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Chromosomal abnormalities after ICSI in relation to semen parameters: results in 1114 fetuses and 1391 neonates from a single center

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Extended abstract

Study question:
Is there a relationship between karyotype abnormalities in fetuses and children conceived by intracytoplasmic sperm injection (ICSI) and their father’s semen parameters?

Summary answer:
The de novo chromosomal abnormality rate in pre-and postnatal karyotypes of ICSI offspring was higher than in the general population and related to fathers’ sperm parameters.

What is known already:
Several studies have reported a higher rate of *de novo* chromosomal anomalies in ICSI fetuses but recent data from large cohorts are limited. Overall, reported prevalences of non-inherited karyotype aberrations are increased in fetuses conceived after ICSI and vary between 1.6% and 4.2%. Only few studies focus on the relation between karyotype anomalies in ICSI offspring and semen parameters of their fathers. Furthermore, an increased incidence of abnormal karyotypes in ICSI neonates has been described, but the rates vary widely across studies.

**Study design, size, duration:**

We report on karyotype results from prenatal testing by means of chorionic villus sampling and amniocentesis and results from postnatal blood sampling in offspring conceived by ICSI in a single center. Ongoing pregnancies resulting from an oocyte retrieval between January 2004 and December 2012 and after transfer of fresh ICSI embryos obtained after ejaculated or non-ejaculated sperm were considered. Pregnancies following frozen embryo transfer, oocyte or sperm donation, IVF, PGT and IVM were excluded.

**Participants/materials, setting, methods:**

From the 4816 ongoing ICSI pregnancies, information on pregnancy outcome was available for 4267 pregnancies. Prenatal testing was performed in 22.3% of the pregnancies, resulting in a diagnosis in 1114 fetuses. A postnatal karyotype was obtained in 29.4% of the pregnancies in which no invasive prenatal diagnosis was performed, resulting in a total of 1391 neonates sampled. The prevalence of chromosomal anomalies according to maternal age and semen quality was analyzed with logistic regression. For definitions of normal semen quality, the World Health Organization reference values for human semen characteristics were adopted.

**Main results and the role of chance:**

An abnormal fetal karyotype was found in 29 singletons and 12 multiples (41/1114; 3.7%; 95%CI: 2.7-4.9%): 36 anomalies were *de novo* (3.2%; 95%CI 2.3-4.4), either numerical (n=25), sex (n=6) or structural (n=5) and 5 were inherited. Logistic regression analysis did not show a significant association between maternal age and a *de novo* chromosomal fetal abnormality (OR 1.05; 95%CI 0.96-1.15; P=0.24). In all but one case, fetuses with an abnormal karyotype were conceived by ICSI using ejaculated sperm.
Abnormal karyotypes were found in 14 (1.0%; 95%CI: 0.6-1.7) out of 1391 postnatal samples of children born after ICSI who were not tested prenatally: 12 were de novo anomalies and 2 were inherited balanced karyotypes. The 14 abnormal karyotypes were all found in children born after ICSI using ejaculated sperm.

The odds of a de novo karyotype aberration increased with maternal age when combining pre- and postnatal data (OR 1.11; 95%CI 1.04-1.19). A higher rate of de novo chromosomal abnormalities was found in fetuses and children of couples with men having sperm concentration <15 million/ml (AOR 2.10; 95%CI 1.14-3.78), sperm concentration < 5 million/ml (AOR 1.9; 95%CI 1.05-3.45) and total sperm count < 10 million (AOR 1.97; 95%CI 1.04-3.74).

Limitations, reasons for caution:

We cannot exclude that the observation of a higher prevalence of karyotype anomalies in ICSI offspring compared to literature data in the general population is due to enhanced surveillance after ART given the lack of a control group. Although we did not find more chromosomal anomalies after non-ejaculated sperm, the small numbers do not allow firm conclusions.

Wider implications of the findings:

The observed increased risk of a de novo karyotype anomaly after ICSI conception in couples with poor sperm warrants continued counseling towards prenatal testing.

The current and widespread use of innovative non-invasive prenatal testing will result in larger datasets, adding to a balanced estimation of the prevalence of karyotype anomalies in ICSI offspring.

