ADJUVANTS IN IVF

British Fertility Society Policy and Practice Committee: Adjuvants in IVF: Evidence for good clinical practice

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Abstract
Optimisation of the environment favourable for satisfactory ovarian response to stimulation and successful embryo implantation remains at the core of assisted conception programmes. The evidence base for the routine use of different adjuvants, alone or in combination, for women undergoing their first in vitro fertilisation (IVF) treatment cycle and for those with poor prognosis is inadequate. The aim of this document is to update the last review of the available literature carried out by the British Fertility Society Policy and Practice Committee (BFS P&P) published in 2009 and to provide fertility professionals with evidence-based guidance and recommendations regarding the use of immunotherapy, vasodilators, uterine relaxants, aspirin, heparin, growth hormone, dehydroepiandrosterone, oestrogen and metformin as adjuvants in IVF. Unfortunately despite the lapse of 5 years since the last publication, there is still a lack of robust evidence for most of the adjuvants searched and large well-designed randomised controlled trials are still needed. One possible exception is metformin, which seems to have a positive effect in women with polycystic ovary syndrome undergoing IVF. Patients who are given other adjuvants on an empirical basis should always be informed of the lack of evidence and the potential side effects.

Keywords: IVF, adjuvants, recurrent implantation failure, auto-immunity, thrombophilia, pregnancy outcome

Introduction
Success rates with in vitro fertilisation (IVF) are variable and having a negative outcome is the most devastating of experiences for the patients. Fertility physicians are faced with the challenge of achieving successful pregnancy outcomes, while emotionally distraught couples seek fertility clinics and specialists who will offer additional treatment to enhance their chance of conception.

Endometrial receptivity is considered critical for successful implantation. Potential mechanisms implicated for embryo implantation failure include endometrial immune hostility, sub-optimal uterine perfusion, inadequate luteal phase support and increased myometrial contractility. Adjuvant therapies have been used in conjunction with IVF regimes in an attempt to counteract causes of repeated implantation failure as well as increase the chances of having a fresh embryo transfer by optimising ovarian response and minimising the risk of ovarian hyperstimulation syndrome (OHSS). However, the quality of studies that have investigated the role of adjuvants is variable; interpretation of the results and routine application in the clinical setting is therefore debatable. It is imperative that the various adjuvant therapies are tested in high-quality, adequately powered randomised controlled trials (RCTs) to provide patients and clinicians with evidence-based information on treatment strategies, which are genuinely effective in enhancing live birth rates after IVF treatment.

Since the last scoping review on behalf of the BFS P&P Committee (Nardo et al., 2009), a number of studies have been conducted on adjuvants in IVF, looking for any effect on outcomes. This document aims to review the available literature and provide fertility professionals with evidence-based guidance regarding the use of immunotherapy, vasodilators, uterine relaxants, aspirin, heparin, growth hormone (GH), dehydroepiandrosterone (DHEA), oestrogen and metformin supplementation. It also includes recommendations for good clinical practice.

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Methodology

A search of online databases (MEDLINE, EMBASE, Cochrane library and Central register of controlled trials) was performed using the Keywords: IVF, ovarian response, OHSS, live birth rate, pregnancy rate, immunotherapy, intravenous immunoglobulin (IVIg), tumour necrosis factor (TNF)-alpha, intralipids, steroids, vasodilators, uterine relaxants, progesterone, aspirin, heparin, GH, DHEA, oestrogen and metformin adjuvants in IVF. The reference list of all included articles (N=155) was examined to identify cited articles not obtained by electronic search. No language restrictions were in place. The literature search and study inclusion were undertaken by all authors, whilst the interpretation of final data and quality assessment was undertaken by the coordinating author (LGN).

The outcome measures included response to ovarian stimulation, clinical pregnancy rate, live birth rate, miscarriage rate and foeto-maternal complication, where data were available.

