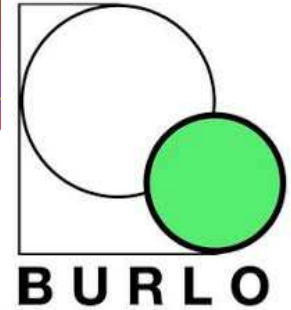




UNIVERSITÀ  
DEGLI STUDI  
DI TRIESTE



***Endometriosi alle porte del  
nuovo decennio***

**13/12/2019**

***Auditorium Museo Revoltella***

***– Via Diaz,27 – Trieste***

# Aspetti patogenetici

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**Department of Life Sciences**  
**University of Trieste**  
**ITALY**

**Just because you can't see it  
Doesn't mean it doesn't exist!**

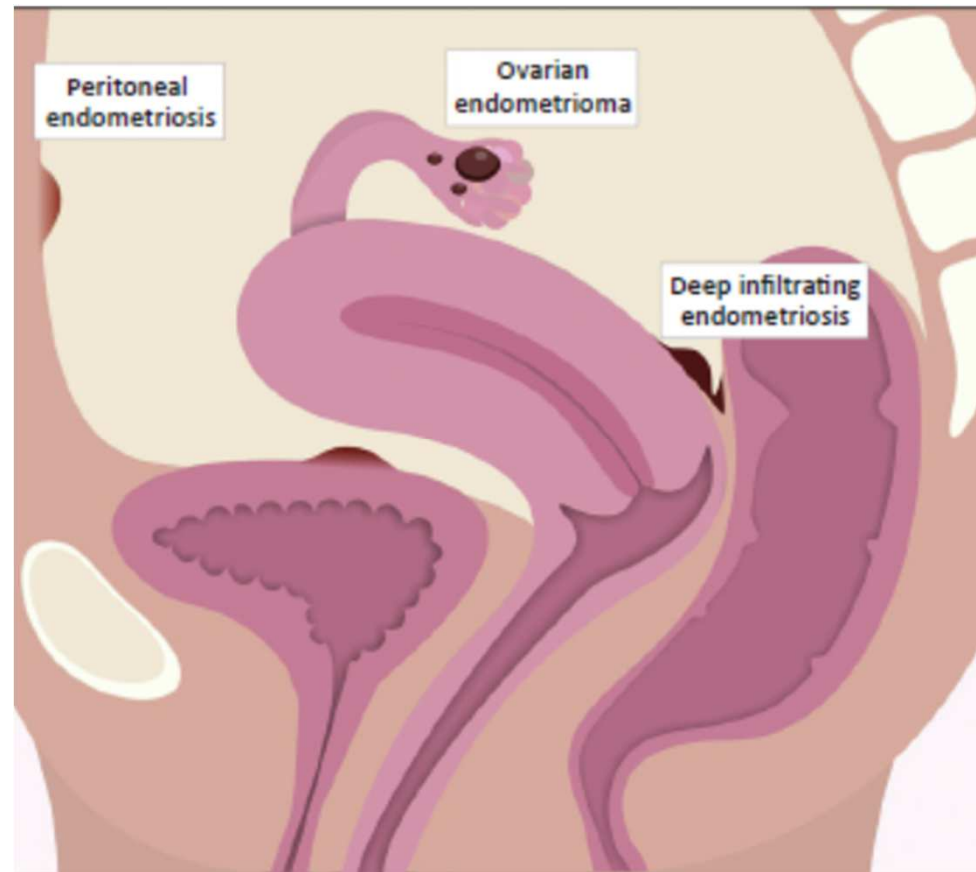
pelvic pain  
dysmenorrhea  
dyspareunia  
dysuria



**If ENDOMETRIOSIS were visible this is how it might look!**

Endometriosis (EM) is a chronic condition that affects about 10% of young women worldwide. Pain and infertility are the two most common features of the disease.

# Endometriosis



- Characterized by the presence of functional endometrial tissue outside the uterine cavity.
- The hormonal cycle induces the bleeding of this ectopic tissue leading to a chronic inflammatory condition.

# Theories of EM pathogenesis

Retrograde Menstruation

Coelomic Metaplasia

Stem Cell Theory

Müllerian Theory or Defective Embryogenesis

All the proposed hypotheses for the cell origin can be categorized into two main theory

- **In situ theory**
- **Transplantation theory**



# The in situ Theory

**Stroma and glands of endometrial-like tissue of endometriosis originate in-situ from the local tissue by metaplasia or by embryological origin**

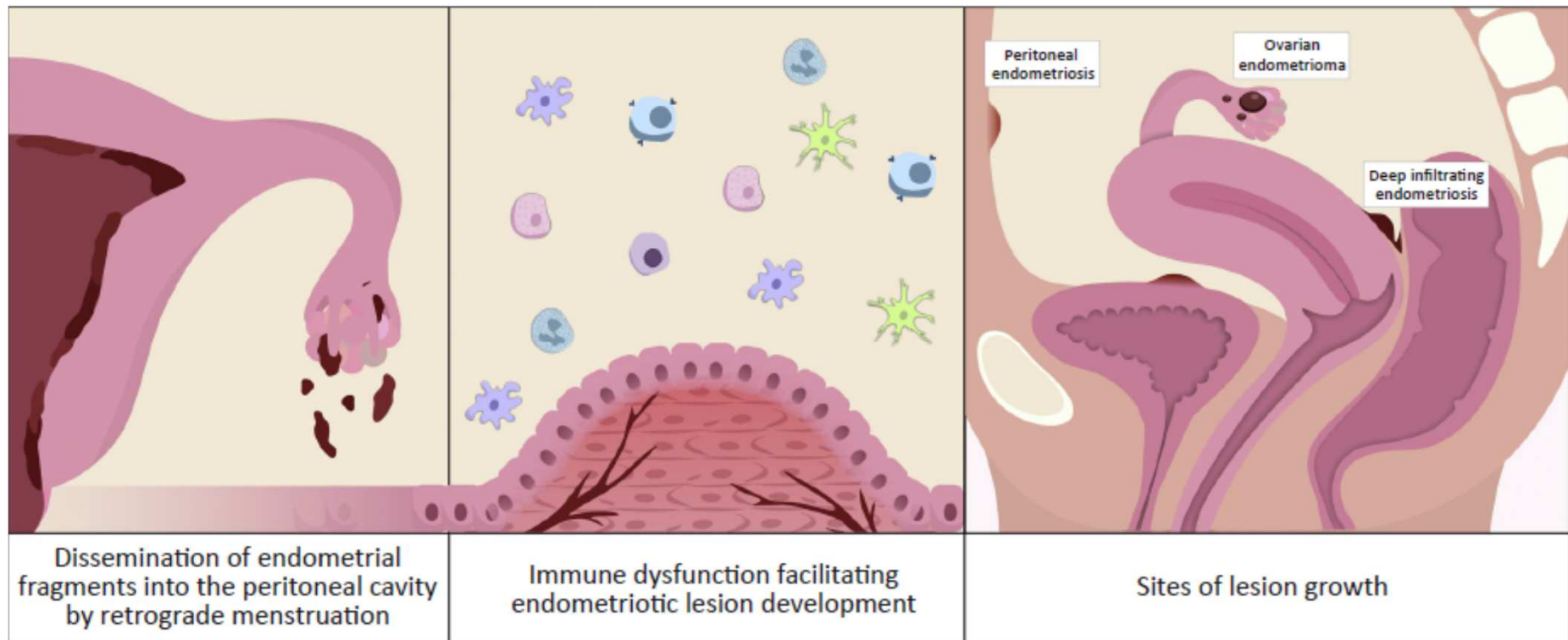


# The Transplantation Theory

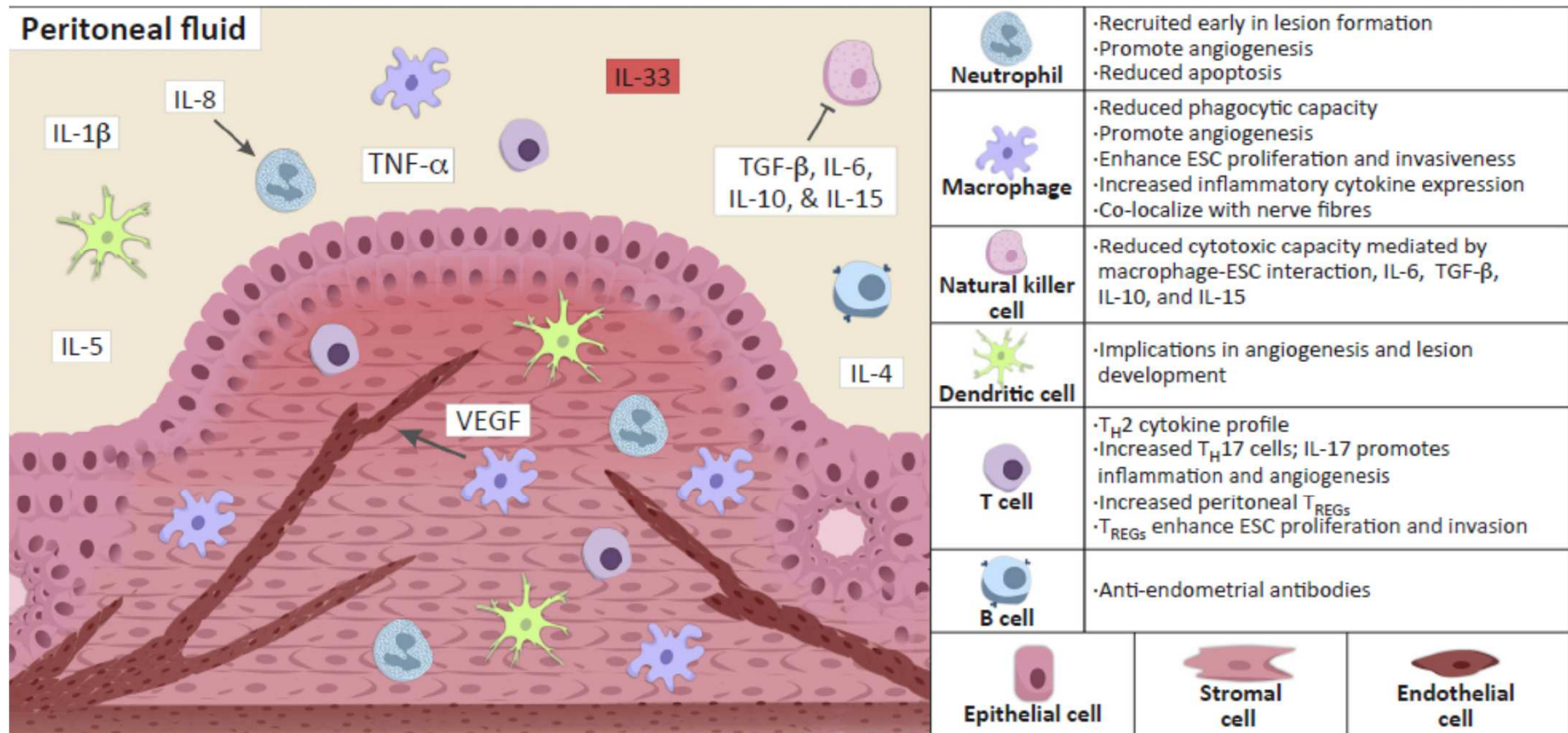
**Stroma and glands of endometrial-like tissue of endometriosis originate from eutopic endometrium.**

