Human Reproduction Update, Vol.15, No.4 pp. 391–408, 2009
Advanced Access publication on April 20, 2009 doi:10.1093/humupd/dmp012

human reproduction update

Approaches to improve the diagnosis and management of infertility

P. Devroey[1,4], B.C.J.M. Fauser[2] and K. Diedrich[3] on behalf of the Evian Annual Reproduction (EVAR) Workshop Group 2008[†]

[1]Center of Reproductive Medicine, Free University Brussels, Laarbeeklaan 101, 1090 Brussels, Belgium [2]Department of Reproductive Medicine and Gynaecology, University Medical Center, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands [3]Department of Obstetrics and Gynecology, University Clinic of Schleswig-Holstein, Campus Luebeck, 23538 Luebeck, Germany

[4]Correspondence address. Tel: +32-2-477-66-60; Fax: +32-2-477-66; E-mail: paul.devroey@uzbrussel.be

TABLE OF CONTENTS

BACKGROUND: Recent advances in our understanding of the causes of infertility and of assisted reproductive technology (ART) have led to the development of complex diagnostic tools, prognostic models and treatment options. The Third Evian Annual Reproduction (EVAR) Workshop Meeting was held on 26–27 April 2008 to evaluate evidence supporting current approaches to the diagnosis and management of infertility and to identify areas for future research efforts.

METHODS: Specialist reproductive medicine clinicians and scientists delivered presentations based on published literature and ongoing research on patient work-up, ovarian stimulation and embryo quality assessment during ART. This report is based on the expert presentations and subsequent group discussions and was supplemented with publications from literature searches and the authors' knowledge.

RESULTS: It was agreed that single embryo transfer (SET) should be used with increasing frequency in cycles of ART. Continued improvements in cryopreservation techniques, which improve pregnancy rates using supernumerary frozen embryos, are expected to augment the global uptake of SET. Adaptation and personalization of fertility therapy may help to optimize efficacy and safety outcomes for individual

[†]The Third Evian Annual Reproduction (EVAR) Workshop Meeting (26–27 April 2008) was organized to discuss personalized approaches to the diagnosis and management of infertility and was supported by an unrestricted educational grant from Merck Serono S.A.—Geneva. The content of this manuscript is based on the presentations and discussions of the EVAR Workshop Meeting. The meeting was chaired by P.D. (Free University Brussels, Brussels, Belgium), K.D. (University Clinic of Schleswig-Holstein, Luebeck, Germany) and B.C.J.M.F. (University Medical Center, Utrecht, The Netherlands). The speakers included Carlo Alviggi (Università degli Studi di Napoli Federico II, Naples, Italy), Esther Baart (University Medical Center, Utrecht, The Netherlands), Christopher Barrat (Ninewells Hospital, Dundee, UK), Christina Bergh (Sahlgrenska University Hospital, Gothenburg, Sweden), Frank Broekmans (University Medical Center, Utrecht, The Netherlands), Johannes L.H. Evers (Maastricht University Medical Center, Maastricht, The Netherlands), Georg Griesinger (University Clinic of Schleswig-Holstein, Luebeck, Germany), Stephen Keay (University of Warwick, Coventry, UK), François Olivennes (ART unit Eylau La Muette, Paris, France), Gamal Serour (Al-Azhar University, Cairo, Egypt), Catherine Staessen (Vrije Universiteit, Brussels, Belgium), Arne Sunde (St Olav's University Hospital in Trondheim, Norway), Christos Venetis (Aristotle University of Thessaloniki, Greece) and Hakan Yarali (Hacettepe University, Ankara, Turkey). Colin M. Howles (Merck Serono S.A.—Geneva) and Julian Jenkins (previously of Merck Serono S.A.—Geneva) were in attendance.

patients. Prognostic modelling and personalized management strategies based on individual patient characteristics may prove to represent real progress towards improved treatment. However, at present, there is limited good-quality evidence to support the use of these individualized approaches.

CONCLUSIONS: Greater quality control and standardization of clinical and laboratory evaluations are required to optimize ART practices and improve individual patient outcomes. Well-designed, good-quality studies are required to drive improvements to the diagnosis and management of ART processes.

Key words: infertility work-up / *in vitro* fertilization / ovarian response / predictive factors / single embryo transfer

Introduction

Infertility can be defined as the failure to achieve a pregnancy within 1 year of regular unprotected intercourse (Evers, 2002; Zegers-Hochschild *et al.*, 2006). Despite the inherent difficulties of estimating the prevalence of infertility (Greenhall and Vessey, 1990), it is generally accepted that one in four women are affected at sometime (Gunnell and Ewings, 1994). Moreover, ~20% of couples consult their general physician because of difficulty conceiving, and half of those couples (10%) require specialist care (Hull *et al.*, 1985; Beurskens *et al.*, 1995).

Assisted reproductive technology (ART) treatment for infertile couples now results in reasonably high pregnancy rates but may be associated with risks of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancy (Van Voorhis, 2006). The variability in patient characteristics and response to ART dictate the need for proven, personalized diagnostic and therapeutic approaches to optimize efficacy and safety outcomes (Fauser *et al.*, 2008).

Advances in our understanding of the causes of infertility and ART have facilitated the development of increasingly complex diagnostic tools, prognostic models and treatment options. It is hoped that the identification of reliable baseline demographic, disease or genetic characteristics that are predictive of treatment outcome will enable selection of the most appropriate management strategy for each couple. Furthermore, new laboratory techniques may facilitate the transfer of single embryos while maintaining existing pregnancy rates (Van Voorhis, 2006).

Personalized management strategies, based on individual patient characteristics, have been proposed and the further development of these strategies may represent real progress towards individually tailored fertility treatment. The Third Evian Annual Reproduction (EVAR) Workshop Meeting was held on 26–27 April 2008 to evaluate the existing evidence to support current approaches to the diagnosis and management of infertility. Here, we report the discussions and expert opinions of the EVAR Workshop Group based on presentations summarizing current literature and identify key areas for future research efforts.

Methods

Prior to the Third EVAR Workshop Meeting in April 2008, specialist reproductive medicine clinicians and scientists prepared presentations based on published literature and ongoing research relating to patient work-up, ovarian stimulation and embryo quality assessment during ART. Following the presentations, group discussion to reach joint conclusions on the topics covered was facilitated by the chairmen: P.D., B.C.J.M.F. and K.D.

The content of this report is based on the expert presentations and subsequent group discussions that took place during the workshop meeting. Given the broad scope of this report, a systematic literature review was not feasible. Instead, the discussions relating to each topic were complemented with electronic literature searches via PubMed for articles of any type that were published in the English language and unlimited by date of publication. Combinations of the following keywords were used to identify relevant articles: 'assisted reproduction', 'ART', 'blastocyst', 'cryopreservation', 'embryo', 'FSH', 'ICSI', 'individualized', 'infertility', 'IUI', 'IVF', 'LH', 'multiple pregnancy', 'outcome', 'ovarian', 'PGS', 'post-coital test', 'prognostic', 'reserve', 'resistance', 'response', 'semen analysis', 'SET', 'stimulation', 'tubal occlusion' and 'work-up'. The literature search was also supplemented with key publications that were known to the authors.

Individualized pretreatment assessment

The accurate detection of underlying reproductive abnormalities helps to guide individual management decisions and maximize ART treatment outcomes. Clinical evaluation of the infertile couple may be grouped into five categories: semen analysis, the post-coital test (PCT), assessment of ovulation, uterine and tubal evaluation, and laparoscopy (Balasch, 2000). Of these, semen analysis, mid-luteal phase serum progesterone level and tubal patency evaluation comprise the initial basic patient work-up (Crosignani and Rubin, 2000). However, the use of several fundamental elements of infertility testing is still contentious, and evidence suggests that the current World Health Organization (WHO) recommendations for the standard investigation of the infertile couple are poorly followed in Europe (Rowe *et al.*, 1993; Balasch, 2000).

Semen analysis

Humans have a low proportion of 'normal' sperm compared with many other species. Although relatively few studies of semen analysis have been performed in men with proven fertility, there is a high degree of overlap in semen characteristics between fertile and infertile men (Guzick *et al.*, 2001). High-quality semen analysis has diagnostic value for gross male infertility conditions (such as azoospermia or globozoospermia), but the predictive value of an individual semen analysis is less robust when moderate numbers of motile sperm are present (Comhaire, 2000).

Semen analysis comprises sperm concentration, motility and morphology. No isolated semen analysis measures have been shown to be diagnostic of infertility in large studies (Guzick *et al.*, 2001). In an effort to increase the value of semen analyses, results have been

incorporated into complex prediction models (Snick *et al*., 1997; Hunault *et al*., 2004). However, the output of these models has large confidence intervals and results must be interpreted cautiously (Snick *et al*., 1997; Hunault *et al*., 2004).

There are various methods for semen analysis, but those recommended by the WHO and the European Society for Human Reproduction and Embryology (ESHRE) provide the most comprehensive and robust methods (World Health Organization, 1999; Kvist and Bjorndahl, 2002). Although a new manual is due to be published in 2009, the WHO currently defines 'normal' as a sperm concentration of $>20 \times 10^6$/ml with >50% of progressively motile sperm and the presence of sperm with standard (no parameters provided) morphology (World Health Organization, 1999).

Evidence suggests that the WHO recommendations for performance of semen analysis and reporting of results are adhered to poorly in routine laboratory practice (Keel *et al*., 2002; Riddell *et al*., 2005). Despite the availability of established systems to improve staff training in semen assessments, such as ESHRE courses (Bjorndahl *et al*., 2002), the majority of laboratories still do not have accurate methods or appropriate training systems. Thus, semen analysis results are often variable. The demonstrated absence of standardization and strict quality control for semen analysis undermines the diagnostic and prognostic value of the test.

Despite the limitations described, semen analysis is routinely used to evaluate the fertilization potential of the male partner in infertile couples. Semen analysis outcomes also guide management decisions and often influence the choice of expectant management, intrauterine insemination (IUI), *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI).

Greater standardization of semen analysis and accurate laboratory evaluation is clearly needed to improve the prognostic value of semen analysis (Ombelet *et al*., 2003). Furthermore, high-quality studies are required to identify threshold levels that are predictive of treatment outcome to assist decision-making for ART treatment. Sperm function tests may offer greater predictive power than traditional semen analysis but require strict validation prior to use in routine clinical practice (Lefievre *et al*., 2007).

Post-coital test

The PCT provides an assessment of the quantity and quality of cervical mucus, sperm–mucus interactions and the presence of antisperm antibodies (Balasch, 2000; Glazener *et al*., 2000). The test involves microscopic examination of extracted endocervical mucus, which should be conducted in the pre-ovulatory phase and 8–12 h after intercourse (Glazener *et al*., 2000). A positive PCT result may be defined as the presence of at least one forward-progressing spermatozoon in more than half of at least five high-power (×400 magnification) microscope fields (Glazener *et al*., 2000).

The PCT may provide an effective predictor of conception for couples with an infertility history of <3 years and no identified female causes of infertility (Glazener *et al*., 2000). However, it has been shown that the outcome of the PCT can be predicted for only half of all infertile couples (in which the female partner has a regular menstrual cycle) using the basic elements of an infertility history and semen analysis results (van der Steeg *et al*., 2004). Furthermore, results of the PCT are subject to considerable intra- and inter-observer variability (Male Infertility Best Practice Policy Committee of the American Urological Association and Practice Committee of the American Society for Reproductive Medicine, 2006). Given the widespread use of ART for couples with a negative PCT, routine use of this test in clinical practice is not recommended (National Institute for Clinical Excellence, 2004; Male Infertility Best Practice Policy Committee of the American Urological Association and Practice Committee of the American Society for Reproductive Medicine, 2006).

Uterine and tubal evaluation

Evaluation of the morphology of the uterus and fallopian tubes is essential prior to initiating ART (Crosignani and Rubin, 2000). Uterine and tubal structures may be visualized using a variety of techniques, including hysteroscopy, hysterosalpingography (HSG), transvaginal ultrasonography (TVS), saline infusion sonohysterography (SIS), magnetic resonance imaging (MRI) or laparoscopy.

Tubal occlusion is estimated to be the cause of infertility for 14% of couples who require specialist treatment (Hull *et al*., 1985). The optimum screening strategy to assess tubal damage is a complex subject with currently no consensus of opinion (National Institute for Clinical Excellence, 2004; den Hartog *et al*., 2008). A recent observational study comparing screening strategies for tubal factor infertility with laparoscopy has provided valuable data (den Hartog *et al*., 2008). *Chlamydia trachomatis* immunoglobulin G serology was shown to discern accurately patients at high versus low risk of tubal pathology and, thus, was suggested to provide a useful initial screening test (den Hartog *et al*., 2008). HSG was worthwhile only for low-risk patients to confirm the absence of tubal pathology and confer potentially beneficial effects of tubal flushing with oil-soluble contrast medium (den Hartog *et al*., 2008). Laparoscopy with dye testing was considered useful for patients with positive *Chlamydia* serology or evidence of bilateral tubal occlusion on HSG (den Hartog *et al*., 2008).

