Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Reproductive Endocrinology Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 2, 2024
Week 13

Reproductive Endocrinology Systems

- Female Reproductive Endocrinology
    - Summary
    - Steroidogenesis and Action
    - Cycle
- Male Reproductive Endocrinology
    - Summary
    - Steroidogenesis and Action
    - Gonadotropins
- Endocrine Regulation
    - Neuroendocrinology
    - Endocrine Disruptors

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Lee S, Kang DW, et al. (2009) Theca-specific estrogen receptor-alpha knockout mice lose fertility prematurely. Endocrinology. 150(8):3855-62.

Pinilla L, et al. (2009) Delayed puberty in spontaneously hypertensive rats involves a primary ovarian failure independent of the hypothalamic KiSS-1/GPR54/GnRH system. Endocrinology. 150(6):2889-97.

Navarro VM, (2009) Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. Endocrinology. 150(5):2359-67.

"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January 9 & 11	Week 1	Systems Biology Introduction
16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
23 & 25	Week 3	Sex Determination Systems
Jan /Feb 30 & 1	Week 4	Male Reproductive Tract Development & Function
February 6 & 8	Week 5	Female Reproductive Tract Development & Function
13 & 15	Week 6	Gonadal Developmental Systems Biology
20 & 22	Week 7	Testis Systems Biology
27 & 29	Week 8	Ovary Systems Biology
March 5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
11 – 15	**Week 10**	**Spring Break**
19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April 2 & 4	Week 13	Reproductive Endocrinology Systems
9 & 11	Week 14	Fertilization & Implantation Systems
16 & 18	Week 15	Fetal Development & Birth Systems
23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May 30 & 2	Week 17	Exam or Grant Review

Spring 2024 – Systems Biology of Reproduction
Lecture Outline – Reproductive Endocrinology Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 2, 2024
Week 13

Reproductive Endocrinology Systems

- Female Reproductive Endocrinology
  - Summary
  - Steroidogenesis and Action
  - Cycle
- Male Reproductive Endocrinology
  - Summary
  - Steroidogenesis and Action
  - Gonadotropins
- Endocrine Regulation
  - Neuroendocrinology
  - Endocrine Disruptors

Spring 2024 – Systems Biology of Reproduction
Discussion Outline – Reproductive Endocrinology Systems
Michael K. Skinner – Biol 475/575
CUE 418, 10:35-11:50 am, Tuesday & Thursday
April 4, 2024
Week 13

Reproductive Endocrinology Systems

Primary Papers:

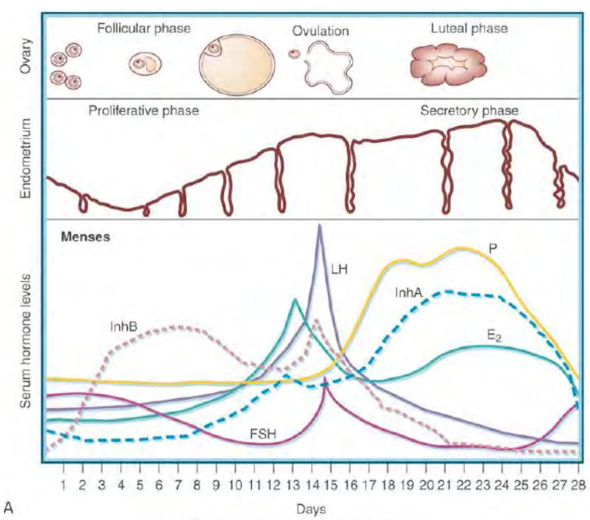
1. Stotzel, et al. (2012) Theriogenology 78:1415-1428
2. Toufaily, et al. (2020) J Endocrinology 244(1):111-122
3. Barban, et al. (2016) Nat Genetics 48:1462

Discussion

Student 9: Reference 1 above
- What endocrine parameters were synchronized and what regulatory agent tested?
- What experimental model was used?
- What model was established and validated?

Student 10: Reference 2 above
- What was the experimental design and technology used?
- Why is the LH surge important?
- What was identified regarding the progesterone regulated phasic LH secretion?

Student 11: Reference 3 above
- What was the experimental design and technology used?
- What reproductive factors were used and what traits were associated?
- What conclusions can be drawn on genomic control of reproduction?



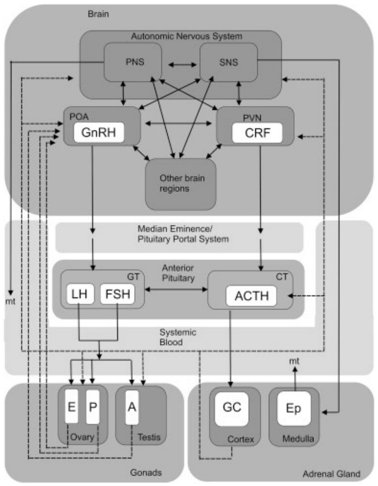
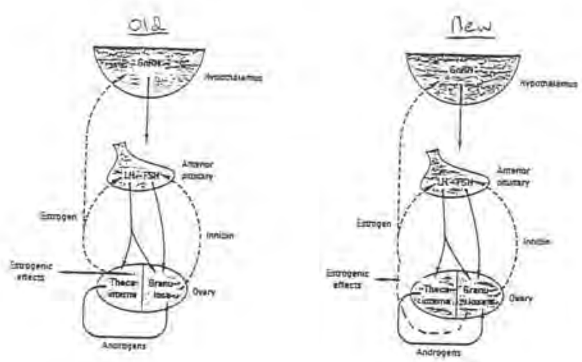
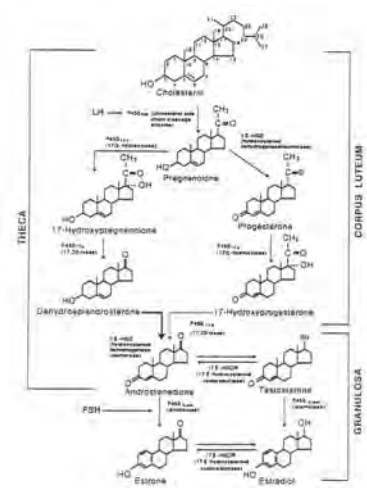
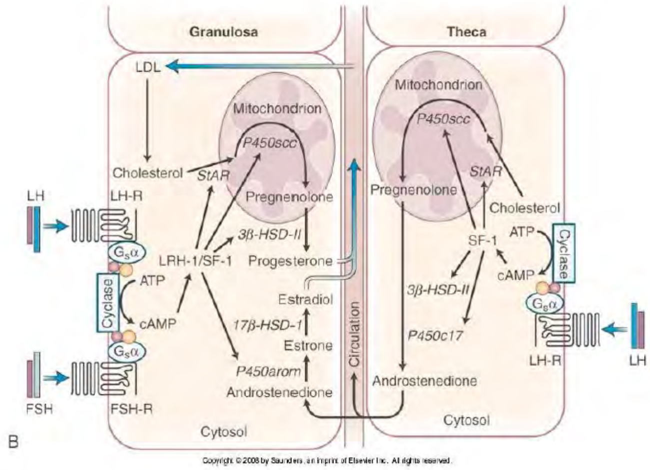


Fig. 1. Simple scheme of the interaction of the stress-response and reproductive systems. These systems are initiated by the release of CRF and GnRH, respectively, after considerable neurological integration. Negative feedback occurs primarily via steroid release from gonadal or adrenal cortex tissue. See text for further details.
Abbreviations: A; androgens, ACTH; adrenocorticotropin-releasing hormone, CRF; corticotropin-releasing factor, CT; corticotropes, E; estrogens, Ep; epinephrine, FSH; follicle-stimulating hormone, LH; luteinizing hormone, GC; glucocorticoids, GnRH; gonadotropin-releasing hormone, GT; gonadotropes, mt; multiple targets, P; progesterone, PNS; parasympathetic nervous system, POA; preoptic area, PVN; paraventricular nucleus, SNS; sympathetic nervous system. Solid line; stimulatory actions, Dashed line; inhibitory actions.

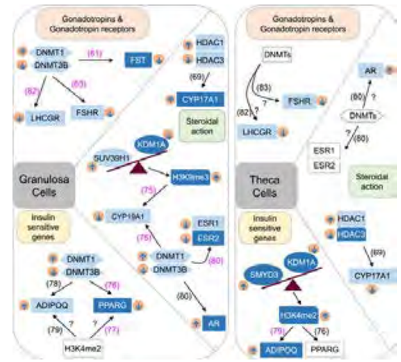
Female Reproductive Endocrinology





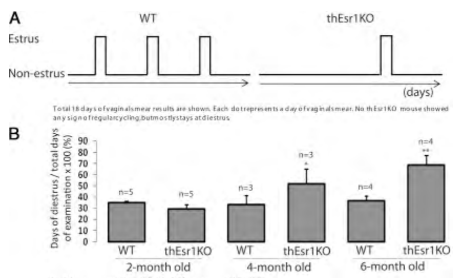


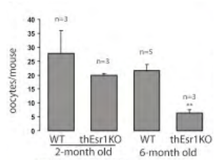
Developmental Programming: Contribution of Epigenetic Enzymes to Antral Follicular Defects in the Sheep Model of PCOS.
Guo X, Puttabyatappa M, Thompson RC, Padmanabhan V.
Endocrinology. 2019 Oct 1;160(10):2471-2484.

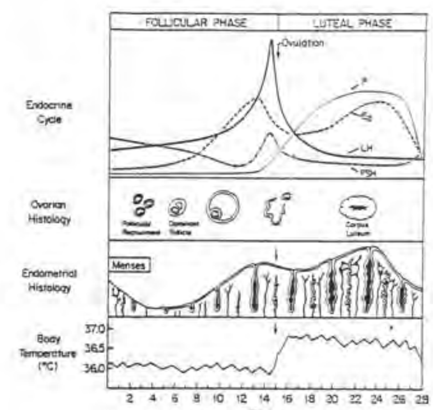


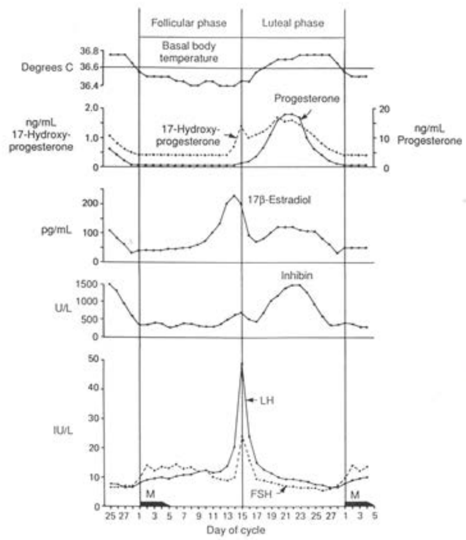
Schematic summarizing the changes in epigenetic-modifying enzymes, histones, and key mediators of ovarian follicular differentiation and function of relevance to PCOS. Changes related to the gonadotropin and gonadotropin receptor, steroidal milieu, and hyperinsulinemic status in (left) granulosa and (right) theca cells are shown. Significant changes in gene expression from the current study are shown with dark-blue shading and the large-magnitude nonsignificant changes (that need further testing) with light-blue shading. Numbers in parentheses point to available evidence supportive of epigenetic-mediated, gene-expression changes, with those that are consistent in prenatal T-treated sheep marked in pink. Question mark symbols (?) indicate that this link remains to be established. The differential role of the histone methylation status, as a result of altered balance (depicted as a maroon-colored balance) of histone de/methylases in granulosa and theca cells governing steroidogenic vs insulin regulation, respectively, is also shown.

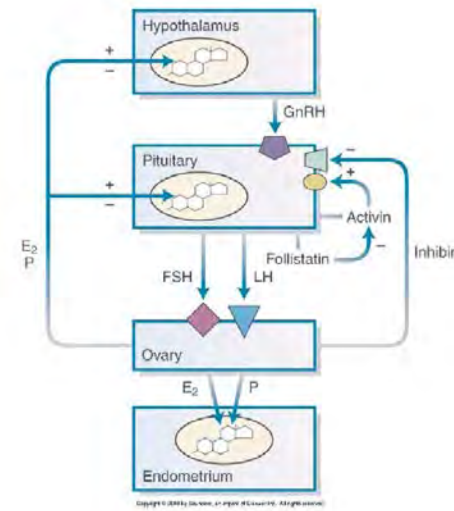
Theca-specific estrogen receptor-alpha knockout mice lose fertility prematurely.
Lee S, et al. Endocrinology. 2009 Aug;150(8):3855-62.



