Malformation rate and sex ratio in 412 children conceived with epididymal or testicular sperm

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BACKGROUND: Follow-up studies of children conceived after ICSI using epididymal or testicular sperm are important due to a still more extensive use of immature male germ cells for ICSI. It is, however, difficult to evaluate the potential risks of malformations of children born after ICSI, overcoming the natural fertilization processes, due to methodological limitations. METHODS: Follow-up study including all children born in Denmark and Norway following ICSI in Denmark, using epididymal or testicular sperm, was done. A questionnaire was sent to the parents between 3 months and 7 years after delivery. RESULTS: Of 341 couples, 329 returned the questionnaire giving a response rate of 96.5%. The study included 412 children, 225 girls and 187 boys, giving a sex ratio (males/males + females) of 45.4% compared with 53.1% in Danish children conceived after conventional IVF without ICSI (P<0.005). Among a total of 14 (3.4%; 95% confidence interval (CI): 1.9%–5.7%) major malformations, three boys with hypospadias were the most remarkable finding (1.6%; 95% CI: 0.33–4.7%). CONCLUSIONS: An increased frequency of hypospadias in the male offsprings was seen compared with the general population. Apart from this, no increased major malformation rate was detected in ICSI children conceived with epididymal or testicular sperm when compared with malformation rates for IVF or spontaneously conceived children reported in the literature. The sex ratio was significantly lower for ICSI children conceived with epididymal or testicular sperm when compared with children conceived with conventional IVF.

Key words: cardiovascular defect/hypospadias/malformation/sex ratio/testicular sperm aspiration/percutaneous epididymal sperm aspiration follow-up

Approximately 300 000 children conceived after IVF or IVF with microinjection (ICSI) are born in Europe every year (Nyboe Andersen et al., 2005). In one of the largest follow-up studies, children born following conventional IVF and ICSI were compared and no differences in malformation rate were observed (Bondeau et al., 2002). Recently, however, two meta-analyses comparing birth defects in IVF and ICSI infants with spontaneously conceived controls were published (Rimm et al., 2004; Hansen et al., 2005). Although most previous studies have been too small to show any significant differences, the two meta-analyses show a statistically significant increased risk of birth defects in infants conceived after IVF or ICSI of the order of 30–40%. Among 25 studies, only 5 reported results for children conceived by ICSI separately (Hansen et al., 2005), and an overall statistically increased occurrence of malformations after ICSI compared with IVF has not been detected (Hansen et al., 2005; Lie et al., 2005).

Particularly, major malformations in the cardiovascular system and the urogenital tract seem to be increased in children born after ICSI (Wennerholm et al., 2000; Ludwig and Katalinic, 2002; Källén et al., 2005). A possible increase in the occurrence of hypospadias seems particularly relevant, as this condition might be associated with male infertility (Sweet et al., 1974).

Since 1994, ICSI has also been done with sperm retrieved from the epididymis or testis (Tournaye et al., 1994). Such sperm are more immature than ejaculated sperm, suggesting a larger potential risk of congenital abnormalities, chromosomal aberrations and imprinting disorders.

A more rapid development has been observed in male mammalian embryos compared with female embryos (Boklage, 2005). This might be due to H-Y antigen acting as a growth factor (Kcro and Goldberg, 1976) and may explain a sex ratio of 55% (males/males + females) found after IVF (Dumoulin et al., 2005). After ICSI, using conventional ejaculated sperm, a sex ratio of 50% was found (Dumoulin et al., 2005). A reduced sex ratio after ICSI compared with IVF might be explained by imprinting defects in the spermatozoa.
Owing to an increased use of immature germ cells for ICSI, a Danish national follow-up study focusing on major malformations, chromosomal aberrations and sex ratio was initiated. This study is, to our knowledge, the largest follow-up study of children conceived using epididymal or testicular sperm published to date. Furthermore, it has the advantage of being a national study including most ‘percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA) children’ conceived in a country.

Materials and methods

Unless the man was vasectomized (and the aetiology therefore obvious), an extensive examination programme including medical history, clinical examination, ultrasonography and hormonal and genetic analyses was recommended prior to the ICSI procedure (Fedder et al., 2004). In some cases, however, the couples were referred from Norway and arrived to Denmark a few days before sperm retrieval. In these cases, the clinics were forced to do a more modest, clinical examination programme.

