Exposure to persistent organochlorine pollutants associates with human sperm Y:X chromosome ratio

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BACKGROUND: During the last decades, there has been concern that exposure to endocrine disruptors, such as persistent organochlorine pollutants (POPs), may contribute to sex ratio changes in offspring of exposed populations. METHODS: To investigate whether exposure to 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and dichlorodiphenyl dichloroethene (p,p′-DDE) affect Y:X chromosome proportion, semen of 149 Swedish fishermen, aged 27–67 years, was investigated. The men provided semen and blood for analysis of hormone, CB-153 and p,p′-DDE levels. The proportion of Y- and X-chromosome bearing sperm in semen samples was determined by two-colour fluorescence in situ hybridization (FISH) analysis. RESULTS: Log transformed CB-153 as well as log transformed p,p′-DDE variables were both significantly positively associated with Y chromosome fractions (P-values = 0.05 and <0.001, respectively). Neither age, smoking nor hormone levels showed any association with Y-chromosome fractions. CONCLUSIONS: This is the first study to indicate that exposure to POPs may increase the proportion of ejaculated Y-bearing spermatozoa. These data add to the growing body of evidence that exposure to POPs may alter the offspring sex ratio.

Key words: polychlorinated biphenyls/POP/p,p′-DDE/sex ratio/sperm

Introduction

Worldwide, the human sex ratio at birth is fairly constant—the male proportion of all births being 51.4% (James, 1996a). However, recent studies indicate that in many countries the proportion of male births has been declining during the past five decades (Allan et al., 1997; van der Pal-de Bruin et al., 1997; Marcus et al., 1998; Møller, 1998; Parazzini et al., 1998). In order to explain this phenomenon several hypotheses have been put forward, including influence of parental hormone levels (James, 1996a, 2001), changes in coital rates (Dickinson and Parker, 1996), the length of inter-pregnancy intervals (James, 1996b), or changes in maternal nutrition (Rosenfeld et al., 2003). It has also been suggested that the time-related reduction in male proportion could be due to an increasing exposure to ‘endocrine disrupters’ such as persistent organohalogen pollutants (POPs) (Toppari et al., 1996).

POPs, such as polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethene (DDT), and dichlorodiphenyl dichloroethene (p,p′-DDE, the most stable daughter compound of DDT), are ubiquitous environmental contaminants. These compounds are highly persistent, which results in bioaccumulation and biomagnification in the food chain. Studies have shown that measurable levels of PCBs and p,p′-DDE are found in a large proportion of the general population (Longnecker et al., 1997). The half-lives of different PCB congeners in the blood range from 1 to 10 or more years, while p,p′-DDE has a half-life of ≈10 years (Brown et al., 2003). Some of these chemicals can disrupt multiple endocrine pathways and induce a wide range of toxic responses (Toppari et al., 1996). A variety of studies have demonstrated their estrogenic, anti-estrogenic, and androgen competing properties (Kelce et al., 1995; Danzo, 1997). Two accidents, which have attracted a lot of attention, are the Yucheng poisoning (Chen et al., 1985; Masuda et al., 1985) and the Seveso disaster (Mocarelli et al., 1996), both of which were associated with an increased proportion of girls born subsequently to paternal exposure to POPs (Mocarelli et al., 1996, 2000; del Rio Gomez et al., 2002). In human populations exposed to more moderate levels of POPs, both increased (Karmaus et al., 2002) and decreased (Rylander et al., 1995) male: female sex ratios have been reported. Therefore, the explanation of the secular trend in sex ratio is still lacking and the mechanisms that can affect the proportion of males to females are not yet understood.

Theoretically, offspring sex ratio determination may be attributed to events that occur before fertilization that favor selection of Y- or X-chromosome bearing spermatozoa,
events that occur after fertilization such as preferential development or survival of embryos of one sex, or a combination of both. In case the paternal exposure is crucial, as supported by all studies to date (Mocarelli et al., 1996; Mocarelli et al., 2000; del Rio Gomez et al., 2002), changes in male germ cell development or function could be causative of the sex ratio changes observed. Although recent human studies have indicated that paternal exposure to POPs has a deleterious effect on sperm parameters (Guo et al., 2000; Hauser et al., 2003; Richthoff et al., 2003) it is not yet known whether these compounds could change the proportion of X- and Y-bearing sperms.