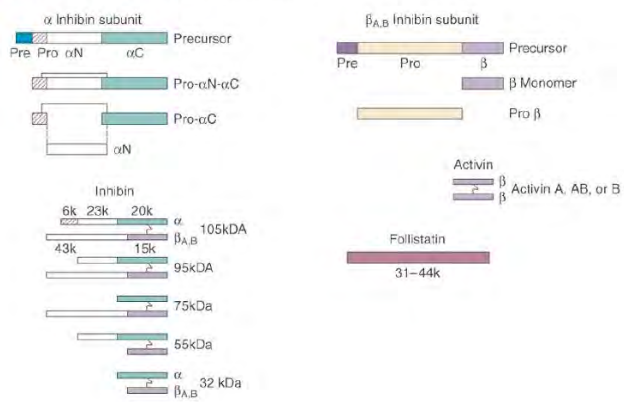




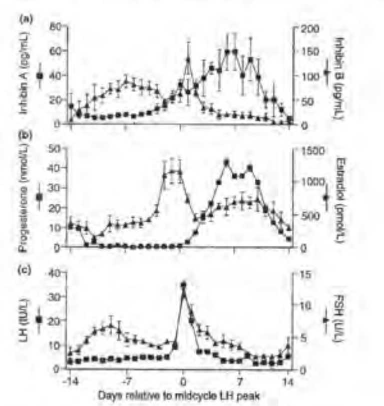


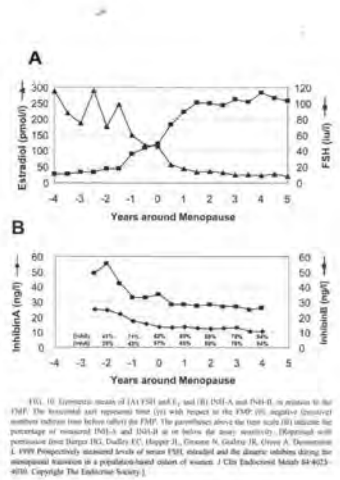


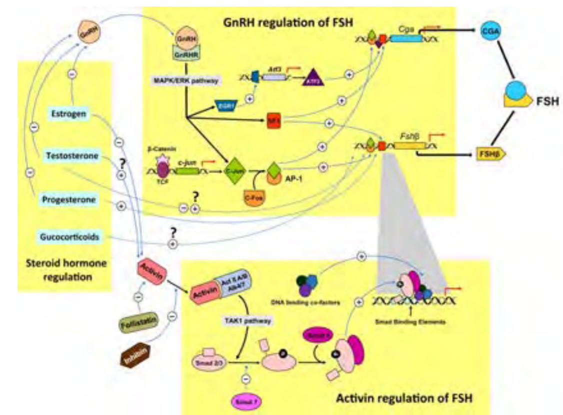
Inhibin, activin and follistatin



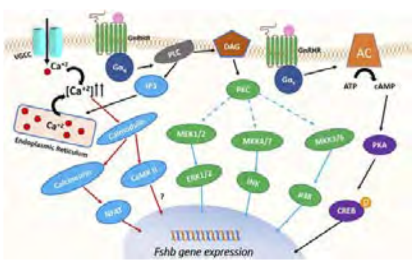
HORMONAL CHANGES IN MENOPAUSE TRANSITION 239

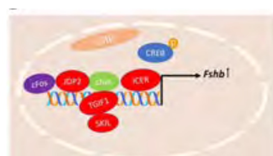


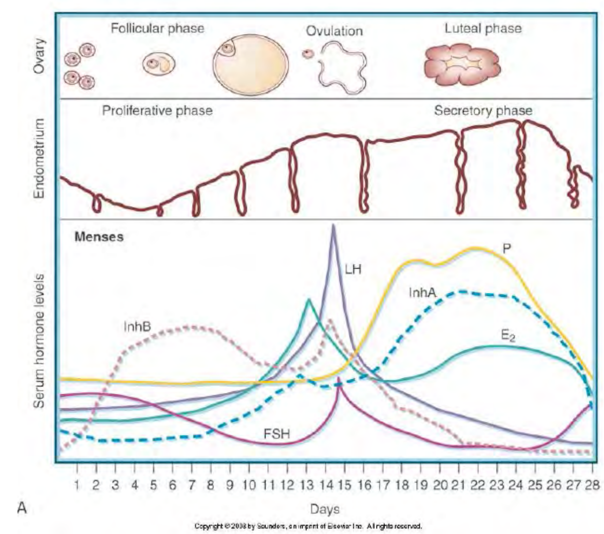


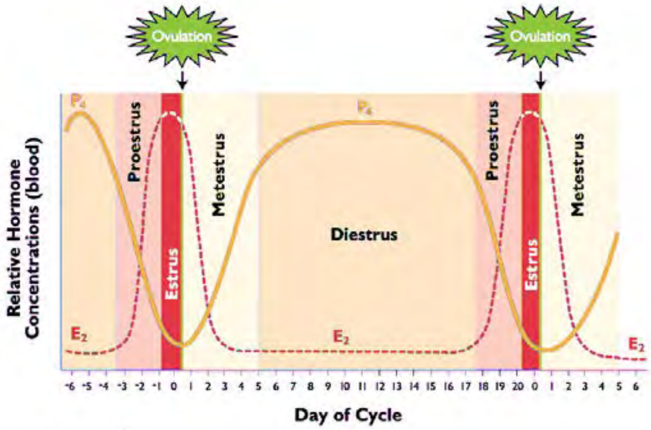


GnRH-A Key Regulator of FSH.
Stamatiades GA, Carroll RS, Kaiser UB.
Endocrinology. 2019 Jan 1;160(1):57-67.



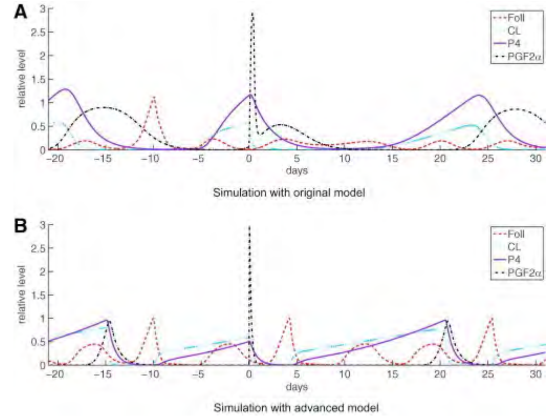


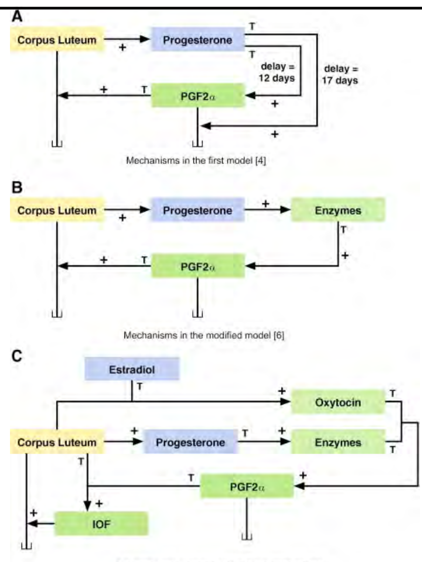




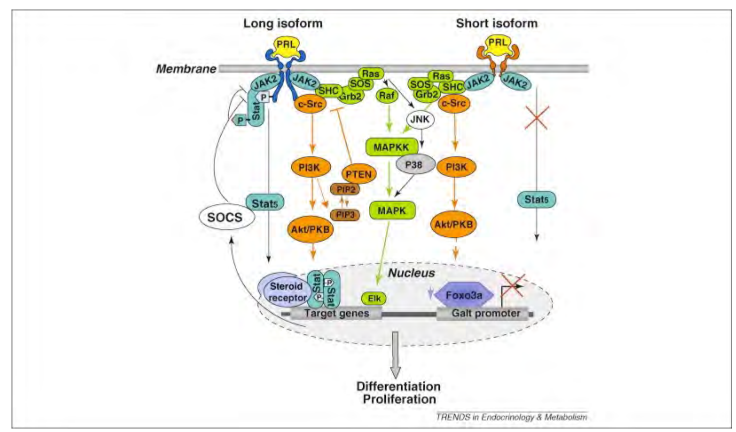
Senger, 2006

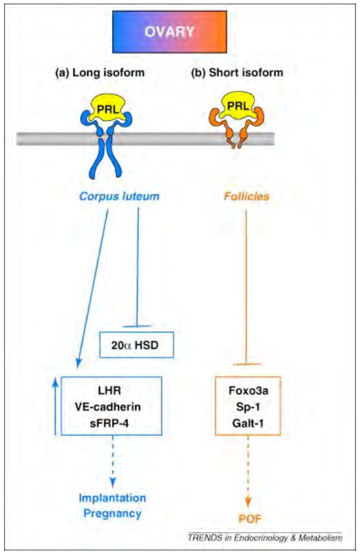
Advances in modeling of the bovine estrous cycle: synchronization with PGF2α.
Stötzel C, Plöntzke J, Heuwieser W, Röblitz S. Theriogenology. 2012 Oct 15;78(7):1415-28.

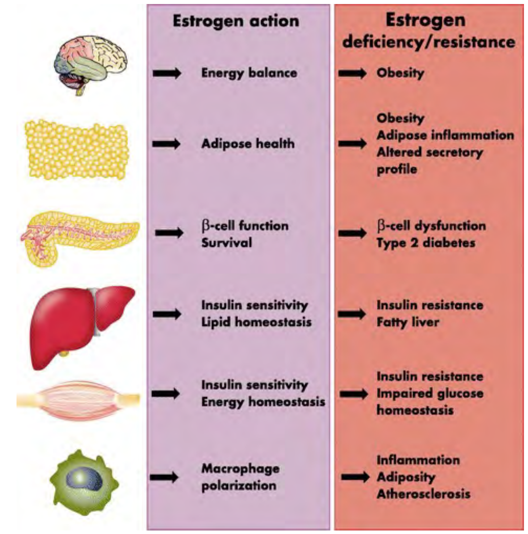




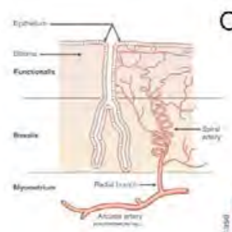
Impact of prolactin receptor isoforms on reproduction.
Binart N, Bachelot A, Bouilly J.
Trends Endocrinol Metab. 2010 Jun;21(6):362-8.

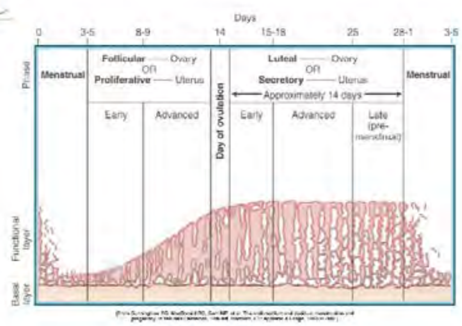


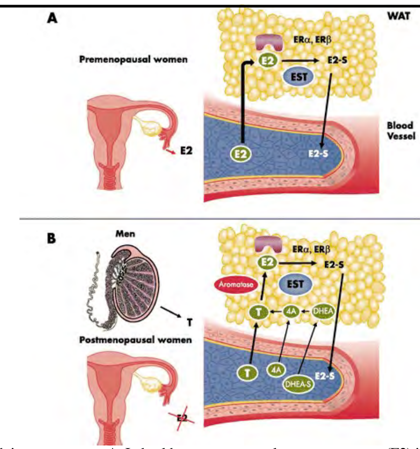




Ovarian steroids support endometrial function





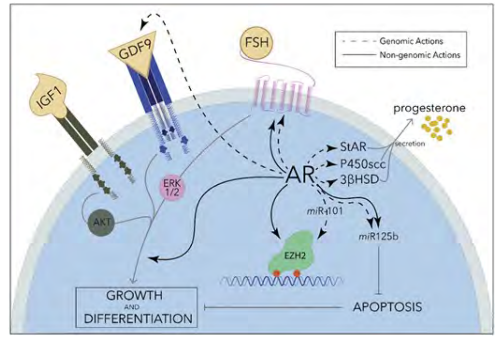


Origin of circulating and tissue estrogens. A, In healthy premenopausal women, estrogen (E2) is produced by the ovaries and functions as a circulating hormone that acts on distant target tissues. Here WAT is represented. B, In postmenopausal women and in men, E2 does not function as a circulating hormone; rather, it is synthesized in extragonadal sites from circulating androgenic precursors such as T, androstenedione (4A), or dehydroepiandrosterone (DHEA). Cellular estrogenic action depends on: 1) the ER signaling and sensitivity; 2) the activity of enzymes like aromatase involved in the biosynthesis of E2 from androgenic precursors; and 3) the inactivation of E2 in E2 sulfate (E2-S) by the estrogen sulfotransferase (EST).

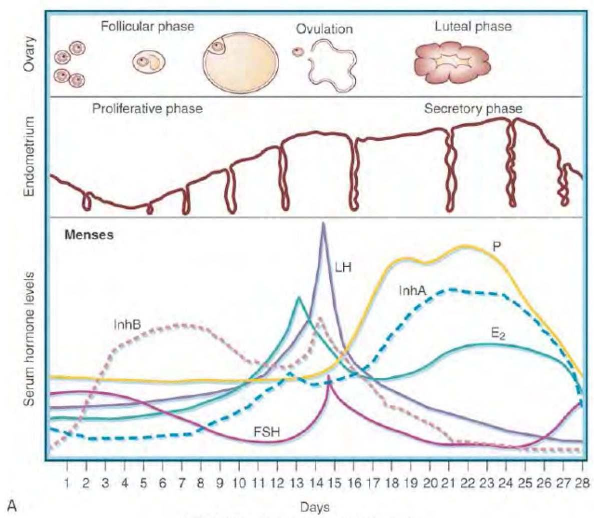
Physiological and Pathological Androgen Actions in the Ovary.
Astapova O, Minor BMN, Hammes SR.
Endocrinology. 2019 May 1;160(5):1166-1174.

Table 1. Summary of Available Evidence From Studies of Androgen Actions in the Ovary

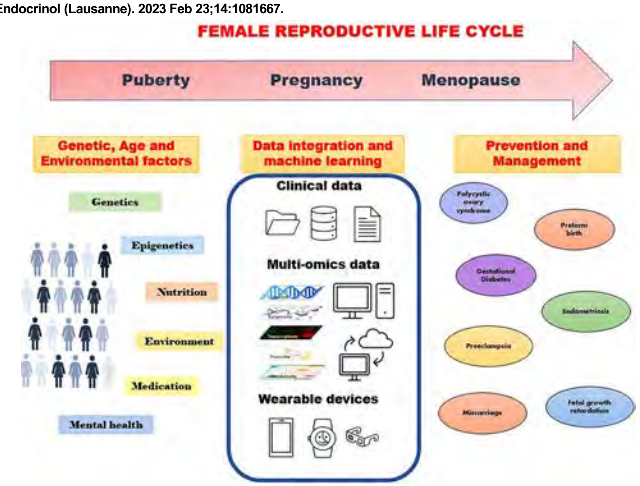
Observed Effect	Experimental Species and Design		DHT Dose	Reference
Increased preantral follicle growth	Rat	*In vitro*	1 nM and higher	(20, 21)
		In vivo	83 μg continuous daily-release pellet	(21)
	Monkey	*In vivo*	145 μg/kg/d for 5 d	(22)
Increased FSH receptor mRNA expression	Rat	*In vitro*	1 nM and higher	(20)
	Monkey	*In vivo*	0.4 or 4 mg/kg of testosterone for 3 d	(23)
Increased FSH receptor protein expression	Mouse, human	*In vitro*	25 nM	(24)
Increased granulosa cell proliferation	Rat	*In vitro*	100 nM	(25)
	Pig	*In vitro*	500 nM	(26, 27)
Reduced apoptosis of follicles	Mouse	*In vitro*	25 nM	(24, 28)
Increased expression of steroidogenic enzymes StAR, P450scc, and 3βHSD in mature granulosa cells	Rat	*In vitro*	100 nM	(25)
Increased expression of cyclooxygenase-2 and	Mouse	*In vivo*	5 μg/g 4 h prior to RNA isolation	(29)
amphiregulin in periovulatory granulosa cells	Human	*In vitro*	100 nM	



AR actions in granulosa cells. AR works through genomic (dashed lines) and nongenomic (solid lines) pathways to promote growth and differentiation of granulosa cells, suppress apoptosis and, in dominant follicles, increase steroid synthesis. The effects of granulosa growth factors IGF1, GDF9, and FSH are all enhanced in the presence of androgens through extranuclear activity of AR. At the gene level, AR induces the expression of antiapoptotic miRNA miR125b, multiple steroidogenic enzymes, GDF9, and FSH receptor, and regulates the activity of DNA methyltransferase Ezh2 through modulation of Ezh2 phosphorylation as well as transcriptional regulation of the miRNA miR101 (24–28, 33).



Multi-omics and machine learning for the prevention and management of female reproductive health.
Kharb S, Joshi A.
Front Endocrinol (Lausanne). 2023 Feb 23;14:1081667.

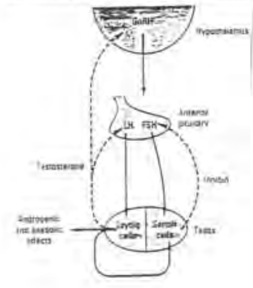


Machine learning approaches using various data to understand genetic and environmental factors towards prevention and management of disorders through the family reproductive life cycle.

Male Reproductive Endocrinology

Table 23–6. Body changes at puberty in boys (male secondary sex characteristics).

External genitalia: Penis increases in length and width. Scrotum becomes pigmented and rugose.
Internal genitalia: Seminal vesicles enlarge and secrete and begin to form fructose. Prostate and bulbourethral glands enlarge and secrete.
Voice: Larynx enlarges, vocal cords increase in length and thickness, and voice becomes deeper.
Hair growth: Beard appears. Hairline on scalp recedes anterolaterally. Pubic hair grows with male (triangle with apex up) pattern. Hair appears in axillas, on chest, and around anus; general body hair increases.
Mental: More aggressive, active attitude. Interest in opposite sex develops.
Body conformation: Shoulders broaden, muscles enlarge.
Skin: Sebaceous gland secretion thickens and increases (predisposing to acne).



