Sex ratio associated with timing of insemination and length of the follicular phase in planned and unplanned pregnancies during use of natural family planning

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This was a multicentred, prospective study of pregnancies among women using natural family planning. The women maintained natural family planning charts of the conception cycle, recording acts of intercourse and signs of ovulation (cervical mucus changes, including peak day and basal body temperature). Charts were used to assess the most probable day of insemination relative to the day of ovulation and length of the follicular phase of the cycle. The sex ratio (males per 100 females) for 947 singleton births was 101.5, not significantly different from the expected value of 105. The sex ratio did not vary consistently or significantly with the estimated timing of insemination relative to the day of ovulation, with the estimated length of the follicular phase or with the planned or unplanned status of the pregnancy. Although these findings may be affected by imprecision of the data, the study suggests that manipulation of the timing of insemination during the cycle cannot be used to affect the sex of offspring.

Key words: follicular phase length/natural family planning/planned and unplanned pregnancies/sex ratio/timing of conception

Introduction

There has long been an interest in the determinants of the sex ratio at birth, in part to explain the universally observed excess of male over female births, and in part because of the interest of some parents in determining the sex of their progeny by manipulating the circumstances of conception (James, 1987; Ruegsegger and Jewelewicz, 1988; Chahnazarian, 1988). A recent review indicates that reliable sex selection is currently possible only by preimplantation diagnosis and transfer of embryos of a given sex, by prenatal diagnosis and abortion of undesired sex, or perhaps by sperm separation using flow cytometry combined with in-vitro fertilization (IVF; Ruegsegger and Jewelewicz, 1988). However, the cost and clinical nature of preimplantation diagnosis and IVF limits their use. There is thus a continued interest in the study of potential sex selection methods that are more under the control of couples, such as timing of intercourse during the menstrual cycle.

Several studies, predominantly of women using natural family planning to plan their pregnancies, have suggested that the sex ratio might vary with timing of conception relative to the day of ovulation. In these studies, the sex ratio generally showed a U-shaped association with fewer male conceptions (i.e. an excess of females) around the time of ovulation, but a predominance of males with both pre- and post-ovulatory conceptions (Guerrero, 1975; Harlap, 1979; Perez et al., 1987). Supporting this hypothesis is the observation that an excess of female births occurs when ovulation is induced using clomiphene citrate or gonadotrophin and the conception follows natural intercourse or artificial insemination (James, 1987; James, 1995). However, with IVF, ova are also exposed to drugs used for ovulation induction, yet the sex ratio at birth shows the expected predominance of males. Presumably this reflects the fact that spermatozoa are not exposed to the hormonal environment in the female genital tract (Kambic and Gray, 1989a).

In a cohort study of young women discontinuing contraception to begin a pregnancy, Weinberg et al. (1995) monitored hormonal status during the conception cycle using urinary radioimmunoassays of steroid and gonadotrophin hormones. Short follicular phases were associated with an excess of male births and longer follicular phases with an excess of female births. This finding has not yet been corroborated by other investigations. These authors reported no association between sex ratio and the timing of intercourse (Weinberg et al., 1995; Wilcox et al., 1995).

In view of the continued controversy over the determinants of sex ratio (Ruegsegger and Jewelewicz, 1988; Simpson, 1995), we used data from a multinational study of pregnancies among natural family planning users to explore further the association between timing of conception or follicular phase...
Materials and methods

All women who became pregnant while using natural family planning between 1987 and 1994 were identified in five centres: two located in Santiago, Chile, and one each in Bogota-Medellin, Colombia, Milan, Italy, and Washington DC, USA. Informed consent was obtained using a common consent form approved by each participating institution. Pregnancies among these natural family planning users were identified early, generally in the 5th week of gestation (3rd week of embryogenesis), and followed up to delivery. The sex ratio of 947 singleton live births was examined in relation to timing of the estimated day of conception relative to ovulation, by the estimated length of the follicular phase of the conception cycle, and by the planned/unplanned status of the pregnancies.

The following definitions were applied. The singleton sex ratio at birth was defined as the number of males per 100 females. The follicular phase length was defined as the number of days from onset of menses to the estimated day of ovulation (Weinberg et al., 1995) and the day of ovulation was estimated from the timing of the mucus peak or basal body temperature shift (Kambic and Gray, 1989b). The act of intercourse that most probably led to conception was determined by natural family planning chart reviewers who were blinded with respect to pregnancy outcome (Simpson et al., 1988; Gray et al., 1995). The time in days between the most probable insemination intercourse and the day of ovulation was used as an estimate of the interval of time the gametes remained exposed in the genital tract prior to fertilization. Pre-ovulatory inseminations reflect prolonged exposure of spermatozoa and post-ovulatory inseminations reflect prolonged exposure of ova (Simpson et al., 1988).

