

Air Force Research Laboratory

Effect of Silver Nanoparticles on SRC Activity in Male Germ-Line Stem Cells

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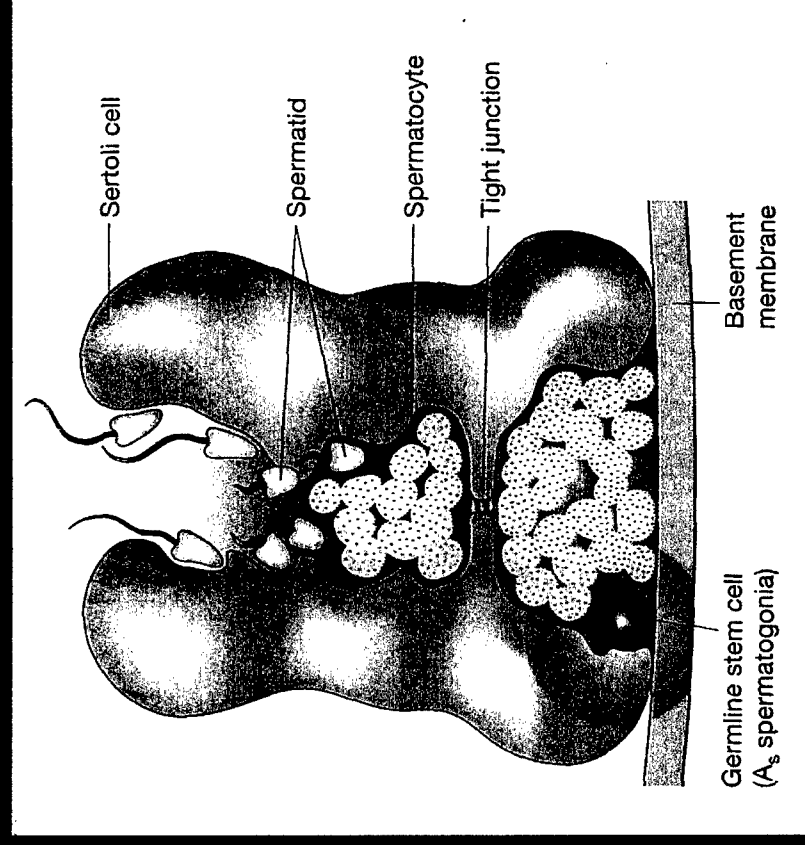
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14. ABSTRACT Gametogenesis is a complex biological process that is particularly sensitive to environmental insults such as chemicals and physical stressors. Exposure to specific chemicals has been shown to inhibit fertility through a negative impact on germ cell proliferation and differentiation that can lower sperm count. In addition, toxicants might produce mutations that could have negative consequences on the development of the offspring. A previous study showed that a spermatogonial stem cell line, called C18-4, provides a sensitive model to assess the cytotoxicity of nanoparticles in the male germ line. We also found that Ag-15nm was more toxic than Al-30nm and MoO3-30nm for spermatogonial stem cell proliferation. The purpose of the present study was to determine the effects of various sizes of Ag nanoparticles on the C18-4 cell line using standard cytotoxic assays. We also assessed the impact of Ag nanoparticles on Src signaling, since the activation of this kinase allows normal spermatogonial stem cells to proliferate. Mitochondrial function (MTS) data showed that Ag-130nm was not toxic to the cells, but smaller particles showed toxicity. Membrane leakage was increased by treatment with the Ag-25nm and Ag-30nm particles, even at low concentrations (5 µg/ml). However, no membrane leakage was apparent when the cells were treated with Ag-130nm. Further, we have found that the activity of Fyn (a member of the Src family of cytoplasmic kinases) is significantly reduced when the cells are treated with Ag nanoparticles. This inhibition increases with the size of the nanoparticles, with no activity detected above a size of 30 nm..					
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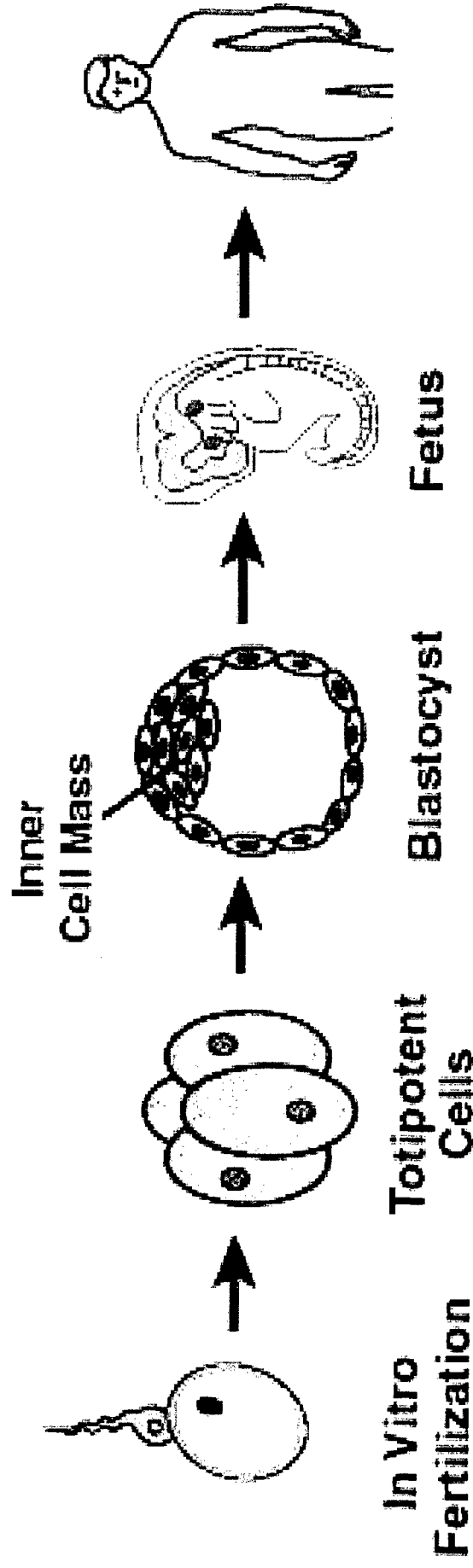
EFFECT OF SILVER NANOPARTICLES ON SRC ACTIVITY IN C18-4 CELLS.

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and Marie-Claude Hofmann¹

Department of Biology, The University of Dayton¹, Operational Toxicology
Branch, Air Force Research Laboratory, Wright-Patterson Air Force Base².



