

Impact of new cryopreservation techniques on clinical management

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New tools

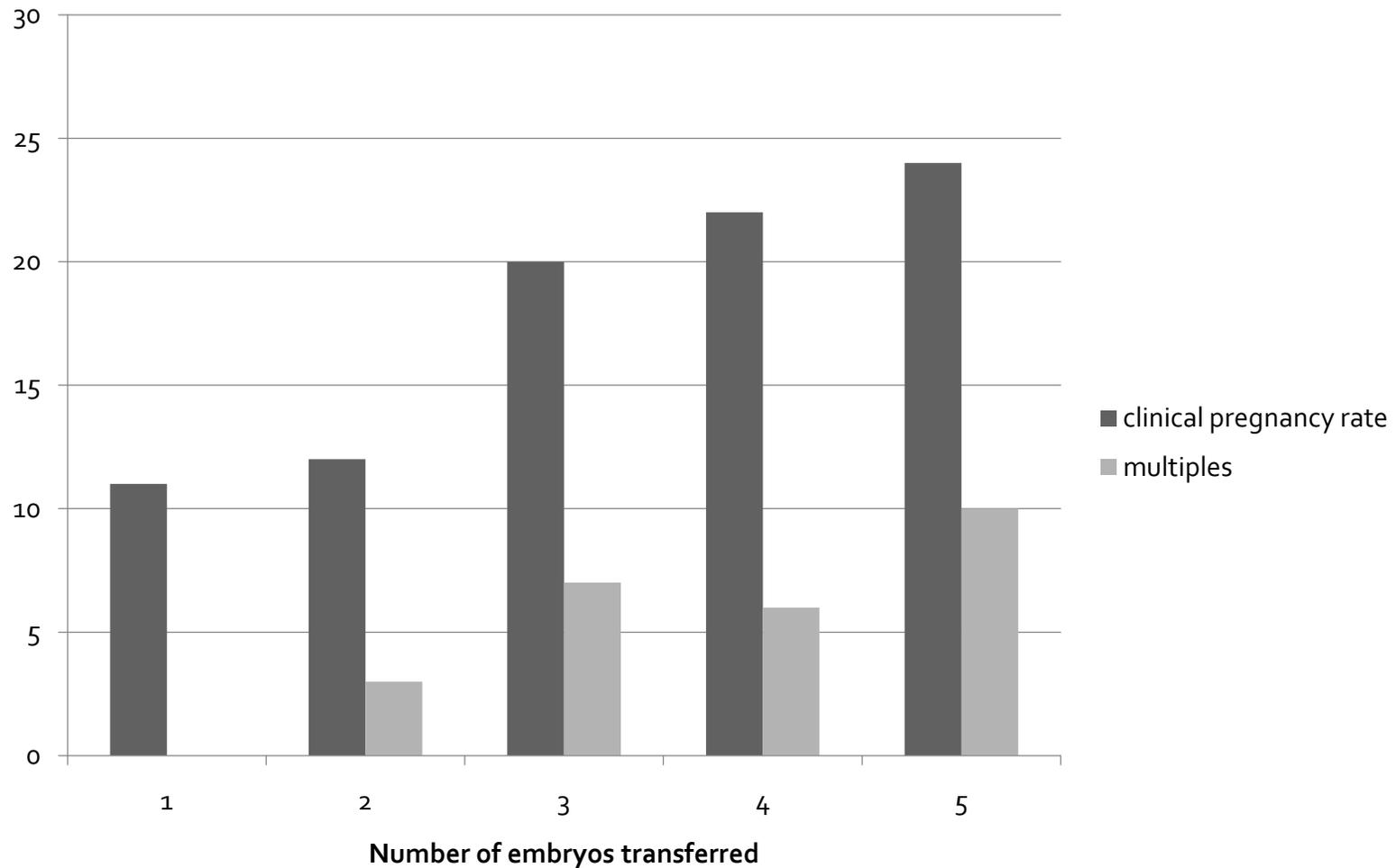


- Vitrification
 - the new multi-tool?

Why do we need highly efficacious freezing methods?



