Survey on intracytoplasmic sperm injection: report from the ESHRE ICSI Task Force

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Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility, since normal fertilization and ongoing pregnancies can be achieved with severely affected spermatozoa. Hence, the application of ICSI is rapidly expanding around the world, necessitating an accurate assessment of the efficacy and safety of this novel technique. The European Society of Human Reproduction and Embryology (ESHRE) Task Force is gathering data annually on the clinical results, the pregnancy outcome and the follow-up of children born after ICSI using ejaculated, epididymal and testicular spermatozoa, in order to be able to provide reliable information on these important issues. During the 3 years 1993–1995, the number of centres performing ICSI increased from 35 to 101, and the total number of ICSI cycles performed per year rose from 3157 to 23 932. The incidence of oocytes damaged by the procedure remained low (<10%) and the fertilization rates obtained with ejaculated, epididymal and testicular spermatozoa in 1995 were 64, 62.5 and 52% respectively. Thus, ~90% of the couples had an embryo transfer and the viable pregnancy rate was 21% for ejaculated, 22% for epididymal and 19% for testicular spermatozoa (with 25–30% multiple pregnancies). Furthermore, 3149 transfers of frozen–thawed embryos were performed and 7–11% of them resulted in a viable pregnancy. The ICSI results were similar during this 3 year period, irrespective of the origin of the spermatozoa. The perinatal outcome of children born after ICSI was not different from those born after in-vitro fertilization (IVF) or natural conception, and was only affected by multiplicity. Moreover, the incidence of major or minor malformations was not increased, but the chromosomal, especially the sex-chromosomal, aberration rate was slightly elevated. To summarize, a very high success rate is obtained by ICSI independently of the source of the spermatozoa, verifying the superiority of ICSI over conventional IVF. The procedure seems to be safe, but further follow-up of the children is necessary in order to be able to assess its safety more accurately.

Key words: ICSI/malformations/pregnancy rate

Introduction

Assisted reproduction technologies (ART) have come a long way since Edwards and Steptoe (1978) pioneered in-vitro fertilization (IVF) and embryo transfer in the human. IVF was also useful as a mode of treatment of male infertility, although the results with this indication were less satisfactory (Tournaye *et al.*, 1992). Partial zona dissection and subzonal insemination have not been able to improve these results substantially, although higher success rates have been reported in selected cases (Fishel *et al.*, 1992).

Nevertheless, the major breakthrough in the treatment of male infertility was the introduction of intracytoplasmic sperm injection (ICSI), because it enabled patients with extremely impaired sperm quality (Palermo *et al.*, 1992), who had not previously been accepted in classical IVF programmes, to achieve high fertilization and pregnancy rates. This has led to the rapid spreading of the application

of ICSI in ART centres, radically changing the treatment of severe male factor infertility and altering the prospects of couples with repeated failed fertilizations in conventional IVF.

Soon after the birth of the first ICSI child, questions were raised about the potential long-term effects of the technique *per se* on the children born, since, using ICSI, the never-proven selection of spermatozoa by the zona pellucida and the oolemma is bypassed (Butler, 1995). Moreover, it has been argued that the genetic defects responsible for sperm impairment could be passed to the male offspring. All these speculations and concerns about ICSI are obviously of crucial importance since they may be associated with significant general and public health problems.

In addition, ICSI involves a high financial cost for both the couple and the health care system, necessitating an accurate assessment of its efficacy.

The ICSI Task Force of the European Society of Human Reproduction and Embryology (ESHRE) was originally established in 1994, with the aim of gathering information about the experience of centres practising ICSI in order to be able to accumulate sufficient data to address the issues of safety and efficacy. The first report on the activities of the ESHRE ICSI Task Force was published in 1996 (Tarlatzis, 1996). In this paper, the ICSI results of 1995 are reviewed and are presented on behalf of all the members of the ICSI Task Force.

Advantages and limitations of the ICSI Task Force

The analysis of cumulative data on ICSI from many different centres has important advantages compared with the results coming from individual centres. Data from a large number of cases can be collected in a short time period, and thus it is easier to assess the average likelihood of fertilization, achievement of pregnancy and incidence of malformations using this procedure. Moreover, the studied population is more representative because of the contribution of centres from different parts of the world, giving the opportunity to have more global estimations.

On the other hand, there are some disadvantages inherent to the method of data collection, including the different degree of experience using ICSI and heterogeneity in the indications, techniques and number of cycles and follow-up procedures in the participating centres. Furthermore, adequate information on pregnancy and neonatal outcome may be lacking since they are not always recorded in some IVF centres. Nevertheless, the advantages outweigh the disadvantages and, hence, this effort is pursued with the aim of stimulating the centres to participate in this endeavour in order to be able to provide reliable information on the efficacy and safety of ICSI.

