Standardisation of embryo evaluation

Ronny Janssens

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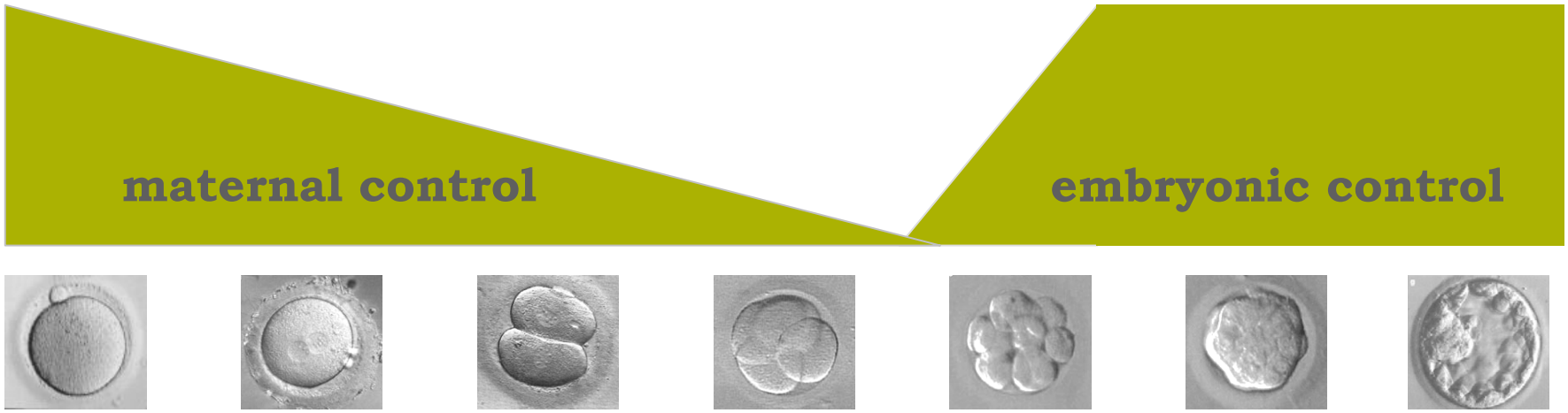
Outline

1. Principles of embryo evaluation
2. Standardization: how?
3. Standardization: why?
4. The future

Normal embryo development



Switch embryonic genome
Monk Oncogene 20, 8085-8091
Cauffman Mol Hum Reprod 2005,11:173-181
Cauffman Mol Hum Reprod 2005,11:405-411

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Embryo evaluation

- Day 1:
  - Fertilisation: scoring of zygotes
  - Early first cleavage
- Day 2-3:
  - Scoring system for multicellular embryos and early compaction
- Day 4:
  - Scoring system for compact embryos
- Day 5-6:
  - Blastocyst scoring system

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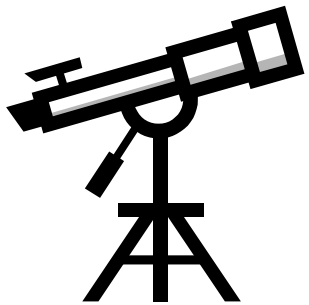
The microscope

- Inverted microscope with high magnification (200 – 400 x) power
- High quality optics
- Hoffman Modulation Contrast
- Heated stage
- Regular maintenance to preserve optical quality



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Embryo evaluation

- Not time consuming
- Non invasive
- Individual traceability
- Frequent - at specific time points

- No deterioration to the embryo
- Exposure to ambient air – pH?

- Parameters highly discriminating
- Grading and selection of the best embryo

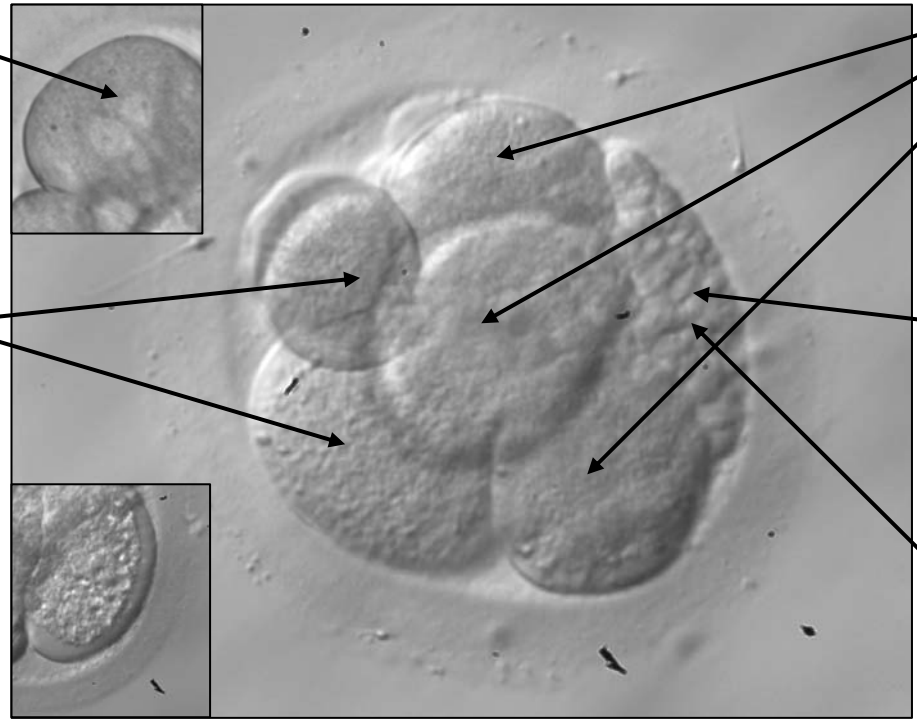
Parameters of Embryo Assessment

Cleavage stage (number of cells)

0,1,2,3,4,5,6,7,8…….

