Human live birth and sperm–sex ratios compared

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The human secondary sex ratio is compared with the percentage of Y-chromosome bearing spermatozoa in human semen. Live birth sex ratio is about 51.3%, whereas the overall percentage of Y-chromosome bearing spermatozoa in our study samples was 50.3%, i.e. 1% closer to the proportion expected by Mendelian segregation. The observed difference between live birth and sperm–sex ratios was significant (P < 0.0001). A possible effect of male age on the percentage Y-bearing spermatozoa was found to be non-significant.

Key words: fluorescence in-situ hybridization/secondary sex ratio/sperm–sex ratio

Introduction

The human secondary sex ratio (percentage of male live births) is slightly male biased (Cavalli-Sforza and Bodmer, 1971). This was probably first noted in the 17th century by Arbuthnott (Kendall and Placket, 1977), whose data on London allow an early estimate of 51.63% (SE 0.05%). Population statistics of the 20th century give estimates for Western countries fluctuating around 51.3%. There is little variation in the secondary sex ratio, and there seems to be little or no genetic variation for the sex ratio (Edwards, 1962; Maynard Smith, 1978). Mendelian segregation theoretically implies a sex ratio of 50%. What could be the cause of this small male bias of 1.3%? With the recent advances in separation of X- and Y-bearing spermatozoa by flow cytometry in mammals (Johnson, 1995) and humans in particular (MicroSort®, Fugger et al., 1998), more data on human semen are becoming available, allowing us to compare the ‘sperm–sex ratio’ (percentage of Y-bearing spermatozoa) with the live birth sex ratio, and to test the hypothesis that a sperm–sex ratio bias underlies a live birth sex ratio bias. Many factors have been postulated to affect the human sex ratio (James, 1987), among which we find parental age and child birth order. The sex ratio has been reported to decrease slightly with increasing parental age and increasing rank number of the child (Garfinkel and Selvin, 1976; Ruder, 1985). Multiparity has also been supposed to affect the sex ratio (Juntunen et al., 1997), though another study found no significant effects (Almagor et al., 1998). Paternal age, maternal age and birth order are all associated, making it difficult to assess their independent effects. Some large scale population studies consider birth order and paternal age to be important factors (Garfinkel and Selvin, 1976; Ruder, 1985). No association between sex ratio and time of insemination (Gray et al., 1998) has been reported. If there is indeed such a paternal effect, then the decrease in sex ratio could be explained by a lower percentage of Y-bearing spermatozoa in older men. We therefore evaluated the effect of male age on the sperm–sex ratio.

Materials and methods

Estimates of live birth sex ratios were obtained from data provided by the national offices of population statistics from several European countries. Live birth data are usually compiled annually, thus allowing a very precise estimate of the secondary sex ratio. Percentages of Y-bearing spermatozoa were determined by FISH (fluorescence in-situ hybridization) studies of semen of men who accessed the Genetics and IVF Institute for the purpose of gender selection (Fugger et al., 1998). Briefly, sperm cells were washed by centrifugation, resuspended in a phosphate buffered saline and air dried on glass slides. The spermatozoa were subsequently treated with 50 mmol/l dithiothreitol and saline sodium citrate (SSC), and hybridized with X and Y specific alpha-satellite DNA probes (Vysis Inc., Downers Grove, IL, USA). Each sample was examined with a Nikon Optiphot-2 fluorescence microscope (Nikon Inc., Melville, New York, USA) equipped with an FITC/rhodamine filter. X-bearing spermatozoa were identified by the presence of a red signal and Y-bearing spermatozoa by the presence of a green signal.

All men were healthy and fertile, aged between 23 and 56 years (one outlying exception). The analysis was based on 176 males of Caucasian background only, as there were too few observations among other races. Semen samples were collected over the years 1994–1998, and observations were more or less uniformly spread over the 12 months in the year. About 200 spermatozoa from each man were analysed by FISH for their sex-chromosomal content (X or Y).

