Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review

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BACKGROUND: For men with azoospermia, it is possible to father their own progeny by intracytoplasmic sperm injection (ICSI) with epididymal or testicular sperm. Some studies show that children born after assisted reproductive technology (ART) are at increased risk of birth defects, other studies suggest that there is no extra concern about ICSI children conceived with epididymal or testicular sperm.

METHODS: Studies about the karyotypes of fetuses, congenital anomalies and the follow-up of the children born after ICSI with non-ejaculated sperm were identified by means of a systematic literature search.

RESULTS: Eight relevant studies were identified; two studies reported karyotype, five reported malformations and one reported follow-up of children after ICSI. In total, there were 55 out of 1973 (2.8\%) abnormal karyotypes in the ICSI with ejaculated sperm group, 0 out of 31 in the ICSI with epididymal sperm group and 5 out of 191 (2.6\%) in the ICSI with testicular sperm group. Major malformations were found in 543 out of 12 377 (4.4\%) in the ICSI with ejaculated sperm group, 17 out of 533 (3.2\%) in the ICSI with epididymal sperm group and 31 out of 670 (4.6\%) in the ICSI with testicular sperm group.

CONCLUSIONS: Although there were no statistical differences, the study groups were small and heterogenic, with a number of potential biases. We therefore recommend a standardized methodology of follow-up studies after ART, with well-defined groups of ICSI with ejaculated sperm, ICSI with epididymal sperm and ICSI with testicular sperm, and a control group of naturally conceived children.

Key words: ICSI / non-ejaculated sperm / karyotyping / congenital anomalies / follow-up

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Introduction

Assisted reproductive technology (ART) is nowadays available worldwide and has been practiced successfully on a large scale. About 1–4% of all live births in Europe are the result of IVF or intracytoplasmic sperm injection (ICSI) (Andersen et al., 2006). Hansen et al. (2005) showed in a systematic review that children born after ART are at increased risk of birth defects compared with spontaneous conceptions. The increase of birth defects in singletons born after ART might be related with the hormonal treatment for infertility or the procedure itself, but the underlying cause of infertility or its determinants might also play a role (Zhu et al., 2006).

Azoospermia is present in ~5% of all investigated infertile couples (Irvine, 1998) and is found in 10% of male infertility cases (De Cróo et al., 2000). Azoospermia can be divided into two groups: obstructive azoospermia (OA) with a normal spermatogenesis and non-obstructive azoospermia (NOA) as the result of a testicular failure. Since the introduction of ICSI, it is possible for these couples to father their own progeny by using sperm retrieved by percutaneous sperm aspiration (PESA), microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE). PESA and MESA are eligible in cases of OA, TESE in cases of NOA or in cases of an azoospermia with a PESA or MESA.

Concerns about the health of the children born after these techniques have been raised, especially in The Netherlands (Johnson, 1998; Te Velde et al., 1998). This led to a moratorium in this country for the application of ICSI in azoospermic men using non-ejaculated sperm from 1996 until 2001. From January 2001, ICSI with sperm retrieved by PESA or MESA in the case of an OA was allowed on the condition that there was a follow-up programme of the children, which was approved by the Central Committee on Human Research (CCMO). From June 2007, ICSI with sperm retrieved by TESE followed, on the same condition.

The goal of this study was to investigate the karyotypes of fetuses, congenital anomalies and the follow-up of the children born after ICSI with epididymal or testicular sperm.

Methods


English was used as a limit.

The first and second author selected papers with categorical data of karyotyping of the fetuses and/or congenital anomalies and/or follow-up of the children conceived by ICSI with epididymal or testicular sperm versus ICSI with ejaculated sperm or IVF or naturally conceived children. By reading the titles and subsequently the abstracts, the first selection was made. Case reports, reports describing less than five children and reviews were excluded, but the references of all these articles were checked. If in more than one paper essentially the same group of infants were discussed, we selected the paper with the larger group or with a control group. If it was not possible to discover the origin of the sperm (ejaculated, epididymal or testicular), the article was excluded. For instance, the paper of Wennerholm et al. (2006) with a long-term follow-up was not included, because there was a combined group of epididymal and testicular sperm. The first and second author (G.H.W. and D.E.B.) read and selected the studies and extracted data separately. Disagreements were solved in discussion with or without the last author (J.A.M.K.).

