



NEW HORMONAL AND INTRACELLULAR REGULATORS OF FEMALE REPRODUCTION

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Background (1)

Regulation of biological processes

Environment



Hormones



Growth factors



Protein kinases



Transcription factors, RNA interference



Genes



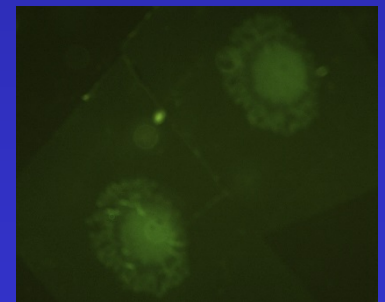
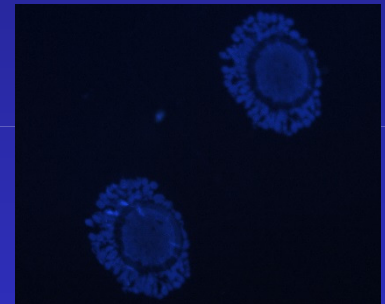
Functions (proliferation, apoptosis, differentiation etc.)



Background (2)

Regulators of reproduction practically used :

- **Environmental factors** (photoperiod, mate)
- **Hormones** (LHRH, gonadotropins, steroids, prostaglandins)
- **Other hormones - none**
- **Growth factors – none**
- **Protein kinases - none**
- **Transcription factors – none**
- **RNA interference – none**
- **Genes - none**



Cause – insufficient knowledge concerning role, effect, mechanisms of action, areas and methods of application

**(1) The role of**

- **hormones** (*GH, nonapeptide hormones, leptin, ghrelin a.o.*),
- **growth factors** (*IGF-I, -II, TGF, TPO*),
- **protein kinases** (*TK, MAPK, CDK a o.*)
- **transcription factors** (*p53, CREB, STAT, NFkB*)
- **RNA interference** (*siRNA, miRNA*)

in control of ovarian functions

(2) Interrelationships between these regulators**(3) Possible areas of practical application**

MATERIAL & METHODS (1)

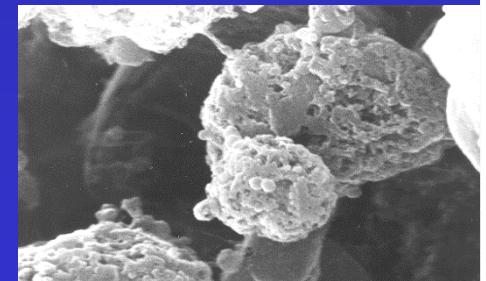
A. Species:

- rabbits
- cows
- pigs
- fowls
- humans



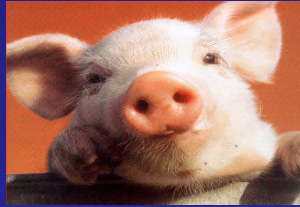
B. Objects:

- living animals
- ovaries, ovarian follicles and their fragments
- ovarian granulosa cells
- oocytes



MATERIAL & METHODS (2)

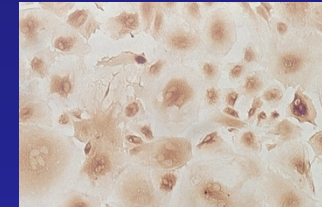
C. Manipulations:



Animals



Isolated cells



Treatment with:

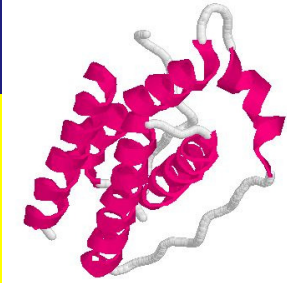
- hormones, growth factors
- antisera against hormones
- protein kinase activators and blockers
- transfection with cDNA, siRNA and miRNA constructs
- combinations of above



Analysis:

reproduction, oocyte maturation, proliferation, apoptosis, secretion, expression of kinases and transcription factors (RIA, Western, immunocytochemistry, TUNEL, RT-PCR a.o.)





THE ROLE OF HORMONES

Hormones *GH, prolactin, nonapeptide hormones, leptin, ghrelin, obestatin a.o.* **control**

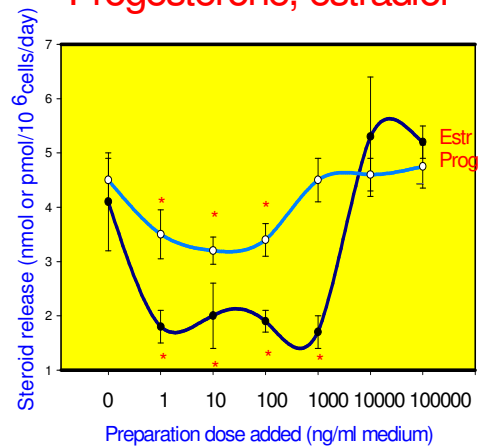
- **proliferation**
- **apoptosis**
- **release of hormones and growth factors**
- **oocyte maturation**
- **intracellular signaling substances**
- **reproductive indexes and fecundity**

Can be used for prediction and improvement of fecundity

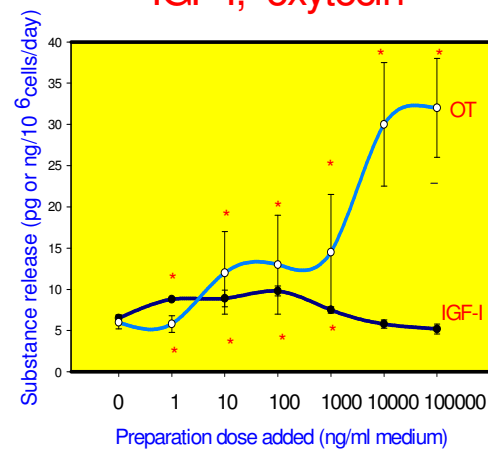
GH regulates secretory activity of human ovarian granulosa cells



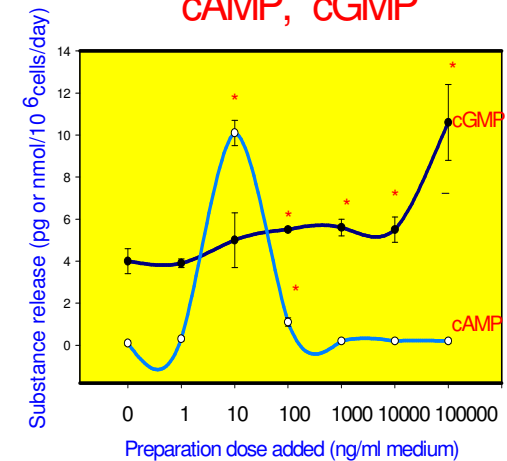
Progesterone, estradiol



IGF-I, oxytocin



cAMP, cGMP

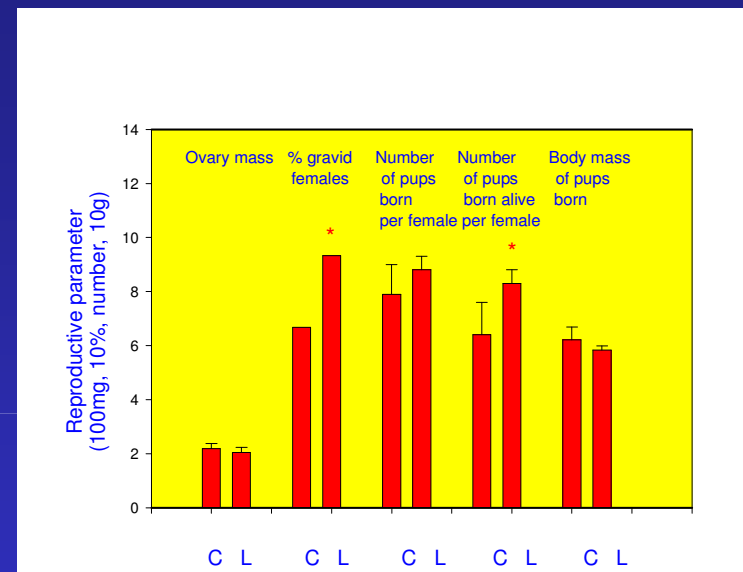
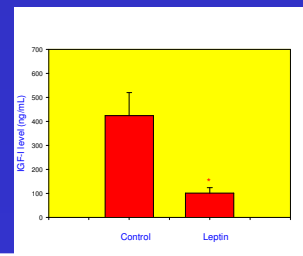
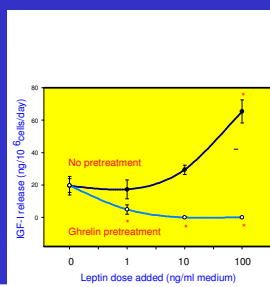
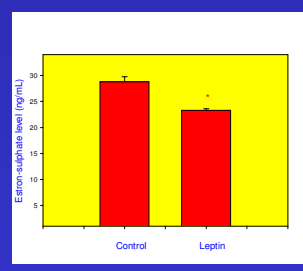
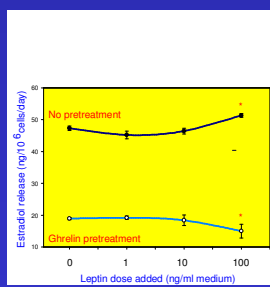
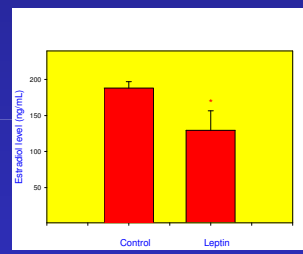
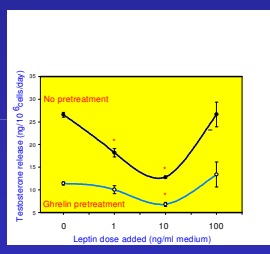
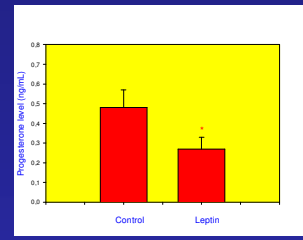
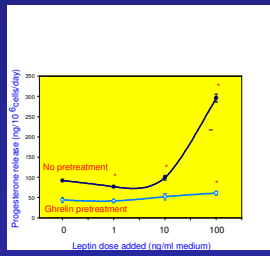


Leptin treatment of rabbits

- affects release of hormones by ovarian cells

- decreases hormones level in blood plasma

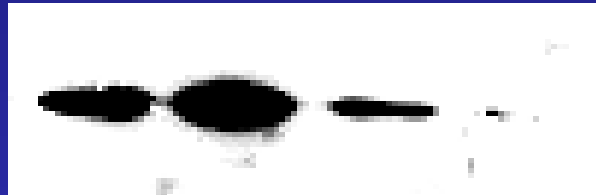
- increases fertility





Leptin affects PKA, MAPK/ERK1,2 and CDK/p34 in chicken ovarian follicles and granulosa cells

PKA 47K



MAPK44K

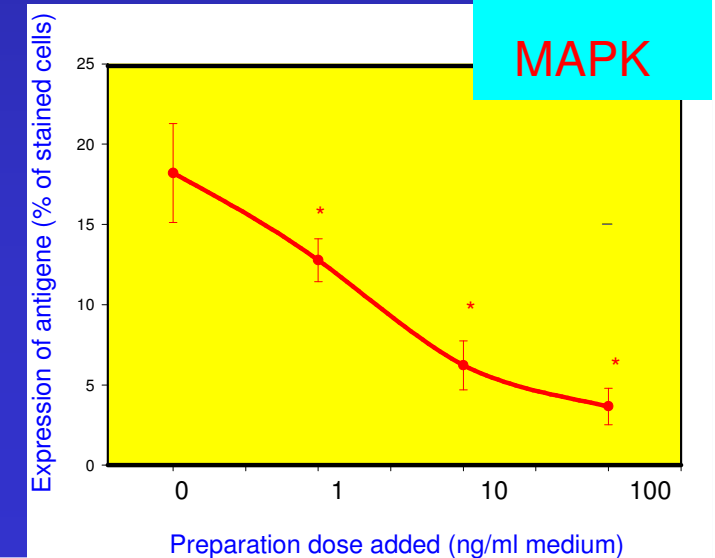
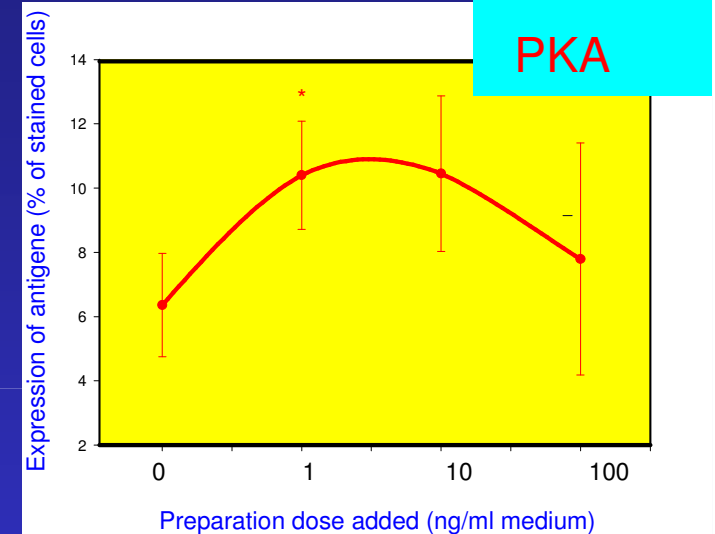


CDK 34K

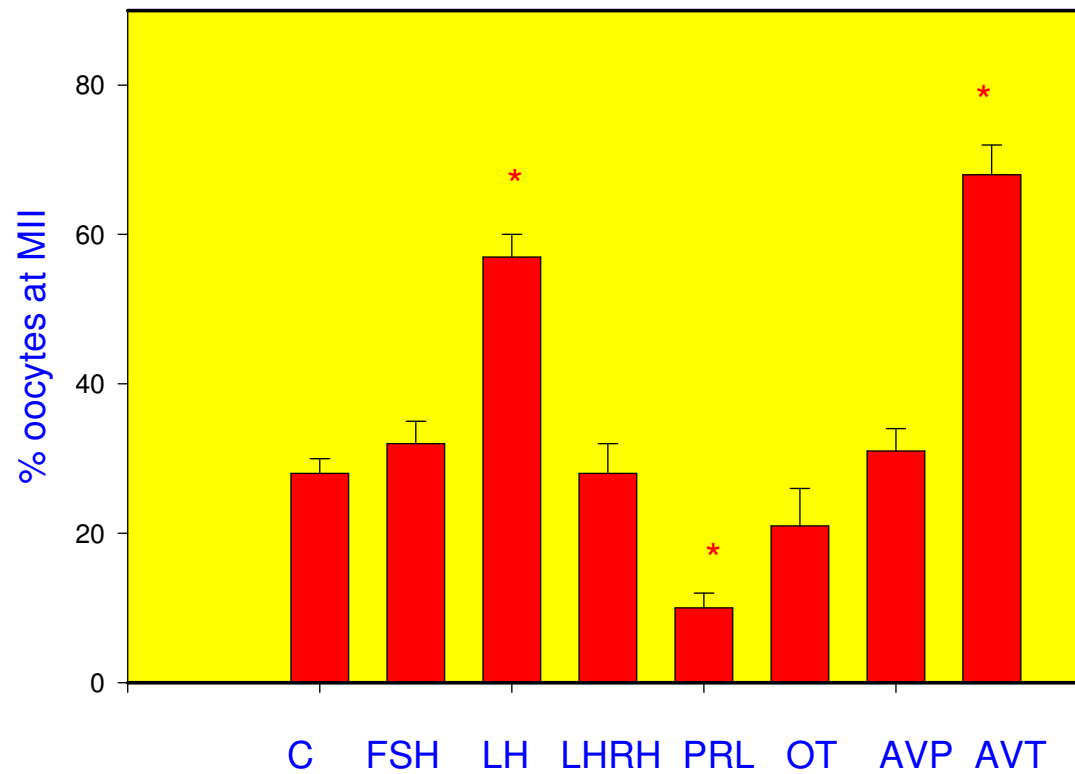


Leptin (ng/ml)

0 1 10 100



Hormones affect maturation of cultured bovine oocytes

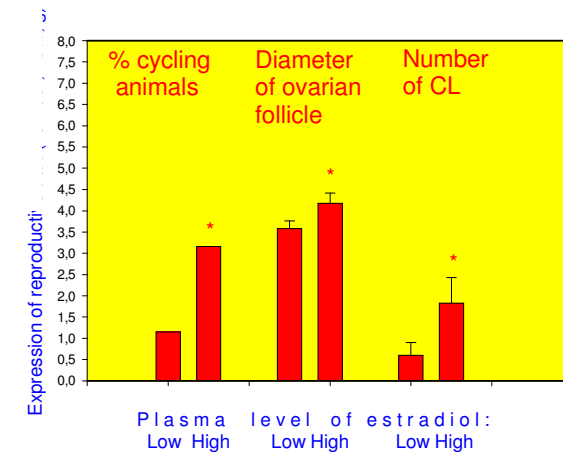
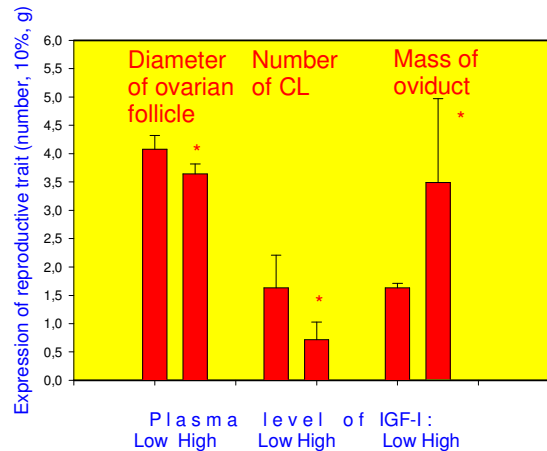
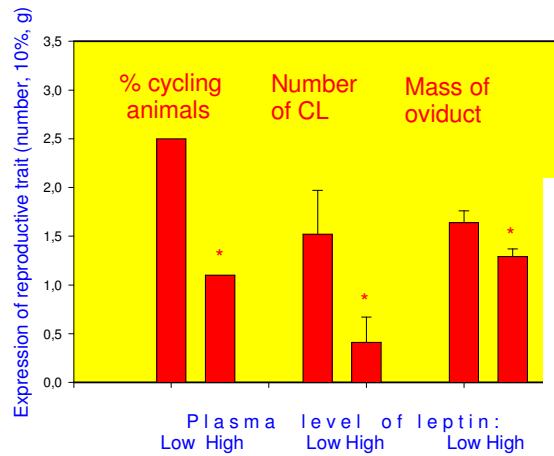


