The sex ratio of fetal deaths and its potential relation with the decline of sex ratio at birth in Greece.

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ABSTRACT: Many factors that affect teratogenesis and fetal death may vary from one region to another as well as in different time periods. Our aim was to identify trends in the sex ratio of intrauterine fetal deaths, and its relationship with the secondary sex ratio (SSR) observed in our country. We reviewed the files of 276 consecutive autopsies performed during the last 16 years. 189 cases were spontaneous abortions and fetal deaths (SAs/FDs); 128 cases in total presented congenital malformations, out of which 87 were pregnancies terminated deliberately due to birth defects detected during the prenatal screening. In the total number of cases studied, 60.6% were male and 39.4% female. In the SAs/FDs group 62.71% were male, and 37.29% female or male to female ratio: 1.68. In the congenital malformations group 59.3% were male and 40.7% females. In addition 71.1% of the cases with intrauterine growth retardation were male and 28.9% female. SSR in Greece shows a trend to decline from 1960 to 2007. This downward tendency in SSR is greater between 1980-2007, \( R^2 = 0.229 \), ANOVA \( p = 0.028 \) and even more significant between 1980-1990 \( R^2 = 0.452 \), ANOVA \( p = 0.023 \). In conclusion, we have found a significant male preponderance in both the spontaneously aborted fetus and the fetuses with congenital malformations and/or intrauterine growth retardation. We suggest that this finding may be related to the decline in SSR observed during the last decades in Greece. Similar trends have been recently recorded in U.S.A., Japan and other westernised countries, and have been attributed to greater prenatal vulnerability of the male fetus, especially at the earlier stages of gestation.

Key Words: Fetal death, Spontaneous abortion, Secondary sex ratio, Birth defects, Growth retardation.

INTRODUCTION

Secondary sex ratio (SSR) is defined as the sex ratio at birth, while primary sex ratio is the sex ratio at the time of conception. Moreover, tertiary sex ratio is the sex ratio in sexually active organisms and quaternary sex ratio is the sex ratio at the post-productive age. Reports from several countries and authorities show that there is great variation between different regions of the world, regarding all of these ratios for various reasons. In addition, it seems that during the last decades, at least in some industrialized countries, there is a decline in SSR\(^1,2\). Data from Greece (as will be discussed further below) show a similar trend in SSR\(^3\). At the same time, unfortunately, there is no official registry operating in Greece for recording fetal deaths and/or birth defects. Due to this fact it is not possible to relate directly the aforementioned trend with the sex ratio observed in fetal deaths and in pregnancies terminated due to congenital malformations detected at the prenatal screening. Still, we have undertaken this task, using sex ratio data from fetal autopsies we performed during the last two decades, in an attempt to investigate if there is any correlation with the declining trend of SSR in Greece.

MATERIAL AND METHODS

During the time period 1993-2009 the authors (D.M. & S.M.) performed 276 consecutive embryonal and fetal autopsies in total. 189 of these represented spontaneous abortions and fetal deaths, while the rest 87 were embryos and fetuses from pregnancies terminated due to birth defects detected during the routine prenatal
screening. We analyzed these autopsies retrospectively, retrieving data recorded in the original pathology reports. These data included the year the autopsy was performed, mother’s age, week of gestation, gender of embryo/fetus, fetal growth or retardation, presence of congenital malformations, and any other pathological findings in the placenta, umbilical cord or fetal membranes. We also analyzed data regarding the total number of live births in Greece during the time period 1960-2007, kindly provided by the National Statistics Agency of Greece (ESYE). These data also included the gender of these births for each year separately. The following statistical methods were used for analysis of our data. Quantitative variables such as sex distribution between the different age or weight groups were analyzed with the chi-square (X²) method. Kolmogorov-Smirnov test (K-S) was conducted to analyze the distribution of quantitative variables such as mother’s age. Finally, in order to analyze the variation of sex ratio at birth during the last 38 years in the general population we used the method of analysis of variance (ANOVA).

RESULTS

1. Observations In the total sample of autopsies. From the total number of 276 autopsies the sex could be identified in 264. Unidentified sex was either due to autolysis of the gonads or very small age of the embryo. 169 cases were male (60.6%) and 104 (39.4%) female. The embryos and fetuses were classified as having either normal growth or growth retardation. Data for fetal growth was recorded for 226/276 cases in the original reports. 71.1% of the cases with growth retardation were male and 28.9% female (Table 1). This difference between the two genders was statistically significant \[X^2 (1) = 8.050, p = 0.005\].

