



Alteration of NRF2 and Glutamate cysteine ligase expression contribute to lesions growth and fibrogenesis in ectopic endometriosis

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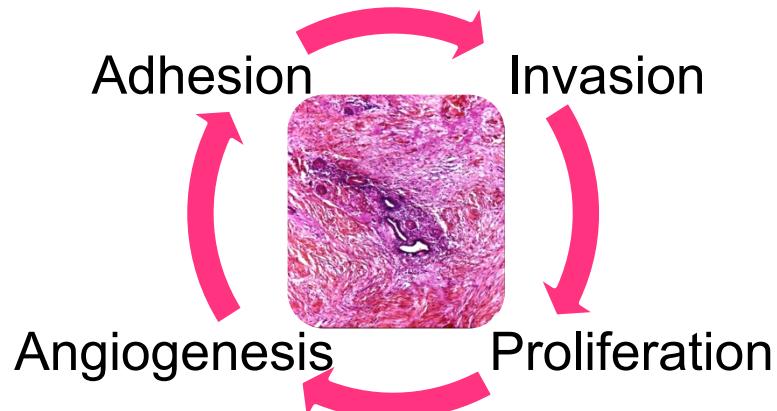
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National Institute for Medical Research | National Council for Scientific Research
Paris Descartes University

Introduction

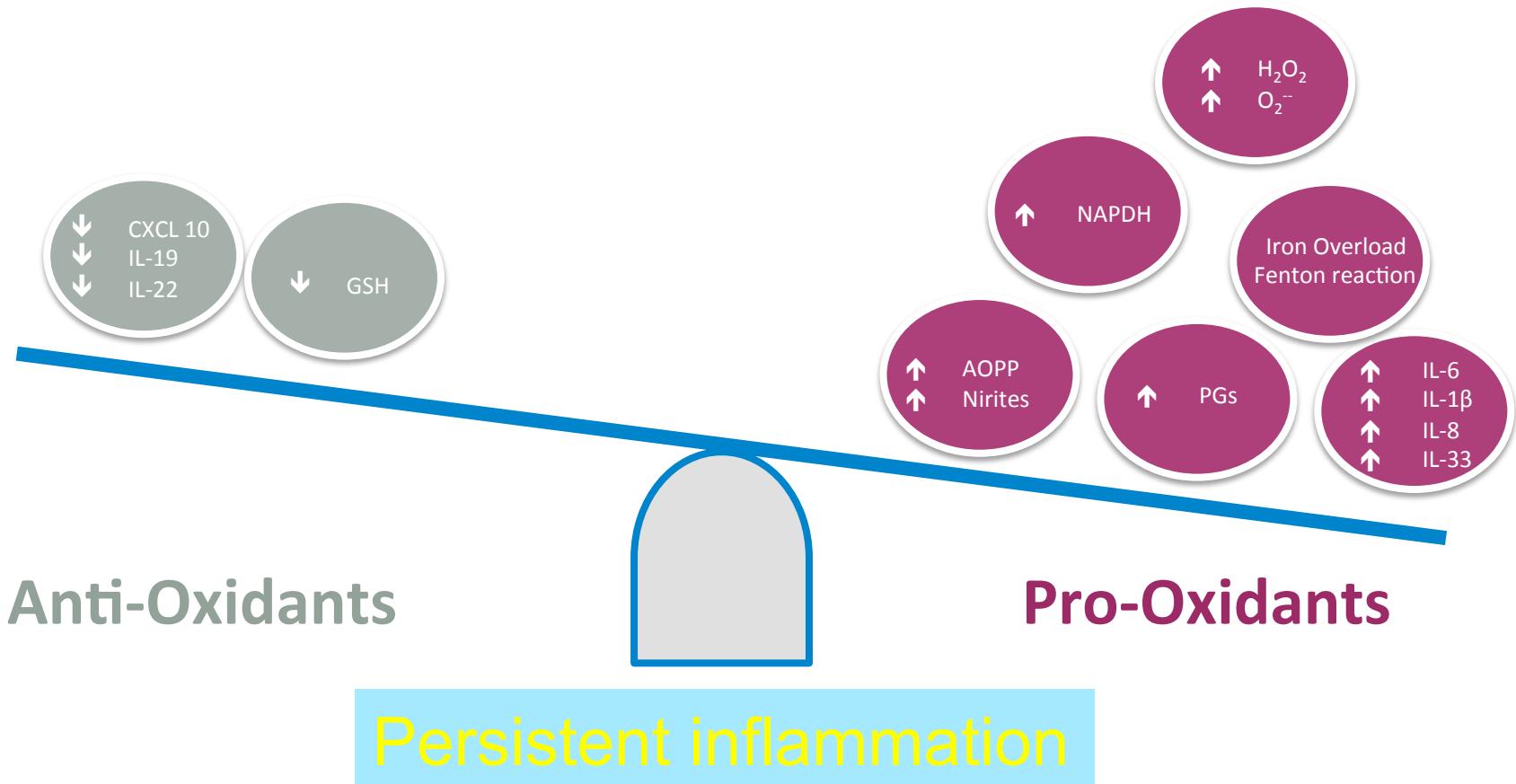
Endometriosis



Chronic
Inflammation
Fibrosis

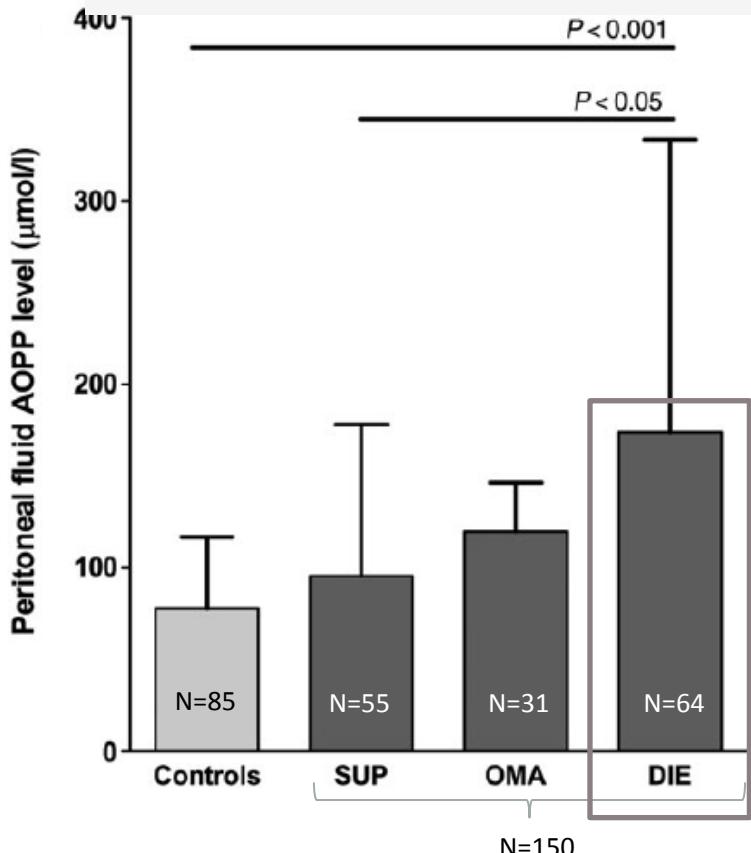
Burney RO and Giudice LC. Fertil Steril. 2012
De Ziegler et al. Lancet. 2010