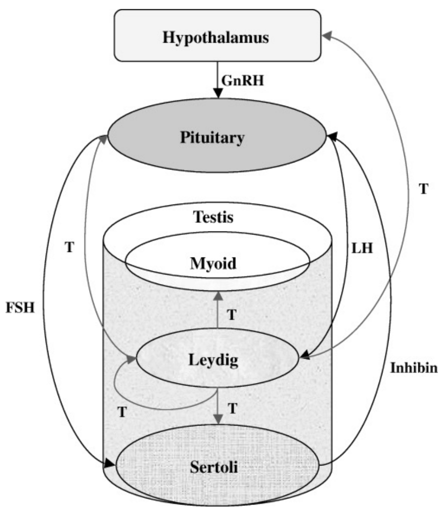


Figure 1. Hormonal regulation of spermatogenesis Most hormones shown can have both positive and negative effects, either at the level of receptor activation/desensitization or through activation and repression of downstream targets. GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone.

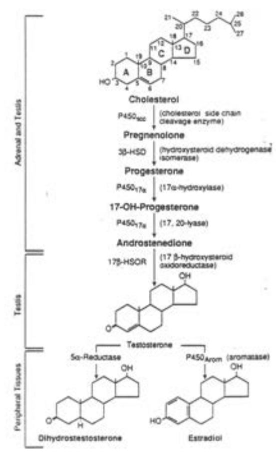


Figure 13–5. Pathway of testosterone formation in the testis and conversion of testosterone to active metabolites in peripheral tissues.

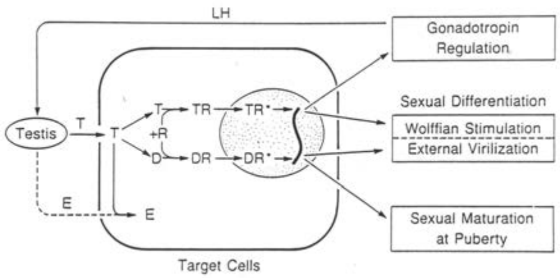


Figure 13–6. Mechanism of androgen action. Abbreviations: T, testosterone; D, dihydrotestosterone; E, 17β-estradiol; R, androgen receptor; R*, transformed androgen receptor; 5α-red, 5α-reductase enzyme; LH, luteinizing hormone. (*Reproduced, with permission, from Wilson JD, Griffin JE. Mutations that impair androgen action. Trends Genet. 1:336, 1985.*)

Targeted gene deletion – endocrine factors regulating spermatogenesis

FSHR -/- quantitative decrease in sperm production and some morphological abnormalities, fertile

FSH beta-/- same as FSHR, phenotypically normal with FSH

LHR -/- low T, cryptorchid, infertile, rescued with T

AR (*tfm -/Y*) – pseudohermaphroditism, cryptorchid, infertile

GnRH -/- (*hpg*) – cryptorchid, rescue with T and FSH

ERα -/- secondary infertile due to efferent duct defect

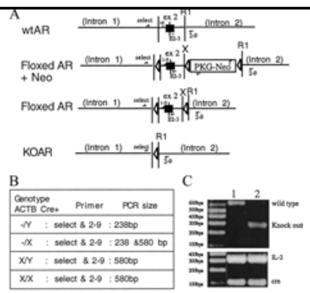


Fig. 4. Genotyping of ARKO mice. We have applied the primers "select" and "2-9" to identify wt and ARKO male mice in our study. (A) Schematic presentation of the DNA construct and primer location in exon 2 area of wt, KO, and floxed AR genes. (B) List of the sizes of PCR product amplified by designed primer pairs. (C) The identification of wt and ARKO mice, by using select and 2-9. We amplified a DNA fragment with 580 bp, which represents wtAR, and with 238 bp, which represents ARKO exon 2. The expression of Cre and internal control IL-2 were confirmed by PCR (Bottom).

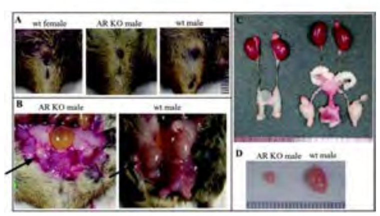


Fig. 5. Phenotype of 8-week-old male ARKO mice. Six 8-week-old male ARKO mice were killed. The results were always compared among siblings. (A) The external genitalia of male ARKO, wt male, and wt female. (B and C) The internal genitalia of male ARKO and wt male mice. Arrows in B identify the testis. (D) The testes of wt male and ARKO mice.

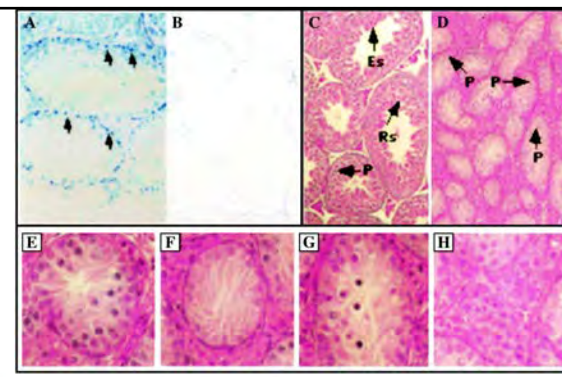


Fig. 6. Immunostaining of AR and histologic analysis of testes from wt and ARKO male mice. Immunostaining of AR in testes from wt (A) and ARKO mice (B). For AR staining, anti-AR antibody and Vectorstain ABC-AP kit were used according to the manufacturer's procedures. Testes from wt show positive signal (A, arrows), and testes from ARKO mice show no positive staining. (Å–40) The histology of the testis from wt (C) and ARKO (D) shown by hematoxylin and eosin stain. Spermatogenesis in ARKO mice arrested in pachytene spermatocyte (P) stage. (Å–40) Rs, round spermatids; Es, elongated spermatids; P, pachytene spermatocytes. In further analysis of testes in complete ARKO mice we obtained the following information (E-H): (E) The tubule contains hypoplastic spermatogonia and pachytene spermatocyte. (F) The tubule contains Sertoli cells only and shows fibrillary degeneration in the cytoplasm of Sertoli cells. (G) Some cells in the tubule contain condensed and pyknotic nuclei. They may represent apoptotic bodies. (H) Leydig cells located in interstitial space are hypertrophic.

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doi: 10.1210/me.2002-0232

Pem Homeobox Gene Promoter Sequences that Direct Transcription in a Sertoli Cell-Specific, Stage-Specific, and Androgen-Dependent Manner in the Testis *in Vivo*

MANJEET K. RAO, CHAD M. WAYNE, MARVIN L. MEISTRICH, AND MILES F. WILKINSON

Departments of Immunology (M.K.R., C.M.W., M.F.W.) and Experimental Radiation Oncology (M.L.M.), The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Although many genes are expressed selectively in Sertoli cells, regulatory sequences sufficient to drive Sertoli cell-specific expression in the postnatal and adult testis *in vivo* have not been identified. In the present study, we identified promoter sequences from the *Pem* homeobox gene that direct Sertoli cell-specific expression in an androgen-dependent and stage-specific manner. Immunohistochemical and RNA analysis demonstrated that 0.6-kb 5′-flanking sequence directed transgene expression specifically in the testis and the epididymis but not in any other tissues tested. In the adult testis, this promoter fragment targeted the transgene expression specifically to Sertoli cells during stages IV–VIII of the seminiferous epithelial cycle, thereby mimicking the expression pattern of the endogenous *Pem* gene. This promoter fragment also recapitulated *Pem*'s normal postnatal expression pattern, as it directed transcript induction between d 6 and d 9 post partum. Deletion of 0.3 kb from the 5′-end of the transgene had no effect on androgen-dependent Sertoli-specific expression but altered stage-specific expression in adult testes and caused premature postnatal expression. Our results suggest that there are at least two regulatory regions in the *Pem* proximal promoter: one that directs androgen receptor-dependent expression specifically in Sertoli cells within the testis and another that confers stage-specific expression in neonates and adults by acting as a negative regulator. To our knowledge, this is the first identification of regulatory regions that direct faithful developmentally regulated gene expression in postnatal and adult Sertoli cells *in vivo*. (*Molecular Endocrinology* 17: 223–233, 2003)

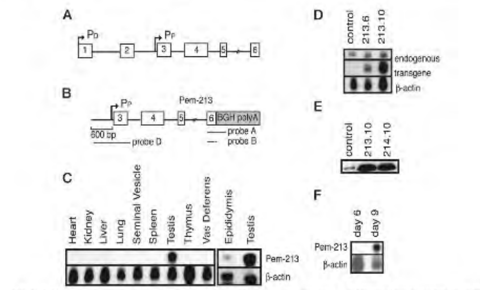


Fig. 1. A Transgene Harboring 0.6-kb *Pem Pp* 5′-Flanking Sequence Is Expressed in a Normal Developmentally Regulated Manner Specifically in Male Reproductive Tissue *in Vivo*

A, Schematic diagram of the *Pem* gene. B, Schematic diagram of the *Pem-213* transgene. Probe A corresponds to the region of the transgene shown; probe B corresponds to the endogenous *Pem* gene and thus is complementary with transgene mRNA over only a short region. C, RPA of total cellular RNA (10 μg) from adult tissues from *Pem-213.6* mice using probe A. The protected RNA fragment was approximately 200 nt. D, RPA of adult testes RNA (10 μg) from two *Pem-213* founder lines using probe B to distinguish between endogenous and *Pem* transgene mRNA. The protected band sizes were approximately 180 nt and approximately 130 nt for endogenous and transgene mRNA, respectively. E, Western blot analysis of Pem protein expression in Pem transgenic mice and control (nontransgenic) mice. The Pem band comigrated with that of recombinant Pem (data not shown). F, RPA of testes RNA (10 μg) from 6- and 9-d-old *Pem-213.6* mice using probe A. A β-actin probe was included in all annealing reactions as a loading control (the protected band was ~35 nt).

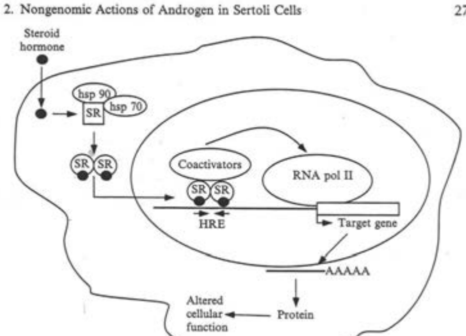
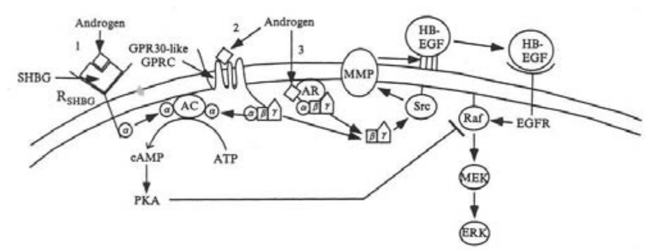


Figure 1 The classical mechanism of steroid action. Steroid hormones diffuse passively into the cell and combine with their cognate receptors in either the cytoplasm or the nucleus. In the cytoplasm, the binding of steroid to the receptor causes conformational changes in the receptor, allowing it to be released from heat shock proteins. The receptors then dimerize and migrate to the nucleus. Once in the nucleus, the steroid-bound receptor binds to specific hormone response elements (HREs) in the promoters of genes and recruits coactivator proteins that in turn alter chromatin structure and recruit RNA polymerase to the transcription initiation site. As a result, mRNA and proteins are produced that regulate cellular functions. Adapted from Onate, 2001, with permission from Humana Press, Inc.



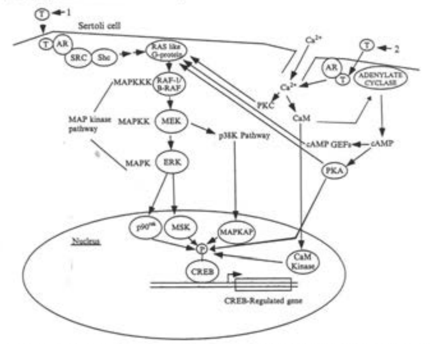
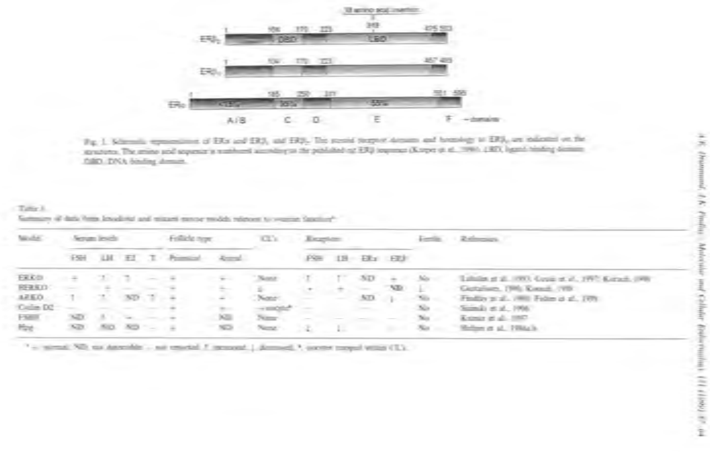
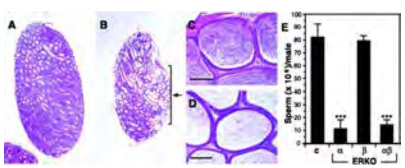


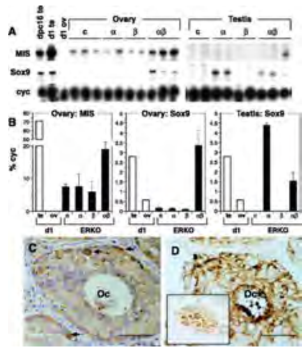
Figure 5 Potential testosterone signaling pathways in Sertoli cells: two potential pathways are proposed for testosterone-induced CREB phosphorylation. In one pathway (left side, 1), testosterone (T) binding to AR allows AR to bind with and activate Src tyrosine kinase, resulting in the stimulation Ras and Raf-1 kinase and the activation of the MAP kinase pathway. In the second pathway (right side, 2), testosterone binding to AR induces Ca^{2+} influx into Sertoli cells, causing calmodulin (CaM) to stimulate CaM kinase to translocate to the nucleus and transiently phosphorylate CREB within 1 minute. Ca^{2+} also induces a slower, more persistent pathway in which protein kinase C (PKC), guanine nucleotide exchange factors (GEFs), or PKA stimulates Ras or a Ras-like GTP binding protein, resulting in the activation of the MAP kinase pathway. Both pathways are capable of inducing CREB phosphorylation and CREB-mediated gene expression.



