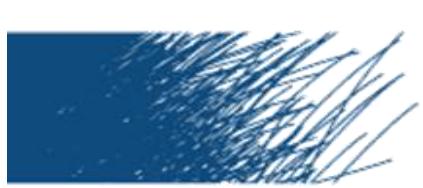

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Why is it more difficult to get high yielding dairy cows pregnant?

Geert Opsomer

Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium

Aims

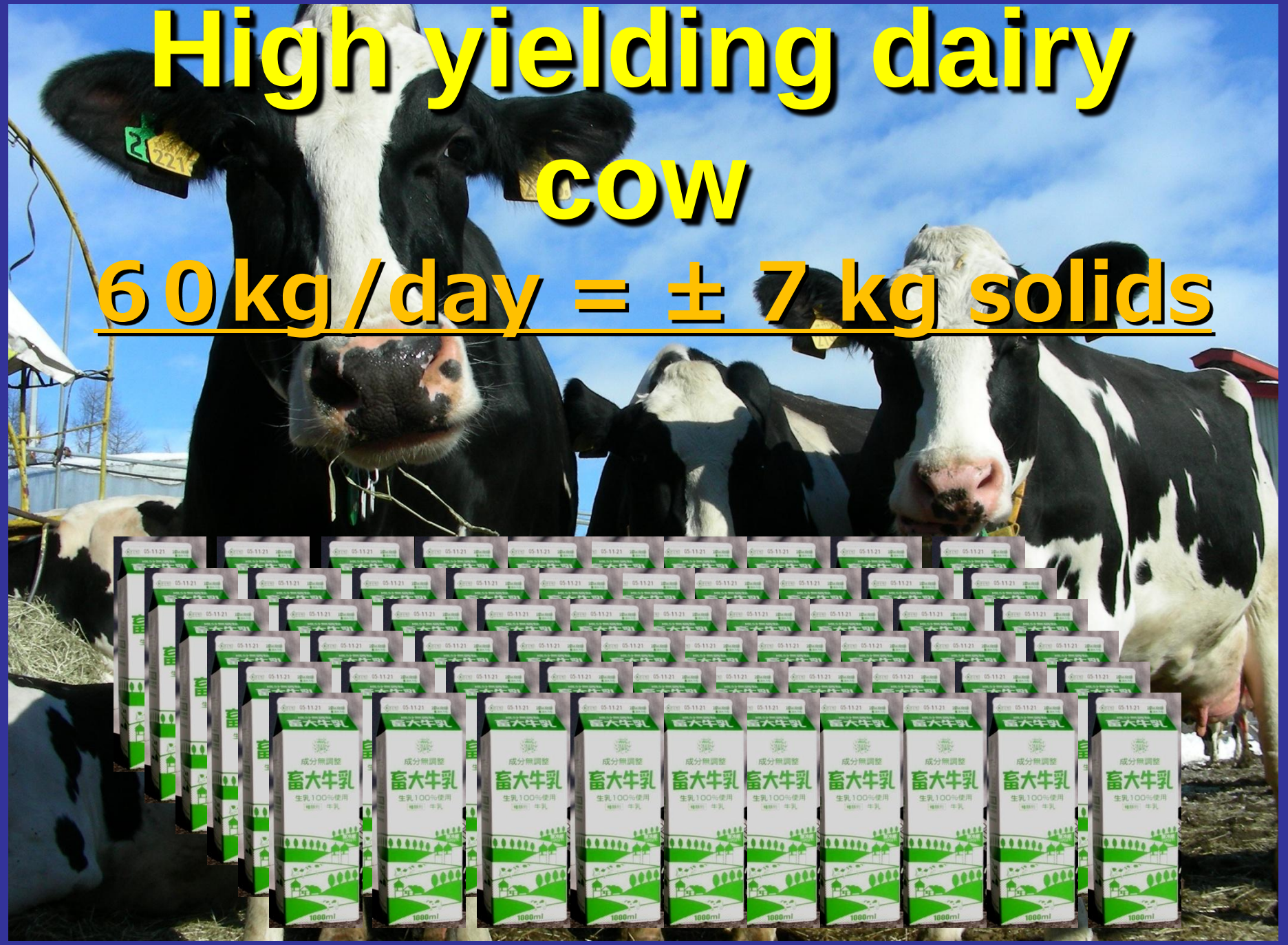
- To overview recent findings about fertility and fertility problems in modern dairy cows
  - evidence based knowledge read through glasses of veterinary practitioners
  - indicating practical points interesting for practitioners
- Two parts:
  - early postpartum (uterine disease)
  - resumption of ovarian activity and how to get the cows pregnant

Major challenges for modern dairy herds

- Increasing milk production per cow
  - milk production >10.000 kg milk/305 days
- Increase in herd size
  - Belgium: average herd size >100 cows – more and more herds >500 cows
- Time
  - finding strategies to optimize work schedules for the personnel – time spent for fertility work, for identifying diseased cows, for separation of cows, for…
- Volatile milk prices
  - reduce costs
- More and more regulations and paper work
  - environment – use of antibiotics -

High yielding dairy cow

60kg/day = ± 7 kg solids



Reproduction

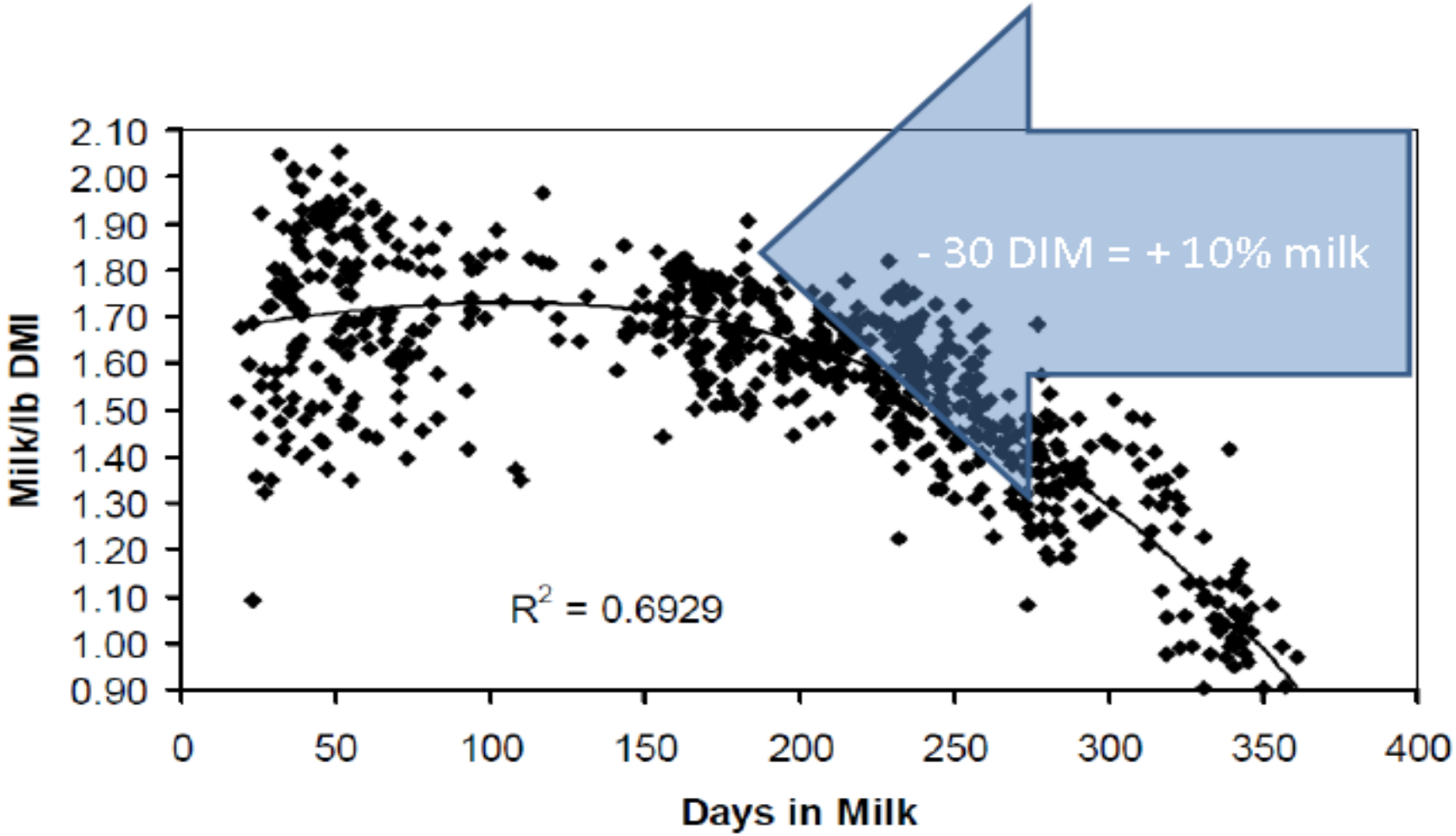
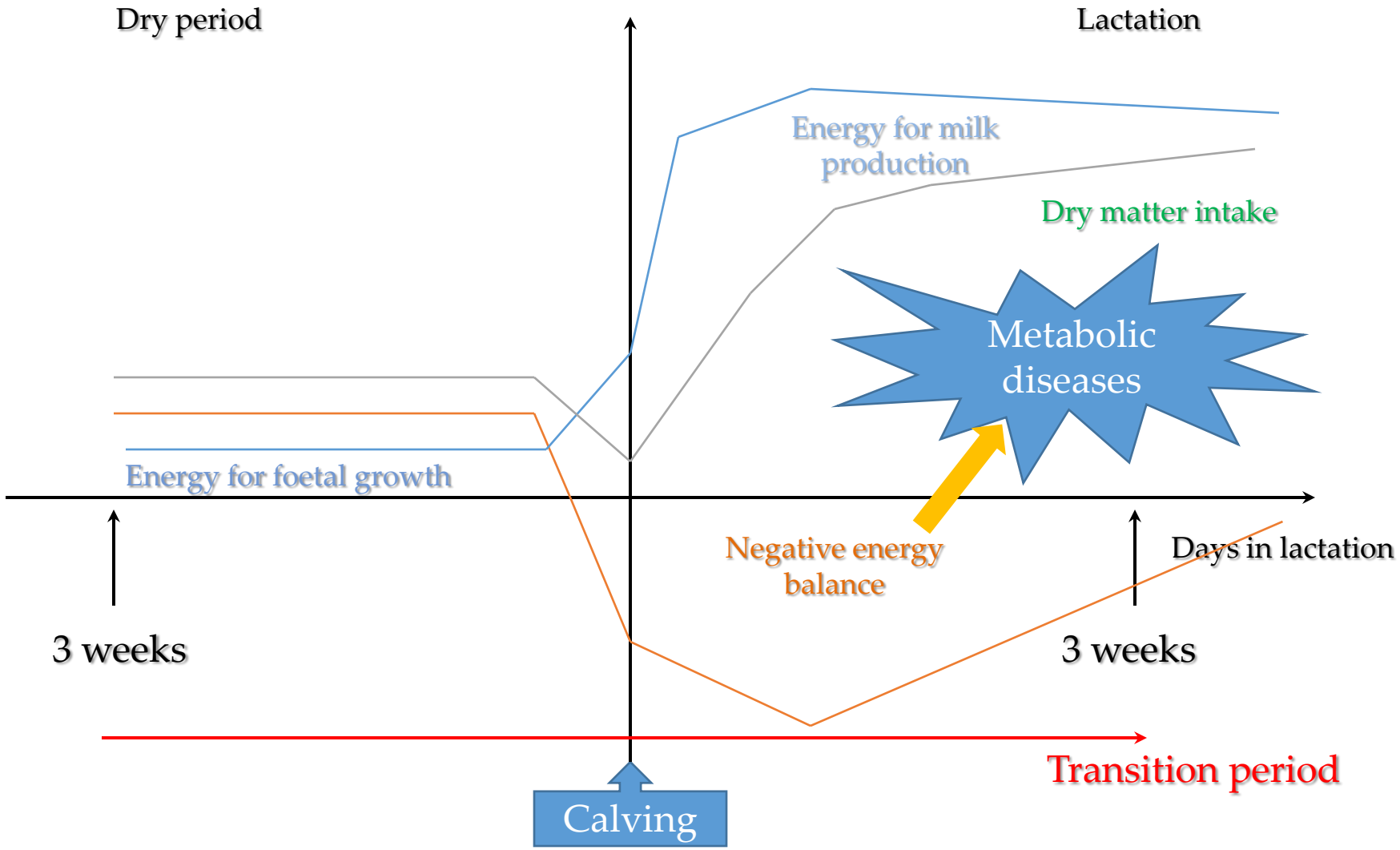


Figure 2. **Relationship between feed efficiency (milk/lb DM intake) and days in milk for 686 pens of Holstein cows.**

To reach optimal calving intervals

- **cows must:**
  - have an undisturbed uterine involution
  - early resume ovarian activity
  - develop healthy follicles containing fertile oocytes
  - coordinate ovulation and (overt) oestrus behaviour
  - maintain a uterine environment that promotes sperm transport and fertilization and finally supports pregnancy
  - remain free from infectious diseases harming the embryo/fetus leading to embryonic death or abortion

Transition period in dairy cows



Transition health problems are common
But healthy cows have good fertility

5719 cows in
7 US herds

Table 2. Impact of health problems in the first 60 d postpartum on pregnancy at first postpartum AI of dairy cows[1]

Health status	Prevalence, %	Pregnant, %	Adjusted OR (95% CI)[2]	*P*
Health problem				
Healthy	56	51.4	1.00	
1 case of disease	27	43.3	0.79 (0.69 – 0.91)	0.001
> 1 case of disease	17	34.7	0.57 (0.48 – 0.69)	< 0.001
Type of health problem[3]				
Calving problem	15	40.3	0.75 (0.63 – 0.88)	< 0.001
Metritis	16	37.8	0.66 (0.56 – 0.78)	< 0.001
Clinical endometritis	20	38.7	0.62 (0.52 – 0.74)	< 0.001
Fever postpartum	21	39.8	0.60 (0.48 – 0.65)	< 0.001
Mastitis	12	39.4	0.84 (0.64 – 1.10)	0.20
Clinical ketosis	10	28.8	0.50 (0.36 – 0.68)	< 0.001
Lameness	7	33.3	0.57 (0.41 – 0.78)	< 0.001
Pneumonia	3	32.4	0.63 (0.32 – 1.27)	0.20
Digestive problem	2	36.7	0.78 (0.46 – 1.34)	0.38

Santos et al RepDomRum 2010

Impacts of ketosis - Reproduction

Subclinical ketosis (serum BHB > 1.0 – 1.4 mmol/L) in early lactation is associated with:

- 3 X Increased risk of metritis (not in all studies)

  Hammon et al 2006; Duffield et al 2009

- 1.4 X greater odds of endometritis (uterine inflammation based on cytology) at 35 DIM

  Dubuc et al 2011

- 1.5 X increased odds of being anovular (not cyclic) at 63 DIM (19% vs. 13% of cows)

  Walsh et al 2007; Dubuc et al 2012

- Decrease in pregnancy at first AI

  Walsh et al, 2007

- Point prevalence (20 cows, 1 test, BHB ≥ 1.4 mmol/L) ≥ 20% associated with herd annual pregnancy at 1st AI < 40%

  Dubuc & Denis-Robichaud, 2017

- BHB > 1.2 mmol/L in any of 1st 5 weeks postpartum associated with lower 6-week in-calf in pasture system (78 vs. 85%)

  Compton et al 2015

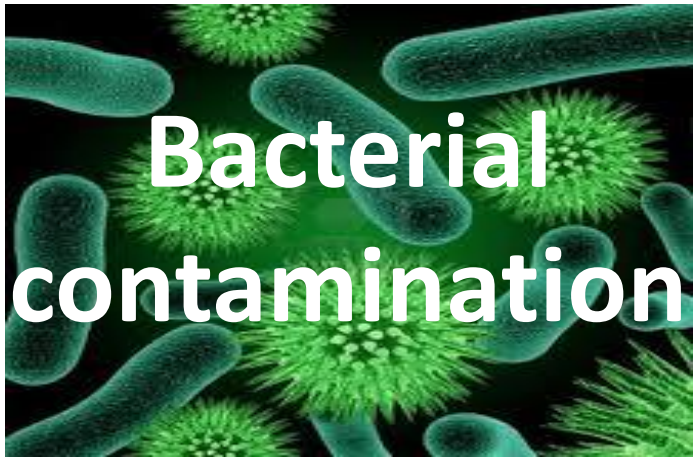
PARTURITION

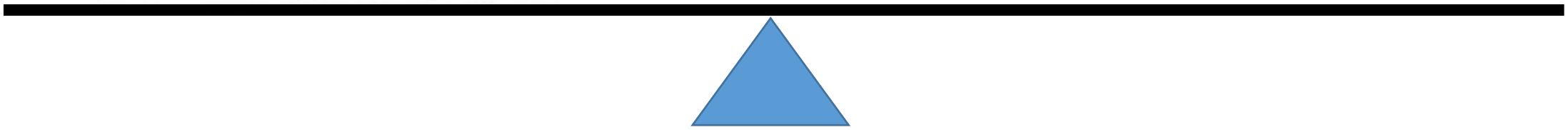
UTERINE INVOLUTION

Caruncular regions of the endometrium are left unprotected

- Regulation of inflammation
- Efficiency of the innate immune system
- Load and pathogenicity of bacteria

Immune and inflammatory response





Year of the study	Authors	(Endo)metritis incidence
1968	Tennant and Peddicord	11%
1977	Bouters and Vandeplassche	10%
1983	Oltenacu et al.	38%
1984	Markusfeld	37%
1986	Whitmore and Anderson	20%
2002	LeBlanc and Kasimanickam	17% (clin) + 37% (subclin)
2005	Gilbert et al.	53%

Defining postpartum uterine disease in cattle

I. Martin Sheldon [a,*], Gregory S. Lewis [b], Stephen LeBlanc [c], Robert O. Gilbert [d]

J. Dairy Sci. 93:5225–5233
doi:10.3168/jds.2010-3428

Definitions and diagnosis of postpartum endometritis in dairy cows

J. Dubuc,*[1,2] T. F. Duffield,* K. E. Leslie,* J. S. Walton,† and S. J. LeBlanc*
*Department of Population Medicine and
†Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

Defining and Diagnosing Postpartum Clinical Endometritis and its Impact on Reproductive Performance in Dairy Cows

