Will my child be normal?

Children's follow-up after ART for male infertility

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Outline lecture

Introduction
Prenatal diagnosis
Perinatal outcome
Malformations at birth
Long-term follow-up studies
Conclusion

Introduction of ICSI and TESE

- 1991 introduction of ICSI at the UZ Brussel
  - Palermo et al. Lancet. 1992 , 340, 17-8
- concerns re-emerged about the health and well-being of the children
- concerns were related
  - to the invasiveness of the procedure
  - to the type of sperm used
- 1995 first child born after TESE (testicular sperm extraction) at the Free University of Brussels
  - Silber et al. Hum Reprod. 1995, 10, 148-52

Follow-up study at the UZ Brussel

- AIM : Evaluate the risk of ICSI (and TESE) to the offspring
  - Overall risk
    - genetic constitution of the fetuses
    - perinatal problems
    - development of the children
  - Procedure-related risk
    - comparison ICSI / IVF
  - Sperm-related risk
    - sperm quality / sperm origin

Study design

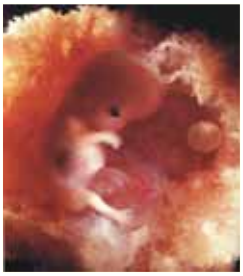
- Informed consent to follow-up study including
  - assessment of genetic risks
  - chromosomal anomalies of parents
  - prenatal testing
  - follow-up of children
- Data on pregnancy and delivery collected
  - prenatal testing (CVS / amniocentesis, ultrasound)
  - written questionnaires on pregnancy and neonatal outcome
- Physical examination of the children
  - at 2m, 1 and 2 years

Materials

- Prenatal diagnostic tests[1]
  - 1586 karyotypes in ICSI (1991-2001)
- Prenatal + neonatal outcome after testicular sperm[2]
  - 61 NOA ICSI children (1994-2000)
  - 196 OA ICSI children (1994-2000)
- Neonatal outcome[1]
  - 2840 ICSI children (1991-1999)
  - 2955 IVF children (1983-1999)
- Developmental outcome (1995-2002)[2]
  - 439 ICSI children
  - 207 IVF children

[1] Fresh embryos, different sources of sperm no cryopreservation or PGD
[2] Fresh and cryopreserved embryos included, different sources of sperm

Prenatal diagnostic testing



Prenatal diagnosis in 1586 ICSI foetuses

Bonduelle et al, 2002

Abnormal results	n	%	Confidence Interval	% General population[1, 2, 3]
■ *De novo*	25	**1.6%***	1.02 - 2.32 %	0.45 - 0.87%
Sex chrom	10	**0.6%***	0.30 - 1.16 %	0.19 - 0.27%
Autosomal	15	0.9%	0.53 - 1.56 %	0.26 - 0.60%
Numerical	8	0.5%	0.22 - 0.99 %	0.14 - 0.33%
Structural	7	0.4%	0.18 - 0.91 %	0.11 - 0.22%
■ Inherited	22	**1.4%***	0.87 - 2.09 %	0.47 - 0.37%
Total	47	3.0%	2.19 - 3.92 %	0.92%

[1] Jacobs, 1992 on 34 910 newborns [2] Ferguson-Smith, 1984 on 52 965 prenatal samples
[3] Hook, 1981, 1984, 1987 on prenatal samples * significant

Prenatal diagnosis in 1586 ICSI foetuses[1]

- Inherited abnormalities 1.4%
- Known risk related to the chromosomal anomalies in the parents (6.3%)
- 17/22 cases paternally inherited
- Preimplantation > prenatal diagnosis

⇨ Informed choice of the parents prior to the procedure

[1]Bonduelle et al. 2002

Prenatal diagnosis in 1586 ICSI foetuses[1]

- Non-inherited (*de novo*) anomalies 1.6%
- Significantly higher than general population (with same age) but absolute risk low
- Related to sperm characteristics
- Severity is variable (termination not always chosen)
- Detectable from 11th week of pregnancy

⇨ Informed choice of the parents
<50% agree to do a prenatal test

[1]Bonduelle et al. 2002

Prenatal diagnosis in 1586 ICSI foetuses[1]
de novo anomalies, sperm parameters / origin

- Sperm count (72%)
  < 20.10^6 / ml ⇒ **2.1 %** chromosomal abnormalities
  Fisher Exact 2 tailed test p < 0.05
- Sperm motility (83%)
  < 50 % N motility ⇒ **1.9 %** chromosomal abnormalities
  Fisher Exact 2 tailed test p < 0.05
- Sperm morphology ⇒ **no influence**
  abn < 14 % N or abn ≥ 14 % N morphology
- Sperm origin ⇒ **no influence**

