Nutritional and metabolic mechanisms in the ovarian follicle

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EXAMPLES OF THE RELATIONSHIPS BETWEEN NUTRITION AND REPRODUCTION IN CATTLE

- In dairy cows, omega 3 PUFA supplementation affects ovarian follicular development (Ponter et al., 2006), steroidogenesis (Oldick et al., 1997), increases small follicle number (Ponter et al., 2006), and improves development of oocytes and embryos

- In sheep, Lupin grain (Kosior-Korzecka et Bobowiec, 2003, (Downing et al., 1995), Glucose **(Downing et al., 1995)** and Branched chain amino acids **(Downing et al., 1995)** infusion increase the ovulation rate

➡ The mechanisms ?

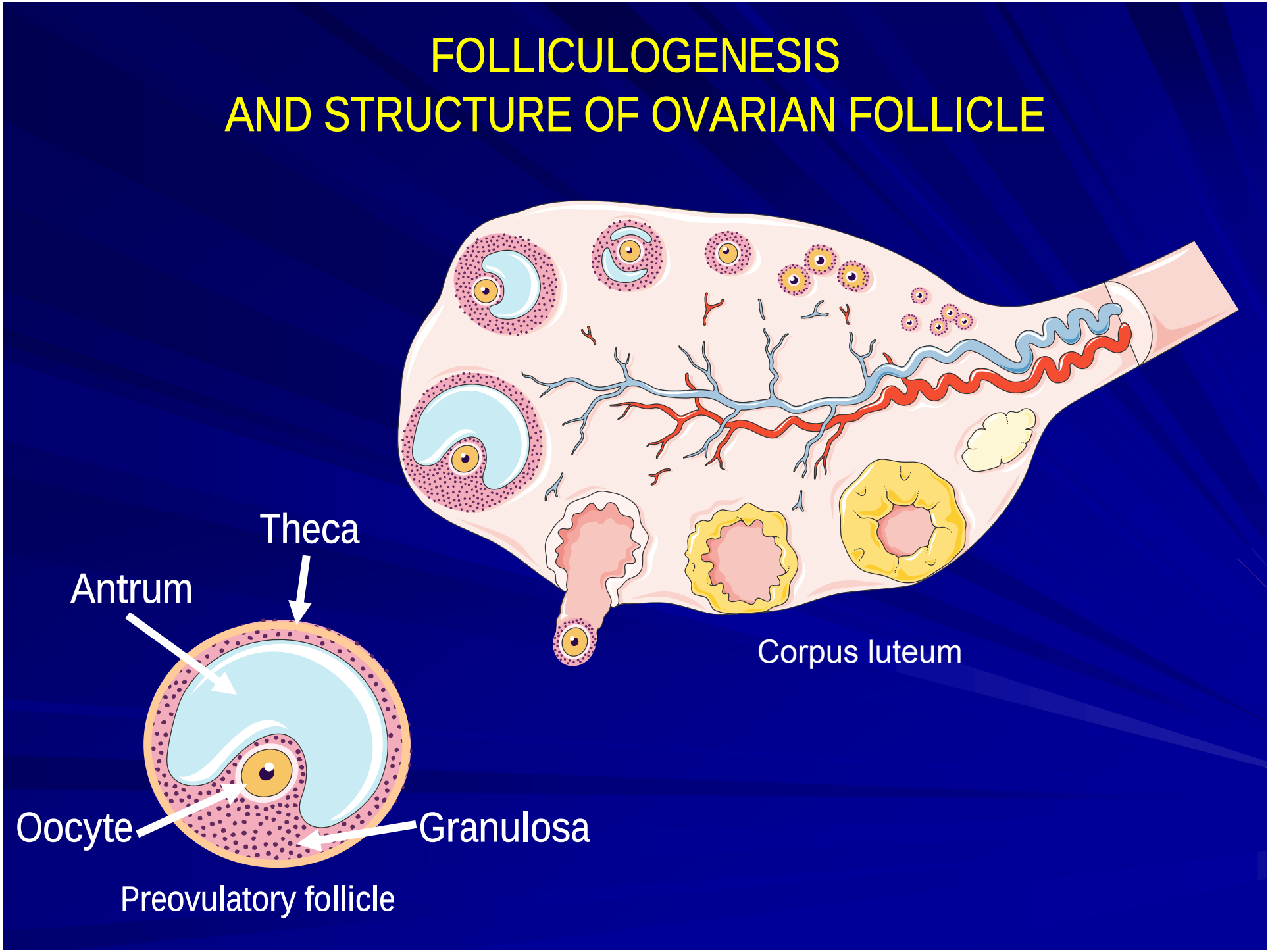
HOW DOES NUTRITION AFFECT FOLLICLE FUNCTIONS ?

- Possible sites of action include:
  - The hypothalamo-pituitary axis (indirect)
  - Other sites (indirect)
  - The follicle (direct)

- The mechanism of the nutritional effect on the follicle can be either static (absence of weight change) or dynamic (in response to weight change)

- The nutritional effect on the follicle can occur at any stage during folliculogenesis

FOLLICULOGENESIS AND STRUCTURE OF OVARIAN FOLLICLE

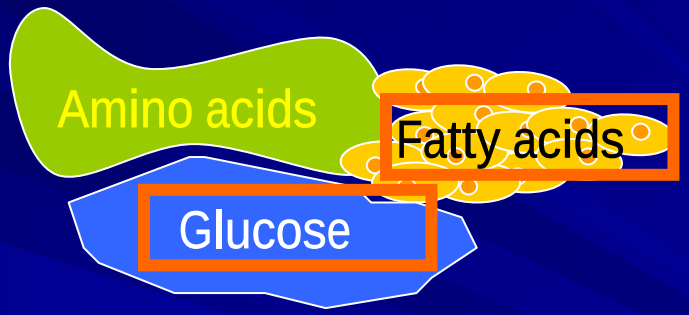


HOW DOES NUTRITION AFFECT REPRODUCTION ?

Although it is self evident that there are biochemical link(s) between metabolism and reproduction, the nature of these link(s) is still a debate.

Some possibilities:

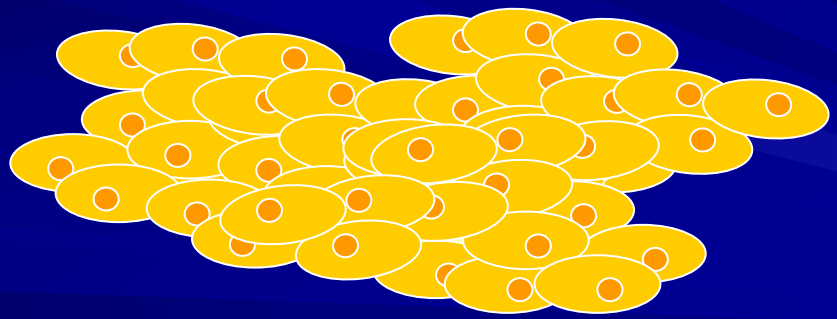
- Metabolites and Nutrients:



- Hormones

Pituitary	Liver	Adipose Tissue	Digestive system
Growth Hormone	IGFs	Leptin	Insulin
Prolactin	IGFBP's	Other adipokines	Glucagon
LH/FSH			Gut peptides

FATTY ACIDS



EFFECT of FATTY ACIDS ON THE FOLLICLE FUNCTIONS IN CATTLE

Several studies have already shown beneficial effects of dietary fat supplementation such as PUFAs on reproductive parameters

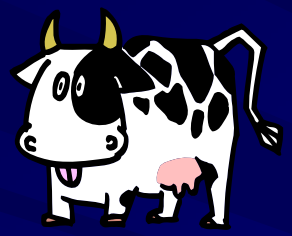
(Mattos et al., 2000, Staples et al., 1998)

