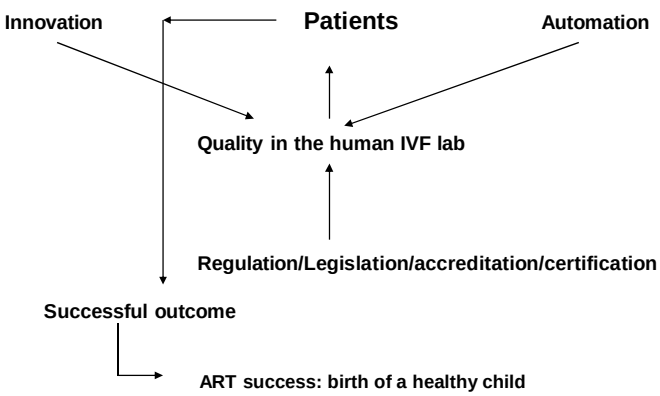
Universitair Ziekenhuis Gent

Blastocyst culture and transfer: a tool to improve the efficiency of ART and to reduce multiple pregnancies?

Prof Dr Etienne Van den Abbeel
Department of Reproductive Medicine, University Hospital Gent, Belgium
KLEM 2014

man, vrouw en kind
Universitair Ziekenhuis Gent

Introduction



man, vrouw en kind
Universitair Ziekenhuis Gent

The IVF lab and the challenges of the 21st century

Improving the efficiency of ART(per oocyte collection cycle) - avoiding multiple pregnancies

Single ET and Single FRET

Optimal embryo selection for transfer and cryopreservation

- Which, how, when and why?

Two questions:

- How many healthy live births are obtained from one stimulation cycle?
- How many stimulation cycles are necessar for a patient to give birth to one healthy baby?

3 titel

man, vrouw en kind
Universitair Ziekenhuis Gent

ART success: birth of one healthy child

Transfer of one embryo: SET and sFRET

Gardner D et al (1998) Culture and transfer of human blastocysts increases implantation rates and reduces the need for multiple embryo transfers. Fertil Steril 69, 84-88.

The road for single embryo transfer = blastocyst transfer?

Yes or No

man, vrouw en kind
Universitair Ziekenhuis Gent

ART success: birth of one healthy child
Transfer of one embryo: SET and sFRET

Day of embryo transfer
Benefit of blastocyst transfer

=

Subject of debate

2014?

man, vrouw en kind
Universitair Ziekenhuis Gent

Rationale for blastocyst culture and ET

- Synchronization of embryo and uterine development
- Provide a mechanism of self-selection of the viable embryos

man, vrouw en kind
Universitair Ziekenhuis Gent

Rationale for blastocyst culture and ET

- Synchronization of embryo and uterine development

Bovine: blastocyst transfer is routine, highly successful and the only viable option because cleaved embryos cannot survive the bovine uterine environment (Thompson and Peterson, 2000)

Primates: pronucleate and early cleaving embryos can survive in the uterine environment (Martson et al, 1977)

Human: First IVF pregnancy = blastocyst transfer, blastocyst transfer was not universally adopted

