

A Specific Determinant of Fertilizability Responsible for Persistent Fertilization Failure: How and Why

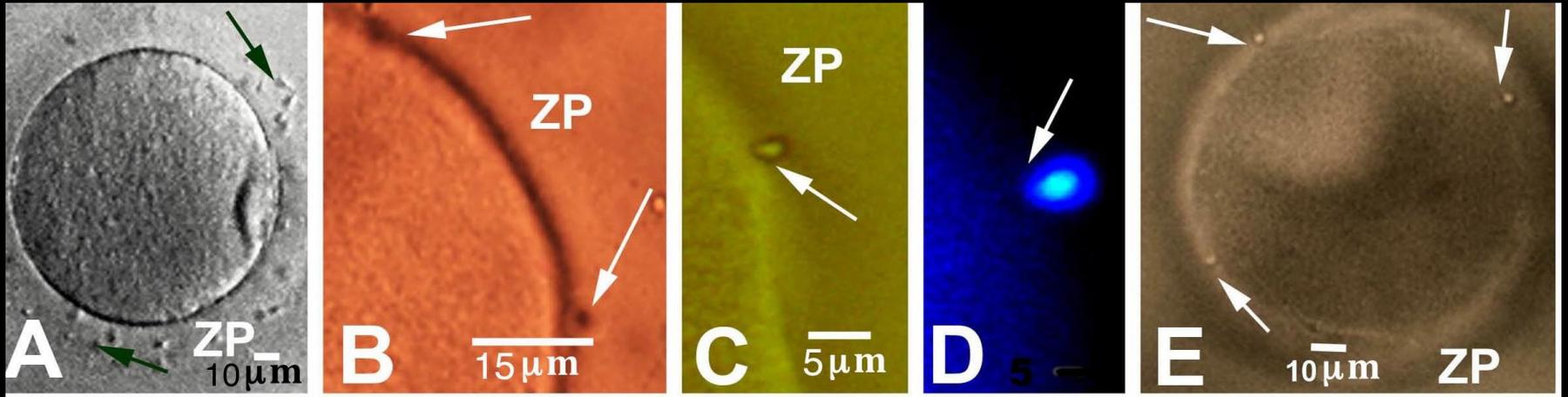
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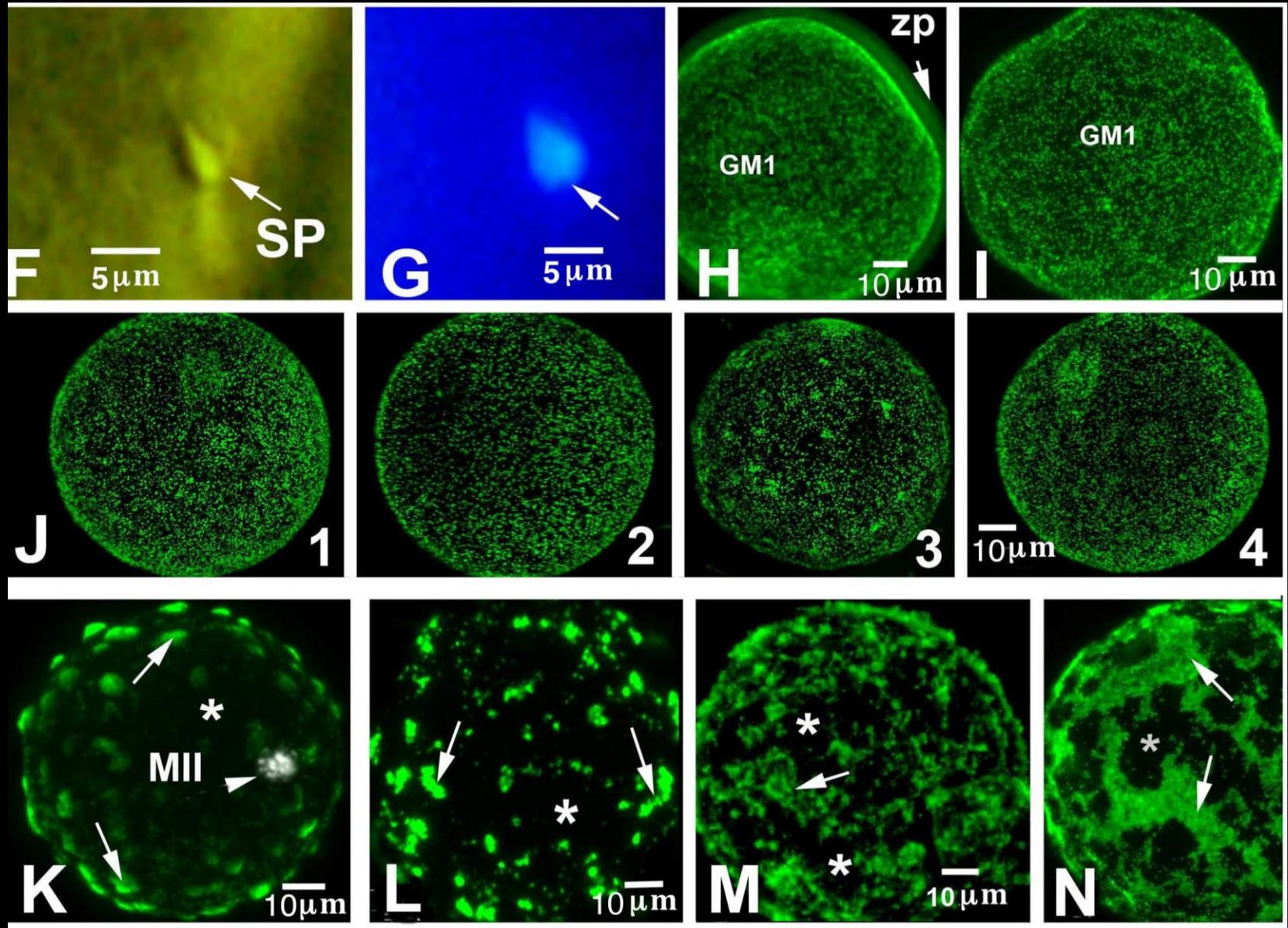
Failure of Sperm to Enter the Oocyte

