高値を示した。その他のパラメーターは対照群と EE 投与群との間で有意差は認められなかった。

## D．考察

成熟雌動物に排卵をもたらす性腺刺激ホルモンの大量放出（サージ）は、視床下部で性腺刺激ホルモン放出ホルモンを上位から直接制御しているキスペプチン分泌ニューロンの局在の性差に起因していることが近年の研究で知られるようになった。このような性差は、視床下部の分化過程で作用する性ステロイドによって決定されるが、性ステロイドが関与し得る時期は限定的で、このような時期は性分化の臨界期と呼ばれている。生殖発生毒性試験に汎用されているラットでは出生直前から生後 5 日ころまでがこの時期に相当し、精巣由来のアンドロゲンが脳内の aromatase によってエストロゲンに変換され、これが、雌に固有の性腺刺激ホルモンサージを制御する AVPV におけるキスペプチンンニューロンを不可逆的に消失させて、キスペプチンンニューロンが ARC のみに局在する雄型の脳になると理解されている。脳の性分化における性ステロイドに対する感受性は雌にも存在し、この時期の雌にアンドロゲンが作用すると、雄と同様のメカニズムで雄型の脳へと分化する。また、胎児および新生児期の肝臓で合成され、循環血中に分泌されて母体由来のエストロゲンと特異的に結合する α-fetoprotein とは結合親和性の低いエストロゲン活性物質も雄型の脳へと分化させることができる。本研究でモデル化合物として用いた EE も内因性 $E_2$ と比べて α-fetoprotein との結合親和性が低いことから、脳の性分化に影響を及ぼすことができると考えられる。

今回我々は、0.4 あるいは 0.08 μg/kg 体重/日の用量の EE を、生後 1 日の雌ラットに反復経口投与し、性周期の回帰停止以前の時期における視床下部／下垂体／性腺軸の変化を検討した。この時期が性周期回帰停止前であることは、平成 25 年度までの研究でも確認され、0.08 μg/kg 体重/日の用量では 26 週齢に至っても、不規則な性周期を回帰するものの、発情の回帰は停止しないことが確認されている。本研究において、EE 投与群では、視床下部 AVPV および ARC のいずれにおいても *kiss1* 遺伝子の発現変化が認められ、また、卵巣では、性腺刺激ホルモン受容体の発現増加、ならびにその下流に位置するステロイド合成酵素などの遺伝子発現増加が認められ、この時期に既に視床下部および性腺のいずれにも明瞭な変化が生じている事が明らかになった。

視床下部における *kiss1* 遺伝子の発現変化は、部位により異なる。すなわち、エストロゲンのポジティブフィードバックを受ける AVPV では低下が認められ、ネガティブフィードバックを受ける ARC では増加が認められた。子宮重量が減少していることから、循環血中 $E_2$ 濃度が 0.4 μg/kg 体重/日群では低下し、フィードバックが減弱したことが *kiss1* 遺伝子の発現増加をもたらしたとも考えられるが、卵巣摘出条件下で測定したパルス状 LH の Amplitude も 0.4 μg/kg 体重/日群で増加していた。パルス状 LH 分泌は ARC の Kndy ニューロンといわれるキスペプチン、ダイノルフィンおよびニューロキニン B を分泌する細胞で発生する刺激によって発生する LH の分泌パターンである。今回、卵巣からのフィードバックを欠く条件でも Amplitude が増加していたことから、ARC の Kndy ニューロン自身に影響を及ぼしている可能性が示唆された。

EE 投与群の卵巣における遺伝子発現は、$E_2$ 合成促進を示唆する変化となっていた。しかし、子宮重量から推測すると、$E_2$ 濃度はむしろ低下しているものと推測された。血中 $E_2$ 濃度の確認が必要とされるが、昨年度の研究において EE 投与群では幼若期の血中 $E_2$ 濃度は卵胞発育や卵巣おける遺伝子発現動態と一致していなかった。幼若期では、卵胞発育は抑制されており、性成熟期とは全く逆の変化を示していた。時期によって卵胞発育に及ぼす影響が異なる理由は

不明である。しかが、いずれの時期も卵巣内部の変化が血中$E_2$濃度に反映されていないと考えられる。

性腺刺激ホルモンによってステロイド合成系が増強されても、それが血中濃度に反映されないと、卵巣の機能的変化に一致しない刺激がさらに卵巣に加えられる結果となる。嚢胞状卵胞の形成にはこのような視床下部／下垂体／性腺軸における情報交換の異常が関与している事が推測された。

嚢胞状卵胞の形成については、10 週齢に観察した実験 2 の対照群でも 1 例に認められていることから、13 週齢での出現が EE 投与の影響かどうかは、さらに例数を追加して検討する必要がある。しかし、本研究から、性周期の回帰を停止しない用量でも卵巣に影響が認められ、性周期の回帰を停止する用量でも、回帰停止前の時期にすでに視床下部／卵巣のいずれにも変化が生じていることが明らかになり、嚢胞状卵胞は閾値の指標として有用であると考えられる。しかし、性周期を回帰している段階での嚢胞状卵胞の毒性学的意義についてはさらに検討が必要である。

以上のように、今年度の研究から、子宮重量を増加させない用量の EE でも、新生児期での経口曝露により嚢胞状卵胞を形成させ、その時期には視床下部／下垂体／性腺軸における情報交換に異常が生じていることが示唆された。

## F．研究発表

1. 論文発表

1) Shirota M, Kawashima J, Ogawa Y, Kamiie J, Yasuno K, Shirota K, Yoshida M.
Delayed effects of single neonatal subcutaneous exposure of low-dose 17α-ethynylestradiol on reproductive function in female rats.
Journal of Toxicological Sciences 37: 681-689 (2012)

2) Shirota M, Kawashima J, Nakamura T, Ogawa Y, Kamiie J, Shirota K.
Vascular Hamartoma in the Uterus of a Female Sprague-Dawley Rat with an Episode of Vaginal Bleeding.
Toxicologic Pathology (in press)

2. 学会発表

1) 川嶋 潤、中村 知裕、菅田 恵理世、鈴木 紗綾、小川 祐布子、吉田 緑： 新生ラットへのエチニルエストラジオール曝露が幼若期の卵巣における卵胞発育関連遺伝子の発現に及ぼす影響 第 40 回日本毒性学会学術年会（2013 年 6 月）

2）代田 眞理子：ラット周生期エストロゲン活性物質曝露による遅発影響—毒性学的視点での解析 第106回日本繁殖生物学会大会シンポジウム（2013年9月）

3) 代田 眞理子、川嶋 潤、森 雅史、立河 紗紀、菅田 恵理世：リプロダクティブヘルスからみた遅発影響 －遅発影響検出のための実践的指標の探索 第 41 回日本毒性学会学術年会（2014 年 7 月）

G．知的財産権の出願・登録状況
（予定を含む。）

1. 特許取得
該当無し
2. 実用新案登録
該当無し
3. その他
無し

参考文献

Aatsinki JT, Pietilä EM, Lakkakorpi JT, Rajaniemi HJ. Expression of the LH/CG receptor gene in rat ovarian tissue is regulated by an extensive alternative splicing of the primary transcript. *Molecular Cellular Endocrinol*ogy 84,127-135 (1992)

Akieda-Asai S, Ohta R, Shirota M, Jaroenporn S, Watanabe G, Taya K. Endocrinological differences between Hatano high- and low-avoidance rats during early two-way avoidance acquisition. *Experimental Animals* 60, 509-516 (2011)

Ayyanan A, Laribi O, Schuepbach-Mallepell S, Schrick C, Gutierrez M, Tanos T, Lefebvre G, Rougemont J, Yalcin-Ozuysal O, Brisken C.