The sperm were obtained by either PESA or TESA. The PESA procedure was performed using a 21-gauge butterfly needle (Neofly, Ohmeda) (Fedder et al., 2001). The TESA procedure was carried out using a Tru-Core biopsy needle (InterV, Medical Device Technologies Inc., USA). Testicular material for diagnostics and treatment was usually obtained during two different procedures. In some clinics, excess material was frozen for new treatments. Testicular biopsies were cut into pieces and shredded using two glass slides in a Petri dish (Becton-Dickinson, Aalst, Belgium). During this procedure, the seminiferous tubules were squeezed and broken, facilitating the sperm to come out into the fluid (Verheyen et al., 1995). ICSI and culture procedures were done according to the routines of the respective labs.

Questionnaire

A questionnaire was sent to all parents of children born after ICSI using epididymal or testicular sperm from February 1996 until 31 December 2004. The questionnaire was sent 3 months to 7 years after delivery and contained questions about the aetiology of the azoospermia (e.g. history according to cryptorchidism, genital infections, trauma and karyotyping), pregnancy, prenatal diagnostics, delivery, sex of the child, birthweight and malformations, as well as numbers of hospital contacts and general health of the child. Furthermore, the parents were asked to give their written informed consent to allow us to call them or to collect further information from other hospitals. If the couples did not reply, the questionnaire was resent. For each couple, the fertility clinics filled out a registration form with laboratory data, including the origin of sperm (epididymal or testis). The gestation age was calculated as the time from a calculated first menstruation day (14 days before aspiration) to the time of birth. Further written information was searched for all hospital contacts reported in the questionnaires, and if any doubt the parents were contacted by phone.

Major malformations were, in this study, defined as malformations requiring surgical treatment or causing essential reduced functionality. This definition has previously been used in several other studies, among them Rimm et al. (2004).

Sex ratio

The sex ratio might be influenced by the IVF culture conditions. According to sex ratio, the children from a Danish national database including girls and boys born after conventional IVF and ICSI, respectively, using ejaculated sperm during the period 1995–2000 were used as comparison cohort (Pinborg et al., 2004a; Pinborg et al., 2004b).

Pregnancy loss

It was not possible to obtain reliable information about the abortion frequency in the patient group examined, but information about the numbers of gestational sacs seen at ultrasonography 5 weeks after embryo transfer was available, and the frequency of vanishing twins could be estimated.

Statistics

According to sex ratio for different subgroups, exact 95% confidence intervals (CI) were calculated on the basis of the binomial distribution. A two-tailed χ²-test with Yates correction was used to compare the relative frequencies of girls and boys after conventional IVF or ICSI in relation to the corresponding frequencies after ICSI in couples where the man showed a specific aetiology or ICSI with only epididymal and/or testicular sperm.

For major malformations generally and hypospadias specifically, exact 95% CI were calculated on the basis of the Poisson distribution.

Results

The questionnaire was sent to 341 couples, and 329 returned the questionnaire giving a response rate of 96.5%. Sixteen couples participated twice (with different children) in the study. No information is available concerning malformations in the 12 couples who did not return the questionnaire.

The study included 412 children, 225 girls and 187 boys, born after ICSI with epididymal or testicular sperm. No stillborn infants were registered in the study group. The treatments were carried out in seven private and seven public fertility clinics (the Danish fertility clinics). The 7 private clinics contributed with 181 children (range: 1–118 per clinic) and the 7 public clinics with 231 children (range: 3–60 per clinic). Ninety-four (~29%) of the 329 couples came from Norway.

Follow-up period

An estimated follow-up period was estimated as the time period from delivery to receipt (in the fertility clinic) of the questionnaire. In none of the cases the questionnaire was sent before at least 3 months after delivery, and for only two children, the follow-up time was 7 years. The mean follow-up period was calculated to 20 months and the median follow-up period 1.5 years.