Transfer on day 0, day 1, day 2, day 3, day 4, …occasionally day 5/6

Since 1998: transfer on day 2/3 and also on day 5, day 6 …

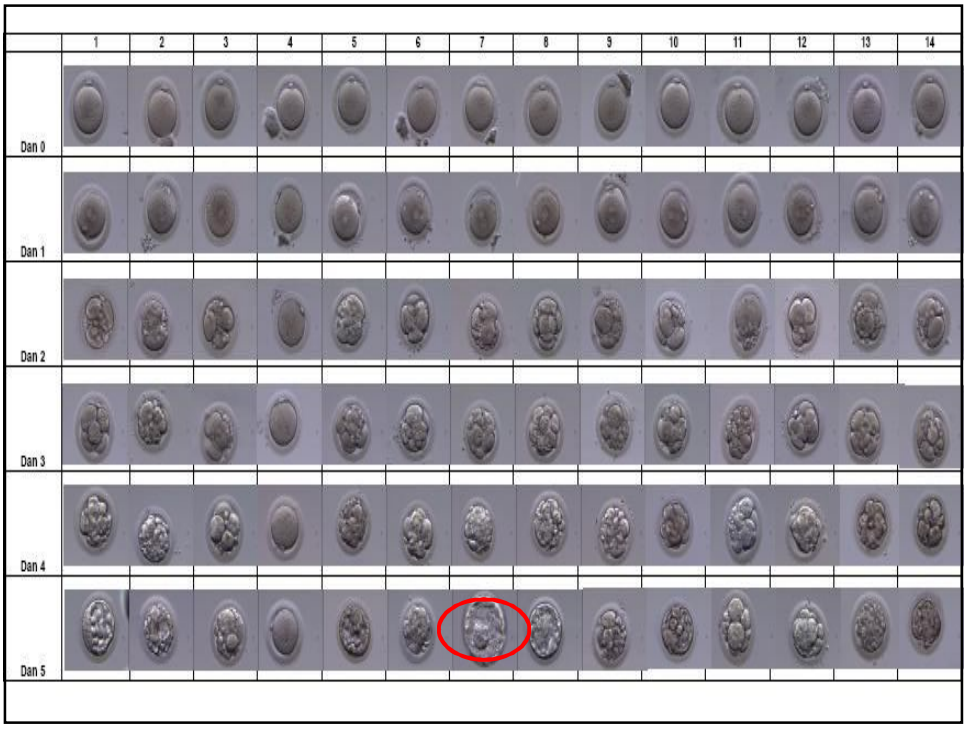
Current debate in the human

Comparison between uterine transfer of cleavage-stage and blastocyst stage embryos

man, vrouw en kind
Universitair Ziekenhuis Gent

Rationale for blastocyst culture and ET

- Provide a mechanism of self-selection of the viable embryos



man, vrouw en kind

Universitair Ziekenhuis Gent

UNIVERSITEIT GENT

Rationale for blastocyst culture and ET

- Provide a mechanism of self-selection of the viable embryos

Adler et al (2013) Blastocyst culture selects for euploid embryos: comparison of blastomere and trophectoderm biopsies. RBM Online , Article in press

- aCGH analysis to assess all 24 chromosomes
- Day 3 blastomeres and day 5 trophectoderm
- 1603 embryos
- A significant larger proportion of embryos were euploid on day 5 biopsy (42%) compared with day 3 biopsy (24%)

man, vrouw en kind
Universitair Ziekenhuis Gent

Can we select embryos with a high developmental potential earlier in development for transfer rather than wait until they develop to the blastocyst stage?

man, vrouw en kind
Universitair Ziekenhuis Gent

Megaset study: objectives and methods (Van den Abbeel et al, RBM Online, 2013, Ziebe et al, Fertil Steril, 2012))

Objective

- **To correlate embryo quality on day 3 and 4 with blastocyst development on day 5**
- **Investigate the relationship between blastocyst quality and pregnancy in a SET trial**

Design

- MEGASET: **Randomized, assessor-blind, parallel group, multicentre trial comparing HP-hMG with rFSH in GnRH antagonist cycles (N=749)**

Methods

- **Central training meetings and scoring sessions for all embryologists, and reference atlas**
- **Quality assessments at fixed, narrow time-points**
- **All oocytes were inseminated using** ICSI **and cultured to** blastocyst stage **(120 ± 2 hours)**
- **Compulsory SET of a blastocyst on Day 5 for all patients (N=618)**
- **All supernumerary blastocysts were vitrified**
- **Pregnancy monitoring:**
  - Serum β-hCG (13–15 days after ET)
  - Clinical pregnancy (5–6 weeks after ET)
  - Ongoing pregnancy (10–11 weeks after ET)

Early pregnancy loss = positive β-hCG, but no ongoing pregnancy

© 2008 Universitair Ziekenhuis Gent

man, vrouw en kind
Universitair Ziekenhuis Gent

Relationship: Day 3 → Day 5

Day 3	Day 5			
	Excellent-quality blastocyst (%)	Good-quality blastocyst (%)	Other-quality blastocyst (%)	Not blastocyst (%)
Top-quality	22	24	27	27
Good-quality	10	14	28	47
Minimum-quality	<1	6	17	76

