

THE FEMALE REPRODUCTIVE SYSTEM: TARGETS FOR TOXICANTS

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Introduction

Mechanisms regulating development of female phenotype are incompletely defined. However, the classic developmental paradigm that genetic sex dictates gonadal sex which, in turn, dictates phenotypic sex, provides a relevant conceptual framework within which to consider the role of genetic, epigenetic and environmental factors that ultimately determine sexual phenotype and the potential for reproductive competence. In this regard it is important to recognize that, while genetic potential for reproductive success is determined at conception, phenotypic potential for reproductive success is determined by the course of down-stream events that regulate the organization of reproductive tissues and organs. These events can be altered by exposure of tissues to pharmaceuticals or environmental chemicals that affect organizational mechanisms during critical periods of development.

Numerous clinical, laboratory and field studies support the idea that environmental chemicals, particularly those that act as steroid receptor agonists and/or antagonists, can alter the course of reproductive development and affect reproductive performance and health in many vertebrate species [1-9]. Whether natural or manmade, environmental chemicals that mimic, block or modulate hormone actions and, thereby, alter the normal course of organizational or physiological events are called 'endocrine disruptors' (EDs). Here, objectives are to identify: (1) events associated with development of the mammalian female reproductive system that are potential targets for EDs; (2) compounds that can act as environmental EDs, domains of exposure through which they may be encountered, and mechanisms through which they can affect reproductive development; and (3) opportunities and challenges for basic and clinical research aimed at identification and remediation of reproductive problems associated with exposure to environmental EDs.

Female Reproductive Development

Components of the mammalian female reproductive system include the ovaries, brain, pituitary, genital tract and mammary glands. Development of these tissues begins in the embryonic period, but is completed postnatally and even, in the case of the mammary gland, post-pubertally. Ultimately, all of these tissues acquire the capacity to respond to steroids and other bioactive factors that support their development and function, making them likely targets for environmental EDs. Following is a brief overview of events and mechanisms that determine gonadal phenotype and support differentiation of the female genital tract and central nervous system (CNS). The reader is directed to recent reviews and associated references for discussions of mechanisms regulating and factors affecting mammary gland development [10-15].

Sexual phenotype in eutherian mammals is acquired through a series of events that begins at conception. Initially, genetic sex is determined by chromosomal contributions of the fertilizing sperm [16]. This event establishes either an XX (female) or XY (male) sexual genotype and determines whether indifferent bipotential tissues of the urogenital ridge become organized into

ovaries or testes. In normal males, presence of the Y chromosome directs testis development, while absence of the Y chromosome in normal females permits development of ovaries. Ovary-determining genes have not been identified [16, 17]. However expression of the X-linked gene DAX-1 and the autosomal gene Wnt-4 is thought to prevent testis formation and, thereby, insure formation of ovaries. In genetic females, conditions that prevent or compromise expression of these 'anti-testis' genes can lead to partial phenotypic sex reversal and various forms of pseudohermaphroditism. Emergence of sex-appropriate phenotype (sexual differentiation) follows gonadal determination [18].

During the indifferent stage of sexual development, male and female genital tracts are indistinguishable. Paired Müllerian and Wolffian ducts form in both sexes and end caudally in the urogenital sinus (UGS). In males, secretion of Müllerian inhibiting substance (MIS) and testosterone by the embryonic testes directs regression of Müllerian ducts, supports sex-appropriate organization of the central nervous system, and insures development of internal and external male genitalia, which differentiate from the Wolffian ducts and UGS [18]. Data for the mouse indicate that autosomal expression of Wnt-4, one of 16 members of a vertebrate gene family involved in regional specification of cell fate, is required for Müllerian duct formation in males and females [13, 19]. Also detectable in pregonadal tissues of both sexes, Wnt-4 expression is down-regulated in males after testes differentiate, but persists in gonadal tissue of females after ovarian differentiation [13, 19, 20]. Wnt-4 deficient female mice display partial female-to-male sex reversal, with gonads that secrete both MIS and testosterone [20]. Thus, Wnt-4 expression stabilizes the organizational program leading to ovary determination and establishment of female phenotype [13, 19]. Taken together with data indicating that ovarian estrogen affects organization of the female brain [21], observations can be interpreted to suggest that complete differentiation of female sexual phenotype does not occur simply by default, in the absence of testes.