Follicular development

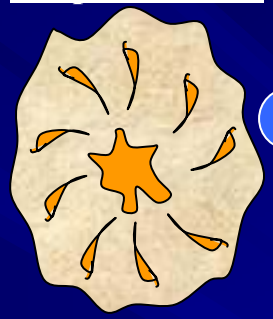
- ↑ in number of ovarian follicles
- ↑ in the size of pre-ovulatory follicle
- ↑ in the plasma concentration of progesterone
- ↑ in the lifespan of the corpus luteum

Number and quality of oocyte and embryo

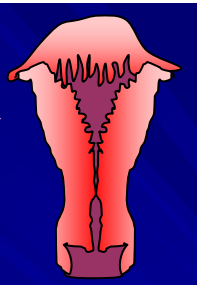
EFFECT of FATTY ACIDS ON FOLLICLE GROWTH, OOCYTE QUALITY AND EMBRYO IN CATTLE



Corpus Luteum



Endometrium



+ PUFA

+/− SFA

− PUFA

Corpus Luteum quality

+ PUFA

+/− SFA

steroids production

Progesterone

+

− Prostaglandins (PGF2α)

Primary Follicle

Follicle growth

Preovulatory follicle

+ PUFA

− SFA

Embryo quality

Embryo

Oocyte quality

Cumulus-oocyte Complexes

PUFA = Poly-Unsaturated Fatty Acids
SFA = Saturated Fatty Acids

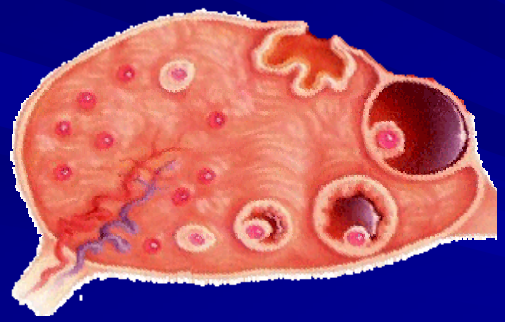
Adapted from Leroy et al., 2008

HOW DO FATTY ACIDS AFFECT FOLLICLE FUNCTIONS IN CATTLE ?

Indirect effects

(hormonal action) ?

(insulin,
adipokines : leptin,
adiponectin and visfatin…)



Direct

Effects ?

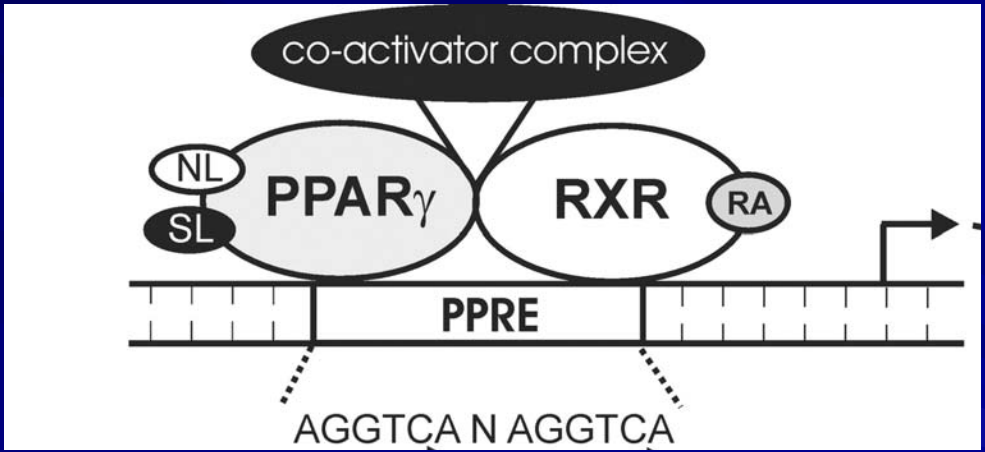
Moreno-Aliaga MJ Proc Nutr Soc. 2010

HOW DO FATTY ACIDS AFFECT FOLLICLE FUNCTIONS IN CATTLE ?

A Fatty acids sensor : PPARs

(Peroxisome proliferator-activated receptors)

3 isotypes (3 genes) : PPARα PPARβ PPARγ



5' PPRE 3'

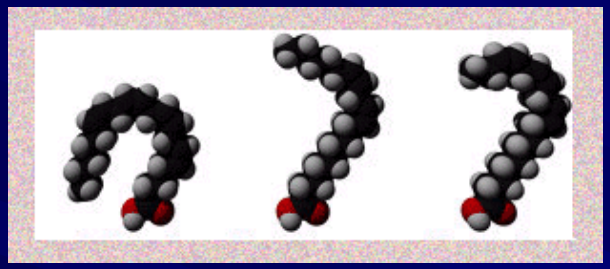
(PPAR Responsive Element)

Dimerisation with RXRα to mediate its action

Brown J M , McIntosh M K J. Nutr. 2003;133:3041-3046

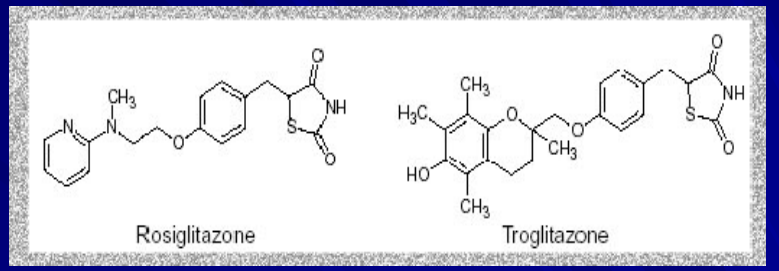
HOW DO FATTY ACIDS AFFECT FOLLICLE FUNCTIONS IN CATTLE ?

Natural Ligands

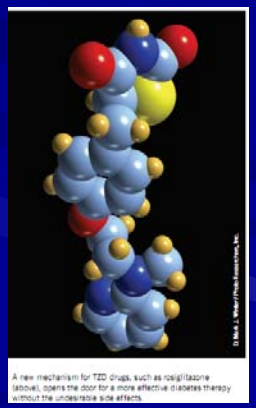


PUFAs
Products from prostaglandins

Synthetic Ligands

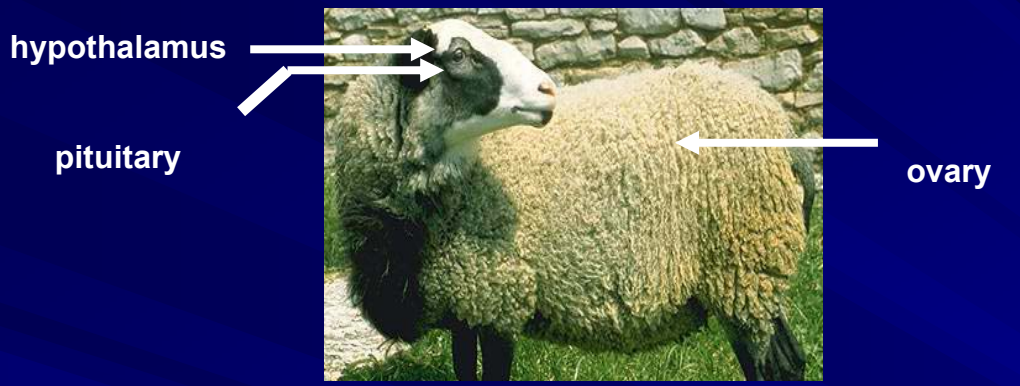


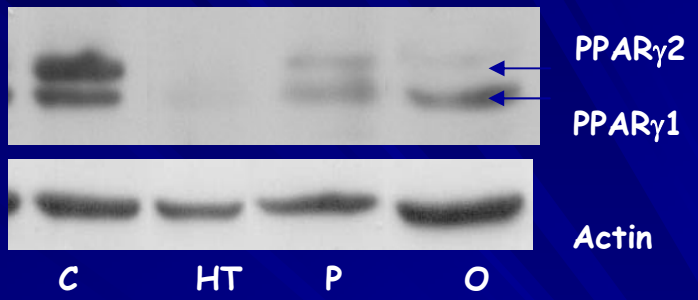
thiazolidinediones
phthalates

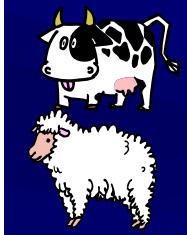


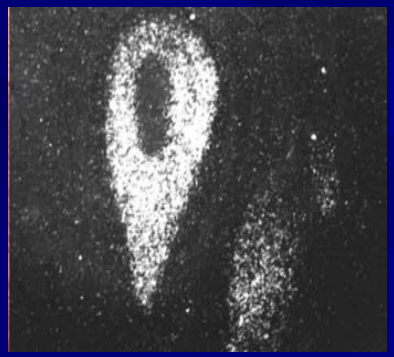
Peroxisome proliferator-activated receptors

