Human Endometrium, Endometrial Stem/Progenitor Cells and their Potential in Regenerative Medicine in Women's Health

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NATIONAL ACADEMIES Sciences Engineering Medicine

Forum on Regenerative Medicine
Forum on Temporomandibular Disorders

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MONASH University

Conflict of Interest Disclosure Form

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AFFILIATION: …Hudson Institute of Medical Research and

Monash University Department of Obstetrics and Gynaecology

DISCLOSURE

I have no potential conflict of interest to report

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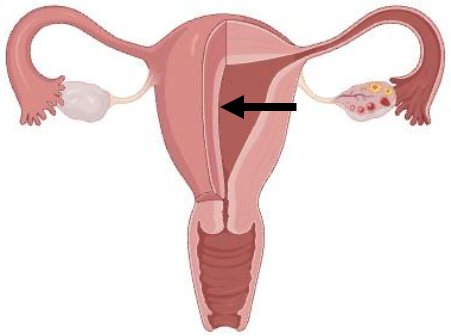
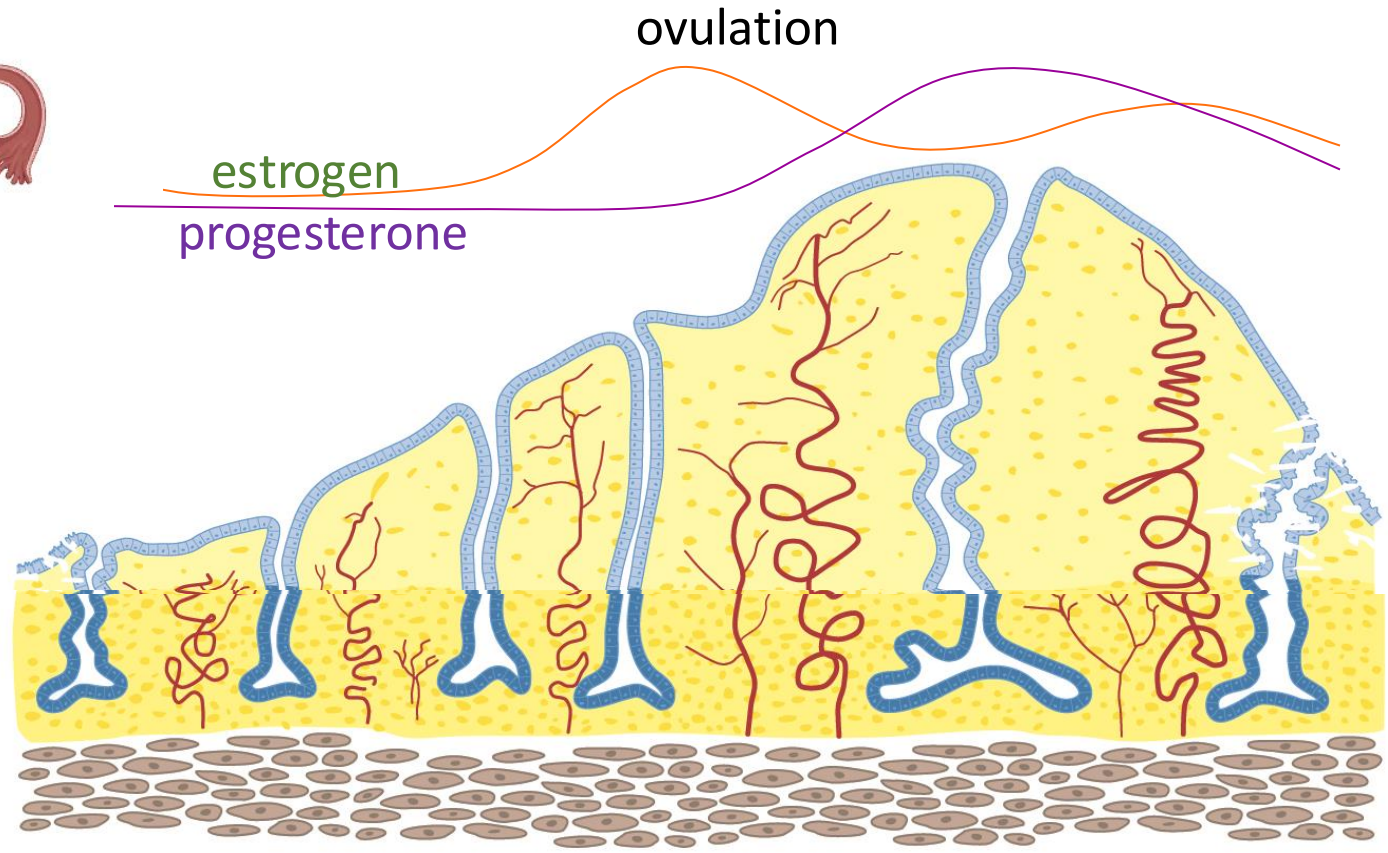
The Remarkable Regenerative Human Endometrium





