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Liposomes of phospholipids, a promising approach for stallion sperm freezing

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Why freezing semen?

Cryopreservation of stallion semen is a very useful biotechnology for:

1°/ Patrimonial conservation of biological resources

2°/ Large diffusion of genetics within and between countries using artificial insemination

A lot of advantages

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

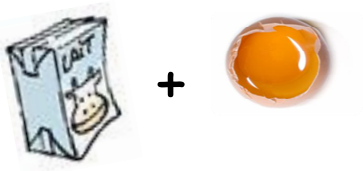
...

Why freezing semen?

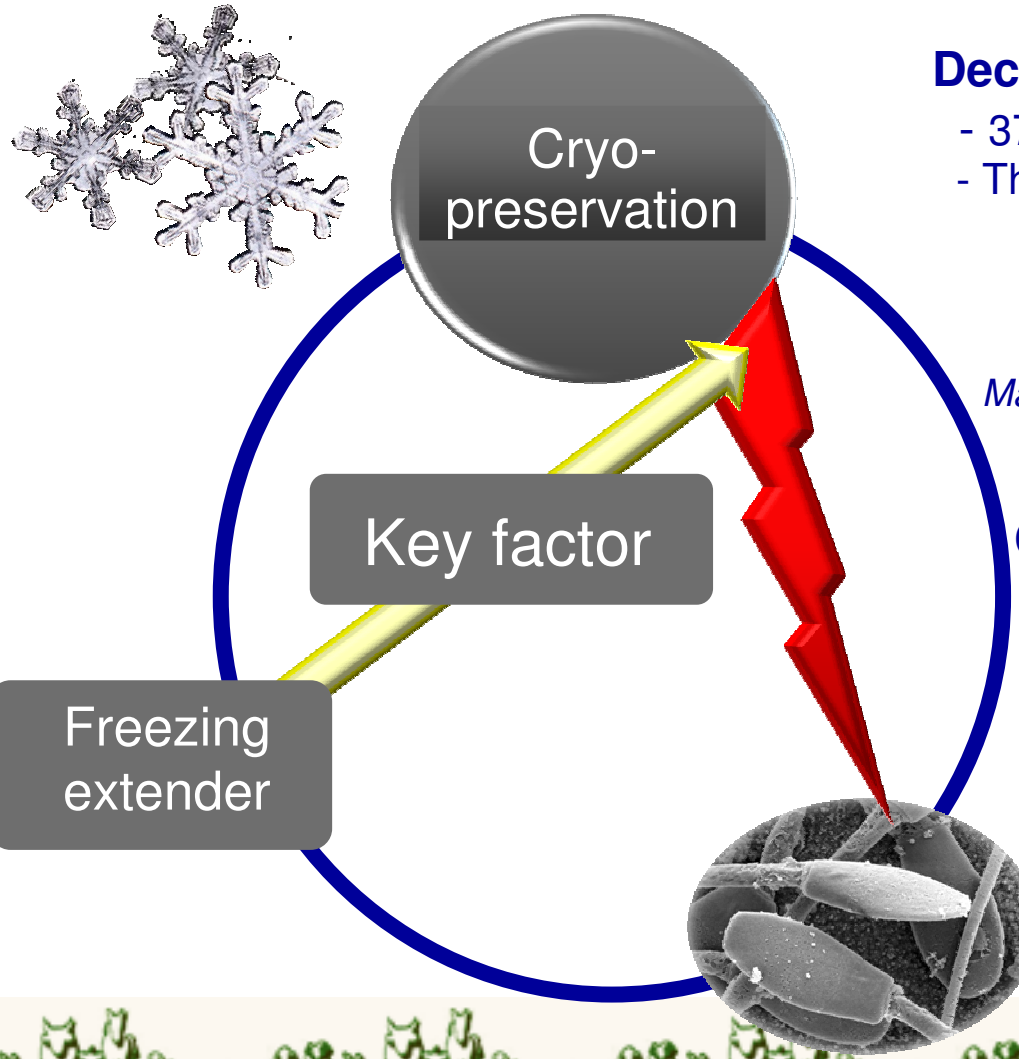
BUT

A few limits as

- Fertility rate is lower than fresh semen because of sperm injury during freeze-thaw
- Freezing extenders:
  - to be optimized in their composition
    - ↳ composed of animal products