Visual sign of multinucleation

- ***Yes***
- ***No***



Degree of fragmentation

- ***0%***
- ***<10%***
- ***11-20%***
- ***21-50%***
- ***>50%***
- ***Totally fragmented***

Blastomere uniformity

- ***Equal***
- ***Unequal (>25% difference)***

Cytoplasmic appearance

- ***Homogeneous***
- ***Dark, granulated, vacuolated***

Localisation of fragments

- ***Local***
- ***Dispersed***

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Parameters of blastocyst scoring Gardner and Schoolcraft (1998)

Expansion

Bl 1 - <50%

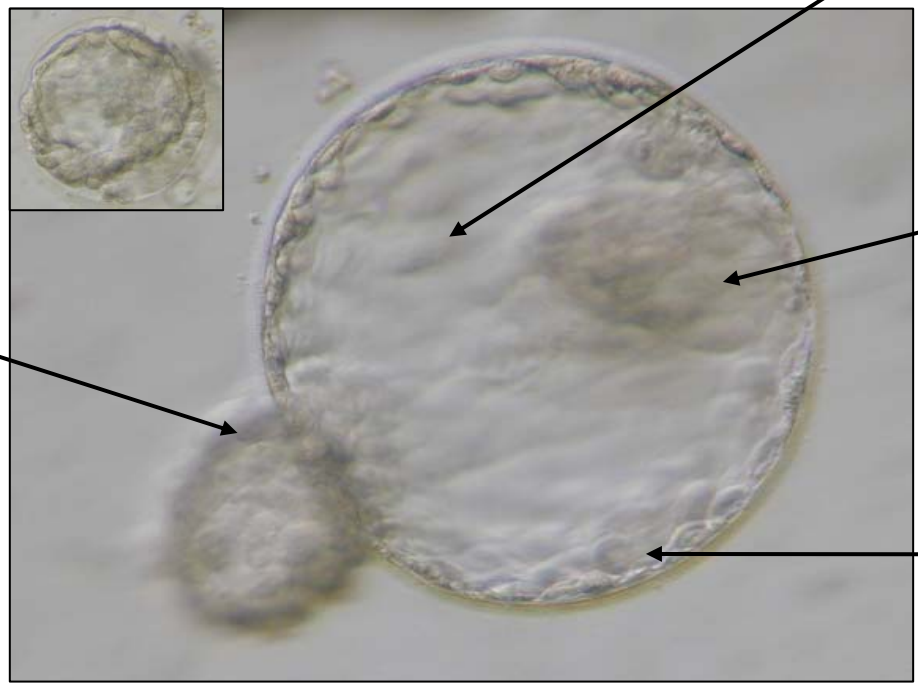
Bl 2 - >50%

Bl 3 - full

Bl 4 - expanded

Collapsed

- ***Bl 8***



Inner cell mass

- ***A***
- ***B***
- ***C***
- ***D***

Hatching

- ***Bl 5 - hatching***
- ***Bl 6 - hatched***
- ***Bl 7 - artificial***

Trophectoderm

- ***A***
- ***B***
- ***D***

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Predictors of embryoquality

- oocyte and zygote morphology, e.g. appearance of the cytoplasm, pronuclei and polar bodies (Scott and Smith, 1998; Ebner et al., 2000)
- Early first cleavage (Shoukir et al., 1997; Sakkas et al., 1998; Lundin et al., 2001; Salumets et al., 2003; Van Montfoort et al., 2004).
- grade of fragmentation, blastomere size, multinucleation, cytoplasmic appearance and embryo cleavage rate (Puissant et al., 1987; Steer et al., 1992; Pelinick et al., 1998; Van Royen et al., 1999, 2001; Hardarson et al., 2001, Ziebe et al., 1997; Van Royen et al., 2001).

Top quality embryo

- Characterisation of a top quality embryo, a step towards single-embryo transfer. *Hum. Reprod*., **14**, 2345–2349. Van Royen, E et al.

Top quality

- 4 or 5 cells (day 2)
- ≥ 7 cells (day 3)
- ≤ 20% fragmentation (day 3)
- equally sized blastomeres (day 3)
- no sign of multi-nucleation (ever)

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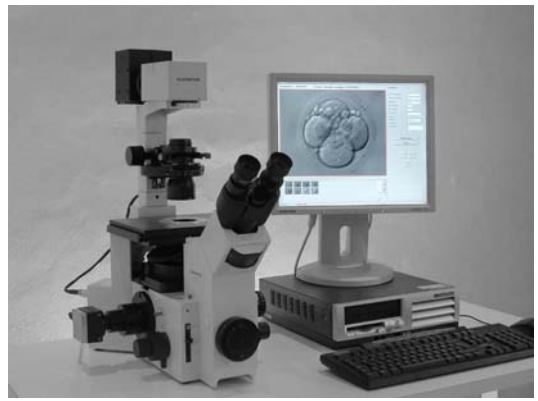
Standardisation: how?

- Training staff – monitor staff performance
- Clear and simple scoring system
- Respect timing
- Interpersonal tuning - embryo evaluation x 2
- Suitable equipment – video – digital imaging
- IQC
- Participate in EQC - PT



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Proficiency Testing (ISO Guide 43: 1997)

- *Determination of laboratory testing performance by means of interlaboratory comparisons*

  → *Qualitative schemes - for example where laboratories are required to identify a component of a test item.*

  → *Data transformation exercises - for example where laboratories are furnished with sets of data and are required to manipulate the data to provide further information.*

Interlaboratory comparisons - ISO Guide 43: 1997

- *Organisation, performance and evaluation of test on the same or similar test items by two or more laboratories in accordance with pre-determined conditions*

- There are specific areas where no interlaboratory comparisons are available. In these circumstances, the laboratory shall use other methods to prove its technical competence such as repeating a test with the same or an other method.

