PRE-CONGRESS COURSE 15

Optimizing IVF outcome

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

London, United Kingdom
7 July 2013

Organised by
The Middle East Fertility Society
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Course coordinators

Mohamed Aboulghar (Egypt)

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 location 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 may 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 prospective randomized trial was to test if a salpingectomy prior to IVF was effective in terms of increased pregnancy rates.
- Patients 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 or 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 stimulation 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 hysterosalpingography 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 patients with 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 treatment while 36%, 33% and 19% recommended treatment weakly, strongly and very strongly respectively. (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 fertility outcomes:50
• 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 endometriosis 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

M. Aboulghar, M.D.

References:


Optimizing Ovarian Stimulation for IVF

Prof. Dr. Bart CJM Fauser
University Medical Center, Utrecht, The Netherlands

Patient tailored ovarian stimulation

Lecture Outline
- Background ovarian stimulation
- Aims ovarian stimulation
- Individualised dosing

Historical milestones

<table>
<thead>
<tr>
<th>Year</th>
<th>Compound</th>
<th>Who did it…</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978</td>
<td>Non</td>
<td>Edwards &amp; Steptoe (UK)</td>
</tr>
<tr>
<td>1982</td>
<td>Clomiphene</td>
<td>Trounson (Austr)</td>
</tr>
<tr>
<td>1984</td>
<td>HMG</td>
<td>Jones (US)</td>
</tr>
<tr>
<td>1986</td>
<td>GnRH agonist</td>
<td>Jacobs, Fleming (UK)</td>
</tr>
<tr>
<td>1991</td>
<td>GnRH antagonist</td>
<td>Friedman (Fr), Dadirizh (Is)</td>
</tr>
<tr>
<td>1992</td>
<td>recFSH (LH, hCG)</td>
<td>Devroey (B), Fauser (N)</td>
</tr>
<tr>
<td>2004</td>
<td>recFSH agonist</td>
<td>Devroey (B), Fauser (N)</td>
</tr>
<tr>
<td>2013</td>
<td>Oral LH</td>
<td>Mannaerts (N)</td>
</tr>
</tbody>
</table>
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?  

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

Downside of ovarian stimulation

- Burden of treatment
- Complexity
- Cost
- Complications
International disparities in access to infertility services

**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??

Patient perspective

Clinical outcomes in relation to the daily dose of recombinant follicle stimulating hormone for oocyte stimulation in in vitro fertilization in promoting minimal responders younger.
Sequela of ovarian stimulation for IVF

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
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?

<table>
<thead>
<tr>
<th>Poor response</th>
<th>Optimal</th>
<th>Disturbed risk/benefit balance</th>
</tr>
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<tbody>
<tr>
<td>1 – 5</td>
<td></td>
<td>8 – 15</td>
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</tbody>
</table>

*(oocyte number)*

Predictors of ovarian response: progesterone levels in relation to estradiol concentrations and ovarian stimulation

Ovarian stimulation for IVF

<table>
<thead>
<tr>
<th>Olivennes, RBM’09</th>
<th>Popovic, HR’03</th>
</tr>
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<tbody>
<tr>
<td>FSH</td>
<td>Total follicle no</td>
</tr>
<tr>
<td>BMI</td>
<td>Total ovarian volume</td>
</tr>
<tr>
<td>Age</td>
<td>Total doppler score</td>
</tr>
<tr>
<td>AFC</td>
<td>Age</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
</tr>
</tbody>
</table>

Biomarkers of ovarian response: current and future applications

<table>
<thead>
<tr>
<th>AFC</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
</tr>
</tbody>
</table>

**Treatment strategy**

- Antegonadotropin
- Antagonist
- LH agonist
- Anti-endometrical
- Anti-estrogens
- Anti-progesterin
- Anti-oestradiol
- Anti-oestradiol / progesterin

**Note:** Specific biomarkers are used by the author. Ovarian response categories.
Ovarian hyperstimulation for IVF
- the bigger picture

- Ovarian stimulation
- Drop out
- Burden of treatment
- Complications (OHSS)
- Contribute to success?

IVF and child health
(less is more?)

“Embryogenesis begins during oogenesis”
(EB Wilson)

PhD’s thesis mild IVF

- 2005
- 2006
- 2007
- 2008
- 2009
- 2010
- 2011
- 2012
Is single embryo transfer going to be the future?

Professor Siladiitya 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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>3.72 (3.26 – 4.25)</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>2.92 (1.23 – 3.33)</td>
</tr>
<tr>
<td>PPH</td>
<td>2.83 (2.45 – 3.26)</td>
</tr>
</tbody>
</table>

Campbell & Templeton 2003

UK cost of IVF births

<table>
<thead>
<tr>
<th></th>
<th>Singleton</th>
<th>Twin</th>
<th>Triplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>£3122</td>
<td>£6058</td>
<td>£11534</td>
</tr>
<tr>
<td>Neonatal</td>
<td>£191</td>
<td>£3064</td>
<td>£20,820</td>
</tr>
<tr>
<td>Cost per</td>
<td>£3313</td>
<td>£9122</td>
<td>£32,354</td>
</tr>
</tbody>
</table>

Ledger et al, BJOG 2006
**Subfertile women: Preference for twins**

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>n</th>
<th>Preference for twins (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleicher</td>
<td>1995</td>
<td>582</td>
<td>67-90</td>
</tr>
<tr>
<td>Grodman</td>
<td>2001</td>
<td>200</td>
<td>67</td>
</tr>
<tr>
<td>Pinczberg</td>
<td>2003</td>
<td>1330</td>
<td>62-85</td>
</tr>
<tr>
<td>Karlin</td>
<td>2003</td>
<td>180</td>
<td>30-38</td>
</tr>
<tr>
<td>Ryan</td>
<td>2004</td>
<td>499</td>
<td>28</td>
</tr>
<tr>
<td>Child</td>
<td>2004</td>
<td>891</td>
<td>41</td>
</tr>
<tr>
<td>Murray</td>
<td>2004</td>
<td>136</td>
<td>40</td>
</tr>
<tr>
<td>Steures</td>
<td>2005</td>
<td>40</td>
<td>77</td>
</tr>
</tbody>
</table>