The presence of hydrosalpinx has a negative effect on clinical pregnancy and live birth rates following IVF (Strandell, 2007). The mechanism by which a hydrosalpinx impairs IVF success is incompletely understood, but its removal prior to IVF is known to significantly improve treatment outcomes (Strandell, 2007). A Cochrane meta-analysis of data from three clinical studies demonstrated superior pregnancy [odds ratio (OR) 1.75, 95% confidence interval (CI) 1.07–2.86] and live birth rates (OR 2.13, 95% CI 1.24–3.65) following salpingectomy compared with no surgical intervention (Johnson *et al*., 2004). Evidence from one of these studies suggested that the benefit of salpingectomy is greatest for patients with fluid-filled hydrosalpinges that are visible on ultrasound examination (Strandell *et al*., 1999). Thus, patients with a hydrosalpinx that is visible on ultrasound should be encouraged to undergo prophylactic salpingectomy prior to IVF.

The optimal management of endometriotic ovarian cysts in infertile patients is less well defined. Recent evidence of reduced responsiveness to gonadotrophins following laparoscopic ovarian cystectomy has challenged the traditional surgical approach to treatment (Somigliana *et al*., 2006). Indeed, it has been suggested that surgery should be undertaken only for the treatment of large endometriomas or pain that is refractory to medical treatment, or to exclude malignancy (Garcia-Velasco and Somigliana, 2009).

Unexpected hysteroscopic abnormalities have been reported in up to 40% of patients during ART work-up (Shamma *et al*., 1992; Doldi

et al., 2005), but there is no compelling evidence that either routine use of hysteroscopy before IVF or correction of identified pathology leads to better treatment outcomes. Compared with hysteroscopy, HSG has high sensitivity (81–98%) but low specificity (23–35%), and high false-negative (10–90%) and false-positive (22–44%) rates.

Although hysteroscopy is considered the gold standard for identification of intrauterine pathology (Bozdag et al., 2008), recent advances have enabled ultrasonographic techniques to increasingly substitute for invasive screening procedures (Ekerhovd et al., 2004). Late follicular phase TVS has proved to be a useful tool for the detection of intrauterine abnormalities such as polyps, synechiae, fibroids and Müllerian anomalies (Van Voorhis, 2008). SIS offers enhanced visualization of the endometrium and better detection of intrauterine pathology than does standard TVS, and may be as effective as hysteroscopy in detecting intracavitary abnormalities (Ragni et al., 2005; Valenzano et al., 2006). MRI may be used for patients with suspected complex Müllerian anomalies (Deutch and Abuhamad, 2008).

Uterine fibroids occur in 20–50% of women aged over 30 years and are the most common benign tumour of the female genital tract (Eldar-Geva et al., 1998; Okolo, 2008). These tumours are heterogeneous in composition, size, location and number (Pritts, 2001), thus complicating the identification of women who would benefit from myomectomy prior to ART treatment.

Retrospective data suggest that the presence of fibroids <4 cm in diameter does not affect the outcome of ART treatment cycles (Vimercati et al., 2007). Additional retrospective analyses suggest that only fibroids that encroach on the uterine cavity negatively affect implantation rates and pregnancy outcomes in ART (Farhi et al., 1995; Eldar-Geva et al., 1998). A meta-analysis suggested that compared with infertile women without fibroids, women with submucosal fibroids have significantly lower rates of clinical pregnancy [relative risk (RR) 0.36, 95% CI 0.18–0.74], implantation (RR 0.28, 95% CI 0.12–0.65) and live birth (RR 0.32, 95% CI 0.12–0.85) (Pritts, 2001). Removal of submucosal fibroids improves clinical pregnancy rates (RR 2.03, 95% CI 1.08–3.83), but the limited available data suggest no improvement in treatment outcomes after removal of intramural fibroids (Pritts, 2001).

Endometrial polyps have been identified by hysteroscopy in 16–27% of women with otherwise unexplained infertility (Kim et al., 2003; de Sa Rosa e de Silva et al., 2005). The benefit of hysteroscopic polypectomy on pregnancy rate has been demonstrated in a prospective, randomized study of women with ultrasonically diagnosed endometrial polyps who were undergoing IUI (Perez-Medina et al., 2005), in which patients who underwent polypectomy had a significantly higher cumulative pregnancy rate than those who underwent hysteroscopy plus polyp biopsy (63.4% versus 28.2%, $P < 0.001$) (Perez-Medina et al., 2005).

An association between polypectomy and improved spontaneous pregnancy rates was also shown in five non-randomized studies (Varasteh et al., 1999; Spiewankiewicz et al., 2003; Shokeir et al., 2004; Stamatellos et al., 2008; Yanaihara et al., 2008). Retrospective data suggest that hysteroscopic polypectomy improves pregnancy rates in previously infertile women, regardless of the number or size of polyps present (Stamatellos et al., 2008), and that resection of polyps located at the utero-tubal junction may improve pregnancy rates in infertile patients (Yanaihara et al., 2008). Although the effect of endometrial polyps on IVF is unclear (Lass et al., 1999; Isikoglu et al., 2006), data suggest that women with otherwise unexplained infertility may still benefit from polypectomy (Stamatellos et al., 2008).

In summary, prophylactic salpingectomy improves ART outcomes for patients with a fluid-filled hydrosalpinx. However, there are currently insufficient high-quality data on the optimum screening modality and management of other uterine and tubal abnormalities prior to ART on which to base personalized patient care. Good-quality, prospective studies are warranted to evaluate the relative merits of uterine and tubal screening tests and management approaches prior to ART treatment.

Evaluation of ovarian reserve

Ovarian stimulation is used in ART to stimulate multifollicular development and enable multiple oocyte retrieval (Fauser et al., 2008). The ovarian response to gonadotrophin stimulation depends primarily on the woman's ovarian reserve (Broekmans et al., 2006) and is a major determinant of the success of IVF (van der Gaast et al., 2006; Shanbhag et al., 2007).

Ovarian reserve represents the remaining population of primordial and resting follicles (Gougeon, 1996) and is generally defined as the quantity and quality of the follicles present in the ovary (Broekmans et al., 2006). For operational purposes, the ovarian reserve can be defined as the number of antral follicles present in the ovaries at a given time that can be stimulated into dominant follicle growth by exogenous follicle-stimulating hormone (FSH). Women with a so-called 'normal' ovarian reserve will develop an average of 8–10 dominant follicles in response to conventional ovarian stimulation, with a corresponding number of oocytes (Broekmans et al., 2006). Although chronological age is the major determinant of ovarian reserve, there is considerable individual variability in the rate of ovarian ageing (Fig. 1) (te Velde and Pearson, 2002). Therefore, accurate tests of ovarian reserve may allow individualized predictions of oocyte yield and ART treatment outcome in terms of ongoing pregnancy (Broekmans et al., 2006).

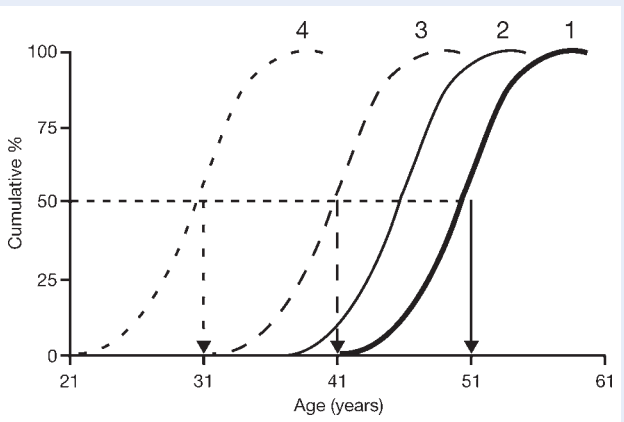

Figure 1 Variation in reproductive ageing.

Adapted with permission from te Velde and Pearson (2002). Curve 1 shows the Gaussian distribution of variation of age at menopause, Curve 2 the variation of age of transition from cycle regularity to irregularity, Curve 3 the variation in age of becoming sterile and Curve 4 the variation in age of becoming infertile.

Serum FSH level and antral follicle count (AFC) assessed by TVS are often used as tests of ovarian reserve (Broekmans *et al*., 2006). The AFC correlates with the number of oocytes retrieved and ART outcomes (Frattarelli *et al*., 2003) and is currently considered to be the best available single predictor of ovarian response to stimulation (Bancsi *et al*., 2002; Hendriks *et al*., 2007). Serum anti-Müllerian hormone (AMH) levels may accurately identify patients at risk of an extreme ovarian response (Nelson *et al*., 2007), but are infrequently measured in routine practice (van Rooij *et al*., 2002; van Rooij *et al*., 2005; Broer *et al*., 2009).

The evaluation of ovarian reserve may enable the identification of patients (with regular menstrual cycles) who will have a better or worse response to gonadotrophin stimulation than would be expected for their chronological age (Broekmans *et al*., 2006). In theory, this would help clinicians to personalize patient management by selecting an appropriate treatment protocol, stressing the need for early initiation of treatment or counselling against initiation of treatment (Broekmans *et al*., 2006).

Studies on the impact of ovarian reserve tests to select the appropriate starting dose of FSH have yielded contradictory results (Popovic-Todorovic *et al*., 2003a, b; Klinkert *et al*., 2005; Olivennes *et al*., 2009).

Although the potential use of AMH needs to be evaluated further, all widely available tests of ovarian reserve are poor predictors of clinical pregnancy and live births after IVF (Hendriks *et al*., 2005; Mol *et al*., 2006). As such, a patient's true ovarian reserve can be determined only after a cycle of ovarian stimulation.

Management strategy selection

Expectant or active therapy

Infertile couples can be divided into two groups: those who are unable to conceive without therapy and those who have reduced fertility but are likely to conceive spontaneously with time (Crosignani and Rubin, 2000). Expectant management is the most appropriate approach for infertile couples with a good prognosis for spontaneous pregnancy, whereas ART provides a valuable treatment option for selected couples with a low probability of natural conception (Hunault *et al*., 2005). A reliable estimate of the likelihood of spontaneous pregnancy is essential to enable clinicians to decide whether expectant or active management is more appropriate for a given couple (Hunault *et al*., 2004).

Prognostic models derive the likelihood of spontaneous pregnancy in an individual couple based on large-sample data (Comhaire, 1987; Eimers *et al*., 1994; Wichmann *et al*., 1994; Collins *et al*., 1995; Snick *et al*., 1997). Statistical analysis of a model developed by Snick *et al*. (1997) demonstrated that it would be predictive of a live birth in 76–79% of infertile couples in a primary care setting. The most important prognostic factors in this model were an abnormal PCT, a tubal defect, an ovulatory defect and infertility of longer than 2 years (Snick *et al*., 1997). Although female age is considered to be one of the most important factors to affect ART outcome (Templeton *et al*., 1996; van Kooij *et al*., 1996), female age (<30 years) was predictive of live birth in this model only when the PCT result was excluded (Snick *et al*., 1997).

The use of such prognostic models in clinical practice requires standardized screening assessments for all patients. Furthermore, there are no strict criteria on which to base management decisions. Hence, the likelihood of spontaneous pregnancy for each individual couple must be weighed against the potential benefits or risks of interventional treatment.

Active ART treatment options

IVF is an effective treatment option for female infertility, whereas ICSI was developed for male infertility. Here, we discuss evidence to support the use of these treatment options in cases of male infertility, bilateral tubal occlusion and unexplained infertility.

Male infertility

Couples affected by severe male infertility related to conditions such as obstructive or non-obstructive azoospermia require treatment with ICSI to achieve pregnancy (National Institute for Clinical Excellence, 2004). However, mild-to-moderate male infertility is a poorly defined concept and treatment strategies are highly variable.

Limited evidence suggests that IUI after clomiphene citrate stimulation (CC-IUI) may be an effective first-line therapy for male infertility when the total inseminating motile sperm count after preparation is $>1 \times 10^6$/ml (Ombelet *et al*., 2003). Furthermore, CC-IUI still represents an effective therapeutic option when the inseminating motile sperm count is $<1 \times 10^6$/ml if the sperm morphology score is at least 4% (Van Waart *et al*., 2001; Ombelet *et al*., 2003). Despite these findings, the authors of a recent Cochrane meta-analysis concluded that there is insufficient evidence to support the use of IUI rather than timed intercourse (with or without ovarian stimulation) for male infertility, and called for additional large, high-quality, randomized controlled trials to investigate this issue (Bensdorp *et al*., 2007).

There are also limited data to support the use of conventional IVF or ICSI for mild-to-moderate male infertility. Clinical pregnancy rates were similar after up to six cycles of IUI (with or without ovarian stimulation) or IVF for male infertility in a prospective, randomized study (Goverde *et al*., 2000). A meta-analysis of data from nine randomized, controlled, sibling oocyte design trials of IVF versus ICSI for couples with 'borderline semen characteristics' demonstrated a pooled relative fertilization rate of 2.2 (95% CI 1.6–3.0) per oocyte in favour of ICSI (Tournaye *et al*., 2002). However, fertilization rates were not significantly different in a sibling oocyte design study of ICSI or high insemination concentration (0.8 sperm $\times 10^6$/ml) IVF (67.6 versus 59.6; $P = 0.066$) (Tournaye *et al*., 2002). There is a need for high-quality trials comparing pregnancy or live birth rates after IVF or ICSI for male infertility.

Given the lack of reliable evidence, the Third EVAR Workshop Group advocated the use of a sibling oocyte technique (i.e. half of the oocytes are inseminated by conventional IVF and half by ICSI) if the likelihood of spontaneous fertilization is uncertain. However, the group acknowledged that use of this technique depends on the retrieval of a sufficient number of oocytes.