**Endometriosis is proposed as a benign metastasis of eutopic endometrium which is displaced from the uterine cavity to another location inside the body through different routes: hematogenous, lymphatic and iatrogenic (mechanical) spread of endometrial or endometriotic cells**

# Retrograde menstruation and immune dysfunction

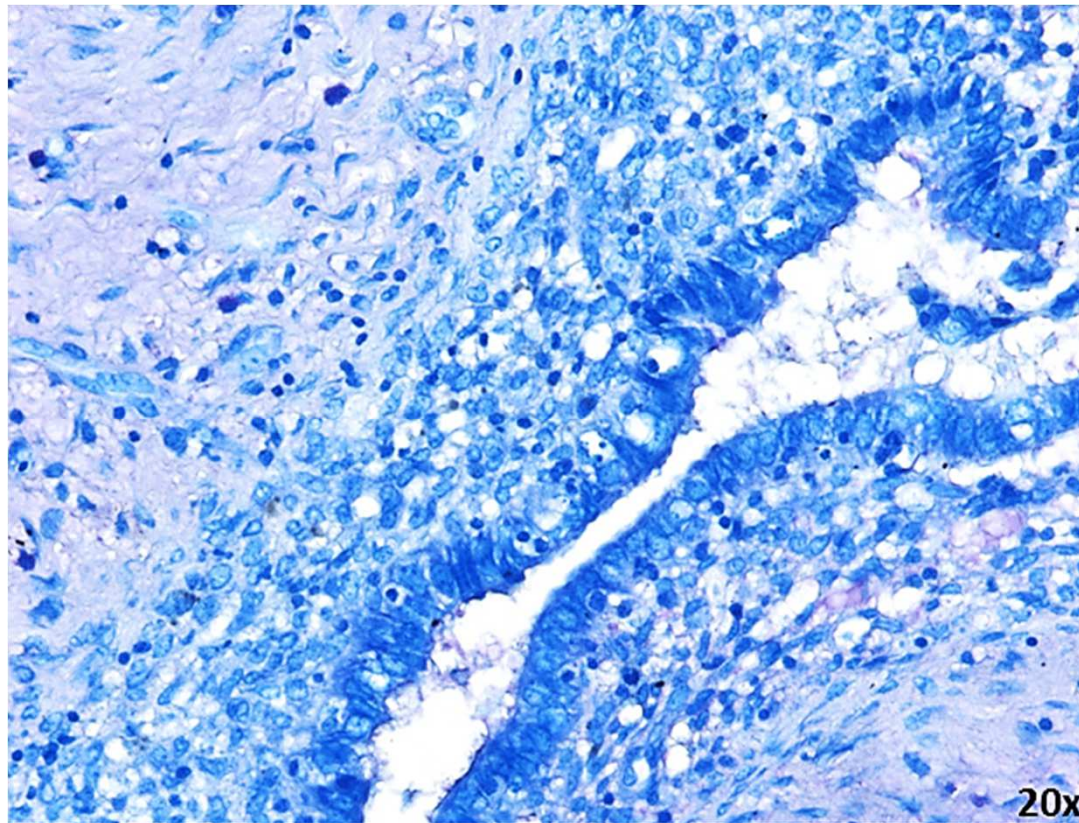


# Endometriotic lesion immune microenvironment

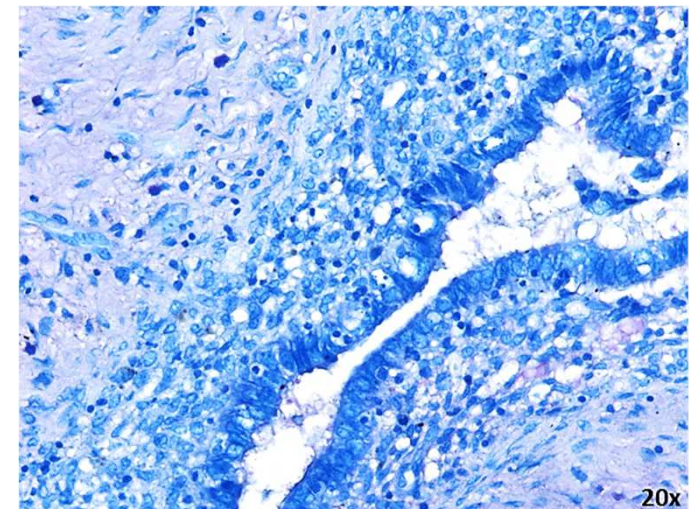
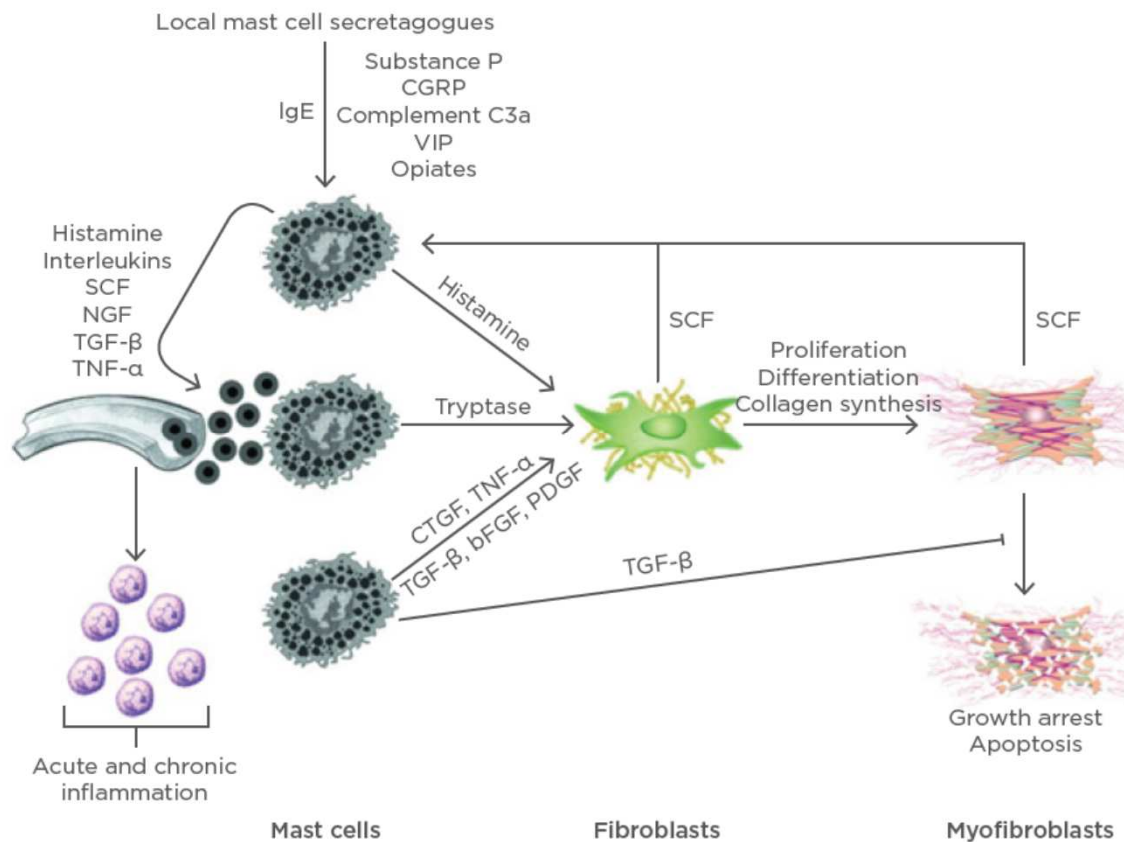




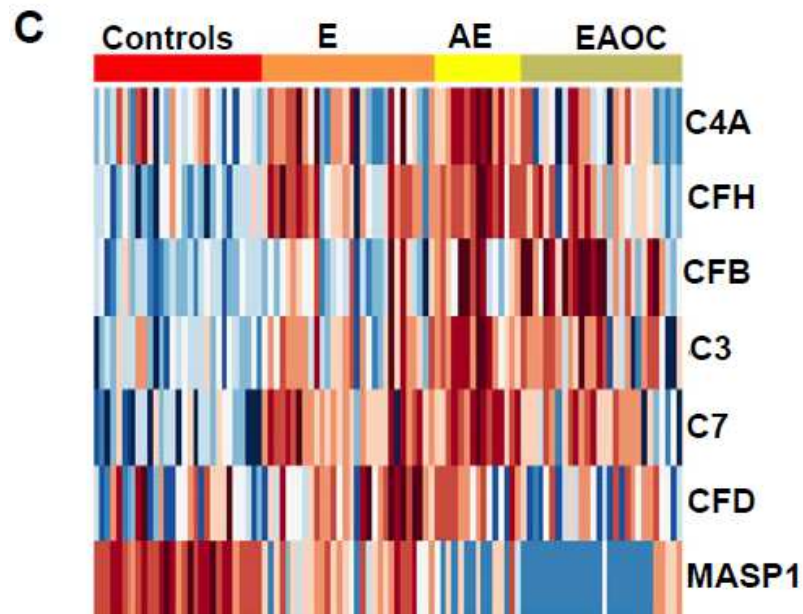
# Mast cells mediate inflammation and fibrosis in endometriosis



