

Ruolo della ICSI in assenza di fattore maschile severo

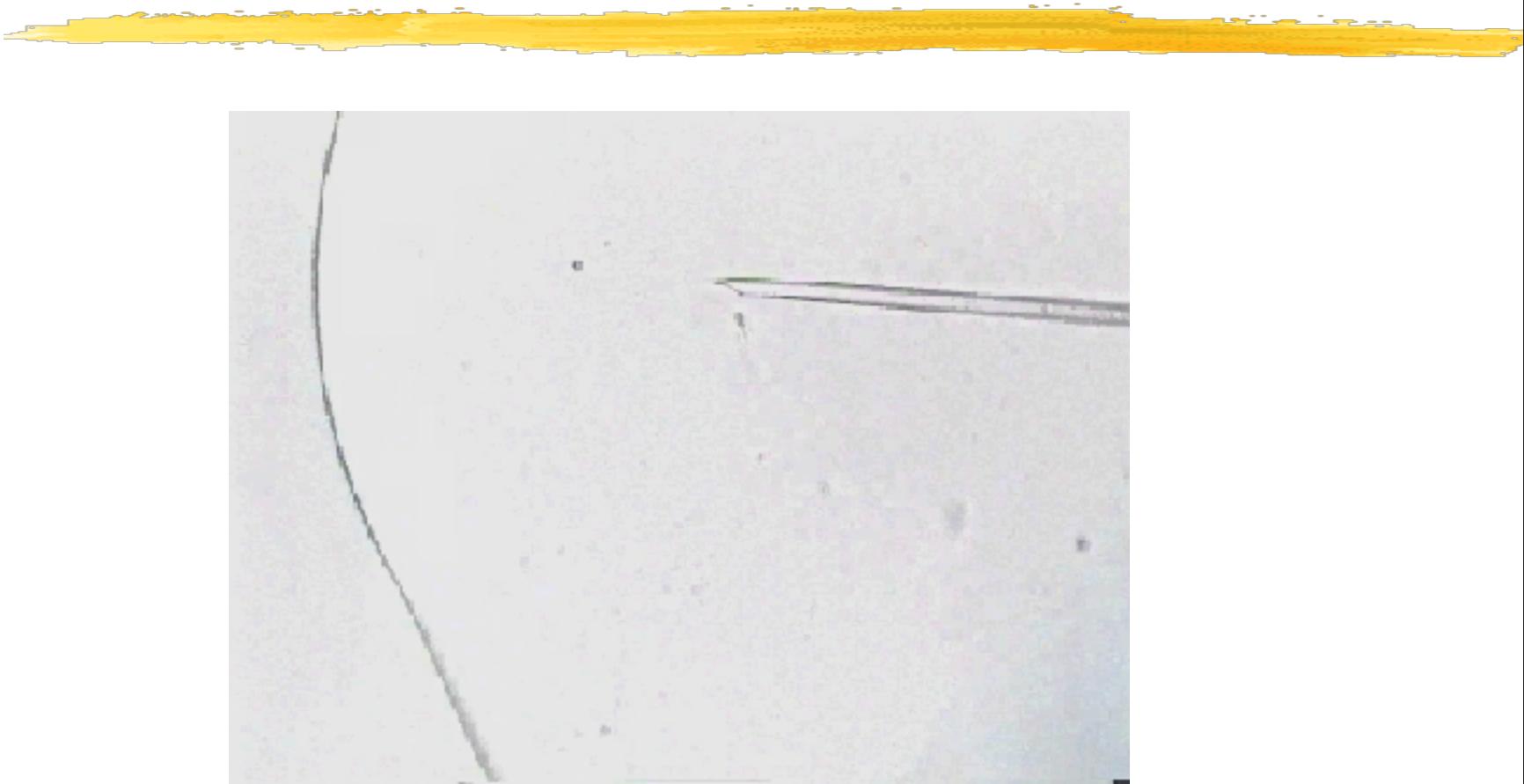


Fecondazione in Vitro: Dai gameti alla Società
Trieste, 22-23 giugno 2001

Carlo Bulletti

U.O. Fisiopatologia della Riproduzione
Ospedale Infermi - Rimini

Tecnica ICSI



Indicazioni

-  fattore maschile
-  diagnosi genetica pre-impianto
-  congelamento oociti
-  precedente fallita fertilizzazione in vitro ?
-  poor responders?
-  normospermia?

