

내분비계 장애물질과 암컷 흰쥐의 비정상적인 사춘기 발달

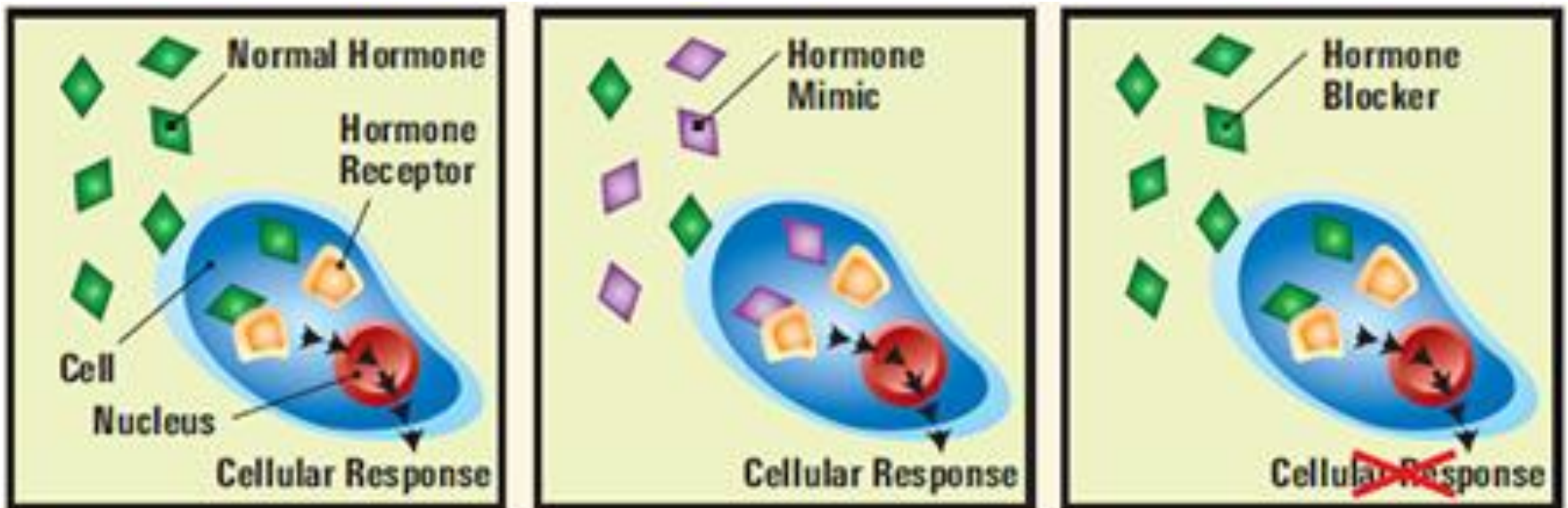
Endocrine disrupting chemicals and
abnormalities of pubertal development
in female rats

상명대학교
발생·생리학 연구실
이 성호

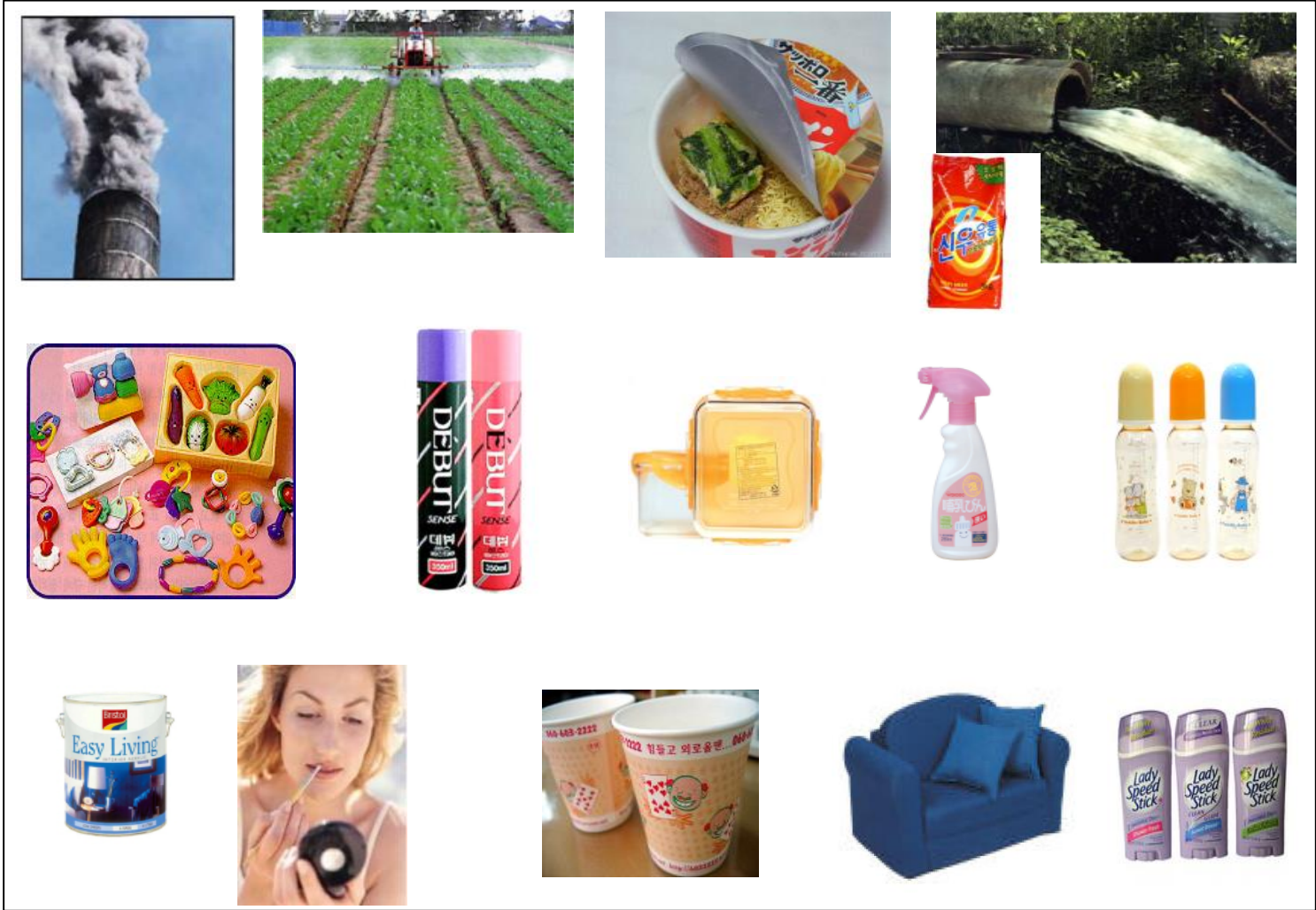
Definition of EDCs

Endocrine disruptors are chemicals that, at certain doses, can interfere with the endocrine (or hormone) system in mammals.

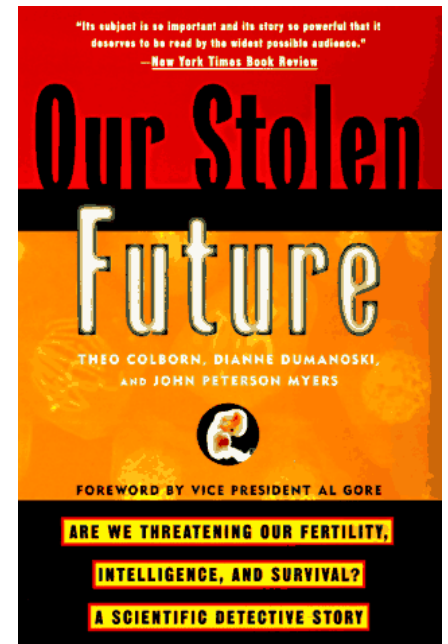
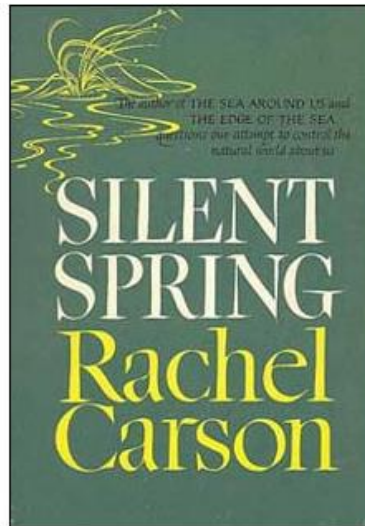
They are sometimes also referred to as **hormonally active agents**, **endocrine disrupting chemicals** or **endocrine disrupting compounds (EDCs)**.



Examples of EDCs



History of EDCs : public concern



History of EDCs : shocking evidence

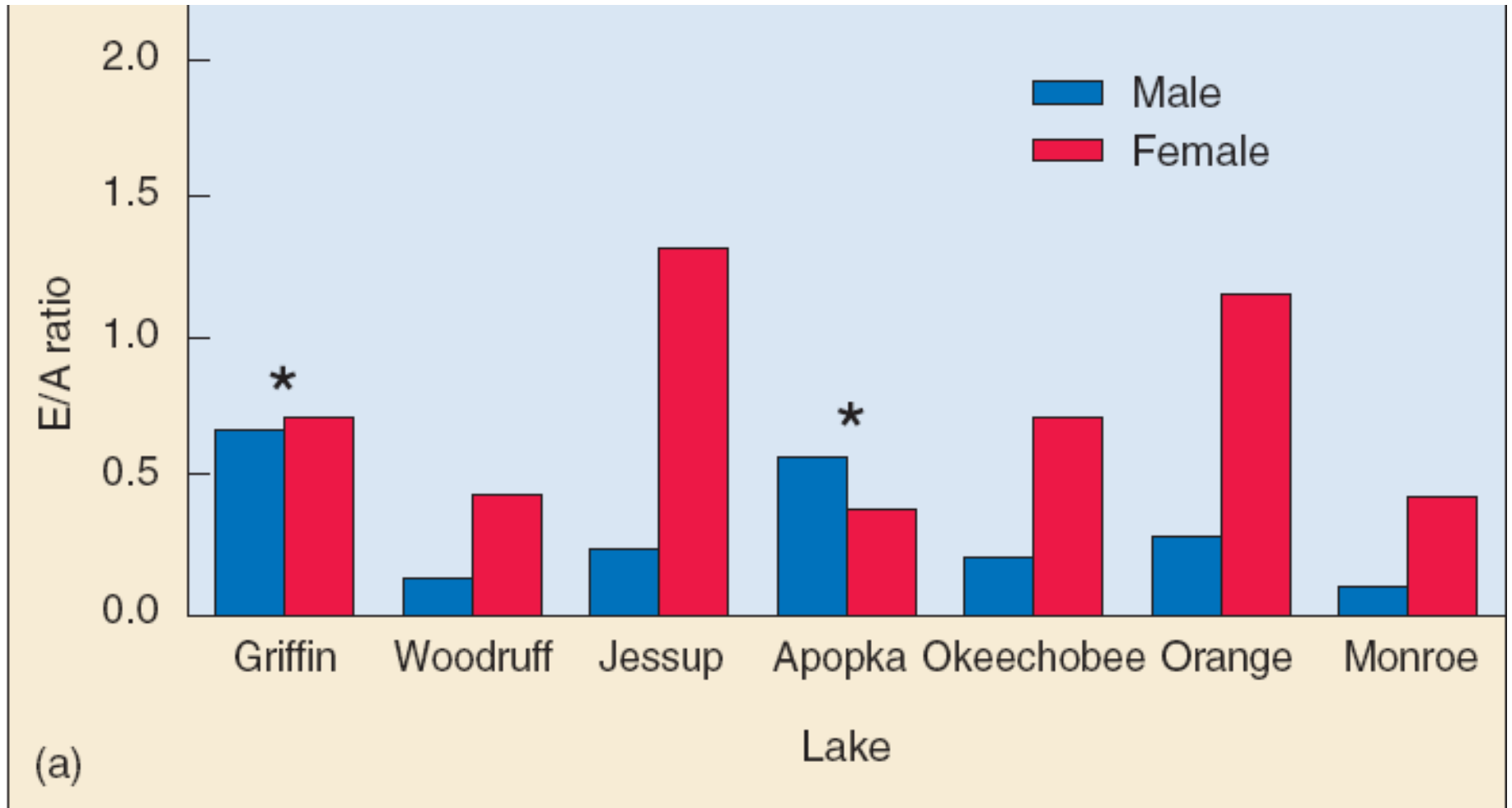


Baby alligator from lake Apopka

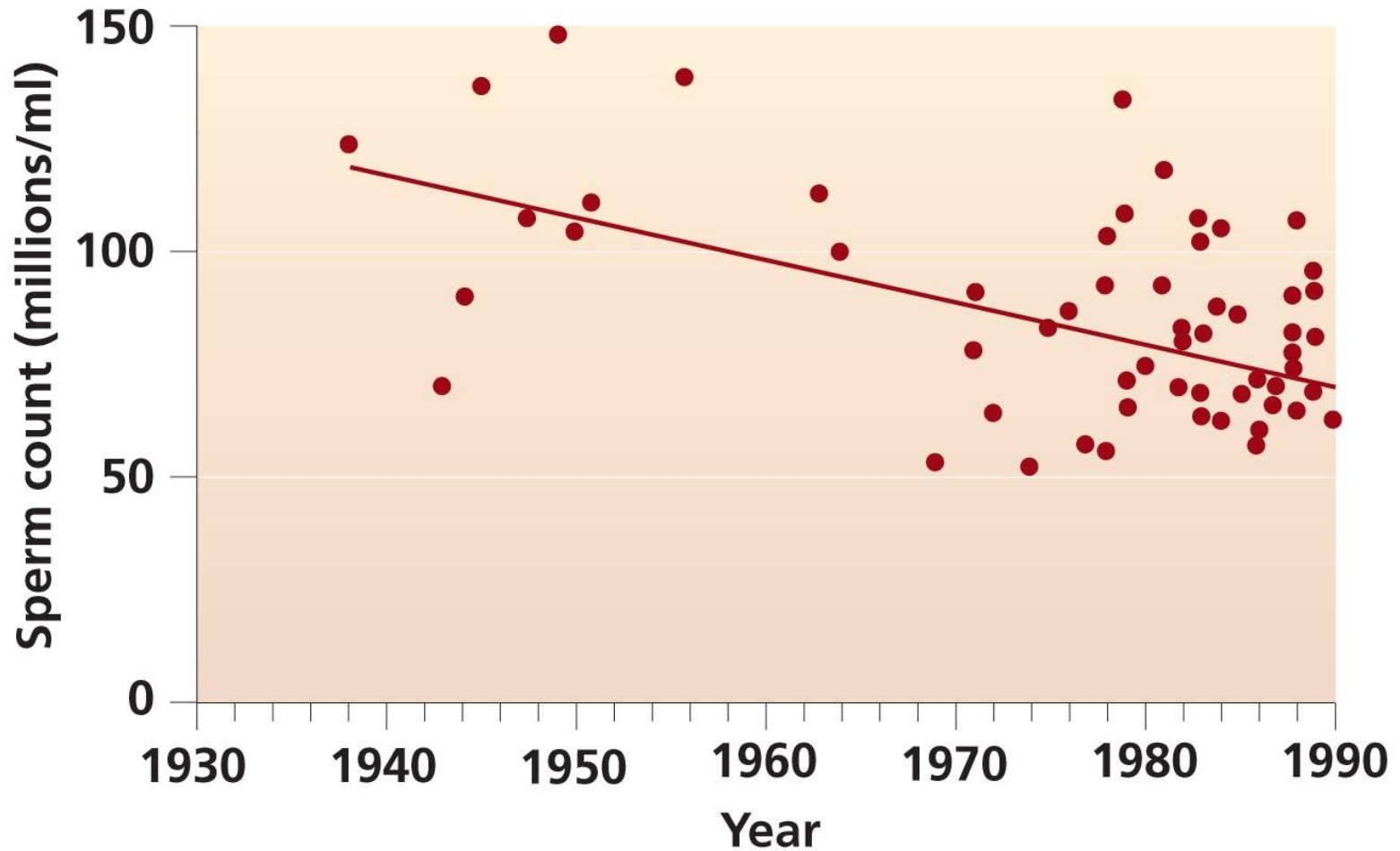


Atrazine-exposed frog

Ratios of **estrogen/testosterone** in male and female Alligators in Florida lakes

