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Our knowledge on the pathogenetic mechanisms could lead to:

- A gender-specific prevention
- A gender-specific diagnosis
- A gender specific therapy

Pathogenetic Mechanisms and gender

- Several gender differences can be explained considering the activity of hormones

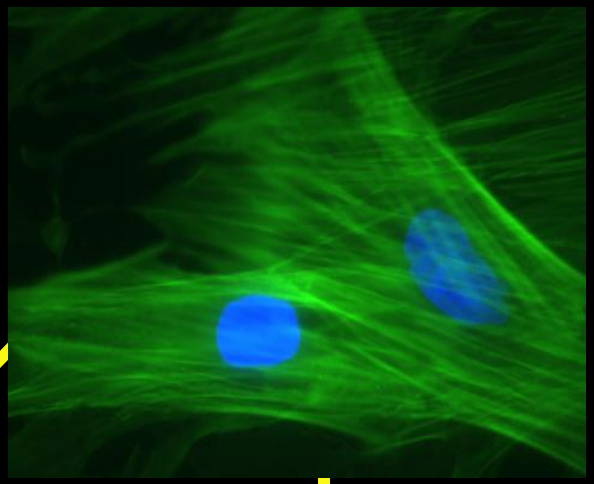
HOWEVER:

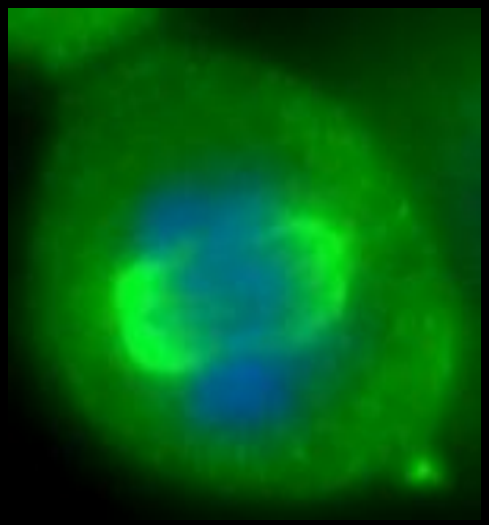
- Studies with animals (es. four core genotypes*) demonstrated that not all gender disparities are due to hormones
- Studies on genetic diseases (e.g. studies on mitochondrial diseases) have demonstrated gender differences
- Several diseases (both communicable and non-communicable) show gender differences in pediatric diseases.
- Differences at cell level (XX and XY cells)

Del Principe D, Marconi M, Matarrese P, Villani A, Malorni W. Gender disparity in pediatric diseases. Curr Mol Med. 2013 May;13(4):499-513.

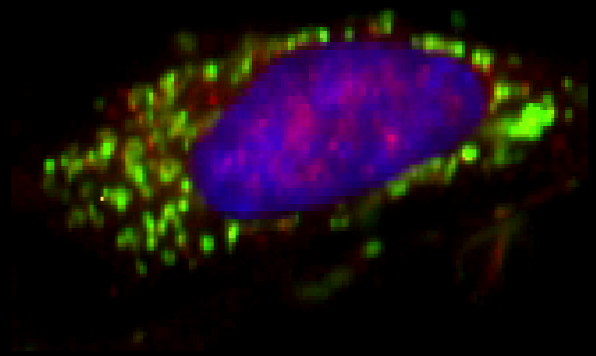
(*) mice in which sex chromosome complement (XX vs. XY) is unrelated to the animal's gonadal sex. The four genotypes are XX gonadal males or females, and XY gonadal males or females

Cell programs

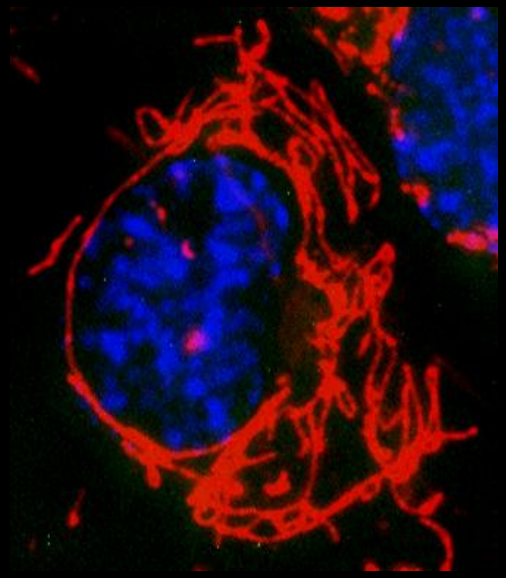




proliferation

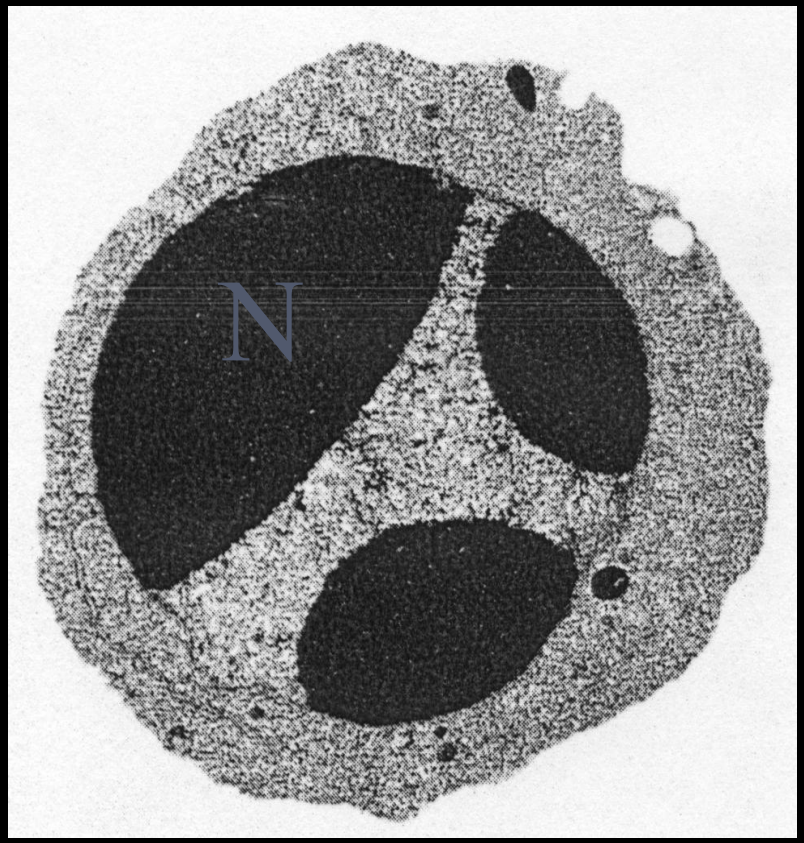


Cell death (e.g. by apoptosis)

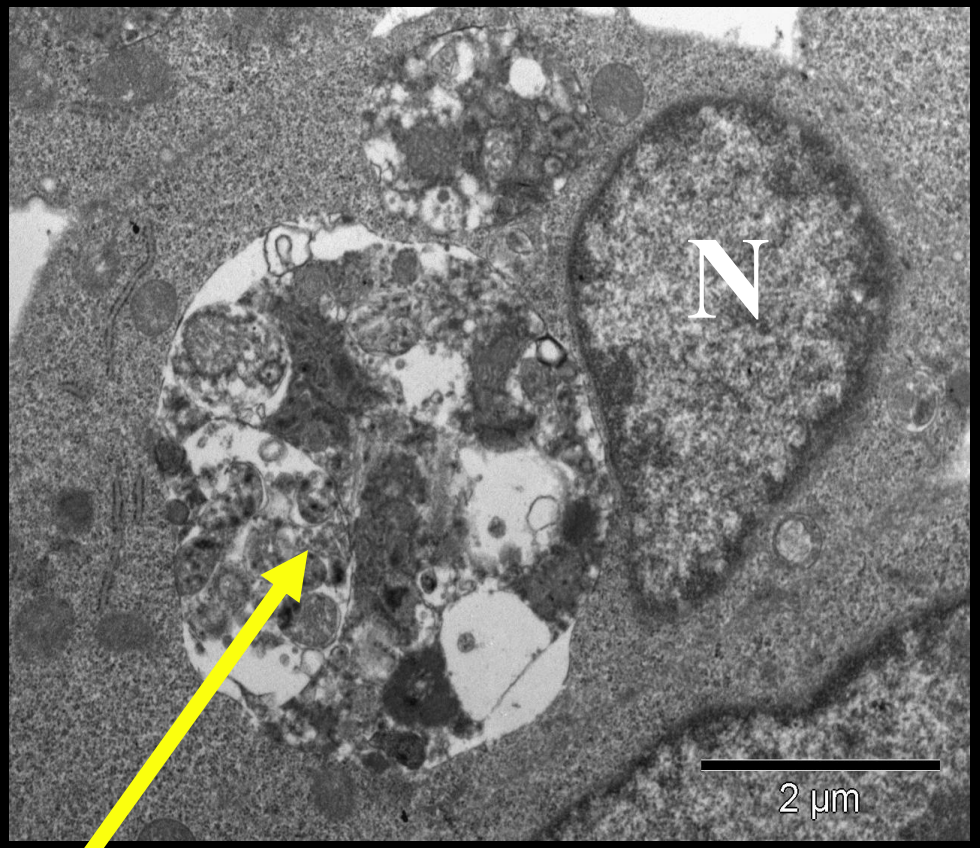


Autophagy and senescence

Cell programs:



apoptosis



autophagy

Apoptosis in human pathology

Cancer
- Colorectal
- Glioma
- Hepatic
- Neuroblastoma
- Leukaemia and lymphoma
- Prostate

·Autoimmune diseases
- Systemic Lupus Erythematosus
- Autoimmune Lymphoproliferative Syndrome
- Thyroid diseases

·Inflammatory diseases
- Bronchial Asthma
- Inflammatory intestinal disease
- Pulmonary inflammation

