

Epigenetic Transgenerational Actions of Endocrine Disruptors

Matthew D. Anway and Michael K. Skinner

Center for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, Washington 99164

Endocrine disruptors have recently been shown to promote an epigenetic transgenerational phenotype involving a number of disease states (e.g. male infertility). The anti-androgenic fungicide vinclozolin was found to act transiently at the time of embryonic sex determination to promote in the F1 generation a spermatogenic cell defect and subfertility in the male. When the animals were allowed to age up to 1 yr, a number of other disease states developed. This phenotype was transferred through the male germ line to all subsequent gen-

erations analyzed (F1–F4). The ability of an environmental factor (i.e. endocrine disruptor) to promote an epigenetic transgenerational phenotype impacts the potential hazards of environmental toxins, mechanisms of disease etiology, and evolutionary biology. The biological importance of the epigenetic actions of environmental agents is reviewed in the context of the primordial germ cell and development of epigenetic transgenerational phenotypes. (Endocrinology 147: S43–S49, 2006)

GENOMIC DNA CONTAINS the core of genetic information of the cell. There is a distinct pattern of gene expression throughout mammalian development that is heritable from parents to offspring. Epigenetics is defined as the molecular phenomena that regulate gene expression without alterations to the DNA sequence (1). The most studied epigenetic modification is DNA methylation of CpG nucleotides that are essential for mammalian development (2–5). DNA methylation of CpG sites is used by mammals to regulate transcription of genes, alter chromosomal positioning, influence X-chromosome inactivation, control imprinted genes, and repress parasitic DNAs (1, 5–9). Alterations in the DNA methylation state can lead to multiple disease states including cancers (10, 11), Rett syndrome, and Prader-Willi/Angelman syndrome (11–13), male infertility (14), autism (12), and Angelman and Beckwith-Wiedemann syndromes (13). Both chemical and environmental toxins have been shown to alter DNA methylation patterns resulting in epigenetic phenotypes (14, 15).

DNA methylation patterns are established at two times during development: the lineage-specific pattern during gastrulation and the germ-line-specific pattern in the gonad after sex determination (16). The lineage-specific pattern establishes the DNA methylation for somatic cell development after fertilization. This epigenetic reprogramming is based on the genetic material transferred from the egg and sperm. Alterations in the lineage-specific epigenetic reprogramming results in developmental defects or embryonic lethality (13, 16). The germ-line DNA methylation pattern is established during gonadal development and is sex specific (16–18). Epigenetic reprogramming of the germ line is critical for imprinting (19–22). Unlike the lineage-specific reprogram-

ming, alterations in the germ-line epigenetic reprogramming can alter the heritable epigenetic information, resulting in a transgenerational phenotype (15) (Fig. 1). The embryonic period is the most sensitive for chemical and environmental effects on the epigenetics of the male germ line (15, 21, 22).

Recent investigations of the DNA methylation state of the primordial germ cells have indicated that as primordial germ cells migrate down the genital ridge, a demethylation (i.e. erasure of methylation) starts, and upon colonization in the early gonad, a complete demethylation is achieved (21–23). This has been primarily observed through the analysis of specific imprinted genes (24). During the period of sex determination in the gonad, the germ cells undergo a remethylation involving a sex-specific determination of the germ cells (Fig. 2). Although the demethylation may not require the gonad somatic cells (21), the remethylation of the germ line appears to be dependent on association with the somatic cells in the gonads (22, 23). Because of this unique property of the germ cells to undergo a demethylation and remethylation during the period of sex determination in the developing gonad, the ability of an environmental agent such as an endocrine disruptor to influence through an epigenetic process the germ line is postulated. This epigenetic effect on the germ line could reprogram the germ cell through an event such as altered DNA imprinting (25, 26). This epigenetic effect could cause a transgenerational effect on subsequent generations through the germ line. Because the remethylation of the germ line appears dependent upon the gonadal somatic cells, an alteration in somatic cell function by an agent such as an endocrine disruptor could indirectly influence the germ cell remethylation (Fig. 2). Epigenetic alterations that lead to transgenerational transmission of specific genetic traits or molecular events (e.g. imprinting) have recently been identified (6, 7, 27). These observations have led to the conclusion that a reprogramming through altered epigenetics of the male germ line is possible (15). The impact this has on human health and evolutionary biology is significant (6, 27).

First Published Online May 11, 2006

Abbreviation: AR, Androgen receptor.