- Menstrual cycle 400X
- Parturition
- Resection
- Postmenopausal

Gargett et al Mol Cell Endocrinol, 2008; Gargett et al Rev Endocrinol Metabol Disorders, 2012

Endometrial stem/progenitor cells likely
- reside in the endometrial basalis and
- responsible for cyclical regeneration of the functionalis

Gargett CE Hum Reprod Update 2007

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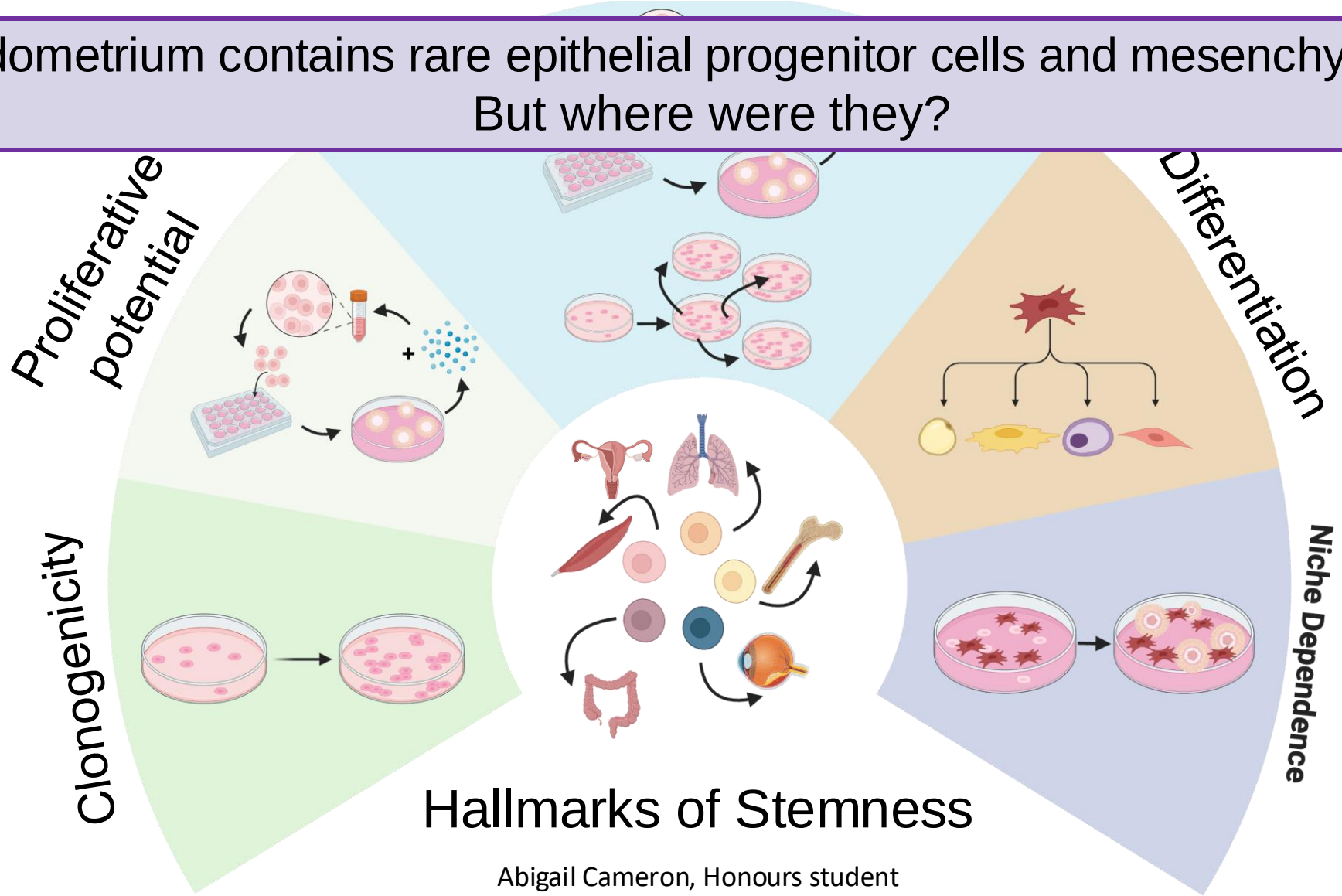
Self-renewal

Human endometrium contains rare epithelial progenitor cells and mesenchymal stem cells. But where were they?

