



# Novel Applications of Agricultural Biotechnology — State of the Science and Societal Acceptance to Transgenic Animals and Regulatory Issues

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## By Way of Example: Biotechnological Defense Against Mastitis Disease

*2006 Agricultural Outlook Forum  
Prospering in Rural America*  
February 17, 2006



# What has been done ?

## Transgenic Livestock goals

1. Create new animal products



2. Improving animal well-being and food safety



3. Improving production efficiency & nutritional quality



4. Xenotransplantation

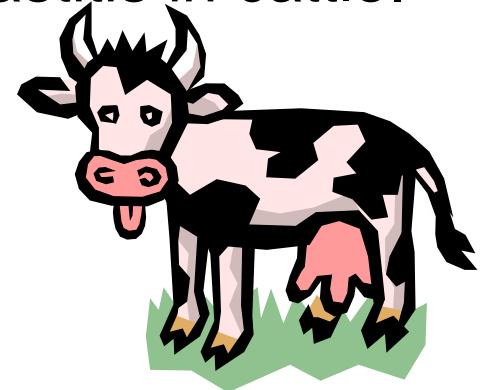


# The Mastitis Project

Goal: Reduce incidence and susceptibility to mastitis in cattle.

## Facts about mastitis

- A third of dairy cows in the U.S. become infected
- Mastitis costs US producers  $\approx \$2$  Billion / year
  - Treatment costs
  - Reduced milk yield, shelf-life, cheese yield
  - Animal well-being diminished, premature culling
- Vaccines, antibiotics and selective breeding are minimally effective

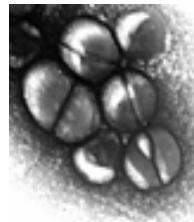
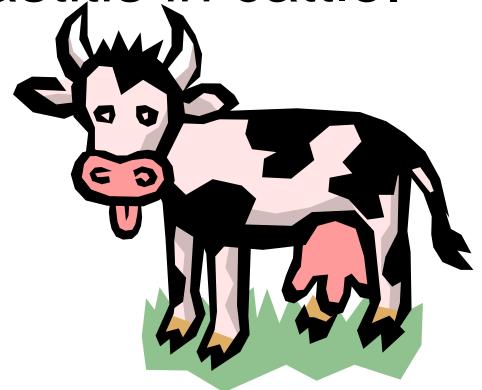


# The Mastitis Project

Goal: Reduce incidence and susceptibility to mastitis in cattle.

Facts about mastitis

- **Disease is on every dairy farm**
- *Staphylococcus aureus* accounts for about a third of cases



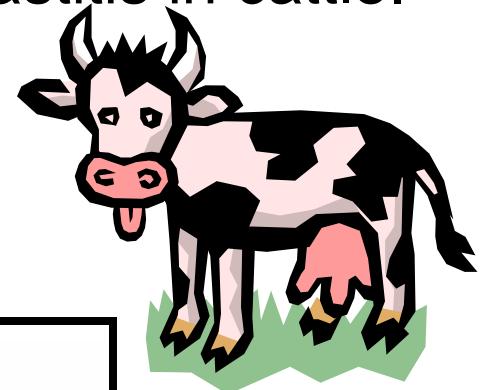
Culling is often the only solution

Staph. aureus

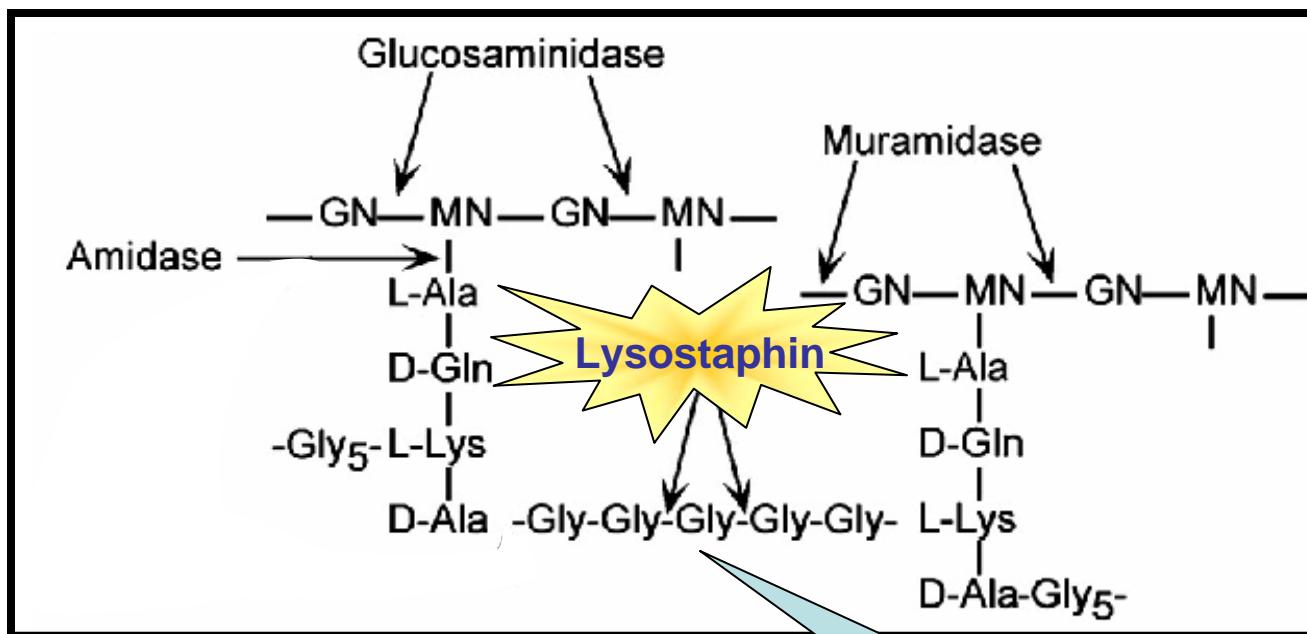


# The Mastitis Project

Goal: Reduce incidence and susceptibility to mastitis in cattle.