In this study, pregnancy intention was ascertained from information on the planning status derived from the woman’s statement at entry into the study and the natural family planning instructor’s assessment, after discussion with the woman and review of her natural family planning chart. Assignment of planning status was made without knowledge of pregnancy outcome. The definition of a planned pregnancy was one in which the natural family planning user stated her intention was to become pregnant. An unplanned pregnancy occurred when the user stated the couple both did not plan or desire a pregnancy, and were also using natural family planning for contraception. Interview information was obtained on sociodemographic characteristics, reproductive history and the circumstances of the index pregnancy. This latter information included the method of natural family planning used, the dates of the last menstrual period, and behaviour such as smoking and alcohol consumption.

Bivariate analysis and graphical displays were used for data manipulation. Stratified analyses were used to search for potential confounders or effect modifiers. Chi-square or Fisher’s exact tests, and $\chi^2$ tests for linear trend in proportions were used to assess statistical significance (Armitage and Berry, 1987).

Results

In the 947 singleton births there were 477 boys and 470 girls, yielding a sex ratio 101.5 males per 100 females. This was not significantly different from the expected sex ratio of 105 males per 100 females ($\chi^2 = 1.37, P = 0.24$) (Chahnazarian, 1988; Ruegsegger and Jewelwicz, 1988).

Table I shows the sex ratio by timing of insemination relative to the day of ovulation. Overall, there was no consistent pattern in the sex ratio for all pregnancies. The sex ratio was 108.8 among 474 unplanned pregnancies, compared to 94.6 in 467 planned pregnancies; however, this difference was not statistically significant. Among unplanned pregnancies, the sex ratio was lower in pre-ovulatory inseminations estimated to have occurred 2 or more days before ovulation, compared with conceptions occurring around the time of ovulation or during the post-ovulatory period. In contrast, among planned pregnancies, we observed no difference in sex ratio associated with timing of conception (Table I). We also examined subgroups by maternal age and parity, but we did not detect any consistent association between timing of insemination and sex ratio in these stratified tabulations (results not shown).

There was no significant association between the estimated follicular phase length and sex ratio (Table II). The mean follicular phase length did not vary with gender of the infant: it was 17.50 days ($\pm$ SE 0.249) for boys and 17.47 ($\pm$ SE 0.268) for girls. Among women with unplanned pregnancies, the sex ratio was highest in those with short estimated follicular phase lengths ($\leq$13 days); however, this was not significantly different from the sex ratio with longer follicular phase cycles.

Discussion

The overall sex ratio in this natural family planning population was 101.5 males per 100 females, a ratio slightly less than the expected range of 104–107 males per 100 females (Chahnazarian, 1988; Ruegsegger and Jewelwicz, 1988), although this difference was not statistically significant. Our results thus do not replicate findings from some natural family planning-based studies which report an excess of male births (Guerrero, 1975; Perez et al., 1985; France et al., 1992). We also observed no association between timing of insemination and sex ratio as has been suggested by some investigations (Guerrero, 1975; Harlap, 1979; Perez et al., 1985; France et al., 1992), nor did we find evidence to support the hypothesis that pregnancies probably conceived around the time of ovulation result in a predominance of female births (James, 1987; James, 1995). Our findings are, however, consistent with a number of previous investigations. Weinberg et al. (1995) conducted a prospective study of 221 women who were planning a pregnancy. Daily urine samples were used for hormonal detection of ovulation and diaries were used to record acts of intercourse. Despite such careful measurements, the investigators observed no association between the sex ratio of offspring and timing of intercourse relative to ovulation (Weinberg et al., 1995). Also, a WHO study of women who experienced method failures during natural family planning found no effect of the timing of insemination intercourse on sex ratio, and no deficit of male infants conceived by inseminations around time of ovulation (World Health Organization, 1984).

Weinberg et al. (1995) also reported an excess of males among conceptions after shorter follicular phase length cycles. They found a significantly shorter follicular phase for conception cycles leading to the birth of boys (15.4 days) as compared...
Sex ratio in natural family planning users

Table I. Sex ratio by timing of insemination relative to ovulation and planning status of the pregnancy

<table>
<thead>
<tr>
<th>Timing of insemination (days)</th>
<th>Unplanned pregnancies</th>
<th>Planned pregnancies</th>
<th>Total pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex ratio M/F</td>
<td>(M/F * 100)</td>
<td>Sex ratio M/F</td>
</tr>
<tr>
<td>≤5</td>
<td>89/87</td>
<td>102.3</td>
<td>12/8</td>
</tr>
<tr>
<td>−2 to −4</td>
<td>52/67</td>
<td>77.6</td>
<td>41/45</td>
</tr>
<tr>
<td>−1</td>
<td>26/16</td>
<td>162.5</td>
<td>66/58</td>
</tr>
<tr>
<td>0</td>
<td>23/16</td>
<td>143.8</td>
<td>87/104</td>
</tr>
<tr>
<td>+1</td>
<td>17/12</td>
<td>141.7</td>
<td>15/18</td>
</tr>
<tr>
<td>≥2</td>
<td>40/29</td>
<td>137.9</td>
<td>6/7</td>
</tr>
<tr>
<td>Total</td>
<td>247/227</td>
<td>108.6</td>
<td>227/240</td>
</tr>
</tbody>
</table>

a The number of days from the most probable insemination intercourse to probable day of ovulation (day 0).

