EAAP
European Federation of Animal Science

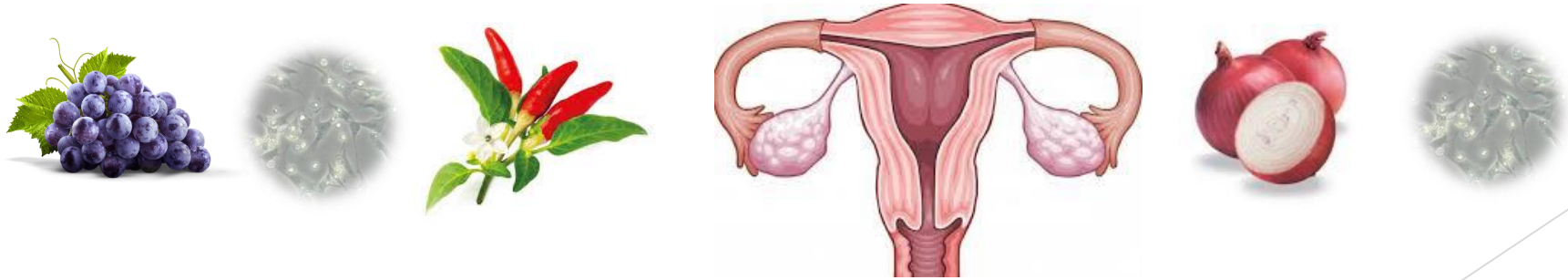
69th Annual Meeting of the European Federation of Animal Science, Dubrovnik, Croatia, 27-31 August 2018

# Flavonoid quercetin as a potential regulator of ovarian functions in vitro

**Adriana Kolesárová, Katarína Michalcová, Simona Baldovská, Marek Halenár**

Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Animal Physiology, Slovak Republic

This work was supported by APVV-16-0170 and VEGA 1/0039/16.



# Flavonoid QUERCETIN and its glycoside ISOQUERCITRIN




# 1. IMPORTANT OF THE ISSUE

# The role of quercetin in female reproduction and fertility

(Yu et al., 2014; Beazley and Nurminskaya, 2015; Maciejczyk and Surowiak, 2013; Wang et al., 2017)