Bilateral tubal occlusion

The use of IVF and ICSI in a sibling oocyte design study of couples with tubal infertility and normal semen showed similar mean fertilization rates of 53% and 62%, respectively (Staessen *et al*., 1999). Moreover, no significant benefit in clinical pregnancy or live birth rates was demonstrated in an analysis of data from two pseudo-randomized controlled trials of

couples with tubal infertility who had been treated with ICSI or IVF (Aboulghar et al., 1996; Bukulmez et al., 2000).

Polycystic ovary syndrome

IVF is currently a third-line treatment option, after ovulation induction and laparoscopic ovarian surgery, for women with anovulatory infertility due to polycystic ovary syndrome (PCOS), but it has been suggested that older women may benefit from earlier use of IVF (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008). Analysis of baseline characteristics may enable identification of a subgroup of women who would benefit from a tailored treatment approach (Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2008). However, further work is required to determine the optimal gonadotrophin stimulation protocol for women with PCOS and the maternal age threshold for deviation from the standard treatment algorithm.

Unexplained infertility

IUI is a commonly used treatment strategy for couples with unexplained infertility (Verhulst et al., 2006). Data have indicated a significantly higher live birth rate with IUI plus ovarian stimulation than with IUI alone for unexplained infertility (OR 2.07, 95% CI 1.22–3.50) (Verhulst et al., 2006).

The use of ovarian stimulation in combination with IUI is a controversial and heavily debated topic (Fauser et al., 2005; Goverde et al., 2005; van Rumste et al., 2006). Biologically, the use of ovarian stimulation would be expected to increase the likelihood of a multiple pregnancy because the development of multiple dominant follicles and ovulation of multiple oocytes is the aim of this intervention. However, published literature contains conflicting reports, which may be related to failure to induce multifollicular development or cycle cancellation after the detection of three or more pre-ovulatory follicles in half of the patients in some studies (Goverde et al., 2005; Verhulst et al., 2006; van Rumste et al., 2006). Nonetheless, a recent systematic literature review of studies of controlled ovarian stimulation and IUI clearly demonstrated that multiple pregnancy rates correlated positively with the number of pre-ovulatory follicles (van Rumste et al., 2008).

Furthermore, the validity of pregnancy rates per cycle for comparisons of different treatment modalities could be questioned (Fauser et al., 2005) when some interventions (e.g. timed intercourse or natural cycle IUI) may be less costly or time-consuming and associated with less patient discomfort and fewer complications. Regardless of the treatment outcome evaluated, the workshop group believes that singleton pregnancy should be the primary aim of ART treatment and, as such, the benefits of ovarian stimulation in combination with IUI must be weighed carefully against the potential risks of a multiple pregnancy.

No differences in live birth rates were demonstrated with IVF or IUI either with (OR 1.15, 95% CI 0.55–2.4) or without (OR 1.96, 95% CI 0.88–4.4) ovarian stimulation in a Cochrane meta-analysis of clinical trials of unexplained infertility (Pandian et al., 2005). ICSI is associated with a significantly lower rate of complete fertilization failure in cases of unexplained infertility than conventional IVF (0.8% versus 19.2%; $P < 0.001$) (Jaroudi et al., 2003). However, data from three randomized controlled trials indicate that clinical pregnancy rates are similar following IVF or ICSI: 11% and 28%, respectively, per oocyte retrieval ($P = 0.09$) (Jaroudi et al., 2003); 32% and 38% (RR 0.83, 95% CI 0.48–1.45) (Bhattacharya et al., 2001); and 50% for both [not significant (*P*-value not provided)] (Foong et al., 2006).

Table I Summary of available evidence on which to base active ART management decisions

Indication	IUI versus IVF	Conventional IVF versus ICSI
Mild-to-moderate male infertility	No difference in clinical pregnancy rates (Crosignani and Walters, 1994; Goverde et al., 2000)	ICSI increases fertilization rates (RR 2.2, 95% CI 1.6–3.0) (Tournaye et al., 2002)
		Decreases fertilization failure (Tournaye et al., 2002)
		There is no high-quality evidence for an effect on pregnancy rates
Tubal infertility	Not applicable	Leads to no difference in clinical pregnancy rates (Aboulghar et al., 1996; Bukulmez et al., 2000)
Unexplained infertility	No difference in live birth rates (Pandian et al., 2005)	Decreases fertilization failure (Jaroudi et al., 2003)
		No difference in clinical pregnancy rates (Bhattacharya et al., 2001; Jaroudi et al., 2003; Foong et al., 2006)

ART, assisted reproductive technology; ICSI, intracytoplasmic sperm injection; IUI, intrauterine insemination; IVF, *in vitro* fertilization.

In summary, evaluation of disease characteristics can help clinicians to select the most appropriate active therapeutic option, thus allowing basic tailoring of ART treatment to the individual. However, there is little convincing evidence on which to base treatment strategies for the majority of infertile couples (Table I). More high-quality data on the relative superiority of each treatment option, and associated adverse events, are needed to guide ART management decisions. Comparison of well-defined outcomes, such as (singleton or term) live births per started treatment strategy rather than per treatment cycle, should also be considered. Moreover, outcomes should be viewed in the wider context of time taken to achieve pregnancy, patient stress, likelihood of complications and overall costs of the intervention and monitoring.

Individualized ovarian stimulation

Ovarian stimulation protocols

The central paradigm of all ovarian stimulation protocols is to maximize the beneficial effects of treatment (relating to high-quality oocyte yield) while minimizing the potential risks associated with

OHSS and multiple pregnancy (Fauser *et al*., 2008). The amount of exogenous FSH required to induce follicle development is related to the so-called FSH threshold and varies widely among women (Brown, 1978; Schoemaker *et al*., 1993; Fauser and Van Heusden, 1997). Conventional daily doses of FSH in ART treatment protocols range from 150 to 225 IU, but close monitoring and dose adjustment is required because of the considerable inter-individual variability in ovarian response (Fauser *et al*., 2008).

A requirement for elevated doses of FSH to induce multifollicular development and increase the oocyte yield may indicate that a patient is at the extreme of ovarian reserve (Tarlatzis *et al*., 2003). Low mitochondrial DNA content has been reported to occur more frequently in women with ovarian insufficiency than in those with normal ovarian function (May-Panloup *et al*., 2005). The consequences of impaired mitochondrial function in human oocytes are currently unknown, but animal studies have shown that replacement of ooplasm in ooplasmic-deficient eggs will restore their developmental capacity (Levron *et al*., 1996). The observed high rate of pregnancy loss in women with reduced ovarian reserve (evidenced by elevated basal FSH levels) indirectly supports the notion of a qualitative reduction in oocyte quality (Levi *et al*., 2001).

The efficacy and safety profile of standard FSH stimulation protocols vary according to individual patient characteristics. Four factors (FSH, body mass index, female age and AFC) have been identified to be predictive of ovarian response to FSH stimulation in women aged <35 years undergoing ART (Howles *et al*., 2006). These factors have been combined into a model that predicts the optimum starting dose of recombinant human (rh) FSH for individual patients (Howles *et al*., 2006; Olivennes *et al*., 2009). Similar factors were previously shown to predict the dose of exogenous FSH required to induce ovulation in normogonadotrophic anovulatory patients (Imani *et al*., 2002).

It is currently unclear from efficacy, safety, tolerability or health economic perspectives whether mild or maximal ovarian stimulation (with cryopreservation of supernumerary embryos) is most beneficial (Fauser *et al*., 2005). Mild stimulation protocols [comprising a gonadotrophin-releasing hormone (GnRH) antagonist for pituitary suppression and low-dose FSH stimulation in the mid-to-late follicular phase] may offer lower multiple pregnancy rates and lower costs than standard ovarian stimulation, despite similar overall treatment efficacy (Heijnen *et al*., 2007). Accordingly, research efforts are ongoing to optimize the efficacy and safety profiles of stimulation protocols.

Evaluation of ovarian response to stimulation

There are no universally accepted definitions of normal, poor or excessive responses to ovarian stimulation and the inconsistent definitions used in different studies have hampered efforts to compare treatment outcomes (Tarlatzis *et al*., 2003; Kailasam *et al*., 2004). Many definitions relate to retrieved oocyte number, which is a crude marker of response when used in isolation. Ovarian response must be evaluated with reference to the stimulation protocol used, including the daily and/or total dose of FSH administered and co-treatment regimens (Kailasam *et al*., 2004; Verberg *et al*., 2009a). Indeed, the retrieval of a small number of oocytes is the very aim of mild stimulation protocols, although this would represent a poor response following conventional ovarian stimulation (Verberg *et al*., 2009b). Furthermore, it has been suggested that ovarian response should be considered in the context of an individual's expected response to gonadotrophin stimulation (Klinkert *et al*., 2004).

Excessive ovarian response

The most serious iatrogenic complication of multifollicular ovarian stimulation is severe OHSS, which is believed to be triggered by human chorionic gonadotrophin (hCG) (Aboulghar and Mansour, 2003). This is a life-threatening condition, in which increased capillary permeability results in haemoconcentration and hypovolaemia (Papanikolaou *et al*., 2005).

There is some evidence that the incidence of early OHSS is limited by the use of a GnRH antagonist (rather than a GnRH agonist) for pituitary down-regulation (Al-Inany *et al*., 2006; Heijnen *et al*., 2007), low-dose and mild FSH-stimulation protocols (Heijnen *et al*., 2007), a GnRH agonist (Griesinger *et al*., 2007b; Engmann *et al*., 2008) or low doses of hCG (Nargund *et al*., 2007) to trigger final oocyte maturation. Although coasting (withholding of gonadotrophins) is often used to prevent OHSS (Mansour *et al*., 2005; Nardo *et al*., 2006), a systematic review concluded that there is a shortage of randomized controlled trials investigating such an approach for the prevention of OHSS (D'Angelo and Amso, 2002). The transfer of frozen–thawed embryos in natural cycles may be used instead to limit the incidence of late OHSS (Mathur *et al*., 2007). However, there are limited data to support the cryopreservation of all embryos (D'Angelo and Amso, 2007). Large randomized trials are required to compare the risks and benefits of different ART treatment strategies for women at risk of OHSS (Aboulghar and Mansour, 2003).

Poor ovarian response

Kailasam *et al*. (2004) attempted to define a poor ovarian response in a retrospective study of IVF cycles in women aged under 40 years. Based on this limited evidence and practical considerations, the workshop group proposed a definition of poor response to stimulation as the retrieval of fewer than four oocytes (or cycle cancellation following the development of fewer than three follicles) in response to an ovarian stimulation protocol of 225 IU FSH per day (which represents maximal ovarian stimulation) (Hoomans *et al*., 1999; Harrison *et al*., 2001; Latin-American Puregon IVF Study Group, 2001; Out *et al*., 2001; Yong *et al*., 2003; Out *et al*., 2004). Parameters of oocyte maturity are not included in this definition.

Signs of diminished ovarian reserve may include age older than 40 years, the presence of fewer than five antral follicles of 2–5 mm in diameter on TVS prior to treatment or an elevated basal FSH level (Klinkert *et al*., 2004; Klinkert *et al*., 2005). A poor response to gonadotrophin stimulation would be unexpected in patients who have no signs of decreased ovarian reserve (Klinkert *et al*., 2004). A retrospective cohort study showed higher cumulative pregnancy rates (after a maximum of three cycles) among patients who had experienced an unexpected versus expected poor response in their first IVF treatment cycle (37–47% versus 16–19%) (Klinkert *et al*., 2004). Thus, patients who have an unexpected poor response to stimulation still have a reasonable chance of pregnancy in subsequent cycles (Klinkert *et al*., 2004; Hendriks *et al*., 2008).

There is little convincing evidence to indicate the most appropriate treatment regimen for patients with an expected or proven poor response to ovarian stimulation (Shanbhag *et al*., 2007). Indeed, prospective, randomized studies have shown little benefit from daily doses of rhFSH of ≥300 IU to induce follicular development (Tarlatzis *et al*., 2003; Kailasam *et al*., 2004). Moreover, although evidence is conflicting, high doses of FSH may stimulate the recruitment of immature or chromosomally abnormal oocytes or have a detrimental effect on the endometrium (Check *et al*., 1999; Katz-Jaffe *et al*., 2005; Kok *et al*., 2006; Baart *et al*., 2007). In addition, chromosomal aneuploidy has been observed in embryos from unstimulated IVF cycles in young women (Verpoest *et al*., 2008).

Future elucidation of relevant genetic markers may allow the identification, prior to stimulation, of patients who are at risk of a poor response or OHSS (Fauser *et al*., 2008).

Ovarian resistance to FSH

Early studies identified a subgroup of normogonadotrophic patients who have normal estimated ovarian reserves but suboptimal responses to FSH stimulation (De Placido *et al*., 2001, 2004, 2005; Mochtar *et al*., 2007). Such women express ovarian resistance to FSH but seem to be distinct from classical poor responders because some investigators suggest that luteinizing hormone (LH) supplementation improves their ART treatment outcomes (Alviggi *et al*., 2006). The incidence of ovarian resistance to FSH is poorly defined in the published literature, but it is believed to affect 10–15% of women with normal ovarian reserve who undergo standard cycles of ART (Alviggi *et al*., 2006). This subgroup can be identified by estradiol levels of <180 pg/ml or the absence of follicles of >10 mm in diameter on Day 8 of a 150–300 IU FSH per day stimulation cycle following GnRH agonist long-protocol pituitary down-regulation (Alviggi *et al*., 2006).

