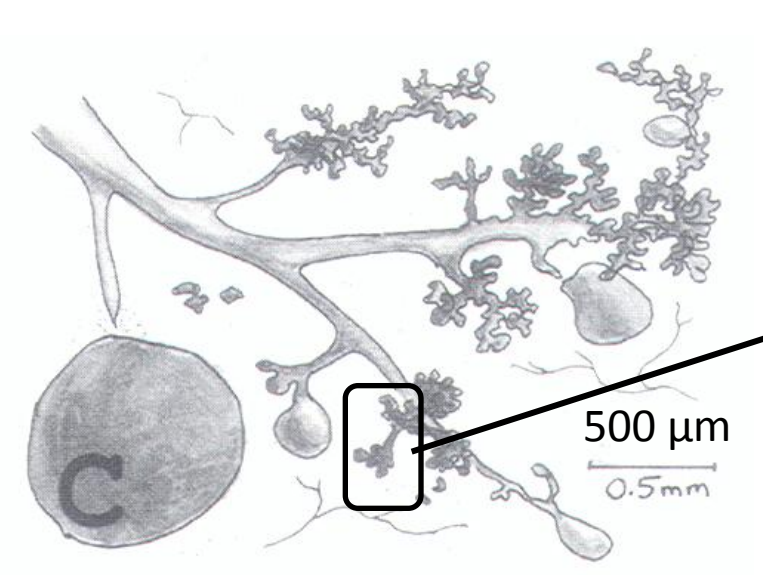
Agricultural Research Organization
The Volcani Center
Institute of Animal Science
Israel

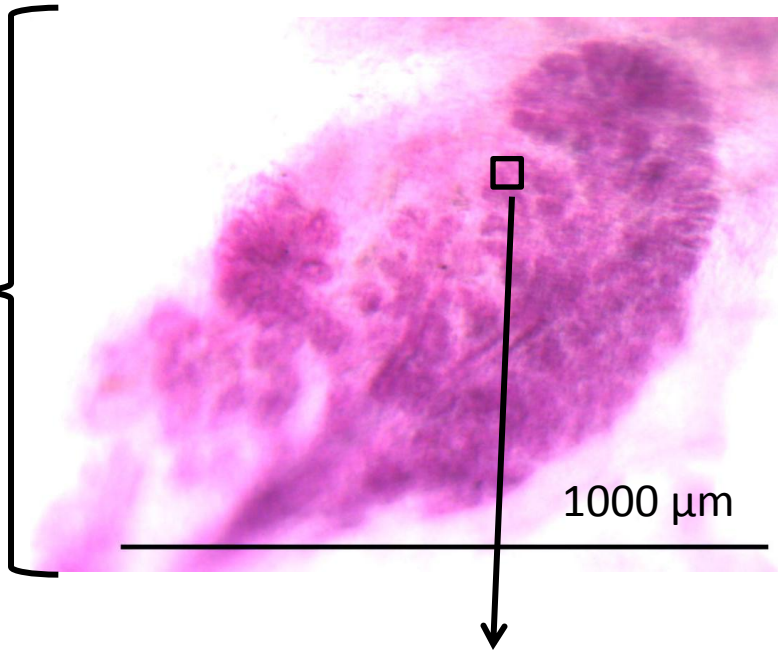


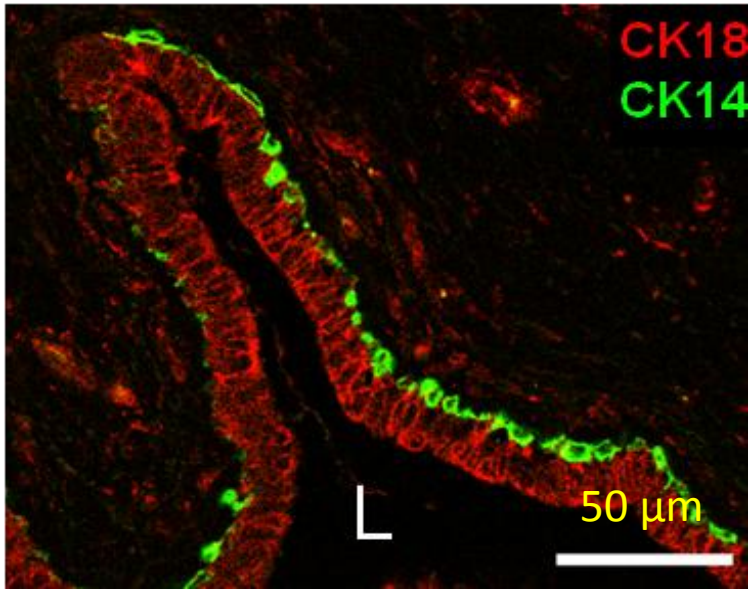
Delineating Stem Cells and Cell Hierarchy in the Bovine Mammary Gland

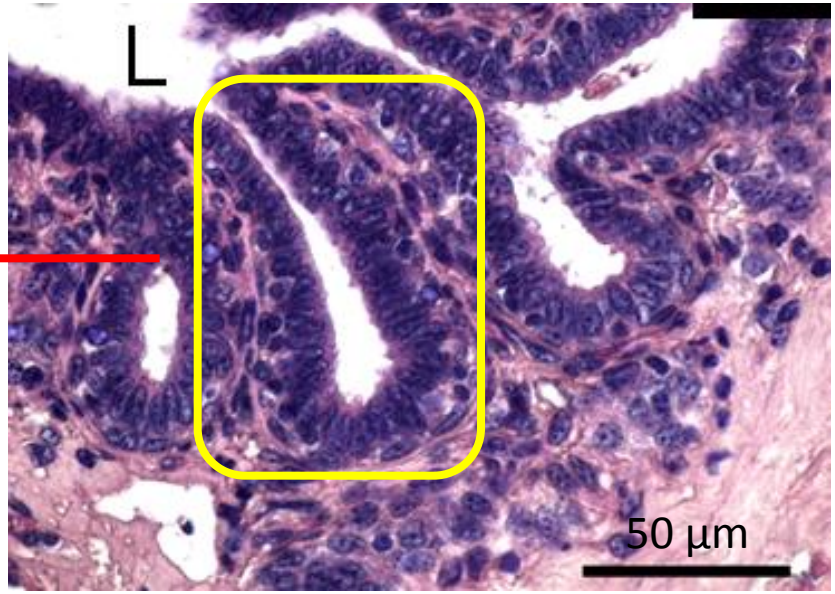
Itamar Barash Ph.D.

Bovine mammary gland morphology









Mammary gland development is synchronized with the reproduction cycle



Virgin

Pregnancy

Lactation

Involution

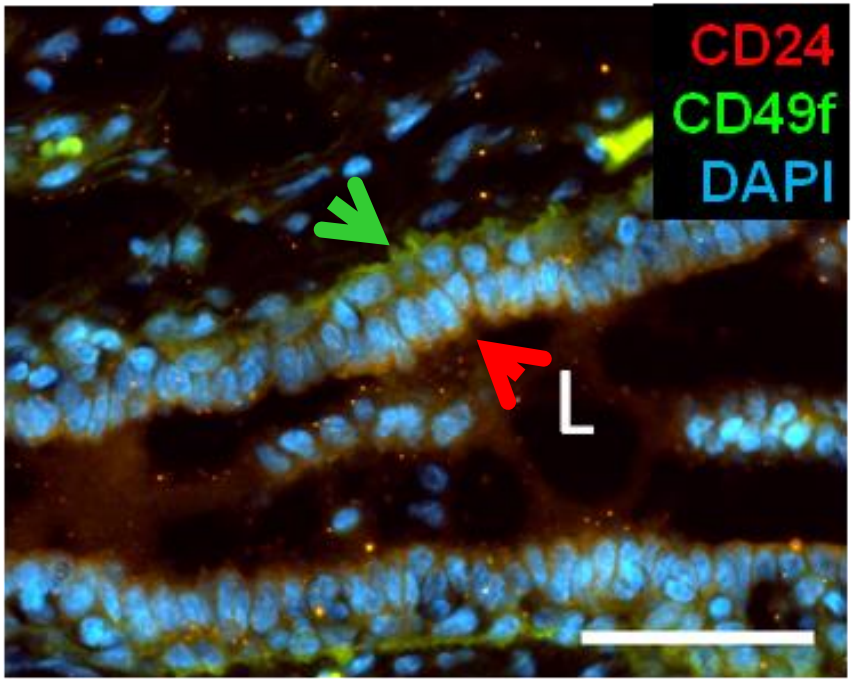
Indeed, in addition to the basal myoepithelial layer and the luminal alveolar or ductal epithelial layers, the mammary gland encompasses a poly-regenerative entity that enables its renewal between successive reproduction cycles

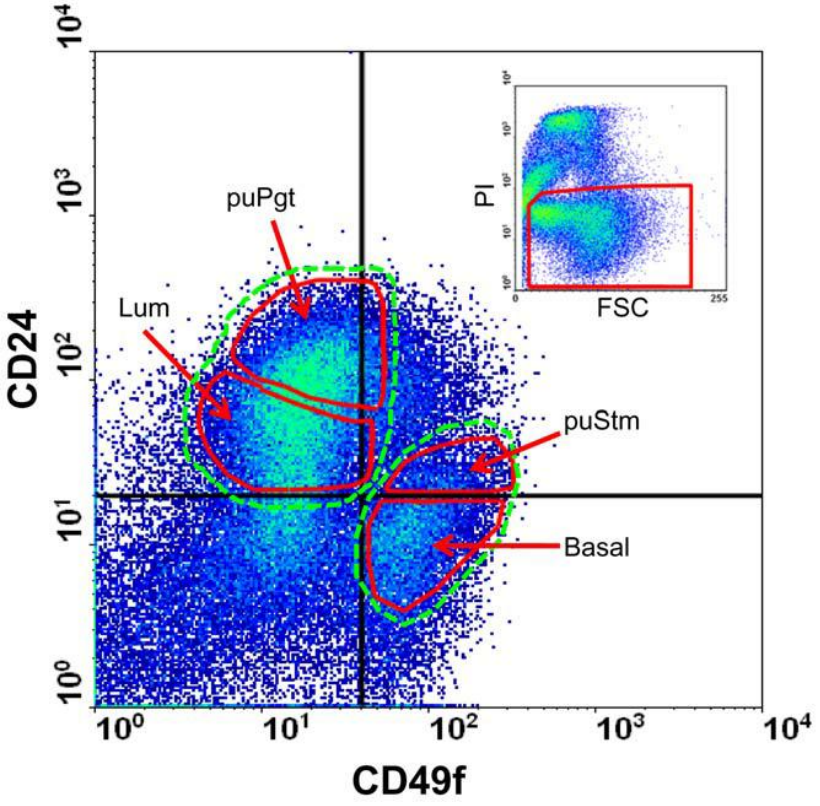
Identifying and harnessing this regenerative entity in order to maintain a highly functional mammary gland in dairy cattle is the goal of our studies

