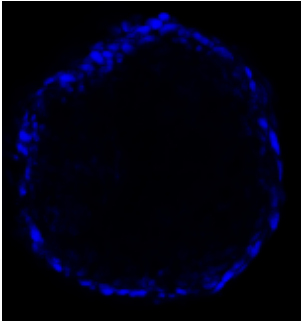
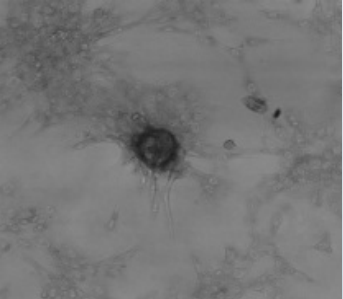
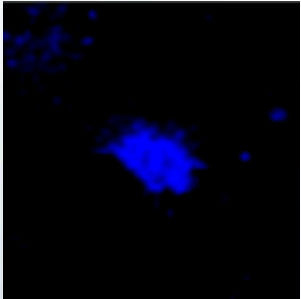
# Approach

Purify mammary extracellular matrix from various phases of lactation (P1, P2A, P2B, P3 and INV)

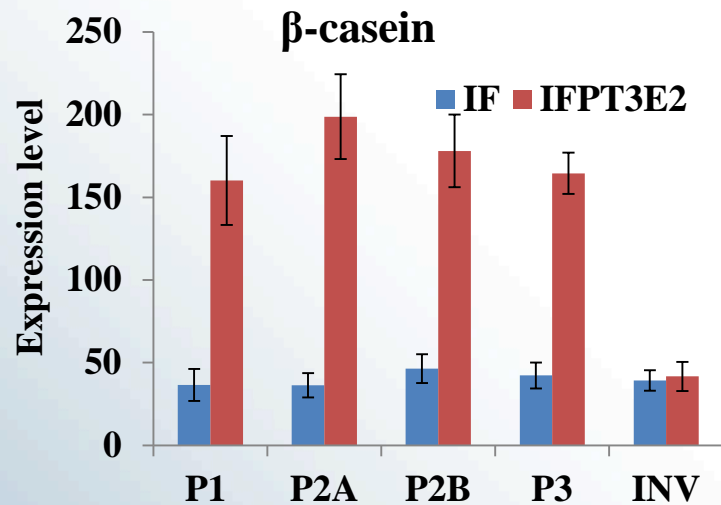
Culture wallaby mammary epithelial cells from P2A and P2B on ECM and stimulate with hormones

Assay expression of milk protein genes by qPCR

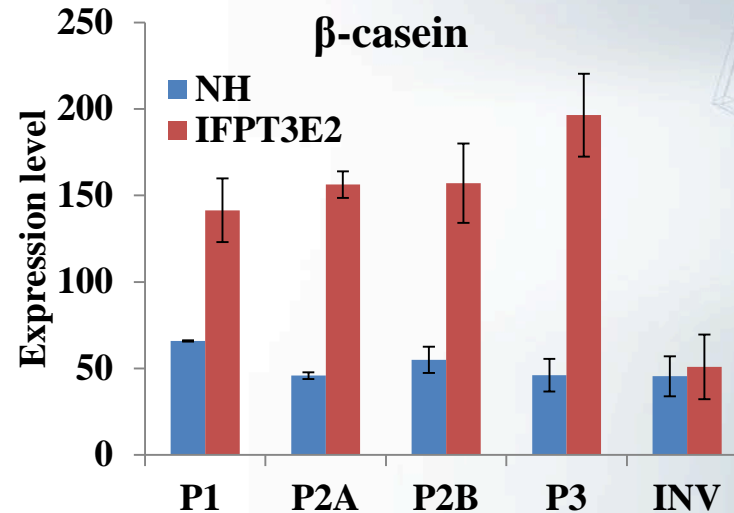
# ECM controls mammosphere morphology

Bright field	Confocal	Lactation Phase of ECM
		<p>P1, P2A, P2B, P3</p>
		<p>INV</p>

