Biology of Lactation in Farm Animals (BOLFA)

Bovine neutrophils' oscillation and transition period

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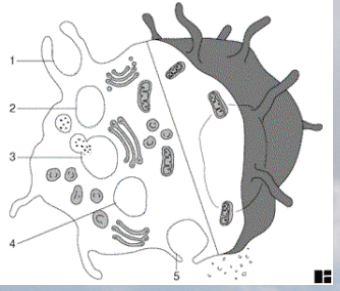
Monday, 27-08-2012, at 14:30
Bratislava, Slovakia



Contents

Location:
Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3800 m above sea level

Introduction

Methodology

Results

General conclusions and some idea

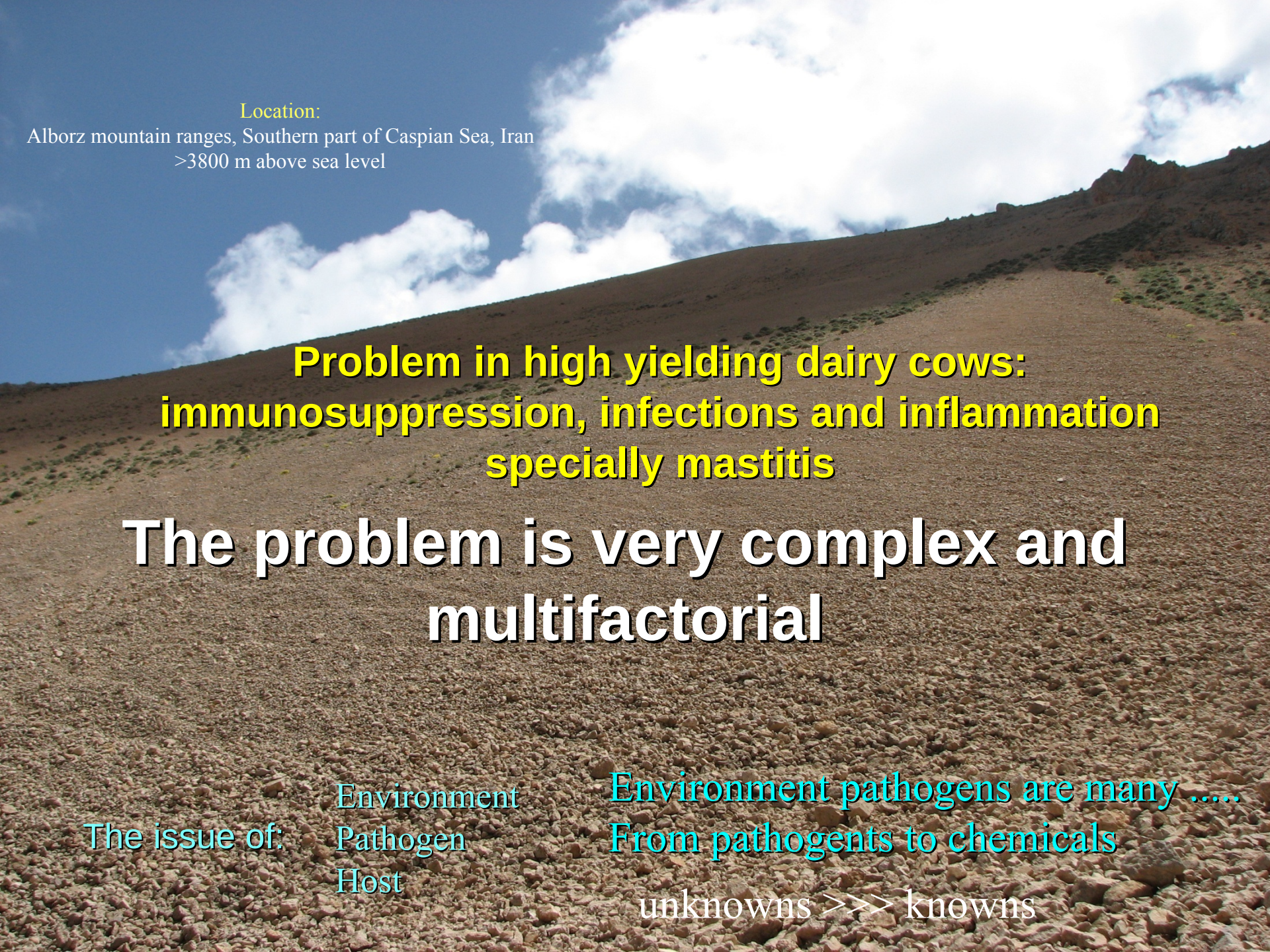
Main emphasis on neutrophils' role in protection of udder in peripartum high yielding dairy cow

Basic aspects of the physioimmunolology of neutrophils in high yielding dairy cow

Neutrophils' oscillatory events in peripartum dairy cows

Attainable way to reverse neutrophils' oscillatory events in peripartum dairy cows


INTRODUCTION



Problem in high yielding dairy cows: immunosuppression, infections and inflammation specially mastitis

The problem is very complex and multifactorial

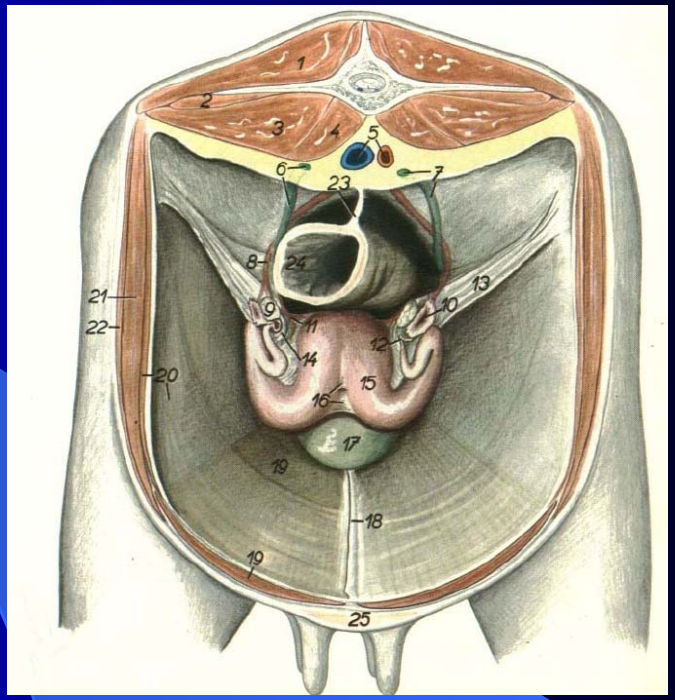
The issue of:

Environment
Pathogen
Host

Environment pathogens are many .....
From pathogents to chemicals

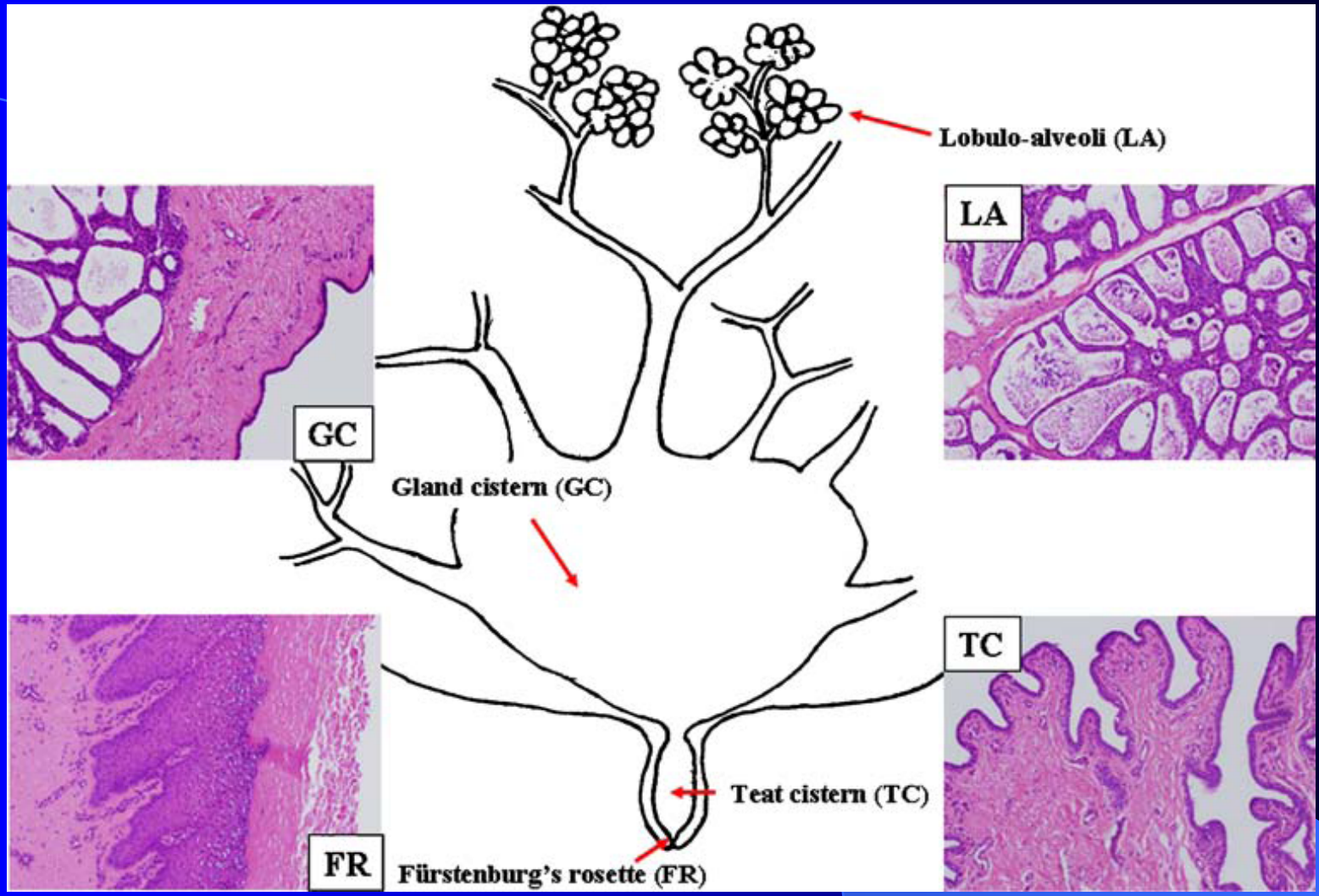
unknowns >>> knowns

Economically, nutritionally and health critical



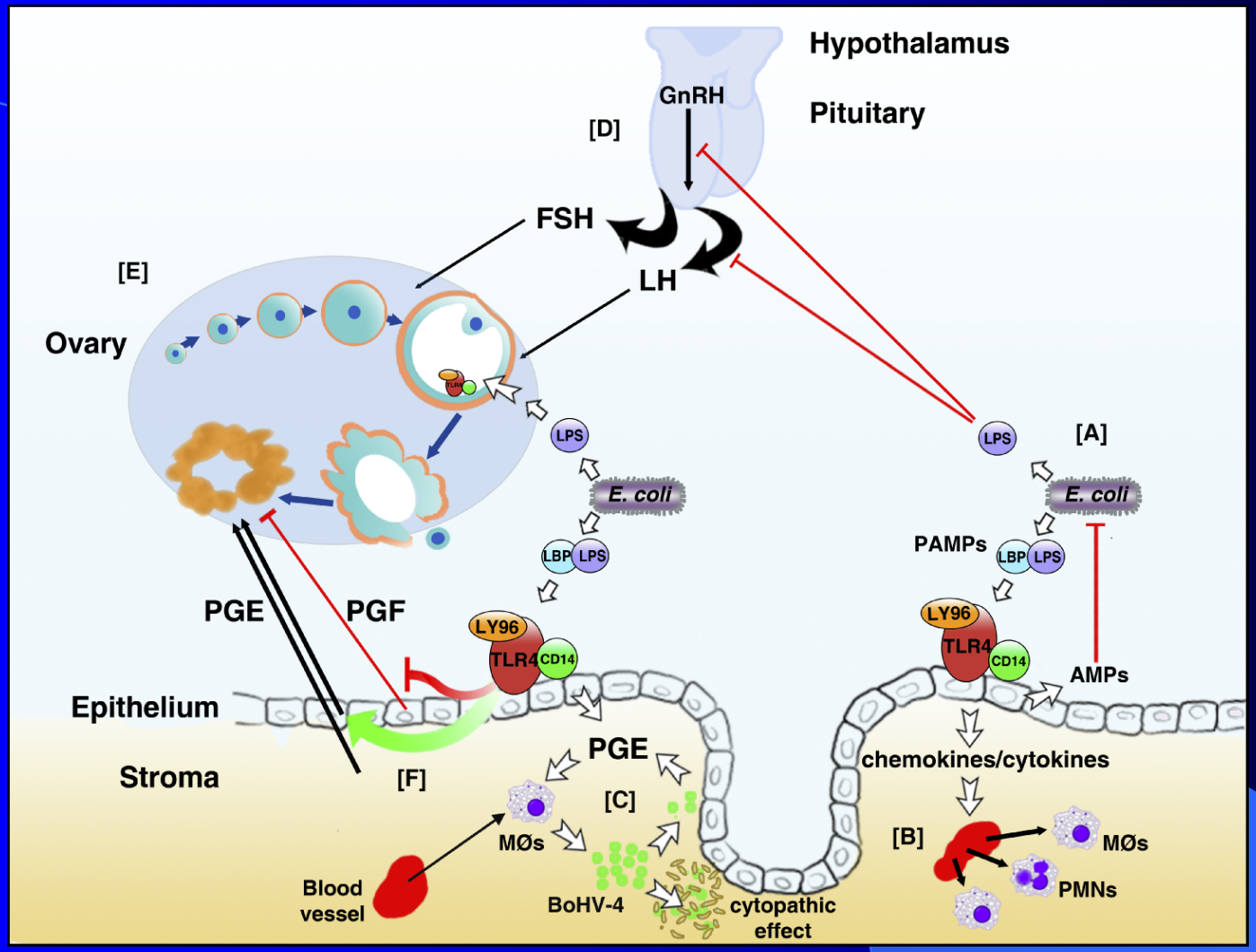
Anatomically, histologically, physioimmunologically very complex!

Example of the complexity of dairy cows' udder



Rinaldi et al., Funct Integr Genomics. 2010

Complex interrelations between udder, uterus, ovary etc.

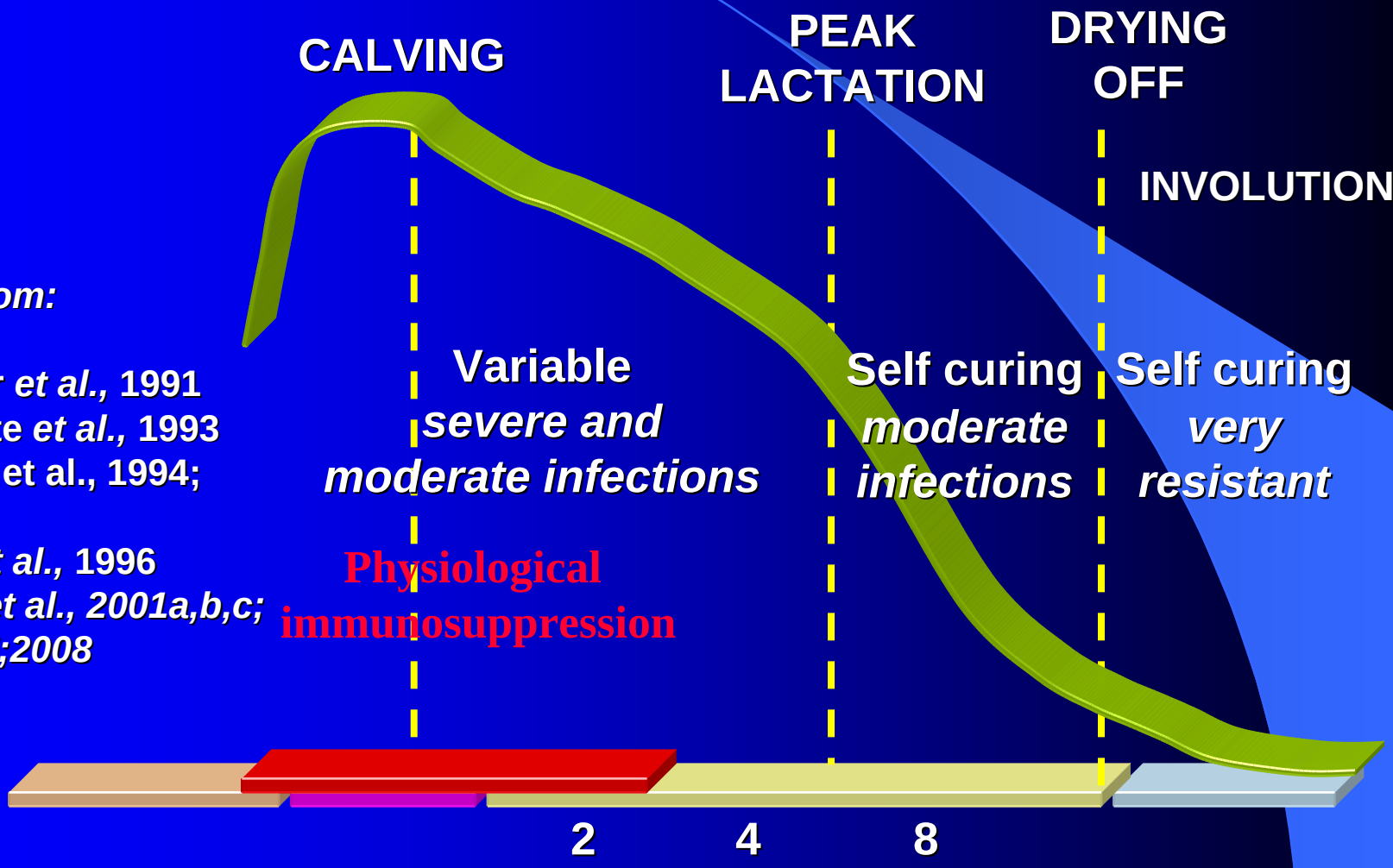


Both in udder and uterus

unknowns >>> knowns

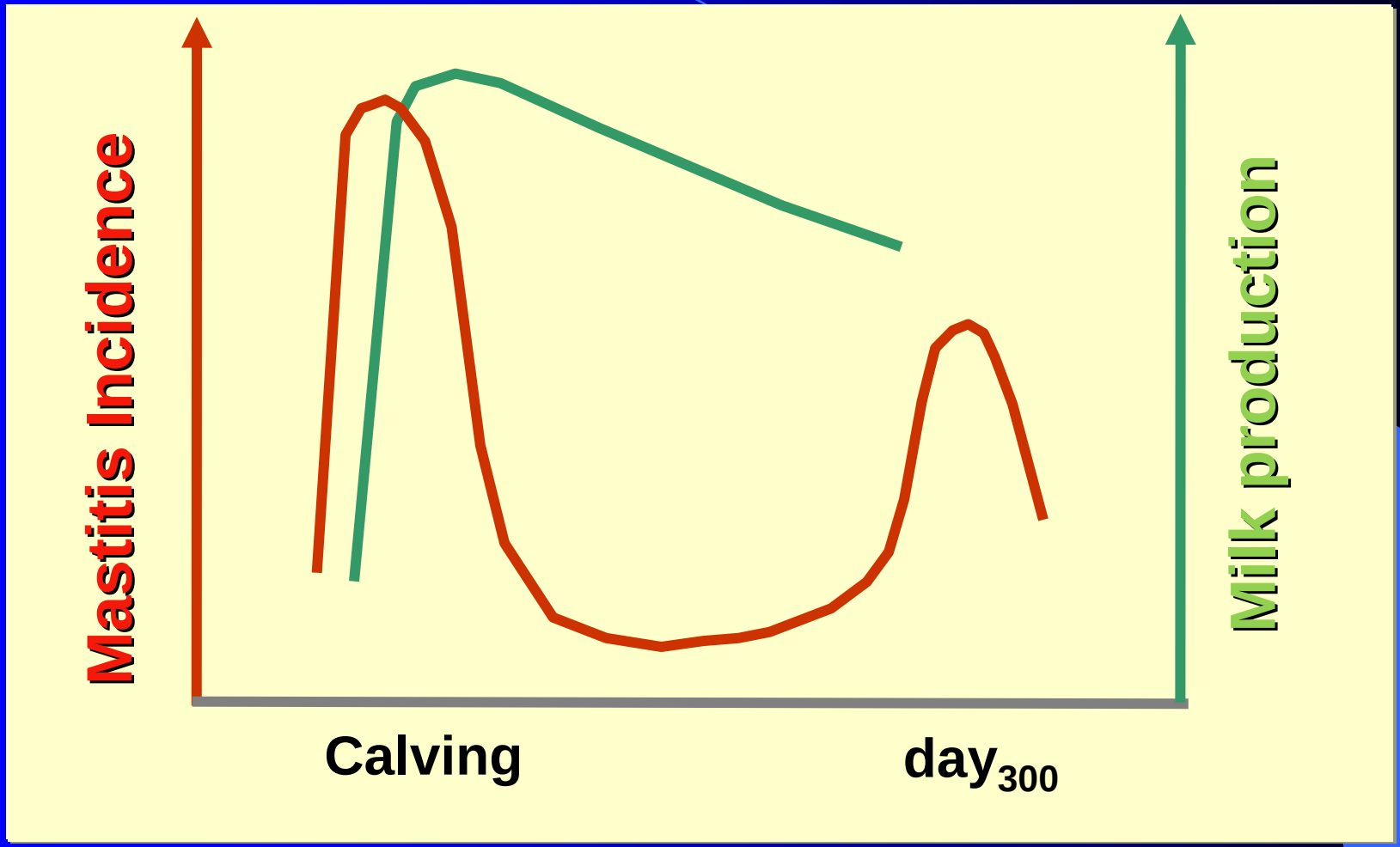
Sheldon et al., Biolo. Reproduc, 2009

Incidence and severity of parturition-and-lactation related mastitis



adapted from:
Hill, 1981
Todhunter *et al.*, 1991
Vandeputte *et al.*, 1993
Burvenich et al., 1994; 2003
Shuster *et al.*, 1996
Mehrzad et al., 2001a,b,c; 2002; 2007;2008

The problem of mastitis in high yielding cow



adapted from
Burvenich et al., 1994; 2003
Shuster *et al.*, 1996
Mehrzad et al., 2001; 2002; 2009; 2010