---

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

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

<table>
<thead>
<tr>
<th></th>
<th>Number of women who ranked</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment failure &lt; disability</td>
<td>Treatment failure = disability</td>
<td>Treatment failure &gt; disability</td>
<td>Total</td>
</tr>
<tr>
<td>Physical disability</td>
<td>47</td>
<td>11</td>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>Cognitive impairment</td>
<td>53</td>
<td>11</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td>Visual impairment</td>
<td>54</td>
<td>11</td>
<td>3</td>
<td>68</td>
</tr>
</tbody>
</table>

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)*

* Wilcoxon signed rank tests

Scotland et al, 2007, BJOG.
### Single vs double embryo transfer: baseline

<table>
<thead>
<tr>
<th></th>
<th>eSET (N = 683)</th>
<th>DET (N = 684)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), mean (SD)</td>
<td>31.3 (3.5)</td>
<td>31.4 (3.4)</td>
</tr>
<tr>
<td>BMI, median (IQR)</td>
<td>23.3 (21.3, 26.1)</td>
<td>23.3 (21.1, 26.3)</td>
</tr>
<tr>
<td>Infertility (yrs) median (IQR)</td>
<td>3.0 (2.1, 4.4)</td>
<td>3.0 (2.0, 4.6)</td>
</tr>
<tr>
<td>Primary infertility</td>
<td>74%</td>
<td>71%</td>
</tr>
<tr>
<td>Cause of infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>41%</td>
<td>40%</td>
</tr>
<tr>
<td>Tubal</td>
<td>14%</td>
<td>14%</td>
</tr>
<tr>
<td>Ovulatory</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Unexplained</td>
<td>16%</td>
<td>25%</td>
</tr>
<tr>
<td>Mixed</td>
<td>15%</td>
<td>11%</td>
</tr>
</tbody>
</table>

*McLernon et al 2010 BMJ*

### Single vs double embryo transfer: treatment details

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

*McLernon et al 2010 BMJ*

### Fresh single vs double embryo transfer: outcomes

<table>
<thead>
<tr>
<th></th>
<th>Single embryo N = 683</th>
<th>Double embryo N = 683</th>
<th>Adj. OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth</td>
<td>27%</td>
<td>42%</td>
<td>0.56 (0.39, 0.63)</td>
</tr>
<tr>
<td>Multiple live birth</td>
<td>2%</td>
<td>29%</td>
<td>0.07 (0.03, 0.17)</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>3%</td>
<td>12%</td>
<td>0.25 (0.16, 0.46)</td>
</tr>
<tr>
<td>Term singleton birth</td>
<td>158 (23%)</td>
<td>169 (24%)</td>
<td>0.91 (0.71, 1.16)</td>
</tr>
</tbody>
</table>

*McLernon et al, 2010 BMJ*
### 1 Fresh + 1 frozen embryo vs 2 fresh embryo transfer

<table>
<thead>
<tr>
<th></th>
<th>eSET</th>
<th>DET</th>
<th>Adj. OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>350</td>
<td>353</td>
<td></td>
</tr>
<tr>
<td>Live birth</td>
<td>38%</td>
<td>42%</td>
<td>0.85 (0.62, 1.15)</td>
</tr>
<tr>
<td>Multiple live birth</td>
<td>1%</td>
<td>32%</td>
<td>0.02 (0.00, 0.13)</td>
</tr>
</tbody>
</table>

McLernon et al, 2010 BMJ

### Subgroup analysis: age and embryo quality

<table>
<thead>
<tr>
<th>Age</th>
<th>&lt; 33 yrs</th>
<th>&gt; = 33 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>eSET N= 456</td>
<td>DET N= 448</td>
</tr>
<tr>
<td>Live birth</td>
<td>29% 40%</td>
<td>23% 35%</td>
</tr>
<tr>
<td>Multiple</td>
<td>2% 25%</td>
<td>0% 29%</td>
</tr>
</tbody>
</table>

- **Embryos**
  - Excellent quality
  - Good / moderate quality

<table>
<thead>
<tr>
<th>Embryos</th>
<th>Live birth</th>
<th>Multiple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>22% 39%</td>
<td>28% 43%</td>
</tr>
<tr>
<td>Good / moderate</td>
<td>4% 28%</td>
<td>1% 30%</td>
</tr>
</tbody>
</table>

* 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
### ANZARD data: live births per transfer (%)

#### 35< years cleavage blastocyst

<table>
<thead>
<tr>
<th>Type</th>
<th>Cleavage</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>eSET</td>
<td>33.6</td>
<td>46.2</td>
</tr>
<tr>
<td>SET</td>
<td>20.6</td>
<td>31.2</td>
</tr>
<tr>
<td>eDET</td>
<td>42.4</td>
<td>44.1</td>
</tr>
<tr>
<td>DET</td>
<td>30.3</td>
<td>33.2</td>
</tr>
</tbody>
</table>

#### 35-39 yrs

<table>
<thead>
<tr>
<th>Type</th>
<th>Cleavage</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>eSET</td>
<td>24.4</td>
<td>37.1</td>
</tr>
<tr>
<td>SET</td>
<td>13.2</td>
<td>21.2</td>
</tr>
<tr>
<td>eDET</td>
<td>29.8</td>
<td>41.3</td>
</tr>
<tr>
<td>DET</td>
<td>21.1</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Wang et al, 2010