S. J. LeBlanc,* T. F. Duffield,* K. E. Leslie,*
K. G. Bateman,* G. P. Keefe,†
J. S. Walton,‡ and W. H. Johnson*

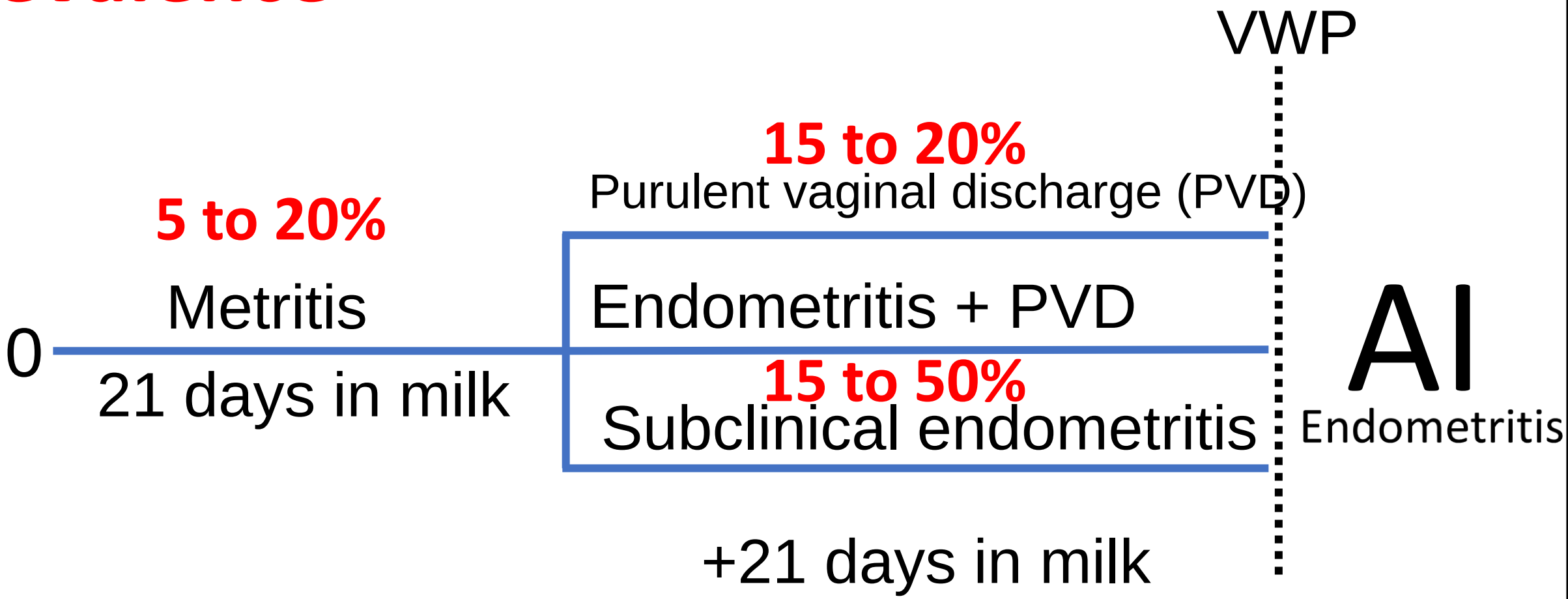
Definitions of uterine diseases

- *Retained fetal membranes*:
  - fetal membranes retained >24 h
- *Metritis (3 grades)*:
  - <21 days pp (most common 10 days pp)
  - enlarged uterus, abnormal discharge, often fetid odour, general health problems depending on the grade
- *Clinical endometritis*:
  - presence of purulent uterine discharge in the vagina >21 days pp or mucopurulent uterine discharge >26 days pp (=PVD + inflammation of the endometrium)
  - PVD = purulent vaginal discharge
- *Subclinical endometritis*:
  - inflammation of the endometrium (an elevated number of PMNs in the uterus) leading to a reduction in reproductive performance in the absence of signs of clinical endometritis
- *Pyometra:*
  - enlarged uterus filled with pus, closed cervix
  - corpus luteum persistens, no heat observed

(Sheldon et al., 2006 and 2009)

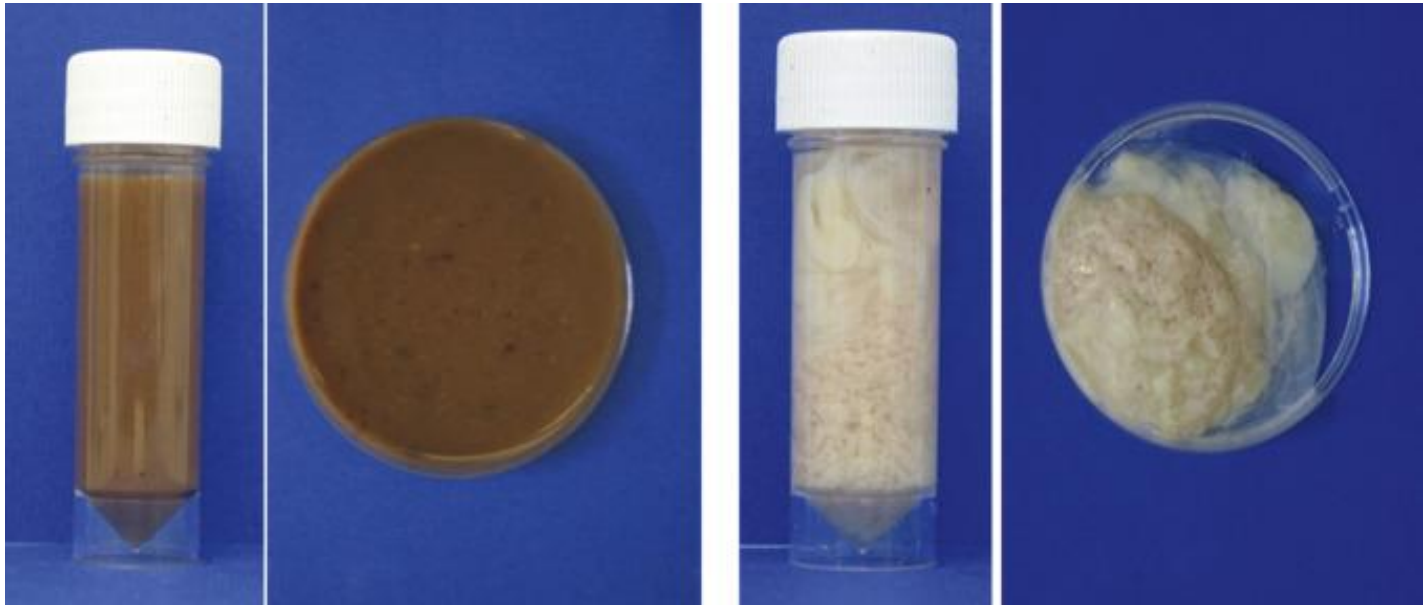
Postpartum uterine disease complex

Prevalence



Around half of postpartum dairy cows experience one or more forms of reproductive tract inflammatory disease

METRITIS

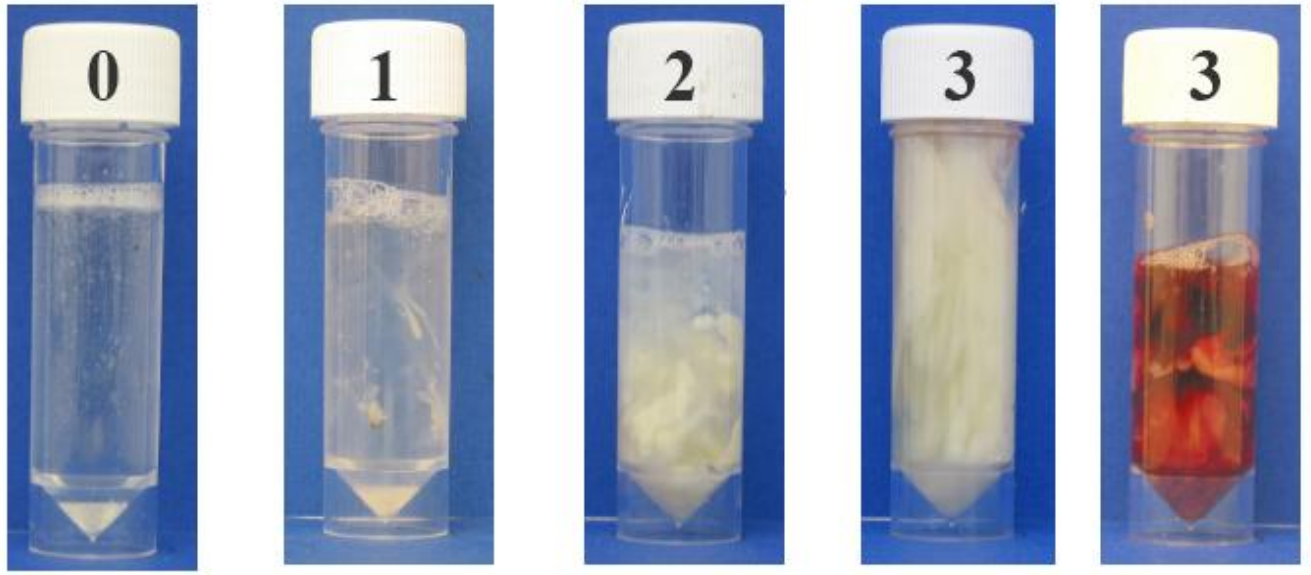


Purulent vaginal discharge

Diagnosis

Endometritis clinical score

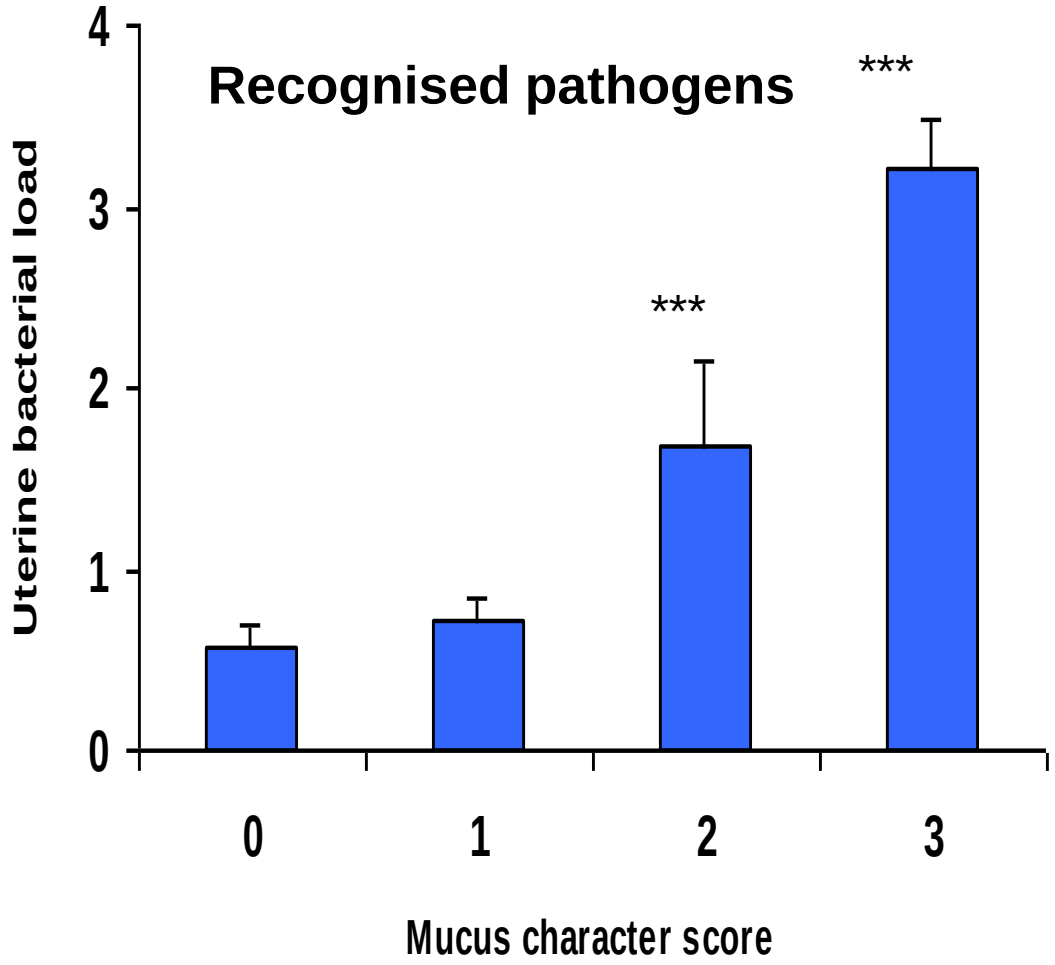
Mucus character

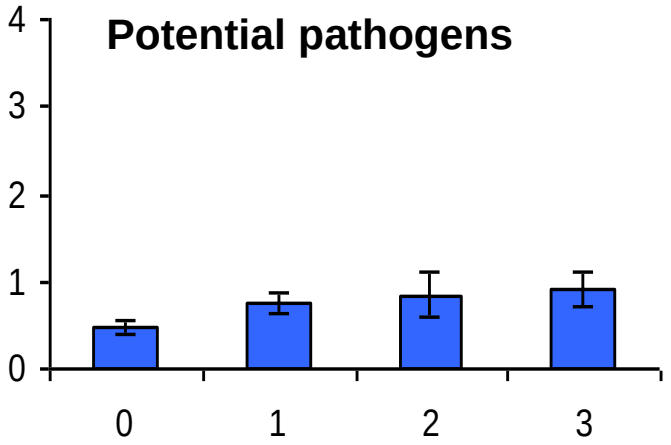


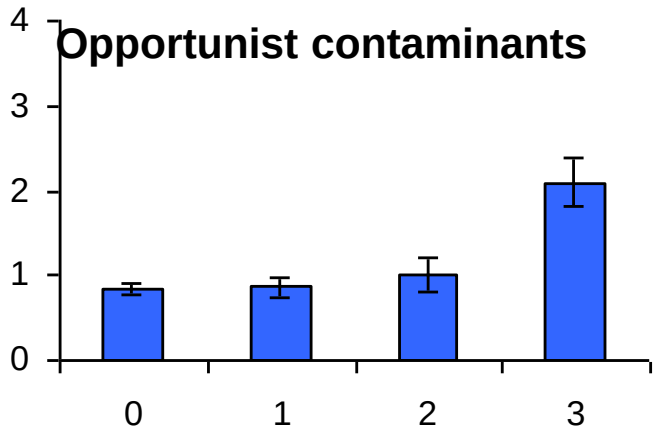
... **AND** mucus odour: Normal 0 Fetid 3

Williams et al *Theriogenology* (2004)

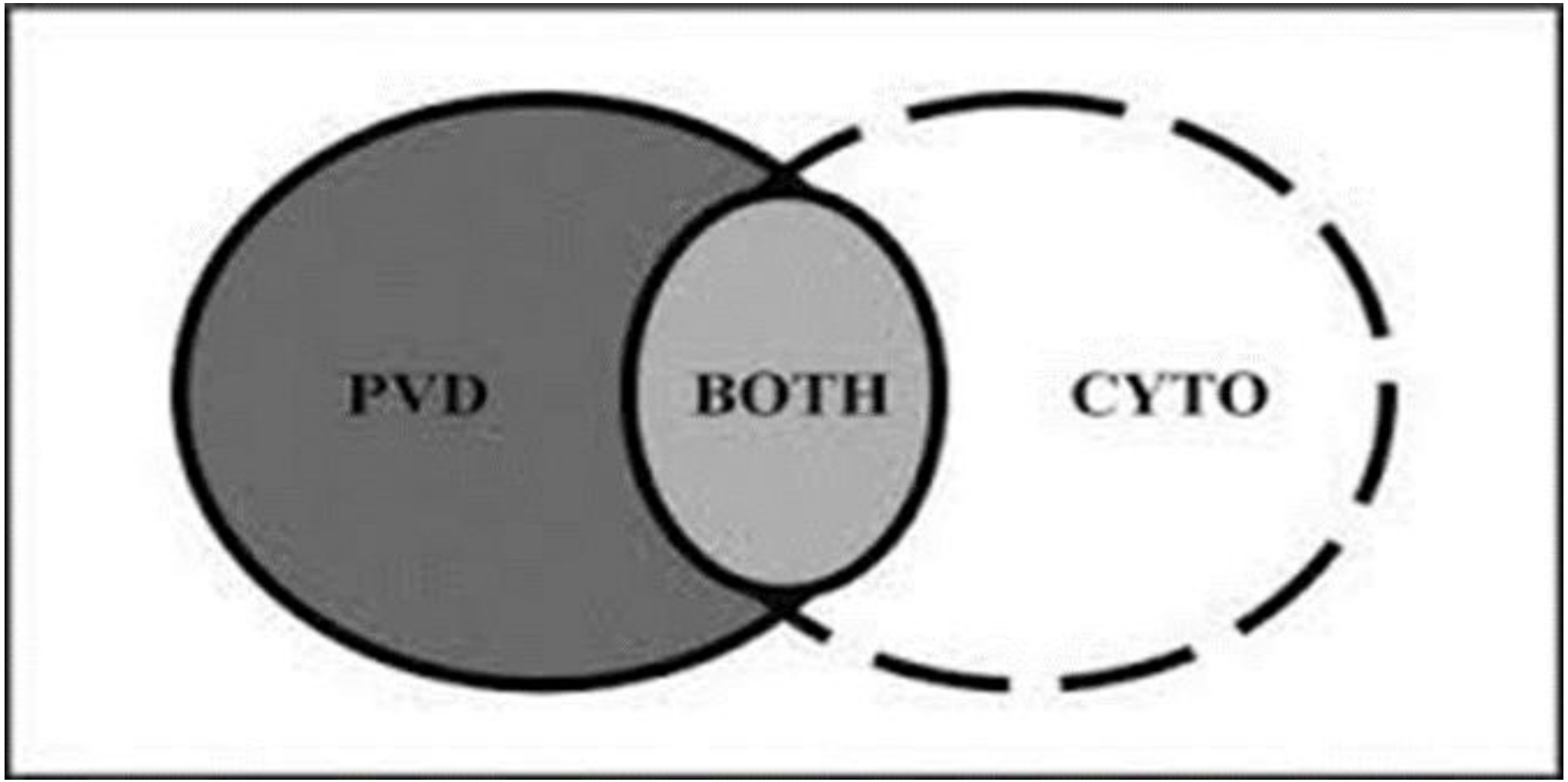
Mucus character reflects uterine bacteria





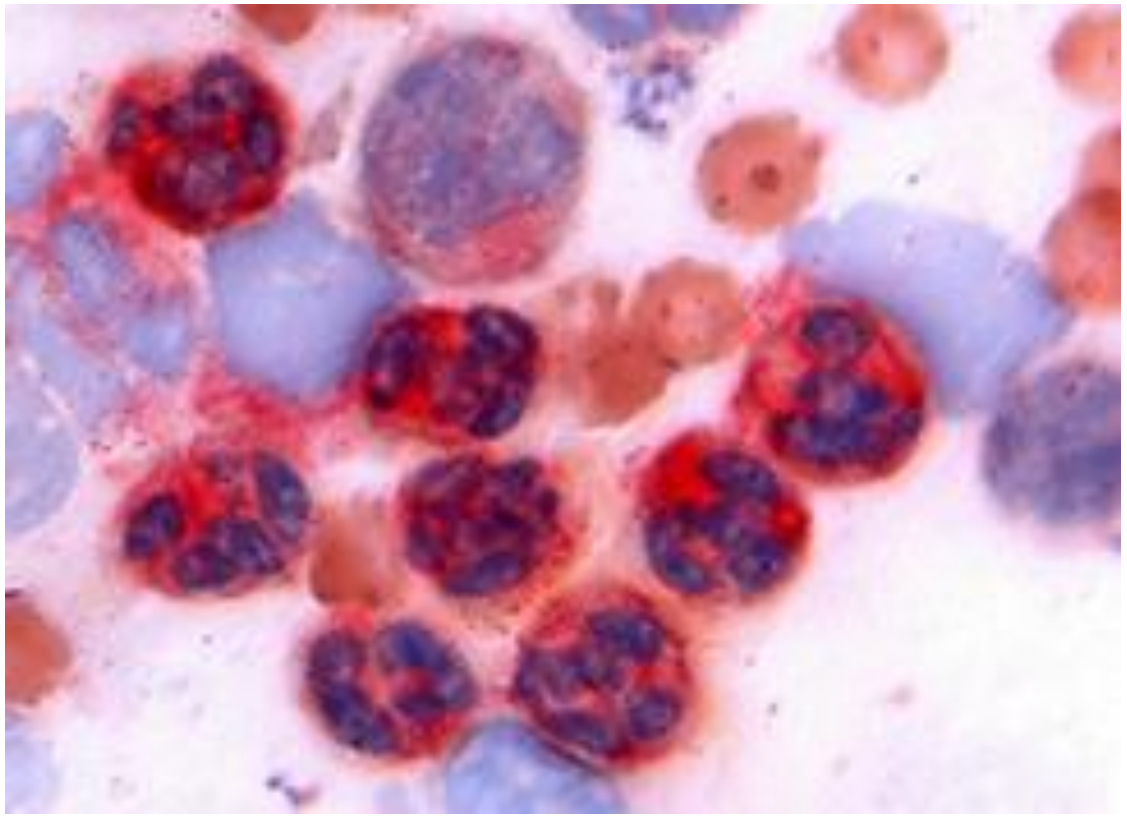


Williams et al., 2005



Dubuc et al., 2010

Clinical endometritis



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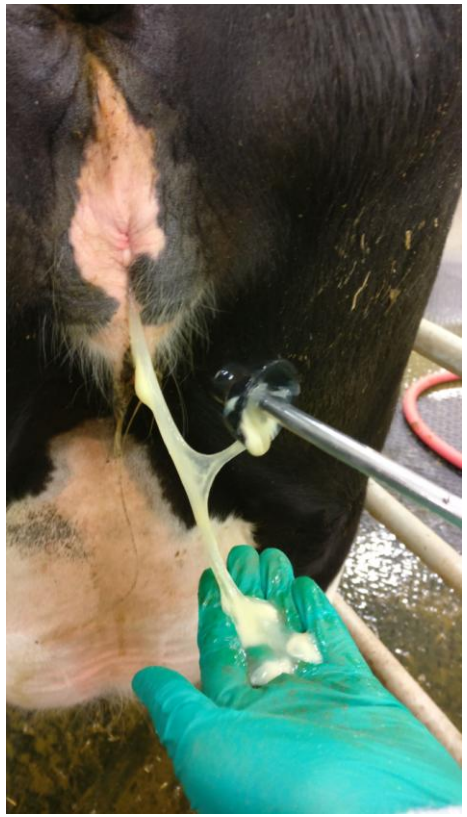


