Do assisted conception twins have an increased risk for anencephaly?

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BACKGROUND: The incidence rates of anterior neural tube defects, anencephaly and encephalocele appear increased among twins compared with singletons. The current study aimed to evaluate whether the etiology of this phenomenon is related to twinning, assisted reproductive technology (ART), or both.

METHODS: The study cohort consisted of parturient women who were referred to our ultrasonography unit between January 1998 and December 2009 due to suspicion of severe fetal abnormality. The study cohort was divided into two subgroups based on mode of conception: spontaneous and ART (including IVF and ICSI). The subgroups were further subdivided into singleton and multiple pregnancies. We also compared pregnancies diagnosed with anencephaly in the study group to all live births in the Department of Obstetrics and Gynecology.

RESULTS: Anencephaly was diagnosed in 43 fetuses out of 1154 (3.7%) pregnancies diagnosed with severe fetal anomaly. Anencephaly was diagnosed in 9 out of 78 twin pregnancies (11.5%); of these, 8 of 45 (17.8%) were ART conceived and 1 of 33 (3%) spontaneously conceived. A significant correlation was found between twinning and anencephaly, with an odds ratio (OR) of 3.4 [confidence interval (CI) = 1.3–8.9, \( P = 0.011 \)], while no significant correlation was found between ART and anencephaly. A significant correlation was found between anencephaly and the combination of ART conception and twinning (OR of 6.6, CI = 2.8–15.3, \( P < 0.01 \)). Analyzing the distribution of pregnancies diagnosed with anencephaly in the study group compared with the total number of live births in the department revealed a significant correlation between twinning and anencephaly, with an OR of 11.4 (CI = 4.9–26.5, \( P < 0.01 \)), with no significant correlation between ART and anencephaly. Among all live births, a significant correlation was found between anencephaly and the combination of ART conception and twinning (OR of 24.6, CI = 11.4–53.2, \( P < 0.01 \)).

CONCLUSIONS: Our data suggest that twin pregnancies conceived by ART constitute a high-risk group for anencephaly, due to a possible synergistic effect of twinning and ART.

Key words: anencephaly / prenatal assisted conception / twin / ultrasound

Introduction

Central nervous system (CNS) anomalies are the most common birth defects (Manning and Archer, 2001). Among them are the anterior neural tube defects (NTDs): anencephaly and encephaloceles, and the posterior NTD: spina bifida. Anencephaly is a uniformly lethal and untreatable anomaly. Since ultrasound enables early prenatal detection of anencephaly in almost 100% of the cases (Johnson et al., 1997; Cameron and Moran, 2009), early elective termination of pregnancy is the most common approach (Obedi et al., 2010).

Twining appears to be associated with a significantly higher rate of anencephaly (~2%) over the population rate (Källén et al., 1994). Advances in assisted reproductive technologies (ARTs) have increased the number of multiple pregnancies. Children from multiple pregnancies are at increased risk for preterm birth, low birthweight, long-term disabilities and early death. Furthermore, these children might be at a higher risk for birth defects (Li et al., 2003b).

The Israeli Ministry of Health (2004) reported an increased rate of twins in pregnancies diagnosed with open NTDs. This observation, together with the high rate of ART cycles in Israel, which is in fact the highest in the world (Collins, 2002), led us to investigate a possible effect of twinning and ART on anencephaly. In a previous study, we found a high rate of anencephaly among ART co-twins to be associated only with twinning, and not with the mode of conception (Ben-Ami et al., 2005). However, that was a relatively small case series, and the subgrouping of ART to IVF and ICSI may have obscured
a statistically significant association between ART and anencephaly (MacMahon and Rothman, 2006). The current study aims to evaluate whether the increased incidence of anencephaly observed among twin pregnancies in Israel is attributed to the twinning, the mode of conception, or both.

Materials and Methods

The study cohort comprises parturient women referred to our ultrasonographic unit between January 1998 and December 2009 due to suspicion of severe fetal abnormality. We divided the cohort into two subgroups based on mode of conception: spontaneous and artificial reproductive technology (ART, including IVF and ICSI). We then subdivided further into singleton and multiple pregnancies.

The ultrasonography unit in our Department of Obstetrics and Gynecology serves as a tertiary referral center for pregnant women with fetuses suspected of severe abnormalities. After completion of the workup (described in detail in Maymon et al., 2003), and confirmation of the severe abnormality, counseling is provided; the aim is either to disseminate more information (e.g. for further genetic studies), or to offer the couple the choice of terminating the pregnancy (Maymon et al., 2003). For twins, first-trimester evaluation includes an accurate determination of chorionicity, the number of sacs and live embryos. Determination of chorionicity is based on the appearance with ultrasound of an extension of placental tissue into the base of the intertwine membrane, forming the lambda sign (Sepulveda et al., 1996, 1997). When severe abnormalities are diagnosed in a dichorionic twin, selective feticide is suggested (Maymon et al., 2001). As generally accepted, we base prenatal ultrasonographic diagnosis of anencephaly late in the first or early in the second trimester of pregnancy on detection of an absent cranial vault and cerebral hemispheres (Campbell et al., 1972).

