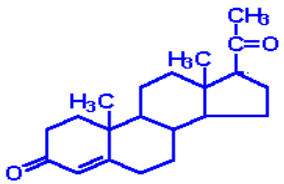
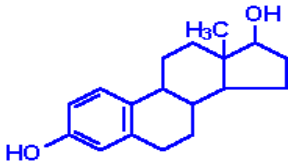
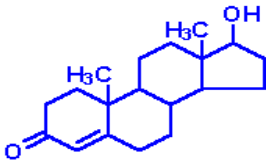
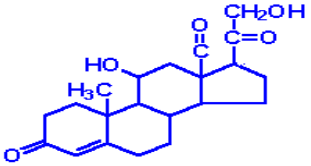
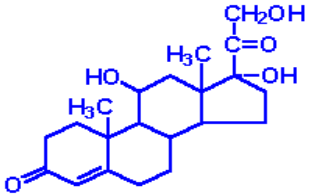
Cellular effect of endocrine disrupting chemicals by affecting estrogen receptor transcription signal pathway

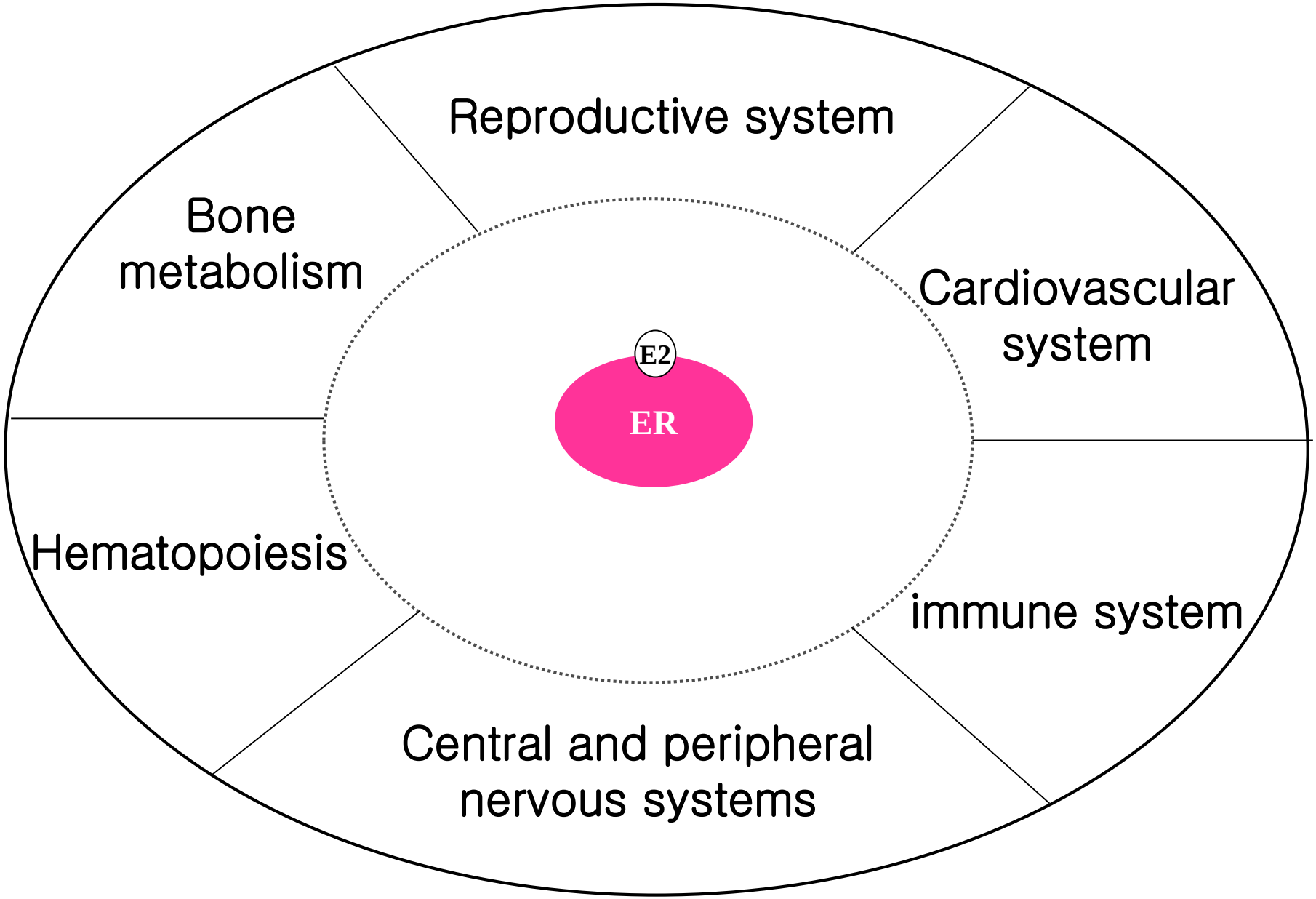
YoungJoo Lee

Sejong University, Seoul, Korea

Physiological roles of the major steroid hormones

	Progesterone: a progestin, produced directly from pregnenolone and secreted from the *corpus luteum*, responsible for changes associated with luteral phase of the menstrual cycle, differentiation factor for mammary glands, maintenance of pregnancy
	Estradiol: an estrogen, principal female sex hormone, produced in the ovary, responsible for secondary female sex characteristics
	Testosterone: an androgen, male sex hormone synthesized in the testes, responsible for secondary male sex characteristics, produced from progesterone
	Aldosterone: the principal mineralocorticoid, produced from progesterone in the *zona glomerulosa* of adrenal cortex, raises blood pressure and fluid volume, regulation of electrolyte balance
	Cortisol: dominant glucocorticoid in humans, synthesized from progesterone in the *zona fasciculata* of the adrenal cortex, involved in stress adaptation, elevates blood pressure and Na^+ uptake, numerous effects on the immune system, regulation of energy utilization

E2 plays an important role in various systems



Structural domains of the human ER

Transcriptional Activation — AF1, AF2

Nuclear Localization

Dimerization

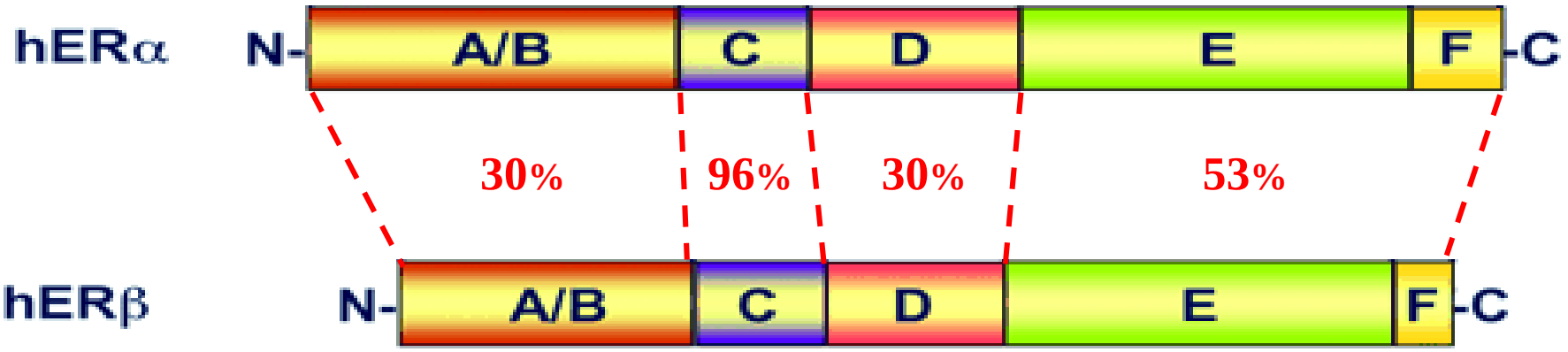
DNA-Binding

Co-Activator Binding

Co-Repressor Binding

Klinge CM. (2000) Steroids 65:227.

The cellular response to estrogen is mediated by two estrogen receptor isoforms, ERα and ERβ



A series of reports strongly indicated that estrogens,

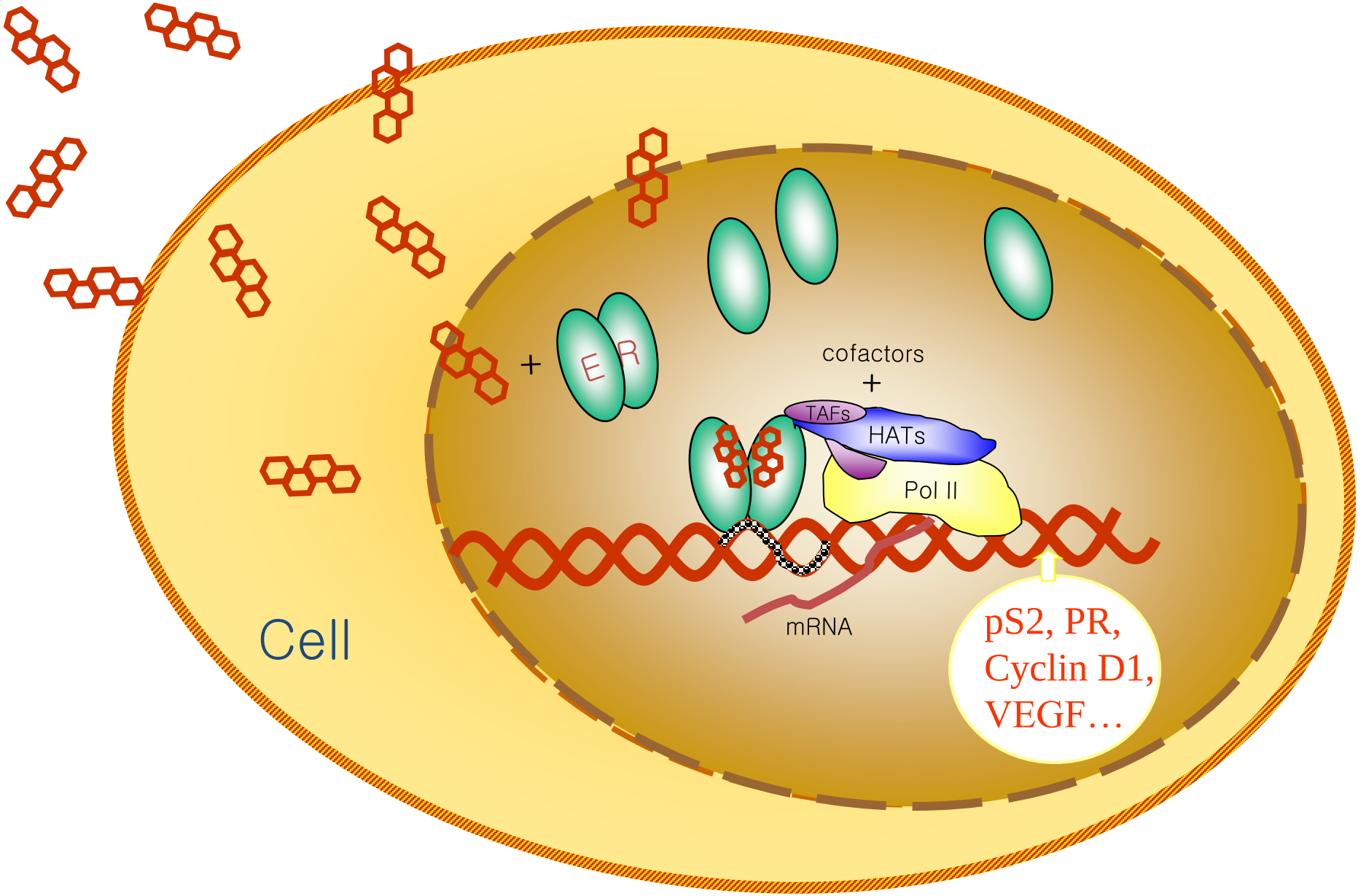
via ERα, stimulate proliferation and inhibit apoptosis,

