In vitro oocyte maturation: towards an improvement?

Michel De Vos, MD PhD
UZ Brussel, Belgium

Universitair Ziekenhuis Brussel

QuickTime™ and a
are needed to see this picture.

Presentation lay-out

- Indications for IVM
- IVM today
- IVM tomorrow

Advantages of IVM as compared to conventional IVF

patient-friendly

- OHSS avoided
- Simplified protocol
- Reduced cost - reduced number of visits
- Drug related side effects avoided (weight gain, bloating, breast tenderness, nausea, mood swings, long-term risks)

Follicular development

Stage	Follicle size (cm)
IVM	

Gougeon, Hum Reprod 1986

Indications

- PCOS
- IVM + vitrification
  - fertility preservation
  - < law restrictions (Italy)
- (low responders/ POI)
- (egg donation)

Lower success rate than conventional IVF ...

- asynchronous maturation of the oocyte
  - cytoplasmic maturation
  - nuclear maturation
- endometrium out of phase
- lower final number of matured oocytes

... but catching up

		IVM 2008	
		<35	
		35%	
		22%	

Chian-Tan protocol, Mc Gill, Montreal, Canada

No. ET IVM = no. ET IVF + 1

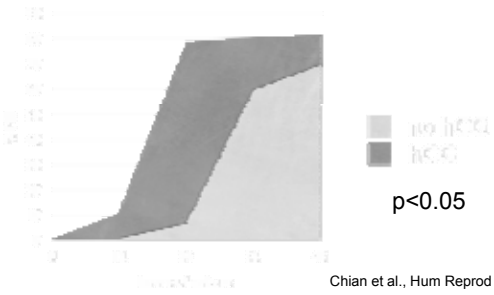
How to improve IVM results?

- optimise the Chian-Tan protocol
- optimise endometrial receptivity
- improve oocyte quality

How to improve IVM results?

- optimise the Chian-Tan protocol
- optimise endometrial receptivity
- improve oocyte quality

hCG priming



p<0.05

Chian et al., Hum Reprod 2000

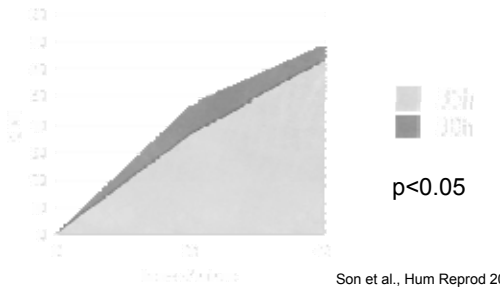
hCG priming

	hCG	no hCG	
Oocytes matured (%)	84,3	69,1	p<0,05

Chian et al., Hum Reprod 2000

However, hCG is disruptive at cumulus-oocyte interface

Adjusting egg retrieval time



p<0.05

Son et al., Hum Reprod 2008

Adjusting egg retrieval time
Diameter of dominant follicle at time of hCG

	<10mm	10-14mm		
	36,0	41,7		
	15,3	14,5		

Son et al., Hum Reprod 2008

dominance induced atresia
(Chian et al., Fertil Steril 2004)

FSH priming

- To increase the number of oocytes?
- To increase the number of MII oocytes?
- To improve endometrium quality?
- To make egg retrieval easier?

FSH priming previous studies

Wynn et al.
Hum Reprod 1998
FSH vs. no FSH
→ efficient in normal ovaries → maturation rate

Mikkelsen et al.
Hum Reprod 2001
FSH vs. no FSH
→ efficient in PCO(S) → maturation rate / implantation rate / CPR

Lin et al.
Hum Reprod 2003
FSH + hCG vs. hCG
→ no benefit in PCO(S)

FSH priming recent studies

Elizur et al.,
Fertil Steril 2008
FSH + hCG vs. E2 + hCG → efficient when endometrial thickness < 6 mm → maturation implantation? CPR?

Fadini et al.
RBM Online 2008
nothing vs. hCG vs. FSH vs. FSH + hCG → in normal ovaries →

The effect of different gonadotrophin priming on in-vitro maturation of oocytes in women with normal ovaries: a prospective randomized study

Fadini et al., RBM Online 2008

				82,0%

FSH enhances the effect of hCG on maturation

The effect of different gonadotrophin priming on in-vitro maturation of oocytes in women with normal ovaries: a prospective randomized study

Fadini et al., RBM Online 2008

		7,6		29,8
		4,0		16,3

Priming in IVM: current recommendations

PCO(S)	⟶	hCG FSH priming
normal ovaries	⟶	no priming or FSH + hCG priming

How to improve IVM results?

