Brief Communication

Decline in sex ratio at birth after 10-day war in Slovenia

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BACKGROUND: We investigated whether the psychological stress related to a short war (26 June–7 July, 1991) in Slovenia induced changes in fertility, sex ratio at birth and semen quality characteristics. METHODS: Sex ratios [i.e. males/(males + females)] for 4966 births in the general population of Slovenia and separately for 1565 births in the Slovenian capital, Ljubljana, from January–March 1992 were compared with the sex ratio calculated for the same time period in 1991 and 1993. Semen analyses for 38 normozoospermic men attending an outpatient infertility clinic from May to September 1991 were also evaluated. RESULTS: In the general population in Slovenia there was a significant fall in the sex ratio at birth in 1992, compared with 1991 (0.504 versus 0.518; P = 0.03). In Ljubljana, the decline in sex ratio in 1992 was even more pronounced: 0.483 versus 0.537 in 1991 (P = 0.0001) and 0.483 versus 0.516 in 1993 (P = 0.005). A decrease in the proportion of sperm that were progressively motile from 56% before the war to 52% after it (P = 0.01) and of those that were rapidly motile from 40 to 36% (P = 0.01) was observed. CONCLUSIONS: Acute psychological stress in relation to a short war in Slovenia resulted 6 to 9 months later in a decrease in the observed sex ratio at birth. Negative changes in sperm motility may be involved in the sex ratio modifications.

Key words: decline in sex ratio at birth/psychological stress/reduced sperm motility/Slovenia/war

Introduction

Many factors have been identified as possibly affecting male/female ratio at birth. Today the exposure to endocrine disruptors or other environmental toxins at the time of sexual differentiation is thought to represent one important cause of the decrease in male births (Møller, 1996; Mocarelli et al., 2000). Psychological stress might also be associated with a decrease of sex ratio at birth (Hansen et al., 1999). On the other hand, after long-lasting wars an increase of sex ratio was observed (Graffelman and Hoekstra, 2000). A decrease of sex ratio may result from an increase of the male/female ratio of fetal deaths (Mizuno, 2000). It has also been hypothesized (Møller, 1996; James, 1997) that semen quality might be connected with lower sex ratio. It has been reported that the decrease of sex ratio observed after the Kobe earthquake might be mediated by a rapid decrease of sperm motility, a consequence of the acute stress resulting from the earthquake (Fukuda et al., 1998).

Between June 26 and July 7, 1991, Slovenia experienced a war, at the end of which the country became independent.

We report on the hypothetical relationship between acute psychological stress induced by war and modifications of fertility and sex ratio at birth in the general population in Slovenia and separately in the capital Ljubljana, 6 to 9 months after the war. Additionally, semen quality parameters in normozoospermic men attending our outpatient clinic were analysed.

Materials and methods

Data on fertility and sex ratio at birth

The data on fertility, concerning live and stillbirths in the general population in Slovenia and in the capital Ljubljana, were obtained from the Institute of Public Health of the Republic of Slovenia. Fertility was defined by the total number of births. The time period studied was the trimester January–March, 1992. We chose this time period because the children conceived during the 10-day war (between June 26 and July 7, 1991) would have been born at that time, i.e. at duration of pregnancy from 6 to 9 months.

The sex ratio in the studied time period was calculated as the male proportion, i.e. males/(males + females) in liveborn infants. It was compared with the sex ratio in 1991 and 1993. Since the latitude of Slovenia is between 45 and 46 degrees north, the sex ratio at birth, according to a previous publication (Grech et al., 2000), was expected to be between 0.516 and 0.513.

Patients

In 1991, 1185 semen analyses were performed for the first time in men from infertile couples attending our outpatient infertility clinic.
Among the 254 carried out from May to June, there were 38 normozoospermic patients in which the control semen analysis was performed in the period following the war till September 1991 (war group). For each patient, we considered two analyses: the first was performed before the war and the second after it. The inclusion criteria were as follows: age of 23–40 years, sexual abstinence of 3–5 days, normozoospermia according to WHO criteria (World Health Organization, 1987), sperm concentration ≥20×10^6/ml, total progressively motile ≥50%, rapidly motile ≥25% and normal morphology ≥50%.