Immune therapy

In recent years, aberrant immune response has gained attention as a potential cause of ‘failure to implant’ and despite the paucity of robust evidence, immune therapies have been administered. A recent web-based IVF-worldwide survey has shown that assays for anti-cardiolipin antibody (ACA), lupus anticoagulant (LA), thyroid peroxidase antibodies (TPA) and anti-nuclear antibody (ANA) are the four most commonly requested tests for women, while repeated implantation failures and cellular immune evaluations are less commonly undertaken (Kwak-Kim et al., 2013). This review systematically examined IVF outcome after IVIg adjuvant therapy (Elram et al., 2011a; Moraru et al., 2012; Virro et al., 2012). This review included 10 studies published between 1994 and 2012, in which 1,477 women received IVIg as adjuvant therapy during their IVF treatment. The authors reported that the use of IVIg was associated with a significantly higher implantation rate relative risk (RR = 2.708, confidence interval 95%CI: 1.302-5.629), clinical pregnancy rate (RR = 1.463, 95%CI: 1.075-1.991) and live birth rate (RR = 1.616, 95%CI: 1.243-2.101), and a significantly lower miscarriage rate (RR = 0.352, 95%CI: 0.168-0.738). However, most of the studies included were retrospective, poorly designed, clinically heterogeneous and had a number of methodological flaws that could introduce bias. Apart from the lack of randomisation, the studies included a mixture of patients with recurrent implantation failure, unexplained infertility and recurrent miscarriage, used multiple adjuvant interventions and a variety of IVIg doses and regimes, and compared their results with historical controls (Li et al., 2013). In addition, cost-effectiveness analysis was not part of any of these studies. Thus, larger randomised trials with strict inclusion and exclusion criteria are warranted to evaluate the role of IVIg adjuvant therapy in improving IVF outcome.

It is also important to emphasise that IVIg is a pooled blood product and therefore its use carries the potential risks of anaphylaxis and infection (Carbone, 2007). IVIg is also associated with other complications in up to 35% of cases (Feldmeyer et al., 2010; Palabrica et al., 2013). Majority of such side effects are mild and transient, including itching, headache, flushing, low backache, nausea, fatigue and skin reaction, and are often related to the rate of infusion, total dose and brand of IVIg infused. Serious side effects such as septic meningitis, severe anaphylactic reaction, acute renal failure and thrombotic events are rare (Souayah et al., 2011).

Recommendation(s): There is no convincing evidence for the use and safety of IVIg as adjuvants in women with recurrent implantation failure embarking on IVF. The use of IVIg in this setting cannot be supported.

Intravenous immunoglobulin

IVIg has been used for the treatment of recurrent implantation failure after IVF either empirically or for patients in whom immunological testing has shown increased number of peripheral natural killer (NK) cells or cytotoxicity, abnormal T helper (Th1:Th2 ratio, positive anti-thyroid antibody or anti-phospholipid antibody (APL) test, or increased TNF-α level or human leukocyte antigen (HLA) antigens similarity, in the hope of improving IVF outcomes (Elram et al., 2005; Clarke et al., 2006).

The proposed mechanisms of action of IVIg include inhibiting NK cell production and/or activity, correcting abnormal Th1:Th2 ratio and non-specific immunomodulation leading to enhancement of immunological tolerance.

Several observational case–control studies have examined IVF outcome after IVIg adjuvant therapy (Winger et al., 2009; Heilmann et al., 2010; Winger et al., 2011a; Moraru et al., 2012; Virro et al., 2012). Recently, an attempt to summarises the results of those studies systematically (Li et al 2013). This review included 10 studies published between 1994 and 2012, in which 1,477 women received IVIg as adjuvant therapy during their IVF treatment. The authors reported that the use of IVIg was associated with a significantly higher implantation rate relative risk (RR = 2.708, confidence interval 95%CI: 1.302-5.629), clinical pregnancy rate (RR = 1.463, 95%CI: 1.075-1.991) and live birth rate (RR = 1.616, 95%CI: 1.243-2.101), and a significantly lower miscarriage rate (RR = 0.352, 95%CI: 0.168-0.738). However, most of the studies included were retrospective, poorly designed, clinically heterogeneous and had a number of methodological flaws that could introduce bias. Apart from the lack of randomisation, the studies included a mixture of patients with recurrent implantation failure, unexplained infertility and recurrent miscarriage, used multiple adjuvant interventions and a variety of IVIg doses and regimes, and compared their results with historical controls (Li et al., 2013). In addition, cost-effectiveness analysis was not part of any of these studies. Thus, larger randomised trials with strict inclusion and exclusion criteria are warranted to evaluate the role of IVIg adjuvant therapy in improving IVF outcome.

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Tumor necrosis factor-alpha

An exaggerated Th1 response is thought to be detrimental to the process of implantation and has been linked to infertility (Clark, 2010). TNF-α is a pro-inflammatory Th1 cytokine implicated in endometriosis-related infertility and IVF failure (Falcorner et al., 2009).

Using a mouse model, Okazaki and colleagues (2005) demonstrated that local TNF-α expression was critical in inducing endometrial cell apoptosis. In addition, exposure to an elevated TNF-α concentration caused a reduction in porcine oocyte maturation and increased the frequency of defective chromosome alignment and cytoskeletal structure (Ma et al., 2010). Thum and colleagues (2007) reported an association between elevated systemic TNF-α level and high levels of activated NK cells. However, the authors as well as others (Fasouliotis et al., 2004; Asimakopoulos et al., 2010) could not find a direct correlation between cir-
calculating TNF-α levels and IVF outcome. By contrast, Kalu and colleagues (2008) reported an increased TNF-α:interleukin-4 ratio in women with recurrent IVF failure and that Th1:Th2 ratio increased following controlled ovarian stimulation.