In normal females, Müllerian differentiation in the anteroposterior axis gives rise to the oviducts (uterine tubes), uterus, cervix and anterior vagina, while the posterior vagina and external genitalia develop from the UGS. Patterning in the radial axis, including differentiation and organization of muscular, stromal and epithelial compartments, establishes tissue-specific histoarchitecture and biochemical phenotype [19, 22, 23]. Tissue patterning and cytodifferentiation require epithelial-mesenchymal interactions that are ultimately influenced by developmentally programmed changes in extracellular matrix biochemistry, as well as by temporospatial patterns of steroid receptor expression and activation that are species-specific [2, 19, 22, 24-26]. Recently, products of at least two tissue patterning gene families, including the clustered homeobox genes *hoxa-9*, *-10*, *-11* and *-13*, and the Wnt gene family members Wnt-4, *-5a* and *-7a*, were implicated as primary mediators of organizationally critical patterning events, as well as markers of genital tract differentiation [13, 19, 23]. Tissue and cell compartment-specific expression of these genes is thought to define and stabilize tissue boundaries and cellular phenotypes in both axes of the female genital tract. For example, in *Hoxa-10*-null female mice a homeotic transformation was described in which the anterior portion of each uterine horn displayed cellular and molecular characteristics of the oviduct [27]. Similarly, Wnt-7a-null female mice display oviductal and uterine morphologies that are effectively reversed. In these animals, uterine glands are absent and the uterine myometrium is disorganized and enlarged [19, 28]. Organizationally critical stromal-epithelial interactions that direct and support differentiation

of tissues in the female genital tract are thought to be stabilized by Wnt signal-dependent Hox-gene expression, patterns of which can be affected by exposure of tissues to steroids or steroidal xenobiotics [19, 23]. Such primary tissue patterning genes are potential targets for environmental EDs.

Mechanisms regulating sexual differentiation of the brain and development of the reproductive neuroendocrine system have been reviewed extensively [21, 29-34]. Mechanistic dogma holds that masculinization of the CNS, necessary for normal male sexual behavior, as well as defeminization of neural centers that regulate patterns of gonadotropin secretion are testis-dependent, while differentiation of the CNS in females is a gonad-independent process that occurs by 'default' in the absence of testes. It is clear that testicular androgens (testosterone) are primary effectors of CNS masculinization, and that exposure to aromatizable androgens or estrogens during organizationally critical periods can masculinize and/or defeminize an otherwise female brain [29, 31, 32]. However, a compelling argument was recently made in support of the idea that ovarian estrogen is necessary for complete differentiation of the female CNS [21]. If this is correct, then sexual differentiation of the CNS may be actively induced through estrogen receptor (ER) -dependent, estrogen-sensitive mechanisms in both males and females.

Citing data for the rat, Fitch and Denenberg [21] submit that sex-related differences in: (1) CNS estrogen levels; (2) critical periods for estrogen action; and (3) ER expression patterns could explain how estrogens might exert masculinizing effects in males and feminizing effects in females. In this model, early (perinatal) high levels of intraneuronal estrogen, typical of males, interact with sex- and age-specific ER populations to masculinize the CNS, while low levels of estrogen, typical of females, interact with sex- and age-specific ER populations to feminize the CNS over a longer and later period of postnatal life [21]. Whether such mechanisms will be proven for the rat or demonstrated in other mammals remains to be seen. Still, critical periods for CNS differentiation have been defined broadly for rodents [33, 35, 36], domestic ungulates [37], and primates [35, 36, 38, 39]. Generally, these periods, during which CNS tissues are uniquely sensitive to the organizational effects of aromatizable androgens or estrogens, occur prenatally in species with comparatively long gestation periods such as the sheep, cow, rhesus monkey, and guinea pig, but perinatally in rats and mice, in which gestation is comparatively short. An exception here is the pig, in which masculinization of the CNS occurs postnatally [37]. The fact that dosage, duration and period of exposure to organizationally active steroids determine the nature and extent of effects observed at morphological and functional levels for both the CNS and other developing steroid target tissues suggests that critical periods are not only species, but trait-specific [21, 31]. Therefore, organizationally active, environmental EDs recognized by developing CNS or other tissues during critical developmental periods are likely to induce effects that are exposure context-dependent (see below).