In the control groups we recruited 30 normozoospermic individuals according to the above criteria with a semen analysis performed in the same time period in 1990 (group 1) and 1992 (group 2). The war group and the control group did not differ in mean age and sexual abstinence.

Semen analysis
In all semen samples seminal volume, sperm concentration, sperm count, total progressive motility, rapid progressive motility, normal morphology and viability were evaluated. The semen analysis techniques used have been described previously. For the war group and the control group, the same standard methods and criteria according to WHO were used by the same three well-trained technicians. Intra- and inter-individual variability were 11 and 17%, and 9 and 11% respectively, for sperm concentration and motility assessments (Zorn et al., 1999).

Statistical analysis
The SPSS (SPSS Inc., Illinois, USA, version 9) and S-Plus (S-Plus 2000, MathSoft Inc., Seattle, Washington, USA) software packages were used for statistical analyses. To evaluate the differences in population fertility and the sex ratio at birth, exact P-values were computed using the binomial distribution. For the evaluation of changes in sex ratio, we compared the numbers of male and female liveborn infants between January and March 1992 to those born in the same trimesters of 1991 and 1993 in the general population of Slovenia and separately in the capital Ljubljana. Moreover, changes in sex ratio throughout the period 1985–1999 were evaluated by Spearman’s test. The changes in sperm quality parameters were analysed by means of the Wilcoxon non-parametric test. Statistical significance was set at P < 0.05.

Results
The data concerning fertility, number of live- and stillbirths, sex ratios at birth in the general Slovene, and separately in the Ljubljana populations in the time period January–March, 1992 as compared with January–March, 1991 and 1993 are presented in Table I. In 1992, fertility declined compared with 1991, but this phenomenon was also observed in 1993 in comparison with 1992.

Among the three observed time periods (January–March 1991, 1992, 1993), we did not observe increased in utero mortality (i.e. stillbirths) in males.

During the time period January–March, 1985–1999, the mean sex ratio at birth in Ljubljana was 0.518. In Slovenia, it was 0.514 (Figure 1). Using the Spearman test, no significant changes in the sex ratio were found in the whole observed period (1985–1999). A significant decline in the sex ratio was observed only in the time period January–March, 1992, in both Slovenia and Ljubljana. The decline was more expressed in Ljubljana than in the whole of Slovenia. The obtained P-value was 0.0001 if the period January–March, 1991 was used for comparison, and 0.005 when compared with the period January–March, 1993. In Slovenia, the sex ratio at birth declined significantly in 1992 in comparison with 1991 (P = 0.03), but not in comparison with 1993 (P = 0.08).

In the war group, we found a significant fall in the total progressive motility (P = 0.01) and rapid progressive sperm motility (P = 0.01) after the war, whereas sperm concentration and morphology did not change (Table II).

In the control groups, there was no significant change of semen quality characteristics except for semen volume in 1990 (Table II).

Discussion
We found an association between acute psychological stress resulting from a short war and decrease in sex ratio at birth. The decrease was time-limited and not accompanied by a decrease in fertility. Yet there was no change in male mortality at birth. In normozoospermic individuals, there was a decrease in sperm motility after the war.

Because of the rather small number, the 38 men in the study group cannot be considered a representative sample of the general male population. However, they were recruited consecutively from infertile couples with a normal semen analysis, which makes the identification of changes in sperm characteristics easier; the control semen analysis was performed after a sufficiently long period to avoid identical stages of spermatogenesis. None of the males were lost from the assessment because of lack of adequate health insurance (in Slovenia both the diagnosis and treatment of infertility are covered by compulsory health insurance) or because of war.

