Embryo/blastocyst cryopreservation in natural and stimulated cycles: clinical aspects

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Maribor, 22.-23.1.2010

Embryofreezing – clinical aspects

- Since the first pregnancy after replacement of frozen-thawed embryos

  (Trounson & Mohr, 1983) embryo freezing has become a routine technique in ART programmes

Important aspects from clinical point of view

- indications for embryofreezing
- current practice in freezing
- strategies used in cryopreservation

  (preparation of the uterus and timing of transfers of frozen-thawed embryos)
- effectivity of embryofreezing in terms of pregnancies
- the health status of babies after embryofreezing

Embryofreezing – clinical aspects

- Indications for embryofreezing expanded over the time now offering a clinician (and couples) a broad variety of strategies for a successful and safe treatment

Embryofreezing - indications

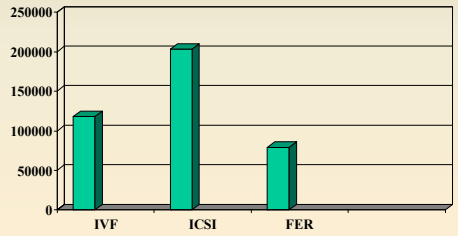
- cost-effective method to increase cumulative pregnancy rates per oocyte retrieval
- strategy to avoid the risks of severe hyperstimulation in women overresponding to ovarian stimulation
- in selected cases a very successful method for fertility preservation in women awaiting cytotoxic therapy

Embryofreezing - indications

- Morover, the current trends towards elective single embryo transfers (eSET) has put cryotechnology on the forefront of research

ART in European countries in 2005

The European IVF-monitoring programme (EIM)



ART in European countries in 2005

The European IVF-monitoring programme (EIM)

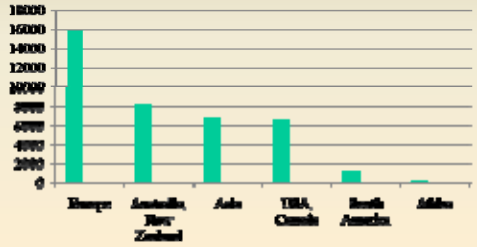
Method	IVF	ICSI	FER
Clinical PR per ET (%)	30,3	30,9	19,0

ART in European countries in 2005

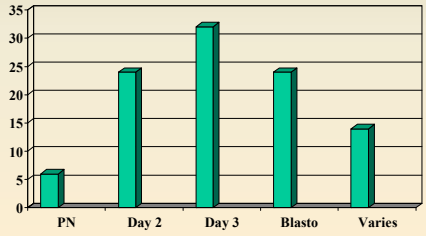
The European IVF-monitoring programme (EIM)

	Singleton	Twins	Triplets
IVF+ICSI	78,2	21,0	0,8
FER	85,6	13,9	0,4

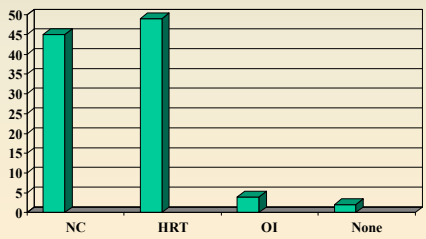
IVF Worldwide survey – FET results
(179 IVF centers, 56 countries)
reporting 133.290 IVF cycles of which 39.152 FET



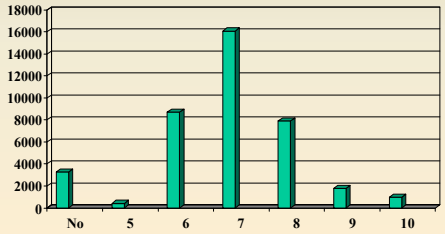
IVF Worldwide survey – FET results
(preferred timing for embryofreezing)



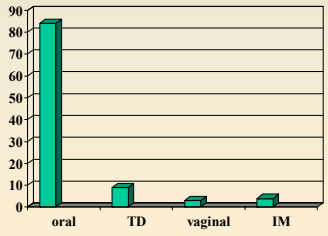
IVF Worldwide survey –FET results
(preferred preparation for FET in ovulatory patients)