>5% PMN

Score 2

Dubuc *et al.*, 2010

Endometrial cytology

- **Cytobrush**

- easier and faster than lavage
- cut off – days in milk??
-only very small sample

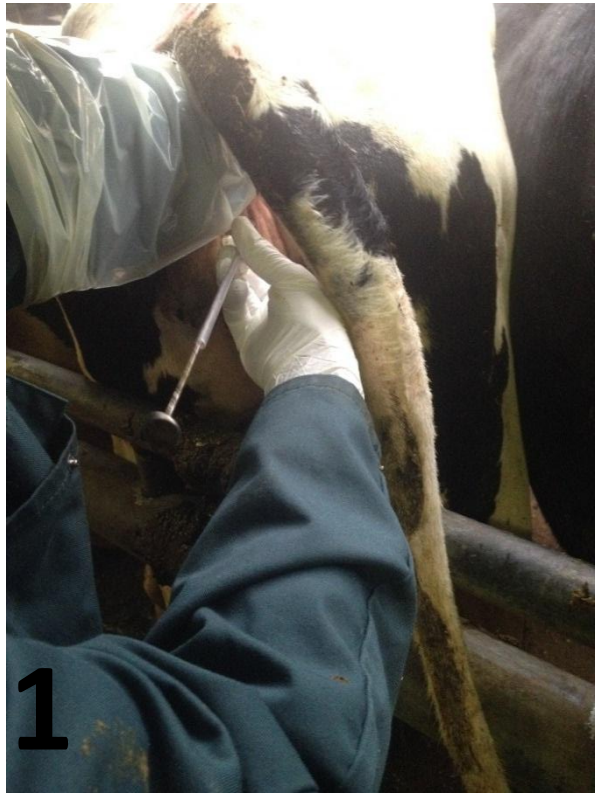
- **Low volume lavage**

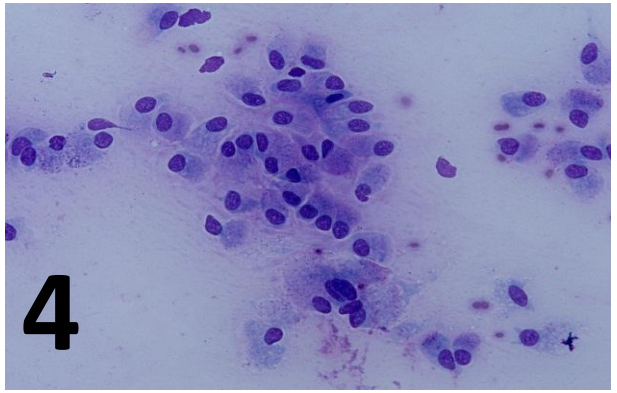
- more complicated than cytobrush (more steps and more difficult to harvest fluid)
- acquires cells from a larger surface?

<u>However, none of them are real cowside tests</u>

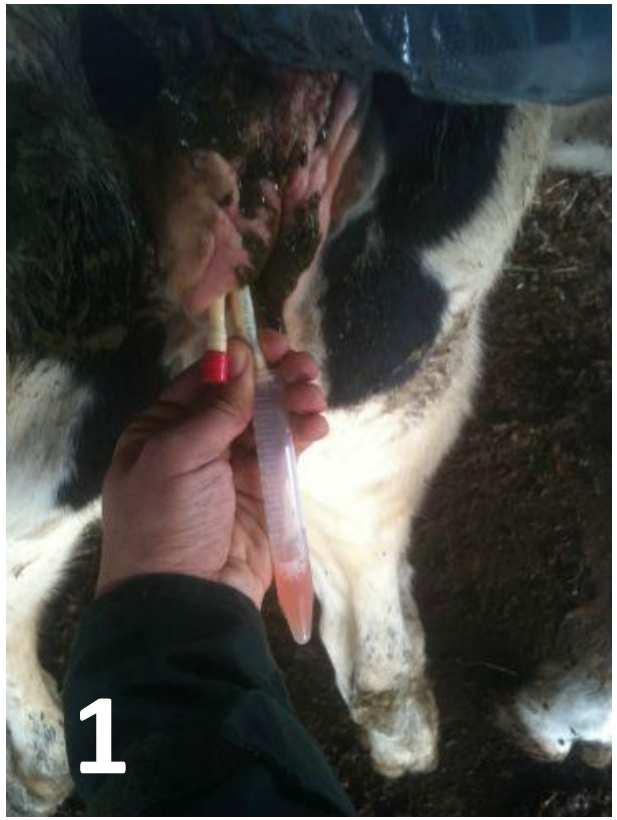
(Kasimanickam et al. 2005)

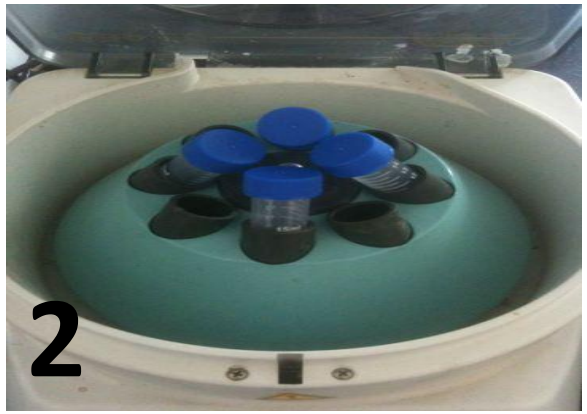
Cytobrush

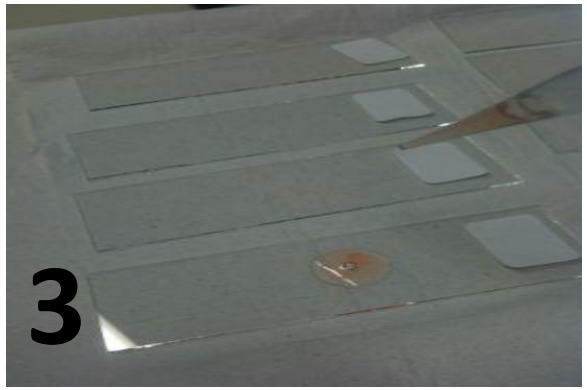


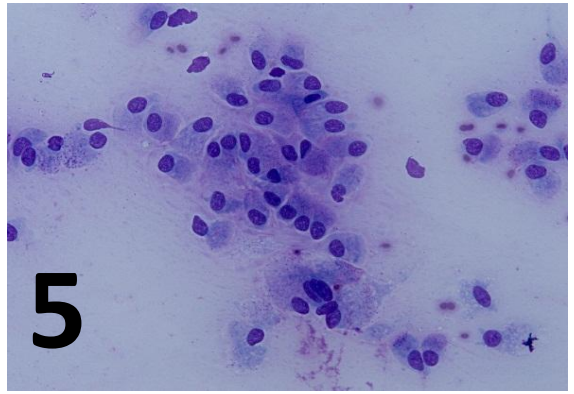


Low volume lavage

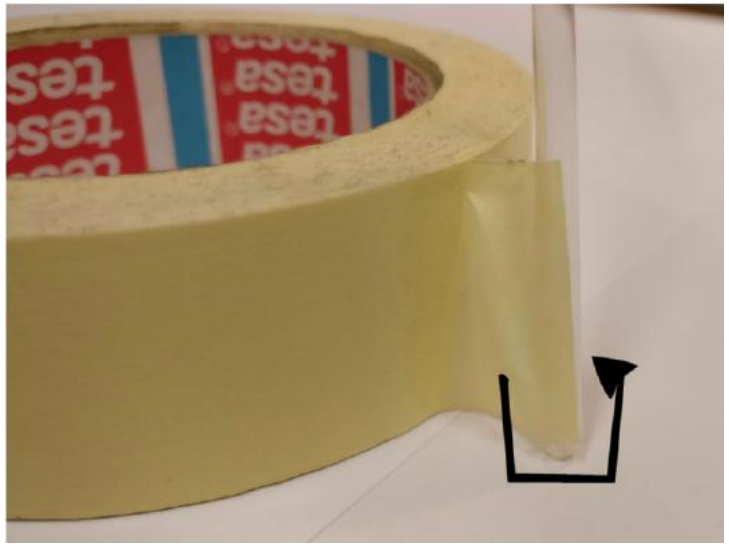


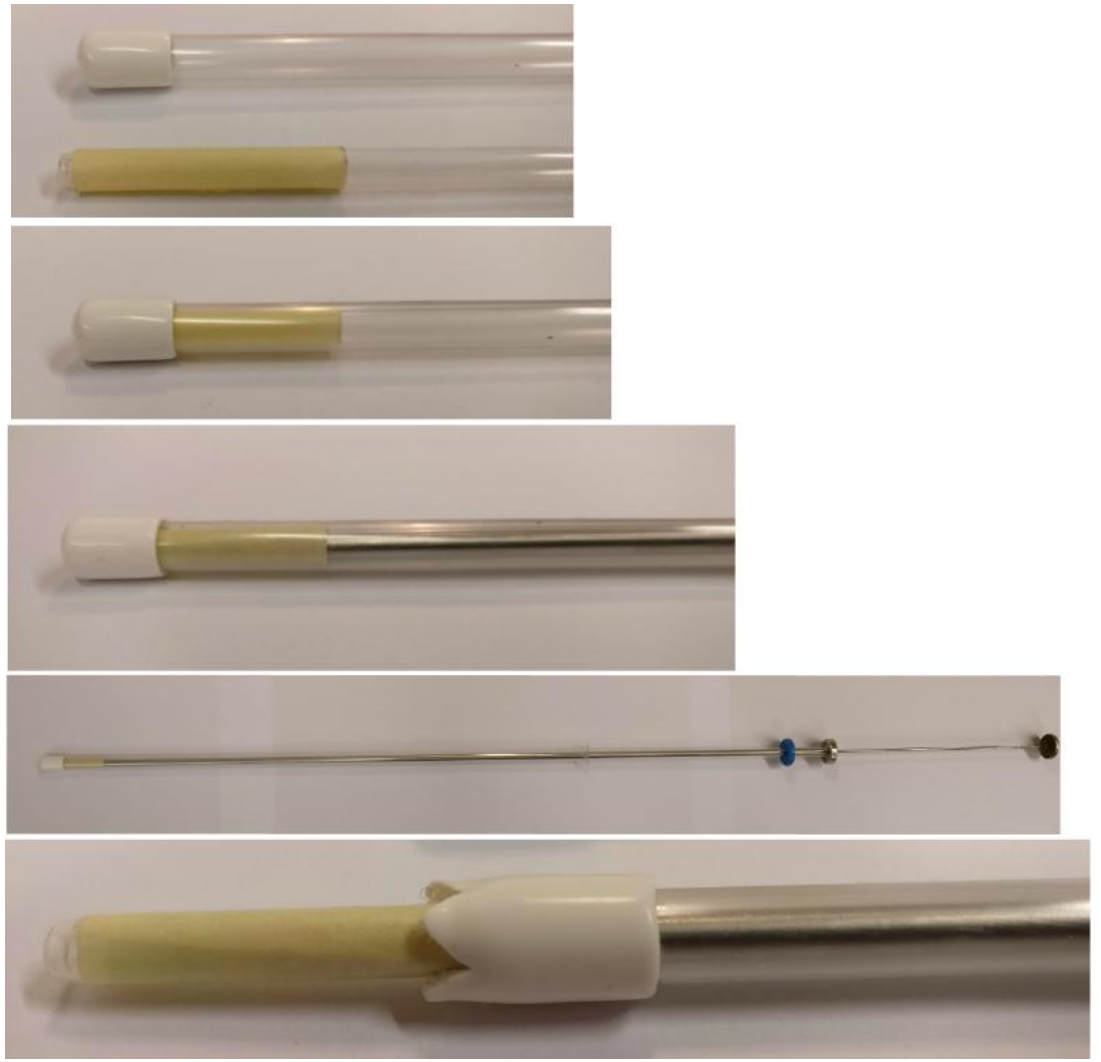






Cyto-tape





(*Pascottini et al., 2015*)

Cytotape: resume

- 1,625 Holstein cows and 512 nulliparous heifers included

- For cows and heifers, ≥1% PMN at AI decreased fertility

- Prevalence in cows was 27% and in heifers it was 7%

- SCE negative cows and heifers had 1.8 and 1.5 more chances of pregnancy per AI, respectively

Conclusions

- LE strips are a simple and efficient way to perform a cow-side diagnostic test for cytological endometritis with reasonable accuracy
- Brix refractometry is a poor diagnostic tool and should not be relied upon for the detection of cytological endometritis in dairy cattle

ELSEVIER

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Comparison of cow-side diagnostic techniques for subclinical endometritis in dairy cows

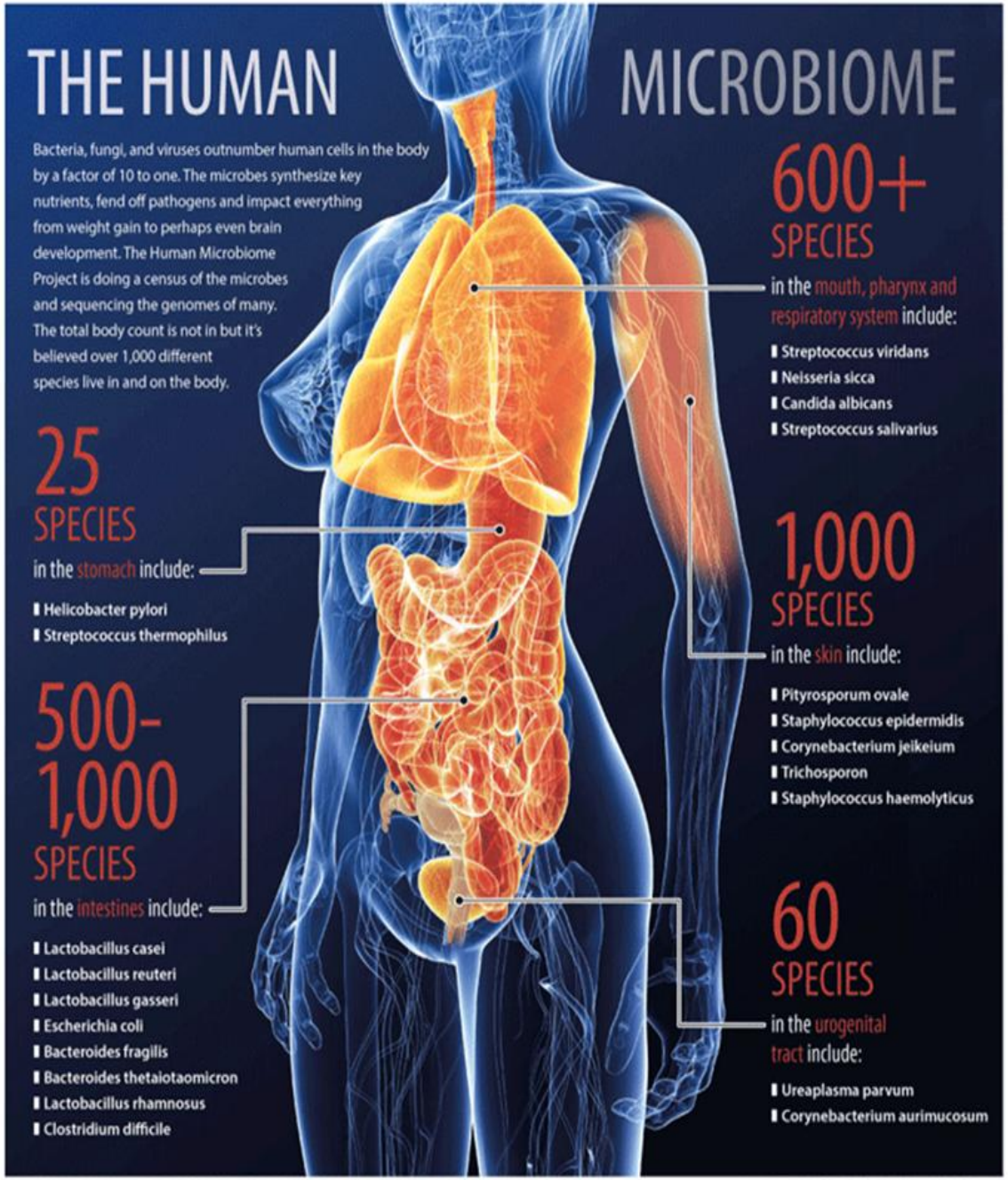
S.J. Van Schyndel*, [1], O. Bogado Pascottini [1], S.J. LeBlanc

Department of Population Medicine, Ontario Veterinary College, University of Guelph, Ont., N1G 2W1, Canada

Coming to a consensus concerning subclinical endometritis

- General treshold of 5% PMN for cows between 21 and 62 days postpartum
- Accuracy and repeatability of counting PMN under the microscope is reliable (counting a total of 300 cells)
- High prevalence: 20 to 40%
  - at insemination: 28%
- Effect on reproductive performance: decreased conception rate, prolonged days to first service, and days open
- Predominant bacteria related to subclinical endometritis?????

(Wagener et al., 2017)



SOURCES: NATIONAL INSTITUTES OF HEALTH, SCIENTIFIC AMERICAN; HUMAN MICROBIOME PROJECT

Dean Tweed • POSTMEDIA NEWS / IMAGE: Fotolia

Bacteria involved in the uterine disease complex

Table 1 – Categorization of bacteria, isolated by aerobic and anaerobic culture of uterine swabs, based on their potential pathogenicity [8–14,17,18]. Categories: (1) pathogens known to cause endometrial lesions; (2) potential uterine pathogens; and (3) bacteria not recognized as uterine pathogens that are likely contaminants of the uterine lumen.