Cause of azoospermia

As shown in Table I, 379 cases (92%) could be categorized as obstructive, non-obstructive or neurological azoospermia. It was not possible to classify the aetiology of azoospermia in only 33 cases. Among the 412 children, 282 cases (68.5%) were due to obstructive azoospermia. Among these, the most prominent cause was not only vasectomy (122 (29.6%)), but also cystic fibrosis transmembrane regulator mutations (70 (17.0%)) and former genital tract infections (30 (7.3 %)), most often with Chlamydia trachomatis, made up larger subgroups. Other aetiologies were rare. Neither aetiology nor the use of epididymal or testicular sperm were associated with maternal age (data not shown) or the frequency of multiple pregnancies (Table I).
Table I. Malformations and sex ratios in relation to the aetiology of the azoospermia of the father

<table>
<thead>
<tr>
<th>Main cause</th>
<th>Specific cause</th>
<th>Total number of children (%)</th>
<th>Children from multiple pregnancies (%)</th>
<th>Children with major malformations</th>
<th>Sex ratio (95% CI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obstructive</td>
<td>Vasectomized</td>
<td>122 (29.6)</td>
<td>46 (37.7)</td>
<td>7</td>
<td>40.2 (31.4–49.4)</td>
</tr>
<tr>
<td></td>
<td>CF carrier</td>
<td>70 (17.0)</td>
<td>30 (42.9)</td>
<td>2</td>
<td>43.3 (32.4–56.7)</td>
</tr>
<tr>
<td></td>
<td>Genital tract infection</td>
<td>30 (7.3)</td>
<td>8 (26.7)</td>
<td>0</td>
<td>43.3 (25.5–62.6)</td>
</tr>
<tr>
<td></td>
<td>Other/Unknown</td>
<td>60 (14.6)</td>
<td>25 (41.7)</td>
<td>3</td>
<td>46.7 (33.7–60.0)</td>
</tr>
<tr>
<td>Non-obstructive</td>
<td></td>
<td>76 (18.4%)</td>
<td>28 (36.8)</td>
<td>0</td>
<td>50.0 (38.3–61.7)</td>
</tr>
<tr>
<td>Neurological cause</td>
<td></td>
<td>21 (5.1%)</td>
<td>10 (47.6)</td>
<td>2</td>
<td>47.6 (25.7–70.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td>33 (8.0%)</td>
<td>18 (54.5)</td>
<td>0</td>
<td>54.5 (36.4–71.9)</td>
</tr>
</tbody>
</table>

*Male offspring/total number of offspring.

*P < 0.01 (χ²-test).

Prenatal diagnostics

Only 14.1% (58) had prenatal diagnostics performed. Ten chorionic villus biopsies and 48 (including eight gemelli) amniocenteses were carried out. In one case, a chromosomal abnormality, a balanced de-novo translocation between chromosome 1 and 3 in a female fetus, was found after amniocentesis. No significant differences were observed by comparing the use of epididymal and testicular sperm.

Pregnancies, mode of delivery and birthweight

By ultrasoundography at week 7, in total 437 gestational sacs were observed, giving rise to 412 children. No fetal reductions were performed in case of twin gestation sacs. The frequency of vanishing twins for this group was 10% (180 children born in 100 cases, each showing two implantations).

The infants were born as 247 singletons, 81 sets of twins and one set of triplets. As shown in Table II, the maternal age did not differ significantly between single and multiple pregnancies. The gestational age and the birthweight were lower and not differ significantly between single and multiple pregnancies. In three cases, twin B had to be delivered with a caesarean section after vaginal birth of twin A. A planned caesarean section was done for multiple pregnancies (%).

Sex ratio

An overall sex ratio of 45.4% (defined as male offspring/male + females) was found in this study. It differs from an average ratio of around 51.3% found in the general population (Danmarks Statistik 1995–2004) and particularly for vasectomized men, the sex ratio is low compared with conventional IVF (40.2%; P < 0.01; Table I).

The five major malformations found in girls were much more variable, ranging from the absence of the thyroid gland to the presence of severe malformations of the lower extremities (Table IV). In the child born without a thyroid gland, a scintigraphy showed total absence of the thyroid tissue on the front of the neck, but some aberrant thyroid tissue was detected near the root of the tongue. The girl became lifeless 1 h after the ICSI control group was between the extremes: sex ratio after IVF (without ICSI) and ICSI with testicular or epididymal sperm, respectively. Particularly, among the 94 children born after treatment with epididymal sperm, only 40.4% (38) were boys (P < 0.025, χ²-test). According to male aetiology, particularly for vasectomized men, the sex ratio is low compared with conventional IVF (40.2%; P < 0.01; Table I).