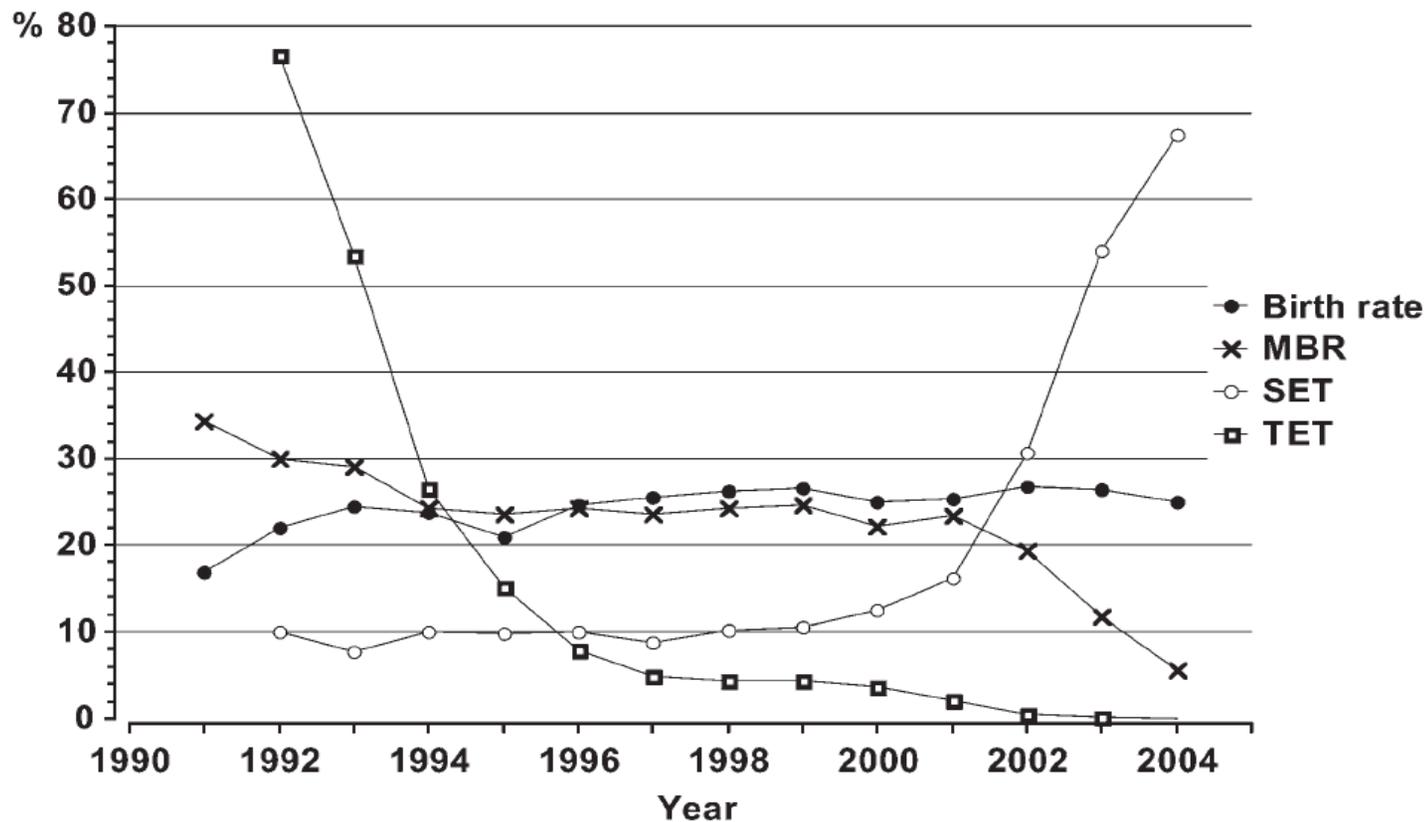
IVF practice in the 1980's: transfer all embryos, immediatly



Dooley et al., 1988

Practice today: eSET + freezing

Results from Sweden, 1991-2004



Ovarian stimulation & endometrium

Implantation failure?

Exposure of the embryo to advanced endometrium

Advanced maturation of endometrium in early luteal phase

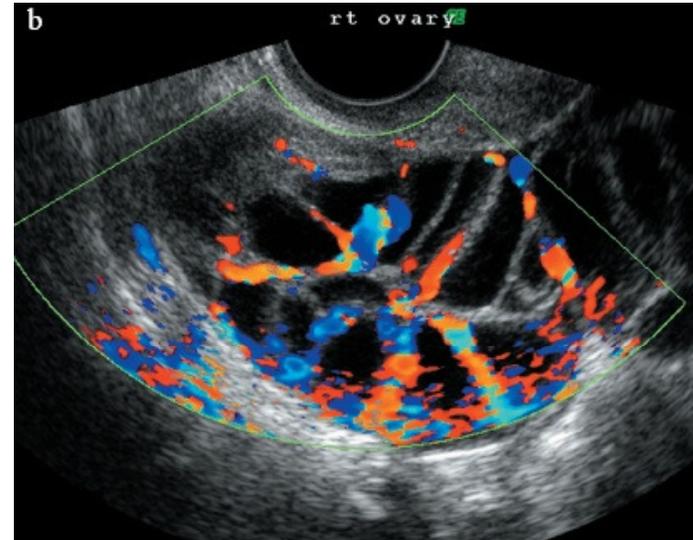
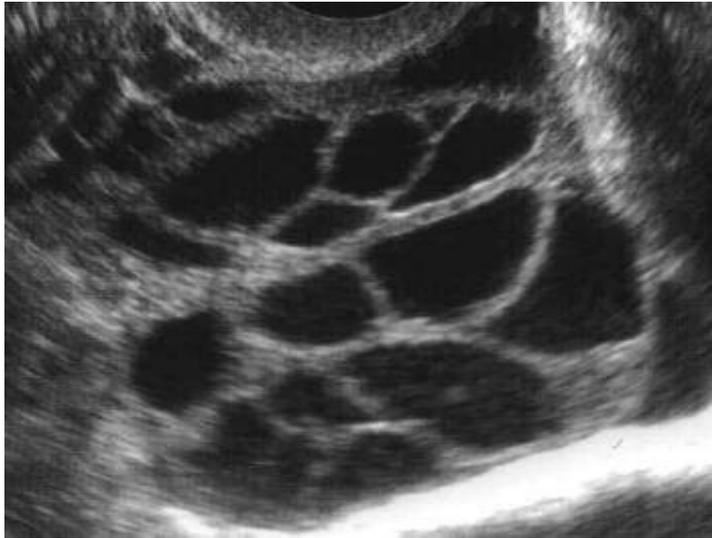
P increase in the follicular phase

Upregulation of the P receptor

High E2 in the follicular phase

- Ubaldi et al., 1997 & 2007
- Kolibianakis et al., 2002
- Tavaniotou et al., 2001
- Fauser & Devroey, 2003
- Devroey et al., 2004
- Horcajadas et al., 2005
- Diedrich et al., 2007
- Bourgain & Devroey, 2007
- Horcajadas et al., 2007
- Martinez et al., 2007
- etc.