Inflammatory response is linked to an altered balance of oxidative stress

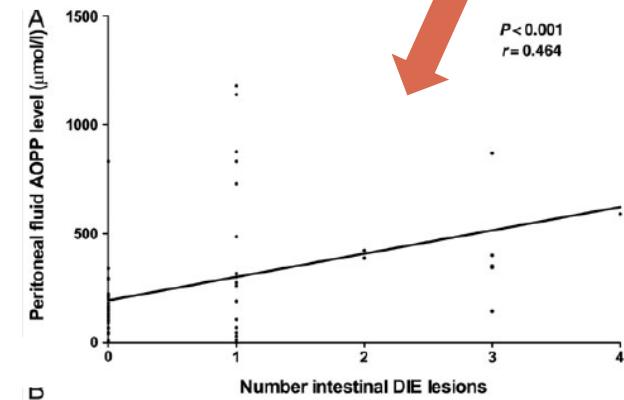
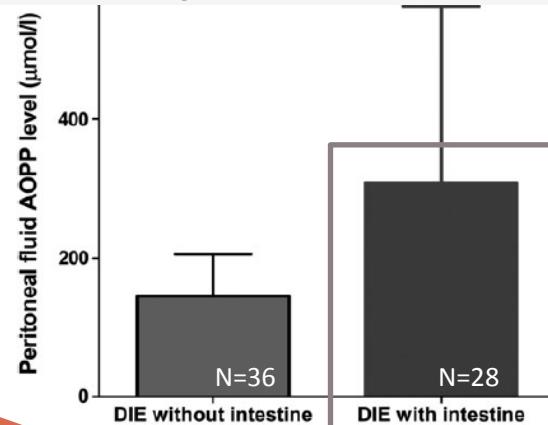


- IL, interleukin; CXCL, chemokine; GSH, glutathione;
- NADPH, nicotinamide adenine dinucleotide phosphate-oxidase;
- AOPP, advanced oxidation protein products; PGs, prostaglandins.

Oxidative stress is increased in endometriosis, especially in DIE



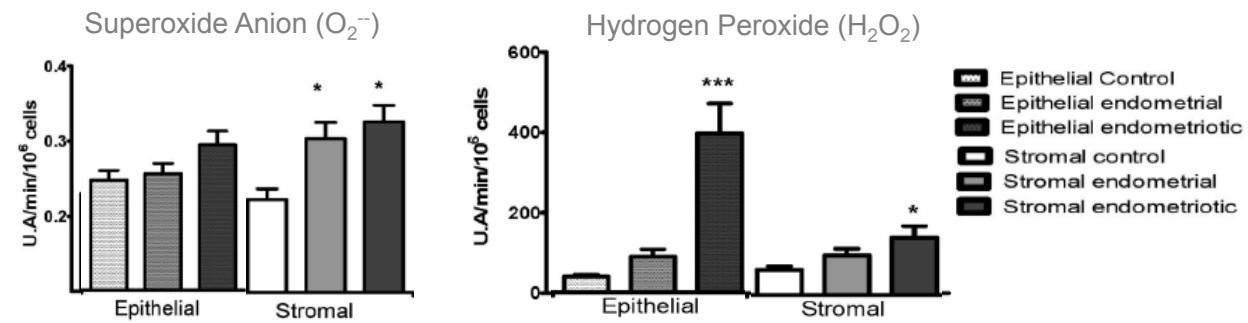
Oxidative stress is increased further in intestinal DIE



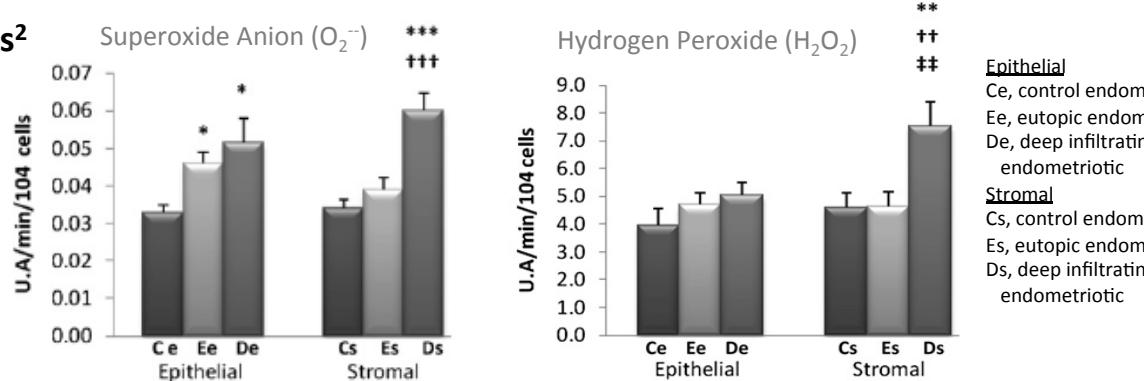
- DIE, deep infiltrating endometriosis; SUP, superficial lesion; OMA, endometrioma;
- AOPP, advanced oxidation protein products.
- Santulli P *et al.* Hum Reprod 2015; 30(1): 49–60.