Perinatal exposure to bisphenol A increases adult mammary gland progesterone response and cell number. *Molecular Endocrinology* 25, 1915-1923 (2011)

Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA.
In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. *Environmental Health Perspectives* 118, 1614-1619 (2010)

Burdick HO, Whitney R. Ovulation induced in mice by single injection of follutein or untreated human pregnancy urine. *American Journal of Physiology* 132, 405-410 (1941)

Bandiera S and Dworschak C. Effects of Testosterone and Estrogen on Hepatic Levels of Cytochromes P450 2C7 and P450 2Cll in the Rat.
*Archives of Biochemistry and Biophysics* 296, 286-295 (1992)

Borgert CJ, LaKind JS, Witorsch RJ. A critical review of methods for comparing estrogenic activity of endogenous and exogenous chemicals in human milk and infant formula. *Environmental Health Perspectives* 111, 1020-1036 (2003).

Boutin JM, Jolicoeur C, Okamura H, Gagnon J, Edery M, Shirota M, Banville D, Dusanter-Fourt I, Djiane J, Kelly PA. Cloning and expression of the rat prolactin receptor, a member of the growth hormone/prolactin receptor gene family. Cell 53, 69-77 (1988).

Boutin JM, Edery M, Shirota M, Jolicoeur C, Lesueur L, Ali S, Gould D, Djiane J, Kelly PA. Identification of a cDNA encoding a long form of prolactin receptor in human hepatoma and breast cancer cells. *Molecular Endocrinology* 3, 1455-1461 (1989)

Byers M, Kuiper GG, Gustafsson JA, Park-Sarge OK. Estrogen receptor-beta mRNA expression in rat ovary: down-regulation by gonadotropins. *Molecular Endocrinology* 11, 172-182 (1997)

Beale KE, Kinsey-Jones JS, Gardiner JV, Harrison EK, Thompson EL, Hu MH, Sleeth ML, Sam AH, Greenwood HC, McGavigan AK, Dhillo WS, Mora JM, Li XF, Franks S, Bloom SR, O'Byrne KT, Murphy KG. The physiological role of arcuate kisspeptin neurons in the control of reproductive function in female rats. *Endocrinology* 155, 1091–1098 (2014)

Banu SK, Samuel JB, Arosh JA, Burghardt RC, Aruldhas MM. Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats. *Toxicology and Applied Pharmacol*ogy **232**,180-189 (2008)

Becú-Villalobos D, González Iglesias A, Díaz-Torga G, Hockl P, Libertun C. Brain sexual differentiation and gonadotropins secretion in the rat. *Cellular and Molecular Neurobiol*ogy **17**, 699-715 (1997)

Biles JE, McNeal TP, Begley TH, Hollifield HC. Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to food simulating liquids. *J Agriculture and Food Chem*istry **45**,3541–3544 (1997)

Blair RM, Fang H, Branham WS, Hass BS, Dial SL, Moland CL, Tong W, Shi L, Perkins R, Sheehan DM. The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands. *Toxicological Sciences* **54**, 138-153 (2000)

Branham WS, Dial SL, Moland CL, Hass BS, Blair RM, Fang H, Shi L, Tong W, Perkins RG, Sheehan DM. Phytoestrogens and mycoestrogens bind to the rat uterine estrogen receptor. *Journal of Nutrition* **132,** 658-664 (2002)

Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released

from lacquer coatings in food cans. *Environment and Health Perspectives* **103**, 608-612 (1995)

Chakraborty TR, Gore AC. Aging-Related Changes in Ovarian Hormones, Their Receptors, and Neuroendocrine Function. *Experimental Biology and Medicine* 229, 977–987 (2004)

Chang L-L, Wun W-S A, Paulus S. Wang PS. *In utero* and neonate exposure to nonylphenol develops hyperadrenalism and metabolic syndrome later in life. I. First generation rats (F1). *Toxicology* 301, 40-49 (2012)

Chen Y, Jefferson WN, Newbold RR, Padilla-Banks E, Pepling ME. Estradiol, Progesterone, and Genistein Inhibit Oocyte Nest Breakdown and Primordial Follicle Assembly in the Neonatal Mouse Ovary *in Vitro* and *in Vivo*. *Endocrinology* 148, 3580–3590 (2007)

Cimafranca MA, Davila J, Ekman GC, Andrews RN, Neese SL, Peretz J, Woodling KA, Helferich WG, Sarkar J, Flaws JA, Schantz SL, Doerge DR, Cooke PS. Acute and chronic effects of oral genistein administration in neonatal mice. *Biology of Reproduction* 83,114-121 (2010)

Clarkson J. Effects of estradiol on kisspeptin neurons during puberty. Frontiers in *Neuroendocrinology* **34**:120-131 (2013)

d'Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB, Aparicio SA, Colledge WH. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. *Proceeding of National Academy Scicience U S A*. **104**,10714-10719 (2007)

Dufau ML, Tsai-Morris CH, Hu ZZ, Buczko E. Structure and regulation of the luteinizing hormone receptor gene. *Journal of Steroid Biochemistry and Molecular Biology* **53**, 283-291 (1995)

Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA, Themmen AP. Anti-Müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology* 143, 1076-1084 (2002)

Edson MA, Nagaraja AK, Matzuk MM. The mammalian ovary from genesis to revelation. *Endocrine Reviews* **30**, 624-712 (2009)

Fernández M, Bianchi M, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol a alters reproductive parameters and gonadotropin releasing hormone signaling in female rats. *Environment and Health Perspectives* **117**,757-762 (2009)

Goldenberg RL, Vaitukaitis JL, Ross GT. Estrogen and follicle stimulation hormone interactions on follicle growth in rats. *Endocrinology* **90**,1492-1498 (1972)

González-Martínez D, De Mees C, Douhard Q, Szpirer C, Bakker J. Absence of gonadotropin-releasing hormone 1 and Kiss1 activation in alpha-fetoprotein knockout mice: prenatal estrogens defeminize the potential to show preovulatory luteinizing hormone surges. *Endocrinology* **149**,2333-2340 (2008)

Gore AC, Walker DM, Zama AM, Armenti AE, Uzumcu M. Early life exposure to endocrine-disrupting chemicals causes lifelong molecular reprogramming of the hypothalamus and premature reproductive aging. *Molecular Endocrinology* **25**, 2157-2168 (2011)

Graham R. Robertson, Geoffrey C. Farrell, Liddle C. Sexually Dimorphic Expression of Rat CYP3A9 and CYP3A18 Genes Is Regulated by Growth Hormone. *Biochemical and Biophysical Research Communications* 242, 57–60 (1998)