Rachel Chan

Kjiana Schwab



Hallmarks of Stemness

Abigail Cameron, Honours student

Chan RWS et al Biol Reprod 2004
Schwab K et al Fertil Steril 2005
Gargett CE et al Biol Reprod 2009

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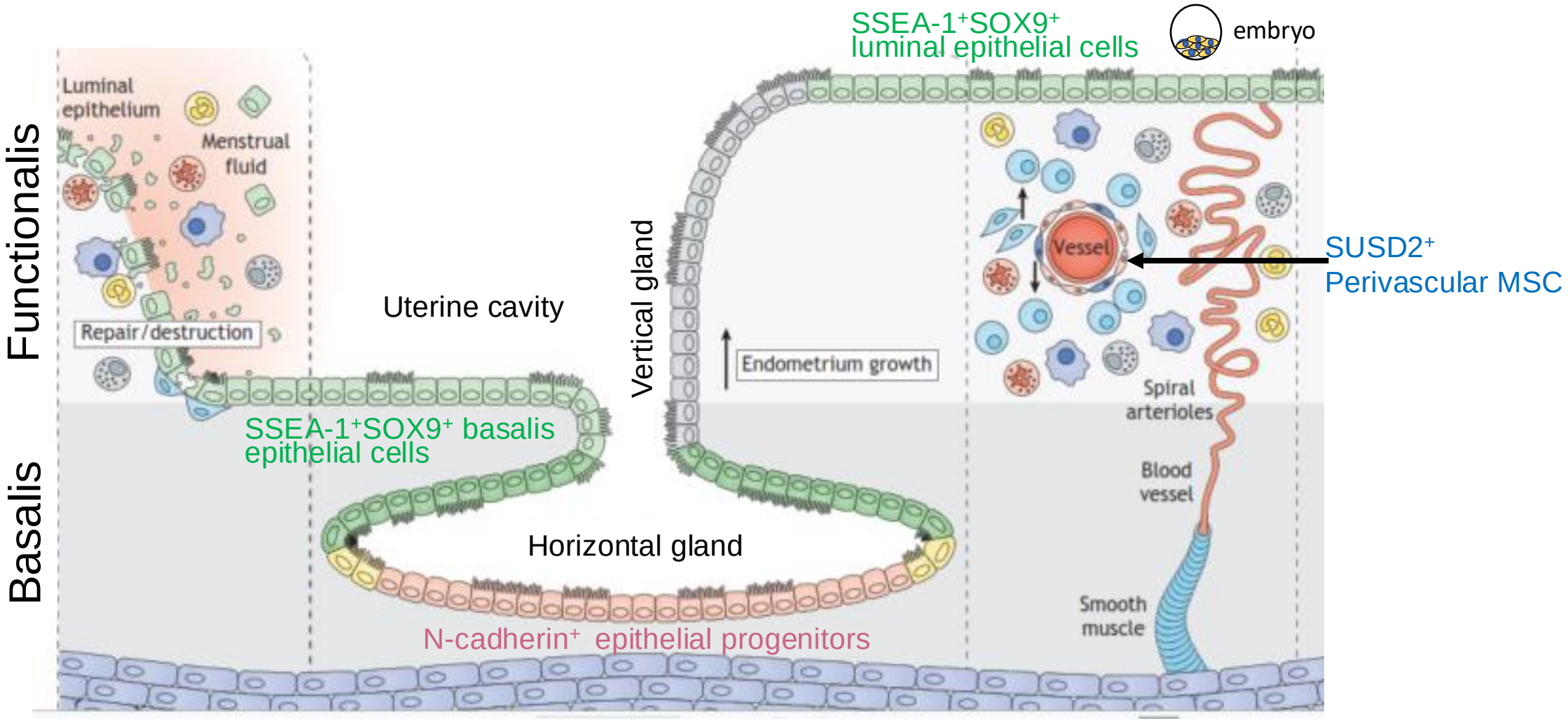
Where are the endometrial epithelial progenitors and MSC? What is their role in endometrial regeneration?



