Comparison of embryo quality between intracytoplasmic sperm injection and in vitro fertilization in sibling oocytes

R. Yoeli · R. Orvieto · J. Ashkenazi · M. Shelef ·
Z. Ben-Rafael · I. Bar-Hava

Received: 9 July 2004 / Accepted: 14 November 2007 / Published online: 19 January 2008
© Springer Science + Business Media, LLC 2007

Abstract

Background The aim of the present study was to compare the quality of embryos derived from sibling oocytes by in vitro fertilization (IVF) or ICSI.

Methods Consecutive patients with a less than 40% fertilization rate in a previous standard IVF cycle or with relative male factor infertility were recruited for the study. The oocytes retrieved from each patient were divided into two groups for either conventional insemination (group A) or ICSI (group B). Power analysis showed that to detect a 25% difference in the rate of high-quality embryos between the groups with a power of 0.8, at least 1,200 oocytes were needed in each group.

Results One hundred seventy-seven patients were included in the study. Group A was comprised of 1,526 oocytes and group B of 1,480 sibling oocytes. As expected, the fertilization rate was significantly higher in group B than group A (67.1 vs. 43.6%, $p < 0.001$). No significant between-group differences were observed in cleavage rate (92.7 and 89.7%, respectively) and the rate of either grade A embryos (22.6 and 23.9%, respectively) or grade A₁ embryos (37.3 and 33.5%, respectively).

However, in the subgroup of patients with relative male-factor infertility ($n = 36$), the rate of grade A₁ embryos was

significantly higher in the IVF than the ICSI group (46.4 vs. 29.0%, respectively, $p = 0.02$).

Conclusions Embryo quality does not seem to be influenced by the mode of fertilization (IVF or ICSI). We assume that embryo quality depends on intrinsic factors of the gametes involved rather than on the fertilization process per se.

Keywords Embryo quality · Intracytoplasmic sperm injection · In vitro fertilization · Sibling oocytes

Introduction

The application of intracytoplasmic sperm injection (ICSI) is becoming more and more popular in assisted reproduction technology (ART) units throughout the world. ICSI is currently being used mostly for two indications: severe male-factor infertility and low fertilization rate in previous standard in vitro fertilization (IVF) treatments. However, the high success rate achieved by ICSI has raised suggestions that it may be performed in all IVF cases [1–3]. At the same time, the lack of natural selection of sperm and the circumvention of most of the known fertilization stages have led to concerns regarding embryo quality and pregnancy outcome.

It is well known that embryo quality is an important factor affecting pregnancy potential [4]. In a previous retrospective case-control study, we reported that embryos obtained after ICSI are inferior to those obtained after standard IVF in terms of both morphology and the likelihood of achieving clinical pregnancy [5]. Similar results were demonstrated by Hsu et al. [6] and Frattarelli et al. [7]. Others, however, found embryo quality to be comparable between the two methods [3, 8–13], or even superior in ICSI [2, 14, 15].

Capsule Embryo quality does not seem to be influenced by the mode of fertilization but depends on intrinsic factors of the gametes involved.

R. Yoeli · R. Orvieto · J. Ashkenazi · M. Shelef ·
Z. Ben-Rafael · I. Bar-Hava (✉)
Department of Obstetrics and Gynecology,
Rabin Medical Center (Golda Campus),
Petah Tikva and Sackler Faculty of Medicine, Tel Aviv University,
7 Kakal St.,
Tel Aviv, Petah Tikva 49372, Israel
e-mail: barhava@gmail.com

It remains unclear if ICSI affects embryo quality. The aim of the present study was to compare the quality of embryos derived from sibling oocytes by IVF or ICSI.

Material and methods

All consecutive women attending our ART unit between February 1997 and December 2000 were considered for the study. Inclusion criteria were either less than 40% fertilization rate in a previous standard IVF cycle or relative male-factor infertility (5 to 10 million total motile sperm in the ejaculate before processing or less than 5 million total motile sperm in the ejaculate on the day of ovum pick up in a couple with above 5 million total motile sperm in the ejaculate in the previous semen sample and normal fertilization rates in a previous conventional IVF cycle), both with at least ten oocytes retrieved per cycle. The ovarian stimulation protocol, ultrasound and hormonal surveillance methods, timing of hCG administration, oocyte retrieval technique, sperm processing and embryo culture methods have been detailed elsewhere [5], as has our laboratory technique for the two ART modes [5].

