

# The Research Imperative

## Arnold Theiler Memorial Lecture

### 2017

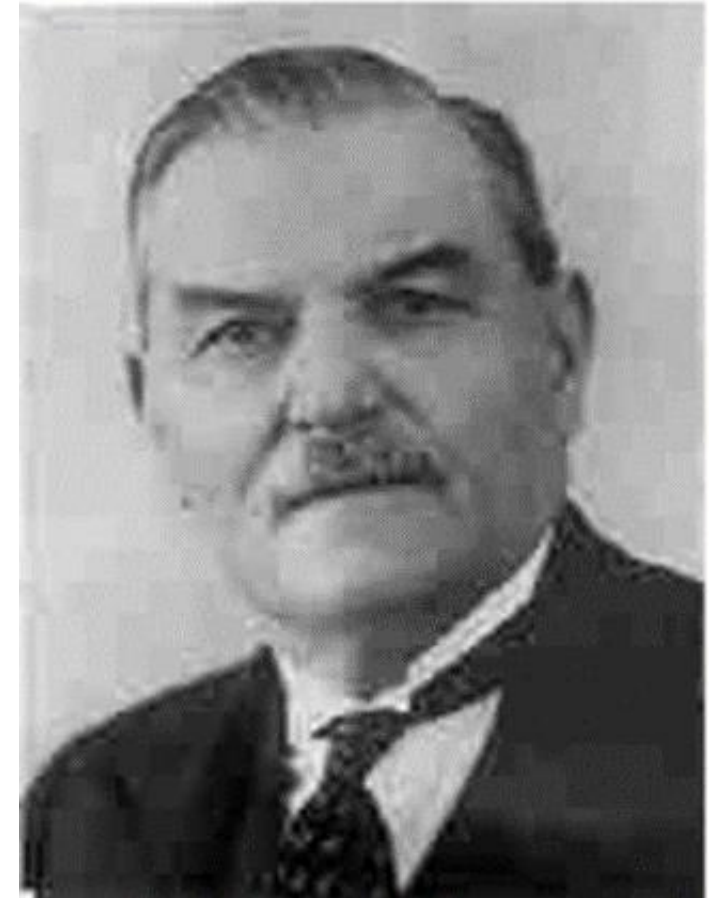
**Robert O. Gilbert, FRCVS**

Professor, Ross University School of Veterinary Medicine  
Professor Emeritus, Cornell University



# Outline

- Personal research in postpartum uterine disease of dairy cows
- Reflections on research more generally
  - Production animal research
  - Research in veterinary practice
    - Academic practice
    - Private practice
- The research imperative
  - For Society and the Profession
  - For Universities
  - For individuals



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Lefapha la Diseense tsa Bongakadiruhwa



# Up to circa 1990

- Most agreed “endometritis” was bad for reproduction
  - Several disagreed
    - Miller HV, et al., Bov.Pract. 1980
    - Griffin, Hartigan & Nunn, Theriogenology, 1974 a,b
- Diagnosis
  - N. America: palpation and visible exudate
    - Incidence < 20 %
  - Europe: vaginoscopy
    - Incidence ~ 40 %





Maurice E. "Pete" White

Editor, *Cornell Veterinarian*  
Creator, "Consultant"

Bovine Endometritis: The burden of proof. *Cornell Vet.*, 1992



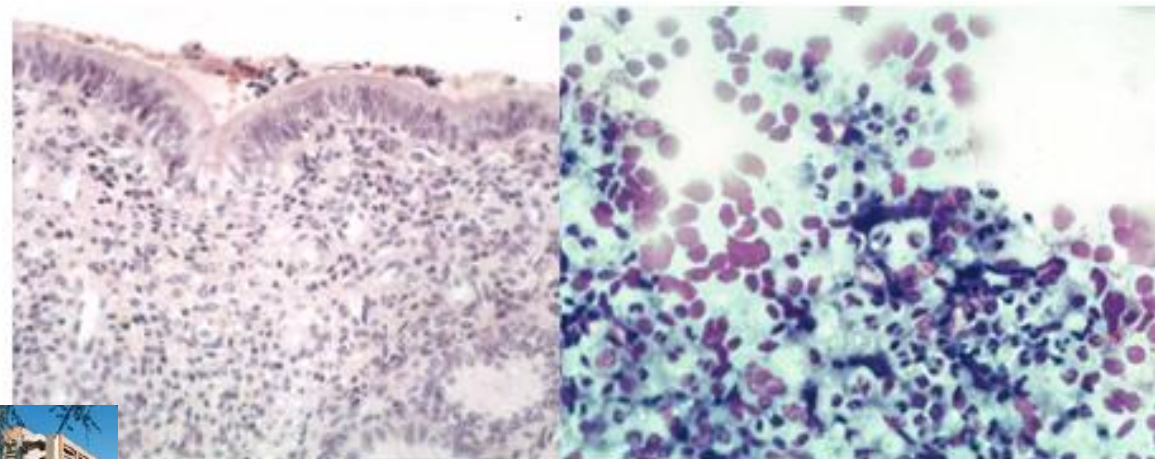
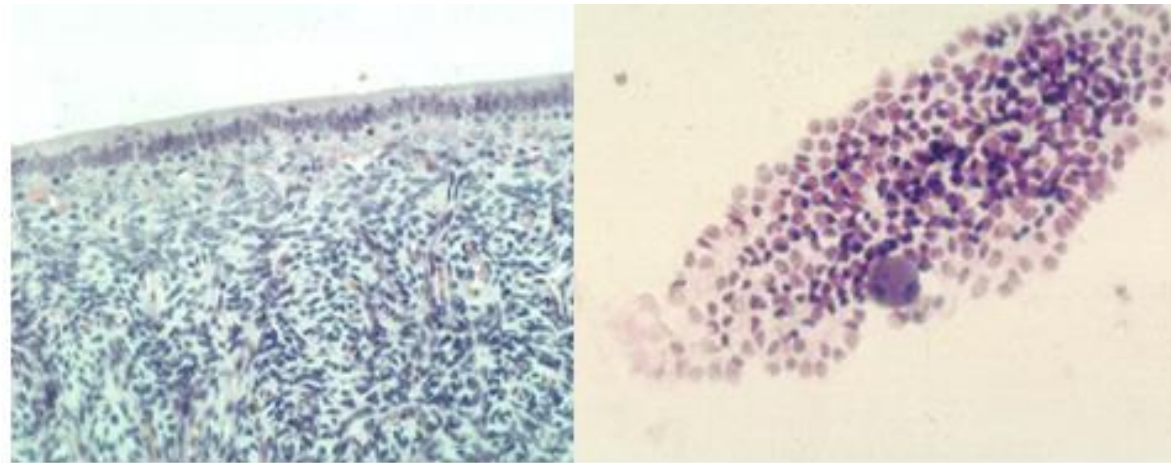
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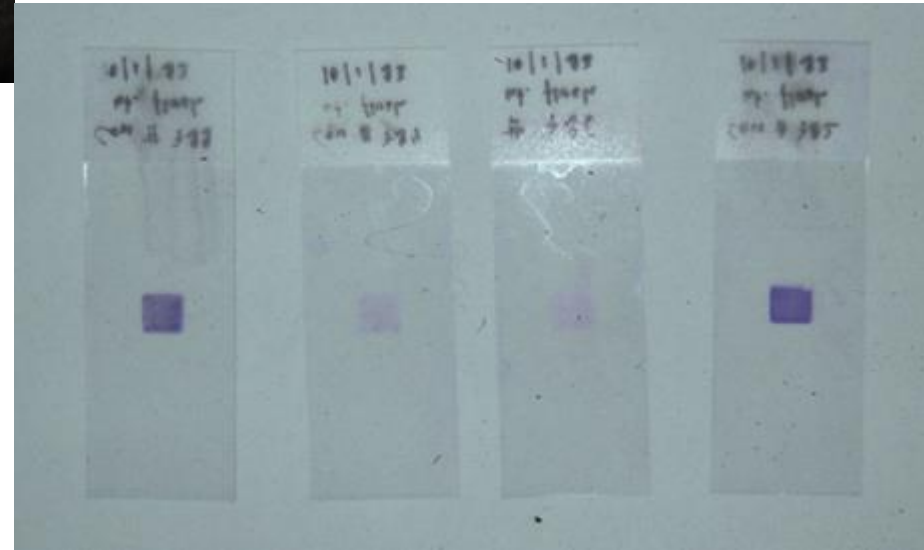




# Endometrial cytology



# Endometrial cytology



# Endometritis

- Cows at 40 – 60 days postpartum
  - End of voluntary waiting period
- 5 dairy herds in Central New York
- Endometrial cytology
- No further involvement in management
- Followed up via dairy records



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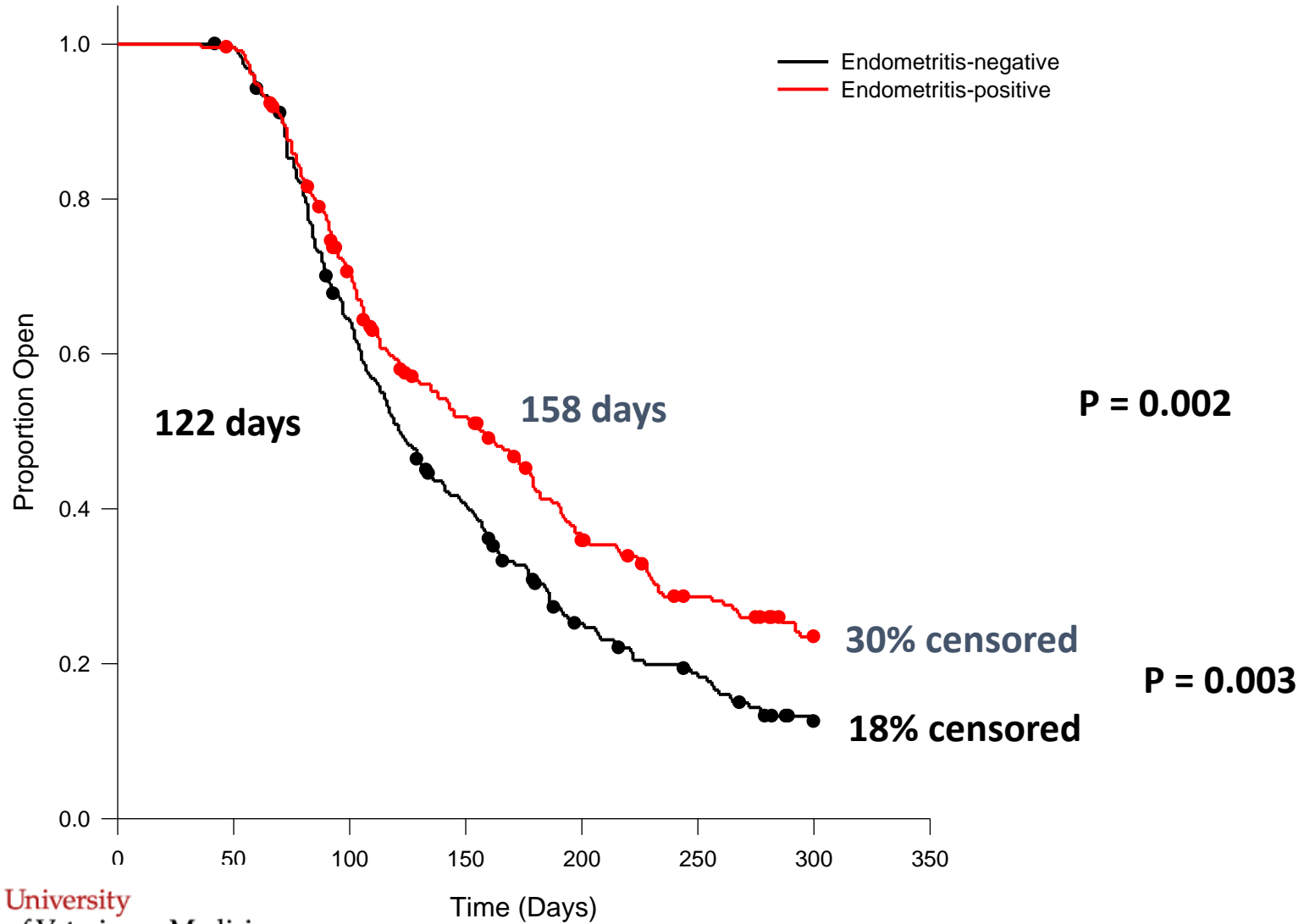
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# Endometritis

## Survival Analysis

### Effect of Endometritis at 7 weeks





# Some central questions

- Which pathogens are primarily responsible for endometritis?
- Which metabolic and immune factors mediate susceptibility to and pathogenesis of endometritis?
- How does endometritis mediate infertility?



# Which pathogens cause endometritis?

- Most cows have postpartum uterine bacterial infection
  - Only a subset develops disease
- Extensively studied by conventional means
- Mixed postpartum population initially judged to be insignificant (Griffin, Hartigan, Nunn, 1974)
  - Persistence of *Trueperella pyogenes* beyond 21 days detrimental to fertility
- Synergistic action of *T. pyogenes* and gram-negative anaerobes established
  - *F. necrophorum*, *P. melaninogenica*, *Bacteroides* spp.