Pigs with good reproductive indexes have in plasma

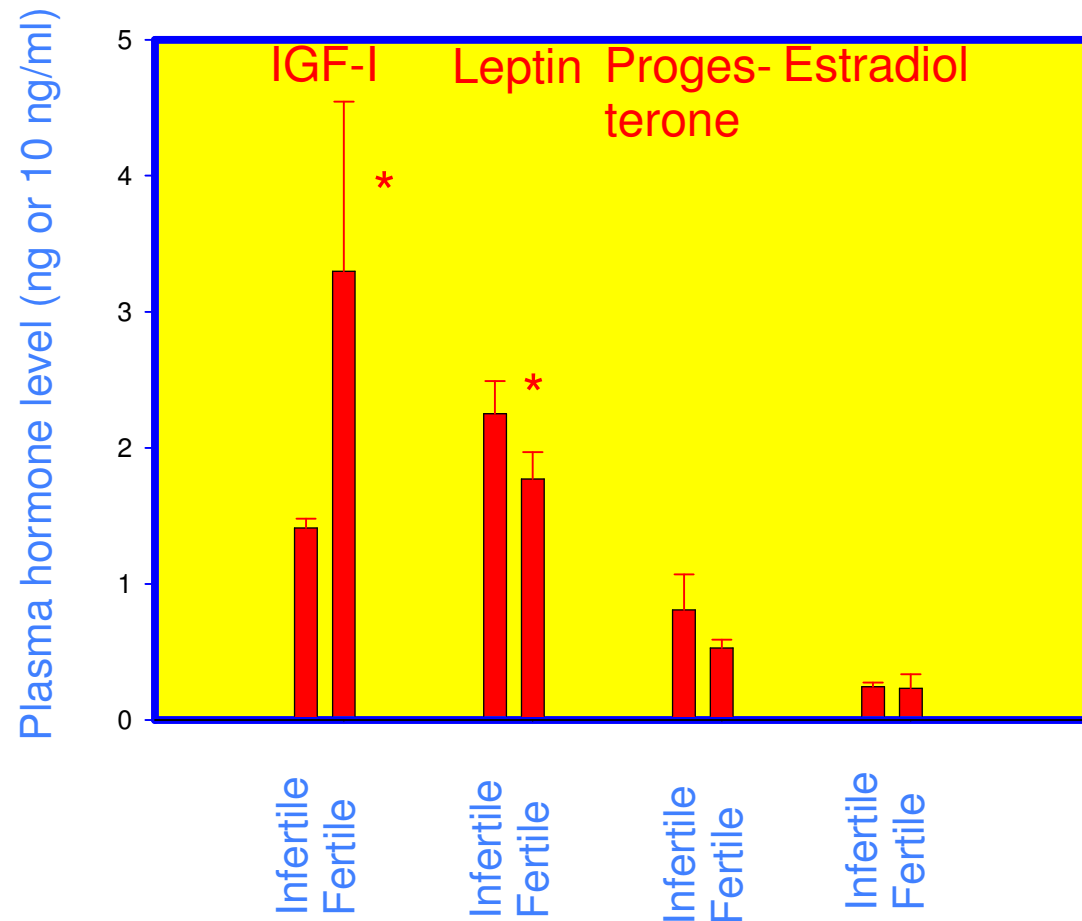
- low leptin level

- low IGF-I level

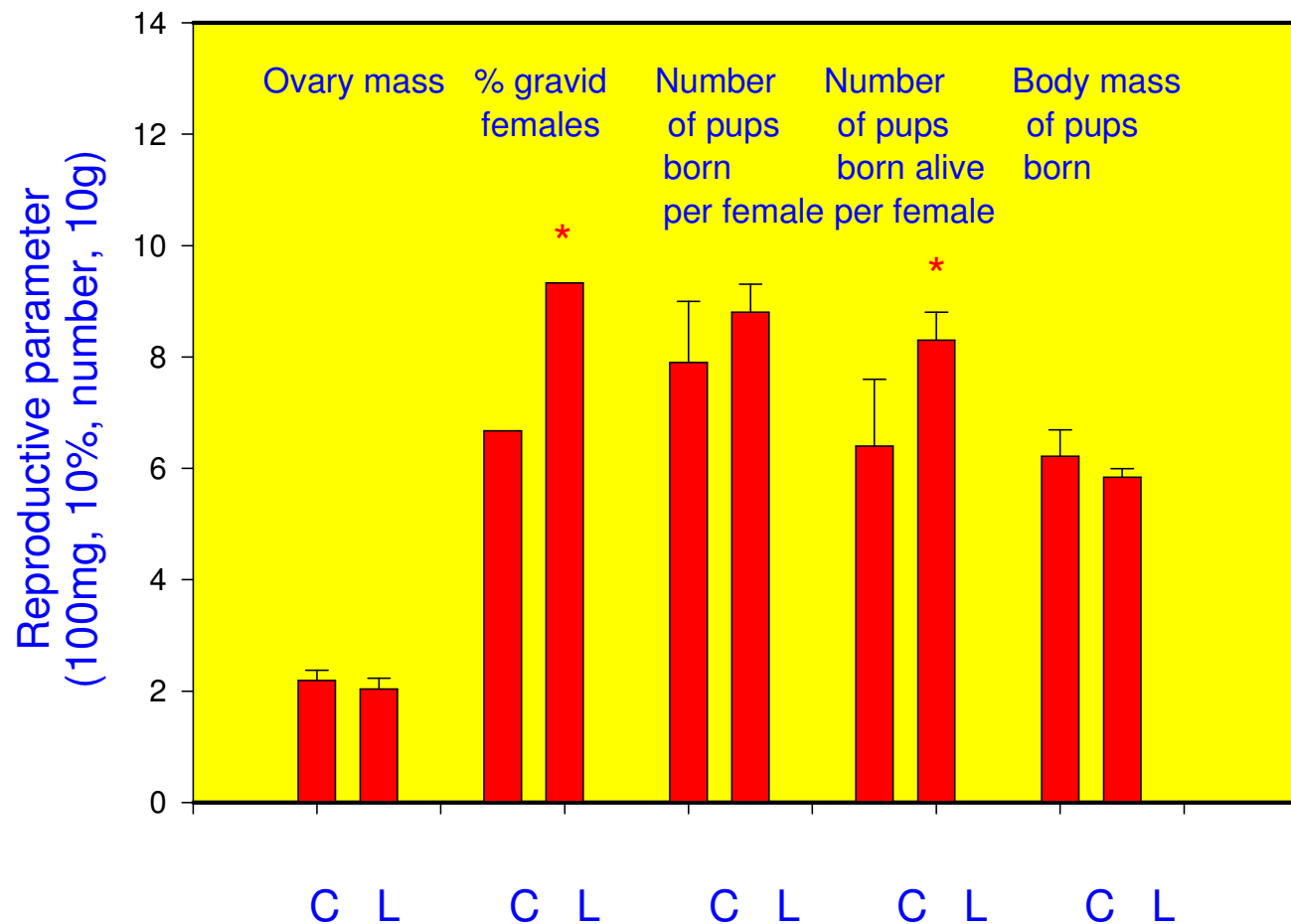
- high estradiol level

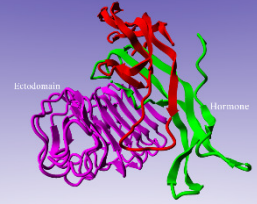


Fertile rabbits have higher IGF-I and lower leptin plasma level, than infertile animals



Leptin increases rabbit gravidity rate and number of pups born alive





THE ROLE OF GROWTH FACTORS

Growth factors *IGF-I, IGF-II, EGF, TGF, TPO*

1. control

- proliferation
- apoptosis
- hormone release
- oocyte maturation
- intracellular signalling substances

2. mediate effect of hormones

Growth factor thrombopoietin stimulates expression of signalling substances in porcine ovarian follicles

PCNA -36K



Bax -23K



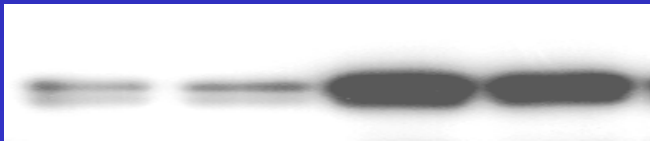
CDK -34K



TK -48K



PKA -47K



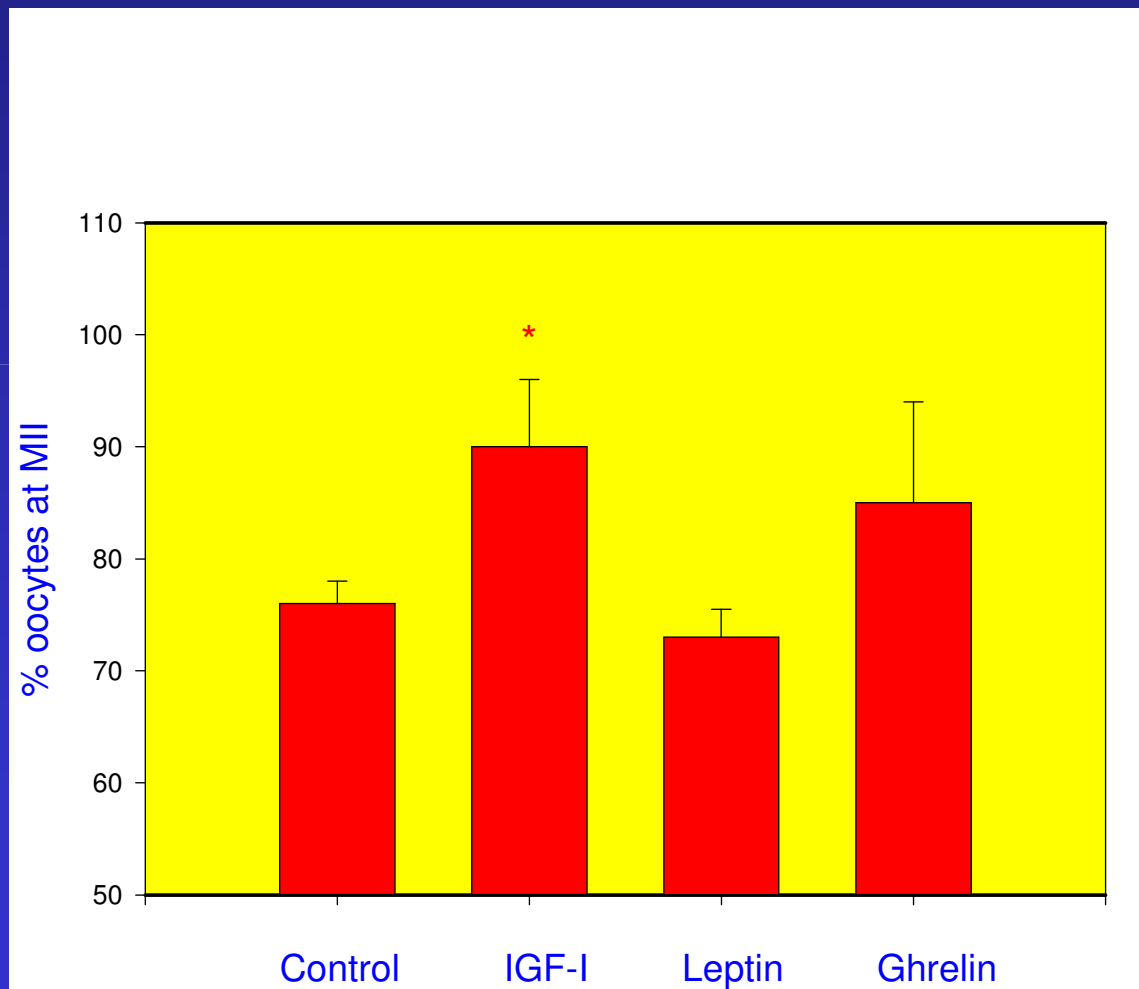
CREB-43K



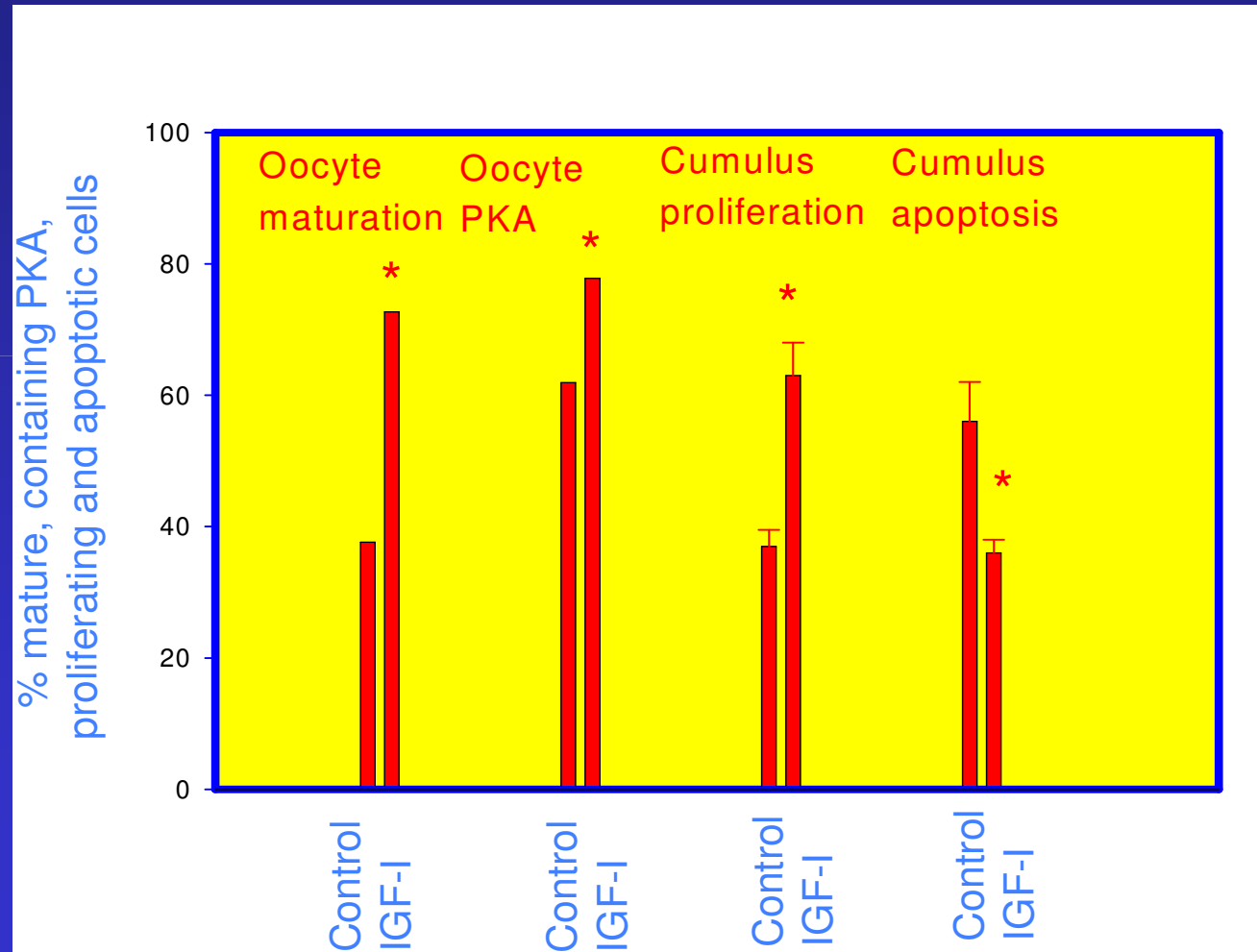
0 1 10 100 ng/ml



IGF-I promotes maturation of bovine oocytes



Growth factor IGF-I promotes porcine oocyte maturation, accumulation of protein kinase A and state of *Cumulus oophorus*





In chicken ovarian follicles IGF-II:

- stimulates proliferation

(PCNA)

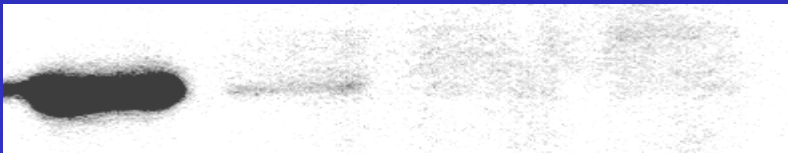
36K



- inhibits apoptosis

(bax)

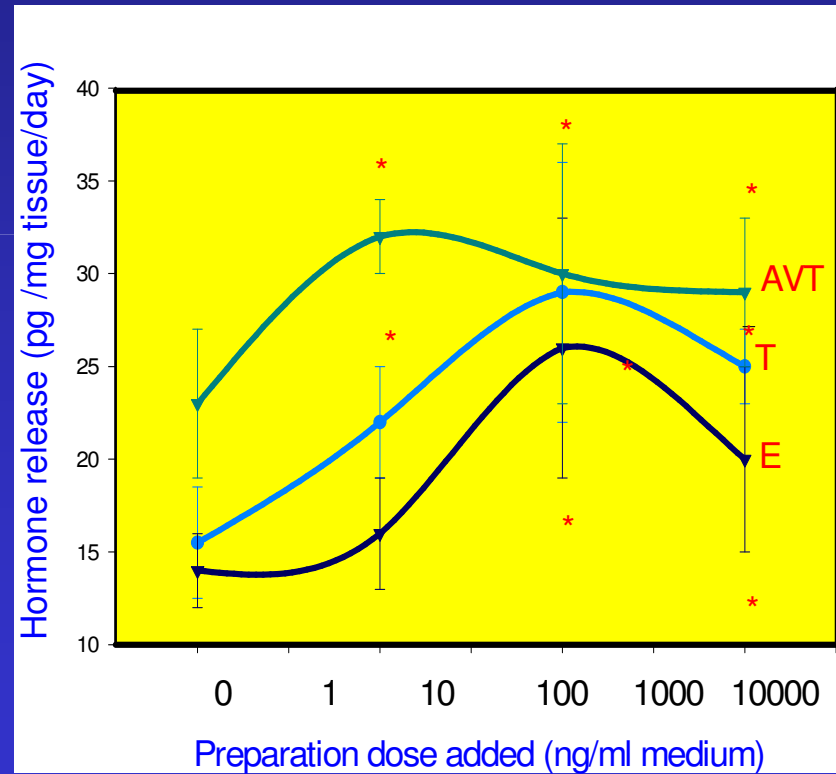
23K



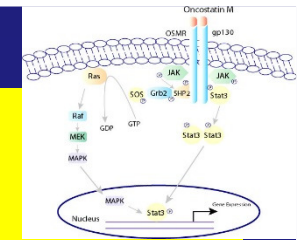
IGF-II dose added (ng-ml)

0 1 10 100

- stimulates AVT, T and E release



RESULTS AND CONCLUSIONS (7)



THE ROLE OF PROTEIN KINASES

1. control

- proliferation
- apoptosis
- hormone and growth factor release
- oocyte maturation
- intracellular signalling substances
- response to hormones and growth factors
- reproductive indexes and fecundity

