

GNOTOBIOTIC PIGLET MODEL OF PRETERM INFANTS

I. Splichal, A. Splichalova and V. Slavikova

Institute of Microbiology of the CAS

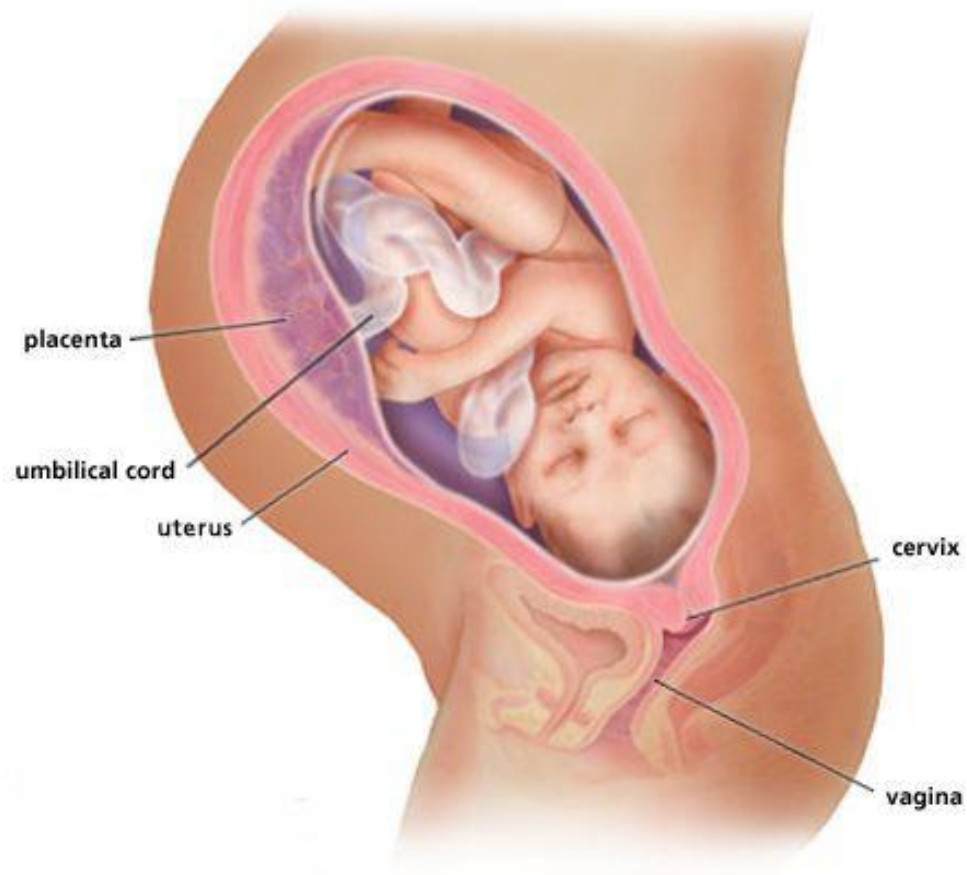
Novy Hradek, Czech Republic

Cork, Ireland

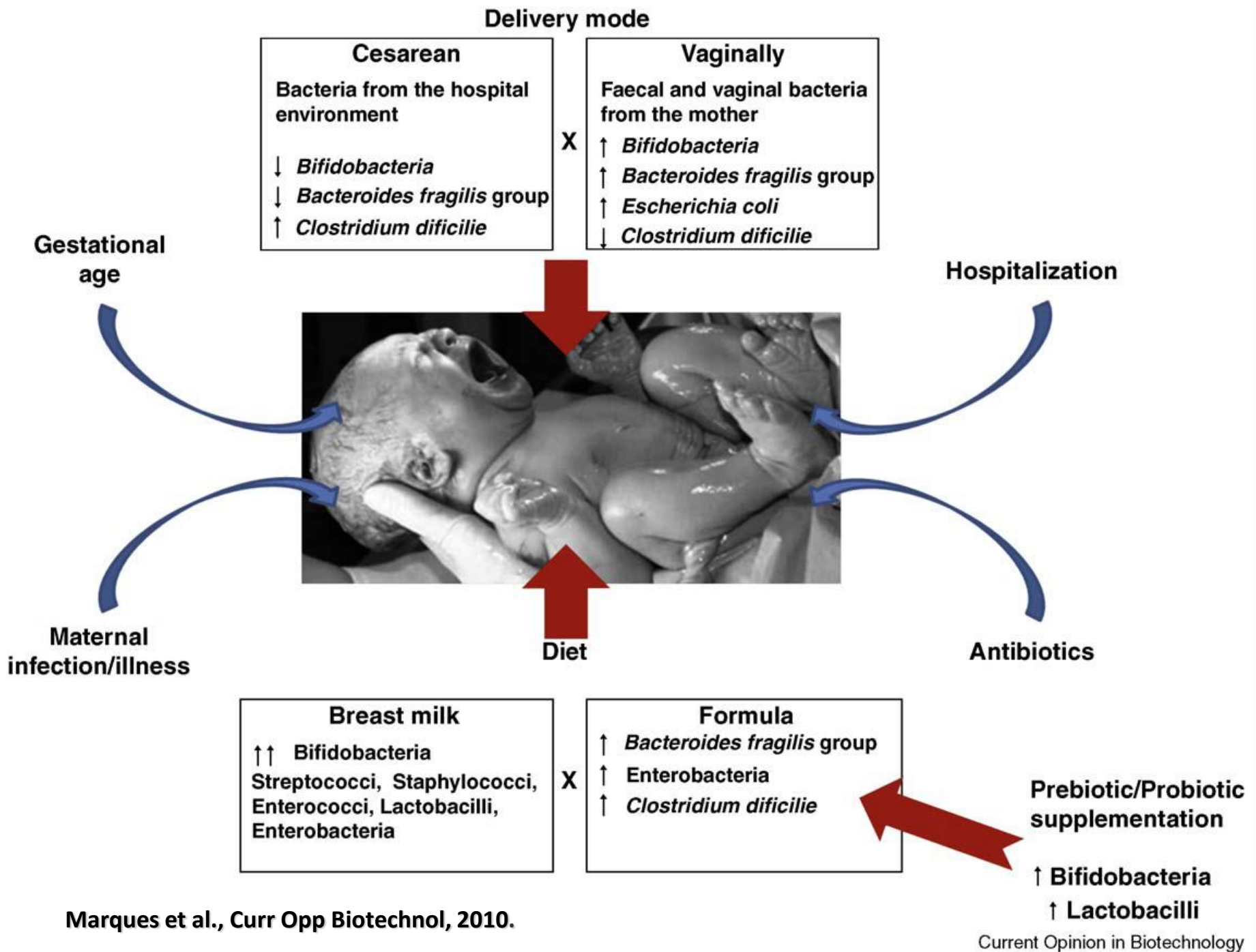
May 4-5, 2017



Embryos and fetuses are developed in sterile conditions



Similar situation is in birds, plants, insects etc.



