



Role of ovarian secretions in mammary gland development and function in ruminants

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Plan

→ Introduction: ovarian steroids and mammary gland

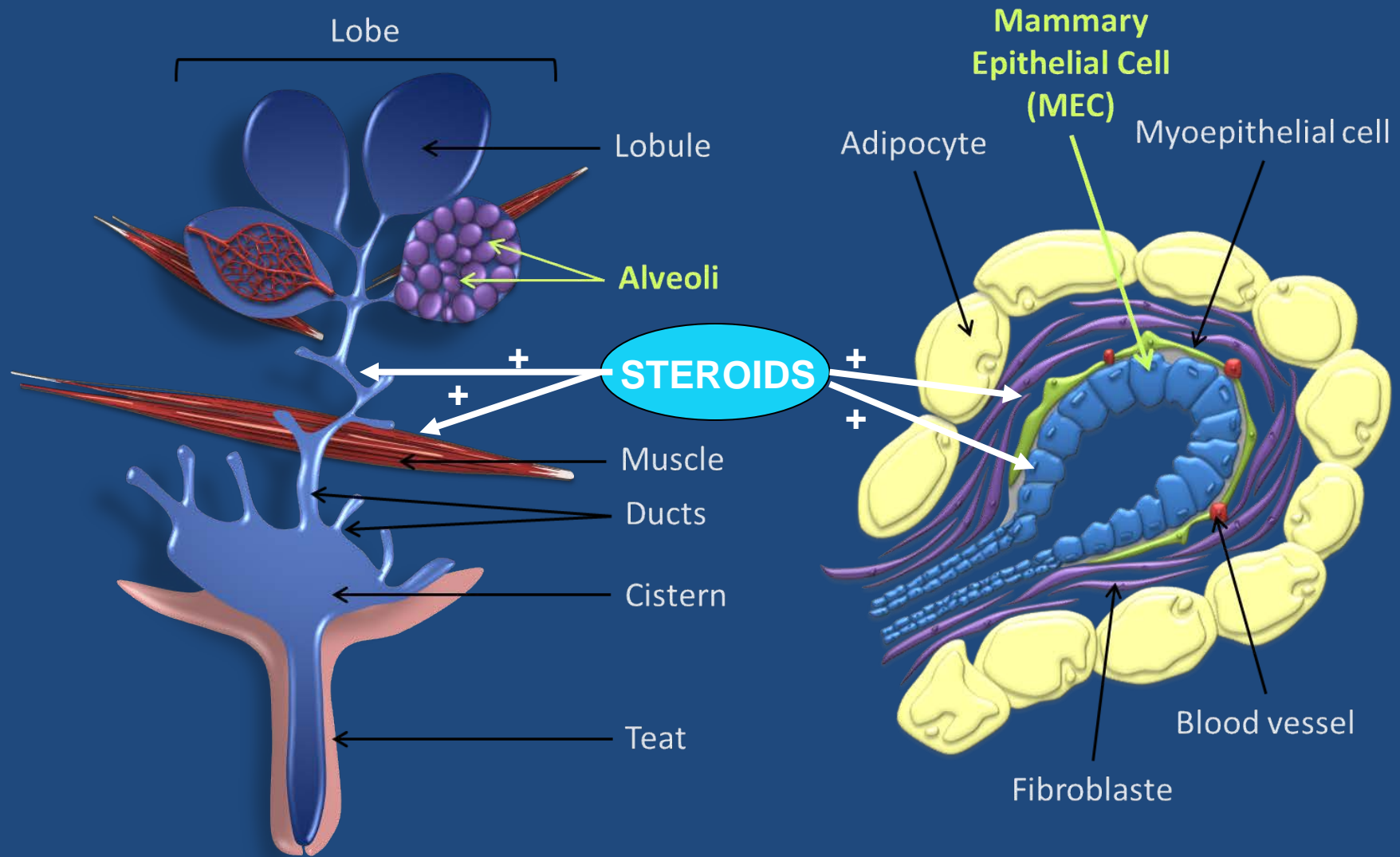
(1) Effects of ovarian steroids on mammary gland development before puberty in goats

(2) Effects of ovarian steroids on mammary function during lactation (lactating cows)

(3) Effects of estradiol on mammary epithelial cells in vitro (example in MAC-T cells)

→ Conclusion

Anatomy of mammary gland in ruminants

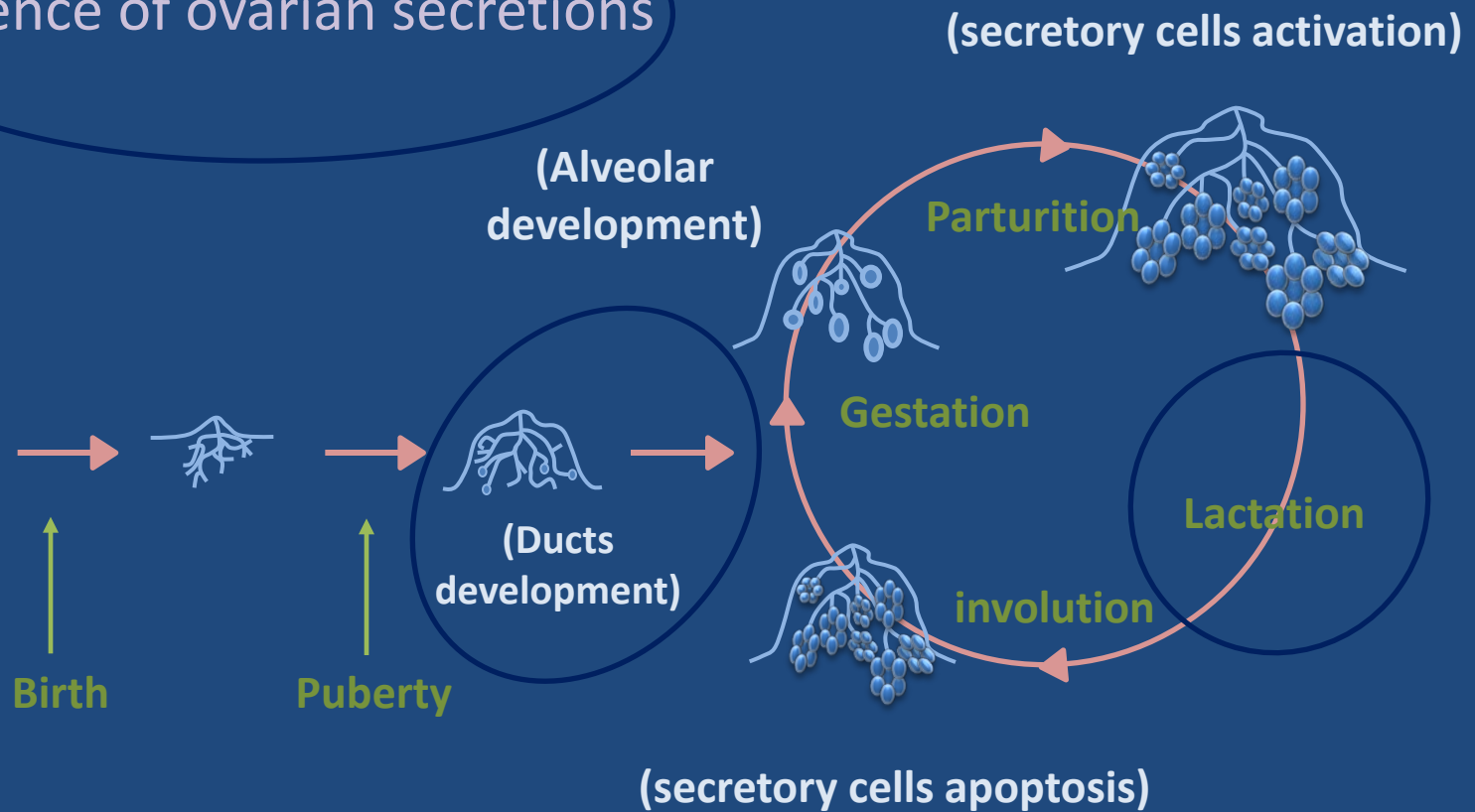


(from Delouis and Richard, 1991)



Mammary gland development cycle

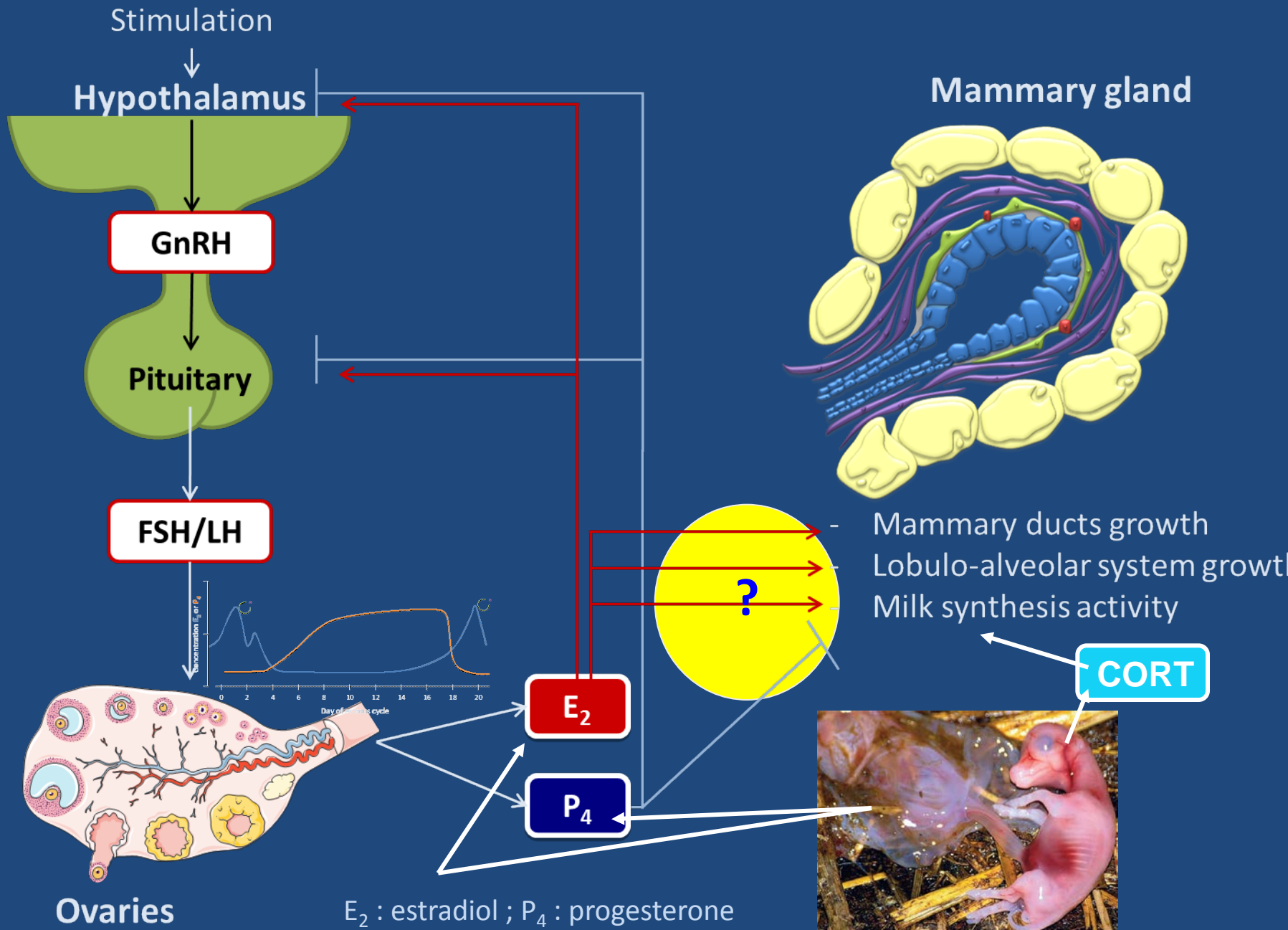
Influence of ovarian secretions



(from Martinet and Houdebine, 1993)



