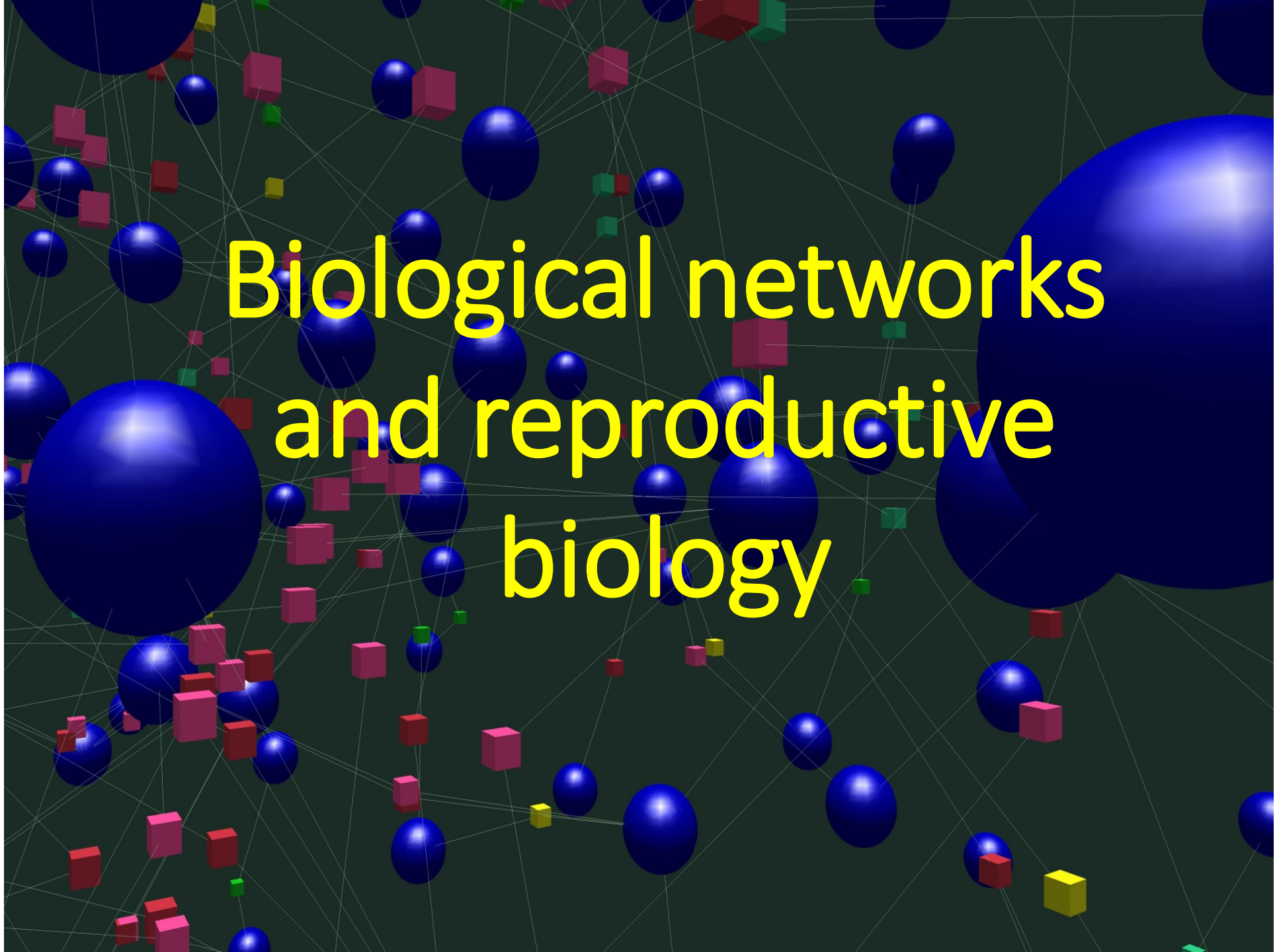
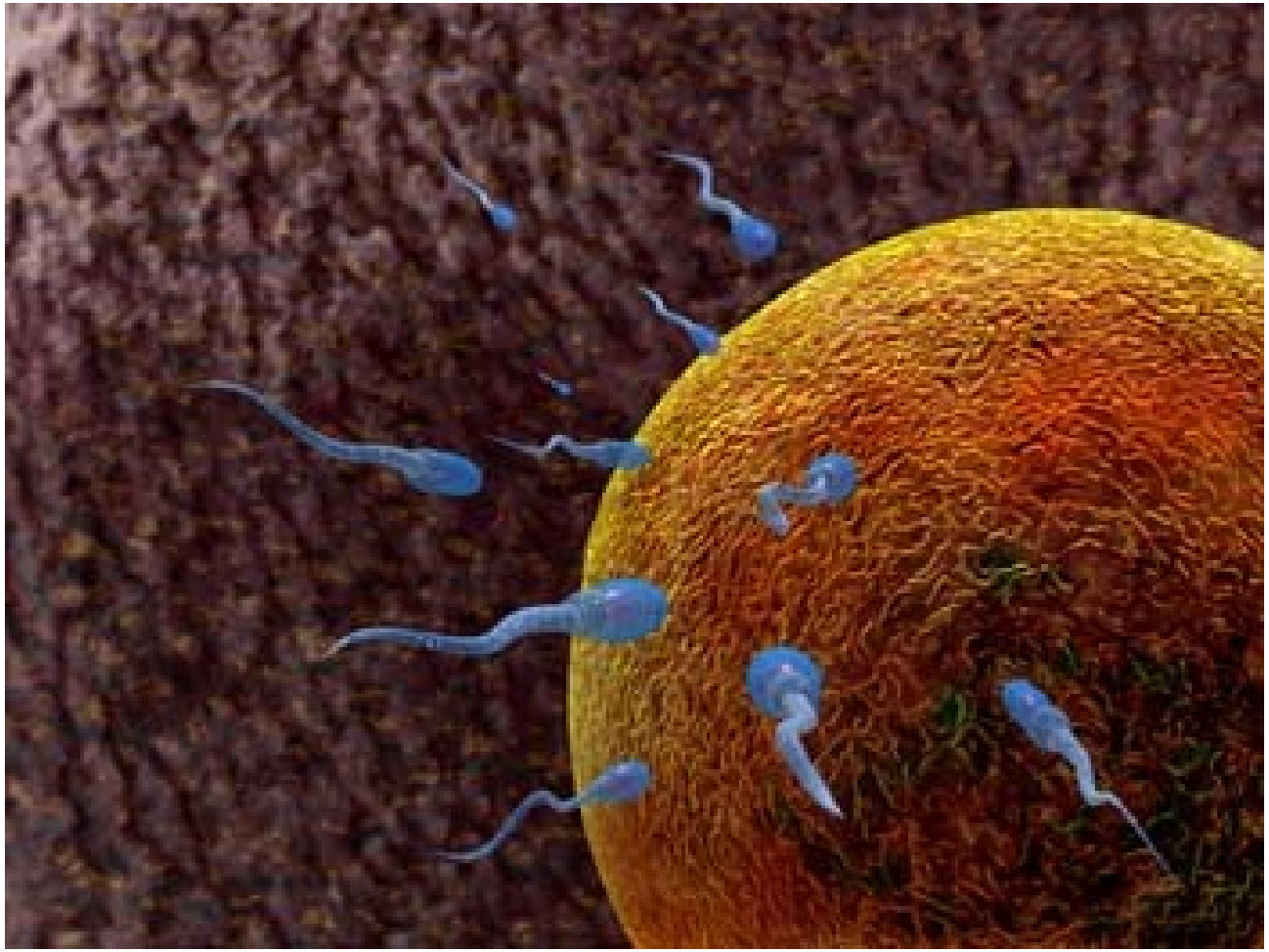
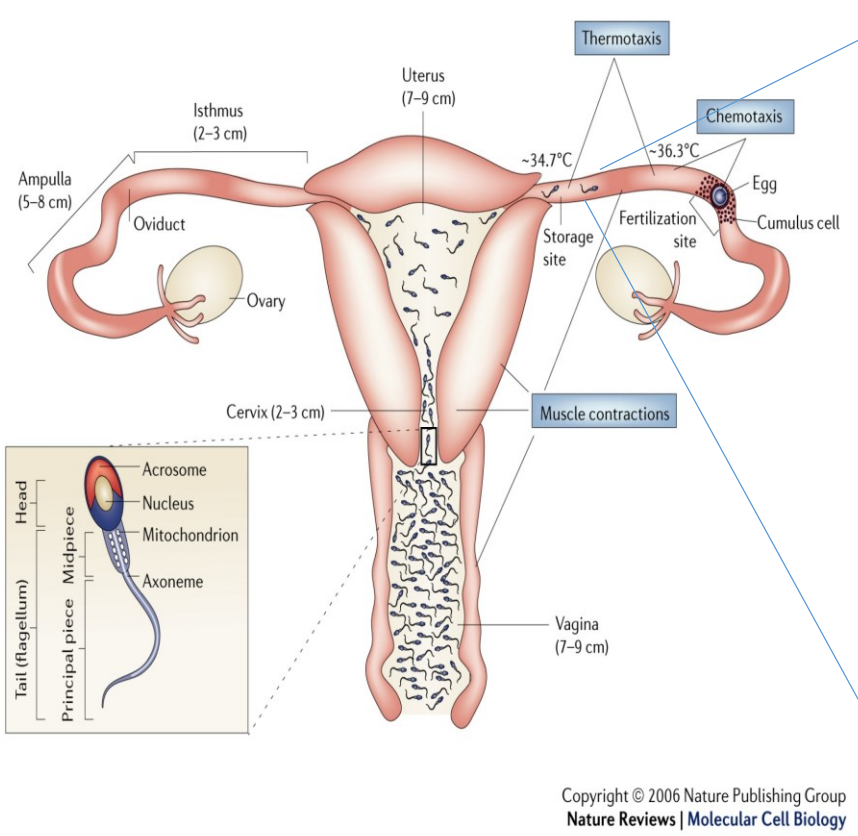
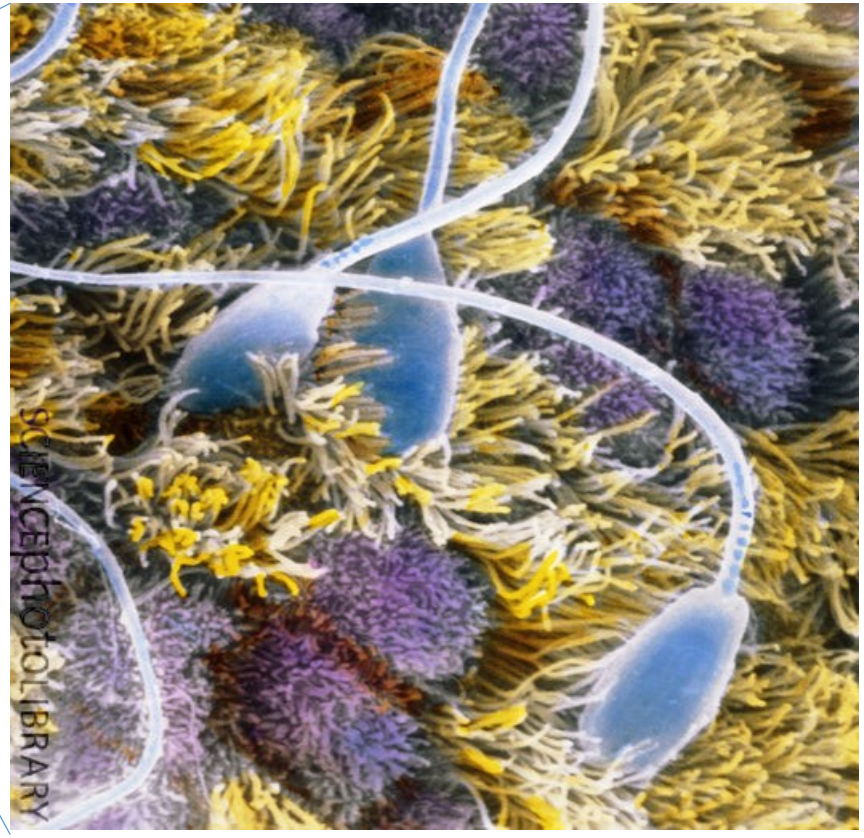