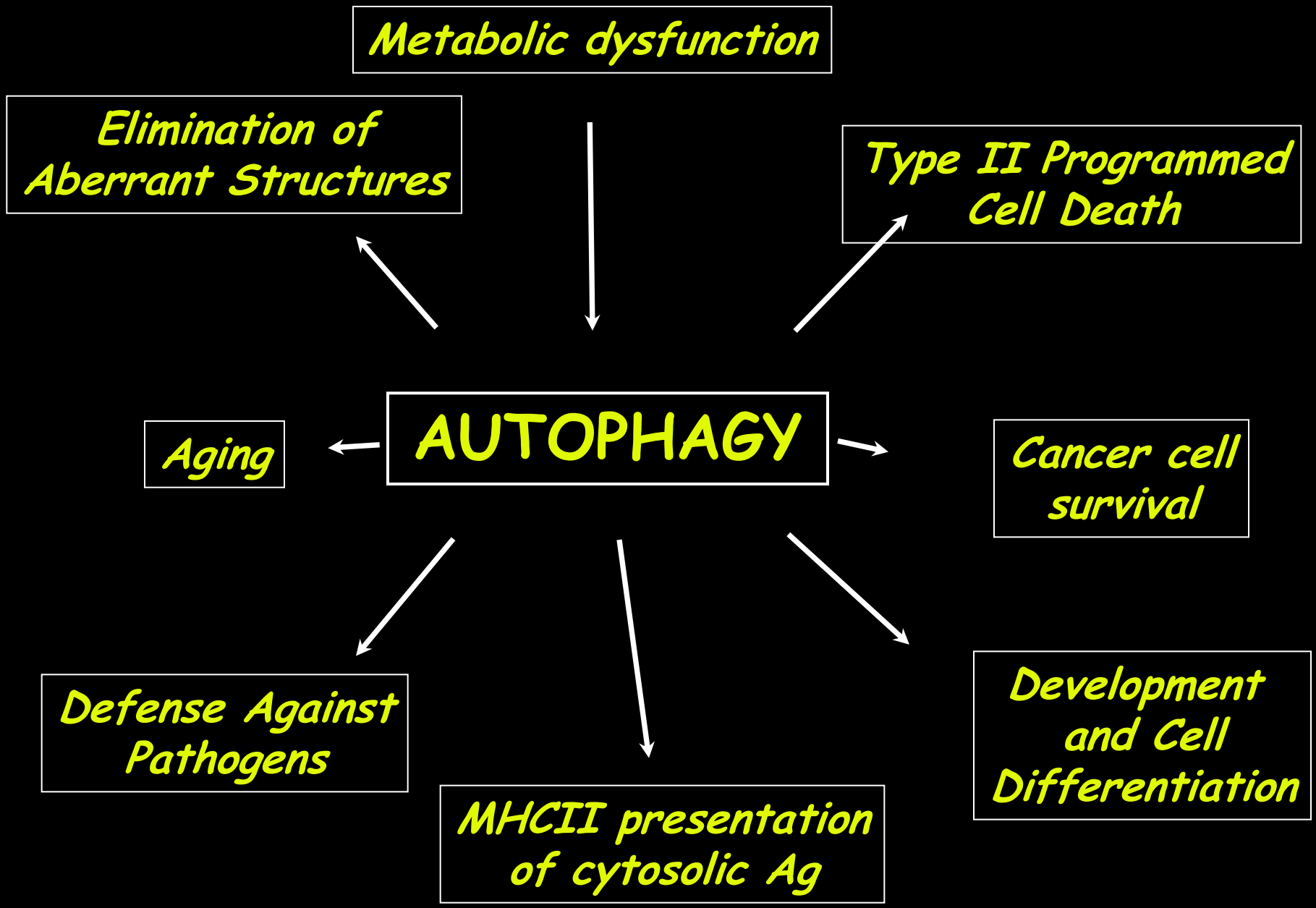
·Viral Infections
- Adenovirus
- Baculovirus

·Neurodegenerative diseases
- Alzheimer's disease
- Amyotrophic Lateral Sclerosis
- Parkinson's disease
- Huntington's disease
- Epilepsy

·Haematologic diseases
- Aplastic anaemia
- Myelodysplastic Syndrome
- T $CD4^+$ lymphocytopenia
- G6PD deficiency

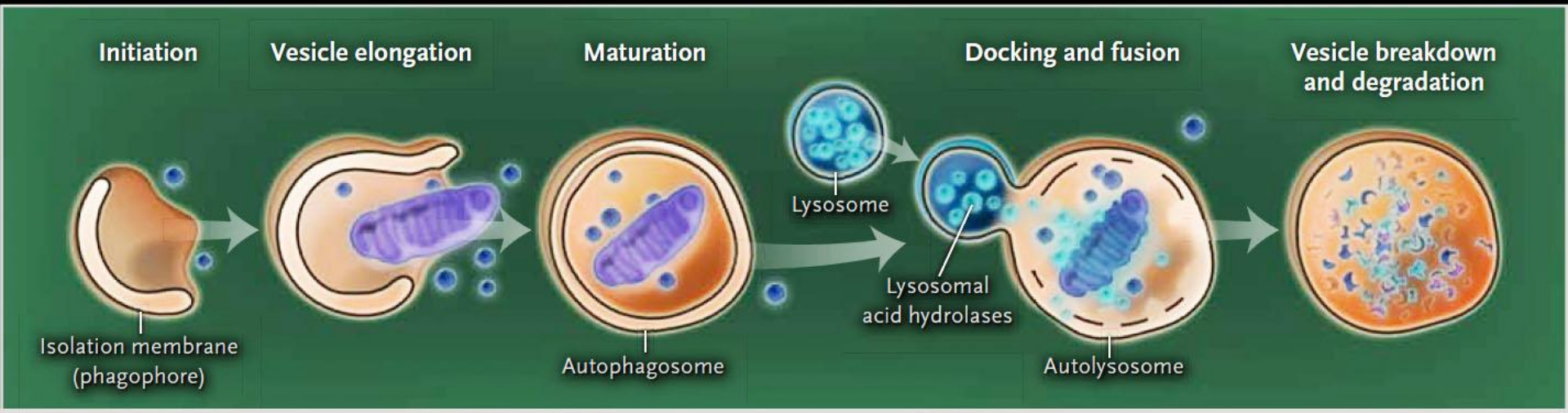
·Cardiovascular diseases
- Myocardial infarction
- Cerobrovascular accident
- Atherosclerosis
- Vasculitis
- Diabetes

·Viral Infections
- AIDS

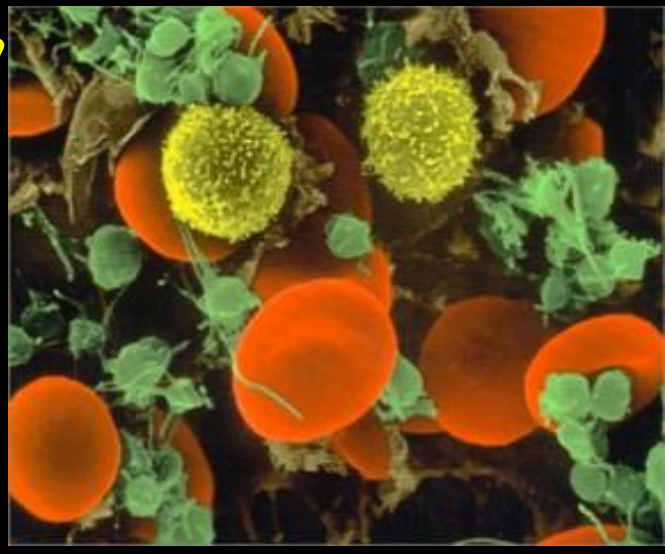

Metabolic dysfunction
Elimination of Aberrant Structures
Type II Programmed Cell Death
AUTOPHAGY
Aging
Cancer cell survival
Defense Against Pathogens
MHCII presentation of cytosolic Ag
Development and Cell Differentiation

Autophagy in human pathology

- Cancer
- Autoimmune diseases
- Viral infections
- Bacterial infections
- Neurodegenerative diseases
- Cardiovascular diseases



Gender cytopathology (peripheral blood)

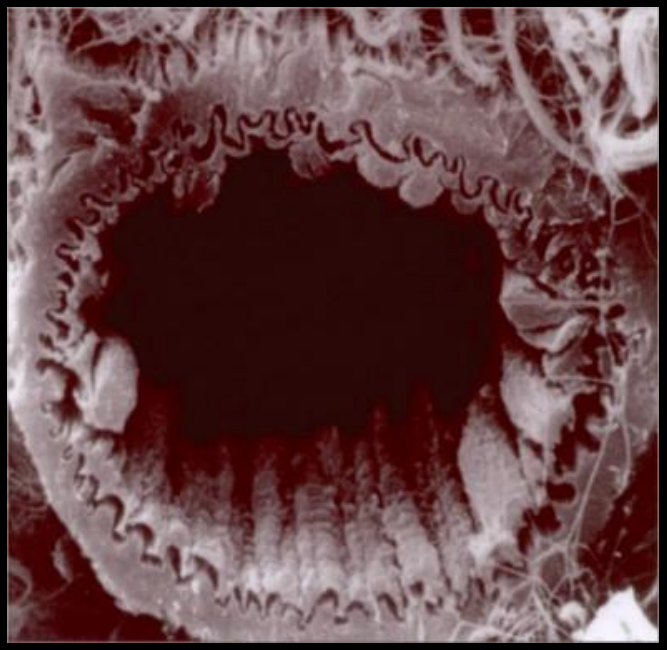


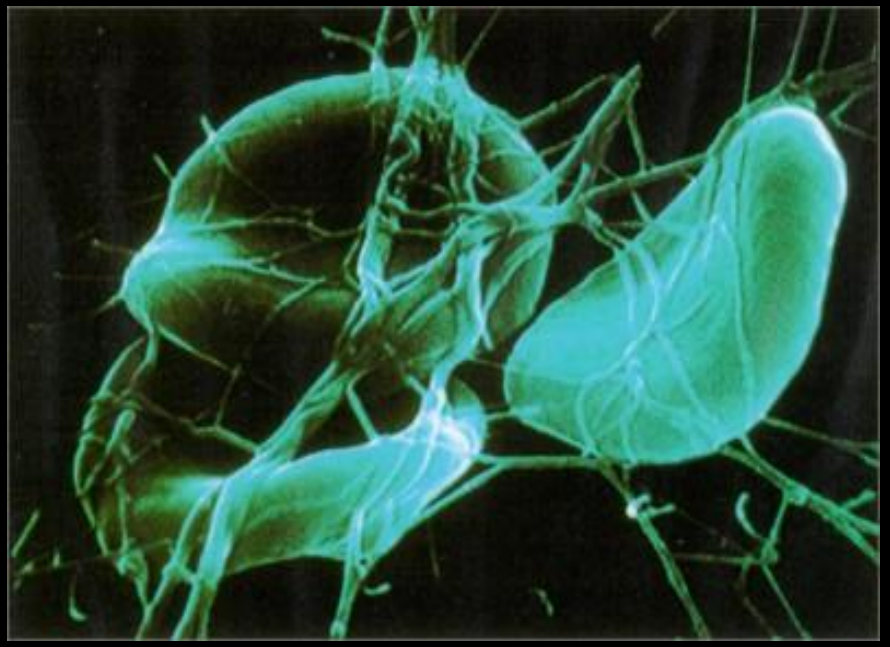
- Cytopathological alterations of RBC, or of other peripheral blood cells, can show a gender disparity
  - This gender disparity can provide useful diagnostic or prognostic tools in certain human diseases
- This gender disparity can contribute to the pathogenesis or the progression of human diseases