Study funding/competing interest(s):

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Keywords

ICSI, karyotype, chromosome, prenatal diagnosis, fetus, chorionic villus sampling, amniocentesis
**Introduction**

Since 1991, intracytoplasmic sperm injection (ICSI) has been performed in couples with male infertility. Notwithstanding the birth of more than 5 million children after ICSI worldwide, the invasive nature of this procedure along with the increased use of non-ejaculated sperm have raised concerns about its safety particularly since it is well known that infertile men have a higher prevalence of chromosomal anomalies (Ferlin et al., 2007). While there is substantial literature on the risk of congenital malformations in ICSI conceived neonates (Källen et al., 2005; Wen et al., 2012), the risk of karyotype anomalies after ICSI in unselected fetuses and children remains largely unknown. Older reports published after the introduction of ICSI cover consequently few cases and/or only selected cases (Van Opstal et al., 1995; Testart et al., 1996; Govaerts et al., 1998). Compared with the general population, an increased frequency of chromosomal abnormalities among fetuses and children conceived by ICSI has been reported. But, even more recent studies complicate a robust conclusion because of the limited numbers of ICSI offspring included (Lam et al., 2001; Causio et al., 1999), the different inclusion criteria being applied (only amniocentesis) (Wennerholm et al., 2000; Samli et al., 2003; Jozwiak et al., 2004) or the combination of pre- and/or postnatal samples (Lam et al., 2001; Aboulghar et al., 2001). Loft et al. (1999) described fetal karyotype anomalies after either chorionic villus sampling (CVS) or amniocentesis: although prenatal karyotyping was only performed in 209 fetuses, abnormal results were found in 7 (3.3%) of them. Furthermore, only few studies included paternal sperm characteristics in their analysis though it is reasonable to expect increased frequencies of karyotype anomalies in ICSI offspring from fathers with impaired semen quality.

We reported previously the largest cytogenetic investigation of ICSI fetuses by means of CVS or amniocentesis and observed abnormal results in 47 samples (3.0%) in a cohort of 1586 ICSI fetuses studied between 1990 and 2001 (Bonduelle et al., 2002). A higher rate of de novo chromosomal anomalies was observed in ICSI fetuses, primarily because of a higher number of sex chromosomal anomalies. Moreover, chromosomal anomalies were associated with a lower sperm concentration and motility of the fathers.
To provide overall risk estimates for karyotype anomalies after ICSI, we analyzed pre-and postnatal data from a second single-center cohort of fetuses and children from pregnancies between 2004 and 2013 after transfer of fresh ICSI embryos. The karyotypes were analyzed in relation to maternal and paternal characteristics, including sperm quality and quantity.

Material and Methods

Study population

All ongoing ICSI pregnancies resulting from an oocyte retrieval between January 2004 and December 2012 were included after their first ultrasound examination at 7-9 weeks. Only pregnancies with a transfer of fresh ICSI embryos obtained either after ejaculated or non-ejaculated (testicular or epididymal) sperm were included in this study. Pregnancies obtained from frozen embryo transfer were excluded in order to avoid confounding by the applied technique (slow-freeze versus vitrification which was implemented during the study period) and by subsequent pregnancies within the same couple ensuing from fresh and subsequent frozen-thawed embryo transfer. Pregnancies obtained after oocyte donation, sperm donation, standard in vitro fertilization (IVF) or mixed IVF-ICSI procedures, preimplantation genetic testing (PGT) and in vitro maturation of oocytes (IVM) were also excluded.

In order to provide an exhaustive overview of the risk of karyotype abnormalities in ICSI pregnancies, all abnormal prenatal results after sampling are reported irrespective of the outcome of the pregnancy (second trimester miscarriage, elective termination and delivery of a live and/or stillbirth). In addition, karyotype abnormalities in neonates that were not prenatally diagnosed are reported.

Study design

Before starting any fertility treatment in UZ Brussel, couples are asked to agree to participate in a prospective clinical follow-up study of the children. Prior to the ICSI treatment, couples are offered counseling for evaluation of possible genetic problems and a parental karyotype analysis is advised. When pregnant, couples are counselled to have a prenatal testing performed based on previous findings in our center (Bonduelle et al., 1994,
Different types of prenatal testing are discussed at approximately 6-8 weeks of gestation. In general, chorionic villus sampling is proposed for multiple pregnancies and amniocentesis for singleton pregnancies. Early pregnancy hormonal and ultrasound monitoring is performed in our hospital until 7-9 weeks gestation; the subsequent monitoring of the pregnancy is performed in the couples’ center of choice. Given that in our center many patients come from abroad for their fertility treatment and their pregnancy is taken care of in their country of origin, results of karyotyping and data on the outcome of the pregnancy are often difficult to obtain.