Postnatal Sex Reversal of the Ovaries in Mice Lacking Estrogen Receptors and

J. F. Couse, 1 S. Curtis Hewitt, 1 D. O. Bunch, 2 M. Sar, 3 V. R. Walker, 1 B. J. Davis, 4 K. S. Korach 1*





Phenotypes of αERKO and βERKO mice

Tissue	Observation: αERKO	Observation: βERKO
Testis	Progressive dilation and degradation of tubules, low sperm count, nonfunctional sperm	Normal structure, normal sperm count and fertility
Uterus	Immature, unresponsive to estradiol	Normal development and response to estrogen
Ovary	Enlarged, hemorrhagic cysts, follicles arrested at preantral stage, no corpora lutea, no ovulation, elevated serum estrogen and T levels	Subfertile, infrequent and inefficient ovulation; normal estrogen and T
Mammary, female	Ducts do not develop beyond epithelial rudiment at nipple, no alveolar development	Normal, fully functional. Able to nurse offspring
Pituitary	FSHβ, LHβ, αGSU, mRNAs all elevated, prolactin mRNA reduced	Normal serum gonadotropin levels
Cardiovascular (male)	Lower basal nitric oxide, estrogen protection in vascular injury not lost. Increase in calcium channels, delayed cardiac depolarization	?
Bone	Shorter; female, smaller diameter; male, lower density	Normal
Brain	Male, no intromission, ejaculation decreased agression; female, no receptive behaviors	Normal sexual behavior

ERKO, Estrogen receptor knockout; FSH, follicle-stimulating hormone; αGSU, gonadotrophin subunit alpha; LH, luteinizing hormone; T, testosterone.

Estrogen Deficiency, Obesity, and Skeletal Abnormalities in Follicle-Stimulating Hormone Receptor Knockout (FORKO) Female Mice*

NATALIA DANILOVICH†, P. SURESH BABU, WEIRONG XING†, MARIA GERDES, HANUMANTHAPPA KRISHNAMURTHY, AND M. RAM SAIRAM

Molecular Reproduction Research Laboratory, Clinical Research Institute of Montréal, Montréal, Québec H2W 1R7, Canada

TABLE 2. Examination of vaginal cytology

Genotype	Length of estrous cycle (days)	Duration of estrus (days)	Presence of epithelial and cornified cells
FORKO	None	None	Occasional
Heterozygous	6.6 ± 3.5[a]	1.5 ± 0.2	Normal
Wild-type	4.4 ± 0.3	2.2 ± 0.4	Normal

Values are expressed as mean SEM. [a], $P < 0.05$.

TABLE 1. Breeding performance

Number of animals	Male × Female	Period between mating and first litter (days)	Number of pups in first litter	Weaning success in first litter (%)
7 × 7	+/+ × +/+	21.4 ± 0.2	9.8 ± 0.5	95.9 ± 0.7
10 × 10	+/+ × +/−	32 ± 2.5[a]	5.6 ± 0.9[a]	75 ± 1.5[a]
16 × 16	+/− × +/−	38.2 ± 3.3[a]	4.7 ± 2.3[a]	68.7 ± 2.5[a]

Weaning success = animals surviving on day 21. All results are expressed as mean SEM.
[a], $P < 0.05$.

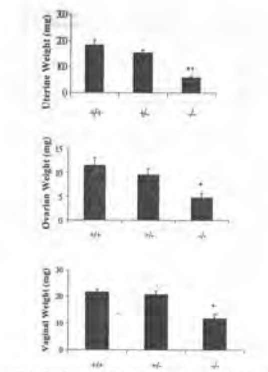


FIG. 2. Growth status of reproductive tissues in wild-type, heterozygous, and FORKO female mice. The wet weight of two ovaries, uterus, and vagina (mg) in +/+ (wild-type), +/− (heterozygous), and −/− (FORKO) are shown. Values represent the mean ± SEM for 10 animals per genotype. Comparisons were made against wild-type animals. *, $P < 0.007$, **, $P < 0.001$.

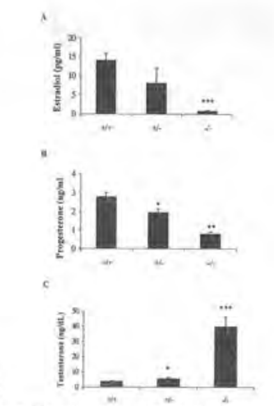
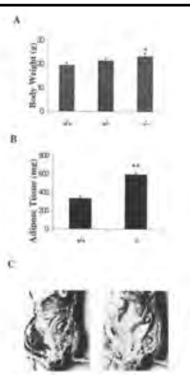
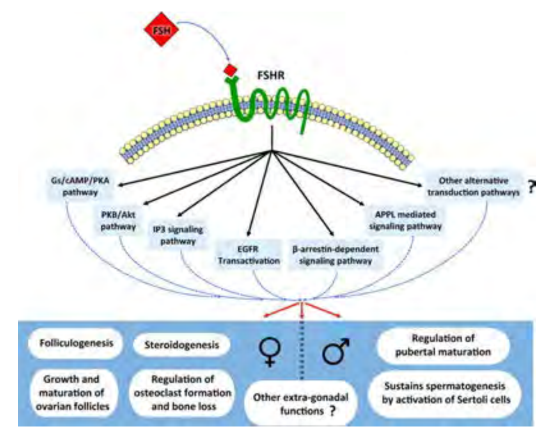


FIG. 3. Steroid hormone levels in serum of wild-type, heterozygous, and FORKO female mice. The levels of estradiol-17β (pg/ml) (A), progesterone (ng/ml) (B), and total testosterone (ng/dl) (C) of individual serum samples of +/+ (wild-type), +/− (heterozygous), and −/− (FORKO) mice determined by respective RIAs are shown. Values represent the mean ± SEM for 7–10 animals per genotype. Comparisons were made against wild-type animals. *, $P < 0.05$, **, $P < 0.005$, ***, $P < 0.002$.





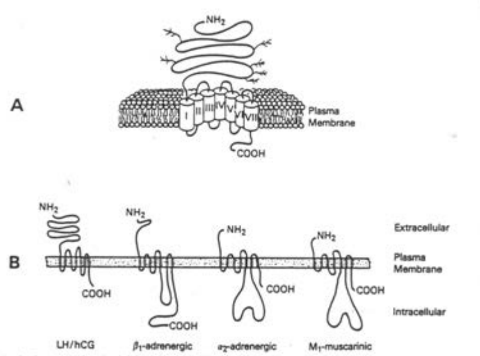


Figure 4–16 ■ Structure of the luteinizing hormone (LH) receptor.
A. General structure of extracellular region and transmembrane domains.
B. Comparison of LH receptor with other G protein–coupled receptors indicating the differences in extracellular regions and third intracellular loops.

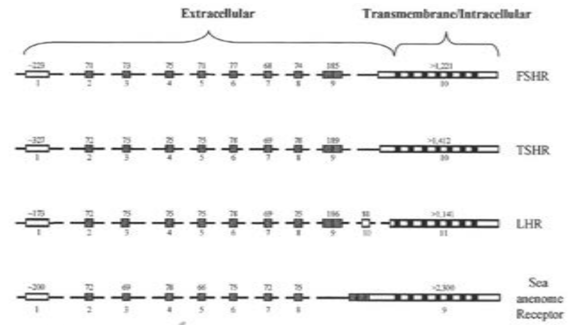


FIG. 1. Comparison of the gene structures for the glycoprotein hormone receptors and sea anemone receptor. Exons are depicted as boxes with their number given below and their size in bp given above. Hatched boxes represent leucine-rich repeats and the black boxes, the seven membrane-spanning domains. For the glycoprotein hormone receptor genes, the structures were derived from the rat FSHR (Heckert *et al.*, 1992), human TSHR (Gross *et al.*, 1991), and rat LHR (Koo *et al.*, 1991; Tsai-Morris *et al.*, 1991). The gene structure for the receptor from sea anemone was derived from studies by Vibede *et al.* (1998) (Accession nos. AF084384-AF084390).

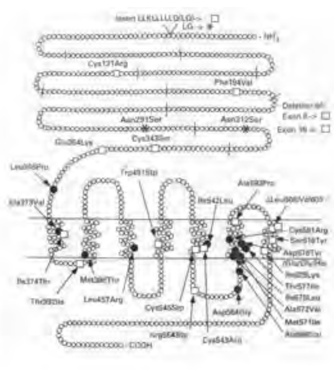


Fig. 3. Locations of the currently known mutations of the human *LHR*. □ = Inactivating mutations; ● = activating mutations; * = polymorphisms. The short lines across the peptide chain depict the exon boundaries [17].

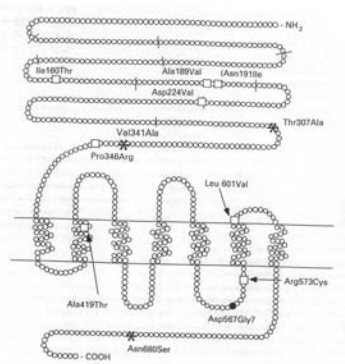


Fig. 4. Schematic structure and currently known mutations of the human *FSHR*. □ = Inactivating mutations; ● = activating mutations; * = polymorphisms. The Asn191Ile mutation has been found only in a heterozygous form in a normal subject. The activating Asp567Gly mutation has marginal constitutive activity in vitro. The short lines across the peptide chain depict the exon boundaries [17].

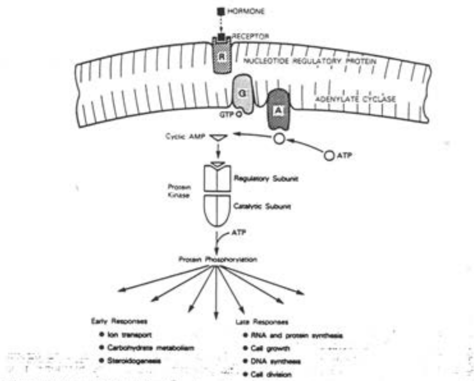


Figure 4–15 ■ Hormonal stimulation of adenylate cyclase in peptide-regulated t... Increased production of adenosine monophosphate (cAMP) leads to activation of protein kinase and pho...rylation of regulatory protei... strates involved in the early and late aspects of hormone action.

Table 1. Natural Loss of Function Mutations of the FSH Receptor: Correlation between Receptor Function and the Phenotype

	Finnish Patients[a]	Patient 2	Patient 1[b]
Pubertal development	± Delayed	Normal	Normal
Amenorrhea	Primary	Primary	Secondary
E_2 (pmol/liter)			
Basal	30	40–80	70–150
After FSH stimulation	NA	40–80	240
Inhibin B (pg/ml)			
Basal	NA	30	50
After FSH stimulation	NA	30	125
Ultrasonography:			
Follicle size (mm)			
Basal	NA	2-3	4-5
After FSH stimulation	NA	2–3	5–8.3
Follicular development	Primordial and primary follicles	Antral follicles	Antral follicles
In vitro activity of FSHR mutants (adenylate cyclase stimulation, % of wild-type receptor)	Nonsignificant	12 ± 3% Leu601Val	24 ± 4% Arg573Cys

NA, Not available.
[a] Ref. 4.
[b] Ref. 8.

Normal Prenatal but Arrested Postnatal Sexual Development of Luteinizing Hormone Receptor Knockout (LuRKO) Mice

Fu-Ping Zhang, Matti Poutanen, Johannes Wilbertz, and Ilpo Huhtaniemi

Department of Physiology (F.-P.Z., M.P., I.H.)
Institute of Biomedicine
University of Turku
FIN-20520, Turku, Finland

Department of Cell and Molecular Biology (J.W.)
Karolinska Institute
S-17177 Stockholm, Sweden

To study further the role of gonadotropins in reproductive functions, we generated mice with LH receptor (LHR) knockout (LuRKO) by inactivating, through homologous recombination, exon 11 on the LHR gene. LuRKO males and females were born phenotypically normal, with testes, ovaries, and genital structures indistinguishable from their wild-type (WT) littermates. Postnatally, testicular growth and descent, and external genital and accessory sex organ maturation, were blocked in LuRKO males, and their spermatogenesis was arrested at the round spermatid stage. The number and size of Leydig cells were dramatically reduced. LuRKO females also displayed underdeveloped external genitalia and uteri postnatally, and their age of vaginal opening was delayed by 5–7 days. The (−/−) ovaries were smaller, and histological analysis revealed follicles up to the early antral stage, but no preovulatory follicles or corpora lutea. Reduced gonadal sex hormone production was found in each sex, as was also reflected by the suppressed accessory sex organ weights and elevated gonadotropin levels. Completion of meiosis of testicular germ cells in the LuRKO males differs from other hypogonadotropic/cryptorchid mouse models, suggesting a role for FSH in this process. In females, FSH appears to stimulate developing follicles from the preantral to early antral stage, and LH is the stimulus beyond this stage. Hence, in each sex, the intrauterine sex differentiation is independent of LH action, but it has a crucial role postnatally for attaining sexual maturity. The LuRKO mouse is a close phenocopy of recently characterized human patients with inactivating LHR mutations, although the lack of pseudohermaphroditism in LuRKO males suggests that the intrauterine sex differentiation in this species is not dependent on LH action. (Molecular Endocrinology 15: 172–183, 2001)

Molecular Endocrinology 15(1): 172–183
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INTRODUCTION

The two gonadotropins, LH and FSH, have a key role in the differentiation and maturation of mammalian sexual organs and functions. After identification of genes for the gonadotropin subunits and gonadotropin receptors (R), their mutations have been discovered in males and females with various types of hypogonadism (for a review, see Ref. 1). Mutations of the FSHβ subunit gene cause infertility with arrested follicular maturation in women and azoospermia in men. Inactivating FSHR mutations in women cause the same phenotype as the ligand mutations, but in men the phenotype is milder with only variable impairment of spermatogenesis (1). Knockout models for both FSHβ and FSHR have been produced (2–4), and they display complete phenocopies of the human FSHR mutations. A discrepant finding is the azoospermia detected in the two men so far described with FSHβ mutation (5, 6), which is not found in the receptor or ligand knockout mice produced (2–4) or in men with an inactivating FSHR mutation (7). Hence, the necessity of FSH for spermatogenesis still remains controversial.

The consequences of inactivation of LH action also remains to be clarified. Only a single man with LHβ mutation has been reported (8); he presented with normal sexual differentiation at birth but total lack of postnatal sexual development. No women with such a mutation have yet been described. Neither are there knockout models for LHβ or LH receptor (LHR). More is known about consequences of inactivating LHR mutations in man (1). Depending on completeness of the

Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the *cyp19* gene

Carolyn R. Fisher, Kathy H. Graves, Albert F. Parlow, and Evan R. Simpson

Table 2. Organ weights, g

	Wild type	ArKO	
Females			
Uterus	[illegible]	[illegible]	[illegible]
Clitoral gland	[illegible]	[illegible]	[illegible]
Males			
Testes	[illegible]	[illegible]	NS
Seminal vesicles	[illegible]	[illegible]	[illegible]
Bladder/prostate	[illegible]	[illegible]	[illegible]

Mice ranging in age from 12–14 weeks were sacrificed, and organs were removed, blotted, and weighed on an analytical balance. Values are reported as means ± SEM. Significance was determined by using the Student's *t* test. NS, not significant.

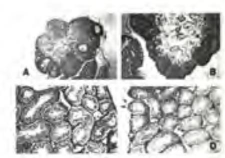

FIG. 3. Optical micrographs of ovaries (A and B) and testes (C and D) of wild-type (A and C) and ArKO (B and D) mice at 12–14 weeks of age. [illegible]

Table 4. Serum gonadotropin concentrations

	Females							Males			
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Wild type	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ND	[illegible]	[illegible]	[illegible]
ArKO	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	ND	[illegible]	[illegible]	[illegible]

Gonadotropins were measured in wild-type female mice that were in estrus as evidenced by labial swelling and reddening of vaginal membranes. ND, not determined.