War in Slovenia consisted mainly of psychological pressure with constant menace of a military attack. Although it is not excluded that minimal stress has positive effects on sperm characteristics (Poland et al., 1986), high stress is presumably linked with negative effects on sperm quality. Stress may increase prolactin (Gerhard et al., 1992) and decrease testosterone concentration (Francis, 1981), and may thereby interfere with spermatogenesis. In soldiers before imminent combat, a lower urinary excretion of testosterone, androsterone, and etiocholanolone than in the normal population has been observed (Rose et al., 1969). In 18 young soldiers in the Officer Candidate School, a significant suppression of plasma testosterone levels during the ‘early part of the course’ as compared with the relaxed period was reported (Kreuz et al., 1972). In prisoners awaiting sentencing, testicular biopsies revealed abnormal spermatogenesis (Stieve, 1952).

Psychological stress and low adaptability to stress are correlated negatively with semen volume and normal sperm morphology (Giblin et al., 1988). Stress associated with work place or family behaviour results in poorer sperm morphology and vitality (Gerhard et al., 1992). However, motility seems to be one of the most sensitive characteristics. In men providing semen samples for an infertility work-up, an inverse relation-ship has been found between the level of perceived job stress and percentage of progressive sperm (Bigelow et al., 1998).
### Table I. Fertility in the general populations of Slovenia and Ljubljana, livebirths, stillbirths and sex ratio at birth during the trimesters January–March 1991, 1992 and 1993

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</thead>
<tbody>
<tr>
<td>Slovenia</td>
<td>2823</td>
<td>2615</td>
<td>2509</td>
<td>2457</td>
<td>2538</td>
<td>2387</td>
</tr>
<tr>
<td>Livebirths</td>
<td></td>
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<tr>
<td>Stillbirths</td>
<td>14</td>
<td>23</td>
<td>18</td>
<td>22</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>0.518&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>0.504&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>0.514</td>
<td></td>
</tr>
<tr>
<td>Ljubljana region</td>
<td>918</td>
<td>791</td>
<td>757</td>
<td>808</td>
<td>795</td>
<td>737</td>
</tr>
<tr>
<td>Livebirths</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>Stillbirths</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sex ratio</td>
<td>0.537&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td>0.483&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>0.516&lt;sup&gt;e&lt;/sup&gt;</td>
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</table>

Statistically significant differences were obtained for the following comparisons: <sup>a</sup> versus <sup>b</sup> (P = 0.03), <sup>c</sup> versus <sup>d</sup> (P = 0.0001), and <sup>d</sup> versus <sup>e</sup> (P = 0.005).

### Figure 1. Male proportion among liveborn infants in Slovenia and the capital Ljubljana in the general population in the trimesters January–March 1985–1999.

### Table II. Semen quality characteristics in the war group and in the two control groups (two semen analyses obtained in the period May–September of each year)

