

# **GNOTOBIOTIC PIGLET MODEL OF PRETERM INFANTS**

**I. Splichal, A. Splichalova and V. Slavikova**

**Institute of Microbiology of the CAS**

**Novy Hradek, Czech Republic**

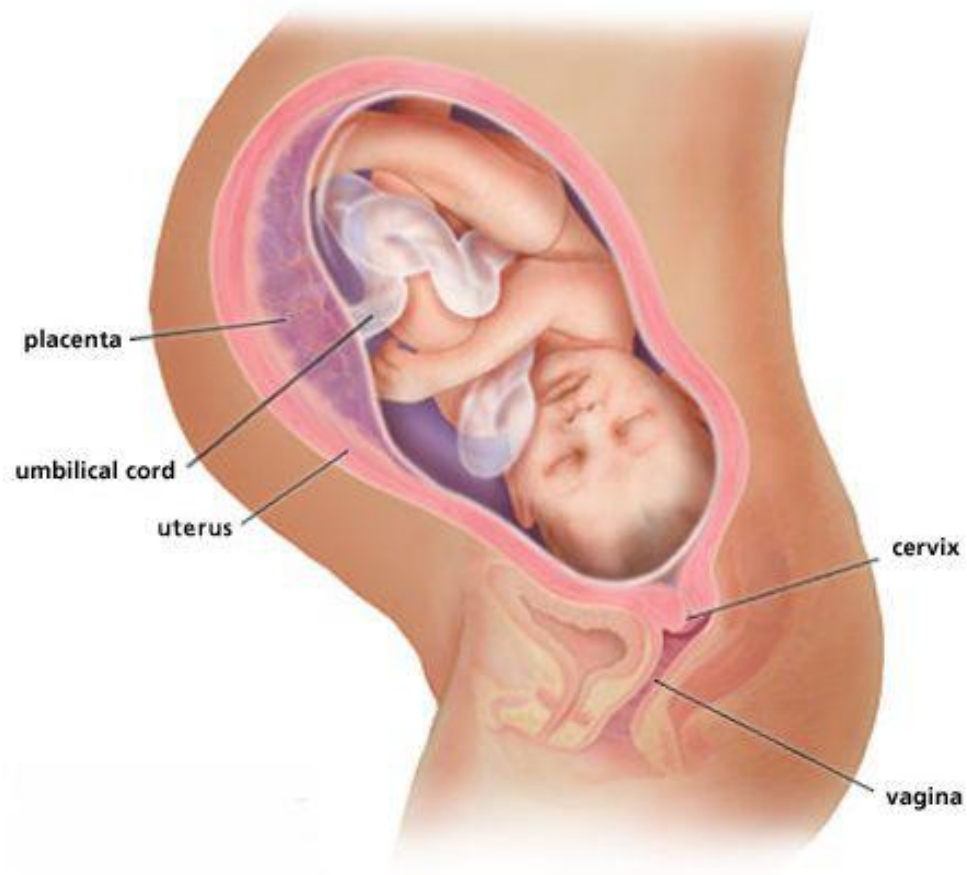
**Cork, Ireland**

**May 4-5, 2017**

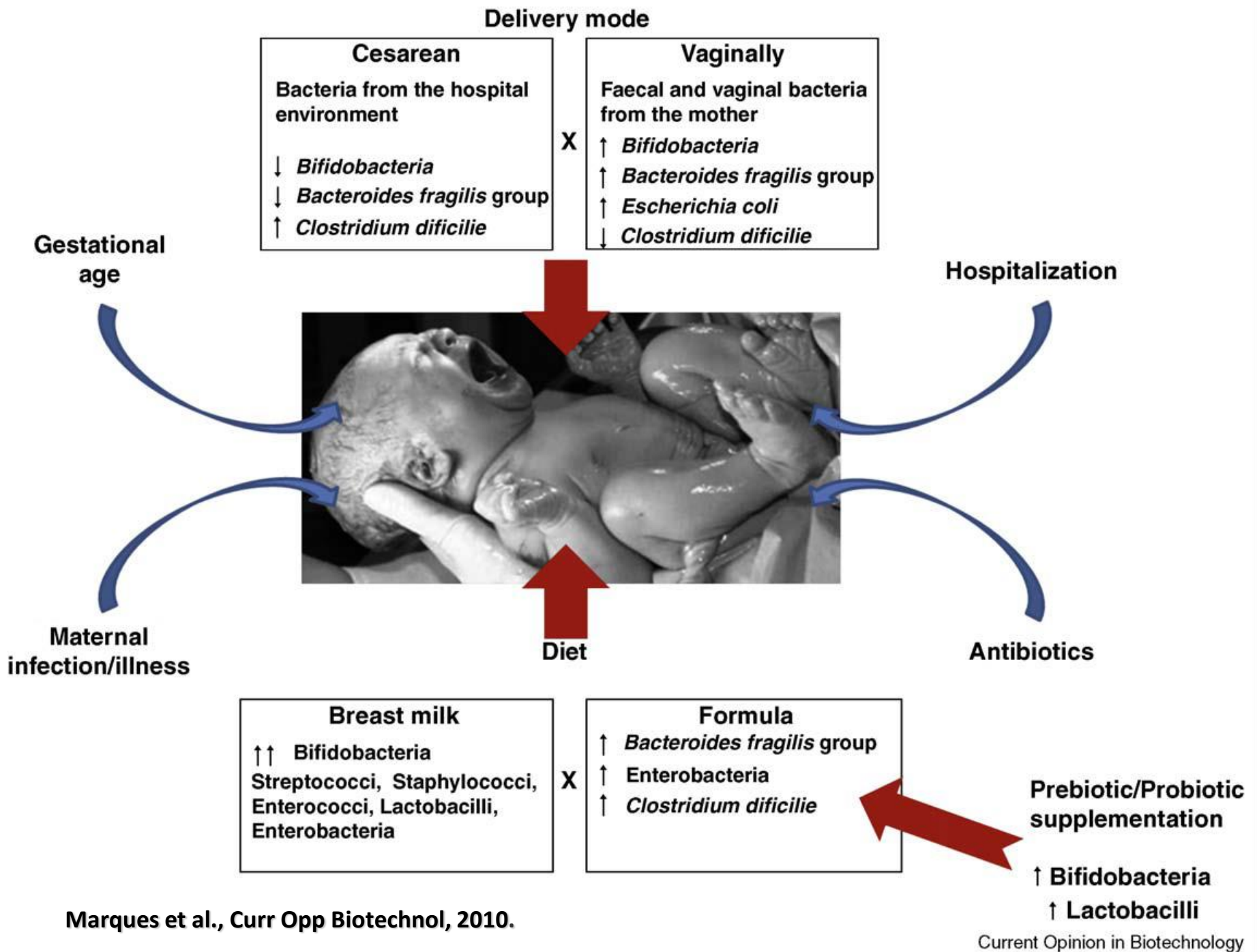


# Embryos and fetuses are developed in sterile conditions

---



Similar situation is in birds, plants, insects etc.



