Session 29

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A new freezing extender for stallion semen

↳ to get high fertility rates

INRA-Freeze®

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G. Delhomme, S. Desherces, E. Schmitt, M. Magistrini.

Artificial insemination with frozen semen

A lot of advantages

- Transport of semen is easier
- Storage can be unlimited
- Choice of stallion is wider

. . .

BUT

A few limits

- Results are lower or more fluctuating than with fresh or chilled semen

  In France, in the National Studs:

  - 2007: Fertility/cycle = **44%** (2557 cycles)
  - 2008: Fertility/cycle = **47%** (2270 cycles)

  (55% with fresh semen in the National Studs)

- Results are lower or more fluctuating than with fresh or chilled semen

In France, in the Nati[...]:

- 2007: Ferti[...]cycles)
- 2008: F[...]es)

(55% wi[...]ls)

For the success
of artificial insemination
Freezing extender
= a key factor

- Results are lower or more fluctuating than with fresh or chilled semen

In France, in the National Studs:

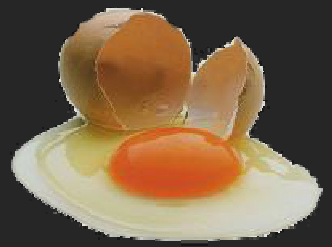
- 2007: Fertility/cycle = **44%** (2557 cycles)
- 2008: Fertility/cycle = **47%** (2270 cycles)

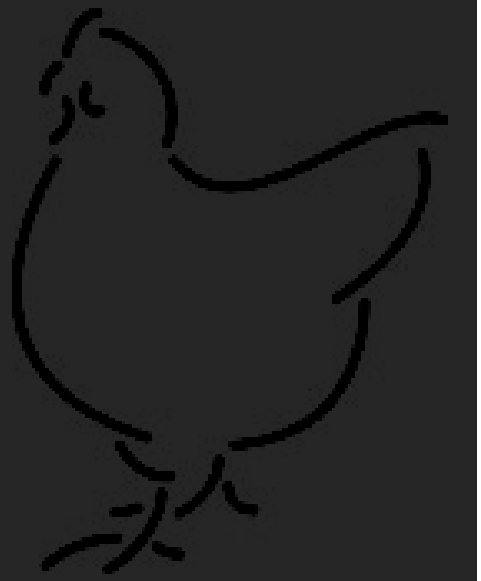
(55% with fresh semen in the National Studs)

- Freezing extenders are not optimal, and not practical

Have to be prepared in the lab

Contain milk and/or egg yolk





Objective :

To develop a freezing extender

- ✓ able to improve fertility results
- ✓ easy to use
- ✓ able to avoid sanitary risks

in vitro analyses + *in vivo trials*

2 steps :

- ► 1) To remove milk
- ► 2) To remove egg yolk (EY)