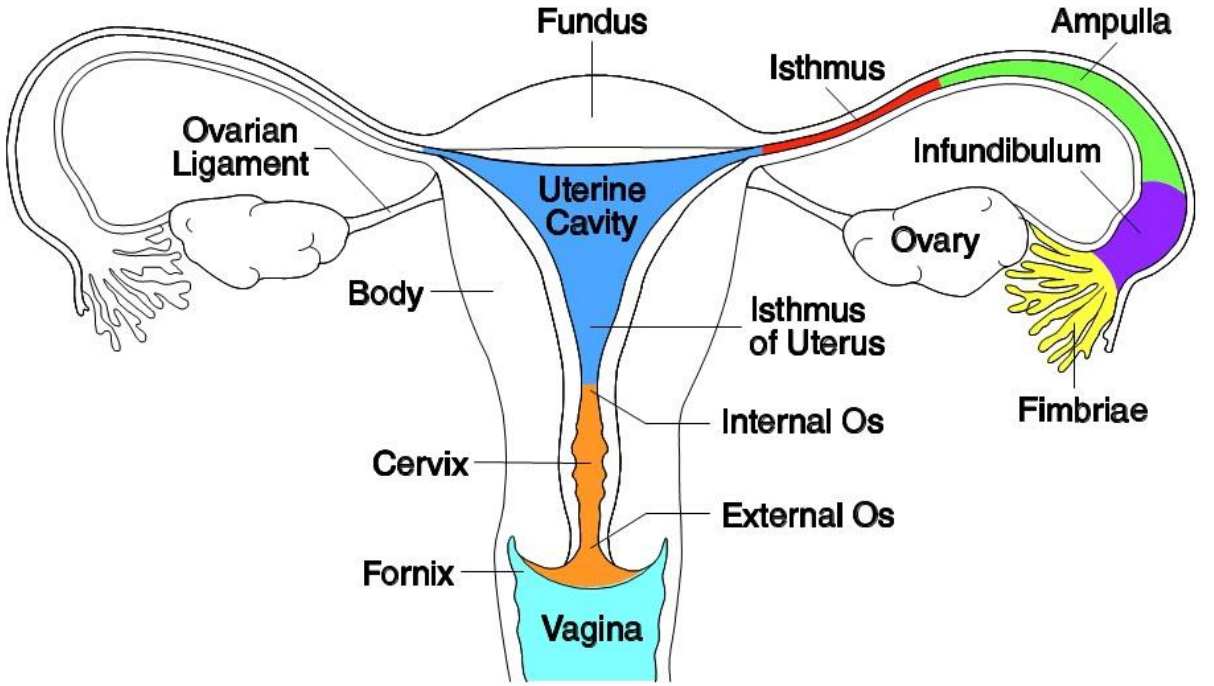
**Ovarium**

**Oviductus**

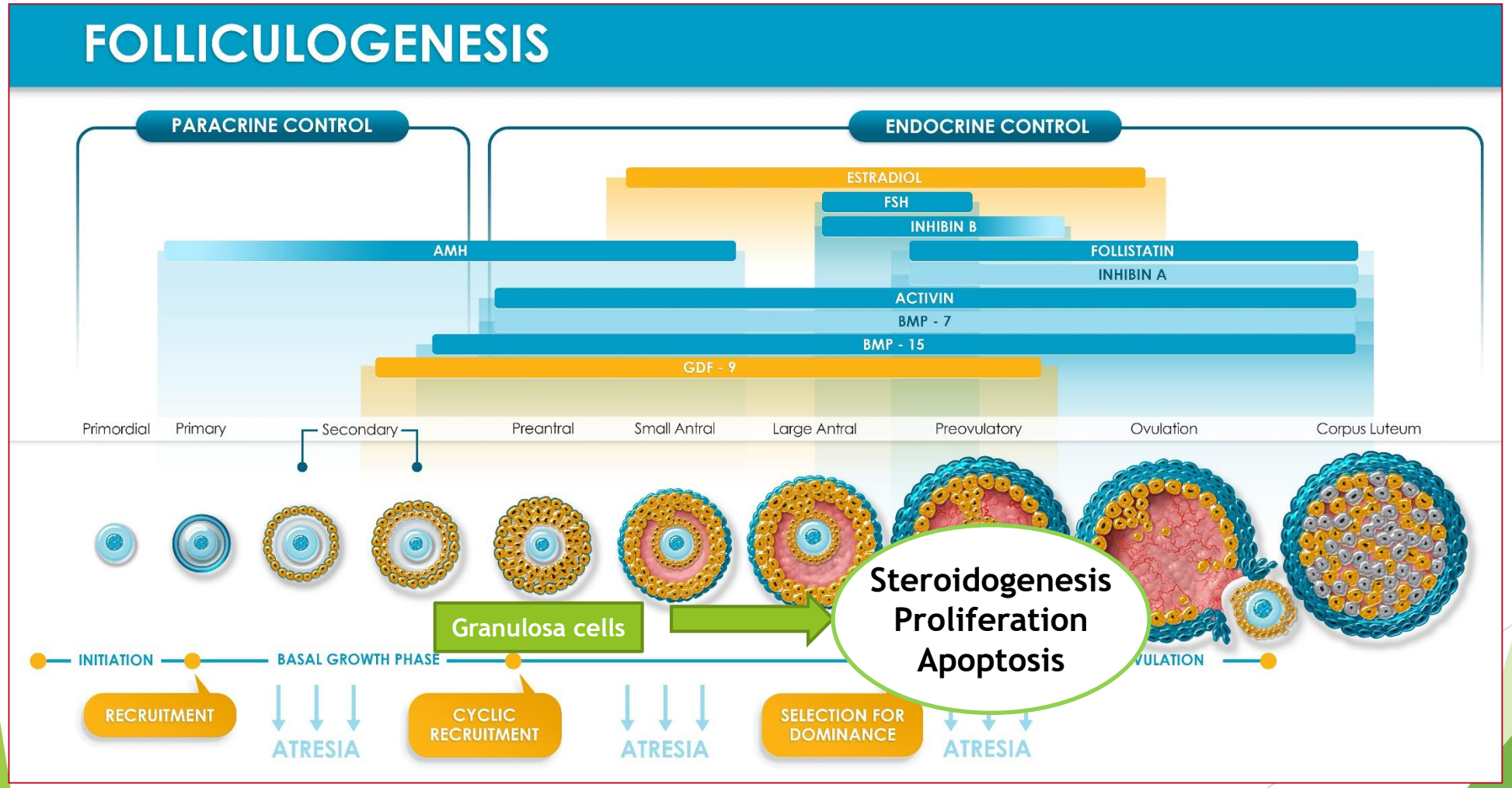
**Uterus**

**Vagina**

**Vulva**



# Foliculogenesis, ovulation and luteogenesis



# Diseases of female reproduction

Amenorea
Dysmenorea
a others

Anovulation

Ovarian cysts

**Ovarian cycle disorders**

**Infertility**
**Sterility**

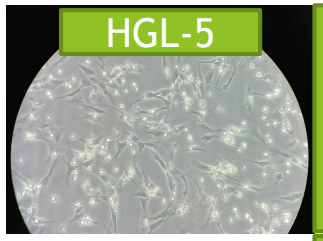
Polycysic Ovary Syndrome (PCOS)

Endometrio-sis

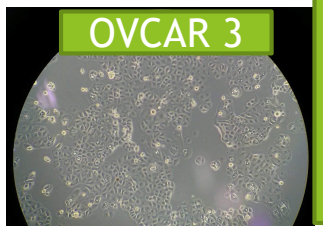
Tumors

Adrenal hyperplasia

**Research**




Ovarian granulosa cells (lines)




Ovarian carcinoma cells (lines)

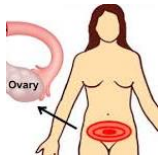
# The aim of the study


The effect of **quercetin/isoquercitrin** after 24h treatment on:

- porcine ovarian granulosa cells
- human ovarian granulosa cell line HGL-5 and
- human ovarian carcinoma cell line OVCAR-3

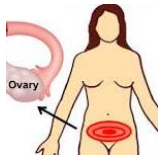
## Steroidogenesis

- progesterone
- 17β-estradiol




## Proliferation

- viability
- PCNA
- cyclin B1
- TGF-β2




## Apoptosis

- caspase-3
- p53

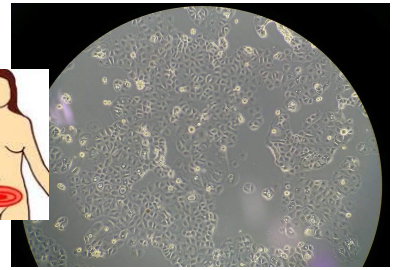
## Oxidative stress

- production of reactive oxygen species (ROS)