Results

The search strategy identified 1662 potentially relevant studies. A flow chart summarizing search results is provided in Fig. 1. In the first selection, 1273 articles were excluded, because they did not fulfil the selection criteria, leaving 389 articles. Following further exclusions, nine
papers with no data overlap were identified for review, and one was eventually excluded from the final analysis.

Two studies discussed karyotyping of fetuses (Bonduelle et al., 2002b; Jozwiak et al., 2004). There were six studies dealing with congenital anomalies (Palermo et al., 2000; Wennerholm et al., 2000; Ludwig and Katalinic, 2002; Bonduelle et al., 2002a; Kallen et al., 2005; Fedder et al., 2007). These studies discussed children born after transfer of embryos fertilized with epididymal sperm or testicular sperm. All studies except the study of Fedder et al. discussed children born after ejaculated sperm as well. Four studies had a control group that consisted of IVF children or naturally conceived children, two studies (Wennerholm et al., 2000; Fedder et al., 2007) had no control groups. Because of the differences between the control groups, we analysed the studies, which described children born after epididymal sperm and after testicular sperm and children born after ejaculated sperm. We used the group with children born after ICSI with ejaculated sperm as the control group. The study of Fedder et al. was excluded from the final analysis, because there was no group of children born after ICSI with ejaculated sperm as a control (comparator) group.

Only one study was found with follow-up of the children until 2 years of age (Bonduelle et al., 1998).

Finally, there were eight articles included in the systematic review. Of these eight studies reviewed, seven originated from Europe and one from the USA. The earliest study was published in 1998, all the others between 2000 and 2005. The size of the study groups ranged between 504 and 4248 children conceived with ejaculated sperm, between 26 and 198 children conceived with epididymal sperm and between 31 and 229 children conceived with testicular sperm. The characteristics of the remaining studies utilized in this systematic review are described in Table I.

**Karyotyping**

Two studies discussed the karyotyping of fetuses (Bonduelle et al., 2002b; Jozwiak et al., 2004). Table II shows the number of fetuses tested and the percentage of abnormal karyotypes per subgroup. Also the relative risks (RRs) and the 95% confidence interval (CI) are given for abnormal karyotypes per study group compared with ICSI with ejaculated sperm. In the study of Jozwiak et al., there were different groups of ICSI with ejaculated sperm: cases of male factor, female factor or unexplained factor. In our analysis, we compared only with the group of ICSI in cases of male factor.

In the study of Bonduelle, prenatal diagnosis was performed in 47% of the ICSI pregnancies. In 37% of the mothers tested, there was an age-related risk (maternal age $\geq 35$ years), the others underwent a prenatal test because of a parental chromosomal anomaly or the possible higher risk related to the ICSI procedure, as explained during the genetic counselling sessions. A total of 1586 ICSI fetuses were tested, of which 47 were abnormal: 25 anomalies were de novo (of these, 10 were sex chromosomal anomalies and 15 were autosomal anomalies) and 22 abnormal karyotypes were inherited (17 of these were transmitted through the father). None of the prenatal chromosomal anomalies was found in fetuses after ICSI with epididymal sperm and three anomalies were found in the fetuses after ICSI with testicular sperm. Two of these anomalies were de novo (3.2%) and one anomaly (1.6%) was inherited. Although the values for de novo anomalies were high, the patient numbers were too small to draw valid conclusions. The chromosomal anomalies such as de novo structural anomalies or sex chromosomal anomalies had a relatively benign character. Abnormal karyotypes were also found in 338 karyotyped children at birth, but none of these was after the use of epididymal or testicular sperm.

In the study of Jozwiak et al., prenatal karyotyping by amniocentesis was recommended universally at 16–20 weeks of gestation for all patients who conceived by ICSI. Only 735 patients of the 1762 (41.7%) underwent amniocentesis, 73% of the mothers were younger than 34 years. They found two (1.5%) abnormal karyotypes (both de novo sex chromosomal) in fetuses after ICSI with testicular sperm. There was no subgroup of fetuses after ICSI with epididymal sperm. No significant difference was found for the frequency of abnormal karyotype between the groups in which ejaculated spermatozoa and testicular spermatozoa were used. Post-natal karyotyping was not described.