2 *in vivo* trials

- 1) To remove milk

Fertility Trial 1

INRA82
(milk based)
+ EY + glycerol

INRA96®
(only caseins of milk)
+ EY + glycerol

(Palmer, 1984) INRA82 → 1st dilution ← INRA96® *(Batellier et al., 1997)*

INRA82
+ 2% EY
+ 2,5% glycerol

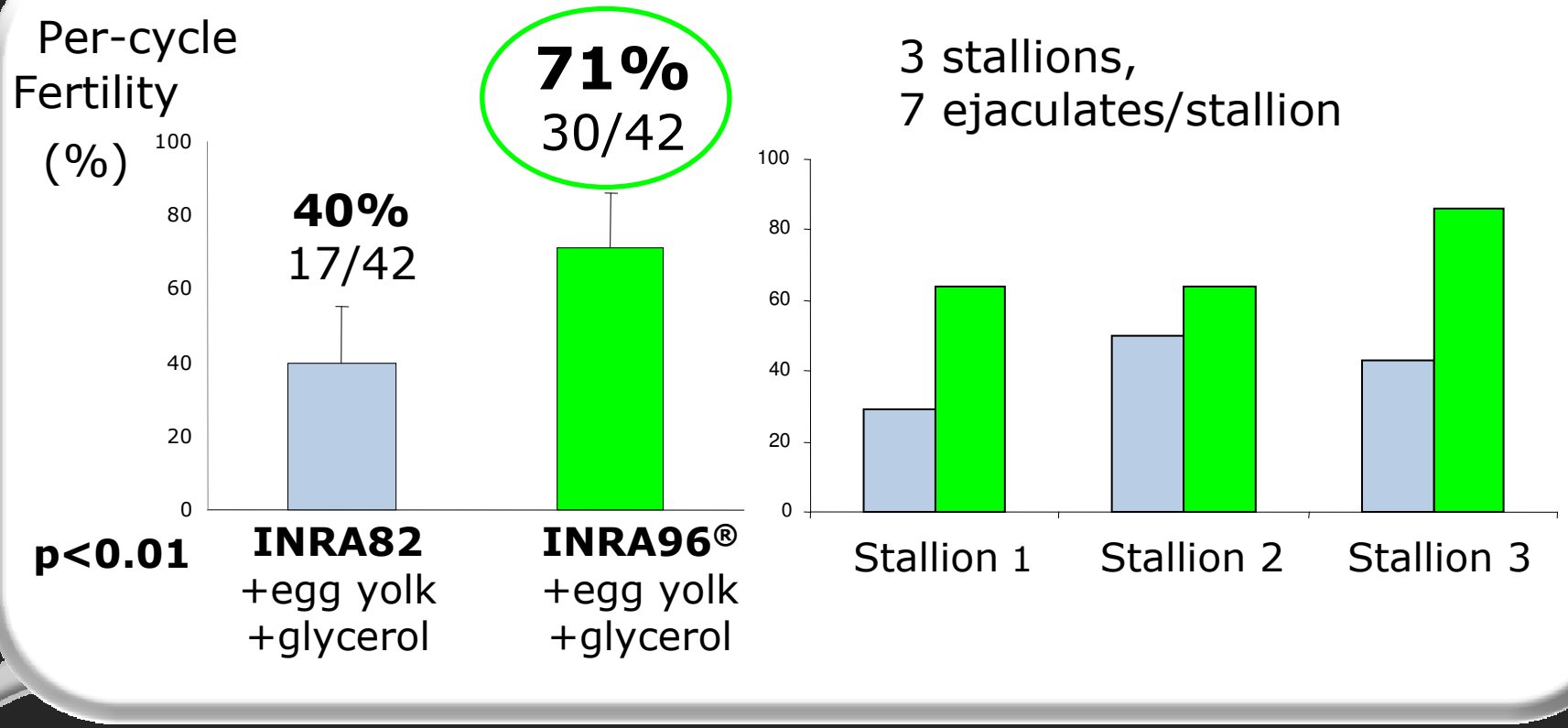
2nd dilution and freezing

INRA96®
+ 2% EY
+ 2,5% glycerol

