During the last 25 years, methods of *in vitro* fertilization (IVF) have become available for most categories of infertile couples. In 1988, Lanzendorf *et al.* reported an experiment with intracytoplasmic sperm injection (ICSI) on human oocytes, and in 1992, ICSI was presented as a successful method of treatment for certain types of male infertility. The ICSI procedure involves the isolation of one particular spermatozoon that is subsequently injected directly into the oocyte with a micropipette. Hardly any experimental knowledge about the ICSI-method was available when the method was first introduced on humans. Several concerns have therefore been raised.

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PERINATAL EPIDEMIOLOGY

**Birth defects in children conceived by ICSI compared with children conceived by other IVF-methods; a meta-analysis**

Rolv T Lie,1* Anita Lyngstadaas,2 Karen Helene Ørstavik,3 Leiv S Bakketeig,4 Geir Jacobsen5 and Tom Tanbo6

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<th>Accepted</th>
<th>15 September 2004</th>
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**Background**

Intracytoplasmic sperm injection (ICSI) is a method of assisted reproductive technology that involves the selection of a single sperm cell and the manual injection of this cell into the egg. The lack of relevant experimental studies, the nature of the technology involving non-natural selection of the fertilizing sperm, and possible damage to the egg have caused concern that ICSI could increase the risk of birth defects. Data from available cohort studies comparing ICSI with standard *in vitro* fertilization (IVF) should be combined to evaluate the risks involved with ICSI.

**Methods**

We reviewed more than 2500 titles and abstracts containing keywords related to ICSI and identified 22 scientific articles with data on birth defects among ICSI-births. A total of four peer-reviewed, non-overlapping prospective cohort studies provided reliable and comparable data on birth defects both for children conceived by ICSI and children conceived by standard IVF. These studies included a total of 5395 children born after ICSI.

**Results**

The pooled estimate of the risk of a major birth defect was a 1.12-fold increase after ICSI when compared with standard IVF (risk ratio = 1.12, 95% confidence interval (CI): 0.97–1.28, *P* = 0.12). There was no marked heterogeneity of risk ratios between these studies (*P* = 0.10). We found no significantly increased risks after ICSI for any of the categories cardiovascular defects, musculoskeletal defects, hypospadias, neural tube defects, or oral clefts.

**Conclusions**

Our analysis does not indicate that the ICSI-procedure represents significant additional risks of major birth defects in addition to the risk involved in standard IVF. The data was limited, particularly on risks of specific categories of defects.

**Keywords**

Birth defects, *in vitro* fertilization, reproduction, meta-analysis

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During the last 25 years, methods of *in vitro* fertilization (IVF) have become available for most categories of infertile couples. In 1988, Lanzendorf *et al.* reported an experiment with intracytoplasmic sperm injection (ICSI) on human oocytes, and in 1992, ICSI was presented as a successful method of treatment for certain types of male infertility. The ICSI procedure involves the isolation of one particular spermatozoon that is subsequently injected directly into the oocyte with a micropipette.

Hardly any experimental knowledge about the ICSI-method was available when the method was first introduced on humans. Several concerns have therefore been raised. Theoretically in the absence of natural selection of the fertilizing...
sperm, any structural damage inflicted by the operation procedure or even the transfer of genes that would not normally have been passed on to a child, could increase the risk of health problems in the children. The risk of birth defects is among these concerns.3,4 If microinjection per se represents a significant risk factor for birth defects one would expect ICSI-babies to have a higher risk of birth defects as compared with other IVF-babies.

There is no general consensus of what constitutes a birth defect. A common, but vague definition of a major birth defect is an anatomical defect that needs treatment or may have functional implications. Traditionally, Down syndrome and other malformation syndromes that may be diagnosed based on their phenotype at birth, are also often included. Diagnostic criteria are, however, vague and may vary by both time and place. For any category of defects, like oral clefts or hypospadias, there is a continuum of severity from serious forms to minor forms with an ambiguous ascertainment. More important, however, are the general problems of ascertaining and reporting of some categories of birth defects. These problems are likely to be reflected in the prevalence of birth defects reported by different studies. A particular concern would be bias due to systematic underreporting of prenatal diagnoses. Attempts to include induced abortions are therefore important.