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Developmental Biology

Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp 19*) gene

Kirsten M. Robertson*, Liza O'Donnell*, Margaret E. E. Jones*, Sarah J. Meachem*, Wah Chin Boon*, Carolyn R. Fisher†, Kathy H. Graves‡, Robert I. McLachlan*, and Evan R. Simpson*§

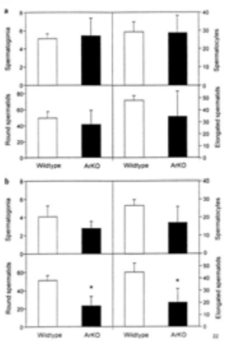


Figure 3.

Germ cells numbers (millions per testis) in ArKO and w/t mice at the two age groups. (*a*) The numbers of spermatogonia, spermatocytes, round spermatids, and elongated spermatids did not differ in the 4.5-month-old ArKO mice as compared with the w/t mice. (*b*) No significant decreases were seen in spermatogonia ($P = 0.06$) and spermatocyte populations ($P = 0.08$) in 1-year-old ArKO mice; however, there were significantly less round and elongated spermatids present in the epithelium of the ArKO mice ($P < 0.05$). Data expressed as mean ± SD.

Table 1. Morphometric data on testicular compartments

Mice	Testis weight, mg	Volume lumen, mm^3	Volume epithelium, mm^3	Volume interstitium, mm^3	Volume Leydig cells, mm^3	Tubule diameter, μm	Tubule length, m
w/t, 4 1/2 mth (*n* = 6)	137.4 ± 6.8	10.2 ± 3.0	106.1 ± 7.7	16.7 ± 3.8	ND	236.1 ± 7.0	2.8 ± 0.3
ArKO, 4 1/2 mth (*n* = 5)	113.4 ± 37.2	8.2 ± 2.8	85.1 ± 29.3	15.2 ± 7.4	ND	229.1 ± 34.9	2.4 ± 1.0
w/t, 1 yr (*n* = 4)	134.5 ± 12.9	16.9 ± 7.4	96.0 ± 10.1	15.8 ± 1.4	3.78 ± 0.43	247.2 ± 11.9	2.4 ± 0.3
ArKO, 1 yr (*n* = 7)	101.5 ± 27.3*	12.4 ± 7.1	63.6 ± 19.1*	21.4 ± 8.2	7.50 ± 2.56*	214.0 ± 31.3	2.0 ± 0.8

There were no differences in the absolute volumes of the various compartments of the testis in the 4.5-month-old ArKO mice compared to w/t. In 1-year-old ArKO mice the absolute volume of seminiferous epithelium was decreased by 34% ($P < 0.05$), and the absolute volume of Leydig cells was increased by 50% ($P < 0.05$). Testicular weight was decreased in the 1-year-old ArKO mice ($P < 0.05$). Data expressed as mean ± SD.
ND = not determined.
*$P < 0.05$.

An Age-Related Ovarian Phenotype in Mice with Targeted Disruption of the *Cyp 19* (Aromatase) Gene*

KARA L. BRITT, ANN E. DRUMMOND, VICTORIA A. COX, MITZILEE DYSON, NIGEL G. WREFORD, MARGARET E. E. JONES, EVAN R. SIMPSON, AND JOCK K. FINDLAY

Prince Henry's Institute of Medical Research and Department of Anatomy, Monash University (N.G.W.), Clayton, Victoria 3168, Australia

ABSTRACT

With the development of a mouse model of estrogen insufficiency due to targeted disruption of the aromatase gene [the aromatase knockout (ArKO) mouse], a new opportunity exists to examine the role of estrogen in ovarian follicular development. Ovaries and serum were collected from wild-type, heterozygous, and ArKO mice at 10–12 and 21–23 weeks and 1 yr of age. The ovaries were assessed histologically and stereologically, with primary, secondary, and antral follicles and corpora lutea counted. The uteri were hypoestrogenic, and serum levels of LH and FSH in ArKO females were elevated above those in heterozygote and wild-type animals at all ages studied. Although estrogen was not a prerequisite for reinitiation of follicle growth, there was a block of follicular development, and no corpora lutea were present in ArKO ovaries. Thus, the ArKO mouse was infertile as a consequence of disrupted folliculogenesis and a failure to ovulate. Hemorrhagic cystic follicles were present by 21–23 weeks of age. The ovarian phenotype degenerated with age, such that by 1 yr there were no secondary or antral follicles, and the primary follicles present were atretic. Extensive interstitial tissue remodeling occurred, exemplified by an influx of macrophages and collagen deposition, coincident with the loss of follicles. In conclusion, the ovarian environment in ArKO mice does not allow the characteristic development of follicles that culminates in ovulation and demonstrates an *in vivo* requirement of estrogen for normal ovarian function in the mouse. (*Endocrinology* **141:** 2614–2623, 2000)

TABLE 1. Uterine weight (milligrams) as a function of age and genotype

Age	Wild-type	Heterozygote	ArKO
10–12 weeks	70.6 ± 4.0[a,b,c]	86.9 ± 8.8[a,c]	53.7 ± 11.3[b]
21–23 weeks	94.2 ± 7.8[c,d]	127.8 ± 27.5[c,d]	*
1 yr	123.3 ± 13.3[a,d]	140.1 ± 51.4[a,d]	41.3 ± 4.6[b]

Mean ± SEM (n = 5–22 organs/group). * Uterine horns were not collected from ArKO mice at 21–23 weeks of age. Means with different letters denote statistical significance. Within a genotype across the age groups, [c] and [d] denote statistical significance; within an age group across the genotypes, [a] and [b] denote statistical significance ($P < 0.02$–0.001 relative to the appropriate control).

TABLE 2. Numbers of corpora lutea per ovary in wild-type, heterozygote, and ArKO mouse ovaries

Age	Wild type	Heterozygote	ArKO
10–12 weeks	8.25 ± 0.5 (4)	5.70 ± 0.7 (3)	0 (4)
21–23 weeks	5.70 ± 0.7 (3)	3.70 ± 0.9 (3)	0 (3)
1 yr	5.70 ± 1.3 (3)	7.00 ± 1.2 (3)	0 (4)

Values are the mean ± SEM; the number of animals is in *parentheses*.

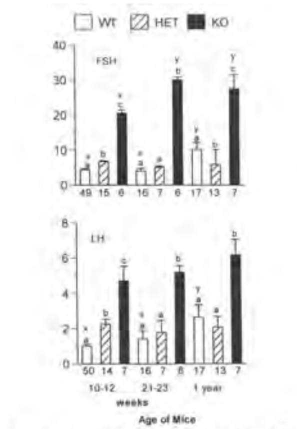


FIG. 1. Serum levels of LH and FSH collected from wild-type, ArKO, and heterozygous mice, at 10–12 and 21–23 weeks and 1 yr of age (mean ± SEM). *Different letters* denote statistical significance: a, b, and c operate within an age group across genotypes, and x and y operate within a genotype across age groups. $P < 0.05$ *vs.* the appropriate control. The number of animals per group is indicated directly below the histograms. All mice were in a stage of the cycle other than estrus.

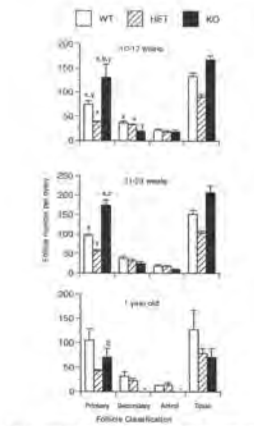


FIG. 5. Effects of genotype and age on ovarian follicle numbers (n = 3–4 ovaries/group, except for the 1-yr-old wild-type antral follicle group, where only two of the three ovaries counted contained antral follicles). *, No follicles of this type were present in the ovary. *Different* letters denote statistical significance: x, y, and z operate within an age group across the genotypes, and a and b operate within a genotype between age groups. $P < 0.05–0.001$ vs. the appropriate control.

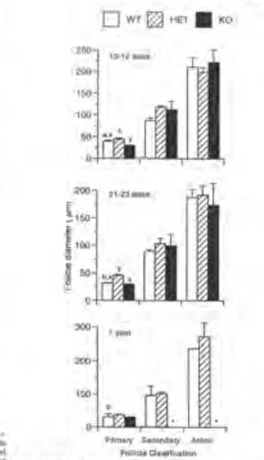


FIG. 6. Effects of genotype and age on oocyte nuclear diameter (n = 3–4 ovaries/group, except for the 1-yr-old wild-type antral follicle group, where only two of the three ovaries counted contained antral follicles). *, No follicles of this type were present in the ovary. *Different* letters denote statistical significance: x and y operate within an age group across the genotypes, and a and b operate within a genotype between age groups. $P < 0.05–0.005$ vs. the appropriate control.

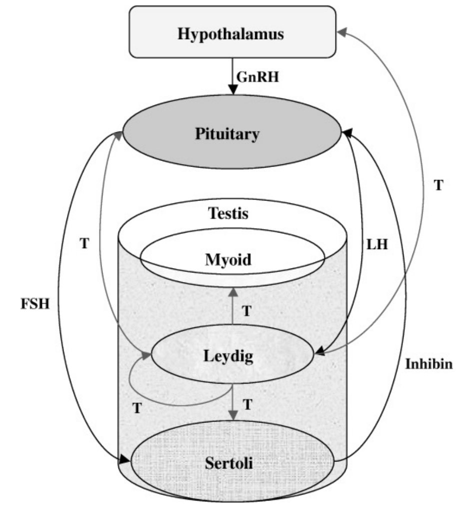


Figure 1.
Hormonal regulation of spermatogenesis Most hormones shown can have both positive and negative effects, either at the level of receptor activation/desensitization or through activation and repression of downstream targets. GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone.

Neuroendocrine

Discovery of LHRH and development of LHRH analogs for prostate cancer treatment.

Prostate. 2017 Jun;77(9):1036-1054.
Schally AV, Block NL, Rick FG.

Abstract
The discovery, isolation, elucidation of structure, synthesis, and initial testing of the neuropeptide hypothalamic luteinizing hormone-releasing hormone (LHRH), which regulates reproduction, is briefly described. The design, synthesis, and experimental and clinical testing of agonistic analogs of LHRH is extensively reviewed focusing on the development of new methods for the treatment of prostate cancer. Subsequent development of antagonistic analogs of LHRH is then faithfully recounted with special emphasis on therapy of prostate cancer and BPH. The concepts of targeted therapy to peptide receptors on tumors are re-examined and the development of the cytotoxic analogs of LHRH and their status is reviewed. The endeavor to develop better therapies for prostate cancer, based on LHRH analogs, guided much of our work.

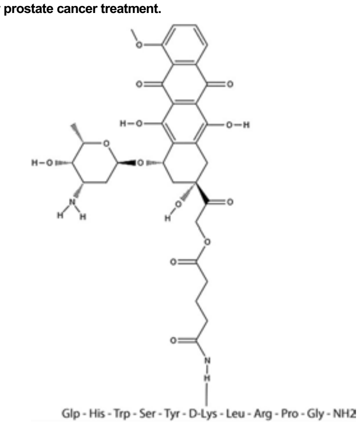
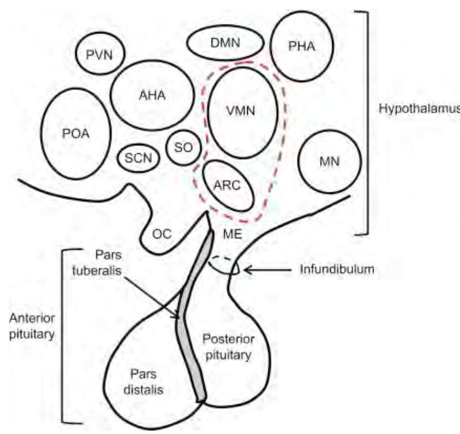


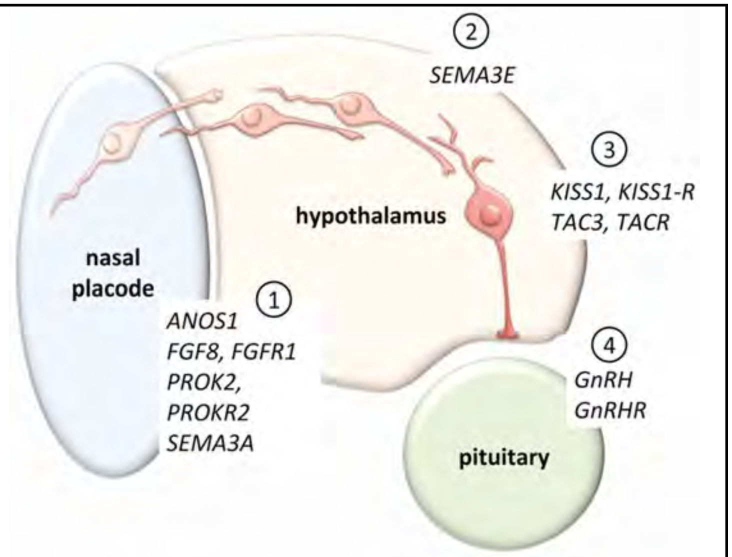
TABLE 1 Structures of agonistic analogs of LHRH in clinical use

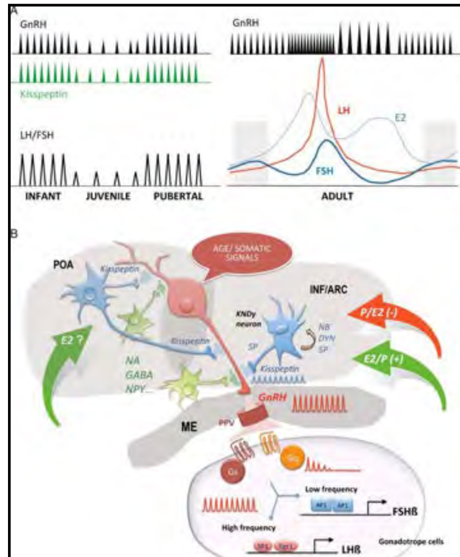
Generic name (brand name)	Chemical structure
leuprolide (Lupron, Eligard)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DLeu^{6}-Leu^{7}-Arg^{8}-Pro^{9}- NHEt
triptorelin (Decapeptyl, Gonapeptyl, Trelstar LA)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DTrp^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}-NH2
buserelin (Suprefact, Suprecor)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DSer(tBu)^{6}-Leu^{7}-Arg^{8}-Pro^{9}-NHEt
goserelin (Zoladex)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DSer(tBu)^{6}-Leu^{7}-Arg^{8}-Pro^{9}-AzGly^{10}-NH2
nafarelin (Synarel)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DNal^{6}-Leu^{7}-Arg^{8}-Pro^{9}- NHEt
histrelin (Supprelin LA, Vantas)	pGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-DHis(Bzl)^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}-NH2

pGlu, pyroglutamic acid; His, histidine; Trp, tryptophan; Ser, serine; Tyr, tyrosine; DLeu, D-leucyl; NHEt, *N*-ethylamide; Gly, glycine; Leu, leucine; Arg, arginine; Pro, proline; DSer(tBu), D-seryl terbutyl; AzGly, Azgly stands for glycine in which the α-CH has been replaced by a nitrogen atom; DNal, D-naphthyl-alanyl; DTrp, D-tryptophyl; DHis(Bzl)$_{6}$, N-benzyl-D-histidine.