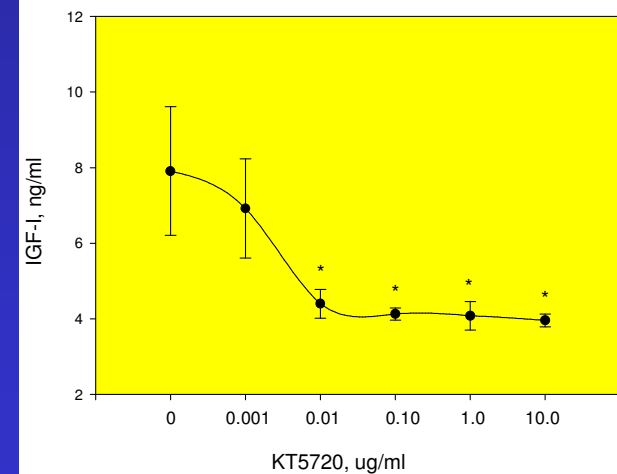
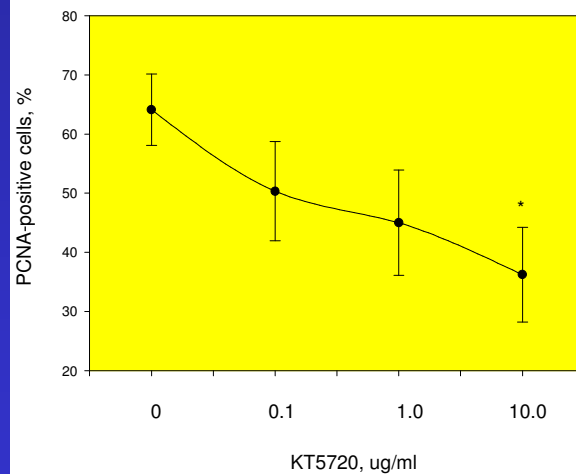
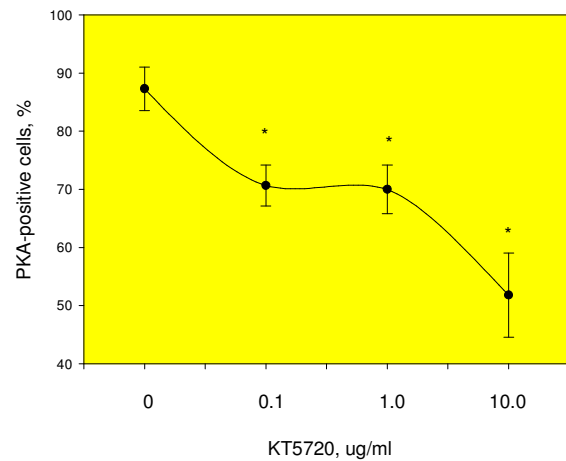
2. mediate effect of hormones and growth factors

In cultured rabbit granulosa cells protein kinase A blocker inhibits:

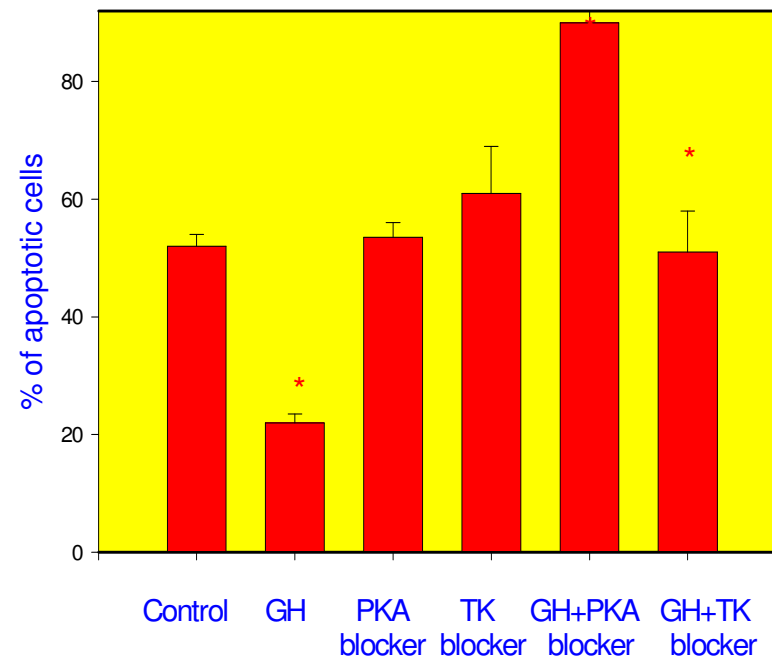
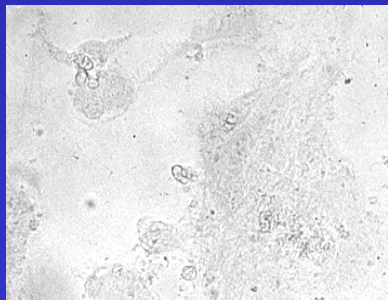
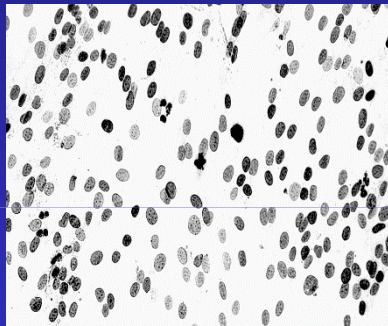
- expression of PKA

- proliferation

- IGF-I release



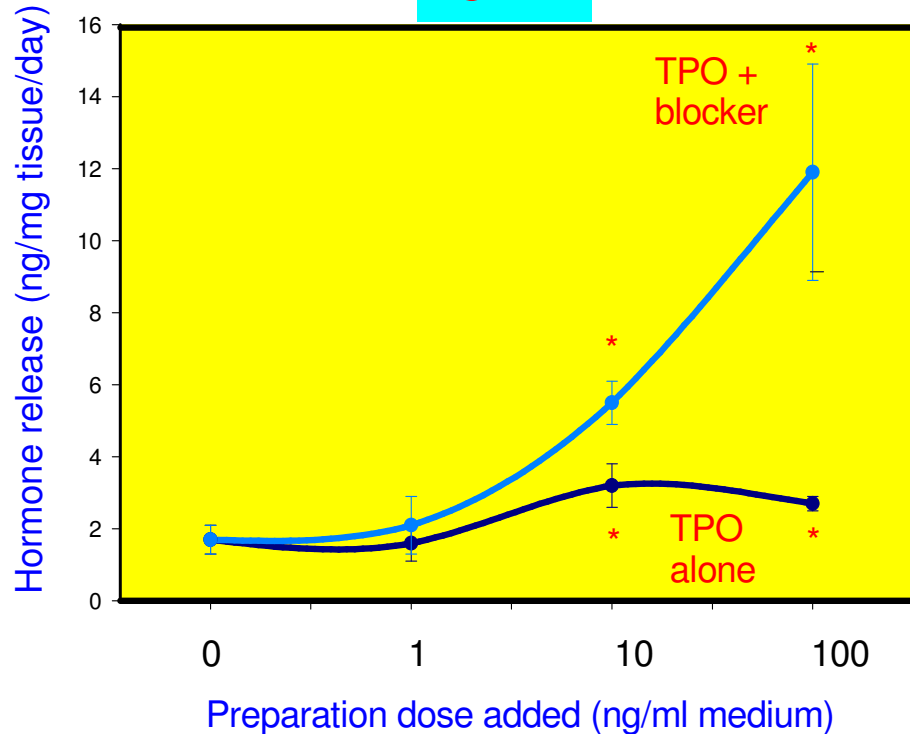
Protein kinase A and tyrosine kinase blockers prevent the anti-apoptotic effect of GH on bovine granulosa cells



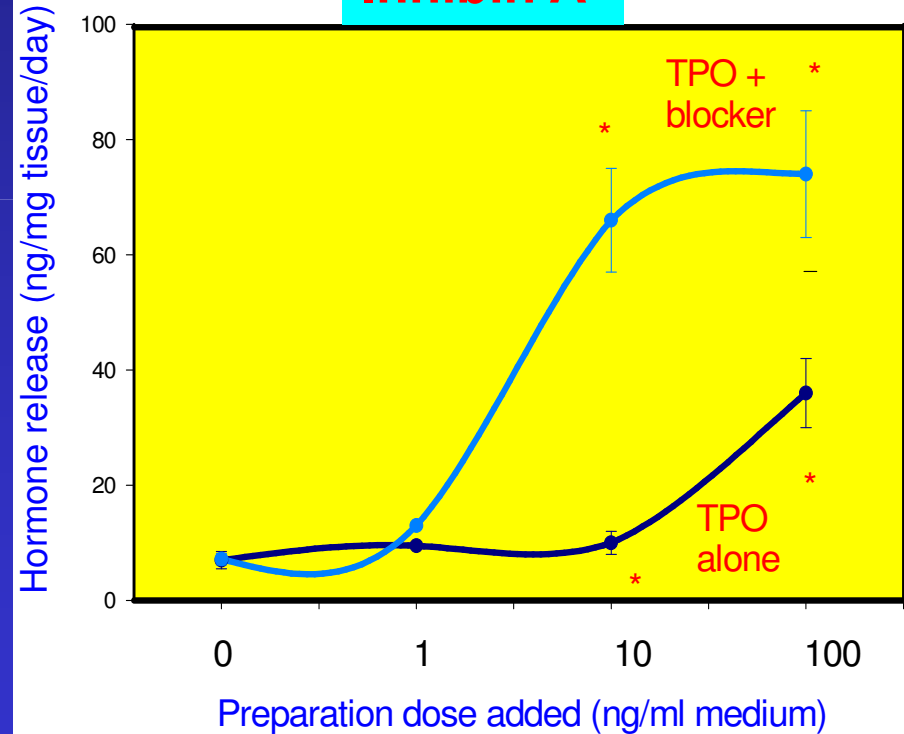
Protein kinase A blocker promotes stimulatory effect of growth factor thrombopoietin on IGF-I and inhibin A by porcine ovarian follicles

(RIA/ELISA)

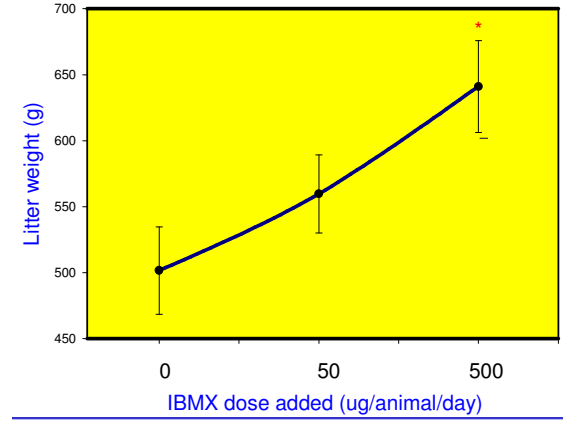
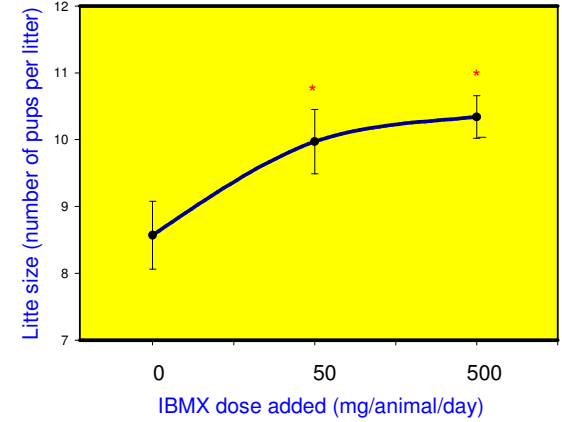
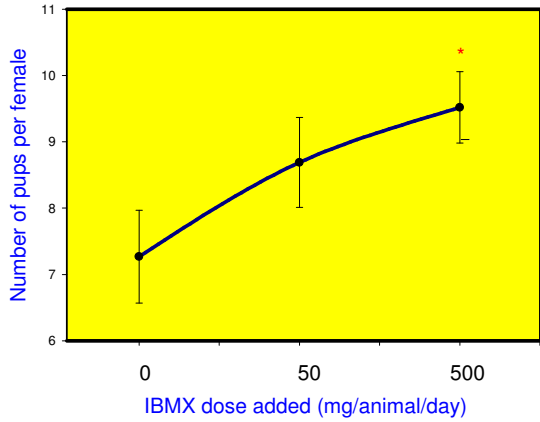
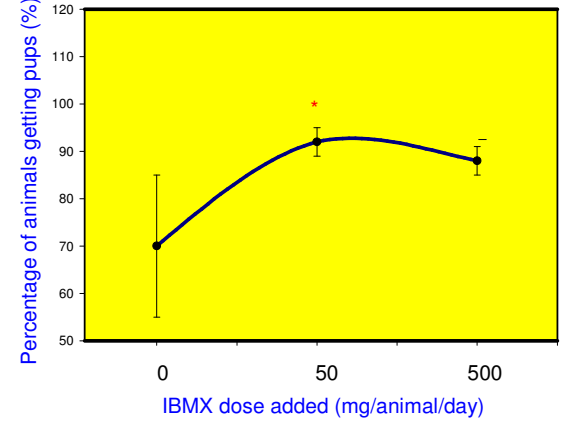
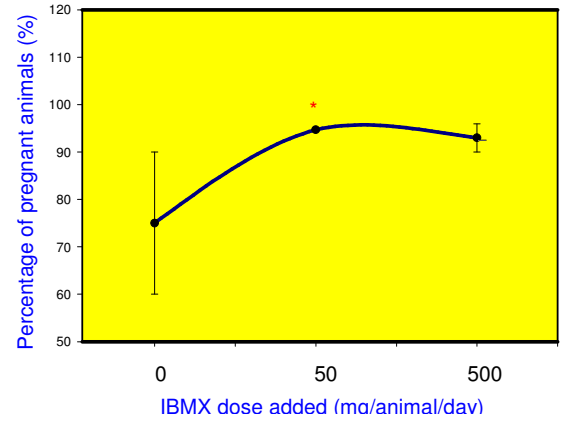
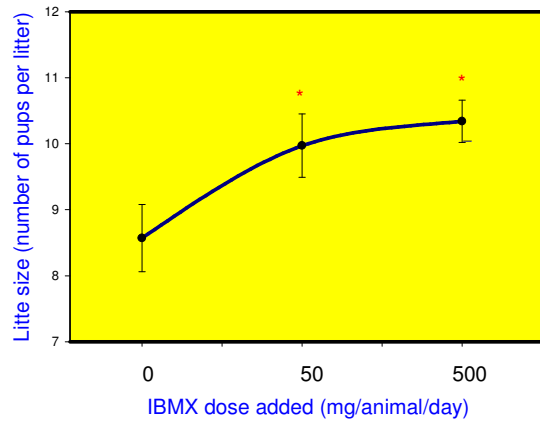
IGF-I

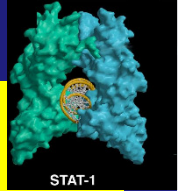


Inhibin A



Protein kinase A stimulator IBMX increases rabbit plasma progesterone level, pregnancy and birth rate, litter size and litter weight





THE ROLE OF TRANSCRIPTION FACTORS

p53, CREB-1, STAT-1, NFkB

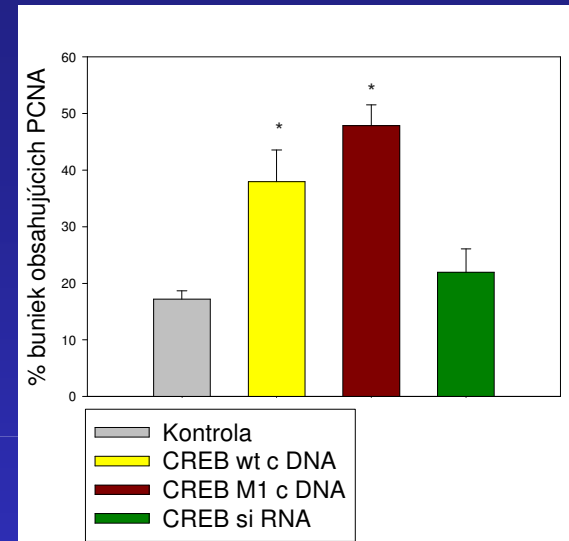
1. control

- proliferation
- apoptosis
- hormone and growth factor release
- intracellular signalling substances

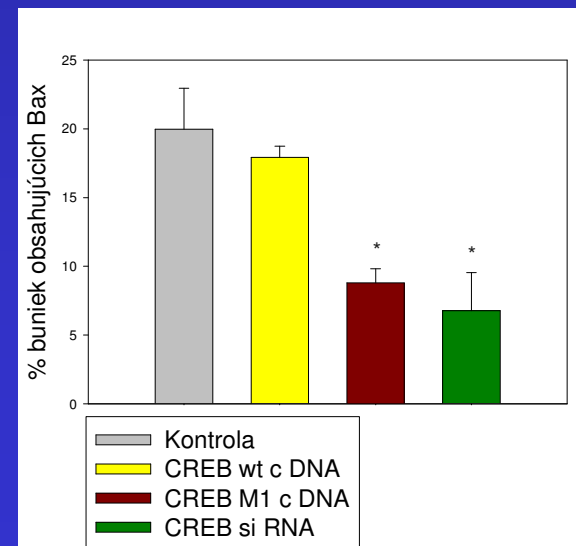
2. can mediate effect of hormones, growth factors and protein kinases

Overexpression of CREB-1 in human ovarian granulosa cells

- promotes proliferation
(*PCNA*)



- inhibits apoptosis
(*bax*)

