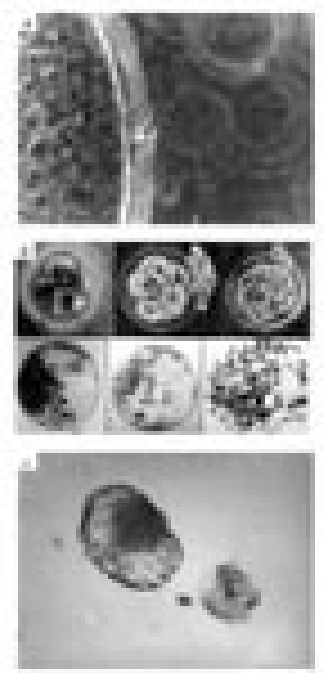
Tecniche di Riproduzione Assistita

Prof. R. Marci

Facoltà di Medicina e Chirurgia
Dpt. Scienze Biomediche e Terapie avanzate

Louise Brown

25 July 1978

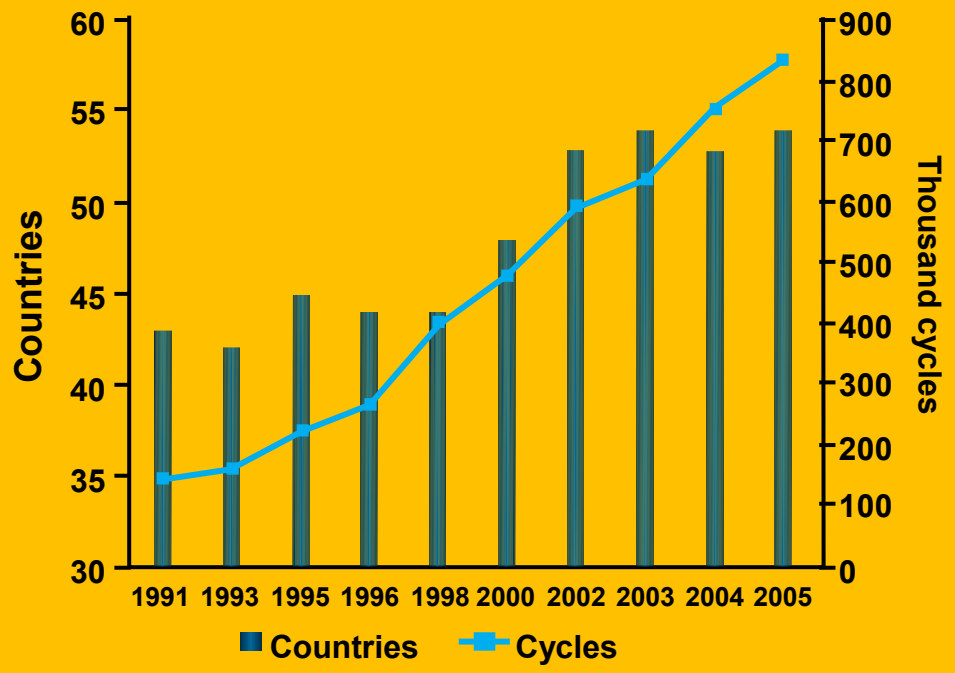


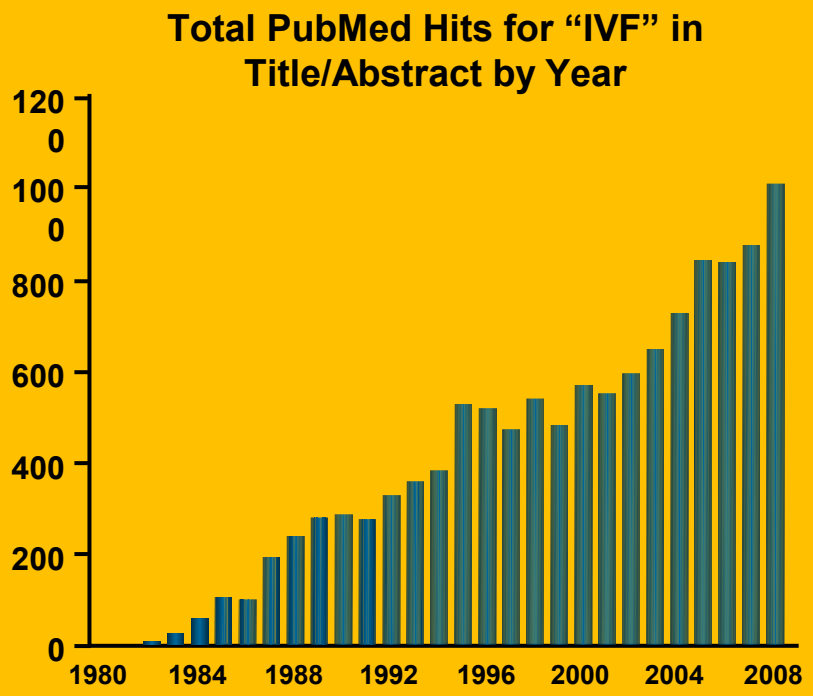
2012 - all roads lead to IVF

The ART Explosion

Explosion in number of people treated!!!

The Growth of IVF





- **2004:** 760,000 cycles
- **2005:** ≈837,850 cycles

- **Increase of 78,000 cycles (10.3%)**

IVF = in vitro fertilization.

ICMART. ESHRE, 2009.

