Special Contribution

Sex Ratio Changes as Sentinel Health Events of Endocrine Disruption

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The production and widespread use of synthetic chemicals since the 1940s have resulted in ubiquitous contamination of fish, wildlife and human populations. Since the 1960s, observers have documented major damage to wildlife reproduction across the globe, and subsequently, damage to reproductive health in exposed humans as well. The sex ratio in human communities and populations can be readily measured to ascertain whether reproductive effects, such as subtle birth defects of the reproductive tract caused by exposures to chemicals, might be occurring. Male to female sex ratios appear to be declining in populations in several parts of the globe, possibly as a result of prenatal exposures to chemicals. Sex ratio data for communities with unusual occupational or environmental exposures can be compiled using traditional epidemiological techniques in pursuit of environmental justice. Local, regional and national population health researchers and occupational hygienists can use health statistics to examine sex ratios as sentinel health events that might portend patterns of subtle structural birth defects of the reproductive tract and functional deficits in neurodevelopment. *Key words*: Reproductive health, Sex ratio, Endocrine disruptors, Pollution, Environment, Human Cancer

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Since the publication of *Silent Spring* by Rachel Carson,[1] humankind has been aware of the widespread effects of synthetic chemicals on populations of fish and wildlife, and of the potential for effects on human health. The exposures to and effects of these risks of "modernity" have not only been difficult to perceive without the instruments of science[2] but also have been highly contested, particularly by industrial interests and the new social movements for health and environment.[3,4] The challenge for both occupational and environmental epidemiologists, as well as the public, in undertaking this task has been to find unequivocal markers of biological effects and to relate the effects unambiguously to specific exposures, in order to make causal statements on which regulatory and remedial actions can be undertaken.

Thirty years after the publication of *Silent Spring*, Dr Theo Colborn formulated the hypothesis of endocrine disruptors.[5] It was based on observations of chemically-induced reproductive and developmental anomalies in Great Lakes gulls[6] and advances in understanding the mechanisms of action of diethylstilbestrol and other xeno-estrogens (synthetic substances with estrogenic activity).[7,4] This provided a unifying explanatory principle for many of the observations of effects on fish, wildlife and human populations, such as the reproductive and developmental anomalies in alligators,[8] and led to an explosion of new experimental, epizootiological and epidemiological research on the effects of chemicals during the past decade. For example, recent studies have documented disturbing reproductive effects of ambient levels of the pesticide atrazine on frogs, characterised by feminization of males exposed to concentrations that can be encountered through permitted uses in the United States.[9] In humans, studies of the effects of chemicals on reproduction and development have been fraught with uncertainties[10] and in some cases have been heavily contested.[11] Concerns have been raised in particular about the effects of prenatal exposures in males, including a predisposition to cryptorchidism and hypospadias at birth, and increased risk of testicular cancer following puberty and declining semen quality.[12] In females, concerns include issues of premature breast development,[13,14] precocious puberty[15] and a predisposition to the development of breast cancer.[16]

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The proximity of many urban and rural populations to chemical landfill sites, incinerators and chemical manufacturing, as well as the common use of chemical products in modern life, result in widespread human exposures to a great variety of compounds, some of which have endocrine-disrupting activity. The challenge is to detect these otherwise imperceptible exposures through the identification of sentinel health events occurring in the exposed populations, particularly those that reliably indicate effects on reproduction, development and population viability in the animal kingdom. In the past thirty years, many communities have become aware that they are highly exposed to chemical wastes and releases and have organized to undertake health surveys through epidemiology studies.[3,4] The measure should be sufficiently simple so that it can be readily used, not only by professional epidemiologists tracking changes in large populations, but also by communities as part of their methods for investigation of the effects of local contamination.

