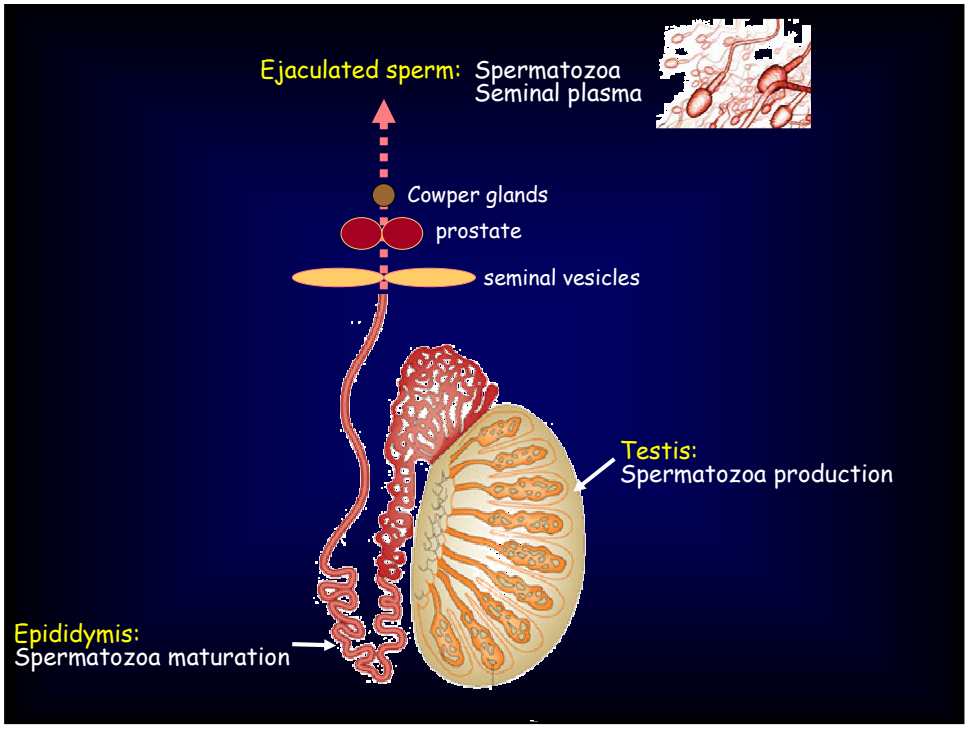
# Seminal plasma and spermatozoa quality in the boar: identification of novel biomarkers

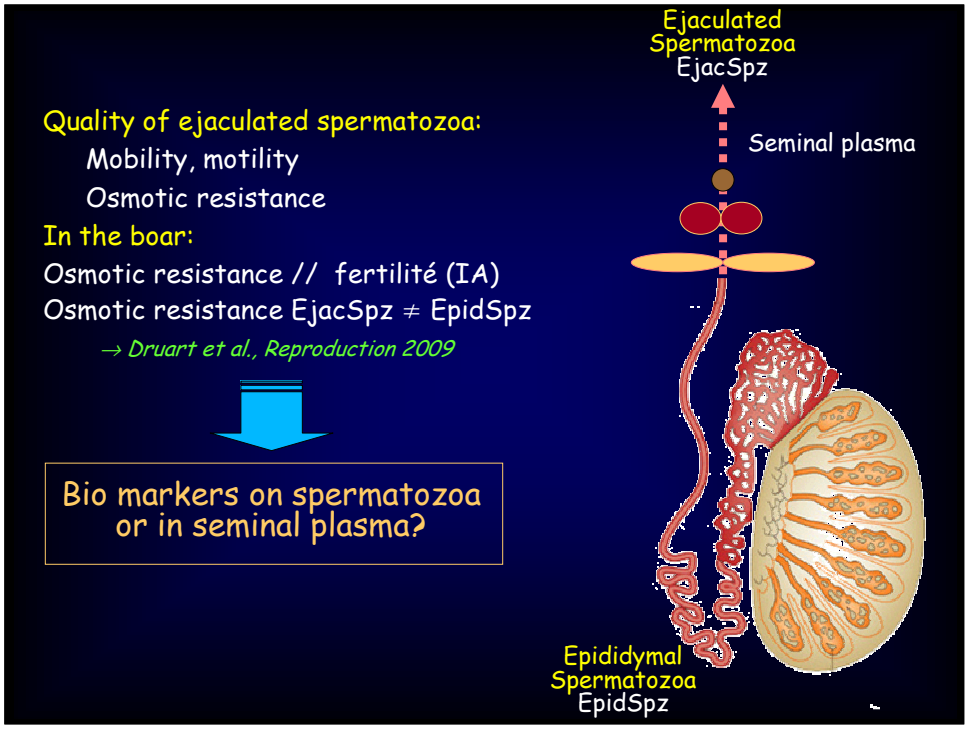
*Laure DEWAELE, Guillaume TSIKIS, Stéphane FERCHAUD, Valérie LABAS, Lucie SPINA, Ana Paula TEIXEIRA, Xavier DRUART & Nadine GÉRARD*