Methodology

Data collection

In order to gather information from the participating centres (listed in Table I), the ICSI Task Force distributed paper data forms on which each clinic could summarize its practice and outcome results. These forms comprised four categories: (i) those referring to the clinical experience with ICSI, (ii) those concerning the follow-up of children born after ICSI, (iii) those aiming to evaluate children born with congenital malformations, and (iv) two forms for recording the results of cryopreservation after ICSI using ejaculated, epididymal and testicular spermatozoa and also the follow-up of children born after the transfer of frozen–thawed ICSI embryos. The forms were completed by each centre's clinicians or biologists and were subsequently sent to the ICSI Task Force Secretariat for data coding and analysis.

The requested entries on the clinical experience form included the indications for using ICSI, the ICSI results obtained using ejaculated, epididymal and testicular spermatozoa, the number of metaphase II oocytes injected, damaged and normally fertilized, the number of embryos transferred or frozen and data on the achievement and outcome of pregnancies after these procedures.

As biochemical pregnancies (or preclinical abortions) were considered to be those showing a moderate and temporary rise in β-human chorionic gonadotrophin (β-HCG) concentrations, clinical pregnancies were identified by the rising of β-HCG concentrations and the presence of gestational sac(s) within the uterine cavity on ultrasonography or by the detection of trophoblastic tissues in

Table I. List of participating centres

No.	Centre	City	Country
1	University Hospital Leiden	Leiden	The Netherlands
2	St Elisabeth Hospital	Tilburg	The Netherlands
3	Univ of Ottawa	Ottawa	Canada
4	Werlin–Zarutskie Fertility Centers	Irvine	USA
5	North Carolina Center for Reproductive Medicine	Cary	USA
6	A.C.H. Ninewells Hospital	Dundee	UK
7	Florence Center of Ambulatory Surgery and Infertility	Firenze	Italy
8	Concept Fertility Centre	Subiaco	Australia
9	Hiroshima Hart Clinic	Hiroshima	Japan
10	University of Virginia	Charlottesville	USA
11	Endokrinologische Praxisgemeinschaft	Hamburg	Germany
12	Fertility Centre Virga Jesse Hospital	Hasselt	Belgium
13	Ideon-Kliniken	Malmö	Sweden
14	Assisted Conception Unit	Leeds	UK
15	Academic Medical Centre	Amsterdam	The Netherlands
16	Institute of Reproductive Medicine	Giessen	Germany
17	Baylor Center for Reproductive Health	Dallas	USA
18	City West IVF	Westmead	Australia
19	University Center for Reproductive Endocrinology and Fertility	New Brunswick	USA
20	BIRTH	Brugge	Belgium
21	Reproductive Technology	Seattle	USA
22	Dept. Human Reproduction, Univ Clinic	Zagreb	Croatia
23	Clinic Dr Fred Maleika	Stuttgart	Germany
24	Centre For Reproductive Medicine	Munich	Germany
25	Advanced Fertility Service	New York	USA
26	Egyptian IVF–ET	Cairo	Egypt
27	Univ Central Hospital of Oulu	Oulu	Finland
28	Clinic of Endocrinology	Zurich	Switzerland
29	Gemeinschaftspraxis Prof Dr Bregula, Dr Hamori, Dr Behrens	Erlangen	Germany
30	Reproductive Resource Center of Greater Kansas Clty	Overland Park	USA
31	Center for Reproductive Health	Cincinnati	USA
32	Cooper Center for IVF	Marlton	USA
33	Noth Shor ART	St Leonards	Australia
34	Center for Reproductive Medicine	Flint	USA
35	Fertility Clinic	Odense	Denmark
36	Sydney IVF Pty Ltd	Sydney	Australia
37	Universitäts Frauenklinik	Tübingen	Germany
38	Centro Procreazione Assistita	Bari	Italy
39	Instituto Bernebeu	Alicante	Spain
40	NYU–Program for *In Vitro* Fertilization	New York	USA
41	Regional IVF Unit	Manchester	UK
42	Hôpital Erasme	Brussels	Belgium
43	Institute for Assisted Reproduction	Charlotte	USA
44	Reproductive Biology Associates	Atlanta	USA
45	Center for Reproductive Medicine	San Ramon	USA
46	Maribor Teaching Hospital	Ljubljanska	Slovakia
47	Medical College of Virginia	Richmond	USA
48	Medicina della Riproduzione	Milano	Italy
49	Reproductive Endocrinology and Infertility	Seoul	Korea
50	Väestöliitto Fertility Clinic	Helsinki	Finland
51	Fertilitetskliniken Cionia	HojBjerg	Denmark
52	Genetics and IVF Institute	Fairfax	USA
53	In Vitro Fertilization Center of South Jersey	Marlton	USA
54	Schoysman Infertility Management	Brussels	Begium
55	Moscow Centre for Infertility Treatment 'EKO'	Moscow	Russia
56	Ärzte für Frauenheilkunde und Geburtshilfe	Düsseldorf	Germany
57	S.I.S.M.E.R.	Bologna	Italy
58	Sevgi Hospital, Assisted Reproductive Techniques and Reproductive Endocrinology Unit	Ankara	Turkey

