HHS Public Access

Author manuscript

Reprod Toxicol. Author manuscript; available in PMC 2019 October 01.

Published in final edited form as:

Reprod Toxicol. 2018 October ; 81: 132–139. doi:10.1016/j.reprotox.2018.07.085.

The Effects of Dietary Levels of Genistein on Ovarian Follicle Number and Gene Expression

Payel Kundu[1], **Shreya Patel**[2], **Daryl D. Meling**[2], **Kassie Deal**[2], **Liying Gao**[2], **William G. Helferich**[3], and **Jodi A. Flaws**[2]

[1]Neuroscience Program, University of Illinois, 405 N Mathews Ave, Urbana, IL 61801

[2]Department of Comparative Biosciences, College of Veterinary Medicine, University of Illinois, 2001 S. Lincoln Ave, Urbana, IL 61802

[3]Food Science and Human Nutrition, University of Illinois, 905 S. Goodwin Ave, Urbana, IL 61801

Abstract

Genistein is a phytoestrogen found in soy. We previously found that adult exposure to dietary levels of genistein affected gestation time, parturition time, litter size, pup weight, and pup mortality in CD-1 mice. The present study investigated the effects of adult genistein exposure on follicle number and gene expression in the ovaries of CD-1 mice. We found that exposure to genistein had no effect on follicle number, but it did affect the expression of apoptotic regulatory genes (*Bax, Bcl-2, Bid, and Dffa*) in the ovary.

1. Introduction

Genistein is an isoflavone found in many plants, but it is particularly abundant in soy. Genistein is an estrogenic isoflavone and has a twenty-fold selectivity for estrogen receptor (ER) β over ERα [1]. Human exposure to genistein can occur either through the diet in the form of soy products like tofu, tempeh, and soy milk, or through dietary supplements.

Women are exposed to genistein primarily through soy-based foods and dietary supplements. Asian populations typically show high serum levels of genistein due to the prevalence of soy in the diet, whereas European and American populations have lower and highly variable serum levels [2]. Dietary supplements containing phytoestrogens are often promoted as safe alternatives to synthetic estrogens and are thus a major route of genistein exposure [2].

Corresponding author: Jodi A. Flaws, Ph.D., Professor, Department of Comparative Biosciences, University of Illinois, 2001 S. Lincoln Ave, Urbana, IL 61802, Phone: 217-333-7933, Fax: 217-244-1652, jflaws@illinois.edu.

Email addresses:

Payel Kundu: kundupayel1@gmail.com, Shreya Patel: shreya.patel214@gmail.com, Daryl Meling: dmeling@illinois.edu, Kassandra Deal: kdeal4@illinois.edu, Liying Gao: lgao@illinois.edu, William G. Helferich: helferic@illinois.edu, Jodi A. Flaws: jflaws@illinois.edu

Genistein has been implicated as a potential preventative agent against various diseases including cancer, postmenopausal bone loss, and cardiovascular disease [3, 4]. However, the broader health effects of genistein are relatively underexplored. Genistein has been shown to affect biological systems via several diverse mechanisms, including inhibition of protein tyrosine kinases, inhibition of topoisomerase II, as well as actions at estrogen receptors [1, 3]. In particular, the ability of genistein to bind to and signal through estrogen receptors is of particular concern to female reproductive health because female reproductive tissues contain many estrogen receptors. Further, previous work has shown that xenoestrogens, including bisphenol A (BPA), the synthetic estrogen diethylstilbestrol (DES), and phytoestrogens can adversely affect measures of female reproductive health [5-8].

Estrogens are known to play important roles in regulating ovarian follicle development and function [9]. Estrogen deficient mice have decreased numbers of primordial and primary follicles as well as impaired antral follicle development [10]. Like estrogen, genistein is known to have activity through the estrogen receptors. Genistein has been shown to stimulate estrogen-responsive pS2 mRNA expression in the estrogen receptor positive human breast cancer cell line MCF-7 *in vitro* [11]. These effects were blocked by the addition of tamoxifen, suggesting that genistein was indeed acting on MCF-7 cells by interacting with estrogen receptors. Interestingly, genistein was found to have proliferative effects at low doses, but anti-proliferative effects at high doses, indicating that like many xenoestrogens, genistein can have non-monotonic effects in biological systems [11]. Genistein has also produced estrogen receptor-mediated changes in the thymus of mice [12]. Previous work has also shown that genistein can affect cognitive changes similar to those of estradiol in ovariectomized rats [13]. The relative binding affinities of genistein for ERα and ERβ are 0.7 and 13, compared to an affinity of 100 for both receptors with estradiol [1]. Thus, given the activity of genistein at the estrogen receptors, and the role of estrogens in regulating follicular growth and development, it is important to investigate the potential effects of genistein on ovarian follicles.

Our previous work showed that genistein exposure inhibited mouse antral follicle growth, altered steroid hormone levels, and changed gene expression *in vitro* [14]. Specifically, genistein exposure increased gene expression of the cell cycle regulators cyclin dependent kinase inhibitor 1A (*Cdkn1a*), cyclin B1 (*Ccnb1*), and cyclin E1 (*Ccne1*). Genistein exposure also increased the expression of the steroidogenic enzymes steroidogenic acute regulatory protein (*Star*) and cytochrome P450 family 11 subfamily A member 1 (*Cyp11a1*), while decreasing the expression of cytochrome P450 family 17 subfamily A member 1 (*Cyp17a1*) and 3beta-hydroxysteroid dehydrogenase/delta(5)-delta(4)isomerase type I (*Hsd3b1*). These results suggest that genistein can affect ovarian function *in vitro*. Thus, the proposed study was designed to investigate whether genistein exposure also affects ovarian function *in vivo*. To closely model human exposure to genistein, we used oral consumption of genistein that resulted in serum genistein levels in mice similar to those observed in humans after exposure to genistein through the diet. We then investigated ovarian follicle number in response to treatment with genistein. We also investigated the expression of genes involved in apoptosis, cell cycle regulation, ovarian steroidogenesis, as well as the expression of estrogen receptor genes.