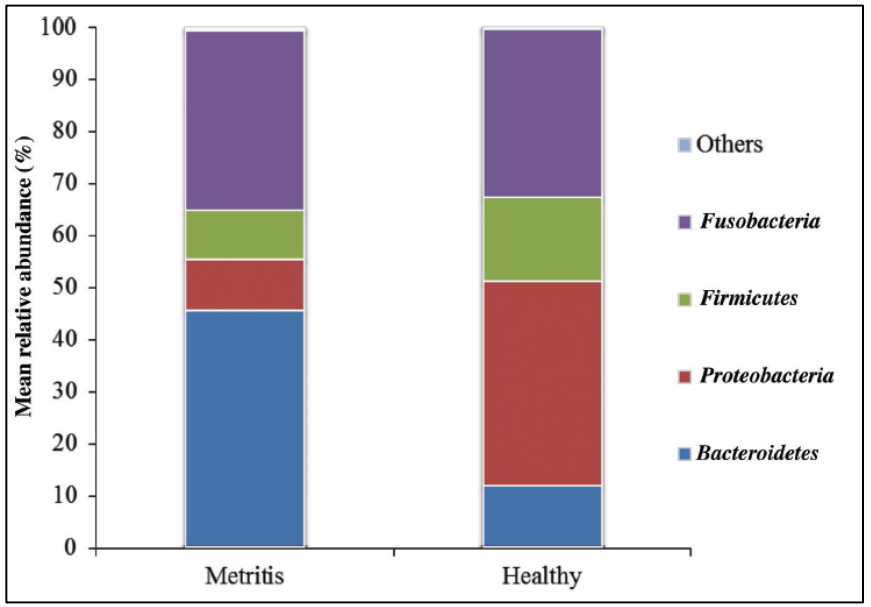
Pathogens	Potential pathogens	Contaminants
Escherichia coli	*Acinetobacter* spp.	*Aerococcus viridans*
Trueperella pyogenes	*Bacillus licheniformis*	*Clostridium butyricum*
Prevotella spp.	*Enterococcus faecalis*	*Clostridium perfringens*
	Haemophilus somnus	
Fusobacterium necrophorum	*Mannhiemia haemolytica*	*Corynebacterium* spp.
Fusobacterium nucleatum	*Pasteurella multocida*	*Enterobacter aerogenes*
	Peptostreptococcus spp.	*Klebsiella pneumoniae*
	Staphylococcus aureus (coagulase +)	*Micrococcus* spp.
	Streptococcus uberis	*Providencia rettgeri*
	Bacteroidetes species	*Providencia stuartii*
	Firmicutes species	*Proteus* spp.
	Fusobacteria species	*Proprionobacterium granulosa*
		Staphylococcus species
		α-haemolyic Stretococci
		Streptococcus acidominimus

Culturable vs non-culturable bacteria → open debate

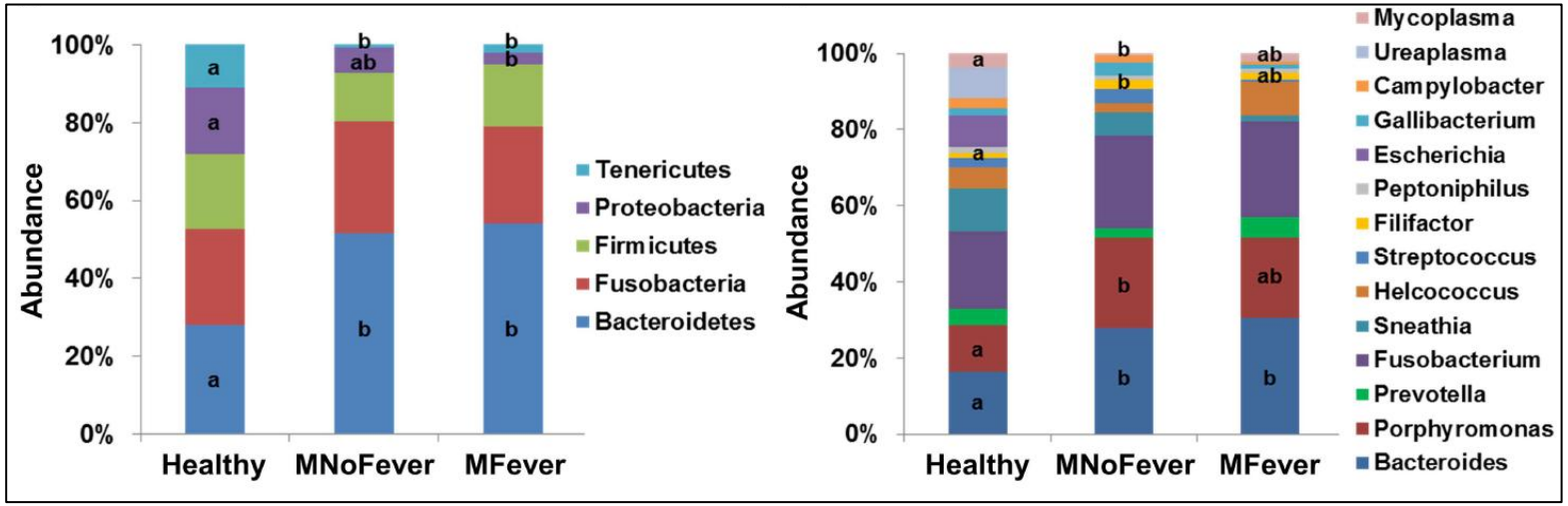
(Caneiro et al. 2016)

Uterine microbiome

- Culture-independent techniques give a different picture than routine bacteriology
- Cows with metritis have lower diversity of uterine microbes than healthy cows



Bicalho et al JDS 2017



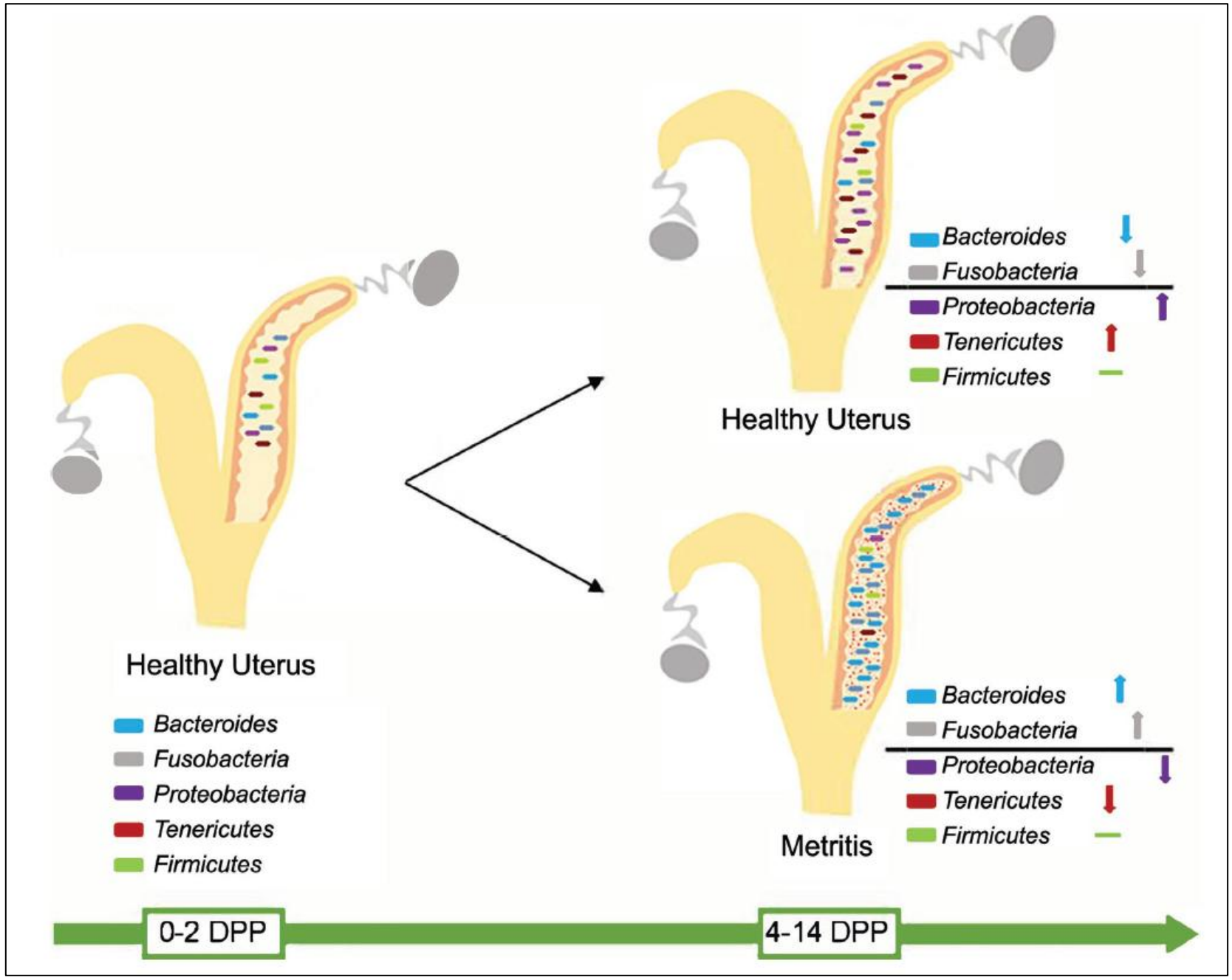
Jeon et al PLoSONE 2016

Uterine microbiota differences in metritis

Important pathogens appear to be:

Fusobacterium *necrophorum*
Porphyromonas *levii*
Bacteroides *pyogenes*

Galvao et al JDS 2019



Postpartum uterine microbiome

-The uterine microbiome of metritis cows and cows with CE was less diverse in comparison to healthy cows

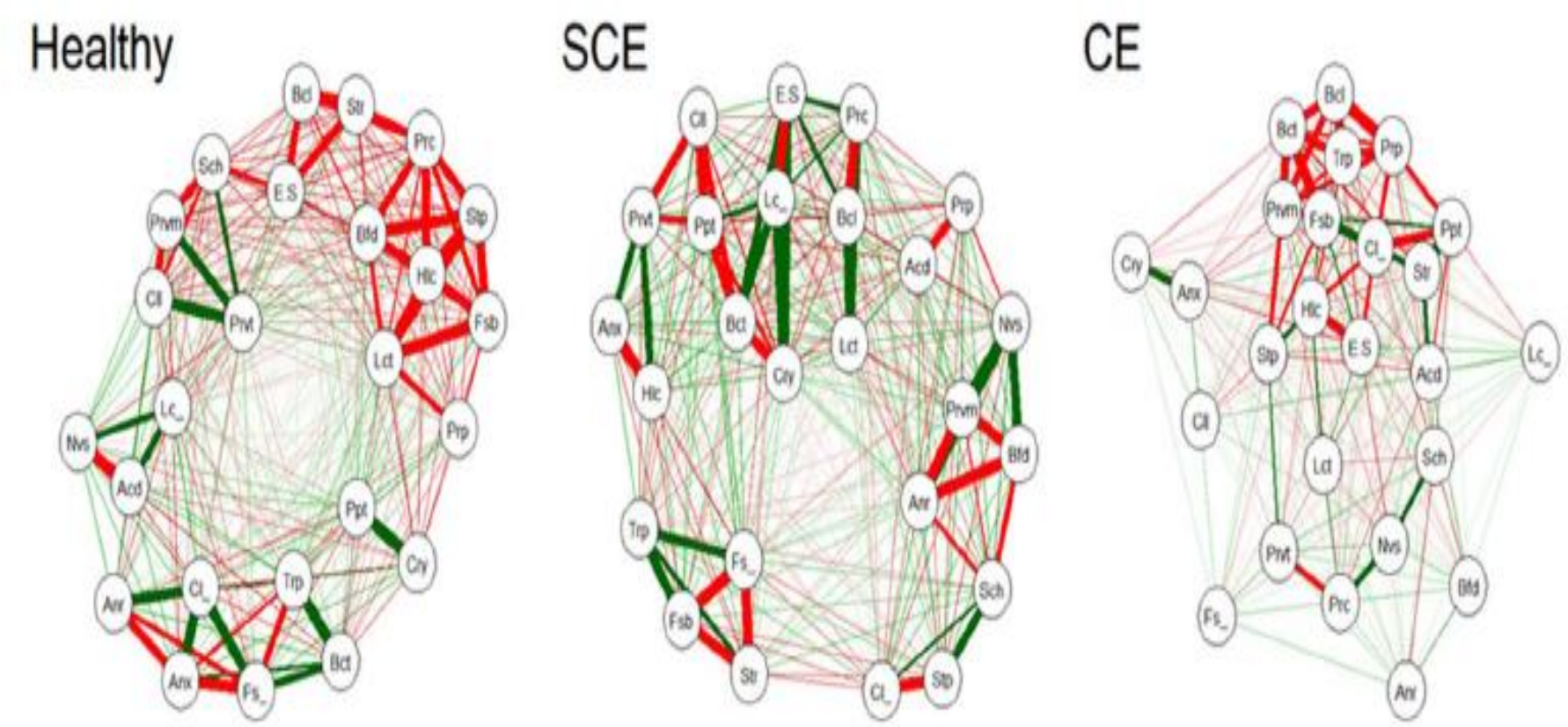
-CE cows had a greater relative abundance of *Fusobacterium* and *Trueperella* in comparison to healthy or SCE cows

- Uterine bacterial composition was not different between healthy and SCE cows

-The uterine microbiota was stable across 10, 21, and 35 DIM for healthy, SCE, and CE cows

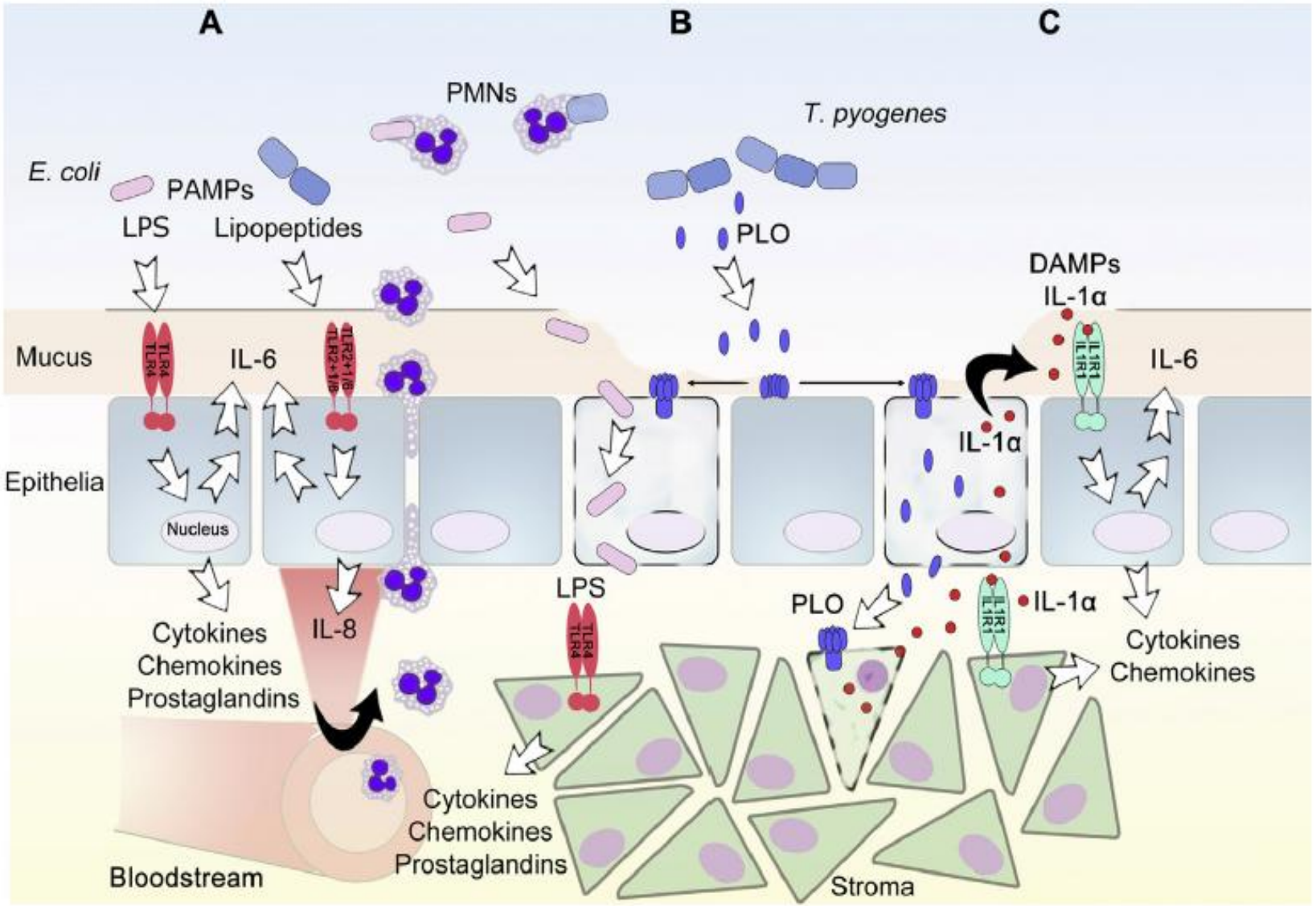
- Bacteria that grew in culture were often present within the most abundant bacteria in the 16S rRNA gene sequencing

Interaction between bacteria in the bovine uterus



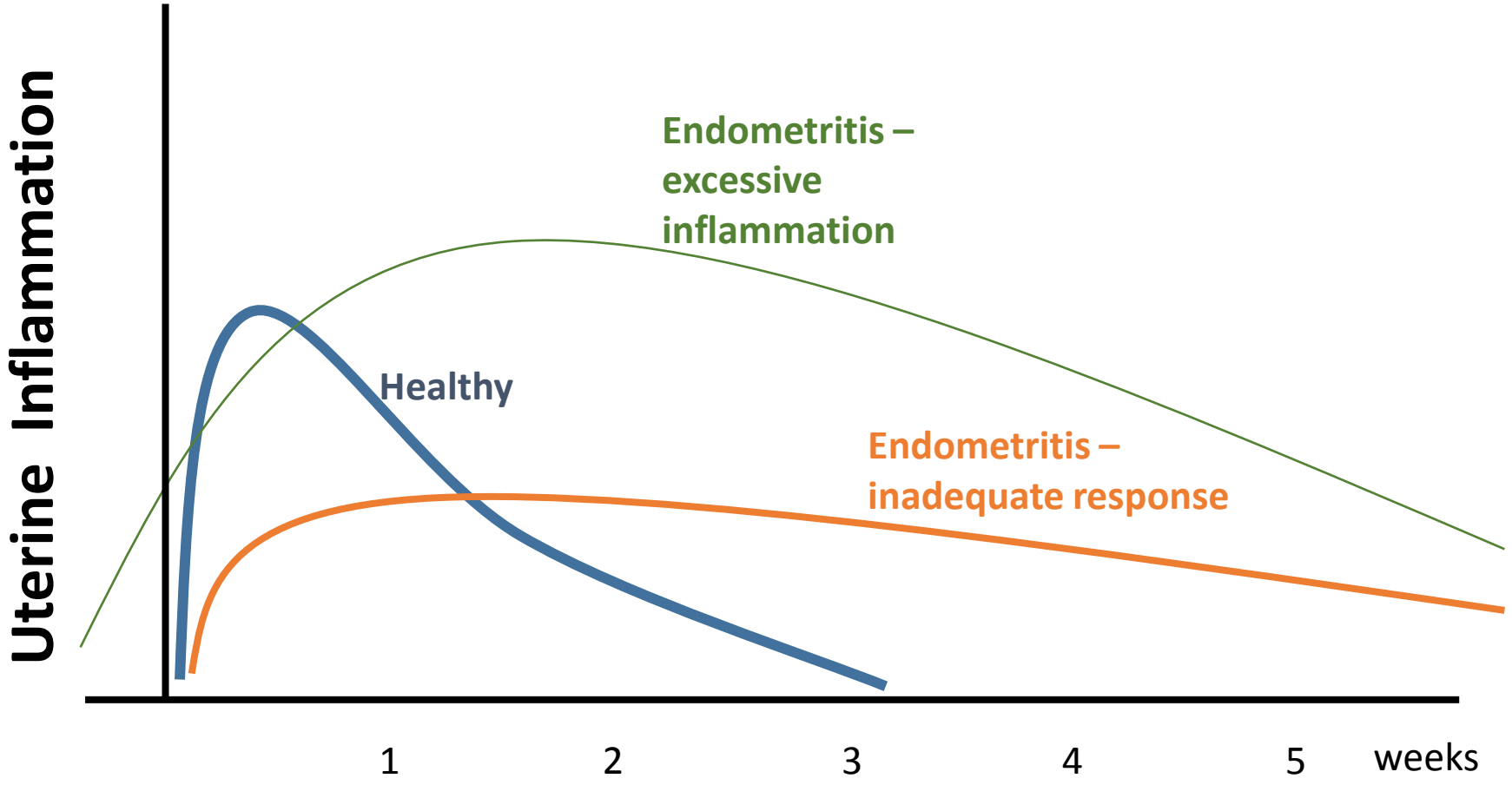
(Pascottini et al., 2020)

The uterine inflammatory response



(Caneiro et al. 2016)

