The safety of intracytoplasmic sperm injection and long-term outcomes

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Abstract

The pioneering of intracytoplasmic sperm injection (ICSI) approximately 25 years ago revolutionized the treatment of infertile couples. Today, ICSI remains an indispensable part of assisted reproductive treatments (ART) and has resulted in the birth of millions of babies. The 25th anniversary of ICSI marks a chronologic landmark in its evolving history. This landmark also serves as an opportunity to thoroughly appraise the safety of ICSI and analyze the long-term outcomes of ICSI-conceived children. In this review, we collate and analyze salient data accrued over the past 25 years pertaining to the long-term safety of ICSI and ICSI conceptions. We also evaluate the effects of ICSI on the perinatal outcomes, congenital malformation rates, cognitive development and reproductive health of ICSI-conceived neonates, children, adolescents and adults, respectively. In doing so, we also highlight the existence of potential confounders and biases that frequently obscure the interpretation of clinical follow-up studies.

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Introduction

The birth of the first babies using intracytoplasmic sperm injection (ICSI) in 1992 marked a new era of assisted reproductive technologies (ART). Although successful clinical and laboratory protocols for *in vitro* fertilization (IVF) were established prior to the inception of ICSI, almost 40% of all IVF cycles were inundated by poor fertilization or complete fertilization failure even in the presence of an adequate number of oocytes (Cohen *et al.* 1984). This was especially evident in couples undergoing IVF for severe male factor infertility (Cohen *et al.* 1989). As highlighted by Rosenwaks and Pereira in this special edition, micromanipulation techniques such as zona drilling, partial zona dissection and sub-zonal insemination were historically utilized to circumvent the zona pellucida and increase fertilization rates in IVF cycles. However, each of these techniques had their share of limitations, which in turn served as the basis for the development of ICSI (Palermo *et al.* 2014a). ICSI not only bypassed the zona pellucida with high precision, but it also increased the fertilizing potential of a single spermatozoon by injecting it directly into the cytoplasm of an oocyte (Palermo *et al.* 1992). The technique was adopted by several clinics throughout the world, and it soon became apparent that ICSI was

capable of fertilizing almost every mature oocyte that was injected, irrespective of the male partner's semen parameters (Palermo *et al.* 1995). Thus, ICSI has become quintessential to modern ART. Global data from the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) estimate that almost 66% of >4,461,309 ART cycles between 2008 and 2010 utilized ICSI (Dyer *et al.* 2016). In the United States, ICSI utilization has increased from 76.3% in 1996 to 93.3% in 2012 for male factor infertility and 15.4% in 1996 to 66.9% in 2012 for non-male factor indications (Boulet *et al.* 2015).

In this special edition, Palermo and coworkers have collated almost 25 years' worth of clinical and research data pertaining to ICSI. They have also highlighted several adjuncts that have not only standardized ICSI globally, but also improved the results obtained with ICSI. However, despite the standardization of ICSI over the past 25 years, concerns about the technique have arisen, even though many are theoretical. These concerns stem from the perception that the injected spermatozoon is selected arbitrarily and that the physiologic steps of zona pellucida binding and oolemma fusion are completely bypassed (Palermo *et al.* 2008, 2014b). Furthermore, there is still concern whether the use of

suboptimal spermatozoa may result in the transmission of undesirable genetic traits, leading to genomic or phenotypic abnormalities in the progeny (Ludwig & Katalinic 2002). In this review, we appraise and analyze the existing medical literature pertaining to the safety of ICSI. In addition to commenting on its technical safety, we scrutinize the long-term clinical data associated with the perinatal, developmental and health outcomes of ICSI children.