a common and usually unrecognized cause of failed fertilization in clinical IVF

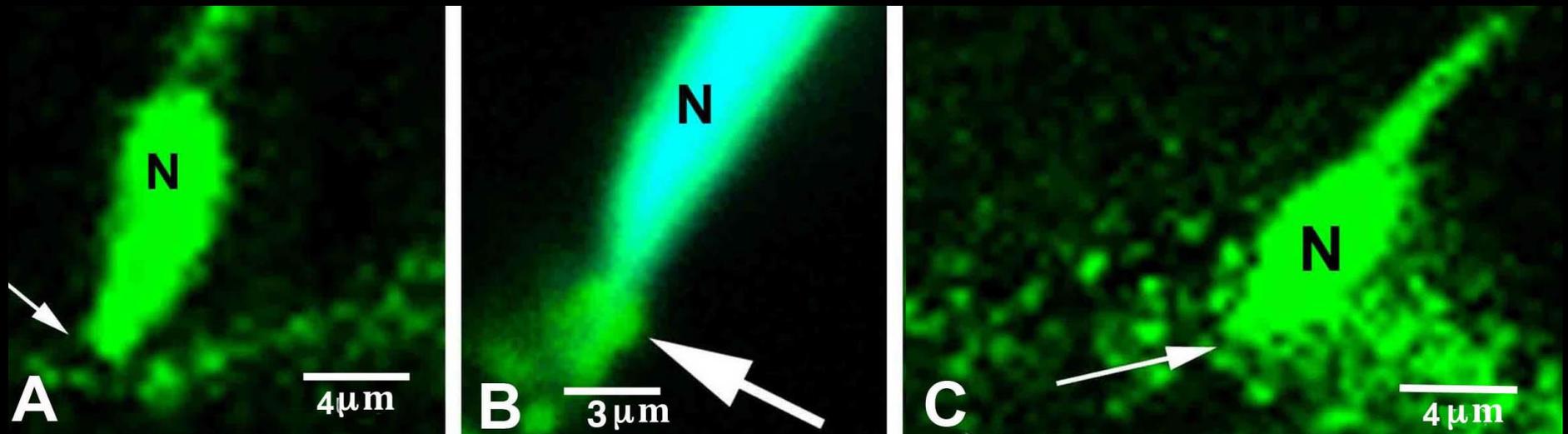


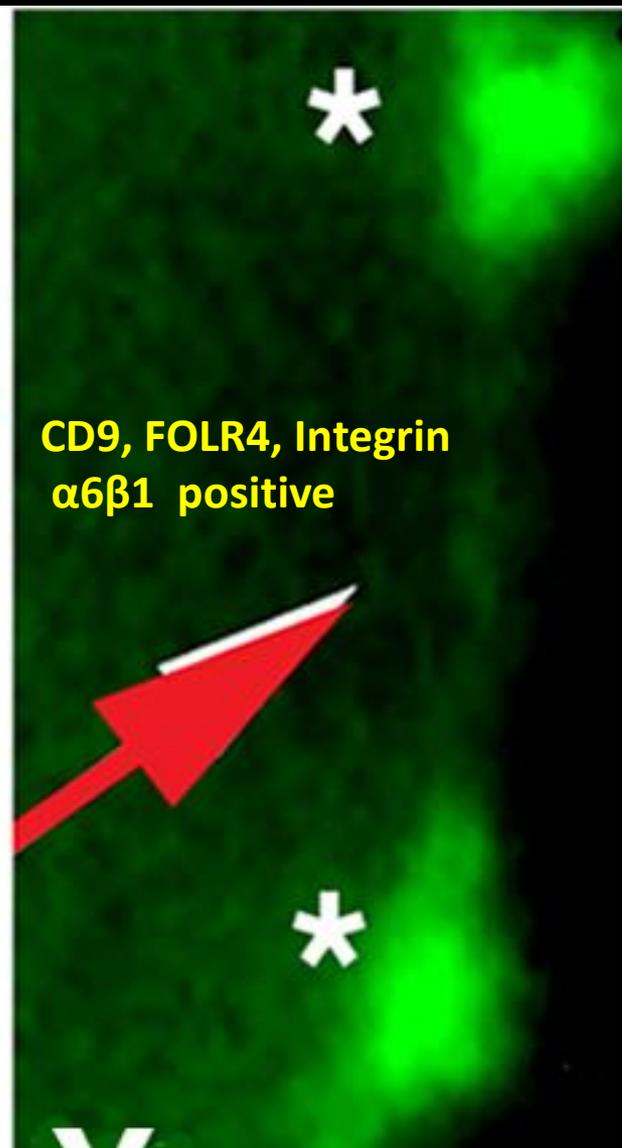
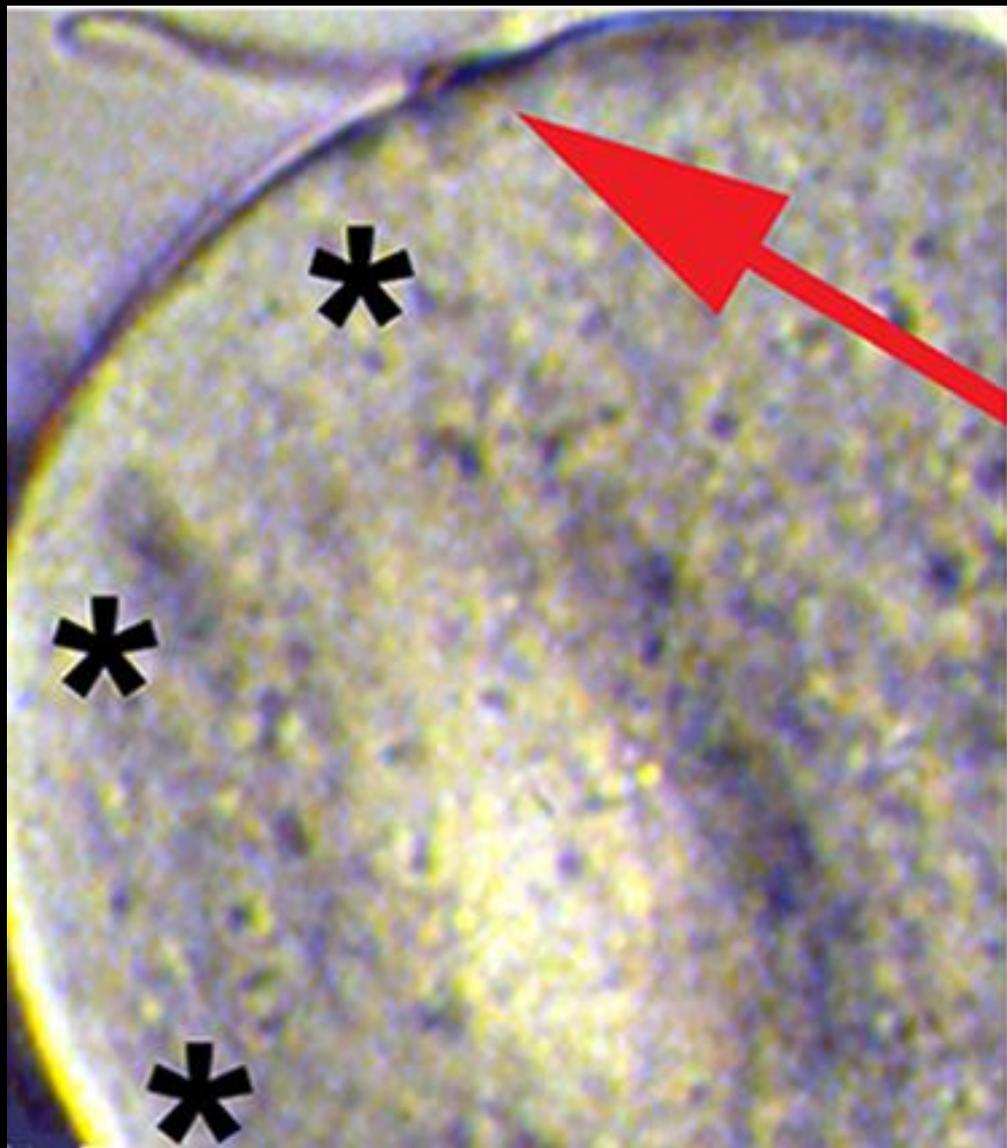
**529 MII UFOs examined to date from conventional IVF-63%
Had a sperm head touching or adjacent to oolemma**