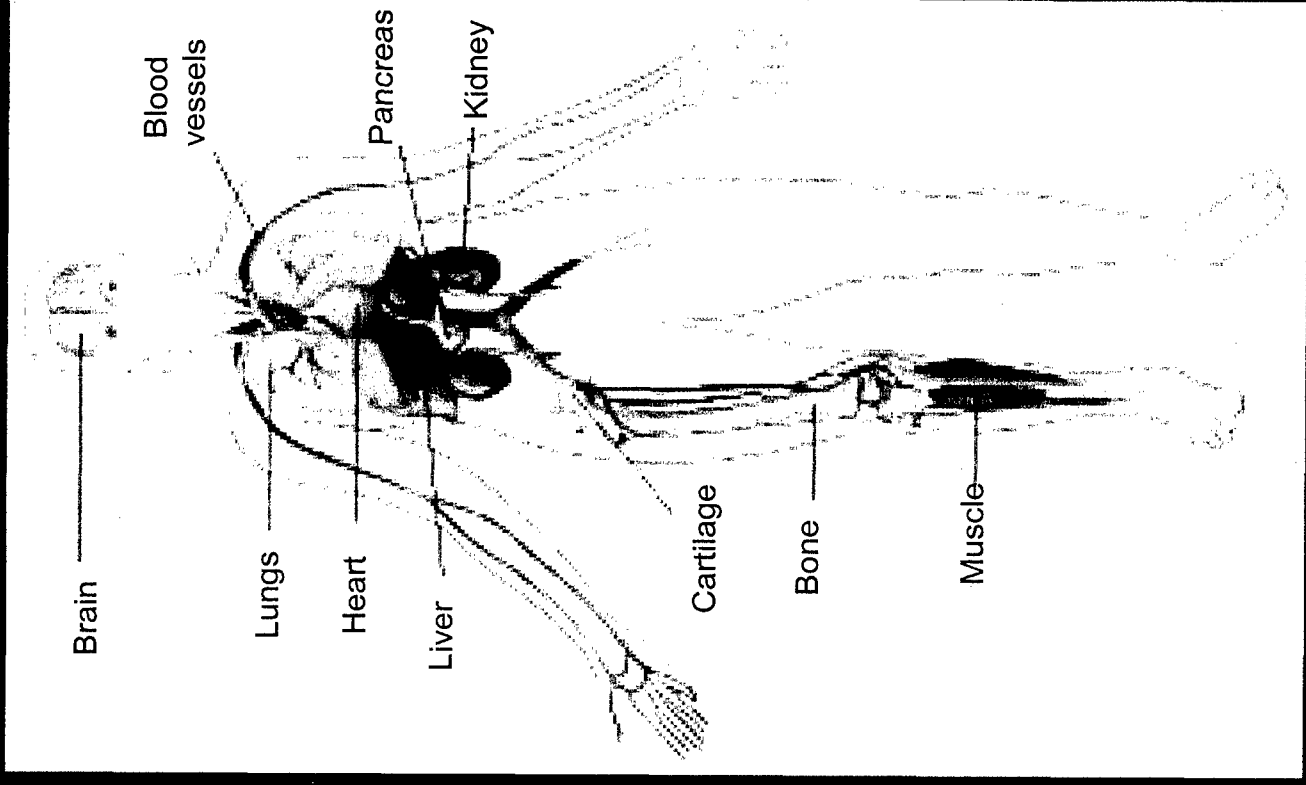
Stem Cells



Totipotent → Pluripotent → Multipotent
Unipotent

ADULT STEM CELLS:

- Multipotent:
 - hematopoietic stem cells
 - mesenchymal stem cells
- Unipotent:
 - epidermal stem cells
 - spermatogonial stem cells

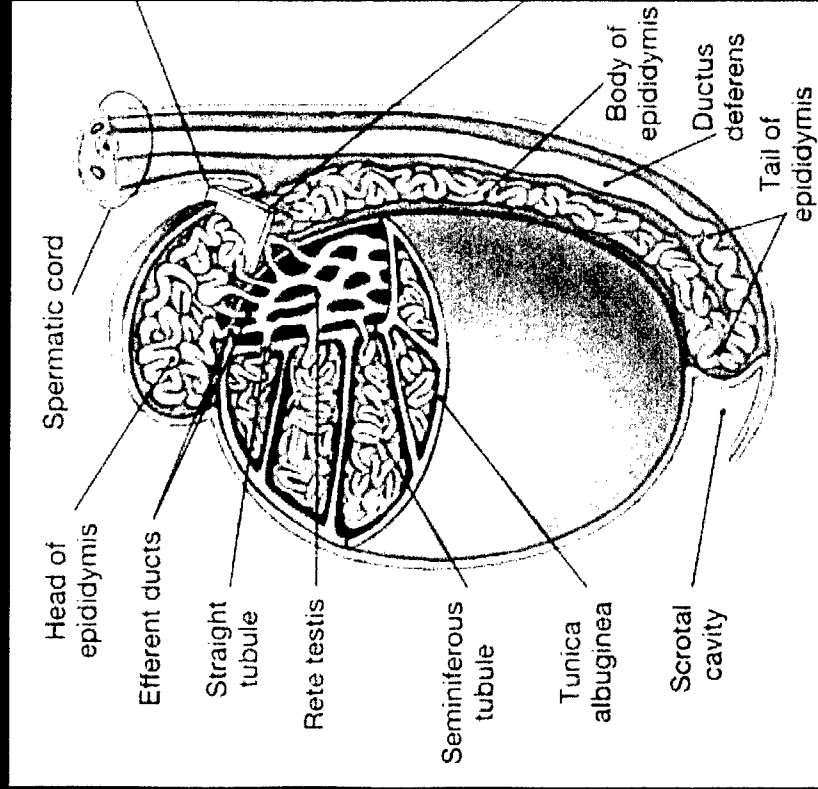


Characteristics of a stem cell:

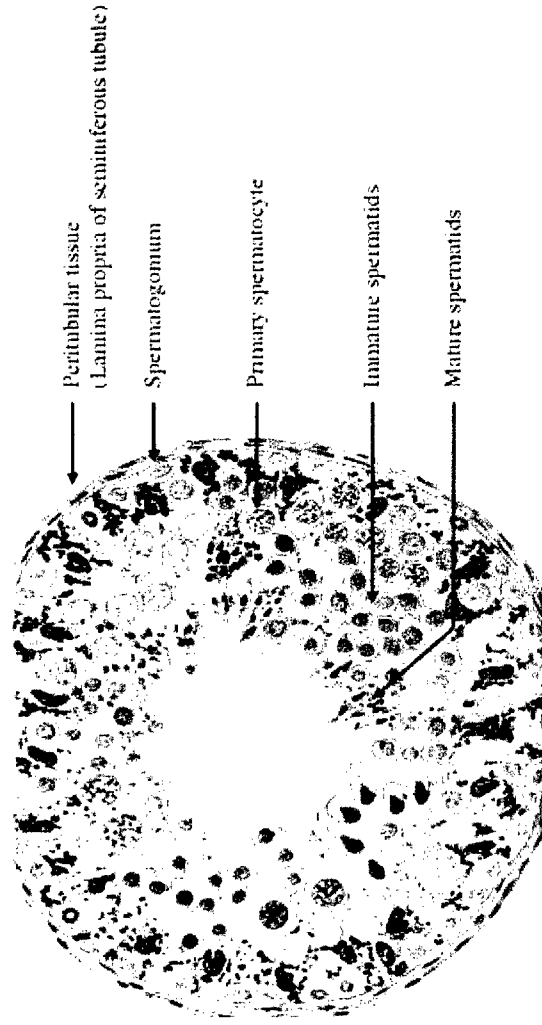
- Undifferentiated
- Capable of self-renewal
- Generates committed progenitor cells
- Regenerates tissues/lineages after transplantation

Adult testis

A



B



Spermatids

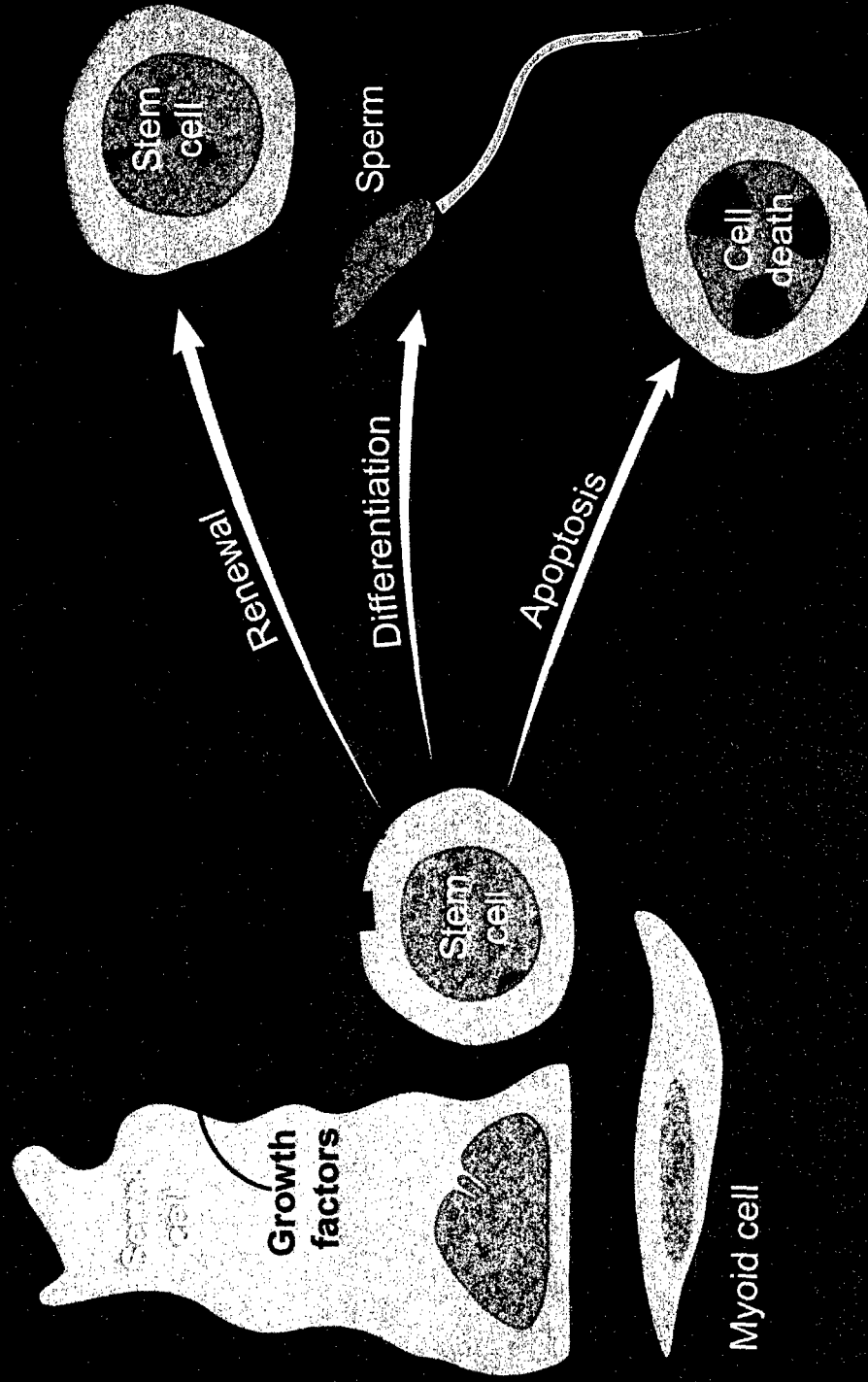
Spermatocytes

Spermatogonia
(include stem cells)

