Human Fertility, March 2009; 12(1): 1–13

informa
healthcare

MEDICAL ADJUNCTS IN IVF

Medical adjuncts in IVF: evidence for clinical practice

LUCIANO G. NARDO[1,2], INGRID GRANNE[3], & JANE STEWART[4]; ON BEHALF OF THE POLICY & PRACTICE COMMITTEE OF THE BRITISH FERTILITY SOCIETY

[1]*Department of Reproductive Medicine, St. Mary's Hospital, Manchester, UK,* [2]*Division of Human Development, University of Manchester, Manchester, UK,* [3]*Oxford Fertility Unit, John Radcliffe Hospital, Oxford, UK, and* [4]*Newcastle Fertility Centre at Life, Newcastle upon Tyne, UK*

Abstract
The cross-talk between the embryo and the endometrium, leading to implantation, is a complex, dynamic and highly controlled phenomenon. Over the last decade, a large amount of translational and clinical research has been carried out in an attempt to increase the likelihood of pregnancy in *in vitro* fertilisation (IVF). The purpose of this article was to review the literature on the effectiveness of adjuvant therapy in IVF and to provide fertility professionals with evidence-based guidance and recommendations. Clinicians who decide to prescribe therapies for which the evidence base is weak, should make patients aware of this lack of knowledge and potential adverse effects. There is a need for good clinical trials in many of the areas surrounding medical adjuncts in IVF to resolve the empirical/evidence divide.

Keywords: *IVF, immune therapy, steroids, aspirin, heparin*

Introduction

Implantation failure is a devastating event for couples undergoing *in vitro* fertilisation (IVF) and a formidable clinical challenge to their fertility specialists. As a consequence of significant emotional distress, couples are often desperate enough to try any procedure that may boost their chance of conceiving or fertility performance. Testing of adjuvant therapies in properly conducted randomised controlled trials (RCTs) should be mandatory so that potential benefits and risks are presented to patients and clinicians, but unfortunately, there is little consensus about which therapeutic strategies are genuinely effective in enhancing the chance of a live birth after IVF.

Endometrial function is complex and dynamic but clearly has a role to play in embryo implantation and thus the outcome of IVF treatment, but reliable diagnostic tests to assess endometrial function both in natural and IVF cycles are lacking. Sub-optimal uterine perfusion, endometrial immune-hostility, increased myometrial activity and luteal phase defect have all been suggested as possible causes of sub-fertility and failed implantation after IVF. The quality of studies which have investigated the role of adjuvant therapies on these conditions is variable, leading to debatable interpretation of the results. Although some studies are graded A based on the available evidence, most are underpowered and further trials are required to produce a clear answer to many of the questions posed.

Aims

This scoping document aims to review the available literature on adjuvant therapies in IVF cycles and to provide fertility professionals with evidence-based guidance. It includes recommendations to enhance good clinical practice.

Scope

The scope of the document includes the effects on IVF success rate and pregnancy outcome of immune testing and therapy, steroids, vasodilators and uterine relaxants, aspirin, heparin, growth hormone (GH) and oestradiol supplementation.

Correspondence: Dr. Luciano G. Nardo, Department of Reproductive Medicine, St. Mary's Hospital, Whitworth Park, Manchester M13 0JH, UK. Fax: +44-161-224-0957. E-mail: luciano.nardo@cmmc.nhs.uk

ISSN 1464-7273 print/ISSN 1742-8149 online
DOI: 10.1080/14647270802692169

Methodology

A search of two online databases (MEDLINE and EMBASE) was performed using the following keywords: IVF, pregnancy, immune testing and therapy, steroids, vasodilators, uterine relaxants, aspirin, heparin, GH and oestradiol (E_2) supplementation. The articles retrieved together with the Proceedings/Abstracts of relevant conferences were hand-searched to identify other potentially relevant studies for inclusion. Additional gross searches were performed using the names of investigators who were the lead authors of at least one previously published randomised trial. The literature search, quality rating of studies and interpretation of data were independently undertaken by all the authors, and then verified by the lead author. The levels of evidence used in this article are shown in Table I.

All the eligible studies that investigated the role of adjuvant therapies in IVF cycles were included. Studies in which adjuncts were given to patients with known medical conditions such as recurrent miscarriage, and in assisted conception treatments other than IVF with or without intracytoplasmic sperm injection (ICSI), were excluded.

Outcome measures

The outcome measures are those usually adopted in IVF cycles, including response to ovarian stimulation treatment, pregnancy rate, live birth rate and fetal–maternal complications, if data are available.

Table I. Levels of evidence.

Hierarchy of evidence

- **1a.** Systematic review and meta-analysis of randomized controlled trials (RCTs).
- **1b.** At least one randomized controlled trial.
- **2a.** At least one well-designed controlled study without randomization.
- **2b.** At least one other type of well-designed quasi-experimental study.
- **3.** Well-designed non experimental descriptive studies, such as comparative studies, correlation studies or case studies.
- **4.** Expert committee reports or opinions and/or clinical experience of respected authorities.

Grade strength of evidence

- **A** Requires at least one RCT as part of a body of literature of overall good quality and consistency addressing the specific recommendations. (Evidence levels 1a, 1b)
- **B** Requires the availability of well-controlled clinical studies but no randomized clinical trials on the topics of recommendation. (Evidence levels 2a, 2b, 3)
- **C** Requires evidence obtained from expert committee reports of opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level 4)
- **GPP** Good practice point

Immune testing and therapy

In recent years, there has been much interest in the possible immune causes and potential immune therapies for sub-fertility. The immunological abnormalities potentially relevant to IVF outcomes as well as the evidence for treating patients found to have such abnormalities are revisited in the literature on a continuous basis (RCOG Scientific Advisory Committee, Opinion Paper 5, 2008).

Immune testing

Natural killer cells

Natural killer (NK) cells are lymphocytes that are part of the innate immune system. They are characterised by their expression of the cell surface antigens, CD56 and CD16, and the lack of expression of CD3. CD-56 is expressed at differing intensity on the cell surface of NK cells; based on this they are divided into two distinct populations. The majority of peripheral blood NK cells are CD-56 dim CD16+ (90%), the remaining peripheral NK cells are CD-56 bright CD16− (10%). The majority of uterine NK cells are CD56 bright CD16−.

Uterine NK cells are the predominant leukocyte population in the uterine mucosa at the time of implantation (Moffett-King, 2002). The NK cells are also present in the endometrium of non-pregnant women and their numbers vary throughout the menstrual cycle. Numbers increase during the secretory phase of the cycle and remain high during early gestation. Despite their name, there is no evidence that uterine NK cells have the ability to kill placental trophoblast (Abadia-Molina et al., 1996). Some authors have found lower percentages of CD56 bright CD16− NK cells and higher percentages of CD56 dim CD16+ NK cells in patients with recurrent miscarriage (Quenby et al., 1999; Dosiou & Giudice, 2005) and poor IVF outcome (Thum et al., 2004). Conversely, other researchers have unable to demonstrate a relationship between uterine NK cells and repeated (three or more) implantation failures after IVF (Matteo et al., 2007). There is a lack of robust evidence to suggest that measuring uterine NK cells is clinically useful in predicting the outcome of IVF treatment. Nevertheless, it has become increasingly common to examine peripheral blood NK cells in women with sub-fertility and a history of recurrent implantation failure. This approach has two main problems. First, uterine NK cells are different from those in peripheral blood. Second, the percentage of CD56+ NK cells in peripheral blood in healthy individuals varies between 5 and 29% (Bisset et al., 2004). Thus, the finding of greater than 12% NK cells in blood of

women with recurrent failed IVF treatment has been arbitrarily defined as abnormally raised (Beer et al., 1996). Other studies (Coulam & Roussev, 2003; Thum et al., 2004) investigating the relationship between the percentage of peripheral blood NK cells and the IVF outcome have reached similar conclusions on the same doubtful premise.

Recommendation(s).
The evidence that measurements of the numbers or percentages of peripheral blood NK cells, or levels of activated peripheral NK cells relate to events in the endometrium at the time of implantation is very weak. (**B**)

Antiphospholipid antibodies

Antiphospholipid antibodies (APA) are a heterogeneous group of about 20 antibodies directed against phospholipid binding proteins. The only antibodies in this group of known clinical significance are the lupus anticoagulant (LA) and the anticardiolipin antibody (ACL) (Wilson et al., 1999).

The antiphospholipid syndrome (APS), characterised by the presence of anticardiolipin antibodies and/or LA, is a recognised and treatable cause of recurrent miscarriage (Miyakis et al., 2006). However, the relevance of finding APS in the context of sub-fertility and IVF failure is much less clear.