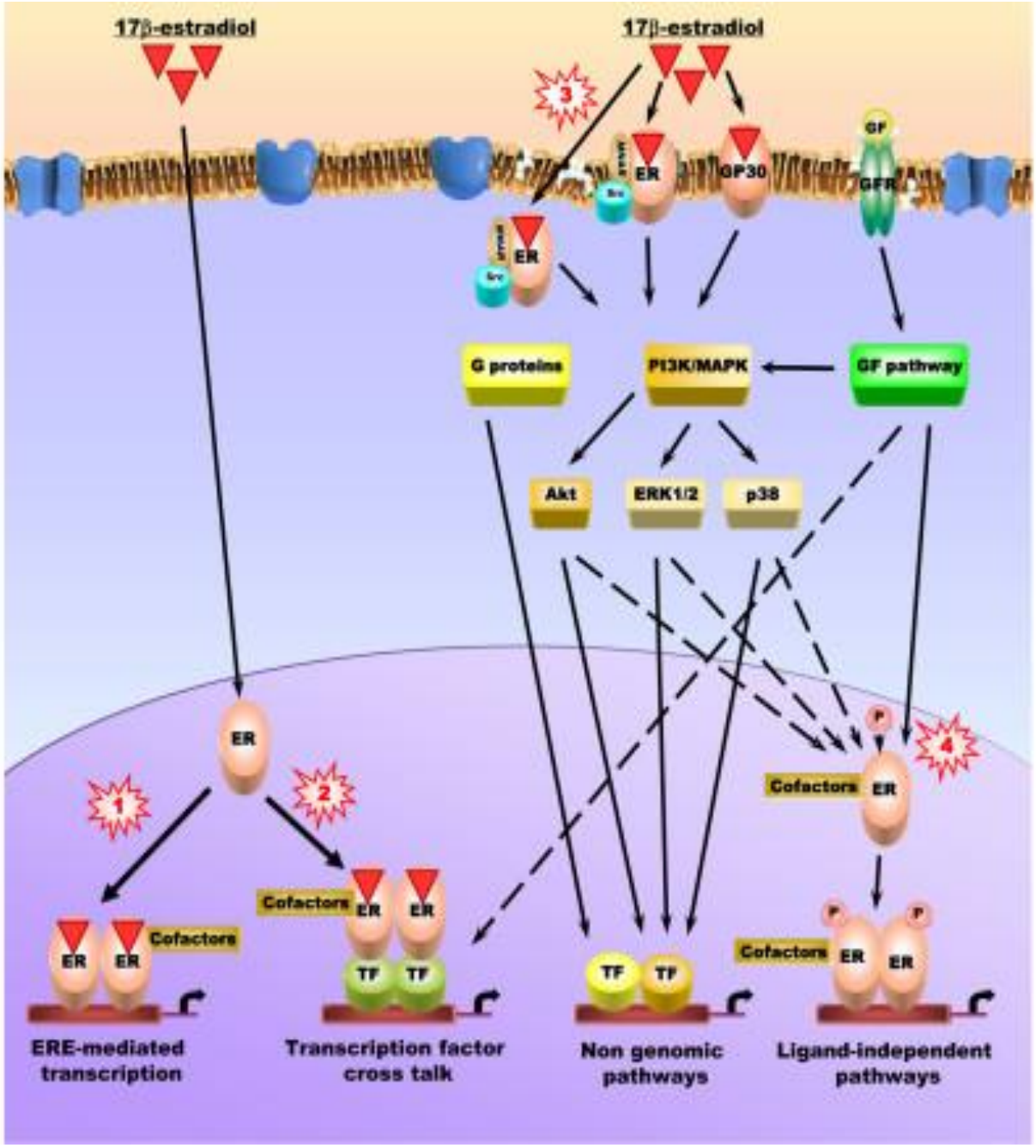
whereas ERβ opposes the proliferative effect of ERα.

Expression of ERβ significantly reduced cancer cell proliferation and tumor growth.

It was suggested that ERbeta is a tumor suppressor and the loss of ERβ expression may be one of the events leading to cancer development.

Action of estrogen in cell




17β-estradiol
17β-estradiol
3
ER
GP30
GF
GFR
ER
G proteins
PI3K/MAPK
GF pathway
Akt
ERK1/2
p38
ER
1
2
4
Cofactors
ER
ER
ER
Cofactors
Cofactors
ER
ER
TF
TF
TF
TF
Cofactors
ER
ER
ERE-mediated transcription
Transcription factor cross talk
Non genomic pathways
Ligand-independent pathways

Distribution of ER alpha and beta

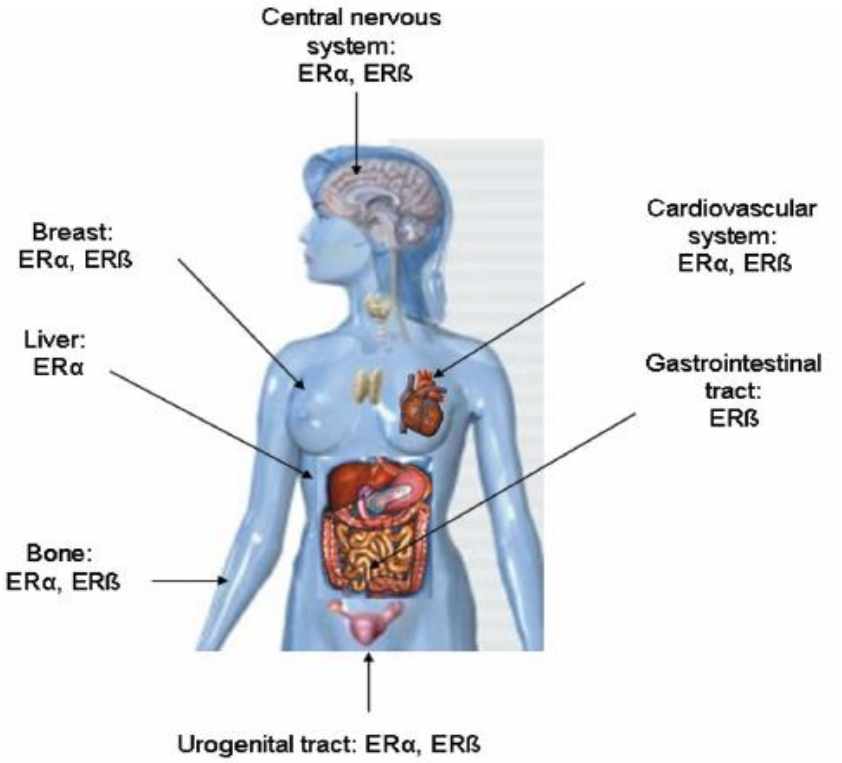


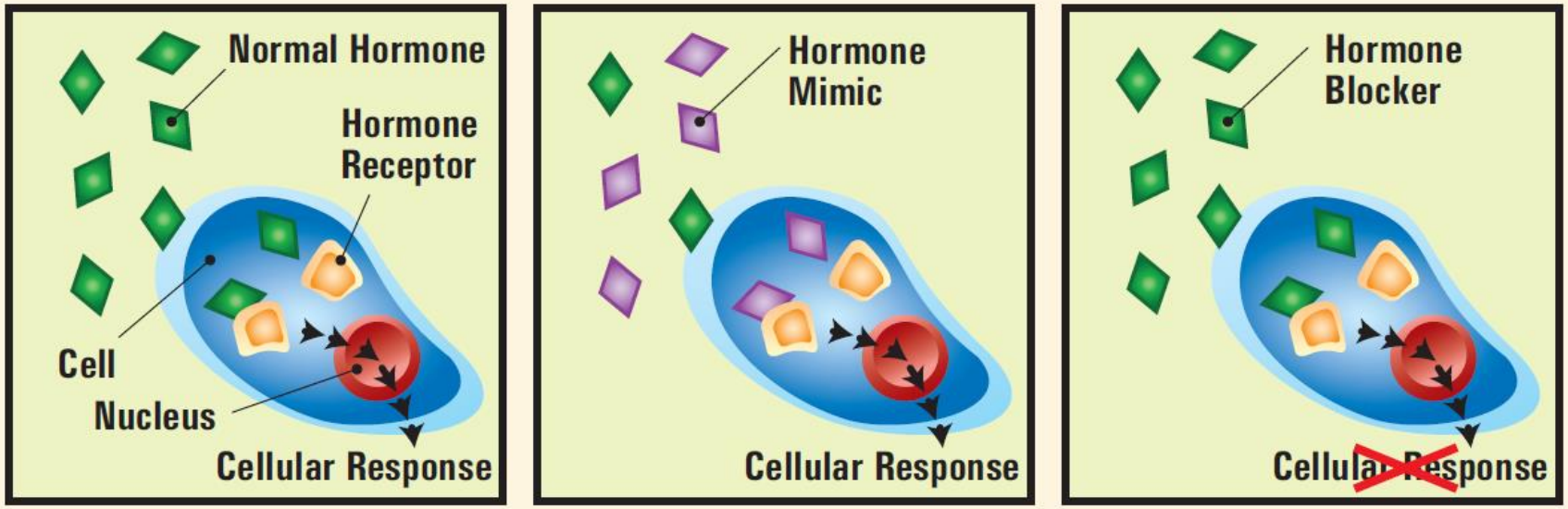
Figure 2. Overall distribution of ERα and ERβ in different tissues from [23].