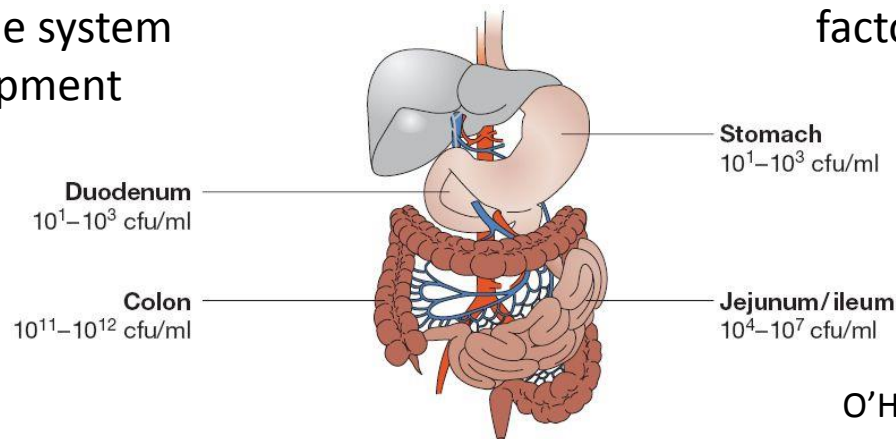
BENEFITS OF MICROBIOTA FOR THEIR HOST

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

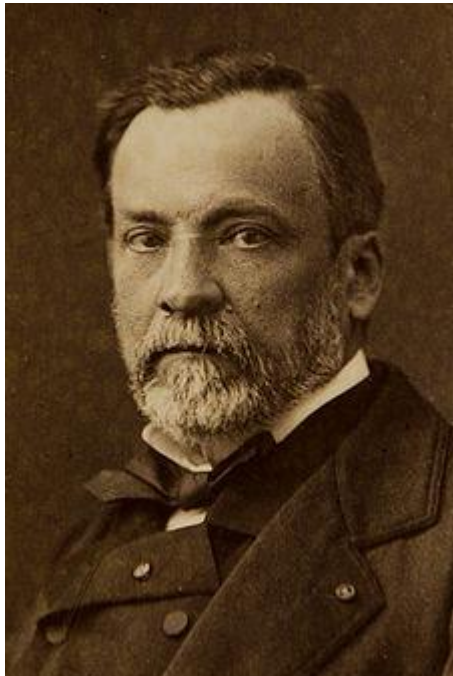
Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
Ion absorption
Salvage of energy

GNOTOBIOLOGY



Is life without microbes possible?

Louis Pasteur

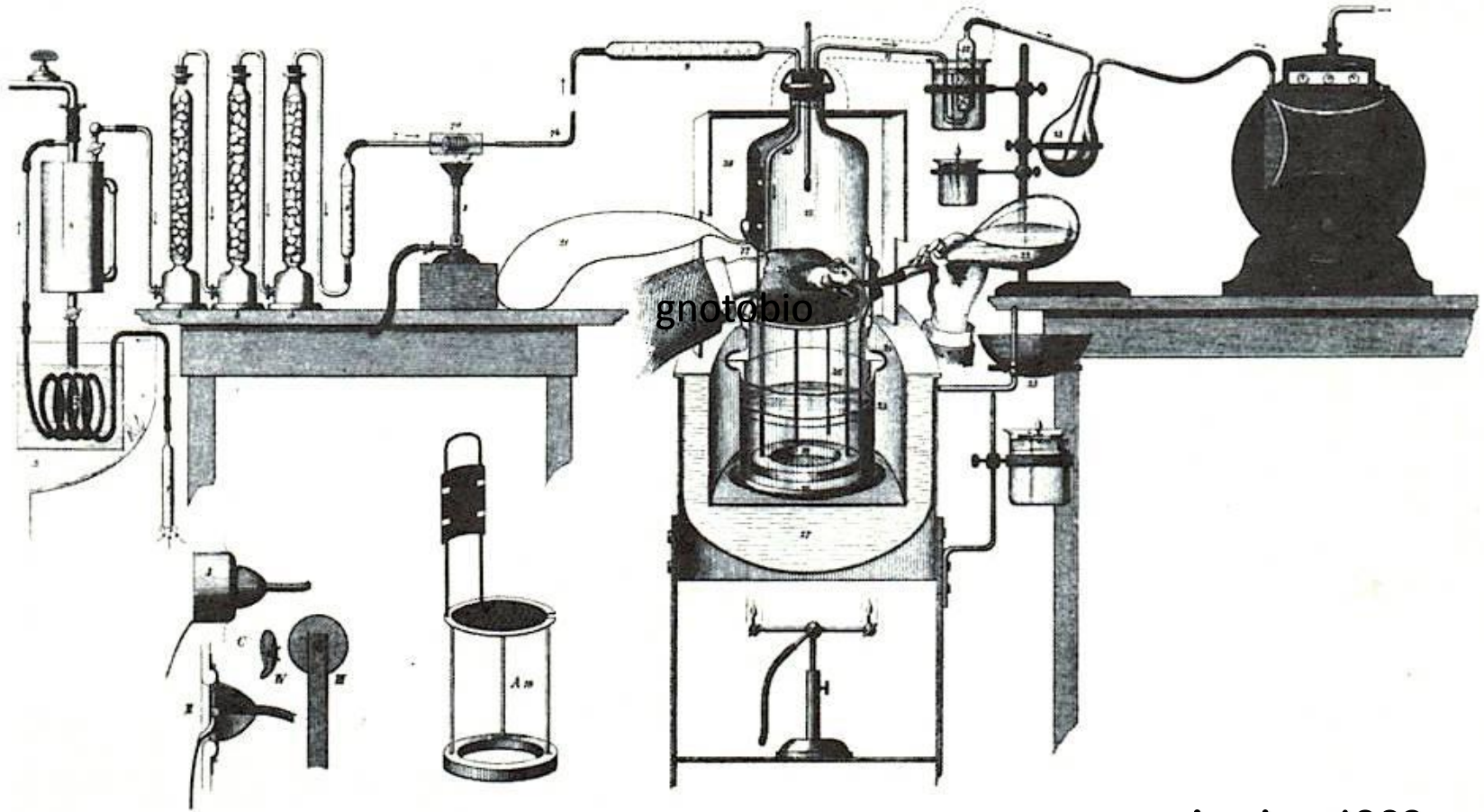


**Theoretical presumptions for an experiment
in germ-free conditions**

1822 - 1895

Nuttall and Thierfelder showed that life without microbes is possible

(1896-1897)



gnotobio

Luckey 1963



GNOTO BIOLOGY

- ❖ First experiment – end of the 19th 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