The safety of ICSI

It is important to note that the technical safety of ICSI is very closely linked to its indications and contraindications. Worldwide, most clinics perform ICSI for moderate-to-severe male factor infertility (Palermo *et al.* 2009, 2015, Chambers *et al.* 2016, Dyer *et al.* 2016). ICMART data between 2008 and 2010 suggest that the overall rates of ICSI utilization were 55% in Asia and 65% in Europe (Dyer *et al.* 2016). The rate of ICSI utilization was 64.8% in Australia based on data from 2013 (Chambers *et al.* 2016). ICSI is the preferred method of fertilizing oocytes in men with ejaculatory dysfunction, retrograde ejaculation or paraplegia-associated complications (Palermo *et al.* 2014a). ICSI is also indicated in couples with a history of poor fertilization or complete fertilization with conventional *in vitro* fertilization (Palermo *et al.* 2014a). However, regional and global disparities in ICSI rates exist. For example, the ICSI utilization rate was almost 100% between 2008 and 2010 in the Middle East (Dyer *et al.* 2016). These data suggest that many clinics utilize ICSI for non-male factor indications, often at their own discretion (Chambers *et al.* 2016). Some of these indications include unexplained infertility, low oocyte yield, advanced age and cryopreservation of embryos for fertility preservation (Palermo *et al.* 2015, Tannus *et al.* 2017).

While the use of ICSI for non-male factor indications remains contentious, there are instances that can jeopardize the technical safety of ICSI; these instances should be considered contraindications. ICSI requires a highly regulated laboratory environment. Following enzymatic removal of the cumulus cells, ICSI should be performed out of the laminar flow hood, on a heated stage outside the incubator (Palermo *et al.* 2015). ICSI also requires technical skills that conventional IVF does not require i.e., injection of oocytes should be performed in an expedited fashion (Palermo *et al.* 2015). Thus, ICSI should not be attempted when the appropriate laboratory environment or technical skills are unavailable. Some investigations have suggested the use of ICSI to reinseminate oocytes that have not fertilized with conventional IVF (Lundin *et al.* 1996, Chen *et al.* 2014, Huang *et al.* 2015). The use of ICSI in such settings is sometimes called rescue ICSI. Studies have shown that rescue ICSI-generated embryos have high rates of polyploidy and frequently arrest at early

developmental stages (Plachot *et al.* 1988, Tucker *et al.* 1991, Tsirigotis *et al.* 1995). Rescue ICSI, therefore, has been considered a contradiction in many clinics (Palermo *et al.* 2015), even though some studies have suggested that early rescue ICSI may have similar clinical pregnancy rates and congenital birth defects when compared to conventional IVF or ICSI (Chen *et al.* 2014, Huang *et al.* 2015).

Long-term outcomes

As seen in Fig. 1, the perinatal, developmental and medical outcomes of ICSI children frequently serve as indicators for the long-term outcomes of ICSI (Shankaran 2014). To facilitate the discussion of long-term outcomes, we divide the current section into four major areas: (a) perinatal outcomes and congenital malformations, (b) developmental outcomes, (c) medical health and (d) reproductive health.

Perinatal outcomes and congenital malformations

Some of the earliest data regarding the perinatal outcomes of ICSI newborns were reported by investigators at the Free University of Brussels, Belgium. In one study, the outcomes of 424 pregnancies between April 1991 and September 1994 resulting from ICSI for severe male factor infertility were analyzed (Wisanto *et al.* 1995). Vanishing twins and triplets, resulting from the transfer of multiple embryos, were found in 36 (8.5%) cases. Overall, 320 (75.5%) pregnancies resulted in live birth, of which 222 (69.3%) were singletons, 93 (29.1%) were twins and 5 (1.6%) were triplets. In general, preterm birth (PTB) and low birth weight (LBW) were

Figure 1 Indicators for the long-term outcomes of ICSI.

related to multifetal gestation i.e., transfer of multiple embryos. There were 3 stillbirths and 5 cases of neonatal mortality, resulting in a perinatal mortality rate of 18.9 per 1000 births. The investigators concluded that the perinatal outcomes of ICSI-conceived newborns in this study were similar to the outcomes of newborns conceived using conventional IVF. In a separate study of 1275 consecutive ICSI treatment cycles in 919 couples, the same group of investigators reported 16 (3.9%) major congenital malformations in ICSI newborns (Tournaye *et al.* 1995). However, this study included couples with male factor infertility who had failed at least 1 IVF cycle, as well as couples where the male partner had semen parameters incompatible with conventional IVF, thereby requiring epididymal sperm aspiration or testicular sperm retrieval.