Further information was obtained from the women’s medical and demographic records, including maternal age, obstetrical history, the week of gestation in which the fetal anomaly was first diagnosed, ultrasonographic findings and karyotype (if performed). Information about live births at our department was obtained by using a computerized-based research, including the week of gestation at delivery, the mode of conception (spontaneous versus ART) and pregnancy type (singleton versus twin).

Statistical analysis

Descriptive parameters are expressed as mean ± SD. Frequencies are presented as percentages. To compare two independent variables, we examined the rate of anencephaly per pregnancy in twins and in singletons. Frequencies were compared using logistic regression analysis and Mantel–Haenszel prevalence ratios. Student’s t-test and χ² test were used to compute demographic variables. Calculations were performed using SPSS software (Version 17, Chicago, IL, USA) by the Tel Aviv University statistical laboratory; P-values of <0.05 were considered statistically significant.

Results

During the study period, 1154 parturient women were referred to our ultrasonographic unit following diagnosis of severe fetal anomaly. Of them, 43 (3.7%) were diagnosed with anencephaly (Fig. 1). Thirty-five of 1053 (3.3%) spontaneous pregnancies versus 8 of 101 (7.9%) ART-conceived pregnancies were diagnosed with anencephaly. Thirty-four of 1076 (3.2%) singleton pregnancies versus 9 of 78 (11.5%) twin pregnancies were diagnosed with anencephaly. Interestingly, 8 of 45 (17.8%) of the ART-conceived twin pregnancies were diagnosed with anencephaly, while only 1 of 33 (3%) of the spontaneous-conceived twin pregnancies was diagnosed with anencephaly.

Logistic regression analysis revealed a statistically significant correlation between twinning and anencephaly, while controlling for ART [odds ratio (OR) of 3.4, confidence interval (CI) = 1.3–8.9, P = 0.011]. In contrast, no significant correlation was found between ART and anencephaly, when controlling for twinning (OR of 1.3, CI = 0.5–3.6, P = 0.58). Interestingly, a statistically significant correlation was found between anencephaly and the combination of ART conception and twinning (OR of 6.6 CI = 2.8–15.3, P < 0.01).

Table I compares spontaneous and ART-conceived twin pregnancies, in this cohort of women carrying fetuses with severe fetal anomaly. The mean parity was significantly higher in women who had spontaneous versus ART-conceived twin pregnancy (P = 0.005). There were no significant differences between the two subgroups in the mean gestational week at which fetal anomaly was diagnosed or

Figure 1 Distribution of pregnancies in which severe fetal anomaly was diagnosed between January 1998 and December 2009 (ART). †For twinning—OR 3.4 (CI = 1.3–8.9, P = 0.011). ‡For combination of twinning and ART—OR 6.6 (2.8–15.3, P < 0.01).
Table I Comparison between spontaneous and ART-conceived twin pregnancies in which severe fetal anomaly was diagnosed between January 1998 and December 2009.

<table>
<thead>
<tr>
<th>Anomaly (%)</th>
<th>Spontaneous twin (n = 33)</th>
<th>ART twin (n = 45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD)</td>
<td>32.6 ± 4.3</td>
<td>32 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Gravity (mean ± SD)</td>
<td>3.04 ± 2</td>
<td>2.1 ± 1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Parity (mean ± SD)</td>
<td>1.5 ± 1.4</td>
<td>0.7 ± 0.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Gestation week at diagnosis (mean ± SD)</td>
<td>15.7 ± 4.8</td>
<td>15.3 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>Gestation week at termination (mean ± SD)</td>
<td>19.2 ± 6.9</td>
<td>18.5 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>Karyotype (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Not performed</td>
<td>63.6</td>
<td>46.7</td>
<td>NS</td>
</tr>
<tr>
<td>Normal</td>
<td>27.2</td>
<td>37.7</td>
<td>NS</td>
</tr>
<tr>
<td>Abnormal</td>
<td>9.2</td>
<td>15.6</td>
<td>NS</td>
</tr>
<tr>
<td>US examination (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>12.1</td>
<td>28.9</td>
<td>NS</td>
</tr>
<tr>
<td>Anomaly (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>6</td>
<td>33.3</td>
<td>0.004</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>6</td>
<td>4.4</td>
<td>NS</td>
</tr>
<tr>
<td>CVS and pulmonary</td>
<td>9.1</td>
<td>11.1</td>
<td>NS</td>
</tr>
<tr>
<td>Skeletal</td>
<td>0</td>
<td>2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Multiple anomalies (%)</td>
<td>3</td>
<td>15.6</td>
<td>0.07</td>
</tr>
<tr>
<td>TTTS (%)</td>
<td>51.5</td>
<td>2.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Others (%)</td>
<td>12.1</td>
<td>22.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

CNS, central nervous system; CVS, cardiovascular system; TTTS, twin-to-twin transfusion syndrome.

