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SCIENCE MOVING
PEOPLE
MOVING SCIENCE

"Working together – theory and practice"

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28 June 2009
Amsterdam
The Netherlands

PRE-CONGRESS COURSE 1

Organised by the Paramedical Group

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PRE-CONGRESS COURSE 1 - PROGRAM

Working together - theory and practice

Organised by the Paramedical Group

Course co-ordinators: Heidi Van Ranst (Belgium) and Heidi Birch (United Kingdom)

Course description: This course is particularly suitable for laboratory, nursing and related staff who wish to have the opportunity to work together. By the end of the day the delegates will have an understanding of how colleagues work which will enhance working relationships and benefit patient care.

Target audience: Nurses, counsellors and affiliated paramedics

Chairman: Heidi Van Ranst (Belgium)

09:00 - 09:10	Introduction - ***Heidi Van Ranst (Belgium)***
09:10 - 09:30	The role of the nurse in The Netherlands - ***Karin Feicke (The Netherlands)***
09:30 - 10:00	The impact of the metabolic fitness on physically inactive obese women with a body mass index (BMI) over 30 and polycystic ovary syndrome (PCOS) - ***Birgitte Raaschou (Denmark)***
10:00 - 10:30	Ultrasound - how it works - ***Ellen van de Vorst (The Netherlands)***
10:30 - 11:00	**Coffee break**
11:00 - 11:45	Patient education and involvement using the internet - ***Wouter Tuil (The Netherlands)***
11:45 - 12:30	Vitrification of human embryos: Will it replace slow controlled-rate freezing? - ***Maureen Wood (United Kingdom)***
12:30 - 13:30	**Lunch**

<u>Afternoon workshops</u>
Group 1: Vitrification
Chair: Heidi Van Ranst (Belgium) & Anneleen Van de Velde (Belgium)
Speakers: ***G. Bocken (Belgium)*** and ***I. De Croo (Belgium)***
Practical demonstrations on vitrification

Group 2: Ultrasound
Chair: Jolieneke Schoonenberg-Pomper (The Netherlands) & Liz Corrigan (United Kingdom)
Speaker: ***E. Van de Vorst (The Netherlands)***
Simple physics, basic training and trouble shooting

Group 3: Patient education and involvement using the internet
Chair: Jolieneke Schoonenberg-Pomper (The Netherlands) & Liz Corrigan (United Kingdom)
Speaker: ***W. Tuil (The Netherlands)***
Going on a tour in a virtual hospital

Amsterdam 2009
Syllabus Paramedical pre-congress course

Sponsors

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The role of the nurse in the Netherlands

Karin Feicke MANP
Fertility clinic
University Medical Center Groningen
The Netherlands

ESHRE Amsterdam 28th june 2009

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Learning objectives

- Dutch facts and numbers
- Nursing and health care teams in NL
- Changes in the health care system
- Example: UMCG Fertility Clinic
  - Nurses tasks and interventions
  - Focus: Health education
  - Focus: Care for emotional well being
- Challenges for nurses

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Dutch facts and numbers

Total population	16.5 million
Age at birth from first child	29.4 years
Number of children per woman	1.7
IVF clinics with laboratory	13
IVF treatments	15000
IVF treatments reimbursed	3
IVF children	1:40
Nurses in reproductive health care	200

J.A.M.Kremer et al 2005 · M. Spelt, Eshre Barcelona 2008
www.cbs.org · www.who.org

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Nursing and health care teams in the Netherlands

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Training and regulations for nurses

Ministry of Education, Culture and Science

Secondary professional education	⇨	2nd level nurse
Higher professional education (bachelor)	⇨	1st level nurse
Post HBO - MANP	⇨	Nurse practitioner

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Levels of nurses

First level nurse (HBO) is a higher level nurse

- Assesses the whole situation and decides the appropriate level of nursing care
- Directs second level nurses (MBO)

National training programme for fertility nurses since 2007

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Nurses and the law

Individual health care professions act (BIG)

- Protects patients from unprofessional treatment
- Authorizes nurses to carry out certain activities under order from authorized professionals
- Nursing practice is regulated by law

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Career possibility for first level nurses: NP

Master of Advanced Nursing Practice

- Since 1997
- 2 years post HBO course

NP operates in the field between cure and care

- Qualified to also treat the patient
- Legally responsible for the treatment

Pioneer role in process of rearranging tasks in a clinic

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Changes in the health care system

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Recent changes in the health care system

Shortage of health care professionals

Costs of the health care increasing

Competition in the health care sector
- Patient's safety
- Patient's satisfaction
- Quality management systems

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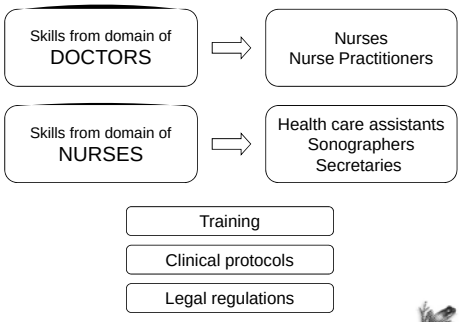
New approaches to organize health care teams



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Nursing in fertility clinics

Many similarities

Many differences
- Design and set up
- Local regulations
- Local management decisions
- Diversity of nurses
- Diversity of other health care professionals

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Example: UMCG fertility clinic

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UMCG – Numbers of cycles in 2008

IVF	490
ICSI	945
Cryo ET	128
IUI	421
Insemination / donorsemen	212

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UMCG – Medical staff

Profession	Individuals	Time
Gynaecologists	4	3.8
IVF doctors	4	3.6
Fertility Nurses	3	2.3
Nurse practitioners	2	1.8
Embryologists	3	2.6
Lab technicians	12	9.1

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Nurses tasks and interventions

UMCG – Fertitily nurses

Competences

- To assess, carry out and evaluate the nursing care during and after the IVF treatment
- Carry out and assist with procedures ordered by the doctor
- Administration
- Participate in working with a quality management system

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Specific nursing care in a fertility center

- Informing about the IVF procedure
- Advising and counselling before, during and after the treatment
- Create empathic relationship to let them talk openly
- Help the couple to cope with differences in communication and the emotional impact
- Ask other professionals in consult if needed

Leerboek O&G verpleegkunde, Voortplantingsgeneeskunde, De Haan, Spelt, Elsevier 2006

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Nursing care interventions (1)

Intake for starters in group sessions

- Logistic in the clinic and laboratory works
- Emotional impact
- Medication, side effects and physical discomfort
- Individual schedules and accessibility
- Chances of the different sorts of treatments
- Time and intimacy, free to ask
- Injection classes

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Nursing care interventions (2)

Daily consulting hour by telephone

- For reassurance and advice
- Nurses inform about patients history and complaints
- Supervision if needed

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Focus: Health education

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Health education (1)

Patients are partners with rights and responsibilities

Teach the patient what they can do themselves

Health education can make the patient feel better and more in control

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Health education (2)

Promote people's health and a healthy lifestyle

- Healthy body weight and healthy diet
- Regular physical exercises
- Reduction alcohol consumption and smoking
- Intake of folic acid supplementation

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Care for emotional well being – why?

Make the patient feel good

- about the whole package of care from the team
- not only about an achieved pregnancy

It's not only the numbers of given life births but the grading of the psychological wellbeing

Not only the cure but also the care we offer

Aftercare

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Care for emotional well being – how?

Patients want to be reassured – Explain to them

Keep it simple and clear

- Offer written information, booklets, cd roms
- Use visible tools, sheets, tables, figures and numbers
- Use no medical abbreviations or terms

Check and evaluate if the patient understands

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Care for emotional well being: how?

Observe the capability of the couple in handling their fertility problems

Try to identify and meet the patient's needs

Listen to them

Notice and detect problems

Speak for their needs and call in other professionals

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Challenges in Nursing

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For us who nurse, our Nursing is a thing, which, unless we are making progress every year, every month, every day… we are going back.

Florence Nightingale, 1872

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Challenge 1

Get ready for lifelong learning!

- Reflect to your actions and thoughts
- Be open to change
- Effective and efficient

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Challenge 2

Participate in rearranging tasks

- Think and be constructive and pro-active
- Where possible and sensible
- In cooperation with other health workers

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Examples

Coordinating and planning the care from the start to the end

Participate and setting up (nursing) research

Individual consultations with nurses

Offering good aftercare

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Take home message

Nurses have crucial role and position

Many opportunities

Do think ambitious!

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References

www.CBS.org

www.WHO.org

'Tien jaar resultaten van IVF in NL, 1996-2005',
J.A.M. Kremer et al, NTG, 2008

Leerboek O&G verpleegkunde, voortplantingsgeneeskunde,
De Haan, Spelt, Elsevier 2006

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The impact of metabolic fitness on physically inactive obese women with a body mass index (BMI) over 30 and polycystic ovary syndrome (PCOS)

Master project in Master of Sport, Physical Activity and Wellness

Head Nurse Birgitte Raaschou Holbæk Fertility Clinic, Holbæk, Denmark

Birgitte Raaschou June 2009

The purpose of the present study is.

- To investigate whether moderate aerobe training as the only intervention can be used as treatment for physically inactive obese (BMI>30) women with PCOS in order to relieve the endocrine and metabolic disorder.
- And how we can implement recommendations of daily phycical activity for inactive obese (BMI>30) women with PCOS in the preconception clinic.

Birgitte Raaschou June 2009

Agenda

- PCOS

”The hidden women's disease”.

- Obesity development in Denmark.
- PCOS and insulin resistance.
- 12-week intervention study.
- Metabolic fitness/training.
- Results of the pilot project.
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- Conclusion.