A comprehensive follow-up program for children born after ART has been set up in our center since the introduction of IVF in clinical practice and has been constant over the years. It consists of the combination of questionnaire and physical data. After the expected delivery date, parents are sent a questionnaire. Demographic data are obtained from the parents and obstetric and neonatal data are obtained from the gynecologist and/or pediatrician. In case of a live birth and a residency in Belgium, the parents are invited for a detailed morphological assessment of their 2-4 month old child(ren) at the Medical Genetics outpatient clinic which is run by certified pediatricians. At the time of the visit, the questionnaire is verified with the parents and completed when necessary. Furthermore, parents are asked for permission for a karyotype analysis of their child(ren) when no prenatal testing had been performed (for instance due to the fact that parents wanted to avoid the risk for miscarriage associated with invasive prenatal testing). Parents who are unable to visit our center are asked to provide a report of the clinical examination of their child performed by their general practitioner or pediatrician at the age of 2-4 months.

In contrast to the postnatal dataset, which contains results from neonates residing in Belgium and in which a physical visit had been performed, the prenatal dataset includes also karyotype results obtained in couples residing abroad.

**Semen sample**

The semen sample was analysed according to the fifth edition of the World Health Organization manual for the examination and processing of human sperm (WHO, 2010). Ejaculate volume was measured by weight of the sample in a pre-weighed container. Sperm concentration was assessed by use of the improved Neubauer hemocytometer, after dilution
with a formalin solution. Total sperm count (semen volume x semen concentration) was reported. For sperm motility assessment, the percentage of progressive motility (A+B) was calculated. For reference values of normal semen quality, the World Health Organization reference values for human semen characteristics were adopted (WHO, 2010; Cooper et al., 2010). More precisely, the threshold values for below-reference values were sperm concentration < 15 million per ml (low sperm concentration) and < 5 million per ml (extremely low concentration), progressive motility < 32% and total sperm count < 39 million. Given the broad range of low total sperm count, this was further stratified arbitrarily into 2 categories: to 0-10 million and 10-39 million.

**Karyotype analysis**

Prenatal karyotyping in all Belgian centers was performed by Giemsa-banding on metaphase chromosomes derived from cells obtained by chorionic villus sampling or amniocentesis based on the approach by Rooney (2001). Information on the applied technique is missing for test results from abroad (<1%).

The General Guidelines and Quality Assurance for Cytogenetics for the degree of resolution required for routine amniotic fluid and chorionic villus culture preparations (minimum G-banding quality is 400 bphs) were adopted (2012). The chorionic villi have an outer layer of trophoblastic cells and an inner core of mesenchymal cells. Cytogenetic analysis of the trophoblastic cells (short-term culture) allows rapid analysis, but placental mosaicism may be observed. Analysis of cultured mesenchymal cells takes 2 to 3 weeks (long-term culture). Both short- (15 metaphases) and long-term (15 metaphases) cultures are performed for each. Amniocytes are a mixture of cells derived from fetal skin and mucosae. These cells are cultured and a G-banded karyotype is obtained after an average of 3 weeks. A minimum of 2 (range: 2-20) metaphases per sample was analyzed but extra cells (if one of the homologue pair is involved in an overlap with another chromosome) may have been counted to exclude mosaicism or to exclude a single cell anomaly according to the General Guidelines and Quality Assurance for Cytogenetics issued by the European Cytogeneticists association (2012).

Postnatal karyotyping was performed by G-banding on peripheral blood and by the same protocol as used for prenatal karyotyping.
All parents gave written informed consent before karyotyping. The study was approved by the Ethics Committee of the UZ Brussel (B.U.N. 143201939543)

Statistical analysis

Descriptive statistics are presented as means with their standard deviation for continuous variables and as frequencies and percentages for categorical variables. The results of the prenatal test (normal or abnormal) were compared with a Student t-test for continuous variables and a Fisher’s Exact test for categorical variables. Additionally, 95% confidence intervals (95%CI) were provided as indicated. Characteristics of the mother or father were compared according to the karyotype (normal, abnormal) in their offspring or subgroup (prenatal, postnatal sample) with a t-test for continuous variables, and Fisher’s Exact test for categorical variables. Data analysis was performed using IBM SPSS Statistics version 26.

The association between an abnormal karyotype and maternal characteristics (age, previous miscarriage) was investigated with univariate logistic regression. In parallel, the association between an abnormal karyotype and paternal characteristics including age, type of sperm (ejaculated versus non-ejaculated), any male factor infertility in the couple (either isolated male factor infertility or combined male-female factor infertility), sperm concentration < 15 million/ml, sperm concentration < 5 million/ml, progressive motility < 32%, total sperm count < 39 million, further stratified as total sperm count < 10 million and total sperm count 10-39 million was investigated with univariate logistic regression. Furthermore, to explore the association between an abnormal karyotype and sperm characteristics, multiple logistic regression analysis was performed with maternal age as an additional covariate, because of the widely known impact of maternal age on karyotype anomalies.