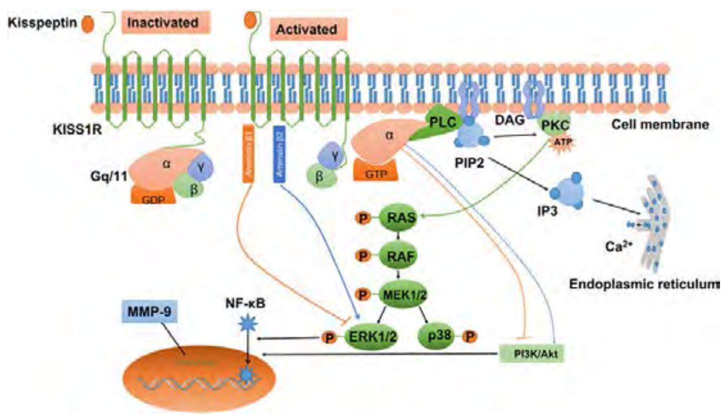


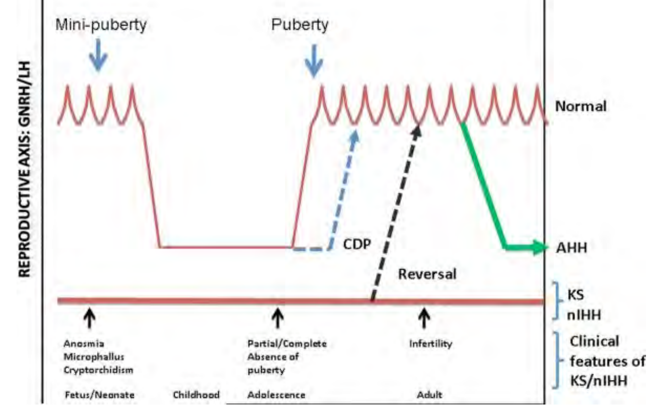
Hypothalamic and pituitary anatomy. Sagittal view of mammalian hypothalamic and pituitary anatomy. The mediobasal hypothalamus is encompassed by red dashed lines. The pars tuberalis, part of the anterior pituitary, is shaded in gray. AHA, anterior hypothalamic area; ARC, arcuate nucleus; DMN, dorsomedial nucleus; ME, median eminence; MN, mammillary nuclei; OC, optic chiasm; POA, preoptic area; PHA, posterior hypothalamic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SO, supraoptic nucleus; VMN, ventromedial nucleus.





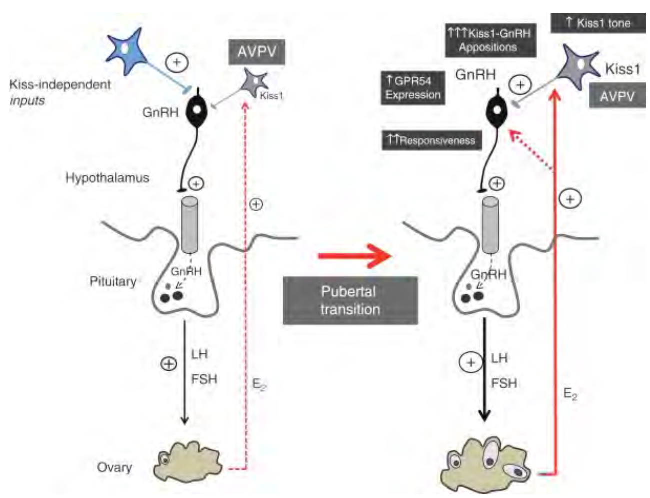
(A) Representation of the variations in pulsatile secretion of GnRH during the human female lifespan. **(B)** Summary of the physiological mechanisms possibly involved in the control of GnRH secretion and its action on gonadotrope cells. (POA: preoptic area; INF: infundibular region; ARC: arcuate nucleus; ME: median eminence; NPY: neuropeptide Y; GABA: gamma aminobutirric acids; NA: noradrenaline; NB: neurokinin B; DYN: dynorphin; SP: substance P; E2: estradiol; P: progesterone; SF1: steroidogenic factor 1 transcription factor; Egr1: early growth response 1 transcription factor; AP1: activating protein 1 transcription factor; Gs: Gs alpha subunit; Gq: Gq/11 subunit).

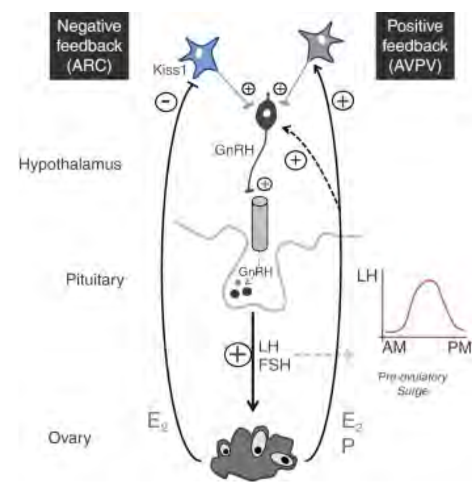




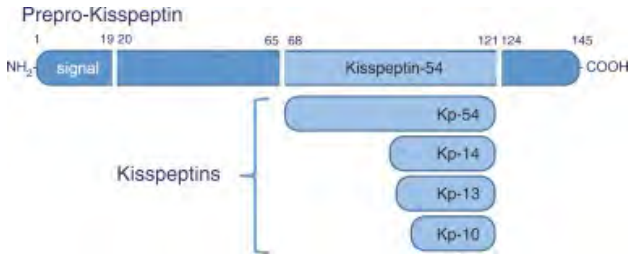
Clinical presentations of isolated GnRH deficiency in humans

Pulsatile GnRH secretion is initiated during the late fetal/early neonatal period ("mini-puberty"), followed by quiescence during childhood and reawakening of the pulsatile secretion in mid-childhood. Presence of anosmia signals a developmental defect in GnRH neuronal migration while, microphallus or cryptorchidism signal fetal/neonatal lack of GnRH secretion. Constitutional delay of puberty (CDP) represents a late activation of the HPG axis while KS and nIHH represent partial or complete failure of pulsatile GnRH secretion. Recovery of pulsatile GnRH secretion in KS/nIHH subjects in adulthood is termed "Reversal", while, AHH (adult-onset hypogonadotropic hypogonadism) refers to the onset of isolated GnRH deficiency during adulthood following a normal mini-puberty and puberty.

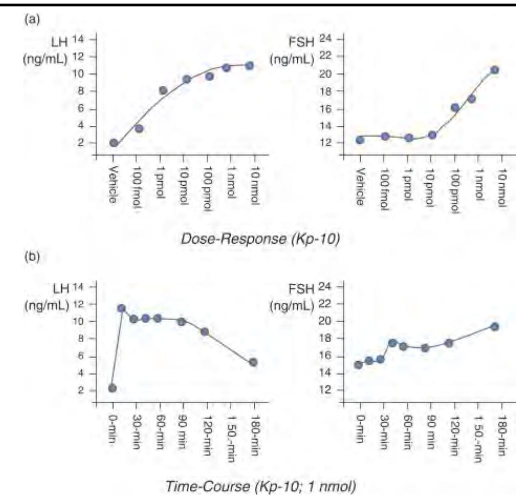




Physiological roles of the kisspeptin/GPR54 system in the neuroendocrine control of reproduction.
Pineda R, Aguilar E, Pinilla L, Tena-Sempere M.
Prog Brain Res. 2010;181:55-77.

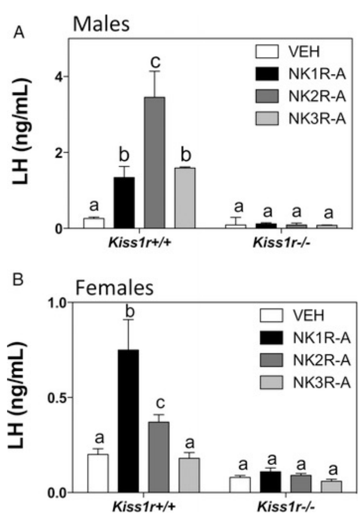


Structure of kisspeptins – the peptide products of the KISS1 gene. Different kisspeptins are generated by proteolytic cleavage from a common precursor of 145 amino acids, prepro-kisspeptin, which contains a 19-amino-acid signal peptide and a central 54-amino-acid region, kisspeptin-54 (Kp-54; formerly termed metastin). Lower-molecular-weight forms of kisspeptins include Kp-14, Kp-13 and Kp-10; the latter corresponds to the common C-terminal 10-amino-acid stretch that contains the RFamide motif and is sufficient to activate GPR54. Adapted from Roa et al. (2008a), with substantial modifications.



Prototypical gonadotropin responses to kisspeptin stimulation in rats. Schematic illustrations are presented of the patterns of LH and FSH responses to intra-cerebral administration of Kp-10. Both dose-dependent (panel A) and time-dependent (panel B) gonadotropic responses are shown. Hormonal values are adapted from original data from Navarro et al., 2005a and Navarro et al., 2005b and our unpublished observations.

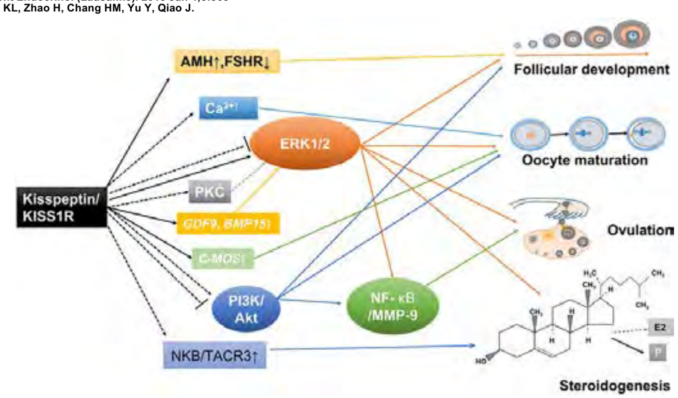
The integrated hypothalamic tachykinin-kisspeptin system as a central coordinator for reproduction.
Navarro VM, Bosch MA, León S, et al.
Endocrinology. 2015 Feb;156(2):627-37.



Serum LH levels in WT (*Kiss1r*$^{+/+}$) and *Kiss1r*$^{-/-}$ adult **(A)** male and **(B)** diestrous female mice 20 minutes after central injection of 600 pmol GR73632 (NK1R-A), GR64349 (NK2R-A), or senktide (NK3R-A).

Two-way ANOVA + Bonferroni's post hoc test. Different letters indicate significant differences between groups ($P < .05$).

Kisspeptin/Kisspeptin Receptor System in the Ovary.
Front Endocrinol (Lausanne). 2018 Jan 4;8:365
Hu KL, Zhao H, Chang HM, Yu Y, Qiao J.



Neuroendocrine regulation of gonadotropins in the male and the female

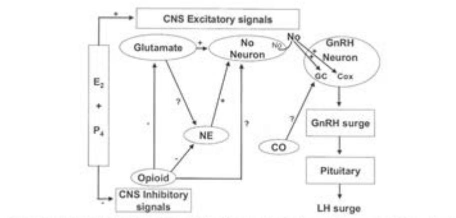


Fig. 5. A proposed model for central role of NO in the preovulatory LH surge (Brann et al., 1997, modified). GC = guanylate cyclase, Cox = cyclooxygenase, NE = norepinephrine, E_2 = estradiol-17β, P_4 = progesterone.

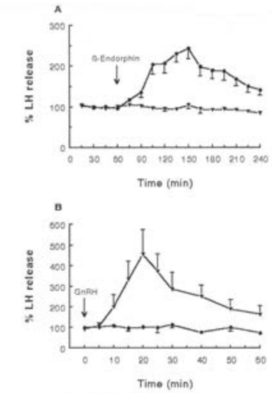
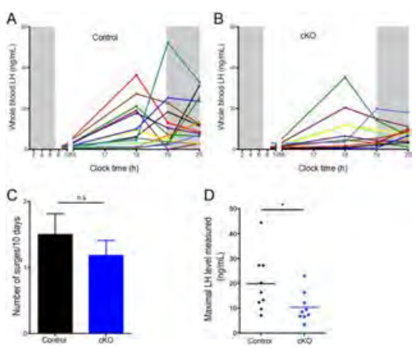


Fig. 4. In vitro LH release from perfused pituitaries (female pigs) in response to β-endorphin (●, panel A). Pituitaries were challenged with GnRH 240 min after β-endorphin (panel B). (▼) Saline + GnRH, (●) saline and β-endorphin.

Impaired LH surge amplitude in gonadotrope-specific progesterone receptor knockout mice.

Toufaily C, Schang G, Zhou X, Wartenberg P, Boehm U, Lydon JP, Roelfsema F, Bernard DJ.

J Endocrinol. 2020 Jan;244(1):111-122.

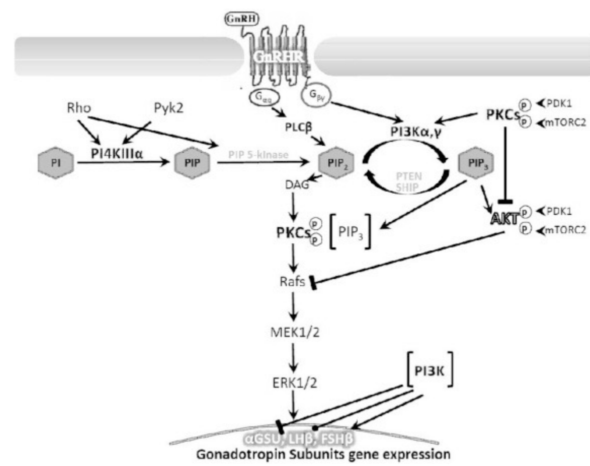


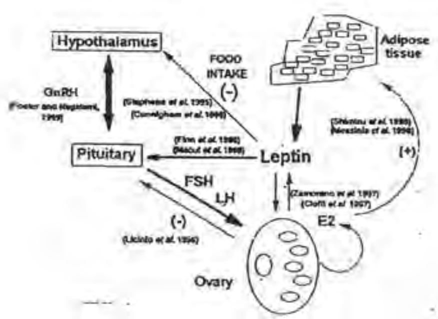
The LH surge is blunted in *Pgr*-knockout females. Blood samples were collected four times daily for 10 consecutive days. Representative profiles of the LH secretion obtained on proestrus from control (A) and cKO (B) female mice. Different colors indicate different mice. Gray areas represent the dark phase of the light/dark cycle. (C) Number of surges observed in each mouse during the 10 days of the experiment. (D) Maximal LH levels measured on proestrus from control ($n = 9$) and cKO ($n = 9$) females. Student *t*-tests were performed for statistical analysis. $*P < 0.05$. n.s, non-significant. Note: maximal values in panel D are lower than in panels A and B because averages were used in panel D in mice that surged more than once (see 'Methods' section).

Role of PI4K and PI3K-AKT in ERK1/2 activation by GnRH in the pituitary gonadotropes.

Bar-Lev TH, Harris D, Tomić M, et al.

Mol Cell Endocrinol. 2015 Nov 5;415:12-23.





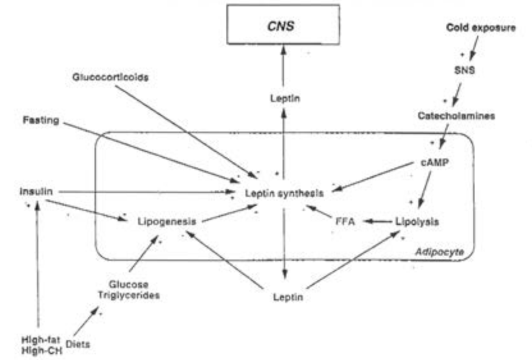


Figure 1 Regulation of leptin production in white adipose tissue. CNS, central nervous system; SNS, sympathetic nervous system; FFA, free fatty acids; cAMP, cyclic adenosine monophosphate; CH, carbohydrate.