2. Spontaneous Abortions and Fetal Deaths (SAs/ FDs): This group of autopsies included 189 cases. The mothers of these embryos and fetuses ranged from 16 to 44 years old (mean = 31.72, SD = 4.778). The mothers’ age follows a normal distribution with K-S (Z) = 0.939 (p > 0.05, Figure 1). The age of embryos and fetuses ranged between 11 and 40 weeks of gestation (mean = 23.90, SD = 8.244). The age of embryos and fetuses does not follow a normal distribution, but presents two peaks that resemble a combination of two normal distributions. The first peak is around 20th week of gestation, starting approximately at 10th week and ending around 28th to 29th weeks. The second peak is around 35th week starting at 29th week, and ending at 40th week. The frequency histogram depicts in illustrative fashion the presence of a double distribution (Figure 2). From the total number of 189 SAs and FDs of our sample the vast majority were males: 62.71%
males, and 37.29% females or male to female ratio: 1.68. More specifically, 111 were males, 66 females, and 12 cases with unidentified gender.

In addition, we categorized further the cases of this group according to the age of gestation (group I < 28 weeks, and group II > 28 weeks), and according to their weight (group I: 0-499 gr, group II: 500-999 gr, and group III: >1000 gr). Out of the 177 embryos and fetuses with identifiable gender, the age of gestation could be retrieved in 168. The vast majority (71.2%) of males were found before the 28th week of gestation, while only 28.8% of male cases was seen after 28th week. The percentage of males before 28th week was 67.3%, while the percentage of females was 32.7%. On the contrary, the female embryos and fetuses presented a much more equal distribution between these two age groups, even though there were still more cases in the group that is smaller than 28 weeks. Hence, 56.2% of the females were < 28 weeks, and 43.8% over 28 weeks. The difference of gender distribution in these two age groups was statistically significant \( \chi^2 (1) = 3.893, p = 0.048 \).

Data regarding weight of SAs/FDs could be retrieved in 172 out of the 177 cases with identifiable gender. 64.2% of the male cases were weighting 0-499 gr, only 9.4% was weighting 500-999 gr, and 26.4% was weighing more than 1000 gr (Table 2). In contrast, female cases presented a much more equal distribution between these three weight groups (40.9%, 25.8%, and 44% respectively). The difference of gender distribution in the three weight groups also reached statistical significance \( \chi^2 (2) = 11.552, p = 0.003 \).

3. Embryos and Fetuses with Congenital Malformations. 128 cases in total presented congenital malformations, out of which 87 were pregnancies terminated deliberately due to birth defects detected during the prenatal screening. The age of the mothers of this group was available for 100 cases. It ranged between 20 and 44 years old (mean: 31.52, SD: 4.98), and followed a normal distribution with K-S (\( Z \)) = 0.701 (\( p > 0.05 \), Figure 3). Gender could be identified in 113 out of the 128 cases. 67 (59.3%) of them were males and 46 (40.7%) females.

4. Observations on sex ratio at birth in Greece. The number of total live births in Greece increased from 157,239 in 1960 to 162,839 in 1967, decreased to 137,526 in 1973, reached 148,134 in 1980, and then on gradually decreased again to 100,718 in 1996 (Figure 4). However, the number of total births in Greece slightly increased to 111,517 in 2007. At the same time period SSR was 1.077 in 1961, and fluctuated between 1.057 and 1.079 until 1985 (Figure 5). Later on SSR decreased to 1.052 in 1990, and 1.046 in 1996, its lowest value in the last 40 years. Sex ratio at birth shows a trend to decline from 1960 to 2007, but \( R^2 \) is 0.029 (ANOVA, \( p = 0.246 \)). However, there is a greater downward tendency in SSR between 1980-
Table 2. Fetal and Embryonal weight distribution in relation to sex.

<table>
<thead>
<tr>
<th>Weight Group</th>
<th>Count</th>
<th>male</th>
<th>female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-499g</td>
<td></td>
<td>68</td>
<td>27</td>
<td>95</td>
</tr>
<tr>
<td>% within Weight Group</td>
<td></td>
<td>71.6%</td>
<td>28.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Sex (male/female)</td>
<td></td>
<td>64.2%</td>
<td>40.9%</td>
<td>55.2%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td>39.5%</td>
<td>15.7%</td>
<td>55.2%</td>
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<tr>
<td>500-999g</td>
<td></td>
<td>10</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>% within Weight Group</td>
<td></td>
<td>37.0%</td>
<td>63.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Sex (male/female)</td>
<td></td>
<td>9.4%</td>
<td>25.8%</td>
<td>15.7%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td>5.8%</td>
<td>9.9%</td>
<td>15.7%</td>
</tr>
<tr>
<td>1000g +</td>
<td></td>
<td>28</td>
<td>22</td>
<td>50</td>
</tr>
<tr>
<td>% within Weight Group</td>
<td></td>
<td>56.0%</td>
<td>44.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Sex (male/female)</td>
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<td>26.4%</td>
<td>33.3%</td>
<td>29.1%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td>16.3%</td>
<td>12.8%</td>
<td>29.1%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>106</td>
<td>66</td>
<td>172</td>
</tr>
<tr>
<td>% within Weight Group</td>
<td></td>
<td>61.6%</td>
<td>38.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% within Sex (male/female)</td>
<td></td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>% of Total</td>
<td></td>
<td>61.6%</td>
<td>38.4%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 3. Distribution of mother’s age in the congenital malformation group.