 Ahlström A et al. Fertil Steril 2011; 96: S244

man, vrouw en kind
Universitair Ziekenhuis Gent

Definitions of Embryo Quality on Day 3

Top-quality embryo*	Good-quality embryo*	Minimum-quality embryo*
4 or 5 blastomeres (day 2) ≥ 7 blastomeres (day 3) ≤ 20% fragmentation (day 3) equally sized blastomeres (day 3) no sign of multinucleation (days 1, 2 and 3)	≥ 6 blastomeres (day 3) no cleavage arrest (i.e. cleavage must have occurred within the last 24h) ≤ 30% fragmentation (day 3) no sign of multinucleation (days 1, 2 and 3)	≥ 4 blastomeres (day 3) no cleavage arrest (i.e. cleavage must have occurred within the last 24h) ≤ 50% fragmentation (day 3) no sign of multinucleation (days 1, 2 and 3)

*** all criteria must be fulfilled**

man, vrouw en kind
Universitair Ziekenhuis Gent

More observations is better embryo selection: Time Lapse imaging

Limitations of Time lapse systems

- Morphokinetic markers different from lab to lab
- TLS does not allow rotation of embryos making visual observations difficult
- Morphokinetic markers algorithms work with quartiles or time range which create very exact and sharp limits and this may not simulate embryo development.
- Morphokinetic markers up to the five cell stage is questionable since embryo development up to that stage is governed by maternal influences only
- Further requirements for innovations in both optics and image analysis
- Can a single culture medium without renewal on day 3 be developed which supports development throughout the pre-implantation period?

man, vrouw en kind
Universitair Ziekenhuis Gent

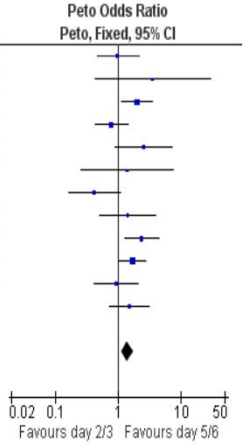
What are we trying to achieve with blastocyst transfer? Increased live birth rates, decreased multiple gestations or both?

man, vrouw en kind
Universitair Ziekenhuis Gent

Live birth rate per couple: Cleavage&Blastocyst transfer
(Glujovski et al, 2012, Cochrane review, following on Blake 2004, 2005, 2007)

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Peto Odds Ratio Peto, Fixed, 95% CI
Brugnon 2010	22	55	21	52	7.8%	0.98 [0.46, 2.12]
Devreker 2000	3	11	1	12	1.0%	3.53 [0.43, 29.14]
Elgindy 2011	52	100	35	100	14.8%	1.99 [1.14, 3.48]
Emiliani 2003	33	82	41	89	12.6%	0.79 [0.43, 1.44]
Frattarelli 2003	15	29	8	28	4.2%	2.57 [0.90, 7.35]
Levitas 2004	3	23	3	31	1.6%	1.40 [0.26, 7.65]
Levron 2002	8	46	15	44	5.2%	0.42 [0.16, 1.08]
Livingstone 2002	14	30	11	29	4.4%	1.42 [0.51, 3.96]
Papanikolaou 2005	38	80	23	84	11.6%	2.35 [1.25, 4.43]
Papanikolaou 2006	56	175	38	176	20.7%	1.70 [1.06, 2.72]
Rienzi 2002	24	50	24	48	7.4%	0.92 [0.42, 2.03]
Van der Auwera 2002	24	70	17	66	8.6%	1.49 [0.72, 3.10]
Total (95% CI)		**751**		**759**	**100.0%**	**1.40 [1.13, 1.74]**
Total events	292		237			

Heterogeneity: Chi² = 18.43, df = 11 (P = 0.07); I² = 40%
Test for overall effect: Z = 3.07 (P = 0.002)