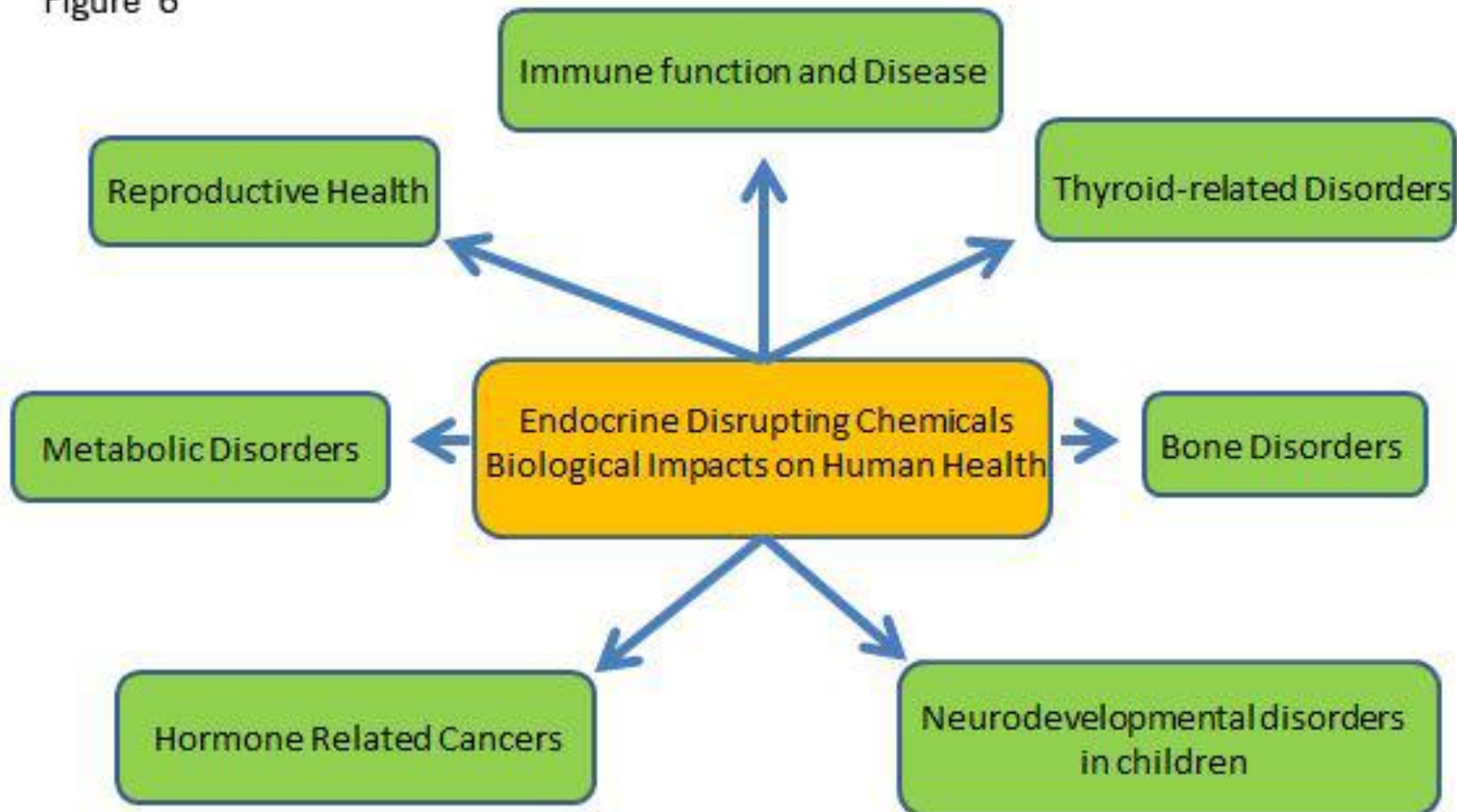


Declining sperm count in humans : based on 61 studies



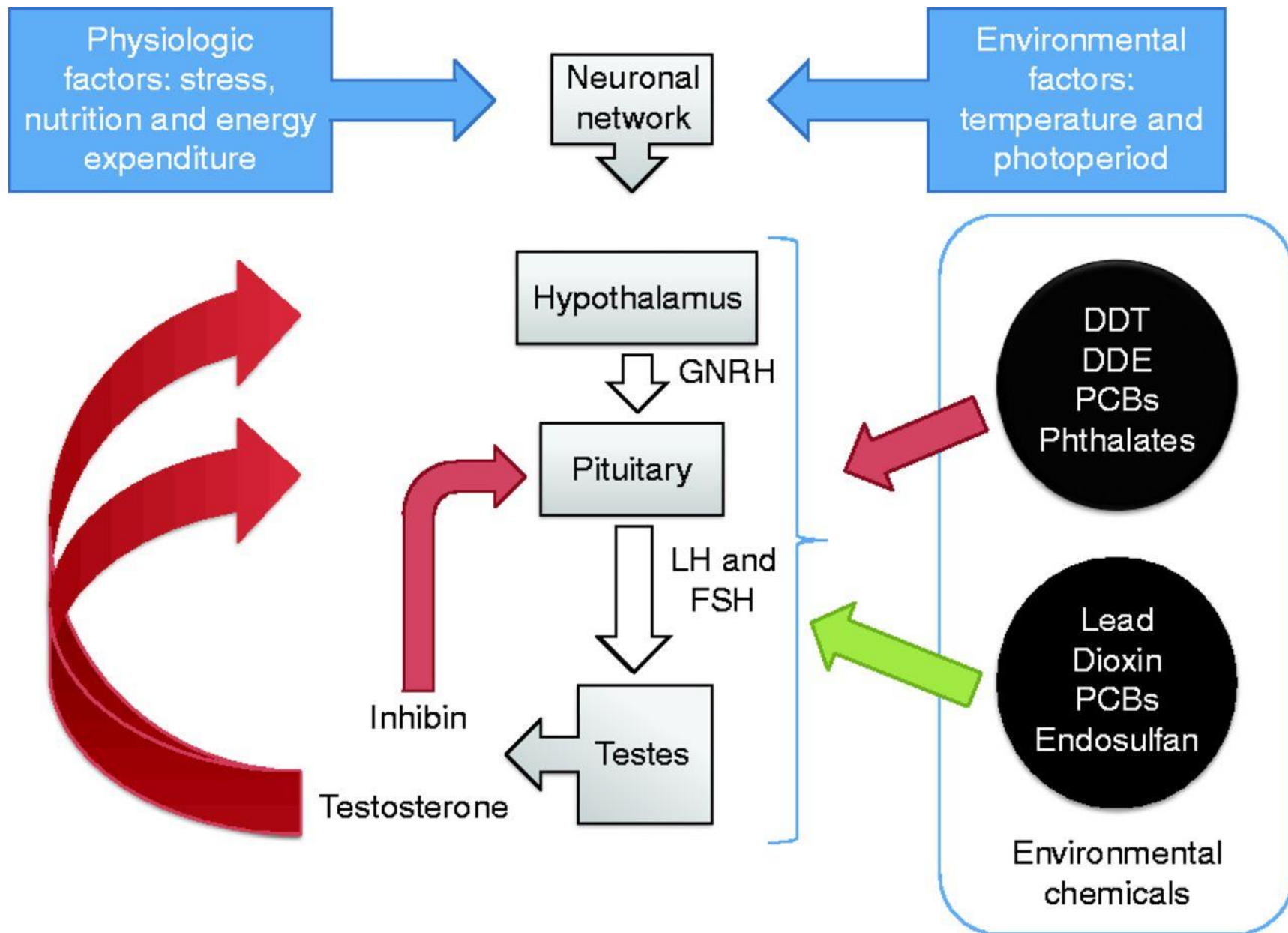
Adverse effects of EDCs

Figure 6



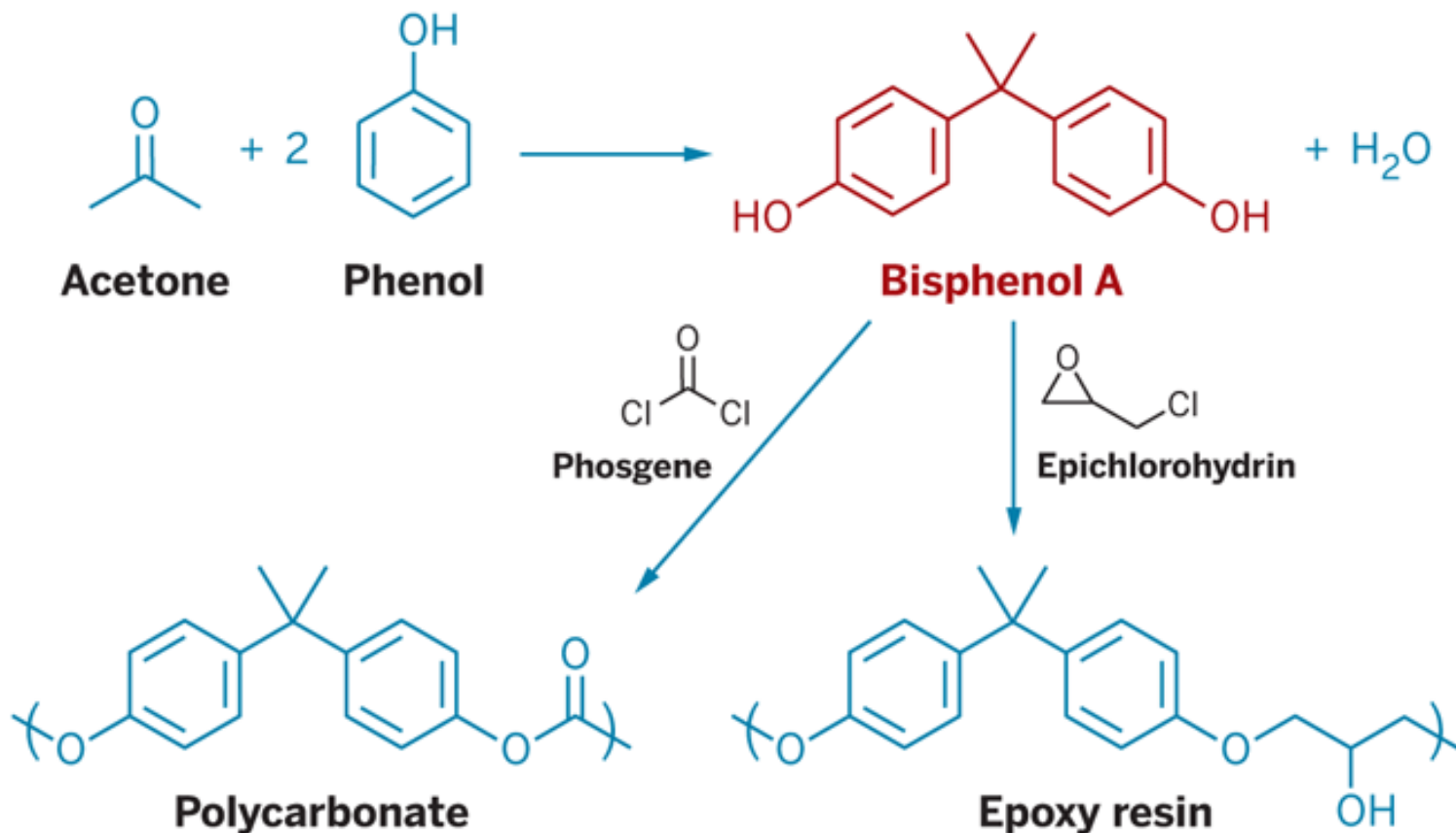
Source: State of the Science of Endocrine Disrupting Chemicals– 2012
Inter-Organization Programme for the Sound Management of Chemicals

EDC & HPG axis



Bisphenol-A : chemistry

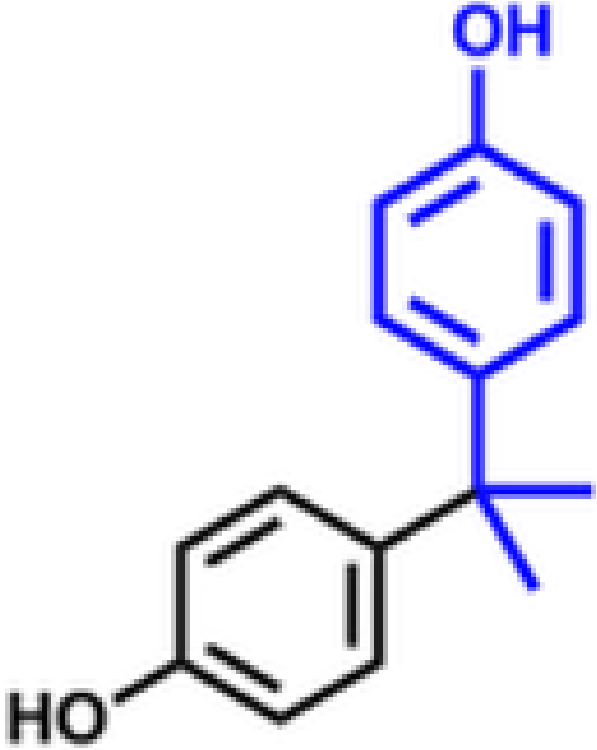
CHEMICAL LINCHPIN Bisphenol A is a commodity chemical and essential component of two classes of polymers.



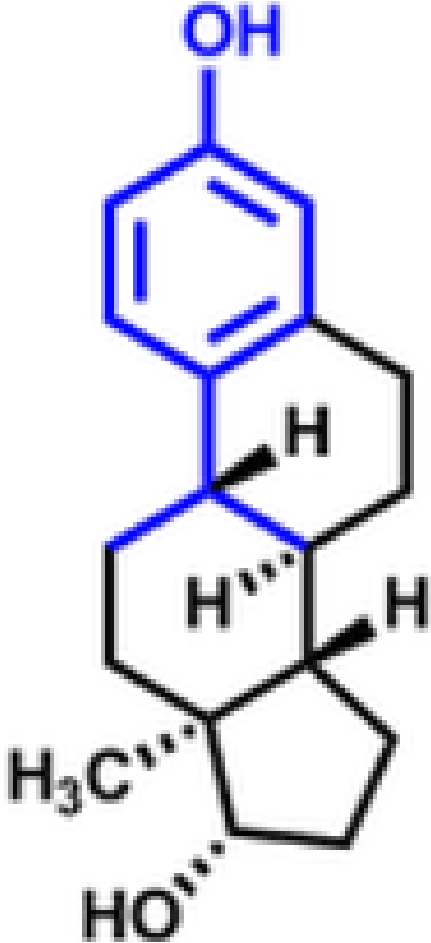
Bisphenol-A case : examples



Bisphenol-A : chemical nature

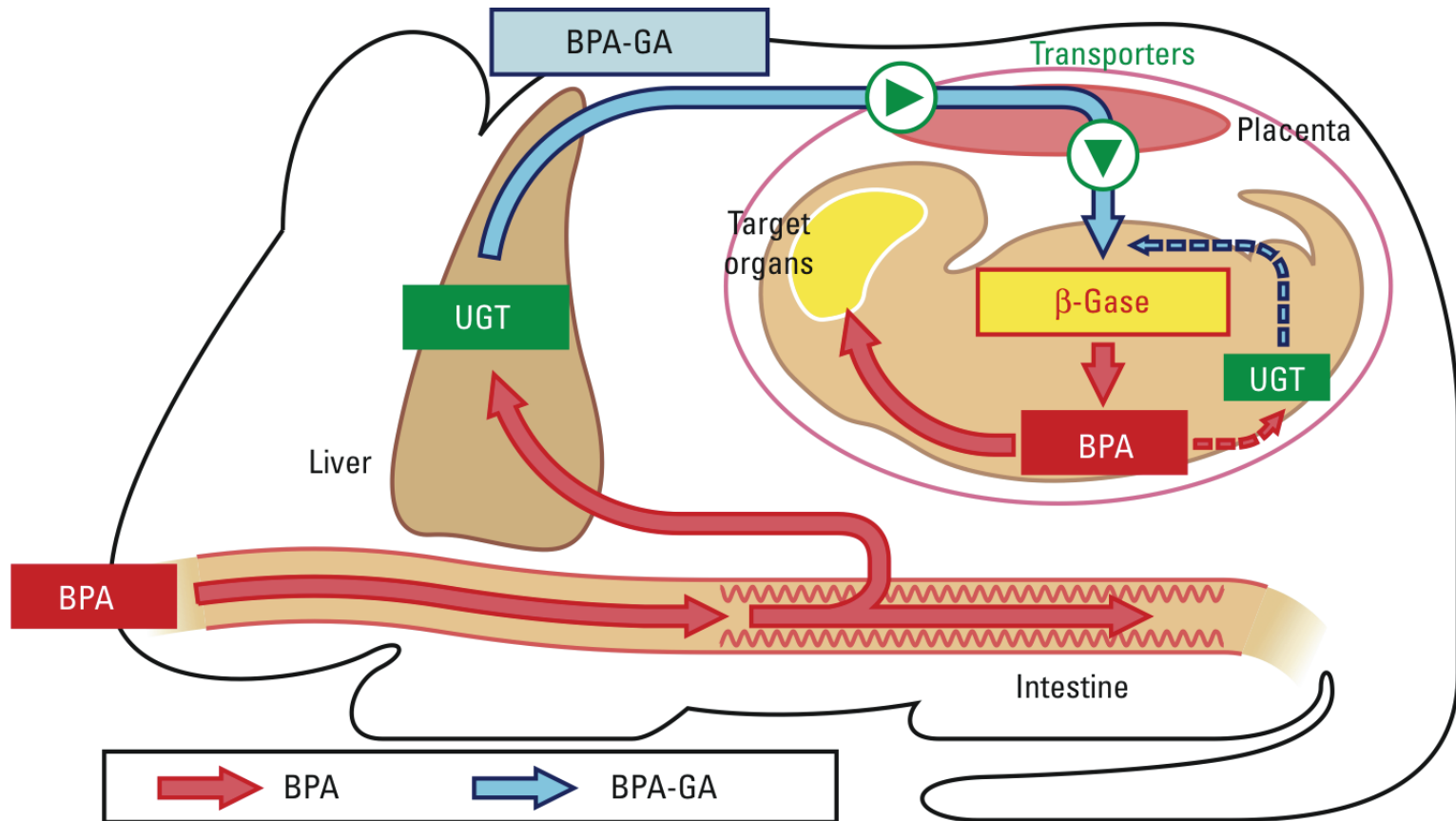


Bisphenol-A (BPA)



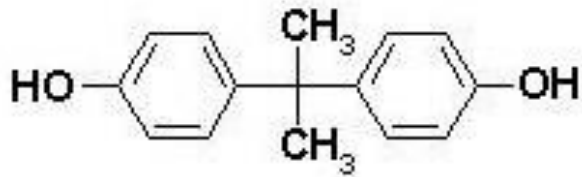
Estradiol

Bisphenol-A : exposure route

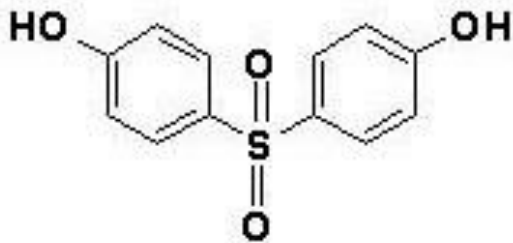


The predicted mechanism of adverse effects on the fetus induced by maternal BPA exposure during pregnancy. BPA-GA in the maternal blood is transferred across the placenta to the fetus and then deconjugated to BPA. Deconjugated BPA may remain in the fetus because of a deficiency in fetal UGT activities. **uridine 5'-diphospho-glucuronosyltransferase (UGT)**

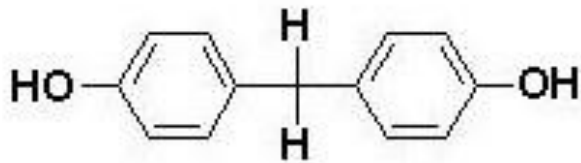
Bisphenol-A : safe?



Bisphenol A (BPA)



Bisphenol S (BPS)



Bisphenol F (BPF)

BPA-FREE
IS A LOAD OF
B (P) S
LITERALLY.

Bisphenol-A has been replaced by its nasty cousin, Bisphenol-S. Sound similar right? Well so do their associated health hazards...

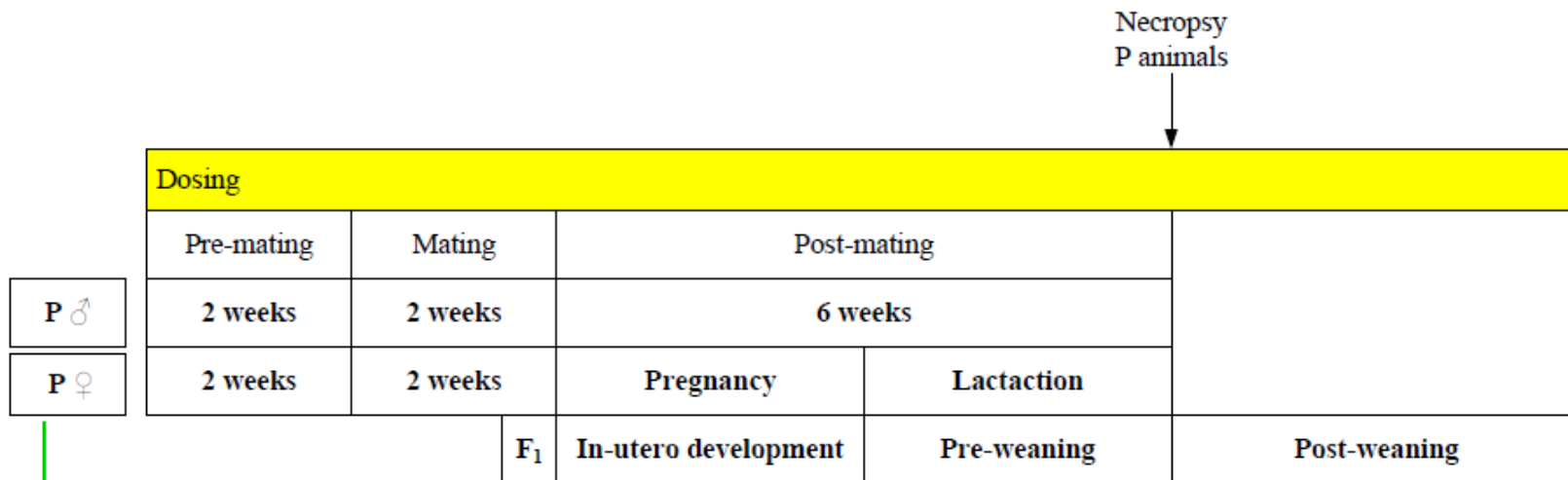
www.bodyunburdened.com

EDCs : Reproductive Toxicological effects

- Neuroendocrine regulation (Normal puberty)**
- Examples (vinclozolin & genistein)**