Biological networks
and reproductive
biology

Fertilization is a complex event

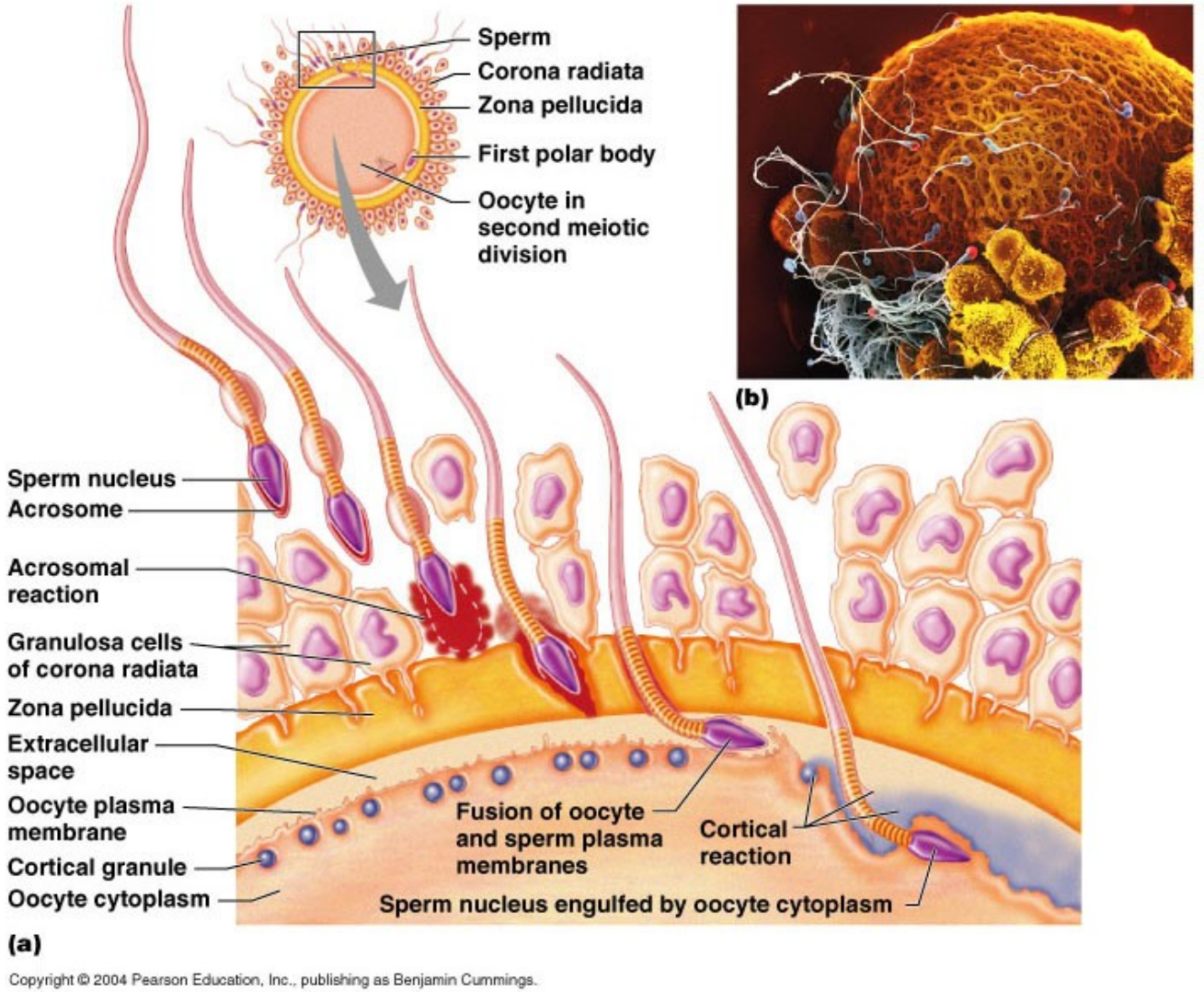


capacitation





Acrosome reaction



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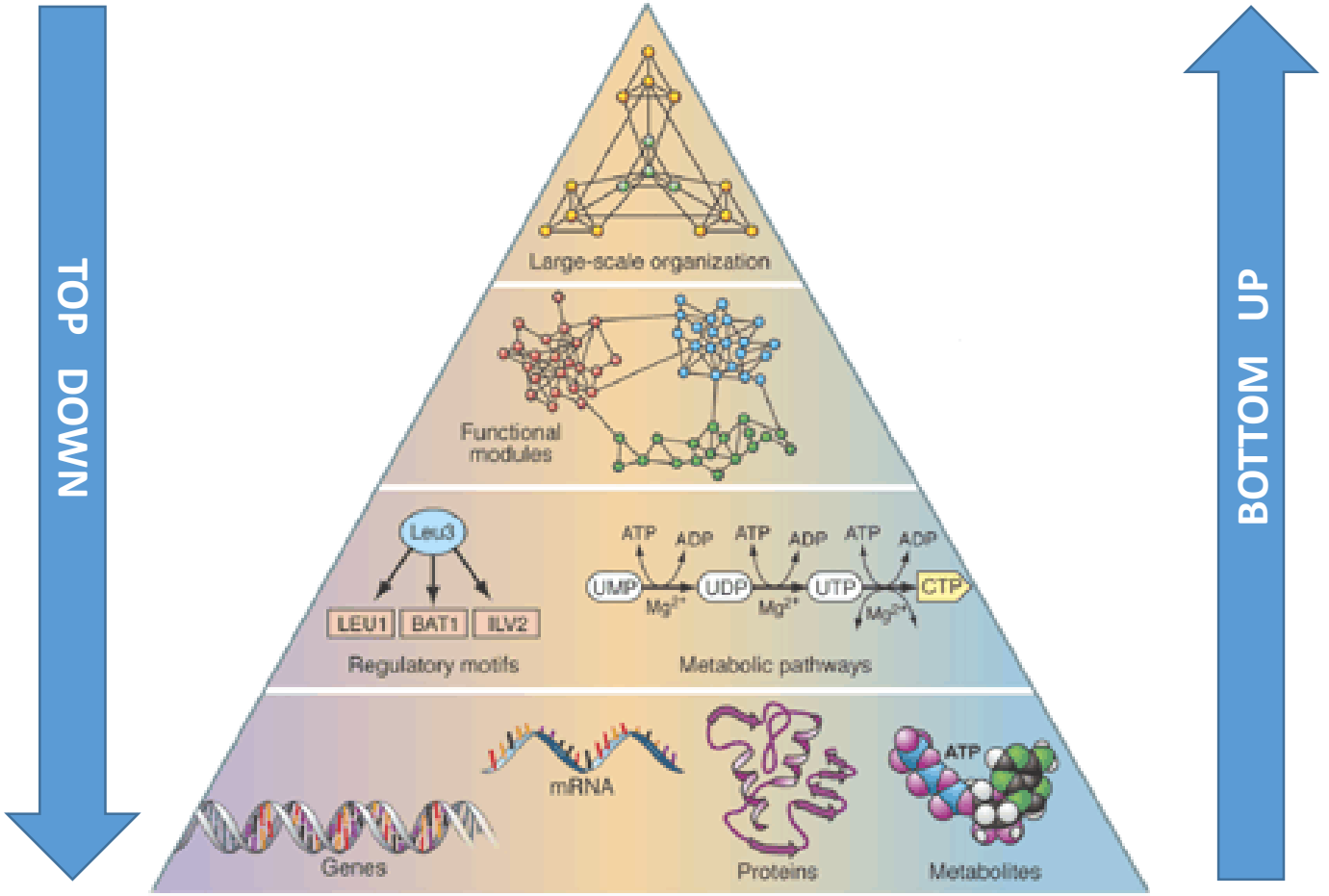
fertilization

Systems biology and reproduction



The model

Bernabò *et al. BMC Systems Biology* 2010, **4**:87
http://www.biomedcentral.com/1752-0509/4/87

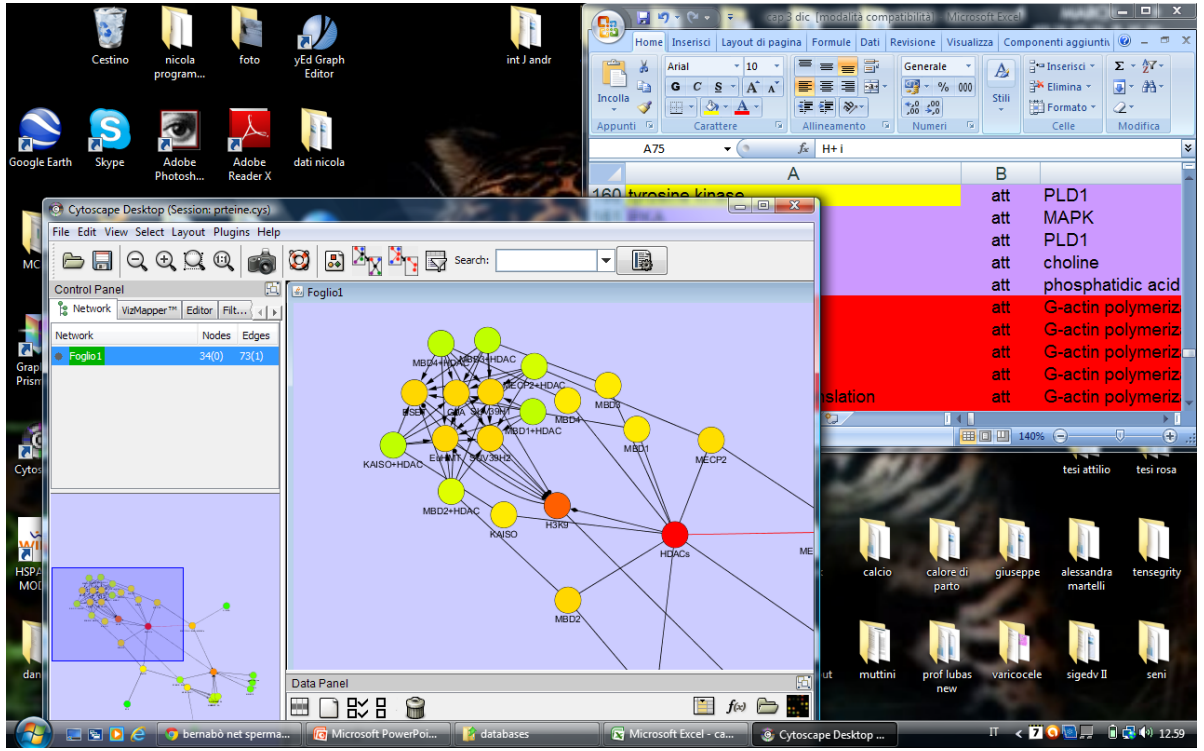
BMC
Systems Biology

RESEARCH ARTICLE Open Access

The spermatozoa caught in the net: the biological networks to study the male gametes post-ejaculatory life