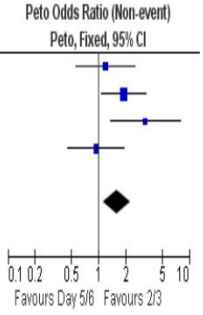
man, vrouw en kind
Universitair Ziekenhuis Gent

Cumulative fresh and frozen

Study or Subgroup	Day 5/6 Events	Total	Day 2/3 Events	Total	Weight	Peto Odds Ratio (Non-event) Peto, Fixed, 95% CI
Brugnon 2010 (1)	24	55	25	52	21.5%	1.19 [0.56, 2.55]
Emiliani 2003	43	99	56	94	39.0%	1.90 [1.08, 3.34]
Rienzi 2002	31	50	41	48	15.5%	3.28 [1.35, 8.02]
Van der Auwera 2002	24	66	22	63	24.0%	0.94 [0.46, 1.93]
Total (95% CI)		**270**		**257**	**100.0%**	**1.58 [1.11, 2.25]**
Total events	122		144			

Heterogeneity: Chi² = 5.54, df = 3 (P = 0.14); I² = 46%
Test for overall effect: Z = 2.55 (P = 0.01)

(1) Study had policy of single embryo transfer



man, vrouw en kind
Universitair Ziekenhuis Gent

Authors' conclusions

- This review provides evidence that there is a ***small*** significant difference in live birth rates in favour of blastocyst transfer (Day 5 to 6) compared to cleavage stage transfer (Day 2 to 3). However, cumulative clinical pregnancy rates from cleavage stage (derived from fresh and thaw cycles) resulted in higher clinical pregnancy rates than from blastocyst cycles. The most likely explanation for this is the higher rates of frozen embryos and lower failure to transfer rates per couple obtained from cleavage stage protocols.

man, vrouw en kind
Universitair Ziekenhuis Gent

Cochrane review *Discussion*

Considerations on Culture media used
Large differences between trials

- Sequential media for blastocyst culture (14 trials)
- Sequential media for both arms (7 trials)
- Different commercial media for the two arms
- In-house made formulations for early embryos

man, vrouw en kind
Universitair Ziekenhuis Gent

Cochrane review *Discussion*

Considerations on Differences in lab policies

- Culture media used
- Culture conditions (must be optimal)
- Minimum criteria for embryo quality for ET/freezing
  - ⇒ ET cancellation rate / embryo freezing rate
- Methodology
- Blastulation rates
- Number of embryos transferred

man, vrouw en kind
Universitair Ziekenhuis Gent

Papanikolaou et al. ***(New Engl J Med 354, 1139,** 2006)*

- July 2003 – Nov 2004
- <36 years
- Rank 1 or 2, IVF or ICSI
- Cycles planned for **SET**
- Inclusion and randomisation at consultation

man, vrouw en kind
Universitair Ziekenhuis Gent

Papanikolaou et al. ***(New Engl J Med 354, 1139,** 2006**)***

	D3 ET n=171	D5 ET n=169	statistics
ET rate (%)	**157 (91.8)**	**150 (88.8)**	**NS**
Clinical PR (%)	**41 (24.0)**	**58 (34.3)**	**P=0.04**
Ongoing PR (%)	**38 (22.2)**	**58 (34.3)**	**P=0.01**
Delivery rate (%)	**38 (22.2)**	**56 (33.1)**	**P=0.03**
Mult. delivery rate (%)	**2 (5.2)***	**0 (0.0)**	**NS**

2 monozygotic twins

man, vrouw en kind
Universitair Ziekenhuis Gent

Further questions

- **Blastocyst grading: Gardner's criteria are based on many subjective observations. Do we need to modify/modulate the system further?**
- **Cryopreservation of blastocysts: will vitrication methods change the picture?**
- **Culture media: how many different formulations do we need? Are further refinements necessary?**

man, vrouw en kind

Universitair Ziekenhuis Gent

UNIVERSITEIT GENT

Blastocyst grading: Gardner's criteria are based on many subjective observations. Do we need to modify/modulate the system further?