In Sweden the main exposure route for POP is through consumption of contaminated fatty fish from the Baltic Sea of the eastern coast of Sweden (Svensson et al., 1995; Rylander and Hagmar, 1999). Swedish fishermen constitute a socio-economically homogeneous group with high fish consumption and previously, east as well as west coast fishermen have been found to eat on average more than twice as much locally caught fatty fish than subjects from the general Swedish population. This has resulted in increased POP levels in plasma among east coast fishermen compared to Swedish population. This has resulted in increased POP levels in plasma among east coast fishermen compared to west coast fishermen as well as the general population (Svensson et al., 1995). In the current study, east coast fishermen constituted ‘the more exposed group’ and west coast fishermen ‘the less exposed group’. The choice of study base ensured us of sufficient variation in POP exposure.

Reliable biomarkers of POP exposure are necessary to establish dose–response relationships. The PCB congener, 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153), found in relatively high concentrations in human serum, was selected as a biomarker for POP exposure due to its very high correlations with the total PCB concentration (Grimvall et al., 1997; Glynn et al., 2000), the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent (TEQ) from PCB, and the total POP derived TEQ (Gladen et al., 1999), respectively. Another relevant biomarker is the anti-androgen compound p,p′-DDE, which is present in relatively high serum concentrations in men consuming fatty fish from the Baltic Sea (Sjödin et al., 2000).

In order to enlighten the mechanism behind possible link between POP exposure and offspring sex ratio, we aimed to investigate whether there is an association between serum levels of CB-153 and p,p′-DDE and the Y:X chromosome ratio in ejaculated sperms of Swedish fishermen.

Materials and methods

Study population

Cohorts of fishermen from the Swedish east and west coasts were established in 1988 (Svensson et al., 1995). In year 2000, a postal questionnaire, mainly focused on fracture incidence, was sent to 3505 west coast fishermen and 1678 east coast fishermen from the original cohorts, born 1935 or later. The questionnaire included a question about whether the subjects were interested in more information on a study of male semen function. Among the 2614 subjects (east $n = 848$ and west $n = 1766$) who responded to this specific question, 479 (east $n = 171$ and west $n = 308$) wanted more information about the semen study. We contacted these subjects as well as another 169 east coast fishermen that had become members of the east coast fishermen’s organization after the closure of the cohorts, with written detailed information. From the east coast, 130 out of 340 men wanted to participate, and from the west coast 136 out of 308. During the field study period, 71 subjects were excluded due to logistical reasons, changes of mind, sickness or recent vasectomy.

The non-participants from the original fishermen’s cohort had similar age distribution as the participants (Rignell-Hydbröm et al., 2004). Circumstantial evidence based on data from the Swedish Medical Birth Register supports that there was no difference in number of fathered children between participants and non-participants.

Out of 195 men who participated in the semen study, 183 donated enough semen for FISH analyses. Out of these 183, 28 samples were excluded due to low number of cells available or failure during analysis. No statistically significant differences regarding age, lipid-adjusted levels of CB-153 and p,p′-DDE, percentage of A + B motile sperms, sperm concentration or total sperm count were found between the 155 participating men with enough semen and the 28 subjects who were excluded due to failure of FISH (21 subjects with hybridization failure and seven with low sperm number). Exposure data were lacking in six men; the results are thus based on 149 subjects.

Mobile laboratory unit, semen and blood sampling, and questionnaire

A mobile laboratory unit was established for collection and analysis of semen and blood samples (Rignell-Hydbröm et al., 2004). The subjects were informed to keep an abstinence period of 3–4 days before collection and in each case the actual length of abstinence period was recorded (median 3.0, range 0.5–15). Sperm motility and sperm concentration were analysed within 1 h after collection according to World Health Organization guidelines (WHO, 1999). Undiluted raw semen was transferred into two tubes and directly put into a box with dry ice. The semen samples were, thereafter, stored in a freezer at –80°C until analysis. Venous blood samples were collected between 7 am and 9 pm. The samples were centrifuged and sera were frozen at –80°C for later analysis.

