The traditional rodent uterotropic response assay has been incorporated into the U.S. Environmental Protection Agency’s screening and testing program for environmental endocrine-disrupting chemicals (EDCs). While much effort continues to focus on determining protocol variables, few studies compare uterotropic responses in rats, a species commonly used in toxicologic testing, with other rodent species. In this study, we compared uterine responses in immature outbred CD-1 mice and Sprague-Dawley rats. After three daily subcutaneous injections with 17β-estradiol (0.1–500 μg/kg/day), immature mice and rats demonstrated a similar dose-response increase in absolute uterine wet weight and uterine weight:body weight ratio. Further, morphologic and biochemical parameters of estrogenicity, including uterine epithelial cell height and number, gland number, and induction of estrogen-responsive proteins lactoferrin and complement C3, mirror wet weight increases. We conclude that mice are as well suited as rats for the uterotropic bioassay. Because of the advantages of using mice, including lower costs, less space required, and smaller and smaller amounts of compound needed for tests, mice should be given appropriate consideration in testing paradigms for EDCs. Key words: complement C3, dose-response modeling, endocrine disruptors, endocrine modulators, environmental estrogens, estrogen, lactoferrin, risk assessment extrapolations, uterotropic bioassay.

Environmental Health Perspectives • VOLUME 109 | NUMBER 8 | August 2001

Address correspondence to R. R. Newbold, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 USA. Telephone: (919) 541-0738. Fax: (919) 541-4634. E-mail: newbold1@niehs.nih.gov

We thank M. Harris for assistance with the rat studies. We also thank M. Sanders and C. Tramp at the National Institutes of Health (NIH) for their help. We also thank M. Sanders and C. Trepma for critical review of the manuscript and W. Stokes, Associate Director for Animal and Alternative Research, NIEHS, for his helpful comments.

Received 19 December 2000; accepted 20 February 2001.
in other groups of mice have been previously reported by this laboratory (16,17).

**Immature rat uterotrophic bioassay.** Timed-pregnant rats (Tac:N(SD)fBR) were obtained from Taconic Laboratories (Raleigh, NC) on gestation day 15. At birth (day 1), all pups were sorted by sex and redistributed so that all litters were standardized to eight female pups per dam. All animals were housed in a temperature-controlled room (21–22°C) with a 12 hr light:12 hr dark cycle. Rats were provided fresh RO/DI water and NIH-31 lab chow and ad libitum. All animals procedures complied with NIH/NIEHS animal care guidelines. Rats were weaned on day 23 and housed four per cage. Starting on the day of weaning, rats (n = 4/treatment group) were subcutaneously injected with varying doses of 17β-estradiol (0, 1, 10, 100, or 500 µg/kg/day) dissolved in corn oil for 3 consecutive days; lower doses were not tested in rats because reports in the literature showed no significant difference between lower doses and control values in uterine wet weight changes (11). Rats were euthanized by cervical dislocation on the day after the last injection (day 26). Body weights and uterine wet weights were determined as described for the mice. Uterine tissues were fixed in Bouin’s fixative and processed for histologic and morphometric examination.

**Uterine wet weight determination.** Uterine wet weight:body weight ratios were calculated for each animal by dividing the uterine wet weight by the body weight and multiplying by 100. We used StatView SE + Graphics (ABACUS Concepts, Inc., Berkeley, CA) to determine the mean and SE to perform analysis of variance (ANOVA) using the Fisher’s test of significance (p < 0.05).

**Histologic observations.** Uterine tissues were processed and embedded in paraffin with one horn placed longitudinally and the other placed cross-wise; sections were cut at 4 µm and stained with hematoxylin and eosin (H&E). We quantitated histologic responses using an Olympus BH-2 microscope (Olympus Corp., New Hyde Park, NY) interfaced with an image analysis system (Southern Micro Instruments, Atlanta, GA) as described previously (22,27).

**Cell height determinations.** To determine cell height, we obtained three measurements from each of three areas from a minimum of three animals (at least 27 measurements per group). We measured cell height in longitudinally embedded tissues, always from the middle of the uterus and away from the oviduct or cervical segments. We calculated the mean for each area and then the mean for each animal. We then used these animal means to determine the mean ± SE for each treatment group.