man, vrouw en kind

Universitair Ziekenhuis Gent

UNIVERSITEIT GENT

Blastocyst classification

Blastocyst expansion and hatching status

1. An early blastocyst with a blastocoel being less than half the volume of that of the embryo
2. A blastocyst with a blastocoel volume half that, or greater than half that, of the embryo
3. A blastocyst with a blastocoel completely filling the embryo
4. An expanded blastocyst with a blastocoel volume larger than that of the early embryo, with a thinning zona
5. A hatching blastocyst with the trophectoderm starting to herniate through the zona
6. A hatched blastocyst, in which the blastocyst has completely escaped from the zona

For blastocysts with expansion and hatching status 3–6, blastocyst inner cell mass grading and trophectoderm grading were evaluated.

Inner cell mass

A. Tightly packed, many cells
B. Loosely grouped, several cells
C. Very few cells

Trophectoderm

A. Many cells forming a cohesive epithelium
B. Few cells forming a loose epithelium
C. Very few, large cells

Gardner DK, et al (eds). Towards reproductive certainty: infertility and genetics beyond. Carnforth: Parthenon Press;1999:378–388

man, vrouw en kind
Universitair Ziekenhuis Gent
UNIVERSITEIT GENT

Literature

- **Blastocyst quality considering composite scores has been linked to pregnancy and/or pregnancy loss (Gardner & Schoolcraft, 1999; Balaban et al, 2000, 2006; Gardner et al, 2000, 2004; Kovacic et al, 2008; Alpha Scientists / ESHRE SIG Embryology, 2011)**
- **Blastocyst quality: individual parameters vs composite score (Dokras et al, 1993; Balaban et al, 2000; Shapiro et al, 2000, 2008; Richter et al, 2001; Ahlström et al, 2011, Hill et al, 2013)**
- **Most of the data in the literature are from multiple embryo transfers, and there are limited data on SET (Ahlström et al, 2011, Hill et al, 2013)**

man, vrouw en kind
Universitair Ziekenhuis Gent
UNIVERSITEIT GENT

Megaset study: objectives and methods

Objective

- **To correlate embryo quality on day 3 and 4 with blastocyst development on day 5**
- **Investigate the relationship between blastocyst quality and pregnancy in a SET trial**

Design

- MEGASET: **Randomized, assessor-blind, parallel group, multicentre trial comparing HP-hMG with rFSH in GnRH antagonist cycles (N=749)**

Methods

- **Central training meetings and scoring sessions for all embryologists, and reference atlas**
- **Quality assessments at fixed, narrow time-points**
- **All oocytes were inseminated using** ICSI **and cultured to** blastocyst stage **(120 ± 2 hours)**
- **Compulsory SET of a blastocyst on Day 5 for all patients (N=618)**
- **All supernumerary blastocysts were vitrified**
- **Pregnancy monitoring:**
  - Serum β-hCG (13–15 days after ET)
  - Clinical pregnancy (5–6 weeks after ET)
  - Ongoing pregnancy (10–11 weeks after ET)

} Early pregnancy loss = positive β-hCG, but no ongoing pregnancy

© 2008 Universitair Ziekenhuis Gent

man, vrouw en kind

Universitair Ziekenhuis Gent

Logistic regression models

- **Identification of statistically significant (p<0.05) predictors of pregnancy and early pregnancy loss**

Approach: Testing of one parameter at a time

Approach: Model including all parameters (expansion and hatching status, inner cell mass, trophectoderm)

Outcome	INDIVIDUAL ANALYSIS Significant predictors	COMBINED MODEL Significant predictors
Positive β-hCG	· Expansion and hatching status · Inner cell mass · Trophectoderm	· Expansion and hatching status
Ongoing pregnancy	· Expansion and hatching status · Inner cell mass · Trophectoderm	· Expansion and hatching status
Early pregnancy loss	· Inner cell mass	*None*