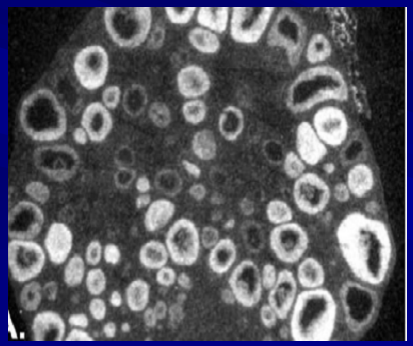
EXPRESSION OF PPARγ IN GONADOTROP AXIS IN SHEEP

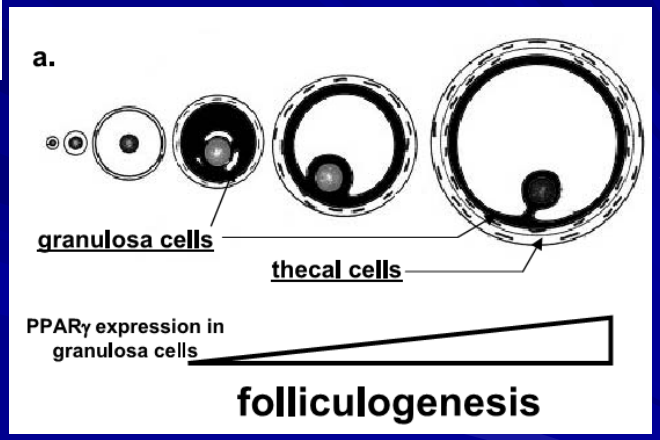








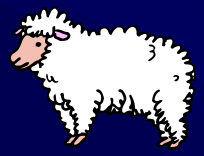




Froment et al. 2005

- High expression in granulosa cells
- No relation with follicle quality (atresia or healthy follicle)

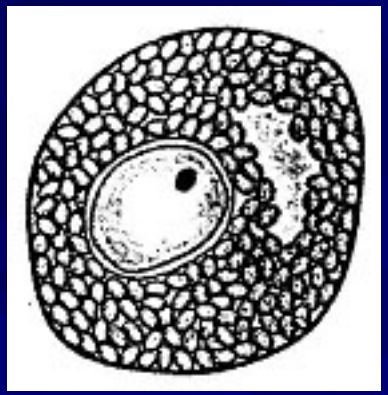
Froment et al. 2003
Komar et al. 2003



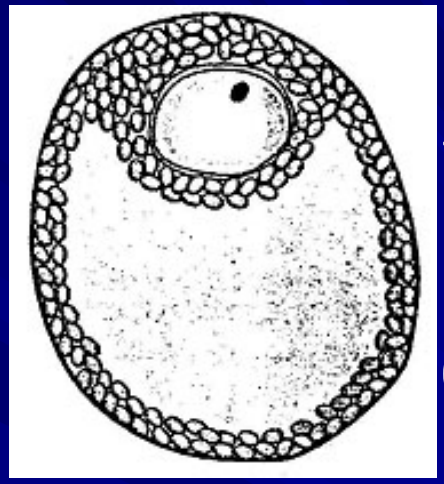
ROLE OF PPARγ IN FOLLICLE DEVELOPMENT?

« Small» Follicle

< 3 mm



maturation



« Large» Follicle

>5 mm

(Ovulation 7mm)

proliferation

steroïdogenesis

proliferation

steroïdogenesis

PPARγ ligands
reduce cell
proliferation

PPARγ ligands
increase steroid production

ROLE OF PPARγ IN OVULATION ?

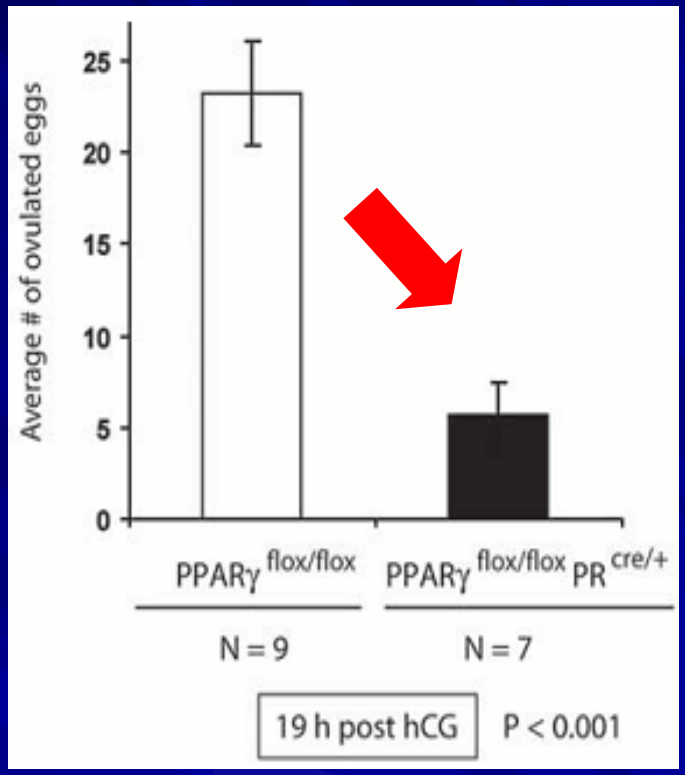
MOLECULAR AND CELLULAR BIOLOGY, Mar. 2008, p. 1770–1782 Vol.
0270-7306/08/$08.00+0 doi:10.1128/MCB.01556-07

Peroxisome Proliferator-Activated Receptor γ Is a Target of Progesterone Regulation in the Preovulatory Follicles and Controls Ovulation in Mice▽

Jaeyeon Kim,[1] Marcey Sato,[1] Quanxi Li,[2] John P. Lydon,[3] Francesco J. DeMayo,[3] Indrani C. Bagchi,[2] and Milan K. Bagchi[1]*

Inactivation of PPARγ gene in granulosa cells from large follicle

Number of oocytes ovulated

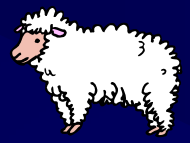


The Fatty acid sensor, PPARγ, could be involved in :