European uptake of IVF

	IVF/ICSI cycles initiated	Cycles per million
UK	40,000	600
Germany	80,000	940
Holland	14,000	890
Sweden	10,000	950
Denmark	8,000	1,600

ART - moving into the mainstream

	IVF livebirths as percentage of total
UK	**1.4%**
Sweden	**2.4%**
Denmark	**6.0%**

Explosion in complexity of treatments

Explosion in complexity of treatments

1985	**IVF**
1988	**embryo freezing**
1990	**donor oocyte**
1992	**blastocyst culture**
1994	**ICSI**
1995	**surrogacy**
1998	**egg sharing**
1999	**IVM**
2000	**PGD**
2000	**GnRH antagonists**
2001	**egg freezing**

Explosion in complexity of treatments

2003 IMSI

2004 ovarian tissue cryopreservation

2004 EU cells and tissues directive

2006 oocyte vitrification

2007 single embryo transfer

2008 chimeric embryo research

Assisted Reproductive Technique

Miscarriage
Multiple pregnancy
Prematurity
Low birthweight
Cesarean section
Perinatal mortality
Congenital malformations

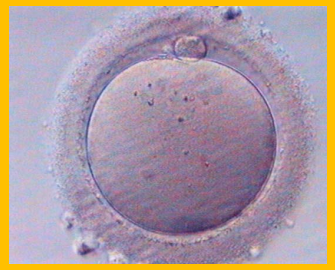
?

newborn infant

Olivennes et al 2002; Wang et al 2005

Induzione della crescita follicolare multipla

ovociti maturi
di qualità elevata



induzione della crescita follicolare multipla

E' necessario sovrastimolare?

Un'elevata percentuale di ovociti presenta delle anomalie morfologiche

Ovociti maturi
di qualità elevata

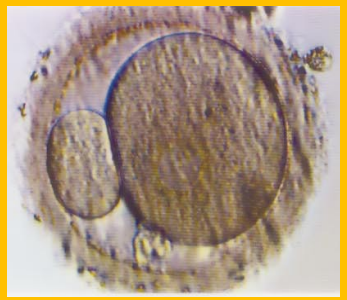
Induzione della crescita follicolare multipla

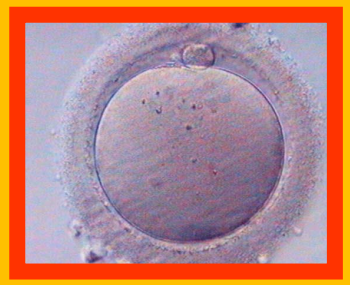
E' necessario sovrastimolare?

Per ottenere 3 ovociti maturi di buona qualità è necessario prelevare almeno 8-10 ovociti

3 ovociti
maturi
di qualità elevata



Intracytoplasmic Morphologically Selected Sperm Injection (IMSI)

Real-Time Fine Morphology of Motile Human Sperm Cells is Associated With IVF-ICSI Outcome

BENJAMIN BARTOOV,* ARIE BERKOVITZ,† FINA ELTES,* AVRAHAM KOGOSOWSKI,‡ YVES MENEZO,§ AND YONA BARAK‡

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ABSTRACT: The aim of the present prospective study was to determine whether subtle sperm morphological characteristics affect the outcome of intracytoplasmic sperm injection (ICSI), and if so, to identify those that are relevant. For this purpose, we developed a new method, the motile sperm organelle morphology examination (MSOME). The examination is performed in real time using an inverted light microscope equipped with high-power Nomarski optics enhanced by digital imaging to achieve a magnification up to 6300×. MSOME was applied to the leftover sperm fraction selected for microinjection in 100 random couples referred for ICSI treatment at 3 major in vitro fertilization centers. We found that the morphological normalcy of the entire sperm cell, according to MSOME criteria, was positively associated with ICSI fertilization rate (area under the receiver operating characteristics [ROC] curve, 88%) but not with pregnancy outcome. The morphological normalcy of the sperm nucleus, defined by MSOME, was significantly and positively associated with both fertilization rate and pregnancy outcome (areas under the ROC curve, 72% and 74%, respectively). These findings indicate that ICSI-associated pregnancy rate may be affected by subtle morphological malformations of the sperm nucleus, which may remain undetected by the embryologist during the routine selection procedure.

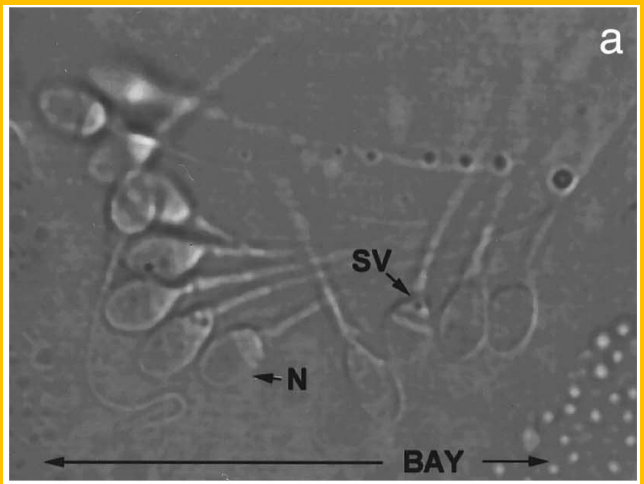
Key words: Motile sperm morphology, ICSI fertilization rate, ICSI pregnancy rate.

J Androl 2002;23:1–8

Table 1. *Specific morphological malformations of the sperm cell subcellular organelles observed by MSOME*

	Sperm Subcellular Organelles						
			Nucleus				
	Acrosome	Postacrosomal lamina	Shape*	Chromatin Content	Neck	Tail	Mitochondria
Specific malformations	Lack Partial Vesiculated	Lack Vesiculated	Small oval Large oval Narrow (<2.9 μm in width) Wide (>3.7 μm in width) Short (<4.2 μm in length) Regional disorder	Vacuolar area >4% of the whole nuclear area	Abaxial Disorder Cytoplasmic droplet	Lack Coiled Broken Multi Short	Lack Partial Disorganization

* Sperm cells with pin, round, amorphous, tapered (cigar-shape), or multinucleated heads were not assessed in this study.