Van den Abbeel E et al. RBM Online, 2013

man, vrouw en kind

Universitair Ziekenhuis Gent

Predicted ongoing pregnancy rate

Blastocyst and expansion	Inner cell mass	Trophectoderm	Ongoing pregnancy (%)
1	-	-	6
2	-	-	12
3	A	A	25
3	A	B	22
3	A	C	21
3	B	A	20
3	B	B	18
3	B	C	17
3	C	A	16
3	C	B	14
3	C	C	14
4–5	A	A	48
4–5	A	B	45
4–5	A	C	44
4–5	B	A	42
4–5	B	B	38
4–5	B	C	37
4–5	C	A	35
4–5	C	B	32
4–5	C	C	31

For blastocyst Stages 3 versus 4–5, selection should be based on blastocyst expansion and hatching status

Van den Abbeel E et al. RBM Online, 2013

man, vrouw en kind
Universitair Ziekenhuis Gent

Cryopreservation of blastocysts: will vitrication methods change the picture?

man, vrouw en kind
Universitair Ziekenhuis Gent

Which method to use: slow freezing or vitrification?

- There are two circumstances in which vitrification methods should definitively be considered: where it is clear that extra-cellular ice is responsible for significant damage, and where the results of classical freezing methods are unsatisfactory (Pegg, 2005)
- Classical freezing of blastocysts is suboptimal (Kolibianakis et al, 2010)

man, vrouw en kind
Universitair Ziekenhuis Gent

Results from literature: blastocysts (open vitrification)

Choi (2000), Yokota (2001), Cho (2002), Reed (2002), Hiraoka (2004), Hu (2004), Stehlik (2005), Huang (2005), Kuwayama (2005), Mukaida (2007), Liebermann (2007), Son (2007), Van der Zwalmen (2007), Hiraoka (2008), Ebner (2009) Liebermann (2009), Rama Raju (2009)

Clin P / ET	Impl /E Transferred	Impl / E Warmed
4974/10197	3124/11117	3124/13629
(48.8%)	(28.1%)	(22.9%)

man, vrouw en kind
Universitair Ziekenhuis Gent

Results from literature: blastocysts (closed vitrification)

Stachecki (2008), Van der Zwalmen (2009, 2013), Liebermann (2009), Van Landuyt (2011), De Croo (2013) (= CBS HS VIT)

Clin P / ET	Impl /E Transferred	Impl / E Warmed
229/435	263/854	263/1004
(52.6%)	(30.8%)	(26.2%)

man, vrouw en kind
Universitair Ziekenhuis Gent

Culture media: how many different formulations do we need? Are further refinements necessary?

man, vrouw en kind
Universitair Ziekenhuis Gent

Embryo culture

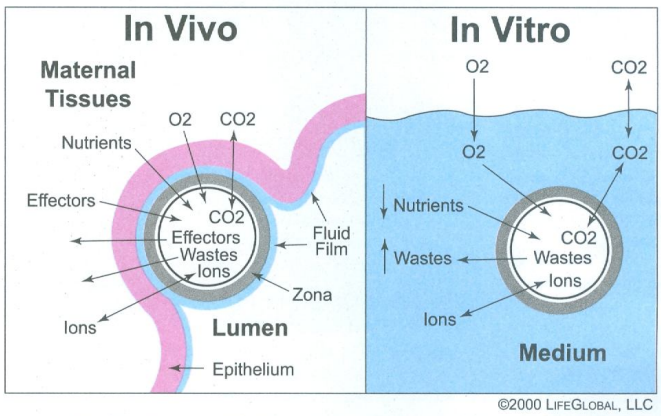


Figure 1. A comparison of the interaction of an embryo with its environment, in vivo *and* in vitro.