# Ovarian cells from pigs, HGL-5 and OVCAR-3

Granulosa cells from ovaries of prepubertal pigs

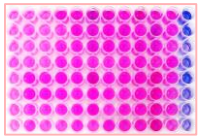
Human ovarian granulosa cell line HGL-5



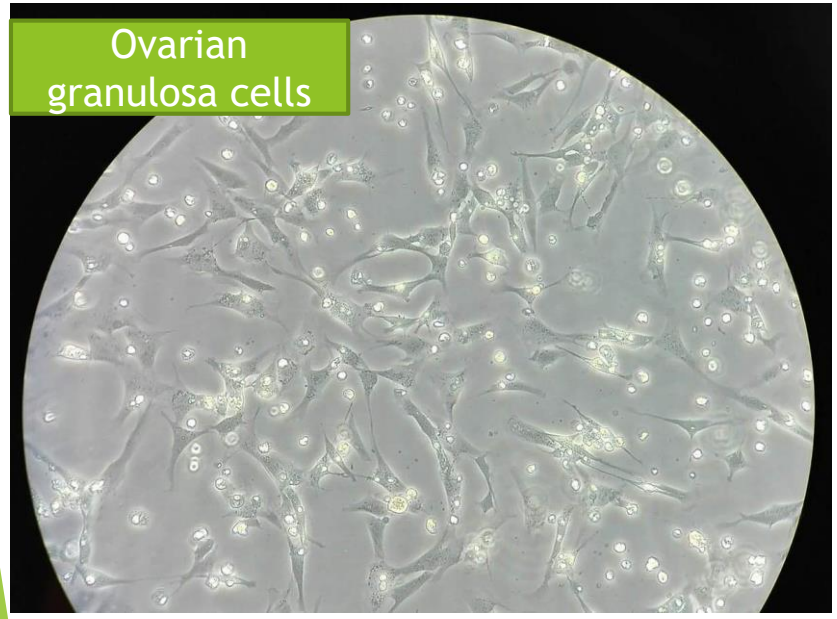
Human carcinoma cell line OVCAR 3

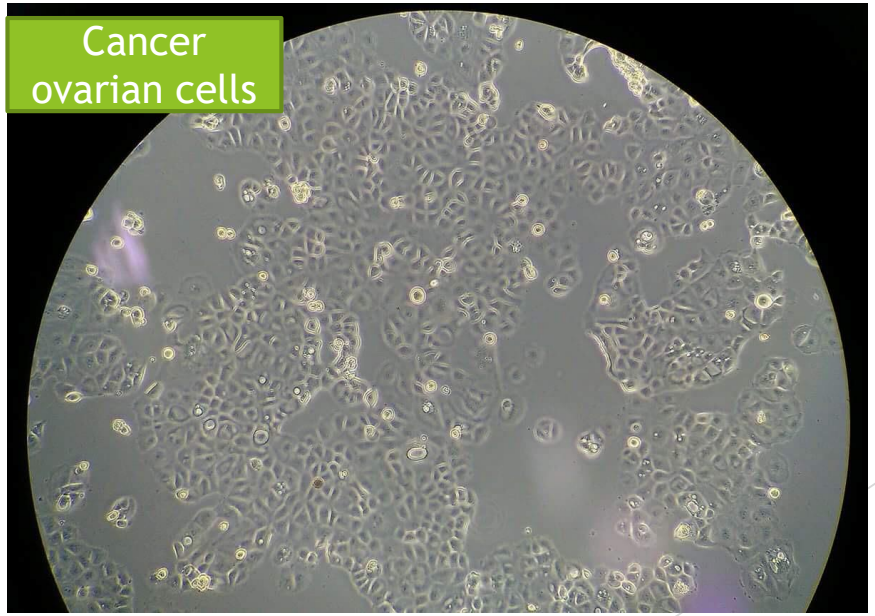
**Quercetin and Isoquercitrin** (cultivation 24h)
at various doses

ELISA
Imunocytochemistry
Chemiluminescence
Flow cytometry
*AlamarBlue™*



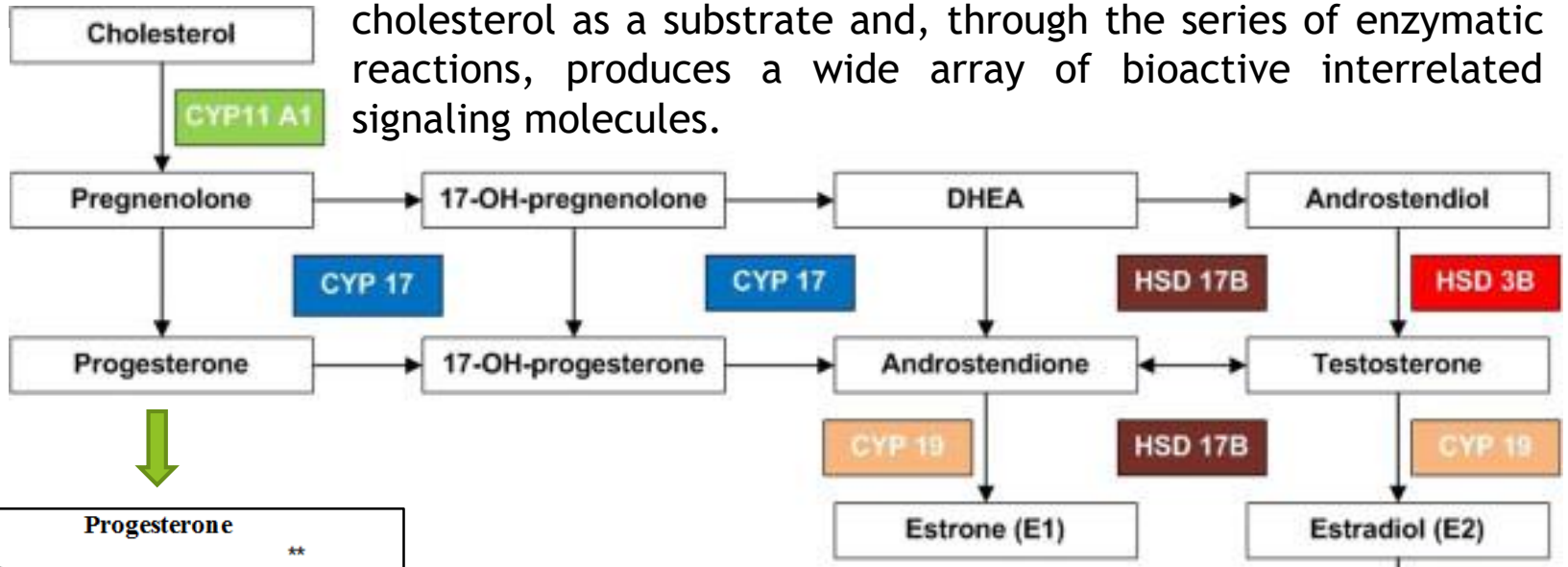
# STEROIDOGENESIS

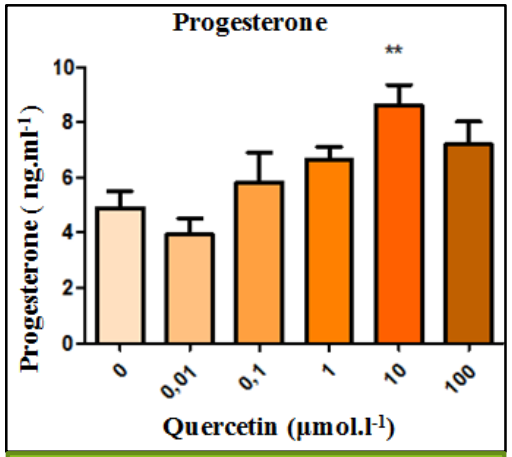


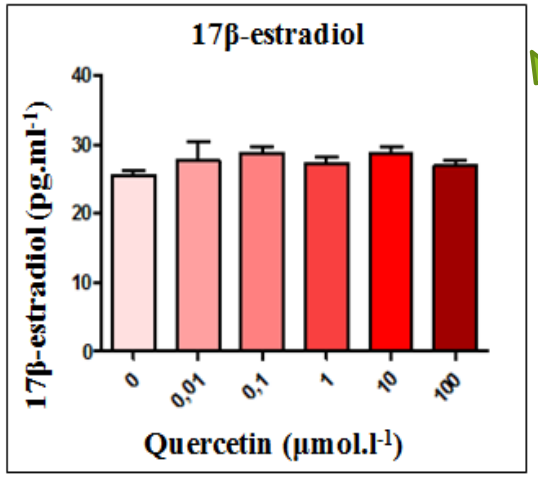
# Quercetin stimulates progesterone but not estradiol

Steroidogenesis is a synthetic process that begins with cholesterol as a substrate and, through the series of enzymatic reactions, produces a wide array of bioactive interrelated signaling molecules.