The oocytes retrieved from each patient were collected into the center of a four well dish which contained medium, and then divided evenly into two (if up to 20 oocytes) or four wells (if more than 20 oocytes). Embryos were divided into two groups by allotting the oocytes from the first well (and, if more than 20 oocytes, the third well) to conventional insemination (group A) or ICSI (group B) in alternating order. Fertilization was confirmed by observation of two pronuclei 18 to 19 h after IVF insemination or ICSI. Total fertilization rate (per group) was calculated as the total number of zygotes divided by the total number of oocytes. Mean fertilization rate was determined by calculating the fertilization rate for each treatment cycle separately and averaging these results. Cleavage rate was calculated as the number of transferable embryos divided by the number of zygotes. Embryo morphology was graded before embryo transfer: embryos with equal-sized blasto-

meres, ideal cleavage rate (four cells on day 2 or eight cells on day 3) and $\leq 10\%$ fragmentation were defined as grade A; similar embryos but with $\leq 20\%$ fragmentation were defined as grade A₁. The rate of grade A or grade A₁ embryos was derived from the total number of zygotes. The fertilization rate, cleavage rate and rate of high-quality embryos (grade A and grade A₁) were compared between the groups. Each patient served as her own control.

Power analysis showed that to detect a 25% difference in the rate of high quality (grade A) embryos between the groups with a power of 0.8, we needed at least 1,200 oocytes in each group. Paired *t*-test, chi-square test and Fisher's exact test were used as appropriate for the statistical analysis. A *p* value of less than 0.05 was considered significant.

Results

One hundred seventy-seven patients were included in the study, 36 with male-factor infertility and 141 with low fertilization rate in a previous standard IVF cycle. Their ages ranged from 21 to 44 years, with a mean ($\pm$ SD) of 31.9 ± 5.2 years. Mean ($\pm$ SD) parameters for the husbands' sperm were as follows: volume 3.13 ± 1.83 mL, motility $48.72\% \pm 18.46\%$, and count $32.56 \pm 41.78 \times 10^6/\text{mL}$. Mean total motile sperm in the ejaculate was $43.93 \pm 60.86 \times 10^6$. A total of 3,006 oocytes were retrieved. Mean number of oocytes retrieved per patient was 17.1 ± 7.2 .

Group A was comprised of 1,526 oocytes and group B of 1,480 sibling oocytes. The fertilization rates are shown in Table 1. In group A, 624 of the 1,526 oocytes (40.9%) were fertilized. Mean ($\pm$ SD) fertilization rate per cycle was $43.6 \pm 30.8\%$. Of the 1,480 oocytes assigned to group B, 1,170 (79%) attained metaphase II and were injected with sperm; of these, 772 were fertilized. The overall fertilization rate per injected oocyte was 66% (772/1,170). On the basis of the number of oocytes assigned to ICSI, the overall fertilization rate was 52.2% (772/1,480). The mean fertilization rate per cycle was $67.1 \pm 24.8\%$ per injected oocyte and $54 \pm 23.6\%$ per retrieved oocyte.

Table 1 Fertilization rate in sibling oocytes assigned to IVF or ICSI in 177 women

	Group A (IVF)	Group B (ICSI)	<i>p</i> value
Total no. of oocytes	1,526	1,480	
No. of mature oocytes		1,170	
Total fertilization rate per injected oocyte	40.9% (624/1,526)	66% (772/1,170)	<0.001 ^a
Total fertilization rate per assigned oocyte	40.9% (624/1,526)	52.2% (772/1,480)	<0.001 ^a
Mean fertilization rate per cycle, per injected oocyte	$43.6 \pm 30.8\%$	$67.1 \pm 24.8\%$	<0.0001 ^b
Mean fertilization rate per cycle, per assigned oocyte	$43.6 \pm 30.8\%$	$54 \pm 23.6\%$	<0.0001 ^b

^a χ^2 test

^b Paired Student's *t*-test

There was a statistically significant difference in the overall number of oocytes fertilized between ICSI and IVF (772/1,480 vs. 624/1,526, respectively, $p<0.001$). Furthermore, the mean fertilization rate was significantly higher per ICSI than per IVF cycle (54 vs. 43.6%, respectively, $p<0.0001$).

