Where the Boys Aren't: Dioxin and the Sex Ratio

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The question of how exposure to dioxins might affect only males and why it affects preferentially male embryos in the Seveso data presented by Mocarelli et al. remains intriguing. This enigma can be explained by the ovopathy concept, which addresses the determination of both the sex and the condition of the progeny. Antiandrogenic properties of dioxin alter the sperm-transit time and mating behavior, which provoke delay of fertilization of the oocyte (postovulatory overripeness of the oocyte). Antiestrogenic properties of dioxin during mid-cycle compromise both mucus liquefaction and maturation of the oocyte (preovulatory overripeness ovopathy). A positive dose–response of male-biased pathologic conceptuses is often followed by a negative one due to "vanishing male conceptuses." This dose–response fallacy is present in animal experiments and explains many otherwise unexplained phenomena related to dioxin contamination and other high-risk conceptions. *Key words*: Seveso, dose–response fallacy, postovulatory overripeness ovopathy, preovulatory overripeness ovopathy, vanishing male conceptuses. *Environ Health Perspect* 110:1–3 (2002). [Online 10 December 2001]
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Mocarelli and colleagues (1,2) compared about 750 people in a cohort from the most contaminated area to 40,000 people from two zones that were less contaminated by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) after the explosion at a herbicide plant in Seveso, Italy. The authors found a lower proportion of male offspring from exposed fathers (77:100) and a tendency to more male offspring (120:100) from exposed mothers. Clapp and Ozonoff (3) describe these results as "intriguing, worrying and provocative" (p. 848). A similar decrease of male offspring was found in an Austrian chloracne cohort, in which only fathers were exposed to dioxin (4).

In the human ejaculate, the proportion of X- and Y-bearing sperm is always equal (5,6), and deviation of this equality has never been identified in animals or humans, regardless of whether the sperm is normal or manipulated (7). However, the normal male-to-female ratio at conception, or primary sex ratio (PSR), is not 100 boys for 100 girls, but is presumed to be much higher, while the secondary sex ratio (SSR) at birth is about 105–106:100 (5).

In previous work we introduced a basic ovopathy concept, which is thoroughly backed by experiments in animals and observations in humans (8,9). It determines both the sex and the condition of the conceptus. After a short delay in fertilization in rabbits, an overrepresentation of male blastocysts or births was observed, but this was followed by sex ratio (SR) reversal after a prolonged delay (10,11). Otherwise, as will be argued further, accelerated as well as postponed ovulations are known to impair the condition of the conceptus (12–15). These phenomena will explain many questions related to the SSR in animals and humans.

Male:Female Ratio in the Offspring of Dioxin-Contaminated Fathers

Lack of knowledge about the physiologic mechanism and the role of dioxin in the reduction of the sex ratio led Moacarelli et al. (2) to hypothesize about the link between the median 1976 body concentration of dioxin in men before and during puberty and the postpubertal effect on the SSR of their offspring. They referred to the antiandrogenic properties *in utero* or during lactation in adult rats exposed to polychlorinated biphenyls (PCBs) and dioxin-like chemicals resulting in permanently altered sperm-transit time through the epididymus and the excurrent duct system (16,17). In experimentally exposed male animals, when mated with untreated, receptive females, dose-related decreases of mature sperm quality (decreased number, abnormal morphology, less motility, and reduced capacity to penetrate hamster oocytes) and reduction of mating behavior and fertility are observed, whereas in exposed men, significantly decreased testosterone (and high gonadotropin) levels are found (18–20). Altered sperm-transit constrains the process of fertilization of the oocyte, leading to postovulatory overripeness ovopathy (PoOO), which leads to defective implantation in animals, transitory retardation in the rate of development, and increased prenatal loss (12,13). Most failures in reproductive success of male rats, exposed to PCB either *in utero* or during lactation, are in fact due to preimplantation loss of their offspring (16).

Because equal proportions of X- and Y-bearing spermatozoa are always found in the human ejaculate, in any condition independent of paternal age (5) or of karyotype, such as XXY or XYY (6), both the XX and the XY conceptuses are expected to be equally affected by PoOO. This concept, according to which inferiority of the oocyte is already determined before fertilization, in combination with the deleterious sublethal X-linked genes [in males not compensated for by a second X-chromosome with normal genes (21)] explains the male sex-specific loss of conceptuses, particularly when already affected and following a dose–response gradient. Such a mechanism is suggested by the Seveso results [odds of a male birth with increasing TCDD serum concentration in the father: odds ratios (ORs) = 0.601 and 0.465)] (2). This PoOO hypothesis, therefore, is more plausible than that of James (22,23), who assumes that the high gonadotropin and low testosterone levels would have injured and skewed the Y-bearing gametes before conception.

