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ARTICLE

Low birth defects by deselecting abnormal spermatozoa before ICSI

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Abstract Consistent evidence from meta-analysis has linked assisted conception by IVF, and particularly intracytoplasmic sperm injection (ICSI), with an increased risk of major birth defects. To compare the risk of major malformations of children born after standard ICSI and after intracytoplasmic injection of morphologically selected spermatozoa (IMSI), a prospective population-based study was conducted from 2005 to 2010. ICSI and IMSI were performed in only one assisted reproduction unit according to its classification of spermatozoa and using fresh semen. Medical data and follow up during 2 years of 1028 infants were collected. Major malformations were identified and classified by an external independent physician. The two groups were similar concerning the parents' age, treatment, number of oocytes recovered, days of transfer, gestational age and birthweight. However, major malformations were significantly lower with IMSI (6/450, 1.33%) versus ICSI (22/578, 3.80%; adjusted odds ratio 0.35, 95% confidence interval 0.14–0.87, $P = 0.014$), mainly affecting boys (adjusted odds ratio 2.84, 95% confidence interval 1.24–6.53, $P = 0.009$). In conclusion, the significantly decreased risk of major birth defects associated with IMSI remained decreased after multivariate adjustment and highlights the beneficial effect of sperm selection before ICSI.

KEYWORDS: birth defects, high magnification, ICSI, IMSI, sperm head

Introduction

Assisted reproduction is associated with an increased risk of pregnancy complications, low birthweight, major malformations and possible imprinting diseases (Cox et al., 2002; Hansen et al., 2002; Hansen et al., 2005; Klemetti et al., 2005). Although intracytoplasmic sperm injection (ICSI) was initially developed in 1991 and has been shown to be an effective treatment for male infertility

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(Palermo et al., 1992), researchers and clinicians have been cautious in its use, especially regarding the outcome of pregnancies. It is generally accepted that the incidence of major malformations in spontaneous conception is about 2.4% (Khoshnood et al., 2010). Most publications report increased risks of birth defects after assisted reproduction treatment compared with spontaneously conceived children (Bonduelle et al., 2005; Rimm et al., 2004). A meta-analysis suggests an increased risk of birth defects among ICSI children compared with those born after IVF or spontaneous conception (Pinborg et al., 2013). The major malformation rate following ICSI is high and can reach 6.5% (Hindryckx et al., 2010). Despite other data reporting an increased risk of birth defects following assisted reproduction treatment, most authors have not emphasized these increased risk estimates because they were not statistically significant (Sutcliffe et al., 2001; Zadori et al., 2003).

Spermatozoa chosen for ICSI may have morphological abnormalities undetectable at 400× magnification, which could decrease the implantation and pregnancy rates (De Vos et al., 2003). However, when using high magnification, several studies have shown an increased blastocyst and pregnancy rates by selecting physiologically normal spermatozoa (Antinori et al., 2008; Bartoov et al., 2003; Vanderzwalmen et al., 2008; Wilding et al., 2011). Moreover, in a recent study, it was shown that, in couples with advanced maternal age, intracytoplasmic injection of morphologically selected spermatozoa (IMSI) performance results in higher blastocyst formation, implantation and clinical pregnancy rates as compared with conventional ICSI (Setti et al., 2012).

The clinical outcomes of children born after ICSI have not been compared with those of children born after IMSI. This work studied prospectively a cohort of 1028 infants, 578 from ICSI and 450 from IMSI with the aim of comparing major malformations in the two groups.

Materials and methods

This study was conducted at the assisted reproduction unit of the Drouot Laboratory. The local ethical committee approved the prospective cohort design on 3 June 2005. The first babies were born in September 2005. All of the participants' parents signed an informed consent before initiation of ovarian stimulation. Data collection was carried out from 2005 to 2010. The studied population of infertile couples included all ICSI and IMSI live births obtained with fresh ejaculated spermatozoa and embryo transfer at days 2, 3 or 5. All pregnant women older than 39 were excluded from the study. All spermatozoa were examined after selection in a bilayer gradient and morphology was assessed by high magnification according to the centre's classification protocol based on (Cassuto et al., 2009). ICSI was first performed if semen parameters were not compatible with IVF, $<$1 million/ml mobile spermatozoa in the centrifuge gradient or after failed IVF attempt. IMSI was done after ICSI failure or when head sperm abnormalities at high magnification scoring 0 according to centre's classification protocol was higher than 40% (Cassuto et al., 2009). Karyotyping was done for all men included in the study with $<$10 million/ml spermatozoa in the ejaculate and for the women in cases of birth defects.