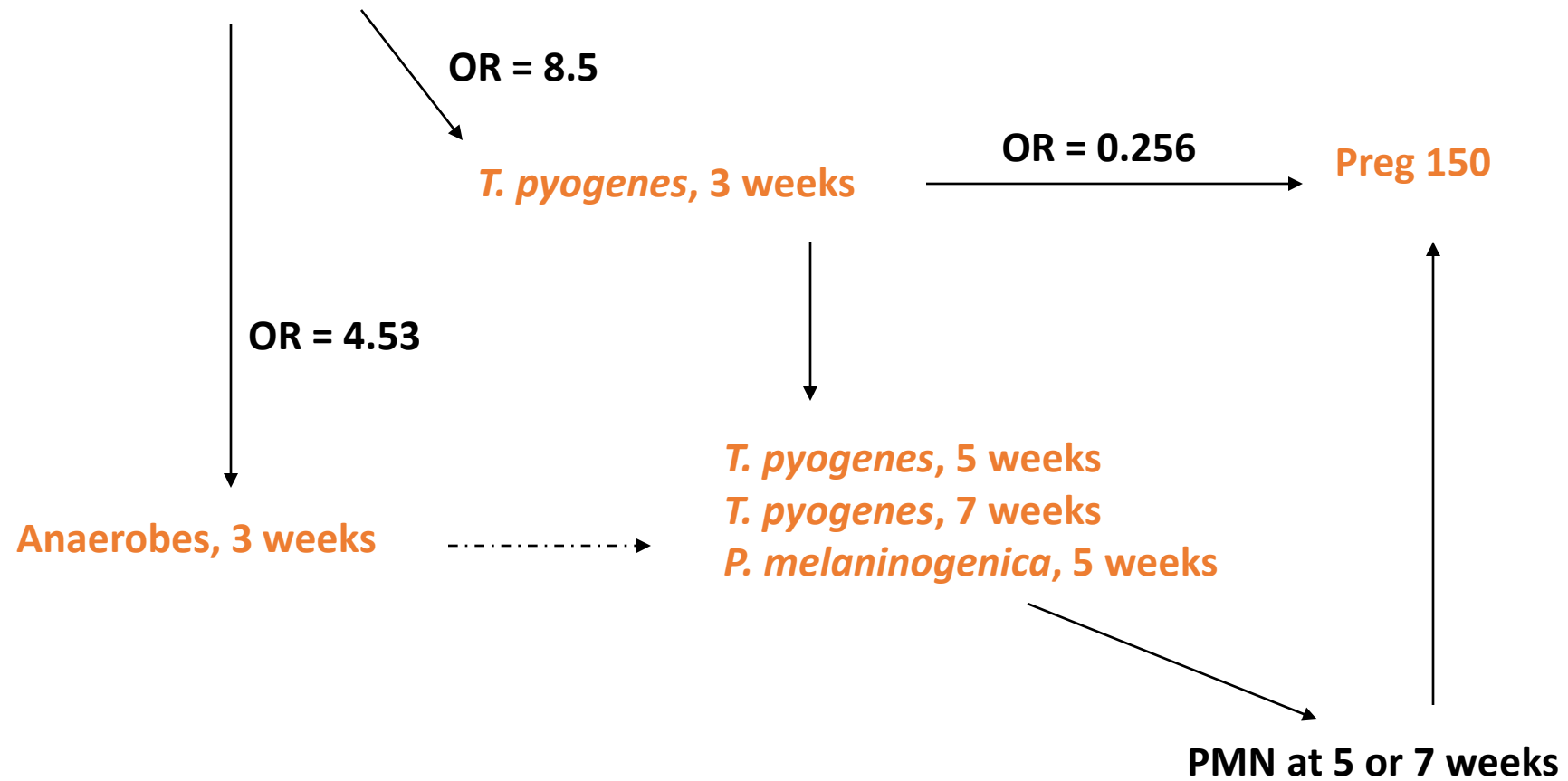


# *E. coli*

- Seldom isolated from diseased uteri
- Early presence seems to predispose to later infection by pathogens



## *E. coli*, 1st week

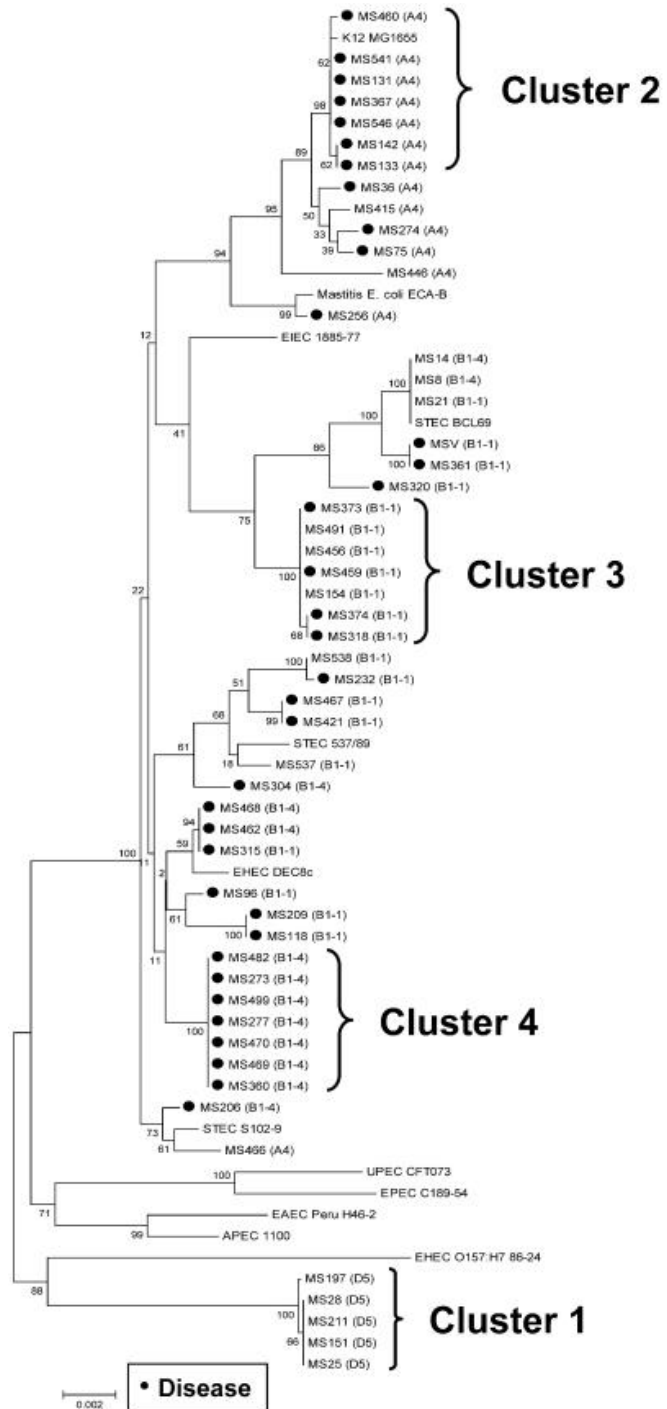


# Risk of Uterine *E. coli*

Risk Factor	n	<i>E. coli</i> (95% CI)	Adj. OR	P
Twin	18	60 (35 – 80)	4.4	<0.01
Stillborn	18	55 (32 - 77)	3.7	
Male alive	138	35 (27 - 42)	1.6	
Female alive	200	25 (19 - 31)	Ref.	
BCS<3	142	36 (26 – 47)	2.3	<0.01
BCS=3	98	42 (32 – 52)	2.8	
BCS>3	134	20 (13 – 29)	Ref.	
Non retained	339	29 (24 – 34)	Ref.	
RFM	35	65 (44 – 81)	4.7	<0.01







## Uterine *E. coli*

- Specific clusters associated with uterine disease
- Pathogenic strains
  - more likely to adhere to uterine cells
  - more likely to be intracellular in uterine cells
  - elicited more reaction from uterine cells
  - caused severe disease in mice

# *E. coli* Virulence Factors and Metritis

- *fimH, astA, ibeA, cdt, hlyA, kpsMII*
  - Significant association with metritis risk
- Apparent synergism between *fimH* and *astA*



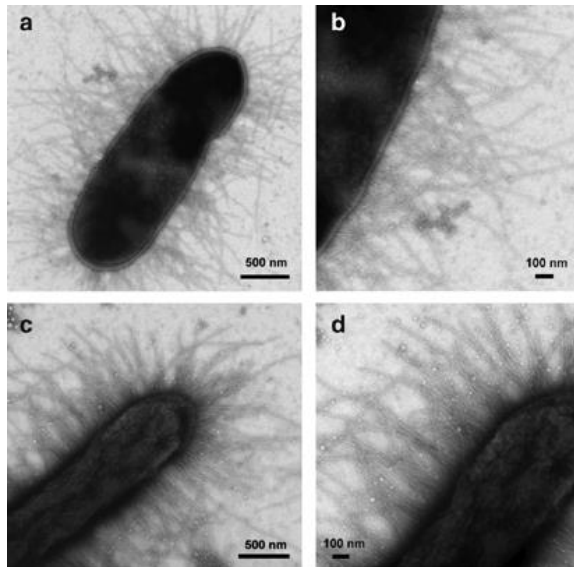
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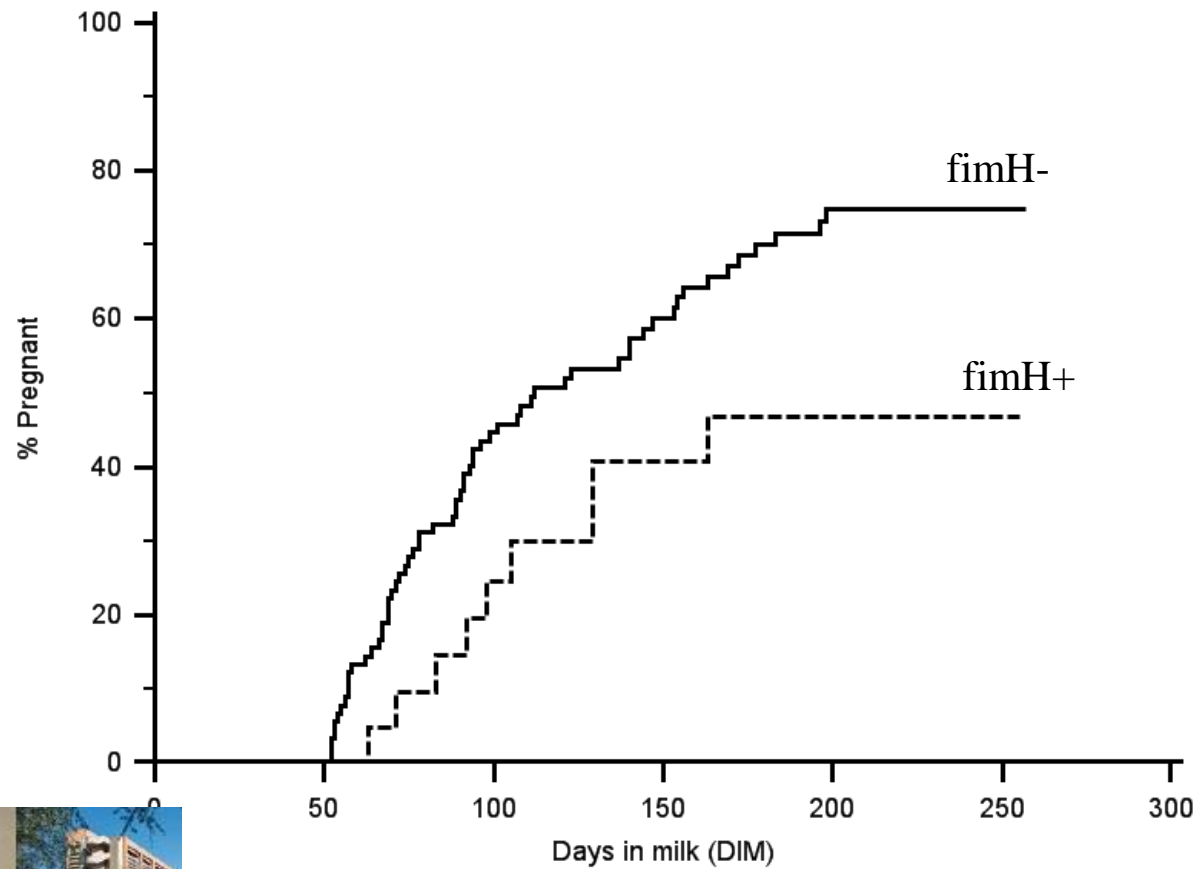
# FimH

- *FimH* was highly prevalent in *E. coli*-infected cows and was the most important predictor of metritis and endometritis
- *FimH* was present in 87% of the *E. coli* positive cows



(Bicalho et al., 2010)

# Association of bacterial species-specific virulence factors ( fimH) with reproductive performance



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# The Application of Metagenomic Methods

- Compare the uterine bacterial composition in healthy and metritic postpartum Holstein dairy cows.
- Construction and Sequencing of 16S rRNA Clone Library
- Group-specific 16S rDNA PCR-DGGE



J. Dairy Sci. 94:291–302  
doi:10.3168/jds.2010-3668

© American Dairy Science Association®, 2011.

**Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows**

T. M. A. Santos,\* R. O. Gilbert,† and R. C. Bicalho\*<sup>1</sup>

\*Department of Population Medicine and Diagnostic Sciences, and

†Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

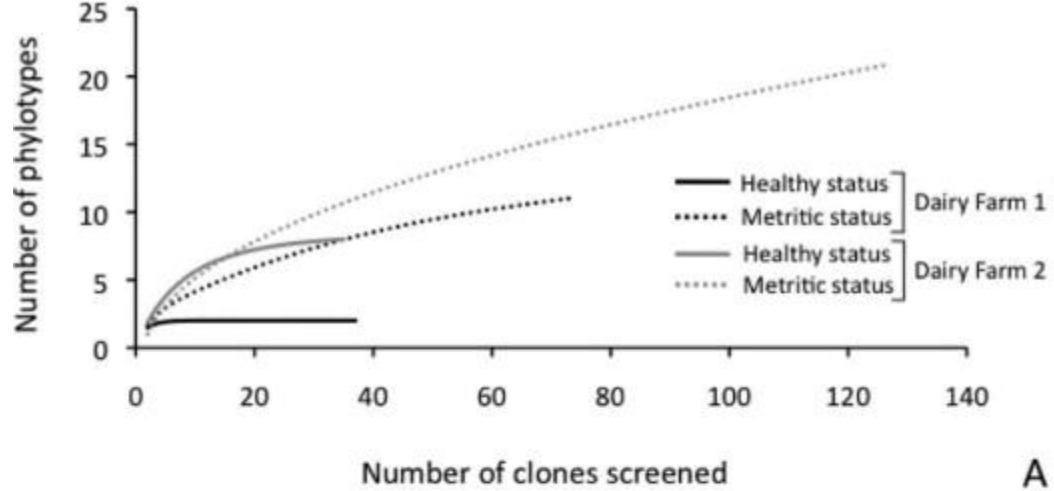


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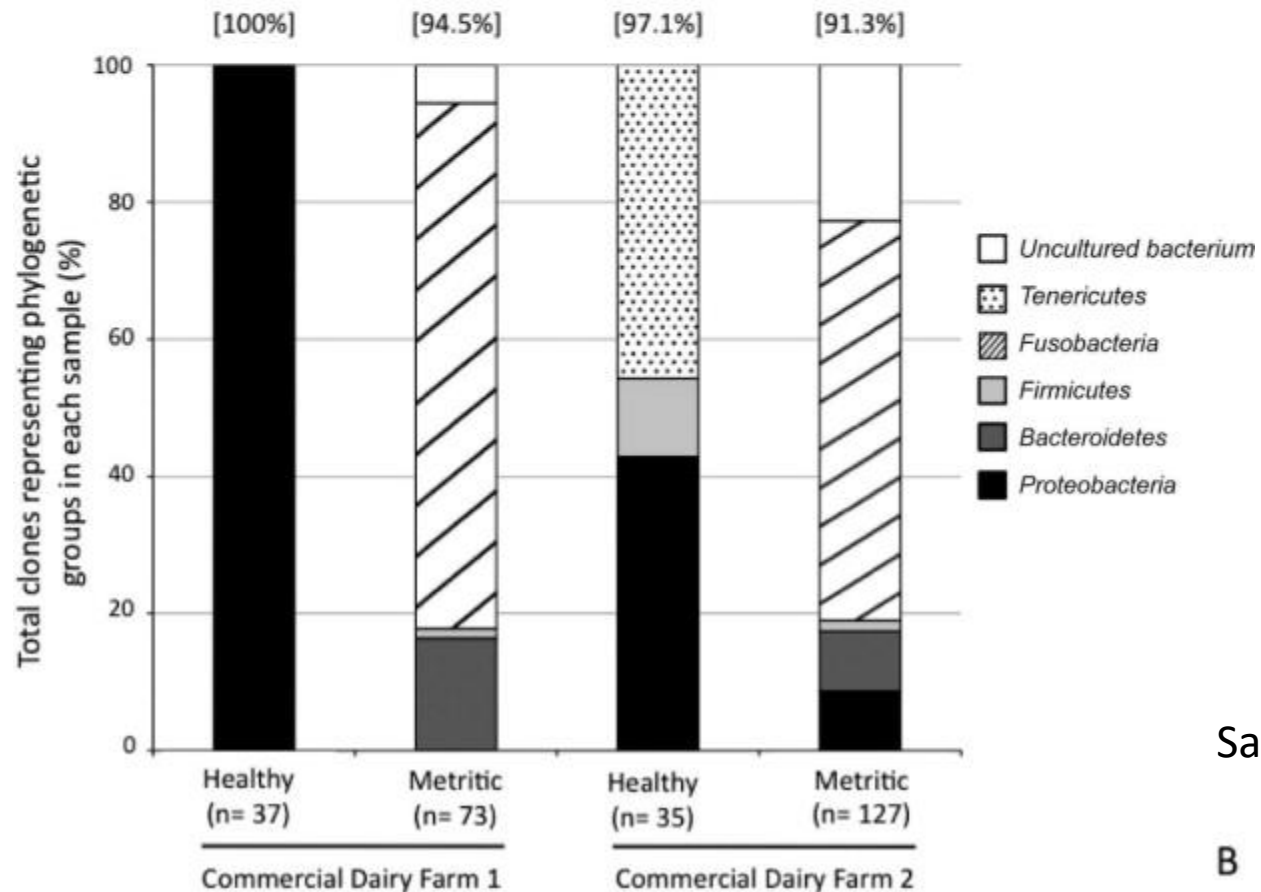






**•Metagenomic Comparison**

- Metritic cows have more diverse microbiota
- Predominantly Gram-neg anaerobes
- No *E. coli!*



Santos, Gilbert & Bicalho, 2011

# More metagenomics

- Compare the uterine bacterial composition in healthy and endometritic postpartum Holstein dairy cows.
- Pyrosequencing of 16S rRNA gene
- *Fusobacterium necrophorum* and *Trueperella pyogenes*

Veterinary Microbiology 159 (2012) 460–469



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Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: [www.elsevier.com/locate/vetmic](http://www.elsevier.com/locate/vetmic)



Investigation of postpartum dairy cows' uterine microbial diversity using metagenomic pyrosequencing of the 16S rRNA gene

V.S. Machado, G. Oikonomou, M.L.S. Bicalho, W.A. Knauer, R. Gilbert, R.C. Bicalho\*

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401, United States



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## Association of bacterial species-specific virulence factors and the prevalence of METRITIS

	<u>Metritis % (n)</u>	Odds ratio	<i>P</i> -value
<i>E. coli fimH</i>			
<i>D1-3</i>			
postpartum			
POSITIVE	76.2% (21)	4.7	<i>&lt; 0.01</i>
NEGATIVE	32.2% (90)	<i>Ref.</i>	
<i>F. necrophorum</i>			
<i>lktA D8-10</i>			
postpartum			
POSITIVE	54.1% (61)	2.6	<i>0.03</i>
NEGATIVE	24.0% (50)	<i>Ref.</i>	

## Association of bacterial species-specific virulence factors and the prevalence of ENDOMETRITIS

	<u>Endometritis % (n)</u>	<b>Odds ratio</b>	<i>P</i> -
<i>E. coli fimH D</i> 1-3 postpartum			
POSITIVE	38.1% (21)	5.4	<i>0.01</i>
NEGATIVE	15.6% (90)	<i>Ref.</i>	
<i>T. pyogenes fimA D</i> 8-10 postpartum			
POSITIVE	33.3% (39)	5.6	< <i>0.01</i>
NEGATIVE	12.5% (72)	Ref.	
<i>T. pyogenes fimA D</i> 34-36 postpartum			
POSITIVE	61.4% (13)	8.8	< <i>0.01</i>
NEGATIVE	14.3% (98)	<i>Ref.</i>	

# Vaccine development

- Recombinant proteins from *E. coli*, *T. pyogenes*, and *F. necrophorum*
- Inactivated whole cells from *E. coli*, *T. pyogenes*, and *F. necrophorum*
- Five formulations
  - 0 = control, 1-3 = systemic vaccine, and 4-5 = intravaginal vaccine



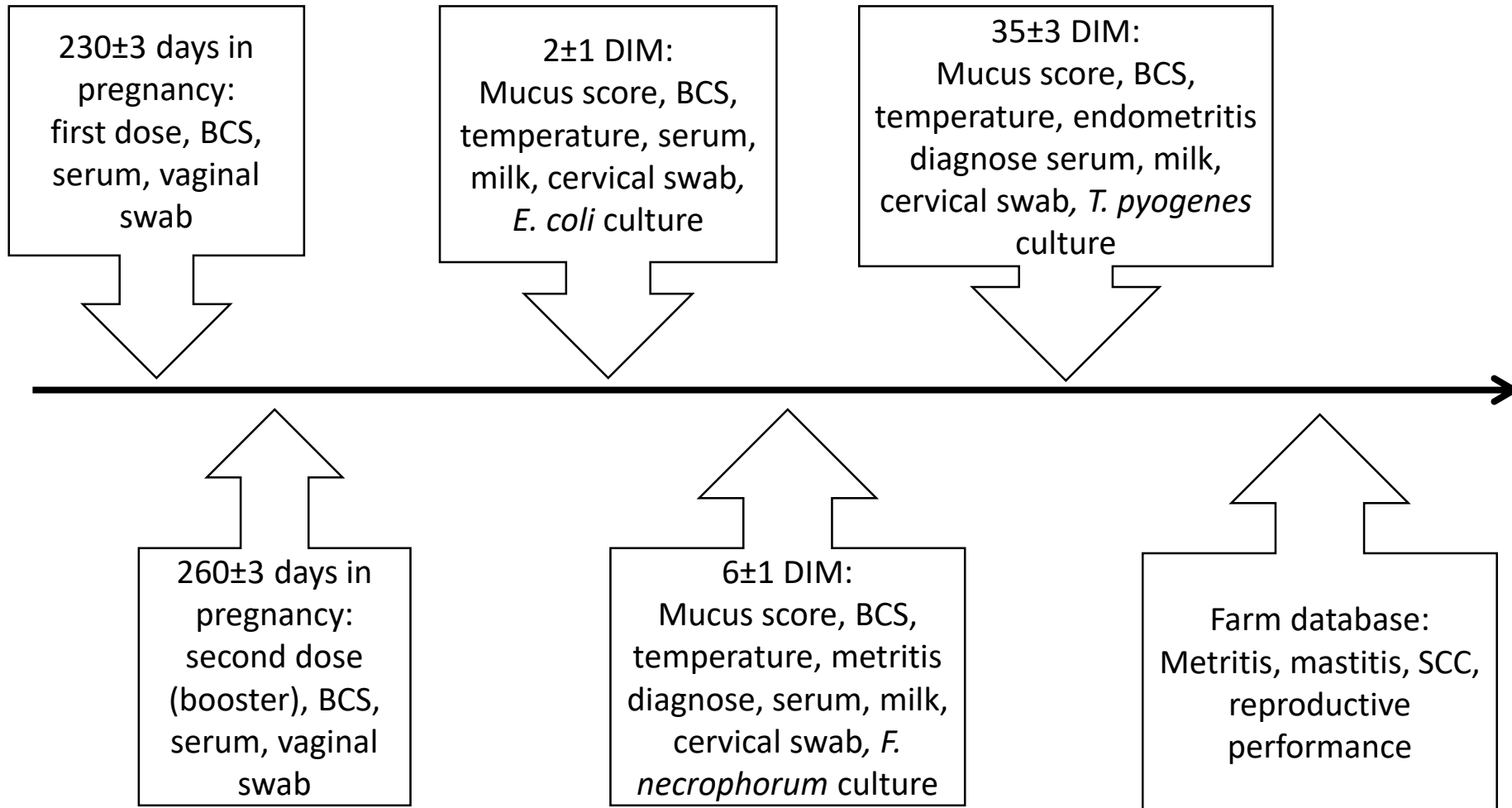
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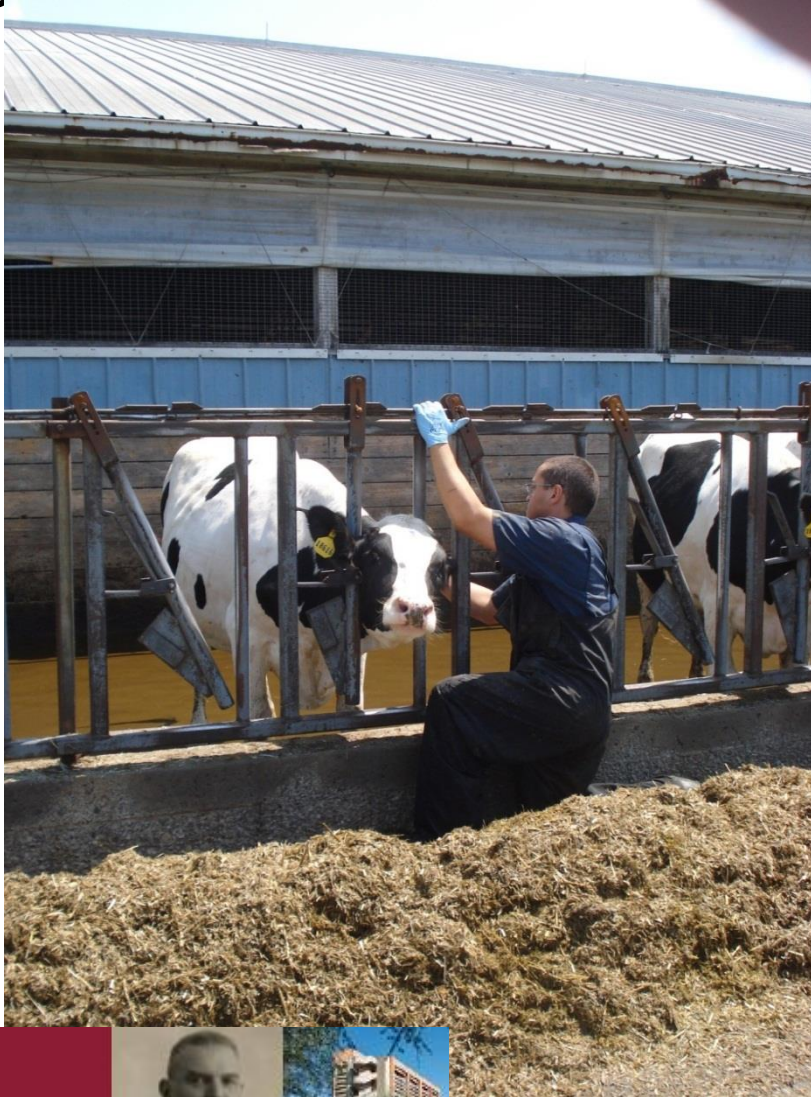




# Summary



# Enrollment



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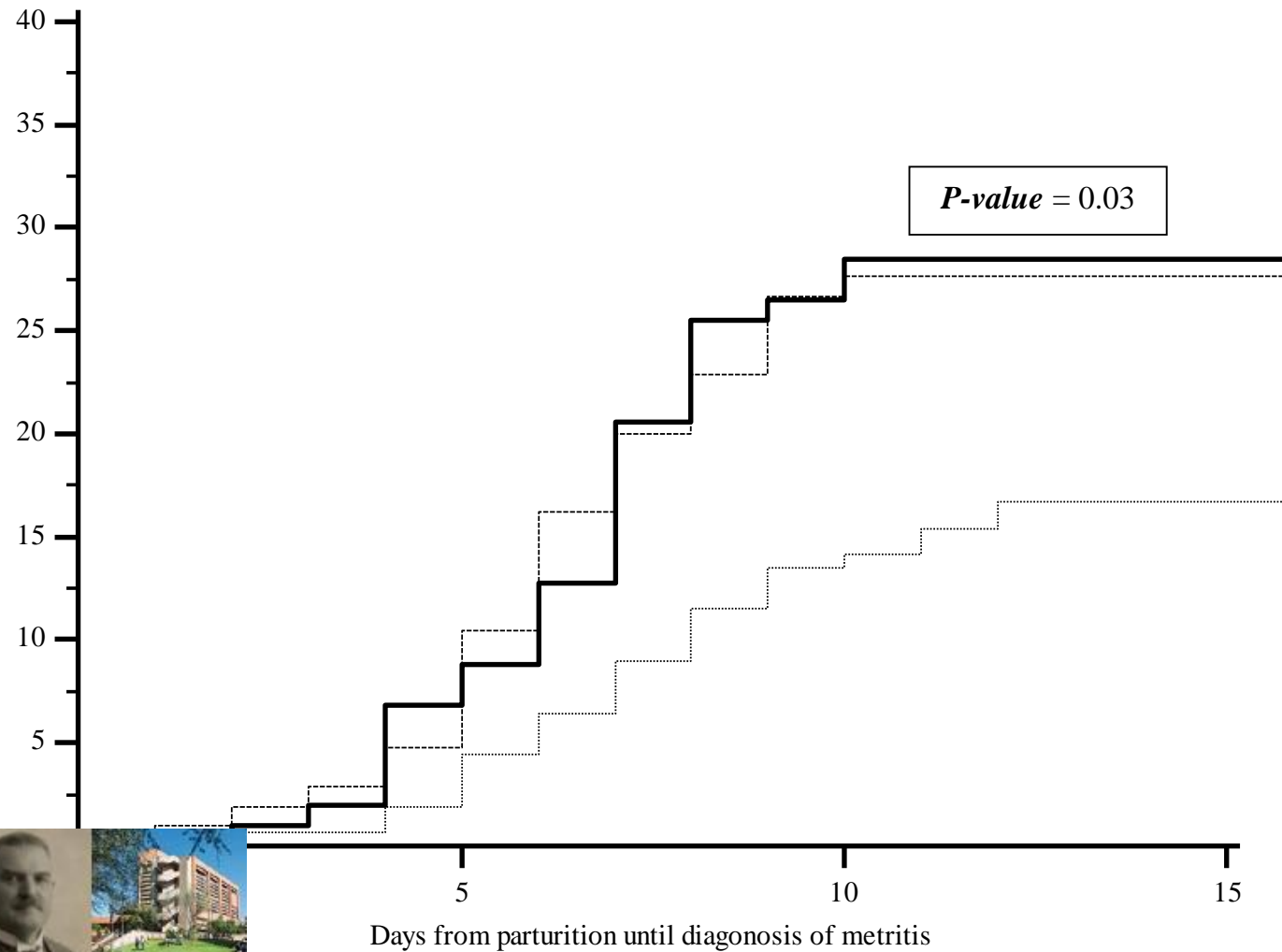
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# Results

**Figure 3:** Kaplan-Meier survival analysis illustrating the effect of vaccination group (Control = solid line, intravaginal = long dashed line, and subcutaneous= short dashed line).