On the basis of this PoOO hypothesis, we suggest that male experimental animals exposed to dioxin, when mated with unexposed females, will experience early loss of male-biased conceptuses, resulting in a reduced number of progeny that are female-biased.

Male:Female Ratio in the Offspring of Dioxin-Contaminated Mothers

The above PoOO concept explains the reduced sex ratios due to exposed fathers, but not the apparently inverse tendency of an increased number of male births in unexposed fathers married to (apparently) unexposed (155:100) or factually exposed (120:100) mothers in the Seveso area (2). Therefore, the female axis should be addressed, too.

In a number of model systems, TCDD inhibits the estradiol-induced receptor protein levels and binding activity (17,24). In rhesus monkeys and several other species, the ovarian function is altered, as indicated by anovulation, suppression of estrous cycles, and infertility. Contaminated women have been reported to have increased rates of abnormal menstrual bleeding, pregnancy loss, stillbirth, and infant death (25,26). In the entire study area, the serum levels of TCDD were shown to be higher in females (27), and the overall mean half-life lasted longer than in men (~9 vs. ~7 years) (28).

We hypothesize that the intricate connections between equal gender proportions

and optimal conceptions at the core of the fertile window of the menstrual cycle and between disproportionate rates of male-biased and pathologic conceptuses outside of it are due to periovulatory hormone modulation, which simultaneously affects cervical liquefaction and oocyte maturation (*8,9*).

Cervical liquefaction plays a pivotal role in the migration of the spermatocytes (*29*), whereas developmental competence of the human oocyte is acquired during follicle formation and meiotic progression (*30*). Before mid-cycle, both liquefaction of the mucus plug and maturation of the oocyte are modulated by estrogens. Concurrence of both, of course, facilitates equal access and fertilization of optimally matured oocytes by X- and Y-bearing spermatocytes, leading to full expression of the genetic potential, good embryo quality, and equal sex ratios.

In contrast, nonoptimal maturation and liquefaction due to hormonal disturbances will occur preferentially at the beginning and the end of the fertile window. The pleiotropic nature of experimentally induced aging of the oocyte in animals before mid-cycle [i.e., preovulatory overripeness ovopathy (PrOO)] depends on molecular, biochemical, and physiologic processes in the nuclear and cytoplasm constituents (*13–15*). As for PoOO, impossibility of fertilization, defective implantation, prenatal loss, transitory retardation in the rate of development, and a spectrum of anomalies and deficiencies in organogenesis or differentiation of various tissues and organ systems are the results. Because the head of Y-bearing spermatozoa, their length, perimeter, and area are significantly smaller than those of X-bearing ones, and their necks and tails are shorter (*31*), differential migration of the Y-bearing spermatozoa (*32*) will be likely at the beginning and at the end of the fertile window. Thus, preferential fertilization of nonoptimally matured oocytes by Y-bearing spermatozoa will occur at these extremes.

We hypothesize that determination of both male sex and pathology of the progeny by PrOO will initially cause a positive male-biased dose response, which, after having reached a threshold, will be followed by a negative one due to disproportional loss of male-specific conceptuses and sublethal X-linked genes (*21*). The resulting SSR inversion can be considered a distortion by differential prenatal loss, or inverted dose–response gradient (*33,34*)—in other words, a dose–response fallacy (*35*). This fallacy is substantiated by animal experiments (*10,11*). The phenomenon of vanishing male conceptuses may be compared with that of vanishing twins, due to twins disappearing before they are clinically recognized [twin conceptions are estimated to be 12% of all natural conceptions, of which only about 2% survive to term as twins and about 12% result in single births (*36*)].

The antiestrogenic action of dioxin provokes periovulatory hormone variation and PrOO and leads to disproportionate rates of male-biased pathologic conceptuses outside the fertile window of the menstrual cycle. We presume that the background exposure in unexposed mothers (< 15 ppt or living outside the contaminated zones) married to unexposed fathers explains the SSR increase (155:100). The decreasing odds of a male birth [OR = 0.722; 95% confidence interval (CI), 0.42–1.24)] in the high exposure category (> 80 ppt) (*2*), as well as the less increased one (OR = 1.05; 95% CI, 0.62–1.79) in the lower exposure category (> 15 but < 80 ppt), may be due to more male-biased loss (i.e., vanishing male conceptuses). An initial incline, followed by a decline of the SSR, obfuscates the true effects, but jeopardizes the alleged insensitivity of exposed females.