# $\beta$ -casein is differentially expressed on ECMs



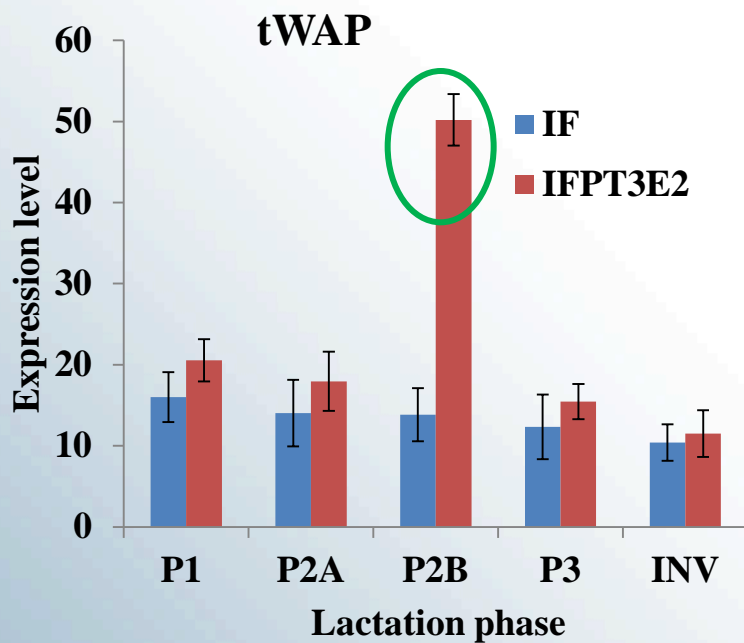
**P2A cells**



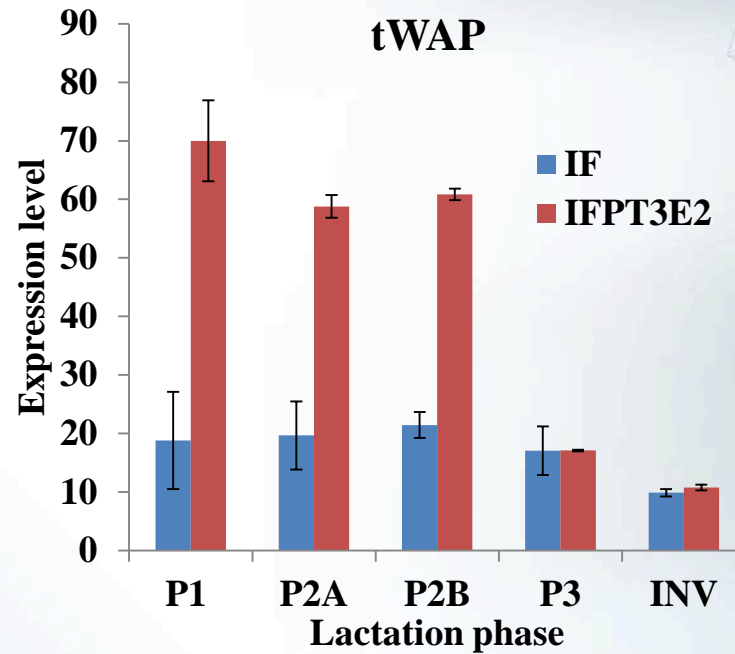
**P2B cells**



# ECM changes lactation phase phenotype of mammary cells

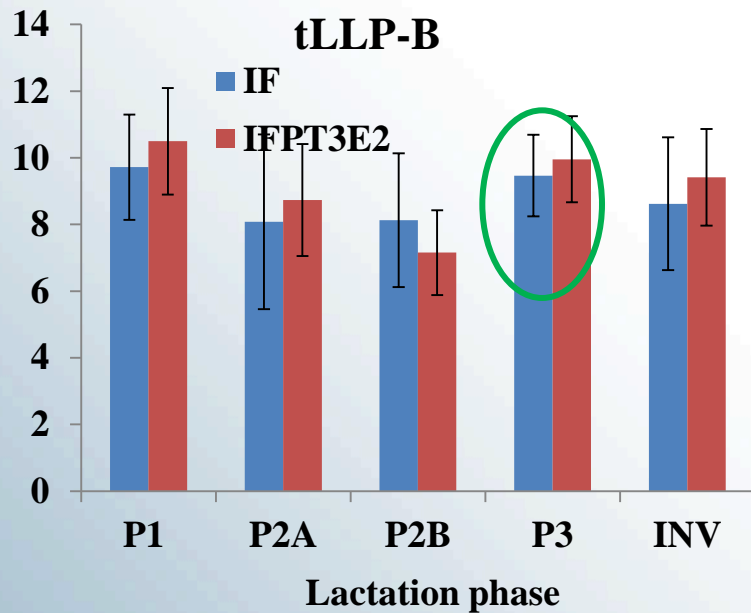


**P2A cells**

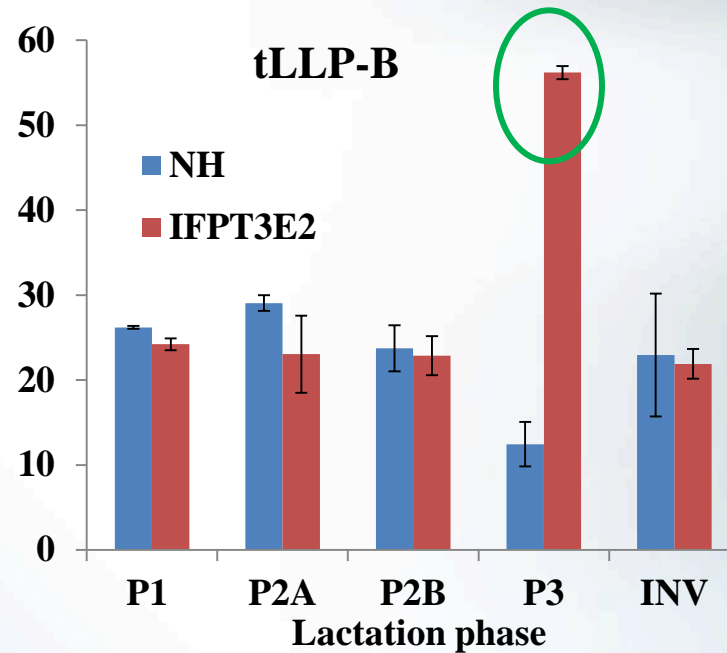


**P2B cells**

# ECM changes lactation phase phenotype of mammary cells

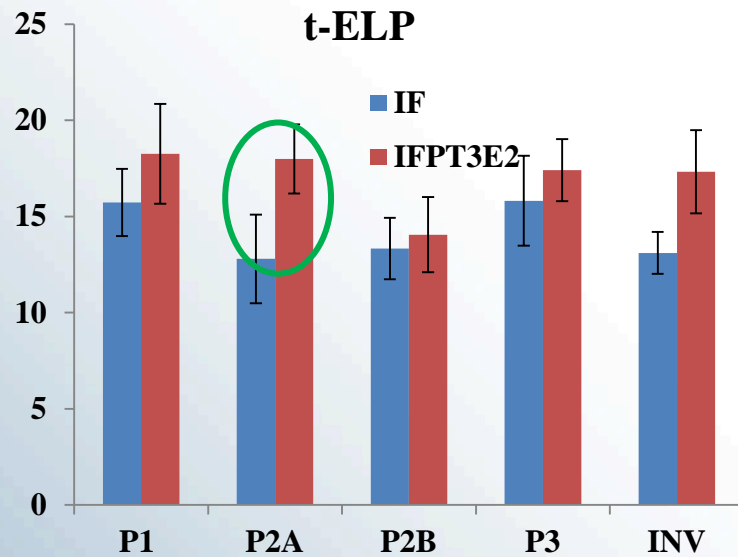