Jiří Trávníček a Leoš Mandel



Rostislav Růžička

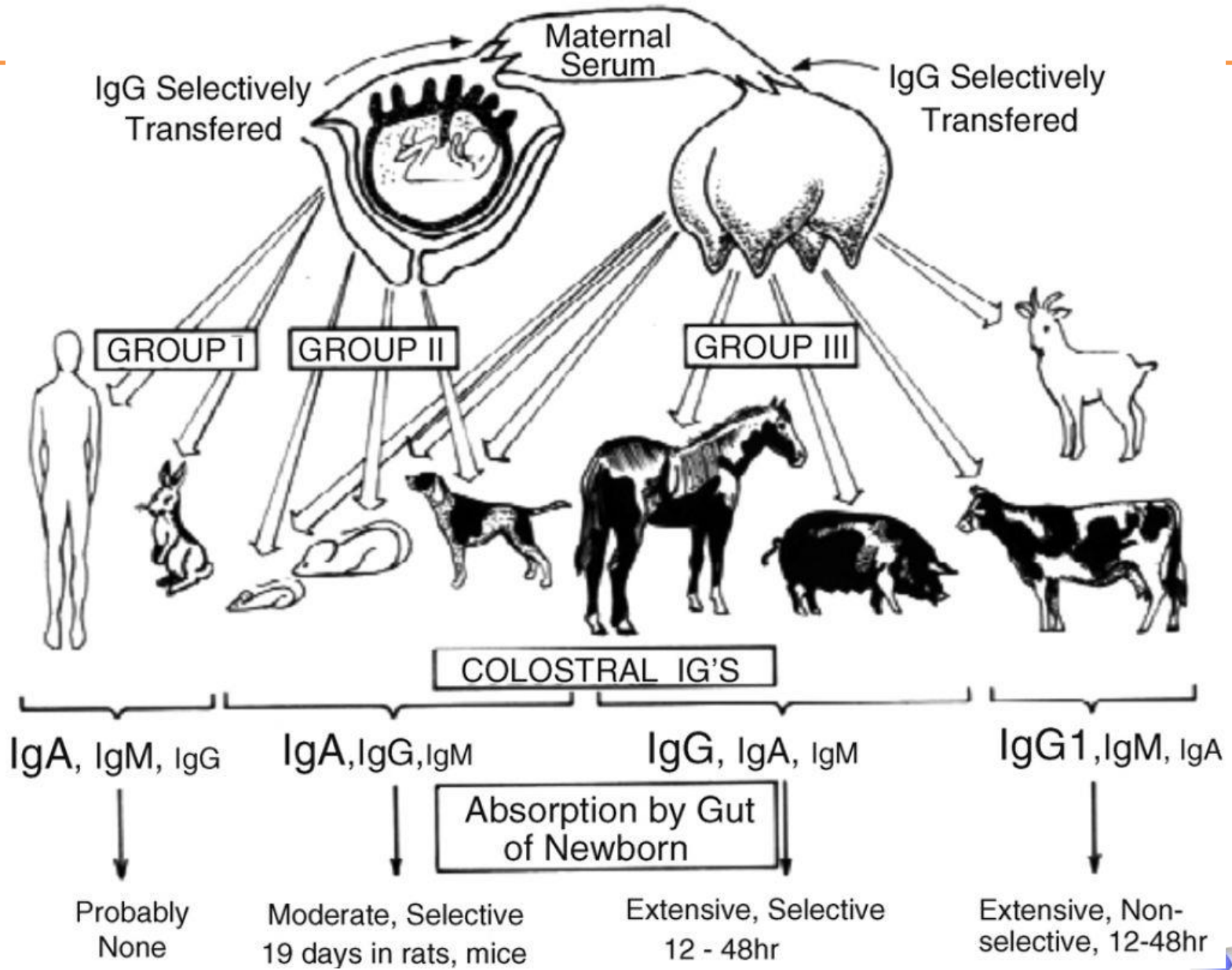


**Practical application of gnotobiotic
technology in medicine.**

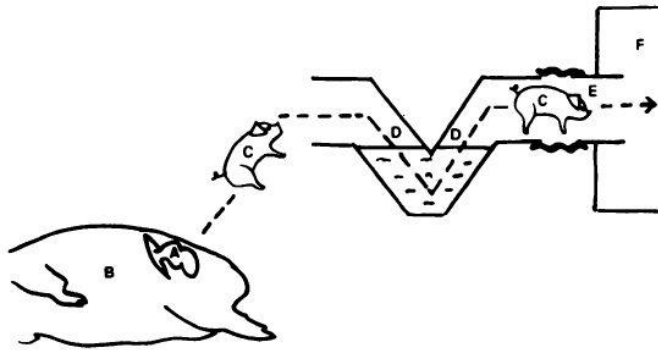
GNOTOBIOTIC PIGLET



PRENATAL TRANSFER OF IMMUNOGLOBULINS

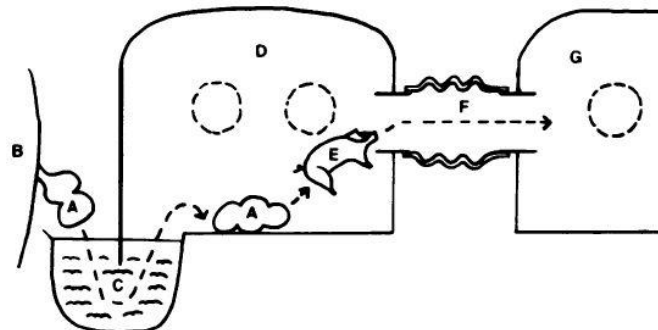
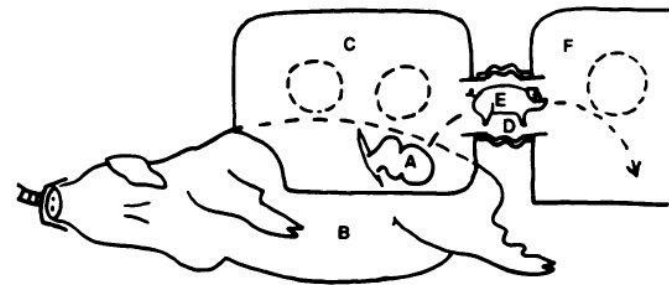