Concepts of the uterine inflammatory response post partum



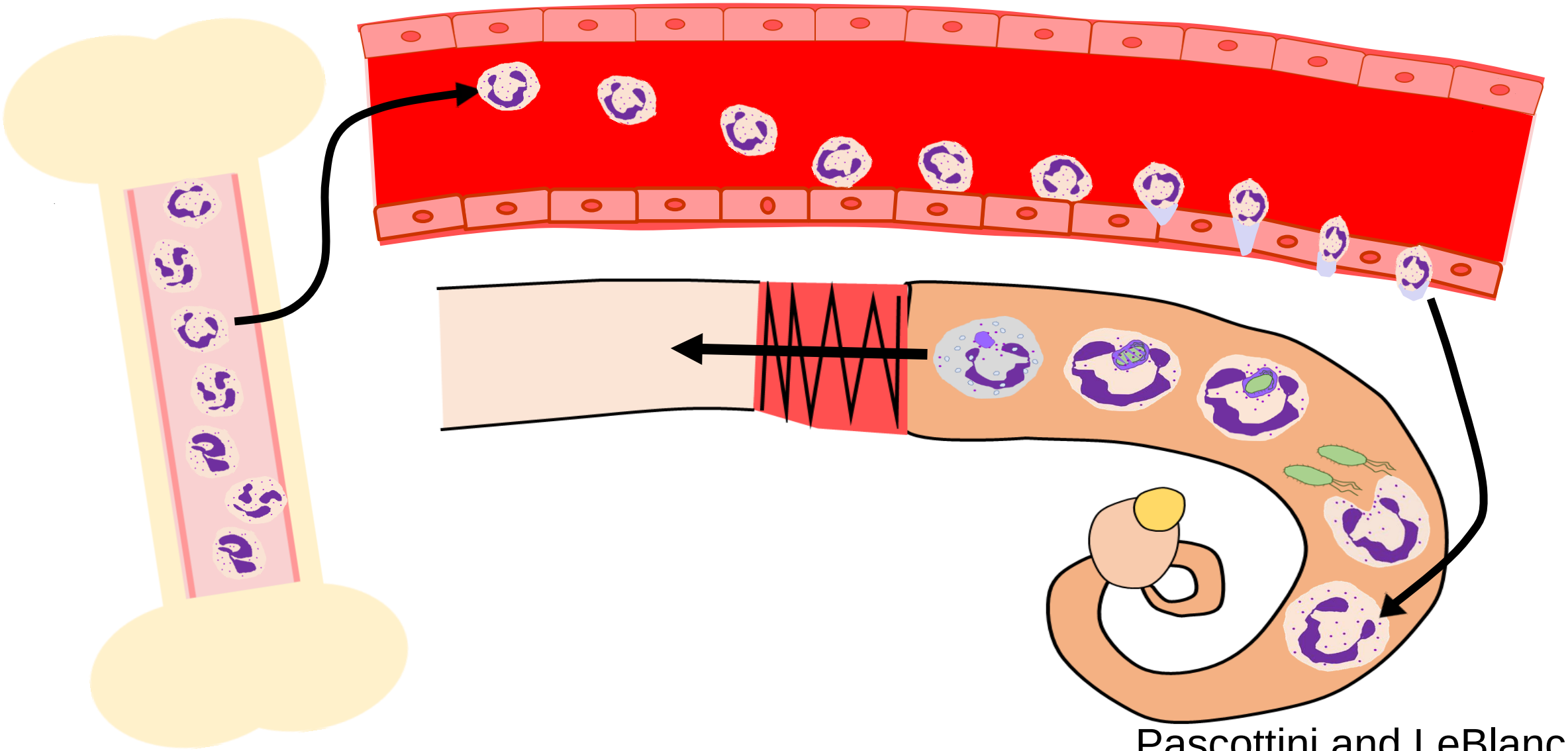
(LeBlanc 2014)

Reasons for blunted inflammatory response after calving

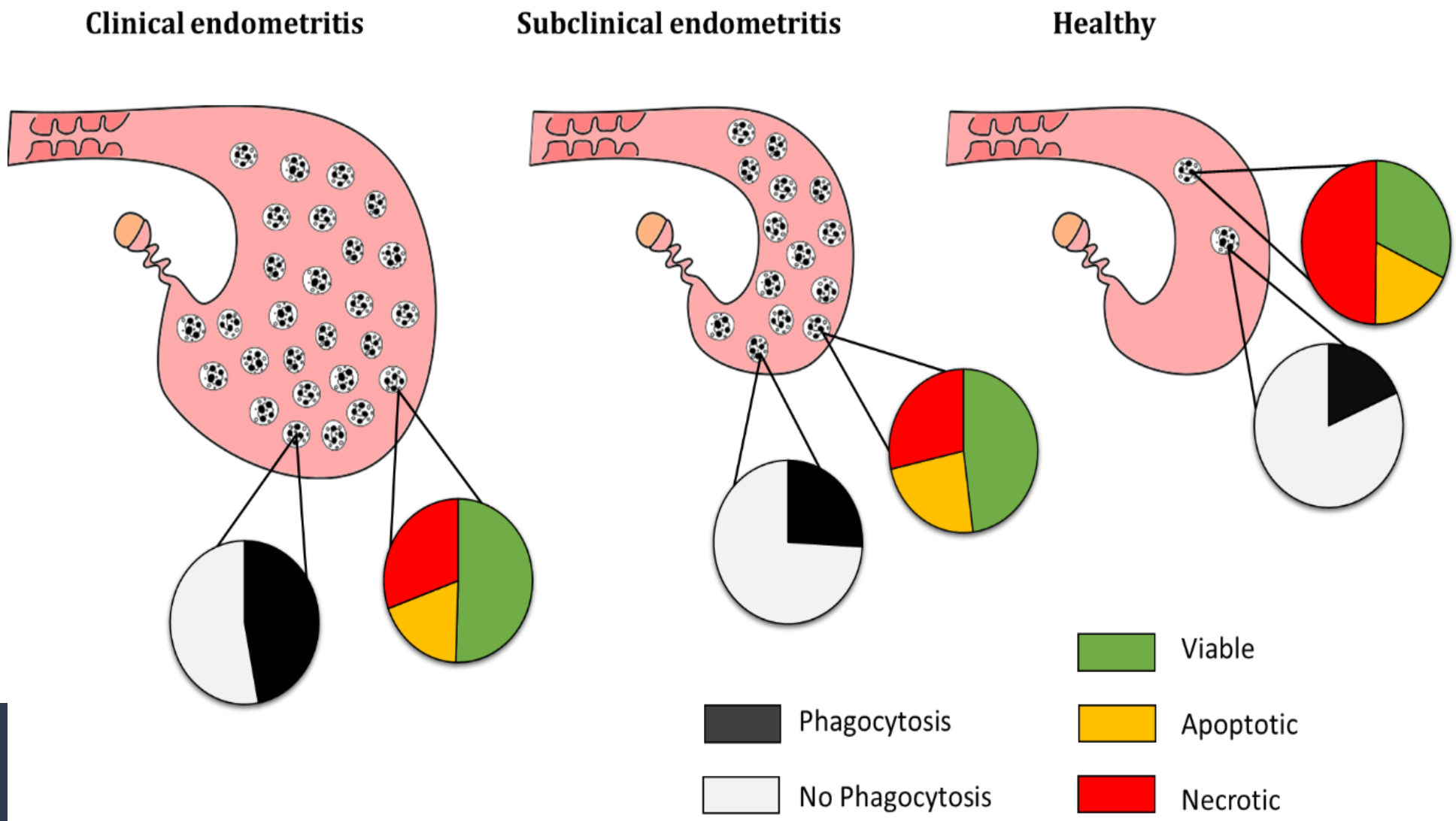
- …*and hence chronic inflammation*
- Attenuated switch from immune tolerance towards an immune reactivation
- Metabolic stress
  - lower abundance of important indispensable ‘fuels’

(Sheldon et al., 2017)

Innate immune function



Pascottini and LeBlanc


Clinical endometritis
Subclinical endometritis
Healthy
Phagocytosis
No Phagocytosis
Viable
Apoptotic
Necrotic

METRITIS

Treatment (new guidelines antibiotic therapy)

- Grade 1:
  - Pain killing – strict follow up (temp during 10 consecutive days)
- Grade 2:
  - Pain and fever treatment
  - Parenteral antibiotic treatment (Gram negative anaerobes)
- Grade 3:
  - Shock treatment + Grade 2 treatment

Clinical approach to (sub)clinical endometritis

- …to treat or not to treat??
  - affected animals have lower pregnancy results
- Prostaglandines: only if CL is present?
- if no CL present: intra-uterine antibiotics
- Clinical endometritis: recent meta-analysis: in favor of intra-uterine treatment with cephalosporins *(Lefebvre and Stock, 2012)*
- although no role for bacteria in SCE, antibiotic treatments for SCE have been described

Innovative therapies for uterine disease in cattle that are currently under research

- Pre- and probiotics
  - certain lactobacillus strains impair growth of pathogenic bacteria in the female reproductive tract
- Vaccination
  - Machado et al., 2014: first positive results of preventing metritis by vaccination but needs further confirmation
- Use of NSAIDs instead of antibiotics
- Phage therapy
  - viral strains with high specificity towards pathogenic bacteria

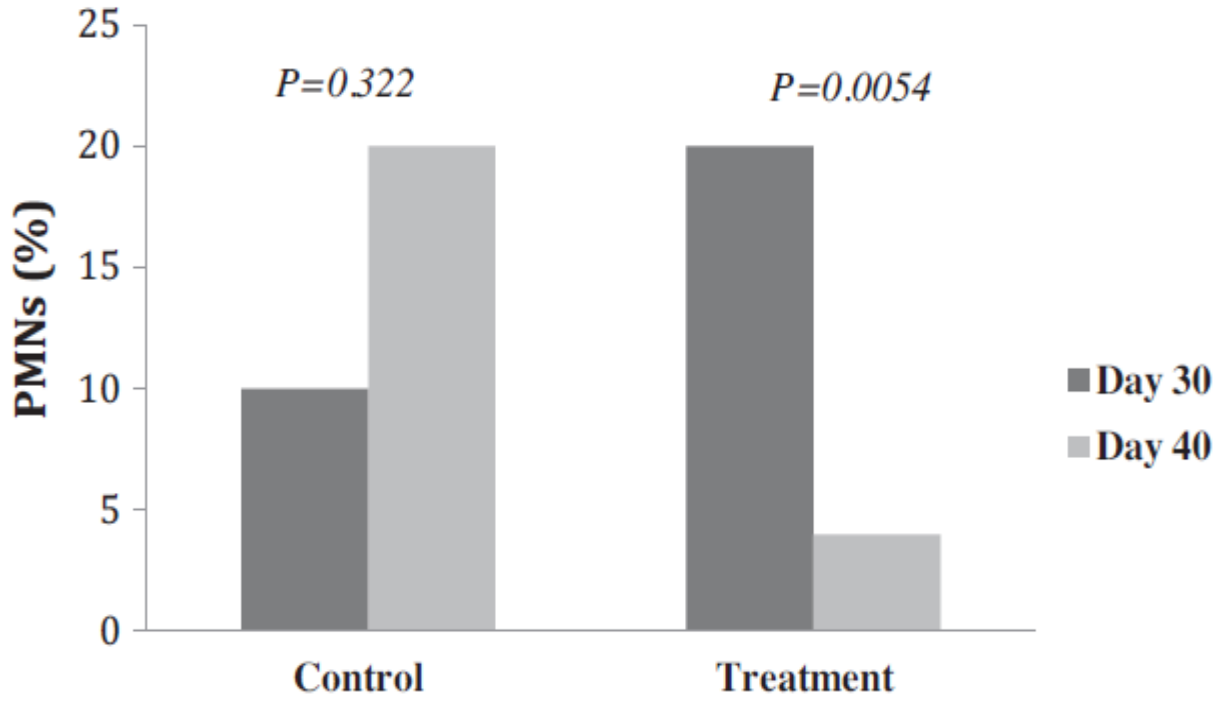
Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Effect of uterine lavage on neutrophil counts in postpartum dairy cows

P. Dini[a,*], M. Farhoodi[c], M. Hostens[b], M. Van Eetvelde[b], O. Bogado Pascottini[b], M.H. Fazeli[d], G. Opsomer[a]



Risk factors for uterine disease

- Species of bacteria
  - Virulence factors
  - Strain
- Level of contamination
  - Diversity of the uterine microbiome

- Dry matter intake
- Energy and lipid metabolic health
- Stressors & hormonal changes
- Hypocalcemia

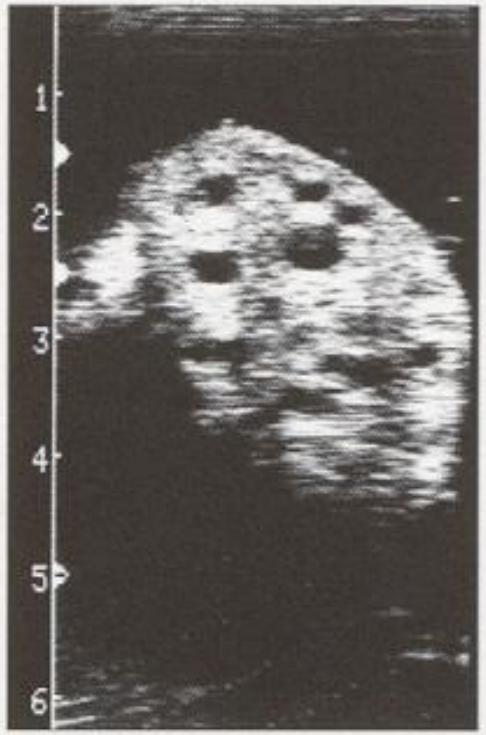
Preventive strategies to reduce (endo)metritis

- Aim for smaller calves and easy calvings
  - choice of bulls
  - use of sexed semen (female/smaller calves)
- Hygiene during parturition
  - calving pen – cow - obstetrician
- Avoid stress around parturition
  - no major changes in housing, nutrition, grouping, …
- Optimize transition management to minimize metabolic disease
  - body condition score – DCAD – optimize dry matter intake and rumen function – minimize negative energy balance


davél 1
/06/04 21:02:31


2
3
4
5
6
7
8
9


0
2
3
4
5
6


1
2
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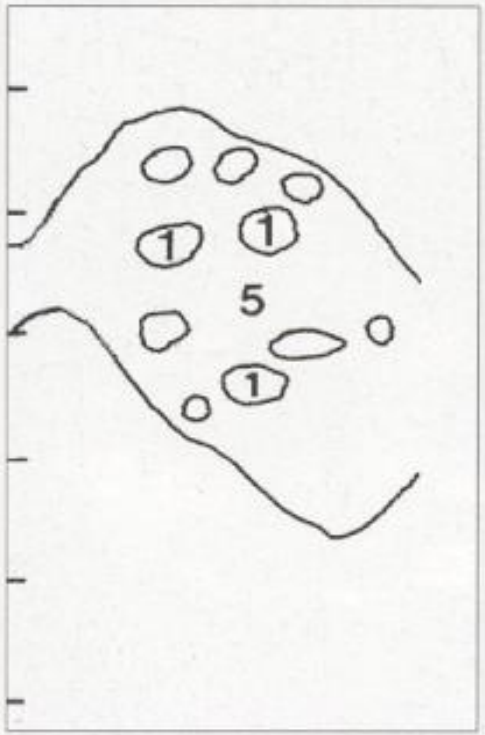
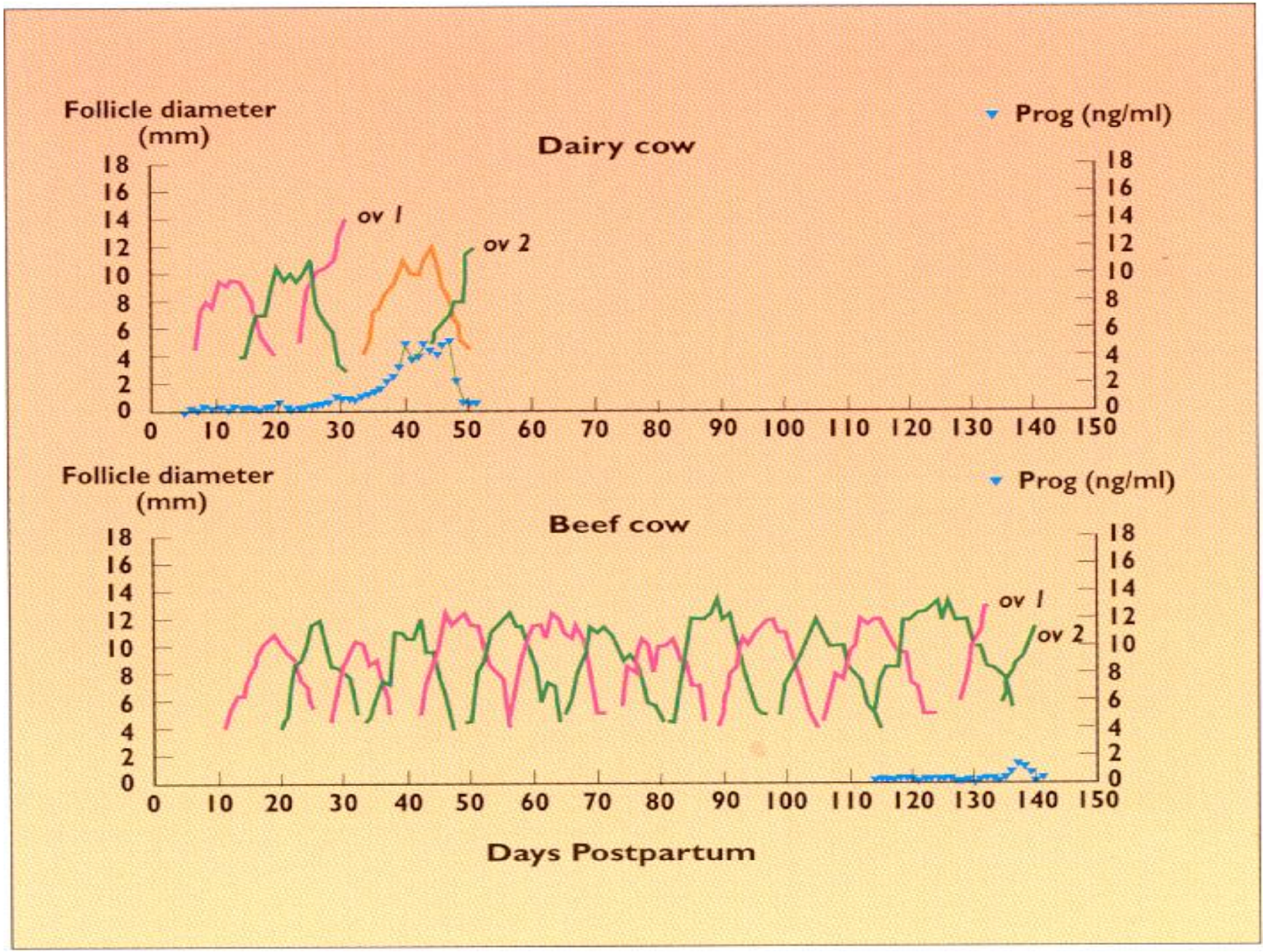
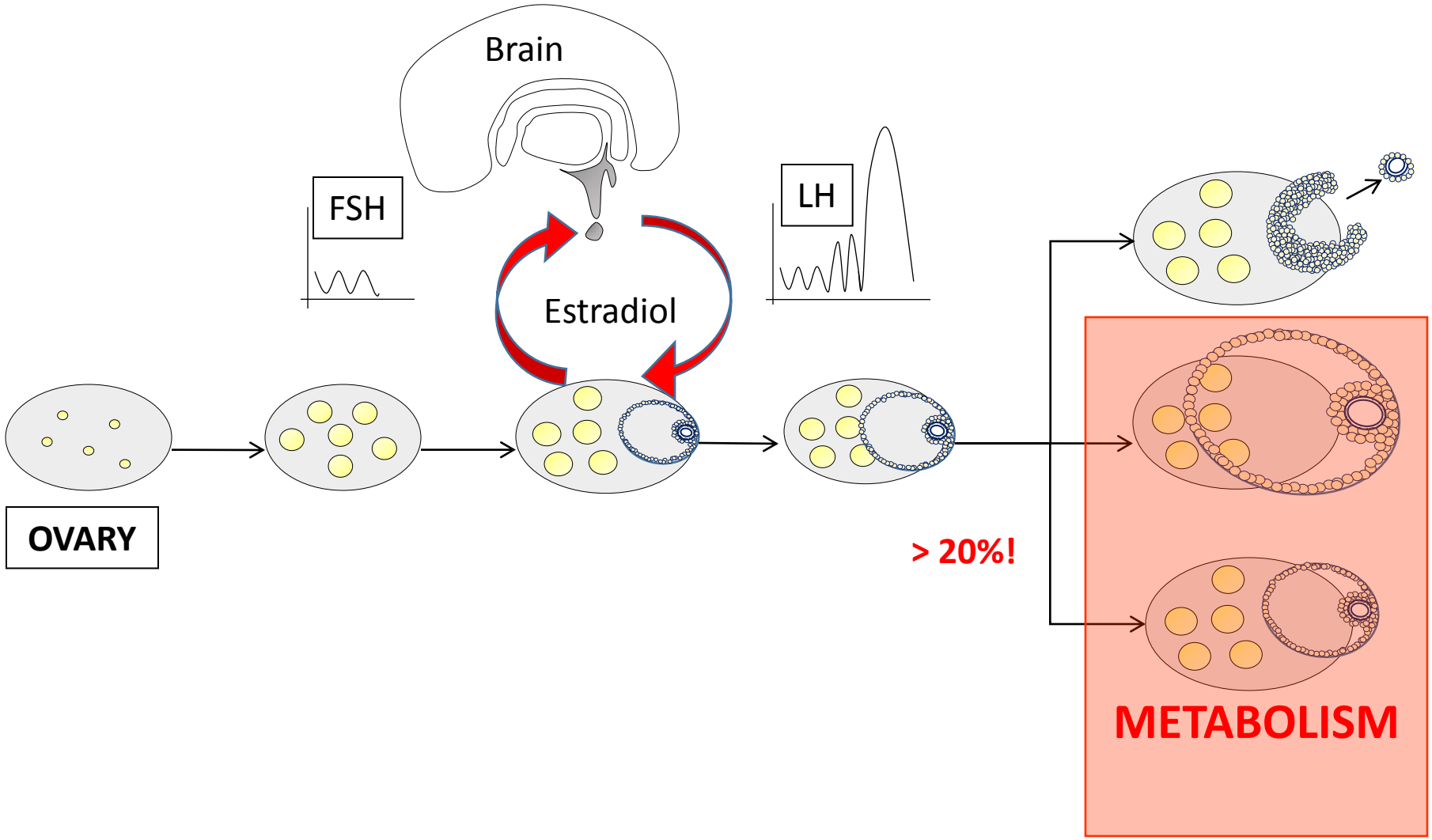
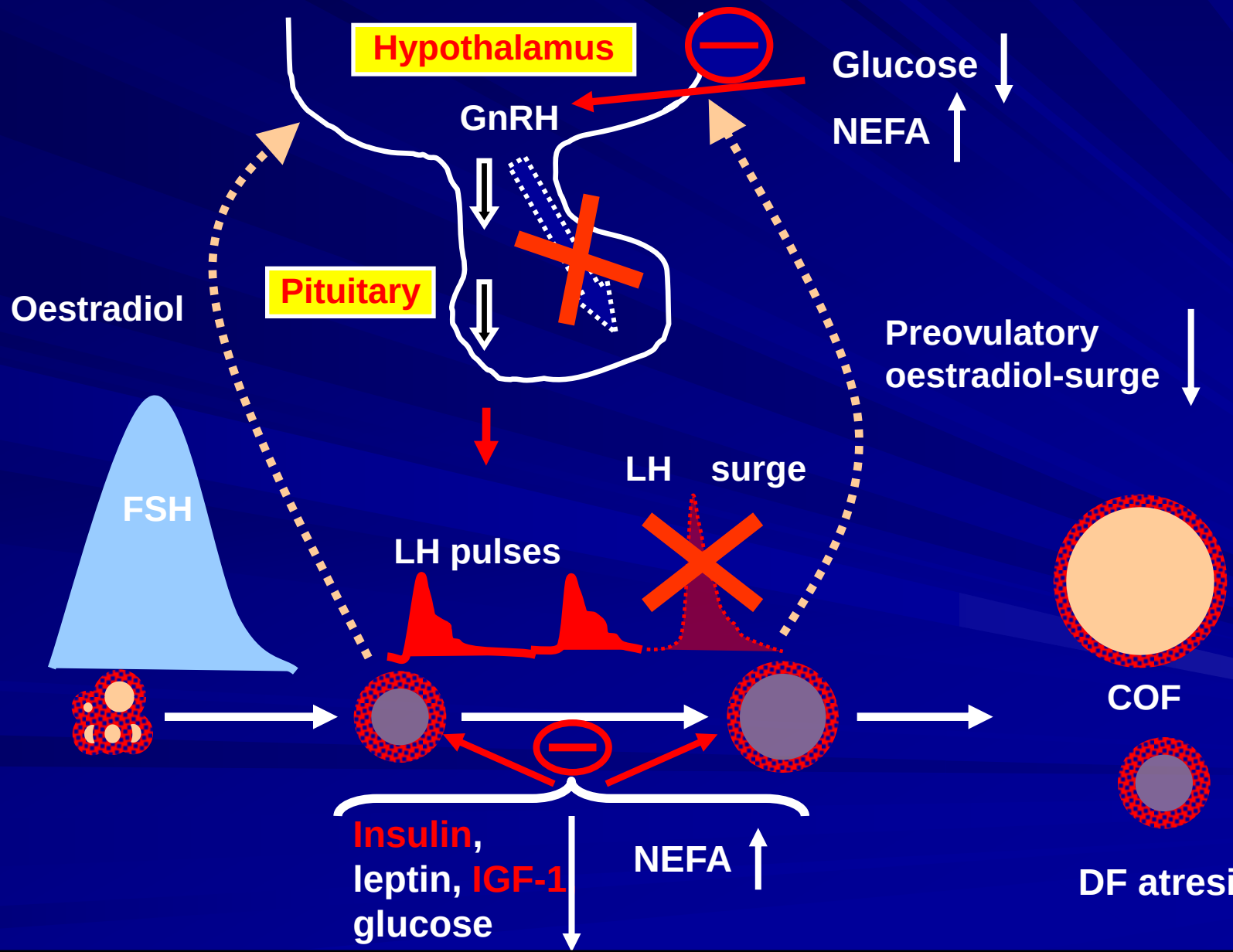