Salamonsen…..Gargett CE Development 2021

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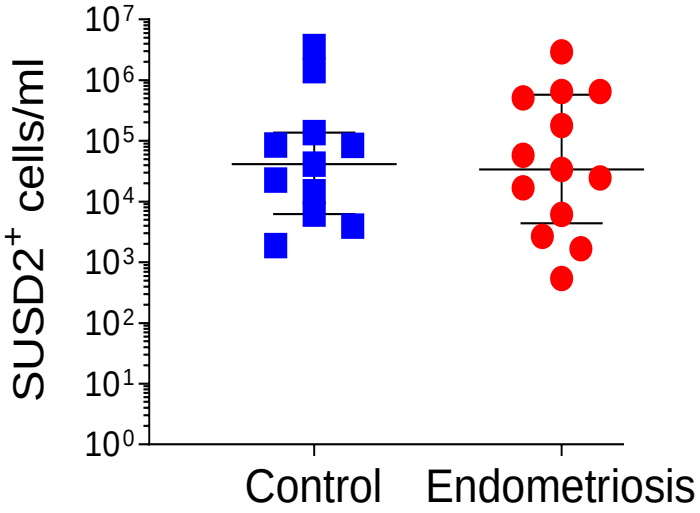
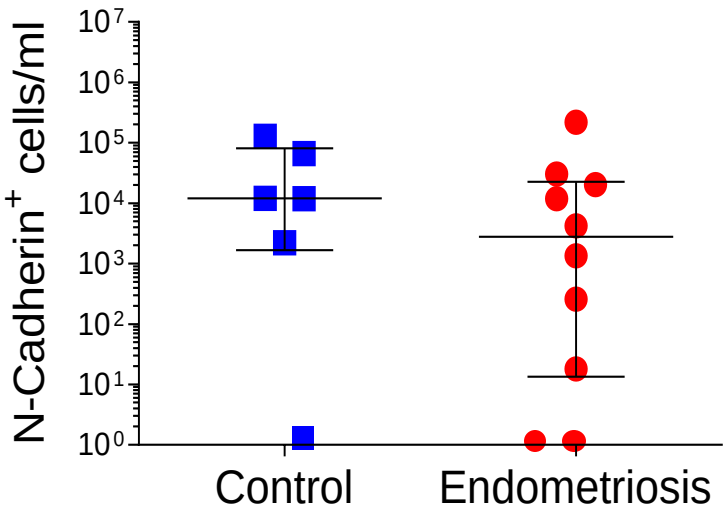
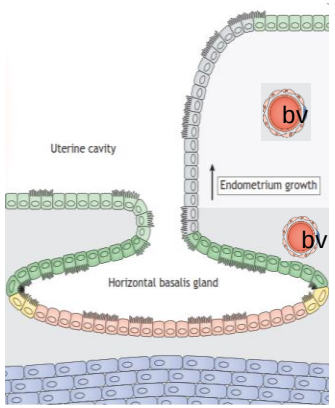
Menstrual Fluid: Source of Stem Cells for Regenerative Medicine







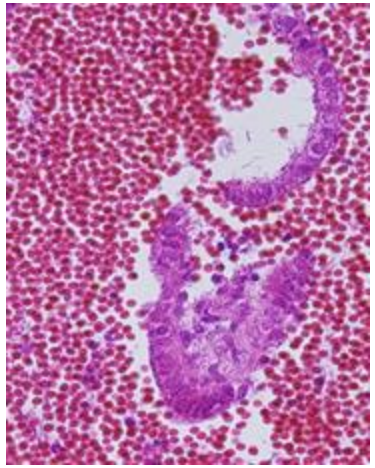
Masuda H, Schwab K et al RBMO 43:3-13, 2021

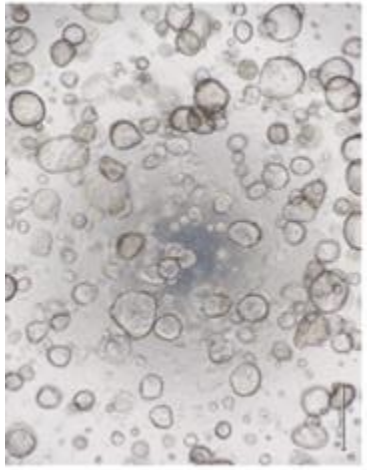
Hiro Masuda

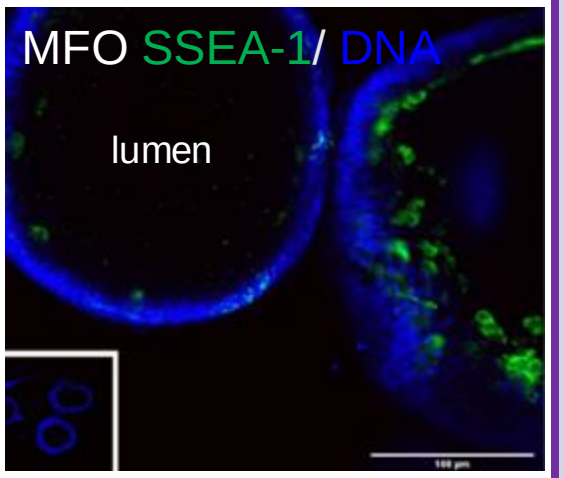
Kjiana Schwab

Menstrual fluid Menstrual fluid organoids

Caitlin Filby







Filby C et al J Personalized Medicine 2021

Menstrual fluid is a non-invasive source of endometrial tissue

- SUSD2+ eMSC
- N-cadherin+ epithelial progenitors
- for generating patient-specific organoids to evaluate drug responses for personalised medicine

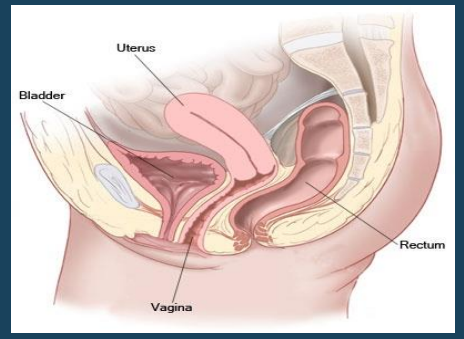
Pelvic Organ Prolapse (POP)