These retrospective studies were followed by prospective follow-up studies of ICSI children. In one such study, the perinatal and pediatric outcomes of 130 newborns born after ICSI were compared with 130 control newborns born after IVF (Bonduelle *et al.* 1995). The follow-up in this study included prenatal karyotyping, ultrasonographic screening, physical examination at birth, developmental milestones, with a follow-up at 2 months and 1 year of age. There were 74 singletons, 50 twins and 6 triplets in each group. Four major malformations were noted in the ICSI group (holoprosencephaly, femur-fibula-ulna syndrome and two cases of palatoschisis); in contrast, 6 major malformations were seen in the IVF group (coarctation of the aorta, palatoschisis, hypospadias, unilateral cryptorchidism, soft tissue syndactyly and 11-beta-hydroxylase deficiency). Overall, there was no difference in the mean birth weight, birth lengths or head circumferences of ICSI or IVF newborns. The pediatric follow-up was also comparable between the two groups. In a different prospective follow-up study, the investigators compared the karyotypes and congenital malformations of ICSI children based on sperm source i.e., ejaculated, epididymal or testicular (Bonduelle *et al.* 1996, 1998a). Of note, two cases of de novo 47,XXY karyotypes were observed, which were attributed to the characteristics of the infertile men rather than the ICSI procedure itself. Four major malformations were noted in this cohort – two in the epididymal group and one each in the ejaculated and testicular groups. Thus, the overall results of this prospective study demonstrated no increase in the incidence of congenital malformations in ICSI newborns, even after considering the sperm source.

In the mid-1990s, clinics from around the world began reporting data pertaining to the early outcomes of ICSI newborns. For example, investigators at the Cornell Medical Center in New York reported the obstetric outcomes and rate of congenital malformations in a large study of 987 ICSI cycles (Palermo *et al.* 1996). The clinical pregnancy and live birth rates per ICSI cycle were 44.3% and 38.7%, respectively. The number of

ICSI newborns born via vaginal or cesarean delivery was comparable. Of the 578 ICSI neonates included in the study, 15 (2.6%) presented with congenital abnormalities (9 major and 6 minor abnormalities). Interestingly, the rate of congenital abnormalities was lower in ICSI than that in IVF neonates, and within the expected range for the general population of New York State. Furthermore, the study showed no difference in pregnancy outcomes or rate of congenital malformations when stratifying the data by sperm source.

Investigators at both the Free University of Brussels and Cornell Medical Center continued to re-assess perinatal outcomes and congenital malformations in larger cohorts of ICSI children during the next 10–15 years to examine any temporal changes or deviations from previously reported rates. A prospective study of 1987 ICSI children in Brussels analyzed obstetric data, karyotypes and congenital malformations (Bonduelle *et al.* 1999). The mean gestational age at birth was 38.7 weeks for singletons, 36.0 weeks for twins and 32.0 weeks for triplets. Eighteen (1.7%) de novo, abnormal karyotypes were noted (9 autosomal and 9 sex chromosomal). Forty-six major malformations (2.3%) were observed at birth. A larger study of 12,866 ICSI cycles in New York compared the pregnancy outcomes of 5891 ICSI newborns to naturally conceived singletons (Neri *et al.* 2006). Overall, the gestational age at birth and the incidence of LBW infants were similar when controlling for maternal age. The rates of congenital malformations in the ICSI and natural conception groups were 6.2% and 6.5%, respectively. However, only 4 major malformations were noted in the ICSI group – ventricular septal defect, tricuspid regurgitation, talipes and trisomy 7 mosaicism. In contrast, the natural conception group had 5 major malformations – ventricular septal defect, patent foramen ovale/atrial septal defect, encephalopathy, polydactyly and severe midshaft hypospadias with penile angulation.