1
1
5
1


Follicle diameter
(mm)
Prog (ng/ml)
Dairy cow
ov 1
ov 2
0 2 4 6 8 10 12 14 16 18
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Follicle diameter
(mm)
Prog (ng/ml)
Beef cow
ov 1
ov 2
0 2 4 6 8 10 12 14 16 18
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Days Postpartum

Resumption of ovarian activity after calving



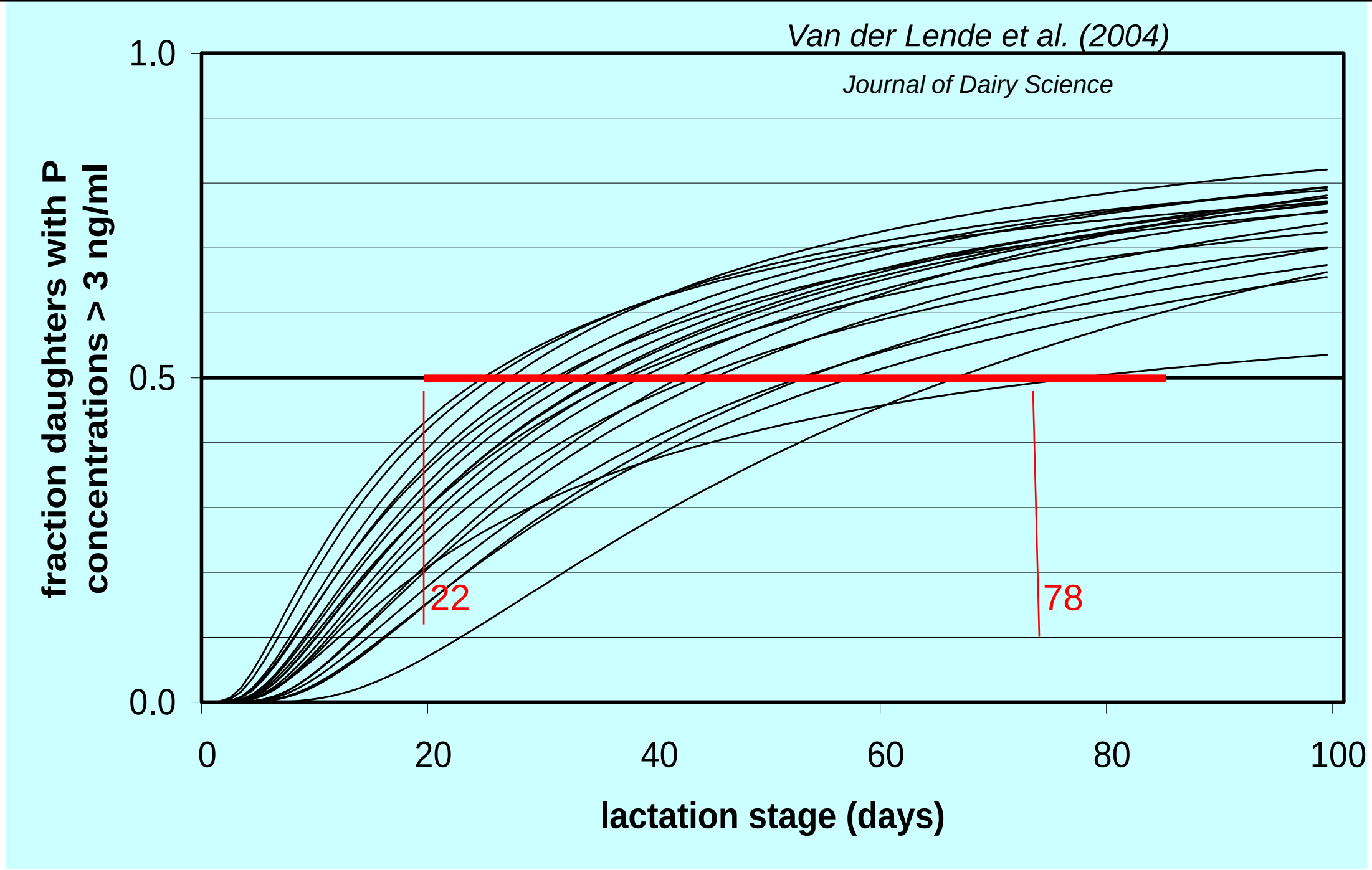

Hypothalamus
GnRH
Pituitary
Glucose
NEFA
Oestradiol
Preovulatory
oestradiol-surge
FSH
LH pulses
LH surge
COF
Insulin,
leptin, IGF-1
glucose
NEFA
DF atresia

Main factors regulating ovarian resumption post partum

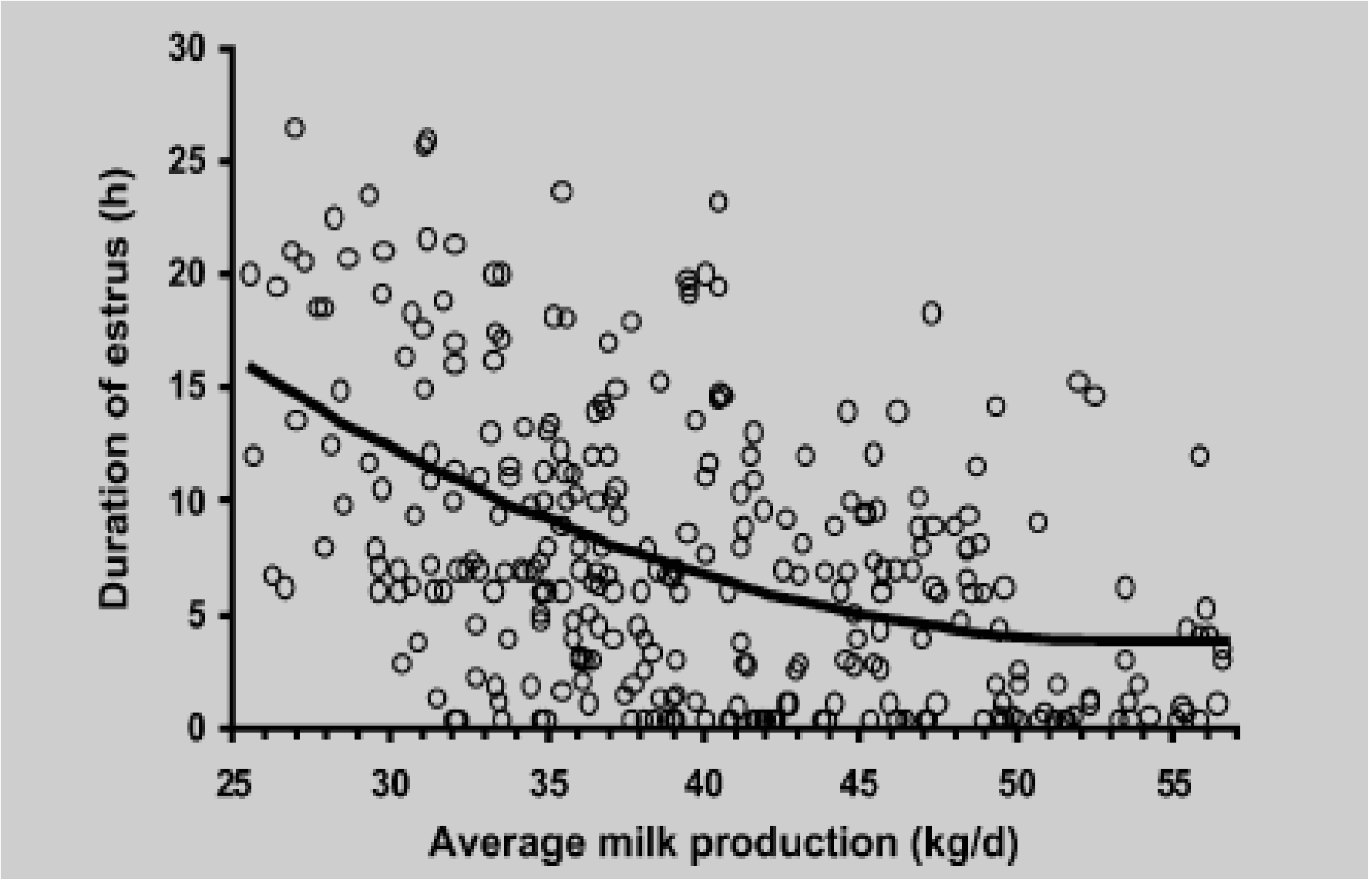
- Peripheral levels of insulin and IGF-1:
  - both have a direct effect on follicular growth and maturation
- Lower insulin levels post partum:
  - associated with higher risk to suffer from cystic ovarian disease (*Vanholder, 2005*)
- Lower bio-availability of IGF-1:
  - generally associated with lower fertility (*Wathes, RVC)*

Nutritional factors to stimulate ovarian function post partum

- Main conclusion of literature: **glucose** is a key component
  - energy early after calving: glucogenic energy
    - lower incidence of cystic ovarian disease
    - earlier resumption of ovarian cyclicity after calving


Van der Lende et al. (2004)
Journal of Dairy Science
1.0
0.5
0.0
fraction daughters with P concentrations > 3 ng/ml
22
78
0
20
40
60
80
100
lactation stage (days)

HEAT DETECTION



Practical approaches to silent heat

°stimulate farmers to optimize heat detection by visual observation

*_Van Eerdenburg_: duration, multiple times daily well spread over the day

*is more and more difficult due to increasing herd size

*is usualy not higher than 50%

°use of heat detection aids (pedometers, activity meters, …):

*increases number of detected cows (from 50 to 70%)

*increases accuracy of heat detection

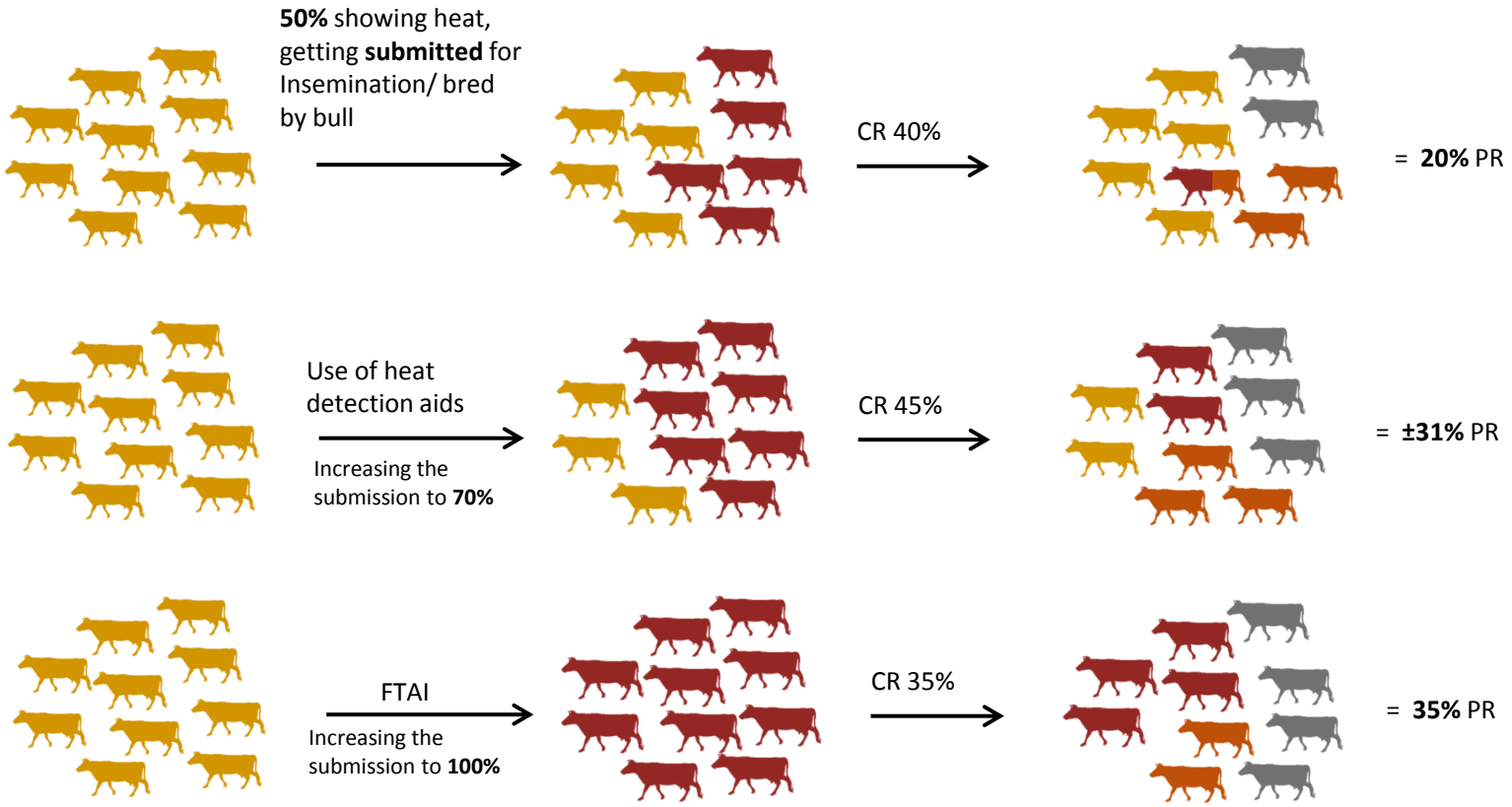
°use of synchronization programmes

*to increase the submission rate

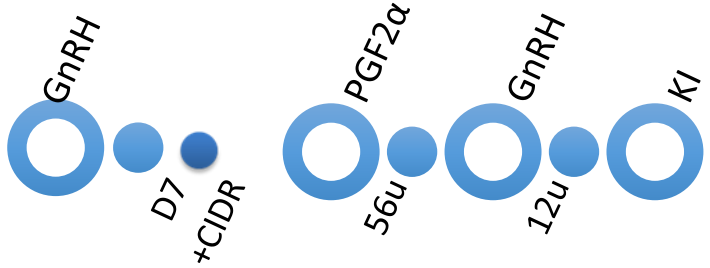
*difficult to accept by EU-consumers

*can be reserved as ultimate solution or help as a ‘treatment option’