Mammary epithelial cells were sorted into 4 populations according to the expression of CD49f and CD24

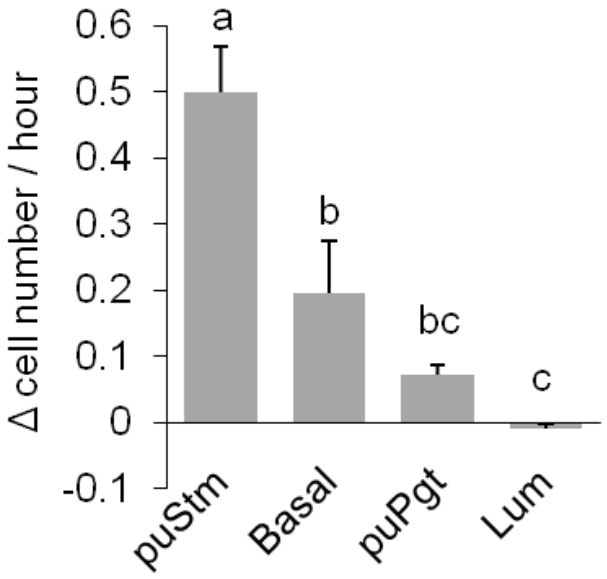


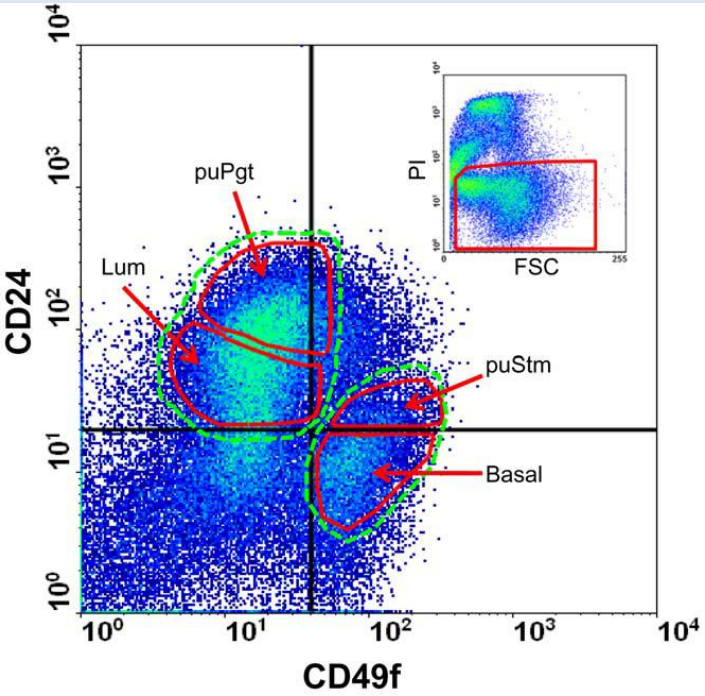
Gat Rauner

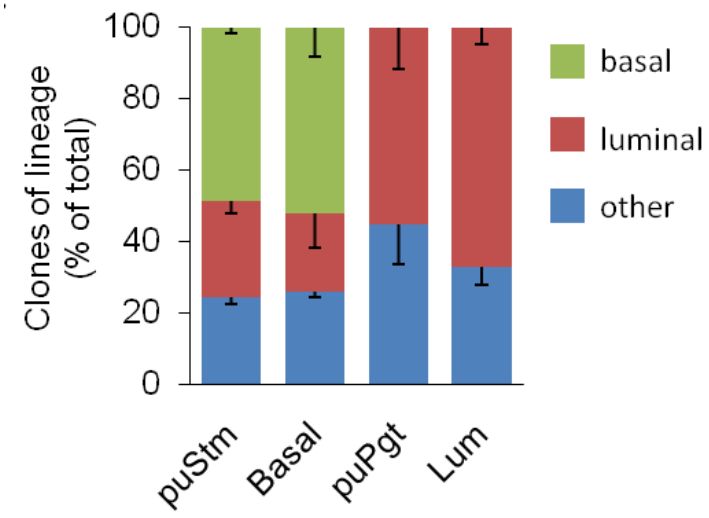


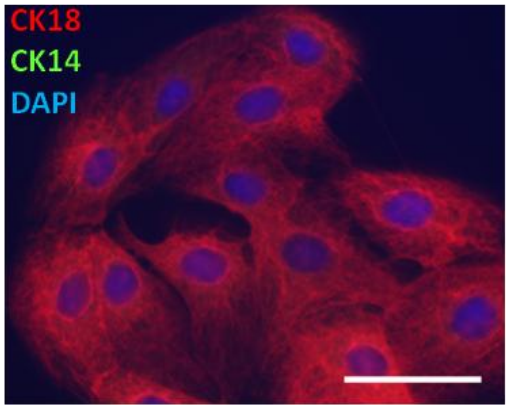


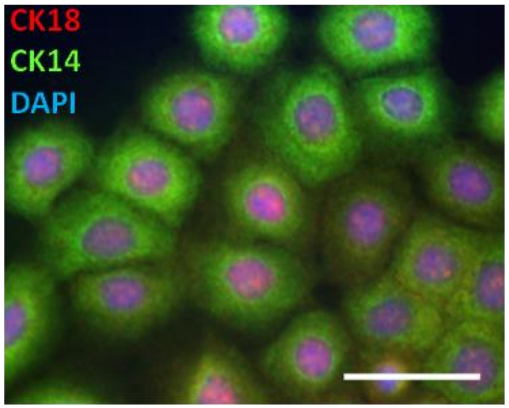
Bovine mammary stem cells generate luminal and basal clones and maintain high propagation potential



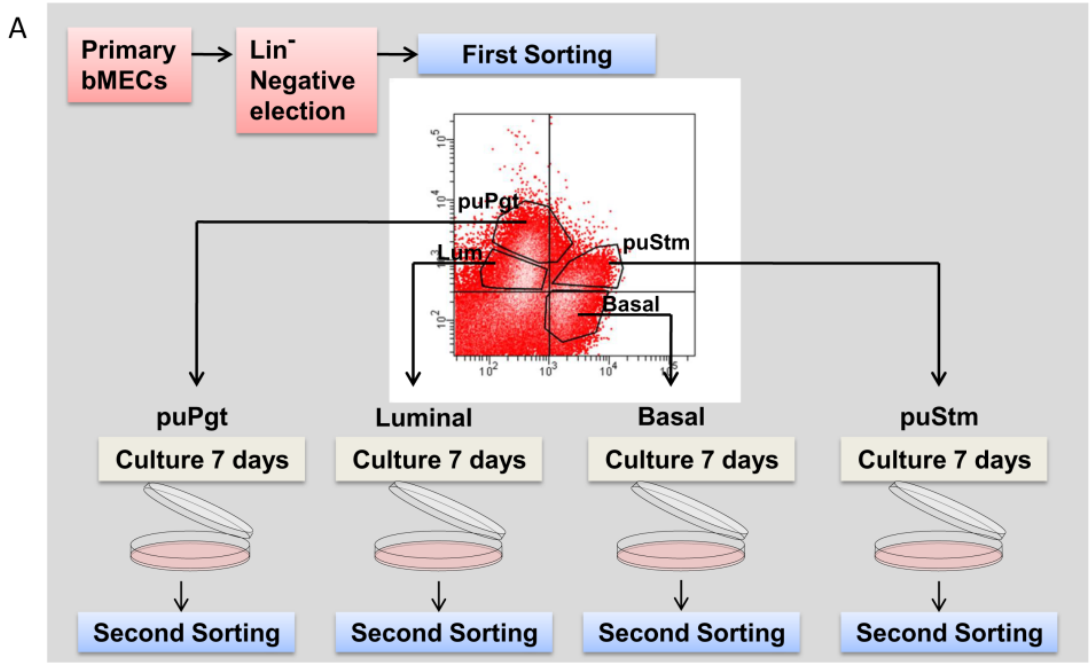


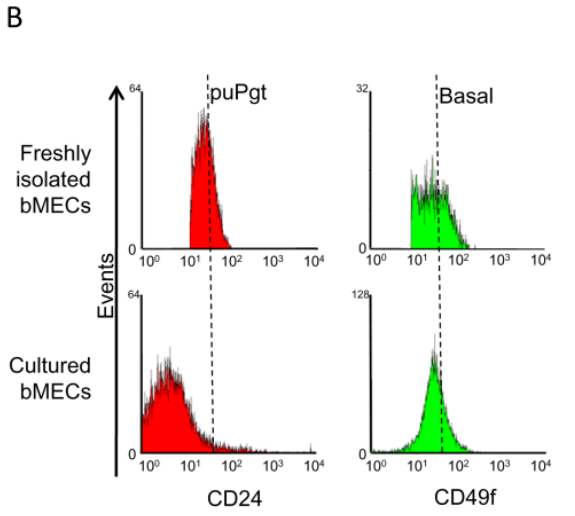


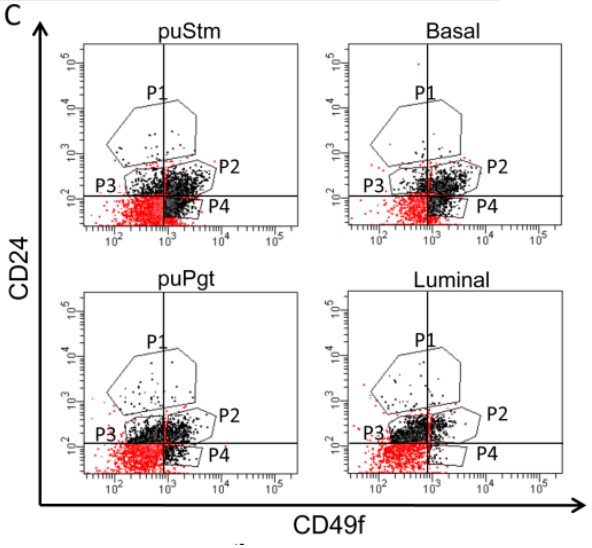


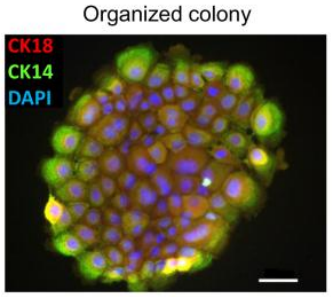


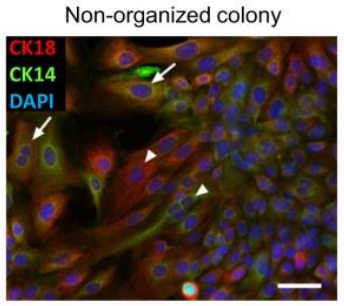
Cultured bMEC populations do not differ in their CD49f/ CD24 expression, but maintain their distinct parental characteristics