**P2A cells**

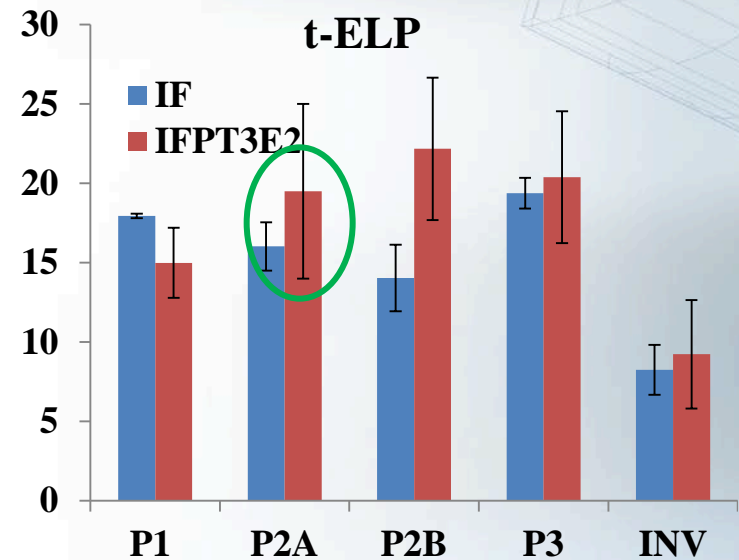


**P2B cells**

# ELP regulated independently of ECM and hormones



**P2A cells**



**P2B cells**

# Summary

- ❑ ECM may regulate the switch from one phase of mammary lactation to another and therefore direct ACL
- ❑ The ECM can only change the phase phenotype in a stepwise manner
- ❑ It may not be possible to reverse the phenotype to an earlier phase

**The ECM could programme ACL by enhancing the response of the mammary gland to hormones**

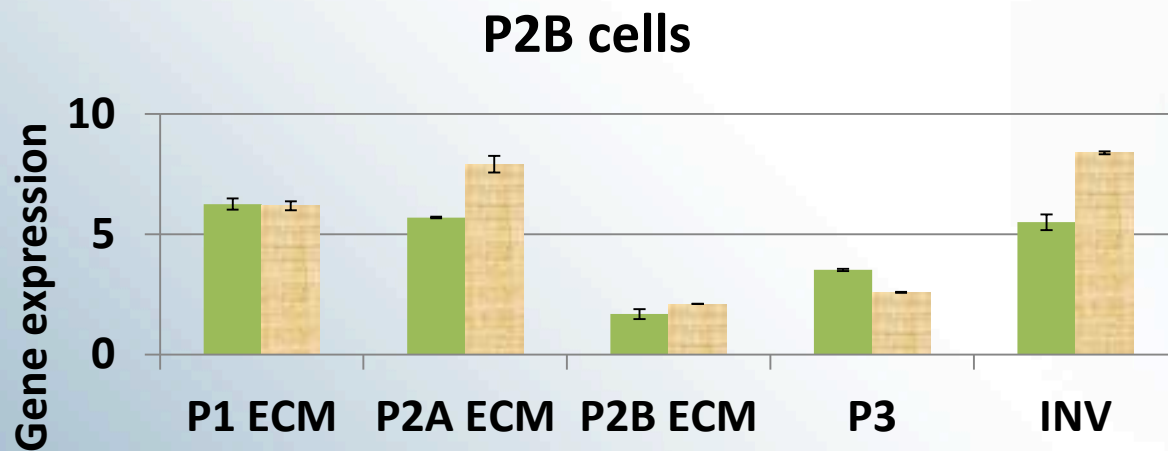