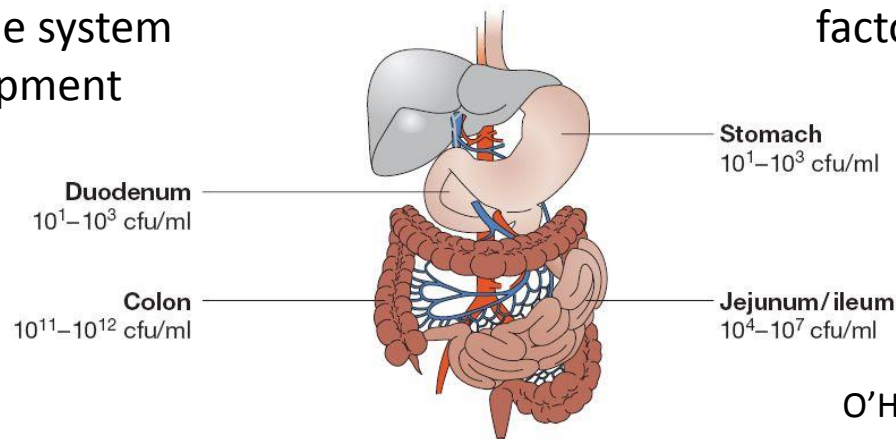
# BENEFITS OF MICROBIOTA FOR THEIR HOST

## Structural functions

Barrier fortification  
Induction of IgA  
Apical tightening of tight junctions  
Immune system development

## Protective functions:

Pathogen displacement  
Nutrient competition  
Receptor competition  
Production of anti-microbial factors e.g., bacteriocins, lactic acids



O'Hara and Shanahan, 2006

## Metabolic functions:

Control intra epithelial cell differentiation and proliferation  
Metabolize dietary carcinogens  
Synthesize vitamins e.g., biotin, folate

Ferment non-digestible dietary residue and endogenous epithelial-derived mucus  
Ion absorption  
Salvage of energy

---

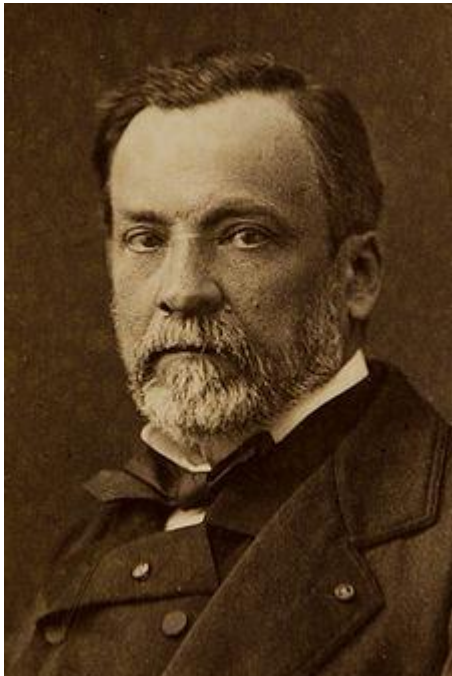
# GNOTOBIOLOGY



# Is life without microbes possible?

---

**Louis Pasteur**

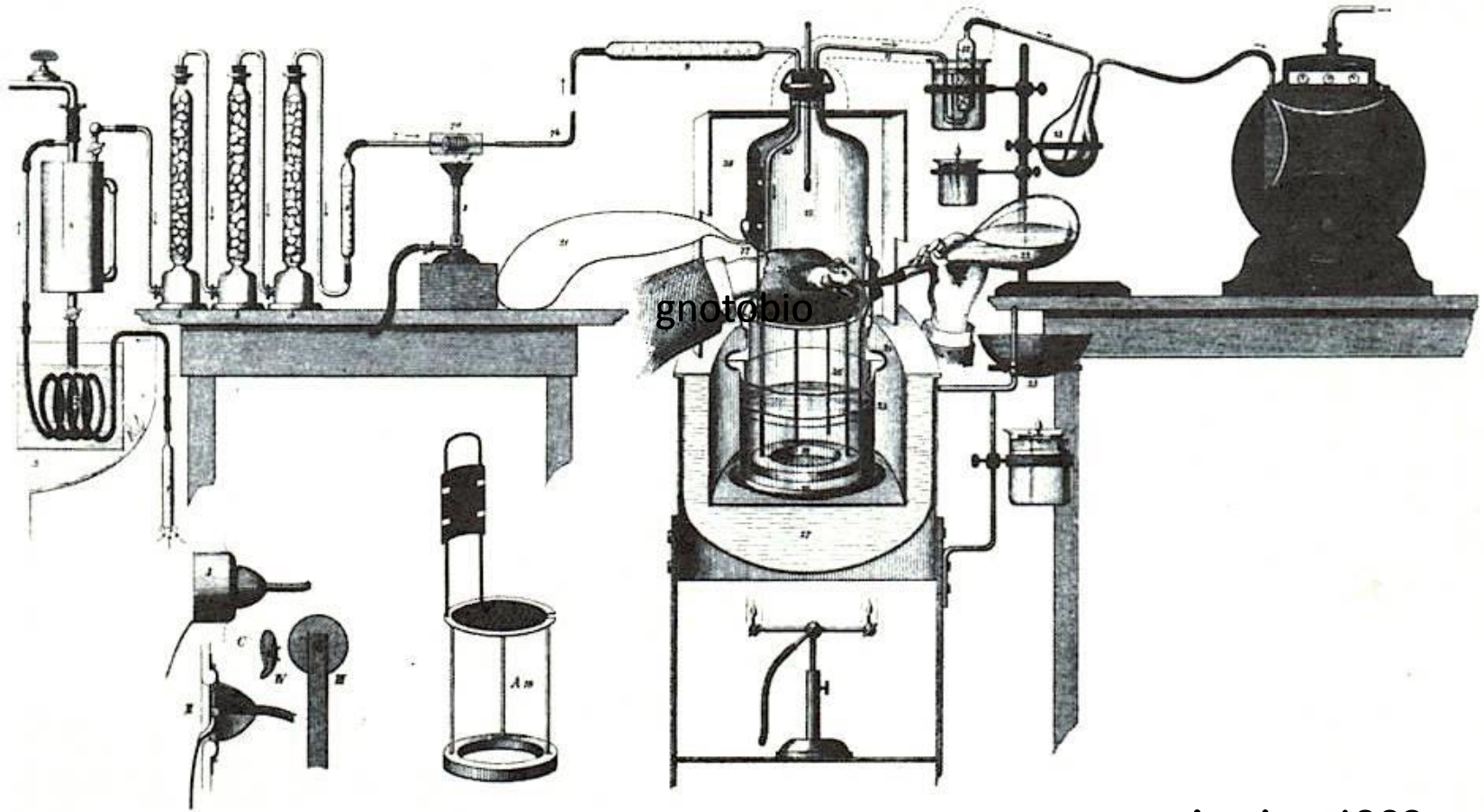


**Theoretical presumptions for an experiment  
in germ-free conditions**

**1822 - 1895**

# Nuttall and Thierfelder showed that life without microbes is possible

(1896-1897)