Greenstein BD. Effects of rat alpha-fetoprotein administration on estradiol free fraction, the

onset of puberty, and neural and uterine nuclear estrogen receptors. *Endocrinology* 130, 3184-3190 (1992)

Kim H, Nakajima T, Hayashi S, Chambon P, Watanabe H, Iguchi T, Sato T. Effects of Diethylstilbestrol on Programmed Oocyte Death and Induction of Polyovular Follicles in Neonatal Mouse Ovaries. *Biology of Reproduction* 81, 1002–1009 (2009)

Herath CB, Yamashita M, Watanabe G, Jin W, Tangtrongsup S, Kojima A, Groome NP, Suzuki AK, Taya K. Regulation of follicle-stimulating hormone secretion by estradiol and dimeric inhibins in the infantile female rat. *Biology of Reproduction* 65,1623-1633 (2001)

Hirshield AH. Overview of Ovarian Follicular Development: Considerations for the Toxicologist. *Environmental and Molecular Mutagenesis* 29,10-15 (1997)

Homma T, Sakakibara M, Yamada S, Kinoshita M, Iwata K, Tomikawa J, Kanazawa T, Matsui H, Takatsu Y, Ohtaki T, Matsumoto H, Uenoyama Y, Maeda K, Tsukamura H. Significance of neonatal testicular sex steroids to defeminize anteroventral periventricular kisspeptin neurons and the GnRH/LH surge system in male rats. *Biology of Reproduction* **81**, 1216-1225 (2009)

Hong H, Branham WS, Dial SL, Moland CL, Fang H, Shen J, Perkins R, Sheehan D, Tong W. Rat α-Fetoprotein binding affinities of a large set of structurally diverse chemicals elucidated the relationships between structures and binding affinities. *Chemical Research of Toxicology* **25**, 2553-2566 (2012)

Huang EJ, Manova K, Packer AI, Sanchez S, Bachvarova RF, Besmer P. The murine steel panda mutation affects kit ligand expression and growth of early ovarian follicles. *Developmental Biology* **157**, 100-109 (1993)

Hutter HS, Gibson MJ. Effect of neonatal androgenization on positive feedback in female mice. *Biology of Reproduction* 38, 636-638 (1988)

Horie K, Takakura K, Taii S, Narimoto K, Noda Y, Nishikawa S, Nakayama H, Fujita J, Mori T. The expression of c-kit protein during oogenesis and early embryonic development. *Biology of Reproduction* 45, 547-552 (1991)

Ieda N1, Uenoyama Y, Tajima Y, Nakata T, Kano M, Naniwa Y, Watanabe Y, Minabe S, Tomikawa J, Inoue N, Matsuda F, Ohkura S, Maeda K, Tsukamura H. KISS1 gene expression in the developing brain of female pigs in pre- and peripubertal periods. *Journal of Reproduction and Development* 60, 312-316 (2014)

Ikeda Y, Nagai A, Ikeda M, AND Hayashi S. Increased Expression of Mullerian-Inhibiting Substance Correlates with Inhibition of Follicular Growth in the Developing Ovary of Rats Treated with E2 Benzoate. Endocrinology 143, 304–312 (2002)

Ikeda Y, Tanaka H, and Esaki M. Effects of Gestational Diethylstilbestrol Treatment on Male and Female Gonads during Early Embryonic Development. *Endocrinology* 149:3970-3979 (2008)

Jager W, Correia MA, Bornheim LM, Mahnke A, WALTER G. Hanstein WG, XUE L, and Benet LZ. Ethynylestradiol-mediated induction of hepatic *CYP3A9* in Female rats: implication for cyclosporine metabolism. *Drug Metabolism and Disposition*, 27, 1505–1511 (1999)

Jefferson W, Newbold R, Padilla-Banks E, and Pepling M. Neonatal Genistein Treatment Alters Ovarian Differentiation in the Mouse: Inhibition of Oocyte Nest Breakdown and Increased Oocyte Survival. *Biology of*

*Reproduction* 74, 161–168 (2006)

Jaroenporn S, Horii Y, Asai S, Wang KM, Nagaoka K, Ohta R, Shirota M, Watanabe G, Taya K. Endocrine mechanisms responsible for different follicular development during the estrous cycle in Hatano high- and low-avoidance rats. *Journal of Reproduction and Development* 57, 690-699 (2012)

Kanno J, Onyon L, Haseman J, Fenner-Crisp P, Ashby J, Owens W. The OECD Program to Validate the Rat Uterotrophic Bioassay to Screen Compounds for in Vivo Estrogenic Responses: Phase 1. *Environmental Health Perspectives* 109,785–794 (2001)

Kelly PA, Boutin JM, Jolicoeur C, Okamura H, Shirota M, Edery M, Dusanter-Fourt I, Djiane J. Purification, cloning, and expression of the prolactin receptor. *Biology of Reproduction* 40, 27-32 (1989)

Kenny HA, Woodruff TK. Follicle size class contributes to distinct secretion patterns of inhibin isoforms during the rat estrous cycle. *Endocrinology* 147, 51-60 (2006)

Kezele P, Skinner MK. Regulation of Ovarian Primordial Follicle Assembly and Development by Estrogen and Progesterone: Endocrine Model of Follicle Assembly. *Endocrinology* 144, 3329–3337 (2003)

Kimura F, Funabashi T. Two subgroups of gonadotropin releasing hormone neurons control gonadotropin secretion in rats. *News of Physiological Sciences* **13**,225-231 (1998)

Kipp JL, Kilen SM, Bristol-Gould S, Woodruff TK, Mayo KE. Neonatal exposure to estrogens suppresses activin expression and signaling in the mouse ovary. *Endocrinology* 148:1968-1976 (2007)

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 139, 4252-4263 (1998)

Lei L, Shiying J, Kelly EM, Woodruff TK. The Interactions Between the Stimulatory Effect of Follicle-Stimulating Hormone and the Inhibitory Effect of Estrogen on Mouse Primordial Folliculogenesis. *Biology of Reproduction* 82, 13–22 (2010)

Mazaud Guittot S, Guigon CJ, Coudouel N, Magre S. Consequences of Fetal Irradiation on Follicle Histogenesis and Early Follicle Development in Rat Ovaries. *Biology of Reproduction* 75, 749–759 (2006)

Melinda E, Wilson R, Handa J. Ontogeny of Gene Expression in the Gonadotroph of the Developing Female Rat. *Biology of Reproduction* 56, 563-568 (1997)

McCarthy MM. Estradiol and the developing brain. *Physiological Reviews* **88**, 91-124 (2008)

McGee EA, Smith R, Spears N, Nachtigal MW, Ingraham H, Hsueh AJ. Müllerian inhibitory substance induces growth of rat preantral ovarian follicles. *Biology of Reproduction* **64**, 293-298 (2001)

Mena MA, Arriaza CA, Tchernitchin AN. Early postnatal androgenization imprints selective changes in the action of estrogens in the rat uterus. *Biology of Reproduction* 46,1080-1085 (1992)

Montano MM, Welshons WV, vom Saal FS. Free estradiol in serum and brain uptake of estradiol during fetal and neonatal sexual

differentiation in female rats. *Biology of Reproduction* 53,1198-207 (1995)