Ganglioside GM1 Phenotypes and Fertilization in Clinical IVF

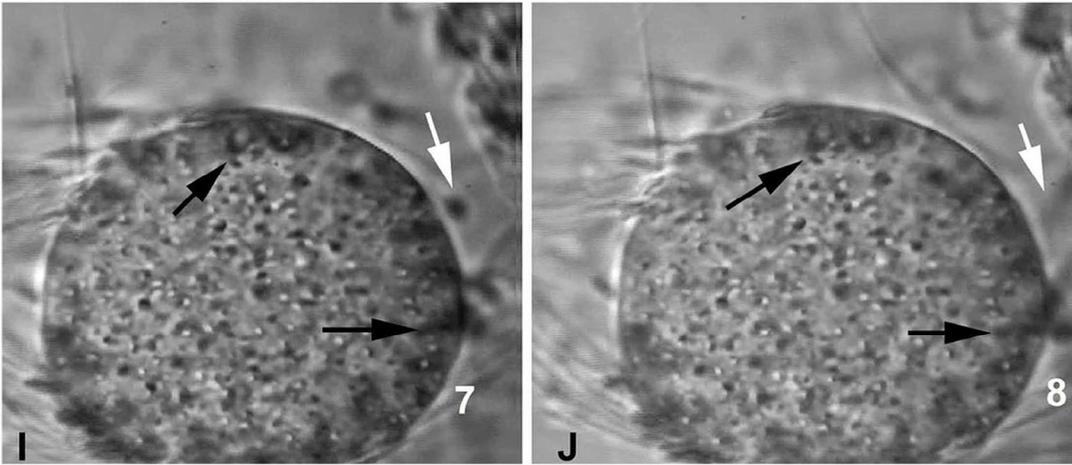
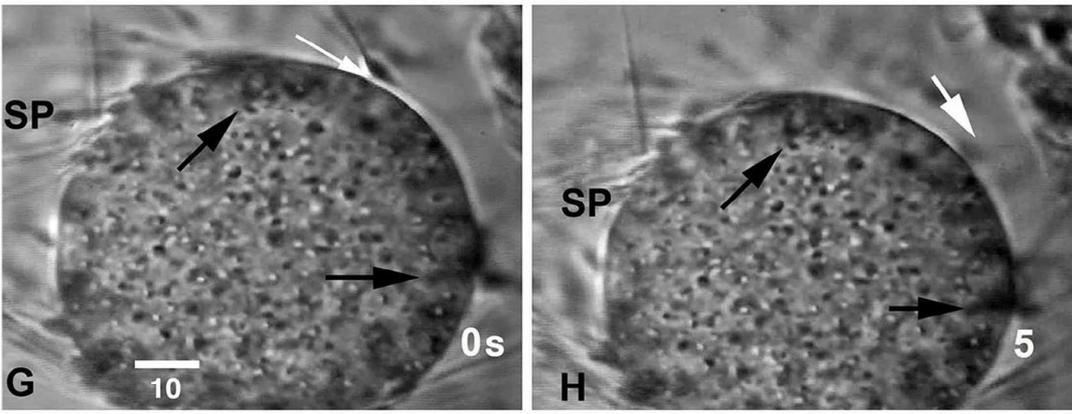
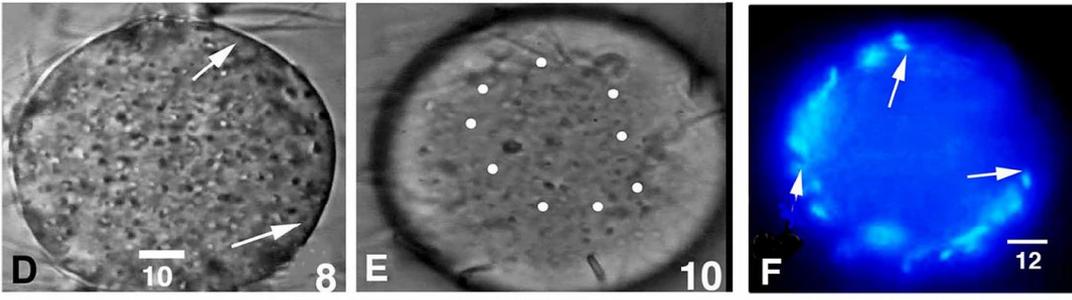
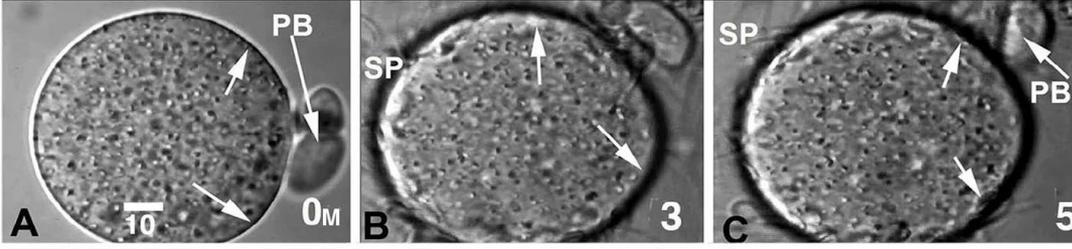