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

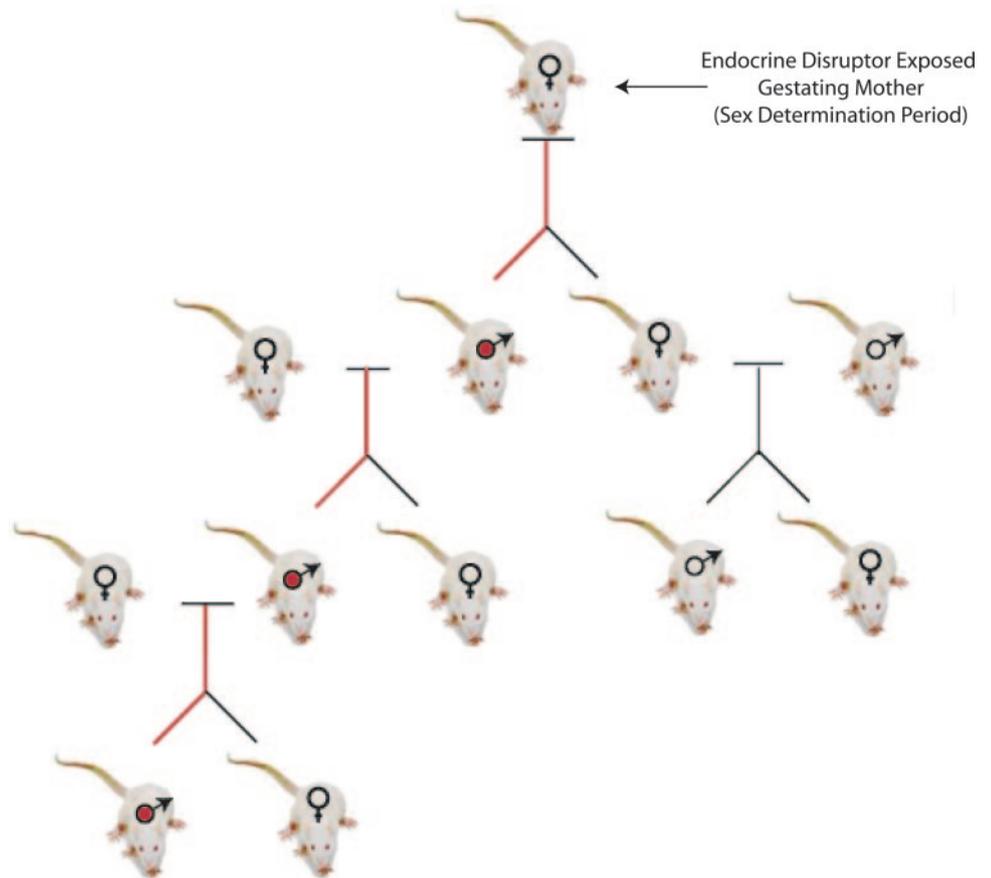


FIG. 1. Epigenetic transgenerational actions of endocrine disruptors through the male germ line.

Transgenerational Phenomena and Environmental Factors

Environmental effects of irradiation, chemical treatments (e.g. chemotherapy), and environmental toxins such as endocrine disruptors have been observed over the past decade. The majority of observations are simply the effects of the agent on the gestating mother (F0) and subsequent actions on the offspring associated with the F1 generation (28-30). Examples of environmental factors during embryonic development that influence the F1 generation include the effects of heavy metals causing cancer (31), abnormal nutrition that causes diabetic and uterine defects (32-34), chemical expo-

sure (i.e. ethoxysulfide and benzpyrene) causing brain and endocrine defects (35, 36), and endocrine disruptors such as diethylstilbestrol (37, 38), phthalates, and dithiothreitol causing reproductive tract and endocrine defects (39-41). Environmental factors have effects on the F1 generation of a number of species including insects (42-44), fish (45, 46), birds (47), and other species (48). Therefore, exposure to a number of environmental factors *in utero* can cause abnormal phenotypes in the F1 generation in a number of different species. Because the F1 generation is exposed to the environmental factor, the F1 effect is not a transgenerational phenotype.

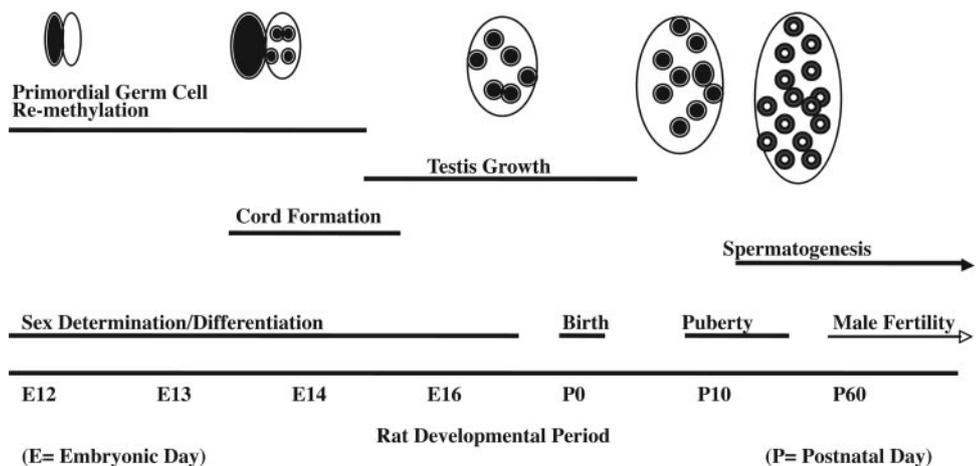


FIG. 2. Rat testis developmental timing and processes.

