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## Day 2-3 versus Day 5 transfer advantages and disadvantages

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### Overview

- Analysis of Cochrane data
- Why do we prefer to transfer blastocyst stage embryo ?
- Analysis of retrospective, non-randomized comparison of day-2 ET versus day-5 ET
- Extended embryo culture in non stimulated cycles, poor, low and normal responders
- To evaluate the clinical efficacy of using day 5 embryo transfer for all patients
- Slow freezing or vitrification ?
- Outcome of blastocyst transfers

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### Dilemmas

- Does the prolonged cultivation in *in vitro* conditions influence the embryo implantation capability?
- Is the five day cultivation the best approach for all patients (including patients with one oocyte or low responders)?
- Is the five day cultivation of any benefit in comparison with two day cultivation ?

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### Dilemas - disadvantages

- Is extended embryo culture appropriate for all patients?
- Worldwide experience with blastocyst cryopreservation is not optimal ?
- Higher rate of cancelled transfers ( having no embryos for transfer ! )
- Question of overall benefit of blastocyst culture (impacts on the final outcome?)
- Monozygotic twinning

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### Dilemas - advantages

- Better synchronization between the embryo and the endometrium
- Better selection of viable embryos and higher implantation rate
- Increased risk of multiple gestation ?
- Higher rate of cancelled transfers ?

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### "The perfect embryo" ?? (based on morphology and development)

Implantation of D5 embryo (SBT) in 49% patients ≤ 36 years



Implantation of Day2 or Day3 embryo (SET) in 33 % of patients ≤36 years



Papanikolaou et al., NEJM, 2006

Van Montfoort et al., Hum Reprod, 2006

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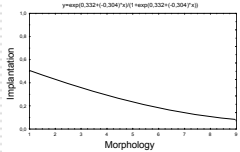
**Maribor blastocyst scoring system**  
Kovacic B. et al. Reproductive BioMedicine Online 2004

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**Four morphologic parameters**

- percentage of embryo, transformed to the blastocyst
- blastocoel expansion
- ICM shape in expanded blastocysts only
- trophectoderm cohesiveness and morphology

**Relationship between blastocyst morphology and implantation ability**



**Eight morphologic categories (B1 - B8)**

Birth rate declined from 45.2% to 7.7% from B1 to B8 quality blastocyst.

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**Article**  
**Developmental capacity of different morphological types of day 5 human morulae and blastocysts**

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- Implantation capacity depending on blastocyst morphology characteristics.
- Birth rate declined from 45.2% to 7.7% from B1 to B8 quality blastocyst.

Kovacic B. et al., Fertil Steril 2008

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**Day 2 vs. Day 5 embryo**  
Randomized prospective trials

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**Ongoing pregnancy rate**

- Day 3 : 32.1% 95%CI 26.4-38.2%
- Day 5 : 33.2% 95%CI 27.3-39.5%
- Lower chance for cryopreservation ( 61.5% for D3 and 50.4% for D5, P<0.02)

Kolbianakis et al.; Hum Reprod 2004

**Implantation rate:**

Day 2-3 embryos vs. day 5 blastocyst: 21.1% vs. 20.9%  
Hreinsson et al. Eur J Obstet Gynecol Reprod Biol 2004

Day 3 vs. hatching blastocyst : 19.1% vs. 21.4 %  
Utsumoriya et al. Hum Reprod 2004

Elective single blastocyst : 60.9%  
Gardner et al. Fertil Steril 2004

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### Cochrane data (2009)

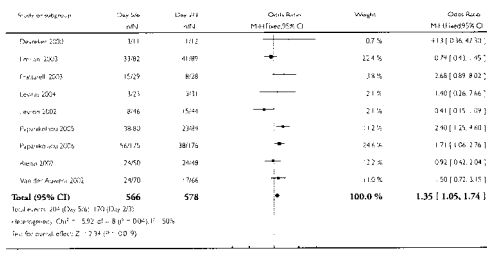
- Primary outcome ( live births per couple)
- Secondary outcome ( clinical pregnancy rate, multiple pregnancy rate, high order MPR, cryopreservation, failure to have any ET per couple )
- Outcomes not appropriate for statistical analysis ( live births per OPU and ET, CPR/OPU&ET, implantation rate