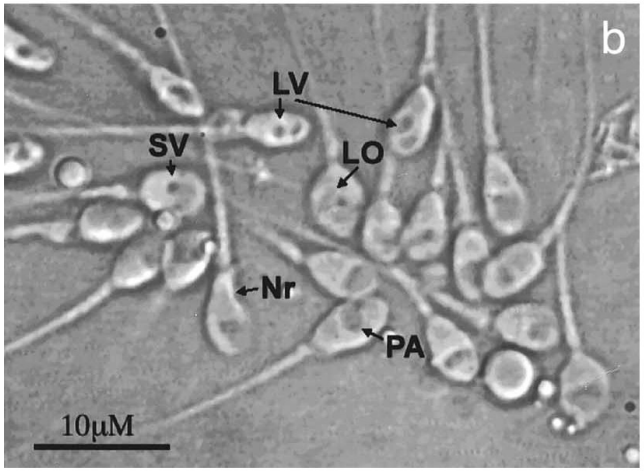


Figure 1. High-power light microscope micrograph of motile sperm (1500×). The sperm heads were captured in small bays (BAY) extruding from the rim of the droplets. N indicates morphologically normal spermatozoa; LV, large nuclear vacuoles; SV, small nuclear vacuoles; LO, large oval sperm cells; Nr, spermatozoa with a narrow postacrosomal region; PA, partial acrosome.

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Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection

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Male Fertility Laboratory, Faculty of Life Sciences, Bar Ilan University, Ramat Gan, and IVF Unit, Herzliya Medical Center, Herzliya-on-the-Sea, Israel

Objective: To verify whether microinjection into retrieved oocytes of motile spermatozoa with morphologically normal nuclei, strictly defined by high power light microscopy (× >6000), improves the IVF/intracytoplasmic sperm injection (ICSI) pregnancy rate in couples with repeated ICSI failures.

Design: Comparative prospective study testing routine IVF/ICSI outcome parameters against those of modified ICSI based on morphological selection of spermatozoa with normal nuclei.

Setting: Male factor fertility laboratory and IVF center.

Patient(s): Sixty-two couples, with at least two previous consequent pregnancy failed ICSI cycles, underwent a single ICSI trial preceded by morphological selection of spermatozoa with normal nuclei. Fifty of these couples were matched with couples who underwent a routine ICSI procedure at the same IVF center and exhibited the same number of previous ICSI failures.

Intervention(s): Standard ICSI and modified ICSI.

Main Outcome Measure(s): ICSI pregnancy rate.

Result(s): The matching study revealed that pregnancy rate after modified ICSI was significantly higher than that of the routine ICSI procedure (66.0% vs. 30.0%).

Conclusion(s): Microinjection into retrieved oocytes of selected spermatozoa with strictly defined morphologically normal nuclei improves significantly the incidence of pregnancy in couples with previous ICSI failures. (Fertil Steril® 2003;80:1413–9. ©2003 by American Society for Reproductive Medicine.)

Table 2. Comparisons between matched intracytoplasmic morphologically selected sperm injection (IMSI) groups and between experimental and control intracytoplasmic sperm injection (ICSI) groups in their biological and clinical outcome parameters.

Reference	*Group and P-value*	*No. of cycles*	*Woman's age (years)*	*No. of injected oocytes*	*No. of transferred embryos*	*Fertilization rate (%)*	*Percentage of top embryos (%)*	*Implantation rate (%)*	*Clinical pregnancy rate (%)*	*Abortion rate (%)*
Bartoov *et al.*, 2003	ICSI control	50	30.3 ± 3.4	10.2 ± 5.5	3.5 ± 1.2	65.5 ± 21.5	31.0 ± 19.5	9.5 ± 15.3	30.0	33.0
	IMSI experimental	50	29.6 ± 3.5	10.6 ± 4.4	3.8 ± 1.1	64.5 ± 17.5	45.2 ± 28.2	27.9 ± 26.4	66.0	9.0
	P-value		NS	NS	NS	NS	≤0.01	≤0.01	≤0.01	≤0.01
Berkovitz *et al.*, 2005	IMSI negative	38	33.3 ± 4.5	9.2 ± 4.0	3.5 ± 1.4	50.3 ± 24.1	19.4 ± 27.1	5.9 ± 12.9	18.4[c]	57.1[d]
	IMSI positive	38	32.3 ± 3.3	10.1 ± 4.5	3.3 ± 1.2	71.3 ± 20.8	34.9 ± 31.3	25.0 ± 25.9	52.6[c]	10.0[d]
	P-value		NS	NS	NS	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Hazout *et al.*, 2006	Conventional ICSI	125	–	9.5 ± 2.7	–	65.2	32.6	0.8	2.4	100.0
	High-magnification ICSI	125	–	9.4 ± 2.5	–	68.1	42.6	20.3	37.6	15.0
	P-value		–	NS	–	NS	NS	<0.001	<0.001	<0.001
Berkovitz *et al.*, 2006a	IMSI control	28	33.5 ± 3.9	8.4 ± 3.2	3.2 ± 0.7	72.8 ± 18.5	27.1 ± 29.4	–	50.0[b]	7.0[a]
	IMSI experimental	28	33.4 ± 3.6	8.1 ± 3.6	3.0 ± 1.3	68.7 ± 20.3	23.0 ± 31.1	–	18.0[b]	80.0[a]
	P-value		NS	NS	NS	NS	NS	–	≤0.01	≤0.01
Berkovitz *et al.*, 2006b	ICSI control	80	33.4 ± 5.6	9.3 ± 5.0	3.1 ± 1.1	69.1 ± 22.6	25.7 ± 28.3	9.4 ± 17.4	25.0[c]	40.0[a]
	IMSI experimental	80	32.3 ± 4.8	9.2 ± 5.0	3.1 ± 1.3	67.4 ± 20.8	38.7 ± 31.6	31.3 ± 36.3	60.0[c]	14.0[a]
	P-value		NS	NS	NS	NS	≤0.05	≤0.05	≤0.05	≤0.05
	IMSI 'best'	70	32.3 ± 4.8	9.5 ± 4.1	3.3 ± 1.3	74.1 ± 20.5	26.7 ± 20.5	26.1 ± 26.8	58.6[b]	9.8[a]
	IMSI 'second best'	70	32.8 ± 4.7	8.9 ± 3.9	3.2 ± 1.4	62.3 ± 24.3	16.2 ± 26.0	8.3 ± 15.1	25.7[b]	33.3[a]
	P-value		NS	NS	NS	≤0.05	≤0.05	≤0.05	≤0.05	≤0.05
Antinori *et al.*, 2008	ICSI	219	31.91 ± 3.30	2.92 ± 0.26	2.37 ± 0.67	94.5	–	11.3	26.5	24.1
	IMSI	227	31.65 ± 3.23	2.90 ± 0.29	2.47 ± 0.68	94.8	–	17.3	39.2	16.9
	P-value		NS	NS	NS	NS	–	= 0.007	= 0.004	NS

Values are mean ± SD. NS = not significantly different.