Complete fertilization failure was observed in 27 patients after conventional IVF and in five patients after ICSI (15.2 vs. 2.8%, respectively, $p<0.001$).

The cleavage rate and the rate of high-quality embryos are shown in Table 2. There was no significant difference between the IVF and ICSI groups in cleavage rate (92.7 ± 21.3 and $89.7\pm 26.5\%$, respectively); or in the rates of grade A embryos (22.6 ± 30.0 and $23.9\pm 30.0\%$, respectively) or grade A₁ embryos (37.3 ± 43.4 and $33.5\pm 39.1\%$, respectively).

For further analysis, the groups were further subdivided by reason for treatment into cycles with relative male factor infertility ($n=36$) and cycles with low fertilization rate in a previous standard IVF cycle ($n=141$). The results are summarized in Table 3. Patient age was similar in the two groups (32.3 ± 5.4 and 31.8 ± 5.2 years, respectively). As expected, there was a significant difference in the husbands' sperm characteristics between the two groups with mean total motile sperm in the ejaculate $5.1\pm 2.5\times 10^6$ vs. $54.3\pm 64.7\times 10^6$, respectively ($p<0.001$). No significant differences were found between the IVF procedures in the two groups in terms of fertilization rate, cleavage rate and rate of grade A or grade A₁ embryos. This was also true for the ICSI procedures.

Within the subgroup of cycles with low fertilization rate in a previous standard IVF cycle ($n=141$), the comparison between IVF and ICSI yielded similar results to those for the whole study group. There was a statistically significant difference between the procedures in overall number of oocytes fertilized (603/1,148 vs. 509/1,199 per ICSI and IVF cycles, respectively, $p<0.0001$); and mean fertilization rate (54.4 vs. 44.2%, respectively, $p<0.001$). Complete fertilization failure was observed in 22 patients after conventional IVF and in two patients after ICSI (15.6 vs.

1.4%, respectively, $p<0.001$). There was no significant difference in cleavage rate (92.7 ± 21.6 and $92.9\pm 17.9\%$, respectively) or rate of grade A embryos (23.3 ± 29.8 and $20.8\pm 28.4\%$, respectively) or grade A₁ embryos (34.6 ± 40.3 and $34.9\pm 42.8\%$, respectively).

Analysis of the cycles with relative male-factor infertility ($n=36$) yielded somewhat different results. There was a statistically significant difference in the overall number of oocytes fertilized with ICSI than with IVF (169/332 vs. 115/327, respectively, $p<0.0001$), but not in the mean fertilization rate per assigned oocyte (52.2 and 41.4%, respectively). The latter finding may be attributed to the small sample size. Complete fertilization failure was observed in five patients after conventional IVF and in three patients after ICSI (13.9 and 8.3%, respectively). Again, the difference was not statistically significant. The cleavage rate was similar in the IVF and ICSI groups (90.0 ± 30.8 and $90.6\pm 25.9\%$, respectively), as was the rate of grade A embryos (29.4 ± 36.9 and $26.7\pm 33.0\%$, respectively). However, the rate of grade A₁ embryos was significantly higher in the IVF group (46.4 ± 45.4 vs. $29.0\pm 34.0\%$, respectively, $p=0.02$).

The best quality embryos were selected for transfer independent of the insemination procedure. Embryo transfer was performed in 176 cycles, resulting in 53 clinical pregnancies (30.1% per embryo transfer).

Because transfers included both IVF- and ICSI-derived embryos, no assessment of the relationship between the treatment procedure and pregnancy rate could be made.

Discussion

By using sibling oocytes where each patient serves as her own control, we were able to control for confounding factors that are inherent to other study designs and to more accurately compare IVF and ICSI embryos. The strength of this study lies in the comparison of the rate of high-quality embryos per couple between IVF and ICSI, which was performed in only one previous sibling oocyte study in the literature [9]. The results demonstrated no significant differences between the IVF and ICSI groups (for the whole study sample) in the rate of high-quality embryos (grade A or grade A₁). Thus, after controlling for possible paternal and maternal effects, we demonstrated that embryo quality does not seem to be influenced by the mode of fertilization (IVF or ICSI). This finding confirms the assumptions raised by others [3, 8–13]. We conclude that embryo quality depends on intrinsic factors of the gametes involved rather than on the fertilization process per se.