Gram-negative outer peptidoglycan layer



Peptidoglycan hydrolase

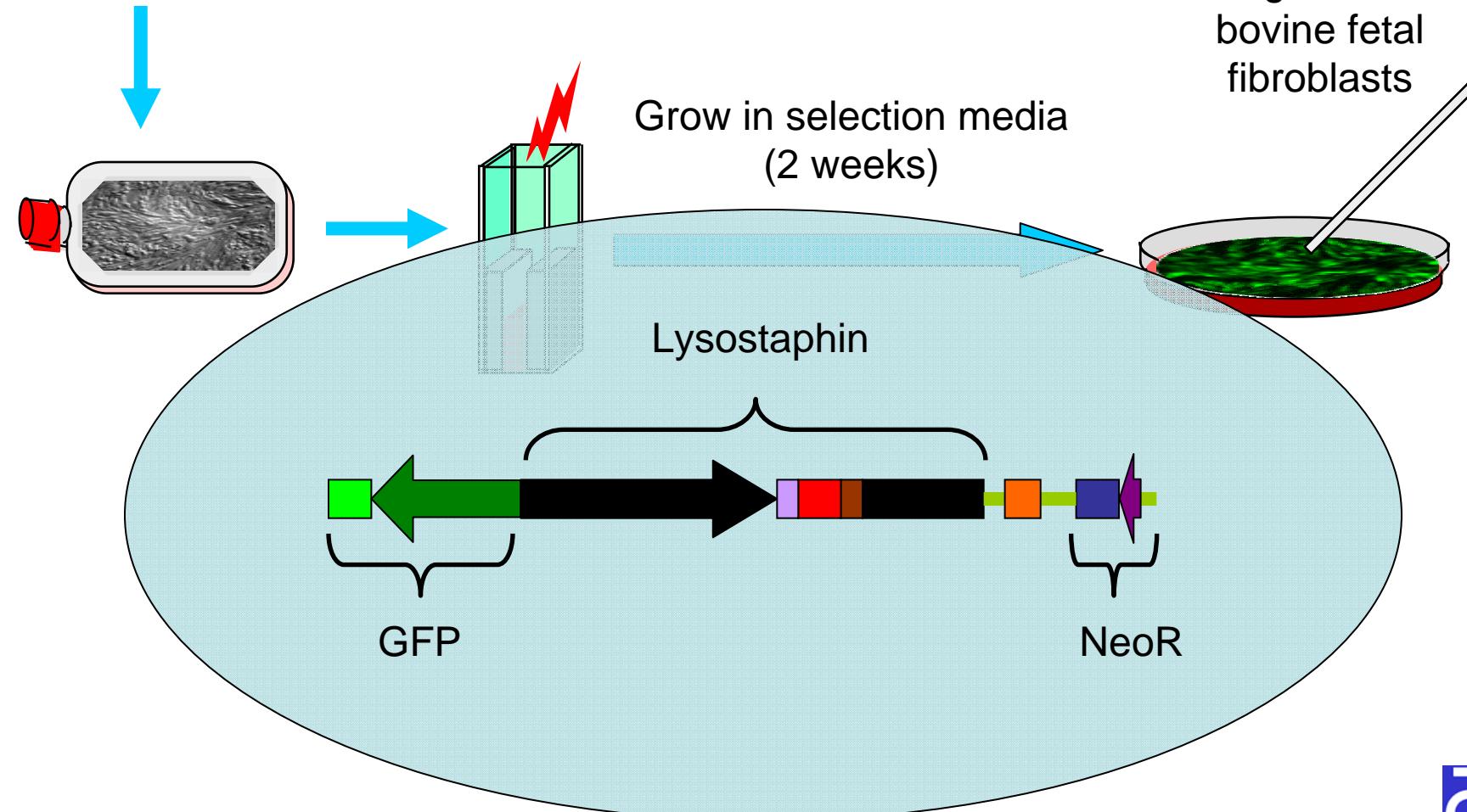
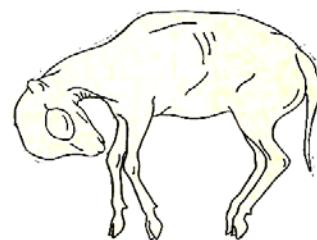
Unique *S.aureus*  
pentaglycin cross link