Synchronization protocols

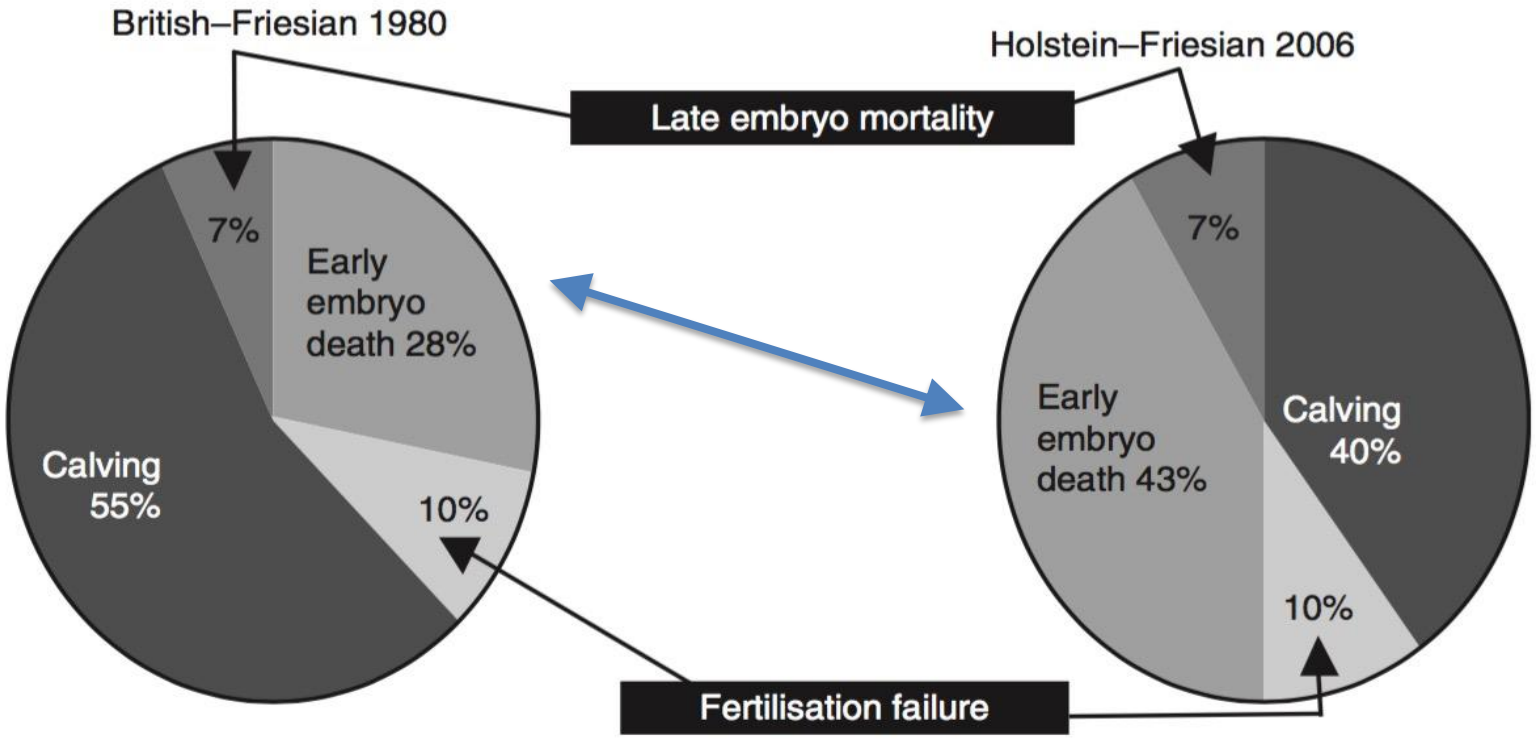


Smart and creative use of synchronization programmes



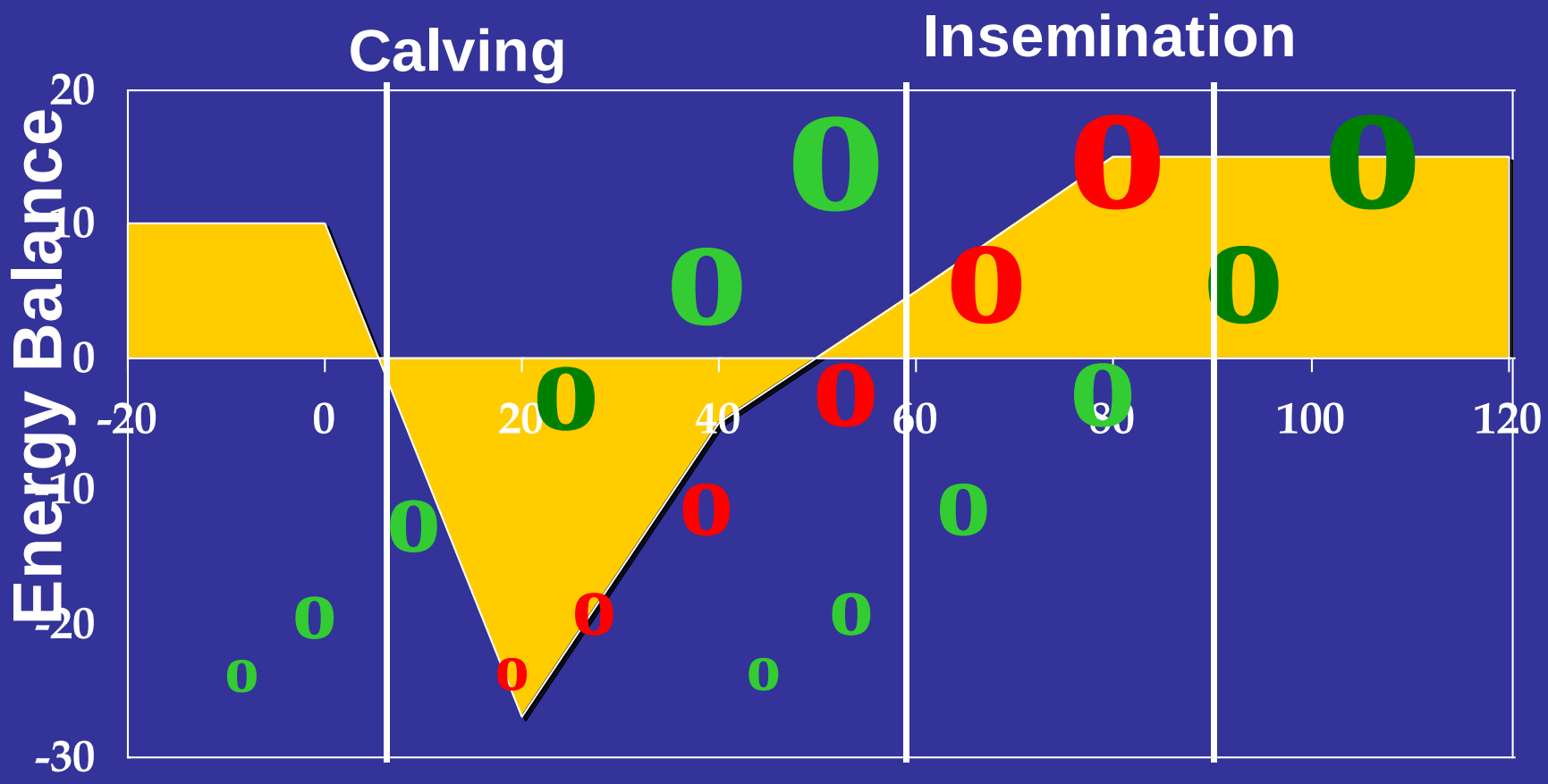
CIDR-synch to treat cows with cystic ovarian disease

Early embryonal death increased over the years

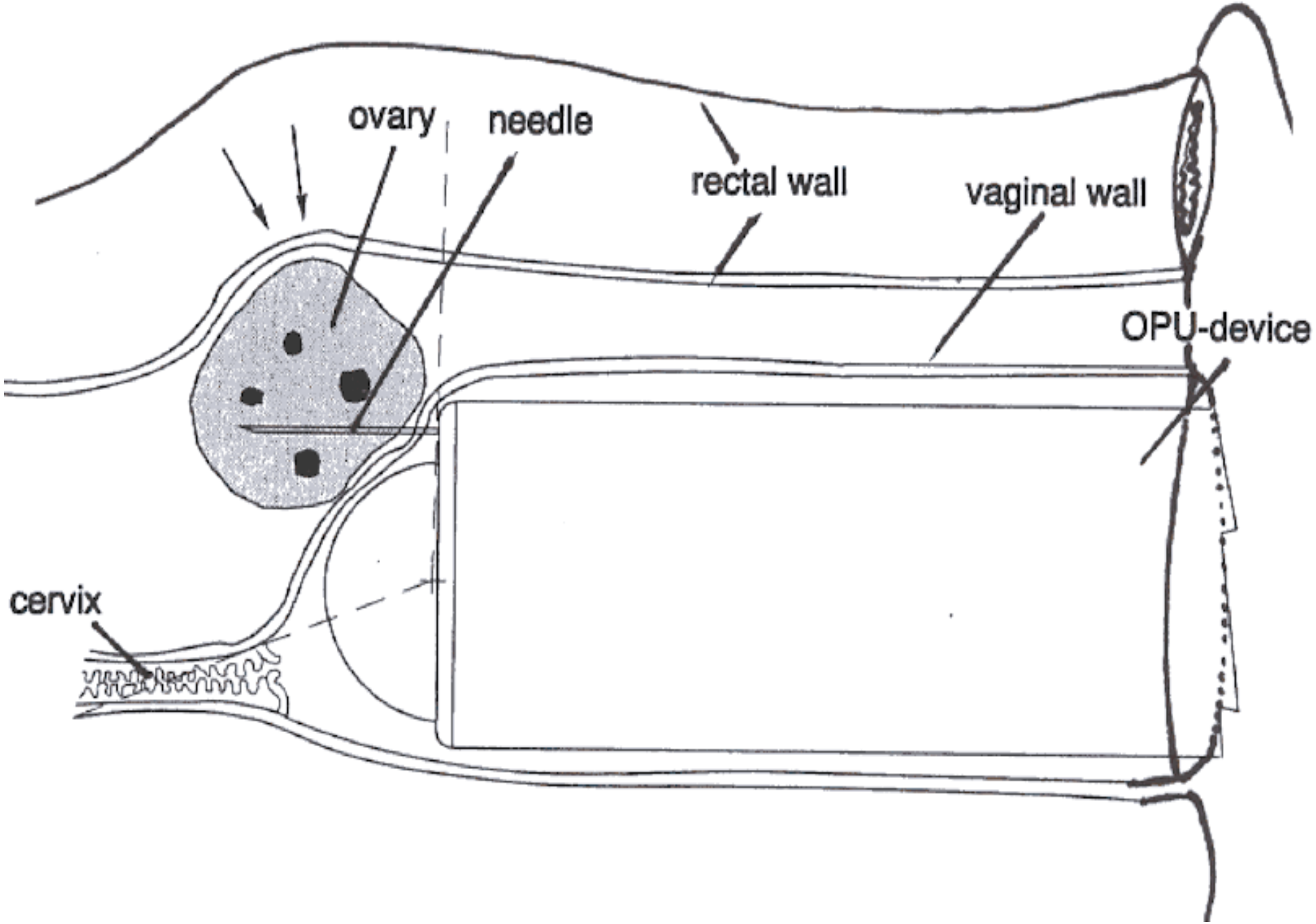


Diskin 2012

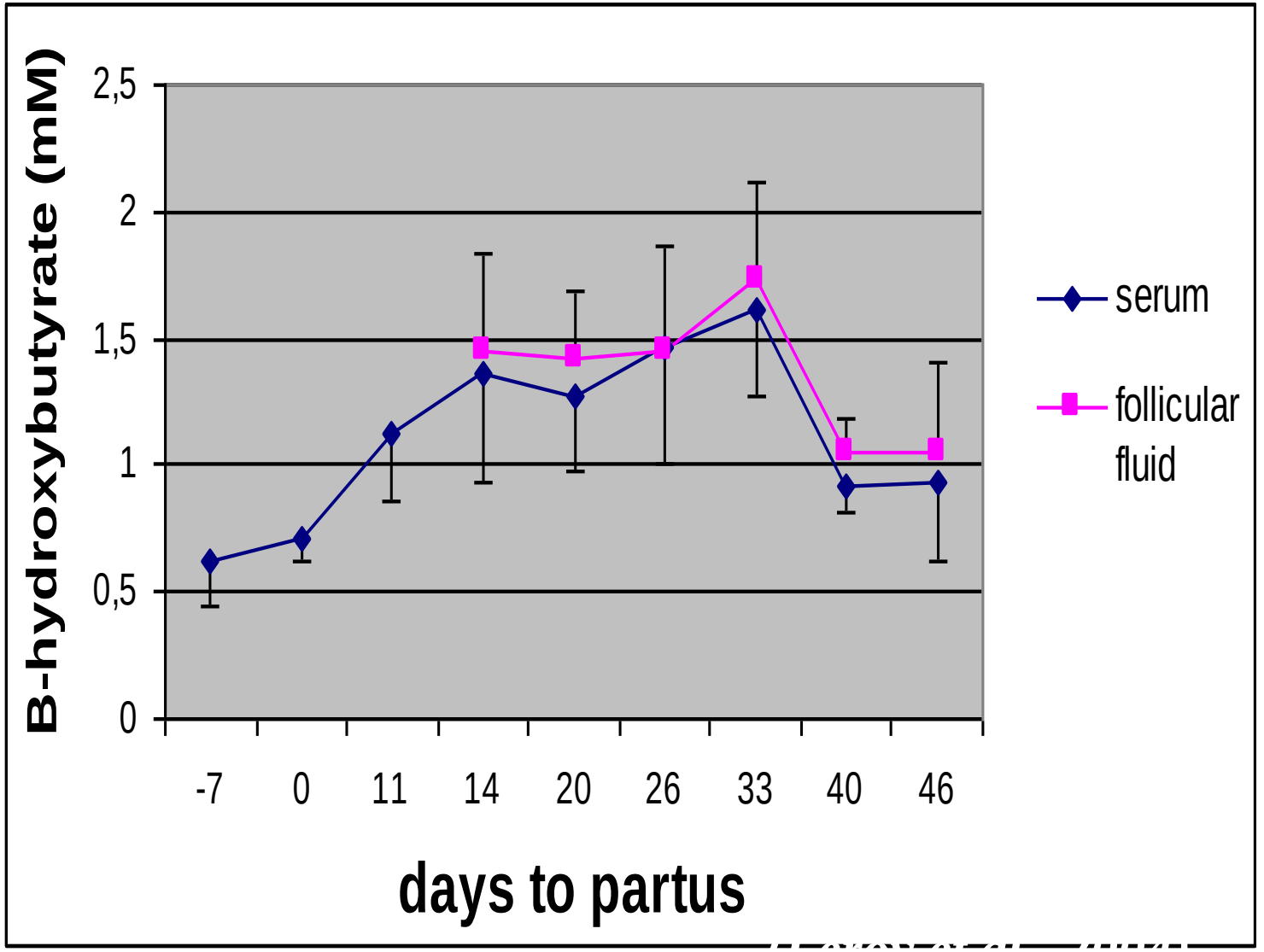
Energy Balance and Reproduction

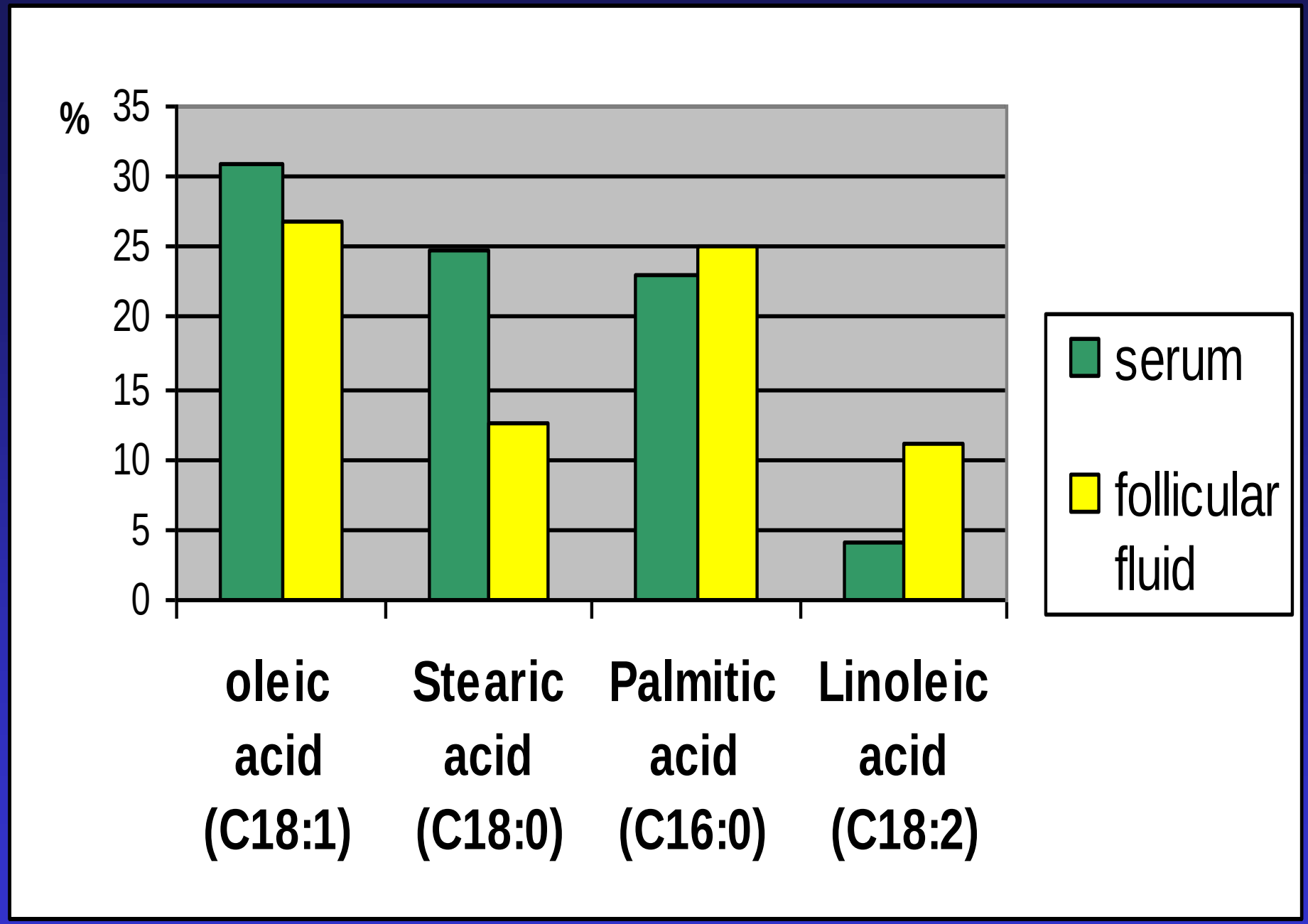


(Britt, 1995)


ovary
needle
rectal wall
vaginal wall
OPU-device
cervix

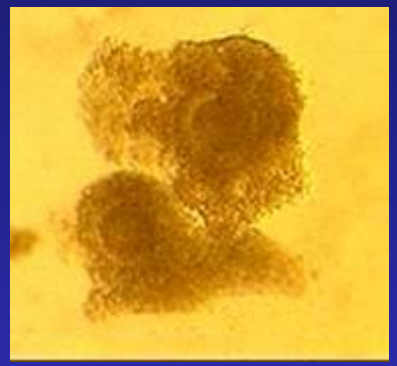
β-hydroxybutyrate




%
35
30
25
20
15
10
5
0
serum
follicular fluid
oleic acid (C18:1)
Stearic acid (C18:0)
Palmitic acid (C16:0)
Linoleic acid (C18:2)

(Leroy , 2005)