2. Methods

2.1 Chemicals and diet formulation

Genistein (98% purified determined via high-pressure liquid chromatography, Botanical Research Center, University of Illinois) was sent to Envigo-Teklad (Madison, WI) to formulate custom diets. The AIN-93G, soy and phytoestrogen free diet was used as the control as well as the base diet. Genistein was added to the base diet at a concentration of 0, 300, 500, or 1000 parts per million (ppm). Previous work has shown that mice consuming these diets have serum genistein levels of 1.0, 1.8, and 2.6 μM respectively [15]. These serum levels are within the range of those observed after human soy supplementation [16].

2.2 Animals

Female CD-1 (30 days old) mice were purchased from Charles River Laboratories (Wilmington, MA) and were allowed to acclimate to the facility prior to adjustment to diet. The mice were housed four per cage at the University of Illinois at Urbana-Champaign Veterinary Medicine Animal Facility and were provided food and water *ad libitum*. Temperature was maintained at 22 ± 1 °C and animals were exposed to 12-h light and dark cycles.

2.3 Study design

This study was conducted as described in Patel et al. [7]. Briefly, beginning on post-natal day (PND) 35, female CD-1 mice were fed diets containing 0, 300, 500, or 1000 ppm genistein *ad libitum* for 30, 60, 150, or 240 days. All mice and food were weighed and recorded twice a week to ensure all the animals were eating similar amounts of food and that they did not experience abnormal weight changes. After each dosing period, three to six mice from each treatment group were euthanized in diestrus. After euthanasia, blood and tissues were collected for analyses as described below.

2.4 Assessment of ovarian follicle numbers

Follicle numbers were quantified as previously described [17]. Ovaries were fixed with paraformaldehyde and transferred into 70% ethanol before being embedded in paraffin wax and sectioned (8 μm) using a microtome. Sections were mounted onto glass slides and then stained with hematoxylin and eosin. Follicle numbers were counted in every 10^{th} section. Follicles were classified as primordial if they contained an oocyte surrounded by a single layer of squamous granulosa cells, primary if they contained an oocyte surrounded by a single layer of cuboidal granulosa cells, preantral if they contained an oocyte surrounded by at least two layers of cuboidal granulosa cells and theca cells, and antral if they contained an oocyte with a fluid-filled antral space surrounded by multiple layers of cuboidal granulosa cells and theca cells. Preantral and antral follicles were only counted if the oocyte contained nuclear material to avoid double counting the larger follicle types that can span multiple sections. Primordial and primary follicles were counted in each section regardless of the presence of nuclear material in the oocyte. Transitioning follicles were classified as the more immature state.

2.5 Gene expression analysis

Gene expression was analyzed as described in Berger et al. [18]. RNA from snap frozen ovaries was extracted using a miRNeasy Micro Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. The samples were treated with DNase (Qiagen, Inc., Valencia, CA) during the process. RNA (100 ng) was reversed transcribed to cDNA and subjected to quantitative real time PCR (qPCR) using the CFX96 Real-Time PCR Detection System (Bio-Rad Inc.) and accompanying software (CFX Manager Software). The initial incubation temperature was 95 °C for 5 min. This was followed by 36 cycles at 95 °C for 10 s, at 60 °C for 10 s, and at 72 °C for 10 s. Melting from 65 °C to 95 °C was followed by final extension at 72 °C for 2 min. Standard curves, melting temperature graphs, and threshold cycle (Ct) values were generated for each run. Primer sequences for each gene are listed in Table 1. All samples were run in duplicate. The expression data from each sample were normalized to the corresponding values of beta-actin (*Actb*). Individual relative fold changes were calculated by the Pfaffl method [19], then average fold changes for each group were represented as a ratio to the average fold change of the control group.

2.6 Statistical analysis

If data met assumptions of normal distribution and homogeneity of variance, data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett (2-sided) *post-hoc* comparisons. If data were not normally distributed, and/or did not meet homogeneity of variance assumptions, the independent sample Kruskal-Wallis H followed by Mann-Whitney U nonparametric tests were performed. Statistical significance was assigned at $P \leq 0.05$.

3. Results

3.1 The effects of genistein on ovarian follicle number

Genistein exposure did not significantly affect the number of primordial, preantral, antral, or total follicles at any of the selected doses (Figure 1). Further, genistein exposure did not significantly affect follicle numbers at any time point (Figure 1).

3.2 The effects of genistein on gene expression in the ovary

3.2.1 Apoptosis genes—After 60 days of exposure, genistein exposure at 500 and 1000 ppm significantly increased the expression of the anti-apoptotic factor known as B-cell lymphoma 2 (*Bcl-2*) (Figure 2, $p < 0.05$). However, genistein exposure did not significantly affect *Bcl-2* expression in the ovary at any other time point. It also did not affect the expression of the anti-apoptotic factor known as Bcl-2-like protein 10 (*Bcl2l10*) at any time point (Figure 2).

After 150 days of exposure, genistein treatment at 300, 500, and 1000 ppm significantly reduced expression of the pro-apoptotic factor *Bcl-2* associated X, apoptosis regulator (*Bax*) compared to control (Figure 2, $p < 0.05$), but it did not significantly affect *Bax* expression in the ovary at any other time point. Genistein exposure also did not affect expression of the pro-apoptotic factor known as apoptosis-inducing factor mitochondrial 1 (*Aifm1*) or the pro-apoptotic factor known as *Bcl-2* related ovarian killer protein (*Bok*) in the ovary at any time point (Figure 2).

Interestingly, genistein exposure reduced the expression of other pro-apoptotic factors known as BH3-interacting domain against death (*Bid*) and DNA fragmentation alpha (*Dffa*) in the ovary (Figure 2, $p<0.05$). Specifically, at 150 days, genistein exposure at 300 ppm reduced *Bid* expression and genistein exposure at 500 ppm and 1000 ppm reduced *Dffa* expression in the ovary compared to control (Figure 2, $p<0.05$).

3.2.2 Cell cycle regulator genes—Genistein did not significantly affect the expression of cyclin A2 (*Ccna2*), *Ccne1*, *Ccnb1*, cyclin dependent kinase 4 (*Cdk4*), *Cdkn1a*, or cyclin D2 (*Ccnd2*) at any dose (Figure 3). Further, it did not affect the selected cell cycle regulators at any time point (Figure 3).

3.2.3 Cytochrome P450 genes—Genistein did not significantly affect the expression of *Cyp11a1, Cyp17a1*, cytochrome P450 family 19 subfamily A member 1 (*Cyp19a1*), cytochrome P450, family 1, subfamily A, polypeptide 1 (*Cyp1a1*), or cytochrome P450 family 1 subfamily B member 1 (*Cyp1b1*) at any dose (Figure 4). Further, it did not affect expression of these cytochrome P450 genes at any time point (Figure 4).