M/F = Number of males per 100 females. There were 6 pregnancies of indeterminant planning status.

Table II. Sex ratio by estimated follicular phase length and planning status of pregnancy

<table>
<thead>
<tr>
<th>Follicular phase length (days)</th>
<th>Unplanned pregnancies</th>
<th>Planned pregnancies</th>
<th>Total pregnancies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sex ratio M/F</td>
<td>(M/F * 100)</td>
<td>Sex ratio M/F</td>
</tr>
<tr>
<td>≤11</td>
<td>20/12</td>
<td>166.7</td>
<td>7/8</td>
</tr>
<tr>
<td>12–13</td>
<td>30/25</td>
<td>120.0</td>
<td>27/35</td>
</tr>
<tr>
<td>14–15</td>
<td>51/51</td>
<td>100.0</td>
<td>63/47</td>
</tr>
<tr>
<td>16–17</td>
<td>51/50</td>
<td>102.0</td>
<td>42/67</td>
</tr>
<tr>
<td>18–19</td>
<td>35/37</td>
<td>94.6</td>
<td>35/30</td>
</tr>
<tr>
<td>20–21</td>
<td>27/19</td>
<td>142.1</td>
<td>21/26</td>
</tr>
<tr>
<td>22+</td>
<td>34/35</td>
<td>97.1</td>
<td>34/28</td>
</tr>
<tr>
<td>Total</td>
<td>248/229</td>
<td>108.3</td>
<td>229/241</td>
</tr>
</tbody>
</table>

M/F = Number of males per 100 females.

to conception cycles leading to the birth of girls (17.6 days). However, our results suggest no consistent association between follicular phase length and sex ratio (Table II), nor did we observe a differential in the mean length of the follicular phase preceding male or female conceptions. In this regard, our results are compatible with data from a Japanese Study (James, 1997) which found no difference in mean cycle length between women who conceived male or female infants. However, aggregate data were used in the Japanese Study and therefore it was not possible to examine the conception cycle per se. Because all the pregnancies studied by Weinberg et al. (1995) were planned, we also examined the sex ratio for planned births in our data, but still observed no association between follicular phase length and sex ratio (Table II).

Our failure to replicate findings of some investigators might be due to random measurement error in our data, causing misclassification of the estimated day of insemination and/or day of ovulation. Such an error would tend to reduce our ability to detect differentials associated with timing of insemination or cycle characteristics. However, it must be noted that such random misclassification error would not only affect our data but also the data of other investigators. We acknowledge that the mucous peak or basal body temperature measurements are imprecise, proxy markers of ovulation, but these parameters have also been used by other investigators who reported an effect of timing of insemination on sex ratio (Guerrero, 1975; Perez et al., 1985). We believe that the chart review procedures used in the present study, in which all chart interpretation was conducted by independent, blinded reviewers, were as rigorous or more rigorous than in other natural family planning investigations (Simpson et al., 1988; Kambic and Gray, 1989b; Gray et al., 1995). Lack of rigour of measurement seems an unlikely explanation for our findings because some previous studies in which an effect on sex ratio was found, such as that by Harlap (1979), used cruder and more indirect measures to infer the probable timing of conception. In contrast, Weinberg et al. (1995) using more precise hormonal measures found no association between timing and sex ratio.

The reporting or recording of acts of intercourse can be problematic, and errors in this variable could affect our findings. However, we do not observe evidence of random error in the distribution of the most probable day of insemination. The distribution of estimated insemination days in the current study was very similar to the distributions of cycle days of probable conception reported in other natural family planning studies (Simpson et al., 1995; Royston, 1991), or in women monitored by hormonal measurements (Carson, 1988; Zarutskie, 1989; Simpson, 1995; Wilcox et al., 1995). Natural family planning users with unplanned pregnancies might be embarrassed to record fully the days on which intercourse occurred, because such acts of intercourse during the fertile phase of the cycle imply they breached the ‘rules’ of abstinence for use of natural family planning as a method of contraception. However, we cannot envisage a mechanism whereby such recording error might affect the sex ratio for planned pregnancies in our study, nor can we visualize why such errors of omission might affect...
our data but not data derived from other natural family planning populations.

The size of this study is a limiting factor, since there was a total of 947 singleton births, and the number of observations for subgroups stratified by estimated timing of insemination or luteal phase length is limited. As discussed by Moore and Gledhill (1988), the variance of the sex ratio is high, and the statistical power to detect a significant difference between two sex ratios is limited. Thus, with 947 births, approximately divided in two equal exposure groups, we would have around 80% power to detect a difference of ±20% in the sex ratio (Moore and Gledhill, 1988). However, sample size limitations cannot explain the conflict between our findings and those of other investigators, since the present study is one of the largest reported in the literature (Gray, 1991).

In summary, we find no consistent pattern in the sex ratio at birth associated with the length of the follicular phase or the timing of conception relative to day of ovulation, although subgroup analyses suggest some minor and statistically non-significant differences in the sex ratios of planned and unplanned pregnancies. Therefore, we conclude that manipulation of the timing of conception or characteristics of the menstrual cycle cannot be used to preselect the sex of offspring.

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References


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