Anomalie cromosomiche dopo ICSI

Table I. Karyotype anomalies in 1082 prenatal diagnoses

Abnormal karyotypes on 1082 prenatal tests	Maternal age (years)	Number	Percentage	95% confidence interval	Percentage in literature ^a (on 56 952 newborns)	Percentage in literature ^b (on 34 910 newborns)
De-novo chromosomal aberrations		18	1.66	1.0–2.7	0.445	
Sex-chromosomal:		9	0.83	0.3–1.6	0.19	0.23 (total sex-chromosomal)
45,X	37					
46,XX/47,XXX	44					
47,XXX (2 children)	32, 37					
47,XXY (4 children)	26, 28, 28, 32					
47,XYY	25					
Autosomal:		9	0.83	0.3–1.6	0.21	0.61 (total autosomal)
Trisomy 21 (5 children)	32, 33, 37, 41, 41	5	0.46		0.14	
structural		4	0.36		0.07	
46,XY,t(4;5)	30					
46,XX,t(2;15)	30					
46,XX,t(2;13)	36					
46,XX, inv(1qh)	39					
Inherited aberrations		10	0.92	0.4–1.7	0.47	
balanced		9	0.83		0.45	
unbalanced		1	0.09		0.023	
Total aberrations <i>de novo</i> + inherited		28	2.5	3.0–5.7	0.92	0.84

^a(Jacobs *et al.*, 1992).

^b(Nielsen and Wohlert, 1991).

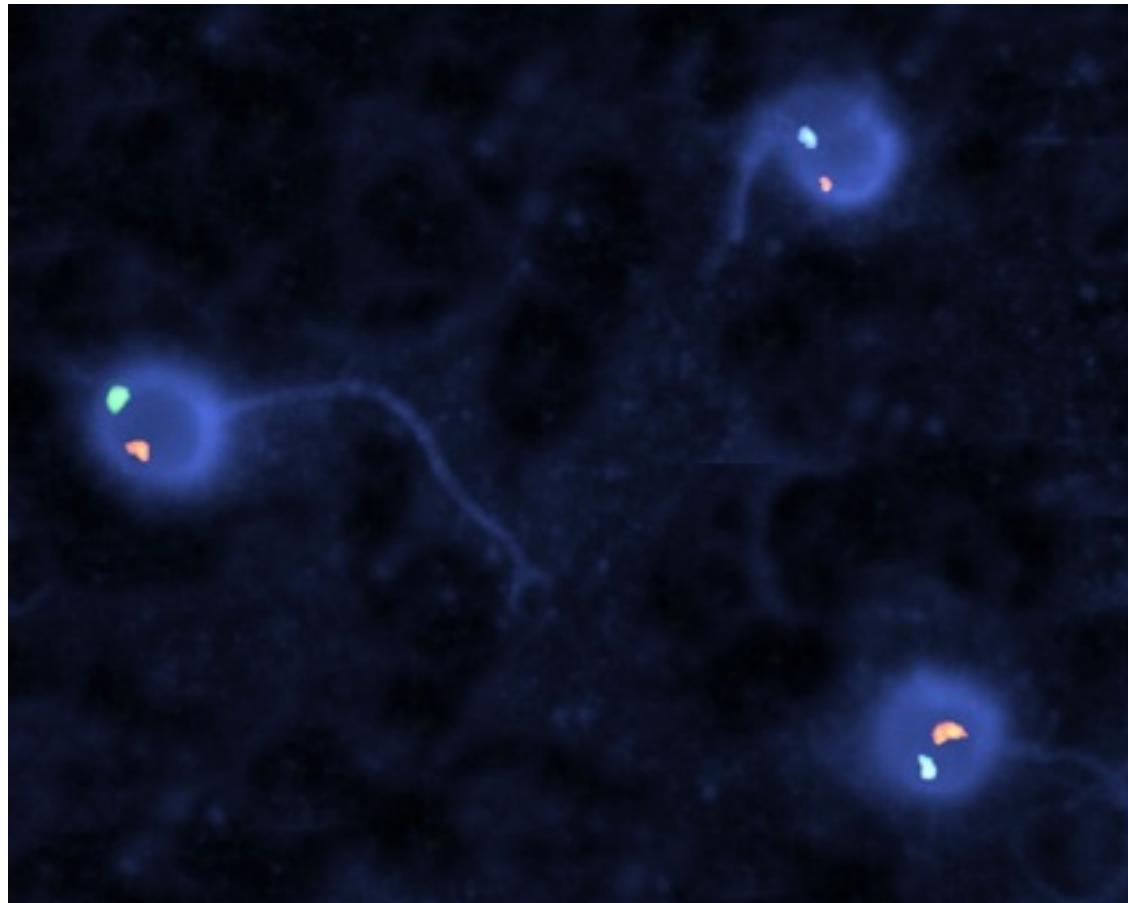


☒ Spermatozoo normale con un segnale per il cromosoma 1 (arancione) ed uno per il cromosoma X (verde).





Spermatozoi normali con un segnale per il cromosoma 1 (arancione) e un segnale per il cromosoma Y (azzurro); uno spermatozoo presenta un segnale per il cromosoma 1 (arancione) e un segnale per il cromosoma X (verde).