To identify studies that compared birth defects among ICSI-births and standard IVF-births an extensive search was performed. We were specifically interested in studies that compared ICSI-births with standard IVF-births. The specific comparison we are making between these groups addresses the question of a possible added risk from the microinjection procedure. It also has the advantage of being less confounded by factors related to a couple’s infertility and the high number of twins among IVF-births than studies using other comparison groups. ICSI-births and other IVF-births may have a relatively similar composition of singletons, monzygous twins, dizygous twins, and different types of other plural births.

**Material and Methods**

**Selection criteria and search strategy**

We decided to include in the meta-analysis only prospective studies that contained data on birth defects from both ICSI- and IVF-pregnancies from the same population. The studies should attempt to include all birth defects diagnoses, also among stillbirths and elective terminations.

A systematic review of the literature was started by identifying a broad set of studies through Medline, Embase and the Cochrane Controlled Clinical Database Register (CCTR) for the period January 1988, when the first experimental study of ICSI on human oocytes was published, until May 2002, and by manual searches of relevant journals and of reference lists of retrieved articles. The electronic search used the terms ‘intracytoplasmic sperm injection’, ‘icsi’, ‘microinjection’, to find all studies of ICSI-births and a combination of the terms ‘male infertility’, ‘in vitro fertilization’, ‘reproduction techniques’, ‘chromosomal aberrations’, ‘chromosome abnormalities’, ‘pregnancy outcome’, and ‘developmental disabilities’ to find all studies of ICSI-births and a combination of the terms ‘intracytoplasmic sperm injection’, ‘icsi’, ‘microinjection’, to perform a broad and relevant search of available literature.

We also contacted the international birth defects monitoring networks EUROCAT and ICBDMS in an attempt to identify other unpublished data. Only studies published in English or with an abstract in English and studies with relevant data on standard IVF for comparison were included for further analysis. Decision about inclusion or exclusion of a particular study in our initial screening of studies was based on independent judgement by at least three experts (clinical fertility expert, medical geneticist, two epidemiologists, and one statistician) and disagreement was solved by discussion in the panel.

**Critical and systematic assessment of available scientific literature**

A broad search yielded more than 2500 potentially relevant titles. A manual review of titles and abstracts identified more than four hundred articles that we thought could contain data on relevant outcomes, including articles in English, French, and German. By reviewing these articles we identified a total of 21 peer-reviewed studies that contained data on birth defects for ICSI-babies. During our review process, relevant Norwegian data were also published and we decided to include this publication in our review. None of the 22 studies were randomized.

Eight studies were excluded because they had no proper comparison group of IVF-births5–12 and two studies comparing ICSI-births with naturally conceived babies13,14 were excluded. Two studies only covering live births and not counting elective abortions were excluded.15,16 Four studies that were not prospective were also excluded.17–20 Finally, two studies with data overlapping with the remaining studies were excluded.3,21 Several studies could have been excluded for more than one reason. The remaining four peer-reviewed papers provided comparable and prospective data on major birth defects for ICSI- and other IVF-births.22–25

Most of the data necessary for our analysis were available from the published papers. We identified the numbers of affected and unaffected babies both in the ICSI- and the IVF-group. Numerators for Swedish data were estimated from rates in the paper and from another report from the Swedish health authorities.22,26

We decided to perform sub-analyses of five more specific subcategories of relatively common defects: cardiovascular defects, musculoskeletal defects, hypospadias, neural tube defects and cleft lip or palate. Except for the more specific category of hypospadias, the other four categories correspond to sections of the ICD codes of birth defects diagnoses. All four studies provided a number of cases for these categories except Hansen et al.24 who provided the necessary numbers upon request (corresponding author Dr Jennifer Kurinczuk).