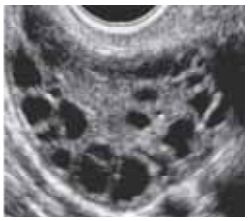
Birgitte Raaschou June 2009

PCOS – definition.

2 out of 3 criteria required.

- Oligo/amenorrhea.
- Hyperandrogenism.
  Clinical and/or biochemical
- Polycystic ovary.
  At least 12 follicles (2-9 mm) in one level and/or ovarian volume >10 cm3.
- Exclusion of other reason.

Rotterdam concensus 2003 ESHRE /ASRM



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PCOS - presence, cause and symptoms

- PCOS affects 5-10% women in the developed world.
- It is the most common endocrine disorder among women of reprodictive age.
- PCOS can be hereditary or may develop because of obesity and physical inactivity.
- The instances of metabolic consequences increases in relation to overweight (40 to 60% are obese): insulin resistance, glucose intolerance, elevated cholesterol and increased risk of cardio vascular diseases.
- Insulin resistance occurs in up to 75% of these cases.

Norman R et al; *Polycystic ovary sydrome.* The Lancet Vol.370 august 25,2007.
Norman R et al; Polycystic ovary syndrome:MJA 2004;180(3).

Birgitte Raaschou June 2009

PCOS – objective and subjective symptoms.

- <u>Objective symptoms.</u>
  Acne, unwanted hair growth, thinning hair in the forehead, abdominal obesity and increased waist/hip ratio. (apple shape)
  Norman R et al; *Polycystic ovary syndrome.* The Lancet Vol. 370, August 25, 2007
- <u>Subjective symptoms.</u>
  Fatigue, head ache, hot flashes, ”sugar-addiction” lack of satiety sateity, sleep problems and mood swings with an increased risk of developing a depression.
  www.pcoinfo.dk; Udengaard, H; *Hvad er Polycystisk ovariesyndrom?* 2007.

Birgitte Raaschou June 2009

Agenda.

- PCOS "The hidden women's disease".
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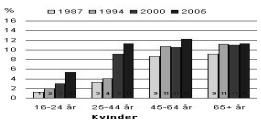
Overweight – an increasing problem in modern society.

- Overweight: BMI>25-29.99
  30 – 40% in Denmark.
- Obesity: BMI≥30
  10-13% in Denmark.
  - Region Sealand 30% higher than the national average 30-40% with a BMI>25.
  1 out of 7 are obese in Region Sealand.

The tables show the development of the share (%) of very overweight adult women from 1987 to 2005 in different ages and the distribution in Denmark.

http://www.sifolkesundhed.dk/Ugens%20tal%20for%20folkesundhed/Ugens%20tal/37_2006.aspx



Overweight – an increasing problem in modern society.

- In 2004 31.7% of the pregnant women were overweight in Denmark.
- In the 1st half of 2008 the number has risen to 33.7%.

http://www.sst.dk/default.aspx?path=%7B47566AC9-C83E-40C9-A63C-0E59F196CEE7%7D&print=1

Overweight and pregnancy complications after IVF/ICSI.

	DM OR (95%)	Hypertension OR (95%)	Pre-eclampsia OR (95%)
BMI< 25	1	1	1
BMI 25-29.9	3.4 (1.7-6.8)	1.9 (0.97-3.7)	1.7 (1.2-2.4)
BMI>30	15.3 (8.2-28.6)	4.8 (2.3-9.9)	2.8 (2.0-4.1)

Rode et al., Obstet Gynecol; 2005 105

Birgitte Raaschou June 2009

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PCOS and insulin resistance.

- " Polycystic ovary syndrome is not only the major cause of ovulatory dysfunction and of hirsutism but is also associated with insulin resistance and is now recognised as an important risk factor for type 2 Diabetes.

Franks Stephen; *How good are we at diagnosing polycystic ovary syndrome?* Clinical Endocrinology Vol. 67 Dec. 2007.

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Weight loss.

- A weight reduction of between 5 and 10% of the initial weight has shown that it can reestablish spontaneous ovulation and increase the insulin sensitivity in 71% of overweight anovular women..

  Clark A.M et al; Weight loss in obese infertile women result in improvement in reproductive outcome for all forms of fertility treatment. Hum. Repr.vol.13 1998
  Norman R et al; *Polycystic ovary sydrome*. The Lancet Vol.370 august 25, 2007.
  Svendsen PF, et al; *Polycystisk ovariesyndrom*. Ugeskrift for læger; 167, 2005

- Such a weight loss is the same as a reduction of viseral fat of about 30% which can explain why even a limited weight loss can enhance metabolic and reproductive functions.

  Balen A et al; *Impact of Obesity on female reproductive health: British Fertility Society, Policy and Practice Guidelines*. Human Fertility,2007;1-12 I First article

Birgitte Raaschou June 2009

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12-week intervention study for physically inactive obese women with a body mass index (BMI) over 30 and polycystic ovary syndrome (PCOS).

Hypothesis:

Increased physical activity increases the insulin sensitivit in physically inactive women with BMI over 30 and PCOS.

- Increased insulin sensitivity can help lower and/or normalise the androgen level.
- Increased physical activity will:
  - Reduce insulin resistance.
  - Have a positive influence of lipid status with ↓ total cholesterol ↓ LDL and ↑ HDL.
  - Reduce abdominal and viseral fat with a decreased waistline.

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12-week intervention study.

Inclusion criteria:

- Age 25-35 (mean 29.8 (25-34))
- BMI ≥ 30 (mean 37.6 (31.4-47.8))
- VO2 max <25 (16-25.1))
- P-FSH< 7,5IUI/L (4.8 (3-6))
- PCOS.

Exclusion criteria:

- Pregnancy.
- Type-1 and Type-2 Diabetes.
- Medical treatmen, birth control pills.
- Competitive illnesses or handicaps.

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12-week intervention study.

The training intervention.

- 2 weekly supervised training of 1 hours duration.
- 30 min daily physical activity with moderate intensity. (slightly out of breath)
- 10,000 paces daily.
- Training intensity: Light to moderate intensity. (slightly out of breath, pulse 120-140)

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12-week intervention study.

- Home training: bicycling, walking, dancing, ball games, swimming and gardening.
  The amount of training was documented in the training dairy and the strain was registrated via a pulse watch.
- Supervised training: Nordic Walking, workout, aerobic, ball exercises, weight-lifting, elastics, circle training and pair exercises.
  The strain of the traning was registrated every 10 minutes via a pulse watch.

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12-week intervention study.

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Metabolic fitness/training. (metabolic condition)

- The capability of the cells to absorb and metabolize nutrients i.e. the mitochondria of the cells.

  "energy stations" are more active and produce more enzymes. (better absorbtion of sugar)

  Sundhedsstyrrelsen - Center for forebyggelse FYSISK AKTIVITET-håndbog om forebyggelse og behandling 2003

- The increased activity can take place regardless of improved physical fitness.

  Kiens.B. et al; Fysisk inaktivitet- konsekvenser og sammenhænge Motions- og ernæringsrådet 2007

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Results:
Amount and intensity of the training.

5 physically inactive obese women with PCOS went through:

- On average 22 out of 24 available supervised training session of the duration of 1 hour.
- On average 66 (60-71) out of 84 available home training sessions incl. supervised training sessions.
- Walked on average 8140 paces daily. (6172-10788)
- Trained with an average pulse of 130. (126-137)

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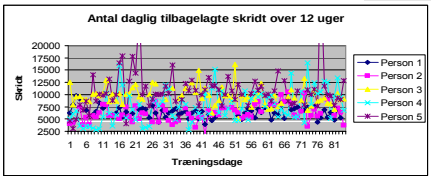
Results:
Number of daily paces.



- The number of average paces daily was 8140 (6172-10788).
- The recommended was 10,000 paces/daily.

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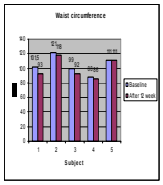
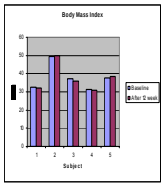
Results:
Waistline and BMI.





- Mean reduction in waistline of 4.1 (0-8.5 cm)
- Mean reduction in BMI of – 0.2 (+ 0.7- -1.1 cm)

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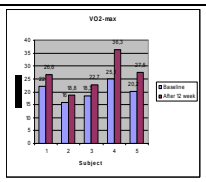
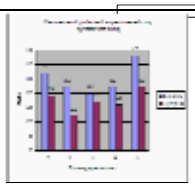
Results:
↑ VO2- max and training intensity (pulse).





- VO2-max 20.3 (16-25.1) ml/kg/min to 26.3 (18.8-36.3) ml/kg/min. Increase in i % 30 (18-45).
- Training intensity (pulse).
  - Structured training 134. (130-143)
  - Unsupervised home training (30 min/day) 127.(122-132)

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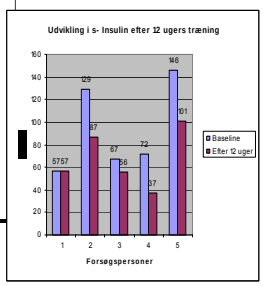
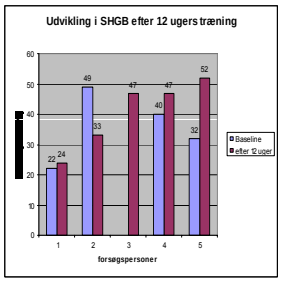
Results:
↓ S-Insulin and ↑ SHGB.





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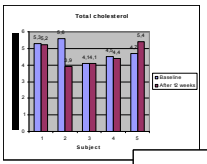
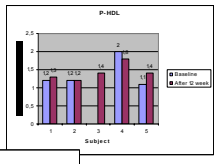
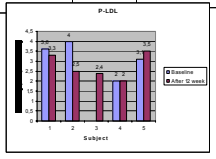
Results:
Total cholesterol, P-HDL and P-LDL.







