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
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”

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
Endometritis

Survival Analysis
Effect of Endometritis at 7 weeks

Time (Days)
0  50  100  150  200  250  300  350

Proportion Open
0.0  0.2  0.4  0.6  0.8  1.0

Endometritis-negative
Endometritis-positive

122 days
158 days

30% censored
18% censored

P = 0.002
P = 0.003

Cornell University
College of Veterinary Medicine
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

OR = 8.5

T. pyogenes, 3 weeks

OR = 4.53

Anaerobes, 3 weeks

T. pyogenes, 5 weeks
T. pyogenes, 7 weeks
P. melaninogenica, 5 weeks

PMN at 5 or 7 weeks

OR = 0.256

Preg 150
### Risk of Uterine *E. coli*

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

Sheldon et al., PLoS One, 2010
E. coli Virulence Factors and Metritis

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

Bicalho et al., JDS, 2010
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

![Graph showing the association of bacterial species-specific virulence factors (fimH) with reproductive performance. The graph plots the percentage of pregnant animals over days in milk (DIM). Two lines are shown: fimH- and fimH+. The fimH- line shows a higher percentage of pregnant animals compared to the fimH+ line.](image-url)
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
• 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*
Association of bacterial species-specific virulence factors and the prevalence of METRITIS

<table>
<thead>
<tr>
<th></th>
<th>Metritis % (n)</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli fimH</strong>&lt;br&gt;D1-3&lt;br&gt;postpartum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>76.2% (21)</td>
<td>4.7</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>32.2% (90)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td><strong>F. necrophorum</strong>&lt;br&gt;<em>lktA D8-10</em>&lt;br&gt;postpartum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>54.1% (61)</td>
<td>2.6</td>
<td>0.03</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>24.0% (50)</td>
<td>Ref.</td>
<td></td>
</tr>
</tbody>
</table>
Association of bacterial species-specific virulence factors and the prevalence of ENDOMETRITIS

<table>
<thead>
<tr>
<th></th>
<th>Endometritis % (n)</th>
<th>Odds ratio</th>
<th>P-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli fimH D 1-3 postpartum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>38.1% (21)</td>
<td>5.4</td>
<td>0.01</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>15.6% (90)</td>
<td>Ref.</td>
<td></td>
</tr>
<tr>
<td><strong>T. pyogenes fimA D 8-10 postpartum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>33.3% (39)</td>
<td>5.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>12.5% (72)</td>
<td>Ref.</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>T. pyogenes fimA D 34-36 postpartum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POSITIVE</td>
<td>61.4% (13)</td>
<td>8.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>14.3% (98)</td>
<td>Ref.</td>
<td>0.01</td>
</tr>
</tbody>
</table>
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
Summary

230±3 days in pregnancy: first dose, BCS, serum, vaginal swab

2±1 DIM: Mucus score, BCS, temperature, serum, milk, cervical swab, \textit{E. coli} culture

35±3 DIM: Mucus score, BCS, temperature, endometritis diagnose serum, milk, cervical swab, \textit{T. pyogenes} culture

260±3 days in pregnancy: second dose (booster), BCS, serum, vaginal swab

6±1 DIM: Mucus score, BCS, temperature, metritis diagnose, serum, milk, cervical swab, \textit{F. necrophorum} culture

Farm database: Metritis, mastitis, SCC, reproductive performance
Enrollment
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).

P-value = 0.03
Results

Figure 4: Effect of vaccination on rectal temperature at 7 days postpartum.
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0091734
Metabolic and Immune Mechanisms Mediating Endometritis
Negative Energy Balance

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

Galvao et al., 2010
Figure 9. Least squares means ± 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 (○).


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


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

Gilbert & Santos, 2016
Endometrial gene expression

Galvao et al., 2011
Gene expression by circulating monocytes stimulated with *E. coli*

Galvao et al., 2012
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

Yale 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
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
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!
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cell count</th>
<th>Inner cell mass</th>
<th>Trophectoderm</th>
<th>Ratio  ICM/Troph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioned - Inflamed</td>
<td>83.1*</td>
<td>30.1</td>
<td>53.0*</td>
<td>0.38*</td>
</tr>
<tr>
<td>Conditioned - Non-Inflamed</td>
<td>99.8</td>
<td>26.5</td>
<td>73.3</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Hill & Gilbert, 2005
Effect of Endometritis on Ovarian Function
Important receptors for responding to LPS are expressed by granulosa cells
## LPS in Bovine Follicular Fluid

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

P = 0.0001

Herath et al., Reproduction, 2007
Intimate vascular exchange

• Uterine veins
• Ovarian artery

Mapletoft & Ginther, AJVR, 1975
Mapletoft et al., Biol.Reprod., 1976
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

Herath et al., Reproduction, 2007
The Research Imperative: Food supply

• 1798: Thomas Malthus predicted population would outstrip food supply by mid 19th century
• 1968: Paul Ehlich predicted famine in 1970s and 1980s
Green Revolution

• World grain production increased 250% from 1950 to 1980

• Depends heavily on fossil fuels
  • Fertilizer
  • Pesticides
  • Energy for irrigation

Norman Borlaug, 1914-2009
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
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%

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
  • ???
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.
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\textsuperscript{a,b}, E.C. Firth\textsuperscript{b}

\textsuperscript{a} Matamata Veterinary Services, Ltd, 26 Tainui Street, Matamata, New Zealand
\textsuperscript{b} 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\textsuperscript{1} and J Cuervo-Arango\textsuperscript{2}

\textsuperscript{1} Equine Fertility Clinic, Warren House Farm, Barracks Lane, West Midlands, UK.\textsuperscript{2} Departamento Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, Moncada, Spain
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
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
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?

RGilbert@rossvet.edu.kn