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On the way to a new defined freezing extender in the equine species

Session 37

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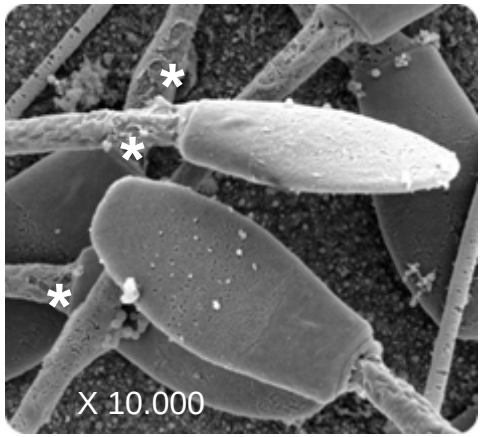
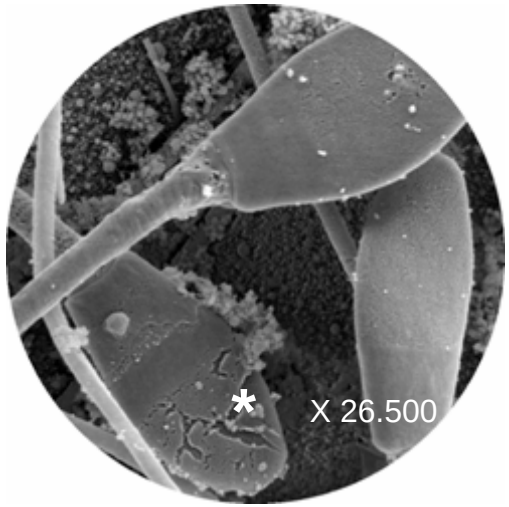
Cell cryopreservation procedures

Sperm cells freezing needs very protective extenders

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



Membranes are the principal target



Decrease of the fertility potential after artificial insemination

Artificial insemination with frozen semen

A lot of advantages

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

...

BUT

A few limits as

- Fertility rate (lower than fresh semen)
- Extenders:
  - to be optimized
  - composed of animal products

Elodie PILLET PhD : *development of a new freezing extender in the equine species*

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

} **EAAP, 2009**

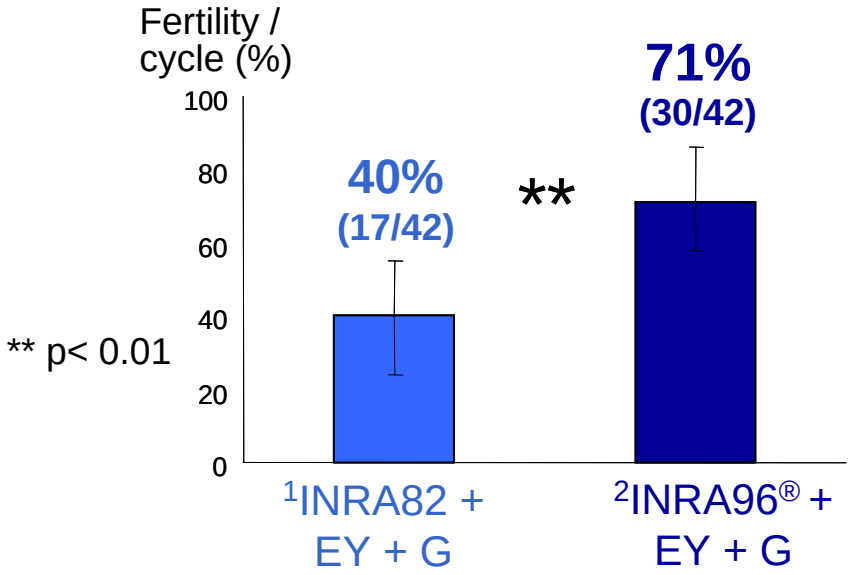
3 - identify the protective fraction in EY plasma : phospholipids