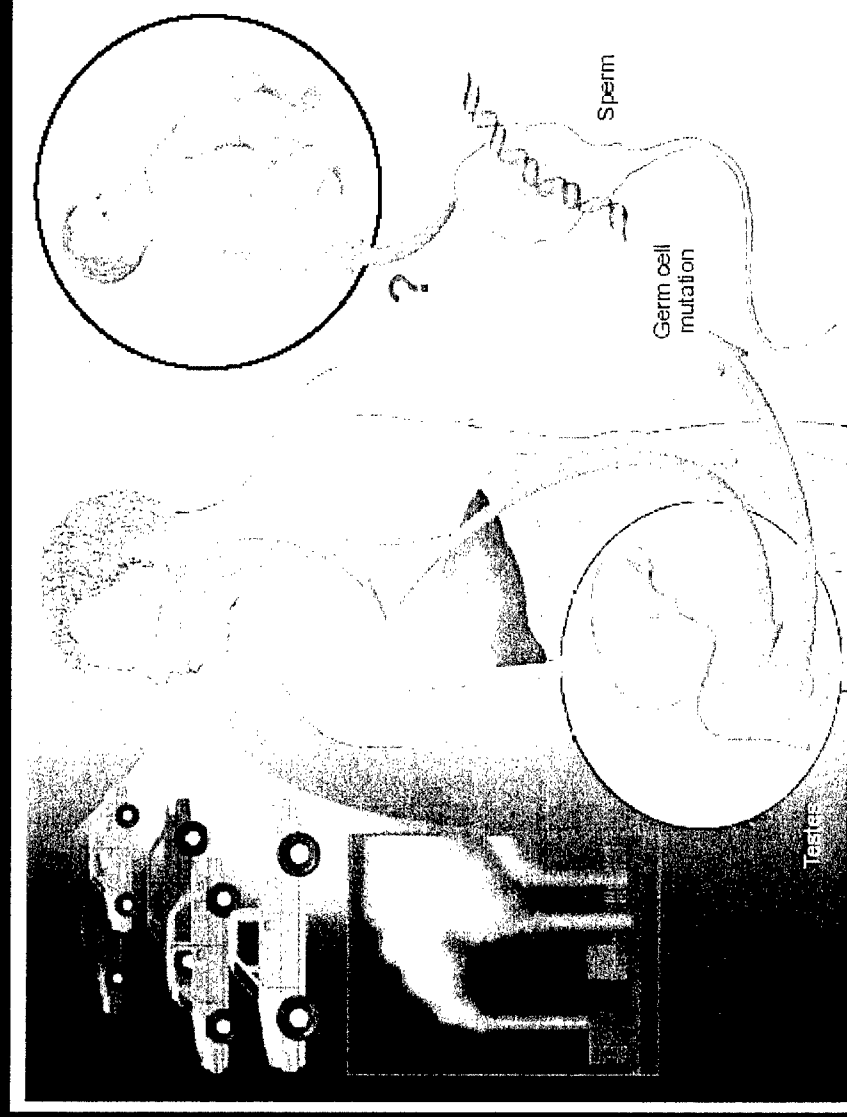


Russell L, *et al*. Histological and Histopathological
Evaluation of the Testis, Cache River Press, 1990

Fate of the spermatogonial stem cell



Are germline stem cells targets for particulate toxicants?

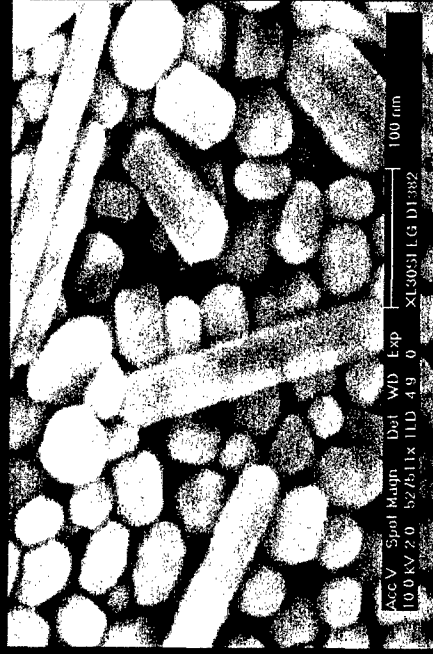


Inhaled air particles and heritable mutations. Airborne particulate pollution is caused primarily by emissions from vehicles, industries, and power stations. Inhalation of airborne particles into the lungs leads to presumptive mutations in mouse male germ cells that can be passed on to the next generation.

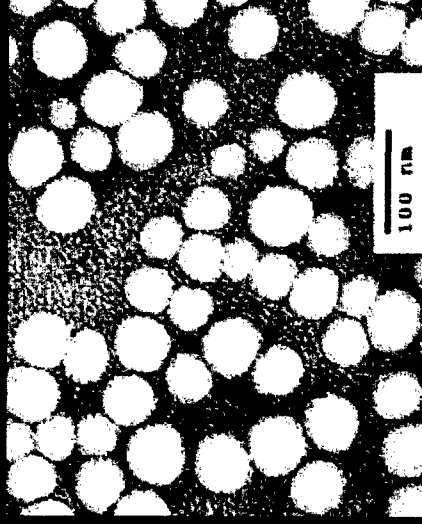
NANOPARTICLES

Nanoparticles are used for a variety of purposes in engineering and medicine (drug delivery)

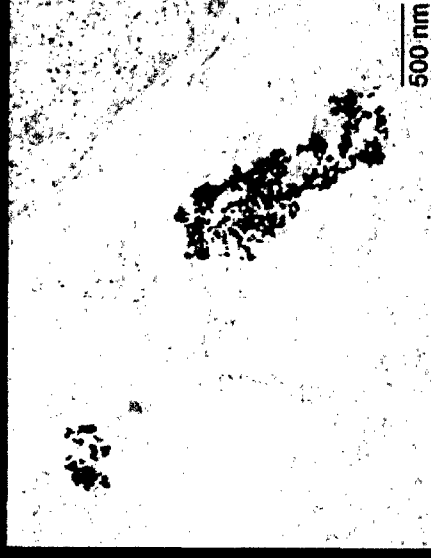
Nanoparticles have a size of <100 nm and can penetrate cellular membranes passively



Ag nanoparticles



Au nanoparticles

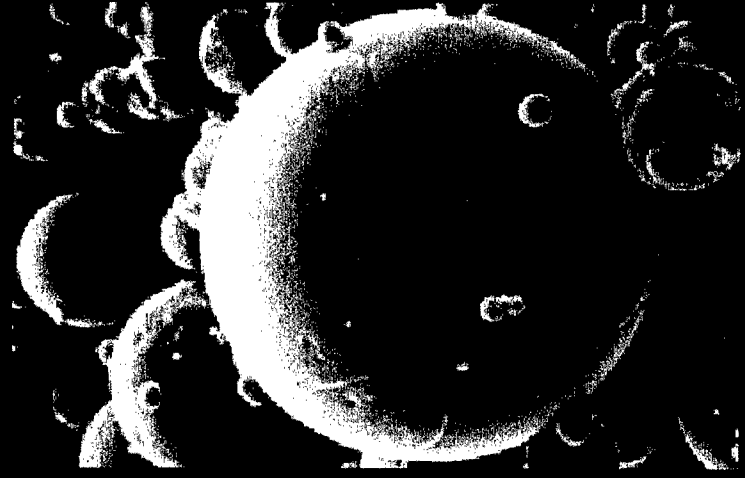


TiO₂ nanoparticles
inside an epithelial cell
(Aitken et al, 2005)

Questions:

Are nanoparticles toxic for germ line stem cells in vitro and in vivo?