The prevalence of APA in the general population is around 10% (Lim & Crowther, 2007). Some researchers have found a high incidence of APA in infertile women undergoing IVF treatment (Birkenfeld et al., 1994; Denis et al., 1997; Kutteh et al., 1997; Yetman et al., 1997; Eldar-Geva et al., 1999). In cases of recurrent implantation failure, defined as three or more failed IVF cycles, Qublan et al. (2006) found that ACL and LA were increased, though not significantly. However, there was a statistically significant increase in combined thrombophilias, including Factor V Leiden mutation, prothrombin mutation, methylenetrahydrofolate reductase mutation, protein C, S and anti-thrombin III, in women with recurrent IVF failure.

A recent small observational study suggested that the pregnancy and live birth rate after IVF is significantly decreased in women with APAs (Lee et al., 2007). A meta-analysis (Hornstein et al., 2000) has compared the outcomes of treatment in APA-positive and APA-negative women who received no immune treatments. In this analysis, 2,053 women were included of whom 34% were found to be APA positive. The combined clinical pregnancy rate was 57% in the APA-positive patients compared with 49.2% in the APA-negative patients. The live birth rate was 46% in the APA patients and 42.9% in the APA-negative patients. In other words, the presence of APA exerted no statistically significant effect on the chance of clinical pregnancy or live birth.

Recommendation(s).
Routine testing for APA before IVF is not necessary. (**A**)

Testing for thrombophilias may be relevant in patients with a history of repeated implantation failure. (**C**)

Other autoantibodies

The prevalence of antinuclear antibodies in patients with recurrent failed implantation after Assisted Reproductive Technologies (ART) is increased in some studies (Cubillos et al., 1997; Stern et al., 1998; Kaider et al., 1999; Taniguchi, 2005), whereas no differences have been found in other reports (Geva et al., 1994; Kikuchi et al., 2003).

A study of 688 women undergoing ART reported that there was no difference in the prevalence of antithyroid antibodies in sub-fertile women compared with fertile normal controls (Kutteh et al., 1999a). Similarly, other studies have found no difference in biochemical pregnancy rate, clinical pregnancy rate or live birth rate between women who are positive or negative for thyroid antibodies (Kutteh et al., 1999b; Negro et al., 2007).

Ovarian autoantibodies are increased in women with sub-fertility (Luborsky et al., 1999) and before assisted conception treatment (Geva et al., 1996). However, the high number of false-positive results and the variable detection methods make tests for ovarian antibodies unreliable (Pires et al., 2007).

Recommendation(s).
The association between nuclear, thyroid and ovarian autoantibodies with IVF outcome is unproven. Testing for these autoantibodies is therefore not recommended. (**B**)

Shared human leucocyte antigen

It has been suggested that couples who share multiple human leucocyte antigen (HLA) may be more likely to have unexplained sub-fertility, failed implantation and miscarriage. The association remains a matter of debate with some authors reporting a link between unexplained sub-fertility and failed IVF with increased sharing of HLA (Ho et al., 1994; Creus et al., 1998; Omu et al., 1999), whereas others have failed to show any association (Nordlander et al., 1983; Martin-Villa et al., 1993; Check et al., 2001).

Recommendation(s).
Alloimmune testing is not necessary because there is lack of studies to confirm that increased sharing of HLA between partners leads to IVF failure. (**C**)

Immune therapies

Intravenous immunoglobulins

Intravenous immunoglobulin (IVIG) therapy has been advocated as *ad-hoc* treatment for recurrent implantation failure after IVF, or associated patients with raised peripheral NK cells, positive antithyroid or APA and shared HLAs. The mode of action of IVIG therapy is far from understood, but possible mechanisms include the downregulation of peripheral NK cells, decreased NK cell activity and downregulation of auto-antibody production. Studies in the available literature suffer from poor design and some of them are methodologically flawed.

A small uncontrolled study has suggested an increase in live birth rate in women defined as having raised peripheral NK cells (Coulam & Goodman, 2000). However, it is difficult to justify the use of IVIG in these cases, as there is no conclusive evidence that raised peripheral NK cells cause implantation failure. Sher et al. (1998a,b,c) have reported an increase in live birth rate after using IVIG in combination with heparin and aspirin in women who were either ACL- or thyroid antibody-positive. In a recent case, a series of 10 patients with multiple shared paternal leukocyte antigens and recurrent IVF implantation failure, the pregnancy rate increased to 50% per treatment cycle after intravenous treatment with immunoglobulins (Elram et al., 2005).

One small uncontrolled study has suggested a benefit in the use of IVIG in women with failed implantation after IVF (Coulam et al., 1994). Conversely, a case series (Balasch et al., 1996) and a placebo-controlled, randomised trial (Stephenson & Fluker, 2000) which included 51 couples, showed no benefit in the use of immunoglobulin therapy in patients with previous unexplained IVF failure. Trials carried out to date have not had sufficient power to detect small differences in pregnancy or live birth rates, and larger RCTs are warranted. Further, the clinical guidelines for immunoglobulin use recently published by the Department of Health (2008) do not support the use of IVIG for IVF failure.

It must be remembered that as immunoglobulins are a pooled blood product, which have an associated risk of anaphylaxis (Sherer et al., 2001) and, presumably, infection. Moreover, IVIG therapy is associated with other complications in up to 36% of cases (Katz et al., 2007). The most adverse effects are mild and transient, and include headache, malaise, flushing, fever, nausea, blood pressure changes and tachycardia. Late adverse effects – renal failure, aseptic meningitis, thromboembolic events and hemolytic anaemia – are rare (Katz et al., 2007).

Recommendation(s).
There is no evidence for the use of IVIG as adjunctive therapy for recurrent failed IVF cycles. (**A**)

Tumor necrosis factor-alpha

A significantly increased T helper 1 (Th1) cytokine response may be the underlying immunological cause for poor reproductive performance and implantation failure after IVF (Ng et al., 2002; Kwak-Kim et al., 2003). *In vitro* experiments have demonstrated that expression of the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) has a role in inducing endometrial cell apoptosis (Okazaki et al., 2005). The authors of an observational study, including 14 women undergoing IVF, concluded that women with a history of recurrent IVF failure have a Th1 bias as shown by an increased TNF-α:IL-4 ratio, and that the Th1:Th2 ratio is increased following controlled ovarian hyperstimulation (Kalu et al., 2008). However, the small sample size makes it impossible to draw any definite conclusions. Thum et al. (2007) found a direct association between systemic TNF-α and activated NK cells, but failed to demonstrate a significant correlation between serum levels of TNF-α and IVF treatment outcome. Similarly, a previous study of 159 IVF patients showed that maternal circulating concentrations of TNF-α do not predict the outcome of IVF cycles (Fasouliotis et al., 2004). Furthermore, anti-TNF-α agents (infliximab and etanercept) may lead to the development of granulomatous disease, lymphoma and demyelinating disease. Well designed, properly powered trials are warranted to establish the effectiveness and safety of immune suppressants in women of reproductive age undergoing IVF.

Recommendation(s).
There is no indication for the use of TNF-α immune therapy in women undergoing IVF because of the lack of studies investigating its role and safety. (**GPP**)

Steroids

Glucocorticoids are steroid hormones with anti-inflammatory and immunosuppressive properties. It has been suggested that the use of glucocorticoids may modulate the uterine environment by altering the cytokine production and decreasing the number

of uterine NK cells. The use of steroids has been advocated routinely in IVF cycles and in women known to have autoantibodies.

Recently, a Cochrane review of 13 properly designed RCTs including 1,759 couples considered whether steroids are effective for increasing clinical outcomes in sub-fertile women undergoing IVF/ICSI, compared with placebo administration or no glucocorticoids (Boomsma et al., 2007). The primary outcome measure was live birth rate per couple, whereas the secondary outcome measures were ongoing pregnancy rate per couple and pregnancy rate per couple. Live birth rate and ongoing pregnancy rate were only reported in three trials and analytical pooling showed no significant difference (OR 1.21, 95% CI 0.67–2.19 and OR 1.15, 95% CI 0.76–1.75, respectively). Analysis of the whole data set (13 RCTs) showed no significant improvement in pregnancy rates (OR 1.15, 95% CI 0.93–1.43). However, a subgroup analysis of six trials, including 650 subjects (335 in the glucocorticoids study group and 315 controls) undergoing conventional IVF alone, demonstrated a significantly higher pregnancy rate (OR 1.5, 95% CI 1.05–2.13) following steroid administration.