Clinical Physiology 18, 5, 399–419 • © 1998 Blackwell Science Ltd

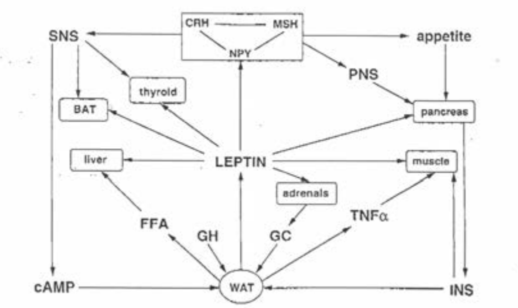


Figure 2 Postulated interrelationships between leptin and different organs. CNS, central nervous system; NPY, neuropeptide Y; CRH, corticotropin-releasing hormone; MSH, melanocyte-stimulating hormone; SNS, sympathetic nervous system; PNS, parasympathetic nervous system; BAT, brown adipose tissue; FFA, free fatty acids; GH, growth hormone; GC, glucocorticoids; TNF-α, tumour necrosis factor α; cAMP, cyclic adenosine monophosphate; WAT, white adipose tissue; INS, insulin.

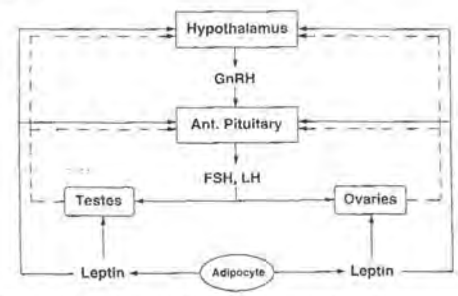


Figure 3 Diagram of the probable involvement of leptin in the hypothalamic–pituitary–gonadal axis. Solid lines indicate enhancement of endocrine secretion; dashed lines indicate hormone release suppression. GnRH, gonadotropin-releasing hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

M.L. Barreiro, M. Tena-Sempere / Molecular and Cellular Endocrinology 226 (2004) 1–9

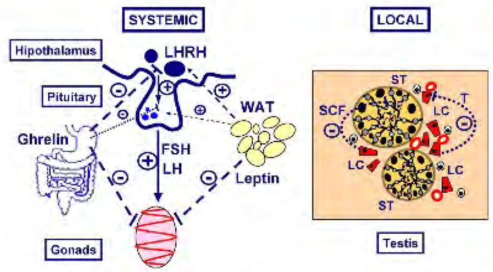
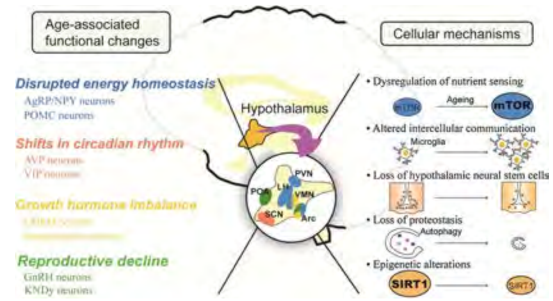


Fig. 1. Tentative model for the 'reproductive' actions of ghrelin, involving two distinct, but probably partially overlapping systems. In the left panel, the potential SYSTEMIC actions of gut-derived circulating ghrelin at different levels of the hypothalamic–pituitary–gonadal axis are depicted. Through these systemic effects, ghrelin likely cooperates with other relevant peripheral signals with key roles in energy homeostasis (e.g. the adipocyte-derived hormone leptin) in the regulation of reproductive function. In addition, LOCAL expression of ghrelin is detected in the gonads. In the right panel, a schematic presentation of the sites of expression and direct biological actions of ghrelin within the testis are presented. In human and rat testis, ghrelin is produced with high selectivity in mature Leydig cells (LC), whereas its putative receptor, the GHS-R1a, presents a wider patter of cellular expression, including Sertoli cells within the seminiferous tubules (ST), and Leydig cells. Direct actions of ghrelin in the testis include inhibition of testosterone (T) secretion (steroidogenesis), and probably, regulation of tubular functions, such as expression of stem cell factor (SCF) gene.

Role of hypothalamus in aging and its underlying cellular mechanisms.
Kim K, Choe HK.
Mech Ageing Dev. 2019 Jan;177:74-79.



The hypothalamus as a regulator of systemic aging. We propose a working model that the hypothalamus controls several aspects of systemic aging. Here, an age-dependent decline in physiological functions, including disruption of energy homeostasis, shifts in the circadian rhythm, imbalance in GH levels, and decline in reproduction, is mediated through age-associated changes in the master regulatory neurons, such as the AgRP/NPY, POMC, AVP, VIP, GHRH, SST, GnRH, and KNDy neurons. Notably, the hypothalamus is also a region where a majority of molecular pathways implicated in aging, such as nutrient sensing, inflammation, neural stem cell, proteostasis, and epigenetic regulation, are altered with aging.

Endocrine Disruptors

PII: S0045-6535(99)00190-3

OVERVIEW OF ENDOCRINE DISRUPTOR RESEARCH ACTIVITY IN THE UNITED STATES

R.J. Kavlock

Reproductive Toxicology Division (MD-71)
National Health and Environmental Effects Research Laboratory
US Environmental Protection Agency
Research Triangle Park, NC 27711, USA

ABSTRACT

The issue of whether environmental contaminants are inducing adverse health effects in humans and wildlife via interaction with endocrine systems has gained increasing interest during the 1990s. Endocrine disruption is one of the highest priority research topics for the US EPA, and a detailed research strategy has been developed to guide the placement of resources over the next several years. To address the deficiency of testing guidelines in detecting and characterizing damage mediated by interaction with the endocrine system, EPA has issued new multi-generation testing guidelines. The new endpoints for monitoring pubertal development, semen quality, and estrous cyclicity will better enable determination of the affected sex, target organ, and life stage following exposure throughout the life cycle. Another major area of effort within EPA is the development of an endocrine disruptor screening program in response to passage of the Food Quality Protection Act of 1996. The current status of these efforts is described. On the federal level, endocrine disruption is one of the five priority research areas for the Committee on the Environment and Natural Resources (CENR) within the Executive Office of President. The CENR has developed a framework to assess research needs for endocrine disruptors, inventoried existing efforts of the federal government (nearly 400 projects were identified as active in FY96), and prioritized additional research needs based upon the needs and gaps in current efforts. It is clear that a great deal of research is underway to clarify the validity of the endocrine disruptor hypothesis and to determine the breadth of chemicals that pose a risk to the endocrine system. The degree of forward research planning and coordination across many organizations should ensure that sufficient data will be available within the next few years to allow a rigorous weight of evidence evaluation that is needed to bring together diverse types of information to make informed decisions regarding risks to humans and wildlife.

INTRODUCTION

The issue of whether humans and domestic and wildlife species have suffered adverse health consequences resulting from exposure to environmental chemicals that interact with the endocrine system has gained increasing prominence throughout the 1990s. However, considerable uncertainty exists regarding the relationship(s) between adverse health outcomes and exposure to environmental contaminants. Collectively, chemicals with the potential to interfere with the function of endocrine systems are called endocrine disrupting

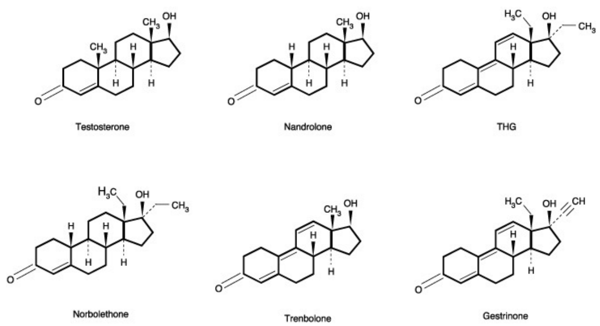
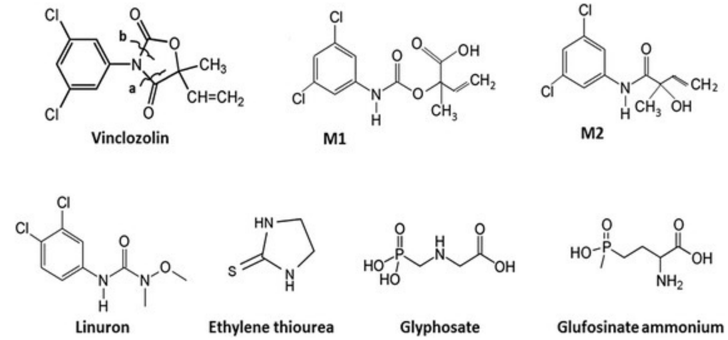
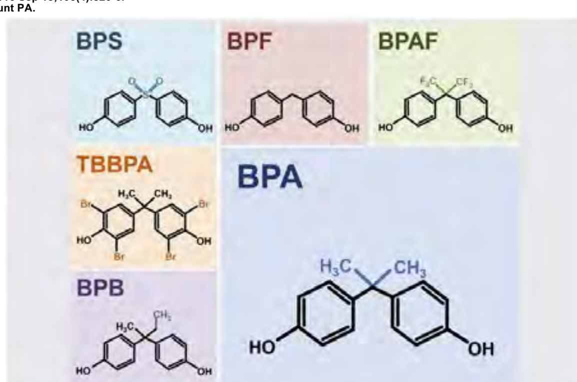


Fig. 1.

THG and related natural and synthetic androgen structures. Note the structural similarities between the two designer androgens norbolethone and THG with THG's parent gestrinone (differing by only a side chain reduction) and the known potent androgens nandrolone and trenbolone.



An old culprit but a new story: bisphenol A and "NextGen" bisphenols.
Fertil Steril. 2016 Sep 15;106(4):820-6.
Sartain CV, Hunt PA.



Molecular shapes and environmental concentrations of test EDCs.

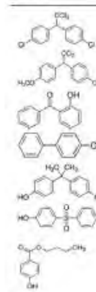
TABLE 1	The test 'phenolic' compounds
	**Dichlorodiphenyltrichloroethane** DDT p,p' isomer. Rapidly metabolized to **phenolic derivative** (Kapoor et al., 1970) Group 2B carcinogen (NTP) Banned … still used in India/Africa for mosquito control. T½ 6 years = 20 year clearance. Conc[a] Serum 2×10^{-7}M; Adipose 5×10^{-5}M (NHANES, 2009; Bosco et al., 2014; Arrebola et al., 2012)
	Methoxychlor MetChlor DDT replacement. Rapidly metabolized to **phenolic derivative** (Kapoor et al., 1970; Hu and Kupfer, 2002) Crop insecticide, carcinogenic Group 2B. Banned 2003. Persistent organic pollutant. Conc[a] Serum 10^{-8}M; Adipose 10^{-6}M (NHANES, 2009; Arrebola, 2012)
	Benzophenone-2 BP-2 2-hydroxybenzophenone. UV blocking agent. Plastics, sunscreen, hair products, printing. Conc[a] Urine 1 mg/l; Serum 5×10^{-8}M; Adipose 5×10^{-8}M (Wan et al., 2015; Schlumpf et al., 2010; Kortenkamp et al., 2014)
	4-Phenylphenol p-phenylphenol 4-PP Biocide Agricultural antifungicide. Stops mould on shelf fruit. Conc[a] Urine 1.7µg/l; Serum 10^{-8}M; Adipose 10^{-8}M (Teixo de Remy-Martin et al., 2014; Arrebola et al., 2012)
	Bisphenol A BPA.Widespread use in epoxy resins & polycarbonate plastics, food/drink can linings, thermal receipt paper. Conc[a] Urine 1-120µg/l; Serum 5×10^{-9}-10^{-8}M; Tissue 10^{-8}-10^{-7}M (NHANES, 2009; Taylor et al. 2011; Hormann et al., 2014; Kortenkamp et al., 2014; Arrebola et al., 2012; Schonfelder et al., 2002)
	Bisphenol S BPS. "BPA substitute/replacement" Used in epoxy resins, glues, dental sealants, cars plastic piping, thermal receipts, ticketing paper. Estrogenic action with potency of BPA. Conc[a] Urine 1-30µg/l; Serum 2×10^{-9}-10^{-8}M; Adipose 10^{-8}M (Liao et al., 2012; NHANES, 2009; Vinas and Watson, 2013)
	n-Butylparaben n-BP butyl 4-hydroxybenzoate Bacteriocide/fungicide cosmetic additive. In 20,000 personal care products & moisturisers. Urine 2-30µg/l; Serum 10^{-10}M; Adipose 10^{-8}M (NHANES, 2009; Sprague et al., 2013; Kortenkamp et al., 2014)

Table 1. Summary of relative sensitivity of endpoints in traditional multi-generation study (fertility, fecundity, and somatic growth) compared with alternate measures of reproduction function or capacity for chemicals that work through the estrogen, androgen, and Ah receptors. The lowest dose level at which a statistically significant effect was observed is noted by symbol (the lower the dose within a chemical, the more the number of "+"'s; see footnotes for chemical, exposure duration, and dose levels).

	WEAK ESTROGEN[1]		ANTI-ANDROGEN[2]		AH AGONIST[3]	
	Male	*Female*	*Male*	*Female*	*Male*	*Female*
Fertility		+	+		–	+ (TTP)
Fecundity		+				
Growth of F1	++	++	–		+	–
Sex differentiation			+++ (AGD)	– (AGD)	– (AGD)	– (AGD)
Puberty	+ (PS)	+++ (VO)			++ (PS)	+ (VO)
Physiology		++ (EC)			+++ (EO)	
Gamete number	++ (CSC)		+ (ESC)		+++ (ESC)	
Accessory sex gland weights	++		+++		+	
Gonad weight	+	+	–		–	
Pituitary hormones	+++ (PL)					
Steroid hormones	– (isT)		– (T)			
Malformations	–		+ (HS)			++ (VT)

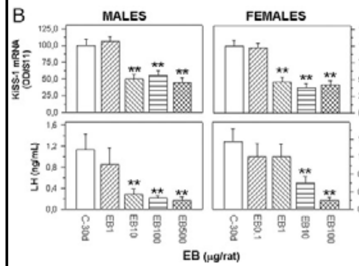
[1] Methoxychlor, GD15-PD21, dose levels: –, 200 mg/kg/d; +, 100 mg/kg/d; ++, 50 mg/kg/d; +++, 25 mg/kg [7, 14, 15]. Fertility and fecundity data are for the parental generation.

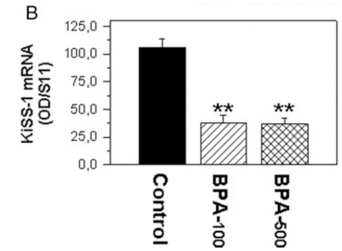
[2] Vinclozolin, GD14-PD3; dose levels: –, 100–200 mg/kg/d; +, 50 mg/kg/d; ++, 12.5–25 mg/kg/d; +++, 3–6 mg/kg/d [8, 16].

[3] "Dioxin", GD 15; dose levels: –, 1 g/kg; +, 0.8 g/kg; ++, 0.2 g/kg; +++, 0.05 g/kg [9, 17–19].