Moral R, Santucci-Pereira J, Wang R, Russo IH, Lamartiniere CA, Russo J. In utero exposure to butyl benzyl phthalate induces modifications in the morphology and the gene expression profile of the mammary gland: an experimental study in rats. *Environmental Health* 10, 5 (2011)

Morohashi KI, Omura T. Ad4BP/SF-1, a transcription factor essential for the transcription of steroidogenic cytochrome P450 genes and for the establishment of the reproductive function. *FASEB Journal* **10**, 1569-1577 (1996)

Murakami T, Sato A, Inatani M, Sakurai H, Yumoto R, Nagai J, Takano M. Effect of neonatal exposure of 17beta-estradiol and tamoxifen on hepatic CYP3A activity at developmental periods in rats. *Drug Metabolism and Pharmacokinetics* 19, 96-102 (2004)

Murray AA, Gosden RG, Allison V, Spears N. Effect of androgens on the development of mouse follicles growing in vitro. *J Reprod Fertility* **113**, 27-33 (1998)

Nagai A, Ikeda Y, Aso T, Eto K, Ikeda MA. Exposure of neonatal rats to diethylstilbestrol affects the expression of genes involved in ovarian differentiation. *Journal of Medical and Dental Sciences* **50**, 35-40 (2003)

Nagaraja AK, Middlebrook BS, Rajanahally S, Myers M, Li Q, Matzuk MM, Pangas SA. Defective gonadotropin-dependent ovarian folliculogenesis and granulosa cell gene expression in inhibin-deficient mice. *Endocrinology* **151**, 4994-5006 (2010)

Navarro VM, Sánchez-Garrido MA, Castellano JM, Roa J, García-Galiano D, Pineda R, Aguilar E, Pinilla L, Tena-Sempere M. Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. *Endocrinology* 150, 2359-2567(2009)

Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, Enmark E, Pettersson K, Warner M, Gustafsson JA. Mechanisms of estrogen action. *Physiological Reviews* 81, 1535-1565 (2001)

Ohta R, Shirota M, Kanazawa Y, Shindo T, Furuya M, Seki T, Ono H, Kojima K, Asai S, Watanabe G, Taya K. Effects of transmaternal exposure to genistein in Hatano high- and low-avoidance rats. *Experimental Animals* 58, 471-479 (2009)

Ojeda SR, Advis JP, Andrews WW. Neuroendocrine control of the onset of puberty in the rat. *Federal Proceedings* **39**, 2365-2371 (1980)

Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, Pedraza V, Soto AM, Sonnenschein C. Estrogenicity of resin-based composites and sealants used in dentistry. *Environment and Health Perspectives* **104**, 298–305 (1996)

Orisaka M, Hattori K, Fukuda S, Mizutani T, Miyamoto K, Sato T, Tsang BK, Kotsuji F, Yoshida Y. Dysregulation of ovarian follicular development in female rat: LH decreases FSH sensitivity during preantral-early antral transition. *Endocrinology* **154**, 2870-2880 (2013)

Osterlund M, Kuiper GG, Gustafsson JA, Hurd YL. Differential distribution and regulation of estrogen receptor-alpha and -beta mRNA within the female rat brain. *Molecular Brain Research* 54, 175-180 (1998)

Pedersen T, Peters H. Proposal hor a classification of oocytes and follicles in the mouse ovary. *Journal of Reproduction and Fertility* **17**, 555-557 (1968)

Pineda R, Garcia-Galiano D, Roseweir A, Romero M, Sanchez-Garrido MA, Ruiz-Pino F, Morgan K, Pinilla L, Millar RP, Tena-Sempere M. Critical roles of kisspeptins in female puberty and preovulatory gonadotropin surges as revealed by a novel antagonist. *Endocrinology* **151**, 722-730 (2010)

Pinilla L, Castellano JM, Romero M, Tena-Sempere M, Gaytán F, Aguilar E. Delayed puberty in spontaneously hypertensive rats involves a primary ovarian failure independent of the hypothalamic KiSS-1/GPR54/GnRH system. *Endocrinology* **150**, 2889-2897 (2009)

Ramirez MC, Luque GM, Ornstein AM, Becu-Villalobos D. Differential neonatal testosterone imprinting of GH-dependent liver proteins and genes in female mice. *Journal of Endocrinology* 207, 301-308 (2010)

Rasier G, Parent AS, Gérard A, Lebrethon MC, Bourguignon JP. Early maturation of gonadotropin-releasing hormone secretion and sexual precocity after wxposure of infant female rats to estradiol or dichlorodiphenyltrichloroethane. *Biology of Reproduction* 77, 734–742 (2007)

Richards JS. Estradiol receptor content in rat granulosa cells during follicular development: modification by estradiol and gonadotropins. *Endocrinology* **97**, 1174-1184 (1975)

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. *Environment and Health Perspectives* **109**, 675-680 (2001)

Ryan BC, Hotchkiss AK, Crofton KM, Gray LE Jr. In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. *Toxicological Sciences* 114, 133-148 (2010)

Sandhoff TW, Hales DB, Hales KH, McLean MP. Transcriptional regulation of the rat steroidogenic acute regulatory protein gene by steroidogenic factor 1. *Endocrinology*. 1998 **139**:4820-4831.

Sakuma Y. Gonadal steroid action and brain sex differentiation in the rat. *Journal of Neuroendocrinology* 21, 410-414 (2009)

Sakurada Y, Sawai M, Inoue K, Shirota M, Shirota K. Comparison of aryl hydrocarbon receptor gene expression in laser dissected granulosa cell layers of immature rat ovaries. *Journal of Veterinary Medical Science* 73, 923-926 (2011)

Schindler R, Nilsson E, Skinner MK. Induction of Ovarian Primordial Follicle Assembly by Connective Tissue Growth Factor CTGF. *PLoS ONE* 5, e12979 (2010)

Schwarz JM, McCarthy MM. The role of neonatal NMDA receptor activation in defeminization and masculinization of sex behavior in the rat. *Hormones and Behavior* 54, 662-668 (2008)

Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. *New England Journal of Medicine* **349**,1614-1627 (2003)

Shirota M, Kawashima J, Nakamura T, Ogawa Y, Kamiie J, Yasuno K, Shirota K, Yoshida M. Delayed effects of single neonatal subcutaneous exposure of low-dose 17α-ethynylestradiol on reproductive function in female rats. *Journal of Toxicological Science* **37**, 681-690 (2012)

Shirota M, Soda S, Katoh C, Asai S, Sato M, Ohta R, Watanabe G, Taya K, Shirota K. Effects of reduction of the number of

primordial follicles on follicular development to achieve puberty in female rats. *Reproduction* 125, 85-94 (2003)

Shirota M, Kurohmaru M, Hayashi Y, Shirota K, Kelly PA. Detection of in situ localization of long form prolactin receptor messenger RNA in lactating rats by biotin-labeled riboprobe. *Endocrine Journal* 42, 69-76 (1995)