- **granulosa cells maturation**
- **ovulation**

GLUCOSE/INSULIN

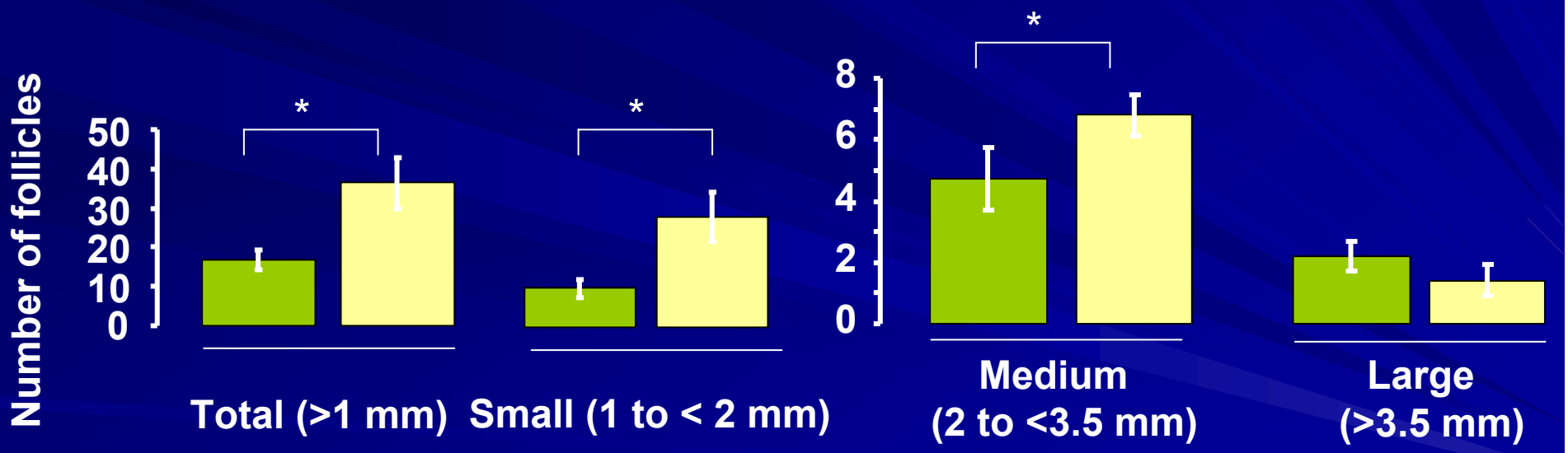
EFFECT OF GLUCOSE IN FOLLICLE FUNCTIONS IN SHEEP ?



Saline or

Glucose infused at 10 mM

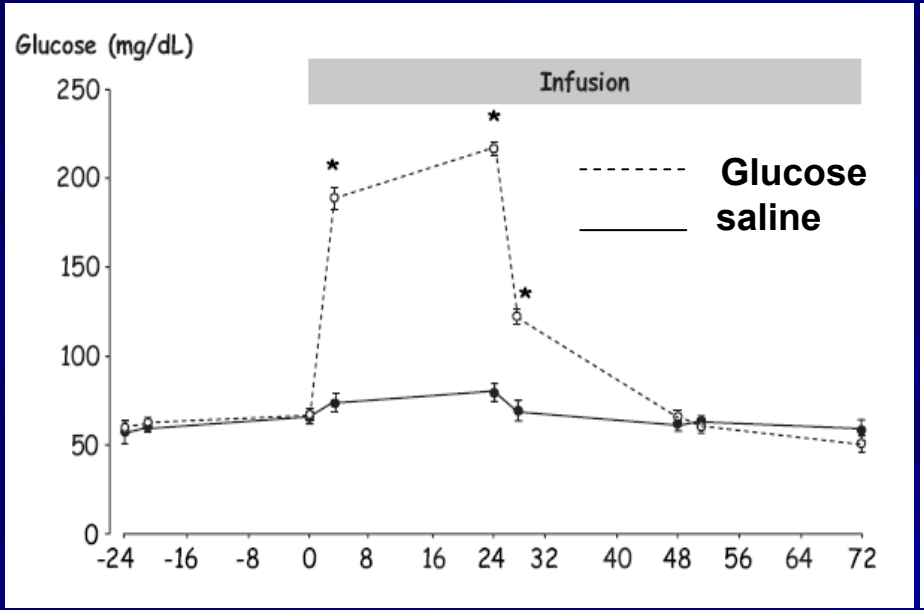
for 72h during the Late luteal phase of the oestrus cycle

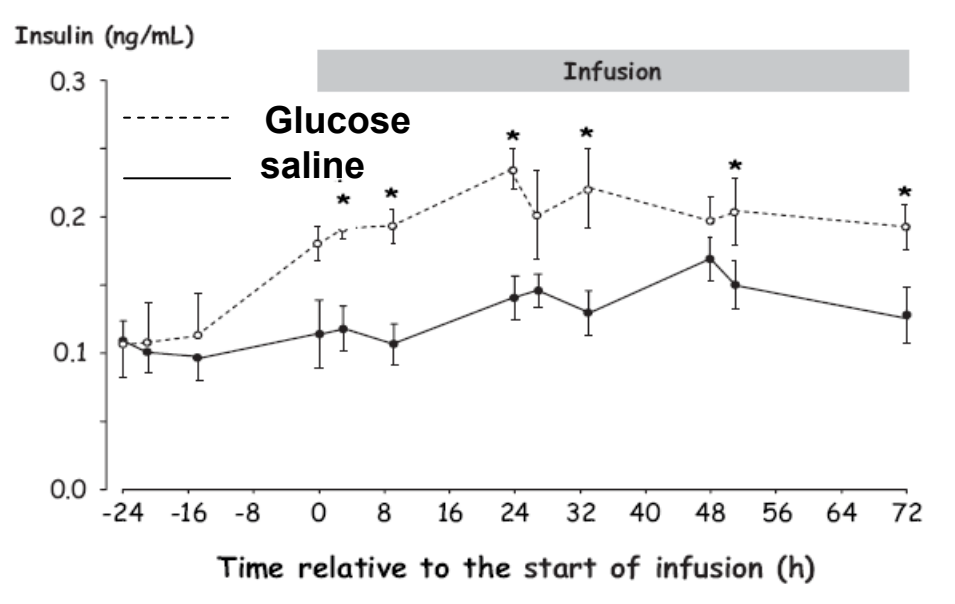


Glucose infusion during the late luteal phase of the oestrus cycle Increases small and medium follicle numbers

(Gallet et al., 2011)

EFFECT OF GLUCOSE IN FOLLICLE FUNCTIONS IN SHEEP ?

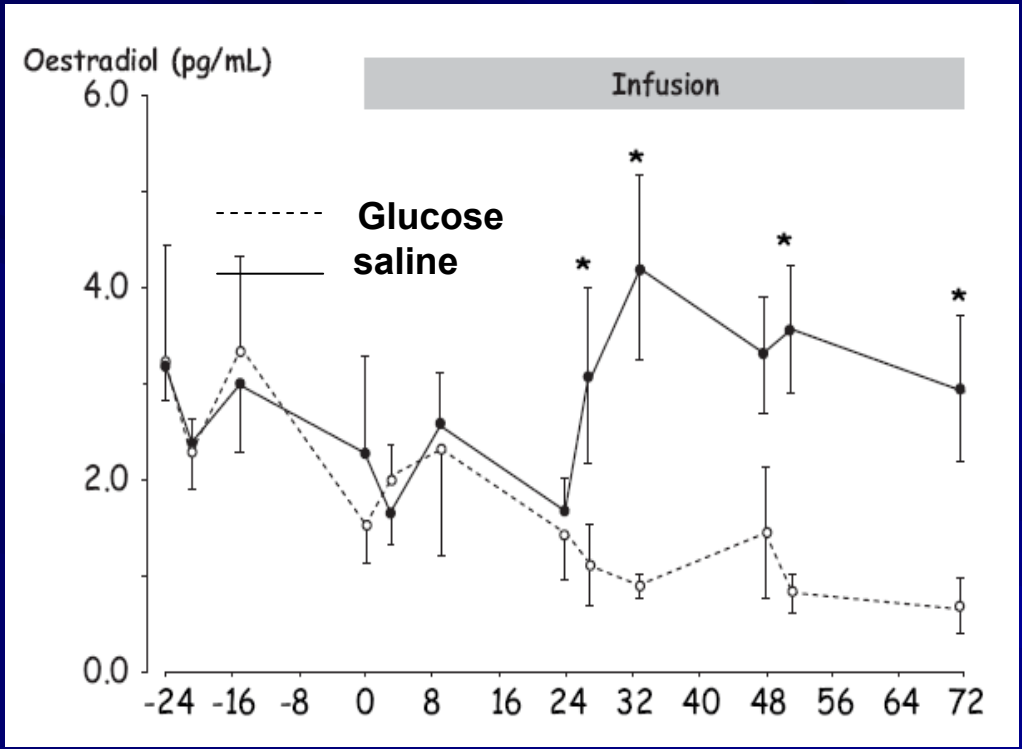




Glucose infusion during the late luteal phase of the oestrus cycle
Increases rapidly glycemia and insulinemia
but has no effect on plasma FSH and LH (basal and pulse frequency)

