TOWARDS
A MORE „PHYSIOLOGICAL“ I.C.S.I. ?

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WHY I ACCEPTED THIS TOPIC „Schwingen“

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What we see …

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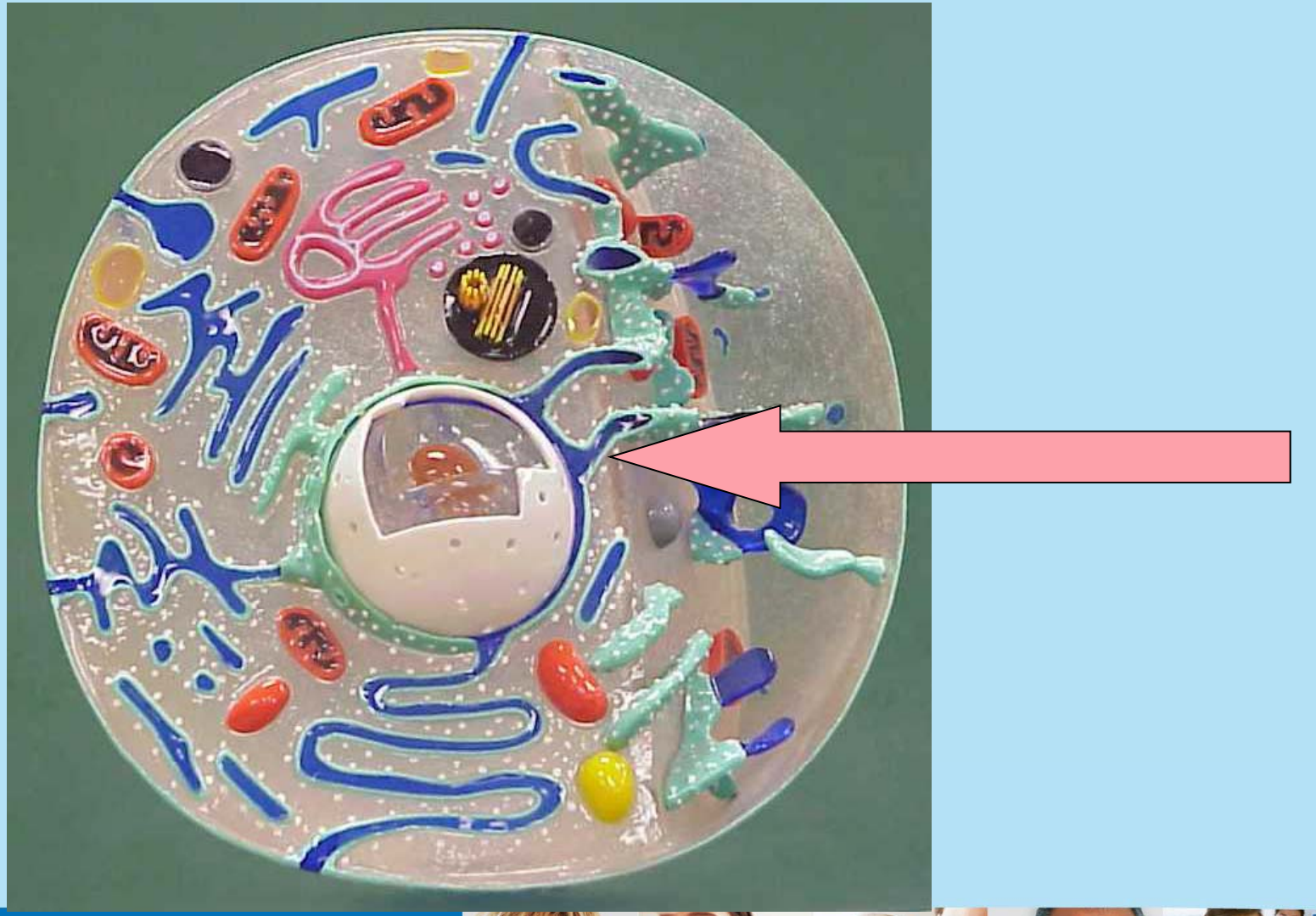
And what is inside.