Transgenerational effects of environmental factors require effects minimally on the F3 generation (15, 49) (Fig. 1). This is because the F3 generation is the first generation not directly exposed to the environmental factor. The ability of an external agent to induce a transgenerational phenotype requires a genetic (*i.e.* DNA sequence) or an epigenetic (*i.e.* DNA methylation) phenomenon mediated through the germ line (50–53). Transgenerational inheritance of an epigenetic state has been shown to occur using several mouse genetic lines and markers (6, 27, 54) and more recently with the use of monozygotic twins with epigenetic differences (55). Irradiation exposure was one of the first transgenerational phenomena observed to be transmitted through the germ line to multiple generations, often associated with mutagenesis and tumor formation (50–53). The chemotherapeutic treatment of cancers has been shown to cause F1 generation effects (31, 35, 46), but the transmission to multiple generations has not been thoroughly investigated. Environmental factors do appear to promote a transgenerational susceptibility to cancer (56, 57). Gestating nutritional deficiency effects on the F1 generation have been observed (34), and recently these nutritional effects on a diabetic condition and growth defects have been shown to be transgenerational to the F2 generation (58–60). Several environmental chemical exposures have also been shown to transgenerationally affect the F2 generation including benzpyrene (36, 61), orthoaminoasotoluol (62), and dioxin (63). Environmental toxins such as endocrine disruptors have also been shown to influence the F1 generation after parental exposure (39, 46, 64–67), but few have demonstrated transgenerational effects on multiple generations (15). Some evidence that diethylstilbestrol has effects in the F2 generation have been reported (68).

Endocrine Disruptors and Reproductive Toxicology

Many reports have suggested that environmental endocrine disruptors, which act to mimic estrogens or act as antiestrogens or antiandrogens, are detrimental to reproduction and may promote abnormalities such as a decrease in sperm count, an increase in testicular cancer (69, 70), and an increase in abnormalities in sex determination for many species (71). Examples of environmental endocrine disruptors that have been targeted for adverse effects on reproductive systems in humans and other animals are pesticides [*e.g.* dichlorodiphenyltrichloroethane (DDT) and methoxychlor] (72), fungicides (*e.g.* vinclozolin) (15, 73), insecticides (*e.g.* trichlorfon) (74), herbicides (*e.g.* atrazine) (75), plastics (*e.g.* phthalates) (76), and a range of xenoestrogens (77). Most of these chemicals are ubiquitous in the environment, resulting in daily exposure for humans and other animals. Many of these compounds and endocrine disruptors can be metabolized into both estrogenic and antiandrogenic activities (78). Recently, methoxychlor and vinclozolin have been used (66, 67) as model endocrine disruptors (72) that have estrogenic, antiestrogenic, and antiandrogenic metabolites (78).

Many environmental endocrine disruptors are weakly estrogenic and elicit their actions through the estrogen receptors. The two mammalian receptors for estrogen (ER- α and ER- β) are widely distributed throughout the reproductive tract (79, 80). ER- β is present in higher concentrations within

the fetal testis and ovary, whereas ER- α is present mainly within the uterus (81, 82). During fetal testis development, ER- β is expressed in Sertoli and myoid cells after seminiferous cord formation (83). In rats, ER- β has also been localized to prespermatogonia, which may explain the proliferative actions of estrogen on early postnatal gonocyte cultures (84). The importance of ER- α was delineated when knockout mice (85) and human males (86) lacking expression of this gene were found to be sterile. Fetal development of the testis in these experiments was not altered; however, fetal testis morphology in a double knockout remains to be examined (87). Neonatal exposure to estrogen alters the ER- α and ER- β expression during postnatal testis and hypothalamic/pituitary development (88, 89). Interestingly, neonatal exposure to the estrogenic compound diethylstilbestrol promotes abnormal testis and male reproductive tract development (90) and leads to changes in gene expression (91). Therefore, actions of estrogenic endocrine disruptors on estrogen receptors may impair normal fetal gonadal development and lead to infertility. Although the estrogen receptors are thought to have a role in testis development (92–94), the specific functions remain to be elucidated. Treatment of males with estrogens during early fetal life may alter responsiveness to androgens by changing androgen receptor (AR) expression patterns (95, 96) and/or Leydig cell function (91).

Antiandrogenic endocrine disruptors can also influence fetal gonad development. AR expression is very similar to ER- β expression in the developing testis (82, 97). AR is detected in Sertoli, myoid, and prespermatogonial cells just after cord formation (98) and in interstitial cells late in fetal development. It is proposed that AR is present in cells that migrate from the mesonephros and enables cord formation to occur (98). Therefore, inappropriate expression or actions of AR through treatment by endocrine disruptors may affect the process of morphological sex differentiation (*i.e.* cord formation). Antiandrogens such as flutamide (99) or cyproterone acetate (100) administered to pregnant rats at different ages of gestation impair fertility in the male offspring. Both flutamide and cyproterone acetate block the ability of androgens and epidermal growth factor to stabilize the Wolffian duct (101). Therefore, perturbation of AR may also cause inappropriate expression and action of growth factors in the testis. A commonly used antiandrogenic endocrine disruptor is vinclozolin, which is used as a fungicide in the wine industry (102, 103). Vinclozolin has been shown to act as an environmental antiandrogen and influence gonad development and fertility (15, 67).

Epigenetic Transgenerational Actions of Endocrine Disruptors

A recent observation demonstrated that the exposure of a pregnant rat transiently to endocrine disruptors caused a spermatogenic cell defect and subfertility in the F1 generation and all subsequent generations examined (F1–F4) (15) (Fig. 1). The endocrine disruptors used were the antiandrogenic fungicide vinclozolin used in the fruit (*e.g.* wine) industry (73) and the pesticide methoxychlor used to replace dichlorodiphenyltrichloroethane (DDT) (78). The critical exposure period was at the time of sex determination, and the

transgenerational phenotype was transmitted through the male germ line (15) (Fig. 1). The phenotype of increased spermatogenic cell apoptosis and decreased sperm numbers and sperm motility was observed in greater than 90% of all males of all the generations examined. When the animals were allowed to age up to 1 yr, additional diseases developed including cancer, prostate disease, kidney disease, and immune cell defects (Anway, M. D., and M. K. Skinner, submitted for publication). A high frequency of transmission was observed in all generations examined for all the disease states.