[a]Abortion rate per pregnancy. [b]Pregnancy rate per cycle. [c]Pregnancy rate per transfer. [d]Abortion rate per cycle.

	N° of cycles		Fertilization rate (%)			Implantation rate (%)			Pregnancy rate (%)			Abortion rate (%)			High-quality embryos		
	IMSI	ICSI	IMSI	ICSI	P	IMSI	ICSI	P	IMSI	ICSI	P	IMSI	ICSI	P	IMSI	ICSI	P
Knez, 2011	20	37	51.2	52.7	NS	17.1	6.8	NS	25	8.1	NS	-	-	-	-	-	-
Setti, 2011	250	250	68	73	=0.013	23.8	25.4	NS	37.2	36.8	NS	18.4	17.9	NS	44.4%	37.3%	-
Oliveira, 2011	100	100	65.4±23.5	62±26.5	NS	13.6	9.8	NS	26	19	NS	15.4	31.6	NS	1.4±0.5 *(mean n°±SD)*	1.5±0.5 *(mean n°±SD)*	NS
Balaban, 2011	87	81	81.6±10.65	80.87±15	NS	28.9	19.5	NS	54	44.4	NS	-	-	-	-	-	-

	ICSI- Ant		IMSI- Ant		P
	count/medium	d.s.	count/medium	d.s.	
N° of cycles	281		51		-
Women age at Pickup	34,98	3,19	35,65	2,98	0,15
Men age at Pickup	37,61	5,47	39,51	5,23	0,02
N° of ICSI attempts	1,61	0,88	1,55	0,87	0,68
N° of injected oocytes	8,12	4,42	8,23	3,78	0,84
Native semen concentration (mil/ml)	23,58	29,34	27,75	32,29	0,39
Native motility(%)	26,27	17,94	27,56	16,55	0,62
Prepared semen concentration (mil/ml)	16,12	23,94	21,1	21,46	0,14
Prepared semen motility (%)	54,43	24,62	60,16	16,95	0,04
2 pronuclei day 1	6,27	3,72	6,59	3,57	0,57
N° of transferred embryos	1,86	0,38	1,87	0,33	0,85
Implantation rate	16,83		16,67		0,97
Fertilization rate	77,27		80,00		0,22
Pregnancy rate	25,30		23,50		0,79
Live birth rate	11,39		13,72		0,23
Ongoing pregnancy rate	7,47		5,88		0,69
Miscarriage rate	17,78		5,26		0,17

Marci et al. Unpublished preliminary data

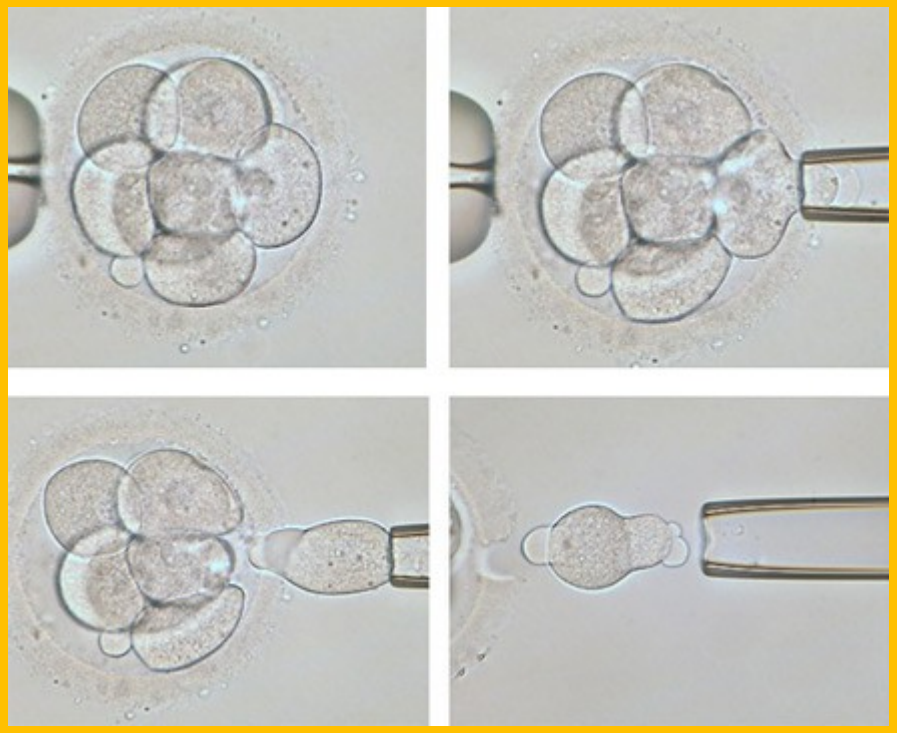
Preimpalntation Genetic Diagnosis
PGD

Sentenza della Corte Costituzionale nr. 151 del 2009

- rimozione del limite imposto dalla legge 40/2004, sul numero massimo di embrioni che secondo la suddetta legge doveva essere “non superiore a tre” il ginecologo stabilisce il numero idoneo di embrioni da creare
- si possano crioconservare gli eventuali embrioni prodotti in eccesso “al fine di tutelare lo stato di salute della donna”
- l’effetto immediato della sentenza della Corte Costituzionale è stato la riapertura alla diagnosi genetica preimpianto (PGD)
- i pazienti hanno il diritto di essere “informati, su loro richiesta, sullo stato di salute degli embrioni prodotti e da trasferire nell'utero”, ai sensi dell’art. 14 comma 5 della Legge 40/2004
- è vietata “ogni diagnosi preimpianto a finalità eugenetica”, come per esempio la selezione del sesso dell’embrione