It is also reassuring to note that several other epidemiologic studies in Denmark, Norway, Israel and the Netherlands have shown no difference in IVF and ICSI newborns in terms of incidence of congenital malformations (Lie *et al.* 2005, Woldringh *et al.* 2011, Farhi *et al.* 2013, Fedder *et al.* 2013). An outlier to the aforementioned trends was a population-based study from Australia that reported major birth defect rates of 8.6% and 9.0% in ICSI and IVF newborns, respectively (Hansen *et al.* 2002). In the same study, only 4.2% of naturally conceived newborns had a major birth defect. However, closer analysis of this study revealed some important confounders, namely maternal age, parity, ethnicity as well as male infertility diagnoses.

Although early studies of ICSI outcomes did demonstrate higher rates of PTB and LBW, these trends were primarily mediated by multifetal gestation due to the transfer of multiple embryos, a clinical practice that was common in early days of IVF and ICSI

(Pinborg *et al.* 2004). In one study comparing ICSI and natural conceptions, the rates of multiple pregnancies, PTB, LBW and early perinatal mortality were higher in the ICSI group than after natural conception (Aytoz *et al.* 1998). In fact, the PTB rates in the ICSI group were as high as 9.9, 56.7 and 96.6% for singletons, twins and triplets, respectively. The overall rate of PTB was 25.6%. It is also important to note that the transfer of multiple embryos in ICSI cycles may exert early in utero effects. In a recent study of 17,415 ICSI cycles resulting in 6281 (39.2%) clinical pregnancies at our center, we reported 2608 (38.2%) multiple gestations – 2038 twins, 511 triplets and 59 quadruplets (Pereira *et al.* 2016). Of these, 18.6% of twin, 54.2% of triplet and 76.3% of quadruplet gestations spontaneously reduced. Intriguingly, ICSI singletons resulting from multiple implantation sites (vanishing twins) had lower birth weights and shorter gestational ages than those from a single implanted embryo. These results suggested that embryonic implantation sites during early gestation set the growth profile of each embryo and that spontaneous reduction of an embryo can confer greater perinatal risk in the form of LBW and PTB to the surviving fetus. Given these findings, caution must be exercised when interpreting the perinatal outcomes of ICSI newborns, especially in the context of multifetal gestation (Pereira *et al.* 2016).

Developmental outcomes

In light of the reassuring perinatal outcomes and congenital malformation rates among ICSI children, many follow-up studies switched their focus to the developmental outcomes of these children. Several strategies have been utilized to evaluate the developmental outcomes of ICSI children including pediatrician visits at regular intervals, assessment of developmental milestones and the use of parent-administered questionnaires. In one of the earliest prospective studies, the medical and developmental outcomes of 89 ICSI children, 84 IVF children and 80 naturally conceived children were compared (Bowen *et al.* 1998). The developmental outcome of these children was measured using the Bayley Scales of Infant Development. Although most ICSI children were found to be healthy and developing normally, there was an increased risk (17%) of mild developmental delay at 1 year when ICSI children were compared with IVF (2%) and naturally conceived children (1%). However, data from Brussels in 2-year-old ICSI and IVF toddlers showed no difference in developmental outcomes when compared to the general population (Bonduelle *et al.* 1998b). The data from this study were notably robust as it included the Bayley Scale, as well as physical examination of the children at 2 months, 1 year and 2 years by a pediatrician who was blinded to the mode of conception. The investigators in Brussels performed a similar analysis of developmental outcomes after accumulating data for an additional

5 years (Bonduelle *et al.* 2003). In this study, the medical and developmental outcomes of 439 ICSI children and 207 IVF children were compared. At age 24–28 months, no significant differences were noted in the Bayley scales of ICSI of IVF toddlers. Furthermore, paternal risk factors associated with male-factor infertility did not seem to contribute to the developmental outcomes of these children. At our center, the physical and psychological outcomes of 5891 five-year-old ICSI children were compared to naturally conceived children (Palermo *et al.* 2008). Although the mean maternal age was higher in the ICSI group, no differences were found in the physical development or intelligence quotient (IQ) assessment of ICSI or naturally conceived children.