☞ Insemination Protocol :

- induction of ovulation (CEG)
- 1 dose of insemination
- 400 x 10^6 sperm cells
- 6 hours before expected ovulation
- insemination in the uterine body

84 mares' cycles inseminated (72 mares)



INRA82 + EY + glycerol << INRA96® + EY + glycerol

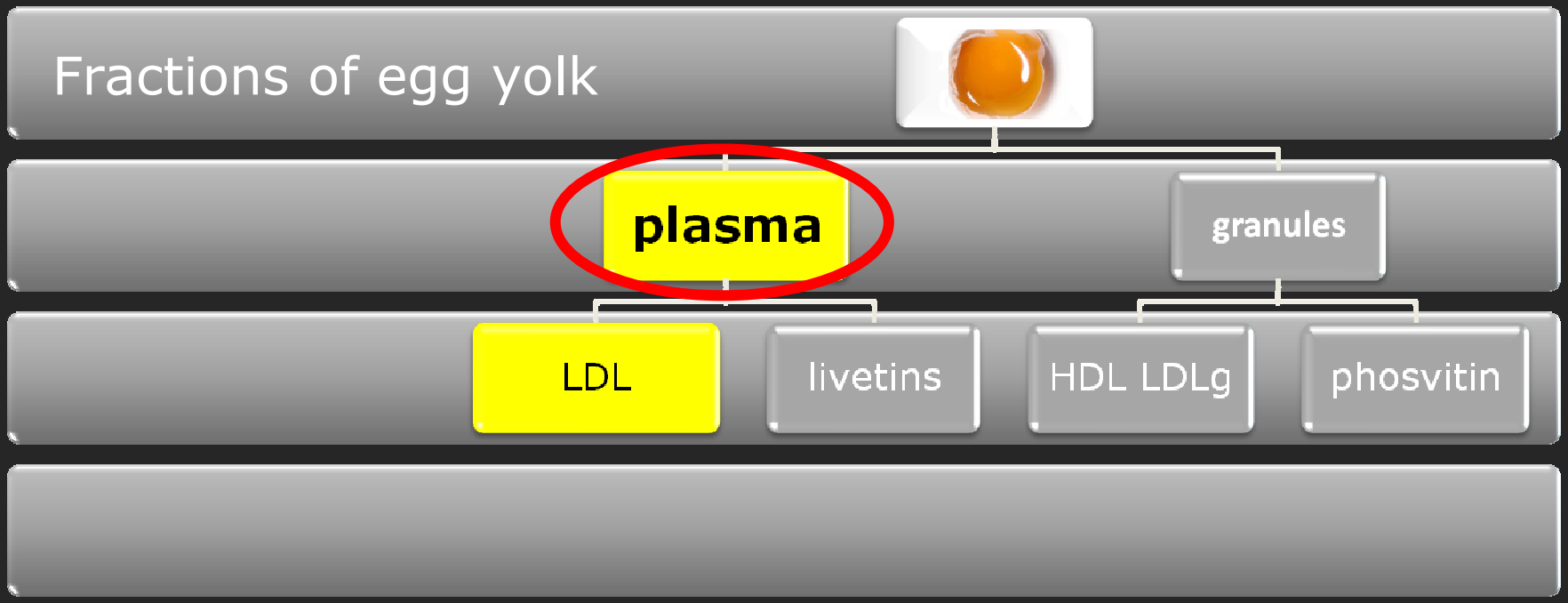
(milk based) (only caseins)

(Pillet et al., 2008)

Fertility Trial 2

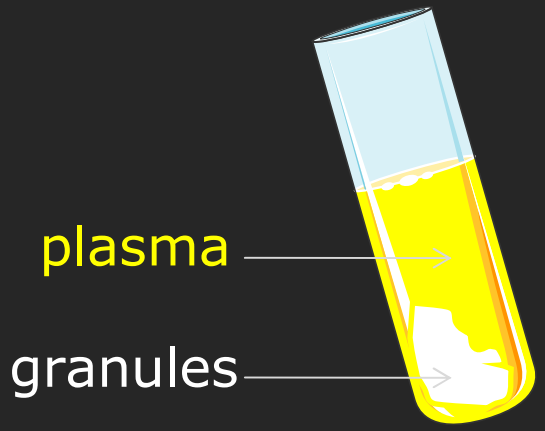
- 2) To remove egg yolk

Sterilized Egg yolk plasma



1) Contains Low Density Lipoproteins LDL

2) Can be sterilized



INRA96®
+ **EY**
+ glycerol

INRA96®
+ **Sterilized EY plasma**
+ glycerol

INRA96®

1st dilution

INRA96®

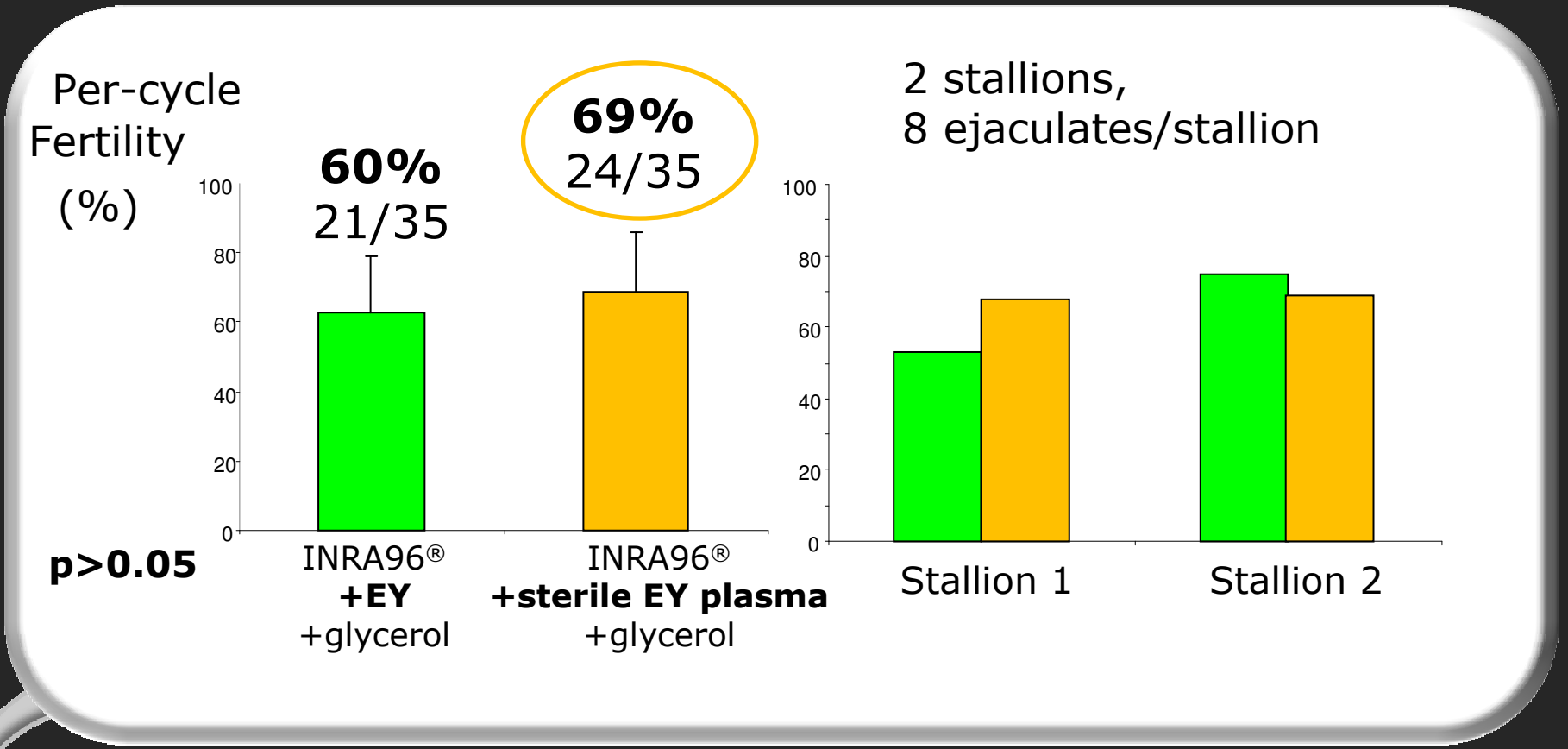
INRA96®
+ 2% EY
+ 2,5% glycerol

2nd dilution and freezing

INRA96®
+ 4% ster. EY plasma
+ 2,5% glycerol

Insemination

70 mares' cycles inseminated (68 mares)



INRA96® + **EY** + glycerol = INRA96® + **Sterilized EY plasma** + glycerol

Conclusion :

Fertility was greatly improved using

INRA96® + egg yolk + glycerol OR

INRA96® + sterilized egg yolk plasma + glycerol

Can be incorporated into
a ready to use extender

2 steps to develop a new freezing extender

69%

(65% with fresh semen with the same experimental herd at INRA)

INRA-Freeze®

Milk and egg yolk

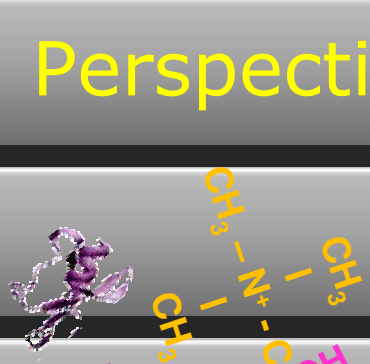
Fertility Trial 1

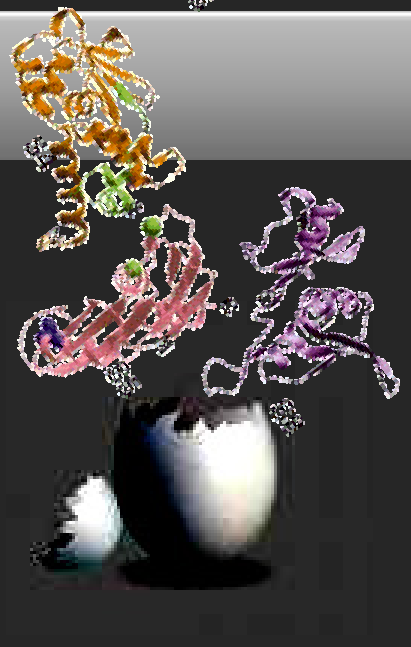
Fertility Trial 2

INRA-Freeze®

- ✓ Increases fertility results
- ✓ Is ready to use
- ✓ Avoids sanitary risks

Perspectives





- plasma
  - LDL
    - phospholipids
  - livetins
- granules
  - HDL LDLg
  - phosvitin

Which phospholipid(s)?

Which mechanism ?

Which molecule(s) ?

Thank you.