Sperm morphology assessment

Sperm selection for ICSI was performed at a magnification of 400× in a bilayer gradient of isolate (99264; Irvine Scientific). Spermatozoa with severe head shape, mid piece and tail defects clearly seen at this magnification were excluded from microinjection into the oocyte. (Cassuto et al., 2007)

In IMSI, motile spermatozoa were examined at high magnification (6100×) after the same sperm preparation process. The motile spermatozoa were selected in three dimensions via a phase-contrast inverted microscope (IX 71; Olympus, Rungis, France) equipped with Nomarski optics. Based on published data, three parameters were taken into account as major abnormalities criteria: head shape, presence of one or several vacuoles in the nucleus and base shape (Cassuto et al., 2009).

ICSI and IMSI procedures

Technical procedures, including ICSI and IMSI, were performed as previously described (Bartoov et al., 2001; Palermo et al., 1992). Briefly, oocytes were cultured in a 30-μl droplet of medium (Complete Early Cleavage, ECM 90140; Irvine Scientific, Santa Ana, CA, USA) under light mineral oil for embryo culture (Irvine Scientific) for 2—4 h until denudation. Oocytes were then transferred to a tissue culture dish (353004; Becton Dickinson Labware, Franklin Lake, NJ, USA). The examination and scoring of spermatozoa and the injection of oocytes were performed in GWST 5040 culture dishes (Will Co., Wells, Amsterdam, The Netherlands) because a glass-embedded dish is necessary for microscopic observation with Nomarski optic systems.

Data collection

After an information appointment, all patients signed and agreed to receive detailed questionnaires filled out by an independent national association dedicated to the monitoring of assisted reproduction children. The first questionnaire was sent to the family at birth. Specified and tailored medical data were developed by paediatricians, at the age of birth and 4, 9, 12 and 24 months for each child. These items are classically used to evaluate the outcome of children and are easily understandable by the parents. If the answers were incomplete or not precise, couples and medical doctors were directly contacted for further information. An external and independent physician reviewed all criteria without knowing the treatment used. A paediatric geneticist, blind for conception mode, categorized the malformations in major or minor. If the major malformations were linked to a genetic disorder, parental and family investigations were performed in order to classify them in inheritable or de-novo birth defects. Hereditary major malformations and genetic disorder were excluded.

This work used the definition of major malformations if they could lead to functional impairment, surgical treatment and/or death on the basis of Smith and Holmes' classifications (Holmes, 1976; Smith, 1975). The remaining malformations were considered as minor. When two or more malformations were reported in a child, only the most

severe one was considered for statistical analysis. Chromosomal analysis was not routinely performed, either during pregnancy or after birth. For each child, type of treatment (ICSI or IMSI), parental age and origin, number of oocytes retrieved, treatment used, biological characteristics, gestational age, birthweight and gender were noted.

Statistical analysis

Statistical analysis was performed using R version 2.10.1 (Ihaka and Gentleman, 1996). When necessary, data were compared using non-parametric tests, Kruskal–Wallis and Fisher's exact test for continuous and categorical data, respectively. The major malformation versus absence outcome was analysed using a logistic regression which included all pertinent variables (i.e. technique, parents' ages, treatment used, oocyte number, day of transfer, gestational age, newborn sex). The R glm function was used and then the R step function was applied to the result in order to retain only significant variables on the basis of the Akaike information criterion.

Statistical significance was reached with *P*-values <0.05. Differences in continuous data were presented as a mean difference and 95% confidence interval (CI). Differences in categorical data were expressed in terms of odds ratios (OR) and 95% CI.

Results

Of 1039 infants conceived after ICSI or IMSI, included in the study and born from 2005 to 2010, 11 (1.09%) were lost to follow up. Finally, 1028 infants were documented and analysed: 578 from ICSI and 450 from IMSI.