Information on lifestyle and reproductive history (e.g. number and sex of children) was collected through telephone interviews. The questionnaire was sent to the participants a couple of weeks before telephone contact. During the telephone contact, an agreement was reached on time and date for collection of semen and blood samples at the subject’s home. The participants received verbal and written information on the procedures for collecting the semen samples.

The fraction of current smokers was 21% ($n = 31$), and of former smokers 42% ($n = 62$) among the participants. Other background characteristics of the study population are given in Table 1. Sperm characteristics of this population have previously been reported (Rignell-Hydbröm et al., 2004). No men presented with azoospermia. Sperm concentration varied between 5.7 and 207 × 10^9/ml (median: 50.1 × 10^9/ml).

Preparation of spermatozoon nuclei

After thawing and mixing, 10 μl of semen was smeared on cleaned microscope slides (Superfrost Plus slides, Menzel Gläser, Germany). The smear was air-dried at room temperature for 24 h. Sperm nuclear decondensation was performed by incubation in 10 mM dithiothreitol (DTT; Saveen Werner AB, Sweden)/0.1 M Tris for 7 min, followed by incubation in 1 mM DTT/4 mM lithium diiodosalicylate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany)/0.1 M Tris for 20 min.
Slides were then washed in 2 × saline sodium citrate (SSC) and air-dried.

**Two-colour fluorescence in situ hybridization (FISH)**

Prior to hybridization, the slides were incubated for 1 min in a pre-treatment solution containing 85 μg/ml pepsin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in 0.01 M HCl, rinsed in TN buffer (0.1 M Tris–HCl, 0.15 M NaCl) for 15 min, and air-dried. To determine the proportion of X and Y chromosomes, two-colour FISH was performed using protein–nucleic acid (PNA) probes (provided by DakoCytomation, Glostrup, Denmark) targeted against the centromeric region of the X chromosome (Rhodamine-labelled) and the q-arm of the Y chromosome (FITC-labelled). The probe mixture was placed on the semen smears within a marked area of a slide, mounted with a clean cover slip and sealed with rubber cement. Subsequently, probe- and target DNA were denaturated simultaneously at 73 °C for 2 min. Following overnight hybridization at 37 °C in a humid chamber, slides were washed twice, 5 min each, at 36 °C in 60% formamide/2 × SSC, in 0.2 × SSC for 5 min at room temperature, and 5 min in TN buffer/0.05% Tween 20. Slides were rinsed in 2 × SSC, and dehydrated in ethanol series (70–95%). Thereafter, the slides were counterstained with 0.1 μg/ml of 4',6-diamidino-2-phenylindole (DAPI) for 30 s, and dehydrated. Finally, the slides were mounted in Vectashield antifade medium (Vector Laboratories Inc., Burlingame, USA).

**Microscopy and scoring criteria**

Slides were examined with an Olympus AX 70 epifluorescence microscope (magnification: 400×) equipped with a single and double band pass filter to detect DAPI, FITC and Rhodamine. The examination was performed blindly, i.e. without knowledge of the exposure levels or other subject characteristics. An X or Y chromosome in a sperm nucleus was recognized by a red or a green fluorescent spot, respectively. Sperm nuclei were only scored when some in a sperm nucleus was recognized by a red or a green fluorochrome, respectively. Sperm nuclei were only scored when an a priori assumption was that an acceptable inter- and intra-observer CV could be achieved with scoring of a lower number of spermatozoa. In the first part of the study, two investigators assessed inter-observer variation by counting ~500 and ~1000 spermatozoa in each of 29 slides. Thereafter, one of the investigators (T.T.) assessed intra-observer variation in 10 slides twice by counting 500 and 1000 cells. Proportions of Y-chromosome bearing spermatozoa from these assessments are based on counts of 1000 cells (median 1042; range 881–1301). Thereafter, inter- and intra-observer CV as considers the proportion of Y-chromosome bearing sperm, was estimated to be 2.3% and 3.3%, respectively, by scoring 500 cells only and this procedure was subsequently applied. However, due to the quality of the samples in six cases the number of nuclei scored was below 500. The proportion of spermatozoa with Y chromosome did not differ between these samples and the remaining 149.