Luckey 1963



# GNOTO BIOLOGY

---

- ❖ First experiment – end of the 19<sup>th</sup> century (Nuttal and Thierfelder)
- ❖ 1930s and 1940s – attempts to develop a sterile workplace for routine maintaining of animals
- ❖ A rigid chamber with rubber gloves - Reyniers and Trexler (1943)
- ❖ A discovery of sporicidal activity of a vapour of peracetic acid (1950)
- ❖ A system of cylindrical pressure vessel sterilized by steam - Myiakawa (1954) and Reyniers (1959)
- ❖ A lightweight stainless steel isolator sterilized in large autoclave – Gustafsson (1948, 1954)
- ❖ A transparent flexible PVC film isolator – Trexler and Reynolds (1957)



# GNOTOBIOLGY IN THE CZECH REP

---

Based in 1953 as a branch of  
the Biological institute in Prague

Gnotobiotic pigs since 1957



Zřícenina hradu Frimburku u Nového Hrádku



**Jiří Trávníček a Leoš Mandel**



**Rostislav Růžička**



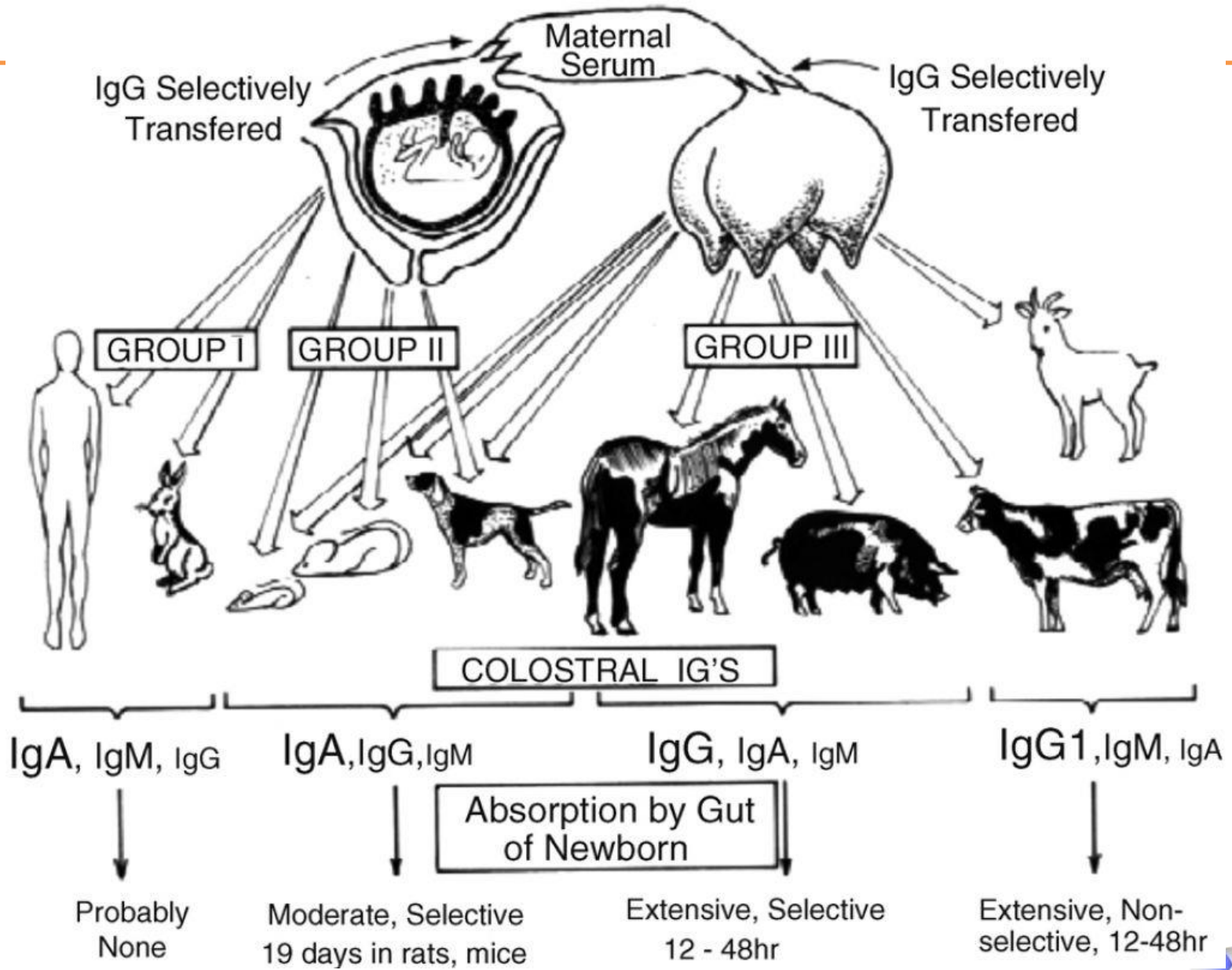
**Practical application of gnotobiotic technology in medicine.**