These sentinel studies of developmental outcomes were followed by multi-center studies. A collaborative study in Brussels, Gothenburg and New York investigated the psychological outcomes of 300 ICSI children at 5 years of age (Ponjaert-Kristoffersen *et al.* 2004). Several assessment scales were utilized including the Wechsler Preschool and Primary scales of intelligence (WPPSI-R), Peabody Developmental Motor Scales, Parenting Stress Index and Child Behavior Checklist. There were no differences in the WPPSI-R verbal and performance scales when comparing ICSI and naturally conceived children. However, a higher proportion of ICSI children scored lower in the visual-spatial subscales of the WPPSI-R. In addition, there were differences in the parenting stress, child behavior problems and motor development scores based on geographic study site. The authors concluded that ICSI did not affect the psychological well-being or cognitive development of children at age 5 years and that the differences observed were likely due to cultural differences and selection biases. In another international study, 511 ICSI children, 424 IVF children and 488 naturally conceived controls were included in Belgium, Denmark, Greece, Sweden and the United Kingdom at 5 years of age (Ponjaert-Kristoffersen *et al.* 2005). Cognitive development was assessed with the WPPSI-R, while motor development was assessed with the McCarthy Scales of Children's Abilities (MSCA) Motor Scale. Although no differences were noted in the WPPSI-R and MSCA scores of ICSI, IVF and control children, the investigators posited that maternal age and maternal education levels may play different roles in the cognitive development of ICSI and IVF children. In fact, independent studies have confirmed that lower mean IQ levels in some ICSI children are likely due to the influence of parental cognitive ability and education levels (Leslie *et al.* 2003, Barnes *et al.* 2004, Knoester *et al.* 2008b, Goldbeck *et al.* 2009).

Subsequent studies have followed the development outcomes of ICSI children until the age of 10 years and have shown that ICSI and naturally conceived children have comparable cognitive and motor development (Leunens *et al.* 2006, Basatemur & Sutcliffe 2008, Knoester *et al.* 2007, 2008b, Leunens *et al.* 2008).

Furthermore, a systematic review of 9 register-based and 14 cohort studies evaluating the neurodevelopmental outcomes of ICSI children concluded that ICSI does not increase the risk for cognitive impairment or neuromotor handicaps (Middelburg *et al.* 2008). A more comprehensive review of 80 studies that included up to 2,446,044 children also concluded that the neurodevelopmental outcomes of ICSI and IVF children were comparable to those of natural conceptions (Bay *et al.* 2013). Indeed, it is encouraging to note that even the most recent studies have confirmed that ICSI and IVF children have similar cognitive, motor and language development as their naturally conceived counterparts (Balayla *et al.* 2017). These results are not only true for ejaculated specimens but also for ICSI performed with testicular sperm (Meijerink *et al.* 2016).

Of note, the long-term developmental outcomes of ICSI children, particularly intelligence and cognitive function, are closely linked with the education level and socio-economic status of the parents. Furthermore, developmental outcomes from countries with coverage for ART would incorporate parents with a broad range of educational and socio-economic levels, thereby increasing its generalizability. In contrast, countries with limited or private ART coverage would reflect a smaller socio-economic group, and therefore, introduce bias in the assessment of long-term developmental outcomes.

Medical health

The medical health of ICSI children has been a growing focus of many investigators. The Collaborative Study Group of Brussels, Gothenburg and New York were one of the first groups to evaluate the growth and general health of ICSI children at age 5 years in three different countries (Bonduelle *et al.* 2004). Three hundred ICSI children were compared to 266 naturally conceived children. The investigators found that the ICSI children demonstrated growth patterns similar to controls. Furthermore, the frequency of common chronic illness such as asthma, food allergies and eczema were similar in the two groups. Independent studies from the Netherlands have also suggested no adverse health outcomes in 5-to-8-year-old ICSI children when compared to IVF and natural conceptions (Knoester *et al.* 2008a). The same study also showed no difference in the consumption of medical care among ICSI, IVF and naturally conceived children.