Environmental Endocrine Disruptors (EDs)

In recent years a great deal of attention has been given to environmental chemicals that can affect development and function of reproductive tissues [1, 3-8, 40]. Among these chemicals, compounds that mimic or modulate the effects of steroid hormones constitute a particularly important class. Whether such compounds pose an immediate and significant health risk to the human population is a subject of some debate [7, 8, 40]. However, it is clear that both domestic

and wild animals can and do encounter these compounds and suffer the consequences of untimely exposures [2, 3, 41, 42].

Categories of environmental EDs likely to be encountered by animals include: (1) pharmaceuticals designed as endocrine modulators for therapeutic purposes, such as growth promotants and oral contraceptives; (2) natural endocrine modulating chemicals, including isoflavones, lignans, coumestans, $\exists$ -sitosterol, and mycotoxins, many of which may act as natural selective estrogen receptor modulators (SERMs); and (3) manmade industrial 'xenochemicals' [42], including pesticides, fungicides, plasticizers, siloxanes, polystyrenes, polychlorinated biphenyls, polychlorinated dibenzodioxins, and alkylphenolic compounds [3, 4, 7-9, 43, 44]. Effectively every steroid receptor-mediated pathway can be activated or otherwise modulated by one or more of these kinds of environmental chemicals [1, 7, 8, 45]. Since therapeutic, natural and industrial domains of exposure can overlap, animals are at risk of simultaneous exposure to multiple EDs beginning *in utero* and continuing throughout life. Many of these compounds can act as either receptor agonists or antagonists, depending on the tissue and exposure context [1, 4, 7, 8]. Moreover, receptors may exhibit promiscuous ligand binding, permitting a single compound to exert more than one kind of receptor-mediated effect [1, 3, 8]. Given that many critical organizational events associated with differentiation of the female reproductive system are steroid receptor-dependent and steroid-sensitive [21, 26, 46, 47], it is not surprising that environmental EDs can induce lesions in developing reproductive tissues directly, via steroid receptor-mediated mechanisms. However, EDs may also act via selective DNA methylation or demethylation (imprinting) of specific genes to 'misprogram' development [9, 48]. Such effects have long-term consequences that may not only be delayed, but inherited [6, 48, 49]. Thus, genes, as well as cells and tissues, are potential targets for environmental toxicants. Overall, effects of exposure to environmental EDs on reproductive development and function are likely to be sex and species-specific. Consequences of exposure to such compounds can also be expected to reflect: (1) the array and chemical nature of EDs to which animals are exposed (exposure profile); (2) dosages, both acute and cumulative; (3) exposure period; (4) exposure duration; and (5) receptor expression patterns.

Obviously, ED exposure profiles are difficult to define, and effects of exposure in the 'real world' even more difficult to predict. For domestic animals, therapeutic and dietary domains of ED exposure can be monitored and controlled to some extent. However, all animals share the biosphere to greater and lesser degrees and, consequently, the risk of exposure to EDs that may be present and accumulate in this domain [7, 42]. When assessing the etiology of disorders or dysfunctions suspected to derive from environmental ED exposure, it is important to obtain a complete exposure profile and history that does not fail to consider potential contributions from any of these domains.

Reproductive Research and EDs

A good deal of modern research aimed at identification of endocrine, cellular and molecular mechanisms governing development and function of the female reproductive system has evolved from studies designed originally to explain the actions of dietary phytoestrogens and the synthetic xenoestrogen diethylstilbestrol (DES) on reproductive function and health in domestic animals and women [6, 7, 41, 43, 44, 50]. Perinatal or later exposure to estrogenic substances in female mammals is known to induce: persistent estrus or other disturbances in estrus or

menstrual cycles; polycystic and polyovulatory follicles; altered oviductal, uterine, cervical and vaginal morphology and function; uterine hypoplasia; and uterine, cervical and vaginal carcinomas [6, 19, 41, 43, 51]. Recognition that pre- or perinatal exposure of humans [52] and rodents to estrogenic EDs could induce permanent changes in the genital tract [6] and CNS [53] reinforced the importance of developmental programming for reproductive success, and catalyzed efforts to identify critical periods of susceptibility to the effects of environmental EDs.