---

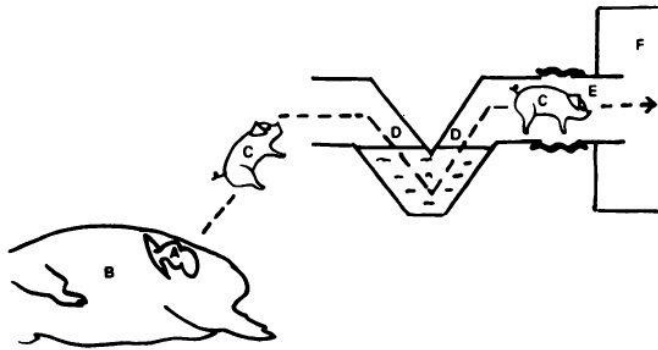
# **GNOTOBIOTIC PIGLET**



# PRENATAL TRANSFER OF IMMUNOGLOBULINS

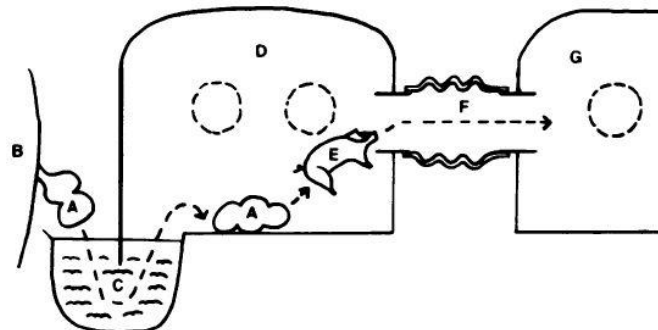
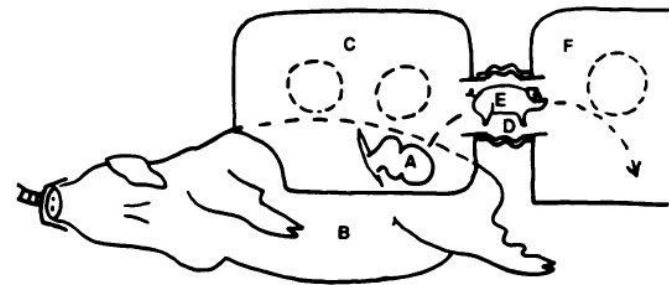


# GERM-FREE PIGLETS



CLOSED HYSTEROTOMY

OPEN HYSTEROTOMY



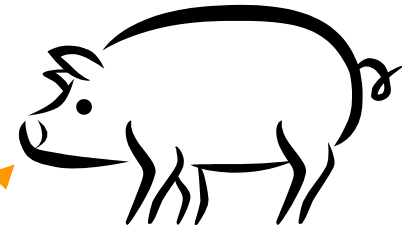
HYSTERECTOMY

Miniats a Jol, 1979

# GNOTOBIOTIC ANIMALS

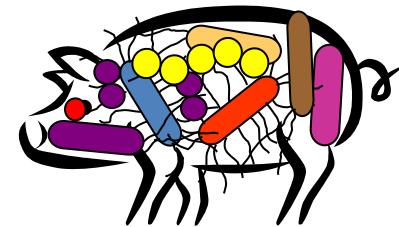
**GF** (germ free)

– without microbes, axenic (sterile host)



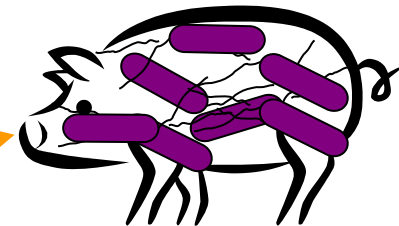
**CV** (conventional)

- a host colonized by normal microbiota after birth



**Associated ...** (mono-, di-, .. poly)

– experimentally colonized host with defined microbes



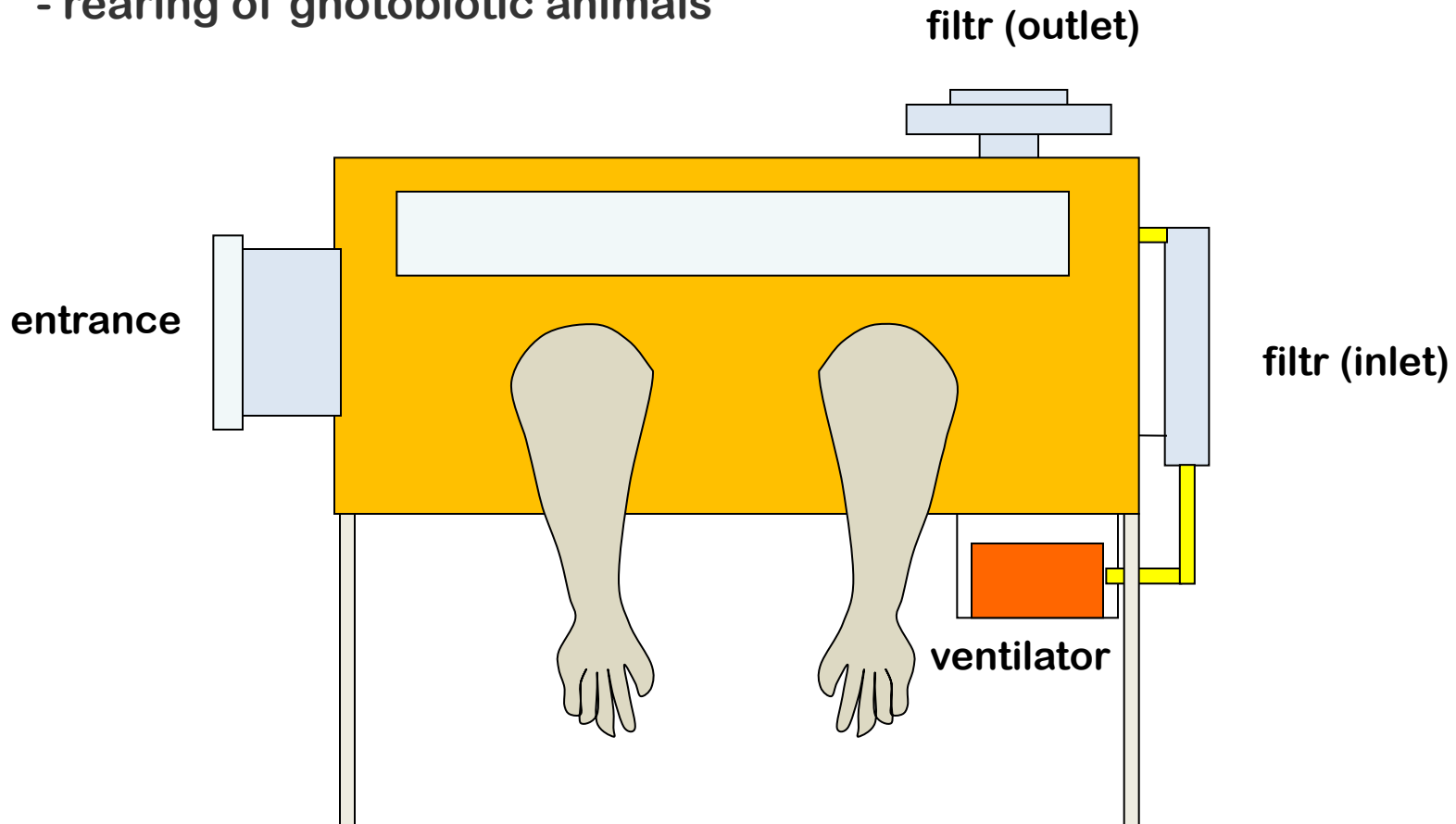
**Gnotobiont**

– mikrobiologisch definiertes Tier  
(steril oder assoziiert)

Examples of gnotobiotic organisms – mammals, birds, insects, plants, etc.