OHSS



Early vs. late-onset OHSS

	Early onset	Late onset
Preovulatory follicles(n)	22.5	17.7
Preovulatory E2 (ng/ml)	3150	2860
OHSS III (%)	23	43
Days in hospital	4.6	7.9
hCG positiv (%)	50	97
Ongoing pregnancy (%)	32	88

Ovarian stimulation & early pregnancy

Human Reproduction Update, Vol.11, No.5 pp. 473–482, 2005
Advance Access publication July 1, 2005

doi:10.1093/humupd/dmi022

Assisted reproduction: the epigenetic perspective

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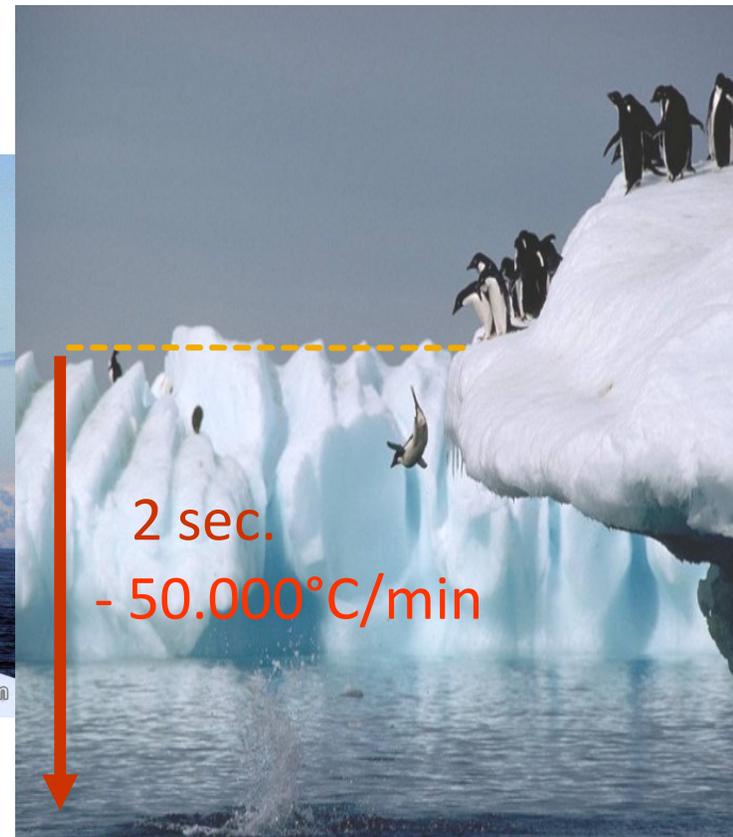
'.....There is increasing evidence that genetic factors as well as environmental factors (hormones and culture media) can have adverse effects on epigenetic processes controlling implantation, placentation, organ formation and fetal growth.'