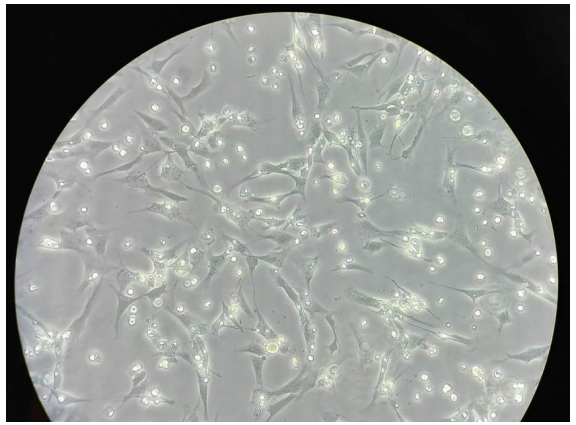



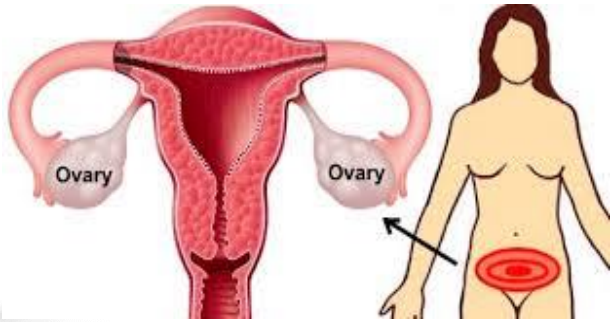

Dose-dependent effect

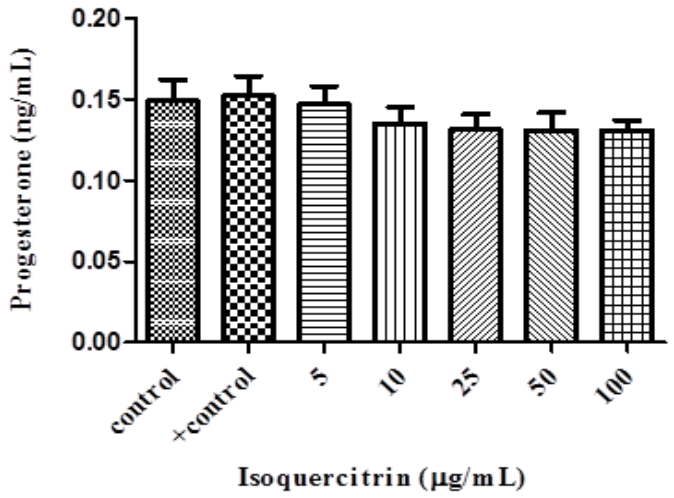


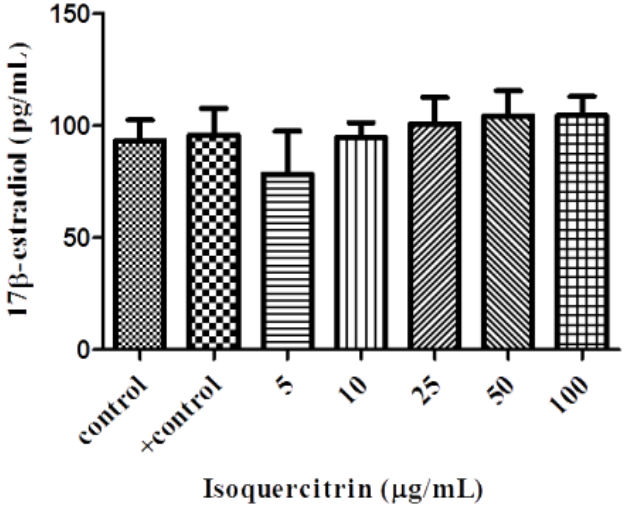
# Isoquercitrin does not affect progesteron and estradiol in human ovarian granulosa cell line HGL-5

