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Children Born After Intracytoplasmic Sperm Injection Compared with Spontaneously Conceived Children a Prospective Study

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Abstract: Objective: To study the outcome of ICSI conceived children in our IVF center; their growth, their psychomotor development and to find out possible birth defects and genetic anomalies. Material and Methods: This is a prospective survey about ICSI conceived children and naturally conceived children in our IVF center over the period between 2007 and 2012. One hundred ICSI children aged between 2 and 6 years were invited in order to get a thorough physical examination and to undergo ultrasound imaging and genetic explorations for birth defects. One hundred naturally conceived children paired by age and sexduring the same period were taken as a control population. Results: The comparison of both ICSI and natural conception groups showed that the rate of prematurity was higher in the ICSI group with 50% *vs* 6% in the natural conception group, p<0.05.The rate of low birth weight was also higher in the ICSI group 35% *vs* 4% in the natural conception group, p<0,05. In the ICSI group, no child had slow growth or slow psychomotor development. 4% of the major birth defects were found in the ICSI group and 2% in the control group, the difference was not significant (p=0.52). These defects were essentially urogenital, orthopedic and facial. In both groups, the affected children were boys. The rate of minor birth defects found in the ICSI children is significantly higher than that in control group (23% *vs* 4%; p<0.05). These malformations were mainly facial, cardiac, gastrointestinal, orthopedic, neurological, urogenital and inguinal hernia type. In the ICSI group, there was a male predominance (sex ratio=1.3) without any significant difference, while in the control group there was no sex predominance. As for genetic anomalies, 3% of balanced genetic defects were detected in the ICSI children, 1% of which were in the sexual chromosomes and 2% in the autosomes. These anomalies were found in 2 boys and one girl without any significant difference. Conclusion: This work is greatly reassuring about the future of ICSI children. Larger series with long term follow-up are needed as an only guarantee in assessing the safety of ICSI technique.

Keywords: Child, Congenital Malformation, Follow-Up, Genetic Anomalies, ICSI

1. Introduction

For a few years, intracytoplasmic sperm injection (ICSI) has been the reference technique to solve the most complex infertility problems. However, this procedure raises many questions about its safety as far the genetic risks and malformation hazards, and it is also the subject of several scientific and ethical debates and it has been dealt with in a considerable amount of publications [1, 2, 3].

The objective of this survey is to study the ICSI children in our In Vitro Fertilization (IVF) center, their growth, and their psychomotor development. We also aimed to find out possible birth defects and genetic anomalies. The results obtained are compared to those of the naturally conceived children and to those reported in the literature.

2. Patients and Methods

2.1. Patients

A total of 100 children from ICSI and whose age varies from 2 to 6 years old were appointed for the study from our computer database during the period from January 2005 to December 2010. Each was included only once. No exclusions were made in connection with the indication of ICSI, the age

of the mother or the course of pregnancy. Were only excluded children whose parents refused to participate in the study. Every child born from ICSI was matched by age and sex to a child from a natural conception (NC) during the same study period. Two groups were obtained after receiving the informed consent of their parents: a study group or ICSI group (n=100) and a control group or CN group (n=100).

2.2. Methods

Each couple was received for three consecutive days by a team composed of a gynecologist, a pediatrician, a radio-pediatrician and a psychologist. The visits' program was established as follows:

First day: Parents are summoned alone with their delivery booklets and the child's health record. A fairly short questionnaire to be used for routine and comprehensive enough to browse through the different aspects that can influence the outcome of pregnancy was developed and has been proposed. The questionnaire was divided into four parts: (1) information about parents (age, lifestyle, medical history, in case of ICSI: origin of infertility, type of infertility, sperm origin and rank attempt); (2) the course of pregnancy (single or multiple, hospitalizations, complications, treatment history, etc.), (3) the course of childbirth (gestational age, premature rupture of membranes, fever, abnormal fetal heart rate, labor length, Apgar score, weight, height at birth, neonatal complications, hospitalization or transfer to intensive care), and (4) subsequent monitoring of children (evaluated on: the growth curve, psychomotor development, possible diseases and frequency of consultations).