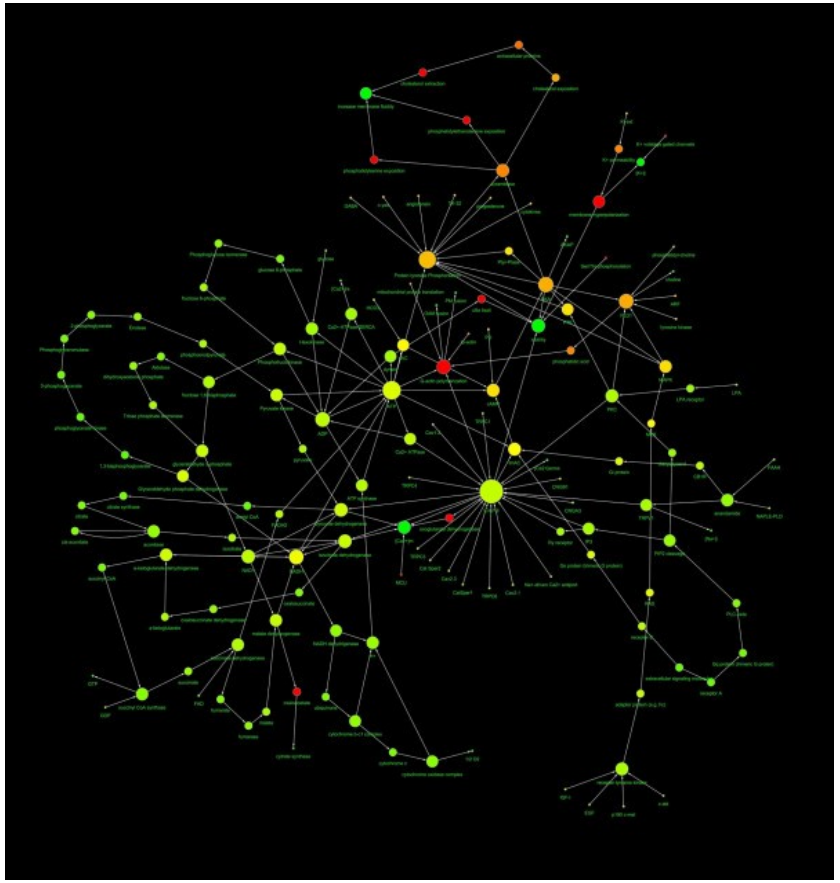
Nicola Bernabò*, Mauro Mattioli and Barbara Barboni

Highly accessed

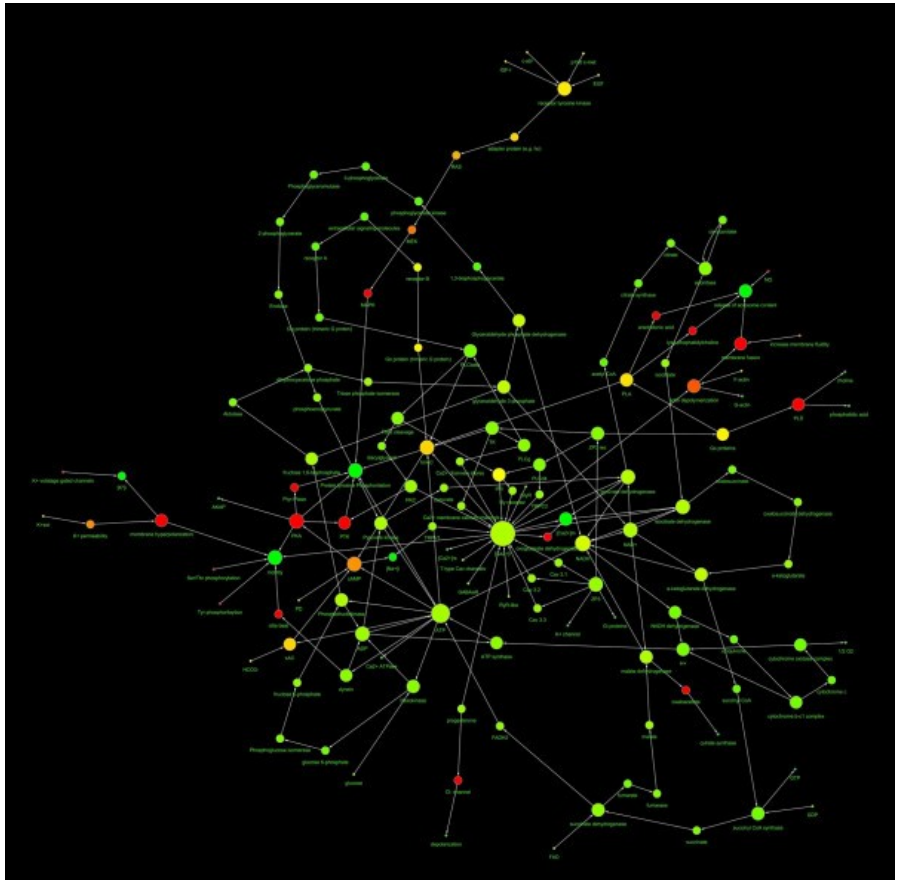


The networks

Capacitaton



Acrosome reaction



Networks topology

Table 1: Main topological parameters of capacitation and AR networks

	capacitation	AR
N°nodes	146	141
N°edges	197	191
Clustering coefficient	0.029	0.026
Diameter	20	20
Averaged n°neighbours	2.667	2.695
Char. path length	6.606	6.736

The number of nodes represent the total number of molecules involved, the number of edges represents the total number of interaction found, the clustering coefficient is calculated as $Cl = 2nl/k(k\text{-}1)$, where nl is the number of links connecting the kl neighbours of node l to each other, the network diameter is the largest distance between two nodes, the Averaged n°neighbours represent the mean number of connection of each node, the Char. path length gives the expected distance between two connected nodes.

Table 2: Result of power law fitting of IN and OUT capacitation and AR networks

	capacitation		AR	
	in	out	in	out
r	0.992	0.997	0.992	0.989
R^2	0.897	0.837	0.906	0.823
b	-1.547	-2.046	-1.657	-2.303

Table 3: Most connected nodes (the hubs) of capacitation and AR networks

Network	Node	Number of links
capacitation	$[Ca^{2+}]_i$	25
capacitation	ATP	14
capacitation	Tyr phosphorylation	13
capacitation	PKA	9
capacitation	ADP	8
capacitation	PLD1	8
AR	$[Ca^{2+}]_i$	23
AR	ATP	13

Hubs removal

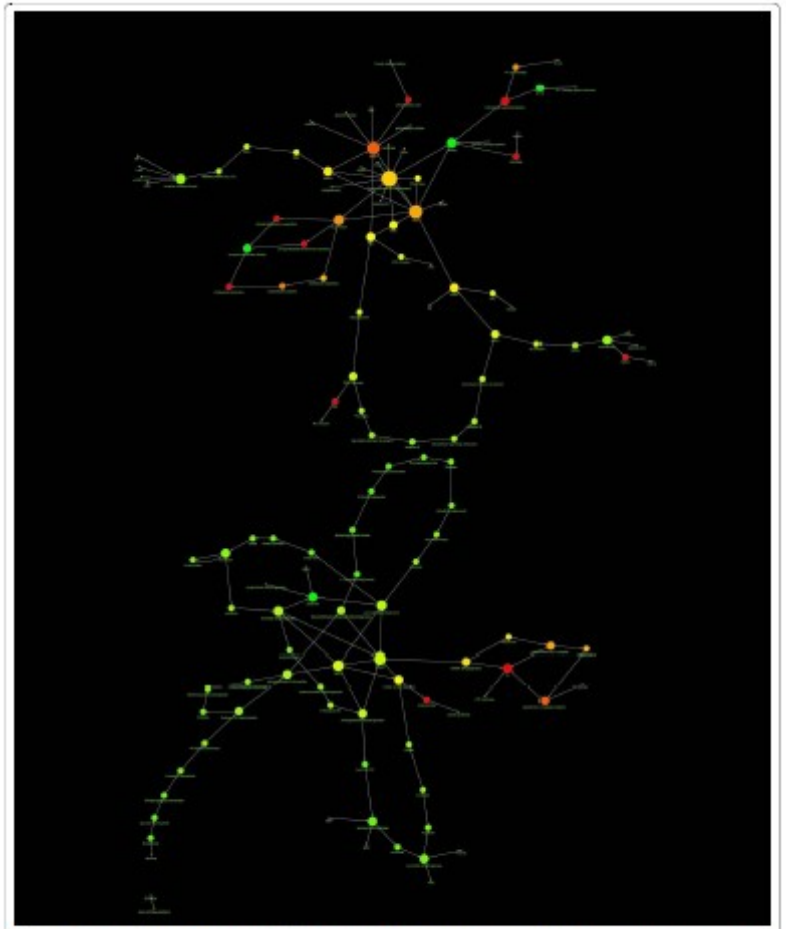


Figure 4 Diagram showing the effect of the elimination from capacitation network of the most linked nodes. The elimination from capacitation network of the most linked nodes ([Ca^{2+}]$_i$ and ATP-ADP) caused the collapse of network structure.