It should be noted, however, that in the subgroup of couples with relative male-factor infertility, the rate of grade A₁ embryos was significantly higher in the IVF than

Table 2 Cleavage rate and rate of high-quality embryos obtained by IVF or ICSI performed on sibling oocytes in 177 women

	Group A (IVF)	Group B (ICSI)	<i>p</i> value (paired <i>t</i> -test)
Cleavage rate	$92.7\pm 21.3\%$	$89.7\pm 26.5\%$	N.S.
Rate of grade A embryos	$22.6\pm 33.0\%$	$23.9\pm 30.0\%$	N.S.
Rate of grade A ₁ embryos	$37.3\pm 43.4\%$	$33.5\pm 39.1\%$	N.S.

Grade A embryos: embryos with equal-sized blastomeres, ideal cleavage rate (four cells on day 2 or eight cells on day 3) and $\leq 10\%$ fragmentation. Grade A₁ embryos: similar embryos but with $\leq 20\%$ fragmentation

Table 3 Results of IVF and ICSI performed on sibling oocytes in cycles with relative male-factor infertility ($n=36$) and cycles with low fertilization rate in a previous standard IVF cycle ($n=141$)

	Cycles with relative male factor infertility		<i>p</i> value	Cycles with low fertilization rate in a previous standard IVF cycle		<i>p</i> value
	IVF	ICSI		IVF	ICSI	
Total no. of oocytes	327	332		1,199	1,148	
No. of mature oocytes		259			911	
Total fertilization rate per injected oocyte	35.2% (115/327)	65.2% (169/259)	<0.0001 ^a	42.5% (509/1,199)	66.2% (603/911)	<0.0001 ^a
Total fertilization rate per assigned oocyte	35.2% (115/327)	50.9% (169/332)	<0.0001 ^a	42.5% (509/1,199)	52.5% (603/1,148)	<0.0001 ^a
Mean fertilization rate per cycle, per injected oocyte (%)	41.4±28.5	65.1±26.6	<0.002 ^b	44.2±31.4	67.6±24.3	<0.001 ^b
Mean fertilization rate per cycle, per assigned oocyte (%)	41.4±28.5	52.2±24.6	N.S. ^b	44.2±31.4	54.4±23.3	<0.001 ^b
Cleavage rate (%)	90.0±30.8	90.6±25.9	N.S. ^b	92.9±17.9	92.7±21.6	N.S. ^b
Rate of grade A embryos (%)	29.4±36.9	26.7±33.0	N.S. ^b	20.8±28.4	23.3±29.8	N.S. ^b
Rate of grade A1 embryos (%)	46.4±45.4	29.0±34.0	0.02 ^b	34.9±42.8	34.6±40.3	N.S. ^b

^a χ^2 test^b Paired Student's *t*-test

Grade A embryos: embryos with equal-sized blastomeres, ideal cleavage rate (four cells on day 2 or eight cells on day 3) and ≤10% fragmentation.
 Grade A₁ embryos: similar embryos but with ≤20% fragmentation

the ICSI group. The main factor characterizing this subgroup was poor semen quality (average total motile sperm in the ejaculate was $5.1 \pm 2.5 \times 10^6$), so that the negative influence on embryo quality may be attributed to the poor quality of the spermatozoa injected by ICSI. When fertilization occurs in IVF, there is still a natural selection of sperm, even if from a smaller, less qualitative cohort. In the ICSI procedure, there is no such selection, and “forced fertilization” can be achieved using spermatozoa that might never have attained natural fertilization. It has been shown that spermatozoa from infertile men have higher rates of defects such as DNA damage [16, 17] and aneuploidy [18–20], which are likely to have an adverse effect on embryo development [21] and thereby decrease embryo quality. These findings were not true for grade A embryos, probably due to the small sample size. These results also support our conclusion that embryo morphology is probably influenced by intrinsic gamete (sperm in this case) factors and not by the mode of fertilization.