The frequency of the transgenerational phenotype was such that a DNA sequence mutational event could not be involved. The random nature of a DNA sequence mutation has a phenotype typically less than 1%, and this often declines in subsequent generations (50, 104). An epigenetic mechanism is involved because of the frequency of the phenotype. To support these conclusions, two genes were identified in the sperm that had altered methylation patterns associated with the transgenerational phenotype discussed (15). Therefore, the endocrine disruptors appear to induce an epigenetic transgenerational disease condition for four generations through the male germ line (15) (Fig. 1). The epigenetics appears to involve altered DNA methylation. Although most genes get reset in early embryonic development, a subset of genes called imprinted genes maintains their DNA methylation pattern that appears to be permanently programmed. In contrast to all somatic cells, the primordial germ cells undergo a demethylation during migration and early colonization of the embryonic gonad, followed by a remethylation starting at the time of sex determination in a sex-specific manner (23, 24, 105). The exposure of the pregnant mother at the time of sex determination appears to have altered the remethylation of the germ line and permanently reprogrammed the imprinted pattern of DNA methylation (15). This provides a unique epigenetic mechanism to promote a transgenerational phenotype induced by an environmental factor.

Summary

The observations that an environmental toxin (*e.g.* endocrine disruptor) can have an epigenetic effect on the germ line and cause a transgenerational effect on male reproduction significantly impacts our understanding of the potential hazards of these compounds to human health as well as all other mammalian species (15). These studies establish a novel mechanism of action not previously appreciated on how environmental toxins may act on a gestating mother to influence her grandchildren and subsequent generations. Elucidation of this phenomenon will allow us to better understand the true hazards of environmental toxins, identify the specific causal agents, and develop appropriate preventative and therapeutic approaches. Independent of the specific compound or agent of interest, the establishment of this potential mechanism of action is critical to our insight into the effects of environmental factors that influence embryonic development and adult reproduction.

The level of endocrine disruptors used in the recent studies (15, 66, 67, 106) (Anway, M. D., and M. K. Skinner, submitted

for publication) is higher than anticipated in the environment, such that conclusions regarding the toxicology of these endocrine disruptors are not possible. However, the important factor is the identification of this novel phenomenon, that an environmental factor can promote an epigenetic transgenerational phenotype (15). Because of this observation, the potential hazards of environmental factors need to be carefully evaluated. If the exposure of your grandmother at midgestation to environmental toxins can cause a disease state in you with no exposure, and you will pass it on to your grandchildren, the potential hazards of environmental toxins need to be rigorously assessed. Transgenerational studies need to be performed in evaluating the toxicology of environmental compounds.

The epigenetic transgenerational phenotype also provides critical insights into disease etiology. Because a number of common disease states are induced (Anway, M. D., and M. K. Skinner, submitted for publication), an epigenetic component of disease now needs to be seriously considered. The fetal basis of adult-onset disease could be a result of epigenetic factors (107, 108). In the event a major epigenetic component exists, the epigenetic background of an individual may be a significant factor in susceptibility to disease development. Therefore, identification of the genes involved with altered methylation may provide essential new diagnostics to assess onset of disease. These epigenetic factors may influence the outcomes of current medical therapies such as assisted reproductive procedures (109, 110). Further analysis of the epigenetic transgenerational phenotypes and identification of specific epigenetic changes will allow new therapeutic targets and therapies to be developed to potentially prevent the onset of disease. This is a new paradigm in disease etiology that needs to be considered.

In a broader biological perspective, the ability of an environmental factor to cause a permanent genetic trait in all subsequent progeny of an effected individual can significantly impact our understanding of evolutionary biology. Currently, a DNA sequence mutation event that allows an adaptation and natural selection is considered the driving factor in evolutionary biology. However, the frequency of specific evolutionary events (110, 111) and regional influences on evolution suggest an additional epigenetic mechanism should be considered (112–115). Although a DNA sequence mutational event will be important for evolutionary biology, an epigenetic component influenced by an environmental factor needs to be considered as an alternate factor that will help explain some aspects of evolutionary biology. Epigenetics is the next layer of complexity beyond the DNA sequence.

Acknowledgments

We acknowledge the assistance of Ms. Jill Griffin in the preparation of this manuscript.

Received August 19, 2005. Accepted October 14, 2005.

Address all correspondence and requests for reprints to: Michael K. Skinner, Center for Reproductive Biology, School of Molecular Biosciences, Washington State University, Pullman, Washington 99164-4231. E-mail: Skinner@mail.wsu.edu.

This research was supported in part by grants from the U.S. Environmental Protection Agency, EPA STAR program, and from the National Institute of Environmental Health Sciences (to M.K.S.).

None of the authors have any conflicts of interest regarding the current publication.