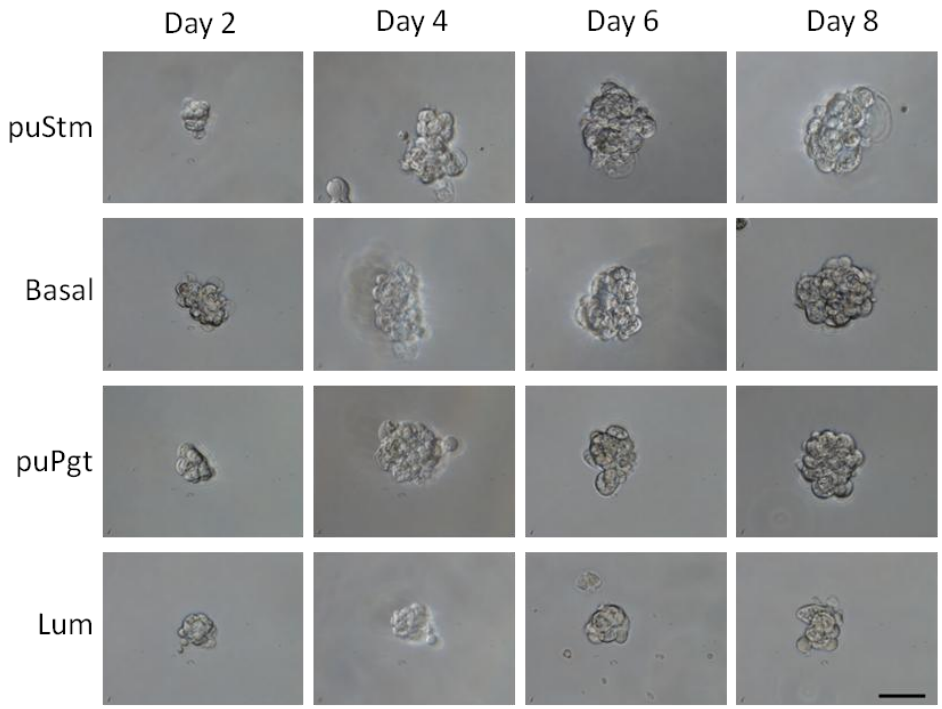


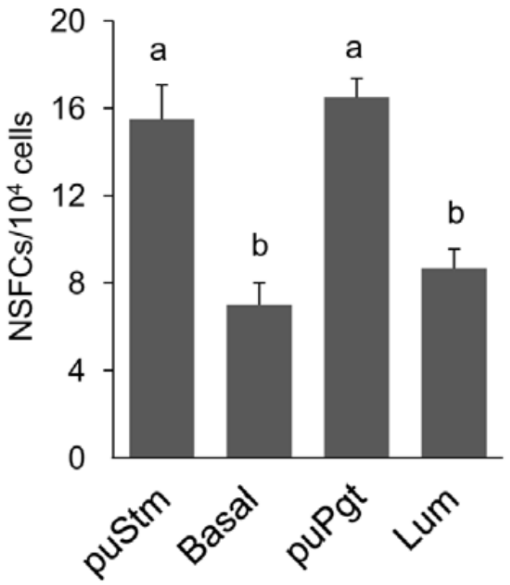


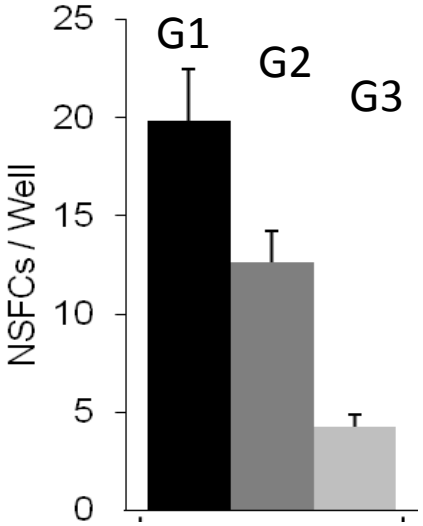




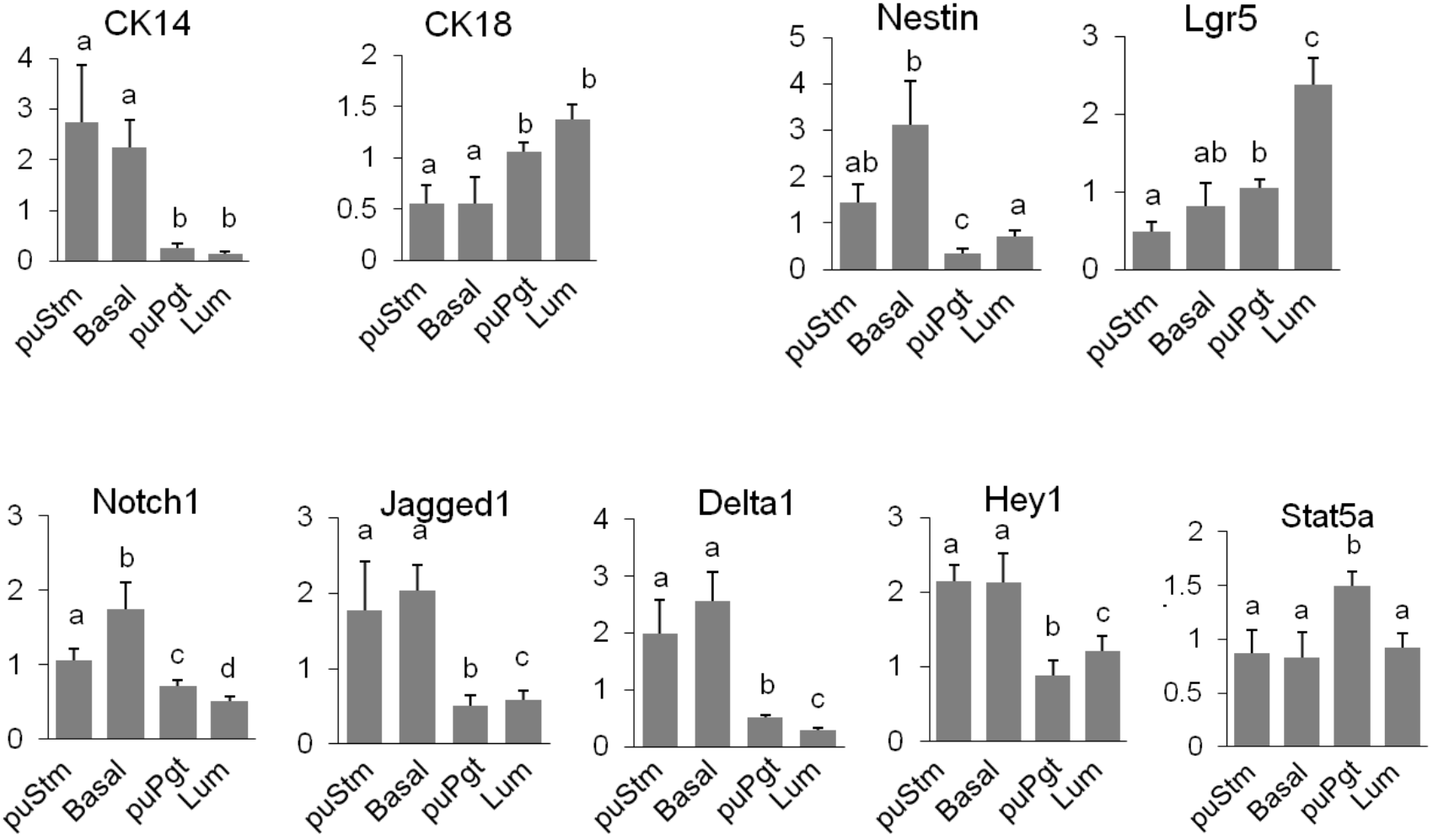
Floating mammosphere formation by bovine mammary epithelial cells