Bai and Gust Arch Pharm Chem Life Sci, 2009

endocrine disrupting chemicals

분류	종류	노출원
식물성 호르몬	이소플라본	콩과 식물
	플라본	각종 과일 및 채소
	쿠머스탄	콩과 식물
	리그난	아마 씨, 참깨 씨
합성호르몬	에스트라디올	경구피임약 등 주로 분포
농약류	DDT/다이아지논	농약에 오염된 음식 및 음용수
환경오염물질	벤조피렌류	태운 식품, 자동차 배기가스, (난로의 연소 시) 가스 및 담배연기
	다이옥신류	소각장의 연기(과거에는 제초제)
	폴리염화페닐류	전기절연체
중금속류	수은	전지, 형광등, 온도계 등
	납	식기류, 유리, 건축자재, 인쇄물 등
	카드뮴	전지, 유리안료, 어패류 등
산업물질	프탈레이트	건축자재, 파이프, 전기전자 부품 등
	비스페놀A	캔 내부 코팅제, 유아용 젖병, 물병류 등

How do endocrine disruptors work?



When absorbed in the body,

an endocrine disruptor can decrease or increase normal hormone levels (left), mimic the body's natural hormones (middle), or alter the natural production of hormones (right).

성조숙증, 조기폐경, 전립선암, 유방암

JULY 2015

THE LEADING MAGAZINE FOR ENDOCRINOLOGISTS

ENDOCRINE news™

Indecent Exposures:

EDCs AND WOMEN'S HEALTH

The evidence is stacking up against endocrine-disrupting hormones and their link to a variety of female reproductive problems. Reducing these problems is going to take more than simply washing fresh produce and avoiding certain pre-packaged foods.

By Kelly Horvath

Persistent Organic Pollutants and Early Menopause in U.S. Women

Natalia M. Grindler[1,2], Jenifer E. Allsworth[3], George A. Macones[4], Kurunthachalam Kannan[5], Kimberly A. Roehl[4], Amber R. Cooper[2]*

1 Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, University of Colorado Denver, Aurora, Colorado, United States of America, 2 Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Washington University in St. Louis, Barnes-Jewish Hospital, St. Louis, Missouri, United States of America, 3 Department of Biomedical and Health Informatics, University of Missouri—Kansas City School of Medicine, Kansas City, Missouri, United States of America, 4 Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Washington University in St. Louis, Barnes-Jewish Hospital, St. Louis, Missouri, United States of America, 5 Wadsworth Center, New York State Department of Health and Department of Environmental Health Sciences, State University of New York at Albany, Albany, New York, United States of America

1999년-2008년
31,575여성 대상 조사

Plos One Jan 28, 2015

AT-A-GLANCE

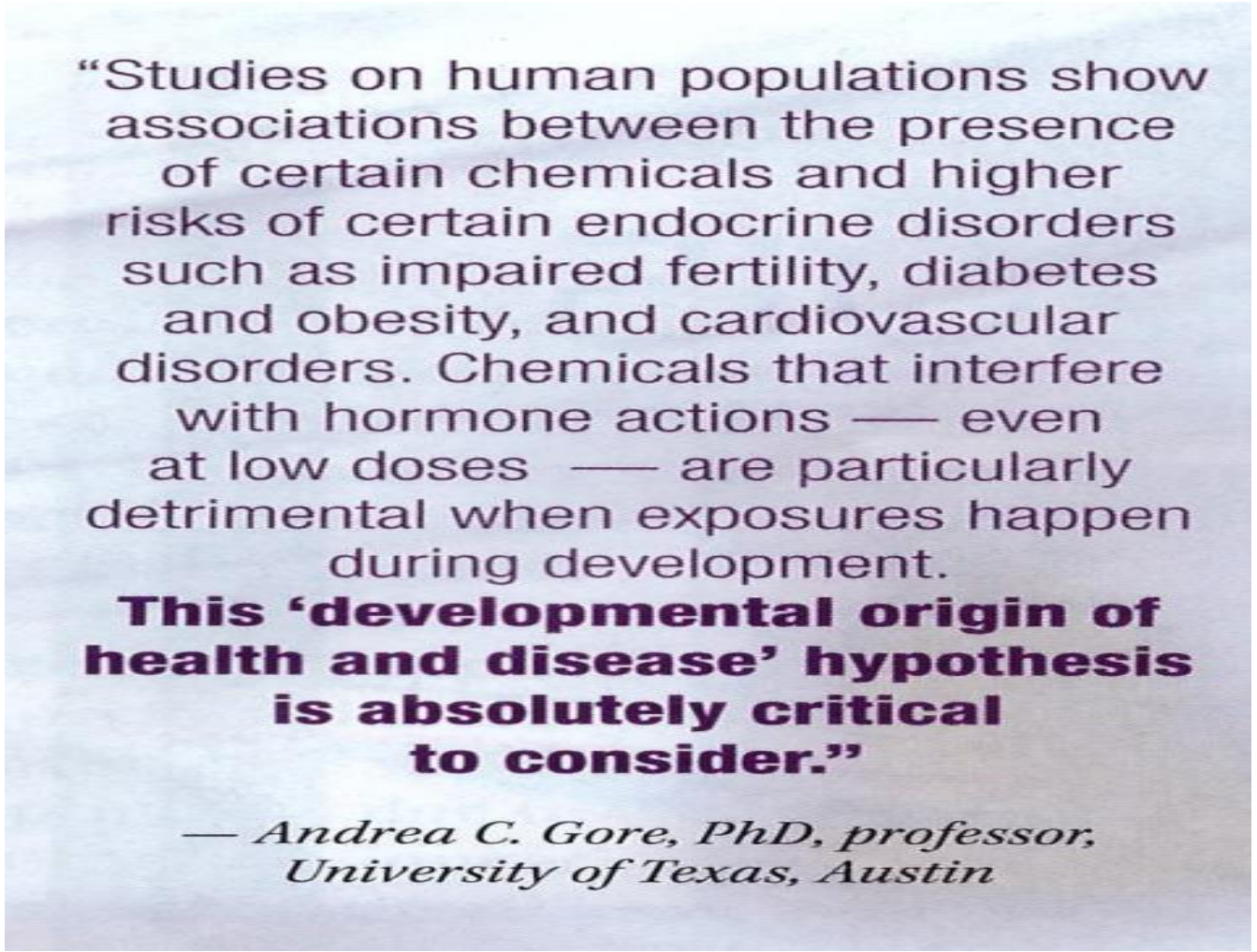
· Biomonitoring demonstrates that nearly 100% of the world's population now carries a chemical body burden due to the astronomical amounts of chemicals produced globally

· Evidence that EDCs are the likely culprits behind increasing incidence of a host of female reproductive problems abounds. Most recently, an association was found between menopause onset 1.9 to 3.8 years earlier with exposure to one or more of nine PCBs, three pesticides, one furan, and two phthalates, as compared to menopause onset in women with lower levels of these chemicals.

· Because intake is a primary route of EDC exposure, a diet of chemical-, hormone-, antibiotic-, and pesticide-free food is recommended to avoid as much new EDC exposure as possible. Washing fresh fruit and vegetables with tap water removes most chemicals.

Phyto-extracts affect the serum lipid profile

	SHAM	OVX (CON)	OVX + E2	OVX + RG
HDL (mg/dl)	43.75±19.93	54.75±5.36	28.50±7.92 *	30.50±4.03 *
LDL (mg/dl)	10.94±7.46 *	32.83±10.17	6.27±2.98 *	14.12±2.48 *
HDL/TC	0.40±0.19	0.40±0.02	0.52±0.04 *	0.28±0.01 *
LDL/TC	0.15±0.07	0.22±0.01	0.14±0.03	0.13±0.01 *
LDL/HDL	0.23±0.09 *	0.54±0.03	0.22±0.01*	0.41±0.03 *



난임, 당뇨, 비만, 심혈관 장애

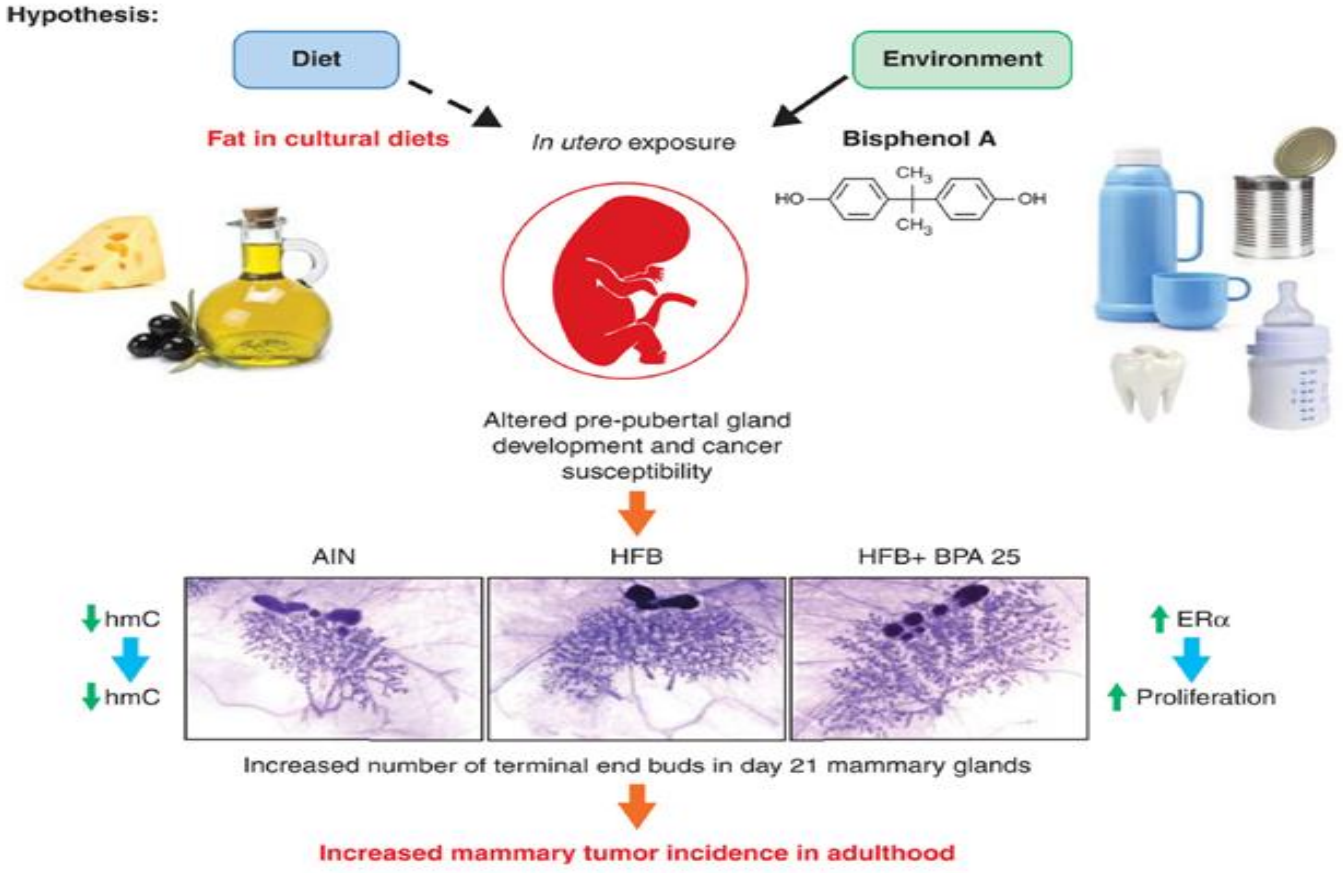
Endocrine disruption of the epigenome: a breast cancer link