Hormonal regulation of Lactation

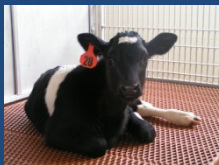
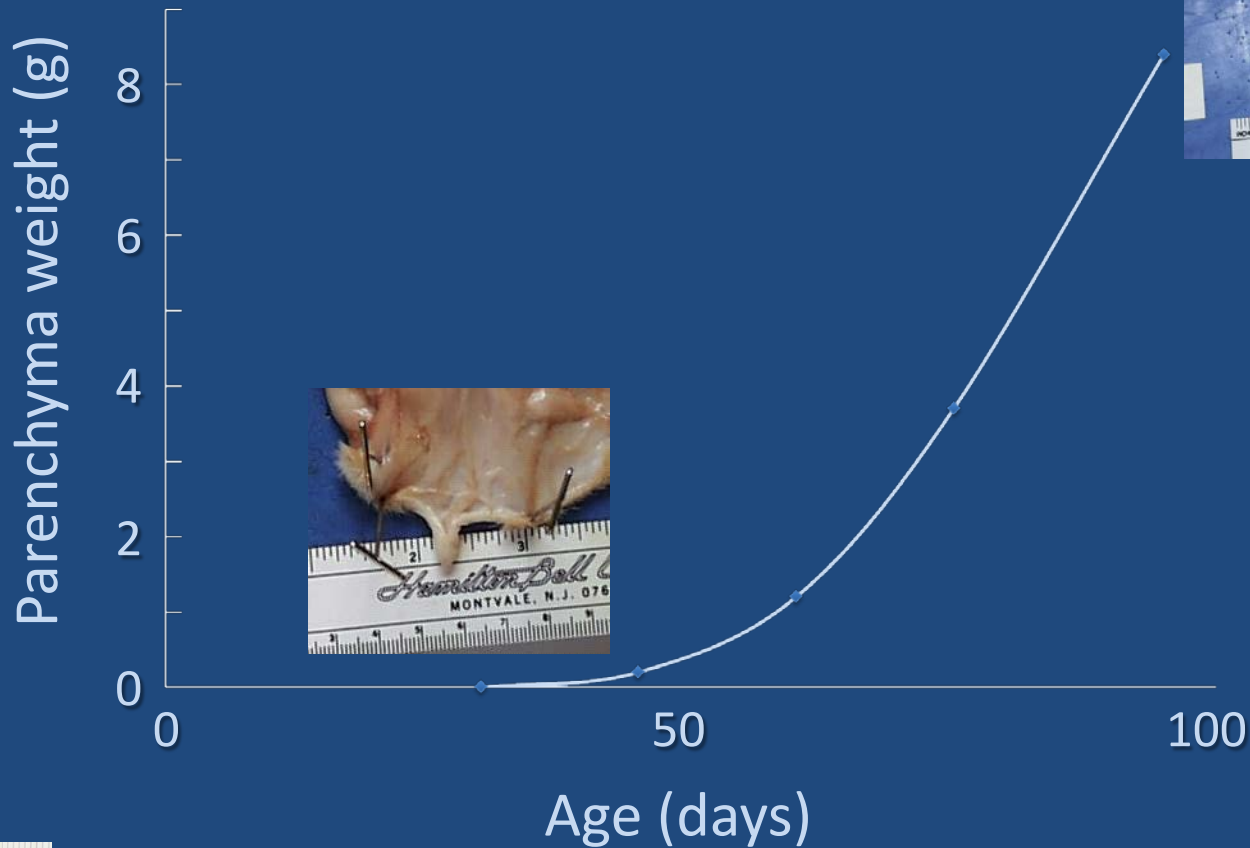




Effects of ovarian steroids on mammary gland development before puberty in goats



Parenchymal tissue weight changes rapidly between 30 and 90 days of age in heifers