**Congenital anomalies**

Three studies (Palermo et al., 2000; Bonduelle et al., 2002a; Ludwig and Katalinic, 2002) defined major malformations as structural defects of the body and/or organs, which affect viability and quality of life requiring medical intervention. Kallen et al. (2005) excluded congenital malformations that are relatively common, variable in registration, and sometimes associated with preterm birth and low birthweight. The following of such conditions were excluded: preauricular appendix, patent ductus arteriosus at preterm birth (37 weeks), single umbilical artery, undescended testicle, congenital hip (sub)luxation and minor skin malformations (mainly nevus). The remaining malformations were classified as ‘weeded’. Wennerholm et al. (2000) applied the following definition of malformation: any congenital malformation defined in the International Classification of Diseases (ICD, 1977, 1992; Chapter 14 in ICD9 and Chapter 17 in ICD 10). In the article of Palermo et al. (2000), it was not clear if the major malformations of the study groups were with or without minor malformations. One article (Ludwig and Katalinic, 2002) described the malformations of live born and stillborn children, as well as of fetuses from spontaneous miscarriages or terminated pregnancies, after the 16th week of gestation. Discrimination for malformations between these groups was not described. In the study, there were 35 (1.0%) spontaneous miscarriages, 18 (0.5%) terminations of pregnancy and 12 (1.4%) stillborns. Three articles (Palermo et al., 2000; Wennerholm et al., 2000; Bonduelle et al., 2002a) stated the number of stillbirths, but they described only the malformations of the live born children. Palermo et al. (2000) report 18 (0.84%) fetal deaths after 20 weeks of gestation and 16 (0.75%) neonatal mortalities. In the study of Wennerholm et al., there was no perinatal death reported in the epididymal retrieved sperm group ($n = 69$), and 1 out of 31 in the testicular retrieved sperm group. Bonduelle et al. presented data on stillbirths in the ICSI group of 49 of 2889 (1.7%) births, and in the IVF group, 40 of 2995 births (1.3%). In the ICSI group, there were abnormal findings at physical examination or autopsy in 8 of the 49 children, but the origin of the sperm was...
<table>
<thead>
<tr>
<th>Authors and publication year</th>
<th>Location</th>
<th>Study design</th>
<th>Study centres</th>
<th>Study groups</th>
<th>Kind of assessment</th>
<th>Age of assessment</th>
<th>Birth years</th>
<th>Maternal age (years, mean ( \pm ) SD</th>
<th>Total sample size</th>
<th>Percentage multiples*</th>
<th>Lost for follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Karyotyping</strong></td>
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<tr>
<td>Bonduelle et al. (2002b)</td>
<td>Belgium</td>
<td>Prospective cohort study</td>
<td>Single centre</td>
<td>ICSI in subgroups</td>
<td>Prenatal karyotyping by chorionic villus sampling or amniocentesis or post-natal karyotyping</td>
<td>Between 12 and 20 weeks of gestation or at birth</td>
<td>1990–2001</td>
<td>33.5 ( \pm ) 4.4</td>
<td>1563</td>
<td>43.1</td>
<td>53%</td>
</tr>
<tr>
<td>Jozwiak et al. (2004)</td>
<td>Turkey</td>
<td>Retrospective case–control analysis</td>
<td>Single centre</td>
<td>ICSI in subgroups</td>
<td>Amniocentesis</td>
<td>Between 16 and 20 weeks of gestation</td>
<td>1992–2002</td>
<td>31.8 ( \pm ) 4.4</td>
<td>632</td>
<td>53.9</td>
<td>58.3%</td>
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<tr>
<td><strong>Congenital anomalies</strong></td>
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<tr>
<td>Bonduelle et al. (2002a)</td>
<td>Belgium</td>
<td>Prospective cohort study</td>
<td>Single centre</td>
<td>IVF and ICSI</td>
<td>Questionnaires and physical examination</td>
<td>At birth and at 2 months</td>
<td>1990–1999</td>
<td>IVF: 32.7 ( \pm ) 4.3; ICSI: 32.2 ( \pm ) 4.1</td>
<td>5743</td>
<td>48.3</td>
<td>1.5%</td>
</tr>
<tr>
<td>Kallen et al. (2005)</td>
<td>Sweden</td>
<td>Retrospective population-based study</td>
<td>Multicentre</td>
<td>IVF and ICSI</td>
<td>Diagnostic codes in Swedish Registry of Congenital Malformations and Swedish Hospital Discharge Register</td>
<td>At birth</td>
<td>1987–2001</td>
<td>NA</td>
<td>14 646</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ludwig and Katalinic (2002)</td>
<td>Germany</td>
<td>Prospective control cohort study</td>
<td>Multicentre</td>
<td>ICSI and natural conceived</td>
<td>Physical examination and ultrasound examination of the kidneys and the hips</td>
<td>Before 8 weeks of age</td>
<td>1998–2000</td>
<td>NA</td>
<td>34 139</td>
<td>39.0**</td>
<td>5.4%</td>
</tr>
<tr>
<td>Palermo et al. (2000)</td>
<td>USA</td>
<td>Prospective cohort study</td>
<td>Single centre</td>
<td>IVF and ICSI</td>
<td>Physical examination</td>
<td>At birth</td>
<td>1993–1999</td>
<td>Ejac.: 36.1 ( \pm ) 5; non-ejac.: 34.4 ( \pm ) 5</td>
<td>3855</td>
<td>41.8</td>
<td>NA</td>
</tr>
<tr>
<td>Wennenholm et al. (2000)</td>
<td>Sweden</td>
<td>Prospective cohort study</td>
<td>Two centres</td>
<td>ICSI in subgroups</td>
<td>Physical examination obtained from medical records</td>
<td>At birth</td>
<td>1993–1998</td>
<td>Epid.: 32.2 ( \pm ) 4.3; test.: 32.7 ( \pm ) 3.7</td>
<td>1034</td>
<td>35.2</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
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<tr>
<td>Bonduelle et al. (1998)</td>
<td>Belgium</td>
<td>Prospective follow-up study</td>
<td>Single centre</td>
<td>ICSI in subgroups</td>
<td>Neurological and psychomotor assessment by a geneticist or paediatrician; a Bayley test</td>
<td>At 2 months, 12 months and 2 years; at ( \sim ) 2 years</td>
<td>1991–1995</td>
<td>Epid.: 32.2 ( \pm ) 4.2; test.: 32.1 ( \pm ) 4.3</td>
<td>163</td>
<td>37.4</td>
<td>77% (at 1 year)</td>
</tr>
</tbody>
</table>