Diametro di un capillare 5µm

Diametro di un eritrocita 7µm

100 billions RBC/day



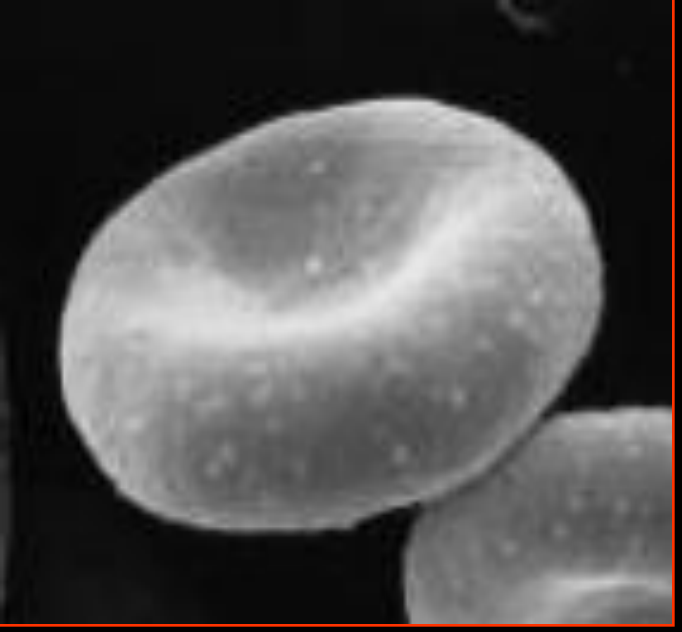


RBC e fibrina

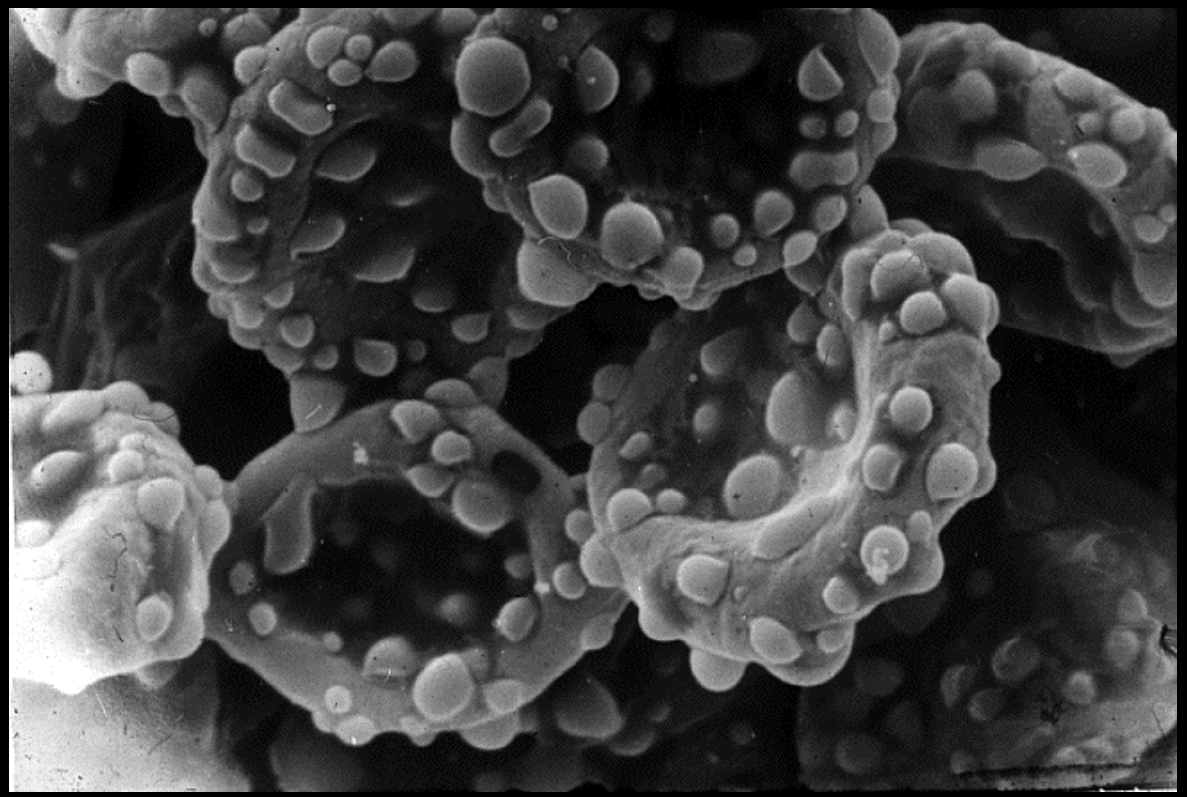
The microenvironment can shift erythrocytes from a friendly to a harmful behavior: they can exert a pathogenetic role in vascular diseases

- Erythrocytes are a potential component in atheromatous lesions and thrombus formation
- Sex hormones may influence the fatty acid composition of human erythrocyte membrane
- Erythrocytes undergoing apoptosis (eryptosis) exposing PS at their surface tend to aggregate. This can lead to changes of rheological properties of RBC *per se* and to a pro-oxidant activity of erythrocytes towards other cells. including RBC-RBC RBC-platelets and RBC-endothelial cells.

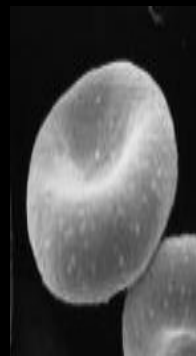
Yunoki et al 2012; Lhoner et al. 2013, Malorni and Minetti 2008



Real-time biomarkers?
Pathogenetic determinants?

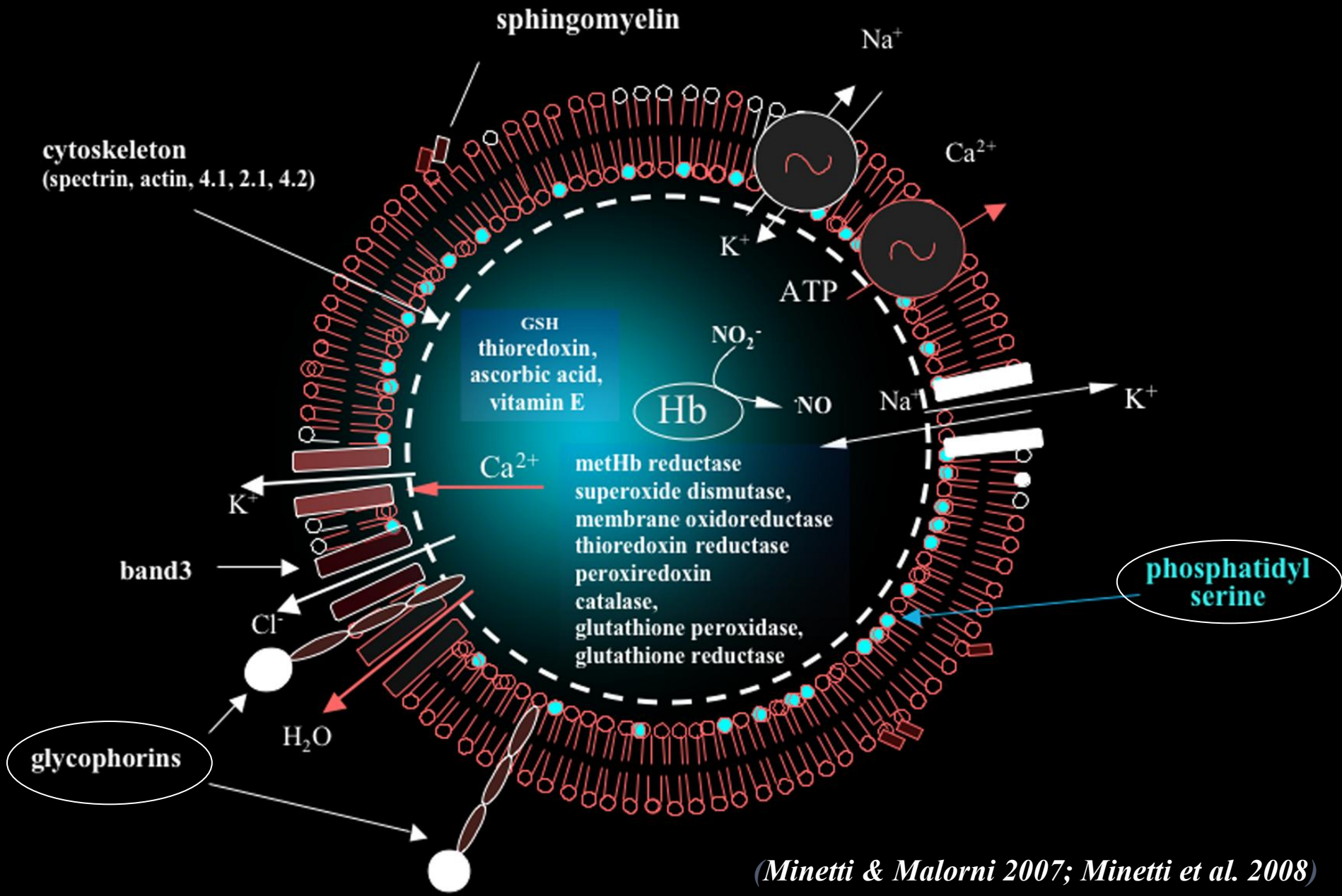


Erythrocytes show a peculiar form of apoptosis



Bednarek-Tupikowska et al. Effects of oestradiol and oestroprogestin on erythrocyte antioxidative enzyme system activity in postmenopausal women. Clin Endocrinol (Oxf). 2006 Apr;64(4):463-8.
Minetti M, Agati L,Malorni W. Cardiovascular Res 2007
Lucantoni et al Antiox Redox Sign 2006

The microenvironment can shift erythrocytes from a friendly to a harmful behavior