The distribution of Asian, African, Caucasian and mixed couples was similar, and no differences in terms of the residence (urban, suburb, countryside and foreign) were noted.

The fathers' and mothers' ages in the ICSI and IMSI groups were not statistically different (36.3 versus 36.4 years and 32.4 versus 32.8 years, respectively; Table 1). Considering the fathers' age, only three fathers older than 45 (46, 48, and 51, respectively with women 37, 28, and 32) had infants with major malformations. In the whole cohort, mothers' age was not significantly different between the ICSI and IMSI groups (according to both Kruskal–Wallis and ANOVA tests; Table 1). However, comparing age groups, there was a greater proportion of younger mothers in the ICSI group (age groups <25 and 25–30 years, $P < 0.01$; Table 1).

The majority of major malformations occurred in women over 30 years of age (24/28, 86%). The combination of high female age with high male age was not informative because there were only three men aged over 45 years. These men were aged 46, 48 and 51 years and their partners were aged 37, 28 and 32 years, respectively.

Treatment characteristics were similar in ICSI and IMSI groups (Table 2). Fertilization rate (77.7% versus 80.6%, $P < 0.01$) was higher in the IMSI group. No difference was found in the proportion of embryo transfers on day 2/3 and day 5/6: although in the ICSI group there were more day 5/6 transfers than in the IMSI group, the difference was not statistically significant.

With the same prolonged culture conditions in the two groups, there were no major malformations in the IMSI group for day 5/6 transfers (Table 2). There were also no differences in terms of induced abortions due to fetal major malformations between the ICSI and IMSI groups: seven (1.2%) with ICSI versus six (1.3%) in IMSI. Finally, the analysis of all the parameters previously described showed a homogeneous population of the two groups.

The results regarding gestational age (38.21 weeks versus 38.25) and birthweight (2867 g versus 2863 g) showed no significant differences between ICSI and IMSI. Compared with ICSI, this work performed more IMSI as the number of oocytes retrieved increased (Figure 1). As shown in Table 3, among 578 children born after ICSI, 22 (3.80%) presented with major malformations mainly affecting the urogenital system (nine cases: 1.6%) and two children had cardiac disease. Among 450 children born after IMSI, six (1.33%) presented with major malformations: two malformations of the urinary tract, two with a polydactyly, one with club feet and one with a diaphragmatic hernia. Children conceived through IMSI had a statistically significantly lower incidence of major malformations when compared

Table 1 Stratification of mothers and fathers of 1028 infants in ICSI and IMSI according to age.

Age (years)	*Mother*		*Father*	
	ICSI (n = 578)	*IMSI (n = 450)*	*ICSI (n = 578)*	*IMSI (n = 450)*
Whole cohort				
Mean ± SD	32.4 ± 4.0	32.8 ± 3.5	36.3 ± 6.3	36.4 ± 5.9
Minimum–maximum	21–39	21–39	23–65	24–65
Age groups				
<25	26 (4.5)[a]	6 (1.3)[a]	1 (0.2)	1 (0.2)
25–30	108 (18.7)[a]	69 (15.3)[a]	64 (11.1)	32 (7.1)
31–35	248 (42.9)	210 (46.7)	182 (31.5)	163 (36.2)
36–39	196 (33.9)	165 (36.7)	186 (32.2)	150 (33.3)
40–50	0	0	121 (20.9)	85 (18.9)
51–70	0	0	24 (4.2)	19 (4.2)

Values are n (%) unless otherwise stated. NS = non significant.
[a]Statistically significant difference between ICSI and IMSI ($P < 0.01$).

Table 2 Ovarian stimulation, biological parameters and delivery characteristics of 1028 infants in ICSI and IMSI.