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Conclusion: Metabolic fitness effect on physically inactive obese women with a BMI over (30) and PCOS.

- S-Insulin ↓ SHGB↑.
  SHGB binds the male hormones → helps to normalize the hormone balance → increase pregnancy chances.
- Modification of body composition with reduction in waistline → reduces the risk of developing metabolic consequences.
- VO2-max improved by 18 to 45% → decreased risk of developing cardiovascular disorders.

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- PCOS "The hidden women's disease".
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- Implementation in Holbæk Fertility Clinic.

Implementation of preconception care in Holbæk Fertility Clinic.

- Preconception care is the first treatment offer for obese and physically inactive women with a BMI>30 and PCOS women with a BMI>27 and/or a waistline > 88 cm.
- Treatment offer:
  Guidance in life style changes in the form of exercise and change of diet.

How to implement preconception care in Holbæk Fertility Clinic.

- 1. Consultation with a fertility doctor in the clinic.
- If BMI>30 and PCOS women with a BMI>27 and/or a waistline > 88 cm.
- Preconception care programme establish.

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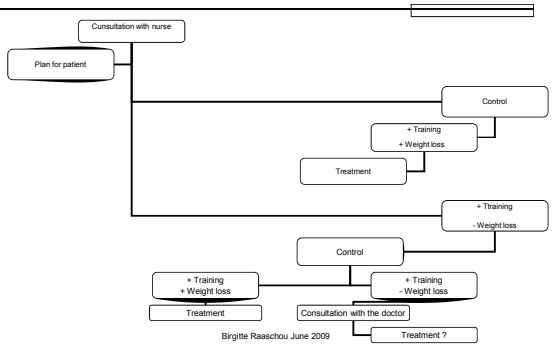
How to implement preconception care in Holbæk Fertility Clinic.



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Preconception care.

- Medical history based on a questionnaire
  - Investigate life style.(diet, exercise, work life, daily rhythm, private life)
  - What is the cause of the overweight? (e.g. PCOS, mental state, nature and nurture)
  - What are their previous experiences with weight loss? (good, bad)
  - Investigate resources. (e.g. personal/family relations)

Birgitte Raaschou June 2009

Preconception care.

Dialogue based on questinnaire.

- What are the consequences of obesity for you and your fertility?
- How do you loose weight?
  - Diet. (”the 8 dietary”)
  - Exercise. (”30 min. daily and 1 hour two times a week”)
  - Other. (Metformin)
- Where to start (partial aim and plan)?
  - INDIVIDUAL GOALS.
  - Clear roles. (responsibility)
  - Plan for future contact. (via telephone and conversations)
- Following conversations
  - Stress that this is a change of life where the low insulin sensitivity is the main focus.
  - How close are we to the goal – what needs to be adjusted?

Thanks.

- Holbæk Fertility Clinic.
- Merck Serono.

Thank you for your time!

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Ultrasound: How it works

Ellen vd Vorst
Nurse reproductive medicine
UMCN St Radboud
Nijmegen, the Netherlands

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Objectives

- Basic anatomy
- Physiological changes during the menstrual cycle
- Follow a IVF cycle/image recognition
- Discuss a few pathologies

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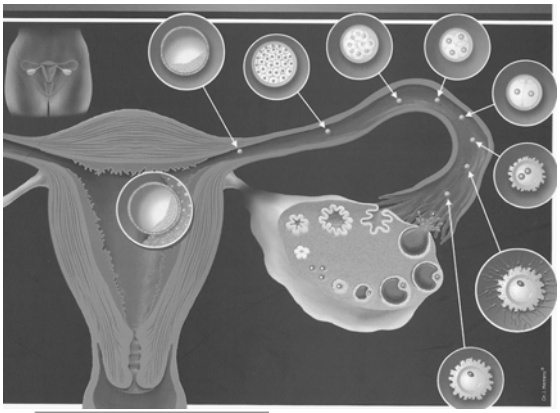


Types of transducers

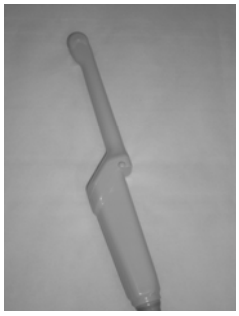
witch we use in gynecology

- Vaginal probe

- Abdominal probe

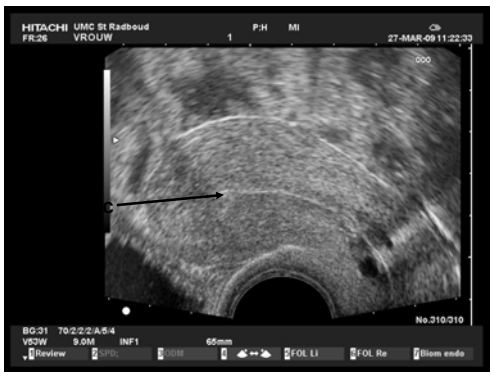
Trans vaginal ultrasound

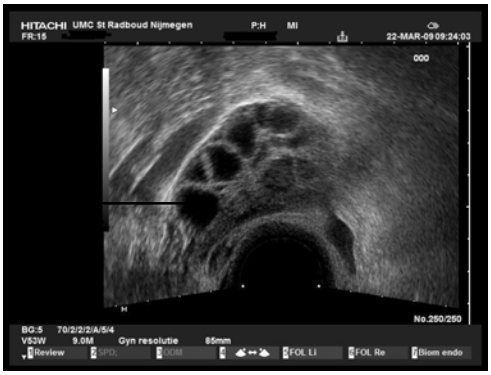
- High resolution
- Loses detail on distance
- Empty bladder

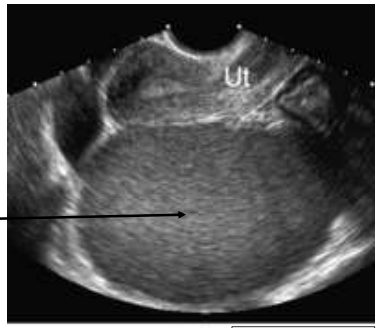


Ultrasound terms

- Echogenic: an image that reflects most ultrasound waves; appears white or bright; hyperechoic
- Anechoic: an image that transmits most ultrasound waves; appears dark or black; hypoechoic
- Isoechoic: an image that both reflects and transmits ultrasound waves; appears grey; homogenous







Isoechoic endometriosis cyst

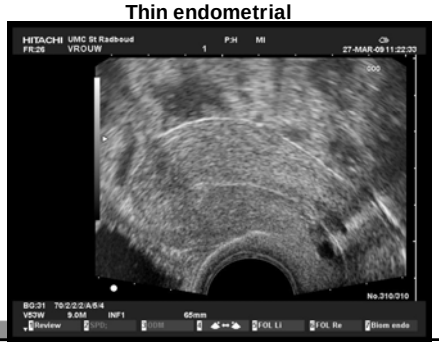
Endometrial

- Composed of basal and functional layer
- Functional layer responds to hormonal changes

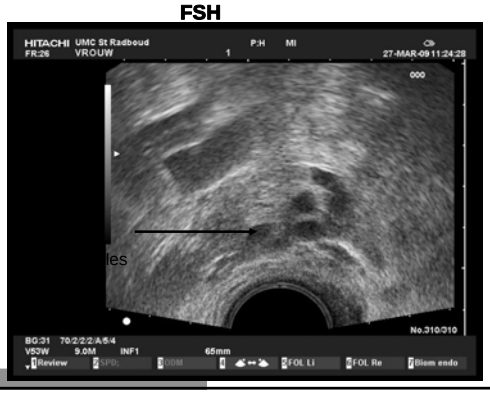
First ultrasound before starting with recombinant FSH

Uterus

Thin endometrial



First ultrasound before starting with recombinant FSH



Endometrium proliferative Phase

- Developing follicle ⇨ Estrogen goes up ⇨ proliferation of the endometrial (8-16 mm)
- Triple layer

Second ultrasound

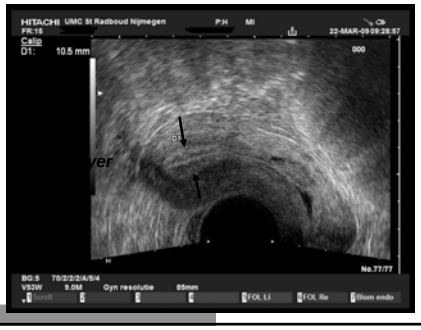
- After stimulation with recombinant FSH
- Ultrasound on day 9-10
- Measuring the endometrial
- Measuring the follicles

Make a new appointment or schedule a follicle puncture

Second ultrasound

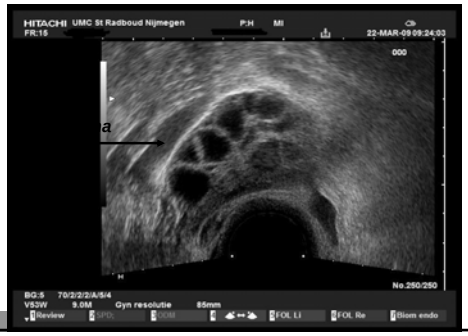
after stimulation with recombinant FSH

Thick endometrial after stimulation



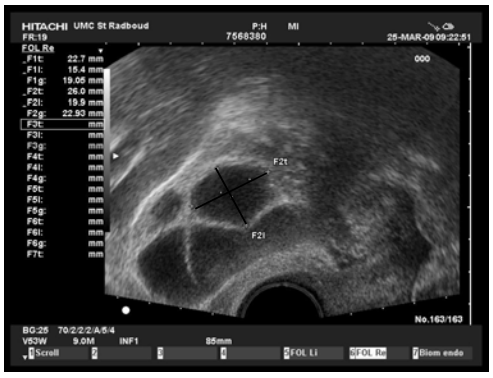
Second ultrasound
after stimulation with recombinant FSH