Cryopreservation

SLOW COOLING

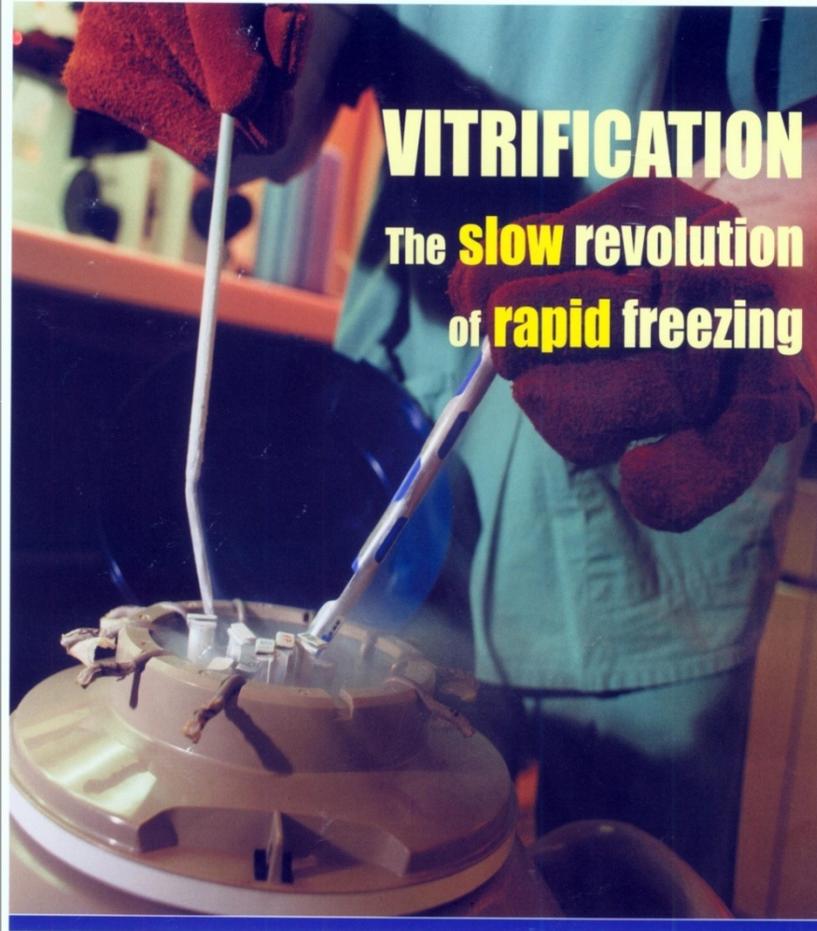


VITRIFICATION



FOCUS ON
Reproduction

EUROPEAN SOCIETY OF HUMAN REPRODUCTION AND EMBRYOLOGY



VITRIFICATION
The **slow** revolution
of **rapid** freezing

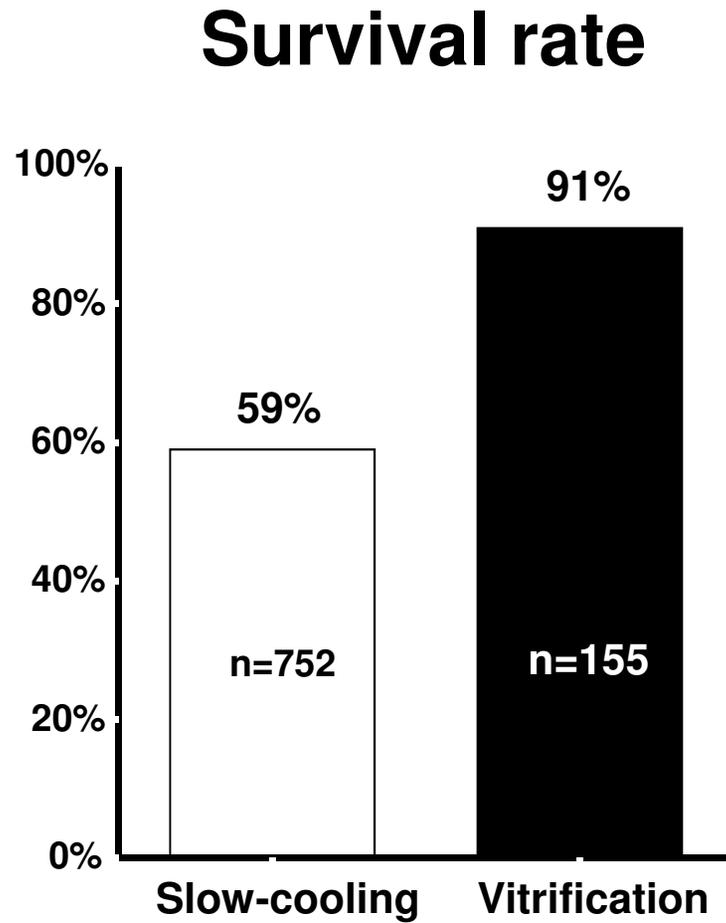


- ESHRE news
- PCOS consensus workshop
- Oocyte donation's huge headway in Spain

Vitrification of oocytes

	<i>Slow-freezing protocol</i>	<i>Vitrification protocol</i>	<i>P-value</i>
No. of patients	208	46	–
No. of thawing/warming cycles	286	59	–
No. of thawed/warmed oocytes	1348	285	–
No. of survived oocytes (%)	780 (57.9)	225 (78.9)	<0.0001 ^a

Lübeck results on 2PN oocytes (up to January 2008)

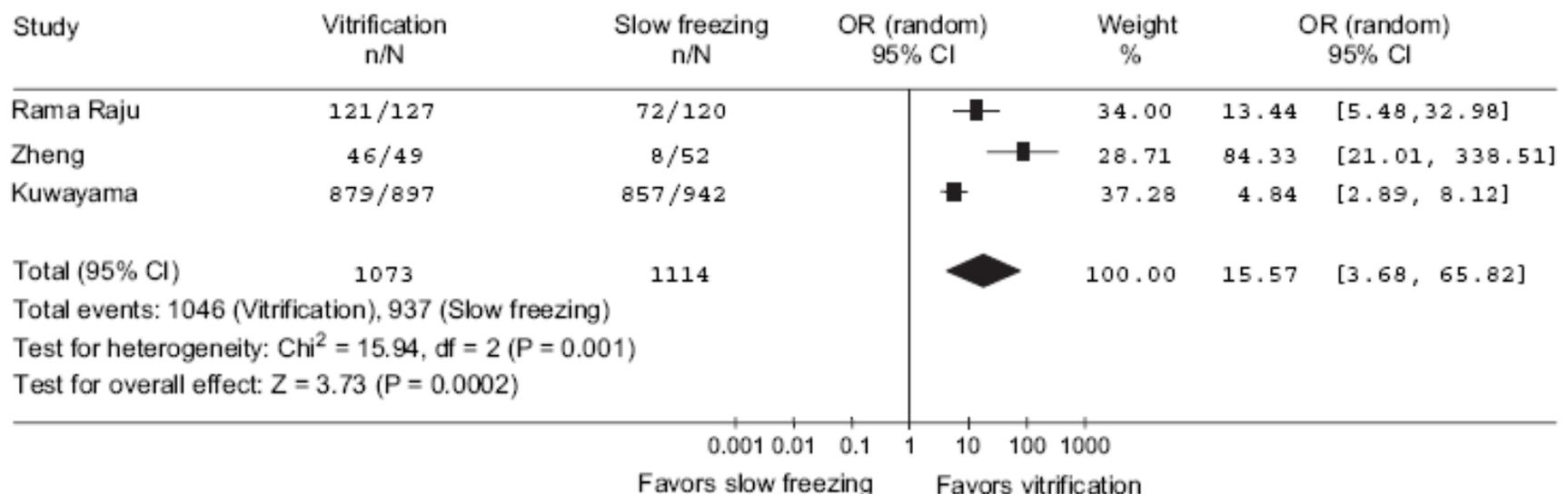


Vitrification:
April 2004- January 2008

Slow cooling:
Historical controls

Vitrification of embryos

Odds ratio of postthawing survival rate of cleavage stage embryos after vitrification and slow freezing.



Loutradi. *Techniques and Instrumentation. Fertil Steril* 2007.