Characteristic	*ICSI* (n = 578)		*IMSI* (n = 450)	
	Healthy (n = 556)	*Major malformation* (n = 22)	*Healthy* (n = 444)	*Major malformation* (n = 6)
Duration of stimulation (days)	11.1 ± 1.6		10.9 ± 1.5	
	11.1 ± 1.6	11.2 ± 1.5	10.9 ± 1.5	10.8 ± 1.8
Starting dose FSH (IU)	200 ± 79		209 ± 101	
	200 ± 80	203 ± 58	209 ± 101	222 ± 70
Total gonadotrophin use (IU)	2076 ± 940		2128 ± 1026	
	2078 ± 950	2032 ± 658	2125 ± 1025	2241 ± 1138
Oocytes retrieved	12.7 ± 6.2		12.4 ± 6.3	
	12.6 ± 6.2	15.6 ± 7.0	12.5 ± 6.4	11.2 ± 3.4
Fertilization rate (%)[a]	77.7 ± 17.6		80.6 ± 16.9	
	77.8 ± 17.6	76.3 ± 18.4	80.7 ± 16.9	73.5 ± 13.4
Day-2/3 transfer	423 (73.2)		354 (78.7)	
	406 (70.2)	17 (2.9)	346 (76.9)	8 (1.8)
Day-5/6 transfer	155 (26.8)		96 (21.3)	
	148 (25.6)	7 (1.2)	96 (21.3)	0 (0)
Intensive care				
No	463 (80.1)	16 (2.7)	385 (85.6)	4 (0.9)
Yes	93 (16.7)	6 (1.0)	59 (13.1)	2 (0.4)

Values are mean ± SD or n (%).
[a] $P < 0.01$.

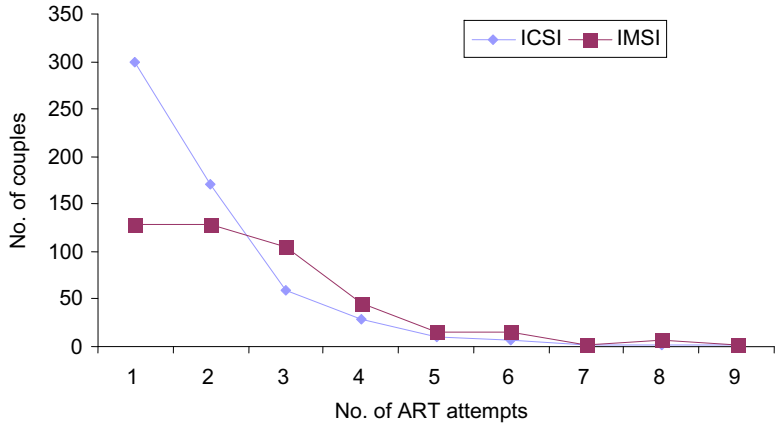

Figure 1 Number of parent couples of 1028 infants in ICSI and IMSI according to the number of ART attempts (S1,S2...S9).

with ICSI group: six (1.33%) versus 22 (3.80%); adjusted OR 0.35 (95% CI 0.14—0.87, $P = 0.014$).

There was no difference in term of gender distribution, (sex ratio 0.92 in ICSI versus 0.84). However, boys had more major malformations than girls in the ICSI group (16 versus six) and the same observation was made in the IMSI group (four versus two). Regarding ICSI and IMSI groups, major malformations significantly more often affected the boys: adjusted OR 2.84 (95% CI 1.24—6.53, $P = 0.009$).

Discussion

As far as is known, this report is the first prospectively analysing major birth defects following IMSI. It has shown a significantly lower incidence of major malformations in children born after IMSI (1.33%) compared with those born after ICSI (3.80%). This work is based on a large cohort of 578 children born after ICSI and 450 IMSI children, both using ejaculated spermatozoa, so the results may be considered overall reassuring.

It is well known that birth defects are more prevalent in children born from IVF compared with those born after spontaneous conception (Bonduelle et al., 2005). These findings are not likely to dissuade many couples for pursuing assisted reproduction. Two recent works suggested that there was a statistically significant increased risk of birth defects in infants conceived using IVF and ICSI, of the order of 30—40%, as compared with birth from spontaneous conception (Davies et al., 2012; Wen et al., 2012). The increased risk of any birth defect is associated particularly with ICSI and remained significant. Differences in male

Table 3 Prevalence of birth defects by organ system in 1028 infants in for ICSI and IMSI.