**Hormone analyses**

Serum concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol were analysed with immunofluorometric techniques. The total assay CVs were 2.9%, 2.6% and 8.1%, respectively. Serum testosterone and sex hormone-binding globulin (SHBG) were measured by commercially available immunoassays. The total assay CVs were 5.5% and 4.6%, respectively. Inhibin B levels were assessed using a specific immunometric method, as previously described (Groome et al., 1996), with a detection limit of 15 ng/l and intra-assay and total assay CVs <7%.

**Determination of CB-153 and p,p’-DDE**

The levels of CB-153 were determined as previously described (Richthoff et al., 2003). In addition, p,p’-DDE was analysed by the same method. Briefly, the CB-153 and p,p’-DDE were extracted from the serum by solid phase extraction (Isolute ENV+; IST, Henggoed, UK) using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry. 13C-labelled CB-153 and 13C-labelled p,p’-DDE were used as an internal standards. The selected ion monitoring of p,p’-DDE was performed at m/z 318 while m/z 330 was used for the internal standard. The relative standard deviations, calculated from samples analysed in duplicate at different days, was 7% at 0.6 ng/ml (n = 76) and 5% at 1.5 ng/ml (n = 37) for CB-153 and 12% at 0.6 ng/ml (n = 56) and 7% at 2.4 ng/ml (n = 50) for p,p’-DDE. The detection limits were 0.05 ng/ml for CB-153 and 0.1 ng/ml for p,p’-DDE. The analyses of CB-153 and p,p’-DDE are part of the Round Robin inter-comparison program (Hans Drexler, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with analysis results within the reference limits.

**Determination of lipids by enzymatic methods**

Serum concentrations of triglycerides, cholesterol and phospholipids were determined by enzymatic methods using reagents from Boehringer–Mannheim (triglycerides and cholesterol; Mannheim, Germany) and Waco Chemicals (phospholipids; Neuss, Germany).
The total lipid concentration in serum was calculated by summation of the amounts of triglycerides, cholesterol and phospholipids. In these calculations, the average molecular weights of triglycerides and phospholipids were assumed to be 807 and 714. For cholesterol, we used an average molecular weight of 571, assuming that the proportion of free and esterified cholesterol in serum was 1:2.

**Statistical analysis**

In linear regression models, we evaluated the effect of the exposure variables CB-153 and p,p'-DDE serum concentrations, respectively, on the fraction of Y chromosomes. The CB-153 and p,p'-DDE variables were analysed as continuous (untransformed and log transformed) as well as categorized variables (into five equally sized groups). Due to the very high correlation between CB-153 and p,p'-DDE (r = 0.73) in the present dataset, both variables were not taken into the models simultaneously. Age (as continuous), current smoking (yes/no), abstinence time (0–2, >2–4, >4–6, and >6 days) and paternal sex hormone levels (testosterone, FSH, LH, SHBG, and testosterone/SHBG ratio, all as continuous variables), were suggested to affect offspring sex ratio (Hilsenrath et al., 1997; James, 2001; Fukuda et al., 2002), and were therefore considered as potential confounders. If these variables showed any association (P < 0.20) with the Y chromosome fraction they were included in the models, one at a time, together with the exposure variable. If the adjusted estimates differed by <15% from the crude estimate, only crude results are presented. The model assumption was checked by means of residual analysis. In addition, to ensure that linear associations were reasonable, scatter plots were evaluated for all bivariate comparisons.

**Results**

The log transformed lipid adjusted p,p'-DDE concentration was significantly (P < 0.001) associated with the Y chromosome fraction (slope [B] for \( \ln[p,p'-DDE] \) 0.66, 95% confidence interval [CI] 0.30, 1.01; Figure 1). According to the regression model this means that a p,p'-DDE concentration of 242 ng/g lipid (the median level) corresponds to a Y chromosome fraction of 51.2% and a p,p'-DDE concentration of 472 ng/g lipid (lower limit value for highest exposure category) corresponds to 51.7%. However, p,p'-DDE explains only 7.5% of the total variance for Y:X chromosome fraction.