For ease of clinical interpretation and in order to allow comparison with previously published data on prevalence rates of karyotype abnormalities (Bonduelle et al., 2002), results are also stratified according to maternal age (< or ≥ 35 years).

Results

Characteristics of the study population
Total cohort

From the 4816 ICSI pregnancies that were ongoing beyond 7-9 weeks, information on pregnancy outcome was available for 4267 (88.6%) pregnancies. Of these, 39 pregnancies were electively terminated and in 24 pregnancies the reason was a karyotype anomaly.

Ninety-eight pregnancies ended in a spontaneous miscarriage: 56 ended before 12 weeks (hence before invasive prenatal testing could have been performed) and 42 after 12 weeks.

In 6 out of these 42 pregnancies ending in a spontaneous miscarriage, prior invasive prenatal testing had been performed resulting in 1 fetus with an abnormal chromosomal result.

Finally, 4130 pregnancies resulted in a delivery: 61 pregnancies ended with one or more stillbirths and 4069 pregnancies resulted in at least one liveborn child.

The mean maternal age at oocyte pick-up was 32.8 ±4.7 years (range 18-44 years) and the paternal age was 36.7 ±6.9 years. Ejaculated sperm was used in 89.9%, testicular sperm in 9.3% and epididymal sperm in 0.8% of the pregnancies to obtain an ICSI embryo. In 70% of the couples, ICSI was performed because of male infertility only (58.2%) or combined male and female infertility (12.0%). The majority of the women (71.8%) were nulliparous. Of all women included in the study, 27.7% had a history of at least one miscarriage.

Couples who opted for karyotype analysis

Pre- and postnatal testing was performed in 950 and 1254 of the 4267 of the pregnancies, respectively. Characteristics of the couples opting for pre- or postnatal karyotype analysis are presented in Table 1.

The mean ages of the women and partners who had undergone prenatal testing were significantly higher than the mean ages of women (32.1 ± 4.5 years; P<0.001) and partners (36.1 ± 6.8 years; P<0.001) who did not opt for invasive prenatal testing. In 67.3% of the couples who had undergone prenatal testing there was also a maternal age-related risk (maternal age ≥ 35 years). In the couples that did not opt for prenatal testing, only 29.8% of the mothers were aged ≥ 35 years. A previous miscarriage was recorded in 29.1% of the women who opted for invasive prenatal testing in the current pregnancy, compared with 27.3 % in women who did not choose such a test (P=0.36).

The mean age of the women whose child was postnatally tested was significantly lower compared to women who opted for invasive prenatal testing (P<0.001).
Karyotype anomalies in prenatal samples

Prenatal testing was performed in 22.3% (950/4267) of the pregnancies, with a total of 1115 fetuses sampled. Prenatal karyotyping with a final diagnosis was available for 1114 fetuses:

781 were singletons and 334 were multiples. Karyotype results of the mothers were available in 55% of tested fetuses of whom 0.65% had an abnormal karyotype. Karyotype results of the fathers were available in 53% of the tested fetuses of whom 1.69% had an abnormal karyotype.

Chorionic villus sampling was performed in 383 fetuses leading to a conclusive result in 372 fetuses, one failure (without further investigation) and 10 inconclusive results for which an amniocentesis was performed.

Amniotic fluid samples were obtained in 740 cases. Of these, 722 were first samples, 14 were repeat samples because of failure or inconclusive results after prior amniocentesis (n=4) or after prior chorionic villus sampling (n=10; see above). In 4 cases the amniocentesis was followed by cord blood sampling and in 2 cases the type of prenatal test was not recorded.

An abnormal fetal karyotype was found in 41 fetuses (29 singletons, 12 multiples) out of 1114 prenatally tested fetuses with a final diagnosis (3.7%): 36 anomalies were de novo (3.2%), either numerical (n=25), sex (n=6) or structural (n=5) and 5 were inherited (0.4%) (Table 2). All inherited anomalies were balanced and 4 out of 5 inherited anomalies were inherited from the father. Ten out of the 41 test results were obtained abroad (Table 3).