Kevin C Knower[1,*], **Sarah Q To**[1,2,*], **Yuet-Kin Leung**[3], **Shuk-Mei Ho**[3], and **Colin D Clyne**[1,2]

[1]Cancer Drug Discovery, MIMR-PHI Institute of Medical Research, PO BOX 5152, Clayton, Victoria 3168, Australia

[2]Department of Molecular Biology and Biochemistry, Monash University, Clayton, Victoria, Australia

[3]Department of Environmental Health, Center for Environmental Genetics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA



Endocr Relat Cancer 2014

Environmental Estrogen Exposure During Fetal Life: A Time Bomb for Prostate Cancer

Jean-Marc A. Lobaccaro and Amalia Trousson

The Holy Frail has been found: environmental estrogen exposure will modify the fate of prostate stem cells, making them more sensitive to estrogen during adulthood and more prone to develop Pca.

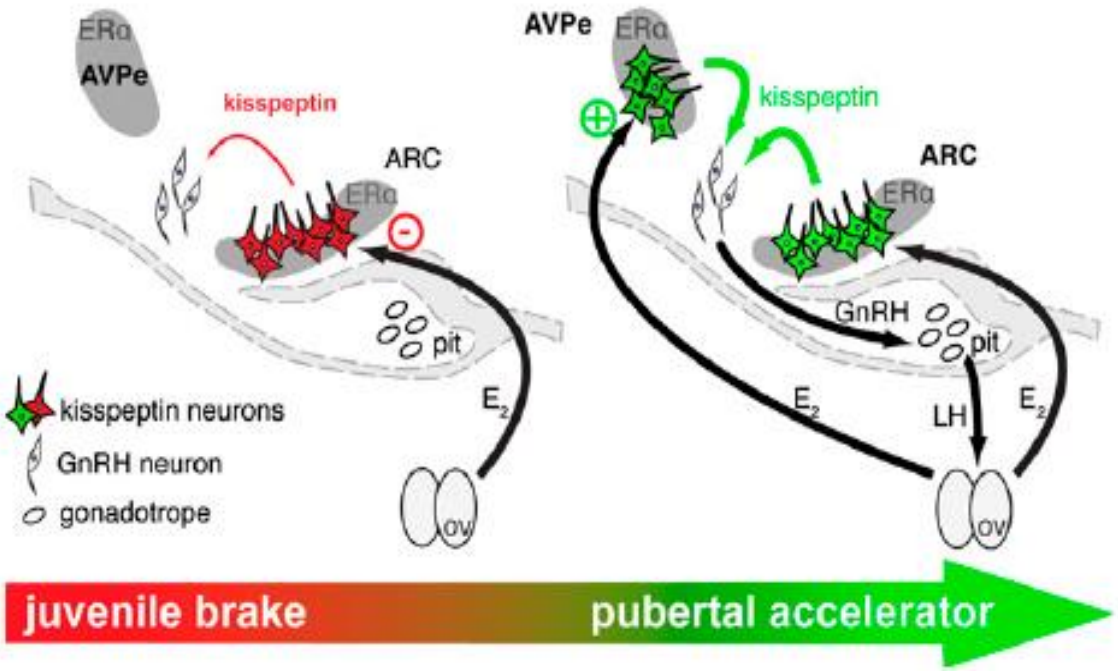
The developmental basis of the adult disease paradigm, extending this to prostate malignancy with aging as a function of early-life exposures to EDC.

Endocrinology 2014

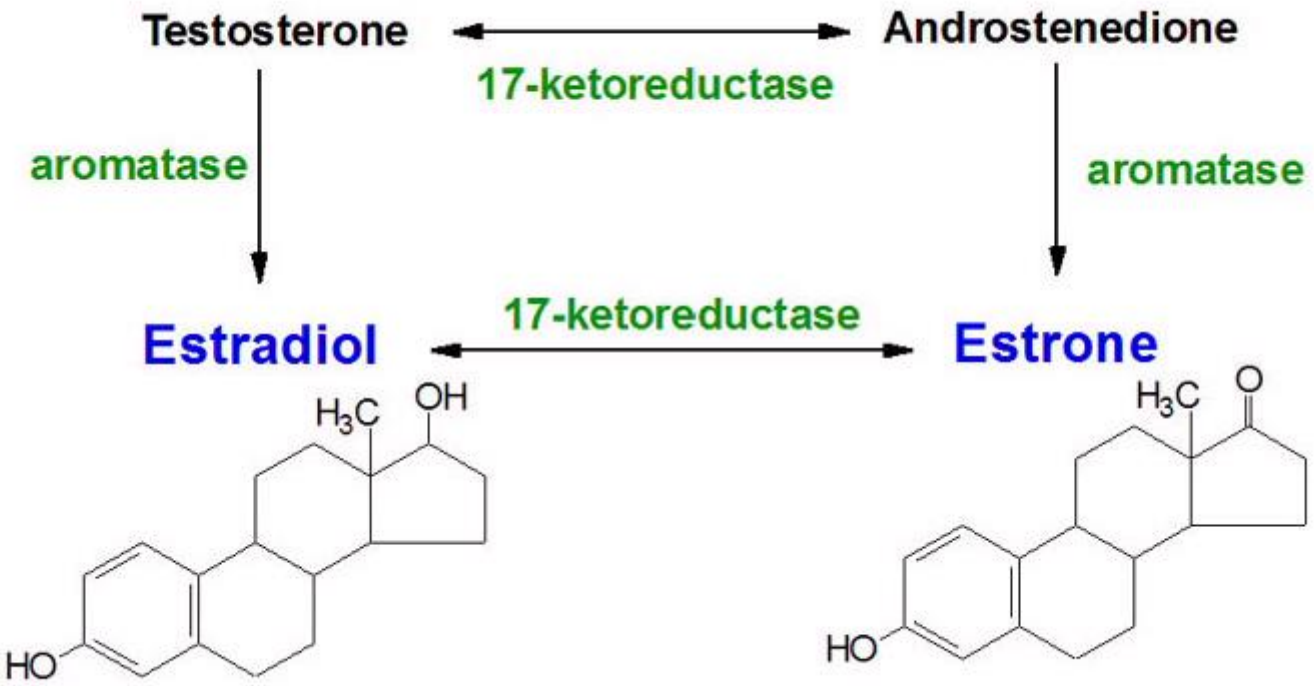
Timing and completion of puberty in female mice depend on estrogen receptor α-signaling in kisspeptin neurons

Christian Mayer[a,1], Maricedes Acosta-Martinez[b,1], Sharon L. Dubois[b], Andrew Wolfe[c], Sally Radovick[c], Ulrich Boehm[a,2], and Jon E. Levine[b,2,3]

[a]Institute for Neural Signal Transduction, Center for Molecular Neurobiology, D-20253 Hamburg, Germany; [b]Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208; and [c]Division of Pediatric Endocrinology, Johns Hopkins University, Baltimore, MD 21287



PNAS 2010


Testosterone
Androstenedione
17-ketoreductase
aromatase
aromatase
17-ketoreductase
Estradiol
Estrone
H3C
OH
HO
H3C
O
HO

OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupting Chemicals

Level 1
Sorting and prioritization based upon existing information (총괄 1 담당)

- Physical & chemical properties, e.g. MW, reactivity, volatility, biodegradability
- Human & environmental exposure, e.g. production volume, release, use patterns
- Hazard, e.g. available toxicological data

Level 2
In vitro assays providing mechanistic data

- ER, AR, TR Transcriptional activation (Stably Transformed Human Estrogen Receptor-a Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals, **TG 455, 457**)
- Aromatase and steroidogenesis in vitro **(TG 456)**
- QSAR

Level 3
In vivo assays providing data about single mechanisms and effects

- Uterotrophic assay **TG 440** (estrogenic related)
- Hershberger assay TG 441 (androgenic related)
- Others (e.g. thyroid)

- Amphibian metamorphosis assay (TG231)

Level 4
In vivo assays providing data about multiple mechanisms and effects

- OECD TG 407 (endpoint based endocrine effects)

Level 5
In vivo assays providing data on effects on endocrine & other mechanisms

- 1-generation assay (TG 415 enhanced)
- Reproductive screening test (TG 421 enhanced)

OECD TG 455

ERα 에 의한 전사활성 평가

세포종류

✓ Stably Transfected TA(STTA) assay: hERα-HeLa-9903 cell line

세포배양

✓ hERα-HeLa-9903 세포는 석탄을 처리한 10% FBS를 포함한 phenol red가 없는 EMEM 배지에서 37℃, 5% CO_2 조건하에서 배양