Definition Downward descent (hernia) of pelvic organs into the vagina

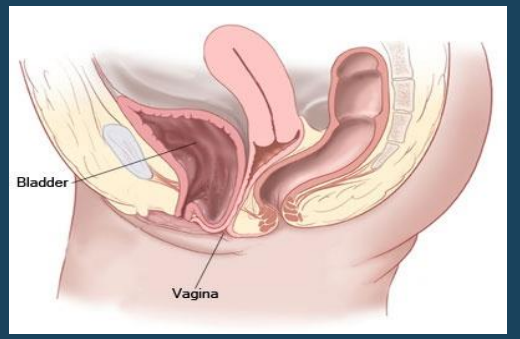
Symptoms Urinary, bowel and sexual dysfunction; Bulge

Etiology

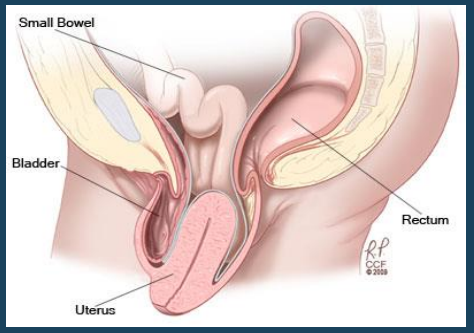
- Vaginal birth injury
- Ageing, exacerbated by obesity



normal pelvis



prolapsed bladder and bowel

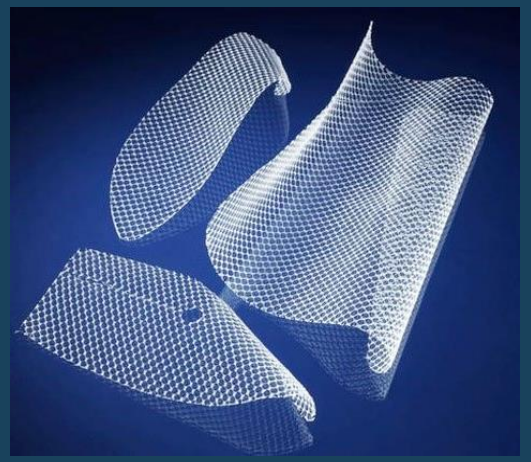


prolapsed uterus

Incidence 25% of all women

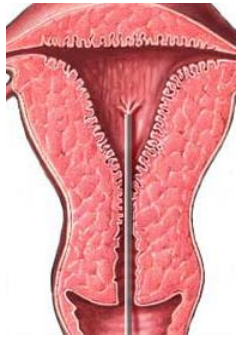
Treatment

- 19% will have POP surgery
- 15-29% risk of multiple repeated operations



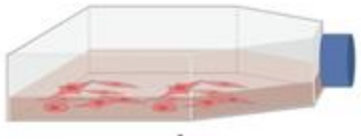
bladder erosion

Tissue Engineering for POP with Autologous Endometrial MSC and Novel Biomaterials



endometrial biopsy

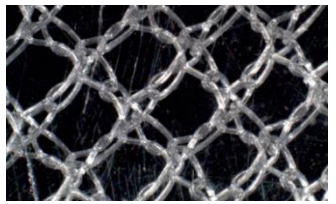
eMSC purification



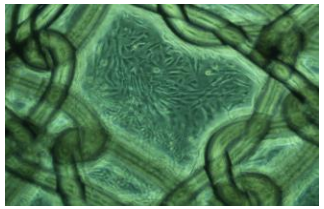
eMSC expansion

+

knitted



fabricate novel scaffolds



tissue engineering construct

eMSC

- ↑angiogenesis
- Modulated the macrophage response and switched M1 to M2 macrophages
- Promoted mesh integration

small animal models

D Ulrich et al, Tissue Engineering A, 2014
S Edwards et al, Acta Biomaterialia, 2015
S Darzi et al Scientific Reports, 2018

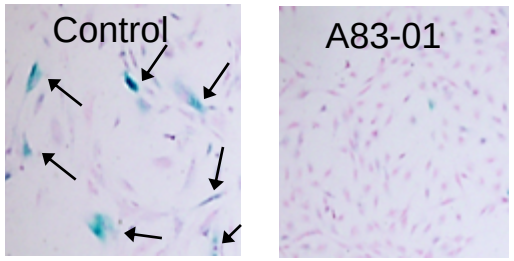
How to Expand SUSD2⁺ eMSC Fit for Clinical Use