Ovary after stimulation

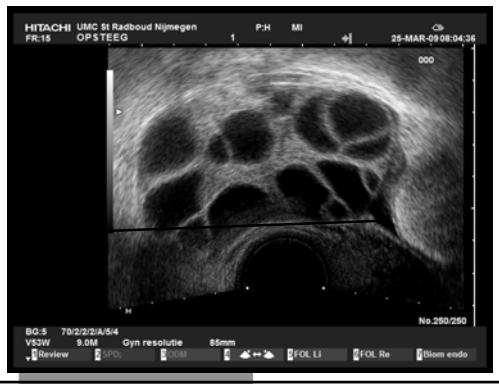


Measure follicles

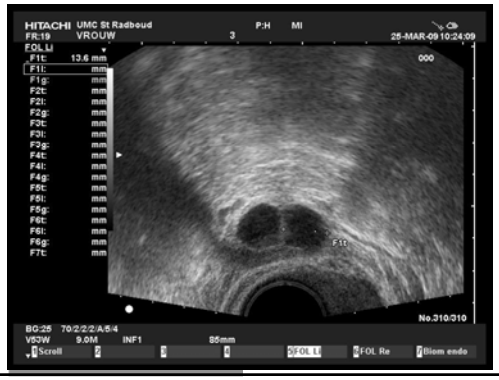
- Follicle < 15 mm measure in 1 direction
- Follicle > 15 mm measure in 2 directions
- Look for free fluid in abdomen especially if there's a risk for ovarian hyper stimulation syndrome (OHSS)



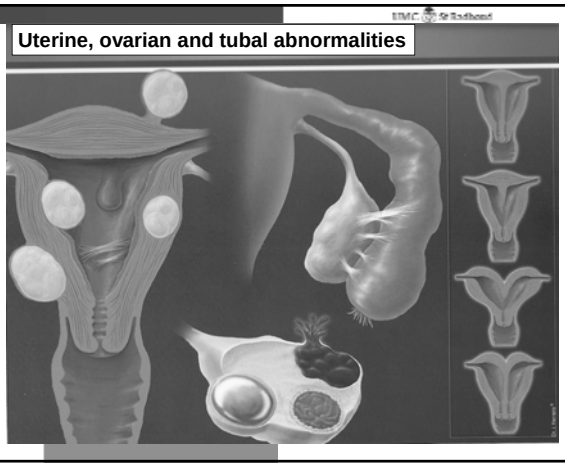
OHSS

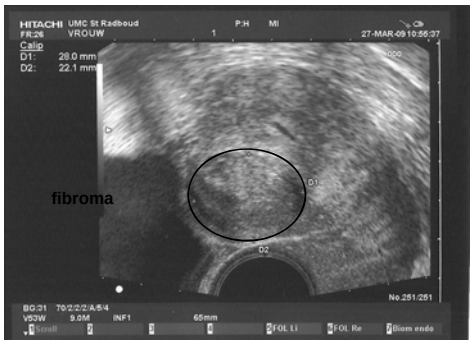


Poor respons

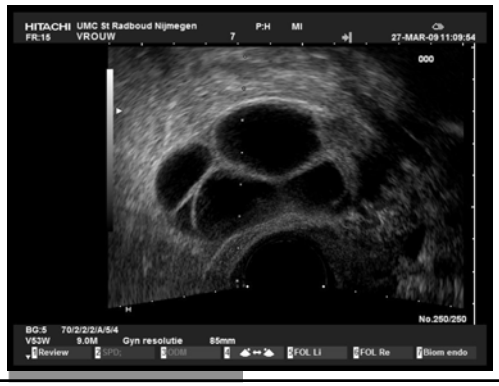


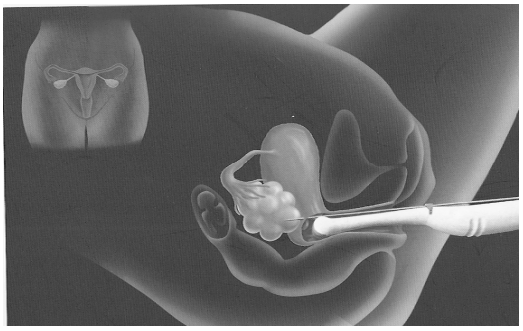
Uterine, ovarian and tubal abnormalities





Follicle puncture



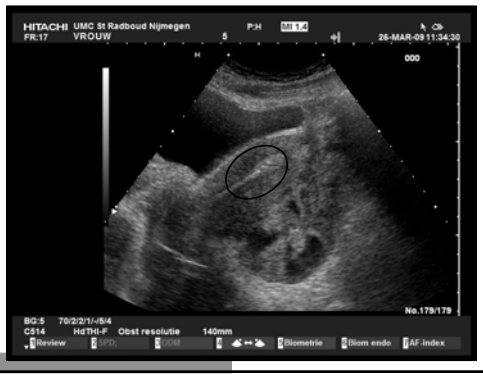


Trans abdominal ultrasound

- Low resolution
- Lose little sharpness in depth
- Full bladder

Trans abdominal ultrasound

during embryo transfer



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PATIENT EDUCATION AND INVOLVEMENT USING THE INTERNET

Wouter Tuil, PhD
Radboud University Medical Centre Nijmegen

ESHRE Annual Meeting 2009, Amsterdam

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Overview

- Introduction
- Patient education
- Patient involvement
- Personalisation
- Virtual IVF clinic
- Getting started
- Conclusion

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Introduction

- Internet is an healthcare revolution
- Patients demand online tools
- Clinic's general website
  - Limited functionality
  - Limited quality
- Great opportunities
  - Counselling
  - Interaction

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PATIENT EDUCATION

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Patient education

- Generic information
  - Disease / illness
  - Examinations
  - Treatment protocols
  - Scientific research programs
  - Clinic's staff
  - Contact information
  - Frequently Asked Questions (FAQ)
- Personal Health Status
  - Diagnostics tests
  - Prognosis
  - Treatment outcome

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Patient education

- Skills
  - Instructions
  - Coping
  - Assertiveness
  - Information management
  - Lifestyle management

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PATIENT INVOLVEMENT

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Patient involvement

- Shared Decision Making
  - Access to medical information
  - Decision support systems
- Record keeping
  - Data entry
    - Intake / evaluation forms
    - Order entry
    - Telecare / home monitoring
  - Data corrections
- Process improvement
  - Guideline adherence
  - Suggestion box

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Patient involvement

- Online peer-to-peer support
  - Exchange experiences
  - Advice
    - Coping
    - Lifestyle
    - Social support
    - Work

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PERSONALISATION

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Personalisation

- Personalised information website
- Personalised health record
- Personalised prognosis
- Personalised protocol

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VIRTUAL IVF CLINIC

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Virtual IVF Clinic

- Content:
  - Generic information
    - About the treatment
    - About the clinic
  - Personal information
    - Access to the medical record
  - Communication
    - Bulletin board
    - Chatroom

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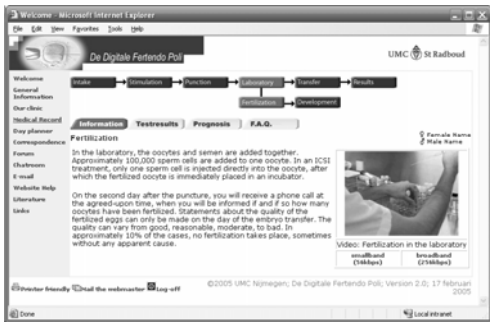
Virtual IVF Clinic



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Virtual IVF Clinic

- Results

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Virtual IVF Clinic – Usefulness

Generic Information	Useful	Not Useful
1. Frequently Asked Queston	88%	12%
2. Information about the clinic	67%	33%
3. Information about the treatment	63%	37%
4. Personal experieces	50%	50%
5. Extrenal links	50%	50%
6. Recommended literature	33%	67%
7. Video fragments	31%	69%

Tuil et al, Human Reproduction 2006

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Virtual IVF Clinic – Usefulness

Patiënt specific information	Useful	Not Useful
1. Personal Health Record	96%	4%
2. Day planner	92%	8%
3. Embryonic photographs	79%	21%
4. Personalised prognosis	78%	22%
5. Correspondence	49%	51%

Communication	Useful	Not Useful
1. E-mail	77%	23%
2. Bulletinboard	75%	25%
3. Chatroom	58%	43%

Tuil et al, Human Reproduction 2006

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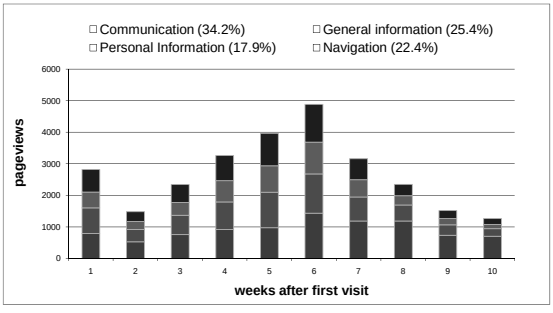
Virtual IVF Clinic – Timeframe



Tuil et al, Human Reproduction 2006

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Virtual IVF Clinic – Styles

- 3 Styles of usage:
  1. Individual information style (33,2%)
  2. Generic information style (29,0%)
  3. Communication style (37,8%)

- Correlations:
  - Style 1 ←→ paid employment (-)
  - Style 1 ←→ emotion coping (-)
  - Style 3 ←→ paid employment (+)
  - Style 3 ←→ anxiety (+)

Tuil et al, Human Reproduction 2008

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GETTING STARTED

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Getting started

① Listen !!!
- Focus group / interviews / questionnaires

② Set attainable goals
- Limited number of goals
- Realistic ambitions
- Solid business case
- Avoid extra workload

③ Measure
- Don't believe the hype

④ Take action
- Improve your service level

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CONCLUSION

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Conclusion

- The internet can help in:
  - Educate patients
  - Involve patients
- Improves
  - Outcome
  - Process
- Beneficial results are in reach
- Best practices are rare

Vitrification of human embryos: Will it replace slow controlled-rate freezing?