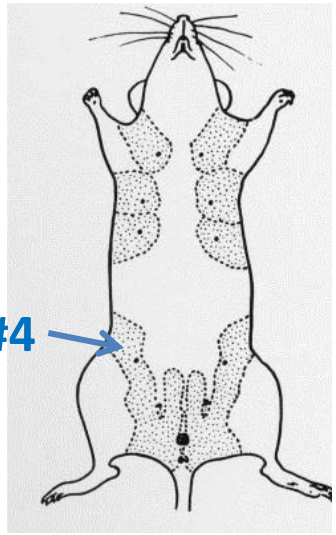


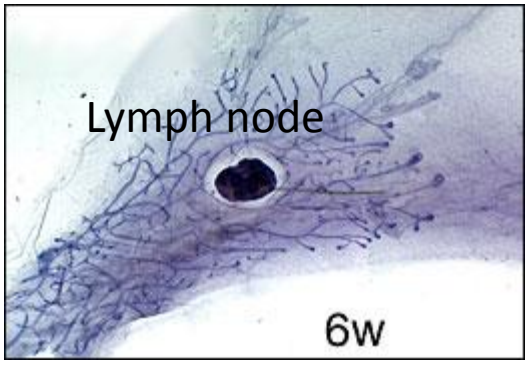


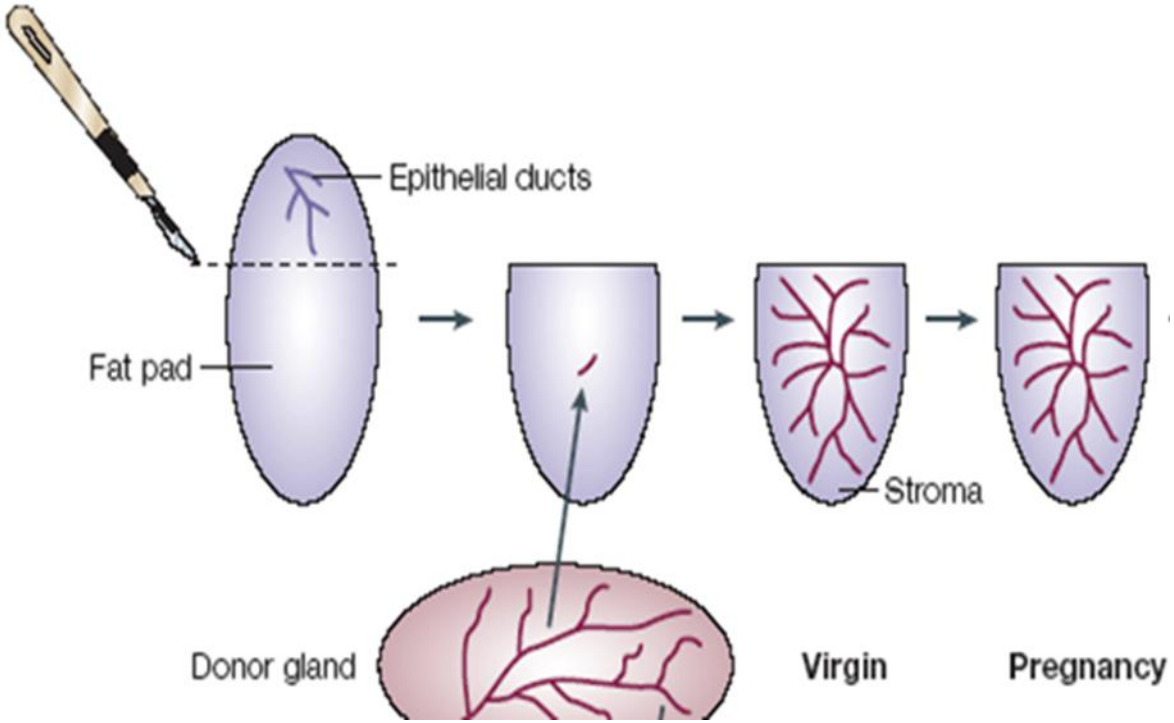
Distinct gene expression pattern in mammary epithelial cell populations



Clearing and cell transplantation into the mouse mammary gland

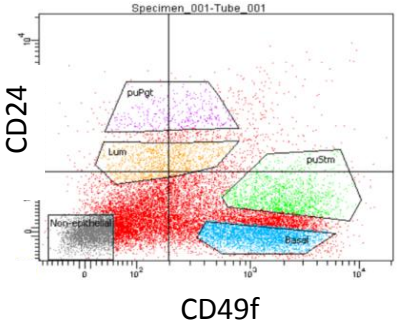






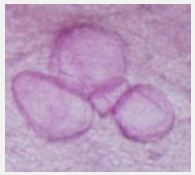
Hennighausen and Robinson, Nature Rev Mol Cell Biol 6, 715 (2005)

Multipotency and self-renwal of mammary epithelial stem cells

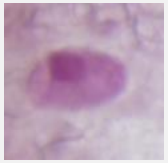


Empty

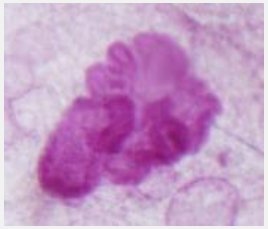
Single layered rings



Spherical outgrowth

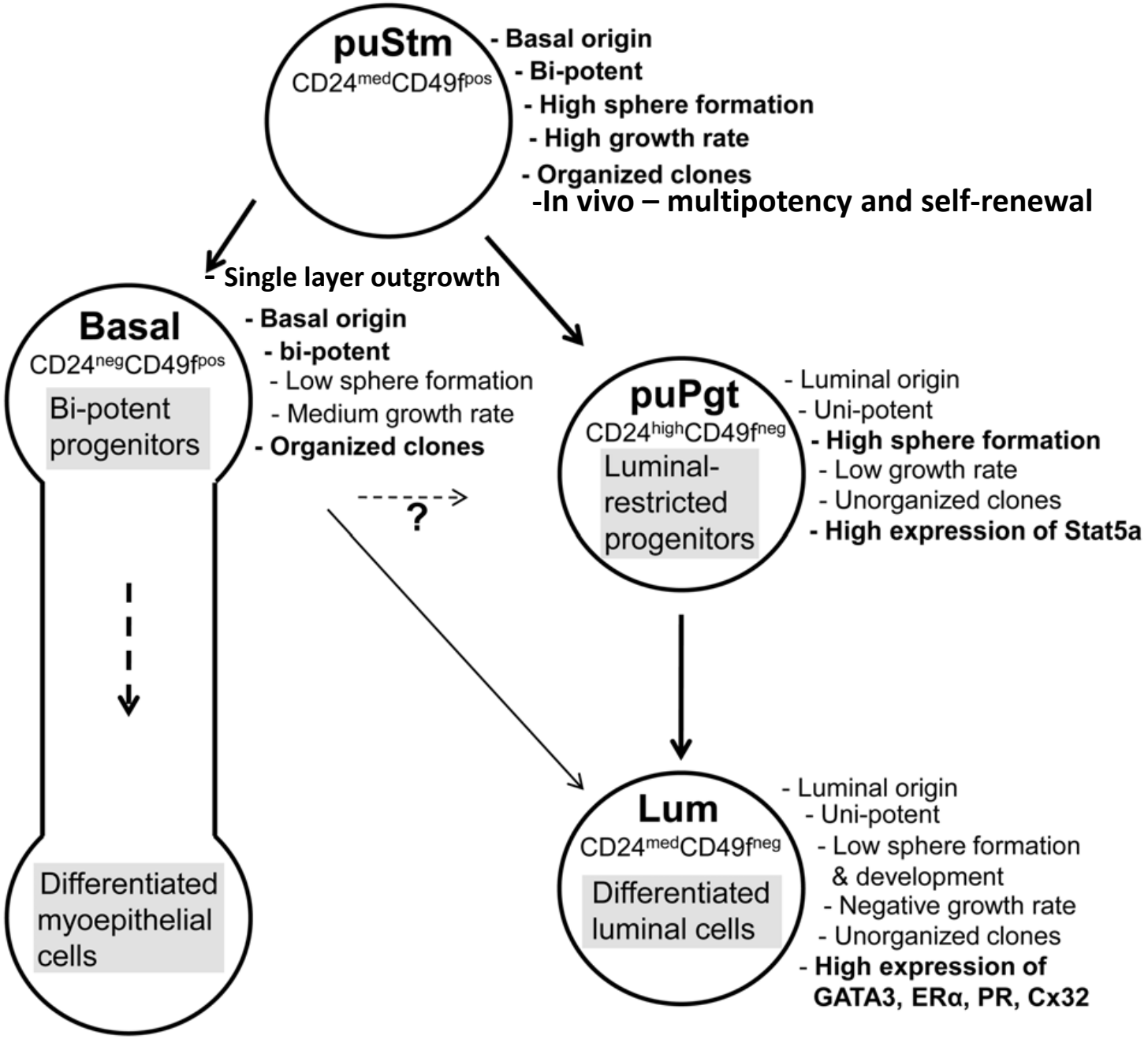


Complex outgrowth

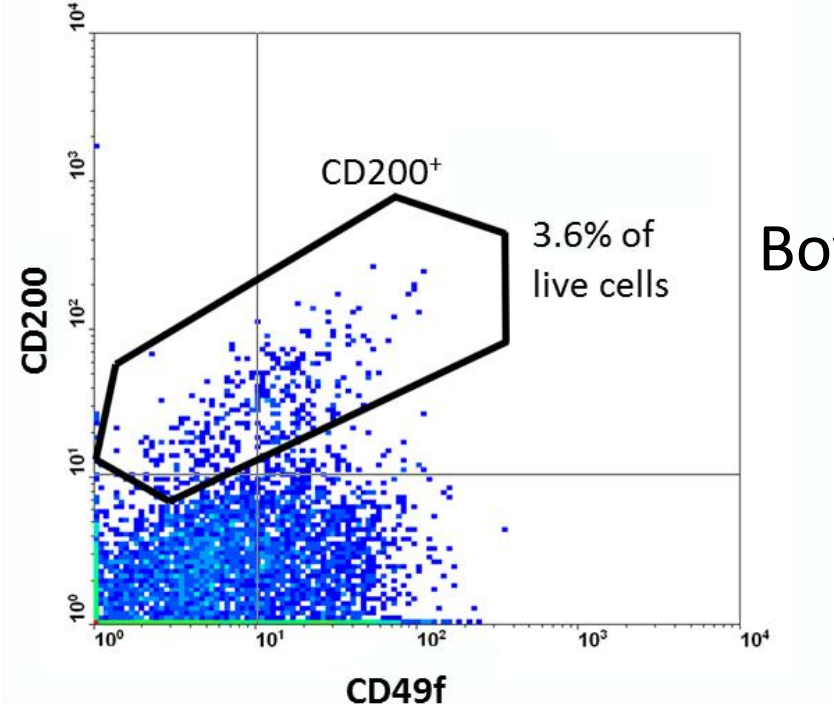


	Group 1	Group 2	Group 3	All groups	Outgrowths (spherical+ complex)
puStm	●●●◉	●●●● ◉◉	●●◉◎	●●●●● ●●●●◉ ◉◉◉◎	13/14 (93%)
Basal	◎◎◎○	●●◉◎	◎◎○○	●●◉◎◎ ◎◎◎◎○ ○○	3/12 (25%)
puPgt	○○○○	●○○	◉◎○○	●◉◎○○ ○○○○○ ○	2/11 (18%)
Lum	○○○○	○○○○ ○○	○○○○	○○○○○ ○○○○○ ○○○○	0/14 (0%)
Non-Epithelial	○○○○	○○○○	○○○○	○○○○○ ○○○○○ ○○	0/12 (0%)