Discussion

Our data on pregnancies with severe anomalies corroborate these obtained from the total number of live births; accordingly there is a significant correlation between twinning per se and anencephaly, while no such association exists between ART and anencephaly. Importantly, the combination of ART and twinning is associated with an extremely high risk ratio for anencephaly, suggesting a possible synergistic effect between twinning and ART.

Twins have a 2.64 risk ratio (95% CI: 1.23–5.67) for anterior NTD: anencephaly and encephalocele, but not for the posterior NTD, spina bifida (Li et al., 2003a,b). This suggests that the etiologic mechanism related to twins or twinning differs from anterior and posterior NTDs (James, 1981).

In the past, Little and Elwood (1992) viewed skeptically the possibility of such an association, partly because of the statistical limitations of the available data. Nevertheless, they summarized possible mechanisms that could account for such: zygotic fission, twin embryogenesis and interactions between gestating co-twins (Little and Elwood, 1992). The causal factor for all NTDs develops between the 17th and 30th post-fertilization days manifesting as developmental delay, as suggested by James (1975).

Certain genetic and environmental factors have also been proposed to explain the susceptibility to twinning and NTDs (Garabedian and Fraser, 1994). This explanation is supported by the high incidence of anencephaly in communities with high rates of consanguinity (Zlotogora 1997a,b; Al-Gazali et al., 1999) and by the detection of chromosomal aberrations in spontaneous abortuses with NTD (Coert et al., 1997; McFadden and Friedman, 1997). Furthermore, spontaneous abortions are often followed by the term infants with more severe anomalies or no anomalies at all in our delivery room admitted to the Department of Obstetrics and Gynecology at our medical center between January 1998 and December 2009.

A total of 81226 live births were documented during the study period, of which 75230 (92.6%) pregnancies were spontaneously conceived and the remaining (5996, 7.4%) conceived by ART (Fig. 2). The mean age of women who had spontaneously conceived twin pregnancies was significantly higher than that of women who had ART-conceived twin pregnancies (31.1 ± 5.2 versus 24.1 ± 3.7, P < 0.05). The distribution of anencephaly cases revealed 1 of 850 (0.1%) spontaneous twin deliveries versus 8 out of 756 (1.1%) ART-conceived twin deliveries (Fig. 2). A logistic regression analysis revealed a significant correlation between twinning and anencephaly, with an OR of 11.4 (CI = 4.9–26.5, P < 0.01), with no significant correlation between ART and anencephaly. A significant correlation was found between anencephaly and the combination of ART conception and twinning (OR of 24.6, CI = 11.4–53.2, P < 0.01).

in the means by which pregnancy was terminated (by selective fetal reduction or elective abortion). Notably, a significantly higher rate of CNS anomalies was diagnosed in ART than in spontaneous twin pregnancies (33.3 versus 6%, respectively, P = 0.004). A significantly higher rate of twin-to-twin transfusion syndrome (TTTS) was diagnosed in spontaneous when compared with ART-conceived twin pregnancies (51.5 versus 2.2%, P = 0.001). This considerable difference could be explained by the significantly higher rate of monozygosity in spontaneous twins when compared with the ART group in Caucasians (35 versus 5%, respectively). A statistical trend of higher rate of multiple anomalies was observed in ART than in spontaneous twin pregnancies (P = 0.07). All of the women carrying fetuses diagnosed with anencephaly underwent either pregnancy termination or early second trimester selective feticide.

Under the assumption of a closed population, and to further support our findings, we compared characteristics of the pregnancies diagnosed with anencephaly in the study group to all live births with other than severe anomalies or no anomalies at all in our delivery room admitted to the Department of Obstetrics and Gynecology at our medical center between January 1998 and December 2009.
An intriguing observation is that during the 3 years following issuance of the folic acid recommendation for women in fertility years in Israel, reduction in NTD rates was more significant for spina bifida than for anencephaly (Zlotogora et al., 2006). For spina bifida, a 41% reduction occurred among Jews (from 4.9 to 2.7 per 10,000 live births) and 35% among non-Jews (from 9.5 to 6.2 per 10,000 live births). However, rates of anencephaly decreased only slightly among both Jews (from 5.3 to 4.9 per 10,000 live births) and non-Jews (from 8.9 to 8.2 per 10,000 live births). One possible explanation for the minimal change in anencephaly rates is that availability of the nuchal translucency test for Down syndrome screening enabled diagnosis of anencephaly at an early stage in pregnancy. Otherwise, many affected pregnancies would have been spontaneously aborted and not diagnosed. Early diagnosis by ultrasonography has increased the total number of diagnoses of anencephaly in recent years, masking any reduction in incidence. However, this explanation accounts for only a small part of the observed differences in incidence rates between anencephaly and spina bifida. Nevertheless, the new prenatal scan of the intracranial translucency for detection of spina bifida at an 11–13 week scan (Chaoui et al., 2009) is also expected to affect incidence rates in forthcoming years.

