PRE-CONGRESS COURSE 15

Optimizing IVF outcome

Middle East Fertility Society Exchange Course
London - UK, 7 July 2013

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Optimizing IVF outcome

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Organised by
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Contents

Course coordinators

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Teaching aims and course description

How to optimize IVF results based on high quality evidence.

Course description and characteristics

This is an advanced course in IVF.
Steps to optimize IVF before starting the procedure by identification of the conditions which may negatively affect the results of IVF, it should recognized and treated. The optimum stimulation protocol for IVF will be discussed. Optimizing culture conditions for oocytes and embryos is essential for generating high quality embryos. Optimum culture conditions will be explained. The number of embryos transferred with emphasis on the current and future role of single embryo transfer is essential for a current IVF program. Results of single embryo transfer will be evaluated.
The technique of embryo transfer is critical to the success of IVF. The methodology of embryo transfer, how to choose the embryo transfer catheter in addition to the importance of mock embryo transfer will be discussed.
The prevention of the most serious complication of ovulation induction which is ovarian hyperstimulation syndrome is an important step to ensure safety of IVF.
Implantation is the critical step for improving pregnancy rate, explanation of the unclear aspects of implantation will be discussed and possible future steps to improve implantation rate will be explored.
Vitrification which gained widespread use in cryopreservation of embryos and oocytes became the method of choice for freezing embryos. Details and advantages will be explained.

Target audience

Physicians, ART specialists, registrars in Ob/Gyn, Fellows in assisted reproduction, embryologists and biologists

Scientific programme

Chairman: Mohamed A. Aboulghar - Egypt

09:00 - 09:30	Identification and management of conditions detrimental to IVF outcome *Mohamed A. Aboulghar - Egypt*
09:30 - 09:45	Discussion
09:45 - 10:15	Optimizing the stimulation protocol for IVF *Bart C.J.M. Fauser - The Netherlands*
10:15 - 10:30	Discussion
10:30 - 11:00	Coffee break
11:00 - 11:30	Is single embryo transfer going to be the future? *Siladitya Bhattacharya - United Kingdom*
11:30 - 11:45	Discussion
11:45 - 12:15	Optimizing culture conditions *Antonio Capalbo - Italy*
12:15 - 12:30	Discussion
12:30 - 13:30	Lunch
13:30 - 14:00	Prevention of OHSS *Mohamed A. Aboulghar - Egypt*
14:00 - 14:15	Discussion
14:15 - 14:45	Techniques and technologies for embryo transfer: Does it really matter? *Johnny Awwad - Lebanon*
14:45 - 15:00	Discussion
15:00 - 15:30	Coffee break
15:30 - 16:00	Improving implantation – The endometrial factor matters *Carlos Simon Valles - Spain*
16:00 - 16:15	Discussion
16:15 - 16:45	Optimizing the outcome of cryopreservation *Safaa Al-Hasani - Germany*

Identification and management of conditions detrimental to IVF

M. Aboulghar, M.D.
Professor, Cairo University
Clinical Director, The Egyptian IVF Center

There are no commercial relationship or other activity related to this lecture which might be perceived as a potential conflict of interest.

Learning Objectives

At the conclusion of this course, the participant should be able to:

- *Evaluate the effect of an intramural fibroid on the outcome of IVF.*
- *Demonstrate the effect of hydrosalpinx on the pregnancy rate after IVF and evaluate lines of treatment necessary to restore the normal chance of IVF outcome.*
- *Find out if surgical excision of endometriomas will be of value in the IVF outcome.*
- *Understand the possible value of excision of uterine septum in improving pregnancy rate after IVF.*

Conditions which may be detrimental to IVF outcome

1. Uterine fibroids.
2. Hydrosalpinx
3. Endometrioma
4. Uterine septum
5. Vaginal infection

Uterine fibroids occur in up to 30% of reproductive age women (Verkauf 1992)

- There is a consensus that submucous fibroids and fibroid polyps affect the outcome of IVF and that they should be removed before starting stimulation.
- Subserous fibroids seem not to affect the outcome of IVF.

(Bernard et al. 2000)

Intramural fibroids and infertility

Intramural fibroids are myomas completely surrounded by muscular wall of the uterus, however, they vary in size, number and distance from endometrial cavity.

The problem of diagnosis of intramural fibroids (1)

- Transvaginal ultrasound:
  - Transvaginal ultrasound can precisely measure the size of the fibroids (Pritts et al 2009).
  - Earlier reports suggested high sensitivity and specificity of vaginal US in excluding uterine cavity involvement (Fedele et al 1991).
  - Recent studies showed sensitivity as low as 69% in locating fibroids by vaginal US (Ayida et al., 1997).

The problem of diagnosis of intramural fibroids (2)

- Hysteroscopy was found to be adequate in evaluating the uterine cavity to exclude a submucosal part of an intramural fibroid (Soares et al., 2000).

The problem of diagnosis of intramural fibroids (3)

- Sonohysterogram can provide 100 percent sensitivity and specificity for identifying the exact local of the fibroid (Pritts et al. 2009).

The problem of diagnosis of intramural fibroids (4)

- Magnetic resonance imaging (MRI) may provide the best means by which to assess whether an intramural fibroid impacts the endometrial cavity either through actual distortion or through its relationship to the junctional zone (the anatomically distinct segment of the uterus that represents the endometrial-myometrial transition) (Somigliana et al 2007).

The problem of diagnosis of intramural fibroids (5)

- Intramural fibroids may disrupt the junctional zone of the myometrium without dramatically altering the contour of the uterine cavity. The junctional zone is structurally and hormonally different from the other layers of the uterine body and further research my elucidate its roles in fertility and how disruptive of this zone by fibroids and/or adenomyosis can reduce implantation (Somigliana et al 2007).

The effect of intramural fibroids on fertility and the outcome of IVF treatment remain poorly understood with studies yielding conflicting results (Sunkara et al 2010)

Patients having subserosal or intramural leiomyomas of <4 cm not encroaching on the uterine cavity have IVF-ICSI outcomes comparable to those of patients without such leiomyomas. Therefore, they might not require myomectomy before IVF. Whether or not women with fibroids > 4 cm would benefit from fibroid treatment remains to be determined.

Oliveira et al. 2004

Epidemiological evidence on the relationship between infertility and intramural fibroids is not conclusive due to methodological limitations.

Why studies on intramural fibroids are inconclusive?

- Most of studies are retrospective.
- Exact position of fibroids in the muscular wall of the uterus is not defined in most studies.
- Number and size of fibroids is not clear in the majority of studies.
- Distance between fibroid and endometrium is not specified in the studies.

In 2001, Pritts et al. performed a systematic review of controlled studies examining the issue of fibroids as a cause of infertility. The analysis failed to demonstrate any effect of fibroids on fertility outcomes except when the tumors deformed the endometrial cavity.

After 8 years, in another meta-analysis, the same authors reported that women with IM fibroids produced significantly lower clinical pregnancy rates, implantation rates, and ongoing pregnancy/live birth rates and significantly higher spontaneous abortion rates (Pritts et al., 2009).

Does myomectomy improve fertility?

Fertility outcomes are decreased in women with submucosal fibroids, and removal seems to confer benefit. Subserosal fibroids do not affect fertility outcomes, and removal does not confer benefit. Intramural fibroids appear to decrease fertility, but the results of therapy are unclear. More high-quality studies need to be directed toward the value of myomectomy for intramural fibroids, focusing on issues such as size, number, and proximity to the endometrium (Pritts et al 2009).

Recent evidence supports the opinion that IVF pregnancy rate is reduced in the presence of intramural fibroids and the pregnancy rate is not affected if the fibroids is removed (Somigliana et al 2007)

If IM fibroids do indeed decrease fertility, it is not a given that their removal will reverse the process and normalize fertility or even be beneficial to the patient. Abdominal or laparoscopic myomectomy can be associated with significant morbidity, including infection
high rate of postoperative adhesion formation (Pritts 2009)

The current results show, there is no clear evidence at this time that myomectomy for IM fibroids is beneficial (Somigliana et al 2007)

Buttram and Reiter (1981) reported a 40% pregnancy rate following abdominal myomectomy (480 out of 1202 cases). A more recent comprehensive review of articles published between 1982 and 1996 on the success rate after abdominal myomectomy confirmed this rate of success. The post-surgical pregnancy rate across prospective studies was 57% (95% CI 48–65) (Sunkara et al 2010).

Patients with intramural fibroids were divided into myomectomy versus expectant treatment, based on their own wish. Each group had 84 patients. The cumulative delivery rate was 25% in myomectomy arm versus 12% in no treatment arm (P=0.01) (Bulletti et al. 2004)

What is the effect of intramural fibroids on IVF outcome? and does myomectomy improve IVF outcome?

The effect of fibroids not distorting the uterine cavity on the outcome of IVF treatment remains poorly understood with studies yielding conflicting results.

Moreover, demonstration of reduction in IVF live births in women with non-cavity-distorting intramural fibroids does not necessarily mean that removal of such fibroids will restore the live birth rates

Sunkara et al (2010) identified 19 observational studies comprising 6087 IVF cycles. Meta-analysis of these studies showed a significant decrease in the live birth (RR = 0.79, 95% CI: 0.70–0.88, $P < 0.0001$) and clinical PRs (RR = 0.85, 95% CI: 0.77–0.94, $P = 0.002$) in women with non-cavity-distorting intramural fibroids compared with those without fibroids, following IVF treatment.

Clinical evidence support the vision that fibroids may interfere with fertility. IVF suggests a detrimental effect on implantation: the delivery rate is reduced in patients with fibroids, while it is not affected in patients who had undergone myomectomy. Second, surgical treatment appears to increase the pregnancy rate (Sunkara 2009)

- A matched follow up study showed that uterine leiomyoma significantly reduced the chance for a clinical pregnancy or delivery. These findings suggest that leiomyomas are associated with a reduction in the efficacy of assisted reproduction cycles. Stovall et al 1998.

- In a prospective controlled study on 112 women with intramural fibroids and 322 controls, it was found that an intramural fibroid halves the chances of an ongoing pregnancy in IVF. Hart et al 2001

The effect of small intramural uterine fibroids on the cumulative outcome of assisted conception (Khalaf et al 2006)

- 322 women without fibroids and 112 women with fibroids underwent 606 IVF/ICSI cycles. Live birth rates in the study group 14.8% compared to 24% in the control group, ($P<0.05$). The cumulative ongoing pregnancy rate was reduced by 43% (HR=0.57, 95% CI=0.35-0.91, P=0.018), and the cumulative live birth rate was reduced by 47% (HR=0.53, 95% CI=0.32-0.87, P=0.013)

The ability to correctly identify the location and size of a patient's fibroids is critical for determining which patients require surgical management, as the current literature suggests that certain fibroids have a greater impact on fertility than others.

The inverse relationship between IVF outcome and the presence of non-cavity distorting intramural fibroid may be explained by altered uterine vascular perfusion, myometrial contractility, endometrial function, gamete migration or myometrial/endometrial gene expression (Arslan et al., 2005)

The Practice Committee of the American Society for Reproductive Medicine (2006) revised their earlier report and recommends surgical treatment after complete evaluation of other potential factors of infertility.

Hydrosalpinx

Hydrosalpinx and IVF outcome

- A prospctive randomized trial was to test if a salpingectomy prior to IVF was effective in terms of increased pregnancy rates.
- Patietns with hydrosalpinx were randomized to either a laparoscopic salpingectomy or no intervention before IVF.
- A total of 204 patients was available for an intention-to-treat analysis and 192 actually started IVF.

(Strandell et al. 1999)

Hydrosalpinx and IVF outcome (Continued)

- Clinical pregnancy rates per included patient were 36.6% in the salpingectomy group and 23.9% in the non-intervention group (not significant, p = 0.045).
- Delivery rates (40% versus 17.5%, p = 0.038) in patients with ultrasound visible hydrosalpinges.
- The delivery rate was increased 3.5-fold in patients with bilateral hydrosalpinges visible on ultrasound (P=0.019)

(Strandell et al. 1999)

Hydrosalpinx and IVF outcome: cumulative results (Continued)

- The results of the cumulative cycles strengthen the recommendation for a laparoscopic salpingectomy prior to IVF in patients with ultrasound-visible hydrosalpinges.

(Strandell et al. 2001)

- In a Cochrane review including 5 randomized studies (646 women). There was evidence that laparoscopic salpingectomy or laparoscopic tubal occlusion improved significantly the pregnancy rate, and surgical treatment should be considered for all women with hydrosalpinges prior to IVF (Johnson et al. 2010)

- To evaluate and compare the clinical impact of proximal tubal occlusion and salpingectomy before IVF in patients with hydrosalpinges, a prospective randomized study was conducted.
- Patients who underwent proximal tubal occlusion before IVF demonstrated ongoing-pregnancy rates compared to those who underwent salpingectomy.
- Proximal tubal occlusion may be viewed as a valid alternative when salpingectomy is technically difficult or not feasible.

(Kintoravdis et al 2006)

Comparison of IVF outcome in patients with hydrosalpinx pretreated with either sclerotherapy of laparoscopic salpingectomy

- Fifty-six patients underwent interventional ultrasound sclerotherapy and the remaining 41 patients received laparoscopic salpingectomy before IVF.
- IVF outcome of the two groups were compared.
- Ultrasound-guided HSF aspiration and sclerotherapy have IVF outcomes comparable to laparoscopic salpingectomy.
- Interventional ultrasound guided sclerotherapy before IVF is an effective and less invasive prophylactic intervention alternative to salpingectomy with hydrosalpinx (Na et al. 2012)

- Pophylactic salpingectomy in women with hydrosalpinx may compromise ovarian response to stimulaiton without affecting pregnancy rates.
- A randomized control trial is recommended to determine the most appropriate laparoscopic procedure in the management of hydrosalpinx before IVF.

(Gelbaya et al 2006)

Does salpingectomy affect the ipsilateral ovarian response to gonadotropin during in vitro fertilization-embryo transfer cycles?

- The observed significant decrease in the ipsilateral ovarian response after salpingectomy, as reflected by the quantity of developing follicles during controlled ovarian hyperstimulation for IVF, should be presented to patients during the decision-making process, before offering salpingectomy for the treatment of hydrosalpinx. (Orvieto et al 2011)

Essure ® hydrosalpinx occlusion prior to IVF as an alternative to laparoscopic salpingectomy

- Prospective clinical study in 20 women with unilateral or bilateral hydrosalpinges (all visible on transvaginal US) laparoscopy was considered to be contraindicated due to extensive pelvic adhesions.
- In all patients the Essure devices were placed in an ambulant setting without any complications. Proximal tubal occlusion was confirmed by hystersalpingography in 19 out of 20 patients (95%). After 45 embryo transfer procedures in 19 patients, 18 pregnancies with 12 live births, 6 miscarriages and 1 immature delivery.
- Essure devices are effective in inducing proximal tubal occlusion in subfertile patientswith hydrosalpinges.

(Mijatovic et al 2012)

- A total of 117 postal survey, anonymous, sealed questionnaires were sent to all IVF center in the UK. There were 75% responders, of which 91 did not recommend treamtnet while 36%, 33% and 19% recommended treatment weakly, strongly and very strongly repectively. (Hammadieh et al 2004)
- Using an anonymous and sealed questionnaire sent to all French IVF center, the current management of hydrosalpinx before of during IVF was evaluated. Laparoscopic salpingectomy was recommended and undertaken in less than half of the centers, even though several other treatments were reported and despite medical evidence for this surgical option (Ducarme et al. 2006)

Ovarian endometriomas

Removal of endometriomas before IVF does not improve fertiliety outcomes: a matched, case-control study

- Laparoscopic cystectomy for endometriomas before commencing an IVF cycle does not improve fertility outcomes. Proceeding directly to controlled ovarian hyperstimulation in women with asymptomatic ovarian endometriomas might reduce the time to pregnancy, the cost of treatment, and the hypothetical complications of laparoscopic surgery (Garcia-Velasco et al. 2004)

Ovarian endometriomas and IVF: a retrospective case-control study

- Ovarian endometriosis does not reduce IVF outcome compared with tubal factor. Furthermore, laparoscopic removal of endometriomas does not improve IVF results, but may cause a decrease of ovarian responsiveness to gonadotropins (Bongioanni et al 2011)

- At laparoscopy, endometriomas > 3 cm were treated by ovarian cystectomy.
- The number of oocytes and embryos obtained was not significantly decreased by laparoscopic cystectomy, suggesting that in experienced hands this procedure may be a valuable surgical tool.
- Great care must be taken to avoid ovarian damage. (Canis et al 2001)

Effects of ovarian endometrioma on the number of oocytes retrieved for IVF

- The numbers of antral follicles and the retrieved oocytes in the ovary that contained endometrioma were compared with those from the contralateral ovary.
- The presence of ovarian endometrioma in a controlled ovarian hyperstimulation cycle for IVF treatment is not associated with a reduced number of oocytes retrieved from the affected ovary (Almog et al 2011)

Evidence-based management of endometrioma

- Except of pelvic clearance, there is insufficient evidence to suggest that surgical treatment of endometrioma is better than medical treatment with respect to the long-term relief of symptoms and quality of life. Laparoscopic excision of ovarian endometrioma prior to IVF does not offer any additional benefit over expectant management (Gelbaya and nardo 2011).

Does ovarian surgery for endometriomas impair the ovarian response to gonadotropin?

- A similar IVF outcome was observed in patients with endometiosis after ablation of endometriomas compared to women with tubal factors. (Donnez et al. 2001)

Effect of endometrioma cystectomy on IVF outcome: a prospective randomized study

- The patients were prospectively randomized into two groups; group I (50 patients) underwent the ICSI cycle directly.
- Ovarian surgery resulted in longer stimulation, higher FSH requirement and lower oocyte number, but fertilization pregnancy and implantation rates did not differ between the groups (Demirol et al. 2006).

The effect of surgical treatment for endometrioma on IVF

- To investigate the effect of surgical treatment of endometrioma on pregnancy rate and ovarian response to gonadotrophin stimulation in women undergoing IVF a systematic review and meta analysis were performed.
- Surgical removal of endometrioma or expectant management.

(Tsoumpou et al 2009)

The effect of surgical treatment for endometrioma on IVF (Continued)

- Meta-analysis was conducted for five studies that compared surgery vs. no treatment of endometrioma. There was no significant difference in clinical pregnancy rate between the treated and the untreated groups.
- Literature shows that surgical management of endometriomas has no significant effect on IVF pregnancy rates and ovarian response to stimulation compared with no treatment.

(Tsoumpou et al 2009)

Impact of ovarian endometrioma on oocytes and pregnancy outcome

- Fewer oocytes were retrieved
- Endometrioma affect oocyte number but not embryo quality or pregnancy outcome, irrespective of the presence of an ovarian endometrioma

(Suzuki et al, 2005)

Uterine Septum

Is hysteroscopic correction of an incomplete uterine septum justified prior to IVF?

- After surgical correction of the septum, IVF results were similar in both groups (Clinical pregnancy and pregnancy loss of 47.8 versus 46.5%)
- A similar pregnancy outcome was found after the incision of the incomplete septum compared with a group with normal uterine cavity.

(Ozgur et al., 2007)

Septate, subseptate and arcuate uterus decrease pregnancy and live birth rates in IVF/ICSI

- A retrospective matched-control study to evaluate the effect of uterine anomalies on pregnancy rates after 2481 embryo transfers.
- The study group of 289 embryo transfers before and 538 embryo transfers following hysteroscopic resection of a uterine septum was compared with two consecutive embryo transfers in the control group. Groups were matched.

(Tomazevic et al. 2010)

Septate, subseptate and arcuate uterus decrease pregnancy and live birth rates in IVF/ICSI (Continued)

- Pregnancy rates after embryo transfer before hysteroscopic metroplasty were significantly lower, both in women with subseptate and septate uterus and in women with acuate uterus compared with controls.
- Negative impact of uterine anomalies on pregnancy and on live birth rates are two important arguments for treating uterine anomalies in infertile women.

(Tomazevic et al. 2010)

Treatment of infection before IVF

- Antibiotic treatment based on seminal culture from asymptomatic male partners in IVF is unnecessary.
  (Liversedge et al 1996)

- Sharara et al (1997) recommended that all couples with elevated titers of chlamedia trachomatis IgG antibodies be treated with Doxycycline prior to IVF.

- IVF patients with bacterial vaginosis and with a decreased vaginal log concentration of hydrogen-peroxide-producing lactobacilli may have decreased conception rates and increased rates of early pregnancy loss.