The medical and growth data of ICSI adolescents have also become available recently. In one study, the adiposity and body fat distribution of 14-year-old ICSI or natural conception adolescents were compared (Belva *et al.* 2012a). The study included 217 ICSI adolescents (116 boys, 101 girls) and 223 controls (115 boys, 108 girls). Body composition data, skinfold thicknesses (peripheral, central, total) and circumferences (waist, mid-upper arm)

were compared. Pubertal ICSI girls were more prone to central, peripheral and total adiposity; however, no differences in body composition were noted in male ICSI adolescents or controls. In general, ICSI adolescents with advanced pubertal stages were found to have more peripheral adiposity. The progression of these body composition patterns to obesity or other chronic illnesses was not gauged. In a separate study of the same cohort, blood pressure (BP) measurements of ICSI adolescents were compared with controls (Belva *et al.* 2012b). ICSI and naturally conceived girls were noted to have comparable resting systolic BP (109 ± 9 mm Hg vs 111 ± 9 mm Hg) and diastolic BP (64 ± 6 mm Hg vs 66 ± 7 mm Hg), even after adjustment for age and height. In contrast, ICSI boys had a slightly lower resting systolic BP (113 ± 10 mm Hg vs 116 ± 9 mm Hg), but comparable resting diastolic BP (64 ± 6 mm Hg vs 65 ± 5 mm Hg). However, after adjustment for age and height, the systolic and diastolic BPs were comparable in ICSI-conceived boys. Current expert opinion suggests that the medical health of ICSI children in terms of growth trajectory as well as common medical illnesses are similar to age- and gender-matched controls in the general population (Fauser *et al.* 2014).

A discussion of epigenetics and imprinting disorders is also warranted within the realm of the medical health of ICSI children. Epigenetic modifications generally involve DNA methylation or histone modification that ultimately cause variations in gene expression, and therefore, phenotypic expression as well (Berger *et al.* 2009, Fauser *et al.* 2014). In this context, imprinting refers to a specific type of epigenetic modification in which DNA methylation occurs in parent-specific manner. In other words, imprinting of a paternal allele would silence paternal expression, resulting in exclusive maternal-specific allele expression, or vice versa. This phenomenon of uni-parental gene expression is critical for the pathogenesis of certain genetic diseases. Recent studies have suggested putative links between ICSI and imprinting disorders (Fauser *et al.* 2014, Hiura *et al.* 2014). Specifically, some epidemiologic studies have indicated a possible association between ART and imprinted disorders such as Beckwith–Wiedemann syndrome (BWS), Angelman syndrome (AS) and Prader–Willi syndrome (PWS) (Maher *et al.* 2003, Thompson & Williams 2005). In a recent meta-analysis, the odds ratio of any imprinting disorder in ART-conceived children was 3.67 (95% confidence interval, 1.39–9.74) when compared to natural conceptions (Lazaraviciute *et al.* 2014). However, several other studies have questioned the strength of this association. A survey of 2492 children born after ART in Ireland and Central England identified only one case of BWS and no cases of AS (Bowdin *et al.* 2007). These data indicated that the absolute risk of imprinted disorders is very small i.e. <1%. In another study, a nationwide survey was distributed throughout

the Netherlands regarding the use of ART in families with a BWS, AS or PWS child (Doornbos *et al.* 2007). Fifteen (6.8%) BWS, AS or PWS children were born to parents with fertility problems compared to 141,340 (3.5%) in the general Dutch population. Furthermore, families with affected children were 3 times more likely to have fertility problems than the general population. Despite these findings, the investigators found that there was no increase in the rate of ART-related BWS, AS or PWS after controlling for baseline fertility issues.