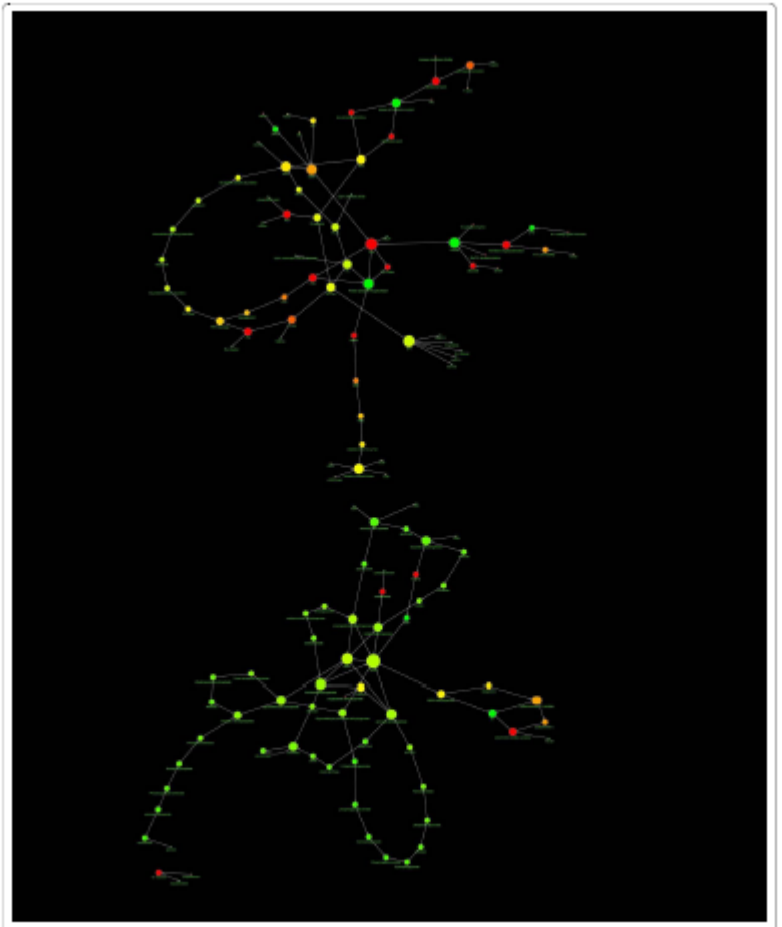


Figure 5 Diagram showing the effect of the elimination from AR network of the most linked nodes.
The elimination from AR network of the most linked nodes ([Ca^{2+}]$_i$ and ATP-ADP) caused the collapse of network structure.

Common elements

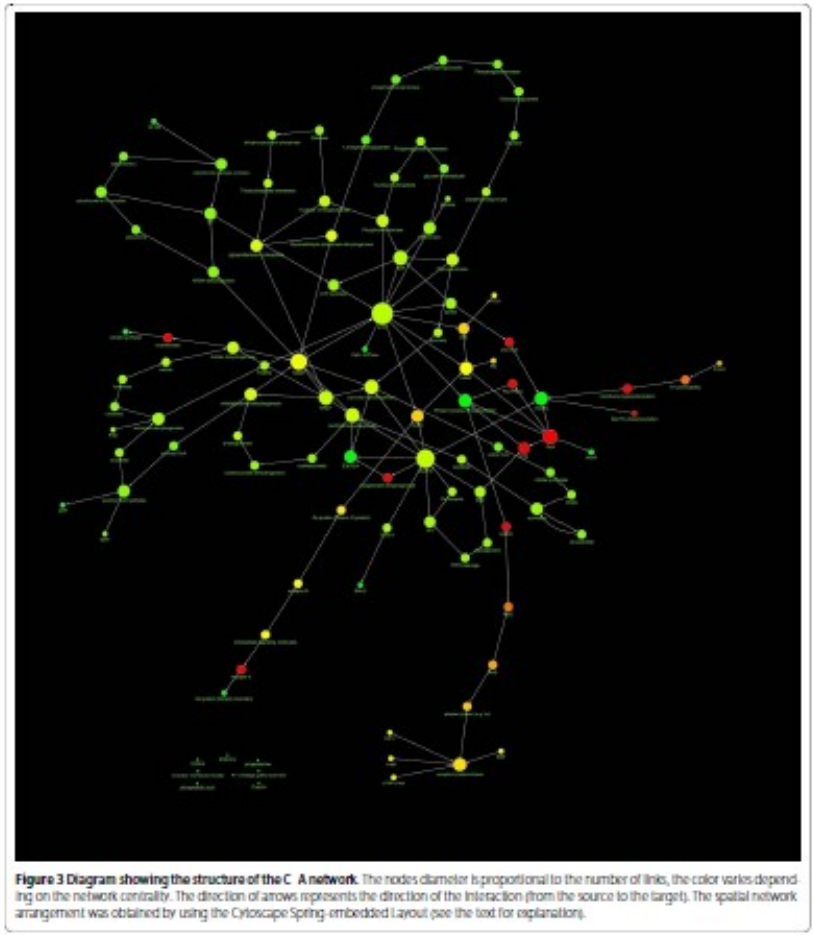


Figure 3 Diagram showing the structure of the C A network. The nodes diameter is proportional to the number of links, the color varies depending on the network centrality. The direction of arrows represents the direction of the interaction (from the source to the target). The spatial network arrangement was obtained by using the Cytoscape Spring-embedded Layout (see the text for explanation).

Table 4: Main topological parameters and the most connected nodes of C A network

	C A
N°nodes	109
N°edges	143
Clustering coefficient	0.036
Diameter	20
Averaged n°neighbours	2.606
Char. path length	6.957
IN degree distribution	b = -1.829 r = 0.997 R^2 = 0.948
OUT degree distribution	b = -2.240 r = 0.992 R^2 = 0.894
Hub (n°edges)	ATP (13); Ca^{2+} (12)

The number of nodes represent the total number of molecules involved, the number of edges represents the total number of interaction found, the clustering coefficient is calculated as $Cl = 2nl/k(k-1)$, where nl is the number of links connecting the kl neighbours of node l to each other, the network diameter is the largest distance between two nodes, the Averaged n°neighbours represent the mean number of connection of each node, the Char. path length gives the expected distance between two connected nodes.

Consequently …

- It is possible to represent the biological events involved in reproduction as networks models;
- They are scale free networks;
- The have a ultra small-world topology;
- It is possible to take important inerences.

Experimental validation of the model

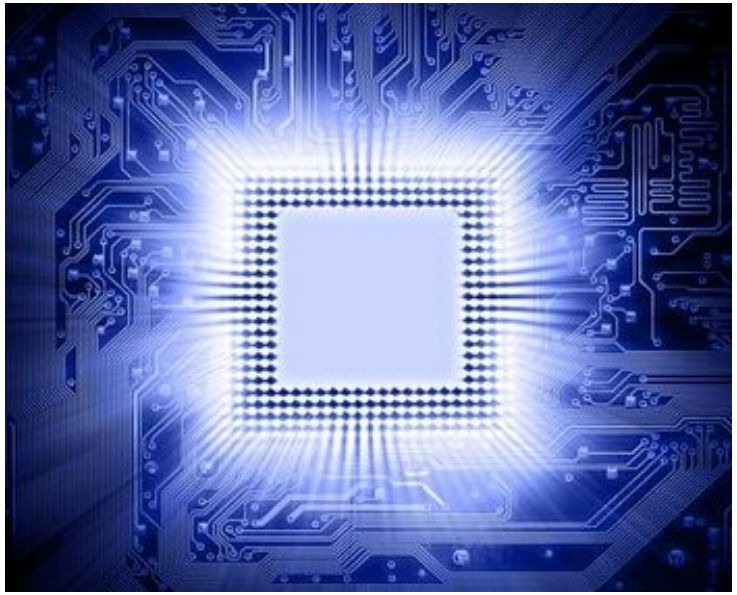
Bernabò *et al. BMC Systems Biology* 2011, **5**:47
http://www.biomedcentral.com/1752-0509/5/47

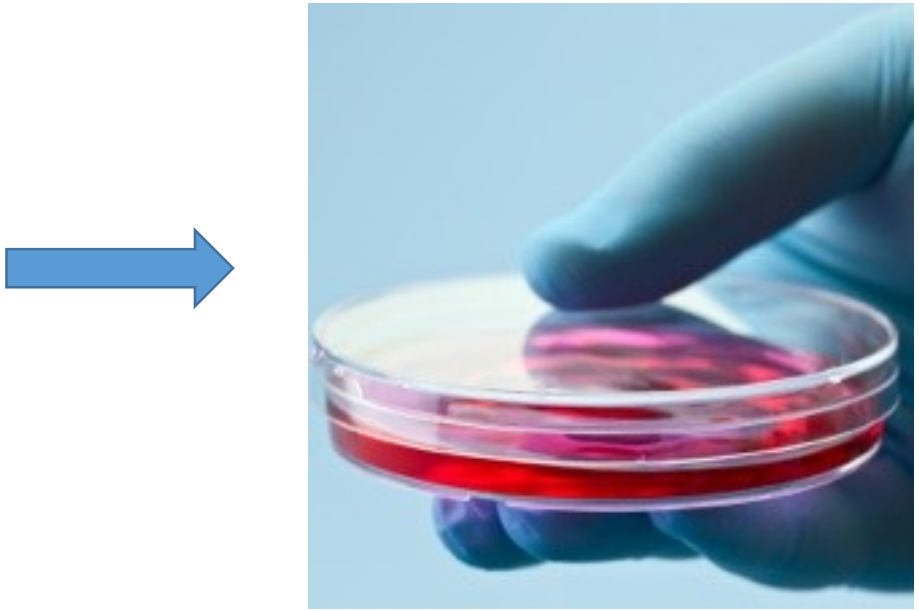
BMC Systems Biology

RESEARCH ARTICLE Open Access

The role of actin in capacitation-related signaling: an in silico and in vitro study

Nicola Bernabò*, Paolo Berardinelli, Annunziata Mauro, Valentina Russo, Pia Lucidi, Mauro Mattioli and Barbara Barboni





Organization of signalig systems

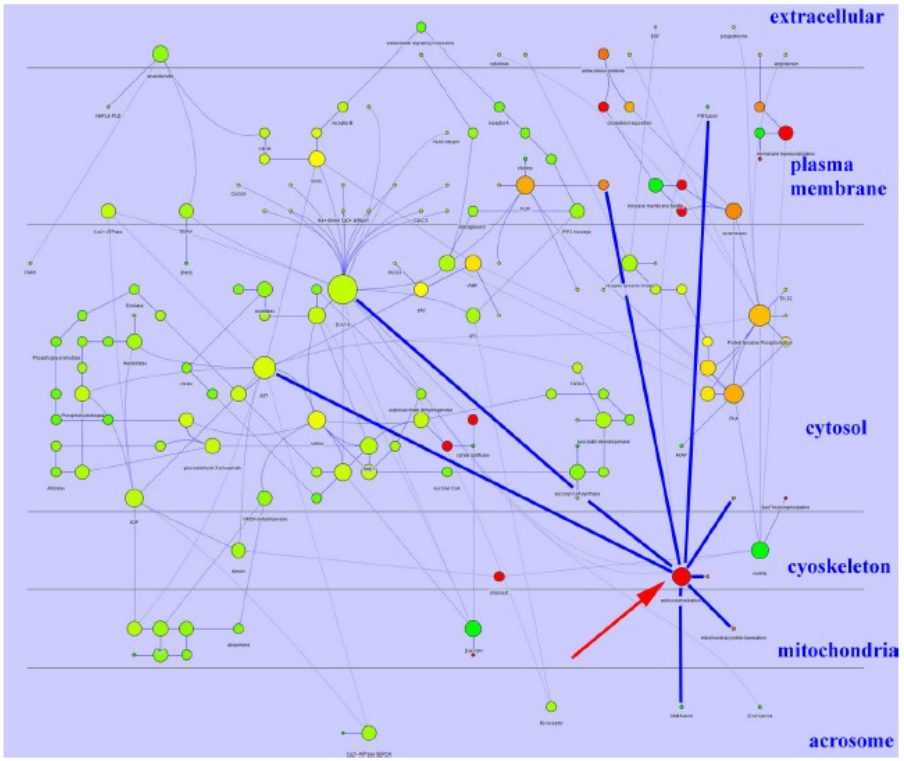


Figure 1 Diagram showing the structure of the boar spermatozoa capacitation network and the subcellular localization of nodes. The node size was proportional to the connection number and the node color gradient was dependent from the closeness centrality. This parameter is computed as: $C_c(n) = 1/\text{avg}(L(n,m))$, were L (n,m) is the length of the shortest path between two nodes *n* and *m*. The closeness centrality of each node ranges from 0 (red) to 1 (green) and it is a measure of how fast the information spreads from a given node to the other nodes. The actn polymerization node is indicated by the red arrow, its links by blue line. All the nodes were localized in their specific subcellular domain (Cerebral V.2).