The distinction between PoOO and PrOO, in connection with dioxin exposure, is that intratubally aged oocytes (PoOO) are fertilized by either Y- or X-bearing spermatozoa, and the XY-zygotes are preferentially lost. Intrafollicularly aged oocytes (PoOO) are preferentially fertilized by advantaged Y-bearing spermatozoa, which results in a positive male-biased, followed by a negative, dose response. In other words, both the male-mediated effect by PoOO and the female mediated effect by PrOO are operative in the female gamete.

On the basis of this PrOO hypothesis, we suggest that female experimental animals exposed to low doses of dioxin, when mated with unexposed males, will experience an increase of male-biased conceptuses and progeny, accompanied by loss of males. However, in the case of high doses, stronger loss of male-biased conceptuses will occur, resulting in female-biased progeny.

Male:Female Ratio in Dioxin-Contaminated Couples

Contamination of both partners (i.e., both PoOO and PrOO) can only aggravate the rate of conceptopathology and the SSR inversion, as seen in exposed fathers married to exposed (79:100) or allegedly unexposed mothers (77:100) (*2*). Similar SSR inversion has been reported in the progeny of subfertile men in which plasma testosterone levels were significantly decreased (*22,23*) and in male and female patients affected by non-Hodgkin's lymphoma during their reproductive period (*37*), who can be supposed to experience a decreased coitus frequency and thus enhancement of delayed fertilization or PoOO.

Male:Female Ratio in Other High-Risk Conceptions

Many other high-risk conditions in animals and humans, in which maturation and/or fertilization are compromised, are connected with excesses in both SSR and pathologic progeny, followed by decreases due to pregnancy loss (*8,9*). This SSR reversal obfuscates the true effects but explains many inconsistencies and controversies in epidemiologic data.

PoOO and PrOO thus reconcile the general tendency of higher SSRs among progeny from compromised conceptions in animals and humans, such as at the extremes of maternal reproductive age, the ovulatory breakthrough and breakdown of the ovulatory seasons (reminiscent to the estrous periods in the animal world), or at war time. In contrast, the lower SSRs or equal gender proportions among progeny coincide with the more optimal conceptions (e.g., at prime reproductive age, at the zenith of ovulatory seasons, and in affluent societies) (*8,9*). In addition, PoOO and PrOO explain the public-health maxim of higher male:female ratios at conception, the relentless loss of male progeny at every age from conception onward up to senescence, and the gap in life expectancy.

We presume that the ovopathy concept, which unites the determination of both sex and quality of the female gamete, elucidates most of the enigmas related to dioxin and sex ratio.

REFERENCES AND NOTES

1. Mocarelli P, Brambilla P, Gerthoux PM, Patterson DG Jr, Needham LL. Change in sex ratio with exposure to dioxin [Letter]. Lancet 348:409 (1996).
2. Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG Jr, Kieszak SM, Brambilla P, Vincoli N, Signorini S, Tramacere P, Carreri V, et al. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858–1863 (2000).
3. Clapp R, Ozonoff D. Where the boys aren't: dioxin and the sex ratio. Lancet 355:1838–1839 (2000).
4. Moshammer H, Neuberger M. Sex ratio in the children of the Austrian chloracne cohort. Lancet 356:1271–1272 (2000).
5. Graffelman J, Fugger EF, Keyvanfar K, Schulman JD. Human live birth and sperm-sex ratios compared. Hum Reprod 14:2917–2920 (1999).
6. Araki Y, Motoyama M, Yoshida A, Kamiyama H. In situ hybridization chromosome analysis of XYY and XYY males' spermatozoa [Letter]. Hum Reprod 12:1604 (1997).
7. Lobel SM, Pomponio RJ, Mutter GL. The sex ratio of normal and manipulated human sperm quantitated by the polymerase chain reaction. Fertil Steril 59:387–392 (1993).
8. Jongbloet PH, Groenewoud JMM, Zielhuis GA. Further concepts on regulators of the sex ratio in human offspring. Non-optimal maturation of oocyte and the sex-ratio. Hum Reprod 11:2–7 (1996).
9. Jongbloet PH, Zielhuis GA, Groenewoud HMM, Pasker-de Jong PCM. The secular trends in male:female ratio at birth in postwar industrialized countries. Environ Health Perspect 109:749–752 (2001).
10. Hammond J. The fertilisation of rabbit ova in relation to time. J Exp Biol 11:140–166 (1934).
11. Shaver EL, Carr DH. The chromosome complement of rabbit blastocysts in relation to the time of mating and ovulation. Can J Genet 11:287–293 (1969).
12. Witschi E. Overripeness of the egg as a cause of twinning and teratogenesis: a review. Cancer Res 12:763–797 (1952).