Healthy cells

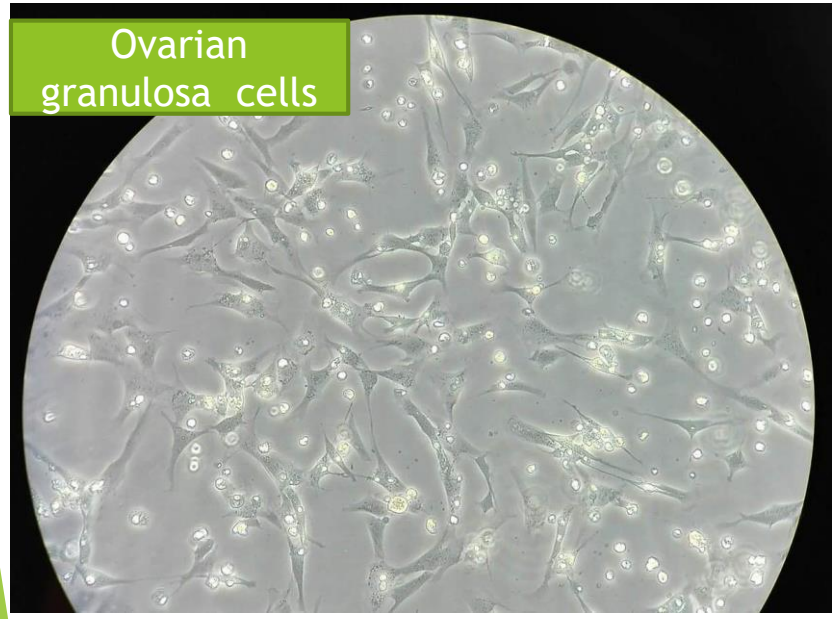


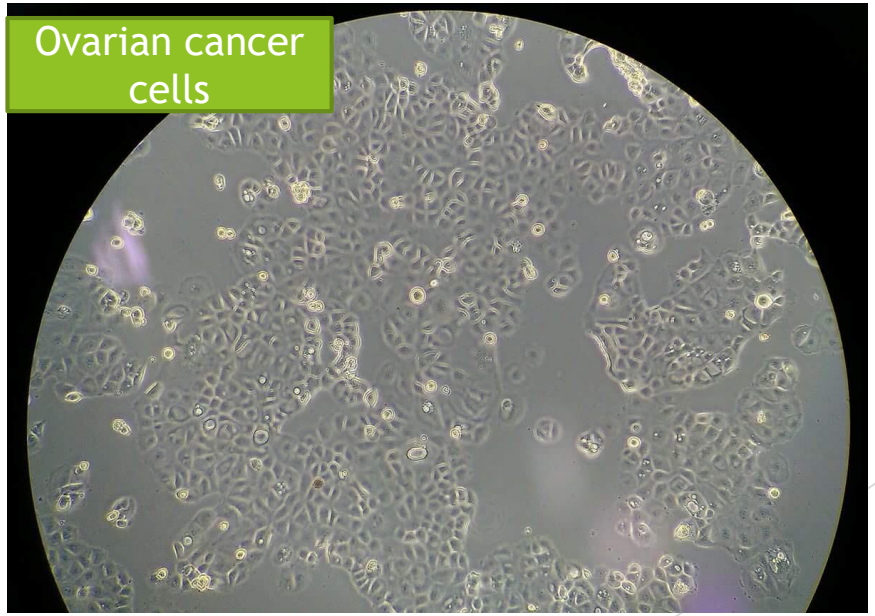


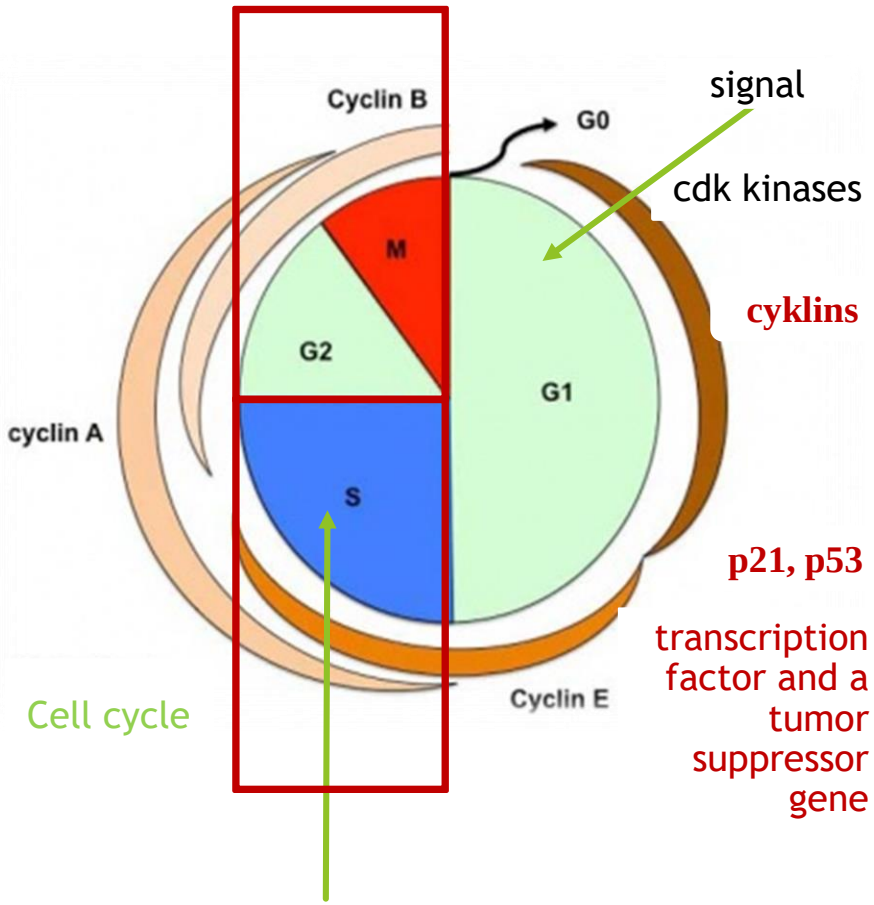



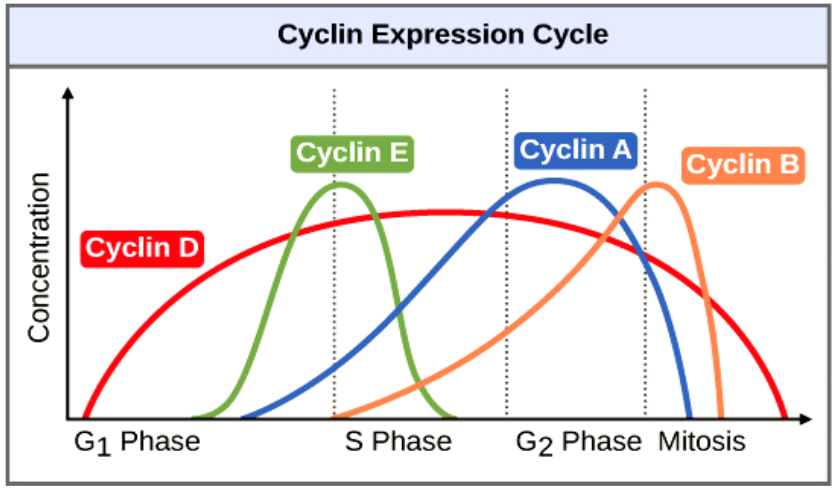
# PROLIFERATION

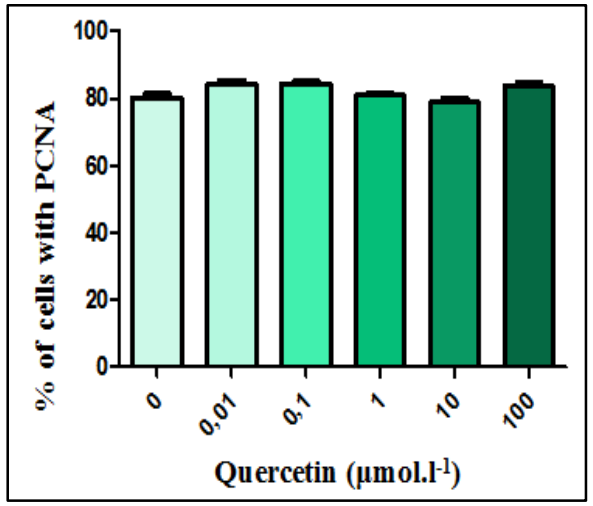


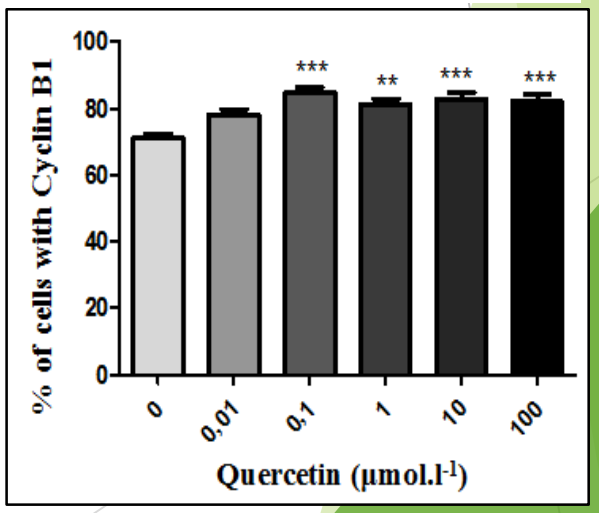



# Quercetin induces marker of proliferation

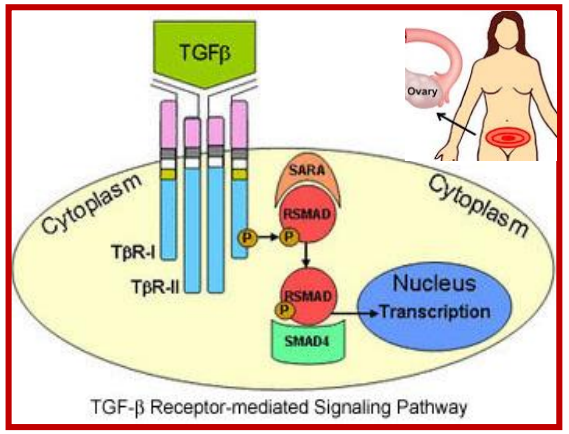


# Dose-dependent effect of isoquercitrin on ROS production

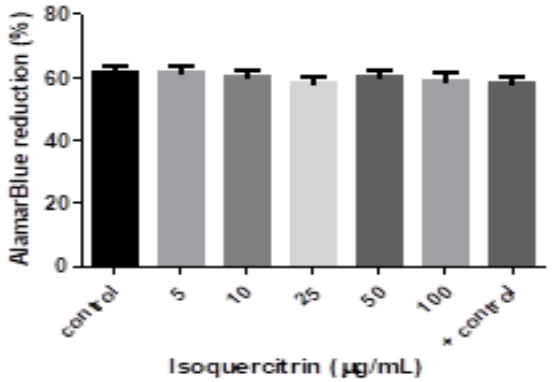


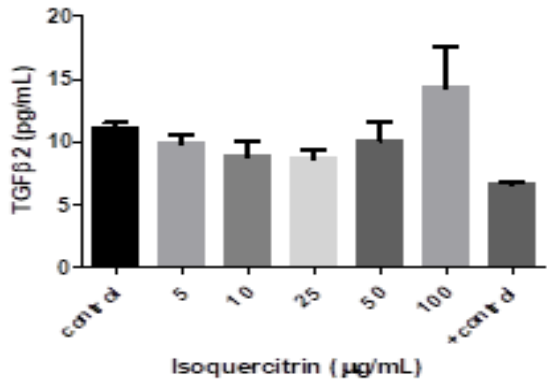