Eckert et al 2003

Conclusion 1

- Treatment of submucous fibroids is essential before IVF.
- Subserous fibroids should be ignored.
- There are no studies to confirm the value of removing intramural fibroids, however there is evidence to suggest a benefit after removing them.

Conclusion 2

- Hydrosalpinx which are visualized by ultrasound reduce IVF outcome and should be removed
- As an alternative cornual ligation could be done.

Conclusion 3

- Removing uterine septum may improve the IVF outcome.
- It seems that excision of endometriomas does not improve IVF outcome and may reduce the number of oocytes retrieved.

Identification and management of conditions detrimental to IVF

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Optimizing Ovarian Stimulation for IVF

Prof.Dr. Bart CJM Fauser
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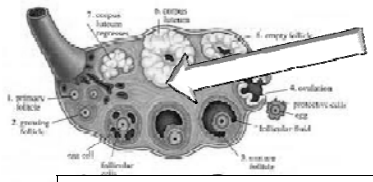
Utrecht, The Netherlands

Patient tailored ovarian stimulation



Lecture Outline
Background ovarian stimulation
Aims ovarian stimulation
Individualised dosing

Historical milestones
ovarian hyperstimulation for IVF

Year	Compound	Who did it..
1978	Non	Edwards & steptoe (UK)
1982	Clomiphene	Trounsen (Austr)
1984	HMG	Jones (US)
1986	GnRH agonist	Jacobs, Fleming (UK)
1991	GnRH antagonist	Frydman (Fr), Diedrich (G)
1992	recFSH (LH, hCG)	Devroey (B), Fauser (N)
2004	recFSH agonist	Devroey (B), Fauser (N)
2013	Oral LH	Mannaerts (N)

Aims of ovarian stimulation

- **Retrieve multiple oocytes for IVF procedure**
- Have multiple embryos available to choose from
- Compensate for suboptimal fertilisation and implantation
- Have access embryos for cryostorage

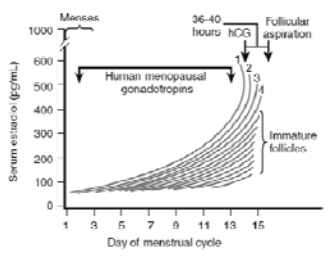
⇒ Improve IVF outcomes

What ovarian stimulation ?

approaches	compounds
Stimulation	Gonadotropins (urine, rec) CC, aromatase inhibitors, insulin sensitizers
Co-treatment (1)	GnRH agonist, antagonist
Co-treatment (2)	LH, hCG, androgens, GH, etc
Oocyte maturation triggering	hCG, GnRH agonist bolus
Pre-stimulation	GnRH agonist flare, OC, Estrogens
Post-stimulation	Prog, Estrogens, hCG

Downside of ovarian stimulation

- Burden of treatment
- Complexity
- Cost
- Complications



International disparities in access to infertility services

FS 2005

Robert D. Nachtigall, M.D.
Institute of Health and Aging, University of California, San Francisco, San Francisco, California

TABLE 3
International utilization of IVF.

IVF cycles/million population per year	% optimal IVF utilization	Countries
<15	1%	China, India, Pakistan, Indonesia, Egypt
<150	10%	United States, Japan, Russia, Argentina, Italy
<500	33%	United Kingdom, Germany, France, Brazil, Switzerland, Iran, Saudi Arabia, Belgium, Australia, Greece
<750	50%	Netherlands, Sweden, Denmark, Iceland
>1,500	100%	Israel

Note. Adapted from Collins (41).

Nachtigall. International infertility disparities. Fertil Steril 2006.

IVF Outcome Assessment

Started cycle

Oocyte Pick-Up

Embryo Transfer

Fresh ET

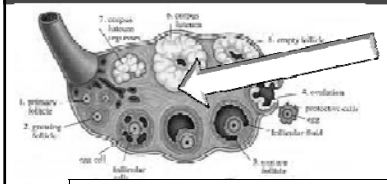
Fresh + Cryo ET

(per fresh ET)

(cum. per oocyte harvest)

(cumulative, per started IVF treatment)

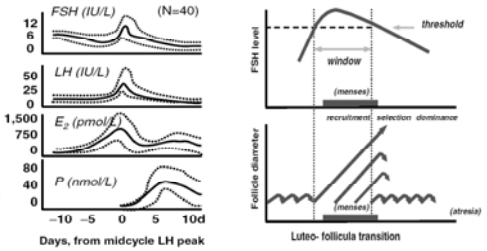
Patient tailored ovarian stimulation



Lecture Outline

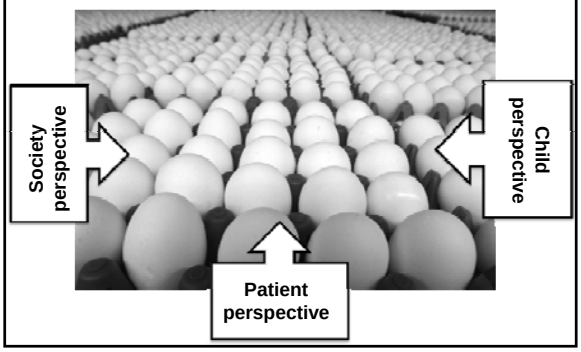
- Background ovarian stimulation
- Aims ovarian stimulation
- Individualised dosing

Follicle recruitment and dominant follicle selection



Pache, FS 1990
vSantbrink, FS 1995
Schipper, FS 1998

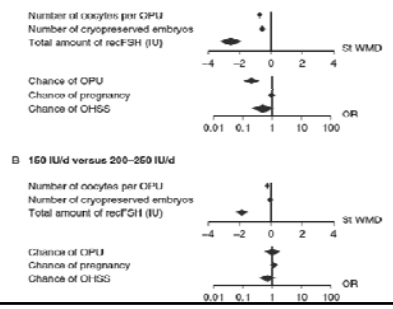
Optimal number of oocytes for IVF
- the more the better ??

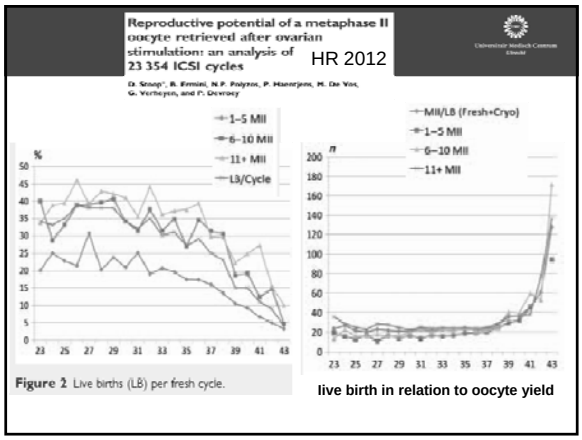


Clinical outcomes in relation to the daily dose of recombinant follicle-stimulating hormone for ovarian stimulation in *in vitro* fertilization in presumed normal responders younger than 39 years: a meta-analysis

human reproduction update

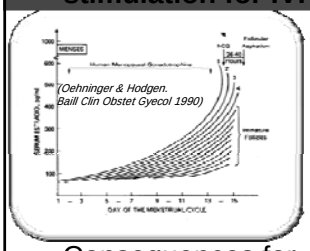
2011



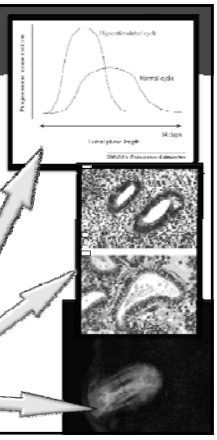


live birth in relation to oocyte yield

Sequela of ovarian stimulation for IVF



(Oehninger & Hodgen. Baill Clin Obstet Gyecol 1990)



Consequences for

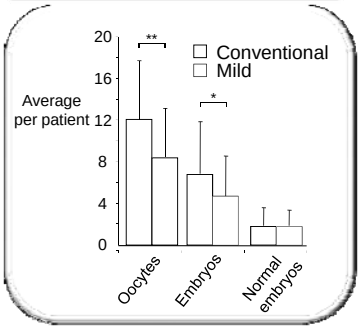
- luteal phase endocrinology
- Endometrial receptivity
- Embryo aneuploidy

Relevant studies in mice

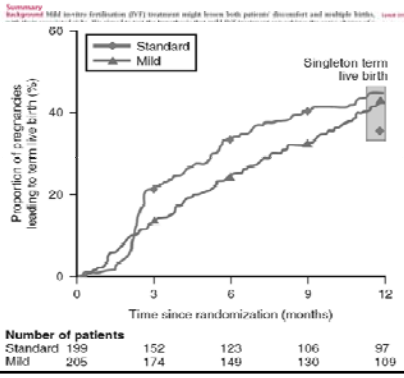
Superovulation in mice causes
Reduced oocyte quality
Reduced embryo quality
Delayed pre-implantation embryo development
Reduced implantation
Fetal growth retardation
Abnormal methylation of imprinted loci
Negative impacts on oviductal and uterine milieu

Laprise, Mol Reprod & Dev 2009

Milder ovarian stimulation for *In-vitro* fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial HR 2007



A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial Lancet 2007



Number of patients

Standard	199	152	123	106	97
Mild	205	174	149	130	109

Mild ovarian stimulation for IVF: 10 years later

human reproduction

DEBATE

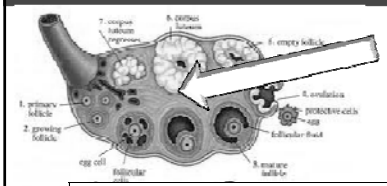
Bart C.J.M. Fauser[1,*], Geeta Nargund[2], Anders Nyboe Andersen[3], Robert Norman[4], Basil Tarlatzis[5], Jacky Boivin[6], and William Ledger[7]

2010

SWOT analysis

S	**Strength**	**Internal**
W	Weakness	
O	Opportunities	External
T	Threats	

Patient tailored ovarian stimulation



Lecture Outline

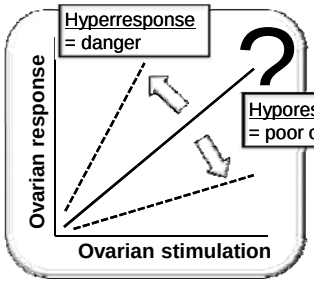
- Background ovarian stimulation
- Aims ovarian stimulation
- Individualised dosing

The paradigm shift in medicine

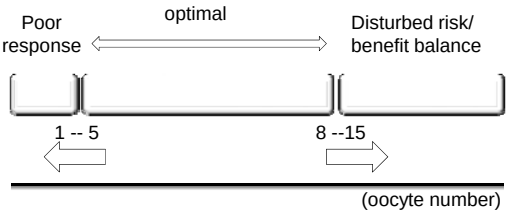
One size fits all

Patient tailored treatment algorithms

The need for more patient tailored ovarian stimulation for IVF



What ovarian response is optimal?



Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation

HRU 2009

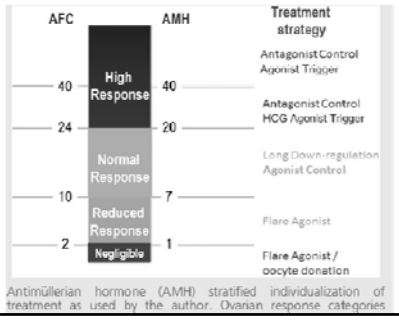
Ovarian stimulation for IVF

Olivennes, RBM'09	Popovic, HR'03
FSH	Total follicle no
BMI	Total ovarian volume
Age	Total doppler score
AFC	Age
	Smoking

Biomarkers of ovarian response: current and future applications

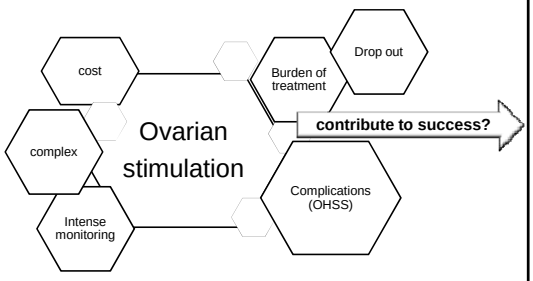
Scott M. Nelson, Ph.D.

F&S, 2013, E Pub



Antimüllerian hormone (AMH) stratified individualization of treatment as used by the author. Ovarian response categories

Ovarian hyperstimulation for IVF - the bigger picture



IVF and child health
(less is more?)

"Embryogenesis begins during oogenesis"

(EB Wilson)

PhD's thesis mild IVF

Is single embryo transfer going to be the future?

Professor Siladitya Bhattacharya FRCOG
University of Aberdeen, U.K.

Conflicts of interest

- **Support from pharmaceutical companies for departmental seminars**
- **Departmental colleagues receive support for conference attendance from pharmaceutical companies**
- **Speaker at conferences which receive support from pharmaceutical companies**

Learning objectives

- **Maternal and perinatal complications in twins**
- **Role of elective single embryo transfer (eSET)**
- **Trends of elective single embryo transfer over time**
- **Patients' preferences regarding number of embryos**
- **Effectiveness of elective single embryo transfer**
- **Outcomes following cleavage stage versus blastocyst**
- **Cost effectiveness of elective single embryo transfer in different age groups**
- **Conclusions**

Prematurity

“The rarity of plural births in women and increased danger to mother and offspring in these circumstances, renders such an event, in a certain limited sense, a disease of abnormality.”

James Matthews Duncan (1865)

Outcome of twins

- **Mortality** **46.8 per 1,000 (8.7)**
- **Prematurity** **47%**
- **Cerebral palsy** **5 fold ↑**
- **Need for prolonged aftercare**

Peterson et al, 1993; Callahan 1994; Yokoyama et al, 1995; Lieberman, 1998; Bergh et al, 1999

Twins: Maternal Morbidity

Twins N = 1,694 Singletons N = 71,851	Relative Risk (95% CI)
Pre-eclampsia	3.72 (3.26 – 4.25)
Placental abruption	2.02 (1.23 – 3.33)
PPH	2.83 (2.45 – 3.26)

Campbell & Templeton 2003

UK cost of IVF births

	Singleton	Twin	Triplet
Maternal cost	£3122	£6058	£11534
Neonatal cost	£191	£3064	£20,820
Cost per family	£3313	£9122	£32,354

Ledger et al, BJOG 2006

Subfertile women: Preference for twins

Author	Year	*n*	Preference for twins (%)
Gleicher	1995	582	67-90
Grobman	2001	200	67
Pinborg	2003	1030	62-85
Kalra	2003	180	30-38
Ryan	2004	449	20
Child	2004	801	41
Murray	2004	200	40
Steures	2005	40	77

Van Wely et al, 2006 Hum. Reprod.

DOI: 10.1111/j.1471-0528.2007.01396.x
www.blackwellpublishing.com/bjog

Fertility and assisted reproduction

BJOG

Safety versus success in elective single embryo transfer: women's preferences for outcomes of *in vitro* fertilisation

GS Scotland,[a] P McNamee,[a] VL Peddie,[b] S Bhattacharya[b]

[a]Health Economics Research Unit, University of Aberdeen, Foresterhill, Aberdeen, UK [b]Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen Maternity Hospital, Foresterhill, Aberdeen, UK
Correspondence: Dr GS Scotland, Health Economics Research Unit, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.
Email g.scotland@abdn.ac.uk

Accepted 16 April 2007. Published OnlineEarly 18 June 2007.

Aim: To assess the values women waiting for IVF treatment place on adverse birth outcomes associated with twin pregnancy compared to never giving birth

Scotland et al, 2007, BJOG.

Treatment failure versus disability

	Number of women who ranked			
	Treatment failure < disability	Treatment failure = disability	Treatment failure > disability	Total
Physical disability	47	11	10	68
Cognitive impairment	53	11	4	68
Visual impairment	54	11	3	68

Scotland et al, 2007, BJOG.

Standard gamble: summary

- **Faced with the prospect of never giving birth, women were willing to accept a significantly greater risk of experiencing the worst perinatal outcome described**
- **All disability outcomes were valued significantly higher than the 'no birth' outcome (all p values < 0.001)***
- **Perinatal death was valued significantly lower than treatment failure (p < 0.001)***

*** Wilcoxen signed rank tests**

Scotland et al, 2007, BJOG.

BMJ

RESEARCH

Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials

D J McLernon, research fellow,[1] K Harrild, research fellow,[1] C Bergh, professor,[2] M J Davies, associate professor,[3] D de Neubourg, medical director,[4] J C M Dumoulin, head of IVF laboratory,[5] J Gerris, sector manager,[6] J A M Kremer, professor in reproductive medicine,[7] H Martikainen, chief physician,[8] B W Mol, professor of obstetrics and gynaecology,[9] R J Norman, professor,[10] A Thurin-Kjellberg, senior consultant,[2] A Tiitinen, professor of reproductive medicine,[11] A P A van Montfoort, research fellow,[12] A M van Peperstraten, resident in obstetrics and gynaecology,[7] E Van Royen, scientific director,[4] S Bhattacharya, professor of reproductive medicine [13]

Single vs double embryo transfer: baseline

	eSET (N = 683)	DET (N = 684)
Age (yrs), mean (SD)	31.3 (3.5)	31.4 (3.4)
BMI, median (IQR)	23.3 (21.3, 26.1)	23.3 (21.1, 26.3)
Infertility (yrs) median (IQR)	3.0 (2.1, 4.4)	3.0 (2.0, 4.0)
Primary infertility	74%	71%
Cause of infertility		
Male	41%	40%
Tubal	14%	14%
Ovulatory	6%	7%
Endometriosis	5%	5%
Unexplained	16%	20%
Mixed	15%	11%

McLernon et al 2010 BMJ

Single vs double embryo transfer: treatment details

	eSET (N = 683)	DET (N = 684)
IVF	53%	57%
ICSI	47%	43%
Median (IQR) embryos	5 (3, 8)	5 (3, 7)
Day of embryo transfer		
Day 2	79%	79%
Day 3	19%	19%
Day 5	1%	1%
Quality of best fresh embryo transferred		
Excellent (D2 > 4, D3 6 cells)	17%	28%
Good (D2 = 4 , D 3 = 6 cells)	67%	60%
Moderate (D2 <4, D3<6 cells)	11%	8%

Fresh single vs double embryo transfer: outcomes

	Single embryo N = 683	Double embryo N = 683	Adj. OR (95% CI)
Live birth	27%	42%	0.50 (0.39, 0.63)
Multiple live birth	2%	29%	0.07 (0.03, 0.17)
Preterm birth	3%	12%	0.25 (0.16, 0.40)
Term singleton birth	158 (23%)	169 (24%)	0.91 (0.71, 1.16)

McLernon et al, 2010 BMJ

1 Fresh + 1 frozen embryo vs 2 fresh embryo transfer

	eSET N = 350	DET N = 353	Adj. OR (95% CI)
Live birth	38%	42%	0.85 (0.62, 1.15)
Multiple live birth	1%	32%	0.02 (0.00, 0.13)

McLernon et al, 2010 BMJ

Subgroup analysis: age and embryo quality

Age	< 33 yrs			> = 33 yrs		
	eSET N= 456	DET N= 448	Odds ratio*	eSET N= 218	DET N= 226	Odds ratio*
Live birth	29%	46%	0.48 (0.36, 0.63)	23%	35%	0.55 (0.36, 0.84)
Multiple	2%	29%	0.05 (0.02, 0.17)	0%	29%	-
Embryos	**Excellent quality**			**Good / moderate quality**		
Live birth	22%	39%	0.35 (0.20, 0.61)	28%	43%	0.50 (0.39, 0.65)
Multiple	4%	28%	0.10 (0.01, 0.84)	1%	30%	0.03 (0.01, 0.12)

** Adjusted for trial*

McLernon et al, 2010 BMJ

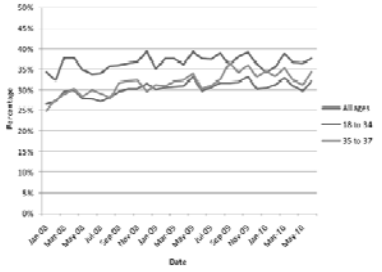
SET vs DET: summary of IPD MA

- Live birth rate lower with eSET in fresh cycle
- Fewer twins and fewer preterm deliveries
- Similar term singleton rate
- Comparable live birth with additional fresh/frozen SET
- Results in fresh cycle hold true for sub-groups (age and embryo quality)
- Higher eSET live birth rates in younger women