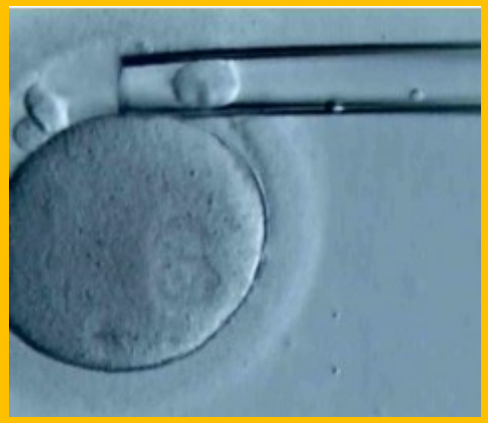
Diagnosi preimpianto-PGD

Identificazione di eventuali problematiche di natura genetica a livello del prodotto del concepimento al fine di evitarne il trasferimento in utero



Biopsia del blastomero

Biopsia del I globulo polare



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Advanced Access publication on October 21, 2010 doi:10.1093/humrep/deq265

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Human Reproduction, Vol.26, No.1 pp. 14–24, 2011
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Human Reproduction, Vol.26, No.1 pp. 33–40, 2011
Advanced Access publication on October 21, 2010 doi:10.1093/humrep/deq231

human reproduction ESHRE PAGES

ESHRE PGD consortium best practice guidelines for amplification-based PGD†

G.L. Harton[1,2,*], M. De Rycke[3], F. Fiorentino[4], C. Moutou[5], S. SenGupta[6], J. Traeger-Synodinos[7], and J.C. Harper[6,8]

[1]Reprogenetics LLC, Livingston, NJ 07039, USA [2]Genetics & IVF Institute, Preimplantation Genetic Diagnosis Laboratory, Fairfax, VA 22031, USA [3]Centre for Medical Genetics, Universitair Ziekenhuis, Brussel, Belgium [4]Genoma Laboratories, Rome, Italy [5]Laboratoire de Biologie de la Reproduction, Université de Strasbourg, Hôpitaux Universitaires de Strasbourg, F-67000 Strasbourg, France [6]UCL Centre for PG & D, Institute for Women's Health, University College London, UK [7]Medical Genetics, University of Athens, 'Aghia Sophia' Children's Hospital

PGD (Preimplantation Genetic Diagnosis)

Elevato rischio di trasmissione di patologie genetiche alla prole (incrementato tasso di aborto)

- Patologie genetiche monogeniche autosomiche recessive, autosomiche dominanti e X-linked (es.beta-talassemia, fibrosi cistica)
- Portatori sani di traslocazioni bilanciate
    - l'utilizzo della PGD nei portatori di traslocazioni bilanciate riduce la percentuale di aborto scende da 62-75% al 15%.

PGS (Preimplantation Genetic Screening)

Utilizzata in coppie sterili o infertili sottoposte ad IVF con l'obiettivo di incrementare il tasso di gravidanza

- Età materna avanzata
- Abortività ricorrente in coppie con cariotipo normale
- Gravi alterazioni dei parametri seminali

ESHRE PGD Consortium/Embriology Special Interest Group- best practice guidelines for polar body and embryo biopsy for preimplantation diagnosis/screening (PGD/PGS). Harton GL et al. Hum Repr 2011

Temi attualmente dibattuti

- Identificazione di eventuali mutazioni genetiche predisponenti allo sviluppo di patologie (es. mutazione gene BRCA-1)
- Patologie neurodegenerative ad insorgenza tardiva (es. Corea di Huntington, morbo di Alzheimer)
- Tipizzazione HLA
- Selezione del sesso del nascituro
- Scelte specifiche di requisiti fisici o QI

PRESERVAZIONE DELLA FERTILITA' FEMMINILE

Is Fertility preservation needded?

- 1/650 children suffer from cancer
- Before 39 y.o, 1/52 women will develop cancer
- Breast ca is diagnosed in 1/9 women and age of onset is decreasing
- Cancer survival is increasing (85%)
- By 2010, 1/250 young people will be cancer survivor

Bleyer et al 1990

Cytotoxic agents-degree of gonadotoxicity

High risk

Cyclophosphamide
Busulfan
Melphalan
Clorambucil
Dacarbazine
Ifosfamide

Intermediate risk

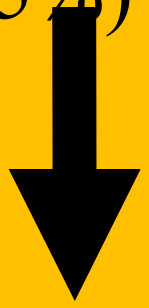
Doxorubicin
Cisplatin
Carboplatin

Low/no risk

Metotrexate
Bleomycin
5-fluorouracil
Vincristine

Is Fertility preservation needded?

Cancer survival is increasing
(85%)



Infertility problems
due to induced premature ovarian failure (POF)

J. Donnez, Human Reprod update 2006

Indications for ovarian tissue cryopreservation

Malignant

Extrapelvic diseases

Bone Cancer
Breast Cancer
Melanoma
Neuroblastoma

Pelvic diseases

Pelvic Sarcoma
Rhabdomyosarcoma
Rectosigmoid tumors

Gynaecological

Early cervical carcinoma
Early vaginal ca
Early vulvar ca
Selected cases of ovarian ca (st IA)
Ovarian borderline tumors

Systemic diseases

Hodgkin's disease
Non-Hodgkin's lymphoma
Leukaemia

Indications for ovarian tissue cryopreservation

Non-Malignant

Uni-bilateral oophorectomy

Benign ovarian tumors
Severe and recurrent endometriosis

Risk of premature menopause

Turner's syndrome
Family history
Benign diseases requiring chemoterapy: autoimmune diseases

Evaluate ovarian function

Clinical parametres:

- Menses
- Regular menses
- Pregnancy

Lab parametres:

- FSH, Estradaiol
- AMH

Ultrasound:

- AFC
- Ovarian volume

Fertility Preservation

- Type and timing of chemoterapy
- Type of cancer
- Patient’s age and partner status

How to preserv Fertility?