# Results

**Figure 4:** Effect of vaccination on rectal temperature at 7 days postpartum.

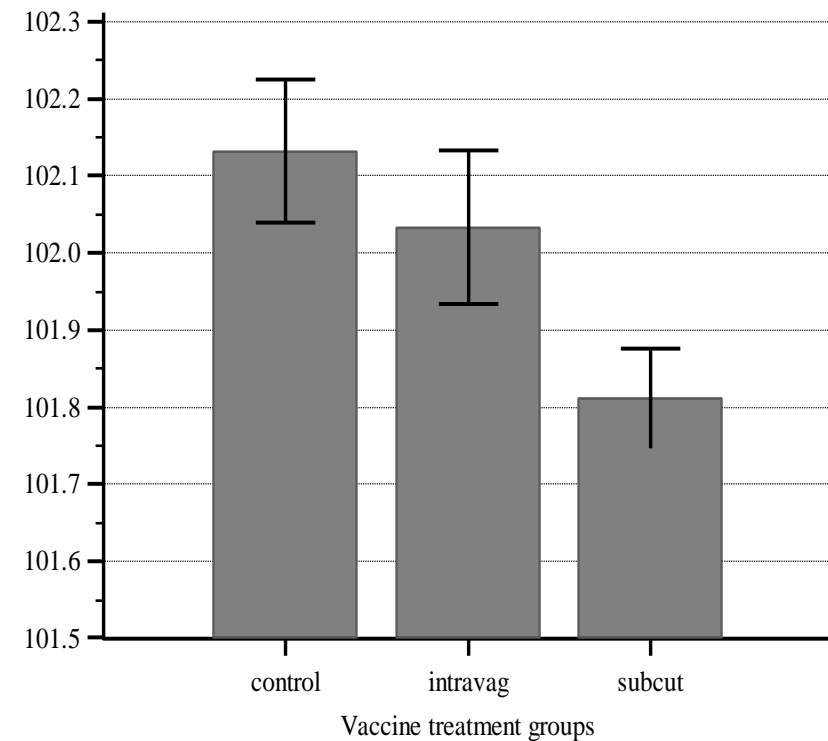
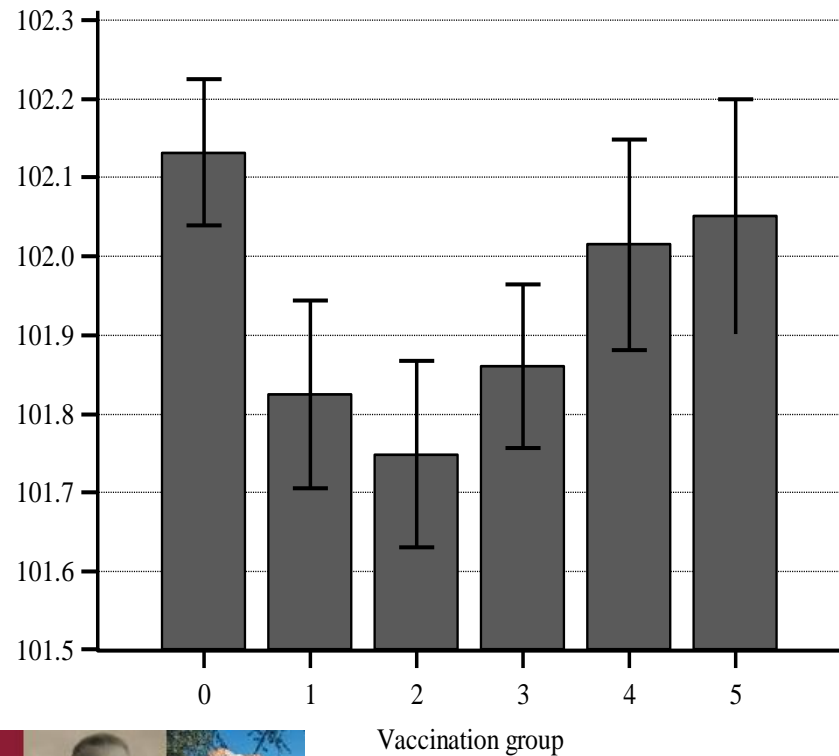
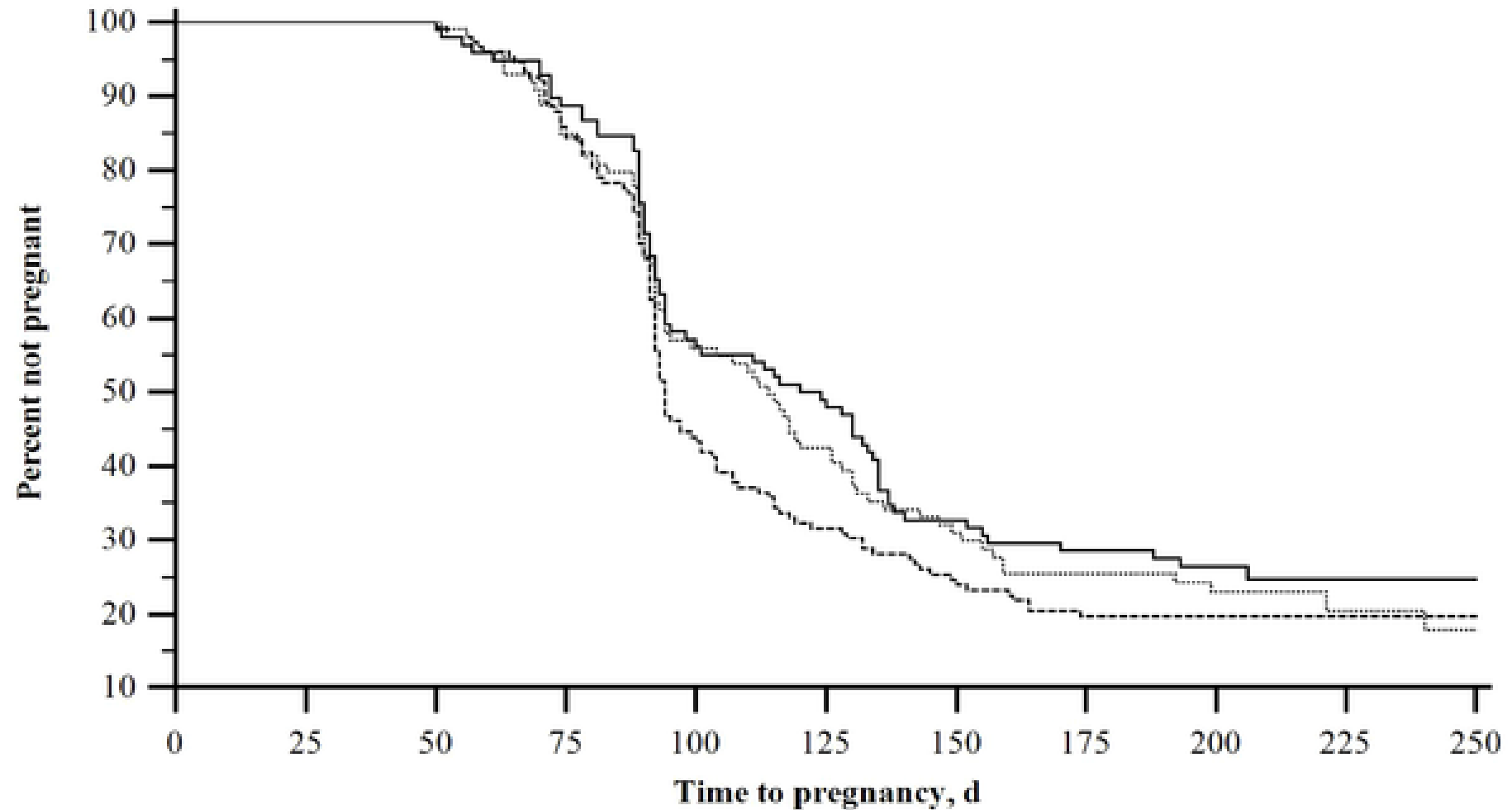


Figure 2. Effect of subcutaneous and intravaginal vaccines on reproduction.



Machado VS, Bicalho MLS, Meira Junior EBdS, Rossi R, Ribeiro BL, et al. (2014) Subcutaneous Immunization with Inactivated Bacterial Components and Purified Protein of *Escherichia coli*, *Fusobacterium necrophorum* and *Trueperella pyogenes* Prevents Puerperal Metritis in Holstein Dairy Cows. PLoS ONE 9(3): e91734. doi:10.1371/journal.pone.0091734  
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0091734>



# Metabolic and Immune Mechanisms Mediating Endometritis



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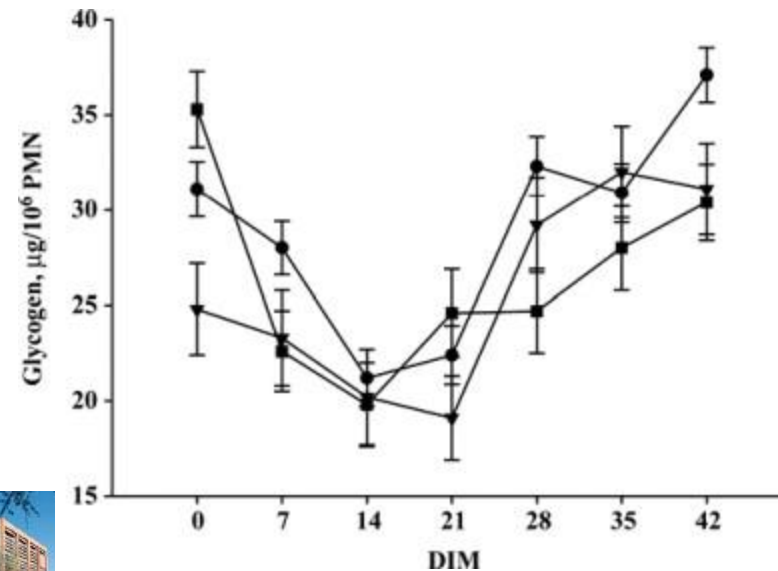
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# Negative Energy Balance

- Consistently linked to postpartum uterine disease
- Cows that develop metritis have reduced neutrophil intracellular glycogen



Galvao et al., 2010



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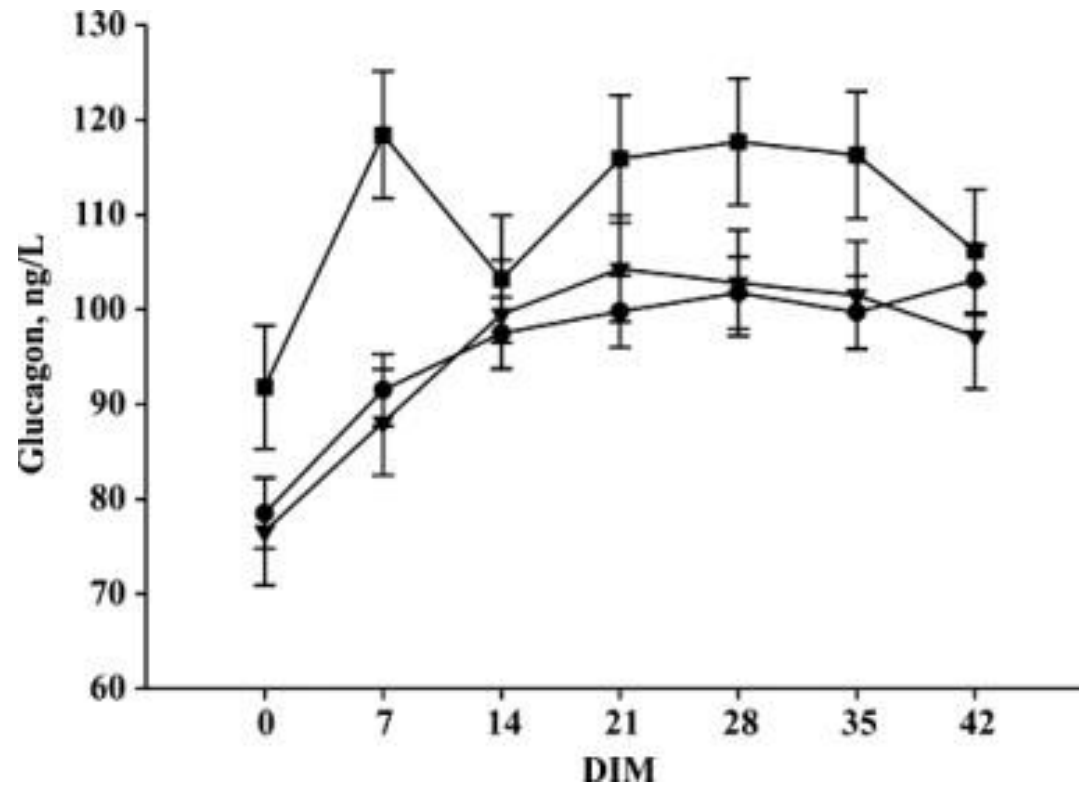


Figure 9. Least squares means  $\pm$  SEM for plasma glucagon concentrations for cows that developed metritis up to 14 DIM (▼), had subclinical endometritis at 42 DIM (■), or remained healthy up to 42 DIM (●).