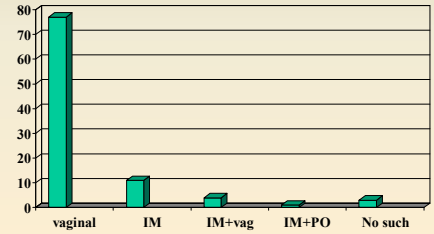
IVF Worldwide survey –FET results
(minimum endometrial thickness for FET)



IVF Worldwide survey –FET results
(preferred route of administration for estrogen)



IVF Worldwide survey –FET results
(preferred route of administration for progesterone)



Implantation potential of human embryos after cryopreservation

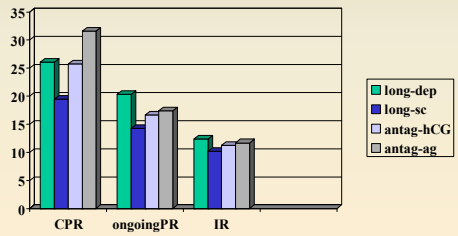
- Cryopreservation adversely affects the implantation potential of human embryos
- Significantly higher implantation rate was obtained after transfer of fully intact embryos than after transfer of partially damaged embryos (Van den Abbeel 1997, Burns 1999)

Is there an influence of stimulation protocol before egg retrieval on FER cycles?

(Eldar-Geva et al., RBMOnline 2007)

- long (depot) protocol ovarian stimulation
- long (s.c.) protocol ovarian stimulation
- GnRH-antagonist/hCG
- GnRH-antagonist/GnRH-agonist

Clinical, ongoing and implantation rates following frozen-thawed embryotransfer according to the ovarian stimulation protocol in the oocyte retrieval group.



Clinical, ongoing and implantation rates following frozen-thawed embryotransfer according to the ovarian stimulation protocol in the oocyte retrieval group.

- The potential to implant and to develop is independent of the GnRH analogue and the final oocyte maturation protocols used in the collection cycle

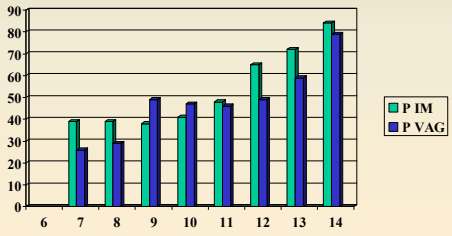
Natural cycles or programmed cycles for FET ?

- **Natural cycles:**
  - physiological process of endometrial preparation for implantation
  - decreased medical interventions
  - less expensive, ovulatory women
- **Hormonally programmed cycles- advantages:**
  - less monitoring
  - very low cancellation rate due to inadequate endometrial reaction, women with anovulatory, irregular or absent cycles

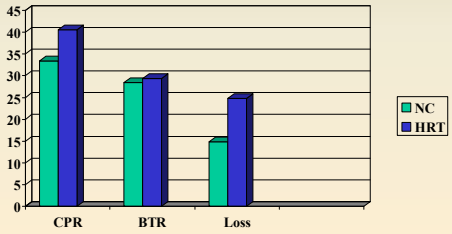
E2 and progesterone treatment in fully suplemented cycles and route of administration

		Follicular phase	Luteal phase	Early pregnancy
E 2	oral	4-8mg/day	4-8mg/day	6-8mg/day
	TD	0.2-0.4mg/day	0.2-0.4mg/day	0.2-0.6mg/day
P	IM.		100mg/day	100mg/day
	Vag.sup.		300-600mg/day	300-600mg/day
	Crinone		90mg/day	90mg/day

First trimester progesteron levels in agonadal pregnant women: i.m. (120mg daily) vers. vaginal (3x200mg) application



Outcomes of natural cycles versus programmed cycles for 1677 frozen-thawed transfers
Givens et al., RBMOnline 2009



Outcomes of natural cycles versus programmed cycles for 1677 frozen-thawed transfers
Givens et al., RBMOnline 2009

- There is no significant difference in LBR for FET in modified natural or programmed cycles

Cycle regimens for frozen-thawed embryo transfer

Ghobara.T.: Cochrane Database 2008 CD003414.

- There is insufficient evidence to support the use of one menstrual cycle regimen over another in frozen-thawed embryo transfer

What is the preferred method for timing natural cycle frozen-thawed embryo transfer?

Weissman et al., RBMOnline 2009

- A: 61 cycles (ovulation triggered with hCG)
- B: 71 cycles (no hCG, monitoring and detection of spontaneous ovulation)

What is the preferred method for timing natural cycle frozen-thawed embryo transfer?