OECD Test Guideline 443

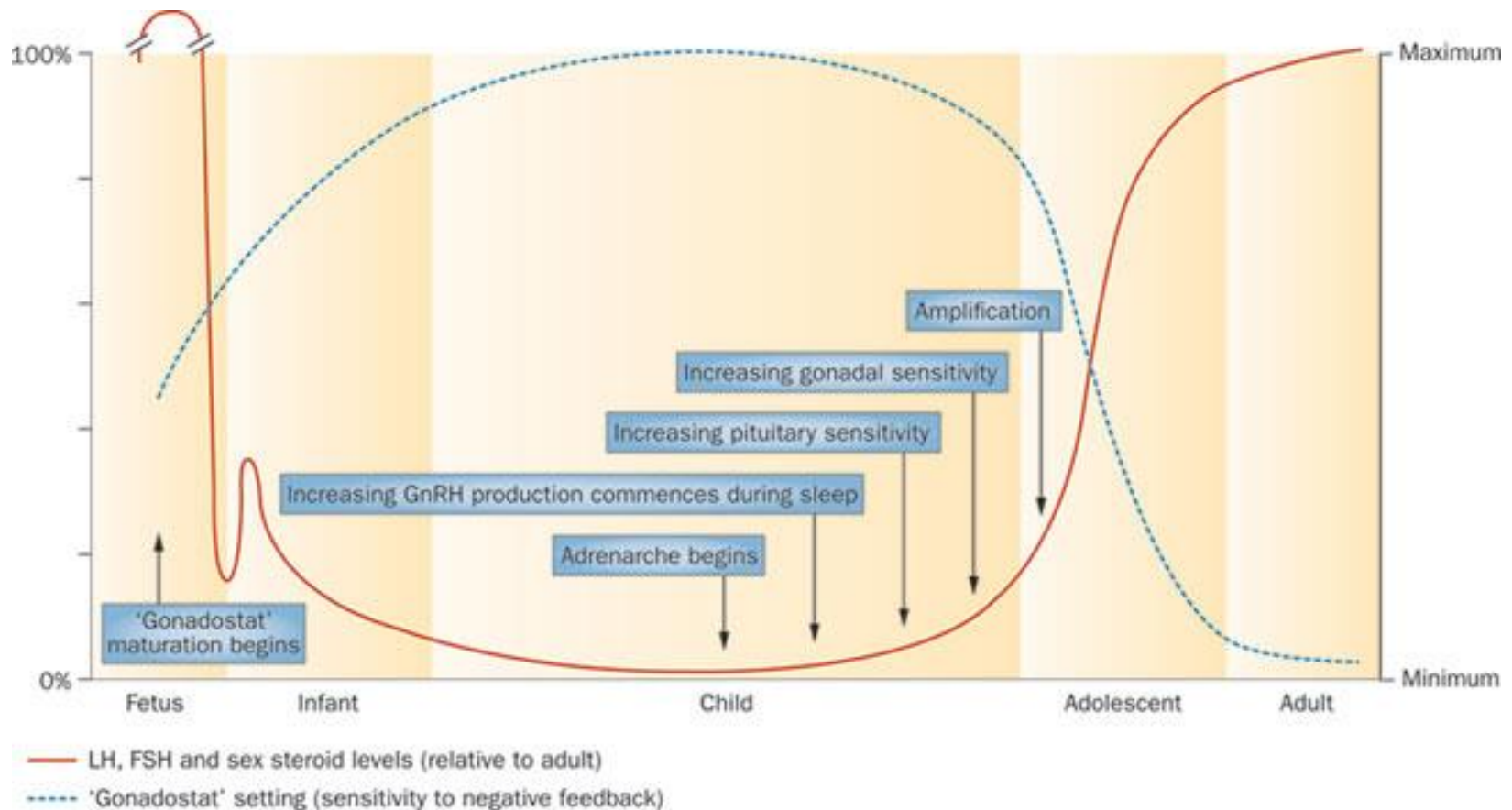
Figure 1: Scheme of the Extended One-Generation Reproductive Toxicity Study



Parental generation	Cohort	Designation	Animals/Cohort	Sexual maturation	Approximate age at necropsy (weeks)
Target is 20 litters per group	1A	Reproductive	20 M + 20 F	Yes	13
	1B	Reproductive	20 M + 20 F	Yes	14 or 20-25 if triggered
	2A	Neurotoxicity	10 M + 10 F @	Yes	11-12
	2B	Neurotoxicity	10 M + 10 F @	No	3
	3	Immunotoxicity	10 M + 10 F @	Yes	8
	Surplus	Spares		No	3

@ one per litter and representative of 20 litters in total where possible

Figure 2 Change in the levels of serum gonadotropins and sex hormones from fetal life to adulthood in relationship to the sensitivity of the central nervous system 'gonadostat' to the negative feedback effect of sex hormones and underlying hormonal changes



Wagner, I. V. *et al.* (2012) Effects of obesity on human sexual development
Nat. Rev. Endocrinol. doi:10.1038/nrendo.2011.241

Normal puberty



Vaginal opening(VO) day (SD rats)

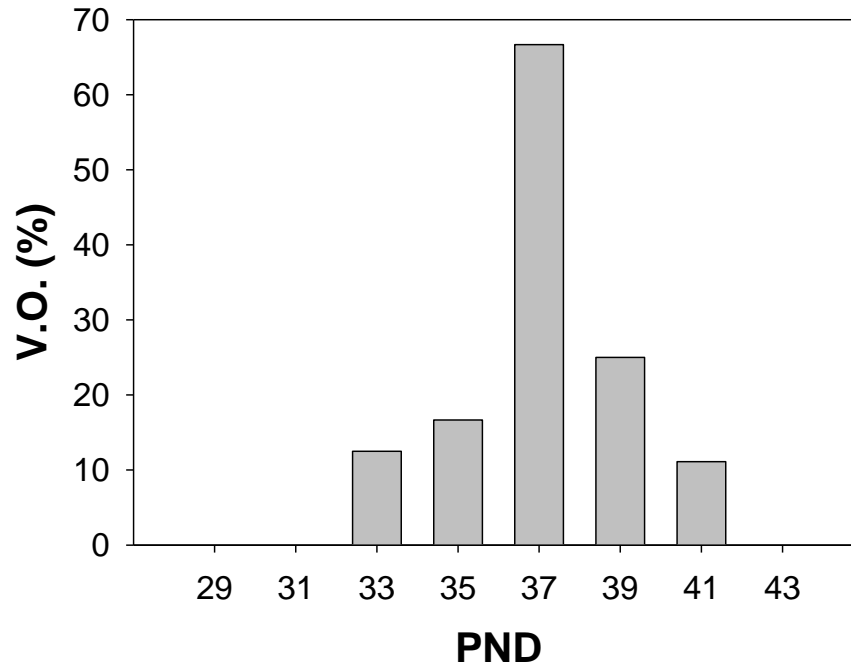


Figure 1. Dates of vaginal opening(VO).

Value are expressed as percentage of total number of animals per experimental group.

Vagina and smeared cells

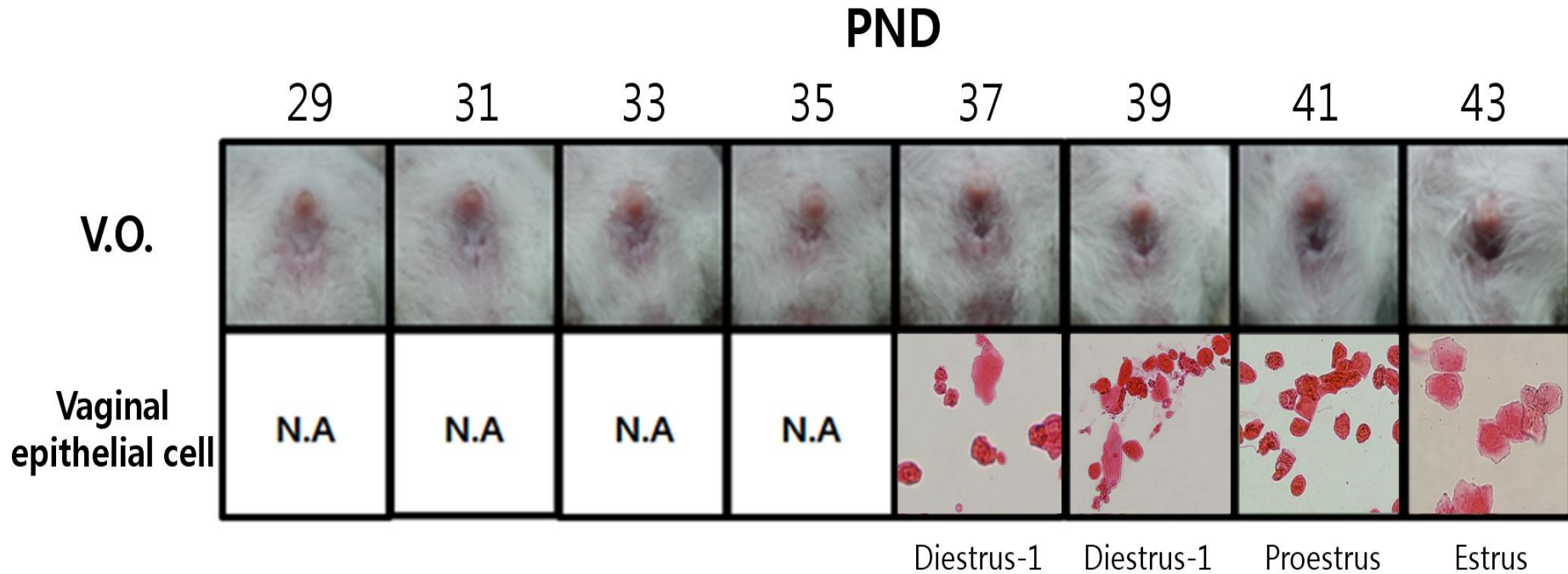


Figure 2. External feature(upper) and smeared epithelial cells(lower) of vagina.
Vaginal epithelial cells stained with eosin. Magnificant, X400.

PND

29

31

33

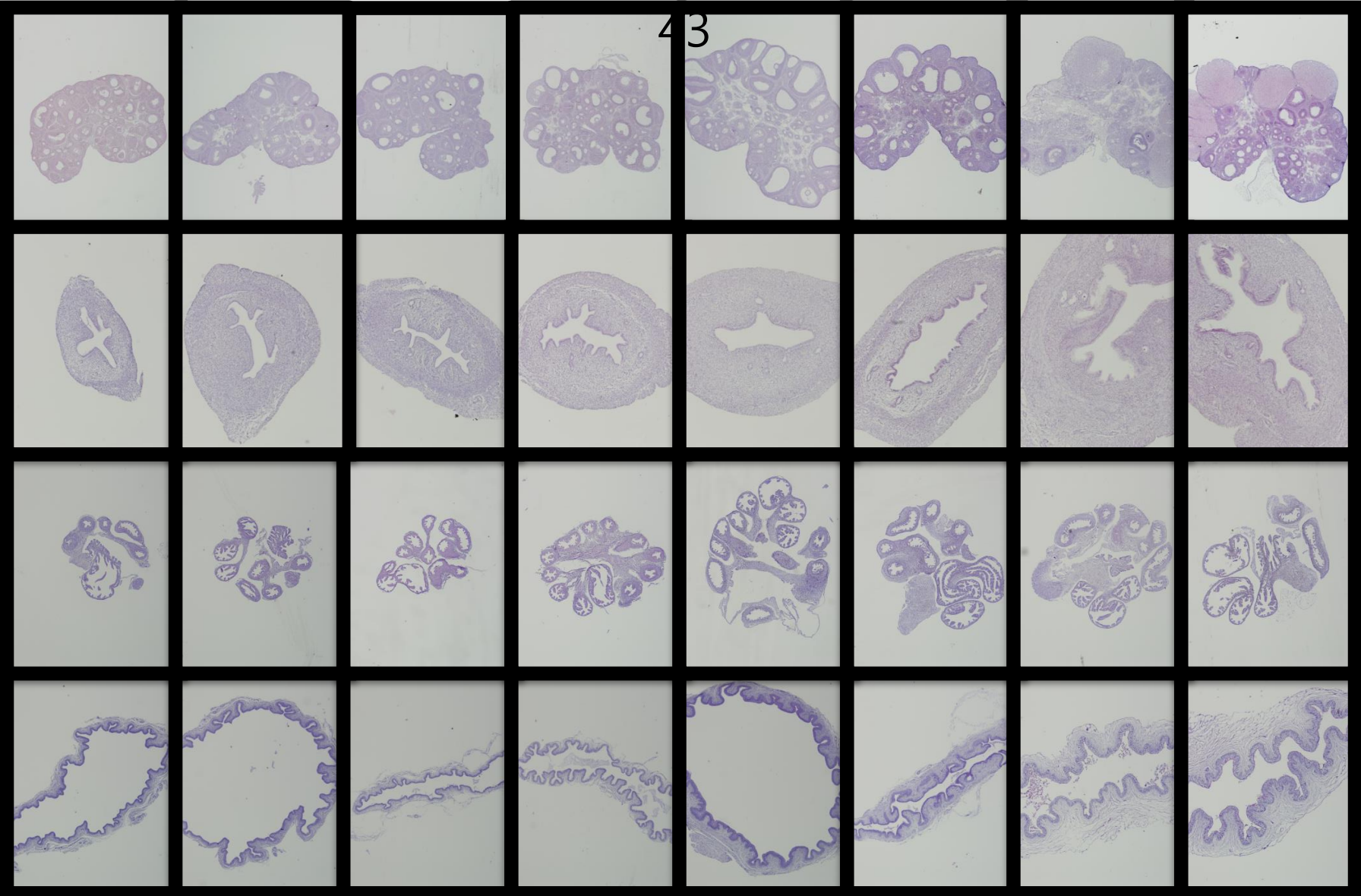
35

37

39

41

Ovary (X40)
Uterus (X100)
Oviduct (X40)
Vagina (X40)



Serum levels of estrogen(E2) and progesterone(P4)

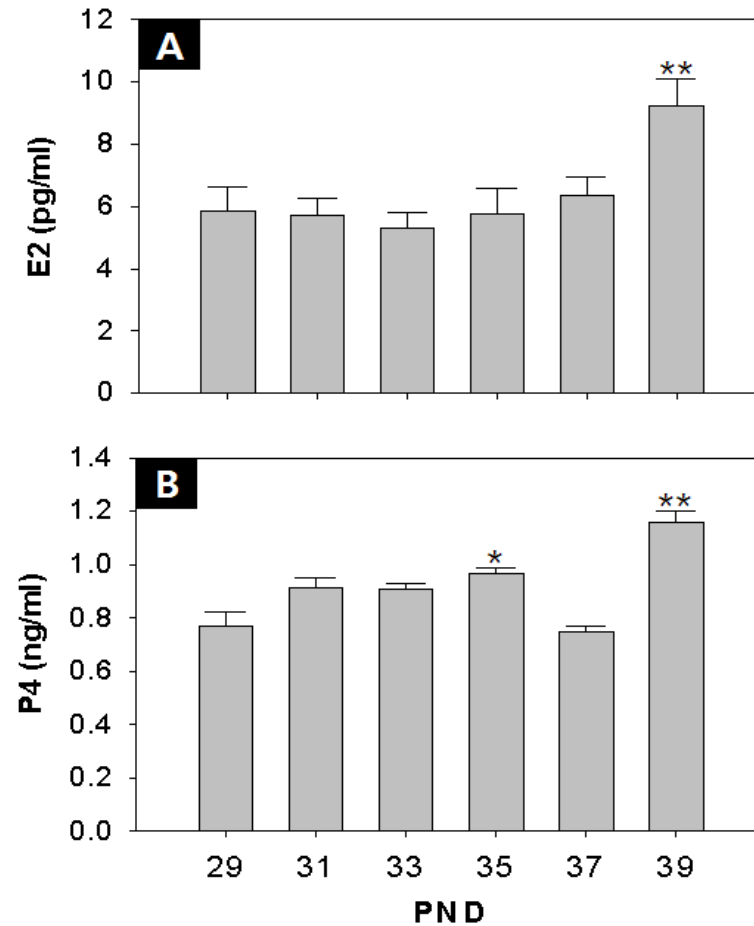
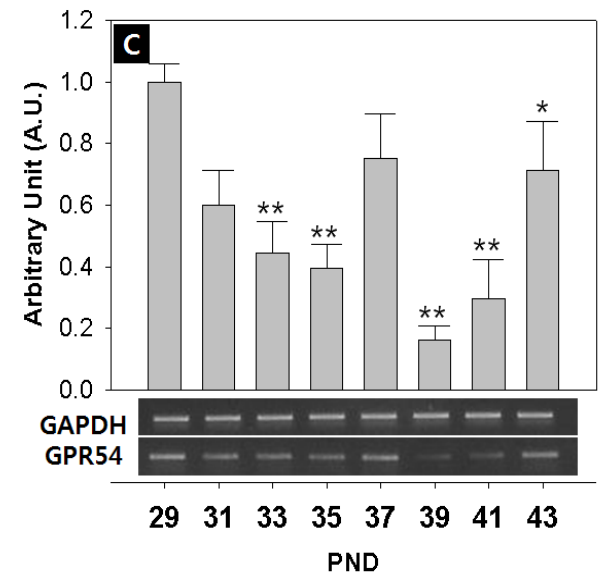
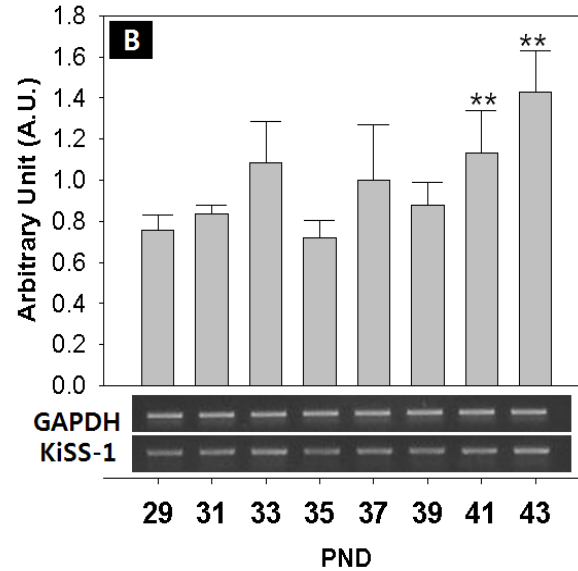
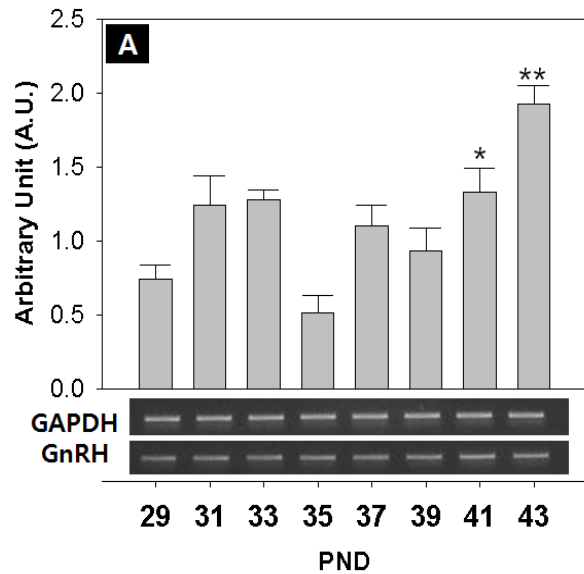


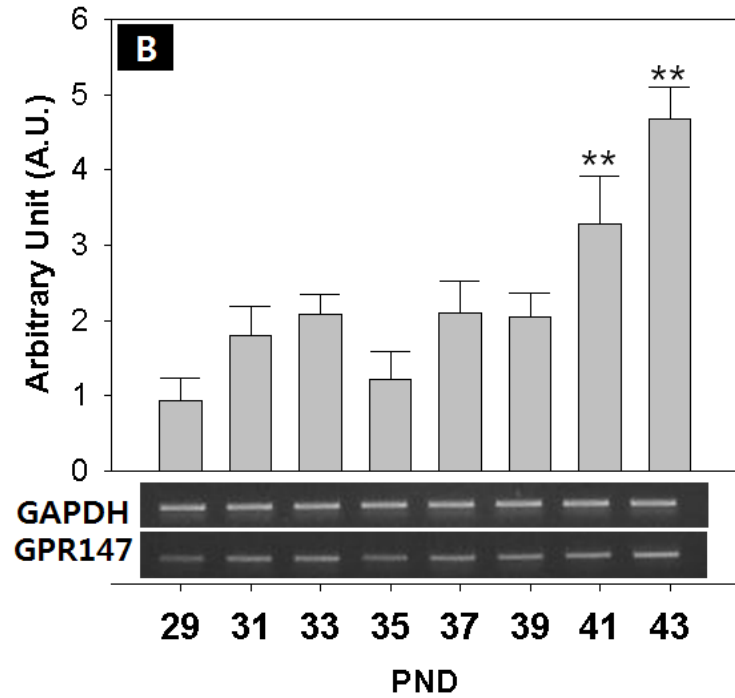
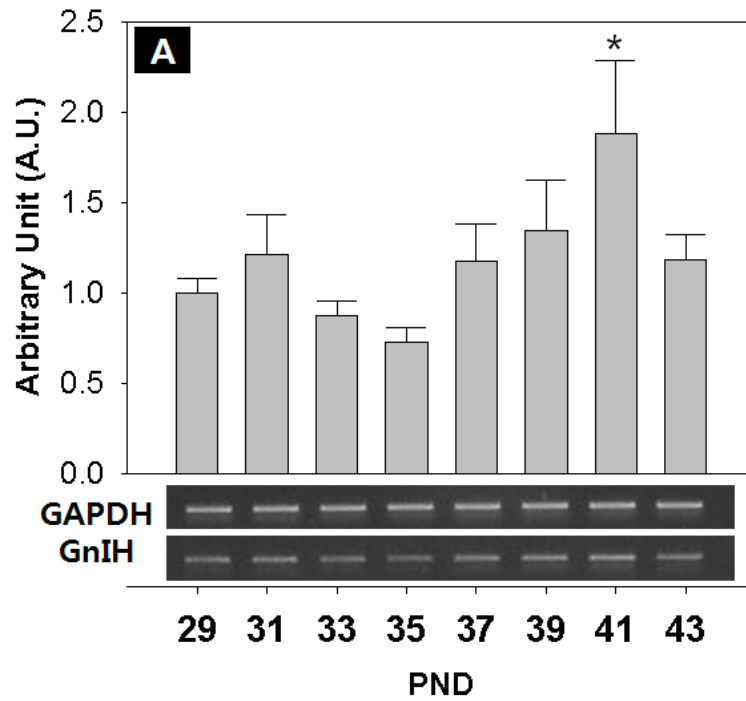
Figure 4. Hormonal changes during puberty in the serum. estrogen level in the serum(pg/ml)(A), progesterone level in the serum(ng/ml)(B).