Many other new synergic issues increase the problem

New Infection Rates by parturition and lactation

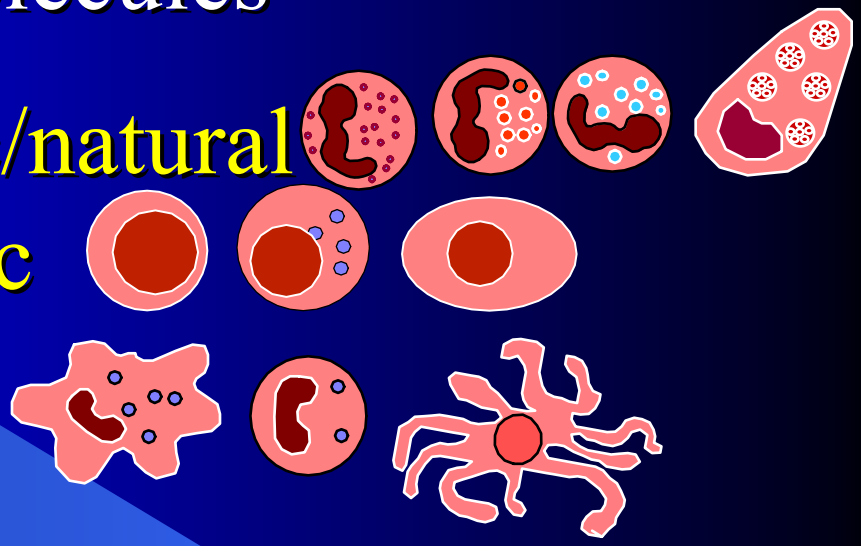
Protection of udder in dairy cows by

Immunne cells and molecules

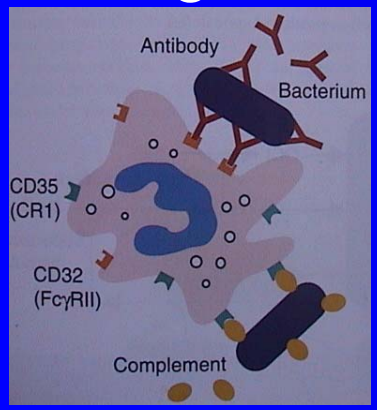
-Innate/native/non-specific/natural

-Acquired/adaptive/specific

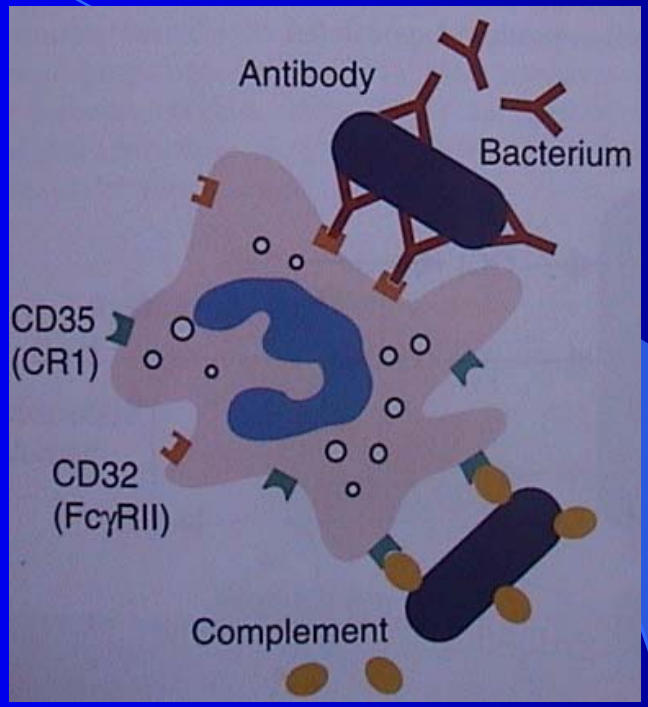
-Interaction between them



Recognition, prime and response against pathogens to protect the udder



Basic aspects of bovine neutrophils (PMN)

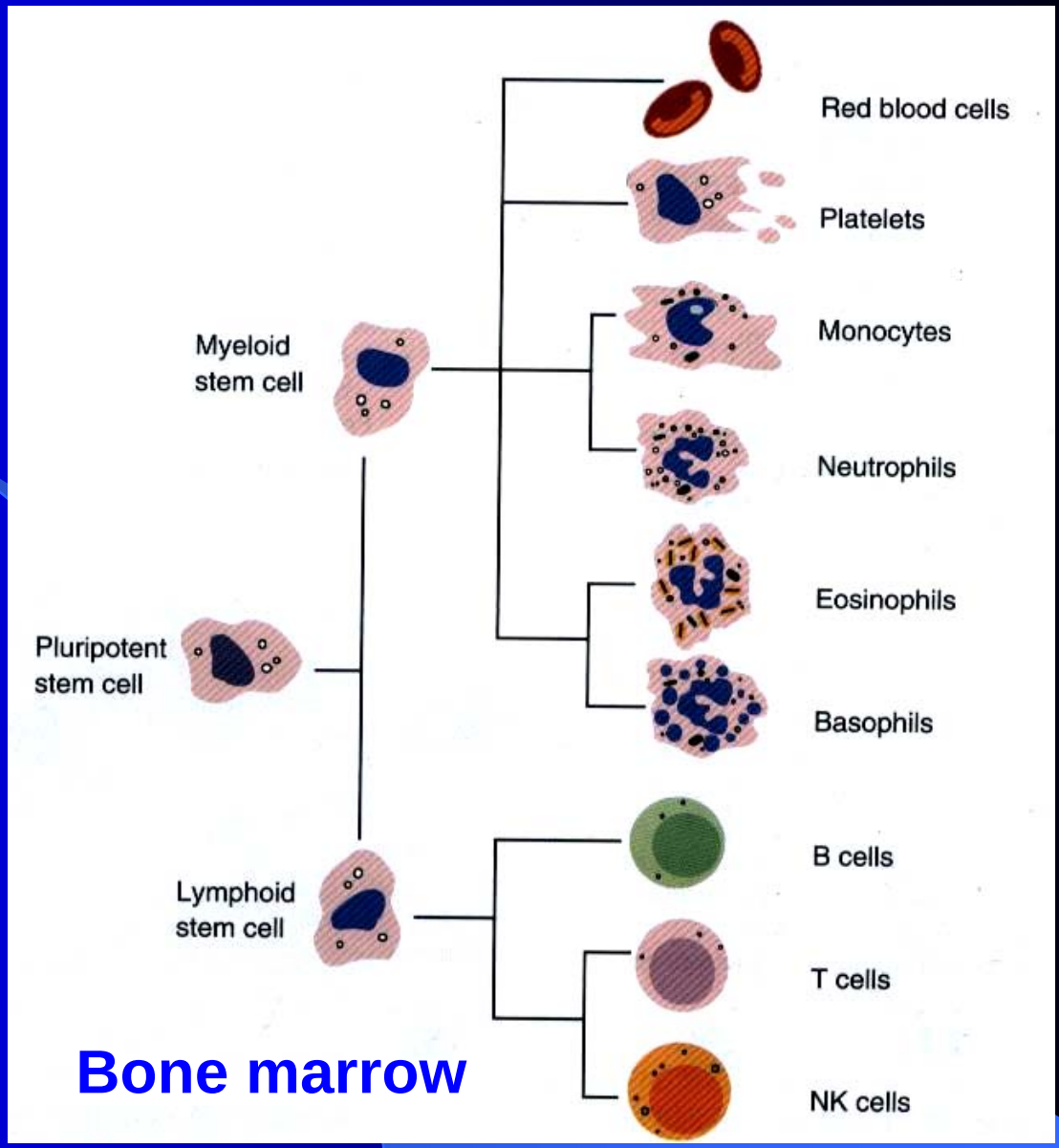


The origin of bovine neutrophils

Myeloid lineage of Hematopoietic stem cells (HSC) or Pluripotential stem cells

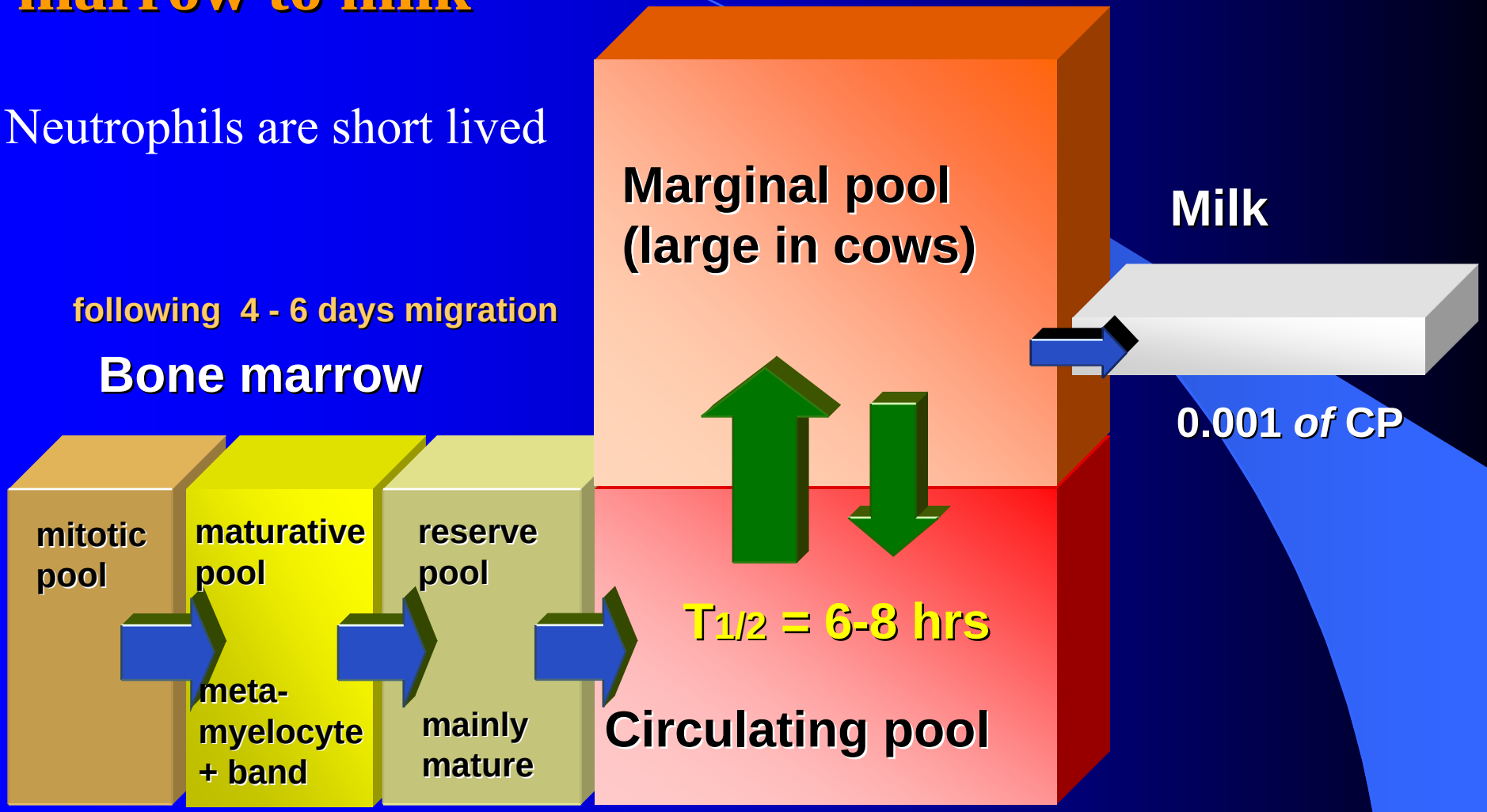
multi-step process of granulopoiesis

Terminally differentiated and cannot proliferate/divide at the site of infection/inflammation.



Life span of the bovine neutrophil from bone marrow to milk

Neutrophils are short lived



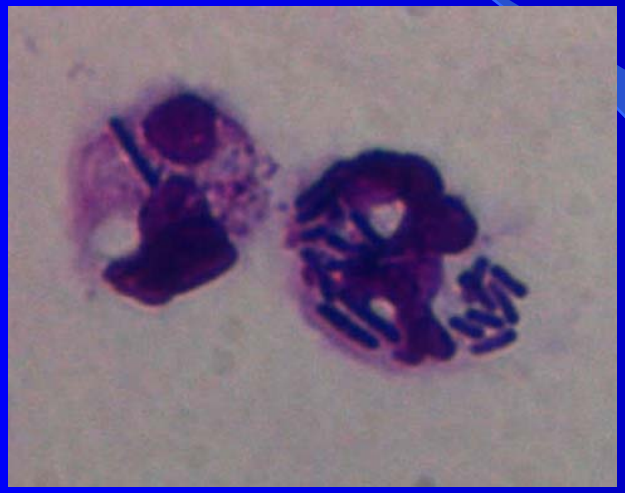
morphologic, biochemical and functional changes

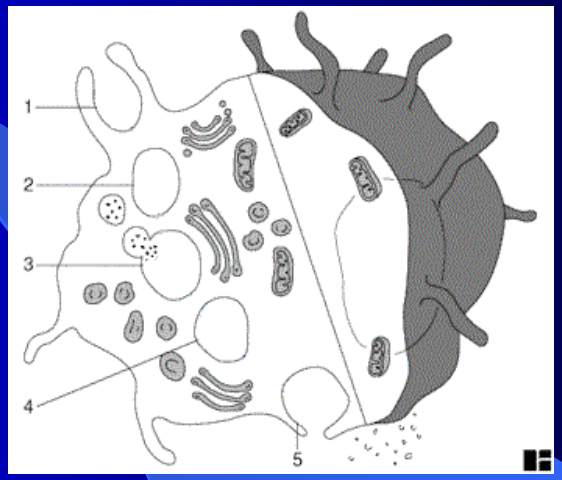
Kehrli *et al.*, 1991
Burvenich et al., 2003

Functional aspects of bovine neutrophils

As a pivotal circulating effector innate immune cells

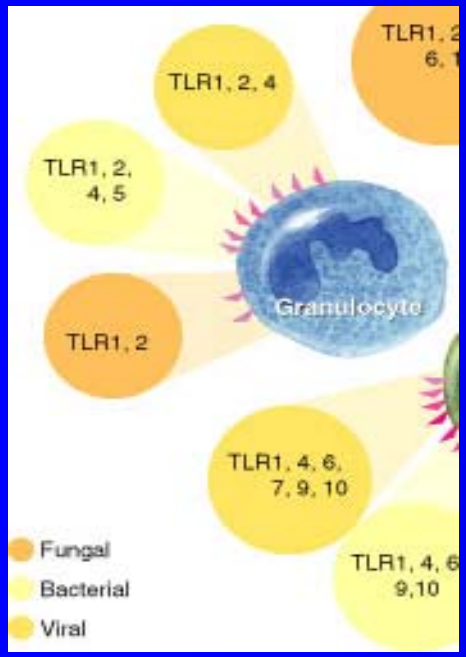
Phagocytosis and microbicidal activity



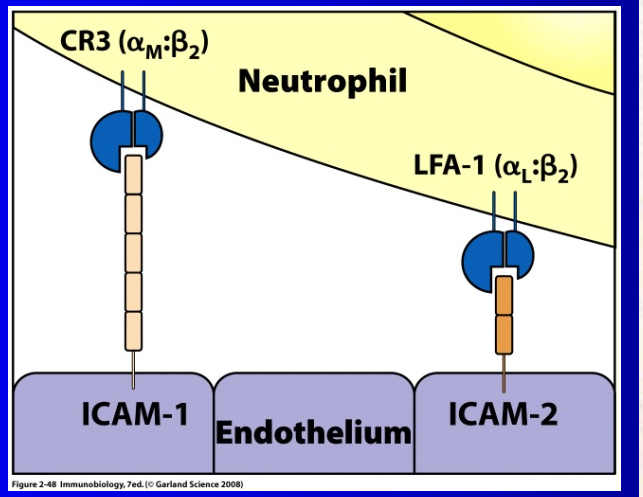


Neutropenic cows with mastitis: severe shock and death but in normal cows!

Bovine neutrophils use variety of receptors to detect/sense and eliminate pathogens



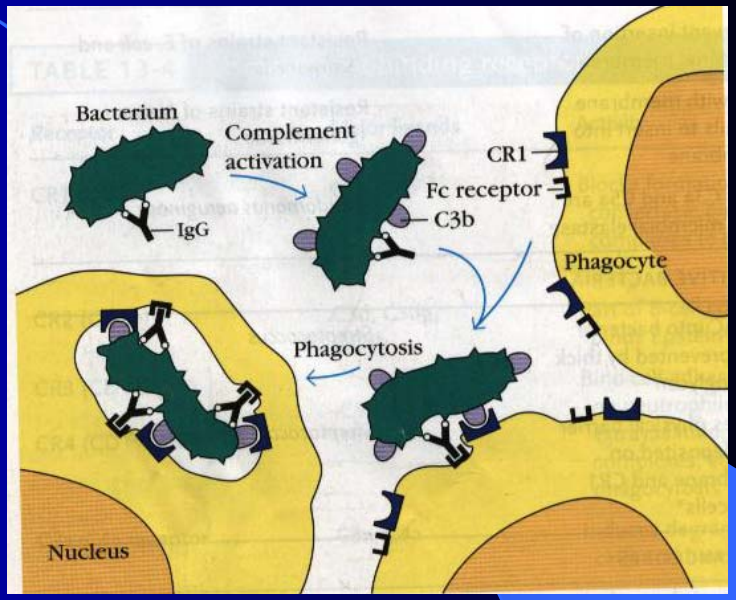
Highly expressed PRRs for early sensing of PAMP



neutrophil endothelium /epithelium/connective tissues interactions

Leukocyte functional antigens (LFA)……

neutrophil extravasation or diapedesis

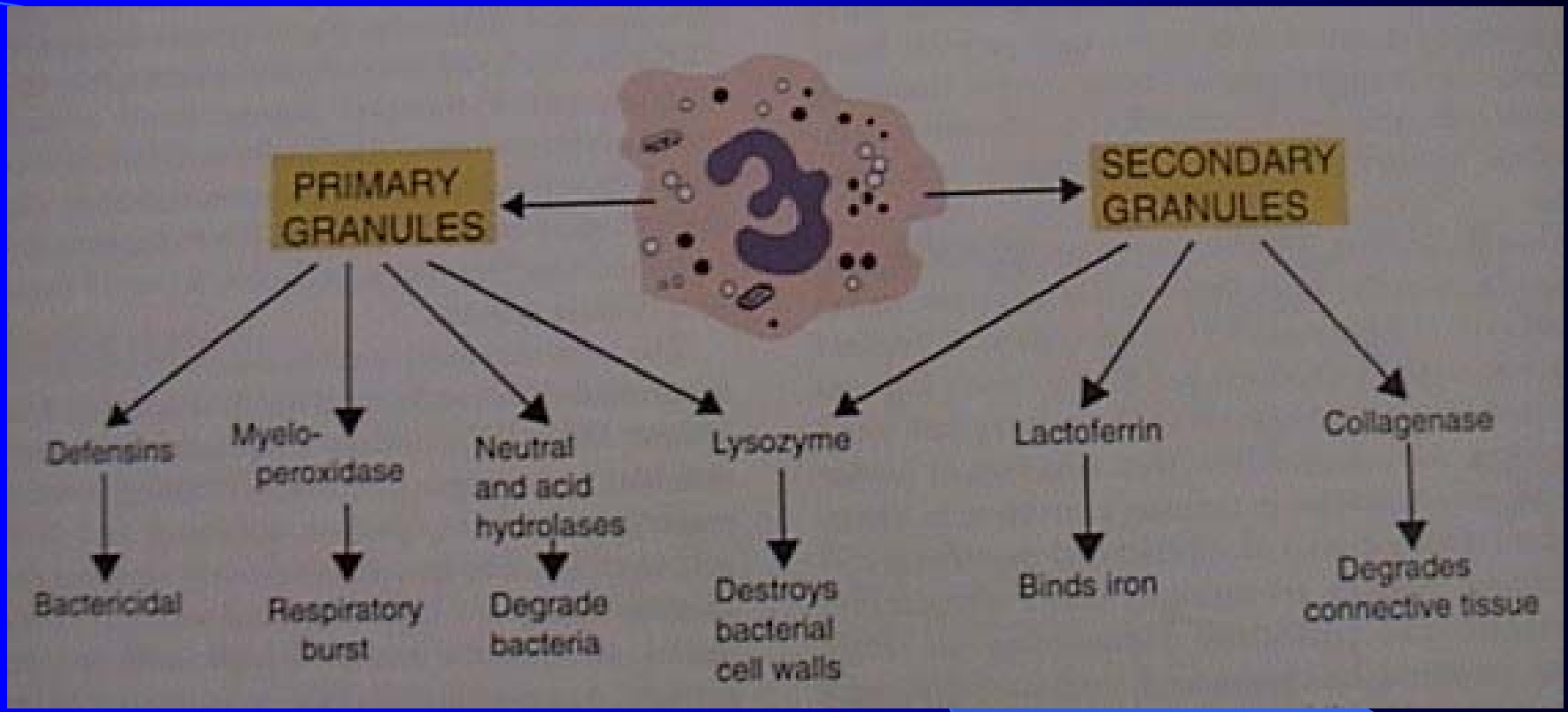


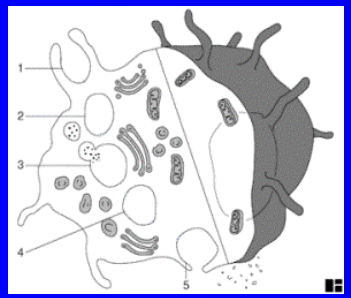
Highly express opsonin receptors, such as FcγRI or complement receptors that enhance phagocytosis.

and other receptors ….

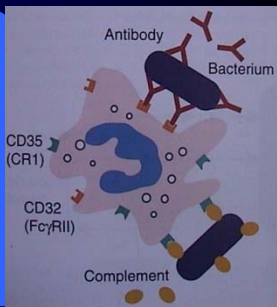
Bovine neutrophils' arsenals

Bovine neutrophils use variety of cytokines, chemokines, adhesion molecules and enzymes to remove pathogens ….