There is also concern about the association of autism with ICSI. For example, a large population-based, prospective cohort study using Swedish national health registers analyzed the relative risks (RR) for autistic disorder (Sandin *et al.* 2013). Of the 2.5 million newborns in the register, 30,959 (1.2%) were conceived by ART and were followed up for about 10 years. There were 6959 children with autistic disorder, of which 1.5% were conceived with ART. Compared to conventional IVF, children born via ICSI with surgically retrieved sperm had an increased relative risk of autistic disorder (RR 4.60, 95% confidence intervals 2.14–9.88). However, when limiting the analysis to only singletons, the risk of autistic disorder associated with ICSI and surgically retrieved sperm was not statistically significant. In a more recent study of 42,383 ART-conceived infants in California (Kissin *et al.* 2015), the incidence of autism was also noted to be higher among ICSI infants (hazard ratio 1.65, 95% confidence intervals 1.08–2.52). These associations of autism with ICSI should be interpreted with utmost caution, given that absolute risks of autism or autistic disorder are small.

Reproductive health

The follow-up studies of ICSI adolescents have also encompassed pubertal development and reproductive health, though to a lesser extent than perinatal or developmental outcomes. The earliest attempts to evaluate the gonadal function of pre-pubertal ICSI boys were through measurements of testicular and penile size (De Schepper *et al.* 2009). In this study, all pre-pubertal boys were found to have normal testicular and penile lengths, except for two boys with micropenis. In 7 boys, median inhibin B levels (69.2 ng/L) were below the lower limit for age (84 ng/L); however, median anti-Müllerian hormone (AMH) levels (64.1 µg/L) were within normal limits. Of note, serum inhibin B and AMH levels in these boys were independent of their father's semen parameters at the time of ICSI treatment. Fifty boys in this study were followed into puberty as well (Belva *et al.* 2010). Serum inhibin B levels were compared at age 14 years, as well as between age 8 and 14 years. The majority of boys showed an increase in inhibin B levels between age 8 years (69 ± 35 ng/L) and age 14 years (145 ± 41 ng/L). The authors also noted that inhibin B

levels did not differ in boys from fathers with or without severe oligozoospermia. In a separate study, pubertal ICSI boys were also found to have testosterone levels similar to naturally conceived controls i.e., 113 ± 42 pg/mL vs 123 ± 56 pg/mL (Belva *et al.* 2011). Subsequent studies have also reported the pubertal development of ICSI-conceived girls (Belva *et al.* 2012c). In a longitudinal comparison of 101 and 108 pubertal ICSI and naturally conceived girls, respectively, the investigators found that mean age of menarche (13.1 ± 1.2 vs 13.1 ± 1.4 years) and pubic hair development was similar between the groups. However, at age 14 years, breast development was lesser in the ICSI group, even after accounting for confounders.

The reproductive health of young ICSI adults has been reported in very recent studies. A comparison of 57 naturally conceived men to 54 men conceived via ICSI showed that the mean levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone and inhibin B were similar in both groups, even after controlling for age and body mass index (Belva *et al.* 2017a). However, the ICSI-conceived men were more likely to have inhibin B levels <10th percentile and FSH levels >90th percentile when compared to controls, though these differences did not reach statistical significance. The semen parameters of this study cohort were also compared (Belva *et al.* 2016). ICSI-conceived men had lower median sperm concentration (17.7 vs 37.0 million/mL), total sperm count (31.9 vs 86.8 million/mL) and total motile sperm count (12.7 vs 38.6 million) than age-matched naturally conceived peers. In addition, ICSI-conceived men were 3 times more likely to have sperm concentrations and 4 times more likely to have total sperm counts below the reference range of World Health Organization. A recent longitudinal study of 18- to 22-year-old women conceived via ICSI reported that these women had similar FSH, LH and AMH levels as naturally conceived women (Belva *et al.* 2017b). The antral follicle counts were also comparable between the two groups. Although the emergence of these results is prodigious, the small sample sizes limit their generalizability.

Role of confounders

While considerable attention has been drawn to potential impacts of ICSI on long-term safety, it is important to note that ICSI is only a part of the ART process. Several patient confounders such as age, parity, ethnicity, educational level and socio-economic status and ART confounders such as baseline ovarian reserve, baseline hormonal parameters, infertility diagnoses, ovarian stimulation parameters, endometrial receptivity and cryopreservation may obscure the analysis and interpretation of clinical studies. Complete controlling of cofounders occurs

Table 1 Summary of confounders and biases associated with clinical studies investigating the long-term outcomes of ICSI-conceived children.