Table 2 Most connected nodes (the hubs) of capacitation network

Node	Number of links
$[Ca^{2+}]_i$	28
ATP	15
Tyr phosphorylation	13
PKA	9
ADP	8
PLD1	8
NADH	8
Actin polymerization	8

networks topology

Table 1 Main topological parameters of capacitation network

Parameter	Value
N° nodes	153
N° edges	204
Clustering coefficient	0.056
Diameter	12
Averaged n° neighbours	2.654
Char. path length	4.995

The number of nodes represent the total number of molecules involved, the number of edges represents the total number of interaction found, the clustering coefficient is calculated as $CI = 2nI/k(k\text{-}1)$, where nI is the number of links connecting the kI neighbours of node I to each other, the network diameter is the largest distance between two nodes, the Averaged n° neighbours represent the mean number of connection of each node, the Char. path length gives the expected distance between two connected nodes.

$$b = -1.578$$

Table 3 Main topological parameters of capacitation network after "actin polymerization" node removal

Parameter	Value
N° nodes	152
N° edges	196
Clustering coefficient	0.056
Diameter	12
Averaged n° neighbours	2.566
Char. path length	6.071
Degree distribution	b = -1.563
	$r = 0.809$ $R^2 = 0.898$

The number of nodes represent the total number of molecules involved, the number of edges represents the total number of interaction found, the clustering coefficient is calculated as $CI = 2nI/k(k\text{-}1)$, where nI is the number of links connecting the kI neighbours of node I to each other, the network diameter is the largest distance between two nodes, the Averaged n° neighbours represent the mean number of connection of each node, the Char. path length gives the expected distance between two connected nodes.

Membrane fusion is impossible

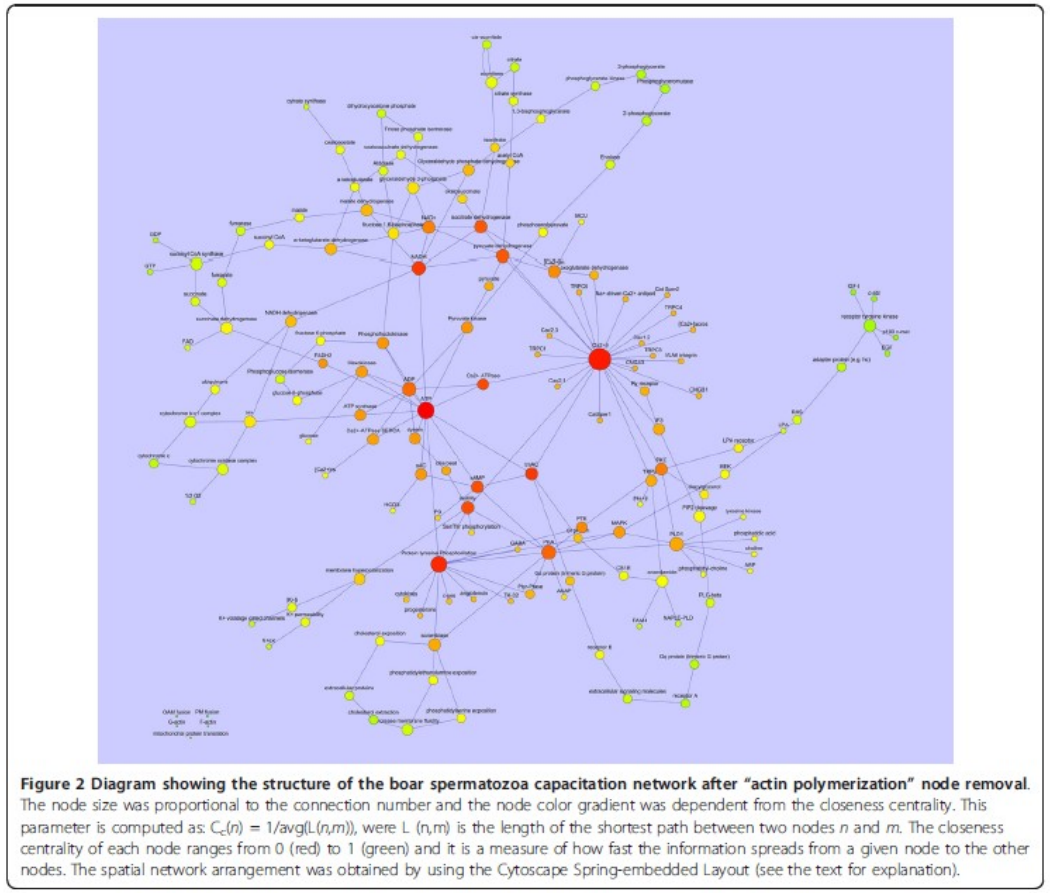


Figure 2 Diagram showing the structure of the boar spermatozoa capacitation network after "actin polymerization" node removal. The node size was proportional to the connection number and the node color gradient was dependent from the closeness centrality. This parameter is computed as: $C_c(n) = 1/\text{avg}(L(n,m))$, were L (n,m) is the length of the shortest path between two nodes *n* and *m*. The closeness centrality of each node ranges from 0 (red) to 1 (green) and it is a measure of how fast the information spreads from a given node to the other nodes. The spatial network arrangement was obtained by using the Cytoscape Spring-embedded Layout (see the text for explanation).

F-actin

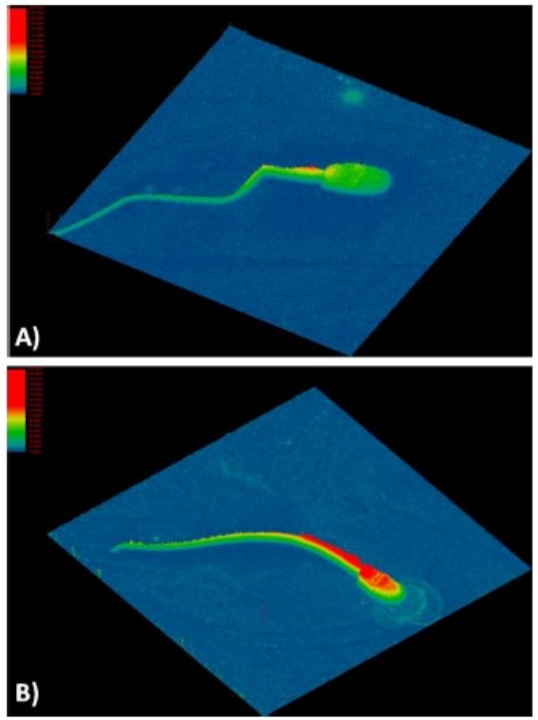


Figure 3 Confocal image representing actin localization in ejaculated and in capacitated spermatozoa. Confocal image representative of TRITC-phalloidin distribution on freshly ejaculated spermatozoon showing a faint fluorescence emission over the midpiece and post-acrosomal region (pattern A; panel A) and of incubated spermatozoon, displaying high fluorescence emission over the whole head (pattern B; panel B).

CTC

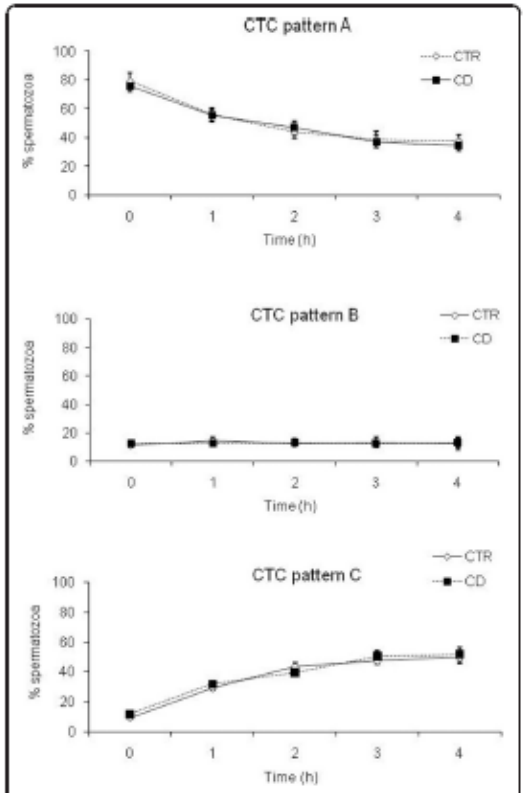


Figure 5 Kinetic of acquisition of CTC staining pattern A, B and C in CTR and CD treated spermatozoa. Graphic showing the percentage of spermatozoa displaying the CTC pattern A, B and C, during the 4 h of incubation under control conditions (dark continue line) or in the presence of CD (dark dot line). All the values are represented as mean ± SD.