*Percentage multiple children of total study groups.
**Percentage only of the ICSI study group.
NA, not available.
In the IVF group, 2 of 40 stillborn children had congenital abnormalities. A differentiation for malformations between singletons and multiples was not made in any of the studies. Only one study (Kallen et al., 2005) made an adjustment for year of birth, maternal age and number of infants in birth for comparison of malformations between IVF and ICSI (with differentiation in source of sperm).

No studies reported statistically significant differences in the rate of major malformations when they compared ICSI children conceived with ejaculated, epididymal or testicular sperm (Table III). In all five studies analysed together, major malformations were found in 543 out of 12,377 (4.4%) in the ICSI with ejaculated sperm group, 17 out of 533 (3.2%) in the ICSI with epididymal sperm group and 31 out of 670 (4.6%) in the ICSI with testicular sperm group. Table IV shows the RR and the 95% CIs of major malformations in children from ICSI with epididymal sperm compared with ICSI with ejaculated sperm and in ICSI with testicular sperm compared with ICSI with ejaculated sperm.

### Table II Abnormal fetal karyotypes per study group (%) with RR (95% CI) compared with ICSI with ejaculated sperm

<table>
<thead>
<tr>
<th>Authors</th>
<th>Abnormal fetal karyotypes</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICSI with ejac. sperm</td>
<td>ICSI with epid. sperm</td>
</tr>
<tr>
<td>Bonduelle et al.</td>
<td>45/1469 (3.1)</td>
<td>0/31 (0.0)</td>
</tr>
<tr>
<td>Jozwiak et al.</td>
<td>10/504 (1.9)*</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values for de novo anomalies were high after ICSI with testicular sperm, but the patient numbers were too small to draw valid conclusions.