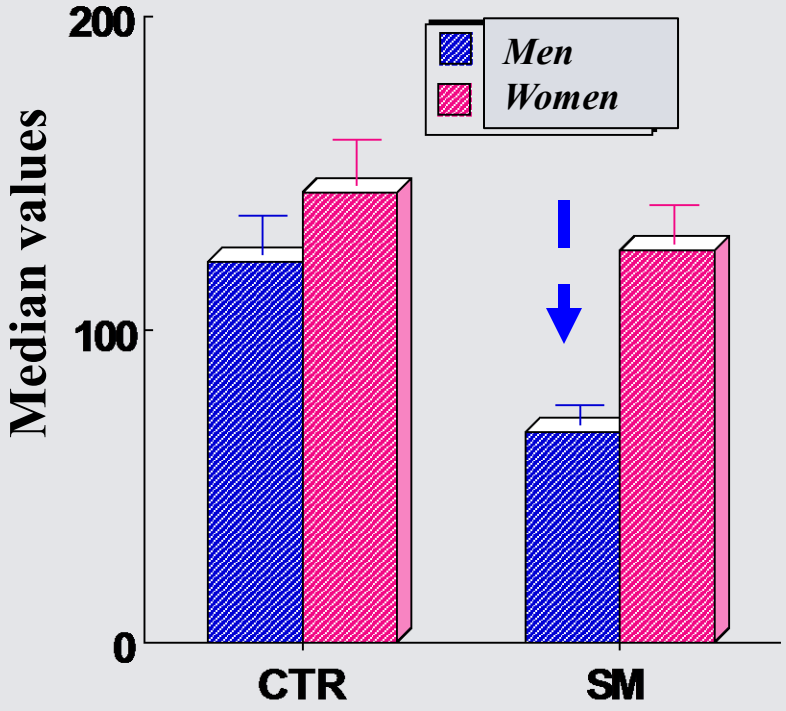


(Minetti & Malorni 2007; Minetti et al. 2008)

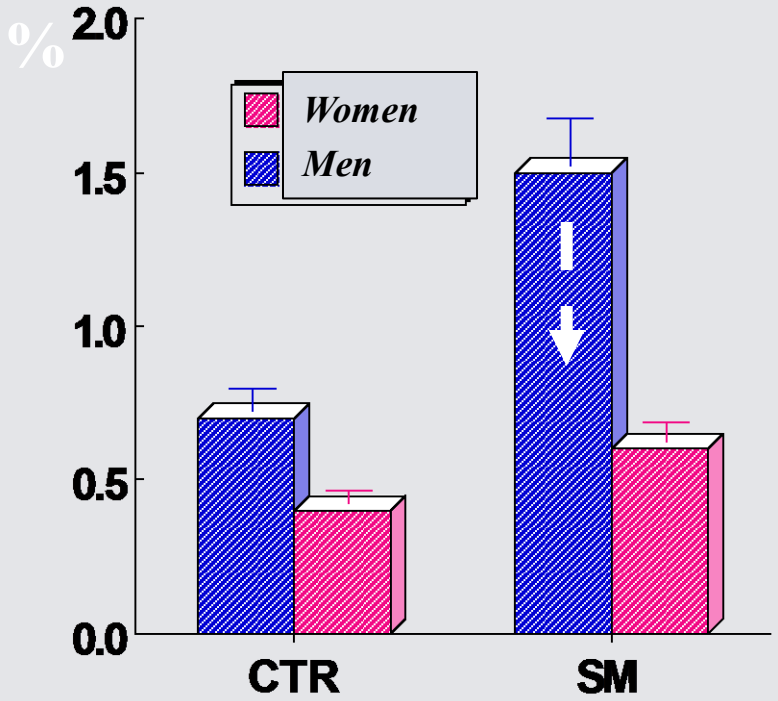
Table 1. Patients' characteristics. Significant differences are in bold.
Data are the mean ± S.D. of 56 MetS patients (31 male and 25 female) and 40 HD (22 male and 18 female).

VARIABLES	56 MetS (M=31 F=25)	40 HD (M=22 F=18)	P-VALUES
Risk factors			
Body Mass Index (Kg/m2)	**31.98 ±4.84**	**21.05±2.01**	**0.0001**
Waist circumference (cm)	**112.7±14.98**	**71.79±6.22**	**0.0001**
Systolic blood pressure (mmHg)	**134.57±17.7**	**120.63±8**	**0.03**
Diastolic blood pressure(mmHg)	**84.13±9.37**	**74.95±5.68**	**0.001**
Glucose (mg/dl)	**122.71±34.87**	**63.84±9.38**	**0.001**
Total cholesterol (mg/dl)	197.62±38.83	178.39±20.23	0.15
LDL-cholesterol (mg/dl)	125.4±36.08	114.27±28.51	0.31
HDL-cholesterol (mg/dl)	44.41±8.47	47.09±8.8	0.78
Triglyceride (mg/dl)	144.8±72.28	118.94±48.96	0.26
Family history of CAD	12 (50%)	4 (21%)	0.60
Family history of Diabetes	13 (54%)	5 (26%)	0.80
Currently smokers	5 (21%)	12 (63%)	0.67
Echocardiography parameters			
LVEF (%)	**52.46±6.16**	**59.68±2.82**	**0.001**
SIV (mm)	**11.58±0.92**	**8.74±1.28**	**0.0001**
PP (mm)	**11.13±0.90**	**9.21±1.22**	**0.001**
LVEDV (ml)	**126.78±31.19**	**115.00±18.93**	**0.05**
LVESV (ml)	**58.57±17.20**	**40.05±6.32**	**0.002**
LVM-I (g)	**114.25±16.84**	**77.36±31.24**	**0.0001**
Carotid echo-color-Doppler parameters			
CCA Sx (mm)	**1.25±0.36**	**0.73±0.22**	**0.0001**
ICA Sx (mm)	**1.68±0.73**	**0.67±0.26**	**0.001**
CCA Dx (mm)	**1.30±0.39**	**0.56±0.28**	**0.001**
ICA Dx (mm)	**1.96±0.79**	**0.66±0.27**	**0.0001**

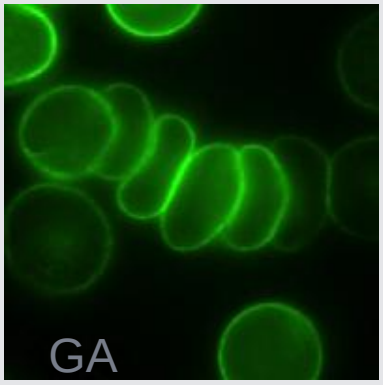
Senescent RBCs (Glic A+)



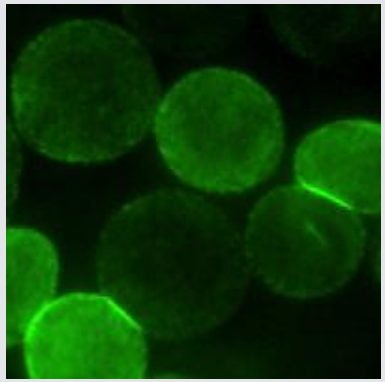
Apoptotic RBCs (AV+)



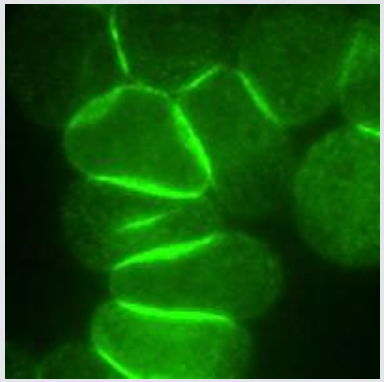
HD



males



females



Senescence and eryptosis Modify RBC rheology (Straface et al. 2010)

Profumo E, et al. Redox imbalance of red blood cells impacts T lymphocyte homeostasis. Thromb Haemost. 2011

Autophagy in Heart Disease

Modulations in autophagy have been associated with diseases of the heart, including cardiomyopathies, cardiac hypertrophy, ischemic heart disease, heart failure, and ischemia–reperfusion injury

- Matsui Y, et al., Distinct roles of autophagy in the heart during ischemia and reperfusion. Circ Res. 2007;100(6):914-22.
- Salabei JK, Conklin DJ. Cardiovascular Autophagy: Crossroads of Pathology, Pharmacology and Toxicology. Cardiovasc Toxicol. 2013 Feb 14. [Epub ahead of print]
- Giricz Z, et al., Autophagy, myocardial protection, and the metabolic syndrome. J Cardiovasc Pharmacol. 2012 Aug;60(2):125-32.
- Yamaguchi O, Taneike M, Otsu K. Cooperation between proteolytic systems in cardiomyocyte recycling. Cardiovasc Res. 2012 Oct 1;96(1):46-52
- Kirshenbaum LA. Regulation of autophagy in the heart in health and disease. J Cardiovasc Pharmacol 2012;60:109.

Autophagy as protective mechanism of cardiomyocyte integrity and function

Dutta D, Xu J, Kim JS, Dunn WA Jr, Leeuwenburgh C. Upregulated autophagy protects cardiomyocytes from oxidative stress-induced toxicity. Autophagy. 2013 Mar 1;9(3):328-44.

Matarrese et al. Induction of autophagy protects cardiomyocytes from stress-induced arrhythmia upregulating membrane estrogen receptors alpha, submitted

17-ß-estradiol

Pro-apoptotic activity

High concentrations (over 10nM)

- Lymphocytes
- Leukemic cells
- Monocytes
- Thymocytes
- Endothelial cells from coronary aorta
- VSMC from thoracic aorta

Anti-apoptotic activity

Low concentrations (below 10nM)