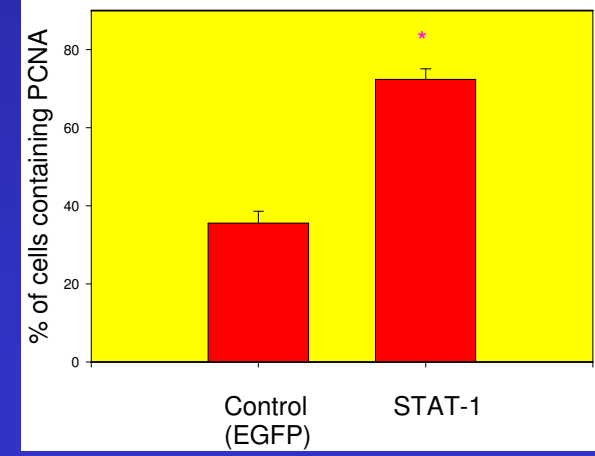
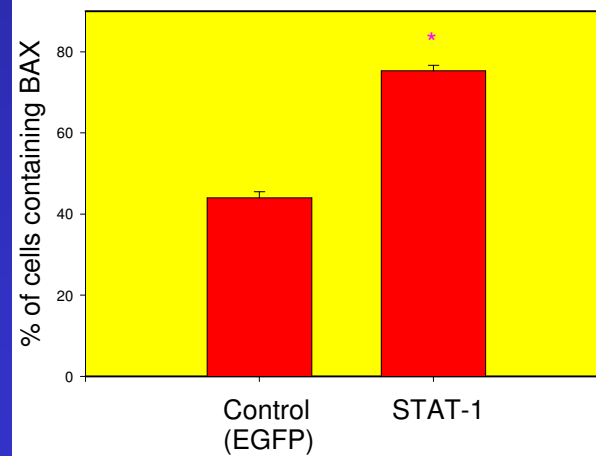
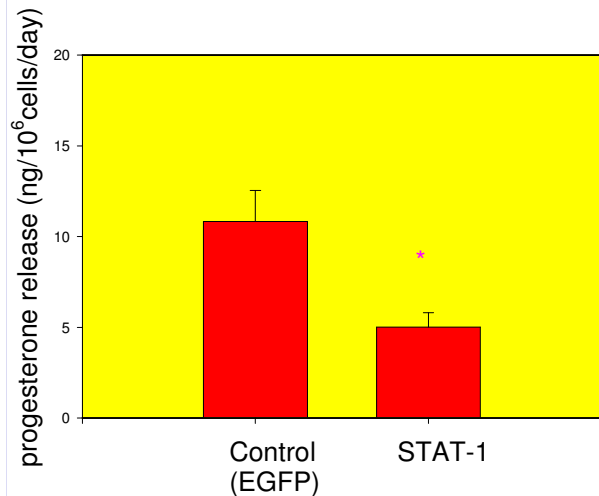


Overexpression of STAT-1 in porcine granulosa cells

- inhibits
progesterone
release

- stimulates
apoptosis
(*bax*)

- stimulates
proliferation
(*PCNA*)

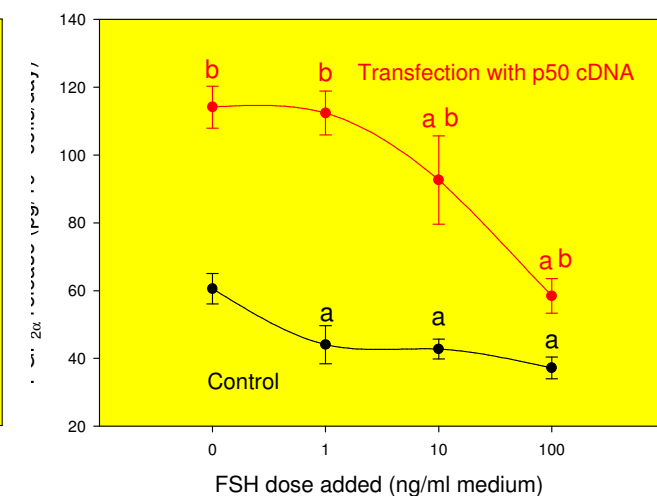
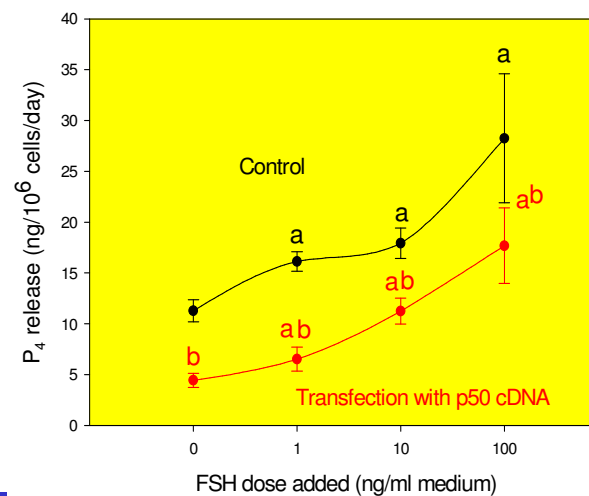
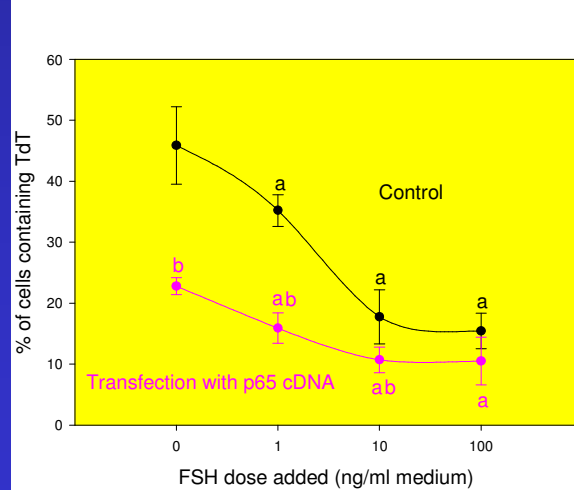


Overexpression of NFkB in porcine granulosa cells

- Inhibits apoptosis (TdT)

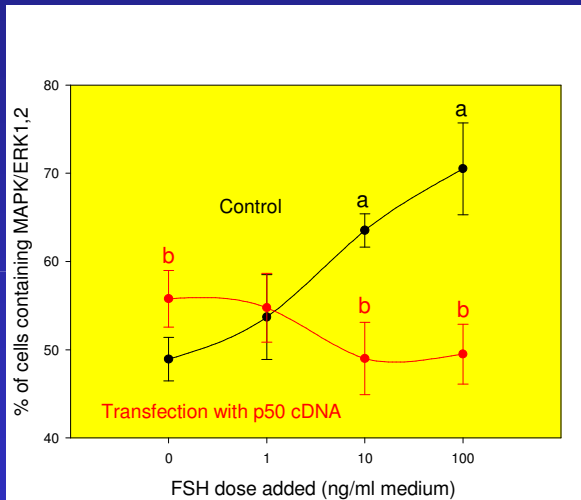
- inhibits progesterone release

- promotes prostaglandin release

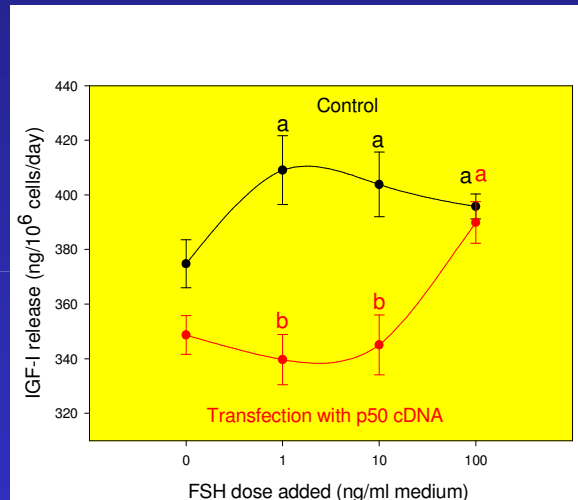


Overexpression of NFkB prevent the effect of FSH on porcine granulosa cell

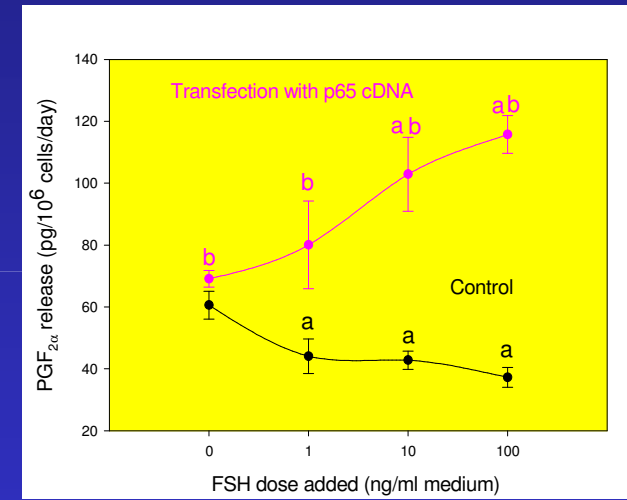
- proliferation
(MAPK)



- IGF-I
release



- prostaglandin F
release



- proliferation (PCNA)



FSH alone FSH+transfection

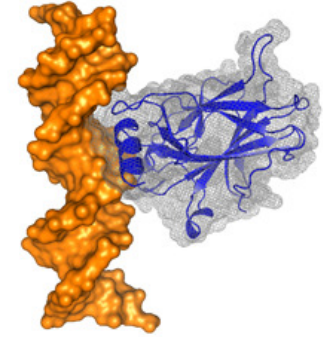
0 1 10 100 0 1 10 100 ng/ml

- apoptosis (bax)



FSH alone FSH+transfection

0 1 10 100 0 1 10 100 ng/ml

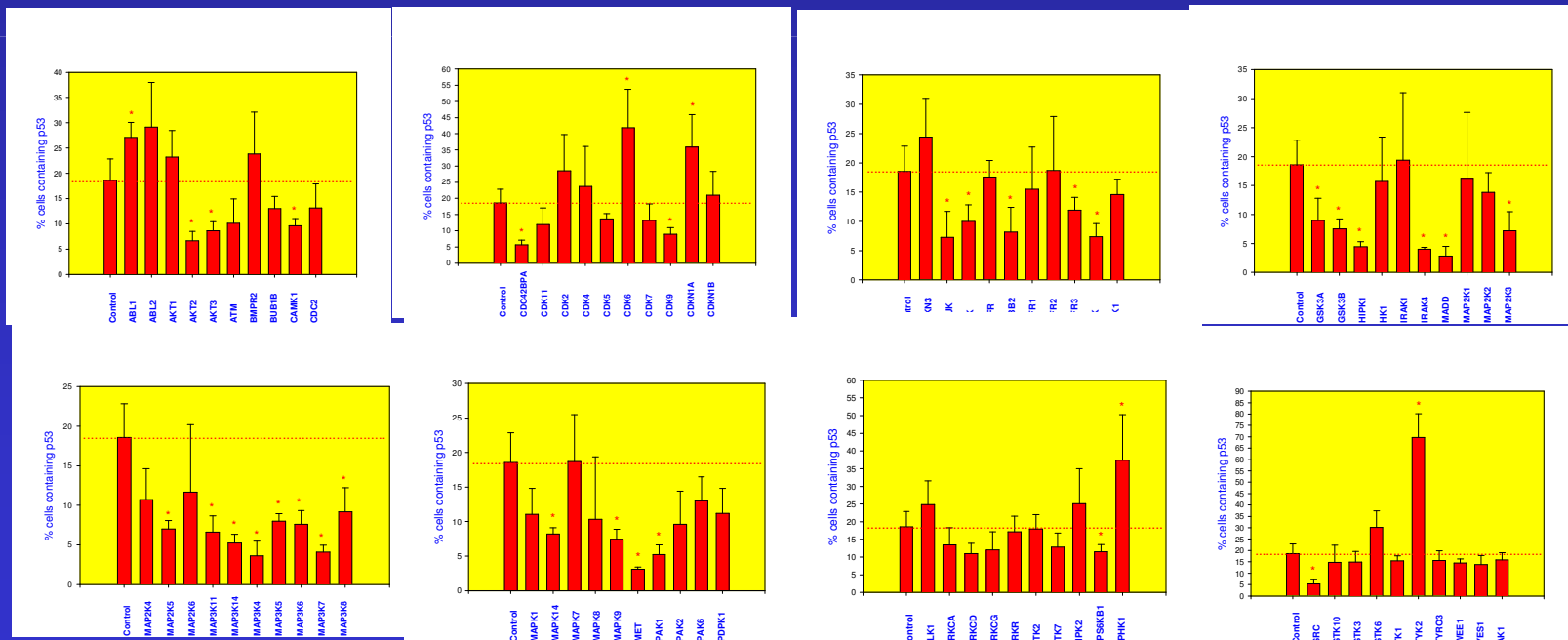
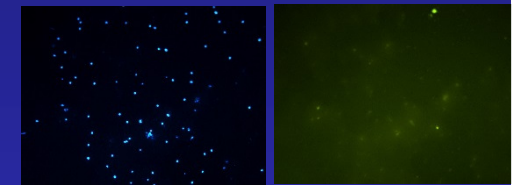


THE ROLE OF RNA INTERFERENCE

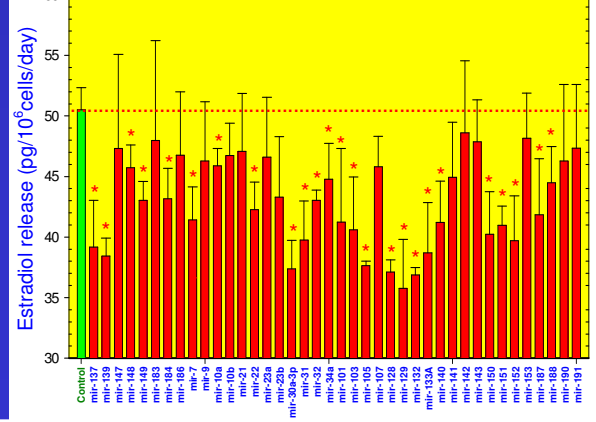
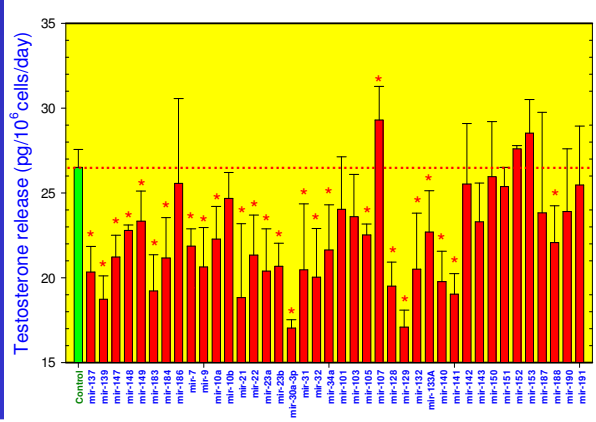
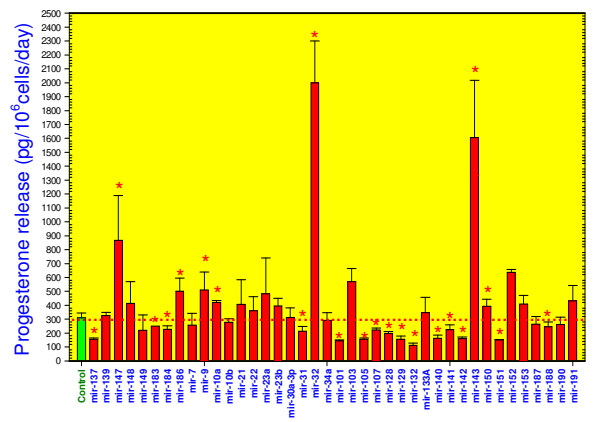
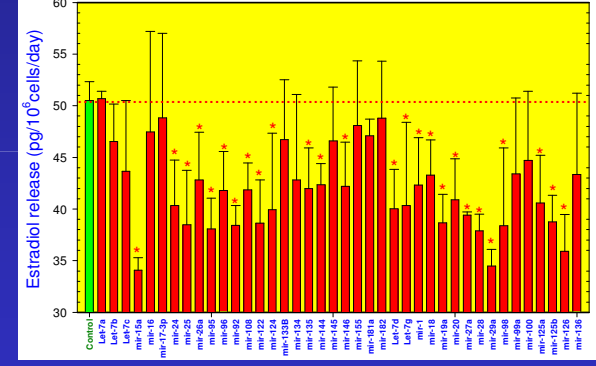
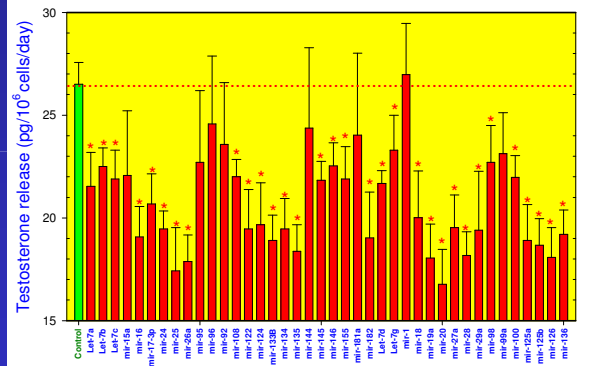
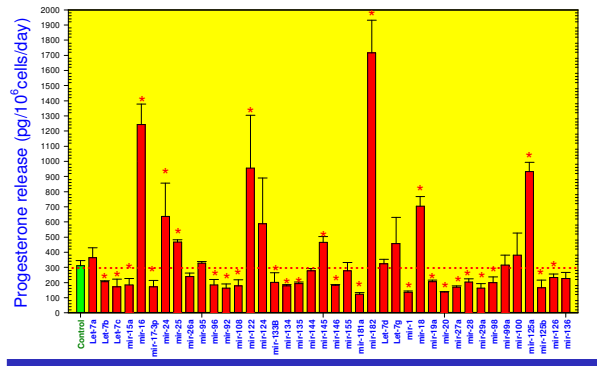
siRNA and miRNA control

- proliferation
- apoptosis
- release of hormones and growth factors
- response to hormones and growth factors
- expression of transcription factors

Blokade of protein kinases by specific siRNAs affects expression of apoptosis-related transcription factor p53 in human ovarian granulosa cells

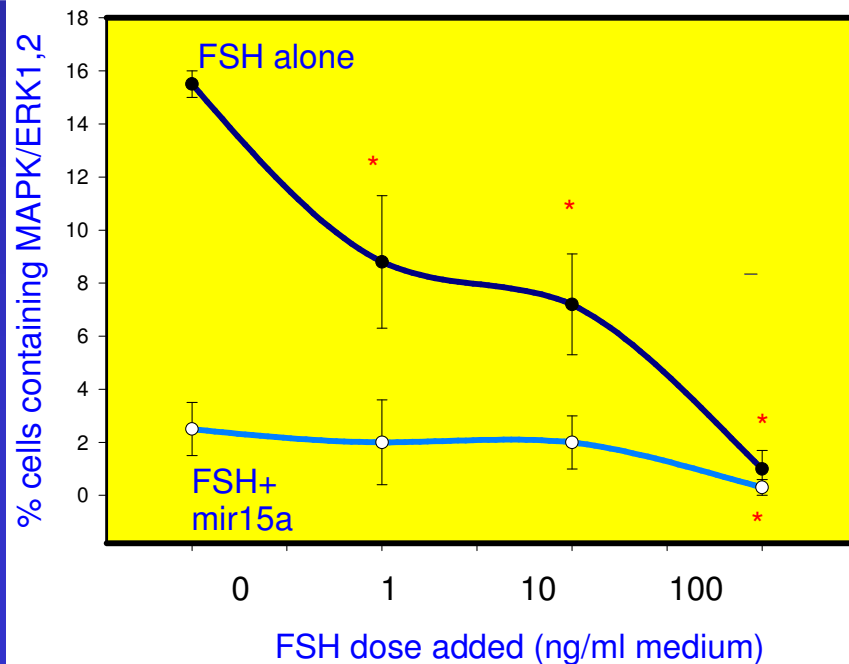


mi RNA affect secretory activity of human granulosa cells: - progesterone - testosterone - estradiol