# ISOLATOR

- separation of animals from outside
- rearing of gnotobiotic animals



Fibreglass isolator for rearing  
of gnotobiotic piglets

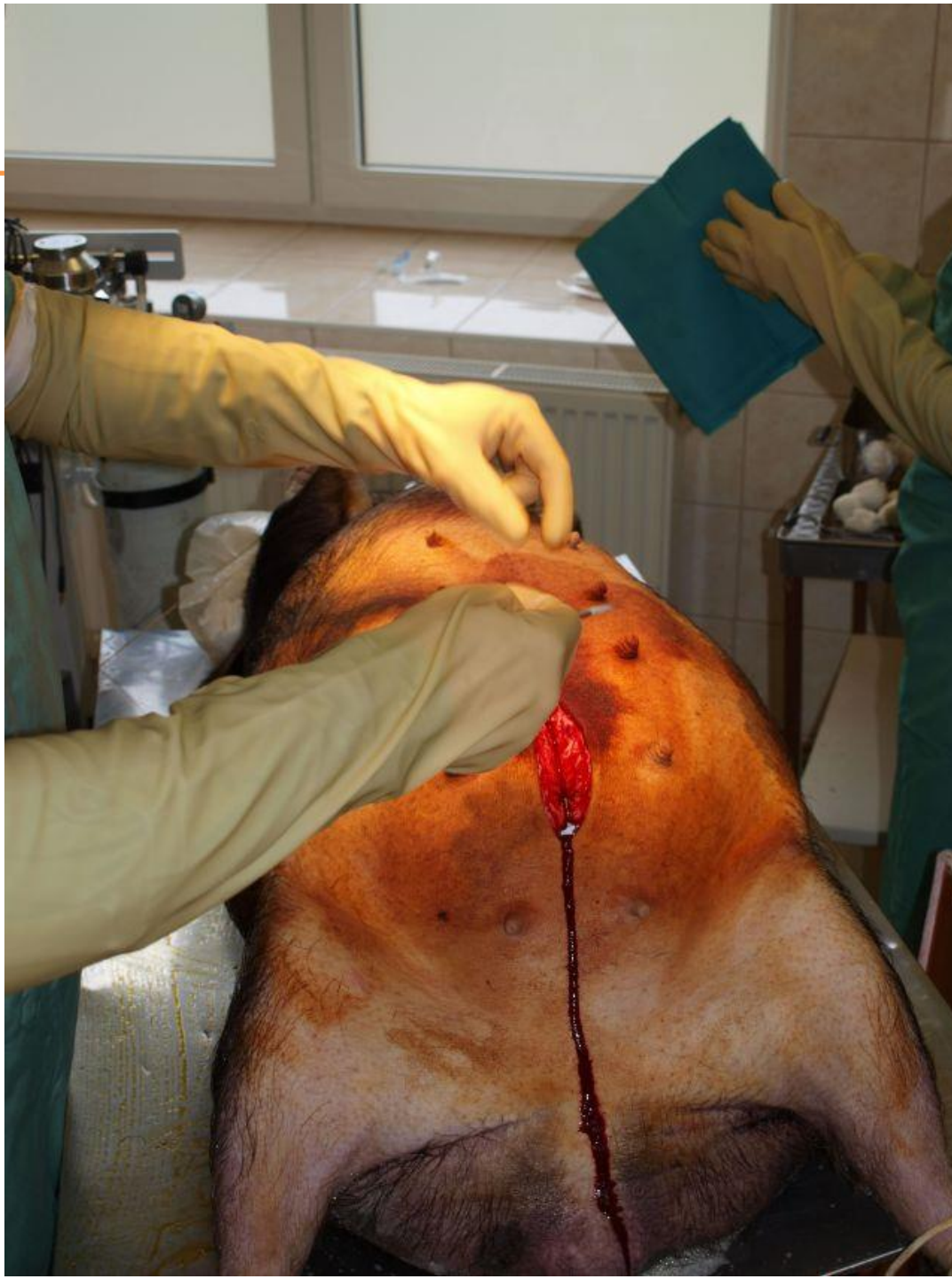
---

# HYSTERECTOMY

All experiments with animals were approved by the Committee for Animal Protection and Use of the Institute of Microbiology.