Shirota M, Banville D, Ali S, Jolicoeur C, Boutin JM, Edery M, Djiane J, Kelly PA. Expression of two forms of prolactin receptor in rat ovary and liver. *Molecular Endocrinology* 4, 1136-1143 (1990)

Sokka T, Huhtaniemi I. Ontogeny of gonadotrophin receptors and gonadotrophin -stimulated cyclic AMP production in the neonatal rat ovary. *Journal of Endocrinology* **127**, 297-303 (1990)

Sokka T, Hämäläinen T, Huhtaniemi L. Functional LH receptor appears in the neonatal rat ovary after changes in the alternative splicing pattern of the LH receptor mRNA. *Endocrinology* **130**, 1738-1740 (1992)

Sokka TA, Huhtaniemi IT. Functional maturation of the pituitary-gonadal axis in the neonatal female rat. *Biology of Reproduction* 52, 1404-1409 (1995)

Sokka TA, Hämäläinen TM, Kaipia A, Warren DW, Huhtaniemi IT. Development of luteinizing hormone action in the perinatal rat ovary. *Biology of Reproduction* **55**, 663-670 (1996)

Shirwalkar H, Modi DN, Maitra A. Exposure of adult rats to estradiol valerate induces ovarian cyst with early senescence of follicles. *Molecular Cellular Endocrinology* 272, 22-37 (2007)

Takumi K, Iijima N, Iwata K, Higo S, Ozawa H. The effects of gonadal steroid manipulation on the expression of Kiss1 mRNA in rat arcuate nucleus during postnatal development. *Journal of Physiological Sciences* **62**, 453–460 (2012)

Tena-Sempere M. Kisspeptin/GPR54 system as potential target for endocrine disruption of reproductive development and function. *International Journal of Andrology* 33, 360–368 (2010)

Thomas FH, Vanderhyden BC. Oocyte-granulosa cell interactions during mouse follicular development: regulation of kit ligand expression and its role in oocyte growth. *Reproductive Biology and Endocrinology* 4, 19 (2006)

Tingen C, Kim A, and Woodruff TK. The primordial pool of follicles and nest breakdown in mammalian ovaries. *Molecular Human Reproduction* 15, 795–803 (2009)

Toran-Allerand CD. Gonadal hormones and brain development: implications for the genesis of sexual differentiation. *Annual New York Academy of Sciences* **435**, 101-111 (1984)

Tsai-Morris CH, Buczko E, Wang W, Xie XZ, Dufau ML. Structural organization of the rat luteinizing hormone (LH) receptor gene. *Journal of Biological Chem*istry **266**, 1355-11359 (1991)

Uenoyama Y, Nakamura S, Hayakawa Y, Ikegami K, Watanabe Y, Deura C, Minabe S, Tomikawa J, Goto T, Ieda N, Inoue N, Sanbo M, Tamura C, Hirabayashi M, Maeda KI, Tsukamura H.Lack of Pulse and Surge Modes and Glutamatergic Stimulation of Luteinising Hormone Release in *Kiss1* Knockout Rats. *Journal of Neuroendocrinology* 27, 187-197 (2015)

Umekita Y, Souda M, Hatanaka K, Hamada T, Yoshioka T, Kawaguchi H, animoto A. Gene expression profile of terminal end buds in rat mammary glands exposed to diethylstilbestrol in neonatal period. *Toxicology Letter* 205, 15-25 (2011)

Uzumcu M, Kuhn PE, Marano JE, Armenti AE, Passantino L. Early postnatal methoxychlor exposure inhibits folliculogenesis and stimulates anti-Mullerian hormone production in the rat ovary. *Journal of Endocrinology* 191, 549–558 (2006)

Vannier B, Raynaud JP. Long-term effects of prenatal oestrogen treatment on genital morphology and reproductive function in the rat. *Journal of Reproduction and Fertility* 59, 43-49 (1980)

Varayoud J, Ramos JG, Bosquiazzo VL, Muñoz-de-Toro M, Luque EH. Developmental exposure to Bisphenol A impairs the uterine response to ovarian steroids in the adult. *Endocrinology* **149**, 5848-5860 (2008)

Vitt UA, McGee EA, Hayashi M, Hsueh AJ. In vivo treatment with GDF-9 stimulates primordial and primary follicle progression and theca cell marker CYP17 in ovaries of immature rats. *Endocrinology* **141**, 3814-3820 (2000)

Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Müllerian hormone: a new marker for ovarian function. *Reproduction* 131,1-9 (2006)

Wang C and Roy SK. Development of Primordial Follicles in the Hamster: Role of Estradiol-17β.*Endocrinology* 148, 1707–1716 (2007)

Watanabe C, Kuwagata M, Yoshimura S, Azegami J, Kojima K, Ono H, Nagao T. An improved technique for repeated gavage administration to rat neonates. *Congenital Anomalies (Kyoto)* 43, 177-179 (2003)

Wu J, Nayudu PL, Kiesel PS, Michelmann HW. Luteinizing hormone has a stage-limited effect on preantral follicle development in vitro. *Biology of Reproduction* **63**, 320-327 (2000)

Yamasaki K, Takeyoshi M, Sawaki M, Imatanaka N, Shinoda K, Takatsuki M. Immature rat uterotrophic assay of 18 chemicals and Hershberger assay of 30 chemicals. *Toxicology* 183, 93-115 (2003)

Yasuno K, Sakashita H, Araki S, Kobayashi R, Sakurada Y, Shirota M, Kamiie J, Shirota K. Cutaneous hybrid cyst in a Sprague-Dawley rats. *Journal of Toxicologic Pathology* 25, 175-178 (2012)

Yeh J, Kim B. Increasing blunting of inhibin responses to dynamic ovarian challenge is associated with reproductive aging in the rat. *Reproductive Sciences* 14, 10-19 (2007)

Yoshida H, Takakura N, Kataoka H, Kunisada T, Okamura H, Nishikawa SI. Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. *Developmental Biology* **184**, 122-137 (1997)

Yoshida M, Takahashi M, Inoue K, Hayashi S, Maekawa A, Nishikawa A. Delayed adverse effects of neonatal exposure to diethylstilbestrol and their dose dependency in female rats. *Toxicologic Pathology* **39**, 823-834 (2011)

Yoshida M, Watanabe G, Shirota M, Maekawa A, Taya K. Reduction of primordial follicles caused by maternal treatment with busulfan promotes endometrial adenocarcinoma development in donryu rats. *Journal of Reproduction and Development* 51, 707-714 (2005)

Zhang FP, Poutanen M, Wilbertz J, Huhtaniemi I. Normal prenatal but arrested postnatal sexual development of luteinizing hormone receptor knockout (LuRKO) mice. *Molecular Endocrinol*ogy **15**, 172-183 (2001)

Zhuang XL, Fu YC, Xu JJ, Kong XX, Chen ZG, Luo LL. Effects of genistein on ovarian follicular development and ovarian life span in rats. *Fitoterapia*. 81, 998-1002 (2010)