Multifunctional cytokine, TGF-β superfamily, which includes three different isoforms (TGF-β1-3) and many other signal proteins produced in all white blood cells. Induces transcription of various target genes for differentiation, chemotaxis, proliferation and activation of many immune cells.

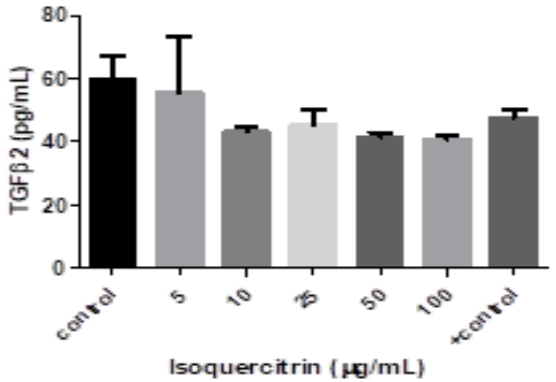
**Viability, TGF-β2 secretion, and the presence of TGF-β2 receptor was not changed.**

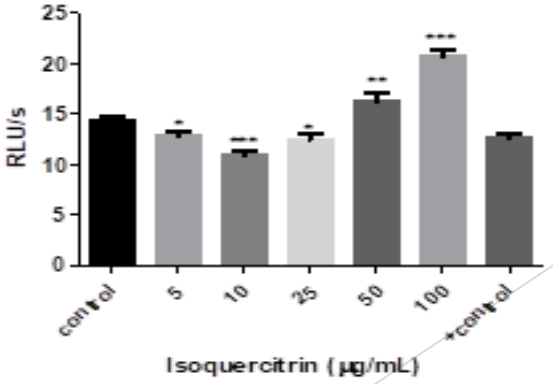
**At the lower concentration: strong antioxidant activity**