Of the 41 fetuses with a chromosomal abnormality, 24 were aborted. One singleton pregnancy ended in a spontaneous miscarriage after a CVS procedure indicated the presence of numerical chromosomal anomaly (trisomy 18). One fetus with an inherited chromosomal abnormality found following amniocentesis was stillborn. The outcome is unknown for 4 fetuses with an abnormal prenatal result because the parents moved or lived abroad. In total, 11 children with a chromosomal abnormality were liveborn. In 7 of these liveborns, no control of the prenatal analysis has been performed: 5 of them are clinically followed in our center and 2 are living abroad and doing well. In 2 children, the (abnormal) prenatal result has been confirmed in a postnatal test and in one child the postnatal control turned out
From one liveborn living abroad we do not have any information on the postnatal course. Details of the parental characteristics, procedures and the pregnancy outcomes in relation to the described chromosomal anomalies are presented in Table 3.

The mean age of mothers of fetuses with a prenatal de novo chromosomal abnormality was 36.6 ±3.5 years. We found a de novo anomaly in 28 of the 763 (3.7%) cases with a maternal age of 35 or above and in 8 of the 351 (2.3%) tested cases with a mother aged < 35 years.

Logistic regression analysis did not show a significant association between maternal age and having a fetus with a de novo chromosomal abnormality (OR 1.05; 95%CI 0.96-1.15; P=0.24).

Stratification of the prenatal karyotype results in relation to maternal age is presented in Table 2.

No statistically significant association was found between the frequency of de novo fetal karyotype anomalies and a previous miscarriage, paternal age and any male factor infertility in the couple (either isolated male factor infertility or combined male-female factor infertility) as an independent variable (univariate logistic regression, data not shown).

**Prenatal karyotype anomalies in relation to sperm characteristics**

The majority (91.8%) of 1073 fetuses with a normal karyotype was conceived after ICSI with use of ejaculated sperm. In all but one case, fetuses with an abnormal karyotype were conceived by ICSI using ejaculated sperm. One numerical chromosomal anomaly was found prenatally after the use of testicular sperm in a total of 89 samples. The frequency of an abnormal de novo prenatal test result was not statistically significantly different after use of ejaculated (35/1021) or non-ejaculated sperm (1/89) (P=0.35). Likewise, the frequency of an abnormal de novo prenatal test result was not statistically significantly different after use of fresh (35/1026) or frozen-thawed sperm (1/83) (P=0.51).

Comparable rates of abnormal de novo fetal karyotypes were found in prenatal samples of couples with men having sperm concentration below versus above the reference limit of 15 million per ml or 5 million per ml. Likewise, comparable frequencies of abnormal fetal karyotypes were found in offspring of men with progressive motility percentages below versus above the reference limit of 32% and in men with total sperm counts below versus above the reference limit of 39 million (Table 4).
Taking maternal age into account, this did not change the results: the frequency of fetal de novo karyotype anomalies was not found higher among men with a sperm concentration below the reference of <15 million per ml (AOR 1.53; 95%CI 0.78-3.02; P=0.22) or <5 million per ml (AOR 1.60; 95%CI 0.80-3.24; P=0.19), nor among men with sperm progressive motility (AOR 1.32; 95%CI 0.66-2.65; P=0.43) or low total sperm count (AOR 1.16; 95%CI 0.59-2.29; P=0.66) (Table 4). A further breakdown of the low total sperm counts showed a non-significant higher rate of de novo chromosomal abnormalities in fetuses of couples with men having a total sperm count below 10 million (AOR 1.60; 0.78-3.28; P=0.20) and a non-significant lower rate in men with a total sperm count between 10 and 39 million (AOR 0.50; 0.14-1.69; P=0.26).

Karyotype anomalies in neonates

A postnatal karyotype was obtained in 1254 (29.4%) out of 4267 of the pregnancies in which no invasive prenatal diagnosis was performed, resulting in a total of 1391 neonates sampled. In 20 pregnancies, both pre- and postnatal karyotyping was performed. Abnormal karyotypes were found in 14 (1.0%; 95%CI: 0.6-1.7) out of 1391 postnatal samples of children born after ICSI who were not tested prenatally: 12 (0.86%; 0.5-1.5) were de novo anomalies and 2 were inherited balanced karyotypes (Table 5). In 6 neonates, an autosomal structural karyotype anomaly was found: one child was diagnosed with Smith-Magenis Syndrome, 1 child displayed global delayed development and 4 were developing normally up to the age of 2 years. In 3 neonates, an autosomal numerical karyotype anomaly was found: 2 children with trisomy 21 and 1 child with a translocation who showed a normal development. In 3 children a de novo sex chromosome abnormality was found.

From the 1391 tested neonates with a normal karyotype, the majority (90.8%) was conceived after ICSI in combination with ejaculated sperm. The 14 abnormal karyotypes were all found in children born after ICSI in combination with ejaculated sperm (n=1265).