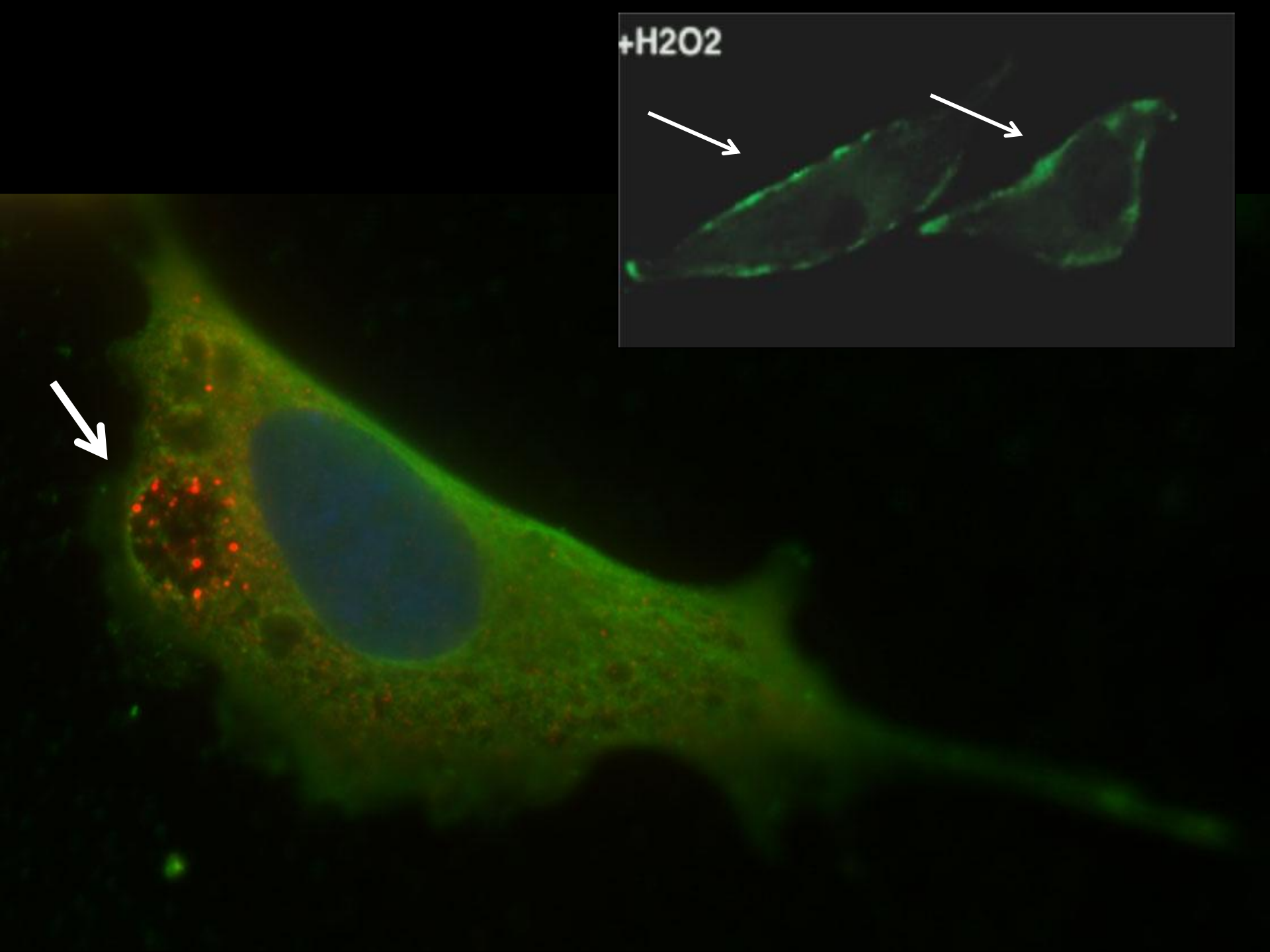
- T and B cells
- Endothelial cells
- VSMC
- Cardiomyocytes

- Neurons
- Platelets

Estrogen receptors at the cell surface (mER)

Under oxidative imbalance (e.g. mimicking an inflammatory state) cell responses seem to be:

i) the up-regulation of mERα, but not mERβ, at the cell surface associated with
ii) rapid functional signals (ERK phosphorylation and p38 dephosphorylation) leading to
iii) apoptosis hindering and autophagic cytoprotection, i.e. to cell survival.


+H2O2

What are the mechanisms underlying gender disparity

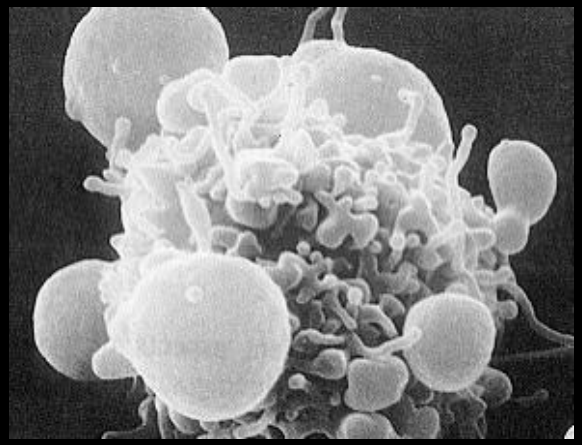
Cultured cells in preclinical studies

Cell "name"	Cell type	Sex	Isolation date
Jurkat	Lymphoid cells	male	1970
CEM	Lymphoid cells	female	1964
Hep-2	epidermoid carcinoma	male	1952
Hela	epidermoid carcinoma	female	1951
U937	Lymphoid cells	male	1974
NCI-H292	mucoepidermoid carcinoma	female	1985
Vero	Kidney (monkey)	unknown	1962
SH-SY5Y	neuroblastoma	female	1970
PC12	pheochromocytoma (rat)	male	1976

The memory of the cells: the birth of "cell sex"

Cell type	Species	N. of passages (with "memory" of their sex)	Investigation tools mainly in:
Fibroblasts	Human, rat, mouse	About 10	Cardiovascular, autoimmune
Vascular Smooth Muscle Cells (VSMC)	Human, rat, mouse	About 10	Cardiovascular, Gastroenterology
Cardiomyocytes	Mainly murine	About 5	Cardiovascular
Resting Lymphocytes	human	-	Immune system and inflammatory diseases
Platelets	human	-	Hematological, Neurodegenerative diseases
Red Blood cells	human	-	Hematological
Freshly isolated cancer cells	human, mouse	10-15	Experimental chemotherapy
Mouse Embrio Fibroblasts (MEFs)	mouse	10-20	Mechanisms of drug toxicity
Keratinocytes	human	10-12	Dermatology
Neuronal cells	mouse, rat	-	Neurodegenerative
Endothelial cells	human	About 10	vascular

Reactive Oxygen Species are
capable of activating
a series of cell functions,
e.g. transcription factors