Increased oxidative stress correlates with increased cellular proliferation

Endometrioma¹

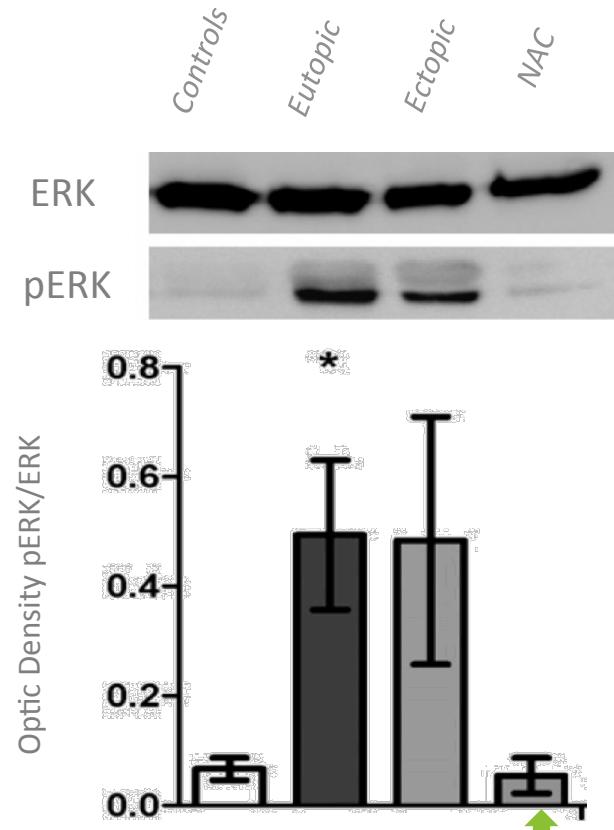
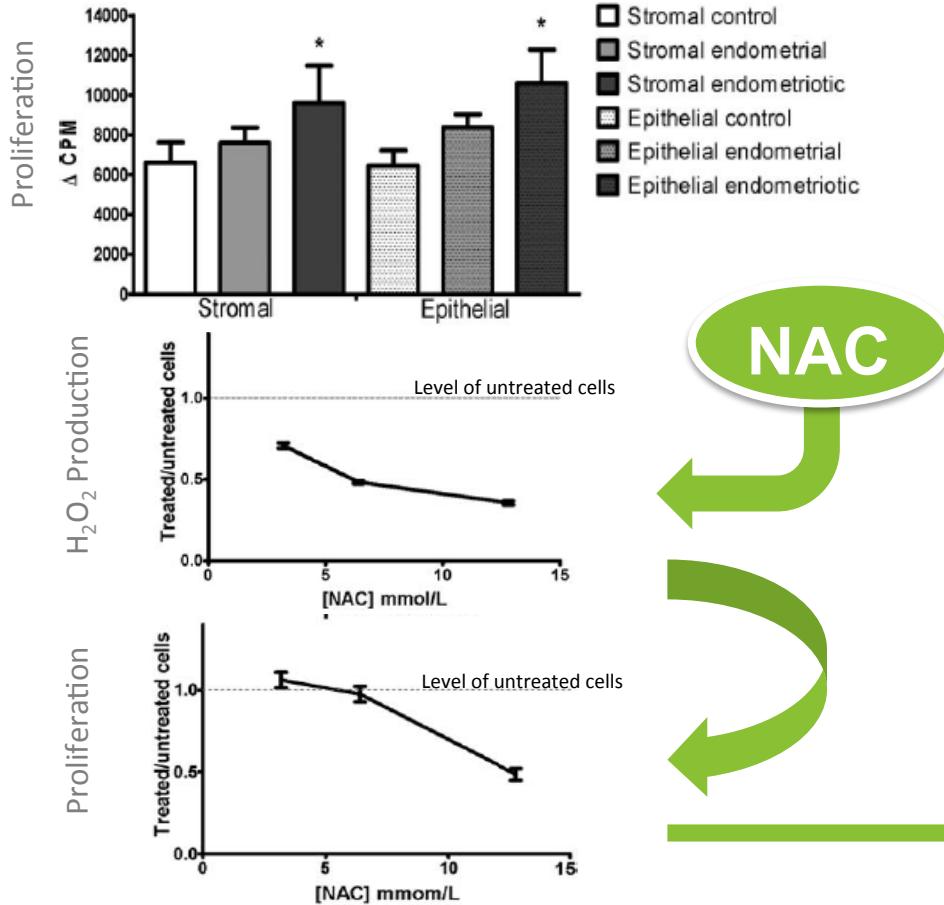


Deep infiltrating endometriosis²



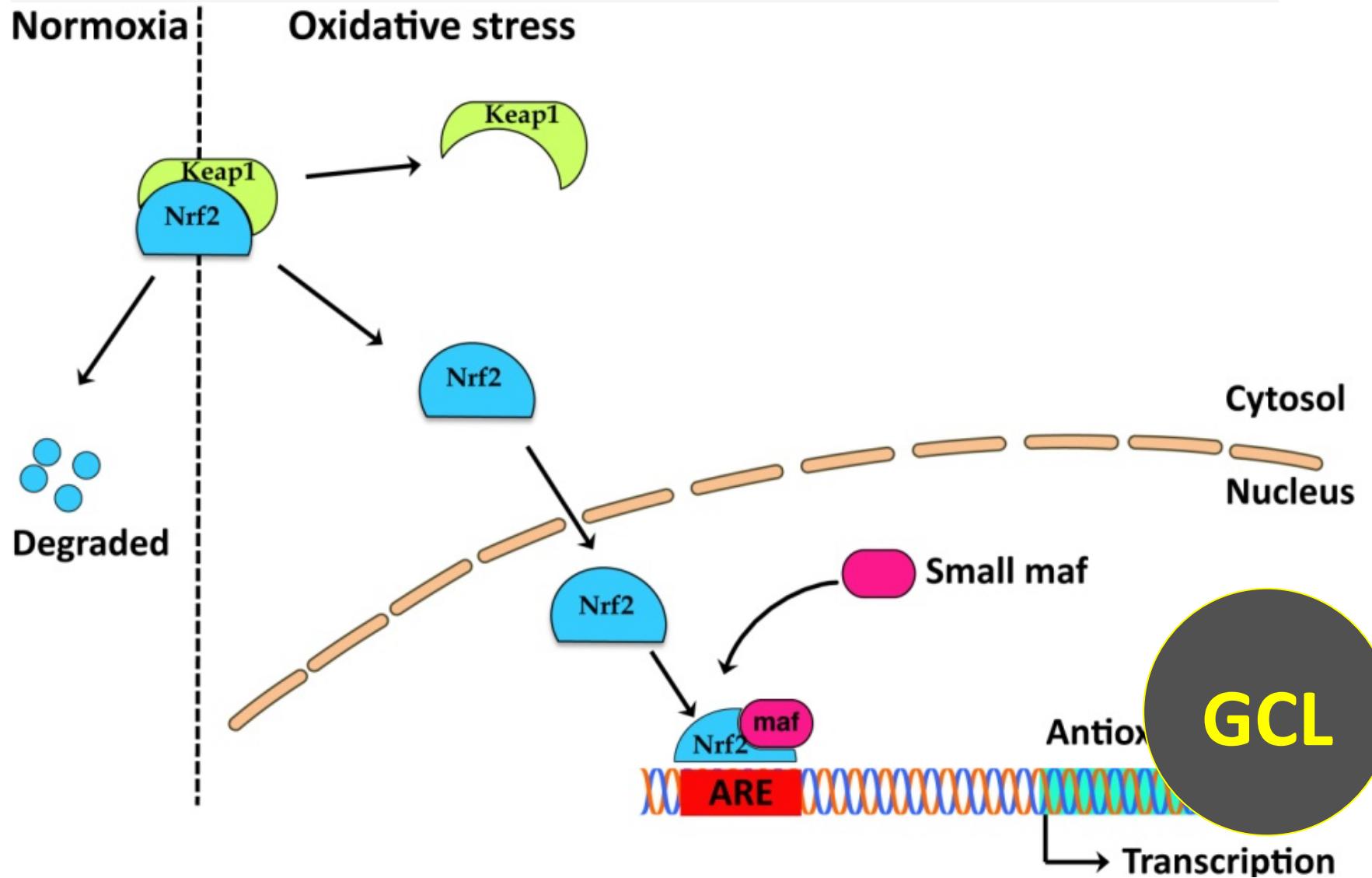
- 1. Ngô C et al. Am J Pathol 2009; 175(1): 225–234.
- 2. Leconte M et al. Am J Pathol 2011; 179(2): 880–889.