interaction between sperm and oocyte plasma membrane first involves ganglioside GM1





CD9, FOLR4, Integrin
 $\alpha 6\beta 1$ positive



occurrence of a nonpermissive domain in MII mouse oocyte

**nonpermissive for sperm binding
in peri-polar body-1 oolemma**

**Van Blerkom and Zimmermann, 2016,
RBMO**

The density and distribution of lipid raft microdomains enriched in the ganglioside GM1 is directly associated with sperm attachment competence.

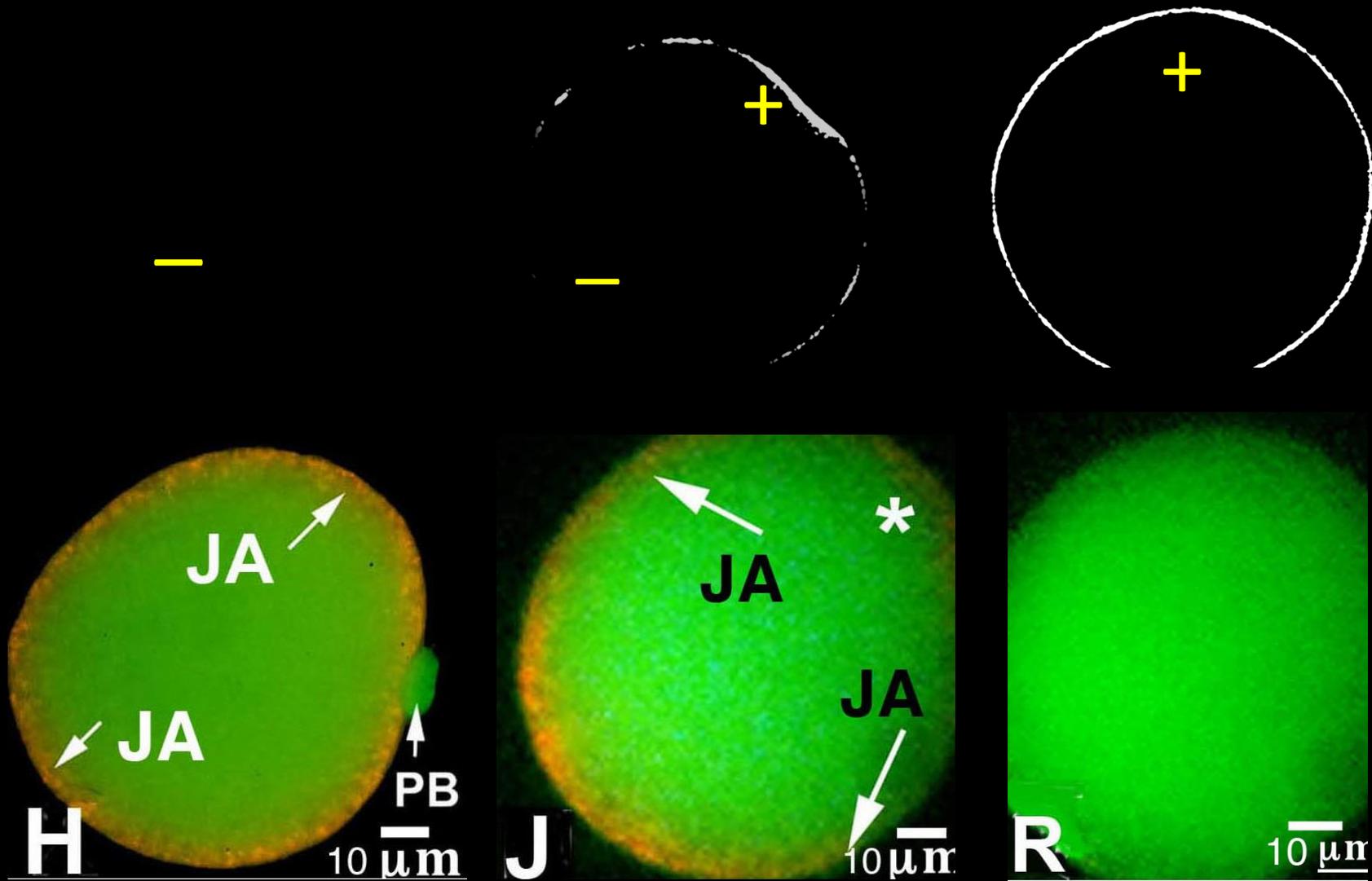
GM1 microdomains may be a common, non species specific site for sperm docking-- similar to human and mouse, detected in pig, rabbit, cow, sheep, dog, cat, deer and bear oocytes

GM1 microdomains and exposure of cell surface proteins associated with robust sperm attachment and binding and penetration (e.g., CD9, FOLR4) –regulated by subplasmalemmal bioenergetics?