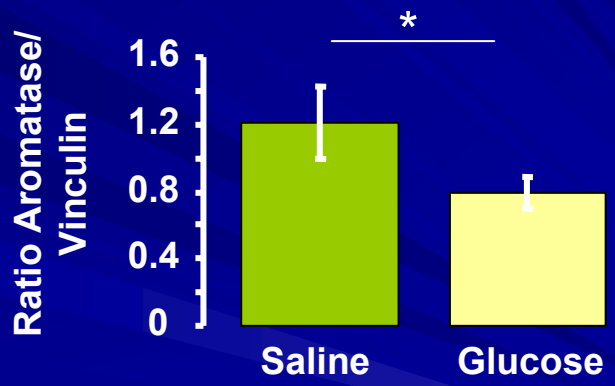
(Gallet et al., 2011)

HOW DOES GLUCOSE AFFECT FOLLICLE FUNCTIONS IN SHEEP ?



(Gallet et al., 2011)

Aromatase Protein amount
in medium follicle (3 mm)



Glucose infusion during the late luteal phase of the oestrus cycle decreases plasma oestradiol and aromatase expression in follicles

Specific effect of insulin or glucose in granulosa cells ?

HOW DOES GLUCOSE AFFECT FOLLICLE FUNCTIONS IN SHEEP ?

Effects of glucose on the number of follicle and plasma oestradiol concentration ?

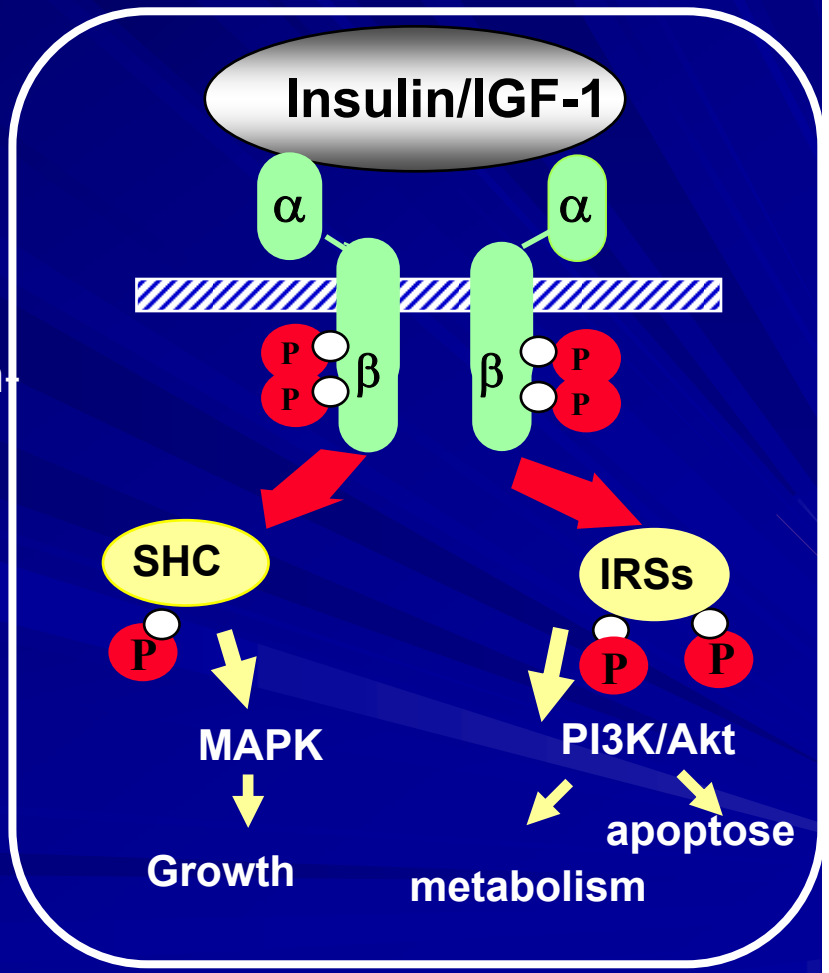
- Indirect through insulin

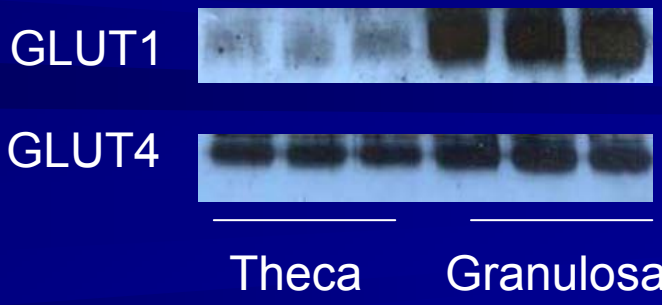
Different components of insulin signaling are expressed in follicular cells

(receptor, substrate … Glucose transporter insulin-sensitive, GLUT4)

- Direct on the follicular cells through :

  - facilitated glucose transporters (GLUT1)
  - and the AMPK kinase

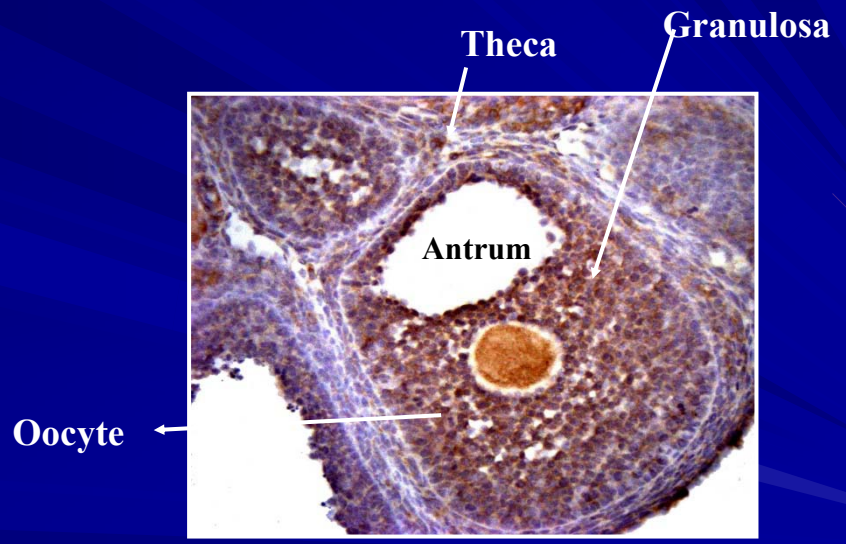




Williams et al., 2001

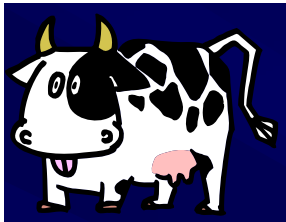
AMPK (Adenosine Monophosphate activated kinase)

- is an energy sensor in the cell (it is a regulator of energy balance), expressed in many cells of several species including bovine, sheep, goat
- is a serine threonine kinase activated in response to low energy status
- is largely expressed in granulosa, oocyte and cumulus cells and less abundantly in theca cells



- is stimulated in response to nutrients such as glucose in sheep granulosa cells