RT PCRs : hypothalamic GnRH, KiSS-1 & GPR54

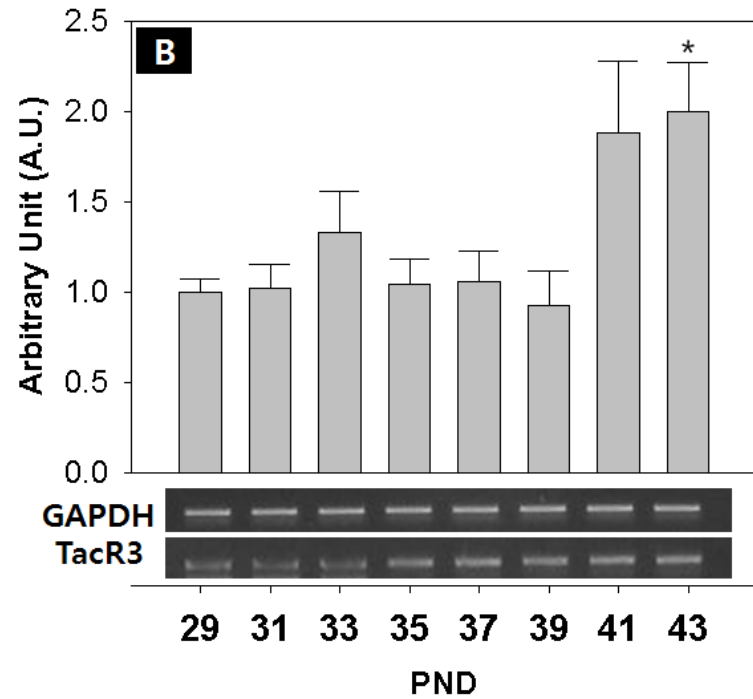
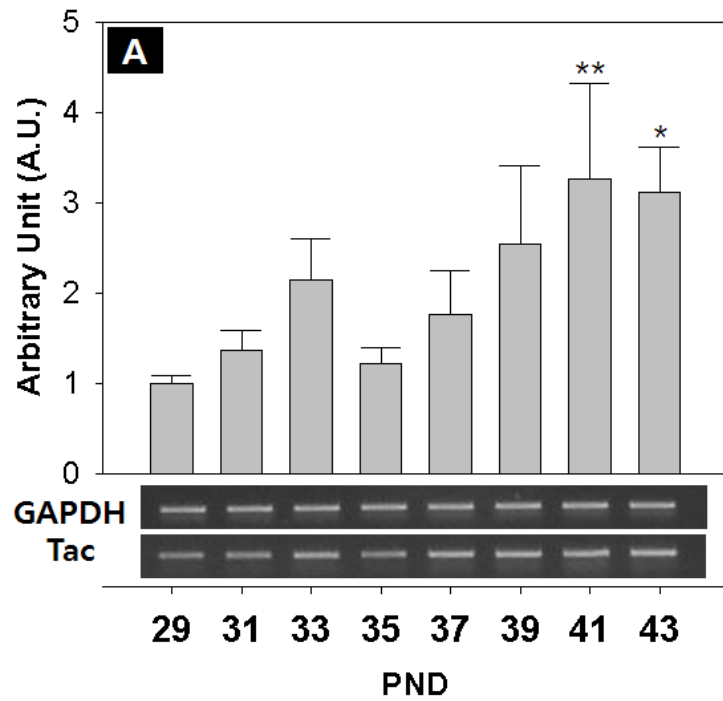


Non-monotonic curve !

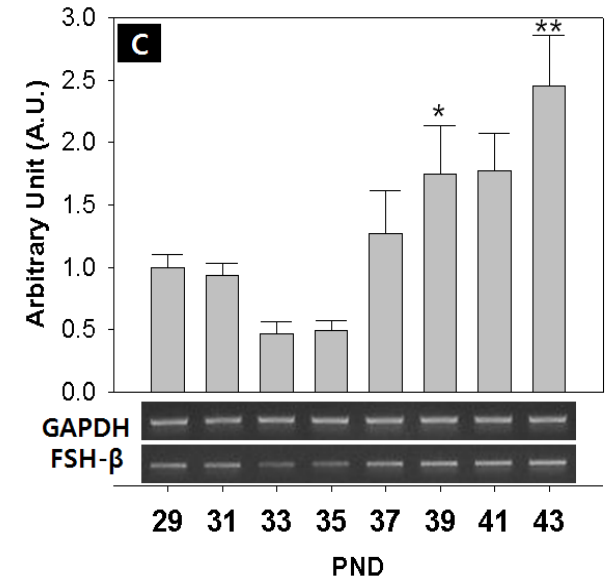
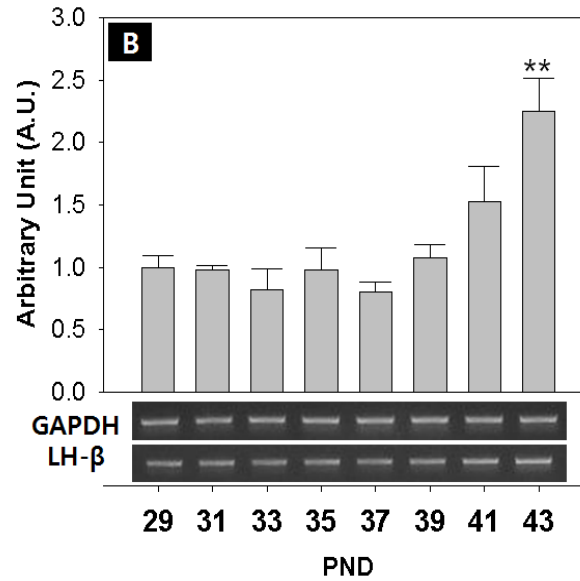
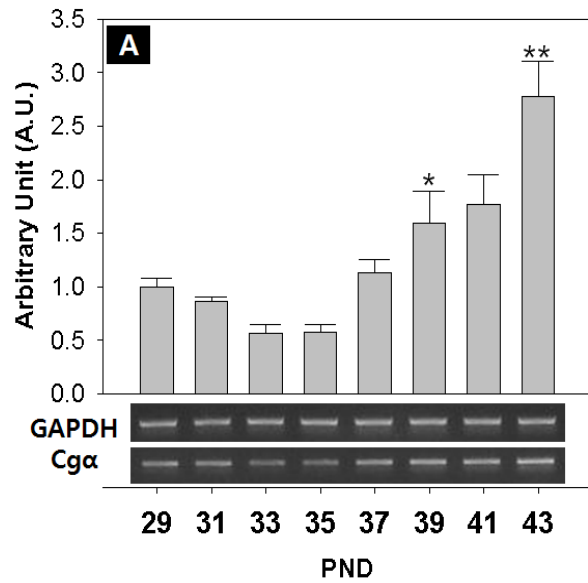
RT PCRs : hypothalamic GnIH & GPR147



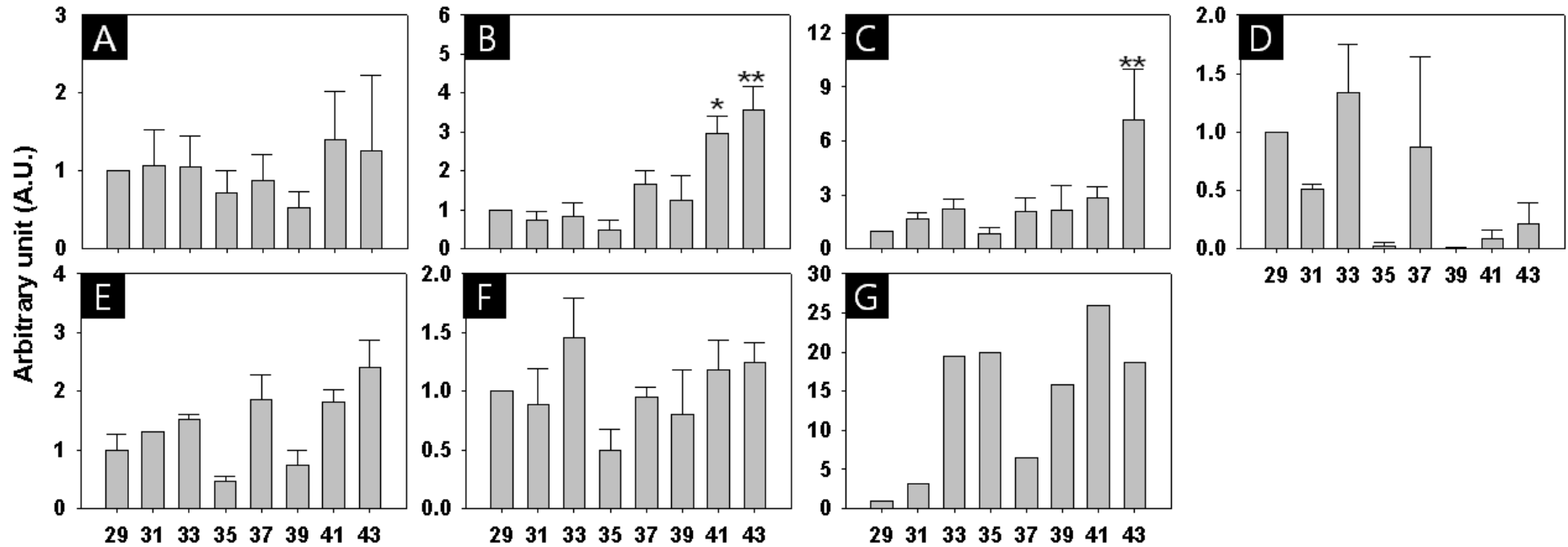
RT PCRs : hypothalamic NKB system



RT PCRs : pituitary gonadotropins



RT PCRs : ovarian steroidogenesis-related genes



(A) LHR (B) StAR (C) P450scc (D) CYP17 (E) 3b-HSD (F) 17b-HSD
(G)Aromatase

LH-R western blot analysis (ovary)

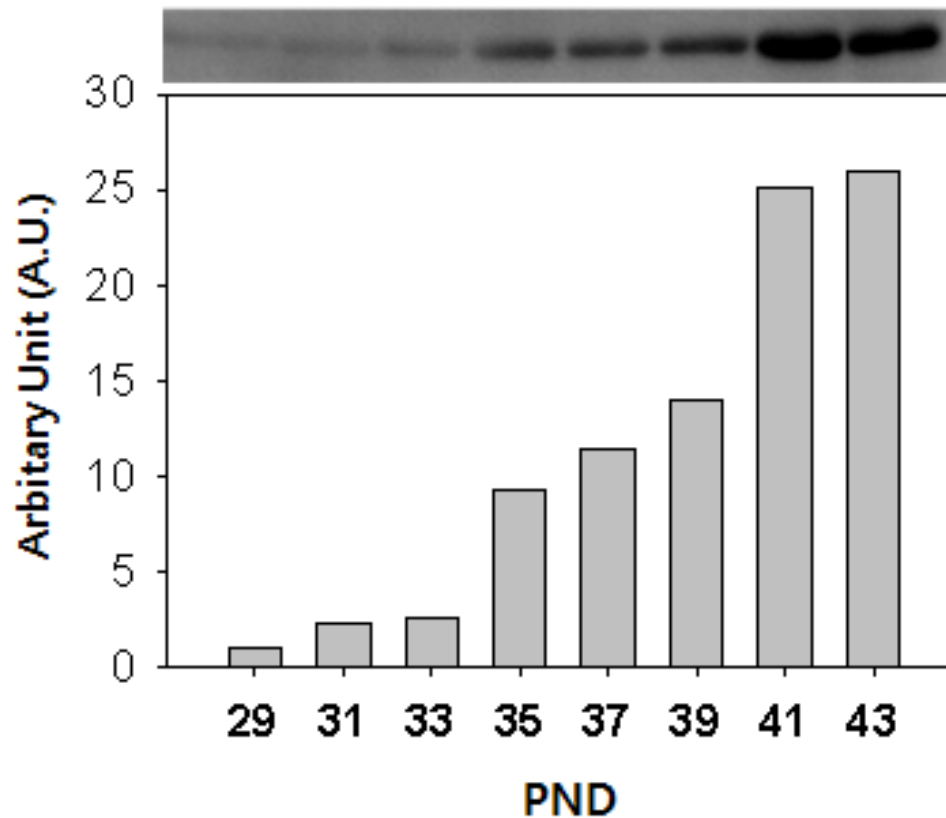


Figure 9. Expression of LH-R protein by Western blot in the ovaries.

Bars indicate the mean value.

Vinclozolin study

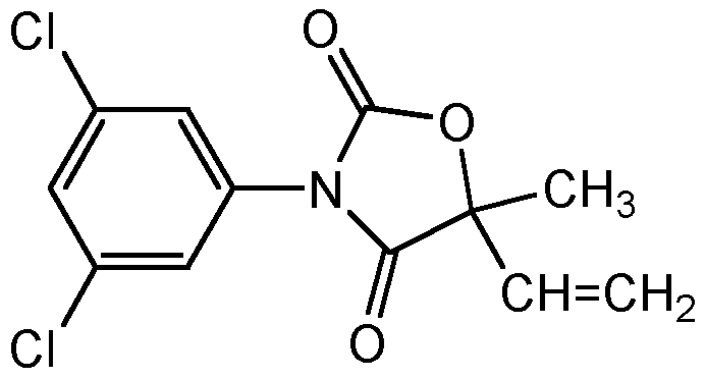
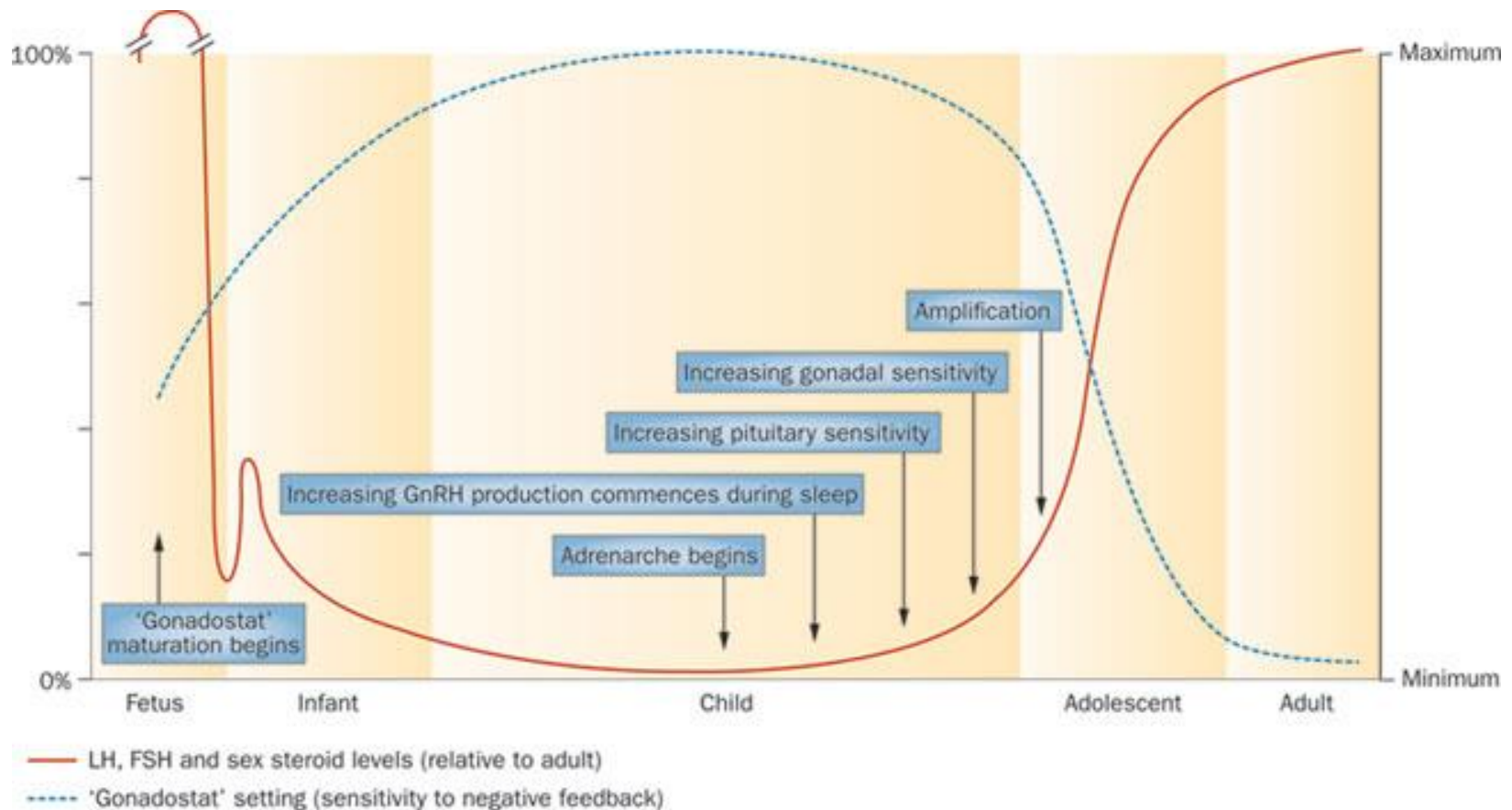


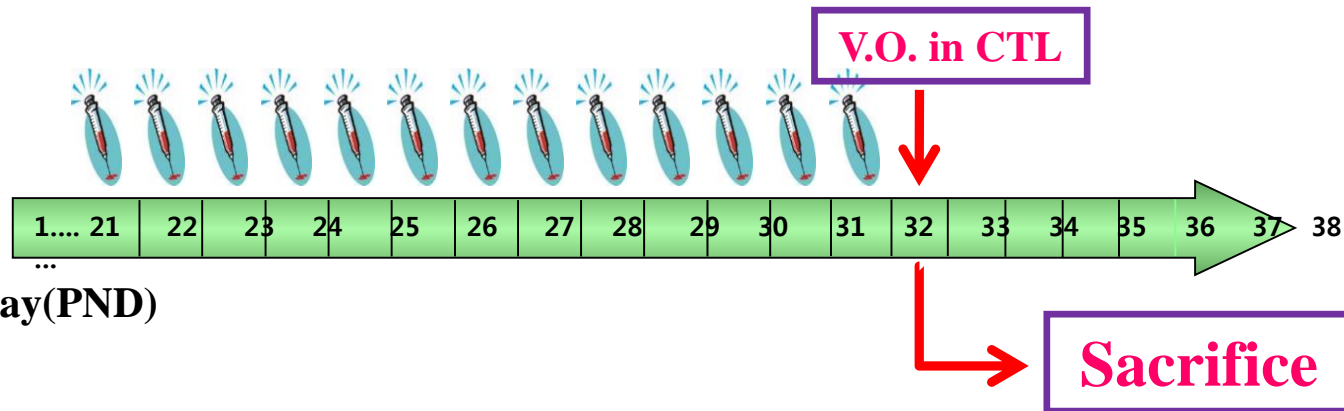
Figure 2 Change in the levels of serum gonadotropins and sex hormones from fetal life to adulthood in relationship to the sensitivity of the central nervous system 'gonadostat' to the negative feedback effect of sex hormones and underlying hormonal changes



