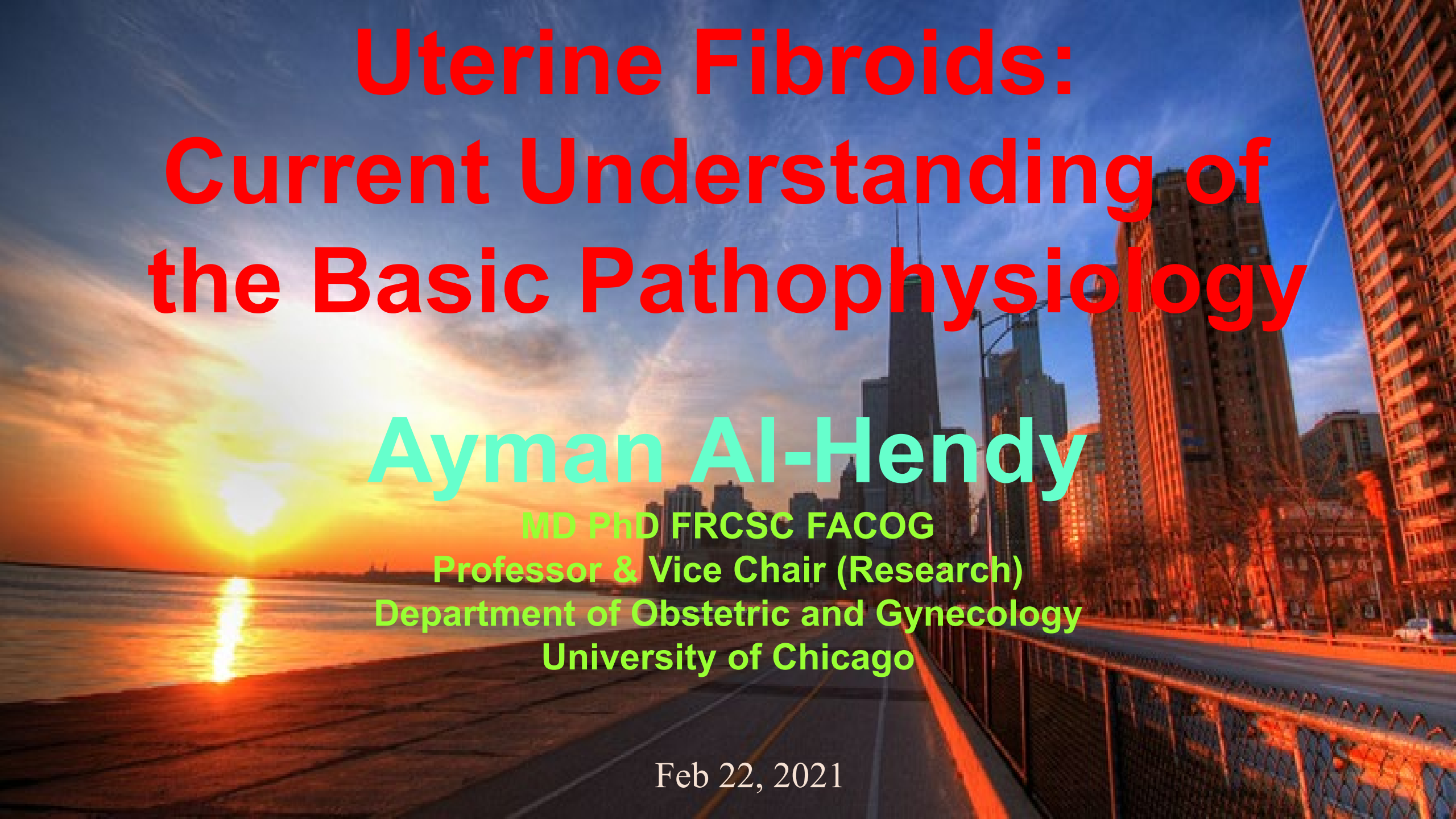

Uterine Fibroids: Current Understanding of the Basic Pathophysiology

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Feb 22, 2021

Disclosures

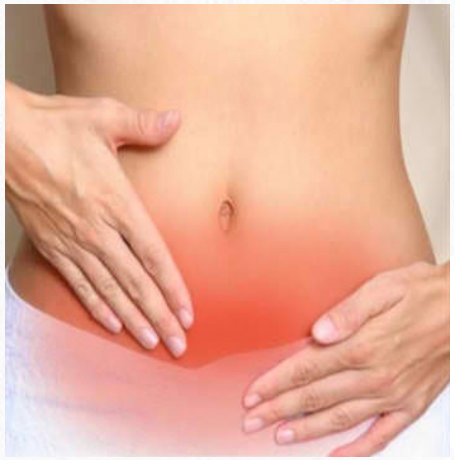
- Receiving research/consultation fund from the following entities
  - Abb-Vie
  - AstraZeneca
  - Bayer
  - MDStemCells Inc.
  - Myovant
  - NIH
  - OBS-EVA
  - Crila Inc.
  - USDA

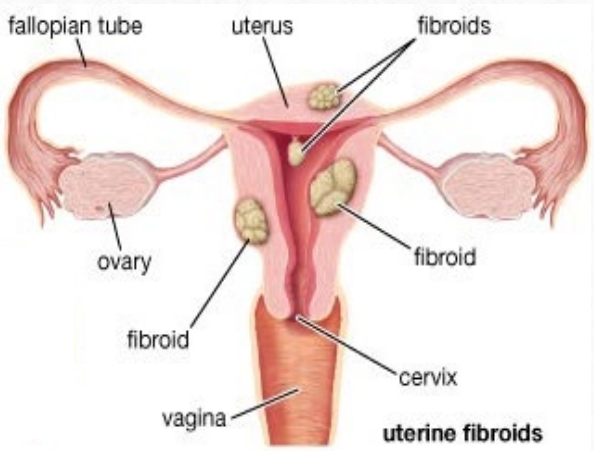
Objectives

- Introduction
- Classification
- Risk factors (who gets uterine fibroids?)
- Pathophysiology
- Knowledge Gaps/Unmet Needs/Future Directions

Uterine Fibroids

- Uterine leiomyomas (UL; fibroids) are ***benign*** smooth muscle tumors originating from the myometrium

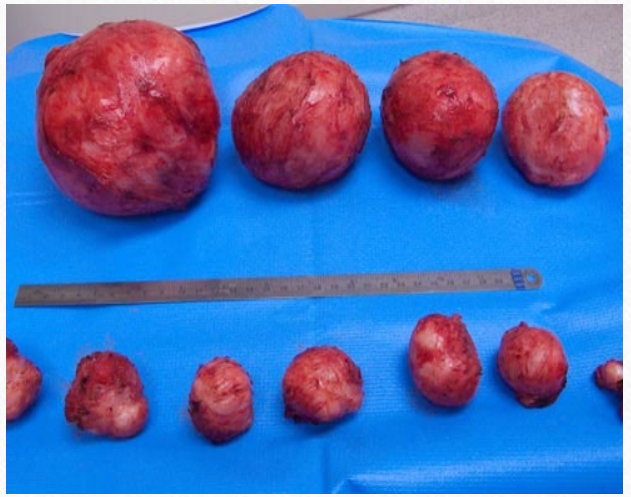


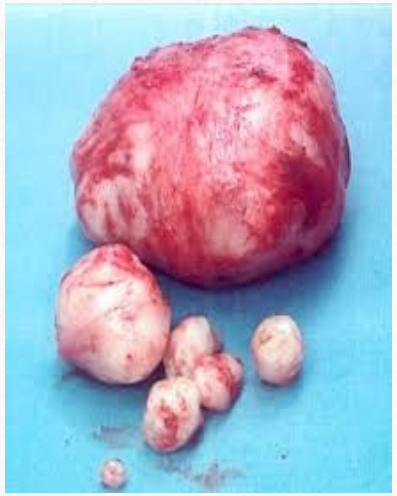


- Most common human tumor:
  - tumors occur in 77% women
  - clinically apparent in 50% by age 45

- Significant source of morbidity:
  - leading indicator of hysterectomy
  - major cause of gynecologic dysfunction:
    - menometrorrhagia and anemia
    - pelvic pressure/bulk symptoms
    - infertility, recurrent miscarriage
    - preterm labor

- Range of clinical disease extraordinary: symptomatic lesions can routinely range from 5 mm-25 cm in size





Patel et al. (2014) *Fertil Steril*, 2014
Moravek et al. *Hum Reprod Update*, 2015

Manifestations of Uterine Fibroids

UF can lead to[1]:

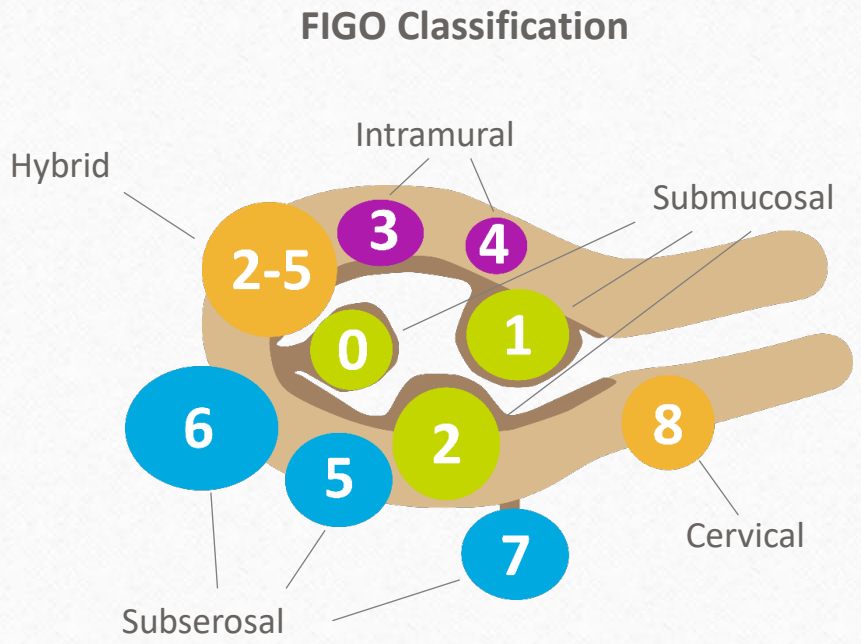
- Heavy menstrual bleeding
- Abdominal distention or distortion
- Anemia and fatigue
- Pressure symptoms and pelvic pain
- Dysmenorrhea
- Infertility/recurrent miscarriage

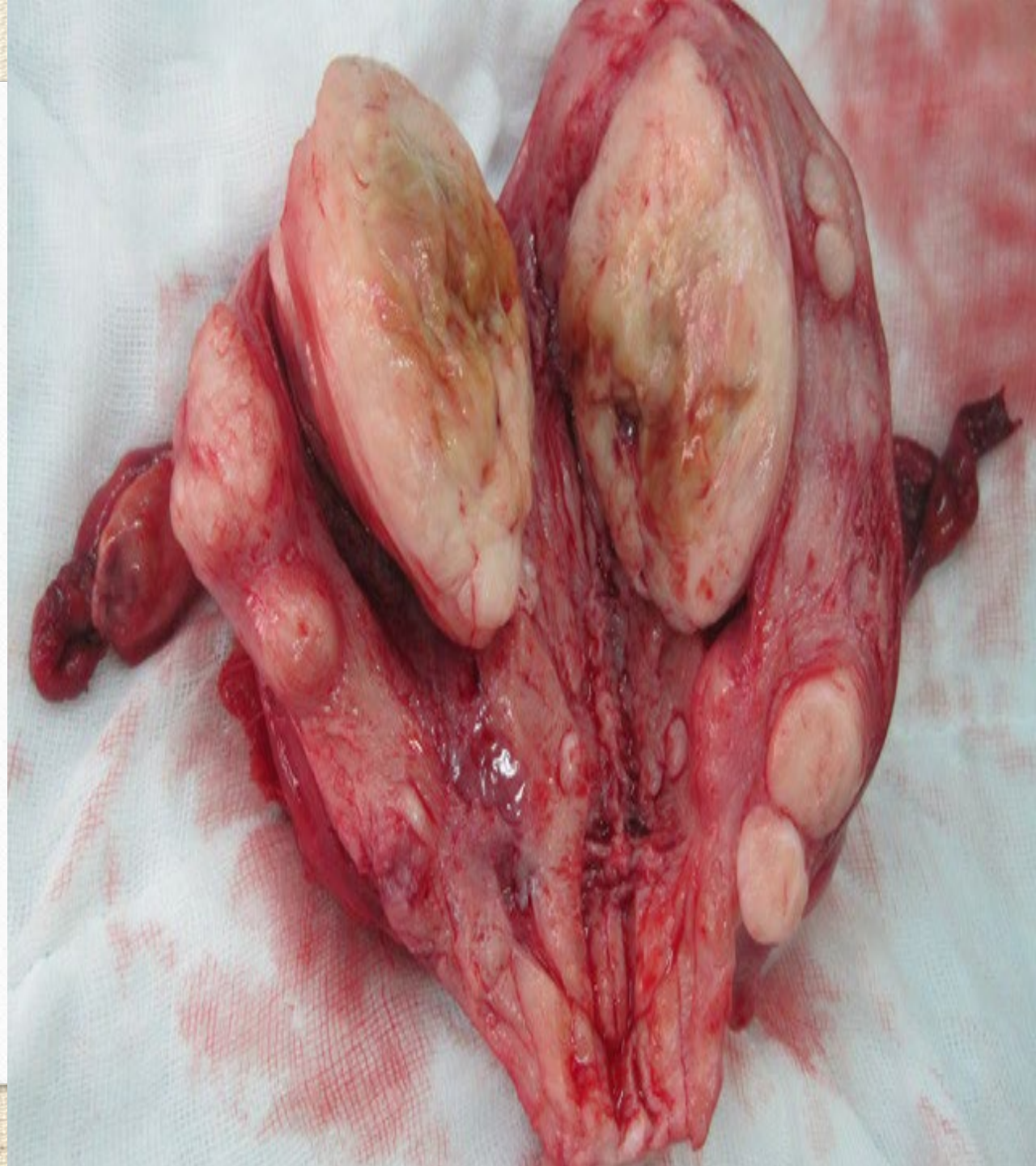
UF has a significant impact on quality of life (QoL) and is the leading indication for hysterectomy in the United States[2-4]

1. Fibroid Treatment Collective website. https://fibroids.com/fibroid-symptoms/. Accessed April 10, 2020. 2. Zimmermann A et al. BMC Womens Health. 2012;12:6. 3. Ghant MS et al. J Psychosom Res. 2015;78(5):499-503. 4. Soliman AM et al. Curr Med Res Opin. 2017;33(11):1971-1978.

Classification

- Most fibroid start as intramural then towards serosa (subserosal), or towards mucosa (submucosal).
- Submucosal most symptomatic → intramural → subserosal



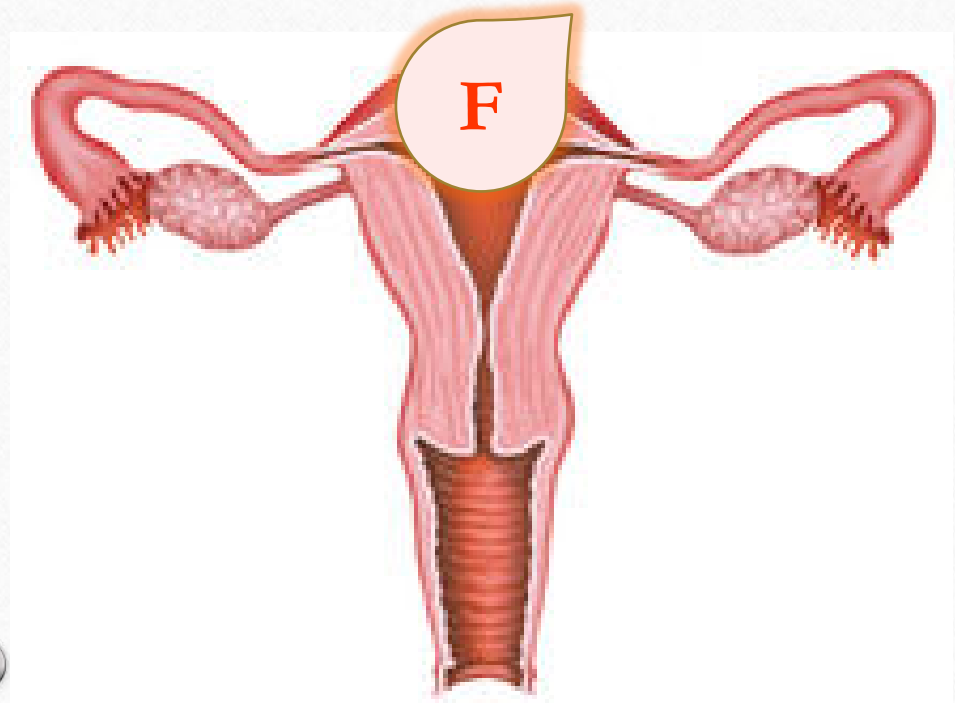


Pritts EA. Obstet Gynecol Surv 2001;56(8):483–491.

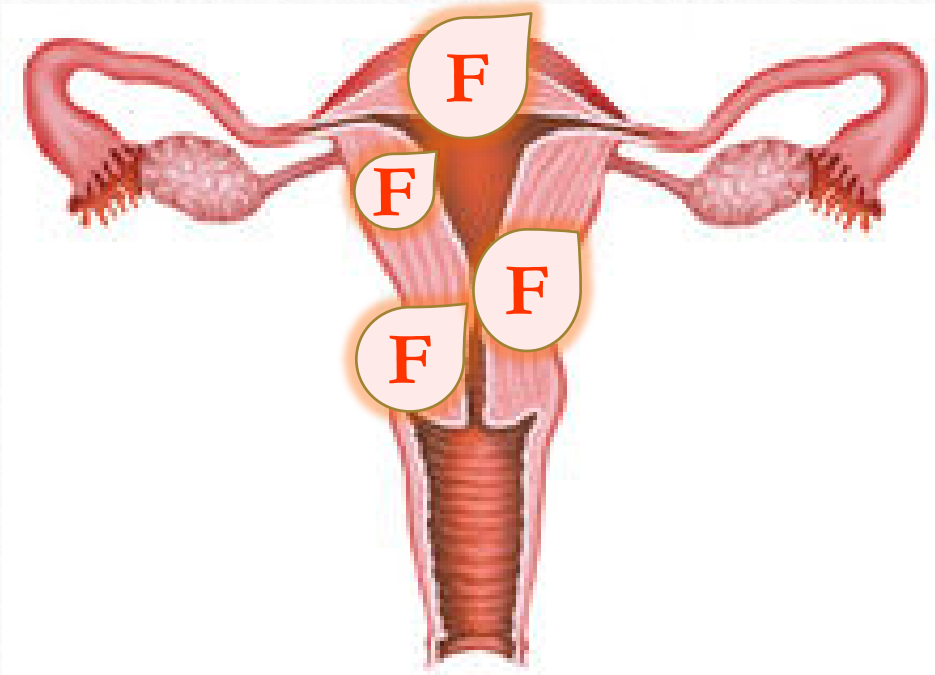
Location…Location…Location…Not in Uterine Fibroids?

We need better classification system….