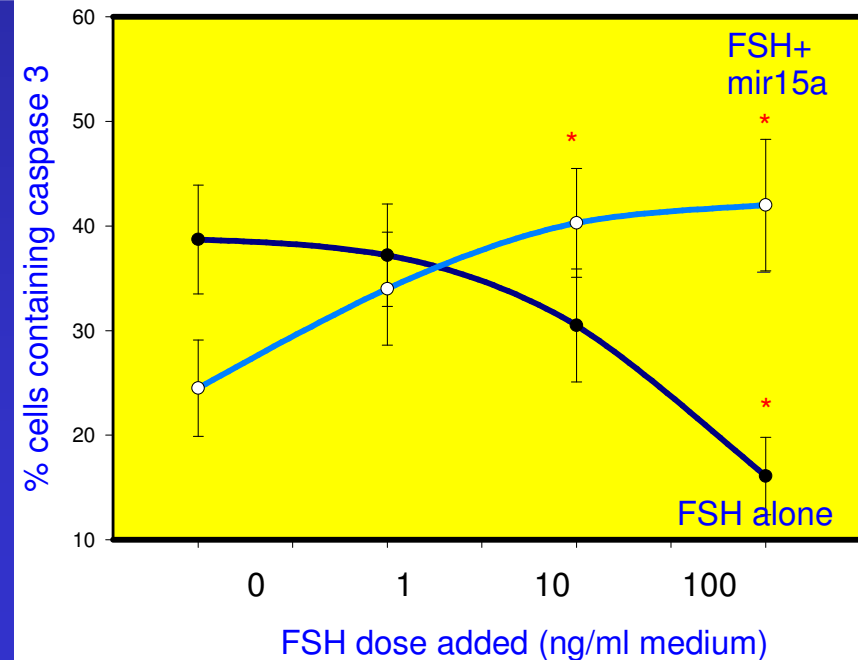


miRNA mir15a stimulates proliferation, inhibits apoptosis and alters response of human granulosa cells to FSH

Proliferation (MAPK/ERK1,2)



Apoptosis (caspase 3)



CONCLUSIONS

- 1. Basic ovarian functions are regulated by “non-classical” reproductive hormones - GH, nonapeptide hormones, leptin, ghrelin a.o.**
- 3. Growth factors IGF-I, IGF-II, EGF, TGF, TPO are important regulators of ovarian functions and mediators of hormone actions.**

CONCLUSIONS

- 3. Protein kinases (TK, PKA, MAPK, CDC2 a o.), transcription factors (CREB, p53, STAT-1, NFkB) and RNA interference (siRNAs and miRNAs) are important regulators of basic ovarian functions and mediators of hormones and growth factors action.**
- 4. These extra- and intracellular regulators are useful for characterisation, prediction and control of reproductive functions.**

Conclusion

Regulation of ovarian functions

External factors



Hormones (leptin, ghrelin, GH, nonapeptide hormones...)



Growth factors (IGF-I,-II, TGF, TPO ...)



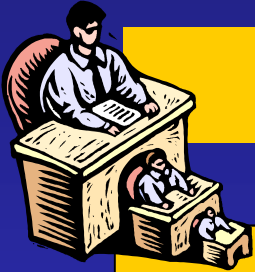
Protein kinases (TK, MAPK, CDK,...)



Transcription factors (p53, CREB, STAT, NFkB, ...),
siRNA, miRNA



Reproductive functions (proliferation, apoptosis, secretion,
oogenesis)



COLLABORATION

P. Chrenek, S. Pavlová, A. Benčo, P. Sanislo, A. Kadasi, A. Balazi (Slovakia)

R. Grossmann, H.-J. Schaeffer (Germany)

J. Kotwica (Poland)

P. Brenaut (France)

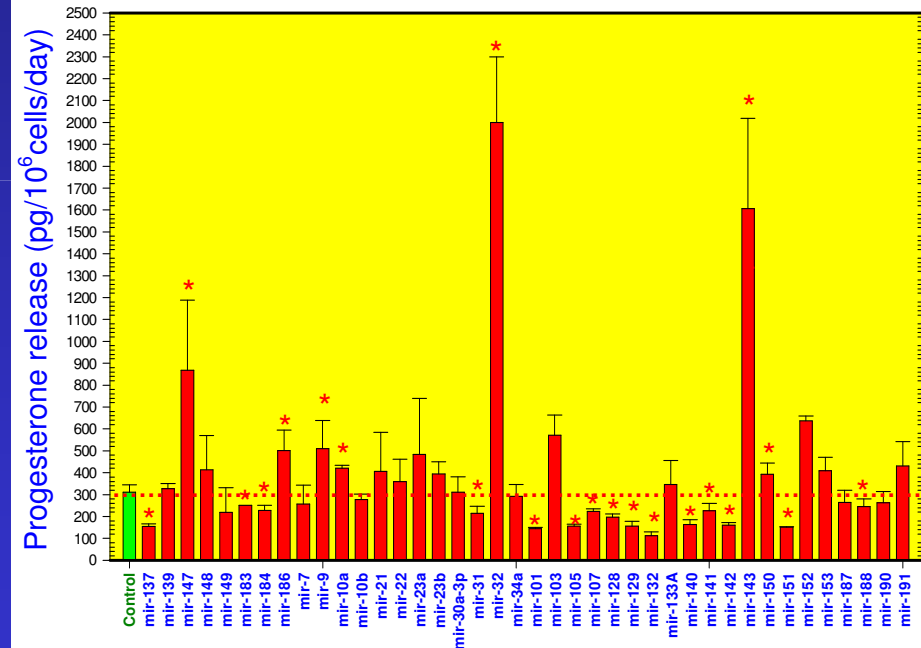
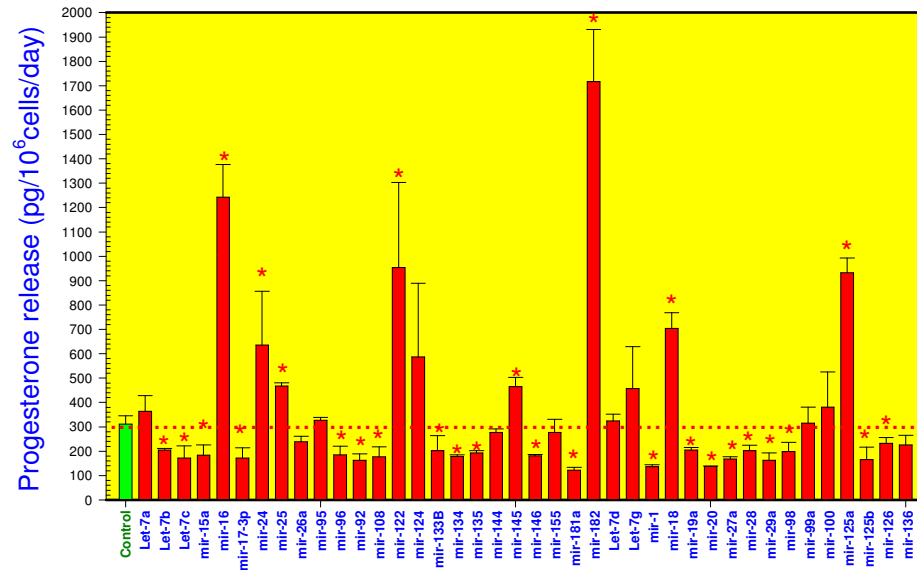
D. Ovcharenko (USA)



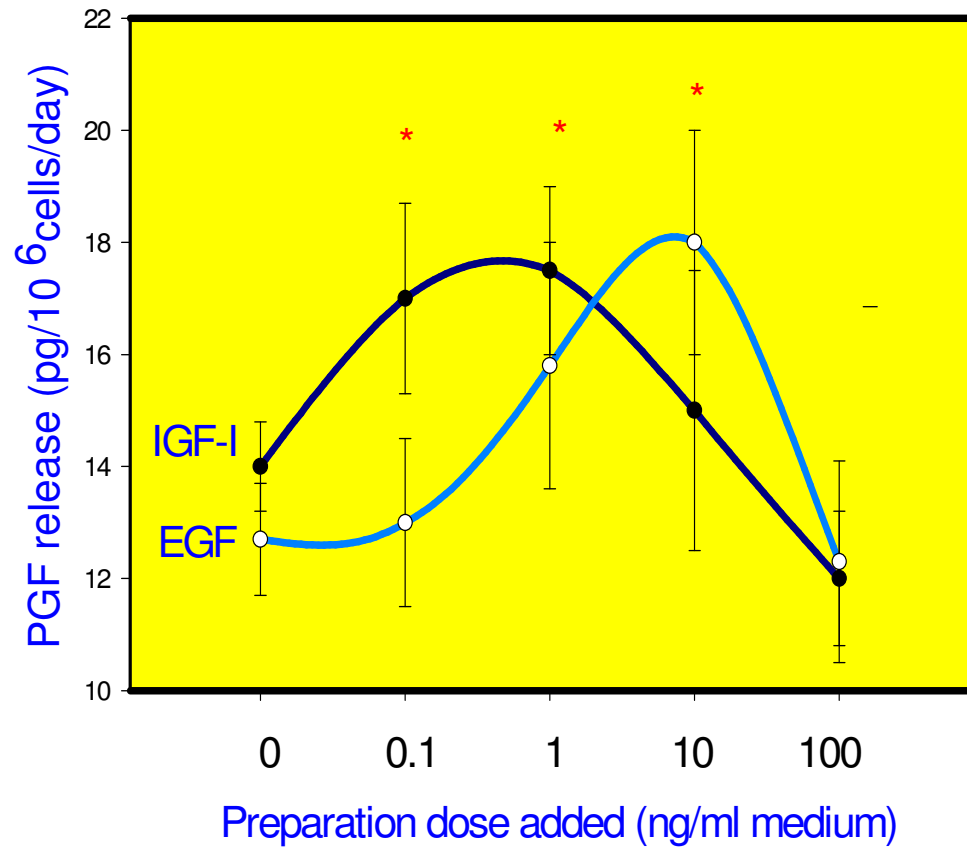
THANK YOU!



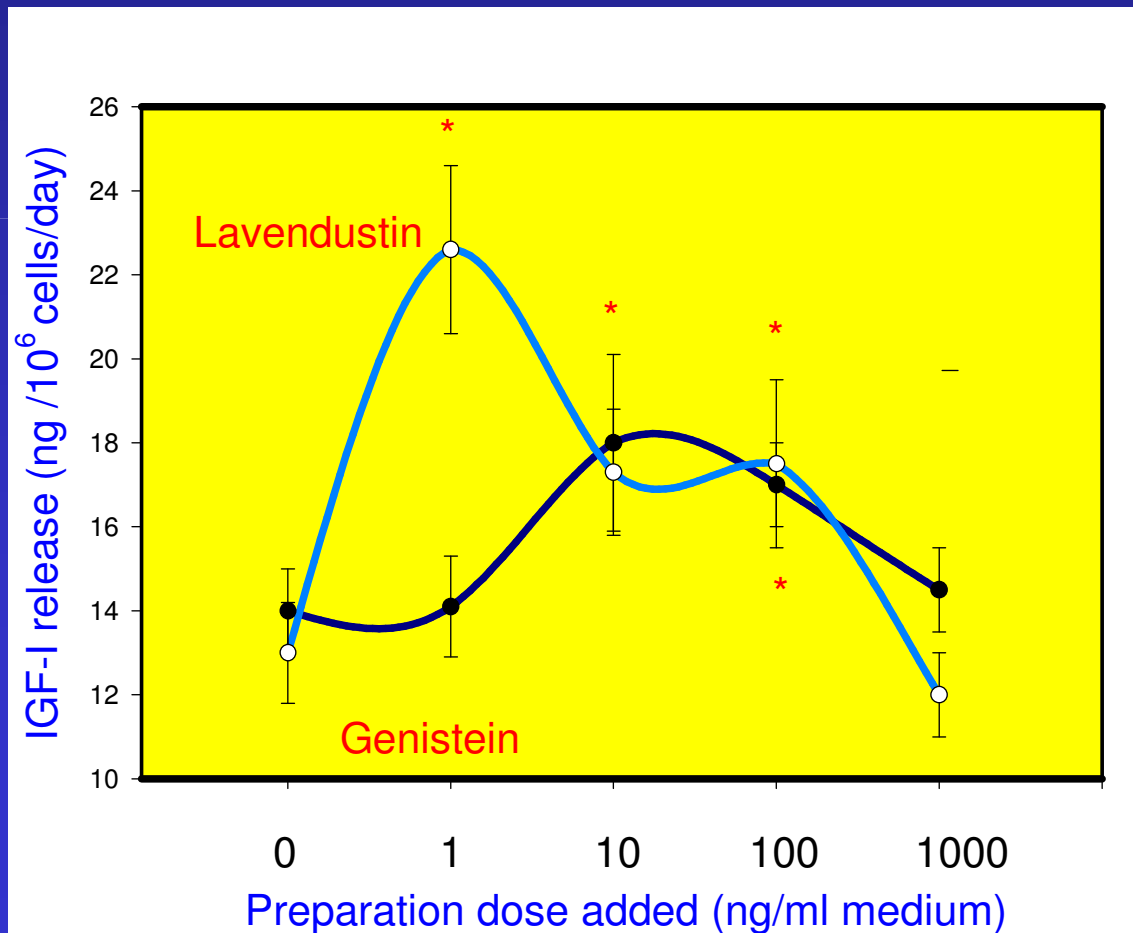
miRNA affect release of progesterone by human granulosa cells



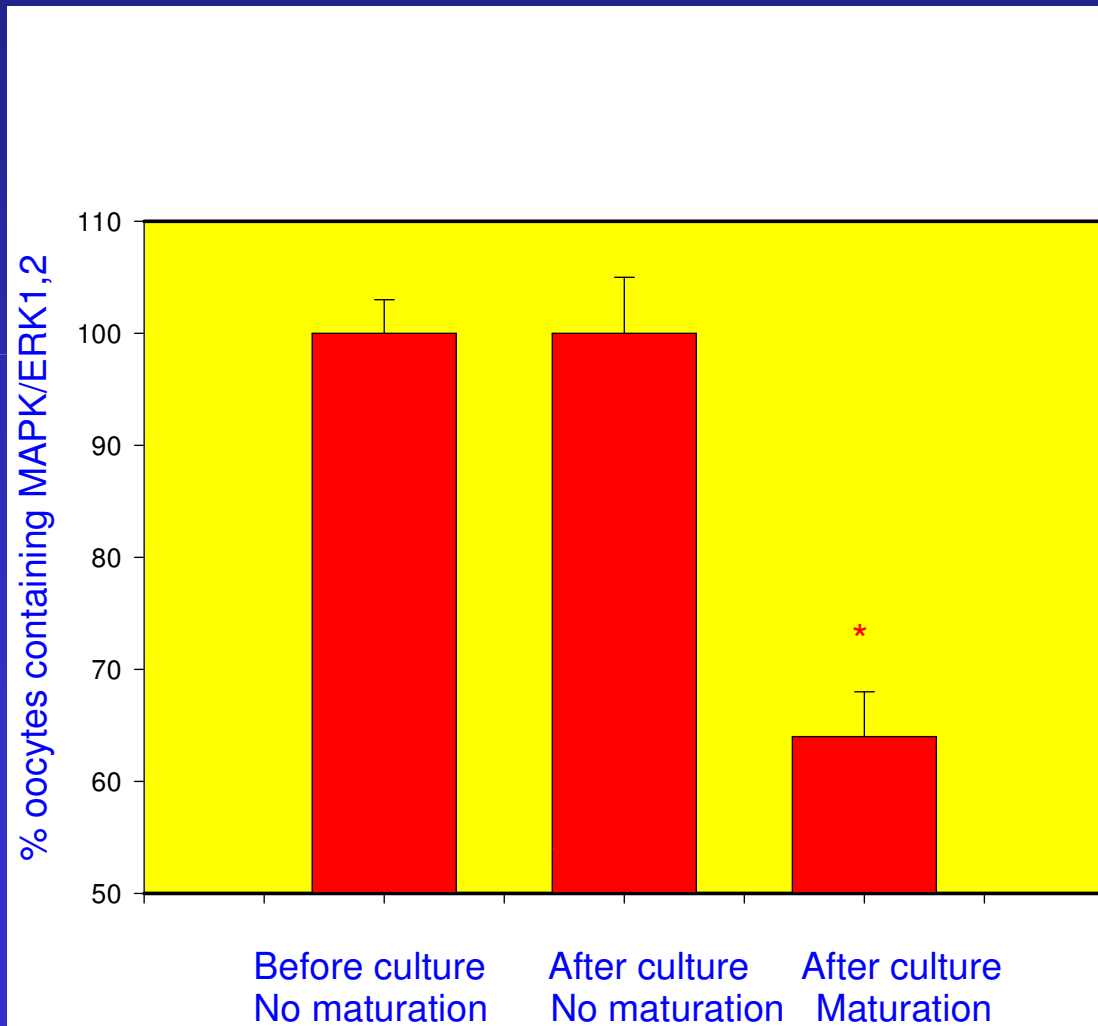
IGF-I and EGF stimulate prostaglandin F release by cultured bovine granulosa cells