References

- Jones PA, Takai D 2001 The role of DNA methylation in mammalian epigenetics. *Science* 293:1068–1070
- Li E, Bestor TH, Jaenisch R 1992 Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69:915–926
- Okano M, Bell DW, Haber DA, Li E 1999 DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 99:247–257
- Holliday R 1989 DNA methylation and epigenetic mechanisms. *Cell Biophys* 15:15–20
- Morgan HD, Santos F, Green K, Dean W, Reik W 2005 Epigenetic reprogramming in mammals. *Hum Mol Genet* 14(Spec No 1):R47–R58
- Roemer I, Reik W, Dean W, Klöse J 1997 Epigenetic inheritance in the mouse. *Curr Biol* 7:277–280
- Lane N, Dean W, Erhardt S, Hajkova P, Surani A, Walter J, Reik W 2003 Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35:88–93
- Waterland RA, Jirtle RL 2003 Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23:5293–5300
- Ruden DM, Xiao L, Garfinkel MD, Lu X 2005 Hsp90 and environmental impacts on epigenetic states: a model for the trans-generational effects of diethylstilbestrol on uterine development and cancer. *Hum Mol Genet* 14(Spec No 1):R149–R155
- Lund AH, van Lohuizen M 2004 Epigenetics and cancer. *Genes Dev* 18:2315–2335
- Esteller M, Herman JG 2002 Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol* 196:1–7
- Muhle R, Trentacoste SV, Rapin I 2004 The genetics of autism. *Pediatrics* 113:e472–e486
- Jiang YH, Bressler J, Beaudet AL 2004 Epigenetics and human disease. *Annu Rev Genomics Hum Genet* 5:479–510
- Cisneros FJ 2004 DNA methylation and male infertility. *Front Biosci* 9:1189–1200
- Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308:1466–1469
- Reik W, Dean W, Walter J 2001 Epigenetic reprogramming in mammalian development. *Science* 293:1089–1093
- Allegrucci C, Thurston A, Lucas E, Young L 2005 Epigenetics and the germline. *Reproduction* 129:137–149
- Kelly TL, Trasler JM 2004 Reproductive epigenetics. *Clin Genet* 65:247–260
- Lucifero D, Mann MR, Bartolomei MS, Trasler JM 2004 Gene-specific timing and epigenetic memory in oocyte imprinting. *Hum Mol Genet* 13:839–849
- Yamazaki Y, Mann MR, Lee SS, Marh J, McCarrey JR, Yanagimachi R, Bartolomei MS 2003 Reprogramming of primordial germ cells begins before migration into the genital ridge, making these cells inadequate donors for reproductive cloning. *Proc Natl Acad Sci USA* 100:12207–12212
- Sato S, Yoshimizu T, Sato E, Matsui Y 2003 Erasure of methylation imprinting of Igf2r during mouse primordial germ-cell development. *Mol Reprod Dev* 65:41–50
- Lee J, Inoue K, Ono R, Ogonuki N, Kohda T, Kaneko-Ishino T, Ogura A, Ishino F 2002 Erasing genomic imprinting memory in mouse clone embryos produced from day 11.5 primordial germ cells. *Development* 129:1807–1817
- Hajkova P, Erhardt S, Lane N, Haaf T, El-Maarri O, Reik W, Walter J, Surani MA 2002 Epigenetic reprogramming in mouse primordial germ cells. *Mech Dev* 117:15–23
- Durcova-Hills G, Ainscough J, McLaren A 2001 Pluripotential stem cells derived from migrating primordial germ cells. *Differentiation* 68:220–226
- Li E 2002 Chromatin modification and epigenetic reprogramming in mammalian development. *Nat Rev Genet* 3:662–673
- Sandovici I, Leppert M, Hawk PR, Suarez A, Linares Y, Sapienza C 2003 Familial aggregation of abnormal methylation of parental alleles at the IGF2/H19 and IGF2R differentially methylated regions. *Hum Mol Genet* 12:1569–1578
- Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, Luu KV, Whitelaw E 2003 Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci USA* 100:2538–2543
- Tsui MT, Wang WX 2004 Maternal transfer efficiency and transgenerational toxicity of methylmercury in *Daphnia magna*. *Environ Toxicol Chem* 23:1504–1511
- Shimada A, Shima A 2004 Transgenerational genomic instability as revealed by a somatic mutation assay using the medaka fish. *Mutat Res* 552:119–124
- Nomura T, Nakajima H, Ryo H, Li LY, Fukudome Y, Adachi S, Gotoh H, Tanaka H 2004 Transgenerational transmission of radiation- and chemically induced tumors and congenital anomalies in mice: studies of their possible relationship to induced chromosomal and molecular changes. *Cytogenet Genome Res* 104:252–260
- Cheng RY, Hockman T, Crawford E, Anderson LM, Shiao YH 2004 Epigenetic and gene expression changes related to transgenerational carcinogenesis. *Mol Carcinog* 40:1–11
- Kaati G, Bygren LO, Edvinsson S 2002 Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet* 10:682–688
- Hemmings DG, Veerareddy S, Baker PN, Davidge ST 2005 Increased myogenic responses in uterine but not mesenteric arteries from pregnant offspring of diet-restricted rat dams. *Biol Reprod* 72:997–1003
- Martin RM, Smith GD, Frankel S, Gunnell D 2004 Parents' growth in childhood and the birth weight of their offspring. *Epidemiology* 15:308–316
- Fujii T 1997 Transgenerational effects of maternal exposure to chemicals on the functional development of the brain in the offspring. *Cancer Causes Control* 8:524–528
- Csaba G, Incze-Gonda A 1998 Transgenerational effect of a single neonatal benzopyrene treatment on the glucocorticoid receptor of the rat thymus. *Hum Exp Toxicol* 17:88–92
- Klip H, Verloop J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE 2002 Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet* 359:1102–1107
- Giusti RM, Iwamoto K, Hatch EE 1995 Diethylstilbestrol revisited: a review of the long-term health effects. *Ann Intern Med* 122:778–788
- Parks LG, Ostby JS, Lambright CR, Abbott BD, Klinefelter GR, Barlow NJ, Gray Jr LE 2000 The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *Toxicol Sci* 58:339–349
- Brucker-Davis F 1998 Effects of environmental synthetic chemicals on thyroid function. *Thyroid* 8:827–856
- Steinhardt GF 2004 Endocrine disruption and hypospadias. *Adv Exp Med Biol* 545:203–215
- Omholt SW, Amdam GV 2004 Epigenetic regulation of aging in honeybee workers. *Sci Aging Knowledge Environ* 2004:pe28
- Garfinkel MD, Sollars VE, Lu X, Ruden DM 2004 Multigenerational selection and detection of altered histone acetylation and methylation patterns: toward a quantitative epigenetics in *Drosophila*. *Methods Mol Biol* 287:151–168
- Seidl MD, Paul RJ, Pirow R 2005 Effects of hypoxia acclimation on morphophysiological traits over three generations of *Daphnia magna*. *J Exp Biol* 208:2165–2175
- Matta MB, Linse J, Cairncross C, Francendese L, Kocan RM 2001 Reproductive and transgenerational effects of methylmercury or Aroclor 1268 on *Fundulus heteroclitus*. *Environ Toxicol Chem* 20:327–335
- Foran CM, Peterson BN, Benson WH 2002 Transgenerational and developmental exposure of Japanese medaka (*Oryzias latipes*) to ethinylestradiol results in endocrine and reproductive differences in the response to ethinylestradiol as adults. *Toxicol Sci* 68:389–402
- Ottinger MA, Wu JM, Hazelton JL, Abdelnabi MA, Thompson N, Quinn ML, Jr, Donoghue D, Schenck F, Ruscio M, Beavers J, Jaber M 2005 Assessing the consequences of the pesticide methoxychlor: neuroendocrine and behavioral measures as indicators of biological impact of an estrogenic environmental chemical. *Brain Res Bull* 65:199–209
- Extavour CG, Akam M 2003 Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. *Development* 130:5869–5884
- Campbell JH, Perkins P 1988 Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. *Prog Brain Res* 73:535–553
- Barber R, Plumb MA, Boulton E, Roux I, Dubrova YE 2002 Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proc Natl Acad Sci USA* 99:6877–6882
- Hoyes KP, Lord BJ, McCann C, Hendry JH, Morris ID 2001 Transgenerational effects of preconception paternal contamination with ⁵⁵Fe. *Radiat Res* 156:488–494
- Mohr U, Dasenbrock C, Tillmann T, Kohler M, Kamino K, Hagemann G, Morawietz G, Campo E, Cazorla M, Fernandez P, Hernandez L, Cardesa A, Tomatis L 1999 Possible carcinogenic effects of x-rays in a transgenerational study with CBA mice. *Carcinogenesis* 20:325–332
- Dubrova YE 2003 Radiation-induced transgenerational instability. *Oncogene* 22:7087–7093
- Morgan HD, Sutherland HG, Martin DI, Whitelaw E 1999 Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 23:314–318
- Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M 2005 Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA* 102:10604–10609
- Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL 2005 Interaction between genetic susceptibility and early-life environmental exposure deter-