Lots of Symptoms



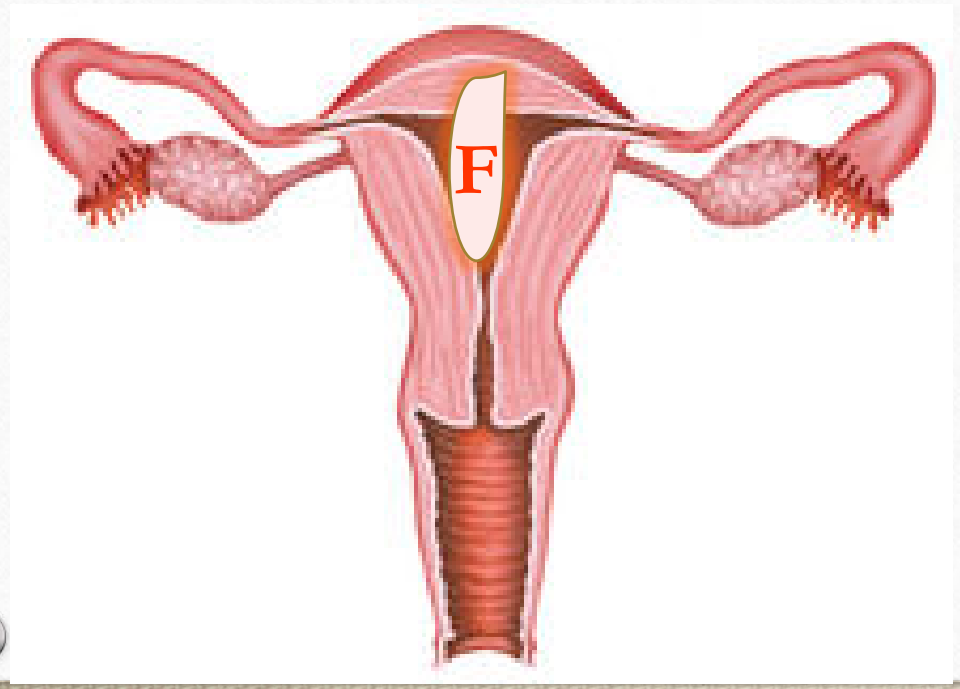
No/Minimal symptoms



Fibroid Size/Number vs. Symptoms Severity…Poor Correlation!

We need better classification system….

Lots of Symptoms



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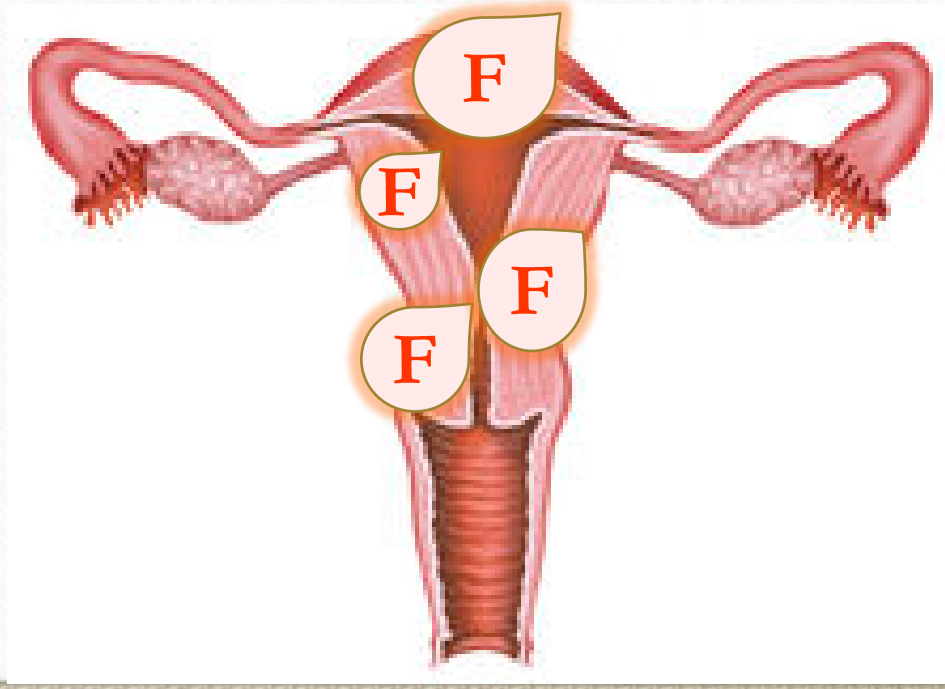
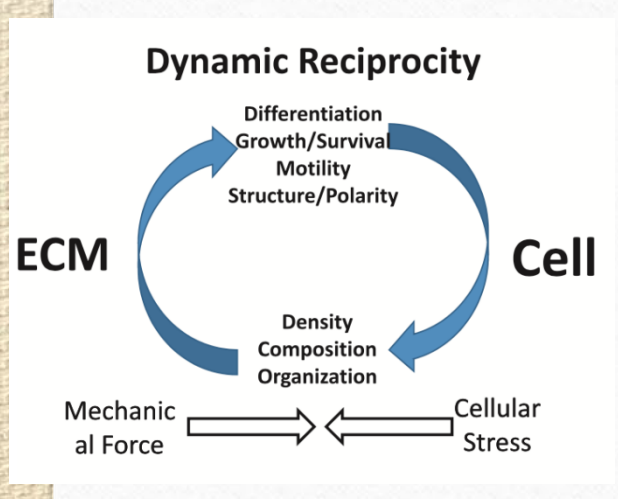


Extracellular matrix (ECM) & Uterine Fibroids

- Extracellular matrix accumulation is a critical event in producing the rigid structure of UF, and ECM stiffness is thought to be a cause of abnormal bleeding and pelvic pain or pressure

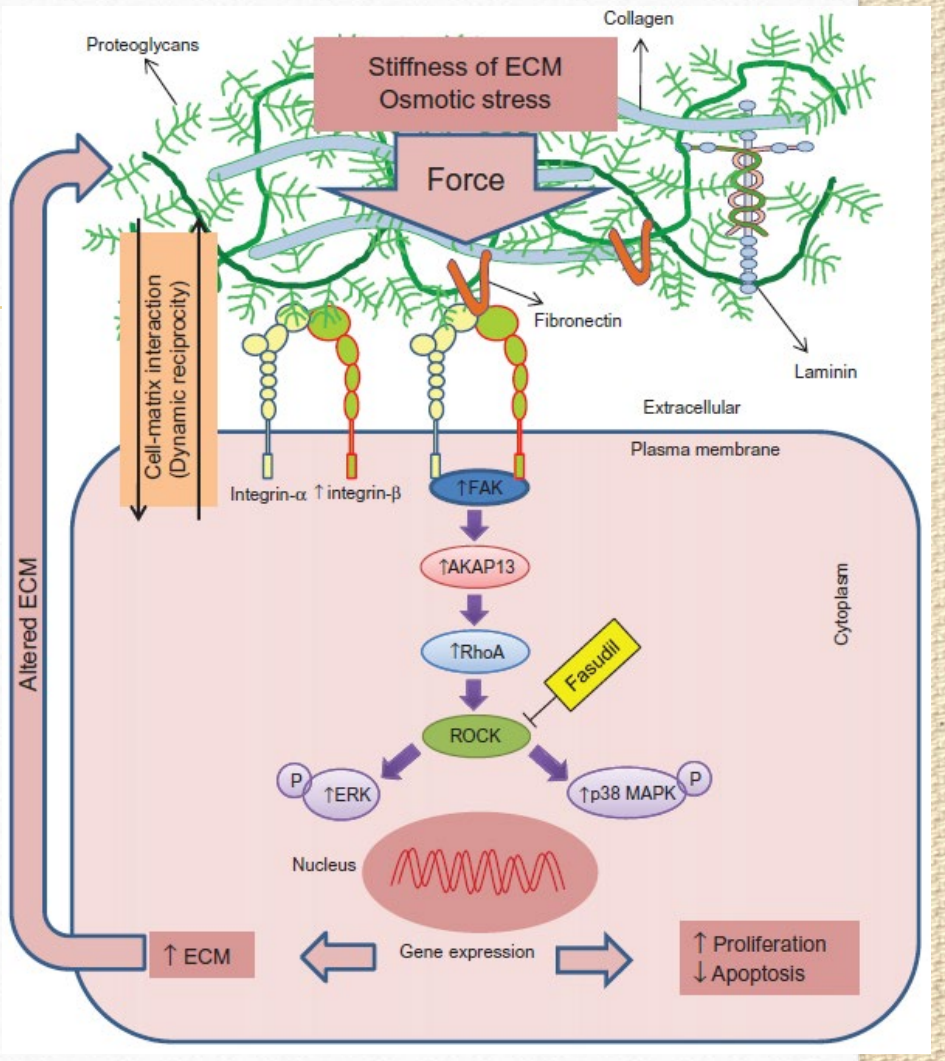
Uterine fibroid expresses a wide variety of ECM components, including collagens, fibronectin, laminins, proteoglycans and integrins as well as metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs)



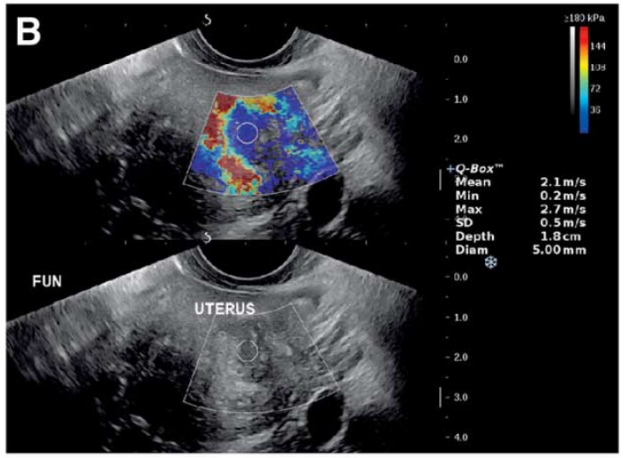
Thorne et al., 2014

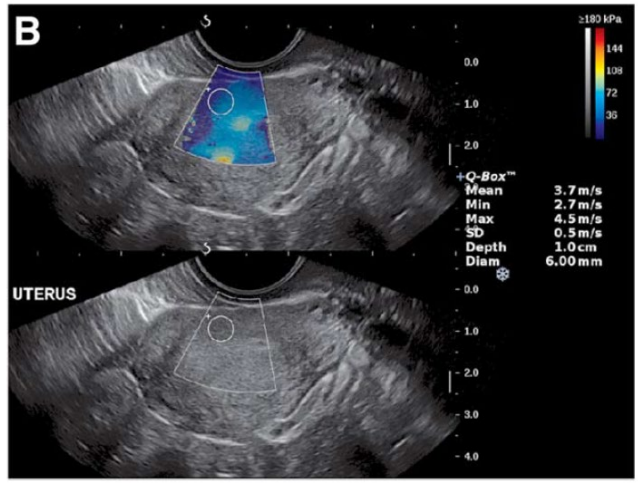
ECM proteins can induce mechanotransduction

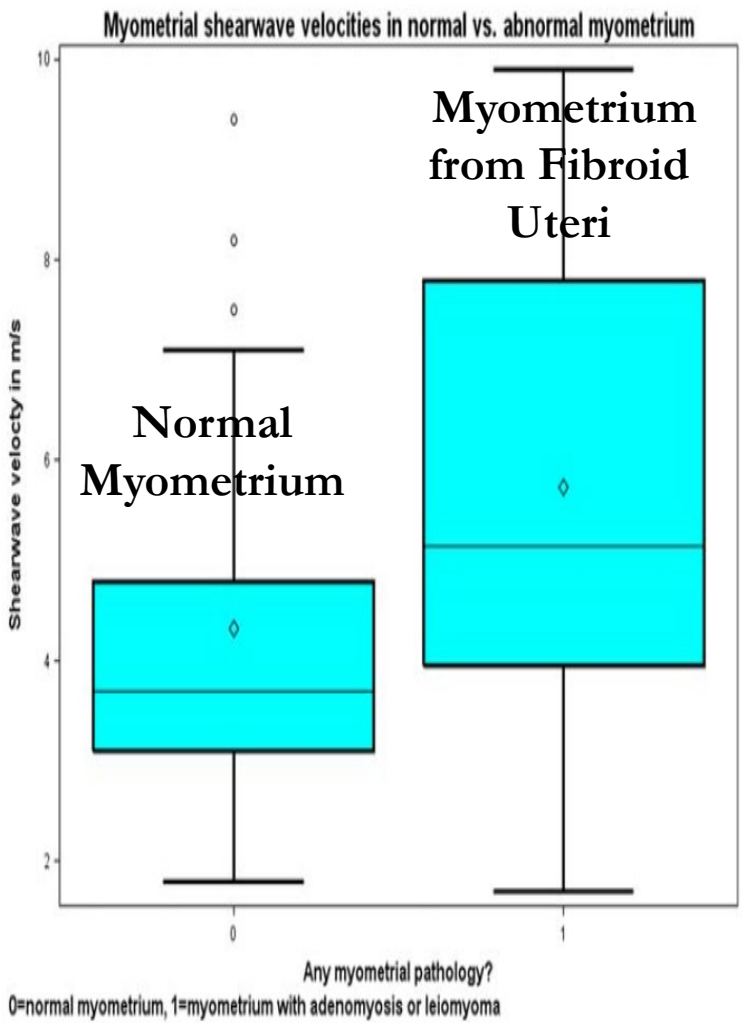
Cells sense their environment and translate mechanical stress into biochemical signals, thus activating pleiotropic intracellular signaling cascades such as the integrin-Rho/p38 MAPK/ERK pathways



Islam, M. S., et al. (2018). "Extracellular matrix in uterine leiomyoma pathogenesis: a potential target for future therapeutics." Hum Reprod Update10.1093/humupd/dmx032 24(1): 59-85







Transvaginal Ultrasound Shear Wave Elastography for the Evaluation of Benign Uterine Pathologies

2019

A Prospective Pilot Study

Man Zhang, MD, PhD, Ashish P. Wasnik, MD, William R. Masch, MD, Jonathan M. Rubin, MD, PhD, Ruth C. Carlos, MD, MS, Elisabeth H. Quint, MD, Katherine E. Maturen, MD, MS

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Obstetrics and Gynecology International
Volume 2014, Article ID 783289, 12 pages
http://dx.doi.org/10.1155/2014/783289

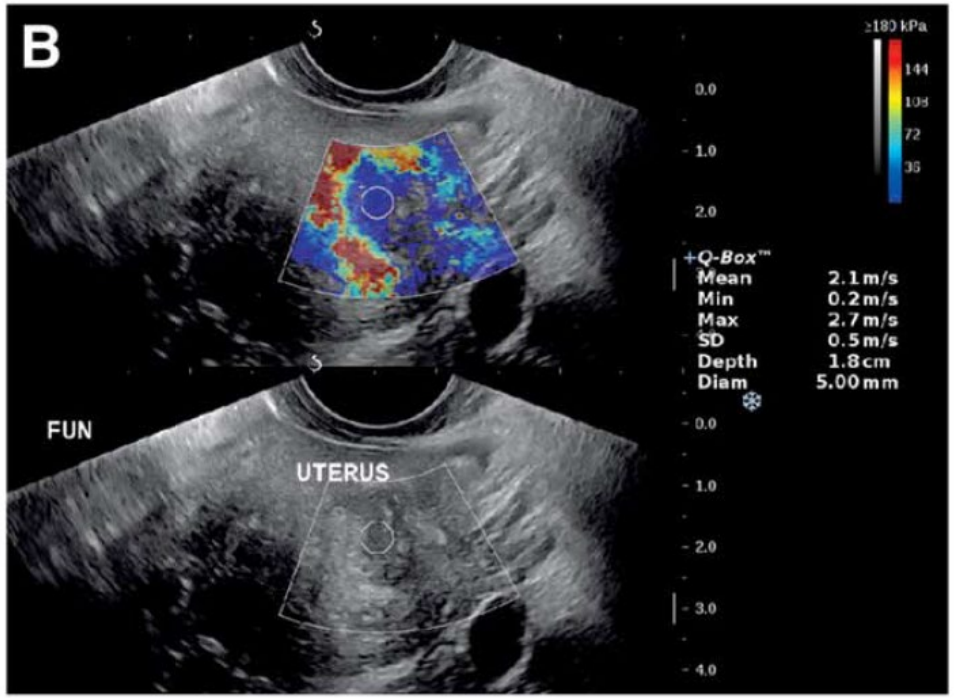
Review Article

The Extracellular Matrix Contributes to Mechanotransduction in Uterine Fibroids

Phyllis C. Leppert,[1] Friederike L. Jayes,[1] and James H. Segars[2]

Proposal for New Uterine Fibroid Classification based on Shear Wave Elastography

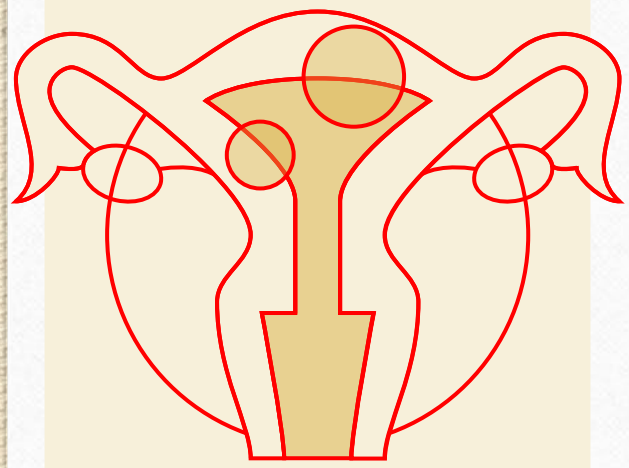
- AL1: Soft
- AL2: Pliable
- AL3: Firm
- AL4: Hard
- Composite SWE score/Uterus
- Correlation to symptoms severity?
- Triage for appropriate therapy (medical, UAE, surgical….etc.)
- Prediction of progression in asymptomatic women
- Targeted preventative strategies



UEFA Champions May 2018

Who develops Uterine Fibroids?

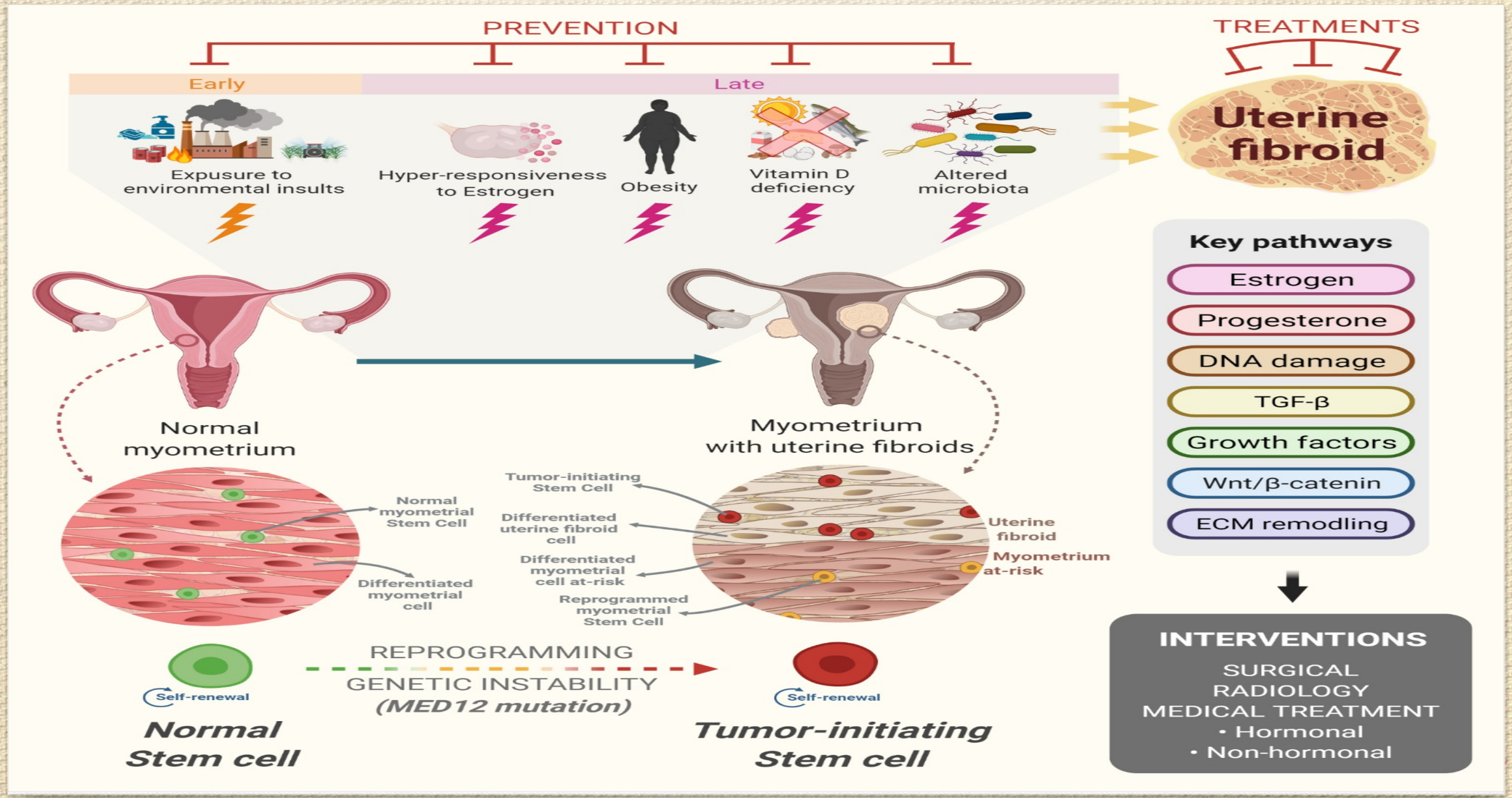
UF-specific risk factors:



- Race/ethnicity: women of color
- Vitamin D deficiency/insufficiency (2010)
- Genetic polymorphic markers (COMT, ERα)
- Weight/BMI
- Parity
- Age at menarche
- Family history of uterine fibroids
- Adolescent persistent menorrhagia
- Age

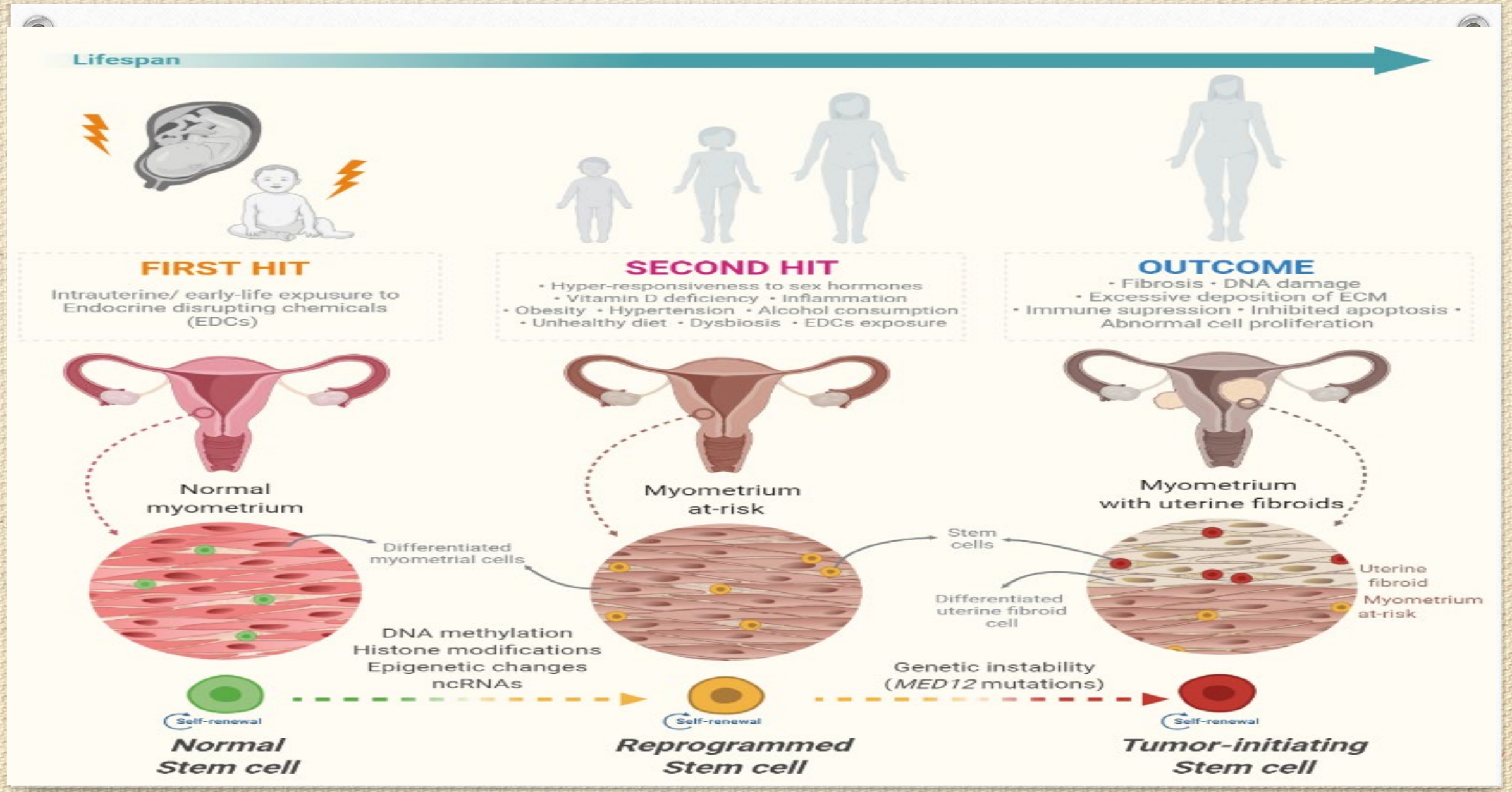
1. Othman EER & Al-Hendy A. Best Pract Clin Obstet Gynecol. 2008;22(4): 589–601. 2. Ali M, et al. Expert Opin Drug Discov. 2018 Feb;13(2):169-177.

Mechanisms underlying Pathogenesis of Uterine Fibroids



Bariani , Yang and Al-Hendy

Developmental Origin of Uterine Fibroids from Myometrial Stem Cells



Bariani , Yang and Al-Hendy

Who develops Uterine Fibroids?

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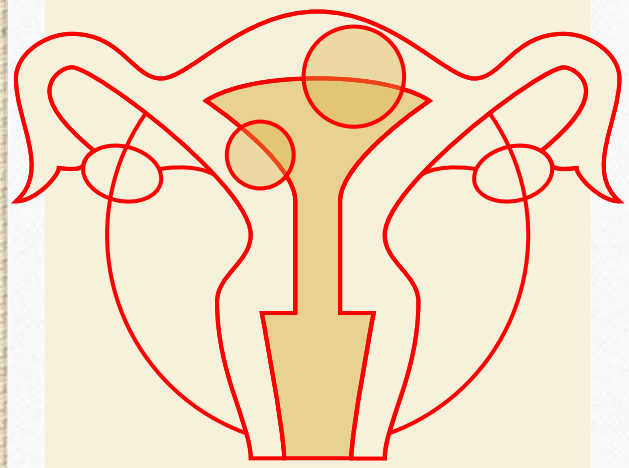

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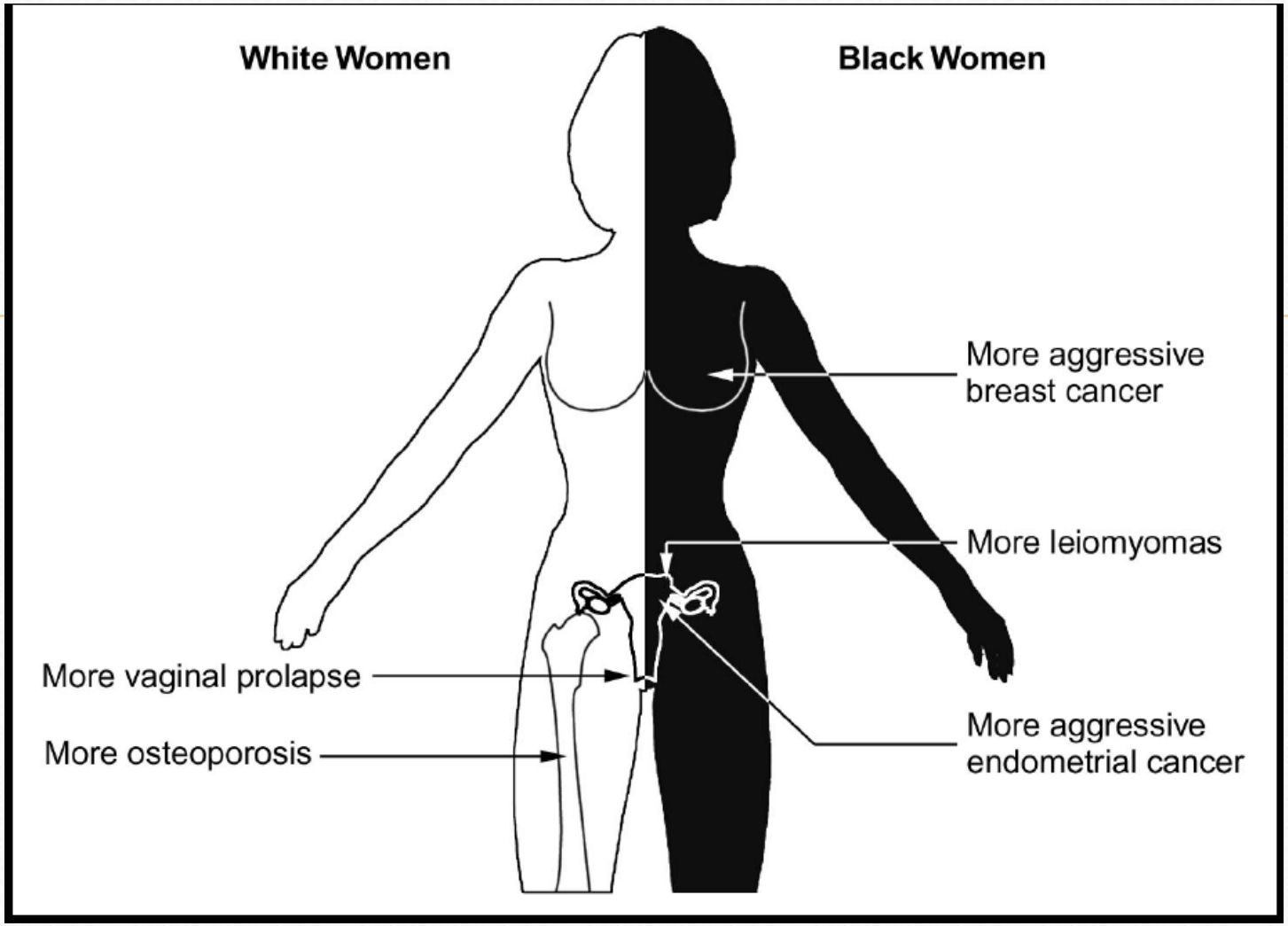
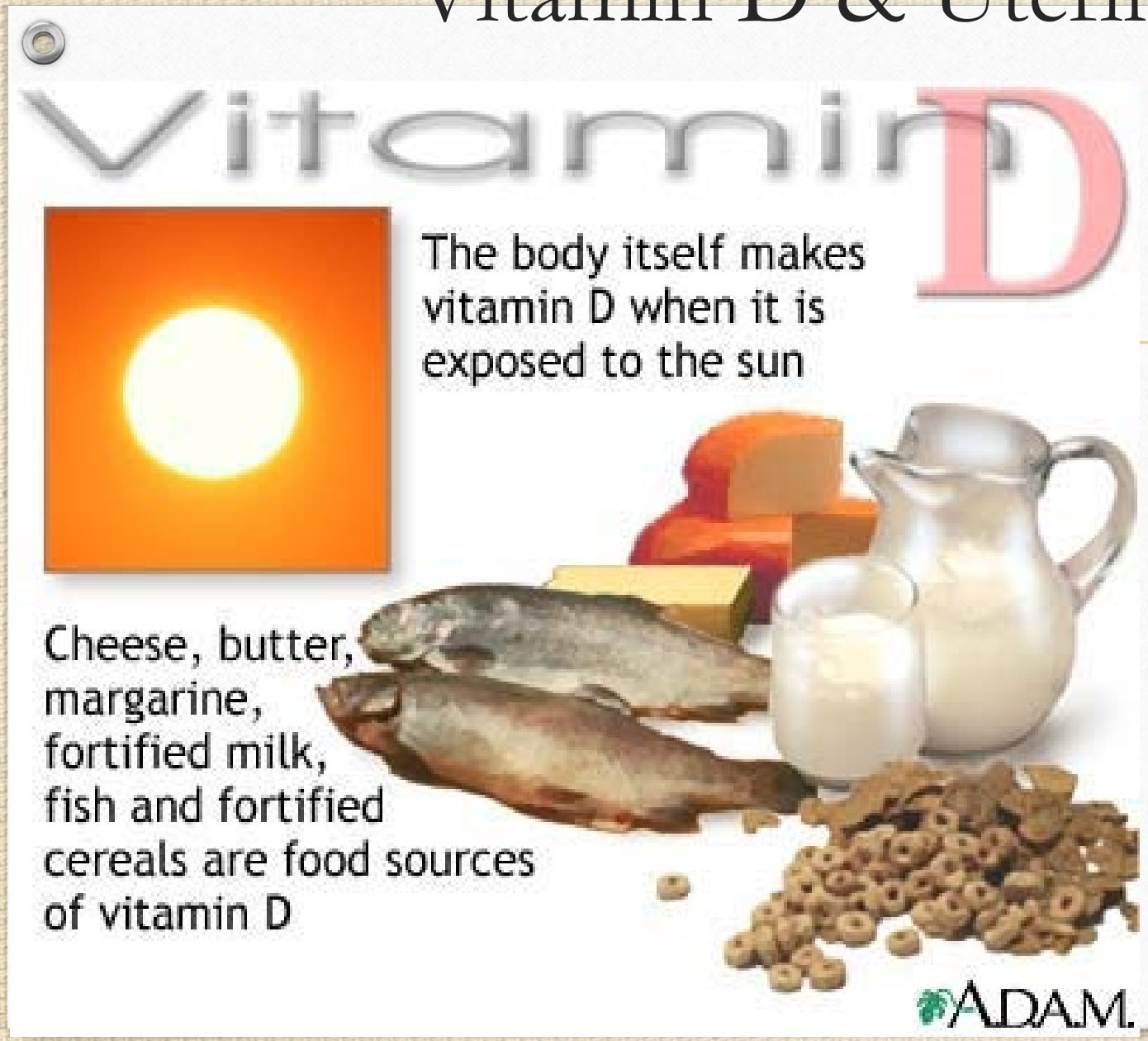
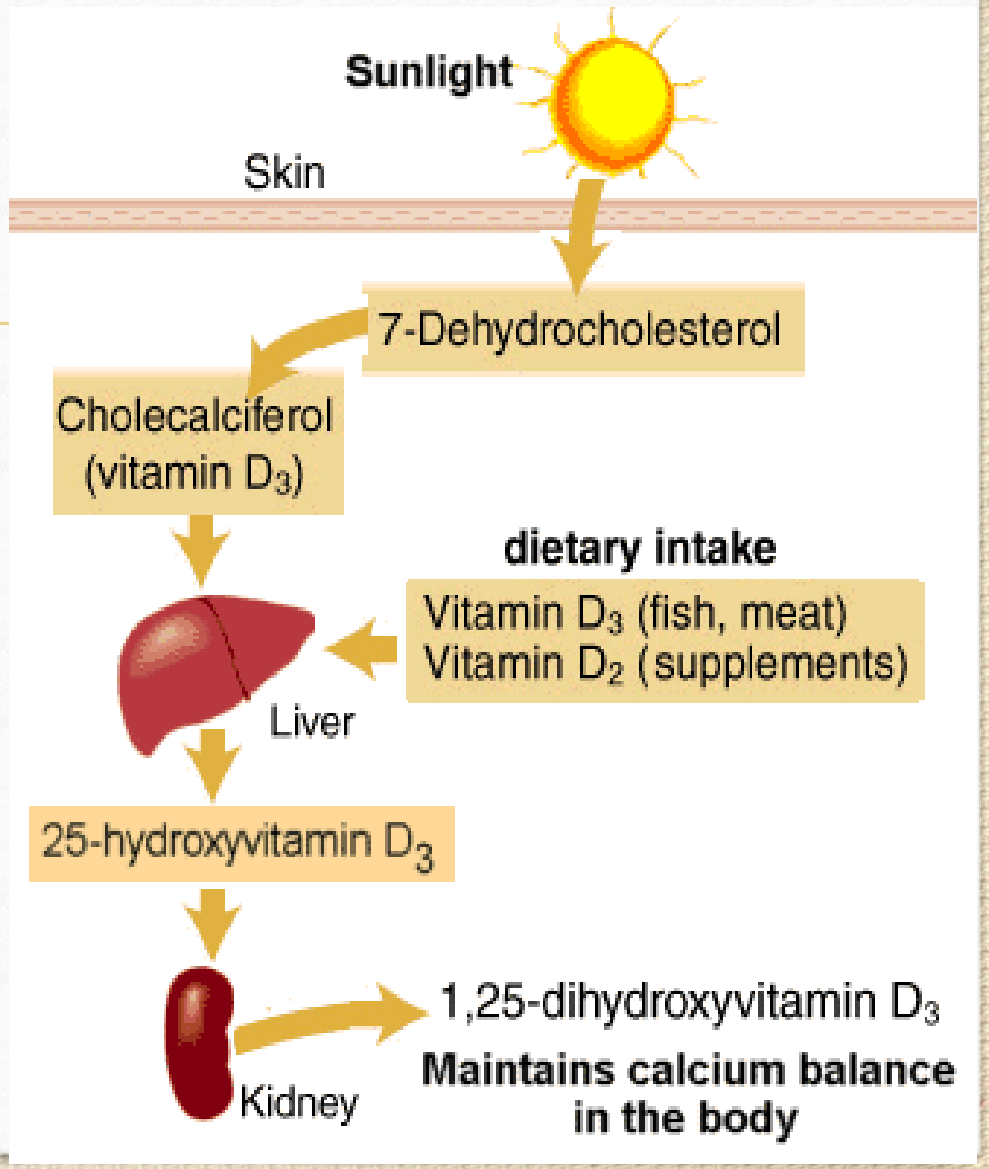
Why are Fibroids More Common in Women of Color?