### The Cochrane Metanalysis 2009 : Live birth rate

Review: Oligo(zig)ospermia & progesterone levels in assisted reproduction

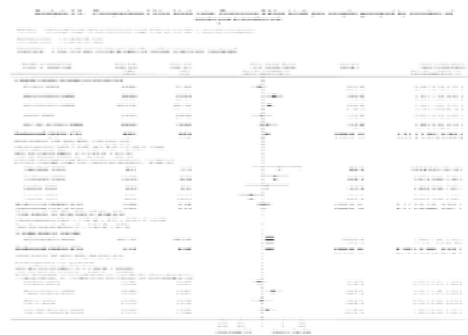
Comparison: Live birth rate

Outcome: Live birth rate

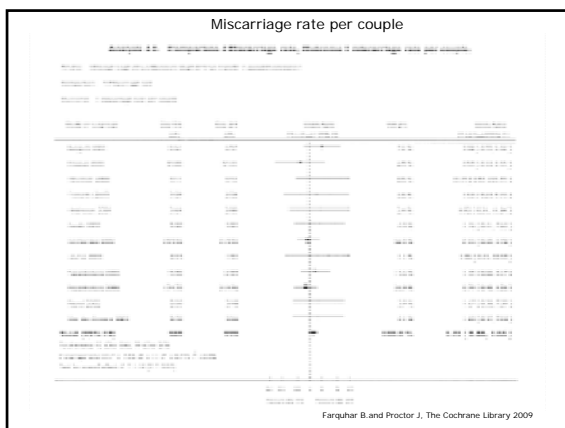
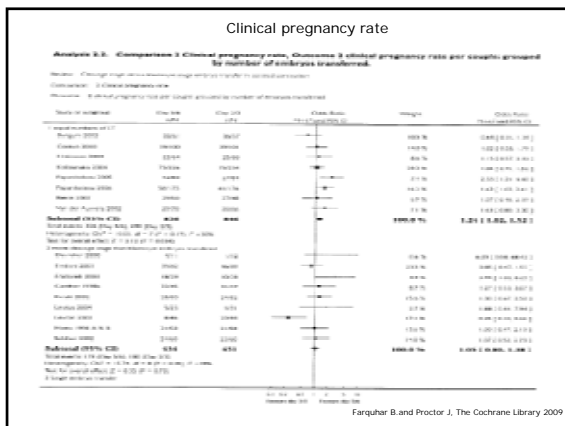


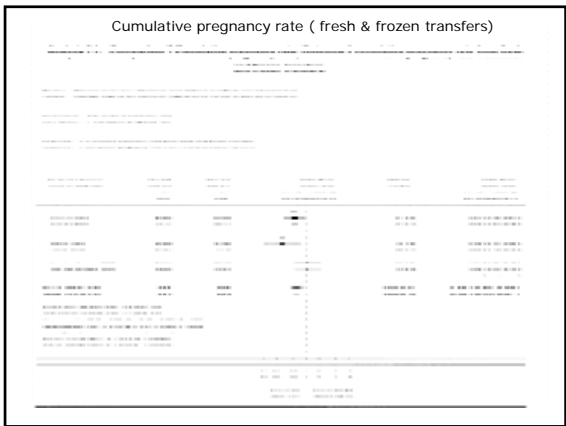
Farquhar B. and Proctor J. The Cochrane Library 2009

### Live birth rate per couple (2)



Farquhar B. and Proctor J. The Cochrane Library 2009






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eSET in daily practice in Slovenia from 2008

- Legal approach** ( 3 embryos for ET from year 2000)
- Professional approach** ( guidelines of national Society for Reproductive Medicine – 2 embryos for ET from year 2000)
- Reimbursement approach** (reimbursement only according to guidelines for SET from year 2008)