Figure 4. Total liveborn births, and male and female births in Greece between 1960-2007.
The expected ratio of male to female births worldwide is generally considered to be 1.05 or otherwise described as male proportion 0.515. Variations of SSR from the common values are thought to be the effects of anomalies in or of the mother’s world around the time of conception, on the order of war, famine, plague and pollution. In addition, reduction from the customary excess of males at birth is taken as a sign of negative influence. Decline in sex ratio at birth has been recently reported in the United States, Japan, Brazil, Mexico, Netherlands, Denmark, and Portugal. On the contrary, in some regions of the world such as Spain, Italy, and Australia an increasing male proportion has been recorded. So it seems that there is no consistent worldwide trend in the sex ratios at birth over the last four decades.

The data from Greece we present in this study, as was also previously reported by Alexopoulos and Alammanos, show that there is a sharp decline of birth rate during the last five decades, while at the same time and especially between 1980 and 2000 there is also a decline of SSR. The birth rate in Greece seems to remain stable during the ’90s, and after 2000 shows a tendency to increase, presumably due to the influx of immigrants after 1990. In contrast to the declining SSR in Greece, the sex ratio in our series of fetal autopsies presents a significant male preponderance in all different groups examined (spontaneous abortions, fetal deaths, pregnancies terminated due to birth defects detected at the prenatal screening). This inverse relation points to a pathogenetic association with the declining SSR, and implies an increased vulnerability of the male fetus to various toxic factors, expressed in the increased male ratio in fetal deaths and in fetuses with birth defects. This vulnerability seems to be more intense in the early fetal period (before 28th week of gestation), and in smaller fetuses (weighting less than 500 gr). In addition, fetuses with growth retardation were much more frequently male than female, a fact that strengthens the hypothesis of increased vulnerability of the male fetus. It is noteworthy that the occurrence of fetal death presented two frequency peaks in our material, one around 20th and one 35th week of gestation. CDC has reported similar distribution of fetal deaths for USA. No plausible explanation can be given for this fact according to the current knowledge.

In order to explain the declining SSR in various regions of the world, several hypotheses and explanations have been suggested in the literature. First of all, it should be clear that sex ratio at birth is not equal with the male to female ratio at the time of concep-
tion. For any single pregnancy, the SRY (sex-determining region Y) gene located on the Y chromosome determines the sex of a fertilized egg. Various endocrine disruptors are thought to affect primary sex ratio. Among them ovarian stimulation with clomiphene or gonadotropins result in more female offsprings, while women receiving diethylstilbestrol or men receiving methyltestosterone or mesterelone give birth to more male children. Men bearing the rheumatoid arthritis associated HLA-B15 have more female offsprings presumably due to lower testosterone levels. Several neoplasms and gynaecologic conditions may also behave as endocrine disruptors: women with premenopausal breast cancer are associated increased SSR, while patients with non-Hodgkin’s lymphoma, and testicular cancer with low SSR. Finally, zplacenta previa, fatty liver of pregnancy, toxemia, and dermatoses of pregnancy have been associated with more male offsprings, while placenta accreta, extraterine pregnancy, and hyperemesis gravidarum with more females.

After conception, the epigenetic environment also affects SSR. Boklage suggests that male embryogenesis is more efficient with females in excess among failures before clinical pregnancy, while there is an excess of male losses during fetal period. Generally speaking, factors affecting sex ratio at birth include parental age, birth order of child, maternal illness, parental hormone levels, stress, natural disaster and period Y) gene located on the Y chromosome determines the sex of a fertilized egg. Various endocrine disruptors are thought to affect primary sex ratio. Among them ovarian stimulation with clomiphene or gonadotropins result in more female offsprings, while women receiving diethylstilbestrol or men receiving methyltestosterone or mesterelone give birth to more male children. Men bearing the rheumatoid arthritis associated HLA-B15 have more female offsprings presumably due to lower testosterone levels. Several neoplasms and gynaecologic conditions may also behave as endocrine disruptors: women with premenopausal breast cancer are associated increased SSR, while patients with non-Hodgkin’s lymphoma, and testicular cancer with low SSR. Finally, zplacenta previa, fatty liver of pregnancy, toxemia, and dermatoses of pregnancy have been associated with more male offsprings, while placenta accreta, extraterine pregnancy, and hyperemesis gravidarum with more females.