田谷一善、渡辺元、笹本修司. $^{125}I$ 標識ホルモンを用いたプロジェステロン、エストラジオール 17β のラジオイムノアッセイについて. 家畜繁殖雑誌 1985 年 31 巻 4 号 186-197 頁

| ターゲット | Accession No. | | | 配列（5'⇒3'） |
|---|---|---|---|---|
| GDF-9 | AF 099912 | プライマー | F | AGCTCAAATGGGACAACTGGAT |
| | | | R | GGGACAGTCCCCTTTACAGTACCT |
| | | プローブ | | CCCCGCACAGATAC |
| FSHR | NM 199237 | プライマー | F | CAAATGATCCTTTCGGGATGA |
| | | | R | CCCGGAACGCCATTGA |
| | | プローブ | | TGAGCACAAACCTC |
| ERß | NM 012754 | プライマー | F | CCTGCCGACTTCGCAAGT |
| | | | R | CCACACCGTTCTCTCCTGGAT |
| | | プローブ | | TTATGAAGTAGGAATGGTCAAGTG |
| Inhibin α | M 36453 | プライマー | F | GCAGCTCTACCAGGGAGCAT |
| | | | R | ACTTGAAAGAGTAGCCTCCATCTGA |
| | | プローブ | | AGGTCCCTACGCGTCCGAACCAC |
| Inhibin ßA | M 37482 | プライマー | F | CCCAGAGGTGCCTGCTATGT |
| | | | R | CATTGCTCCCTCTGGCTATCA |
| | | プローブ | | CTTGGGCACTCACCCTCACAATAGTTGG |
| Inhibin ßB | NM 080771 | プライマー | F | CCCTGGGCCGGTGAA |
| | | | R | CGTCATCAAAGTAGAGCATAGACATAGA |
| | | プローブ | | TTGCTGCATCCCCACCAAGCTGAG |
| LHR | NM 012978 | プライマー | F | CCGTCAGGGTGTAGACAGAGAGT |
| | | | R | CGGTGCAGCTGGCTTCTT |
| | | | | CACTGGCAAACACAG |
| StAR | NM 031558 | プライマー | F | GGCATGGCCACACACTTTG |
| | | | R | AGTGGATGAAGCACCATGCA |
| | | プローブ | | AGATGCCTGAGCAAAG |
| P450scc | J 05156 | プライマー | F | TCCCAGCGGTTCATCGA |
| | | | R | GAAATAAGTCTGGAGGCATGTTGA |
| | | プローブ | | CCGTCTACCAGATGTT |
| P450c17 | NM 012753 | プライマー | F | TGGCTTTGGTGGTGCACAATC |
| | | | R | TGAAAGTTGGTGTTCGGCTGAAG |
| | | プローブ | | ATCCAAAAGGAGATTGACCA |
| Aromatase | M 33986 | プライマー | F | GAAACGGTCCGCCCTTTCT |
| | | | R | TGGATTCCACACAGACTTCTACCA |
| | | プローブ | | ATGAAAGCTCTGACGGGC |
| 3ß-HSD | L 17138 | プライマー | F | AAGTATGCAATGTGCCACCATTT |
| | | | R | GCAAAAAGATGGCCGAGAA |
| | | プローブ | | CATTGGCTGCCAGCAC |

表1　リアルタイムPCRに用いたプライマーおよびプローブ

表 2　1 日齢から 5 日間エチニルエストラジオールの反復経口投与を受けた雌ラットの 13 週齢における器官重量（平均±標準偏差）

| EE (μg/kg/day) | 0[a] | 0.08 | 0.4 |
|---|---|---|---|
| N | 9 | 10 | 9 |
| Pituitary (mg) | 13.8 ± 1.1 | 17.1 ± 1.0 | 21.3 ± 3.6** |
| Ovaries (mg) | 94.4 ± 18.6 | 98.3 ± 14.7 | 57.8 ± 6.1** |
| Uterus (mg) | 525 ± 56 | 493 ± 19 | 441 ± 93** |

[a] corn oil 10 mL/kg/day

**p<0.01

| EE (μg/kg/day) | 0[a] | 0.4 | 2 |
|---|---|---|---|
| N | 3 | 3 | 3 |
| Mean LH | 6.72 ± 1.22 | 7.20 ± 2.06 | 4.91 ± 1.88 |
| Basal LH | 5.36 ± 1.12 | 5.56 ± 1.88 | 3.64 ± 1.77 |
| Frequency | 4.0 ± 0.0 | 5.0 ± 1.0 | 3.3 ± 1.2 |
| Amplitude | 3.65 ± 0.28 | 5.35 ± 1.18* | 4.16 ± 0.32 |

表 3　1 日齢から 5 日間エチニルエストラジオールの反復経口投与を受けた雌ラットの 14 週齢におけるパルス状 LH 分泌のパラメーター（平均±標準偏差）

動物は10週齢で両側卵巣を摘出し、13週齢で装着した頚静脈カニューレから頻回採血した。

[a] corn oil 10 mL/kg/day

*$p<0.05$

図１　１日齢から５日間エチニルエストラジオールの反復経口投与を受けた雌ラットの 8-9 週齢における性周期

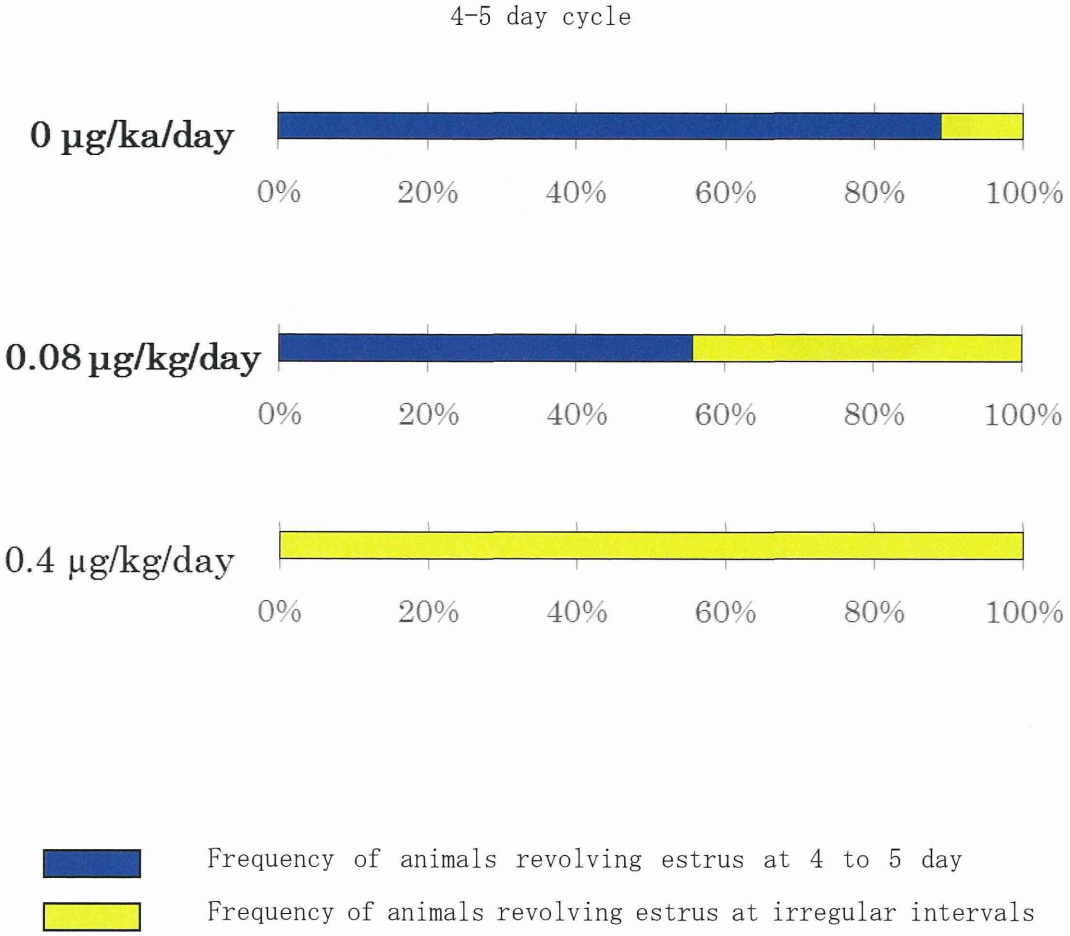
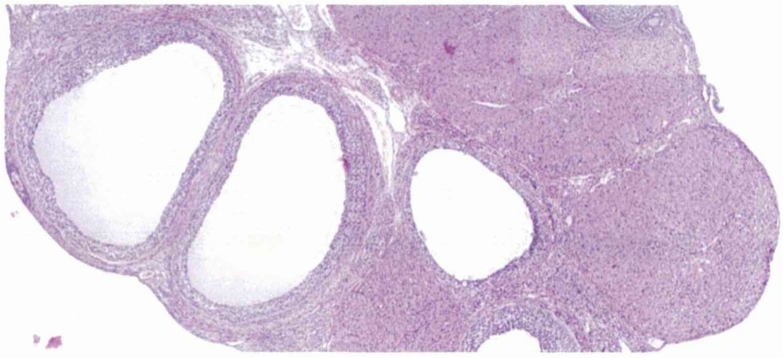