Essam-Eldin & A-Hendy, *Best Pract Res Clin Ob Gyn*, 2008

Vitamin D & Uterine Fibroids

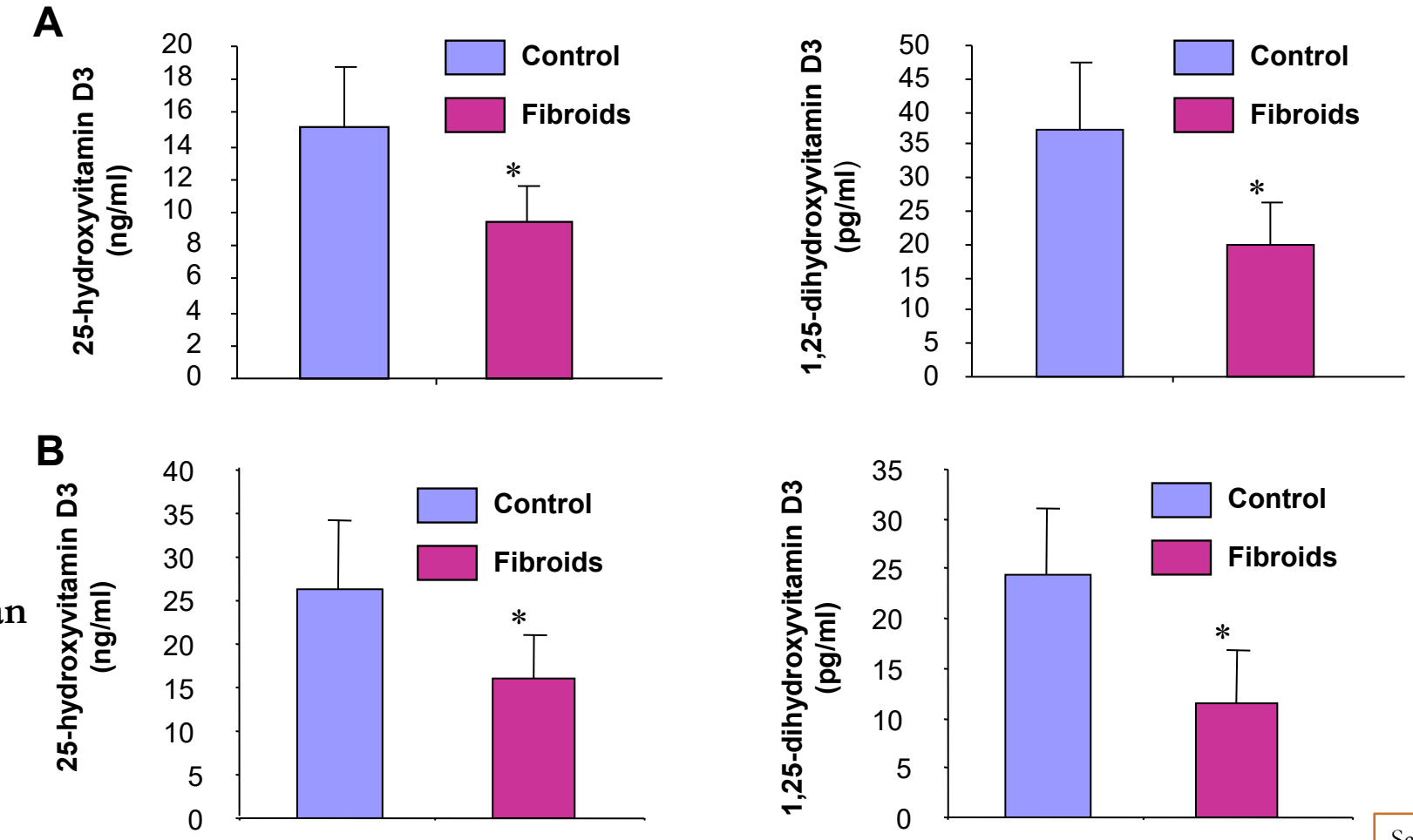




Vitamin D Deficiency is a Novel Risk Factor for Uterine Fibroids

African-American

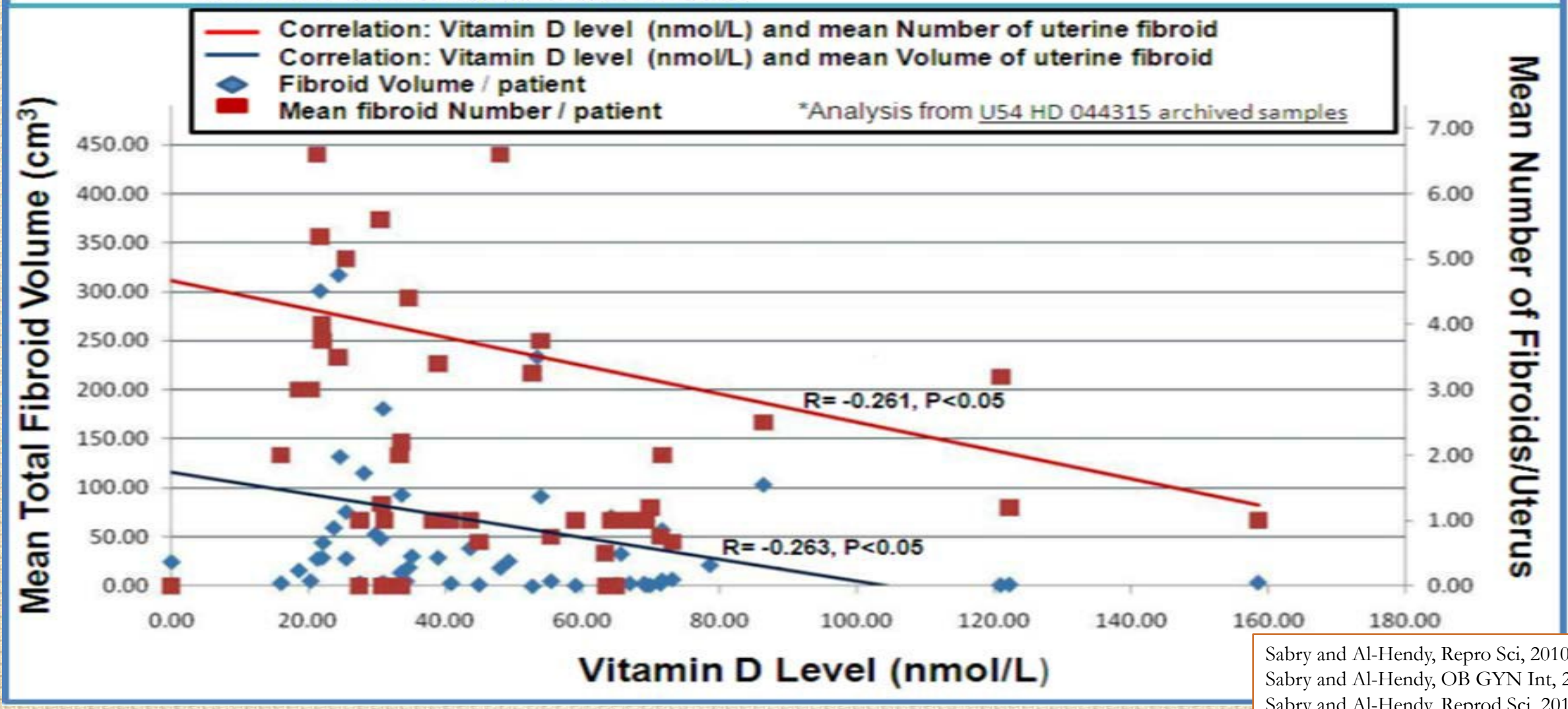
European-American



Sabry and Al-Hendy, Repro Sci, 2010
Sabry and Al-Hendy, OB GYN Int, 2012
Sabry and Al-Hendy, Reprod Sci, 2012
Sabry et. al, IJWH, 2013

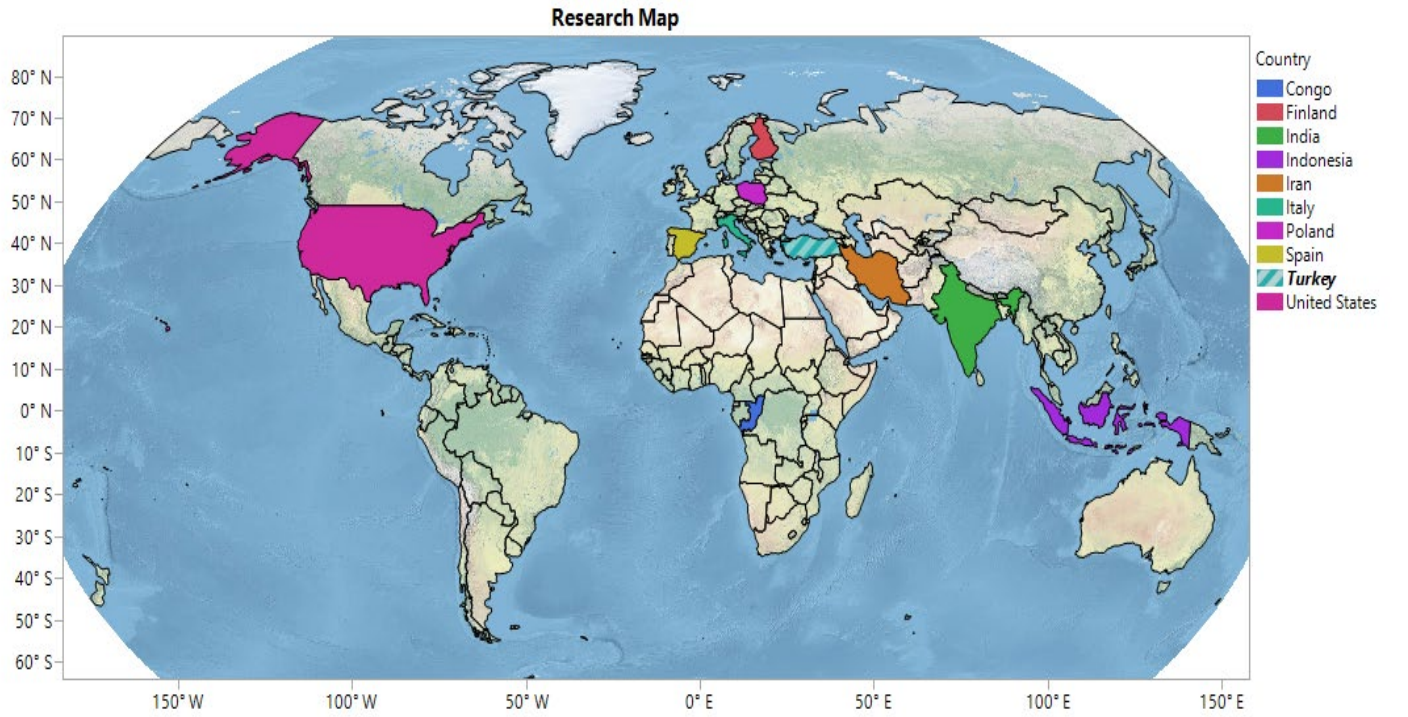
FIGURE 3 Serum Vitamin D3 level (nmol/L) inversely correlates with both mean volume and number of uterine leiomyomas

Mohamed.S. & Al-Hendy A Innovative Oral Treatments of Uterine Leiomyoma , Ob Gyn Intl., 2011
http://www.hindawi.com/journals/ogi/aip/943635/



Sabry and Al-Hendy, Repro Sci, 2010
Sabry and Al-Hendy, OB GYN Int, 2012
Sabry and Al-Hendy, Reprod Sci, 2012
Sabry et. al, IJWH, 2013

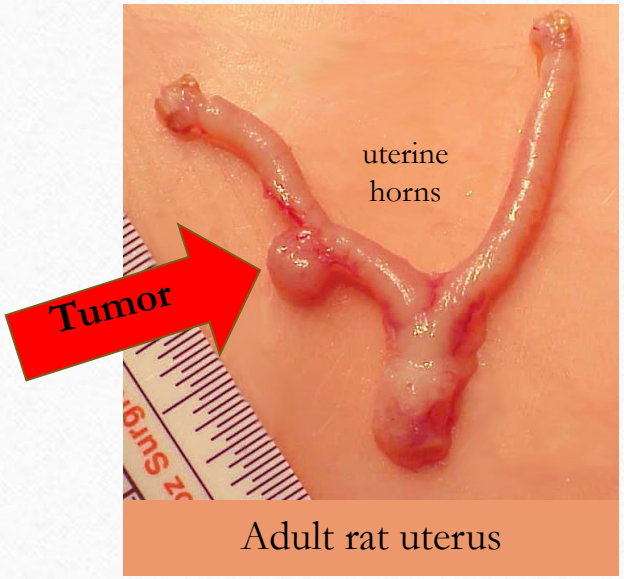
Vitamin D Deficiency & Uterine Fibroids: A Global Phenomenon



Source	Year	Data
Srivastava et al. JOG	2019	VD deficiency = UF occurrence „+”, size „+”
Singh et al. JOGI	2019	VD deficiency = UF occurrence „+”, size „+”
Oskovi Kaplan et al. TJOG	2018	VD deficiency = UF occurrence „+”
Ciebiera et al. Fertil Steril	2016	VD deficiency = UF occurrence „+”
Mitro et al. Reprod Toxicol	2015	VD deficiency = UF occurrence none (white „+””)
Paffoni et al. JCEM	2013	VD deficiency = UF occurrence „+”
Baird et al. Epidemiol	2013	VD deficiency = UF occurrence „+”
Sabry and Al-Hendy, Reprod Sci	2010	VD deficiency = UF occurrence „+”, size „+”

Eker Rat: Model for Gene-Environment Interactions

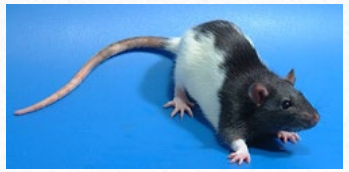
- Eker rats carry a germline genetic defect in Tsc2 ($Tsc2^{Ek/+}$)
- Females develop multiple, proliferative smooth muscle lesions (leiomyoma) in the uterus when ***Other Tsc2 allele is mutated or deleted (LOH)***
  - **60% of female $Tsc2^{Ek/+}$ rats by 16 mo**
- Tumors are hormone dependent with molecular/biochemical correlates to human UL



Adult rat uterus

Everitt et al. , *AJP*, (1995)
Howe et. al., *AJP*, (1995)

Vitamin D3 Treatment Shrinks Uterine Leiomyoma Tumors in the Eker Rat Model



14-16 months old with visible leiomyoma tumors

Control group (n=6)

Ethylene glycol (vehicle) for 3 week

Treated group (n=6)

Vitamin D3 delivered by micro-osmotic pumps (0.5lg/kg) per day for 3 week

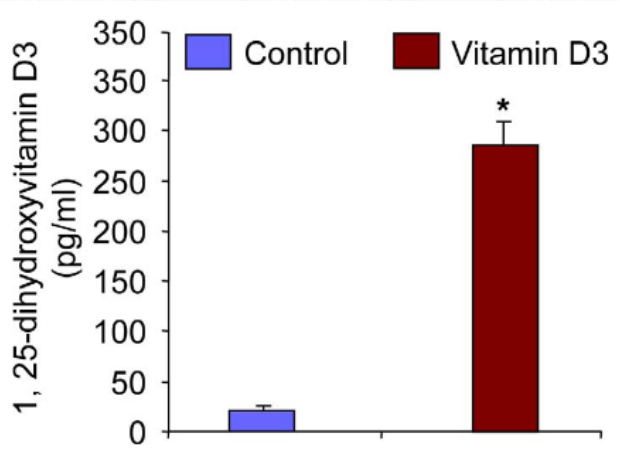
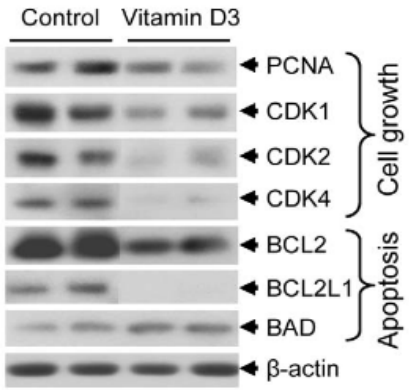
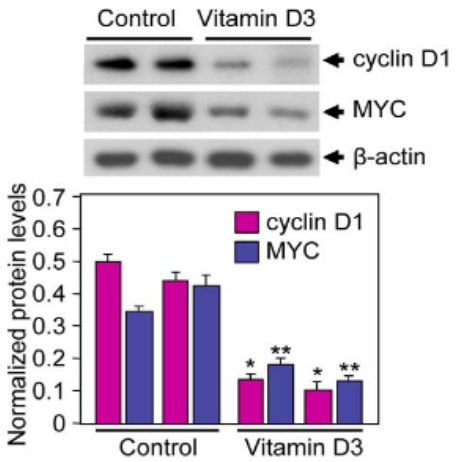
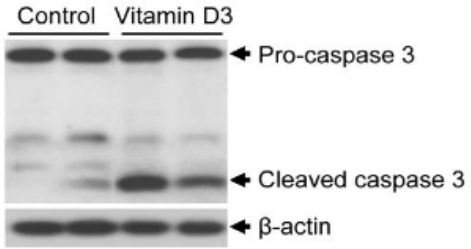
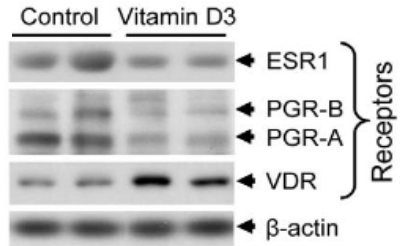


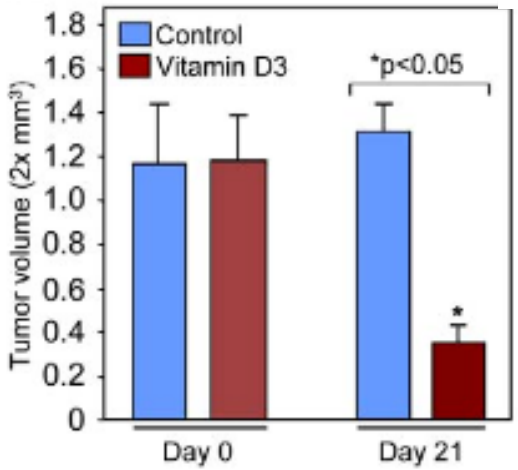
FIG. 1. Serum levels of 1,25-dihydroxyvitamin D3 (vitamin D3) in treated Eker rats. Serum samples from vehicle-treated control and vitamin D3-treated Eker rats were used to determine the concentration of vitamin D3 using RIA. Elevated levels of serum vitamin D3 were found in treated Eker rats compared with vehicle-treated control rats (n = 6 each group). *$P < 0.05$ when compared with corresponding control.

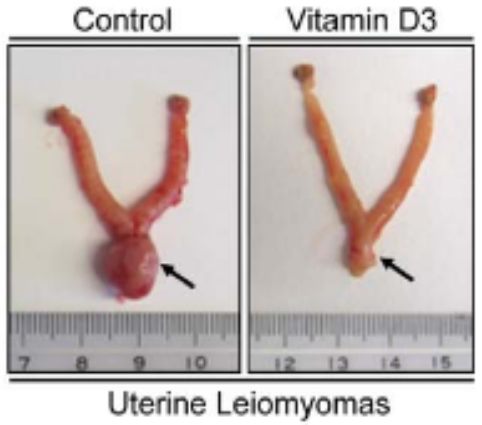


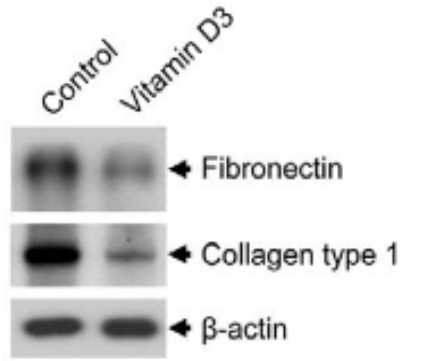






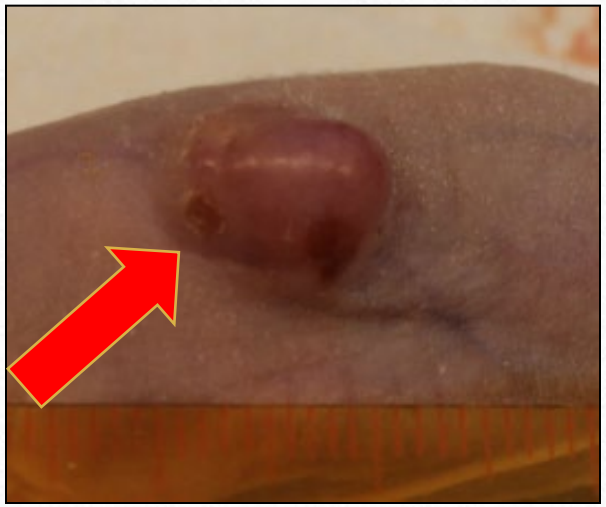


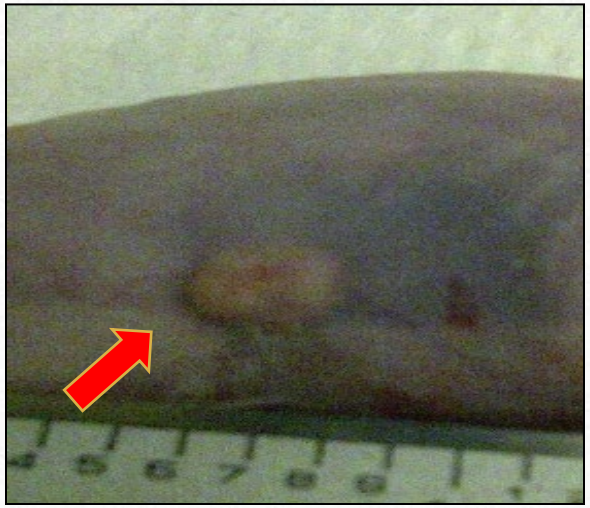




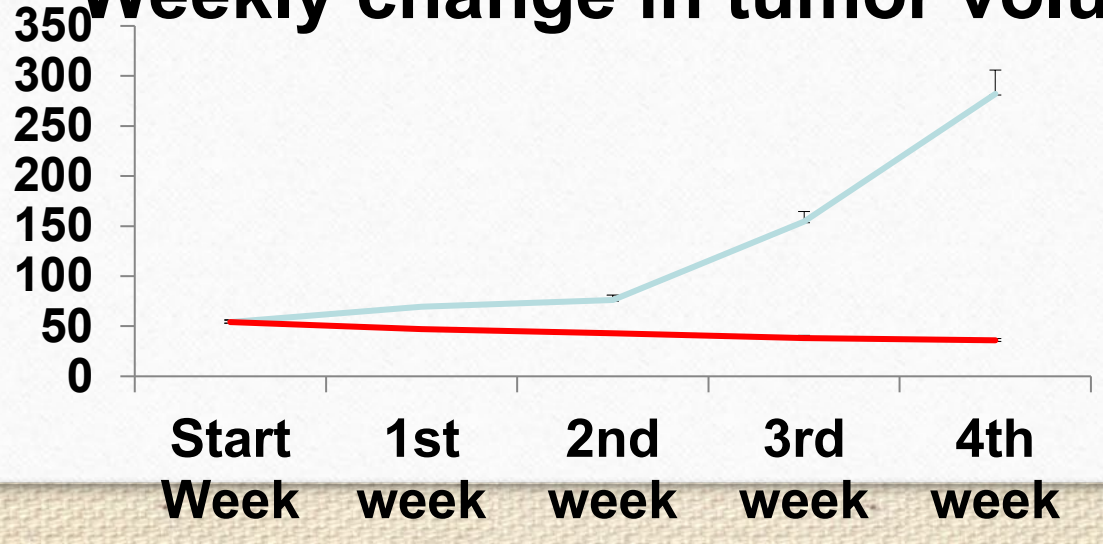
Halder SK, Sharan C, Al-Hendy A. 1, 25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. Biol Reprod. 2012;86:116.