Malformations

Using the present definition, major malformations were found in 14 (3.4%; 95% CI: 1.9–5.7%) of the 412 children. As shown in Table IV, the nine major malformations found in boys included severe coronary-vascular and urogenital malformations. The frequency of congenital heart malformations was 2.7% of 187 boys or 1.2% of 412 children. These heart diseases were cured surgically (Kromann Andersen, 1999).

According to the urogenital system, an occurrence of 3 of 187 boys (1.6%; 95% CI: 0.33–4.7%) with hypospadias was most prominent. This is about five times an expected frequency of 0.28%, calculated as twice the incidence of 0.14% in the general (male + female) population (Toppari et al., 2001). Two of the boys with hypospadias were dizygotic twins. None of the fathers to the present boys with hypospadias suffered from the same condition.

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Table II. Obstetric data: mean ± SD (range) according to pregnancy multiplicity

<table>
<thead>
<tr>
<th></th>
<th>Singletons (n = 247)</th>
<th>Gemelli (n = 162)</th>
<th>Triplet (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>31.7 ± 4.0 (19–41)</td>
<td>31.2 ± 3.3 (23–38)</td>
<td>34</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>279 ± 11 (228–303)</td>
<td>257 ± 16 (218–283)</td>
<td>242</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3476 ± 573 (1570–5150)</td>
<td>2515 ± 483 (1239–3540)</td>
<td>2198 ± 167 (2070–2400)</td>
</tr>
<tr>
<td>Caesarian section, acute (%)</td>
<td>19 (7.7)</td>
<td>29 (17.9)</td>
<td>—</td>
</tr>
<tr>
<td>Caesarian section, planned (%)</td>
<td>16 (6.5)</td>
<td>44 (27.2)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>Caesarian section, totally (%)</td>
<td>35 (14.2)</td>
<td>73 (45.1)</td>
<td>3 (100)</td>
</tr>
</tbody>
</table>

*aSex ratio/

In three cases, twin B was delivered with a caesarean section after vaginal birth of twin A.


Table III. Distribution of girls and boys according to conception procedure

<table>
<thead>
<tr>
<th>Patient categories</th>
<th>Girls</th>
<th>Boys</th>
<th>Children (total)</th>
<th>Sex ratio (95% CI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICSI using testicular or epididymal sperm</td>
<td>225</td>
<td>187</td>
<td>412</td>
<td>45.4 (40.5–50.3)</td>
</tr>
<tr>
<td>ICSI using testicular sperm</td>
<td>169</td>
<td>149</td>
<td>318</td>
<td>46.9 (41.3–52.5)</td>
</tr>
<tr>
<td>ICSI using epididymal sperm</td>
<td>56</td>
<td>38</td>
<td>94</td>
<td>40.4 (30.4–51.0)</td>
</tr>
<tr>
<td>ICSI using ejaculated sperm (national database)</td>
<td>1026</td>
<td>1044</td>
<td>2070</td>
<td>50.4 (48.3–52.6)</td>
</tr>
<tr>
<td>Conventional IVF (national database)</td>
<td>3009</td>
<td>3403</td>
<td>6412</td>
<td>53.1 (51.5–54.3)</td>
</tr>
<tr>
<td>*General Danish population</td>
<td>322762</td>
<td>340514</td>
<td>663276</td>
<td>51.3 (51.2–51.5)</td>
</tr>
</tbody>
</table>

The comparison cohorts make up children born after IVF or ICSI, respectively, in Denmark 1995–2000 and the part of the general Danish population born 1995–2004.

CI, Confidence interval.

*Source: Danmarks Statistik

delivery. She had cardiac arrest, but was resuscitated and given thyroxin. Today, except for the congenital hypothyreosis, the child is completely healthy and showed normal psychomotoric development during the whole follow-up period (4 years).

The three children with bowing tibia/severe pes calcaneovalgus, cleft lip and torticollis, respectively, were operated upon using conventional surgery. A girl was born with an ovarian cyst, 3 cm in diameter, which disappeared spontaneously after 5 weeks. The cause of the ovarian cyst might have been maternal hormone production. In addition, two cases with hip instability were found. Using the chosen definition, they were considered only as minor malformations, as surgery was not indicated. They were treated with Dennis-Brown splint for 2 and 3 months, respectively.