3.2.4 Steroidogenesis genes—Genistein did not significantly affect the expression of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 (*Hsd3b2*), hydroxysteroid 17-beta dehydrogenase 1 (*Hsd17b1*) or *Star* at any dose (Figure 5). Further, it did not affect expression of these steroidogenesis genes at any time point (Figure 5).

3.2.5 Estrogen receptor genes—Genistein did not significantly affect the expression of estrogen receptor 1 (*Esr1*) or estrogen receptor 2 (*Esr2*) at any dose (Figure 6). Further, it did not affect expression of these genes at any time point (Figure 6).

Discussion

This study investigated the effects of chronic oral exposure to dietary levels of genistein on ovarian follicle numbers and gene expression in the ovary. The results show that exposure to 300, 500, or 1000 ppm genistein did not affect follicle numbers at any time-point in mice. The results also show that exposure to 300, 500 or 1000 ppm genistein did not affect the expression of several cell cycle regulatory genes (*Ccna2, Ccne1, Ccnb1, Cdk4, Cdkn1a, Ccnd2*), members of the cytochrome P450 superfamily of genes (*Cyp11a1, Cyp17a1, Cyp19a1, Cyp1a1, Cyp1b1*), key genes involved in steroidogenesis (*Hsd3b2, Hsd17b1, Star*), or estrogen receptor genes (*Esr1, Esr2*) in the ovary. However, genistein exposure did increase ovarian expression of the anti-apoptotic factor *Bcl-2* (500 and 1000 ppm) and decrease ovarian expression of the pro-apoptotic factors known as *Bax* (300, 500, and 1000 ppm), *Bid* (500 ppm), and *Dffa* (500 and 1000 ppm).

We previously found that exposure to genistein in the same paradigm as the current study resulted in the disruption of several indices of fertility. Mice treated with 500 or 1000 ppm genistein for 30 days had significantly decreased gestation times compared to control mice. Mice treated with 500 ppm genistein for 60 days had decreased litter size and increased average pup weight compared to control mice. Additionally, mice treated with 300 ppm genistein for 150 days had an increased risk of prolonged parturition compared to control

mice [7]. Interestingly, Patel et al. [7] showed that genistein exposure also had a non-linear effect on fertility in mice, with the 300 ppm genistein group showing reduced fertility at 240 days compared to control mice, but the 500 and 1000 ppm genistein groups showing higher fertility at 240 days compared to control mice. Genistein exposure did not affect levels of estradiol or progesterone in Patel et al. [7]. Taken together with the results of the current study, these data suggest that chronic oral exposure to environmentally relevant levels of genistein can affect female reproductive health, but that these effects are likely not mediated by changes in follicle number, changes in estradiol or progesterone biosynthesis, or changes in the expression of the cell cycle regulatory genes *Ccna2, Ccne1, Ccnb1, Cdk4, Cdkn1a*, or *Ccnd2* in the ovary. They are also not likely due to changes in ovarian steroidogenesis or in the number of estrogen receptors in the ovary, as shown by the lack of changes in gene expression of key members of the cytochrome P450 superfamily (*Cyp11a1, Cyp17a1, Cyp19a1, Cyp1a1*, and *Cyp1b1*) as well as the lack of changes in gene expression of *Esr1* and *Esr2*.

Given the changes in *Bcl-2, Bax, Bid*, and *Dffa* with exposure to genistein that were found in the present study, it is possible that some of the effects of genistein in the ovary are mediated by changes in apoptotic regulation. *Bcl-2* is generally considered to be anti-apoptotic [20,22], whereas *Bax, Bid*, and *Dffa* are generally considered to be pro-apoptotic [21, 22]. Thus, the pattern of gene expression found in the present study suggests that chronic exposure to genistein might act in an anti-apoptotic manner in the ovary, providing a possible mechanism for the increased fertility found in aged mice given high levels of genistein in Patel et al. [7]. However, it is important to note that this study did not find any effect of genistein exposure on the expression other selected regulators of the apoptotic pathway (*Bok*, *Aifm1*, and *Bcl2l10*). It is possible that the current study missed changes in expression of these genes because potential changes occurred earlier or later than the times examined in this study. It is also possible that genistein exposure alters the protein levels, but not the transcript levels of apoptotic regulators. Thus, future studies should examine the effects of genistein exposure on protein levels of regulators of apoptosis.

The results from the present study are in contrast with our previous work in which genistein, while not altering levels of *Bax* or *Bcl-2*, did alter the expression of the cell cycle regulatory genes *Cdkn1a, Ccnb1*, and *Ccne1* as well as the steroidogenic enzymes *Star, Cyp11a1*, *Hsd3b1* and *Cyp17a1* at the highest dose (36μM) [14]. However, the Patel et al. study [14] was conducted *in vitro* and the doses used in that study were higher than the doses found *in vivo* in rodents after dietary exposure to genistein. Additionally, the *in vitro* paradigm exposed the follicles to genistein directly, which differs from genistein exposure to the ovaries *in vivo* after oral dosing due to hepatic metabolism. Additionally, it should be noted that in the present study, due to small sample sizes, variability in gene expression was high, thus potentially obscuring small changes in gene expression between exposure groups.

Other studies have also shown effects of genistein on reproductive health in female rodents. Genistein exposure early in life was shown to cause hyperplasia of the mammary glands, abnormal cellular maturation in the vagina as well as abnormal ovarian antral follicles in female rats [23]. It was also shown to alter the numbers of corpora lutea and ovulated oocytes in mice [24]. Neonatal exposure to environmentally relevant doses of genistein was

shown to increase estrous cycle length and decrease the percentage of live pup births in exposed females compared to control female mice [24]. Further, it was shown to inhibit oocyte nest breakdown in mice [25, 26] and increase the number of multi-oocyte follicles in rats and mice [27, 28].