Tosca et al., 2007



Involvement of AMPK in oocyte maturation

24h MIV

pAMPK ↓ (oocyte)

pAMPK ↓ (Cumulus cells)

oocyte

Cumulus cells

Métaphase II

expansion of cumulus cells

24h MIV
+ Metformin
(AMPK activator)

pAMPK ↑ (oocyte)

pAMPK ↑ (Cumulus cells)

oocyte

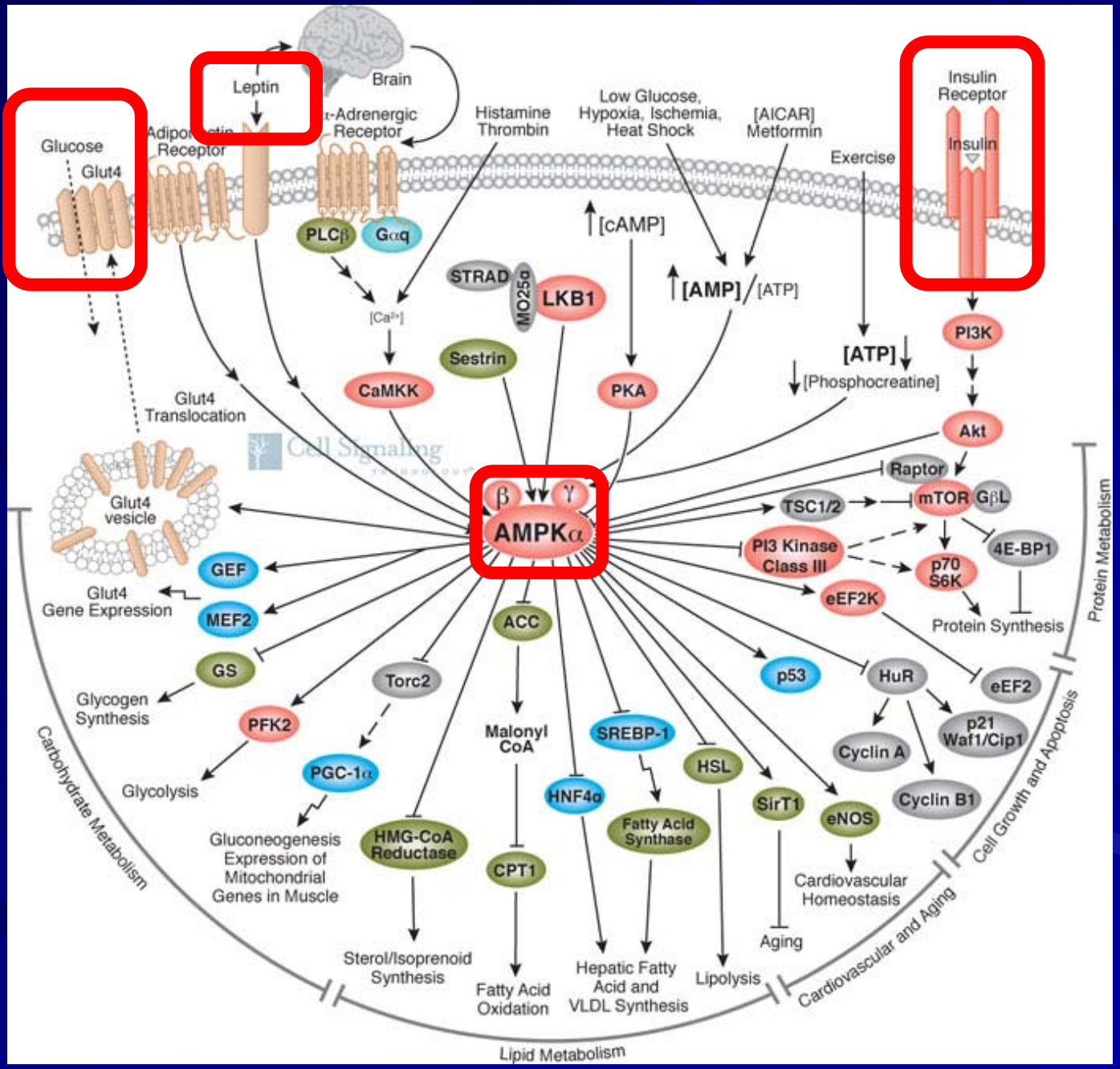
Cumulus cells

~~Métaphase II~~

~~expansion of cumulus cells~~

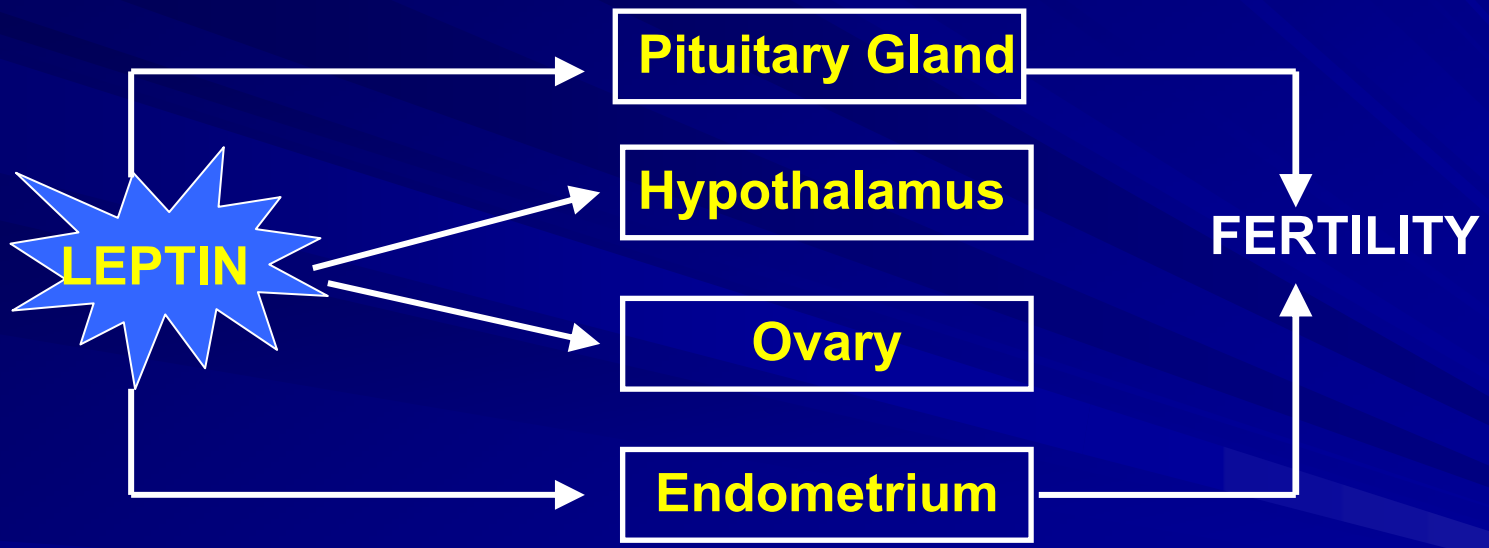
Tosca et al., Biol Reprod. 2007, 2008

AMPK activation reduced oocyte maturation



From Cell Signaling Technology, Inc.

LEPTIN



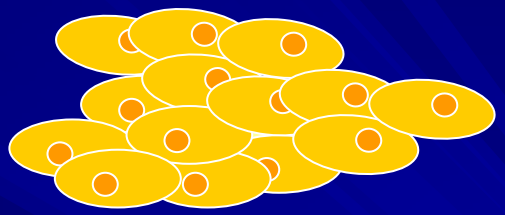
Action of leptin on hypothalamo-pituiray-gonadal axis

Pinelli, MINERVA GASTROENTEROL DIETOL 2007

HOW DOES LEPTIN AFFECT FOLLICLE FUNCTIONS IN CATTLE ?