# Mast cells mediate inflammation and fibrosis in endometriosis



# Complement System Pathway is altered in EM



## A. Control vs. Endometriosis

ID	Fold change*	P-Value
BST2	2.916337	7.86E-12
C3	5.257982	6.84E-22
C4B	2.028549	0.000174
C4BPA	0.161936	2.47E-09
C7	23.71404	3.39E-61
CCL19	4.040469	8.03E-07
CD22	3.169821	1.31E-09
CD24	0.40247	2.78E-09
CD97	0.491109	3.02E-07
CFB	2.818664	3.36E-08
CFD	2.5879	2.73E-08
CFH	7.166509	2.49E-28

Gene expression analysis revealed the complement pathway as most prominently involved in endometriosis

# Complement cascade Activation

## Alternative way

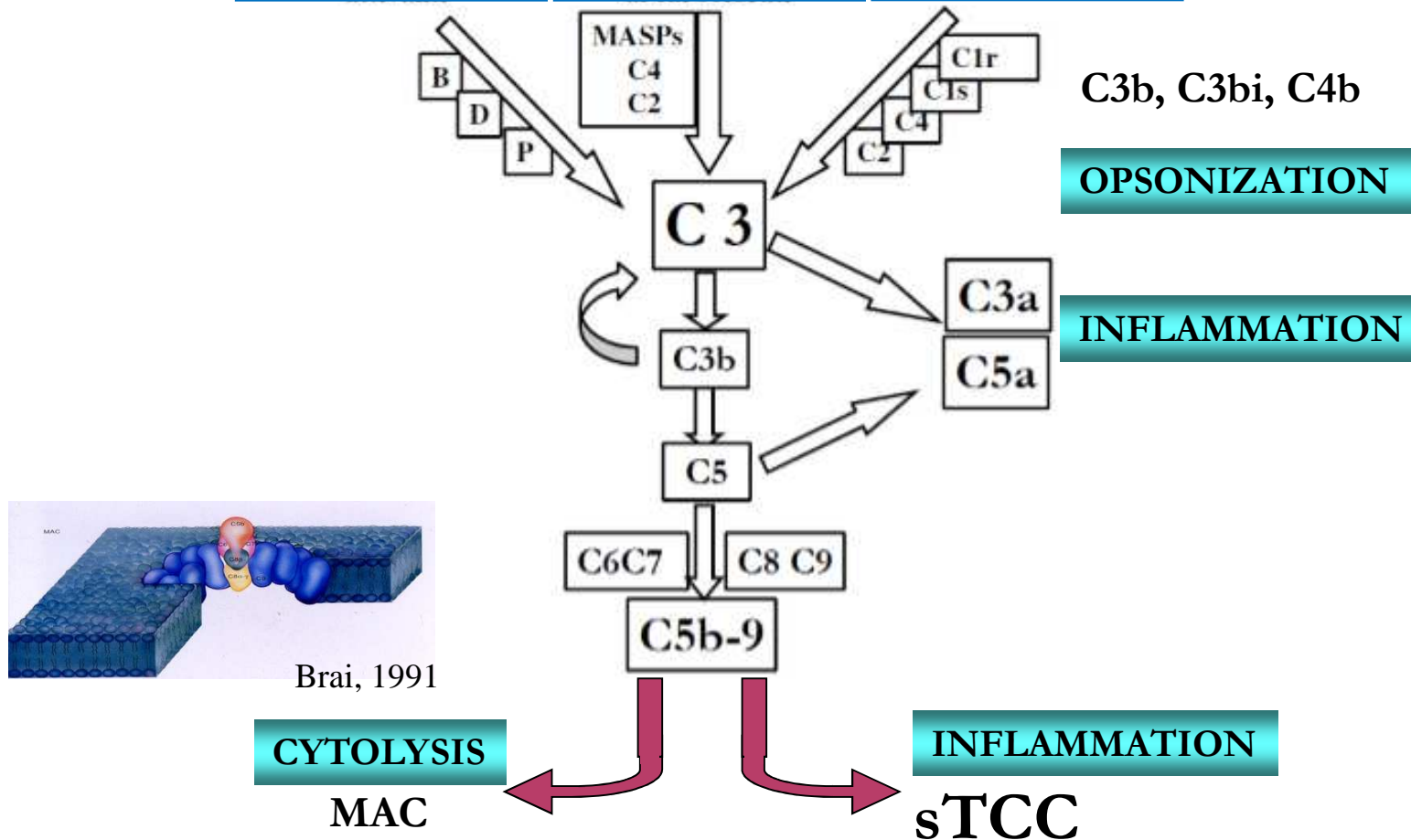
**C<sub>3</sub>**<sub>H<sub>2</sub>O</sub> to several activating surfaces

## Lectinin way

**MBL**, ficolins or collectin 11 bind to carbohydrates

## Classical way

**C1q** binds to immunocomplexes





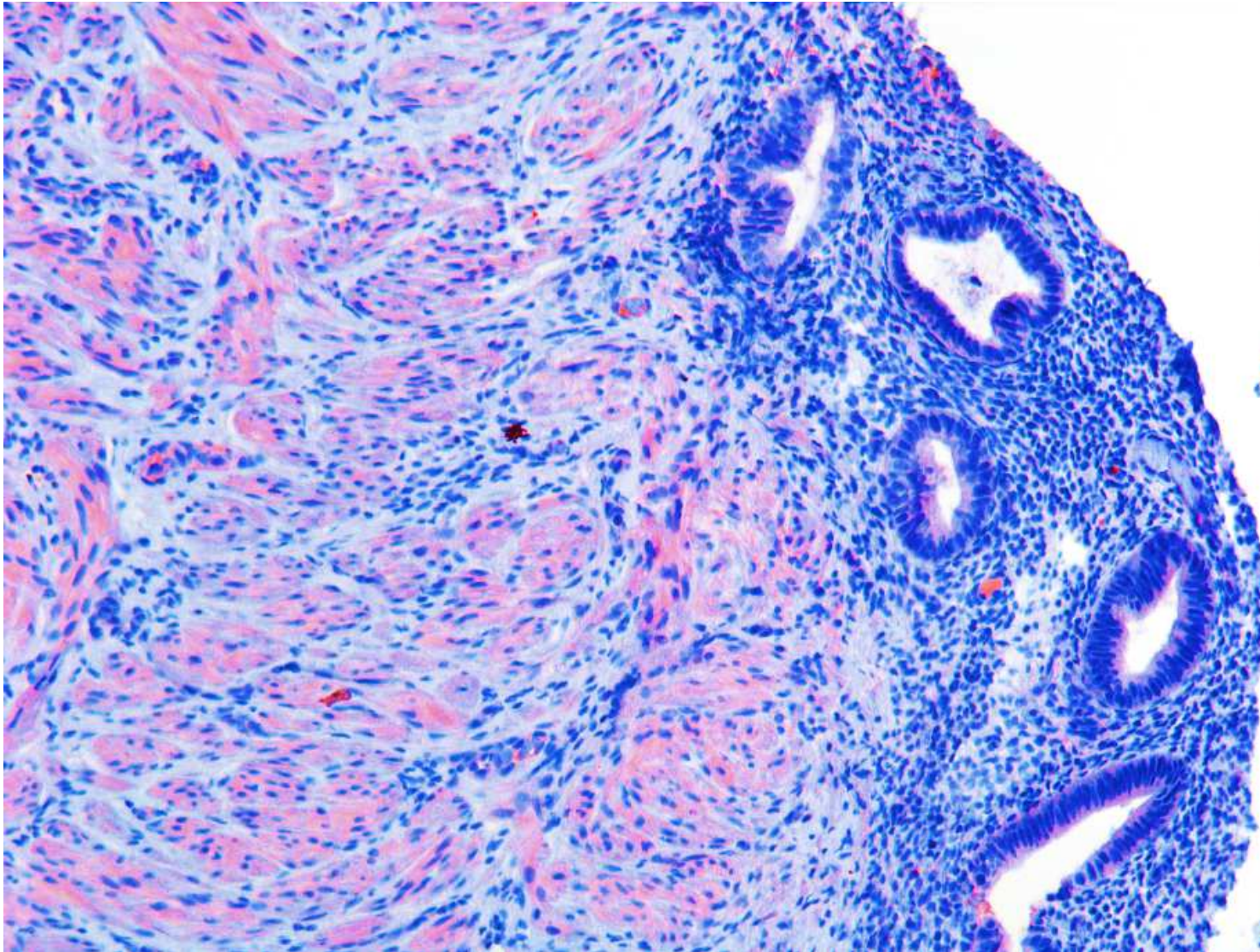
# AIM

Several groups\* demonstrated that the glandular epithelial and stromal cells found in endometriotic implants produce and secrete the C component C3.

The aim of this work was to confirm the presence of C3 in the ectopic tissue in comparison to the eutopic one, and to investigate the direct role of C3 in the pathogenesis of EM.

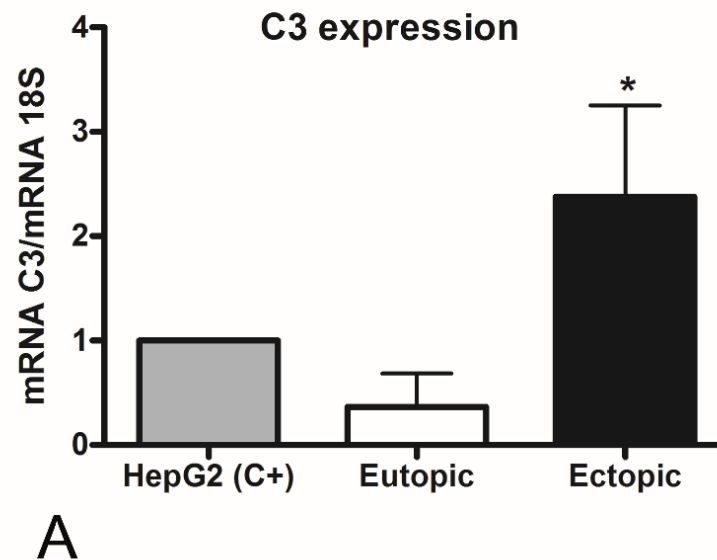
\*Weed and Arquembourg 1980; Bartosik D. et al., 1987; Isaacson K.B. et al., 1989; Bischof P. et al., 1994; Ruiz LA et al., 2011; Signorile P.G. et al., 2014, Suryawanshi S. et al., 2014; Rekker et al., 2017