Except for the child with malformations of the lower extremities, no one had multiple malformations.

Discussion

This study including 412 children born after ICSI using epididymal or testicular sperm is, to our knowledge, the largest study focusing on this particular population. Furthermore, it is unique, including an almost complete cohort of a nation.

Azoospermia may be caused by several different conditions including genetic abnormalities, infections, cryptorchidism and vasectomy or other surgical operations (Fedder et al., 2004). Different aetiologies might require different treatment. Therefore, it is relevant to classify the children according to the exact diagnosis of the azoospermic father or at least according to whether the azoospermia is on an obstructive or a non-obstructive basis.

Most remarkable a low sex ratio of 45.4% boys was found. During the whole study period, excess females were born. This was particularly remarkable when compared with ‘the IVF comparison cohort’. A sex ratio of 53.1% males found for the IVF comparison cohort in this study is close to the sex ratio of 55% found by Dumoulin et al. (2005). The explanation of this sex ratio may be that morphological scoring criteria of male embryos are better with less fragmentation 2 and 3 days after fertilization. This might be due to H-Y antigen acting as a growth factor (Krco and Goldberg, 1976; Fedder and Hjort, 1989; Boklage, 2005). Therefore, a positive selection of male embryos is expected, giving a higher sex ratio in conventional IVF.

The Danish national children material used as comparison cohort according to sex ratio consisted of children born during the first half of the study period, during which only about one-third of the children born after ICSI using epididymal or testicular sperm were born. However, a smaller local database from the fertility clinic, Braedstrup Hospital, including 744 ‘IVF children’ and 206 ‘ICSI children’ born during the whole period showed very similar results (data not shown) as the national comparison cohort.

The lower sex ratio after ICSI with ejaculated sperm (50.4%) compared with IVF might be due to genetic abnormalities causing male infertility and decreasing chances of male embryos to develop. So far, an association between severe male infertility and a higher proportion of male embryos without a potential to develop might be hypothesized. However, it has recently been hypothesized that imprinting defects (according to X-inactivation) may be the reason, and it is suggested that such imprinting failures are related to the ICSI technique itself, resulting possibly from the introduction of the sperm acrosome and its digestive enzymes into the ooplasm or from the mechanical stress that may damage cellular structures (Boklage, 2005). In the light of this, an even lower sex ratio (45.4%) found after ICSI with testicular and particularly epididymal sperm is therefore very interesting.

Table IV. Major malformations found in the 187 boys and 225 girls according to ICSI with testicular and epididymal sperm, respectively

<table>
<thead>
<tr>
<th>Testicular sperm</th>
<th>Epididymal sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys</strong> n = 149</td>
<td><strong>n = 38</strong></td>
</tr>
<tr>
<td>Hypospadias (n = 3, three cases), ventricular septal defect, coarctation of the aorta, patent ductus arteriosus during neonatal life, pyloric stenosis</td>
<td>Classical coronar transposition of the great vessels (tetralogy of Fallot), ventricular septal defect</td>
</tr>
<tr>
<td><strong>Girls</strong> n = 169</td>
<td><strong>n = 56</strong></td>
</tr>
<tr>
<td>Absence of the thyroid gland, double left kidney and ureteroceles</td>
<td>Malformations of the legs &amp; severe pes calcaneovalgus (bowing tibia), cleft lip, torticollis</td>
</tr>
</tbody>
</table>
Currently, it is impossible to say whether such a low sex ratio is due to the use of more immature germ cells or rather to an increased stress of the male cells, having been extracted from the epididymis or testicular tissue.

One explanation for a particularly low sex ratio (40.2%) found for vasectomized men might be that epididymal sperm were mainly used in couples with a vasectomy aetiology. As vasectomized men should be healthy except for the iatrogenic cause, probably the use of epididymal sperm is the reason for the reduced sex ratio rather than the aetiology itself, unless the sperm could be stressed due to the obstruction caused by the vasectomy.