Weissman et al., RBMOnline 2009

	hCG	No hCG
No. cycles	61	71
No. embryos	2,7	2,6
CPR/ET %	37	33,9
LBR/ET %	31,5	27,4
IR %	15,9	16,3

What is the preferred method for timing natural cycle frozen-thawed embryo transfer?
Weissman et al., RBMOnline 2009

- Triggering of ovulation with hCG is as efficient as serial monitoring until ovulation detection in patient preparation for non-stimulated FET

Cleavage stage versus blastocyst stage embryo transfer - metaanalytic studies -

- **Blake,D., Farquhar,C., Johnston,N. et al.: Cochrane Database of Systematic Reviews CD002118**
  (no significant difference between the two treatment groups in live birth rate, CI 0.80-3.15)
- **Papanikolaou E., Kolibianakis,E., Tournaye,H.: Hum. Reprod., 23, 2008, 91-99.**
  (Live birth rate is significantly higher when embryotransfer was delayed from day 2-3 to day 5)

Impact of developmental stage at cryopreservation and transfer on clinical outcome of frozen embryo cycles
(Noyes et al., RBMOnline 2009)

	No. cycles	CPR/ET %	LBR %
2PN CS	9	44	33
2PN BL	15	47	27
Day 2-3 CS	152	31	25
Day 2-3 BL	43	40	35
Day 5 BL	51	41	33
Day 6 BL	205	22	17

Impact of developmental stage at cryopreservation and transfer on clinical outcome of frozen embryo cycles
(Noyes et al., RBMOnline 2009)

- Although no single embryonic freezing stage is definitely superior in terms of outcome, it appears that day 5 blastocyst transfer may offer benefits when setting up a FET protocol

Cumulative live birth rates after transfer of cryopreserved ICSI embryos
Osmanagaoglu et al., RBM Online, 2004

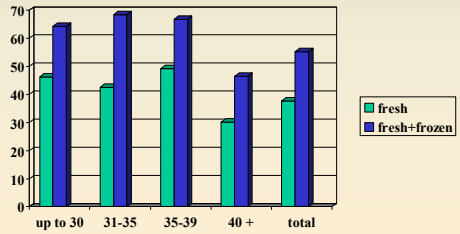
- 11.082 egg retrievals
- 9.963 fresh ET
- 4.587 cycles with at least one embryo frozen

- Overall birth rate in fresh ICSI cycles was 17%
- FET conferred additional 8% LBR / cycle

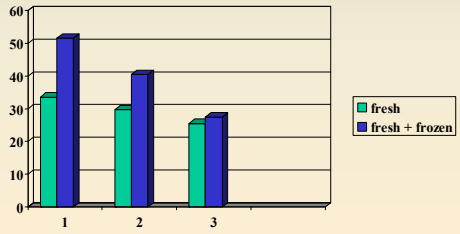
Cumulative live birth rates after transfer of cryopreserved ICSI embryos
Osmanagaoglu et al., RBM Online, 2004

- At least 8,3% live birth rate can be obtained from cryopreservation in addition to those ocurring from a fresh cycle

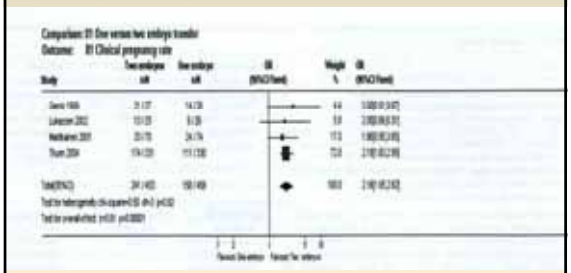
Cumulative pregnancy rates observed in 500 couples after 3years they have entered IVF programme (according to the age)
T.Mardesic, unpublised data



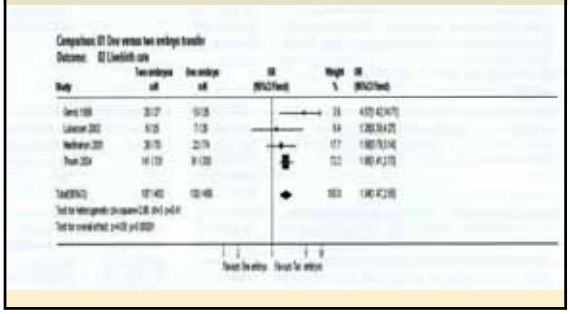
Cumulative pregnancy rates observed in 500 couples after 3years they have entered IVF programme (according to treatment cycle)
T.Mardesic, unpublished data