It is believed that androgens, which are endogenously produced in response to LH stimulation, are involved in sensitizing small antral follicles to FSH (Durnerin *et al*., 2008). Profound suppression of endogenous LH by GnRH agonists combined with the use of rhFSH may cause LH activity to fall below a hypothetical threshold value in some women. An observational trial by Alviggi *et al*. (2006) showed that a common polymorphism of LH (v-LH) was present in a higher proportion of patients who required >3500 IU compared with <3500 IU FSH for follicular maturation (35% versus 2.6%). v-LH has a short half-life and may be ineffective in supporting FSH-stimulated multifollicular growth.

Despite these data, a meta-analysis showed no significant difference in live birth rates with or without LH supplementation of FSH stimulation in a non-selected patient population (OR 0.92, 95% CI 0.65–1.31); subgroup analyses also produced similar findings (Kolibianakis *et al*., 2006). However, some of the studies reported above were excluded from this meta-analysis because of co-treatment with variable doses of FSH. Indeed, in a recent Cochrane systematic review, a subanalysis of data from three clinical trials showed a significantly higher pooled estimate of ongoing pregnancy in poor responders who had received co-treatment with rhLH than FSH alone (OR 1.85, 95% CI 1.10–3.11) (Mochtar *et al*., 2007). Additional data are awaited, and pharmacogenomic studies may provide evidence of associated biological pathways.

Embryo assessment

The use of embryo morphology scoring was prospectively evaluated by Holte *et al*. The group assessed the ability of five real-time visual embryo scoring variables to predict implantation of an individual embryo after double-embryo, Day 2 transfer (Holte *et al*., 2007). Cleavage stage, variation in blastomere size and the presence of multinucleated cells were found to be independent markers of embryo competence following the application of a conditional multiple-regression model.

An increased understanding of the metabolic needs of embryos and improvements in cell culture media have enabled prolonged *in vitro* culture of embryos (Fig. 2) (Papanikolaou *et al*., 2008). Extension of the *in vitro* culture period now allows the transfer of Day 5 blastocyst-stage rather than early cleavage-stage embryos (Blake *et al*., 2007). A recent meta-analysis showed significantly higher rates of clinical pregnancy (OR 1.27, 95% CI 1.03–1.55; $P = 0.02$) and live births (OR 1.39, 95% CI 1.10–1.76; $P = 0.005$) following the transfer of blastocyst-stage compared with cleavage-stage embryos (Papanikolaou *et al*., 2008). Given the speed at which laboratory techniques have evolved, it is essential to compare procedures and outcomes to elucidate the best practices for maximal embryo viability.

The scientific rationale for blastocyst transfer is to increase implantation rates by improving uterine and embryonic synchronicity and allowing self-selection of embryos with greater implantation potential (Wilson *et al*., 2002; Blake *et al*., 2007). However, there is little level I evidence on which to base recommendations for morphology screening or blastocyst selection.

Greater standardization of blastocyst morphology scales is required to allow comparison of study findings. In addition, validated, objective measurements of blastocyst morphology and growth kinetics throughout the culture period are required to increase the reproducibility of results. Operator-independent computer-processing programmes have shown promising ability to objectively assess blastocyst morphology (Lemmen *et al*., 2008), but require further validation prior to use in routine practice.

Selection of culture conditions

Individual laboratory culture strategies are complex and highly varied, and there are few data on which to base the selection of a specific culture strategy. For example, each culture fluid contains up to 80 components that will influence embryo development and assessments. Greater standardization of culture conditions is required to permit accurate comparisons of embryo and/or blastocyst quality-assessment tools.

Chromosomal evaluation

There is a high incidence of chromosomal abnormalities in human embryos cultured *in vitro* (Munne, 2006) and the incidence of aneuploidy increases with maternal age (Munne *et al*., 1995; Marquez *et al*., 2000). Theoretically, identification of chromosomal abnormalities would permit selection of the healthiest embryos, and thus increase live birth rates (Gianaroli *et al*., 1997; Wells and Delhanty, 2000). Unfortunately, embryo morphology correlates only partially with its chromosomal status (Wells and Delhanty, 2000).

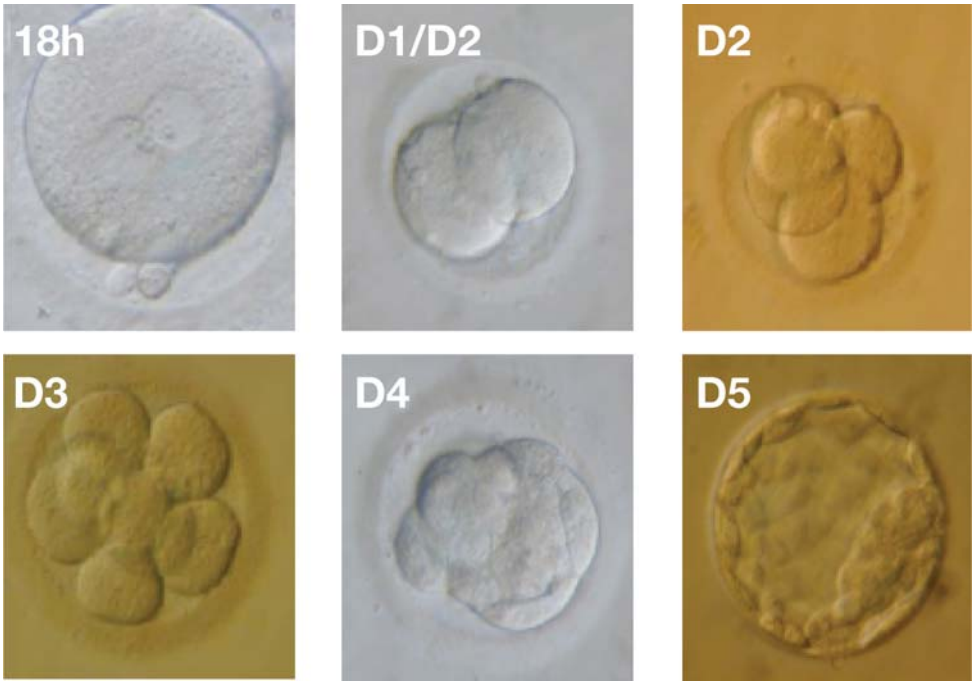

Figure 2 Images of *in vitro* embryonic development showing morphology changes from culture Day 0 to Day 5.

Preimplantation genetic screening (PGS) for aneuploidy can currently be performed in up to 15 chromosomes using fluorescence *in situ* hybridization (Munne, 2006). PGS requires removal and analysis of a single blastomere from a Day-3 embryo (Gianaroli *et al*., 1997; Wells and Delhanty, 2000). Embryos with a normal genetic constitution are subsequently selected for transfer and those with an abnormal number of chromosomes are discarded (Mastenbroek *et al*., 2007).

The success of PGS depends on the assumption that the chromosomal constitution of a single blastomere is representative of the entire embryo. However, mosaicism is now known to occur frequently in early cleavage-stage embryos (Baart *et al*., 2006; Coulam *et al*., 2007; Frumkin *et al*., 2008). The chromosomal constitution of a preimplantation embryo may evolve during early cleavages, allowing some mosaic embryos to develop normally (Baart *et al*., 2006; Frumkin *et al*., 2008). Self-corrective mechanisms may include the growth advantage of normal cells within a mosaic embryo (Bielanska *et al*., 2002) or, in cases of trisomy, the active loss of the additional chromosome (Munne *et al*., 2005; Frumkin *et al*., 2008).

Despite the promising results of early observational studies (Gianaroli *et al*., 1999; Munne *et al*., 1999; Munne *et al*., 2003; Montag *et al*., 2004), recent randomized controlled trials have shown no improvement in pregnancy outcomes after PGS (Staessen *et al*., 2004; Twisk *et al*., 2006; Mastenbroek *et al*., 2007; Hardarson *et al*., 2008; Staessen *et al*., 2008; Fauser, 2008). Indeed, the use of PGS may even reduce resulting pregnancy and live birth rates for couples with advanced maternal age (Mastenbroek *et al*., 2007; Hardarson *et al*., 2008; Fauser, 2008).

In short, although evidence is conflicting (Goossens *et al*., 2008), there is no good-quality evidence to support a benefit of routine PGS in the prediction of pregnancy outcomes (Staessen *et al*., 2004; Baart *et al*., 2006; Twisk *et al*., 2006; Coulam *et al*., 2007). Thus, current methods of PGS for aneuploidy have no place in current ART treatment protocols (Twisk *et al*., 2006; Mastenbroek *et al*., 2007; Fauser, 2008; Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine, 2008b).

Cryopreservation techniques

Cryopreservation of gametes, embryos and blastocysts is an essential component of modern ART (Youssry *et al*., 2008). Successful cryopreservation maximizes cumulative pregnancy rates per oocyte retrieval by storing unused embryos for future use (Youssry *et al*., 2008) and allows delayed embryo transfer during a natural menstrual cycle (Loutradi *et al*., 2008). Two cryopreservation techniques are available: the conventional slow cooling method and the recently introduced rapid procedure known as 'vitrification' (Youssry *et al*., 2008).

Vitrification results in significantly improved survival of frozen–thawed cleavage stage blastocysts and pregnancy rates, compared with standard cryopreservation (Fig. 3) (Loutradi *et al*., 2008; Youssry *et al*., 2008). However, there are concerns regarding the high concentrations of potentially cytotoxic cryoprotectants used during the vitrification process (Loutradi *et al*., 2008).

Chromosomal abnormalities have been reported in rapidly frozen mouse embryos (Shaw *et al*., 1991). Thus, there is a potential risk of serious malformations in human embryos following cryopreservation. Although no adverse effects of the transfer of cryopreserved embryos on IVF outcomes, including malformations, were shown in two European registry studies (Kallen *et al*., 2005; Pinborg *et al*., 2008), more high-quality studies are required to assess long-term

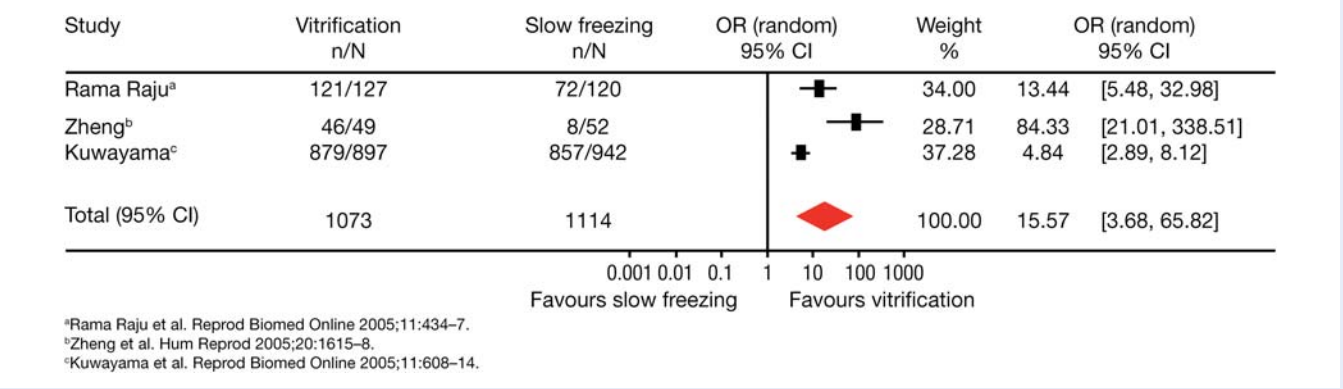

Figure 3 The odds of post-thawing survival rate of cleavage-stage embryos after vitrification or slow freezing.

CI, confidence interval; OR, odds ratio. Total events: 1064 (vitrification), 937 (slow freezing). Test for heterogeneity: $\chi^2 = 15.94$, df = 2 ($P = 0.001$). Test for overall effect: $Z = 3.73$ ($P = 0.0002$). Reprinted from Loutradi *et al.* (2008) with permission from Elsevier.

safety, given the recent and rapid development of cryopreservation techniques (Shaw *et al.*, 1991).

Frozen–thawed embryo or blastocyst transfer schedule

Transfer of frozen–thawed embryos or blastocysts can be performed during spontaneous ovulatory cycles, cycles of ovulation induction, cycles in which the endometrium is artificially prepared using estrogen and progesterone, and all with or without the use of a GnRH agonist (Ghobara and Vandekerckhove, 2008). Despite these numerous options, a recent Cochrane review found insufficient evidence to support the use of a particular transfer schedule (Ghobara and Vandekerckhove, 2008).

Laboratory standards and procedures

High laboratory standards and adherence to correct procedures are critical to the outcome of ART. Unfortunately, there is currently little good-quality evidence to support the use of specific ART-related laboratory processes, including culture strategy and selection of blastocysts for transfer. Furthermore, there is considerable variation in quality and outcomes between IVF clinics and laboratories. This is reflected in the considerable variation in cycle outcomes recorded at individual UK clinics despite a country-wide policy of a maximum of two embryos transferred (Human Fertilization and Embryology Authority, 2008).

