



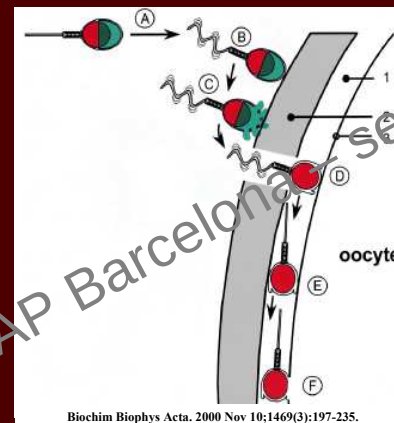
# “Functional characterization of equine sperm: a molecular evaluation of stallion fertility”

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## • Fertilization



Functionally  
intact



## Variability

- between breeds  
PSL  $\approx$  Others  
PSL  $\neq$  Sorraia



- between stallions  $\rightarrow$  intra-stallions

## Reproductive indexes

- sperm motility and vitality



- A single ejaculate is not enough to define sperm quality

- One ejaculate/Stallion:

N=152 ejaculates

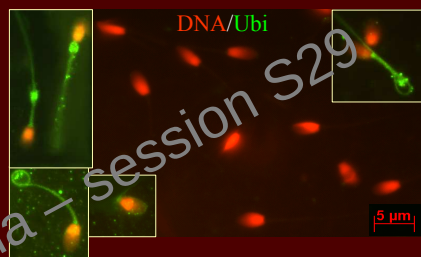
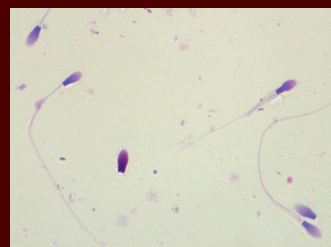


- Multiple ejaculates/stallion:

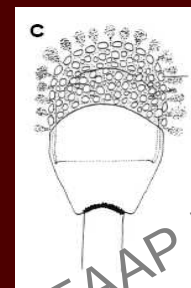
371 sperm samples (PSL)  
112 sperm samples (Sorraia)  
361 sperm samples (Others)

PMS AC ( $P < 0.001$ )

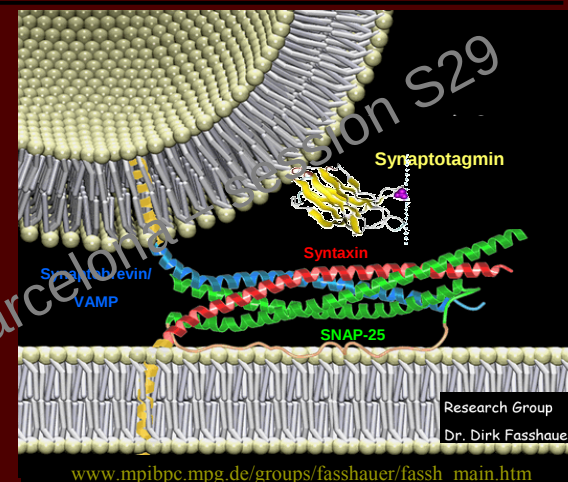
Morphology ( $P < 0.001$ )



| Spermatozoa     | A<br>Fertile              | B<br>Fertile             | C<br>Sub-fertile         | D<br>Infertile           |
|-----------------|---------------------------|--------------------------|--------------------------|--------------------------|
| Ubiquitin stain | 12.95±7.47 <sub>a,b</sub> | 8.50±2.70 <sub>a,b</sub> | 37.68±12.43 <sub>c</sub> | 30.44±16.51 <sub>b</sub> |
| Negative stain  | 16.09±3.86 <sub>b</sub>   | 11.33±3.5 <sub>b</sub>   | 55.82±10.85 <sub>c</sub> | 27.55±7.97 <sub>b</sub>  |

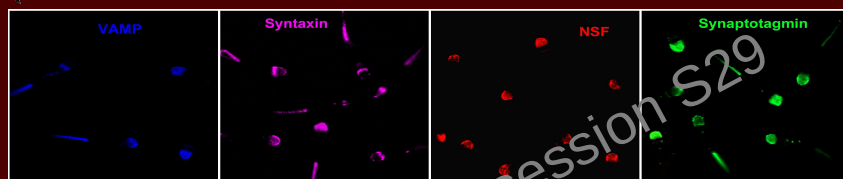


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Research Group  
Dr. Dirk Fasshauer

[www.mpibpc.mpg.de/groups/fasshauer/fassh\\_main.htm](http://www.mpibpc.mpg.de/groups/fasshauer/fassh_main.htm)

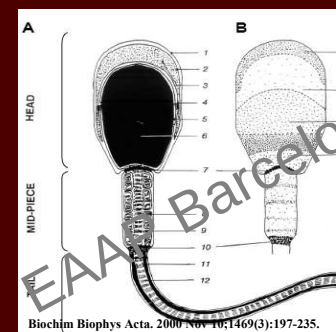


| Stallion       | VAMP/<br>Synaptobrevin | Syntaxin      | NSF                | Synaptotagmin      |
|----------------|------------------------|---------------|--------------------|--------------------|
| A              | 74.47±11.59 b          | 72.68±11.64 c | 66.46±8.97         | 85.43±8.20 d       |
| B              | 34.80±6.38             | 43.62±7.72    | 48.60±8.17         | 81.00±8.83 d,e     |
| C              | 19.17±8.13             | 29.95±5.45    | 32.00±3.95         | 56.20±9.26         |
| D              | 60.39±11.09 b          | 68.75±6.09 c  | 19.33±5.86         | 71.50±1.92 e       |
| Fertile        | 56.44±22.64            | 59.26±18.70   | 58.34±12.40<br>*** | 83.39±8.45<br>***  |
| In(sub)fertile | 35.74±25.50            | 43.07±22.81   | 27.78±7.65<br>***  | 63.00±10.45<br>*** |