No significant difference between the groups in which ejaculated spermatozoa and testicular spermatozoa was used.

### Table III Major malformations per study group (%)

<table>
<thead>
<tr>
<th>Authors</th>
<th>Major malformations</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICSI with ejac. sperm</td>
<td>ICSI with epid. sperm</td>
</tr>
<tr>
<td>Bonduelle et al.</td>
<td>84/2477 (3.4)</td>
<td>4/105 (3.8)</td>
</tr>
<tr>
<td>Källen et al.</td>
<td>139/4248 (3.3)</td>
<td>5/135 (3.7)</td>
</tr>
<tr>
<td>Ludwig and Katalinic</td>
<td>248/2944 (8.4)</td>
<td>1/26 (3.8)</td>
</tr>
<tr>
<td>Palermo et al.</td>
<td>33/1774 (1.9)</td>
<td>4/198 (2.0)</td>
</tr>
<tr>
<td>Wennerholm et al.</td>
<td>39/934 (4.2)</td>
<td>3/69 (4.3)</td>
</tr>
</tbody>
</table>

No statistical difference (ejaculated sperm versus non-ejaculated sperm; testicular sperm versus epididymal sperm; ICSI versus IVF).

No significant difference (between different methods of ICSI, between standard IVF and ICSI)*

No influence of sperm origin; increased risk after ICSI compared with natural conceived children*

No difference in frequency (between IVF and ICSI; between ejaculated, epididymal and testicular sperm).

Similar rate in different subgroups.

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### Table IV RRs with 95% CI for major malformations of ICSI children with epididymal or testicular sperm compared with major malformations of ICSI children with ejaculated sperm

<table>
<thead>
<tr>
<th>Authors</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epid/ejac</td>
</tr>
<tr>
<td>Bonduelle et al.</td>
<td>1.12 (0.42–2.98)</td>
</tr>
<tr>
<td>Källen et al.</td>
<td>1.13 (0.47–2.72)</td>
</tr>
<tr>
<td>Ludwig and Katalinic</td>
<td>0.46 (0.07–3.13)</td>
</tr>
<tr>
<td>Palermo et al.</td>
<td>1.09 (0.39–3.03)</td>
</tr>
<tr>
<td>Wennerholm et al.</td>
<td>1.04 (0.33–3.28)</td>
</tr>
</tbody>
</table>

Ejac., ejaculated; Epid., epididymal; Testic., testicular; NA, not available.
ICSI with ejaculated sperm. In conclusion, the differences in rate of malformations between the children conceived with ejaculated, epididymal or testicular sperm were similar in all the studies regarding their 95% CI. Note that the study of Ludwig and Katalinic (2002) deviates in the direction of the estimates compared with the other studies. They found a lower RR for major malformations between ICSI with epididymal sperm (based on only 26 infants) and ejaculated sperm and a higher RR between ICSI with testicular sperm and ejaculated sperm than the other studies. They also found an increased risk of major malformations of ICSI children in comparison with naturally conceived children (data were taken from a prospective population-based birth registry in Germany). It was not clear if this increased risk was related to ICSI with ejaculated sperm or to ICSI with non-ejaculated sperm as well. Furthermore, malformations in stillbirths were included in the major malformations, and finally, although the relative size of the ICSI subgroups are quite different between the five studies, the study of Ludwig and Katalinic (2002) deviates most at this point with the smallest group of ICSI with epididymal sperm \( (n = 26) \) and a more than eight times larger group of ICSI with testicular sperm. This again may illustrate the diversity in protocols used in the studies.

Minor malformations were mentioned in two of the studies (Palermo et al., 2000; Wennerholm et al., 2000). In one study (Palermo et al., 2000), it was not possible to discover the origin of the sperm and in the other study (Wennerholm et al., 2000), the minor malformations were distributed evenly in the subgroups with the same incidence as in the general population.

**Follow-up**

Only one article described the follow-up of children born from ICSI with epididymal or testicular sperm (Bonduelle et al., 1998). In the group of children born from ICSI with epididymal sperm, 14 out of 55 children were seen at the age of 1 year for a physical, neurological and psychomotor assessment. There was a large group (77%) lost for follow-up at 1 year and most of the children were still <2 years of age. Two children had a minor developmental problem, one child had an axial hypotonia at 2 months, but was normal at 2 years and one child had a language delay at 2 years. Ten out of 50 children born from ICSI with testicular sperm were seen at 1 year. No problems were described.