# Somatic Cell Nuclear Transfer

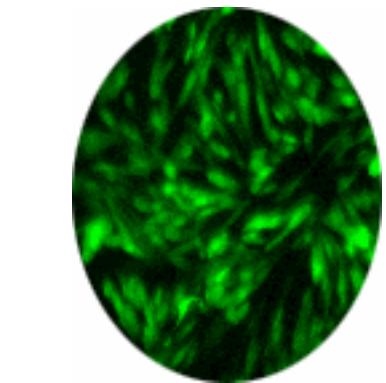
## - Preparing the nuclear donor cells

Jersey fetus

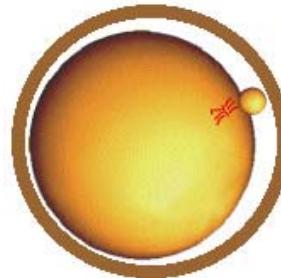


# Somatic Cell Nuclear Transfer

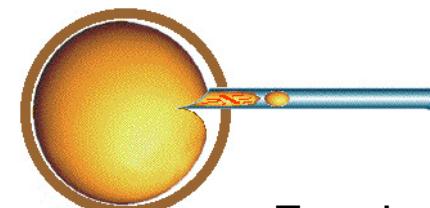
- Producing embryos



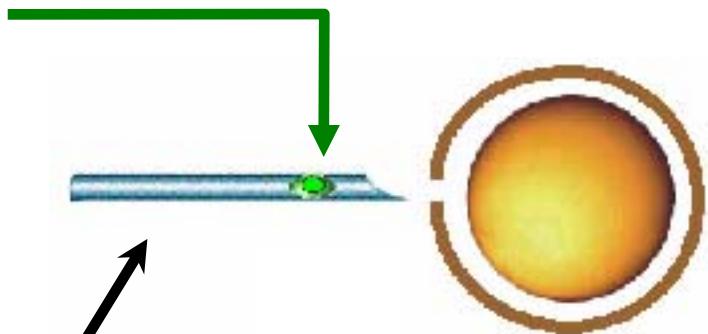
Genetically  
engineered  
donor cells



Slaughterhouse  
derived eggs

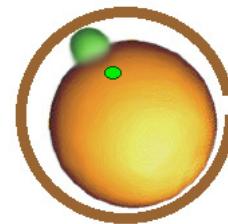


Enucleate  
unfertilized  
eggs

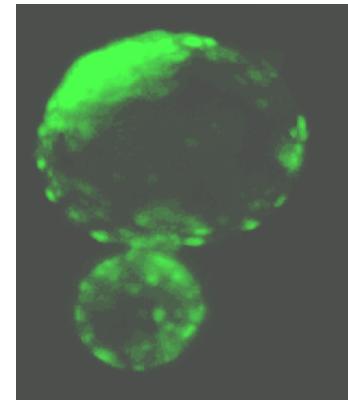