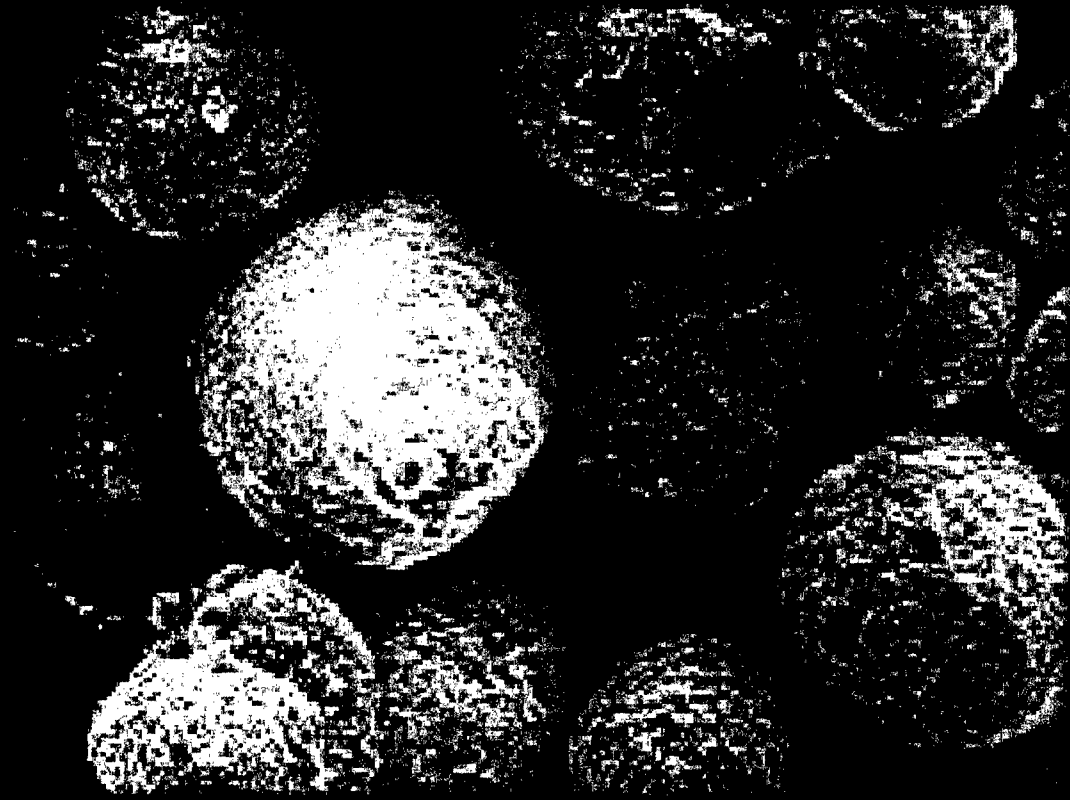
Will nanoparticles disrupt signaling pathways in live germ line stem cells?



TiO₂ (Swiss Re)

Nanoparticles are able to
penetrate cell membranes
passively.

They also are able to penetrate the
blood-brain and **blood-testis**
barriers



In vitro toxicity studies

Material:

Cell line: C18-4 spermatogonial stem cell line (Hofmann et al, Stem Cells, 2005)

Nanoparticles:

Composition:

Au

Mo

TiO₂

Al

Ag

Size:

3 nm

30 nm

20-30 nm

30 nm

15-30 nm

Toxicity in liver cells:

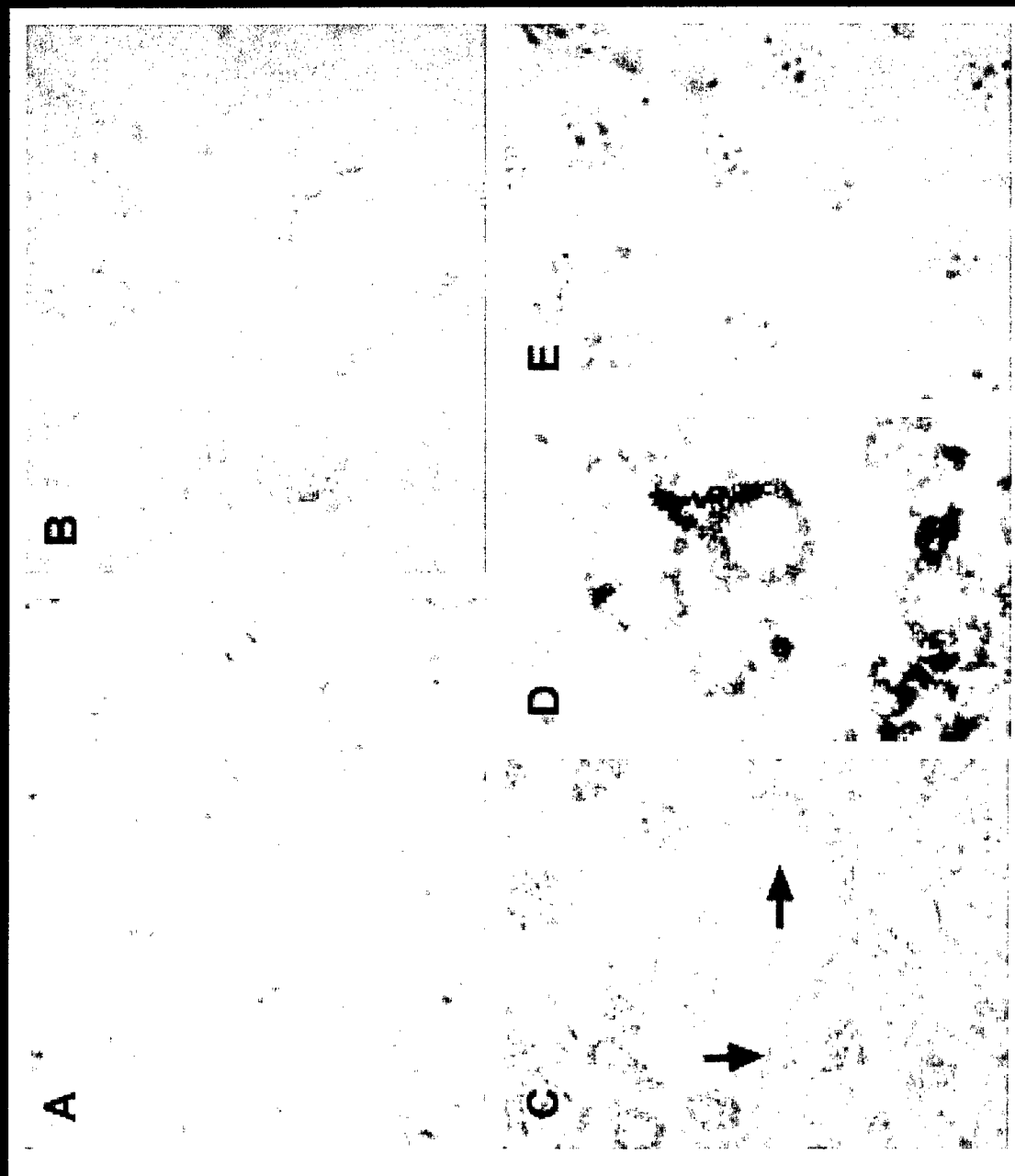


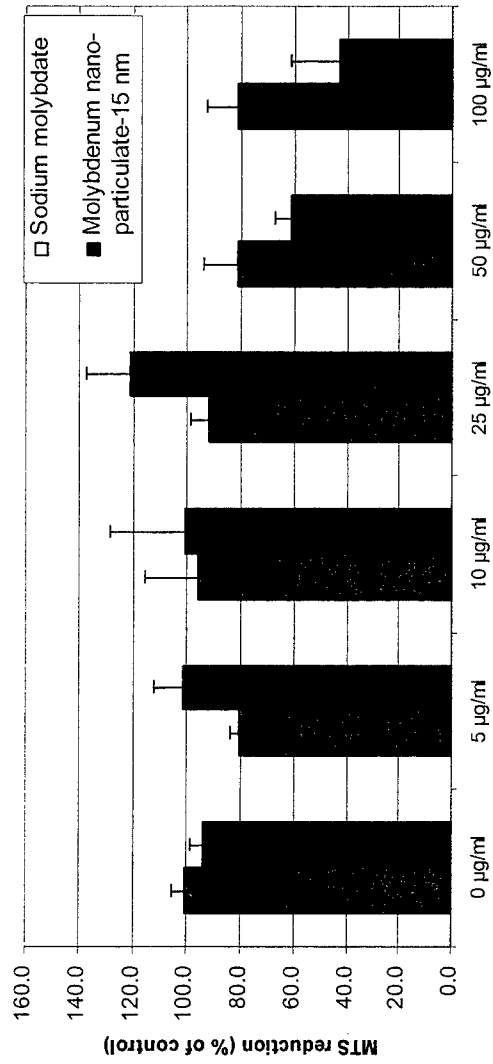
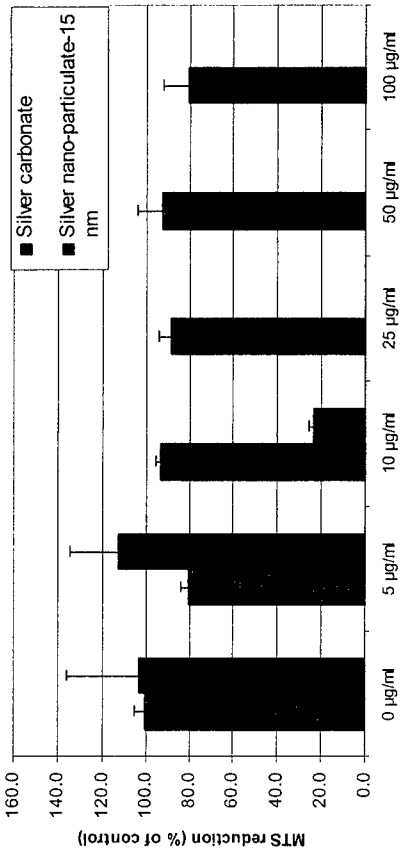
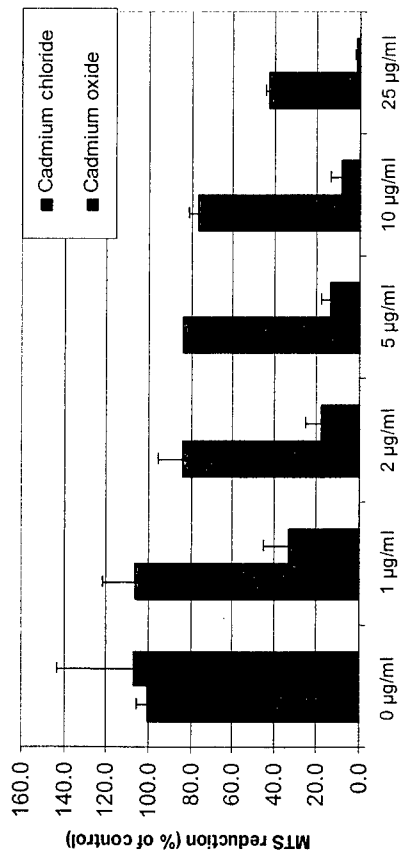
Methods:

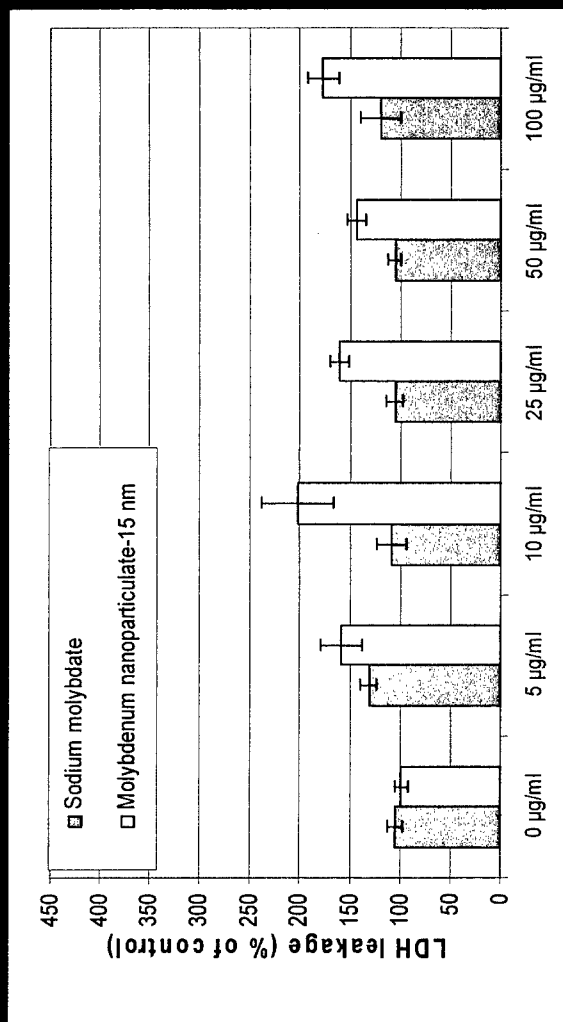
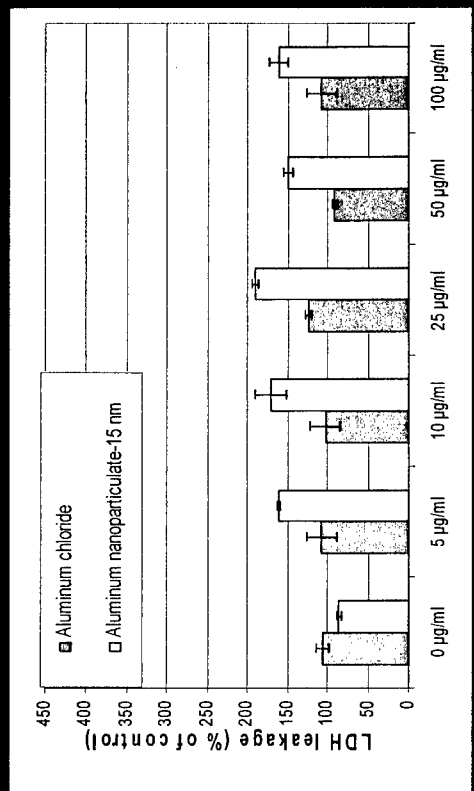
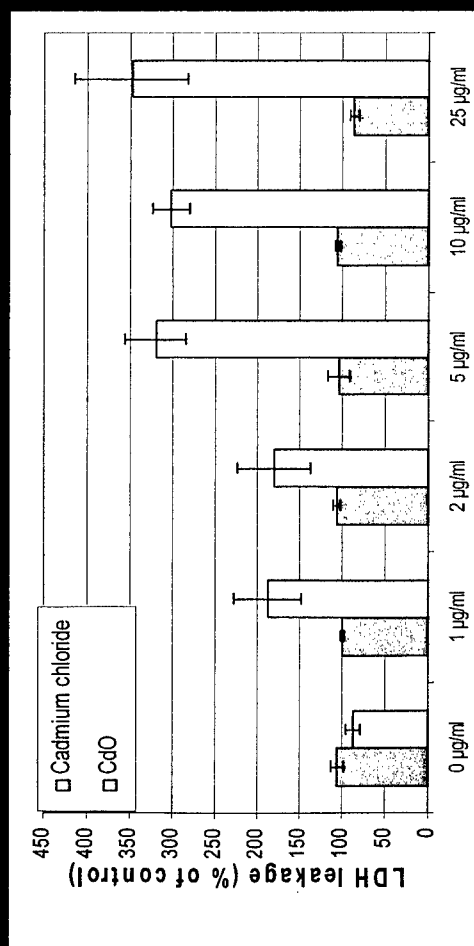
1) Culture the C18-4 cells with different nanoparticles concentrations