The observed inter-centre variability hampers agreement on, and uptake of, best clinical and laboratory practices. In an attempt to standardize IVF laboratory techniques, the ESHRE has developed guidelines for good practice that require robust quality-management programmes for each ART centre (Magli *et al.*, 2008). Well-powered studies to investigate ART-related laboratory practices and monitor adherence to the recently published guidelines are warranted, and we encourage professional bodies to promote such research efforts.

Single embryo transfer

Despite recommendations to limit the number of embryos transferred (The ESHRE Capri Workshop Group, 2000; Land and Evers, 2003; Wright *et al.*, 2003; Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine, 2008a), at least 20–30% of IVF-related pregnancies are twin or higher-order multiple gestations (Reddy *et al.*, 2007; Andersen *et al.*, 2008). Furthermore, approximately half of all babies born from IVF globally are from multiple pregnancies (Adamson *et al.*, 2006).

Multiple pregnancy and birth is associated with a high incidence of maternal and neonatal complications (Pandian *et al.*, 2004; Wood, 2008) and, thus, considerable fetal morbidity and costs (ESHRE Campus Course Report, 2001; Land and Evers, 2003). Indeed, whenever possible, elective single embryo transfer (eSET) is recommended for young patients (aged <35 years) (Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine, 2008a).

Randomized controlled trials have clearly shown that when only fresh transfer cycles are evaluated, double embryo transfer (DET) leads to higher pregnancy and live birth rates than eSET (Gerris *et al.*, 1999; Martikainen *et al.*, 2001; Thurin *et al.*, 2004; Lukassen *et al.*, 2005; van Montfoort *et al.*, 2006). However, comparable rates of cumulative clinical pregnancy (52.6% versus 47.9%; $P = 0.24$) and live births (42.9% versus 38.8%; $P = 0.30$) have been demonstrated following DET or eSET, respectively, when a single frozen–thawed embryo transfer is also considered as part of the same stimulation cycle (Thurin *et al.*, 2004). Unsurprisingly, a recent Cochrane meta-analysis demonstrated a significantly lower multiple pregnancy rate in women who underwent SET than DET (OR 9.97, 95% CI 2.61–38.19; $P = 0.0008$) (Pandian *et al.*, 2004). Health economic data also support the superior cost-efficacy of SET over DET when the number of deliveries with at least one live-born child and all complications are considered (Kjellberg *et al.*, 2006; Polinder *et al.*, 2008; Wood, 2008).

The group proposed that an acceptable rate of ART-related multiple pregnancies would be up to 10%. However, the worldwide

incidence of ART-related multiple births remains considerably higher (Reddy *et al*., 2007; Andersen *et al*., 2008). The observed resistance to universal uptake of eSET in some societies is related to its perceived low efficacy; factors including advanced maternal age and few or poor-quality oocytes retrieved are cited frequently to justify the transfer of multiple embryos (ESHRE Campus Course Report, 2001; van Montfoort *et al*., 2006; Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine, 2008a). This is confounded by insufficient awareness of the risks and costs associated with multiple pregnancy among the general public and policy makers, the inability to select the best-quality embryo, suboptimal cryopreservation processes, constrictive cryopreservation legislation, the costs related to repeated treatment cycles (particularly in low- and middle-income countries) and competition between ART centres based on pregnancy/birth rates per cycle (Serour *et al*., 1991; ESHRE Campus Course Report, 2001; Adashi *et al*., 2003; Fauser *et al*., 2005; Karlstrom and Bergh, 2007).

Evaluation of ART treatment outcomes

The single most relevant measure of success in ART is a controversial and heavily debated topic (Land and Evers, 2003; Dickey *et al*., 2004; Griesinger *et al*., 2004; Heijnen *et al*., 2004; Pinborg *et al*., 2004). Previously proposed criteria to evaluate successful ART treatment outcomes include live birth rate per ovarian stimulation started (Griesinger *et al*., 2004), healthy live birth rate per treatment cycle (Dickey *et al*., 2004), singleton and multiple live birth rates per started treatment cycle (Vayena *et al*., 2001) or term live birth per started treatment strategy, which may include multiple cycles (Heijnen *et al*., 2004). Because of the lack of a consistent definition of ART success, national criteria for evaluation of treatment outcomes tend to reflect the local economic and legal frameworks.

The Third EVAR Workshop Group concurred with the ESHRE recommendations and believes that the birth of a single healthy child should be the aim of ART (Land and Evers, 2003). Therefore, the group advocated the use of singleton delivery rates as the gold standard expression of ART treatment outcome. Nonetheless, cumulative delivery rates per retrieved oocyte cohort using fresh and frozen embryos, or per treatment strategy, must also be considered. The group also believed that all comparisons of treatment modalities must incorporate efficacy and safety data, in addition to health-economic evaluations.

The future of ART

As described, the Third EVAR Workshop Group believes that optimization of singleton delivery rates should be the common aim of all ART clinicians. Reproductive medicine specialists have a responsibility to educate policymakers and the wider society on the risks of multiple pregnancies and births. Furthermore, the group calls on national and international professional bodies to issue guidelines promoting a responsible attitude to eSET, and to help raise awareness of the greater cost-efficacy of SET compared with DET among healthcare providers and policymakers.

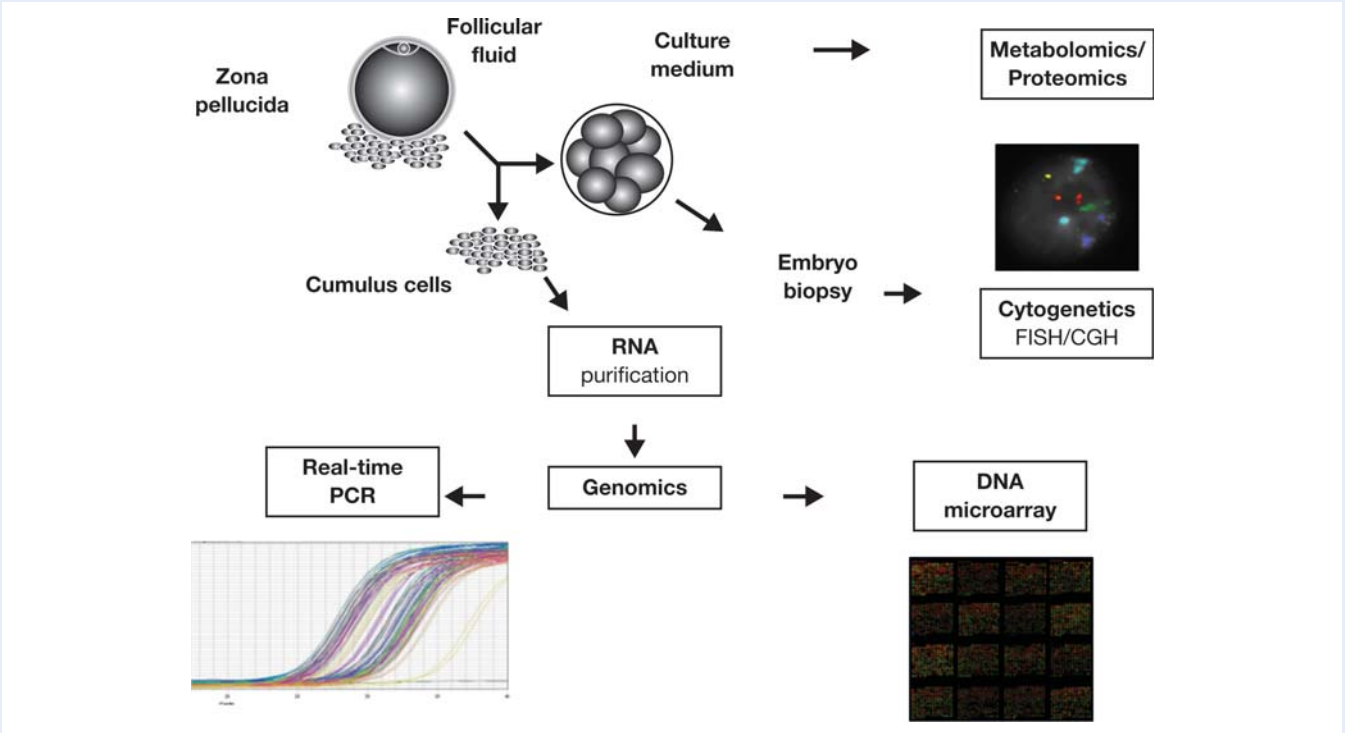

Figure 4 New techniques for assessment of oocyte and embryo quality.
CGH, comparative genomic hybridization; FISH, fluorescence *in situ* hybridization.

Future refinements in cryopreservation and vitrification techniques are expected to have a huge impact on ovarian stimulation protocols and increase the acceptance and uptake of SET around the world. Improvements in cryopreservation outcomes may also encourage the use of thawed embryos in subsequent natural cycles. This process may help to avoid the consequences of gonadotrophin stimulation on endometrium and late-onset OHSS.

New techniques in assessing oocyte and embryo quality could also improve pregnancy and delivery rates per embryo transfer, thus encouraging greater uptake of SET (Fig. 4). This may be achieved by studying oocyte zona birefringence (Montag *et al*., 2008), gene expression profiling of oocyte cumulus cells (McKenzie *et al*., 2004; Assou *et al*., 2006; Feuerstein *et al*., 2007), evaluation of spent culture fluid by proteomic analysis (Katz-Jaffe *et al*., 2006) or metabolic profiling using near-infrared spectroscopy (McKenzie *et al*., 2004; Vergouw *et al*., 2008). Good-quality studies of these techniques will help evaluate their potential clinical application and contribute to our understanding of basic oocyte and embryo biology, and the effects of iatrogenic ovarian stimulation.

The use of GnRH agonists to trigger final oocyte maturation during GnRH antagonist cycles is another promising approach to ART (Griesinger *et al*., 2007a). Results of a small, observational, proof-of-concept study suggest that GnRH agonist triggering (in combination with elective cryopreservation of two pronucleate oocytes) leads to acceptable cumulative pregnancy rates while reducing the risk of moderate-to-severe OHSS. Further investigation and validation of these new ART treatment strategies in prospective studies are eagerly awaited. Accurate identification of (genetic) markers of ovarian response to gonadotrophin stimulation would strengthen the development of predictive models of response and may enable the use of truly individualized ART stimulation treatment protocols (Fauser *et al*., 2008).

Conclusions

In conclusion, members of the Third EVAR Workshop Group agreed that SET should be the primary aim of many ART treatment cycles and supported the use of singleton live birth rate as the reported outcome measure from clinical trials and routine practice. However, the group acknowledged that improved cryopreservation techniques are required to further increase the global uptake of SET. Within this standard ART framework, adaptation and personalization of therapy may help to optimize efficacy and safety outcomes for individual patients.

Management decisions, including expectant therapy versus IUI, IVF or ICSI, reflect a rudimentary individualization of therapy but are currently based on limited available evidence. Greater quality control and standardization of clinical and laboratory evaluations are needed to optimize ART practices and improve individual patient outcomes. Furthermore, additional well-designed, good-quality studies are required to drive improvements in the diagnosis and management of ART processes in future years.

Acknowledgements

The authors thank Hannah Wills of Caudex Medical (supported by Merck Serono S.A.—Geneva) for her assistance in the preparation of this manuscript.

Funding

The workshop and preparation of the manuscript were sponsored by an unrestricted educational grant from Merck Serono S.A.—Geneva. P.D. has received grant support from Ferring, Merck Serono S.A., Organon (now part of Schering-Plough) and Besins. B.C.J.M.F. has received fees and grant support from the following companies: Andromed, Ardana, Ferring, Genovum, Merck Serono S.A.—Geneva, Organon (now part of Schering-Plough), Pantarhei Bioscience, PregLem, Schering, Schering-Plough, Serono (now Merck Serono S.A.—Geneva) and Wyeth.

References

Aboulghar MA, Mansour RT. Ovarian hyperstimulation syndrome: classifications and critical analysis of preventive measures. *Hum Reprod Update* 2003;**9**:275–289.

Aboulghar MA, Mansour RT, Serour GI, Amin YM, Kamal A. Prospective controlled randomized study of in vitro fertilization versus intracytoplasmic sperm injection in the treatment of tubal factor infertility with normal semen parameters. *Fertil Steril* 1996; **66**:753–756.

Adamson GD, de Mouzon J, Lancaster P, Nygren KG, Sullivan E, Zegers-Hochschild F. World collaborative report on in vitro fertilization, 2000. *Fertil Steril* 2006;**85**:1586–1622.

Adashi EY, Barri PN, Berkowitz R, Braude P, Bryan E, Carr J, Cohen J, Collins J, Devroey P, Frydman R *et al.* Infertility therapy-associated multiple pregnancies (births): an ongoing epidemic. *Reprod Biomed Online* 2003;**7**:515–542.

Al-Inany HG, Abou-Setta AM, Aboulghar M. Gonadotrophin-releasing hormone antagonists for assisted conception. *Cochrane Database Syst Rev* 2006;**3**:CD001750.

Alviggi C, Mollo A, Clarizia R, De Placido G. Exploiting LH in ovarian stimulation. *Reprod Biomed Online* 2006;**12**:221–233.

Andersen AN, Goossens V, Ferraretti AP, Bhattacharya S, Felberbaum R, de Mouzon J, Nygren KG. Assisted reproductive technology in Europe, 2004: results generated from European registers by ESHRE. *Hum Reprod* 2008;**23**:756–771.