Increased oxidative stress correlates with increased cellular proliferation: Link to MAPK pathway



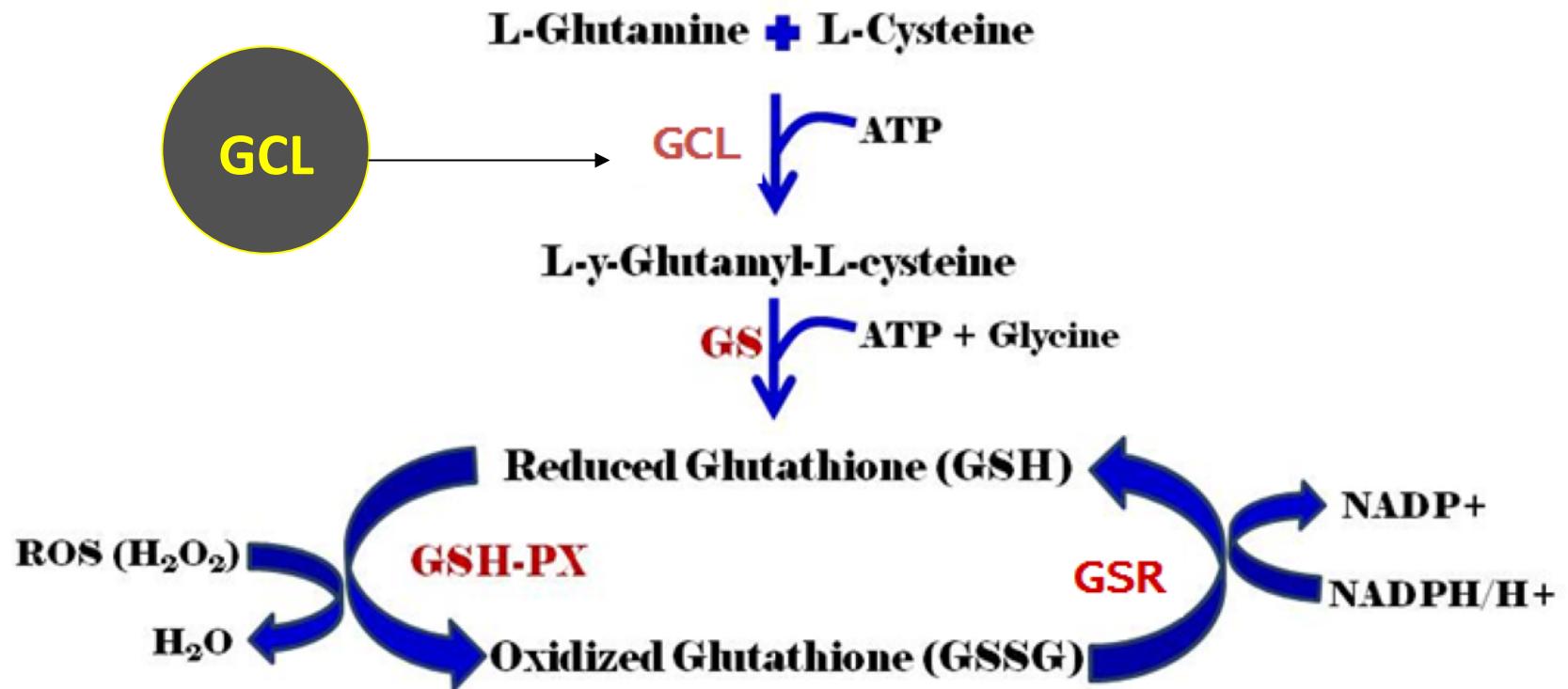
- NAC, N-acetyl-cysteine; ERK, Extracellular signal-regulated kinases; pERK, phosphorylated-ERK.
- Ngô C et al. Am J Pathol 2009; 175(1): 225–234.

NRF2 Pathway



Glutamate cystein ligase

First enzyme of Glutathion biosynthesis



The purpose of the present study...

- 1- Is to evaluate whether the NRF2 and GCL are differentially expressed in endometriosis
- 2 -to explore the consequence of a drop in NRF2 expression in a mouse model of endometriosis

Methods

January 2012

Tertiary accademic center

September 2014

Women Submitted to surgery

Controls

Endometriosis

Eutopic endometrium

Eutopic endometrium

Ectopic endometrium

Real Time PCR

Immunohisto-chemistry

Western Blot

Mouse Model

Patient baseline characteristics

	Patients with endometriosis (n= 31)	Controls (n= 30)	P value
Age, mean ± SD	33.9 ± 3.4	29.6 ± 4.2	0.005
BMI, mean ± SD	22.6 ± 2.9	24.8 ± 1.6	0.06
Parity, mean ± SD	0.3 ± 0.4	0.3 ± 0.3	0.89
Gravidity, mean ± SD	0.8 ± 0.3	1.5± 0.8	0.43
Cycle n (%)			
Hormonal treatment	20 (64.5%)	11 (36.6%)	
Proliferative phase	5 (16.1%)	9 (30.0%)	0.09
Secretory phase	6 (19.4%)	10 (33.3%)	
DIE ^a	18 (58.1 %)	-	
Total ASRM score, mean ± SD	36.2 ± 19.3	-	