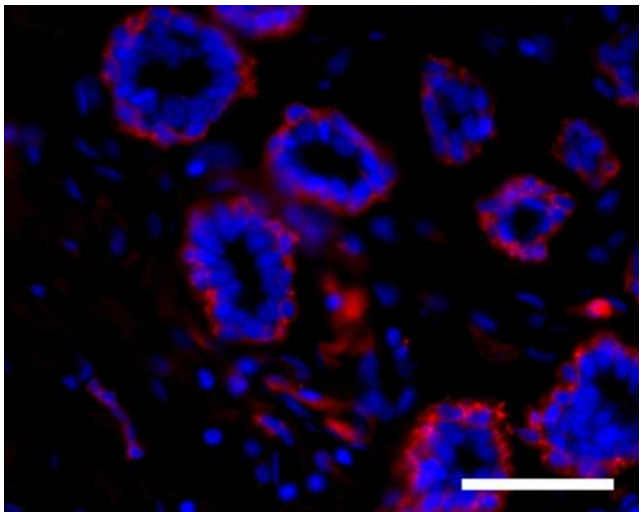
Proposed bovine mammary epithelial cell hierarchy



Other populations with specific functions exist in the mammary gland: for example CD200+ cells (CD200 restrains the immune system)



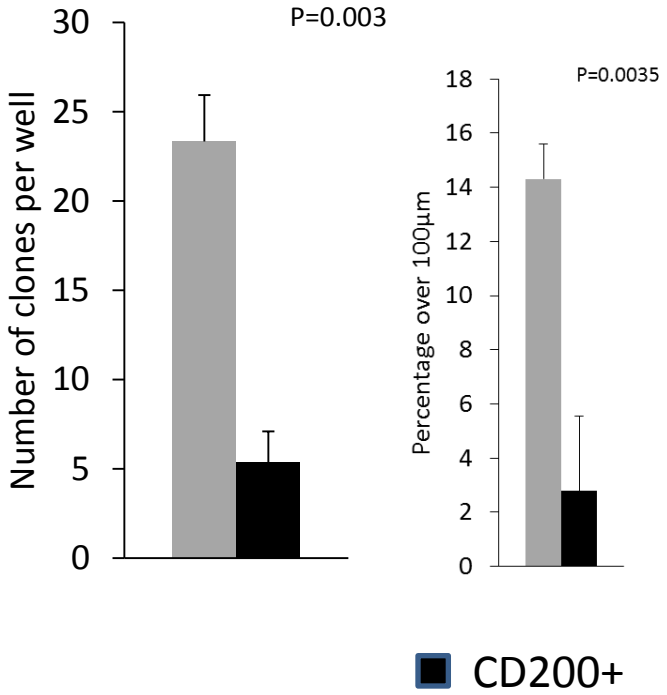
Bovine



Human

Clones

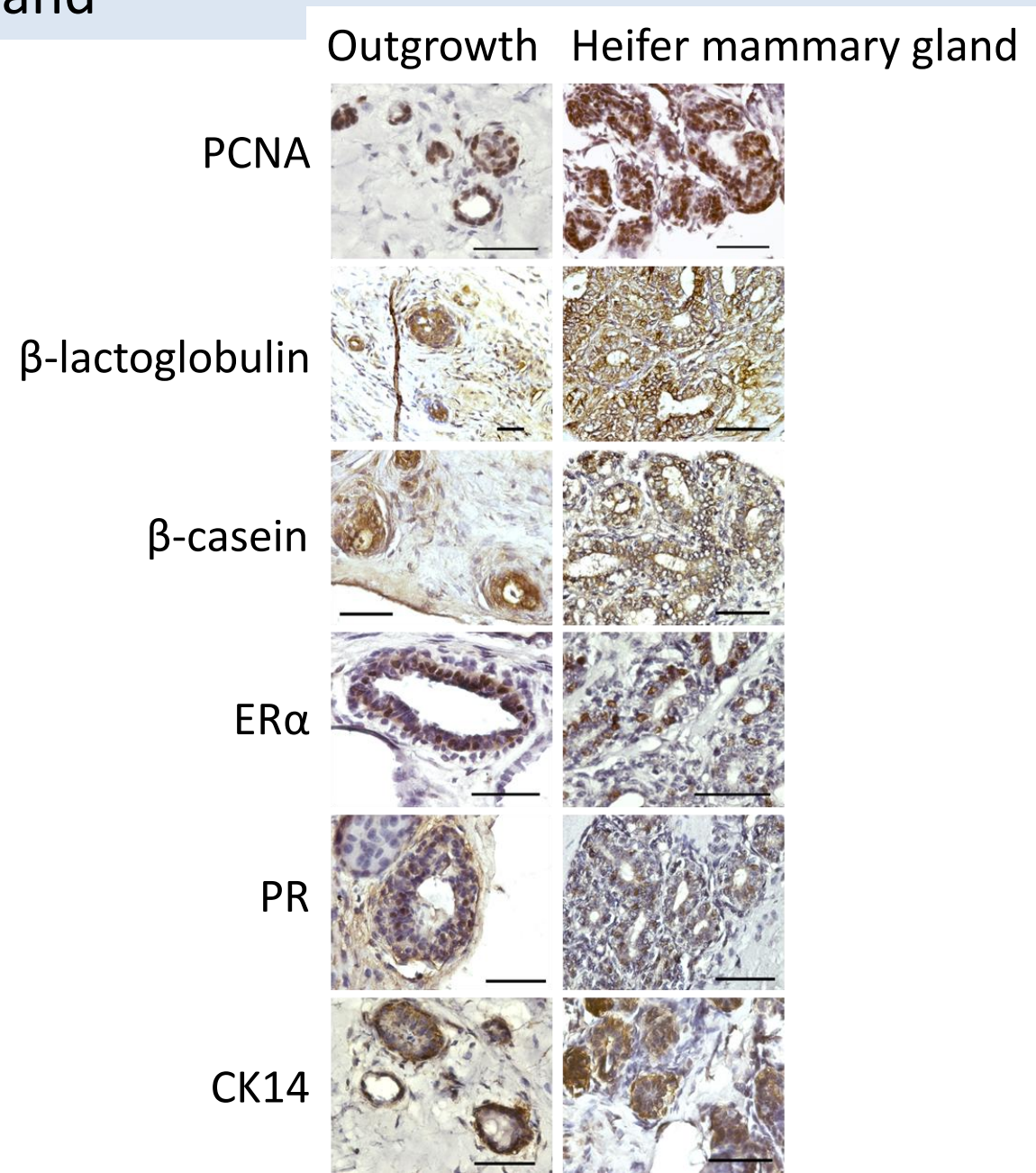
Memospheres



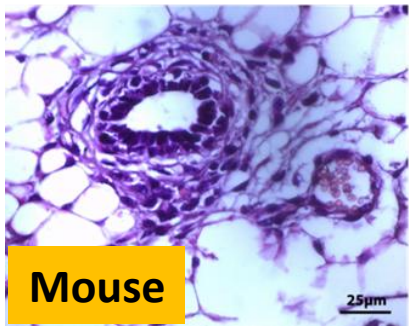
Stem cell transplantation results in outgrowths that contain lumen but do not expand via duct formation

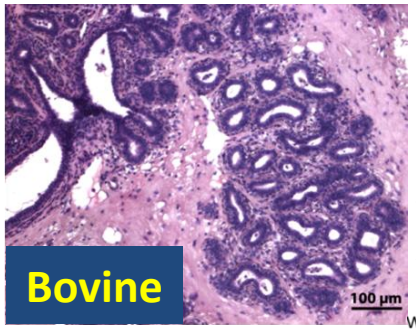


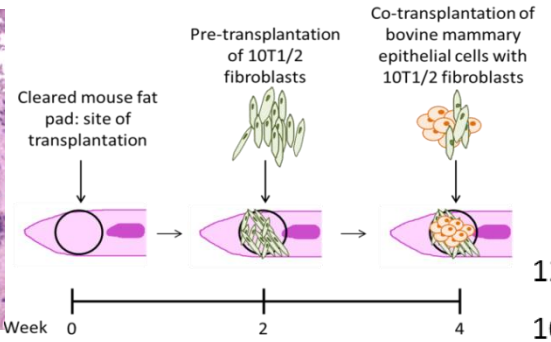
Outgrowths maintained the characteristics of the bovine mammary gland

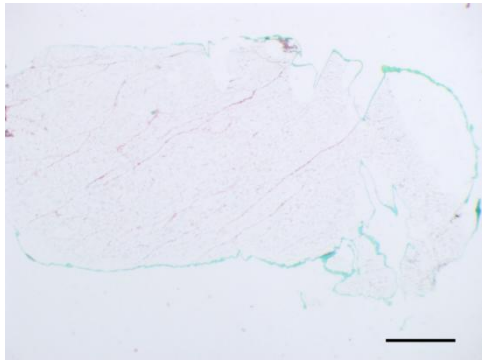


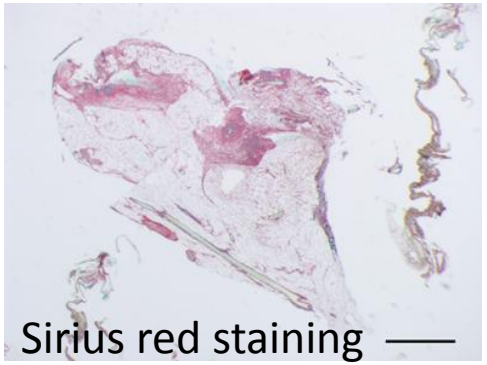
Promoting fibrotic characteristic in the mouse mammary fat pad improved bovine outgrowth Frequency but not representative bovine morphology