A number of articles have examined the use of corticosteroids in women known to have autoantibodies. Steroids alone or in combination with low-dose aspirin have been shown to have a beneficial effect on pregnancy rate in Anti Nuclear Antibodies (ANA)-positive women undergoing IVF (Ando et al., 1996; Hasegawa et al., 1998; Geva et al., 2000; Taniguchi, 2005). However, the only study to report on live birth rate did not find any statistically significant improvement in success rate (Taniguchi, 2005).

Recommendation(s)

There is no evidence to support the routine use of peri-implantation steroids in ART cycles. (**A**)

There is limited evidence that peri-implantation steroid administration may improve pregnancy rates in women undergoing IVF alone. However, the results are of borderline statistical significance and need to be confirmed in a suitably powered, RCT designed specifically to address this issue. (**A**)

Vasodilators

As inadequate endometrial thickness may be a cause of implantation failure, it has been speculated that an appropriate increase in endometrial thickening may enhance the success of IVF treatment. It is further argued that since endometrial receptivity may relate to a poor endometrial blood supply, enhanced endometrial vascularity might increase implantation rates through endometrial development, particularly in women with apparent sub-optimal endometrial development.

Mechanisms for vasodilatation

Nitric oxide. Nitric oxide (NO) has a significant local regulatory role in vascular physiology. On exposure to nitric oxide synthetase inhibitor, post-ovulation increases pregnancy failure (Sengupta et al., 2005). NO acts directly as a vasodilator via a cyclic GMP (cGMP)-dependent mechanism or via the induction of matrix metalloproteases, which have a role in remodelling the extracellular matrix during implantation (Zhang et al., 2004).

Nitroglycerine. Following a promising pilot study, Ohl et al. (2002) randomised 138 IVF patients with a history of implantation failure to receive either the NO donor nitroglycerine (NTG) (70 subjects) or placebo (68 subjects). The women had an average of 3.9 previous treatment failures. The NTG patches were applied regularly from the day before embryo transfer until the treatment outcome was known. There were no significant differences in treatment response (oocytes retrieved, oocyte maturity, embryo cleavage rates, number of good quality embryos at transfer), implantation rate, biochemical and clinical pregnancy rates, live birth rates or pregnancy complications.

Viagra. Viagra (sildenafil citrate) augments the vasodilatory effect of NO. As a Type-5 specific phosphodiesterase inhibitor, it blocks the degradation of cGMP through which NO acts. Sher and Fisch (2000) administered sildenafil citrate followed by placebo, then sildenafil citrate and oestradiol valerate to four women in a cross-over trial while they were being down-regulated with a gonadotropin-releasing hormone (GnRH) analogue. The findings were that uterine artery Pulsatility Index (PI) fell in all of the women during administration of sildenafil and sildenafil plus oestrogen during down-regulation and reverted to baseline during the placebo phase. Endometrial thickness in three women increased to >10 mm during the oestrogen/sildenafil phase and this was reproduced in the treatment cycle, resulting in a pregnancy in each case. The fourth woman had no increased endometrial thickness and failed to conceive. Check et al. (2004) found that the addition of Viagra to an oestrogen-supplemented regime had no effect on endometrial thickness or blood flow in women who had previously failed to achieve an endometrial thickness of greater than 8 mm in fresh IVF or frozen embryo transfer cycles.

Recommendation(s).
Neither NTG nor sildenafil citrate have been shown to have significant beneficial effects on IVF outcome and their use cannot be supported. (**A**)

Uterine relaxants

Uterine contractile activity occurs throughout the menstrual cycle but varies with cycle stage (van Gestel et al., 2003). It has been hypothesised that adverse uterine contractility at the time of embryo transfer may occur as a result of the early timing of transfer in the luteal phase, the supraphysiological hormonal milieu and the mechanical stimulus of the transfer procedure itself (Morizaki et al., 1989). Lesny et al. (1998) reported increased uterine activity during IVF as opposed to natural cycles, and an earlier study by Knutzen et al. (1992) concluded that contractions result in potentially expulsive activity, which may have a detrimental effect on embryo implantation. Interestingly, it has been suggested that abnormal contractile waves may also increase the rate of ectopic pregnancy and that increased uterine tone may contribute to difficult transfers (Shaker et al., 1993). Some authors have hypothesised that the administration of uterine muscle relaxants might improve success following embryo transfer.

Nitroglycerine

NTG is a NO donor vasodilator that by virtue of its smooth muscle relaxant properties also relaxes uterine muscle. Shaker et al. (1993) found no benefit of NTG administration 3 min before embryo replacement on the ease of transfer or pregnancy rate.

β_2-adrenergic antagonists

Ritodrine, terbutaline and salbutamol have all been used as muscle relaxants in preterm labour and for extrauterine fetal manipulation. Pinheiro et al. (2003) compared the effects of ritodrine and terbutaline, administered for 2 weeks following oocyte retrieval. There was no benefit in the implantation and pregnancy rates. As with premature labour, the use of these agents was limited in some women by unacceptable side-effects.

Progesterone

Progesterone promotes uterine quiescence and is believed to have a role in preventing the premature onset of labour. Fanchin et al. (1998), while undertaking ultrasound assessment of uterine contractions on the day of embryo transfer in 209 women, found that increased contractile activity was associated with reduced implantation rate, clinical pregnancy rate and ongoing pregnancy rate, and that high levels of activity correlated negatively with serum progesterone concentrations. The authors considered their data a stimulus to further investigation of uterine relaxants in IVF cycles.

Recommendation(s).
There is no good evidence to support the use of uterine relaxants in IVF cycles as their effect(s) remains to be defined.

Uterine relaxants should not therefore be used to improve IVF outcome. (**GPP**)

Aspirin

Low-dose acetylsalicylic acid (aspirin) irreversibly inhibits the enzyme cyclo-oxygenase in platelets, preventing the synthesis of the vasoconstrictive agent thromboxane. The daily administration of aspirin in low doses induces a shift from thromboxane A_2 to prostacyclin, leading to vasodilatation and increased peripheral blood perfusion (Patrono et al., 2005).

Impaired uterine perfusion has been suggested as a possible cause of sub-fertility (Goswamy & Steptoe, 1988) because of a negative effect on endometrial receptivity, which in turn may cause embryo implantation failure (Steer et al., 1992). The evidence supporting the effect of low-dose aspirin in women undergoing IVF is, however, poor and controversial. Although some papers have reported some beneficial effects of aspirin (Rubinstein et al., 1999; Waldenstrom et al., 2004), others have failed to confirm these findings (Urman et al., 2000; Hurst et al., 2005; Päkkilä et al., 2005; Frattarelli et al., 2008). One RCT demonstrated that adjuvant therapy with aspirin and prednisolone did not improve uterine blood flow, implantation and pregnancy rates (Revelli et al., 2008).

In a recent meta-analysis, Gelbaya et al. (2007) included six RCTs that investigated the effects of aspirin on IVF outcome. Of these, four studies reported on the effect of low-dose aspirin in women undergoing IVF/ICSI (Rubinstein et al., 1999; Urman et al., 2000; Waldenstrom et al., 2004; Päkkilä et al., 2005), one study reported on the effect of low-dose aspirin on poor responders (Lok et al., 2004) and a further one the effect of low-dose aspirin in patients undergoing embryo transfer from donated oocytes (Weckstein et al., 1997). Waldenstrom et al. (2004) found that pregnancy rate per Embryo Transfer (ET) was significantly higher in patients who received low-dose aspirin from the day of ET than in those who received no treatment. Conversely, the meta-analytical pooling of three

trials (Rubinstein et al., 1999; Urman et al., 2000; Päkkilä et al., 2005) reporting on clinical pregnancy rate per cycle did not show a significant difference between the two groups (RR 1.12, 95% CI 0.78–1.59). In keeping with these findings, meta-analysis of the randomised trials included (Rubinstein et al., 1999; Urman et al., 2000; Waldenstrom et al., 2004; Päkkilä et al., 2005) failed to show a statistically significant difference between the two groups in clinical pregnancy rate per embryo transfer (RR 1.12, 95% CI 0.91–1.37). Furthermore, a meta-analysis of the two trials (Waldenstrom et al., 2004; Päkkilä et al., 2005) that reported on live birth rate per embryo transfer found no difference (RR 1.08, 95% CI 0.83–1.40). Low-dose aspirin had no effect on clinical pregnancy rate per cycle or embryo transfer or on cycle cancellation in poor responders undergoing IVF (Lok et al., 2004) and had no beneficial effects on pregnancy outcome in recipients of donated oocytes (Weckstein et al., 1997). Another systematic review and meta-analysis on the use of aspirin in IVF cycles published by Khairy et al. (2007) reached the same conclusions. By contrast, other authors have reported opposite findings after meta-analytical pooling (Ruopp et al., 2008). Discrepancies between manuscripts, including the statistical approach and the validity of the meta-analysis, have been addressed in a letter-to-the-editor (Gelbaya et al., in press). The main concern with the analysis of Ruopp et al. (2008) was that the use of fixed-effects model, instead of the random-effects model, in the presence of heterogeneity between the studies shrinks the confidence interval of the summary estimate leading to an erroneous, statistically significant, result. Further, in contrast to the study of Ruopp et al. (2008), Gelbaya et al. (2008) excluded studies that were not randomised and those that investigated the use of low-dose aspirin only in specific subgroups.