Table I. *Continued*

No.	Centre	City	Country
59	PIVET Medical Centre	Perth	Australia
60	Fertility Center Scandinavia	Göteborg	Sweden
61	UAMS ART Program	Little Rock	USA
62	University Hospital Nijmegen	Nijmegen	The Netherlands
63	University Hospital Utrecht	Utrecht	The Netherlands
64	Oregon Health Sciences University Embryology Lab	Portland	USA
65	Helsinki University Central Hospital	Helsinki	Finland
66	International Hospital IVF Center	Istanbul	Turkey
67	Genker Institute for Fertility Technology	Genk	Belgium
68	Assisted Reproduction Unit	Aberdeen	UK
69	IVF Centre	Lübeck	Germany
70	Reproductive Medicine Unit	Woodville	Australia
71	Felicitas Infertility Centre	Helsinki	Finland
72	Centre for Reproductive Medicine	Edegem	Belgium
73	Centre for Reproductive Medicine	Newcastle/Tyne	UK
74	The Churchill Clinic	London	UK
75	Carl Von ...kliniken	Uppsala	Sweden
76	Center for Advanced Reproductive Care	Redondo Beach	USA
77	Foothills Hospital, Regional Fertility Program	Calgary	Canada
78	Al Salama Hospital	Jeddah	Saudi Arabia
79	AVA-clinic	Tampere	Finland
80	Centre for Reproductive Medicine, Dutch-speaking Brussels Free University	Brussels	Belgium
81	Clinic In-Tiimi	Kuopio	Finland
82	Assisted Procreation Centre	Pisa	Italy
83	The Midwest Center for Reproductive Health, P.A.	St Louis Park	USA
84	Flinders Reproductive Medicine	Bedford Park	Australia
85	St Mother Obstetrics and Gynaecology Clinic	Kitakyushu City	Japan
86	Centre For Fertility and Reproductive Medicine	Newark	USA
87	Kaali Institute	Budapest	Hungary
88	The Infertility Center of St Louis	St Louis	USA
89	Academic Hospital	Maastricht	The Netherlands
90	California Fertility Associates	Santa Monica	USA
91	University of Berne, Division of Endocrinology	Berne	Switzerland
92	The Center for Reproductive Medicine and Infertility Cornell Medical Center	New York	USA
93	Fertility Institute	Athens	Greece
94	Embryogenesis	Athens	Greece
95	Centre for Human Reproduction	Athens	Greece
96	Euromedica IVF Infertility and IVF Center	Athens	Greece
97	Geniki Kliniki	Thessaloniki	Greece
98	IVF Unit Assaf Harofeh	Zerifin	Israel
99	Medisch Centrum voor Vruchtbaarheidsdiagnostiek en ET	Leuven	Belgium
100	IVF-Unit University of Göteborg	Göteborg	Sweden
101	Instituto Universitario Dexeus	Barcelona	Spain

the uterine curettage material (de Mouzon and Lancaster, 1995). Clinical pregnancies that were spontaneously terminated before the 20th week of gestation were considered to be clinical abortions, whereas those remaining comprised the ongoing clinical pregnancies and the deliveries, which as a whole constitute the viable pregnancies (Tarlatzis, 1996).

The children follow-up form was filled-in for the total number of children born after ICSI, their mean gestational age and birthweight, as well as the minor and major neonatal malformations observed. Major malformations were considered those causing functional impairment and requiring surgical correction; all the others were classified as minor (Holmes, 1976). Moreover, the information obtained from prenatal ultrasound and prenatal or postnatal karyotyping was recorded. A special part

Table II. Indications for intracytoplasmic sperm injection (ICSI) reported for 1995 by the European Society of Human Reproduction and Embryology (ESHRE) Task Force

Indication for performing ICSI	No. of centres
Abnormal semen	99
Failed in-vitro fertilization	96
Obstructive azoospermia	73
Non-obstructive azoospermia	63
Preimplantation diagnosis	8
Other (globozoospermia, antisperm antibodies, idiopathic infertility etc.)	23

of this form was devoted to the methodology and the length of time of the follow-up of the children.

Another form was completed individually for every child born with congenital malformations, including information on the pregnancy, the delivery and the physical examination of the child system-by-system, as well as the treatment and the prognosis.

Participating centres

Data forms were sent to all centres that had already taken part in the previous ICSI Task Force survey, as well as to new ICSI centres known from the literature or from national registries. Up to 31 December 1995, 101 centres had submitted their clinical results based on 2 to 2507 cycles (see Table I). Their participation in this endeavour was of paramount importance and is greatly appreciated by the ESHRE ICSI Task Force.

Analysis of data

All data were entered into two different programs of a personal computer. Centre identification and address were stored in filemaker pro 2.1 Bv1 and the data in Excel spreadsheets (Microsoft Excel version 5.0a). Analyses and graphs were also done using the same program, as previously described (Tarlatzis, 1996).