Step 1: successful replacement of

1st fertility trial - comparison of 2 freezing extenders : INRA82 *vs.* INRA96

[1]INRA82 extender contains milk

[2]INRA96 extender contains only the purified fraction of native milk caseins



+ glycerol

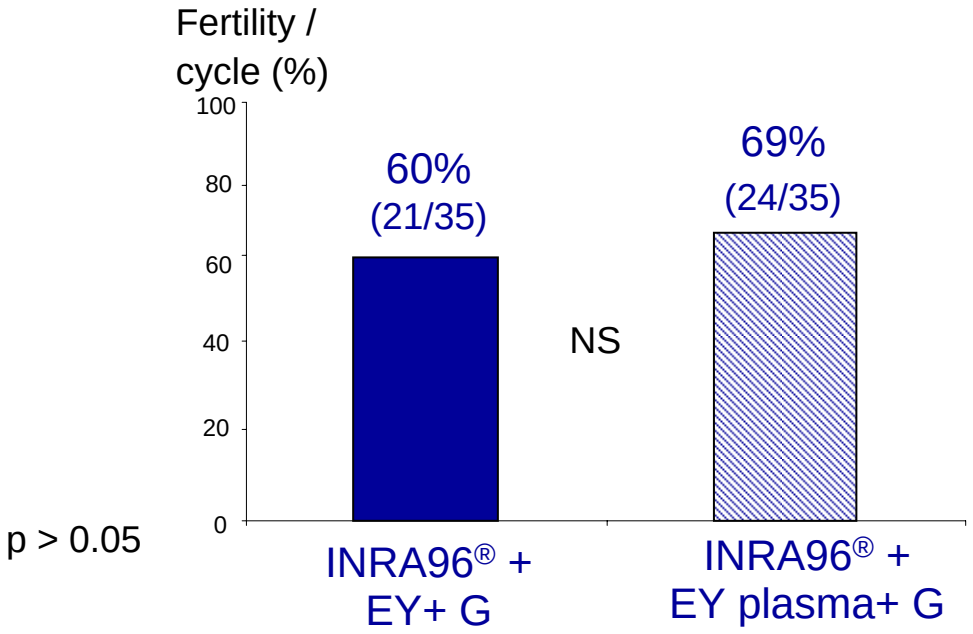
↳ excellent freezing extender

EY : egg yolk
G : glycerol

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

Step 2 : successful replacement of

2nd fertility trial - comparison of egg yolk *vs.* sterilized egg yolk plasma



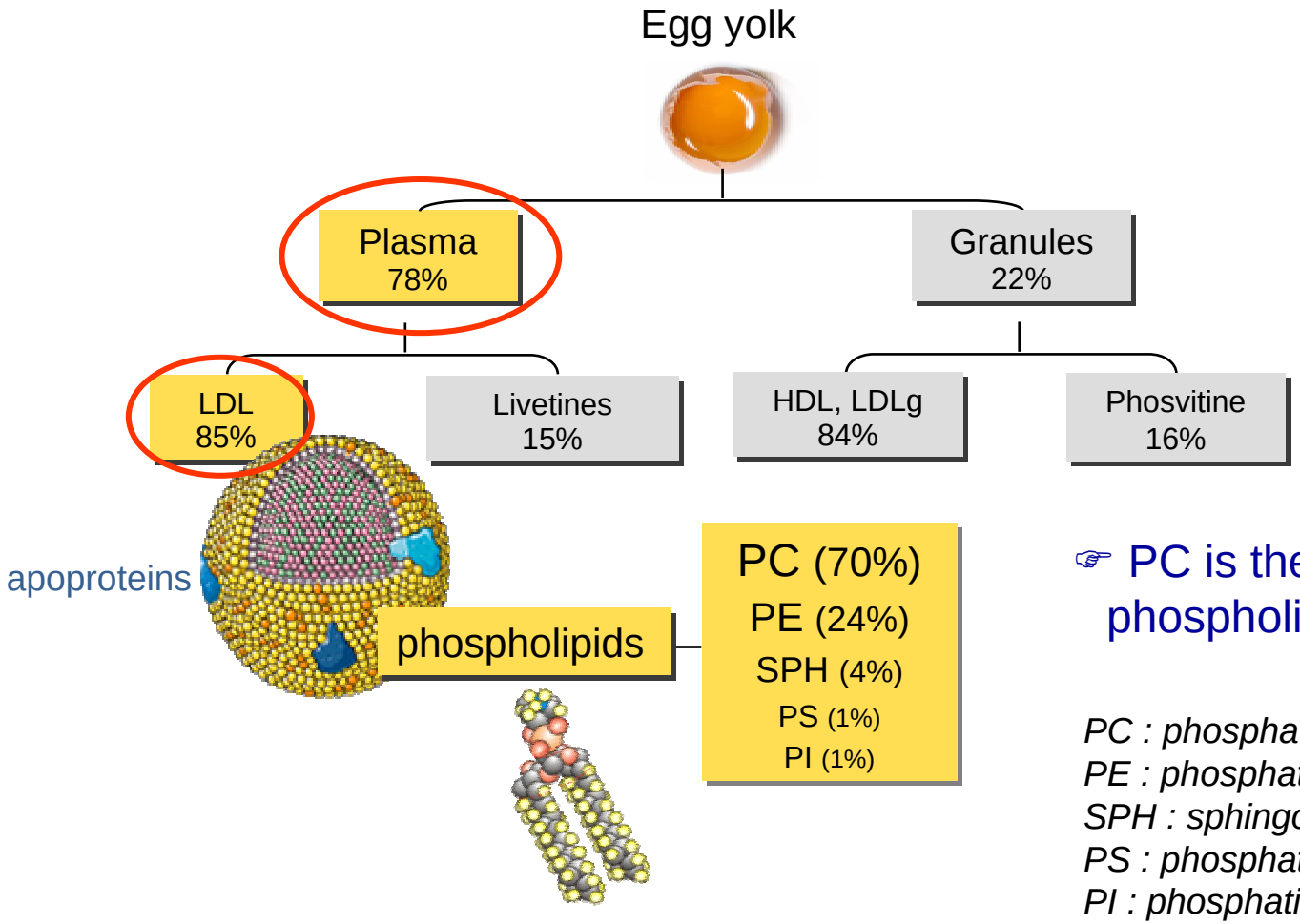
Sterilized egg yolk plasma can replace egg yolk in freezing extender

↳ INRA Freeze extender, ready to use extender

EY : egg yolk
EY plasma : sterilized egg yolk plasma
G : glycerol

Pillet et al., 2010, Theriogenology, in press

Step 3: protective fraction in EY plasma?