Genetically  
engineered  
donor cells

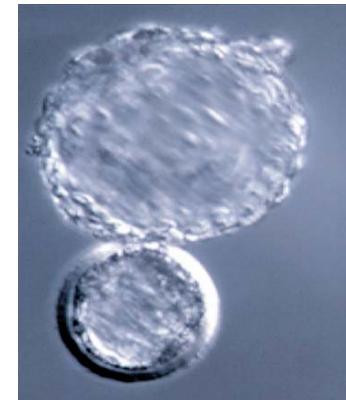
Fuse donor cell  
to empty egg



Simulate  
fertilization



Culture for a week



# First genetically modified cows carrying a disease resistance gene

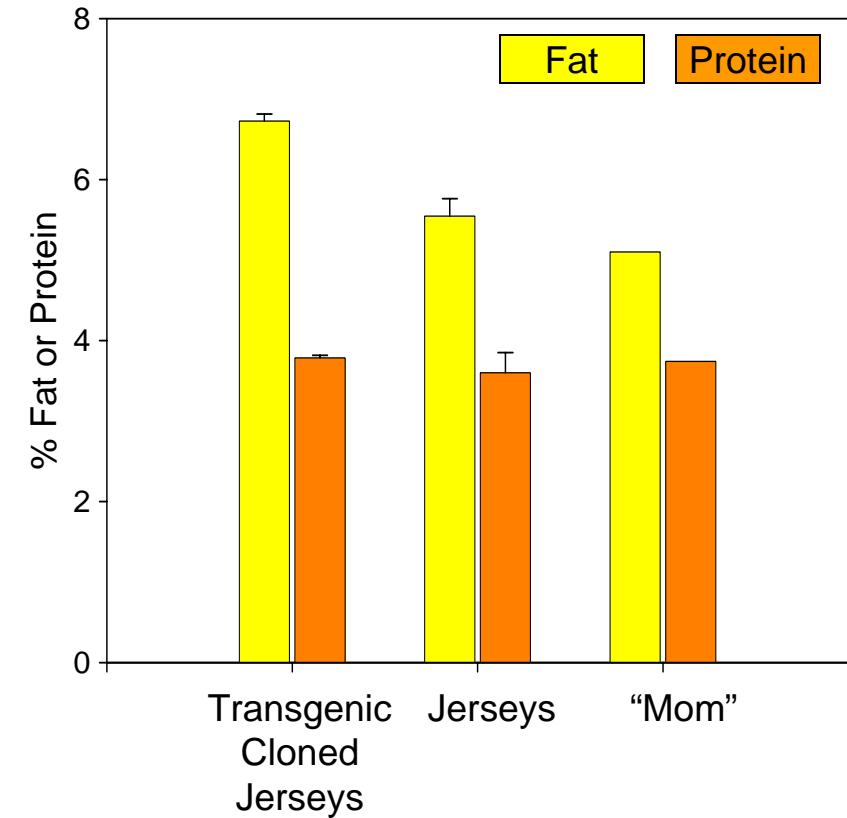
Annie



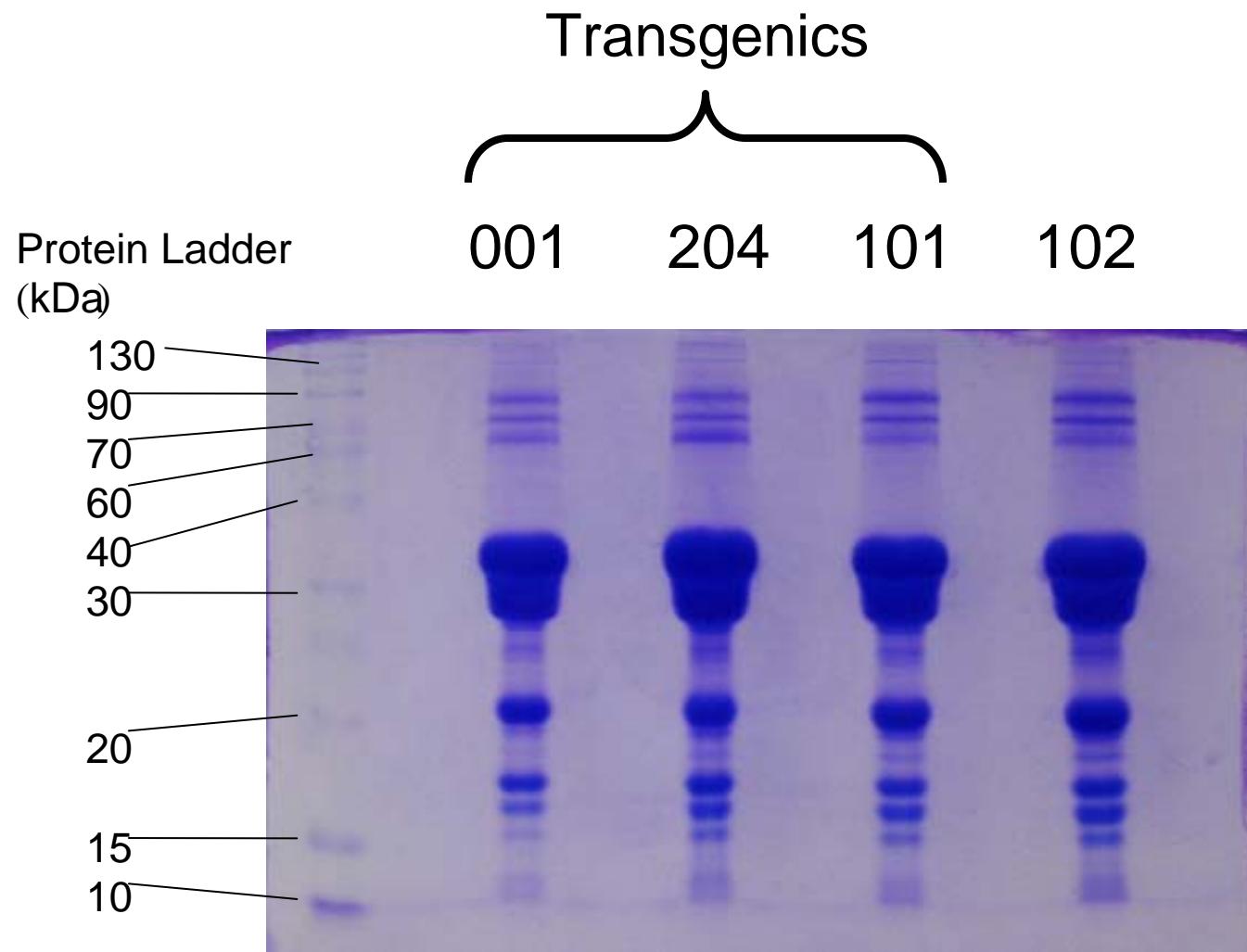
GEM



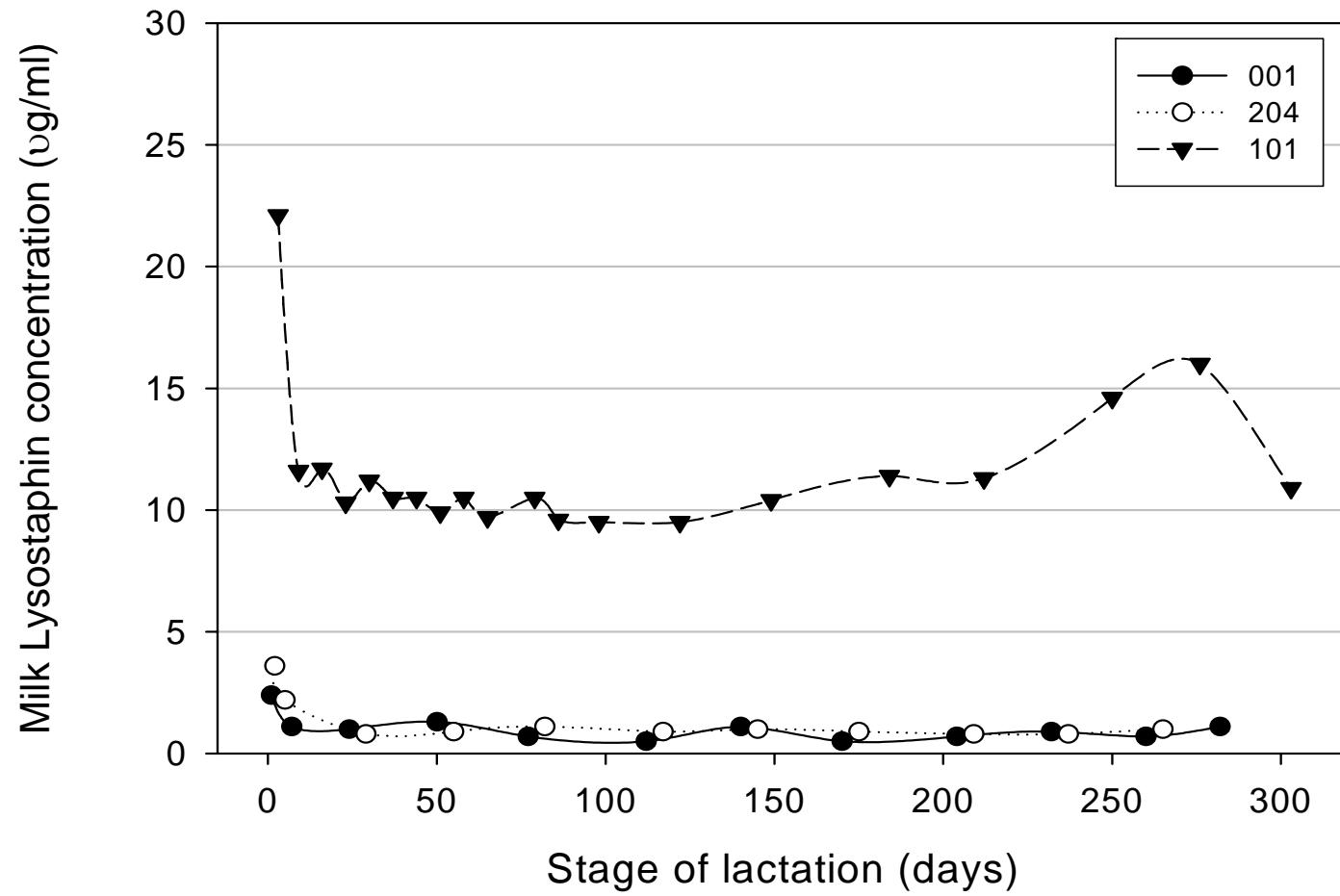
# Estimated adult performance (305 Mature Equivalents) of Transgenic clones and their “Mother”