The above observations have led to elevated TNF-α levels being targeted for therapeutic correction (Clark, 2009). The potential reproductive benefit of anti-TNF-α agents [Adalimumab (Humira), Enantercept (Enbrel) and Infliximab (Remicaid)] has been studied in infertile patients with a raised Th1:Th2 cytokine ratio (Jerzak et al., 2010; Winger et al., 2011b; Winger et al., 2012). A small retrospective case–control study of women with Th1:Th2 cytokine elevation reported a live birth rate of 73% in 41 patients who received IVIg and Adalimumab; 50% in 6 patients who received Adalimumab alone and no live birth in 5 patients who received neither (Winger et al., 2009).

Any advantage gained from the use of anti-TNF-α agents has to be balanced against the potential adverse effects of immune suppression. Prolonged use of anti-TNF-α agents has been linked with an increased risk of developing lymphoma, skin cancer and granulomatous infections (Bongartz et al., 2006; Bongartz et al., 2009); however, the risk profile of short-term use of such medication is far from clear. Well-designed, appropriately conducted large clinical trials are needed to elucidate the role and safety of using anti-TNF-α agents as an adjunctive treatment to improve IVF outcome.

Recommendation(s): There is a lack of evidence to indicate the effectiveness and safety of using anti-TNF-α agents as an adjuvant in IVF cycles. The use of anti-TNF-α agents in this setting cannot therefore be supported.

Intravenous lipids

It has recently been suggested that 20% intravenous lipid solution can improve implantation in women with repeated implantation failure and raised Th1 response (Ndewke, 2011). Whilst the precise immune-modulatory mechanism of action remains unknown, it is postulated that intralipids inhibit pro-inflammatory factors such as Th1 cytokines (Granato et al., 2000) and that the fatty acids work as ligands to activate peroxisome proliferator-activated receptors expressed by the NK cells, which in turn reduces the cytotoxic effects of NK cells (Roussev et al., 2008). However, there is paucity of evidence regarding immune-modulatory action and efficacy of intralipid infusion in women undergoing IVF treatment. Large well-designed clinical trials are needed.

Recommendation(s): There is a lack of evidence to recommend intralipid infusion therapy as an adjuvant in IVF cycles. The use of intravenous lipids in this setting cannot therefore be supported.

Corticosteroids

Immunological dysfunction or autoimmunity has been associated with reproductive failure and lower IVF success rates. Corticosteroids have anti-inflammatory and immune-suppressive activity and might therefore improve the intrauterine environment by reducing endometrial pro-inflammatory cytokines production and NK cell activity (Boomsma & Macklon, 2008).

Corticosteroids have been proposed as an adjuvant to IVF. A Cochrane review (Boomsma et al., 2012) investigated whether empirical use of corticosteroids in women undergoing IVF or intracytoplasmic sperm injection (ICSI) could improve treatment outcomes. Fourteen studies involving a total of 1879 couples were included. Pooling of the results showed no evidence that corticosteroids improved the clinical pregnancy rate odds ratio (OR: 1.16, 95%CI: 0.94–1.44) or live birth rate (OR: 1.21, 95%CI: 0.67–2.19). A subgroup analysis of patients undergoing IVF, rather than ICSI, revealed a significantly higher pregnancy rate in women taking corticosteroids (OR: 1.5, 95%CI: 1.05–2.13).

The use of corticosteroids in women known to have an abnormal immunological test, such as elevated NK cells or presence of auto-antibodies including ACA, anti-thyroid, ANA and anti-ovarian antibodies, has also been examined. A very recent systematic review (Polanski et al., 2014) of the use of corticosteroids in women with elevated NK cells undergoing IVF found only one study which suggested that oral prednisolone could increase the clinical pregnancy rate (OR: 1.63, 95%CI: 1.00–2.66). The conclusions did not support the use of prednisolone during IVF in this subset of women due to paucity of evidence.

In a retrospective study (Ying et al., 2012), 56 women with high ACA undergoing IVF were treated with adjuvant methylprednisolone and low-dose aspirin and compared with 60 women with high ACA who were not given adjuvant therapy and 518 women negative for ACA. The pregnancy and implantation rates (46.4% and 25.4%, respectively) in the treated ACA group were significantly higher than those in the untreated ACA group (33.3% and 17.9%, respectively), but similar to the ACA negative group (53.9% and 32.3%, respectively). A further retrospective study (Revell et al., 2009) evaluated the role of combined corticosteroids, aspirin and levothyroxine therapy in 76 women with anti-thyroid antibodies undergoing IVF. Higher pregnancy and implantation rates were reported in the anti-thyroid-antibodies-treated group (25.6% and 17.7%, respectively) compared with those in the untreated group (7.5% and 4.7%, respectively).