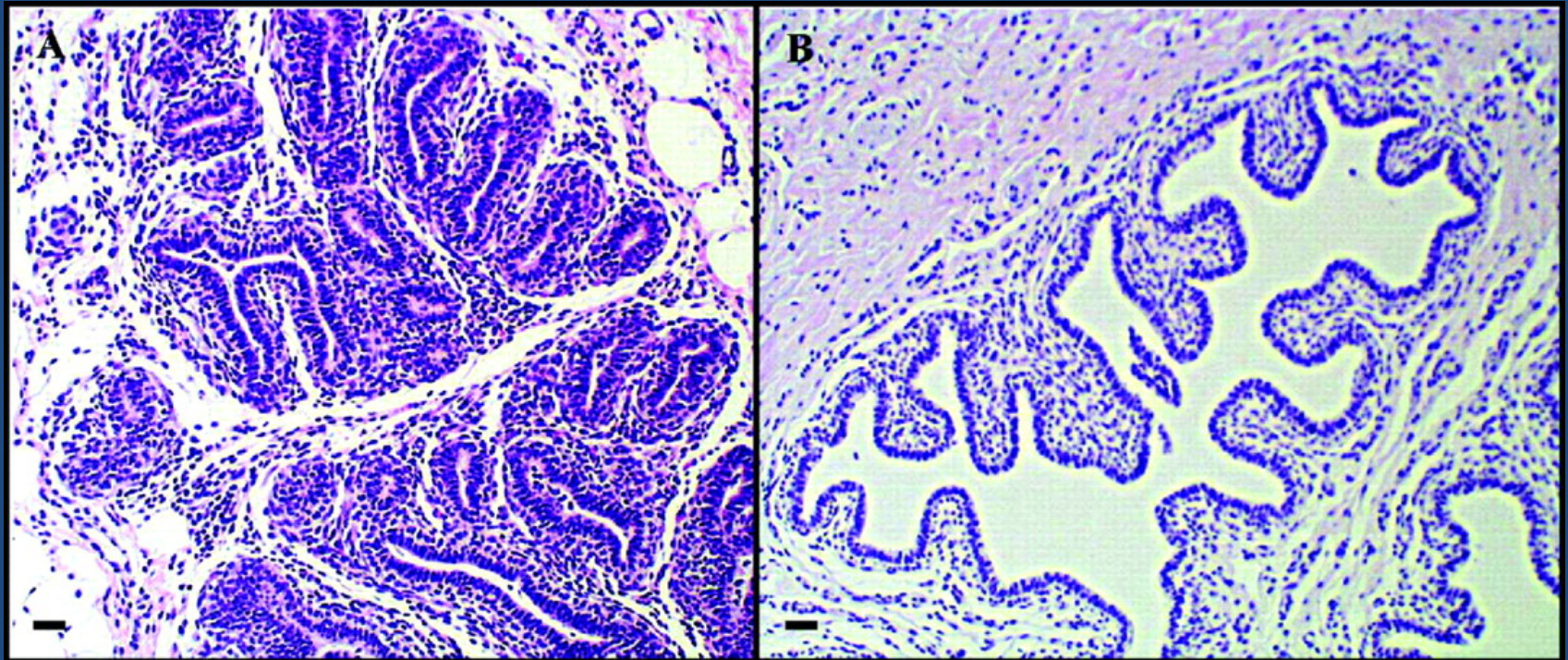




Ovariectomy strongly affects mammary parenchyma development in heifers

Int

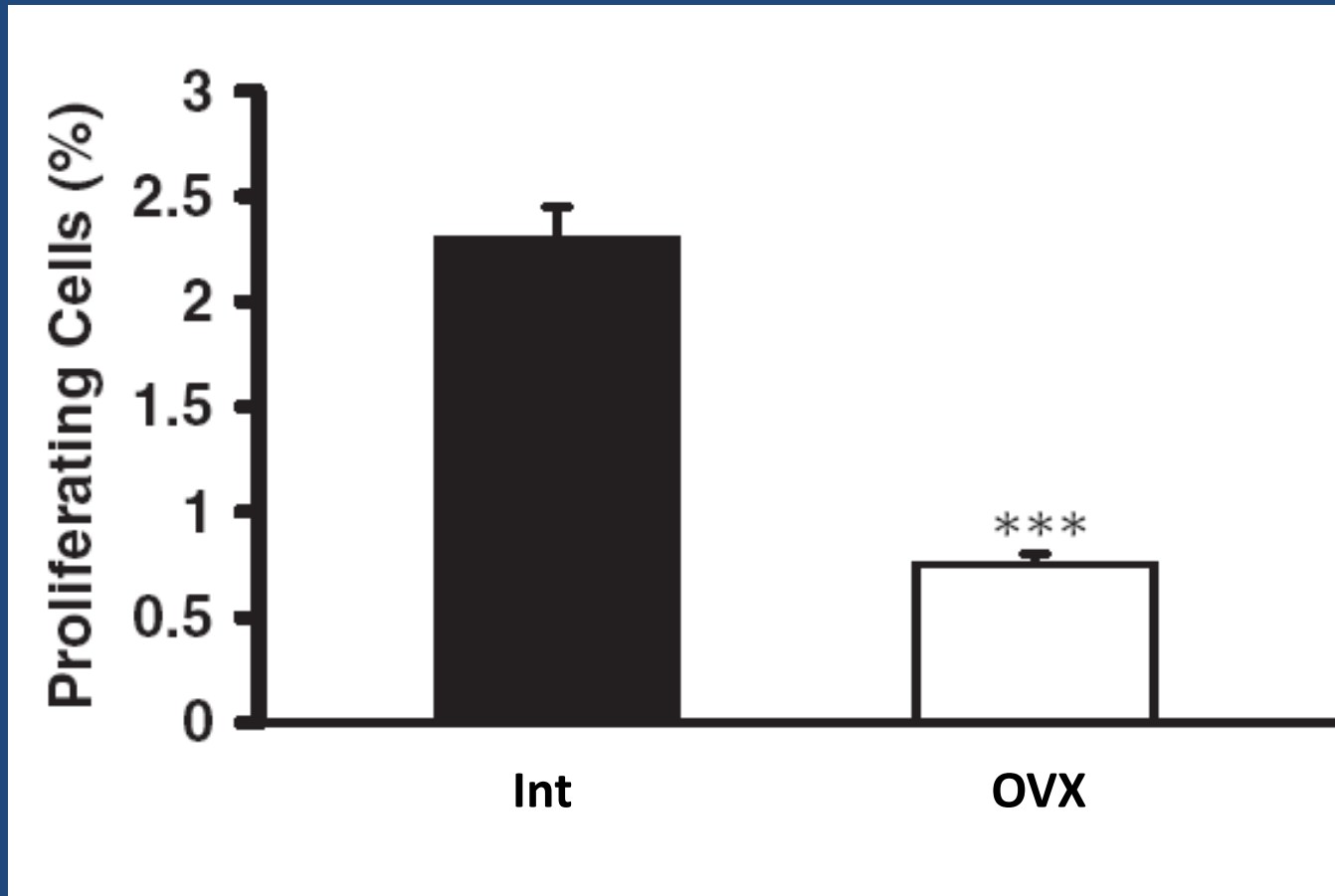
OVX



Hematoxylin and Eosin histological staining – Bars represent 20µm



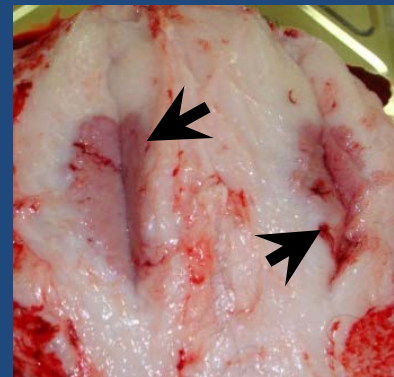
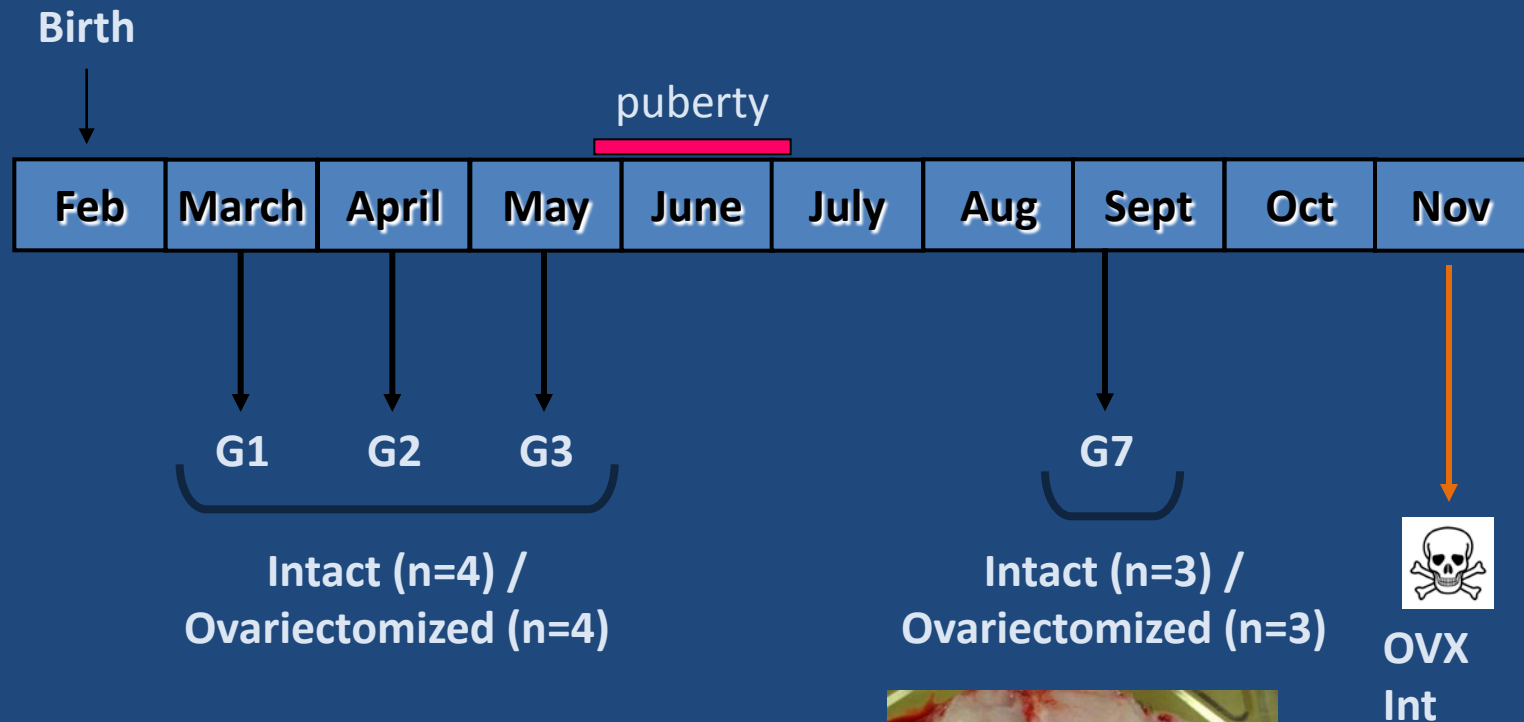
Ovariectomy negatively affects mammary cell proliferation in heifers



*BrdU incorporation into DNA - *** $p < 0.001$*

Effect of ovariectomy on mammary gland development in prepubertal goats

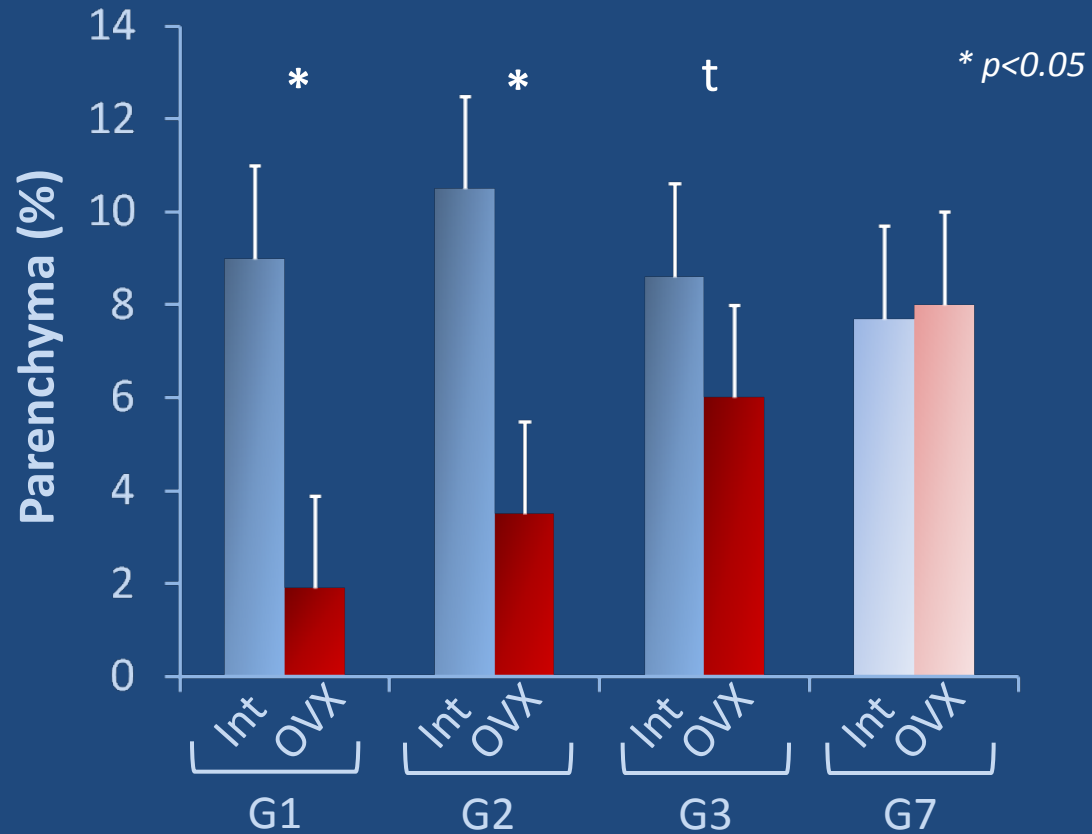
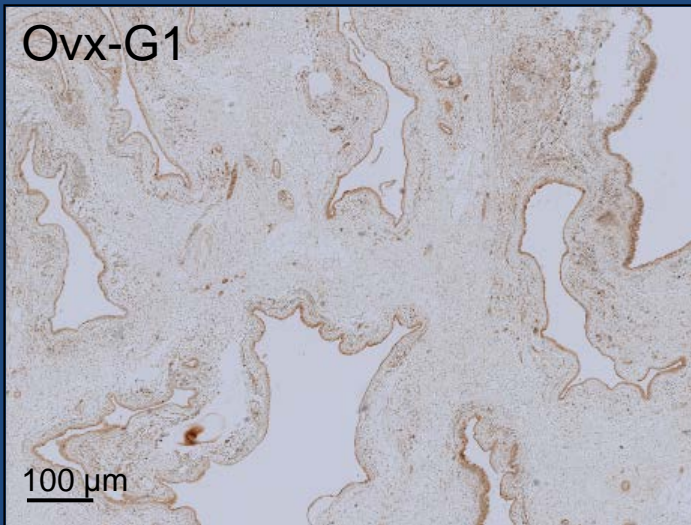
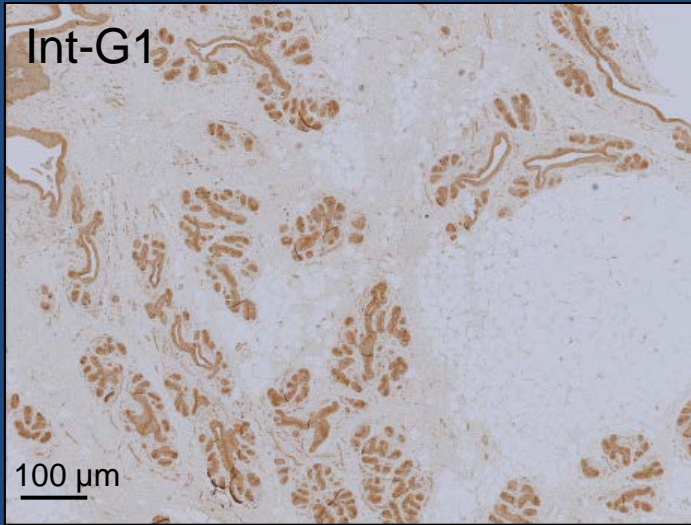
Experimental design