K.N. Galvão, M.J.B.F. Flaminio, S.B. Brittin, R. Sper, M. Fraga, L. Caixeta, A. Ricci, C.L. Guard, W.R. Butler, R.O. Gilbert

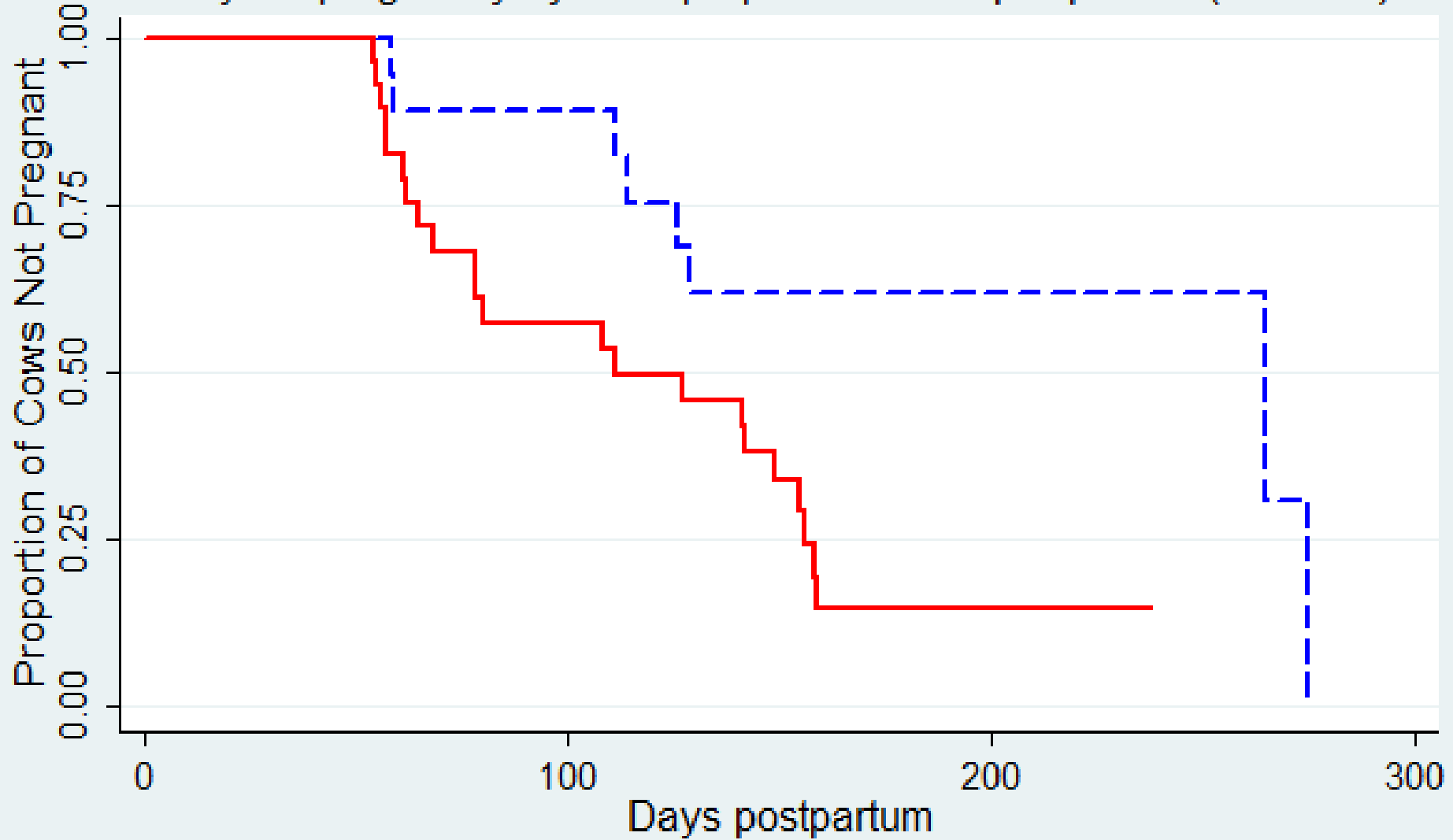
**Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows**

Journal of Dairy Science, Volume 93, Issue 7, 2010, 2926–2937

<http://dx.doi.org/10.3168/jds.2009-2551>

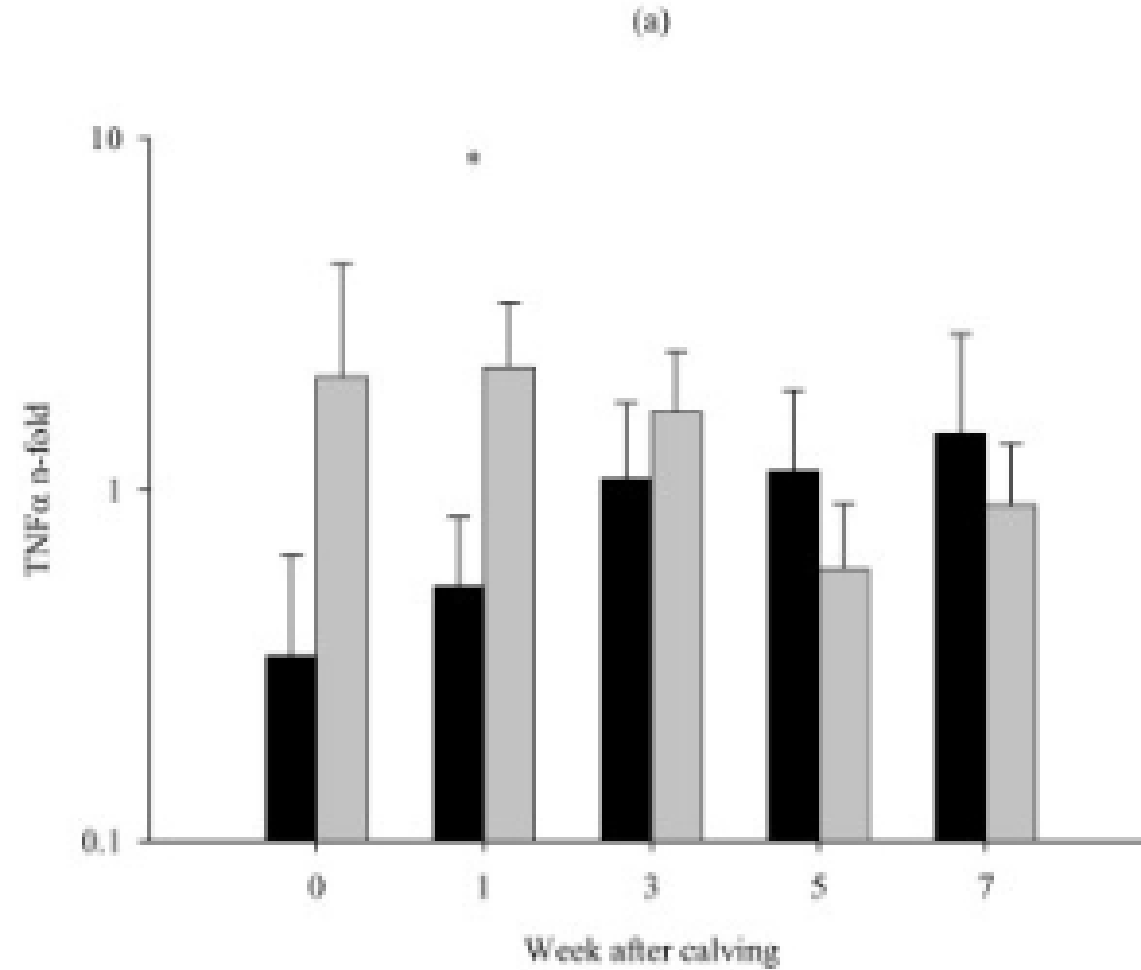
# Kaplan-Meier survival estimates

Days to pregnancy by PMN proportion at 7 d postpartum ( $P < 0.01$ )



--- PMN < 40 %, n = 20      — PMN > 40 %, n = 32

# Endometrial gene expression



Galvao et al., 2011

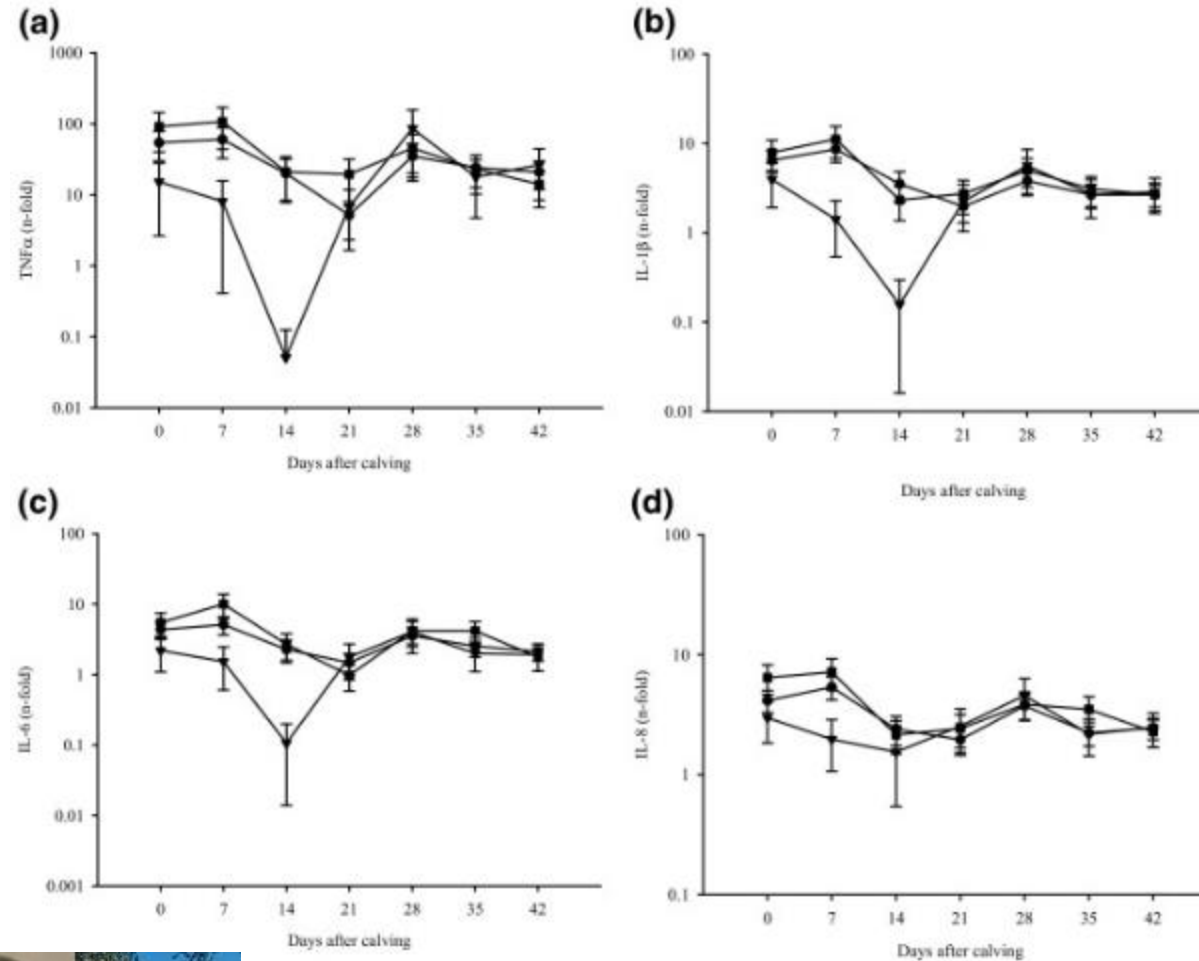


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# Gene expression by circulating monocytes stimulated with *E. coli*



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# Postpartum Endometrial Gene Expression

- Pro-inflammatory in early postpartum period
  - Th2 bias in pregnancy
- Changes to predominantly tissue remodeling by D 21
- Failure to make switch characteristic of cows with endometritis  
Foley et al., 2015
- Negative energy balance associated with sluggish recruitment of inflammatory cells to uterus  
Yasui et al., 2014



# How does endometritis mediate infertility?

- May seem obvious
- Infertility persists after resolution of inflammation



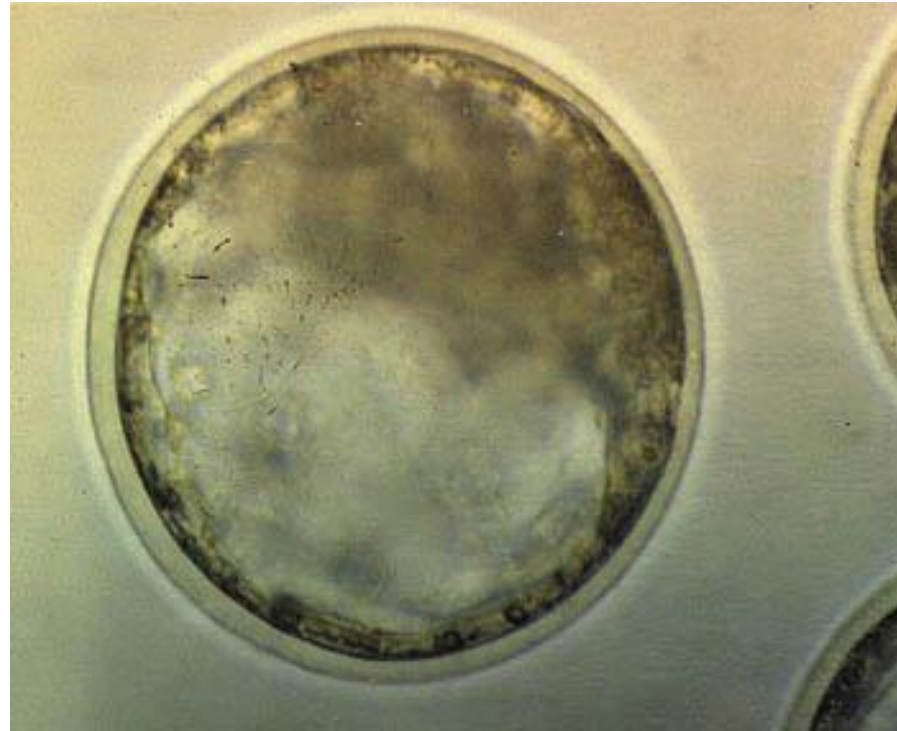
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# Effects of Inflammation on Embryo Development



# Superovulated Heifers

- Crossover experiment (n = 7)
  - Inflammation induced on Day 6 by infusion of glycogen (15 ml, 1%) vs. sham infusion
  - Embryo recovery 16 h later
- Embryo recovery 3.8 vs. 2.9 embryos/ heifer (n.s.)
- Median embryo quality 1 vs. 3



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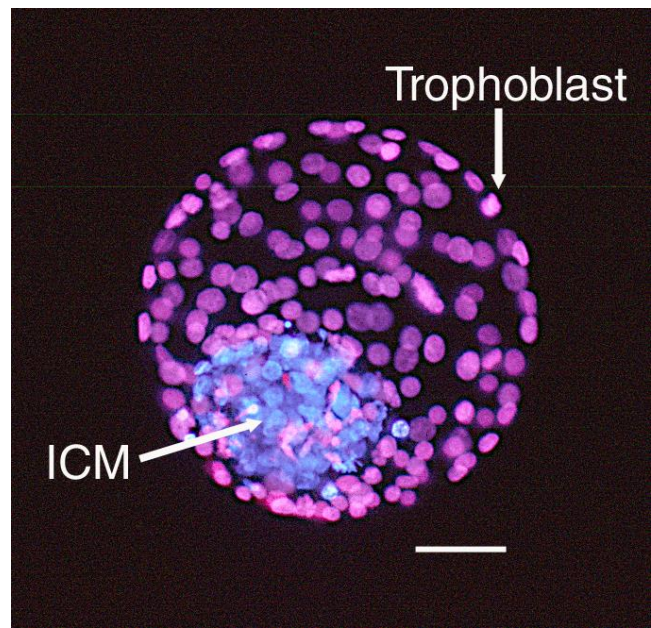
# Exposure of bovine embryos to PMN in vitro

- Exposure of approx. 850 embryos to PMN for different intervals (up to 6 days) and at different developmental stages:
  - Minimal to no effect on development!