One such sentinel marker that is proving valuable to health researchers and the public is the simple expedient of comparing the number of males with the number of females born and calculating the sex ratio. This metric has been used extensively to document declines in the proportion of males born in many countries (reviewed by Davis et al[17]), most recently in United States and Japanese populations.[17] In addition, it has been used in a small Chippewa community surrounded by chemical manufacturing plants in Sarnia, Ontario, Canada.[18] The purpose of this commentary is not only to explore the scientific aspects of this epidemiological endpoint as a marker of toxicological effects within the wider context of the science of endocrine disruptors, but also to locate this science within social, economic and political contexts.[19, 20]

ENDOCRINE DISRUPTORS: A CONTESTED AREA OF SCIENCE

For more than a decade, the hypothesis of endocrine disruption has been a contested area of science.[21] Some scientists have argued that isolated incidents of high levels of chemical contamination have affected the health and reproduction of non-human organisms, but that the levels of contamination have generally been far too low to have had effects on human beings. In the late 1990s, the U.S. National Academy of Sciences[11] set up a committee to examine the evidence on endocrine disruptors and the claims that there were irreversible developmental effects from exposures to low-levels of contamination with certain chemicals. The committee was characterised by an extreme polarization of views based not only on the evidence but also on the threat the evidence posed to the continuing profitability of the chemical products in question. The Academy finally concluded that while there were clear indications of reproductive and developmental disruption in animals, the evidence regarding such effects in humans was equivocal. The Academy explained that xeno-estrogens were at least a thousand times less powerful than endogenous hormones, and held that the levels encountered by humans were low and so were unlikely to wield any serious effects on human health.

NEW EPIDEMIOLOGICAL STUDIES

In the past decade, there have been several new epidemiological papers (reviewed by Aitken et al.[22]) that point towards widespread declines in sperm quality and increases in testicular cancer incidence, and show that these likely have an endocrine etiology.[23] Researchers have recently reported declining levels of testosterone in U.S. males of 1% per year, the same rate of decline seen for sperm concentrations.[24] There has also been speculation that testicular dysgenesis syndrome[25] originates from conception and can result in a cascade of defects in Sertoli and Leydig cells that ultimately affect maldescent of the testes, cryptorchidism, fertility and the risk of testicular cancer.[26] It seems likely that early exposures, occurring in a critical time window during fetal life, are implicated in this testicular dysgenesis syndrome and might also contribute to the risk of other cancers.[27] Others have noted that mothers with higher levels of phthalates in their blood during pregnancy have a significantly greater probability of bearing male infants with reduced ano-genital distance, a marker for testicular dysgenesis in rodents.[28] Residues of anabolic steroids and other xenobiotics used in food production may pose long-term risks for developmental processes in males. For example, in a large study of sperm concentration and fertility in American men, there was a negative association with the number of servings of beef their mothers ate per week while pregnant.[29] While the negative association is likely attributable to animals treated with hormonal growth promoters, other interpretations of these findings are possible.[30]

NEW MECHANISTIC STUDIES

In addition to these new epidemiological studies, research on mechanisms of action has reduced many of the uncertainties formerly associated with the endocrine disruptor hypothesis.[11] Replication of studies undertaken at extremely low doses have convincingly demonstrated the inverted-U-shaped dose-response curves familiar to endocrinologists, with the decreases in effects at higher doses. For example, vom Saal and Welshons[31] have shown that extremely low concentrations of xeno-hormones, such as bisphenol A, cause hormone-like effects that are often not seen at higher concentrations, which can however lead to other detrimental effects. These low-dose findings have

challenged all of traditional toxicology, which has been based on high dose testing and linear dose-response relationships as a reflection of the prevailing paradigm of "*dosis facit venenum*": the dose makes the poison. Another anomalous mechanism that supports the endocrine disruptor hypothesis concerns receptor binding. Receptors bound to xenobiotic ligands do not have exactly the same influence on gene expression as receptors bound by endogenous ligands.[32, 33] In reviewing the state of evidence on bisphenol A, vom Saal and Weshons[31] pointed out that blood levels in many human populations exceed levels that affect some cell functions and cause adverse effects in animals.

Since the contentious report of the U.S. National Academy of Science,[11] further mechanistic evidence concerning the physiological timing and the critical windows of development has accumulated, boosting the credibility of the endocrine disruptor hypothesis. A new paradigm of the "developmental origins of human health and disease" (DOHaD) has been proposed,[34,30] and is playing an important role in understanding the effects of low-dose exposures to endocrine disruptors. Of utmost importance is the realization that intrauterine programming of physiological systems occurs at the gene, cell, tissue, organ, and system levels and causes permanent structural and functional changes, which can lead to overt disease, particularly with increasing age.[35] This programming rests in part on DNA modification and covalent modifications to histones.[36] During critical periods of intrauterine development when epigenetic programming determines tissue differentiation, very low doses of endocrine disruptors might lead to permanent changes in gene expression underlying infertility and increased risk of cancer later in life.[37,38,39,30]