Single embryo transfers in UK

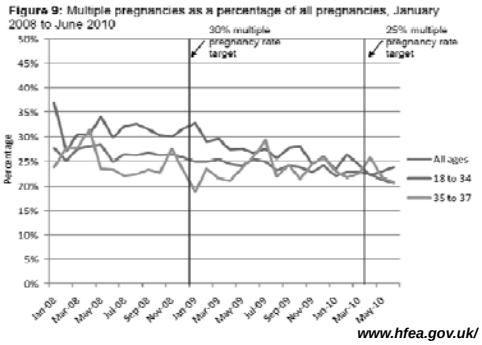
www.hfea.gov.uk/

Pregnancy rate per embryo transfer



www.hfea.gov.uk/

UK multiple pregnancies



www.hfea.gov.uk/

ANZARD data: live births per transfer (%)

35< years	cleavage	blastocyst
eSET	33.6	46.2
SET	20.6	31.2
eDET	42.4	44.1
DET	30.3	33.2
35-39 yrs		
eSET	24.4	37.1
SET	13.2	21.2
eDET	29.8	41.3
DET	21.1	13.0

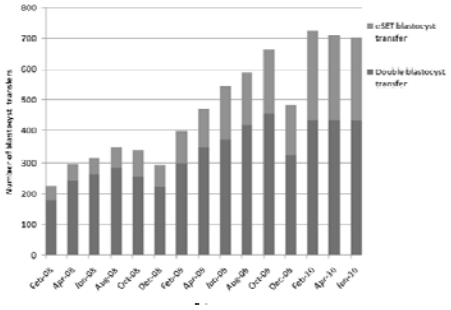
Wang et al, 2010

ANZARD data: live births per transfer (%)

> 40 years	cleavage	blastocyst
eSET	16.2	22.7
SET	7.1	8.6
eDET	21.7	26.1
DET	14.4	13.0

Wang et al, 2010

Blastocyst transfer in the UK



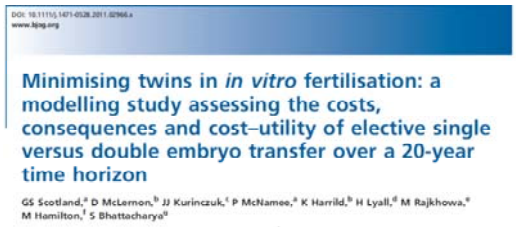
www.hfea.gov.uk/

Elective single cleavage vs single blastocyst transfer: cumulative outcomes

	Elective single cleavage N= 243	Elective single blastocyst SBT N = 235
Fresh cycles	243	235
Frozen cycles	143	76
Fresh birth per woman	61	80
Frozen birth per woman	22	9
Total births per woman	83 (34.2%)	89 (37.9%)*
Multiples	4 (4.8%)	3 (3.4%)

* P > 0.05

Guerif et al, 2009



Minimising twins in *in vitro* fertilisation: a modelling study assessing the costs, consequences and cost–utility of elective single versus double embryo transfer over a 20-year time horizon

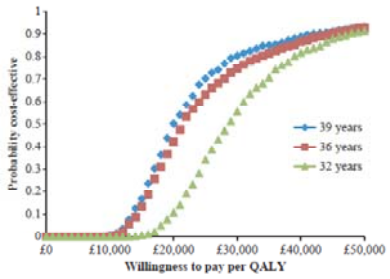
GS Scotland,[a] D McLernon,[b] JJ Kurinczuk,[c] P McNamee,[a] K Harrild,[b] H Lyall,[d] M Rajkhowa,[e] M Hamilton,[f] S Bhattacharya[g]

Incremental cost per live birth and per QUALY (double vs single embryo transfer)

Age (yrs)	ICER per livebirth	ICER per Qualy
32	£ 27,356	£ 28,263
36	£18,580	£21,722
39	15,539	£20,278

Scotland et al, BJOG, 2011

Cost effectiveness acceptability curves for DET vs SET



Scotland et al, BJOG, 2011

Single embryo transfer: is it the future?

- Risk of twins reduced by eSET
- Strong preferences exist
- In good prognosis women:
  - Fewer live births per fresh cycle
  - Similar term singleton live birth
  - Similar cumulative live birth rates
- Single blastocysts:
  - higher livebirth per fresh cycle
  - but comparable cumulative birth rate
- Cost effective in younger women
- Selective approach to eSET

Pre-congress course 15
Middle East Fertility Society exchange course

Optimizing culture conditions

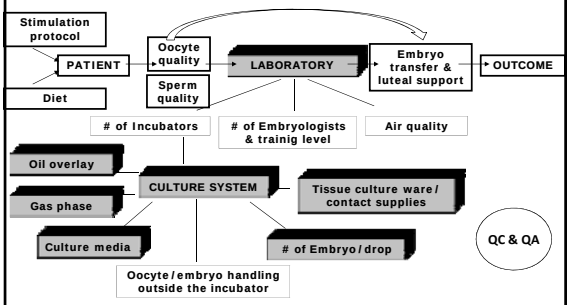
Antonio Capalbo, Ph.D.
Geneticist, Clinical Embryologist
GENERA Centres for Reproductive Medicine
Rome, Marostica, Umbertide, Italy

I disclose no conflict of interest and/or commercial relationships or other activities that might be perceived as a potential conflict of interest.

Agenda

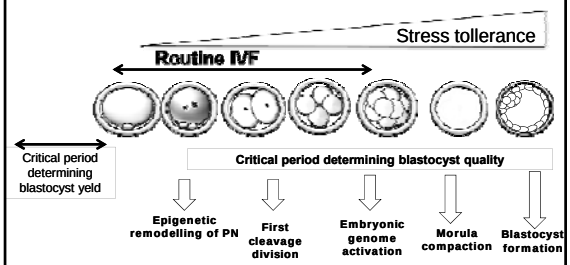
1. Overview of preimplantation embryo development;
2. Review of standard Lab technologies that should be utilized for gamete and embryo culture;
3. In vivo versus in vitro microenvironment;
4. New technologies to increase efficacy for the in vitro culture.

The central role of the laboratory



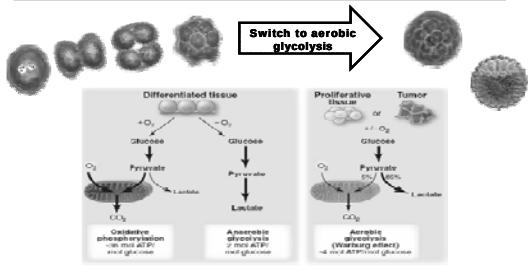
Gardner and Lane, 2007

Preimplantation embryo development



Dynamic nature of the preimplantation embryo

Embryo culture system should better feet with changing of embryo physiology and requirments during development.

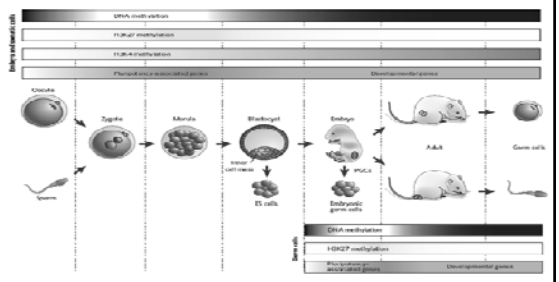


Otto Warburg, Science. 1956

g.en.e.r.a.

Genotype to phenotype and the journey of reproductive cells

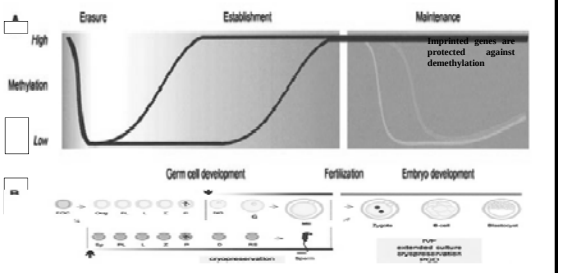
"Development is, by definition, epigenetic"



Swain and Pool.,HRU2008

g.en.e.r.a.

Epigenetic modification in relation to IVF procedures



Lucifero et al., 2004

Agenda

1. Overview of preimplantation embryo development;
2. Review of standard Lab technologies that should be utilized for gamete and embryo culture;
3. In vivo versus in vitro microenvironment;
1. New technologies to increase efficacy for the *in vitro* culture.

g.en.e.r.a.

Appropriate culture conditions have been identified and must be maintained

Temperature: 37°C ±0.2°C

pH

ROS production and REDOX state

g.en.e.r.a.

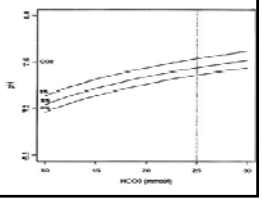
Buffer System and Hydrogen Ion Concentration (pH)

pHi in somatic cells is implicated in:

- **metabolism**
- **activity of regulatory enzymes**
- **maintenance of gap junctions and the cyto-skeleton**
- **modulation of calcium levels**
- **proliferation**

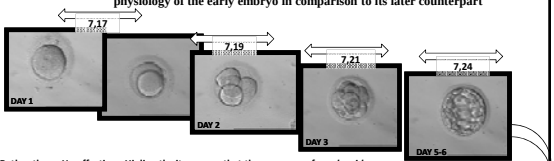
The pH and hence the [H+] can be approximately estimated using the Henderson-Hasselbach equation: pH = 6.1 + log [[NaHCO3]/(PaCO2)]

$HCO_3^- + H^+ \leftrightharpoons H_2CO_3 \leftrightharpoons CO_2 + H_2O$

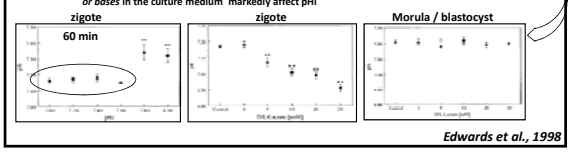


Intracellular (pHi) and extracellular pH (pHe)

This elevation in pHi observed as development proceeds may reflect the very different physiology of the early embryo in comparison to its later counterpart



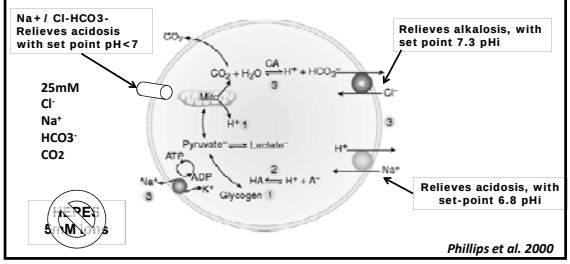
Rather than pHe affecting pHi directly, it appears that the presence of *weak acids or bases* in the culture medium markedly affect pHi



Edwards et al., 1998

The presence of ions in the media is required for cellular buffer systems

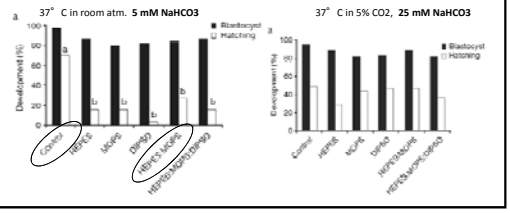
Three transporters collectively maintain the pHi of blastomeres at a set point that falls between 7.0 and 7.3, provided that HCO3 and CO2 are present, not as conjugates of the buffer system, but as components of the cellular ion exchangers.



Phillips et al. 2000

The presence of ions in the media is required for cellular buffer systems

- ✓ HCO_3^- in the media is essential to the functionality of transport mechanisms
- ✓ Lower mouse blastocyst formation and hatching rates when culturing with reduced bicarbonate concentration



Phillips et al, 2000; Swain and Pool, 2009; Swain JE, 2011

pHi alterations significantly affect embryo development

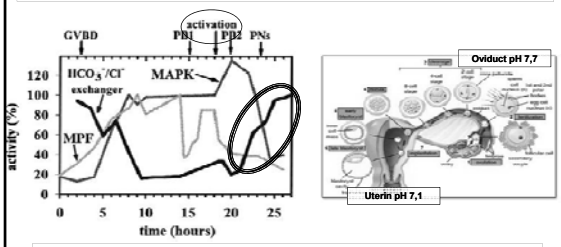
It has been established that even relatively small fluctuations in pHi can significantly retard subsequent developmental competence

Compound concentration[a]	pHi[b]	% Morula and blastocyst	% Blastocyst
TMA 0 mM	7.186 ± 0.01	97.3	73.3
TMA 5 mM	7.285 ± 0.07	82.6[c]	34.7[d]
TMA 10 mM	7.383 ± 0.04[c]	49.1[d]	6.0[d]
TMA 20 mM	7.421 ± 0.06[c]	5.17[d]	2.6[d]
TMA 40 mM	7.578 ± 0.04[d]	0[d]	0[d]
TMA 80 mM	7.685 ± 0.05[d]	0[d]	0[d]
DMO 0 mM	7.242 ± 0.04	90.58	74.7
DMO 5 mM	7.221 ± 0.02	82.1	61.1[c]
DMO 10 mM	7.09 ± 0.05[c]	46.3[d]	21.6[d]
DMO 20 mM	6.87 ± 0.05[d]	0[d]	0[d]

Bavister et al 2001, Edwards et al,1998, Squirrell et al. 2001

Oocyte lacks functional tansport systems to regulate iPH

It has been determined that the oocyte lacks any functional transport systems to regulate pHi in either the acid or the alkaline ranges around 6 h following fertilisation



Following the denudation procedure oocytes and early embryos cannot regulate their ionic homeostasis

Phillips and Baltz 1999

Lab setting outside incubator to minimize pH fluctuation and avoid the use of HEPES

FOR HANDLING: chambers

CCOCs screening, denudation, dishes change, embryo transfer

FOR INVERTED MICROSCOPE: stage top incubators

Oocyte evaluation, oocyte micromanipulation, embryo evaluation

Appropriate culture conditions have been identified and must be maintained

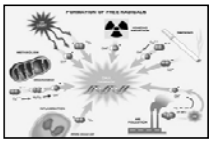
Temperature: 37°C ±0.2°C

ROS production and REDOX state

Oxygen concentration

Under atmospheric oxygen conditions the main contributor to poor embryo development is supposed to be ROS production. However a 'cause and effect' mechanism is yet to be demonstrated.

- **There are some specific events in reproductive system that are positively associated with ROS production**
- **Increase in antioxidant gene expression under 20% oxygen has not been observed**
- **Reducing O2 tension is more effective in promoting embryo development *in vitro* than is treatment with detoxifying enzymes (superoxide dismutase and catalase)**



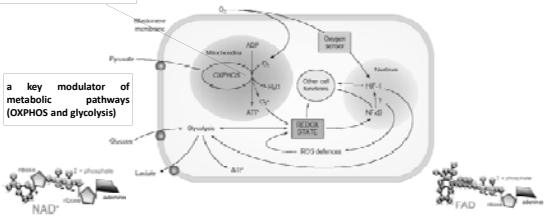
Harvey et al., 2002; Thomas et al., 1997

Oxygen regultates the cellular REDOX state

Oxygen is an essential key energy substrate for oxidative phosphorylation

Other cellular functions, such as apoptosis and cell cycle control, are also significantly influenced by oxygen availability and REDOX state, via transcription factors such as NFkB

a key modulator of metabolic pathways (OXPHOS and glycolysis)



Harvey et al., 2004

Oxygen regulates gene expression via Hypoxia-inducible factors

Members of the hypoxia inducible factor family are influenced directly by the intracellular oxygen concentration, and are important for embryonic development within the hypoxic reproductive tract

Under normoxic conditions, HIF- 1a protein is degraded rapidly by the ubiquitin–proteasome system

Under hypoxic conditions, the HIF-1a protein is not targeted for degradation and can translocate to the nucleus to form the active DNA-binding complex

[O₂] ⟶ **Correct gene expression**

Core consensus sequence (A/G)CGTG in the hypoxia response element (HRE) regulatory region

Semenza et al., 2000

Oxygen acts as physiological signal for blastocyst differentiation

Embryos encounter a decreasing O2 concentration gradient as they progress down the reproductive tract

O2 from a 5% atmosphere would be able to sustain OXPHOS throughout preimplantation development

[O2] ≈ 7%

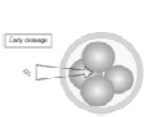
[O2] ≈ 2%

Shift from glycolysis

post-compaction development is associated with a significant shift in the REDOX state to a more reduced state

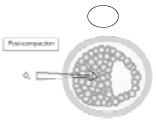
glycolytic enzyme activity

glucose uptake



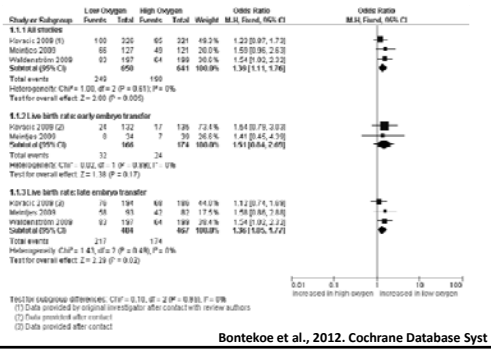
Oxigen gradient provide spatial information to cells within the embryo

ICM ⇄ **TE**



Harvey et al., 2002; Thomas et al., 1997

Low oxygen concentration improve IVF clinical outcomes

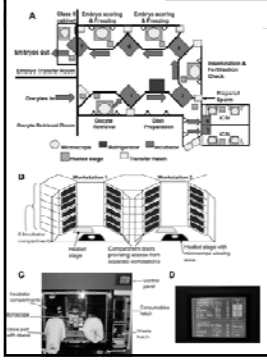


Bontekoe et al., 2012. Cochrane Database Syst Rev

TAKE-HOME MESSAGES: standard requirments of embryo culture

- ✓ At least strict temperature control
- ✓ To assure optimum intracellular pH, CO2 and bicarbonate concentration:
  - o Routinely check the pH in culture media;
  - o Minimize or possibly avoid the use of HEPES or MOPS buffers;
  - o closed systems are necessary.
- ✓ Low oxygen tension **must** be used;
- ✓ Closed systems are necessary.
- ✓ **Rely on new instruments for a better control of the culture environment**

Fully enclosed workstation for gametes and embryo culture



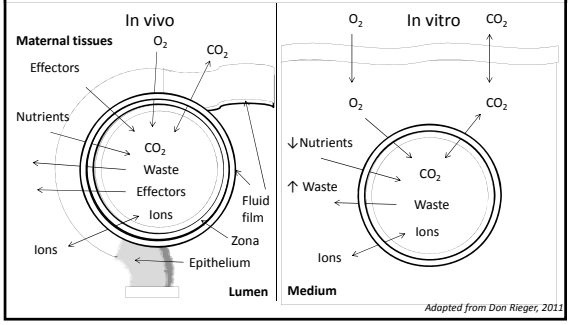
- **Less fluctuations of pH and T;**
- **No need of zwitterionic buffered media, HEPES and MOPS for oocyte retrieval and ICSI;**
- **More consistent and reproducible outcomes;**
- **Clinical and laboratory data suggested improvement in cell proliferation and viability of human embryos.**

Hyslop et al., 2012

Agenda

1. Overview of preimplantation embryo development;
2. Review of standard Lab technologies that should be utilized for gamete and embryo culture;
3. In vivo versus in vitro microenvironment;
1. New technologies to increase efficacy for the *in vitro* culture.

Microdrop under oil: significantly different from in vivo conditions



Adapted from Don Rieger, 2011

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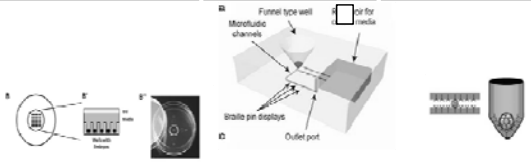
g.en.e.r.a.

INNOVATIVE TECHNOLOGIES TO IMPROVE EMBRYO CULTURE

New static culture platforms:	Dynamic platforms:	Specialized surfaces:
▪ Microdrops ▪ Ultramicrodrops ▪ Submicroliter platforms ▪ Microwells ▪ Micro channels	•Shaking/rotation •Tilting •Vibration •Controlled fluid flow	•Agarose •Matrigel •Hyaluronic acid •Co-culture •3-Dimensional matrix



g.en.e.r.a.

Reduced culture volume

The successful development of embryos in the absence of paracrine or endocrine embryotrophins, may infer that their actions overlap, to a significant degree, the actions of autocrine factors

Since autocrine mediators are released by the embryo in vitro, embryos are exposed to these ligands even in simple defined media.

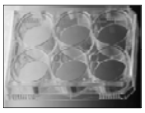
Table I. Ligands released by the preimplantation embryo.

