Outcomes for offspring of men having ICSI for male factor infertility

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Since the introduction of intracytoplasmic sperm injection (ICSI) using single sperm isolated from testicular tissue in men with obstructive and non-obstructive azoospermia, or using ejaculated sperm in those with poor semen quality, there have been concerns that this might have adverse effects on the offspring compared to conventional in vitro fertilisation (IVF) and natural conceptions. ICSI is done for reasons other than male factor infertility, and on the whole has not been shown to have any more negative effects than those seen with IVF. There have however, been few very studies of ICSI with a focus on, or large enough numbers to examine, the specific outcomes associated with male factor infertility. From the limited information available in relation to the source of the sperm and aetiology of infertility in the presence of ICSI, there appears to be no increased risk of congenital malformations. There is, however, a small increase in both de novo and inherited chromosome abnormalities. In terms of growth and neurodevelopment, there are very few studies, and so far, no adverse outcomes have been found in young children whose fathers have a sperm defect. The origin of the sperm used in ICSI does not have a major influence on the early life outcomes for the offspring, but transgenerational and epigenetic effects remain unknown. When the male factor infertility is known or thought to be due to a Y-chromosome deletion, this information should be given to the young male offspring at a time that will ensure his own reproductive health and plans are optimized.

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INTRODUCTION

The use of intracytoplasmic sperm injection (ICSI), with both ejaculated and non-ejaculated sperm, circumvents natural selection barriers against sperm with physiological or genetic abnormalities. There is direct injection of the sperm into the vitellus of the oocyte during metaphase II, which is technically invasive to the oocyte and may cause chemical or mechanical damage, such that errors occur in chromosome pairing because of disturbances of the meiotic spindle.

Although there are many articles written about outcomes associated with ICSI as a whole, as ICSI is being used increasingly in the absence of abnormal semen parameters or male factors, the role of male infertility per se in the analysis of outcomes has become less evident. There is often a masking of data pertaining to male-factor infertility as ICSI is being used to increase the odds of successful live birth in couples with combined causes. The source of the sperm and type of infertility are seldom examined and the results are not stratified according to these potentially important exposures.

The major subgroups of male infertility, obstructive azoospermia (OA), non-obstructive azoospermia (NOA) and severe oligozoospermia (SO) seem likely to have different implications in terms of outcomes for the offspring. In the case of OA, there will be normal spermatogenesis but a mechanical problem preventing passage of the sperm, such as in men who have had damage to the epididymis because of infection or inflammation or have had vasectomies. OA can also be the result of congenital bilateral absence of the vas deferens in men with or without clinically evident cystic fibrosis. With NOA on the other hand, there is abnormal spermatogenesis as a result of testicular dysfunction. This is often due to genetic defects which affect production of sperm, such as Klinefelter’s syndrome, Y-chromosome microdeletions and some translocations. For those with oligozoospermia, the actual sperm concentration is indicative of the potential presence of a genetic disorder, specifically a Y-chromosome microdeletion in those with \( <1 \times 10^6 \) sperm ml\(^{-1}\). Overall, there is variation in reports of the frequency of abnormal karyotypes in infertile men, most estimating between 8% and 10%. This variation persists when specifically looking for the Yq deletions, but it appears that 5%–10% of men with NOA or SO may have such a deletion. There are also many unrecognized genetic and unknown non-genetic causes of NOA and oligozoospermia.

Using the ICSI procedure, it is possible for males with OA, NOA and SO to father their own offspring using non-ejaculated sperm retrieved by microsurgical testicular sperm extraction (TESE) or epididymal sperm aspiration (MESA). A number of adverse outcomes have been postulated as a result of using sperm retrieved in these ways. First, TESE may result in extraction of immature sperm, which in turn might lead to an increased miscarriage rate and adverse effects on embryo development. This has in fact been refuted in a recent study which did not demonstrate an increased miscarriage rate in pregnancies resulting from TESE over and above that seen with MESA, both
being 17%–18%.\textsuperscript{10} Use of immature sperm may also result in imprinting errors associated with disruption of normal methylation erasure and re-establishment at critical stages of development.\textsuperscript{11–13} Another purported problem associated with the extraction of individual sperm is the potential use of aged epididymal sperm, which may lead to chromosomal errors. The third and most likely cause of adverse outcomes is the non-natural selection of a single sperm (ejaculated or non-ejaculated) which may lead to the passage of genetic defects that have caused the male infertility in the first place.\textsuperscript{4,14}