The log transformed lipid adjusted CB-153 concentration was also significantly (P = 0.05) associated with the Y chromosome fraction (B for \( \ln[CB-153] \) 0.42, 95% confidence interval [CI] 0.01, 0.83; Figure 2). According to the regression model this means that a CB-153 concentration of 200 ng/g lipid (the median level) corresponds to a Y chromosome fraction of 51.3% and a CB-153 concentration of 328 ng/g lipid (lower limit value for highest exposure category) corresponds to 51.5%. However, CB-153 explains only 2.0% of the total variance for Y:X chromosome fraction.

For CB-153, and especially for p,p'-DDE, the log transformed variables better fulfilled model assumptions as compared with the untransformed ones. When the exposure variables were divided into five categories, subjects in the category with the lowest concentration of p,p'-DDE had a significantly lower Y chromosome fraction as compared with the category with the highest exposure (mean difference 1.6%, 95% CI 0.8, 2.5, P < 0.001; Figure 3). A corresponding comparison for CB-153 showed a tendency of a lower fraction of Y chromosomes among subjects within the lowest exposure category as compared with the subjects in the category with the highest exposure (mean difference 0.8%, 95% CI –0.1, 1.7, P = 0.07; Figure 4).

Neither smoking (P = 0.24) nor hormone parameters (all P-values >0.40) showed any association with the Y
chromosome fractions and the same was noted regarding age, irrespective of whether age was considered as a continuous ($P = 0.47$) or categorical ($P = 0.30$) variable with the cut-off points at median age. Hence, none of these parameters were included in the multivariate models. On the other hand, men with the longest abstinence period tended to have a somewhat lower proportion of Y-chromosome bearing sperms compared with the rest. When including abstinence period in the models, the effect estimates were, however, only changed by 4–10%.

The Y chromosome fractions did not differ ($P = 0.36$) between men who had fathered only/more boys ($n = 59$) as compared with men who had fathered only/more girls ($n = 37$).

**Discussion**

Our main finding was that a higher exposure to POPs, expressed as concentrations of CB-153 and p,p'-DDE in serum, was associated with a slightly higher proportion of Y-chromosome bearing sperms. Due to the strong correlation between the serum levels of the two POP biomarkers, we can, however, not assess whether the observed effect was linked to one or both of these exposure markers, or to any other correlated POP.

When evaluating the results of the current study, several potential biases need to be considered. It has been shown that the participation rate in sperm studies is related to both age and experienced fertility (Larsen et al., 1998). In the present study, the age-distributions as well as the mean number of children were very similar among the participants and the non-participants (Rignell-Hydbom et al., 2004). Therefore, we do not consider that selection bias is of major concern. Moreover, we believe that residual confounding is probably not an issue of great concern, as we have considered all potential confounders known to us in the analyses. However, we cannot exclude that imperfect measurements of the confounders have caused some residual confounding. Only having a single semen sample could be a limitation of this study. However, there are no data in the literature on possible temporal variation in chromosomal fractions. Even if such a phenomenon exists, this variability would most likely dilute and not magnify the associations found in the present study.

Another potential bias, which needs to be considered, was the lack of hybridization signal in up to 5% of spermatozoa. This could be attributed to insufficient hybridization. However, a hybridization efficiency of 95% or more is in good accordance with the hybridization efficiency reported by other groups (e.g. Martin et al., 1996; Johannisson et al., 2002). Since the proportion of unlabelled spermatozoa did not correlate to the proportion of those with Y chromosome, it seemed probable that cell-based hybridization failure shared equally between the Y bearing and X bearing cells, and hence no additional probe for any autosomal chromosome was applied. Moreover, the results of our study could hardly be influenced by sex chromosomal aneuploidies, since previous studies have shown that sex chromosomal aneuploidies are rare, occurring in <0.5% of cells (Martin et al., 1996). Twenty-eight cases were excluded due to insufficient labelling. However, there was no significant difference considering age, exposure level, and seminal parameters between subjects from whom FISH data were obtained and those excluded from the study.