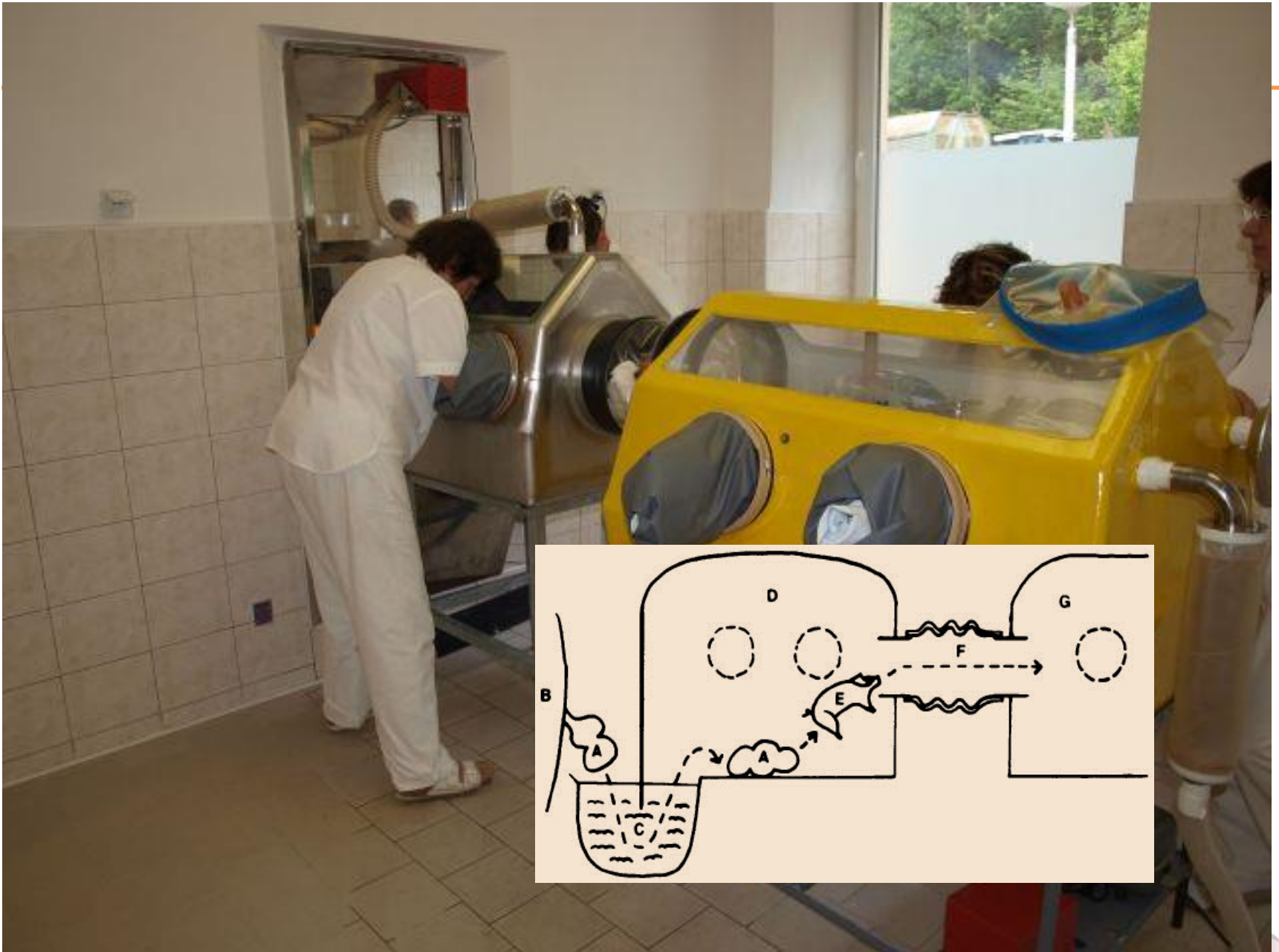












# MATERIAL AND METHODS

One-week-old hysterectomy-derived GF piglets were infected/colonized with  $10^8$  bacterial CFU

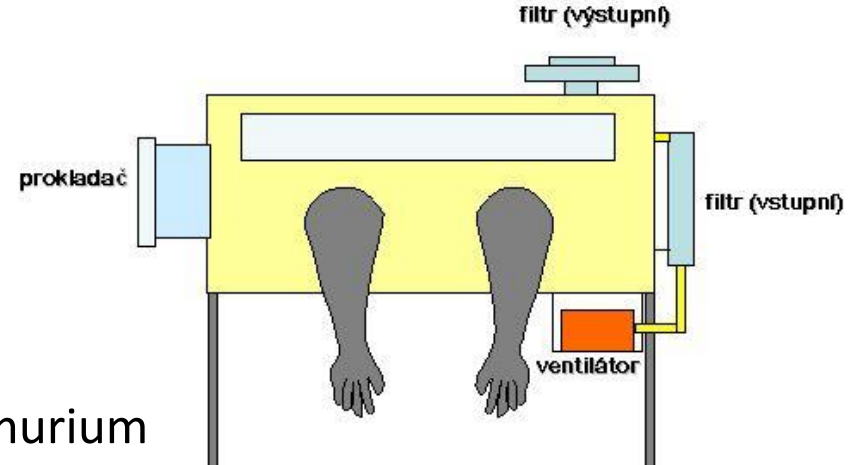
**GF** – control group

**LT2** – *Salmonella enterica* serovar Typhimurium

**rfaG** – *Salmonella enterica* isogenic mutant

**EcN** – probiotic *E.coli* Nissle 1917

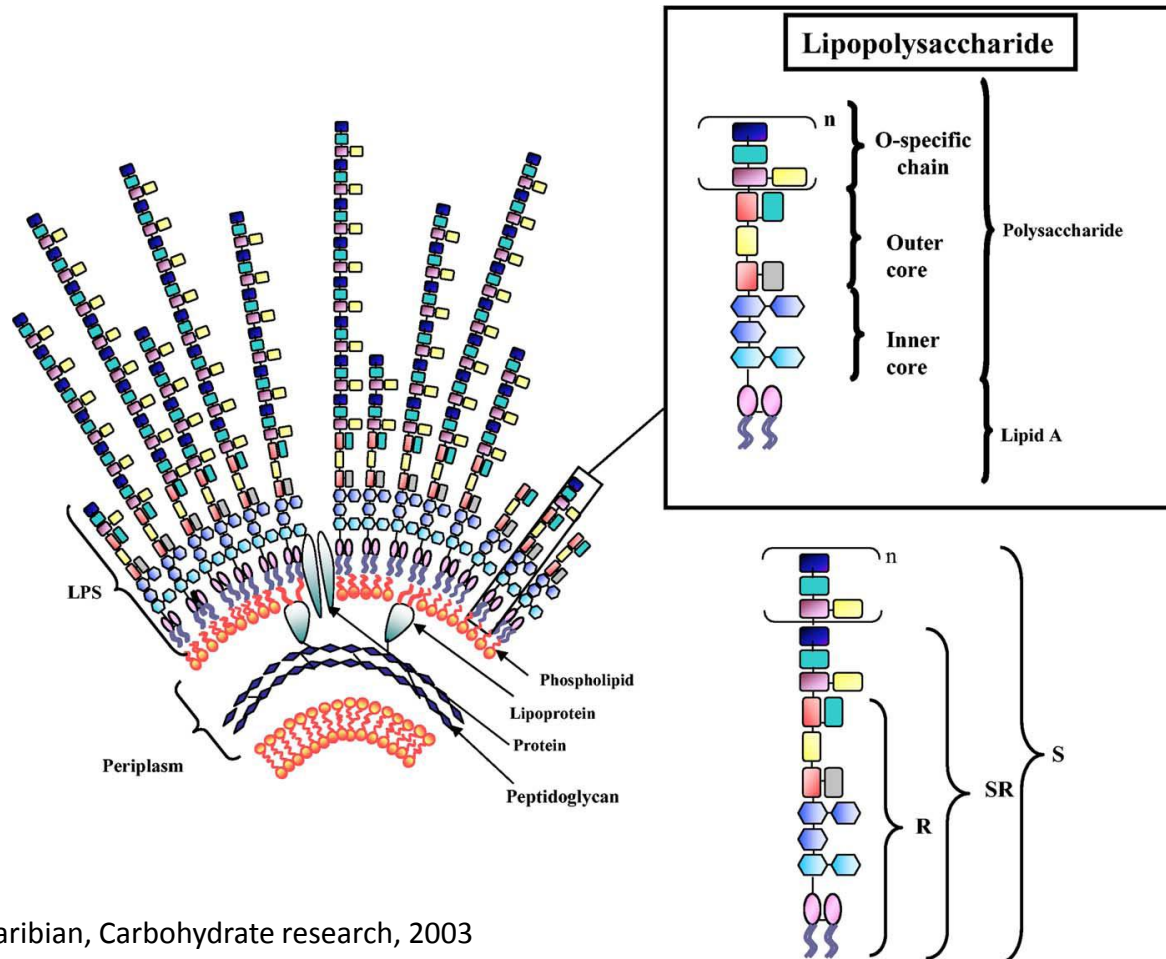
**EcN+ST** – piglets colonized with EcN and later infected with ST