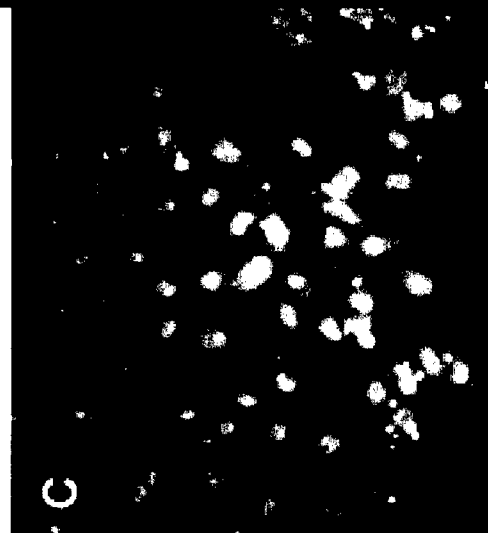
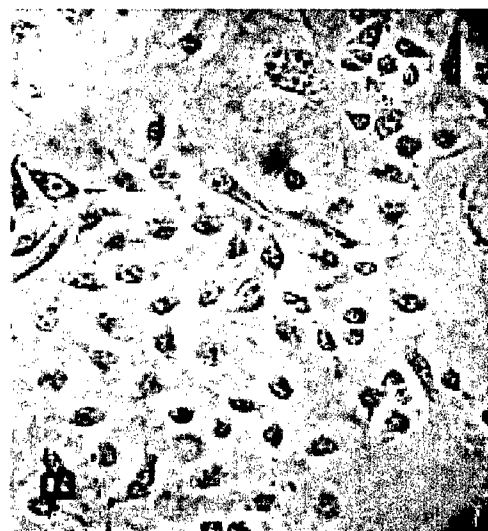
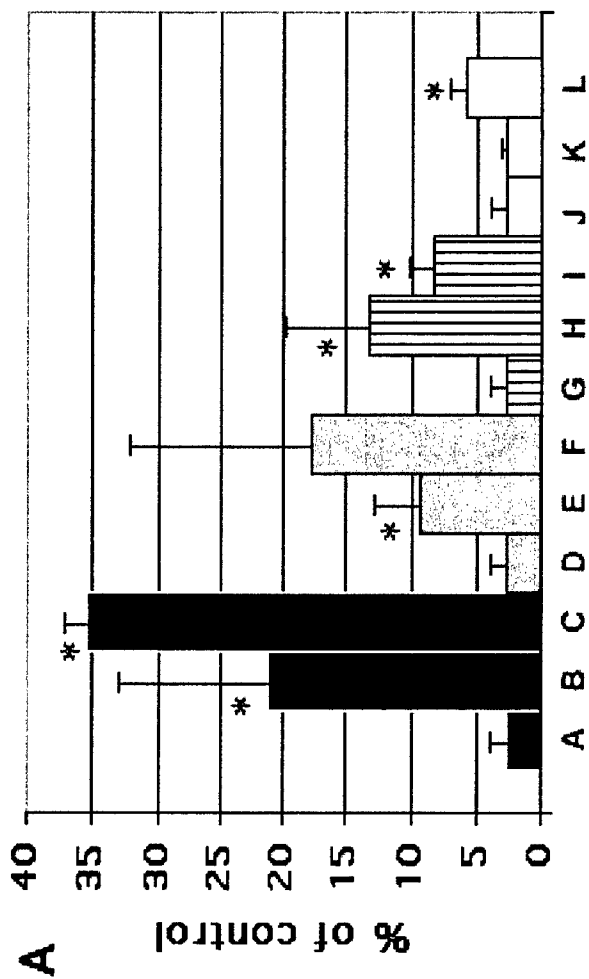
2) Standard cytotoxicity assays:

- morphology
- MTT reduction (mitochondrial activity)
- LDH membrane leakage
- apoptosis









Conclusions:

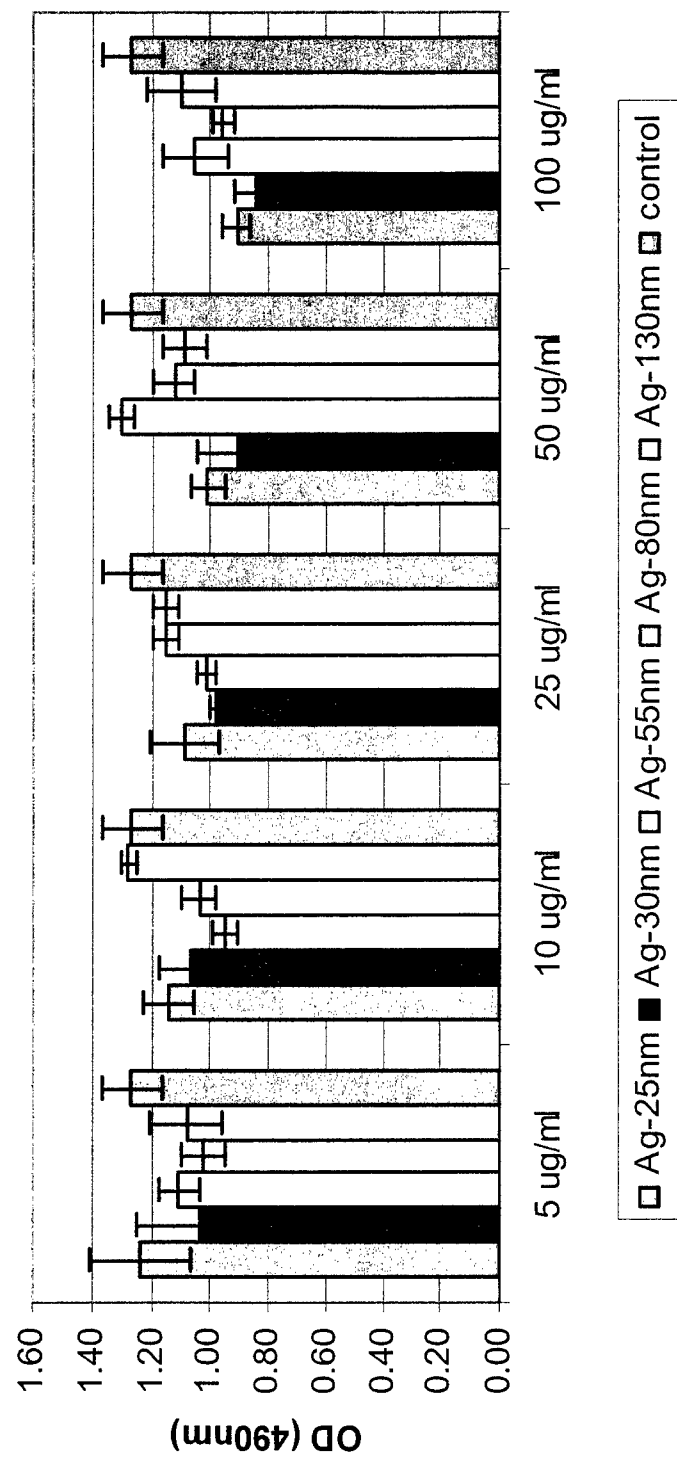
- At low concentrations nanoparticles induce apoptosis in the C18-4 cells, and at high concentrations they induce necrosis.
- CdO showed high cellular toxicity even at low concentrations ($EC_{50}=0.5\text{ }\mu\text{g/ml}$) and LDH leakage was apparent.
- In comparison, the other particles were less toxic than the CdO. Ag-15nm was the most toxic ($EC_{50}=7.5\text{ }\mu\text{g/ml}$) and MoO_3 -30nm was the least toxic ($EC_{50}=75\text{ }\mu\text{g/ml}$), and LDH leakage occurred around $5\text{ }\mu\text{g/ml}$.

Question: Are the Ag-15nm nanoparticles more toxic than the Al-30nm & MoO₃-30nm because they are smaller?