Impact of cryopreservation on sperm cells



Decrease of temperature:

- 37°C- 4°C : "Cold Shock"
- Then 4°C to -196°C

↳osmotic stress
solution effect
ice crystals

Mazur, 1963 ; Amann et Pickett, 1987

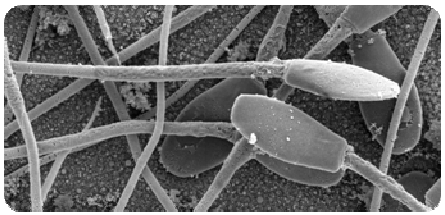
Cellular damages can be reversible or not

Watson, 2000

Elodie Pillet, 2009

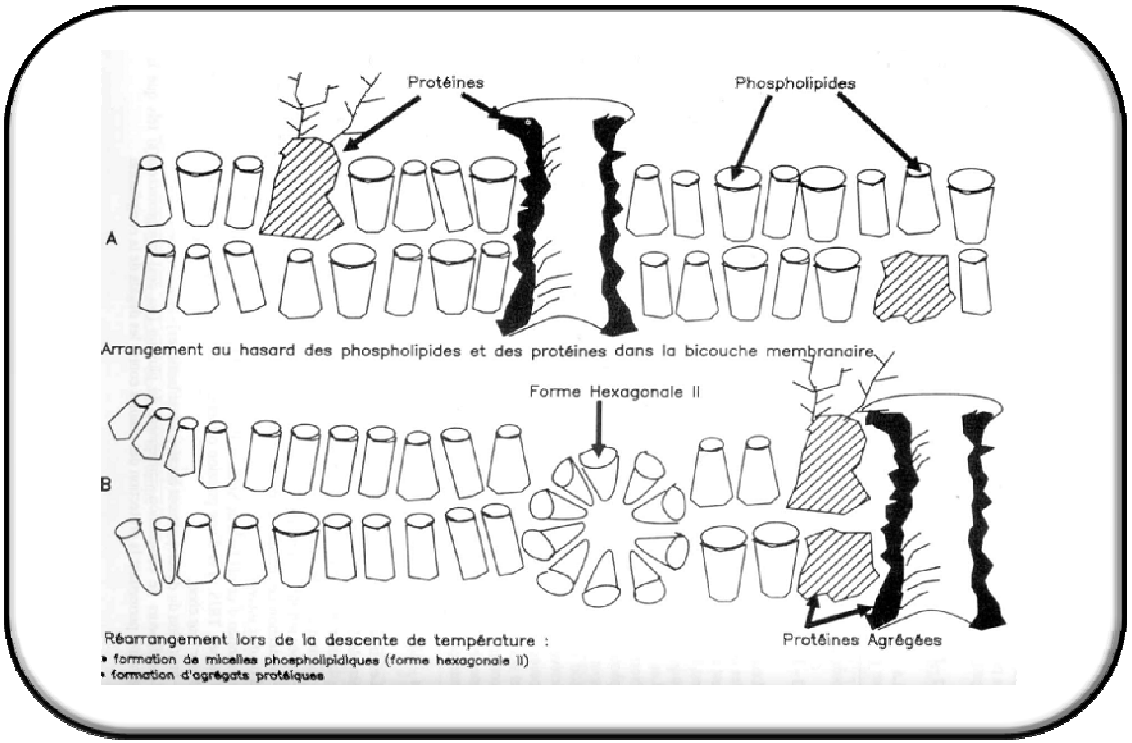
Impact of cryopreservation on sperm cells

↳ Cellular damages (especially membranes)