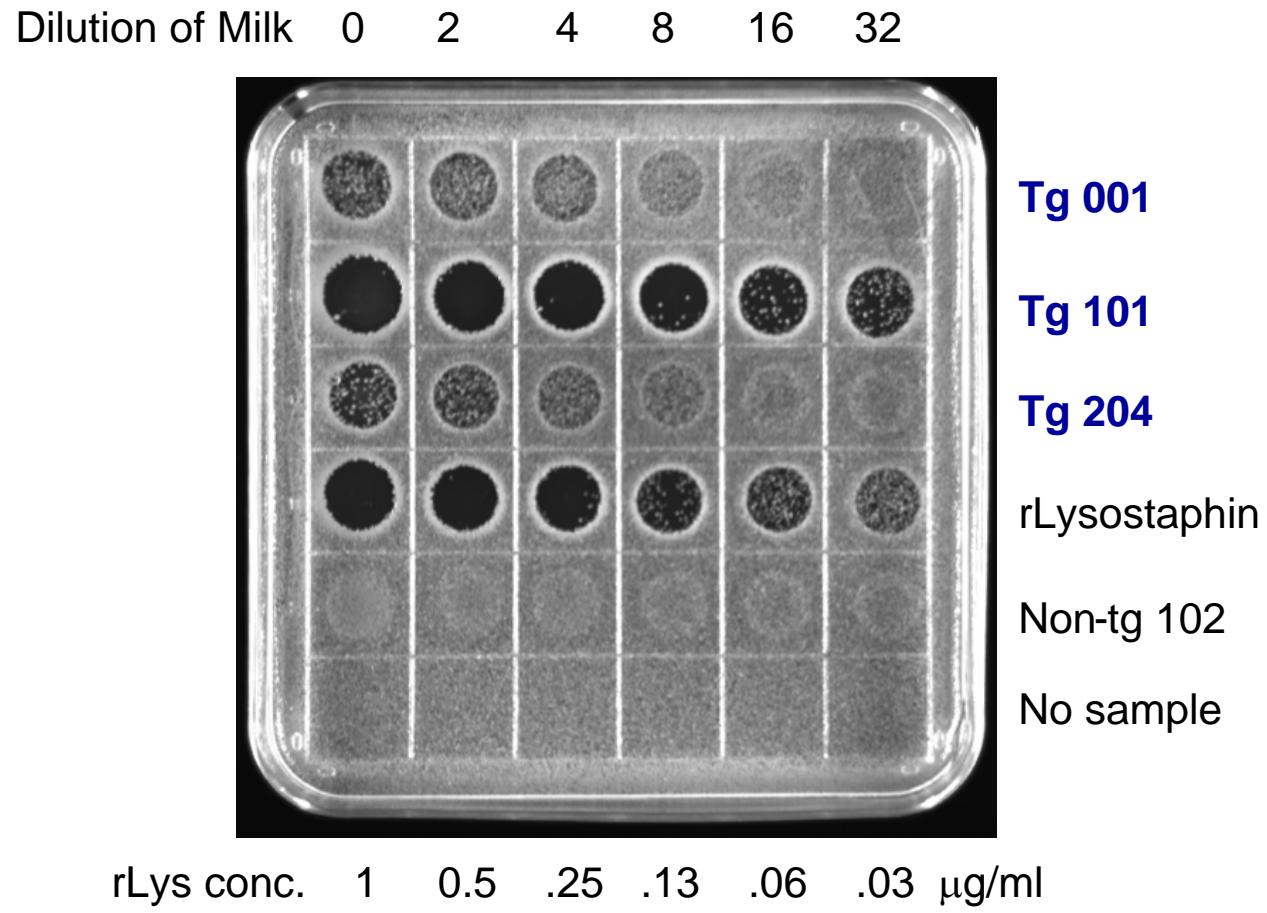
# Milk Protein from Transgenic and Non-tg Jerseys



# Lysostaphin in milk (1st lactations) of transgenic cloned Jerseys



# Lysis of *S. aureus* by Milk from Transgenic Cows



Lawn  $\approx 10^5$  c.f.u.



# *S. aureus* Challenge – cow assignment schedule



3 *S. aureus* strains:  
1 per gland  
Saline in the 4th

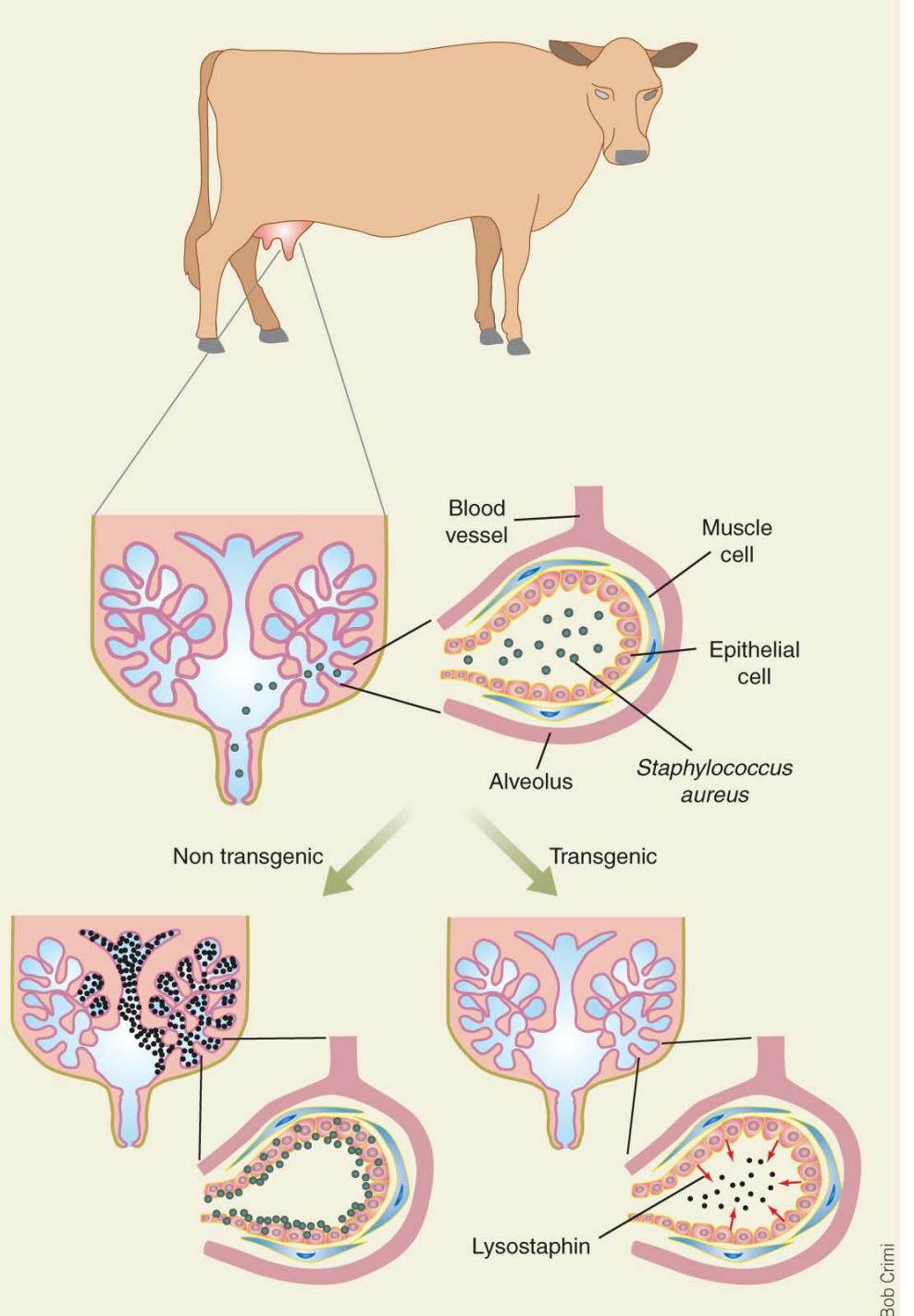
Type	Cow	Stage of Lactation (days)			
		60	150	240	270
Transgenic Jersey	001	✓	✓		
Transgenic Jersey	101	✓	✓		✓
Transgenic Jersey	204	✓	✓		
Non-transgenic Jersey	102	✓	✓	✓	
Non-transgenic Jersey	207	✓			
Non-transgenic Jersey	208	✓			
Non-transgenic Holstein	2227	✓			
Non-transgenic Holstein	2234		✓		✓
Non-transgenic Holstein	2262	✓		✓	
Non-transgenic Holstein	2264	✓	✓		
Non-transgenic Holstein	2287	✓			
Non-transgenic Holstein	2288	✓			
Non-transgenic Holstein	2291	✓	✓		