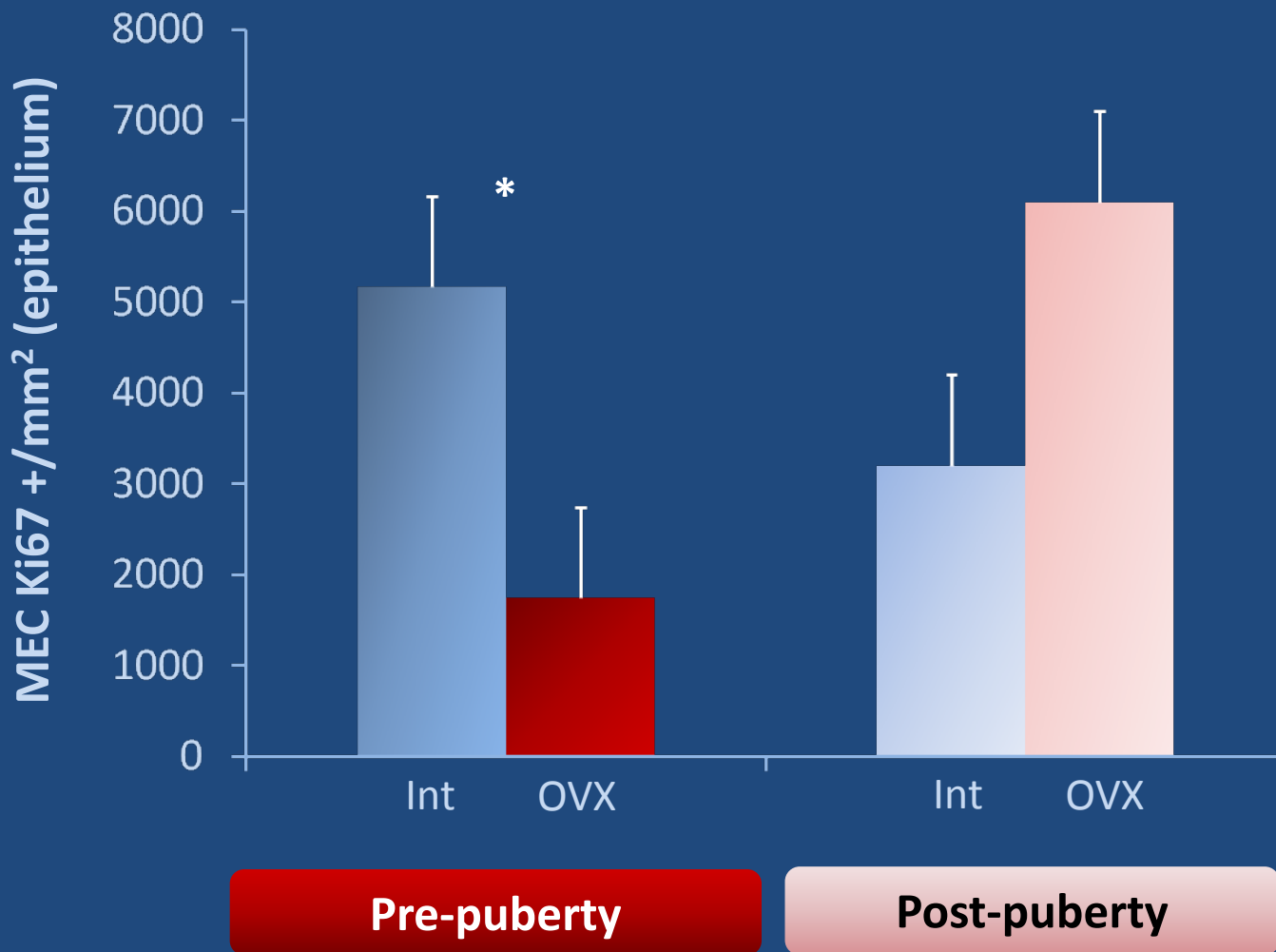
Early ovariectomy affects mammary gland parenchyma development in young goats

63rd Annual Meeting EAAAP 2012 – Bratislava – Slovak Republic





Ovariectomy affects mammary epithelial cell proliferation in young goats



* $p < 0.05$



Conclusion

Effects of ovarian steroids on mammary gland development before puberty

Ovarian secretions of estradiol and progesterone are essential for parenchyma development and cell proliferation in both heifer and goat mammary gland.

In young goat, there is a critical period between birth and 2 months of age – before the onset of ovarian cyclicity - during which the lack stimulation of mammary epithelial cell proliferation by ovarian steroids dramatically affects the subsequent mammary development.

Preliminary results demonstrate that a longer stimulation of mammary growth after puberty could negatively affect succeeding lactation. The window length remains to determine

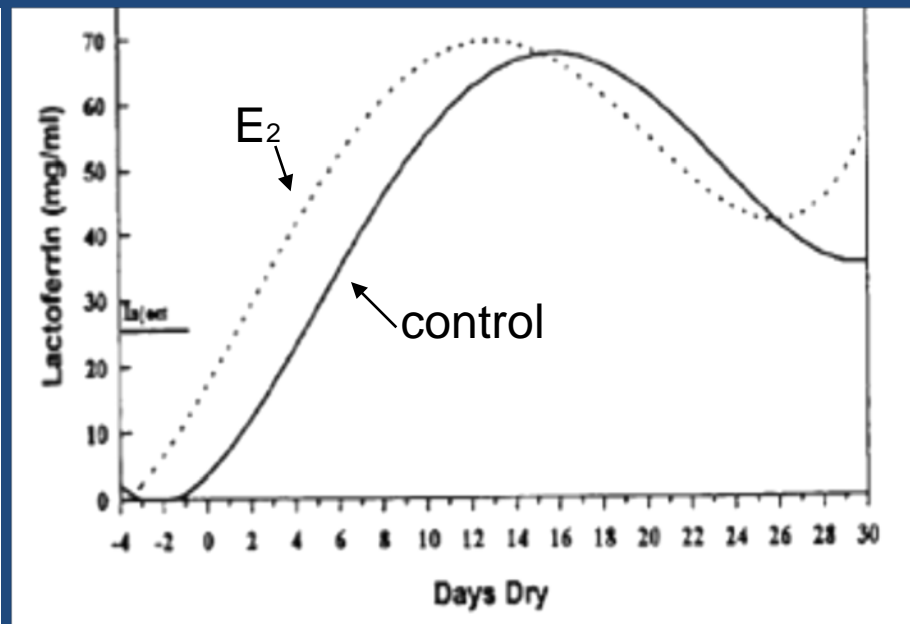
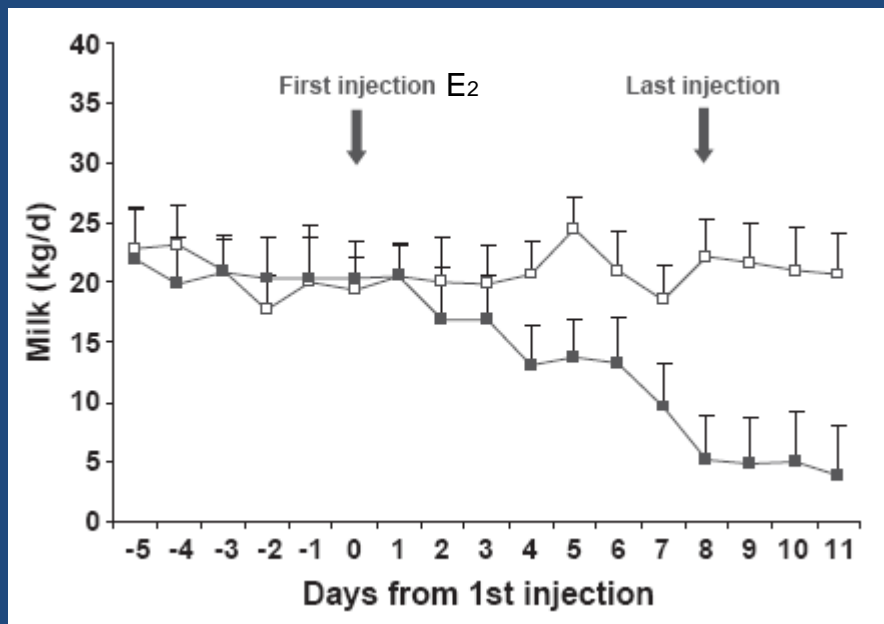


Effects of ovarian steroids on mammary function during lactation

Estradiol treatment of lactating cows induces a decrease in milk yield and accelerates mammary gland involution

Effect of E₂ on milk yield in mid-lactation cows

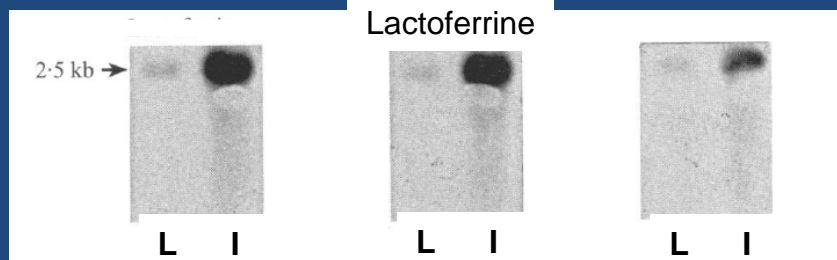
Effect of E₂ on lactoferrin concentration in mammary secretions during involution