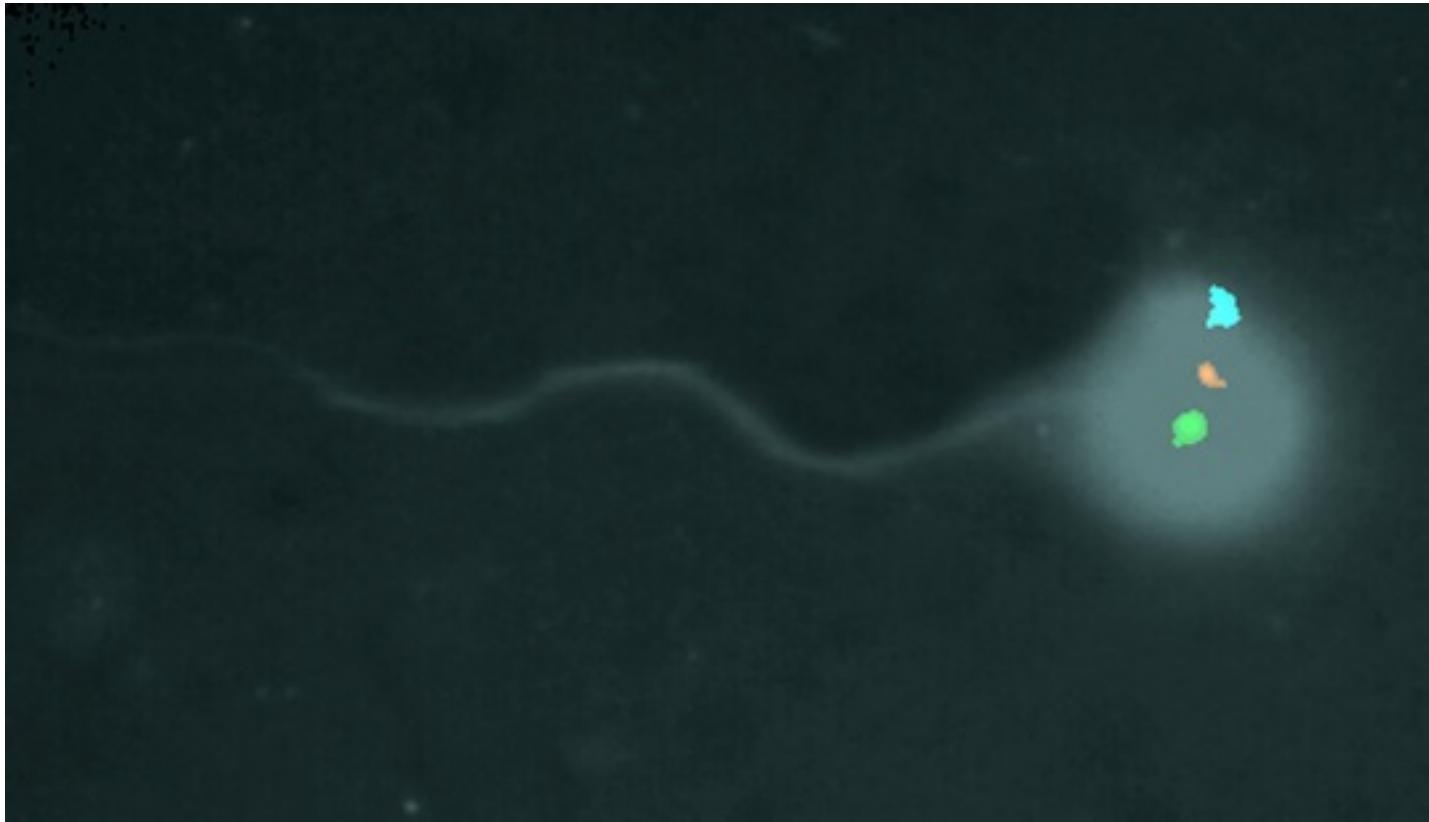


☒ Spermatozoo con disomia dei cromosomi sessuali. Notare la presenza di due cromosomi X (verde) ed un cromosoma 1 (arancione).