SEX RATIO AS AN INDICATOR OF ENDOCRINE DISRUPTION

Mammalian sex ratios at birth are partially controlled by parental hormone levels around the time of conception.[40,41] That endocrine disruptors can induce changes in sex ratio has been shown through experiments in many animal species including mammals.[42–49] But human data also indicate probably causal link between endocrine disruption and changes in sex ratio.[50,51] Men exposed to endocrine disruptors known to cause low testosterone/ gonadotropin ratios have been reported to sire significant excesses of daughters.[52–54] Incidence of testicular cancer has been rising in many countries over the past decades, with evidence of a cohort effect, suggesting that an in utero exposure, possibly to endocrine disruptors, might have been involved. Men with testicular cancer have a low testosterone/gonadotropin ratio,[55] and sire a significantly higher proportion of daughters both before and after the onset of disease.[56,57] The low sex ratio before the disease suggest that this hormone profile is a potential cause of testicular cancer.

As to mechanisms, hormones and xeno-hormones might affect sex ratio through the induction of a change in testosterone/gonadotropin ratio in men. Alternatively or more generally, they might affect sex ratio through a change in imprinting of gametes or through a change in DNA methylation affecting the embryo resulting in differential mortality of male versus female embryos. Gametic (genomic) imprinting of some genes is regulated through an imprinting control region that contains sequences binding nuclear hormone receptors,[58] so it seems possible that sex hormones could affect genomic imprinting. Hormones and xeno-hormones have been shown to affect DNA methylation and gene expression in embryos.[59,60]

NEW SEX RATIO STUDIES IN HUMANS

Endocrine disruptors, particularly xeno-estrogens, pose special problems for males, and there is new epidemiological evidence of effects occurring in specific populations. Recently Canadian researchers McKenzie et al.[18] investigated the male-to-female sex ratio in the Aamjiwnaang First Nation in Sarnia, Ontario. This Great Lakes community, which lives amid a large number of petrochemical plants, has experienced a dramatic and unprecedented decline in sex ratio: almost 40% of the boys had been lost. The recent study by Davis *et al.*[17] shows that a decline in sex ratio is not restricted to a limited community residing in an area with very particular exposures, but that steady reductions in the births of male infants have occurred in the U.S. and Japan over the past four decades. According to this analysis, declines in the proportion of males born in Japan 1970-1999 and to whites in the United States 1970-2002 are equivalent to a shift from male to female births of 127,000 and 135,000 births, respectively.

The MacKenzie and Davis studies used different methodologies and were undertaken at very different scales. Davis *et al.*[17] used national databases to calculate the small but statistically significant declines in the proportion of boys born, whereas MacKenzie *et al.*[18] used a very small database to demonstrate the statistically-significant large losses of potential male members of the Aamjiwnaang First Nation. The similarity of the findings despite the very different study sizes shows the need to undertake calculations of sex ratio at different scales from the local to the regional, national and international levels. The findings also indicate that less-conventional sources of data may be useful to document a local phenomenon. Different kinds of epidemiologists may be needed for each scale. At the regional and larger scales, trained epidemiologists are required for accessing massive databases and for undertaking calculations. But, as the example of the Aamjiwnaang First Nation demonstrated, participatory action research untaken by

a collaboration of staff of a local occupational health clinic and members of the local native community rapidly identified the change in sex ratio as a sentinel health event. The observation of the anomaly in sex ratio led to further investigation of health concerns, initially using qualitative methods, followed by quantitative surveys of contamination and health effects. Through such collaborations in population-based epidemiology, the true extent of the health issues within contaminated communities can be established, the underlying causes of disease or condition can be hypothesized for further investigation, and the community can empower itself to bring about social change.[61] Such methods have been used in Canada and in other countries[62,63] to overcome the limitations of traditional scientific approaches in responding to occupational hazards and the contamination of communities.[64]

The rapid identification of the sex ratio anomaly resulted in an immediate, deep rapport between the researchers from the clinic and the members of the community. In our societies, such research is necessary but, too frequently at the local level, the boundaries around professions and the loyalties to particular institutions can undermine respect and lead to distrust between those affected and those charged with protecting public health.[3,4] For example, a local anomaly discovered by civil society researchers can be made to "disappear" by officials measuring the same outcome at a larger scale, thereby diluting the local exposed population within a larger unexposed or less-exposed population, leading to exposure misclassification and reducing the likelihood of detecting the effect.[65]