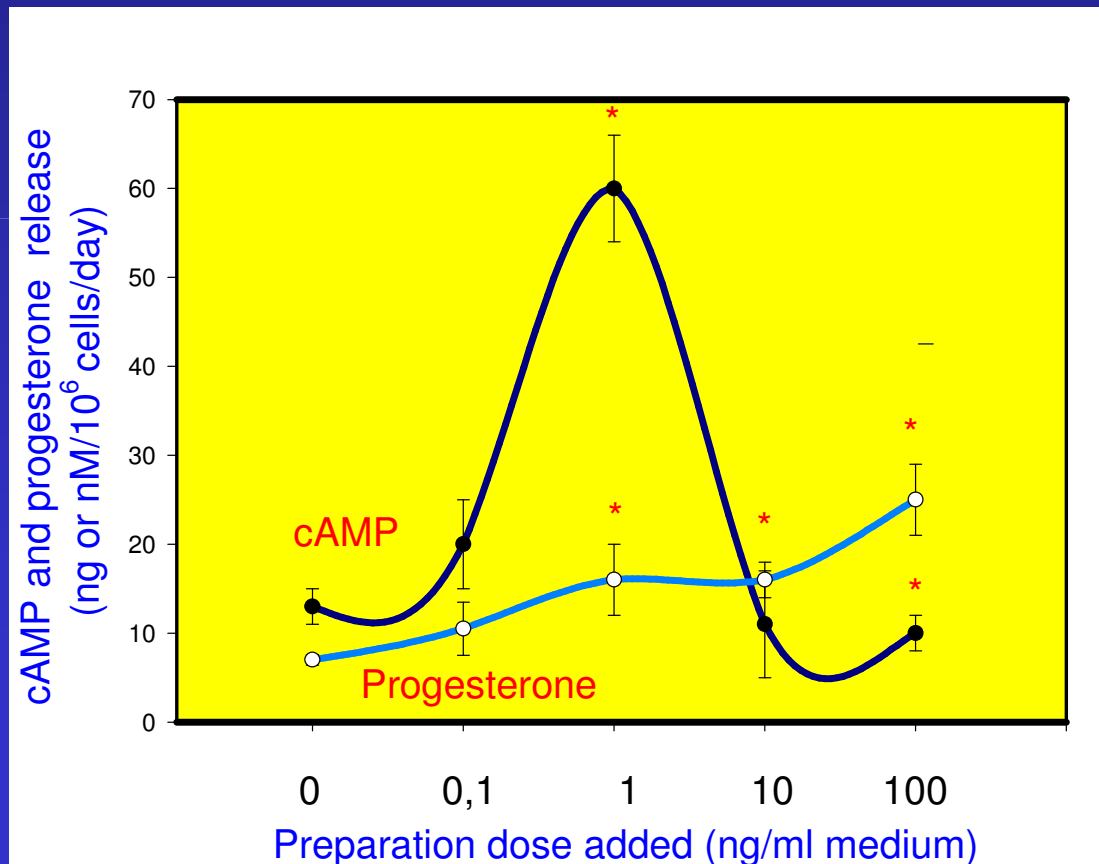
Blockers of tyrosine kinases promote release of IGF-I by cultured bovine granulosa cells



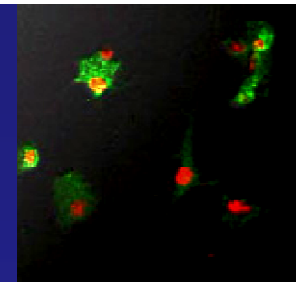
Maturation of cultured bovine oocytes is associated with decrease in accumulation of ERK1,2 MAP kinase



In cultured bovine ovarian granulosa cells IGF-I stimulates release of cAMP and progesterone



V ovariálnych bunkách sliepok IGF-II:

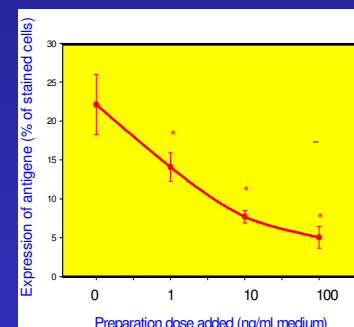


- stimuluje proliferáciu
- inhibuje nukleárnu apoptózu

(PCNA)

(TdT)

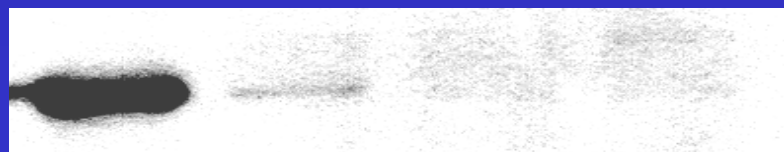
36K



- inhibuje cytoplazmatickú apoptózu (*bax*)

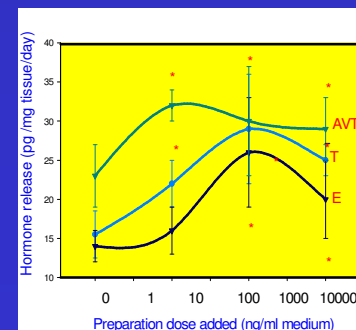
- stimuluje vylučovanie hormónov (*P, T, E*)

23K



Prídaná dávka IGF-II (ng/ml)

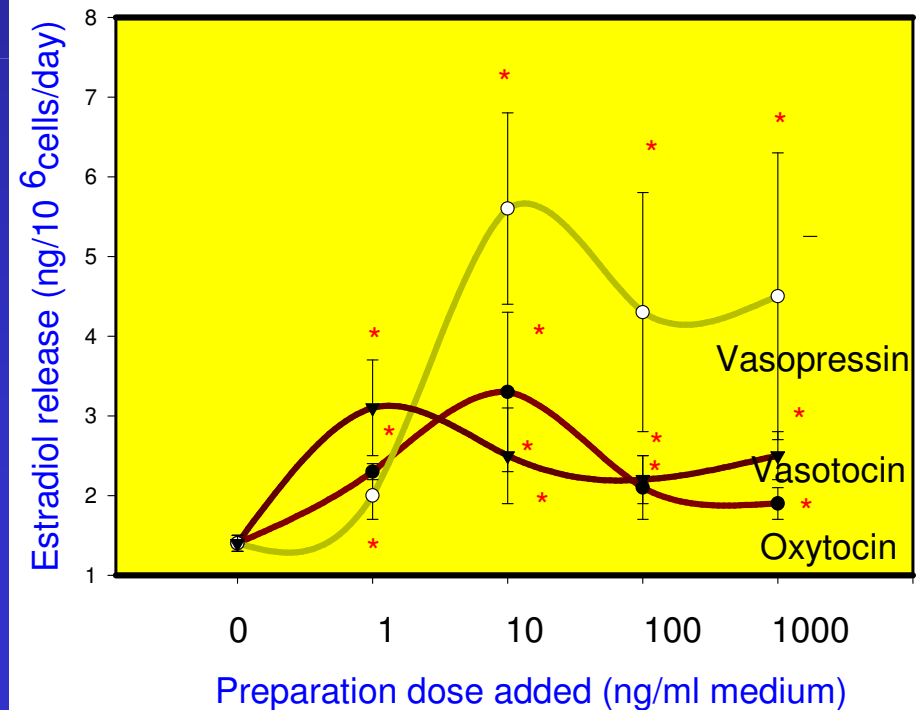
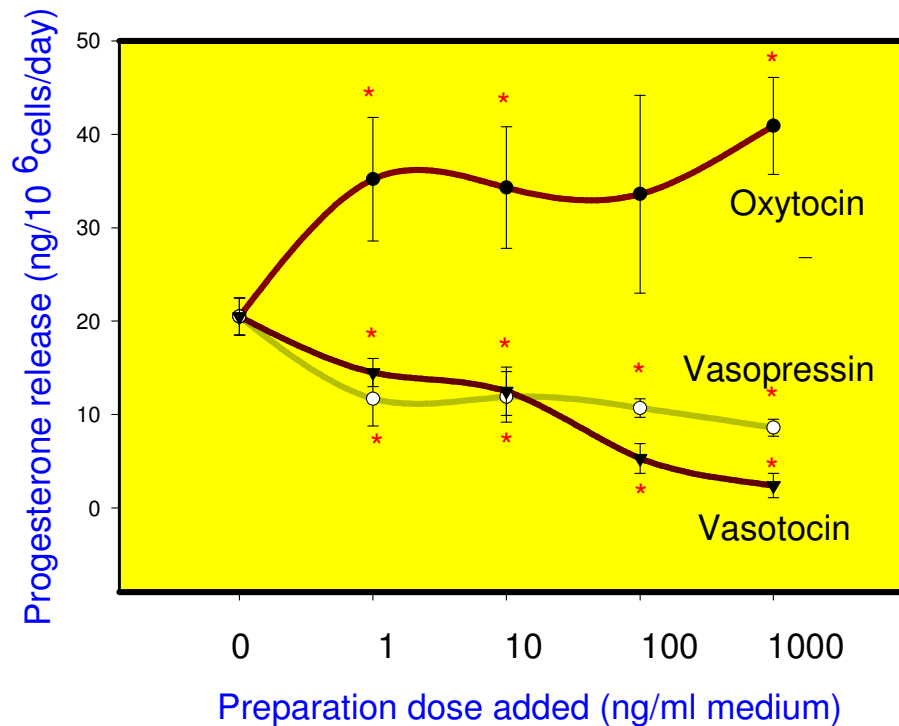
0 1 10 100



In cultured bovine ovarian granulosa cells nonapeptide hormones regulate release of

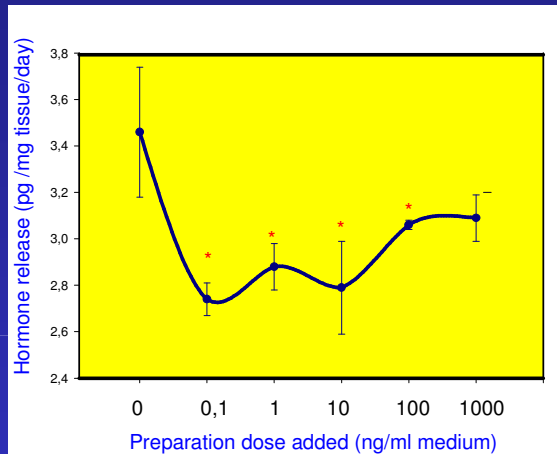
- progesterone

- estradiol

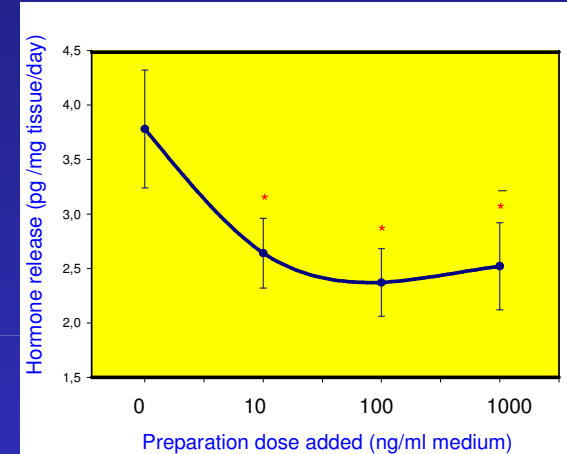


Ghrelin inhibits secretory activity of porcine ovarian granulosa cells:

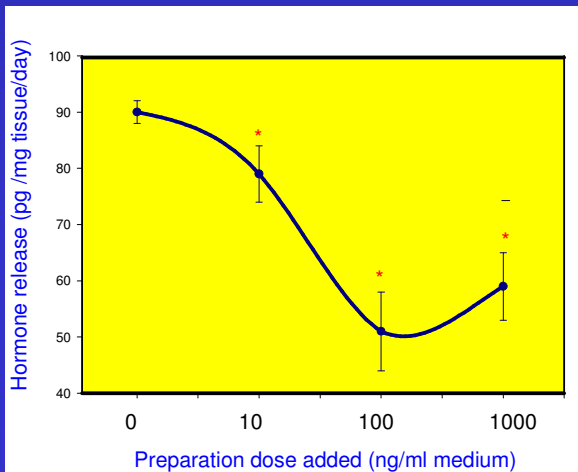
- progesterone



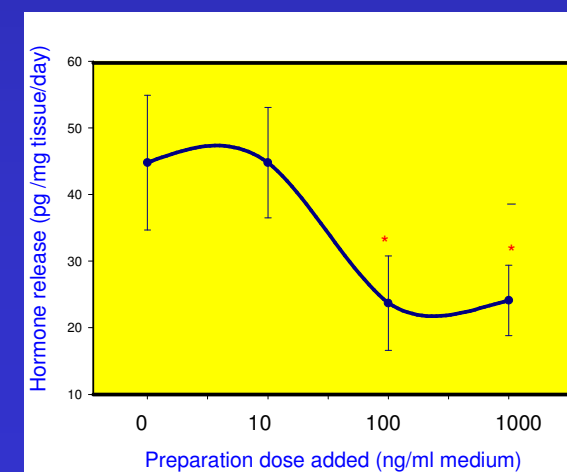
- testosterone



- oxytocin



- prostaglandin F



Rastový faktor IGF-I sprostredkuje efekty hormónu leptínu na ovariálne bunky ľudí:

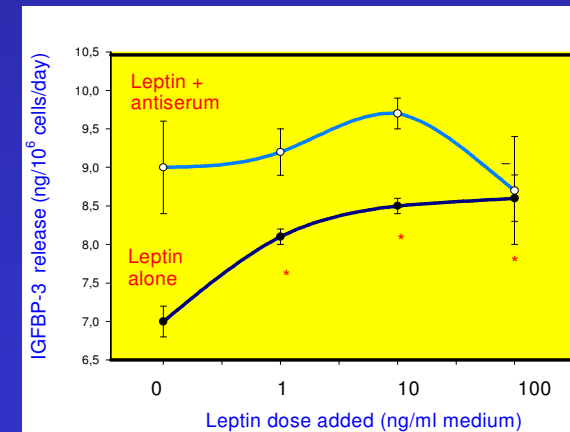
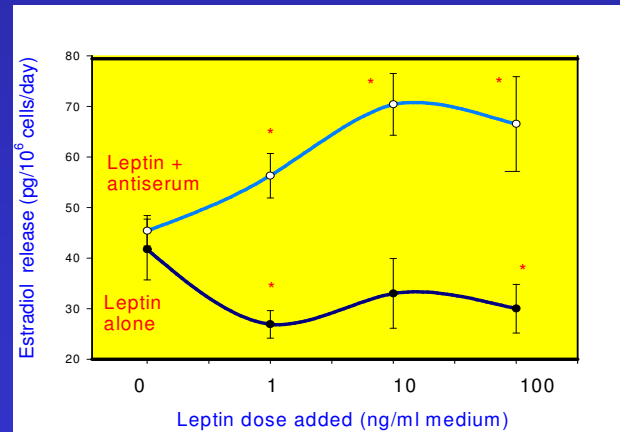
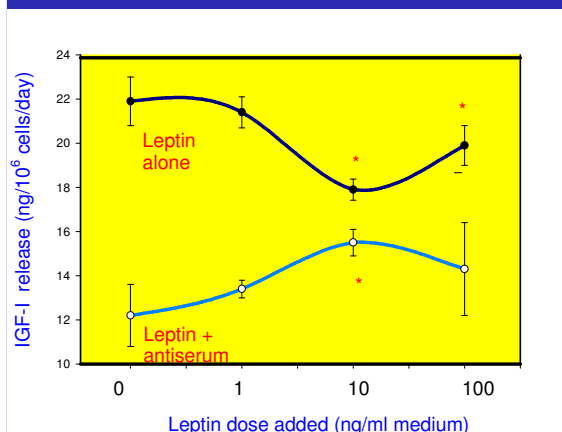
Leptín reguluje vylučovanie IGF-I.

Blokáda IGF-I zabraňuje a mení efekty leptínu na :

- IGF-I

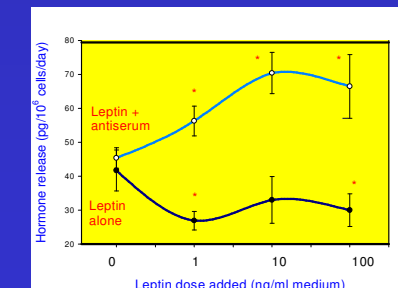
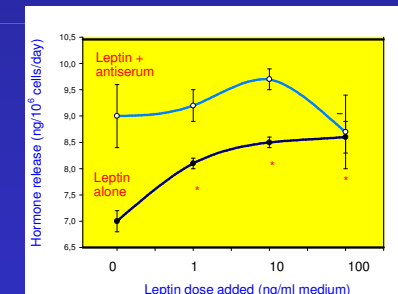
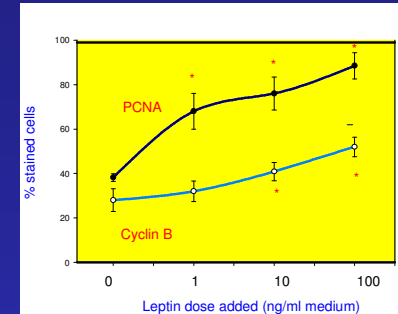
- estradiol

- IGFBP-3

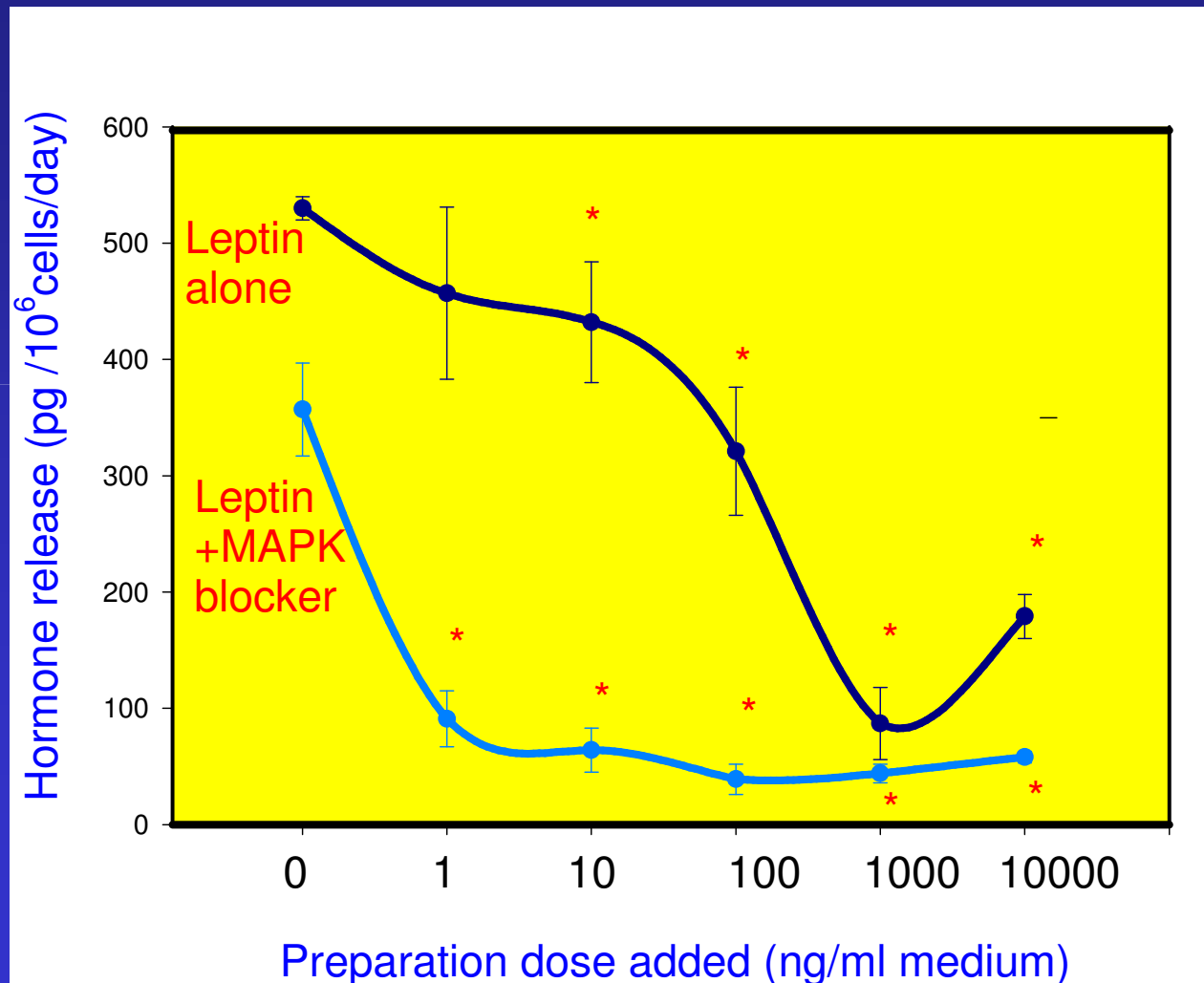


Prídavky leptínu ku kultivovaným granulóznym bunkám ľudí:

- Stimuluje proliferáciu (expresiu PCNA a cyklínu B1)
- Stimuluje vylučovanie oxytocínu, a IGFBP-3
- Inhibuje vylučovanie estradiolu a IGF-I



Blokátor MAP kinázy napodobňuje a zvyšuje efekt leptínu na vylučovanie vazotocínu granulóznymi bunkami sliepok



Blokátor CDK mení efekt leptínu na ovariálne bunky ošípaných

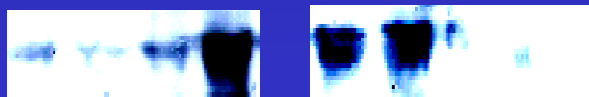
ASK-1 160K-



Cyclin 55K-



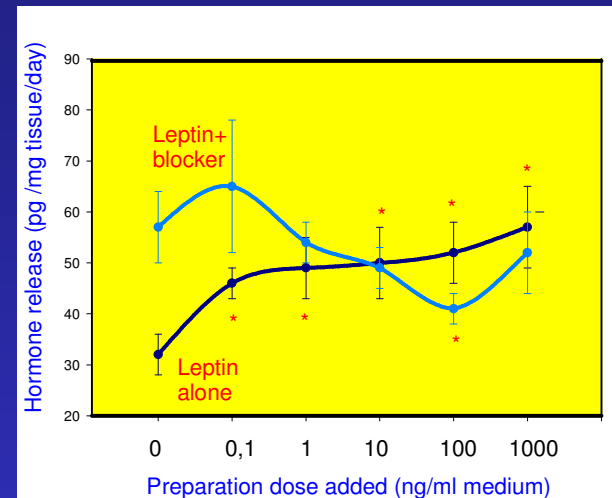
MAPK 44K-



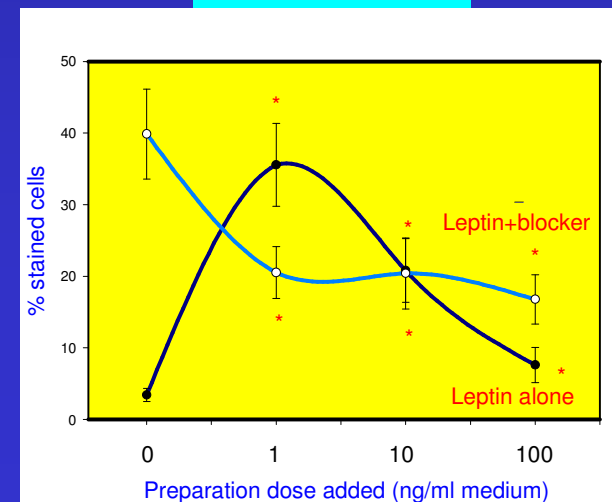
Leptin alone Leptine+blocker

0 1 10 100 0 1 10 100

Oxytocin

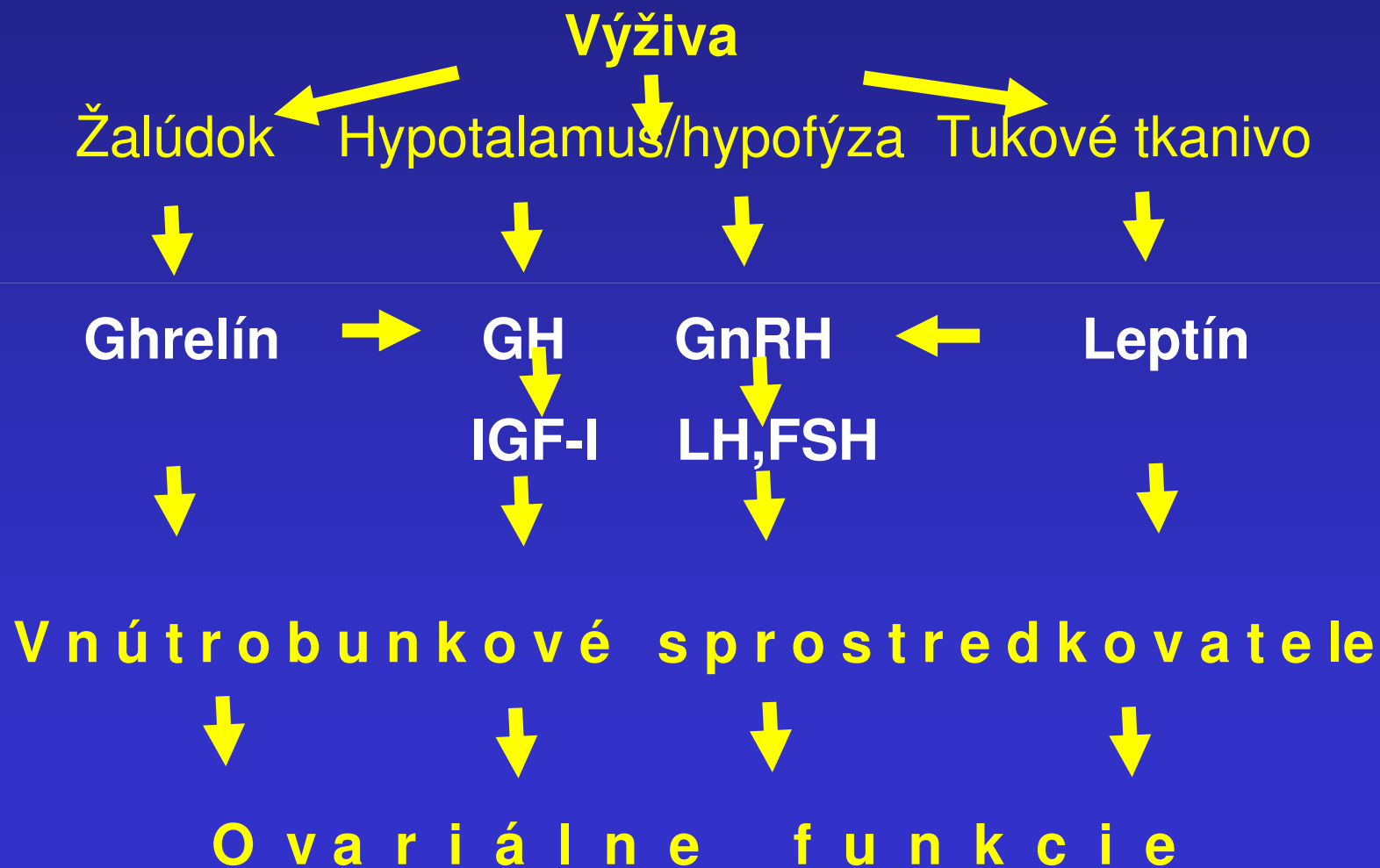


Caspase

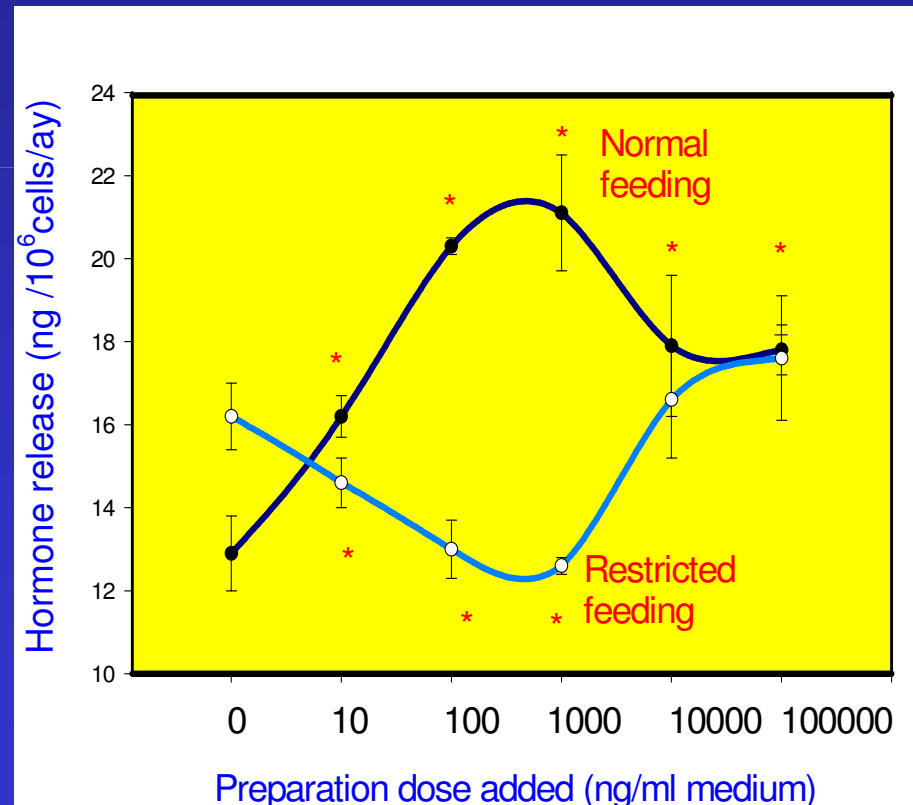




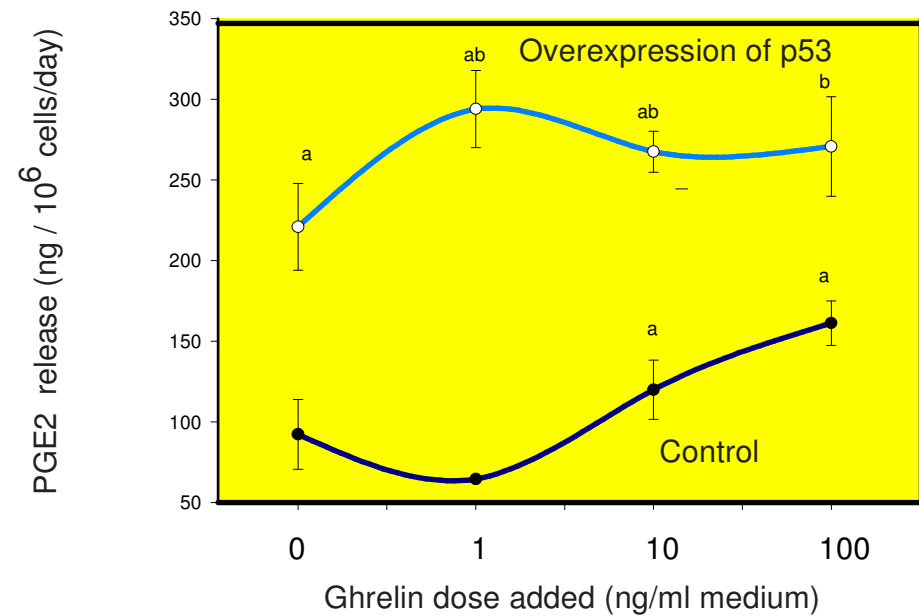
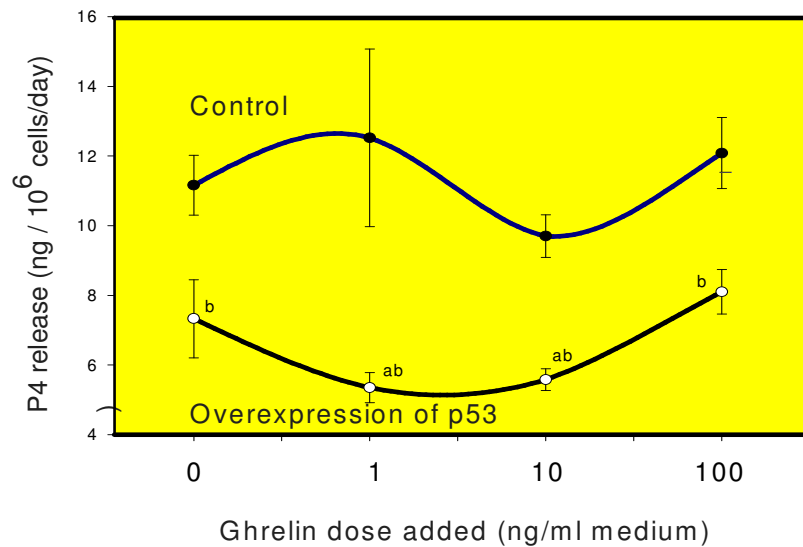
Hormonálne sprostredkovatele vplyvu výživy na ovária:

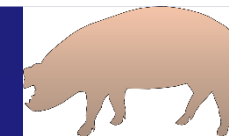


Reštrikcia výživy mení produkciu IGF-I granulóznymi bunkami králikov a jej odozvu na leptín



Overexpressia p53 reguluje vylučovanie progesterónu (z ľava) a prostaglandínu E2 (z prava) a mení efekty ghrelínu na granulózne bunky ošípaných



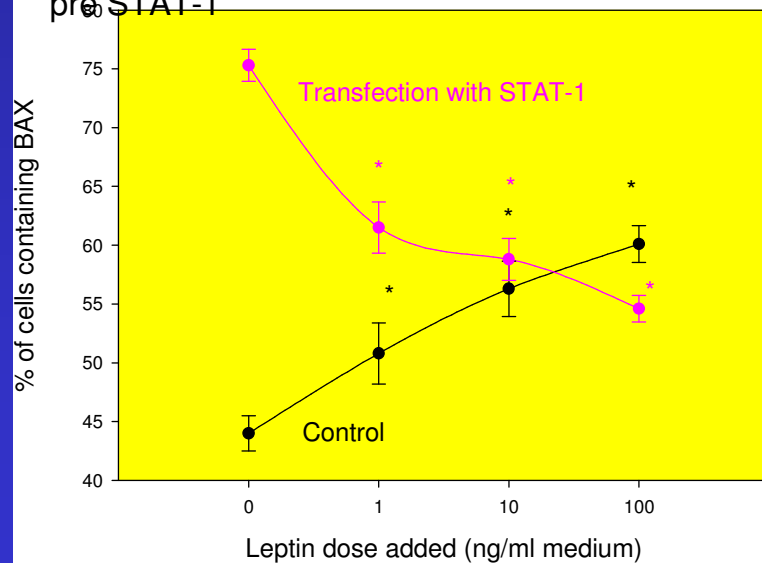


Overexpresia STAT-1 v granulóznych bunkách ošípaných zabraňuje účinku leptínu na

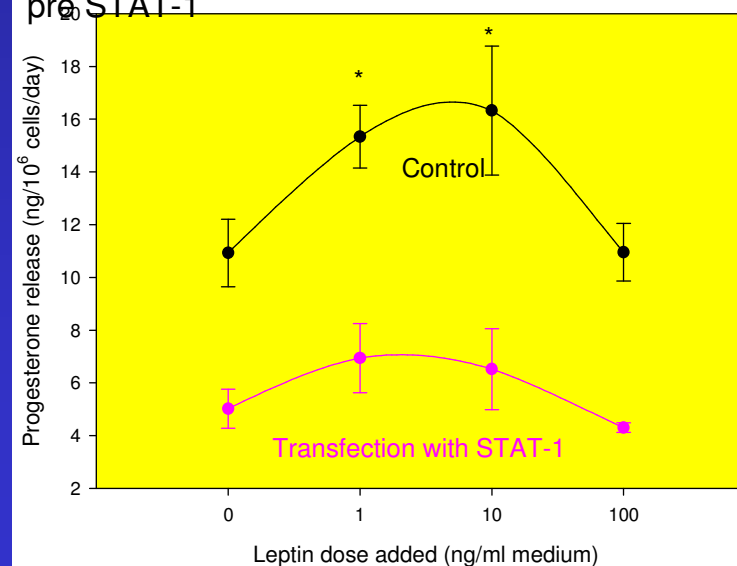
- apoptózu (bax)

- vylučovanie progesterónu

Obr.è.2: Vplyv leptínu na expresiu BAX v bunkách bez(control) a po transfekcii génovou konštrukciou pre STAT-1

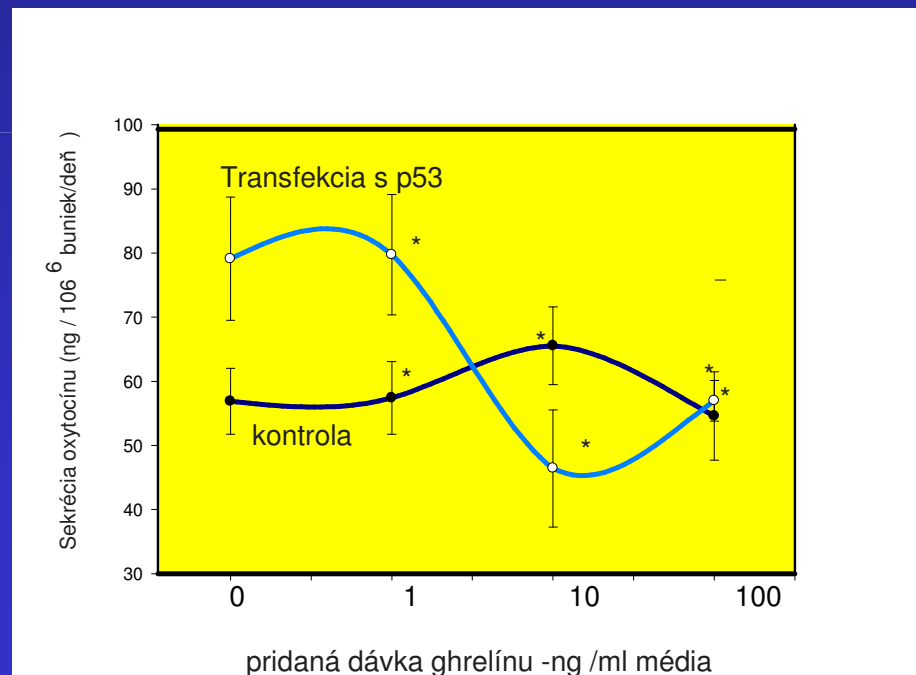


Obr.è.5: Vplyv leptínu na produkciu progesterónu v bunkách bez(control) a po transfekcii gén.konštr. pre STAT-1



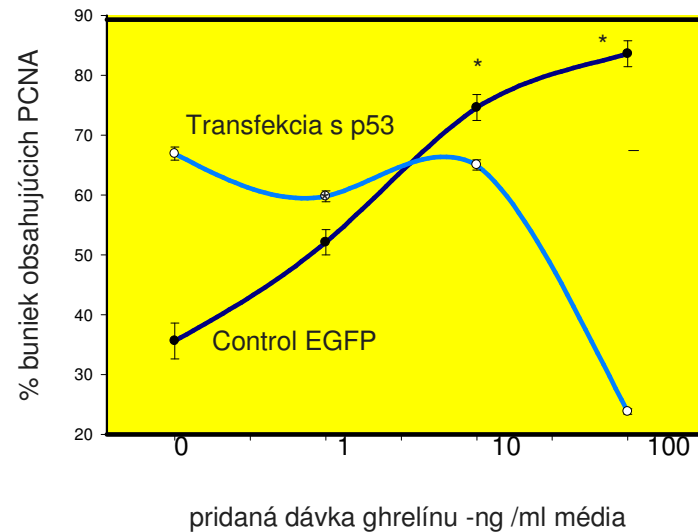
Ghrelín stimuluje sekréciu oxytocínu granulóznymi bunkami ošípaných.