**Discussion**

The goal of this study was to review the literature about abnormal karyotypes, congenital anomalies and the follow-up of the children born after ICSI using non-ejaculated sperm. In doing this, we were aware of the differences in study design and study groups. All studies were cohort or case–control studies, none of them was matched. Some were retrospective, by identifying health registers (Kallen et al., 2005), and some were prospective, by physical examination (Palermo et al., 2000; Wennerholm et al., 2000; Ludwig and Katalinic, 2002) or by physical examination as well as by sending questionnaires (Bonduelle et al., 2002a). Only one study (Kallen et al., 2005) made adjustment for some variables, which could act as confounders: year of birth, maternal age and parity, years of involuntary childlessness and maternal smoking in early pregnancy.

Identifying the studies, none of them was more methodologically superior or reliable regarding inclusion and exclusion criteria, sample size, statistical adjustment and kind of assessment. The study of Ludwig and Katalinic (2002) was superior with inclusion of stillbirths and the study of Kallen et al. (2005) was superior with statistical adjustment. Most reliable for kind of assessment was the study of Bonduelle et al. (2002a), by sending questionnaires and physical examination. The studies suggest that the risk ratios may be similar in the subgroups of the ICSI procedures. Points of concern are the possible heterogeneity and the low number of children in some subgroups resulting in large CIs. Possible heterogeneity between the study groups in the articles is related to the age of the mother, indication for PESA or TESE, difference in OA and NOA, the use of fresh or frozen-thawed sperm or embryos and difference in percentage of singletons and multiples.

There is a maternal age-related risk (>35 years) for aneuploidy (Marquez et al., 2000); therefore, it is important to eliminate this possible bias in studies for karyotyping. In the study of Bonduelle et al. (2002b), they found 2.2% de novo anomalies in the age-related group (≥34 years) and 1.2% in the group of the fetuses with mothers aged ≤35 years. The study of Jozwiak et al. (2004) did not find a significantly correlation between the maternal age and the frequency of abnormal karyotypes: 1.4% abnormal karyotypes in the group mothers ≤34 years and 1.6% in the group mothers ≥35 years. Furthermore, there is still a risk for miscarriage after chorionic villus sampling or amniocentesis (Alfirevic et al., 2000). Because of this risk not all women do the test, especially considering the long time they waited to get pregnant. Therefore, a small number of children were karyotyped post-natally (Bonduelle et al., 2002b). The percentage of patients who underwent prenatal diagnosis by chorionic villus sampling or amniocentesis was <50%, although all the patients were recommended to do these tests. In the case of a higher percentage of patients undergoing prenatal karyotyping, especially of the patients with an age of ≤34 years, the real figures of abnormal karyotypes will possibly be lower.

In all studies, there was a classification in epididymal sperm and testicular sperm, but testicular sperm was used in cases of OA and NOA. The study of Ludwig and Katalinic (2002) had an apparently larger group of children conceived by ICSI using testicular sperm than children conceived by ICSI using epididymal sperm. It was not possible to discover in what cases testicular sperm was used and why there were more children after ICSI with testicular sperm. In the case of NOA (severe testicular failure), the spermatozoa are known to show a higher chromosomal aneuploidy rate (Bernardini et al., 2000; Martin et al., 2000; Mateizel et al., 2002; Burrello et al., 2005). The aneuploidy frequency in embryos obtained from NOA is also very high, but similar to embryos from OA (Platteau et al., 2004). Furthermore, it is assumed that genomic imprinting may be less complete when immature gametes are used (Tesarik and Mendoza, 1996). However, all these concerns are theoretical, and the only study that compared the prevalence of congenital malformations in live born children obtained with testicular sperm of NOA men \( (n = 54) \) and children obtained with testicular sperm of OA men \( (n = 188) \) has not shown a difference (Vernaeve et al., 2003).