**A higher concentration: stimulation**



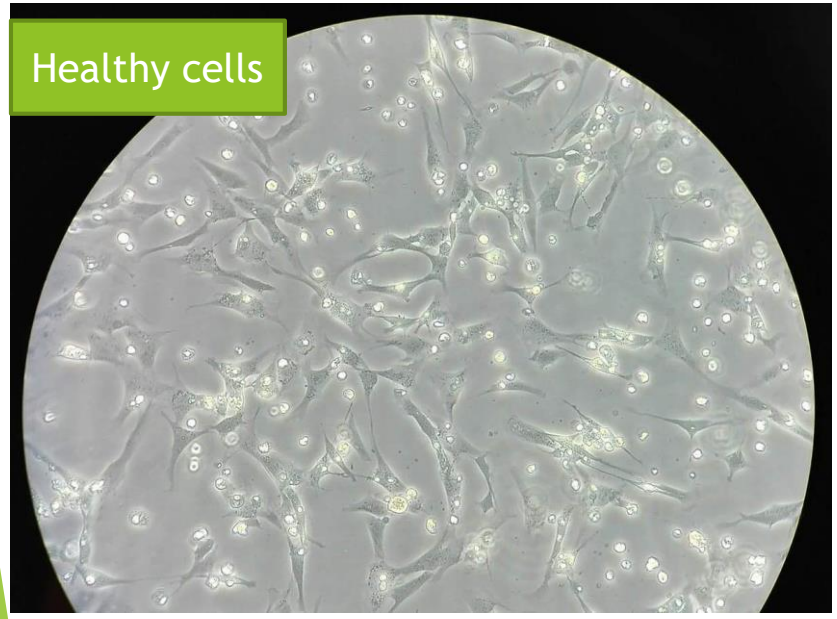



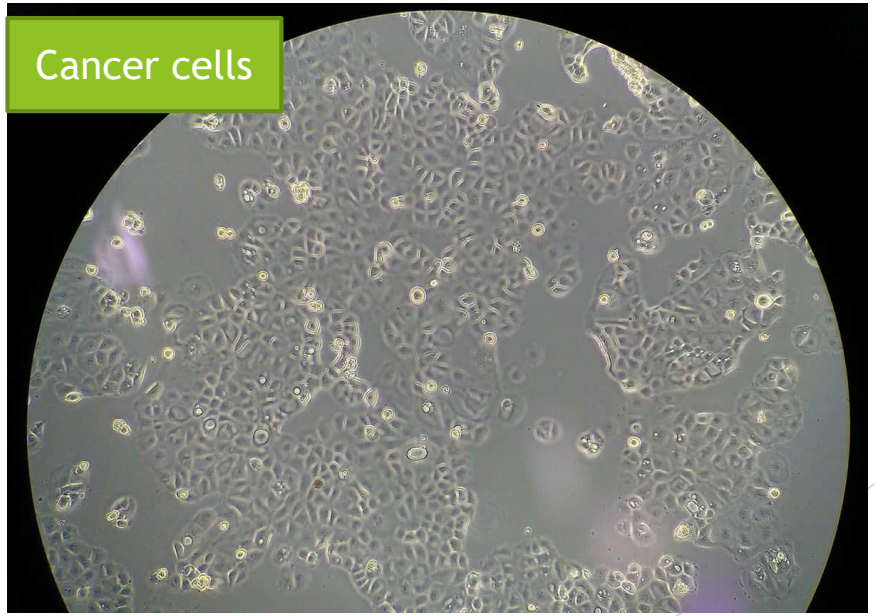



# APOPTOSIS

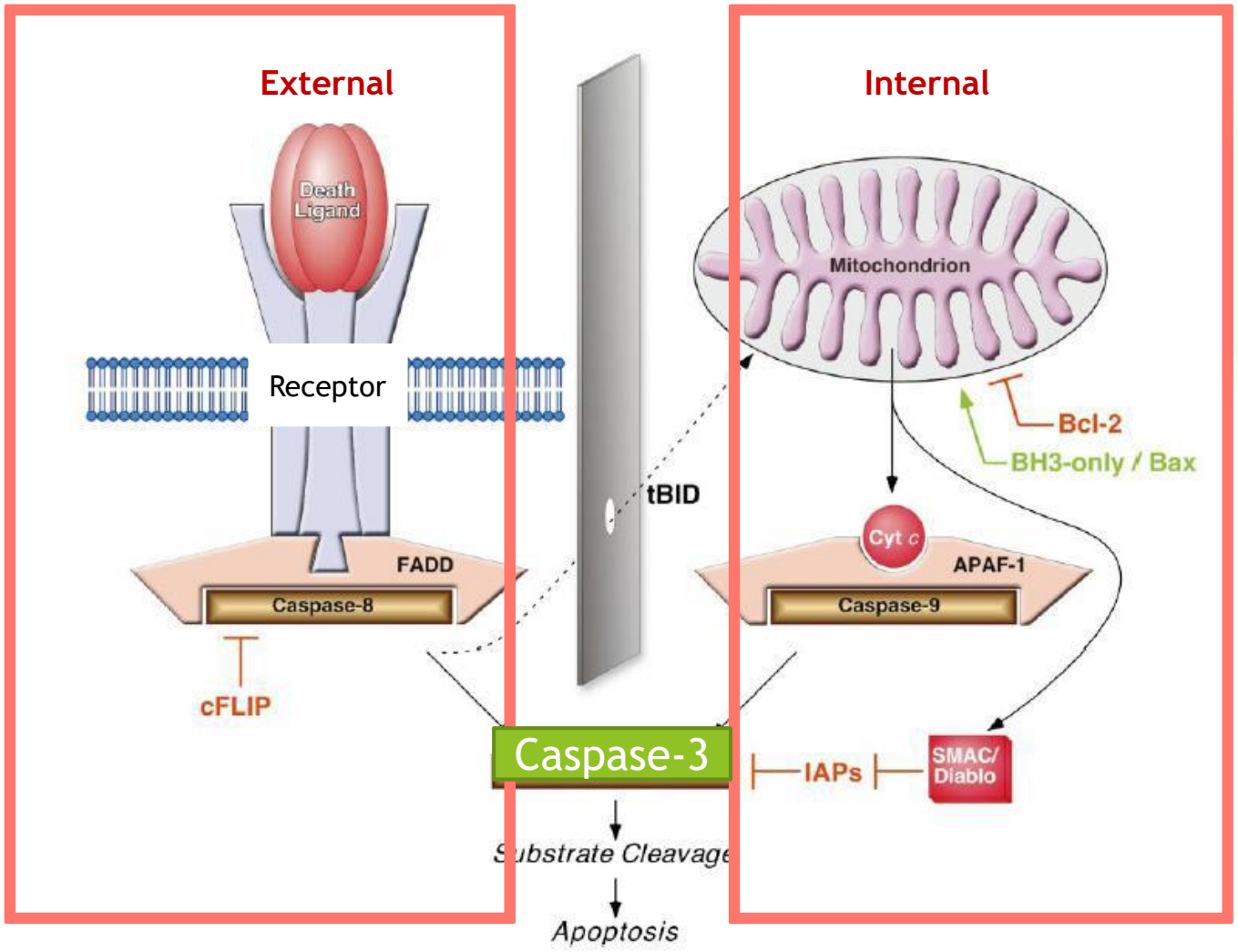
Healthy cells

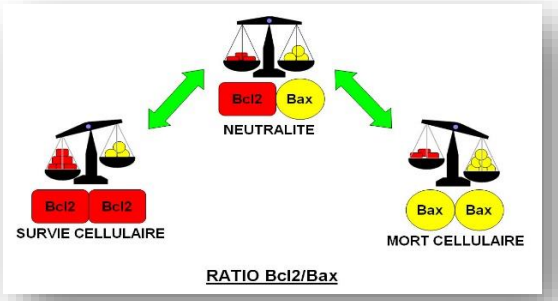


Cancer cells



# Apoptosis - physiological process



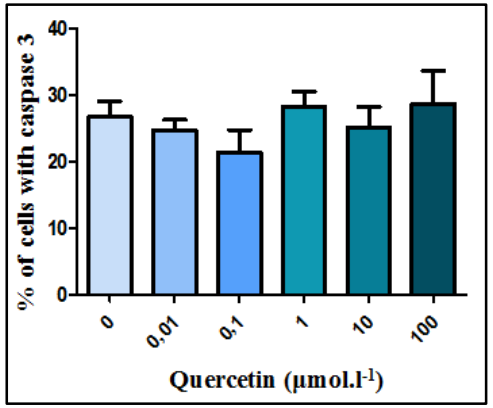



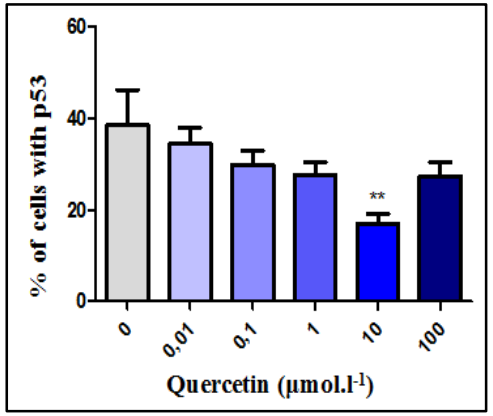

anti-apoptotic

pro-apoptotic

# Dose-dependent effect of quercetin on apoptotic marker p53

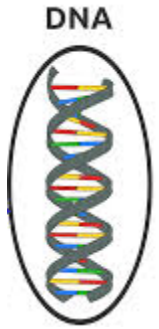
- Targeted process, genetically determined
- Elimination of the cells that are damaged, aging, potentially harmful or unnecessary for the organism
- therapeutic target (tumor therapy)