In the USA, as in Israel, the incidence of spina bifida decreased considerably, and that of anencephaly more moderately following the folic acid recommendation (Stevenson et al., 2000; Feldkamp et al., 2002). However, in Ireland, for instance, a significant decline occurred in the 15 years preceding the folic acid recommendation for both anencephaly and spina bifida (McDonnell et al., 1999). In Chile, constant incidence rates of NTD before folic acid supplementation were followed by a decline in both malformations subsequent to the recommendation (Lopez-Camelo et al., 2005). Differences in nutritional status, socioeconomic conditions and genetic background may explain diverse observations between populations. It has been suggested that higher quantities of folic acid are required to prevent the more severe defects (Wald et al., 2001).

An association between NTD and either clomiphene citrate treatment or controlled ovarian hyperstimulation has been discussed for several years (Elwood et al., 1992; Van Loon et al., 1992; Greenland and Ackerman, 1995; Ericson and Källén, 2001; Elizur and Tulandi, 2008). The possibility of increased risk after conventional IVF has been suggested (Lancaster et al., 2000). However, though the Swedish rate of anencephaly in spontaneous twins is twice as high as in singletons (Källén et al., 1994), and the rate of twins in IVF children is above 40%, the expected number of IVF children with anencephaly in Sweden is only marginally increased (Ericson and Källén, 2001). In a study of 694 cases of NTD and a comparator group, Whiteman et al. (2000) found no evidence that either subfertility (OR = 1.2, 95% CI: 0.7, 2.1) or its treatment (OR = 0.9, 95% CI: 0.4, 2.0) increased the risk of NTD-affected pregnancies. After adjustment for potentially confounding variables, NTD-affected pregnancies were also found to have some influence on the overall increased risk of malformations in IVF infants (Pinborg, 2005). In light of the expanded use of ART, many clinics have recently adopted policies of elective single embryo transfer (SET) to minimize the rate of twin pregnancies associated with IVF (reviewed in McLernon et al., 2010). Selective fetal reduction of a desired pregnancy because of fetal abnormality is a very painful decision in modern society. Early diagnosis of fetal anomalies and/or genetic disorders presents the couple with an agonizing choice, especially among ARTs-achieved pregnancies. According to the current study, at least one possible additional advantage of the SET policy is a probable reduction of anencephaly in twins.

The present study has a number of limitations. The most obvious is the reliance upon the retrospective data, subjecting the findings to bias. A prospective study from a single center could, however, be difficult to achieve due to the rarity of this event. Another disadvantage of this study is the limited information that was available for the IVF procedures. While some specified variants could be analyzed, thereby enabling analysis according to standard IVF and ICSI, to fresh and cryopreserved embryos, and to source of sperm at ICSI, detailed information about the nature of fertility problems was not available. Still, we believe that our sample is representative of the affected fetuses and the normal population.

Anencephaly in assisted conception twins

**Figure 2** Distribution of live births admitted to our department between January 1998 and December 2009. †For twinning—OR 11.4 (CI = 4.9–26.5, P < 0.01). ‡For combination of twinning and ART—OR 24.6 (11.4–53.2, P < 0.01).
Our data suggest that twin pregnancies conceived by ART constitute a high-risk group for anencephaly in one of the co-twins. This may be secondary to a synergistic effect of twinning and ART. Additional studies are needed to validate this possibility, and to elucidate a more specific etiologic explanation for such a phenomenon. Ideally, a national registry of anencephaly should be established. Using such data would enable us to address, at least in part, some of the points that are necessary to define causality between the combination of ART, twinning and anencephaly.

**Authors’ roles**

I.B. assisted with the study design, data acquisition and wrote the manuscript. Y.E. assisted with the data acquisition and analysis (This research was performed in partial fulfillment of the MD thesis requirements of the Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel.). O.B. and Z.V. assisted with the data acquisition of the study. A.H. participated in the study design. R.M. designed the study, wrote the manuscript and revised it, providing comments on its content and has given final approval of the version to be published.

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