None of the studies made a difference in using fresh or frozen-thawed sperm or embryos. There was no difference between using fresh or frozen-thawed epididymal or testicular sperm on
the outcome of ICSI, in spontaneous miscarriage rate, pregnancy and delivery rate (Friedler et al., 1997; Habermann et al., 2000; Cayan et al., 2001). No congenital anomalies were found; however, group sizes were too small to draw any valid conclusions. A recent study found a significantly higher major malformation rate in the ICSI cryo embryo live born group than in the fresh ICSI embryo live born group (Belva et al., 2008). Other data suggest that malformation rates after cryopreservation seem to be comparable with those of fresh ICSI and fresh IVF (Aytoz et al., 1999; Wennerholm et al., 1997, 1998). More de novo karyotype anomalies were found prenatally in the cryo ICSI group compared with the fresh ICSI group, but this difference was not statistically significant (Belva et al., 2008).

The total malformation rate (major + minor) has been shown to be higher in IVF/ICSI twins than in IVF/ICSI singletons, which is strongly associated with preterm birth of the twins (Pinborg et al., 2004). Furthermore, the physical health of twins is poorer compared with singletons, because of prematurity and low birthweight (Pinborg et al., 2003; Pinborg, 2005); therefore, especially in follow-up studies, it is important to know whether the children are singleton or a part of a multiple.

There are a number of limitations and sources of potential bias in this review; the studies described here, with the exception of Ludwig and Katalinic (2002), did not mention the stillborn and neonatal deaths and their cause of death and malformations, although this is important to know to draw valid conclusions. Thus, there might be an underestimation of the number of malformations. This is one of the possible biases in the studies. Secondly, the children born after IVF/ICSI are possibly more carefully investigated and malformations are more carefully recorded than in non-IVF/ICSI children; however, in none of the studies, blinding was applied, this will give another bias.

Not all major malformations are found at birth, but will be identified up to 12 months of age; two-thirds of major malformations were detected within the first 7 days of life and about 90% within the first 6 months (Hansen et al., 2002). Only in three of the studies, there was an investigation of the children at birth and at an older age; Bonduelle et al. and Ludwig et al. up to 2 months and Fedder et al. up to 3 months–7 years (mean 20 months). It is therefore possible that in the other studies not all the malformations were identified within the study timeframe. Further, a proportion of children were lost to follow-up (Table I), because not all of them will come for examination or response the questionnaire, introducing further bias.

Although in the studies analysed a large number of ICSI children were included, most subgroups were small, even to detect a 2-fold increase (or decrease) in malformations. For example, if epididymal sperm retrievals account for only 5% of the ICSI children and if the prevalence of birth defects in the ejaculated ICSI group was 6%, you would need 168 children in the epididymal ICSI group in order to detect an RR of 2.0 and 431 to detect an RR of 0.5, with 80% power at 5% level of significance. On the basis of this example, only one-fifth epididymal sperm subgroups and two-fifths testicular sperm subgroups were large enough (n > 168) to detect a 2-fold increase in birth defect risk, if such a large increase should exist. None of the subgroups was large enough (n > 431) to detect a decreased risk of 0.5. In the Netherlands, 500 ICSI children were born between January 2002 and June 2009 after ICSI with epididymal sperm; only 35 children were born between March 2008 and June 2009 after ICSI with testicular sperm, because of a moratorium on this treatment until 2007 (unpublished data). This indicates that the subgroup of epididymal sperm is large enough to detect an RR of 0.5, but it will take over 5 years to reach such a large subgroup of testicular sperm.

Unfortunately, there was only one study about the physical, neurological and psychomotor development of children with a large lost for follow-up rate. So it is not possible to draw conclusions about this item.

**Conclusions**

Although there are no statistical differences in abnormal karyotypes, major malformations and follow-up of the children found in the studies we analysed, it should be considered that the study groups are small and heterogenic with numerous potential biases. We therefore call for standardized methodology for follow-up studies after ART, with a physical examination at birth and psychomotor assessment in childhood. Well-defined groups are necessary, such as ICSI with ejaculated sperm, ICSI with epididymal sperm and ICSI with testicular sperm and a control group of naturally conceived children.

Maybe ESHRE could play a role in this process, by making standards for the methodology of follow-up of children after reproductive technologies.

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**References**


Karyotype, congenital anomalies and follow-up of children born after ICSI


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