# IHC analysis of C3 in human EM cysts



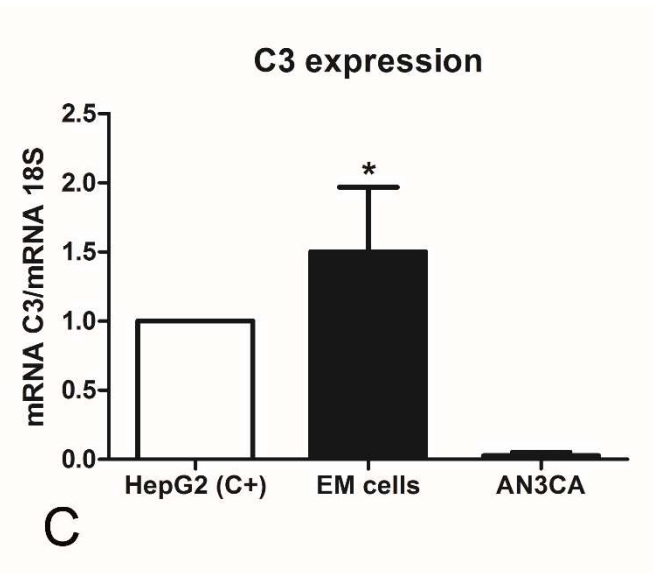
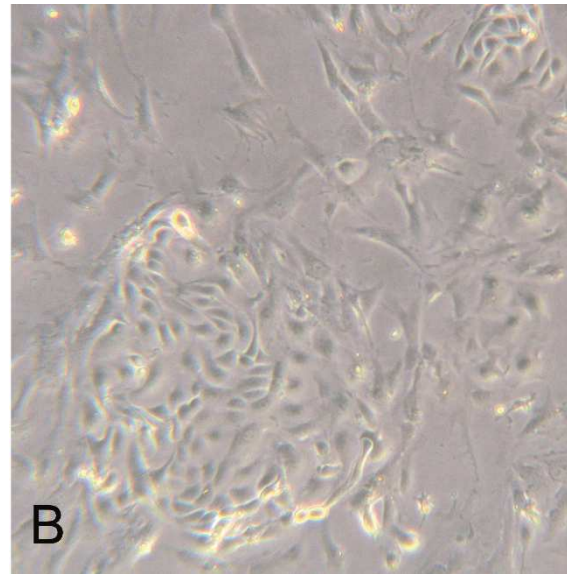
AEC

# Analysis of C3 in ectopic endometrial tissue and normal uterus



endometriotic cyst tissue presents higher level of C3 (but not C4 or C5) mRNA compared to normal uterus.

# C3 expression in endometrial cells isolated from cysts



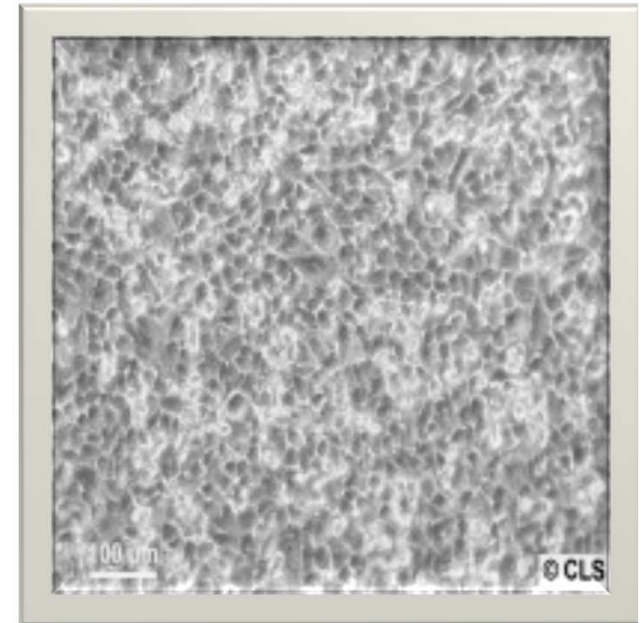
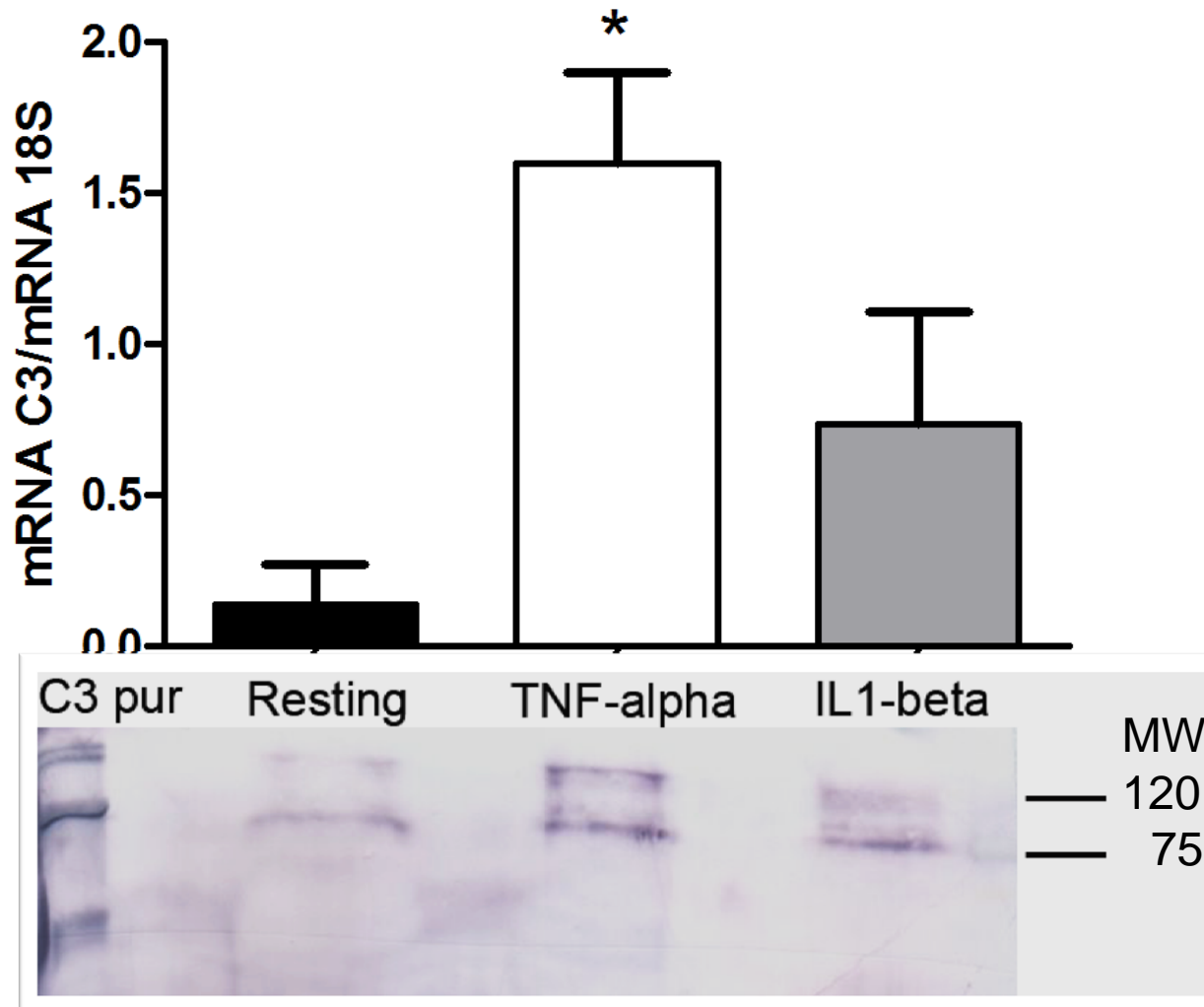
Endometriotic cells isolated from cysts and cultured in vitro, were able to synthesized C3.

-RESULTS-



# C3 expression by normal endometrial cells

## C3 expression by AN3CA

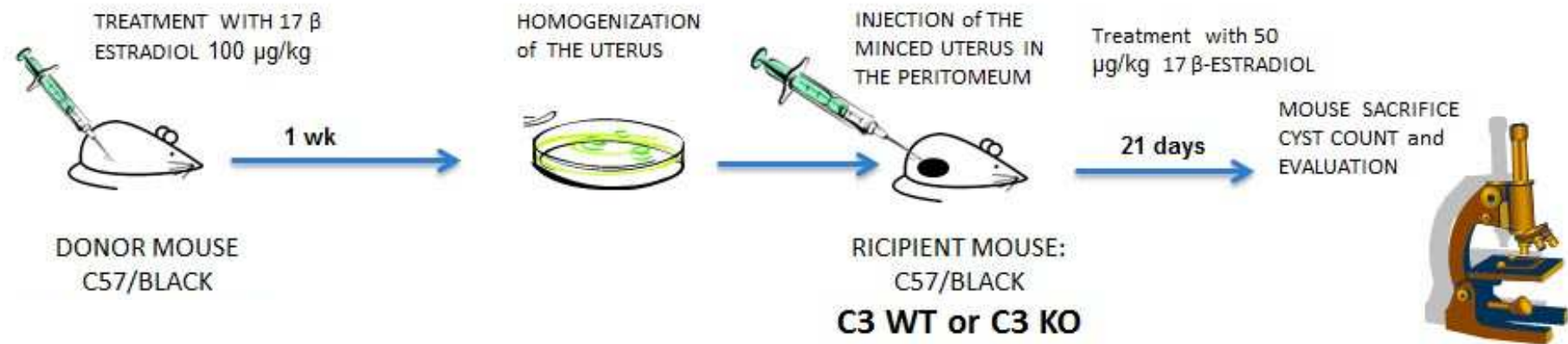


**AN3CA**

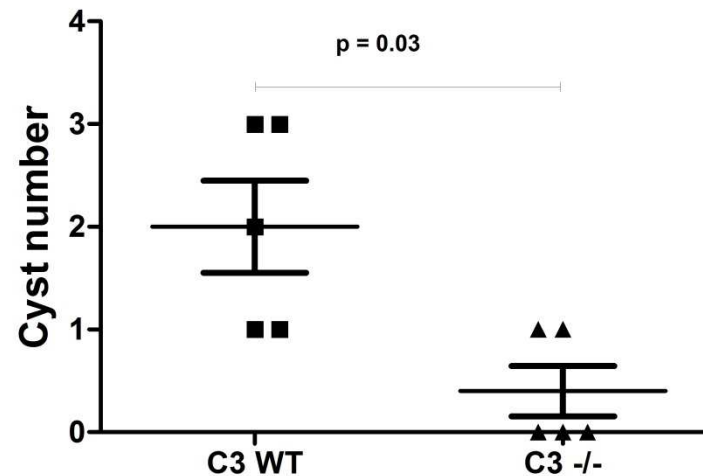
(human endometrial cell line)