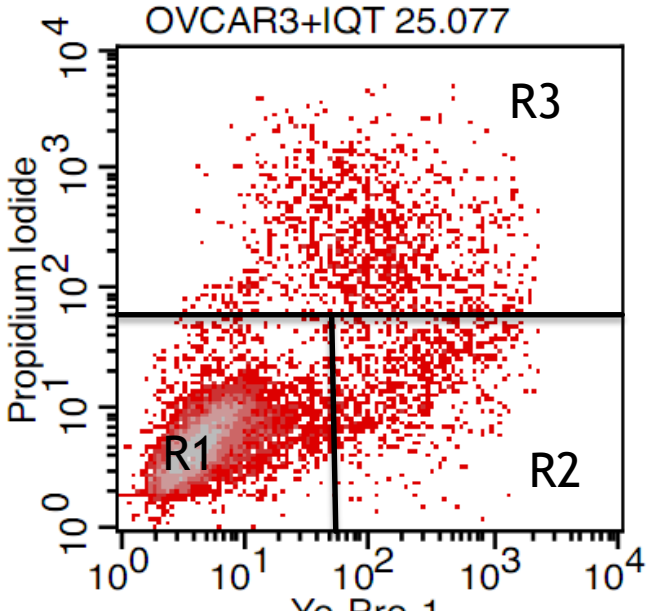



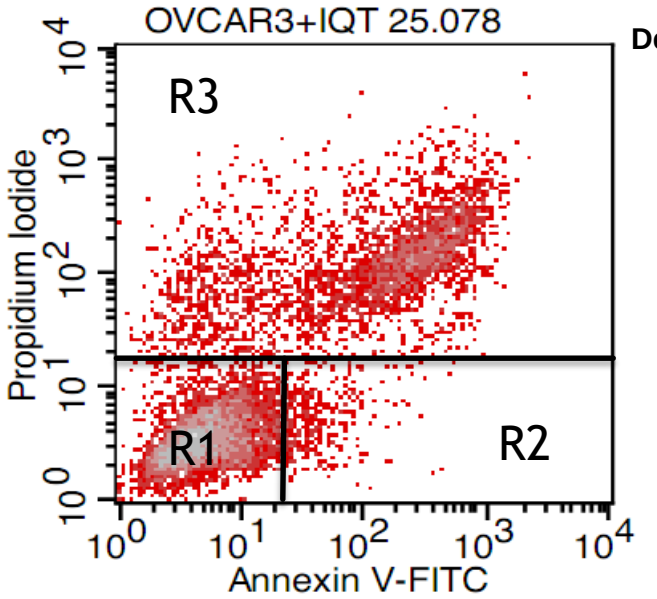

p53

apoptosis

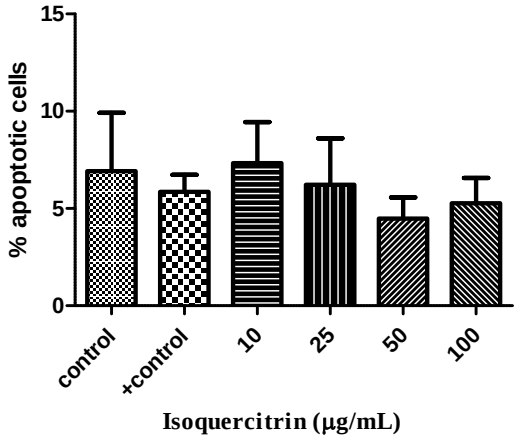


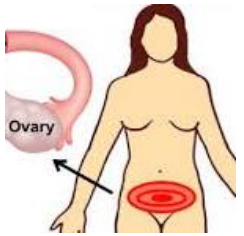
# Isoquercitrin does not affect apoptotic cells



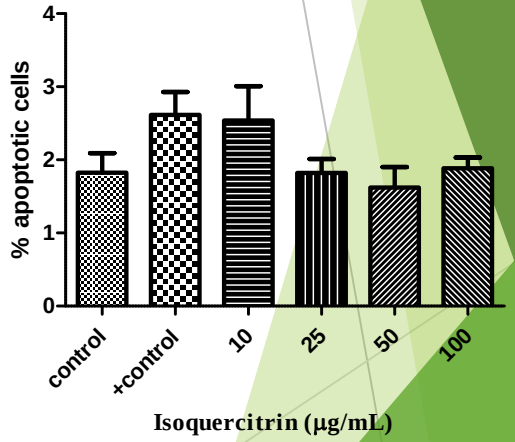




**Detection of positive apoptotic OVCAR-3 cells with Yo-Pro-1 conjugates (Yo-Pro-1$^{+}$/PI$^{-}$)**

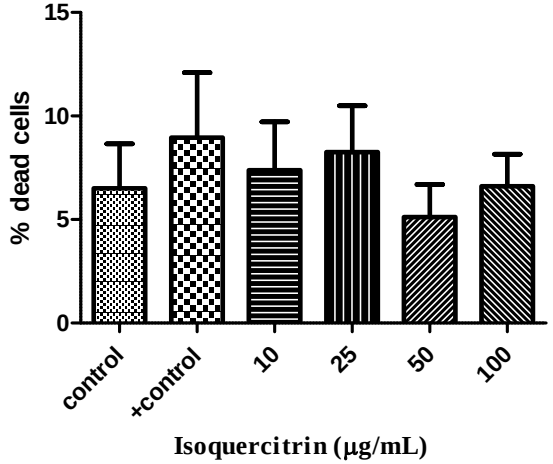



**Detectin of positive apoptotic OVCAR-3 cells with Annexin V conjugates (Annexin V$^{+}$/PI$^{-}$)**



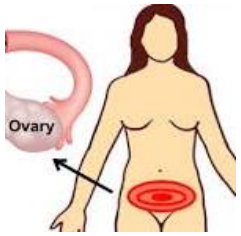

**Detection of positive dead OVCAR-3 cells with Yo-Pro-1 conjugates (Yo-Pro-1$^{+-}$/PI$^{+}$)**



# CONCLUSION

**(1) Quercetin** is able to increase the secretion of progesteron by porcine ovarian granulosa cells and influences steroidogenesis in the cells.

(2) Possible dose-dependent influence of **quercetin** on ovarian proliferation and apoptosis through pathway that may include activation of cyclin B1 and p53.




**(1) Dose-dependent effects of ISOQUERCITRIN** on the secretory activity and oxidative balance in ovarian carcinoma cell line OVCAR-3 was found.

(2) Knowledge gained from this *in vitro* study could lead to **a better understanding of the mechanism of quercetin and isoquercetin action in healthy ovarian cells and ovarian cancer.**

**(3) Quercetin and isoquercitrin are potential regulators of ovarian functions.**

# CONCLUSION



**Biotechnologies**

A through control of the sex cycle and ovulation ensures the gain of sufficient oocytes and early embryos.

**Bioregulators**

Folliculogenesis stimulating regulators influence growth, follicular maturation, and ovulation of oocytes.

On the other hand, substances with the opposite effect inhibit follicular maturation.

**Nutraceuticals**

The new hormonal, pharmacological and gene regulators of reproduction and the application of alternative natural substances can be designed to regulate and treat disorders of reproductive processes.

# Thank you for your attention

Adriana Kolesárová

69th Annual Meeting of the European Federation of Animal Science, Dubrovnik, Croatia, 2018