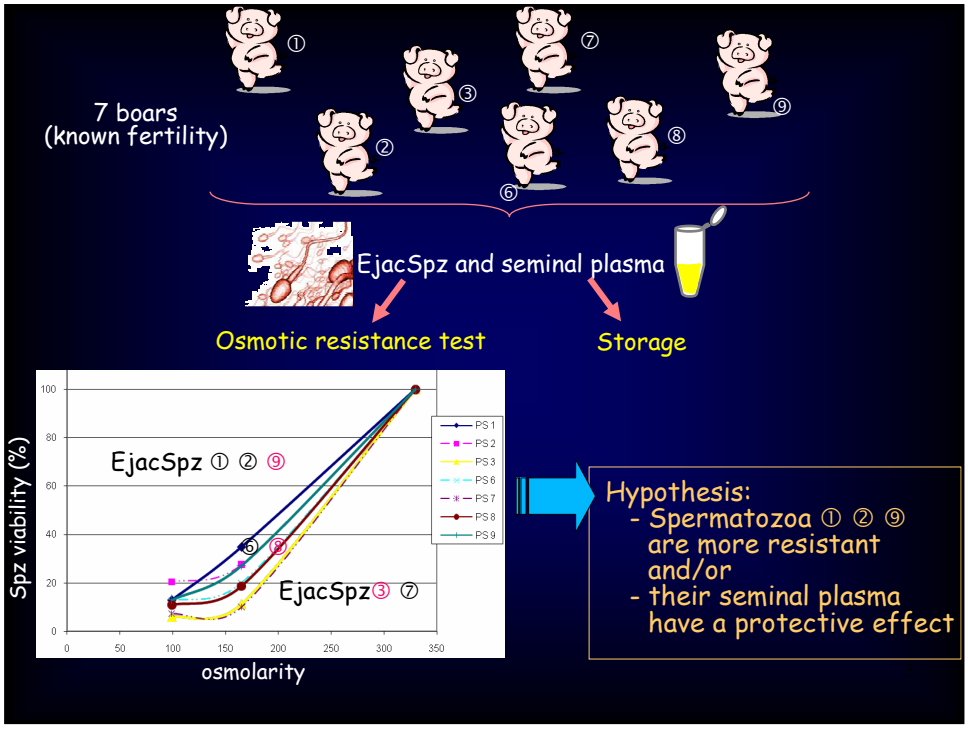
INRA
37380 Nouzilly, France

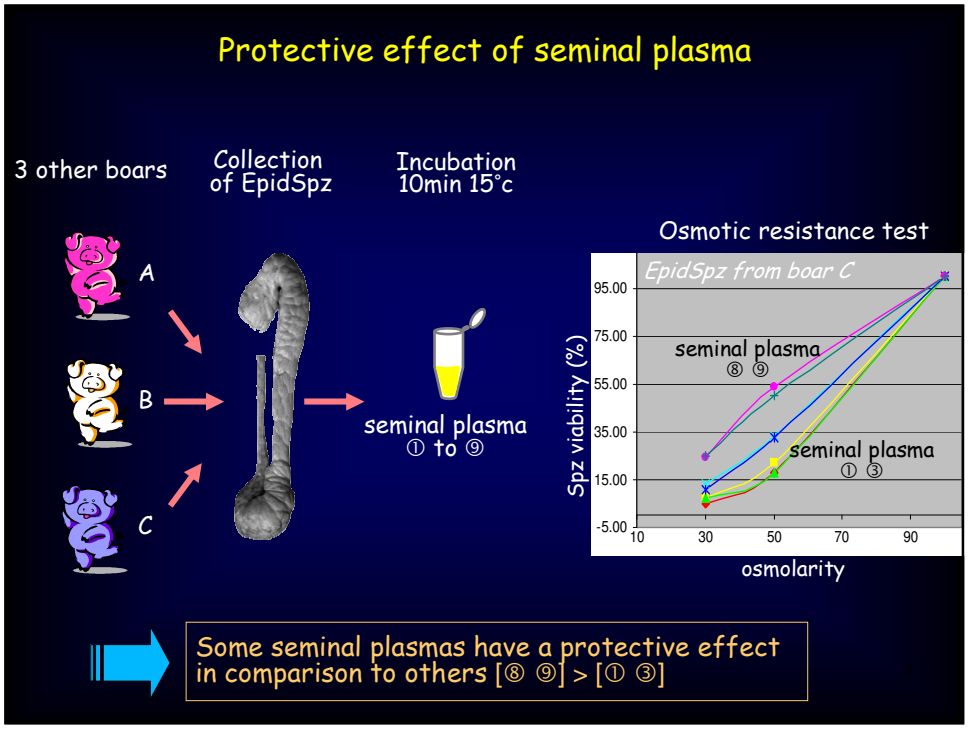
Abstract:

Sperm fertility is progressively acquired during maturation in the genital tract, under the influence of biochemical interactions with paracrine factors secreted within the tract. Thus, it is characterized with modifications of membrane properties, such as fluidity and composition. It has been recently demonstrated that in the boar, hypotonic resistance of ejaculates is positively correlated with in vivo fertility. The aim of the present study was to analyze the effect of seminal plasma on the hypotonic resistance of boar spermatozoa, by using mass spectrometry.
For this purpose, seminal plasma (SP) were collected from several boars with known fertility and stored. Epididymal spermatozoa (EpSpz: n=3 boars) were recovered from cauda epididymis by microperfusion. EpSpz were incubated in the presence of saline or SP for 10min at 37°c before being assessed for hypotonic resistance by flow cytometry, and analyzed by Intact Cells Matrix Mass Spectrometry (ICM-MS) that allow to achieve direct protein profiling.
Our results showed that hypotonic resistance of the 3 populations of EpSpz displayed similar profiles, in relation to seminal plasma in which they had been incubated. Thus, two SP that induced the lowest, and two other that induced the best, hypotonic resistance to EpSpz were selected (SP low and SP high, respectively). EpSpz incubated with SP high or SP low displayed different ICM-MS profiles, some molecular species being increased and other decreased (or absent) between the two groups of SP. Identification of these peptides/proteins is under analysis.
In conclusion, our results demonstrate that hypotonic resistance induced by SP to EpSpz is related to their ICM-MS profiles, and that this approach may be useful to identify novel biomarkers of fertility in the boar.


Ejaculated sperm: Spermatozoa
Seminal plasma
Cowper glands
prostate
seminal vesicles
Testis:
Spermatozoa production
Epididymis:
Spermatozoa maturation


Quality of ejaculated spermatozoa:
Mobility, motility
Osmotic resistance
In the boar:
Osmotic resistance // fertilité (IA)
Osmotic resistance EjacSpz ≠ EpidSpz
→ Druart et al., Reproduction 2009
Bio markers on spermatozoa or in seminal plasma?
Ejaculated Spermatozoa EjacSpz
Seminal plasma
Epididymal Spermatozoa EpidSpz