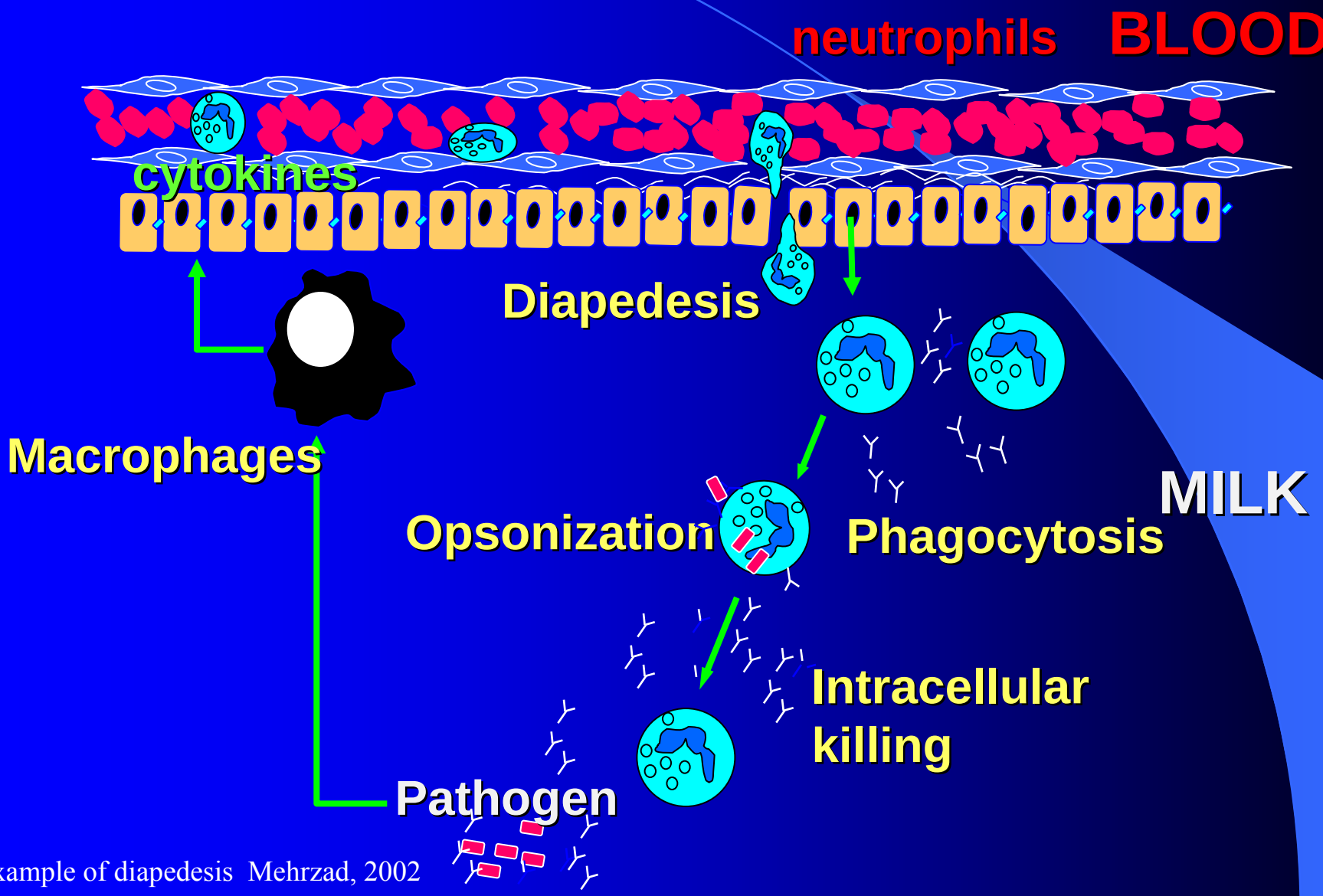




Bovine neutrophils' arsenals

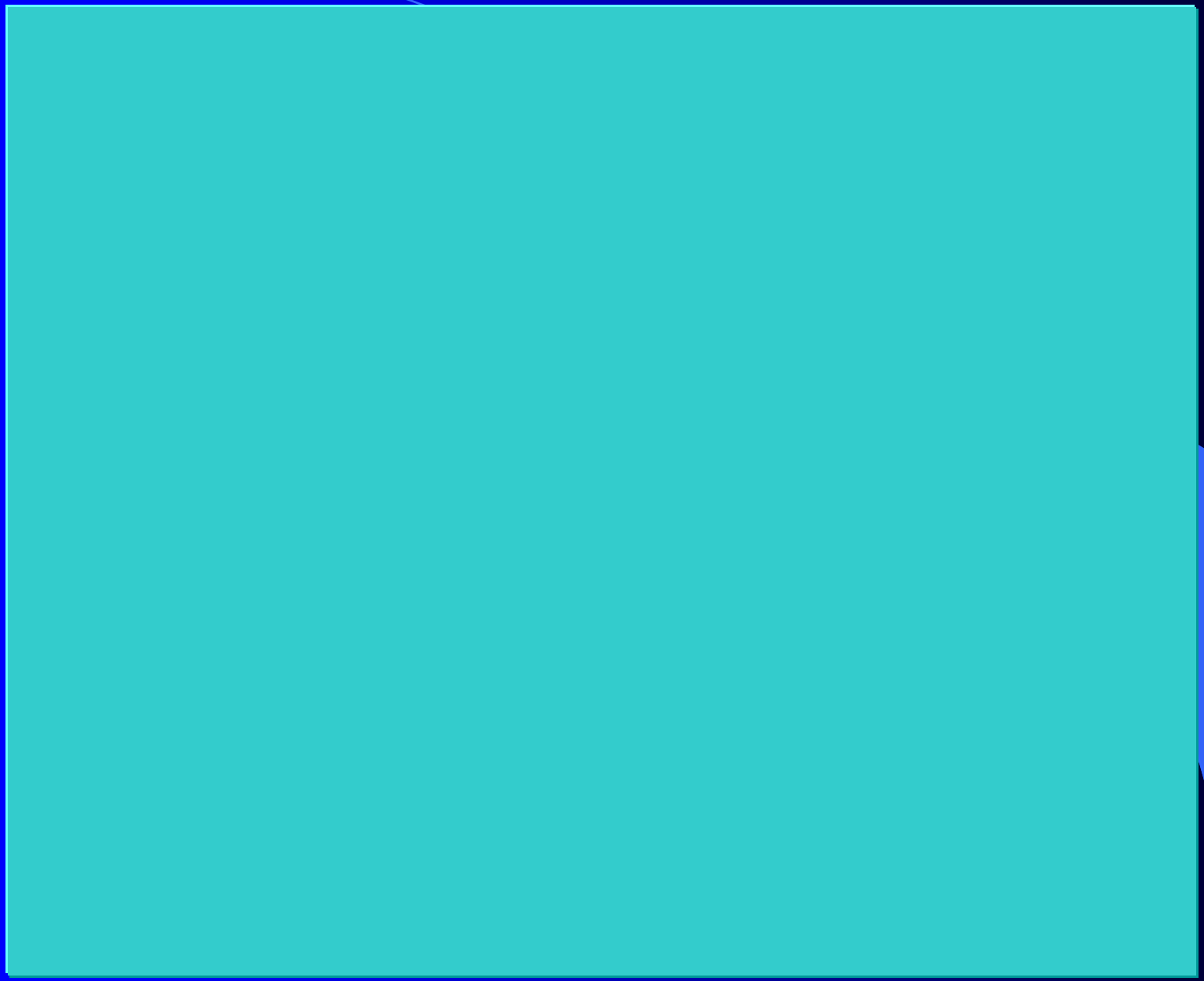


Neutrophil transmigration through blood-milk barrier in dairy cow



Example of diapedesis Mehrzad, 2002

Factors in 3 locations affect neutrophils' arsenals in blood and udder

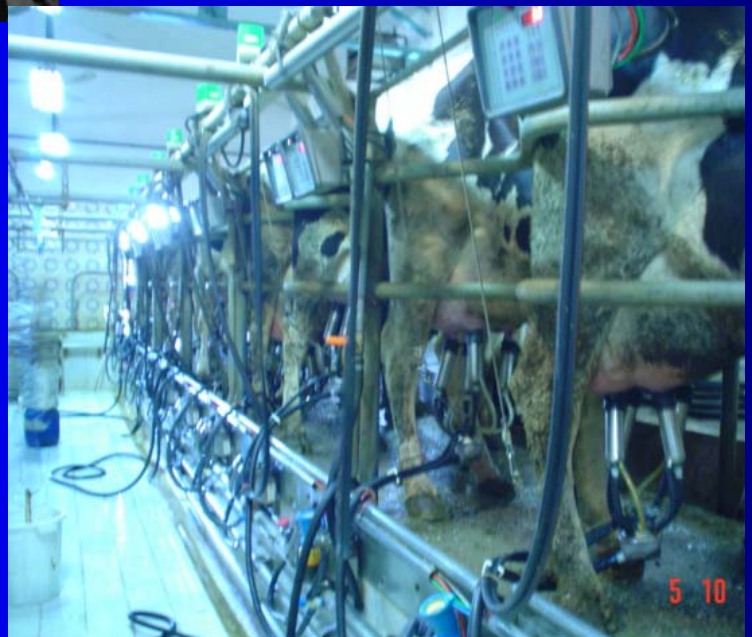


Mehrzad et al., 2001; 2004; 2010

Determine the outcome of mastitis


Functional assays/analyses on bovine neutrophils
Location: Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3500 m above sea level

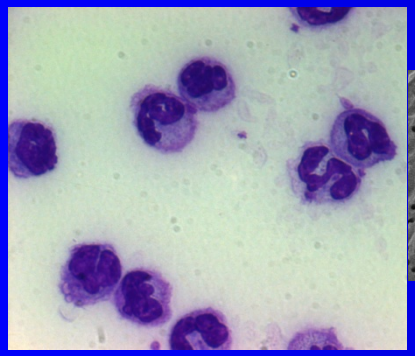
Blood and milk samples from many Holstein dairy cows

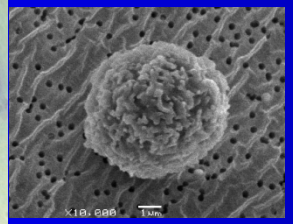


Bovine blood and milk neutrophil isolation procedure

Blood

Hypotonic lysis
& centrifugation

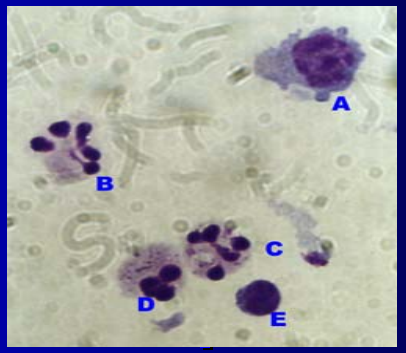


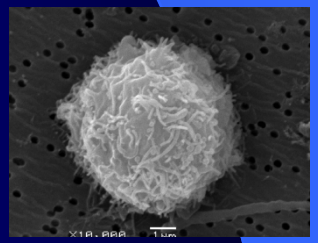


Milk

60% PBS

centrifugation



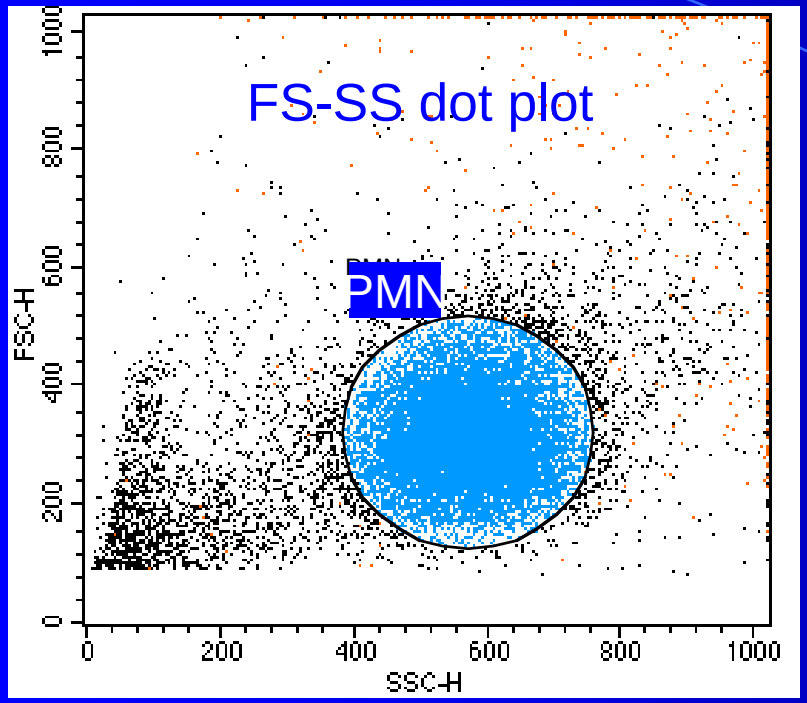


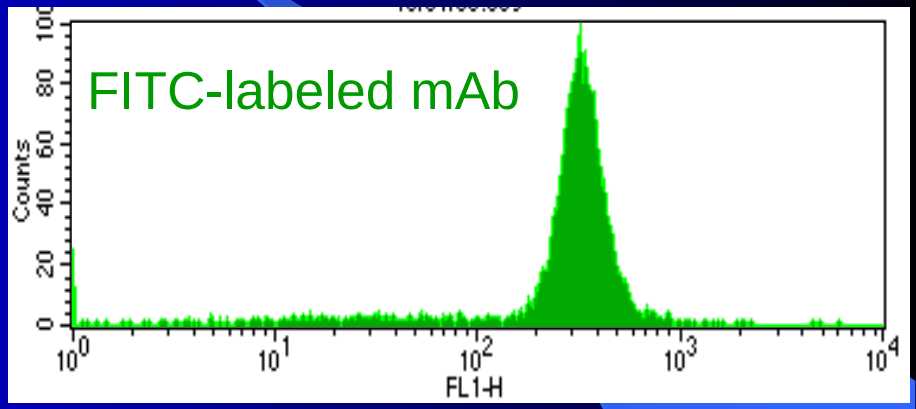
mastitis and non-mastitis samples

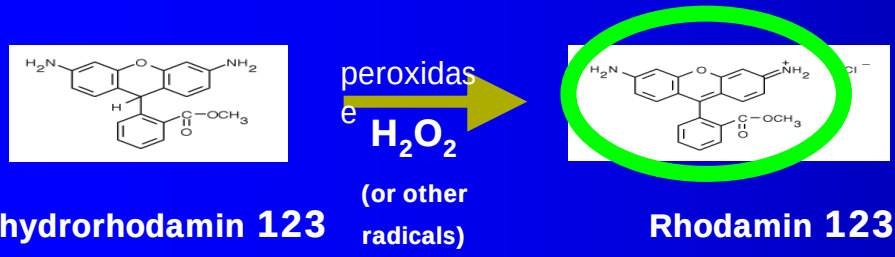
adapted from
Mehrzad et al., J Dairy Res. 2001; J. Dairy Sci. 2004; Vet. Res. 2005

Many analyses on these neutrophils

Flow cytometric analysis of neutrohil functions



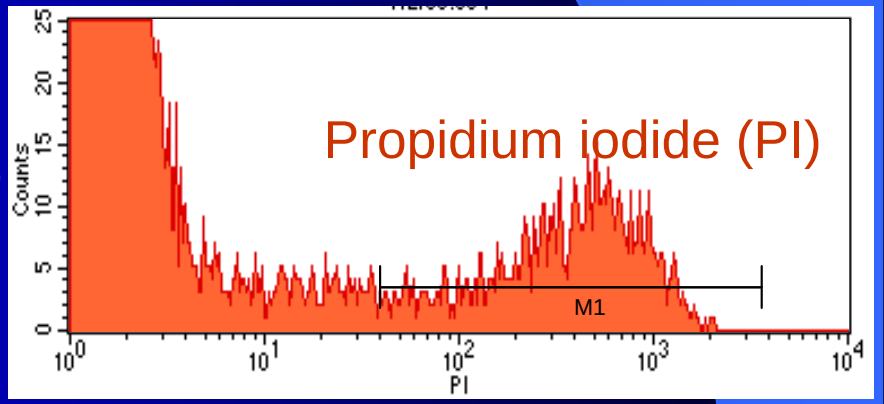




Dihydrorhodamin 123

DHR 123

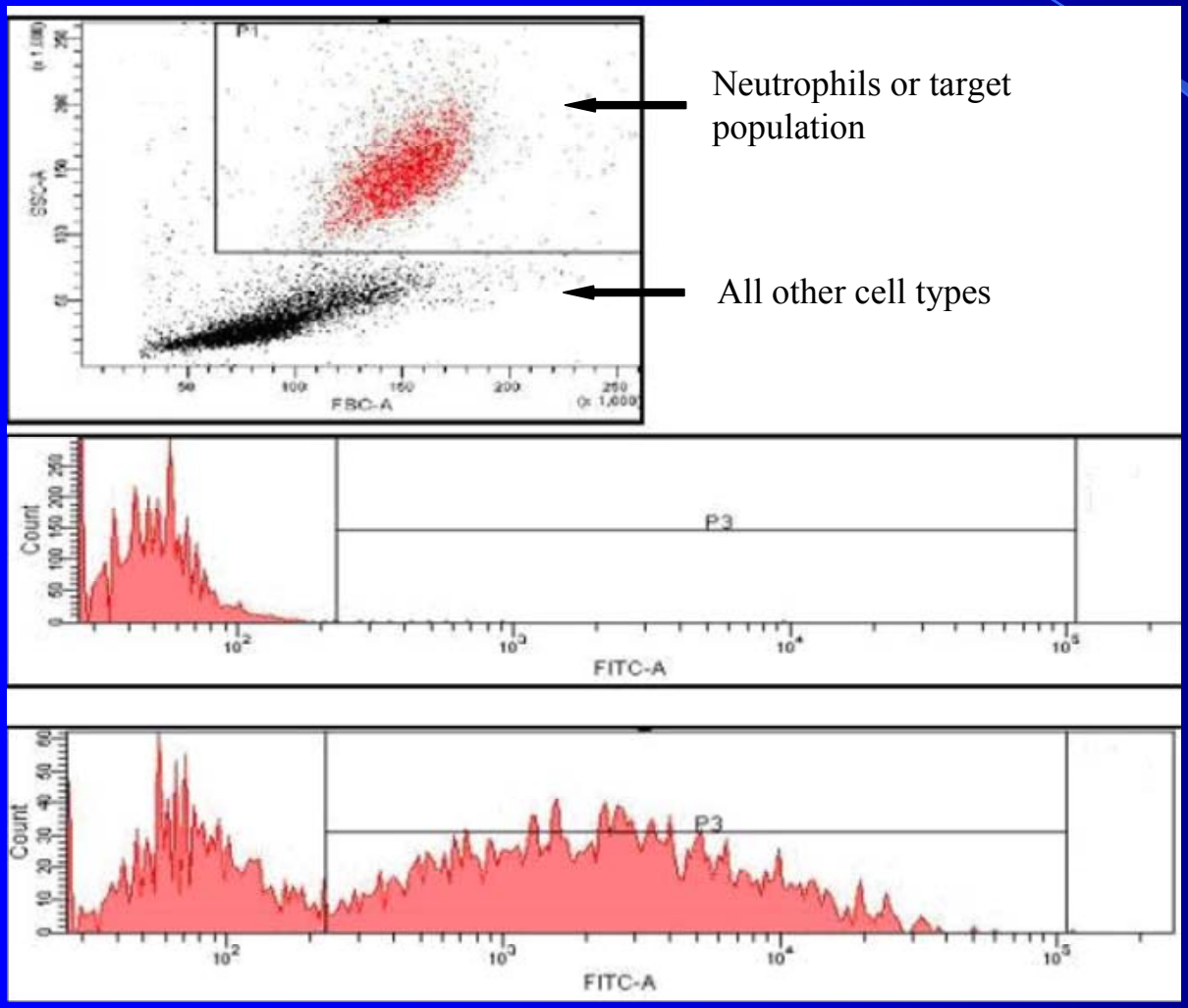
Rhodamin 123



adapted from

Mehrzad et al., J Dairy Res. 2001; J. Dairy Sci. 2004; 2008

Flow cytometric analyses of bovine whole blood neutrophils gated in FSC-SCC dot plot



The gate P3 was applied to determine the % phagocytosis (fluorescien isothiocyanate, FITC-labled E. coli) from the P1.

Or, PI +ve, for PMN necrosis.

Annexin-V +ve for PMN apoptosis.

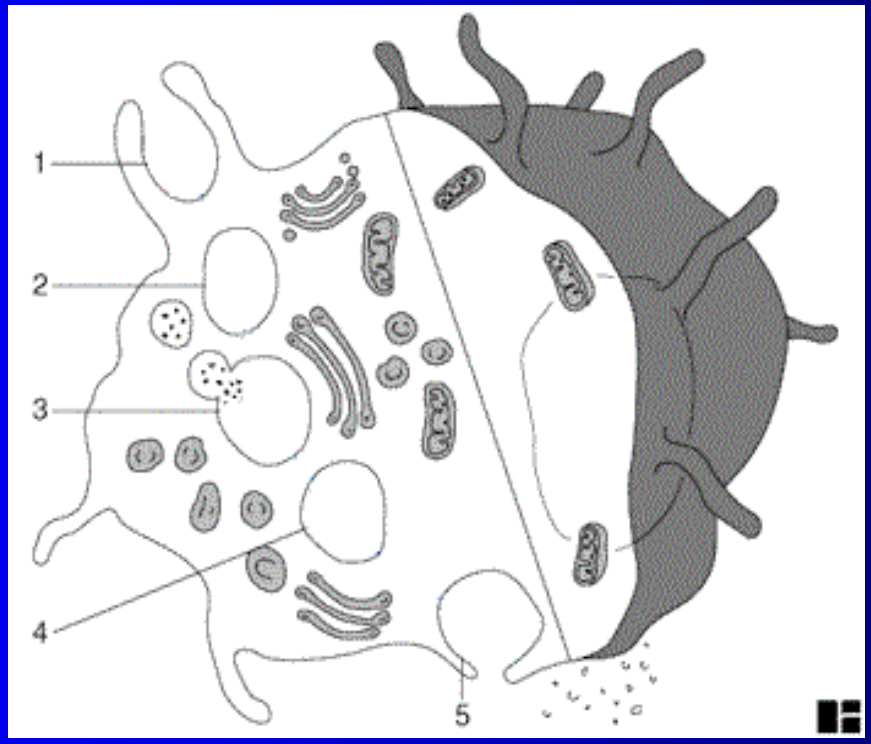
And conversion of DHR-123 to HR (green fluorescent) for respiratory burst activity of PMN.