Assou S, Anahory T, Pantesco V, Le Carrour T, Pellestor F, Klein B, Reyftmann L, Dechaud H, De Vos J, Hamamah S. The human cumulus–oocyte complex gene-expression profile. *Hum Reprod* 2006; **21**:1705–1719.

Baart EB, Martini E, van den Berg I, Macklon NS, Galjaard RJ, Fauser BC, Van Opstal D. Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. *Hum Reprod* 2006;**21**:223–233.

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007;**22**:980–988.

Balasch J. Investigation of the infertile couple: investigation of the infertile couple in the era of assisted reproductive technology: a time for reappraisal. *Hum Reprod* 2000;**15**:2251–2257.

Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertil Steril* 2002;**77**:328–336.

Bensdorp AJ, Cohlen BJ, Heineman MJ, Vandekerckhove P. Intra-uterine insemination for male subfertility. *Cochrane Database Syst Rev* 2007; CD000360.

Beurskens MP, Maas JW, Evers JL. Subfertility in South Limburg: calculation of incidence and appeal for specialist care. *Ned Tijdschr Geneeskd* 1995; **139**:235–238.

Bhattacharya S, Hamilton MP, Shaaban M, Khalaf Y, Seddler M, Ghobara T, Braude P, Kennedy R, Rutherford A, Hartshorne G *et al.* Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. *Lancet* 2001;**357**:2075–2079.

Bielanska M, Tan SL, Ao A. Chromosomal mosaicism throughout human preimplantation development in vitro: incidence, type, and relevance to embryo outcome. *Hum Reprod* 2002;**17**:413–419.

Bjorndahl L, Barratt CL, Fraser LR, Kvist U, Mortimer D. ESHRE basic semen analysis courses 1995–1999: immediate beneficial effects of standardized training. *Hum Reprod* 2002;**17**:1299–1305.

Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2007;CD002118.

Bozdag G, Aksan G, Esinler I, Yarali H. What is the role of office hysteroscopy in women with failed IVF cycles? *Reprod Biomed Online* 2008;**17**:410–415.

Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006;**12**:685–718.

Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. *Fertil Steril* 2009;**91**:705–714.

Brown JB. Pituitary control of ovarian function—concepts derived from gonadotrophin therapy. *Aust N Z J Obstet Gynaecol* 1978;**18**:46–54.

Bukulmez O, Yarali H, Yucel A, Sari T, Gurgan T. Intracytoplasmic sperm injection versus in vitro fertilization for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. *Fertil Steril* 2000;**73**:38–42.

Check JH, Choe JK, Katsoff D, Summers-Chase D, Wilson C. Controlled ovarian hyperstimulation adversely affects implantation following in vitro fertilization-embryo transfer. *J Assist Reprod Genet* 1999;**16**:416–420.

Collins JA, Burrows EA, Wilan AR. The prognosis for live birth among untreated infertile couples. *Fertil Steril* 1995;**64**:22–28.

Comhaire FH. Simple model and empirical method for the estimation of spontaneous pregnancies in couples consulting for infertility. *Int J Androl* 1987;**10**:671–680.

Comhaire FH. Clinical andrology: from evidence-base to ethics. The 'E' quintet in clinical andrology. *Hum Reprod* 2000;**15**:2067–2071.

Coulam CB, Jeyendran RS, Fiddler M, Pergament E. Discordance among blastomeres renders preimplantation genetic diagnosis for aneuploidy ineffective. *J Assist Reprod Genet* 2007;**24**:37–41.

Crosignani PG, Rubin BL. Optimal use of infertility diagnostic tests and treatments. The ESHRE Capri Workshop Group. *Hum Reprod* 2000; **15**:723–732.

Crosignani PG, Walters DE. Clinical pregnancy and male subfertility; the ESHRE multicentre trial on the treatment of male subfertility. European Society of Human Reproduction and Embryology. *Hum Reprod* 1994;**9**:1112–1118.

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

D'Angelo A, Amso N. Embryo freezing for preventing ovarian hyperstimulation syndrome. *Cochrane Database Syst Rev* 2007;CD002806.

De Placido G, Mollo A, Alviggi C, Strina I, Varricchio MT, Ranieri A, Colacurci N, Tolino A, Wilding M. Rescue of IVF cycles by HMG in pituitary down-regulated normogonadotrophic young women characterized by a poor initial response to recombinant FSH. *Hum Reprod* 2001;**16**:1875–1879.

De Placido G, Alviggi C, Mollo A, Strina I, Ranieri A, Alviggi E, Wilding M, Varricchio MT, Borrelli AL, Conforti S. Effects of recombinant LH (rLH) supplementation during controlled ovarian hyperstimulation (COH) in normogonadotrophic women with an initial inadequate response to recombinant FSH (rFSH) after pituitary downregulation. *Clin Endocrinol (Oxf)* 2004;**60**:637–643.

De Placido G, Alviggi C, Perino A, Strina I, Lisi F, Fasolino A, De Palo R, Ranieri A, Colacurci N, Mollo A. Recombinant human LH supplementation versus recombinant human FSH (rFSH) step-up protocol during controlled ovarian stimulation in normogonadotrophic women with initial inadequate ovarian response to rFSH. A multicentre, prospective, randomized controlled trial. *Hum Reprod* 2005;**20**:390–396.

de Sa Rosa e de Silva A, Rosa e Silva J, Candido dos Reis FJ, Nogueira AA, Ferriani RA. Routine office hysteroscopy in the investigation of infertile couples before assisted reproduction. *J Reprod Med* 2005;**50**:501–506.

den Hartog JE, Lardenoije CM, Severens JL, Land JA, Evers JL, Kessels AG. Screening strategies for tubal factor subfertility. *Hum Reprod* 2008; **23**:1840–1848.

Deutch TD, Abuhamad AZ. The role of 3-dimensional ultrasonography and magnetic resonance imaging in the diagnosis of mullerian duct anomalies: a review of the literature. *J Ultrasound Med* 2008;**27**:413–423.

Dickey RP, Sartor BM, Pyrzak R. What is the most relevant standard of success in assisted reproduction? No single outcome measure is satisfactory when evaluating success in assisted reproduction; both twin births and singleton births should be counted as successes. *Hum Reprod* 2004;**19**:783–787.

Doldi N, Persico P, Di Sebastiano F, Marsiglio E, De Santis L, Rabellotti E, Fusi F, Brigante C, Ferrari A. Pathologic findings in hysteroscopy before in vitro fertilization-embryo transfer (IVF-ET). *Gynecol Endocrinol* 2005; **21**:235–237.

Durnerin CI, Erb K, Fleming R, Hillier H, Hillier SG, Howles CM, Hugues JN, Lass A, Lyall H, Rasmussen P *et al.* Effects of recombinant LH treatment on folliculogenesis and responsiveness to FSH stimulation. *Hum Reprod* 2008;**23**:421–426.

Eimers JM, te Velde ER, Gerritse R, Vogelzang ET, Looman CW, Habbema JD. The prediction of the chance to conceive in subfertile couples. *Fertil Steril* 1994;**61**:44–52.

Ekerhovd E, Fried G, Granberg S. An ultrasound-based approach to the assessment of infertility, including the evaluation of tubal patency. *Best Pract Res Clin Obstet Gynaecol* 2004;**18**:13–28.

Eldar-Geva T, Meagher S, Healy DL, Maclachlan V, Breheny S, Wood C. Effect of intramural, subserosal, and submucosal uterine fibroids on the outcome of assisted reproductive technology treatment. *Fertil Steril* 1998;**70**:687–691.

Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril* 2008;**89**:84–91.

ESHRE Campus Course Report. Prevention of twin pregnancies after IVF/ICSI by single embryo transfer. *Hum Reprod* 2001;**16**:790–800.

Evers JL. Female subfertility. *Lancet* 2002;**360**:151–159.

Farhi J, Ashkenazi J, Feldberg D, Dicker D, Orvieto R, Ben RZ. Effect of uterine leiomyomata on the results of in-vitro fertilization treatment. *Hum Reprod* 1995;**10**:2576–2578.

Fauser BC. Preimplantation genetic screening: the end of an affair? *Hum Reprod* 2008;**23**:2622–2625.

Fauser BC, Van Heusden AM. Manipulation of human ovarian function: physiological concepts and clinical consequences. *Endocr Rev* 1997; **18**:71–106.

Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. *Lancet* 2005;**365**:1807–1816.

Fauser BC, Diedrich K, Devroey P. Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. *Hum Reprod Update* 2008;**14**:1–14.

Feuerstein P, Cadoret V, Dalbies-Tran R, Guerif F, Bidault R, Royere D. Gene expression in human cumulus cells: one approach to oocyte competence. *Hum Reprod* 2007;**22**:3069–3077.

Foong SC, Fleetham JA, O'Keane JA, Scott SG, Tough SC, Greene CA. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. *J Assist Reprod Genet* 2006;**23**:137–140.

Frattarelli JL, Levi AJ, Miller BT, Segars JH. A prospective assessment of the predictive value of basal antral follicles in in vitro fertilization cycles. *Fertil Steril* 2003;**80**:350–355.

Frumkin T, Malcov M, Yaron Y, Ben-Yosef D. Elucidating the origin of chromosomal aberrations in IVF embryos by preimplantation genetic analysis. *Mol Cell Endocrinol* 2008;**282**:112–119.

Garcia-Velasco JA, Somigliana E. Management of endometriomas in women requiring IVF: to touch or not to touch. *Hum Reprod* 2009; **24**:496–501.

Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M. Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: a prospective randomized clinical trial. *Hum Reprod* 1999;**14**:2581–2587.

Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* 2008;CD003414.

Gianaroli L, Magli MC, Munne S, Fiorentino A, Montanaro N, Ferraretti AP. Will preimplantation genetic diagnosis assist patients with a poor prognosis to achieve pregnancy? *Hum Reprod* 1997;**12**:1762–1767.

Gianaroli L, Magli MC, Ferraretti AP, Munne S. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. *Fertil Steril* 1999;**72**:837–844.

Glazener CM, Ford WC, Hull MG. The prognostic power of the post-coital test for natural conception depends on duration of infertility. *Hum Reprod* 2000;**15**:1953–1957.

Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, Van Steirteghem A, Bertrand C, Liebaers I, Devroey P *et al.* Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. *Hum Reprod* 2008;**23**:481–492.

Gougeon A. Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocr Rev* 1996;**17**:121–155.

Goverde AJ, McDonnell J, Vermeiden JP, Schats R, Rutten FF, Schoemaker J. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: a randomised trial and cost-effectiveness analysis. *Lancet* 2000;**355**:13–18.

Goverde AJ, Lambalk CB, McDonnell J, Schats R, Homburg R, Vermeiden JP. Further considerations on natural or mild hyperstimulation cycles for intrauterine insemination treatment: effects on pregnancy and multiple pregnancy rates. *Hum Reprod* 2005;**20**:3141–3146.

Greenhall E, Vessey M. The prevalence of subfertility: a review of the current confusion and a report of two new studies. *Fertil Steril* 1990; **54**:978–983.

Griesinger G, Dafopoulos K, Schultze-Mosgau A, Felberbaum R, Diedrich K. What is the most relevant standard of success in assisted reproduction? Is BESST (birth emphasizing a successful singleton at term) truly the best? *Hum Reprod* 2004;**19**:1239–1241.

Griesinger G, Kolibianakis EM, Papanikolaou EG, Diedrich K, Van Steirteghem A, Devroey P, Ejdrup Bredkjaer H, Humaidan P. Triggering of final oocyte maturation with gonadotropin-releasing hormone agonist or human chorionic gonadotropin. Live birth after frozen-thawed embryo replacement cycles. *Fertil Steril* 2007a;**88**:616–621.

Griesinger G, von Otte S, Schroer A, Ludwig AK, Diedrich K, Al-Hasani S, Schultze-Mosgau A. Elective cryopreservation of all pronuclear oocytes after GnRH agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective, observational proof-of-concept study. *Hum Reprod* 2007b;**22**:1348–1352.

Gunnell DJ, Ewings P. Infertility prevalence, needs assessment and purchasing. *J Public Health Med* 1994;**16**:29–35.

Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA *et al.* Sperm morphology, motility, and concentration in fertile and infertile men. *N Engl J Med* 2001;**345**:1388–1393.

Hardarson T, Hanson C, Lundin K, Hillensjo T, Nilsson L, Stevic J, Reismer E, Borg K, Wikland M, Bergh C. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. *Hum Reprod* 2008;**23**:2806–2812.

Harrison RF, Jacob S, Spillane H, Mallon E, Hennelly B. A prospective randomized clinical trial of differing starter doses of recombinant follicle-stimulating hormone (follitropin-beta) for first time in vitro fertilization and intracytoplasmic sperm injection treatment cycles. *Fertil Steril* 2001;**75**:23–31.

Heijnen EM, Macklon NS, Fauser BC. What is the most relevant standard of success in assisted reproduction? The next step to improving outcomes of IVF: consider the whole treatment. *Hum Reprod* 2004;**19**:1936–1938.

Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, Broekmans FJ, Passchier J, te Velde ER, Macklon NS *et al.* A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. *Lancet* 2007;**369**:743–749.

Hendriks DJ, Mol BW, Bancsi LF, te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril* 2005;**83**:291–301.