In a recent study using sibling oocytes from 35 couples with non-male-factor infertility, Khamisi and colleagues [15] demonstrated increased formation of good-quality embryos per retrieved oocyte after ICSI than after conventional IVF (64.4 vs. 47.1%, respectively). However, there was no significant difference in the formation of good-quality embryos per fertilized oocyte. These findings indicate that ICSI does not improve or diminish the formation of good embryos; rather, the difference between IVF and ICSI in good embryo-formation per oocyte was related to the better fertilization rate obtained with ICSI [15]. Yang et al [14] found that more

grade A embryos were obtained by ICSI than by conventional IVF performed on sibling oocytes. However, their study group was comprised of only 13 couples.

The major advantage of performing ICSI is that cases complicated by total fertilization failure can almost be eliminated. Several studies have attempted to demonstrate the superiority of ICSI over IVF based on total fertilization failure rates. Aboulghar and his colleagues [22] performed a study on sibling oocytes of 22 patients with unexplained infertility and 24 patients with borderline semen. They found a clear benefit of ICSI over IVF in both groups (total fertilization failure in 22.7 and 45.8% for IVF vs. 0 and 0 for ICSI, respectively). Ruiz and his colleagues [8] in a controlled study of 70 couples with unexplained infertility or mild endometriosis, again, demonstrated an advantage of ICSI over IVF (failed fertilization rates of 0 vs. 11.4%, respectively). Verheyen and his colleagues demonstrated similar results in 20 couples with relative male factor infertility (failed fertilization rates of 0 vs. 50%) [11]. Piskara et al. [9], Staessen et al. [10] and Khamisi et al. [15] demonstrated a similar trend towards lower rates of total fertilization failure in ICSI than IVF. Reported rates ranged between 10 to 27.8% for IVF vs. 0–5.5% for ICSI. In the current study, which is the largest to date, the rate of complete fertilization failure was 15.2% after conventional IVF (27 of 177 patients) and 2.8% after ICSI (5 of 177 patients). Since this difference was found to be highly significant ($p < 0.001$) it confirms the advantage of the ICSI procedure in minimizing the risk for cycle cancellation due to total fertilization failure.

Unexplained lack of fertilization is a known phenomenon in IVF. Some authors suggest that it does not tend to recur in subsequent cycles [23–26]. According to these authors total fertilization failure in these cycles may be explained by non-repetitive factors such as treatment-related inadequate ovarian stimulation producing fewer good oocytes or temporarily inadequate laboratory conditions. However, other studies demonstrate that total fertilization failure in IVF is a repetitive phenomenon indicating a possible underlying, undiagnosed sperm or oocyte pathology [27, 28]. In order to avoid cases of total fertilization failure, a combination between IVF and ICSI on sibling oocytes has been advised in the literature.

Nevertheless, this application has some disadvantages. They include higher costs, more technical difficulties, unavailability of the technique in some centers and performing an unnecessary invasive technique in some cases. Moreover, since the ICSI procedure itself is more invasive questions regarding safety issues have been raised. Several studies have demonstrated that there is no increase in the incidence of congenital anomalies in children born after ICSI compared with standard IVF or with the general population [29–36]. However, others showed that infants born after ICSI might indeed have an excess of major birth defects [37–39] and an increased incidence of chromosomal abnormalities [31, 35]. It is possible that the increases in congenital abnormalities observed in some ICSI children, such as hypospadias or sex chromosome abnormalities, are due to factors related to paternal infertility and the use of suboptimal male gametes. It is also possible that the technique itself plays a role. Furthermore, the long-term outcome of the ICSI procedure in terms of development and transfer of genetic disorders is still unknown.

The limitation of the study is its limited power, designed to detect only a 25% difference in the rate of high quality (grade A) embryos between the two methods of fertilization. Still, this is the largest study to date. Moreover, according to the mean number of oocytes retrieved per patient and the mean fertilization and cleavage rates the study has sufficient power to detect a difference of at least one embryo between the two groups. A lesser difference would not be of clinical importance.

In conclusion, embryo quality does not seem to be influenced by the mode of fertilization (IVF or ICSI). We suggest that embryo quality depends on intrinsic factors of the gametes involved rather than on the fertilization process per se. Conventional IVF should be the option of choice for every couple requiring ART treatments, to avoid the disadvantages of ICSI. It may be concluded that it is not justified to perform ICSI for all couples requiring in vitro conception. A possible strategy might be to perform a diagnostic comparison between conventional IVF and ICSI in all first treatment cycles. This would be especially

beneficial in preventing total fertilization failure in unexplained and relative male factor infertility cases.