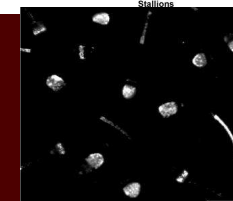
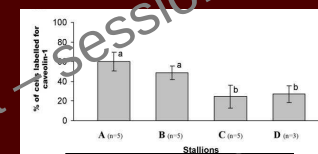
b,g,d,e Within a column, same letter denotes no differences between stallions (P<0.05)  
\*\*\* denotes differences (<0.001) between groups (P<0.05)

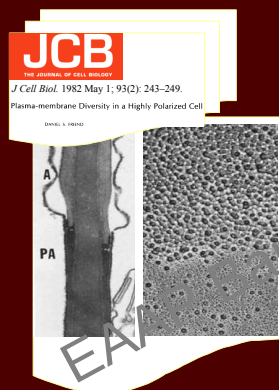


- Highly regionalized  
Macro/Micro- membrane domains



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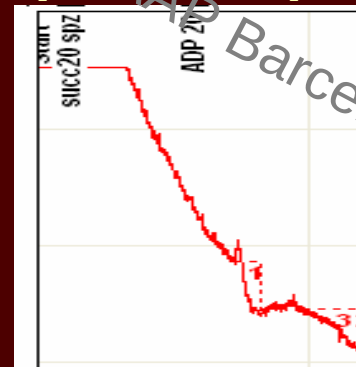




- Substrates  
(maintenance of energy status)
  - Glucose: [2.97 – 6.21] mg mL<sup>-1</sup>
  - Fructose: [0.04 – 0.36] mg mL<sup>-1</sup>



Energy production (ATP):  
O<sub>2</sub> consumption (% O<sub>2</sub>/sec)



MMP (JC-1)



↑Δψ<sub>m</sub>

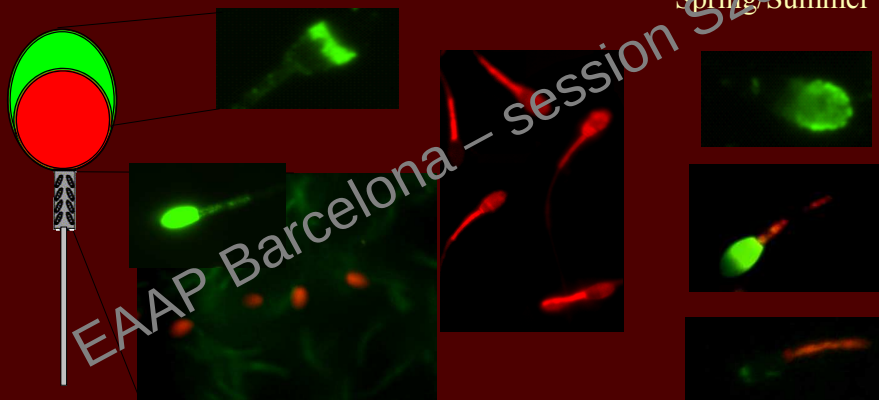


↓Δψ<sub>m</sub>



Autum/Winter

Spring/Summer



• ROS - Lipid (TBARS) and Protein (DNPH) oxidation

•fertile animals

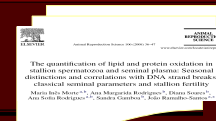
TBARSSp - sperm morphology (r=0.45, p<0.001)

DNPHspz - sperm vitality (r=0.39, p<0.001)

•Sub-fertile animals

TBARSSp - sperm morphology (r=0.48, p<0.001)

DNPHspz - sperm motility (r=0.34, p<0.05)





## • ROS

## • DNA damage



The quantification of lipid and protein oxidation in stallion spermatozoa and seminal plasma: Spermatozoal distinctions and correlations with DNA strand breaks, classical seminal parameters and stallion fertility. Aires-Brito, Manuel<sup>1,2</sup>, Ana Margarida Rodrigues<sup>3</sup>, Diana Soares<sup>2</sup>, Ana Sofia Santos<sup>2</sup>, Cátia Baptista<sup>2</sup>, Liliana Henriques<sup>2</sup>, Maria Inês Morte<sup>2</sup>, Margarida Rodrigues<sup>2</sup>, Patrícia Cortês<sup>2</sup>, Conceição Cravo<sup>2</sup>, Manuel Vaz de Castro<sup>2</sup>, Miguel Moura<sup>2</sup>, Fernando Serrador<sup>2</sup>, Pedro Neto<sup>2</sup>, Jorge Oliveira<sup>2</sup>, Pedro Pinto Bravo<sup>2</sup>, Rosa Rebordão<sup>2</sup>.



## ↑Fertility

IP- JC1+ PSA-  
55.68% spz

## ↓Fertility

IP- JC1+ PSA-  
48.52% spz

|            | FC (%) | FFE (%) |
|------------|--------|---------|
| ↑Fertility | 68.42  | 100.00  |
|            | 78.57  | 100.00  |
|            | 64.29  | 96.43   |
|            | 35.09  | 67.06   |
| ↓Fertility | 31.25  | 55.56   |
|            | 7.69   | 25.00   |



- Discrete data
  - different subpopulations
  - complexity and heterogeneity
  - levels of functionality of sperm cells
- Stain measurements of cell structure/function
  - biochemical & physiological characteristics of individual cells
  - heterogeneity present in a population
  - several sperm properties in a single sample



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