Real Time PCR

Immunohisto-
chemistry

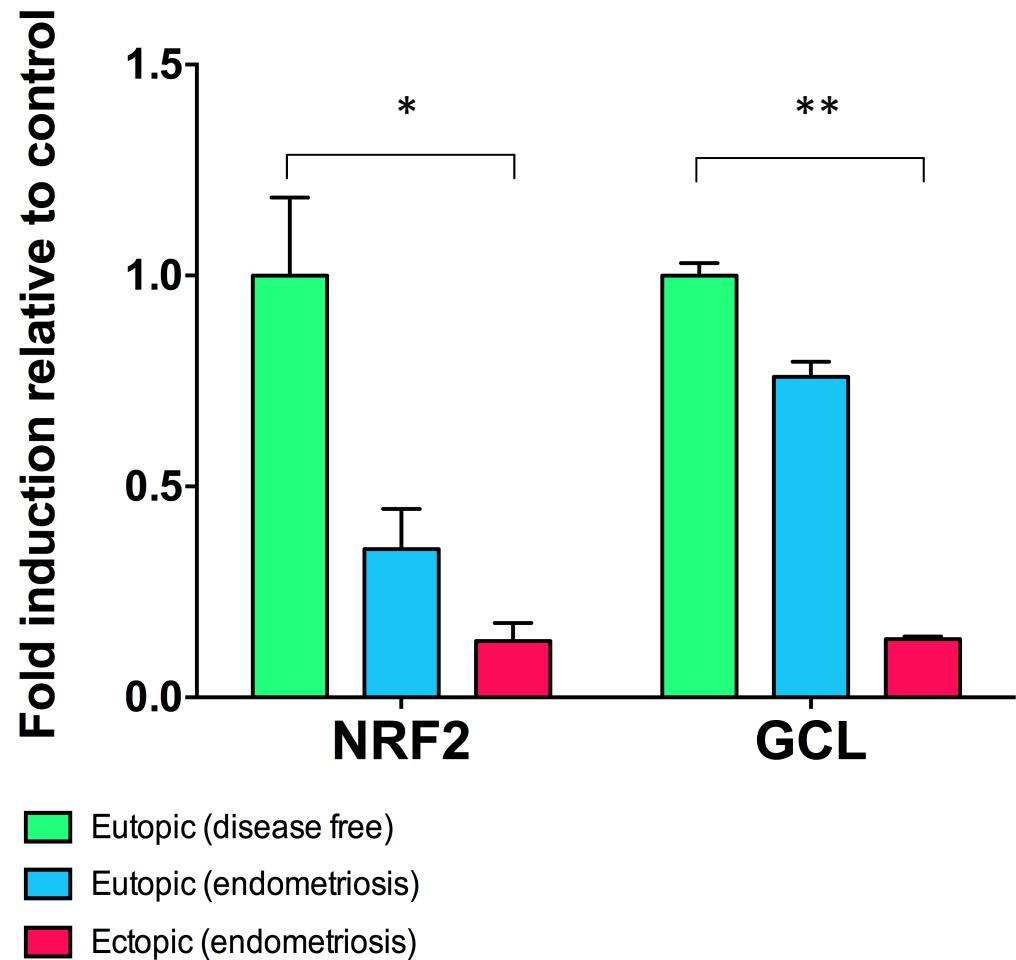
Western Blot

RT-qPCR NRF2 / GCL

16 patients with endometriosis (both eutopic and ectopic endometrium).
15 control patients matched

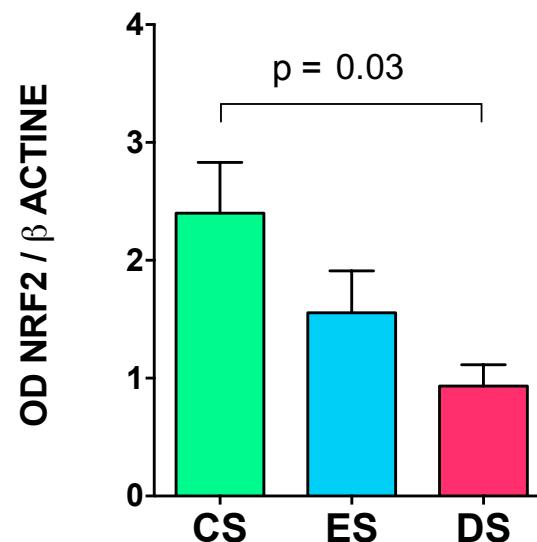
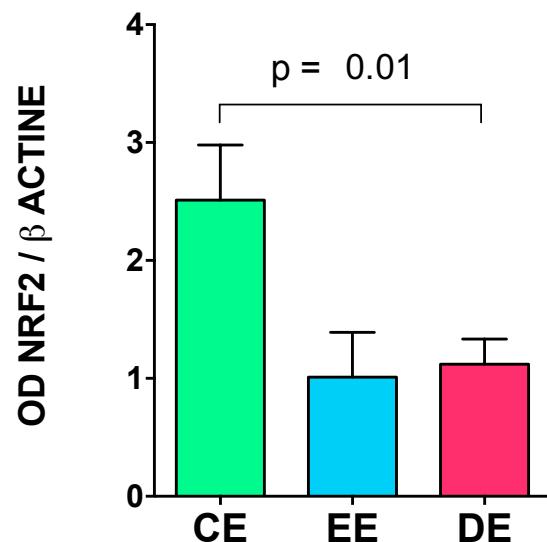
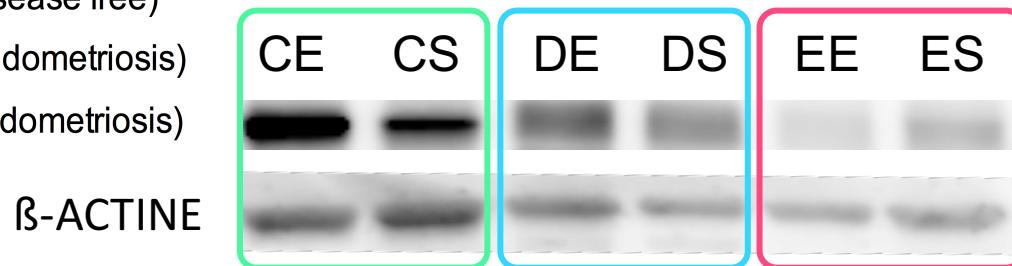
Real Time PCR

	Patients with endometriosis (n=16)	Controls (n=15)
Eutopic endometrium endometriosis free woman	-	15
Eutopic endometrium endometriosis affected woman	4	-
Ectopic endometrium endometriosis affected woman	9	-
Endometrioma	6	-
DIE	3	-



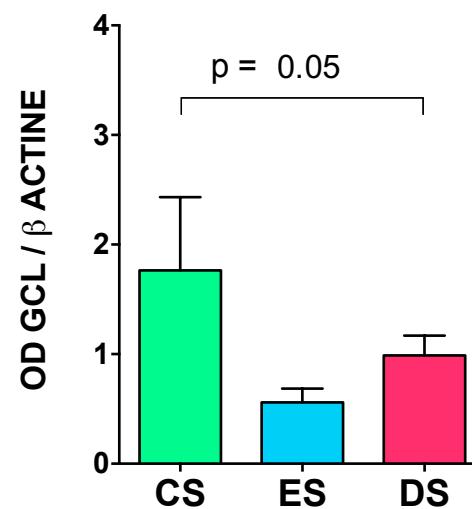
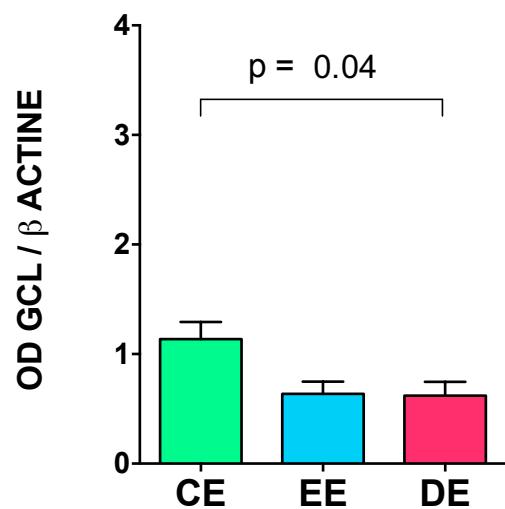
Western Blot – NRF2

- Eutopic (disease free)
- Eutopic (endometriosis)
- Ectopic (endometriosis)