-Embryo cryopreservation
-In vitro maturation-IVM
-Oocyte cryopreservation
-Ovarian tissue cryopreservation

Ovarian tissue cryopreservation

-Fragments of ovarian cortex
-Entire ovary
-Isolated follicles

Human ovarian cryopreservation and transplantation procedures have been limited to avascular cortical fragments

Donnez et al 2004

Ovarian tissue cryopreservation:3 options

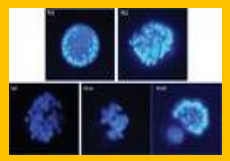
Cortical ovarian biopsy

Fragments

Avascular transplantation

6 live birth

Isolated follicles



In vitro culture

Avoid the trasmission of malignant cells

Whole ovary

Vascular transplantation

Avoid follicular loss due to ischemia

Ovarian tissue cryopreservation

The aim of this strategy is to reimplant cortical ovarian tissue into the pelvic cavity (orthotopic site), or a heterotopic site like the forearm or the abdominal wall once treatment is completed and the patient is desease-free

For patients who need immediate chemotherapy is the only possile alternative

Donnez et al 2000,2004,2006

Live birth after orthotopic transplantation of cryopreservedovarian tissue

Ovarian cortical biopsies were isolated from a patient with stage IV Hodgking lymphoma and cryopreserved prior the initation of chemotherapy.
Six years after biopsies were thawed and transplanted back to the patient orthotopically

- Spontaneous ovulation 5 months post-trasplant

- Spontaneous single intrauterine pregnancy

Donnez et al Lancet 2004

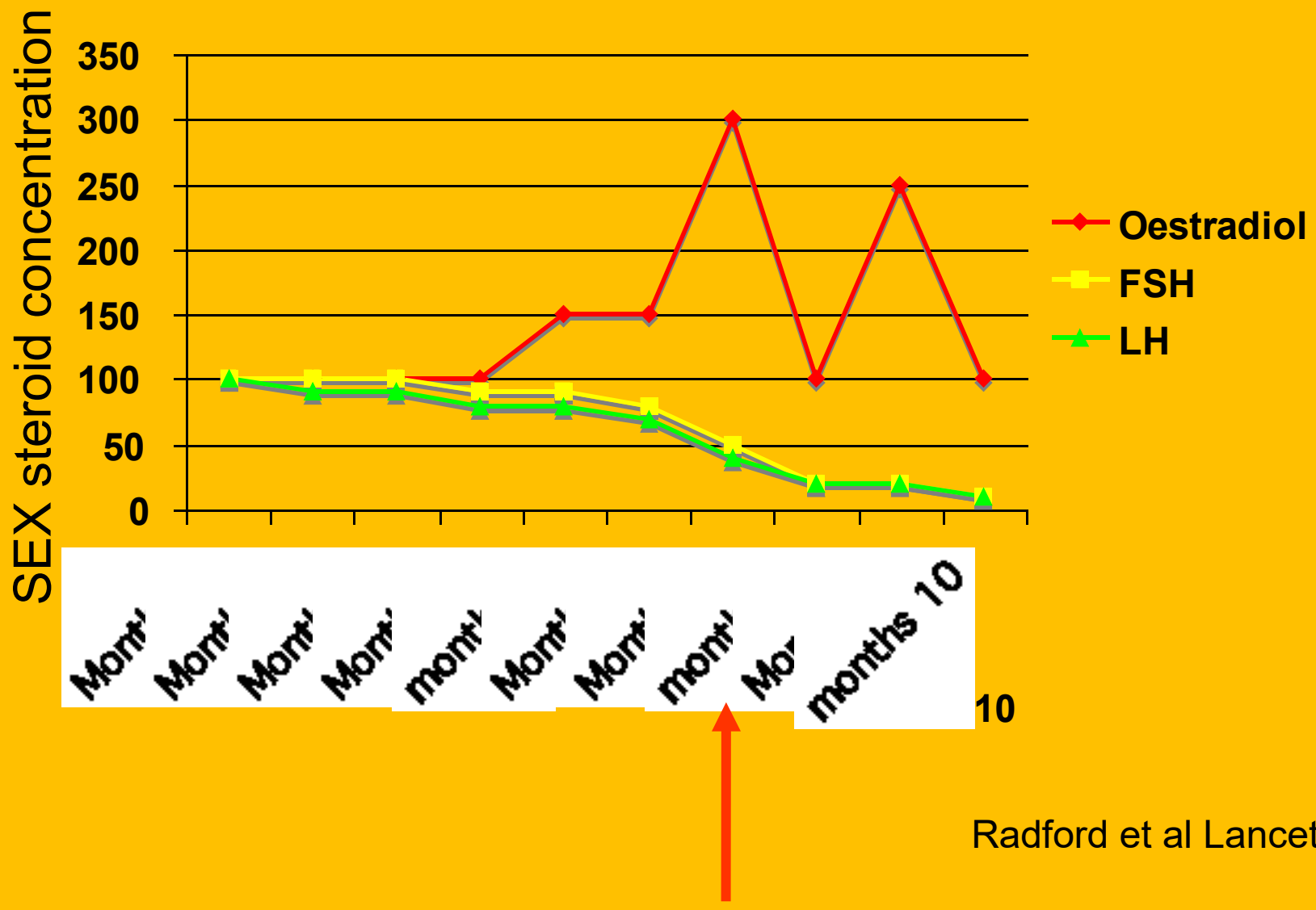
4 months after ortothopic transplantation

QuickTime™ e un
decompressore
sono necessari per visualizzare quest'immagine.