1-o-alkyl-2-acetyl-sn-glycero-3-phosphocholine (PAF) (Collier *et al.*, 1988; O'Neill, 1985)
Activin and Inhibin sub units (Albano *et al.*, 1993; Ouhibi *et al.*, 1990)
α-Interferon (Jones *et al.*, 1992)
Growth Hormone (GH) (Pantaleon *et al.*, 1997b)
Human Leukocyte Antigen-G (HLA-G) (Shaw *et al.*, 2005)
Insulin-like growth factor 1 (IGF-1) (Kaye *et al.*, 1992; Lighten *et al.*, 1997)
Insulin-like growth factor 2 (IGF-2) (Hemmings *et al.*, 1992; Rappolee *et al.*, 1992)
Leukaemia Inhibitory Factor (LIF) (Baker *et al.*, 1993)
Transforming Growth factor-α (TGF-α) (Rappolee *et al.*, 1988)
Platelet derived growth factor subunits (PDGF) (Osterlund *et al.*, 1996; Rappolee *et al.*, 1988)
Prostaglandin F2 (PGF2) (Niimura and Ishida, 1987; Tan *et al.*, 2004)
Transforming growth factor-β (TGF-β) (Babalola and Schultz, 1995; Rappolee *et al.*, 1988)

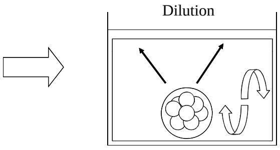
When designing embryo culture systems, a priority should be given to the maintenance of the milieu that embryos create themselves. O'neill et al 2008

Single Embryo culture in large volume

With the traditional methods, tools and dishes (established originally for somatic cells), this microenvironment cannot be properly maintained in vitro.



800 µl of medium

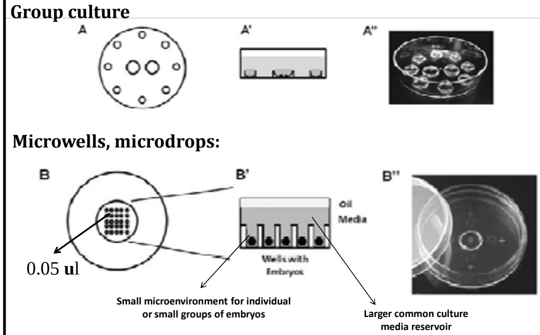


Any autocrine factors produced by the developing embryo will be diluted and may therefore become ineffectual (Gardner and Lane 2007)

Specialized Microdrops and Ultralow Volume Microwells

Group culture

Microwells, microdrops:



The WOW approach

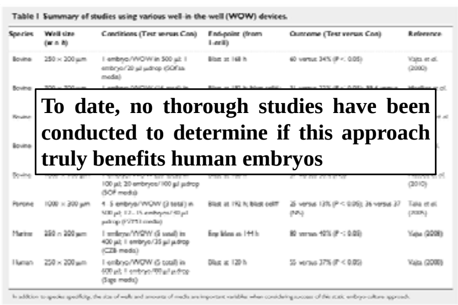


Table 1 Summary of studies using various well-in-the-well (WOW) devices.

Species	Well size (w × h)	Conditions (Test versus Con)	End-point (from 1-cell)	Outcome (Test versus Con)	Reference
Bovine	250 × 200 µm	1 embryo/WOW in 500 µl; 1 embryo/20 µl µdrop (SOFaa media)	Blast at 168 h	60 versus 34% (P < 0.05)	Vajta et al. (2000)
Bovine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bovine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bovine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bovine	[illegible]	[illegible] 100 µl; 20 embryos/100 µl µdrop (SOF media)	[illegible]	[illegible]	[illegible] (2010)
Porcine	1000 × 200 µm	[illegible]	[illegible]	[illegible]	[illegible]
Murine	250 × 200 µm	1 embryo/WOW (5 total) in 400 µl; 1 embryo/35 µl µdrop (CZB media)	[illegible]	80 versus 40% (P < 0.05)	Vajta (2008)
Human	250 × 200 µm	1 embryo/WOW (5 total) in 600 µl; [illegible] (Sage media)	Blast at 120 h	55 versus 37% (P < 0.05)	Vajta (2008)

To date, no thorough studies have been conducted to determine if this approach truly benefits human embryos

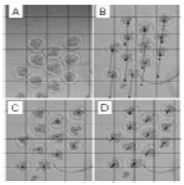
Swain and Smith, 2011

Advances in embryo culture: dynamic culture platforms

Dynamic culture platforms: culture devices purposely engineered to stimulate controlled media flow/movement in order to create **continuous or stepwise medium exchange** and to induce a **sheer mechanical stimulation** to the embryo. Gentle stimulation of embryos could activate beneficial mechano-receptors or signaling pathways to promote embryo growth.

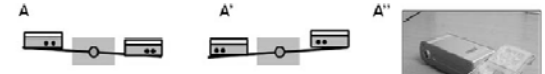
Dynamic culture platforms

- Shaking/rotation
- Tilting
- Vibration
- Controlled fluid flow

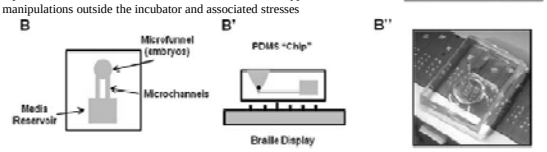


Two promising dynamic culture approaches

Tilting embryo culture system: compatible with current culture dishes and provides a relatively simple way for provide gentle physical stimulation to embryos (estimated sheer force of 0.7 dyn/mm2).



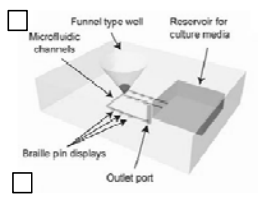
Dynamic microfunnel culture: Perfusion devices offer the ability to replenish culture media and remove harmful byproducts without manipulations outside the incubator and associated stresses

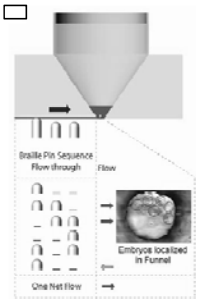


Swain and Smith, 2011

Dynamic microfunnel culture

Medium is added and removed via a microfluidic channel connected to the bottom of the funnel via actions of a Braille actuator. Braille pins are raised and lowered in a controlled fashion to create pulsatile media flow through the bottom of the funnel.

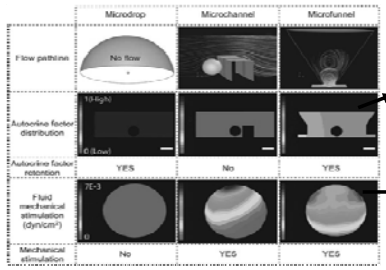




Heo et al., 2010

Dynamic microfunnel culture

Three-dimensional modelling suggests that the funnel design utilized helped retain any potential localized autocrine factors, but also allowed mechanical stimulation, thus combining benefits of microdrop and microchannel approaches

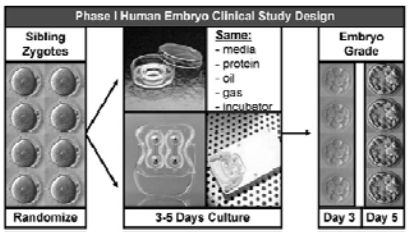


Retention of significant amounts of autocrine factors

Fluid mechanical stimulation to the embryo

Heo et al., 2010

Dynamic microfunnel culture: ongoing RCT human embryos



The formation of good quality embryos on day 3 in a dynamic culture system was 1.63 times higher than in static culture (p=0,038)

Smith et al, 2012

Conclusion 2: advances in embryo culture platforms

Several criteria must be met before any new culture system receives widespread implementation, and these include biocompatibility, ease-of-use and lab compatibility and cost.

Conclusion2: on new culture devices

Low volume culture system: novel devices are now being developed that alter the physical culture environment to manipulate embryo spacing and take advantage of any autocrine/paracrine effect. However:

- The same concentrating effect would occur for metabolic and/or secreted waste products.
- Require extreme attention to media properties because shifts in ph and osmolality are common and can have a profound impact on embryo development.
- Handling such small volumes of liquid is technically challenging (risk for embryo loss) and can be highly variable;
- Future studies on the potential benefit of group culture and/or paracrine/autocrine biomolecule signalling in human embryos are significantly needed.

Conclusion2: on new culture devices

Dynamic culture platforms:

- Relative complexity and requirement to operate efficiently and safely within the humidified and warmed environment of the incubator.
- Biocompatibility is paramount. Sheer stress over 1.2 dyn/cm2 results embryo degeneration within 12 h (Xi et al., 2006).
- To date, simpler approaches such as rotation or tilting devices that can be utilized with traditional culture dishes may be easier to implement in the clinical laboratory and are already commercially available.
- Microfluidic devices perhaps offer the most potential in improving in vitro embryo culture, as they provide a means of controlled fluid flow, while also providing the potential for integration of bioanalytic assays.
- Interesting technology to improve cryopreservation procedures!

Concluding remarks: optimizing culture conditions

Does the present efficiency of in vitro embryo culture approach the natural limits?

1. We do not know where these limits are.

2. There are possibilities outside the present frames, that may result in considerable increase in efficiency.

3. A lot of things to consider, answer, resolve, improve, *and create...*

In vitro embryo culture is not an imperfect copy, is an artificial process, with its own frames, limitations, *and possibilities.* The embryologist task is to modify the frames, eliminate the limitations, *exploit fully the possibilities*

Vajta, 2011

Thanks for your attention

Prevention of Ovarian hyperstimulation syndrome

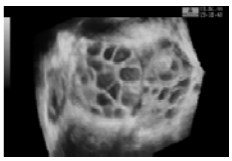
Mohamed Aboulghar, M.D.
Professor, Cairo University
Clinical Director, The Egyptian IVF Center
Cairo, Egypt

There are no commercial relationship or other activity related to this lecture which might be perceived as a potential conflict of interest.

Learning Objectives

At the conclusion of this course, the participant should be able to:

1. *Detect the prevalence and predisposing factors of OHSS*
2. *Discuss the efficacy of each preventive measure of OHSS*
3. *Summarize the different measures to prevent OHSS*



OHSS is the most serious complication

of ovulation induction.

In its severest forms, it is complicated by hemoconcentration, venous thrombosis, electrolyte imbalance and renal and hepatic failure

(Schenker and Weinstein 1978; Navot et al. 1992; Aboulghar et al. 1993)

How to prevent OHSS?

1. *Identifying patients at risk before ovulation induction.*
2. *Low dose FSH / hMG*
3. *GnRH antagonist protocol*
4. *Careful monitoring of ovarian response: US / E2*
5. *Metformin*
6. *Patients at risk during ovulation induction:*
    - *Canceling the cycle*
    - *Coasting*
    - *GnRH antagonist*
    - *hCG dose and alternatives*
    - *Cryopreservation of all embryos*
    - *Albumin / starch*
    - *Dopamin agonist*
    - *Triggering ovulation by GnRHa*

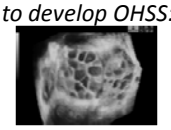
First

Identifying patients at risk before ovulation induction:

- *History of previous OHSS*
- *PCOS patients are more liable to develop OHSS*

(Schenker and Weinstein 1978; Navot et al. 1992; Bider et al. 1989; Rizk et al. 1992; Aboulghar et al. 1992)

PCOS patients are more liable to develop OHSS:



1- Oligomenorrhea or periods of amenorrhea

2- Hormonal profile (FSH/ LH) high LH

3- Ultrasonography

- *Increased ovarian volume (Danning et al. 1996; Lass et al. 2000)*
- *Number of antral follicles and necklace appearance (Navot et al. 1992)*

5- Young age < 35 years old (Navot et al. 1992)

6- Lean body (Asch et al., 1991; Ayhan et al., 1996)

Stimulation protocols
for non IVF cycles

- Low dose step-up protocol
  - *(Homburg and Howles, 1999)*
  - 225 women with PCO stimulated by low dose protocol for 934 cycles resulted in 109 pregnancies, 7 twin pregnancies and no OHSS *(White et al. 1996).*

Stimulation protocols
for IVF cycles

- Lower doses of gonadotrophins *(Homburg and Insler 2002)*
- GnRH agonist protocol increases the incidence of OHSS *(Rizk and Smitz 1992)*.
- GnRH antagonist protocol *(Al-Inany et al 2011)*.

Livebirth after IVF: agonist / antagonist: a meta analysis (2006)

- 22 RCT
- 3176 Subjects
- Livebirth (from manuscript in 10 studies and by convenrsion of pregnancy rate to live birth rate using special formula in 12 studies
- Both long and flare up agonist protocols were included
- No significant difference between PR in agonist and antagonist protocols (OR, 0.86; 95% CI, 0.72-1.02)

Kolibianakis et al Hum Reprod Update. **2006** Nov-Dec;12(6):651-71.

New Cochrane review (1)
(Al-Inany et al. 2011)

- In a recent Cochrane review
- 46 RCT = 7511 cycles
- Comparing long GnRHs versus GnRH antagonist
- There was no significant difference in the livebirth rate (9 RCT OR: 0.086, 95% CI 0.69-1.88)

Cochrane Database Syst Rev. 2011 May 11;(5):CD001750

Meta-analyses Confirm That GnRH Antagonists Have a Better Safety Profile vs GnRH Agonists

	Kolibianakis	Al-Inany
Risk of severe OHSS	RR 0.46* (0.26, 0.82; P=.01)	OR 0.61 (0.42, 0.89; *P*=.01)
Interventions to prevent OHSS		OR 0.44 [0.21, 0.93] vs. agonist; *P*=.03

*For every 59 women treated with a GnRH agonist vs GnRH antagonist, one additional case of severe OHSS will occur.

OR = Odds ratio; RR = Risk ratio

1. Al-Inany et al. *Cochrane Database Syst Rev*. 2006;3:CD001750.
2. Kolibianakis et al. *Hum Reprod Update*. 2006;12:651.

Low Gonadotropin doses

Starting with 150 IU for all patients at a possible risk irrespective of age is recommended (Golan et al., 1988; Homburg and Insler 2002; El-Sheikh et al., 2001)

Type of gonadotropins: Urinary or Recombinant

No significant difference in the occurrence of OHSS (van Wely et al. et al., 2003)

Careful monitoring of ovarian response to diagnose patients at risk

1. *US:*
   - *PCOS pattern*
   - *Large number of follicles*
   - *Increase in the fraction of very small follicles and decrease in the fraction of dominant follicles* (Blankstein et al 1987)
2. *E2*
   - *Log E2 and Slope E2 increment was a good predictor to OHSS* (Delvigne et al 1993)

Patients at risk of OHSS during ovulation induction

I. *Stop hMG and continue down regulation. (Complete prevention)* *(Nardo et al. 1992;* Rizk and Aboulghar, 1999; Aboulghar and Mansour 2003)

II. *Coasting.*

III. *GnRH antagonist.*

Withholding hCG and cycle cancellation

- After the introduction of other different modalities for prevention of OHSS in high risk patients and in particular coasting, withholding hCG with cyclic cancellation is seldom used *(Orvieto 2005)*.

Cryopreservation of all embryos: a Cochrane review

Seventeen studies were identified, two of which met the inclusion criteria. When elective cryopreservation of all embryos was compared with fresh embryo transfer no difference was found in all the outcomes examined between the two groups. There is insufficient evidence to support routine cryopreservation for prevention of OHSS. *(D'Angelo and Amso (2002)*

Coasting

It is stoppage of FSH stimulation and monitoring of E2 level

Coasting

- Coasting for non-IVF cycles.
  - *Rabinovici et al., (1987)*
  - *Urman et al., (1992).*

Coasting for IVF cycles

- *Sher et al., (1993)* suggested that prolonged coasting in GnRH-a/hMG.FSH cycles could prevent live-endangering complications of OHSS.
- *Sher et al., (1995)* treated 51 women at great risk of developing OHSS by coasting until the plasma E2 fell to <3000 pg/ml There were 21 clinical pregnancies (41%/oocyte retrieval). None of the women developed severe OHSS.

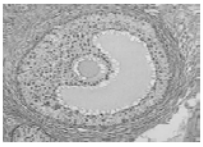
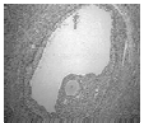
Coasting

- When to start coasting:

  When the mean diameter of the follicles reaches 16 mm in diameter and the E2 level is above 3500 pg/ml *(Mansour et al 2005).*

What happens when you start coasting?

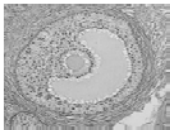
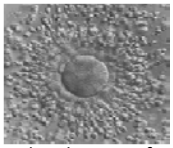
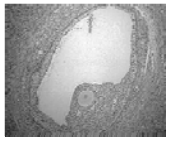
- Follicular growth will continue with the same rate.
- E2 will continue to rise then will platform and then decline.

Mature follicles can survive for a few days without exogenous FSH/hMG while small follicles will undergo apoptosis / necrosis

(Garcia-Velasco et al., 2004)

Coasting diminishes the granulosa cell cohort


E2 ↓↓

In the absence of gonadotropin stimulation, dominant follicles will continue their growth, while intermediate and small ones will undergo atresia.

How to monitor coasting cycles?

- Daily E2 assays.
- Daily folliculometry.

When to give hCG

When E2 levels drop to 3000 pg/ml

Problems with coasting

- Occasionally E2 drops markedly to very low levels and cycle is canceled.
- Difficulty in identification of oocytes in aspirated follicular fluid after prolonged coasting.

Are there specific Lab precautions ?

Yes

Extra time and care is needed in order to find the oocytes due to the diminished amount of granulosa cells

The experience of the Egyptian IVF Center (Mansour et al. 2005)

- From January 2000 till December 2004, 12,494 ICSI/IVF cycles were performed at the Egyptian IVF-ET Center. Coasting was done for 1223 patients that were diagnosed to be at risk of developing OHSS during that period.

ICSI outcome according No. of coasting days

	Group I up to 3 days	Group II 4 days or more	P-value * Significant
Cycles	983	240	
E2 level on day of hCG (pg/mL)	2674±1789	2801±1930	P=0.88
Oocytes retrieved	16.45±6.26	14.93 ±6.01	P=0.002*
MII oocytes	12.94 ±5.58	11.6 ±5.61	P=0.003*
Fertilization rate	62.67%	64.92%	P=0.06
Embryo per transfer	2.99 ±0.69	3.03 ± 0.66	P=0.27
Implantation rate	26.32%	18.16%	P=0.0001*
Clinical PR	51.96%	35.88%	P=0.0002*

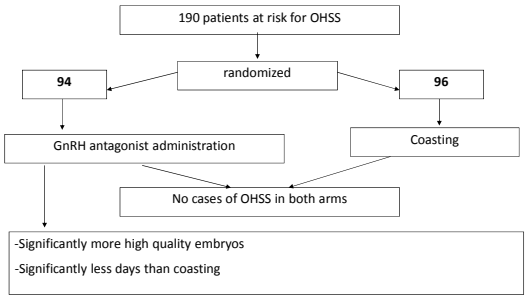
Severe OHSS occurred in 16 cases which is 0.13% (16/12,494) of stimulated cycles as compared to 1.8% in our report before introducing coasting (Aboulghar et al 1993) and 1.3% (16/1223) of patients at risk of developing OHSS who underwent coasting (Mansour et al. 2005)

GnRH antagonist during stimulation of high risk patients

Forty-seven patients at high risk for OHSS because of markedly elevated E_2 were treated with ganirelix acetate. Despite being pretreated with GnRH agonist and without withholding gonadotropins, serum E_2 decreased by 49.5% of pretreatment value after initiation of ganirelix, and 68.1% of the patients became pregnant *(Gustofson et al 2006)*.

Antagonist for prevention of OHSS

Aboulghar et al 2007



Metformin and IVF in PCOS patients a Cochrane review
(TSO et al 2009)

- No evidence that metformin improves pregnancy or live birth rate.
- Metformin significantly reduces OHSS rates (OR 0.27, 95% CI 0.16-0.47)

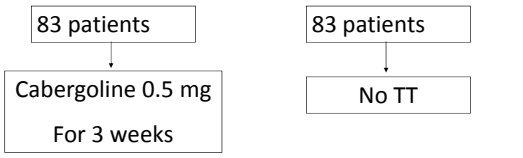
IV albumin for prevention of OHSS: A Cochrane review

IV albumin does not significantly reduce the incidence of OHSS *(Youssef et al 2011)*.