Organ system	*ICSI (n = 578)*	*IMSI (n = 450)*
Urogenital	9 infants (1.6%) Inguinal hernia (3) Hypospadias (3) Urinary malformations (2) Ectopic testis	2 infants (0.4%) Urinary malformations (2)
Musculoskeletal	3 infants (0.5%) Silver–Russell syndrome Hip dysplasia and dysmorphic features Syndactyly	4 infants (0.9%) Polydactyly of thumbs or toes (2) Club feet Diaphragmatic hernia
Cardiac	2 infants (0.3%) Aortic valvular dysplasia Single jugular vein	
Facial	5 infants (0.9%) Hearing loss: deafness Congenital cataract Blepharophimosis Ptosis Epicanthus Inversus syndrome Unilateral blindness Brown syndrome	
Respiratory	1 infant (0.2%) Congenital cystic adenomatoide	
Brain	1 infant (0.2%) Mental retardation speech delay	
Skin	1 infant (0.2%) Epydermolysis bullosa	
Total	22 infants (3.80%)	6 infants (1.33%)

infertility factors that lead to the use of ICSI may also underlie this association.

The use of high magnification of one spermatozoon before microinjection was introduced more than 10 years ago, highlighting the impact of sperm head morphology in the outcome of ICSI (Bartoov et al., 2001; Berkovitz et al., 2006a). Detailed sperm examination, especially sperm head, at high magnification in real time allows the selection of the best morphological spermatozoa before oocyte injection. According to the Cassuto–Barak sperm classification (Cassuto et al., 2009), there was a clear positive correlation between the head morphology of the spermatozoon, fertilization and expanded blastocyst rate. Ultramorphological criteria with a scoring scale have been established according to the head, vacuole and base of the spermatozoon, which appear to be not related with chromosomal abnormalities (Cassuto et al., 2011) but related to DNA damage, particularly with chromatin decondensation which may affect embryo development (Cassuto et al., 2012). By deselecting the worst spermatozoon, undetectable at low magnification, IMSI seems to provide less major malformations.

In fact, according to this centre's experience of IMSI, it seems more reasonable not to propose the microinjection of the best spermatozoon, whose definition is not clear, but rather to discard the spermatozoa with the highest number of anomalies. A recent study of Tanaka et al. (2012) shows that most vacuoles were not a cave of the plasma membrane or a hollow on the acrosome, but a cavity in the nucleus. The authors suggest that vacuole formation occurs naturally during the process of condensation of sperm nuclei and should not be regarded as degeneration but as physiological changes.

Some reports using IMSI pointed out that human spermatozoa with vacuoles are as inferior in quality as male gametes without vacuoles (Berkovitz et al., 2006b; Vanderzwalmen, 2008) and accordingly pregnancy rate should increase with IMSI. The significance of the universal occurrence of vacuoles in the human sperm head is still not completely known but, in this centre's experience, what is more important is the size of vacuoles and, in accordance with Tanaka et al. (2012), the use of spermatozoa with especially large vacuoles should be avoided. When this centre embarked on its IMSI programme, it was convinced to choose the best spermatozoa on the basis of the work of Bartoov et al. (2001). According to Tanaka et al. (2012), the definition of the best spermatozoon is not clear and still conflicting. Moreover, after having established its own classification protocol, this centre knows that a so-called 'normal spermatozoon' does not necessarily reach the blastocyst stage. On the contrary, the 'worst spermatozoon' with the highest number of anomalies (score 0 in this centre's classification) has never reached the blastocyst stage and must be discarded (Cassuto et al., 2009).

While the presence of birth defects was assessed only at birth in the majority of the studies, here are reported major malformations in two groups of infants according to their conception status (ICSI and IMSI) and a follow up for 2 years, assuming the fact that some malformations become symptomatic only later in life.

The abnormalities in the ICSI group were not detectable by amniocentesis or chorionic villous sampling test. However, the cases of syndactyly in the ICSI group and polydactyly in the IMSI group were detected by prenatal sonography (with no history in the families). PGD was not performed but all pregnancies received usual prenatal screening (tri-test and sonography determination of nuchal translucency).

This study excluded mothers older than 39 because the genetic risk linked to their oocytes is high (Gill et al., 2012). Moreover an oocyte's mitochondria lose a quarter of their

genome, exposing to epigenetic disorders (Bentov et al., 2010).