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Abstinence (days)</th>
<th>Volume (ml)</th>
<th>Sperm conc. (10&lt;sup&gt;6&lt;/sup&gt;/ml)</th>
<th>Total progressive motility (%)</th>
<th>Rapid progressive motility (%)</th>
<th>Morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991 War Group</td>
<td>2001 Before war</td>
<td>Mean 29.4</td>
<td>3.9</td>
<td>3.4</td>
<td>75.4</td>
<td>55.8</td>
<td>39.6</td>
</tr>
<tr>
<td>(38 men)</td>
<td>Median 29.0</td>
<td>4.0</td>
<td>3.0</td>
<td>68.0</td>
<td>55.0</td>
<td>40.0</td>
<td>63.0</td>
</tr>
<tr>
<td></td>
<td>Mean as above</td>
<td>3.9</td>
<td>3.6</td>
<td>79.6</td>
<td>52.5</td>
<td>36.1</td>
<td>60.7</td>
</tr>
<tr>
<td></td>
<td>Median as above</td>
<td>4.0</td>
<td>3.8</td>
<td>79.5</td>
<td>52.5</td>
<td>35.0</td>
<td>63.0</td>
</tr>
<tr>
<td></td>
<td>Range as above</td>
<td>2–7</td>
<td>2–6.5</td>
<td>25–275</td>
<td>25–70</td>
<td>10–55</td>
<td>6–80</td>
</tr>
<tr>
<td></td>
<td>Difference</td>
<td></td>
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</tr>
<tr>
<td>1991 Control</td>
<td>1st testing</td>
<td>Mean 30.1</td>
<td>3.5</td>
<td>3.9</td>
<td>70.7</td>
<td>55.3</td>
<td>39.0</td>
</tr>
<tr>
<td>Group 1</td>
<td>Median 29.0</td>
<td>3.0</td>
<td>3.8</td>
<td>69.0</td>
<td>55.0</td>
<td>40.0</td>
<td>61.5</td>
</tr>
<tr>
<td></td>
<td>Mean as above</td>
<td>3.6</td>
<td>3.3</td>
<td>71.1</td>
<td>55.0</td>
<td>37.0</td>
<td>61.7</td>
</tr>
<tr>
<td></td>
<td>Median as above</td>
<td>4.0</td>
<td>3.1</td>
<td>71.0</td>
<td>55.0</td>
<td>37.5</td>
<td>62.0</td>
</tr>
<tr>
<td></td>
<td>Range as above</td>
<td>2–6</td>
<td>1.5–5</td>
<td>23–131</td>
<td>40–70</td>
<td>25–60</td>
<td>55–75</td>
</tr>
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<td></td>
<td>Difference</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1992 Control</td>
<td>1st testing</td>
<td>Mean 28.5</td>
<td>3.8</td>
<td>3.1</td>
<td>91.4</td>
<td>61.3</td>
<td>46.0</td>
</tr>
<tr>
<td>Group 2</td>
<td>Median 28.0</td>
<td>4.0</td>
<td>4.0</td>
<td>90.0</td>
<td>60.0</td>
<td>45.0</td>
<td>68.5</td>
</tr>
<tr>
<td>(30 men)</td>
<td>Range 23–36</td>
<td>2.5–5</td>
<td>1.8–4.5</td>
<td>16–260</td>
<td>40–75</td>
<td>30–60</td>
<td>41–80</td>
</tr>
<tr>
<td></td>
<td>Mean as above</td>
<td>3.3</td>
<td>2.8</td>
<td>82.6</td>
<td>60.0</td>
<td>44.3</td>
<td>65.3</td>
</tr>
<tr>
<td></td>
<td>Median as above</td>
<td>3.0</td>
<td>2.0</td>
<td>62.5</td>
<td>60.0</td>
<td>45.0</td>
<td>66.0</td>
</tr>
<tr>
<td></td>
<td>Range as above</td>
<td>2–5</td>
<td>1.5–5</td>
<td>18–320</td>
<td>50–75</td>
<td>25–60</td>
<td>50–86</td>
</tr>
<tr>
<td></td>
<td>Difference</td>
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</table>

S = difference in medians statistically significant at the 5% level as evaluated by the Wilcoxon signed-ranks test.
In men involved in IVF, anxiety and perceived importance of producing a semen sample are related to impaired sperm motility. Sperm motility decreased significantly at the time of oocyte retrieval compared with pre-IVF baseline values (Clarke et al., 1999). In a retrospective study of the semen of 2343 healthy Slovene men (Zorn et al., 1999), semen volume, sperm concentration, sperm count and total sperm motility did not change between 1983 and 1996, whereas between 1988 and 1996 rapid progressive sperm motility decreased by 0.95% per year on average. This could be related to stress or negative environmental factors in this time period. It has been observed that a recent death of a close family member was associated with a reduction in straight-line velocity and percentage of progressively motile sperm (Fenster et al., 1997). The catastrophic earthquake of Kobe was related to a reduction of environmental factors in this time period. It has been observed that a recent death of a close family member was associated with a reduction in straight-line velocity and percentage of progressively motile sperm (Fenster et al., 1997). The catastrophic earthquake of Kobe was related to a reduction of environmental factors in this time period.