Dopamine agonist in prevention of OHSS

Cabergoline was administered in 20 women at risk of OHSS. No OHSS developed in all patients The authors believe that the drug may be even more effective if administrated immediately after oocyte retrieval. Cabergoline may work through the relation between VEGF/VEGFr and its relation with the neurotramitter dopamin
(Manno et al 2004)

Cabergoline reduces early onset of moderate OHSS: a randomized study
(Carizza et al 2008)

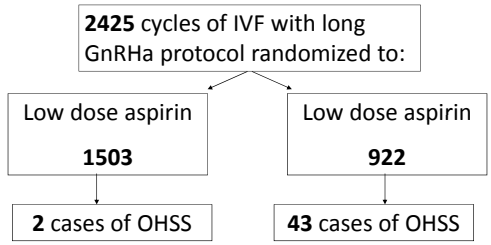


Cabergoline reduces the incidence of early OHSS. A larger study on high risk patients is required
(Alvarez et al 2007)

- In a non-randomized double blind placebo controlled trial, it was found that quinagolide appears to prevent moderate/severe early OHSS (Russo et al 2010)

- In a Cochrane review (Tang et al 2012) which included two studies showed that cabergoline reduces the incidence of moderate OHSS and it does not affect pregnancy outcome.

Low dose aspirin to prevent OHSS (Varnagy et al 2009)



Conclusion: low dose aspirin is effective for prevention of OHSS

Triggering ovulation and OHSS

- Reducing the hCG trigger dose. *(Abdalla 1987)*
- The use of recombinant hCG to trigger ovulation. *(Driscoll et al 2000)*
- Recombinant LH for triggering ovulation.
- Gonadotrophin releasing hormone agonist to trigger ovulation.

- In a meta analysis of three studies comparing triggering ovulation in antagonist cycles, it was found that GnRHa triggering is associated with significantly lower OHSS rate and also lower ongoing pregnancy rate as compared with triggering with hCG (Griesinger et al 2006).

- Several non-randomized studies suggest that trigger ovulation with GnRHa supplement with small doses of hCG or high doses of progesterone can result in a smimilar pregnancy rate as hCG triggering (Humaidan et al 2012, Humaindan 2012, Kol et al, 2011).

Conclusions

The most effective measures in preventing OHSS:

I. *Identifying patients at risk of developing OHSS before ovulation induction:*

   ** Previous history of OHSS*

   ** PCOS*

Conclusions (cont.)

II. *Low doses of hMG*

III. *Patients during ovulation induction and at risk of OHSS:*

   1- Coasting

   2- GnRH antagonist protocol

   3- hCG 5000 IU only to triger ovulation

Conclusions *(cont.)*

4- GnRH agonist to trigger ovulation

5- Progesterone only for luteal phase support

6- Other Measures:

- *For complete prevention: Stop hMG and continue GnRH agonist or antagonist (cancellation)*
- *Cryopreservation of all embryos: There is insufficient evidence for its value.*
- *IV albumin or Starch:*
  No evidence of beneficial effect.
- *Corticosteroids may help, but no sufficient evidence.*

M. Aboulghar, M.D.

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CONFLICT OF INTEREST

NOTHING TO DECLARE

Techniques and Technologies for Embryo Transfer: Does it Really Matter

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American University of Beirut Medical Center

Learning Objectives

- **Understand** the dynamics involved in the process of ET
- **Evaluate** the evidence for/against common practices and techniques
- **Develop** a standardized ET process in view of supporting evidence

Background

- ET is the least sophisticated, yet the most vulnerable step of the In Vitro Fertilization process
- ET performance remains largely operator-dependent
- Despite major advances in the field, the process of ET remains the most unchanged

Hypothesis

- Developing **a standardized embryo transfer approach** is expected to maintain higher pregnancy success rates and lower adverse effects

Parameters affecting ET outcome

Anatomy	• Uterine position • Utero-cervical angle • Cervical mucus • Bacterial inoculation • Uterine contractions
Technique	• Catheter guidance • Catheter tip location • Injection mode
Catheter	• Soft versus firm • Internal diameter • Tip shape
Transfer medium	• Medium/air volume • Viscosity • Adherence compounds

OBJECTIVE I

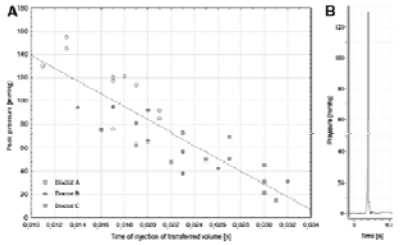
UNDERSTANDING THE DYNAMICS

Laboratory and computational simulation models

- Recommendations based on experimental findings:
  - Positioning of the patient to keep the **fundus at the highest point in the sagittal cross-section above the horizon**
  - Delivery of the load over a course of **10 s or more**
  - Placement of the catheter tip at **mid cavity** about 2.0 cm from the fundal end

Eytan O et al. 2007

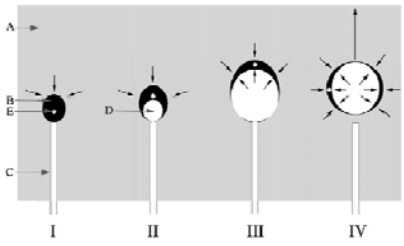
Laboratory simulation uterine model with pressure sensor



The pressure buildup in the transferred liquid is proportional to the **ejection speed** of the transferred load

Grygoruk C et al. 2011

Laboratory simulation uterine model with pressure sensor

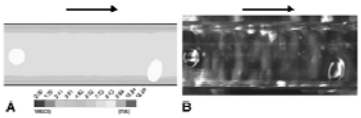


To avoid abrupt pressure fluctuations, ET ought to occur **as slowly as possible** to avoid embryo damage

Grygoruk C et al. 2011

Computational fluid dynamics model

- Recommendations based on experimental findings:
  - To reduce the strength of the shear stress and the pressure changes
    - Transfer embryos with **minimal injection speed**
    - **Eliminate any narrowing** of the catheter lumen



Grygoruk C et al. 2011

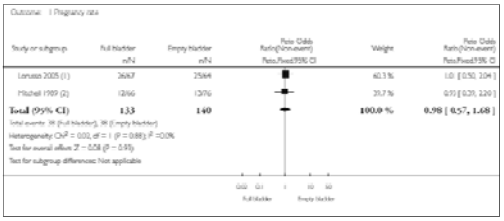
OBJECTIVE II

EVALUATING COMMON PRACTICES

Preparations Prior to ET

I. **Straightening of the utero-cervical angle**
II. **Preparation of the cervix**
III. **Antibiotics administration**
IV. **Acupuncture**
V. **Mock transfer**

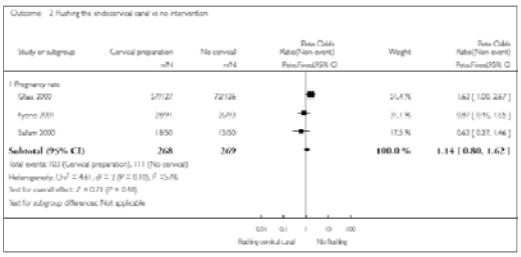
I. Straightening of the utero-cervical angle - Full bladder



There was **no evidence of an effect** on the pregnancy rate for women undergoing ET with a full bladder compared with an empty bladder

Derks RS et al. 2010

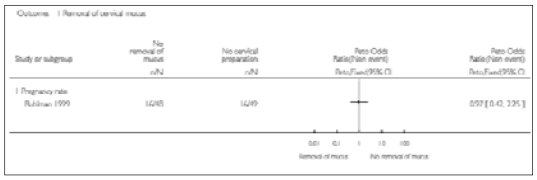
II. Preparation of the cervix - Flushing of the endocervical canal



There was **no evidence of an effect** on the pregnancy rate for flushing of the endocervical canal using culture medium prior to ET compared with no flushing

Derks RS et al. 2010

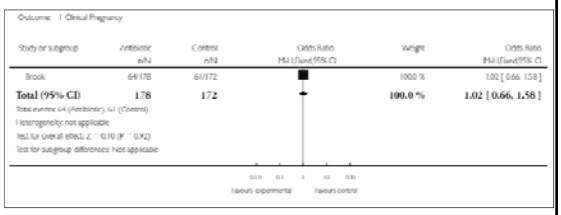
II. Preparation of the cervix - Removal of the cervical mucus



There was **no evidence of an effect** on the pregnancy rate for removal of the cervical mucus prior to ET compared with no removal

Derks RS et al. 2010

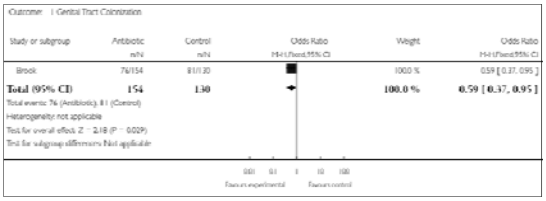
III. Antibiotic administration



The use of amoxycillin/clavulanic acid had **no effect** on clinical pregnancy rate

Kroon B et al. 2012

III. Antibiotic administration



The use of amoxycillin/clavulanic acid **reduced significantly** bacterial colonization of the genital tract

Kroon B et al. 2012

IV. Acupuncture

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live Birth	4	767	Odds Ratio (M-H, Fixed, 95% CI)	2.02 [1.45, 2.82]
1.1 Sham acupuncture	2	425	Odds Ratio (M-H, Fixed, 95% CI)	1.91 [1.22, 3.00]
1.2 No sham acupuncture	2	342	Odds Ratio (M-H, Fixed, 95% CI)	2.17 [1.37, 3.54]
2 Ongoing pregnancy	6	1080	Odds Ratio (M-H, Random, 95% CI)	1.77 [0.91, 3.42]
2.1 Sham acupuncture	3	644	Odds Ratio (M-H, Random, 95% CI)	1.79 [1.24, 2.58]
2.2 No sham acupuncture	3	436	Odds Ratio (M-H, Random, 95% CI)	1.66 [0.35, 7.92]
3 Clinical pregnancy	8	1341	Odds Ratio (M-H, Random, 95% CI)	1.39 [0.94, 2.06]
3.1 Sham acupuncture control	3	652	Odds Ratio (M-H, Random, 95% CI)	1.71 [1.11, 2.65]
3.2 No sham acupuncture	5	689	Odds Ratio (M-H, Random, 95% CI)	1.18 [0.64, 2.18]
4 Miscarriage	3	634	Odds Ratio (M-H, Fixed, 95% CI)	1.14 [0.63, 2.05]
4.1 Sham acupuncture	2	452	Odds Ratio (M-H, Fixed, 95% CI)	1.15 [0.59, 2.24]
4.2 No sham acupuncture	1	182	Odds Ratio (M-H, Fixed, 95% CI)	1.11 [0.33, 3.76]

There is evidence that acupuncture performed **on the day of ET** is **associated with improved** live birth rate, but not when performed around the time of oocyte retrieval or during the luteal

Cheong YC et al. 2011

V. Mock transfer

- No RCTs found on the effects of mock transfer on reproductive outcome

Derks RS et al. 2010

Table 1. Uterine position at actual ET compared with mock ET

Uterine position at mock ET (no. patients) (%)	AV at fresh ET (no. cycles) (%)	RV at fresh ET (no. cycles) (%)	Total at fresh ET (no. cycles)	AV at thawed ET (no. cycles) (%)	RV at thawed ET (no. cycles) (%)	Total thawed ET (no. cycles)
AV 434 (74)	608 (98)	15 (2)	623	100 (88)	14 (12)	114
RV 151 (26)	118 (35)	95 (65)	213	15 (33)	31 (67)	46

ET = embryo transfer; AV = anteverted; RV = retroverted.

There is evidence of a **lack of consistency** between uterine position at mock and actual embryo transfer

Henne MB, Milki AA. 2004

Techniques during ET

I. **Ultrasound catheter guidance**
II. **Soft versus firm embryo transfer catheters**
III. **Site of embryo deposition**
IV. **Adherence compounds**
V. **Interval loading discharging embryos**
VI. **Physician factor**

I. Ultrasound catheter guidance

Comparison 1. Pregnancy

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth (per woman randomised)	3	2264	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.14 [0.93, 1.39]
2 Ongoing pregnancies (per woman randomised)	7	2472	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.38 [1.16, 1.64]
3 Clinical pregnancies (per woman randomised)	17	6415	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.31 [1.18, 1.46]

Ongoing and clinical pregnancies per woman randomized associated with "ultrasound guided" ET was **significantly higher** than for "clinical touch" technique

Brown J et al. 2010

I. Ultrasound catheter guidance

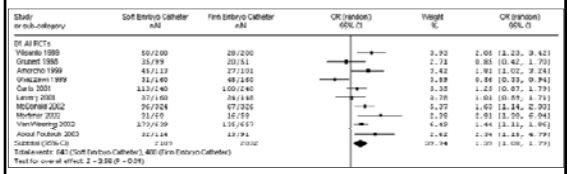
Comparison 2. Adverse events

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Multiple pregnancies (per woman randomised)	6	3346	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.37 [0.93, 1.75]
2 Ectopic pregnancy (per woman randomised)	7	2908	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.62 [0.26, 1.47]
3 Spontaneous miscarriage (per woman randomised)	8	2930	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.95 [0.65, 1.38]
4 Blood su saiheit	2	2789	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.09 [0.77, 1.59]
5 Not considered an easy transfer	8	3262	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.87 [0.68, 1.11]

There was **no significant difference** in multiple pregnancies, ectopic pregnancies and miscarriages per woman randomized for "ultrasound guided" ET and "clinical touch" embryo transfer

Brown J et al. 2010

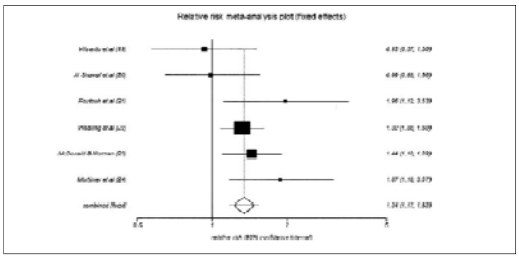
II. Soft versus firm embryo transfer catheters



The use of soft embryo transfer catheters for embryo transfer was associated with a **significantly higher** clinical pregnancy rates as compared to firm catheters

Abou-Setta AM et al. 2005

II. Soft versus firm embryo transfer catheters



The use of soft ET catheters is associated with **a higher chance** of clinical pregnancy when compared with the use of firm catheters

Buckett W. 2006

III. Site of embryo deposition

	DTC II	DTC III	DTC IV	DTC III/DTC IV
Live-birth rate				
DTC-I	OR = 0.76*, 95% CI = 0.47–1.23*	NA	NA	OR = 0.70, 95% CI = 0.51–0.97
DTC-II		OR = 0.62, 95% CI = 0.39–1.30	OR = 0.42, 95% CI = 0.30–0.88	OR = 0.92 95% CI = 0.64–1.32
DTC-III			OR = 0.69, 95% CI = 0.33–1.42	NA
DTC-IV				NA
Ongoing pregnancy rate				
DTC-I	OR = 0.77*, 95% CI = 0.48–1.25*	NA	NA	OR = 0.71, 95% CI = 0.57–0.98
DTC-II		OR = 0.62, 95% CI = 0.29–1.30	OR = 0.43, 95% CI = 0.29–0.89	OR = 0.92, 95% CI = 0.64–1.32
DTC-III			OR = 0.69, 95% CI = 0.33–1.42	NA
DTC-IV				NA
Clinical pregnancy rate				
DTC-I	OR = 0.88*, 95% CI = 0.57–1.36*	NA	NA	OR = 0.86, 95% CI = 0.64–1.15
DTC-II		OR = 0.67, 95% CI = 0.33–1.38	OR = 0.43, 95% CI = 0.21–0.90	OR = 0.98, 95% CI = 0.69–1.38
DTC-III			OR = 0.64, 95% CI = 0.31–1.33	NA
DTC-IV				NA

CI = confidence interval, DTC-I = <7.25 mm, DTC-II = 10 ± 2.5 mm, DTC-III = 15 ± 2.5 mm, DTC-IV = 20 ± 2.5 mm, NA = not applicable, OR = odds ratio
*Estimated indirect analysis.

Live-birth, ongoing pregnancy and clinical pregnancy rates are **significantly improved** when the tip of the catheter is positioned in the middle area of the endometrial cavity

Abou-Setta AM. 2007

IV. Adherence compounds - Fibrin sealant

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per randomised woman	1	211	Odds Ratio (M-H, Fixed, 95% CI)	0.98 [0.54, 1.78]
2 Ectopic pregnancy rate per randomised woman	1	211	Odds Ratio (M-H, Fixed, 95% CI)	5.55 [0.26, 117.06]

There was **no evidence** of an effect of using a fibrin sealant in improving the clinical pregnancy rate over control

Abou Setta AM et al. 2010

IV. Adherence compounds – Hyaluronic acid

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth rate	3	324	Odds Ratio (M-H, Fixed, 95% CI)	1.35 [0.86, 2.12]

There was **no evidence** of a treatment effect of using medium enriched with hyaluronic acid versus medium devoid of hyaluronic acid in improving live birth rate

Bontekoe S et al. 2010

IV. Adherence compounds – Hyaluronic acid

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6 Ongoing pregnancy rate	4	425	Odds Ratio (M-H, Fixed, 95% CI)	1.75 [1.15, 2.65]
7 Ongoing pregnancy rate (grouped by timing of intervention)	4	425	Odds Ratio (M-H, Fixed, 95% CI)	1.75 [1.15, 2.65]
7.1 Early transfers	3	343	Odds Ratio (M-H, Fixed, 95% CI)	1.85 [1.15, 2.91]
7.2 Late transfers	1	82	Odds Ratio (M-H, Fixed, 95% CI)	1.42 [0.34, 5.68]
8 Ongoing pregnancy rate (grouped by frozen-thawed or fresh embryos)	4	425	Odds Ratio (M-H, Fixed, 95% CI)	1.75 [1.15, 2.65]
8.1 Frozen-thawed embryos	1	80	Odds Ratio (M-H, Fixed, 95% CI)	1.50 [0.62, 3.61]
8.2 Fresh embryos	3	345	Odds Ratio (M-H, Fixed, 95% CI)	1.82 [1.14, 2.92]
9 Ongoing pregnancy rate (grouped by exposure time to HA)	4	425	Odds Ratio (M-H, Fixed, 95% CI)	1.75 [1.15, 2.65]
9.1 Exposure time ≤ 10 minutes	2	181	Odds Ratio (M-H, Fixed, 95% CI)	2.88 [1.42, 5.87]
9.2 Exposure time > 10 minutes	2	244	Odds Ratio (M-H, Fixed, 95% CI)	1.30 [0.77, 2.20]

Pooled data showed **evidence of an increased** ongoing pregnancy rate with medium enriched with hyaluronic acid

Bontekoe S et al. 2010

IV. Adherence compounds – Hyaluronic acid

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
12 Clinical pregnancy rate	6	886	Odds Ratio (M-H, Fixed, 95% CI)	1.97 [1.46, 2.67]
13 Clinical pregnancy rate (grouped by timing of intervention)	6	886	Odds Ratio (M-H, Fixed, 95% CI)	1.97 [1.46, 2.67]
13.1 Early transfer	5	590	Odds Ratio (M-H, Fixed, 95% CI)	2.41 [1.69, 3.45]
13.2 Late transfer	1	296	Odds Ratio (M-H, Fixed, 95% CI)	1.19 [0.67, 2.09]
14 Clinical pregnancy rate (grouped by frozen-thawed or fresh embryos)	5	609	Odds Ratio (M-H, Fixed, 95% CI)	1.63 [1.16, 2.28]
14.1 Frozen-thawed	2	204	Odds Ratio (M-H, Fixed, 95% CI)	1.26 [0.72, 2.22]
14.2 Fresh	4	405	Odds Ratio (M-H, Fixed, 95% CI)	1.89 [1.23, 2.90]
15 Clinical pregnancy rate (grouped by exposure time to HA)	5	699	Odds Ratio (M-H, Fixed, 95% CI)	1.60 [1.14, 2.25]
15.1 Exposure time ≤ 10 minutes	3	241	Odds Ratio (M-H, Fixed, 95% CI)	2.42 [1.34, 4.36]
15.2 Exposure time > 10 minutes	2	458	Odds Ratio (M-H, Fixed, 95% CI)	1.29 [0.85, 1.97]

Pooled data showed **evidence of an increased** clinical pregnancy rate with medium enriched with hyaluronic acid

Bontekoe S et al. 2010

IV. Adherence compounds – Hyaluronic acid

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
18 Multiple pregnancy rate	2	181	Odds Ratio (M-H, Fixed, 95% CI)	3.14 [1.14, 8.65]

There was **evidence of an increased** multiple pregnancy rate with medium enriched with hyaluronic acid

Bontekoe S et al. 2010

V. Interval loading discharging embryos

Table II. Duration of the interval loading discharging embryos (ILDE), cycle characteristics and cycle results