GERM-FREE PIGLETS



CLOSED HYSTEROTOMY

OPEN HYSTEROTOMY



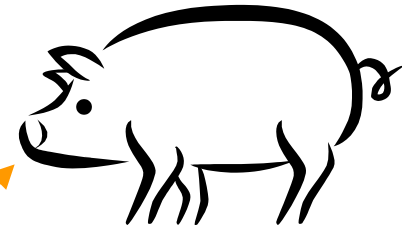
HYSTERECTOMY

Miniats a Jol, 1979

GNOTOBIOTIC ANIMALS

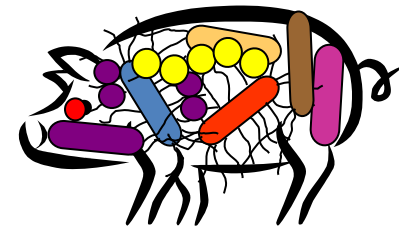
GF (germ free)

– without microbes, axenic (sterile host)



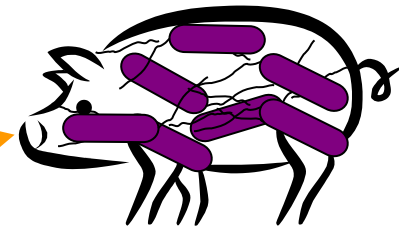
CV (conventional)

- a host colonized by normal microbiota after birth



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

– experimentally colonized host with defined microbes



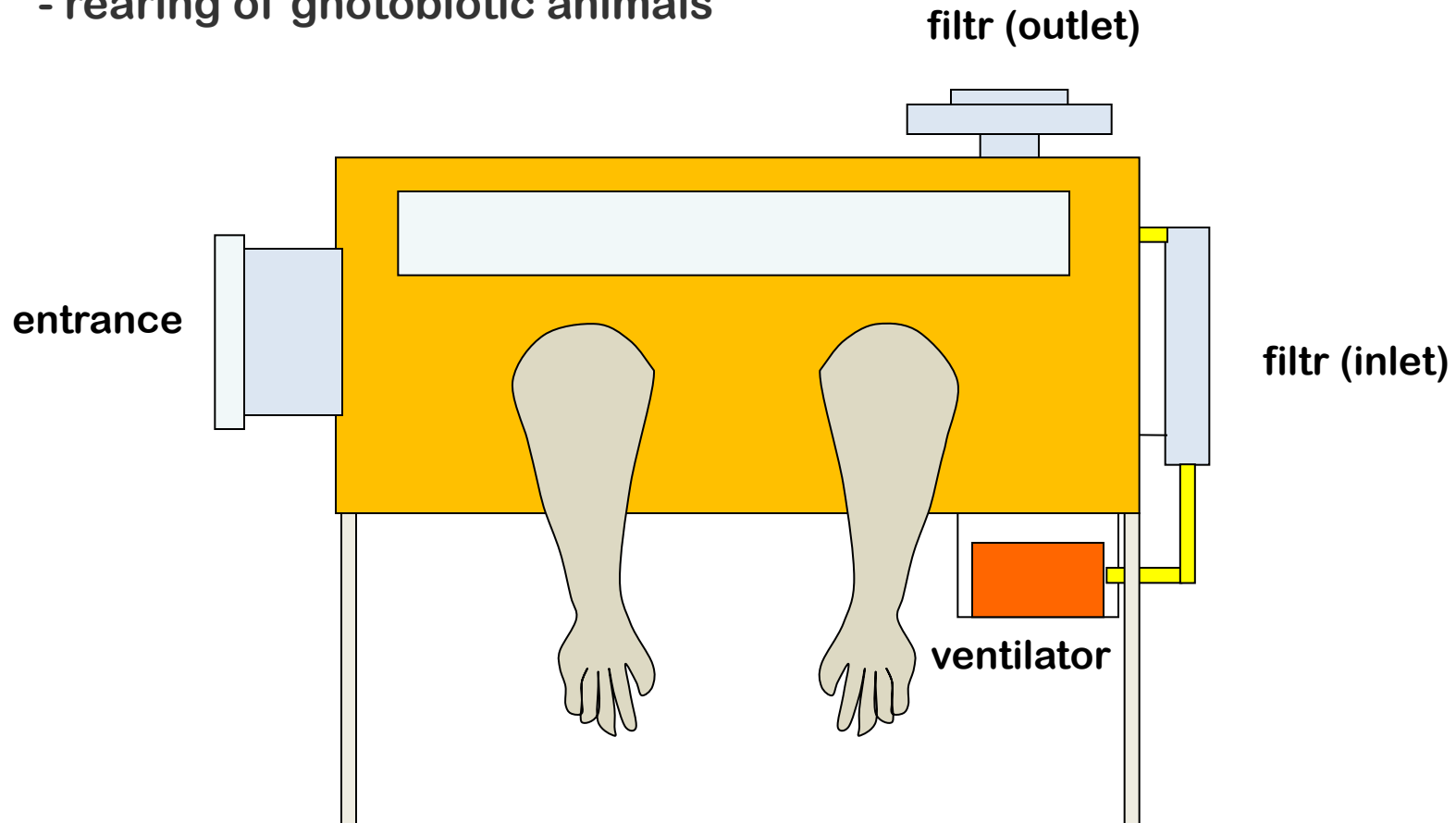
Gnotobiont

– mikrobiologisch defined host
(sterile or associated)