We also identified three sources of large non-peer-reviewed reports with relevant data on birth defects for ICSI- and standard IVF-births. The Australian Institute of Health and Welfare has provided data for Australia and New Zealand since 199827,28 and the Human Fertilization and Embryology Authority has provided data for UK also since 1998.29–31 The association FIVNAT has provided data for France.32 We decided to include these data in addition to the peer-reviewed data in a sensitivity analysis of our results. Provisional case data from Germany were also identified,33 but when we contacted the group, they considered their current data too incomplete for estimation of birth defects risks.

We verified that our meta-analysis complied with the checklist of the MOOSE-guidelines.34
Statistical methods
The meta-analysis program Metan available in the STATA package was used for the main analysis of major birth defects. Analyses were performed with a fixed effects model, but consistency was checked with a random effects model. Analyses involved the calculation of a common estimate of risk ratio between the ICSI- and the IVF-group with 95% confidence interval and a chi-squared test of heterogeneity between the studies. For analyses of specific categories of birth defects with smaller numbers we used methods for exact analyses of categorical data available in the program StatXact. The odds ratios (OR) estimated are close approximations of risk ratios.

Results
The data from the studies that contributed to the meta-analysis on risks of major birth defects are summarized in Table 1. Reported prevalences of major defects varied from 3.0–9.0% across studies. Among the four studies included in our analysis, only one estimated an increased risk of major birth defects after ICSI compared with standard IVF.

In the meta-analysis, the overall risk of a major birth defect was estimated at 1.12-fold for ICSI as compared with standard IVF (Risk Ratio = 1.12, 95% confidence interval (CI): 0.97–1.28, \( P = 0.12 \)) (Figure 1). We found no evidence of heterogeneity of risk ratios between the four studies (\( P = 0.10 \)).

Data from the four studies on cardiovascular and musculoskeletal defects, hypospadias, neural tube defects and cleft lip or palate are presented in Table 2. In meta-analyses of these data we found little evidence of increased risk for any of the five categories. For cardiovascular defects we estimated a 0.85-fold risk in the ICSI-group (OR = 0.85, 95% CI: 0.61–1.16). For musculoskeletal defects (OR = 1.18, 95% CI: 0.80–1.73), hypospadias (OR = 1.13, 95% CI: 0.65–1.92), neural tube defects (OR = 1.12, 95% CI: 0.53–2.34) and cleft lip or palate

### Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ericson (2001)</td>
<td>1.35 (1.11,1.65)</td>
</tr>
<tr>
<td>Bonduelle (2002)</td>
<td>0.93 (0.73,1.18)</td>
</tr>
<tr>
<td>Hansen (2002)</td>
<td>0.96 (0.63,1.48)</td>
</tr>
<tr>
<td>Oldereid (2003)</td>
<td>1.02 (0.60,1.75)</td>
</tr>
<tr>
<td>Overall (95% CI)</td>
<td>1.12 (0.97,1.28)</td>
</tr>
</tbody>
</table>

Figure 1: Meta-analysis of data from selected well-documented studies comparing risks of major birth defects after ICSI and standard IVF. Squares indicate estimated risk ratios from each study and horizontal lines the corresponding confidence intervals. Size of the square corresponds to sample size of the study. A risk ratio higher than one indicates higher risk among ICSI-babies. The diamond indicates the pooled estimate with confidence interval.

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of study</th>
<th>Pregnancy inclusion criteria</th>
<th>Time period</th>
<th>Induced abortions</th>
<th>Follow-up and ascertainment of birth defects</th>
<th>Number of children</th>
<th>Number of children with major birth defects</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ericson (2001)</td>
<td>Population based registry</td>
<td>Not known</td>
<td>1982–97</td>
<td>Yes</td>
<td>One year, centralized registry with multiple sources of ascertainment</td>
<td>1,052</td>
<td>118 (11.1%)</td>
<td>0.93 (0.73,1.18)</td>
</tr>
<tr>
<td>Bonduelle (2002)</td>
<td>Population based registry</td>
<td>Yes</td>
<td>1983–99</td>
<td>Yes</td>
<td>Up to 2 months with different sources</td>
<td>2,905</td>
<td>121 (4.2%)</td>
<td>0.96 (0.63,1.48)</td>
</tr>
<tr>
<td>Hansen (2002)</td>
<td>Population based registry</td>
<td>Yes</td>
<td>1993–97</td>
<td>Yes</td>
<td>One year, centralized registry with multiple sources of ascertainment</td>
<td>301</td>
<td>26 (8.6%)</td>
<td>0.96 (0.63,1.48)</td>
</tr>
<tr>
<td>Oldereid (2003)</td>
<td>Clinical cohort</td>
<td>Yes</td>
<td>1996–98</td>
<td>Yes</td>
<td>One week by centralized registry plus clinical follow-up</td>
<td>553</td>
<td>17 (3.1%)</td>
<td>1.12 (0.97,1.28)</td>
</tr>
</tbody>
</table>
defects with a 5% significance level. However, we found no risk ratios significantly different from one, for our categories. There are differences in the prevalence of birth defects between the different studies. This could be due to general problems of defining what constitutes a birth defect. Inclusion criteria and definitions of major defects probably also differed between the studies included in our analysis.

### Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Total number of children</th>
<th>Children with five specific types of birth defects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ICSI</td>
<td>IVF</td>
</tr>
<tr>
<td>Ericson (2001)</td>
<td>1652</td>
<td>7523</td>
</tr>
<tr>
<td>Bonduelle (2002)</td>
<td>2889</td>
<td>2995</td>
</tr>
<tr>
<td>Hansen (2002)</td>
<td>301</td>
<td>837</td>
</tr>
<tr>
<td>Oldereid (2003)</td>
<td>553</td>
<td>1731</td>
</tr>
</tbody>
</table>

a Total number of ICSI-children and IVF-children was estimated.
b Data obtained from the corresponding author (Dr Jennifer Kurinczuk).

### Table 3

<table>
<thead>
<tr>
<th>Report</th>
<th>ICSI</th>
<th>IVF</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFEA (1998)</td>
<td>1438</td>
<td>20 (1.4%)</td>
<td>1.07 (0.88,1.31)</td>
</tr>
<tr>
<td>Hurst et al. (1999)</td>
<td>4237</td>
<td>118 (2.8%)</td>
<td>1.09 (0.87,1.38)</td>
</tr>
<tr>
<td>HFEA (1999)</td>
<td>2491</td>
<td>41 (1.6%)</td>
<td>2.79 (1.90,4.08)</td>
</tr>
<tr>
<td>FIVNAT (2000)</td>
<td>5478</td>
<td>126 (2.3%)</td>
<td>0.76 (0.48,1.19)</td>
</tr>
<tr>
<td>HFEA (2000)</td>
<td>3232</td>
<td>54 (1.7%)</td>
<td>1.35 (1.11,1.65)</td>
</tr>
<tr>
<td>Hurst et al. (2001)</td>
<td>1830</td>
<td>31 (1.7%)</td>
<td>0.93 (0.73,1.18)</td>
</tr>
<tr>
<td>Ericson (2001)</td>
<td>1652</td>
<td>20 (1.4%)</td>
<td>1.64 (0.99,2.70)</td>
</tr>
<tr>
<td>Oldereid (2003)</td>
<td>553</td>
<td>31 (1.7%)</td>
<td>1.02 (0.60,1.75)</td>
</tr>
</tbody>
</table>

(OR = 1.15, 95% CI: 0.56–2.27) estimated odds ratios were higher than one, but none of the odds ratios were significantly different from one. A test for heterogeneity was significant only for hypospadias ($P = 0.03$), and reflected the apparently opposite tendencies in the studies by Ericson and Bonduelle.

When we included data from non-peer-reviewed reports from Australia and New Zealand (Hurst et al.) and France (FIVNAT) included in a supplementary analysis (Figure 2) to look for consistency with our more restricted overall analysis, a 1.20-fold increased risk of major birth defects was estimated for ICSI-babies (95% CI: 1.09–1.31) (Figure 2). The increase was notable only in the UK data and there was significant heterogeneity in risk ratios between the studies ($P < 0.001$).