Proficiency Testing

- Competence of organisator : accreditation or according to ISO/IEC 43
- Type of material: as close as possible to the reality
- Parameters: as much as possible or those used in the laboratory
- Range: as close a possible to the reality in the laboratory (zygote – embryo – blastocyst)
- Frequency : high enough to meet the laboratory needs and efficient to manage the quality of the results
- Statistical analysis: clearly defined

QAP online - EQC

- Internet based Quality Assessment system QAP-online (WWW.fertaid.com)
- Subscription based
- Schemes for andrology – embryology – follicular measurements…
- Monthly series of questions
- Images or videos, participants are asked to score and rank embryos

- Anonymous - independent
- Report generator - statistics

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QAP online QA schemes

- Andrology
  - → Concentration
  - → Motility
  - → Morphology (WHO & Strict)
  - → Antisperm Antibodies
- Embryology
  - → Pronuclear
  - → Fragmentation
  - → Early cleavage
  - → Late cleavage
- Ultrasound
  - → Follicle Size
  - → Endometrial thickness

QAP online

Code: HEF2006.02-1 - [Title:Single Human Embryo] Released:2/2006 Page Speed= FAST

QAP Comment:"Assess as a Day 5 embryo -

What stage of blastocyst formation is evident from the image? Select Question

Your QAP Supervisor requires you to reply to the questions marked with an *. The remainder may be replied to at your discretion.

1.	QHE013	What stage of blastocyst formation is evident from the image?
2.	QHE011	Describe the appearance of the inner cell mass
3.	QHE053	Identify the location of the inner cell mass.
4.	QHE012	Describe the appearance of the trophectoderm
5.	QHE016	Is the rate of cleavage normal?
6*.	QHEBISc1	QAP What is the embryo score for this blastocyst
7.	QHE040	How would you describe this embryo?

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Why?

- **Proficiency testing remains one of the most transparent methods of demonstrating competence, with little opportunity to falsify or manipulate results.**
- **To meet certification or accreditation requirements (ISO 9001:2000 or ISO 15189:2002)**

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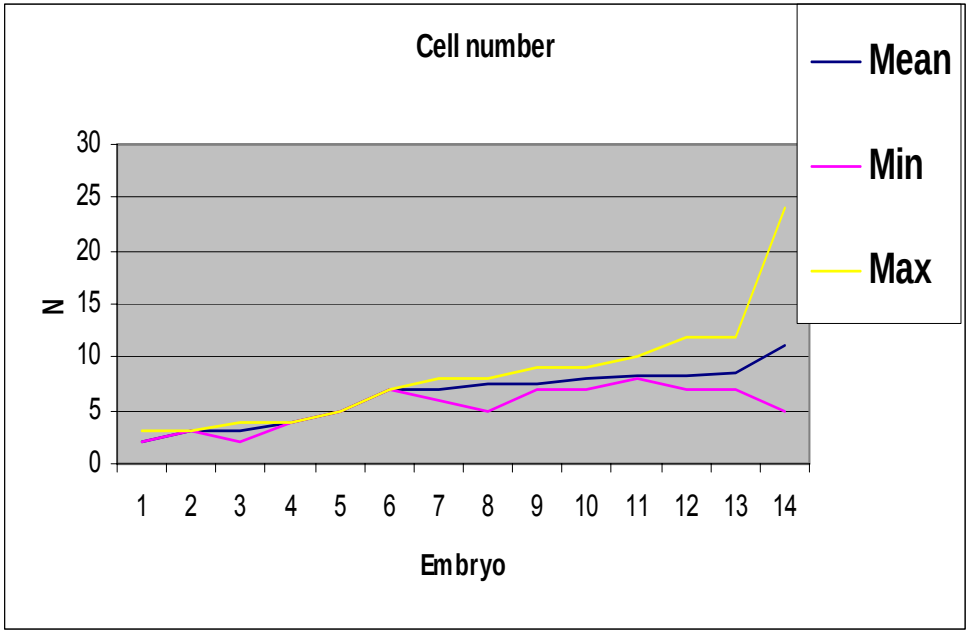
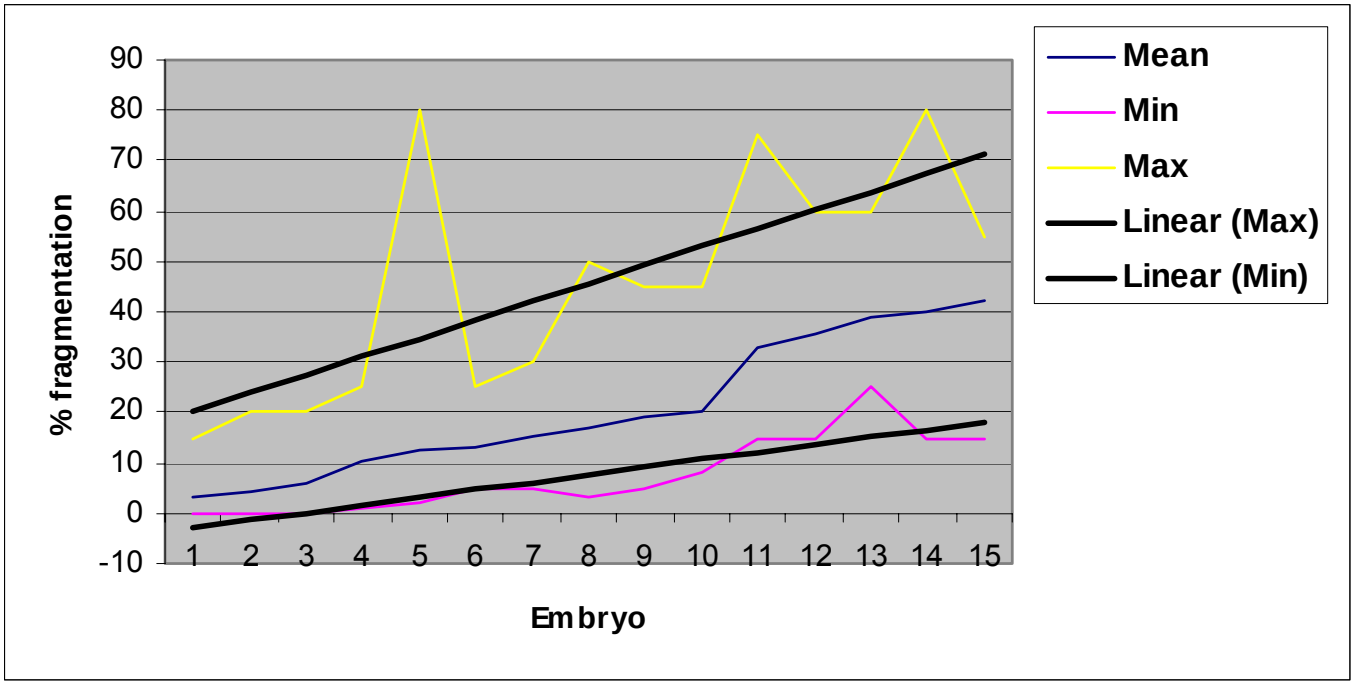
Cell number – QAP online 2004



- High agreement if less than 7 blastomeres
- SD on 8 cells = 0.75 blastomeres

The poorer the quality (fragmentation), the more variable the assessment.