Growing follicle at the site of reimplantation

Donnez et al 2004

Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemoterapy for hodgikin's lymphoma



Radford et al Lancet 2001

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QuickTime™ e un
decompressore
sono necessari per visualizzare quest'immagine.

QuickTime™ e un
decompressore
sono necessari per visualizzare quest'immagine.

Donnez J 2008

Cancer and Fertility: Ethical and Legal Challenges

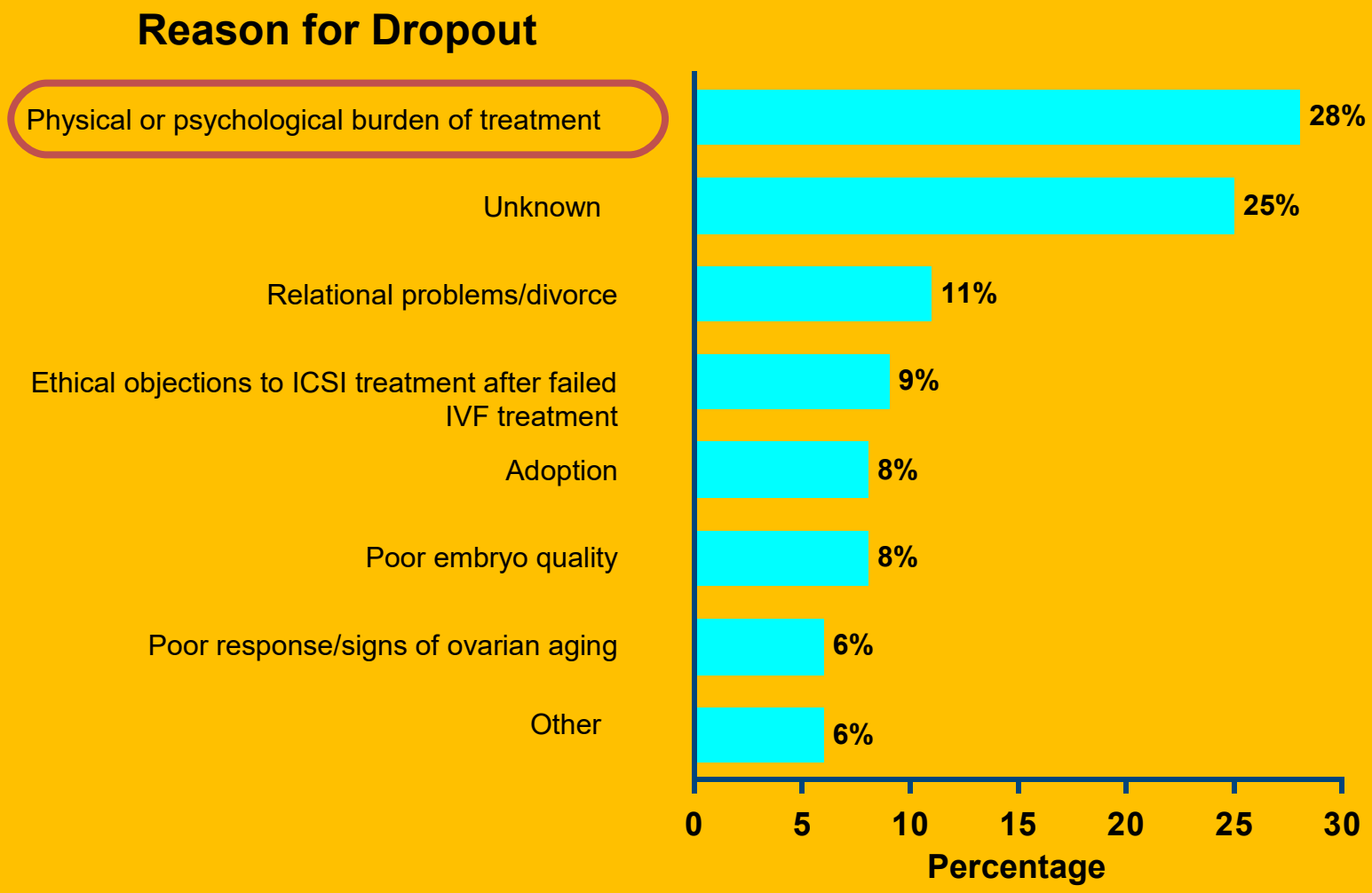
-Experimental
-Cost
-Minors
-Informed Consent
-Recurrence of the cancer