protein tyrosine phosphorylation

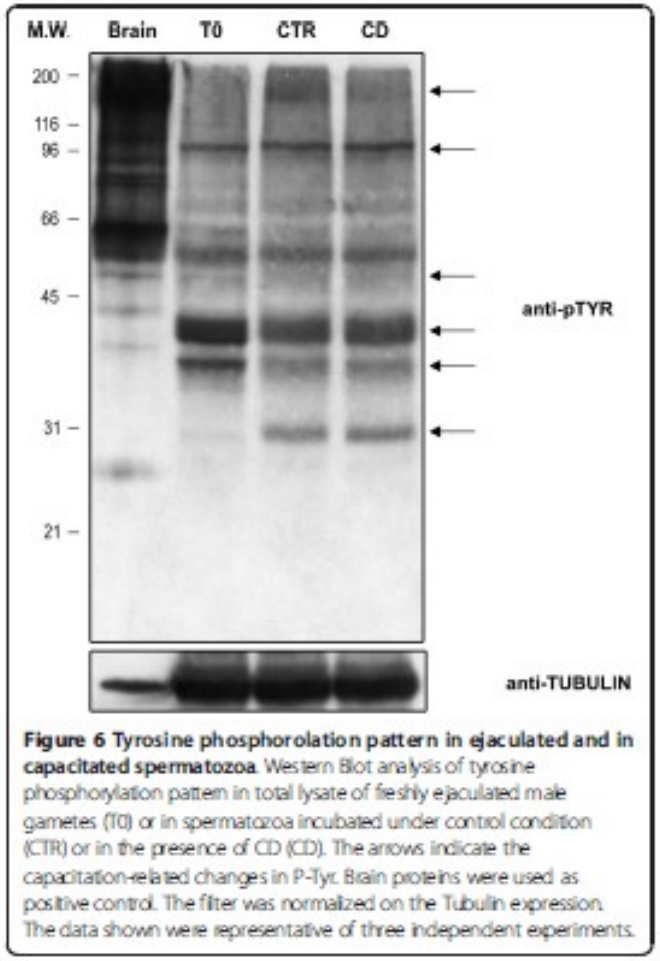
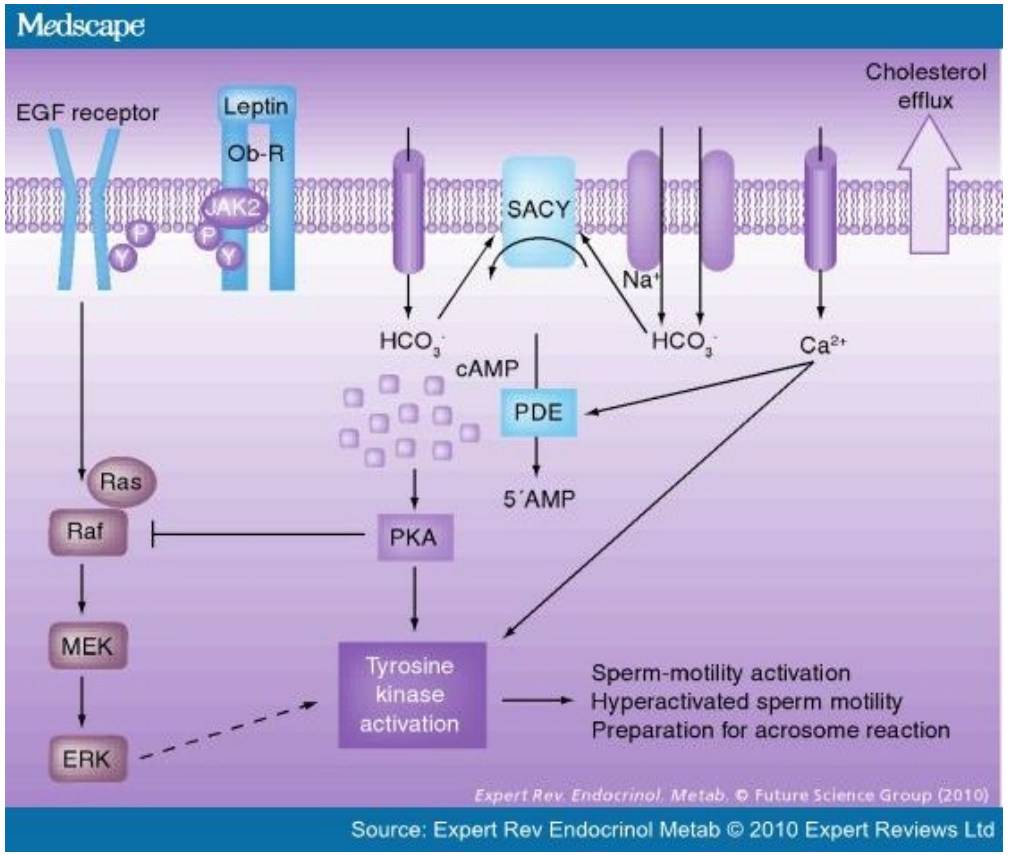
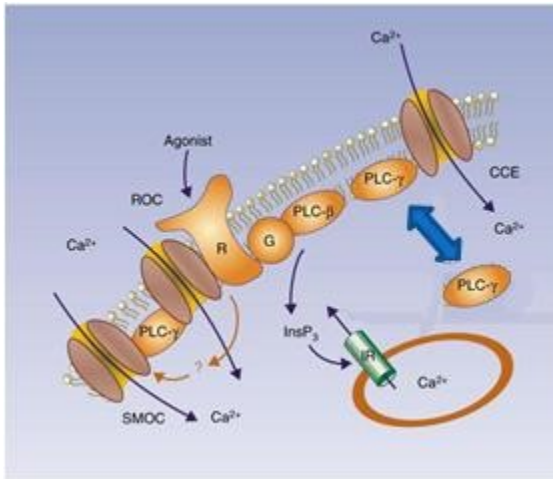


Figure 6 Tyrosine phosphorolation pattern in ejaculated and in capacitated spermatozoa. Western Blot analysis of tyrosine phosphorylation pattern in total lysate of freshly ejaculated male gametes (T0) or in spermatozoa incubated under control condition (CTR) or in the presence of CD (CD). The arrows indicate the capacitation-related changes in P-Tyr. Brain proteins were used as positive control. The filter was normalized on the Tubulin expression. The data shown were representative of three independent experiments.



PLC-γ1



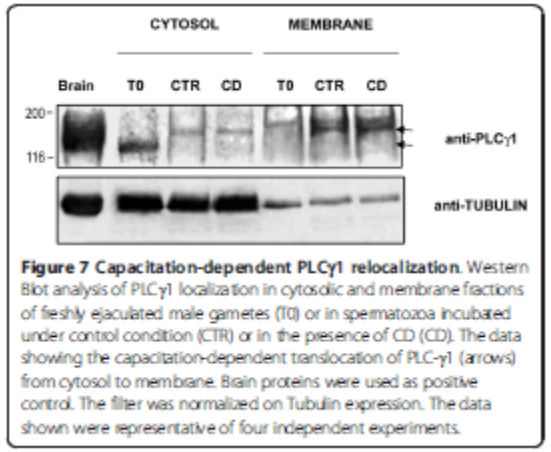


Figure 7 Capacitation-dependent PLCγ1 relocalization. Western Blot analysis of PLCγ1 localization in cytosolic and membrane fractions of freshly ejaculated male gametes (T0) or in spermatozoa incubated under control condition (CTR) or in the presence of CD (CD). The data showing the capacitation-dependent translocation of PLCγ1 (arrows) from cytosol to membrane. Brain proteins were used as positive control. The filter was normalized on Tubulin expression. The data shown were representative of four independent experiments.

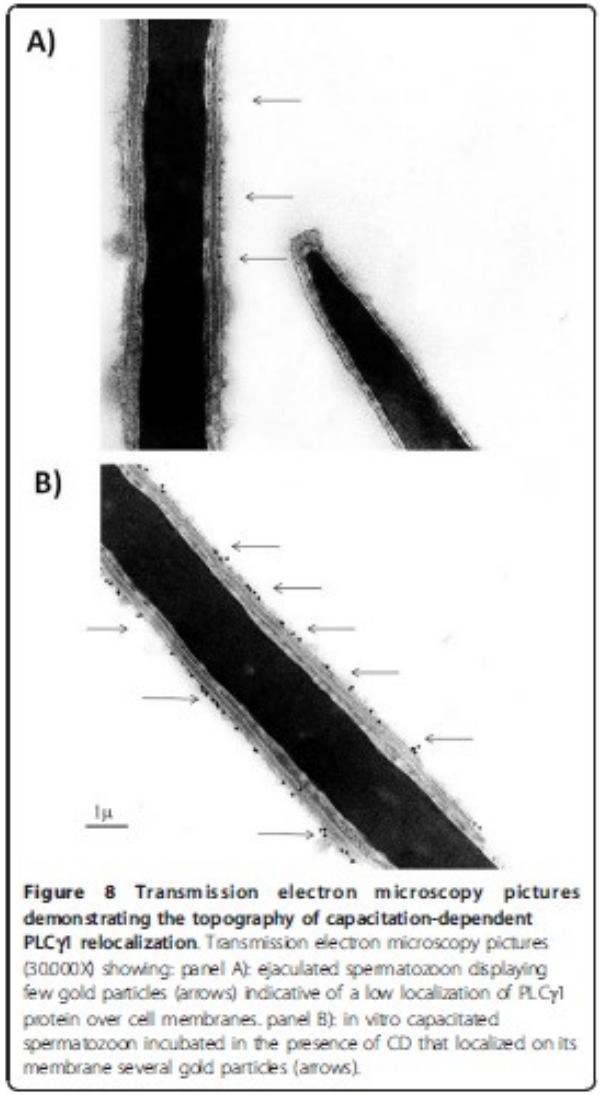


Figure 8 Transmission electron microscopy pictures demonstrating the topography of capacitation-dependent PLCγ1 relocalization. Transmission electron microscopy pictures (30.000X) showing: panel A): ejaculated spermatozoon displaying few gold particles (arrows) indicative of a low localization of PLCγ1 protein over cell membranes. panel B): in vitro capacitated spermatozoon incubated in the presence of CD that localized on its membrane several gold particles (arrows).