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

ISOLATOR

- separation of animals from outside
- rearing of gnotobiotic animals

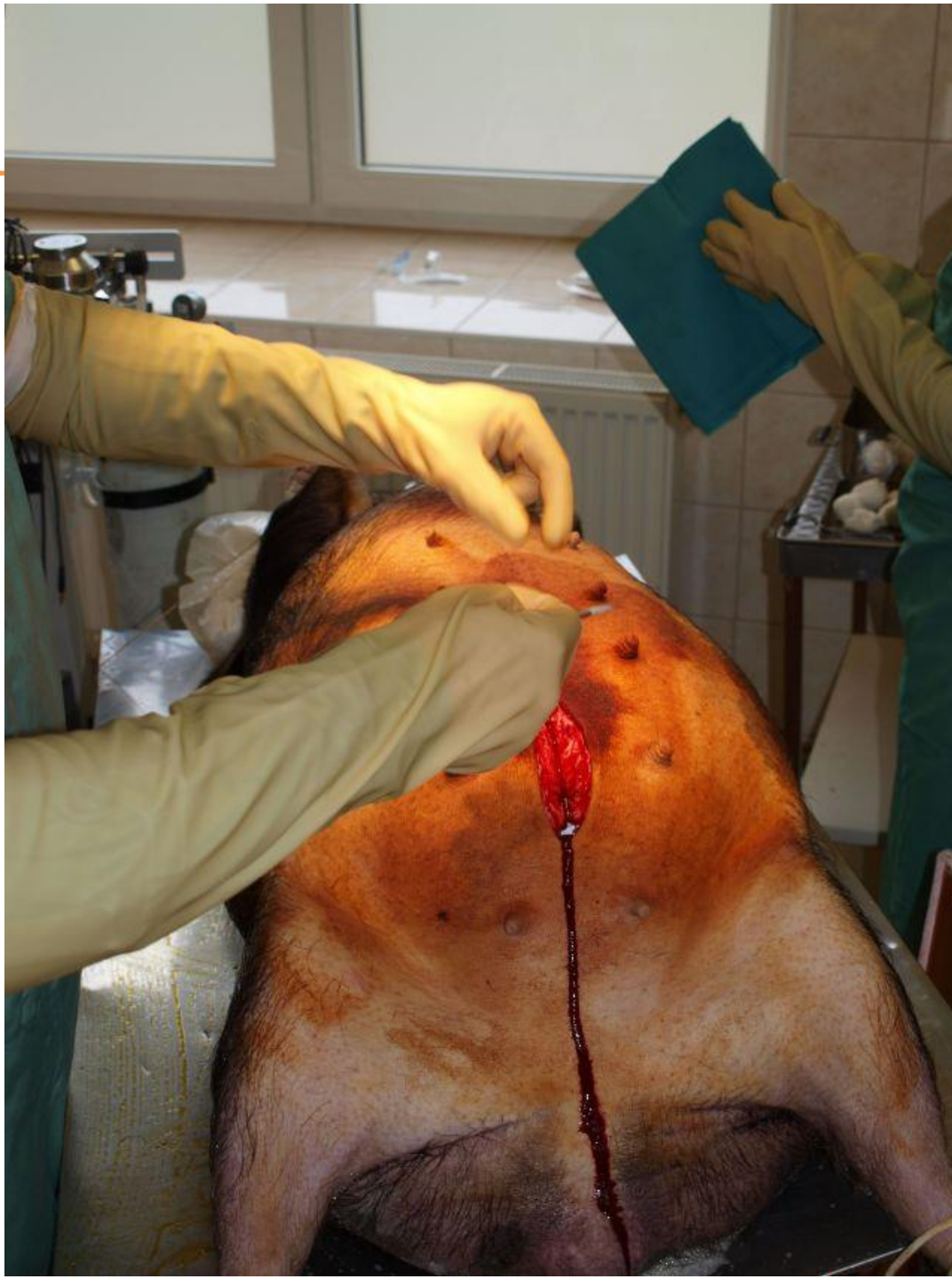


Fibreglass isolator for rearing
of gnotobiotic piglets

HYSTERECTOMY

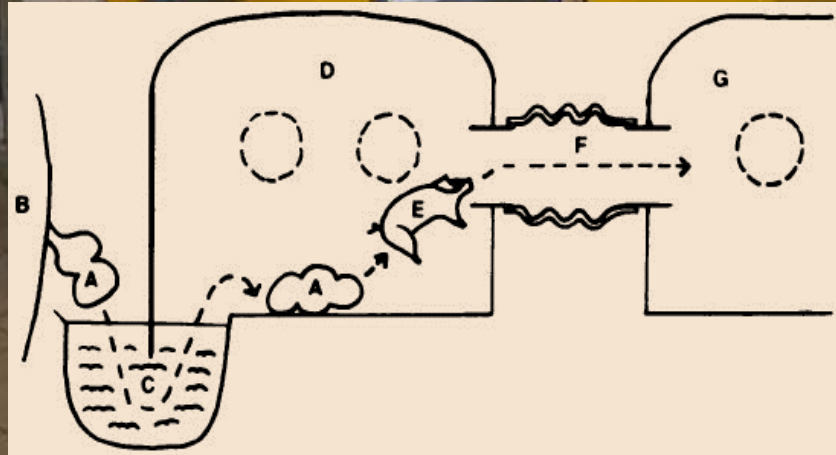
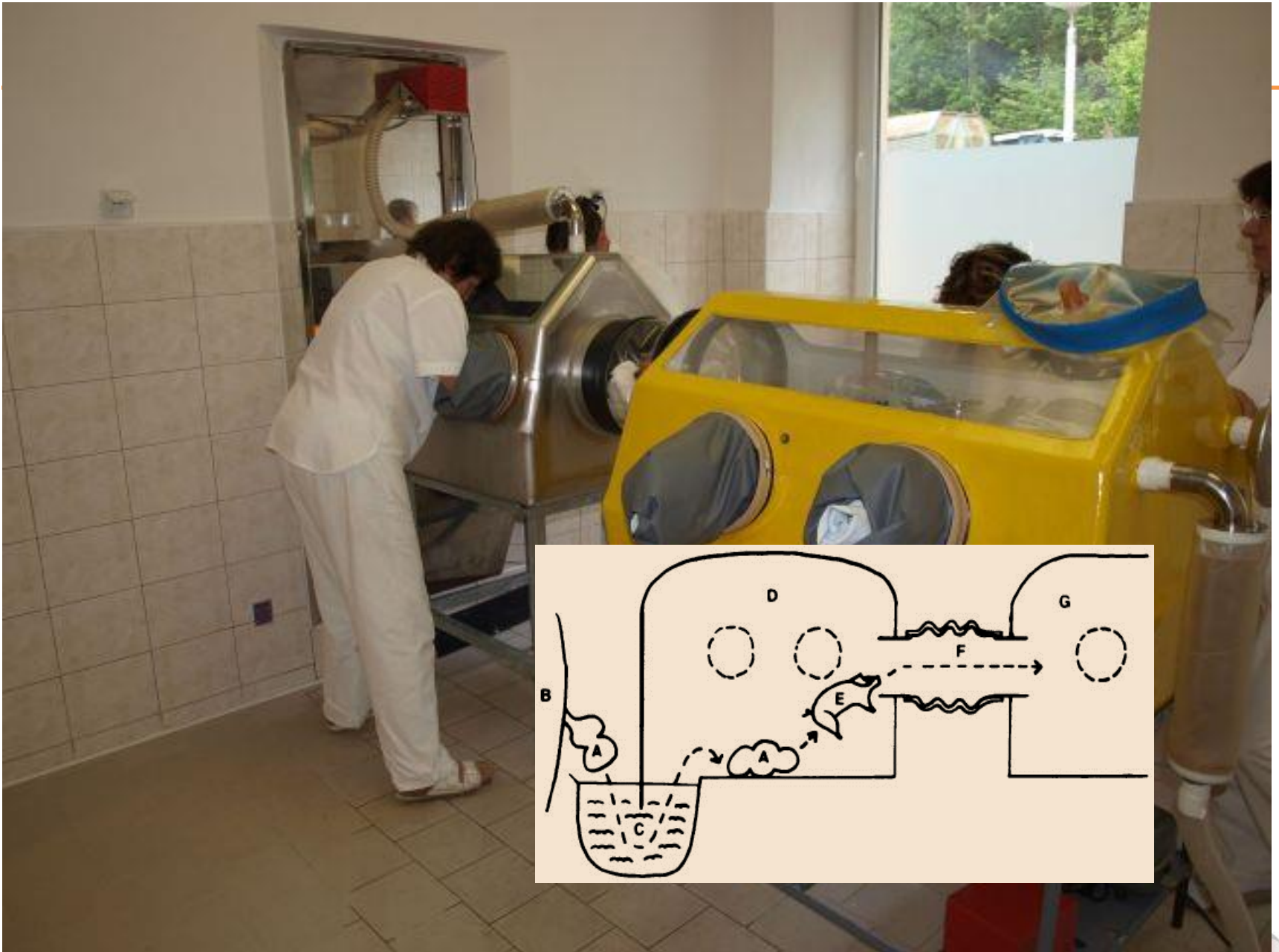
All experiments with animals were approved by the Committee for Animal Protection and Use of the Institute of Microbiology.