The male-to-female ratio at birth may be a useful indicator of male reproductive hazard. Chronic exposure to substances with toxic properties on the male reproductive system could lead to lower male/female ratios (Møller, 1996; Bromen and Jockel, 1997; van der Pal-de Bruin et al., 1997). Mocarelli et al. reported fewer male offspring of fathers who were intoxicated by dioxin when young (Mocarelli et al., 2000). The negative effect of environmental agents on human sex ratio is either non-existent (Van der Broek, 1997) or expressed differently in various countries (Parazzini et al., 1997). Nevertheless, in humans this relationship requires further investigation. Interestingly, in animals fetotoxic dioxin did not lower the sex ratio (Melaine and Jégou, 2000). Other factors are likely to be involved in sex ratio changes. Climate characteristics such as high ambient temperatures could affect not only fertility, but also sex ratio at birth (Grech et al., 2000). The ratio of male births in the south of Europe is higher than in the north. Birth order, race and parental age may also affect the sex ratio. However, in Slovenian society these factors could not change so dramatically within a few days to induce the change of sex ratio observed in the first trimester of 1992.

Moreover, psychological stress might be related to a lower sex ratio. Natural catastrophes such as volcano eruptions, cyclones and floods (Lyster, 1974) have been associated with decreased male births. A decline in sex ratio at birth 9 months after the Kobe earthquake was reported (Fukuda et al., 1998). Women who were exposed to intensive psychological stress (hospitalization or death of a partner or a child because of a severe disease) bore more girls than boys (Hansen et al., 1999). The phenomena, especially for exposures around the time of conception, might be related to differential conception or differential abortion of male embryos. In Japan, the male/female ratio of fetal deaths (after 12 weeks) was reported to be increasing from the 1970s: this trend suggests a particular prenatal vulnerability of the male fetus (Minuzo, 2000). Psychological stress related to severe life events may also alter the sex ratio through bad nutrition, lower frequency of sexual activity, longer sexual abstinence that may result in a higher proportion of X-bearing sperm (Hilsenrath et al., 1997) and changes in paternal hormones around the time of conception (James, 1997; Hansen et al., 1999). Finally, it should be emphasised that females are also involved in the process. As maternal condition declines, the female tends to produce a lower ratio of males to females (Trivers and Willard, 1973). Some authors have hypothesized that poorer semen quality—lower motility in particular—might be connected with a lower sex ratio (Møller, 1996; James, 1997; Fukuda et al., 1998). However, in a recent retrospective study (Jacobsen et al., 2000) no association was found between semen characteristics in subfertile men and changes in sex ratio.

Our report is in contradiction with the observation of increased sex ratio at birth during and immediately after World Wars I and II (Van der Broek, 1997; Graffelman and Hoekstra, 2000; Lerchl, 2000). The explanation for this increase is mainly based on the time of conception: when the time of conception is either early or late in the fertile phase of the menstrual cycle—a frequent condition in wartime when non-programmed copulation and high coital rates co-exist—more boys are born (James, 1980). In Slovenia the war was brief, yet a decrease in sperm motility was observed. As already noted, due to the small sample size of the control group, our findings—the observed decrease in sperm motility after war—should be interpreted with caution and taken only as an indication, with a need for other studies on a large population. Nevertheless, the delayed time of conception and lower sperm motility might need to be combined to lead to a decreased sex ratio. Because of decreased sperm motility, conception would be expected to occur some time after sexual intercourse, closer to ovulation, which is a condition favourable to the birth of females.

In Slovenia, after psychological stress induced by a short war, we observed a fall in sex ratio at birth. The mechanisms involved are far from being elucidated. The negative changes in sperm motility explain the phenomenon only partially. Considering that the fall in sex ratio was more pronounced in the capital Ljubljana, where the menace was the most intense with possibility of air raids and constant threat of sabotage or direct assault from the Yugoslav army forces, we presume that a gradation in subjective stress and adaptation argues for the direct effect of psychological stress on the changes in sex ratio.

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