✓ 96well-plate에 plating 후 약물 처리 전 3시간 동안 배양

OECD TG 457

ERα와 ERβ 에 의한 전사활성 평가

세포종류

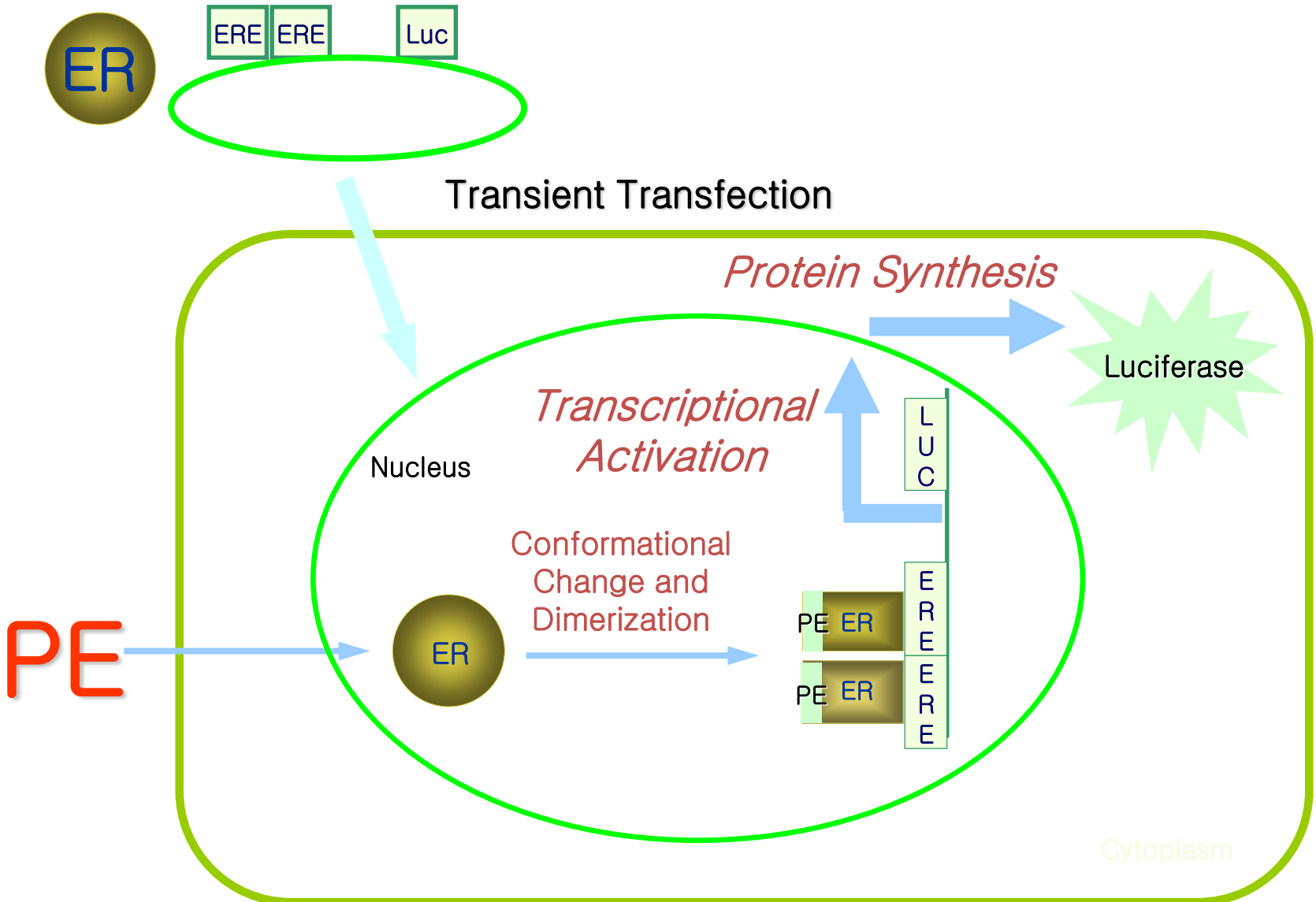
✓ The BG1Luc ER TA(Transactivation) assay: BG1Luc4E2 cell line 사용

세포배양

✓ BG1Luc4E2 세포는 8.0% FBS을 포함한 RPMI1640 배지에서 37℃, 5% CO_2 조건하에서 배양

✓ 48시간 동안 석탄을 처리한 4.5% FBS을 포함한 phenol red가 없는 DMEM에서 배양 후 96-well plate에 plating 하여 시험물질 처리

Model System to Screen Estrogen Activities of Phytoestrogens



OECD TG 457

적합기준

✓ 시험의 적합, 부적합 여부는 실험으로 얻은 참고물질과 대조군을 이용하여 검증

Agonist Test

Range Finder Test

✓ 참고물질인 E2의 가장 높은 농도의 RLU(Relative Light Unit) 평균값을 DMSO 대조군의 RLU 평균으로 나누어 측정 하며 4배 혹은 그 이상이 유도되어야 함

✓ DMSO 대조군의 결과: 용매 대조군의 RLU값은 표준편차가 2.5배 이내여야 함

Comprehensive test

✓ 참고물질의 결과: E2 참고물질의 농도-반응 곡선은 S자형 곡선

✓ 양성 대조군의 결과: 대조군 RLU 값은 DMSO 대조군 평균값에 표준편차의 3배를 더한 값보다 높아야 함

Substance	BG1Luc ER TA Mean EC_{50} (M)	Product Class
Bisphenol A	5.33×10^{-7}	Chemical Intermediate, Flame Retardant, Fungicide
Genistein	2.71×10^{-7}	Natural Product, Pharmaceutical
17α-Estradiol	1.40×10^{-9}	Pharmaceutical, Veterinary Agent

Activation of Estrogen Receptor-α by the Heavy Metal Cadmium

Binding of Cadmium to the ER

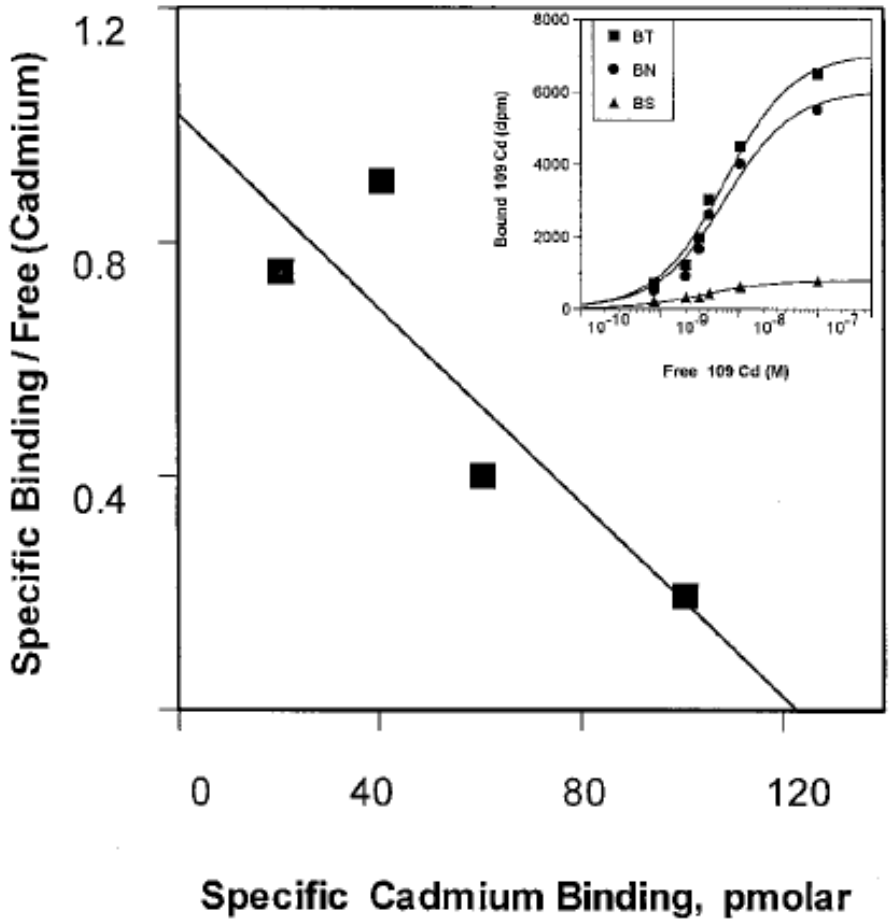
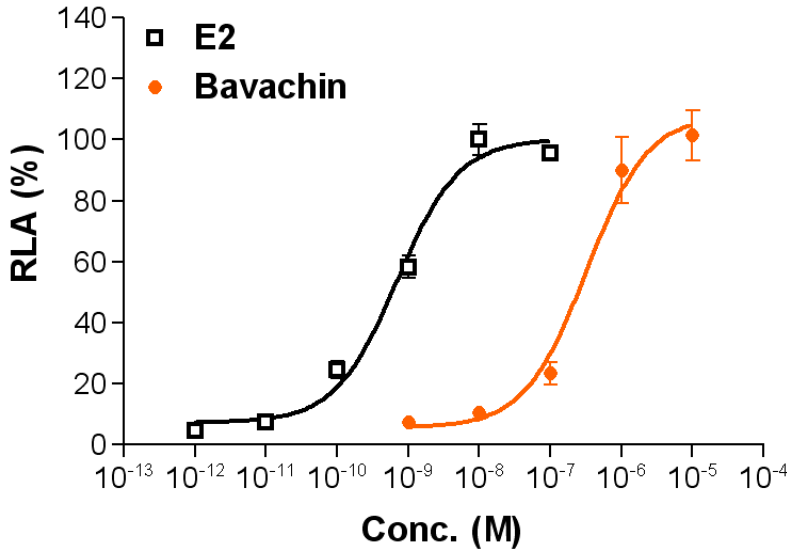
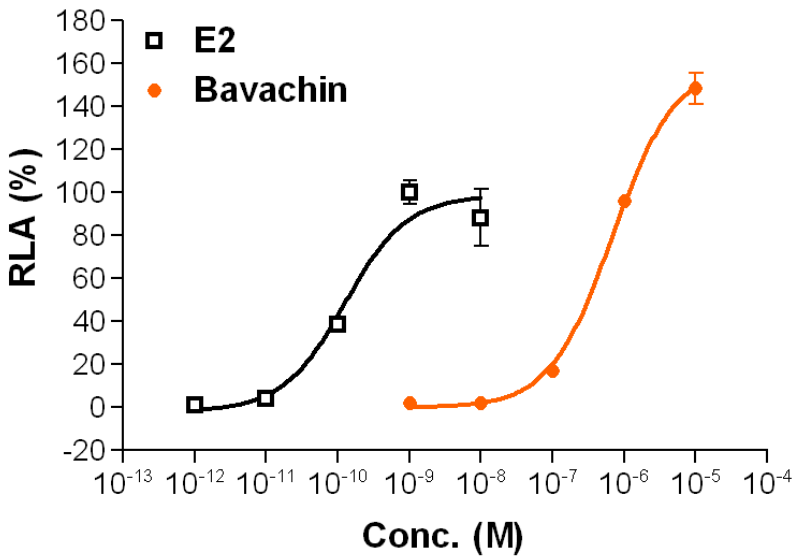