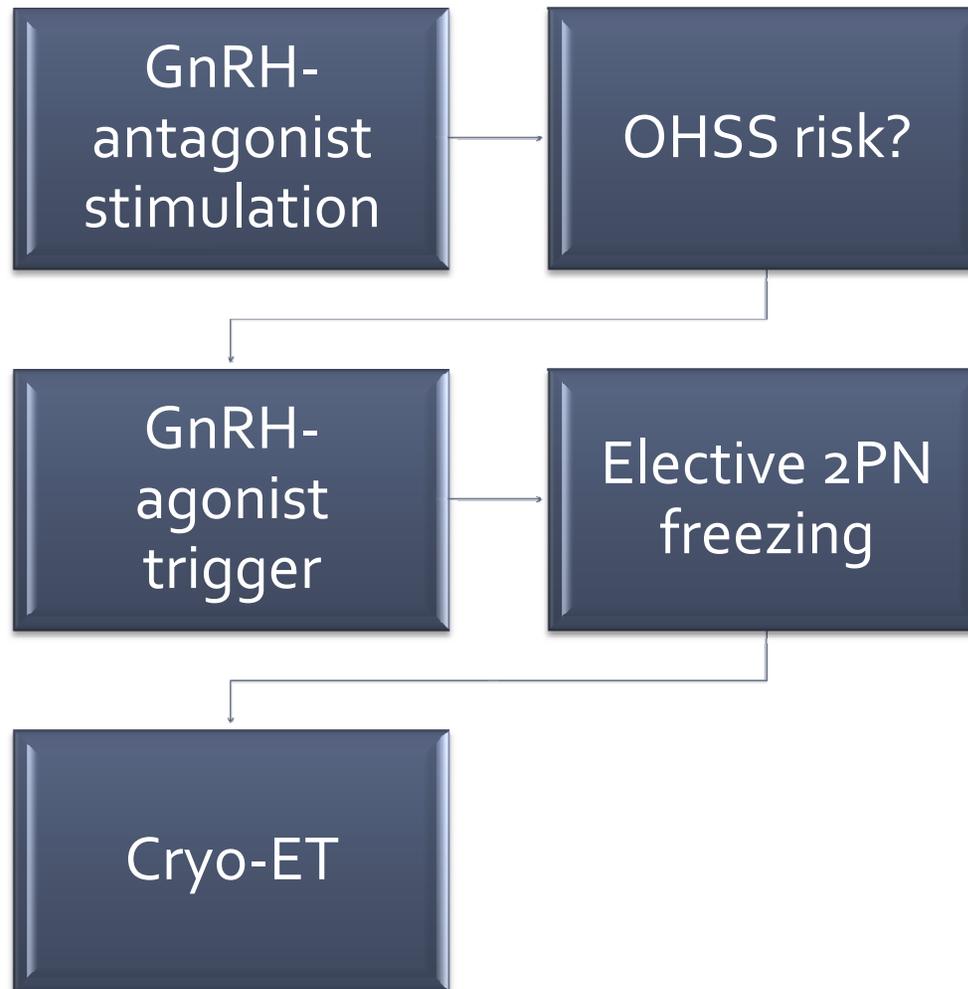
Loutradi et al., *Fertil Steril*. 2008 Jul;90(1):186-93

Temporally splitting stimulation & embryo transfer

- Vitrification of 2PN oocytes/embryos
- Implications:
 - *to test for oocyte competence independent of endometrial receptivity post stimulation*
 - *to avoid consequences of stimulation on endometrium & course of pregnancy*
 - *to avoid late-onset OHSS*
 - *To allow efficacious eSET programs*



OHSS prevention



Patients with GnRH-
agonist triggering, n = 40



Patients with 2 PN
oocytes frozen, n = 39



Patients with at least one
embryo transfer, n = 39



Number of (cryo) embryo
transfers, n = 87

Cumulative live birth rate (95% confidence interval)

35.0% (14/40)

23.9-48.0%

Live birth rate per embryo transfer

16.1% (14/87)

10.6-23.6%

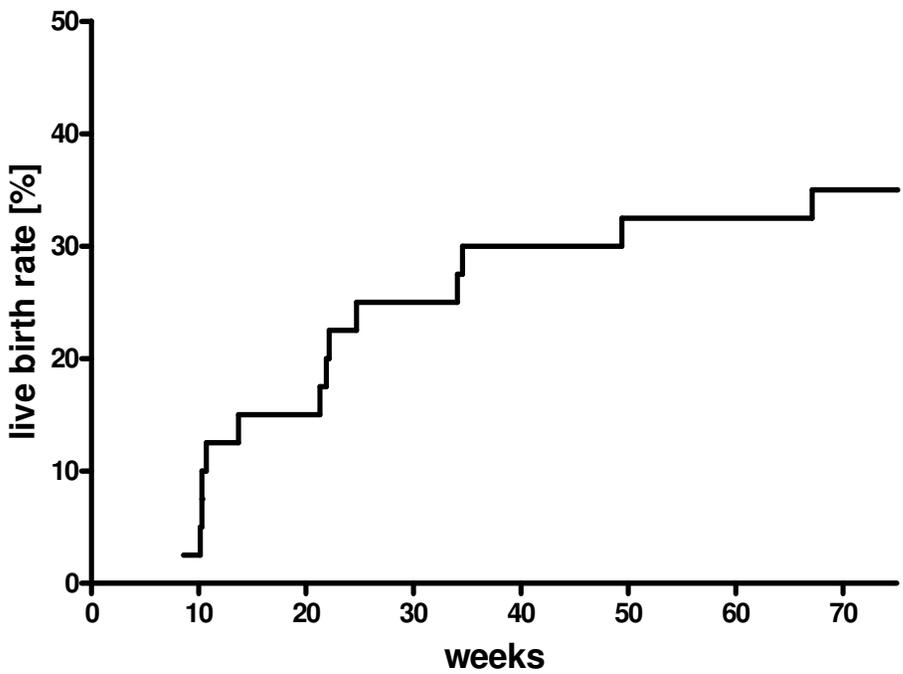
Live birth rate per first embryo transfer

17.9% (7/39)

10.0 – 30.1

OHSS incidence: 0% (0.0 – 6.2)

Cumulative incidence of positive hCG test leading to live birth



Mean number of ETs:

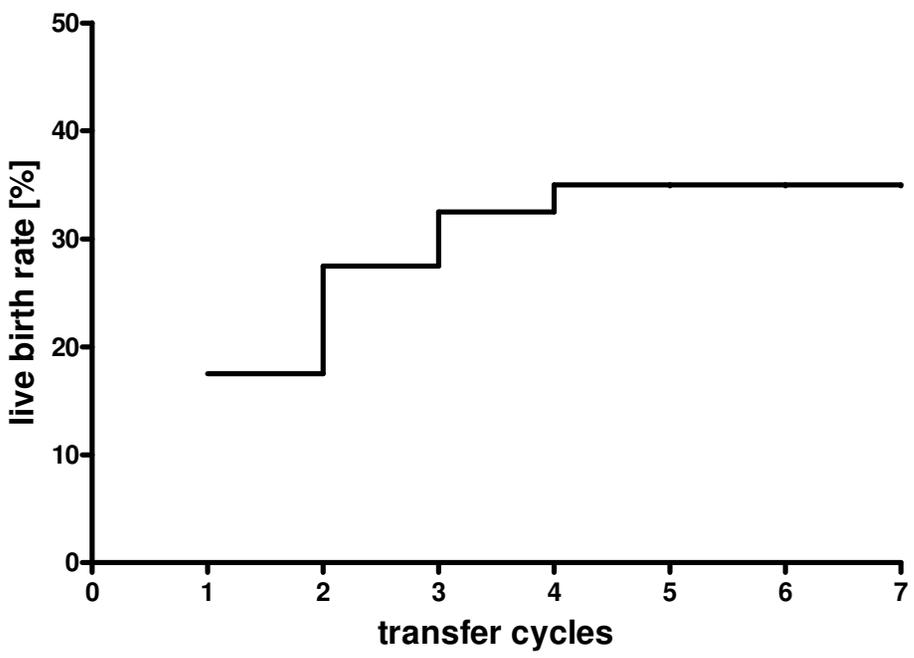
2.2

Mean number of embryos transferred

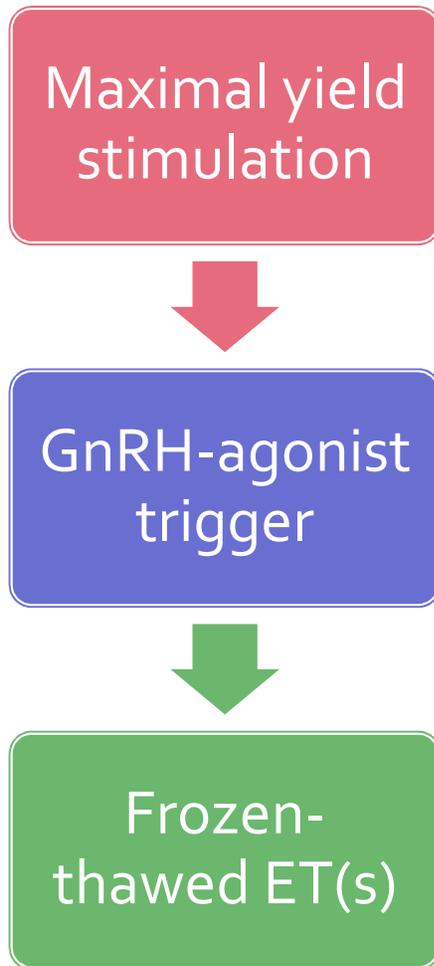
2.2

Mean time-to-conception

24 weeks

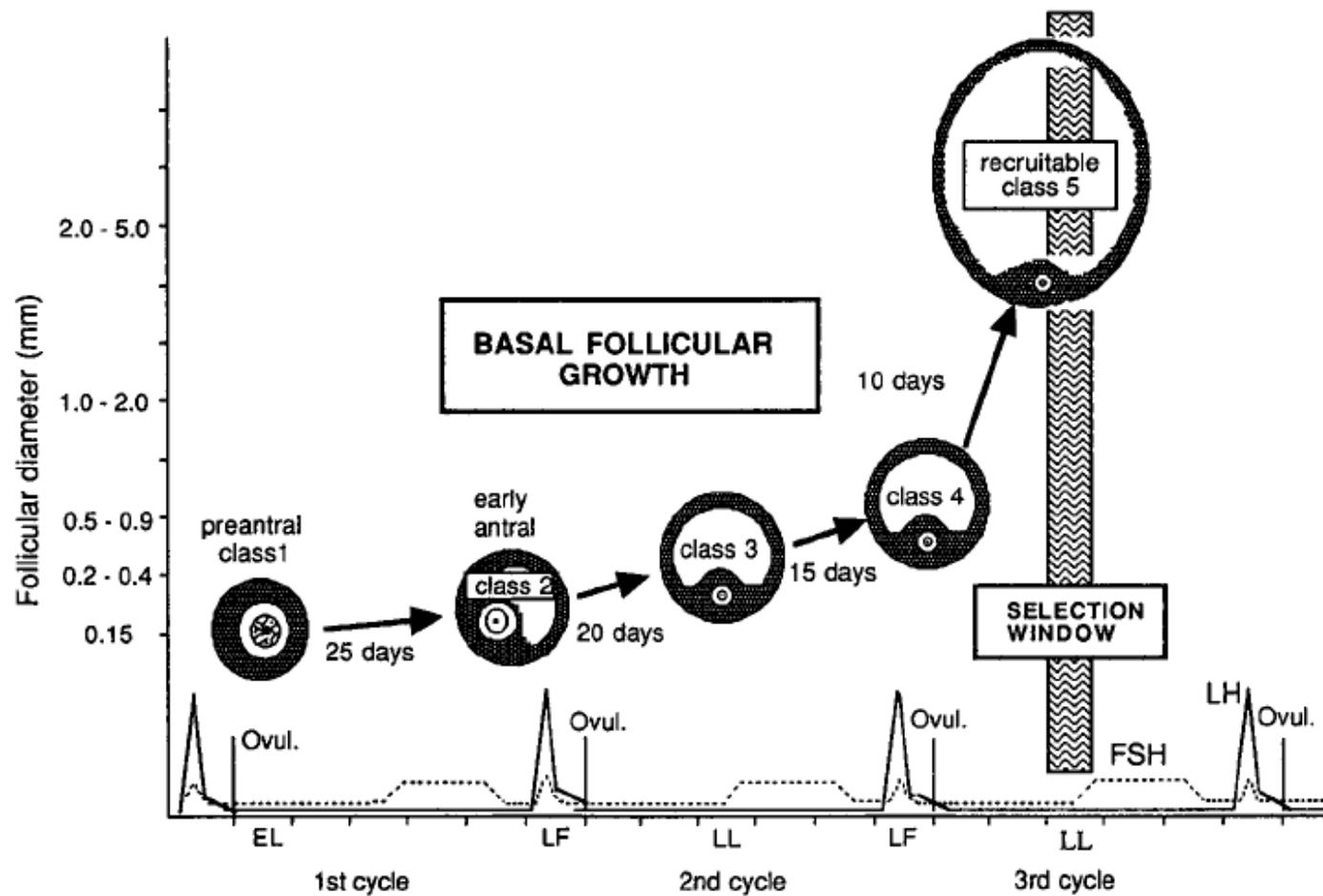


Splitting ovarian stimulation and ET as a routine

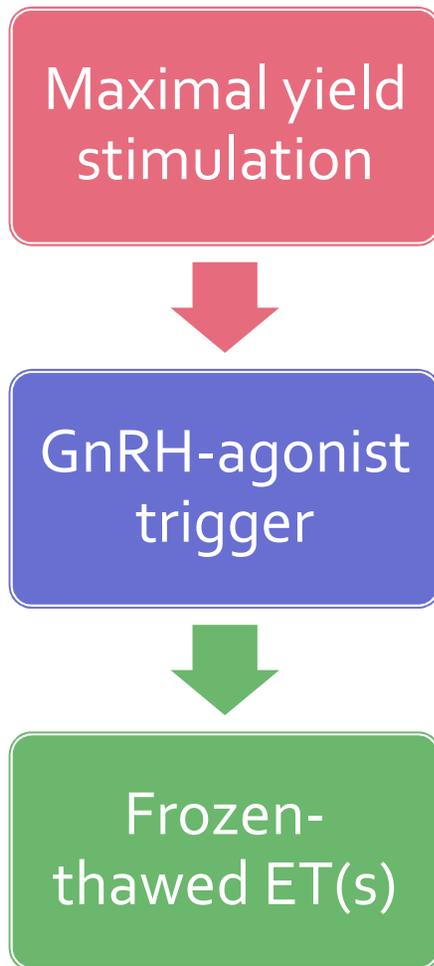


- **Prospective, observational pilot study**
- Is it feasible? (discomfort)
- Is it safe? (OHSS)
- What is the # fertilized & frozen oocytes?
- What number of frozen-thawed cycles can be expected?
- What is the clinical outcome? (pregnancy)

„Maximal yield’ stimulation



Experimental protocol



- 300-375 IU FSH/d
- Antagonist protocol
- Age < 37a, 2 ovaries, ICSI
- Triptorelin 0.2 mg sc