Indications and number of cycles

Although ICSI was originally developed to treat male infertility, it is now also used for other disorders (Table II). Abnormal semen quality was the main indication for ICSI in 1995 (99 centres),

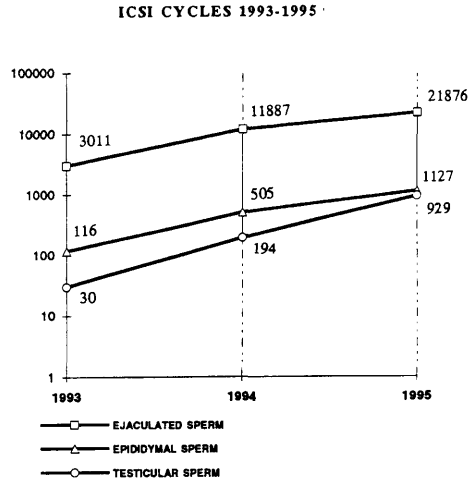

Figure 1. Numbers of intracytoplasmic sperm injection (ICSI) cycles for 1993, 1994 and 1995 as reported to the European Society of Human Reproduction and Embryology (ESHRE) Task Force.

Table III. Distribution of centres according to the number of intracytoplasmic sperm injection (ICSI) cycles performed per year between 1993 and 1995 inclusive

No. of cycles/year	No. of centres performing ICSI		
	1993	1994	1995
<100	25	33	43
100–200	9	10	22
200–300		9	10
300–400		5	7
400–500		5	5
500–1000		1	10
>1000	1	2	4
Total	35	65	101

followed by failed IVF (96 centres), obstructive azoospermia (73 centres), non-obstructive azoospermia (63 centres), preimplantation diagnosis (eight centres) and globozoospermia, antisperm antibodies, idiopathic infertility, etc (23 centres). A similar distribution of ICSI indications was also observed in 1994 (Tarlatzis, 1996).

In 1995, 101 clinics performing ICSI reported a total of 23 932 cycles (21 876 with ejaculated, 1127 with epididymal and 929 with testicular spermatozoa), which represents an almost two-fold increase over 1994. Thus, the impressive rise in the application of this technique between 1993 and 1995 seems to be continuing (Figure 1). Similarly,

Table IV. Intracytoplasmic sperm injection (ICSI) results using ejaculated, epididymal and testicular spermatozoa

	Ejaculated	Epididymal	Testicular
No. of oocytes (MII) injected	143 598	9197	7834
No. (%) of oocytes damaged	13 374 (9.3)	762 (8.3)	668 (8.5)
No. (%) of oocytes fertilized	91 898 (64)	5745 (62.5)	4051 (51.7)
No. (%) of good embryos (transferred/frozen)	62 444 (43.5)	4202 (45.7)	3046 (38.9)
No. (%) of embryo transfers	15 407 (86)	952 (88.1)	731 (90)
No. (%) of cycles with freezing	4 154 (23.2)	235 (21.8)	216 (26.6)

the number of centres performing a larger number of cycles per year also increased in 1995, although variations between centres still existed (Table III).

Fertilization and embryo transfer

Using ejaculated spermatozoa from the 143 598 metaphase II (MII) oocytes injected, 13 374 (9.3%) were damaged during the ICSI procedure and 91 898 (64%) were normally fertilized, creating 62 444 good quality embryos that could be transferred or frozen (43.5% of injected oocytes and 67.9% of fertilized oocytes). These led to 15 407 (86.0%) embryo transfers and 4154 cycles (23.2%) with embryo freezing (Table IV).

In the cycles with epididymal spermatozoa, of the 9197 MII oocytes injected, 762 (8.3%) were damaged and 5745 (62.5%) fertilized, leading to 4202 embryos that were available for transfer or freezing (45.7% of injected oocytes and 73.1% of fertilized oocytes). Thus, 952 embryo transfers (88.1%) and 235 cycles with freezing (21.8%) were accomplished (Table IV). Moreover, the ICSI results were similar when classified according to the aetiology of obstruction, i.e. congenital or acquired (Table V).

When testicular spermatozoa were used, of the 7834 MII oocytes injected, 668 (8.5%) were damaged and 4051 (51.7%) fertilized, giving rise to 3046 good quality embryos that could be transferred or frozen (38.9% of injected oocytes and 75.2% of fertilized oocytes). As a result, 731 (90.0%) embryo transfers were performed and there were 216 (26.6%) cycles with transfer of frozen–thawed embryos (Table IV). On the other hand, when the ICSI data were analysed according to the aetiology of azoospermia, patients with non-obstructive azoospermia tended to have lower fertilization and embryo transfer rates than the obstructive cases (Table VI). This is probably due to the lower chances of finding spermatozoa, at all or in sufficient numbers, in patients with non-obstructive lesions. According to Tournaye *et al.* (1997), this is possible in ~50% of these patients.

Therefore, it is evident that the fertilization rates after ICSI, even with severely impaired spermatozoa, are significantly higher that those with classical IVF in cases of male infertility (Tournaye *et al.*, 1992). Moreover, most of the fertilized oocytes (67.9–75.2%) developed into high quality embryos that could be either transferred or frozen. Thus, even in cases of non-obstructive or obstructive azoospermia, 84.7 and 91.1% of the patients respectively will have an embryo transfer, an impossible rate for these patients with classical IVF or any other assisted reproduction technique, and 22.6 and 26.2% respectively are expected to have a frozen/thawed embryo transfer.