Previous research also suggests that soy isoflavone exposure, especially early in life, can affect reproductive health in human populations [29]. Infants fed soy formula have exhibited premature development of breast buds as well as the vaginal epithelium when compared to their cow milk or breast milk fed counterparts [30, 31]. Additionally, one study has found that women who were fed soy formula as infants report slightly longer menstrual bleeding duration as well as more menstrual discomfort than those fed milk-based formulas [32]. A recent large study of adult women found that women who were fed soy-based formulas as infants were more likely to develop smooth muscle tumors in the uterus than women fed milk-based formulas [33]. Infants fed soy-based formulas have serum genistein levels of 1.4–4.5 μM [34], which are comparable to the serum genistein levels (1, 1.8, and 2.6 μM) in the present study. Thus, the results of the present study may be applicable to this population. However, it should be noted that exposure in the present study started when mice were 35 days old, not at an age comparable to human infancy.

Collectively, genistein exposure has been shown to affect reproductive outcomes in rodents as well as in humans. Women continue to be exposed to genistein through the consumption of soy-based food products as well as through dietary supplements, yet much remains unknown about this potent phytoestrogen. Future studies should further investigate the mechanisms by which genistein could be affecting female reproductive health. This study and others like it are critical for understanding the broader physiological effects of genistein.

Acknowledgements

This work was supported by NIH R03ES023972 (JAF), NIH R03ES023972S1, the Botanical Estrogen Research Center at the University of Illinois (WGH), a Billie A. Field Fellowship (SP), and a Toxicology Scholar Award (PK).

References:

[1]. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998;139:4252–63. [PubMed: 9751507]

[2]. Jefferson WN, Patisaul HB, Williams CJ. Reproductive consequences of developmental phytoestrogen exposure. Reproduction. 2012;143:247–60. [PubMed: 22223686]

[3]. Polkowski K, Mazurek AP. Biological properties of genistein. A review of in vitro and in vivo data. Acta Pol Pharm 2000;57:135–55. [PubMed: 10934794]

[4]. Russo M, Russo GL, Daglia M, Kasi PD, Ravi S, Nabavi SF, et al. Understanding genistein in cancer: The "good" and the "bad" effects: A review. Food Chem 2016;196:589–600. [PubMed: 26593532]

[5]. Maffini MV, Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: the case of bisphenol-A. Mol Cell Endocrinol 2006;254-255:179–86. [PubMed: 16781053]

[6]. Palter SF, Tavares AB, Hourvitz A, Veldhuis JD, Adashi EY. Are estrogens of import to primate/human ovarian folliculogenesis? Endocr Rev 2001;22:389–424. [PubMed: 11399749]

[7]. Patel S, Hartman JA, Helferich WG, Flaws JA. Preconception exposure to dietary levels of genistein affects female reproductive outcomes. Reprod Toxicol 2017;74:174–80. [PubMed: 28970133]

[8]. Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A, Flaws JA. Bisphenol A Exposure, Ovarian Follicle Numbers, and Female Sex Steroid Hormone Levels: Results From a CLARITY-BPA Study. Endocrinology. 2017;158:1727–38. [PubMed: 28324068]

[9]. Britt KL, Findlay JK. Estrogen actions in the ovary revisited. J Endocrinol 2002;175:269–76. [PubMed: 12429025]

[10]. Britt KL, Saunders PK, McPherson SJ, Misso ML, Simpson ER, Findlay JK. Estrogen actions on follicle formation and early follicle development. Biol Reprod 2004;71:1712–23. [PubMed: 15269096]

[11]. Wang TT, Sathyamoorthy N, Phang JM. Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis. 1996;17:271–5. [PubMed: 8625449]

[12]. Cooke PS, Selvaraj V, Yellayi S. Genistein, estrogen receptors, and the acquired immune response. J Nutr 2006;136:704–8. [PubMed: 16484547]

[13]. Pisani SL, Neese SL, Doerge DR, Helferich WG, Schantz SL, Korol DL. Acute genistein treatment mimics the effects of estradiol by enhancing place learning and impairing response learning in young adult female rats. Horm Behav 2012;62:491–9. [PubMed: 22944517]

[14]. Patel S, Peretz J, Pan YX, Helferich WG, Flaws JA. Genistein exposure inhibits growth and alters steroidogenesis in adult mouse antral follicles. Toxicol Appl Pharmacol 2016;293:53–62. [PubMed: 26792615]

[15]. Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, et al. The soy isoflavone genistein decreases adipose deposition in mice. Endocrinology. 2003;144:3315–20. [PubMed: 12865308]

[16]. Albertazzi P, Pansini F, Bottazzi M, Bonaccorsi G, De Aloysio D, Morton MS. Dietary soy supplementation and phytoestrogen levels. Obstet Gynecol 1999;94:229–31. [PubMed: 10432133]

[17]. Hannon PR, Niermann S, Flaws JA. Acute exposure to di(2-ethylhexyl) phthalate in adulthood causes adverse reproductive outcomes later in life and accelerates reproductive aging in female mice. Toxicol Sci 2015.

[18]. Berger A, Ziv-Gal A, Cudiamat J, Wang W, Zhou C, Flaws JA. The effects of in utero bisphenol A exposure on the ovaries in multiple generations of mice. Reprod Toxicol 2015;60:39–52. [PubMed: 26746108]

[19]. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29:e45. [PubMed: 11328886]

[20]. Belka C, Budach W. Anti-apoptotic Bcl-2 proteins: structure, function and relevance for radiation biology. Int J Radiat Biol 2002;78:643–58. [PubMed: 12194748]

[21]. Pawlowski J, Kraft AS. Bax-induced apoptotic cell death. Proc Natl Acad Sci U S A. 2000;97:529–31. [PubMed: 10639111]

[22]. Matsuda F, Inoue N, Manabe N, Ohkura S. Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. J Reprod Dev 2012;58:44–50. [PubMed: 22450284]

[23]. Delclos KB, Bucci TJ, Lomax LG, Latendresse JR, Warbritton A, Weis CC, et al. Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats. Reprod Toxicol 2001;15:647–63. [PubMed: 11738518]

[24]. Jefferson WN, Padilla-Banks E, Newbold RR. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. Biol Reprod 2005;73:798–806. [PubMed: 15930323]

[25]. 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. 2007;148:3580–90. [PubMed: 17446182]

[26]. Jefferson W, Newbold R, Padilla-Banks E, Pepling M. Neonatal genistein treatment alters ovarian differentiation in the mouse: inhibition of oocyte nest breakdown and increased oocyte survival. Biol Reprod 2006;74:161–8. [PubMed: 16192398]