Chemiluminescence (CL) analysis

Burvenich et al., 2003; Mehrzad et al., 2004; 2005; 2009; 2011; 2012

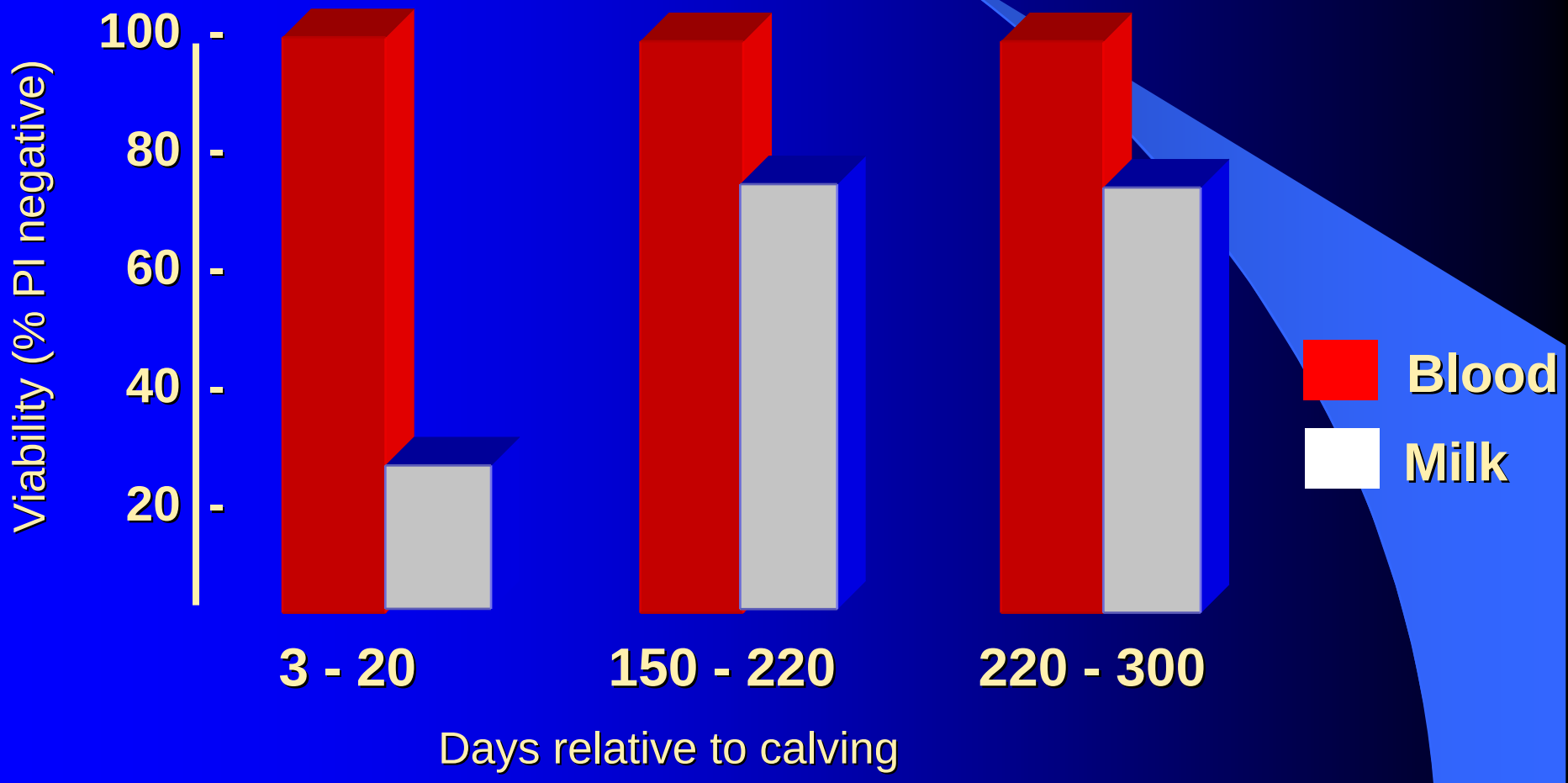

Some results and discussions
Location: Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3500 m above sea level

Stage of lactation vs blood and milk neutrophils' oscillation



Viability of bovine neutrophils

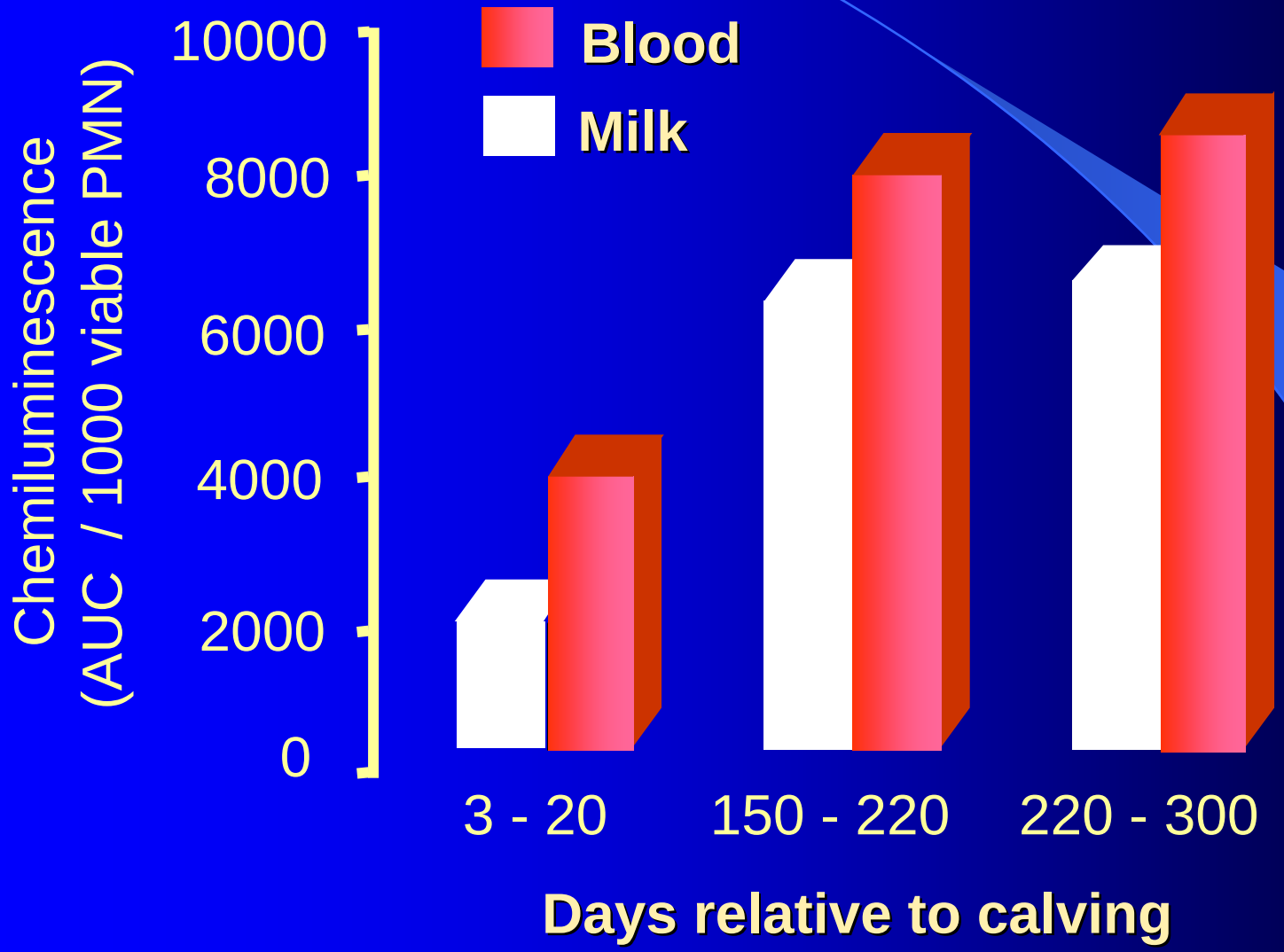
Milk PMN oscillation



Mehrzad *et al.*, 2001; 2002

ROS production by bovine neutrophils

Huge PMN oscillation



Mehrzad *et al.*, 2001; 2002

Kinetics of ROS production by neutrophils

Key slide

Blood

Milk

30 min

30 min

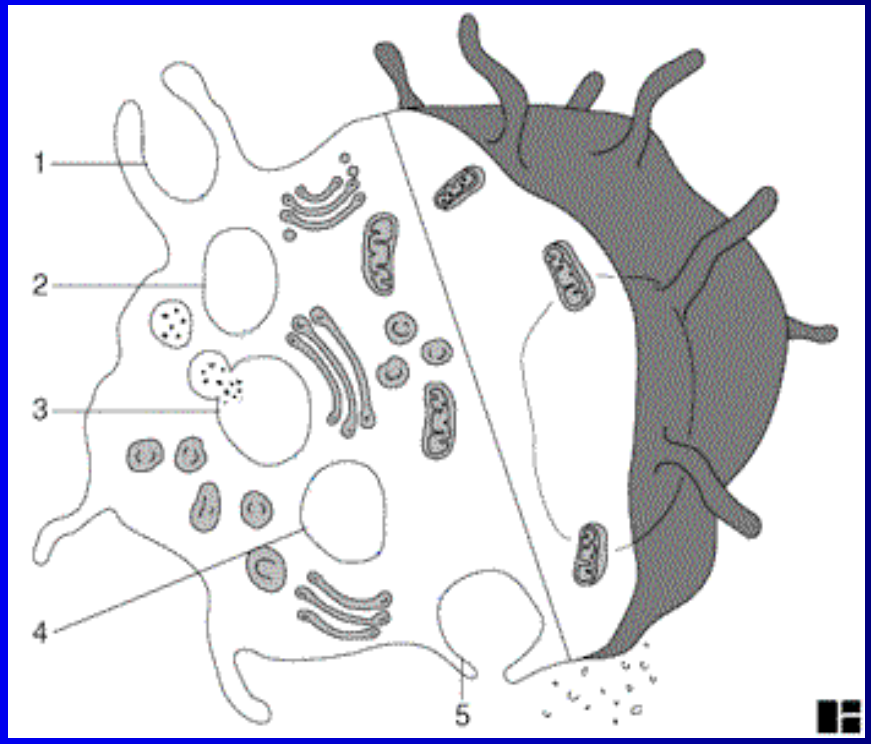
A detailed comparison intracellular vs extracellular ROS (early vs mid lactation)

Huge PMN oscillation

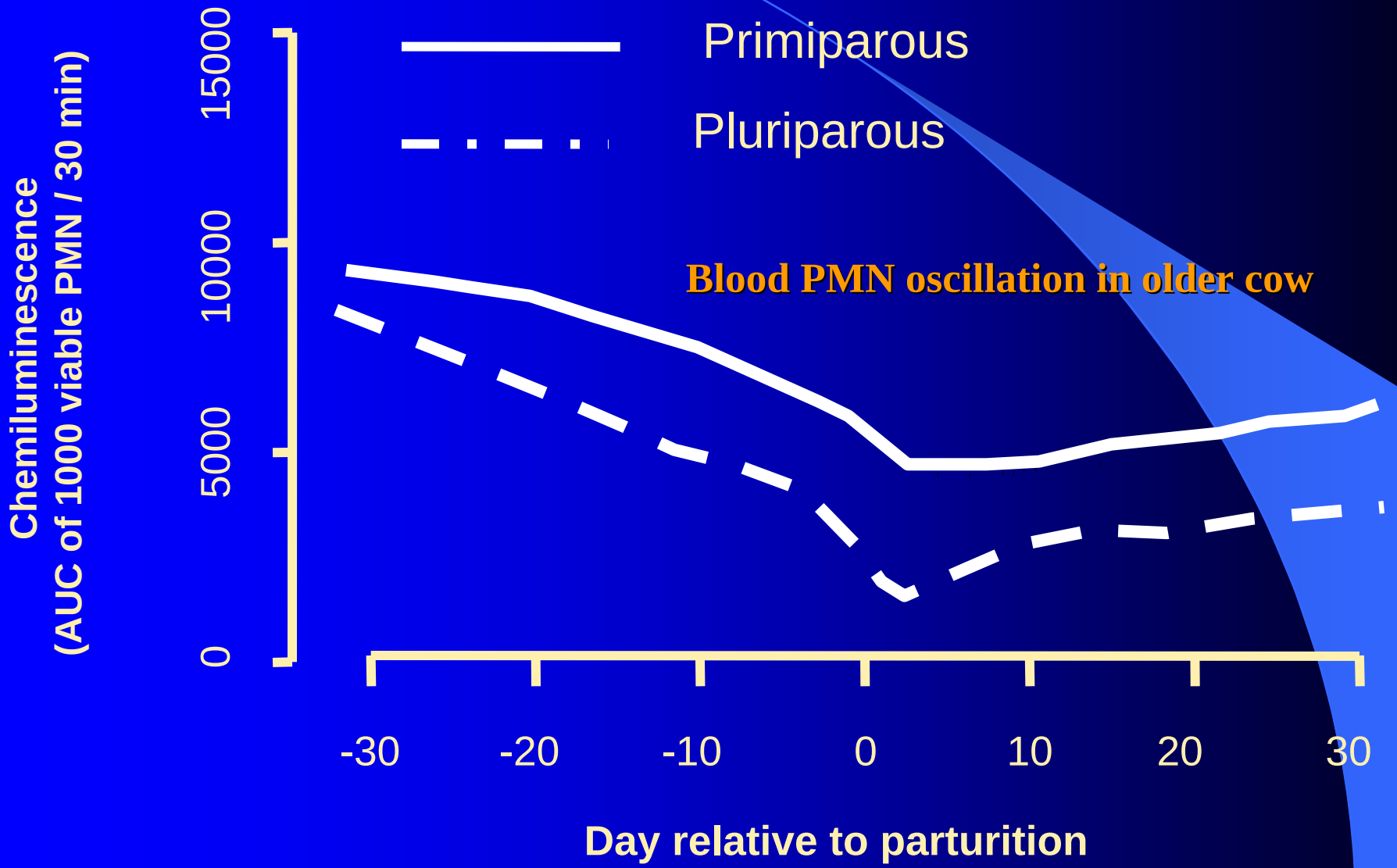
adapted from

Mehrzad et al., J Dairy Res. 2001; Vet. Res. 2005, 2009; J Dairy Sci. 2004; 2011;2012

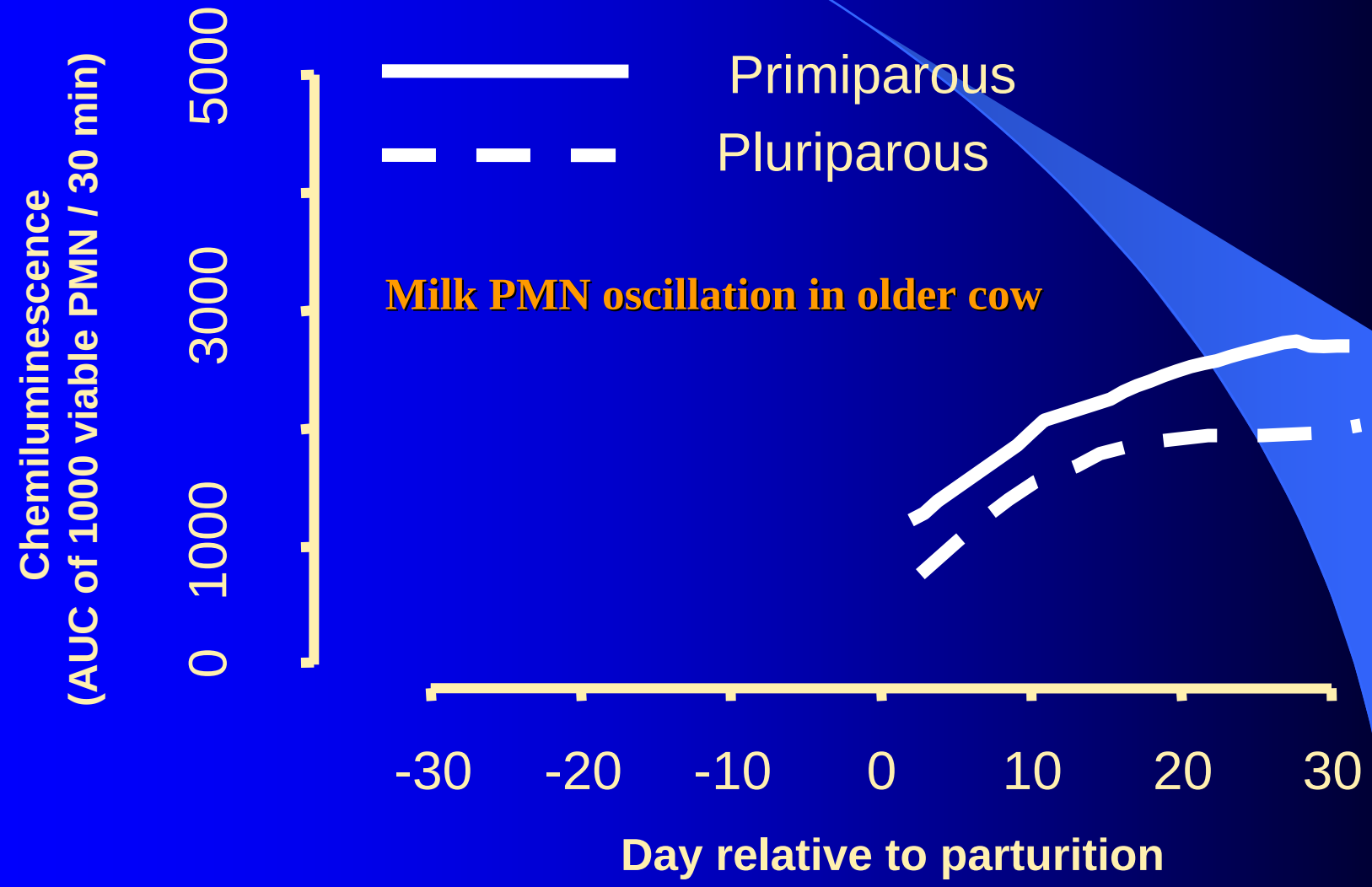
Age/calving /parity vs blood and milk neutrophils oscillation



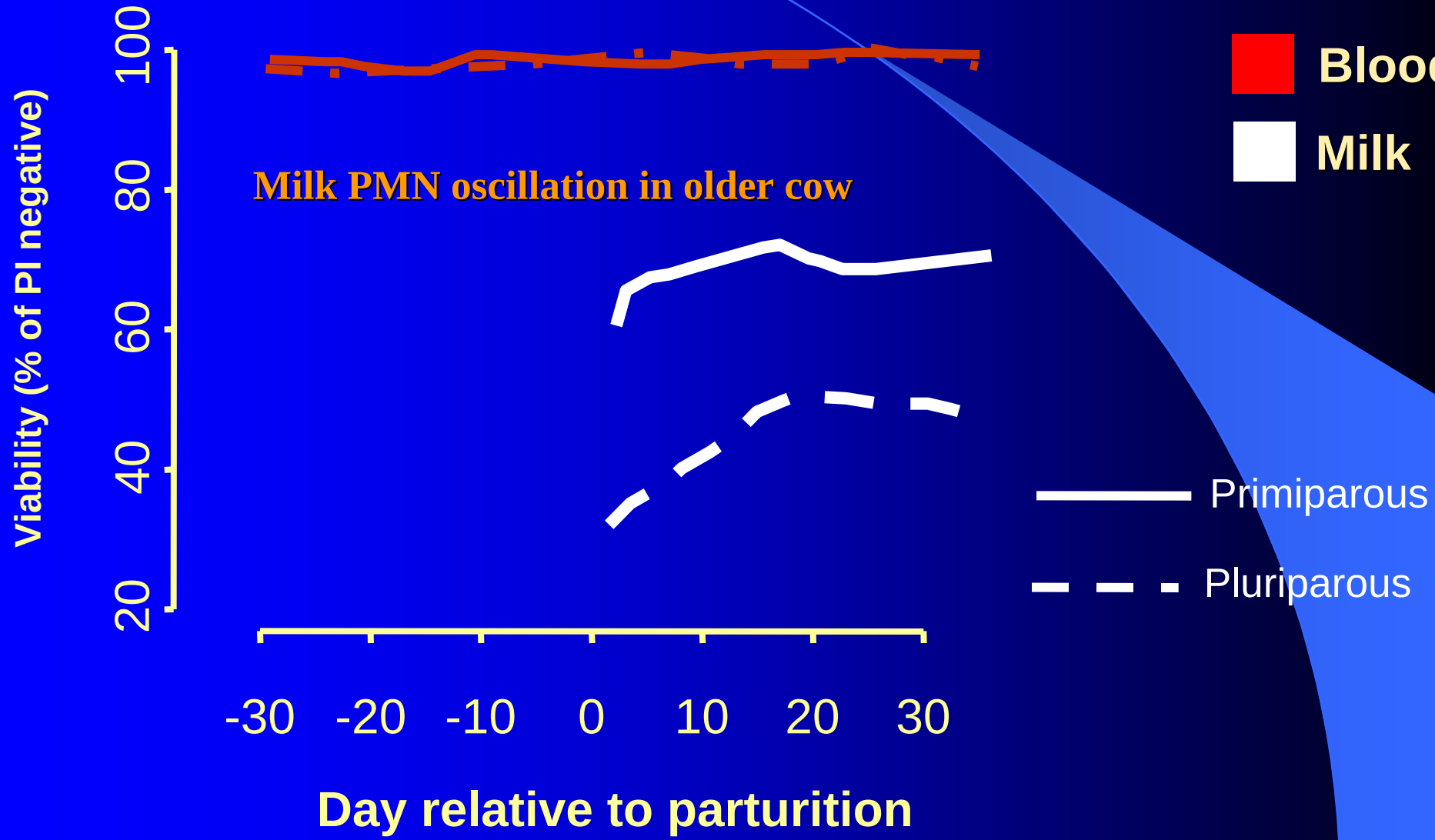
Blood neutrophils ROS production vs age during peripartum period



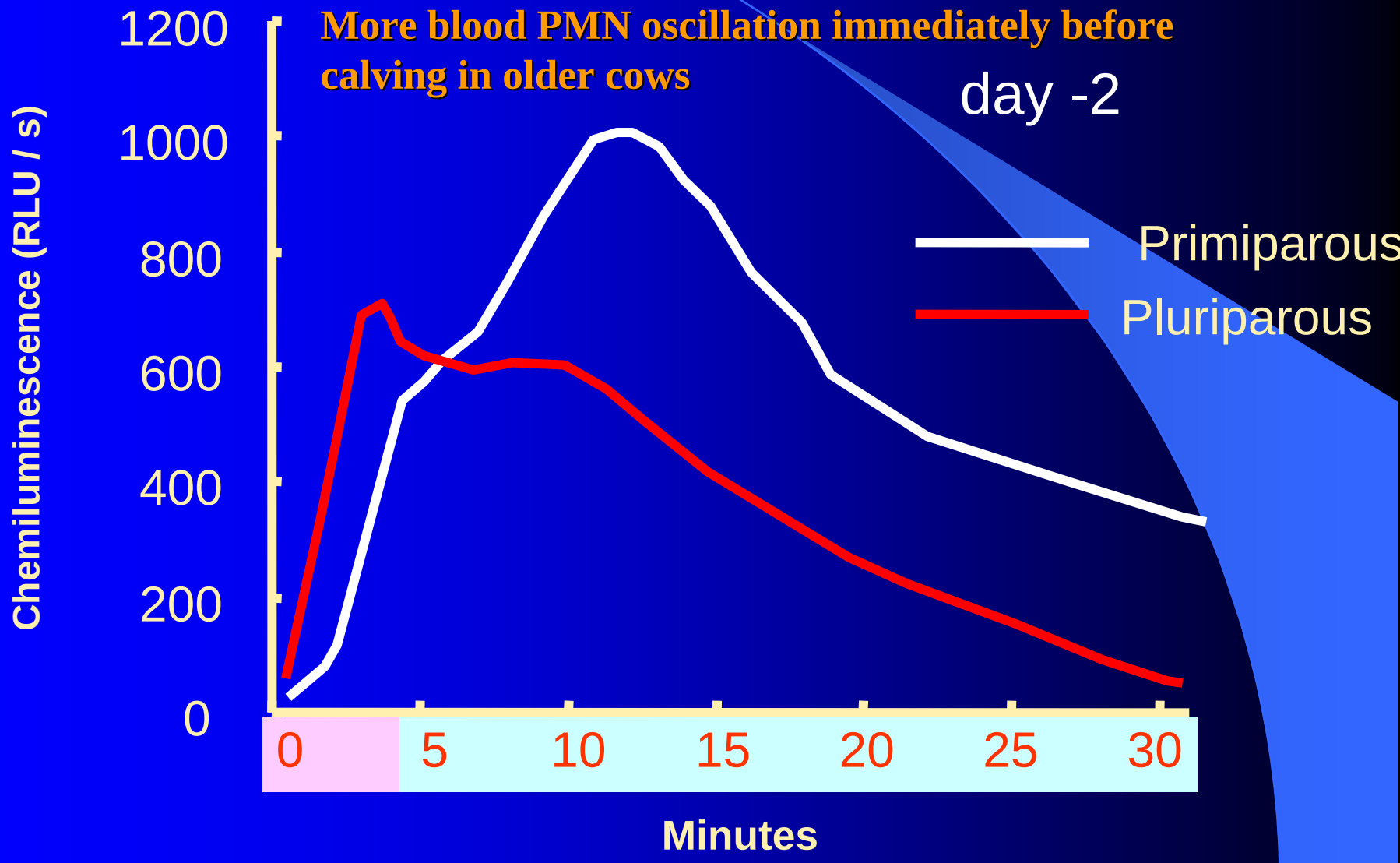
Milk neutrophils ROS production vs age during early lactation