Dramatic Shrinkage of Fibroid Lesions in Nude Mice after 4 weeks of Paricalcitol Treatment





Weekly change in tumor volume



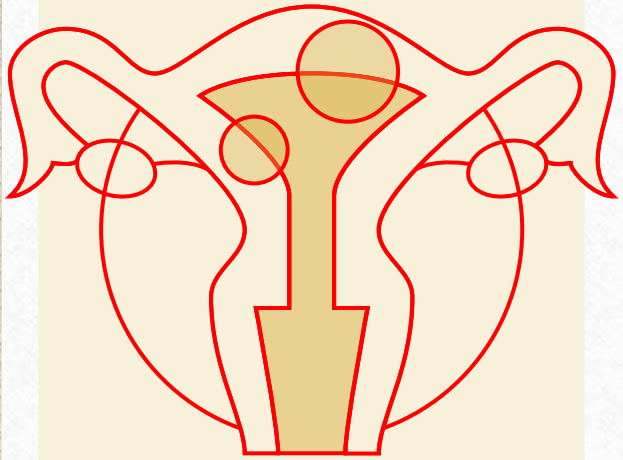
Halder et. al., RS, 2013

Vitamin D Anti-Uterine Fibroids in Pilot Clinical Trials

Source	Year	Data
Hajhashemi et al. CJIM	2019	Human - 50,000 IU every 2 weeks for 10 weeks Leiomyomas size in vitamin D group significantly decreased as compared to placebo group (52.58 vs 61.11 mm, respectively, $P<0.05$)
Ciavattini et al. Medicine	2016	Human (53 women) A significant increase in the 25-OH-D3 serum level was observed after 12 months of supplementation, and a lower rate of surgical or medical treatment due to the "progression to extensive disease" was reported (13.2% vs 30.9%)

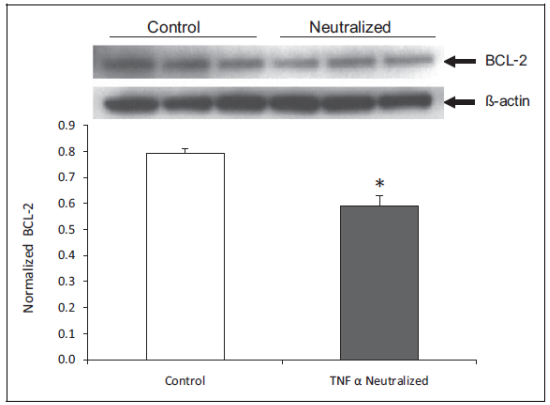
Who develops Uterine Fibroids?

UF-specific risk factors:



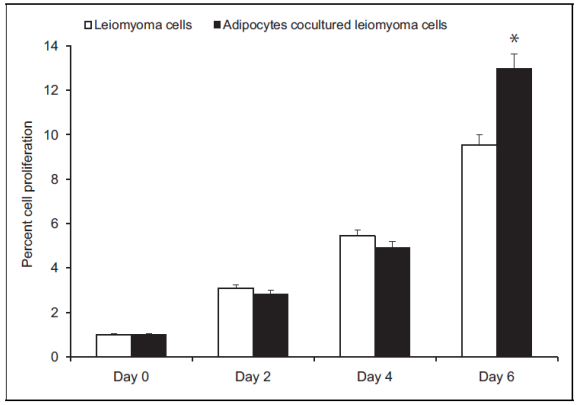
- Race/ethnicity: women of color
- Vitamin D deficiency/insufficiency (2010)
- Genetic polymorphic markers (COMT, ERα)
- Weight/BMI
- Parity
- Age at menarche
- Family history of uterine fibroids
- Adolescent persistent menorrhagia
- Age

1. Othman EER & Al-Hendy A. Best Pract Clin Obstet Gynecol. 2008;22(4): 589–601. 2. Ali M, et al. Expert Opin Drug Discov. 2018 Feb;13(2):169-177.



Adipocytes Enhance the Proliferation of Human Leiomyoma Cells Via TNF-α Proinflammatory Cytokine

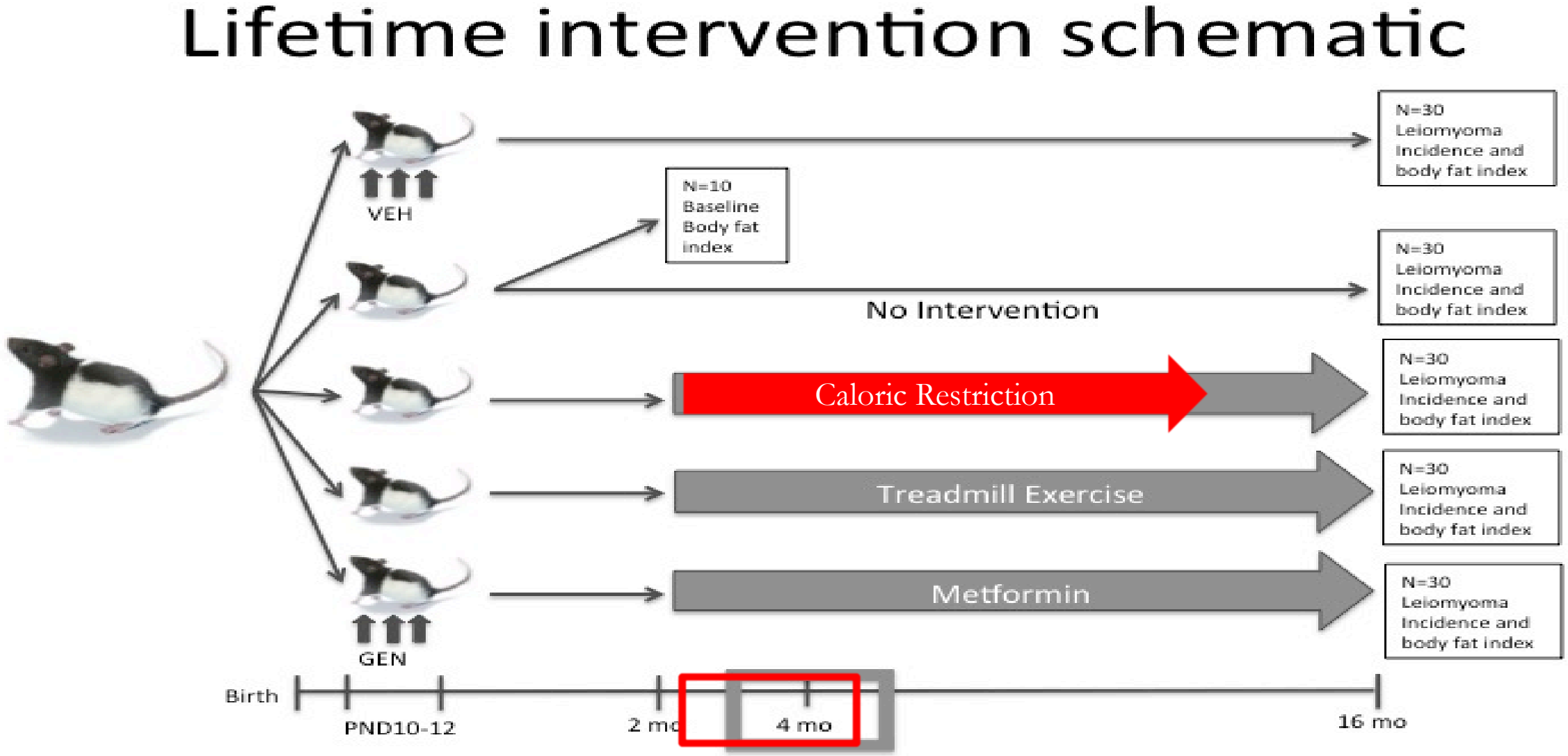
Sangeeta Nair, DVM, MS[1] and Ayman Al-Hendy, MD, PhD[1]



RS, 2011

Obesity and Increased Risk of Uterine Fibroids

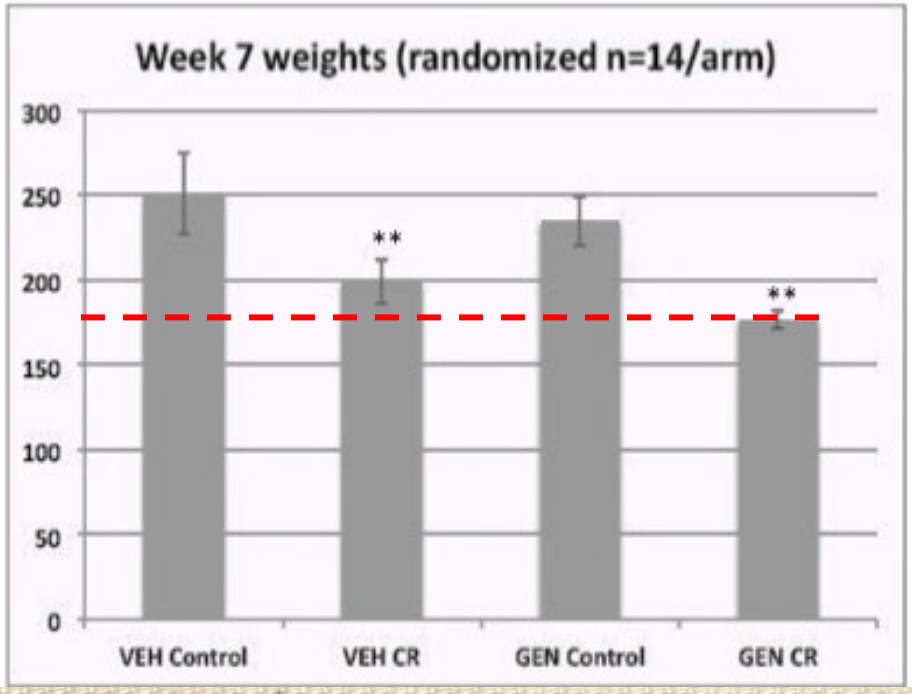
Caloric Restriction and Uterine Fibroids

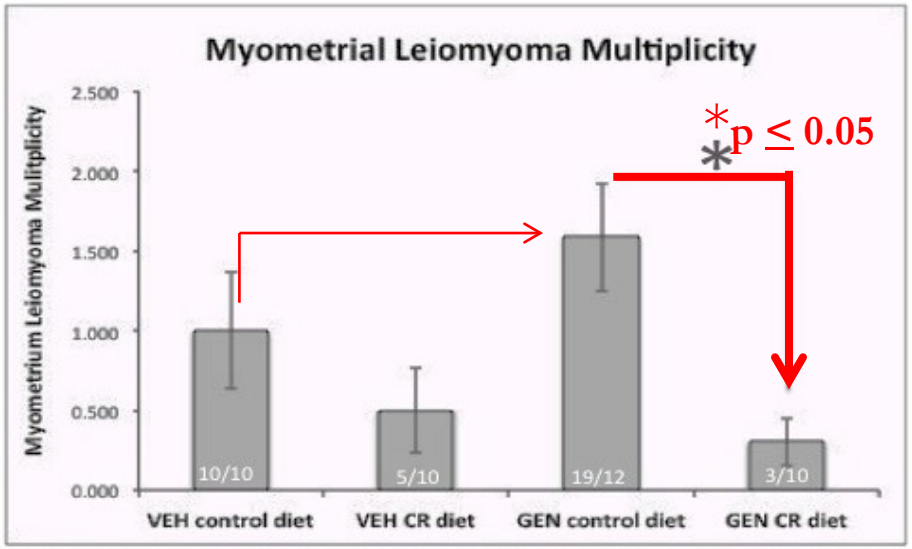


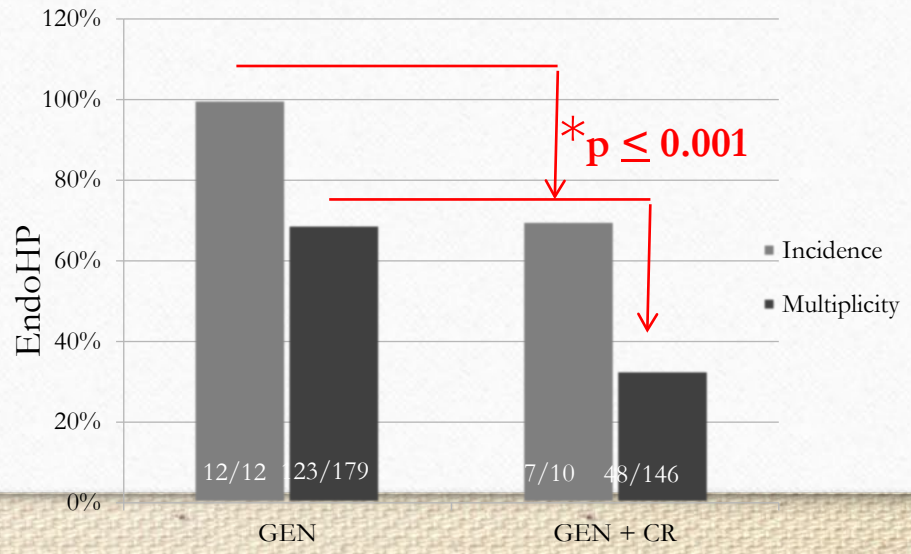
GEN dose = 50 mg/kg, resulting in a plasma level of 6-8 uM.
Infant consumption of soy formula results in a plasma level of 1-6 uM.

Caloric Restriction and Uterine Fibroids

- CR from 2-4 mo of age caused a decrease in weight
- EndoHP was reduced from 100% to 70% incidence, and from a multiplicity (number of lesions/histological section) of 69% to 33%
- Uterine leiomyomas were significantly reduced from a multiplicity of 1.58 to 0.30
- Thus, CR reversed the increase in susceptibility caused by developmental exposure to EDCs and developmental (re)programming

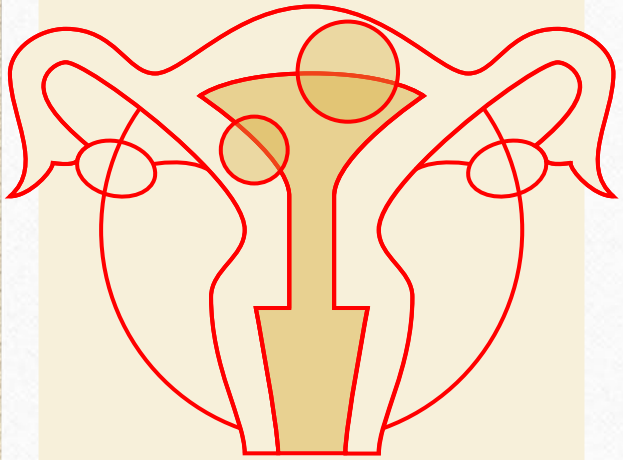






Who develops Uterine Fibroids?

UF-specific risk factors:



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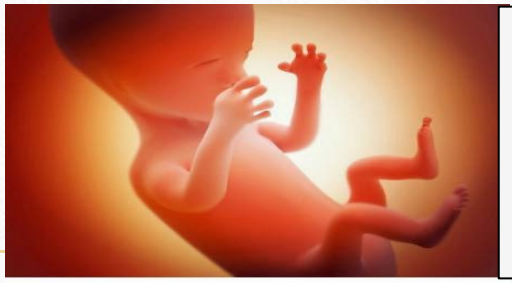
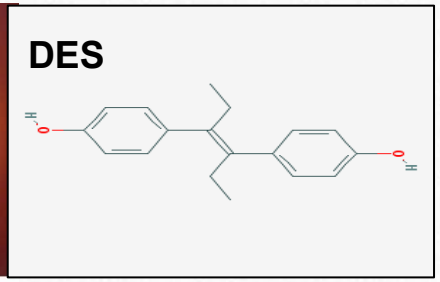
Does Early Life Exposure to Environmental Estrogen Increase Occurrence of Uterine Fibroids in Women?

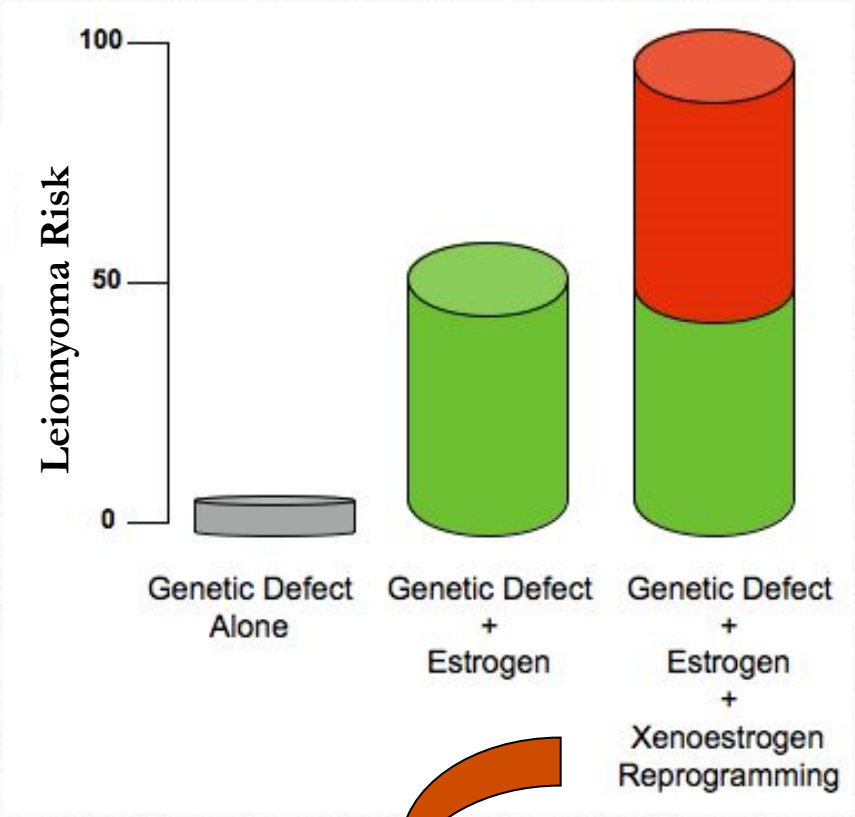
- In Utero exposure to DES increases risk of uterine fibroids later in adult life
- NIEHS sister study showed increased risk of uterine fibroids after early life exposure to genistin and other estrogenic isoflavones (soy formula)
- Hair relaxers use is associated with increased risk if uterine fibroids in African Americans
- Minority communities are particularly at higher risk of hazardous environmental exposures (might contribute to high prevalence of uterine fibroids in African Americans)

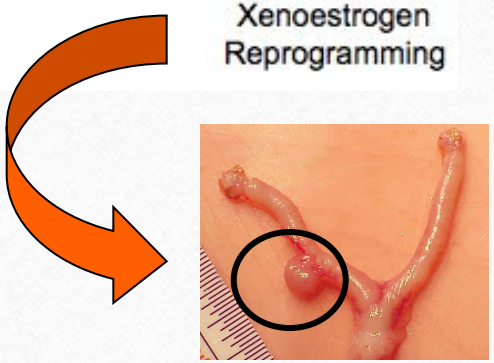




Aimee et al. , *Environ Health perspect* (2010)
Wise et. al., *Am J Epidemiol* (2012)
Cook et.al., *PNAS* (2005)
Silbergeld et. al., *AJOG* (2005)

Eker Rat: Model for Gene-Environment Interactions





Environmental exposures increase incidence from 65% to 100% (Cook et al *PNAS* 2005)

- Tsc2 gene defect combined with endogenous hormones: 65% of animals develop hormone-dependent uterine leiomyoma (i.e. 60% gene penetrance)
- Brief early life exposure to environmental estrogens (e.g. DES) increases tumor incidence in adulthood to 100% (i.e. 100% Tsc2 gene penetrance). Also increases tumor size and multiplicity 3-5 fold

Where do *Uterine Fibroids* come from?