In vitro effect of elevated NEFA levels on oocyte and embryo quality

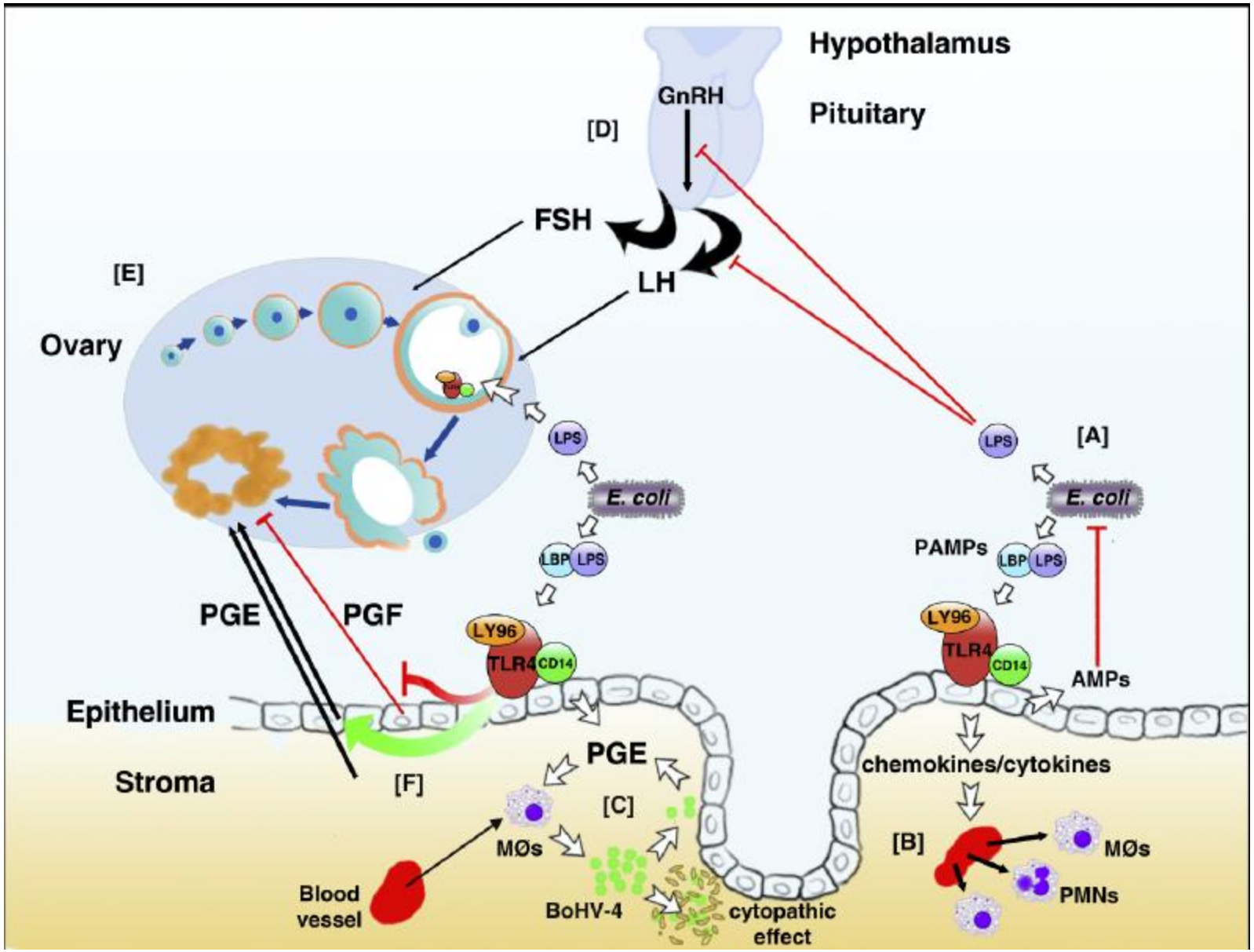


24 hr with
- -palmitic acid
- -stearic acid
- -oleic acid

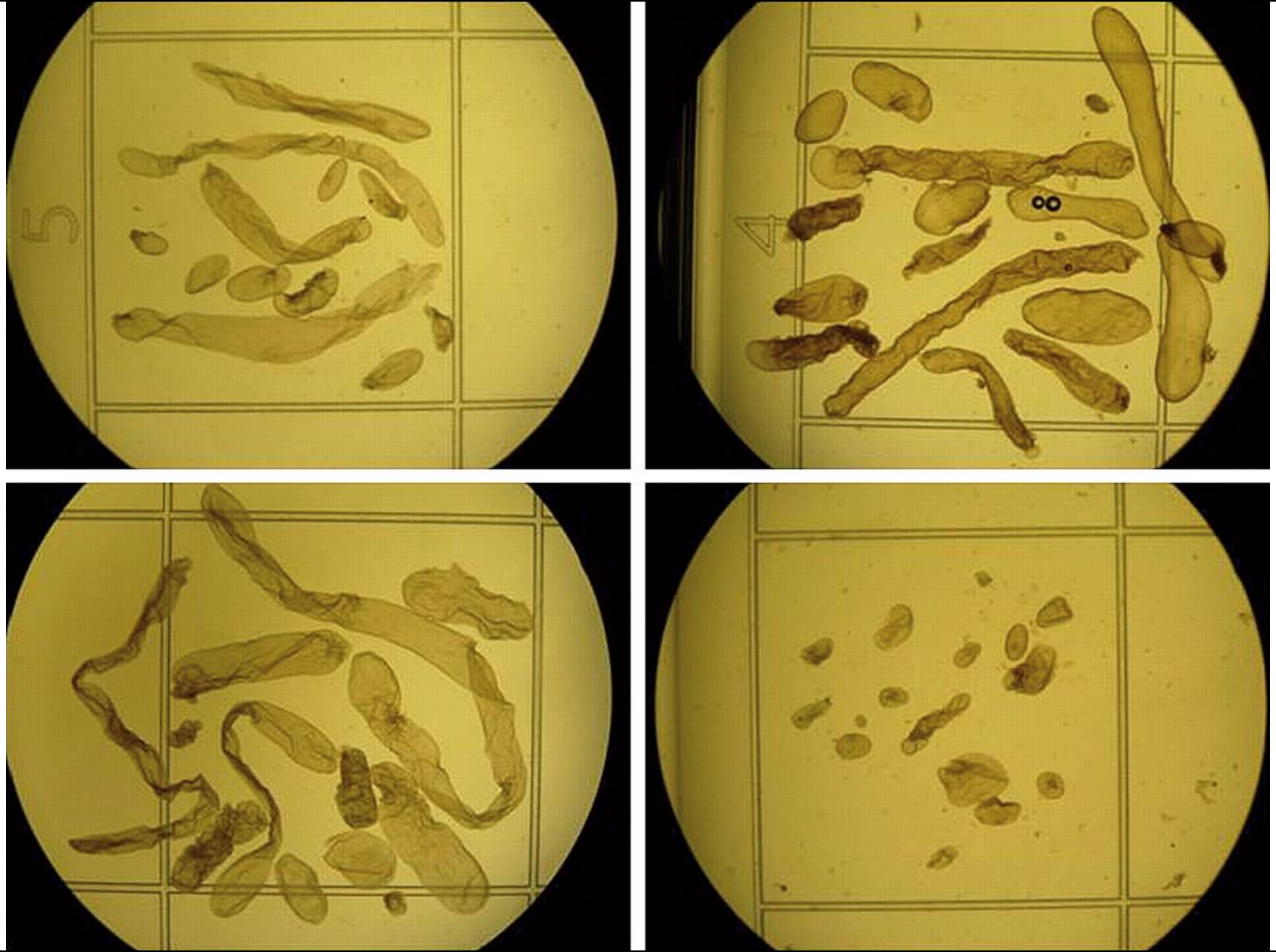
- parameters: oocyte maturation, fertilisation rate, cleavage rate and blastocyst formation

- palmitic and stearic acid: negative effect on all parameters, oleic acid: no effect

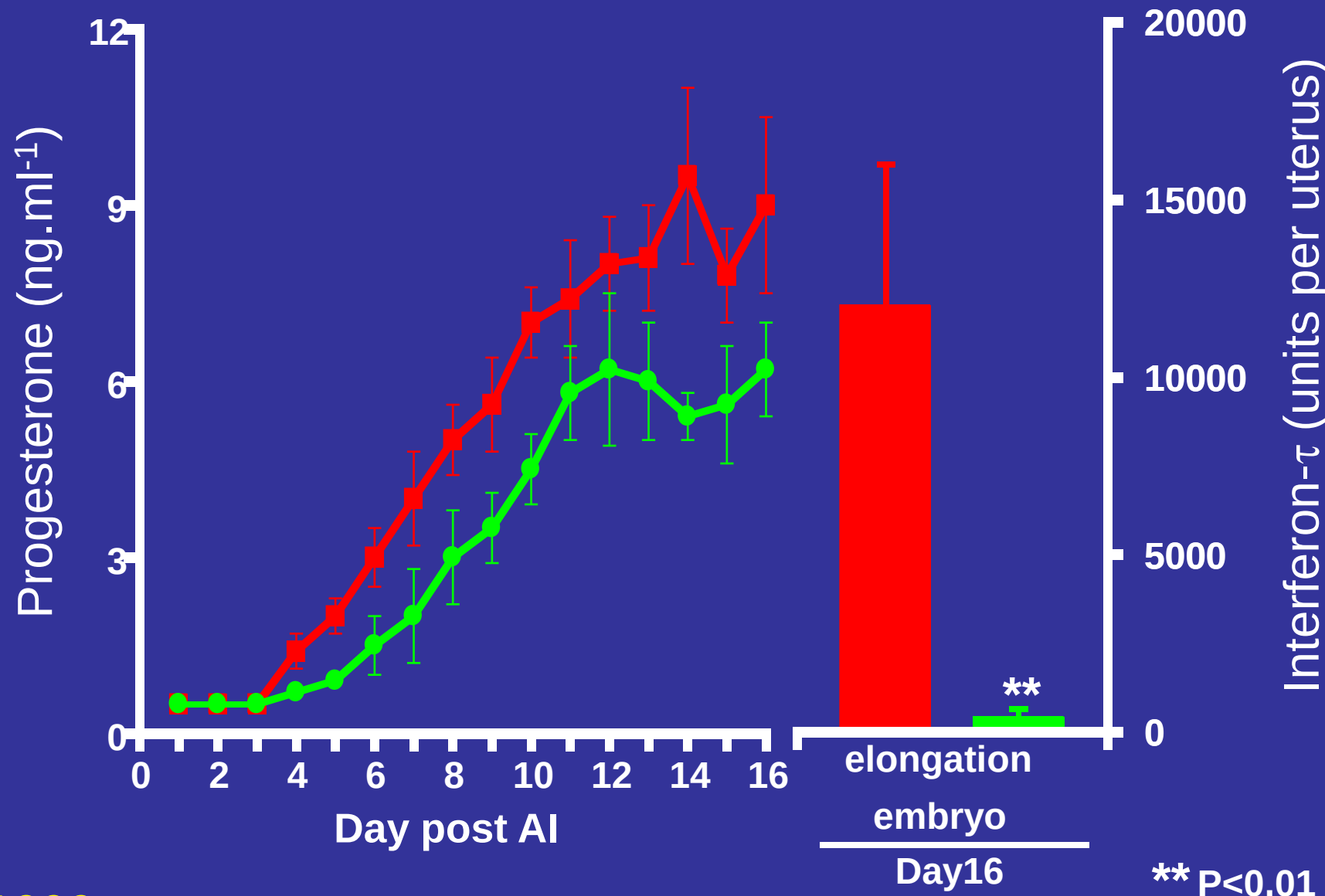
(Leroy, 2005)



(Sheldon et al., 2009)

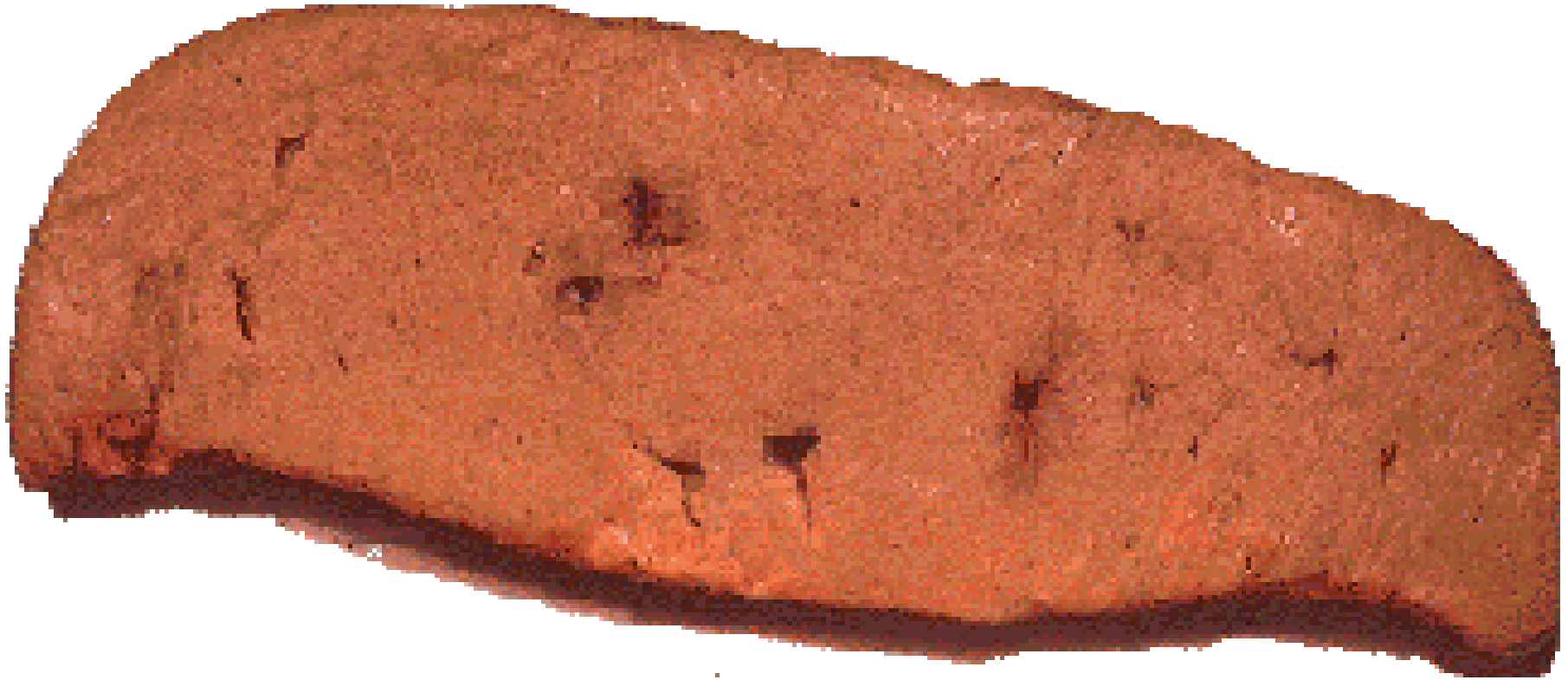


Progesterone concentration and embryo elongation



Mann et al., 1999

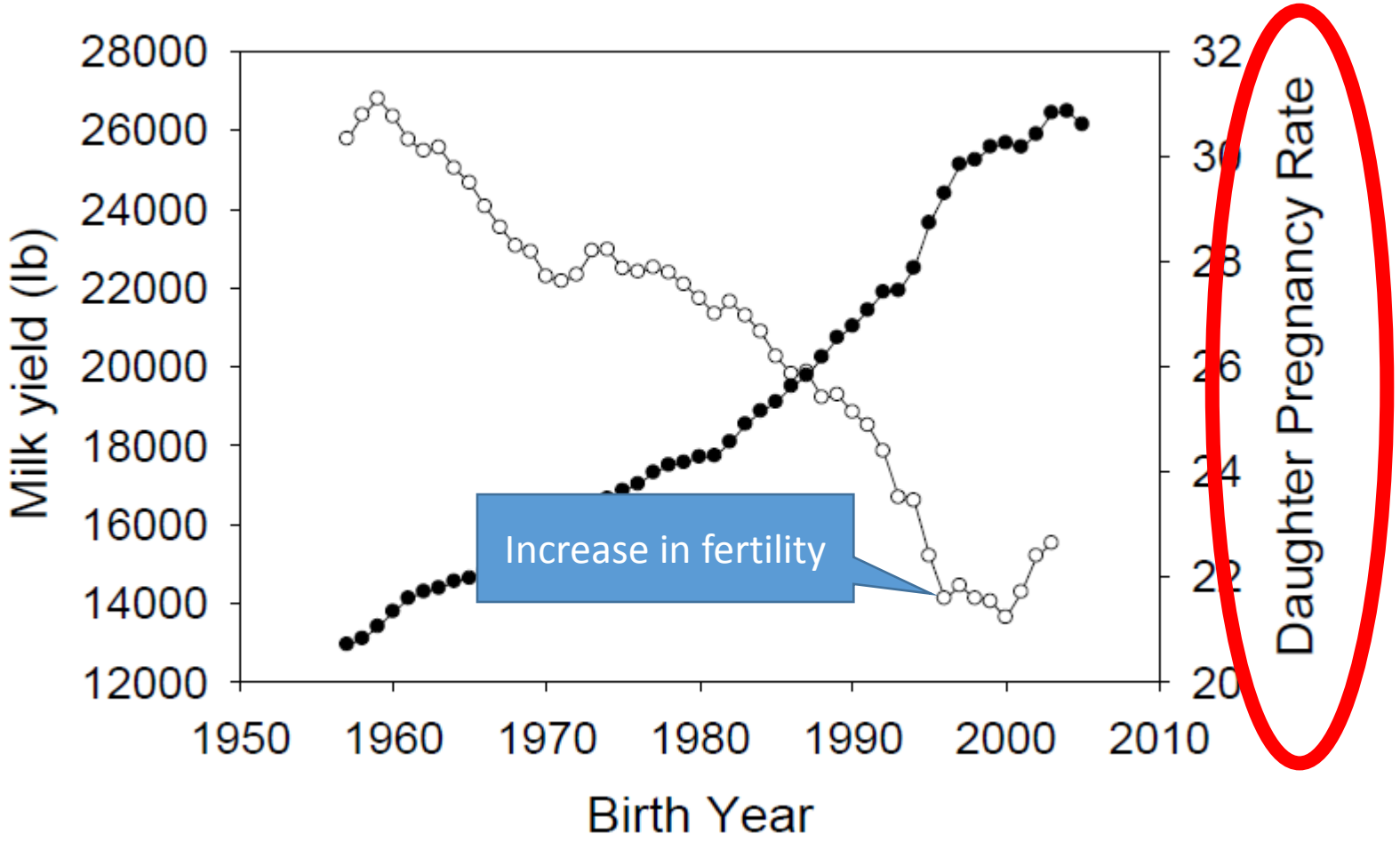
Liver as central reproductive organ



Daily hepatic blood flow for a 40 liter cow:

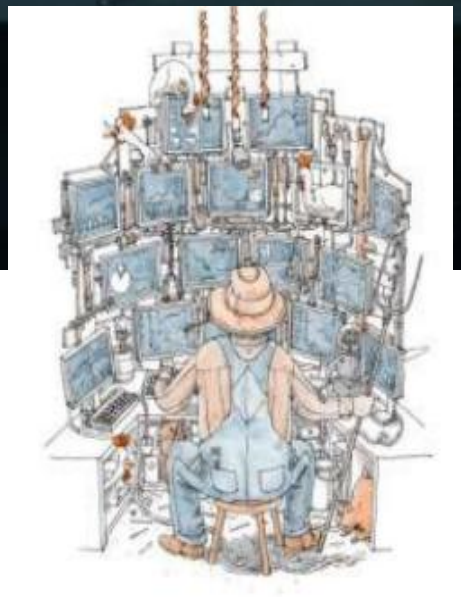
50.000 liters

Fertility


Milk yield (lb)
28000
26000
24000
22000
20000
18000
16000
14000
12000
1950
1960
1970
1980
1990
2000
2010
Birth Year
32
30
28
26
24
22
20
Daughter Pregnancy Rate
Increase in fertility

Fertility management in the future

- Increased use of cow side tests (automated??)
- Increased use of automatic devices (activity meters, health monitoring, …)
- Genetics: genomics to select animals that are able to combine high yield with good fertility
- Smart use of all available data → Big Data
- Further optimization of nutrition and transition management
- Innovative treatments: probiotics, vaccinations, phage therapy
- Smart and well reasoned use of hormones/treatment protocols
- Economics of inseminating animals later in lactation??




Thank you