**Cell number determinations.** We determined the number of uterine epithelial cells per micrometer of basement membrane by obtaining three counts per area (in longitudinally embedded tissues, as described above) from three areas per animal from a minimum of three animals (at least 27 measurements per treatment group). We counted the epithelial cells in a length of approximately 100 µm uterine epithelium and calculated the number of cells per micrometer of basement membrane. We calculated the mean for each area and then the mean for each animal. We used these animal means to determine the mean ± SE for each treatment group.

**Gland number determinations.** We counted the number of glands in cross-sections of uterine tissues obtained from the middle region of the uterus. We counted glands in three sections per animal from a minimum of four animals per treatment group (a minimum of 12 counts per group). The counts for each animal were averaged, and these means were used to determine the mean ± SE for each group.

---

**Table 1. Comparison of the immature mouse and rat in the uterotrophic bioassay.**

| 17β-Estradiol (µg/kg/day) | Body weight (g) | Uterine wet weight (g) | Uterine wet weight:body weight ratio |
|--------------------------|-----------------|------------------------|------------------------------------|
|                          |                 | Actual | Fold induction over control | Relative | Fold induction over control |
| Mouse                    |                 |        |                          |          |                          |
| 0                       | 9.58 ± 0.374    | 0.011 ± 0.001 | 1.00                     | 0.111 ± 0.006 | 1.00                     |
| 0.1                      | 9.28 ± 0.428    | 0.012 ± 0.001 | 1.09                     | 0.131 ± 0.012 | 1.18                     |
| 1                        | 10.38 ± 0.504   | 0.025 ± 0.002* | 2.27                     | 0.242 ± 0.009 | 2.18                     |
| 10                       | 9.63 ± 0.809    | 0.044 ± 0.006* | 4.00                     | 0.450 ± 0.046 | 4.06                     |
| 100                      | 10.58 ± 0.301   | 0.062 ± 0.008* | 5.64                     | 0.578 ± 0.059 | 5.21                     |
| 500                      | 11.01 ± 0.221*  | 0.073 ± 0.007* | 6.84                     | 0.725 ± 0.008 | 6.54                     |
| Rat                      |                 |        |                          |          |                          |
| 0                        | 77.31 ± 1.87    | 0.047 ± 0.004 | 1.00                     | 0.061 ± 0.006 | 1.00                     |
| 0.1                      | ND              | ND      | ND                      | ND        | ND                      |
| 1                        | 70.50 ± 3.48    | 0.085 ± 0.003* | 1.81                     | 0.121 ± 0.010 | 1.98                     |
| 10                       | 55.47 ± 2.02    | 0.108 ± 0.008* | 2.30                     | 0.206 ± 0.014 | 3.41                     |
| 100                      | 55.57 ± 2.17    | 0.150 ± 0.007* | 3.19                     | 0.283 ± 0.010 | 4.64                     |
| 500                      | 67.27 ± 0.56*   | 0.217 ± 0.017* | 4.62                     | 0.344 ± 0.028 | 5.64                     |

ND, not determined. Numbers represent mean ± SE.

*Statistically significant at p < 0.05.

---

**Figure 1.** Uterine weight:body weight ratio of mice (n = 5) and rats (n = 4) that received varying subcutaneous doses of 17β-estradiol once daily for 3 days. See “Materials and Methods” for details. Values shown are the mean ± SE. The shaded areas represent control values (mean ± SE). The slope is similar for both mice and rats, but the sensitivity of mice is higher than that of rats at all doses tested.

**Figure 2.** Epithelial cell height in the uterotrophic bioassay of immature mice and rats. Values represent the mean ± SE of three measurements from three areas from three animals (total of 27 counts/group). For doses ≥ 1 µg/kg/day in both mice and rats, there was a significant increase over the corresponding control values. *Statistically significant differences between treated mice and rats and their respective controls (p < 0.05).
Uterine biochemical observations. Lactoferrin (LF) and complement C3 have been shown to be estrogen-responsive proteins in the mouse and rat uterus (18). After exposure to estrogen, both proteins were induced in uterine epithelial cells, and LF was shown to increase with increasing doses of estrogen (28). Thus, we examined these two proteins to determine the sensitivity of each species to increasing estrogenic doses and to determine the expression of these two estrogen-inducible proteins.