Second day: The children were received, accompanied by one or both parents, to radio-pediatric ward for a complete physical examination (performed by the same pediatrician into the examination room) before they propose a cardiac and abdominopelvic ultrasound (performed by the same radio pediatrician) in search of birth defects.

Third day: A blood karyotype was performed in children in the cytogenetic service, 15 metaphases taken at random were analyzed according to the recommendations and the international nomenclature (ISCN). Karyotypes revealing a chromosomal abnormality were systematically supplemented by a blood karyotype of the parents and a complete genetic survey.

Both groups (ICSI and NC) were compared for all clinical, biological and radiological investigations. All parents signed their informed consent. The study was approved by the Institutional Ethics Committee.

2.3. Statistical Analysis

Data are expressed as the mean ± standard deviation (SD) or percentages. Statistical analysis was performed using the χ^2 test, Fisher's exact test, and Student's *t*-test. A p-value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS software, version 17.0 for Windows (SPSS, Inc, Chicago, IL, USA).

3. Results

ICSI was performed in most cases for male factor infertility problems (71%). The female, mixed or unexplained origins of infertility were much less frequent (respectively, 13%, 5% and 11%). The sperm used for ICSI were ejaculated in 92% of cases, and testicular in 8% of cases. Table 1 summarizes the characteristics of the study population, pregnancy outcomes and neonatal complications.

Table 1. *Characteristics of the study population, pregnancy outcomes and neonatal complications.*

	ICSI Group n = 100	**NC Group n = 100**	***P*-value***
Mothers' age (years), mean ± SD	31.8±3.7	31.2±3.5	NS
Twins			
Singleton; n (%)	56(56)	100(100)	<0.05
Twins; n (%)	31(31)	0(0)	<0.05
Triplets; n (%)	13(13)	0(0)	<0.05
Mode of delivery			
Vaginally; n (%)	33(33)	88(88)	<0.05
Cesarean section; n (%)	67(67)	12(12)	<0.05
[a]Children' age (years), mean ± SD	3.2±0.9	3.2±0.9	NS
[a]Sex			
Male; n (%)	48(48)	48(48)	NS
Female; n (%)	52(52)	52(52)	NS
Weight at birth (gram), mean ± SD	2774 ± 605	3460 ± 459	<0.05
Low birth weight< 2500g; n(%)	35(35)	4(4)	<0.05
Acute fetal distress; n(%)	2(2)	2(2)	NS

ICSI: intra-cytoplasmic sperm injection; NC: natural conception; NS: not significant; SD: standard deviation.
[a]matched for the study
*Significance level set at p<0.05.

Table 2. *Major congenital malformations.*

Malformations	**ICSI Group n = 100**		**NC Group n = 100**		***P*-value***
	n(%)	**sex**	**n(%)**	**sex**	
Facial					
Cleft lip and palate	1(1)	M	-	-	
Orthopedic					
Bilateral clubfoot	1(1)	M	-	-	
Right valgus	-	-	2(2)	M	
Urogenital					
Hypospadias	1(1)	M	-	-	
Cryptorchidism	1(1)	M	-	-	
Total	4(4)		2(2)		0.52

ICSI: intra-cytoplasmic sperm injection, M: male; MF: malformation; NC: natural conception.
*Significance level set at p<0.05.

All children had normal weight, height and psychomotor development. Disease history children were generally similar between the two groups. Nevertheless, two anomalies need to be cited in the ICSI group: a case of hydrocephalus in a girl aged 5 years whose diagnosis was made by ultrasound during pregnancy with a subsequent normal psychomotor development; and one case of a left unilateral retinoblastoma in a girl of 6 years, for which she had an enucleation of the eye

associated with chemotherapy at the age of 3 years. The major malformations rate was two times higher in the ICSI group, but the difference was not significant (4% vs 2%, p = 0.52); all these defects concerned had male children (Table 2).

The rate of minor malformations was significantly higher in the ICSI group (23% *vs* 4%, p<0.05), with a male non-significant predominance (sex ratio=1.3) (Table 3).