Maureen Wood PhD
Department of Obstetrics and Gynaecology
University of Aberdeen

Disclosure

The speaker has no commercial relationships or other conflicts of interest to declare

Learning objectives

- To understand the differences between freezing and vitrification
- To evaluate the evidence that vitrification should replace freezing, including:
  - Practical aspects
  - Outcome (embryo survival, pregnancy and livebirths)
  - Risks for children born from vitrified and frozen embryos

Introduction

- Embryo storage vital in assisted conception
- Increases cumulative pregnancy rates
- Demand growing
  - Improved embryo quality
  - Introduction of eSET
  - PGD/PGS
- Fertility conservation

Dilemma

- Slow controlled freezing
  - Outcome variable
  - Embryo viability reduced?
- Should vitrification replace freezing?

Learning objectives

- To understand the differences between freezing and vitrification
- To evaluate the evidence that vitrification should replace freezing, including:
  - Practical aspects
  - Outcome (embryo survival, pregnancy and live births)
  - Risks for children born from vitrified and frozen embryos

Outline

- History of embryo storage
- Principles of freezing and vitrification
- Compare:
  - Practical aspects
  - Outcome
  - Risks

Chronology of embryo freezing

1972 Mouse embryos frozen
1984 Human 8-cell embryos: DMSO
1984 Human blastocysts: glycerol
1984 Mouse embryos: PrOH
1985 Human embryos: PrOH

Chronology of embryo vitrification

1985 Mouse embryos vitrified
1987 Live mice from vitrified embryos
Less toxic solutions developed
1994 Vitrification = freezing in mouse

Vitrification *vs* Freezing Mouse 8-cell embryos

	Vitrification	Freezing
No embryos	206	157
% Survived	97	99
% Implanted	76	85
% Foetuses/Live Births	65	77
Overall survival (%)	63	76

Rall & Wood, 1994

Chronology of embryo vitrification

1985 Mouse embryos vitrified
1987 Live mice from vitrified embryos
Less toxic solutions developed
1994 Vitrification = freezing in mouse
1998 Birth from vitrified human 8-cell
1999 Birth from vitrified blastocyst

Principles of freezing and vitrification

Cryopreservation

Freezing: ice

Cryopreservation

Freezing: ice

Vitrification: glass

Cryopreservation

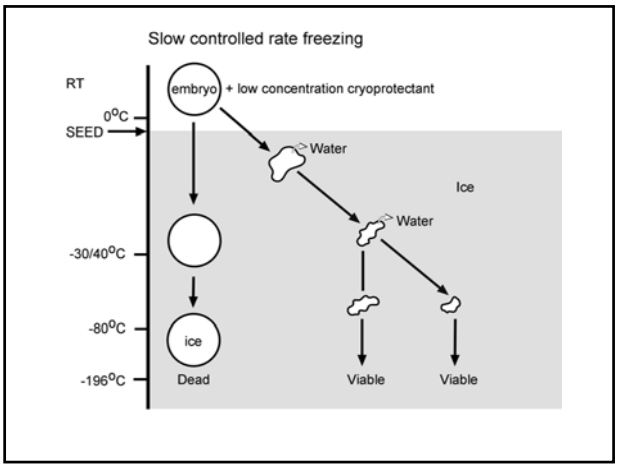
Freezing: ice

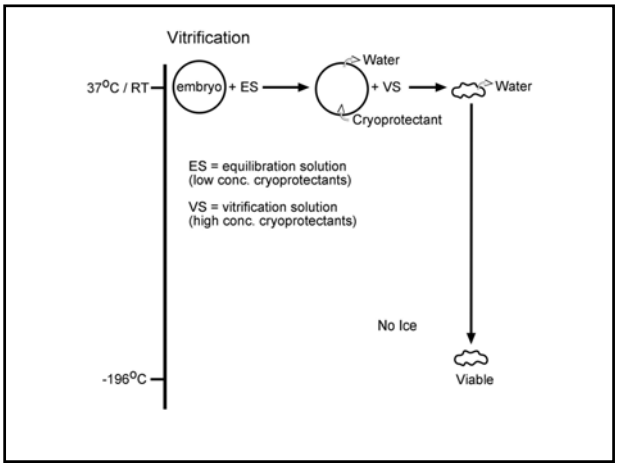
Vitrification: glass

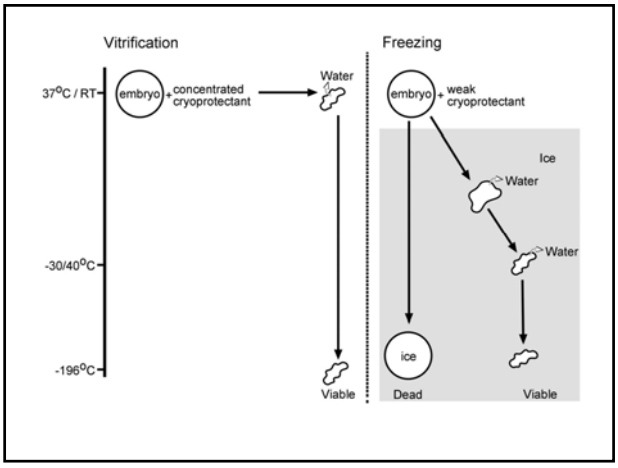
AIM

Prevent internal freezing

Remove intracellular water


Slow controlled rate freezing
RT
0°C
SEED
-30/40°C
-80°C
-196°C
embryo
+ low concentration cryoprotectant
Water
Ice
Water
ice
Dead
Viable
Viable


Vitrification
37°C / RT
-196°C
embryo
+ ES
Water
+ VS
Water
Cryoprotectant
ES = equilibration solution
(low conc. cryoprotectants)
VS = vitrification solution
(high conc. cryoprotectants)
No Ice
Viable


Vitrification
Freezing
37°C / RT
-30/40°C
-196°C
embryo
concentrated
cryoprotectant
Water
Viable
embryo
weak
cryoprotectant
Ice
Water
Water
ice
Dead
Viable

Recent breakthrough in vitrification

- Cooling in ultra-small volumes
  - Increases cooling rate
- Decreased concentration of cryoprotectant
  - Minimises toxicity

Vitrification containers

These include:

- Copper grid
- Metal loop
- Finely pulled straw (OPS)
- CryoTop
- CryoTip
- CryoLeaf
- HSV straw

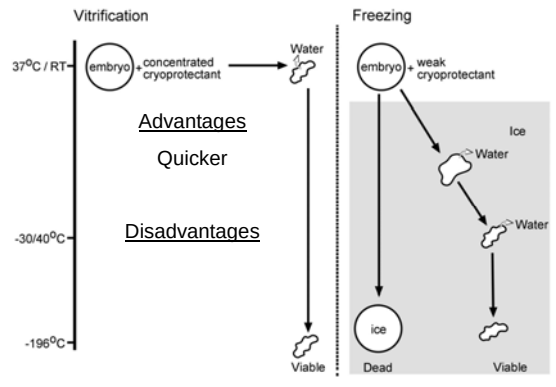
Evidence that vitrification should replace freezing?

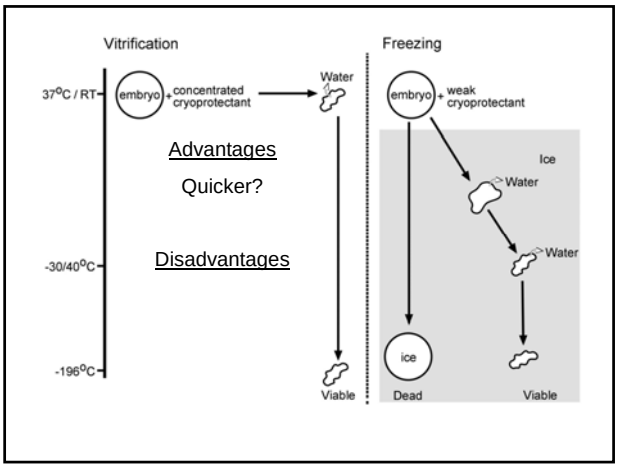
Vitrification *vs* Freezing

- Quicker
- Simpler
- Less costly
- Better outcome
- An open and shut case?

How successful is vitrification in practice?

- Practical advantages?
- Robust?
- Improved survival?
- Live births per embryo?
- Safe?