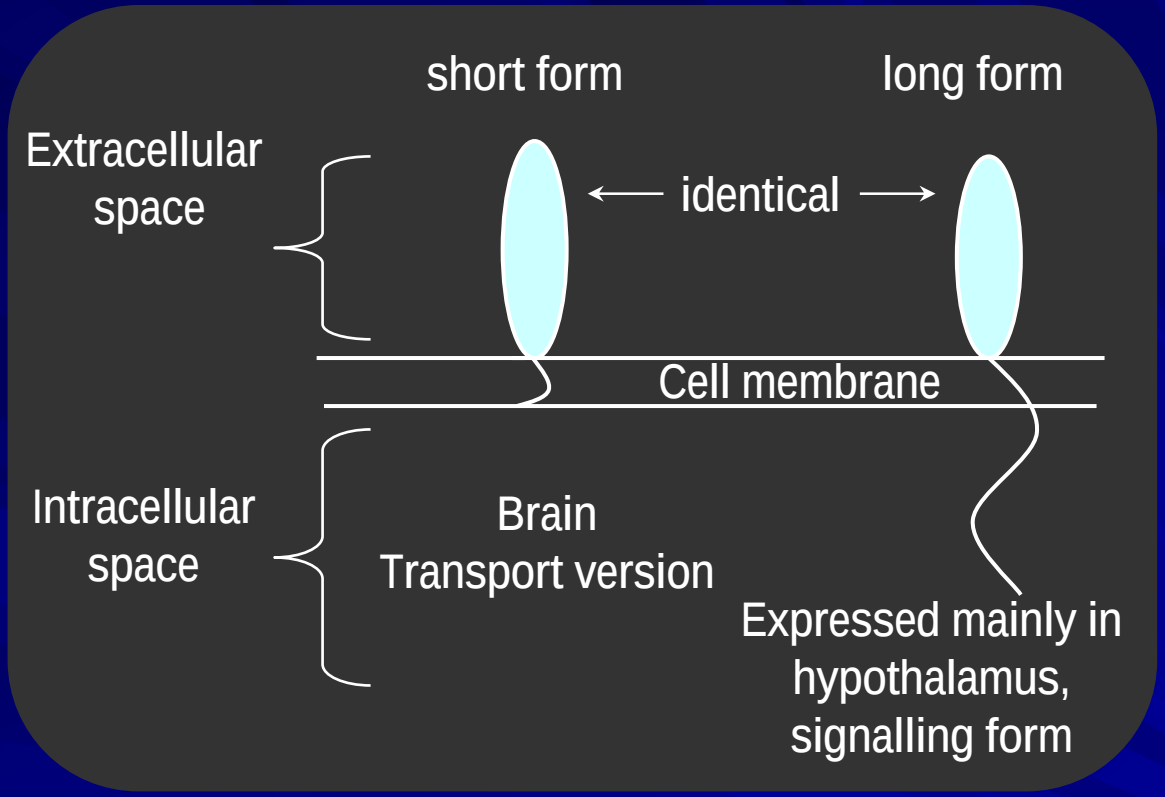
Leptin:

- -produced in adipose cells but mRNA found also in other tissues including Ovary
- -found in plasma, CSF, milk and follicular fluid



- -plasma leptin concentrations reflect not only body fat content but also availability of metabolic fuel => it's a « metabolic signal »
- -correlation with fat depot
- -diurnal variation
- -control food intake

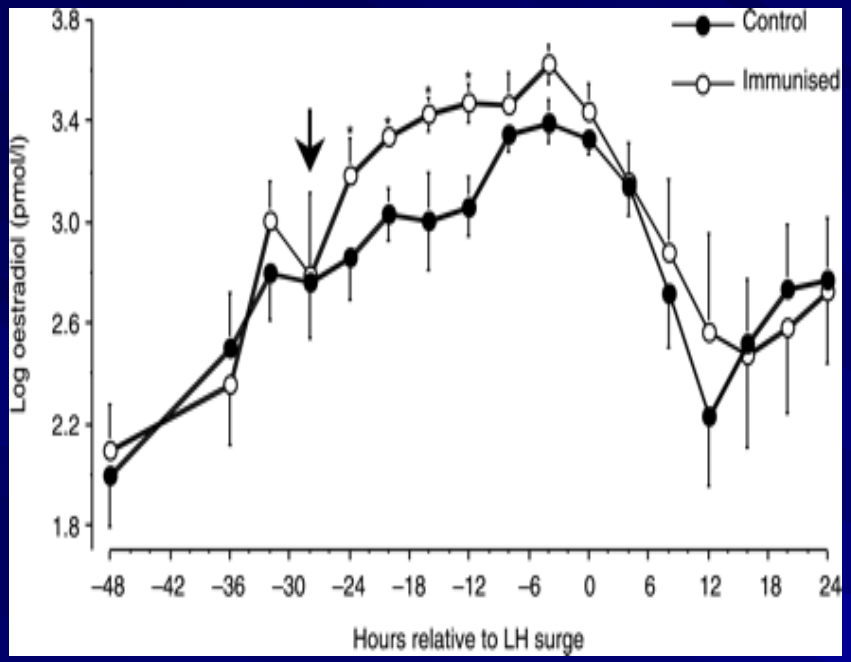
Expression of leptin and its receptor in ovarian cells ?



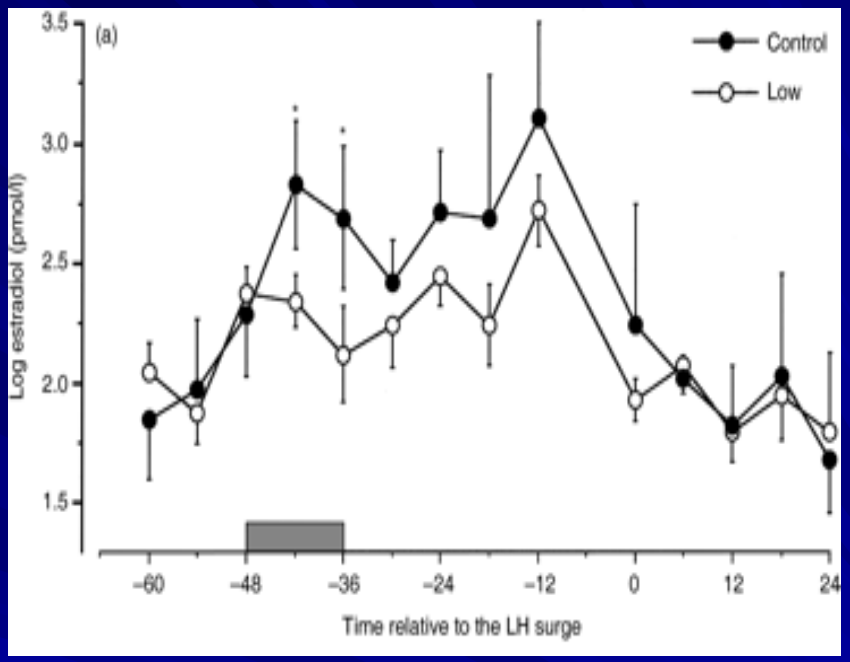
- **leptin is expressed in oocyte and cumulus cells**
- **leptin receptor is expressed in granulosa and theca cells, cumulus cells and oocyte in cattle and sheep**

- Leptin receptor mRNA is present in all ovarian follicular cells

Effect of LEPTIN in vivo on oestradiol secretion?