# *S. aureus* Challenge

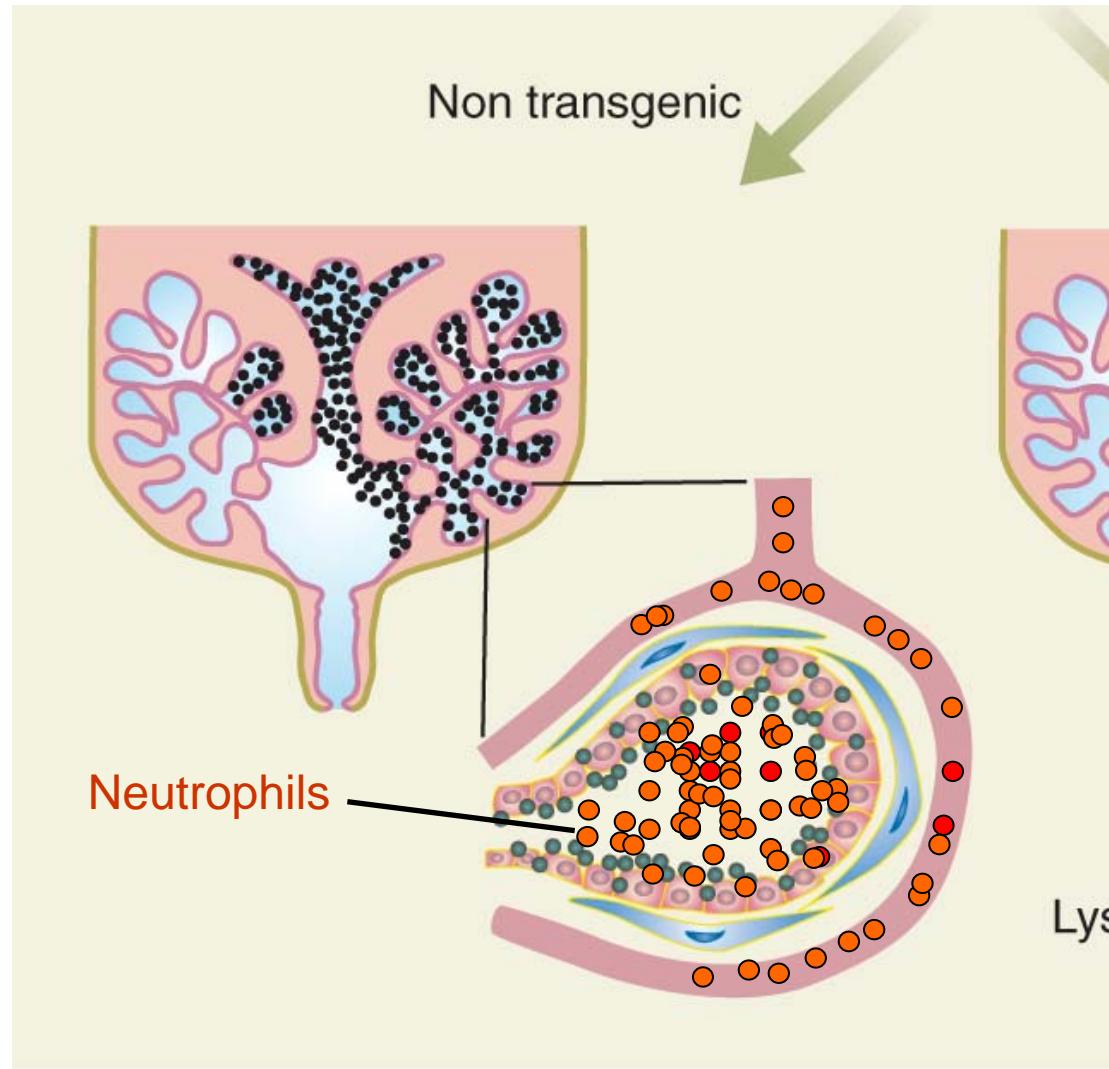


3 *S. aureus* strains:  
1 per gland  
Saline in the 4th

Type
Transgenic
Transgenic
Transgenic
Non-transgenic

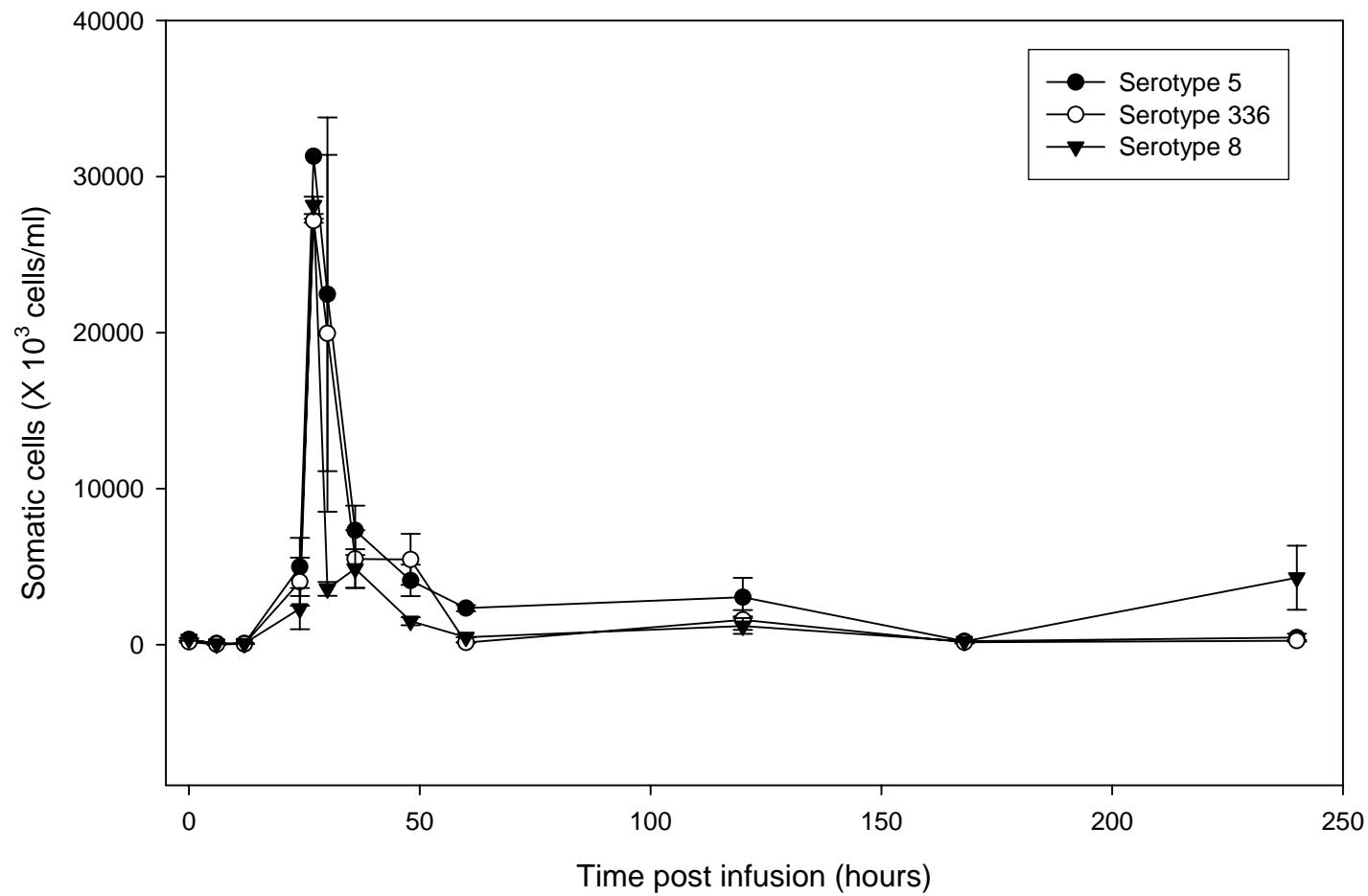


# Milk “somatic cells” infiltrate gland in response to infection



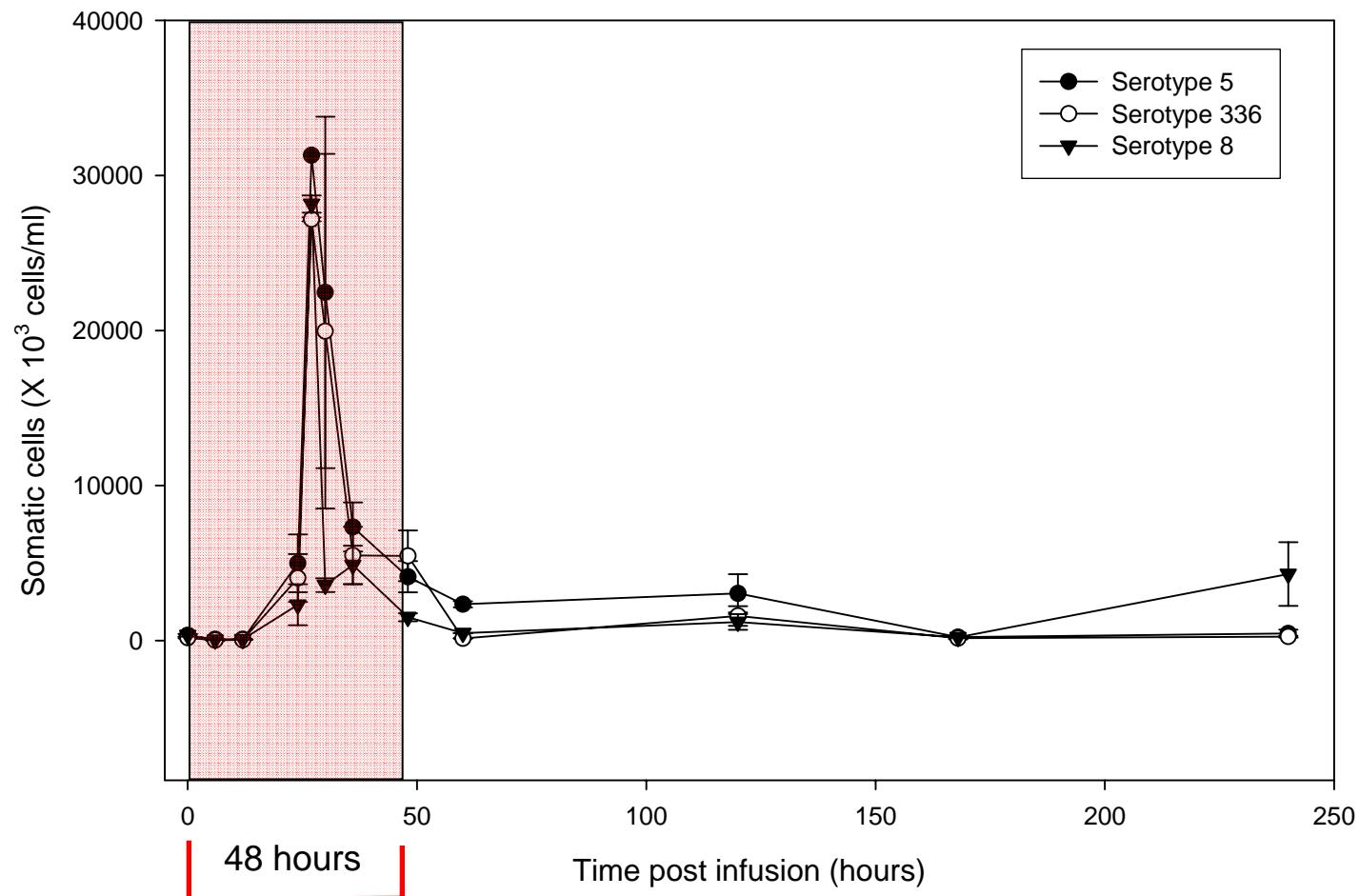
# *S. aureus* Milk somatic cell response