図２　　１日齢から５日間エチニルエストラジオール（EE）の反復経口投与を受けた雌ラットの卵巣にみられた嚢胞状卵胞の代表的画像



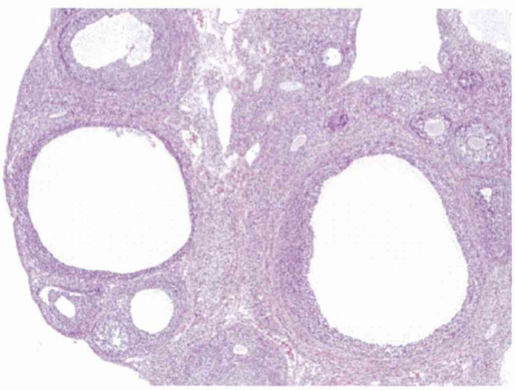
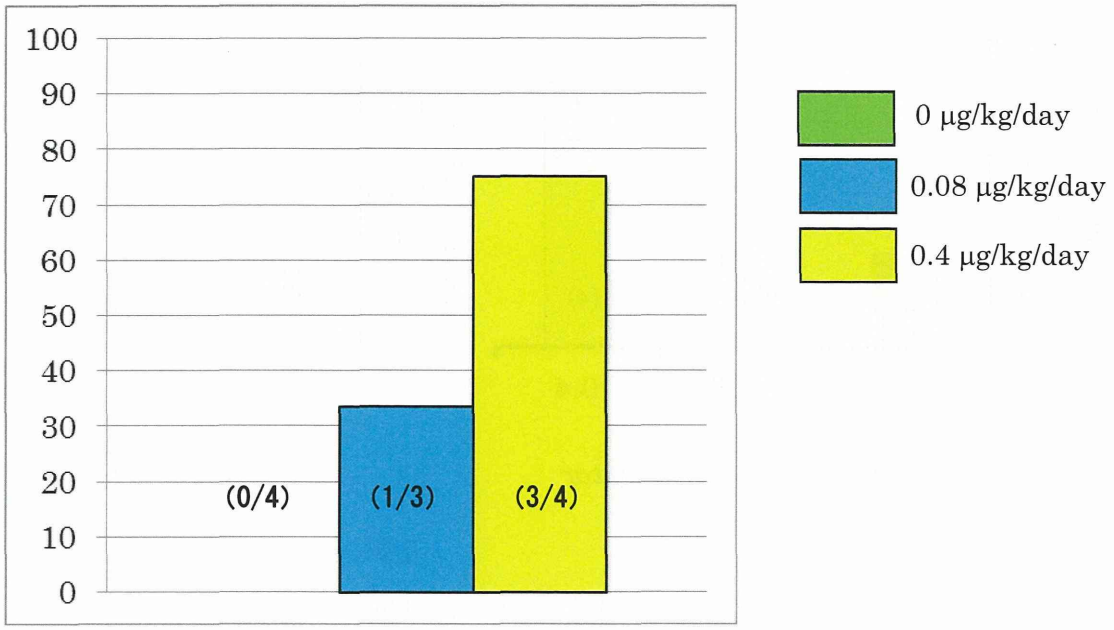