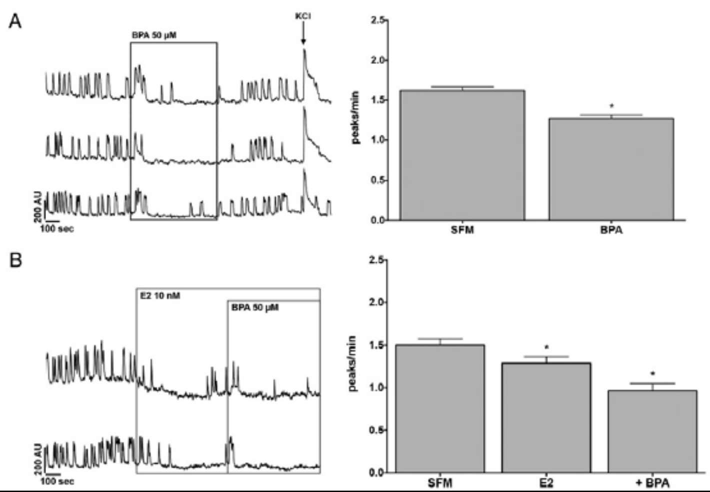
Abbreviations: AGD, anogenital distance; CSC, caudal sperm count; EC, estrous cyclicity; EO, age at eye opening; ESC, ejaculated sperm count; GD, gestation day; HS, hypospadias; isT, *in vitro* stimulated testosterone release from testes; PD, postnatal day; PL, prolactin; PS, age at preputial gland separation; VO, age at vaginal opening; VT, vaginal thread; T, serum testosterone; TTP, time-to-pregnancy.

Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation.
Navarro VM, et al.
Endocrinology. 2009 May;150(5):2359-67.

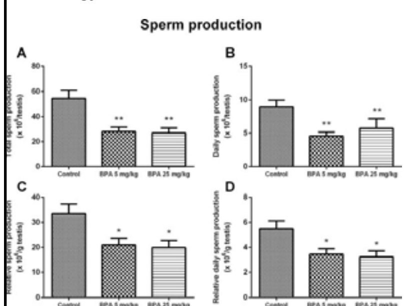




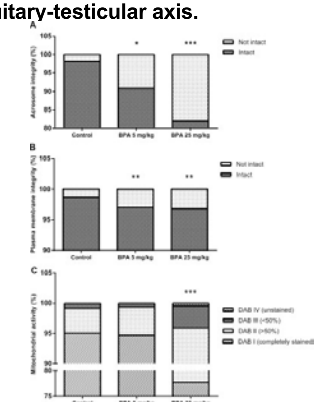
BPA directly decreases GnRH neuronal activity via non-canonical pathway.
Klenke U, Constantin S, Wray S.
Endocrinology. 2016 Mar 2:en20151924. [Epub ahead of print]



Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary-testicular axis.
Wisniewski P, Romano RM, Kizys MM, et al.
Toxicology. 2015 Mar 2;329:1-9.

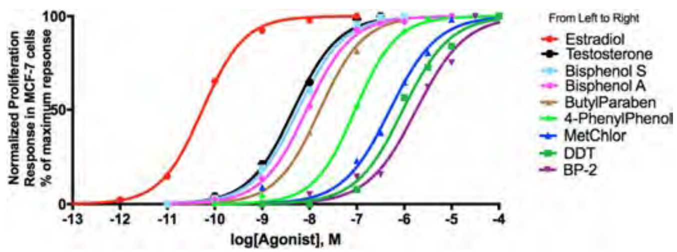


Total sperm production **(A)**, daily sperm production **(B)**, relative sperm production **(C)** and relative daily sperm production **(D)** in rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M., n = 10 animals/group, ANOVA followed by Tukey HSD test, *P < 0.05 and ** P < 0.01 vs. control.

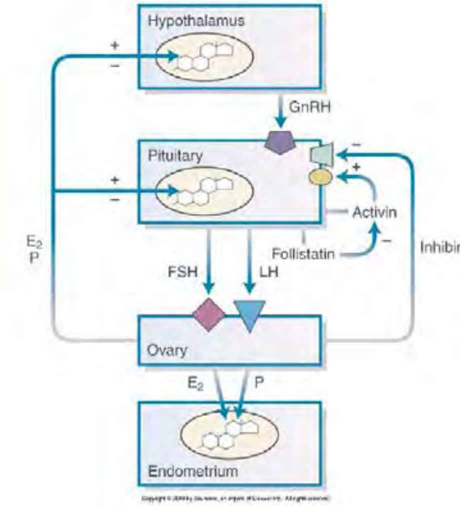


Sperm parameters in male rats exposed to bisphenol A (BPA). Mean values for the frequency of **(A)** acrosome integrity, **(B)** plasma membrane integrity and **(C)** mitochondrial activity for the control, 5 mg/kg BW and 25 mg/kg BW BPA-exposed groups. Data are shown as the mean ± S.E.M., n = 10 animals/group, ANOVA followed by Tukey HSD test, *P < 0.05, **P < 0.01 and ***P < 0.001 vs. control.

Low-dose environmental endocrine disruptors, increase aromatase activity, estradiol biosynthesis and cell proliferation in human breast cells.
Williams GP, Darbre PD.
Mol Cell Endocrinol. 2019 Apr 15;486:55-64.



Proliferative growth-response curves of MCF-7 cells treated with serial dilutions of the test compounds, plotted as concentration-response sigmoidal curves with log concentration of molarity vs. proliferative cell growth normalized to the percentage of maximal response. The cells were grown in phenol red-free DMEM, 5% DCFCS, with a nil addition/vehicle (negative control) in the presence of the serial test concentrations, for 7 day at 37°, in humidified air containing 10% carbon dioxide. Technical triplicate cell counts were averaged and expressed as a percentage of the maximal response relative to the untreated control, with normalization and curve smoothing using GraphPad Prism 8.



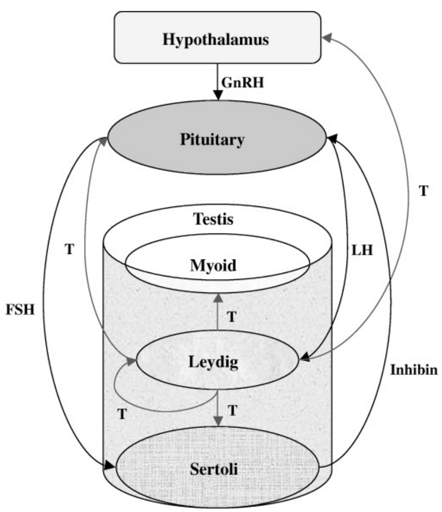
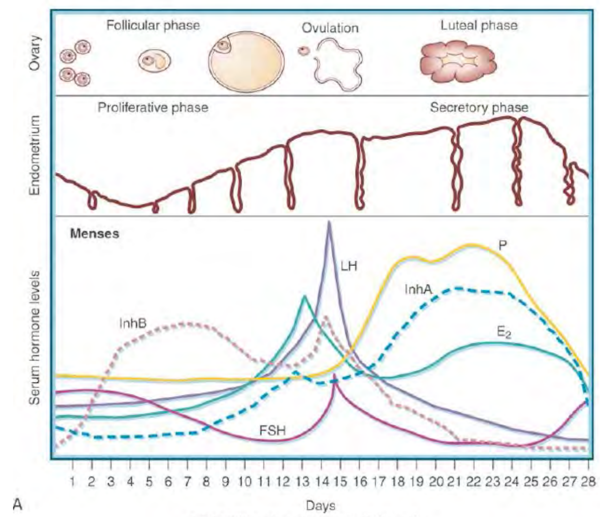


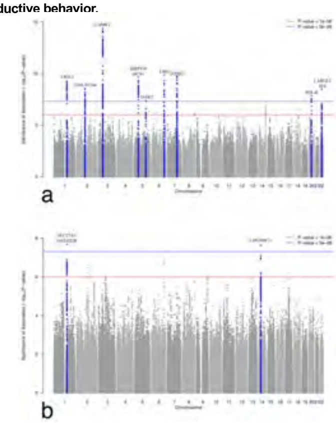
Figure 1.
Hormonal regulation of spermatogenesis Most hormones shown can have both positive and negative effects, either at the level of receptor activation/desensitization or through activation and repression of downstream targets. GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone.



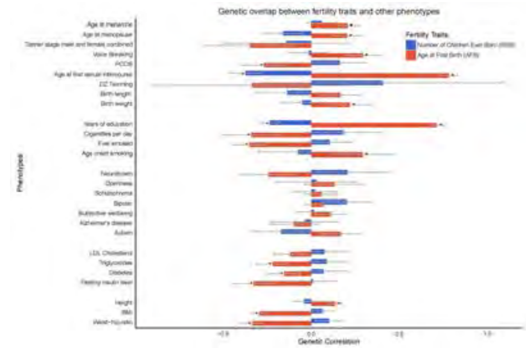
Genome-wide analysis identifies 12 loci influencing human reproductive behavior.
Nat Genet. 2016 Dec;48(12):1462-1472.
Barban N, Jansen R, de Vlaming R, et al.

Abstract

The genetic architecture of human reproductive behavior-age at first birth (AFB) and number of children ever born (NEB)-has a strong relationship with fitness, human development, infertility and risk of neuropsychiatric disorders. However, very few genetic loci have been identified, and the underlying mechanisms of AFB and NEB are poorly understood. We report a large genome-wide association study of both sexes including 251,151 individuals for AFB and 343,072 individuals for NEB. We identified 12 independent loci that are significantly associated with AFB and/or NEB in a SNP-based genome-wide association study and 4 additional loci associated in a gene-based effort. These loci harbor genes that are likely to have a role, either directly or by affecting non-local gene expression, in human reproduction and infertility, thereby increasing understanding of these complex traits.

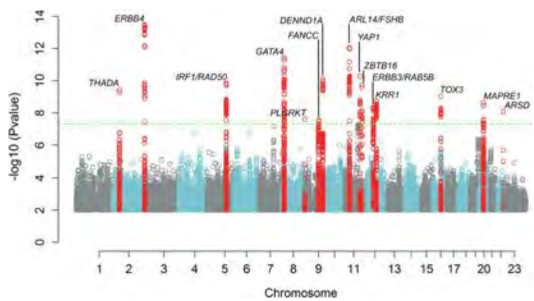


Manhattan plots of SNPs for AFB (age at first birth) and NEB (number of children ever born) in single genomic control meta-analysisSNPs are plotted on the x-axis according to their position on each chromosome against association with AFB (panel a) and NEB (panel b). The solid blue line indicates the threshold for genome-wide significance ($P<5x10^{-08}$) and the red line, the threshold for suggestive hits ($P<5x10^{-06}$). Blue points indicate SNPs in a ±100 KB region around genome-wide significant hits. Gene labels are annotated as the nearby genes to the significant SNPs.

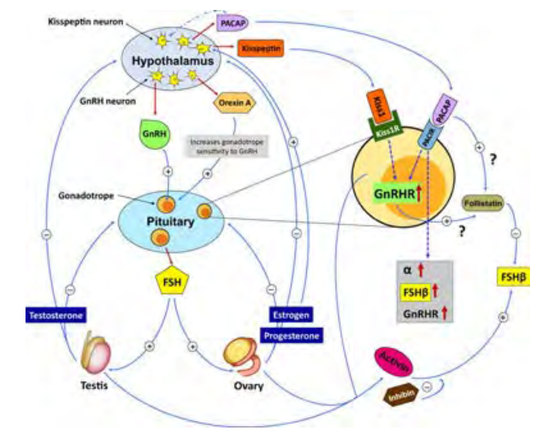


Genetic overlap between AFB and NEB and other related traits Results from Linkage-Disequilibrium (LD) Score regressions: estimates of genetic correlation with developmental, reproductive, behavioral, neuropsychiatric and anthropometric phenotypes for which GWAS summary statistics were available in the public domain. The length of the bars indicates the estimates of genetic correlation. Grey error bars indicate 95% confidence intervals. The mark "*" indicates that the estimate of genetic correlation is statistically significant after controlling for multiple testing ($P<0.05/27=1.85x10^{-3}$).

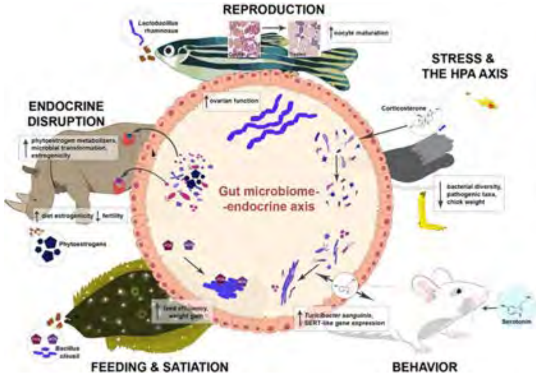
Large-scale genome-wide meta-analysis of polycystic ovary syndrome suggests shared genetic architecture for different diagnosis criteria.
Day F, Karaderi T, Jones MR, Meun C, et al.
PLoS Genet. 2018 Dec 19;14(12):e1007813.



Manhattan plot showing results of meta-analysis for PCOS status, adjusting for age.
The inverse log10 of the *p* value (-log10(*p*)) is plotted on the Y axis. The green dashed line designates the minimum *p* value for genome-wide significance ($<5.0 \times 10^{-8}$). Genome wide significant loci are denoted with a label showing the nearest gene to the index SNP at each locus. SNPs with *p* values $\leq 1.0x10^{-2}$ are not depicted.



Regulation of endocrine systems by the microbiome: Perspectives from comparative animal models.
Williams CL, Garcia-Reyero N, Martyniuk CJ, Tubbs CW, Bisesi JH Jr.
Gen Comp Endocrinol. 2020 Feb 12;292:113437.



"Systems Biology of Reproduction"

Spring 2024 (Even Years) – Course Syllabus
Biol 475/575 Undergraduate/Graduate (3 Credit)
SLN: (475) – 06763, (575) – 06764
Time - Tuesday and Thursday 10:35 am-11:50 am
Course Lectures in person and recorded on Canvas/Panopto and Discussion Sessions live in person and on WSU Zoom for all campuses (Hybrid Course)
Room – CUE 418
Course Director – Michael Skinner, Abelson Hall 507, 335-1524, skinner@wsu.edu
Co-Instructor – Eric Nilsson, Abelson Hall 507, 225-1835, nilsson@wsu.edu
Learning Objective -
Current literature based course on the Systems Biology of Reproduction. Learning Systems approaches to the biology of reproduction from a molecular to physiological level of understanding.
Schedule/Lecture Outline –

January 9 & 11	Week 1	Systems Biology Introduction
16 & 18	Week 2	Molecular/ Cellular/ Reproduction Systems
23 & 25	Week 3	Sex Determination Systems
Jan /Feb 30 & 1	Week 4	Male Reproductive Tract Development & Function
February 6 & 8	Week 5	Female Reproductive Tract Development & Function
13 & 15	Week 6	Gonadal Developmental Systems Biology
20 & 22	Week 7	Testis Systems Biology
27 & 29	Week 8	Ovary Systems Biology
March 5 & 7	Week 9	Epigenetics and Transgenerational Gonadal Disease
11 – 15	**Week 10**	**Spring Break**
19 & 21	Week 11	Gametogenesis/ Stem Cells/ Cloning
26 & 28	Week 12	Hypothalamus-Pituitary Development & Function
April 2 & 4	Week 13	Reproductive Endocrinology Systems
9 & 11	Week 14	Fertilization & Implantation Systems
16 & 18	Week 15	Fetal Development & Birth Systems
23 & 25	Week 16	Assisted Reproduction/Contraception
Apr/May 30 & 2	Week 17	Exam or Grant Review