Vitrification
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embryo
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Water
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Advantages
Quicker?
Disadvantages
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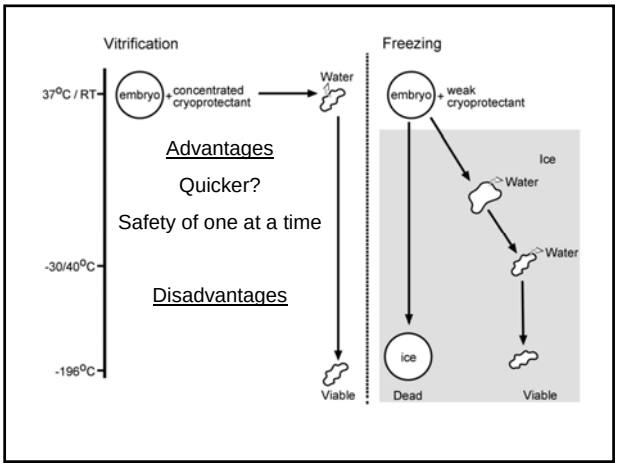
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Safety of one at a time
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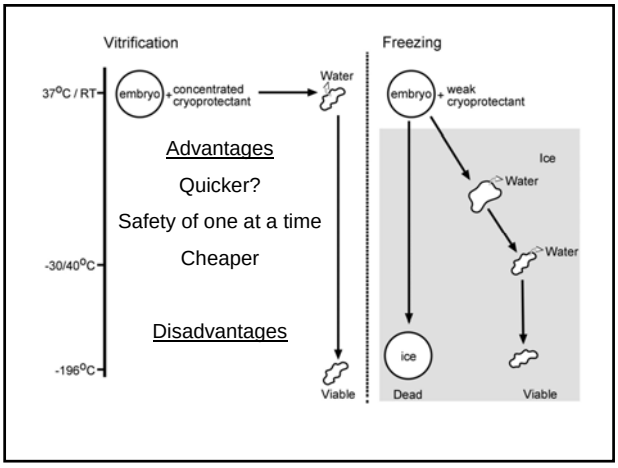
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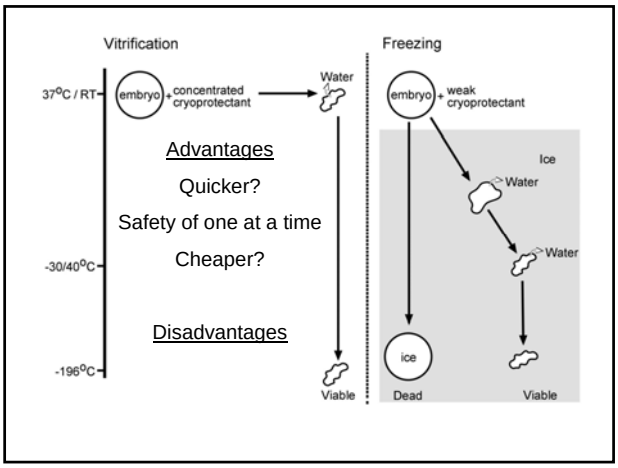
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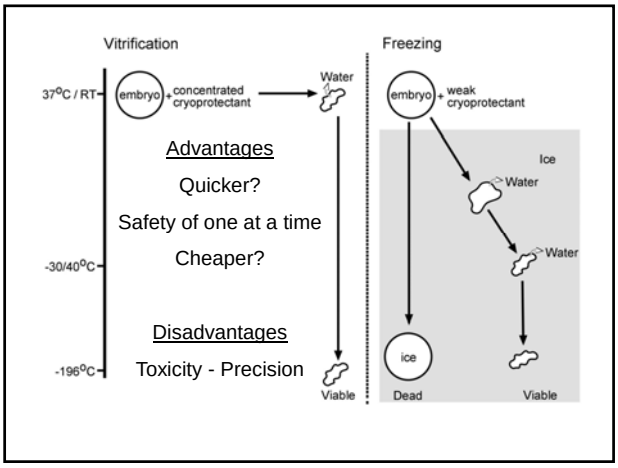
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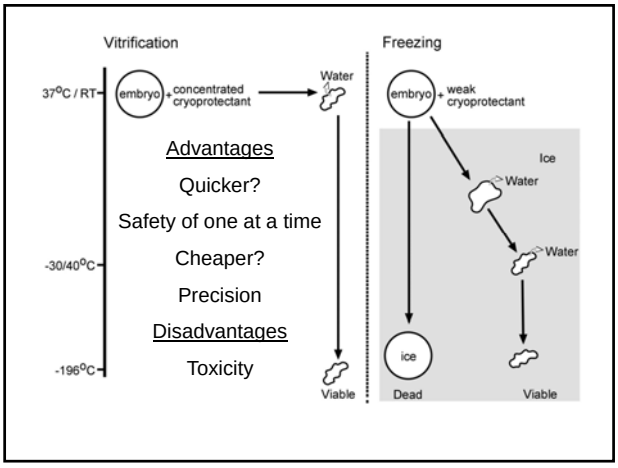
37°C / RT
-30/40°C
-196°C
embryo
concentrated cryoprotectant
Water
Viable
Advantages
Quicker?
Safety of one at a time
Cheaper?
Disadvantages
Toxicity - Precision
embryo
weak cryoprotectant
Ice
Water
Water
ice
Dead
Viable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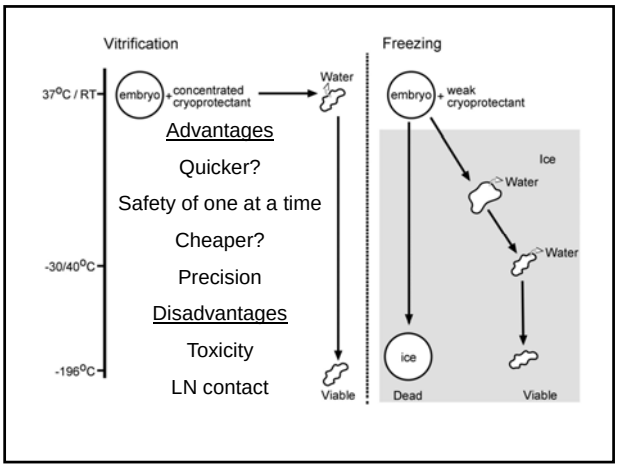


Advantages

Quicker?

Safety of one at a time

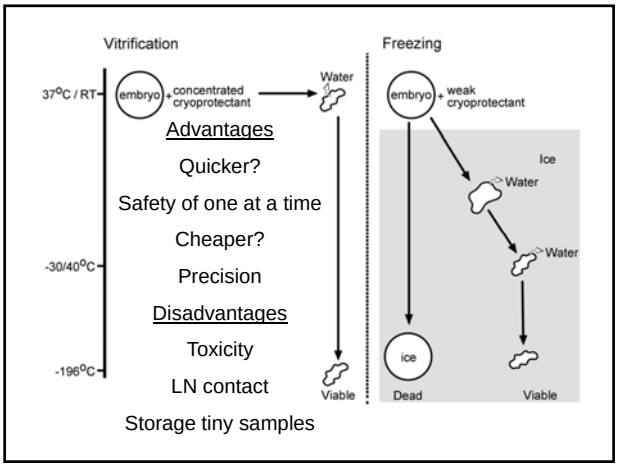
Cheaper?

Precision

Disadvantages

Toxicity

LN contact



Advantages

Quicker?

Safety of one at a time

Cheaper?

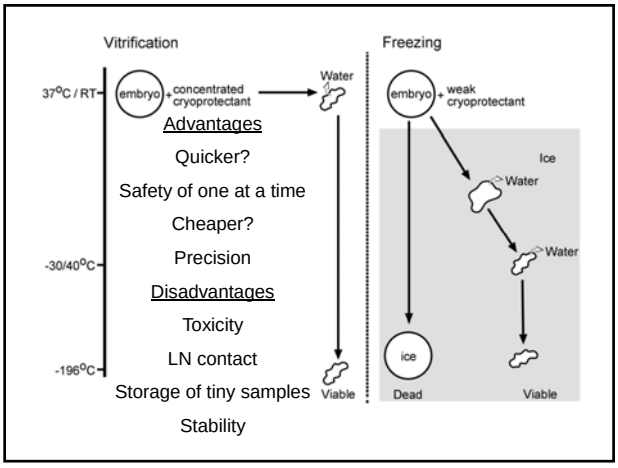
Precision

Disadvantages

Toxicity

LN contact

Storage tiny samples



Advantages

Quicker?

Safety of one at a time

Cheaper?

Precision

Disadvantages

Toxicity

LN contact

Storage of tiny samples

Stability

Incident

- Supplying clinic vitrifies
- Receiving clinic freezes
- No embryos survived
- Transport temperature?
- Error in warming?

Safeguards

- Controlled transport
- *Precise* protocol in advance
- Correct warming solutions
- Practise warming procedure

How successful is vitrification in practice?

- Practical advantages?
- Robust?
- Improved survival?
- Live births per embryo?
- Safe?

Freezing *vs V*itrification†

	Frozen	Vitrified
No. embryos warmed	232	234
% Survived	89	95*
% Blastocysts	50	60*
Overall survival	44	57*

Metabolism significantly reduced after freezing

†Data from donated supernumerary day 3 embryos (Balaban *et al* 2005)

Survival of biopsied embryos

Method	Embryos (n)	% Survived	% Blastocyst
Slow freeze	Control (53)	85	20
Slow freeze	Biopsied (52)	16	2
Modified freeze	Biopsied (52)	75	23
Modified thaw	Biopsied (50)	76	14
Vitrification	Biopsied (49)	94	18

Zheng *et al* 2005

Vitrification *vs* Freezing in clinical practice

Better outcome?

- embryo survival
- implantation
- pregnancy
- live birth

Vitrification *vs* Freezing

	Frozen	Vitrified	No. reports vitrified> frozen
% Survival	60-92	90-100	4/5
% Pregnancy per ET	17-51	27-53	2/5

Kuwayama *et al* 2005; Raju *et al* 2005; Stehlik *et al* 2005; Liebermann & Tucker 2006

Freezing *vs* vitrification Embryo survival

	% Embryos surviving (n)				
	4-cell	6-8-cell	Blastocyst		
Frozen	91 (942)	60 (120)	84 (156)	86 (147)	92 (570)
Vitrified	98* (897)	95* (127)	90* (6328)	100* (77)	97 (547)

Kuwayama *et al* 2005; Raju *et al* 2005; Stehlik *et al* 2005; Liebermann & Tucker 2006

Freezing *vs* vitrification Pregnancy rates

	% Pregnancy per ET (no of ET)				
	4-cell	6-8-cell	Blastocyst		
Frozen	32 (536)	17 (23)	51 (98)	18 (51)	46 (254)
Vitrified	27 (504)	35* (40)	53 (4745)	43* (35)	43 (254)

Kuwayama *et al* 2005; Raju *et al* 2005; Stehlik *et al* 2005; Liebermann & Tucker 2006

Outcome of freezing *vs* vitrification

- Lack of prospective randomisation
- Unequal/small samples
- Definition of survival
- Definition of pregnancy
- Lack of live birth data
- Inadequate freezing protocols

Vitrification *vs* freezing

Does the method of cryopreservation carry any risk for the children conceived from the embryos?