Normal membrane ⇒

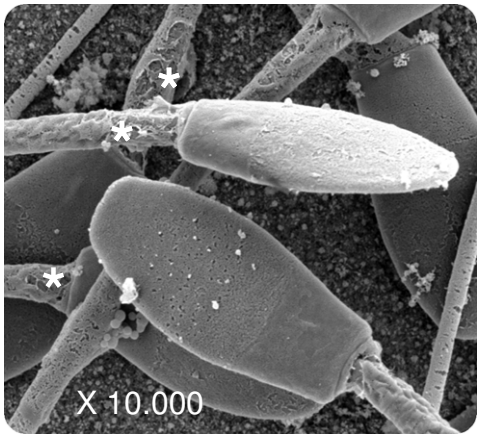
After decrease of temperature ⇒

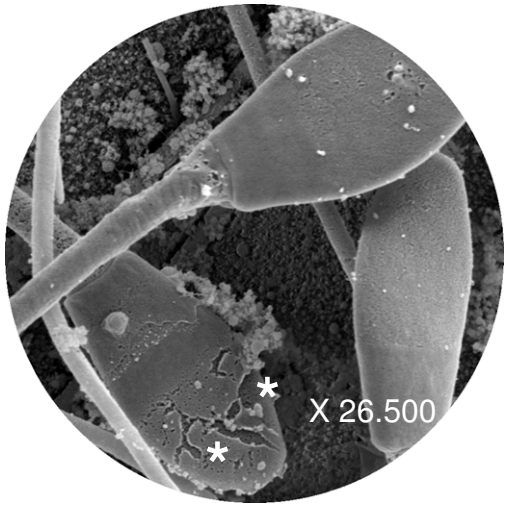


Amann et Pickett, 1987

Impact of cryopreservation on sperm cells

To limit membrane damages induced by low temperatures (-196°C)





↳ increase of membrane permeability
↳ decrease of the fertility potential after artificial insemination

⇩

Very protective freezing extenders are needed

Development of a new freezing extender in the equine species (Elodie PILLET PhD, 2006-2009)

Our objective was to develop a new freezing extender :

- able to improve fertility rates after AI with frozen sperm
- easy to use
- able to avoid sanitary risks (without animal products)

↳ 3 different steps were conducted (*in vitro* and *in vivo* studies) :

1 - remove  from the composition of the extender

2 - replace whole  (EY) by egg yolk plasma

3 - identify the protective fraction in EY plasma : phospholipids

Development of a new freezing extender in the equine species (Elodie PILLET PhD, 2006-2009)

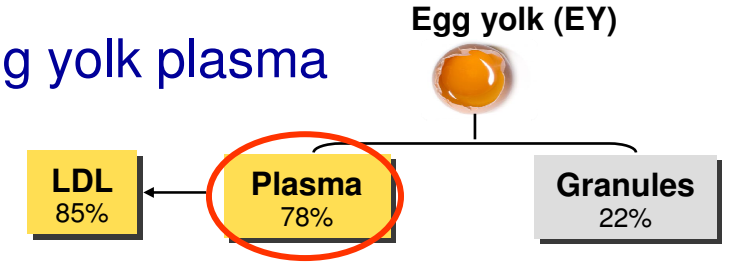
Step 1: successful replacement of  by INRA96* extender

*INRA96 contains only the purified fraction of native milk caseins

 +  + glycerol ⇨ excellent freezing extender

Pillet et al., DST, 88 (2), 2008
Magistrini et al., 2007, Patent FR-07 09145

Step 2: successful replacement of  by egg yolk plasma



 +  sterilized plasma + glycerol ⇨ INRA Freeze extender ready to use

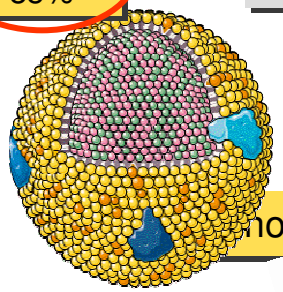
Pillet et al., Theriogenology, 75 (1), 2011

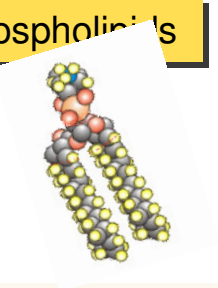
Development of a new freezing extender in the equine species (Elodie PILLET PhD, 2006-2009)

Step 3: phospholipids the protective fraction in EY plasma ?

Egg yolk (EY)

- **Plasma** 78%
  - LDL 85%
  - Livetines 15%
- **Granules** 22%
  - HDL, LDLg 84%
  - Phosvitine 16%





PC (70%)
PE (24%)
SPH (4%)
PS (1%)
PI (1%)

☞ PC is the most representative phospholipid (PL) in egg yolk

PC : phosphatidylcholine
PE : phosphatidylethanolamine
SPH : sphingosine
PS : phosphatidylserine
PI : phosphatidylinositol

Phospholipids : the protective fraction in EY plasma ?