Shanti Gurung

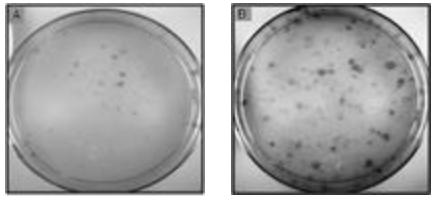

Raffaella Lucciola

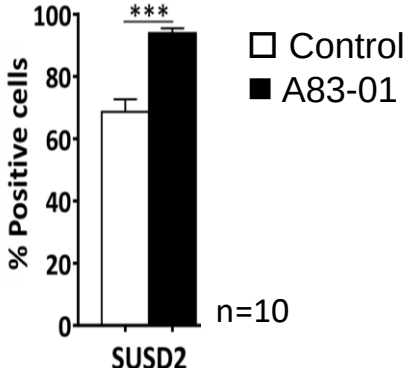
A83-01, a TGFβ Receptor Inhibitor

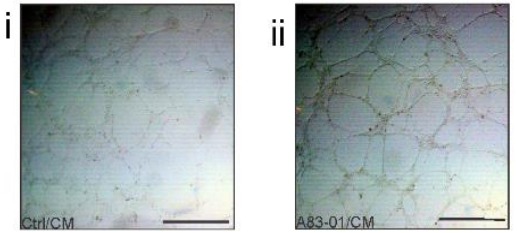
- Maintains the proportion of SUSD2⁺cells
- Maintains clonogenicity
- Promotes proliferation
- Represses senescence genes at chromatin and transcript levels and blocks apoptosis and senescence
- Upregulates angiogenesis genes and promotes angiogenesis
- Upregulates MSC potency genes *TWIST1, TWIST2*
- Induces anti-fibrotic genes
- No pluripotency genes
- Survive *in vivo*

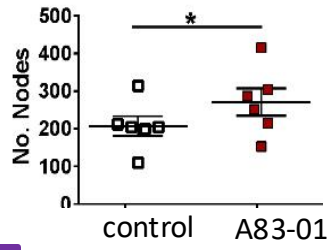


β-galactosidase

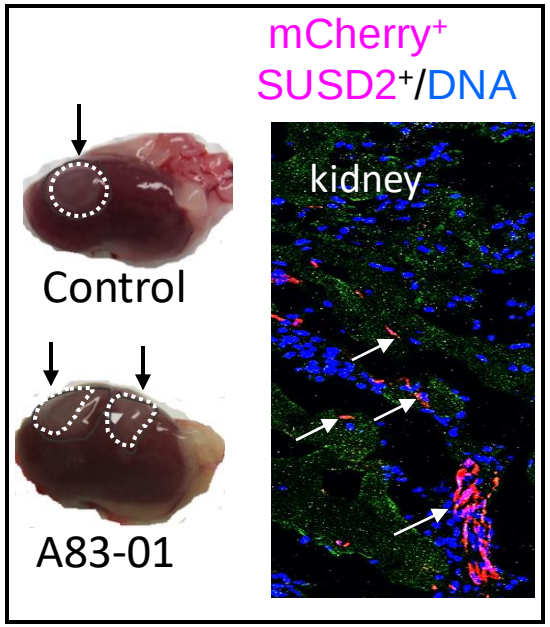








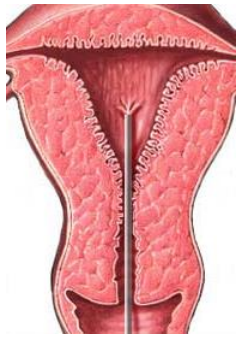
S Gurung et al Front Cell Dev Biol 2018
R Lucciola et al Front Cell Dev Biol 2020



S Gurung et al, Sci Reports 2015
S Gurung et al, Stem Cells Devel 2018

A83-01-treated eMSC have a desirable gene expression profile and function for clinical translation

Tissue Engineering for POP with endometrial MSC and Novel Biomaterials



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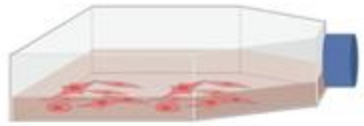
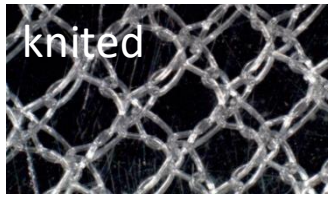
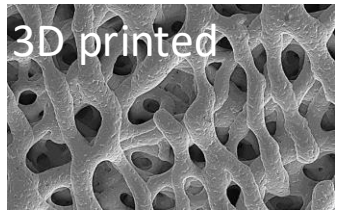