Risks for the children?

Freezing	Vitrification
• >350,000 children	• >3000 children
• Follow-up studies	• No follow-up
• Long term effects?	• Long term effects?

Conclusions

- Vitrification very promising
- Before replacing freezing:
  - Prospective randomised comparisons
  - Assess “robustness” in various clinics
  - Live birth data
  - Safety during storage
  - Examine freezing protocols

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Vitrification

Working together – theory and practice

G. Bocken and I. De Croo

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Learning Objectives

- Basic principles of slow freezing
- Basic principles of vitrification
- Technical challenges of vitrification
- Short overview literature
- Closed vitrification procedure (by Geertrui Bocken)

Introduction

Cryopreservation of reproductive cells

↓

Stopping biological time

↓

-196°C

Equilibrium cooling
Slow freezing

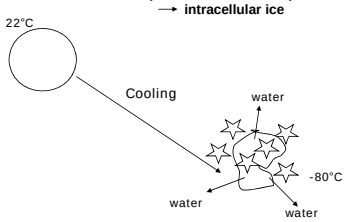
non-equilibrium cooling
vitrification

Freezing proces

extracellular ice formation : rise of osmolarity by

1) efflux of cell water
→ cell shrinkage

2) formation of a solid phase in the cell
→ intracellular ice

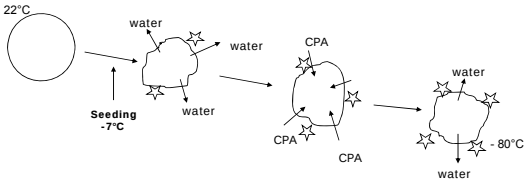


Freezing proces : equilibrium cooling

How ? : - adding a cryoprotective agent (CPA) (Dimethylsulfoxide, glycerol,1,2- propanediol, etc ...)

- optimal slow cooling rate

- inducing extracellular ice crystals (seeding)



Cryoprotective agent (CPA)

A **cryoprotectant** is a substance that is used to protect **biological tissue** from **freezing damage** (damage due to ice formation).

Conventional **cryoprotectants** are glycols such as **ethylene glycol**, **propylene glycol** and **glycerol**. Ethylene glycol is commonly used as **automobile antifreeze** and **propylene glycol** has been used to reduce ice formation in ice cream.

Dimethyl sulfoxide (DMSO) is also regarded as a conventional cryoprotectant. Glycerol and DMSO have been used for decades by cryobiologists to reduce ice formation in sperm and embryos that are cold-preserved in liquid nitrogen.

Examples of slow freezing protocols

Slow freezing protocol for embryos with DMSO

- Equilibration in 0.75 M and 1.5 M for 10 minutes each
- Transfer to a biological freezer

0°C
-2°C/min
seeding → -7°C hold for 10 minutes
-0.3°C/min
-80°C
-25°C/min
-196°C
plunge into liquid nitrogen (-196°C)

Total : ± 4 hours 30 minutes

Examples of slow freezing protocols

Slow freezing protocol for embryos with 1,2 PrOH

- Equilibration in 0 M and 1.5 M PrOH for 10 minutes for 10 minutes each
- Load straws in 1.5M + 0.1 M surcose
- Transfer to a biological freezer

22°C
-3°C/min
seeding → -7°C hold for 10 minutes
-0.3°C/min
-30°C
-25°C/min
-196°C
plunge into liquid nitrogen (-196°C)

Total : ± 2 hours

Equilibrium cooling

Vitrification

Non-equilibrium cooling or vitrification

Definition

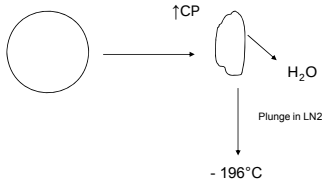
Vitrification is a process by which a liquid is solidified into a non-crystalline (glassy) phase by lowering rapidly the temperature below the 'glass transition' temperature and greatly increasing the viscosity

Vitrification

•Dehydratation before cooling

•Very high concentrations of CPA

•Very fast cooling (-25 000°C/min) to avoid damage by 'chilling'



Example of vitrification protocol

* Equilibration for 10 minutes
* Exposure to vitrification medium for 20 sec in total (2 x 5 sec and 1x10 sec) and load the specimens to the carrier (max 90 sec)
* Plunge into liquid nitrogen

Total : +/- 12 minutes

But only 1 or 2 specimens at a time

Vitrification

Vitrification

Variables of vitrification

- Effect of cooling and warming rates
  Concentration of CPA low or near zero: cooling and warming rates
  > 1 000 000°C/min

-permeability of cells to water and CPA
  *Glycerol> EG>DMSO>PG
  *Oocytes<zygotes<embryos<blastocysts

-toxicity of CPA
  *type and concentration of CPA
    PG>EG>DMSO>Glycerol
  * temperature of exposure

Vitrification

Vitrification and slow freezing : a comparison

Slow freezing	Vitrification
slow cooling (0.3°C/min)	very fast cooling (25 000°C/min)
low conc CPA (1.5M)	high conc CPA (5-7M)
ice crystallization	no ice crystallization
big volumes 250µl	very small volumes < 1µl
dehydration during cooling	dehydration before cooling
expensive equipment	expensive devices and vitrification solutions

Technical challenges of vitrification

•Small volumes : well trained embryologists

•High cooling rates : open systems
direct contact with liquid nitrogen : cross contamination

•Search for an efficient closed carrier

•Concentration and type of CPA
succesfull vitrification depends on 'sufficient' penetration of permeating CPA's and 'sufficient' dehydration by non-permeating CPA's

*Permeability characteristics of cells to water and CPA
- temperature and time dependency
- variability amongst cells

•Risk of crystallization during storage or warming

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Vitrification

Vitrification of :

Oocytes

Embryos

Blastocysts

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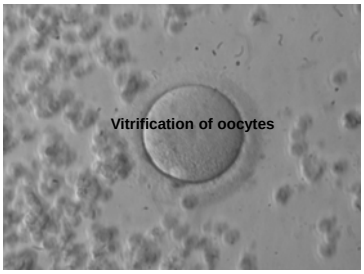
Vitrification



Vitrification of oocytes

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Vitrification of oocytes

Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method

Ana Cobo, Ph.D.,* Mauralejos Bernardo, Ph.D.,* Sonia Pérez, Ph.D.,* Augusto Buxaderas, M.D.,* Antonio Beltrán, M.D.,* and José Remohí, M.D.*

* IVI Universidad de Valencia, Valencia, Spain, and † Kato Lahav Clinic, Hadera Hospital, Hadera, Haifa, Israel

TABLE 3

Oocyte distribution, survival, and fertilization.

	Vitrified	Fresh	*P* value
MII oocytes No. (%)	221 (67.2)	218 (66.7)	.360
MI oocytes No. (%)	76 (7.2)	11 (3.4)	.202
GV oocytes No. (%)	12 (5.1)	4 (2.7)	.671
Retrieval No. (%)	229/251 (89.0)		
No. of injected oocytes	22.4	27.9	
Normal fertilization No. (%)	171 (75.1)	165 (82.2)	.138
Abnormal fertilization No. (%)	9 (4.0)	12 (5.4)	.499
Degenerated oocytes No. (%)	7 (3.1)	6 (2.7)	.809

Cobo. Clinical outcome of oocyte vitrification. Fertil Steril 2008

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Vitrification of oocytes

TABLE 4

Clinical results.

	Vitrified	Fresh	Mixed
No. of transfers	23	1	4
No. of embryos transferred (mean ± SD)	49 (2.1 ± 1.2)	2 (2 ± 0)	9 (2.1 ± 0.1)
Pregnancy rate (per transfer	15/23 (65.2)	1 (100)	2 (50)
Implantation rate (No. of sacs/ No. of embryos transferred)	20/49 (40.8)	2/2 (100)	3/9 (33)
Multiple pregnancy rate (twin)	6/15 (33.3)	1 (100)	0
Miscarriage rate	2/15 (20)	0	0
Biochemical pregnancy rate	1/15 (6.6)	0	0
Ongoing pregnancy rate	11/23 (47.8)	1 (100)	2 (100)

Note: The chance in parentheses are percentages.

Cobo. Clinical outcome of oocyte vitrification. Fertil Steril 2008

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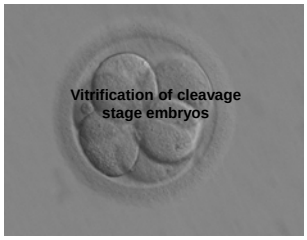
Vitrification



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Vitrification of cleavage stage embryos

A randomized controlled study of human Day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism and blastocyst formation[1]

E. Balaban[1], B. Urman[1,2], B. Ata[1], A. Isikoglu[1], M.G. Larman[1,3], K. Hamilton[1] and D.K. Gardner[1,3]

Table II. Outcomes of vitrification and slow freezing.