### ANZARD data: live births per transfer (%)

#### > 40 years cleavage blastocyst

<table>
<thead>
<tr>
<th>Type</th>
<th>Cleavage</th>
<th>Blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>eSET</td>
<td>16.2</td>
<td>22.7</td>
</tr>
<tr>
<td>SET</td>
<td>7.1</td>
<td>8.6</td>
</tr>
<tr>
<td>eDET</td>
<td>21.7</td>
<td>26.1</td>
</tr>
<tr>
<td>DET</td>
<td>14.4</td>
<td>13.0</td>
</tr>
</tbody>
</table>

Wang et al, 2010

### Blastocyst transfer in the UK

Blastocyst transfer in the UK

www.hfea.gov.uk/
Elective single cleavage vs single blastocyst transfer: cumulative outcomes

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

* P > 0.05

Scotland et al, BJOG, 2011

Minimising twins in *in utero* fertilisation: a modelling study assessing the costs, consequences and cost utility of elective single versus double embryo transfer over a 50-year time horizon

Scotland et al, BJOG, 2011

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

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>ICER per livebirth</th>
<th>ICER per Qualy</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>£ 27,356</td>
<td>£ 28,263</td>
</tr>
<tr>
<td>36</td>
<td>£18,580</td>
<td>£21,722</td>
</tr>
<tr>
<td>39</td>
<td>15,539</td>
<td>£20,278</td>
</tr>
</tbody>
</table>

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
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

- Stimulation protocol
- Quality of oocytes
- Laboratory conditions
- Air quality

OUTCOME

- Embryo transfer & luteal support

CULTURE SYSTEM

- Oil overlay
- Gas phase

Culture medium:

- Oocyte/embryo handling outside the incubator

QC & QA

Gardner and Lane, 2007

Preimplantation embryo development

Routine IVF

Stress tolerance

Critical period determining blastocyst quality

Epigenetic remodeling of PN

First cleavage division

Embryonic genome activation

Morula compaction

Blastocyst formation

Otto Warburg, Science, 1956

Dynamic nature of the preimplantation embryo

Embryo culture system should better meet the changing demands of embryo physiology and requirements during development.

Switch to aerobic glycolysis

Otto Warburg, Science, 1956
Genotype to phenotype and the journey of reproductive cells

"Development is, by definition, epigenetic"

Epigenetic modification in relation to IVF procedures

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.
Appropriate culture conditions have been identified and must be maintained

Temperature: 37°C ± 0.2°C

Buffer System and Hydrogen Ion Concentration (pH)

The pH and hence the [H+] can be approximately estimated using the Henderson-Hasselbach equation: 

\[ \text{pH} = pK_a + \log \left( \frac{[\text{base}]}{[\text{acid}]} \right) \]

Buffer System and Hydrogen Ion Concentration (pH)

Buffer System and Hydrogen Ion Concentration (pH)

Buffer System and Hydrogen Ion Concentration (pH)

Intracellular (pHi) and extracellular pH (pHe)

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

Rather than pH directly, it appears that the presence of weak acids or bases in the culture medium markedly affects pH.
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.

Relieves acidosis, with set point pH<7

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

The presence of ions in the media is essential to the functionality of transport mechanisms.

Lower mouse blastocyst formation and hatching rates when culturing with reduced bicarbonate concentration.

pHi alterations significantly affect embryo development.

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

Oocyte lacks functional transport systems to regulate pH

It has been determined that the oocyte lacks any functional transport systems to regulate pH in either the acid or the alkaline range around 6 h following fertilization.

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

Philippe 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 regulates the cellular REDOX state

Oxygen is an essential key energy substrate for oxidative phosphorylation
- Other cellular functions, such as apoptosis and cell cycle controls, are also significantly influenced by oxygen availability and REDOX state, via transcription factors such as NFkB

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-1α protein is degraded rapidly by the ubiquitin-proteasome system
- Under hypoxic conditions, HIF-1α protein is not degraded and can translocate to the nucleus to form the active DNA-binding complex

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

Low oxygen concentration improve IVF clinical outcomes

Low oxygen tension must be used;
Closed systems are necessary.
Rely on new instruments for a better control of the culture environment

TAKE-HOME MESSAGES: standard requirements of embryo culture

Low oxygen concentration improve IVF clinical outcomes

Oxygen gradient provide spatial information to cells within the embryo

ICM TE

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

Bontekoe et al., 2012. Cochrane Database Syst Rev
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
INNOVATIVE TECHNOLOGIES TO IMPROVE EMBRYO CULTURE

New static culture platforms:
- Microdrops
- Ultramicrodrops
- Submicroliter platforms
- Microwells
- Microchannels

Dynamic platforms:
- Shaking/rotation
- Tilting
- Vibration
- Controlled fluid flow

Specified surfaces:
- Agarose
- Matrigel
- Hyaluronic acid
- Co-culture
- 3-Dimensional matrix

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.

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.

Dilution

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:

0.05 ul

Small microenvironment for individual or small groups of embryos

Larger common culture media reservoir

The WOW approach

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 shear mechanical stimulation to the embryo. Gentle stimulation of embryos could activate beneficial mechanoreceptors 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 to provide gentle physical stimulation to embryos (estimated shear 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. 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.

---

Dynamic microfunnel culture

---

---
Three-dimensional modeling 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

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

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.

Conclusion 2: advances in embryo culture platforms
**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.