10 Non-transgenic controls



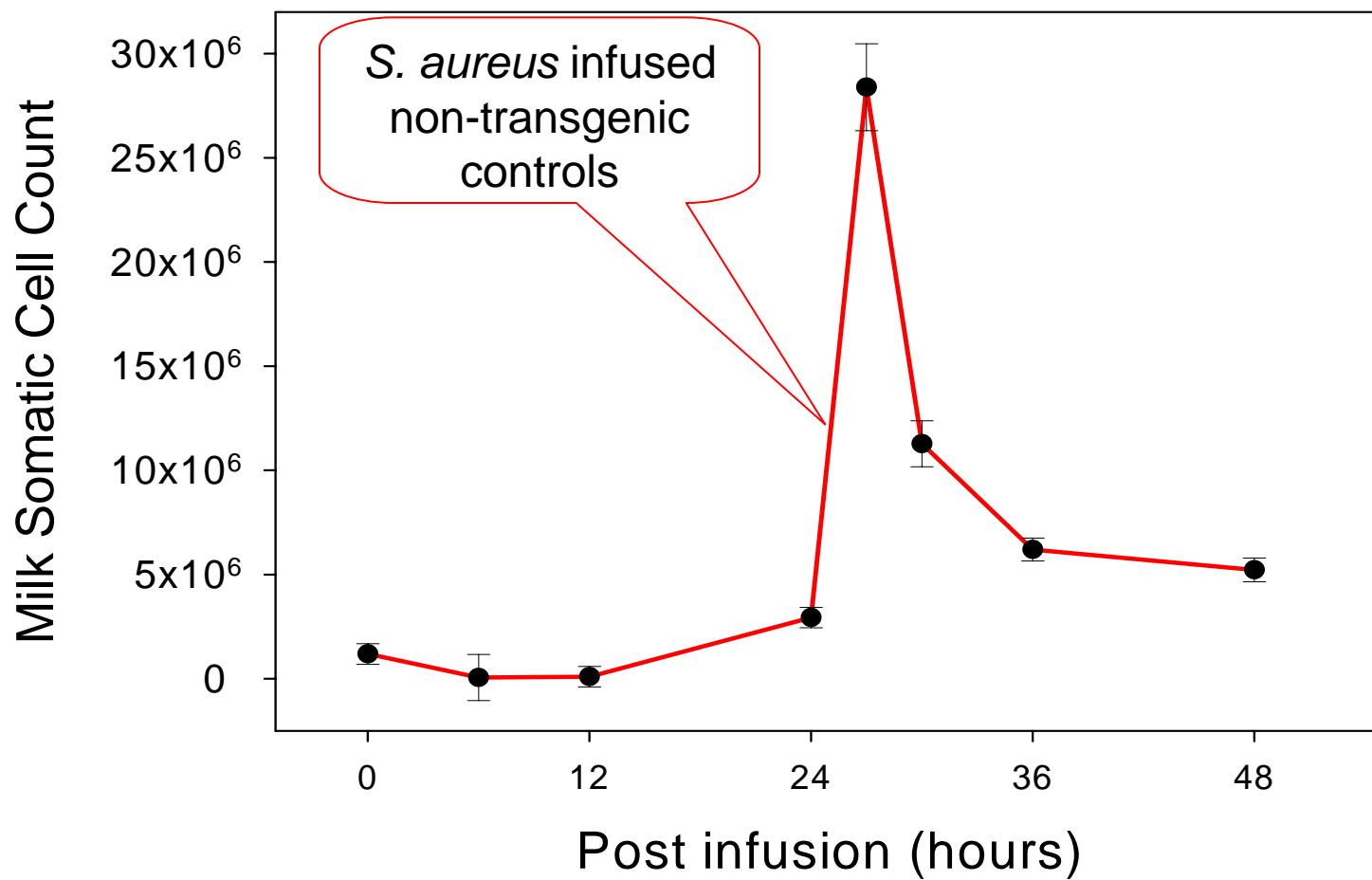
# *S. aureus* Milk somatic cell response