- mines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci USA* 102:8644–8649
57. **Yamasaki H, Loktionov A, Tomatis L** 1992 Perinatal and multigenerational effect of carcinogens: possible contribution to determination of cancer susceptibility. *Environ Health Perspect* 98:39–43
 58. **Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, Rodriguez-Gonzalez GL, Guzman C, Larrea F, Nathanielsz PW** 2005 Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. *J Physiol* 566:225–236
 59. **Portha B** 2005 Programmed disorders of β -cell development and function as one cause for type 2 diabetes? The GK rat paradigm. *Diabetes Metab Res Rev* 6:495–504
 60. **Cesani MF, Orden B, Zucchi M, Mune MC, Oyhenart EE, Pucciarelli HM** 2003 Effect of undernutrition on the cranial growth of the rat. An intergenerational study. *Cells Tissues Organs* 174:129–135
 61. **Turusov VS, Nikonova TV, Parfenov Yu D** 1990 Increased multiplicity of lung adenomas in five generations of mice treated with benz(a)pyrene when pregnant. *Cancer Lett* 55:227–231
 62. **Popova NV** 1989 Transgenerational effect of orthoaminoasotoluol in mice. *Cancer Lett* 46:203–206
 63. **Ikeda M, Tamura M, Yamashita J, Suzuki C, Tomita T** 2005 Repeated in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure affects male gonads in offspring, leading to sex ratio changes in F₂ progeny. *Toxicol Appl Pharmacol* 206:351–355
 64. **Kang IJ, Yokota H, Oshima Y, Tsuruda Y, Oe T, Imada N, Tadokoro H, Honjo T** 2002 Effects of bisphenol A on the reproduction of Japanese medaka (*Oryzias latipes*). *Environ Toxicol Chem* 21:2394–2400
 65. **Schwaiger J, Mallow U, Ferling H, Knoerr S, Braunbeck T, Kalbfus W, Negele RD** 2002 How estrogenic is nonylphenol? A transgenerational study using rainbow trout (*Oncorhynchus mykiss*) as a test organism. *Aquat Toxicol* 59:177–189
 66. **Cupp AS, Uzumcu M, Suzuki H, Dirks K, Phillips B, Skinner MK** 2003 Effect of transient embryonic in vivo exposure to the endocrine disruptor methoxychlor on embryonic and postnatal testis development. *J Androl* 24:736–745
 67. **Uzumcu M, Suzuki H, Skinner MK** 2004 Effect of the anti-androgenic endocrine disruptor vinclozolin on embryonic testis cord formation and postnatal development and function. *Reprod Toxicol* 18:765–774
 68. **Blatt J, Van Le L, Weiner T, Sailer S** 2003 Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J Pediatr Hematol Oncol* 25:635–636
 69. **Carlsen E, Giwercman A, Keiding N, Skakkebaek NE** 1992 Evidence for decreasing quality of semen during past 50 years. *BMJ* 305:609–613
 70. **Sharpe RM, Fisher JS, Millar MM, Jobling S, Sumpter JP** 1995 Gestational and lactational exposure of rats to xenoestrogens results in reduced testicular size and sperm production. *Environ Health Perspect* 103:1136–1143
 71. **Facemire CF, Gross TS, Guillette Jr LJ** 1995 Reproductive impairment in the Florida panther: nature or nurture? *Environ Health Perspect* 103(Suppl 4):79–86
 72. **Cummings AM** 1997 Methoxychlor as a model for environmental estrogens. *Crit Rev Toxicol* 27:367–379
 73. **Kelce WR, Monosson E, Gamsick MP, Laws SC, Gray Jr LE** 1994 Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. *Toxicol Appl Pharmacol* 126:276–285
 74. **Voccia I, Blakley B, Brousseau P, Fournier M** 1999 Immunotoxicity of pesticides: a review. *Toxicol Ind Health* 15:119–132
 75. **Cooper RL, Goldman JM, Stoker TE** 1999 Neuroendocrine and reproductive effects of contemporary-use pesticides. *Toxicol Ind Health* 15:26–36
 76. **Fisher JS** 2004 Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* 127:305–315
 77. **Sultan C, Balaguer P, Terouanne B, Georget V, Paris F, Jeandel C, Lumbroso S, Nicolas J** 2001 Environmental xenoestrogens, antiandrogens and disorders of male sexual differentiation. *Mol Cell Endocrinol* 178:99–105
 78. **Kelce WR, Lambright CR, Gray Jr LE, Roberts KP** 1997 Vinclozolin and *p,p'*-DDE alter androgen-dependent gene expression: in vivo confirmation of an androgen receptor-mediated mechanism. *Toxicol Appl Pharmacol* 142:192–200
 79. **Pettersson K, Gustafsson JA** 2001 Role of estrogen receptor β in estrogen action. *Annu Rev Physiol* 63:165–192
 80. **Lee KH, Hess RA, Bahr JM, Lubahn DB, Taylor J, Bunick D** 2000 Estrogen receptor α has a functional role in the mouse rete testis and efferent ductules. *Biol Reprod* 63:1873–1880
 81. **Brandenberger AW, Tee MK, Lee JY, Chao V, Jaffe RB** 1997 Tissue distribution of estrogen receptors α (ER- α) and β (ER- β) mRNA in the midgestational human fetus. *J Clin Endocrinol Metab* 82:3509–3512
 82. **Saunders PT, Maguire SM, Gaughan J, Millar MR** 1997 Expression of oestrogen receptor β (ER β) in multiple rat tissues visualised by immunohistochemistry. *J Endocrinol* 154:R13–R16
 83. **Goyal HO, Bartol FF, Wiley AA, Khalil MK, Chiu J, Vig MM** 1997 Immunolocalization of androgen receptor and estrogen receptor in the developing testis and excurrent ducts of goats. *Anat Rec* 249:54–62