It has been interesting to see over the years that most studies comparing ICSI with \textit{in vitro} fertilisation (IVF) generally report no increased risks associated with ICSI over and above those associated with IVF in any particular outcomes for the offspring.\textsuperscript{15–19} There has been a recent study from Norway reporting that ICSI pregnancies have a lower risk of iatrogenic moderate preterm delivery, than IVF pregnancies.\textsuperscript{20} Although the preterm IVF pregnancies (8.6% overall) had more congenital malformations than ICSI and may have contributed to the obstetric intervention (14% compared with 9.6%), there is no convincing reason for this. In previous studies, an increased rate of preterm deliveries has not generally been reported as being significantly different.

In terms of obstetric outcomes, a retrospective cohort record linkage study from Australia in 2010 showed that singleton ICSI pregnancies have less adverse outcomes (post-partum haemorrhage, placenta praevia and antepartum haemorrhage) than IVF, although the difference is of borderline significance.\textsuperscript{21} This study highlighted the findings that are now emerging consistently, showing that adverse outcomes are most frequently associated with fresh embryo transfers in stimulated cycles as distinct from embryo transfers in natural menstrual cycles, as is the usual case with frozen–thawed embryo transfers.\textsuperscript{22} This may ultimately be a far more important predictor of outcomes than the IVF or ICSI technologies.

Although there are no marked differences between ICSI and IVF outcomes, the same differences between ICSI and spontaneous conceptions exist as between IVF and spontaneous conceptions. The over-arching obstetric and perinatal concerns for all twin and higher order pregnancies remain for ICSI as well as for IVF pregnancies.\textsuperscript{23} Overall, compared with spontaneous conceptions, both ICSI and IVF are associated with lower birth weight, preterm delivery and an increased prevalence of congenital malformations.\textsuperscript{24,25} There have also been a variety of medical and psychosocial outcomes examined beyond the perinatal period demonstrating no specific differences between IVF- and ICSI-conceived children in growth,\textsuperscript{26} neurological,\textsuperscript{27,28} cognitive\textsuperscript{29} and socio-emotional development\textsuperscript{30} and physical health.\textsuperscript{31}

From here on, this review will present studies that have specifically examined aspects of male infertility, noting that there is limited published material available on offspring outcomes in the presence of male infertility. The outcomes of interest are congenital malformations overall and chromosomal defects specifically, the inheritance of male infertility as indicated by non-chromosomal markers and some sparse data on growth and neurodevelopment.

CONGENITAL MALFORMATIONS

Overall defects

Research into determining the risk of congenital malformations in babies conceived after IVF or ICSI has demonstrated clearly an overall increased risk, but is limited in terms of the ability to identify risks for specific birth defects, because of the inherent problem of small sample sizes. In addition, some studies do not report on prenatally diagnosed conditions for which there may be a termination as they have no record of events prior to 20 weeks of gestation; this creates a major underascertainment of real risks. Some believe there is overvigilance in clinical review of babies conceived by IVF or ICSI and therefore, overascertainment of less severe defects. Probably the biggest problems in comparing findings and coming up with meaningful risk figures are: the use of different definitions for congenital malformations, major versus minor; different coding systems; and different inclusion/exclusion criteria on the basis of such categorisation. We have recently published on another method of classification identifying congenital malformations (blastogenesis defects) that are most likely to be caused by events in the first 4 weeks of life.\textsuperscript{25} With all this in mind, there have been very few studies that have examined congenital malformations in relation to male infertility that provide convincing figures on the exact risks.