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Several pathogenetic mechanisms have been proposed to explain the incidence of lower SSR. There are many clinical, biochemical, evolutionary and epidemiologic evidence that lower SSR is a result of increased death rate of male fetuses. In addition, lower sperm motility due to paternal exposure to various stress factors seems to offer advantage to X-sperms. Diminished frequency of sexual intercourse during periods of socioeconomic stress (like war, earthquake or deterioration of economic conditions) is also believed to have the same effect. Other factors that may affect SSR include race/ethnicity, time of conception during the menstrual cycle and temperature at the time of conception. For example, sex ratio at birth has declined significantly in U.S. whites, but not for African Americans, for whom sex ratio remains significantly lower than that of whites. Intercourse and conception early in the fertile period favors conception of a male embryo, while during the peak of LH secretion (ovulation) favors conception a female embryo. Catalano et al have suggested cold ambient temperature at the time of conception intercourse predicts lower SSR.

Another reason of decreasing sex ratio at birth is the increasing number of induced abortions due to detection of birth defects. Since most congenital malformations are more frequent in males than in females, an increasing availability of perinatal diagnosis would cause a decrease of male liveborns and, consequently, a decrease in male:female ratio. However, the availability of prenatal diagnosis is probably different in different parts of the world, and in any case the effect of prenatal diagnosis should not have affected the proportion of males before the early 1980s.

A role of ionizing radiation on sex ratio at birth has also been proposed. Ionizing radiation can produce lethal mutations on chromosome X. Some studies suggest that paternal radiation leads to increased sex ratio, while maternal radiation leads to the opposite effect. However, data from Hiroshima & Nagasaki nuclear bombings, and from nuclear plant workers do not support this view. Increased male proportion has been reported after the Chernobyl accident. In general the data from chronic or acute exposure to radiation has failed to provide unequivocal evidence for an effect of radiation on the sex ratio.

In conclusion, as in other westernized countries, a decline in SSR has been recorded in Greece during the last decades. Our results provide evidence that this trend is at least due to increased male rate of fetal deaths, especially in the early fetal period (<28 wks.). Factors acting at the pre-conception period may also be implicated. Literature points at several chemicals.
as well as socioeconomic stress as potential aetiologic factors of decreasing SSR. However, these investigations do not always provide clear-cut results on a cause and effect basis. More focused and intensive research is needed in this field, in small groups of population, exposed to specific substances, in order to understand better which materials may be involved in this sex imbalance process, and the underlying mechanisms of their action.

ΠΕΡΙΛΗΨΗ: Οι παράγοντες που προκαλούν τερατογένεση και εμβρυϊκό θάνατο μπορεί να ποικίλουν από περιοχή σε περιοχή, καθώς σε διαφορετικές χρονικές περιόδους. Σκοπός μας ήταν να εντοπίσουμε τάσεις στην αναλογία φύλου στους ενδομήτριους εμβρύους που εκτίθενται στις διαφορετικές συγκροτήματα. Επιπρόσθετα, ήταν βασικό να διαπιστωθούν διαφορές σε ταχύτητα εμφάνισης των συγκροτήματων ανωμαλιών. Στην ομάδα των ανωμαλιών, 59.3% των εμβρύων ήταν αρρενία και 40.7% θήλεα. Επιπρόσθετα, ήταν βασικό να διαπιστωθούν διαφορές σε ταχύτητα εμφάνισης των συγκροτήματων ανωμαλιών. Στην ομάδα των ανωμαλιών, 59.3% των εμβρύων ήταν αρρενία και 40.7% θήλεα. Επιπρόσθετα, ήταν βασικό να διαπιστωθούν διαφορές σε ταχύτητα εμφάνισης των συγκροτήματων ανωμαλιών. Στην ομάδα των ανωμαλιών, 59.3% των εμβρύων ήταν αρρενία και 40.7% θήλεα. Επιπρόσθετα, ήταν βασικό να διαπιστωθούν διαφορές σε ταχύτητα εμφάνισης των συγκροτήματων ανωμαλιών. Στην ομάδα των ανωμαλιών, 59.3% των εμβρύων ήταν αρρενία και 40.7% θήλεα. Επιπρόσθετα, ήταν βασικό να διαπιστωθούν διαφορές σε ταχύτητα εμφάνισης των συγκροτήματων ανωμαλιών.

Λέξεις Κλειδιά: Εμβρυϊκός θάνατος, Αυτόματη αποβολή, Δευτερογενής αναλογία φύλου, Συγγενείς ανωμαλίες, Καθυστέρηση της ανάπτυξης.

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