The effect of aspirin administration in the first trimester of pregnancy on the risk of congenital malformations and specific fetal anomalies has been analysed. A meta-analysis including 22 studies incorporating 16,138 exposed and 48,980 controls did not demonstrate an overall increase risk in congenital malformations. A subgroup analysis of 261 cases found that the use of aspirin in the first trimester of pregnancy was associated with a significantly increased risk of gastroschisis (OR 2.37, 95% CI 1.44–3.88) (Kozer et al., 2002). However, this meta-analysis was limited by the heterogeneity of the studies available in the literature, including their design and methodologies. For instance, low socioeconomic status, lack of information regarding the dose of aspirin given and the use of other drugs at the same time of aspirin could be regarded as confounding factors. Further, rigorously designed adequately powered studies are required to investigate the association between aspirin treatment and the risk of fetal malformations.

Recommendation(s)

Given the lack of proven efficacy on likelihood of pregnancy aspirin as adjunctive therapy in IVF should not be prescribed. (**A**)

Heparin

Heparin is an established anti-coagulant treatment option for women with recurrent early pregnancy loss and APS (Empson et al., 2005). Whether heparin alone or in combination with low-dose aspirin improves the pregnancy outcome in subfertile autoantibody-positive women with IVF implantation failure is uncertain. The up-to-date evidence suggests a significant relationship between anticardiolipin antibodies and recurrent miscarriage (Opatrny et al., 2006), however, the mechanisms of implantation failure by APA and ANA require further investigation. On the assumption that the altered thrombotic status leading to coagulation disorders could interfere with the different stages of embryo implantation (Chamley et al., 1998; Di Simone et al., 2000), heparin therapy is sometimes given in women undergoing IVF. Moreover, data in the literature show that activation of the coagulation cascade and impairment of fibrinolysis occur during controlled ovarian stimulation and are more pronounced in women with ovarian hyperstimulation syndrome who have unsuccessful pregnancy outcomes (Rogolino et al., 2003).

Interestingly, there are only a few heterogenous studies investigating the role of anticoagulant therapy in women with acquired thrombophilia undergoing IVF. Two studies reported no significant differences in IVF implantation and pregnancy rates in APA-positive patients treated with heparin and low-dose aspirin (Schenk et al., 1996; Kutteh et al., 1997). In a double-blind cross-over RCT, Stern et al. (2003) randomised 143 autoantibody-positive women who had $\geq$10 failed ET cycles. All subjects received unfractionated heparin 5,000 U twice daily and low-dose aspirin 100 mg or placebo from the day of embryo transfer until 14 weeks of gestation or fetal demise. Implantation rate and pregnancy rate per transfer were similar between treatment and placebo groups. However, the study has been criticised for its crossover design, which did not allow for the exclusion of the potential hangover effects of aspirin in the selected cohort of patients. Nelson and Greer (2008) in their systematic review of the role of heparin in ART suggested that women with

either definitive APS (as defined by Miyakis et al., 2006) or repeated IVF implantation failure and antiphospholipid seropositivity should be started on Low Molecular Weight Heparin (LMWH) and aspirin at the time of ovarian stimulation and continued throughout pregnancy. The authors identified six studies, two of which were RCTs, including a total of 1,792 subjects with APA. The pregnancy rate per patient was 41.8% (563/1,344) in the heparin and aspirin arm and 27.2% (122/448) in the control arm ($p < 0.001$). However, one of the studies ($n = 81$) was only a personal communication, not published at the time of writing, and again all the studies included were heterogeneous in design and entry criteria.

Recommendation(s)

Given the lack of trials investigating the role of heparin alone and in combination with low-dose aspirin in the general IVF population, treatment should be avoided. (**C**)

Pragmatic treatment with LMWH and low-dose aspirin would be appropriate in women with APS and in those with APA and repeated implantation failure. (**C**)

Growth hormone

The expression of GH and its receptor has been reported in human ovaries (Abir et al., 2008), suggesting that this hormone may be involved in primordial follicular growth in humans. Interestingly, these data follow previous studies that have investigated the efficacy of GH supplementation during controlled ovarian hyperstimulation with exogenous gonadotrophins for IVF. In a cohort of poor responders, Kim et al. (1999) found that co-treatment with pyridostigmine, a GH releasing agent, enhanced the ovarian response to stimulation and resulted in a non-statistically significant higher clinical pregnancy rate. Recently, similar findings were reported by Kucuk et al. (2008) in a small RCT where daily subcutaneous injections of GH were given throughout the stimulation cycle. In contrast, another RCT failed to demonstrate a beneficial effect of GH adjuvant therapy for IVF in poor responders (Dor et al., 1995). An updated Cochrane review of nine trials, including a total of 401 subjects, demonstrated that in patients with no history of poor response to stimulation, the use of GH therapy did not affect live-birth rate, while in poor responders adjuvant GH treatment resulted in an increase in live-birth rate that just reached statistical significance (Harper et al., 2003). The authors therefore concluded that before giving GH in IVF cycles further studies are warranted, and that until they are completed, this compound should only be used in the context of clinical research. Furthermore, in 2004, (spell out for non-UK-based practitioners) – the UK National Institute of Clinical Excellence (NICE) recommended that GH treatment should not be used as adjuvant to IVF because of the lack of significant improvements in pregnancy rates (NICE, 2004).

Recommendation(s)

Currently, there is no indication for the use of GH adjuvant therapy in IVF cycles. (**GPP**)

Oestradiol supplementation

Oestradiol (E_2) is essential for endometrial priming, but is also responsible for proliferation of the uterine surface epithelium, glands, stroma and blood vessels. The role of E_2 in the luteal phase, including the preparation of the endometrium for embryo implantation, remains unclear, while its depletion in the human luteal phase does not appear to affect the morphological developmental capacity of the endometrium adversely (Younis et al., 1994).

Early studies suggested that in the luteal phase of an IVF cycle, serum E_2 and progesterone (P4) drop to low levels resulting in reduced implantation and pregnancy rates (Hutchinson-Williams et al., 1989). The defect in luteal phase is more pronounced in GnRH-agonist (GnRH-a) long protocols compared with short protocols (Devreker et al., 1996), and is present even after an early cessation of GnRH-a administration (Beckers et al., 2000). The benefit of additional luteal supplementation with E_2 is however controversial. Some reports (Fahri et al., 2000; Gorkemli et al., 2004; Lukaszuk et al., 2005) have found that combined luteal phase support with E_2 and P4 was associated with higher pregnancy rates per embryo transfer, while others (Smitz et al., 1993; Lewin et al., 1994; Tay & Lenton, 2003) failed to observe any beneficial effect on IVF outcomes. This was recently confirmed in a series of RCTs (Engmann et al., 2008a,b; Serna et al., 2008). Furthermore, a meta-analysis of 10 RCTs published between 1993 and 2007 demonstrated that the implantation rate (RR 1.05, 95% CI 0.81–1.36), clinical (RR 1.19, 95% CI 0.74–1.90) and ongoing (RR 1.37, 95% CI 0.81–2.30) pregnancy rates per embryo transfer were not significantly different between women who had combined E_2 and P4 therapy and those who had P4 supplementation alone for luteal phase support (Gelbaya et al., 2008). Subgroup analyses according to the dose of E_2 administered (2, 4 and 6 mg) suggested similar trends toward favourable outcomes in the group given E_2 and P4, but conclusions regarding the optimal dose and the length of treatment were not

possible because of the limited number of trials (Gelbaya et al., 2008).

Recommendation(s)

Current evidence shows no beneficial effects of luteal E_2 supplementation in IVF. (**A**)

Oestradiol supplementation should only be used in recipients of donated oocytes and in patients with hypogonadotrophic hypogonadism. (**GPP**)

Recommendations for practice

Fertility practitioners should be aware, and always inform their patients, of the best available evidence about the safety and the clinical effectiveness of diagnostic tests and treatments offered. Clinicians who decide to prescribe unproven therapeutic approach are obliged to discuss with their patients the lack of evidence for clinical benefits and all the potential adverse effects to mother and fetus.