LF Immunocytochemistry. Mouse and rat uterine tissue sections were immunostained for the presence of LF using horseradish peroxidase immunocytochemistry as previously described (28). Briefly, sections were incubated with rabbit anti-mouse LF (28) for 2 hr at room temperature, or with normal rabbit IgG (Accurate Chemical Co., Westbury, NY) diluted 1:200 or without LF antibody for negative controls. We used the image analysis system (Southern Micro Instruments) to score LF intensity. We measured staining intensity in a minimum of three areas (three measurements per area) for each of three animals. The intensity scale ranged from 0 (no stain) to 50 (very intense stain).

Complement C3 immunocytochemistry. Mouse and rat uterine tissue sections were immunostained for complement C3. Tissue sections were incubated with goat anti-mouse complement C3 (primary antibody) diluted 1:5,000 (Cappel, Durham, NC) for 2 hr at room temperature. We used negative controls as described for LF immunolocalization. The immunostain was detected using an avidin:biotinylated enzyme complex (ABC) method. Briefly, tissue sections were rinsed with Tris-buffered saline containing 0.6% Tween-20 (TBS-T), incubated with biotinylated anti-goat antibody diluted 1:500 for 30 min at room temperature, rinsed with TBS-T, then incubated with ABC-AP (ABC-AP kit; Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Tissue sections were washed with TBS-T and rinsed briefly with TBS (pH 9.5) before the alkaline phosphatase (AP) substrate was applied. We prepared the color enhancement according to directions in the AP substrate kit IV (blue; Vector Laboratories) and incubated the tissue sections for approximately 10 min. Tissues were rinsed in distilled water, dehydrated, and coverslipped. We determined the intensity of C3 intensity as described for LF.

Statistical analysis. We performed statistical analysis by ANOVA (StatView SE + Graphics); values were determined to be significant at p < 0.05.

Results

Uterotrophic wet weight. The results of the uterotropic bioassays in mice and rats are shown in Table 1. Although there are differences in body weight in mice and rats after estradiol treatment, none of the animals appeared ill; thus, differences in body weight most likely did not have any confounding effect on this study. We observed a similar dose-response increase in uterine wet weight in both species with increasing doses of 17β-estradiol. We expressed data as the uterine weight to body weight ratio (Table 1, Figure 1). Doses ranging from 1 to 500 µg/kg/day were statistically significant for both mice and rats compared to their corresponding controls.

Uterine histologic observations. Epithelial cell height. Figure 2 shows the epithelial cell height results for the immature mouse and rat. The increase in cell height was higher in immature rats treated with ≥ 1 µg/kg/day 17β-estradiol than in their corresponding controls. Uterine epithelial cell height in the mouse followed the same pattern of increase as in the rat, but the rat demonstrated a greater increase over controls with this end point than the mouse at given doses. In Figure 3, photomicrographs show the effects of 17β-estradiol treatment (500 µg/kg/day) on epithelial cell height in mice (Figure 3B) and rats (Figure 3D). Epithelial cell number. Figure 4 shows an increase in cell number in response to ≥ 10 µg/kg/day 17β-estradiol in both mice and rats. There was a greater increase over controls in mice than in rats at all doses. The increase in cell number is also shown in Figure 3.

Uterine gland number. The number of glands is increased in mice in response to ≥ 1 µg/kg/day 17β-estradiol (Figure 5). However, there is no apparent increase in gland number in rats after any dose of estradiol used in this study. Thus, there is a striking difference between these two species in the ability of 17β-estradiol to elicit gland formation in rodents of this age.