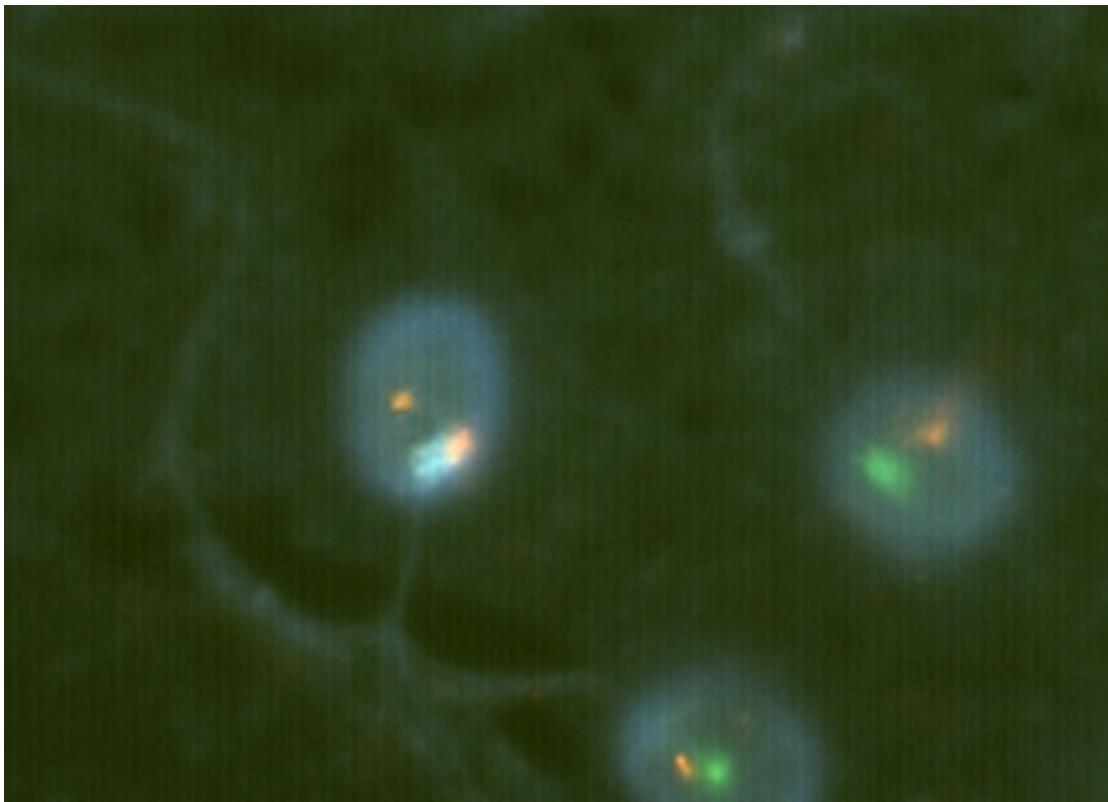


- ▣ Spermatozoo con disomia dei cromosomi sessuali. Notare la presenza dei due cromosomi sessuali X e Y (verde e azzurro) ed un cromosoma 1 (arancione).





Spermatozoo con disomia del cromosoma 1: presenza di due segnali per il cromosoma 1 (arancione) e un segnale per il cromosoma Y (azzurro). Sono inoltre presenti due spermatozoi normali, con un segnale per il cromosoma 1 (arancione) e un segnale per il cromosoma X (verde).



Anomalie cromosomiche negli spermatozoi in presenza di cariotipo periferico normale

TABELLA XII: Aneuploidie, calcolate come somma delle disomie e delle nullisomie, nei Pazienti ICSI

Pazienti	n. cellule	Aneuploidie 1(%)	Aneuploidie X/Y(%)
An.	933	6(0.64)	8(0.85)
St.	771	5(0.64)	11(1.41)
Gi.	1047	6(0.57)	5(0.47)
Ca.	805	6(0.74)	9(1.11)
Pi.	932	4(0.42)	2(0.21)
Fe.	922	7(0.75)	6(0.65)
Ca.	994	9(0.90)	8(0.80)
Va.	846	8(0.94)	1(0.11)
Bi.	980	9(0.91)	6(0.61)
Da.	1022	4(0.39)	4(0.39)
Totale	9252	64	60
%		0.69	0.64

ICSI e precedente fallita fertilizzazione in vitro

Table I. Intracytoplasmic sperm injection (ICSI) performed due to previous IVF failure or known male factor

	No. patients	Aspiration	Transfer	Pregnancy ^a	Pregnancy/asp. (%)	Pregnancy/transf. (%)
Male factor	220	258	248	111	43	45
IVF failure	162	189	180	26	14	14

^aOngoing pregnancies with at least one fetal heart detectable by ultrasound. The mean number of embryos transferred in each group was 2.45.

Table II. Intracytoplasmic sperm injection (ICSI) performed due to previous IVF failure or known male factor

	No. oocytes aspirated	No. at MII	No. PN	No. cleaved	Cleaved/MII (%)	PN/MII (%)	Cleaved/PN (%)
Male factor	2521	1905	1709	1599	84 ^a	90 ^a	94 ^a
IVF failure	1349	1070	882	779	73 ^a	82 ^a	88 ^a

^a $P < 0.05$ for comparison of male factor with IVF failure.

MII: metaphase II eggs; PN: eggs with 2 pronuclei.

ICSI e precedente fallita fertilizzazione in vitro

Table II. Overall results of intracytoplasmic sperm injection performed because of previous failed fertilization or a low fertilization rate (without male factor) in in-vitro fertilization (IVF) or due to a primary male factor

	Previous failed fertilization or low fertilization rate in IVF	Primary male factor
No. of cycles	65	219
No. of oocytes collected	579	2147
No. of mature oocytes injected	430 (74)	1657 (77)
No. of oocytes fertilized	300 (70)	1156 (70)
Two pronuclei	279 (65)	1076 (65)
Three pronuclei	19 (4)	61 (4)
No. of oocytes cleaved	258 (92)	1011 (94)
No. of embryo transfers	56	197
No. of embryos transferred	115	390
Average no. of embryos per transfer	2.1	2.0
No. of pregnancies (% per transfer)	11 (19.6)	66 (33.5) ^a
No. of embryos implanted	11 (9.6)	76 (19.5) ^a
No. of cycles with frozen embryos	27 (42)	111 (51)
No. of frozen embryos	108	548
Average no. of frozen embryos per patient	4.0	4.9

Values in parentheses are percentages.