Another possible hypothetical explanation of the particularly low sex ratio using epididymal sperm from vasectomized men might be the presence of anti-H-Y antibodies on the ‘male sperm’, reducing the chances of this sperm population to fertilize the eggs. Production of anti-H-Y antibodies may be triggered by the breakdown of the blood-testis barrier (Hoppe and Koo, 1984).

Incidences of malformations and chromosomal abnormalities depend upon maternal age, frequency of multiple pregnancies and examination intensity: clinical examination, echocardiography, prenatal diagnostics and so on. Although the most severe malformations usually are diagnosed at birth, the follow-up period is essential. A major malformation frequency of 3.4% might seem similar to a suggested frequency of 1–6% in the general population.

At present, few controlled, but many non-controlled, studies examining the risk of birth defects after IVF and ICSI have been published, and they all tend to show a modest increased risk of malformations after IVF and ICSI. However, results for ‘ICSI children’ are reported separately in only five controlled studies (Hansen et al., 2005). The present material is too small to draw clear conclusions according to malformation risks after ICSI with epididymal and testicular sperm compared with IVF or ICSI children conceived with ejaculated sperm or spontaneously conceived children. Ludwig and Katalinic (2002) reported that among 3372 ‘ICSI children’, only 229 children were conceived with testicular sperm and 26 children with epididymal sperm. No significant differences according to malformation rate were found comparing children conceived with ejaculated, epididymal or testicular sperm.

Among the 187 boys of the present study, five major malformations in the cardiovascular system, and three in the urogenital tract were found (Table IV). The incidence of congenital heart disease (CHD) in different studies varies from 4/1000 to 50/1000 live births (Hoffman and Kaplan, 2002). In the present study, two cases with ventricular septal defects (VSD), one case with tetralogy of Fallot (classical coronary transposition of the great vessels) and one case with coarctation of the aorta were found. These findings do not differ significantly from the frequencies reported in the literature. VSD is the most frequent acyanotic anomaly and the most frequent CHD of all, and tetralogy of Fallot is the most frequent cyanotic anomaly. VSD may be divided into perimembranous and muscular defects, and particularly, the last type is likely to close spontaneously during early childhood. Thus, the highest frequencies of VSD are found in neonates (Lin et al., 2001).

In conclusion, the frequency of CHD found in this study does not differ from the frequency found in the general population.

Consistent with Wennerholm et al. (2000), examining children conceived by ICSI generally, in this study, a relatively high incidence of hypospadias was found. Three boys with hypospadias out of 187 (1.6%; 95% CI: 0.33–4.7%) represent an increase in frequency of about five times the expected incidence of 0.28% in Denmark (Toppari et al., 2001). An increased incidence of hypospadias might be due to an increased occurrence of hypospadias in general or due to particular conditions in boys conceived by ICSI. In a recent study from the Netherlands, 53 (0.73%) of 7292 consecutive newborn boys were found to have hypospadias (Pierik et al., 2002). This was six times higher than a previously reported rate from the same area. However, the incidences of hypospadias seem to have shown a far more modest increase in the Nordic countries during a 20-year period ranging until 1990 (Toppari et al., 2001).

Many multiple pregnancies are included in this study, which may also be of importance. Thus, several studies have documented an association between hypospadias and low birthweight (Fredell et al., 2002). Interestingly, of 18 monozygotic twin pairs discordant for hypospadias, the twin with the lowest birthweight had hypospadias (Fredell et al., 1998).

Contrary to CHD and genitourinary malformations, the congenital absence of the thyroid gland is an extremely seldom finding. The least seldom agenesia related to the thyroid gland is the absence of the left lobe.

The results of the present study are based on a questionnaire. Almost all questionnaires were filled out completely. In the very few cases in which something was missing, it was typically the Central Personal Register number of the child. The reason might be that the parents were afraid that we or others would later contact the child without their consent. We had to assure that this would not be the case, and birth date, which is important for calculation of, for example gestational age, was obtained for all children. According to other data, we had good experiences with the design of the questionnaire and registration form.

In conclusion, the findings of this study are in accordance with the results in the relatively large study by Ludwig and Katalinic (2002). It is still too early to conclude that the frequency of major malformations generally or in the cardiovascular system is increased after ICSI using epididymal or testicular sperm. The study, however, supports the hypothesis of an increased frequency of hypospadias in boys conceived by ICSI due to severe male factor infertility.

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