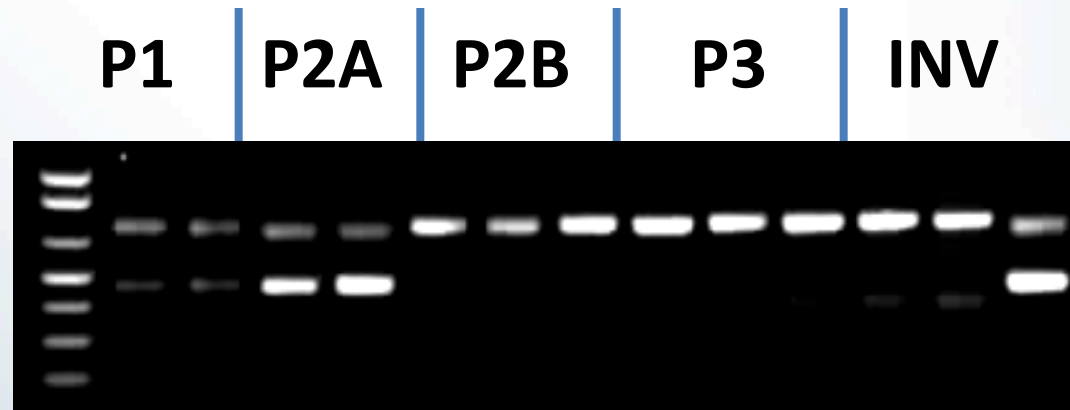
# Future questions

- 1. Can we deliberately change the phenotype of early lactation cells in a stepwise manner into a late lactation phenotype?**
- 2. What are the molecular factors in the ECM that drive ACL?**

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*Thank you*

# Tammar milk cathelicidins regulated by the ECM







# MMP activity is increased during P3 and involution