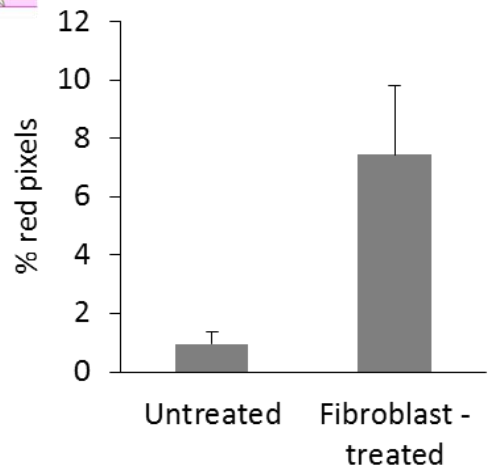




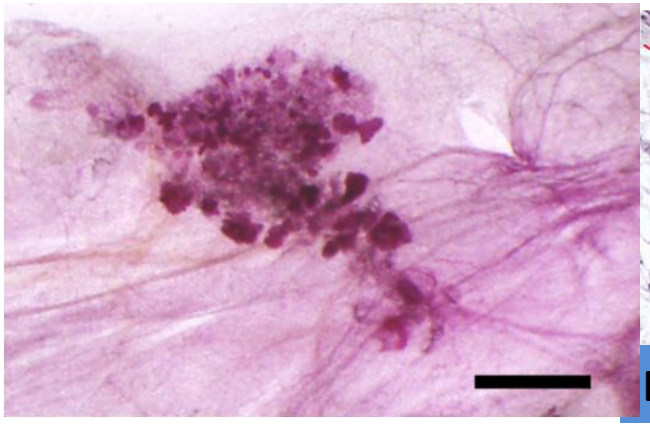






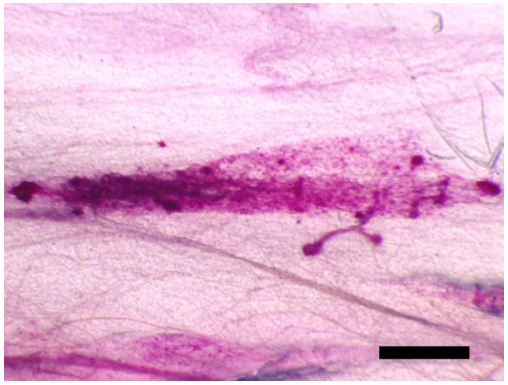


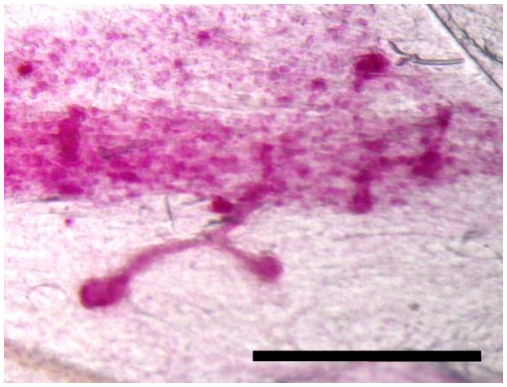
Pre-transplantation of fibroblasts	Co-transplantation of fibroblasts	Outgrowth frequency
-	-	19/29 (65%)
+	+	16/30 (53%)
+	-	16/19 (84%)

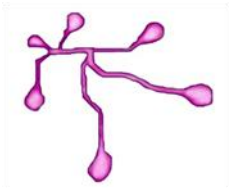




Attempts to apply the spreading capability of the mouse cells by generating chimeric gland failed since cells of each origin developed separately

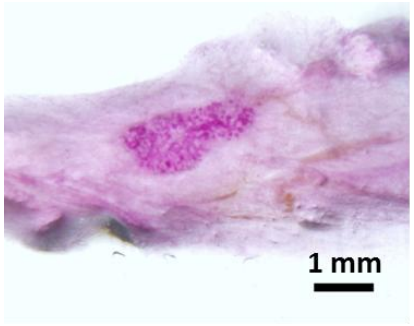




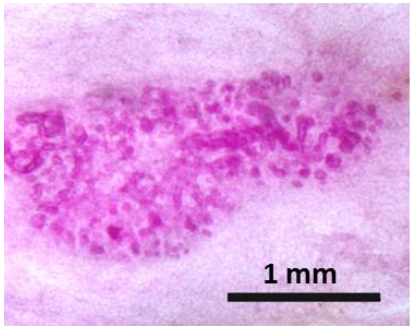




BLG$^+$	1/14	10/14



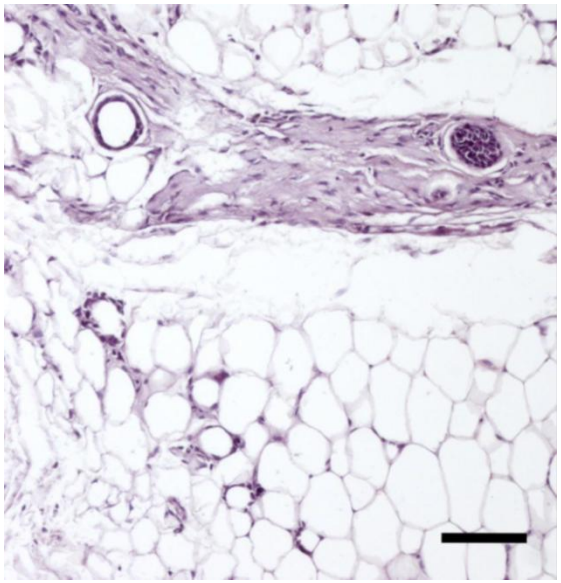






Estrogen and progesterone induced TDLU's formation, but not in a consistent manner

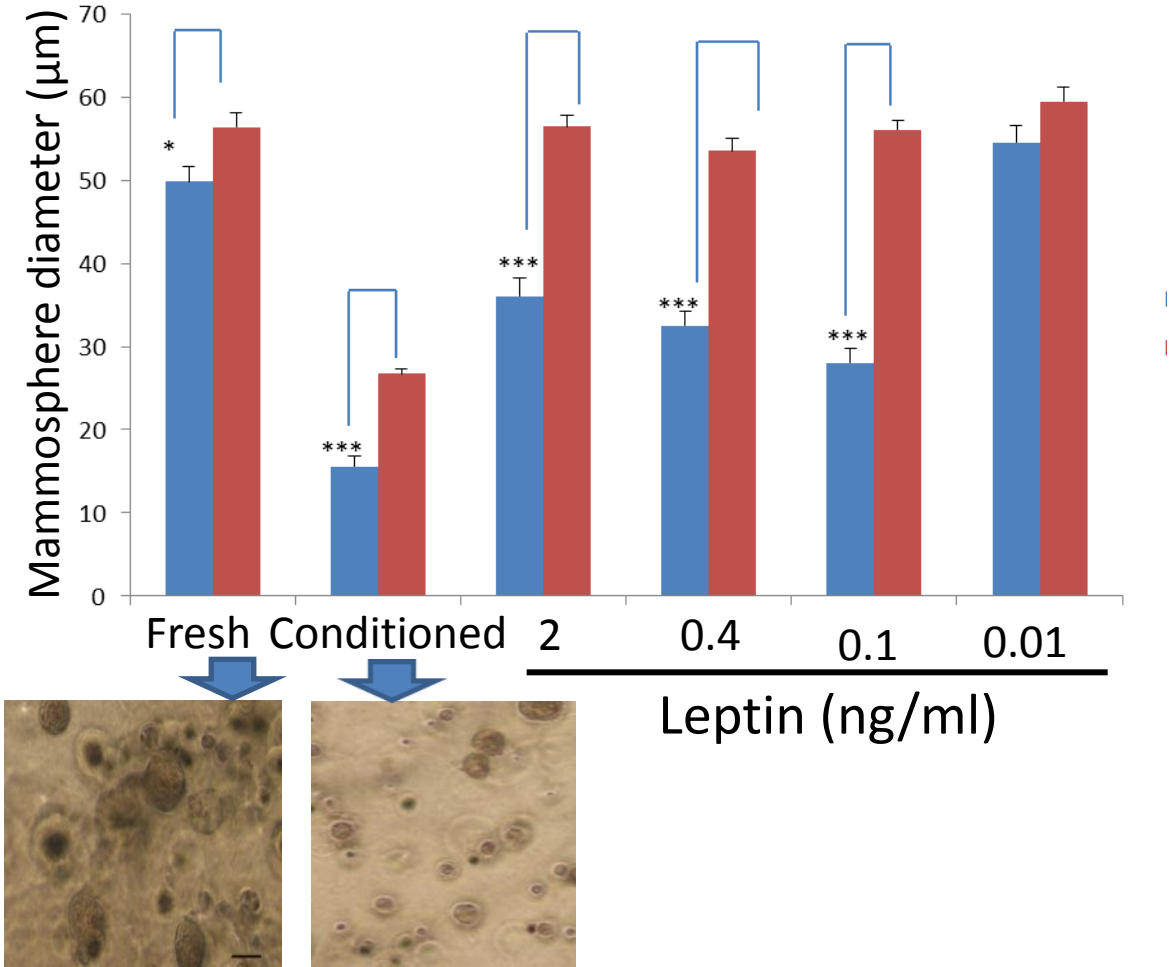
<u>Hypothesis</u>: the mouse mammary fat pad may actively prevent growth of foreign epithelium



<u>Test</u>: development of bovine mammary mammospheres in 3D culture supplemented with adipocyte condition medium