**Dynamic culture platforms:**

- Relative complexity and requirement to operate efficiently and safely within the humidified and warmed environment of the incubator.
- Biocompatibility is paramount. Shear 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
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 GnRH

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 (Gzimmer et al. 1996; Lass et al. 2000)
   - Number of ovarian follicles and necklace appearance (Navot et al. 1992)
4. Young age < 35 years old (Navot et al. 1992)
5. Lean body (Ash 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)

- 22 RCT
- 3176 Subjects
- Livebirth (from manuscript in 10 studies and by conversion 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)


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

<table>
<thead>
<tr>
<th>Kolibianakis</th>
<th>Al-Inany</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of severe OHSS</td>
<td>OR 0.46* (0.26, 0.82; P=.01)</td>
</tr>
<tr>
<td>Interventions to prevent OHSS</td>
<td>OR 0.44 [0.21, 0.93] vs. agonist; P=.03</td>
</tr>
</tbody>
</table>

*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.
**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)

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

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.

<table>
<thead>
<tr>
<th>ICSI outcome according No. of coasting days</th>
<th>Group I up to 3 days</th>
<th>Group II 4 days or more</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles</td>
<td>983</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>E2 level on day of hCG (pg/ml)</td>
<td>2674±1789</td>
<td>2801±1930</td>
<td>P=0.88</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>16.45±6.26</td>
<td>14.93 ±1.01</td>
<td>P=0.002*</td>
</tr>
<tr>
<td>MII oocytes</td>
<td>12.94±5.58</td>
<td>11.6±5.61</td>
<td>P=0.003*</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>62.67%</td>
<td>64.92%</td>
<td>P=0.06</td>
</tr>
<tr>
<td>Embryo per transfer</td>
<td>2.99±0.69</td>
<td>3.03±0.66</td>
<td>P=0.27</td>
</tr>
<tr>
<td>Implantation rate</td>
<td>26.32%</td>
<td>18.16%</td>
<td>P=0.0001*</td>
</tr>
<tr>
<td>Clinical PR</td>
<td>51.96%</td>
<td>35.88%</td>
<td>P=0.0002*</td>
</tr>
</tbody>
</table>

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 E₂ were treated with ganirelix acetate. Despite being pretreated with GnRH agonist and without withholding gonadotropins, serum E₂ 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

190 patients at risk for OHSS

94 randomized

GnRH antagonist administration

No cases of OHSS in both arms

96 Coasting

- Significantly more high quality embryos
- Significantly less days than coasting

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 administered immediately after oocyte retrieval. Cabergoline may work through the relation between VEGF/VEGFr and its relation with the neurotransmitter dopamin (Manno et al 2004)
Cabergoline reduces early onset of moderate OHSS: a randomized study (Carizza et al 2008)

<table>
<thead>
<tr>
<th>83 patients</th>
<th>83 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabergoline 0.5 mg</td>
<td>No TT</td>
</tr>
<tr>
<td>For 3 weeks</td>
<td></td>
</tr>
</tbody>
</table>

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)

2425 cycles of IVF with long GnRHa protocol randomized to:

<table>
<thead>
<tr>
<th>Low dose aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1503</td>
</tr>
<tr>
<td>2 cases of OHSS</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low dose aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>922</td>
</tr>
<tr>
<td>43 cases of OHSS</td>
</tr>
</tbody>
</table>

Conclusion: low dose aspirin is effective for prevention of OHSS

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**Triggering ovulation and OHSS**

- Reducing the hCG trigger dose. *(Abdallo 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 similar 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 trigger ovulation
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.
Prevention of Ovarian hyperstimulation syndrome

M. Aboulghar, M.D.

References:


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

D’Angelo A, Amso N. "Coasting" (withholding gonadotrophins) for preventing ovarian hyperstimulation syndrome. Cochrane Database Syst Rev. 2002; (3):CD002811


Techniques and Technologies for Embryo Transfer: Does it Really Matter

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Professor of Obstetrics and Gynecology
Head, Division of Reproductive Endocrinology and Infertility
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

<table>
<thead>
<tr>
<th>Anatomy</th>
<th>Technique</th>
<th>Catheter</th>
<th>Transfer medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine position</td>
<td>Catheter guidance</td>
<td>Soft versus firm</td>
<td>Medium/or volume</td>
</tr>
<tr>
<td>Cervico-cervical angle</td>
<td>Catheter tip location</td>
<td>Internal diameter</td>
<td>Viscosity</td>
</tr>
<tr>
<td>Cervical mucus</td>
<td>Injection mode</td>
<td>Tip shape</td>
<td>Adherence compounds</td>
</tr>
</tbody>
</table>
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
To avoid abrupt pressure fluctuations, ET ought to occur as slowly as possible to avoid embryo damage.

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

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

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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

<table>
<thead>
<tr>
<th>Outcomes subgroups</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Live birth</td>
<td>4</td>
<td>62</td>
<td>N/A</td>
<td>130 (1.49, 3.5)</td>
</tr>
<tr>
<td>2. Live birth</td>
<td>4</td>
<td>44</td>
<td>N/A</td>
<td>198 (1.15, 3.0)</td>
</tr>
<tr>
<td>3. Pregnancy rate</td>
<td>4</td>
<td>10</td>
<td>N/A</td>
<td>197 (0.14, 2.0)</td>
</tr>
</tbody>
</table>

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

Cheung 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>
<thead>
<tr>
<th>Mock transfer position</th>
<th>% at actual ET</th>
<th>% at mock ET</th>
<th>% at abort ET</th>
<th>Surviving ET</th>
<th>Total number of ET</th>
<th>Differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine position</td>
<td>60 (12)</td>
<td>60 (12)</td>
<td>60 (12)</td>
<td>60 (12)</td>
<td>60 (12)</td>
<td>N/A</td>
</tr>
<tr>
<td>No. of transfer cycles</td>
<td>11 (26)</td>
<td>11 (26)</td>
<td>11 (26)</td>
<td>11 (26)</td>
<td>11 (26)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Outcome</th>
<th>Study ID</th>
<th>No. of patients randomized</th>
<th>No. of pregnancies per woman</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound guided ET</td>
<td>1,800</td>
<td>Brown et al. 2010</td>
<td>8,400</td>
<td>Yes/No</td>
<td>Risk Ratio (95% CI)</td>
<td>0.68 (0.59, 0.78)</td>
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</tr>
</tbody>
</table>

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

Brown et al. 2010

---

I. Ultrasound catheter guidance

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Outcome</th>
<th>Study ID</th>
<th>No. of patients randomized</th>
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<td>Brown et al. 2010</td>
<td>8,400</td>
<td>Yes/No</td>
<td>Risk Ratio (95% CI)</td>
<td>0.68 (0.59, 0.78)</td>
</tr>
</tbody>
</table>

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 et al. 2010

---

II. Soft versus firm embryo transfer catheters

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Outcome</th>
<th>Study ID</th>
<th>No. of patients randomized</th>
<th>No. of pregnancies per woman</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft catheter</td>
<td>1,800</td>
<td>Abou-Setta et al. 2005</td>
<td>8,400</td>
<td>Yes/No</td>
<td>Risk Ratio (95% CI)</td>
<td>0.68 (0.59, 0.78)</td>
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<tr>
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<td>Abou-Setta et al. 2005</td>
<td>8,400</td>
<td>Yes/No</td>
<td>Risk Ratio (95% CI)</td>
<td>0.68 (0.59, 0.78)</td>
</tr>
</tbody>
</table>

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

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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

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

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

<table>
<thead>
<tr>
<th>Outcome or subgroup title</th>
<th>No. of patients</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall pregnancy rate</td>
<td>166</td>
<td>166</td>
<td></td>
<td>1.37 (1.26, 1.49)</td>
</tr>
<tr>
<td>Ongoing pregnancy rate</td>
<td>166</td>
<td>166</td>
<td></td>
<td>1.37 (1.26, 1.49)</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>166</td>
<td>166</td>
<td></td>
<td>1.37 (1.26, 1.49)</td>
</tr>
</tbody>
</table>

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**

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**

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

<table>
<thead>
<tr>
<th>Outcome to subgroup title</th>
<th>No. of studies</th>
<th>No. of participants</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiphasic pregnancy rate</td>
<td>5</td>
<td>101</td>
<td>Odds Ratio (95% CI)</td>
<td>0.47 [0.31, 0.71]</td>
</tr>
</tbody>
</table>

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

Bonnetob S et al. 2010

V. Interval loading discharging embryos

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

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

Ciray HN et al. 2007
VI. Physician factor

<table>
<thead>
<tr>
<th>Physical</th>
<th>No. ETs</th>
<th>Age of patient, y</th>
<th>BMI mean±SD</th>
<th>Age of partner, y</th>
<th>BMI mean±SD</th>
<th>No. of embryos</th>
<th>Mean grade of embryos per patient</th>
<th>Pregnancy rate (CD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>107</td>
<td>1.5±0.2</td>
<td>22.7±0.2</td>
</tr>
<tr>
<td>B</td>
<td>22</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>107</td>
<td>1.5±0.2</td>
<td>22.7±0.2</td>
</tr>
<tr>
<td>C</td>
<td>22</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>107</td>
<td>1.5±0.2</td>
<td>22.7±0.2</td>
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<tr>
<td>D</td>
<td>22</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
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<td>1.5±0.2</td>
<td>22.7±0.2</td>
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<td>E</td>
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<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>28.4±1.4</td>
<td>20.9±4.3</td>
<td>107</td>
<td>1.5±0.2</td>
<td>22.7±0.2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Outcome or subgroup title</th>
<th>No. of outcomes</th>
<th>No. of patients</th>
<th>Statistical method</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing pregnancy (whick was not excluded from the analysis)</td>
<td>1</td>
<td>194</td>
<td>Odds Ratio (95% CI)</td>
<td>1.0 (0.74, 1.35)</td>
</tr>
<tr>
<td>Clinical pregnancy per embryo transfer</td>
<td>2</td>
<td>542</td>
<td>Odds Ratio (95% CI)</td>
<td>1.15 (0.87, 1.53)</td>
</tr>
<tr>
<td>Clinical pregnancy per transferred embryo</td>
<td>2</td>
<td>542</td>
<td>Odds Ratio (95% CI)</td>
<td>1.62 (0.93, 2.99)</td>
</tr>
<tr>
<td>Twin pregnancy per transferred embryo</td>
<td>3</td>
<td>39</td>
<td>Odds Ratio (95% CI)</td>
<td>2.85 (0.87, 10.64)</td>
</tr>
<tr>
<td>Single pregnancy per transferred embryo</td>
<td>1</td>
<td>378</td>
<td>Odds Ratio (95% CI)</td>
<td>3.14 (0.81, 12.15)</td>
</tr>
<tr>
<td>Multiple pregnancy per transferred embryo</td>
<td>2</td>
<td>542</td>
<td>Odds Ratio (95% CI)</td>
<td>1.62 (0.87, 3.03)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Outcome in utero estrus</th>
<th>No. of studies</th>
<th>No. of pregnancies</th>
<th>Statistical method</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancy rate</td>
<td>1</td>
<td>639</td>
<td>Odds Ratio: 20.11, 95% CI 3.42</td>
<td>3.42 (1.46, 8.01)</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>1</td>
<td>639</td>
<td>Odds Ratio: 20.11, 95% CI 3.42</td>
<td>2.37 (1.85, 3.61)</td>
</tr>
</tbody>
</table>

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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- Kroon B, Hart RJ, Wong BM, Ford E, Yazdani A. Antibiotics prior to embryo transfer in ART. Cochrane Database of Systematic Reviews 2012, Issue 3
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Henne MB and Miki AA. Uterine position at real embryo transfer compared with mock embryo transfer. Human Reproduction 2006;19:570-572

Uyar A, Bener A, Ciray HN, Bahceci M. Physician experience in performing embryo transfers may affect outcome. Fertility and Sterility 2011;95:1860-1861

References
Improving Implantation
The endometrial factor matters!!!

Prof. Carlos Simón MD; PhD
Professor Obst/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?*

<table>
<thead>
<tr>
<th></th>
<th>E</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>P1</td>
<td>P2</td>
<td>P3</td>
<td>P4</td>
<td>P5</td>
<td>P6</td>
<td>P7</td>
<td>P8</td>
</tr>
</tbody>
</table>

Epithelial PR

Progesterone

**DIAGNOSIS OF ENDOMETRIAL RECEPTIVITY**

**ANATOMICAL MEDICINE**

*ENDOMETRIAL DATING*


**MOLECULAR MEDICINE. TRANSCRIPTOMICS**

*ENDOMETRIAL RECEPTIVITY ARRAY (ERA)*


*De press*

**PROTEOMICS OF ENDOMETRIAL RECEPTIVITY**


*Gonzalez, F et al. (2006) FASEB J.*

**SECRETOMICS. NEXT GENERATION ENDOMETRIAL DIAGNOSTICS**


Dating the endometrial biopsy

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

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


---

The age of -OMICS

Transcriptomics

DNA → Transcription
RNA → mRNA → Protein

Transcription regulation
Alternative splicing

Proteomics
Protein → Metabolites

Metabolomics
METABOLITES

---

Transcriptomics

Microarray Analysis and Tumor Classification

DNA microarray experiments are being carried out in order to investigate the role of transcriptional changes in cancer. The microarray technology allows the simultaneous measurement of mRNA expression levels for thousands of genes in a single experiment. This approach has been used for the identification of genes that are differentially expressed in cancerous and normal tissues, which can be potential targets for therapeutic intervention. Microarray data can be analyzed using various computational tools to identify gene expression patterns that correlate with clinical outcomes. The integration of microarray data with clinical information can help in the development of personalized treatment strategies.
**Gene expression profiling during the WOI in natural versus COH**

**EXPERIMENTAL DESIGN**

50 WOMEN

AGAFFIMETRIX

HG-133A

>22,000 genes

50 WOMEN

**NATURAL**

LH Day 1 2 3 4 5 6 7 8 9

n=5 n=5 n=5 n=5 n=5

**COH**

hCG Day 1 2 3 4 5 6 7 8 9

n=5 n=5 n=5 n=5 n=5

Evaluated Fertile Caucasian women with normal cycles, 23–39 years.

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

\[ \downarrow \]

Bioinformatic analysis of data obtained by the customized microarray

\[ \downarrow \]

Classification and prediction from gene expression.

Predictors: Characterization of RECEITIVITY transcriptome

Receptive Profile
Predictor classifies the molecular receptivity status of the endometrium
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 preferable).

**Hormone Replacement Therapy Cycle**
Endometrial biopsy must be taken on day P+5, after proper E2 priming.

---

Endometrial Receptivity Array (ERA) - Interpretation of Results

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

→ It is recommended 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 recommended 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.
## Endometrial Receptivity Array (ERA) - Consistency

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

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


---

**ERA Gene Topography**

- Pre-Receptive vs. Receptive
- Over-expressed in Pre-Receptive
- Under-expressed in Pre-Receptive

35 genes differentially expressed
**Over-expressed in Receptive**

**Under-expressed Receptive**

88 genes differentially expressed

Endothelial cell

Epithelial cell

Stromal cell

**Tcell**

**Cell cycle regulators**

- DLG7: Potential cell cycle regulator
- PRC1: Protein regulator of cytokinesis
- KNTC2: Spindle checkpoint signaling
- CENPE: Maintenance of chromosomal stability
- PBK: G2/M checkpoint
- KIF20A: Kinesin-like protein
- TACC3: A role in the microtubule-dependent coupling of the nucleus and the centrosome

**ERA Gene Topography**

Ruiz M et al. Fertil Steril 2013 In press

**Clinical Outcome**

<table>
<thead>
<tr>
<th></th>
<th>RIF</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nº. of patients</td>
<td>91</td>
<td>27</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.7 ± 4.6</td>
<td>38.9 ± 5.3</td>
</tr>
<tr>
<td>Ave. # of previous cycles</td>
<td>4.8 ± 2.1</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Receptive ERA/total analyzed (%)</td>
<td>67/91 (73.6)</td>
<td>23/27 (85.2)</td>
</tr>
<tr>
<td>Patients with pET after Receptive ERA</td>
<td>25</td>
<td>9</td>
</tr>
<tr>
<td>Implantation rate 1st transfer post-ERA (%)</td>
<td>15/49 (30.6)</td>
<td>7/17 (41.2)</td>
</tr>
<tr>
<td>Pregnancy rate 1st transfer post-ERA (%)</td>
<td>13/25 (52.0)</td>
<td>5/9 (55.6)</td>
</tr>
<tr>
<td>Biochemical pregnancies (%)</td>
<td>-</td>
<td>1/7 (14.3)</td>
</tr>
<tr>
<td>Clinical abortions (%)</td>
<td>1/16 (6.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

> ≥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
First Embryo transfer outcome after RECEPTIVE ERA in patients with RIF

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>DAY#1</th>
<th>RESULT#1</th>
<th>PROFILE#1</th>
<th>DAY#2</th>
<th>RESULT#2</th>
</tr>
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<tbody>
<tr>
<td>4007</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>4769</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>5451</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>6272</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>7351</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>2917</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>3334</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
<tr>
<td>1020</td>
<td>LH+7</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>LH+9</td>
<td>R</td>
</tr>
<tr>
<td>3475</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
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<tr>
<td>4801</td>
<td>P+5</td>
<td>NR</td>
<td>Pre-Receptive</td>
<td>P+7</td>
<td>R</td>
</tr>
</tbody>
</table>

ERA Clinical Outcome in Non Receptive patients

Displacement of the WOI

Example of Case 6272
Clinical Outcome in Non Receptive ERA patients

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

ERA in RIF

- Receptive (73.6%) P+5/LH+7
- Non-Receptive (26.4%) P+5/LH+7

pWOI Receptive

Personalized Embryo Transfer (pET)
Window of Endometrial Receptivity (WOI)

- 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 international multicenter RCT.

Personalized Embryo Transfer (pET)

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

Conclusions
Randomized Clinical Trial Profile

**IVF Patients**

**Randomization**

- Group A: ET in COS  
  - N=640
- Group B: ET in HRT  
  - N=640

**Reproductive Follow-up**

- Receptive  
  - N=576
- Non-receptive  
  - N=64

**ET in COS vs ET in HRT**

**pET vs. ET**

SECRETOMICS, NEXT GENERATION ENDOMETRIAL DIAGNOSTICS

- Aspiration of endometrial secretion does not affect pregnancy rates  
  Van der Gaast et al. RBmOnline 2002
- Glycoalkyl 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  
  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
REFERENCES


REFERENCES


Optimizing the Outcome of Cryopreservation

Safaa Al-Hasani
Department of Gynecology and Obstetrics
Reproductive Medicine
University of Schleswig-Holstein, Campus Lübeck
Germany

Introduction

- In 1937, Luyet wrote that "crystallization is incompatible with living systems and should be avoided whenever possible" [Luyet, Biodynamica 1937; 1: 1]

- 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 (LN₂ 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 LN₂
- 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 H₂ 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 \( \text{CH}_3\text{OH} \) 32
  - Ethanol \( \text{C}_2\text{H}_5\text{OH} \) 46
  - Ethylene glycol \( \text{C}_2\text{H}_2\text{(OH)}_2 \) 62
  - 1-2 Isopropanol \( \text{C}_3\text{H}_6\text{(OH)}_2 \) 76
  - Glycerol \( \text{C}_3\text{H}_5\text{(OH)}_2 \) 92
  - DMSO \( (\text{CH}_3)_2\text{SO} \) 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)

<table>
<thead>
<tr>
<th>Replaced with</th>
<th>Replaced with</th>
<th>Replaced with</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% DMSO</td>
<td>5% DMSO</td>
<td>10% DMSO</td>
</tr>
</tbody>
</table>

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


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


• 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

<table>
<thead>
<tr>
<th>Slow freezing versus ultra-rapid freezing</th>
<th>Traditional</th>
<th>Vitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPA concentration</td>
<td>1.5 M</td>
<td>3.0–5.0 M</td>
</tr>
<tr>
<td>Volume</td>
<td>0.3–1.0 mL</td>
<td>&lt;1 µL</td>
</tr>
<tr>
<td>Contact between N₂ and cell</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Cooling rate</td>
<td>~0.5°C/min</td>
<td>~25,000–50,000°C/min</td>
</tr>
<tr>
<td>Freezing</td>
<td>Slow</td>
<td>Ultra-rapid</td>
</tr>
<tr>
<td>Thawing / warming</td>
<td>Slow</td>
<td>Rapid</td>
</tr>
<tr>
<td>Time consuming</td>
<td>≥180 min</td>
<td>1 sec</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Not controlled</td>
<td>Controlled</td>
</tr>
</tbody>
</table>
Slow freezing versus ultra-rapid freezing

<table>
<thead>
<tr>
<th></th>
<th>Traditional</th>
<th>Vitrification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced osmotic injury</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Zona pellucida fracture</td>
<td>Possible</td>
<td>No</td>
</tr>
<tr>
<td>Ice crystal formation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Seeding</td>
<td>Yes</td>
<td>No need</td>
</tr>
<tr>
<td>Procedure</td>
<td>Complicated</td>
<td>Simple</td>
</tr>
<tr>
<td>Device</td>
<td>Yes</td>
<td>No need</td>
</tr>
<tr>
<td>Costs</td>
<td>High</td>
<td>Less</td>
</tr>
<tr>
<td>Liquid nitrogen amount</td>
<td>High</td>
<td>Much less</td>
</tr>
<tr>
<td>Duration out of incubator</td>
<td>3-4 hr.</td>
<td>10-15 min.</td>
</tr>
</tbody>
</table>

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

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 LN₂

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 protocols 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.
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
• Worldwide 5.5 million children born through ART
• 20-25% of the children born through cryopreservation procedure

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 LN₂)

Cryotop
### Cryoleaf (McGill)

![Image of Cryoleaf (McGill)]

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

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

<table>
<thead>
<tr>
<th></th>
<th>Vitrified / warmed group</th>
<th>Update up to July 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of transfers</td>
<td>120</td>
<td>270</td>
</tr>
<tr>
<td>No. of transferred embryo</td>
<td>295</td>
<td>624</td>
</tr>
<tr>
<td>No. of embryos per transfer</td>
<td>2.45</td>
<td>2.31</td>
</tr>
<tr>
<td>No. of clinical pregnancies</td>
<td>39 (32.5%)</td>
<td>76 (28.1%), 5 twins</td>
</tr>
<tr>
<td>No. of ongoing pregnancies</td>
<td>--</td>
<td>24</td>
</tr>
<tr>
<td>No. of abortions</td>
<td>8 (20.5%)</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>No. of deliveries</td>
<td>31</td>
<td>36, all singleton</td>
</tr>
<tr>
<td>IR per transferred embryo</td>
<td>13.2 %</td>
<td>12.9%</td>
</tr>
<tr>
<td>IR per thawed oocyte</td>
<td>11.8%</td>
<td>11.6</td>
</tr>
</tbody>
</table>
**Oocyte Donation & Vitrification**