10 Non-transgenic controls

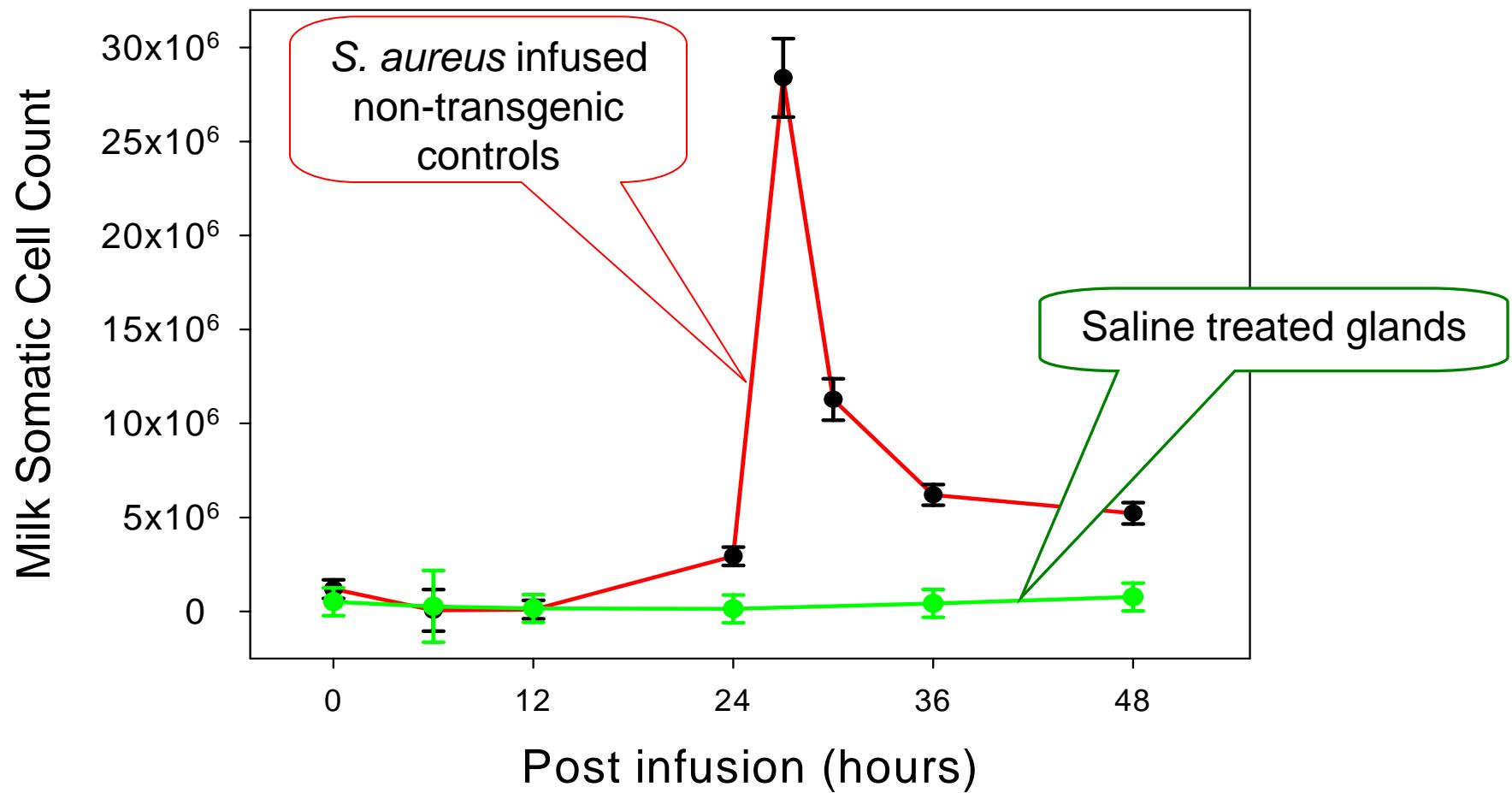


# *S. aureus* Milk somatic cell response

10 Non-transgenic controls

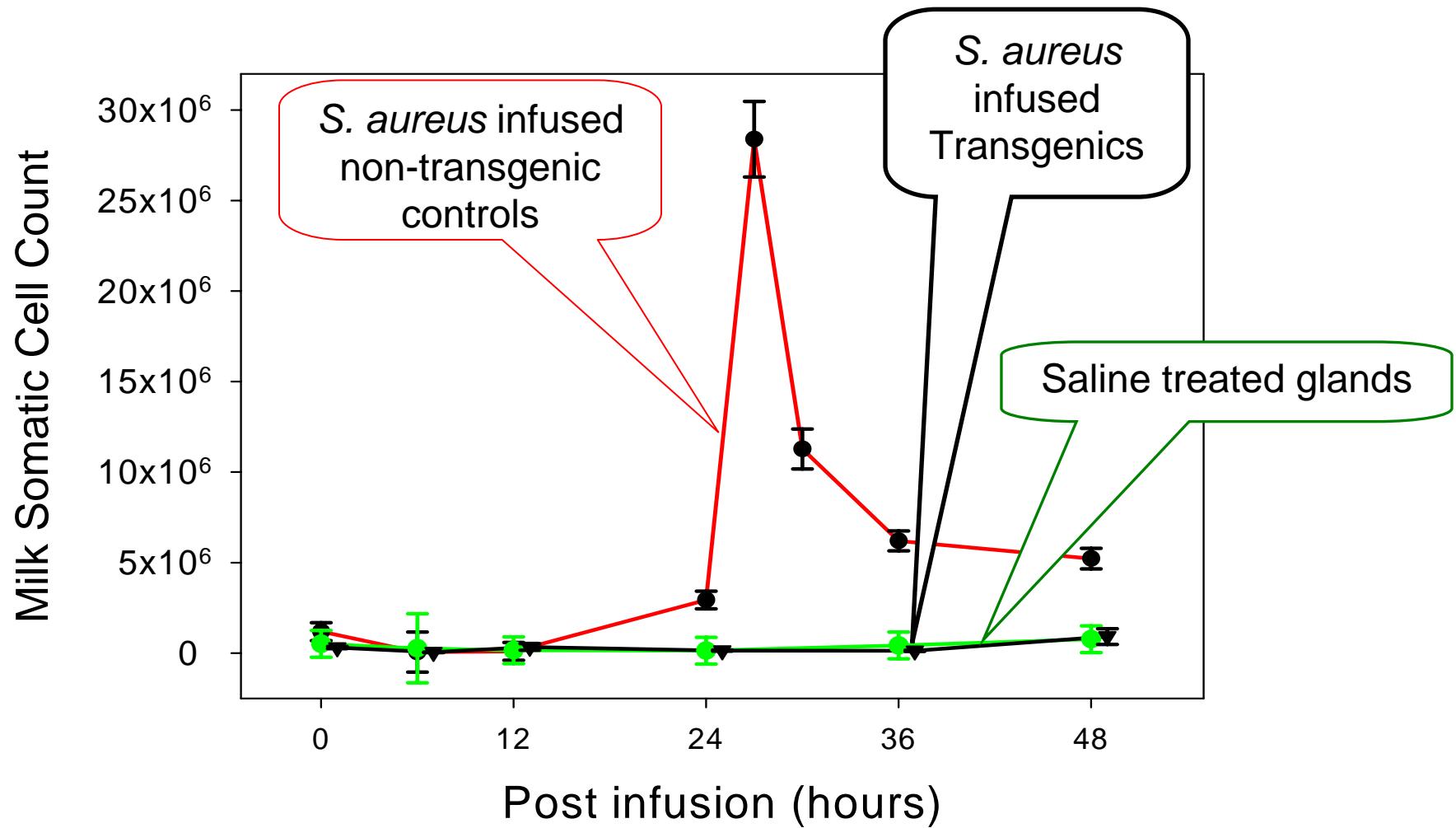


## *S. aureus* Milk somatic cell response



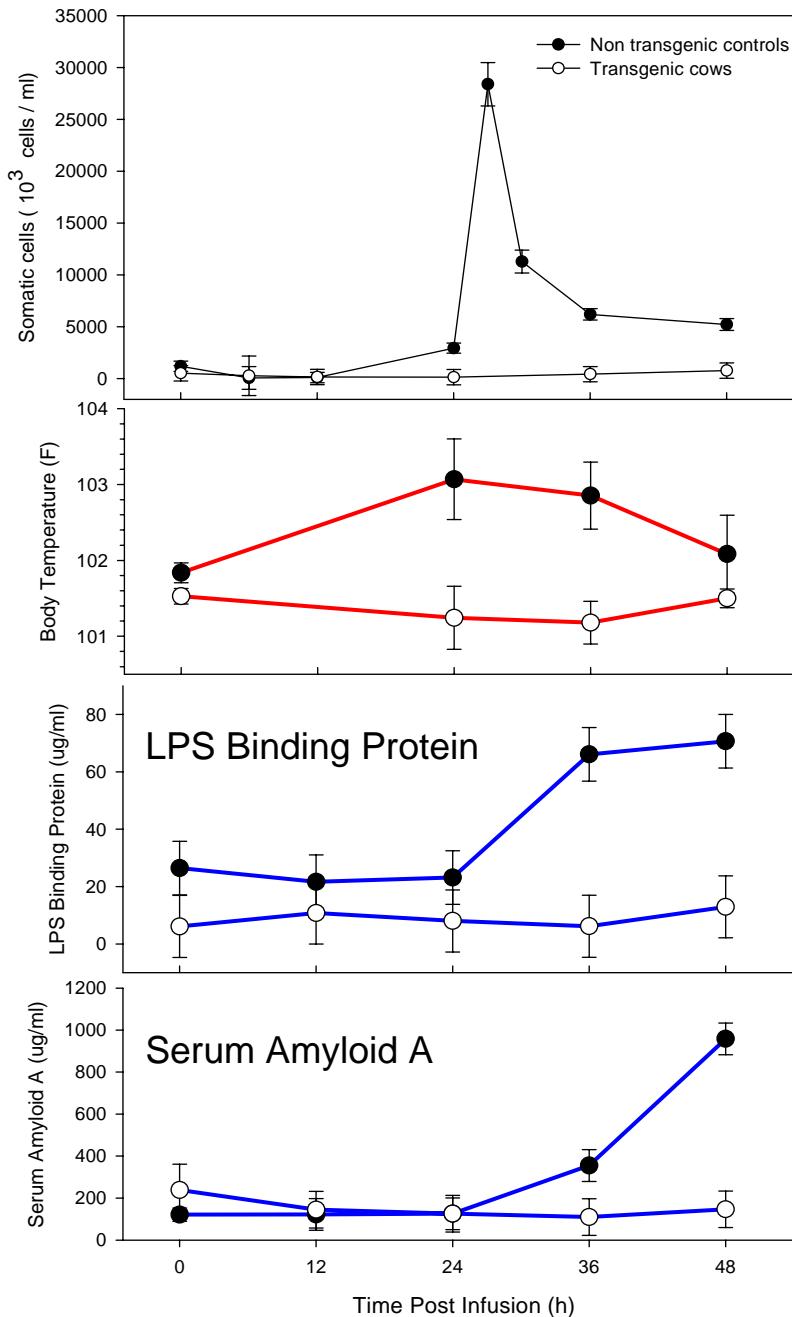
# *S. aureus* Milk somatic cell response

3 Transgenic, 10 Non-transgenic controls



# Immune Responses to *S. aureus*

## Systemic response



Local response

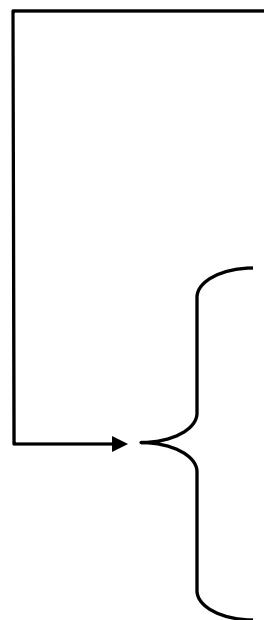
Body temp

Acute phase proteins



## Infection rate (Infection = 2 consecutive milk samples with live *S. aureus*)

Cows		Glands	
Genotype	No.	Infused No.	Infected No. (%)
TG	3	21	3 (14%)
Non-TG	10	48	30 (63%)



Transgenic 001 (0.7)	6	2 (33%)
Transgenic 101 (12)	9	0
Transgenic 204 (0.6)	6	1 (17%)
Saline infused glands	23	1 (4%)

# Summary

- Lysostaphin can protect against infection (3 µg/ml is probably enough)
- Lysostaphin seems to cause no harm



# Future plans

- Immediate: Is the lysostaphin milk OK ?
- Identify additional anti-mastitis peptides
- Can we avoid resistant strains ?

# Just the Beginning