Table 3. *Minor congenital malformations.*

Malformations	ICSI Group n=100			NC Group n=100			*P*-value*
	n (%)	M	F	n (%)	M	F	
Facial							
Auricular detachment	2 (2)	1(1)	1(1)	-	-	-	
Cardiac							
Pulmonary artery dilatation	1(1)	0(0)	1(1)	-	-	-	
Gastro-intestinal							
GER	3(3)	1(1)	2(2)	-	-	-	
Dolichocolon	1(1)	1(1)	0(0)	-	-	-	
Urogenital							
Pelvic ectasia	7 (7)	4 (4)	3 (3)	2 (2)	1 (1)	1 (1)	
Phimosis	2 (2)	2 (2)	0 (0)	-	-	-	
Swinging testicles	2 (2)	2 (2)	0 (0)	-	-	-	
Orthopedic							
Congenital hip dislocation	1 (1)	0 (0)	1 (1)	2 (2)	1 (1)	1 (1)	
Abnormal implantation of the secondtoe	1 (1)	1 (1)	0 (0)	-	-	-	
Neurologic							
Spina bifida	1(1)		1(1)	-	-	-	
Inguinal hernia	2(1)	1(1)	1(1)	-	-	-	
Total	23(23)	13(13)	10(10)	4(4)	2(2)	2(2)	<0.05

F: female; GER: gastroesophageal reflux, ICSI: intra-cytoplasmic sperm injection, M: male; MF: malformation; NC: natural conception.
*Significance level set at p<0.05.

The blood karyotyping performed in all children revealed three balanced chromosomal abnormalities (all occurred in the ICSI group; 3%), of which 2 were autosomal abnormalities and one gonosomal:

- A pericentric inversion of the Y chromosome (Figure 1): in a boy of 3 years; from a multiple pregnancy (1 boy and 2 girls). Physical examination showed the presence of phimosis. No genetic, clinical or ultrasound abnormality was detected in the two twin sisters. The parents karyotype was normal.

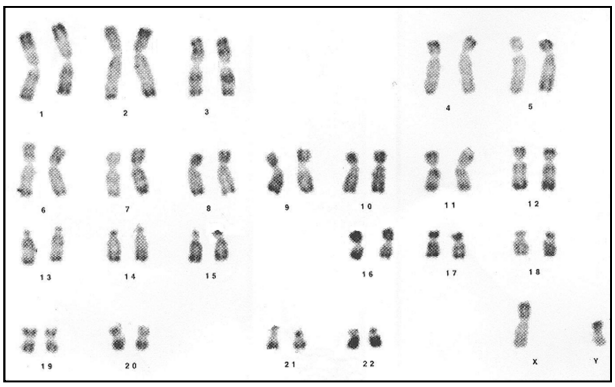

Figure 1. *Blood karyotype in RHG tape showing a 46, X inv (Y) formula.*

- A balanced translocation t(2;8) (Figure 2): in a boy aged 4 years, from a twin pregnancy, the child had no associated malformations outside a bilateral inguinal hernia for which he was operated on at the age of six months. The parents karyotype showed a Klinefelter syndrome in the father (47, XXY).

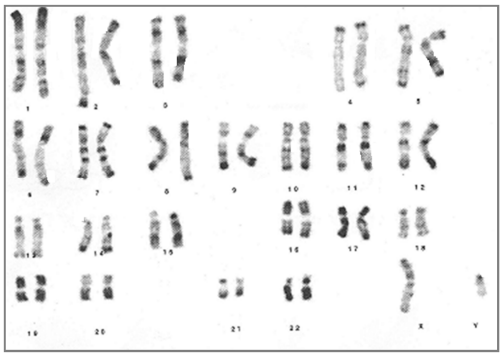

Figure 2. *Blood karyotype in RHG tape showing a 46, XY, t (2;8) (q14;q12) formula.*

- A duplication of chromosome 8 (Figure 3): in a girl of 5 years. No birth defects were detected on clinical examination or on ultrasound with a strictly normal parental karyotypes.