- optimise the Chian-Tan protocol
- optimise endometrial receptivity
- improve oocyte quality

Optimise endometrial receptivity

Endometrial thickness >10 mm predictor of pregnancy (Child, Fertil Steril 2003)

Preliminary study of endometrial development:
Lindenberg, pers. communication
biopsy 7 days after egg retrieval
Results:
9 biopsies analysed
2 biopsies in phase
7 biopsies out of phase

Need for larger study

Optimise endometrial receptivity

IVM	→	ICSI + ET in same cycle
	vs	
IVM + vitrification	→	ICSI + ET in natural cycle
	vs	
IVM + ICSI + embryo vitrification	→	ET in natural cycle

need for studies

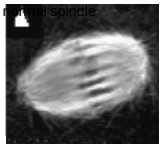
Developmental potential of embryos after IVM

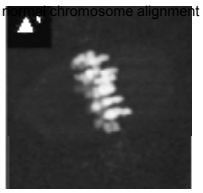
- aneuploidy?
- spindle ultrastructure?
- epigenetics?

Aneuploidy?

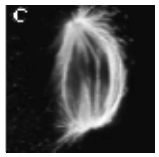
- Abnormalities of chromosome segregation in IVM of horse and pig oocytes (Sosnowskiet al., Theriogenology 2003)
- Higher proportion of spindle abnormalities in oocytes matured in vitro (43.7%) than in oocytes matured in vivo (13.6%)(Li et al., Fertil Steril 2006)
- No significant difference of spindle organization, chromosomal alignment and aneuploidy between in vivo and in vitro matured oocytes in mice. (Xu et al., Systems biology in reproductive medicine 2008)
- Obstetric outcomes after IVM cycles are comparable to those after IVF/ICSI (Buckett et al., Obstet Gynecol 2007)

Confocal imaging of mouse MII spindles and chromosomes





disorganised spindle



chaotic chromosome alignment

Aneuploidy?

- Abnormalities of chromosome segregation in IVM of horse and pig oocytes (Sosnowskiet al., Theriogenology 2003)
- Higher proportion of spindle abnormalities in oocytes matured in vitro (43.7%) than in oocytes matured in vivo (13.6%)(Li et al., Fertil Steril 2006)
- No significant difference of spindle organization, chromosomal alignment and aneuploidy between in vivo and in vitro matured oocytes in mice. (Xu et al., Systems biology in reproductive medicine 2008)
- Obstetric outcomes after IVM cycles are comparable to those after IVF/ICSI (Buckett et al., Obstet Gynecol 2007)

Aneuploidy?

- IVM does not lead to increased incidence of aneuploid oocytes (Chian, unpublished)
- lower developmental potential results from DNA fragmentation (TUNEL assay) (Gianaroli, unpublished)

Epigenetics?

- Dynamic CpG methylation of the KCNQ1OT1 gene during maturation of human oocytes (Khoureiry et al., J Med Genet 2008)
  - About 60% of alleles were fully methylated in GV oocytes and that full imprint is acquired in most MII oocytes. Similarly to in vivo, de novo methylation of DNA occurred in vitro during oocyte maturation.

How to improve IVM results?

- optimise the Chian-Tan protocol
- optimise endometrial receptivity
- improve oocyte quality

How to improve oocyte quality in IVM?

Synchronise

- Cytoplasmic maturation
- Nuclear maturation

Preserve

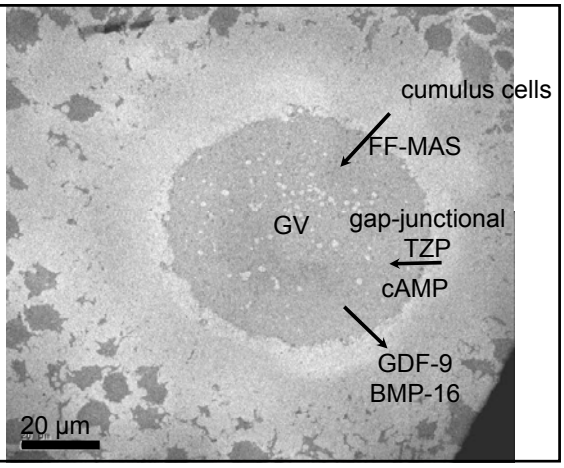
- oocyte - granulosa cell connection

Nuclear maturation

- Oocytes resume meiosis once removed from the follicle (extrusion of first polar body)
- In vivo: GV to MII only takes 24 hours after LH surge - cytoplasmic maturation takes longer

Cytoplasmic maturation

- Developing competence to regulate fertilisation
- Growth of organelles, accumulation of RNA and proteins to build up energy for downstream embryonic development
- Enable communication between cumulus/granulosa cell and oocyte (gap junctions): transzonal connection



How to synchronise nuclear and cytoplasmic maturation in IVM?