Endometrial cells, when stimulated with pro-inflammatory stimuli (in particular with TNF- $\alpha$ ) start to express C3 (but not C4 or C5)

# Syngeneic in vivo model: C3<sup>-/-</sup> mice are resistant to develop EM cycts

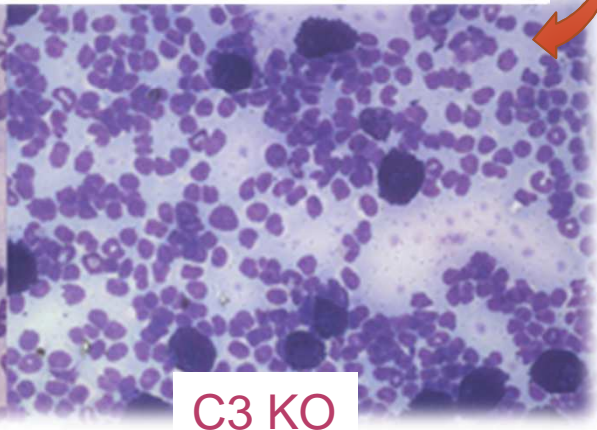
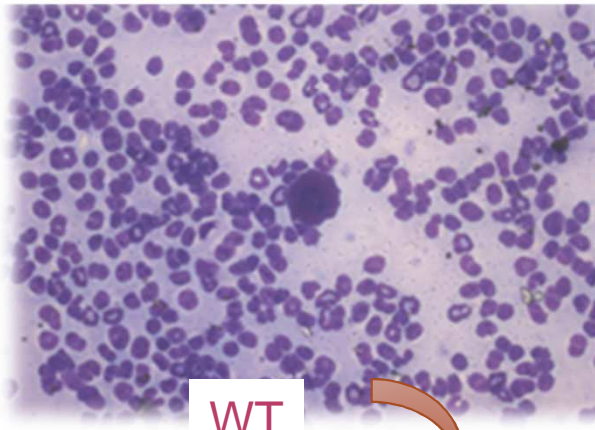
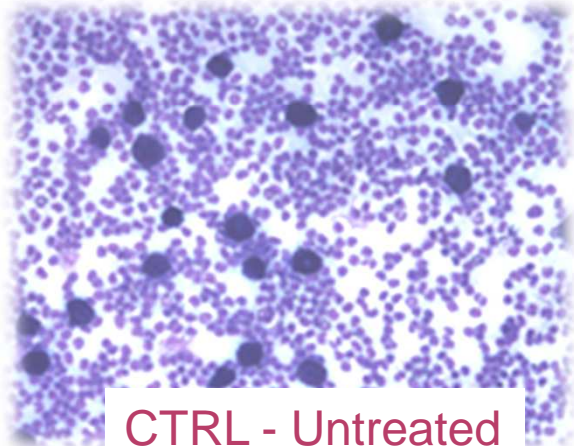
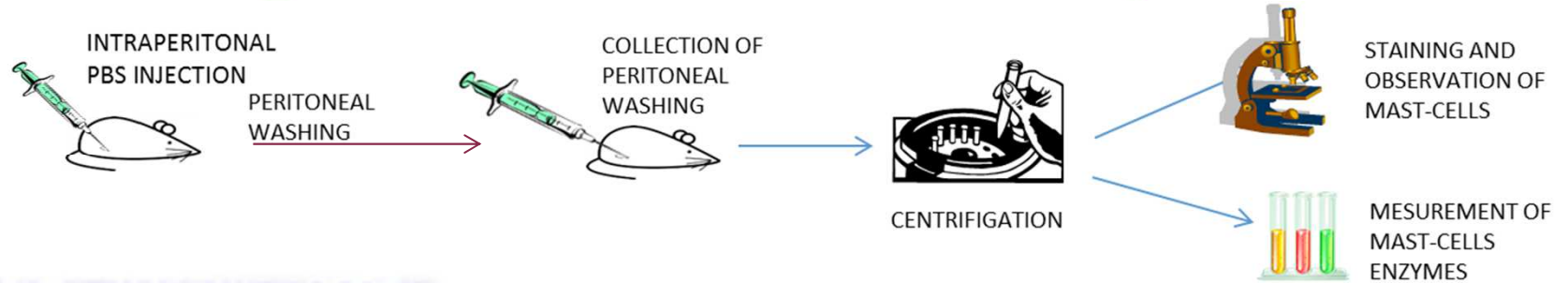


The engraftment and the dimensions of endometriotic lesions is reduced in C3 deficient mice compared to WT mice

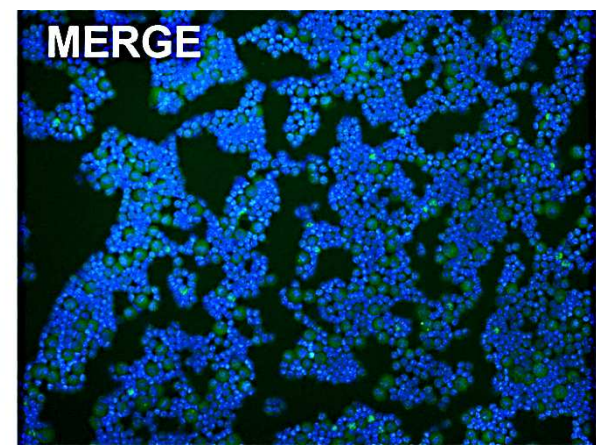
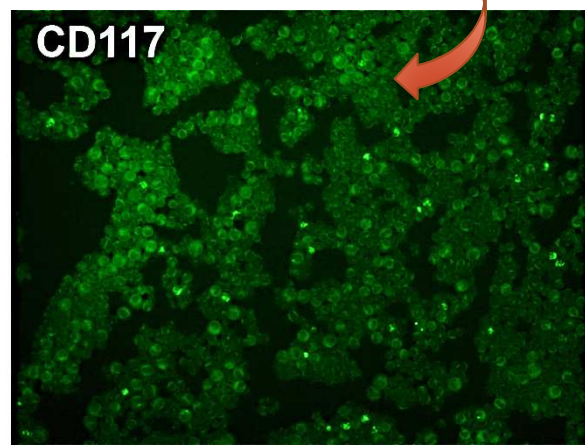
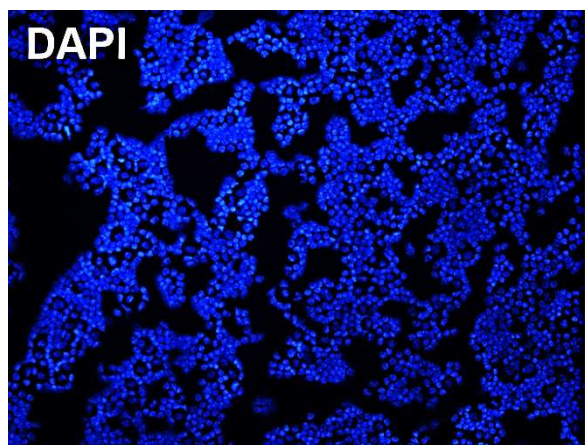


-RESULTS-

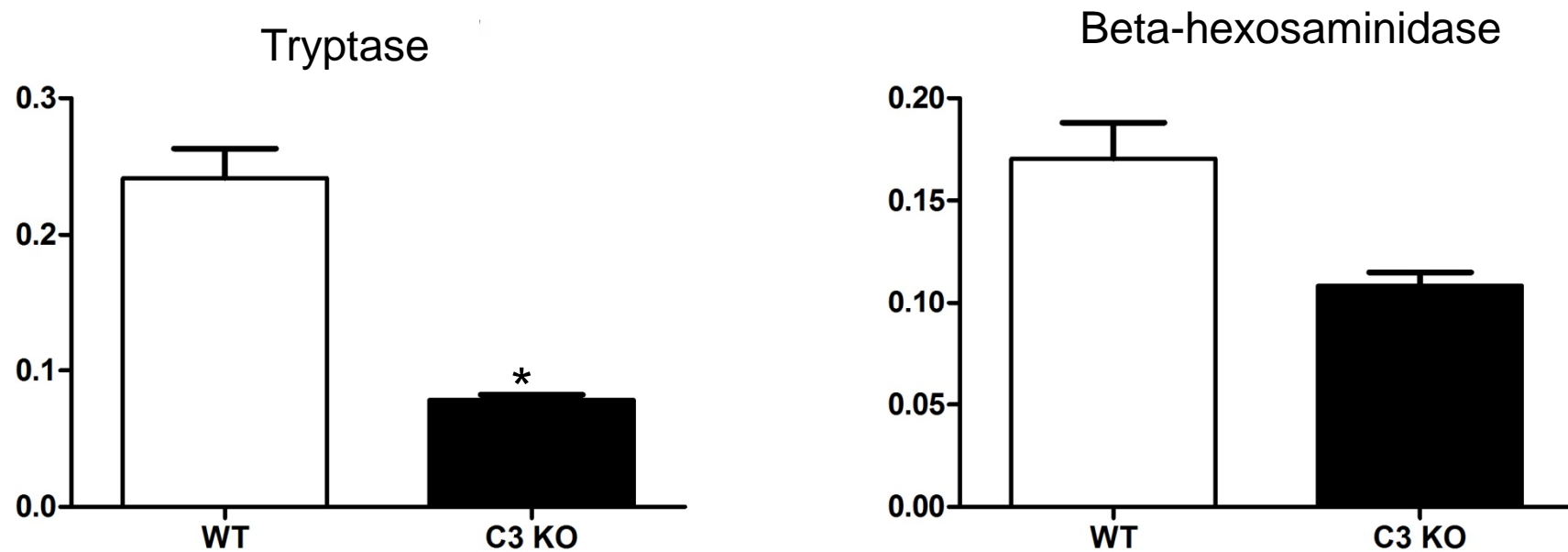
# Peritoneal liquid from WT mice with EM present more degranulated mast cells compared to C3 KO



WT Mouse Peritoneal washing

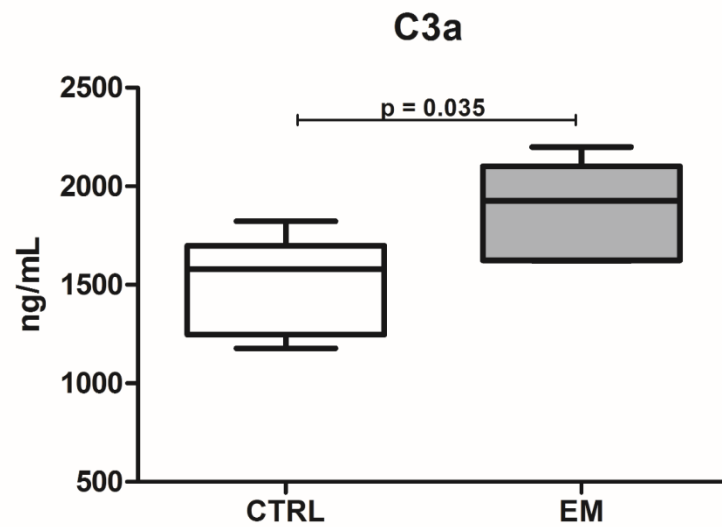


# Peritoneal liquid from WT mice with EM present higher levels of mastcell enzymes compared to C3 KO



The analysis of the peritoneal liquids, collected from endometriotic mice, revealed an increase of tryptase and  $\beta$ -hexosaminidase, enzymes present in the mast-cell granules, in WT animals compared to C3<sup>-/-</sup> mice.