The fact that developing tissues are targets for EDs, and that susceptibility to the organizationally disruptive effects of specific compounds changes in time over the course of development, has been exploited as an experimental strategy for the production of both CNS [29, 31] and genital tract [2, 6, 19] lesion models. Comparisons of normal and lesioned (ED-exposed) tissues at structural and functional levels, both during and after ED exposure, have contributed to the identification of tissue patterning mechanisms and factors required for normal reproductive function.

The ovine uterine gland knock-out (UGKO) model [2, 54-57] provides a recent, novel example of the utility of this kind of experimental system. Adult UGKO ewes possess hypoplastic uteri that lack endometrial glands [2]. This extreme, stable adult uterine phenotype is created by transient exposure of ewe lambs to a progesterone receptor (PR) agonist (norgestomet) for a defined period after birth. This organizationally disruptive condition prevents normal gland genesis, which occurs postnatally in sheep [54, 58, 59]. Histologically, effects of postnatal norgestomet exposure on adult genital tract tissues were confined to the uterus [54], suggesting a tissue-specific critical period for this PR ligand. Comparative gene expression analyses revealed dozens of known and several novel transcripts that were expressed differentially by uterine endometrium obtained from adult UGKO and control ewes during diestrus, the majority of which were absent from UGKO tissues [60]. Consistently, embryo survival was severely curtailed in UGKO ewes [55]. Further comparisons established that patterns of conceptus survival and development could be related directly to endometrial phenotype and the presence and state of development of endometrial glands [55]. Thus, an animal model established through strategic disruption of critical developmental events mediated, in this case, by a PR agonist, can now be used as a tool for identification of uterine gene products and mechanisms required to support pregnancy. Similar strategies, with complementary aims, involving targeted disruption of estrogen sensitive organizational events during perinatal life, have been exploited extensively in rodents [6] and are being developed for the pig [51, 61-64].

If exploitation of concepts derived from the study of ED effects on development has created opportunities for advancement of reproductive sciences and medicine, substantial challenges remain in terms of defining real ecotoxicological risks associated with ED exposure in wild and domestic animal populations [1, 5, 7, 8]. The extent of this challenge becomes even more daunting when the potential diversity of species responsiveness to endocrine modulators is considered. Assuming that contact (exposure) between the organism and environmental chemical in question is confirmed, and the level of exposure (dosage and duration) can be defined, exposure hazard (toxicity) is determined by the sensitivity of the organism to the chemical and the biological context of exposure. Risk arising from exposure to a potential ED can then be assessed by comparing degree of toxicity with level of exposure [5]. Owing to the complex nature of organismal responses to environmental chemicals and the danger of

extrapolating observations from one species to another, there is broad international agreement that comprehensive hazard and risk assessment strategies for environmental EDs should require in vivo testing and be multi-tiered, involving mammals, birds, fish, amphibia and invertebrates [5]. Farm animals, a category often overlooked by toxicologists, represent yet another potentially important sentinel animal population.

New 'toxicogenomic' screening procedures permit global, qualitative and quantitative assessment of many classes of molecular responses to environmental toxicants in tissues and cells [65, 66]. Use of these procedures will expedite efforts to identify reliable biomarkers of both ED exposure and effect, generate novel molecular reagents that will be invaluable in development of diagnostic screens, and identify mechanistic linkages between ED exposure and reproductive dysfunction and disease.

Summary

The female reproductive system provides many potential targets for environmental EDs, both through the course of development and in adulthood. Owing to the ubiquity of environmental endocrine modulating compounds of pharmaceutical, industrial and natural origin, animals are at risk of exposure to multiple EDs simultaneously, throughout life. Effects of exposure to such compounds in adulthood can be problematic, but are typically transient. However, exposure during critical pre- and postnatal periods, when developing tissues are uniquely sensitive to the organizationally disruptive effects of steroid receptor modulating chemicals, can alter the course of development and induce permanent changes in reproductive tissues with lasting consequences for reproductive performance and health. Critical exposure periods are likely to be compound-, species-, tissue-, context- and trait-specific. These important developmental periods remain largely undefined for most animals. Targeted ED-mediated disruption of reproductive development during critical organizational periods has been employed successfully as a strategy for induction of stable, definable lesions in both the female CNS and genital tract. Structural, biochemical and functional comparisons of normal and lesioned tissues can provide important insights into mechanisms regulating development and function of the female reproductive system. Establishing clear mechanistic linkages between ED exposures and reproductive dysfunctions and disease will facilitate the task of designing environments and refining management guidelines for domestic animals in order to insure that genetic potential for reproductive performance is realized and reproductive health is optimized.