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Retrospective analysis - IVF Maribor

- Retrospective analysis of 6056 stimulated and 391 nonstimulated cycles was performed
- Selection of embryos for blastocyst culture on day 3  
*BlastAssist System ( MedCult Denmark)*
- Embryotransfer catheter *Labotect (Germany)*
- No more than two blastocyst transferred at the time
- Surplus blastocyst frozen  
*Blast Freez (MediCult, Denmark)*  
*Vitrification Kit (IrvineScientific, USA)*
- Vaginal progesteron for luteal support  
*Utrogestan (Iscovesco, France)*

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Patients selection strategy for blastocyst culture:  
**NUMBER OF FOLLICLES OR OOCYTES**

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- Good responders on COH during the stimulation
- Patients with 5 oocytes and more after OPU

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Patients selection strategy for blastocyst culture:  
**FERTILIZATION**

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- Patients with more than 4 fertilized oocytes
- Patients with more than three good quality embryos on day 3.

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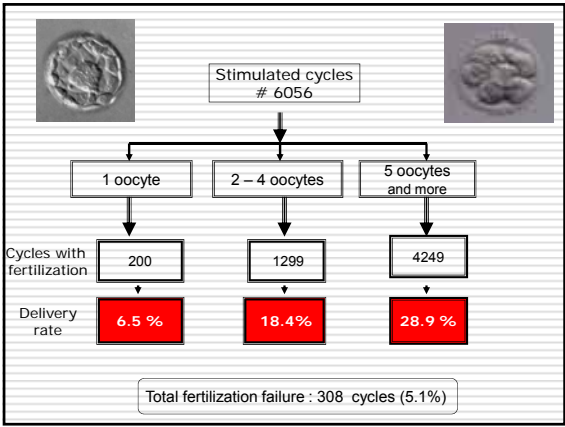
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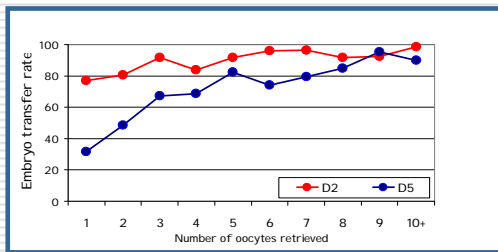
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### Embryo transfer rate on Day 2 and Day 5 in patients younger than 40



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### Results

- Non stimulated cycle
- Poor responders ( one oocyte)
- Low responders ( 2-4 oocytes)
- Normal responders ( 5 oocytes and more)
- Slow freezing and vitrification

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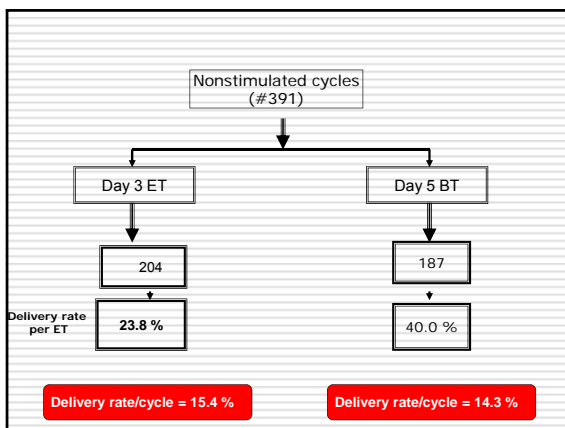
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Hum Reprod 2001; 26(11): 2795-2802

### Is there any benefit from the culture of a single oocyte to a blastocyst-stage embryo in unstimulated cycles?

Veljko Vlaisavljević<sup>1,2</sup>, Borna Kovačić<sup>2</sup>, Milan Rajčić<sup>2</sup>, Vida Gavrić Luvrić<sup>1</sup> and Maja Čirk Šajko<sup>2</sup>

<sup>1</sup>Department of Obstetrics, Gynecology and Neonatology, Maternity Hospital, Ljubljana, S. M. 2000, Matična Slovenija