Passive immunisation against leptin results in an acute increase in ovarian oestradiol secretion (no effect on LH/FSH)

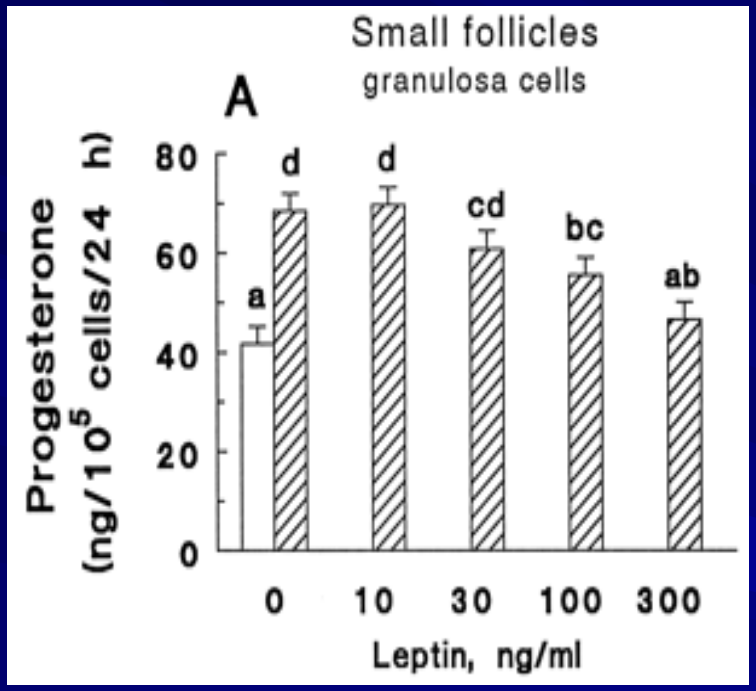


Conversely, direct ovarian arterial infusion of the low dose of leptin results in a decline in ovarian oestradiol secretion (no effect on LH/FSH)

- Leptin decreases in vivo plasma oestradiol

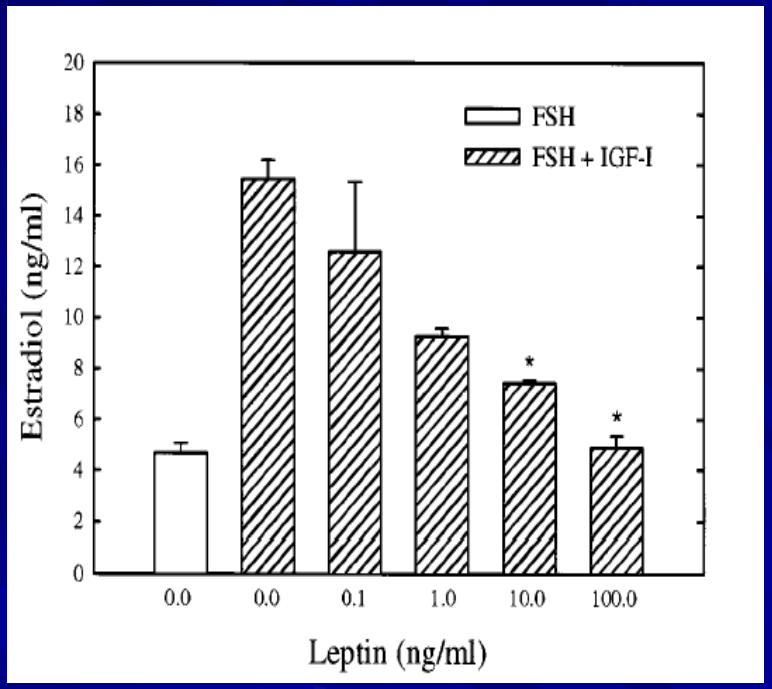
(Kendall NR et al., 2004)

EFFECTS OF LEPTIN ON GRANULOSA CELL steroidogenesis in vitro ?



Leptin inhibits insulin-induced progesterone by granulosa cells from small follicles

(Spicer LJ, Francisco CC, 2007)

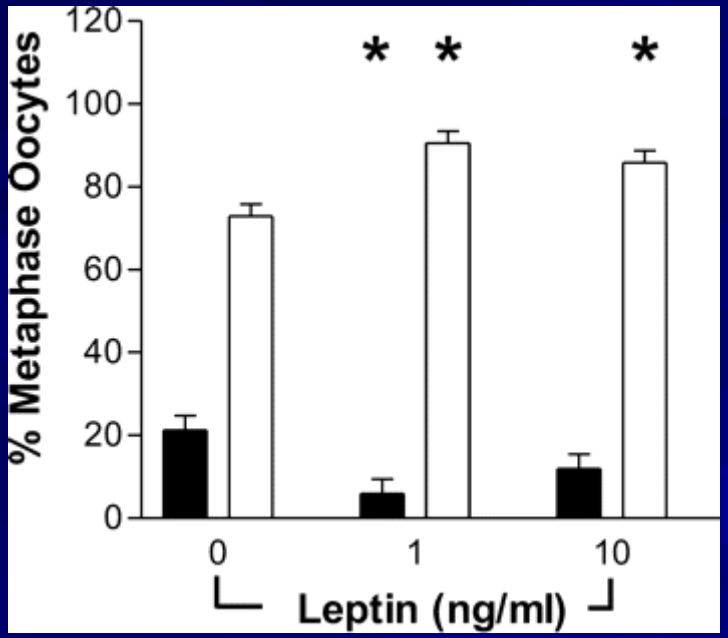


Leptin antagonizes the IGF-1 increase in steroidogenesis in granulosa and theca cells

Agarwall et al, 1999)

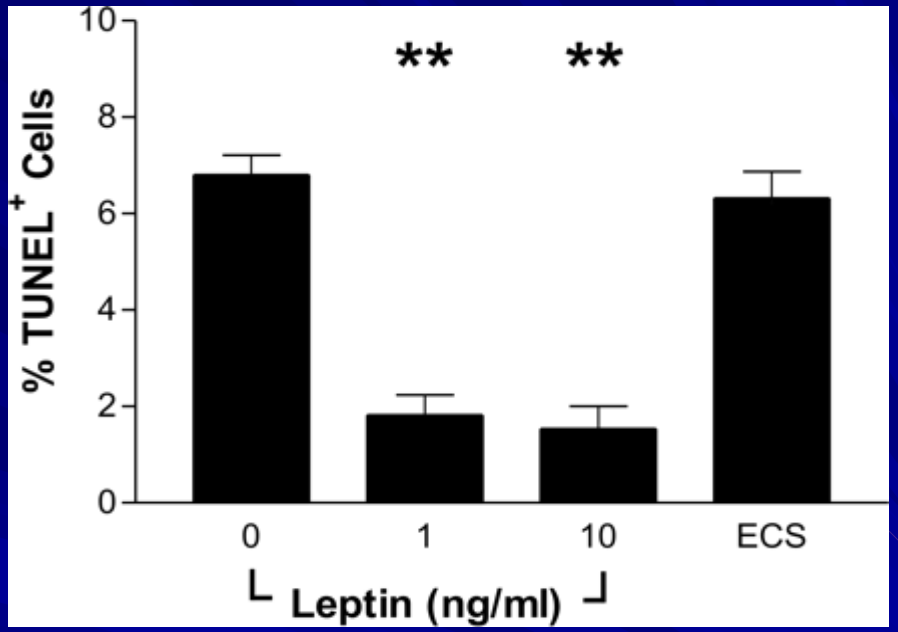
- Leptin decreases in vitro steroid production in granulosa and theca cells

HOW DOES LEPTIN and oocyte maturation in vitro ?



Leptin increases the proportion of oocytes at metaphase II

(Paula-Lopes et al, 2007)



Leptin reduces the proportion of TUNEL-positive cumulus cells

(Paula-Lopes et al, 2007)

- Leptin improves in vitro oocyte maturation

CONCLUSIONS

⇨ There are direct effects of nutrition on the follicle (fatty acids, amino acids, glucose...)

Identification of fatty acid sensors and energy sensors (PPARg, AMPK.....)

⇨ The IGF system, insulin and glucose are all involved

⇨ The effect of insulin is probably via insulin-mediated glucose uptake

⇨ Evidence for other mediators e.g. adipokines (other than leptin, adiponectin...) is emerging

Thanks to :

PhD students:
Lucie Tosca
Christine Chabrolle
Stéphanie Coyral-Castel

Technician:
Christelle Ramé

Claire Gallet
Rex Scaramuzzi

INSERM, Paris:
Pascal Ferré
Fabienne Foufelle
(dominant negative of AMPK)

Institut Cochin, Paris:
Benoît Viollet
(KO AMPK alpha 1…)