One of the first attempts to record congenital malformations associated with TESE and MESA came from the Brussels group, published in 1996.\textsuperscript{32} They found none among 29 children conceived using TESE, 1 out of 29 (3.4%) MESA and 21 of 797 (2.6%) standard ICSI. Such small numbers for the non-ejaculated sperm groups are almost meaningless in the context of congenital malformations and it has taken many years for numbers to build up at IVF centres and within registries so that comparisons can be made.

In 2001, a case–control study in the United Kingdom comparing rates of both major and minor abnormalities in 2-year-old offspring of oligozoospermic and non-oligozoospermic men was published.\textsuperscript{1} There was a large difference in overall rate of congenital malformations between the two groups, with the oligozoospermic group faring much worse (25 of 121, 21%) than the non-oligozoospermic group (7 of 87, 8%) ($P=0.02$). Their data suggested that male urogenital problems may have been higher in the former group, but the authors stressed the need for larger registry-based studies.

Another study published in 2003, this time from Germany, focused on the source of the sperm (i.e., epididymal or testicular) and indication for ICSI.\textsuperscript{33} This study had slightly higher numbers, comparing outcomes for children conceived using 1785 ejaculated sperm with 147 testicular, but only 12 epididymal sperm. They reported no statistically significant difference in major malformations between different sperm sources, and in fact the only congenital malformations found in singletons were among the ejaculated sperm group (8.5%). No individual malformations were analysed. They also examined their data by indication for ICSI, the vast majority being for severe idiopathic oligozoospermia, 68 for OA, 86 for NOA, 204 for failed IVF and 207 for other indications. They found no significant differences in risk of major malformations between the groups, with the OA rate being 8.4% and the NOA being 7.1%.

A later (2005) study from Singapore stratified results on the basis of sperm count, motility and morphology for major malformations.\textsuperscript{34} They too, like the United Kingdom group, found slight evidence of an increase in adverse outcomes with severe oligozoospermia, but the numbers are far too small to place importance on.

In a European multicentre study reporting a follow-up of 300 5-year-old children conceived using ICSI, including 266 whose fathers were infertile, a general statement was made that the rate of major malformations was no greater than that in the spontaneously conceived group, 14/252 (5.6%) compared with 8/266 (3.0%). In fact, there is almost a doubling in the odds ratio (1.90; 95% confidence interval: 0.73–5.04) and, with a larger sample size, this may have become significant.\textsuperscript{35}

A study from Norway (2007) analysed malformation data in children conceived using epididymal or testicular sperm and they
observed an increased rate of hypospadias compared with the general population, with three boys out of 187 having hypospadias (1.6%; 95% confidence interval: 0.33%–4.7%). This study included both twins and singletons and the numbers were again very small. Male urogenital abnormalities have always been suspected of being increased because of the possible perpetuation via ICSI of fertility problems in the offspring of men with compromised spermatogenesis. Interestingly, however, important earlier studies of ICSI in general did not find such an increase in male urogenital abnormalities and neither have recent ones. Several studies followed the ICSI outcomes for children conceived using testicular sperm, and these were compared to each other and combined for a comparison with ejaculated sperm outcomes. They compared outcomes for these 370 children with those conceived using ejaculated sperm in 1168 IVF and 1105 ICSI. No differences were observed in live and stillbirth rates, neonatal deaths or deaths up to 1 year of age. Neither were there any marked differences in rates of plurality, low birth weight, average gestation at birth or congenital malformations. The actual proportions of major malformations in 243 singletons were 3.3% in the epididymal group and 4.1% in the ejaculated ICSI group. An additional 8% and 12% of minor malformations were identified, respectively.

Another publication in 2011, from the Belgium cohort, compared outcomes for children conceived using testicular or epididymal sperm, including congenital malformations. The Dutch multicentre study collected detailed information on a comparatively large number of children (n = 370) conceived with epididymal sperm. They compared outcomes for these 370 children with those conceived using ejaculated sperm in 1168 IVF and 1105 ICSI. No differences were observed in live and stillbirth rates, neonatal deaths or deaths up to 1 year of age. Neither were there any marked differences in rates of plurality, low birth weight, average gestation at birth or congenital malformations. The actual proportions of major malformations in 243 singletons were 3.3% in the epididymal group and 4.1% in the ejaculated ICSI group. An additional 8% and 12% of minor malformations were identified, respectively.