Mandel a Travnicek, Nahrung, 1986

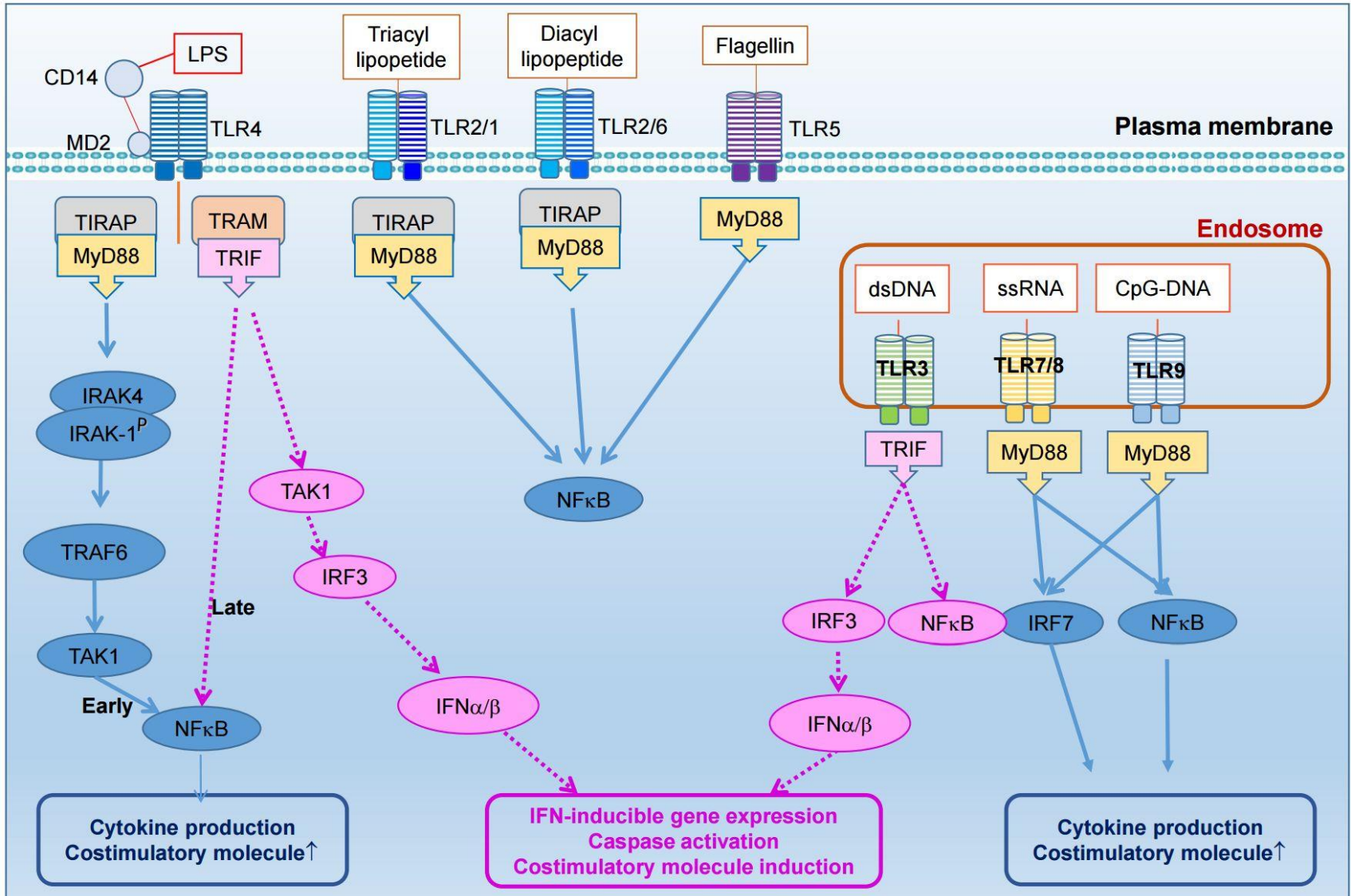
# LIPOPOLYSACCHARIDE

Virulence factor of G- bacteria



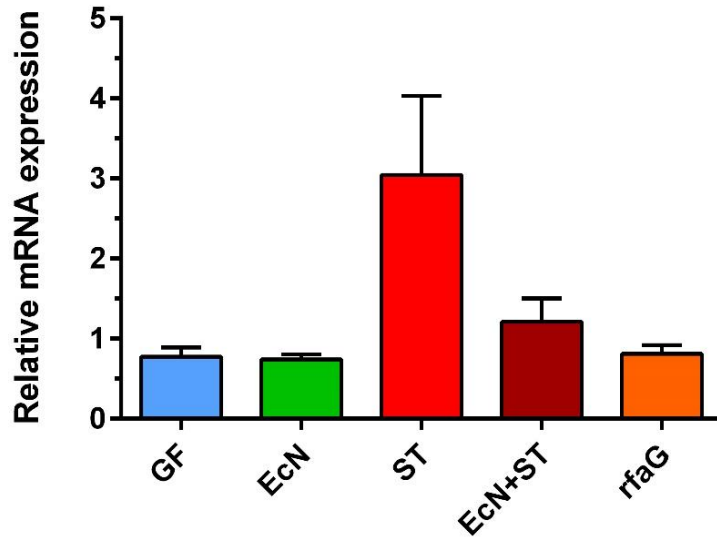
Caroff and Karibian, Carbohydrate research, 2003