Figure 3. Photomicrograph of uterine tissues from immature mice (A,B) and rats (C,D). (A) The unstimulated uterus from an immature mouse shows a single layer of cuboidal or low columnar cells lining the uterine lumen; there is minimal differentiation in the stroma. (B) The stimulated uterus from an immature mouse treated with 17β-estradiol (500 µg/kg/day) for 3 days shows tall columnar epithelial cells; the stromal cells have round nuclei and more space between nuclei. (C) The unstimulated uterus from an immature rat shows low cuboidal epithelium lining the uterine lumen, and stromal cells are undifferentiated and unresponsive as in the immature mouse. (D) The stimulated uterus from an immature rat treated with 17β-estradiol (500 µg/kg/day) for 3 days shows tall pseudostratified columnar cells and differentiated responsive stromal cells. Magnification, 200× for all photomicrographs.
Uterine LF and C3 immunohistochemistry. The results of LF quantitation are shown in Figure 6. We detected LF in all of the 17β-estradiol–treated groups of immature mice and rats. We previously reported a dose-dependent increase in LF immunohistochemistry over a tighter dose-response curve than is shown in Figure 6 (28). For all of the doses tested, the highest measurements were seen in mice. Figure 7 shows the pattern of LF immunostaining for both mice and rats. The cellular distribution of LF in the stimulated mouse uterus (Figure 7B) is different from that in the stimulated rat uterus (Figure 7D); LF is evenly distributed over the entire uterine epithelial cell in the mouse (Figure 7B), whereas LF in the rat is more apical (Figure 7D). Negative control slides showed no staining.

Tissue sections immunostained for complement C3, another estrogen-inducible protein, are shown in Figure 8. Although 17β-estradiol induced C3 in the same cell type, uterine epithelial cells, the cellular distribution was different in mice and rats: C3 was expressed in the apical portion of luminal epithelial cells of the 17β-estradiol–treated mouse (Figure 8B), whereas C3 was more evenly distributed throughout the uterine epithelial cells of the 17β-estradiol–treated rat. Image analysis results of the C3 immunostained tissue sections were similar to those of LF-immunostained tissue (data not shown). Thus, both the mouse and the rat show a strong staining reaction for both LF and C3 in uterine epithelial cells in response to 17β-estradiol, but the pattern of staining varied between the two species. The differential distribution of these two proteins in mice and rats has been discussed in more detail in a recent report from our laboratory (24).

Discussion

The data presented in this paper shows the sensitivity and degree of uterine response of the CD-1 mouse and the Sprague-Dawley rat to 17β-estradiol in the in vivo uterotrophic bioassay. Of particular interest is the difference in some end points between the strains, whereas other end points are similar. Some of these differences have been noted in the past and go back at least 40 years (29). However, with renewed interest in using the rodent uterotrophic bioassay as a screen for endocrine-disrupting substances, a more direct comparison is of interest to laboratories desiring to use an in vivo assay to determine the estrogenicity of compounds. Therefore, we conducted the present study to compare the estrogenic responses in mice and rats and to provide additional morphologic and biochemical end points that may be useful for increasing the information that can be obtained from the uterotrophic bioassay. For the purposes of this study, we compared the responses of rats and mice in the linear portion of the uterotrophic dose-response curve; higher doses show an S-shaped response for uterine wet weight (16), as well as other estrogen-responsive end points (30).

LF and C3 are strongly expressed in uterine luminal epithelial cells of both rats and mice after 17β-estradiol treatment. It is interesting to note the specific pattern of expression of these two proteins and the cellular distribution of each protein within the epithelial cells of rats and mice. In the mouse, LF is distributed throughout the cytoplasm of the uterine epithelial cells, and C3 localizes primarily at the apical tip. In the rat, LF localizes at the apical tip, and C3 is distributed throughout the cytoplasm. This pattern of protein distribution agrees with an earlier report (24). The differential cellular distribution of both proteins in rats and mice for each dose (total of 27 counts per group).

*Statistically significant differences between each mice and rats and their respective controls; there is a marked response in mice with this end point, whereas few uterine glands are present in the rat regardless of the 17β-estradiol dose (p < 0.05).

Figure 5. Number of uterine glands in the uterotropic bioassay of immature mice and rats. Values shown are the number of glands per section (mean ± SE) for three sections from three animals for each dose (total of 27 counts per group).

Figure 7. Immunohistochemical localization of LF in the uterotropic bioassay of immature mice and rats. (A) The unstimulated uterus from an immature mouse shows no apparent LF staining. (B) The stimulated uterus from an immature mouse treated 3 days with 500 µg/kg/day 17β-estradiol shows intense immunostaining in uterine epithelial cells; note the localization of LF throughout the cytoplasm. (C) The unstimulated uterus from an immature rat shows no apparent LF staining. (D) The stimulated uterus from an immature rat treated 3 days with 500 µg/kg/day 17β-estradiol shows specific LF staining in the luminal epithelial cells; there are fewer intensely stained cells than in the stimulated mouse, and the immunostaining is primarily at the apical surface of the epithelial cells. Negative controls, as described in "Materials and Methods," demonstrated no immunostaining. Magnification, 200× for all photomicrographs.
and mice may be because of different patterns of gene expression within each species.