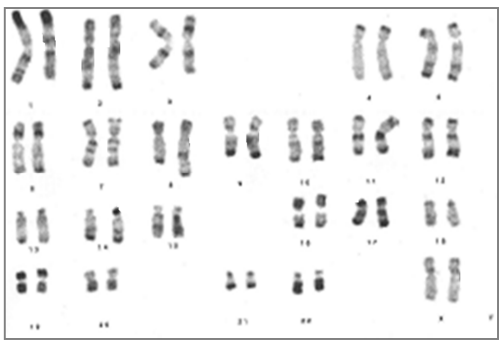

Figure 3. *Blood karyotype in RHG tape showing a 46, XY, dup (8) formula.*

4. Discussion

Prematurity rate in the ICSI group was higher than that of CN (50% *vs* 6%, p<0.05); with consequently low birth weight (LBW): 4% *vs* 35% (p<0.05). Similar results were reported by Hansen *and al.* [4] (prematurity: 31% *vs* 7%; LBW: 31% *vs* 6%). Generally, this high rate of prematurity and LBW found in ICSI can be explained by a higher incidence of multiple pregnancies. Furthermore, advanced maternal age and deleterious effect engendered BY hormonal stimulation on the endometrium may explain the increase in prematurity rate among singletons.

In our series, all ICSI children had a normal height and weight development compared to those conceived naturally. Bonduelle *and al.* [5] compared the measurements at the age of 5 years in 3 groups of children: ICSI, IVF and natural conception. The results were comparable for the weight (19.5 Kg *vs* 19.3 Kg and 19.7 Kg), height (111 cm for the three groups), and head circumference (51.6 cm *vs* 51.8 cm and 51.5 cm). Studies evaluating the growth curves in these children remain rare, and unfortunately there are no sufficient data that could indicate a different kinetics of ICSI children compared to IVF and natural conception.

No child had a delay in psychomotor acquisitions. The published data show that children conceived by ICSI showed no difference compared to conventional IVF and natural conception [6, 7]. In a multicenter study of Ponjaert-Kristoffersen *and al.* [8] on 300 Belgian, Swedish and American children aged 5 years old, cognitive development evaluated by the WPPSI (Wechsler Preschool and Primary scales of intelligence) score was similar to that of children conceived naturally. Motor development assessed by the MSCA (McCarthy Scales of Children's Abilities) score and emotional behavior assessed by the CBCL (Child Behavior Checklist) score were different from naturally conceived children, with lower scores in ICSI (p<0.05). In another multicenter study [7], covering 1,423 Belgian, Danish, Greek, Swedish and English children conceived by ICSI, IVF and naturally, the authors demonstrated (using the same scores) a similar psychomotor development in the three groups.According to a study by Leunens *and al.* [9] performed on children aged 8 years, cognitive development was evaluated by the WPPSI score and better in ICSI compared to NC, while motor development assessed by the ABC (Assessment Battery for Children) test was comparable. The same author reported similar results in another study on children aged 10 years old [10]. These results show that the technique of ICSI has no direct impact on psychomotor development of children. The differences may be related to the intellectual and socio-economic status of the parents.

Our results showed a lower rate of major defects in natural conception (2% *vs* 4%, p=0.52). According to Palermo *and al.* [11], major defects rates were higher in conventional IVF and natural conception *versus* ICSI: 3.5%, 3.1% and 1.6%, respectively. The same findings were found by Bonduelle *and al.* [12], 3.38% (ICSI) *vs* 3.79% (IVF) in a total of 2,889 and 2,995 live births, respectively. In another more recent study [6], covering 540 children conceived by ICSI, 437 children conceived by IVF and 538 naturally, the proportions were reversed with a malformation rate in ICSI higher than IVF (1.9% *vs* 0.9%), and NC (1.5% *vs* 0.37%). According to Hansen *et al.* [4], the malformation rate in ICSI is lower than IVF (8.6% *vs* 9%), but higher than NC (8.6% *vs* 4.2%), for the respective sample sizes of 301, 837 and 4,000 children. This difference in results can be explained by inhomogeneity of the surveyed effectives.