References

- Hall J, Fishel S, Green S. Intracytoplasmic sperm injection versus high insemination concentration in cases of very severe teratozoospermia. *Hum Reprod* 1995;10:493–6.
- Oehninger S, Kruger TF, Simon T, Jones D, Mayer J, Lanzendorf S, et al. A comparative analysis of embryo implantation potential in patients with severe teratozoospermia undergoing in-vitro fertilization with a high insemination concentration or intracytoplasmic sperm injection. *Hum Reprod* 1996;11:1086–9.
- Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. *Fertil Steril* 1996;65:899–908.
- Schwartz LB, Chiu AS, Courtney M, Krey L, Schmidt-Sarosi C. The embryo versus endometrium controversy revisited as it relates to predicting pregnancy outcome in in vitro fertilization-embryo transfer cycles. *Hum Reprod* 1997;12:45–50.
- Bar-Hava I, Ashkenazi J, Shelef M, Schwartz A, Brengauz M, Feldberg D, et al. Morphology and clinical outcome of embryos after in vitro fertilization are superior to those after intracytoplasmic sperm injection. *Fertil Steril* 1997;68:653–7.
- Hsu MI, Mayer J, Aronshon M, Lanzendorf S, Muasher S, Kolm P, et al. Embryo implantation in in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. *Fertil Steril* 1999;72:679–85.
- Frattarelli JL, Leondires MP, Miller BT, Segars JH. Intracytoplasmic sperm injection increases embryo fragmentation without affecting clinical outcome. *J Assist Reprod Genet* 2000;17:207–12.
- Ruiz A, Remohi J, Minguez Y, Guanes PP, Simon C, Pellicer A. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril* 1997;68:171–3.
- Pisarska MD, Casson PR, Cisneros PL, Lamb DJ, Lipshultz LI, Buster JE, et al. Fertilization after standard in vitro fertilization versus intracytoplasmic sperm injection in subfertile males using sibling oocytes. *Fertil Steril* 1999;71:627–32.
- Staessen C, Camus M, Clasen K, De Vos A, Van Steirteghem A. Conventional in vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum Reprod* 1999;14:2474–9.
- Verheyen G, Tournaye H, Staessen C, De Vos A, Vandervorst M, Van Steirteghem A. Controlled comparison of conventional in-vitro fertilization and intracytoplasmic sperm injection in patients with asthenozoospermia. *Hum Reprod* 1999;14:2313–9.
- Bukulmez O, Yarali H, Yucel A, Sari T, Gurgan T. Intracytoplasmic sperm injection versus in vitro fertilization for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. *Fertil Steril* 2000;73:38–42.
- Fishel S, Aslam I, Lisi F, Rinaldi L, Timson J, Jacobson M, et al. Should ICSI be the treatment of choice for all cases if in-vitro conception? *Hum Reprod* 2000;15:1278–83.
- Yang D, Shahata MA, Al-Bader M, Al-Natsha SD, Al-Flamerzia M, Al-Shawaf T. Intracytoplasmic sperm injection improving embryo quality: comparison of the sibling oocyte of non-male-factor couples. *J Assist Reprod Genet* 1996;13:351–5.
- Khamsi F, Yavas Y, Roberge S, Wong JC, Lacanna IC, Endman M. Intracytoplasmic sperm injection increases fertilization and good-quality embryo formation in patients with non-male factor indications for in vitro fertilization: a prospective randomized study. *Fertil Steril* 2001;75:342–7.