Add

- Maturation enhancers
- Meiosis inhibitors

to the culture medium

Maturation enhancers

- FSH, LH, IGF-1, BMP, GDF9, GH, FGF, statins, neurotrophin
- FF-MAS (Follicular Fluid Activating Sterol)
- LIF/IL6 (Leukemia Inhibiting Factor/Interleukin 6)
- VEGF (Vascular Endothelial Growth Factor)
- EGF (Epidermal Growth Factor)
- other

Problems with proprietary issues (patents)

How to synchronise nuclear and cytoplasmic maturation in IVM?

Add

- Maturation enhancers
- Meiosis inhibitors

to the culture medium

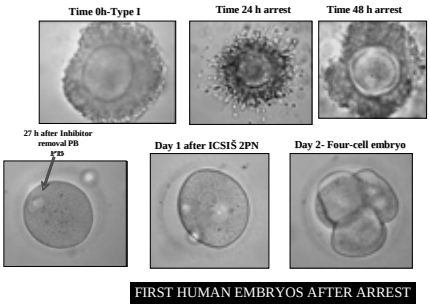
Keep the oocyte meiotically arrested

- High levels of cAMP (analogues) prevent meiotic resumption (Dekel, Ann NY Acad Sci 1988)
- Cilostamide (Phosphodiesterase 3-inhibitor) (Tsafriri et al., Dev Biol 1996)
- Pre-maturation system (PMC)

BIOLOGY OF REPRODUCTION 74, 177–184 (2006)
Published online before print 5 October 2005.
DOI 10.1095/biolreprod.105.040485

Meiotic Arrest In Vitro by Phosphodiesterase 3-Inhibitor Enhances Maturation Capacity of Human Oocytes and Allows Subsequent Embryonic Development[1]

D. Nogueira,[2,3] R. Ron-El,[4] S. Friedler,[4] M. Schachter,[4] A. Raziel,[4] R. Cortvrindt,[3] and J. Smitz[3]



FIRST HUMAN EMBRYOS AFTER ARREST

Temporary inhibition of meiosis

Evidence that maturation progresses :

- during butyrolactone-1 inhibition:
  - continued RNA synthesis (Pavlok, 2000)
  - RNA transcription ongoing (Sui et al., 2005)
- during PDE3 A inhibition:

GV chromatin undergoes NSN - SN transformation (Nogueira et al., Biol Reprod 2003)

Effect of PMC on fertilisation and embryonic developmental rates in mice

Cilostamide		52,3		57,3
		20,6		50,8
		81,8		85,0

Vanhoutte et al., Mol Reprod Dev 2008

Keep the oocyte meiotically arrested

- High levels of cAMP (analogues) prevent meiotic resumption (Dekel, Ann NY Acad Sci 1988)
- Pre-maturation system (PMC)
- Cilostamide (Tsafriri et al., Dev Biol 1996)
- Forskolin (adenylate cyclase activator) + PDE3-I improves fertilisation (Shu et al., Hum Reprod 2008)

Maintain transzonal connection between granulosa cells and oocyte

- Three-dimensional culture + PDE3-I
  - Development beyond the blastocyst stage is lower in cumulus-free oocytes compared to cumulus-intact counterparts (Ali et al., Reprod Biomed Online 2006)
  - Improved fertilisation and embryonic development in rescue IVM in a three-dimensional co-culture system (Vanhoutte et al., Hum Reprod 2008)

Conclusions/ Future prospects

- In most centres, IVM will not replace IVF
- Valuable technique in selected patients
- Focus on improvement of culture media
  - more competent MII oocytes
  - embryos with higher implantation potential
- Focus on endometrium
- IVM + vitrification = promising

Acknowledgements

Johan Smitz MD PhD, Laboratory of Follicular Biology
Paul Devroey MD PhD, Centre for Reproductive Medicine
Josiane Van der Elst MD PhD, Centre for Reproductive Medicine
Dominic Stoop MD PhD, Centre for Reproductive Medicine
Claire Bourgain MD PhD, Dept. of Histopathology
Catherine Staessen, Dept. of Cytogenetics
Sandy Lenie BSc, Laboratory of Follicular Biology
Tom Adriaenssens BSc, Laboratory of Follicular Biology