 84. **Li H, Papadopoulos V, Vidic B, Dym M, Culty M** 1997 Regulation of rat testis gonocyte proliferation by platelet-derived growth factor and estradiol: identification of signaling mechanisms involved. *Endocrinology* 138:1289–1298
 85. **Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB, Korach KS** 1996 Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. *Endocrinology* 137:4796–4805
 86. **Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS** 1994 Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N Engl J Med* 331:1056–1061
 87. **Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ, Korach KS** 1999 Postnatal sex reversal of the ovaries in mice lacking estrogen receptors α and β . *Science* 286:2328–2331
 88. **Tena-Sempere M, Navarro J, Pinilla L, Gonzalez LC, Huhtaniemi I, Aguilar E** 2000 Neonatal exposure to estrogen differentially alters estrogen receptor α and β mRNA expression in rat testis during postnatal development. *J Endocrinol* 165:345–357
 89. **Tena-Sempere M, Gonzalez LC, Pinilla L, Huhtaniemi I, Aguilar E** 2001 Neonatal imprinting and regulation of estrogen receptor α and β mRNA expression by estrogen in the pituitary and hypothalamus of the male rat. *Neuroendocrinology* 73:12–25
 90. **Odum J, Lefevre PA, Tinwell H, Van Miller JP, Joiner RL, Chapin RE, Wallis NT, Ashby J** 2002 Comparison of the developmental and reproductive toxicity of diethylstilbestrol administered to rats in utero, lactationally, preweaning, or postweaning. *Toxicol Sci* 68:147–163
 91. **Guyot R, Odet F, Leduque P, Forest MG, Le Magueresse-Battistoni B** 2004 Diethylstilbestrol inhibits the expression of the steroidogenic acute regulatory protein in mouse fetal testis. *Mol Cell Endocrinol* 220:67–75
 92. **Atanassova N, McKinnell C, Walker M, Turner KJ, Fisher JS, Morley M, Millar MR, Groome NP, Sharpe RM** 1999 Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood. *Endocrinology* 140:5364–5373
 93. **Nielsen M, Bjornsdottir S, Hoyer PE, Byskov AG** 2000 Ontogeny of estrogen receptor α in gonads and sex ducts of fetal and newborn mice. *J Reprod Fertil* 118:195–204
 94. **Ebling FJ, Brooks AN, Cronin AS, Ford H, Kerr JB** 2000 Estrogenic induction of spermatogenesis in the hypogonadal mouse. *Endocrinology* 141:2861–2869
 95. **Prins GS, Birch L** 1995 The developmental pattern of androgen receptor expression in rat prostate lobes is altered after neonatal exposure to estrogen. *Endocrinology* 136:1303–1314
 96. **McKinnell C, Atanassova N, Williams K, Fisher JS, Walker M, Turner KJ, Saunders TK, Sharpe RM** 2001 Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. *J Androl* 22:323–338
 97. **Wilson CM, McPhaul MJ** 1996 A and B forms of the androgen receptor are expressed in a variety of human tissues. *Mol Cell Endocrinol* 120:51–57
 98. **Majdic G, Millar MR, Saunders PT** 1995 Immunolocalisation of androgen receptor to interstitial cells in fetal rat testes and to mesenchymal and epithelial cells of associated ducts. *J Endocrinol* 147:285–293
 99. **Silversides DW, Price CA, Cooke GM** 1995 Effects of short-term exposure to hydroxyflutamide in utero on the development of the reproductive tract in male mice. *Can J Physiol Pharmacol* 73:1582–1588
 100. **Nambu A, Kumamoto Y** 1995 [Studies of spermatogenic damages induced by anti-cancer agent and anti-androgenic agents in rat testes]. *Nippon Hinyokika Gakkai Zasshi* 86:1221–1230 (Japanese)
 101. **Gupta C, Chandorkar A, Nguyen AP** 1996 Activation of androgen receptor in epidermal growth factor modulation of fetal mouse sexual differentiation. *Mol Cell Endocrinol* 123:89–95
 102. **Turner KJ, Barlow NJ, Struve MF, Wallace DG, Gaido KW, Dorman DC, Foster PM** 2002 Effects of in utero exposure to the organophosphate insecticide fenitrothion on androgen-dependent reproductive development in the Crl:CD(SD)BR rat. *Toxicol Sci* 68:174–183
 103. **Hotchkiss AK, Ostby JS, Vandenberg JG, Gray Jr LE** 2002 Androgens and environmental antiandrogens affect reproductive development and play behavior in the Sprague-Dawley rat. *Environ Health Perspect* 110(Suppl 3):435–439
 104. **Shi BS, Cai ZN, Yang J, Yu YN** 2004 N-methyl-N'-nitro-N-nitrosoguanidine sensitivity, mutator phenotype and sequence specificity of spontaneous mutagenesis in FEN-1-deficient cells. *Mutat Res* 556:1–9
 105. **Reik W, Walter J** 2001 Genomic imprinting: parental influence on the genome. *Nat Rev Genet* 2:21–32
 106. **Skinner MK, Anway MD** 2006 Seminiferous cord formation and germ cell programming: epigenetic transgenerational actions of endocrine disruptors. *Ann NY Acad Sci* 1061:18–32
 107. **Heindel JJ** 2005 The fetal basis of adult disease: role of environmental exposures—introduction. *Birth Defects Res A Clin Mol Teratol* 73:131–132