Hendriks DJ, Kwee J, Mol BW, te Velde ER, Broekmans FJ. Ultrasonography as a tool for the prediction of outcome in IVF patients: a comparative meta-analysis of ovarian volume and antral follicle count. *Fertil Steril* 2007;**87**:764–775.

Hendriks DJ, te Velde ER, Looman CWN, Bancsi LFJMM, Broekmans FJM. Expected poor response in predicting cumulative ongoing pregnancy rates: a powerful tool. *Reprod Biomed Online* 2008;**17**:727–736.

Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, Bergh T. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. *Hum Reprod* 2007;**22**:548–557.

Hoomans EH, Andersen AN, Loft A, Leerentveld RA, van Kamp AA, Zech H. A prospective, randomized clinical trial comparing 150 IU recombinant follicle stimulating hormone (Puregon(R)) and 225 IU highly purified urinary follicle stimulating hormone (Metrodin-HP(R)) in a fixed-dose regimen in women undergoing ovarian stimulation. *Hum Reprod* 1999;**14**:2442–2447.

Howles CM, Saunders H, Alam V, Engrand P. Predictive factors and a corresponding treatment algorithm for controlled ovarian stimulation in patients treated with recombinant human follicle stimulating hormone (follitropin alfa) during assisted reproduction technology (ART) procedures. An analysis of 1378 patients. *Curr Med Res Opin* 2006;**22**:907–918.

Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. *Br Med J (Clin Res Ed)* 1985;**291**:1693–1697.

Human Fertilization and Embryology Authority. *Live Birth Rate (per Cycle Started)—2005 Data*. http://guide.hfea.gov.uk/guide/Default.aspx (15 September 2008, date last accessed).

Hunault CC, Habbema JD, Eijkemans MJ, Collins JA, Evers JL, te Velde ER. Two new prediction rules for spontaneous pregnancy leading to live birth among subfertile couples, based on the synthesis of three previous models. *Hum Reprod* 2004;**19**:2019–2026.

Hunault CC, Laven JS, van Rooij I, Eijkemans MJ, te Velde ER, Habbema JD. Prospective validation of two models predicting pregnancy leading to live birth among untreated subfertile couples. *Hum Reprod* 2005;**20**:1636–1641.

Imani B, Eijkemans MJ, Faessen GH, Bouchard P, Giudice LC, Fauser BC. Prediction of the individual follicle-stimulating hormone threshold for gonadotropin induction of ovulation in normogonadotropic anovulatory infertility: an approach to increase safety and efficiency. *Fertil Steril* 2002;**77**:83–90.

Isikoglu M, Berkkanoglu M, Senturk Z, Coetzee K, Ozgur K. Endometrial polyps smaller than 1.5 cm do not affect ICSI outcome. *Reprod Biomed Online* 2006;**12**:199–204.

Jaroudi K, Al-Hassan S, Al-Sufayan H, Al-Mayman H, Qeba M, Coskun S. Intracytoplasmic sperm injection and conventional in vitro fertilization are complementary techniques in management of unexplained infertility. *J Assist Reprod Genet* 2003;**20**:377–381.

Johnson NP, Mak W, Sowter MC. Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. *Cochrane Database Syst Rev* 2004;CD002125.

Kailasam C, Keay SD, Wilson P, Ford WC, Jenkins JM. Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. *Hum Reprod* 2004;**19**:1544–1547.

Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: risk for congenital malformations after different IVF methods. *Birth Defects Res A Clin Mol Teratol* 2005;**73**:162–169.

Karlstrom PO, Bergh C. Reducing the number of embryos transferred in Sweden-impact on delivery and multiple birth rates. *Hum Reprod* 2007;**22**:2202–2207.

Katz-Jaffe MG, Trounson AO, Cram DS. Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. *Fertil Steril* 2005;**84**:634–643.

Katz-Jaffe MG, Schoolcraft WB, Gardner DK. Analysis of protein expression (secretome) by human and mouse preimplantation embryos. *Fertil Steril* 2006;**86**:678–685.

Keel BA, Stembridge TW, Pineda G, Serafy NT Sr. Lack of standardization in performance of the semen analysis among laboratories in the United States. *Fertil Steril* 2002;**78**:603–608.

Kim MR, Kim YA, Jo MY, Hwang KJ, Ryu HS. High frequency of endometrial polyps in endometriosis. *J Am Assoc Gynecol Laparosc* 2003;**10**:46–48.

Kjellberg AT, Carlsson P, Bergh C. Randomized single versus double embryo transfer: obstetric and paediatric outcome and a cost-effectiveness analysis. *Hum Reprod* 2006;**21**:210–216.

Klinkert ER, Broekmans FJ, Looman CW, te Velde ER. A poor response in the first in vitro fertilization cycle is not necessarily related to a poor prognosis in subsequent cycles. *Fertil Steril* 2004;**81**:1247–1253.

Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. *Hum Reprod* 2005; **20**:611–615.

Kok JD, Looman CW, Weima SM, te Velde ER. A high number of oocytes obtained after ovarian hyperstimulation for in vitro fertilization or intracytoplasmic sperm injection is not associated with decreased pregnancy outcome. *Fertil Steril* 2006;**85**:918–924.

Kolibianakis EM, Collins J, Tarlatzis BC, Devroey P, Diedrich K, Griesinger G. Among patients treated for IVF with gonadotrophins and GnRH analogues, is the probability of live birth dependent on the type of analogue used? A systematic review and meta-analysis. *Hum Reprod Update* 2006;**12**:651–671.

Kvist U, Bjorndahl L. *Manual on Basic Semen Analysis. ESHRE Monographs (2)*. Oxford, UK: Oxford University Press, 2002.

Land JA, Evers JL. Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. *Hum Reprod* 2003;**18**:455–457.

Lass A, Williams G, Abusheikha N, Brinsden P. The effect of endometrial polyps on outcomes of in vitro fertilization (IVF) cycles. *J Assist Reprod Genet* 1999;**16**:410–415.

Latin-American Puregon IVF Study Group. A double-blind clinical trial comparing a fixed daily dose of 150 and 250 IU of recombinant follicle-stimulating hormone in women undergoing in vitro fertilization. *Fertil Steril* 2001;**76**:950–956.

Lefievre L, Bedu-Addo K, Conner SJ, Machado-Oliveira GS, Chen Y, Kirkman-Brown JC, Afnan MA, Publicover SJ, Ford WC, Barratt CL. Counting sperm does not add up any more: time for a new equation? *Reproduction* 2007;**133**:675–684.

Lemmen JG, Agerholm I, Ziebe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes. *Reprod Biomed Online* 2008;**17**:385–391.

Levi AJ, Raynault MF, Bergh PA, Drews MR, Miller BT, Scott RT Jr. Reproductive outcome in patients with diminished ovarian reserve. *Fertil Steril* 2001;**76**:666–669.

Levron J, Willadsen S, Bertoli M, Cohen J. The development of mouse zygotes after fusion with synchronous and asynchronous cytoplasm. *Hum Reprod* 1996;**11**:1287–1292.

Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, Tarlatzis BC. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertil Steril* 2008;**90**:186–193.

Lukassen HG, Braat DD, Wetzels AM, Zielhuis GA, Adang EM, Scheenjes E, Kremer JA. Two cycles with single embryo transfer versus one cycle with double embryo transfer: a randomized controlled trial. *Hum Reprod* 2005;**20**:702–708.

Magli MC, Van den Abbeel E, Lundin K, Royere D, Van der Elst J, Gianaroli L. Revised guidelines for good practice in IVF laboratories. *Hum Reprod* 2008;**23**:1253–1262.

Male Infertility Best Practice Policy Committee of the American Urological Association and Practice Committee of the American Society for Reproductive Medicine. Report on optimal evaluation of the infertile male. *Fertil Steril* 2006;**86**:S202–S209.

Mansour R, Aboulghar M, Serour G, Amin Y, Abou-Setta AM. Criteria of a successful coasting protocol for the prevention of severe ovarian hyperstimulation syndrome. *Hum Reprod* 2005;**20**:3167–3172.

Marquez C, Sandalinas M, Bahce M, Alikani M, Munne S. Chromosome abnormalities in 1255 cleavage-stage human embryos. *Reprod Biomed Online* 2000;**1**:17–26.

Martikainen H, Tiitinen A, Tomas C, Tapanainen J, Orava M, Tuomivaara L, Vilska S, Hyden-Granskog C, Hovatta O. One versus two embryo transfer after IVF and ICSI: a randomized study. *Hum Reprod* 2001;**16**:1900–1903.

Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, Vogel NE, Arts EG, de Vries JW, Bossuyt PM *et al.* In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007;**357**:9–17.

Mathur R, Kailasam C, Jenkins J. Review of the evidence base of strategies to prevent ovarian hyperstimulation syndrome. *Hum Fertil (Camb)* 2007; **10**:75–85.

May-Panloup P, Chretien MF, Jacques C, Vasseur C, Malthiery Y, Reynier P. Low oocyte mitochondrial DNA content in ovarian insufficiency. *Hum Reprod* 2005;**20**:593–597.

McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, Amato P, Matzuk MM. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. *Hum Reprod* 2004;**19**:2869–2874.

Mochtar MH, van der Veen F, Ziech M, van Wely M. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. *Cochrane Database Syst Rev* 2007;CD005070.

Mol BW, Verhagen TE, Hendriks DJ, Collins JA, Coomarasamy A, Opmeer BC, Broekmans FJ. Value of ovarian reserve testing before IVF: a clinical decision analysis. *Hum Reprod* 2006;**21**:1816–1823.

Montag M, van der Ven K, Dorn C, van der Ven H. Outcome of laser-assisted polar body biopsy and aneuploidy testing. *Reprod Biomed Online* 2004;**9**:425–429.

Montag M, Schimming T, Koster M, Zhou C, Dorn C, Rosing B, van der Ven H, van der Ven K. Oocyte zona birefringence intensity is associated with embryonic implantation potential in ICSI cycles. *Reprod Biomed Online* 2008;**16**:239–244.

Munne S. Chromosome abnormalities and their relationship to morphology and development of human embryos. *Reprod Biomed Online* 2006;**12**:234–253.

Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;**64**:382–391.

Munne S, Magli C, Cohen J, Morton P, Sadowy S, Gianaroli L, Tucker M, Marquez C, Sable D, Ferraretti AP *et al.* Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Hum Reprod* 1999;**14**:2191–2199.

Munne S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, Cohen J, Sable D. Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reprod Biomed Online* 2003;**7**:91–97.

Munne S, Velilla E, Colls P, Garcia BM, Vemuri MC, Steuerwald N, Garrisi J, Cohen J. Self-correction of chromosomally abnormal embryos in culture and implications for stem cell production. *Fertil Steril* 2005;**84**:1328–1334.

Nardo LG, Cheema P, Gelbaya TA, Horne G, Fitzgerald CT, Pease EH, Brison DR, Lieberman BA. The optimal length of 'coasting protocol' in women at risk of ovarian hyperstimulation syndrome undergoing in vitro fertilization. *Hum Fertil (Camb)* 2006;**9**:175–180.

Nargund G, Hutchison L, Scaramuzzi R, Campbell S. Low-dose HCG is useful in preventing OHSS in high-risk women without adversely affecting the outcome of IVF cycles. *Reprod Biomed Online* 2007; **14**:682–685.

National Institute for Clinical Excellence. Clinical Guideline 11. *Fertility: Assessment and Treatment for People with Fertility Problems*. 2004. http://www.nice.org.uk/nicemedia/pdf/CG011niceguideline.pdf (February 2005, date last accessed).

Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles—implications for individualization of therapy. *Hum Reprod* 2007;**22**:2414–2421.

Okolo S. Incidence, aetiology and epidemiology of uterine fibroids. *Best Pract Res Clin Obstet Gynaecol* 2008;**22**:571–588.

Olivennes F, Howles CM, Borini A, Germond M, Trew G, Wikland M, Zegers-Hochschild F, Saunders H, Alam V. Individualizing FSH dose for assisted reproduction using a novel algorithm: the CONSORT study. *Reprod Biomed Online* 2009;**18**:195–204.

Ombelet W, Deblaere K, Bosmans E, Cox A, Jacobs P, Janssen M, Nijs M. Semen quality and intrauterine insemination. *Reprod Biomed Online* 2003; **7**:485–492.

Out HJ, David I, Ron-El R, Friedler S, Shalev E, Geslevich J, Dor J, Shulman A, Ben-Rafael Z, Fisch B *et al.* A randomized, double-blind clinical trial using fixed daily doses of 100 or 200 IU of recombinant FSH in ICSI cycles. *Hum Reprod* 2001;**16**:1104–1109.

Out HJ, Rutherford A, Fleming R, Tay CC, Trew G, Ledger W, Cahill D. A randomized, double-blind, multicentre clinical trial comparing starting doses of 150 and 200 IU of recombinant FSH in women treated with the GnRH antagonist ganirelix for assisted reproduction. *Hum Reprod* 2004;**19**:90–95.

Pandian Z, Bhattacharya S, Ozturk O, Serour GI, Templeton A. Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. *Cochrane Database Syst Rev* 2004;CD003416.

Pandian Z, Bhattacharya S, Vale L, Templeton A. In vitro fertilisation for unexplained subfertility. *Cochrane Database Syst Rev* 2005;CD003357.

Papanikolaou EG, Tournaye H, Verpoest W, Camus M, Vernaeve V, Van SA, Devroey P. Early and late ovarian hyperstimulation syndrome: early pregnancy outcome and profile. *Hum Reprod* 2005;**20**:636–641.

Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, Devroey P. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. *Hum Reprod* 2008;**23**:91–99.

Perez-Medina T, Bajo-Arenas J, Salazar F, Redondo T, Sanfrutos L, Alvarez P, Engels V. Endometrial polyps and their implication in the pregnancy rates of patients undergoing intrauterine insemination: a prospective, randomized study. *Hum Reprod* 2005;**20**:1632–1635.

Pinborg A, Loft A, Ziebe S, Nyboe AA. What is the most relevant standard of success in assisted reproduction? Is there a single 'parameter of excellence'? *Hum Reprod* 2004;**19**:1052–1054.

Pinborg A, Loft A, Rasmussen S, Nyboe Andersen A. Danish national controlled cohort study on neonatal outcome of 1267 children born after transfer of cryopreserved IVF and ICSI embryos in 1995 to 2006. *Hum Reprod* 2008;**23**:i51.

Polinder S, Heijnen EM, Macklon NS, Habbema JD, Fauser BJ, Eijkemans MJ. Cost-effectiveness of a mild compared with a standard strategy for IVF: a randomized comparison using cumulative term live birth as the primary endpoint. *Hum Reprod* 2008;**23**:316–323.

Popovic-Todorovic B, Loft A, Bredkjaeer HE, Bangsboll S, Nielsen IK, Andersen AN. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a 'standard' dose of 150 IU/day in 'standard' patients undergoing IVF/ICSI treatment. *Hum Reprod* 2003a;**18**:2275–2282.

Popovic-Todorovic B, Loft A, Lindhard A, Bangsboll S, Andersson AM, Andersen AN. A prospective study of predictive factors of ovarian response in 'standard' IVF/ICSI patients treated with recombinant FSH. A suggestion for a recombinant FSH dosage normogram. *Hum Reprod* 2003b;**18**:781–787.

Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine. Guidelines on number of embryos transferred. *Fertil Steril* 2008a; **90**:S163–S164.

Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril* 2008b;**90**:S136–S143.

Pritts EA. Fibroids and infertility: a systematic review of the evidence. *Obstet Gynecol Surv* 2001;**56**:483–491.

Ragni G, Diaferia D, Vegetti W, Colombo M, Arnoldi M, Crosignani PG. Effectiveness of sonohysterography in infertile patient work-up: a comparison with transvaginal ultrasonography and hysteroscopy. *Gynecol Obstet Invest* 2005;**59**:184–188.

Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive

summary of a National Institute of Child Health and Human Development workshop. *Obstet Gynecol* 2007;**109**:967–977.

Riddell D, Pacey A, Whittington K. Lack of compliance by UK andrology laboratories with World Health Organization recommendations for sperm morphology assessment. *Hum Reprod* 2005;**20**:3441–3445.

Rowe PJ, Comhaire FH, Hargreave TB, Mahmoud A. *WHO Manual for the Standardized Investigation of the Infertile Couple*. Cambridge, UK: Cambridge University Press, 1993.

Schoemaker J, van Weissenbruch MM, Scheele F, van der Meer M. The FSH threshold concept in clinical ovulation induction. *Baillière's Clin Obstet Gynaecol* 1993;**7**:297–308.

Serour GI, el Ghar M, Mansour RT. In vitro fertilization and embryo transfer in Egypt. *Int J Gynaecol Obstet* 1991;**36**:49–53.

Shamma FN, Lee G, Gutmann JN, Lavy G. The role of office hysteroscopy in in vitro fertilization. *Fertil Steril* 1992;**58**:1237–1239.

Shanbhag S, Aucott L, Bhattacharya S, Hamilton MA, McTavish AR. Interventions for 'poor responders' to controlled ovarian hyperstimulation (COH) in in-vitro fertilisation (IVF). *Cochrane Database Syst Rev* 2007;CD004379.

Shaw JM, Kola I, MacFarlane DR, Trounson AO. An association between chromosomal abnormalities in rapidly frozen 2-cell mouse embryos and the ice-forming properties of the cryoprotective solution. *J Reprod Fertil* 1991;**91**:9–18.

Shokeir TA, Shalan HM, El-Shafei MM. Significance of endometrial polyps detected hysteroscopically in eumenorrheic infertile women. *J Obstet Gynaecol Res* 2004;**30**:84–89.

Snick HK, Snick TS, Evers JL, Collins JA. The spontaneous pregnancy prognosis in untreated subfertile couples: the Walcheren primary care study. *Hum Reprod* 1997;**12**:1582–1588.

Somigliana E, Vercellini P, Vigano P, Ragni G, Crosignani PG. Should endometriomas be treated before IVF-ICSI cycles? *Hum Reprod Update* 2006;**12**:57–64.

Spiewankiewicz B, Stelmachow J, Sawicki W, Cendrowski K, Wypych P, Swiderska K. The effectiveness of hysteroscopic polypectomy in cases of female infertility. *Clin Exp Obstet Gynecol* 2003;**30**:23–25.

Staessen C, Camus M, Clasen K, De Vos A, Van Steirteghem A. Conventional in-vitro fertilization versus intracytoplasmic sperm injection in sibling oocytes from couples with tubal infertility and normozoospermic semen. *Hum Reprod* 1999;**14**:2474–2479.

Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, Devroey P, Liebaers I, Van Steirteghem A. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;**19**:2849–2858.

Staessen C, Verpoest W, Donoso P, Haentjens P, Van der Elst J, Liebaers I, Devroey P. Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer. *Hum Reprod* 2008;**23**:2818–2825.

Stamatellos I, Apostolides A, Stamatopoulos P, Bontis J. Pregnancy rates after hysteroscopic polypectomy depending on the size or number of the polyps. *Arch Gynecol Obstet* 2008;**277**:395–399.

Strandell A. Treatment of hydrosalpinx in the patient undergoing assisted reproduction. *Curr Opin Obstet Gynecol* 2007;**19**:360–365.

Strandell A, Lindhard A, Waldenstrom U, Thorburn J, Janson PO, Hamberger L. Hydrosalpinx and IVF outcome: a prospective, randomized multicentre trial in Scandinavia on salpingectomy prior to IVF. *Hum Reprod* 1999;**14**:2762–2769.

Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: a systematic review. *Hum Reprod Update* 2003;**9**:61–76.

te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update* 2002;**8**:141–154.

Templeton A, Morris JK, Parslow W. Factors that affect outcome of in-vitro fertilisation treatment. *Lancet* 1996;**348**:1402–1406.

The ESHRE Capri Workshop Group. Multiple gestation pregnancy. *Hum Reprod* 2000;**15**:1856–1864.

Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Fertil Steril* 2008;**89**:505–522.

Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, Bergh C. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. *N Engl J Med* 2004;**351**:2392–2402.

Tournaye H, Verheyen G, Albano C, Camus M, Van Landuyt L, Devroey P, Van Steirteghem A. Intracytoplasmic sperm injection versus in vitro fertilization: a randomized controlled trial and a meta-analysis of the literature. *Fertil Steril* 2002;**78**:1030–1037.

Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. *Cochrane Database Syst Rev* 2006;CD005291.

Valenzano MM, Mistrangelo E, Lijoi D, Fortunato T, Lantieri PB, Risso D, Costantini S, Ragni N. Transvaginal sonohysterographic evaluation of uterine malformations. *Eur J Obstet Gynecol Reprod Biol* 2006;**124**:246–249.

van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, Fauser BC, Macklon NS. Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online* 2006;**13**:476–480.

van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Van der Veen F, Bossuyt PM, Hompes PG, Mol BW. Should the post-coital test (PCT) be part of the routine fertility work-up? *Hum Reprod* 2004;**19**:1373–1379.

van Kooij RJ, Looman CW, Habbema JD, Dorland M, te Velde ER. Age-dependent decrease in embryo implantation rate after in vitro fertilization. *Fertil Steril* 1996;**66**:769–775.

van Montfoort AP, Fiddelers AA, Janssen JM, Derhaag JG, Dirksen CD, Dunselman GA, Land JA, Geraedts JP, Evers JL, Dumoulin JC. In unselected patients, elective single embryo transfer prevents all multiples, but results in significantly lower pregnancy rates compared with double embryo transfer: a randomized controlled trial. *Hum Reprod* 2006;**21**:338–343.

van Rooij I, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002;**17**:3065–3071.

van Rooij I, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril* 2005;**83**:979–987.

van Rumste MM, den Hartog JE, Dumoulin JC, Evers JL, Land JA. Is controlled ovarian stimulation in intrauterine insemination an acceptable therapy in couples with unexplained non-conception in the perspective of multiple pregnancies? *Hum Reprod* 2006;**21**:701–704.

van Rumste MM, Custers IM, Van der Veen F, van Wely M, Evers JL, Mol BW. The influence of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: a meta-analysis. *Hum Reprod Update* 2008;**14**:563–570.

Van Voorhis BJ. Outcomes from assisted reproductive technology. *Obstet Gynecol* 2006;**107**:183–200.

Van Voorhis BJ. Ultrasound assessment of the uterus and fallopian tube in infertile women. *Semin Reprod Med* 2008;**26**:232–240.

Van Waart J, Kruger TF, Lombard CJ, Ombelet W. Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. *Hum Reprod Update* 2001;**7**:495–500.

Varasteh NN, Neuwirth RS, Levin B, Keltz MD. Pregnancy rates after hysteroscopic polypectomy and myomectomy in infertile women. *Obstet Gynecol* 1999;**94**:168–171.

Vayena E, Rowe PJ, Griffin PD. Recommendations. Medical, ethical and social aspects of assisted reproduction: current practices and controversies in assisted reproduction. *Report of a WHO meeting*. WHO publications, Geneva, Switzerland, 2001.

Verberg MF, Eijkemans MJ, Macklon NS, Heijnen EM, Baart EB, Hohmann FP, Fauser BC, Broekmans FJ. The clinical significance of the retrieval of a low number of oocytes following mild ovarian stimulation for IVF: a meta-analysis. *Hum Reprod Update* 2009a;**15**:5–12.

Verberg MF, Macklon NS, Nargund G, Frydman R, Devroey P, Broekmans FJ, Fauser BC. Mild ovarian stimulation for IVF. *Hum Reprod Update* 2009b;**15**:13–29.

Vergouw CG, Botros LL, Roos P, Lens JW, Schats R, Hompes PG, Burns DH, Lambalk CB. Metabolomic profiling by near-infrared spectroscopy as a tool to assess embryo viability: a novel, non-invasive method for embryo selection. *Hum Reprod* 2008; **23**:1499–1504.

Verhulst SM, Cohlen BJ, Hughes E, te Velde E, Heineman MJ. Intra-uterine insemination for unexplained subfertility. *Cochrane Database Syst Rev* 2006;CD001838.

Verpoest W, Fauser BC, Papanikolaou E, Staessen C, Van Landuyt L, Donoso P, Tournaye H, Liebaers I, Devroey P. Chromosomal aneuploidy in embryos conceived with unstimulated cycle IVF. *Hum Reprod* 2008;**23**:2369–2371.

Vimercati A, Scioscia M, Lorusso F, Laera AF, Lamanna G, Coluccia A, Bettocchi S, Selvaggi L, Depalo R. Do uterine fibroids affect IVF outcomes? *Reprod Biomed Online* 2007;**15**:686–691.

Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. *Mol Hum Reprod* 2000; **6**:1055–1062.

Wichmann L, Isola J, Tuohimaa P. Prognostic variables in predicting pregnancy. A prospective follow up study of 907 couples with an infertility problem. *Hum Reprod* 1994;**9**:1102–1108.

Wilson M, Hartke K, Kiehl M, Rodgers J, Brabec C, Lyles R. Integration of blastocyst transfer for all patients. *Fertil Steril* 2002;**77**:693–696.

Wood L. Costs of multiple pregnancy. *BJOG* 2008;**115**:416.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 4th edn. Cambridge: Cambridge University Press, 1999.

Wright V, Schieve L, Reynolds M, Jeng G. Assisted reproductive technology surveillance—United States, 2000. *Morb Mortal Wkly Rep* 2003;**52**:1–16.

Yanaihara A, Yorimitsu T, Motoyama H, Iwasaki S, Kawamura T. Location of endometrial polyp and pregnancy rate in infertility patients. *Fertil Steril* 2008;**90**:180–182.

Yong PY, Brett S, Baird DT, Thong KJ. A prospective randomized clinical trial comparing 150 IU and 225 IU of recombinant follicle-stimulating hormone (Gonal-F*) in a fixed-dose regimen for controlled ovarian stimulation in in vitro fertilization treatment. *Fertil Steril* 2003; **79**:308–315.

Youssry M, Ozmen B, Zohni K, Diedrich K, Al-Hasani S. Current aspects of blastocyst cryopreservation. *Reprod Biomed Online* 2008;**16**: 311–320.

Zegers-Hochschild F, Nygren KG, Adamson GD, de Mouzon J, Lancaster P, Mansour R, Sullivan E. The ICMART glossary on ART terminology. *Hum Reprod* 2006;**21**:1968–1970.

Submitted on November 5, 2008; resubmitted on February 19, 2009; accepted on March 18, 2009