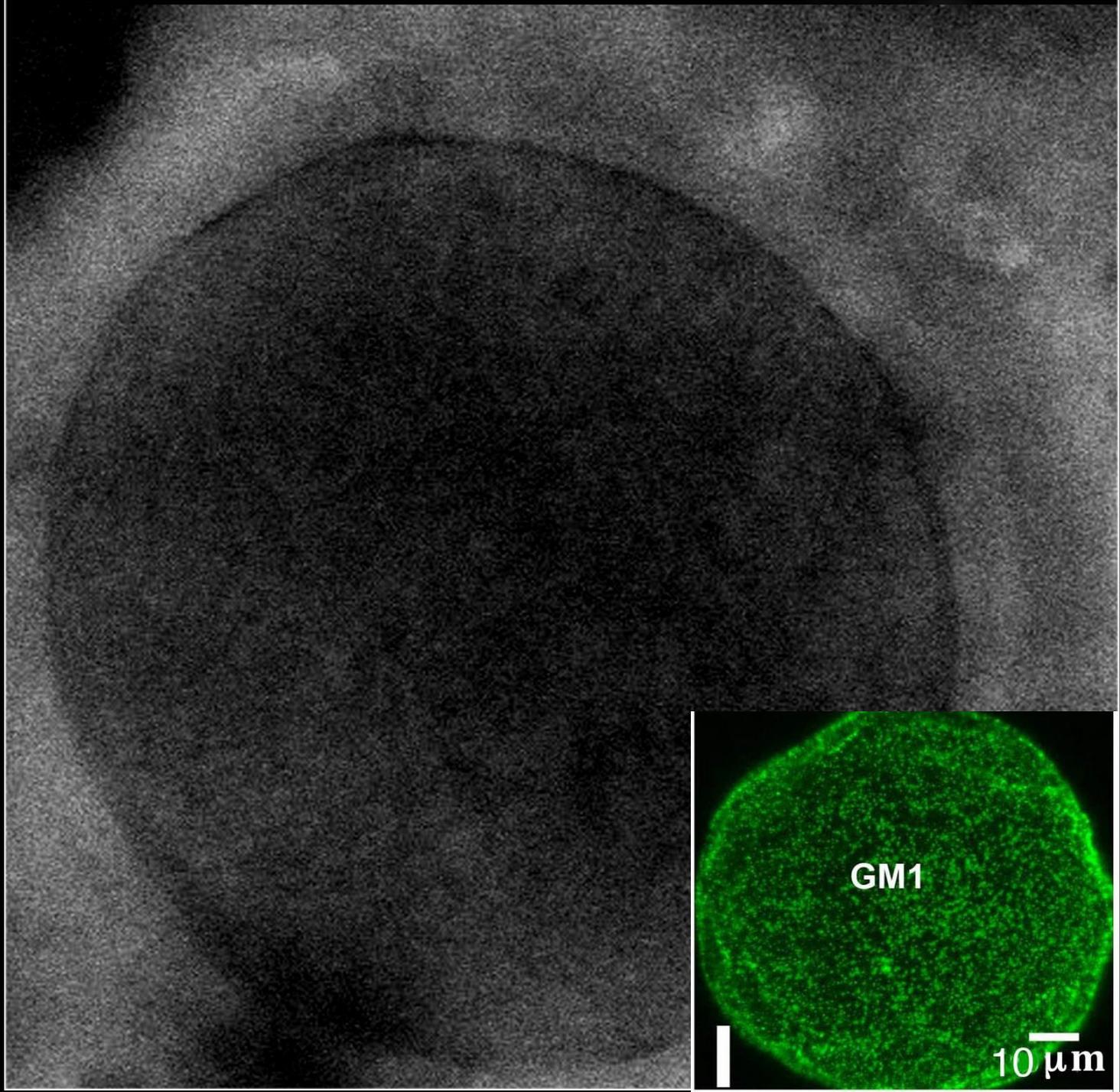
NO in subplasmalemmal cytoplasm and $\Delta\Psi_m$?