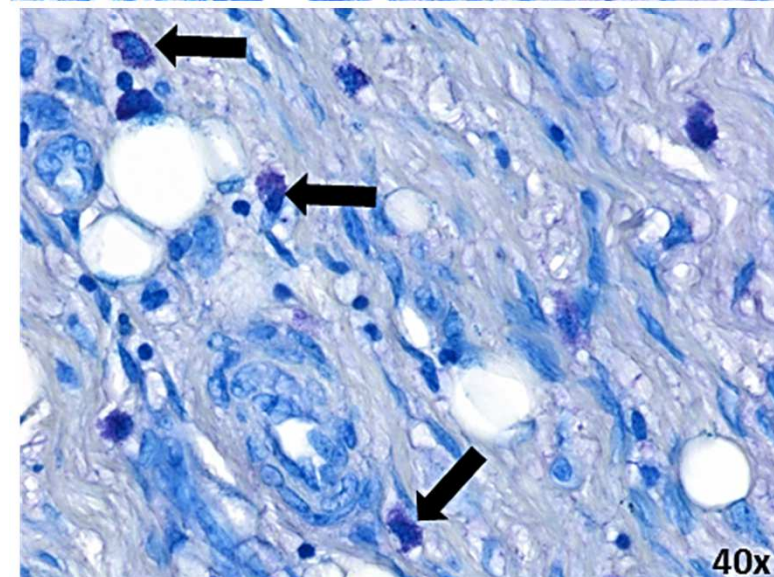
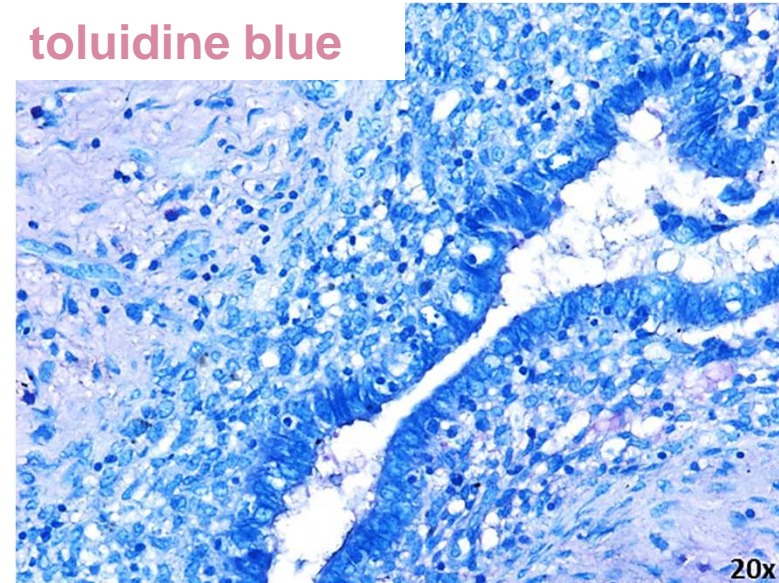
# C3a is higher in peritoneal fluid of EM patients



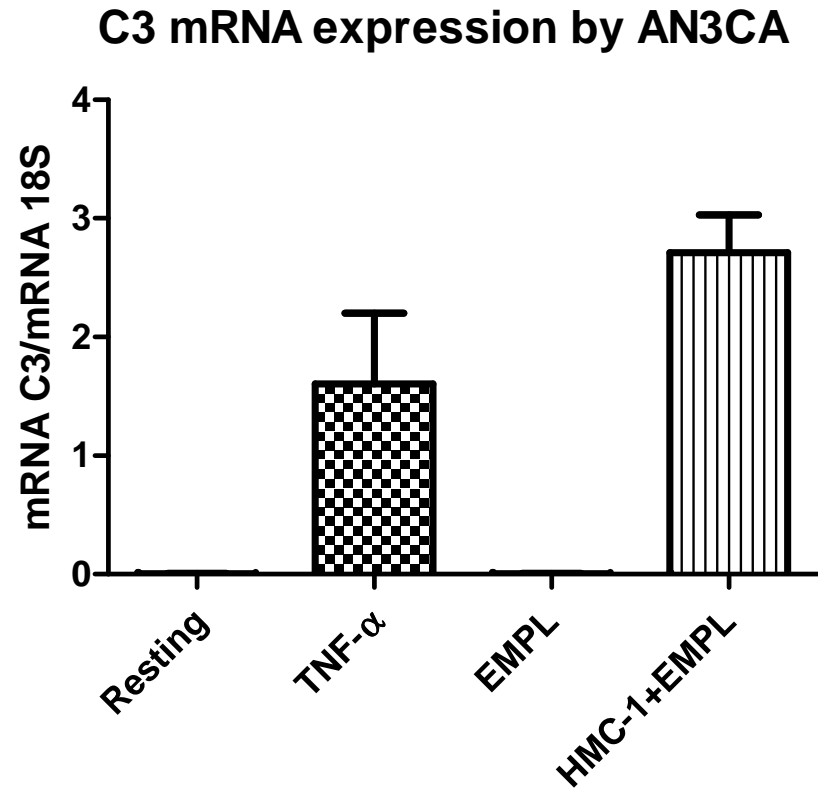
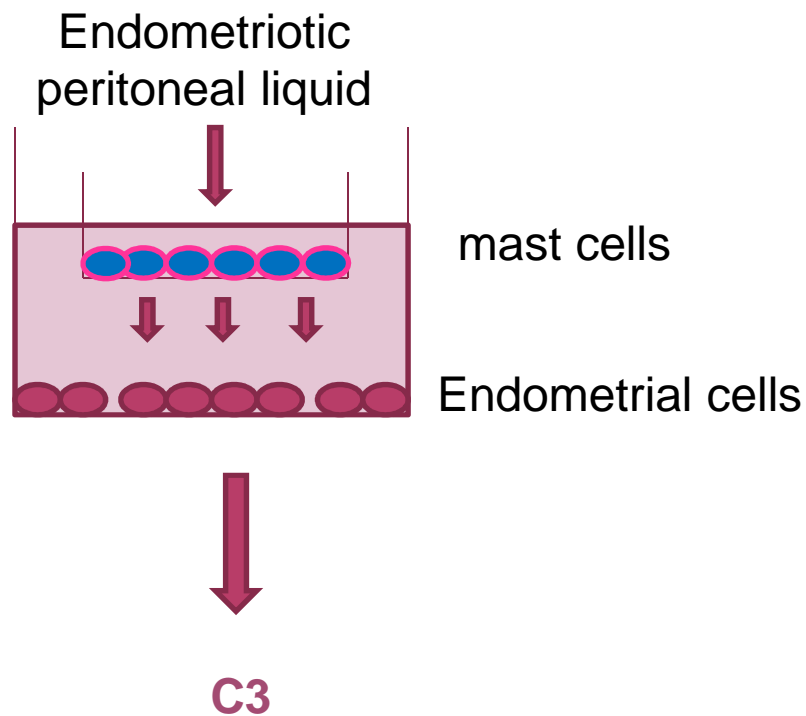
CTRL from explorative laparoscopy