Possible indicators of inherited male infertility

Serum inhibin B. To further examine any possibility of male infertility in the father being passed on, patients having ICSI were compared with non-ejaculated men having ICSI. It is known that about 1 in every 1000 males has a Y-chromosome submicroscopic deletion rendering him infertile, but with the use of testicular sperm aspiration and MESA, their fertilisation or pregnancy rates are not decreased compared with non-Y-deleted men having ICSI. It is known that there is no increase in size of the deletion and the fertility problem will likely be the same as in the father.

Digit length and azoospermia. A study was designed to look for transmitted fertility problems in ICSI-conceived children who may show perturbations in finger development, thus reflecting the suggested genetic link between digit length and azoospermia. Digit measures were taken on 211 ICSI and 195 controls, and indeed shorter finger lengths than expected for their height were seen in both females and males conceived by ICSI. The importance of this in terms of fertility potential is not known.
GROWTH AND NEURODEVELOPMENT
The Singapore cohort of ICSI-conceived children were analysed on the basis of sperm parameters in their fathers for differences in the Bayley scales measuring cognitive, language and motor development. They were approximately 2 years of age and, like another contemporaneous study using the Griffith scale of mental development, no problems were found in relation to sperm concentration, morphology or motility. In other words, the severity of the sperm defect in the father did not affect neurodevelopmental outcome in these young children.

In 2006, the European multicentre study examined the growth and cognitive development in 5-year-old children and revealed no differences in height and weight or IQ with epididymal and testicular sperm groups or with paternal sperm concentration.55

Most recently, the Dutch multicentre study cited earlier on 370 children conceived with epididymal sperm,56 had excellent response rates to a questionnaire sent to mothers and to an invitation for clinical review of children to 1 year and even some who reached 4 years of age in the study period. This allowed them to report on psychomotor development using the Bayley scales and again there were no differences found comparing outcomes to children conceived using ejaculated sperm in 1168 IVF and 1105 ICSI.

CONCLUSIONS
After careful review of the literature, it is evident that there is limited information available pertaining to offspring outcomes attributable specifically to male infertility and sperm parameters. Many of the studies have very small sample size for offspring conceived using epididymal sperm in particular. In summary, however, it appears that the origin of the sperm used in ICSI does not have a major influence on early-life outcomes for the offspring. The longer-term effects, such as transgenerational passage of male infertility through genetic or epigenetic processes, remain unresolved. It is recommended that genetic counselling should occur before treatment if there is a risk of transmitting a genetic defect, albeit very slight, and that embryo or fetal testing may also be ‘appropriate in selected cases’. Such cases are those where there is use of non-ejaculated sperm for severe oligo- and azoospermia.

Boys who have been conceived using sperm from fathers with a Y-chromosome deletion should be advised at a suitable age of their inherited infertility so that they can instigate their own reproductive planning process. The ‘suitable’ age may vary from child to child, and more research is needed to inform best practice in terms of age of disclosure of ICSI and particularly where there is a Y-chromosome deletion known or suspected.

Of course, the advice to parents having ICSI for any reason must include information about the risks of the well-described and –recognized adverse perinatal outcomes, particularly the increased risk of congenital malformations compared to spontaneously conceived children. This risk could be about 30% greater than the general population, giving a frequency of as high as 8%–10% depending on the categorisation of the malformations. It is probable that this information will seldom trouble the prospective parents enough to deter them from going ahead with ICSI, but at best they are doing so with some knowledge of the possible short-term risks. They must also understand that there is very little knowledge about the longer-term risks associated with assisted reproductive technology, especially in the presence of male infertility.

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The author declares no competing financial interests.


46 Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. Hum Reprod 2009; 24: 1330–8.