# Effect of Inflammation on Embryo Development in vitro

Treatment	Total cell count	Inner cell mass	Trophectoderm	Ratio ICM/Troph
Conditioned -Inflamed	83.1*	30.1	53.0*	0.38*
Conditioned- Non-Inflamed	99.8	26.5	73.3	0.28





# Effect of Endometritis on Ovarian Function



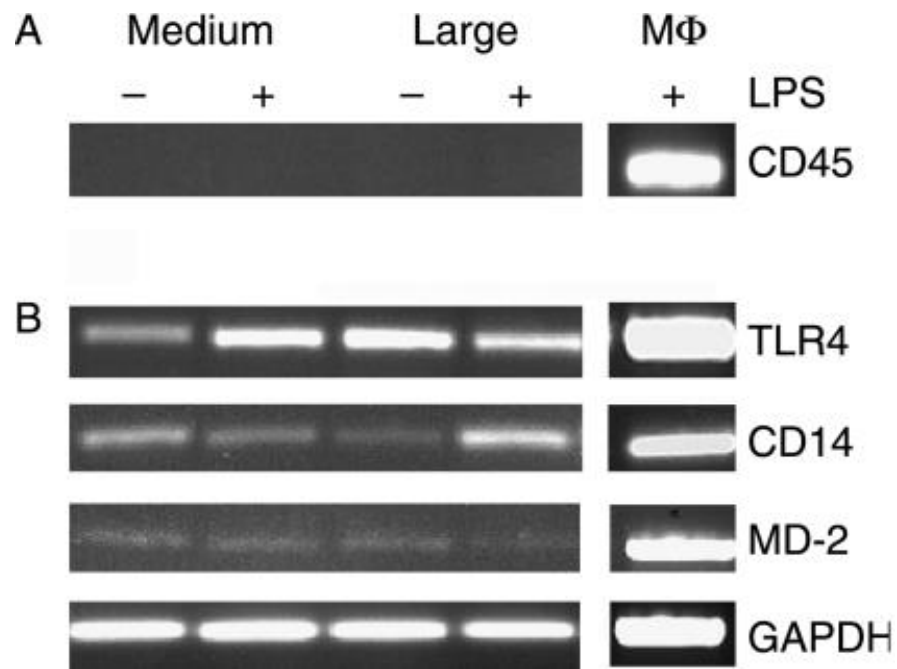
[www.cytochemistry.net](http://www.cytochemistry.net)



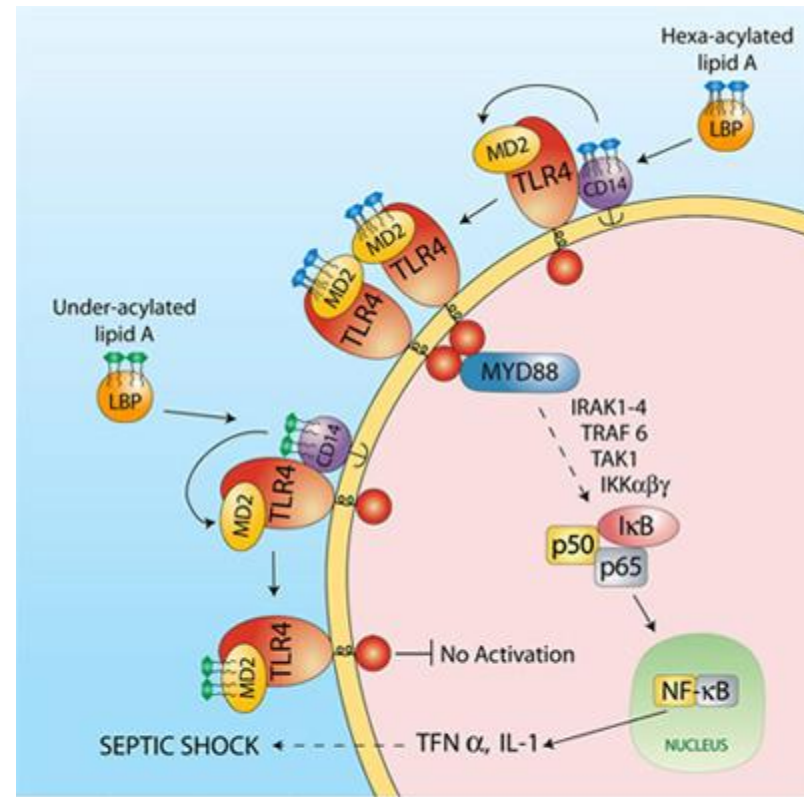
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Herath et al., Reproduction, 2007



**Agonist and antagonist activities of lipid A.** Hexa-acylated but not under-acylated lipid A bound to MD-2 induce oligomerization of TLR4 leading to the production of inflammatory cytokines.

Important receptors for responding to LPS are expressed by granulosa cells

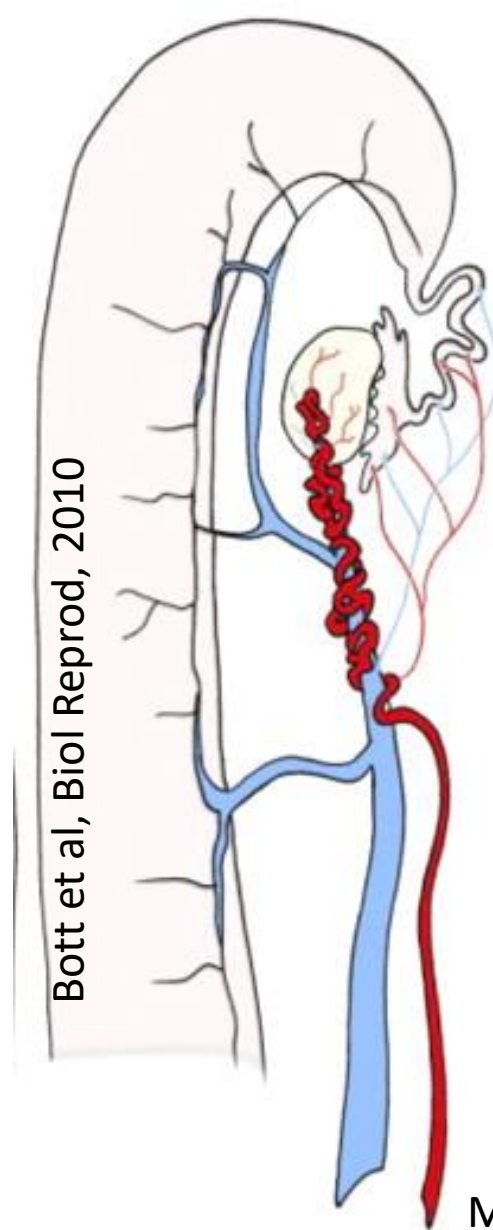
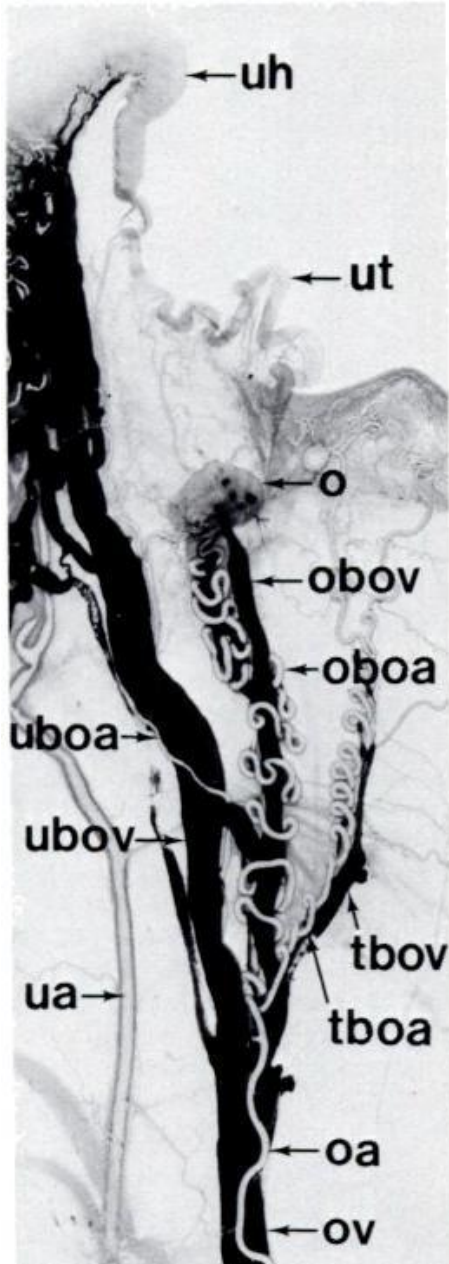
# LPS in Bovine Follicular Fluid

Category	Cows with detectable LPS	LPS concentration (Range; ng/ml)	Mean LPS Concentration (ng/ml)
Normal	3 / 19 (16 %)	0 – 0.8	0.06 ± 0.04
Mild endometritis	4 / 8 (50 %)	0 – 0.8	0.7 ± 0.3
Moderate endometritis	16 / 24 (67 %)	0 – 40.0	4.8 ± 1.8
Severe endometritis	7 / 7 (100 %)	4.3 – 875.2	176.1 ± 112.0

P = 0.0001







## Intimate vascular exchange

- Uterine veins
- Ovarian artery

Mapletoft & Ginther, AJVR, 1975

Mapletoft et al., Proc.Soc.Exp.Biol.Med., 1975

Mapletoft et al., Proc.Soc.Exp.Biol.Med., 1976

Mapletoft et al., Biol.Reprod., 1976



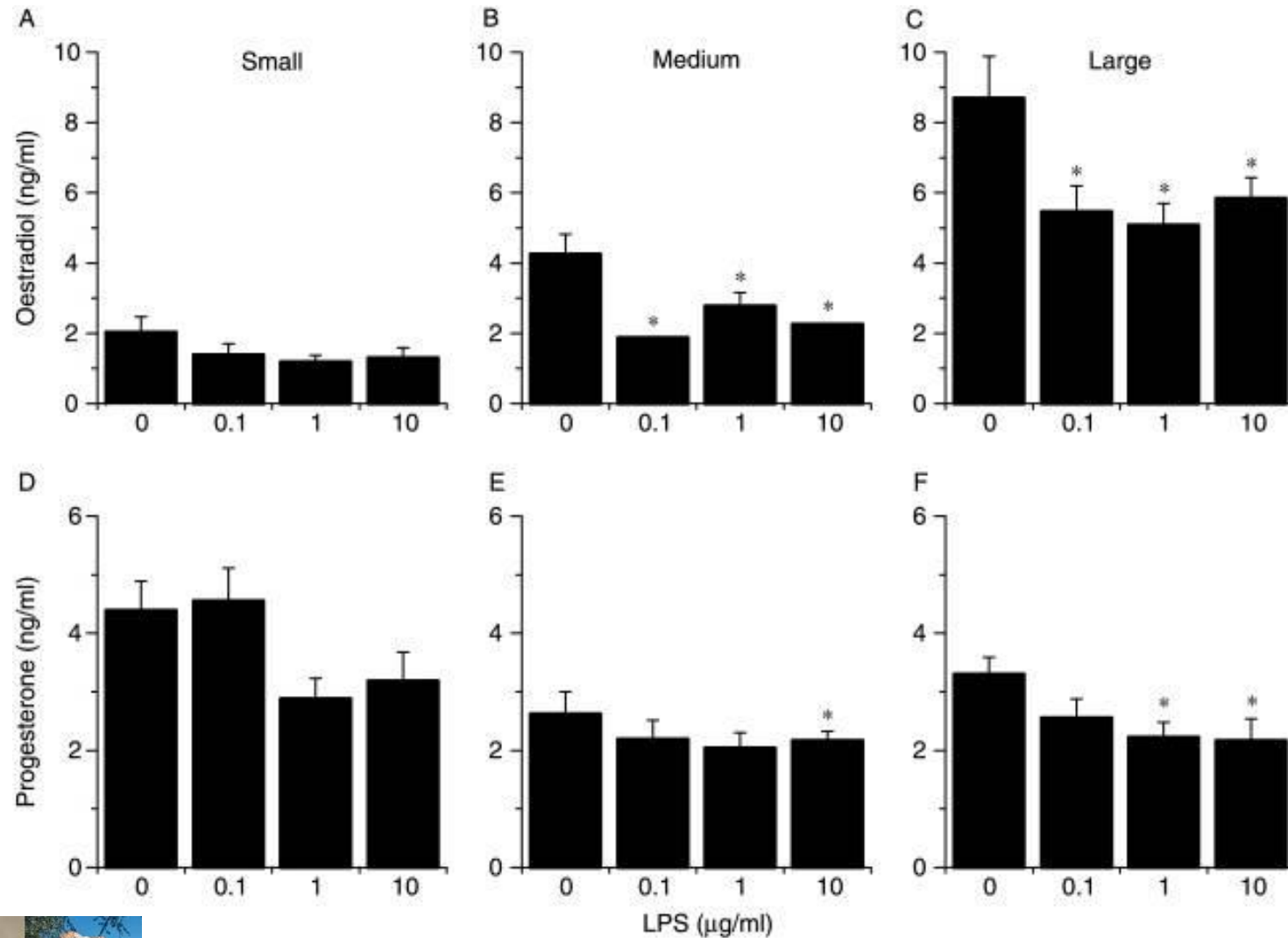
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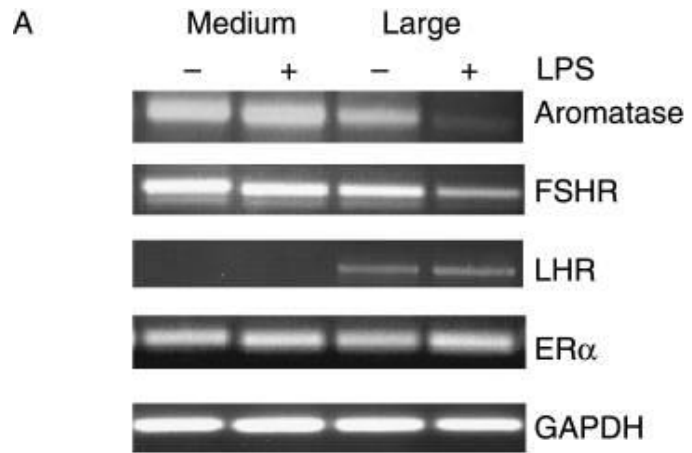