[27]. Cimafranca MA, Davila J, Ekman GC, Andrews RN, Neese SL, Peretz J, et al. Acute and chronic effects of oral genistein administration in neonatal mice. Biol Reprod 2010;83:114–21. [PubMed: 20357267]

[28]. Losa SM, Todd KL, Sullivan AW, Cao J, Mickens JA, Patisaul HB. Neonatal exposure to genistein adversely impacts the ontogeny of hypothalamic kisspeptin signaling pathways and ovarian development in the peripubertal female rat. Reprod Toxicol 2011;31:280–9. [PubMed: 20951797]

[29]. Hooper L, Madhavan G, Tice JA, Leinster SJ, Cassidy A. Effects of isoflavones on breast density in pre- and post-menopausal women: a systematic review and meta-analysis of randomized controlled trials. Hum Reprod Update. 2010;16:745–60. [PubMed: 20511398]

[30]. Bernbaum JC, Umbach DM, Ragan NB, Ballard JL, Archer JI, Schmidt-Davis H, et al. Pilot studies of estrogen-related physical findings in infants. Environ Health Perspect 2008;116:416–20. [PubMed: 18335112]

[31]. Zung A, Glaser T, Kerem Z, Zadik Z. Breast development in the first 2 years of life: an association with soy-based infant formulas. J Pediatr Gastroenterol Nutr 2008;46:191–5. [PubMed: 18223379]

[32]. Strom BL, Schinnar R, Ziegler EE, Barnhart KT, Sammel MD, Macones GA, et al. Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. Jama. 2001;286:807–14. [PubMed: 11497534]

[33]. D'Aloisio AA, Baird DD, DeRoo LA, Sandler DP. Association of intrauterine and early-life exposures with diagnosis of uterine leiomyomata by 35 years of age in the Sister Study. Environ Health Perspect. 2010;118:375–81. [PubMed: 20194067]

[34]. Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet. 1997;350:23–7. [PubMed: 9217716]

Highlights:

- Genistein exposure does not affect ovarian follicle number
- Genistein exposure does not affect expression of cell cycle regulators
- Genistein exposure does not affect expression of steroidogenic enzymes
- 2Genistein exposure alters expression of apoptotic regulator genes

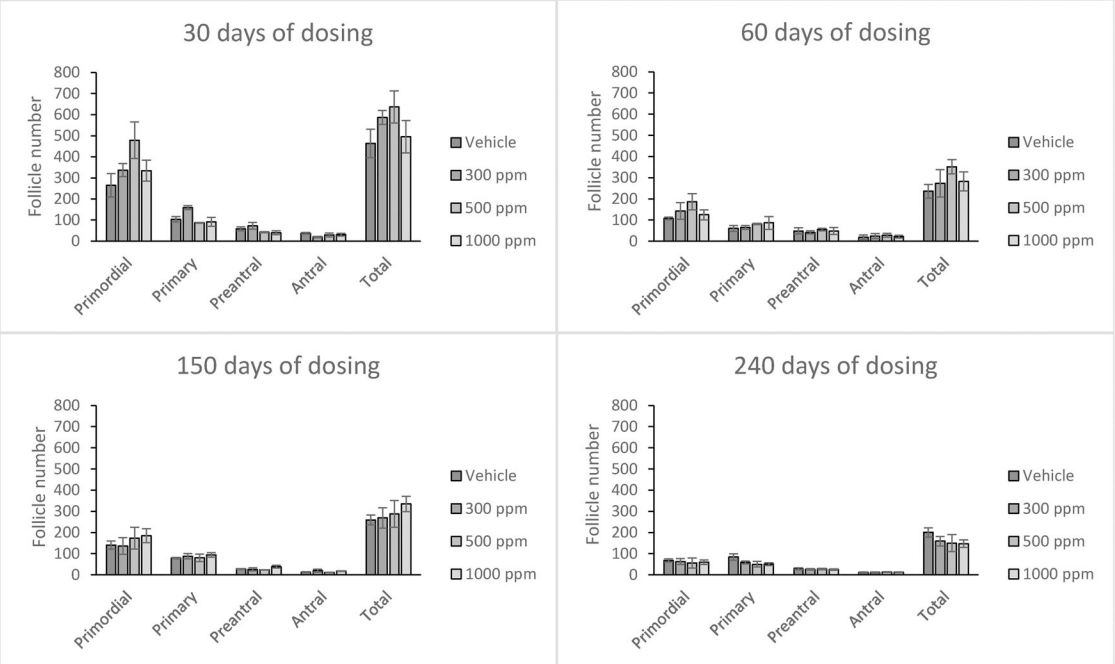

Figure 1:

Effects of genistein exposure on follicle numbers in mice treated with 0, 300, 500, or 1000 ppm genistein. The numbers of primordial, primary, preantral, antral, and total ovarian follicles were counted at 30, 60, 150, and 240 days of age. Graphs represent means ± SEM from 4-6 females per treatment group in the F1 generation.