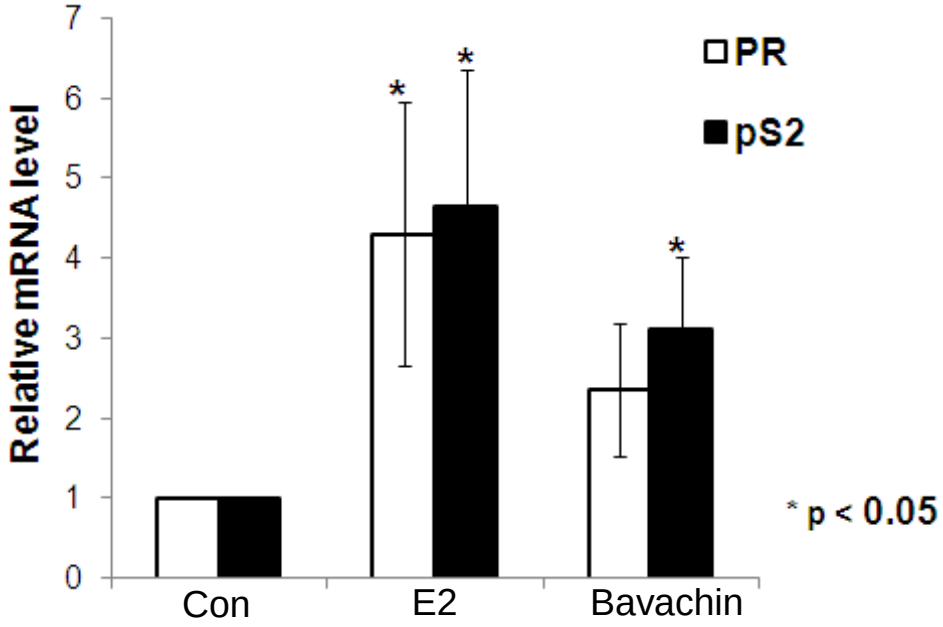
Fig. 5. Binding of Cadmium to Recombinant Human ER-α

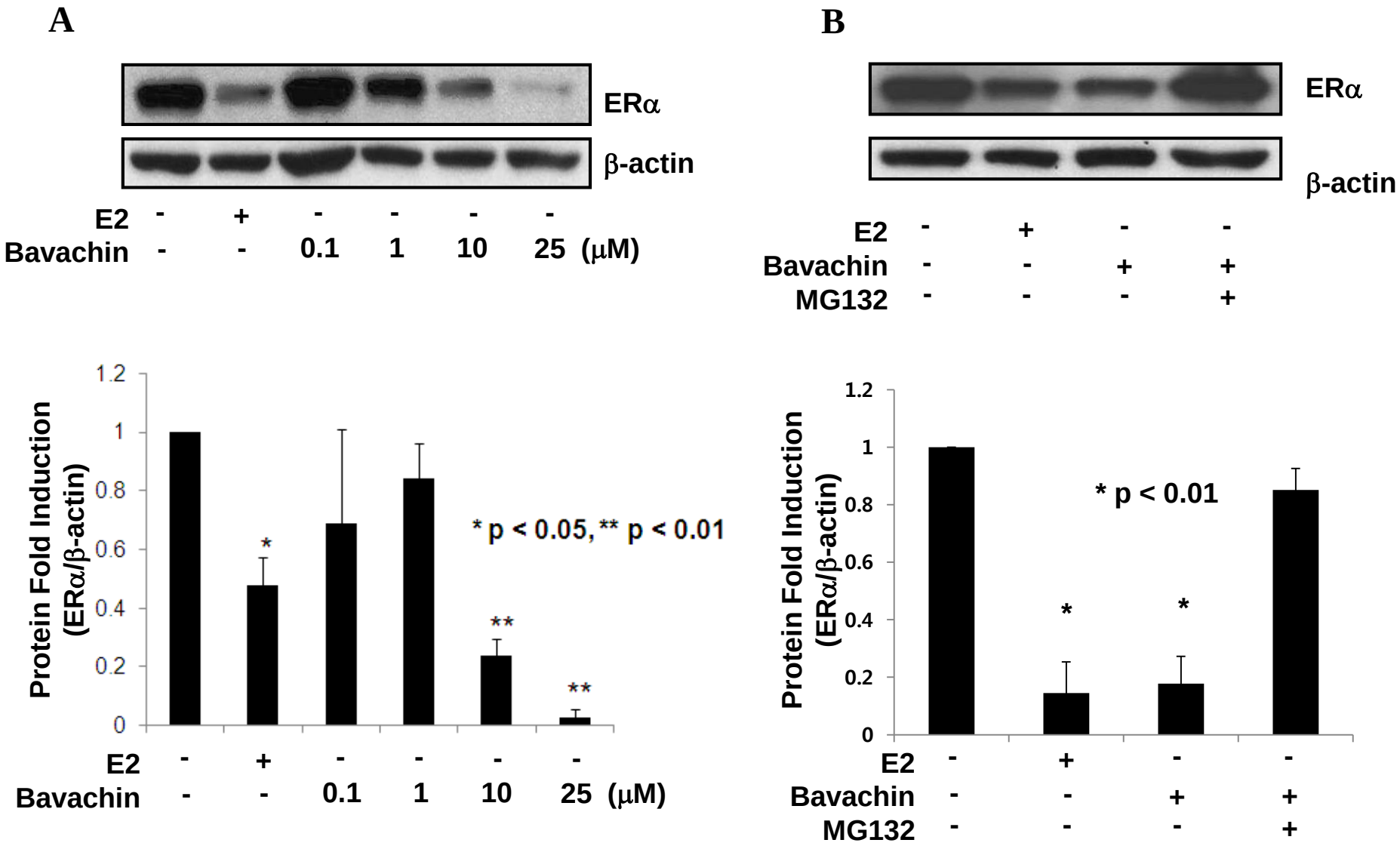
A


E2
Bavachin
RLA (%)
Conc. (M)

B


E2
Bavachin
RLA (%)
Conc. (M)


PR
pS2
Relative mRNA level
Con
E2
Bavachin
* p < 0.05


A
ERα
β-actin
E2 - + - - - -
Bavachin - - 0.1 1 10 25 (μM)
Protein Fold Induction (ERα/β-actin)
1.2
1
0.8
0.6
0.4
0.2
0
* p < 0.05, ** p < 0.01
*
**
**
E2 - + - - - -
Bavachin - - 0.1 1 10 25 (μM)
B
ERα
β-actin
E2 - + - -
Bavachin - - + +
MG132 - - - +
Protein Fold Induction (ERα/β-actin)
1.2
1
0.8
0.6
0.4
0.2
0
* p < 0.01
*
*
E2 - + - -
Bavachin - - + +
MG132 - - - +

OECD TG 440

동물실험 및 난소 측정

동물의 상태와 수

- ✓ 각 그룹은 최소 6 동물을 포함해야 함
- ✓ 투여는 출생 후 18일에 지난 후 시작

난소 절개 절차

- ✓ Rat과 mouse는 6주에서 8주 사이에 난소를 절제함
- ✓ Rat은 , 최소14 일이 지난 후 자궁 최소 안정된 상태로 유지하고 첫날 기준으로 잡으며, 마우스의 경우 최소 7 일 후 첫 날로 투여함
- ✓ 난소 조직 소량의 상당한 순환 에스트로겐 수준을 생산하기에 적어도 5 일부터 채취 상피 세포를 관찰하여, 사용 전에 검사되어야 하며 발정된 동물은 사용할 수 없음
- ✓ 동물을 제대로 마취한 상태에서 난소를 제거하며, 멸균된 제품을 기구를 이용해서 난관과 자궁 본체의 접합부에서 분리 되어야 함. 출혈이 발생되지 않도록 확인한 후 봉합을 하고 봉합에 의해 폐쇄된 피부까지 폐쇄해야 함

체중

- ✓ 자궁 중량은 에스트로겐과 같은 호르몬뿐만 아니라 신체 크기를 조절하는 성장인자의 영향을 받으므로, ovx-adult method 에서는 체중 및 자궁 중량은 상관하지 않고, 투여는 출생 후 18일에 지난 후 시작함
- ✓ 미성숙된 모델에서는 체중과 자궁 중량 변화를 체크하고 최소한 마리 수와 평균 체충은 ± 20 %를 초과하지 않아야 함

OECD TG 440

약물 처리 방법

- ✓ 구강 투여와 피하 주사를 이용해서 투여 되며,
- ✓ 대부분 수용액 및 현탁액을 사용하며, 에스트로겐 리간드 또는 소수성 전구체의 경우 오일용액을 사용함
- ✓ 시험 물질은 위장 튜브 또는 기관삽관에 캐뉼라를 사용하여 동물에 단일 투여량에서 수행해야 하며, 투여 될 수 있는 최대의 액체 부피는 시험 동물의 크기에 의존한다.
- ✓ 부피는 10 ㎖/kg 체중이 사용될 수 있는 수용액의 경우를 제외하고 5 ㎖/kg 체중을 초과하지 않아야 한다.
- ✓ 피하 주사에 의해서 투여될 경우는 당일 투여량으로 수행하여야 하며, 투여 후 적어도 하루에 한번 상태를 체크 해야 함
- ✓ 관찰은 매일 같은 시간에 확인하는 것이 바람직하고 투여 후 피크 예상 효과의 기간을 고려해야 함
- ✓ 동물의 행동의 변화, 피부, 털, 눈, 점막, 분비물과 배설물 또는 자율활동의 방생으로 사망률 등의 증상을 관찰해야 한다.

음식 소비

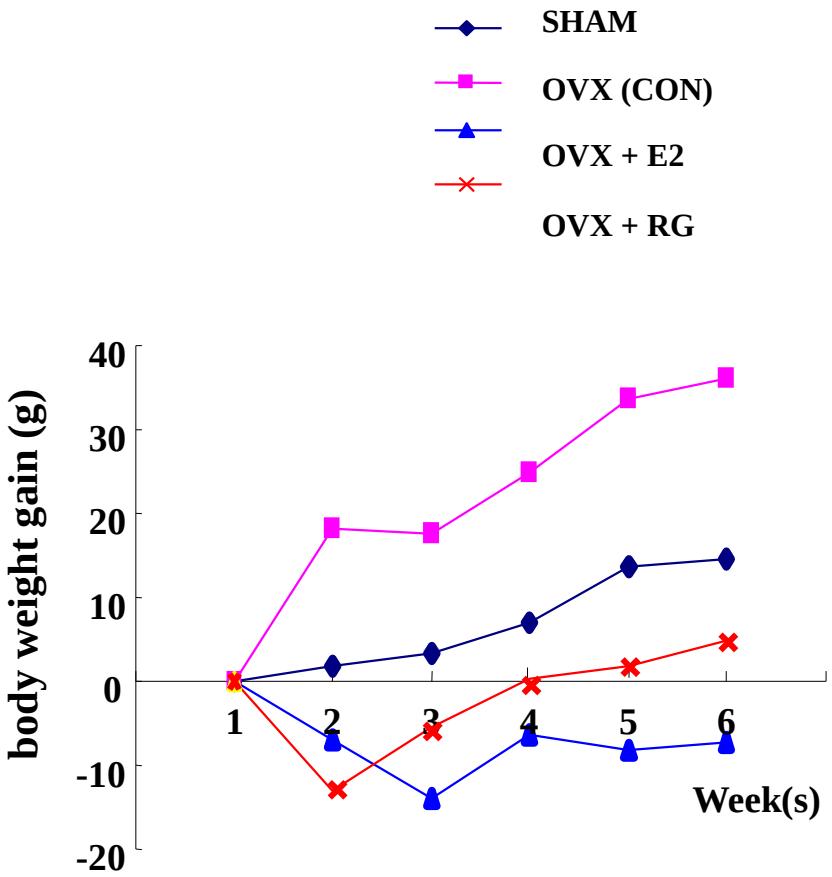
- ✓ 음식의 소비 결과는 g/day로 표현하고 소비된 음식의 양을 계량하여서 체크한다.