MATERIAL AND METHODS

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

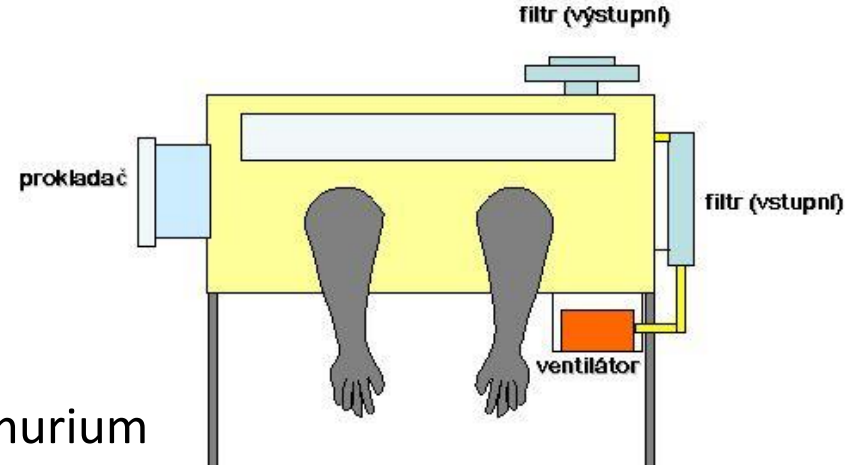
GF – control group

LT2 – *Salmonella enterica* serovar Typhimurium

rfaG – *Salmonella enterica* isogenic mutant

EcN – probiotic *E.coli* Nissle 1917

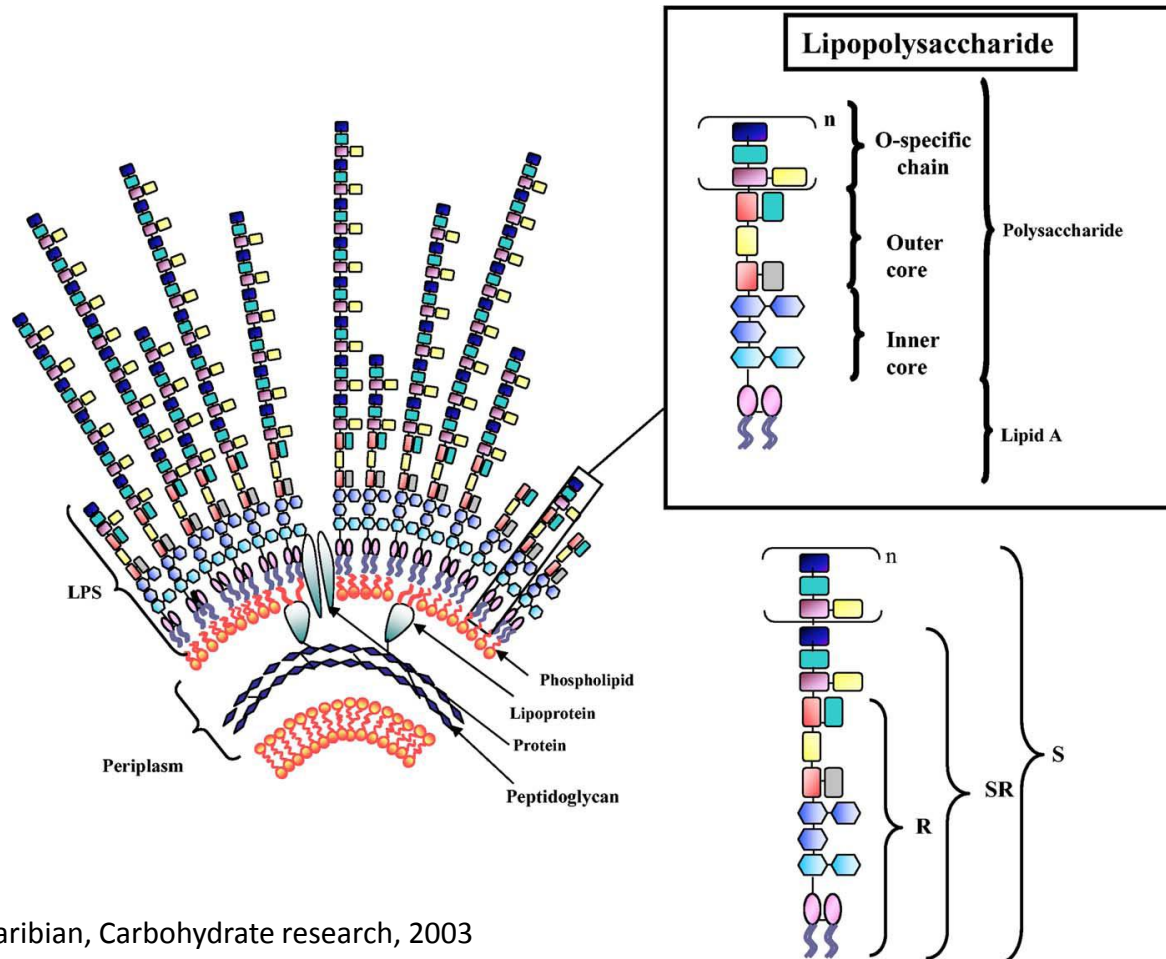
EcN+ST – piglets colonized with EcN and later infected with ST