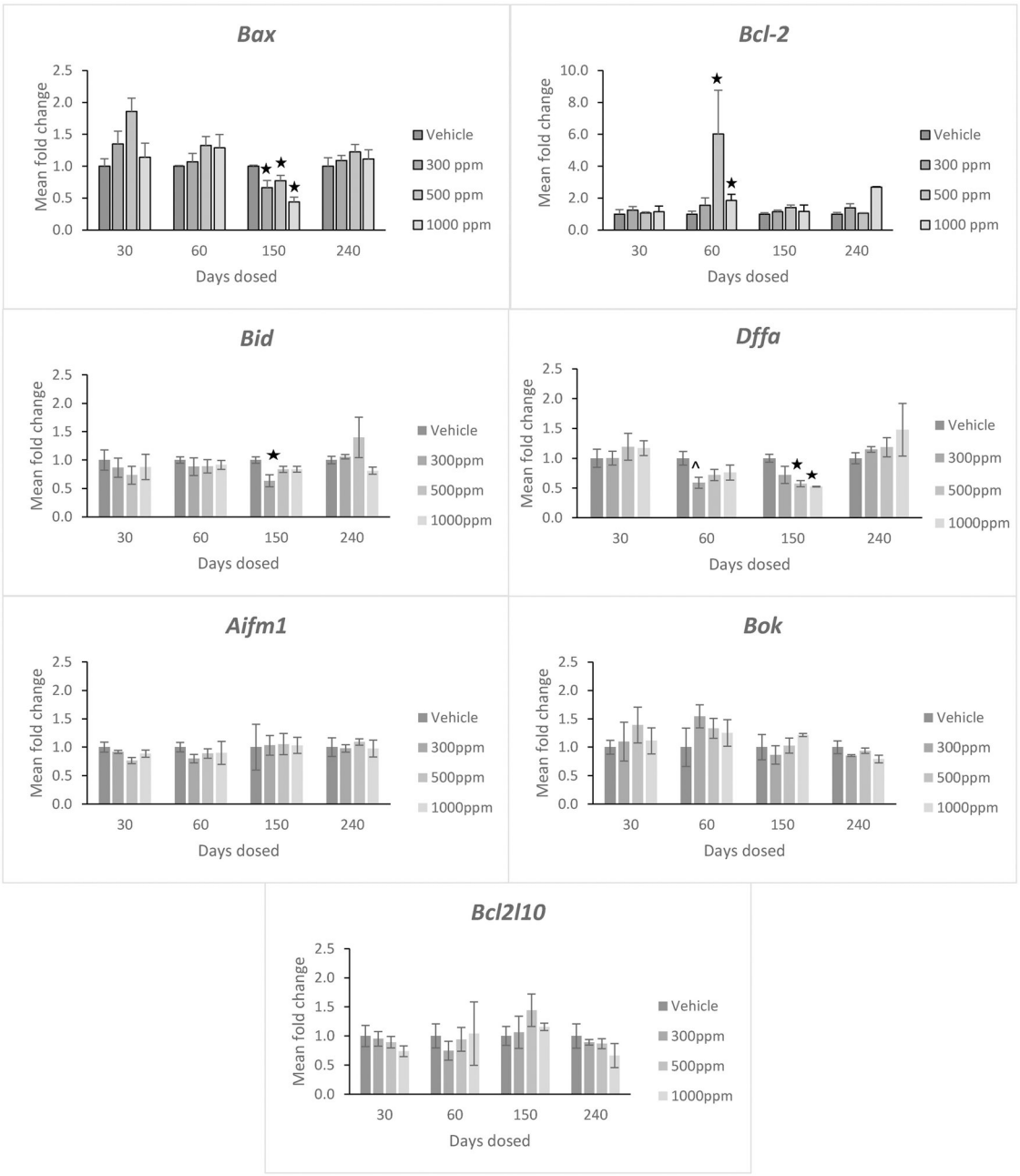

Figure 2:

Effects of genistein exposure on gene expression of *Bax, Bcl-2, Bid, Dffa, Aifm1, Bok*, and *Bcl2l10* in mice treated with 0, 300, 500, or 1000 ppm genistein. Gene expression was measured after 30, 60, 150, and 240 days of exposure to genistein. RNA was extracted from snap-frozen ovaries, reversed transcribed to cDNA, and subjected to quantitative real time PCR. Graphs represent means ± SEM from 3 females per treatment group. Asterisks indicate significant differences compared to the control ($p \leq 0.05$). ^indicates $p = 0.064$.

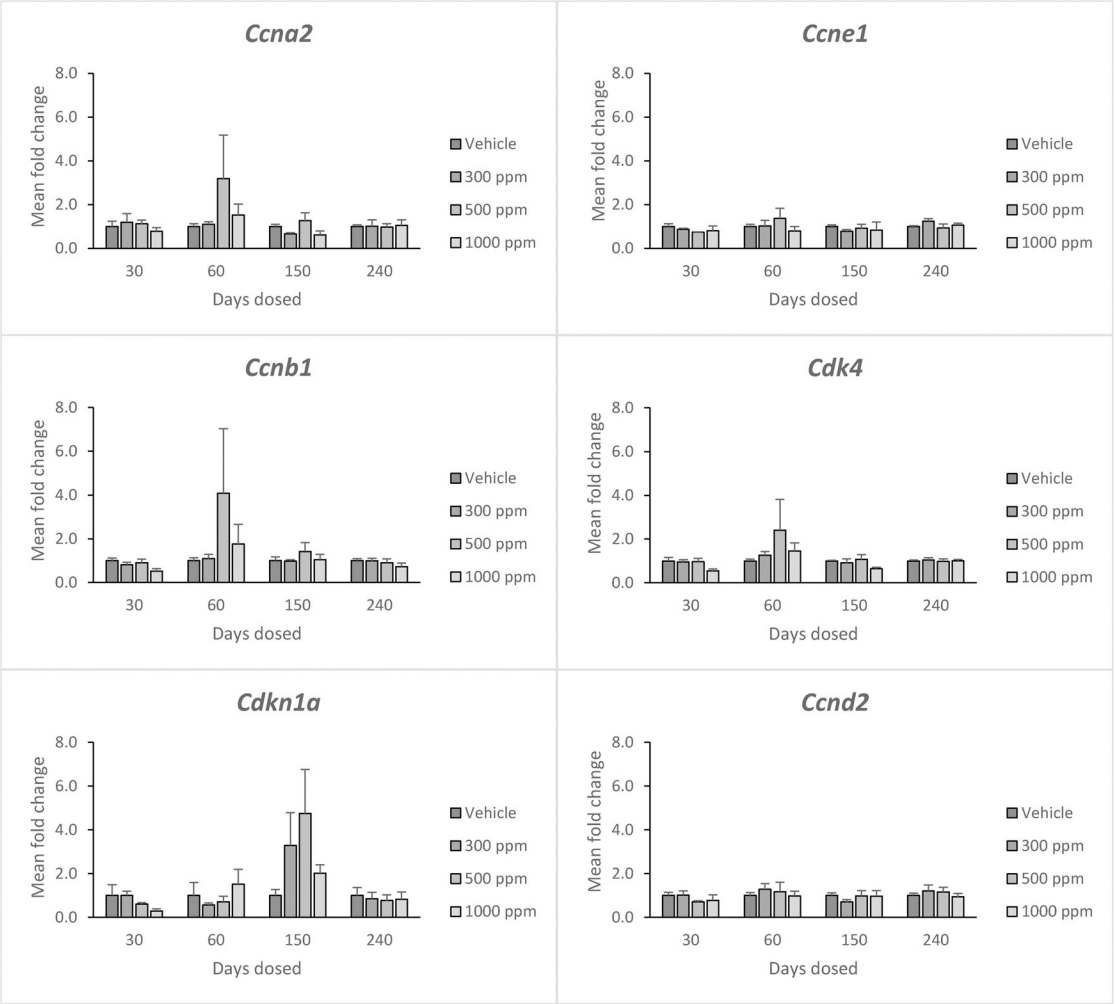

Figure 3:

Effects of genistein exposure on gene expression of *Ccna2, Ccne1, Ccnb1, Cdk4, Cdkn1a*, and *Ccnd2* in mice treated with 0, 300, 500, or 1000 ppm genistein. Gene expression was measured after 30, 60, 150, and 240 days of exposure to genistein. RNA was extracted from snap-frozen ovaries, reversed transcribed to cDNA, and subjected to quantitative real time PCR. Graphs represent means ± SEM from 3 females per treatment group.