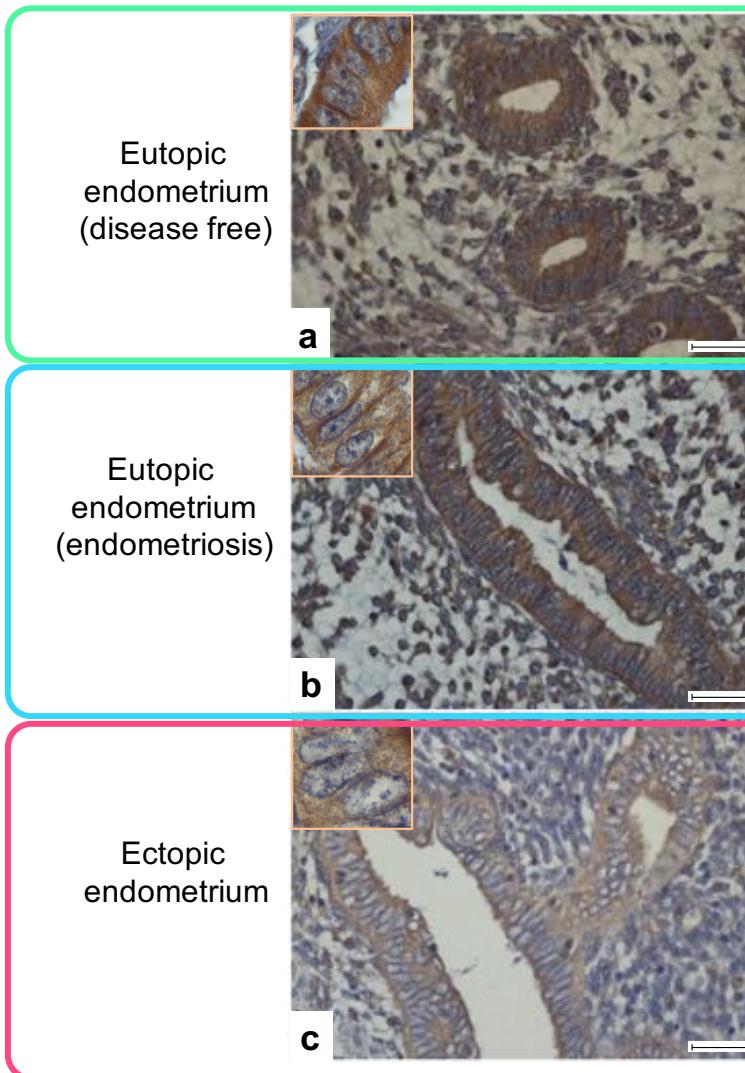
Western Blot – GCL

- [Green square] Eutopic (disease free)
- [Blue square] Eutopic (endometriosis)
- [Red square] Ectopic (endometriosis)