Combined results: Karyotypes in pre-and postnatal samples and relation to sperm characteristics

Pre- or postnatal testing was performed in 51.7% (2204/4267) of the pregnancies, with a total of 2505 cases sampled.
Abnormal karyotype results were observed in 55 (2.2%) out of 2505 cases. Of these chromosomal aberrations, 48 (1.9%) were *de novo* and 7 (0.3%) were inherited anomalies. The rate of sex chromosomal anomalies was 0.3% (Table 2).

The mothers of fetuses and children with an abnormal *de novo* karyotype anomaly had a mean age of 35.3 ± 4.4 years, while mothers of fetuses and children without a karyotype anomaly were on average 33.0 ± 4.7 years (P<0.002). We found a *de novo* anomaly in 31 of the 1061 (2.9%) cases with a maternal age of 35 or above and in 17 of the 1444 (1.2%) tested cases with a mother aged < 35 years (P<0.002).

The rate of *de novo* karyotype anomalies increased with maternal age (OR 1.11; 95%CI 1.04-1.19; P=0.002) and higher rates were also found in fetuses and children of couples with men having sperm concentration below versus above the reference limit of 15 million per ml (OR 1.87; 95% CI 1.03-3.38; P=0.03) (Table 4). Tendency to a higher rate of abnormal *de novo* anomalies was found in fetuses and children of couples with men having sperm concentration below versus above 5 million per ml (OR 1.67; 95%CI 0.93-3.02; P= 0.08).

Comparable frequencies of *de novo* chromosomal anomalies were found in offspring of men with progressive motility percentages below versus above the reference limit and in men with total sperm counts below versus above the reference limit.

When taking into account maternal age, a higher rate of *de novo* chromosomal abnormalities was found in fetuses and children of couples with men having low sperm concentration (AOR 2.10; 95%CI 1.14-3.78; P=0.02) and extremely low sperm concentration (AOR 1.9; 95%CI 1.05-3.45; P=0.03) and below-reference sperm counts (AOR 1.70; 95%CI 0.95-3.06; P=0.07), but not in men with below-reference progressive motility (AOR 1.52; 95%CI 0.84-2.74; P=0.16) (Table 4). A further breakdown of the low total sperm counts showed a higher rate of *de novo* chromosomal abnormalities in fetuses and children of couples with men having a total sperm count below 10 million (AOR 1.97; 1.04-3.74; P=0.04) but not in men with a total sperm count between 10 and 39 million (AOR 1.20; 0.52-2.73; P=0.67).

No statistically significant association was found between the frequency of *de novo* karyotype anomalies and a previous miscarriage, paternal age, type of sperm (ejaculated versus non-ejaculated) and any male factor infertility in the couple (either isolated male
factor infertility or combined male-female factor infertility) as an independent variable (univariate logistic regression, data not shown).

Discussion

In the present study we have assessed the prevalence of prenatally and postnatally detected karyotype anomalies in an unselected single-center cohort of ICSI pregnancies obtained after fresh embryo transfer between 2004 and 2012.

In the 1114 fetuses sampled, an abnormal prenatal karyotype rate was found in 3.7%. In a previous report from our group (Bonduelle et al., 2002), abnormal karyotypes were found in 3.0% of the tested ICSI fetuses. The overall study design of both studies is similar but the previous study only included ongoing pregnancies after 12 weeks of gestation, while the present study included pregnancies that were ongoing from the first ultrasound at 7-9 weeks of gestation and thus also included early elective terminations after prenatal testing. As such, the current study provides a better estimate of cytogenetic aberrations after ICSI because results of early performed CVS were also included. This early testing showed 6 chromosomal numerical anomalies (three fetuses with Trisomy 18, two with Trisomy 21, one with Trisomy 13) for which the pregnancy was terminated, but which often end in a spontaneous miscarriage. Noteworthy is that there was a maternal age risk in 4 out of these 6 terminated pregnancies.