Although most end points showed similarities between mice and rats, there was an increase in uterine gland formation after estrogen treatment in the mouse but not in the rat. Previous studies have shown that during postnatal development, uterine gland differentiation is sensitive to estrogen perturbation, resulting in inhibition of gland differentiation in both rats (31) and mice (32). However, after postnatal differentiation, the response of uterine glands to estrogen in the immature animal is quite different in rats and mice. Similar to humans, mouse uterine glandular epithelium responds to estrogen by becoming more convoluted and by increasing secretions.

Values for control and 17β-estradiol-treated rat tissues reported in this study are consistent with those from other laboratories (33). Furthermore, uterotrophic responses in immature Alpk:AP rats (8) is similar to responses observed in Sprague-Dawley rats in this study. In the Alpk:AP rats (8), the uterine wet weight (mean ± SE) was 38.2 ± 7.3 mg in controls and 95.4 ± 17.0 mg in rats treated with 10 µg/kg/day 17β-estradiol. In the Sprague-Dawley rats in the present study, the uterine wet weight (mean ± SE) was 47 ± 4 mg in controls and 108 ± 8 mg in rats treated with 10 µg/kg/day 17β-estradiol. This suggests similar estrogenic responses in two different strains of rats. Our data are also consistent with control values reported in other studies using immature rats (8,10,12). Further comparison after estrogen treatment is not possible because these studies only reported “blotted” uterine weight instead of actual uterine wet weight, which includes uterine luminal fluid. We specifically included uterine luminal contents because fluid imbibition is an important early estrogenic response.

Although we did not compare different routes of exposure between mice and rats in our study, a previous report showed a similar magnitude of uterine response in rats between oral gavage and subcutaneous injection (10). Although we used a small number of rats in this study, the data are consistent with other studies in the literature; this leads us to believe that our data are representative of responses seen in rat uterotrophic bioassays.

The present study was designed to compare species differences in uterine response between immature mice and rats. We did not compare immature and adult ovarioctomized animals because earlier studies demonstrated that immature animals are more sensitive to stimulation with estrogens than adult ovarioctomized animals (34). Kang et al. (35) has recently reconfirmed this. Thus, the age of the animals must be considered in designing the most sensitive screening methods for estrogenic chemicals.

As reported by other laboratories (13,36), there was a greater variation in body weights in all of the treatment groups in rats compared to mice. Also, rats showed a general decrease in body weight after 17β-estradiol treatment, and mice showed an increase with similar treatment. This greater variation in body weight in the rat can decrease the significance of the results and thus make the estrogenic effect of test chemicals less apparent, so that weak estrogens may not be detected. Therefore, decreased variability afforded by the mouse may result in decreased numbers of animals needed for testing. The potentially smaller numbers of animals and the certainty of lower animal husbandry costs and smaller amounts of compound needed for tests (due to the smaller body size of mice) support the use of mice in this screen until substitute in vitro models can be validated.