^a $P < 0.05$.

Tomas C et al, *Hum Reprod*, 1998, 13(1):65-70.

ICSI e precedente fallita fertilizzazione in vitro

Table I. Results with conventional in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) of sibling oocytes in 23 cycles

	Conventional IVF	ICSI	
	n	n	%
MII oocytes	85	143	
Oocytes with:			
2 pronuclei	0	90 ^c	62.9
3 pronuclei	0	10 ^c	7.0
1 pronucleus	0	9 ^c	6.3
no fertilization	85	13 ^c	9.1
Cleaved embryos		82	91.1
Replacements		23	
Replaced embryos		48	
Implantation rate		11	22.9 ^a
Pregnancies		8	34.8 ^b
Ongoing pregnancies		6	26.1 ^b
Live birth rate		9	18.8 ^a

^aPer embryo replaced.

^bPer embryo replacement.

^cSignificantly different from IVF result ($P < 0.001$).

Kastrop PMM et al, *Hum Reprod*, 1999, 14(1):65-9.

ICSI e precedente fallita fertilizzazione in vitro

TABLE 1

Outcomes of IVF after ICSI in 25 patients who had previous idiopathic fertilization failure with conventional IVF (group A) compared with 87 patients who underwent ICSI for male factor indications (group B).

Variable	Group A (n = 25)	Group B (n = 87)
No. of cycles	38	118
Mean (\pm SD) no. of eggs retrieved	14.6 \pm 9.7	14.9 \pm 7.2
Fertilization rate (%)	68	64
Implantation rate per embryo (%)	22.6	20.0
Delivery rate (%) (no. of deliveries/ no. of cycles)	47.3 (18/38)	49.1 (58/118)

Note: All P values were not statistically significant.

Benadiva. Intracytoplasmic sperm injection. *Fertil Steril* 1999.

ICSI e precedente fallita fertilizzazione in vitro

TABLE 2

Outcomes of IVF after ICSI in the subgroup of patients who had previous complete fertilization failure (0) with conventional IVF versus patients who had a fertilization rate of $\leq 20\%$ but >0 .

Variable	No fertilization (n = 14)	Fertilization $>1\%$ but $\leq 20\%$ (n = 11)
No. of cycles	22	16
Fertilization rate (%)	69	66
Implantation rate per embryo (%)	20.4	25.8
Delivery rate (%) (no. of deliveries/no. of cycles)	45.4 (10/22)	50 (8/16)
Delivery rate per patient (%) (no. of deliveries/no. of patients)	71.4 (10/14)	72.7 (8/11)

Note: All *P* values were not statistically significant.

Benadiva. Intracytoplasmic sperm injection. *Fertil Steril* 1999.

ICSI e poor/low responders

Table I. Results of in-vitro fertilization with standard IVF and ICSI

	IVF	ICSI
Cycles	52	52
Age ^{a,b} (years)	36.7 ± 0.6	35.3 ± 0.6
No. of FSH ampoules ^{a,b}	27.0 ± 1.7	27.7 ± 1.9
No. of HMG ampoules ^{a,b}	19.2 ± 1.6	21.0 ± 2.0
Motile spermatozoa ^{a,b} ($\times 10^6$)	87.2 ± 10.6	78.0 ± 11.0
Oocytes per patient ^{a,b}	4.5 ± 0.2	4.4 ± 0.2
Fertilization per inseminated oocyte ^{a,b} (%)	70.2 ± 4.9	77.7 ± 4.5
Fertilization per obtained oocyte ^{a,b} (%)	58.8 ± 4.6	56.5 ± 4.1
Complete fertilization failure ^b	6 (11.5%)	6 (11.5%)
Embryos per patient ^{a,b}	2.5 ± 0.2	2.2 ± 0.2
No. blastomeres per embryo ^{a,b}	2.7 ± 0.1	3.0 ± 0.2
Degree of fragmentation per embryo ^{a,b}	1.6 ± 0.1	1.4 ± 0.1
Embryos per transfer ^{a,b}	2.4 ± 0.2	2.1 ± 0.2
Pregnancy rate/cycle ^b (%)	17.3	21.1
Implantation rate ^b (%)	11.1	14.0
Miscarriages ^b (%)	33.3	36.4

FSH = follicle stimulating hormone; HMG = human menopausal gonadotrophin; IVF = in-vitro fertilization; ICSI = intracytoplasmic sperm injection.

^aValues are expressed as a mean ± SEM.

^bInter-group differences were not statistically significant.