- Artificial cycle (trans-dermal E2 and vaginal P)

Results from pilot study (n=30):

- Stimulation (mean, SD)

➤ FSH dose, IU	2630	650
➤ Duration, d	9.8,	2.0
➤ E2, pg/ml	3630,	2150

- Embryology

➤ COCs	17.2,	8.5
➤ MII oocytes	13.5,	6.6
➤ 2 PN oocytes	8.3,	4.6
➤ 2PN frozen/total COC	48%	17%

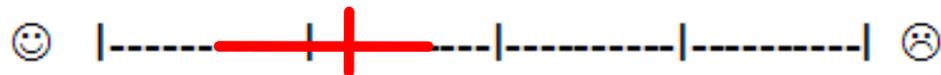
- Ongoing pregnancy (>12 GW)

- 6/28 = 21.4%

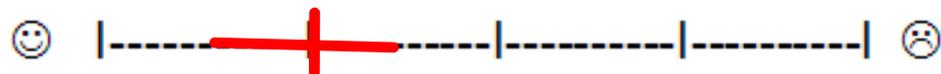
Is it feasible?

patient discomfort on OPU + day 3

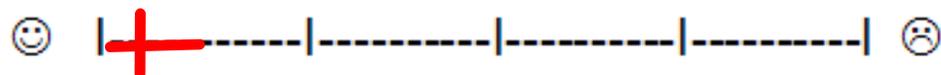
Abdominal pain?



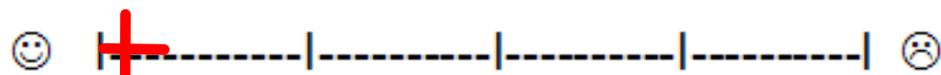
Abdominal tension?



Nausea/vomiting?



Headache?



■ n=30 patients

■ Mean

■ 95% confidence interval of the mean

Is it safe: OHSS?