eMSC expansion

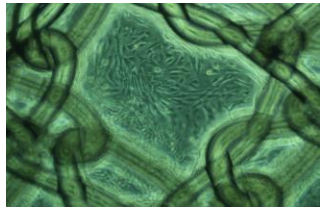
+



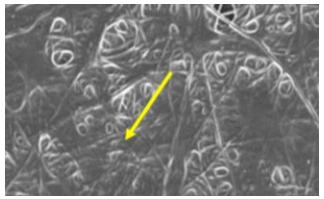


Kallyan Paul

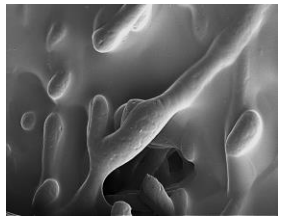
fabricate novel scaffolds

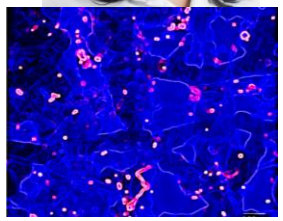


Jerome Werkmeister



Shayanti Mukherjee

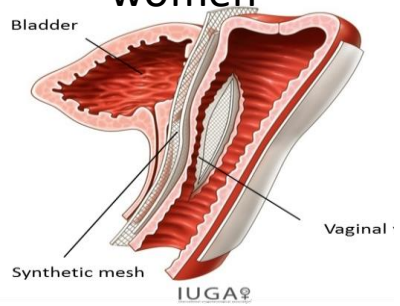




tissue engineering construct

small animal models

women



Emmerson et al, World J Stem Cells 2016
Gargett et al Curr Opin Urol 2019
Mukherjee et al Interface Focus 2019
Hennes et al J Personalized Med 2021

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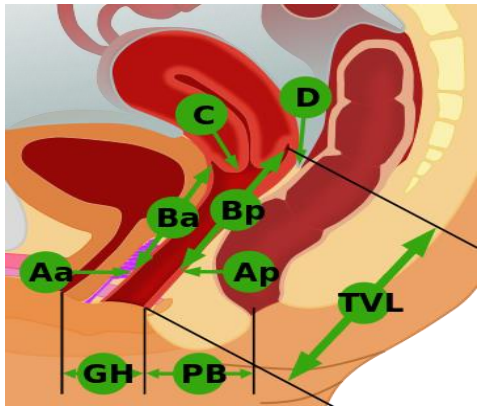
Developing a Pre-Clinical Animal Model for Treating POP with eMSC/Mesh Constructs - Parous Ewes

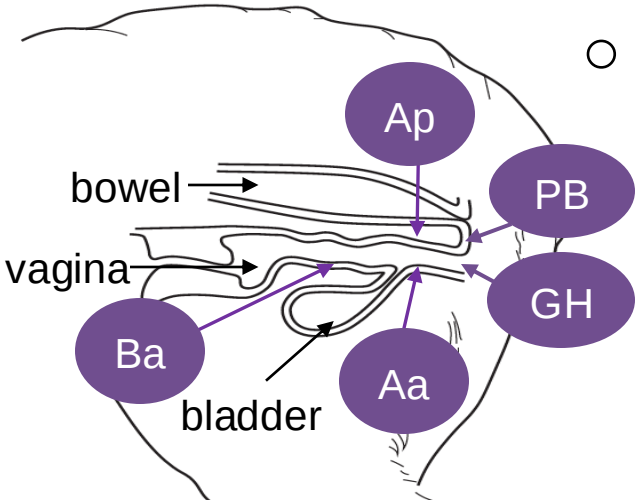

Anna Rosamilia


Natharnia Young

POP-Q



- Adapted a clinical measure of POP severity – the POP-Q to measure vaginal wall weakness
- On a flock of 58 ewes at 3 POP-Q points
  - Multiparous > Primiparous > Nulliparous (P<0.001) for vaginal wall weakness
  - Contributing factors resulting in ovine “POP” were Age OR 3.0 and Parity OR 7.6



- Highlights the importance of clinical collaborators – urogynecologists
- Allowed us to develop a vaginal surgery model to asses our constructs

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N Young et al Internat J Urogynecol, 2017