	ET on day 2 (204)	ET on day 5 (187)	p-value
Oocyte recovery rate	79.4 (162/204)	82.3 (154/187)	NS
Fertilization rate	73.8 (113/153)	77.7 (115/148)	NS
ET rate per aspiration	51.5 (105/204)	29.4 (55/187)	<0.05
Pregnancy rate per ET	23.8 (25/105)	40.0 (22/55)	<0.05
PR calculated /day 2 ET	23.8 (25/105)	22.2 (22/99)	NS
PR/ aspirated oocyte	15.4 (25/162)	14.3 (22/154)	NS

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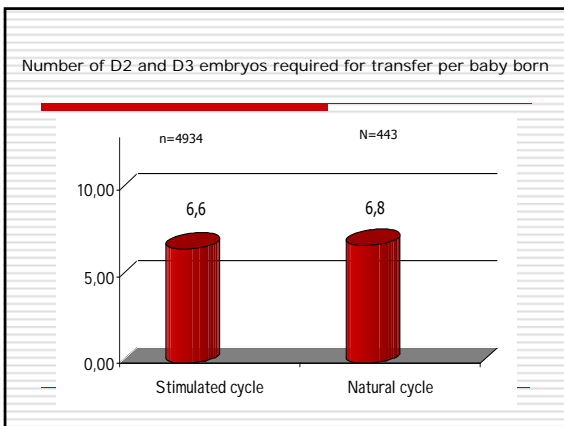
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Hum Reprod 2001; 26(11): 2795-2802

### Results of Intracytoplasmic Sperm Injection of Single Oocyte in 362 Unstimulated Cycles

Veljko Vlaisavljević<sup>1,2</sup>, Borna Kovačić<sup>2</sup>, Milan Rajčić<sup>2</sup>, Vida Gavrić Luvrić<sup>1</sup> and Maja Čirk Šajko<sup>2</sup>

Probability for implantation of a single embryo does not depend on cycle type (nonstimulated and stimulated cycles), but on morphologically assessed quality

Vlaisavljevic et al. Hum Reprod 2001

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## Results

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- Poor responders ( one oocyte)
- Low responders ( 2-4 oocytes)**
- Normal responders ( 5 oocytes and more)
- Slow freezing and vitrification

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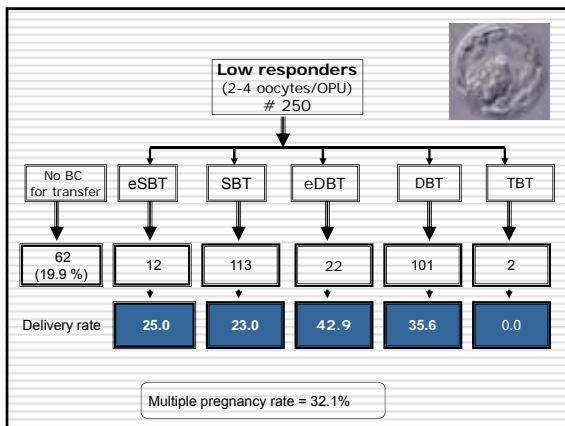
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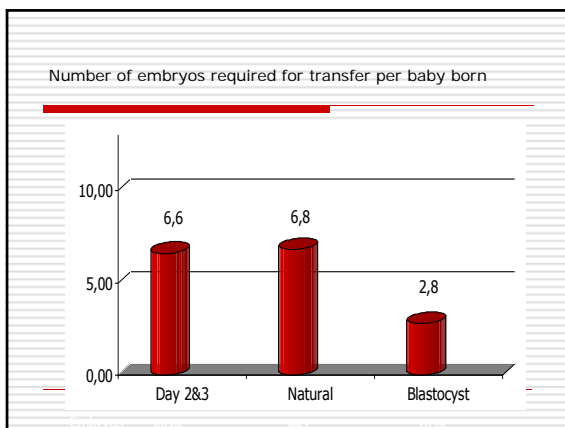
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**IN VITRO FERTILIZATION**

**Clinical outcome of day 2 versus day 5 transfer in cycles with one or two developed embryos**

Shoval Elimelech, PhD, Dr. Hagit Mervin-Ben-David, PhD, Dr. Miriam Hegerl, PhD, and Yulia Shmida-Kornet, MSc, Department of Reproductive Medicine and Gynecology, Hadassah University Hospital, Jerusalem, Israel

- Embryo transfer rate per cycle was higher when day 2 embryos were transferred
- Expected pregnancy rate in poor responders calculated per embryo(s) available on day 2 is not affected by oocyte culture to the blastocyst stage.