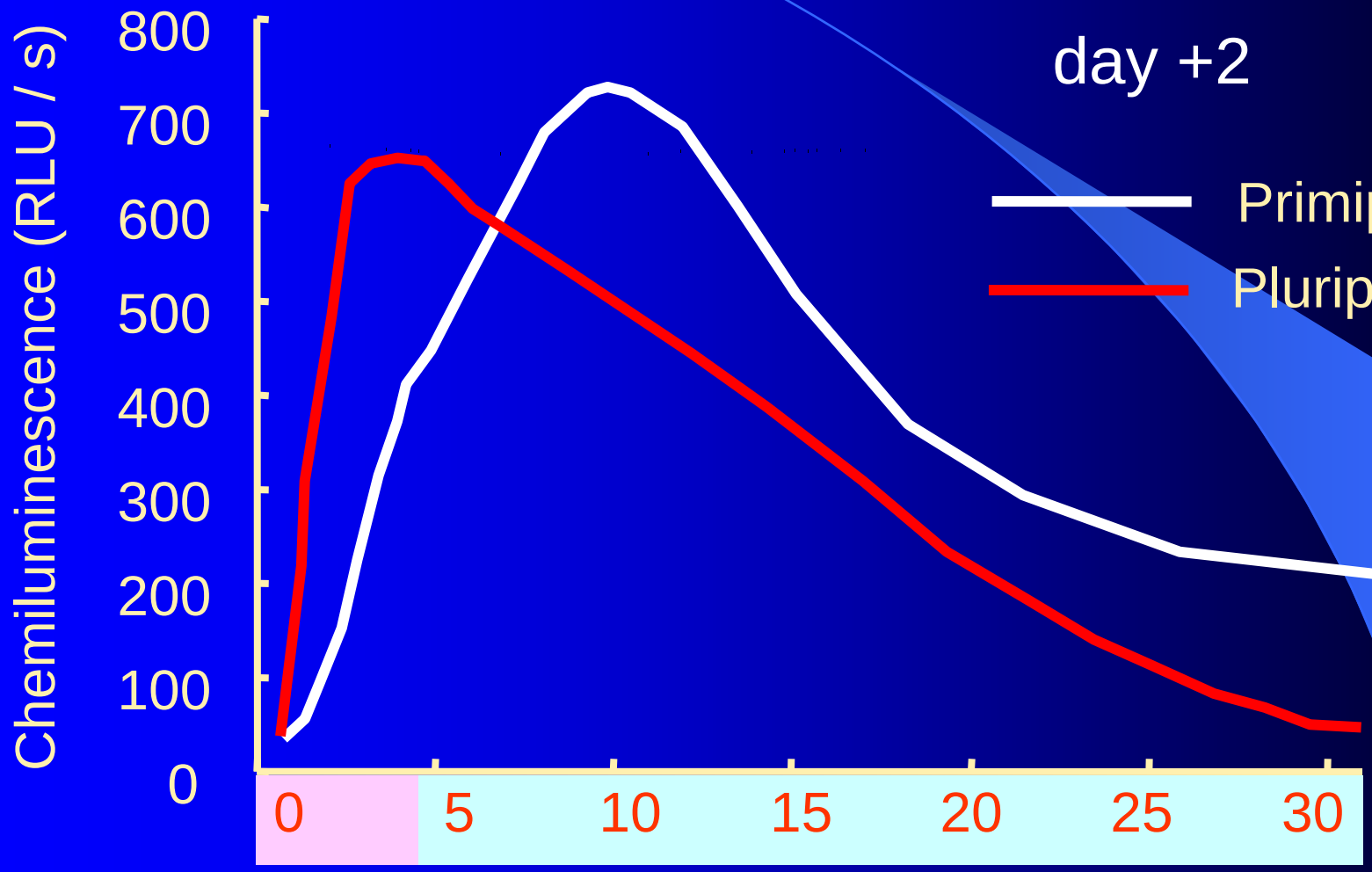
Blood and milk neutrophils viability vs age during peripartum period



Kinetics of ROS production by blood neutrophils vs age immediately before calving

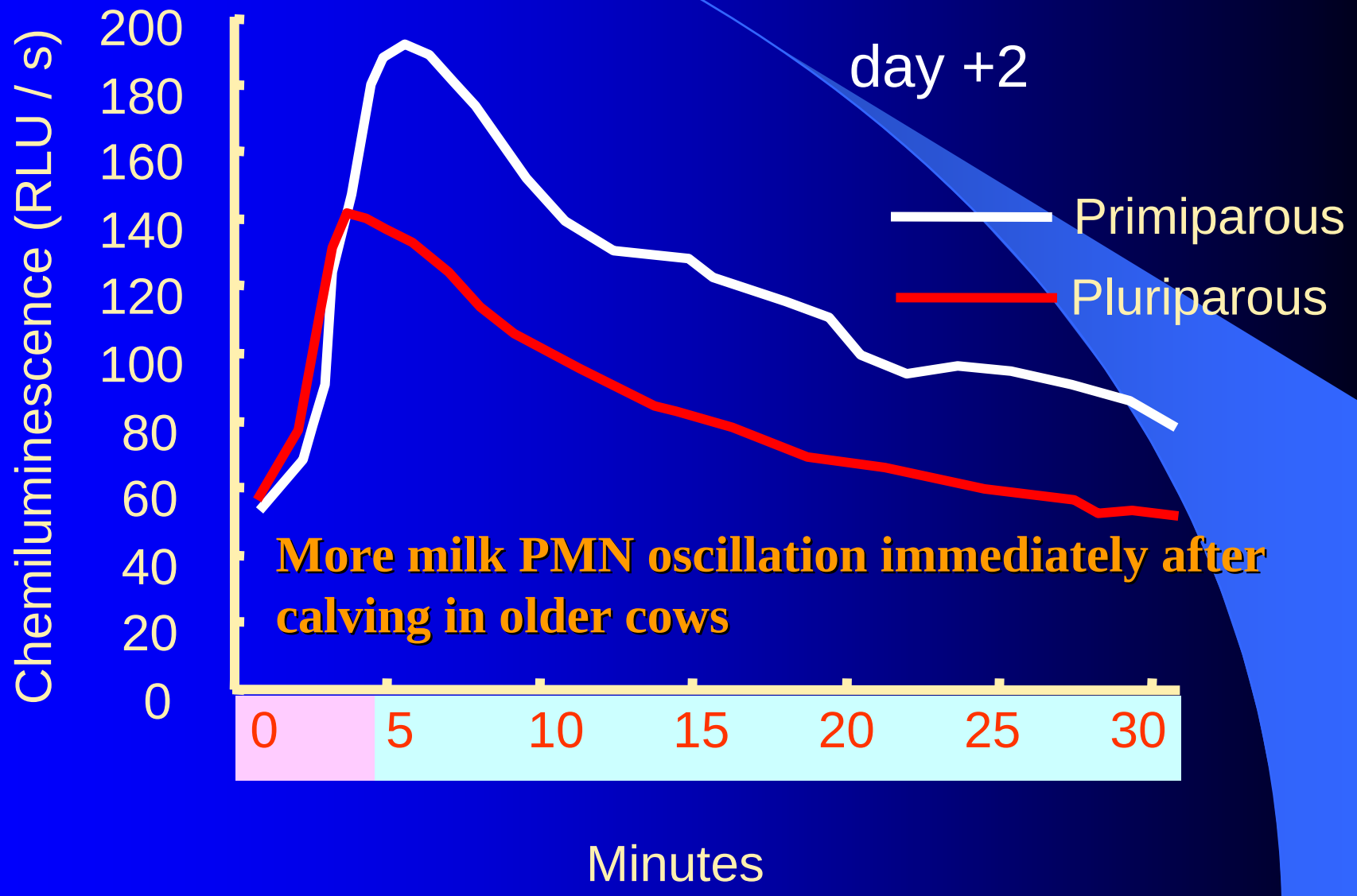


Kinetics of ROS production by blood neutrophils vs age immediately after calving

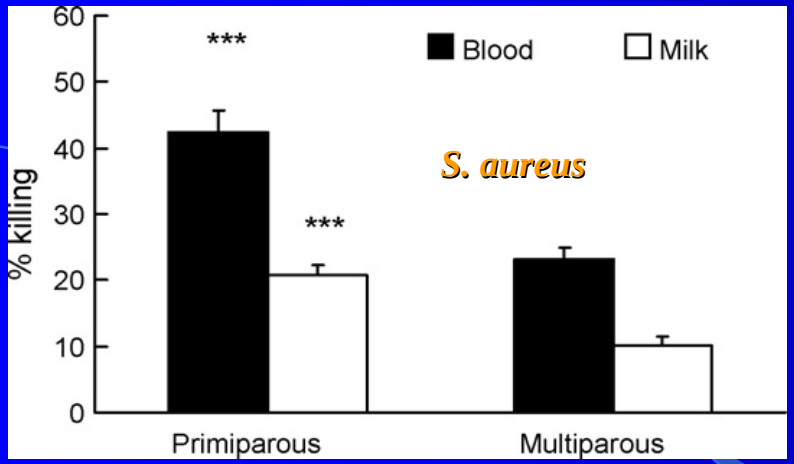


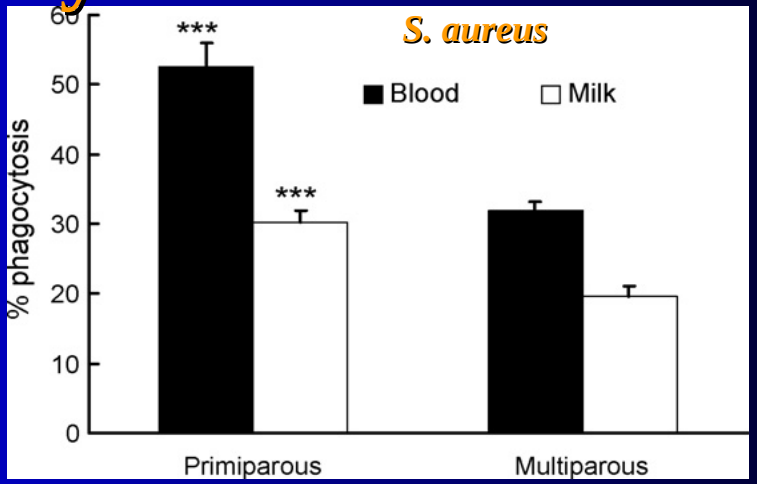
More blood PMN oscillation immediately before calving in older cows

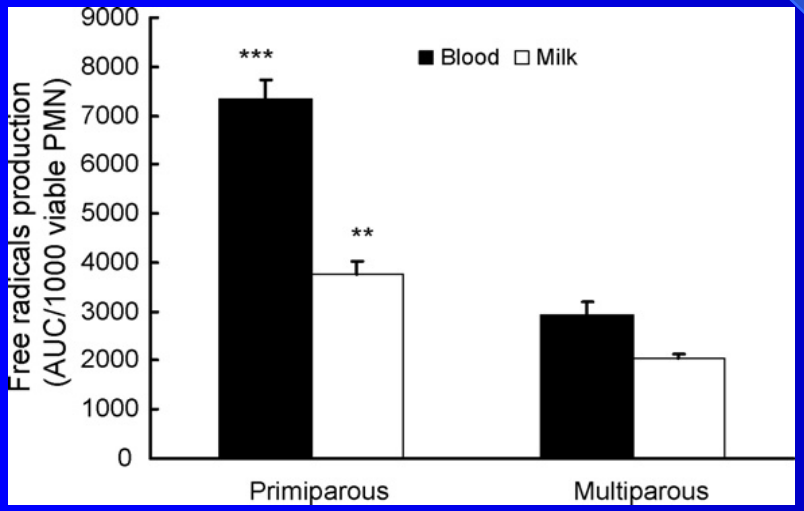
Kinetics of ROS production by milk neutrophils vs age immediately after calving

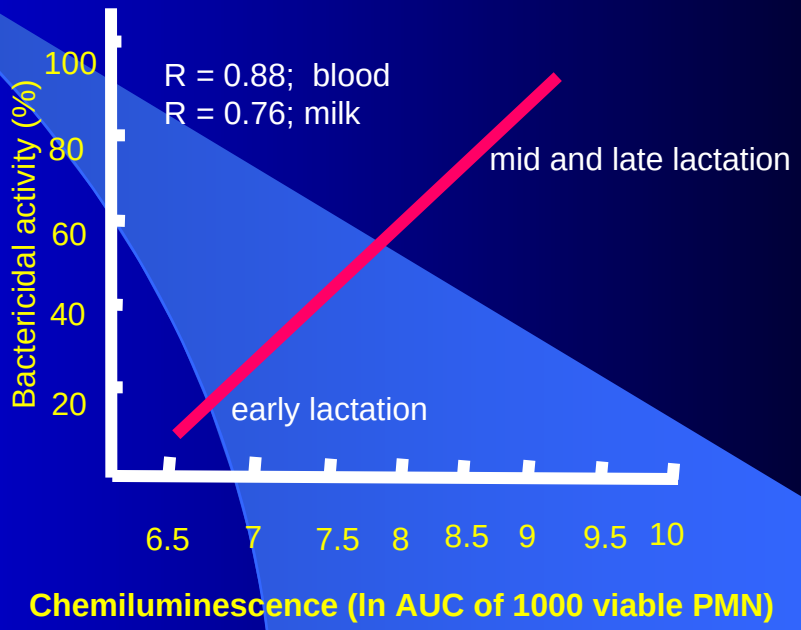


Neutrophils' oscillatory events more pronounced in old dairy cows



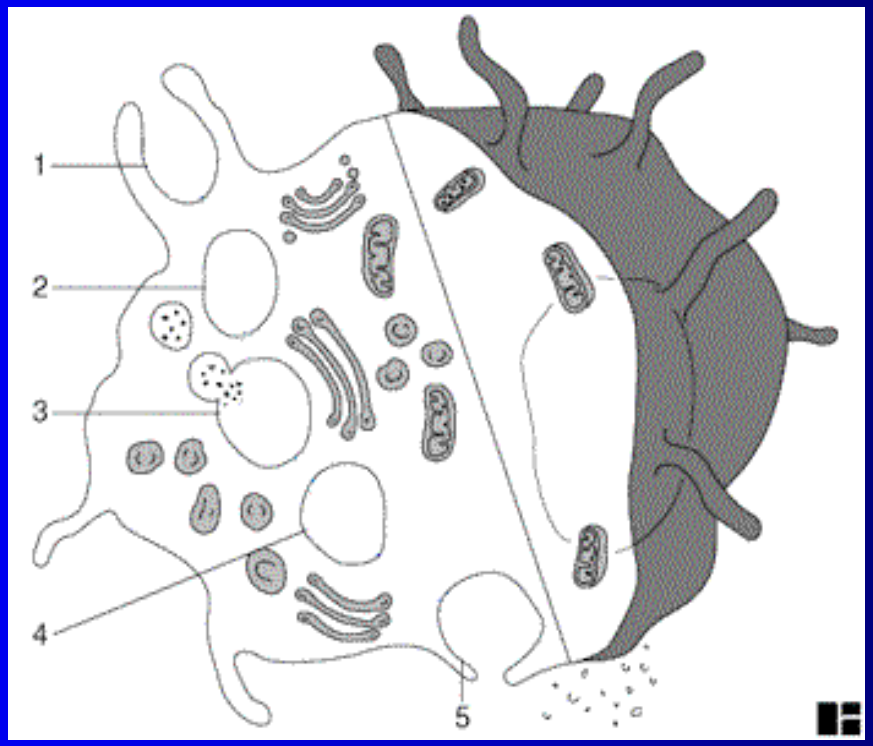




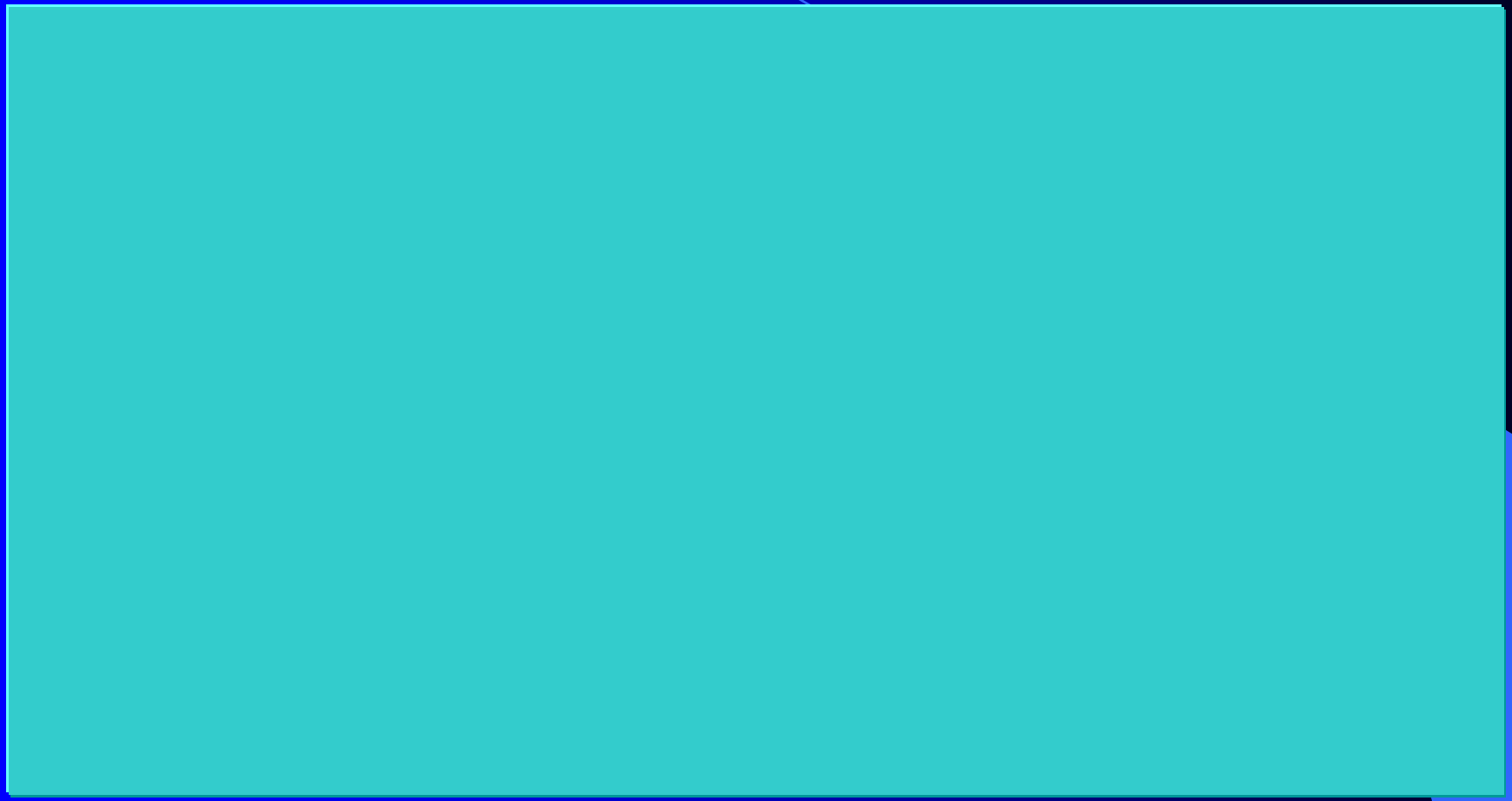


Mehrzad et al., Luminescence, 2001; J Dairy Res. 2001; Vet Microbiol. 2008

Blood/post- diapedetic neutrophils' oscillation leads to higher severity of mastitis



Neutrophils' oscillation vs. sever and moderate response to i.mam



diapedesis rate, postdiapedetic neutrophil ROS production capacity and *E. coli CFU dynamics in moderate and* severe responders of high yielding dairy cows

Mehrzad et al., 2001b, 2004; 2005; 2010

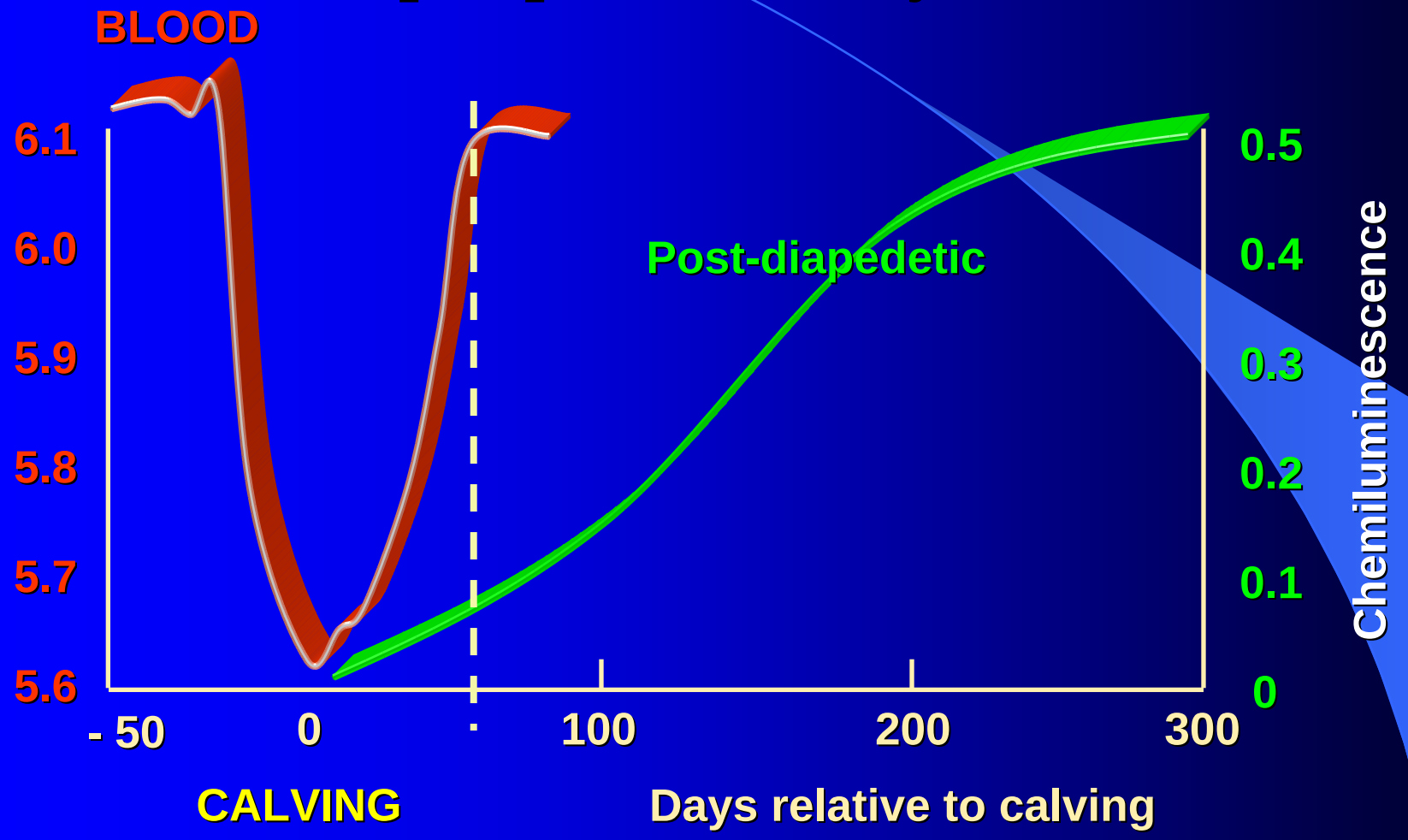
Oscillation in neutrophils at three locations