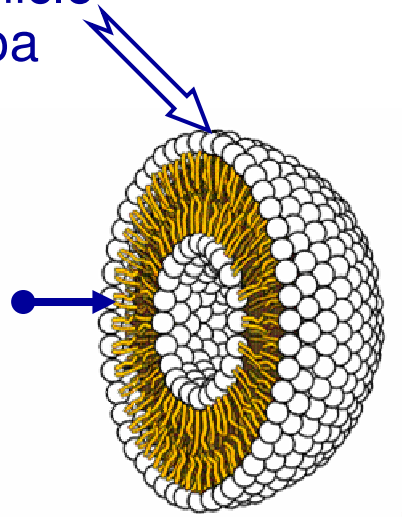
- Which EY phosholipids :PC, PE, PS% ?

  ⇨ Commercial EY phospholipids : PL E80 (Lipoïd)

  83,6% PC
  9,1% PE
  2% SPH
  1,8 LPC
  0,5 % LPE

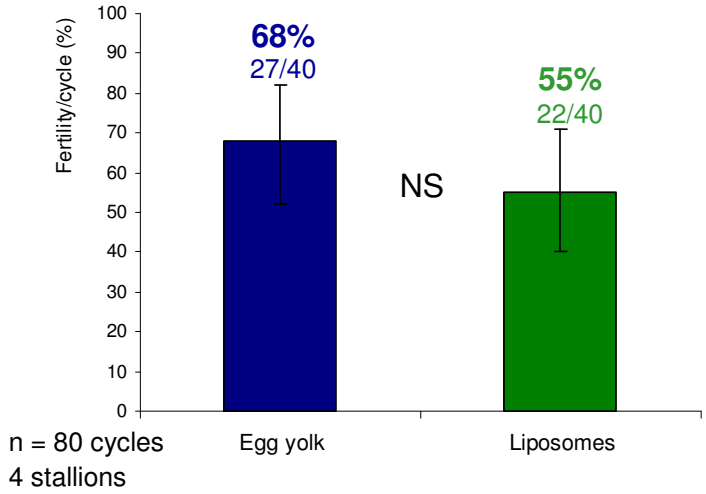
- Which arrangement of phospholipids ?

  ⇨ Liposomes were chosen as the ”vehicle” to transport PL up to spermatozoa



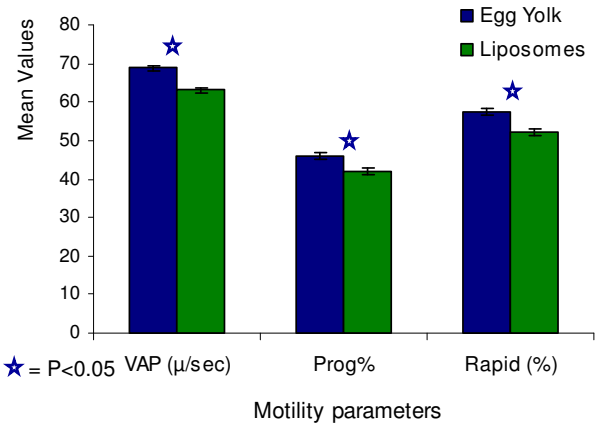
Phospholipids : the protective fraction in EY plasma ?

1 °/ Comparison of INRA96 + EY + G *vs.* INRA96 + PLE80 liposomes + G (2009)

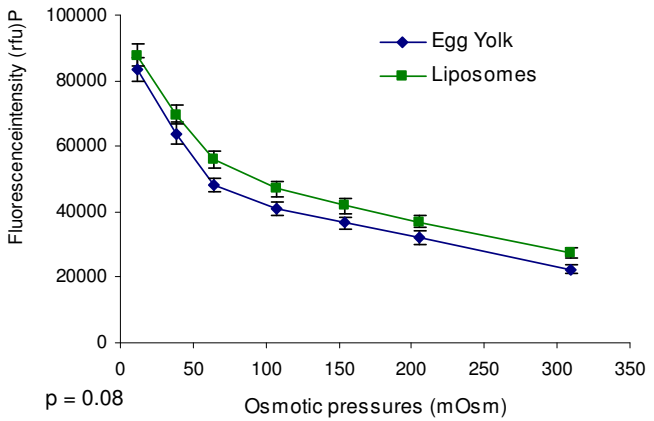


In vitro parameters

Motility parameters analyzed by CASA (computer assisted analysis)