The oncologist’s role in preserving fertility

Evolution of clinical practice

Physical or Psychological Treatment Burden Is a Primary Reason for Dropout

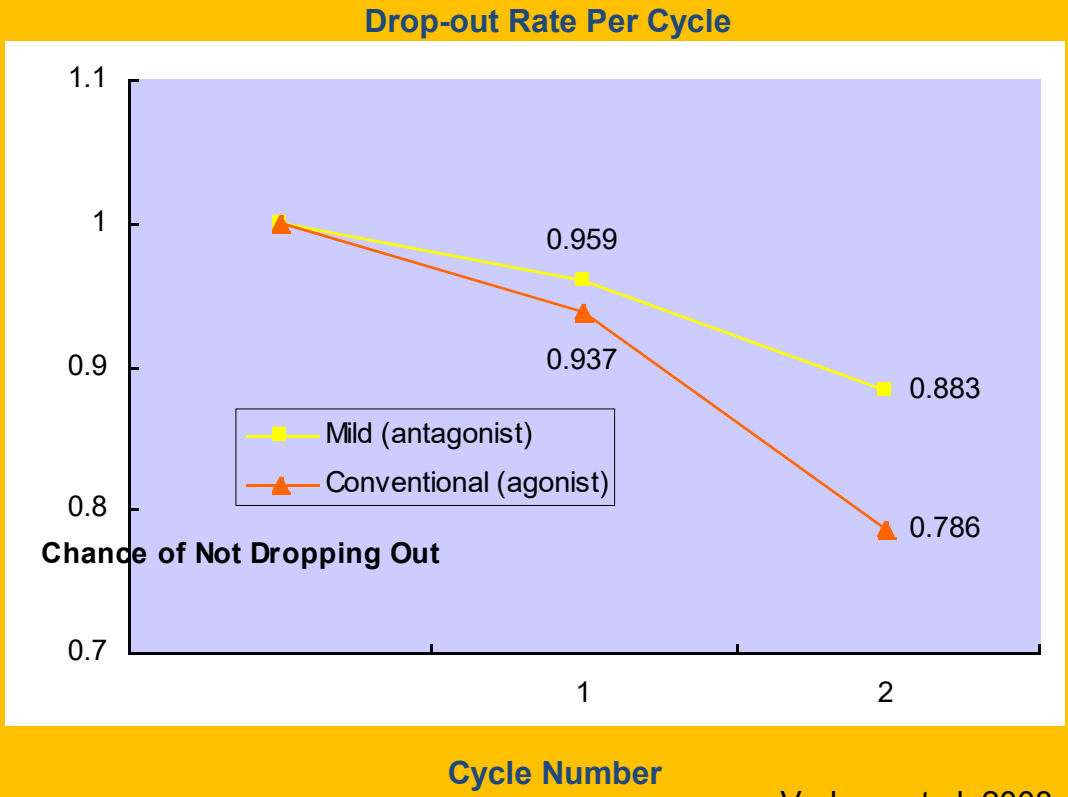
Among 384 couples undergoing IVF treatment, 65 (17%) dropped out



Verberg et al. *Hum Reprod*. 2008;23:2050.

Making IVF more acceptable

- Most IVF patients are employed full time
- Patient friendly IVF should be……
  - Quicker
  - Less hospital visits
  - Less side effects
  - Cheaper
  - Less stressful
    - Less injections



Verberg et al, 2008

Evolution of clinical practice

The present…….

- Simpler
- More patient friendly
- Safer
- Quicker
- Cheaper
- As effective

The future?

The Future of IVF

- Making IVF safer
  - Reducing risk of OHSS
  - Reducing risk of multiple pregnancy
- Making IVF more acceptable
  - Simplified user-friendly stimulation
  - Improved success rates
  - Lower cost

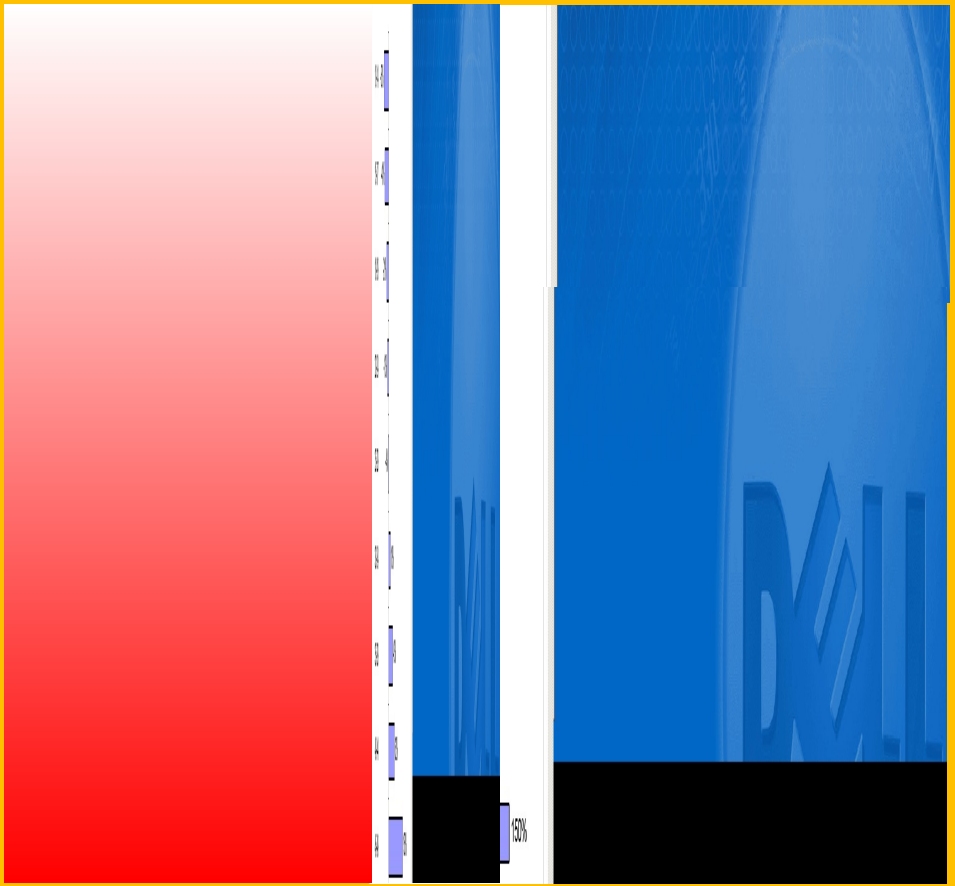
Reducing risk of multiple pregnancy

- Single blastocyst transfer
- Vitrification
- Frozen embryo replacement in natural cycle where possible
- Patient/public education

The future:
IVF for older and older patients

1990 - 2004 change in birth cohorts - USA

Infertility **Age** **Birth Rate (% Change)**



US Vital National Statistics Report, 2006

The future of fertility preservation

- Fertility preservation strategies will be used more frequently
  - *This will include preservation of embryos, sperm and oocytes before cancer treatment and preservation of oocytes for single women*
- New approaches will be adopted to allow pregnancy after menopause
  - *Treatment for premature ovarian failure may also be applied to pregnancy after natural menopause*

And further …..

- Routine storage of umbilical cord stem cells
- Routine storage of gametes for healthy young people
- Routine screening of embryos
- Routine selection of desirable characteristics – including embryonic sex

Sexual intercourse purely for recreation!!!