A number of investigators have reported beneficial effects of using steroids alone or in conjunction with low-dose aspirin in women with positive ANA undergoing IVF (Ando et al., 1996; Hasegawa et al., 1998; Geva et al., 2000), whereas others have not found any statistically significant improvement in success rates (Taniguchi, 2005).

The combination of prednisolone and low-molecular-weight heparin (LMWH) as adjuvant therapy in women who previously had one or more unexplained IVF or ICSI failure has recently been studied (Siristadis
Nitric oxide and nitroglycerine

Nitric oxide (NO) is a vasodilator with a significant regulatory role on the smooth muscle of blood vessels. It has been shown that NO is involved in the fertilisation process and plays a role in deciduation and implantation (Telfer et al., 1997; Chwalisz et al., 1999). Its vasodilating effect is mediated via a cyclic guanosine monophosphate (c-GMP)-dependent pathway or through induction of metalloproteases that help with remodeling the extracellular matrix during implantation (Zhang et al., 2004). Exposure to NO production has been associated with pregnancy failure (Sengupta et al., 2005).

Nitroglycerine (NTG) is an NO donor, and has been investigated in a RCT which assessed its efficacy in inducing uterine vasodilatation and thereby endometrial receptivity (Ohl et al., 2002). A total of 138 women with history of two previous implantation failures and good-quality embryos were randomised the day before embryo transfer into receiving a 5-mg NTG patch, or placebo, daily until pregnancy test. The authors found no significant difference in the implantation and pregnancy rates or uterine artery Doppler between the NTG and placebo groups.

Sildenafil citrate

Sildenafil citrate (Viagra) has been used empirically to improve endometrial thickness in women undergoing IVF treatment. It is a type-5 phosphodiesterase inhibitor that prevents breakdown of c-GMP and potentiates the effect of NO on vascular smooth muscle. Studies in the literature have shown controversial results. In a prospective study (Takasaki et al., 2010) of 61 patients with a thin endometrium (<8mm) and high uterine radial artery resistance index (RA-RI: ≥ 0.81) it was found that sildenafil improved RA-RI in 12 patients and endometrial thickness in 11 of those 12 patients. In some small studies with a cross-over study design and using sildenafil–placebo–sildenafil with oestradiol valerate, uterine artery pulsatility index (PI) reduced with sildenafil and reverted to normal during placebo use. Similarly, endometrial thickness increased to more than 10 mm with improvement in pregnancy rates (Sher & Fisch, 2000; Sher & Fisch, 2002). By contrast, Check and colleagues (2004) failed to demonstrate an increase in endometrial thickness or blood flow following addition of sildenafil to an oestrogen supplemented regime in women undergoing fresh IVF or frozen embryo transfer.

Recommendation(s): Both NTG and sildenafil have been shown to have significant beneficial effects on IVF outcome, and their routine use as adjuvants in IVF cycles is not recommended.

Uterine relaxants

Throughout a normal menstrual cycle, the uterine smooth muscle shows variation in its contractility (van Gestel et al., 2003). However, at the time of IVF uterine activity is increased compared with that in natural cycle conception (Lesny et al., 1998). This adverse uterine activity at the time of embryo transfer can occur as a result of a number of factors, including early timing of transfer in the luteal phase, mechanical stimulation and supraphysiological hormonal milieu (Morizaki et al., 1989). At the time of embryo transfer increased uterine tone can make the process more difficult and increased contractility can lead to ectopic pregnancy (Shaker et al., 1993). In an attempt to optimise IVF success rates, different investigators have used uterine smooth muscle relaxants.

Nitroglycerine

As already discussed, NTG is a NO donor and along with its vasodilatation action, it relaxes the uterine smooth muscle. No difference has been observed with its use 3 min before embryo transfer in the ease of transfer or the pregnancy rates (Shaker et al., 1993).

β2-Adrenergic antagonists

Selective β2-adrenergic blockers (Ritodrine, Terbutaline and Salbutamol) are known uterine smooth muscle relaxants used in obstetric for management of pre-term labour and before external cephalic version. Administration of these agents for two weeks following oocyte retrieval fails to improve implantation and pregnancy rates (Pinheiro et al., 2003), whilst causing adverse effects such as hypotension and tachycardia.