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

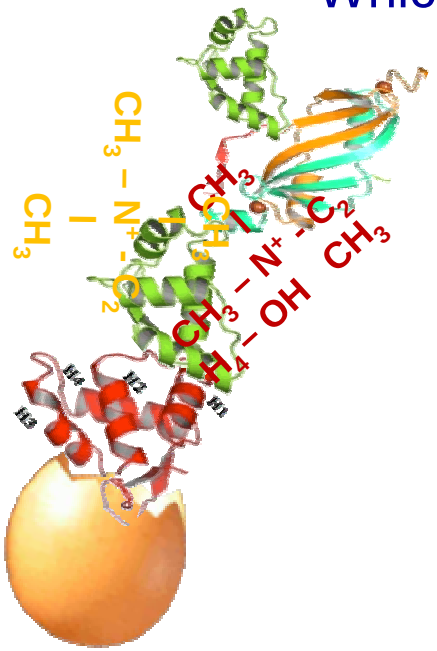
PC : phosphatidylcholine
PE : phosphatidylethanolamine
SPH : sphingosine
PS : phosphatidylserine
PI : phosphatidylinositol

Step 3: phospholipids as the protective fraction ?

Which EY phosholipids?

Which combination of PC, PE, PS…..?

Which arrangement of phospholipids ?



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

Commercial EY phospholipids

83,6% PC

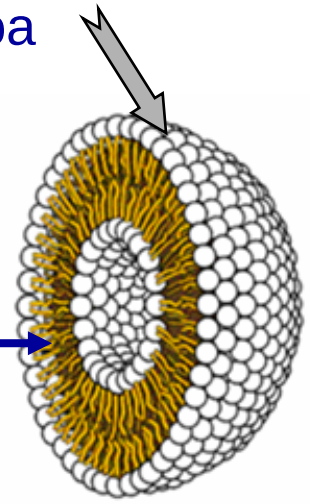
9,1% PE

2% SPH

1,8 LPC

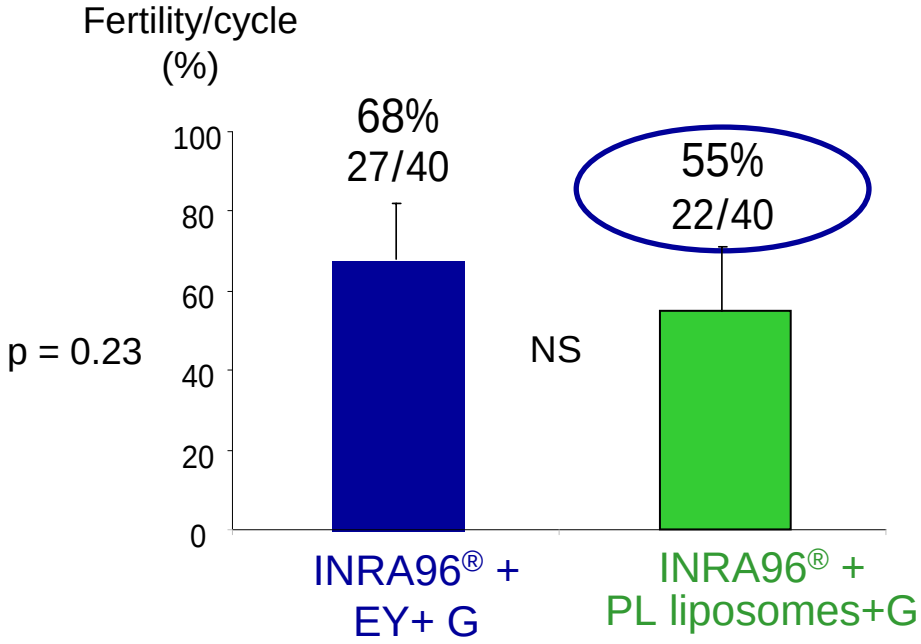
0,5 % LPE

Double layer of PL



Step 3 : phospholipids as the protective fraction ?

3rd fertility trial - comparison of egg yolk *vs.* egg yolk phospholipid liposomes



Liposomes of egg yolk PL can replace egg yolk in freezing extender

↳ liposomes of egg yolk PL are a promising approach but the ratio of PL in liposomes have to be optimized

EY : egg yolk
PL : phospholipids
G : glycerol

Pillet et al, submitted

In Summary

Our results have demonstrated that :

- the fertility rate can be improved using an optimal freezing extender
- the extender is a key factor for the success of AI with frozen semen

A “ready to use” extender which complies with biosafety rules is already available

But……ongoing studies have demonstrating that liposomes of EY phospholipids are a promising alternative to EY or EY plasma

↳ a defined freezing extender composed of :

- INRA96 extender
- glycerol
- liposomes of identified PL

Thanks for attention !

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