Myometrial Stem Cell

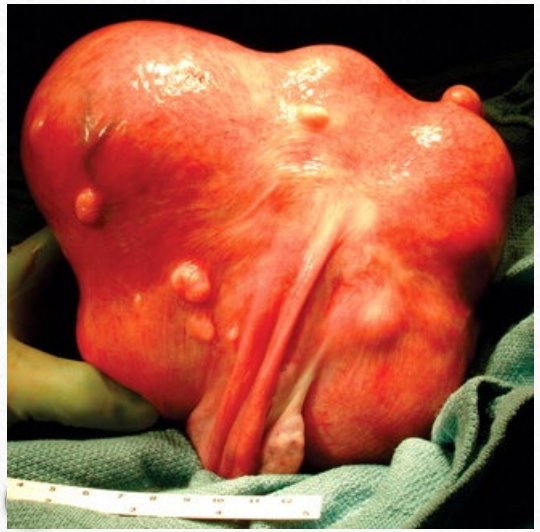
Tumorigenic Driver Mutation

Leiomyoma Tumor Forming Stem Cell

Self-Renewal

- Abundant E2/P4
- Limited Vitamin D
- Retinoic acid
- COMT Over-expression
- Others

Proliferation
Differentiation

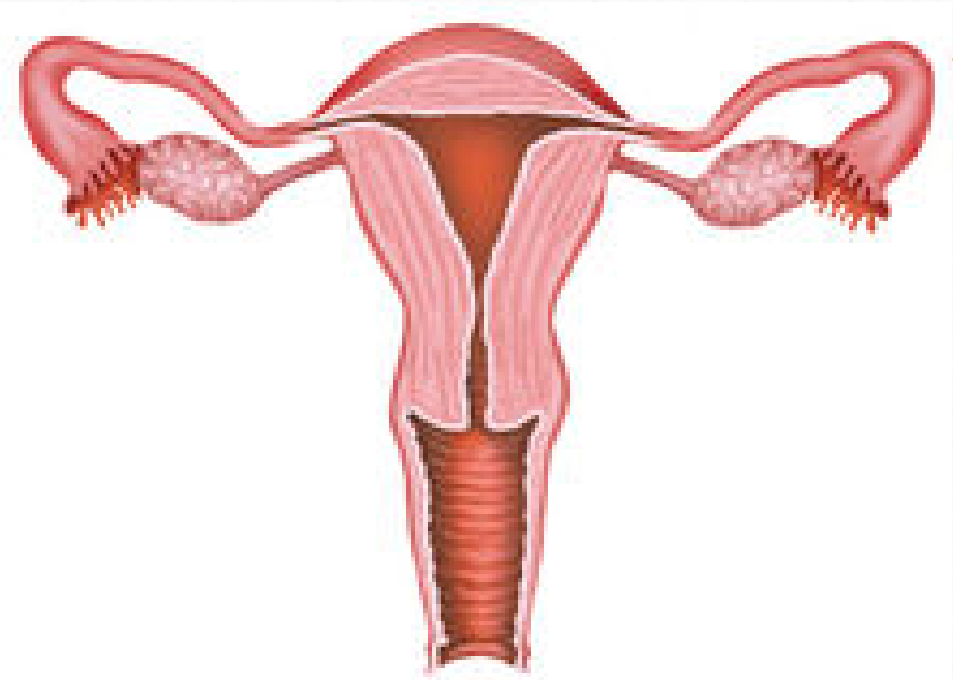


Fibroid Tumor cells

Mas et. al., *Hum Reprod*, 2015
Bulun. *NEJM*, 2013
Moravek et. al., *Hum Reprod Update*, 2015

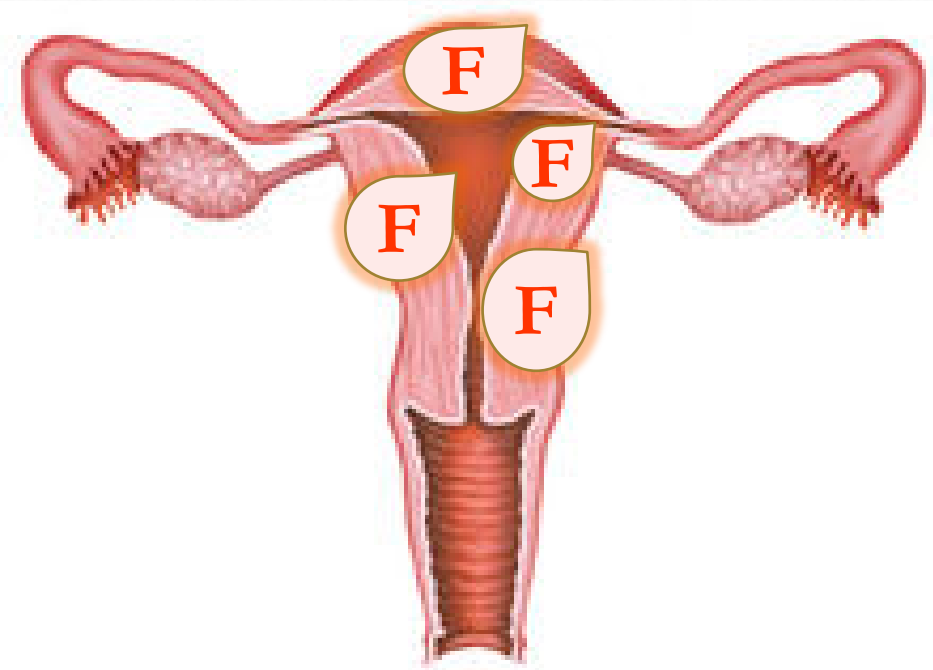
How does Early Life Exposure to Environmental Estrogen Increase Occurrence of Uterine Fibroids in Women?

MyoN



- Prolapse (pre-menopausal)

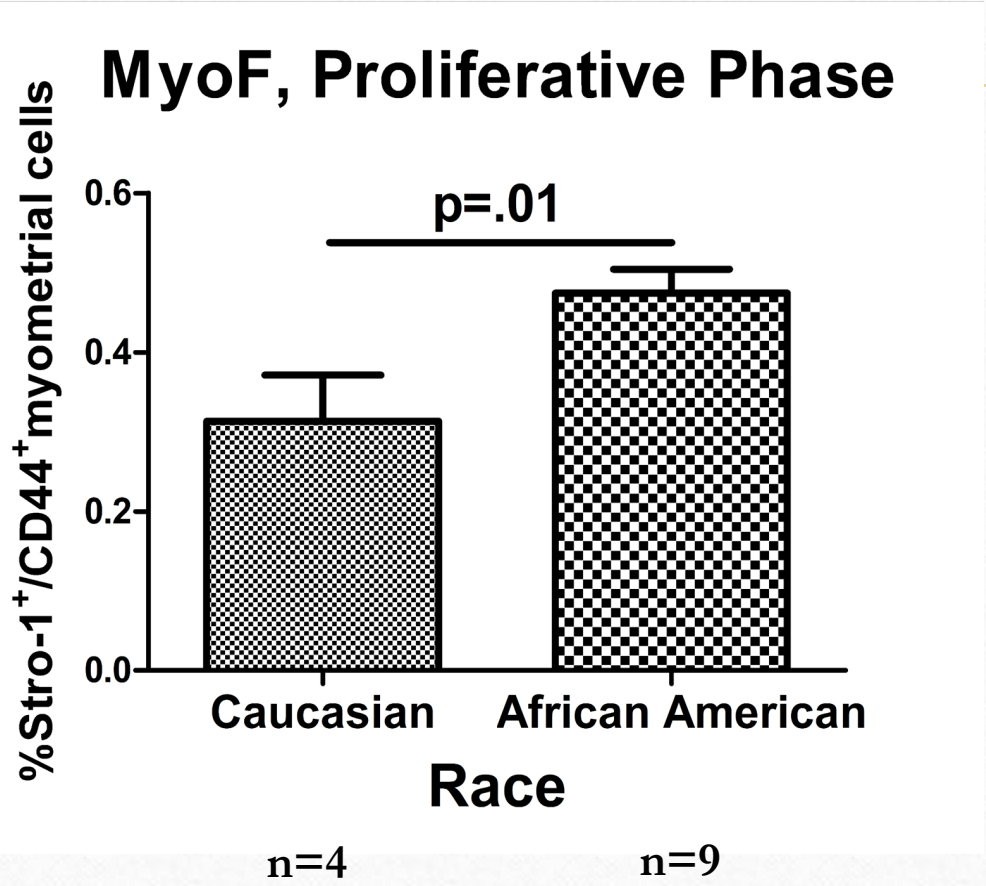
MyoF



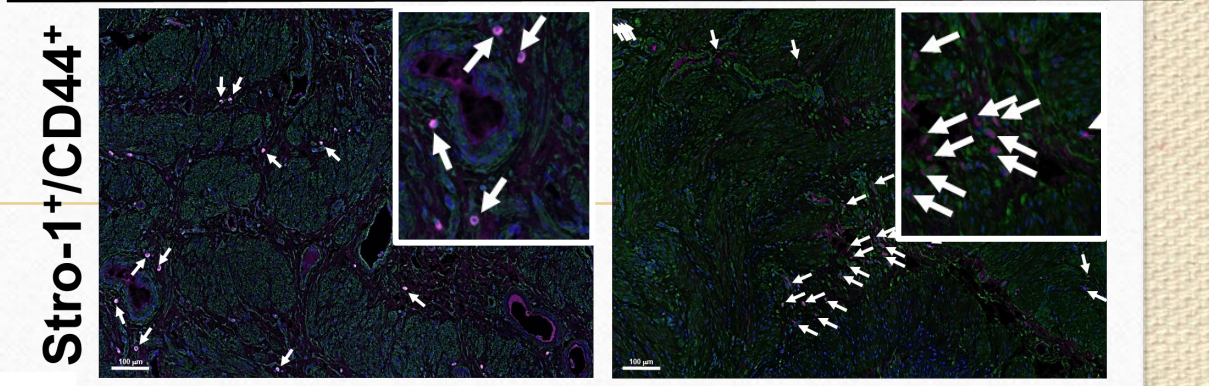
- Fibroid Uteri

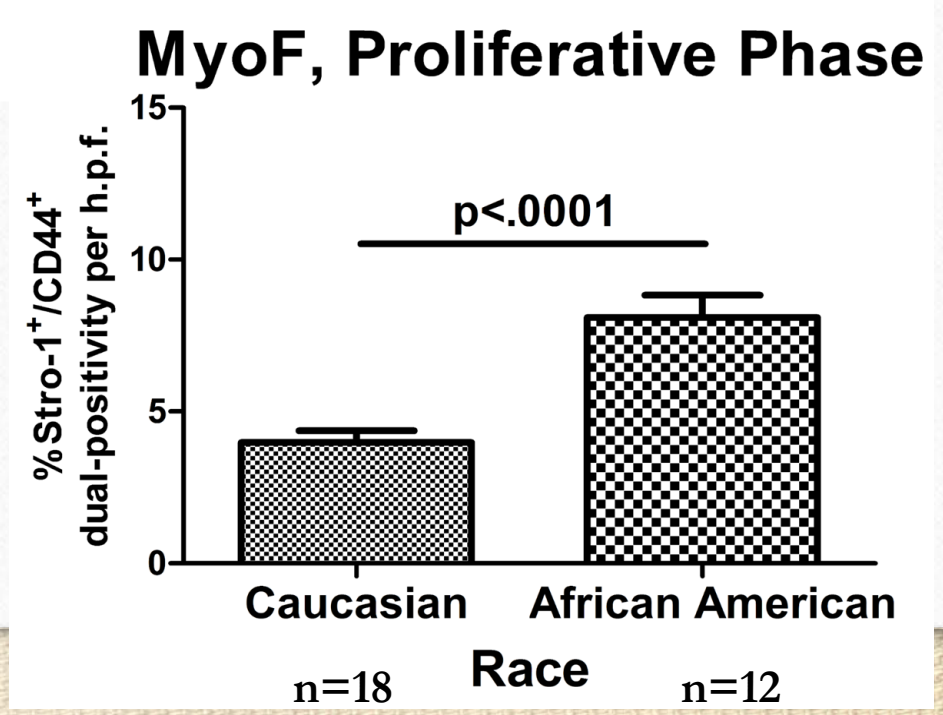
Stro-1$^+$/CD44$^+$ myometrial cells are increased in myometrium from African American women

Flow cytometry



IHC



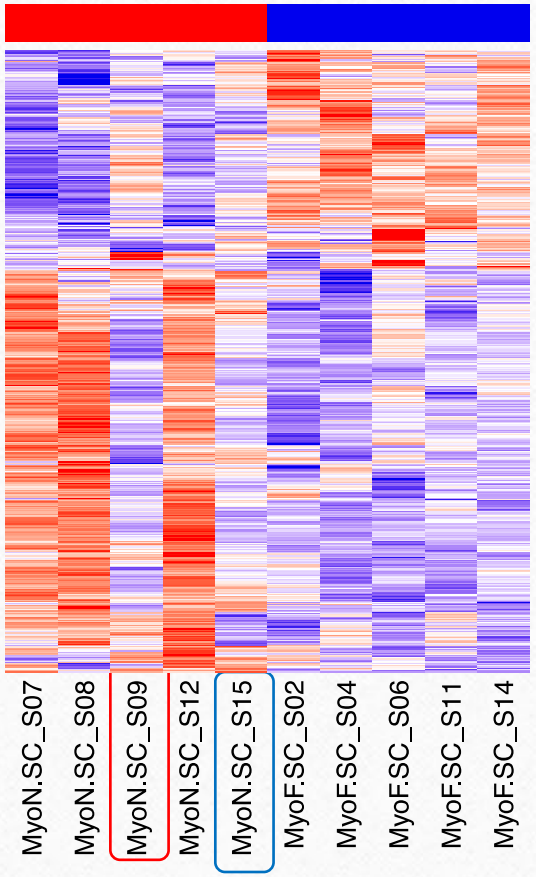


Prusinski et. al., Am J Path, 2018

At-Risk MyoF Genomic Profile Resembles Fibroid Profile and is Distinct from Healthy MyoN

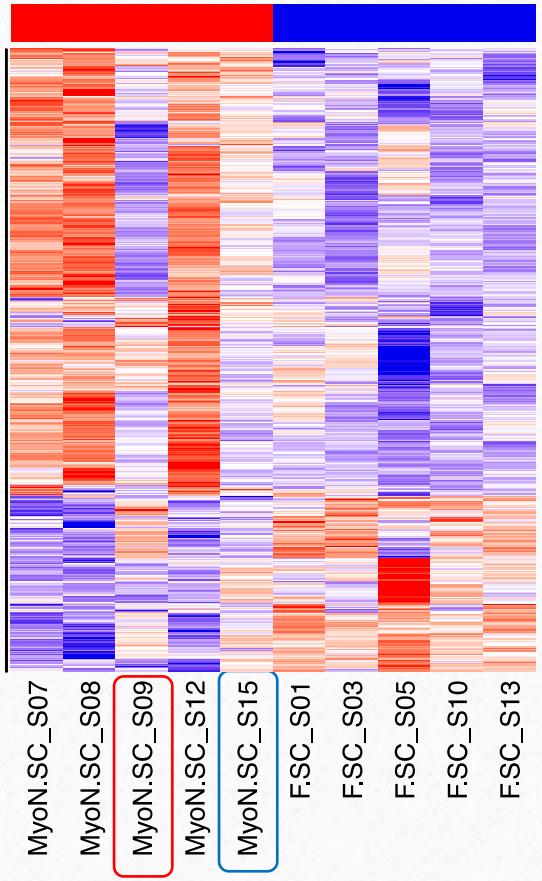
MyoF over MyoN

185 up
365 down



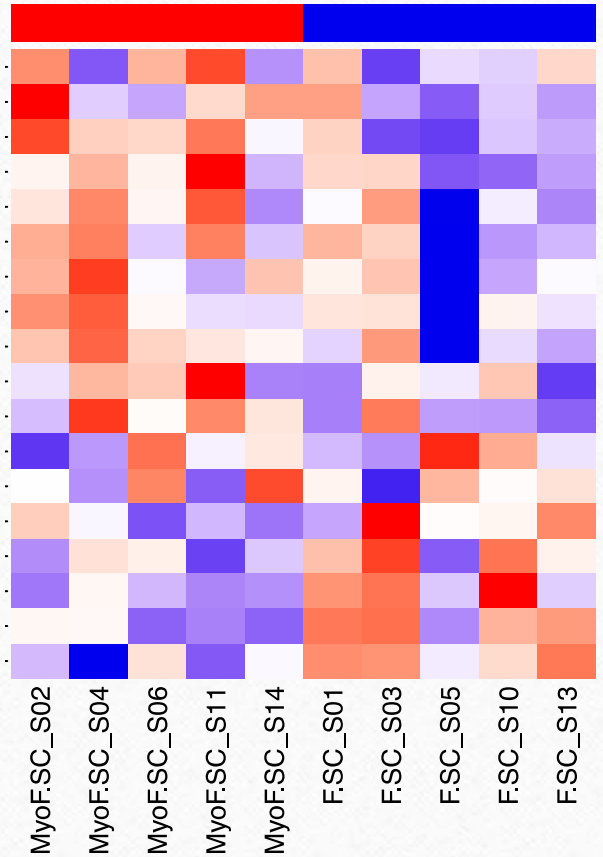
F over MyoN

149 up
384 down



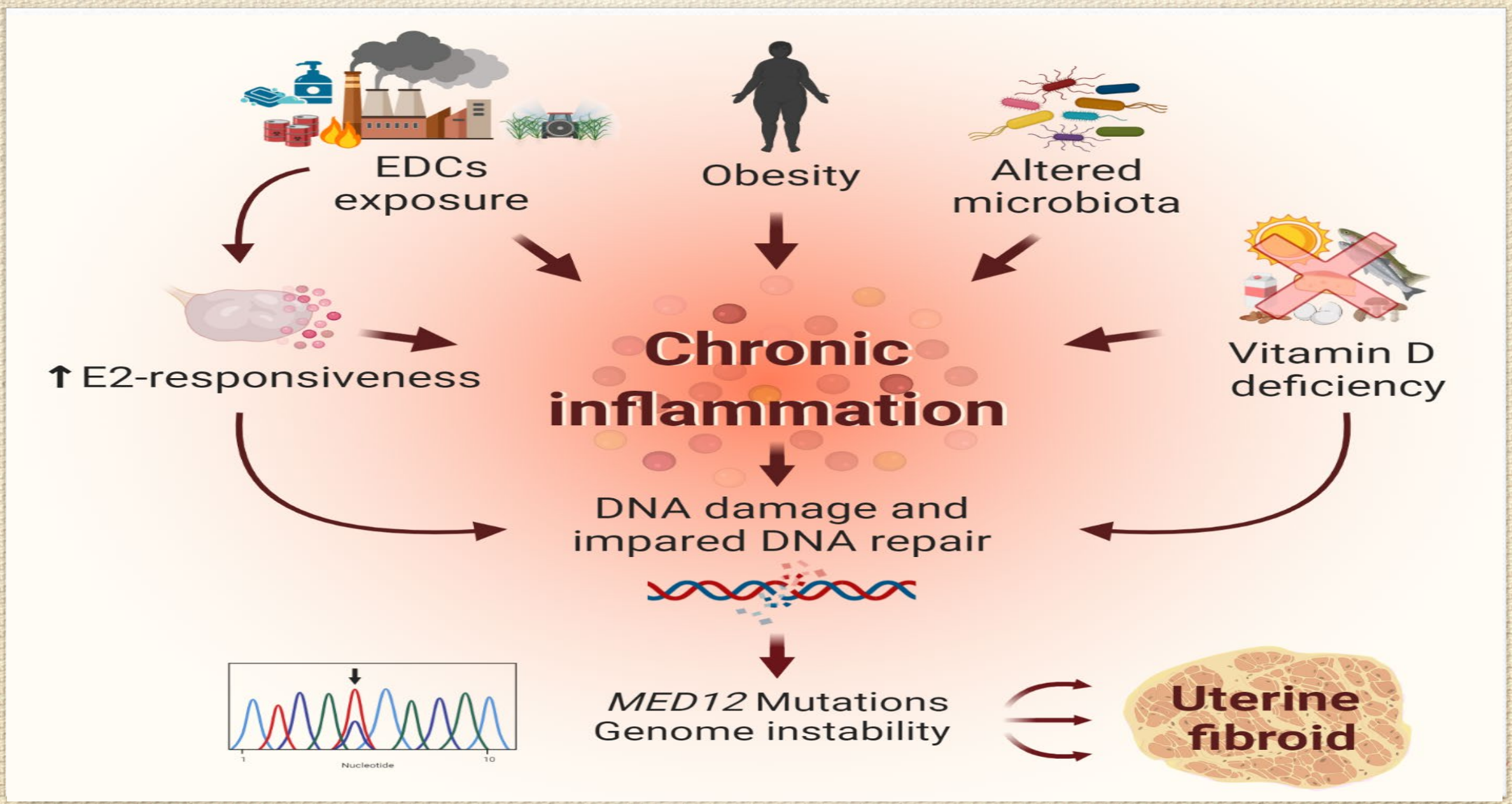
F over MyoF

6 up
12 down



EdgeR upper quartile LRT RUVr
FC>1.5, FDR<0.05
cpm>1

10.7.20



Bariani , Yang and Al-Hendy

Knowledge gaps/Unmet needs

- Why fibroids are more common in women of color?
- Role of adverse environmental exposures (direct, developmental, transgenerational)
- Role of uterine fibroid exosomes
- Role of uterine microbiome
- Role of epitranscriptomics
- Fertility friendly therapy
- Prevention of uterine fibroids