REFERENCES AND NOTES

1. McLachlan JA. Estrogens in the Environment. New York:Elsevier Science, 1985.
2. Colborn T, Clement C, eds. Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection. Princeton, NJ:Princeton Scientific Publishing, 1992.
3. McLachlan JA, Korach K. Introduction: Symposium on Estrogens in the Environment, III. Environ Health Perspect 103(suppl 7): 3-4 (1995).
4. Colborn T, Dunamski D, Myers J P. Our Stolen Future. New York:Penguin Books, 1996.
5. Leder N. Royal Society warns on hormone disrupters. Nature 404:4 (2000).
6. McLachlan A. Endocrine disrupter verdict left open. Nature 400:507 (1999).
7. U.S. EPA. Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) Final Report. Washington, DC:U.S. Environmental Protection Agency, 1998.
8. Ashby J, Odum J, Foster J. Activity of raloxifene in immature and ovarioctomized rat uterotrophic assays. Regul Toxicol Pharmacol 25:226-231 (1997).
9. Odum J, Lefere PA, Tittensor S, Paton D, Routledge EJ, Beresford NA, Sumpfer J, P. Ashby J. The rodent uterotrophic assay: critical protocol features, studies with nonphenois, and comparison with a yeast estrogenicity assay. Regul Toxicol Pharmacol 25:176-188 (1997).
10. Ashby J, Tinwell H. Oestrogenic activity of burger bread, to female rats. Hum Exp Toxicol 17:598-599 (1998).
11. O’Connor JC, Frame SR, Biegel LB, Cook J C, Davis LG. Sensitivity of a Tier I screening battery compared to an in utero exposure for detecting the estrogen receptor agonist 17beta-estradiol. Toxicol Sci 44:169-184 (1998).
12. O’Connor JC, Cook J C, Stone TW, Makovec GT, Frame SR, Davis LG. An ongoing validation of a Tier I screening battery for detecting endocrine-active compounds (EACs). Toxicol Sci 46:45-60 (1998).
13. Christian MS, Oberman AM, Bachmann S, Hellwig J. Variability in the uterotrophic response assay (an in vivo estrogenic response assay) in untreated control and positive control (DES-DP, 2.5 microg/kg, bid) Wistar and Sprague-Dawley rats. Drug Chem Toxicol 21:51-100 (1998).
14. Korach KS, Mebaker M, McLachlan JA. Estrogenic activity in vivo and in vitro of some diethylstilbestrol metabolites and analogs. Proc Natl Acad Sci USA 75:468-471 (1978).
15. Isenhower WD J, Newbold RR, Cefalo RC, Korach K, McLachlan JA. Absence of estrogenic activity in some drugs commonly used during pregnancy. Biol Reprod Pregnanv Perinatol 7:6-10 (1986).
16. Shelby WD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of in vivo and in vivo assays. Environ Health Perspect 104:1296-1300 (1996).
17. Jefferson WN, Padilla-Burgos E, Miller C, Newbold RR. Endocrine modulating effects of various phytoestrogens.
18. Jefferson WN, Newbold RR. Potential endocrine-modulating effects of various phytoestrogens in the diet. J Nutr 16:658–662 (2000).

19. Sundstrom SA, Komi B S, Ponce-de-Leon H, Yi Z, Teuscher C, Lyttle CR. Estrogen regulation of tissue-specific expression of complement C3. J Biol Chem 264:16941–16947 (1989).

20. Teng CT, Pentecost BT, Chen YH, Newbold RR, Eddy EM, McLachlan JA. Lactotransferrin gene expression in the mouse uterus and mammary gland. Endocrinology 124:992–999 (1989).

21. Teng C. Mouse lactoferrin gene: a marker for estrogen and epidermal growth factor. Environ Health Perspect 101:386–387 (1993).

22. Jefferson WN, Voelker CR, Newbold RR. Increased sensitivity of Western blots using various secondary antibody detection methods. Toxicol Methods 3:100–108 (1993).

23. Jefferson WN, Hanson RB, Newbold RR. Detection of lactoferrin in paraffin-embedded sections of mouse uterus: comparison of fixation and immunohistochemical techniques. Cell Vis 3:329–334 (1996).

24. Teng CT, Beckman WC Jr, Jefferson WN, Hanson RB, Newbold RR. Detection of lactoferrin in paraffin-embedded sections of mouse uterus: comparison of fixation and immunohistochemical techniques. Cell Vis 3:329–334 (1996).

25. Hisaw FL. Comparative effectiveness of estrogens on fluid imbibition and growth of the rat's uterus. Endocrinology 54:276–289 (1959).

26. Newbold RR, Teng CT, Beckman WC J r, J efferson WN, Hanson RB, Miller J V, MClachlan J A. Fluctuations of lactoferrin protein and messenger ribonucleic acid in the reproductive tract of the mouse during the estrous cycle. Biol Reprod 47:903–915 (1992).

27. Newbold RR, Hanson RB, J efferson WN. Ontogeny of lactoferrin in the developing mouse uterus: a marker of early hormone response. Biol Reprod 56:1147–1157 (1997).

28. Newbold RR, Padilla-Banks E, Newbold RR. Lactoferrin is an estrogen responsive protein in the uterus of mice and rats. Reprod Toxicol 14:103–110 (2000).

29. Bern H. The fragile fetus. In: Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection (Colborn T, Clement C, eds). Princeton, NJ:Princeton Scientific Publishing, 1992;9–16.