16. Hughes CM, Lewis SEE, McKelvey-Martin VJ, Thompson W. A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. *Mol Hum Reprod* 1996;2:613–9.
17. Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG, Bianchi U. Origin of DNA damage in ejaculated human spermatozoa. *Rev Reprod* 1999;4:31–7.
18. Bernardini L, Martini E, Geraedts JP, Hopman AH, Lanteri S, Conte N, et al. Comparison of chromosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by in-situ hybridization. *Mol Hum Reprod* 1997;3:431–8.
19. Colombero LT, Hariprashad JJ, Tsai MC, Rosenwaks Z, Palermo GD. Incidence of sperm aneuploidy in relation to semen characteristics and assisted reproductive outcome. *Fertil Steril* 1999;72:90–6.
20. Pfeffer J, Pang MG, Hoegerman SF, Osgood CJ, Stacey MW, Mayer J, et al. Aneuploidy frequencies in semen fraction from ten oligoasthenoteratozoospermic patients donating sperm for intracytoplasmic sperm injection. *Fertil Steril* 1999;72:472–8.
21. Ola B, Afnan M, Sharif K, Papaioannou S, Hammadieh N, Barratt CLR. Should ICSI be the treatment of choice for all cases of in-vitro conception? *Hum Reprod* 2001;16:2485–90.
22. Aboulghar MA, Mansour RT, Serour GI, Sattar MA, Amin YM. Intracytoplasmic sperm injection and conventional in vitro fertilization for sibling oocytes in cases of unexplained infertility and borderline semen. *J Assist Reprod Genet* 1996;13:38–42.
23. Molley D, Harrison K, Breen T, Hennessey J. The predictive value of idiopathic failure to fertilize on the first in vitro fertilization attempt. *Fertil Steril* 1991;56:285–9.
24. Ben-Shlomo I, Bider D, Dor J, Levran D, Mashiach S, Ben-Rafael Z. Failure to fertilize in vitro in couples with male factor infertility: what next? *Fertil Steril* 1992;58:187–9.
25. Lipitz S, Rabinovici J, Ben-Shlomo I, Bider D, Ben-Rafael Z, Mashiach S, et al. Complete failure of fertilization in couples with unexplained infertility: implications for subsequent in vitro fertilization cycles. *Fertil Steril* 1993;59:348–52.
26. Lipitz S, Rabinovici J, Goldenberg M, Bider D, Dor J, Mashiach S. Complete failure of fertilization in couples with mechanical infertility: implications for subsequent in vitro fertilization cycles. *Fertil Steril* 1994;61:863–6.
27. Calderon G, Belil I, Aran B, Veiga A, Gil Y, Boada M, et al. Intracytoplasmic sperm injection versus conventional in-vitro fertilization: first results. *Hum Reprod* 1995;10:2835–9.
28. Roest J, Van Heusden AM, Zeilmaker GH, Verhoeff A. Treatment policy after poor fertilization in the first IVF cycle. *J Assist Reprod Genet* 1998;15:18–21.
29. Tarlatzis BC. Report on the activities of the ESHRE Task Force on intracytoplasmic sperm injection. *Hum Reprod* 1996;11(Suppl 4):160–85.
30. Wennerholm UB, Bergh C, Hamberger L, Nilsson L, Reisner E, Wennergren M, et al. Obstetric and perinatal outcome of pregnancies following intracytoplasmic sperm injection. *Hum Reprod* 1996;11:1113–9.
31. Bonduelle M, Wilikens A, Buysse A, Van Assche E, Wisanto A, Devroey P, et al. A follow-up study of children born after intracytoplasmic sperm injection (ICSI) with epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum Reprod* 1998;13(suppl 1): 196–207.
32. Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E, et al. Seven years of intracytoplasmic sperm injection and follow up of 1987 subsequent children. *Hum Reprod* 1999;14 (Suppl 1):243–64.
33. Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and 2995 infants born after IVF (1983–1999). *Hum Reprod* 2002;17:671–94.
34. Loft A, Petersen K, Erb K, Mikkelsen AL, Grinsted J, Hald F, et al. A Danish national cohort of 730 infants born after intracytoplasmic sperm injection (ICSI) 1994–1997. *Hum Reprod* 1999; 14:2143–8.
35. Tarlatzis BC, Grimbizis G. Pregnancy and child outcome after assisted reproduction techniques. *Hum Reprod* 1999;14(Suppl 1):231–42.
36. Palermo GD, Neri QV, Hariprashad JJ, Davis OK, Veeck LL, Rosenwaks Z. ICSI and its outcome. *Semin Reprod Med* 2000; 18:161–9.
37. Kurinczuk JJ, Bower C. Birth defects in infants conceived by intracytoplasmic sperm injection: an alternative interpretation. *Br Med J* 1997;315:1260–6.
38. Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M, et al. Incidence of congenital malformations in children born after ICSI. *Hum Reprod* 2000;15:944–8.
39. Ericson A, Kallen B. Congenital malformations in infants born after IVF: a population-based study. *Hum Reprod* 2001;16:504–9.