man, vrouw en kind
Universitair Ziekenhuis Gent

Composition of culture medium: debate

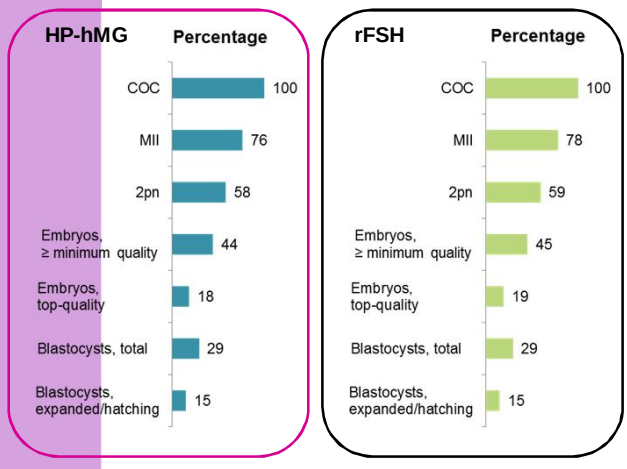
Questions

- Simple basic media used for day 2/3 culture are suboptimal for blastocyst culture?
- Should the composition of the medium be changed to correspond with the physiological changes which occur as development proceeds?
- Can a single culture medium be developed which supports development throughout the pre-implantation period?

man, vrouw en kind
Universitair Ziekenhuis Gent

Embryo development: MEGASET STUDY

Overall development from COC to blastocyst



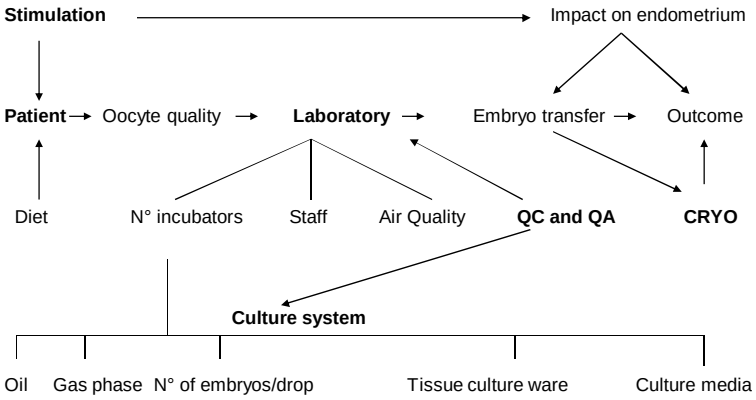
Blastocyst expansion and hatching status

	HP-hMG	rFSH
1	8%	10%
2	9%	8%
3	16%	18%
4	41%	42%
5	26%	23%
6	0%	0%

© 2008 Universitair Ziekenhuis Gent

man, vrouw en kind
Universitair Ziekenhuis Gent

2013: Holistic approach of IVF



man, vrouw en kind
Universitair Ziekenhuis Gent

Additional Considerations

- **Higher cost related to blastocyst culture – transfer**
  - Additional incubators
  - Optimized laboratory environment
  - Additional culture media
  - More laboratory staff members
- Cost – benefit analysis ?
- **Can patients and ARTologists accept the psychological trauma of no ET if blastocysts do not develop in vitro?**

man, vrouw en kind
Universitair Ziekenhuis Gent

The IVF lab and the challenges of the 21st century
Single embryo transfer avoiding multiple pregnancies

- Ecological clinical embryology?
  - Transfer embryos early D2/D3
  - Embryo Selection?
- Blastocyst transfer?
  - Benefits of blastocyst culture and ET
  - Risks of long term culture?

41 titel

man, vrouw en kind
Universitair Ziekenhuis Gent

- **Blastocyst culture and single blastocyst transfer is a powerful tool to improve the efficiency of ART and to reduce multiple pregnancies**
- **Vitrification of blastocysts works and should be regarded as an established technique**
- **Time for an optimized RCT?**

man, vrouw en kind
Universitair Ziekenhuis Gent

The IVF lab and the challenges of the 21st century
Single embryo transfer avoiding multiple pregnancies

- Ecological clinical embryology?
  - Transfer embryos early D2/D3
  - Embryo Selection?
- Blastocyst transfer?
  - Benefits of blastocyst culture and ET
  - Risks of long term culture?
- Freeze all but at the blastocyst stage?
  - Transfer in natural cycle
  - Transfer all embryos one at the time
  - No embryo selection
  - Optimized cryopreservation
  - Time to pregnancy?

43 titel