Overexpresia p53 zvyšuje sekréciu oxytocínu a mení stimulačný účinok ghrelínu na inhibičný



Ghrelín stimuluje proliferáciu (expresiu PCNA) granulóznych buniek ošípaných.

Overexpresia p53 zvyšuje expresiu PCNA a mení stimulačný účinok ghrelínu na inhibičný



Efekt ghrelínu na PCNA :

34 K



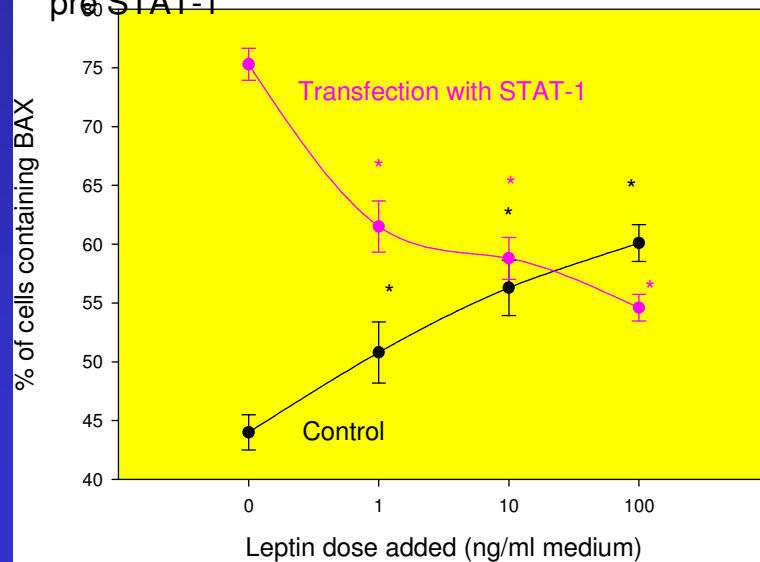
**0 1 10 100
ng/ml**

Overexpresia STAT-1 v granulóznych bunkách ošípaných ovplyvňuje a zabraňuje účinku leptínu na

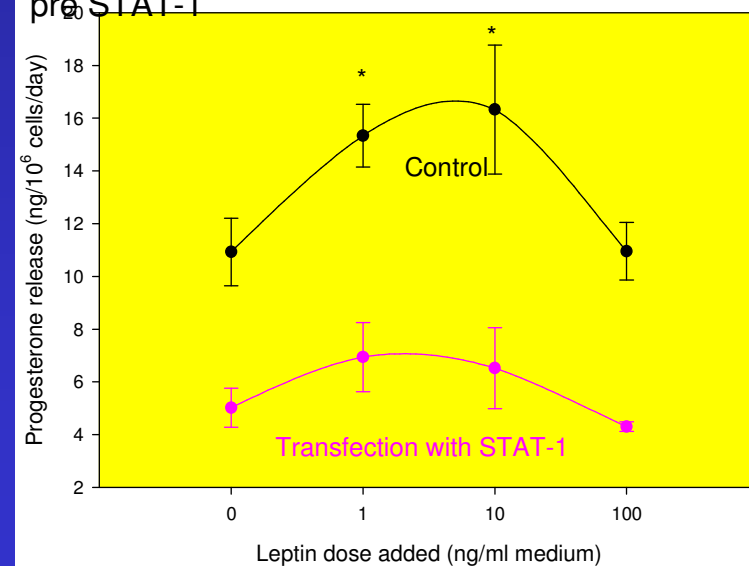
- apoptózu (bax)

- vylučovanie progesterónu

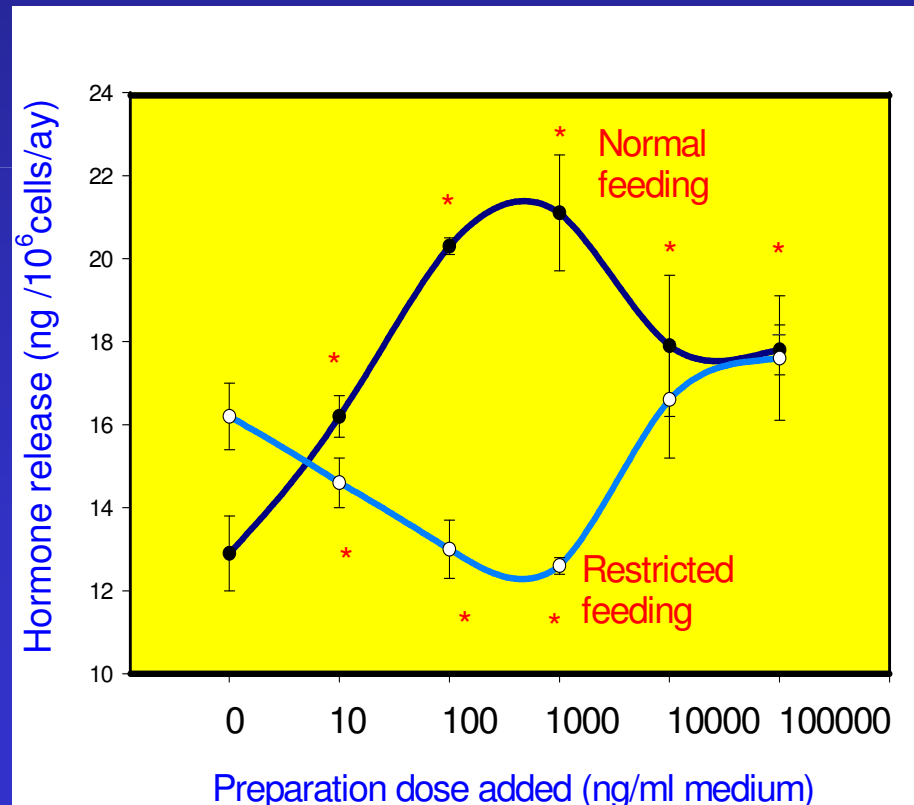
Obr.è.2: Vplyv leptínu na expresiu BAX v bunkách bez(control) a po transfekcii génovou konštrukciou pre STAT-1



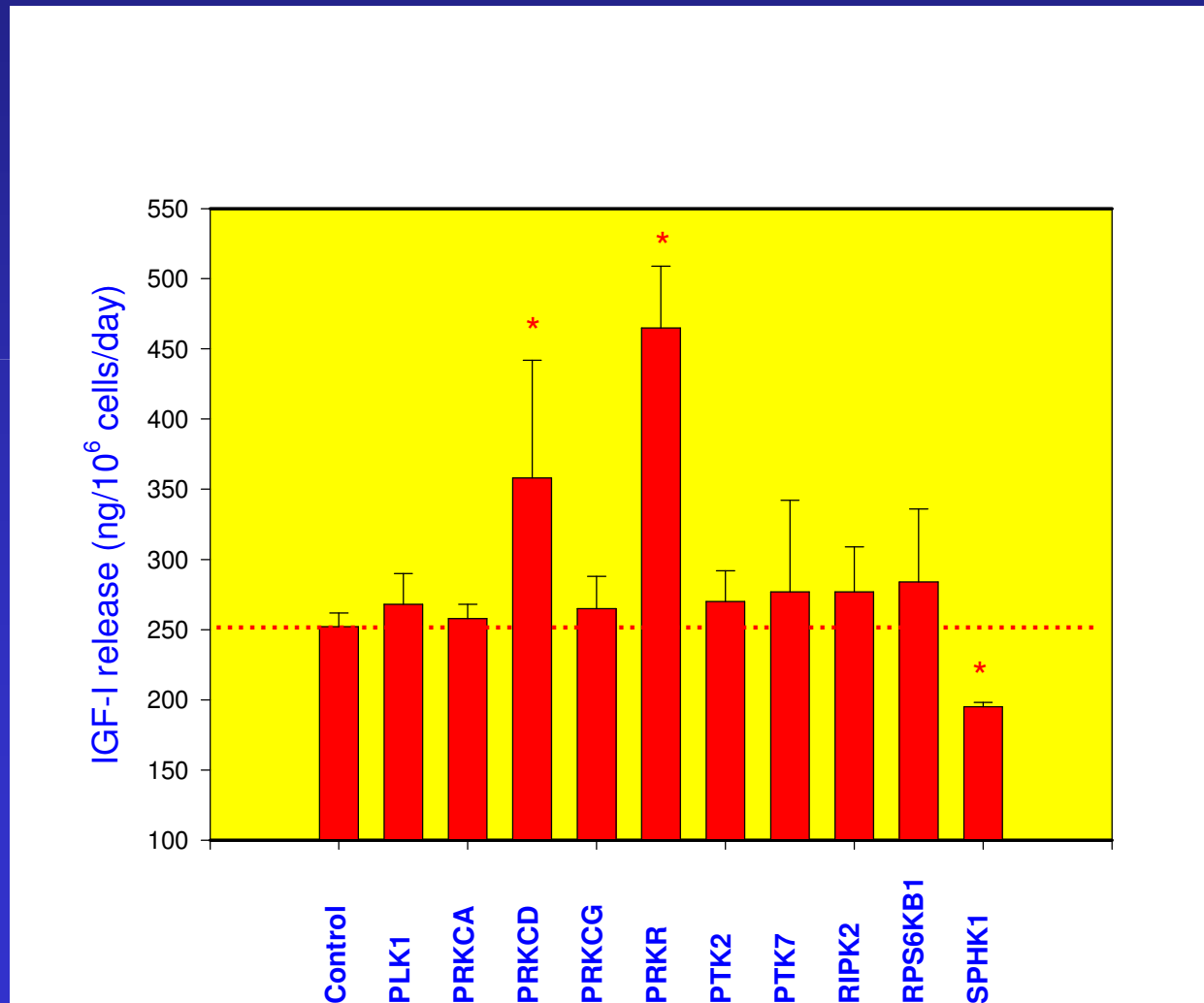
Obr.è.5: Vplyv leptínu na produkciu progesterónu v bunkách bez(control) a po transfekcii gén.konštr. pre STAT-1



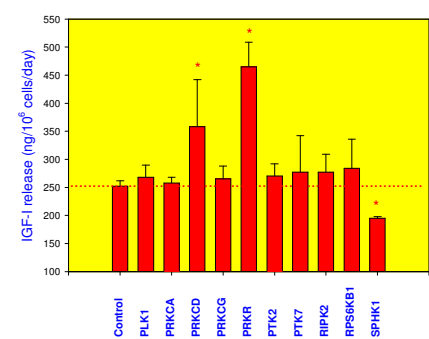
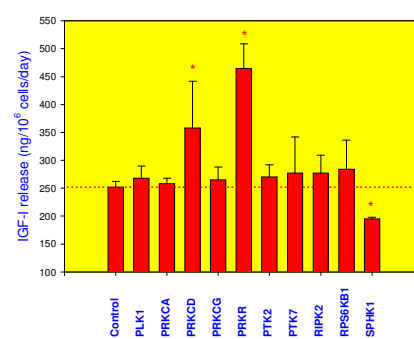
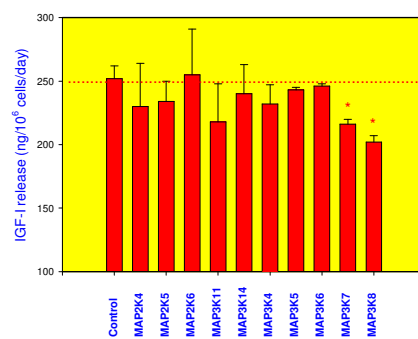
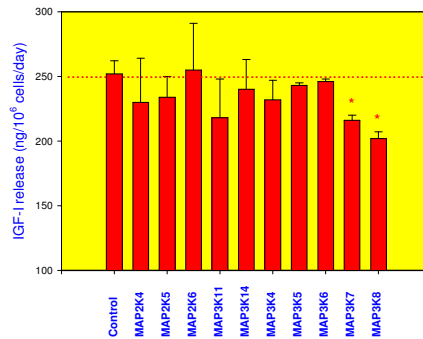
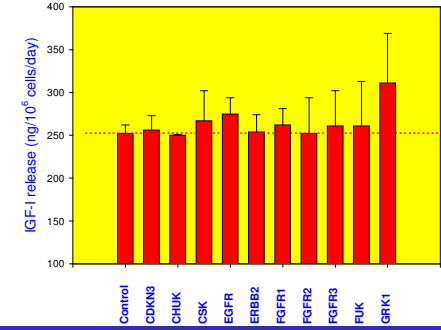
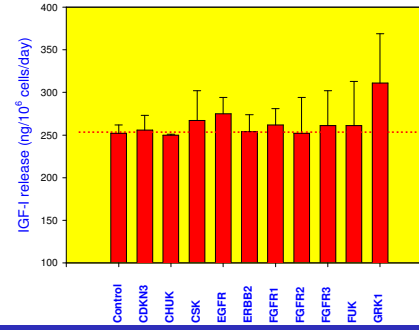
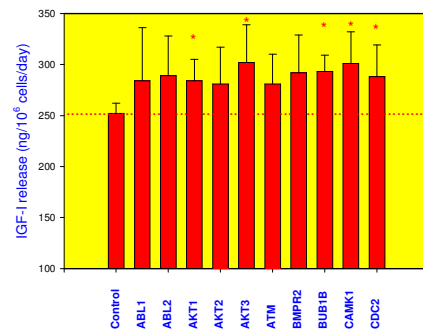
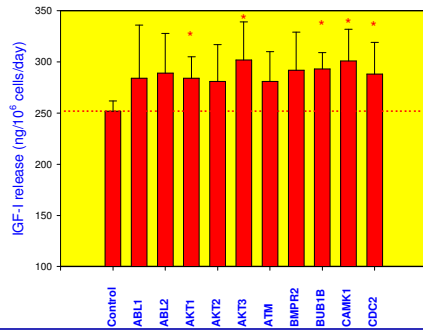
Food restriction alters IGF-I release by rabbit ovarian cells and their response to leptin



siRNA targeting protein kinases affect IGF-I release by human granulosa cells

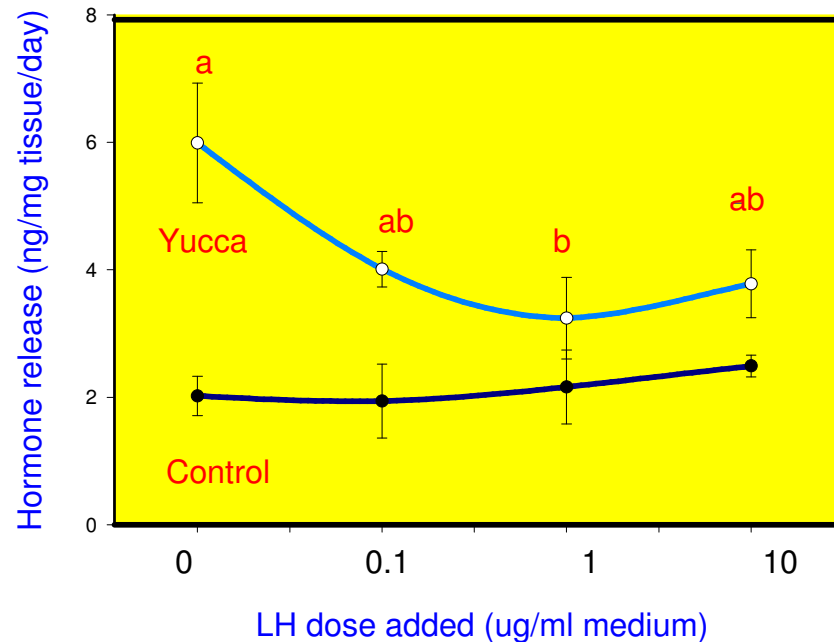


siRNA targeting protein kinases affect IGF-I release by human granulosa cells

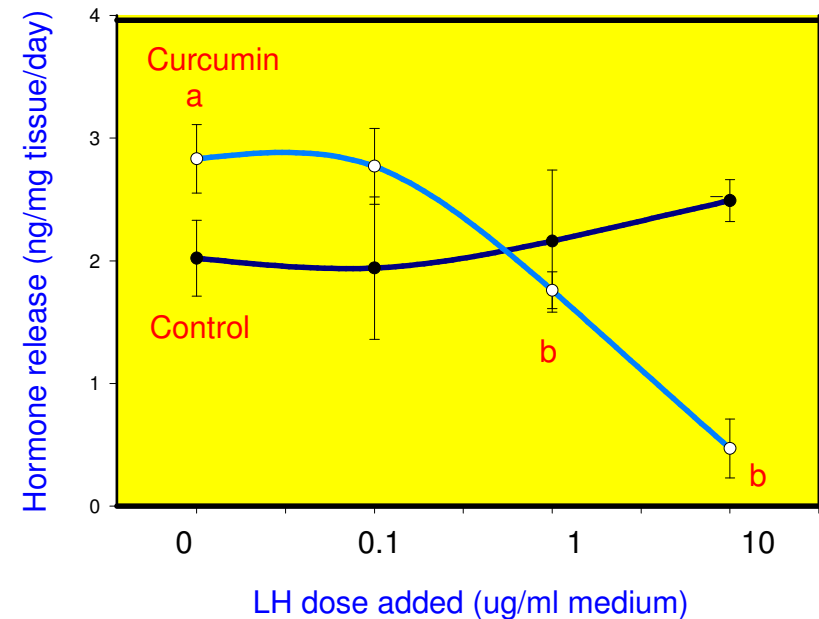


Yukka (left) and curcumin (right) promote leptin release and invert LH action on cultured rabbit ovarian cells

Exp. 603. Effect of LH and yucca additions on leptin release by cultured rabbit ovarian fragments



Exp. 603. Effect of LH and curcumin additions on leptin release by cultured rabbit ovarian fragments



Metabolic hormones - possible mediators of effect of nutrition on the ovary:

