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# Best practices of ASRM and ESHRE: a journey through reproductive medicine†‡

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**BACKGROUND:** The American Society for Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) are the two largest societies in the world whose members comprise the major experts and professionals working in the field of reproductive medicine and embryology. These societies have never before had a joint scientific meeting.

**METHODS:** A 3-day meeting was planned and took place in March of 2012. The goal was to present and debate key topics, as well as modes of practice in reproductive medicine and to discuss recent developments in the field.

**RESULTS:** Presentations by members of ASRM and ESHRE were of three types: 'state of the art' lectures, 'back-to-back' presentations of two points of view and debates.

**CONCLUSIONS:** For the first time, ASRM and ESHRE held a joint meeting where a special emphasis was given to presentations on the hottest topics in the field. Although different opinions and approaches sometimes exist on the two sides of the Atlantic, an appreciation and acceptance of these differences was evident, and there was more commonality than divergence of opinion.

**Key words:** assisted reproduction / embryo implantation / menopause / ovarian cancer / reproductive endocrinology

## Introduction

In March 2012, American Society for Reproductive Medicine (ASRM) and European Society of Human Reproduction and Embryology (ESHRE) held their first joint meeting with the theme of Best Practices in Reproductive Medicine. The goal was to assess and present the evidence for both established and emerging approaches to the science and art of reproductive healthcare. Faculty from both ASRM and ESHRE presented specific topics, focusing on different approaches and points of view in a wide range of areas of importance for reproductive health. This 3-day workshop was comprised of 'back-to-back' sessions where different topics were presented from both the American and European points of view, debates where two experts discussed more controversial issues and several 'cutting-edge' lectures. What follows is a summary of the major themes of the workshop, divided into sections rather than specifically reporting on the way in which they were presented. The summaries found below do not constitute the official positions of either ASRM or ESHRE. The list of the presenters may be found at the end of the review.

## Reproductive endocrinology

This section focused on current concepts and approaches to ovarian stimulation, particularly for IVF, the diagnosis and management of polycystic ovary syndrome (PCOS) and the poor response to ovarian stimulation.

### Ovarian stimulation

How to best carry out ovarian stimulation for IVF was a topic of debate. It was stated that there are several advantages to using conventional ovulation induction including: (i) cycle programming to

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optimize IVF clinic efficiency, (ii) achieving the highest success rate from the fewest number of IVF cycles, (iii) creating a larger number of embryos from which the 'best' embryos can be transferred and (iv) allowing spare embryos to be cryopreserved in order to have several potential transfer cycles. However, some disadvantages have to be considered, such as reduced quality and viability of some oocytes, the increased burden of treatment and potential complications, higher costs and the possibility of negative clinical outcomes. The efficiency of oocyte utilization after controlled ovarian stimulation for IVF has been shown to be poor (25.1 oocytes needed per live birth and 6–16 retrieved oocytes required for women <38 years; Inge *et al.*, 2005). In addition, a high oocyte yield has been associated with an increased aneuploidy rate (51% in women <35 years having more than 10 oocytes retrieved, compared with 35% when there were 6–10 oocytes; Haaf *et al.*, 2009).

More recently, what has been considered to constitute a successful IVF treatment has moved away from the outcome of a single cycle toward the concept of the singleton birth rate per initiated cycle over a given time period, including patient distress, complications and costs (Heijnen *et al.*, 2004). In view of these considerations, milder stimulation protocols have been proposed, which are more 'patient-friendly' causing less stress and side effects and reducing costs.

Mild ovarian stimulation is defined as the administration of fixed, low doses of gonadotrophins in GnRH antagonist cycles and/or the use of anti-estrogens or aromatase inhibitors with the aim of limiting the outcome to no more than eight oocytes retrieved (Baart *et al.*, 2007; Nargund *et al.*, 2007).

The combination of mild stimulation protocols with elective single embryo transfer (eSET) may provide the same delivery rate per treatment cycle by reducing the negative aspects of a more aggressive stimulation. It has been reported that over 1 year of treatment, the cumulative live birth rates are similar for mild ovarian stimulation with single embryos transferred compared with the standard stimulation and two embryos transferred. A milder IVF treatment protocol also reduces the multiple pregnancy rate and overall costs (Heijnen *et al.*, 2007).

A recent meta-analysis showed that 150 IU/day of rec FSH in normal responders <39 years is the optimal daily dose for the best balance between a high pregnancy rate and a low risk of complications, thus maximizing the cost-effectiveness of an ART cycle (Sterrenburg *et al.*, 2011). Although evidence in favor of milder ovarian stimulation for IVF is accumulating, it also has been argued that this protocol is associated with lower ongoing pregnancy rates and higher cancelation rates (Hohmann *et al.*, 2003; Heijnen *et al.*, 2007). In the USA, where cycle-specific data are reported to the Society for Assisted Reproductive Technology (SART), reporting a lower pregnancy rate using a mild stimulation would not be acceptable in a competitive marketplace. Further, the concept of reporting cumulative pregnancy rates is not acceptable to most couples who have to pay out of pocket for all treatment.

Ten years after the introduction of mild ovarian stimulation, there is still room for improvement. A more patient-tailored stimulation should take into account several factors such as age, BMI, smoking status, the status of ovarian reserve markers such as anti-Müllerian hormone (AMH) and antral follicle counts (AFCs; Fauser *et al.*, 2010). With such an approach, mild stimulation may be an attractive option for both good and poor responders.

## Polycystic ovarian syndrome

The two lectures on PCOS were focused on the different definitions of PCOS and a review of evidence-based treatment. Of the three definitions of PCOS, the one suggested by NIH, as well as the definition put forward by the Androgen Excess and PCOS Society, includes the presence of hyperandrogenism as a required criterion. The Rotterdam definition (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) is the most widely used and allows for several possible phenotypes, being defined by two of three of the following criteria: menstrual irregularity, polycystic ovaries on ultrasound and hyperandrogenism. Thus, it is possible for PCOS to be diagnosed in the absence of documented hyperandrogenism. Although controversial, the importance and requirement of hyperandrogenism in the definition was the position taken by one of the speakers for the following reasons: it is the most prominent diagnostic feature, it is most associated with the metabolic features of the disorder, it serves as a prognostic feature of treatment outcomes and having hyperandrogenism may be used as a criterion for inclusion of these women into multicenter trials studying PCOS.

The Thessaloniki consensus meeting focused on the treatment of PCOS (Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008). It was agreed that prior to initiation of any intervention, emphasis should be given to the importance of lifestyle management, especially weight reduction. The recommended first-line treatment for ovulation induction remains the anti-estrogen clomifene citrate (CC). The use of aromatase inhibitors for routine ovulation induction seems to be as effective as CC, but insufficient evidence is currently available on its efficacy and safety (Requena *et al.*, 2008). Metformin alone is less effective than CC in inducing ovulation in women with PCOS. The addition of metformin to CC may be indicated in specific subgroups of women with PCOS, but its use should be restricted to women with glucose intolerance (Legro *et al.*, 2007; Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008). Based on recent data in the literature, the routine use of this drug in ovulation induction is not recommended (Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008).

In cases of failure of ovulation or no pregnancy, the proposed second-line intervention includes either exogenous gonadotrophins or laparoscopic ovarian surgery (LOS).

The use of exogenous gonadotrophins is reported to be highly effective, but it is associated with increased chances for multiple pregnancies and, therefore, careful monitoring of ovarian response is needed. LOS alone is usually as effective as gonadotrophins for ovulation induction and achievement of pregnancy, but is associated with a significantly lower risk of multiple pregnancy (in one RCT: odds ratio, 0.11; 95% confidence interval, 0.01–0.86; Bayram *et al.*, 2004).

Recommended third-line treatment is IVF. Specific patient-tailored approaches for ovulation induction should be based on initial screening characteristics of the patient. IVF appears to be a reasonable option, as the risk of multiple pregnancies can be controlled by SET. Nevertheless, even singleton pregnancies in women with the classic features of PCOS are associated with increased health risks, for both the mother and the fetus (Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008).

## Poor ovarian response

In a retrospective analysis, the performance of women with a poor ovarian response (POR) was evaluated. POR in a single cycle with conventional stimulation was defined as one which was canceled because there were less than three growing follicles or from which three or less oocytes were retrieved in response to conventional stimulation. With these criteria, it was found that the majority of patients, up to an age of 40 years, exhibited a normal response in a subsequent stimulation cycle with higher doses of gonadotrophins leading to a cumulative ongoing pregnancy rate that was not different compared with normal responders (Ferraretti *et al.*, 2010). It was noted that after two consecutive cycles with POR, the risk of POR in further cycles was close to 100% with a cumulative ongoing pregnancy rate significantly lower than normal responders.

In an attempt to assess ovarian reserve before starting stimulation, several tests have been proposed. Generally, ovarian reserve testing (ORT) has moderate accuracy in predicting quantitative responses but low accuracy for qualitative predictions, unless very high thresholds are used. The AFC and AMH appear to have the best sensitivity and specificity (Hendriks *et al.*, 2005; Broer *et al.*, 2009), but even the best ORT is associated with a 20–25% false-positive rate. The use of more than one test does not lead to significant improvement.

Several risk factors have been postulated to have a role in determining POR (Younis, 2011), the most relevant being age, genetic factors related to premature ovarian ageing, previous ovarian surgery and chemotherapy/radiotherapy. Possibly, chronic smoking, pelvic infection, short menstrual cycle length and endometriomas could also be involved.

There are no data on the quality of the oocytes generated in POR cycles, but alterations in oocyte quality influencing the prospects of pregnancy is likely to be involved in some cases (Oudendijk *et al.*, 2012).

Most of the treatments proposed to improve the clinical outcome in women with POR are not supported by sufficient evidence to be recommended with the possible exception of the addition of growth hormone, as well as performing embryo transfer on Day 2 versus Day 3 (Kyrou *et al.*, 2009). Similarly, no indication seems to favor any particular intervention either for down-regulation, ovarian stimulation or adjuvant therapy (Pandian *et al.*, 2010; Musters *et al.*, 2012). Evaluation of the results yielded by different clinical trials is confounded by the heterogeneous definition of POR. Thus, patient selection alone might have altered any true effect of some interventions (Polyzos and Devroey, 2011).

A recent consensus meeting sought to find an appropriate definition for POR. It concluded that at least two of the following features must be present: (i) advanced maternal age or any other risk factor for POR, (ii) documented POR in a previous stimulation and (iii) an abnormal ORT (Ferraretti *et al.*, 2011). In addition, two episodes of POR after conventional stimulation are sufficient to define a patient as a poor responder even in the absence of advanced maternal age or abnormal ORT (Ferraretti *et al.*, 2011). Patients with a risk factor for POR and an abnormal ORT might be classified as an 'expected poor responder', since they have not undergone a stimulated cycle.

# Evaluation and treatment of the infertile female

Discussed in some detail was: the role the clinician plays as a member of the IVF team, newer surgical techniques and the management of endometriosis.

## Role of the clinician

It was emphasized here that the clinician of the IVF team has an important role in assuring that an adequate work up has been carried out, which otherwise might affect outcomes.

Multiple studies have identified that between 26 and 50% of patients who fail IVF have uterine abnormalities, suggesting that hysteroscopy could serve as a prognostic factor for the IVF outcome, especially in the case of fibroids which distort the endometrial cavity (Bozdag *et al.*, 2008; Sunkara *et al.*, 2010). Specific hysteroscopic interventions have been reported to significantly improve the pregnancy rate (Demirol and Gurgan, 2004).

The presence of hydrosalpinges also has a negative effect on the ART outcome, and surgery has been shown to improve pregnancy rates (Johnson *et al.*, 2010).

The testing of ovarian reserve performed on Day 2/3, including FSH and E2 levels, should be used for determining an age-adjusted initial stimulation protocol and not to exclude treatment. Moreover, being aware that the best markers of ovarian reserve, AMH and AFC, have limited value in predicting 'non-pregnancy'; these markers therefore should not be used to exclude treatment.

## New surgical techniques

Laparoscopic surgery in Gynecology is widely used, but requires a steep learning curve and deals with long instruments that impose fixed entry points, different tactile sensations and a 2D image. Robotics have been proposed to improve surgical skills through technology, thus overcoming the limitations of human performance. However, no RCTs are available on the application of robotics, and we only have comparative observational studies reporting that it facilitates laparoscopic surgery, shortens operating time and hospital stay, resulting in less major complications while providing comparable results (Caillet *et al.*, 2010; Ercoli *et al.*, 2012).

The new approach in laparoscopy is to decrease both the number of access ports and their size to avoid scars. Therefore, a wide range of new terminology has emerged, including SILS (single incision laparoscopic surgery), SPA (single port access), LESS (endoscopic single site surgery), NOTES (natural orifice transluminal endoscopic surgery), NOTUS (natural orifice transumbilical surgery) and TUES (transumbilical endoscopic surgery). The current literature on the use of these techniques in Gynecology is still rather limited.

Transvaginal endoscopy includes the procedure of ovarian capsule drilling and reconstructive ovarian surgery in endometriosis. Transvaginal ovarian drilling in women with PCOS is an easy intervention with minimal trauma and low morbidity that is performed in a watery environment. Ovarian electrocautery has been stated to be second-line treatment for PCOS (Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008). The clinical outcome after ovarian drilling depends on the number of punctures

per ovary. Based on animal studies evaluating tissue damage after ovarian drilling, the optimal case is to perform the procedure via transvaginal hydrolaparoscopy (THL) with a bipolar diathermy needle making 10–15 punctures with 70 w for 10 s (Amer et al., 2003).

The laparoscopic removal of endometriomas is associated with a significant decrease in residual ovarian volume, which may result in diminished ovarian reserve and function; this does not happen in the case of ovarian dermoids (Hirokawa et al., 2011).

The myometrial junctional zone is structurally and functionally different from the outer endometrium and plays an important role in reproduction, especially in gamete transportation and implantation. During placentation, trophoblast invasion is preceded by decidual remodeling of endometrium and the junctional zone is essential for implantation. For this reason, evaluation of the uterus should include visualization of the myometrial junctional zone, considering that subtle lesions, such as adenomyosis, may be detected by magnetic resonance imaging. A novel approach for the investigation of the junctional zone could be performed with an ultrasound guided myometrial biopsy during diagnostic hysteroscopy using the spirotome, a device specifically designed for endometrial sampling.

### The medical treatment of endometriosis

The main principles of treatment for Endometriosis-Associated Chronic Pelvic Pain are that: (i) initial surgery is more effective than medical treatment, but multiple surgeries should be avoided; (ii) all hormone-altering medications have equivalent efficacy but have different side effects; (iii) 3 months of initial treatment is as effective as 6 months; (iv) 3 months re-treatment is as effective as 6 months of initial treatment; (v) shorter courses of medication have fewer side effects including bone loss; (vi) the ideal add-back regimen after GnRH agonist therapy has not been established; (vii) oral contraceptives are effective for maintenance in patients; (viii) different medical therapies can be used sequentially in the same patient; (ix) since hysterectomy and oophorectomy frequently may become necessary, attention to fertility issues is mandatory and (x) biopsychosocial issues are a critical component of effective care.

For the treatment of infertility in endometriosis, data are insufficient to recommend a specific strategy such as ovarian suppression (Allen et al., 2005) or ovarian suppression and surgery (Hughes et al., 2007). Conversely, controlled ovarian stimulation has been reported to be effective in minimal/mild endometriosis before laparoscopy (Yap et al., 2004).

A new staging system has been developed, the endometriosis fertility index, which is a simple, robust and validated clinical tool that predicts pregnancy rates for patients following surgical staging of endometriosis (Adamson and Pasta, 2010). Its use is especially valuable in developing treatment plans for infertile endometriosis patients.

The effect of endometriosis on the IVF outcome is controversial with several confounding factors typically making it difficult to draw conclusions. However, there are consistent data showing that IVF is a valid alternative for women with stage III/IV endometriosis who (i) fail to conceive following conservative surgery or because of advancing reproductive age; (ii) have compromised tubal function in the presence of male factor infertility and/or (iii) have failed other treatments. No data support an effect of the stage of endometriosis on the IVF outcome with the exception of an overall lower pregnancy rate in endometriosis. Similar statements can be made for the presence of endometriomas. Regarding the role of ovarian suppression before IVF, there are no RCTs with adequate controls, but some evidence suggests improved pregnancy rates when suppression precedes IVF (Sallam et al., 2006).

Several different innovations are also under study to improve the IVF outcomes.

One of these, lipiodol, has been shown to improve live birth rates slightly in women with endometriosis who are attempting natural conception (Johnson et al., 2004).

## Unexplained infertility

This section deals with treatment options for couples with 'unexplained infertility'. While various treatments tend to be empiric, there has been a vast contrast in the approaches used in Europe and in the United States.

Over the past two decades the overall success of IVF has increased significantly, while results of IUI have remained stable and have been associated with an unpredictable occurrence of multiple pregnancies (Reindollar et al., 2010). More recently, the cost-effectiveness of prescribing gonadotrophin/IUI has been questioned. Two large NIH-sponsored trials have been completed, one in women younger than 40 years and the other in women older than 38 years. The first trial, known as the FASTT trial, compared the standard IUI treatment approach (three cycles of CC/IUI followed by three cycles FSH/IUI and then with up to six IVF cycles) with an accelerated treatment that omitted FSH/IUI cycles. The findings showed that the accelerated approach to IVF resulted in a shorter time to pregnancy with an equivalent cumulative percentage of pregnancies occurring in less treatment cycles and with cost savings, suggesting that FSH/IUI was of no added value (Reindollar et al., 2010). According to the FASTT hypothesis, an accelerated approach to IVF (that eliminates FSH/IUI, but starts with CC/IUI) represents the best treatment option. The reason to start with CC/IUI is because, with normal stimulation and no cancelation of cycles, it allows the most fertile couples with the highest chance for multiple pregnancies to become pregnant before IVF is attempted.

The results of the second study, the FORT-T Trial, supported the hypothesis that immediate treatment with two cycles of IVF for older women (38–42 years) results in significantly higher pregnancy and live birth rates compared with two cycles of either CC/IUI or FSH/IUI (Reindollar et al., 2011).

In other studies, mostly European, observational arms with no treatment had been included, and these studies suggested that initial expectant management provides considerable cost savings, without jeopardizing the time to pregnancy or chances of pregnancy. The use of IUI with controlled ovarian stimulation provided similar pregnancy results to the expectant management group, suggesting that expectant management for 6 months is justified in these couples (Steures et al., 2006). A commentary on this approach is that the controlled stimulation in these trials was milder, with many cycles being canceled because of multiple follicular recruitment, thereby resulting in pregnancy rates not statistically different from natural cycles with timed intercourse.

In patients with unexplained or mild male subfertility, it has been suggested that one cycle of IVF-eSET as first-line treatment seems

to be as effective as three stimulated IUI cycles (Custers *et al.*, 2012). However, there are data to suggest that use of clomifene alone or IUI alone is unlikely to offer superior live birth rates compared with expectant management (Bhattacharya *et al.*, 2008). In the view of the presenter regarding the European experience, initial expectant management provides considerable savings without jeopardizing time to pregnancy or the chance of pregnancy.

# Environment and reproduction

This section encompassed a review of known and emerging data regarding the epidemiology of reproduction, the role of culture media, whether there are perinatal risks with various interventions and the potential effects of environmental toxins.

## Epidemiology of reproduction

A declining age at menarche has been observed in industrialized European countries, and in the USA, over the last 100 years. Puberty in girls is now 5 years earlier than historically reported (Parent *et al.*, 2003). This change could have an effect on the risk of reproductive cancer, age of menopause and reproductive senescence. There is an association between obesity and PCOS, and environmental exposures may increase the risk of endometriosis and fibroids.

Despite the methods available for family planning, 50% of pregnancies currently are unplanned (Finer and Henshaw, 2006).

The concept of natural fecundity in recent years has changed, especially when considering the influence of population density, environmental exposure, ethnicity, obesity and social constructs. Similarly, factors like nutrition, various exposures and concomitant illnesses could affect the reproductive life span.

Unexplained infertility is one of the most intriguing challenges for reproductive medicine, often involving undiagnosed issues of ovarian reserve and male factor, or failure of implantation.

Some ART-related risk factors are modifiable, such as time to conception, ovulation induction, an altered endocrine environment at implantation, incubation and manipulation of gametes and embryos including the use of ICSI and extended culture.

Generally speaking, the possible effect of ART on the childhood outcome is of great concern. Although the etiology of various morbidities is not completely understood, it is believed that the underlying infertility, time to conception and ovulation induction (independent of IVF) all are contributing factors. However, some data also support a negative contribution of laboratory factors such as extended embryo culture (Kansal Kalra *et al.*, 2010) and of the hormonal environment at the time of transfer (Kansal Kalra *et al.*, 2011). Very recent data also suggest that the rate of HCG rise is faster following the transfer of cryopreserved embryos, that the HCG rise is positively associated with birthweight but not with gestational age at delivery and that subjects with a faster HCG rise were less likely to have an infant of low birthweight (Morse *et al.*, 2011).

The possibility exists that adult diseases have a developmental origin. If the epigenome is altered by ART and/or by the environment, then the corresponding effect could start as early as conception or implantation. Co-morbidities such as PCOS, endometriosis and obesity may affect pregnancy (Barnhart *et al.*, 2002). PCOS elevates the risk for gestational diabetes mellitus, pre-eclampsia and possibly adult cardiovascular disease.

There is little understanding of the high rate of early fetal loss in humans and, for this reason, diagnosis and management of women with ectopic pregnancy and miscarriage have not changed dramatically in decades. There is a strong need to identify and validate molecular markers specific for ectopic pregnancy, miscarriage and women who need surgical treatment.

During the late reproductive years, decreased ovarian reserve could represent a window into premature aging. There are reports about the presence of altered telomerase activity in gametes and of greater risk of cardiovascular disease, hypertension, altered metabolism and cancer. Male reproductive senescence is associated with erectile dysfunction and apparently with the risk of autism in children.

A final consideration about premature ovarian failure, primary ovarian insufficiency and premature menopause and the possible association with Fragile X premutation, suggests that genetic markers could predict fecundity in the normal population as well as in response to therapy.

## Culture media

Commercially available culture media for clinical IVF contain between 11 and more than 30 different components plus some type of protein supplement such as human serum albumin, synthetic serum substitute or plasmanate. Due to the limited access to human oocytes/zygotes for research, it is clear that the optimal formulation for ART media does not exist. When designing a culture medium, zygote/embryo physiology has been studied, and research with animal embryos, and analyses of the constituents of the human fallopian tube and uterus have been undertaken. Then modifications to the media attempt to compensate for the adverse conditions *in vitro* compared with *in vivo*. Animal models, mostly mice, have provided much of our understanding of early embryo physiology under culture conditions (Biggers *et al.*, 1971). Using simplex optimization in which media components were systematically tested at specific concentrations and combinations, a superior medium for mouse embryo culture was developed (Lawitts and Biggers, 1991). This medium has since been modified for use in clinical IVF and is one of the most popular 'single-step medium' used today for culturing human embryos from the pronuclear stage on Day 1 through to the blastocyst stage on Day 5. In contrast, the design of sequential media originated from the observation that zygotes and embryos change metabolically and uptake/secrete differently during different stages of development (Gardner *et al.*, 1996). However, no convincing data have proven that sequential media, two-step media (used in sequence from Day 1 to Day 3, and then from Day 3 to Day 5) support embryo quality better than modern monophasic and one-step media.

Surprisingly, there are large differences between formulations and usually only marginal scientific data supporting more recent formulations. Some culture media contain growth factors like insulin, and one medium is supplemented with GM-CSF (granulocyte-macrophage colony-stimulating factor). Besides any possible advantage, insulin induces abnormal methylation patterns in mice embryos, while GM-CSF, by reducing apoptosis, could have an effect on the epigenome.

Culture media can influence the growth rate and implantation of human embryos, but it is totally unknown whether a faster growth is

synonymous with better growth. In a comparison of two media, the one promoting faster growth resulted in pregnancies where HCG rose earlier and with higher levels, and the children were 200 g heavier at birth compared with the other medium (Dumoulin et al., 2010; Nelissen et al., 2011). Although there was no appropriate control, the dissimilarity in birthweights suggests that different formulations may have different effects on the epigenome of the embryo. However, at least one other study failed to show any significant association between type of culture medium and birthweight (Eaton et al., 2012).

With these concepts in mind, ESHRE set up a working group on culture media whose aim was to focus on the relationship between culture media (and culture conditions) and the phenotype of the offspring. The working group positions were: (i) companies should disclose the composition and preferably the formulation of each medium that is used clinically, (ii) new formulations should have a scientific foundation, (iii) a standard minimum QC certificate should be shared by all companies that should use the same SOPs, (iv) a more relevant test than the mouse embryo assay should be designed and (v) great caution should be taken regarding the addition of growth factors or hormones to culture media until more is known about possible effects on the epigenome.

## Perinatal outcome

The analysis of more than 30 years of IVF has revealed that there are short-term consequences of ART. There is increased morbidity for children conceived with IVF, with less than 50% of pregnancies having a full-term infant without any complications. The disease process appears to be altered both in the child (low birthweight noted even when not premature) and in the mother (Shevell et al., 2005). Poor perinatal outcome and more episodes of long hospitalization have been reported in children born after ovulation induction, suggesting that either ovulation induction treatment or the reasons for the treatment increase the risk of health problems in early childhood (Klemetti, 2010). Several questions are still unanswered about the clinical implications of the short-term outcomes and possible long-term consequences. Also unknown is how IVF may confer risk. Does ART result in abnormal placentation or is this inherently due to infertility or subfertility (Raatikainen et al., 2010)? When trying to determine the effect of IVF on the risk of perinatal morbidity, multiple births are a major variable, but after adjusting for multiple births, ovarian hyperstimulation syndrome and suboptimal endometrial development appear to be significant factors.

It is unclear why frozen embryo transfer reduces morbidity (Kansal Kalra et al., 2011) and how extended culture increases morbidity as preliminary evidence tends to suggest.

The subspecialty of reproductive endocrinology and infertility needs to take the lead in addressing the issues related to the risks in ART for which an important step is to change the informed consent process. Minimizing multiple pregnancy is the foremost goal, as well as understanding what part of ART is associated with adverse outcomes. To be avoided is (i) underestimating the magnitude of this issue in the eyes of the public by simply blaming it on the disease or the patient, (ii) let another discipline (or the government) dictate standard of care, (iii) use unproven technology without careful consideration and full disclosure and (iv) dismiss the risk to infertile patients.

## Environmental toxins

The hypothesis that toxicants could negatively affect reproduction arose many years ago when endocrine disrupting compounds (EDCs) were suspected to have an impact (Colburn, 1995) and have a role in the decrease in sperm count over the past 50 years (Carlsen et al., 1992), acting as estrogen disruptors (Sharpe and Skakkebaek, 1993). Potential sites of disruption are spread throughout the whole reproductive system, from hormone receptors, to germ cell development, the process of fertilization and embryo development by affecting genetic and epigenetic mechanisms, implantation and pregnancy. Consequently, disorders may occur in females: reproductive tract abnormalities, precocious puberty, premature thelarche, infertility, endometriosis, PCOS and breast abnormalities/cancer. In males, reproductive tract disorders such as hypospadias and cryptorchidism, abnormal semen indices, infertility, testicular and prostate cancer have been observed leading to what is known as 'testicular dysgenesis syndrome'. The factors influencing the outcome are age at exposure, latency from exposure, importance of mixtures and non-traditional dose–response dynamics. The effects may be trans-generational including epigenetic changes.

The analyses of data implicating environmental factors affecting reproduction are complex. For example, the suggested decline in sperm quality during the past decade is not supported by convincing evidence (Axelsson et al., 2011).

Chemicals implicated as EDCs are heavy metals, such as lead, agricultural chemicals like DBCP (1,2-dibromo-3-chloropropane) and vinclozalin, and industrial chemicals, such as phthalates and BPA (Bisphenol A).

Lead can be found in plastics, mirrors, paint, transmissions and gasoline, soil and ceramics. Children are the most vulnerable and contact usually happens through inhalation and ingestion. Animal studies revealed that in males it causes decreased levels of testosterone, LH and FSH; defective spermatogenesis and sperm function as well as epigenetic changes (Sokol, 2002).

DBCB has been proven to cause irreversible sperm toxicity (Whorton et al., 1977) and is currently prohibited in Europe and the USA. Vinclozalin in animal studies showed serious effects in males including androgen receptor disruption, undescended testes, hypospadias, delayed puberty and transgenerational effects (Anway and Skinner, 2006).

Phthalates are ubiquitous (personal care products, food packing and processing materials, building materials and medication coating) and have been shown in animals to cause cryptorchidism, oligospermia and reduced anal-genital distance. Clinical studies performed in the USA reported higher urine levels and lower sperm count, while a study in newborns found a possible increased incidence of hypospadias and cryptorchidism and possibly reduced anal-genital distance (Swan et al., 2005).

BPA in male animals has been associated with decreased anal genital distance and prostate abnormalities, while in females there were changes in mammary glands and early puberty. Clinical studies do not provide definitive evidence, but there is a possible association in males with erectile dysfunction and in females with PCOS.

In summary, exposure to chemicals is increasing and animal studies support the hypothesis that some chemicals disrupt reproduction. Although some clinical data suggest that EDCs disrupt the

reproductive system, a cause and effect connection has not yet been clearly established and, therefore, further clinical studies are needed. However, based on the available evidence both the EU and the USA issued regulations of chemical exposure: lead was regulated in EU and the USA; DBCP was banned in many countries, but not all; phthalates were banned from cosmetics and baby toys in the EU, while in the USA only limited amounts were allowed in toys; BPA was banned from baby bottles in the EU, the USA and Canada.

# The core of ART: the process of IVF and embryo transfer

With the constant improvement of laboratory procedures and culture systems, the rates of implantation have increased significantly leading to a reconsideration of the number of embryos to transfer, with the specific aim of decreasing the incidence of high-order multiple gestations. With the awareness that morphology alone does not reflect the physiology of the embryo, non-invasive assays have been proposed to identify markers of viability in individual embryos. Included in this discussion are sections on the gametes, embryos, the day of transfer and implantation as well as data on oocyte cryopreservation and pre-implantation genetic screening.

## The gametes

Unfortunately, the assessment of oocyte viability remains rather superficial and a general morphological evaluation leads to the discarding only of those oocytes with nuclear immaturity or with significant degeneration or gross abnormalities. Thus, the great majority of metaphase II oocytes are inseminated, and the selection of the embryo destined for transfer is entirely based on its morphology and growth irrespective of the quality of the corresponding oocyte.

There are data which show that the analysis of the birefringence properties of the meiotic spindle and the zona pellucida are indicative of good health of the oocyte (Magli *et al.*, 2011; Montag *et al.*, 2011), although there is no agreement regarding its clinical applicability (Petersen *et al.*, 2009). Novel data are coming from the application of studying gene expression from cumulus cells, using microarrays, as biomarkers for oocyte viability (Menezo *et al.*, 2010; Assidi *et al.*, 2011). The metabolomic profiling of oocyte spent culture media by mass spectroscopy has found differences related to oocyte maturation, embryo development and implantation success (Nagy *et al.*, 2009). Similar results have been shown by the measurement of oocyte oxygen consumption (Tejera *et al.*, 2011). These data are very preliminary, but demonstrate an increasing interest toward the oocyte as the cell that determines embryo development and viability.

Significant progress has also been made recently in defining the role of the sperm cell in guiding the oocyte to resume meiosis and undergo fertilization and cleavage. Following progesterone release by the cumulus cells, a progesterone-induced $Ca^{2+}$ influx in spermatozoa triggers hyperactivation, the acrosome reaction and chemotaxis toward the oocyte (Lishko *et al.*, 2011). Once released into the oocyte, the sperm PLC-zeta factor mobilizes the $Ca^{2+}$ signal that induces egg activation and embryo development with a tight association reported between the type of calcium oscillations and embryo development (Ajduk *et al.*, 2011). Accordingly, abnormalities in PLC-zeta negatively affect fertilization rates and possibly embryo development, confirming the key role of this factor in triggering oocyte activation. This finding suggests that certain types of infertility could be caused by failure of the sperm cell to properly activate the oocyte due to a defective PLC-zeta factor (Heytens *et al.*, 2009; Kashir *et al.*, 2011).

Once entered into the oocyte, sperm chromatin and DNA integrity are necessary to ensure normal embryo development (Barratt *et al.*, 2010). Although it is now clear that DNA damage in spermatozoa has a negative influence on blastocyst development and the ICSI outcome, there is a strong need to standardize the specific tests used to quantify the extent of DNA damage (Barratt *et al.*, 2010). Similarly, centrosome integrity is critical for successful fertilization and embryo development, and new data indicate that the replacement of defective centrosomes, which are responsible for specific forms of male infertility, with functional donor sperm centrosomes can restore normal functionality (Schatten and Sun, 2009).

A great deal of research has recently emerged providing evidence regarding novel aspects in sperm cell DNA structure, namely the presence of epigenetic information in the form of post-translational modifications (e.g. histones), which may impart specific imprints especially in developmentally important genes and passing on the genetic information to the oocyte (Hammoud *et al.*, 2009). Sperm from infertile patients show different patterns of epigenetic marking compared with fertile men, particularly at certain imprinted and developmental loci (Hammoud *et al.*, 2011).

The sperm cell also contains various forms of RNA, e.g. mRNA, miRNA, siRNA (Cappallo-Obermann *et al.*, 2011; Krawetz *et al.*, 2011) as well as more than 2000 proteins with unknown roles.

Following gamete interaction by conventional IVF or ICSI, even in cases with apparently normal oocytes and spermatozoa, fertilization failure occurs 20–30% of the time. The incidence of total fertilization failure (TFF) after conventional IVF using sperm of normal quality has been reported to range from 5% (Bhattacharya *et al.*, 2001) to as high as 15–20% (Barlow *et al.*, 1990; Liu and Baker, 2000). While ICSI has overcome many fertilization problems, it does not completely eliminate TFF. In a randomized clinical trial comparing outcomes after ICSI or IVF for cases of non-male factor infertility, Bhattacharya *et al.* (2001) documented a TFF rate of 2 versus 5% for ICSI and IVF, respectively. Indeed, several large studies using ICSI for a variety of infertility diagnoses reported TFF at rates of 1.3% in 1779 cycles (Esfandiari *et al.*, 2005) and 3% in 2732 cycles (Liu *et al.*, 1995).

The specific causes are unknown, but failure in most ICSI cycles is ascribed to defective activation, while with conventional IVF, lack of sperm penetration is the leading reason of failure. In an analysis of specific cases of failures, oocyte maturation arrest was associated with highly abnormal spindle/chromosomal structures (Heindryckx *et al.*, 2011). In some cases of oocyte spontaneous activation or activation failure, experiments with heterologous gametes may help determine the contributing factor (Combelles *et al.*, 2010, 2011). The etiology of fertilization failure is complex and heterogeneous and requires more specific research.

## The embryo

The improvement of culture systems has provided the possibility of extending embryo cultures to Days 5 and 6, making the day of transfer a flexible choice. The reasons supporting extended cultures to Days 5

and 6 are (i) the identification of those embryos capable of developing to the blastocyst stage *in vitro*, (ii) a modest selection against aneuploidy and (iii) a uterine environment that is likely more favorable for blastocyst transfer (Fanchin *et al*., 2001). Potential downsides are (i) increased risk of having no transfer when applied in unselected patients (Glujovsky *et al*., 2012), (ii) increased occurrence of monozygotic/monochorionic twins, especially after ICSI (Skiadas *et al*., 2008) (iii) fewer embryos available for cryopreservation (Glujovsky *et al*., 2012) and (iv) the risk that prolonging duration of culture could cause epigenetic disorders, as suggested by animal and perinatal outcome data (Kallen *et al*., 2010; Park *et al*., 2011).

A special advantage associated with blastocyst transfer is related to the practice of PGD/PGS, both when the biopsy is performed at earlier stages giving more time for the analysis before blastocyst transfers, and when it is done from trophectoderm cells, a strategy that seems to be very promising in terms of the accuracy of the results with improved implantation rates (Schoolcraft *et al*., 2010).

It is clear that the day of transfer should be individualized for each patient (Glujovsky *et al*., 2012). The capacity to identify an embryo that develops into a viable blastocyst requires a robust scoring system. Besides the classical morphological scoring criteria, novel tools come from the observation of dynamic parameters on Day 2 that can predict with high accuracy blastocyst formation. It has also been shown that imaging phenotypes reflect the molecular program of the embryo, where individual blastomeres develop autonomously towards embryo genomic activation (Wong *et al*., 2010). Prospective trials are now warranted to determine whether such time-lapse imaging improves implantation rates compared with current standard morphological assessment.

The procedure of embryo transfer is critical in determining successful implantation and a skillful operator and the use of a soft catheter are associated with the best results (Yao *et al*., 2009).

There is a general consensus that elective Single Blastocyst Transfer (eSBT) should be indicated in young, good-prognosis patients with good quality embryos thus promoting a reduced twin rate without decreasing the chances of pregnancy. In addition, the high survival rate of cryopreserved blastocysts greatly contributes to good cumulative pregnancy rates (Mesut *et al*., 2011).

The maternal and neonatal risks of multiple gestations and deliveries associated with their socioeconomic costs, promoted the adoption of advocating elective single-embryo transfer (eSET) by some countries, particularly in Northern Europe. After almost a decade of this experience, the generalized use of eSET in a fresh cycle combined with the subsequent transfer of a frozen embryo provides outcomes that are similar to one fresh cycle with dual embryo transfer (DET) (McLernon *et al*., 2010). The main difference resides in the incidence of multiple live birth rates that drops significantly from 22–29% after DET to 1% in eSET (McLernon *et al*., 2010).

The measure of treatment success is crucial to the acceptance of eSET, for which the focus needs to be shifted away from the results of a single fresh cycle to results of cumulative cycles. In this case, eSET matches or outperforms DET (Practice Committee of Society for Assisted Reproductive Technology; and Practice Committee of American Society for Reproductive Medicine, 2012). As a prerequisite to this strategy, the cryopreservation program must be efficient and reliable. Patients' characteristics should be carefully evaluated when deciding on the transfer policy, since poor prognosis factors such as advanced female age, poor embryo quality and some infertility factors may dictate the need for DET (Lawlor and Nelson, 2012).

The situation is somewhat different in the US. Live birth rates in the US are higher than in those countries with a greater percentage of eSET cycles. These differences cannot be explained by the larger number of embryos transferred (Gleicher *et al*., 2007). However because of the expectation of couples in the US for higher pregnancy rates and the fact that most cycles have to be paid for by the couple, there has been some reluctance in adopting a strict eSET program (Practice Committee of Society for Assisted Reproductive Technology; and Practice Committee of American Society for Reproductive Medicine, 2012). Nevertheless there is keen awareness that eSET should be seriously considered in good prognosis patients with good embryo quality (Practice Committee of Society for Assisted Reproductive Technology; and Practice Committee of American Society for Reproductive Medicine, 2012). Not to be forgotten is patient autonomy, and the couples' choice. A survey a few years ago reported that many couples strongly favor twin pregnancies (Practice Committee of American Society for Reproductive Medicine, 2012). However, proper education and information given to patients significantly changed this position (Newton *et al*., 2007).

## Implantation

Successful implantation requires that the transferred embryo be viable and that the uterus is receptive. In this regard it was reaffirmed that both embryologists and clinicians contribute significantly to this end. A successful IVF program requires the update of professionals on new advances and knowledge as well as high laboratory QC/QA standards.

Unfortunately a high proportion of transferred embryos fail to implant, and there is a group of patients where implantation failure occurs repeatedly even under optimal conditions. Several strategies have been adopted to overcome recurrent implantation failure (RIF), addressing both the embryo, using techniques like assisted hatching, and the endometrium by intensifying the investigation of the female reproductive tract. The administration of intravenous immunoglobulins (IVIG) in women suspected of having an immunological cause of RIF did not improve the live birth rates when compared with placebo (Stephenson and Fluker, 2000). Similarly, in the presence of ACA (anti-cardiolipin antibodies), the use of heparin and aspirin showed no benefit (Stern *et al*., 2003). Attempts of administering steroids (Boomsma *et al*., 2007) or low-dose aspirin (Gelbaya *et al*., 2007) were also negative.

In a novel approach, repeated endometrial biopsies in the cycle immediately preceding IVF treatment has been shown significantly to increase implantation, pregnancy and live birth rates in women who had at least one previous IVF failure (Barash *et al*., 2003). A positive outcome was also found after the intrauterine administration of autologous PBMCs (peripheral blood mononuclear cells) (Yoshioka *et al*., 2006) especially when pretreated with CRH (corticotropin-releasing hormone) that acts by regulating apoptosis of activated T-lymphocytes at the implantation site (Makrigiannakis *et al*., 2001). As a proposed mechanism of action, CRH added to primary cultures of PBMCs significantly increases IL-6 release (Th2-type immunity) and decreases IFN-γ (Th1-type immunity) levels in a dose dependent manner. In this way, CRH induces stromal decidualization and

potentiates the decidualizing effect of progesterone (Zoumakis *et al.*, 2000). More work is still needed in this challenging area.

## Oocyte cryopreservation

Oocyte cryopreservation has been used for i) fertility preservation for medical or social reasons, ii) use of cryo-banked oocytes for egg donation, iii) storage of spare oocytes avoiding the production of supernumerary embryos, iv) storage of oocytes in cases of no sperm availability, and v) aspiration of excess oocytes in IUI cycles.

Several factors influence the clinical efficiency of oocyte cryopreservation including female age (which might be more important than in fresh cycles), quality of oocytes, and, most importantly, the technique used - slow freezing or vitrification. The critical evaluation of results should be based on the implantation rate, calculated on the basis of thawed/warmed oocytes (Gook and Edgar, 2007).

Widespread use of oocyte cryopreservation began with the introduction of vitrification after the birth of the first baby from warmed oocytes (Kuleshova *et al.*, 1999). The most recent publications demonstrate a performance that is comparable to that obtained with fresh oocytes in young women (Rienzi *et al.*, 2010; Cobo *et al.*, 2011) and possibly a superiority over the slow-freezing method (Smith *et al.*, 2010; Cobo and Daz 2011). Nevertheless, proper randomized controlled trials are still lacking and when comparisons are made taking into consideration the number of implantations per thawed/warmed oocyte, the two techniques have generally shown similar performance (Kim *et al.*, 2010; Bianchi *et al.*, 2012). In addition, it must be considered that the great majority of data on oocyte vitrification derive from oocytes from fertile patients as used in donor egg programs, and not from infertile women, particularly those who are older.

Neonatal data on more than 900 children demonstrate no increase in spontaneous miscarriage, chromosomal anomalies or birth defects (Noyes *et al.*, 2009). These data are considered by many as a good argument to remove the label of 'experimental' from the technique of oocyte cryopreservation. Nevertheless, a meticulous follow-up of pregnancies, especially from infertile patients and on the health of children are considered to be mandatory to finally prove the safety of the technique.

## Preimplantation genetic screening (PGS)

To date, randomized controlled trials have not shown that PGS by FISH on blastomeres from cleavage-stage embryo has improved the live birth rate compared with a control group (Mastenbroek *et al.*, 2011). Possible reasons could be that i) the biopsied blastomere is not truly representative of the embryo at the 8-cell stage because of mosaicism; ii) the biopsy procedure itself might cause harm and negatively influence the developmental potential of the embryo; iii) not all chromosomes were tested by FISH; and iv) the contribution of aneuploidy to implantation failure may be overestimated. Therefore, two possible alternatives were proposed, namely trophectoderm biopsy and polar body biopsy for the analysis of all chromosomes (Bisignano *et al.*, 2011; Handyside, 2011). With trophectoderm biopsy, both maternal and paternal abnormalities can be studied without touching the future embryo, but possible disadvantages are the limited amount of time available for this analysis, the presence of mosaicism, although at a level lower than at the 8-cell stage, and the fact that the trophectoderm might not be representative of the inner cell mass. For the polar body biopsy, advantages are that polar bodies are external to the embryonic mass and do not exhibit mosaicism. However, this approach only permits the testing of maternal chromosomes and does not evaluate mitotic errors, including the possible correction of meiotic errors following mitosis.

When deciding to perform PGS, two aspects play an important role, namely the validation of the protocol to be used for the chromosomal analysis and the safety of the biopsy procedure.

ESHRE recently organized a pilot study to verify the feasibility and reliability of full-karyotype testing on polar bodies by CGH array technology with confirmation of results on the corresponding oocytes. It was shown that the analysis of both polar bodies could be completed within a time period that allows for fresh transfer with a reliable identification of the chromosomal status in about 90% of biopsy attempts, and a concordance of results between polar body and oocyte of 94% (Geraedts *et al.*, 2011; Magli *et al.*, 2011). From a biological point of view, the study of meiosis demonstrated that MII anomalies predominate over MI, with chromatid predivision being the most frequent mechanism of aneuploidy-causing mechanism, and chromosome losses prevailing over chromosome gains (Fragouli and Wells, 2011; Handyside *et al.*, 2012).

The results of the pilot study promoted the organization of a multicentre, randomized double-blind controlled trial with an intention-to-treat analysis including women with advanced maternal age. The trial, called the ESHRE Study into The Evaluation of oocyte Euploidy by Microarray analysis (ESTEEM), has two primary aims i) to assess the predictive value of having no euploid oocytes in future ART cycles; and ii) to improve live birth rates. The results are expected within two years.

An alternative approach proposed is the use of single nucleotide polymorphism (SNP) microarrays whose validity has been tested in single cells from cell lines, with a demonstrated 98.6% accuracy for overall assignment of aneuploidy status (Treff *et al.*, 2010a). An experimental comparison with FISH based aneuploidy screening showed that SNP microarray is significantly more reliable for providing interpretable results (Treff *et al.*, 2010b), and can be achieved in only four hours (Treff *et al.*, 2012). In this way, the transfer can be done in a fresh cycle, although the process of vitrification does not seem to impair the implantation of euploid blastocysts (Schoolcraft *et al.*, 2011).

To investigate whether the procedure of biopsy may be critical for embryo selection or what stage is optimum, data on DNA fingerprinting that permits the tracking of the implanted embryo suggest that blastomere biopsy decreases implantation, whereas this does not happen with trophectoderm biopsy (Treff *et al.*, 2011, 2012). In addition, it has been shown that the chromosomal analysis on blastocysts has an excellent negative and positive predictive value with the eventual clinical outcome (Scott *et al.*, 2012).

# Ovarian cancer and menopause

Reproductive medicine encompasses concerns regarding cancer as well as the management of menopause. In this section, theories of ovarian carcinogenesis are explored as well as the 'timing' hypothesis regarding the use of hormones in women after menopause.

## Ovarian cancer: the incessant menstruation hypothesis

Epithelial ovarian cancers are the most lethal form of gonadal malignancies for which routine screening has no effect on mortality. The most common form is serous carcinoma, followed by endometrioid, clear cell, and mucinous carcinoma. The vast majority of ovarian cancers arise from non ovarian cells; serous carcinomas from tubal epithelial cells, and endometrioid cell carcinoma from endometrial cells.

It has been suggested that retrograde menstruation may have a role in carcinogenesis through the generation of reactive oxygen species (ROS) which results from the action of free iron derived from the lysis of refluxed erythrocytes in the peritoneal cavity (Toyokuni, 2009). ROS promote the activation of M1-macrophages, which favor tumorgenesis by inducing epithelial cell transformation sustained by M2-macrophages (Mantovani and Sica, 2010). Refluxed blood collects in the pouch of Douglas, close to the distal tube, and chronically exposes the epithelium of fimbria to heme, iron, and macrophages, possibly contributing to the development of serous carcinoma. Similarly, the high concentration of free iron in endometriotic cysts could cause carcinogenesis through persistent oxidative stress (Yamaguchi *et al*., 2008).

The number of lifetime menstruations, pelvic endometriosis and the use of the IUD are considered risk factors for ovarian cancers, whilst hormonal contraception and tubal ligation are protective factors (Collaborative Group on Epidemiological Studies of Ovarian Cancer, 2008).

Bilateral salpigoophorectomy results in a reduced risk of ovarian cancer (Domchek *et al*., 2010), but a higher incidence of coronary heart disease (Parker *et al*., 2009). A possible strategy could be hysterectomy with ovarian preservation (Moorman *et al*., 2011) or the post-reproductive removal of fallopian tubes (Dietl *et al*., 2011). Despite some evidence supporting the tubal hypothesis, some concern and doubt remain (Collins *et al*., 2011). At the present time, a preventative strategy should be the use of hormonal contraception for prolonged periods of time, especially in women with endometriosis. In addition, bilateral salpingectomy may be considered in women not desirous of future pregnancies.

## Menopause: The Timing Hypothesis

Several RCTs in the past 10 years showed increased cardiovascular (CV) risk in women initiating hormones at regular doses, up to 20 years after menopause. The putative protective effects of hormonal therapy (HT) were detected in observational studies in women initiating HT close to menopause; similar observations have been made for Dementia risk. The timing of initiation of HT may be critical in determining risks and benefits.

Clinical and animal studies have demonstrated that early and continued estrogen treatment has beneficial effects that can arrest atherosclerotic lesion development. However, after a vascular plaque has been established, which occurs with natural aging or in an accelerated fashion with risk factors such as hypercholesterolemia, the vascular effects of estrogen are no longer beneficial (Mendelsohn and Karas, 2005). In addition, when initiated late in the atherosclerotic process, HT could have adverse effects, potentially destabilizing existing plaques and triggering a coronary event. Oral estrogens increase the expression of matrix metalloproteinase (MMPs) that are produced by the inflammatory cells in the atheroma.

It is postulated that standard doses of oral estrogens may cause early cardiovascular events in older women through up-regulation of MMPs, disruption of the fibrous cap, and subsequent rupture of plaque. In recent RCTs, administration of conjugated equine estrogens (CEE) for up to 6 years provided no protection against myocardial infarction (MI) or coronary death in generally healthy post-menopausal women who were in the wide age span of 50–79 years, but there was a statistical reduction in coronary heart disease with CEE among women in the 50 to 59 year old age group (Hsia *et al*., 2006).

Also in the WHI, women 50 to 59 years old receiving estrogen had less calcified-plaque burden in their coronary arteries compared with placebo (Manson *et al*., 2007). A more recent case control study also suggested that early initiation of HT reduced the prevalence of abnormal coronary angiograms (Shufelt *et al*., 2011).

In a series of meta-analyses HT was found to reduce CHD events as well as mortality in younger post-menopausal women, whilst in older women, HT initially increases and then decreases risk over time (Salpeter *et al*., 2006, 2009).

However, the 'timing' hypothesis has not been tested in a RCT as hard end-points of MI or CVD mortality are not possible in a young healthy population. Use of 'intermediate' end-points with a high correlation to CHD have been proposed, and have been incorporated into the design of on- going RCTs, namely KEEPS and ELITE.

Estrogens have several important effects on the brain being neuroprotective and neurotrophic, and act especially on neurotransmitters, glial cells, proteins- amyloid, Tau and apo E, and regulate organizational activity (Henderson, 1997).

Many observational studies and meta-analyses agree that the use of estrogens in mid-life reduces a woman's risk of subsequent dementia, whereas HT initiation in late life could have deleterious effects (Whitmer *et al.,* 2011) as was also shown in a substudy of WHI. In cases with Alzheimer's disease, estrogens seem to have an effect on cognition that is most apparent on tasks of semantic memory.

In conclusion, RCT data regarding the 'timing' hypothesis are sorely needed. KEEPS and ELITE will be available shortly, but in the meantime young, healthy, symptomatic women near the onset of menopause should not be fearful of HT in terms of CV or dementia risk. Nevertheless, it is unlikely that we will ever see an HT indication for either coronary disease or Alzheimer's risk.

# Conclusion

Many diverse topics were discussed and debated at this first- of- a-kind meeting of ASRM and ESHRE. In the final analysis, we wished to convey current best practices in reproductive medicine, realizing that there are differences in approaches in the US and Europe. It is hoped that the summary above captures at least some of the valuable exchange of ideas which will foster better reproductive healthcare.

# Presenters at Best Practices of ASRM and ESHRE

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