Progesterone

By analogy with its use in pre-term labour, progesterone has been used in IVF to promote uterine quiescence. In
the IVF setting, increased uterine contractility has been linked with a reduction in positive outcomes (Fanchin et al., 1998) and progesterone therapy has been used to promote uterine quiescence.

**Recommendation(s):** There is a lack of strong evidence to support the use of uterine relaxants (NTG, selective β2-adrenergic blockers and progesterone) as adjuvants in IVF cycles.

**Aspirin**

Acetylsalicyclic acid (Aspirin) is a non-steroidal anti-inflammatory agent that works by inhibition of cyclo-oxygenase enzyme in platelets and reduction of prostaglandin synthesis (Vane et al., 1990). Patrano and colleagues (2005) observed that daily administration of aspirin caused a shift from thromboxane $A_2$ to prostacyclin, thereby leading to vasodilation and increased peripheral blood flow. Aspirin also increases uterine blood flow (Wada et al., 1994) that in turn may enhance endometrial receptivity and improve implantation rates.

Although use of aspirin has a beneficial effect in women with APL syndrome and recurrent miscarriage and in prevention of pre-eclampsia, the evidence supporting its use in women undergoing IVF cycles is controversial. Various studies and seven meta-analyses (Gelbaya et al., 2007; Khairy et al., 2007; Poustie et al., 2007; Ruopp et al., 2008; Groeneveld et al., 2011; Siristatidis et al., 2011; Dentali et al., 2012) have provided conflicting evidence. However, a Cochrane review (Siristatidis et al., 2011) has confirmed that the use of aspirin in IVF does not improve pregnancy rates. In this meta-analysis 13 trials with a total of 2653 subjects were included. No significant differences were found between aspirin and control group for live birth rate (RR: 0.91, 95%CI: 0.72–1.15), clinical pregnancy rate (RR: 1.03, 95%CI: 0.91–1.17) and miscarriage rate (RR: 1.10, 95%CI: 0.68–1.77). Interestingly, similar results have been obtained after performing individual patient data meta-analysis (IPD meta-analysis) using published data (Groeneveld et al., 2011).

Geva and colleagues (2000) concluded that low-dose aspirin and prednisolone administered before ovarian stimulation improves pregnancy rate in women with positive auto-antibodies and repeated IVF cycle failures. A recent IPD meta-analysis of 268 pregnancies from four studies showed that pre-conceptual administration of low-dose aspirin in IVF patients does not confer benefits in sustaining pregnancy (Groeneveld et al., 2013).

**Recommendation(s):** There is lack of proven efficacy for routine use of aspirin as an adjuvant in IVF cycles.

**Heparin**

Heparin is a poly-sulphated glycosaminoglycan that interacts with positively charged amino acids. LMWH is the depolymerised form of unfractionated heparin with similar action but increased bioavailability and half-life. Heparin exerts its anti-thrombotic effect by inhibition of factor Xa and thrombin (Linhardt et al., 1992). In women with APA and ACL, the antibodies bind to human trophoblast cell β2-glycoprotein and lead to defective placentation (Chamley et al., 1998; Di Simone et al., 2000). It has also been observed that during the process of controlled ovarian stimulation the activation of the coagulation cascade and impaired fibrinolysis are associated with unsuccessful pregnancy outcome (Rogolino et al., 2003). Two research groups found that thrombophilia is more common in women with repeated implantation failure compared with that in healthy fertile controls (Coulam et al., 2006; Qublan et al., 2006).

Consistent with the above scientific reasoning, heparin improved pregnancy outcomes in a proportion of women with thrombophilia undergoing assisted conception treatment. It is postulated that thrombophilia causes micro-thrombi at the site of implantation leading to impaired invasion of maternal vessels by the syncytiotrophoblast (Geva et al., 1995; Azem et al., 2004). In these women, heparin can potentially improve implantation; however, observational studies reached conflicting conclusions (Sher et al., 1994; Schenk et al., 1996; Kutteh et al., 1997; Sher et al., 1998; Stern et al., 2003; Qublan et al., 2008). Some studies (Schenk et al., 1996; Kutteh et al., 1997; Stern et al., 2003) showed that unfractionated heparin and low-dose aspirin do not improve pregnancy rates, whereas others demonstrated a significant difference in pregnancy rates in women with thrombophilia receiving heparin treatment with or without low-dose aspirin (Sher et al., 1994; Sher et al., 1998; Qublan et al., 2008).