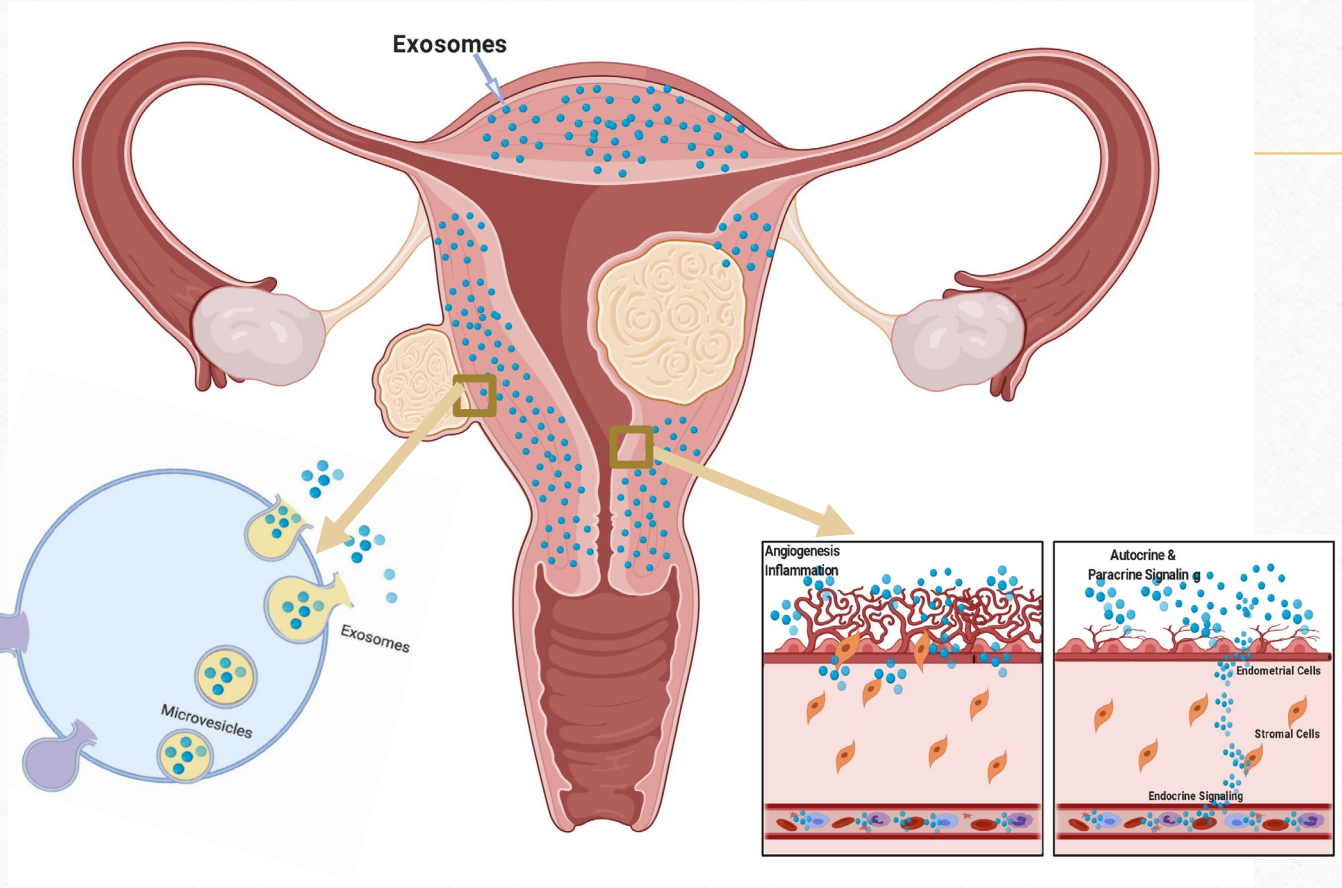
THE UNIVERSITY OF CHICAGO

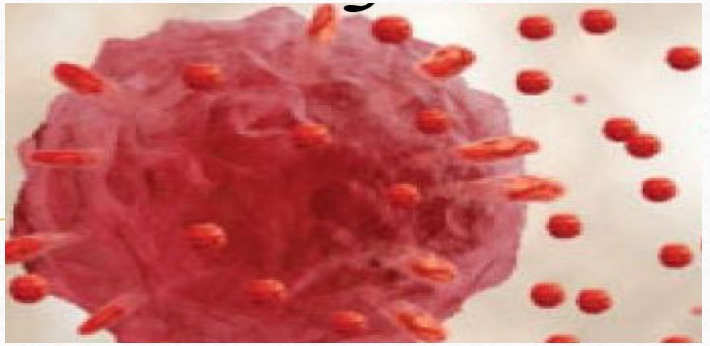
Acknowledgement

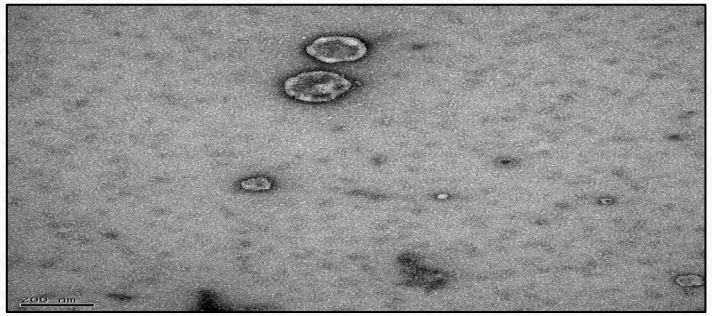
NIH, NICHD, NIEHS, NIMHS, CRWH

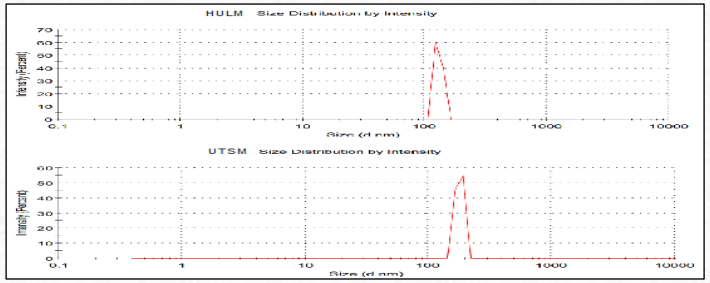
UEFA Champions May 2017

Leiomyoma-Derived Exosomes: Impact on Human Endometrium

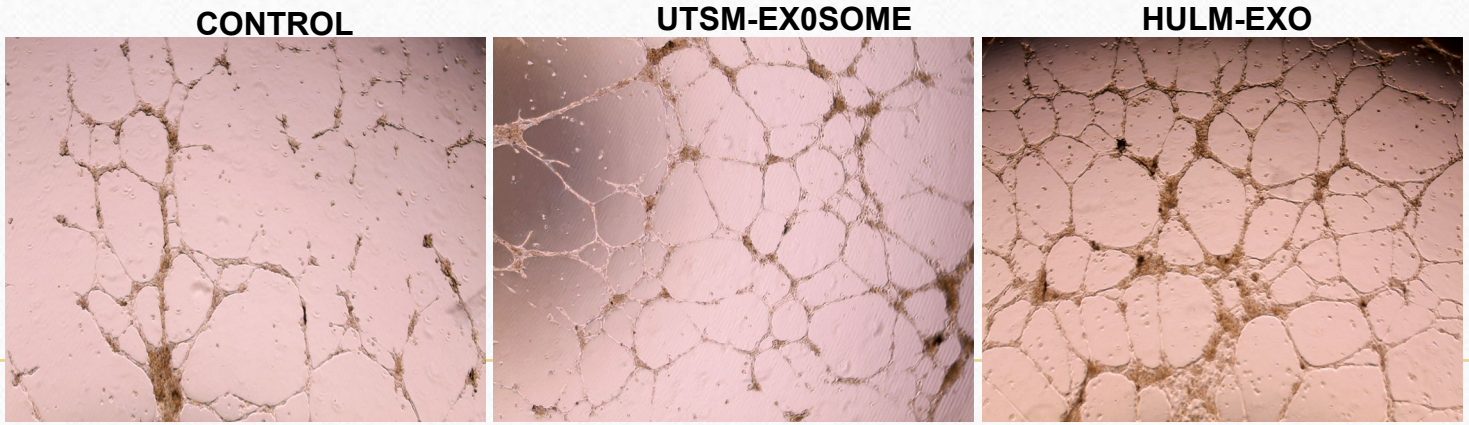


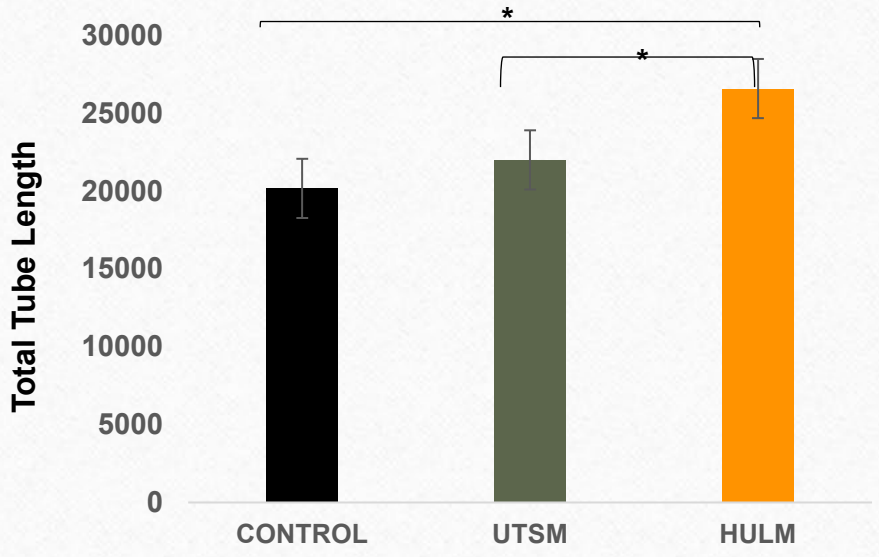






Uterine Fibroid-Derived Exosomes Have Enhanced Angiogenic Properties





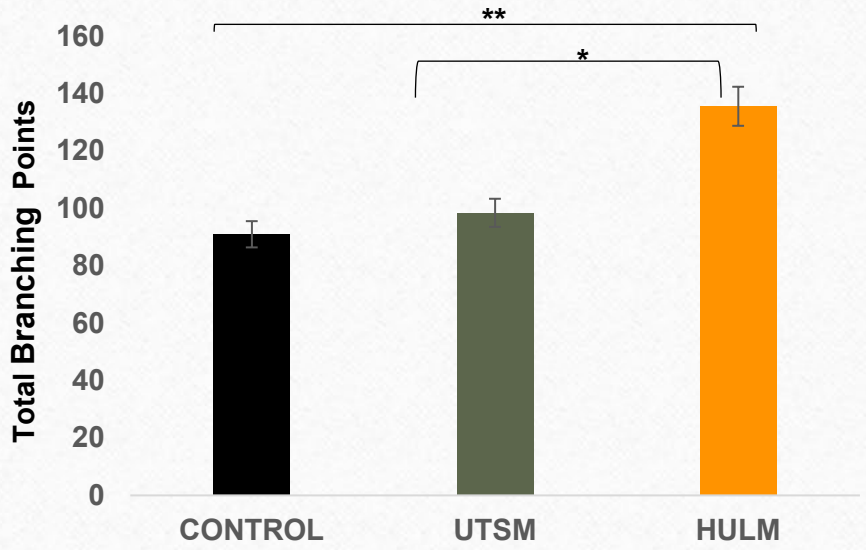
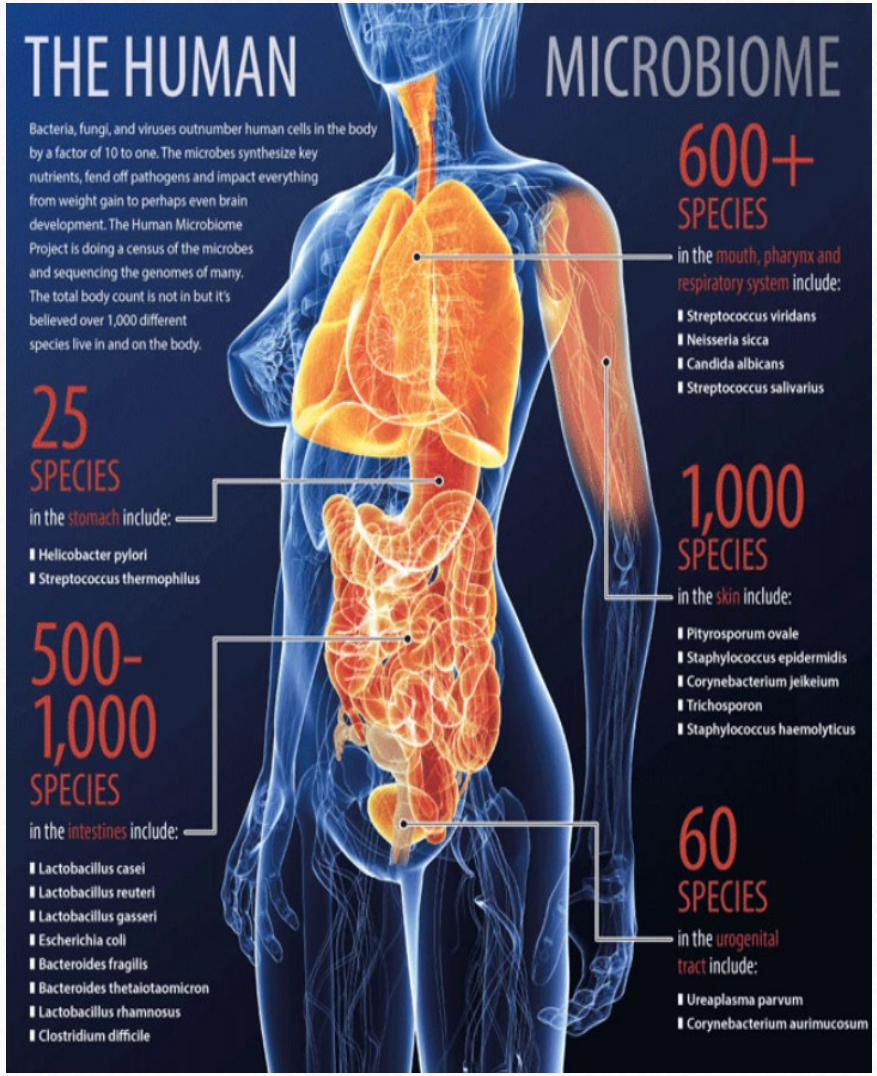
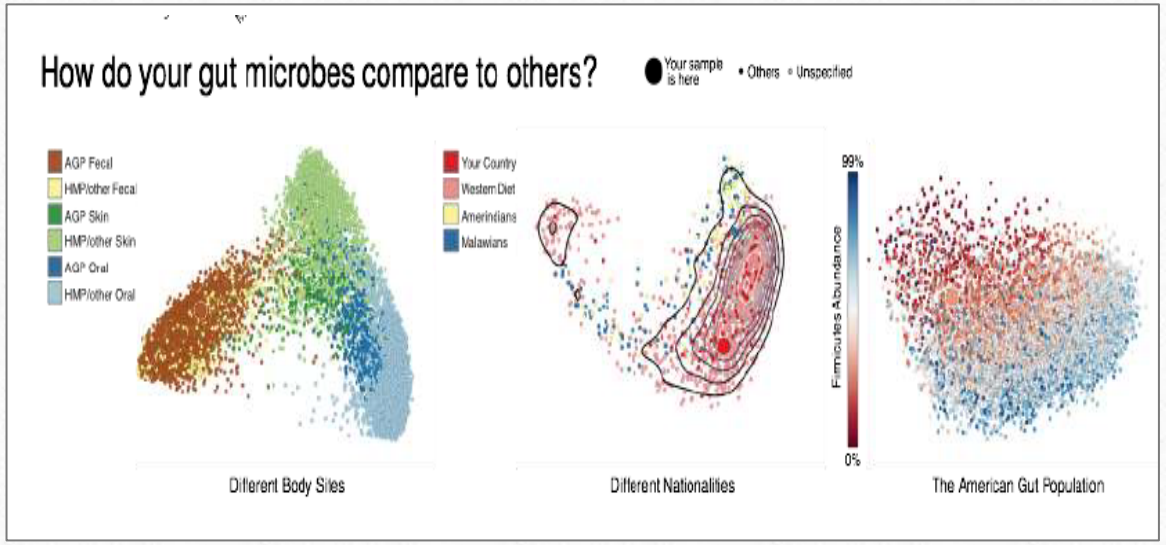
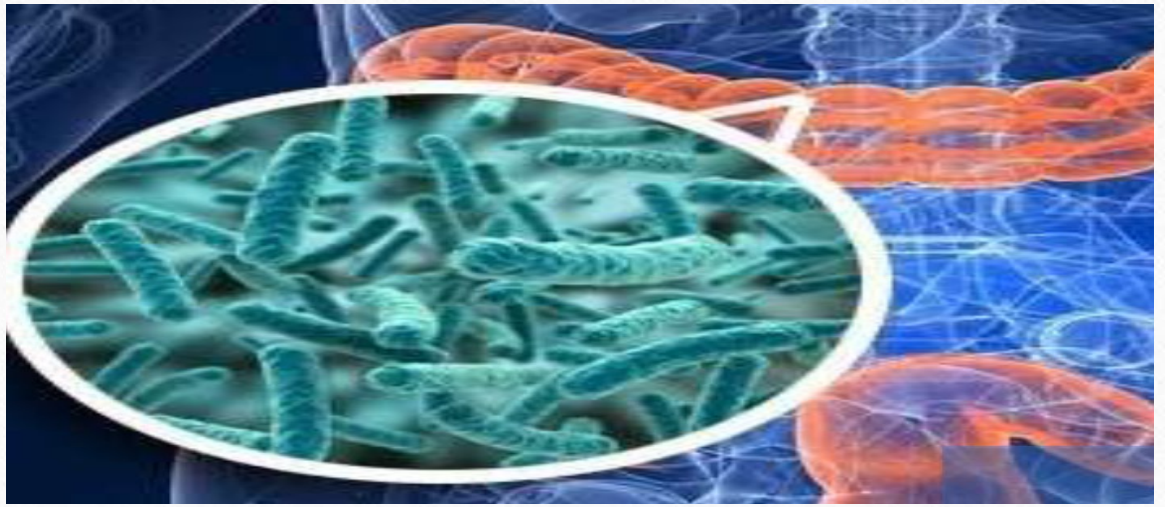