References

1. Cooper RL, Kavlock RJ. Endocrine disruptors and reproductive development: a weight-of-evidence overview. *Journal of Endocrinology* 1997; 152: 159-166.
2. Bartol FF, Wiley AA, Floyd JG, Ott TL, Bazer FW, Gray CA, Spencer TE. Uterine differentiation as a foundation for subsequent fertility. *Journal of Reproduction and Fertility* 1999: 287-302.
3. Danzo BJ. The effects of environmental hormones on reproduction. *Cellular and Molecular Life Sciences* 1998; 54: 1249-1264.
4. Sharara FI, Seifer DB, Flaws JA. Environmental toxicants and female reproduction. *Fertility & Sterility* 1998; 70: 613-622.

5. Hutchinson TH, Brown R, Brugger KE, Campbell PM, Holt M, Lange R, McCahon P, Tattersfield LJ, van Egmond R. Ecological risk assessment of endocrine disruptors. *Environmental Health Perspectives* 2000; 108: 1007-1014.
6. Iguchi T, Sato T. Endocrine disruption and developmental abnormalities of female reproduction. *American Zoologist* 2000; 40: 402-411.
7. Nilsson R. Endocrine modulators in the food chain and environment. *Toxicologic Pathology* 2000; 28: 420-431.
8. Hoyer PB. Reproductive toxicology: current and future directions. *Biochemical Pharmacology* 2001; 62: 1557-1564.
9. McLachlan JA, Newbold RR, Burow ME, Li SF. From malformations to molecular mechanisms in the male: three decades of research on endocrine disrupters. *Apmis* 2001; 109: 263-272.
10. Hovey RC, McFadden TB, Akers RM. Regulation of mammary gland growth and morphogenesis by the mammary fat pad: a species comparison. *Journal of Mammary Gland Biology & Neoplasia* 1999; 4: 53-68.
11. Akers RM, McFadden TB, Purup S, Vestergaard M, Sejrsen K, Capuco AV. Local IGF-I axis in peripubertal ruminant mammary development. *Journal of Mammary Gland Biology & Neoplasia* 2000; 5: 43-51.
12. Richert MM, Schwertfeger KL, Ryder JW, Anderson SM. An atlas of mouse mammary gland development. *Journal of Mammary Gland Biology & Neoplasia* 2000; 5: 227-241.
13. Heikkila M, Peltoketo H, Vainio S. Wnts and the female reproductive system. *Journal of Experimental Zoology* 2001; 290: 616-623.
14. Knight CH, Sorensen A. Windows in early mammary development: critical or not? *Reproduction* 2001; 122: 337-345.
15. Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, Nandi S. Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging. *Journal of Steroid Biochemistry & Molecular Biology* 2002; 80: 137-148.
16. Migeon CJ, Wisniewski AB. Human sex differentiation: from transcription factors to gender. *Hormone Research* 2000; 53: 111-119.
17. Parker KL, Schedl A, Schimmer BP. Gene interactions in gonadal development. *Annual Review of Physiology* 1999; 61: 417-433.
18. Hughes IA. Minireview: sex differentiation. *Endocrinology* 2001; 142: 3281-3287.
19. Kitajewski J, Sassoon D. The emergence of molecular gynecology: homeobox and Wnt genes in the female reproductive tract. *Bioessays* 2000; 22: 902-910.
20. Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. *Nature* 1999; 397: 405-409.
21. Fitch RH, Denenberg VH. A role for ovarian hormones in sexual differentiation of the brain. *Behavioral & Brain Sciences* 1998; 21: 311-327; discussion 327-352.
22. Cunha GR. Epithelial-stromal interactions in development of the urogenital tract. *International Review of Cytology* 1976; 47: 137-194.
23. Taylor HS. The role of HOX genes in the development and function of the female reproductive tract. *Seminars in Reproductive Medicine* 2000; 18: 81-89.
24. Couse JF, Korach KS. Contrasting phenotypes in reproductive tissues of female estrogen receptor null mice. *Annals of the New York Academy of Sciences* 2001; 948: 1-8.