108. **Basha MR, Wei W, Bakheet SA, Benitez N, Siddiqi HK, Ge YW, Lahiri DK, Zawia NH** 2005 The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and β -amyloid in the aging brain. *J Neurosci* 25:823–829
109. **Horsthemke B, Ludwig M** 2005 Assisted reproduction: the epigenetic perspective. *Hum Reprod Update* 11:473–482
110. **Penny D** 2005 Evolutionary biology: relativity for molecular clocks. *Nature* 436:183–184
111. **Balter M** 2005 Evolutionary genetics. Are humans still evolving? *Science* 309:234–237
112. **Johnston TD, Gottlieb G** 1990 Neophenogenesis: a developmental theory of phenotypic evolution. *J Theor Biol* 147:471–495
113. **Guerrero-Bosagna C, Sabat P, Valladares L** 2005 Environmental signaling and evolutionary change: can exposure of pregnant mammals to environmental estrogens lead to epigenetically induced evolutionary changes in embryos? *Evol Dev* 7:341–350
114. **Schmitt S** 2005 From eggs to fossils: epigenesis and transformation of species in Pander's biology. *Int J Dev Biol* 49:1–8
115. **Chong S, Whitelaw E** 2004 Epigenetic germline inheritance. *Curr Opin Genet Dev* 14:692–696

Endocrinology is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.