[1]Bonduelle et al. 2002

Prenatal diagnosis in 1586 ICSI fetuses / anomalies in relation to sperm origin[1]

	de novo	inherited
• Ejaculated sperm	1.7%*	1.4%
• n =1469	(25)	(22)
• Epididymal sperm	0%*	0.0%
• n = 31	(0)	(0)
• Testicular sperm[2]	2.3%*	1.2%
• n = 85	(2)	(1)

[1] Bonduelle et al., 2002 * not significant

Prenatal diagnosis after use of testicular sperm[1] in non-obstructive azospermia (NOS) and obstructive azospermia (OA)[2]

	de novo	inherited
• Testicular sperm	2.3%	1.2%
• n = 85 (of 257)	(2)	(1)
Non-obstructive (NOA)	6.7%*	0.0%
• n = 15 (of 61)	(1)	(0)
Obstructive (OA)	1.4%*	1.4%
• n = 70 (of 196)	(1)	(1)

[1] Bonduelle et al. 2002
[2] Vernaeve et al. 2003

* not significant

Prenatal diagnosis in Belgium and France

	Epididymal de novo + inherited	Testicular de novo + inherited	ICSI total de novo + inherited
Belgium	n = 31 (0) 0%	n = 85 (3) 3.5%[1]	n = 1586 (47) 3.0%[1]
France	n = 546 (1) 0.2%	n = 201 (5)* 2.5%[2]	n= 2332 (16) 0.7%[2]

[1] n.s. [2] p = 0.02 *most *de novo* and terminated

Chromosomal anomalies in relation to sperm origin / quality

- Increased aneuploidy rate in sperm when severe testicular failure
  - Levron et al., 2001; Burello et al., 2002; Palermo et al., 2002
  - Gianarolli et al. 2005. Higher aneuploidy compared to the general population in testicular sperm compared to ejaculated sperm
- Higher aneuploidy rate in MESA / TESE embryos compared to ICSI embryos from normospermic patients
  - Gianarolli et al. 2000
- Higher incidence of mosaicism in TESE embryos
  - Silber et al. 2003 Immature centrosome leading to errors in mitosis?

Chromosomal anomalies in relation to sperm origin / quality

- No difference in non-obstructive azospermia / normal spermatogenesis azospermia patients
  - Mateizel et al. 2002. n =17 NOA; 26 OA
    NO difference in chromosomal abnormality in patients with severe testicular failure vs normal spermatogenesis except for more aneuploidy for chromosome 18
- Higher aneuploidy rate in preimplantation NOA embryos
  - Silber et al. 2003
- Lower implantation rate of NOA embryos compared to OA
  - Vernaeve at al., 2002

Perinatal outcome

Literature : ICSI and IVF are a risk factor of adverse perinatal outcome

- Higher risk in singletons[1,2]
  - LBW OR 1.8 (CI 1.4 - 2.2)
  - VLBW OR 2.7 (CI 2.3 - 3.1)
  - prematurity OR 2.O (CI 1.7 - 2.2)
  - perinatal mortality OR 2.2 (CI 1.6 – 3.0)
- Related to high rates of multiples
- Perinatal outcome in ART twins comparable to the general population or better[2]
- No obvious difference between IVF and ICSI

[1]Meta analysis, R Jackson et al. 2004 [2] Helmerhorst F et al. 2004

Perinatal outcome: pregnancy data / evolution of pregnancies UZ Brussel

	ICSI[1]	Testicular[2]
Pregnancies	3073	299
Biochemical	8.2%	12.0%
Ectopic	1.6%	0.7%
Miscarriage	13.9%	14.2%
Termination	0.6%	0.0%
Birth	**73.3%**	**72.6%**
Lost to FU	2.4%	8.3%

1 Bonduelle et al 2002 2 Vernaeve et al 2003

Perinatal outcome: Testicular NOA / OA

	ICSI	Testicular		
		Total	NOA	OA
Singletons	n = 1499	n = 148	n = 38	n =110
Prematurity	8.4%	16.2%	**24.3%**[1]	13.1%[1]
All births	n = 2840	n = 257	n = 61	n = 196
LBW	27.1%	49.4%	33.9%[1]	30.7%[1]
VLBW	4.5%	7.8%	**10.2%**[2]	3.6%[2]

[1] **not significant,** neither for singletons or twins individually
[2] **significant p<0.05,** not significant in singletons or twins individually

