Cyclin/CdK COMPLEXES ARE INVOLVED IN CONTROL OF ACTIN DYNAMICS DURING BOAR SPERM CAPACITATION

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INTRODUCTION

Mammalian spermatozoa are virtually infertile immediately after ejaculation and will only reach their full fertilizing ability after they reside within the female genital tract for hours to days, depending on the species. This process of capacitation implies marked changes in the whole biochemical machinery expressed by spermatozoa. Thanks to the adoption of high-throughput technologies (Chronowska, 2014) it was demonstrated that male gametes express proteins involved in cell cycle control, that are thought to be not present or active in sperm cells (Hydbring et al., 2016). Here, we used an in silico approach based on the application of networks theory to identify specific proteins that could play a central role in signal transduction active during sperm capacitation and afterwards we tested the hypothesis with an in vitro model.

MATERIALS AND METHODS

- In silico experiments were carried out using Reactome as a data source and Cytoscape 3.3.0 to represent the molecules involved in cell cycle.
- **Semen samples** were collected and processed using a validated protocol and spermatozoa were incubated under capacitated conditions with or without Aminopurlavanol A (AA) during 4 hours
- **PSA** and **Phalloiding** staining were performed to evaluate under fluorescence microscopy, respectively, the increased induction of **acrosome reaction** and the effects on **actin polymerization** produced by AA.
- DilC12(3) staining and **FRAP** (fluorescence recovery after photobleaching) analysis with confocal microscopy were carried out to assess changes in **membrane fluidity**
- **IVF** experiments were performed by obtaining healthy oocytes of follicules isolated from boar ovaries and incubating them with healthy boar spermatozoa treated and untreated with AA. Oocytes were stained with HOECHST 3324 to check the success of fertilization.
- Western blotting analysis using anti-Phospho-p44/42 MAPK (Erk1/2) and HRP-conjugated anti-rabbit IgG antibodies were performed with sperm homogenates obtained at different times. Proteins were detected by ECL and immunoblot bands were analysed by ImageQuant™ TL.

IN SILICO EXPERIMENTS

Network realization, analysis and visualization

We realized a network (CCN, Cell Cycle Network) representing the molecules involved in cell cycle and its control. There were two connected components, one of 2 nodes and 1 edge and the other of 331 nodes and 10419 edges. The most linked node was CDK1 (cdk1 protein) and the nodes showing a higher score were CDK1, CCNB1 and CCNA2. To predict the effects of Aminopurvalanol A on network topology we removed from CCN the following records: CDK1/CCNB; CDK2/CCNA; CDK5/p25; CDK4/CCND.

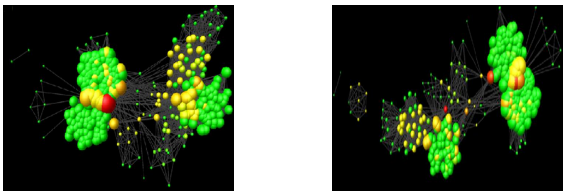
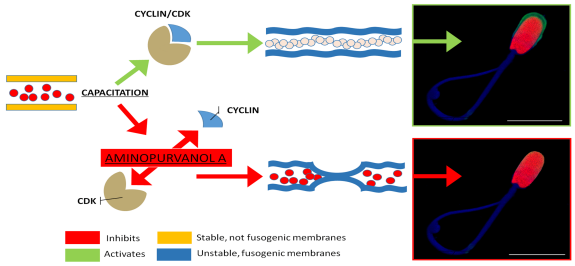


Figure 1. Network models realized. At left, CCN. At right, network resulting after the removal of the nodes affected by AA from CCN. Represented with the Perfuse Force Directed Layout, and the node diameter proportional to the node degree. The node color depends on the betweenness centrality (green = lower values, red = higher values).

EXPERIMENTAL DESIGN



IN VITRO EXPERIMENTS AND RESULTS

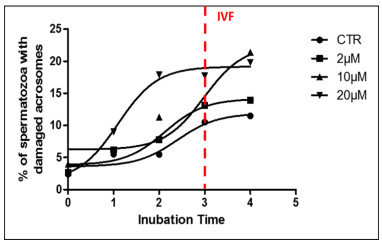


Figure 2. Effects of AA in acrosome integrity. Effects of Aminopurvalanol A at different concentration and incubation times on the percentage of spermatozoa with absent or damaged acrosome.

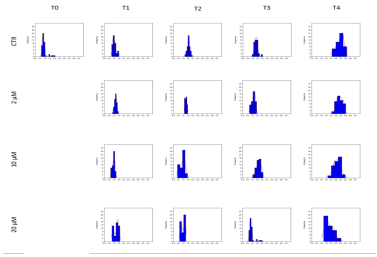


Figure 3. Actin polymerization kinetics. AA negatively affect the increase of F-actin amount during spermatozoa incubation under capacitation conditions.

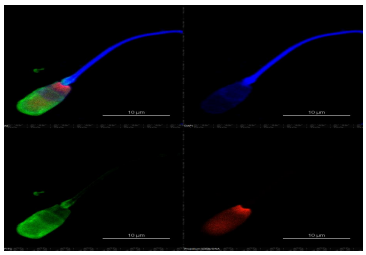


Figure 4. Confocal analysis of actin subcellular localization. AA affect the increase of fluorescence emission in the anterior area of sperm head.

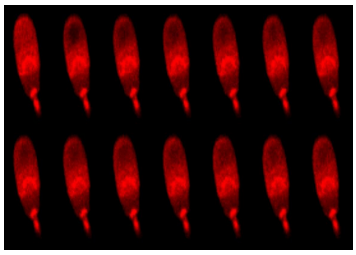


Figure 5. FRAP analysis. AA seems to do not affect the physico-chemical properties of sperm membranes.

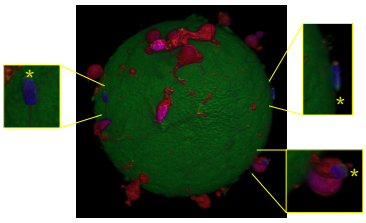


Figure 6. Sperm binding oocyte.

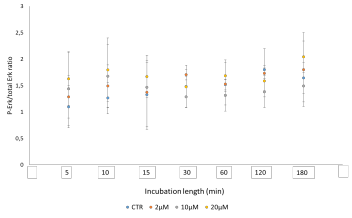


Figure 7. Erk1/2 activation. AA seems to do not affect Erk1/2 activation by analysing P-Erk/total Erk ratios.

CONCLUSIONS

- The adoption of a **biological network-based approach** allowed us to infer new and interesting **processes involved in sperm capacitation,** demonstrating the utilizy of computational modelling strategies in exploring cell signalling systems.
- AA effects on actin cytoskeleton rearrangement during capacitation were documentated, suggesting **Cyclin/Cdk complexes as a new element in control system of actin polymeryzation**
- These findings open the way for further studies that could give new information related to the physiology and pathology of sperm capacitation.

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