Delbecchi et al., 2005

Athie et al., 1996

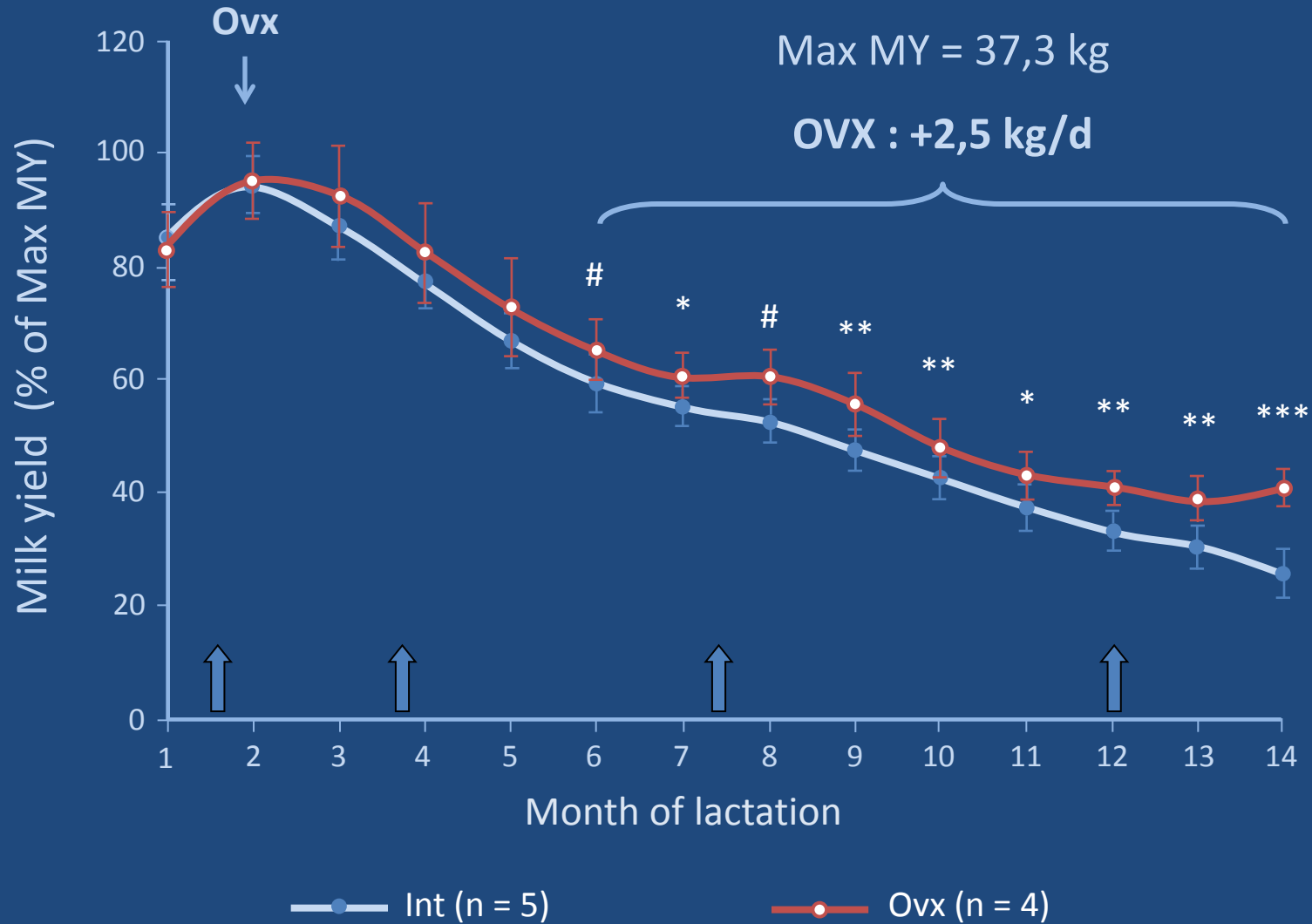
Wilde et al., 1997



L = lactating I = Involuting



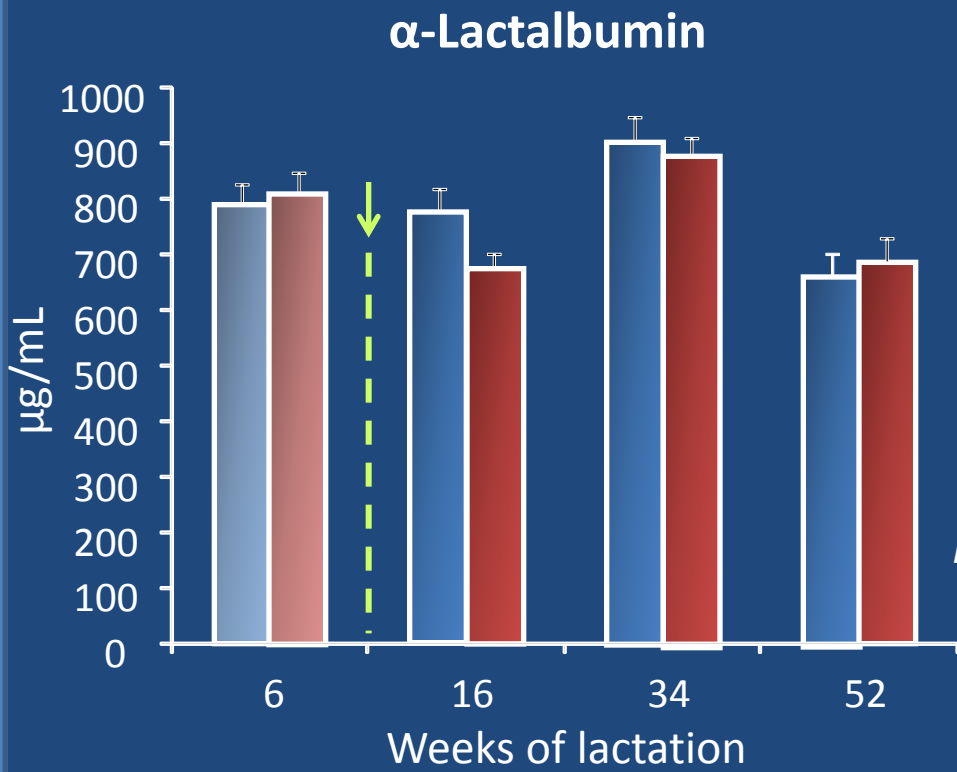
Ovariectomy of lactating cows at the time of lactation peak limits the decline in milk production



$p < 0,1$; * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$



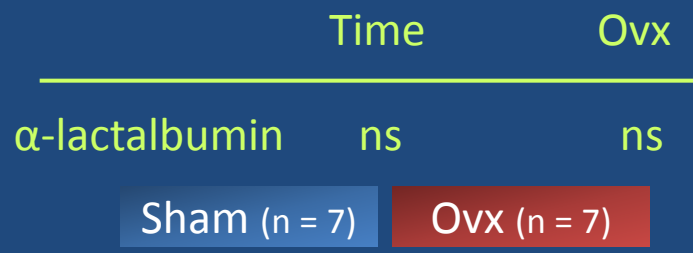
Ovariectomy of lactating cows at the time of lactation peak does not modify milk composition



Milk composition

	Sham (n = 7)	Ovx (n = 7)	P
Protein yield (g/d)	791	827	*
Fat yield (g/d)	915	1006	***
Lactose yield (g/d)	1081	1094	***

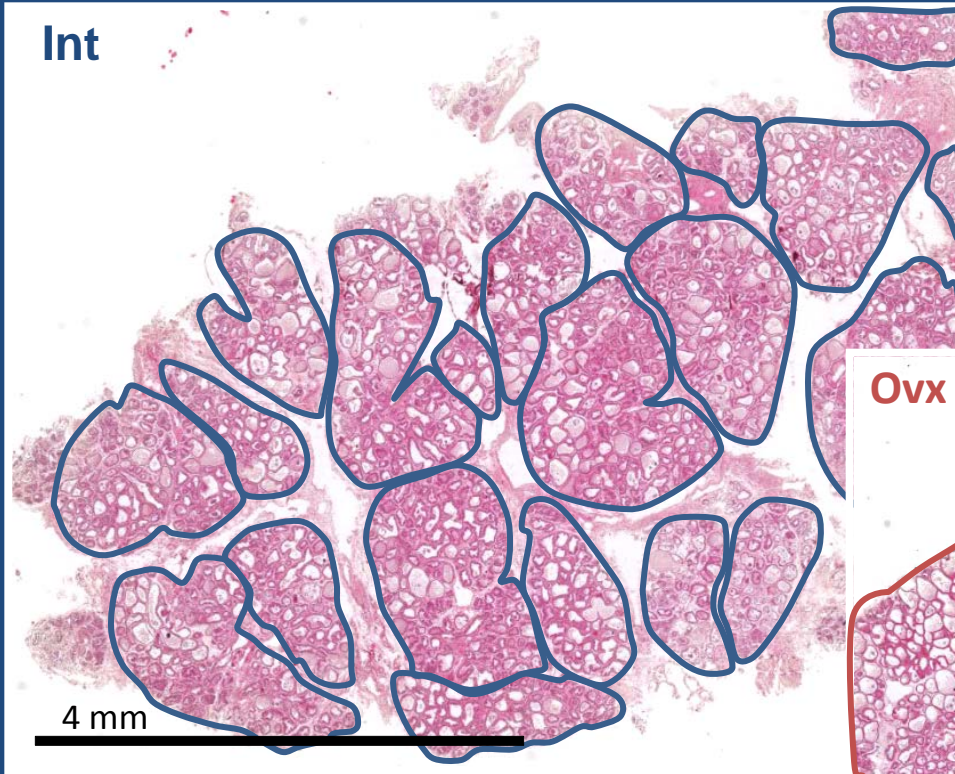
Mean values from 16 to 52 weeks of lactation



* $p < 0,05$; *** $p < 0,001$



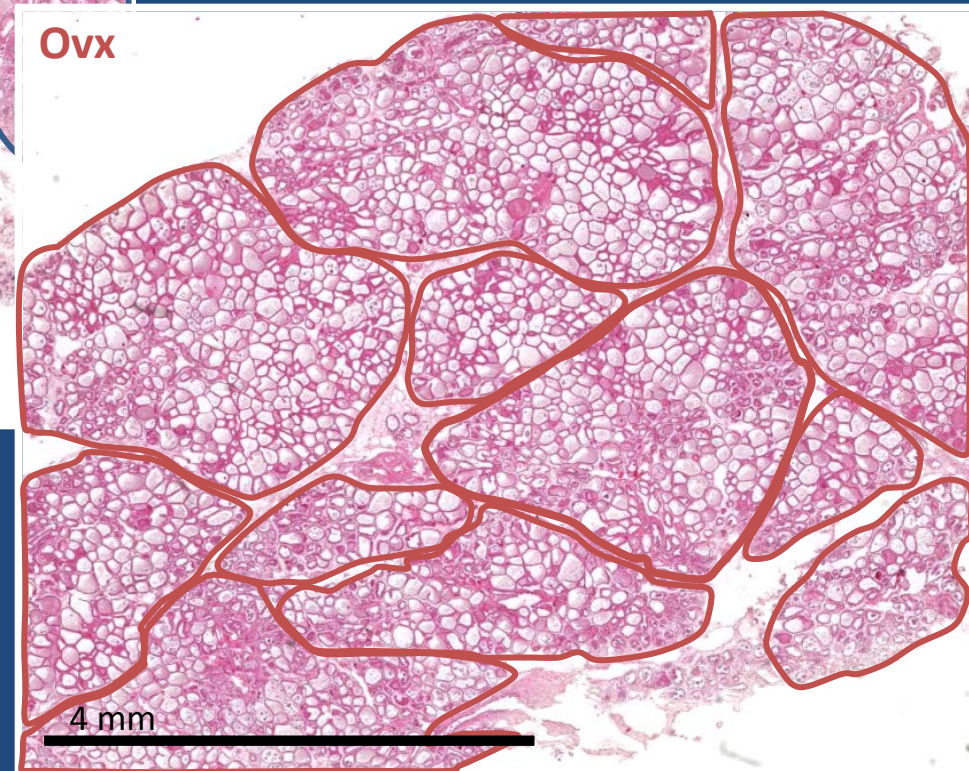
Ovariectomy of lactating cows at the time of lactation peak increases the proportion of mammary secretory tissue



Secretory tissue proportion:

Ovx : 87 %**

Int : 76 %



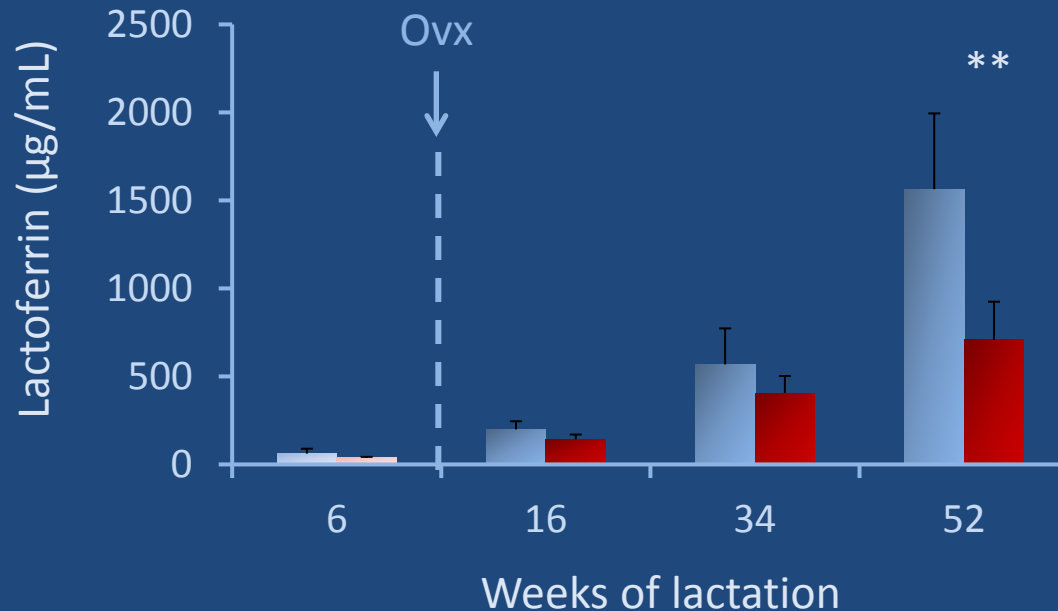
** $p < 0,01$

Nanozoomer images, Hamamatsu technology



Ovariectomy of lactating cows at the time of lactation peak decreases concentration in involution markers in milk

Lactoferrin concentration in milk

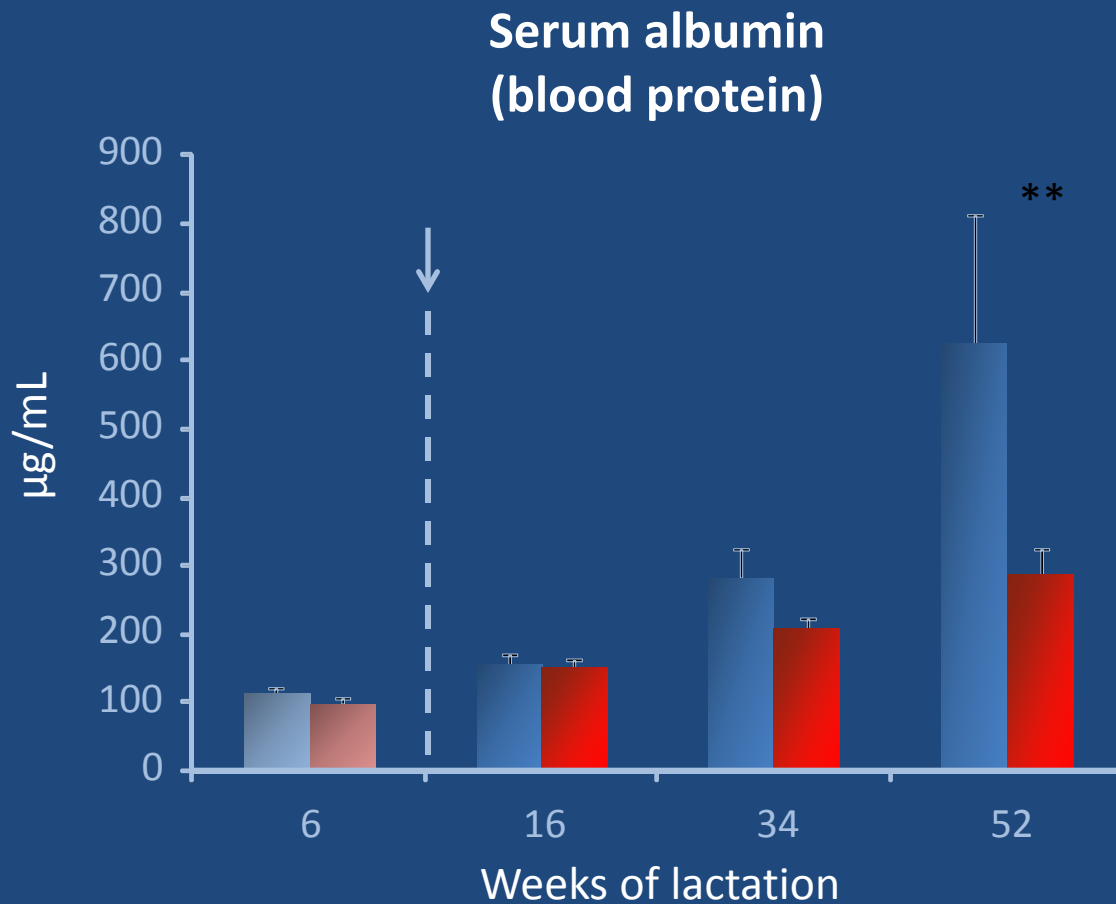
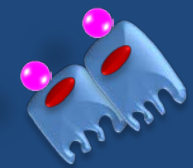


	Time	Ovx
Lactoferrin	**	*
Int (n = 7)		Ovx (n = 7)

* $p < 0,05$; ** $p < 0,01$



Ovariectomy of lactating cows at the time of lactation peak limits the decline in milk production



Time Ovx

Serum albumin ** *

Sham (n = 7)

Ovx (n = 7)

* $p < 0,05$; ** $p < 0,01$

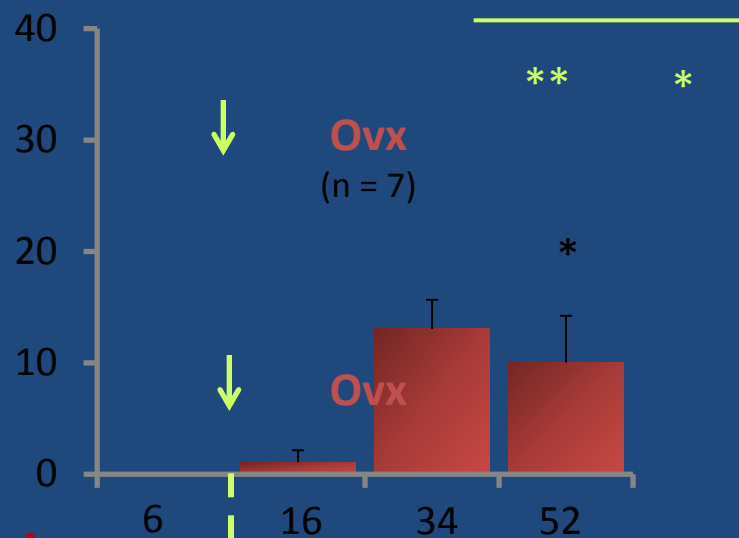
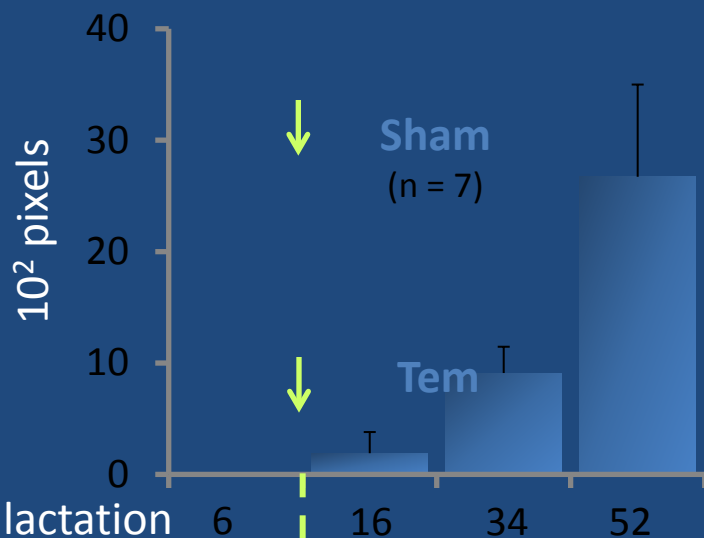


Ovariectomy of lactating cows at the time of lactation peak decreases mammary tissue remodeling

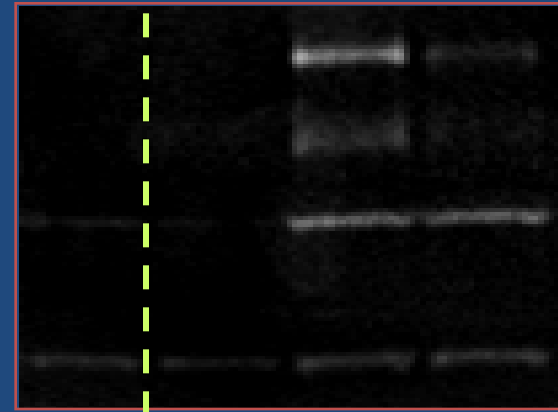
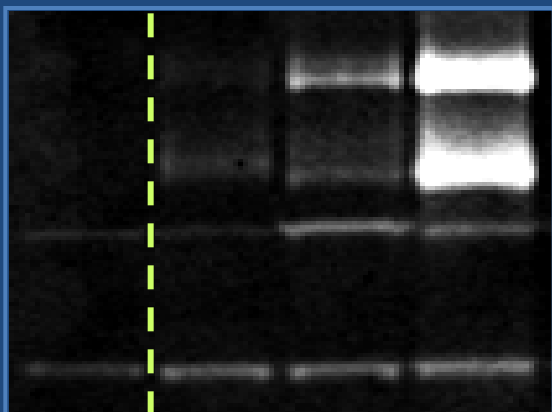


Evolution of **MMP activity in milk** during lactation.