	Vitrification	Slow freezing	95% CI of difference	*P*-value
Cryosurvival (%)	222/234 (94.8)	206/232 (88.7)	+1% to +11%	0.02
Embryos with 100% blastomere survival (%)	171/222 (77.9)	106/206 (51.4)	+18% to +35%	<0.01
Blastocyst formation (%)	134/222 (60.3)	102/206 (49.5)	+1% to +20%	0.02
Blastocysts ≥ 3AA (%)	70/134 (52.2)	43/102 (42.1)	−2.7% to +22.8%	0.12
Hatching blastocysts (%)	42/134 (31.3)	22/102 (21.5)	−1.4% to +20.9%	0.09

CI, confidence interval.

Vitrification of cleavage stage embryos

Table III: Clinical outcome of vitrified-warmed embryo transfers.

No. of women	52
No. of embryos vitrified (mean)	344 (6.2)
No. of embryos warmed (mean)	240 (5.8)
Cryosurvival (%)	222/240 (92.5)
No. of embryos with 100% blastomere survival (%)	169/222 (72.1)
No. of women on Day 6 (%)	146/222 (65.8)
No. of embryos transferred (mean)	160 (2.9)
No. of women E2 (%)	146/160 (91.3)
Clinical pregnancy rate (%)	56/74 (49.3)
Implantation rate	59/98
Ongoing pregnancy rate (%)	53/74 (45.2)
Multiple pregnancy rate (%)	13/56 (36.1) (1 triplet, 12 twins)
Abortions (%)	3/56 (6.2)
Deliveries[a] (%)	8/53 (14.2) (2 twins, 6 singletons)

[a]Deliveries until the time of writing the manuscript.

Vitrification

Vitrification of blastocysts

Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application

Zeynep Kuleshovska, M.D.,* and Michael J. Tucker, M.D.**
*Fertility Centers of Illinois, Chicago, Illinois; and **Chicago Reproductive Specialist Associates, Chicago

TABLE 1

Retrospective data from the blastocyst cryopreservation program (Fertility Centers of Illinois, Chicago) where both vitrification (VIT) and conventional (CONV) technologies were applied from January 2004 to December 2005.

Technique	VIT	CONV
Patients' age (y)	34.2 ± 5.0	35.1 ± 4.7
No. of thawed cycles	257	259
No. of transfers	254	254
No. of blastocysts warmed/thawed	547	570
No. of blastocysts survived (%)	529 (96.5)	525 (92.1)
No. of blastocysts transferred	523	518
Mean no. of blastocysts transferred	2.0	2.0
No. of implantations (%)	160 (30.6)	152 (28.9)
No. of positive pregnancy/thaw (%)	132 (51.4)	135 (52.1)
No. of positive pregnancy/FET (%)	132 (51.9)	135 (53.1)
No. of clinical pregnancy/thaw (%)	117 (45.5)	109 (42.1)
No. of clinical pregnancy/FET (%)	117 (46.1)	109 (42.9)
Ongoing pregnancies (%)	117 (88.6)	109 (79.6)
No. of livebirths	54	79

Note: $P > .05$ for every comparison. FET = frozen embryo transfer.

Kuleshovska. Comparison of vitrification and conventional cryopreservation of blastocysts. Fertil Steril 2006.

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Conclusion

Vitrification seems to be superior to slow freezing for oocytes, embryos and blastocysts regarding post-thaw survival

BUT

more prospective trials are needed to confirm this and to evaluate pregnancy outcomes and follow-up of the children

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Vitrification

Vit Kit TM – Freeze Technique

UZ man, vrouw en kind Universitair Ziekenhuis Gent Universitair Ziekenhuis Brussel

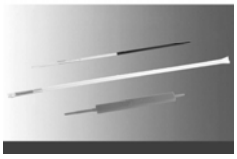
Materials Required for Vitrification

- Stereomicroscope (NIKON)
- Mini Incubator (K system)
- Stopwatch or Timer
- Liquid nitrogen (LN_2) (Air Liquide)
- Dewar Agil 3 (Air Liquide)
- Sealer (Cryo Bio System)

- Flexipet^R manipulation pipette (Cook)
  - Denuding pipette flexipet – K-FPIP-1300-10BS
- Stripper^R (Cook)
  - Adjustable handle for all flexipet sizes – K-MPH-1000
- Petri-dish Falcon^R
  - (Becton Dickinson) 351006 50 x 9 mm
- Eppendorf Research (20 -200 µl)
- Eppendorf biopur 200 µl

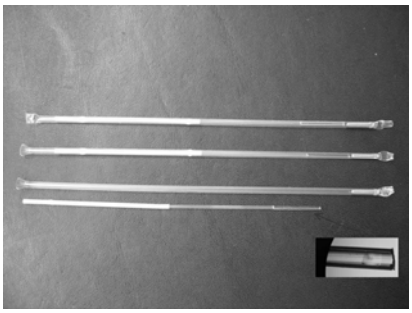
High Security Vitrification kit (Cryo Bio System)

- Composed of 3 parts:
  - A High Security ionomeric recin straw
  - A capillary tube with pre-formed gutter and colour-coded holder
  - A blue plastic insertion and removal device



- Sterilized by irradiation

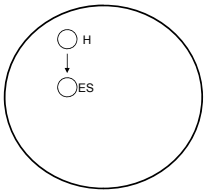
High Security Vitrification kit (Cryo Bio System)



Vit Kit™ – Freeze (Irvine Scientific)

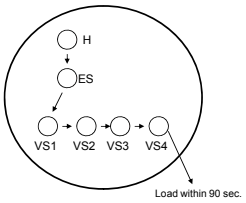
- Equilibration Solution (ES)
  - 7.5% v/v DMSO
  - 7.5 % v/v ethylene glycol
  - 20% Dextran Serum Supplement (DSS)
  - In M199-H
- Vitrification Solution (VS)
  - 15% v/v ethylene glycol
  - 15% v/v DMSO
  - 0.5 M sucrose
  - 20% Dextran Serum Supplement (DSS)
  - In M199-H

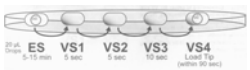
Vitrification Protocol



- Prepare a Petri-dish with a droplet of 20 µl HEPES buffered Culture Medium (H)
  - Hold at 37°C
- Transfer the specimen into the droplet
  - Hold the dish at room temperature
- Transfer the specimen in a droplet of 20 µl ES solution
  - Expose to ES for 5 – 15 min
  - The specimen will shrink and then gradually return to its original size which indicates that the equilibration is complete.

Vitrification Protocol





- Prepare four droplets of 20 µl VS
- Rinse the specimen in the first droplet of VS (vs1) and transfer it Quickly into the second (vs2) and third (vs3)one
- Finally, transfer the specimen to the fourth drop of VS (vs4)
- The specimen is now ready to be loaded on the straw and plunge into liquid nitrogen within 90 sec.

High Security Vitrification kit (HSV) Loading


Fig. 1

- Deposit the specimen into the gutter a millimetre from the end. (fig.1)


Fig. 2

- Place the capillary rod an handler into the straw and push until the rectangular portion of the handler comes in contact with the flared end of the straw (fig. 2)


Fig. 3

- Seal the open end using a SYMS sealer (fig. 3)


Fig. 4

- Quickly plunge the entire straw into the liquid nitrogen (fig. 4)

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Vitrification

Vit Kit TM – Thaw Technique

UZ man, vrouw en kind Universitair Ziekenhuis Gent
Universitair Ziekenhuis Brussel

Materials Required for Vitrification

- Stereomicroscope (NIKON)
- Mini Incubator (K System)
- Stopwatch or Timer
- Liquid nitrogen (LN_2) (Air Liquide)
- Dewar Agil 3 (Air Liquide)
- Sharp scissors (sterile)
- Flexipet^R manipulation pipette (Cook)
  - Denuding pipette flexipet – K-FPIP-1300-10BS
- Stripper^R (Cook)
  - Adjustable handle for all flexipet sizes – K-MPH-1000
- Petri-dish Falcon^R
  - (Becton Dickinson) 351006 50 x 9 mm
- Eppendorf Research (20 -200 µl)
- Eppendorf biopur 200 µl

Vit Kit™ – Thaw (Irvine Scientific)

- Thawing Solution (TS)
  - 1.0 M Sucrose
  - 20% DSS
  - In M199-H
- Dilution Solution (DS)
  - 0.5 M Sucrose
  - 20% DSS
  - In M199-H
- Washing Solution (WS)37
  - 20% DSS
  - In M199-H

Thaw protocol

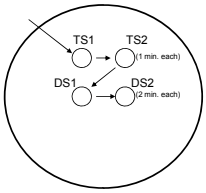
Fig. 1

Fig. 2

Fig. 3

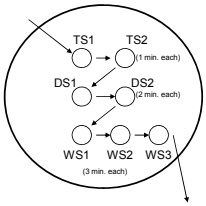
- Prepare a Petri-dish with 2 droplets of 20 µl TS
  - Hold at 37°C
- Lift the straw to expose the coloured rod. (fig.1)
  - Make sure that the end with the sample remains immersed in the liquid nitrogen.
- Cut the straw above the handling rod. (fig.2)
- Insert the short end of the removal device into the coloured handler and pull the capillary out of the straw (fig. 3)

Thaw protocol



- Put immediately the gutter into the first 20 µl TS droplet.
  - The specimen will be floating and bring it down.
  - Hold the Petri-dish at room temperature
- After 1 min. transfer the specimen in the other 20 µl TS droplet for another min.
- Prepare two DS droplets of 20 µl each
- Transfer the specimen to DS1 and DS2 drops for 2 min. each

Thaw protocol



- Prepare three WS droplets of 20 µl each
- Transfer the specimen to WS1, WS2 and WS3 drops for 3 min. each
- After the last 3 min. In WS transfer the specimen in a culture system and evaluate the survival rate

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