	<30 s (n = 113)	31–60 s (n = 214)	61–120 s (n = 76)	>120 s (n = 47)	P*
Woman's age (years)	34.01 ± 3.04	34.40 ± 2.83	33.97 ± 3.57	33.93 ± 3.73	Ns
Infertility duration (years)	5.21 ± 2.54	5.07 ± 2.66	5.19 ± 2.54	5.20 ± 2.61	Ns
Primary infertility (%)	91.1	89.7	89.5	89.4	Ns
ICSI (%)	53.1	50.9	57.8	57.5	Ns
Obtained oocytes	11.00 ± 6.13	12.28 ± 6.22	12.22 ± 6.20	13.23 ± 5.83	Ns
Inseminated oocytes	9.30 ± 5.30	10.29 ± 5.30	9.92 ± 5.08	10.46 ± 4.03	Ns
Fertilized oocytes	5.30 ± 3.97	6.03 ± 4.07	5.34 ± 3.63	5.74 ± 3.16	Ns
Transferred embryos	3.05 ± 1.06	3.12 ± 0.99	3.07 ± 1.09	3.14 ± 0.85	Ns
Transferred class I embryos	1.95 ± 1.00	2.10 ± 0.98	2.37 ± 0.98	2.17 ± 0.99	Ns
% of non easy transfers	2.6	0.5	1.3	19.1	<0.001
Pregnancy rate (%)	38.9	33.2	31.6	19.1	<0.05
Implantation rate (%)	21.2	15.4	15.9	9.4	<0.01
Pregnancy rate excluding non-easy transfers (%)	40.0	33.3	32.0	19.4	<0.05
Implantation rate excluding non easy transfers (%)	21.4	15.4	16.2	8.8	<0.01

Longer interval loading discharging embryos is associated with **significant decrease** in pregnancy and implantation rates

Matorras R et al. 2004

V. Interval loading discharging embryos

Embryo quality	Good		Poor	
Transfer	Easy	Difficult	Easy	Difficult
Cycles (N)	187	34	71	8
Mean age of women ± SD (years; range)	31.0 ± 4.3 (18–39)	31.5 ± 4.5 (21–39)	31.6 ± 4.8 (20–39)	30.3 ± 5.9 (23–39)
Mean day 3 FSH ± SD (mIU/mL)	6.9 ± 3.2	6.7 ± 3.7	8.2 ± 3.5	9.9 ± 4.3
Mean peak E_2 ± SD (pg/mL)	2791 ± 1639	3037 ± 1989	1942 ± 1201	1989 ± 1104
Mean thickness of endometrium at transfer ± SD (mm)	10.7 ± 2.3	11.2 ± 2.8	10.5 ± 3.3	11.6 ± 2.7
Mean number of retrieved oocytes ± SD (N)	17.5 ± 10.0 (3,277)	17.9 ± 7.7 (610)	11.7 ± 8.2 (834)	16.6 ± 13.7 (133)
% Mean M2/retrieved oocytes ± SD (N)	81.3 ± 15.8 (2,640)	82.5 ± 9.6 (497)	80.8 ± 15.3 (417)	81.8 ± 15.1 (106)
% Mean 2PN/M2 oocytes ± SD (N)	76.5 ± 16.9 (1,973)	72.9 ± 13.0 (355)	68.7 ± 21.0 (417)	68.5 ± 27.2 (64)
Mean number of good quality embryos ± SD (N)	5.9 ± 4.4 (1,097)	6.6 ± 4.5 (225)	1.2 ± 2.1 (85)	0.9 ± 1.4 (7)
Mean number of embryos transferred ± SD (N)	2.8 ± 0.5 (526)	2.9 ± 0.3 (100)	2.5 ± 0.9 (178)	2.9 ± 0.4 (23)
Mean ET duration ± SD (seconds; range)	86.1 ± 41.6 (29–318)	223.7 ± 108.2 (87–580)	91.8 ± 59.0 (30–342)	263.6 ± 86.7 (130–378)
N positive β-hCG cycles	135	25	41	2
N biochemical pregnancy (% positive cycles)	5 (3.7)	1 (4.0)	12 (29.3)	0
N clinical pregnancy (% [illegible])	130 (69.5)	24 (70.6)	29 (40.8)	2 (25.0)
N implantation (%)	184 (34.9)	36 (36.0)	37 (21.0)	2 (8.7)
N ongoing pregnancy (cycles; % ET)	103 (55.1)	20 (58.8)	24 (33.8)	2 (25.0)

Good-quality embryos are **less vulnerable** to prolonged transfer duration compared with poor-quality ones

Ciray HN et al. 2007

VI. Physician factor

Cycle characteristics and pregnancy rate per physician.

Physician ID	No. ET cycles	Age of women, (y)[a]	FSH amount[b]	Peak E_2[c]	No. of embryos	Mean no. of embryos per cycle[d]	Mean grade of embryos per cycle[e]	Pregnancy rate (%)[f]
1	633	34.4 ± 5.2	3,432 ± 1478	1,962 ± 1158	1,760	2.8 ± 0.9	1.7 ± 0.6	43.1 (273/633)
2	608	34.2 ± 5.6	3,267 ± 1725	2,026 ± 1232	1,742	2.9 ± 0.9	1.7 ± 0.6	45.6 (277/608)
3	233	34.2 ± 5.6	3,525 ± 1627	2,101 ± 1277	667	2.9 ± 1.0	1.7 ± 0.6	41.6 (97/233)
4	317	33.4 ± 5.5	2,602 ± 1609	2,409 ± 1298	912	2.9 ± 0.8	1.5 ± 0.6	46.1 (146/317)
5	173	33.9 ± 5.7	3,763 ± 1710	1,967 ± 1113	488	2.8 ± 0.9	1.8 ± 0.7	38.7 (67/173)
6	248	33.7 ± 5.7	3,857 ± 1717	2,184 ± 1339	662	2.7 ± 0.9	1.7 ± 0.7	49.2 (122/248)

The level of physician experience appears to be **a significant determining factor** in the reproductive outcome

Uyar A et al. 2011

Precautions following ET

I. Bed rest following ET

II. Mechanical closure of cervix following ET

I. Bed rest following ET

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Ongoing pregnancy/ live-birth rate per randomised woman	1	164	Odds Ratio (M-H, Fixed, 95% CI)	1.0 [0.54, 1.85]
2 Clinical pregnancy rate per randomised woman	2	542	Odds Ratio (M-H, Fixed, 95% CI)	1.13 [0.77, 1.67]
3 Multiple pregnancy per randomised woman	2	542	Odds Ratio (M-H, Random, 95% CI)	1.62 [0.33, 7.90]
4 Twin pregnancy per randomised woman	1	378	Odds Ratio (M-H, Fixed, 95% CI)	2.83 [0.74, 10.84]
5 Triplet pregnancy per randomised woman	1	378	Odds Ratio (M-H, Random, 95% CI)	7.34 [0.38, 143.15]
6 Miscarriage rate per randomised woman	2	542	Odds Ratio (M-H, Fixed, 95% CI)	1.63 [0.79, 3.35]

There was **no evidence of an effect** of bed rest in improving the rate of ongoing pregnancies, clinical pregnancies, multiple pregnancies, or miscarriages

Abou Setta AM et al. 2010

II. Mechanical pressure on the cervix

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Clinical pregnancy rate per randomised woman	1	639	Odds Ratio (M-H, Fixed, 95% CI)	1.92 [1.40, 2.63]
2 Multiple pregnancy per randomised woman	1	639	Odds Ratio (M-H, Fixed, 95% CI)	2.33 [1.53, 3.56]

There was a significantly **higher probability** of clinical and multiple pregnancy following mechanical pressure on the cervix compared with no intervention

Abou Setta AM et al. 2010

OBJECTIVE III

DEVELOPING A STANDARDIZED PROCESS

ET practices with supported proof of benefit

- **Use of soft embryo transfer catheters**
- **Ultrasound guidance**

ET practices with limited proof of benefit

- **Mid-uterine position of catheter tip**
- **Mechanical closure of the cervical canal following ET**
- **Acupuncture during ET**
- **Use of hyaluronic acid during ET**
- **Shortening of the loading discharging interval time of embryos**

ET practices with no proof of benefit

- **Use of mock transfer**
- **Full bladder during ET**
- **Use of cervical tenaculum**
- **Removal or flushing of the cervical mucus**
- **Antibiotic administration during ET**
- **Bed rest following ET**
- **Use of fibrin sealants during ET**

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ESHRE Annual Meeting 2013
Pre-congress course 15
Optimizing IVF outcome

Improving Implantation
The endometrial factor matters!!!

Prof. Carlos Simón MD; PhD
Professor Obs/Gyn, University of Valencia.
Scientific Director, Fundación IVI and IVIOMICS.

Disclosure

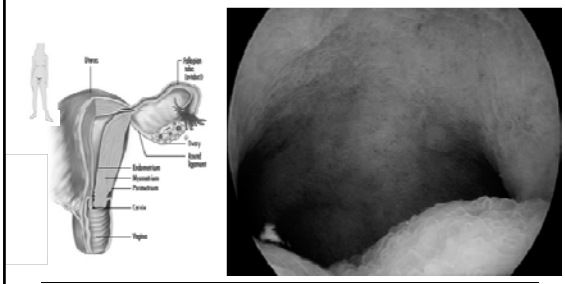
Shareholder:
IVI & IVIOMICS

Consultant:
MERCK SERONO

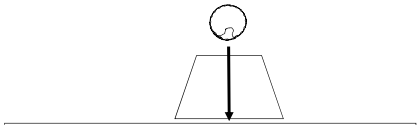
Learning Objectives

- ✓ To acknowledge the relevance of the endometrial factor
- ✓ To learn the different diagnostic methods of endometrial receptivity
- ✓ To discuss the clinical efficiency of personalized embryo transfer (pET) according to the endometrial status
- ✓ To introduce future non-invasive diagnostic methods.

Human endometrium.The last challenge



Window of Endometrial Receptivity (WOI) Does one size fit all?

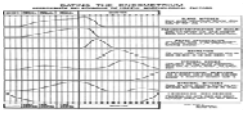


E	17	18	19	20	21	22	23
P	P_{+4}	P_{+5}	P_{+6}	P_{+7}	P_{+8}	P_{+9}	P_{+10}

DIAGNOSIS OF ENDOMETRIAL RECEPTIVITY

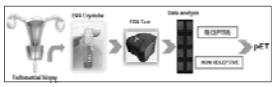
ANATOMICAL MEDICINE
ENDOMETRIAL DATING

Noyes, *et al.* (1950) *Fertil Steril.*
Coutifaris, *et al.* (2004) *Fertil Steril.*
Murray, *et al.* (2004) *Fertil Steril.*



MOLECULAR MEDICINE. TRANSCRIPTOMICS
ENDOMETRIAL RECEPTIVITY ARRAY (ERA)

Diaz-Gimeno, et al. (2011) Fertil Steril.
Diaz-Gimeno, et al. (2013) Fertil Steril.
Ruiz, et al. (2013) Fertil Steril. In press



PROTEOMICS OF ENDOMETRIAL RECEPTIVITY

Dominguez F et al. (2009) Hum Reprod
Garrido T et al. (2012) FASEB J.

SECRETOMICS. NEXT GENERATION ENDOMETRIAL DIAGNOSTICS

Berlanga O, et al. (2011) Placenta.
Vilella F , et al. (2013) Science Translational Med Submitted

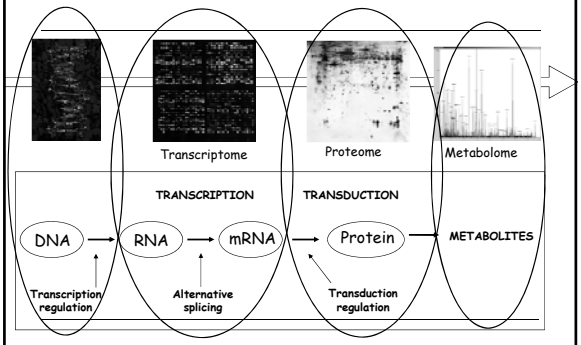
Dating the endometrial biopsy[1]

- Randomized studies
  - Interobserver and cycle-to-cycle (60%) variations[2]
  - Endometrial dating is not related to fertility status[3]

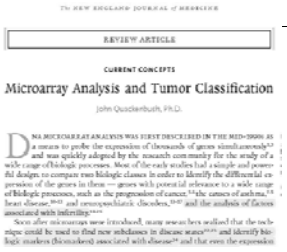
Histological dating is not a valid method for the diagnosis of luteal phase deficiency neither guidance throughout clinical management in infertility

1. Noyes, et al. Fertil Steril 1950
2. Murray, et al. Fertil Steril 2004
3. Coutifaris, et al. Fertil Steril 2004

The age of -OMICS



Transcriptomics


REVIEW ARTICLE

CURRENT CONCEPTS

Microarray Analysis and Tumor Classification

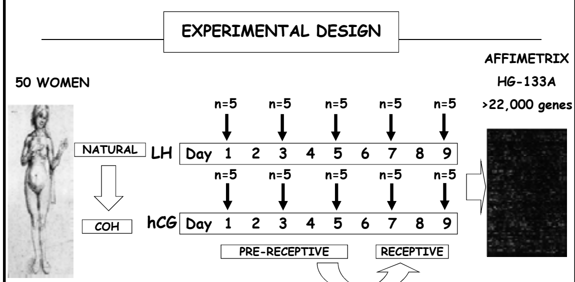
John Quackenbush, Ph.D.

Table I. Summary of studies performed in human endometrium using microarray analysis

Process studied	Microarray	Company	Number of gene targets	Study	
Decidualization	Clontech Atlas array	Stanford University	589	Popovici et al. (2000)	
Decidualization	Incyte human GEM-V	Incyte Genomics	6918	Brar et al. (2001)	
Endometrial cancer	Hu6800	Affymetrix	6000	Mutter et al. (2001)	
WOI	HG-U95A	Affymetrix	12 686	Kao et al. (2002)	
WOI	HG-U95A	Affymetrix	12 686	Carson et al. (2002)	
Endometriosis	Human gene genefilter GF211	Research Genetics	4133	Eyster et al. (2002)	
Endometriosis	Atlas human cDNA expression Array	Clontech	597	Lebovic et al. (2002)	
Endometrial cancer	Oncochip	Centro Nacional de Investigaciones Oncológicas	6386	Moreno-Bueno et al. (2003a)	
WOI	Human cytokine expression array	R&D Systems	375	Dominguez et al. (2003)	JCEM
Decidualization	HU95A	Affymetrix	12 686	Tierney et al. (2003)	
Endometriosis	Home-made	University of Tokio	23 040	Arimoto et al. (2003)	
WOI	HG U95 A-E	Affymetrix	>60 000	Borthwick et al. (2003)	
WOI	HG-U95A	Affymetrix	12 686	Riesewijk et al. (2003)	MHR
RU486	Home-made	University of Cambridge	~1000	Catalano et al. (2003)	
Endometriosis	HG-U95A	Affymetrix	12 686	Kao et al. (2003)	
Endometrial cancer	Home-made	National Cancer Institute	9984	Risinger et al. (2003)	
Progesterone effect	Human Chip 1K set 1	Takara Shuzo	1000	Okada et al. (2003)	
Endometriosis	Atlas human 1.2 cDNA expression array	Clontech	1172	Matsuzaki et al. (2004)	
Endometrial cancer	GEMarray clones	Incyte Genomics	18 098	Cao et al. (2004)	
Endometrial cancer	Home-made	University of Cambridge	1056	Saidi et al. (2004)	
Endometrial cancer	U133A	Affymetrix	>22 000	Ferguson et al. (2004)	
Stimulated cycles	HG U95Av2	Affymetrix	12 686	Mirkin et al. (2004)	
Menstrual cycle	Home made	Peter MacCallum Cancer Institute	10 500	Ponnampalam et al. (2004)	
Endometrial cancer	U133A	Affymetrix	>22 000	Ferguson et al. (2005)	
WOI	HG U95Av2	Affymetrix	12 686	Mirkin et al. (2005)	
Proliferative phase	BD Atlas nylon cDNA expression array	BD Biosciences Clontech	1200	Yanaihara et al. (2005)	
Stimulated cycles	HG_U133A	Affymetrix	>22 000	Horcajadas et al. (2005)	MHR
Stimulated cycles	HG_U133A	Affymetrix	>22 000	Simón et al. (2005)	HR
Menstrual cycle	HGU133 Plus 2.0	Affymetrix	54 600	Talbi et al. (2005)	
Menstrual cycle	HU-133A	Affymetrix	>22 000	Punyadeera et al. (2005)	
RU486	Home-made	University of Cambridge	>12 000	Sharkey et al. (2005)	
Endometriosis	Atlas human 1.2 cDNA expression array	Clontech	1172	Matsuzaki et al. (2005)	
IUD	Home-made	University of Cambridge	>16 000	Horcajadas et al. (2006)	JCEM

Horcajadas JA, Pellicer A, Simón C. Hum Reprod Update 2007.

Gene expression profiling during the WOI in natural versus COH



Caucasian
Fertile women with normal cycles
23-39 years
body mass index:19-25 kg/m2

Horcajadas JA/ Simon C. JCEM 2008

Endometrial Receptivity Array (ERA)

GENETICS

A genomic diagnostic tool for human endometrial receptivity based on the transcriptomic signature

Patricia Díaz-Gimeno, Ph.D., José A. Horcajadas, Ph.D., José A. Martínez-Conejero, Ph.D., Francisco J. Esteban, Ph.D., Pilar Alamá, M.D., Antonio Pellicer, M.D., and Carlos Simón, M.D.

Fundación IVI-Instituto Universitario IVI, University of Valencia, Valencia; Instituto de Investigación, Hospital Clínico de Valencia, Valencia University, Valencia; iGenomix, Valencia; Department of Experimental Biology, University of Jaén, Jaén; and Centro de Investigación Príncipe Felipe, Valencia, Spain

Objective: To create a genomic tool composed of a customized microarray and a bioinformatic predictor for endometrial dating and to detect pathologies of endometrial origin. To define the transcriptomic signature of human endometrial receptivity.

Design: Two cohorts of endometrial samples along the menstrual cycle were used: one to select the genes to be included in the customized microarray (endometrial receptivity array [ERA]), and the other to be analyzed by ERA to train the predictor for endometrial dating and to define the transcriptomic signature. A third cohort including pathological endometrial samples was used to train the predictor for pathological classification.

Setting: Healthy oocyte donors and patients.

Patient(s): Healthy fertile women (88) and women with implantation failure (4) or hydrosalpinx (7).

Intervention(s): Human endometrial biopsies.

Main Outcome Measure(s): The gene expression of endometrial biopsies.

Result(s): The ERA included 238 selected genes. The transcriptomic signature was defined by 134 genes. The predictor showed a specificity of 0.8857 and sensitivity of 0.99758 for endometrial dating, and a specificity of 0.1571 and a sensitivity of 0.995 for the pathological classification.

Conclusion(s): This diagnostic tool can be used clinically in reproductive medicine and gynecology. The transcriptomic signature is a potential endometrial receptivity biomarkers cluster. (Fertil Steril® 2011;95:50–60. ©2011 by American Society for Reproductive Medicine.)

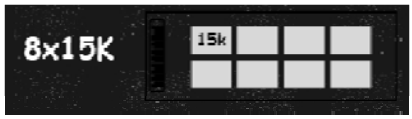
Key Words: Endometrial receptivity, endometrial dating, microarray, transcriptomic signature, predictor, diagnostic tool

Fertility and Sterility® Vol. 95, No. 1, January 2011 0015-0282/$36.00

doi:10.1016/j.fertnstert.2010.04.063

Endometrial Receptivity Array (ERA)

Agilent e-array: https://earray.chem.agilent.com/earray/



-**238 Genes**
-Probe selection: "Cross-linking"
-569 probes
-8 copies per probe
-Controls

Patented in 2009: PCT/ES 2009/000386

Endometrial Receptivity Array (ERA)

Customized microarray

⇩

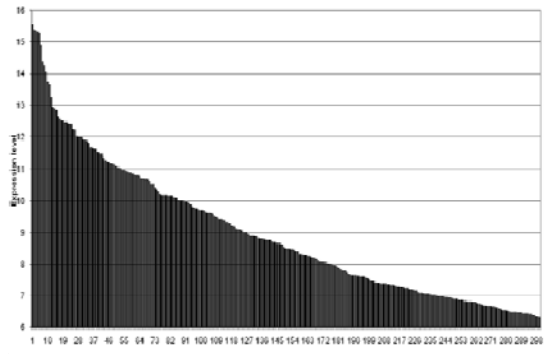
Bioinformatic analysis of data obtained by the customized microarray

⇩

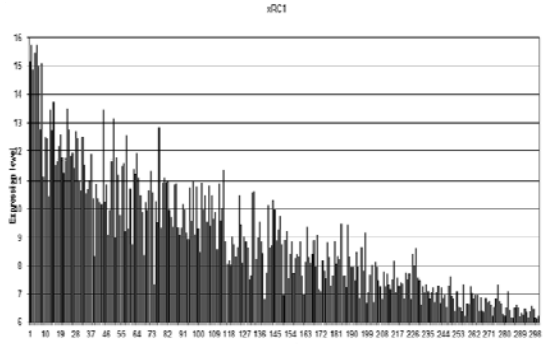
Classification and prediction from gene expression.