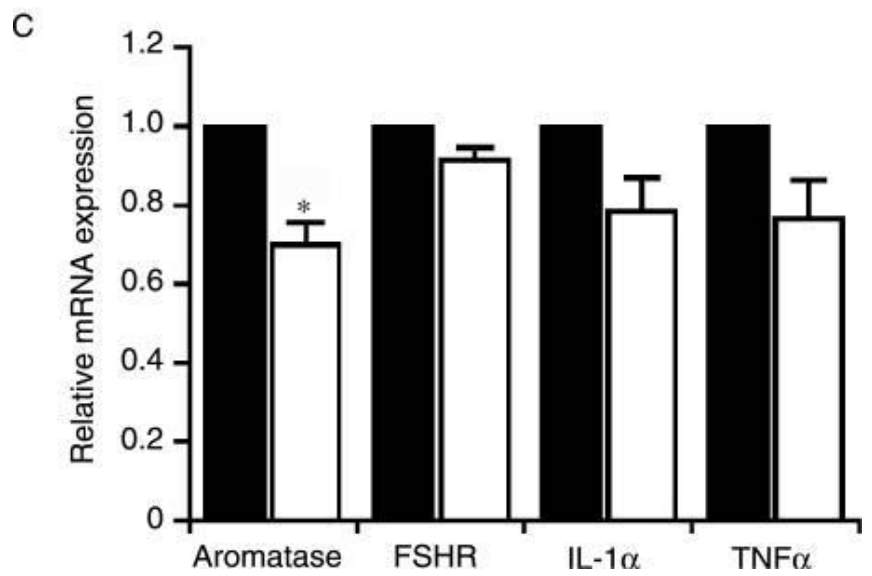
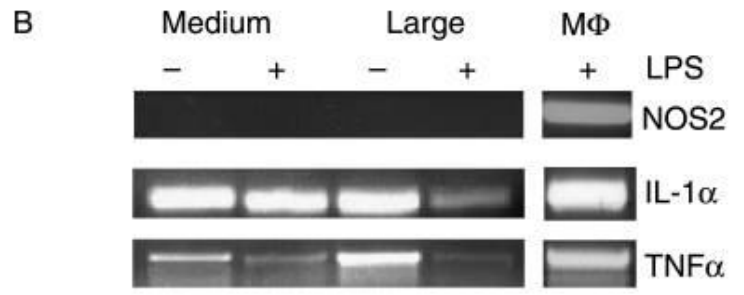
# LPS mediates reduced production of estradiol by granulosa cells in vitro



Herath et al., Reproduction, 2007



LPS decreases expression of aromatase by granulosa cells of dominant follicles



# The Research Imperative: Food supply

- 1798: Thomas Malthus predicted population would outstrip food supply by mid 19<sup>th</sup> century
- 1968: Paul Ehlich predicted famine in 1970s and 1980s



Australian Broadcasting Corporation

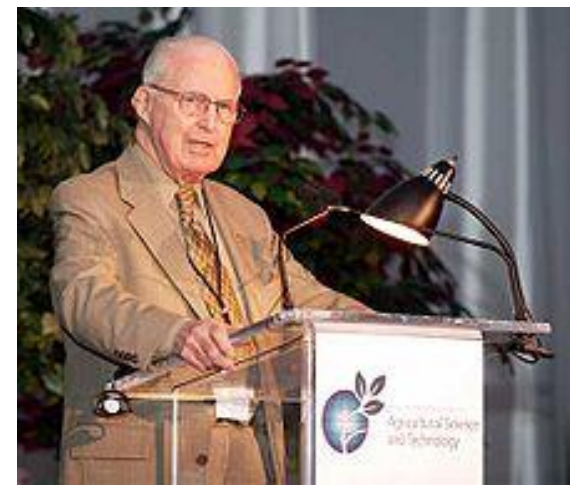


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# Green Revolution



Norman Borlaug, 1914-2009

- World grain production increased 250% from 1950 to 1980
- Depends heavily on fossil fuels
  - Fertilizer
  - Pesticides
  - Energy for irrigation

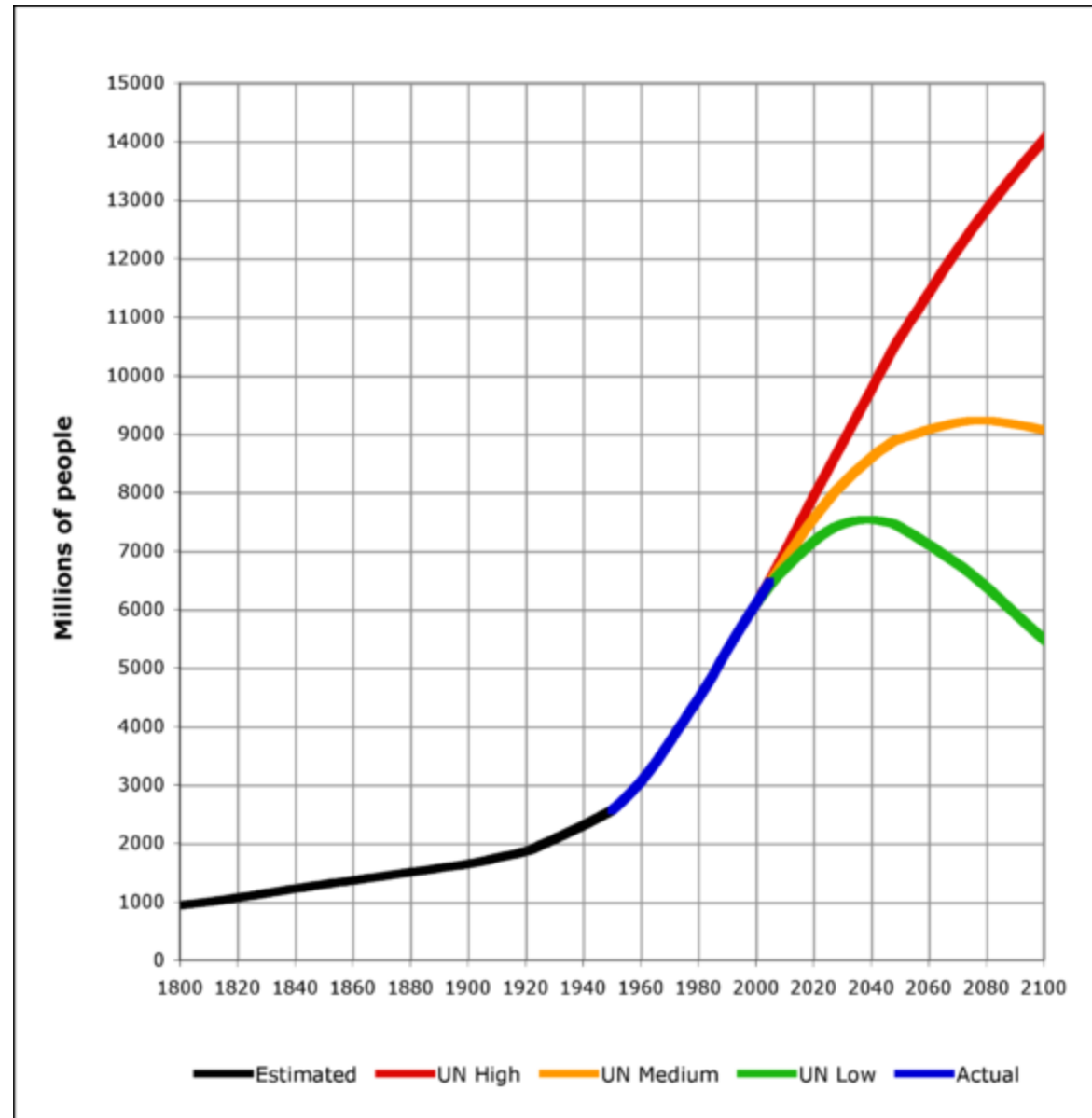


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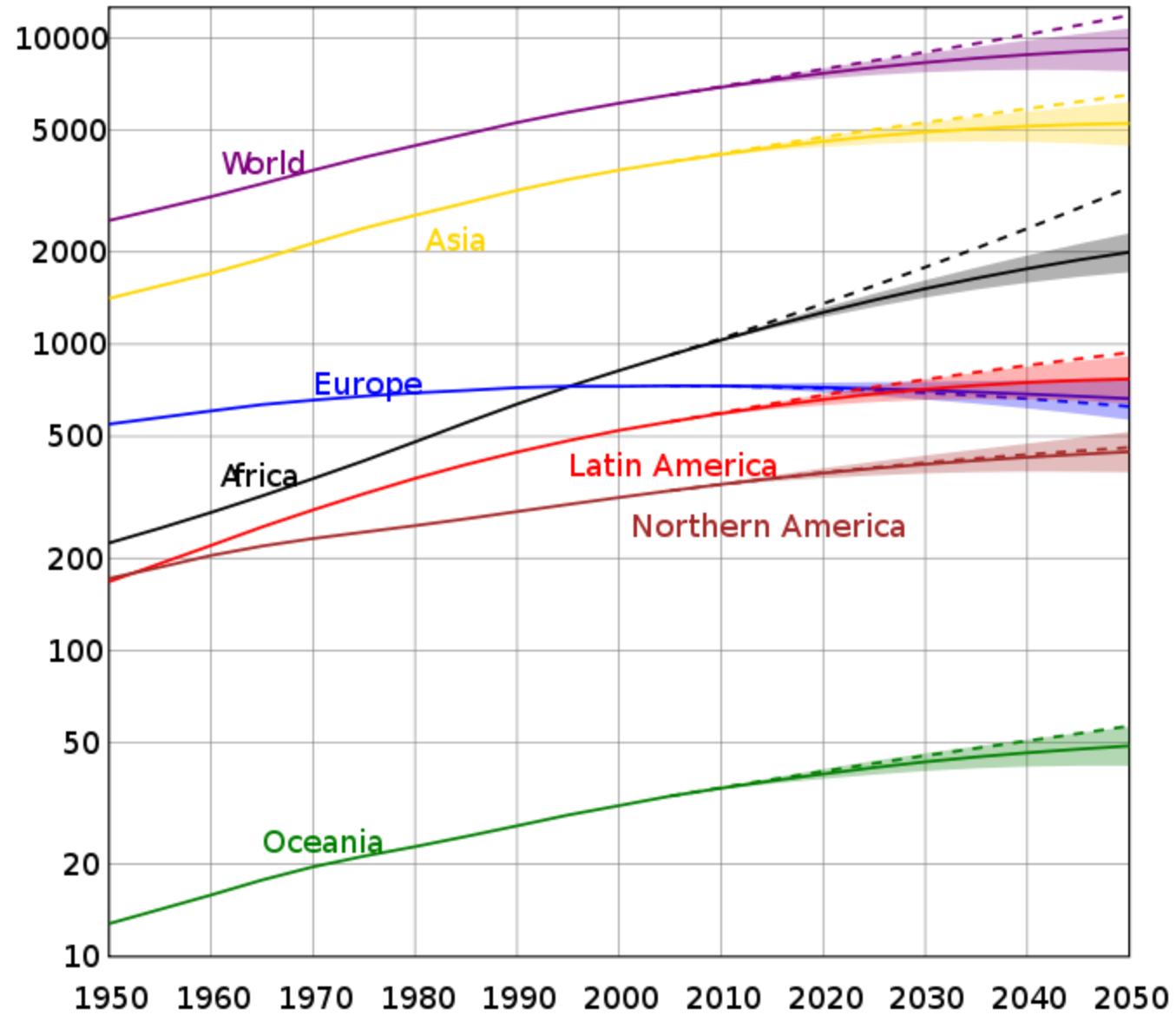


# World Population



Based on UN projections and US census Bureau historical estimates





# Over 1 billion undernourished people

- FAO, 2009: 1.02 billion
- World Bank, 2005: 1.4 billion
- About 2 billion lack food security
- 6 million children die of hunger every year



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26% of children in the developing world are undernourished (UN)





# Food Security

- Hunger and poverty
- Perpetuation of hunger and poverty
  
- In USA, food insecurity exceeds 15%
- In CANADA, over 10% are food insecure



# Future Production

- World food production will have to increase by 70 % by 2050
  - Increasing world population
  - Increasing income
- Increasing population and decreasing energy availability
  - A perfect storm?



# Novel Diseases

- Malicious introduction of disease
  - FMD
- Spontaneous disease
  - BSE
- Previously unrecognized diseases

Mad Cow Blog, Marler Clark, LLP, PS



# Animal Welfare

- Farrowing crates
- Tail docking
- Layer housing
- Foie gras
- Intensive agriculture in general



# “Livestock’s Long Shadow”

- FAO, 2006
- Environmental impact of livestock production
- Climate change
- Land degradation
- Water pollution / water shortage
- Air pollution
- Biodiversity





# Environmental Impact of Dairying

- Comparison of 1944 and 2007 in USA
- Per billion pounds of milk:
  - 21% of cows
  - 23% of feedstuffs
  - 35% of water
  - 10% of land
  - 24% of manure
  - 43% of methane
- Carbon footprint approx. 37%

Colo. State Univ.



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Capper, Cady & Bauman, 2009

# Environmentally Responsible Livestock Production

- Innovative
- Evaluate and adopt new technologies
- Maintain an independence
  - Preserve credibility
- Educate the public



# Recombinant Bovine Somatotropin

- “Green” technology?
  - Reduced nutrient input and waste output per unit of milk produced
  - Reduced water use, cropland area, greenhouse gas production, N and P excretion, fossil fuel use



Capper et al., PNAS, 2008





# Challenges

- Feed the population
  - Safe, nutritious
- Preserve environment
  - Prevent deforestation
  - Prevent surface water contamination
  - Maintain sustainability
- Enhance animal welfare



# Research in Clinical Practice

- Academic clinicians
- Private practice



# Academic Clinicians

- Part of the job!
- Advance the state of the art in your discipline
- Depend on observations, retrospective and prospective studies
- Coordinate multicenter studies
- Perform systematic reviews and meta-analyses
- Collaborate with basic scientists
- Collaborate with private practitioners
- Research focus
  - ???