Results

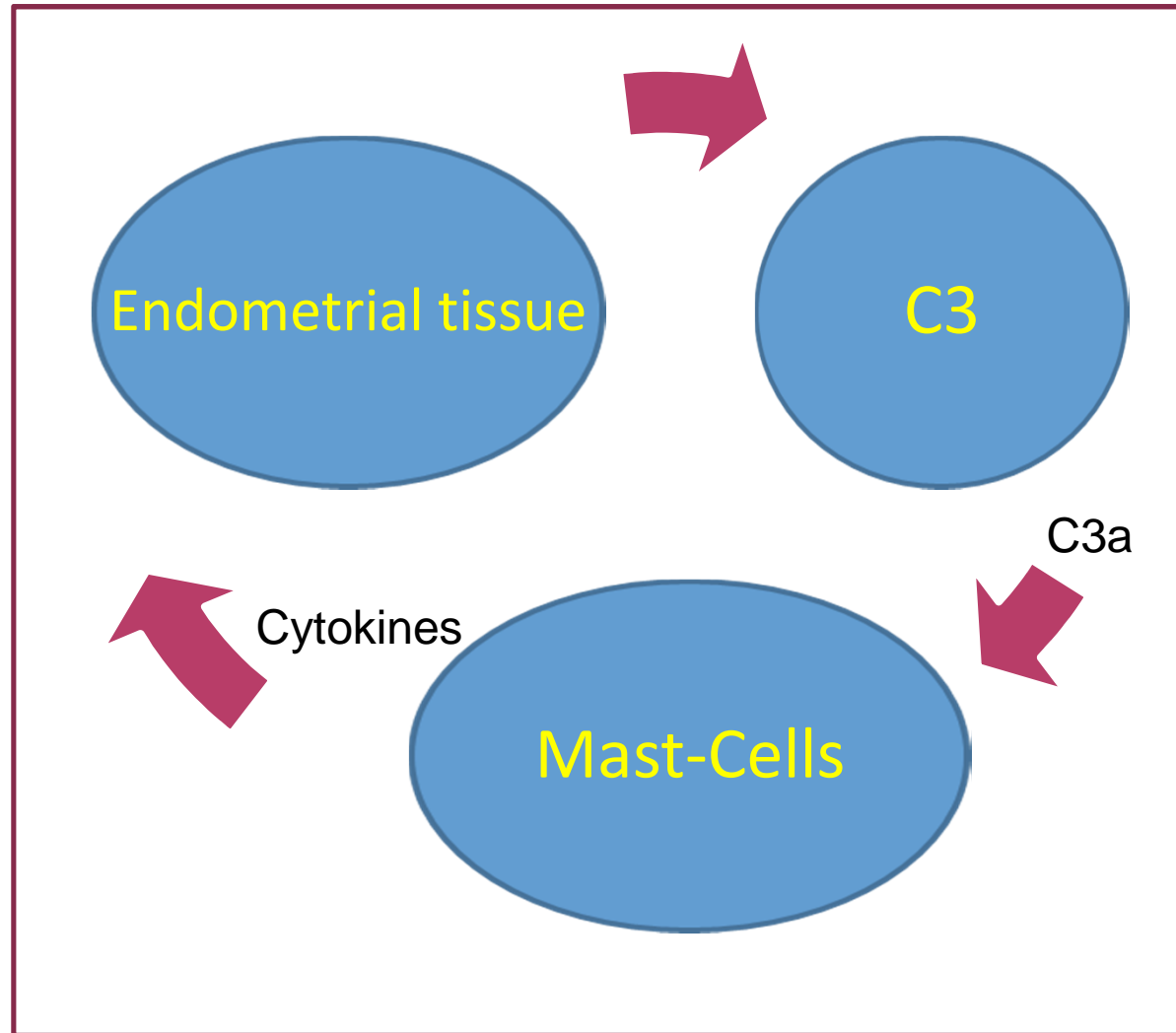
toluidine blue



# Peritoneal liquid from endometriotic patients stimulated mast cells to produce proinflammatory cytokines



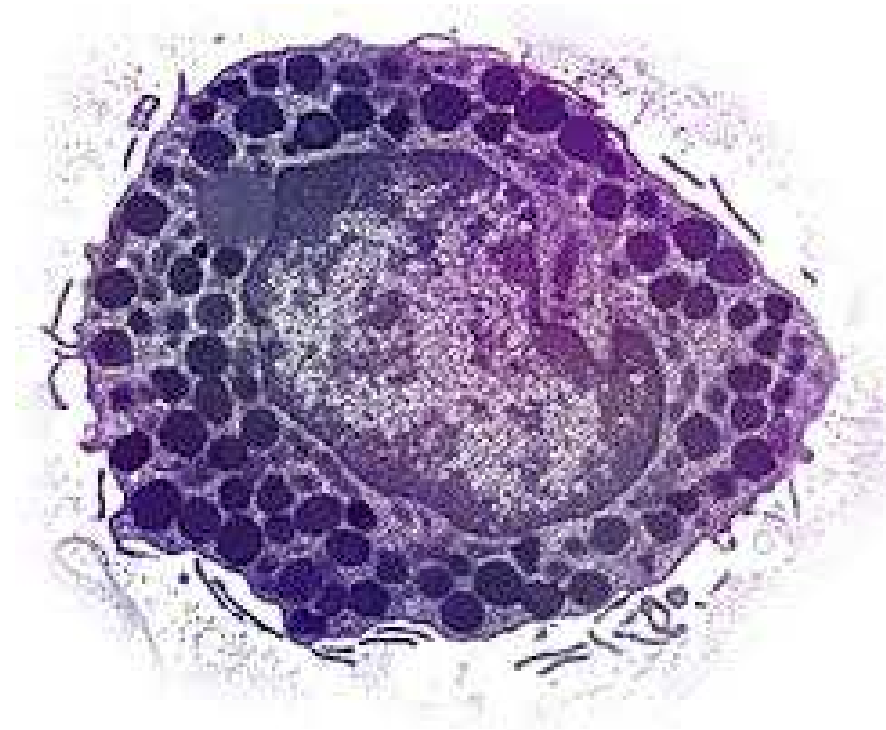
# ENDOMETRIOSIS and C3



-Conclusions-

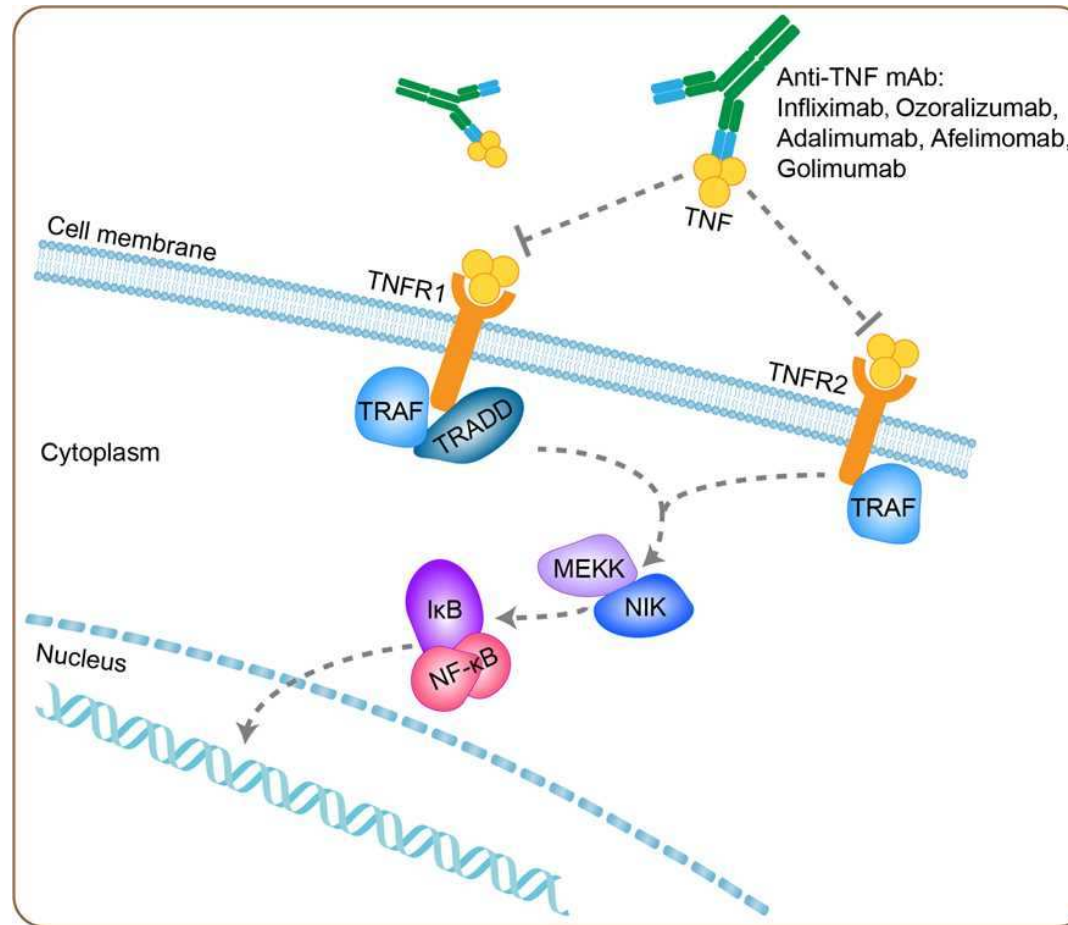
# Endometriosis treatment

**Sodium  
cromoglycate**

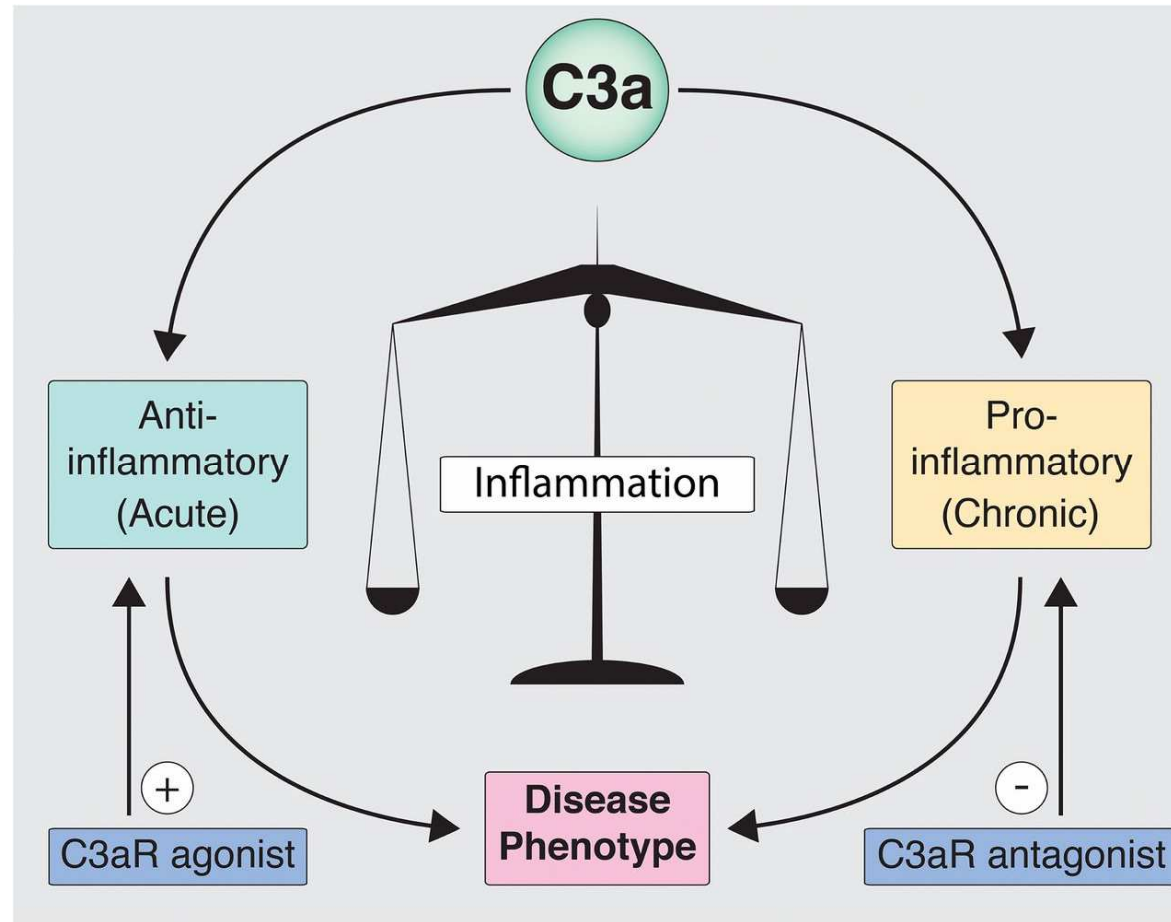




# Endometriosis treatment

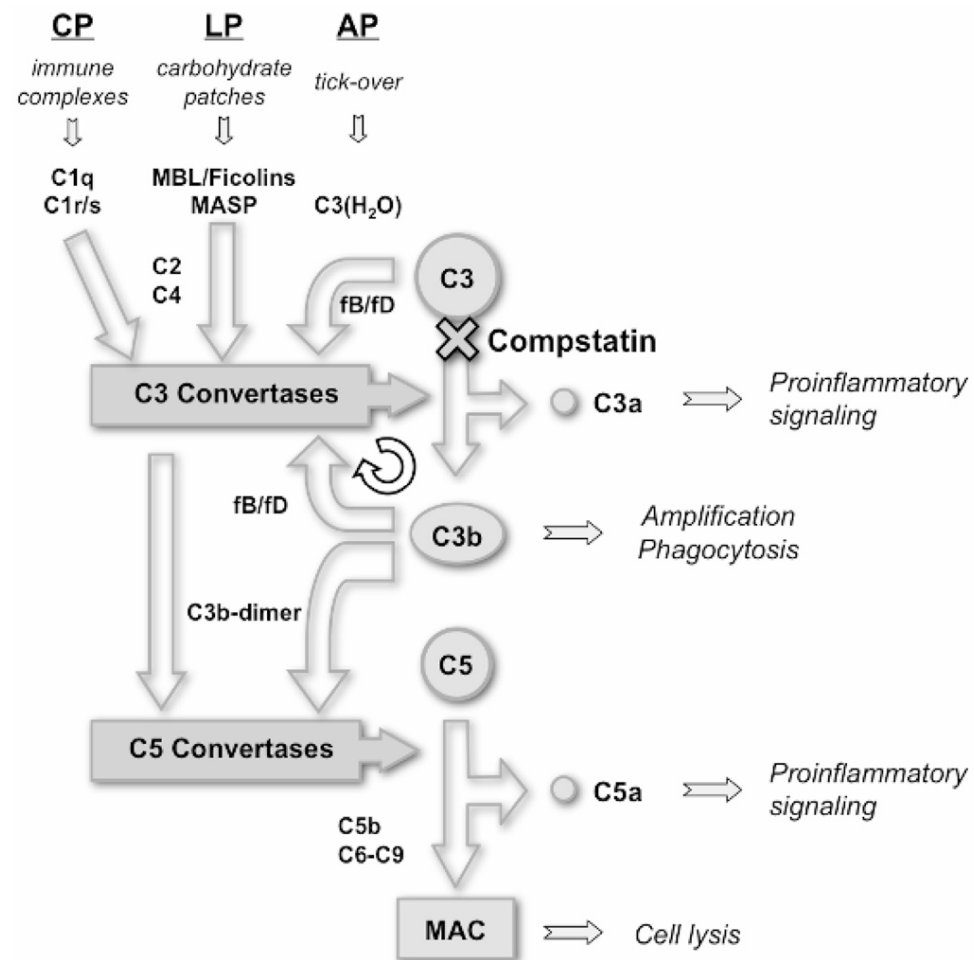


# Endometriosis treatment



**C3a antagonists**

# Endometriosis treatment



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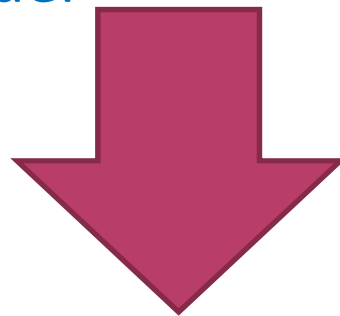