Patient confounders

Age
Parity
Body mass index
Socioeconomic status
Educational level

ART confounders

Baseline ovarian reserve
Baseline hormonal parameters
Infertility diagnosis
Ovarian stimulation protocol and response
Cryopreservation of gametes or embryos

Study biases

Questionnaire completion – telephone vs self vs physician
Definitions of major and minor malformations
Selection bias of study or control population
Surveillance bias when following ICSI children
Source of data – self-reported vs registry
Source of data – regional vs national
ART coverage and population included – global vs limited

infrequently. Clinical studies of ICSI children also suffer from some biases (Fauser *et al.* 2014). For example, ICSI children are monitored more closely and frequently than naturally conceived children, thereby introducing a surveillance bias. Inclusion of control populations may be influenced by selection bias. These confounders and biases have been summarized in Table 1. Thus, despite the accumulation of critical follow-up data pertaining to the birth, growth, development and well-being of

ICSI children over the past 25 years, one must remain cognizant about the existence of potential confounders and biases when interpreting clinical follow-up studies (Fauser *et al.* 2014).

Conclusions

In this review, we have attempted to collate and analyze salient data pertaining to the long-term outcomes of ICSI children (Table 2). Our discussion has encompassed perinatal, developmental, medical and reproductive outcomes. Current evidence suggests no difference in perinatal outcomes or congenital malformation risks in ICSI children when compared to naturally conceived children. While studies reporting adverse perinatal outcomes in ICSI children do exist, the transfer of multiple embryos is almost always a major confounder. Similarly, higher malformation rates have been noted infrequently and only in the national registries of very few countries. The growth, development and cognitive function of ICSI children mirror their naturally conceived counterparts. However, maternal age and maternal cognitive function should be factored in such studies. The overall medical and reproductive health of ICSI children also seems reassuring, albeit in small study populations. The association of imprinted disorders and autism with ICSI is an ongoing area of investigation, which will require arduous appraisal and surveillance.

Table 2 Summary of long-term ICSI outcomes reviewed in the current paper.

Perinatal outcomes

1. The perinatal outcomes of ICSI-conceived singletons (birth weight, gestational age at delivery) are similar to singletons born after conventional IVF. Multiple births can frequently confound these results
2. No difference in perinatal outcomes based on sperm source

Congenital malformations

1. Most retrospective and prospective data indicate no increase in the rate of major or minor malformations in ICSI newborns. However, occasional regional studies have indicated slightly higher rates of major birth defects
2. Increased trend towards de novo and abnormal sex chromosomal karyotypes in ICSI newborns have been noted in some studies
3. No difference in congenital malformations based on sperm source

Developmental outcomes

1. Data from multicenter studies have shown no differences in physical, psychological and cognitive outcomes between ICSI and naturally conceived children until adolescence
2. Studies reporting lower intelligence scores, child behavior problems or motor development issues are often confounded by the educational level and socioeconomic status of parents, as well as cultural and selection biases
3. No difference in physical, psychological and cognitive outcomes based on sperm source

Medical health

1. The medical health of ICSI children in terms of growth trajectory, common medical illnesses and blood pressure are similar to age- and gender-matched controls in the general population
2. Occasional studies reporting that pubertal ICSI girls may be more prone to central, peripheral and total adiposity
3. Epidemiologic studies have reported associations between ART and imprinting disorders, particularly Beckwith–Wiedmann syndrome. However, these disorders may be related to the underlying infertility diagnosis
4. Slightly increased incidence of autism in ICSI children, though the association should be interpreted with caution given the small absolute risk of autism

Reproductive health

1. Onset of puberty and pubertal development is similar between ICSI and naturally conceived boys and girls. Breast development may be lesser in ICSI-conceived girls
2. ICSI-conceived men may have lower sperm concentration, total sperm count and total motile sperm count when compared to age-matched naturally conceived peers
3. Ovarian reserve parameters including antral follicle counts, anti-Müllerian hormone levels and follicle-stimulating hormone levels are similar between women conceived naturally or via ICSI

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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