### Discussion

We found only weak evidence of an increased risk of major birth defects after ICSI as compared with standard IVF. Our CIs even with the extended data, excluded increases of more than 30% corresponding to a risk ratio of 1.3. This indicates that the microinjection procedure used in ICSI does not represent a large increase in risk of birth defects in addition to the risks involved in IVF in general. The risk of birth defects after IVF in general (including ICSI) may still be higher than for other births as indicated by the study of Hansen et al.

Our study does not eliminate the possibility that risks after an ICSI-procedure could be significantly increased for smaller categories of specific defects. The sub-analyses of five categories of birth defects had limited power to detect small risk differences. We had 80% power to detect a risk ratio of 1.4 for cardiovascular defects, 1.6 for musculoskeletal defects, 1.8 for hypospadias, 2.0 for cleft lip or palate and 2.2 for neural tube defects with a 5% significance level. However, we found no risk ratios significantly different from one, for our categories.

There are differences in the prevalence of birth defects between the different studies. This could be due to general problems of defining what constitutes a birth defect. Inclusion criteria and definitions of major defects probably also differed between the studies included in our analysis. A particular study
could have had problems detecting differences among types of birth defects that were not included in their follow-up. Since follow-up time differed between the studies, some studies may have included fewer birth defects that are difficult to ascertain shortly after birth, like some cardiac defects.

It is, however, also possible that the different prevalences reflected that under-ascertainment of defects was larger for some studies. General under ascertainment that equally affected all cases among both ICSI- and IVF-births would not necessarily distort the risk ratio. If, hypothetically, poor ascertainment selectively removed case types with different risks for ICSI- and IVF-birth, the difference would be masked by ascertainment bias. If ascertainment was different for the ICSI- and for the IVF-babies, bias could also be created. Ascertainment of birth defects is a general problem and may have created bias in our analysis.

Some of the studies included in our analysis show data for the categories single births and multiple births separately. The rate of birth defects is higher among multiple births. However, the proportion of children from multiple births is similar for ICSI-births and IVF-births (38 vs 37% for Hansen et al. and 47 vs 47% for Bonduelle et al.). The proportion of monozygotic twins may also be similar for the two groups. Confounding from the proportion of multiple births is therefore probably small. Information on other potential confounders like smoking, maternal age, etc. was not available from most studies, but chances of confounding may also be small from these sources. Adjustment did not alter the comparison of risks between the ICSI- and the IVF-groups in Hansen's study. The meta-analysis was therefore based on the crude numbers obtained from the different studies.

Our main analyses were performed with a statistical model assuming fixed effects. Estimates and CIs were only marginally different in a random effects model. The risk ratio for the restricted data was estimated at 1.09 (CI: 0.86–1.36, P = 0.48).

A potentially important type of confounding in this analysis is confounding by indication. ICSI is mostly used in cases of male infertility while other IVF-techniques are mostly used for female infertility. There are therefore differences between couples receiving ICSI treatment and couples receiving standard IVF. There are also other differences than the use of microinjection between ICSI and standard IVF treatment.

Ericson and Kallen and Bonduelle et al. included births ten years before ICSI was introduced in their IVF groups. This could have introduced bias since risks may have changed over time.

Non-peer-reviewed data from Australia, New Zealand, and France were fairly consistent with the data of our meta-analysis (Figure 2). Data from UK however, still raise some concern that the risks of ICSI are different from those of standard IVF. It is not unlikely that there are other non-peer-reviewed reports with large numbers available from similar organizations. In our opinion they should still carry much less weight than data from peer-reviewed papers. Careful description of data and critical evaluation is essential to establish credibility of any such dataset.

Several studies have compared ICSI-children with naturally conceived children. The study by Hansen et al. suggested higher risks for ICSI- as well as IVF-babies when compared with naturally conceived babies and a recent study of Katalinic et al. suggested a slightly increased risk for ICSI-births. It is not unlikely that there are similarly increased risks for both ICSI- and other IVF-babies.

This meta-analysis did not find a significantly increased risk for major birth defects in general for babies conceived by the microinjection procedure of ICSI when we compared with standard IVF. There are still concerns related to risks of specific types of defects after ICSI and more well-conducted observational studies are needed. There is also still concern for risks involved with IVF in general.

Acknowledgements

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References