Table II. Results obtained with IVF and ICSI in patients with three or fewer retrieved oocytes

	IVF	ICSI
Cycles	15	11
Age ^{a,b} (years)	37.7 ± 1.1	36.3 ± 1.2
No. of oocytes per patient ^{a,b}	2.2 ± 0.2	2.2 ± 0.3
Fertilization per inseminated oocyte ^{a,b} (%)	61.1 ± 12.1	72.3 ± 11.7
Fertilization per obtained oocyte ^{a,b} (%)	48.9 ± 10.6	65.1 ± 11.9
Complete fertilization failure ^b	5 (33.3%)	2 (18.9%)
No. of embryos per transfer ^{a,b}	0.9 ± 0.2	1.2 ± 0.3

IVF = in-vitro fertilization; ICSI = intracytoplasmic sperm injection.

^aValues were expressed as mean ± SEM.

^bInter-group differences were not statistically significant.

ICSI e normospermia



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Prospective controlled randomized study of in vitro fertilization versus intracytoplasmic sperm injection in the treatment of tubal factor infertility with normal semen parameters*

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Conclusions: “ICSI does not offer a higher pregnancy rate as compared with IVF in the treatment of tubal factor infertility with normal semen”

ICSI e normospermia

Table I. 2PN-fertilization in sibling oocytes treated by conventional IVF or by ICSI

	IVF (n = 334)	ICSI (n = 328)	Statistics
All patients (n = 56)			
No. of OCC ^a	6.0 ± 2.2	5.9 ± 2.3	
2PN/OCC (%) ^a	53.0 ± 31.2	62.0 ± 26.6	^b Not significant
Without FF (n = 47)			
No. of OCC	6.1 ± 2.2	5.9 ± 2.3	
2PN/OCC (%)	60.3 ± 25.6	63.8 ± 25.2	^b Not significant
With FF in IVF (n = 7)			
No. of OCC	5.9 ± 1.8	6.2 ± 1.9	
2PN/OCC (%)	-	69.2 ± 15.3	
With FF in ICSI (n = 2)			
No. of OCC	4.0 ± 1.4	3.5 ± 0.7	
2PN/OCC (%)	56.6 ± 33.0	-	

^aExpressed as mean ± SD per patient.

^bPaired t-test.

FF = fertilization failure; NS = not significant.

Table II. Comparison of embryonic cleavage and development in 47 couples with fertilization after ICSI and after conventional IVF

	IVF	ICSI	P-value
Total number of zygotes	174	205	
Embryo cleavage			
Mean ± SD (%)			
Type A	10.7 ± 17.9	12.4 ± 25.3	NS
Type B	57.3 ± 34.6	57.3 ± 32.0	NS
Type C	19.0 ± 25.8	19.8 ± 26.7	NS
Developmental stage (42 h after insemination)			
Mean ± SD (%)			
2-cell	29.4 ± 31.8	18.6 ± 23.4	<0.02
3-cell	9.4 ± 18.5	9.5 ± 19.1	NS
4-cell	35.1 ± 30.6	51.8 ± 34.4	<0.001
≥5-cell	10.8 ± 20.1	10.9 ± 20.8	NS

Paired t-test used for statistical evaluation.

NS = not significant.

There is no difference in implantation potency of the embryos obtained with either technique after the non-randomized transfers

ICSI e normospermia

TABLE 2

Cycle parameters of the patients randomized to undergo ICSI or IVF.

Variable	Procedure	
	ICSI (n = 38)	IVF (n = 38)
E ₂ level on the day of hCG administration (pmol/L)	5,193.7 ± 3,764.0	5,071.0 ± 3,060.0
No. of oocytes retrieved	9.08 ± 6.22	8.11 ± 3.70
No. of metaphase II oocytes/mature cumulus-oocyte complexes retrieved	7.32 ± 4.34	7.00 ± 3.50
Normal (two-pronuclei) fertilization rate per injected/inseminated oocyte (%)	69.03 ± 22.12	67.32 ± 25.01
Cleavage rate per fertilized oocyte (%)	66.87 ± 23.04	69.84 ± 23.73
No. of transferable embryos obtained	4.97 ± 3.06	5.00 ± 3.17
No. of grade I embryos obtained	3.45 ± 2.43	3.13 ± 2.54
No. of embryos transferred	3.39 ± 1.39 (4)	3.61 ± 1.59 (4)
No. of grade I embryos transferred	2.71 ± 1.71 (2)	2.50 ± 1.43 (3)

Note: Values are means ± SD (median). The differences between the two groups were not statistically significant.

Bukulmez. ICSI vs. IVF. *Fertil Steril* 2000.

“The type of procedure performed is not a significant predictor of clinical pregnancy”

Bukulmez O et al, *Fertil Steril*, 2000, 73(1): 38-42.

ICSI e normospermia

TABLE 3

Treatment results in the patients randomized to undergo ICSI or IVF.