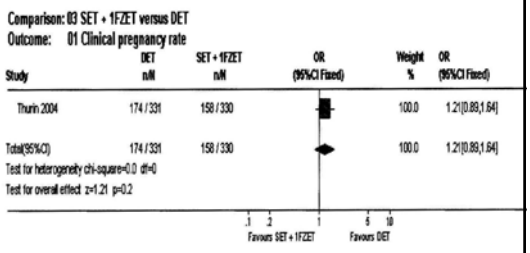
Number of embryos for transfer after IVF and ICSI:
A Cochrane review (**clinical pregnancy rate**)



Number of embryos for transfer after IVF and ICSI: A Cochrane review (**livebirth rate**)



Elective single embryo transfer + single frozen and thawed embryotransfer vers. elective double embryo transfer (**clinical pregnancy rate)**



eSET + FER = DET
(1+1 = 2)

- These data emphasize the importance of a well functioning cryopreservation programme.
- The addition of cryopreserved embryo results in similar cumulative live birth rates for SET and DET. By lowering the multiple birth rate dramatically the outcome for IVF children will improve.

Children born after cryopreservation of embryos: a systematic review of outcome data

Wennerholm et al., Hum.Reprod., 2009

- The health of children born after ART has always been of concern.
- A growing proportion of the children born after ART are now born after cryopreservation.

Children born after cryopreservation of embryos : a systematic review of outcome data

(early cleavage stage embryos)

Wennerholm et al., Hum.Reprod., 2009

Cryo n=11.000 Fresh n= 37.000	Cryo singletons	Fresh IVF/ICSI singletons
Preterm birth	9,2-12,0%	7,4-14.0%
	Cryo twins	**Fresh IVF/ICSI twins**
	33-62%	48-61%

Children born after cryopreservation of embryos : a systematic review of outcome data

(early cleavage stage embryos)

Wennerholm et al., Hum.Reprod., 2009

Cryo n=11.000 Fresh n= 37.000	Cryo singletons	Fresh IVF/ICSI singletons
Low birth weight	6,2 – 10,5%	7,2-13,6%
	Cryo twins	**Fresh IVF/ICSI twins**
	38-50%	45-56%

Children born after cryopreservation of embryos : a systematic review of outcome data
(early cleavage stage embryos)
Wennerholm et al., Hum.Reprod., 2009

	Cryo singletons + twins	Fresh IVF/ICSI singletons + twins
Birth defects and chromosomal abnormalities	0,7 – 8,6%	0,7 – 8,7%

Children born after cryopreservation of embryos : a systematic review of outcome data
(vitrification of blastocysts)
Wennerholm et al., Hum.Reprod., 2009

- Four retrospective studies and four case reports have presented information on 252 children born after vitrification of blastocysts
- No statistical differences in the mean gestational age, birthweight, preterm birth rate or congenital birth defect rates as compared with fresh blastocyst transfer

Conclusion (1)

- Successful freezing and thawing of embryos along with tailored patient preparation schemes can enhance significantly the cumulative pregnancy rates from an oocyte retrieval

Conclusion (2)

- The potential to implant and to develop is independent of the GnRH analogue and the final oocyte maturation protocols used in the collection cycle
- There is no conclusive evidence that the stage of development at the time of freezing provides a clear advantage for the outcome of FER

Conclusion (3)

- There is insufficient evidence to support the use of one menstrual cycle regimen over another in frozen-thawed embryo transfer

Conclusion (4)

- For early cleavage embryos at least as good obstetric outcome for cryo children as compared with children after fresh cycles is documented
- No differences in malformation rates were found between cryo children and children from fresh transfers
- For newly techniques (vitrification) very limited data have been reported on obstetric and neonatal outcomes so far

Conclusion (5)

- Slow freezing of embryos has been used for 25 years and all the data available seems reassuring.
- With new techniques emerging new informations and strategies for the preservation of human gametes and embryos are critical to the expansion of safe and effective clinical services