The recognition of a sentinel event, such as a change in sex ratio, poses serious questions about the implications of the event for futher research or action. Many factors in sex ratio changes have been identified, and the etiology of the long-term trends is likely to be multicausal.[17] Further research is needed to identify the contribution of the great variety of known risk factors to the changes in sex ratio calculated from national population statistics. This contrasts with the Aamjiwnaang situation, in which the proportion of boys to girls was close to the national ratio during the 1980s and early 1990s.[18] In this cases, there was a well-defined point of inflection potentially indicating exposure to a specific risk factor that started around 1995. In contrast to other locations, such as Seveso, Italy, where a 2,4,5,trichlorophenol explosion in 1976 resulted in an immediate loss of males with subsequent recovery,[66] there has been a gradual progressive decline in the sex ratio among the Aamjiwnaang over more than a decade. Because the likely agents are known to be teratogenic, these observations also indicate the need to look for other co-morbidities such as neuro-developmental deficits or congenital abnormalities. For example, the Japanese community at Minamata was exposed to methyl mercury in the 1950s resulting not only in a higher mortality of males resulting in a decrease in sex ratio but also in selective neuro-developmental anomalies in males.[67]

It has to be acknowledged that factors other than environmental ones play a role in sex ratio alterations. Increased age of parents,[68] parents' cohabitation before conception,[69] stress due to war or unfavourable economic conditions,[70] greater use of pharmaceuticals, in particular drugs used for treatment of childhood cancer,[71] as well as alcohol and tobacco consumption[72] also play a role which may differ by country and social class. Yet, these factors are unlikely to explain marked changes in otherwise stable communities, such as the one observed in Sarnia. At a national level in the U.S., the global alcohol and tobacco per capita consumption went down at the time the Davis study[17] was carried out, although these findings do not necessarily reflect the trend for women and pregnant women in particular.[73,74] Whereas it is reassuring to note there has been a decrease in alcohol-related conditions in newborns,[75] smoking rates have increased among pregnant women from African American and low socioeconomic status populations. [76,77]

Successful forensic research to identify and locate the source of exposures to endocrine disruptor chemicals requires the involvement of many disciplines and a willingness of investigators to cross traditional disciplinary boundaries. For example, in addition to epidemiologists, chemists and toxicologists, an observation of a change in the sex ratio has immediate psycho-social consequences, and trained sociologists, anthropologists and psychologists are needed as an integral part of research teams in "toxic-assaulted communities."[61] These disciplines are needed to reveal the power relationships within the affected community and between the affected community and the larger community, its health authorities and other institutions. For it is not only a matter of solving the toxicological riddles, but also finding the technical and political means to resolve the situation. For those seeking environmental justice, the simplicity of the sex ratio metric is a means of empowering disadvantaged communities in their struggles against powerful interests.

CONCLUSIONS

In both animals and humans, changes in sex ratio are associated with certain pathological conditions known to be at least in part due to endocrine disruption. Observations based on animal experiments and human epidemiology indicate that the sex ratio of a population is a valuable indicator of endocrine disruption. Most effects of endocrine disruption, such as testicular cancer, hypospadias or cryptorchidism, are rare events. In contrast, the births of boys and girls are routine daily occurrences. The calculation of the sex ratio is a useful parameter in epidemiological studies at scales ranging

from the local to national, and is a useful tool for studying the effects of long-term, low level, and long-past exposures to endocrine disruptors.[50]

In light of these observations, it is important for population health researchers to conduct both routine surveillance and specific studies using the following three approaches. First, systematic examination of temporal and geospatial patterns of sex ratio and subtle birth defects of reproductive tract should be routinely carried out by national health statistical agencies. Second, more detailed evaluations of sex ratio should be carried out in populations with unusual workplace or environmental exposures. Third, more sophisticated toxicological investigations should examine the effect on sex ratio of low levels of widely used pollutants. Changes in sex ratio are matters that merit serious consideration, whether or not some as yet unexplained environmental factors are contributing to these patterns. The capacity of the human species to sustain itself partly depends on the detection of sentinel health events operating at the community and population levels. A change in the normal sex ratio within a community or population appears to be a simple and reliable metric for detecting threats to human sustainability.

Notes

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