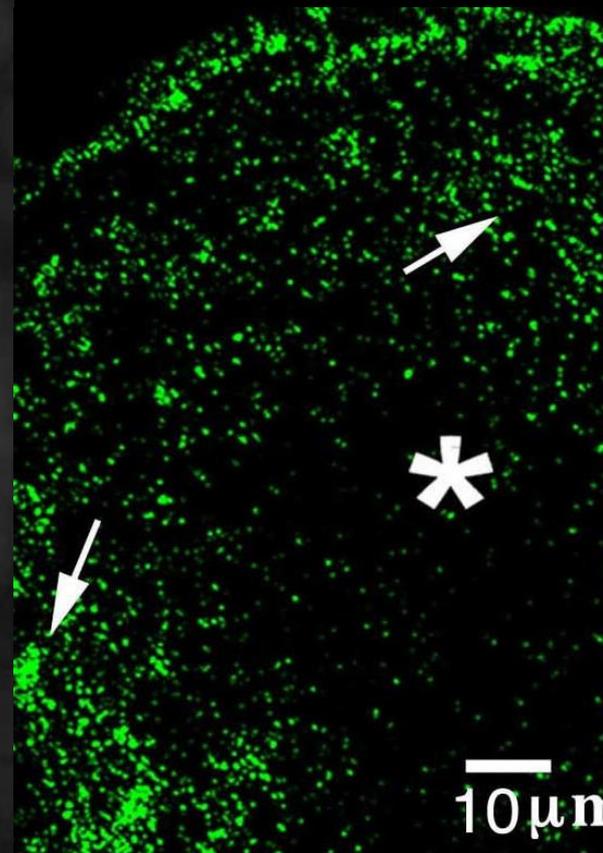
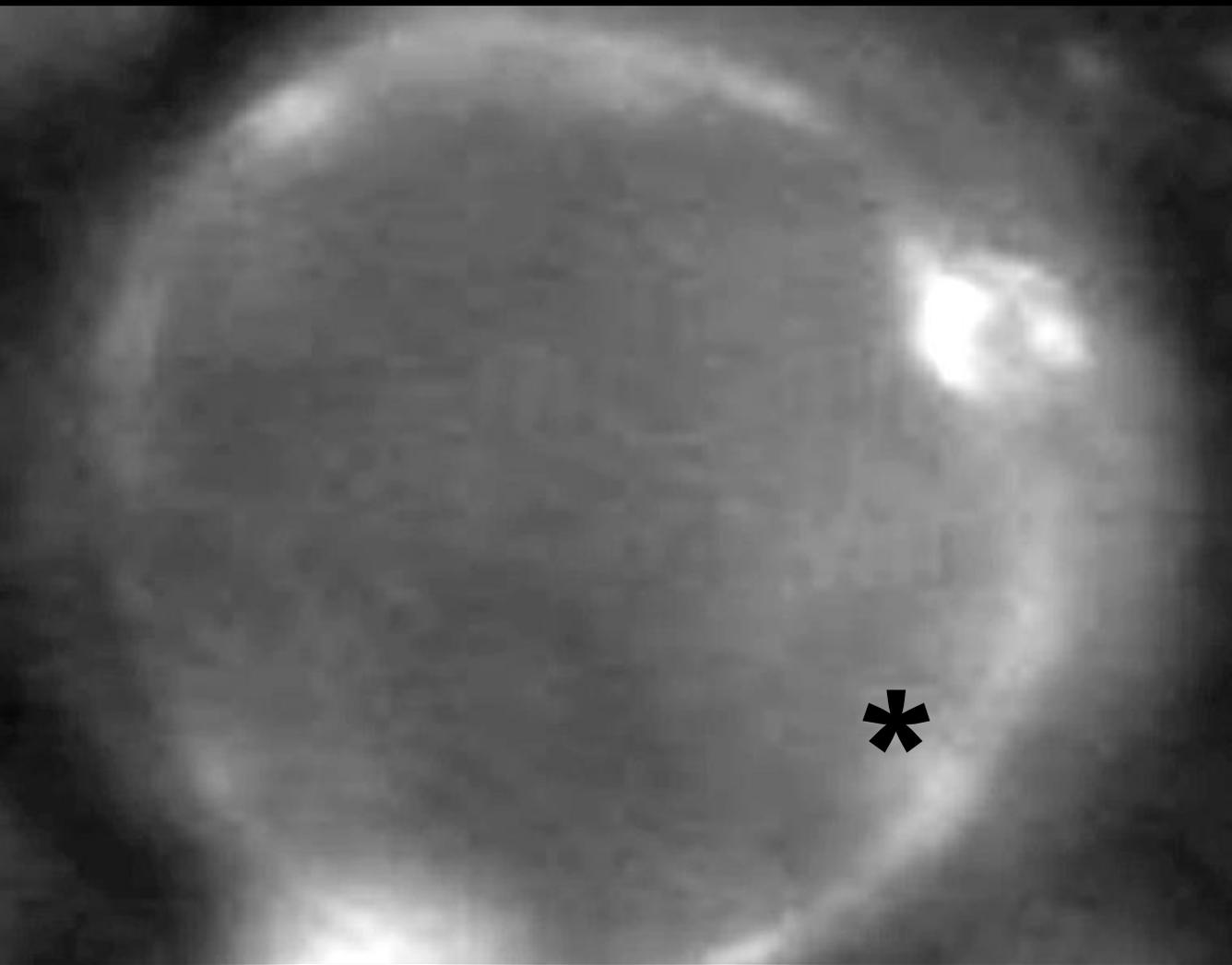
Could NO compete O₂ at level of cytochrome oxidase to reduce electrical potential across inner mitochondrial membrane, which drives ATP production—resulting in a highly local ATP deficit which may include reduced focal concentration of Pi in subplasmalemmal cytoplasm that effects kinase activities and proteins involved in formation of lipid rafts including those containing GM1?

Van Blerkom et al , 2008, Mol Hum Reprod



Cell BioLabs—fluorescent intracellular NO assay-



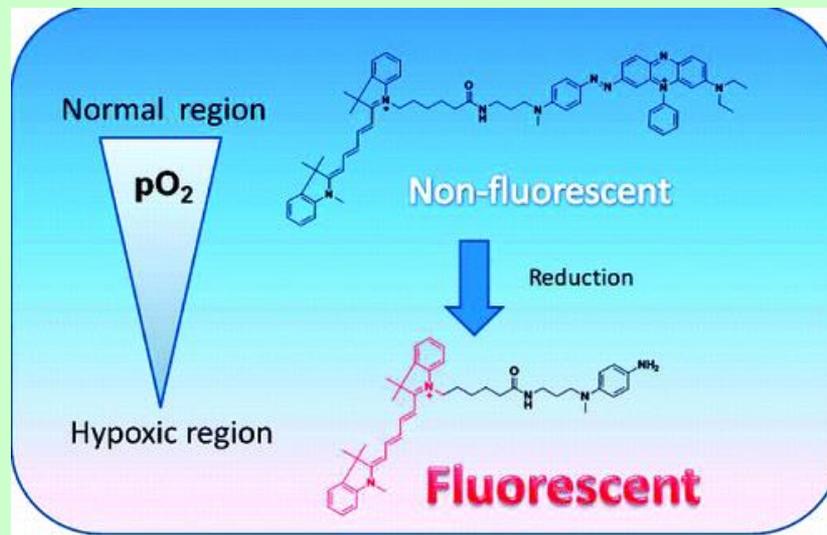


does the level of intrafollicular hypoxia influence subplasmalemmal bioenergetics in the presence of NO from extrinsic and intrinsic eNOS?