図 3　1 日齢から 5 日間エチニルエストラジオール（EE）の反復経口投与を受けた雌ラットの嚢胞状卵胞の保有率

A. 13 週齢における保有率（実験 1）




B. 10 週齢における保有率（実験 2）

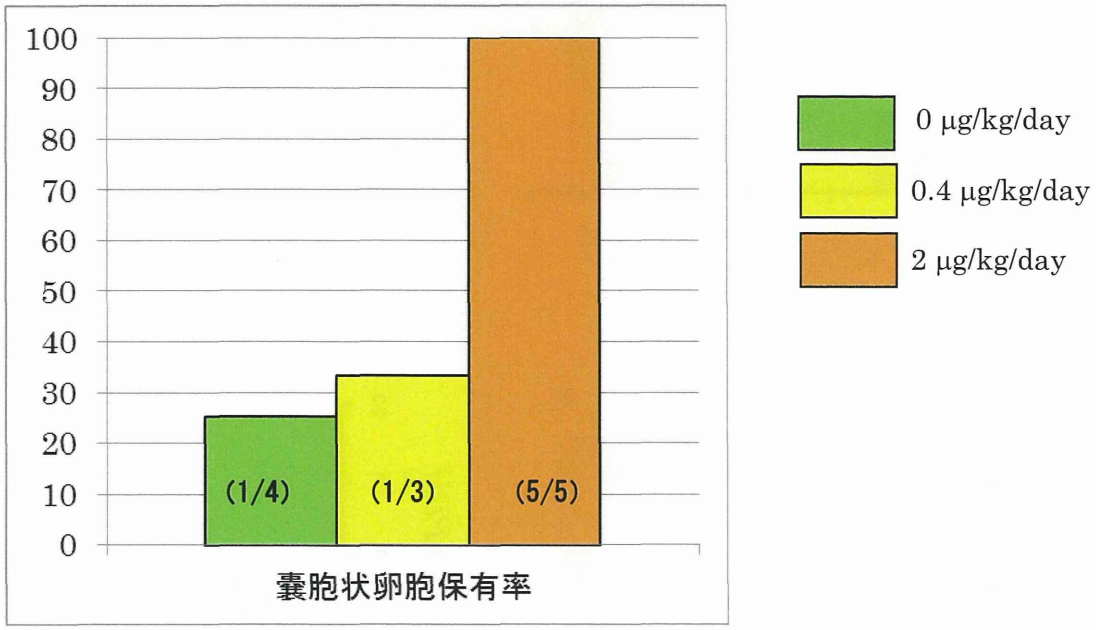


図４　１日齢から５日間エチニルエストラジオール（EE）の反復経口投与を受けた雌ラットの 13 週齢卵巣における卵胞発育関連遺伝子の発現（＊ p<0.05）

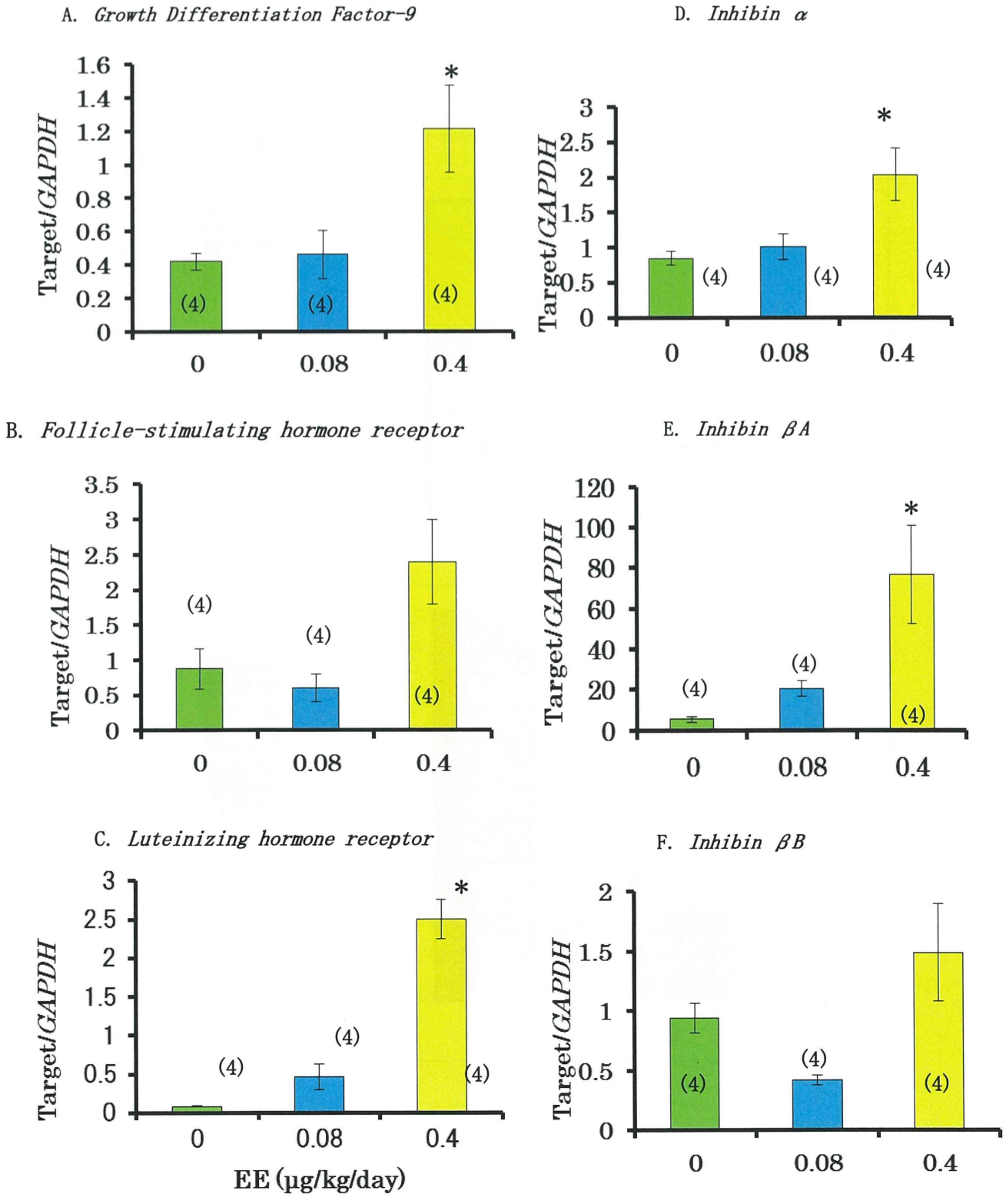
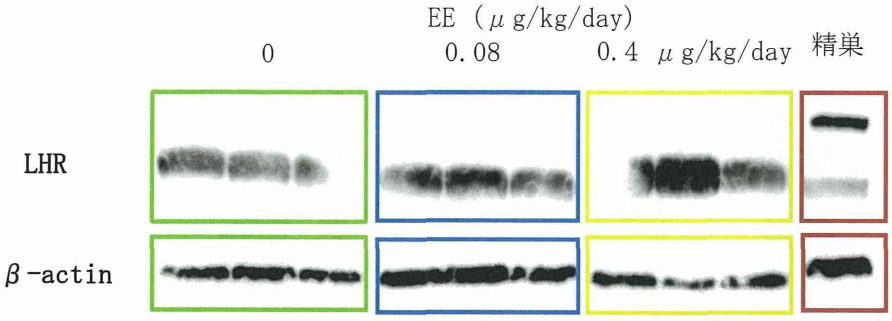


図5　1日齢から5日間エチニルエストラジオール（EE）の反復経口投与を受けた雌ラットの13週齢卵巣における luteinizing hormone 受容体(LHR)タンパク質の発現

A. Western blotting




B. LHR のβ-actin に対する相対発現量

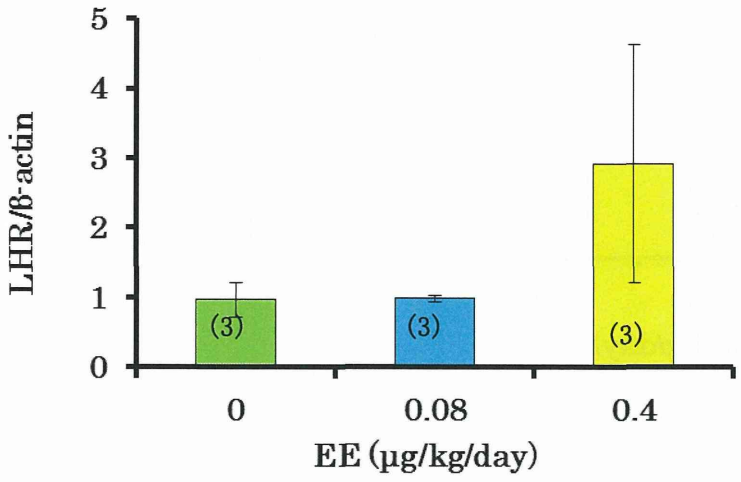


図6　1日齢から5日間エチニルエストラジオール（EE）の反復経口投与を受けた雌ラットの13週齢卵巣におけるステロイドホルモン合成関連遺伝子の発現（* p<0.05）