The rate of de novo chromosomal abnormalities was 3.2% in all tested fetuses, which is higher than our previously reported rate of 1.6% and is higher than the expected rate of 0.45% in a general newborn population (Jacobs et al., 1992). While the number of de novo anomalies in our previous study grossly consisted of a higher rate of sex chromosome aberrations, this was not the case in the current study. The majority of the de novo chromosomal anomalies reported here were autosomal (30/36), more specifically numerical, and this rate should therefore be interpreted considering the inclusion of early testing (as stipulated above) and also the increased surveillance in ART pregnancies. In addition, the prevalence of de novo chromosomal anomalies in fetuses from mothers without an age risk should be interpreted with caution because of the relatively small sample size. While our previous study included nearly 1000 mothers younger than 35 years, there were only 351
below this age in the current study. The sex chromosomal aberration rate of 0.5% in
the present study is in line with our previous data (0.6% in Bonduelle et al., 2002) but still
higher than the range of 0.23% - 0.27% reported in the general population (Ferguson-Smith

In contrast, the overall karyotype anomaly rate (1.0%) and particularly the sex chromosomal
anomaly rate (0.2%) in our postnatal samples of ICSI children were in line with results from a
large cohort of 56952 newborns from the general population (0.92% and 0.19%
respectively), which is reassuring.

The total rate of 3.7% of prenatally detected abnormal karyotypes cannot be directly
compared to other studies involving ICSI pregnancies. Samli et al. (2003) reported 4.2%
(6/142) fetuses with a chromosomal abnormality, but only 98 out of 1500 couples gave
consent for amniocentesis, which might indicate a potential bias of preferential inclusion of
high-risk couples. Also in the study of Jozwiak et al. (2004) only a small proportion of
patients (735 out of 4405) underwent amniocentesis, but this is still one of the few studies
with a large dataset of prenatal samples. Jozwiak et al. (2004) found a rate of 1.5% of fetal
chromosomal abnormalities after amniocentesis but did not report the abnormal karyotypes
that were detected and possibly aborted earlier in pregnancy. It is therefore possible that
the ICSI related risk is underestimated. Not surprisingly, studies that include prenatal testing
at early stage of the pregnancy report higher cytogenetic abnormality rates. In view of this,
the study of Loft et al. (1999), who included all clinical pregnancies with an intrauterine
gestational sac identified on ultrasound, is comparable to our study-design. They found
abnormal prenatal karyotypes in 3.3%, which is comparable to our rate of 3.7%.

In the same way, comparing the rate of 1.0% postnatally detected karyotype anomalies in
our study with other studies in literature is somewhat troublesome. Aboulghar et al. (2001)
sampled cord blood in a large cohort of 430 ICSI babies and 430 babies born after
spontaneous conception but only 30% of the couples had a maternal age risk and notably
nearly 10% of the ICSI couples were consanguineous. Nevertheless, the authors reported a
significantly higher karyotype anomaly rate in ICSI babies (3.5%) compared to controls (0%;
RR 31.0; 95%CI 1.86-516.45), but the wide confidence interval points to substantial
uncertainty.
In the present study, prenatal testing was performed in 22.3% of the pregnancies, which is considerably lower than our previously reported rate of 47%. The decrease cannot be attributed to a switch to prenatal cell-free DNA screening, as this was only introduced after 2017, but improved non-invasive prenatal examinations including hormonal assays and ultrasound might have played a role.

Not only the total number of invasive prenatal tests has declined, but also the population choosing for this test has changed over the years. In our previous report, 62% of the prenatal tests were obtained at a maternal age ≤35 years, but in the current study this has decreased to 33%. Interestingly, in more than half of the couples opting for invasive prenatal diagnosis in our study, both partners were older than 35 years. Furthermore, a tendency towards less severe male infertility could be noticed in couples opting for invasive prenatal testing: in our previous report, nearly three quarters of the men had sperm concentrations < 20 x 10^6, but this has reduced to only half of the men in the present study. Taken together, we hypothesize that apart from parental age, a previous miscarriage or male factor infertility, other factors make couples opt for or being counselled to prenatal testing which might explain the higher karyotype abnormality rate in this particular population.

While in our previous study nearly half (22/47) of the karyotype abnormalities were inherited, the proportion of inherited anomalies in the present study was lower (5/41). This observation most likely reflects that parents with a risk of transmitting chromosomal aberrations were in the past decade counselled towards preimplantation genetic testing (PGT) which was previously not always possible. Nevertheless, our estimate of 0.4% inherited chromosomal anomalies is in line with results from the literature where the frequency of prenatally detected karyotype anomalies ranges between 0.29 and 0.37% (Hook et al., 1984; Hook and Cross, 1987).