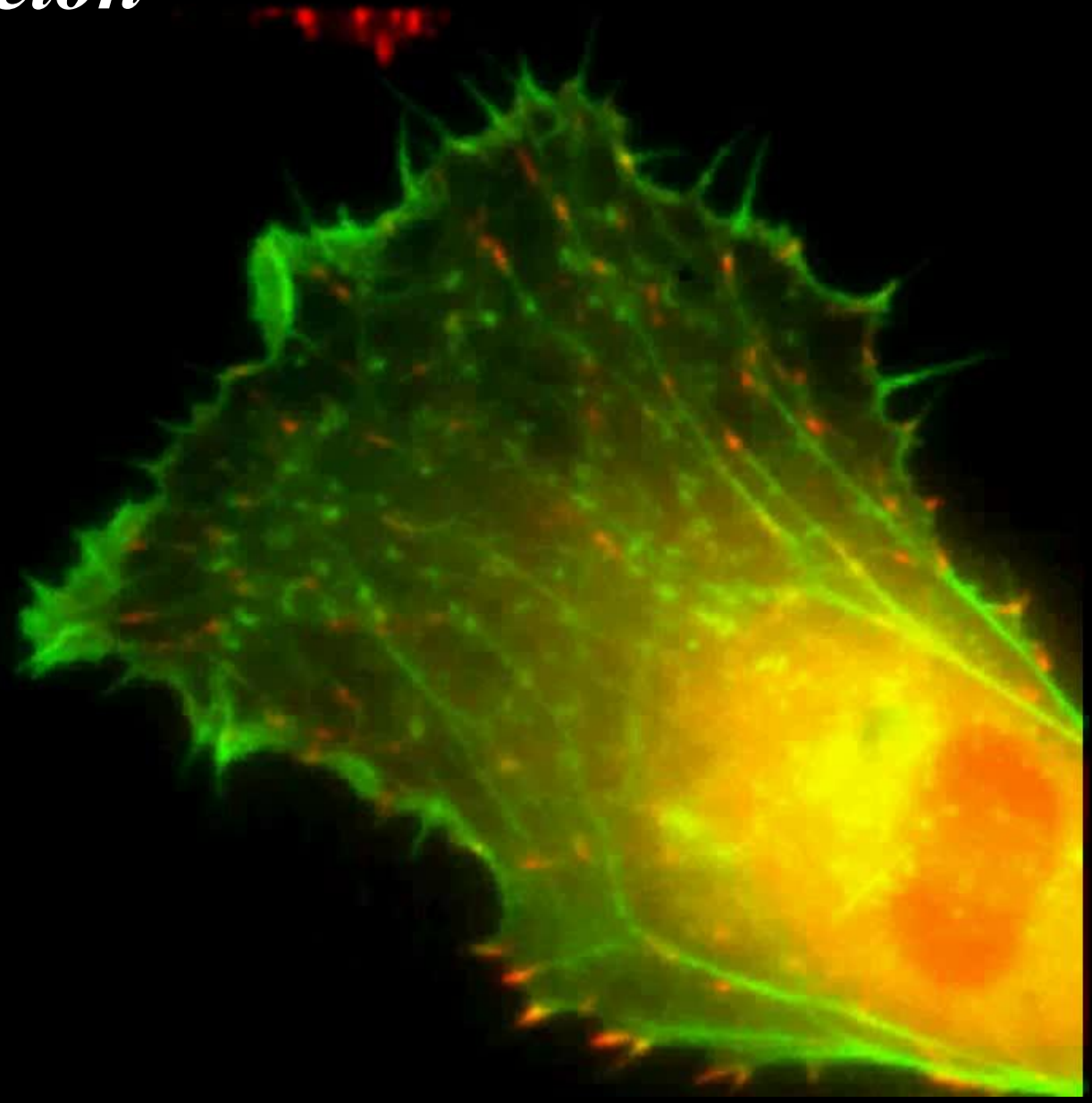


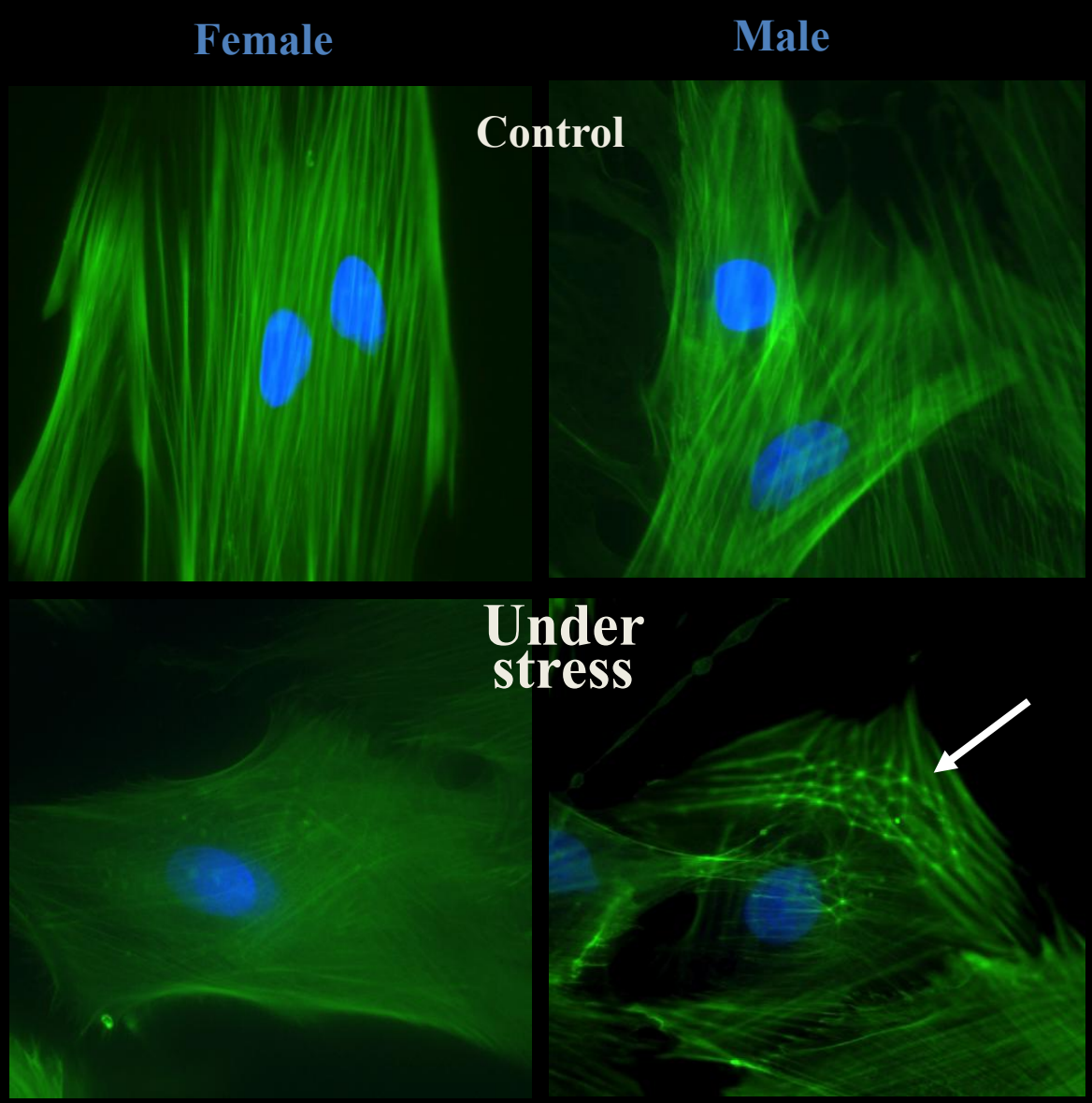
But, also, free radicals, mainly ROS,
are known to induce
a number of intracellular lethal and sublethal
alterations including:

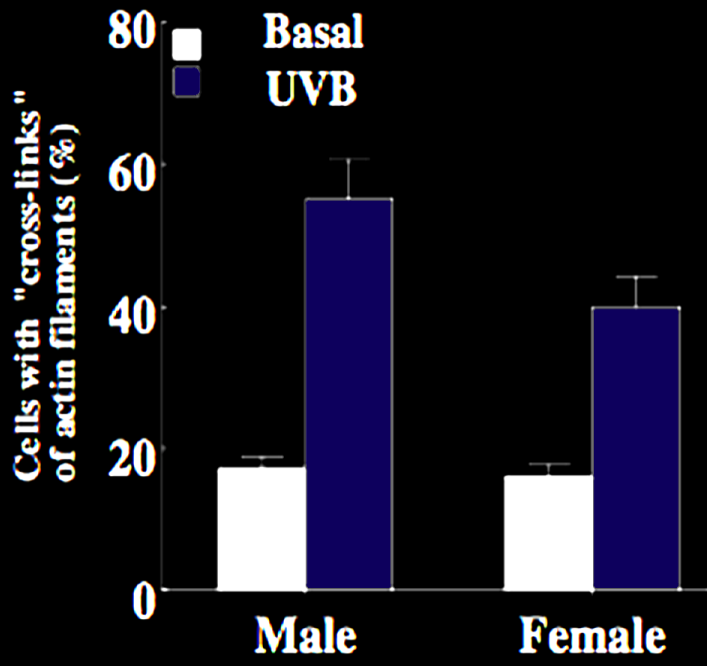
DNA damage
Oxidative alterations of proteins
Oxidative alterations of (membrane) phospholipids
and