The data were adjusted or matched for newborn sex, plurality, fathers' age, birthweight and gestational age. Consistent with the literature, no difference regarding birth parameters and incidence of prematurity, low birthweight and very low birthweight rates was found between the two groups.

The major malformations affected mainly the boys in both groups. One explanation might be the hereditary paternal subfertility associated with ICSI and IMSI. This is in accordance with previously published data, where boys from IVF, both singletons and multiples, had major malformations more often than IVF girls (Klemetti et al., 2005). Considering the ovulation induction treatment before ICSI and IMSI, the current study did not find any difference in terms of starting dose, duration of stimulation and total gonadotrophin used, suggesting the homogeneity of the two groups. This is in accordance with some authors who argued that the excess risk of birth defects found in infants born after assisted reproduction treatment may be due to the underlying infertility of the couples seeking treatment rather than the treatments themselves. (Ericson and Kallen, 2001).

The distribution of gestational age was related with birthweight. Urogenital anomalies (hypospadias, ectopic testis, urinary malformations) were the most frequently found, affecting six children in ICSI, which is in accordance with another study (Silver et al., 1999), and only two urinary malformations in IMSI. The use of progesterone during IVF treatment has been offered as one explanation for the increased risk of hypospadia (Hemminki et al., 1999). In the current study, all pregnancies in the two groups were exposed to the same progesterone treatment (600 mg/day) from oocyte retrieval to the human chorionic gonadotrophin test. However, a significantly decreased risk of urogenital malformations was found in infants born after IMSI: this suggests no direct influence of hormonotherapy in the occurrence of these malformations. In the ICSI group, the major malformations included a cardiac disease in two neonates, which was a common finding in other studies (Wen et al., 2012).

One de-novo Silver–Russell syndrome was detected in this study. The syndrome involves hypomethylation of paternally derived H19 and IGF2 locus. These epimutations, mainly affecting paternal alleles, might be associated with assisted reproduction treatment (Ludwig et al., 2005; Niemitz and Feinberg, 2004; Shiota and Yamada, 2005) and was not an uniparental disomy.

Except for the Silver–Russell syndrome, which could be influenced by epigenetic factors and thus be secondary to the assisted reproduction process, epigenetic analysis of children born after assisted reproduction found minor imprinted gene expression imbalances (Palermo et al., 2008). Hearing loss, Blepharophimosis Ptosis Epicanthus Inversus (BPEI) syndrome and hip dysplasia cases were not inherited. Slight delay in psychomotor and language was not associated with Fragile X syndrome. While epidermolysis bullosa is mostly dominant autosomal, one case was tagged *de novo*. One congenital cataract was not inherited and not due to maternal infection.

Since neonatal outcomes of singleton pregnancies appeared to be dependent upon maternal age, the great majority of the 2-year-old children in the ICSI and IMSI groups had normal development, cognitive abilities and motor skill scores, and this may be because the mothers' age was limited to 39 or younger. The major limitation of this study is the low number of anomalies in the two groups. Moreover, this study did not have a control group because spontaneous conceptions are very rare with spermatozoa such as those used in ICSI/IMSI. Further studies are needed to confirm these results.

In conclusion, the use of IMSI minimizes the risk of major malformations in offspring. This follow-up study emphasizes the importance of spermatozoon selection before ICSI. Large-scale studies on the prevalence of IMSI-associated birth defects and long-term follow up of the infants are needed in order to estimate the particular birth defects risks.

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References

Antinori, M., Licata, E., Dani, G., Cerusico, F., Versaci, C., d'Angelo, D., Antinori, S., 2008. Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. Reprod. Biomed. Online 16, 835–841.

Bartoov, B., Berkovitz, A., Eltes, F., 2001. Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection (letter). N. Engl. J. Med. 345, 1067–1068.

Bartoov, B., Berkovitz, A., Eltes, F., Kogosovsky, A., Yagoda, A., Lederman, H., Artzi, S., Gross, M., Barak, Y., 2003. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil. Steril. 80, 1413–1419.