7 boars
(known fertility)
①
②
③
⑥
⑦
⑧
⑨
EjacSpz and seminal plasma
Osmotic resistance test
Storage
Spz viability (%)
osmolarity
EjacSpz ① ② ⑨
EjacSpz③ ⑦
PS 1
PS 2
PS 3
PS 6
PS 7
PS 8
PS 9
Hypothesis:
- Spermatozoa ① ② ⑨ are more resistant and/or
- their seminal plasma have a protective effect


Protective effect of seminal plasma
3 other boars
Collection of EpidSpz
Incubation 10min 15°c
A
B
C
seminal plasma ① to ⑨
Osmotic resistance test
EpidSpz from boar C
seminal plasma ⑧ ⑨
seminal plasma ① ③
Spz viability (%)
osmolarity
Some seminal plasmas have a protective effect in comparison to others [⑧ ⑨] > [① ③]

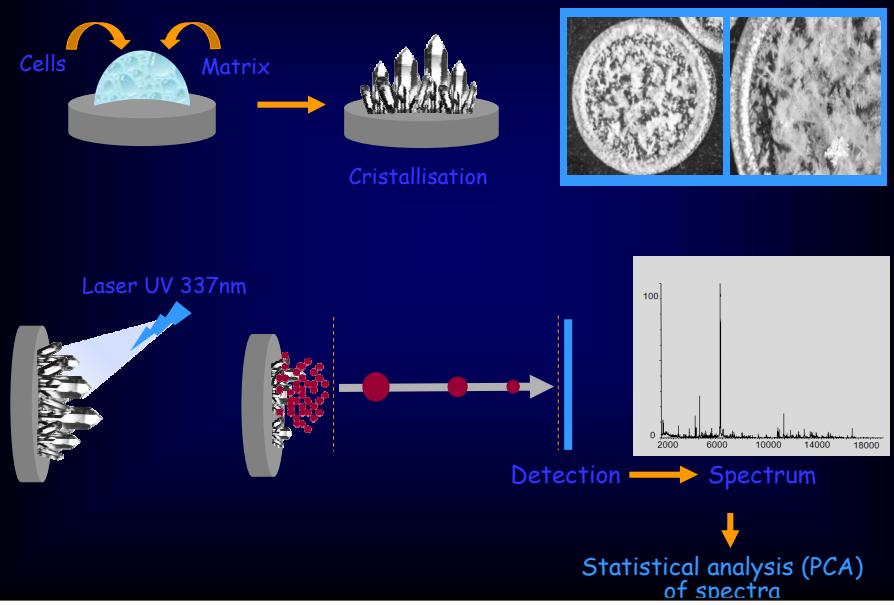
## Where does the protective effect come from ?

Hypothesis:

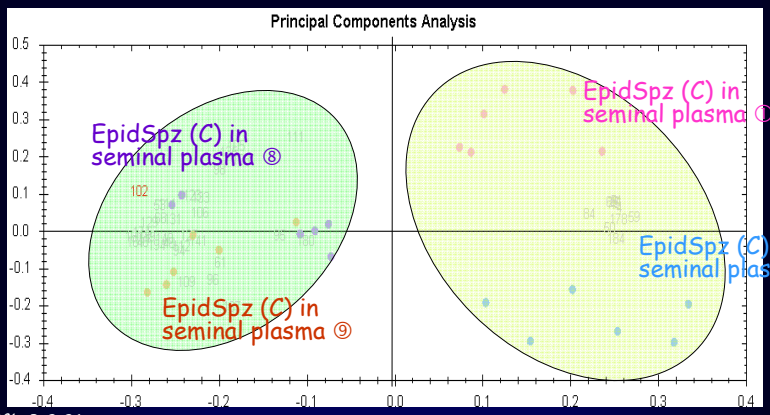
Molecular species are present/absent

⇒ Mass Spectrometry analysis of spermatozoa (ICM-MS) after incubation in seminal plasmas ①③⑧⑨
⇒ SDS-PAGE analysis of seminal plasmas