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# Research in Private Veterinary Practice

- Not as easy; requires dedication / obsession
  - Arnold Theiler and African Horse Sickness
- Well placed to perceive priorities
- Often have higher case loads for specific types of cases
  - E.g. Assisted reproductive technologies
    - John Hasler
    - Jon Hill
- Practitioner can coordinate multicenter studies
  - E.g. cancer therapy.



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# Research in Private Veterinary Practice

- Chris Marlow (South Africa)
- Dave Hanlon (New Zealand)
- John Newcombe (UK)
- Ryan Cavanaugh (USA)
- Others
  - JSAVA, 2015
  - 106 authors, 20 in private practice!

A field study of patterns of unobserved foetal loss as determined by rectal palpation in foaling, barren and maiden Thoroughbred mares

R. O. GILBERT and C. H. B. MARLOW\*

The reproductive performance of Thoroughbred mares treated with intravaginal progesterone at the start of the breeding season

D.W. Hanlon<sup>a,\*</sup>, E.C. Firth<sup>b</sup>

<sup>a</sup> *Matamata Veterinary Services, Ltd, 26 Tainui Street, Matamata, New Zealand*

<sup>b</sup> *Massey University, Veterinary Teaching Hospital, Palmerston North, New Zealand*

**The Effect of Time of Insemination With Fresh Cooled Transported Semen and Natural Mating Relative to Ovulation on Pregnancy and Embryo Loss Rates in the Mare**

JR Newcombe<sup>1</sup> and J Cuervo-Arango<sup>2</sup>

<sup>1</sup>*Equine Fertility Clinic, Warren House Farm, Barracks Lane, West Midlands, UK;* <sup>2</sup>*Departamento Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, Moncada, Spain*



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# Ph.D. training for veterinarians

- Imperative for new knowledge
- Urgent need for trained researchers
  
- Time investment
- Cost / Student debt
- Gender issues







# Nobel Prize (Physiology and Medicine)

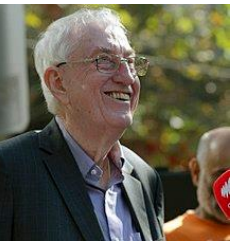
## • Medical degree only

- Harold Varmus, 1989
- Stanley Prusiner, 1997
- Arvid Carlsson, 2000
- Erik Kandel, 2000
- Richard Axel, 2004
- Barry Marshall, 2005
- John Robin Warren, 2005
- Harold zur Hausen, 2008
- Bruce Beutler, 2011
- Ralph Steinman, 2011
- Tu Youyou, 2015



## • Medical/Veterinary degree and PhD (equiv.)

- Alfred Gilman, 1994
- **Peter Doherty, 1996**
- Rolf Zinkernagel, 1996
- Fred Murad, 1998
- Shinya Yamanaka, 2012



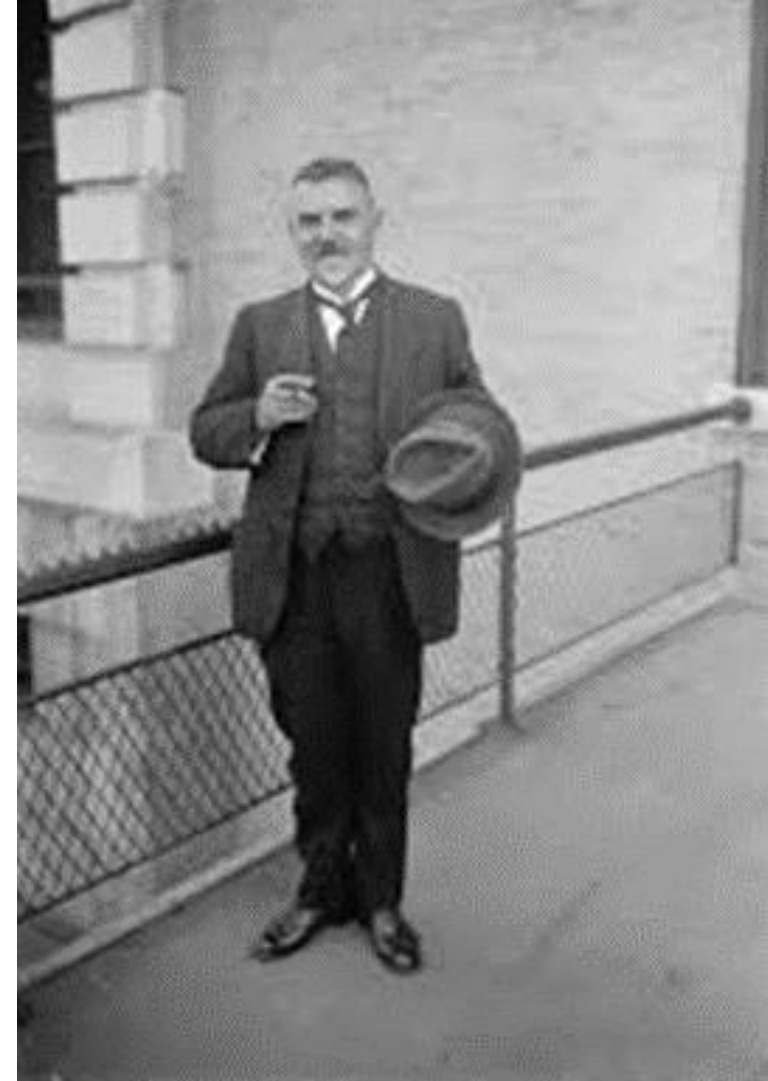


# What does it take to do research?

- Formal PhD helps, but not essential
- Intimate knowledge of the discipline is important
- Distractions
  - Generally avoid them, but keep your eyes open!
- Most of all:

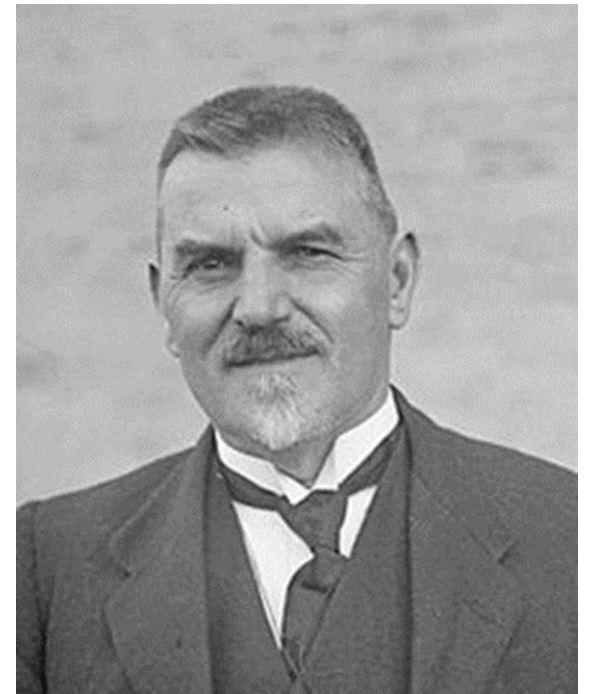
**ENTHUSIASM and DETERMINATION**

**Passion!**



# Research imperative for society

- Easy to understand
- Foundation of public funding of universities and university research



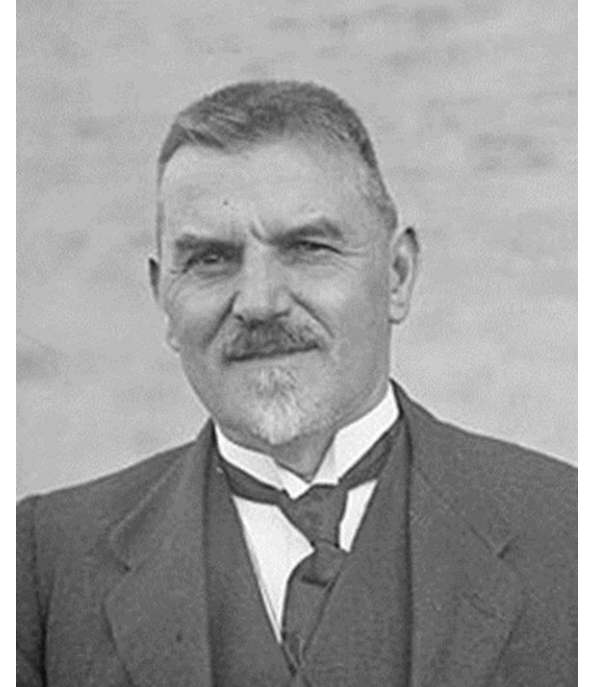
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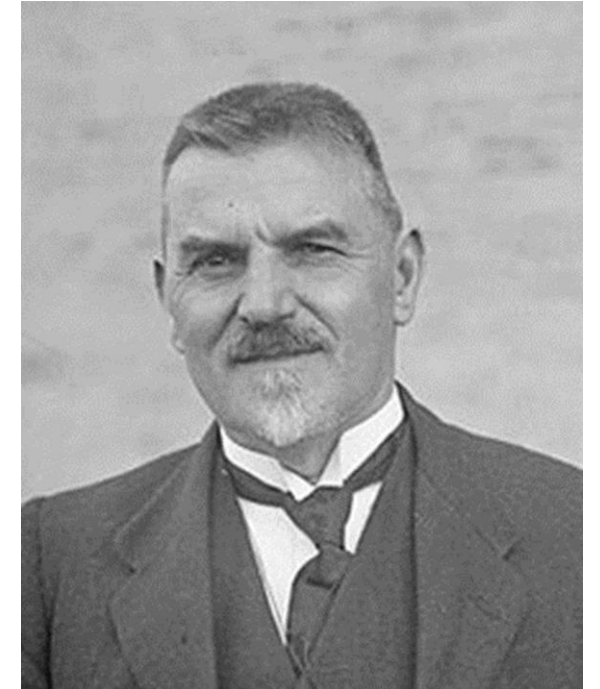
# Research Imperative for Universities

- Mission
  - Universities create and disseminate knowledge
- Prestige
  - “Currency” for universities
- Education
  - Environment of discovery
  - Equip students for 50 years of practice
- How?
  - Reward research productivity
  - Remove obstacles
    - Funding
  - The professors make the university, but the university makes the professors



# Research imperative for academics

- Academic success
- Satisfaction in an academic career
- Personal satisfaction



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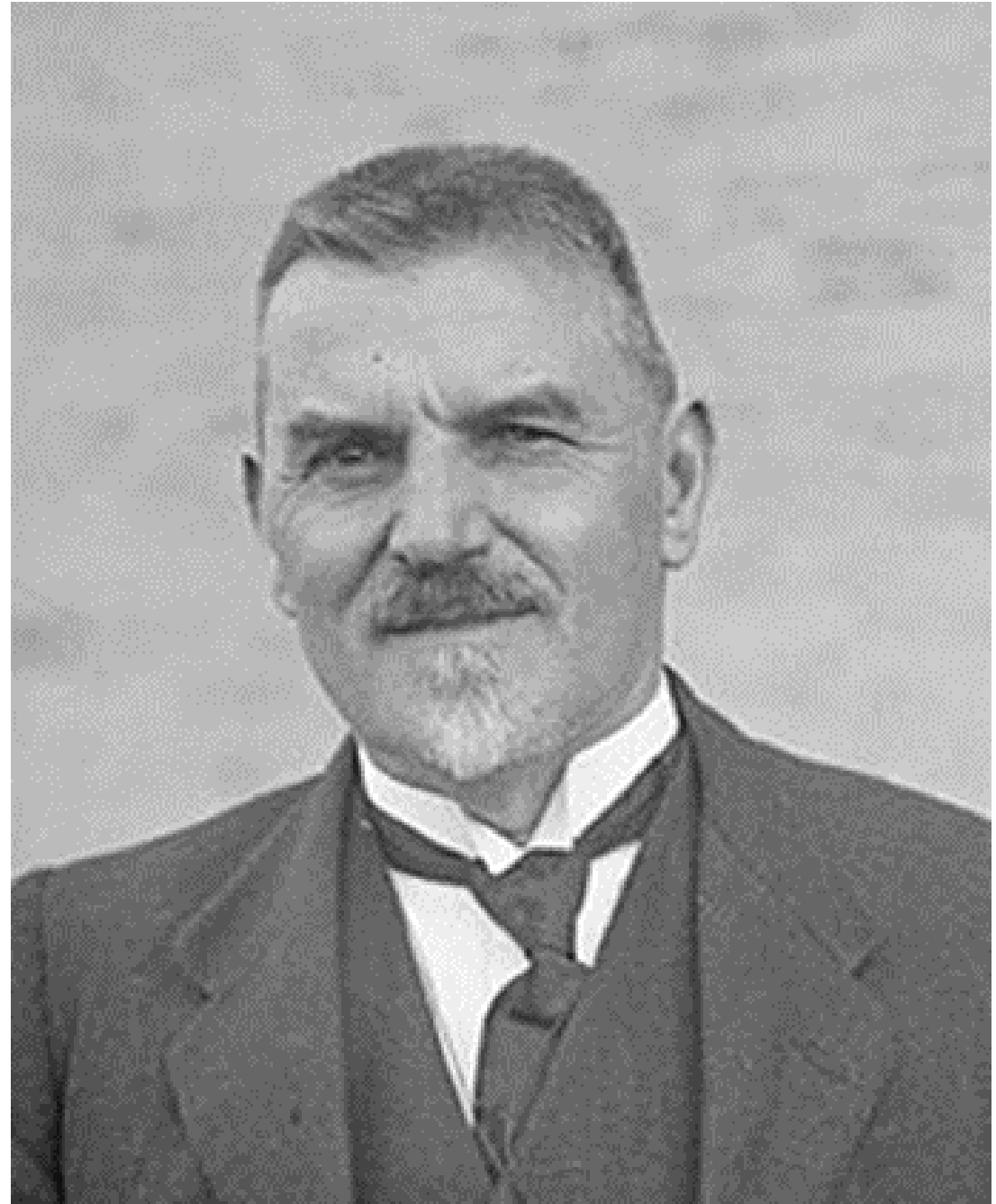
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# The Research Imperative

- For Society
- For Universities
  - “The professors make the university but the university makes the professors”
- For individual academics
  - Passion
- For privately practicing veterinarians
  - Passion

**For successful researchers this passion translates into a personal imperative to discover new knowledge.**





# Thank you! Questions?



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