Fragmentation – QAP online 2004



1. <10% fragmentation: SD 4.2% (range 0 – 20%)
2. 10-20% of fragmentation: SD 7.8% (average difference between minimum and maximum = 40%)

Blastomere size – QAP online 2004

How even are the size of the blastomeres?

Mr. Ronny Janssens	Question: How even are the size of the blastomeres.
AZ-VUB	Scheme: Early Human Embryology QAP-2004; Embryology HEE2004 -[53]
15/01/2005- Remove menu	Report #1 Individual Summary - Data:Professional

The List below contains your reply to the selected question when it appeared in an Issue for QAP Scheme HEE2004 -[53]. The % agreement indicates the incidence other subscribers choose the same option from subcsribers actively involved in the subject of the Scheme . This will change as the number of replies increases.

[HEE2004.01-3]	The blastomeres differ only marginally in size % Agreement= 67.44% -[29/43]	
[HEE2004.02-1]	The blastomeres differ significantly in size % Agreement= 95.74% -[45/47]	
[HEE2004.03-1]	The blastomeres differ only marginally in size % Agreement= 65.12% -[28/43]	
[HEE2004.04-1]	The blastomeres appear of equal size % Agreement= 55.00% -[22/40]	
[HEE2004.04-3]	The blastomeres differ only marginally in size % Agreement= 58.54% -[24/41]	
[HEE2004.05-1]	The blastomeres differ only marginally in size % Agreement= 44.19% -[19/43]	
[HEE2004.06-1]	The blastomeres differ only marginally in size % Agreement= 80.49% -[33/41]	
[HEE2004.07-1]	The blastomeres differ significantly in size % Agreement= 7.69% -[3/39]	
[HEE2004.08-1]	The blastomeres differ significantly in size % Agreement= 92.50% -[37/40]	
[HEE2004.09-1]	The blastomeres differ only marginally in size % Agreement= 83.78% -[31/37]	
[HEE2004.10-1]	The blastomeres differ only marginally in size % Agreement= 81.82% -[27/33]	
[HEE2004.11-1]	The blastomeres appear of equal size % Agreement= 58.06% -[18/31]	
[HEE2004.12-1]	The blastomeres differ significantly in size % Agreement= 81.82% -[18/22]	
[HEE2004.12-3]	The blastomeres differ significantly in size % Agreement= 16.67% -[2/12]	
Summary	% Agreement= 65.63% -[336/512] Date:15/01/2005	

65.6% agreement

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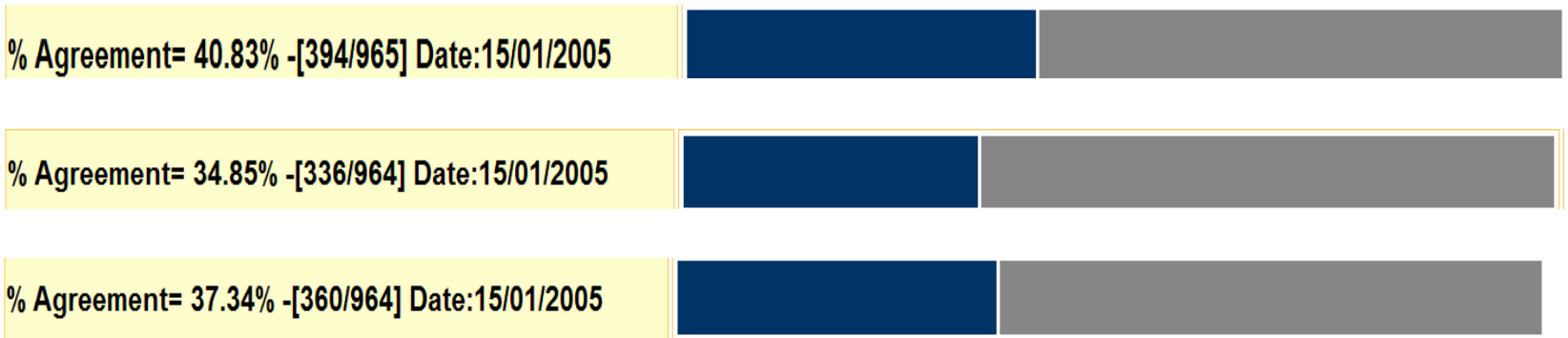
Blastocyst scoring – QAP online 2004

- blastocyst stage 40.8 % agreement
- ICM 34.8 % agreement
- TE 37.5 % agreement



Embryo scoring – conclusions

- Embryo scoring and selection is highly variable
  - → Semi quantitative
  - → No reference – standard

  - → EQC: different scoring parameters as used in daily practice?
  - → Different selection rules?
  - → Other way of visualization?
- At regular intervals embryologist performance should be evaluated statistically

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UZ Brussels – Fragmentation 2004 (QAP)

Confidential Report: This Report should be considered confidential by your laboratory.

QAPonline Report: AZ-VUB [Attention:Mr. Ronny Janssens] **Date:5/02/2005**

EXTERNAL QAP: Year to Date Printed on: **5/02/2005**

Laboratory:IVF laboratory AZ-VUB - Lab Number: 25

Scheme - HEC2004-Embryo Fragmentation-2004

Key QAP Question: What is the % fragmentation in this embryo.