Ca^{2+}

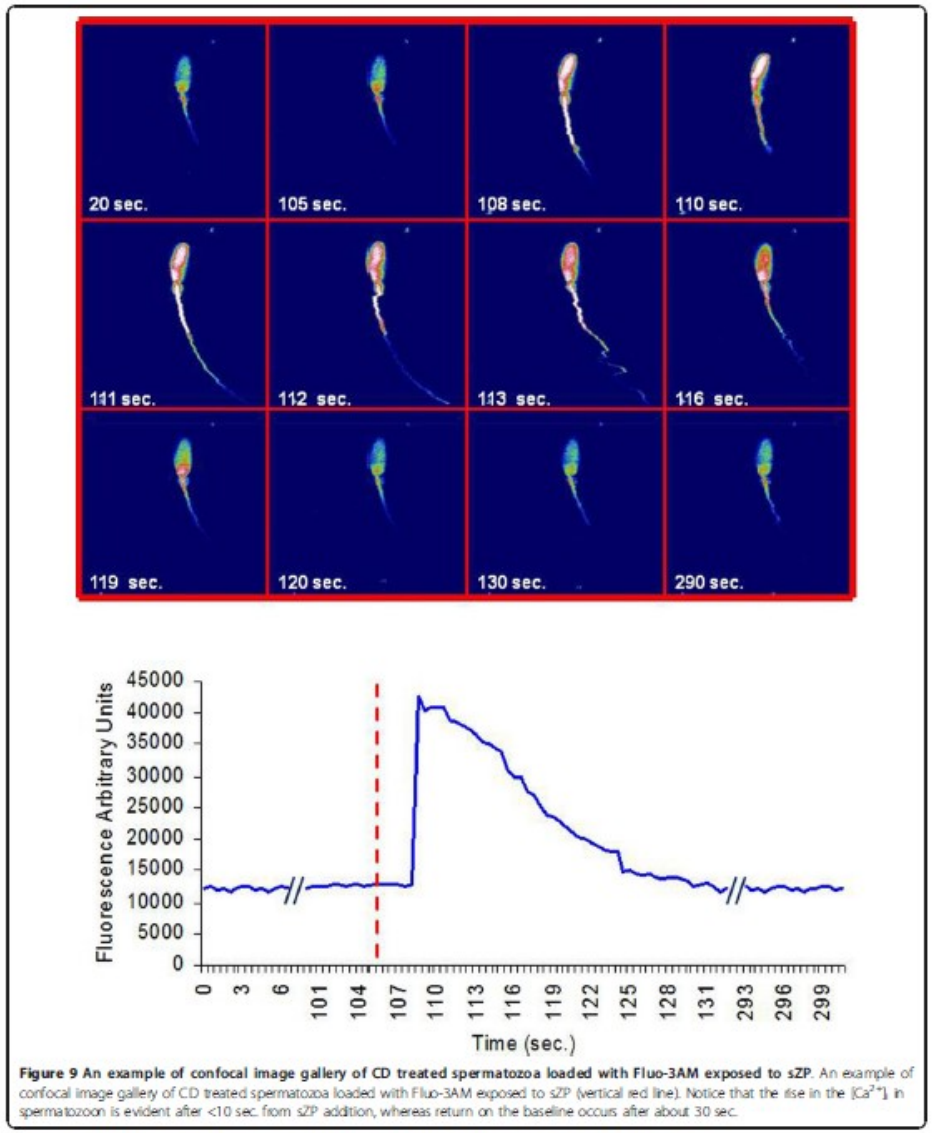


Figure 9 An example of confocal image gallery of CD treated spermatozoa loaded with Fluo-3AM exposed to sZP. An example of confocal image gallery of CD treated spermatozoa loaded with Fluo-3AM exposed to sZP (vertical red line). Notice that the rise in the $[Ca^{2+}]_i$ in spermatozoon is evident after <10 sec. from sZP addition, whereas return on the baseline occurs after about 30 sec.

Acrosome reaction

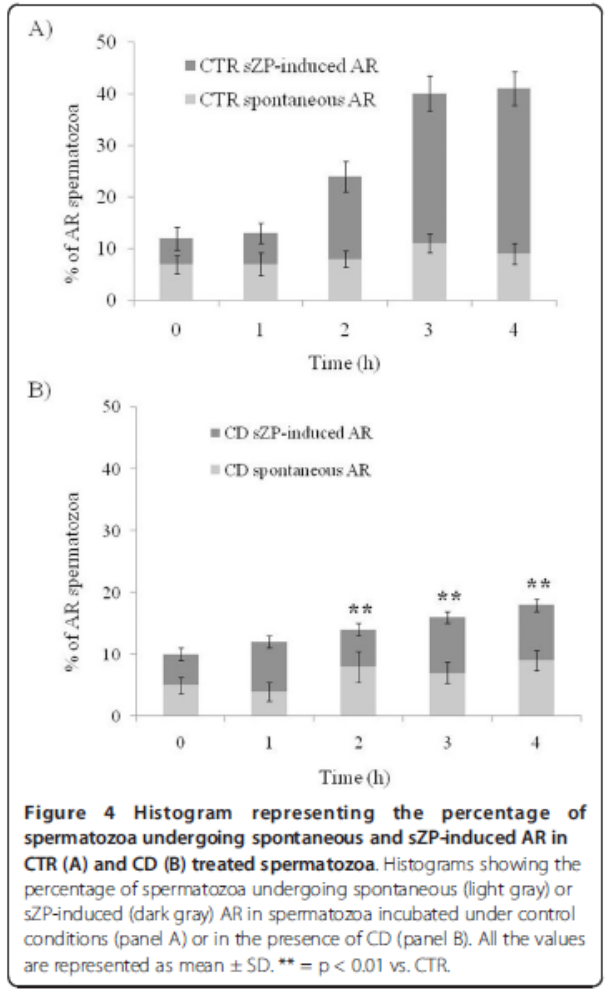
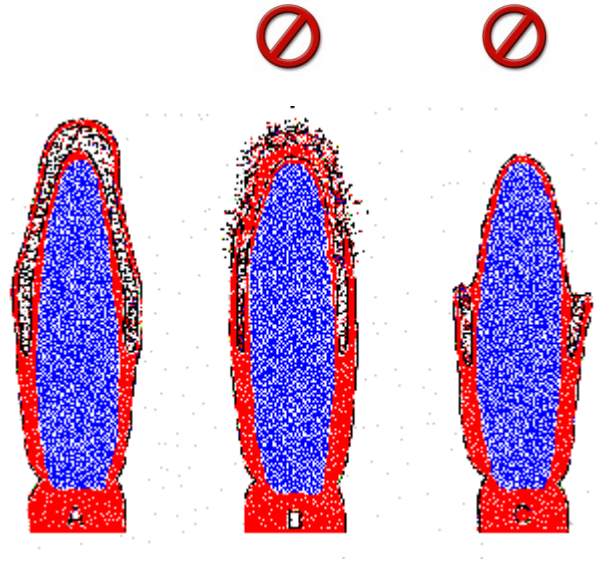


Figure 4 Histogram representing the percentage of spermatozoa undergoing spontaneous and sZP-induced AR in CTR (A) and CD (B) treated spermatozoa. Histograms showing the percentage of spermatozoa undergoing spontaneous (light gray) or sZP-induced (dark gray) AR in spermatozoa incubated under control conditions (panel A) or in the presence of CD (panel B). All the values are represented as mean ± SD. ** = $p < 0.01$ vs. CTR.



In conclusion

- The model has been validated;
- A new role of actin during sperm capacitation has been proposed.

Evolution of the system

Bioengineering & Biomedical Science

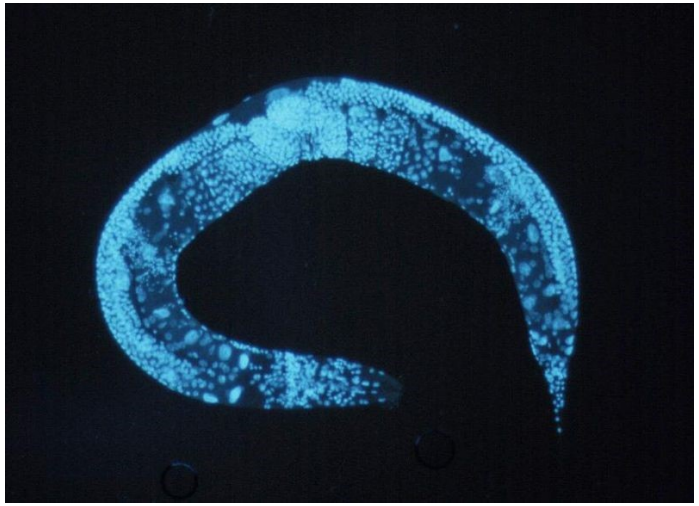
Bernabò et al., J Bioengineer & Biomedical Sci 2012, S3
http://dx.doi.org/10.4172/2155-9538.S3-001

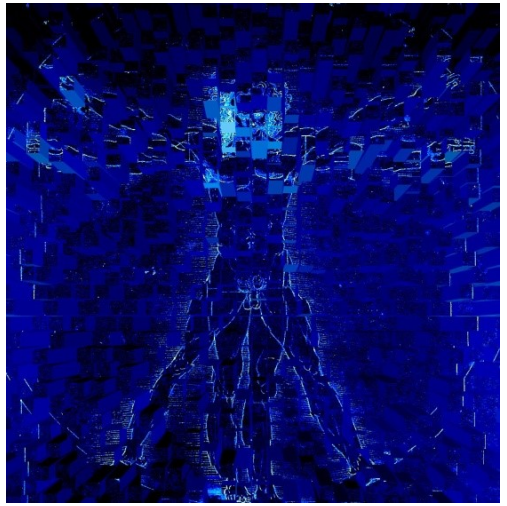
Research Article Open Access

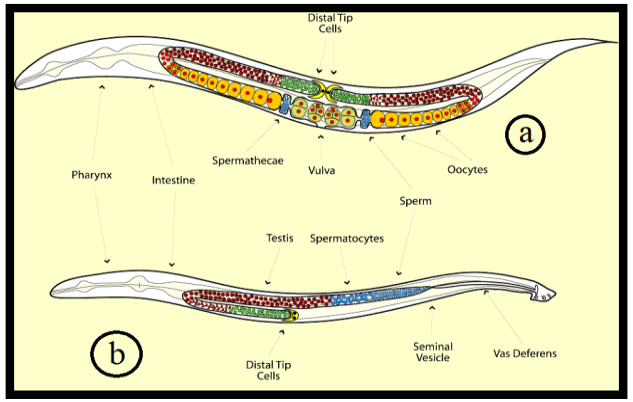
Signaling Strategy in Spermatozoa Activation of Sea Urchin, *C. elegans* and Human: Three Different Players for the Same Melody

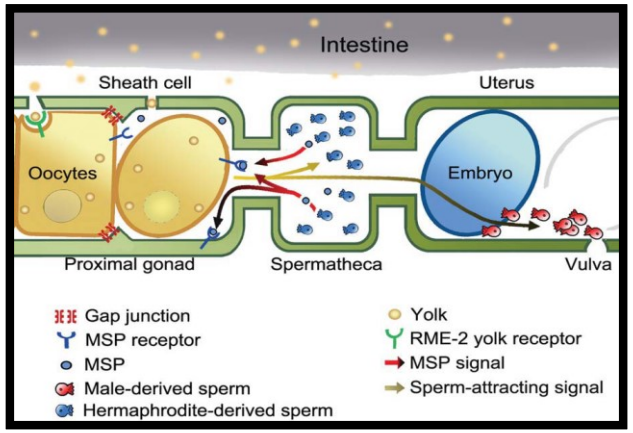
Nicola Bernabò*, Ilaria Saponaro, Mauro Mattioli and Barbara Barboni

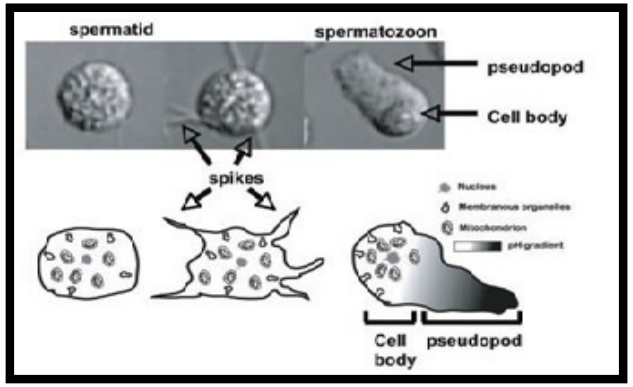
Department of Comparative Biomedical Sciences, University of Teramo, Teramo, Italy