Results

Live birth sex ratios calculated from data aggregated over time give estimates of about 51.3% (e.g. Austria 51.39%, Belgium 51.27%, Denmark 51.38%, England and Wales 51.26%, France 51.18%, Germany 51.47%, Italy 51.35%, Netherlands 51.38%, Spain 51.44%, USA 51.38%; SE ≤0.02%). The combination of these data sets gives us a secondary sex ratio of 51.33%, which we use as a point estimate. Estimates of the sperm–sex ratio of 176 Caucasian men (repeated samples of the same...
man being aggregated), are shown in Figure 1, where dots indicate the 95% confidence limits. The confidence intervals (CI) always contain the Mendelian proportion and practically always (two exceptions) the live birth sex ratio, 51.3%. For an individual man, the sample size is too small to allow an appreciation of small bias from Mendel or the live birth sex ratio. However, the pooled data of all men provides us with a more precise estimate: 50.31% (95% CI: 49.86–50.76) which differs significantly from the live birth sex ratio (P = 5.5 × 10^-6), but not from the Mendelian ratio. Computer-intensive methods confirm this result: bootstrap resampling (Noreen, 1989) with 2000 samples also gives a CI for the overall sperm-sex ratio excluding the live birth sex ratio: 50.31% (95% CI: 50.00–50.61), where the 2.5 and 97.5 percentiles of the bootstrap distribution are used to construct this interval.

In order to assess a possible effect of male age on sperm-sex ratio, we regressed sperm-sex ratio on age, and found the slope to be insignificant [a = 0.498 (t = 52.66, P = 0.00), b = 0.0001 (t = 0.51, P = 0.61)]. We performed weighted regression, weights being total sperm counts. The regression line is shown in Figure 2. We omitted one outlier exerting high leverage on the slope of the regression line [estimates including the outlier: (a = 0.499, P = 0.00, b = 0.00008, P = 0.72)].

Discussion

Our data suggest that the male bias observed in live births cannot be ascribed to any systematic semen sex ratio bias. Uterine environment factors may possibly play a role in sex ratio bias and include differential motility or survival of X and Y spermatozoa in utero, differential survival of male and female embryos or fetuses, or a combination of these factors. A developmental synchrony between uterus and faster developing male blastocysts has been postulated to cause male bias at birth in mammals (Krackow, 1995). Other findings also suggest biased sex ratios arise in utero: still births have a higher sex ratio than live births, e.g. Austria 54.27% (SE 0.23), Netherlands 53.87% (SE 0.14) and Spain 57.80% (SE 0.24). The high reported rates of spontaneous abortion (Boklage, 1990; Forbes, 1997) also show that prenatal mortality is important from a quantitative point of view, and suggest that changes in mortality in the womb might affect the sex ratio observed at birth. Our finding is also corroborated by a FISH study (Chevret et al., 1995) where few men were sampled, but a large number of cells scored. The pooled X and Y counts from their study give a sperm-sex ratio of 49.67%, and also differs significantly from the live birth sex ratio at the 5% level.

As for the effect of age, our data failed to reveal a relationship between male age and semen sex ratio. Observed effects of paternal age on live birth sex ratio are based on population studies with enormous sample size, and effects on sex ratio were of a tiny order of magnitude (Garfinkel and Selvin, 1976; Ruder, 1985). Our own sample size is probably too small to detect such tiny effects, if present, in the sperm-sex ratio.

Acknowledgements

This work was partially supported by the Spanish DGICYT grant PB96-0300.

References


Figure 1. Sperm sex ratio of Caucasian males. Percentages of Y-bearing spermatozoa (open circles) are plotted for 176 men. Dots indicate 95% confidence limits. Mendelian (solid line) and live birth (dashed line) sex ratios are indicated for reference.

Figure 2. Sperm sex ratio versus age. Weighted regression of sperm-sex ratio on age. Dot size roughly proportional to total sperm count. Slope is insignificant (b = 0.0001, SE 0.0002, P = 0.611, R^2 = 0.00015).


Received on May 10, 1999; accepted on August 24, 1999