Plot Type= // Data:Professional

Scheme:HEC2004	Embryo Fragmentation-2004		Print Report
Name	Company	#QAP/Total QAP	
[redacted]	AZ-VUB	4/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for [redacted]			
Total . -3SD . . . . . -2SD . . . . . 1 1 . . 1 . . . . . X . . . . . 1 . . . . . +2SD . . . . . +3SD .			
-Janssens, Ronny	AZ-VUB	15/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for Ronny Janssens			
Total . -3SD . . . . . -2SD . . . . . 1 . . -1SD . . . 2 . . 4 . 1 X 2 2 . . . . . 1 +1SD . 1 . . . . . 1 . . . . . +3SD .			
[redacted]	AZ-VUB	9/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for [redacted]			
Total . -3SD . . . . . -2SD . . . . . 2 . . . . . X . . 1 . . 1 . . . 1 . 2 1 . . . . . +2SD . . . . . +3SD 1			
[redacted]	AZ-VUB	11/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for [redacted]			
Total . -3SD . . . . . -2SD . . . . . 1 2 . 2 . . 1 2 . . X . . . . . 1 . . . +1SD . 1 1 . . . . . +2SD . . . . . +3SD .			
[redacted]	AZ-VUB	15/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for [redacted]			
Total . -3SD . . . . . -2SD . . . . . -1SD . . 1 . . . . 1 1 X . . . . . 2 1 . . 2 . 1 1 . . 1 . . . 1 . 1 . . . . . 1 1			
[redacted]	AZ-VUB	0/15	Lab: IVF laboratory AZ-VUB
[redacted]	AZ-VUB	15/15	Lab: IVF laboratory AZ-VUB
HEC2004:Embryo Fragmentation-2004 - Results for [redacted]			
Total . -3SD . . . . . -2SD . . . . . 1 . . 2 1 . 1 . . 1 . . . X 2 . 1 . . 1 . 1 . +1SD . . 2 . 1 1 . . . +2SD . . . . . +3SD .			

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UZ Brussels – Blastocysts 2004 (QAP)

EXTERNAL QAP: Year to Date Printed on: **5/02/2005**

Laboratory:IVF laboratory AZ-VUB - Lab Number: 25

Scheme - HEF2004-Blastocyst QAP - 2004

Key QAP Question: What is the embryo score for this blastocyst

Plot Type= // Data:Professional

Scheme:HEF2004	Blastocyst QAP - 2004		Print Report
Name	Company	#QAP/Total QAP	
-Janssens, Ronny	AZ-VUB	28/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for Ronny Janssens

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
		1 1 . 2 . . 2 . .	1 1 1 4 1 2 2 . 1 3 . . . . . 2 . .	1 1 1 1		

[redacted]	AZ-VUB	14/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for [redacted]

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
		1	1 . 1 . 1 . 2 . 1 2 . 1 . 2 . . 1	1 . .		

[redacted]	AZ-VUB	21/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for [redacted]

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
		1 1	1 1 1 . . . . 2 1 3 3 3 . . . . 2 1 1	+1SD		

[redacted]	AZ-VUB	30/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for [redacted]

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
1		. . . . . 1 2 1 .	1 2 . . . 2 2 . 1 4 6 1 1 1 . . 1 . .	. 1 . . 1 . .		

[redacted]	AZ-VUB	2/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for [redacted]

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
			. . . . 1 . . . . X . . 1	. . . . .		

[redacted]	AZ-VUB	28/30	Lab: IVF laboratory AZ-VUB

HEF2004:Blastocyst QAP - 2004 - Results for [redacted]

Total	-3SD	-2SD	-1SD	+1SD	+2SD	+3SD
1	1 . 1 1	. . 2 1 . 1 2 1 2	2 . 3 2 1 2 3 . 1 X 1	. . . . .		

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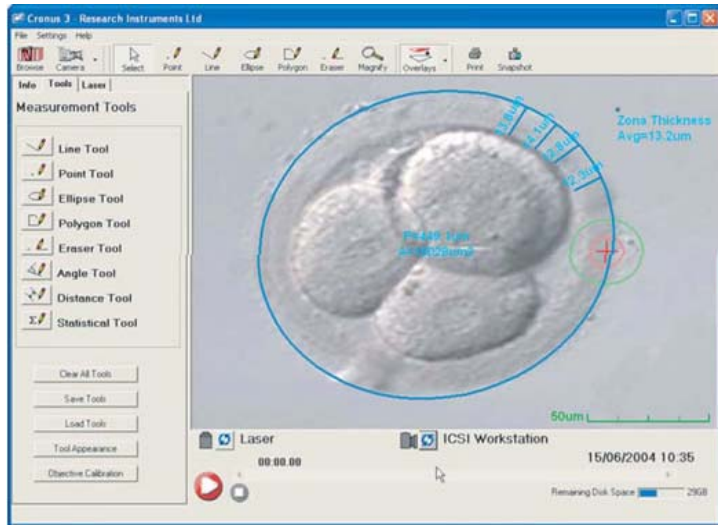
Digital imaging

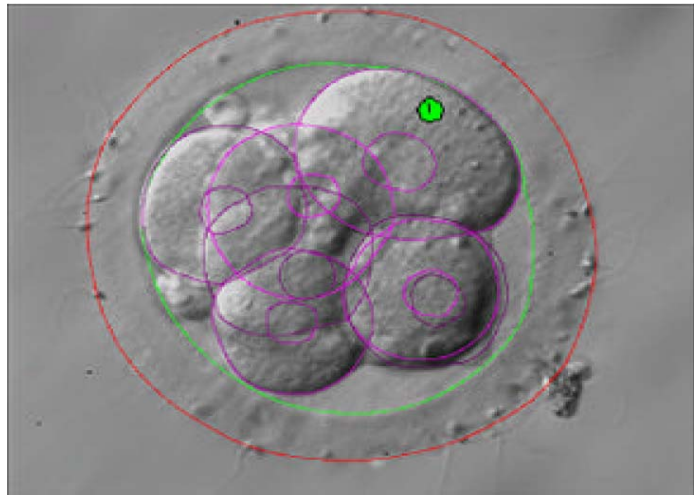
- Imaging software
  - → Cronus
  - → Fertigrab/Fertimorph
  - → …
- Live cell imaging
  - → Time lapse microscopy
  - → Embryoguard
  - → Biostation