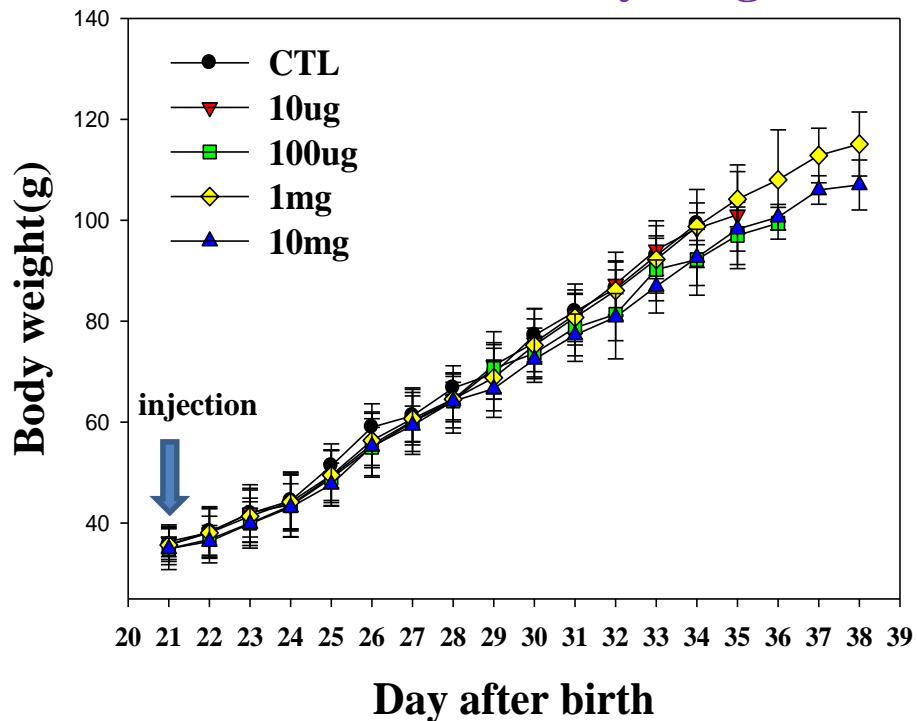
Wagner, I. V. *et al.* (2012) Effects of obesity on human sexual development
Nat. Rev. Endocrinol. doi:10.1038/nrendo.2011.241

Experimental design

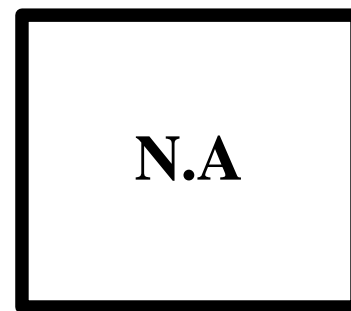
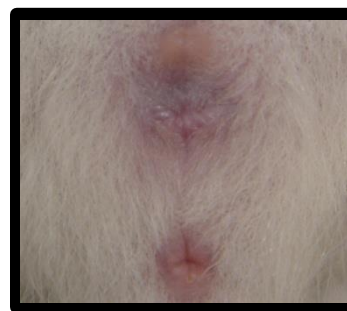
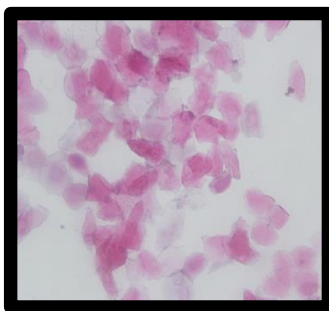
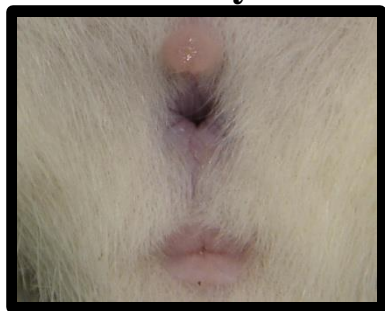
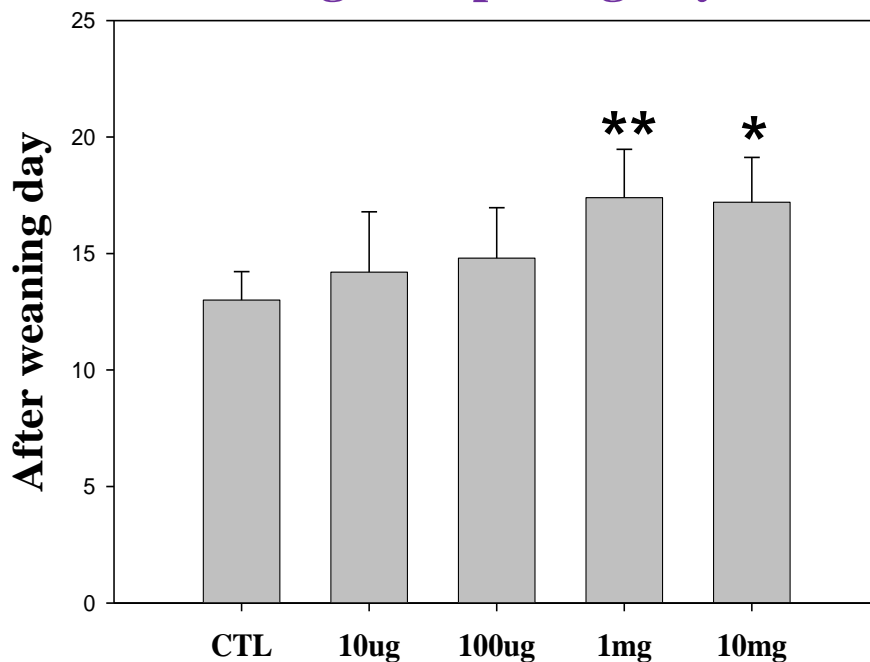
- ❖ Group 1 → Control(CTL)
- ❖ Group 2 → 10ug/kg/day VCZ i.p.
- ❖ Group 3 → 100ug/kg/day VCZ
- ❖ Group 4 → 1mg/kg/day VCZ
- ❖ Group 5 → 10mg/kg/day VCZ



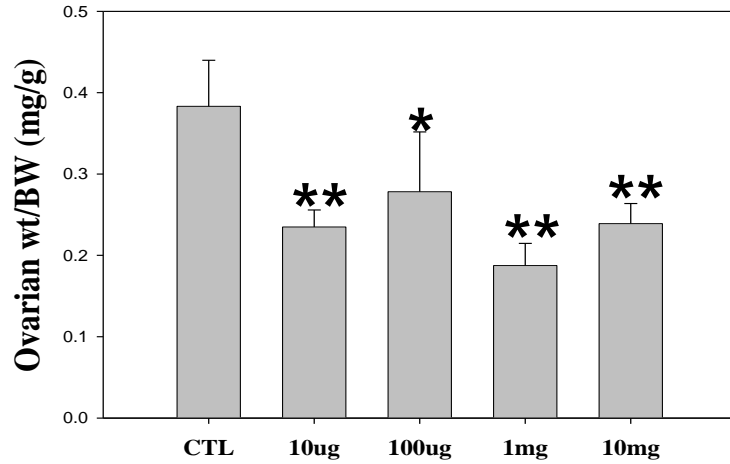
Increment of body weight



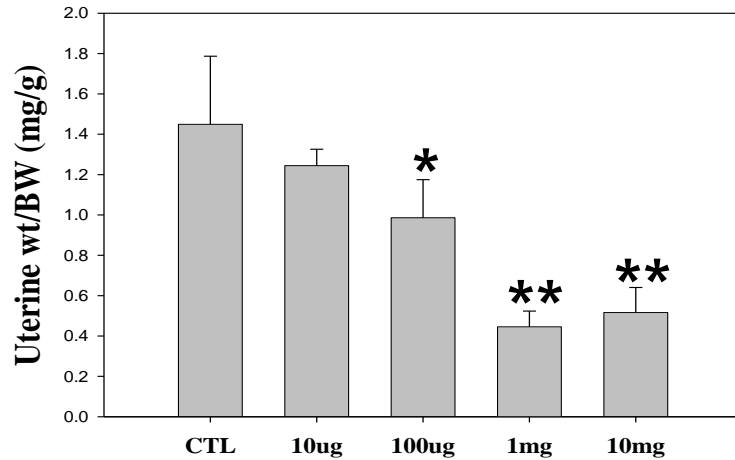
Vaginal opening day



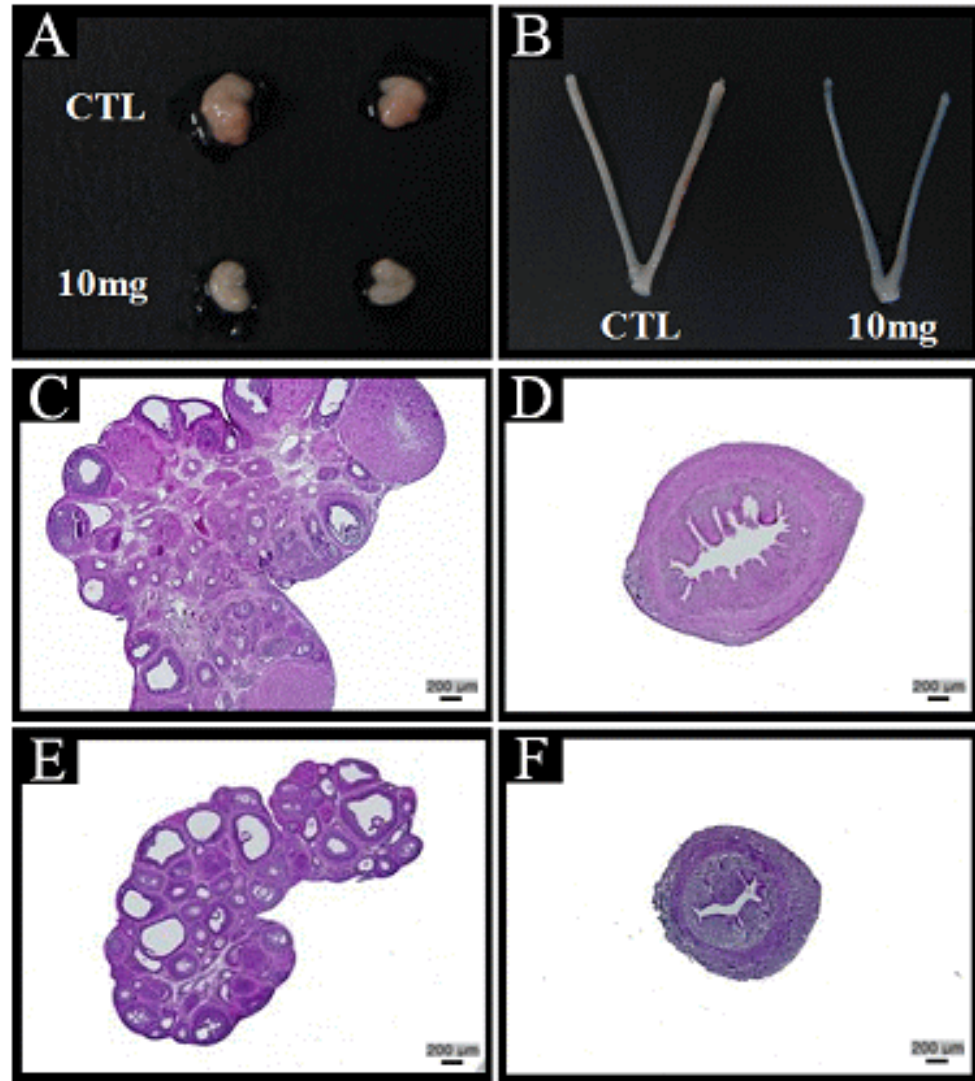
Comparison of ovarian weights



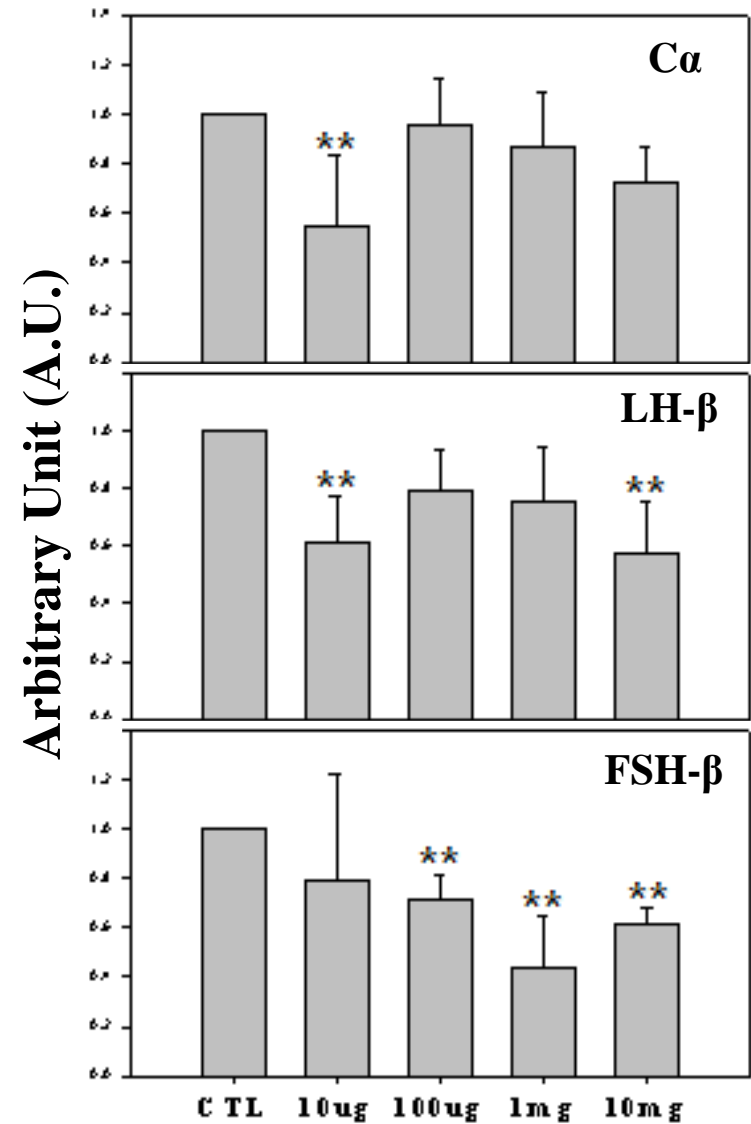
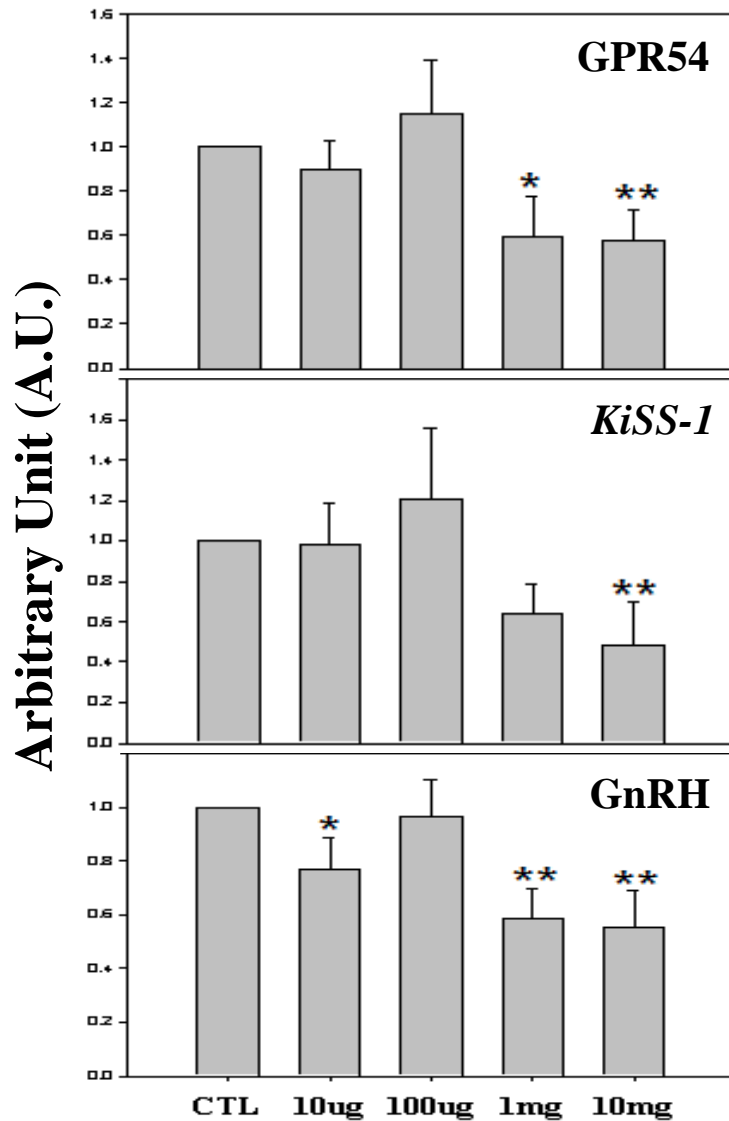
Comparison of uterine weights



Histological comparison of ovary and uterus

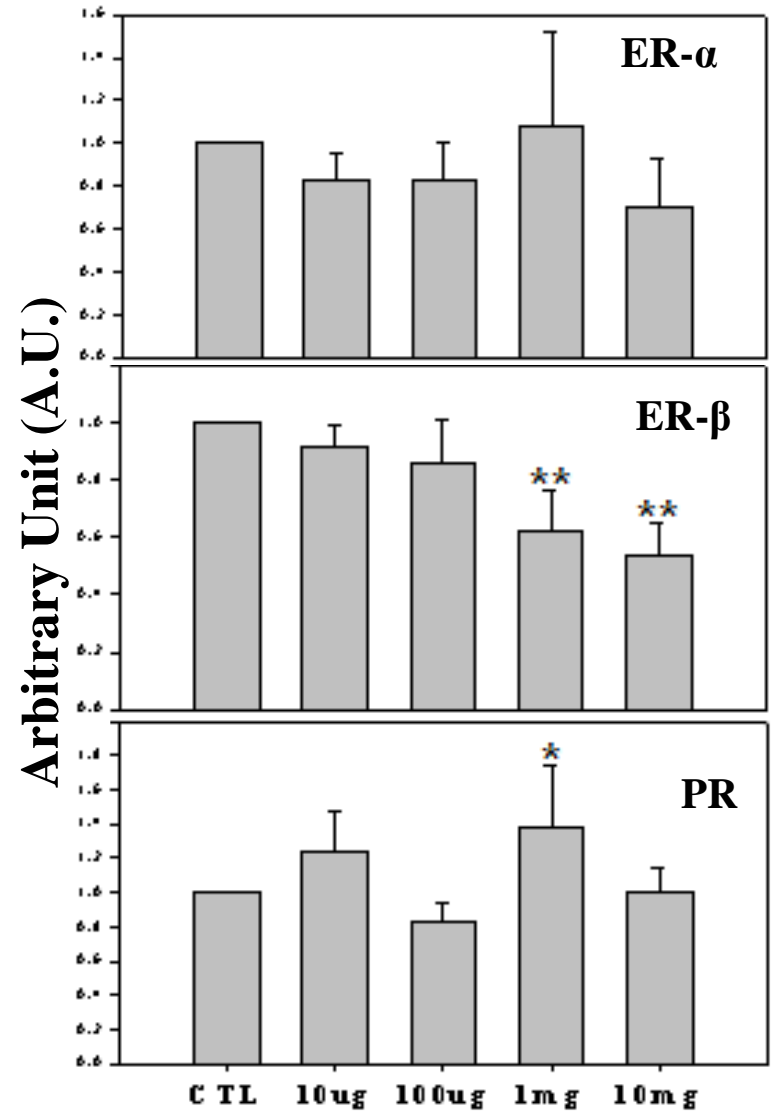
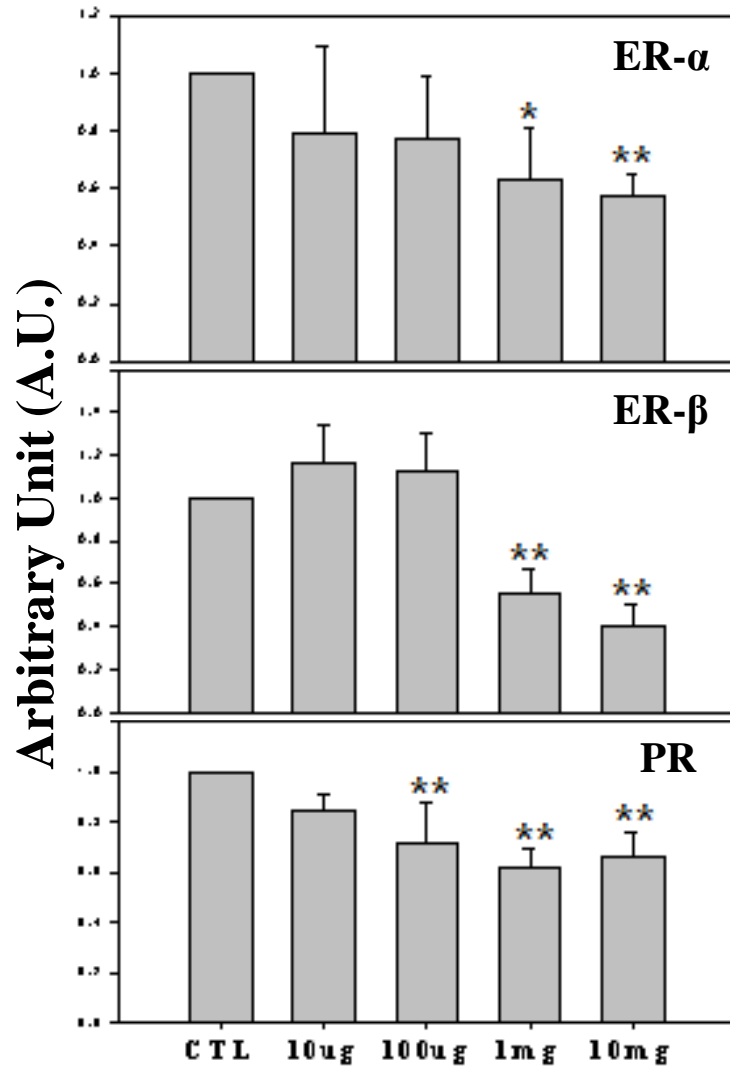


RT PCRs : hypothalamus-pituitary axis genes

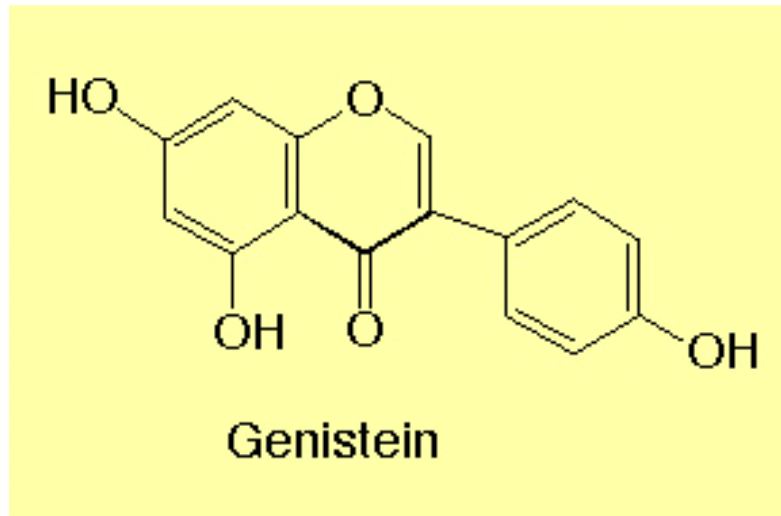


Non-monotonic curve !