apoptosis as well as autophagy

Cytoskeleton

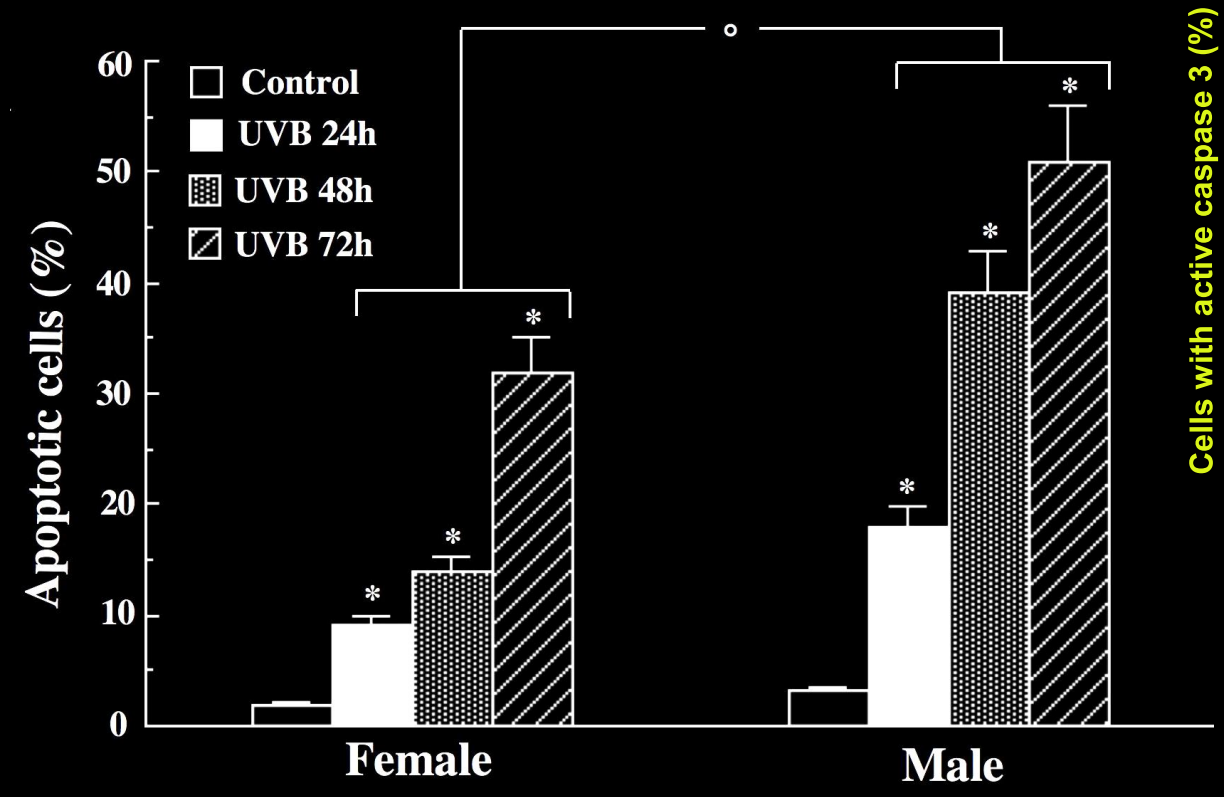


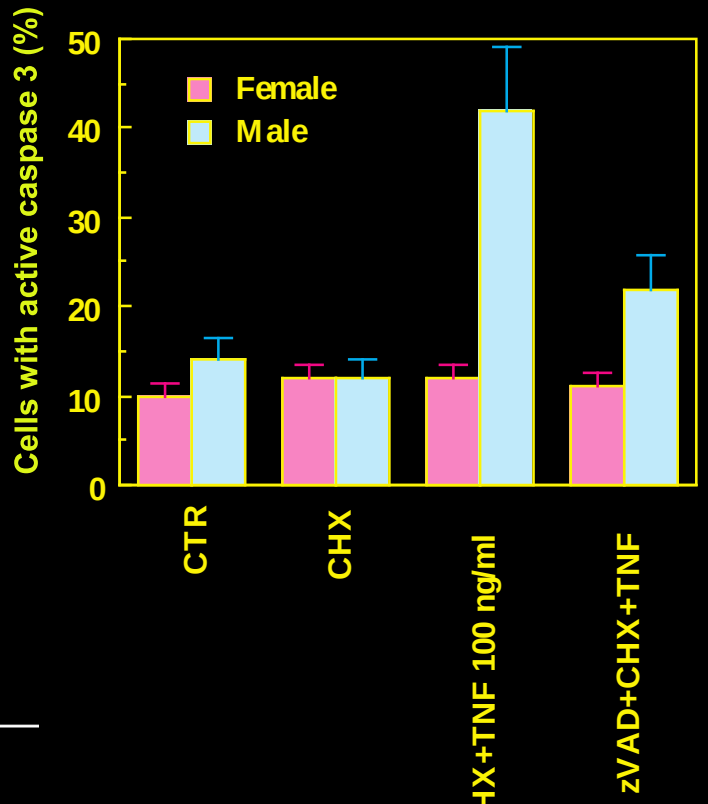


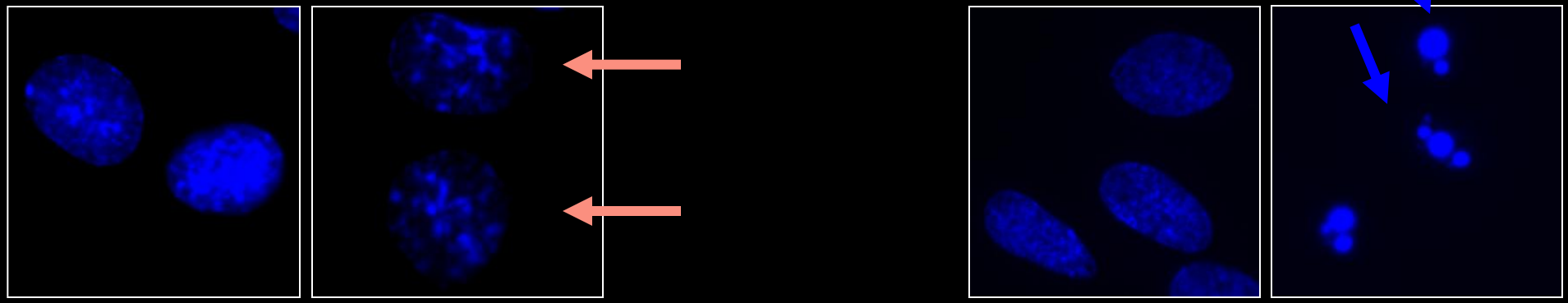


Actin filaments

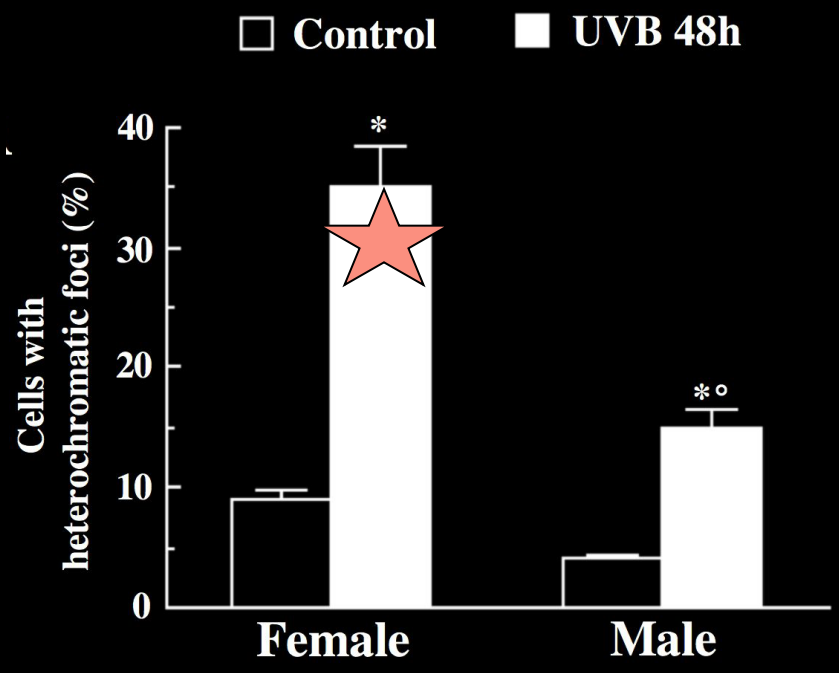
"male cells" are more susceptible to death than "female cells" …

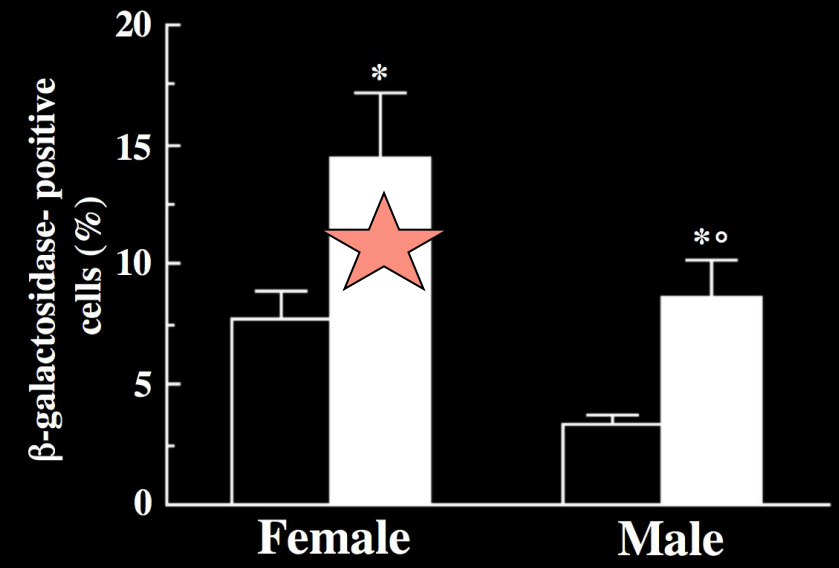


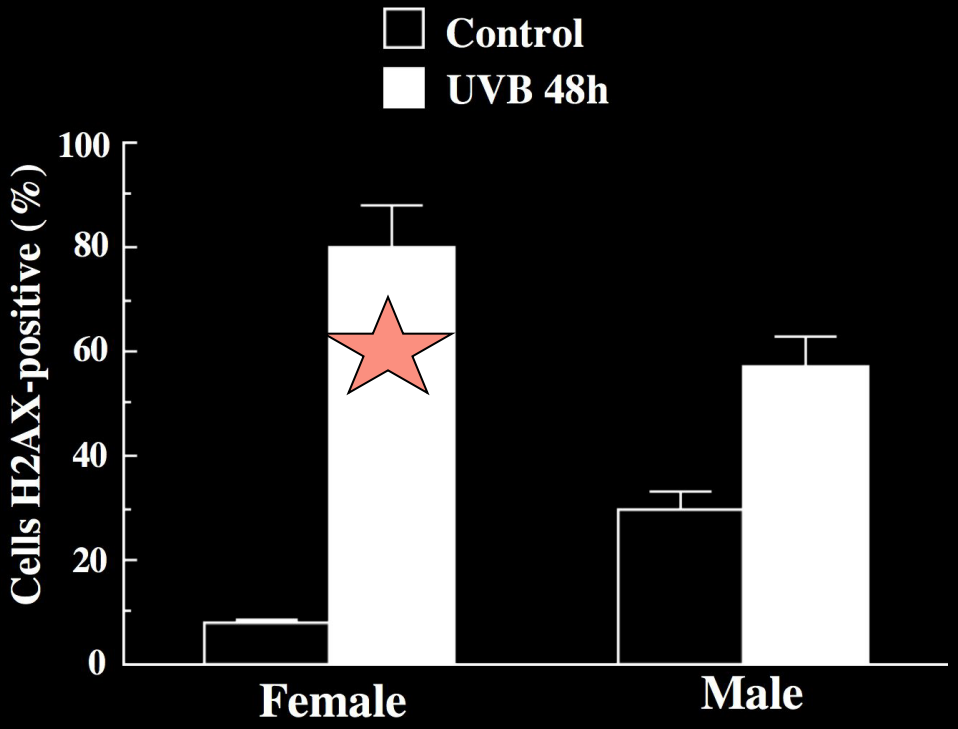


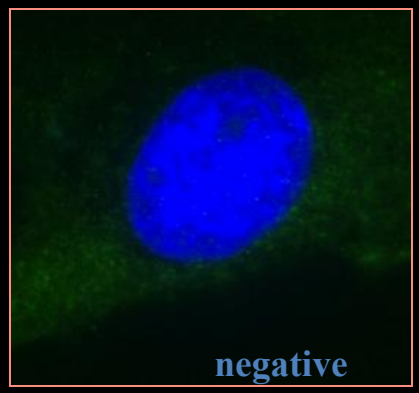


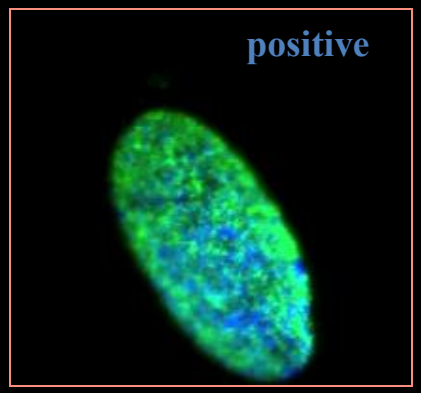
...whereas female cells easier undergo senescence



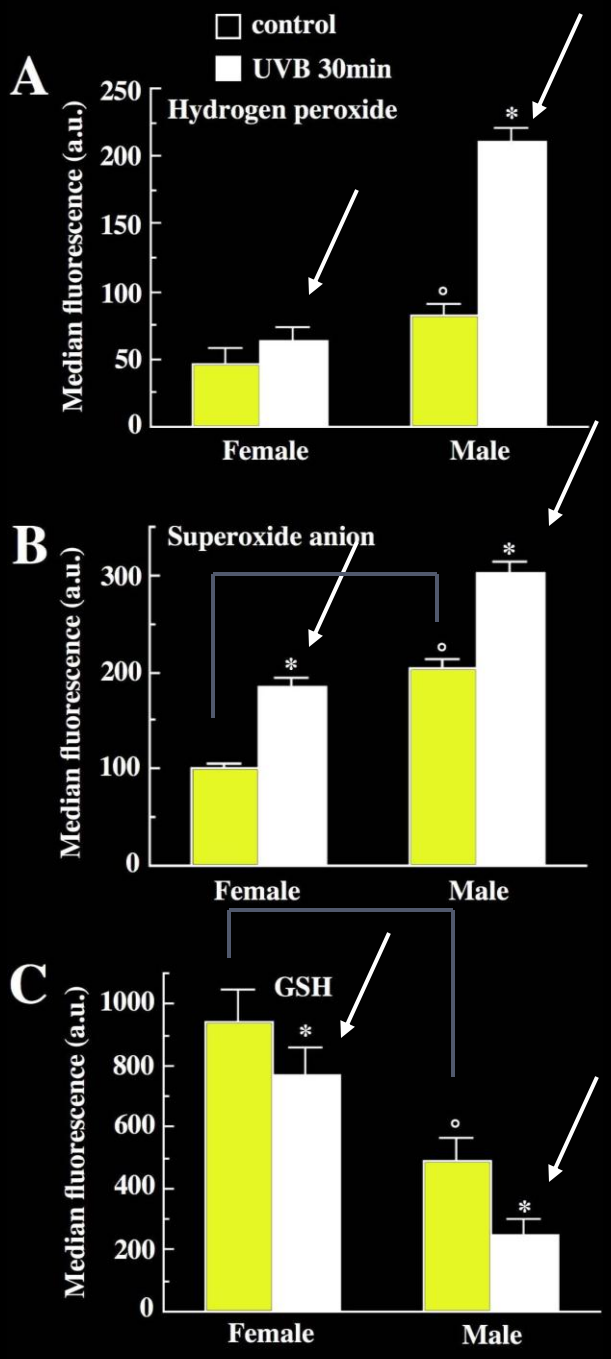






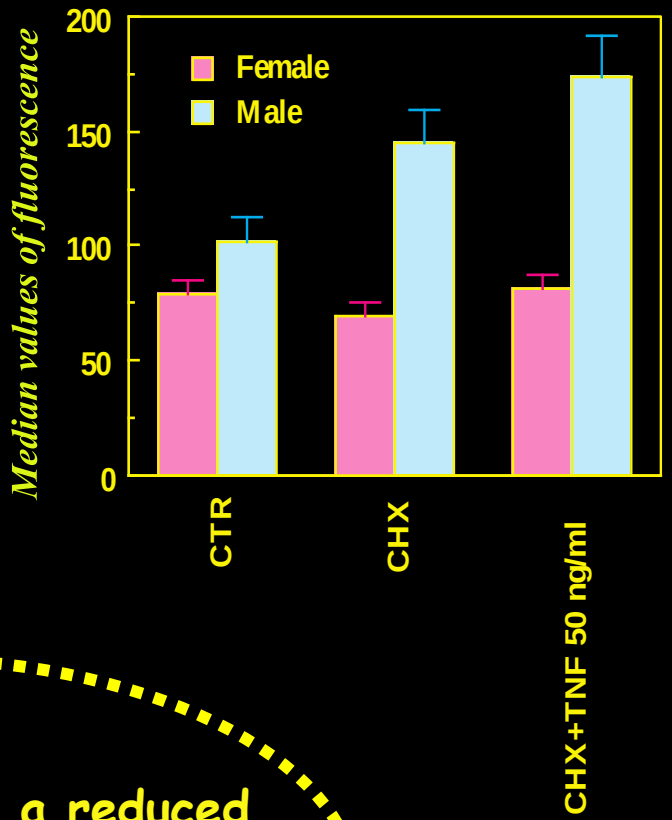


Evaluation of
histone H2AX phosphorylation



Effects of oxidative stress on VSMCs:

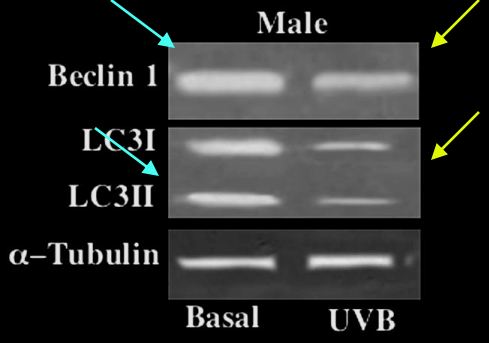
"male cells" are more susceptible



"female cells" have a reduced ROS production and/or "more" antioxidant defenses than "male cells"

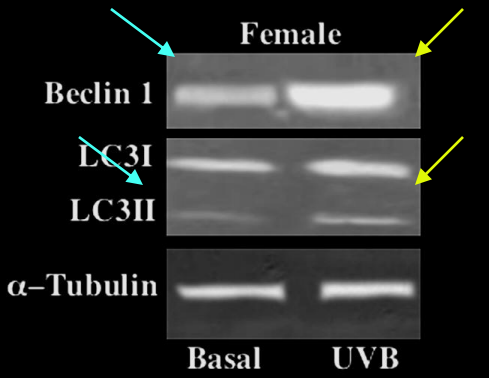
Note different "basal" levels

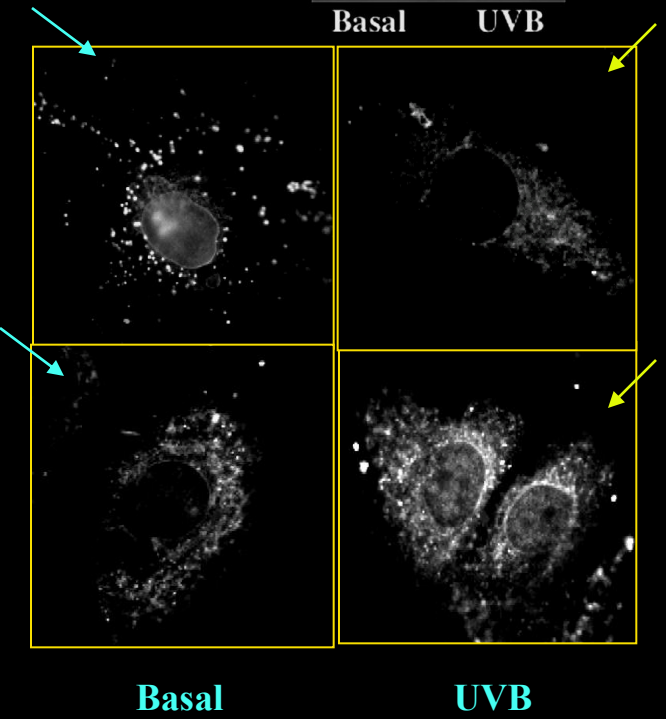
Straface et al. 2010

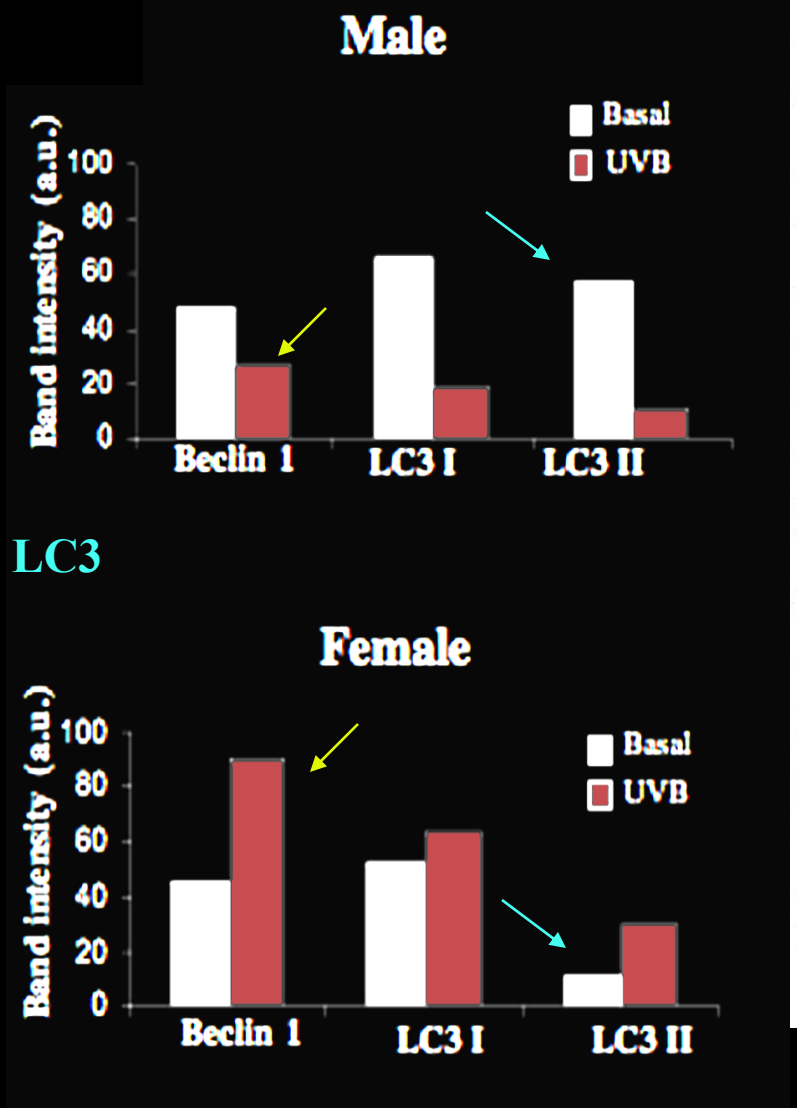


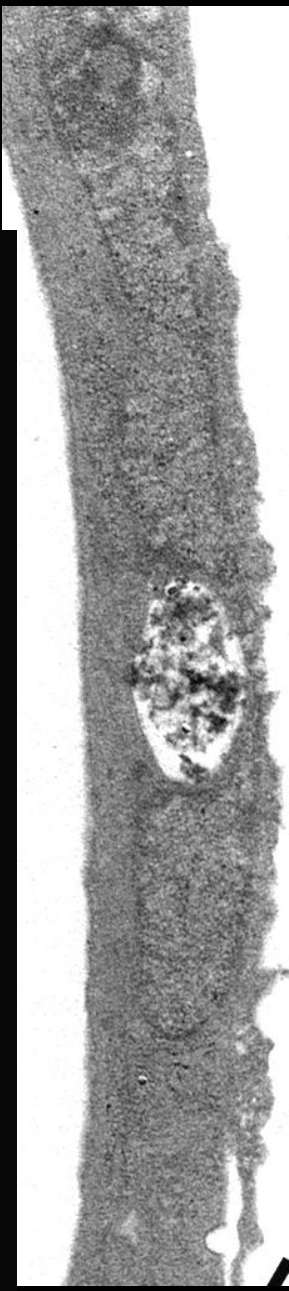
autophagy induction
is normally higher in male cells.

Under stress female cells
develop a more pronounced autophagic
(protective) response









Conclusions

- Freshly isolated vessel cells, e.g. smooth muscle and endothelial cells (3-8th passage), mantain a sort of "memory" of their sexual origin. In particular:

- i) a different "basal" redox state.

- ii) a different susceptiblity to stress.

- iii) this susceptibility appears associated with a different fate: "male cells" appear more "apoptotic-prone" whereas "female cells" survive better and appear more "autophagy-prone" cells

A higher ability of "female cells" to adapt to environmental changes (behavioral plasticity)?

Some recent reviews published by our group

- Del Principe D, et al. Gender disparity in pediatric diseases. Curr Mol Med. 2013 May;13(4):499-513.
- Spoletini I, et al. Sex differences in drug effects. Handb Exp Pharmacol. 2012;(214):91-105.
- Straface E, et al. Sex differences at cellular level: "cells have a sex". Handb Exp Pharmacol. 2012;(214):49-65.
- Giammarioli AM, et al. Integrating gender medicine into the workplace health and safety policy in the scientific research institutions: a mandatory task. Ann Ist Super Sanita. 2012;48(3):311-8.
- Pierdominici M, et al. Gender specific aspects of cell death in the cardiovascular system. Curr Pharm Des. 2011;17(11):1046-55.
- Lista P, et al. On the role of autophagy in human diseases: a gender perspective. J Cell Mol Med. 2011 Jul;15(7):1443-57
- Marino M, et al. Nutrition and human health from a sex-gender perspective. Mol Aspects Med. 2011 Feb;32(1):1-70.
- Paggi MG, et al. Gender-related disparities in non-small cell lung cancer. Cancer Lett. 2010 Dec 1;298(1):1-8.
- Straface E, et al. Gender-specific features of plasmatic and circulating cell alterations as risk factors in cardiovascular disease. Fundam Clin Pharmacol. 2010 Dec;24(6):665-74.
- Ruggieri A, et al. Cellular and molecular mechanisms involved in hepatocellular carcinoma gender disparity. Int J Cancer. 2010 Aug 1;127(3):499-504.
- Maselli A, et al. Cell sex: a new look at cell fate studies. FASEB J. 2009 Apr;23(4):978-84.
- Ortona E, et al. Redox state, cell death and autoimmune diseases: a gender perspective. Autoimmun Rev. 2008 Jul;7(7):579-84.