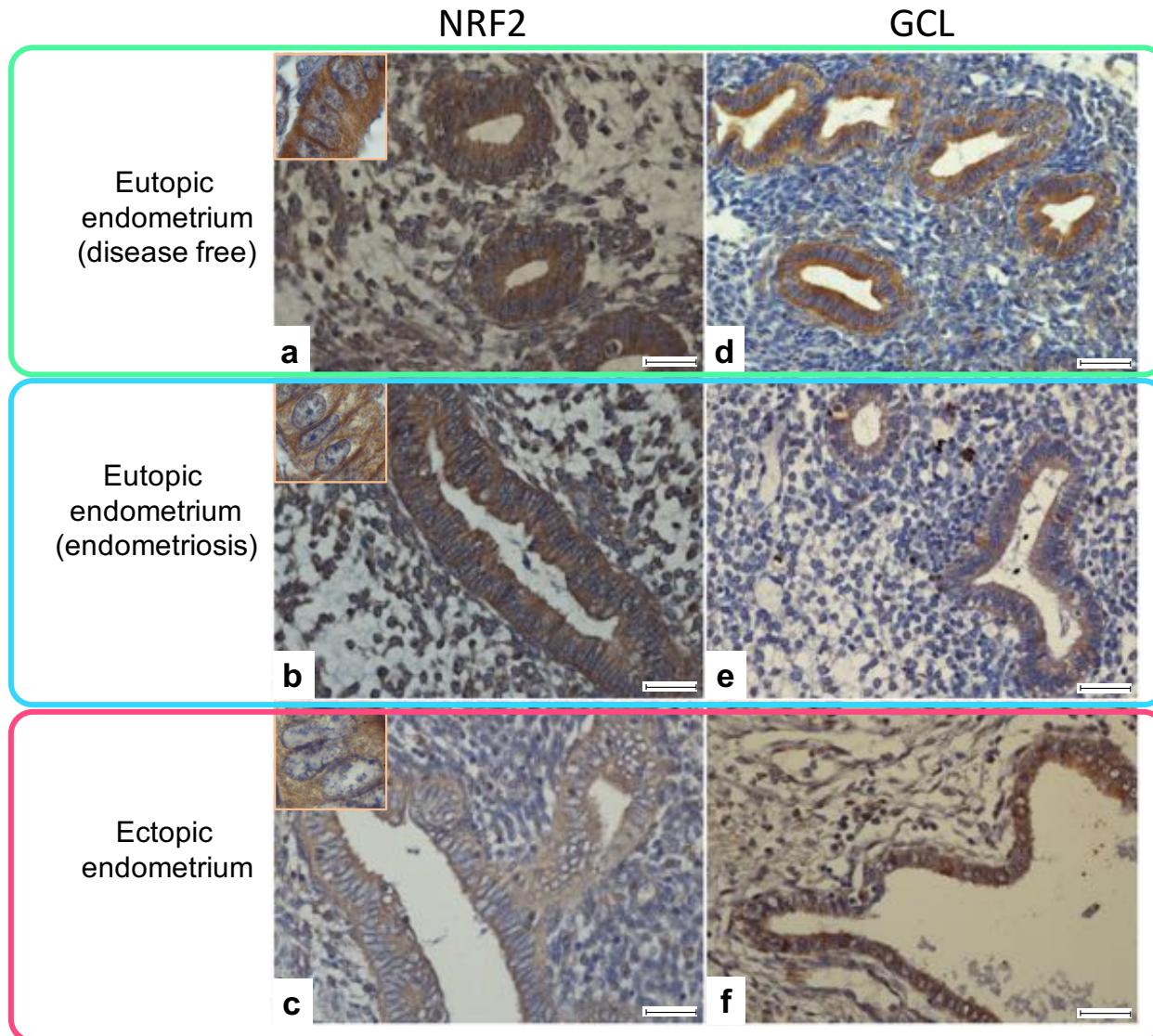


Immunostaining

NRF2

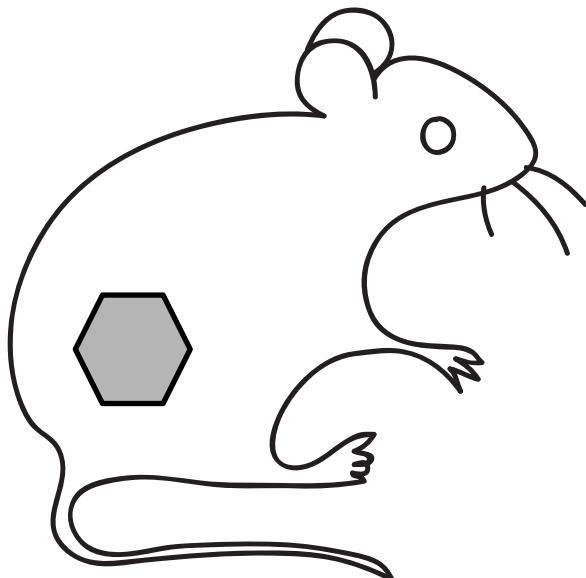


Immunostaining

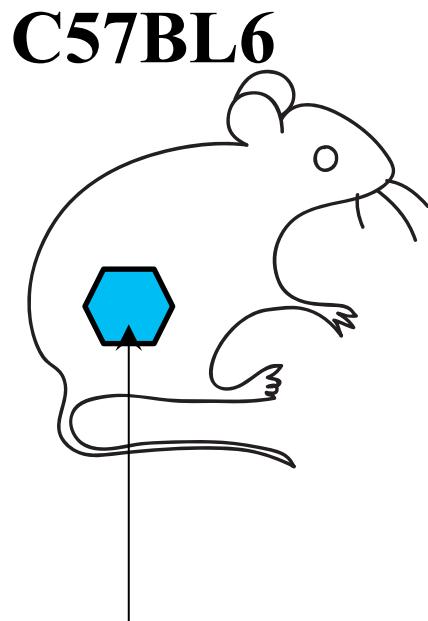


Mouse Model

C57BL6



Mouse Model

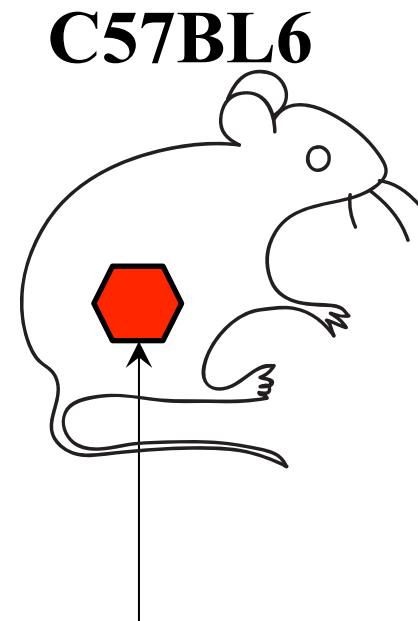


Controls
NRF2 +/+

N = 10



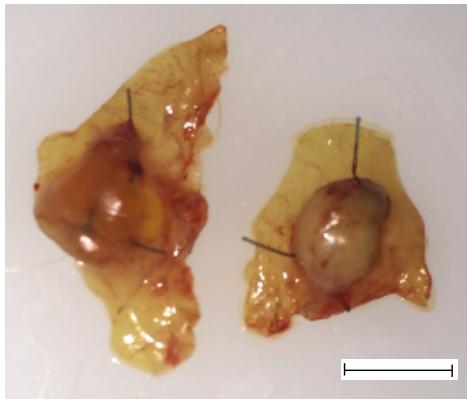
donor horn
C57BL6
(peritoneal implant)