RT PCRs : ovary & uterus



Genistein study



Model I



GS Oral Gavage : 10, 100, 500mg/kg/day
& Control (vehicle-sesame oil)

Birth

Weaning

VO open(GS 500mg)

control VO open

0d

21day

25day

34day

sacrifice

Model II



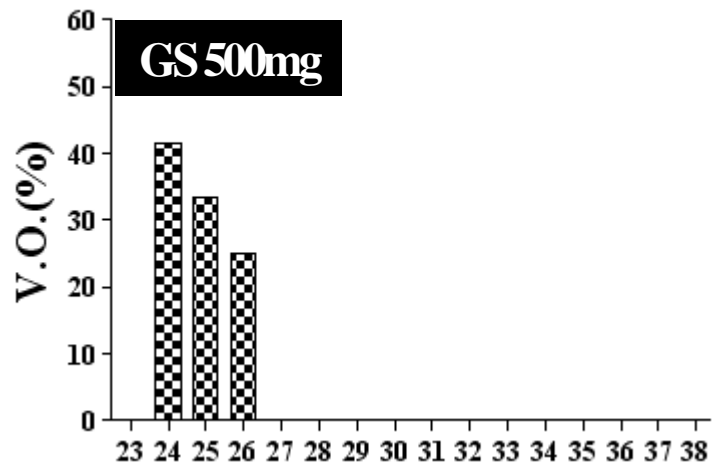
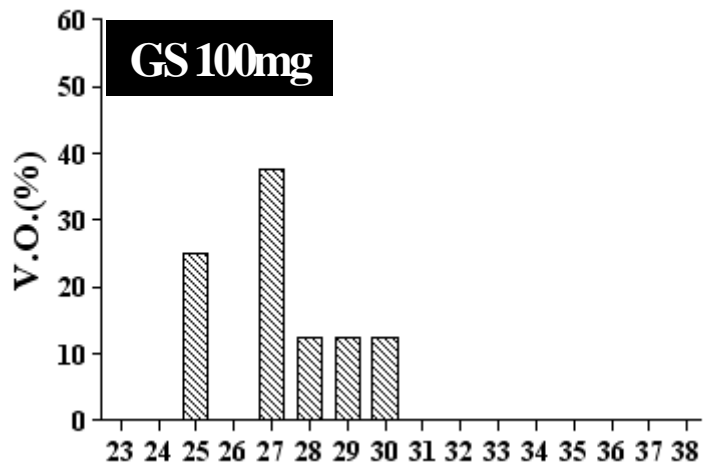
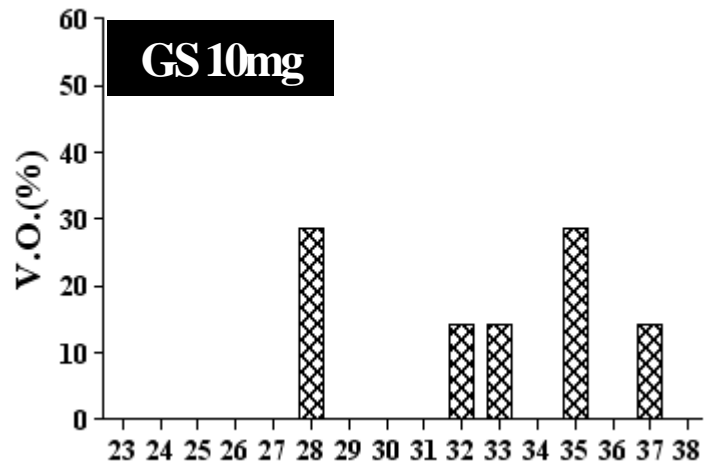
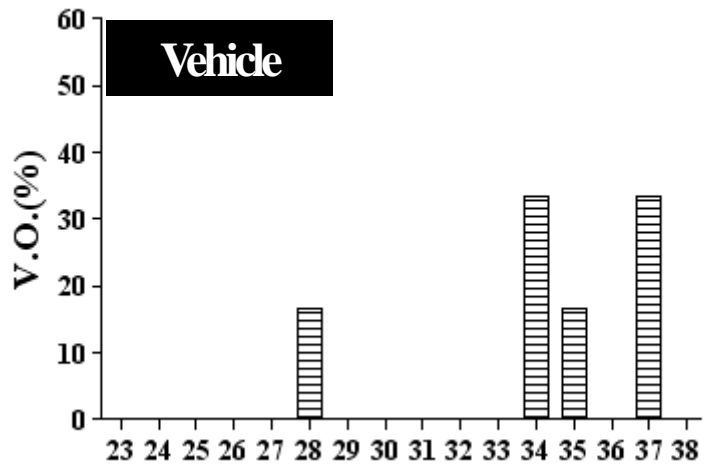
ICV injection

<12주령 암컷 흰쥐 사용>

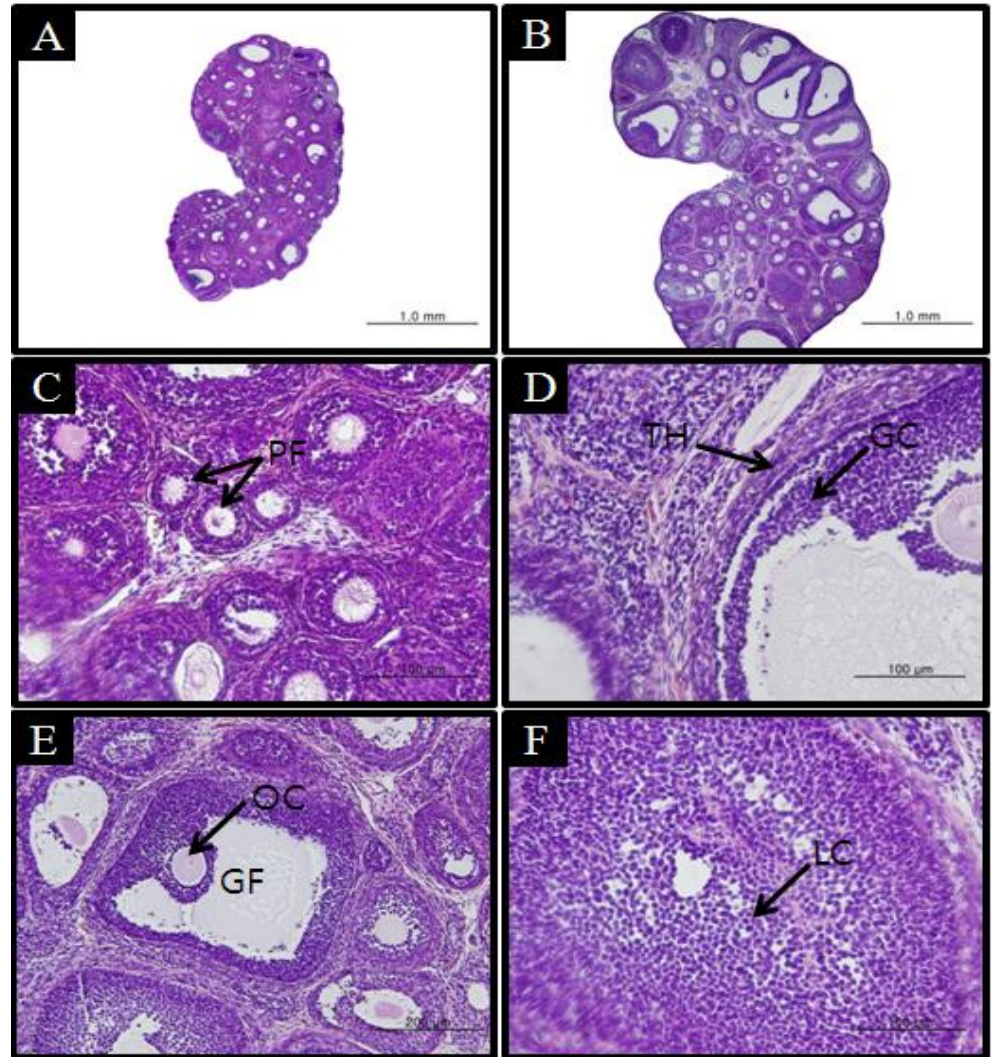
뇌실에 GS 3.4ug 투여

3시간 후, 희생시켜 시상하부와
뇌하수체 적출

total RNA 추출

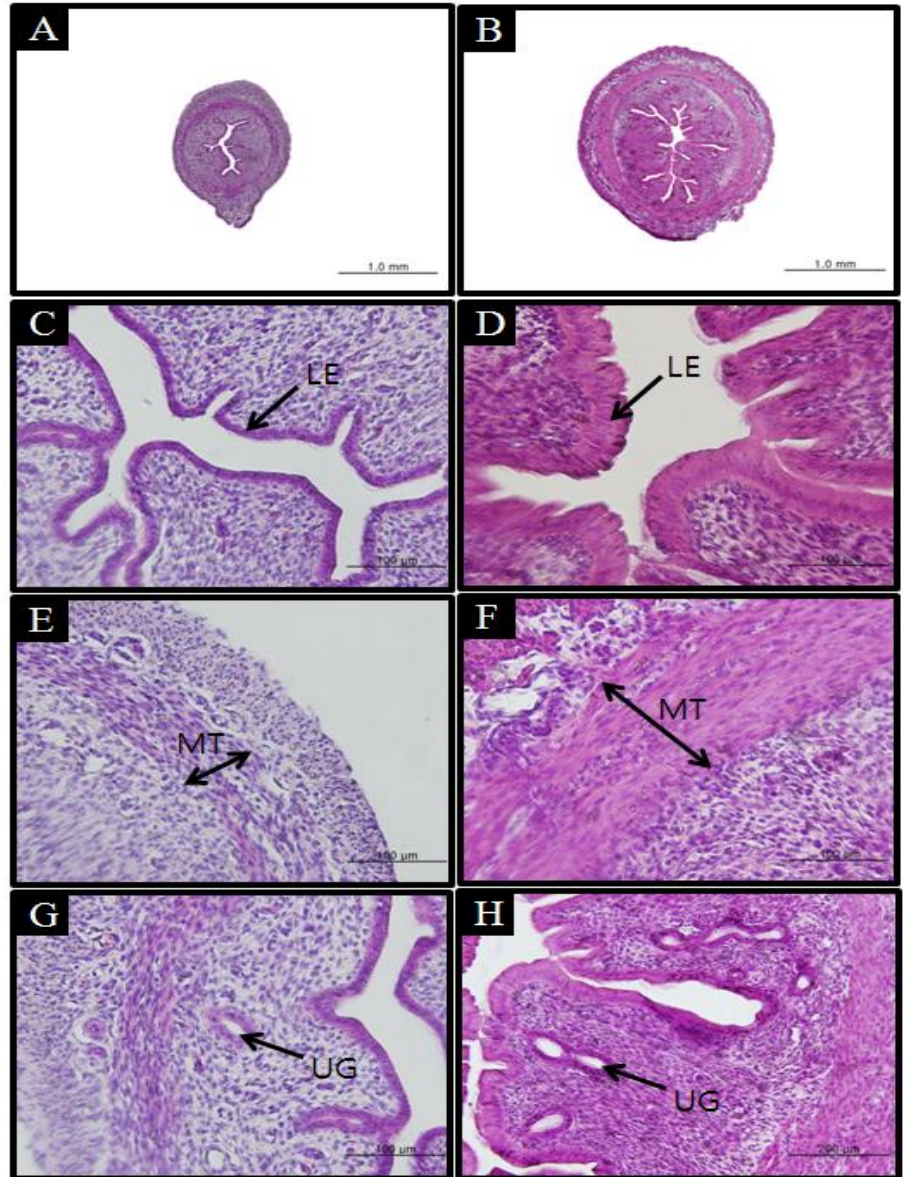


GS 투여군과 대조군의 난소의 조직 구조 비교



A and B, ovaries from treated with vehicle and 100mg GS (x40); C, primary and secondary follicles in control group (x400); D and E, Graafian follicles in 100mg GS group (D, x400; E, x200); F, corpus luteum in 100mg GS group; PF, primordial follicle; TH, theca cell; GC, granulosa cell; OC, oocyte; GF, graafian follicle; LC, lutein cell.

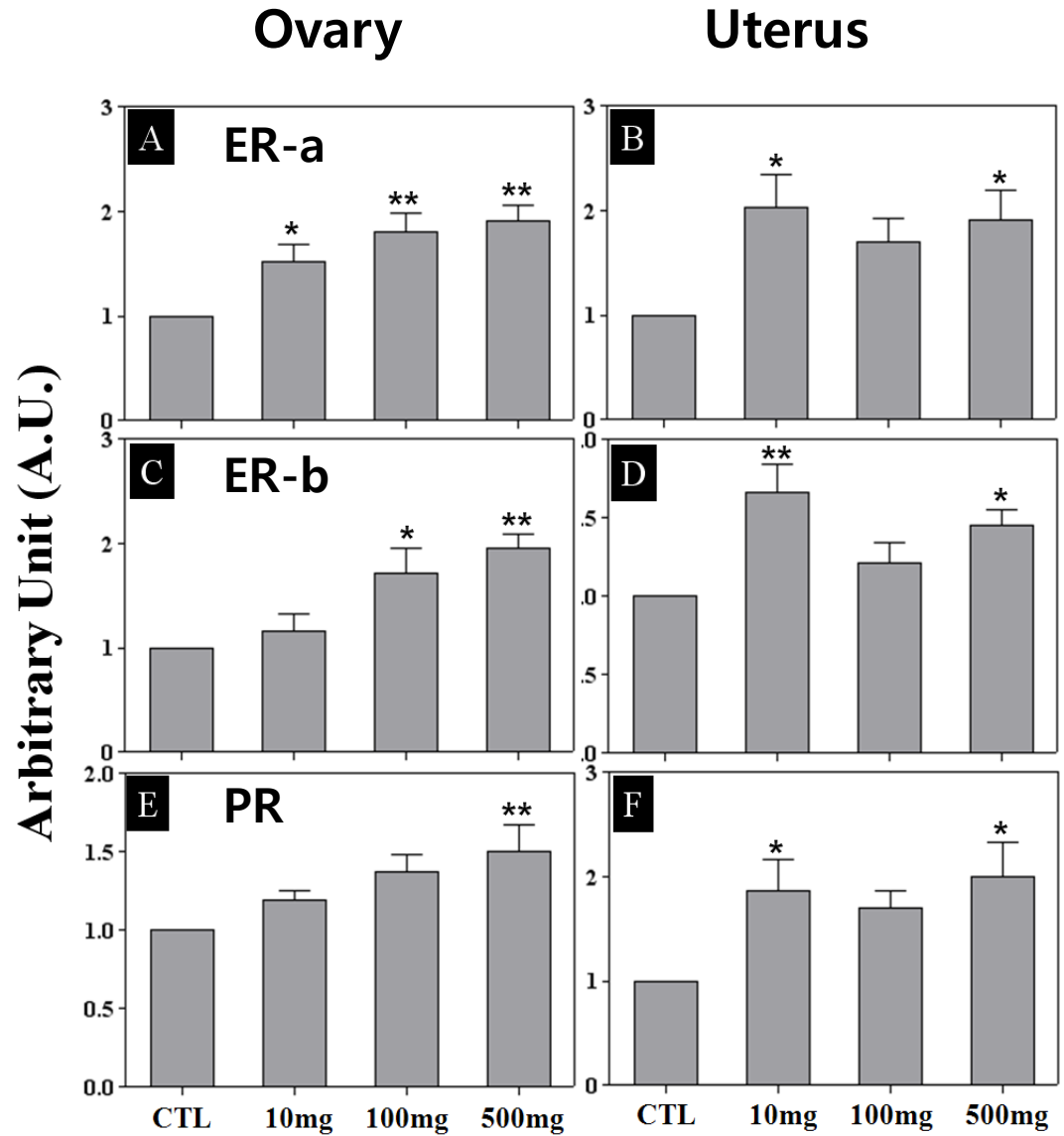
GS 투여군과 대조군의 자궁의 조직 구조 비교



A and B, uteri from treated with vehicle and 100mg GS (x40); C and D, uterine lumen in control and 100mg GS group; E and F, myometrium layer in control and 100mg GS group; G and H, uterine glands in control and 100mg GS group. LE, luminal epithelium; MT, myometrium; UG, uterine gland.

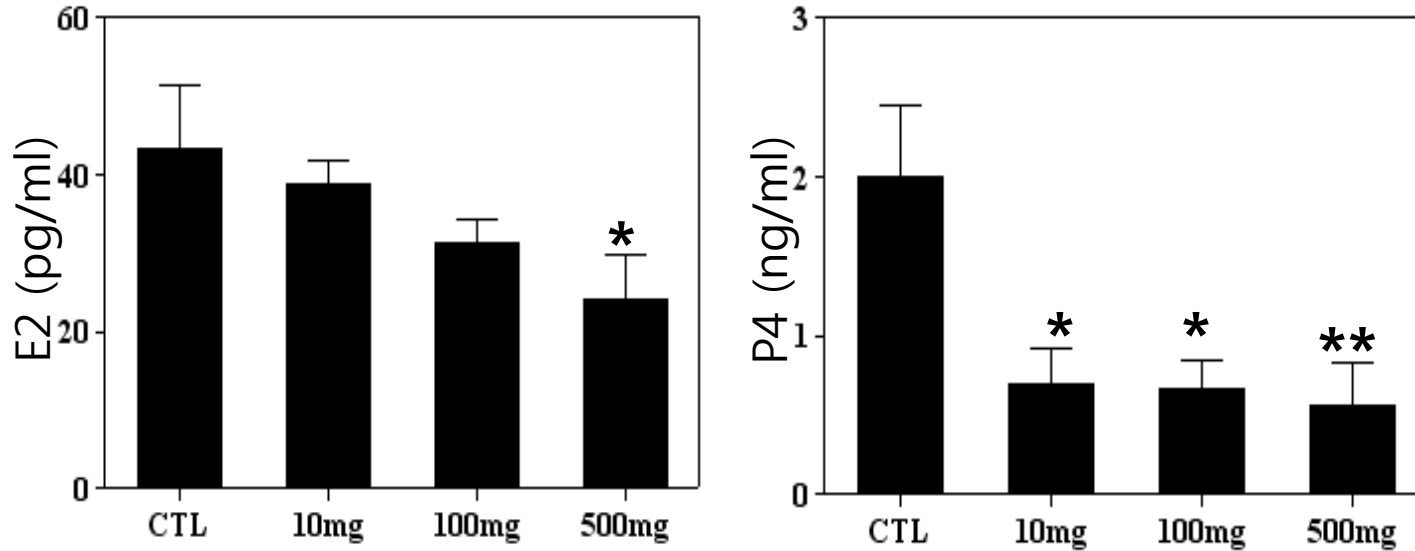
RT PCRs

난소와 자궁의
스테로이드 호르몬
수용체 발현 양상



A and B, The relative ratio of ER- α transcript levels in ovary and uterus of each groups, respectively;
C and D, The relative ratio of ER- β transcript levels in ovary and uterus of each groups, respectively;
E and F, The relative ratio of PR transcript levels in ovary and uterus of each groups, respectively.