Perinatal outcome in ICSI literature / testicular sperm

	Belgium singleton		Germany[2] singleton		Sweden[3] singleton	
	ICSI n = 1499	Test[1] n = 326	ICSI n = 1944	Test n = 147	ICSI n = 773	Test n = 23
Prema <37w	8.4%	4.9%	-	-		
LBW <2500	7.2%	6.8%	10.8%[4]	6.1%[4]	7.6%[6]	17.4%[6]
VLBW <1500	1.5%	0.93%	3.0%[5]	0.7%[5]	1.4%[7]	0%[7]
GA	-	38.7W	38.9w[8]	39.1w[8]	40w	40w

[1]De Schrijver et al. Update 10/2007
[2]Ludwig et al. Hum. Reprod.,18, 2003
[3]Wennerholm et al., Hum Reprod, 2000
[4,5,6,7,8] **Not significant**

Perinatal death in ICSI literature / testicular sperm

	Stillbirth		Perinatal death	
	ICSI	Testicular	ICSI	Testicular
Belgium[1]	n = 2889 1.7%	n = 257 2.3%	n = 2889 1.9%	n = 257 2.7%
Germany[2]	n = 3199 0.3%[4]	n = 229 0%[4]	n = 3199 1.4%[5]	n = 229 1.3%[5]
Sweden[3]	n = 1192 0.5%[4]	n = 31 0%[4]	n = 1192 1.2%[5]	n = 31 3.2%[5]

[1]Bonduelle et al., Hum Reprod, 2002
[2]Ludwig et al., Hum Reprod, 2003
[3]Wennerholm et al., Hum Reprod, 2000
[4,5]**not significant**

Stillbirth and perinatal death in Brussels study

	ICSI	Testicular		
		Total	NOA	OA
Total children	2889	257	61	196
Stillborns[1]	1.7%	2.3%	4.9%	1.5%
Perinatal death	1.9%	2.7%	**6.6%**[2]	1.5%[2]

[1] gestational age of ≥ 20 weeks or a birthweight of ≥ 500g
[2] FE $p < 0.05$

Congenital malformations

Major malformations definitions

- ICD-10 codes for malformations
- Major malformation defined as malformation causing functional impairment and/or requiring surgical correction

  Remaining malformations were classified as minor
- Internal guidelines to code for major/minor

Major malformations in Brussels study

	ICSI	IVF
Maj. malform.	96 **(3.4%)**[1]	112 **(3.8%)**[1]
Number	2840	2955

[1] Cochram Mantel Haenzel test p = 0.402 n.s

The same in both groups

Malformations in ICSI: sperm parameters / origin

• Sperm conc.	≥ 5.10^6 /ml	2.8 %[1]
Sperm conc.	< 5.10^6 /ml	3.8 %[1]
• Ejaculated sperm	n = 2477	3.4 %[1]
• Testicular[2]	n = 525	5.1 %[1]
• Epididymal	n = 105	3.8 %[1]

[1]Fisher's Exact Test n.s
[2]De Schrijver et al. update 10/2007 of children born after TESE

Malformations in ICSI: sperm origin

	Liveborns	Major malf
ICSI	2840	3.4 %[1]
Testicular[2]	525	5.1 %[1]
• NOA[3]	58	3.7 %[1]
• OA	193	2.1 %[1]

[1]Fisher's Exact Test **non significant**
[2]De Schrijver et al. update 10/2007 of children born after TESE
[3]Vernaeve et al. 2003

Malformations in ICSI literature / testicular sperm

	Epididymal	Testicular	ICSI	Statistics
Belgium	n = 105 liveb 3.8%	n = 251 liveb 2.4%	n = 2840 liveb 3.4%	**n.s.**
France A France B	n = 546 preg 2.2% OR=1.30 [0,95-1,84]	n = 201 preg 4.0%[2]	n = 2332 preg 2.5%[2]	[2]**n.s.** **significant**
Germany	n = 26 liveb 3.8%	n = 229 liveb 9.1%[3]	n = 3199 liveb 8.4%[3]	[3]**n.s.**

France A De Mouzon et al oral comm., 2005 B de Mouzon et al. 2007
Germany Ludwig et al., Hum Reprod, 2003

Long term FU studies on ICSI

- Multicentre EU study at 5y
  - on 1515 ICSI, IVF and SC children
  - Bonduelle et al. 2004

Major malformations at 5 years[1]

	ICSI n 540	IVF n 437	Control n 538	p-value
Neonatal	3.3%	2.1%	1.9%	ns
Childhood	**3.0 %** [1]	2.3%	0.4%[1]	[1]0.001
Total major malformation	**6.3 %** [2]	4.3%	2.2%[2]	[2]0.001