This document is based on the interpretation of the best currently available data. There is a clear need for further, appropriately powered, RCTs to provide robust answers. Nevertheless, though there is concerted agreement that further studies are required, at this point in time consideration should be given to the following recommendations:

1. There is lack of robust evidence to suggest that measuring uterine NK cells is clinically useful in predicting the outcome of IVF treatment (Grade B).
2. Routine testing for APA before IVF is not necessary (Grade A).
3. Testing for thrombophilias may be relevant in patients with a history of repeated implantation failure (Grade C).
4. The association between nuclear, thyroid and ovarian autoantibodies with IVF outcome is unproven. Testing for these autoantibodies is of no benefit (Grade B).
5. There is no evidence that alloimmune testing is useful in IVF management (Grade C).
6. There is no evidence for the use of IVIG as adjunctive therapy for recurrent failed IVF cycles (Grade A).
7. Because of the lack of studies investigating the role and the safety of TNF-alpha immune therapy in women undergoing IVF, there is no indication for its use (Grade C).
8. There is no evidence to support the routine use of peri-implantation steroids in ART cycles (Grade A).
9. There is limited evidence that peri-implantation steroid administration may improve pregnancy rates in women undergoing IVF alone. These results need to be confirmed in a suitably powered, RCT designed specifically to address this issue (GPP).
10. Neither NTG nor sildenafil citrate has beneficial effects on IVF outcome, and should not be used (Grade A).
11. Uterine relaxants are not to be used to improve IVF outcome as their effect remains unknown (GPP).
12. Given the lack of proven efficacy on likelihood of pregnancy aspirin as adjunctive therapy in IVF should not be prescribed (Grade A).
13. There is no evidence that heparin alone and in combination with low-dose aspirin improves IVF treatment success rate (Grade C).
14. Pragmatic treatment with LMWH and low-dose aspirin would be appropriate in women with APS and in those with APA and repeated implantation failure (Grade C).
15. Because of the limited evidence on the role of GH adjuvant therapy in IVF, there is no indication for its use (Grade A).
16. The current evidence shows no beneficial effects of luteal E_2 supplementation in IVF (Grade A).
17. Oestradiol supplementation should only be used in recipients of donated oocytes and in patients with hypogonadotrophic hypogonadism (Grade C).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Abadia-Molina, A. C., Ruiz, C., Montes, M. J., King, A., Loke, Y. W., & Olivares, E. G. (1996). Immune phenotype and cytotoxic activity of lymphocytes from human term decidua against trophoblast. *Journal of Reproductive Immunology*, *31*, 109–123.

Abir, R., Garor, R., Felz, C., Nitke, S., Krissi, H., & Fisch, B. (2008). Growth hormone and its receptor in human ovaries from fetuses and adults. *Fertility and Sterility*, *90*(4 Suppl), 1333–1339.

Ando, T., Suganuma, N., Furuhashi, M., Asada, Y., Kondo, I., & Tomoda, Y. (1996). Successful glucocorticoid treatment for patients with abnormal autoimmunity on *in vitro* fertilization and embryo transfer. *Journal of Assisted Reproduction and Genetics*, *13*, 776–781.

Balasch, J., Creus, M., Fabregues, F., Font, J., Martorell, J., & Vanrell, J. A. (1996). Intravenous immunoglobulin preceding in vitro fertilization-embryo transfer for patients with repeated failure of embryo transfer. *Fertility and Sterility*, *65*, 655–658.

Beckers, N. G. M., Laven, J. S., Eijkemans, M. J. C., & Fauser, B. C. (2000). Follicular and luteal phase characteristics following early cessation of gonadotrophin-releasing hormone agonist during ovarian stimulation for *in-vitro* fertilization. *Human Reproduction*, *15*, 43–49.

Beer, A. E., Kwak, J. Y., & Ruiz, J. E. (1996). Immunophenotypic profiles of peripheral blood lymphocytes in women with recurrent pregnancy losses and in infertile women with multiple failed *in vitro* fertilization cycles. *American Journal of Reproductive Immunology*, *35*, 376–382.

Birkenfeld, A., Mukaida, T., Minichiello, L., Jackson, M., Kase, N. G., & Yemini, M. (1994). Incidence of autoimmune antibodies in failed embryo transfer cycles. *American Journal of Reproductive Immunology*, *31*, 65–68.

Bisset, L. R., Lung, T. L., Kaelin, M., Ludwig, E., & Dubs, R. W. (2004). Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. *European Journal of Haematology*, *72*, 203–212.

Boomsma, C. M., Keay, S. D., & Macklon, N. S. (2007). Peri-implantation glucocorticoid administration for assisted reproductive technology cycles. *The Cochrane Database of Systematic Review*, Art no.: CD005996.

Chamley, L. W., Duncalf, A. M., Mitchell, M. D., & Johnson, P. M. (1998). Action of anticardiolipin and antibodies to β2-glycoprotein-I on trophoblast proliferation as a mechanism for fetal death. *Lancet*, *352*, 1037–1038.

Check, J. H., Choe, J. K., Ubelacker, L., & DiAntonio A. (2001). Maternal/paternal sharing of DQ-α type II histocompatibility antigens not associated with pregnancy outcome following *in vitro* fertilization (IVF)-embryo transfer (ET). *Clinical and Experimental Obstetrics & Gynecology*, *28*, 142–143.

Check, J. H. Graziano, V., Lee, G., Nazari, A., Choe, J. K., & Dietterich, C. (2004). Neither sildenafil nor vaginal estradiol improves endometrial thickness in women with thin endometria after taking oral estradiol in graduating dosages. *Clinical and Experimental Obstetrics & Gynecology*, *31*, 99–102.

Coulam, C. B. & Goodman, C. (2000). Increased pregnancy rates after IVF/ET with intravenous immunoglobulin treatment in women with elevated circulating C56+ cells. *Early Pregnancy*, *4*, 90–98.

Coulam, C. B., & Roussev, R. G. (2003). Correlation of NK cell activation and inhibition markers with NK cytoxicity among women experiencing immunologic implantation failure after *in vitro* fertilization and embryo transfer. *Journal of Assisted Reproduction and Genetics*, *20*, 58–62.

Coulam, C. B., Krysa, L. W., & Bustillo, M. (1994). Intravenous immunoglobulin for *in-vitro* fertilization failure. *Human Reproduction*, *9*, 2265–2269.

Creus, M., Balasch, J., Fabregues, F., Martorell, J., Boada, M., Penarrubia, J., et al. (1998). Parental human leukocyte antigens and implantation failure after *in-vitro* fertilization. *Human Reproduction*, *13*, 39–43.

Cubillos, J., Lucena, A., Lucena, C., Mendoza, J. C., Ruiz, H., Arango, A., et al. (1997). Incidence of autoantibodies in the infertile population. *Early Pregnancy*, *3*, 119–124.

Denis, A. L., Guido, M., Adler, R. D., Bergh, P. A., Brenner, C., & Scott, R. T. Jr. (1997). Antiphospholipid antibodies and pregnancy rates and outcome in *in-vitro* fertilization patients. *Fertility and Sterility*, *67*, 1084–1090.

Department of Health. (2008). *Clinical guidelines for immunoglobulin use*, 2nd ed. Department of Health, UK.

Devreker, F., Govaerts, I., Bertrand, E., Van den Bergh, M., Gervy, C., & Englert, Y. (1996). The long acting gonadotrophin releasing hormone analogues impaired the implantation rate. *Fertility and Sterility*, *65*, 122–126.

Di Simone, N., Meroni, P. L., de Papa, N., Raschi, E., Caliandro, D., De Carolis, C. S., et al. (2000). Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered β2-glycoprotein I. *Arthritis and Rheumatism*, *43*, 140–150.

Dor, J., Seidman, D. S., Amudai, E., Bider, D., Levran, D., & Mashiach, S. (1995). Adjuvant growth hormone therapy in poor responders to *in-vitro* fertilization: a prospective randomized placebo-controlled double-blind study. *Human Reproduction*, *10*, 40–43.

Dosiou, C., & Giudice, L. C. (2005). Natural killer cells in pregnancy and recurrent pregnancy loss: endocrine and immunologic perspectives. *Endocrine Reviews*, *26*, 44–62.

Eldar-Geva, T., Wood, C., Lolatgis, N., Rombauts, L., Kovacs, G., Fuscaldo, J., et al. (1999). Cumulative pregnancy and live birth rates in women with antiphospholipid antibodies undergoing assisted reproduction. *Human Reproduction*, *14*, 1461–1466.