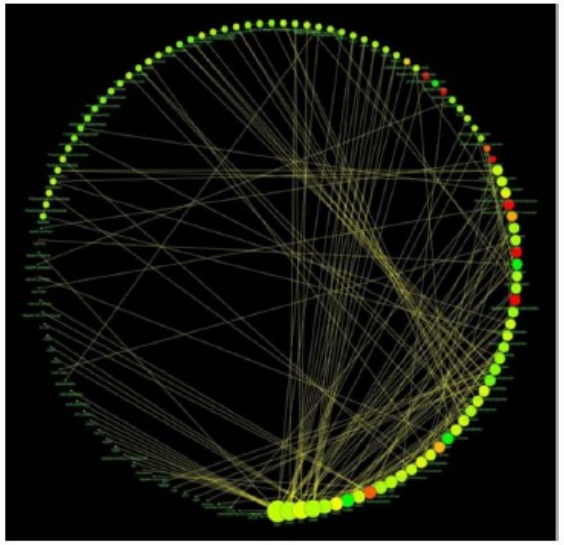

Distal Tip Cells
a
Pharynx
Intestine
Spermathecae
Vulva
Oocytes
Sperm
Testis
Spermatocytes
b
Distal Tip Cells
Seminal Vesicle
Vas Deferens


Intestine
Sheath cell
Uterus
Oocytes
Embryo
Proximal gonad
Spermatheca
Vulva
Gap junction
MSP receptor
MSP
Male-derived sperm
Hermaphrodite-derived sperm
Yolk
RME-2 yolk receptor
MSP signal
Sperm-attracting signal


spermatid
spermatozoon
pseudopod
Cell body
spikes
Nucleus
Membranous organelles
Mitochondrion
pH gradient
Cell body
pseudopod

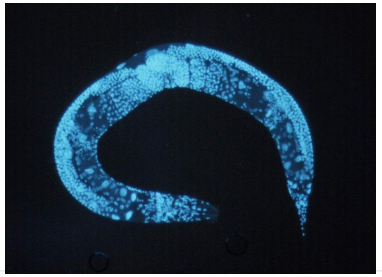
	Sea urchin	*C. elegans*	Human
Symmetry	Fivefold	Bilateral	Bilateral
Sexes	Male/Female	Hermaphrodite/ Male	Male/Female
Fertilization	External	Internal	Internal
Sperm motility	Flagellum	Amoeboid	Flagellum
Acrosome reaction	Yes	No	Yes
Membrane remodeling	No	Yes	Yes
Cytoskeleton remodeling	Actin	MSP	Actin
Time for sperm activation	Seconds	Days	Hours to days

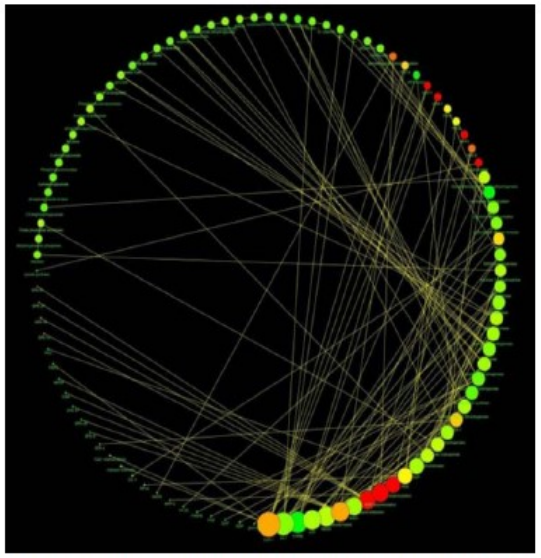
Table 5: Main biological characteristics of reproduction and spermatozoa in sea urchin, *C. elegans* and Human.



The nodes diameter is proportional to the number of links, the color varies depending on the closeness centrality (see text for explanation). The networks were spatially represented using the Cytoscape Degree Sorted Circle Layout: all nodes woth the same number of links are located together around the circle (see Cytoscape's User Manual).

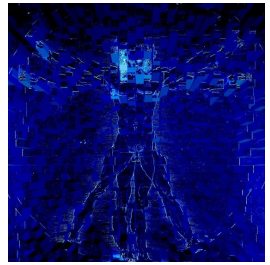
Figure 1: Diagram showing the sea urchin spermatozoa activation network.

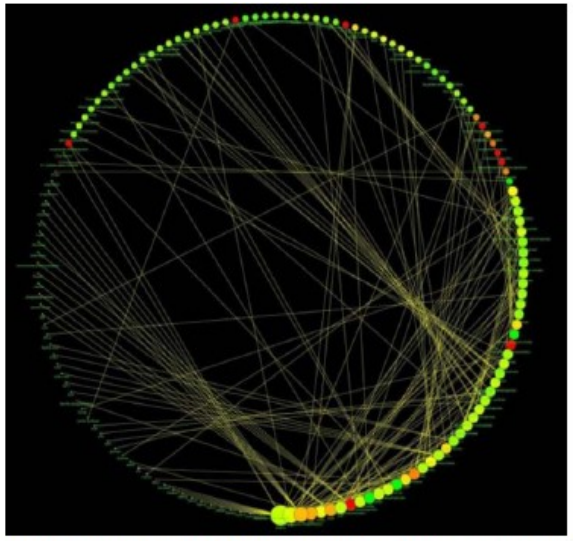




The nodes diameter is proportional to the number of links, the color varies depending on the closeness centrality (see text for explanation). The networks were spatially represented using the Cytoscape Degree Sorted Circle Layout: all nodes with the same number of links are located together around the circle (see Cytoscape User Manual).

Figure 2: Diagram showing the *C. elegans* spermatozoa activation network.





The nodes diameter is proportional to the number of links, the color varies depending on the closeness centrality (see text for explanation). The networks were spatially represented using the Cytoscape Degree Sorted Circle Layout: all nodes with the same number of links are located together around the circle (see Cytoscape User Manual).

Figure 3: Diagram showing the Human spermatozoa activation network.

	Sea urchin	*C. elegans*	Human
N° nodes	127	100	151
N° edges	175	132	202
Clustering coefficient	0.023	0.032	0.028
Diameter	23	23	20
Avg. n° neighbours	2.740	2.620	2.662
Char. path length	8.128	7.816	6.546

The number of nodes represent the total number of molecules involved; the number of edges represents the total number of interactions; the clustering coefficient is calculated as $CI=2nI/k(k-1)$, where nI is the number of links connecting the kI neighbours of node I to each other; the network diameter is the largest distance between two nodes; the Averaged n° neighbours represents the mean number of connections of each node; the Char. path length gives the expected distance between two connected nodes.

Table 1: Main topological parameters of Sea urchin, *C. elegans* and Human spermatozoa activation networks.

	Sea urchin		*C. elegans*		Human	
	IN	**OUT**	**IN**	**OUT**	**IN**	**OUT**
R	0.998	0.967	0.992	0.971	0.988	0.997
R^2	0.748	0.924	0.866	0.884	0.890	0.828
b	-1.589	-2.421	-2.067	-2.127	-1.542	-1.993

Table 2: Results of power law fitting of IN and OUT sea urchin, *C. elegans* and Human spermatozoa activation networks.

Sea urchin		*C. elegans*		Human	
Node	**N° of links**	**Node**	**N° of links**	**Node**	**N° of links**
$[Ca^{2+}]_i$	19	**$[Ca^{2+}]_i$**	10	**$[Ca^{2+}]^i$**	25
$[H^+]_i$	14	**$[H^+]_i$**	9	**Tyr phosph.**	13
ATP	9	**ATP**	7	**ATP**	15
cGMP	15	**Motility**	8	**PKA**	9
cAMP	13	**Vesicle fusion**	7		
		NADH	7		
		NAD^+	6		
		Pseudopod extension	6		

Table 3: Most connected nodes (the hubs) of sea urchin, *C. elegans* and Human spermatozoa activation networks.

In conclusion

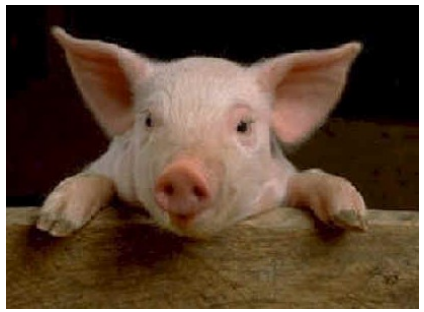
- Different organisms share the same topology

Sptz as model

- Disposable cells;
- Transcriprionally silent;
- IVF to evaluate their function.

Possible applications

- Contraception;
- unexplained infertility;
- Personalized medicine;
- in vivo – in vitro - in silico systems



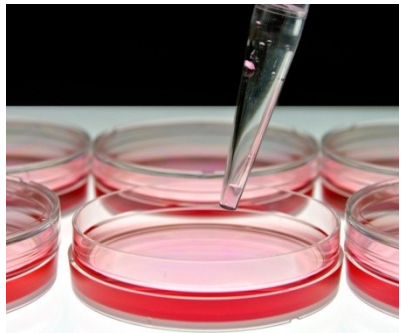


Table 1 Main topological parameters of examined networks

		Topological parameters								
		No. nodes	*No. edges*	*CC*	*Diameter*	*Avg. no. neighbours*	*Char. path length*	*Nodes degree exponent (γ)*	*r*	R^2
SAN	SU	130	179	0.074	13	2.738	5.205	−1.418	0.716	0.825
	CE	102	132	0.063	15	2.569	5.865	−1.572	0.680	0.815
	Hu	151	202	0.057	12	2.662	5.014	−1.588	0.817	0.852
OSN	MC	134	139	0	12	2.075	5.59	−1.579	0.958	0.731
	NRC	238	356	0.059	11	2.798	5.465	−1.294	0.977	0.776
	VP	143	158	0	15	2.14	9.928	−2.015	0.964	0.857
	INS	346	428	0.02	18	2.416	6.435	−1.428	0.993	0.868
	p53	802	917	0.002	16	2.217	6.044	−1.464	0.907	0.748
	pRb	291	323	0	15	2.151	6.814	−1.782	0.974	0.848
	ATP	186	210	0.033	12	2.183	6.83	−1.532	0.983	0.841
	GLU	260	346	0.025	17	2.723	6.502	−2.127	0.795	0.842
	c-Kit	248	311	0	27	2.177	9.035	−1.799	0.677	0.852
	CC	125	126	0	12	2.016	5.781	−1.590	0.978	0.934
	SFN	130	130	0.004	10	2.000	4.857	−1.598	0.991	0.854

Notes: The number of nodes represents the total number of molecules involved, the number of edges represents the total number of interaction found, the Clustering Coefficient (CC) is calculated as $CI = 2nI/k(k-1)$, where nI is the number of links connecting the kI neighbours of node I to each other, the network diameter is the largest distance between two nodes, the averaged no. neighbours represent the mean number of connection of each node, the Char. path length gives the expected distance between two connected nodes, node degree exponent ($-\gamma$) and r represents the exponent and the correlation coefficient of power law equation of node distribution, respectively. SAN = sperm activation networks; SU = sea urchin; CE = *C. elegans*; Hu = human; OSN = other signalling networks; MC = smooth and striated muscular contraction; NRC = 6 neurotransmitters release cycle; VP = visual phototransduction (rods); INS = insulin signalling pathway; p53 = p53 pathway; pRb = regulation of retinoblastoma protein; ATP = mitochondrial ATP metabolism; GLU = glucose metabolism; c-Kit = signalling events mediated by stem cell factor receptor c-Kit; CIRC = circadian clock; SFN = randomly computer generated scale free network.