혈중 에스트로겐과 프로게스테론 농도



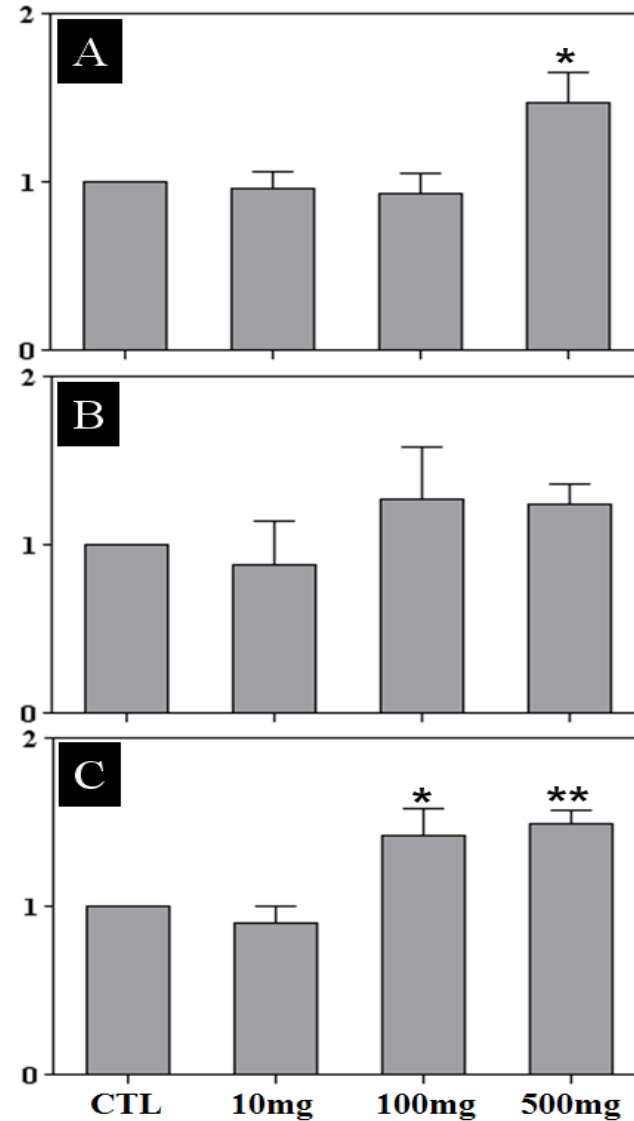
A, The E2 levels in serum of each groups, respectively; B, The P4 levels in serum of each groups, respectively.

시상하부

KISS-1, GPR54, GnRH
mRNA 변화 양상

A, The relative ratio of *Kiss-1* transcript levels in hypothalamus of each groups, respectively;
B, The relative ratio of *GPR54* transcript levels in hypothalamus of each groups, respectively;
C, The relative ratio of *GnRH* transcript levels in hypothalamus of each groups, respectively.

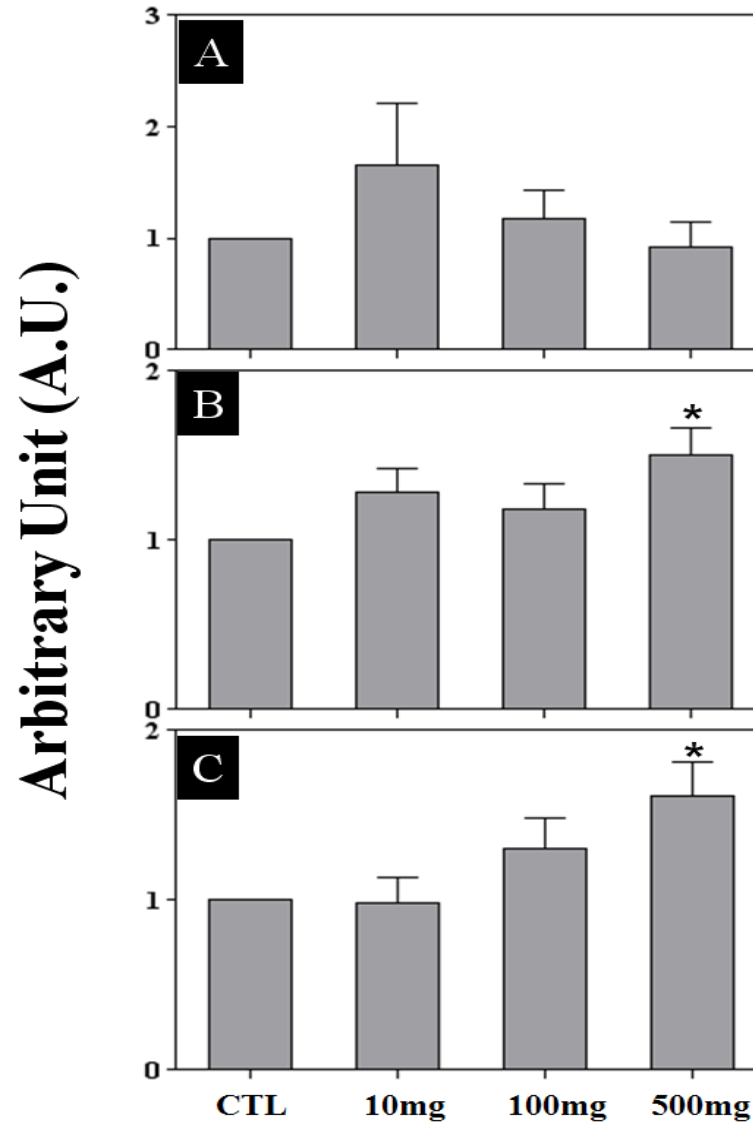
Arbitrary Unit (A.U.)



뇌하수체

Ca, LH- β , FSH- β
mRNA 변화 양상

A, The relative ratio of Ca transcript levels in pituitary of each groups, respectively;
B, The relative ratio of LH- β transcript levels in pituitary of each groups, respectively;
C, The relative ratio of FSH- β transcript levels in pituitary of each groups, respectively.

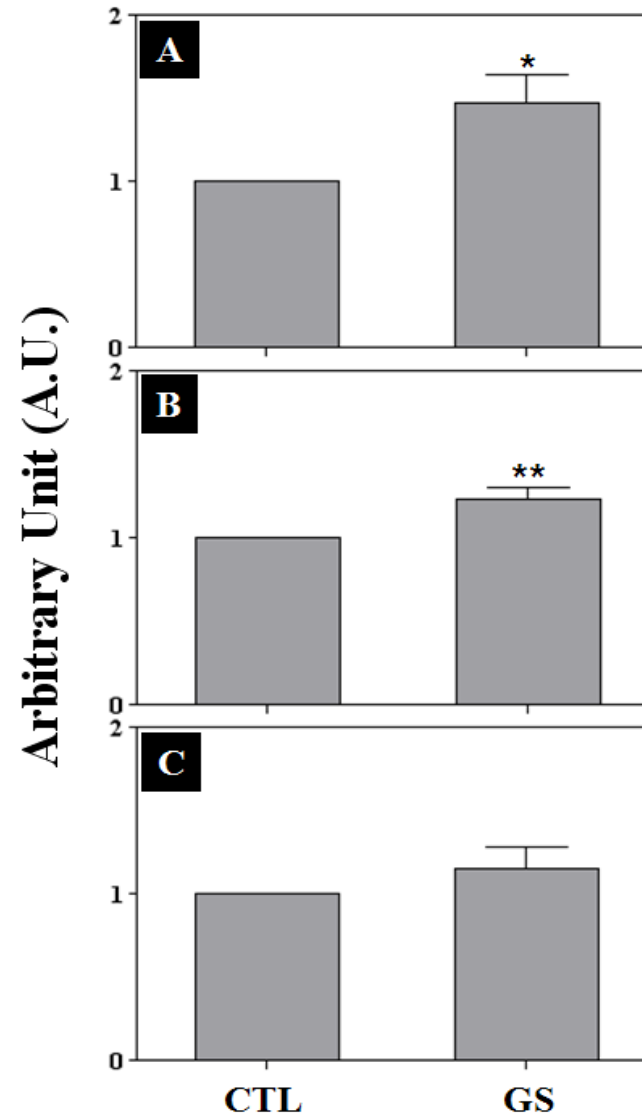


<icv injection>

시상하부

EAP-1, GAD67, NOS-2

mRNA 변화 양상



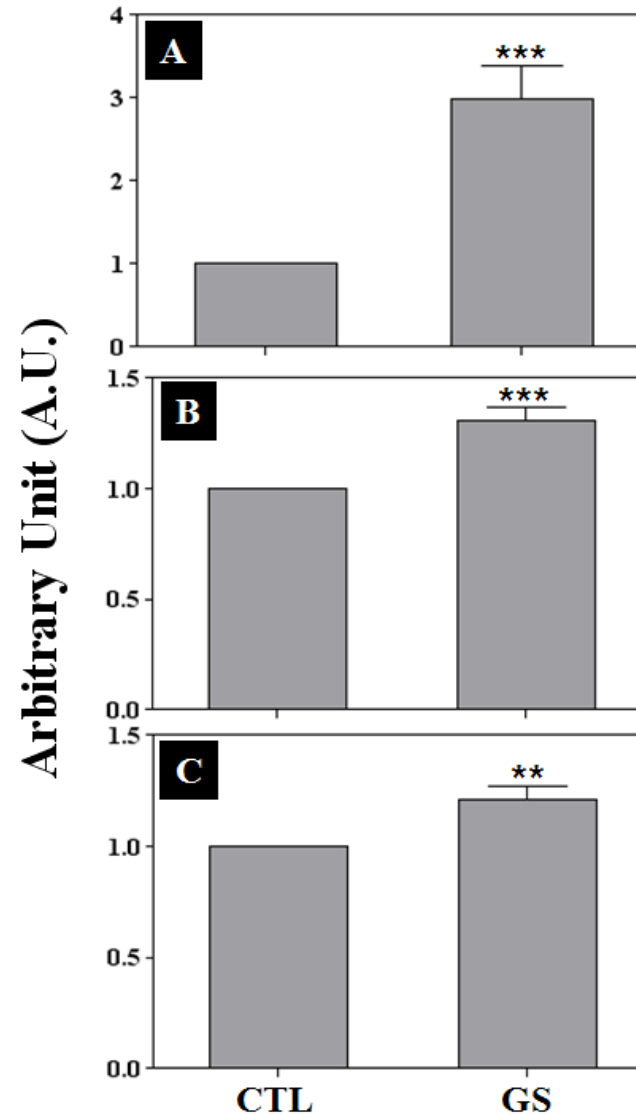
A, The relative ratio of EAP-1 transcript levels in hypothalamus of each groups, respectively;
B, The relative ratio of GAD67 transcript levels in hypothalamus of each groups, respectively;
C, The relative ratio of NOS-2 transcript levels in hypothalamus of each groups, respectively.

<icv injection>

시상하부

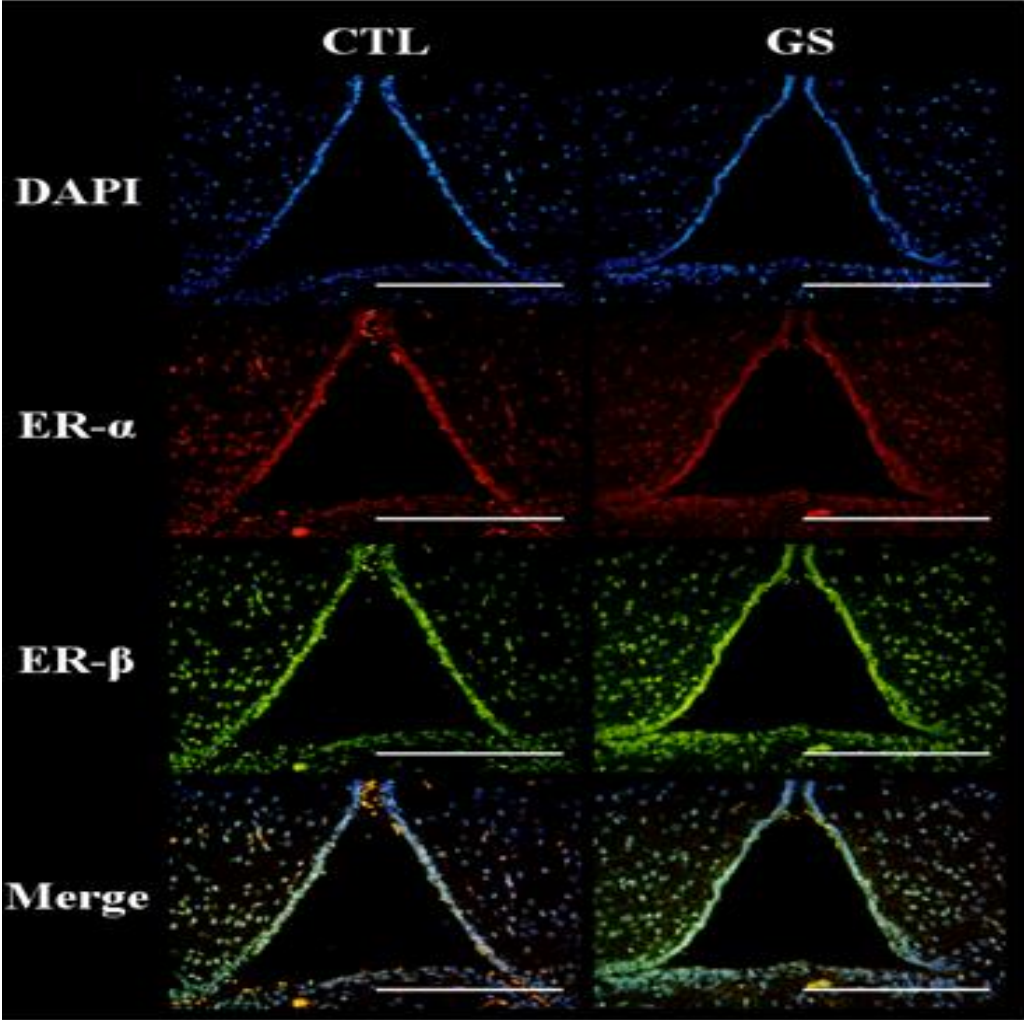
KiSS-1, GPR54, GnRH

mRNA 변화 양상



A, The relative ratio of *KiSS-1* transcript levels in hypothalamus of each groups, respectively;
B, The relative ratio of *GPR54* transcript levels in hypothalamus of each groups, respectively;
C, The relative ratio of *GnRH* transcript levels in hypothalamus of each groups, respectively.

ER immunohistochemistries : hypothalamus



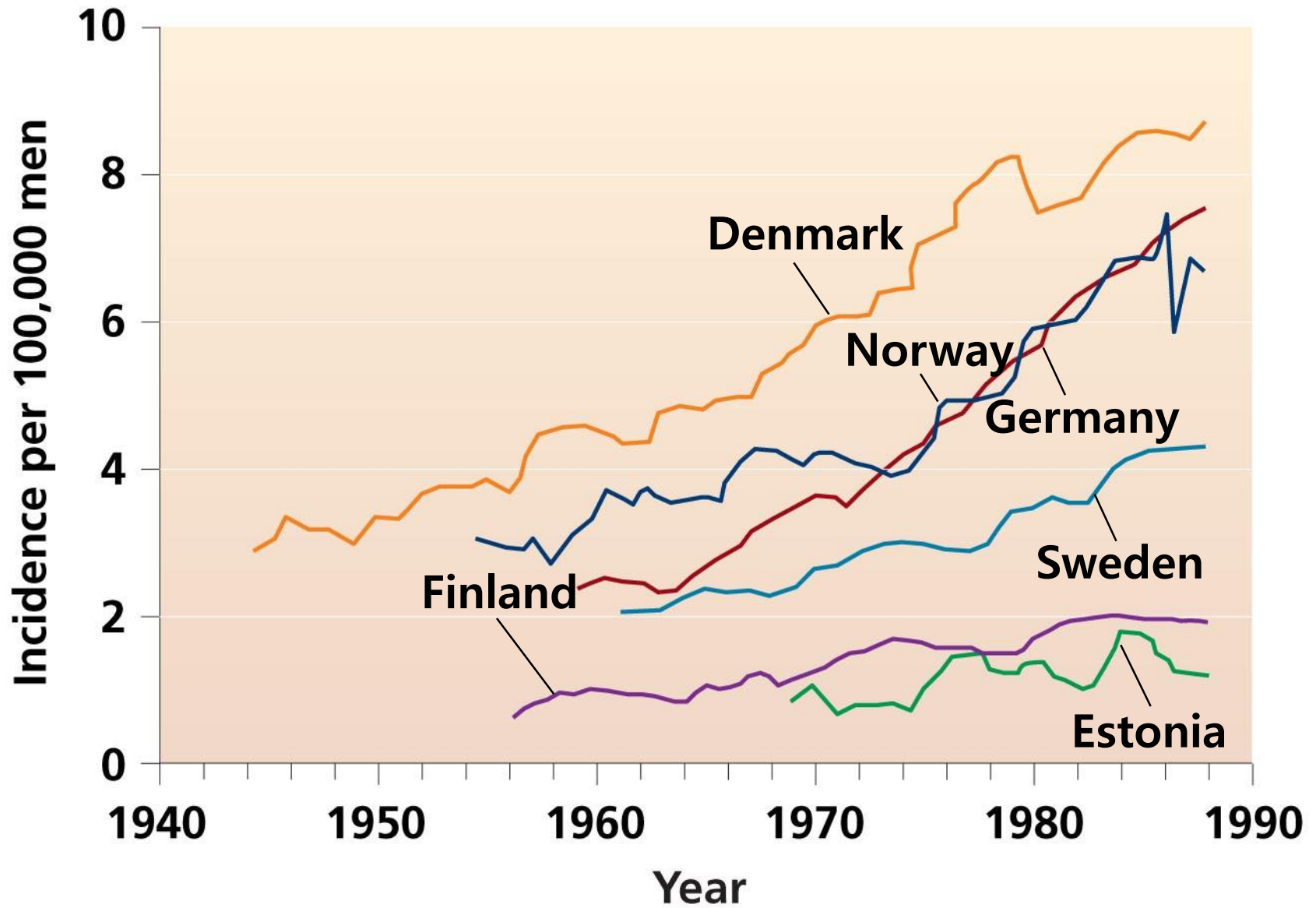
Summary of our previous researches

EDC	VO	Ovary	Uterus	GnRH	Gn	nature
Genistein	←	↑	↑	↑	↑	Pro-estrogenic
DEHP	→	↓	↓	↓	↓	Anti-androgenic
Vinclozolin	→	↓	↓	↓	↓	Anti-androgenic
Methoxychlor	←	↑	↑	↑	↑	Pro-estrogenic
Nonylphenol	←	↑	↑	↑	↑	Pro-estrogenic

← Advanced; → Delayed; ↑ Increased; ↓ Decreased.