To our knowledge, this is the first study to elucidate the impact of POP exposure on sex chromosome ratio in sperm and our results indicate that sperm Y:X ratio is influenced by exposure to POPs. The design of our study does not, however, allow us to clarify the mechanisms behind the observed association. One hypothesis could be that testicular apoptosis, which was reported to be sex hormone regulated (Billig et al.,...
1996), might also be controlled through the effect of POPs mediated via androgen or estrogen signalling, as POPs are able to act as sex hormone receptor agonists or antagonists (Kelce et al., 1995; Danzo, 1997). Furthermore, it was recently shown that the aryl hydrocarbon receptor (AHR) which mediates the toxic effect of dioxins and regulates apoptosis of germ cells was highly expressed in human testis (Schultz et al., 2003). However, it is not clear how or whether apoptosis might specifically change the ratio between Y- and X-chromosome bearing spermatids. Another possible mechanism could be a loss of the X chromosome through an organochlorine effect on formation of micronuclei during the process of meiosis. Organochlorines were shown to have an effect on micronucleation in somatic cells both \textit{in vitro} and \textit{in vivo} (Cicchetti et al., 1999; Gauthier et al., 1999; Lu et al., 2000), whereas a similar effect on meiotic cells has not yet been shown.

It is a reasonable hypothesis, as yet unproven however, that the Y:X chromosome sperm ratio will affect the male: female offspring ratio. There are several epidemiological studies evaluating the effect of different POPs on the sex ratio outcome, giving a rather ambiguous picture. Accidental high paternal TCDD exposure from the Seveso disaster significantly lowered the sex ratio among the offspring several years later (Mocarelli et al., 1995, 2000), but on the other hand more long-term but lower exposure levels to dioxins among US Vietnam veterans showed a tendency towards an increased proportion of boys fathered (Michalek et al., 1998). The Yucheng poisonings by PCB and PCDF lead to a decrease in the proportion of boys born, but only among offspring to men exposed before adulthood (del Rio Gomez et al., 2002), whereas on the other hand, there was among Michigan fish eaters an association between paternal serum PCB concentrations and increased odds ratio of fathering a boy (Karmaus et al., 2002). On a cohort basis, Baltic Sea fishermen’s families from the Swedish east coast had higher POP levels in blood than fishermen’s families from the west coast (Rylander and Hagmar 1995; Svensson et al., 1995). The proportion of boys was significantly lower among the offspring from the east coast cohort as compared to the west coast cohort; none of these figures differed, however, significantly from the sex ratio of the overall Swedish population (Rylander et al., 1995). It is possible that the inconsistent associations between POP exposure and sex ratio are due to different impact on the male reproductive system of low-level continuous and major, but short-term, exposures, respectively. Furthermore, apart from the dose and the duration of exposure, differing sex hormone mimicking effects of the various POPs, as shown \textit{in vitro}, might contribute to diverging \textit{in vivo} effects (Bønnefeld-Jørgensen et al., 2001). The relative composition of POP compounds has varied considerably between the different exposure situations where the sex ratio has been assessed. Our findings on positive correlation between the proportion of Y-bearing sperms and serum levels of \textit{p,p'-DDE} and CB-153 could be considered to be in concordance with the work of Karmaus et al. (2002), linking low-level continuous paternal POP exposure to offspring sex ratio.

In the current study, the Y chromosome fraction did not differ between the 59 men who fathered only/more boys as compared to those 37 men who fathered only/more girls. However, it should be kept in mind that the number of children of exposed fishermen in the study is small and even if there were a direct link between sperm Y:X ratio and sex ratio of the offspring, the statistical power would be insufficient for detecting any effect on sex ratio of the presently observed weak association between POP exposure and sperm Y:X ratio. Furthermore, the semen samples were collected several years after the birth of the children and it is unclear how stable Y:X ratios are over time.

In conclusion, our observed effects of a slightly higher Y:X sperm chromosome ratio among men with higher POP concentrations in serum do not give any circumstantial evidence in support for the hypothesis that the trend of declining sex ratio in several societies over past decades (Parazzini et al., 1998) has been due to increasing exposure to POP. However, the relation between paternal Y:X sperm chromosome ratio and offspring sex ratio may be quite complicated. Even factors like timing of exposure in sensitive periods like fetal life and puberty need to be taken into consideration. Elucidation of the intratesticular mechanisms affecting the distribution of Y and X sperm may not only add to our understanding of the biological mechanisms regulating the offspring sex ratio but also contribute to better understanding of the process of spermatogenesis.

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