(structure and functions, MPO-H2O2-Halide system and viability,) in healthy and mastitis dairy cows

Mehrzad et al., 2001b, 2004; 2005; 2010

ROS even higher than blood

Why huge neutrophils' oscillatory events in peripartum dairy cows?



adapted from Hallén Sandgren *et al.* , 1991
Hoeben *et al.*, 1999, Burvenich et al.,, 2003
Mehrzad *et al.* , 2001, 2004; 3005; 2009; 2012

Hypotheses & reasons behind neutrophil oscillatory events and mastitis severity in peripartum dairy cows

During the periparturient period

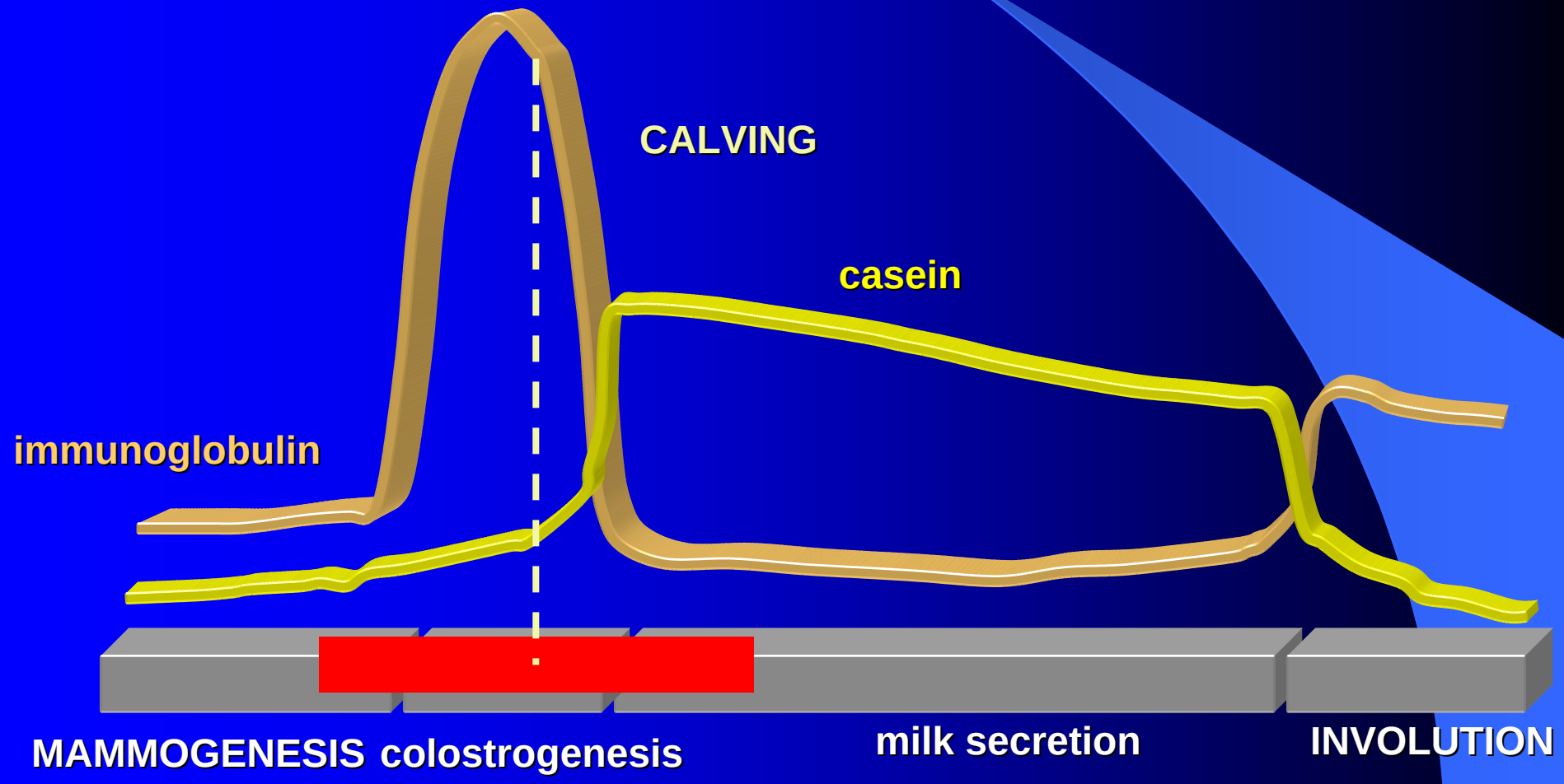
- low circulating neutrophil number
- decreased chemotaxis
- decreased diapedesis towards i.mam. LPS
- decreased oxygen burst (superoxide,...)
- impaired CD11/CD18 expression
- impaired CD62L expression
- left shift of neutrophils (immature)
- decreased phagocytosis
- decreased LPS detoxification
- decreased C5a production
- *etc,...*

In relation with severity of mastitis (0 - 8 wks)

- IgG1 inhibits opsonic activity of IgG2 & IgM
- blastogenesis (lymphocyte) depressed
- loss in T-helper cell function
- IgM production is impaired
- total Ig level decreased in blood & milk
- complement hemolytic activity impaired
- colostrum increases growth of E.coli
- lactoferrin not efficient

Relative local changes in *milk protein* by stage of lactation

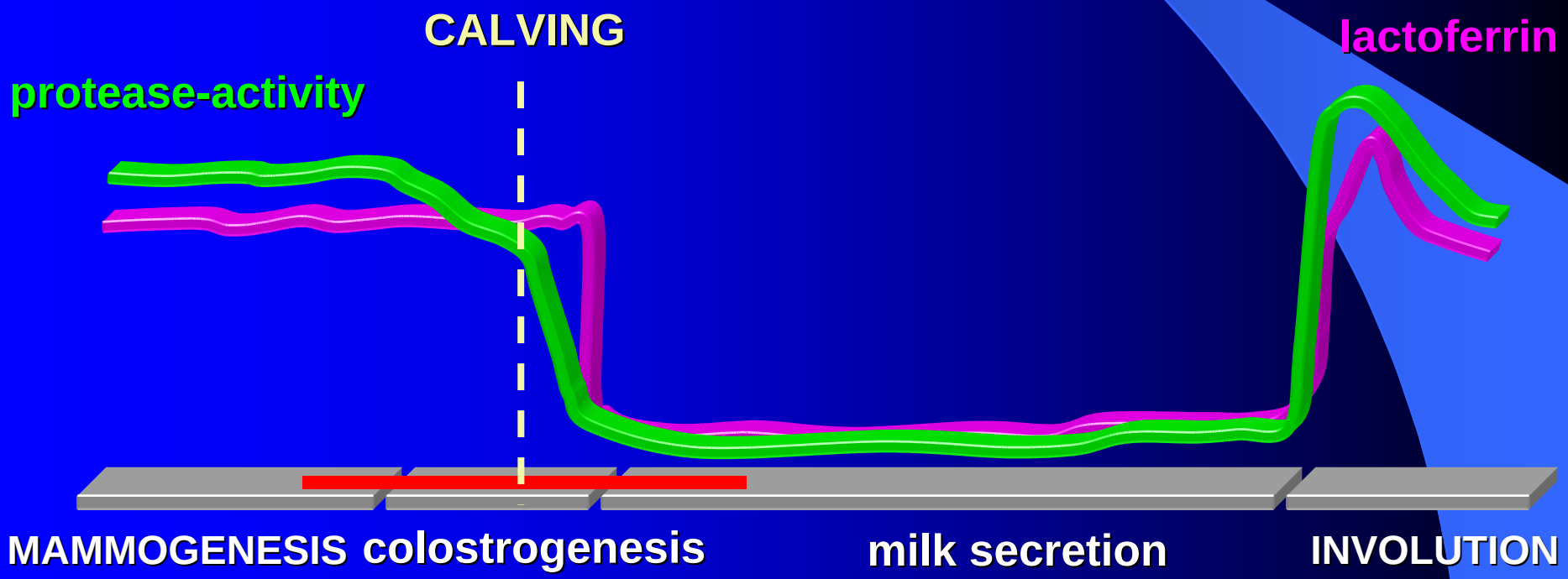
adapted from
Schanbacher *et al*, 1993
Schanbacher & Smith, 1975



Burvenich et al.,, 2003

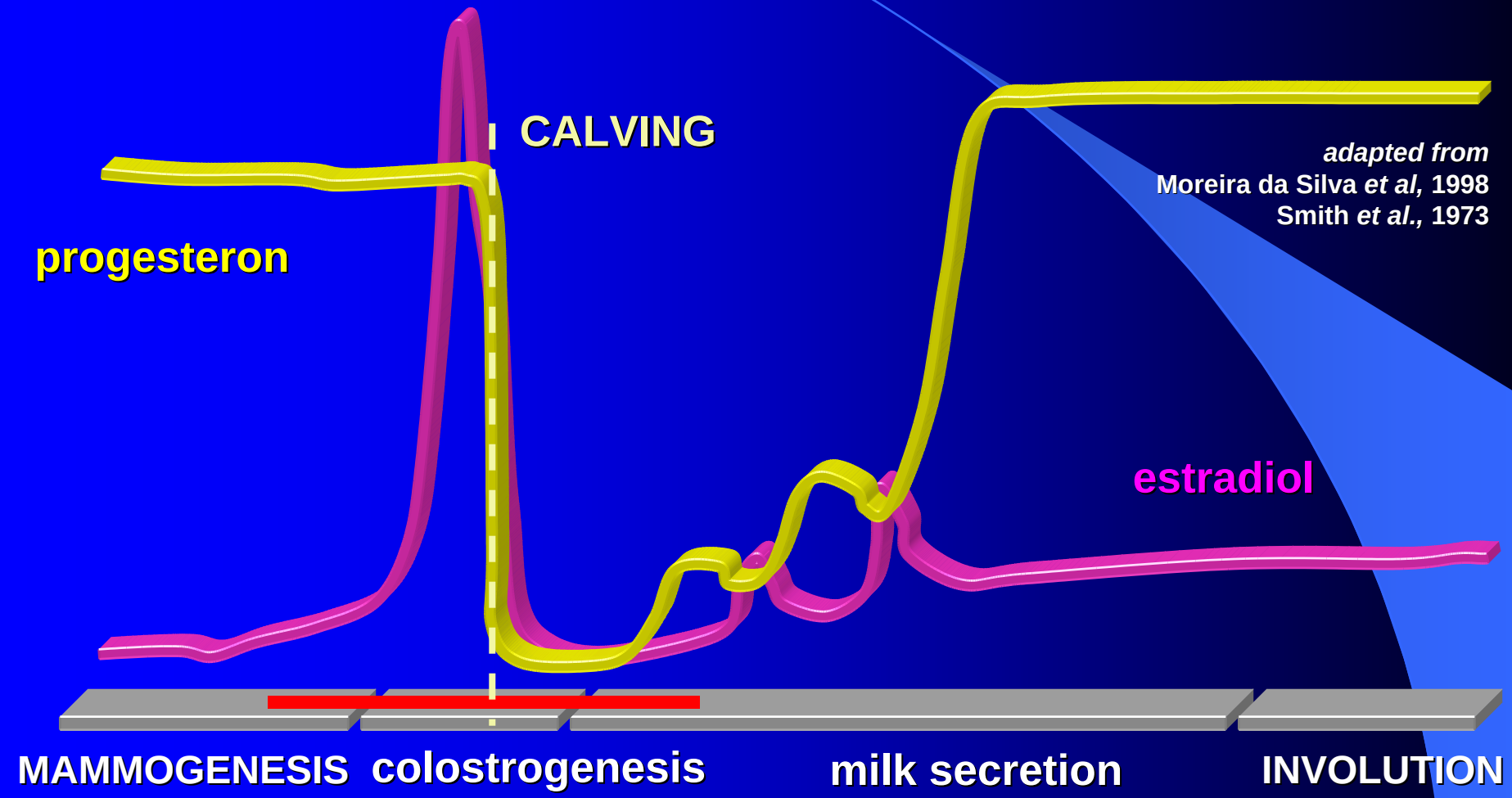
Relative local changes in *milk protease* by stage of lactation

adapted from
Zavizion *et al,* 1996
Schanbacher & Smith, 1975



Burvenich et al.,, 2003

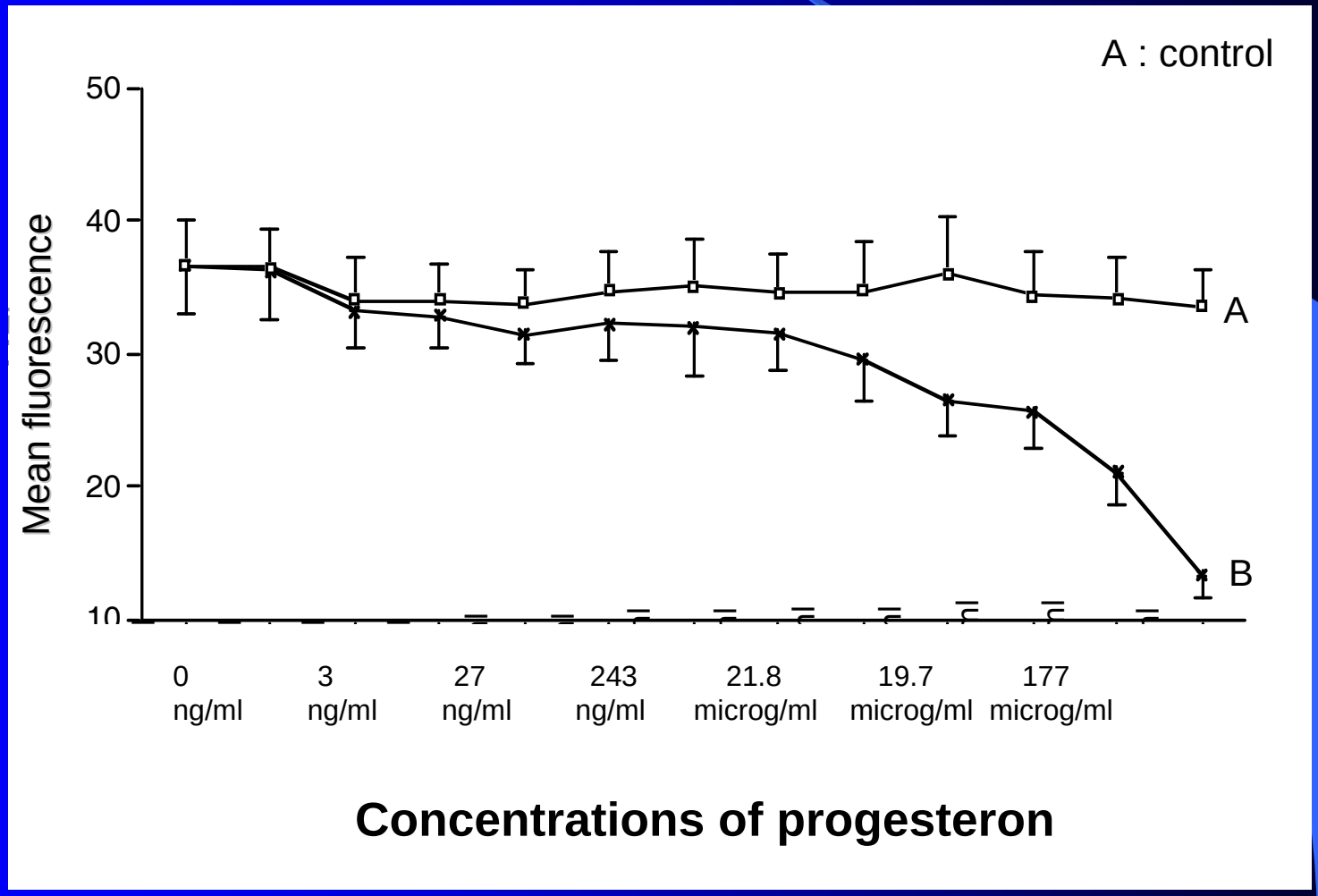
Relative systemic changes in *blood sex steroids* by stage of lactation



Burvenich et al.,, 2003

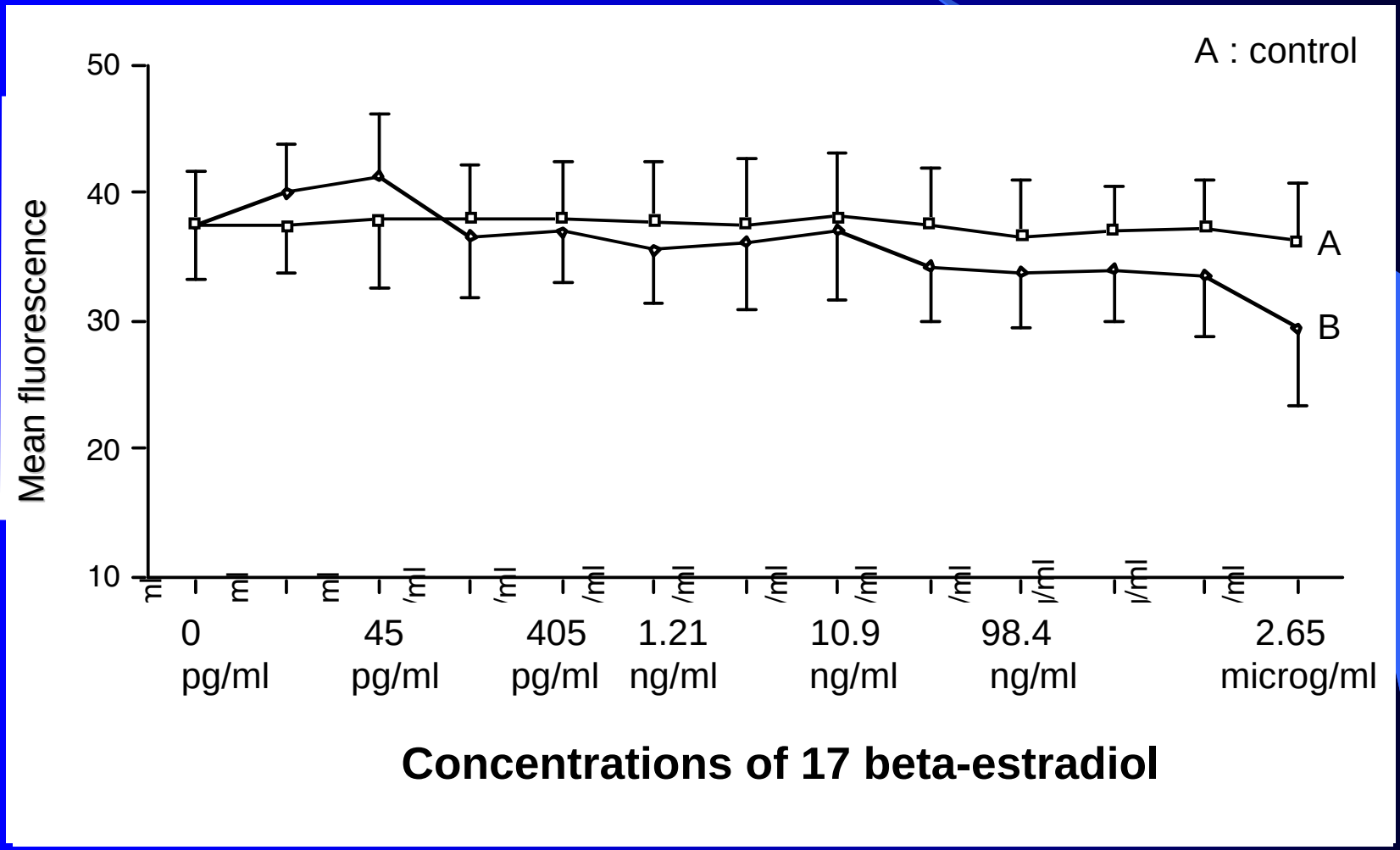
Effect of *progesteron* on respiratory burst of blood neutrophils