<table>
<thead>
<tr>
<th>M II oocytes</th>
<th>Survival</th>
<th>Fertilization</th>
<th>No. of transfers</th>
<th>Mean number of embryos</th>
<th>Ongoing pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>231</td>
<td>96.9%</td>
<td>76.3%</td>
<td>23</td>
<td>2.1</td>
<td>48%</td>
</tr>
</tbody>
</table>

Cobo et al., 2008

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

<table>
<thead>
<tr>
<th>No of oocytes</th>
<th>Fresh ICSI (%)</th>
<th>Vitrified/Warmed ICSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fertilization (2PN) per sibling oocyte</td>
<td>Fertilization (2PN) per injected oocyte</td>
</tr>
<tr>
<td></td>
<td>Normal 2PN morphology</td>
<td>1PN-oocytes</td>
</tr>
<tr>
<td>120</td>
<td>83.3%</td>
<td>83.3%</td>
</tr>
<tr>
<td>124</td>
<td>76.6%</td>
<td>79.2%</td>
</tr>
</tbody>
</table>

Renzi et al., Sept. 2010

**Conclusion:** 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 insemination. We believe that these results will help the spread of vitrification for human oocytes.

Renzi et al., 2010

Human Reprod., 25, 66-73
Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomised, controlled, clinical trial

<table>
<thead>
<tr>
<th></th>
<th>Egg-bank</th>
<th>Fresh</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of subjects</td>
<td>295</td>
<td>289</td>
</tr>
<tr>
<td>Oocytes received</td>
<td>3039</td>
<td>3185</td>
</tr>
<tr>
<td>Survival rate</td>
<td>92.5%</td>
<td>-</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>74.2%</td>
<td>73.3%</td>
</tr>
<tr>
<td>Top quality Day-2 cleaved embryos</td>
<td>43.6%</td>
<td>43.8%</td>
</tr>
<tr>
<td>Top quality Day-3 cleaved embryos</td>
<td>58.4%</td>
<td>60.7%</td>
</tr>
<tr>
<td>Ongoing pregnancy rate / transfer</td>
<td>49.1%</td>
<td>48.3%</td>
</tr>
</tbody>
</table>

Cobo et al. Sept 2010
Human Reprod. 25

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

Comparison between fresh and frozen-thawed embryo transfer

Vitrification of Zygotes (Luebeck)

<table>
<thead>
<tr>
<th></th>
<th>Fresh ET</th>
<th>Frozen-Thawed ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>52</td>
<td>59</td>
</tr>
<tr>
<td>No of cycles</td>
<td>53</td>
<td>61</td>
</tr>
<tr>
<td>No of vitrified Zygotes</td>
<td>/</td>
<td>259</td>
</tr>
<tr>
<td>No of survived zygotes</td>
<td>/</td>
<td>250 (96.5)</td>
</tr>
<tr>
<td>No of transferred embryos</td>
<td>114 (2.5)</td>
<td>240 (2.6)</td>
</tr>
<tr>
<td>No of embryo transfers</td>
<td>53</td>
<td>83</td>
</tr>
<tr>
<td>No of pregnancies</td>
<td>13 (24.5)</td>
<td>29 (34.1)</td>
</tr>
</tbody>
</table>

Cobo et al. Sept 2010
Human Reprod. 25
Our Results in Avoiding Hyperstimulation
Patients Triggered with GnRH-Agonist

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>No. of Zygotes vitrif.</th>
<th>No. of Zygotes re-warmed</th>
<th>No. &amp; (%) Zygotes survived</th>
<th>No. &amp; (%) Preg.</th>
<th>No. Of Children born</th>
<th>(% of live birth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>433</td>
<td>163*</td>
<td>158 (97)</td>
<td>25 (42)</td>
<td>13**</td>
<td>(25)</td>
</tr>
</tbody>
</table>

* 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.

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

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)_

<table>
<thead>
<tr>
<th></th>
<th>Vitrification</th>
<th>Slow freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos, n</td>
<td>436</td>
<td>420</td>
</tr>
<tr>
<td>Embryos thawed, n</td>
<td>127</td>
<td>120</td>
</tr>
<tr>
<td>Embryos survival, n (%)</td>
<td>121 (95.3)</td>
<td>72 (60)</td>
</tr>
<tr>
<td>Pregnancy, n (%)</td>
<td>14 (35)</td>
<td>4 (17.4)</td>
</tr>
</tbody>
</table>

40% ethylene glycol + 0.6 mol sucrose, nylon loop

“Ethylene glycol is a good cryoprotectant 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.”

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

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of vitrified blastocysts</th>
<th>Survival rate %</th>
<th>Implantation rate %</th>
<th>Pregnancy rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hiraoka et al. 2003</td>
<td>40</td>
<td>98</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>Stehlik et al. 2005</td>
<td>41</td>
<td>100</td>
<td>NA</td>
<td>50</td>
</tr>
<tr>
<td>Kuwayama et al. 2005</td>
<td>6484</td>
<td>90</td>
<td>NA</td>
<td>53</td>
</tr>
</tbody>
</table>

---

### Obstetric 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
Conclusions: 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 anesthesia 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

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

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.


Impact on Health and Outcomes for the Future Child

The optimal way to avoid the occurrence 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”)  

Contact us at info@eshre.eu