A consistent relation between de novo chromosomal anomalies in ICSI offspring and poor sperm parameters in their fathers could be observed. Combining the results of prenatal and postnatal samples, we found a statistically significant higher risk of a de novo karyotype anomaly when the father had low total sperm counts or low or extremely low sperm concentrations. More specifically, men with a low or extremely low sperm concentration and men with a total sperm count below 10 million have a 2-fold risk of having an offspring with a de novo chromosomal anomaly. This finding is in line with previous results from our center.
These results clearly demonstrate that couples with severe male factor infertility, if not all couples requiring ICSI to conceive, should be counselled towards prenatal testing. Although a variety of karyotype aberrations can be detected nowadays by the highly accurate and non-invasive cell-free DNA prenatal tests, the limitations of these tests and the reporting policy (e.g. in view of sex-chromosomal anomalies) should be explained to the patients. Also, the option of preimplantation genetic testing should be discussed since severe male infertility is known to impair early embryonic competence (Mazzilli et al., 2017). More specifically, since the use of preimplantation genetic testing for aneuploidy (PGT-A) has been associated with reduced miscarriage rates and with the avoidance of the termination of an ongoing chromosomally abnormal pregnancy, its application in patients with severe male factor infertility should be considered.

The strengths of this study are a large sample of karyotyped fetuses from a single center and the inclusion of a large sample of postnatal karyotypes from unselected ICSI children. However, since prenatal karyotyping is evidently only performed after consent, an overestimation of the reported number of prenatal karyotype anomalies can not be excluded as couples with a low risk might have refrained from invasive testing. Anyhow, the current widespread use of non-invasive prenatal testing will probably partly overcome this concern. But since also non-invasive prenatal testing is not compulsory, residual bias will always remain, both in ICSI and in spontaneous pregnancies. Likewise, postnatal sampling was performed in the large majority of neonates solely because they were born after ICSI and rarely because of suspected chromosomal abnormalities. In order to overcome a possible bias, we only included postnatal karyotype samples from children up to the age of 6 months and whom were investigated in our center.

While an overestimation of the reported abnormal karyotypes can not be excluded, one could also argue for an underestimation of the reported karyotype anomalies after ICSI conception. It is estimated that about half of first trimester pregnancy losses are associated with chromosome aberrations in the developing fetus, regardless the mode of conception (Goddijn and Leschot, 2000). Contrary to first trimester pregnancy losses, where no invasive prenatal testing could have been performed, second trimester miscarriages were included. Even though in only 6 of the 42 second trimester pregnancy losses prior invasive testing had been performed showing an abnormal result in one single fetus, there is no indication that
the chromosomal abnormality rate in miscarriages after ICSI conception would be lower than in the general population. But given the few data on karyotypes of miscarriage material after ICSI, we can only speculate that karyotype anomalies were the underlying cause of most of these pregnancy losses which would eventually increase our reported chromosomal anomaly rates. Taken together, any karyotype analysis was performed in 51.7% of the initial cohort of 4267 pregnancies with a known outcome. Pooling the pre- and postnatal karyotype samples adds to the generalizability of our findings regarding the ICSI-related risk.

A limitation of our study is that even our overall large cohort included few fetuses and children born after ICSI with use of non-ejaculated sperm. Only one fetus with an abnormal karyotype was conceived by ICSI with use of non-ejaculated sperm from a total of 89 samples and none of the 126 children born after non-ejaculated sperm was diagnosed with an abnormal karyotype.

The main shortcoming of our study is the lack of a control population. In line with the majority of the available reports, we compared our data with available registers dating back to the 80s and which describe results in mostly younger mothers. Moreover, it is generally known that more intense prenatal karyotype screening is performed after ART compared to the general population. To rule out if an increased frequency of karyotype anomalies after ICSI is linked to increased surveillance, the ideal comparison group would be drawn from a population of spontaneous and unselected pregnancies with a similar screening rate. An alternative is to compare results to outcomes in other ART pregnancies, e.g. conventional IVF (without ICSI) pregnancies, but an increased prevalence of karyotype anomalies in such a comparison is not guaranteed to be attributable to the effect of the invasive ICSI procedure rather than to the infertility status of the couple.

In conclusion, we found a de novo fetal karyotype anomaly rate of 3.2%, mainly related to a higher number of de novo autosomal anomalies rather than to sex chromosomal anomalies. The 1.9% de novo chromosomal anomaly rate in the combined set of pre- and postnatally sampled ICSI offspring is higher compared to the general population and related to the sperm quantity of the father. A higher rate of de novo chromosomal abnormalities was found in fetuses and children of couples with men having below-reference sperm concentration and below-reference total sperm counts.
Author’s roles

The study was designed by F.B., M.B., H.T. and F.H. Data collection was performed by A.B. and F.B. Data of the sperm parameters were provided by G.V. and H.T. Statistical analysis was performed by F.B. and M.R. All co-authors interpreted the data. F.B. drafted the paper and all co-authors contributed to the critical discussion and approved the definitive version of the manuscript.

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