Predictors: Characterization of RECEPTIVITY transcriptome

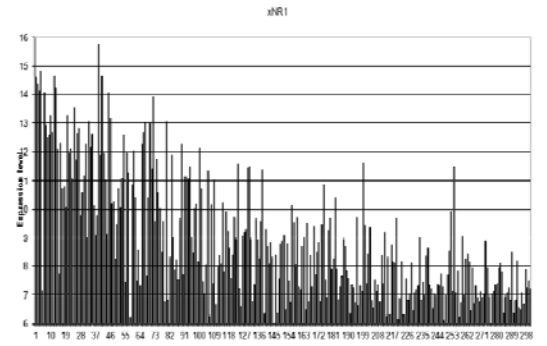
Receptive Profile



Receptive Sample RC1

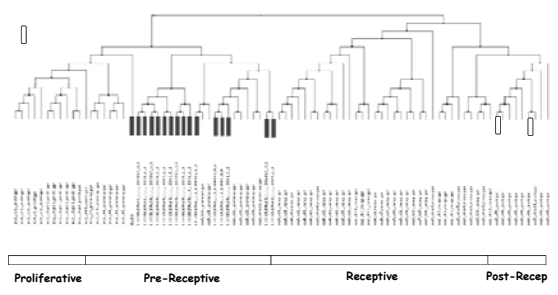


IF Sample NR1



Predictor classifies the molecular receptivity status of the endometrium

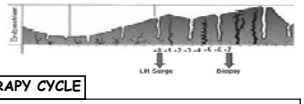
Non Receptive samples



Endometrial Receptivity Array (ERA) - Timing of the biopsy

NATURAL CYCLE

Endometrial biopsy must be taken on the 7th day after the LH surge (LH+7) (urine or serum preferible).

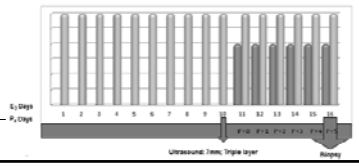


HORMONE REPLACEMENT THERAPY CYCLE

Endometrial biopsy must be taken on day P+5, after proper E_2 priming

E_2 :6 mg/day

P_4 :800 mg/day



Endometrial Receptivity Array (ERA)

INTERPRETATION OF RESULTS

Receptive (R):
The gene expression profile corresponds to a normal receptive endometrium.

⇒ **It is recomended to proceed with the embryo transfer at the indicated WOI**

Non Receptive (NR):
The gene expression profile does not correspond to a normal receptive endometrium.

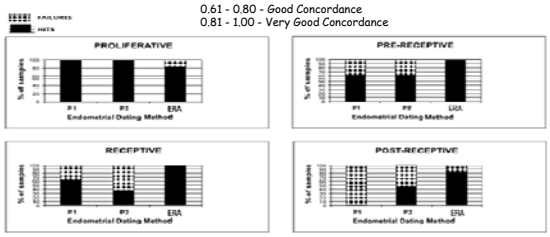
⇒ **It is not recomended to proceed with the embryo transfer at the indicated WOI and a personalization of the WOI is advised.**

Endometrial Receptivity Array (ERA) – Accuracy

In a blinded study ERA classifies better than Noyes criteria

	Pathologist 1 (P1)	Pathologist 2 (P2)	P1 vs P2	ERA
Kappa value	**0.618** (0.446-0.791)	**0.685** (0.545-0.824)	**0.622** (0.435-0.839)	**0.922** (0.815-1.000)

0.61 - 0.80 - *Good Concordance*
0.81 - 1.00 - *Very Good Concordance*



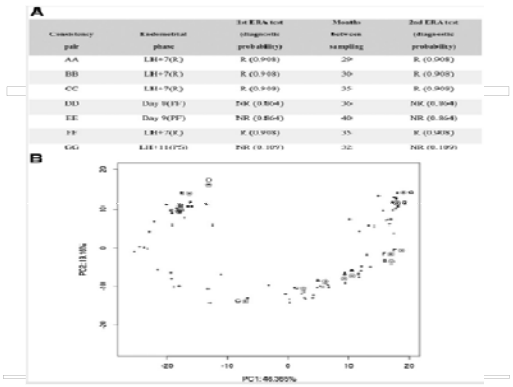
Díaz P et al Fertil Steril. 2013

Endometrial Receptivity Array (ERA) – Consistency

ERA TEST ANALYZED IN THE SAME PATIENT in two endometrial biopsies two years apart

Code	Date First Biopsy	Date Second Biopsy	Months between	First Biopsy Results	Second Biopsy Results
CON1	09/2009	02/2012	29	Receptive	Receptive (0.908)
CON2	09/2009	03/2012	30	Receptive	Receptive (0.908)
CON3	05/2009	04/2012	35	Receptive	Receptive (0.908)
CON4	05/2009	05/2012	36	Proliferative	Non Receptive (0.864)
CON5	01/2009	05/2012	40	Proliferative	Non Receptive (0.864)
CON6	07/2009	05/2012	35	Receptive	Receptive (0.908)

Díaz P et al Fertil Steril. 2013



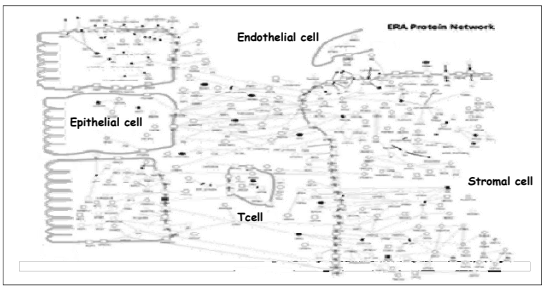
Díaz P et al Fertil Steril. 2013

ERA Gene Topography

Pre-Receptive *vs.* Receptive

35 genes differentially expressed

☐ Over-expressed in Pre-Receptive
☐ Under-expressed in Pre-Receptive

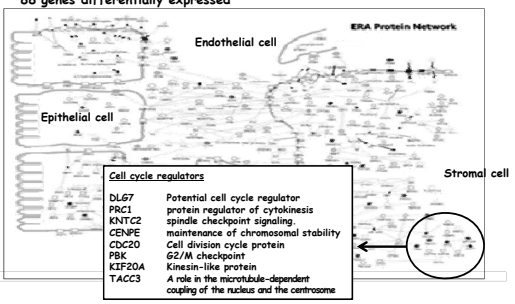


ERA Gene Topography

Receptive *vs.* Post-Receptive

☐ Over-expressed in Receptive
☐ Under-expressed Receptive

88 genes differentially expressed



ERA in patients with RECURRENT IMPLANTATION FAILURE (RIF)

≥3 unsuccessful embryo transfers in patients < 40 years old undergoing IVF

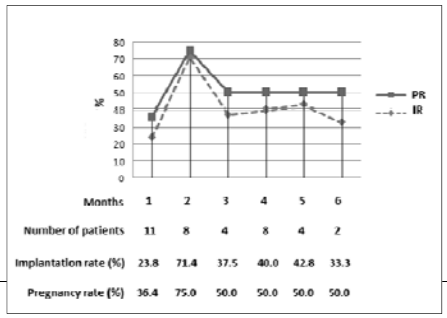
≥3 unsuccessful embryo transfers in patients undergoing ovum donation

Ruiz M et al Fertil Steril 2013 In press

Clinical Outcome

	RIF	CONTROL
N°. of patients	91	27
Age (years)	37.7 ± 4.6	38.9 ±5.3
Ave. # of previous cycles	4.8 ± 2.1	0.4 ±0.5
Receptive ERA/total analyzed (%)	67/91 (73.6)	23/27(85.2)
Patients with pET after Receptive ERA	25	9
Implantation rate 1st transfer post-ERA (%)	15/49 (30.6)	7/17 (41.2)
Pregnancy rate 1st transfer post-ERA (%)	13/25 (52.0)	5/9 (55.6)
Biochemical pregnancies (%)	-	1/7 (14.3)
Clinical abortions (%)	1/16 (6.2)	-

First Embryo transfer outcome after RECEPTIVE ERA in patients with RIF



Months	1	2	3	4	5	6
Number of patients	11	8	4	8	4	2
Implantation rate (%)	23.8	71.4	37.5	40.0	42.8	33.3
Pregnancy rate (%)	36.4	75.0	50.0	50.0	50.0	50.0

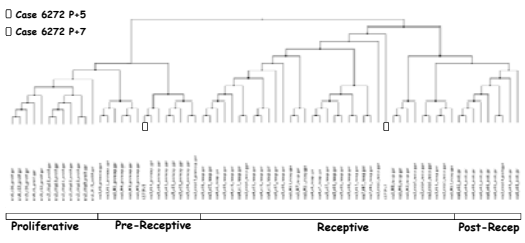
ERA Clinical Outcome in Non Receptive patients

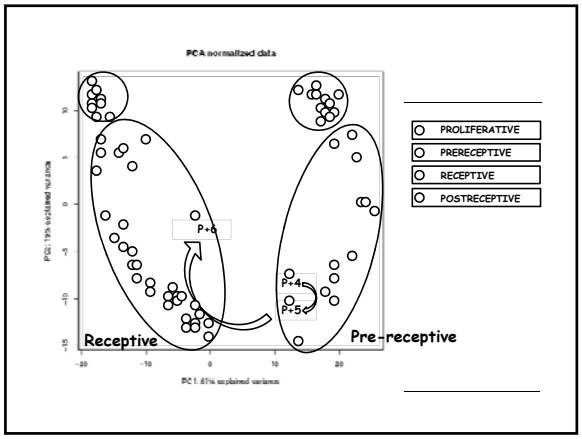
Displacement of the WOI

	1° BIOPSY			2° BIOPSY	
PATIENT	DAY#1	RESULT#1	PROFILE#1	DAY#2	RESULT#2
4007	P+5	NR	Pre-Receptive	P+7	R
4769	P+5	NR	Pre-Receptive	P+7	R
5451	P+5	NR	Pre-Receptive	P+7	R
6272	P+5	NR	Pre-Receptive	P+7	R
7351	P+5	NR	Pre-Receptive	P+7	R
2917	P+5	NR	Pre-Receptive	P+7	R
3334	P+5	NR	Pre-Receptive	P+7	R
1020	LH+7	NR	Pre-Receptive	LH+9	R
3475	P+5	NR	Pre-Receptive	P+7	R
4801	P+5	NR	Pre-Receptive	P+7	R

ERA Clinical Outcome in Non Receptive patients

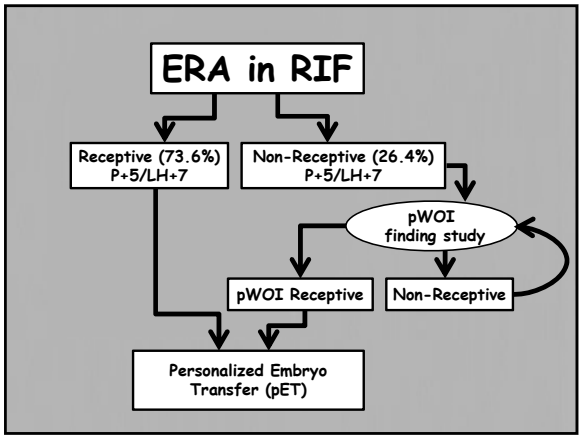
- Example of Case 6272



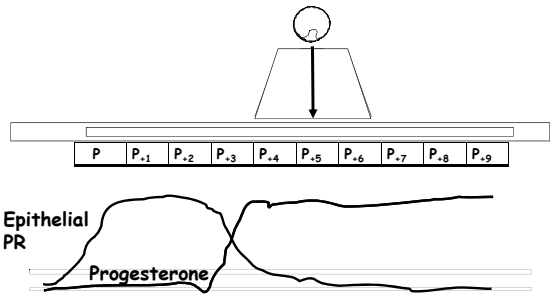


Clinical Outcome in Non Receptive ERA patients

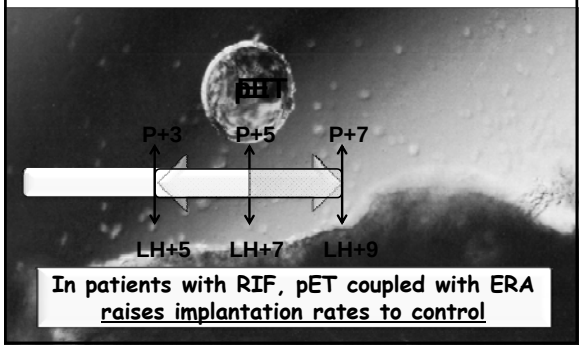
No of patients (RIF / Controls)	28 (24/4)
Ave. # of previos cycles RIF / Control Patients	5.3±2.0/ 0.3±0.5
ERA Prediction: Pre-receptive (%) Post-receptive (%)	26/28 (92.8) 2/28 (7.2)
2nd ERA at the specified day (P+4;P+6;P+7;LH+9)	16
Months between 1st and 2nd ERA	2.8±2.9
2nd ERA Receptive at the specified day	14
Patients with pET after 2nd RECEPTIVE ERA**	10
Months between 2nd RECEPTIVE ERA and pET	2.3±1.5
Implantation rate using pET (%)	10/21 (47.6)
Pregnancy rate using pET (%)	8/10 (80.0)
Biochemical pregnancies (%)	1/8 (12.5)
Clinical abortions (%)	1/8 (12.5)



Window of Endometrial Receptivity (WOI)



Personalized Embryo Transfer (pET)

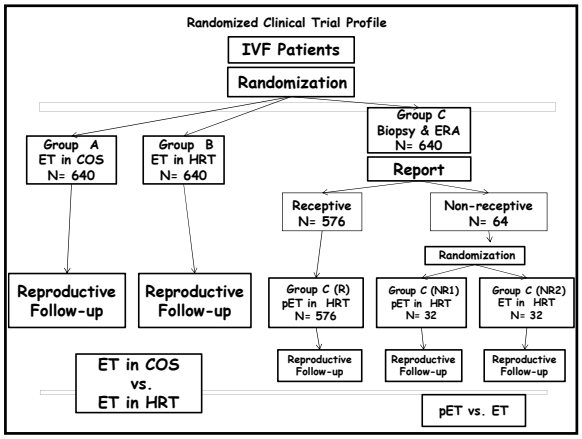


In patients with RIF, pET coupled with ERA raises implantation rates to control

Conclusions

- **ERA test diagnoses the timing of the endometrial WOI.**
- **The PR in RIF patients with R ERA is comparable to the general ART population.**
- **NR ERA patients have the WOI displaced and personalized Embryo Transfer (pET) improve clinical results.**

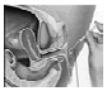
WE ARE WORKING ON THE CLINICAL VALIDATION OF THE ERA TEST IN a Internationl Multicenter RCT



SECRETOMICS. NEXT GENERATION ENDOMETRIAL DIAGNOSTICS

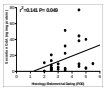
- ✓ Aspiration of endometrial secretion does not affect pregnancy rates

  Van der Gaast et al. RBmOnline 2002



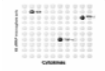
- ✓ Glycodelin levels correlate with the menstrual cycle phase of endometrial aspirations

  Van der Gaast MH, et al. BJOG 2009



- ✓ The profile of cytokines can be determined in endometrial secretions

  Simón C, et al. J Reprod Immunol 1996
  Boomsma CM et al. RBmOnline 2009



- ✓ *Non-invasive*
- ✓ *Embryo transfer must be performed in the same cycle*
- ✓ *Implantation rate itself should be the end-point*

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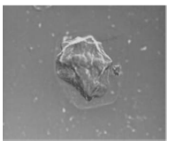
Optimizing the Outcome of Cryopreservation



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- In 1937, Luyet wrote that “crystallization is incompatible with living systems and should be avoided whenever possible”

Luyet. Biodynamica 1937; 1: 1–

Introduction

- Cryopreservation of human oocytes, zygotes, cleavage stage embryos and blastocysts has progressed to become a useful adjunct to human IVF-ET programmes
- Vitrification, an ultra-rapid cooling technique, offers an interesting perspective in the attempts to develop the optimal cryopreservation procedure for human oocytes and embryos
- Until recently, scientific results have proved that vitrification is at least equal or significantly better than results obtained by the traditional slow-cooling method

Conventional cryopreservation versus ultra-rapid vitrification

- Basic principles of cryopreservation
- Traditional method versus rapid freezing
- Vitrification cooling rates
- Difficulties or disadvantages of vitrification
- Safety of the procedure and straws and vials (LN_2 contamination)
- Cryoprotectants used in vitrification
- State of the art

Main principles of vitrification in ART

- Guarantee fertilisation (oocyte)
- High survival rate after warming
- Increasing the success rate by achieving a significantly high cumulative pregnancy rate

Steps of cryopreservation

- Equilibration in the cryoprotectant
- Freezing process
- Storage in LN_2
- Thawing (warming) process
- Removal of the cryoprotectant
- Culture in the physiological *milieu*

Factors influencing the success of cryopreservation

- Possible temperature shocks (+15°C to –5°C)
- Possible changes in the plasma membrane
- Selection of the right cryoprotectant
- Dehydration: intensity and time
- Critical cell volume
- Solute concentration
- Cooling rate
- Thawing rate

Temperature shock

- This happens if the cells are cooled too fast (without ice crystallisation)
- Temperature shock starts at the plasma membrane due to:
  - Shrinkage of different parts of the membrane
  - Mechanical effect
  - Volume reduction

Characteristics of cryoprotectants

- High solubility in water
- Relative low molecular weight (<400)
- Fast cell permeability
- Combine with water to build stable H_2 bridges
- Non-toxic at high concentrations
- Reduce the freezing point of extracellular fluid
- Allow low influx of intracellular water to avoid sudden cell shrinkage

Cryoprotectants

- Permeable (Mw <400)
  - Methanol CH_3OH 32
  - Ethanol C_2H_5OH 46
  - Ethylene glycol $C_2H_4(OH)_2$ 62
  - 1-2 Isopropanol $C_3H_6(OH)_2$ 76
  - Glycerol $C_3H_5(OH)_2$ 92
  - DMSO $(CH_3)_2SO$ 78
- Non-permeable (MG >10,000)
  - Polyethylene glycol 8000
  - Polyvinylpyrrolidone 40,000
  - Ficoll 70,000 or 400,000
  - Sucrose –

Cryoprotectant concentration and solute concentration during freezing

Isotonic saline solution (NaCl 9.0 g/L)

Replaced with 1% DMSO	Replaced with 5% DMSO	Replaced with 10% DMSO

It will reach a concentration of 50 g/L by

–5°C	–20°C	–50°C

Cryoprotectant action

Penetrate into cell

↓

Partially replace intracellular water

↓

Dehydration of cell

Cooling rate

- Avoid temperature shock
- Avoid cell damage during dehydration
- Avoid damaging colloidal *milieu* of cell

Cooling rate

- Optimal cooling rate
  - If cell gives the maximum amount of intracellular water to avoid intracellular ice crystal formation

Cooling rate

- Optimal cooling rate is dependent on the critical volume of the cell, which can be defined by:
  - Permeability of the cell membrane to water
  - Large membrane surface
  - Relationship between cell surface and cell volume
- Each cell has a unique cooling rate, depending on these parameters

Thawing rate

- The thawing rate is closely related to the cooling rate
- In general, fast thawing is preferred
- Thawing rate has no influence on slow freezing

- The most important principle of cryopreserving oocytes and embryos is:
- Avoid the formation of ice crystals during the freezing process
  - Intracellular crystal formation creates lethal factors through unwanted physical and chemical events that may injure the cell during the cryopreservation process

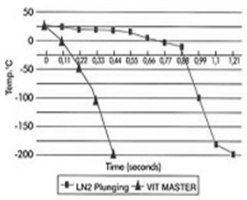
Two techniques have been developed

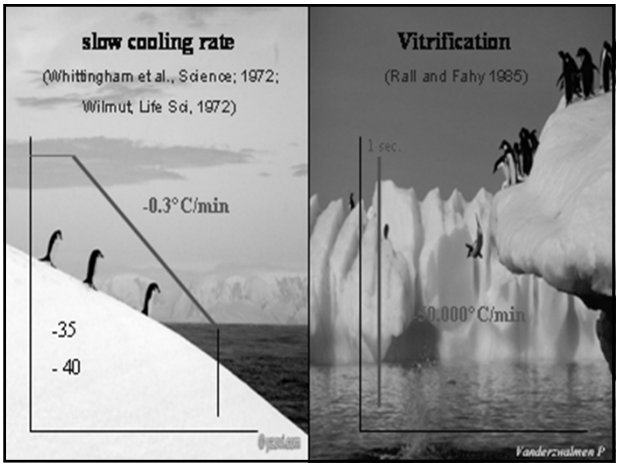
- Controlled, slow freezing
  - Slow-rate freezing: Whittingham et al., 1972
- Ultra-rapid freezing
  - Vitrification procedure: Rall & Fahy, 1985

Whittingham et al. Science 1972; 178 (59): 411–414
Rall & Fahy. Nature 1985; 313 (6003): 573–575

Freezing in liquid nitrogen (Vitrification)

- Physical definition:
  - Solidification of a solution to a state similar to that of glass





"The physical definition of vitrification is the solidification of a solution (water is rapidly cooled and formed into a glassy, vitrified state from the liquid phase) at low temperature, not by ice crystallization but by extreme elevation in viscosity during cooling"

Fahy, 1984

Fahy et al. Cryobiology 1984; 21: 407–426

- In contrast to slow-rate freezing protocols, during vitrification the entire solution remains unchanged and water does not precipitate, so no ice crystals are formed

Slow freezing versus ultra-rapid freezing

	Traditional	Vitrification
CPA concentration	1.5 M	3.0–5.0 M
Volume	0.3–1.0 mL	<1 µL
Contact between N_2 and cell	No	Yes
Cooling rate	~0.5°C/min	~25.000–50.000°C/min
Freezing	Slow	Ultra-rapid
Thawing / warming	Slow	Rapid
Time consuming	≥180 min	1 sec
Dehydration	Not controlled	Controlled

Slow freezing versus ultra-rapid freezing

	Traditional	Vitrification
Reduced osmotic injury	No	Yes
Zona pellucida fracture	Possible	No
Ice crystal formation	Yes	No
Seeding	Yes	No need
Procedure	Complicated	Simple
Device	Yes	No need
Costs	High	Less
Liquid nitrogen amount	High	Much less
Duration out of Incubator	3-4 hr.	10-15 min.