Three meta-analyses evaluating the efficacy of heparin in women undergoing IVF treatment cycles (Seshadri et al., 2012; Potdar et al., 2013; Akhtar et al., 2013), albeit having a different objective, gave similar results. Potdar and colleagues (2013) determined the effect of LMWH on live birth and implantation rates in women with recurrent implantation failure (RIF) embarking on IVF: Two RCTs (Qublan et al., 2008; Urman et al., 2009) and one quasi-RCT were included (Berker et al., 2011). One study (Qublan et al., 2008) included women with thrombophilia, whereas the other two studies (Urman et al., 2009; Berker et al., 2011) included women with unexplained RIF. Pooled risk ratios in all women with ≥ 3 RIF showed significant improvement in live birth rate (RR = 1.79, 95%CI: 1.10–2.90, P = 0.02) and a reduction in miscarriage rate (RR = 0.22, 95%CI: 0.06–0.78, P = 0.02). Implantation rate for ≥ 3 RIF (N = 674) showed a non-significant trend towards improvement (RR = 1.73, 95%CI 0.98–3.03, P = 0.06). Of note, the beneficial effect of LMWH was not significant when studies with unexplained RIF only were pooled. The authors concluded that in women with ≥3 RIF, the use of adjunct LMWH significantly improves live birth rate by 79% and reduces the risk of miscarriage by 78% compared with that in the control group. Although
the authors stated that routine use of LMWH as an adjuvant in IVF should not be advocated, they highlighted the strong need to evaluate the role of LMWH with adequately powered multi-centre RCTs. The Cochrane systematic review (Akhhtar et al., 2013) included three studies of which two were similar to the above meta-analysis, but the third was an RCT which used LMWH in women in their first cycle of IVF (Noci et al., 2011) rather than repeated implantation failure. The study by Berker and colleagues (2011) was excluded because it was quasi-randomised (subjects were women with repeated implantation failure). Despite this difference the observed live birth rate was very similar to the meta-analysis (OR: 1.77, 95%CI: 1.07–2.90).

Interestingly, there is emerging evidence that heparin modulates endometrial receptivity and decidualisation of endometrial stromal cells, and improves implantation. It is postulated that these effects are exerted by various mechanisms including inhibition of production of insulin-like growth-factor-binding protein (IGF-BP) (Fluhur et al., 2010), regulation of heparin-binding epidermal growth factor (hb-EGF) (Tamada et al., 1999), reduction in expression of adhesion molecules such as E-cadherin which promotes trophoblast invasion (Erden et al., 2006) and blockage of complement activation and modulation of inflammatory responses (Girardi et al., 2004). Further research using adequately powered RCTs is required to confirm or refute the beneficial effects of heparin.

**Recommendation(s):** Evidence for the efficacy of LMWH is weak such that its routine use in the wide population of women undergoing IVF treatment is not warranted. However, it should be carefully considered in women with thrombophilia.

**Growth hormone**

GH is a requisite of normal puberty and continues to have a role in ovarian function (Spiliotis, 2003) and, most likely, a modulatory role in follicular development with effects on both steroidogenesis and gametogenesis. It is recognised that many GH-deficient women require ovulation induction to achieve fertility (Homburg & Ostergaard, 1995).

GH regulates the effect of FSH on the granulosa cells of the ovary by increasing the synthesis of IGF-1 and has roles in ovarian function including follicular development, oestrogen synthesis and oocyte maturation (Howles et al., 1999; Kucuk et al., 2008). In women with poor ovarian response, co-treatment with pyridostigmine, a GH releasing agent, enhanced the ovarian response to stimulation (Kim et al., 1999). Other investigators reported that high concentration of GH in follicular fluid is related to embryo morphology and increases embryo implantation (Mendoza et al., 1999; Mendoza et al., 2002).

The Cochrane review of Harper and colleagues in 2003 showed that the increase in IVF live birth rates in poor responders receiving GH adjuvant therapy just reached significance (Harper et al. 2003). The recommendation was that more studies were required before it was considered suitable for clinical use. At that time, the UK National Institute for Clinical Excellence (NICE, 2004) upheld this view. Since then a meta-analysis (Kolibianakis et al., 2009) and the updated Cochrane review (Duffy et al., 2010) have been published. In the meta-analysis by Kolibianakis and colleagues (2009) pooled results of six RCTs with a total of 169 subjects demonstrated that GH addition significantly improved clinical pregnancy rate (rate difference: +16%, 95%CI: +4 - +28) and live birth rate (rate difference: +17%, 95%CI: +5 - +30), and a higher proportion of women reached embryo transfer stage (rate difference: +22%, 95%CI: +7 - +36). However, owing to the small number of subjects in the meta-analysis the authors concluded that further RCT’s were required to confirm the findings. The Cochrane review by Duffy and colleagues (2010) included 10 RCTs with a total of 440 subjects; two trials (Tesarik et al., 2005; Kucuk et al., 2008) post-dated previous recommendations. Considerable heterogeneity in the definitions, protocols and outcomes was observed. No benefit was seen in the use of GH supplementation in women considered as normal responders, whereas those considered to be poor responders based on researchers’ definitions had a significant benefit in both clinical pregnancy rate (OR: 3.28, 95%CI: 1.74–6.20) and live birth rate (OR: 5.39, 95%CI: 1.89–15.35). Only four studies (Owen et al., 1991; Bergh et al., 1994; Suikkari et al., 1996; Kucuk et al., 2008) assessed the effect of GH in women who had a poor response in previous cycles and found a significant increase in pregnancy rate but no apparent improvement in live birth rate. The conclusions drawn were not dissimilar to those of the 2003 Cochrane review, and NICE again supported these (NICE, 2013). Furthermore, a recent RCT showed no difference in clinical pregnancy rate and live birth rate when GH was added to a gonadotrophin-releasing hormone (GnRH) antagonist protocol in poor responders (Effekhar et al., 2013).