해부 및 자궁무게 측정

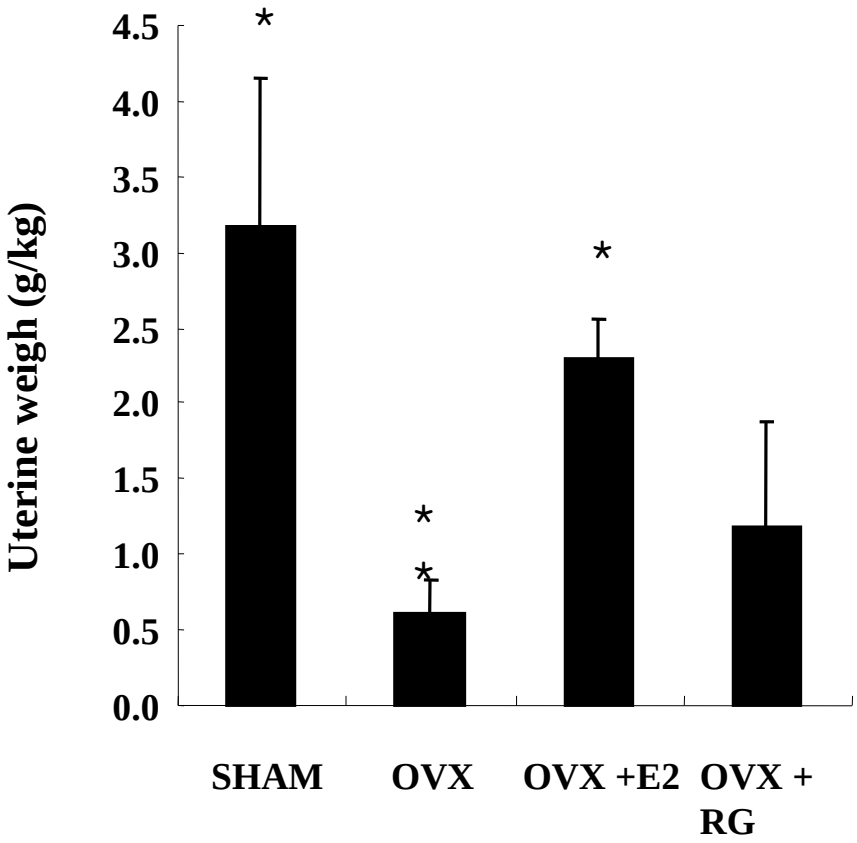
- ✓ Sacrifice 24시간 전에 실시하며, 부검 순서는 미묘하게 데이터에 영향을 미칠 수 있으므로 그룹 단위로 무작위로 해부함
- ✓ OECD 지침 문서에 따라서 관리 후 3일이 지난 후부터 자궁절제가 가능하며, 마지막 약물 투여 후 24시간 후에 마지막 무게를 측정

Effects on the body and uterine weights

A.



B.

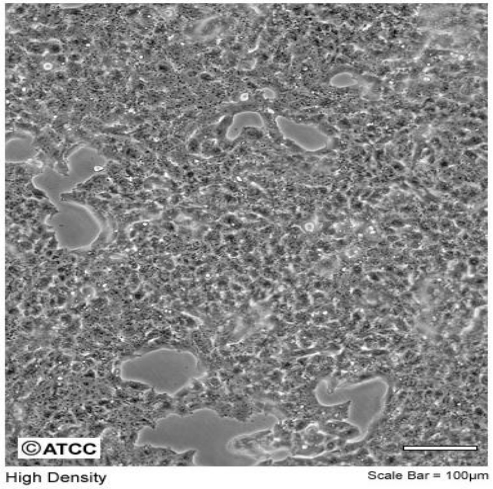


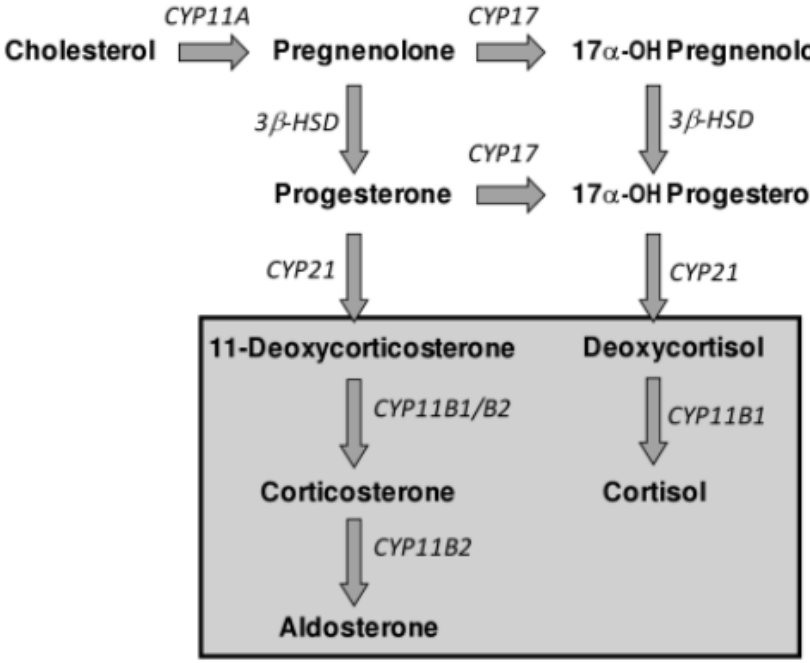
OECD TG 456

H295R세포를 이용한 스테로이드호르몬 합성시험법

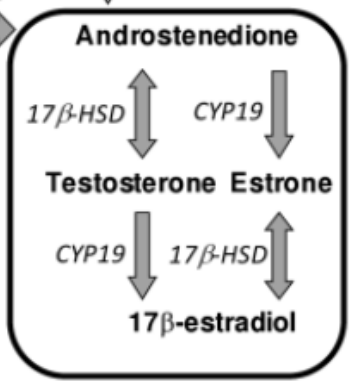
- ✓ H295R 세포는 American Type Culture Collections(ATCC) 를 통한 구입이 용이
- ✓ 성호르몬과 코르티코스테로이드호르몬을 포함한 모든 호르몬의 합성효소를 발현
- ✓ OECD 내분비계장애물질 검색시험법 가이드라인으로 채택

ATCC Number: **CRL-2128**
Designation: **NCI-H295R**





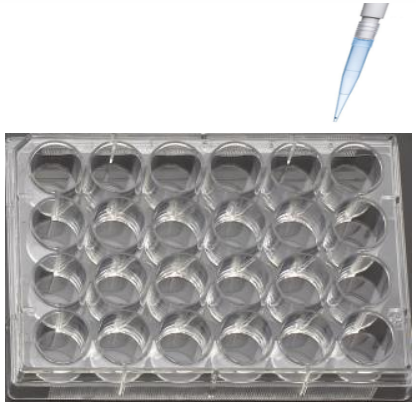
Corticosteroid pathways



Sex steroid pathways

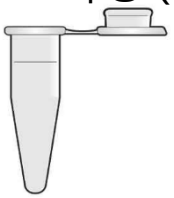
OECD TG 456

24well plate에 24시간 배양

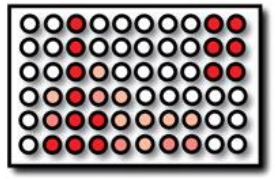


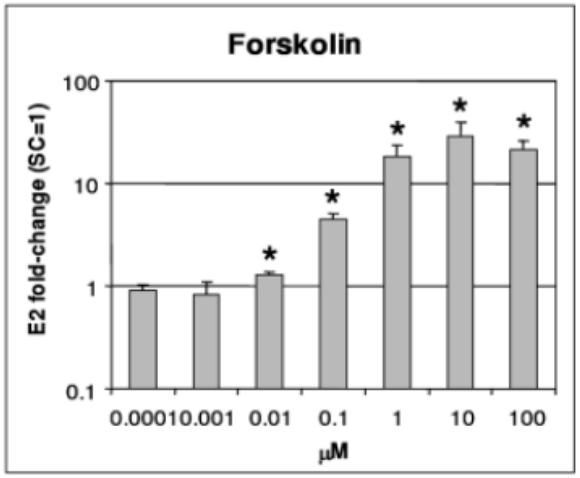
48시간 약물처리

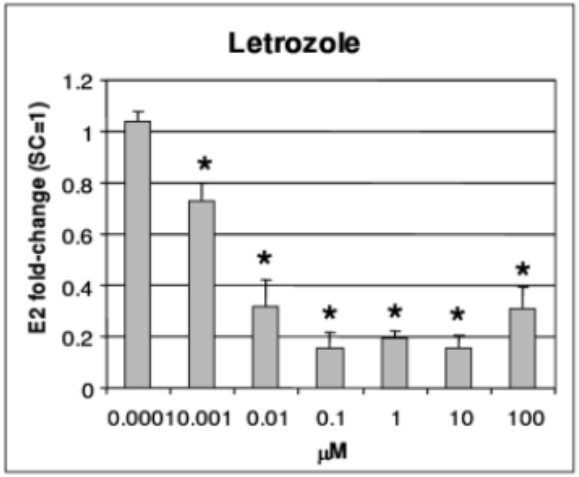
호르몬 농도 측정 (E2 & T)