Elram, T., Simon, A., Israel, S., Revel, A., Shveiky, D., & Laufer, N. (2005). Treatment of recurrent IVF failure and human leukocyte antigen similarity by intravenous immunoglobulin. *Reproductive BioMedicine Online*, *11*, 745–749.

Empson, M., Lassere, M., Craig, J., & Scott, J. (2005). Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database Systematic Reviews*, Art no.: CD002859.

Engmann, L., Diluigi, A., Schmidt, D., Nulsen, J., Maier, D., & Benadiva, C. (2008a). The use of gonadotropin-releasing hormone (GnRH-a) 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. *Fertility and Sterility*, *89*, 84–91.

Engmann, L., Diluigi, A., Schmidt, D., Benadiva, C., Maier, D., & Nulsen, J. (2008b). The effect of luteal phase vaginal estradiol supplementation on the success of *in vitro* fertilization treatment: a prospective randomized study. *Fertility and Sterility*, *89*, 554–561.

Fahri, J., Weissman, A., Steinfeld, Z., Shorer, M., Nahum, H., & Levran, D. (2000). Estradiol supplementation during the luteal phase may improve the pregnancy rate in patients undergoing *in vitro* fertilization-embryo transfer cycles. *Fertility and Sterility*, *73*, 761–765.

Fanchin, R., Righini, C., Olivennes, F., Taylor, S., de Ziegler, D., & Frydman R. (1998). Uterine contractions at the time of embryo transfer alter pregnancy rates after *in-vitro* fertilization. *Human Reproduction*, *13*, 1968–1974.

Fasouliotis, S. J., Spandorfer, S. D., Witkin, S. S., Schattman, G., Liu, H. C., Roberts, J. E., et al. (2004). Maternal serum levels of interferon-γ and interleukin-2 soluble receptor-α predict the outcome of early IVF pregnancies. *Human Reproduction*, *19*, 1357–1363.

Frattarelli, J. L., McWilliams, G. D., Hill, M. J., Miller, K. A., & Scott, R. T. Jr. (2008). Low dose aspirin use does not improve *in vitro* fertilization outcomes in poor responders. *Fertility and Sterility*, *89*, 1113–1117.

Gelbaya, T. A., Kyrgiou, M., Li, T. C., Stern, C., & Nardo, L. G. (2007). Low-dose aspirin for *in vitro* fertilization: a systematic review and meta-analysis. *Human Reproduction Update*, *13*, 357–364.

Gelbaya, T. A., Kyrgiou, M., & Nardo, L. G. Letter to the editor – reply: 'Is it time for meta-analysis?' *Human Reproduction Update*, in press.

Gelbaya, T. A., Kyrgiou, M., Tsoumpou, I., & Nardo, L. G. (2008). The use of estradiol for luteal phase support in *in-vitro* fertilization/intracytoplasmic sperm injection cycles: a systematic review and meta-analysis. *Fertility and Sterility*, *90*, 2116–2125.

Geva, E., Yaron, Y., Lessing, J. B., Yovel, I., Vardinon, N., Burke, M., et al. (1994). Circulating autoimmune antibodies may be responsible for implantation failure in *in vitro* fertilization. *Fertility and Sterility*, *62*, 802–806.

Geva, E., Vardinon, N., Lessing, J. B., Lerner-Geva, L., Azem, F., Yovel, I., et al. (1996). Organ-specific autoantibodies are possible markers for reproductive failure: a prospective study in an *in-vitro* fertilization-embryo transfer programme. *Human Reproduction*, *11*, 1627–1631.

Geva, E., Amit, A., Lerner-Geva, L., Yaron, Y., Daniel, Y., Schwartz, T., et al. (2000). Prednisone and aspirin improve pregnancy rate in patients with reproductive failure and autoimmune antibodies: a prospective study. *American Journal of Reproductive Immunology*, *43*, 36–40.

Gorkemli, H., Ak, D., Akyurek, C., Aktan, M., & Duman, S. (2004). Comparison of pregnancy outcomes of progesterone or progesterone + estradiol for luteal phase support in ICSI-ET cycles. *Gynecologic and Obstetric Investigation*, *58*, 140–144.

Goswamy, R. K. & Steptoe, P. C. (1988). Doppler ultrasound studies of the uterine artery in spontaneous ovarian cycles. *Human Reproduction*, *3*, 721–726.

Harper, K., Proctor, M., & Hughes, E. (2003). Growth hormone for *in vitro* fertilization. *Cochrane Database Systematic Review*, Issue 3, Art no.: CD000099.

Hasegawa, I., Yamanoto, Y., Suzuki, M., Murakawa, H., Kurabayashi, T., Takakuwa, K., et al. (1998). Prednisolone plus low-dose aspirin improves the implantation rate in women with autoimmune conditions who are undergoing *in vitro* fertilization. *Fertility and Sterility*, *70*, 1044–1048.

Ho, H. N., Yang, Y. S., Hsieh, R. P., Lin, H. R., Chen, S. U., Chen, H. F., et al. (1994). Sharing of human leukocyte antigens in couples with unexplained infertility affects the success of *in vitro* fertilization and tubal embryo transfer. *American Journal of Obstetrics and Gynecology*, *170*, 63–71.

Hornstein, M. D., Davis, O. K., Massey, J. B., Paulson, R. J., & Collins, J. A. (2000). Antiphospholipid antibodies and *in vitro* fertilization success: a meta-analysis. *Fertility and Sterility*, *73*, 330–333.

Hurst, B. S., Bhojwani, J. T., Marshburn, P. B., Papadakis, M. A., Loeb, T. A., & Matthews, M. L. (2005). Low-dose aspirin does not improve ovarian stimulation, endometrial response, or pregnancy rates for *in vitro* fertilization. *Journal of Experimental & Clinical Assisted Reproduction*, *31*, 2–8.

Hutchinson-Williams, K., Lunenfeld, B., Diamond, M., Lagy, G., Boyers, S., & De Cherney, A. (1989). Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and non-conception cycles in an *in vitro* fertilization program. *Fertility and Sterility*, *52*, 441–445.

Kaider, A. S., Kaider, B. D., Janowicz, P. B., & Roussev, R. G. (1999). Immunodiagnostic evaluation in women with reproductive failure. *American Journal of Reproductive Immunology*, *42*, 335–346.

Kalu, E., Bhaskaran, S., Thum, M. Y., Vishwanatha, R., Croucher, C., Sherriff, E., et al. (2008). Serial estimation of Th1:Th2 cytokines profile in women undergoing *in-vitro* fertilization-embryo transfer. *American Journal of Reproductive Immunology*, *59*, 206–211.

Katz, U., Achiron, A., Sherer, Y., & Shoenfeld, Y. (2007). Safety of intravenous immunoglobulin (IVIG) therapy. *Autoimmunity Reviews*, *6*, 257–259.

Khairy, M., Banerjee, K., El-Toukhy, T., Coomarasamy, A., & Khalaf, Y. (2007). Aspirin in women undergoing *in vitro* fertilization treatment: a systematic review and meta-analysis. *Fertility and Sterility*, *88*, 822–831.

Kikuchi, K., Shibahara, H., Hirano, Y., Kohno, T., Hirashima, C., Suzuki, T., et al. (2003). Antinuclear antibody reduces the pregnancy rate in the first IVF-ET treatment cycle but not the cumulative pregnancy rate without specific medication. *American Journal of Reproductive Immunology*, *50*, 363–367.

Kim, C. H., Chae, H. D., & Chang, Y. S. (1999). Pyridostigmine cotreatment for controlled ovarian hyperstimulatiom in low responders undergoing *in vitro* fertilization embryo transfer. *Fertility and Sterility*, *71*, 652–657.

Knutzen, V., Stratton, C. J., Sher, G., McNamee, P. I., Huang, T. T., & Soto-Albors, C. (1992). Mock embryo transfer in early luteal phase, the cycle before *in vitro* fertilization and embryo transfer: a descriptive study. *Fertility and Sterility*, *57*, 156–162.

Kozer, E., Nikfar, S., Costei, A., Boskovic, R., Nulman, I., & Koren, G. (2002). Aspirin consumption during the first trimester of pregnancy and congenital anomalies: a meta-analysis. *American Journal of Obstetrics & Gynecology*, *187*, 1623–1630.

Kucuk, T., Kozinoglu, H., & Kaba, A. (2008). Growth hormone co-treatment within a GnRH-agonist long protocol in patients with poor ovarian response: a prospective, randomized, clinical trial. *Journal of Assisted Reproduction and Genetics*, *25*, 123–127.