13. Butcher RL. Experimentally induced gametopathies. In: Principles and Practice of Obstetrics & Perinatology (Iffy L, Kaminetzky HA, eds). New York:John Wiley & Sons, 1981;339–349.

14. Mikamo K. Intrafollicular overripeness and teratologic development. Cytogentics 7:212–233 (1968).

15. Mattheij JAM, Swarts JJM, Hurks HMH, Mulder K. Advancement of meiotic resumption in Graafian follicle by LH in relation to preovulatory ageing of rat oocytes. J Reprod Fertil 100:65–70 (1994).

16. Roman E, Peterson RE. Developmental male reproductive toxicology of 2,3,77,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and PCBs. In: Reproductive and Developmental Toxicology (Korach KS, ed). New York:Marcel Dekker, 1998;593–624.

17. Webster T, Commoner B. The dioxine debate. In: Dioxins and Health (Schecter A, ed). New York:Plenum Press, 1994;1–50.

18. Potashnik G, Yanai-Inbar I. Dibromochloropropane (DBCP): an 8-year reevaluation of testicular function and reproductive performance. Fert Steril 47:317–323 (1987).

19. Egeland GM, Sweeney MH, Fingerhut MA, Wille KK, Schnorr TM, Halperin WE. Total serum testosterone and gonadotropins in workers exposed to dioxin. Am J Epidemiol 139:272–281 (1994).

20. Guo YL, Hsu P-C, Hsu C-C, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. Lancet 356:1240–1241 (2000).

21. Smith DWE. Is greater female longevity a general finding among animals? Biol Rev 64:1–12 (1989).

22. James WH. The hypothesized hormonal control of human sex ratio at birth—an update. J Theor Biol 143:555–564 (1990).

23. James WH. Total serum testosterone and gonadotropins. Am J Epidemiol 141:467–468 (1995).

24. De Vito MJ, Birnbaum LS. Toxicology of dioxins and related chemicals. In: Dioxins and Health (Schecter A, ed). New York:Plenum Press, 1994;139–162.

25. Nicholson WJ. Landrigan PJ. Human health effects of polychlorinated biphenyls. In: Dioxins and Health (Schecter A, ed). New York:Plenum Press 1994;496–501.

26. Yu M-L, Guo YL, Hsu C-C, Rogan WJ. Menstruation and reproduction in women with polychlorinated biphenyl (PCB) poisoning: long-term follow-up interviews of the women from the Taiwan Yucheng cohort. Int J Epidemiol 29:672–677 (2000).

27. Landi MT, Consonni D, Patterson DG Jr, Needham LL, Lucier G, Brambilla P, Cazzaniga MA, Mocarelli P, Pesatori AC, Bertazzi PA, et al. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plasma levels in Seveso 20 years after the accident. Environ Health Perspect 106:273–277 (1998).

28. Needham LL, Gerthoux PM, Patterson DG, Brambilla P, Turner WE, Beretta C, Pirkle JL, Colombo L, Sampson EJ, Tramacere PL, et al. Serum dioxin levels in Seveso, Italy, population in 1976. Teratog Carcinog Mutagen 17:225–240 (1997–98).

29. Moghissi KS. Sperm migration through the human cervix. In: Cervical Mucus in Human Reproduction (Elstein M, Moghissi KS, Borth R, eds). Copenhagen:Scriptor 1973;128–152.

30. Schramm RD, Bavister BD. A macaque model for studying mechanisms controlling oocyte development and maturation in human and non-human primates. Hum Reprod 14:2544–2555 (1999).

31. Cui KH. Size differences between human X and Y spermatozoa and prefertilization diagnosis. Mol Hum Reprod 3:61–67 (1997).

32. Broer KH, Winkhaus I, Sombroek H, Kaiser R. Frequency of Y-chromatin bearing spermatozoa in intrauterine postcoital tests. Int J Fertil 21:181–185 (1976).

33. Khoury MJ, Flanders WD, James LM, Erickson JD. Human teratogens, prenatal mortality and selection bias. Am J Epidemiol 130:361–370 (1989).

34. Khoury MJ, James LM, Flanders WD, Erickson JD. Interpretation of recurring weak associations obtained from epidemiologic studies of suspected human teratogens. Teratology 46:69–77 (1992).

35. Selevan SG, Lemasters GK. The dose-response fallacy in human reproductive studies of toxic exposures. J Occup Med 29:451–455 (1987).

36. Boklage CE. Survival probability of human conceptions from fertilization to term. Int J Fert 35:75–94 (1990).

37. Olson H, Brandt L. Sex ratio in offspring of patients with non-Hodgkin lymphoma. N Engl J Med 306:367–368 (1982).