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ICSI IS INVASIVE

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- **WHAT HAPPENS IN NATURE**
- **WHAT HAPPENS DURING I.C.S.I.**

Natural Fertilisation

- Interaction between fallopian tube and sperm
- Sperm capacitation
- Interaction between cumulus
- Interaction Zona Pellucida and Sperm
- Acrosome reaction
- Interaction Ollema Sperm
- Nuclear decondensation
- Fate of Sperm mitiochondria

Fallopian Tube Interactions

- Sperm has to remain for a finite period in the fallopian tube before acquiring fertilizing capacity.

- Motility enhancement
- Modification in ciliar beat frequency
- Increased sperm survival
- Delay sperm capacitation

Chang M.C. Nature 1951, 168, 697-698
Murray S.C. Fertil . Steril. 1997, 68, 2, 351-357
Morales P. Hum. Reprod. 1996, 11,7,1504-1509

Oviductal Sperm Reservoir

- Observed in cattle, sheep, mice, pig and HUMAN

- Binding sites with high affinity for oligomanose-hybrid-type N-glycans
- Spermadhesins
- Annexins containing Fucose
- HSPA8
- Heparin binding sites and disulfide bridges

- Progesteron possible sperm releasing factor
- Sulfated glycoconjugates, disulfide-reductants induce sperm release

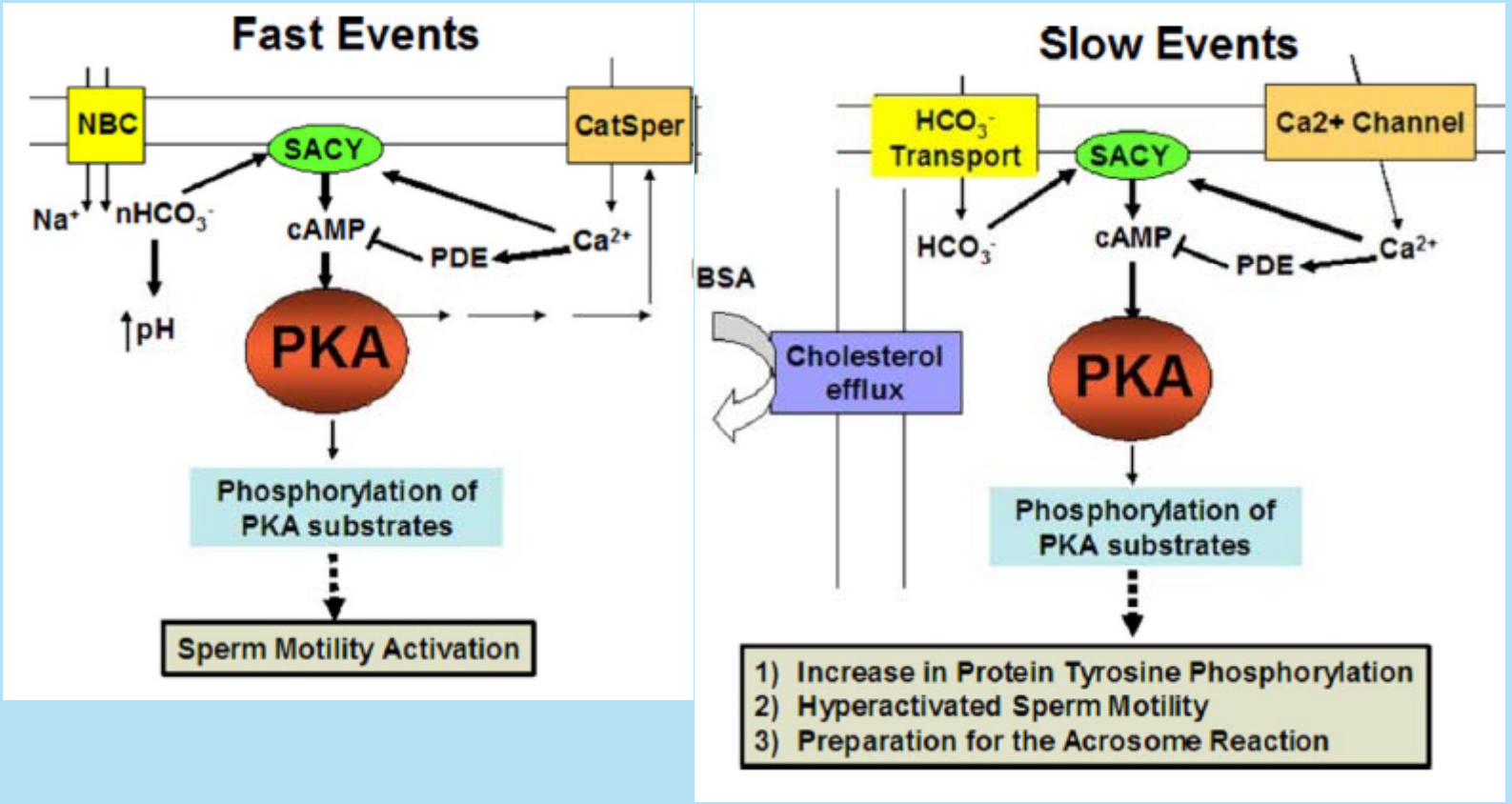
- Downregulation of oviductal redox pathways