MMP 120 kDa activity



Weeks of lactation



← MMP 120
← MMP 102
← MMP 89
← MMP 57

* $p < 0,05$; ** $p < 0,01$

Conclusion

Effects of ovarian steroids on mammary function during lactation

Estradiol treatment of lactating cows induces a decrease in milk yield and accelerates mammary gland involution

Ovariectomy of lactating cows at the time of lactation peak:

- improves lactation persistency by limiting the decline in MY, and cell turnover
- Reduce the relative decrease of mammary secretory tissue and the tissue remodeling
- decreases the concentration in involution markers in milk

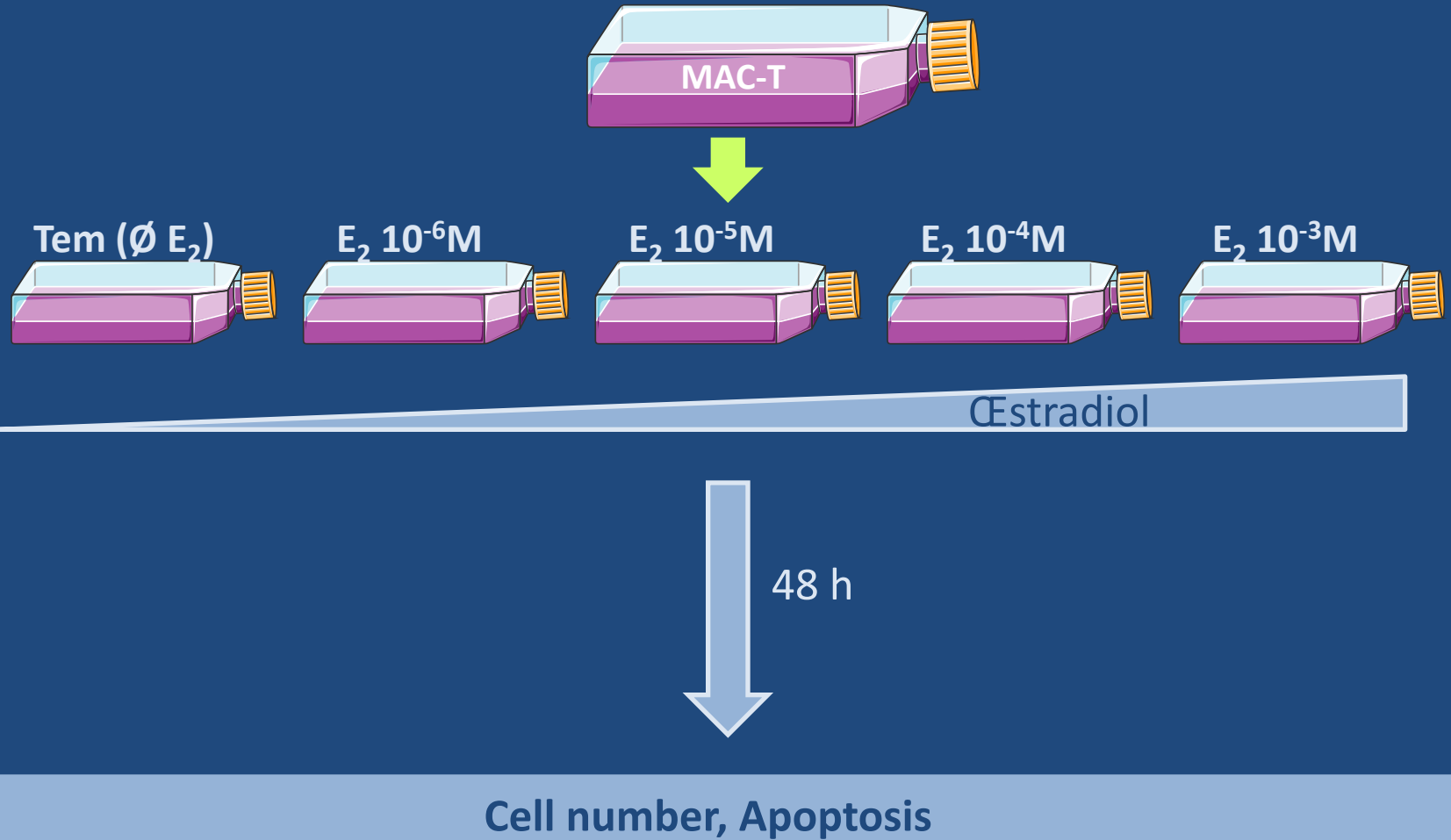
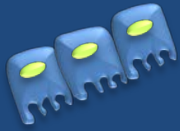




Effects of estradiol on mammary epithelial cells in vitro (example in MAC-T cells)

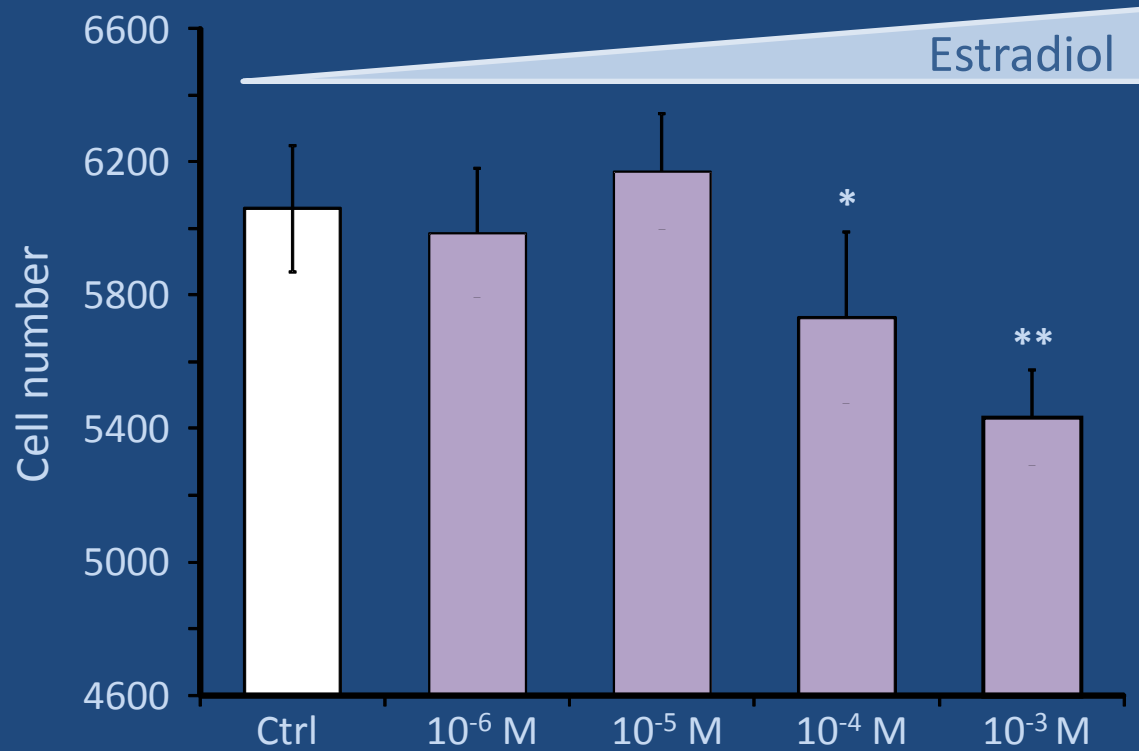
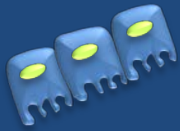


Effects of estradiol on MEC *in vitro*





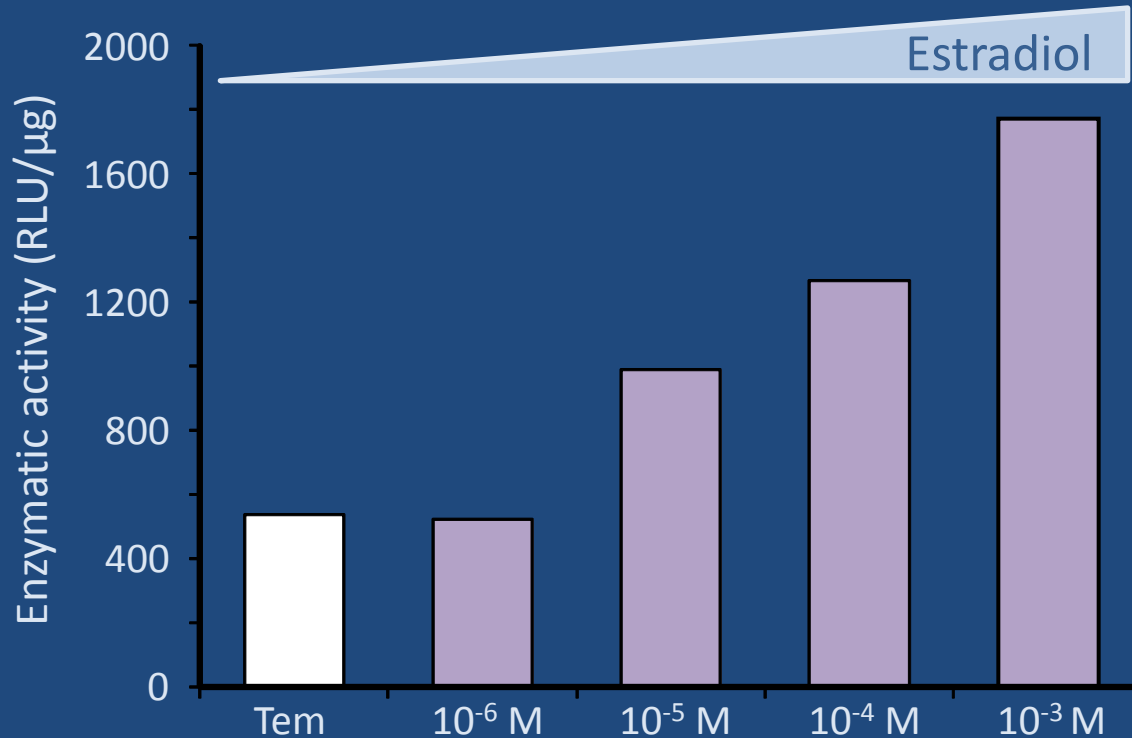
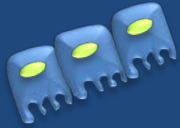
Estradiol treatment induces a decrease in cell viability



↑ [estradiol] → ↓ Cell number



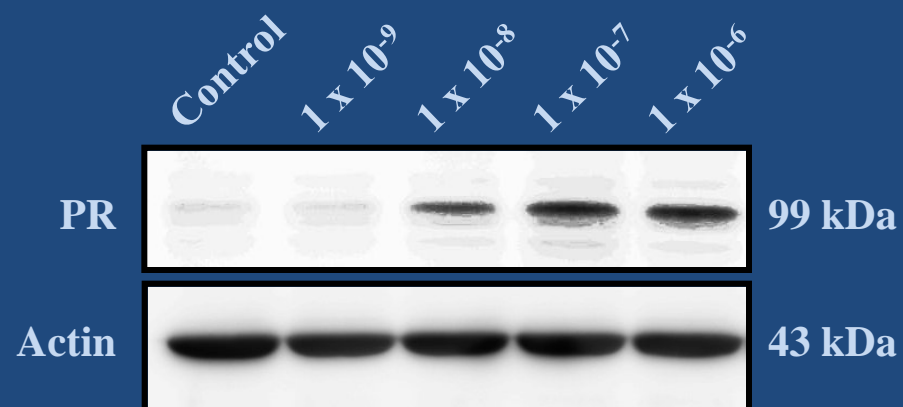
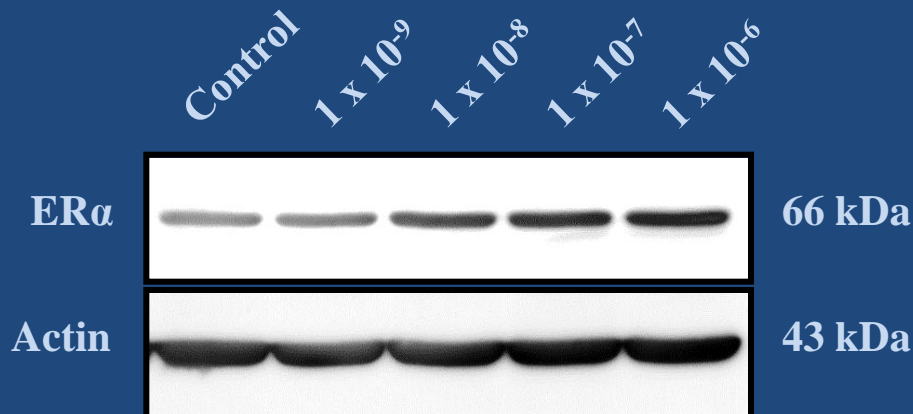
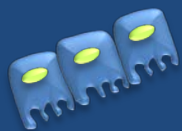
Estradiol treatment induces apoptosis in MAC-T cells