## ICM-MS …



## Seminal plasmas ⑧⑨ and ③① were discriminated




*Power 100%; P<0,01*

49 discriminant pics
between the 2 « groups » of seminal plasmas
[⑧ ⑨ vs ① ③]

| m/z | Anova (p) | Tags |
|---|---|---|
| 13211,57 | <1.1E-16 | A |
| 13356,79 | 2,55E-015 | A |
| 9217,3 | 1,47E-014 | A |
| 7621,56 | 1,96E-012 | D |
| 5107,19 | **1,80E-010** | D |
| 6797,03 | **2,03E-010** | D |
| 12875,45 | 3,92E-010 | A |
| 3443,46 | 4,41E-010 | A |
| 8910,01 | 1,33E-009 | A |
| 8704,97 | 3,33E-009 | A |
| 7784,72 | 1,35E-008 | D |
| 7736,86 | 1,92E-008 | D |
| **3311,84** | 2,55E-008 | A |
| 4351,74 | 2,80E-008 | A |
| 5970,88 | 6,21E-008 | D |
| **12434,91** | 6,22E-008 | A |
| **8464,03** | 7,65E-008 | A |
| 2156,64 | 1,30E-007 | D |
| 13063,63 | 1,46E-007 | A |
| 7898,94 | 1,98E-007 | D |
| 6097,06 | 4,19E-007 | D |
| 8888,26 | 6,04E-007 | A |
| 13914,26 | 7,87E-007 | A |
| 8563,56 | 9,95E-007 | D |
| 7020,57 | 1,25E-006 | D |
| 10271,35 | 1,44E-006 | A |
| 4818,39 | 1,63E-006 | D |
| 10261,01 | 2,05E-006 | A |
| 7003,16 | 2,73E-006 | D |
| 3398,32 | 3,14E-006 | D |
| 5618,44 | 3,88E-006 | D |
| 6146,01 | 1,39E-005 | A |
| 4741,71 | 1,46E-005 | D |
| 16793,57 | 1,61E-005 | A |
| 5267,09 | 2,38E-005 | A |
| 4285,93 | 2,97E-005 | D |
| 6325,49 | 3,56E-005 | D |
| 5380,77 | 3,89E-005 | D |
| 4016,17 | 5,50E-005 | D |
| 11950,85 | 6,74E-005 | D |
| 5862,1 | 0,00013 | A |
| 8099,63 | 0,00047 | D |
| **6855,23** | 0,00055 | A |
| 5489 | 0,00093 | A |
| 4489,34 | 0,00097 | D |
| 3796,44 | 0,00102 | D |
| 9637,18 | 0,00113 | A |
| 8511,89 | 0,00662 | A |
| 12491,47 | 0,00732 | D |

49 discriminant pics

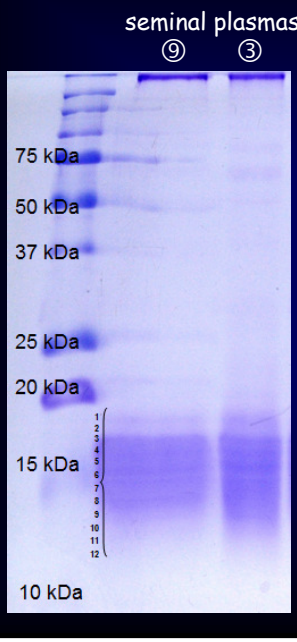
24 ■ : seminal plasmas ⑧⑨ > ①③
with 9 → specific pics to ⑧⑨

25 ■ : seminal plasmas ⑧⑨ < ①③
with 4 → specific pics to ①③

Analysis / identification

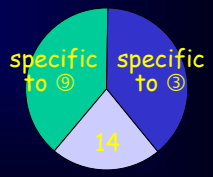
*Spermadhesins*

## SDS-PAGE of seminal plasmas




Analysis
Identification
of 64 proteins
by MS


Spermadhesins (9/64)




# Conclusion

In the boar osmotic resistance of Spz // fertilité (IA)

Osmotic resistance EpidSpz ≠ seminal plasmas ① to ⑨

Protective effect of seminal plasmas [⑧ ⑨] > [① ③]

Disciminated by osmotic resistance test, ICM-MS (PCA) and SDS-PAGE

Osmotic resistance / ICM-MS profiles (discriminant and specific pics) / SDS-PAGE profiles (bands)

Involvement of spermadhesins

Identification and functional studies