Recapitulating Human POP Surgery in an Ovine Model

Preclinical vaginal surgery model

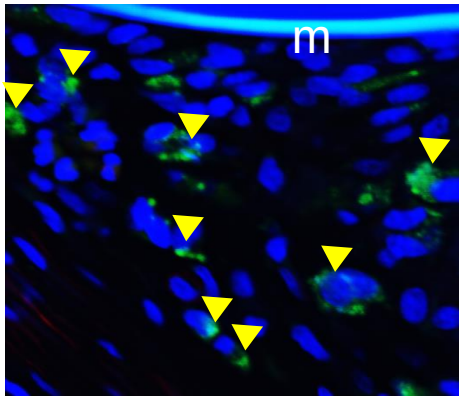
multiparous ewes
≥ 3 deliveries

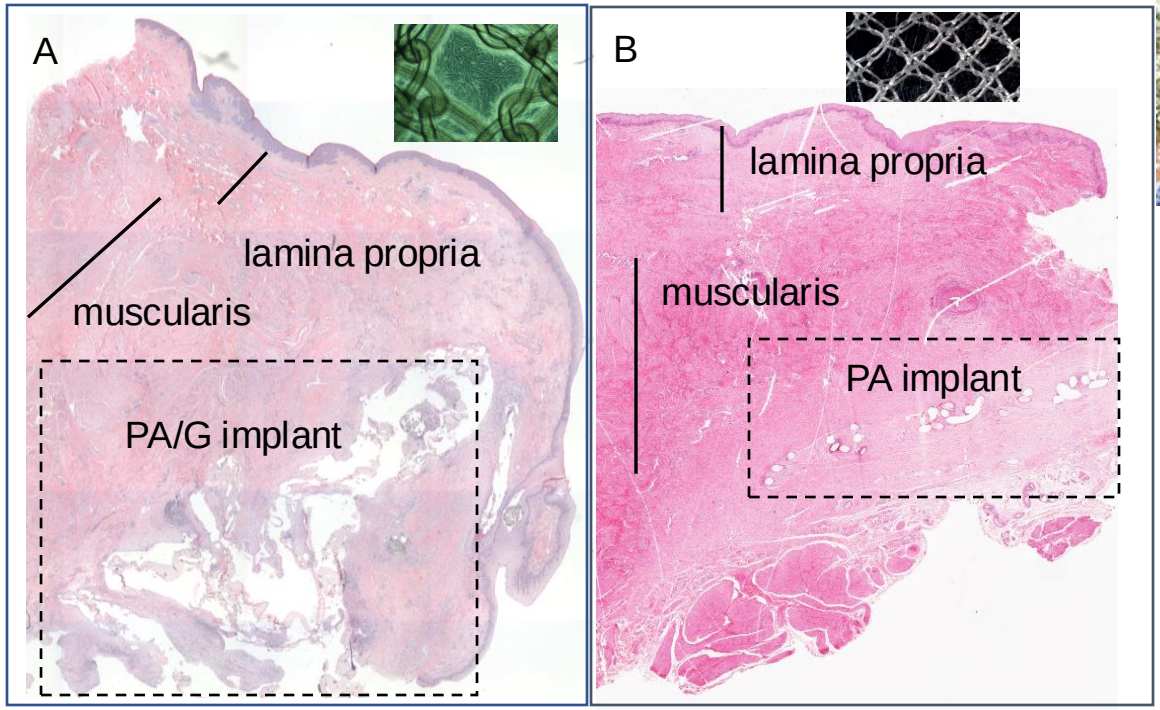
Diagnosis of "POP"

- **Modified POP-Q**
  Ap -1 (-2 to 0)
  Aa 0 (-2 to 0)

Ovine eMSC - 2 step surgical procedure

- Autologous eMSC persisted 30 days
- Modulated the inflammatory response to mesh





Exposures 5/12 0/12

Emmerson S et al Biomaterials 2019

Stuart Emmerson

- Ovine vaginal surgery model recapitulates human condition
- Nondegradable PA mesh format determines mesh exposure and tissue integration

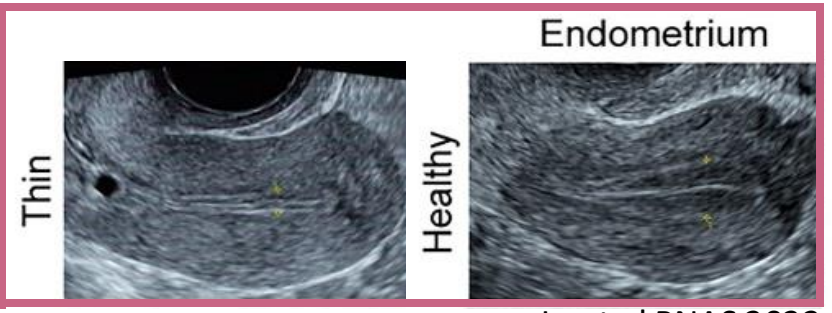
Regenerating Endometrium for Endometrial Disorders

Asherman's Syndrome/IUA

Damage/loss of normal stem/progenitor cells

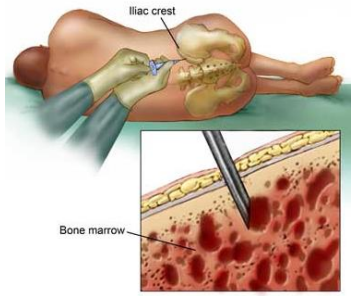
Thin unresponsive endometrium (TUE)

Diminished activity of normal stem/progenitor cells



Lv et al PNAS 2022

Stem Cells to Regenerate Endometrium



Cell type	BMMSC
AS/IUA	4 instil 4 inject
TUE	2 inject

Rodriquez-Eguren et al Human Reprod Update, 2024

Issues

- MSC vs Fibroblasts
- Method of delivery
- Tracking delivered cells
- Survival of delivered cells
- Mechanism of action
  - Paracrine
  - Cell incorporation
  - Stimulate endogenous SC
- Epithelial vs stromal cell type to be regenerated

Acknowledgements

Endometriosis and Vaginal Stem Cell Team

POP Team Principal Investigators

Australian Government
National Health and Medical Research Council
NHMRC

DEPARTMENT OF DEFENSE
UNITED STATES OF AMERICA

The Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Excellence in Women's Health

MAGEE-WOMENS
RESEARCH INSTITUTE & FOUNDATION

CASS
The CASS Foundation

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SCIENCE AND INDUSTRY ENDOWMENT FUND

Rebecca L. Cooper
Medical Research Foundation

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