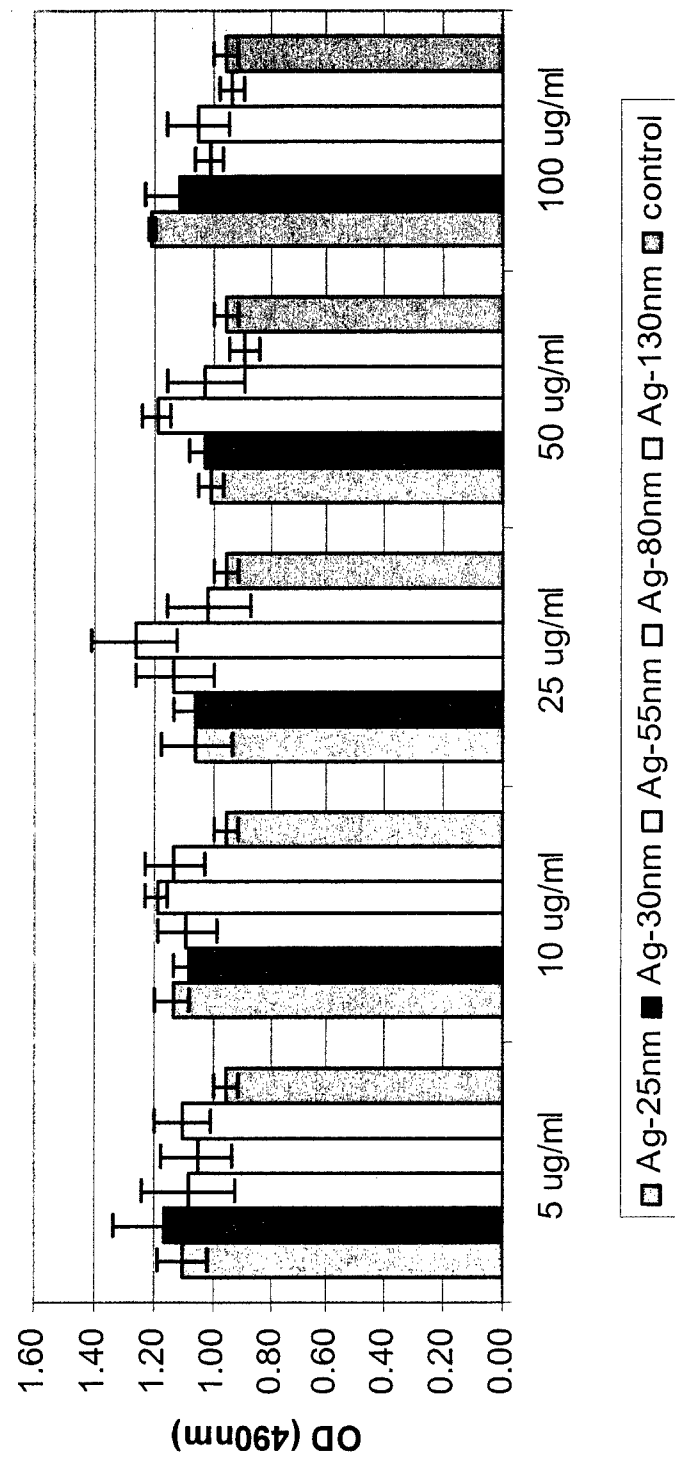
Methods:

- 1) Culture the C18-4 cells with different sized Ag nanoparticles
- 2) Standard cytotoxicity assays:
 - MTT reduction (mitochondrial activity)
 - LDH membrane leakage

Nanoparticles



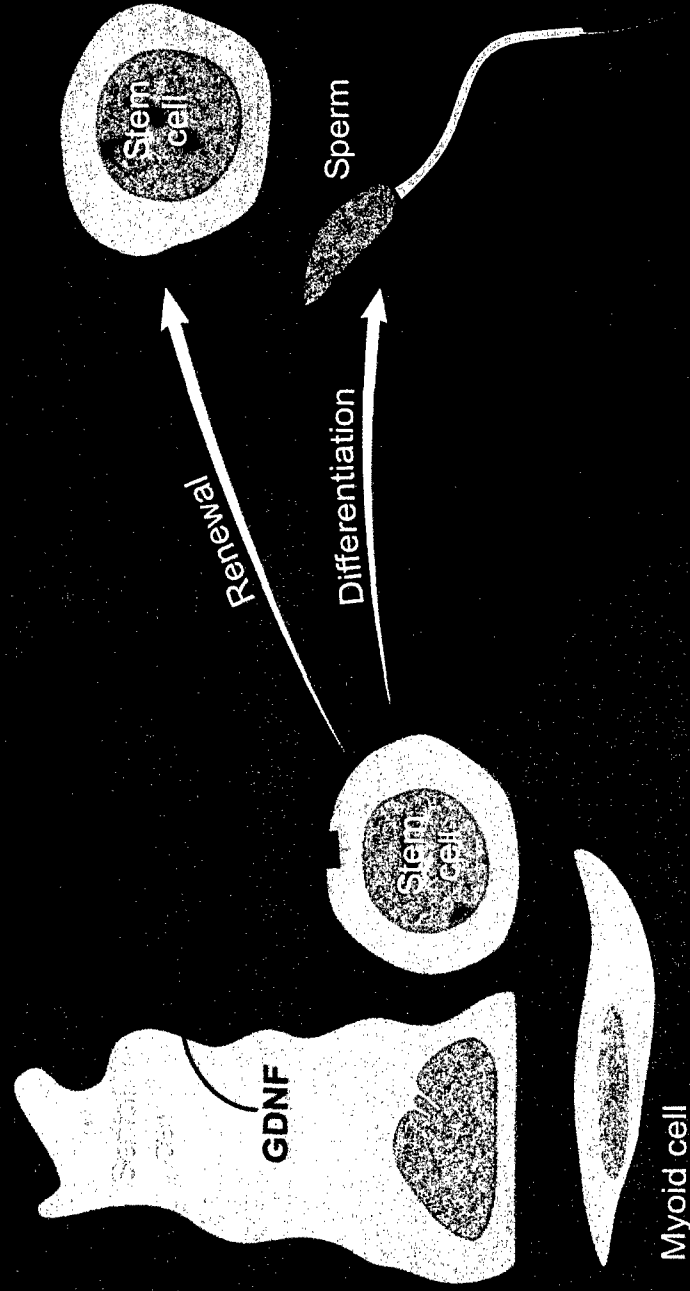
Membrane Leakage in C18-4 Cells After Treatment with Ag Nanoparticles



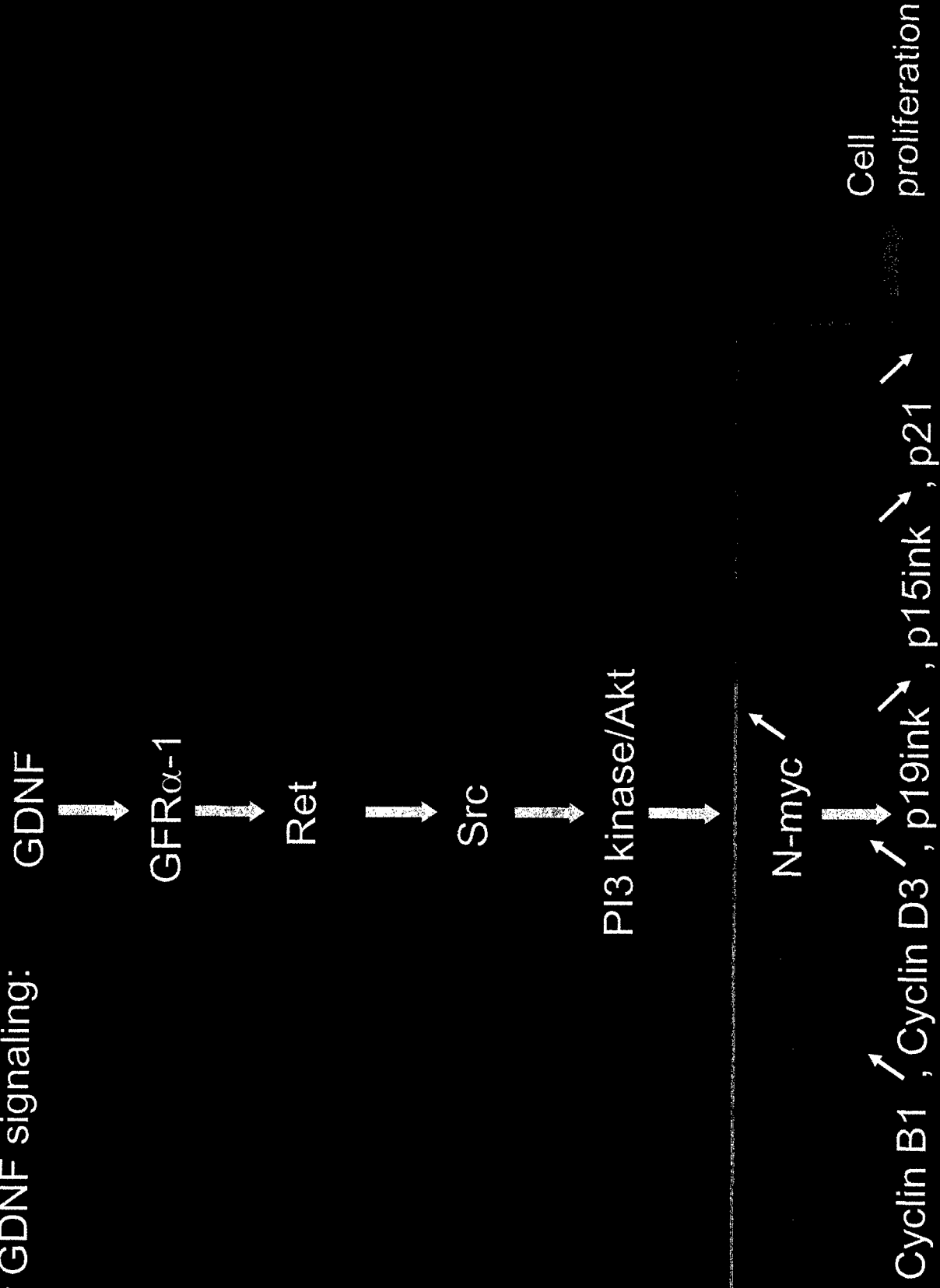
Conclusions:

- Ag-55nm, Ag-80nm, and Ag-130nm were not toxic to the cells, but for Ag-25nm $EC_{50}=100\mu\text{g/ml}$, Ag-30nm $EC_{50}=25\mu\text{g/ml}$.
- In the Ag-25nm particles, LDH leakage is apparent at $5\mu\text{g/ml}$, in the other nanoparticles (30nm-80nm) LDH leakage begins to occur at $10\mu\text{g/ml}$.
- This suggests that size may play a role in the mechanism of toxicity.