Thank you for your attention

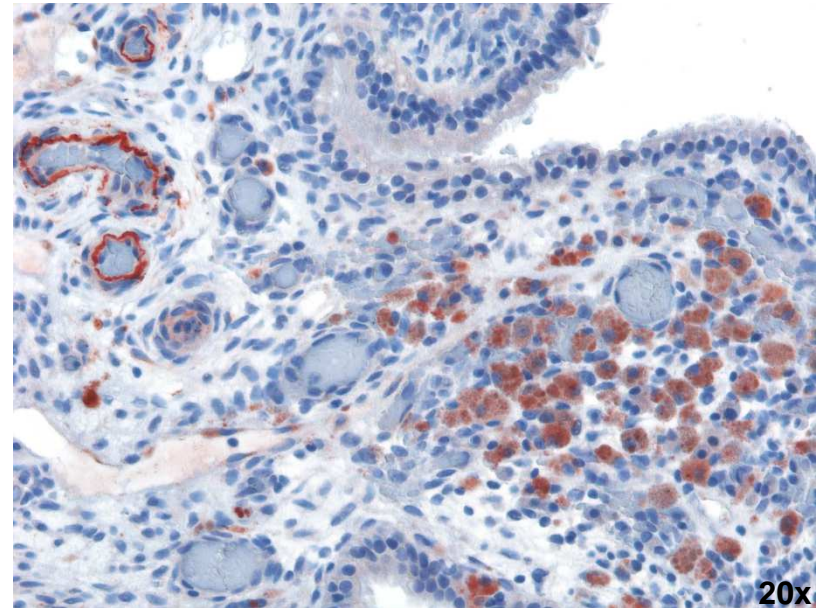
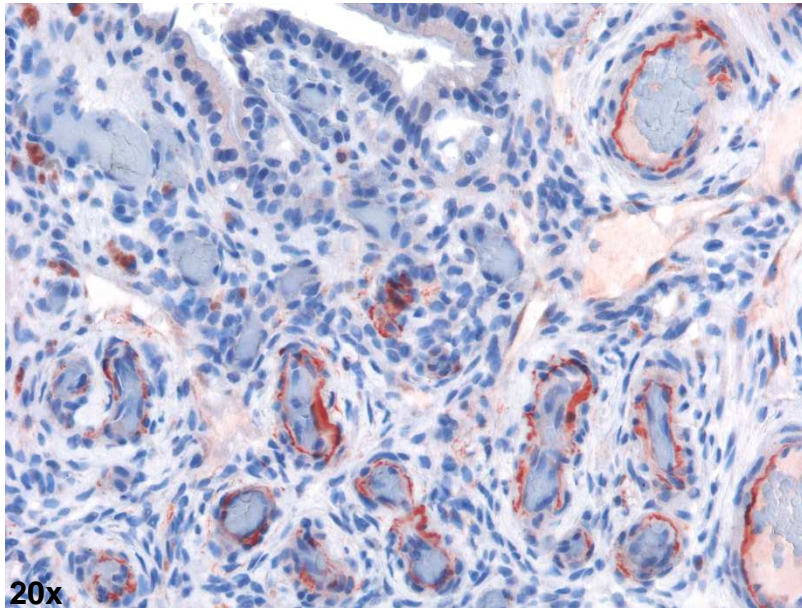
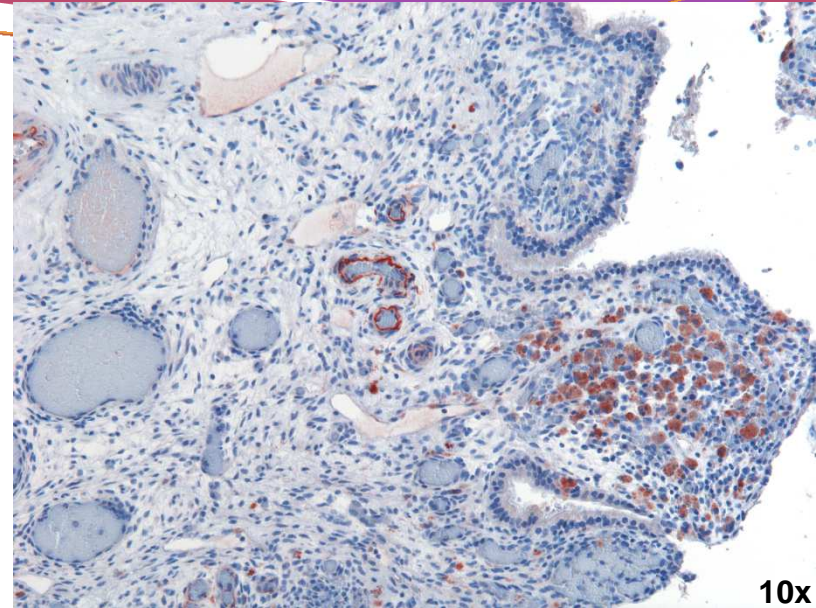
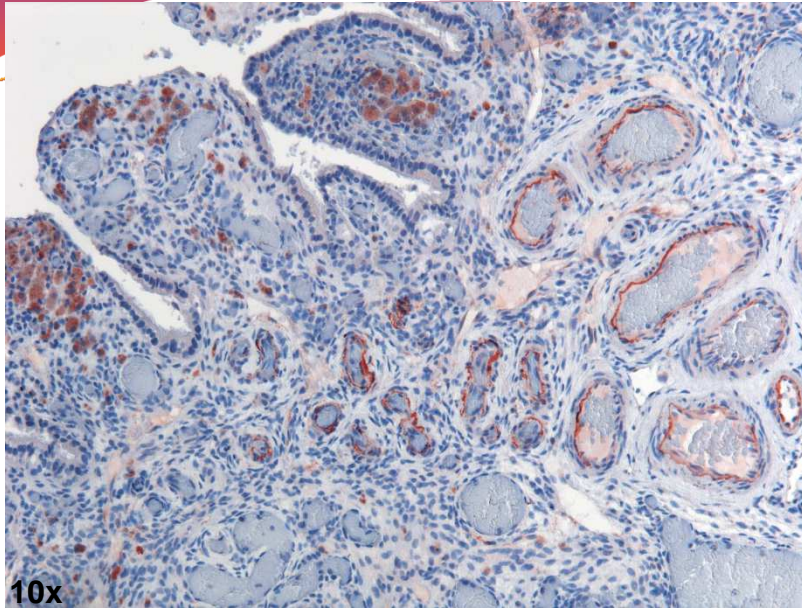


## CONCLUSIONS

- The complement component C3 is locally synthesized by ectopic endometrial tissue.
- Normal endometrial cells under pro-inflammatory stimuli are able to produce C3
- C3 deficient mice present less endometriotic lesions in a syngeneic EM mouse model



C3 is involved in the pathogenesis of EM



IHC analysis of **C3** in human EM cysts