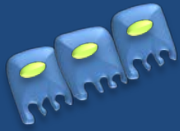


↑ [estradiol] → ↑ caspase 3 activity (Apoptosis)

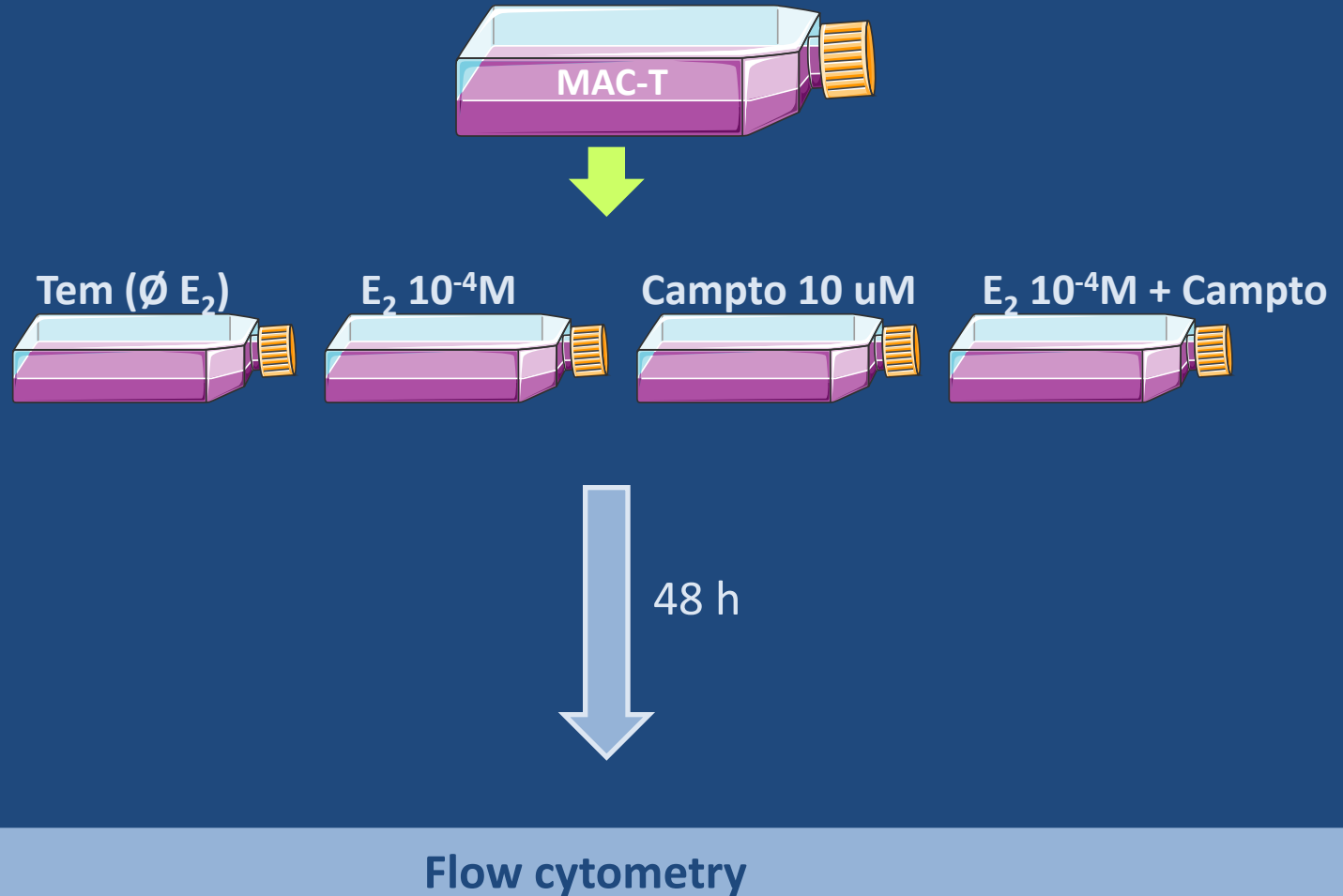


Estradiol treatment induces ER α and PR expressions



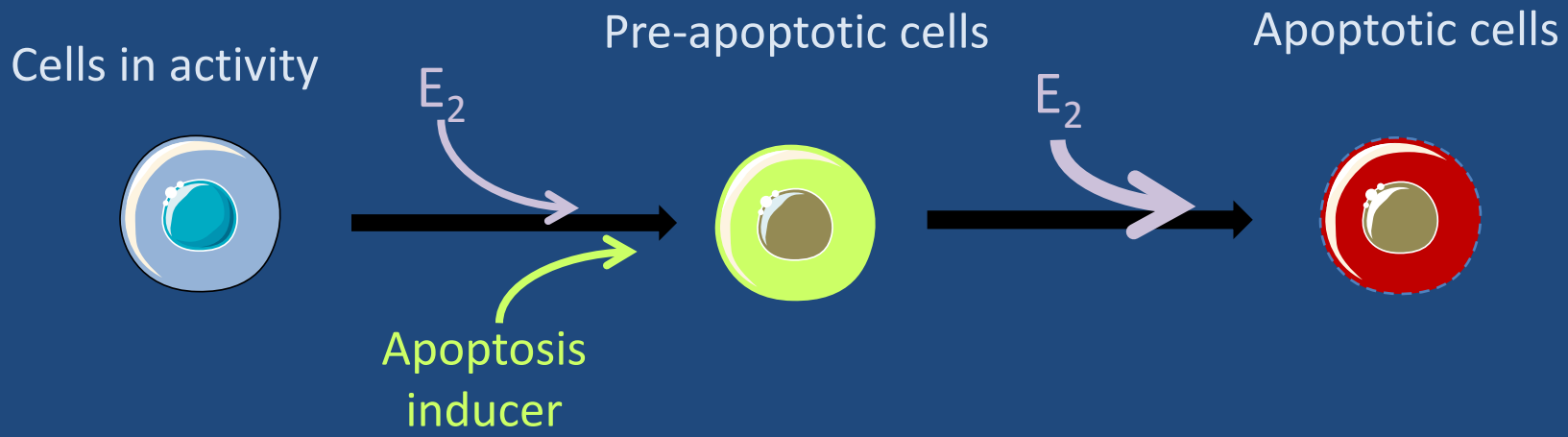
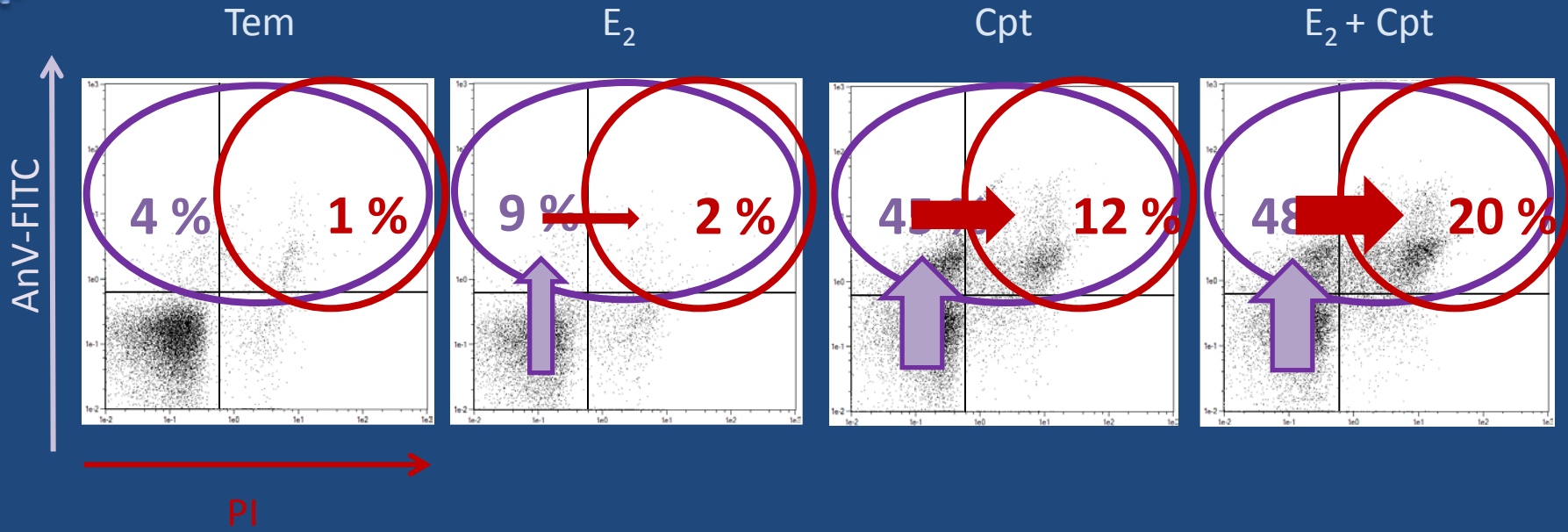
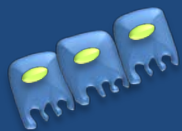


Effects of estradiol on MEC *in vitro*





Estradiol treatment enhances apoptosis in pre-apoptotic MAC-T cells





Conclusions

Effects of ovarian steroids on mammary function during lactation

Estradiol treatment induces a decrease in cell viability

Estradiol treatment induces an increase in steroid receptors

Estradiol treatment induces apoptosis in MAC-T cells

Estradiol treatment enhances apoptosis in pre-apoptotic MAC-T cells



General Conclusions

Ovarian steroids, estradiol and progesterone, are **key factors** for mammary **development** and **function**

Before puberty:

Ovarian steroids stimulate growth and morphogenesis by enhancing MECs proliferation, in heifer and in goat.

But they are not required for mammary development in lamb. (*Ellis et al., 1998*)

During lactation:

Ovarian steroids have a negative effect on milk production in cows:

- by accelerating mammary secretory tissue involution as lactation stage increase
- and by enhancing apoptosis in pre-apoptotic (senescent) MECs



Thank you for your attention !

QUESTIONS ?