Bentov, Y., Esfandiari, N., Burstein, E., Casper, R.F., 2010. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. Fertil. Steril. 93 (1), 272–275.

Berkovitz, A., Eltes, F., Lederman, H., Peer, S., Ellenbogen, A., Feldberg, B., Bartoov, B., 2006a. How to improve IVF–ICSI outcome by sperm selection. Reprod. BioMed. Online 12, 634–638.

Berkovitz, A., Eltes, F., Ellenbogen, A., Peer, S., Feldberg, D., Bartoov, B., 2006b. Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Hum. Reprod. 21 (7), 1787–1790.

Bonduelle, M., Wennerholm, U.B., Loft, A., Tarlatzis, B.C., Peters, C., Henriet, S., 2005. A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. Hum. Reprod. 20, 413–419.

Cassuto, G.N., Vanderzwalmen, P., Plouchart, J.M., Balet, R., Larue, L., Bouret, D., 2007. How to improve the rate of good quality blastocysts after ICSI in a new approach for classification of motile human spermatozoa. Fertil. Steril. 88, S90.

Cassuto, N.G., Bouret, D., Plouchart, J.M., Jellad, S., Vanderzwalmen, P., Balet, R., Larue, L., Barak, Y., 2009. A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. Fertil. Steril. 92, 1616–1625.

Cassuto, N., Le Foll, N., Chantot-Bastaraud, S., Balet, R., Bouret, D., Rouen, A., Bhouri, R., Hyon, C., Siffroi, J.P., 2011. Sperm fluorescence in situ hybridization study in nine men carrying a Robertsonian or a reciprocal translocation: relationship between segregation modes and high-magnification sperm morphology examination. Fertil. Steril. 96 (4), 826—832.

Cassuto, N.G., Hazout, A., Hammoud, I., Balet, R., Bouret, D., Barak, Y., Jellad, S., Plouchart, J.M., Selva, J., Yazbeck, C., 2012. Correlation between DNA defect and sperm-head morphology. Reprod. Biomed. Online 24, 211—218.

Cox, G.F., Bürger, J., Lip, V., Mau, U.A., Sperling, K., Wu, B.L., Horsthemke, B., 2002. Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am. J. Hum. Genet. 71, 162—164.

Davies, M.J., Moore, V.M., Willson, K.J., Van Essen, P., Priest, K., Scott, H., Haan, E.A., Chan, A., 2012. Reproductive technologies and the risk of birth defects. N. Engl. J. Med. 366 (19), 1803—1813.

De Vos, A., Van De Velde, H., Joris, H., Verheyen, G., Devroey, P., Van Steirteghem, A., 2003. Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. Fertil. Steril. 79, 42—48.

Ericson, A., Kallen, B., 2001. Congenital malformations in infants born after IVF: a population-based study. Hum. Reprod. 16, 504—509.

Gill, S.K., Broussard, C., Devine, O., Green, R.F., Rasmussen, S.A., Reefhuis, J., the National Birth Defects Prevention Study, 2012. Association between maternal age and birth defects of unknown etiology — United States, 1997—2007. Birth Defects Res. A Clin. Mol. Teratol. 94 (12), 1010—1018.

Hansen, M., Kurinczuk, J.J., Bower, C., Webb, S., 2002. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N. Engl. J. Med. 346, 725—730.

Hansen, M., Bower, C., Milne, E., de Klerk, N., Kurinczuk, J.J., 2005. Assisted reproductive technologies and the risk of birth defects — a systematic review. Hum. Reprod. 20 (2), 328—338.

Hemminki, E., Gissler, M., Toukomaa, H., 1999. Exposure to female hormone drugs during pregnancy: effect on malformations and cancer. Br. J. Cancer 80, 1092—1097.

Hindryckx, A., Peeraer, K., Debrock, S., Legius, E., de Zegher, F., Francois, I., Vanderschueren, D., Demyttenaere, K., Rijkers, A., D'Hooghe, T., 2010. Has the prevalence of congenital abnormalities after intracytoplasmic sperm injection increased? The Leuven data 1994—2000 and a review of the literature. Gynecol. Obstet. Invest. 70 (1), 11—22.

Holmes, L., 1976. Congenital malformations. N. Engl. J. Med. 295, 204—207.