25. Conneely OM, Lydon JP, De Mayo F, O'Malley BW. Reproductive functions of the progesterone receptor. *Journal of the Society for Gynecologic Investigation* 2000; 7: S25-32.
26. Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, Spencer TE. Developmental biology of uterine glands. *Biology of Reproduction* 2001; 65: 1311-1323.
27. Benson GV, Lim H, Paria BC, Satokata I, Dey SK, Maas RL. Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. *Development - Supplement* 1996; 122: 2687-2696.
28. Miller C, Sassoon DA. Wnt-7a maintains appropriate uterine patterning during the development of the mouse female reproductive tract. *Development - Supplement* 1998; 125: 3201-3211.
29. Gorski RA. The 13th J. A. F. Stevenson memorial lecture. Sexual differentiation of the brain: possible mechanisms and implications. *Canadian Journal of Physiology & Pharmacology* 1985; 63: 577-594.
30. Dohler KD. Influence of hormones and hormone antagonists on sexual differentiation of the brain. *Archives of Toxicology. Supplement* 1998; 20: 131-141.
31. Wood RI, Foster DL. Sexual differentiation of reproductive neuroendocrine function in sheep. *Reviews of Reproduction* 1998; 3: 130-140.
32. Pinckard KL, Stellflug J, Resko JA, Roselli CE, Stormshak F. Review: brain aromatization and other factors affecting male reproductive behavior with emphasis on the sexual orientation of rams. *Domestic Animal Endocrinology* 2000; 18: 83-96.
33. Woodson JC GR. Structural Sex Differences in the Mammalian Brain: Reconsidering the Male/Female Dichotomy. In: Matsumoto (ed.) *Sexual Differentiation of the Brain*. Boca Raton, Fla: CRC Press; 2000: 229-255.
34. Schlinger BA, Soma KK, London SE. Neurosteroids and brain sexual differentiation. *Trends in Neurosciences* 2001; 24: 429-431.
35. MacLusky NJ NF. Sexual Differentiation of the Central Nervous System. *Science* 1981; 211: 1294-1302.
36. Resko JA, Roselli CE. Prenatal hormones organize sex differences of the neuroendocrine reproductive system: observations on guinea pigs and nonhuman primates. *Cellular & Molecular Neurobiology* 1997; 17: 627-648.
37. Ford JJ DOM. Differentiation of Sexual Behavior in Cattle, Sheep and Swine. *J Anim Sci* 1989; 67: 1816-1823.
38. Conte FA GM. Bearing Abnormalities of Sex Differentiation on the Hypothalamic-Pituitary-Gonadal Axis at Puberty. In: Serio (ed.) *Sexual Differentiation: Basic and Clinical Aspects*. New York, NY: Raven Press; 1984: 275-286.
39. Resko JA EW. Sexual Differentiation of the Brain of Primates. In: Serio (ed.) *Sexual Differentiation: Basic and Clinical Aspects*. New York, NY: Raven Press; 1984: 169-182.
40. Safe SH. Environmental and dietary estrogens and human health: is there a problem? *Environmental Health Perspectives* 1995; 103: 346-351.
41. Adams NR. Detection of the effects of phytoestrogens on sheep and cattle. *Journal of Animal Science* 1995; 73: 1509-1515.
42. Guillette LJ, Jr., Guillette EA. Environmental contaminants and reproductive abnormalities in wildlife: implications for public health? *Toxicology & Industrial Health* 1996; 12: 537-550.