Kutteh, W. H., Yetman, D. L., Chantilis, S. J., & Crain, J. (1997). Effect of antiphospholipid antibodies in women undergoing *in-vitro* fertilization: role of heparin and aspirin. *Human Reproduction*, *12*, 1171–1175.

Kutteh, W. H., Yetman, D. L., Carr, A. C., Beck, L. A., & Scott, R. T. Jr. (1999a). Increased prevalence of antithyroid antibodies identified in women with recurrent pregnancy loss but not in women undergoing assisted reproduction. *Fertility and Sterility*, *71*, 843–848.

Kutteh, W. H., Schoolcraft, W. B., & Scott, R. T. Jr. (1999b). Antithyroid antibodies do not affect pregnancy outcome in women undergoing assisted reproduction. *Human Reproduction*, *14*, 2886–2890.

Kwak-Kim, J. Y., Ching-Bang, H. S., Ng, S. C., Ntrivalas, E. I., Mangubat, C. P., Beaman, K. D., et al. (2003). Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Human Reproduction*, *18*, 767–773.

Lee, S. R., Park, E. J., Kim, S. H., Chae, H., Kim, C. H., Kang, B. M. (2007). Influence of antiphospholipid antibodies on pregnancy outcome in women undergoing *in vitro* fertilization and embryo transfer. *American Journal of Reproductive Immunology*, *57*, 34–39.

Lesny, P., Killick, S. R., Tetlow, R. L., Robinson, J., & Maguiness, S. D. (1998). Uterine junctional zone contractions during assisted reproduction cycles. *Human Reproduction Update*, *4*, 440–445.

Lewin, A., Benshushan, A., Mezker, E., Yanai, N., Schenker, J., & Goshen, R. (1994). The role of estrogen support during the luteal phase of *in vitro* fertilization-embryo transplant cycles: a comparative study between progesterone alone and estrogen and progesterone support. *Fertility and Sterility*, *62*, 212–225.

Lim, W. & Crowther, M. A. (2007). Antiphospholipid antibiodies: a critical review of the literature. *Current Opininon in Hematology*, *14*, 494–499.

Lok, I. H., Yip, S., & Cheung, L. P. (2004). Adjuvant low-dose aspirin therapy in poor responders undergoing *in vitro* fertilization: a prospective, randomized, double-blind, placebo-controlled trial. *Fertility and Sterility*, *81*, 556–561.

Luborsky, J., Llanes, B., Davies, S., Binor, Z., Radwanska, E., & Pong, R. (1999). Ovarian autoimmunity: greater frequency of autoantibodies in premature menopause and unexplained infertility than in the general population. *Clinical Immunology*, *90*, 368–374.

Lukaszuk, K., Liss, J., Lukaszuk, M., & Maj, B. (2005). Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing *in vitro* fertilization-embryo transfer cycles. *Fertility and Sterility*, *83*, 1372–1376.

Martin-Villa, J. M., De Juan, D., Vicario, J. L., Luque, I. M., Alvarez, E., Cortes-Prieto, J., et al. (1993). HLA class I, class II, and class III antigen sharing is not found in couples with unexplained infertility. *International Journal of Fertility and Menopausal Studies*, *38*, 280–288.

Matteo, M. G., Greco, P., Rosenberg, P., Mestice, A., Baldini, D., Falagario, T., et al. (2007). Normal percentage of CD56bright natural killer cells in young patients with a history of repeated unexplained implantation failure after *in vitro* fertilization cycles. *Fertility and Sterility*, *88*, 990–993.

Miyakis, S., Lockshin, M. D., Atsumi, T., Branch, D. W., Brey, R. L., Cervera, R., et al. (2006). International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *Journal of Thrombosis and Haemostasis*, *4*, 295–306.

Moffett-King, A. (2002). Natural killer cells and pregnancy. *Nature Reviews Immunology*, *2*, 656–663.

Morizaki, N., Morizaki, J., Hayashi, R. H., & Garfield, R. E. (1989). A functional and structural study of the innervation of the human uterus. *American Journal of Obstetrics and Gynecology*, *160*, 218–228.

National Institute for Health and Clinical Excellence. (2004). Fertility: assessment and treatment for people with fertility problems. *Clinical Guideline 11*, 12.3.

Negro, R., Formoso, G., Coppola, L., Presicce, G., Mangieri, T., Pezzarossa, A., et al. (2007). Euthyroid women with autoimmune disease undergoing assisted reproduction technologies: the role of autoimmunity and thyroid function. *Journal of Endocrinological Investigation*, *30*, 3–8.

Nelson, S. M. & Greer, I. A. (2008). The potential role of heparin in assisted conception. *Human Reproduction Update*, *14*, 623–645.

Ng, S. C., Gilman-Sachs, A., Thaker, P., Beaman, K. D., Beer, A. E., & Kwak-Kim, J. (2002). Expression of intracellular Th1 and Th2 cytokines in women with recurrent spontaneous abortion, implantation failures after IVF/ET or normal pregnancy. *American Journal of Reproductive Immunology*, *48*, 77–86.

Nordlander, C., Fuchs, T., Hammarstrom, L., & Smith, C. I. (1983). Human leukocyte antigens group A in couples with unexplained infertility. *Fertility and Sterility*, *40*, 60–65.

Ohl, J., Lefebvre-Maunoury, C., Wittemer, C., Nisand, G., Laurent, M. C., & Hoffmann, P. (2002). Nitric oxide donors for patients undergoing IVF. A prospective, double-blind, randomized, placebo-controlled trial. *Human Reproduction*, *17*, 2615–2620.

Okazaki, M., Matsuyama, T., Kohno, T., Shindo, H., Koji, T., Morimoto, Y., et al. (2005). Induction of epithelial cell apoptosis in the uterus by a mouse uterine ischemia-reperfusion model: possible involvement of tumor necrosis factor-alpha. *Biology of Reproduction*, *72*, 1282–1288.

Omu, A. E., al-Qattan, F., Ismail, A. A., al-Taher, S., & al-Busiri, N. (1999). Relationship between unexplained infertility and human leukocyte antigens and expression of circulating autogeneic and allogeneic antisperm antibodies. *Clinical and Experimental Obstetrics and Gynecology*, *26*, 199–202.

Opatrny, L., David, M., Kahn, S. R., Shrier, I., & Rey, E. (2006). Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a meta-analysis. *Journal of Rheumatology*, *33*, 2214–2221.

Päkkilä, M., Räsänen, J., Heinonen, S., Tinkanen, H., Tuomivaara, L., Mäkikallio, K., et al. (2005). Low-dose aspirin does not improve ovarian responsiveness or pregnancy rate in IVF and ICSI patients: a randomized, placebo-controlled double-blind study. *Human Reproduction*, *20*, 2211–2214.

Patrono, C., Garcia Rodriguez, L. A., Landolfi, R., & Baigent, C. (2005). Low-dose aspirin for the prevention of atherosclerosis. *New England Journal of Medicine*, *353*, 2373–2383.

Pinheiro, O. L., Cavagna, M., Baruffi, R. L. R., Mauri, A. L., Petersen, C., & Franco, J. G. Jr. (2003). Administration of β2-adrenergic agonists during the peri-implantation period does not improve implantation or pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles. *Journal of Assisted Reproduction and Genetics*, *20*, 513–516.

Pires, E. S., Meherji, P. K., Vaidya, R. R., Parikh, F. R., Ghosalkar, M. N., & Khole, V. V. (2007). Specific and sensitive immunoassays detect multiple anti-ovarian antibodies in women with infertility. *The Journal of Histochemistry and Cytochemistry*, *55*, 1181–1190.

Qublan, H. S., Eid, S. S., Ababneh, H. A., Amarin, Z. O., Smadi. A. Z., Al-Khafaji, F. F., et al. (2006). Acquired and inherited thrombophilia: implication in recurrent IVF and embryo transfer failure. *Human Reproduction*, *21*, 2694–2698.

Quenby, S., Bates, M., Doig, T., Brewster, J., Lewis-Jones, D. I., Johnson, P. M., et al. (1999). Pre-implantation endometrial leukocytes in women with recurrent miscarriage. *Human Reproduction*, *14*, 2386–2391.

Revelli, A., Dolfin, E., Gennarelli, G., Lantieri, T., Massobrio, M., Holte, J. G., et al. (2008). Low-dose acetylsalicylic acid plus prednisolone as an adjuvant treatment in IVF: a prospective, randomized study. *Fertility and Sterility*, *90*, 1685–1691.