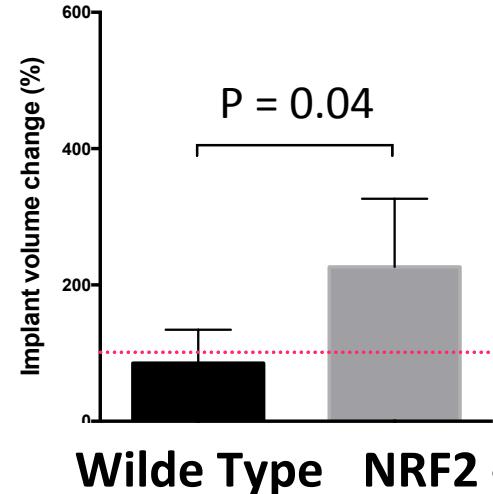
Cases
NRF2 -/-

N = 10

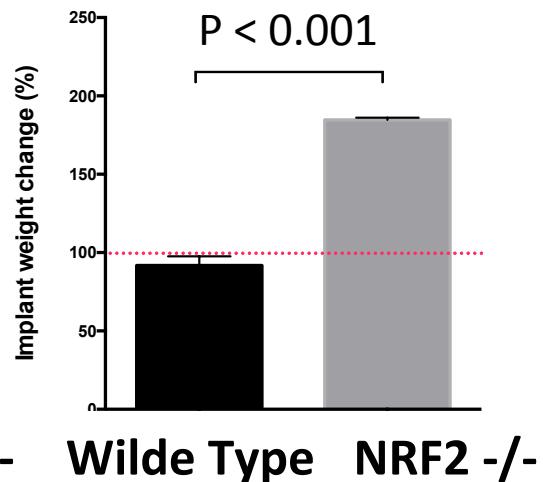
Mouse Model



Wilde Type NRF2 -/-

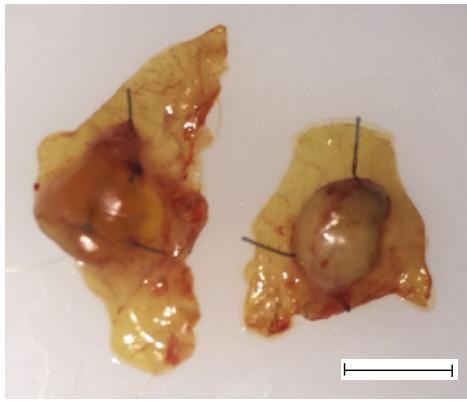


Wilde Type NRF2 -/-

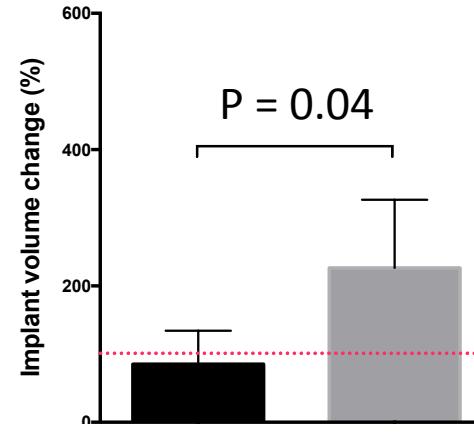


Wilde Type NRF2 -/-

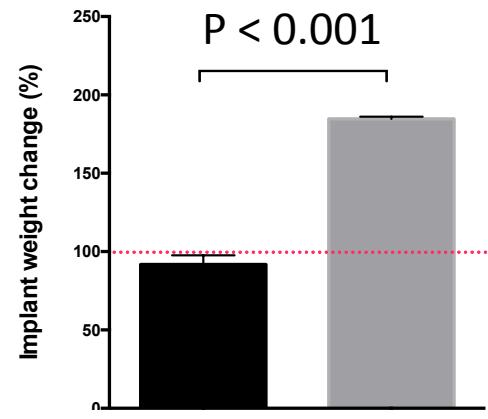
Mouse Model



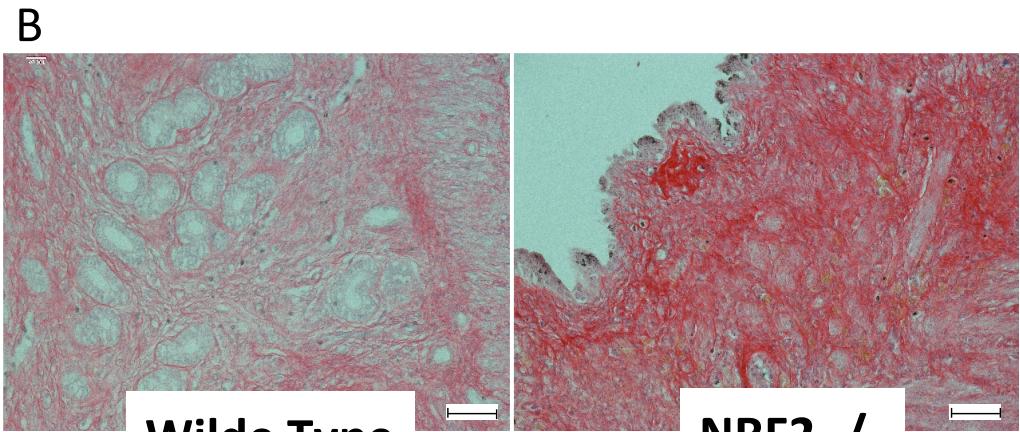
Wilde Type NRF2 -/-



Wilde Type NRF2 -/-

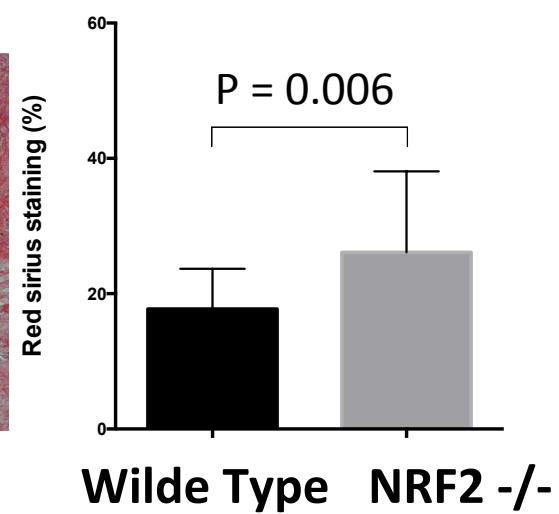


Wilde Type NRF2 -/-



Wilde Type

NRF2 -/-



Wilde Type NRF2 -/-



Conclusion



- First study evaluating
 - **NRF2 / GCL** pathway in endometriosis:
 - Which were **decreased** at both mRNA and proteic level in ectopic lesions
 - Our Mouse model confirms the main role of NRF2 in fibrogenesis mediated by oxydative stress .
 - This mouse model may help to test new molecules



Gynecology

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G Pierre, M Even, MC Lamau,
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C Maignien, FX Aubriot

Intestinal surgery

B Dousset, S Gaujoux, M Leconte

Radiology

AE Millischer, L Maitrot

Laboratory: *Genetic*

D Vaiman, F Mondon, S Barbaux

Laboratory: *Immunology*

F Batteux, S Chouzenoux
C Nicco, C Chéreau, B Weill

Laboratory: *Reproductive biology*

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JM Kuntzman, C Chalas

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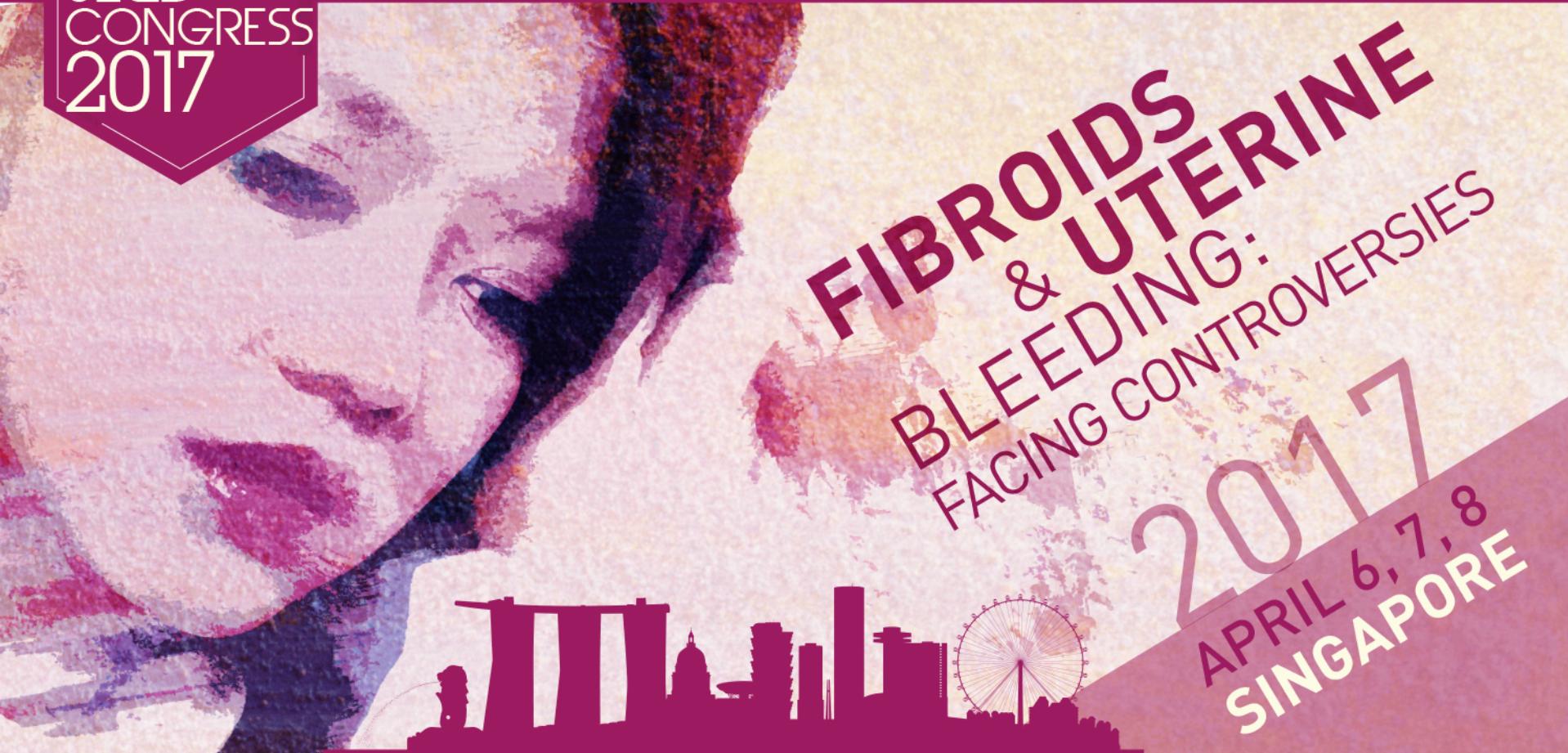
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A. Gompel, Professor and Head, Medical Gynecological unit,

C. Chapron, Professor and Chair, Gynecology Obstetrics II and Reproductive Medicine

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