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**Results**

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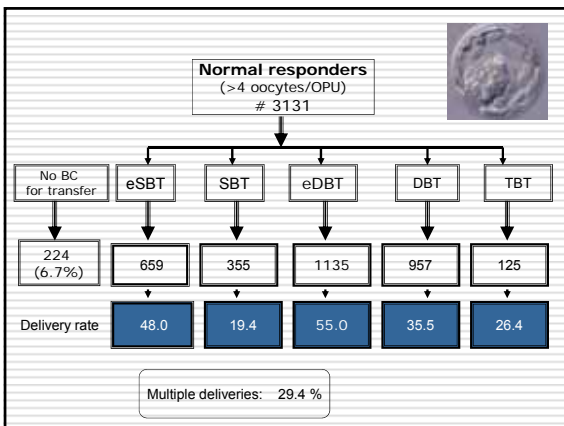
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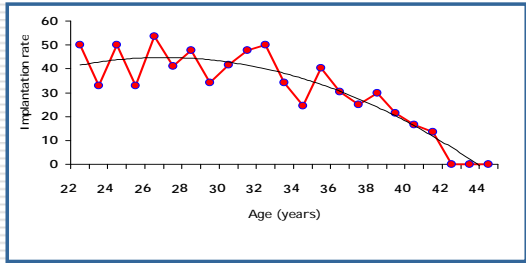
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Implantation rate per cycle in single blastocyst transfer




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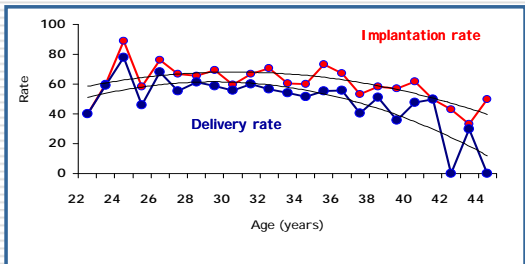
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Implantation and delivery rate in cycles with good prognosis (892 fresh cycles with frozen spare blastocysts)




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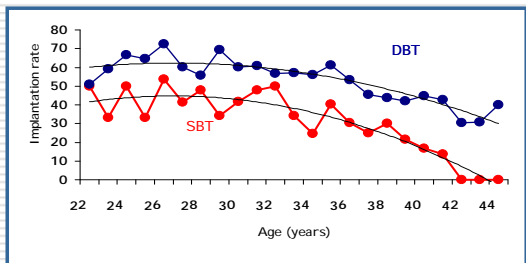
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Pregnancy rate per single (SBT) and double (DBT) blastocyst transfer




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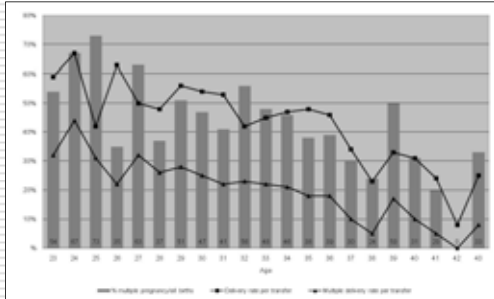
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Should the practice of double blastocyst transfer be abandoned?

Vlaisavljevic et al., RBM Online, 2008; 16:671.