43. Cunha GR, Forsberg JG, Golden R, Haney A, Iguchi T, Newbold R, Swan S, Welshons W. New approaches for estimating risk from exposure to diethylstilbestrol. *Environmental Health Perspectives* 1999; 107: 625-630.
44. Cassidy A, Faughnan M. Phyto-oestrogens through the life cycle. *Proceedings of the Nutrition Society* 2000; 59: 489-496.
45. Safe S, Wang F, Porter W, Duan R, McDougal A. Ah receptor agonists as endocrine disruptors: antiestrogenic activity and mechanisms. *Toxicology Letters* 1998; 102-103: 343-347.
46. Conneely OM, Mulac-Jericevic B, Lydon JP, De Mayo FJ. Reproductive functions of the progesterone receptor isoforms: lessons from knock-out mice. *Molecular & Cellular Endocrinology* 2001; 179: 97-103.
47. Rosenfeld CS, Roberts RM, Lubahn DB. Estrogen receptor- and aromatase-deficient mice provide insight into the roles of estrogen within the ovary and uterus. *Molecular Reproduction & Development* 2001; 59: 336-346.
48. McLachlan JA, Burow M, Chiang TC, Li SF. Gene imprinting in developmental toxicology: a possible interface between physiology and pathology. *Toxicology Letters* 2001; 120: 161-164.
49. Rakyan VK, Preis J, Morgan HD, Whitelaw E. The marks, mechanisms and memory of epigenetic states in mammals. *Biochemical Journal* 2001; 356: 1-10.
50. Herbst AL. The effects in the human of diethylstilbestrol (DES) use during pregnancy. *Princess Takamatsu Symposia* 1987; 18: 67-75.
51. Tarleton BJ, Wiley AA, Bartol FF. Neonatal estradiol exposure alters uterine morphology and endometrial transcriptional activity in prepubertal gilts. *Domestic Animal Endocrinology* 2001; 21: 111-125.
52. Herbst AL, Ulfelder H, Poskanzer DC. Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *New England Journal of Medicine* 1971; 284: 878-881.
53. Gorski RA. Sexual dimorphisms of the brain. *Journal of Animal Science* 1985; 61: 38-61.
54. Gray CA, Bazer FW, Spencer TE. Effects of neonatal progestin exposure on female reproductive tract structure and function in the adult ewe. *Biology of Reproduction* 2001; 64: 797-804.
55. Gray CA, Taylor KM, Ramsey WS, Hill JR, Bazer FW, Bartol FF, Spencer TE. Endometrial glands are required for preimplantation conceptus elongation and survival. *Biology of Reproduction* 2001; 64: 1608-1613.
56. Spencer TE, Stagg AG, Joyce MM, Jenster G, Wood CG, Bazer FW, Wiley AA, Bartol FF. Discovery and characterization of endometrial epithelial messenger ribonucleic acids using the ovine uterine gland knockout model. *Endocrinology* 1999; 140: 4070-4080.
57. Taylor KM, Gray CA, Joyce MM, Stewart MD, Bazer FW, Spencer TE. Neonatal ovine uterine development involves alterations in expression of receptors for estrogen, progesterone, and prolactin. *Biology of Reproduction* 2000; 63: 1192-1204.
58. Wiley AA, Bartol FF, Barron DH. Histogenesis of the ovine uterus. *Journal of Animal Science* 1987; 64: 1262-1269.
59. Bartol FF, Wiley AA, Coleman DA, Wolfe DF, Riddell MG. Ovine uterine morphogenesis: effects of age and progestin administration and withdrawal on neonatal endometrial development and DNA synthesis. *Journal of Animal Science* 1988; 66: 3000-3009.

60. Spencer TE, Bartol FF, Bazer FW, Johnson GA, Joyce MM. Identification and characterization of glycosylation-dependent cell adhesion molecule 1-like protein expression in the ovine uterus. *Biology of Reproduction* 1999; 60: 241-250.
61. Bartol FF, Wiley AA, Spencer TE, Vallet JL, Christenson RK. Early uterine development in pigs. *Journal of Reproduction & Fertility - Supplement* 1993; 48: 99-116.
62. Vallet JL, Christenson RK, Bartol FF, Wiley AA. Effect of prepubertal retinyl palmitate treatment on uterine development and subsequent uterine capacity in swine. *Journal of Animal Science* 1996; 74: 603-609.
63. Tarleton BJ, Wiley AA, Bartol FF. Endometrial development and adenogenesis in the neonatal pig: effects of estradiol valerate and the antiestrogen ICI 182,780. *Biology of Reproduction* 1999; 61: 253-263.
64. Tarleton BJ, Wiley AA, Spencer TE, Moss AG, Bartol FF. Ovary-independent estrogen receptor expression in neonatal porcine endometrium. *Biology of Reproduction* 1998; 58: 1009-1019.
65. Hamadeh HK, Bushel P, Paules R, Afshari CA. Discovery in toxicology: mediation by gene expression array technology. *Journal of Biochemical & Molecular Toxicology* 2001; 15: 231-242.
66. Aardema MJ, MacGregor JT. Toxicology and genetic toxicology in the new era of "toxicogenomics": impact of "-omics" technologies. *Mutation Research* 2002; 499: 13-25.