Imaging software - Fertimorph

- Microscopic embryo monitoring
- Digital image (2D – 3D)
- Embryo Analysis
- IVF Database Integration

- Remote access
- Quality Assurance
- Ideal tool for training and standardization





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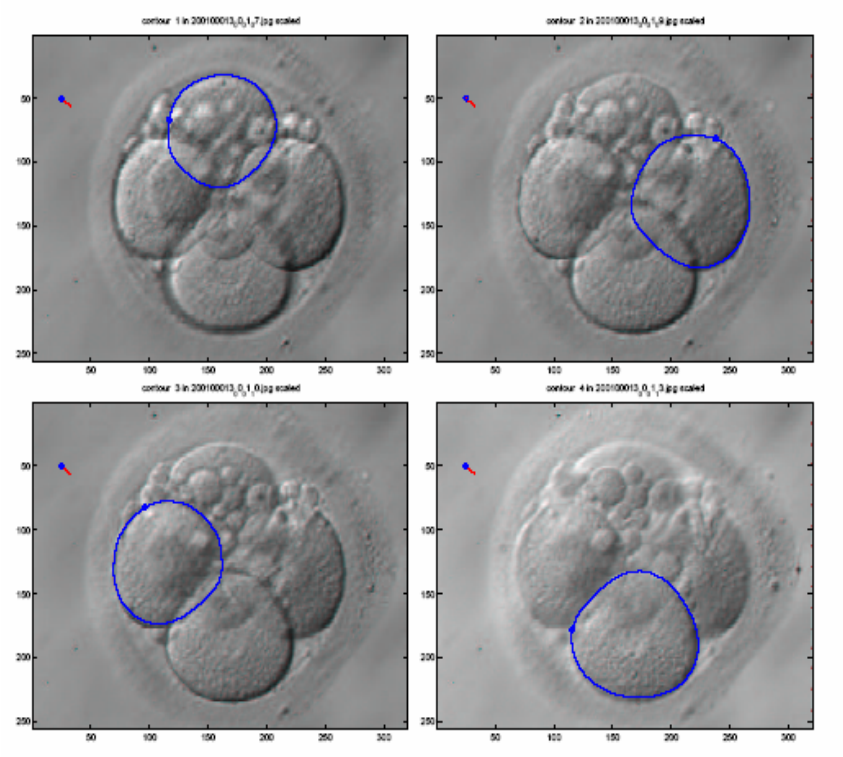
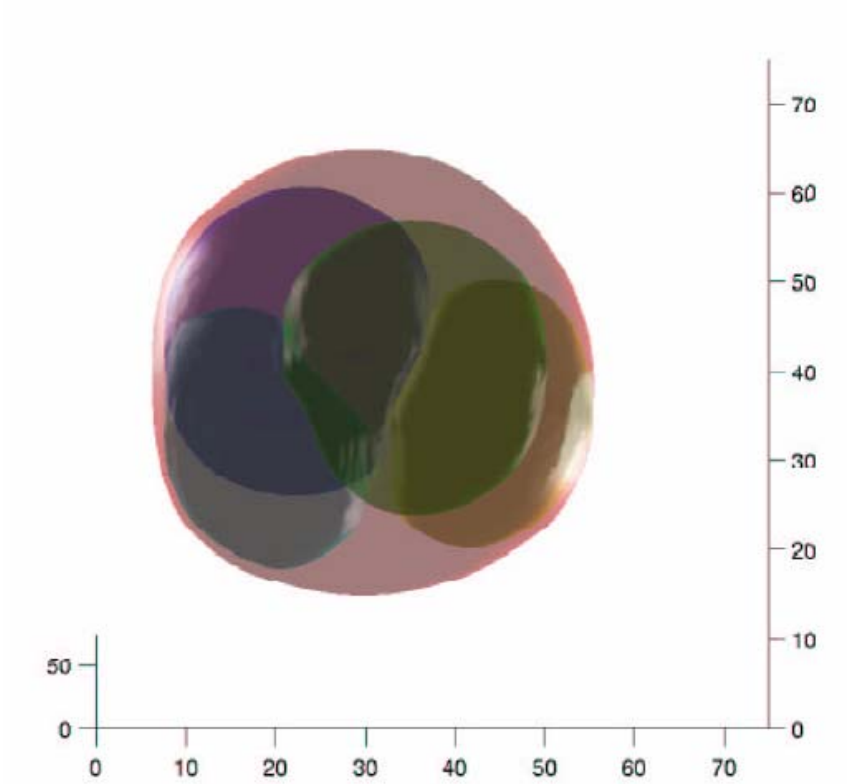
Software