Van Blerkom et al 1997, HR: measured pO₂ in follicular fluid aspiration --<1% to 4-4.5%.

Related to aneuploidy:

confirmed by Gregory and colleagues, and others .



Reduction, Oxidation
Loss of Quenching

Hypoxyprobe-1 or pimonidazole HCl, produces pimonidazole adducts in hypoxic tissues designed to be introduced in vivo, and is highly water soluble and can be used in vitro.

Adducts form with thiol groups in proteins such that side-chain of the 2-nitroimidazole is Retained and visualized by anti-adduct immunofluorescence.

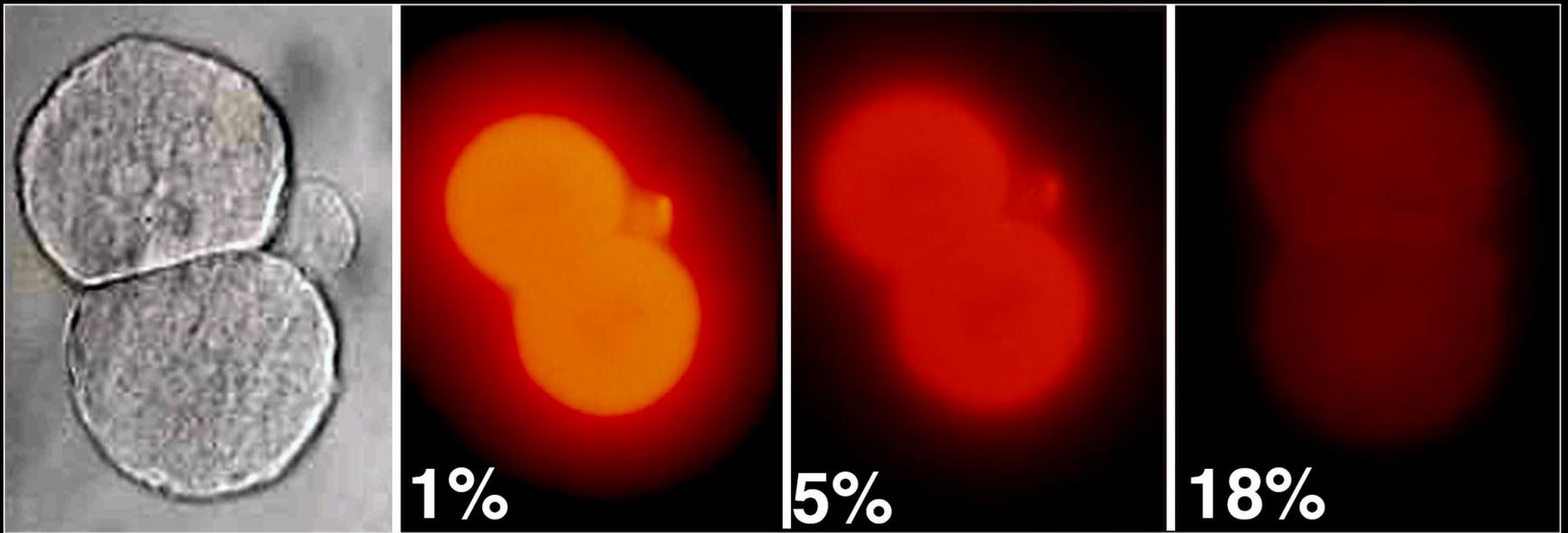
Hypoxia Probe LOX-1 (Scivax Life Sciences), **rapid response to changing pO_2 in vitro -added to culture medium**

Image-iTTM Hypoxia Probe (Thermo- Fisher) **iridium-based for in vitro use**

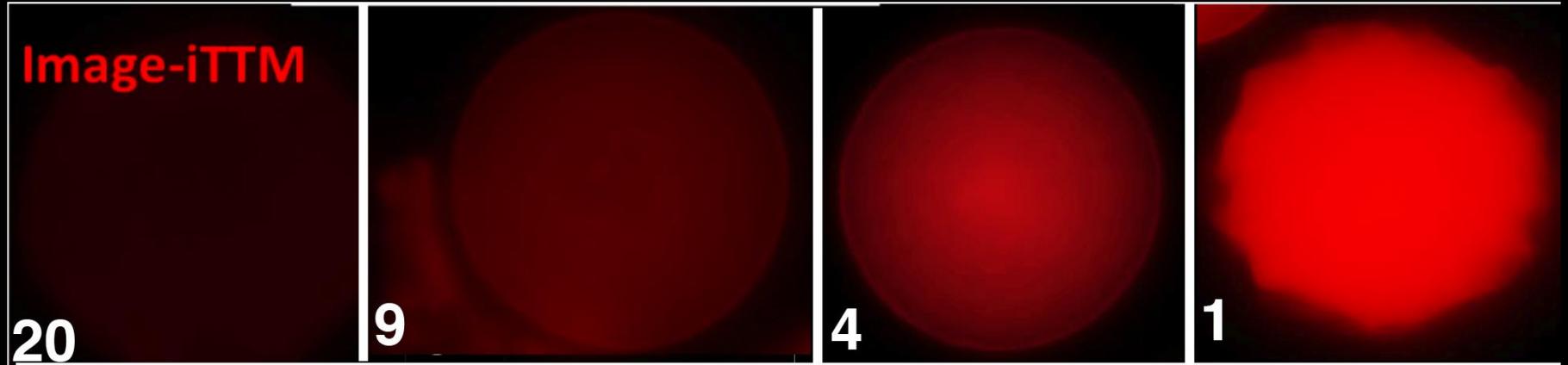


fluorescent determination of the relative degree of hypoxia in situ in human COCs at aspiration