Clinical implications





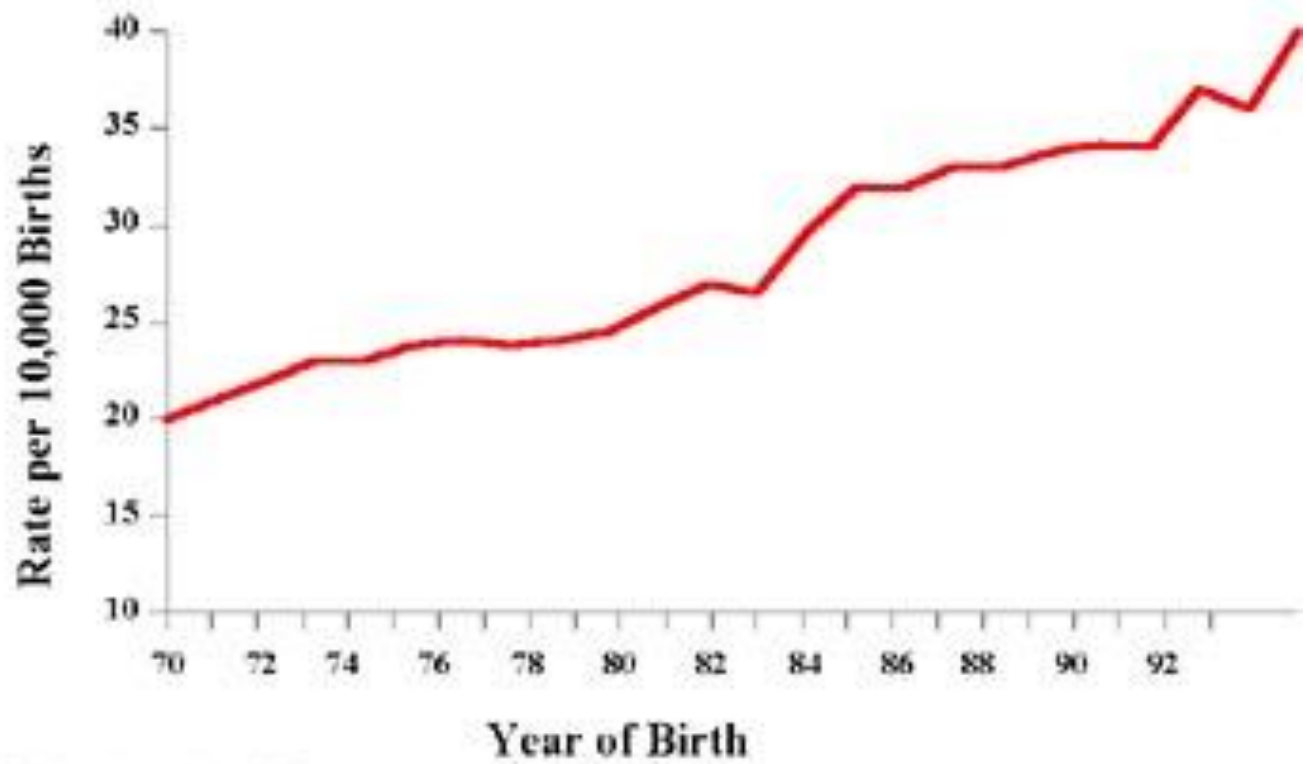
(b) Increasing incidence of testicular cancer

Penoscrotal type hypospadias
www.vghttp.e.gov.tw/~peds/lecture/pedsintr/73.jpg

Are Endocrine Disruptors Causal?



HYPOSPADIAS in the US



Paulozzi et al., 1997

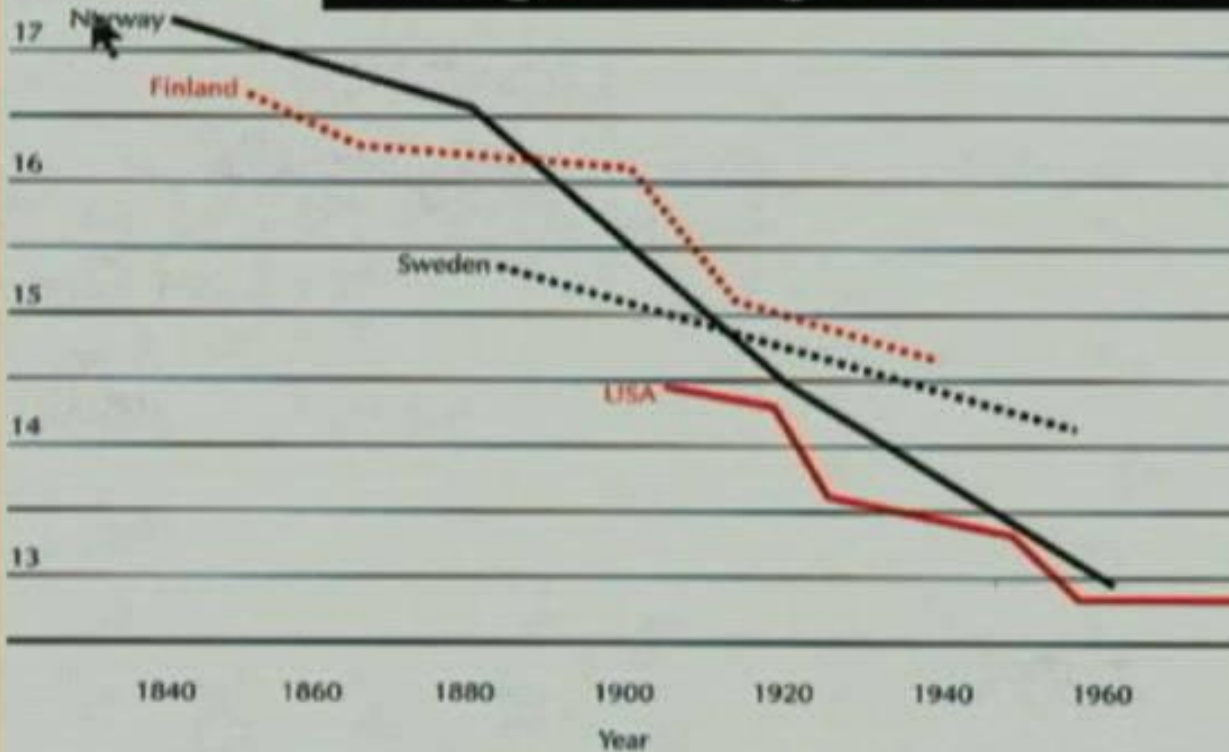
www.ourstolenfuture.org/Images/graphs/hypospadias.jpg

Precocious puberty

Delayed puberty

Age at menarche (years)

Changes in Age of Menarche



Speroff, 5th ed, p. 369



A sample growth chart of a girl with **central precocious puberty**

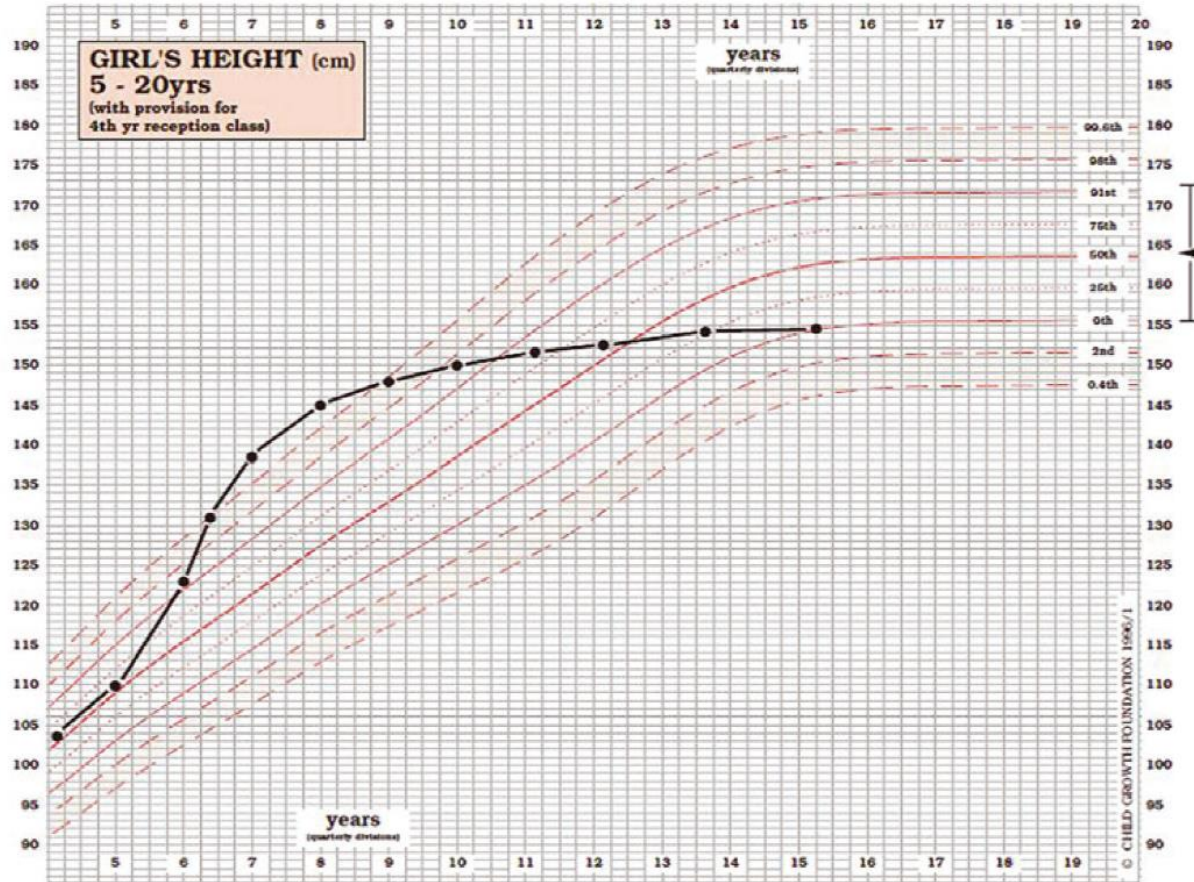


Figure 2. A sample growth chart of a girl with central precocious puberty, demonstrating a premature growth spurt resulting in early cessation of linear growth and final short stature. This figure was reproduced with permission from the Child Growth Foundation.

short stature & quality of life

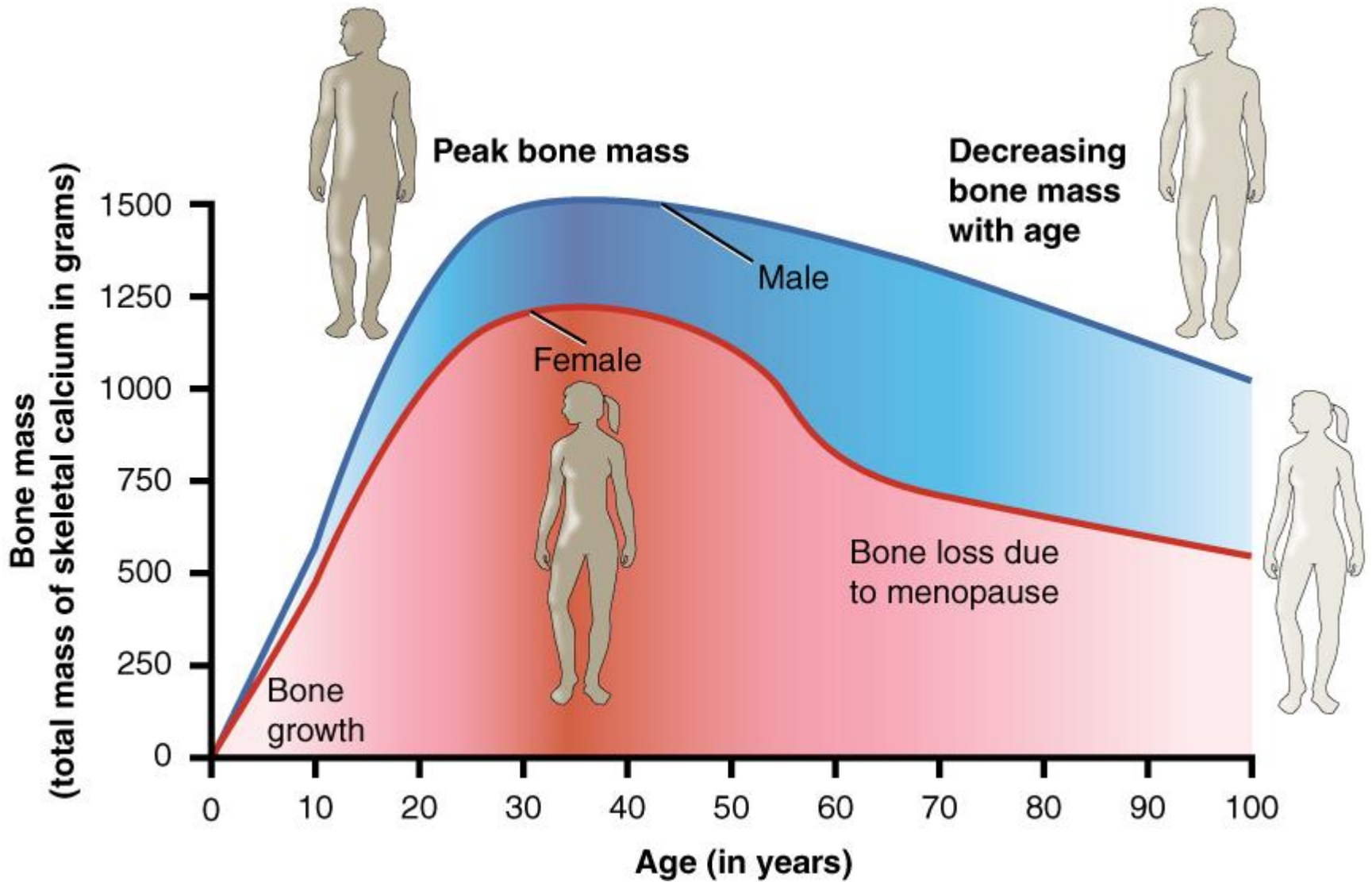


[Redacted]

또래보다
키가 작은 자녀를 보며
속상해 하실 부모님들께

[Redacted] **한의원**의 성장보약,
키 크는 한약을 소개해 드립니다.

Osteoporosis



Becoming a mother at 11 (?)





Got a good job?
I cost thousands of dollars each year.

THINK BEING A TEEN PARENT WON'T COST YOU? EXPECT TO SPEND MORE THAN \$10,000 A YEAR TO RAISE A CHILD.

Text "NOTNOW" to 877877 for the real price of teen pregnancy.
Responsible pregnancy like the adult. That's the only way to go.

NYC Department of Health & Mental Hygiene




Dad, you'll be paying to support me for the next 20 years.

THINK BEING A TEEN PARENT WON'T COST YOU? NY STATE LAW REQUIRES A PARENT TO PAY CHILD SUPPORT UNTIL A CHILD IS 21.

Text "NOTNOW" to 877877 for the real price of teen pregnancy.
Responsible pregnancy like the adult. That's the only way to go.

NYC Department of Health & Mental Hygiene




Honestly Mom... chances are he won't stay with you. What happens to me?

ARE YOU READY TO RAISE A CHILD BY YOURSELF? 90% OF TEEN PARENTS DON'T MARRY EACH OTHER.

Text "NOTNOW" to 877877 for the real price of teen pregnancy.
Responsible pregnancy like the adult. That's the only way to go.

NYC Department of Health & Mental Hygiene




I'm twice as likely not to graduate high school because you had me as a teen.

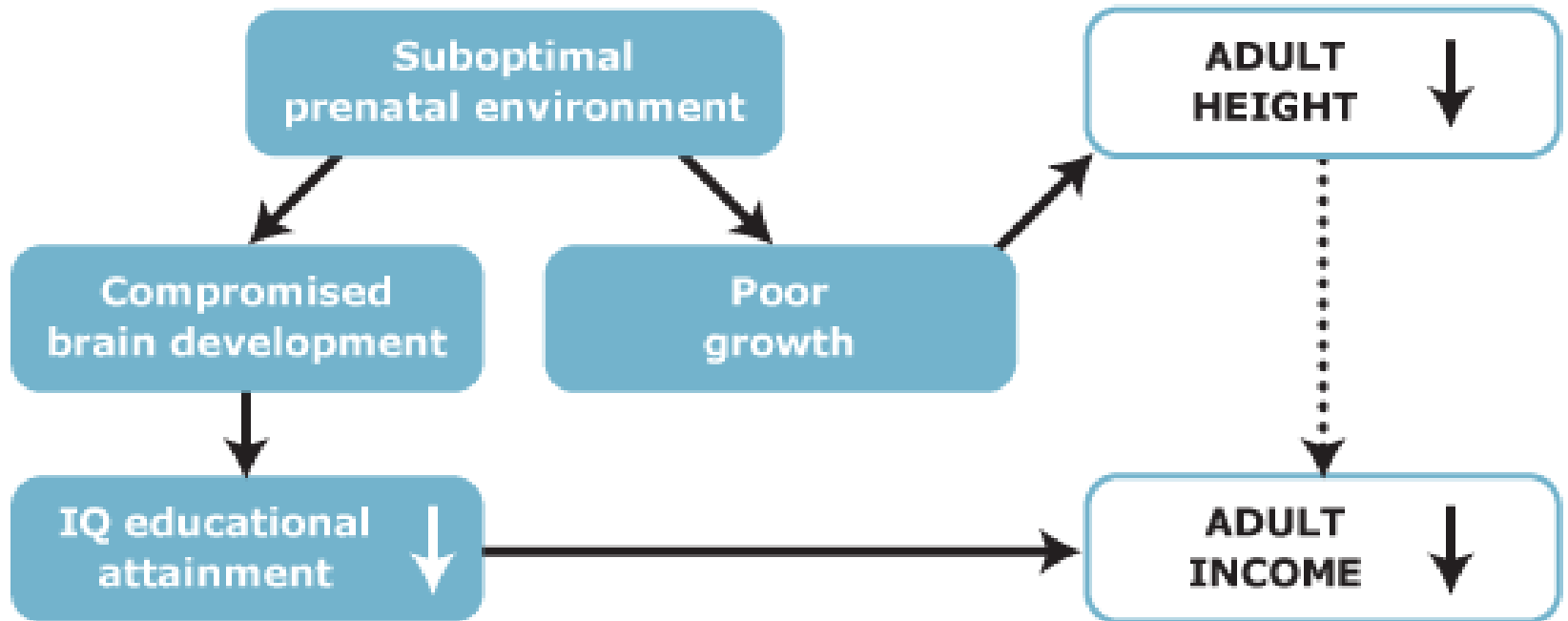
KIDS OF TEEN MOMS ARE TWICE AS LIKELY NOT TO GRADUATE THAN KIDS WHOSE MOMS WERE OVER AGE 22.

Text "NOTNOW" to 877877 for the real price of teen pregnancy.
Responsible pregnancy like the adult. That's the only way to go.

NYC Department of Health & Mental Hygiene



Origin of huge social problems ...





4-year-olds



5-year-olds



Drawings by children in the foothills



4-year-olds



5-year-olds



Drawings by children in the valley



State of the Science of Endocrine Disrupting Chemicals - 2012

Edited by
Åke Bergman, Jerrold J. Heindel, Susan Jobling,
Karen A. Kidd and R. Thomas Zoeller



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among FAO, ILO, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

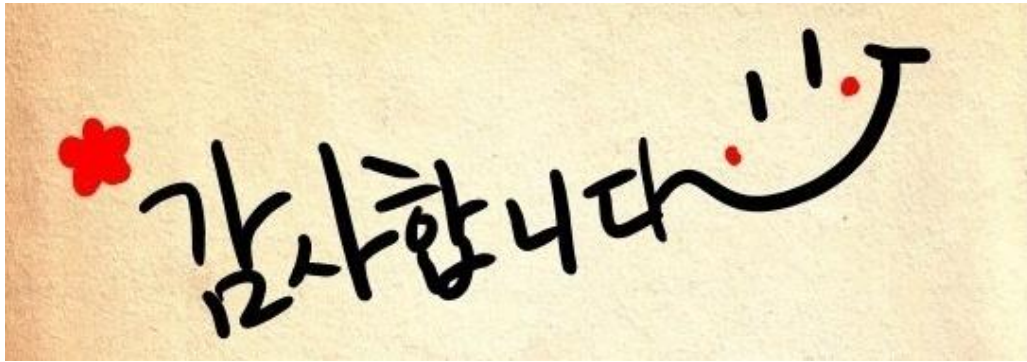


INTRODUCTION TO ENDOCRINE DISRUPTING CHEMICALS (EDCs) A GUIDE FOR PUBLIC INTEREST ORGANIZATIONS AND POLICY-MAKERS



Andrea C. Gore, PhD
David Corman, PhD
Janette L. Boon, PhD
Nicole La Merrill, PhD, MPH
Reather Pothouard, PhD
Jim Zito, ScD, MS

December 2014



서울대학교 김경진 교수
전남대학교 (차병원) 안련섭 교수
경희대학교 조세형 교수
상명대학교
이경엽
전 윤
손혁준
이우철
안나경
이송이
전은영
곽병국
유다경
변혜림