Membrane integrity evaluated by a range of osmotic pressures (330mOsm to 10mOsm)

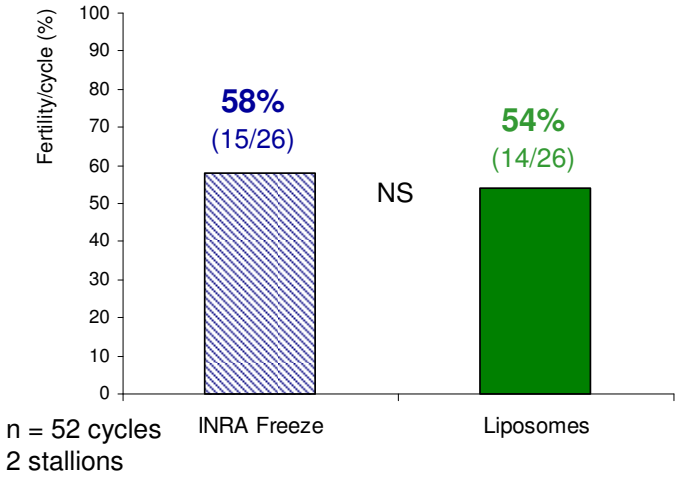


⇨ Liposomes of egg yolk PL (E80) can replace egg yolk in the freezing extender

Pillet et al, Theriogenology, in press

Phospholipids : the protective fraction in EY plasma ?

2°/ Comparison of EY plasma (INRA Freeze extender) *vs.* INRA96 +PLE80 liposomes + G (2010)



In vitro parameters

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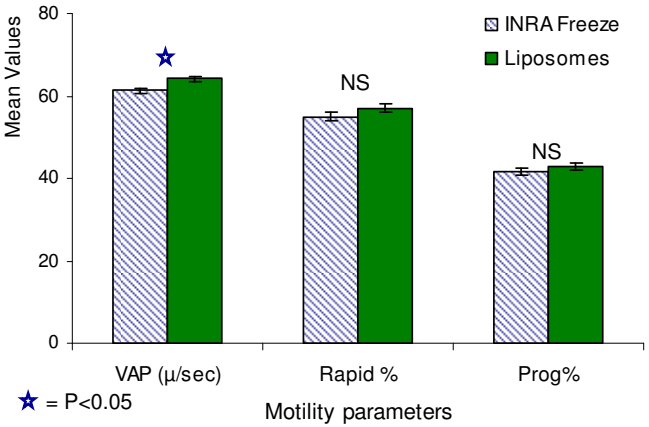

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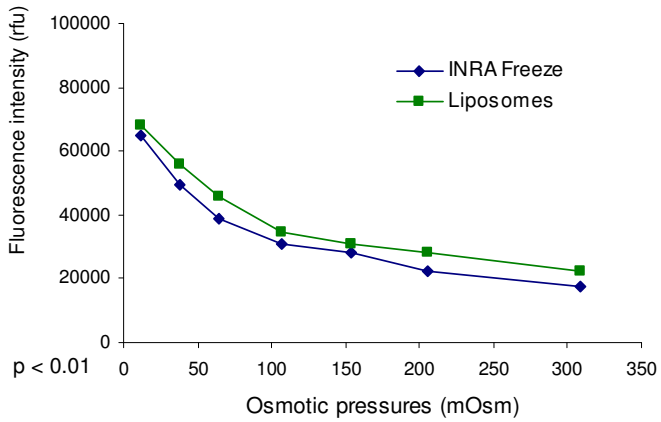

⇨ Liposomes of egg yolk PL (E80) can replace egg yolk plasma in the freezing extender

In Summary

Our results demonstrate that **liposomes of egg yolk phospholipids** (commercial PL E80) can replace egg yolk or egg yolk plasma in stallion sperm freezing extender

More **liposomes** are a very promising approach since it is possible:

to modulate - the **composition** in **phospholipids**
- the **diameter**

to **sterilize** them

↳These conditions are essential to optimize the freezing extender

Thanks for attention !

Unité expérimentale équine de Nouzilly
G. Duchamp et al.

Jean-Marie, Yvan, Thierry, Philippe etc.….

...and V. Beaumal & M. Anton (INRA, Nantes)