Mandel a Travnicek, Nahrung, 1986

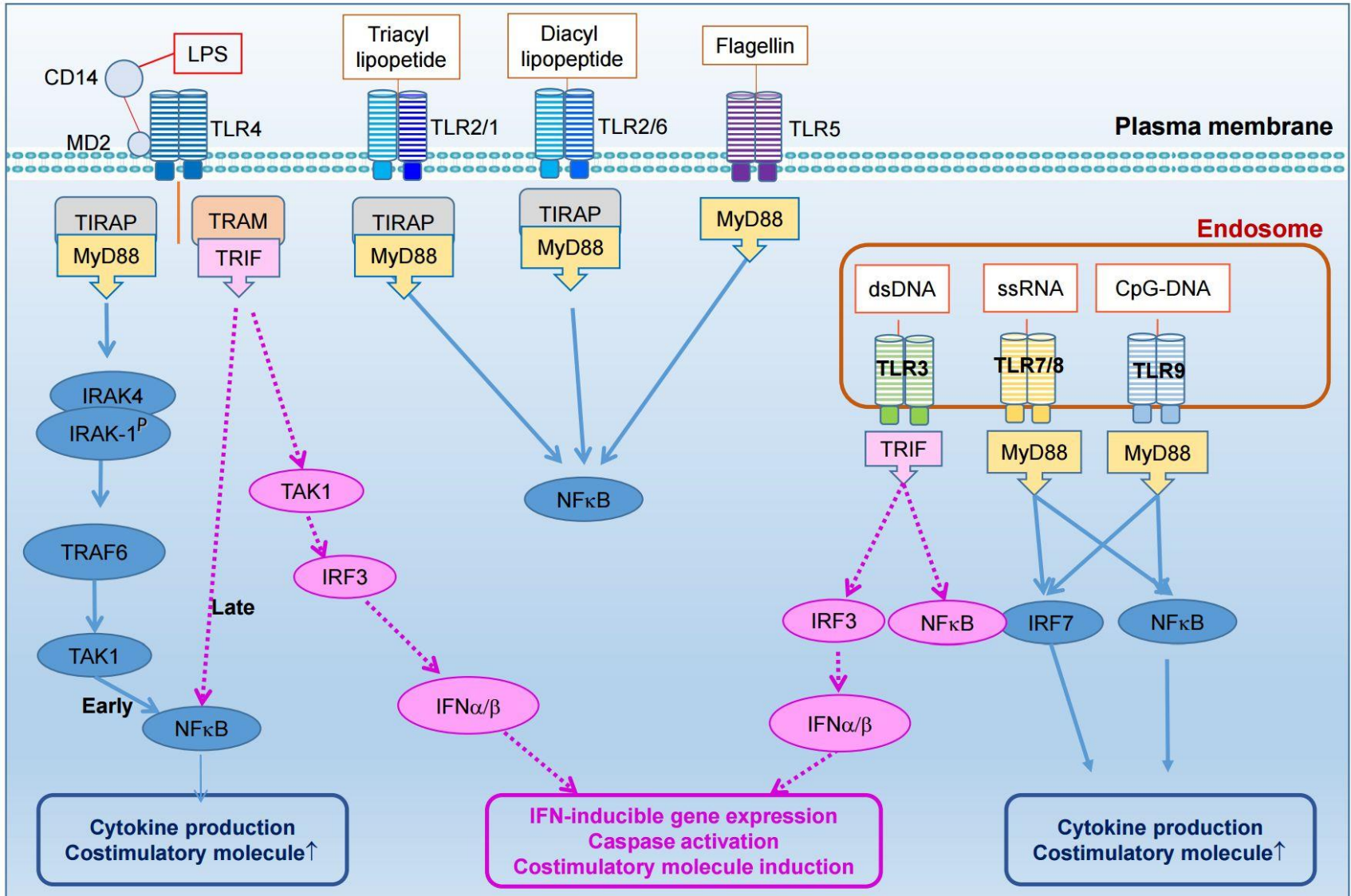
LIPOPOLYSACCHARIDE

Virulence factor of G- bacteria



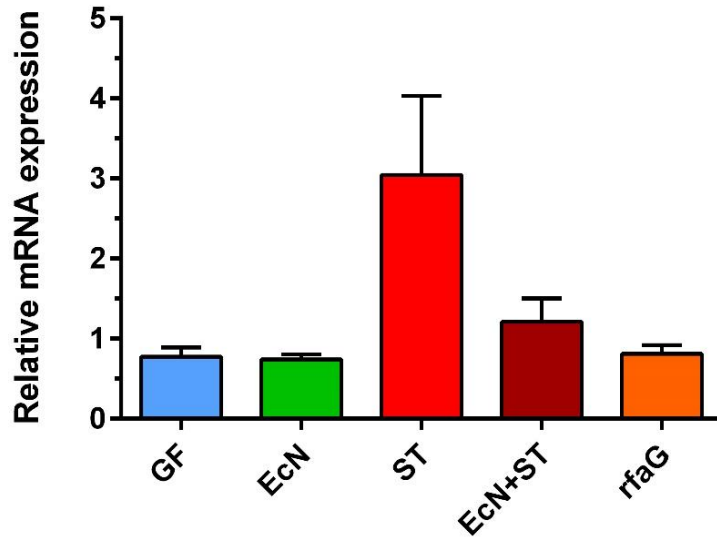
Caroff and Karibian, Carbohydrate research, 2003