- OHSS incidence: 0% (0/30)
- CRP 5.0 mg/l (range 0.5 – 28.5)
- Hct 37.4 (range 28-43)
- WBC 9,000/ μ l (range 5,000 – 16,700)
- Ascites: largest diameter of fluid in douglas' pouch 1,3 cm (range: 0 – 3,5)

Spatially splitting stimulation & embryo transfer: oocyte donation

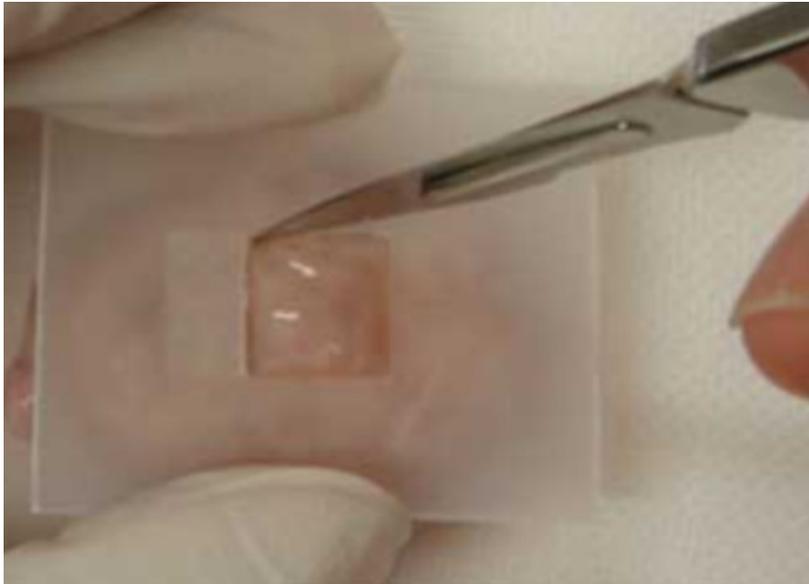
- Vitrification of oocytes
- Implications:
 - *to timely and spatially disconnect donor and recipient schedules*
 - *to allow repetitive serological testing of the donor and thus increase safety of the program*
 - *To facilitate an eSET strategy in oocyte donation programs*

Oocyte donation & vitrification

	Vitrified
M II oocytes	231
Survival	96.9%
Fertilization	76.3%
No. of transfers	23
Mean number of embryos	2.1
Ongoing pregnancy rate	48%

Ovarian cortex cryopreservation

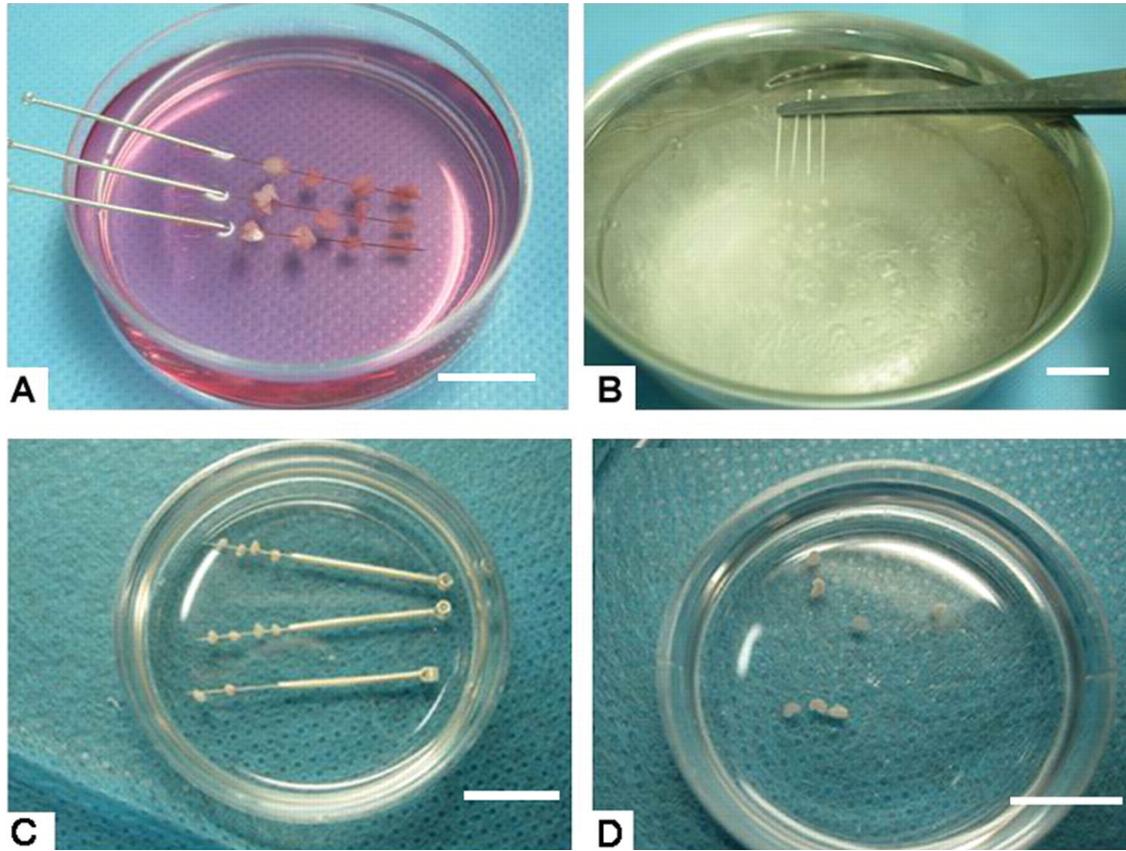
- Cryotissue method



Kagawa et al., RBMonline 2007
Kagawa et al., RBMonline 2009

Ovarian cortex cryopreservation

- Needle immersion method



Clinical perspective on new cryopreservation methods

- Safety of procedure: child health
- Safety of procedure: cross-contamination
- Efficacy as compared to fresh fertilization and embryo transfer
- Efficacy in re-transplantation of autografts