As for urogenital malformations, some studies report an increased risk of hypospadias in the offspring in case of male factor infertility [13, 14]. In opposite, other studies show the absence of a relationship between the quality and origin of the sperm, and the rate of congenital malformations [15, 16]. In our study, children with major malformations in ICSI (4%) were all boys. Studies concerning the sex ratio are unfortunately poor and no gender predominance has been affirmed to date.

The rate of minor defects were statistically lower in NC (4% *vs* 23%, p<0.05). Bonduelle *and al.* [5] reported a lower rate of minor defects in NC compared to IVF and ICSI (respectively 3.7% *vs* 5.3% *vs* 7%). In literature, the rate of minor malformations varies from 0.71% to 20% [17, 18]. This variability in results could be explained by the diversification of classifications. According to the Australians authors, certain heart defects are classified as major malformations, whereas the Belgians authors consider them as part of minor defects. Similarly to major malformations, our study showed a male predominance for minor malformations with a sex ratio of 1.3 (13/10). These results are due, in part, to the high prevalence of urogenital malformations in boys.

In our study, genetic anomalies were discovered on blood karyotypes performed on peripheral blood, taken on the day of the exam. In almost the majority of published studies, karyotypes were performed on amniotic fluid obtained by amniocentesis during pregnancy. Thus, the anomalies detected were either viable, resulting in live births, or lethal resulting in intrauterine fetal death or therapeutic terminations of pregnancy [19, 20]. Of all blood karyotypes performed in children born by ICSI, 3 balanced chromosomal abnormalities were revealed in 2 boys and one girl (3%), including 2 autosomal and 1 gonosomal abnormalities.

The chromosomal abnormalities rate varies according to the authors from 0.3% to 5.4% [21]. In the study by Bonduelle et al. [12], covering 1,425 fetal karyotypes, autosomal abnormalities were more frequent (1.8% *vs* 0.5%). The same author in another series on 293 fetal karyotypes reported as much gonosomal as autosomal anomalies (0.68%) [22]. Wennerholm *and al.* [23], Loft *and al.*[24], and Westergaard *and al.* [23] reported only autosomal anomalies.

The three anomalies found in our study were formed *de novo*. Loft *et al.* [24] reported a rate of *de novo* anomalies higher than inherited abnormalities (2.8% *vs* 0.5%). However in other studies [12, 22], the inherited abnormalities rates were higher. Wennerholm *and al.* [17] reported comparable rates.

No studies have shown a gender predominance, especially as autosomal anomalies were more frequent [25, 26]. However, it is very likely that inherited abnormalities are most often transmitted from the father, which predicts a higher prevalence in boys [27, 28].

Most authors report genetic abnormality in ICSI rates relatively high compared to IVF. Over 1,008 children born by ICSI and 5,446 born by IVF, Vennerholm and *al.* [17] reported a genetic abnormality rate of 0.3% in ICSI *vs* 0.2% in IVF. According to Westergaard *and al.* [21], the rates were 5.4% in ICSI *vs* 3.4% in IVF for sample sizes of 56 and 207 children, respectively. In the study by Bonduelle *and al.* [12], the rates were 2.3% (ICSI) v*s* 1% (IVF). This higher prevalence of genetic abnormalities in ICSI, compared to conventional IVF appears, to be related to the origin of infertility, more than the technology itself. Indeed, the main indication for ICSI is male infertility with the whole range of genetic abnormalities that may be associated with a pathological semen. These anomalies are then likely to be transmitted to the offspring, either in balanced or unbalanced states which can cause a malformation syndrome and even intrauterine fetal death.

5. Conclusion

Like most studies published about the future of ICSI children, this work is greatly reassuring, and our results are comparable to those found in the literature. We think that this prospective survey would gain importance in being completed by a large series. This programme might be carried out if all the children in both groups (the ICSI group and the control group) could be invited and investigated at regular intervals, thus allowing medium and long-term follow up, which represents the only guarantee in assessing the safety of ICSI technique. However such a follow up requires a considerable amount of work, moreover it sets the problem of confidentiality about the conception and that of the psychological impact that an increased monitoring can have on the children.

Authors' Contributions

MK and JD carried out the statistic tests and drafted the manuscript. FZ and AZ participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.

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