Figure 3. HULM-derived exosomes demonstrated increased angiogenic properties compared to exosomes from normal myometrial cells

Uterine Microbiome







IGut. 2020 Aug 5:gutjnl-2020-321153. doi: 10.1136/gutjnl-2020-321153. Online ahead of print.PMID: 32759302d data

Profiling the Functional Inferences of Endometrial Microbiome in Women With and Without Uterine Fibroids

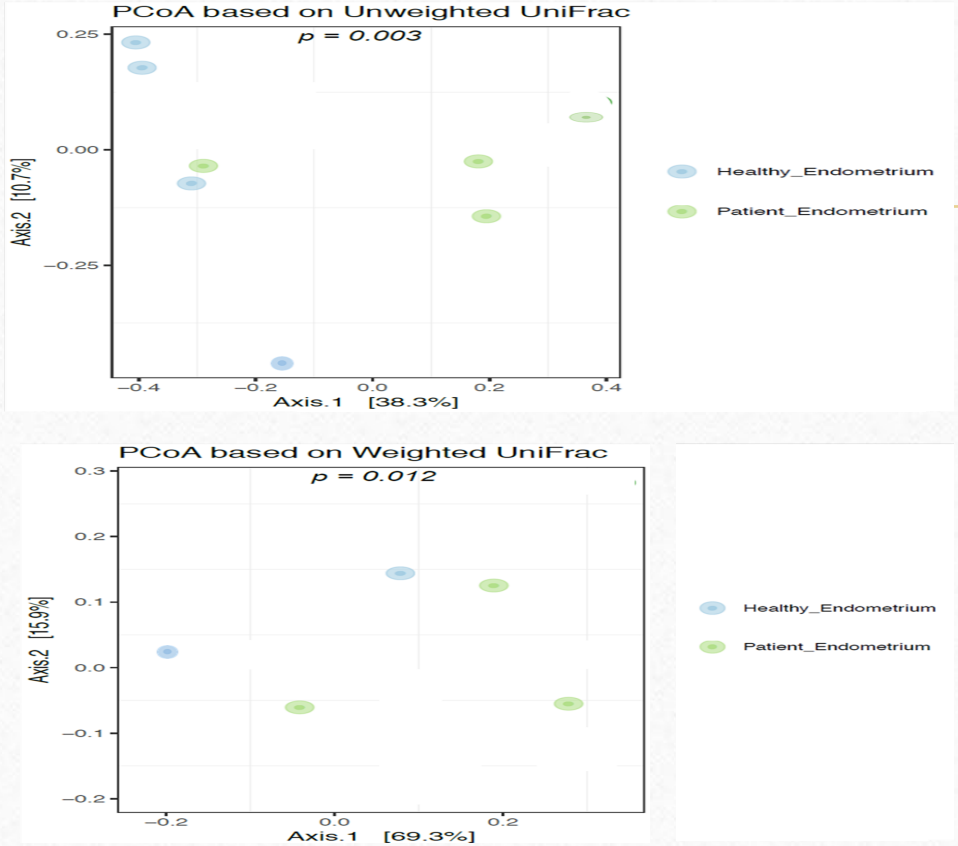


Figure 1: PCoA shows membership variation as determined by the Jaccard diversity calculator. To prevent potential bias from samples with few sequencing reads. Each colored symbol corresponds to an individual sample as indicated. The variation represented by each axis (PC1 or PC2) is indicated as %. Samples grouped by body site in patients versus healthy controls.

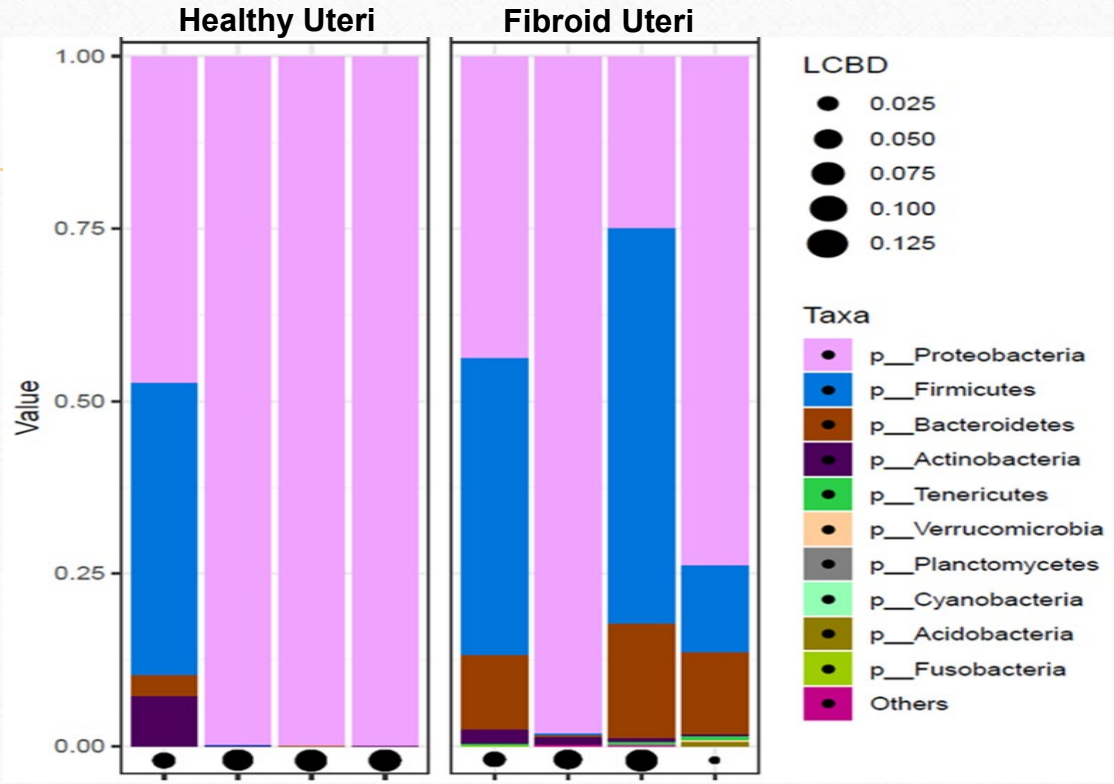


Figure 2. Distribution of phyla by body site in uterine fibroids patients versus healthy controls. Samples from each patient are grouped by individual body site. Each column represents the percent abundance of the indicated bacterial phyla present at each designated body site for an individual patient. Data for the five most common phyla are presented; all remaining phyla were grouped and classified as "other."

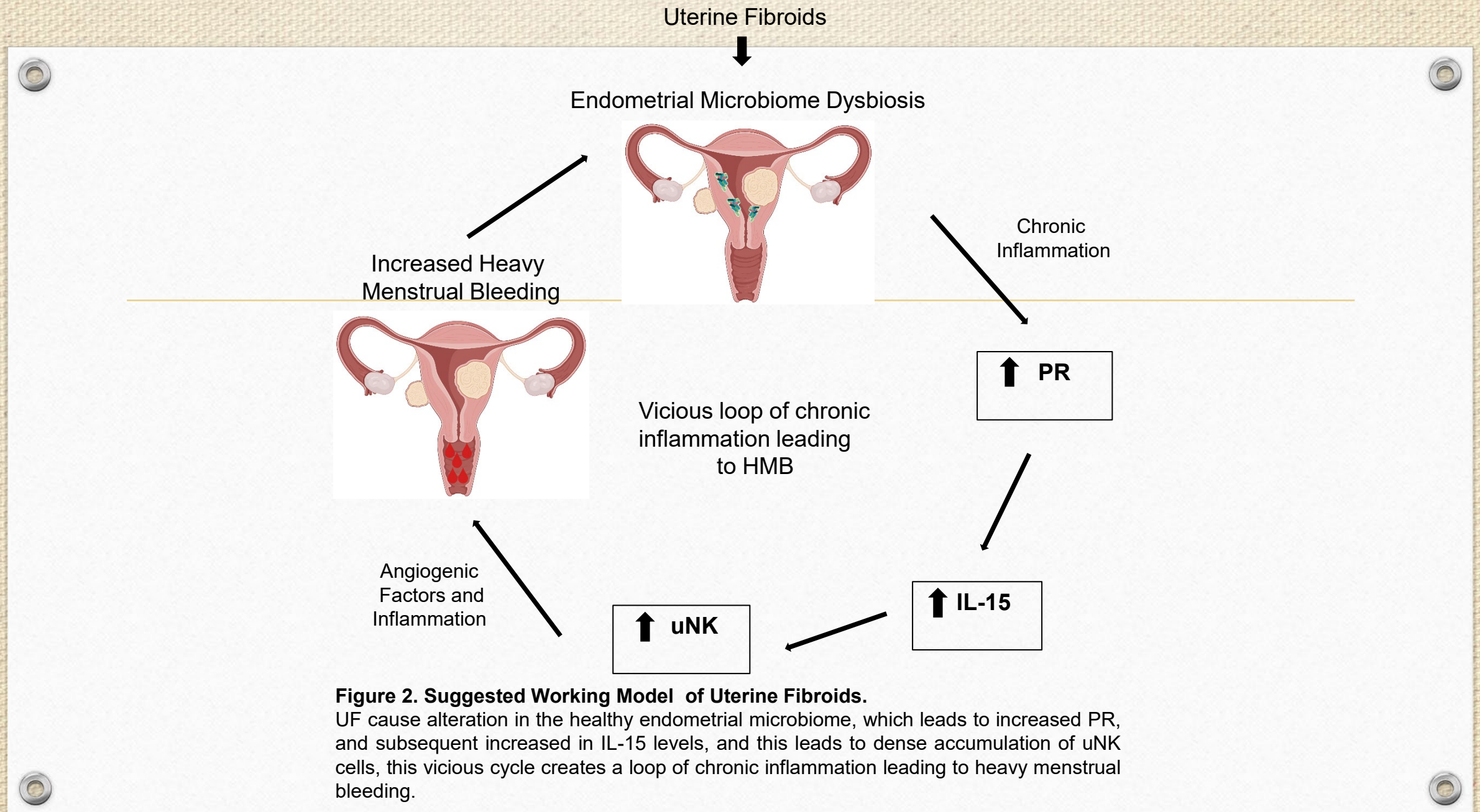


Figure 2. Suggested Working Model of Uterine Fibroids.
UF cause alteration in the healthy endometrial microbiome, which leads to increased PR, and subsequent increased in IL-15 levels, and this leads to dense accumulation of uNK cells, this vicious cycle creates a loop of chronic inflammation leading to heavy menstrual bleeding.

In vitro Three Dimensional Uterine Fibroid Model Fibroid Organoids

Uterine Fibroid Organoids

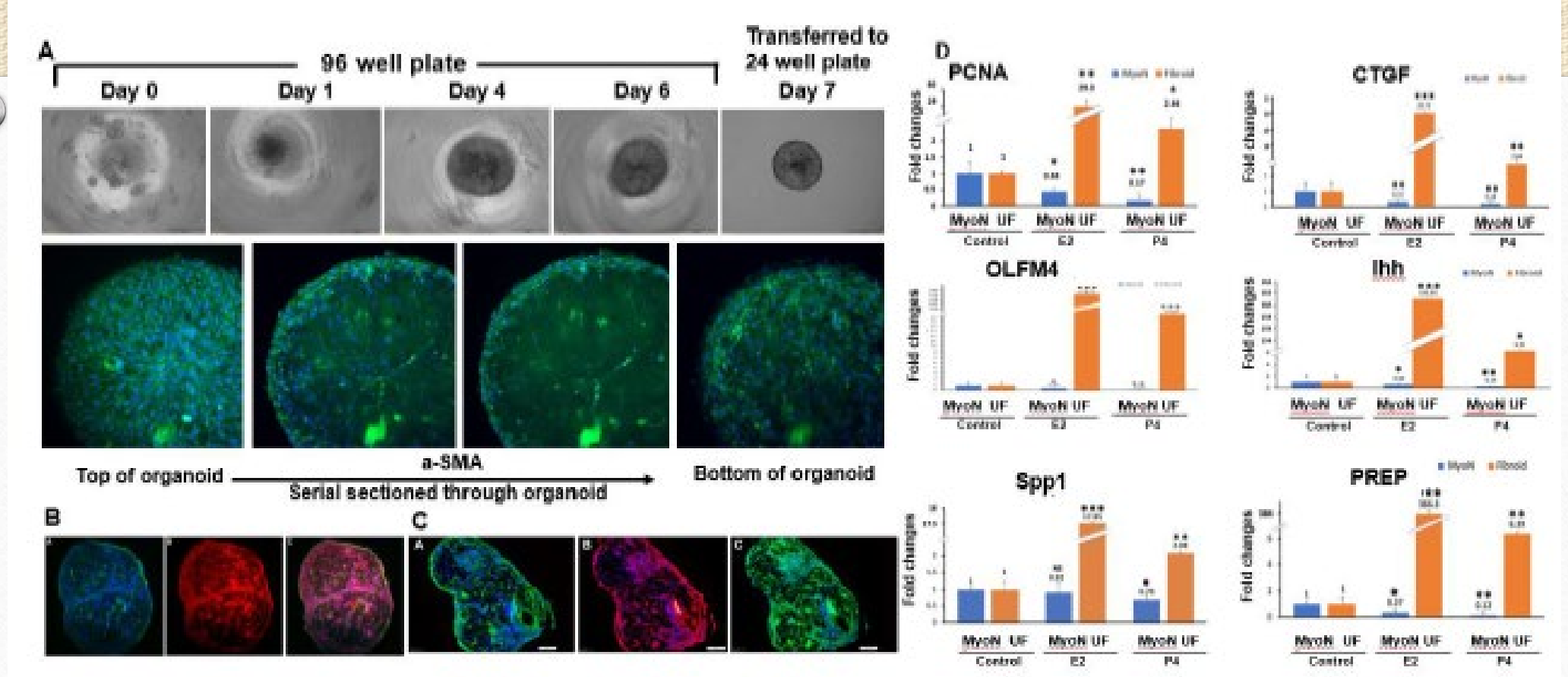
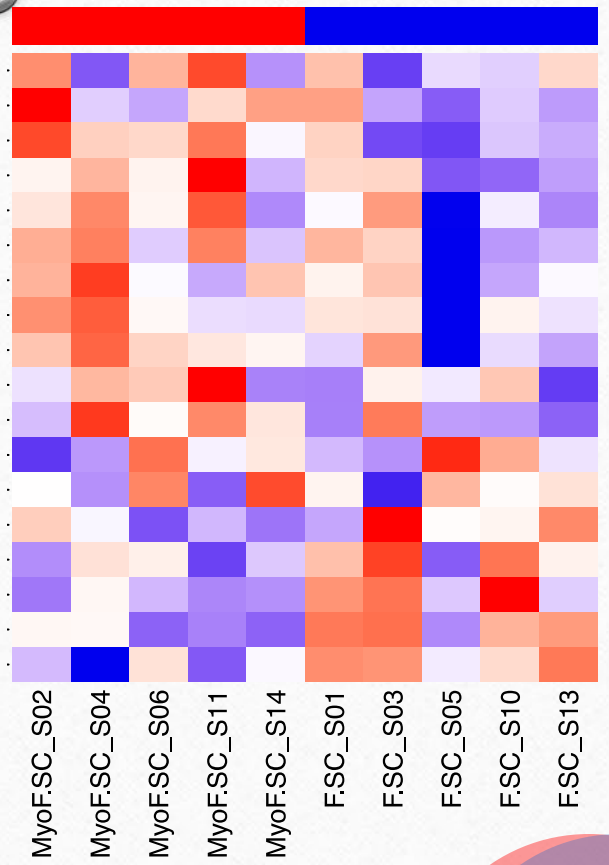


Figure 5. Myometrial and fibroid organoids in culture. (A) Organoid development and Smooth muscle actin cells are organized to the exterior of the organoid. MMSCs were grown in 96-well plate. After 6 days, Matrix/MMSCs were transferred to 24 well plates for further growth (Top). Matrigel was removed and cultured till day 7. After day 7, whole intact organoids were fixed, and immunofluorescence stained with alpha- smooth muscle actin antibody. As shown, serial confocal images, smooth muscle actin cells are organized to the exterior of the organoid with few labeled cellular structures within the interior (right) (N=4, n=6). **(B) Smooth muscle and fibroblast cells in organoids.** The organoids on day 7 stained for alpha-smooth muscle (green) and vimentin (red) (N=4, n=6). smooth muscle cells intercalated with fibroblast cells. (**C) The expression of ERa and PgR in the organoids.** Day 7 organoids were treated with estradiol (E2, 10 ng/ml) only and progesterone (P4, 10 ng/ml) for 72 hr. Organoids from myometrial cells were cryosectioned and processed for immunostaining for ERa and PgR (N=4, n=6). myometrial organoids respond to steroid hormone treatment by the presence of both ERa (Left, green) and PgR (middle, red) in the cytoplasm and nucleus of positive cells. Smooth muscle cells are revealed (green, right). **(D) Organoids derived from TIC showed more hormone responsiveness with identified E2 and P4-stimulated genes compared to organoids derived from low-risk MMSCs** (MyoN, N=4, n=24). The proliferation marker PCNA, the profibrotic markers CTGF, OLFM-4, an extracellular matrix protein, Ihh, a major mediator of progesterone, P4-responsive genes *Spp1* and *PAEP* were significantly up-regulated in both E2 (10 ng/mL) and P4 (10 ng/mL) treated TIC derived organoids compared to MyoN. n=individual organoids per independent experiment (N).*p<0.05, **p<0.01, ***p<0.001. MyoN: myometrium from uterus without fibroids, MyoF: myometrium from uterus with uterine fibroids.

OMICS-Based Discovery of Novel Therapeutics

F over MyoF 6 up, 12 down



ConnectivityMap

Library of Integrated Network-Based Cellular Signatures (*LINCS*)

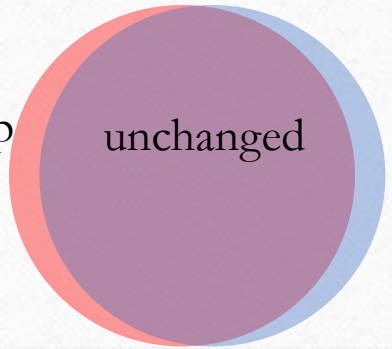
Run using these 18 genes, with all considered "up" for this query

CMap Classes

PHARMACOLOGIC

Sets of compound perturbagens with enrichment scores above 90 (similar) and below -90 (opposing).

DNA dependent protein kinase inhibitor x 4	96.41
Vitamin D receptor agonist x 6	94.37
JAK inhibitor x 5	93.65
TGF beta receptor inhibitor x 4	93.02
HRH1 antagonist x 3	91.60
Progesterone receptor antagonist x 5	91.32
Aurora kinase inhibitor x 14	90.48
Bacterial DNA gyrase inhibitor x 5	-90.64
Glucocorticoid receptor agonist x 44	-95.37
Norepinephrine reuptake inhibitor x 4	-96.10
Sigma receptor antagonist x 3	-96.43
Calmodulin antagonist x 3	-96.48
Bacterial 30S ribosomal subunit inhibitor x 5	-99.45
MDM inhibitor x 4	-99.77



LINCS_F.over.MyoF_18up.txt

Rank	Score	Type	ID	Name
285	96.49	cc		Interleukin receptors LOF
290	96.41	cc		DNA dependent protein kinase inhibitor
406	94.37	cc		Vitamin D receptor agonist
445	93.65	cc		JAK inhibitor
480	93.02	cc		TGF beta receptor inhibitor
549	91.76	cc		BMP Signaling LOF
558	91.6	cc		HRH1 antagonist
569	91.36	cc		MCM family LOF
571	91.32	cc		Progesterone receptor antagonist
587	91.03	cc		Structural maintenance of chromosomes proteins LOF

10.23.20

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