Ihaka, R., Gentleman, R.R., 1996. A language for data analysis and graphics. J. Comput. Graph. Stat. 5, 299—314.

Khoshnood, B., Lelong, N., Vodovar, V., Thieulin, A.C., Goffinet, F., 2010. Registre des malformations congénitales de Paris. Evolution sur vingt sept ans (1981—2007). INSERM U953 Université Pierre et Marie Curie, pp. 22—26.

Klemetti, R., Gissler, M., Sevón, T., Koivurova, S., Ritvanen, A., Hemminki, E., 2005. Children born after assisted fertilization have an increased rate of major congenital anomalies. Fertil. Steril. 84 (5), 1300—1307.

Ludwig, M., Katalinic, A., Gross, S., et al., 2005. Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. J. Med. Genet. 42, 289—291.

Niemitz, E.L., Feinberg, A.P., 2004. Epigenetics and assisted reproductive technology: a call for investigation. Am. J. Hum. Genet. 74, 599—609.

Palermo, G., Joris, H., Devroy, P., Van Steirteghem, A.C., 1992. Pregnancies after intracytoplasmic sperm injection of single spermatozoon into an oocyte. Lancet 340, 17—18.

Palermo, G.D., Neri, Q.V., Takeuchi, T., Squires, J., Moy, F., Rosenwaks, Z., 2008. Genetic and epigenetic characteristics of ICSI children. Reprod. Biomed. Online 17 (366), 820—833.

Pinborg, A., Henningsen, A., Malchau, A.K., Loft, A., 2013. Congenital anomalies after assisted reproductive technology. Fertil. Steril. 99 (2), 327—332.

Rimm, A.A., Katayama, A.C., Diaz, M., Katayama, K.P., 2004. A meta-analysis of controlled studies comparing major malformation rates in IVF and ICSI infants with naturally conceived children. J. Assist. Reprod. Genet. 21 (12), 437—443.

Setti, A.S., Figueira, R.d.C., de Almeida Ferreira Braga, D.P., Iaconelli, A., Borges, E., 2012. IMSI is beneficial in cases of advanced maternal age: a prospective randomized study. Reprod. Biomed. Online. http://dx.doi.org/10.1016/j.rbmo.2012.10.020.

Smith, W., 1975. Classification, nomenclature and naming of morphologic defects. J. Pediatr. 87, 162—163.

Shiota, K., Yamada, S., 2005. Assisted reproductive technologies and birth defects. Congenit. Anom. 45, 39—43.

Silver, R.I., Rodriquez, R., Chang, T.S.K., Gearhart, J.P., 1999. In vitro fertilization is associated with an increased risk of hypospadias. J. Urol. 161, 1954—1957.

Sutcliffe, A.G., Taylor, B., Saunders, K., Thornton, S., Leiberman, B.A., Grudzinskas, J.G., 2001. Outcome in the second year of life after in-vitro fertilization by intracytoplasmic sperm injection: a UK case-control study. Lancet 357, 2080—2084.

Tanaka, A., Nagayoshi, M., Tanaka, I., Kusunoki, H., 2012. Human sperm head vacuoles are physiological structures formed during the sperm development and maturation process. Fertil. Steril. 98 (2), 315—320.

Vanderzwalmen, P., Hiemer, A., Rubner, P., Bach, M., Neyer, A., Stecher, A., Uher, P., Zintz, M., Lejeune, B., Vanderzwalmen, S., Cassuto, G., Zech, N.H., 2008. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reprod. Biomed. Online 17, 617—627.

Wen, J., Jiang, J., Ding, C., Dai, J., Liu, Y., Xia, Y., Liu, J., Hu, Z., 2012. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil. Steril. 97, 1331—1337.

Wilding, M., Coppola, G., di Matteo, L., Palagiano, A., Fusco, E., Dale, B., 2011. Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. J. Assist. Reprod. Genet. 28 (3), 253—262.

Zadori, Z.J., Kozinszky, Z., Orvos, H., Katona, M., Kaali, S.G., Pal, A., 2003. The incidence of major birth defects following in vitro fertilization. J. Assist. Reprod. Genet. 20, 131—132.

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