It is noteworthy that the incidence of oocytes damaged during the procedure in 1995 ranged between 8.3 and 9.3% and did not differ from that observed in previous years (Tarlatzis, 1996). Hence, oocyte damage seems to be an inherent drawback of the ICSI procedure *per se*. Moreover, the incidence of fertilization and the rate of development of good quality embryos were similar in 1994 and 1995, except for the cases with epididymal spermatozoa, where more oocytes fertilized (52.5 and 62.5% respectively) and more good quality embryos were available in 1995 (61.7 and 73.1% of fertilized oocytes respectively).

Achievement of pregnancy

The main factor determining the effectiveness of an assisted reproduction technique, such as ICSI,

Table V. Intracytoplasmic sperm injection (ICSI) results for 1995 with epididymal spermatozoa according to the aetiology of obstruction

	Aetiology of obstruction		
	Unspecified	Congenital	Acquired
No. of centres	8	30	37
No. of cycles	161	341	578
No. of patients	131	245	461
No. of oocytes (MII) injected	1677	2826	4694
No. (%) of oocytes damaged	177 (10.6)	229 (8.1)	356 (7.6)
No. of oocytes fertilized	986 (58.8)	1729 (61.2)	3030 (64.5)
No. of embryos transferred/frozen	601	1292	2309
% per injected oocyte	35.8	45.7	49.2
% per fertilized oocyte	60.9	74.7	76.2
No. (%) of embryo transfers	150 (93.2)	283 (83)	519 (89.8)
No. (%) of cycles with freezing	52 (32.3)	63 (18.5)	120 (20.8)

Table VI. Intracytoplasmic sperm injection (ICSI) results for 1995 with testicular spermatozoa according to the aetiology of azoospermia

	Aetiology of azoospermia		
	Unspecified	Obstructive	Non-obstructive
No. of centres	7	32	30
No. of cycles	186	339	287
No. of patients	153	298	247
No. of oocytes (MII) injected	1686	3401	2747
No. (%) of oocytes damaged	131 (7.8%)	302 (8.9%)	235 (8.5%)
No. (%) of oocytes fertilized	907 (53.8%)	1860 (54.7%)	1284 (46.7%)
No. of embryos transferred/frozen	720	1336	990
% per injected oocyte	42.7	39.3	36.0
% per fertilized oocyte	79.4	71.8	77.1
No. of embryo transfers	179 (96.2%)	309 (91.1%)	243 (84.7%)
No. (%) of cycles with freezing	62 (33.3)	89 (26.2)	65 (22.6)

is the achievement of a pregnancy and especially of a viable pregnancy.

In 1995, a fresh embryo transfer was performed in 15 407 (86.0%) ICSI cycles with ejaculated spermatozoa, resulting in 5012 positive β-HCG tests (28.0% per cycle) and 3808 viable pregnancies (1908 ongoing and 1900 delivered; 21.3% per cycle; Table VII). In the ICSI cases with epididymal spermatozoa, a fresh embryo transfer was done in 952 (88.1%) cycles, leading to 322 positive β-HCG tests (29.8% per cycle) and 236 viable pregnancies (83 ongoing and 153 delivered; 21.8% per cycle; Table VII).

On the other hand, in cases of ICSI with testicular spermatozoa, 731 (90.0%) fresh embryo transfers were accomplished, resulting in 218 positive β-HCG tests (26.8% per cycle) and 152 viable pregnancies (18.7% per cycle; Table VII).

The overall results for ejaculated, epididymal and testicular spermatozoa in 1995 were similar to those recorded in 1993–1994. Nevertheless, the results using testicular spermatozoa showed a slight decline in the number of positive β-HCG tests and in the viable pregnancy rate (Figure 2), possibly due to the larger number of cycles performed and to the wider application of this procedure to less favourable cases.

Regarding frozen–thawed embryo transfers after ICSI, a total of 3363 cycles were accomplished during 1995 in 57 centres (Table VIII). Of those, 2990 embryo transfers were done in 3146 cycles after ICSI using ejaculated spermatozoa, giving

Table VII. Pregnancy outcome after intracytoplasmic sperm injection (ICSI) using ejaculated, epididymal and testicular spermatozoa in 1995

	Source of spermatozoa		
	Ejaculated	Epididymal	Testicular
No. of positive β-HCG tests	5012 (28%)	322 (29.8%)	218 (26.8%)
No. (%) of viable pregnancies	3808 (21.3)	236 (21.8)	152 (18.7)
No. of ongoing pregnancies	1908	83	82
No. of delivered pregnancies	1900	153	70
No. (%) of biochemical pregnancies	464 (9.3)	41 (12.7)	22 (10.1)
No. (%) of clinical abortions	723 (14.4)	34 (10.6)	34 (15.6)
No. (%) of ectopic pregnancies	70 (1.4)	0 (0)	1 (0.5)

