Membrane remodelling events occurring during mammalian spermatozoa capacitation: A systems biology study

Nicola Bernabò[a], Luana Greco[a], Alessandra Ordinelli[a], Mauro Maccarrone[b,c*], Barbara Barboni[a*]

[a] Faculty of Veterinary Medicine, University of Teramo, Teramo, 64100, Italy
[b] Center of Integrated Research, Campus Bio-Medico University of Rome, Rome, 00128, Italy
[c] European Center for Brain Research (CERC), Santa Lucia Foundation, Rome, 001743, Italy
*Equally senior authors. E-mail: nbernabo@unite.it

Keywords: spermatozoa , capacitation, biological networks.

Immediately after ejaculation, mammalian spermatozoa are unable to fertilize the homologous oocyte. They reach their fertilizing ability only after they reside for hours to days, depending on the species, within the female genital tract. Here a complex process, the capacitation, takes place as a consequence of the functional dialogue of activating (hormones, bicarbonate, calcium ions, etc…) and inhibiting (seminal plasma, endocannabinoids) factors present in female genital tract fluids. This process involves virtually all the component of male gametes: the intracellular pH rises, the ionic composition of cytosol changes, the proteins tyrosine phosphorylation pattern modifies, the actin cytoskeleton reorganizes, and the sperm membranes change their physical/chemical proprieties[1]. In particular, the cholesterol/phospholipids ratio decreases, the microdomains architecture reorganizes, and the plasma membrane (PM) anisotropy decreases, then PM becomes more instable and fluid[2]. These lasts events attracted the attention of researchers because of their possible implication in the determinism of "idiopathic infertility" of male origin, which is at the present one of the most important causes of fertilization failure. In addition, recently, has emerged the intriguing question of interconnection of signaling systems involved in capacitation and those expressed in apoptosis[3].

To study the structure of signaling systems involved in the lipid remodeling process that occurs during capacitation, we applied a systems biology approach. In particular, we realized a computational model of lipid remodeling, by using a biological networks-based modeling strategy: each molecule involved in that process was represented as a node, each interaction between molecules was represented as a link[4].

The database representing the lipid remodeling of spermatozoa and endocannabionid system have been *de novo* manually compiled. The one concerning apoptosis has been downloaded from Reactome, version v51 (http://www.reactome.org/), a free, open-source, curated and peer reviewed pathways database. All the networks have been realized with Cytoscape 3.1.1 (http://www.cytoscape.org/), an open source software for visualizing and integrating complex networks. All the analysis have been carried out with the plug-in Network Analyzer (http://apps.cytoscape.org/apps/networkanalyzer).

As a result, we obtained a network that contains a single connected component composed by 244 nodes and 418 links. The in- and out-node degree, i.e. the probability distribution of the number of connection per node, followed the exponential law (exponent = -1.522 and -1.792 respectively), while the clustering coefficient, i.e. the measure the network tendency to form clusters, was near 0 (0.061). The node degree was correlated with the centrality of nodes within the network (r=0.802), expressed as beetweenness centrality. i.e. as the number of shortest paths from all vertices to all others that pass through a node. The values of characteristic path length was 6.373, while that of the average number of neighbors was 3.287.

The statistical analysis of the obtained network clearly showed that it has a scale free topology and a small word structure. This specific feature could lead to take some important biological inferences. Firstly, it implies that the most of nodes is scarcely linked, while only a few nodes, the hubs, are highly connected. In other words the network is robust against random failure[4]. The low value of clustering coefficient, together with the values of averaged number of neighbors and of characteristic path length suggest that the messages will spread within the networks quickly and efficiently. Finally, it is possible to identify the nodes that show a higher level of control within the networks, i.e. the most connected ad central ones, listed in decreasing order: [Ca2+]I, ATP, Protein tyrosine Phosphorilation, PKA, cholesterol efflux, apoptosis, scramblase, acquisition of fertilizing ability, PKC, cAMP, AEA, membrane symmetry, oxysterols, sAC, cholesterol, pHi rise, PLD1, CaV, acrosome reaction, CB1, NADH.

In our opinion, these findings could contribute to the knowledge of such an important event, which leads the spermatozoa to gain their fertilizing ability, and allows identifying the molecular mechanisms involved in the final fate of spermatozoa and in the check points involved.

[1] Gadella and Luna C, Theriogenology. 81 (2014) 74-84.
[2] Barboni et al., PLoS One 2011;6:e23038.
[3] Aitken Biol Reprod. 85 (2011) 9-12.
[4] Bernabò et al.,. BMC Syst Biol. 4 (2010) 87.

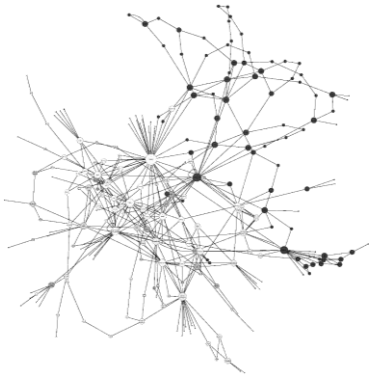

Figure 1. network representing the lipid remodeling that occurs during mammalian capacitation in spermatozoa

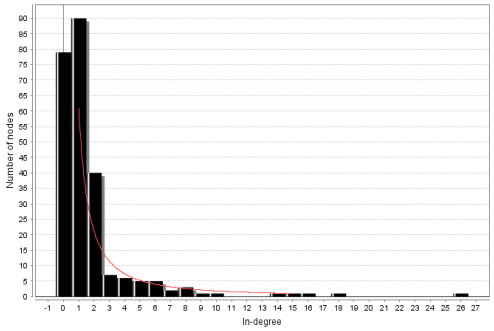

Figure 2. In-degree distribution.

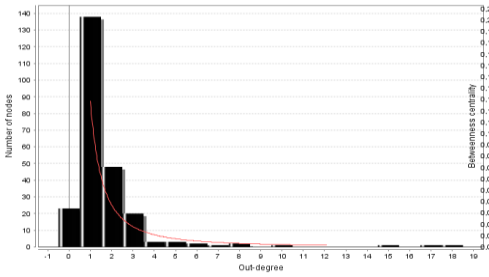

Figure 3. Out-degree distribution.

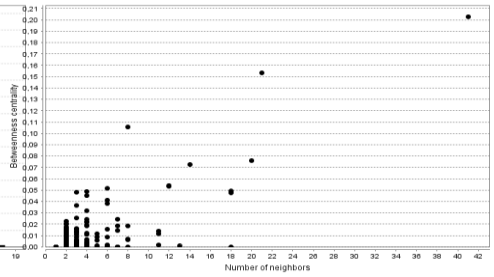

Figure 4. Betweenness centrality vs. degree.