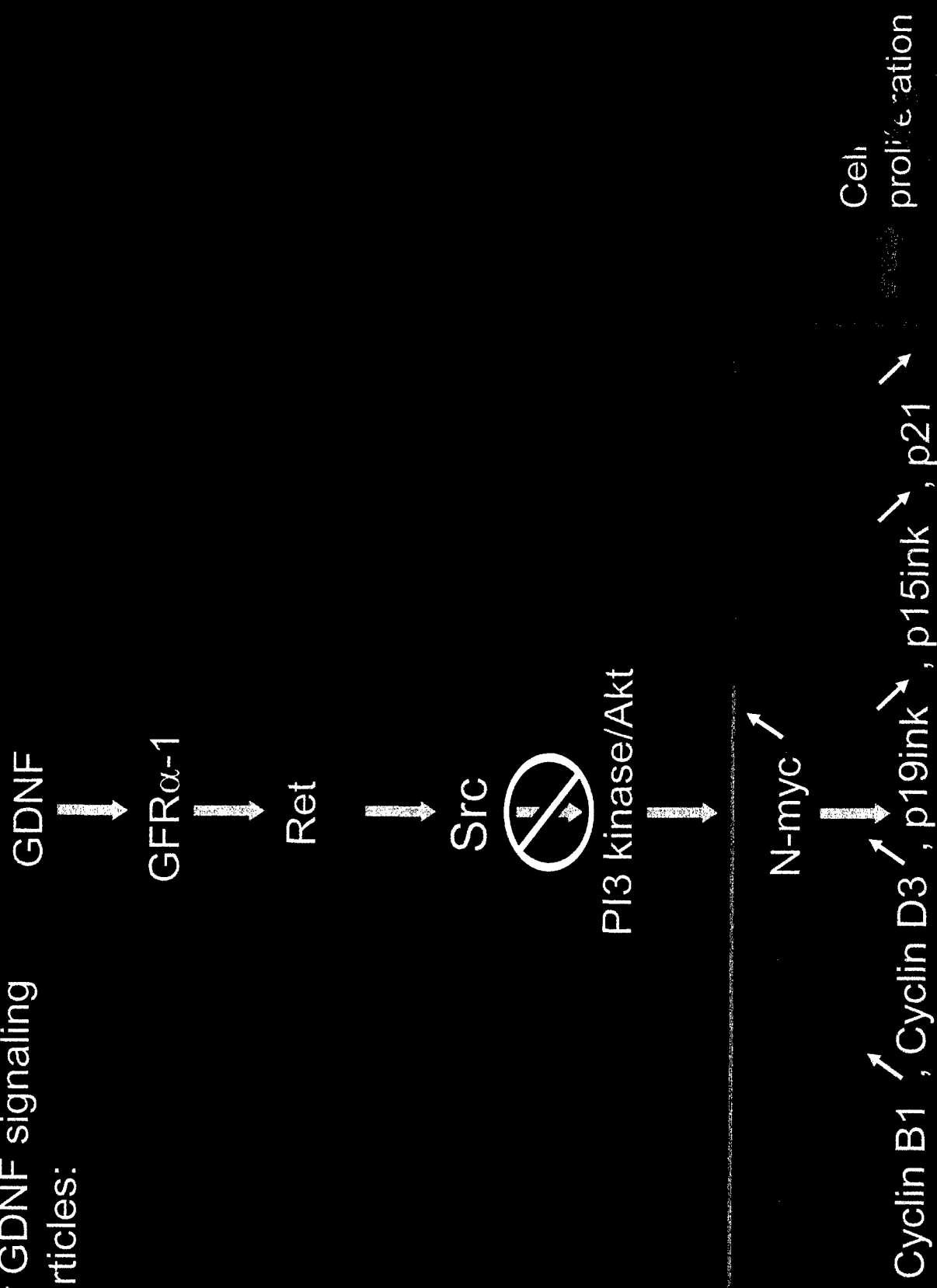
Fate of Spermatogonial Stem Cells



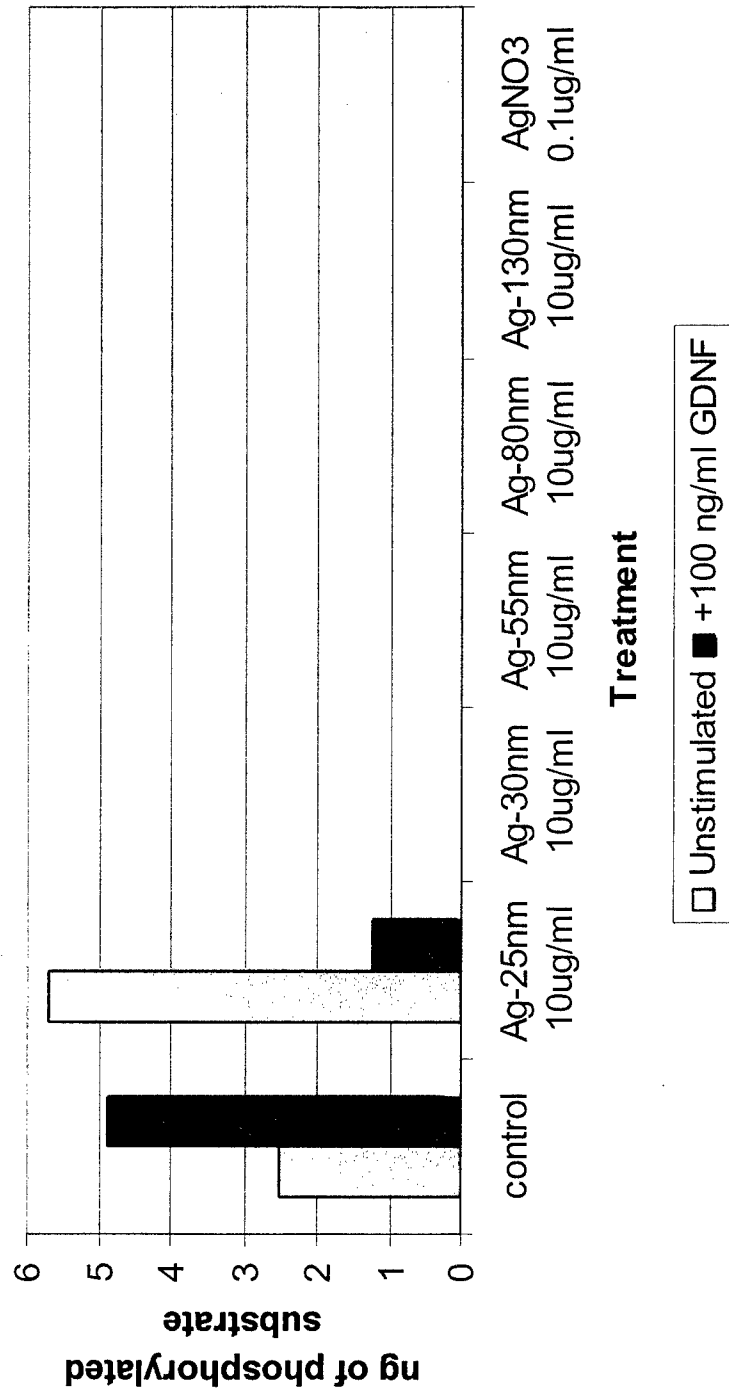
Model for GDNF signaling:



Model for GDNF signaling
+ nanoparticles:



Fyn Kinase Activity in C18-4 cells after Exposure to Ag Nanoparticles



Conclusions:

Ag nanoparticles larger than 30nm show diminished Fyn kinase activity.

What is causing the disruption of the Src kinase:

Nanoparticles inhibit GDNF from binding to receptor and prevent upstream activation of Fyn?

Nanoparticles interfere with Fyn kinase?

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