Rogolino, A., Coccia, M. E., Fedi, S., Gori, A. M., Cellai, A. P., Scarselli, G. F., et al. (2003). Hypercoagulability, high tissue factor and low tissue factor pathway inhibitor levels in severe ovarian hyperstimulation syndrome: possible association with clinical outcome. *Blood Coagulation & Fibrinolysis*, *14*, 277–282.

Royal College of Obstetricians and Gynaecologists. Scientific Advisory Committee Opinion Paper 5. (2008). Immunological testing and interventions for reproductive failure. Available at: http://www.rcog.org.uk/resources/Public/pdf/SAC_Paper_5

Rubinstein, M., Marazzi, A., & Polak, D. F. (1999). Low dose aspirin treatment improves ovarian responsiveness, uterine and ovarian blood flow velocity, implantation and pregnancy rates in patients undergoing *in vitro* fertilization: a prospective, randomized double blind placebo controlled assay. *Fertility and Sterility*, *71*, 825–829.

Ruopp, M. D., Collins, T. C., Whitcomb, B. W., & Schisterman, E. F. (2008). Evidence of absence or absence of evidence? A re-analysis of the effects of low-dose aspirin in *in-vitro* fertilization. *Fertility and Sterility*, *90*, 71–76.

Schenk, L. M., Butler, L., Monis, J. P., Cox, B., Leete, J., Abuhamed, A., et al. (1996). Heparin and aspirin treatment yields higher implantation rates in IVF patients with antiphospholipid antibody seropositivity compared to untreated seronegative patients. In: Fifty-Second Annual Meeting of the American Society for Reproductive Medicine, Boston, MA, USA, Nov 2–6, 1996.

Sengupta, J., Dhawan, L., Lalitkumar, P. G. L., & Ghosh, D. (2005). Nitric oxide in blastocyst implantation in the rhesus monkey. *Reproduction*, *130*, 321–332.

Serna, J., Cholquevilque, J. L., Cela, V., Martinez-Salazar, J., Requena, A., & Garcia-Velasco, J. A. (2008). Estradiol supplementation during the luteal phase of IVF-ICSI patients: a randomized, controlled trial. *Fertility and Sterility*, *90*, 2190–2195.

Shaker, A. G., Fleming, R., Jamieson, M. E., Yates, R. W., & Coutts, J. R. (1993). Assessments of embryo transfer after *in-vitro* fertilization: effects of glyceryl trinitrate. *Human Reproduction*, *8*, 1426–1428.

Sher, G. & Fisch, J. D. (2000). Vaginal sildenafil (Viagra): a preliminary report of a novel method to improve uterine artery blood flow and endometrial development in patients undergoing IVF. *Human Reproduction*, *15*, 806–809.

Sher, G., Maassarani, G., Zouves, C., Feinman, M., Sohn, S., Matzner, W., et al. (1998a). The use of combined heparin/aspirin and immunoglobulin G therapy in the treatment of *in vitro* fertilization patients with antithyroid antibodies. *American Journal of Reproductive Immunology*, *39*, 223–225.

Sher, G., Matzner, W., Feinman, M., Maassarani, G., Zouves, C., Chong, P., et al. (1998b). The selective use of heparin/aspirin therapy, alone or in combination with intravenous immunoglobulin G, in the management of antiphospholipid antibody-positive women undergoing *in vitro* fertilization. *American Journal of Reproductive Immunology*, *40*, 74–82.

Sher, G., Zouves, C., Feinman, M., Maassarani, G., Matzner, W., Chong, P., et al. (1998c). A rational basis for the use of combined heparin/aspirin and IVIG immunotherapy in the treatment of recurrent IVF failure associated with antiphospholipid antibodies. *American Journal of Reproductive Immunology*, *39*, 391–394.

Sherer, Y., Levy, Y., Langevitz, P., Rauova, L., Fabrizzi, F., & Shoenfeld, Y. (2001). Adverse effects of intravenous immunoglobulin therapy in 56 patients with autoimmune diseases. *Pharmacology*, *62*, 133–137.

Smitz, J., Bourgain, C., Van Waesberghe, L., Camus, M., Devroey, P., & Van Steirteghem, A. (1993). A prospective randomized study on estradiol valerate supplementation in addition to intravaginal micronized progesterone in buserelin and HMG induced superovulation. *Human Reproduction*, *8*, 40–45.

Steer, C. V., Campbell, S., Tan, S. L., Crayford, T., Millis, C., Mason, B. A., et al. (1992). The use of transvaginal color flow imaging after *in vitro* fertilization identifies optimum uterine conditions before embryo transfer. *Fertility and Sterility*, *57*, 372–376.

Stephenson, M. D. & Fluker, M. R. (2000). Treatment of repeated unexplained *in vitro* fertilization failure with intravenous immunoglobulin: a randomized, placebo-controlled Canadian trial. *Fertility and Sterility*, *74*, 1108–1113.

Stern, C., Chamley, L., Hale, L., Kloss, M., Speirs, A., & Baker, H. W. (1998). Antibodies to beta2 glycoprotein I are associated with *in vitro* fertilization implantation failure as well as recurrent miscarriage: results of a prevalence study. *Fertility and Sterility*, *70*, 938–944.

Stern, C., Chamley, L., Norris, H., Hale, L., & Baker, H. W. (2003). A randomized, double-blind, placebo-controlled trial of heparin and aspirin for women with *in vitro* fertilization implantation failure and antiphospholipid or antinuclear antibodies. *Fertility and Sterility*, *80*, 376–383.

Taniguchi, F. (2005). Results of prednisolone given to improve the outcome of *in vitro* fertilization-embryo transfer in women with antinuclear antibodies. *Journal of Reproductive Medicine*, *50*, 383–388.

Tay, P. Y. S. & Lenton, E. A. (2003). Inhibition of progesterone secretion by oestradiol administration in the luteal phase of assisted conception cycles. *Medical Journal of Malaysia*, *58*, 187–195.

Thum, M. Y., Bhaskaran, S., Abdalla, H. I., Ford, B., Sumar, N., Shehata, H., et al. (2004). An increase in the absolute count of CD56dimCD16+CD69+ NK cells in the peripheral blood is associated with a poorer IVF treatment and pregnancy outcome. *Human Reproduction*, *19*, 2395–2400.

Thum, M. Y., Abdalla, H. I., Bhaskaran, S., Harden, E. L., Ford, B., Sumar, N., et al. (2007). The relationship of systemic TNF-α and IFN-γ with IVF treatment outcome and peripheral blood NK cells. *American Journal of Reproductive Immunology*, *57*, 210–217.

Urman, B., Mercan, R., Alatas, C., Balaban, B., Isiklar, A., & Nuhoglu, A. (2000). Low dose aspirin does not increase implantation rates in patients undergoing intracytoplasmic sperm injection: a prospective randomized study. *Journal of Assisted Reproduction and Genetics*, *17*, 586–590.

van Gestel, I., Ijland, M. M., Hoogland, H. J., & Evers, J. L. (2003). Endometrial wave-like activity in the non-pregnant uterus. *Human Reproduction Update*, *9*, 131–138.

Waldenström, U., Hellberg, D., & Nilsson, S. (2004). Low-dose aspirin in a short regimen as standard treatment in *in vitro* fertilization: a randomized, prospective study. *Fertility and Sterility*, *81*, 1560–1564.

Weckstein, L. N., Jacobson, A., Galen, D., Hampton, K., & Hammel, J. (1997). Low-dose aspirin for oocyte donation recipients with a thin endometrium: prospective, randomized study. *Fertility and Sterility*, *68*, 927–930.

Wilson, W. A., Gharavi, A. E., Koike, T., Lockshin, M. D., Branch, D. W., Piette, J. C., et al. (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis and Rheumatism*, *42*, 1309–1311.

Yetman, D. L., Kutteh, W. H., Castorena, R., Brown, C., & Baskin, L. (1997). Prevalence of elevated anticardiolipin antibodies in pregnant women with unexplained elevations of alpha-fetoprotein. *Journal of Reproductive Immunology*, *33*, 71–81.

Younis, S., Ezra, Y., Sherman, Y., Simon, A., Schencker, G., & Laufer, N. (1994). The effect of estradiol depletion during the luteal phase on endometrium development. *Fertility and Sterility*, *62*, 103–107.

Zhang, X., Wang, H. M., Lin, H. Y., Liu, G. Y., Li, Q. L., & Zhu, C. (2004). Regulation of matrix metalloproteinases (MMPS) and their inhibitors (TIMPS) during mouse peri-implantation: role of nitric oxide. *Placenta*, *25*, 243–252.