Thus there is no clear evidence of benefit in supplementing IVF cycles routinely with GH. There may be a positive effect in the use of GH supplementation in poor responders; however, studies have been weak and heterogeneous, and larger trials are needed.

**Recommendation(s):** The available evidence does not recommend routine use of GH as an adjuvant in IVF cycles. The use of GH in this setting cannot therefore be supported.

**Dehydroepiandrosterone**

DHEA is an androgen produced primarily in the adrenal glands and also in the gonads and brain. It is a precursor of androstenedione, testosterone and oestradiol, and is difficult to distinguish from its sulphated product. DHEA is implicated in the onset of puberty and is known to decline with chronological age such that
there has been speculation about the benefits of supplementation in menopausal women. Whilst unlicensed for prescription in Europe, DHEA is categorised as a food product in the USA and an industry of on-line DHEA has grown up around the putative benefits.

With regard to ovarian performance, there has been much discussion about the potential benefits of DHEA supplementation to enhance fertility in older women and those with low ovarian reserve. Many of the online preparations of the hormone have been targeted at the fertility market and the uptake has been widespread (IVF Worldwide Survey, 2010).

The postulated mechanisms of action of DHEA for improving ovarian response include a) an increase in IGF-1 concentrations, which have a positive effect on follicular development and oocyte quality; b) an effect via the intra-ovarian androgen receptors (Hillier & Tetsuka, 1997; Walters et al., 2010) which enhances follicular development through the growth promoting effect of IGF-1; c) the regulation of (luteinising hormone) LH-stimulated follicular androgen and oestrogen production (Barad & Gleicher, 2006) and increase in expression of FSH receptor resulting in an increase in the number of pre-antral and small antral follicles (Walters et al., 2008; Nielsen et al., 2011). There is a suggestion that DHEA can improve oocyte quality via the GH axis, promote DNA repair in oocytes (Ménéo et al., 2010) and affect mitochondrial function beneficially in follicular cells and oocytes (Pitteloud et al., 2005).

It has been suggested that DHEA improves the number of antral follicles available for stimulation, since it is believed to have a role in antral follicle development (Sunkara et al., 2011); however, reports on the potential benefits have been mixed. Initial observational studies showed improved ovarian response (Casson et al., 2000), increased numbers of oocytes retrieved (Barad & Gleicher, 2006) and higher clinical pregnancy rates (Barad et al., 2007). A number of subsequent trials have been undertaken which suggested benefit; however, the cohorts were significantly heterogeneous and generally small.

The controversy surrounding DHEA has led to a plethora of publications. In a randomised study which included 33 subjects, Wiser and colleagues (2010) showed that there was a benefit in live birth rates following DHEA administration. The quality and size of the study have been criticised and the findings have been disputed since cumulative data were used to show benefit. When only the first interventional cycles were considered, no increase in live birth rate was demonstrated (Kolibianakis et al., 2011, Yakin & Urman, 2011).

Narkwichean and colleagues (2013) identified two suitable studies: one an RCT (Wiser et al., 2010) and one a non-RCT (Barad et al., 2007). Their conclusions were that evidence was lacking for the clinical use of DHEA in women with diminished ovarian reserve undergoing controlled ovarian stimulation.

Several authors have challenged the validity of the claims for DHEA in the face of lack of robust evidence of benefit (Bosdou et al., 2012, Narkwichean et al., 2013, Fouany & Sharara, 2013). Two publications post-date the most recent of these reviews (Yilmaz et al., 2013; Kara et al. 2014). The observational study by Yilmaz and colleagues (2013) included 41 women considered to be poor responders and supplemented with DHEA for 6 weeks before IVF treatment. Whilst the authors’ conclusion is emphatic in recommending DHEA treatment for IVF in poor responders before moving to donor oocytes, it adds little weight to the argument. The largest RCT thus far (Kara et al., 2014) investigating 12-week DHEA supplementation in 104 poor responders compared with that in 104 controls found an insignificantly higher pregnancy rate in the control group.