Table VIII. Results for 1995 of frozen–thawed embryo transfers after intracytoplasmic sperm injection (ICSI) using ejaculated, epididymal and testicular spermatozoa

	Source of spermatozoa		
	Ejaculated	Epididymal	Testicular
No. of cycles	3146	144	73
No. of zygotes/embryos thawed	12041	466	336
No. (%) of zygotes that survived	7669 (63.7)	292 (62.7)	213 (63.4)
No. (%) of zygotes transferred	6574 (54.6)	241 (51.7)	191 (56.8)
No. (%) of embryo transfers	2990 (95)	91 (63.2)	68 (93.1)
No. (%) of positive β-HCG tests	525 (16.7)	22 (15.3)	8 (11)
No. (%) of viable pregnancies	341 (10.8)	13 (9)	5 (6.8)

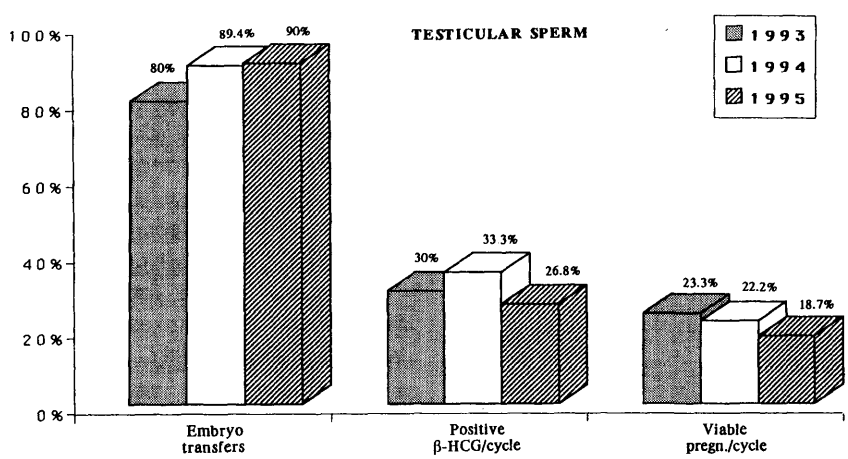

Figure 2. Achievement of pregnancy using testicular spermatozoa for 1993, 1994 and 1995 as reported to the European Society of Human Reproduction and Embryology (ESHRE) Task Force. β-HCG = β-human chorionic gonadotrophin.

rise to 525 (16.7%) positive β-HCG tests and 341 (10.8%) viable pregnancies. With epididymal spermatozoa, 91 transfers of frozen/thawed embryos were performed in 144 cycles, leading to 22 (15.3%) positive β-HCG tests of which 13 (9.0%) were viable pregnancies. On the other hand, 68 frozen–thawed embryo transfers were performed in 73 cycles with testicular spermatozoa,

resulting in 8 (11.0%) positive β-HCG tests and 5 (6.8%) viable pregnancies.

Early pregnancy losses and ectopic pregnancies

The implantation rates after IVF and ICSI, even in cases of good quality embryos, remain relatively low despite the progress in ovarian stimulation and culture conditions. Moreover, the implantation as well as the miscarriage rates are significantly affected by the woman's age, primarily due to oocyte and embryo quality (Lancaster *et al.*, 1995).

In the cases where ejaculated spermatozoa were used in 1995, of the 5012 positive β-HCG tests, 464 (9.3%) were biochemical pregnancies, 723 (14.4%) were clinical abortions and 70 (1.4%) were ectopic pregnancies (Table VII). On the other hand, in cases using epididymal spermatozoa, of the 322 positive β-HCG tests, 41 (12.7%) were biochemical pregnancies and 34 (10.6%) clinical abortions, and no ectopics were observed (Table VII). With testicular spermatozoa, of 218 positive β-HCG results, 22 (10.09%) were biochemical pregnancies, 34 (15.59%) were clinical abortions, and 1 (0.45%) was an ectopic (Table VII). It is noticeable that the incidence of early pregnancy loss after ICSI is similar to that after IVF (Lancaster *et al.*, 1995), whereas the incidence of ectopic pregnancies (0–1.4%) is lower than that observed in standard IVF (4.3%). This difference is probably due to the fact that most women undergoing ICSI have normal tubes, in contrast with the patients undergoing classical IVF (Marcus and Brinsden, 1995).

The pregnancy outcome after frozen–thawed embryo transfers was similar to that of fresh transfers. Thus, using frozen–thawed embryos from ejaculated spermatozoa, 72 (13.7%) biochemical pregnancies, 83 (15.8%) clinical abortions and 10 (1.9%) ectopic pregnancies were observed, whereas with epididymal spermatozoa, 3 (13.6%) biochemical pregnancies, 2 (9.1%) clinical abortions and no ectopics were obtained (Figure 3). In addition, using embryos from testicular spermatozoa, 22 (10.1%) biochemical pregnancies, 34 (15.6%) clinical abortions and 1 (0.5%) ectopic pregnancy were recorded.