Variable	Procedure	
	ICSI (n = 38)	IVF (n = 38)
Individual implantation rate (%)	38.75 ± 24.46	34.58 ± 16.97
No. of clinical pregnancies/total no. of patients (%)	8/38 (21.05)	8/38 (21.05)
No. of women who gave birth/total no. of patients (%)	7/38 (18.42)	6/38 (15.79)

Note: Values are means ± SD. The differences between the two groups were not statistically significant.

ICSI e normospermia

TABLE 2

Fertilization rate and good-quality embryo formation in sibling oocytes allotted to IVF or ICSI in 35 women.

Variable	Treatment		<i>P</i> value
	IVF	ICSI	
Fertilization rate per assigned oocyte (%)	57.2 (107/187)	71.3 (134/188) ^a	.005
Good-quality embryos per assigned oocyte (%)	47.1 (88/187)	64.4 (121/188)	.001
Good-quality embryos per fertilized oocyte (%)	82 (88/107)	90 (121/134)	NS

Note: NS = not significant.

^a The fertilization rate was 81.7% (134/164) when it was based on the number of metaphase II oocytes injected with sperm.

Khamsi. *Intracytoplasmic sperm injection*. *Fertil Steril* 2001.

ICSI e normospermia

Table II. Comparison between incidence of fertilization achieved by conventional IVF with high insemination concentration (HIC) and by ICSI using donor spermatozoa on the sibling oocytes retrieved from patients ($n = 18$) with failed IVF with HIC cycle

	IVFa	ICSI ^a
No. of oocytes	80	81
No. of oocytes fertilized	48 (60%)	64 (79%)

^aSignificantly different, $P < 0.015$.

ICSI e normospermia



nessuna differenza in:

- -
 -
 -
 -
- percentuale di fertilizzazione
sviluppo embrionale
qualità degli embrioni
percentuali di impianto e gravidanza



ridotto rischio di cicli con completa mancanza di
fertilizzazione (10%)

ICSI e sviluppo embrionale

Table III. Comparison between treatment groups of blastocyst development and quality

Parameter	ICSI (n = 32)	Insemin (n = 31)	P value
No. blastocyst stage embryos	2.16 ± 1.97	5.29 ± 3.64	<0.001
Percent blastocyst stage embryos	30.3 ± 21.5	51.9 ± 27.9	0.003
No. high quality blastocysts	1.09 ± 1.82	3.33 ± 3.21	0.002
Percent high quality blastocysts	13.6 ± 21.3	28.2 ± 25.7	0.011

Data are expressed as mean ± SD. Differences between means were tested for significance by Kruskal-Wallis one way ANOVA on Ranks. High quality blastocysts were defined as expanded blastocyst stage embryos with a well-defined trophectoderm layer and adequate inner cell mass (see text for details).

“Progressive motility and sperm morphology were significantly correlated with diminished blastocysts development and quality”

Table IV. Correlation between various semen parameters and blastocyst development and quality

Regression analysis	r ²	P value
Concentration versus % blastocyst development	0.0845	0.042
Percent motility versus % blastocyst development	0.0345	NS
Percent progressive motility versus % blastocyst development	0.1130	0.039
Percent normal morphology versus % blastocyst development	0.1390	0.006
Concentration versus % high quality blastocysts	0.0217	NS
Percent motility versus % high quality blastocysts	0.0188	NS
Percent progressive motility versus % high quality blastocysts	0.0807	0.041
Percent normal morphology versus % high quality blastocysts	0.0748	0.048

Correlations were tested for significance by ANOVA. High quality blastocysts were defined as expanded blastocyst stage embryos with a well-defined trophectoderm layer and adequate inner cell mass (see text for details). NS = not significant.

ICSI e sviluppo embrionale

Table II. Development to the blastocyst stage *in vitro* of ICSI and IVF embryos (series I)

	Embryos cultured (n)	Mean spare embryos/cycle	Blastocysts (%)	Hatched blastocysts (%)
ICSI	446	4.6	40 (8.9)	8 (20)
IVF	748	7.4	176 (23.5)	69 (39)
P value		< 0.001	< 0.001	< 0.05

Table III. Fertilization and blastocyst formation of sibling oocytes from eight cycles, allocated non-selectively to ICSI or IVF (series II)

	ICSI	IVF	P value
Oocytes obtained (n)	91	67	
Oocytes inseminated (n)	78	67	
2PN zygotes (n)	57	48	
2PN zygotes/oocyte obtained (%)	62.6	71.6	NS
2PN zygotes/oocyte inseminated (%)	73	71.6	NS
Embryos transferred (n) (number of cycles)	8 (4)	9 (4)	
Embryos cultured (n)	45	40	
Blastocysts (%)	9 (20)	20 (50)	< 0.01

The % embryos developing to the blastocyst stage was lower after ICSI

ICSI procedure contributes to a reduced capacity for blastocysts formation *in vitro*

ICSI e sviluppo embrionale

Table IV. Effect of the technician performing the ICSI procedure on fertilization and embryonic development to the blastocyst stage