# TOLL-LIKE RECEPTORS

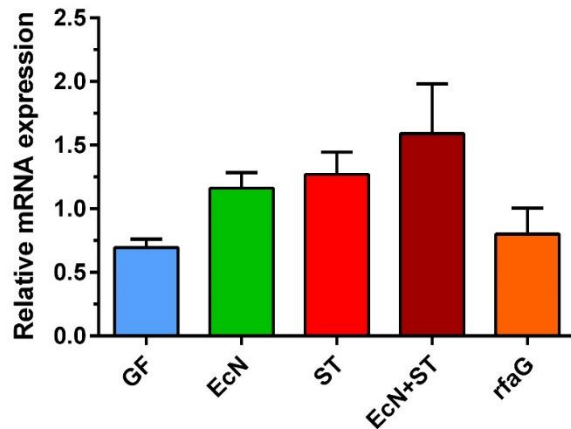


# TLR4 SIGNALING

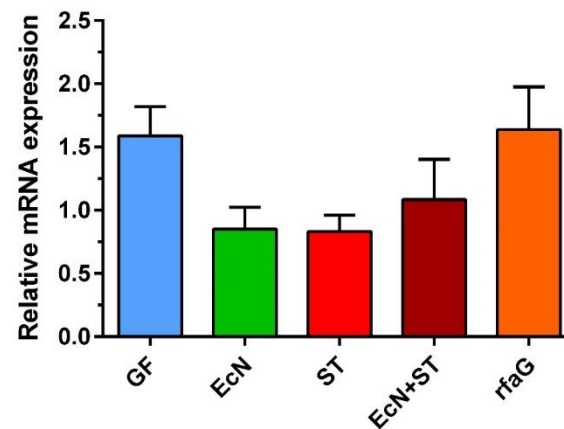
## TLR4



## MyD88



## TRIF





# CONCLUSIONS

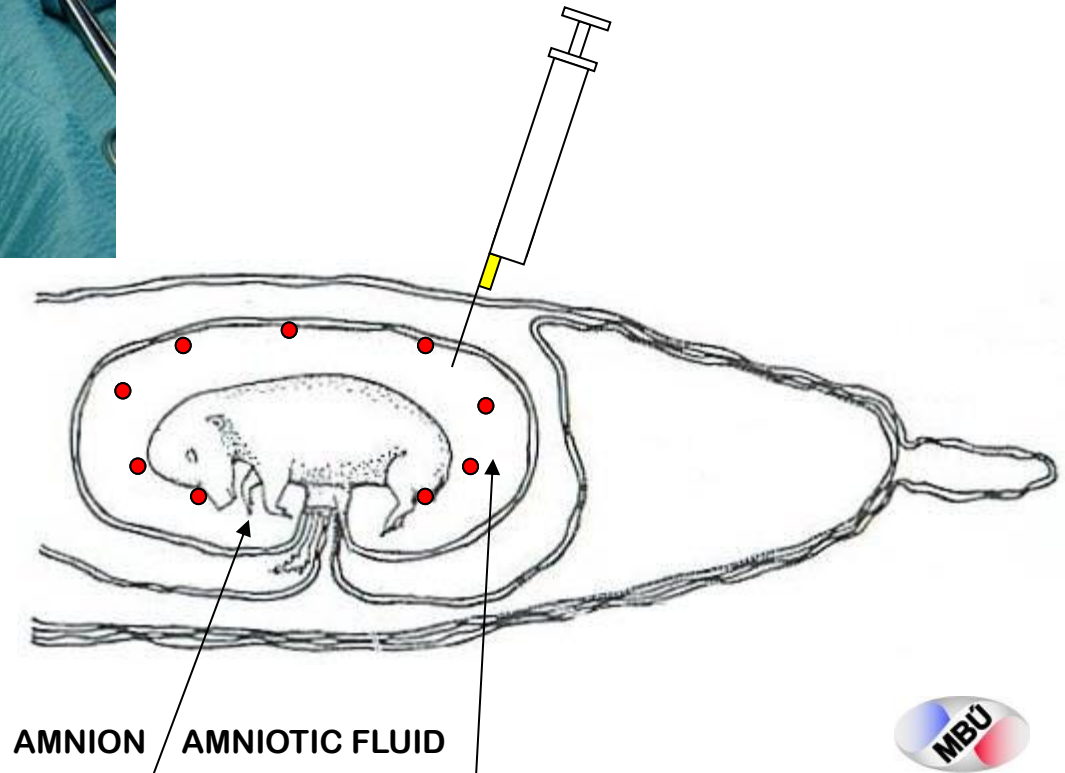
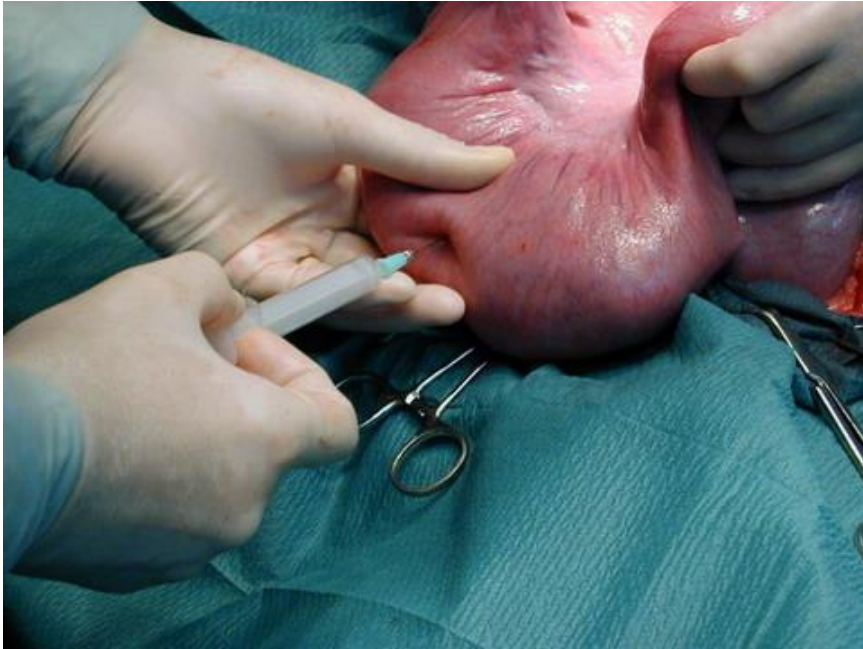
---

One of possible unique probiotic properties of *E. coli* Nissle 1917 consists in its semirough type of LPS. The gnotobiotic piglet is the suitable model to study action of probiotics and their possible candidates in a low birth weight preterm infants to evaluate their opportunistic pathogenicity. The gnotobiotic piglets has been highly required animal model of many international collaborative studies.

This work was supported by grants COST LD15090 of the Ministry of Education, Youth and Sports, 13-08803S of the Czech Science Foundation and the Institutional Research Concept RVO: 61388971 of the Institute of Microbiology.



# INTRAAAMNIOTIC INFECTIONS



# INSTITUTE OF MICROBIOLOGY

## NOVY HRADEK, CZECH REPUBLIC

---



# THANK YOU FOR YOUR ATTENTION

This work was supported by grants COST LD15090 of the Ministry of Education, Youth and Sports, 13-08803S of the Czech Science Foundation and the Institutional Research Concept RVO: 61388971 of the Institute of Microbiology.