[1]Bonduelle et al. 2004

Major malformations at 5 years

Increase in ICSI > IVF > control children

- Not detected at birth
- Partially due to increased defects in uro-genital system
- Higher malformation rate in ICSI boys 8.2% > girls 3.6%

Growth at 5 years

- Subgroups
  - Epididymal and testicular
  - Ejaculated <1 million/ml
  - Ejaculated 1- 4.99 million/ml
  - Ejaculated 5- 19.9 million/ml
  - Ejaculated > 5 million/ml
- No difference in growth and cognitive development

[1] Wennerholm et al. H Reprod 2005

Major malformations
after epididymal and testicular sperm[1]

- Questionnaire sent to the parents
- Age children 3m -7y
- 96 % return rate
- Major malformation rate not increased
- Hypospadias more frequent OR 1.6% 95 CI 0.3-5.7

[1] Fedder et al. 2007

Major malformations at 8 years
in 150 ICSI and SC children[1]

- Medical or neurological outcome similar
- Physical examination
  - Weight, heigth, HC and Body Mass Index similar
- Major congenital malformation were more frequent in ICSI
  - RR 2.94 (95% CI 1.09-7.89)
- Pubertal staging was similar in both groups
- Genital examination :similar

[1] Belva et al. 2007

Long term development

Long term
psychomotor / behavioral development

- Review (9 publications) on ICSI behavioral and cognitive development (9 publications)
  Leslie et al. 2005
  - Majority of ICSI children have normal mental development
  - Most have found no increased risk for developmental delay
  - Demographics, maternal education and social class are important determinants

EU study on 1515 ICSI, IVF and controls children at the age of 5 years (EU study)[1]

- Ponjaert et al, Ped 2005
  - ICSI is not associated with mental and psychomotor abnormalities
- Barnes et al, H Reprod 2004
  - Couples benefiting from successful ICSI have no adverse effects on their relationships with their child or between themselves
  - Indeed they appear to be more dedicated to parenting. This may have implications for the future, as their children mature

UZ Brussel study on 151 ICSI and control children at age 8 and 10

- Leunens et al. 2006
  - ICSI and SC children show a comparable cognitive and motor development until the age of 8 and 10 years
- Leunens et al. 2007
  - Couples benefiting from successful ICSI have no adverse effects on their relationships with their child or between themselves when the children are 8 years

Conclusion

Conclusions (1)

- Prenatal diagnosis in ICSI
  - A slight increase (1.6%) in *de novo* anomalies
  - Related to sperm concentration and motility
  - Indications for prenatal testing if concentration < 20.10^6 / ml or abnormal motility
- Prenatal diagnosis after testicular sperm
  - Comparable rate in Belgium

Possible higher risk in NOA→ Further analysis TO DO

Conclusion (2)

- Adverse neonatal outcome in ART / ICSI
- Higher risk in singletons for
  - LBW x2
  - VLBW x2-3
  - prematurity x2
  - perinatal mortality x2
- Mainly related to high rates of multiples

No obvious difference between IVF and ICSI

Conclusion (3)

- Neonatal data after ICSI + testicular sperm
  - No different pregnancy course and neonatal outcome in Belgium, German and Swedish study
  - Higher rate of VLBW, in the NOA group (1study)
- Perinatal death after ICSI + testicular sperm
  - No statistical increase in limited series
  - Higher perinatal death in severely defective sperm (NOA) in 1 study

Conclusion (4)

- Malformation rate in ICSI
  - At birth comparable in ICSI to IVF
  - Malformation rate in ICSI 20-30% higher compared to the general population, based on controlled studies and meta analysis
  - No influence of sperm parameters or sperm origin
- Malformation rate in ICSI + testicular sperm
  - No difference in Belgium, France, Sweden and Germany

Take home messages

- Prenatal diagnosis is advisable in pregnancies after ICSI with male factor infertility (< 20.10^6 / ml or abnormal motility)
- A possible higher risk of chromosomal anomalies in ICSI embryos after use of sperm from NOA patients needs to be further investigated
- Outcome of children after use of different sperm sources /quality seems satisfactory
- Further investigations needed on neonatal outcome and further development of ICSI–NOA children

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Germany	n = 26 liveb 3.8%	n = 229 liveb 9.1%[3]	n = 3199 liveb 8.4%[3]	[3]n.s.

A including chromosomal aberrations which were interrupted
B major malformations excluding chromosomal anomalies
[3] Ludwig et al., Hum Reprod, 2003