세포 증식 측정

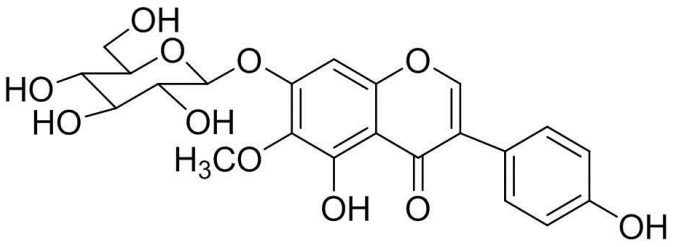


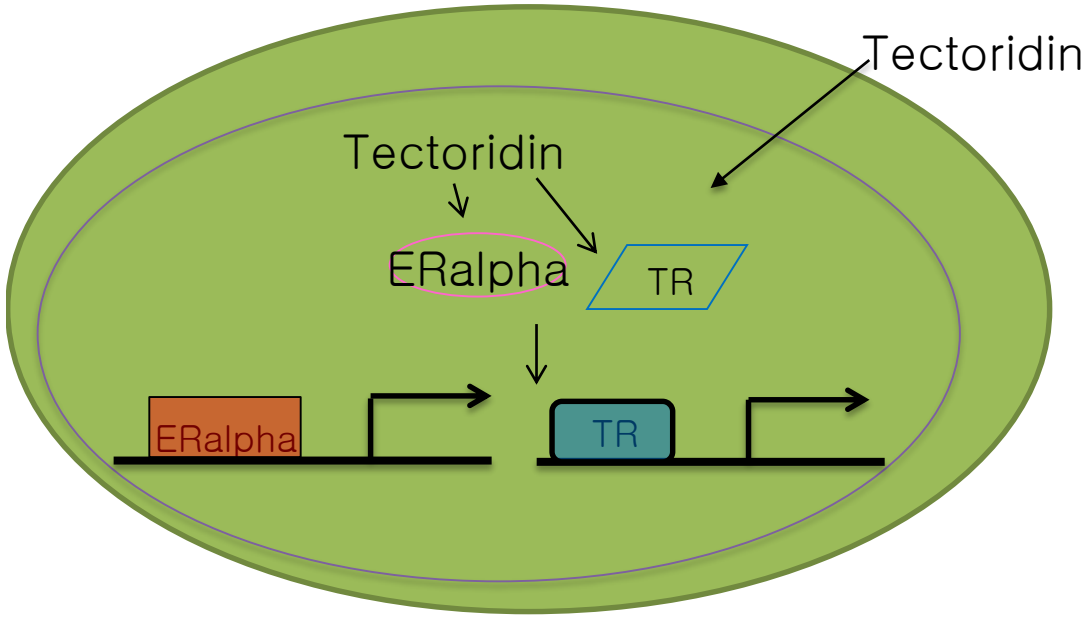




Chemical	*LOEC*	*Max Change*
Forskolin	0.01	29-fold
Letrozole	0.001	0.15-fold

✓ 최소 2회 이상의 독립된 실험으로 검증되어야 하며 대조군 대비 최소효과농도와 최대변화량을 산출함





Tectoridin Induces Estrogen and Thyroid Hormone Responses

Fig 2.

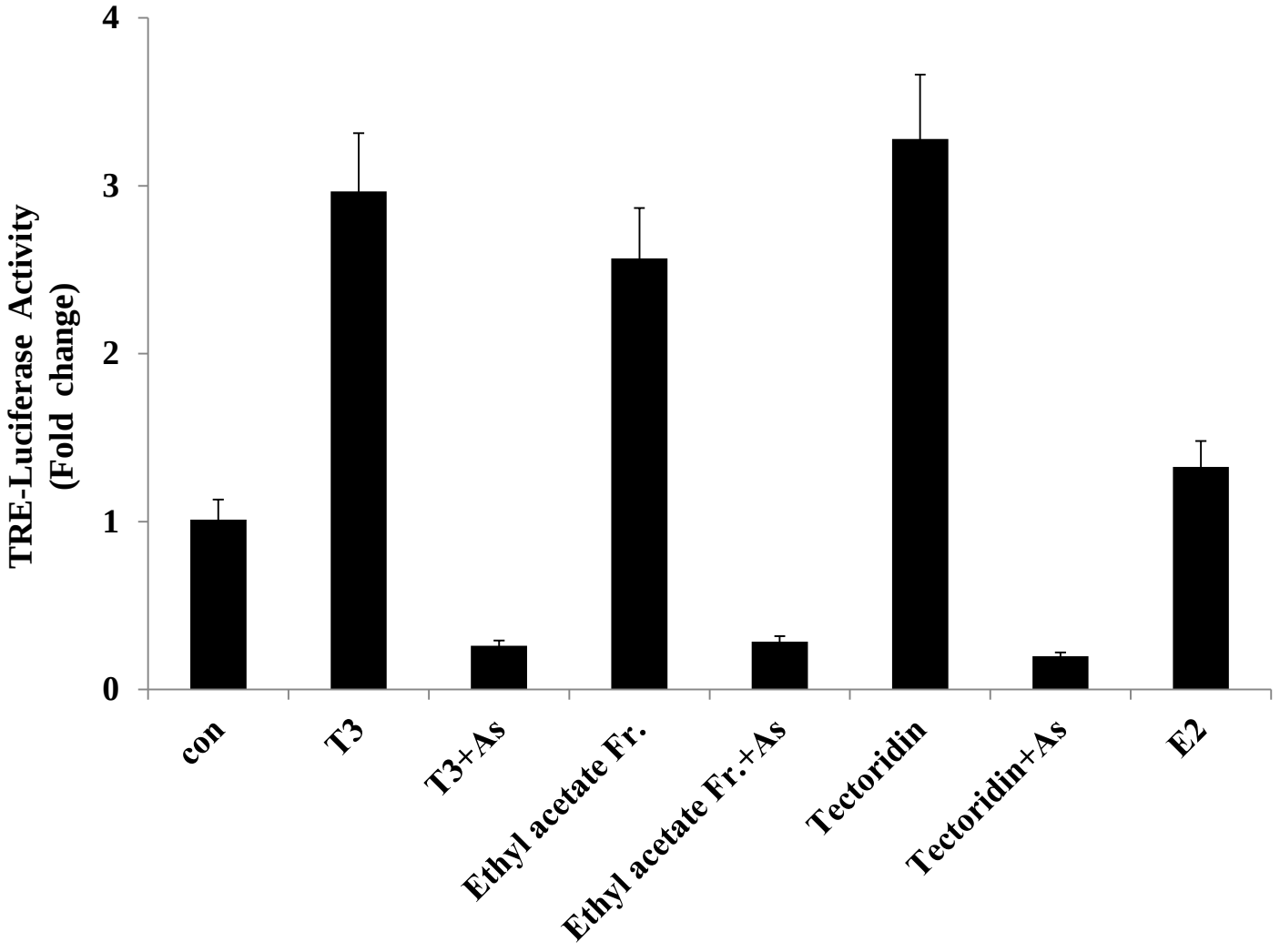
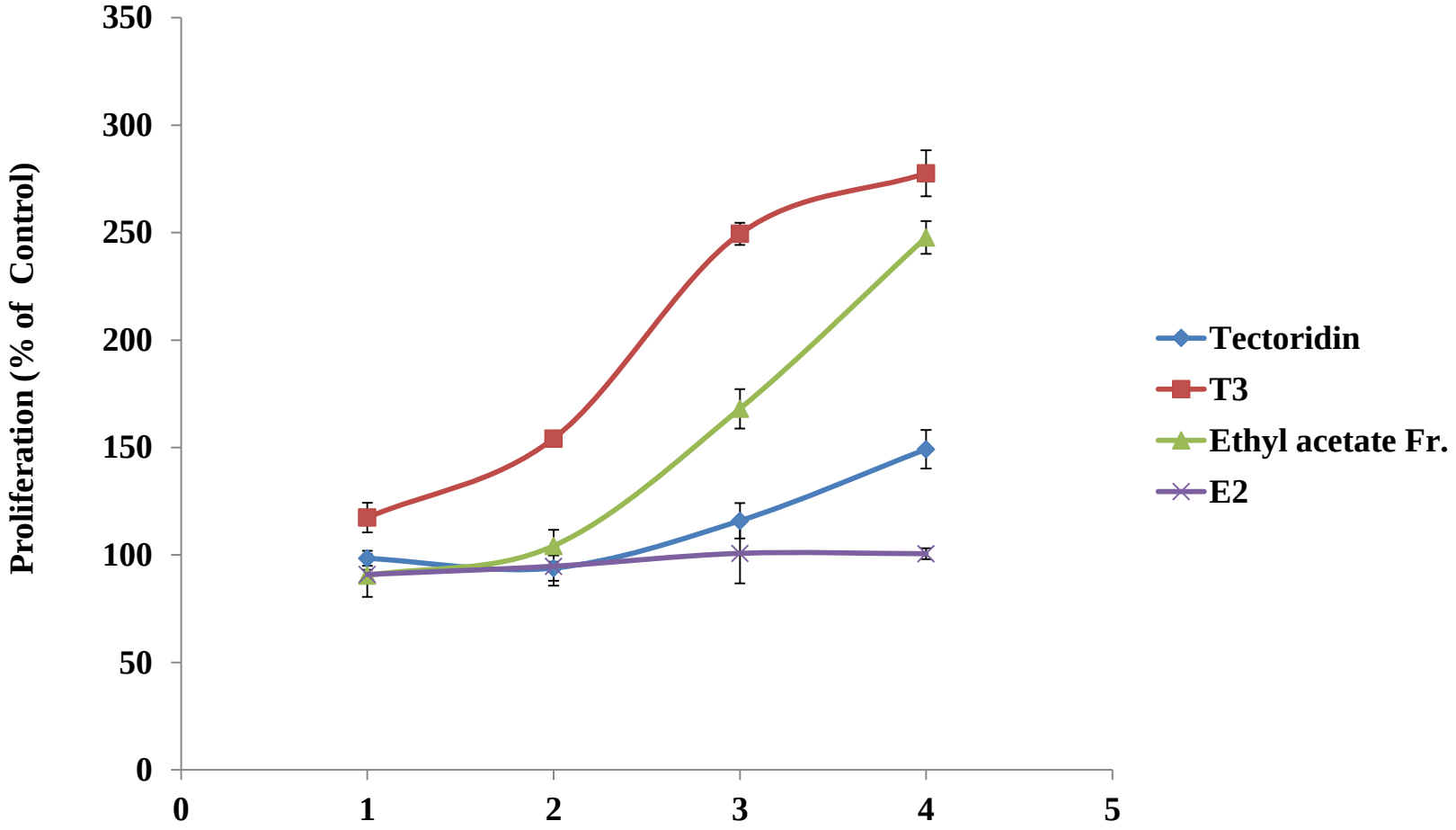

TRE-Luciferase Activity
(Fold change)
4
3
2
1
0
con
T3
T3+As
Ethyl acetate Fr.
Ethyl acetate Fr.+As
Tectoridin
Tectoridin+As
E2

Fig 3.


Proliferation (% of Control)
350
300
250
200
150
100
50
0
0
1
2
3
4
5
Tectoridin
T3
Ethyl acetate Fr.
E2