- Hyaluronan from cumulus cells

Oviductal Sperm Reservoir

- Ensure the availability of fertilisation competent sperm at the time of ovulation
- Modulation of capacitation
- Extension of sperm life span

Sperm Capacitation



Viscont P. Proceedings National Academy Sciences U.S.A 2009,106,3,667-668

Sperm Capacitation in Vivo

FAST EVENTS.

1. HCO_3^- Ca^{2+} Isotonic solution => Flagellar mouvement
2. Increased PKA activity
   1. HCO_3^- Ca^{2+} mediated
   2. Cordination atypical Adenyl Cyclase
   3. Na^+ HCO_3 Cotransporter (NBC)
   4. Sperm Specific Ca^{2+} Channel

SLOW EVENTS.

Down stream increased PKA activity , Cholesterol dependend with an increase in tyrosine phosphorylation**.**

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Sperma capacition in vitro

- „ Sperm capacitating conditions „

  – Crude semen overlayered with 1 ml Ham F10
  – Top layer diluted with Ham F10 (35mg/ml BS)
  – 6 to 22 H in 5% CO2 5% O2 in presence of increasing concentration of conditioned medium.
  – Measured protein tyrosine phosphorylation

Proteins from human oviductal tissue-conditioned medium modulate sperm capacitaion. Zumoffen C. Hum. Reprod. 2010,25,6,1504-1512

Sperm capacitation

MEMBRANE LIPID DYNAMICS

- **Essential is the removal of cholesterol from the acrosome membrane**
- **Albumin and high density lipoproteins present in the uterine fluid act as cholesterol receptors.**
- **Change in distribution and composition of membrane phospholipids**

Capacition

- How can we make „capacitating media“ when many of the regularatory mechanisms are only partially, poorly understood or elucidated ??

- Albumin induced calcium increase during capacitation requires the CATSPER channel
- (calcium transient potential channels)

- SMART1® protein –free medium for Sperm Capacitation 1998

Acrosome Reaction

- ZP-domain of human zona pellucida glycoprotein-1 binds to human spermatozoa and induces acrosomal exocytosis.
  - **ZP4 binds to anterior head of capacitated sperm**
  - **ZP1 induces acrosome reaction (Acrosomal Exocytose)**
  - **ZP2 binds to acrosome reacted sperm as a secondary receptor but does not induce acrosome reaction.**
  - **ZP domain 260 Animo Acids with 8 conserved Cys residues important for extracellular matrix polymerisation and is also found in other proteins,TGF-β-IIIG, DMBT-1**

Acrosome Reaction

INDUCTION

- Physiological
  - Follicular fluid
  - Granulosa Cells
  - Zona Pellucida
  - Progestin, progesteron, 17 -α-hydroxyprogesteron
  - Cumulus cells