Technician performing the ICSI	No. of oocytes	Degeneration after ICSI (%) ^a	Fertilization results			Culture of surplus embryos to the blastocyst stage				
			No. of 1 PN zygotes (%) ^a	No. of 2 PN zygotes (%) ^a	No. of >2 PN zygotes (%) ^a	No. of surplus embryos	No. of blastocysts (%) ^a	No. of embryos fixed	No. of blastocysts of ≥25 cells	Cells per blastocyst ^{b,c} (%)
1	1126	88 (8) ¹	85 (8) ¹	716 (64) ¹	36 (3) ¹	272	50 (18) ^{1,2}	35	22 (63)	43.1 ± 3.9
2	599	51 (9) ¹	28 (5) ¹	361 (60) ¹	20 (3) ¹	163	29 (18) ^{1,2}	22	13 (59)	44.5 ± 3.7
3	585	39 (7) ¹	40 (7) ¹	364 (62) ¹	12 (2) ¹	188	26 (14) ¹	19	12 (63)	53.3 ± 7.8
4	614	35 (6) ¹	63 (10) ¹	358 (58) ¹	35 (6) ¹	155	43 (28) ²	17	16 (94)	50.7 ± 5.1

^aIn each column, proportions with different superscripts^{1,2} are significantly different (χ^2 -test, 4×2 table, Bonferroni correction, $P < 0.05$).

^bMean (± SEM) number of cells per blastocyst that consisted of ≥25 cells.

^cNo significant differences (ANOVA).

Certain Technical aspects if the ICSI procedure cab affect subsequent embryonic development to the blastocyst stage

Rischi genetici della iniezione

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Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracytoplasmic human sperm injection (ICSI) into golden hamster eggs: Does ICSI itself introduce chromosomal anomalies?

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Rischi genetici della iniezione

Table II. The incidence of sperm sex-chromosome nullisomy and disomy in the swim-up motile fractions (SU), the pellet fractions (Pellet), and the spermatozoa–hemizona complexes (S-HZ) in 41 ICSI patients

	Total spermatozoa	Nullisomy (%)	Disomy (%)				Sex-chromosome aneuploidy (%)
			XX	YY	XY	Total	
SU	21 839	0.70	0.27	0.29	0.14	0.70	1.41 ^a
Pellet	21 936	0.85	0.33	0.32	0.19	0.84	1.69 ^a
S-HZ	4081	0.27	0.15	0.12	0.07	0.34	0.61 ^b

^{a,b}Comparisons between a and b showed significant difference ($P < 0.0001$).

Table III. The incidence of sperm chromosome 18 nullisomy and disomy in the swim-up motile fractions (SU), the pellet fractions (Pellet), and the spermatozoa–hemizona complexes (S-HZ) in 41 ICSI patients

	Total spermatozoa	Nullisomy (%)	Disomy (%)	Chromosome 18 aneuploidy (%)
SU	21 839	0.29	0.31	0.60 ^a
Pellet	21 936	0.41	0.31	0.72 ^a
S-HZ	4081	0.07	0.02	0.10 ^b

^{a,b}Comparisons between a and b showed significant difference ($P < 0.0001$).

Rischi genetici della iniezione

Table IV. The incidence of sex-chromosome aneuploidy and the incidence of chromosome 18 aneuploidy in the swim-up motile fractions (SU), in the pellet fractions (Pellet), and in the spermatozoa-hemizona complexes (S-HZ) in 41 ICSI patients

Sample	Sex-chromosome (%)	Chromosome 18 (%)	P
SU	1.41	0.60	<0.0001
Pellet	1.69	0.72	<0.0001
S-HZ	0.61	0.10	<0.0001

Table V. The incidence of diploid spermatozoa in the swim-up motile fractions (SU), in the pellet fractions (Pellet), and in the spermatozoa-hemizona complexes (S-HZ) in 41 ICSI patients

Total spermatozoa	Diploid (%)			Total diploid (%)	
	46,XX	46,YY	46,XY		
SU	21 839	0.10	0.07	0.08	0.26
Pellet	21 936	0.12	0.09	0.15	0.35 ^a
S-HZ	4081	0.05	0.02	0.00	0.07 ^b

^{a,b}Comparisons between a and b showed significant difference ($P < 0.0001$).

Conclusioni



- La Fecondazione in Vitro si conferma la procedura di elezione nel trattamento della sterilità da fattore tubarico.
- L'uso della ICSI, già utilizzata per risolvere problemi di sterilità inspiegata, liquido seminale borderline, sterilità immunologica e precedenti fallimenti di FIVET, evoca perplessità e conflitti tra gli autori per l'efficacia, i rischi di contaminazione e di perpetuazione dei problemi genetici della coppia.
- La ICSI provvede forse ad una più elevata fertilizzazione ed alla formazione di una quota più elevata di embrioni di buona qualità per ovocita recuperato, nelle coppie in assenza di un fattore maschile severo, ma la FIVET deve continuare ad essere il trattamento di prima scelta, lasciando alle coppie con reiterato fallimento l'opzione ISCI nel trattamento della loro sterilità.