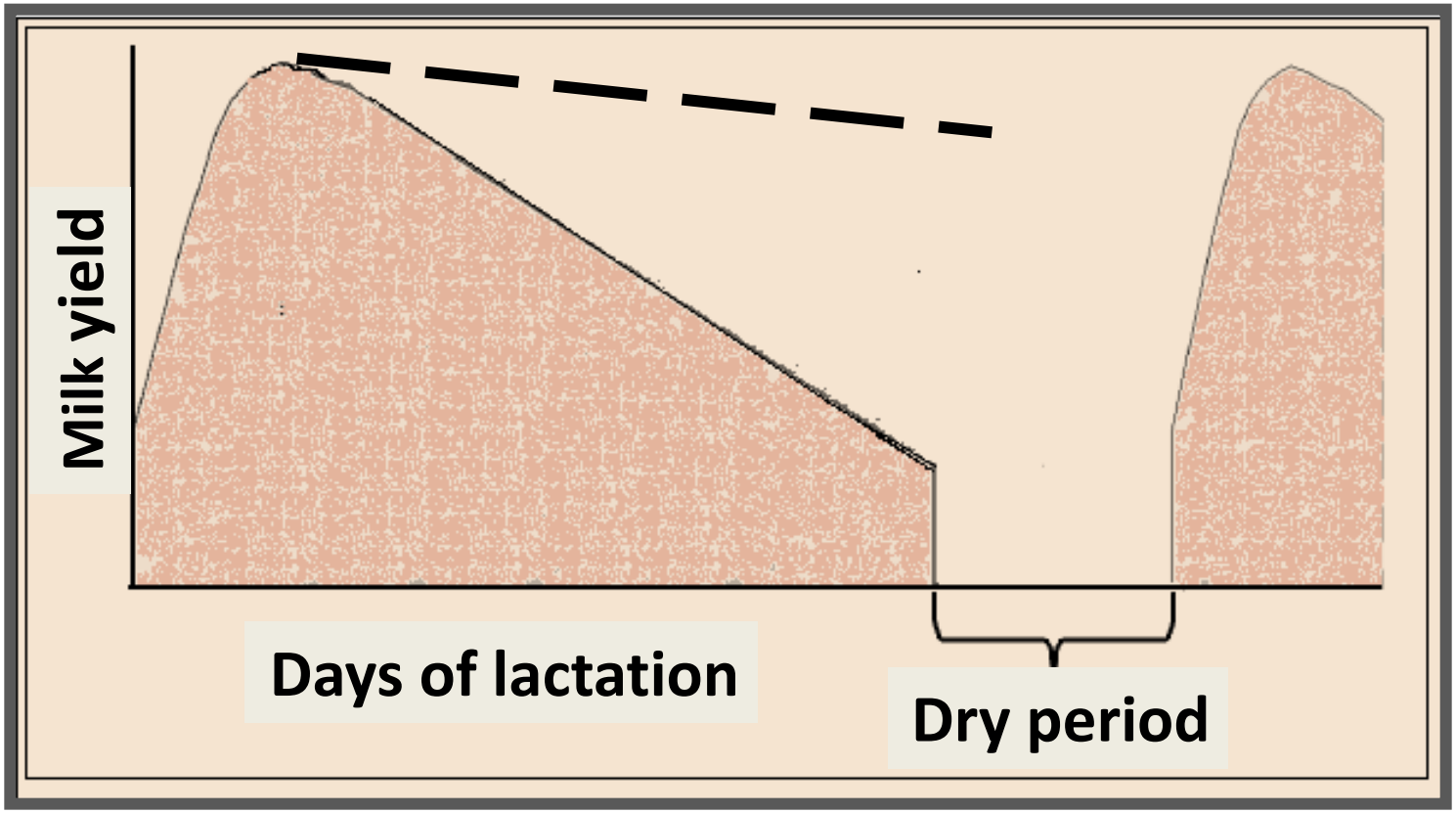
Results: mouse leptin, secreted from the mammary fat pad inhibits bovine mesosphere formation *in vitro*

- Bovine mammospheres developed in the presence of various leptin concentrations for 3 days
- Leptin was removed and development continued for additional 3 days

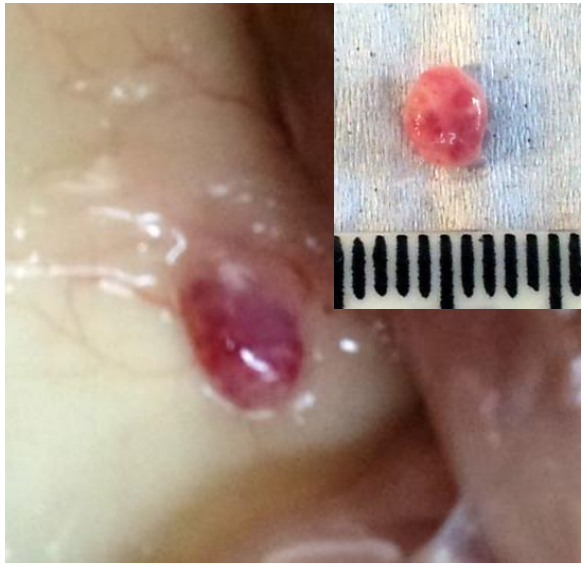


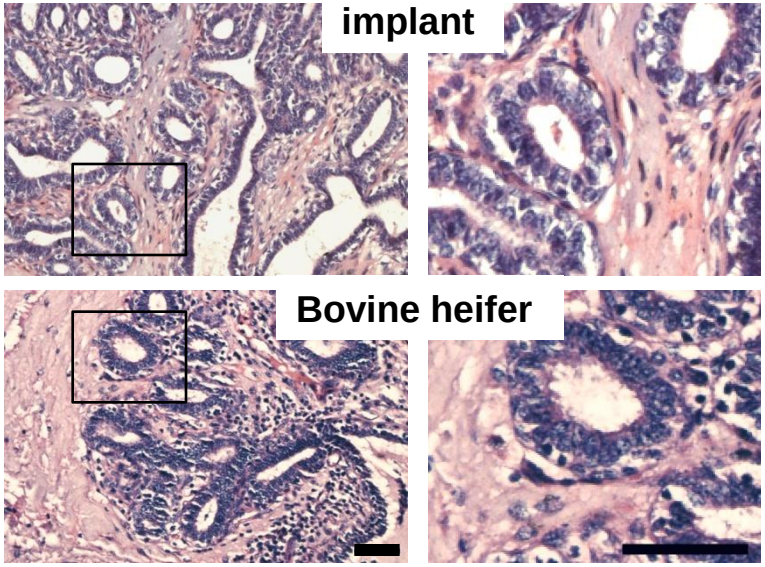
Xanthosine involvement in augmenting BrdU retaining cells was reported in the mammary gland of 3 month old calves

Such improvement could fit our strategy to prevent the reduction in milk production by augmenting stem cell number at the onset of lactation

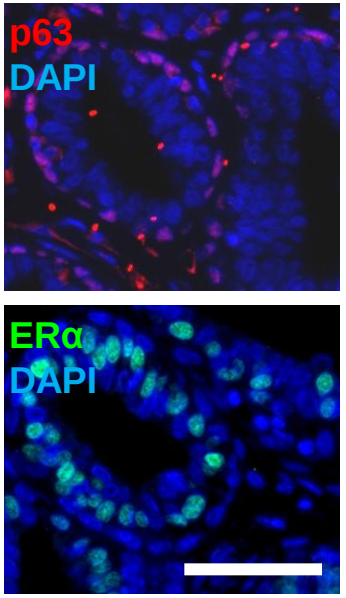


Bovine mammary tissue was implanted into the cleared fat pad of immunocompromised mice and maintained viable for 4 months