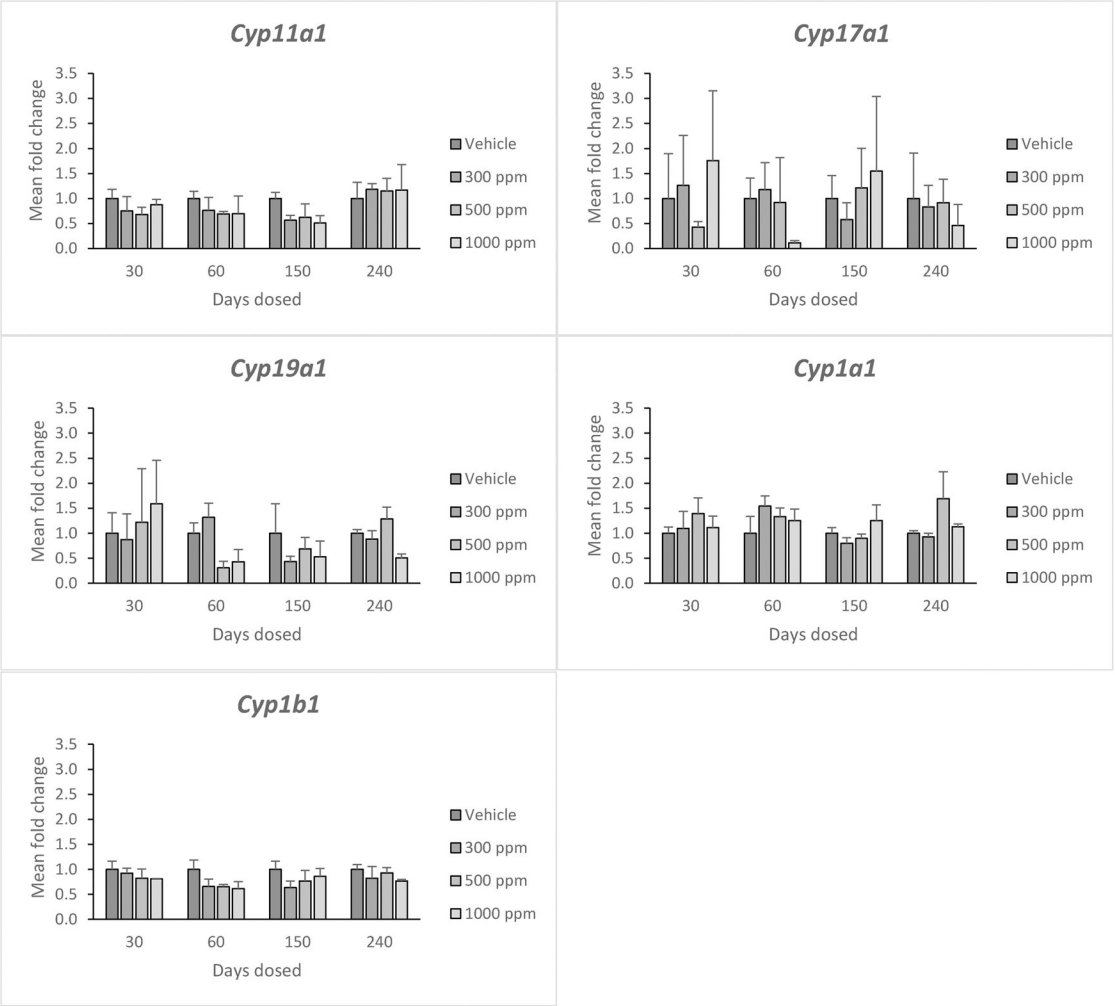

Figure 4:

Effects of genistein exposure on gene expression of *Cyp11a1, Cyp17a1, Cyp19a1, Cyp1a1*, and *Cyp1b1* in mice treated with 0, 300, 500, or 1000 ppm genistein. Gene expression was measured after 30, 60, 150, and 240 days of exposure to genistein. RNA was extracted from snap-frozen ovaries, reversed transcribed to cDNA, and subjected to quantitative real time PCR. Graphs represent means ± SEM from 3 females per treatment group.

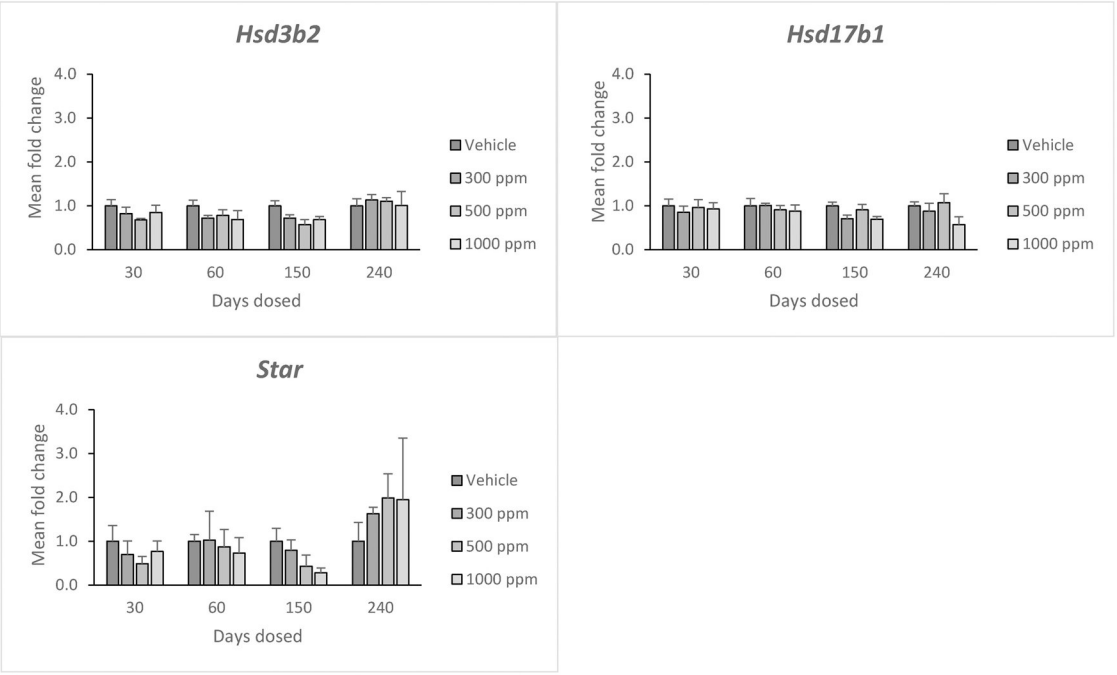

Figure 5:

Effects of genistein exposure on gene expression of *Hsd3b2, Hsd17b*, and Star in mice treated with 0, 300, 500, or 1000 ppm genistein. Gene expression was measured after 30, 60, 150, and 240 days of exposure to genistein. RNA was extracted from snap frozen ovaries, reversed transcribed to cDNA, and subjected to quantitative real time PCR. Graphs represent means ± SEM from 3 females per treatment group.

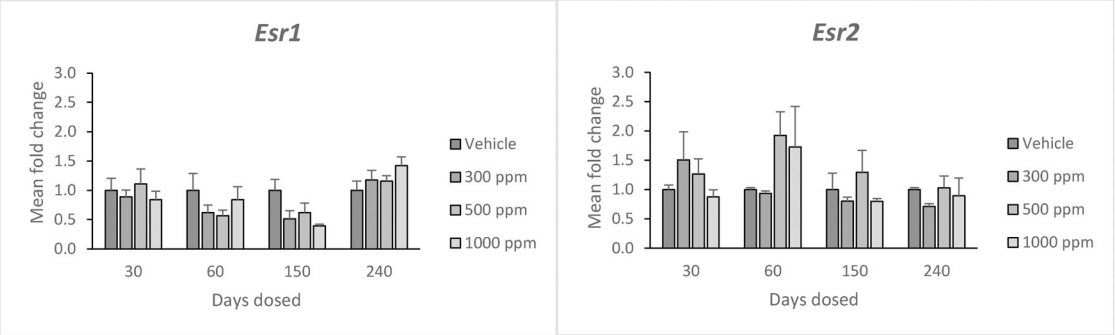

Figure 6.

Effects of genistein exposure on gene expression of *Esr1* and *Esr2* in mice treated with 0, 300, 500, or 1000 ppm genistein. Gene expression was measured after 30, 60, 150, and 240 days of exposure to genistein. RNA was extracted from snap-frozen ovaries, reversed transcribed to cDNA, and subjected to quantitative real time PCR. Graphs represent means ± SEM from 3 females per treatment group.

Table 1

Gene	Species	Forward sequence	Reverse sequence
Actb	mouse	AGC ACA GCT TCT TTG CAG CTC CTT	CAG CGC AGC GAT ATC GTC ATC CAT
Aifm1	mouse	AGG ACG GTG AGC AAC ATG AA	GTT CTA TCC ACC CAT CCC GC
Bax	mouse	TGA AGA CAG GGG CCT TTT TG	AAT TCG CCG GAG ACA CTC G
Bcl-2	mouse	ATG CCT TTG TGG AAC TAT ATG GC	GGT ATG CAC CCA GAG TGA TGC
Bcl2l10	mouse	CGC TAC ACA CAC TGA CTG GA	CTT TAG GAT CCC CTG CCC TG
Bid	mouse	AGC AAA TGT TCC CTC CGC TTC TGT	GTA GGC TGT GGC GGC TCG TG
Bok	mouse	CTG CCC CTG GAG GAC GCT TG	CCG TCA CCA CAG GCT CCG AC
Ccna2	mouse	GCT CTA CTG CCC GGA GGC TGA	TGG CCT ACA TGT CCT CTG GGG AA
Ccnb1	mouse	TGC ATT CTC TCA GTG CCC TCC ACA	AGA CAG GAG TGG CGC CTT GGT
Ccnd2	mouse	CCT TTG ACG CAG GCT CCC TTC T	ACC CTG GTG CAC GCA TGC AAA
Ccnel	mouse	GGT GTC CTC GCT GCT TCT GCT T	CCG GAT AAC CAT GGC GAA CGG A
Cdk4	mouse	AGA AAC CCT CGC TGA AGC GGC A	TGG GGG TGA ACC TCG TAA GGA GA
Cdkn1a	mouse	TTA GGC AGC TCC AGT GGC AAC C	ACC CCC ACC ACC ACA CAC CAT A
Cyp1a1	mouse	TGT CAG ATG ATA AGG TCA TCA CG	TCT CCA GAA TGA AGG CCT CCA G
Cyp1b1	mouse	GCG ACG ATT CCT CCG GGC TG	TGC ACG CGG GCC TGA ACA TC
Cyp11a1	mouse	AGA TCC CTT CCC CTG GTG ACA ATG	CGC ATG AGA AGA GTA TCG ACG CAT C
Cyp17a1	mouse	CCA GGA CCC AAG TGT GTT CT	CCT GAT ACG AAG CAC TTC TCG
Cyp19a1	mouse	CAT GGT CCC GGA AAC TGT GA	GTA GTA GTT GCA GGC ACT TC
Dffa	mouse	GCC AGA TCC TTA CCA CAC TGA	TTA TGT CCC AGC TCA GAG CGA
Esr1	mouse	CCG TGT GCA ATG ACT ATG CC	GTG CTT CAA CAT TCT CCC TCC TC
Esr2	mouse	GGA ATC TCT TCC CAG CAG CA	GGG ACC ACA TTT TTG CAC TT
Hsd17b1	mouse	AAG CGG TTC GTG GAG AAG TAG	ACT GTG CCA GCA AGT TTG CG
Hsd3b2	mouse	CAG GAG AAA GAA CTG CAG GAG GTC	GCA CAC TTG CTT GAA CAC AGG C
Star	mouse	CAG GGA GAG GTG GCT ATG CA	CCG TGT CTT TTC CAA TCC TCT G