Moreira da Silva *et al*, 1998

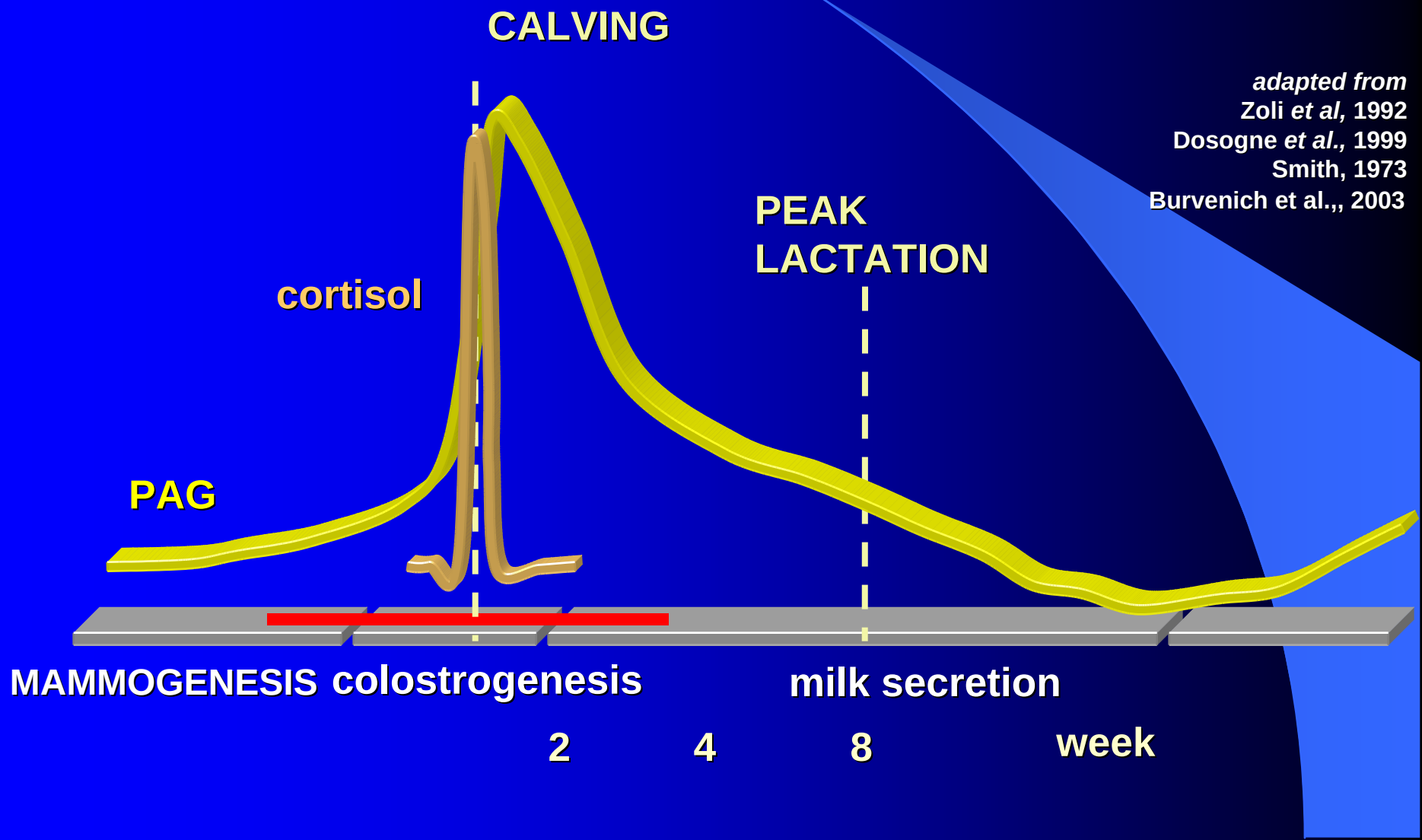


Effect of *17-beta estradiol* on respiratory burst of blood neutrophils

Moreira da Silva *et al,* 1998

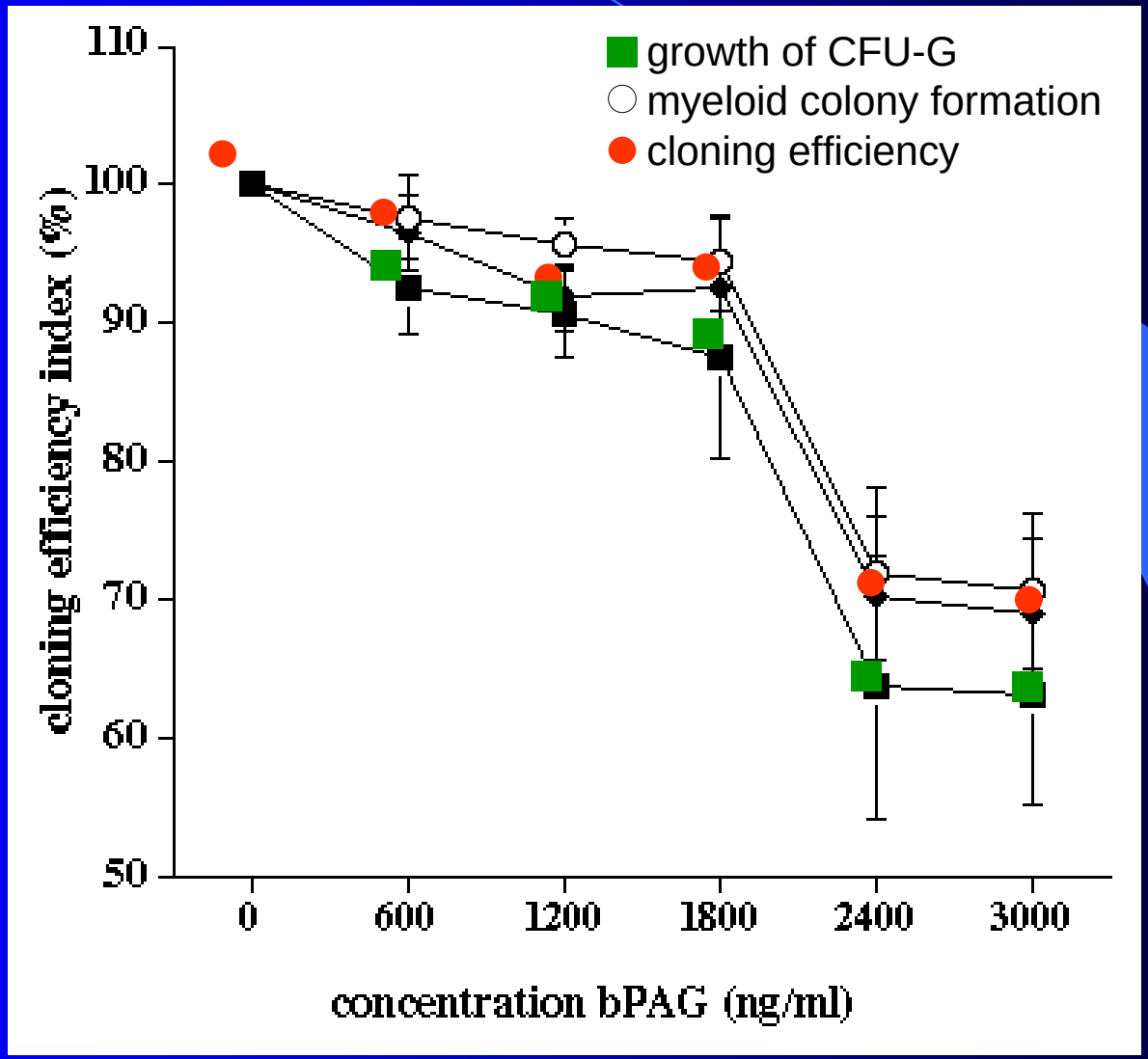


Relative systemic changes in *plasma* *PAG & cortisol* by stage of lactation



adapted from
Zoli *et al,* 1992
Dosogne *et al.,* 1999
Smith, 1973
Burvenich et al.,, 2003

In Vitro effect of *pregnancy associated glycoprotein* on cloning efficiency in the bone marrow

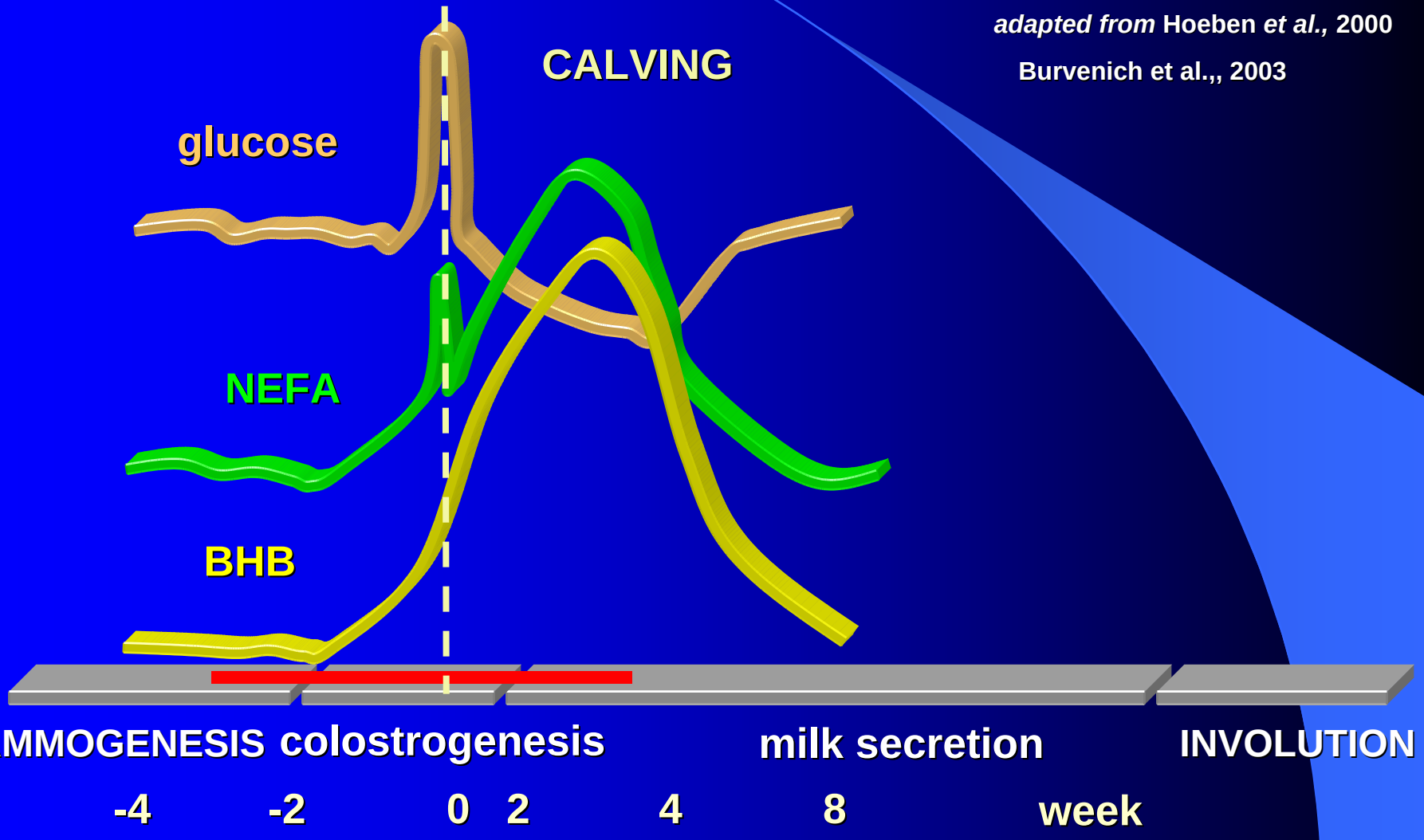


Hoeben *et al.*, 1999

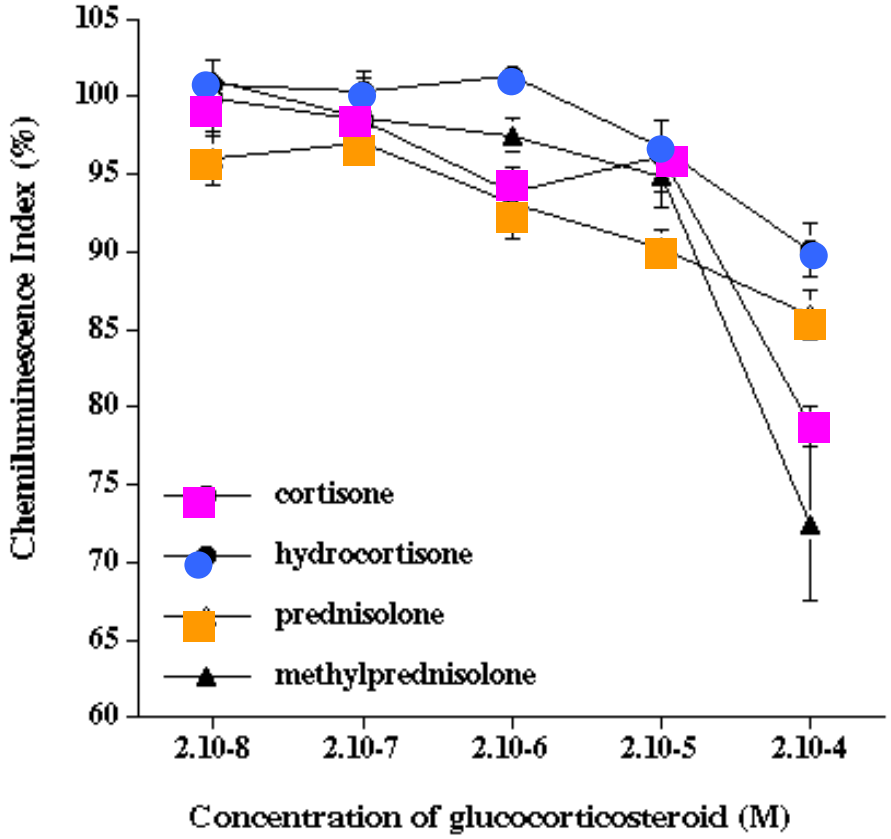
Relative systemic changes in *plasma glucose, BHB, NEFA* by stage of lactation

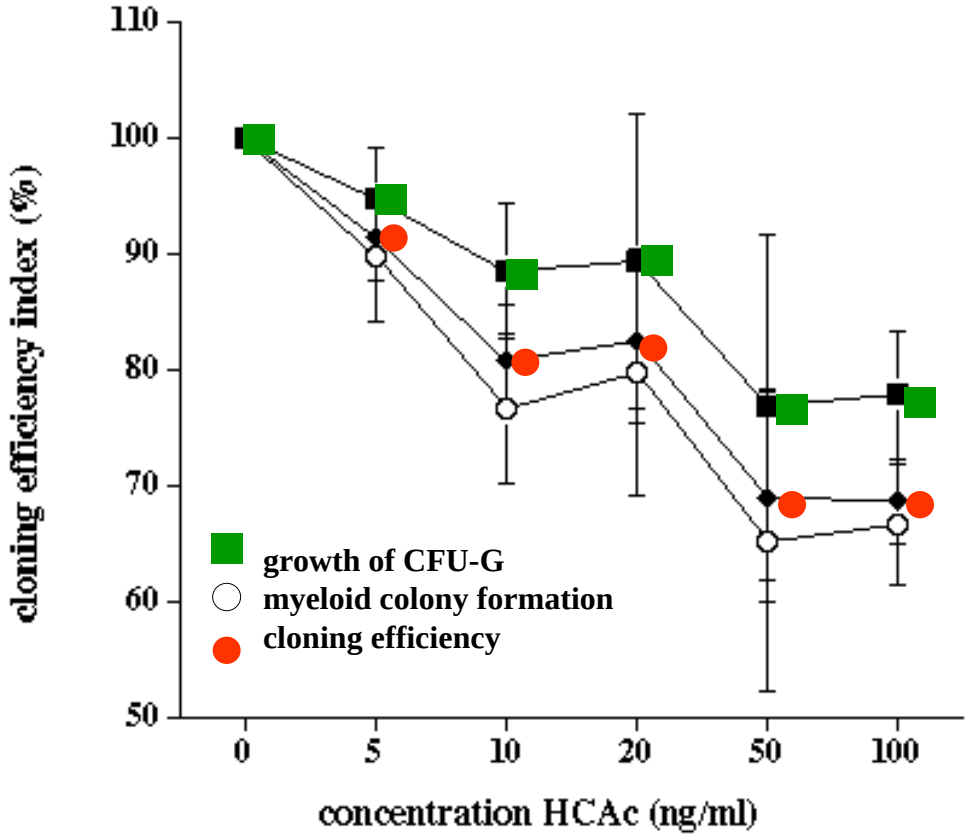
adapted from Hoeben *et al.,* 2000

Burvenich et al.,, 2003



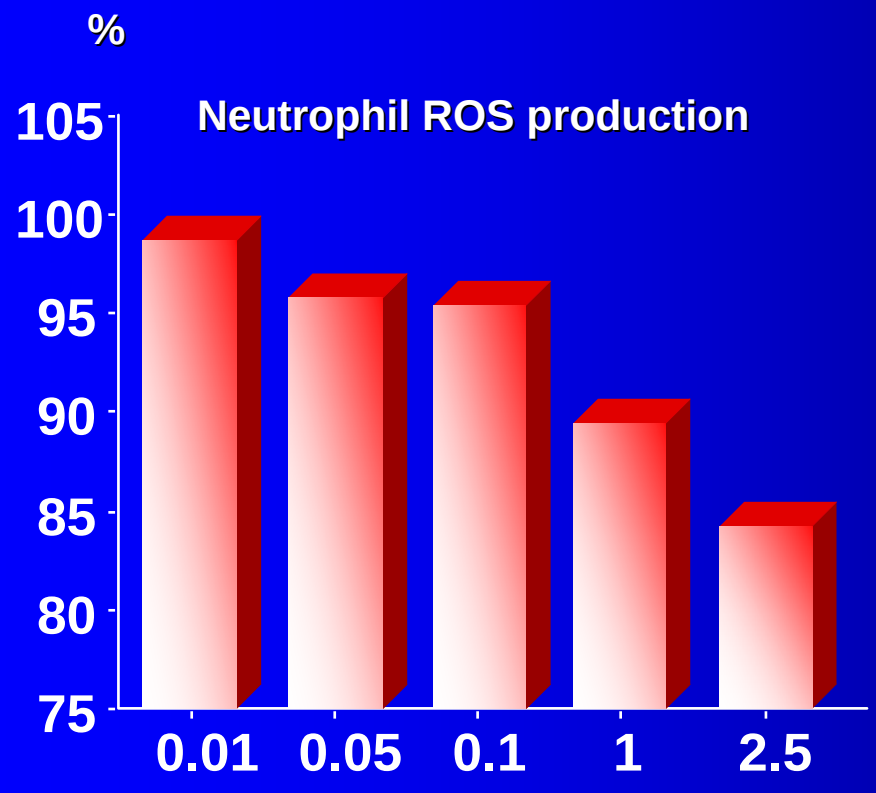
In Vitro effect of *glucocorticosteroids* on respiratory burst of blood neutrophils and cloning efficiency in bone marrow

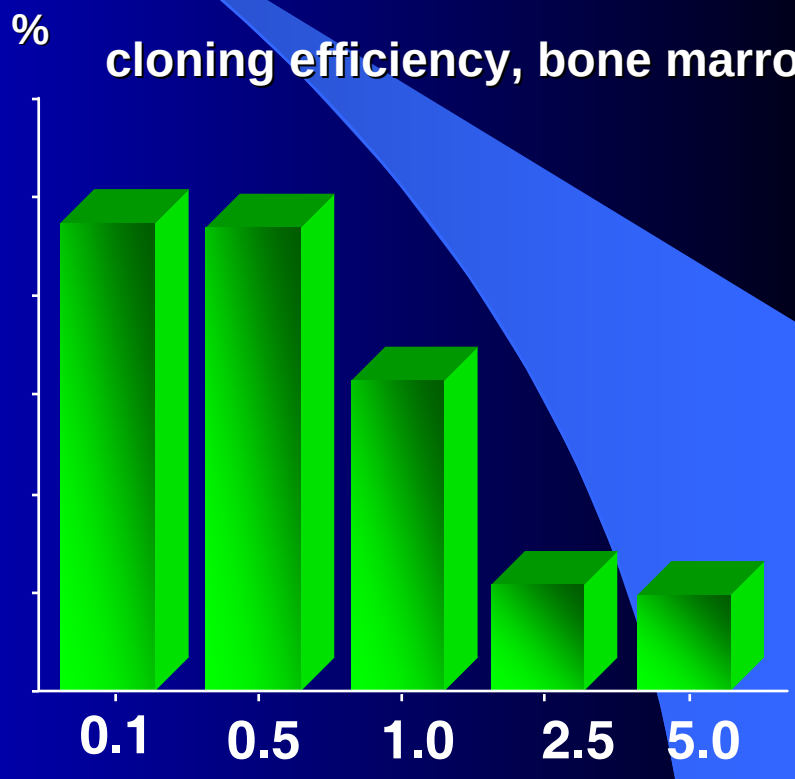




Hoeben *et al.*, 1998 and 1999

In Vitro effect of *beta-hydroxybutyrate* on Physioimmunology of neutrophils





mM

Hoeben *et al.*, 1997 & 1999

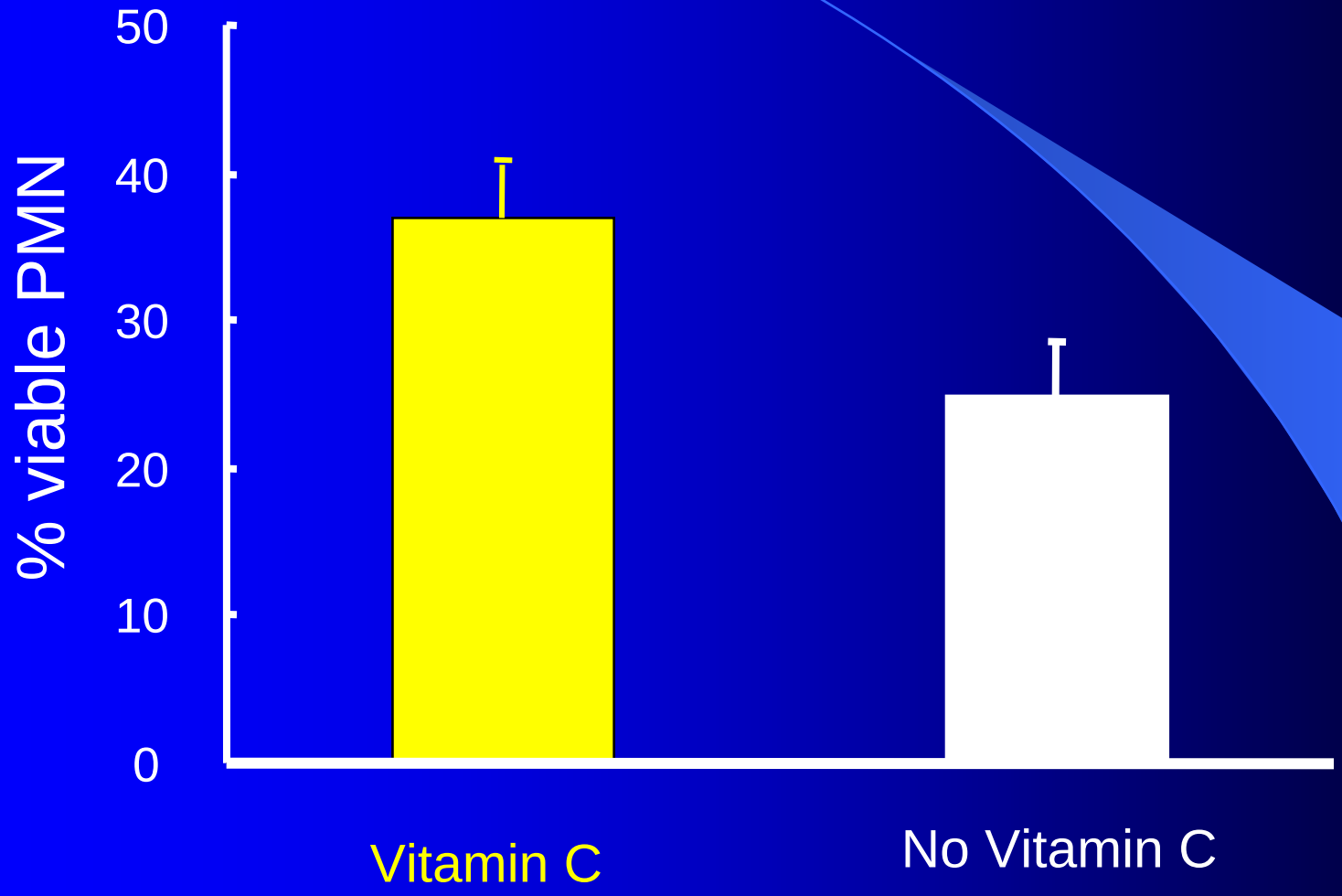
Attainable way to reverse neutrophils' oscillatory events in peripartum dairy cows

Here two issues in peripartum dairy are exempified: good and bad products ……

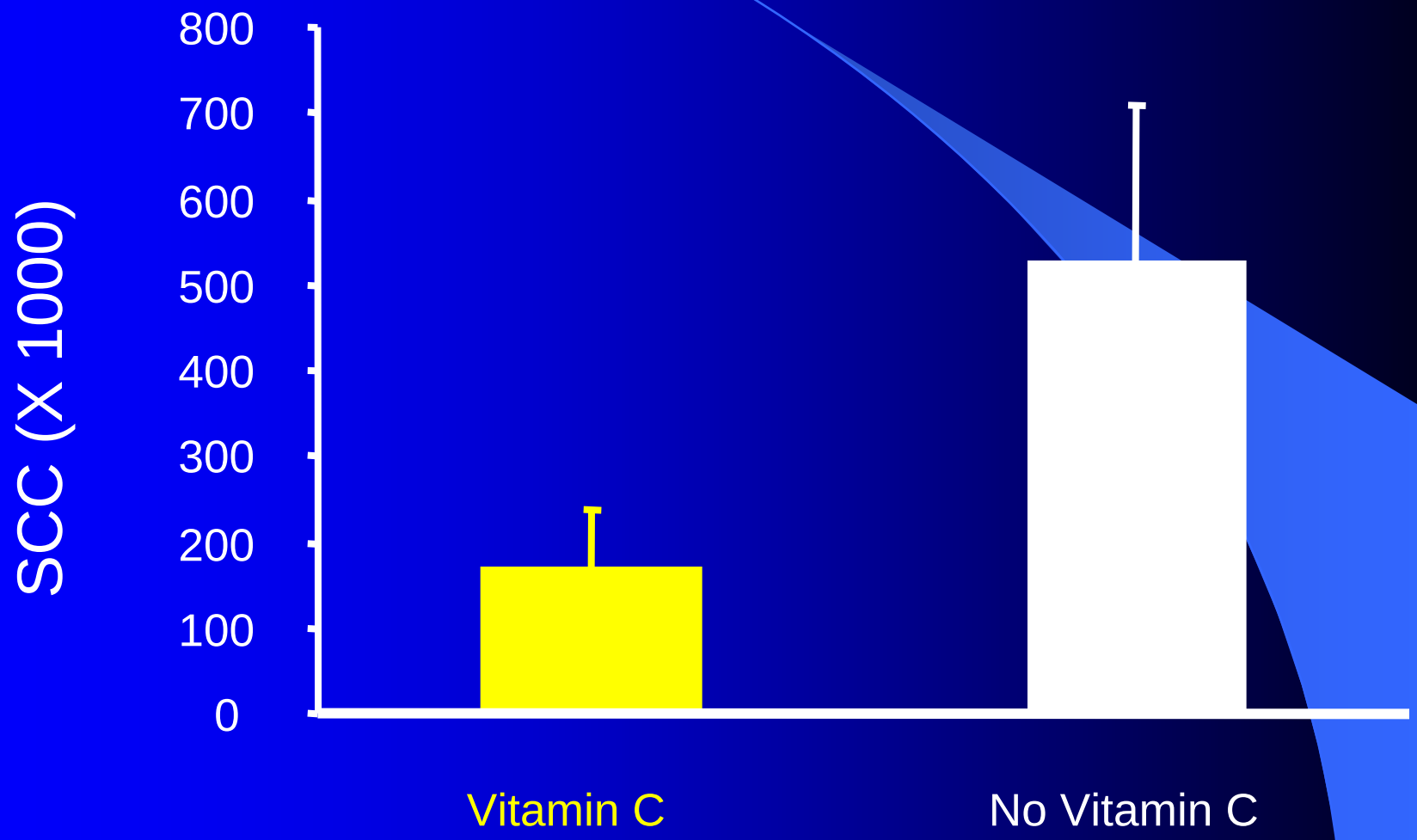
Vitamin C and neutrophil's phagocytosis & killing capacity

About 10 days before parturition, Holstein cows devided in 2 groups they were fed diets that provided 0 (n = 10) or 50 (n = 10) gm/d of supplemental vitamin C…. blood samples were collected for neutrophils' functional assays.