- Artificial
  - A23187
  - Ionomycin

Acrosome Reaction

- The acrosome is a Golgi-derived organelle, a secretory granule situated at the apex of the sperm head.
- Acrosme reaction resembles exocytose with some unusual features.
  - Multiple openings
  - Increasing fenestration
  - Particle free clearings in the plasma membrane
  - Aggregation of intramemnabranous particles
- Acrosin and hyluronidase release

Acrosome Reaction

- 95 kDa protein tyrosine kinase „p95“ on the sperm surface associated with ZP3 binding
  - ***2 Hypotheses***
    - p95 itself is a protein tyrosine kinase receptor
    - p95 serves as a ZP3 receptor and is separate from a protein kinase activated during gamete interaction

Sperm Oolemma Interaction

- **Fusion between equatorial segment of the sperm and the microvillar region of the oocyte.**

- **Which molecules are involved.**
  - **SLLP1 (sperm lysozyme like protein) in the acrosome**
  - **ADAM1,2,3 (A desintegrin metalloproteinase) on the sperm surface and the integrins of the oolemma**
  - **Sperm DE (CRISP 1) , cystein rich secretory protein**
  - **GPI anchored proteins (Glycophosphaditylinositol)**
  - **CD 9 Transmembrane family 4**

  - **Exact mechanisms and involved molecules ???**

Nuclear decondensation

- Shortly after fertilistion protamines are replaced by histones
  - But not necessaraly in regions were histones remained present
  - Some histones in the paternal nucleus are propably never replaced
  - Transmission of sperm histones allows the oocyte to inherit histone-based chromatin structural organisation from the sperm.

WHAT HAPPENS DURING I.C.S.I. ?

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Sperm Damage

- PVP has a detrimental action on:
  - Plasma membrane
  - Acrosomal & mitochondrial membranes

- Deterioration of:
  - axonemal tubules
  - Chromatin
  - Fibrous sheath
  - Accessory fibres

Baccetti B. et al. 1995. J. Androl.16,356-71
Strehler E. et al. 1998. Hu. Reprod. 13.1,120-23

Sperm Damage

- Importance of breaking a spermatozoon‘s tail before intracytoplasmic injection.

Van den Bergh M. et al. 1995, Hum. Reprod.10,11,2819-20,

Sperm Damage

- Partial membrane damage is required to ensure normal sperm condensation.

Dozortsev. D. 1995, Hum. Reprod,9,2139-44.

Sperm damage

- The Method of Sperm Immobilisation influences the onset of the Ca2+ oscillations after ICSI.

  1. Pipetting in and out
  2. Squeezing
  3. Piezo

  Piezo better fertilisation and cleavage.

  Yanagadi K. et al Hum. Reprod. 2001,16,1,148-152

Sperm Damage

- Increased disruption of the sperm plasma membrane at the sperm immobilisation promotes dissociation of pronuclear theca .(Pig)

- Piezo with H intensity gives

  – Better fertilisation
  – Accelerated dissociation of subacrosomal sperm perinuclear theca (SAR-PT)

- *Katayama M, Reproduction,2005,130,907-916*

Sperm Damage

- Immobilised sperm undergo ultrastructural damage and acrosomal disruption.
  - Loss of plasma membrane of the flagellum
  - Dissamblage of the axoneme
  - Morphological changes of the head
    - Membrane fracture line
    - Patch membrane loss
    - Grayish halos around the head

Gomez-Tores MJ, et al. 2007, Fertil. Steril. 88,3,702-5

Mechanical Damage

- **Membrane-bound vacuoles and oolemma inclusions at the site of injection**
- **Evidence of plasma membrane damage**
- **Increased oocyte exchanges processes with**
- **multivesiculated bodies**
- **No gamete membrane fusion**

Bourgain C. et al. Hum. Reprod. Vol.1 Supplement 1 1998. 107-116

Mechanical Damage

ICSI results in abnormal nuclear remodeling during sperm decondensation due to the presence of sperm acrosome and perinuclear theca normally removed at the oolemma.