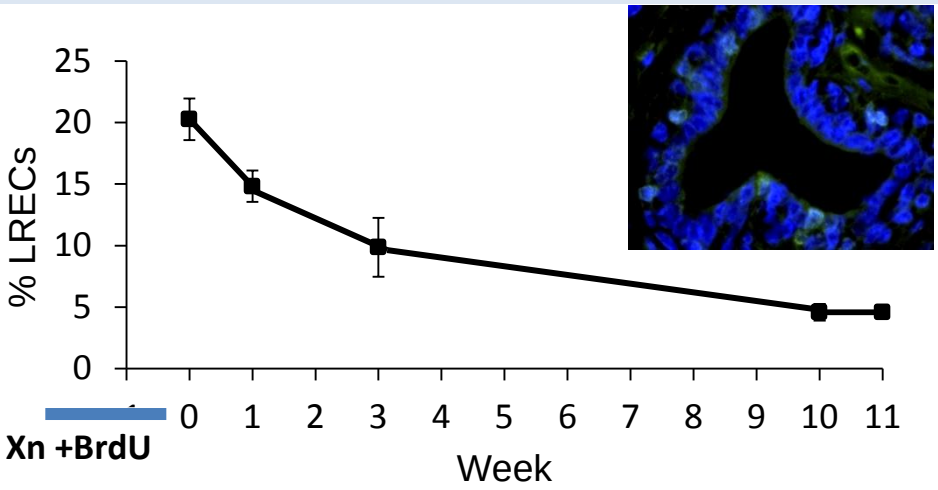


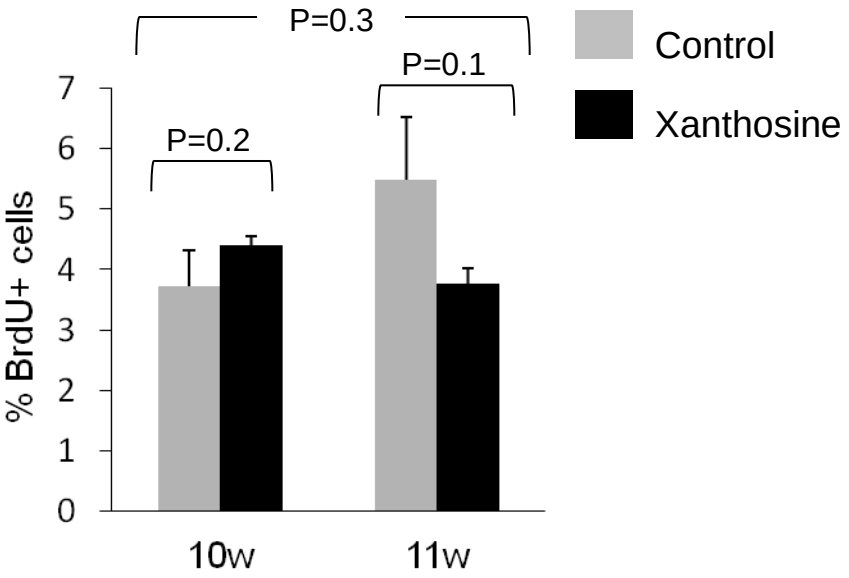


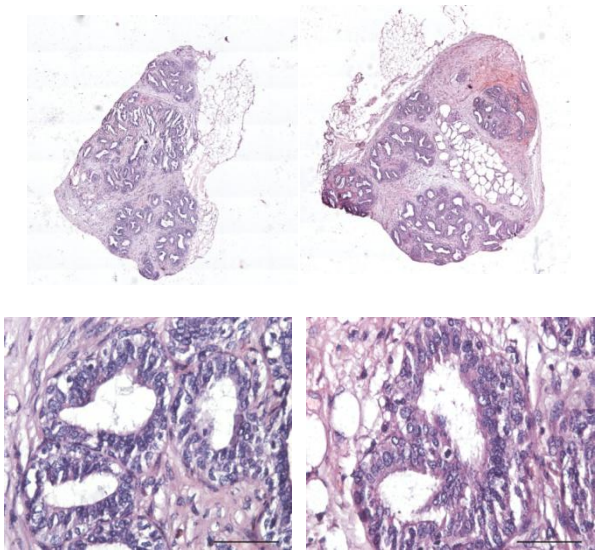
Bovine implant



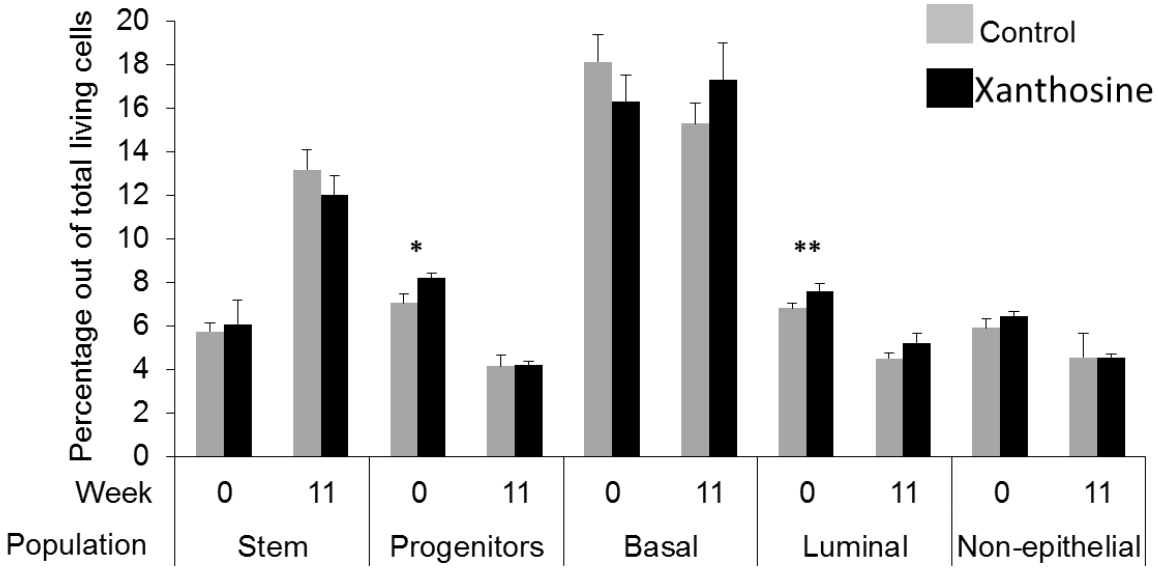
BrdU retaining cells are not affected by Xanthosine administration

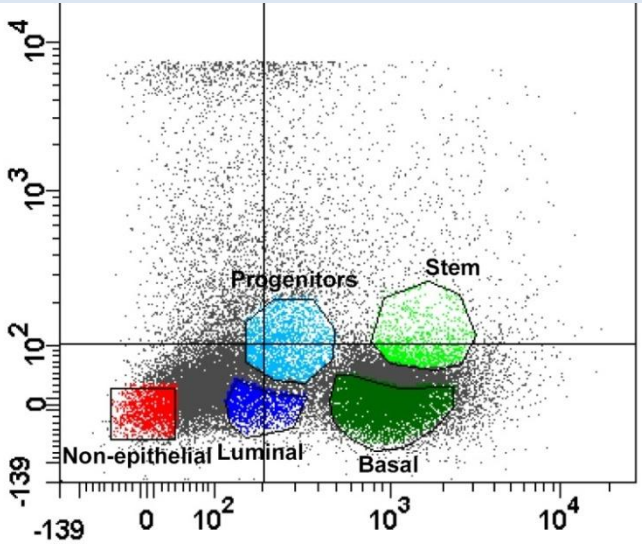


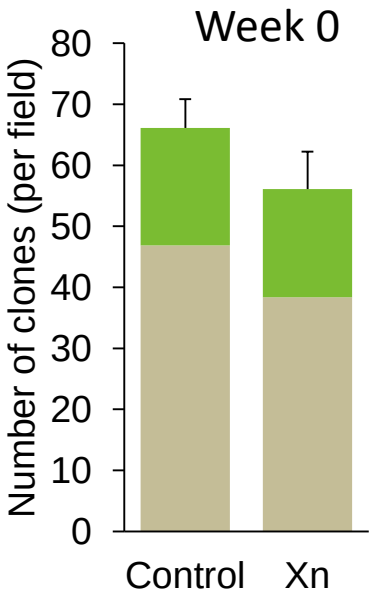


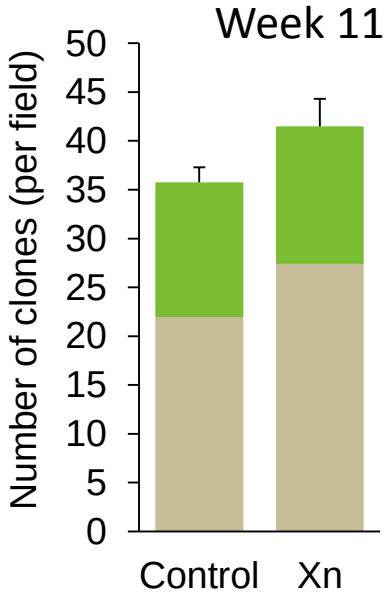


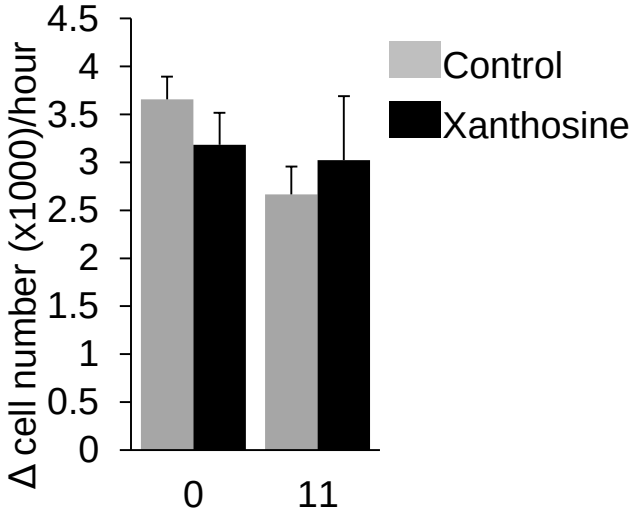
Xanthosine does not have a long-term effect on bovine mammary cell populations



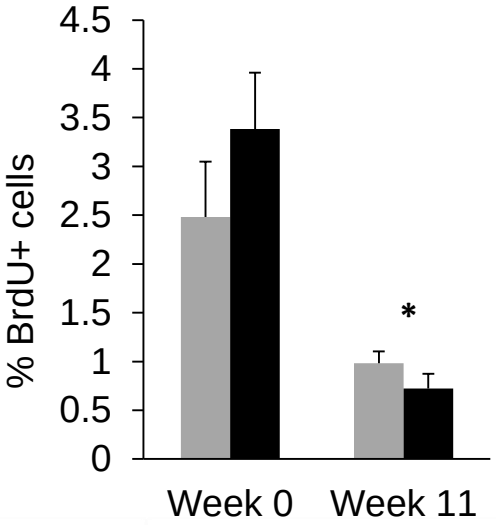


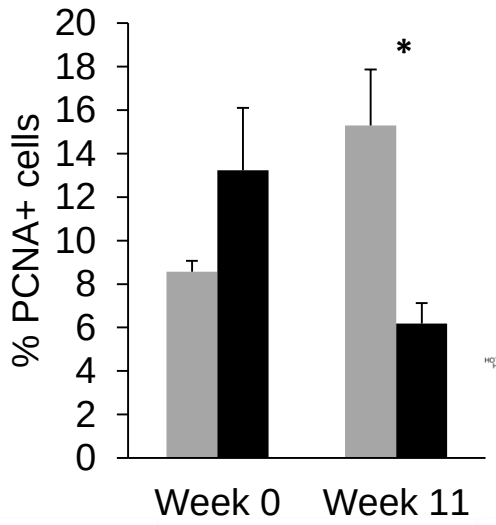


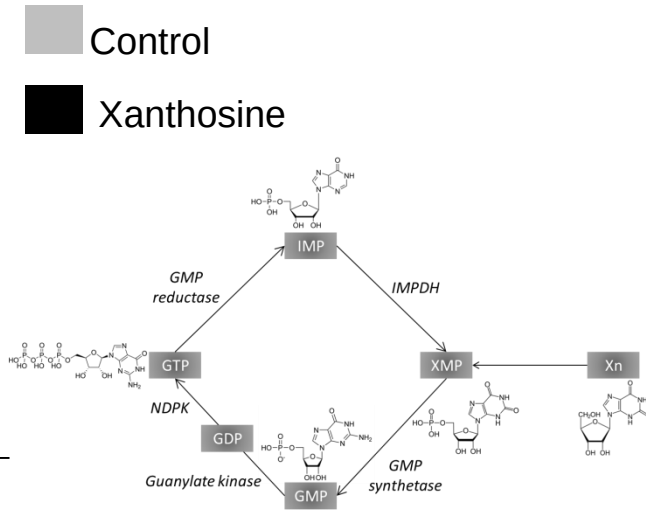




Xanthosine exerts a latent negative effect on cell proliferation in the mammary gland







Gene	Description	Week 0 Xn-induced change (fold control)	Week 0 p	Week 11 Xn-induced change (fold control)	Week 11 p
Ki67	Markers of proliferation.	0.88	0.33	**0.55**	0.003
Cyclin D1		**0.72**	0.004	**0.36**	0.02
IMPDH2	IMPDH catalyzes the formation of inosine mono-phosphate (IMP) to XMP, which is the rate-limiting step in guanine nucleotide biosynthesis.	**0.74**	0.03	1.05	0.36

In summary:

-Distinct cell populations: stem cells, Basal, luminal progenitors and differentiated luminal cells participate in the bovine mammary epithelial cell hierarchy. Other populations with specific properties were also identified.

-Transplantation of stem cells into the mouse cleared fat pad resulted in multilayered outgrowths with self renewal capabilities. They resembled the alveoli, but lacked expansion properties via ductal elongation. Leptin, secreted from the mouse fat pad may serve as a local protecting agent against foreign tissue expansion.

-Estrogen and progesterone contributed to the development of TDLUs.

-Xanthosine does not affect stem cell number and has a latent anti-proliferative effect. Therefore it does not qualify for inducing lactation persistency. Alternative methodologies for stem cell augmentation are under investigation.