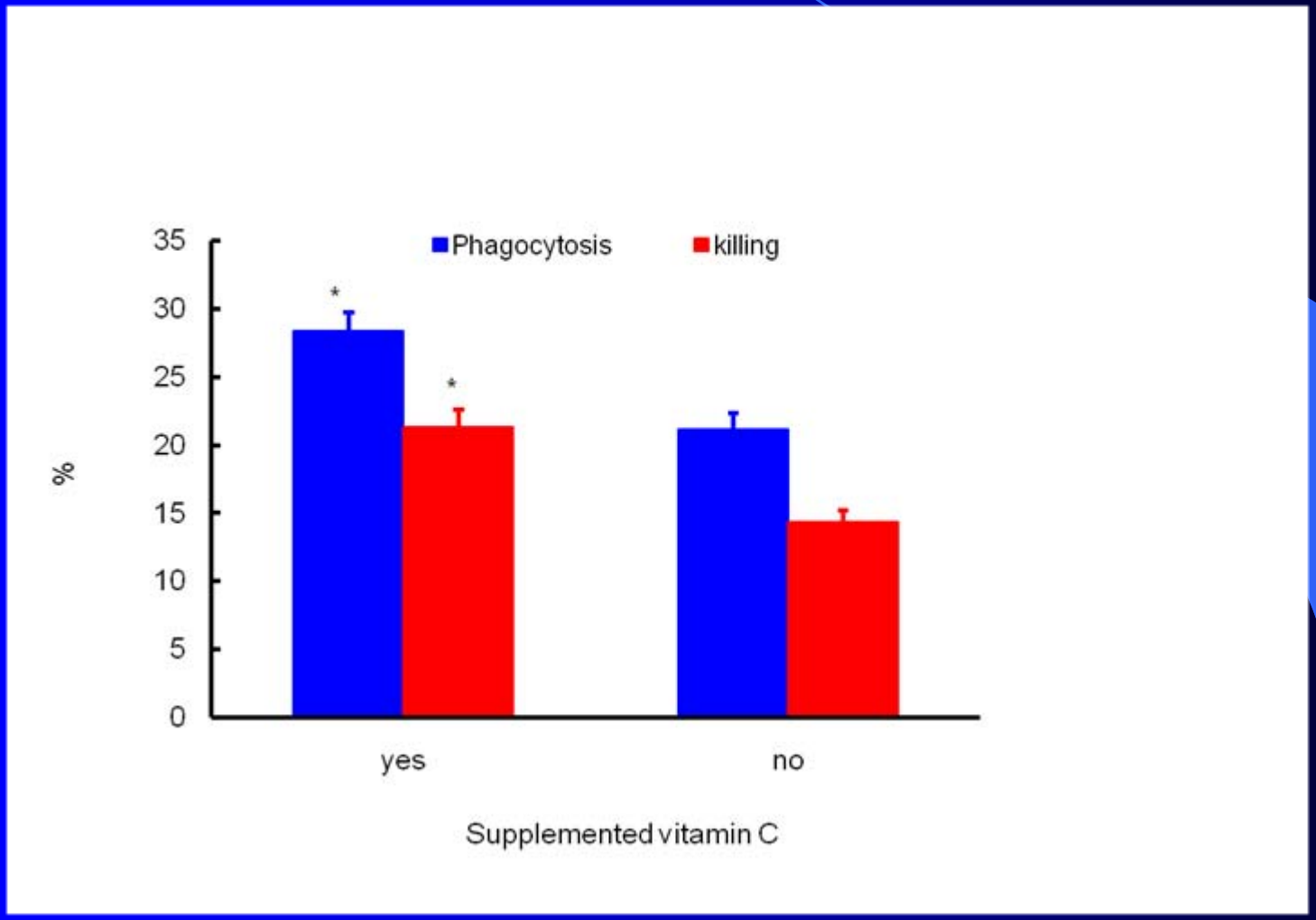
Dietary vitamin improves milk PMN viability in early lactating cows



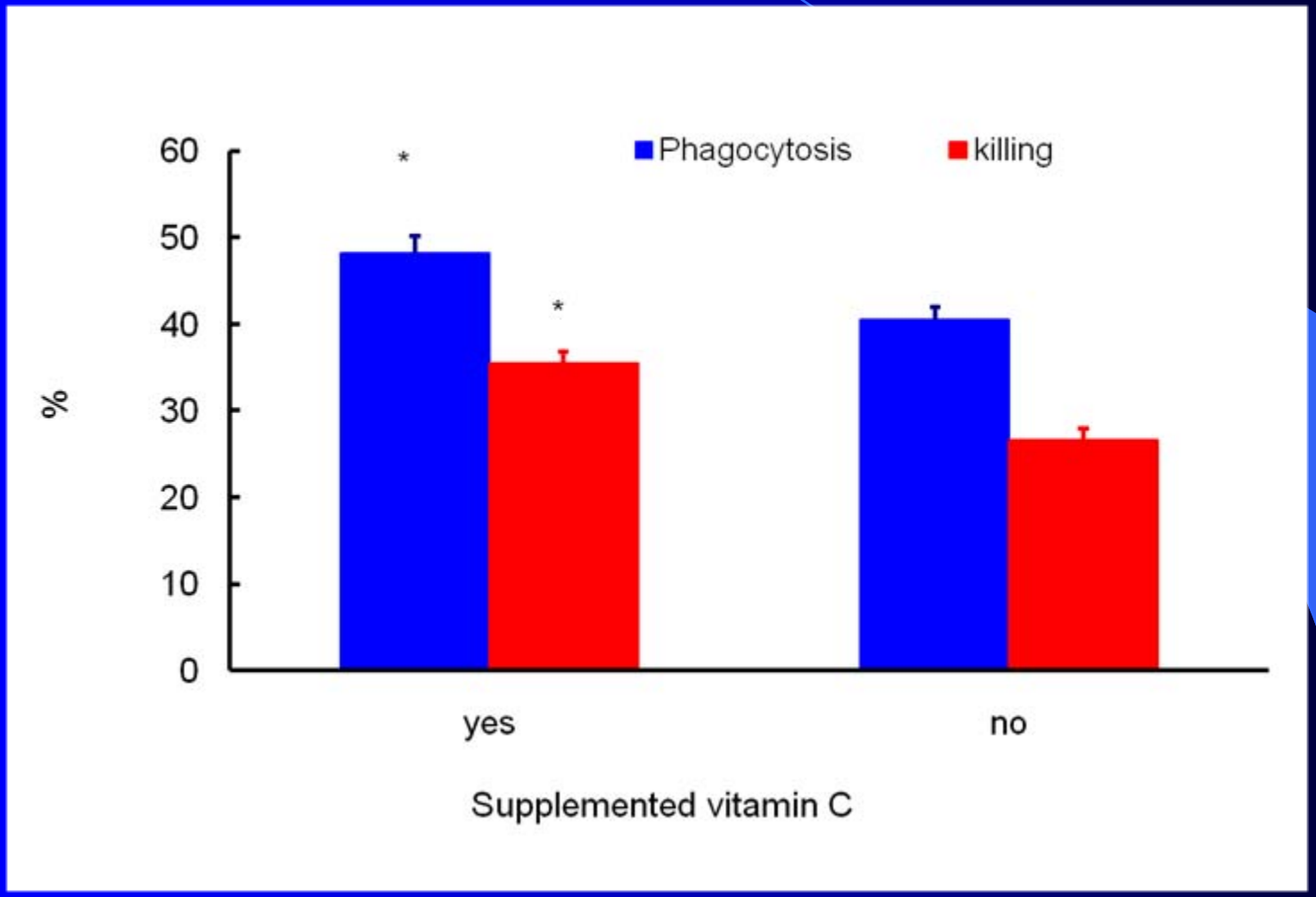
Dietary vitamin C improves milk SCC



Killing & phagocytosis of *S. aureus* by blood PMN vs vitamin C



Killing & phagocytosis of *E. coli* by blood PMN vs vitamin C

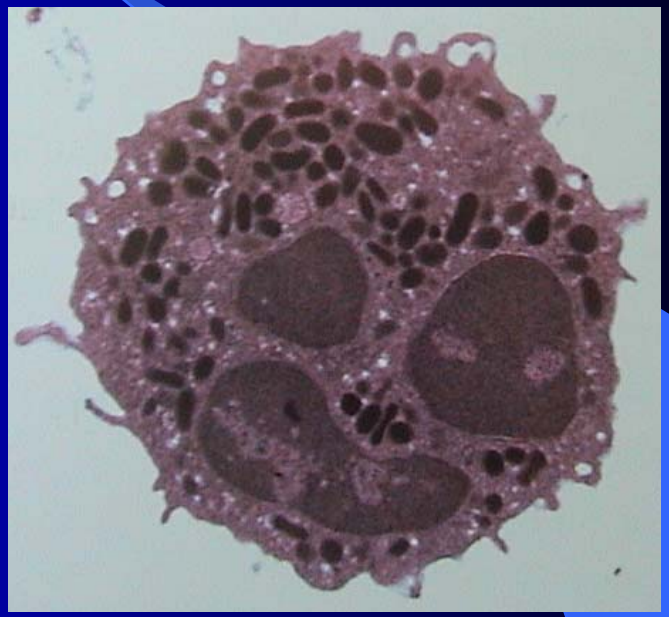


Nneutrophils' oscillation

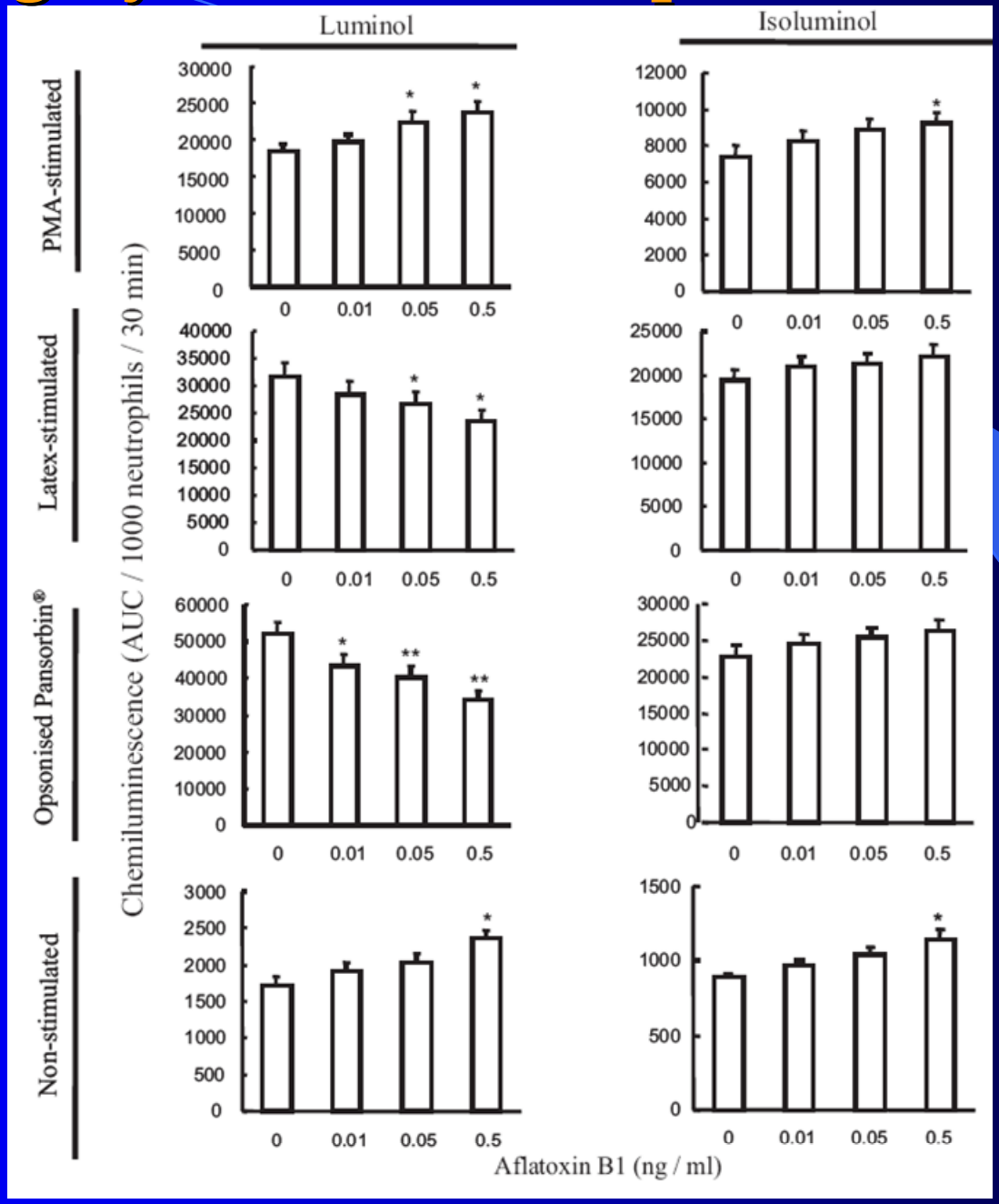
Vitamin C reverse neutrophil disarmament in high yielding dairy cows

Similarly, we have observed on Se, Deferoxamine (DFO), probionts

Aflatoxin B1 & neutrophil's oscillation

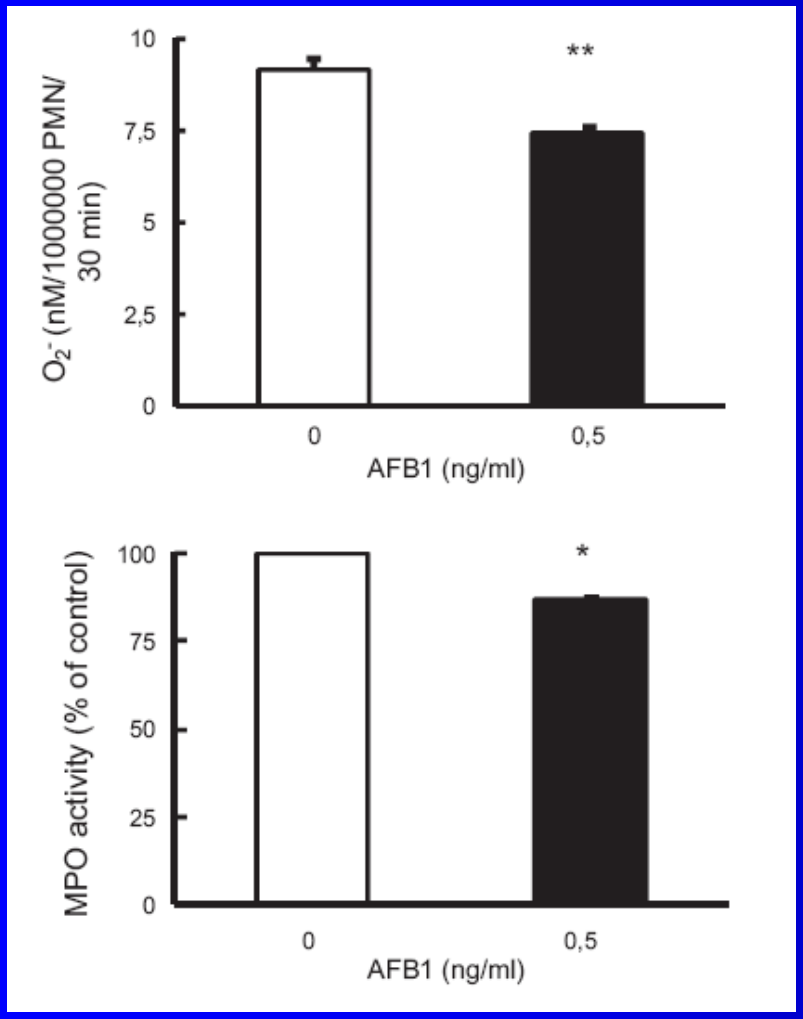


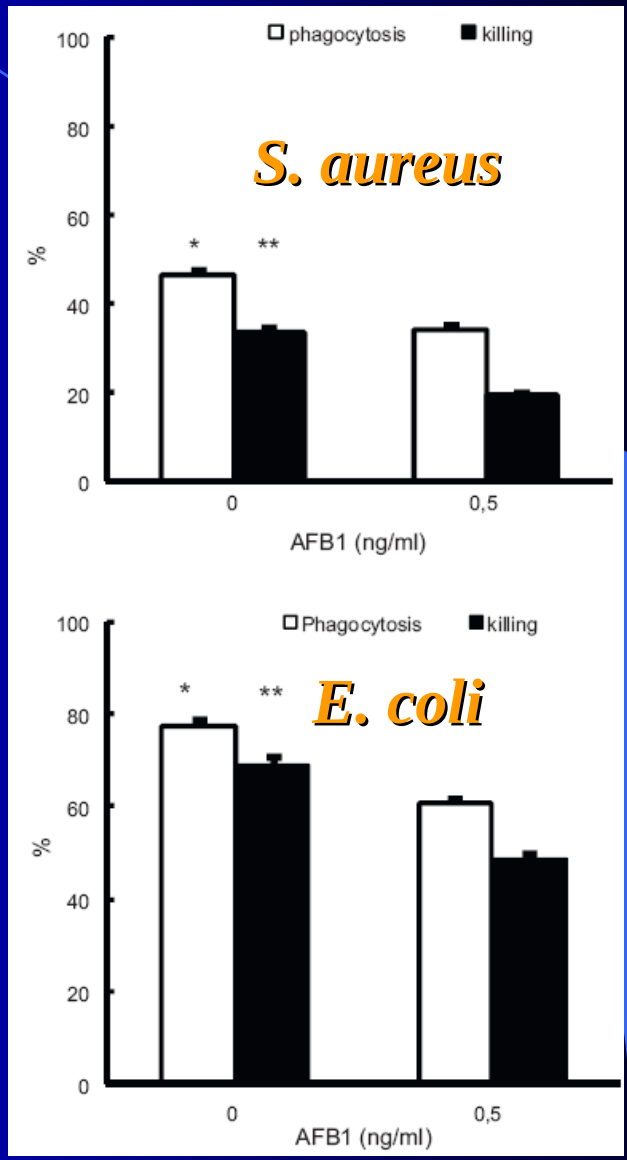
PMN phagocytosis and ROS production vs AFB1



Mehrzad et al.,
Vet Immunolo.
Immunopatholo. 2011

Killing & phagocytosis of *S. aureus* and *E. coli* by neutrophils vs AFB1





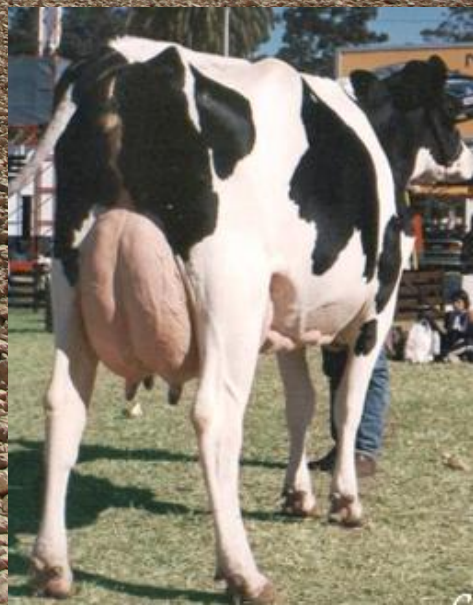
Mehrzad et al., Vet Immunolo. Immunopatholo. 2011

Nneutrophils' oscillation

AFB1 disarms neutrpphils' arsenals in high yielding dairy cows

Similarly, we have observed on As..

We have done much more works eg, AFM1, probiotics (see today's poster of session 09 # 7) ……



Conclusions

Neutrophils' oscillation occurs at 3 levels: 1) the bone marrow, 2) the circulation and 3) the udder

The oscillatory events on neutrophil is related to 2 phenomena: 1) calving factor and, 2) lactogenesis factor

Severe clinical mastitis is typical for the transition period and early lactation of highly yielding cows and is highly related to neutrophils' oscillation

Removing excessive and unwanted ROS, especially $OH^{\cdot}$ from the body of high yielding cows during transition period is very helpful to reverse neutrophils' oscillation, and AFB1, As…. can be very harmful, but vitamin C, Se, probionts, DFO …

Thanks for your attention

Location: Alborz mountain ranges, Southern part of Caspian Sea, Iran
>3500 m above sea level