Rhesus Monkey

Hewitson L. et al. Semin. Reprod Med. 2000; 18(2).151-9

Mechanical Damage

- Aspiration of > 6 picoliter cytoplasm compromises the development to the blastocyst stage.
- Position of the polar body and aspirated volume do not influence the rate of chromosal abnormalities
- *Dumoulin J. Hum. Reprod. 2001, 16,2,306-312*

Mechanical Damage

- Possible spindle damage to be avoided by spindle imaging may increase the yield of euploid embryos.

- *Eichenlaub-Ritter U. Repod Biomed Online, 2002,2.117-24*

RELEASE SPERM OOCYTE ACTIVATING FACTORS

- **Dr. Richard Oko** from Queen's University and **Dr. Peter Sutovsky** from University of Missouri, have identified PAWP (postacrosomal sheath WW domain binding protein, gene WBP2NL, also known as PT32) as an oocyte-activating factor that induces fertilization events including meiotic resumption, pronuclear formation, and egg cleavage.
-
- Through protein binding inhibition, peptide competitive assays, localization, and expression patterns, our researchers now have compelling evidence that ***PAWP is the primary initiator of zygotic development***.
-
- Microinjection of recombinant PAWP into metaphase arrested Xenopus oocytes also induces intracellular calcium release; a key step in oocyte activation.

RELEASE SPERM OOCYTE ACTIVATING FACTORS

Postacrosomal sheath WW domain binding protein (PAWP) (Xenopus laevis)

Aarabi et al. 2010,Mol. Reprod. Dev. 77,3,249-56.

Sperm Born Oocyte Activating Factor. (SOAF)

SOAF in the perinuclear theca

- **is the first part of the cytosol enterring the oocyte**
- **is needed for complete DNA decondensation**
- **activates the oocyte**
- **completes meiotic cell cycle**
- **enables pronuclear development**
- **block for polyspermy**

Sutovsky P, et al. Microsc. Res. Tech. 2003, 61,4,362-78.

Polyvinyl Pyrrolidone

Daily subcutaneous injections of polyvinyl-pyrrolidone-vasopressin in a woman with diabetes insipidus for six years led to a papular dermatosis. Polyvinylpyrrolidone was detected in biopsy material

La Chapelle, J. M. (1966) Dermatologia (Basel), 132, 476

Polyvinyl Pyrrolidone

- **There is a large amount of experience available on the parenteral administration of PVP to man. I.v. injected PVP having a molecular weight exceeding 40 000 is stored in the body for a long time, mainly in the RES.**

- *WHO Food additives Series 5 Geneva 1974*

Hyaluronidase Acrosome Injection

- **Effects of different hyaluronidase concentrations and mechanical procedures for cumulus cell removal.**
  - As low as 10 IU/ml are effective
  - Pipettes 250 Micrometer.

Van de Velde H, et al. 1997, Hum. Reprod. 12,10,2246-250

Hyaluronidase

- Human Recombinant Hyaluronidase.
  - Increased normal fertilisation
  - Decreased oocyte damage

Evison M. et al. R.B.M. Online 2009,18,6,811-

Hyaluronidase Acrosome Injection

- Injection of several acrosome free spermatozoa did not induce oocyte lysis.
- Injection of a single acrosome intact spermatozoon induced lysis in many oocytes in species with large acrosomes (hamster, cattle,pig)
- Removal of acrosomes is theoretically preferable also in human.

Morozumi K. and Yanagimach R. PNAS,2005,102.40,14209-214

Hyaluron Bound Spermatozoa

- Are devoid from DNA-fragmentation
- Are devoid from active caspase 3

Cayli S. et al. Mol. Hum. Reprod 2004,10,365-72

Hyaluron Bound Spermatazoa

- Only mature spermazozoa without cytoplasmic retention bind to the Z.P.