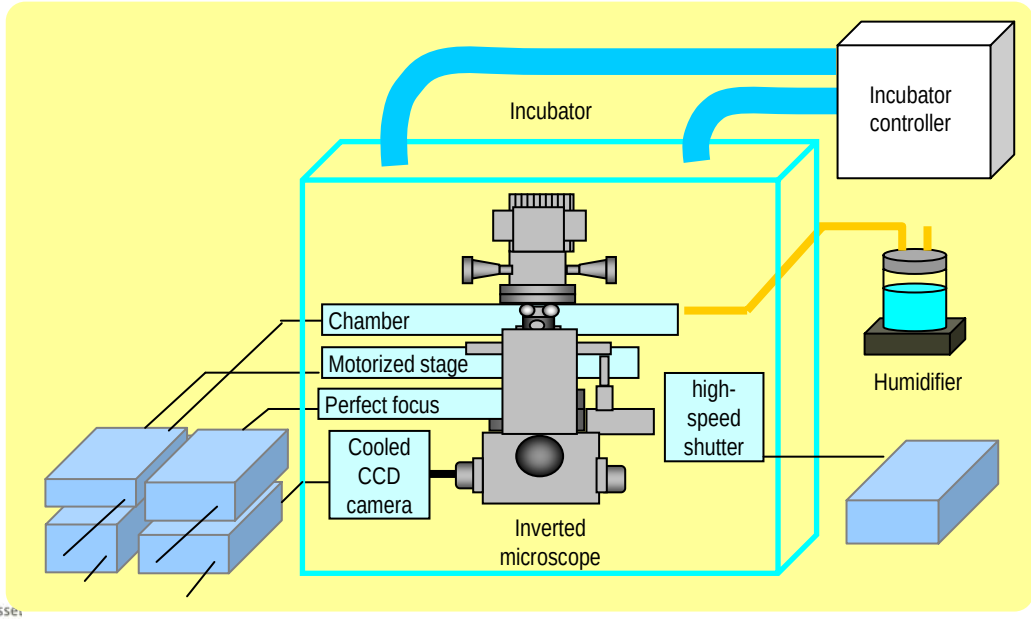




From Pedersen U, Olsen N, A multiphase variational level set approach for modelling human embryos

Live cell imaging

- Microscope installed inside an incubator
- Avoids the need to open the incubator for periodic check-up, exposing the embryos to different environmental conditions (different temperature, humidity, etc.).
- On-line monitoring of the embryos and selection of embryos by cleavage timing



Time-lapse photography

- Cinematography technique whereby each frame is captured at a rate much slower than it will be played back. When replayed at normal speed, time appears to be moving faster and thus lapsing.
- Processes that would normally appear subtle to the human eye become very pronounced.

- Limited capacity
- Single cell analysis

- **Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography**
  Payne et al. Human Reproduction vol.12 no.3 pp.532–541, 1997

- **Internalization of cellular fragments in a human embryo: time-lapse recordings.**
  Hardarson et al. Reprod Biomed Online. 2002 Jul-Aug;5(1):36-8

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Time lapse photography

30 frames/hour

15 frames/second

1h = 2 seconds

The near (?) future

CO2 incubator

Clean Bench

Microscope

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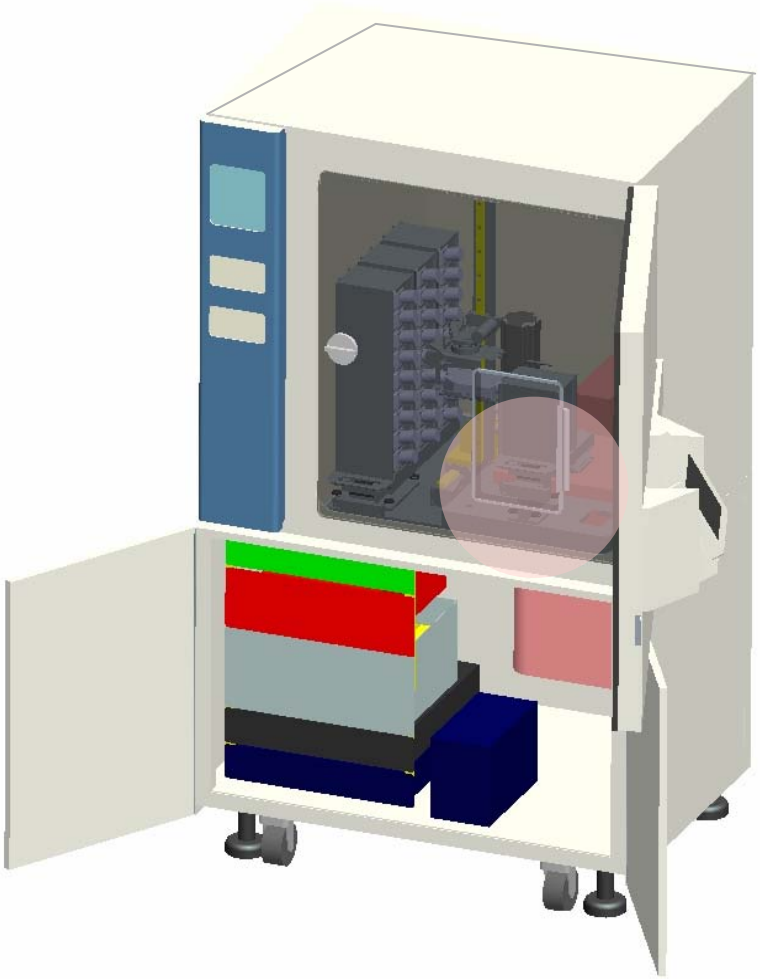
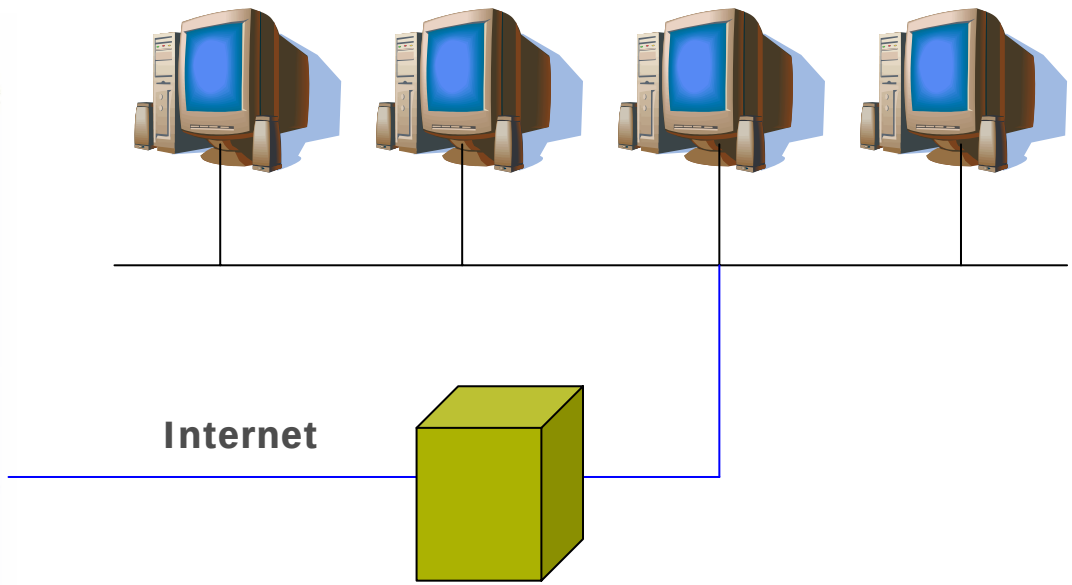
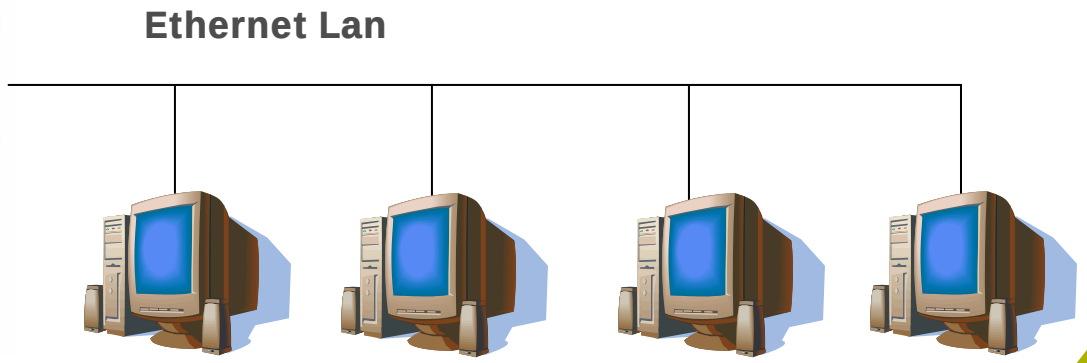
Remote Access Observation