Multiple gestations

The incidence of multiple pregnancies after ICSI in 1995 was approximately the same as in 1993 and 1994, ranging from 27.9 to 30.4%, and similar results have also been reported after the transfer of frozen–thawed ICSI embryos. These findings confirm the good quality of ICSI embryos and further support the need to reduce the number of embryos replaced (Staessen *et al.*, 1995).

The avoidance of multiple pregnancies, especially those of high order, is currently one of the most pressing problems not only for ICSI but also for classical IVF, since multiple gestations are associated with poor fetal outcome, which impairs the ultimate purpose of having a healthy baby. Obtaining a pregnancy is not enough, since the gestation must be maintained until a healthy live baby is born at term. However, many programmes, especially new ones, are unwilling to reduce the number of transferred embryos, in order to ensure high pregnancy rates. On the other hand, recent studies have shown that the elective transfer of two good quality embryos does not compromise the success rate of IVF or ICSI (Staessen *et al.*, 1995).

Perinatal outcome

Despite the important advances in prenatal and neonatal care, preterm delivery remains a major problem in everyday obstetric practice, ranging between 5 and 10% of all births in developed countries (Creasy, 1989). Assisted reproduction techniques are associated with a 25% preterm delivery rate as well as a 33% low birth weight rate (Doyle *et al.*, 1992). This high incidence of preterm birth and low birth weight in IVF pregnancies has been noted not only in multiple pregnancies but also in the singletons (Doyle *et al.*, 1992; Lancaster, 1996).

This pattern does not seem to be the same for the ICSI babies, since the mean gestational age and the mean birth weight for singleton pregnancies were similar to those observed in the general population, whereas they were significantly lower in high order multiple pregnancies (Table IX). These findings for the ICSI babies are in agreement with those observed by Wisanto *et al.* (1995, 1996), and, also, in certain countries concerning the IVF babies (Lancaster, 1996).

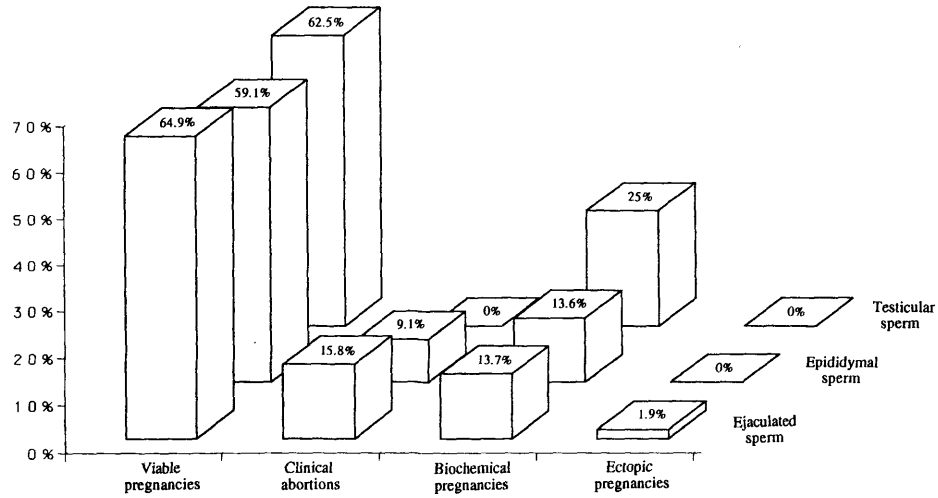

Figure 3. Pregnancy outcome for 1995 of frozen–thawed embryo transfers after intracytoplasmic sperm injection (ICSI) using ejaculated, epididymal and testicular spermatozoa, as reported to the European Society of Human Reproduction and Embryology (ESHRE) Task Force.

Table IX. Perinatal outcome of children born after intracytoplasmic sperm injection (ICSI), up to 31 December 1995

	Source of spermatozoa		
	Ejaculated	Epididymal	Testicular
No. of centres	60	14	7
No. of children born	2486	119	63
Singleton	1384	59	27
Twins	534	25	15
Triplets	39	5	2
Quadruplets	1		
Mean gestational age (weeks)	37.6	38.2	36.5
Singleton	38.7	38.4	38.3
Twins	36.0	36.9	36.2
Triplets	31.4	33.0	32.3
Quadruplets	32.0		
Mean birth weight (g)	2918	3172	2809
Singleton	3201	3229	3396
Twins	2423	2731	2420
Triplets	1732	1636	1859
Quadruplets	1763		

It is noteworthy that no significant differences in perinatal outcome were observed between children born after ICSI using ejaculated, epididymal or testicular spermatozoa (Table IX).

Genetic and malformation risks

ICSI, as a new treatment modality in reproductive medicine, has raised concerns on the possible genetic and malformation risks associated with this procedure.