TOLL-LIKE RECEPTORS

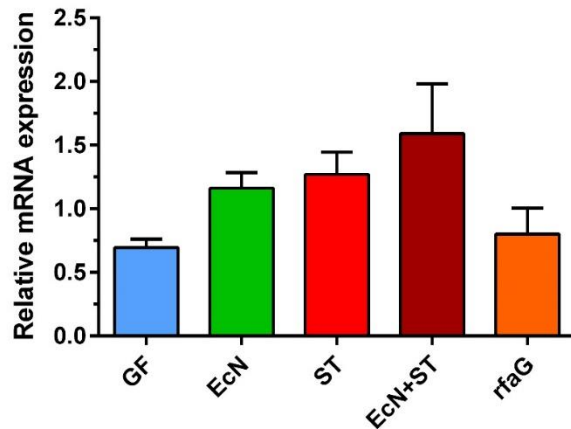


TLR4 SIGNALING

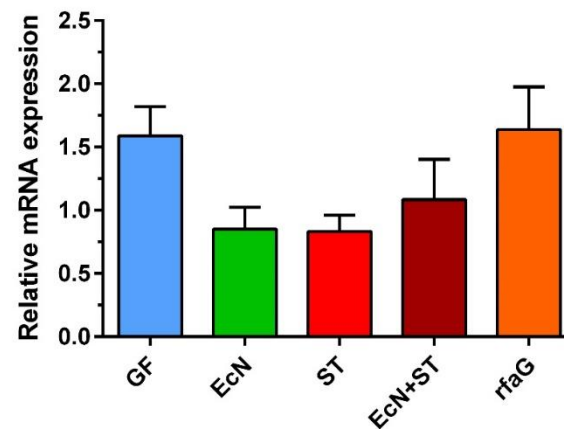
TLR4



MyD88



TRIF



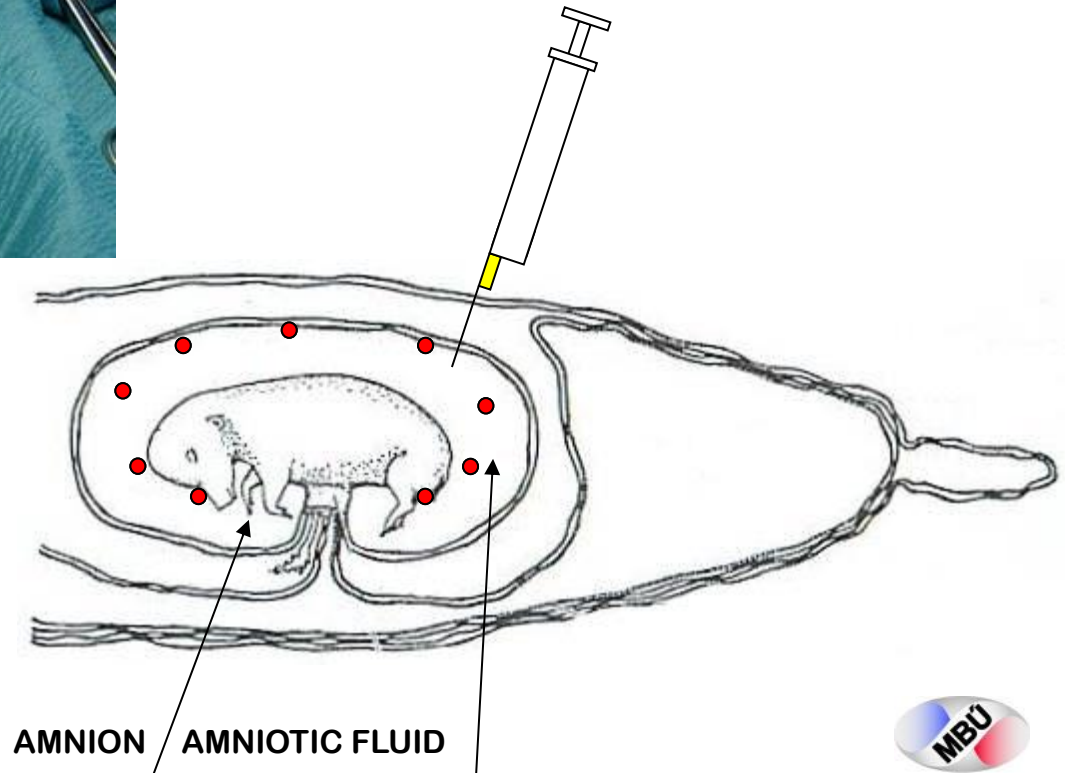
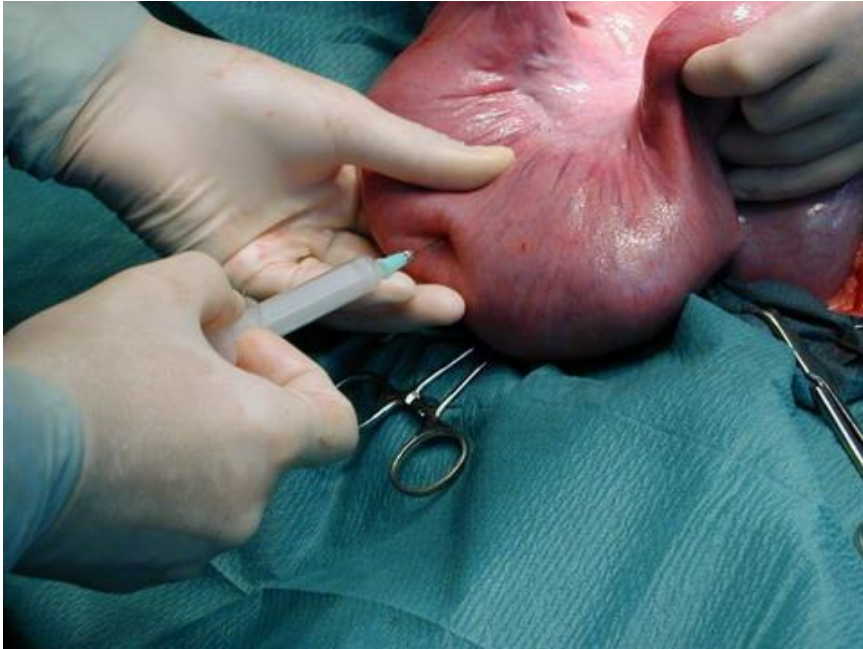
CONCLUSIONS

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.