Vlaisavljevic et al. RBM Online 2008

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Delivery rate per cycle after blastocyst transfers




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Results

- Non stimulated cycle
- Poor responders ( one oocyte)
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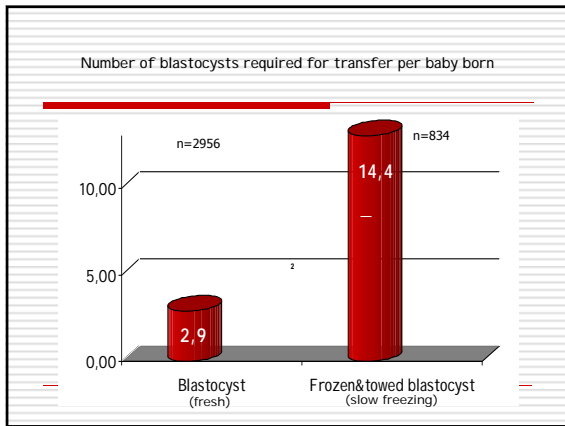
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Retrospective analysis of nonselective blastocyst slow freezing (n=667 cycles)

	At least 1 optimal blastocyst (n=317)	Only nonoptimal blastocysts (n=350)
Survival rate (%)	77.3	65.6
Transfers (%)	100	91.3
Transferred blastocysts	1.6 ± 0.5	1.3 ± 0.7
Positive beta hCG (%)	33.3	22.0
Ongoing pregnancies rate (%)	<b>19.7*</b>	<b>12.7</b>

Statistical significance: \*\* P<0.01

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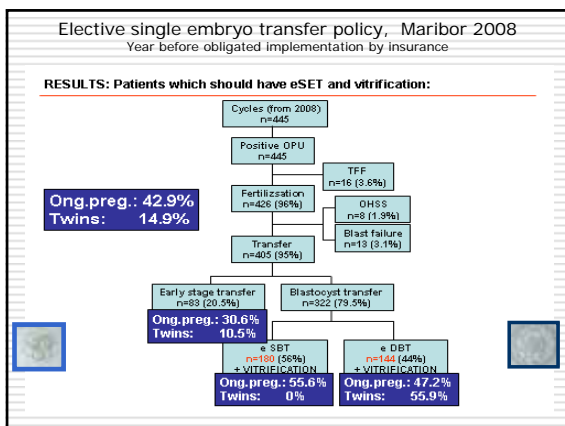
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### Conclusion (1) Cochrane review 2009

- ❑ Significant difference in live-birth rate per couple in favour of blastocyst transfer particularly in:
  - a. Good prognosis patients
  - b. Equal number of embryos transferred ( including single embryo transfer)
  - c. Randomization after Day3
- ❑ Rate of embryo freezing for couple was significantly higher on Day 2 to 3 transfers
- ❑ Failure to transfer any embryo was significantly higher in the blastocyst group, but not different in good prognosis patients.

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### Conclusions (2)

- Necessary improvement of:
  - patient selection
  - embryo selection
  - more successful cryopreservation programmes
- Implementation of prospective randomized studies is necessary to select effective strategies.
- Absence of international standard procedures for eSET may hinder full implementation of eSBT.

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### Conclusions (3)

- ❑ Same number of pregnancies with lower number of fresh transferred embryos?

YES

- ❑ Major barriers for eSBT seem to be:
  - patients' lack of knowledge.
  - actual reimbursement system.
  - cryopreservation technique ?

YES

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In 2007 3.8% of all babies born in Slovenia were conceived by ART

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### Reproductive Medicine Maribor



<b>Hospital:</b>	<b>ART Laboratory:</b>
Veljko Vlaisavljević	Borut Kovarič
Vida Gavrić Lovrec	Nina Hojnik
Milan Rejlić	Martin Ivec
Vilma Kovač	Barbara Breznik
Lea Mlakar	Martina Zafošnik
Polona Kores Testen	Petra Robič
Ksenija Rakić	Marjan Taborin
Marko Došen	Naca Herceg
<b>Nurses:</b>	<b>Secretary:</b>
Božena Rodeš	Suzana Kruplež
Daniela Hanžel	Marina Kokoi
Marija Kristovič	
Jasna Muršič	
Marija Piperski	

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