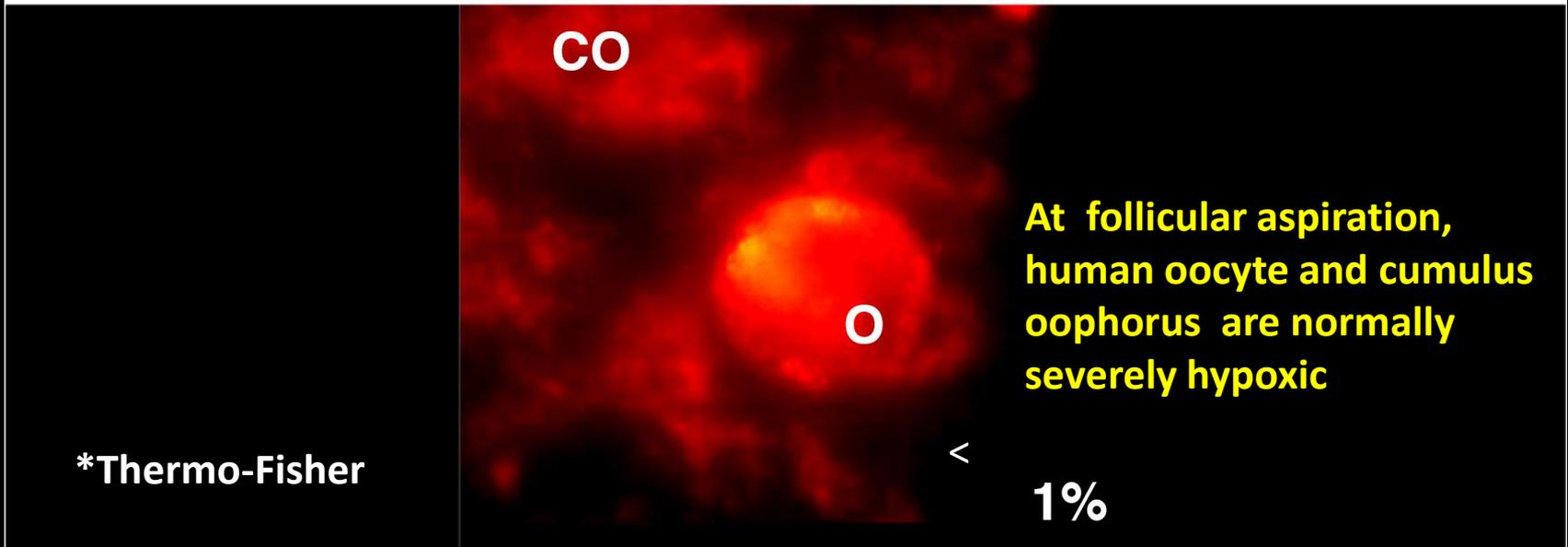
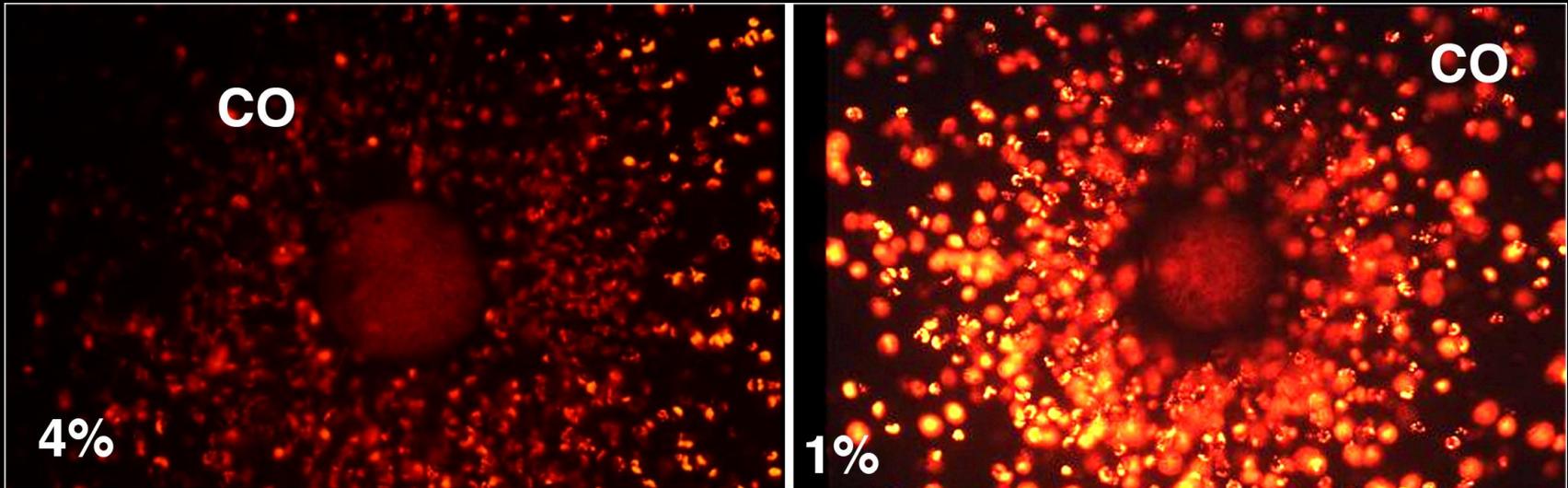
In vitro modulation of O₂ detected with Hypoxia Probe LOX-1 in 2 cell mouse embryo



Detection of hypoxia level in oocytes denuded and stained in follicular fluid at known oxygen concentrations



Detection of relative hypoxia level at follicular aspiration with Image-iTTM Hypoxia Probe*



Cumulus oophorus outgrowth phenotypes in 6-12 h: potential bio-indicator of hypoxia level and ganglioside GM1 organization consistent with fertilization