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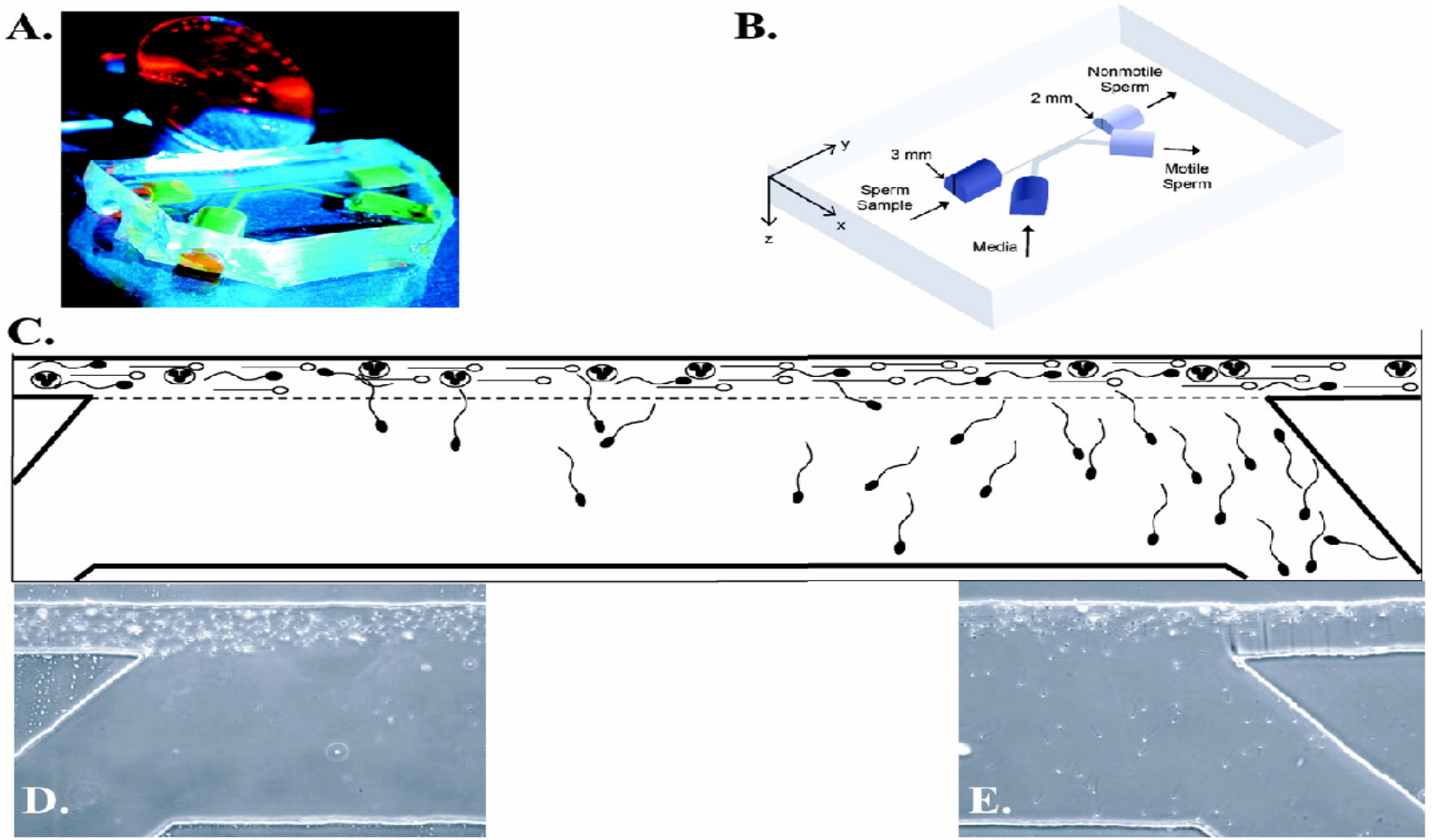
Microfluidic technology



From: Suh et al, Journal of Andrology, Vol. 26, No. 6, November/December 2005

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Conclusions

- Embryo evaluation is highly variable
- Every centre should participate in PT to evaluate individual embryologists performance
- New developments in software and technology will facilitate and improve embryo evaluation
- Automatisation 200?

Thanks

- Hilde Van de Velde
- Etienne Van den Abbeel
- Lisbet Van Landuyt
- Anick de Vos
- Greta Verheyen

- Josiane Van der Elst
- Paul Devroey

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