It has been generally claimed that fertilizing spermatozoa are somehow selected and that only normal spermatozoa achieve fertilization (Yanagimachi, 1995). Yet, with the possible exception of sperm morphology and motility, there is no evidence in humans or in animals supporting this 'selection' procedure (Yanagimachi, 1995). Never-

Table X. Congenital malformations in children born after intracytoplasmic sperm injection (ICSI) using ejaculated, epididymal and testicular spermatozoa

	Source of spermatozoa		
	Ejaculated	Epididymal	Testicular
No. of children born	2486	119	63
No. (%) of malformations			
Major	47 (1.9)	0	3 (4.8)
Minor	185 (7.4)	3 (2.5)	2 (3.2)
No. of abnormalities detected by ultrasound	17	0	0
No. of therapeutic abortions	17	0	0

theless, if such a selection mechanism exists, it is important to examine what the implications might be when these selective barriers are bypassed using ICSI. Thus, the follow-up of children born after ICSI is of great significance, although it is a very difficult project since it requires special arrangements at the centres and substantial funding in order to be done properly. Hence, only 17 of the 101 centres that have submitted ICSI results for 1995 are performing a prospective follow-up of the children and only nine as a part of a special project, while another 46 centres are trying to collect information by contacting the infertility specialist, the paediatrician, or the nurses.

Concerning the incidence of congenital malformations, among 2486 children born after ICSI using ejaculated spermatozoa, 47 (1.9%) major and 185 (7.4%) minor malformations were reported, whereas no major and 3 (2.5%) minor ones were observed in 119 babies born after the use of epididymal spermatozoa and there were 3 (4.8%) major and 2 (3.2%) minor malformations in 63 babies resulting from testicular spermatozoa (Table X). Similar results have been also recorded in the children born with frozen–thawed ICSI embryos, although the numbers for epididymal and testicular spermatozoa were too small for comparison (Table XI). These incidences of major and minor congenital malformations using spermatozoa of all categories are consistent with those reported previously for ICSI (Wisanto *et al.* 1995; Bonduelle *et al.*, 1994, 1995), and for IVF (MRC Working Party, 1990; Rizk *et al.*, 1991), but are also within the range observed in the general population (Office of Population Censuses and Surveys, 1988; New York State Department of Health, 1990).

Furthermore, the prenatal genetic screening of 539 fetuses after ICSI with ejaculated, epididymal and testicular spermatozoa revealed 11 (2%) abnormal karyotypes, while the postnatal screening of 99 babies showed 2 (2%) abnormal karyotypes (Table XII). On the other hand, in 27 prenatal karyotypes of fetuses from frozen–thawed embryos in five centres, one (3.7%) was abnormal, whereas one postnatal screening revealed one normal karyotype. Obviously, these numbers are too small to draw any conclusions.

Recently, Bonduelle *et al.* (1996) studied a total of 486 karyotypes in 877 children born after ICSI and found that 6 (1.2%) were de-novo chromosomal abnormalities (mainly of the sex chromosomes) and 6 (1.2%) were familial structural aberrations, values that are higher than expected in the general population (Jacobs *et al.*, 1992). This distinction of chromosomal abnormalities cannot be applied to the data collected by the ICSI Task Force, since they were not recorded separately. However, the total incidence of 2% observed by the ICSI Task Force, which probably includes both types of abnormalities, is similar to the 2.4% reported by Bonduelle *et al.* (1996). It seems, therefore, that the rate of chromosomal aberrations in children born after ICSI is slightly elevated and this is probably related to the problem of male infertility *per se*. For this reason, it is recommended that karyotypes of the male partners are performed in order to detect the pre-existing aberrations and to counsel the couples for prenatal screening until this issue is resolved with larger amounts of data.

Table XI. Congenital malformations in children produced from frozen–thawed intracytoplasmic sperm injection (ICSI) embryos according to the type of spermatozoa used for ICSI

	Source of spermatozoa		
	Ejaculated	Epididymal	Testicular
No. of children born	139	1	2
No. (%) of malformations			
Major	3 (2.2)	0	0
Minor	13 (9.3)	0	0
No. of abnormalities detected by ultrasound	0	0	0
No. of therapeutic abortions	1	0	0

Table XII. Karyotypes of children born after intracytoplasmic sperm injection (ICSI) using ejaculated, epididymal and testicular spermatozoa

Prenatal diagnosis	
No. of centres	19
No. of karyotypes	539
No. of 46,XX	259
No. of 46,XY	266
No. of others	11 (2%)
Postnatal diagnosis	
No. of centres	6
No. of karyotypes	99
No. of 46,XX	49
No. of 46,XY	48
No. of others	2 (2%)

Conclusions

All gathered data concerning ICSI in 1995 showed a high success rate of fertilization and achievement of pregnancy irrespective of sperm origin. Moreover, there did not appear to be an increased risk of major or minor congenital malformations, although a slight increase of chromosomal aberrations, especially of the sex chromosomes, was observed. Undoubtedly, this database is not large enough to allow definite conclusion, and this further supports the need to continue the follow-up of children born after ICSI. Centres should be encouraged to join collective forces, such as the ESHRE Task Force on ICSI.

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