- Immature sperm are unable to bind to immobilized HA

- *Husar G. et al. Fertil. Steril. 1994,61,136-42*
- *Husar G. et al. Biol. Reprod. 1997,56,1020-40*

Hyaluron Bound Spermatozoa

- Reduced frequence of disomy
- Reduced diploidy
- Reduced sex chromosome diploidy

- *Jakab A. et al. Fertil. Steril. 2005, 84,6,1665-73*

Hyaluron Bound Spermatzoa

- No relation between Hyaluron selected Spermatozoa and pronuclear morphology.

- *Van den Bergh et al. 2009. RB.M. online,2009,19,6,796-801*

Sperm Selection

- Faster Spermatozoa (VSL) fertilise better.

Van den Bergh M.1998, Hum. Reprod.13,11,3103-107

Sperm Selection

- MSOME
  - Motile Sperm Organellar Morpholgy Examination

- IMSI
  - Intracytoplasmic morphological selected sperm injection

Berkovitz A. 2005 Hum. Reprod.20,1,185-190

Fate of the Sperm Mitochondria

- Paternal inherited mtDNA is as well found in polyploid IVF as in ICSI embryos.
- Equivocal concerns about paternal mtDNA inheritage
- Paternal mtDNA may survive in the human embryo in association with an altered genetic environnement.

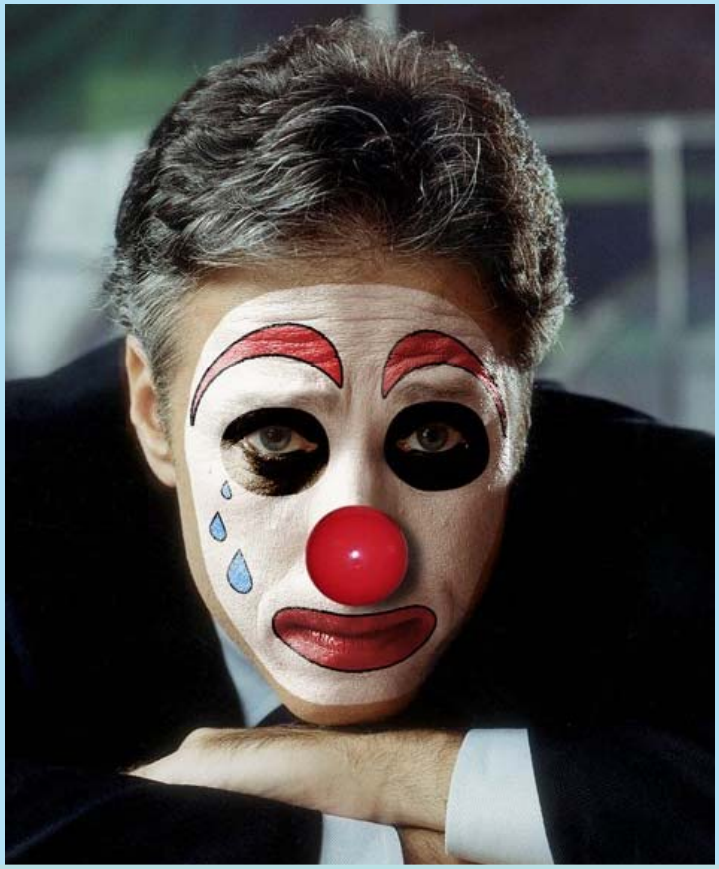
Fate of the Sperm Mitochondria

- Ubiquitination of sperm mitochondria is the proposed mechanism for targeted degradation of paternal mitochondria to promote predominantly maternal inheritance of mitochondria

- Prohibitin is one of the ubiquitinated substrates that makes sperm mitochondria recognizable by the egg‘s ubiquitin-protesome dependent proteolytic machinery after fertilisation.

Fate of Sperm Mitochondria

- No evidence für paternal mtDNA transmission to offspring or exra-embryonic tissues after ICSI
- No paternal mtDNA detected in blood,buccal swabs, placenta or umbilical cord of children born after ICSI.



ICSI IS AN EXTREMELY NON PHYSIOLOGIC MANNER TO FERTILISE OOCYTES WITH HIGH SUCCESS RATES

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NEXT TIME „Fight of the Queens“

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