Historical review

- It was described at the end of the 18th Century
  Tammann, 1898
- Vitrification of mouse embryos at –196°C
  Rall & Fahy, 1985; Ali & Shelton, 1993
- Blastocyst development from bovine oocytes
  Martino et al., 1996
- Blastocyst development, pregnancies, deliveries from human vitrified oocytes, zygotes, cleaved eggs and blastocyst

Tammann. Z phys Chem 1898; 25: 441-479
Rall & Fahy. Nature 1985; 313 (6003): 573–575
Ali & Shelton. J Reprod Fertil 1993; 98 (2): 459–465
Martino et al. Biol Reprod 1996; 54 (5): 1059–1069

Why do we prefer the vitrification procedure now?

- No mechanical injury (extracellular crystal formation)
- Less osmotic stress to cells
- No intracellular crystal formation
- Less labour in laboratory daily work
- Simple protocol
- Useful for oocytes and blastocyst, which have less success with slow freezing
- No need for expensive devices

Example of cooling rates

- ~ 2500°C/min by using 0.25 mL straws
  - Thick straws and large volumes of medium do not allow a high cooling rate and thawing rate
- ~ 25.000–50.000°C/min by using a carrier that allows very small volumes
  - Direct contact with LN_2

What are the different solutions for vitrification?

- Cell membrane-permeable cryoprotectant
  - Glycerol
  - Ethylene glycol
  - DMSO
- Non-permeable cryoprotectant
  - Sugar
  - Proteins
  - Polymer

Vitrification with DMSO protects embryo membrane integrity better than solutions without DMSO

Conclusion: The two standard vitrification protocols, DMSO-containing and DMSO-free, did not differ in embryo survival rates and were equally efficient in both mouse and human embryo models.

Extended exposure to vitrification solutions using both vitrification protoclos showed that the DMSO-containing vitrification solutions were milder and did not lead to cell membrane damage and death as quickly as the DMSO-free vitrification solutions.

Kartberg et al. RBM online 17. Sept. 2008

Non-permeating sucrose

- Disaccharide additives of large molecular weights
  - Do not penetrate the cell membrane
  - Can significantly reduce the amount of cryoprotectant required
  - Reduce the toxicity of ethylene glycol
  - Act as an osmotic buffer to reduce the osmotic shock that might occur as a result from the dilution of the cryoprotectant after cryo-storage

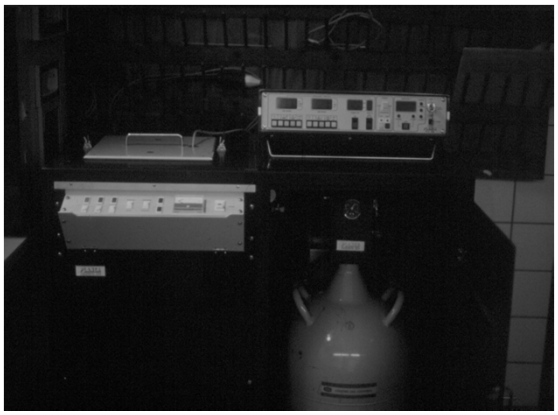
Successful vitrification

- High cooling rate (> –50.000°C/min)
- Fast cooling period (<1 sec.)
- Low volume (<1 µL)
- High concentration of cryoprotectants

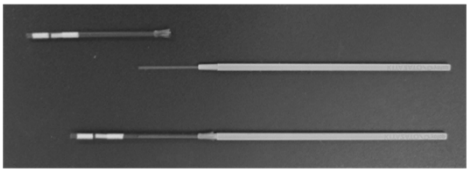
=> This will avoid crystal formation

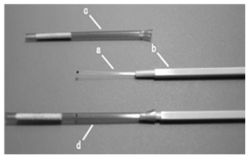
Why do we prefer the vitrification procedure now?

- No mechanical injury (extracellular crystal formation)
- Less osmotic stress to cells
- No intracellular crystal formation
- Less labour in laboratory daily work
- Simple protocol
- Useful for oocytes and blastocysts, which have less success with slow freezing
- No need for expensive devices



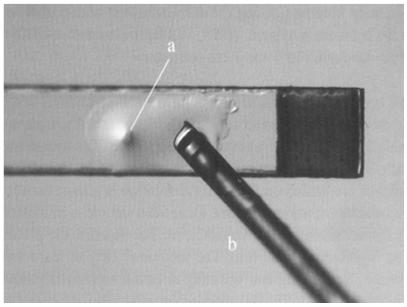
Ultra-rapid vitrification container: Cryotop



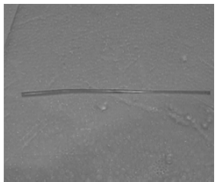


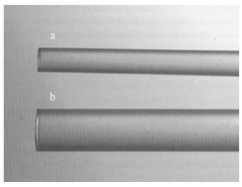
a: Transparent fine polypropylene sheet (0.8 mm x 2 cm)
b: Plastic handle
c: Cover cap
d: Cover top part (during storage in LN_2)

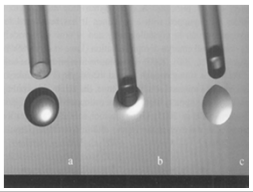
Cryotop



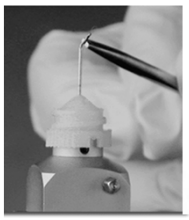
Open- pulled straws (OPS)



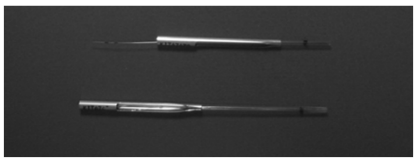




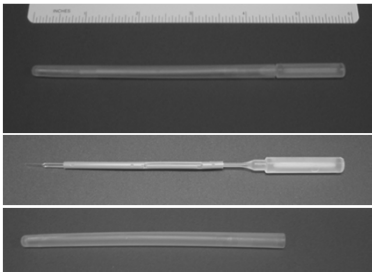
Cryoloop



Cryotip



Cryoleaf (McGill)



Antinori et al. RBM Online 2007; 14 (1): 72-79

	Vitrified / warmed group	Update up to July 2007
No. of cycles	120	270
No. of warmed oocytes	330	707
No. of oocytes survived (%)	328 (99.3%)	699 (98.8%)
No. of injected oocytes	328	699
No. of fertilised oocytes (2PN)	305 (92.9%)	639 (91.4%)
No. of cleaved oocytes	295 (96.7%)	624 (97.6%)

Antinori et al. RBM Online 2007; 14 (1): 72-79

	Vitrified / warmed group	Update up to July 2007
No. of transfers	120	270
No. of transferred embryo	295	624
No. of embryos per transfer	2.45	2.31
No. of clinical pregnancies	39 (32.5%)	76 (28.1%), 5 twins
No. of ongoing pregnancies	–	24
No. of abortions	8 (20.5%)	16 (21%)
No. of deliveries	31	36, all singleton
IR per transferred embryo	13.2 %	12.9%
IR per thawed oocyte	11.8%	11.6

Oocyte Donation & Vitrification

	Vitrified
M II oocytes	231
Survival	96.9%
Fertilization	76.3%
No. of transfers	23
Mean number of embryos	2.1
Ongoing pregnancy rate	48%

Cobo et al., 2008

Embryo Development of Fresh „Versus“ Vitrified Metaphase II Oocytes after ICSI: A Prospective Randomised Sibling-Oocyte Study

	Fresh ICSI (%)	Vitrified/Warmed ICSI (%)
No of oocytes	120	124
Fertilization (2PN) per sibling oocyte	83.3%	76.6%
Fertilization (2PN) per injected oocyte	83.3%	79.2%
Normal 2PN morphology	96 %	90.5 %
1PN oocytes	2.5 %	5 %
3PN	0.83 %	1.66 %
Degenerated oocytes post-ICSI	0.83 %	3.34 %
Day 2 embryo development	100 %	97.9 %
Excellent quality embryos	52 %	51.6 %
Good quality embryos	38.0 %	43.2 %
Fair/poor quality embryos	10 %	3.16%

Renzi et al. Sept. 2010

Embryo Development of Fresh „Versus“ Vitrified Metaphase II Oocytes after ICSI: A Prospective Randomised Sibling-Oocyte Study

- Conclussion: Our results indicate that oocyte vitrification procedure followed by ICSI is not inferior to fresh insemination procedure, with regard to fertilization and embryo developmental rates. Moreover, ongoing clinical pregnancy is comparable with this procedure, even with a restricted number of oocytes available for inseminat-ion. We believe that these results will help the spread of vitrification for human oocytes

Rienzi et al., 2010
Human Reprod., 25, 66-73

Use of cryo-banked oocytes in an ovum donation programme: a prospective, radomized, controlled, clinical trial

	Egg-bank	Fresh
No of subjects	295	289
Oocytes received	3039	3185
Survival rate	92.5%	-
Fertilization rate	74.2%	73.3%
Top quality Day-2 cleaved embryos	43.6%	43.8%
Top quality Day-3 cleaved embryos	58.4%	60.7%
Ongoing pregancy rate / transfer	49.1%	48.3%

Cobo et al. Sept 2010
Human Reprod. 25

Use of cryo-banked oocytes in an ovum donation programme: a prospective, radomized, controlled, clinical trial

- Statment: The ongoing pregnancy rate obtained in this study after oocyte vitrification/storage demonstates that cryo-banking can provide successful clinical outcome in oocyte donation programmes.
- It allowes to overcome the traditional drawbacks associated with use of fresh oocytes.

Cobo et al. Sept. 2010

Human Reprod. 25

Comparison between fresh and frozen-thawed embryo transfer Vitrification of Zygotes (Luebeck)

	Fresh ET	Frozen-Thawed ET
No of patients	52	59
No of cycles	53	61
No of vitrified Zygotes	/	259
No of survived zygotes	/	250 **(96.5)**
No of transferred embryos	114 (2.5)	240 (2.6)
No of embryo transfers	53	83
No of pregancies	**13 (24.5)**	**29 (34.1)**

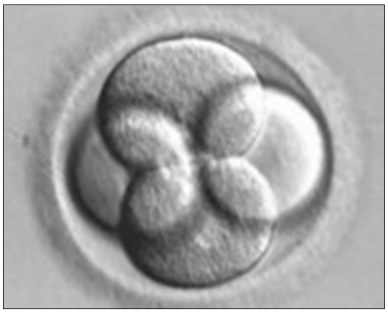
Our Results in Avoiding Hyperstimulation

Patients Triggered with GnRH-Agonist

No. of Patients	No. of Zygotes vitrif.	No. of Zygotes re-warmed	No. & (%) Zygotes survived	No. & (%) Preg.	No. Of Children born	(%) of live birth
59	433	163*	158 (97)	25 (42)	13**	(25)

* No. of Patients received warmed Zygotes 45

** Two Twins



Can fresh embryo transfers be replaced by cryo-preserved-thawed embryo transfers in assisted reproductive cycles?
A prospective controlled trial.

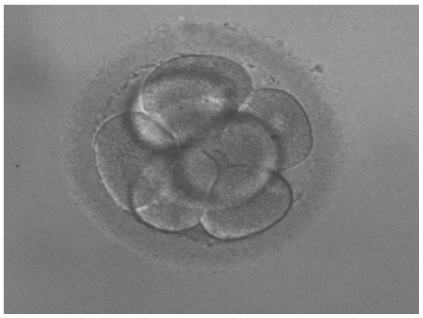
	Fresh ET (n=191)	FET (n=184)	p value
No. of oocytes retrievd	14.2	14	NS
No. Of M II oocytes retrieved	11	10.8	NS
E2 day of hCG (pg/ml)	2861.2	2793.4	NS
Fertilization rate	72.7	73	NS
No. of embryos transferred	2.2 $\pm$ 0.4	2.1 $\pm$ 0.3	NS
Implantation rate (%)	14.1	23.0	0.004
Clinical pregnancy rate (%)	24.6	36.4	0.013
Ongoing pregnancy rate (%)	22.5	34.2	0.012
Multiple pregnancy rate (%)	14.9	26.4	NS

Aflatoonian et al. 2010, JARG.

Can fresh embryo transfers be replaced by cryo-preserved-thawed embryo transfers in assisted reproductive cycles?

- **CONCLUSIONS:**
- Controlled ovarian hyperstimulation has been shown to advance endometrial maturation and adversely affects implantation in ART. It has been reported that there is a better embryo-endometrium synchrony in frozen-thawed embryo transfer cycles than fresh embryo transfer cycles
- Frozen/thawed embryo transfer can be performed instead of fresh ETs to improve the outcome of ART in highly selected patients

Aflatoonian et al. 2010, . JARG



Vitrification of human 8-cell embryos, a modified protocol for better pregnancy rates Rama Raju et al. (2005)

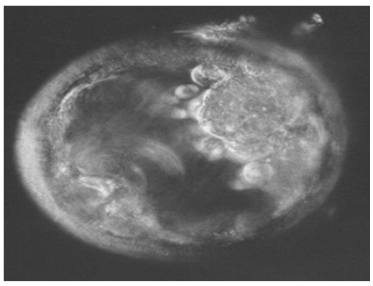
	Vitrification	Slow freezing
Embryos, n	436	420
Embryos thawed, n	127	120
Embryos survival, n (%)	121 (95.3)	72 (60)
Pregnancy, n (%)	14 (35)	4 (17.4)

40% ethylene glycol + 0.6 mol sucrose, nylon loop

"Ethylene glycol is a good croyprotectant to preserve 8-cell embryos because of its low toxicity as shown by the high survival rate, and vitrification is a promising alternate to the conventional slow-freezing method."

Rama Raju et al. Reprod Biomed Online 2005; 11 (4): 434–437

The blastocyst is characterized by early cavitation resulting in the formation of an eccentric and then expanded cavity lined by a distinct inner cell mass region and trophectoderm layer. The blastocele is less than half the volume of the embryo



Outcome of Blastocyst Vitrification by using the "Cryotop" Method

Reference	No. of vitrif. blastocysts	Survival rate %	Implantation rate %	Pregnancy rate %
Hiraoka et al. 2003	49	**98**	33	**50**
Stehlik et al. 2005	41	**100**	NA	**50**
Kuwayama et al. 2005	**6484**	90	NA	**53**

Obsteric And Perinatal Outcome in 200 Infants Conceived From Vitrified Oocytes

Statement: These preliminary findings may provide reassuring evidence that pregnancies and infants conceived following oocyte vitrification are not associated with increased risk of adverse obstetric and perinatal outcomes.

Chian et al, RBM online 16, May 2008

Outcomes after ART mainly occur with fresh not frozen embryo transfers: Significance and implications

Coclusion: These results suggest the adverse birth outcomes of ART are associated with fresh embryo transfers and therefore embryology laboratory procedures affecting the embryos are not the cause because they are not seen with frozen embryo transfer. The adverse effects must operate via the woman. If these involve ovarian stimulation or ansthesia for oocyte collection they may be able to be modified to improve birth outcomes.
The other implication is that frozen embryo transfer should be more widely used.

Baker et al., Fertil. & Steril., Abstract S29, 2008 FRSM

Viable pregnancies following fresh versus frozen embryo transfer: Is there a difference in the rate of serum Human Chorionic Gonadotropin (HCG) rise?

Conclusion: The rate of hCG rise following transfer of frozen embryos is significantly greater than that seen with transfer of fresh embryos. This difference may reflect that the most healthy embryos survive the freeze-thaw process or may be secondary to the more physiologic endocrine environment at the time of implantation. Future study with a larger sample size may confirm that the standard curve for the rate of hCG rise is not applicable to pregnancies following frozen embryo transfer.

Kansal Kalra,etal. 2008, Fertil. & Steril., abst., S205

Impact on Health and Outcomes for the Future Child

The optimal way to avoid the occurence of OHSS and multiple pregnancies could be:

- Downregulation with LHRH antagonist
- LHRH agonist instead of hCG to induce final egg maturation
- Vitrification of all embryos
- Replacement of 1 embryo in a thaw cycle

In conclusion, the combination of FSH and LHRH antagonist stimulation, along with GnRH agonist triggering and replacing only 1 embryo in the thaw cycle, will totally avoid the occurrence of OHSS and multiple pregnancies. Further research on the children born using this protocol is mandatory.

Paul Devroy, MD, PhD

Current Aspects

- Avoiding hyperstimulation syndrome in patients with PCOS by vitrification of all 2PN or embryos and replaced in a programmed cycle
- Cancelling of fresh ET in case of more than 10 Follicles
- Vitrification of all zygotes or embryos resulted from IVM programme
- An option for cancer patients to vitrify the oocytes instead of ovarian tissue
- In oocytes donation programme
- Vitrification of the oocytes to postpone fertility

Summary

Vitrification

... is easy to perform

… is a low cost method

… has very high survival rates of oocytes and embryos at all stages of development

… requires a skilled embryologist

… is the current and future first choice procedure

... is a standard method for cryopreservation

… it improves the implantation rate

Thank you for your attention!

You can now register for these upcoming ESHRE Campus events:

- Application and challenges of emerging technologies in preimplantation and prenatal diagnosis
  12-13 September 2013 - Prague, Czech Republic
- Female genital tract congenital malformations: new insights in an old problem
  27-28 September 2013 - Thessaloniki, Greece
- Introducing new techniques into the lab
  4-5 October 2013 - Barcelona, Spain
- Polycystic ovary syndrome: A new look at an old subject
  25-26 October 2013 - Rome, Italy
- Infections from conception to birth: role of ART
  7-8 November 2013 - Berlin, Germany
- Endoscopy in reproductive medicine
  20-22 November 2013 - Leuven, Belgium
- From early implantation to later in life
  28-29 November 2013 - Brussels, Belgium

Mark your calendar for:

- Premature ovarian insufficiency
  6-7 December 2013 - Utrecht, The Netherlands

www.eshre.eu
(see "Calendar")

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