Interestingly, Narkwichean and colleagues (2014) have begun to consider the issue of DHEA and ovarian physiology as part of a translational research project. In a sheep model, ovarian tissue grafts and normal ovary tissue were examined after a 10-week period of DHEA supplementation. Although there were no non-treatment controls, there appeared to be an increase in antral follicle development, cell proliferation and functional markers, such as Ki67 and AMH. The authors’ conclusions were that DHEA may yet have a role in ovarian ageing and may be a useful IVF adjunct.

Potential side effects of long-term androgen supplementation in women seeking fertility have not been widely addressed.

Recommendation(s): The available evidence does not support the routine use of DHEA as an adjuvant in IVF cycles. The use of DHEA in this setting cannot therefore be supported.

Oestrogen

Oestrogen has been used as an adjuvant for follicular priming and endometrial development in women undergoing IVF cycles.

Asynchronicity of follicular recruitment in ovarian stimulation cycles is thought to be responsible for the high numbers of follicles produced in some women at a high risk of OHSS and may contribute to low numbers of mature follicles in women at risk of poor response. One rationale for the use of oestrogen priming before the commencement of ovarian stimulation is that this may synchronise antral follicle development allowing for the use of reduced gonadotrophin doses in high responders and improved follicle numbers in poor responders. A Cochrane review (Smulders et al., 2010) evaluated the effect of the combined oral contraceptive pill (COCP), oestrogen only and progesterone only supplementation in IVF cycles on pregnancy outcomes and the incidence of OHSS. Twenty-three studies involving a total of 2596 subjects were included. The studies were heterogeneous in design and outcome measures, and the authors concluded that there was insufficient evidence to comment
on the primary outcome measure of live birth rates for any of the interventions and too little data to understand any effect on OHSS. The use of priming could not be recommended on the basis of the review and, moreover, a suspicion of negative effects attributable to oestrogen priming (COCP or alone) was mooted; namely, longer and higher dose gonadotrophin requirements and possible lower clinical pregnancy rates when COCP was used. Adverse effects were also noted in a review of pooled data (Griesinger et al., 2010). Whilst some practitioners use hormone priming, particularly COCP to co-ordinate treatment regimes conveniently (Huirne et al., 2006), the potential negative effects should be born in mind (Nardo et al., 2013). Since the publication of these reviews, there appears to have been little objective interest in the subject.

Chang and colleagues (2012) reported retrospective data suggesting that oestrogen priming in the luteal phase before ovarian stimulation may result in increased numbers of oocytes retrieved and improved embryo quality. They observed a non-significant increase in clinical pregnancy rates but only when the oestrogen was continued throughout the stimulation phase of the cycle. This work does not alter the overriding conclusion that there is lack of evidence for the use of oestrogen priming or the COCP in IVF cycles.

Oestradiol when used for endometrial priming causes proliferation of the uterine surface epithelium, glands, stroma and blood vessels; however, the role in the luteal phase for the preparation of a favourable endometrium is unclear. Earlier studies showed that a drop in oestradiol and progesterone levels in the luteal phase of IVF cycles was associated with reduced pregnancy and implantation rates (Hutchinson-Williams et al., 1989). Subsequently, some investigators reported that luteal phase support with oestradiol and progesterone was associated with higher pregnancy rates per embryo transfer (Gorkemli et al., 2004; Lukaszuk et al., 2005); however, others failed to observe any beneficial effects (Smitz et al., 1993; Tay & Lenton, 2003).

A meta-analysis of nine RCTs to assess whether adding oestradiol to standard luteal progesterone supplementation is beneficial in GnRH agonist and antagonist IVF cycles showed no statistically significant difference in the outcomes of implantation rate, clinical pregnancy rate, ongoing pregnancy rate, miscarriage rate and ectopic rate (Jee et al., 2010). Subsequently, other studies have reported similar findings (Tonguc et al., 2011; Lin et al., 2013). Aghahosseini and colleagues (2011) in an RCT further investigated oestradiol supplementation during the luteal phase in poor responders undergoing IVF and found no significant difference in the outcomes. A recent RCT of 110 infertile women undergoing IVF using GnRH antagonists showed reduction in luteal vaginal bleeding and increase in embryo implantation rate in patients receiving vaginal progesterone gel and 4-mg oestradiol valerate tablets in the luteal phase compared with that in women receiving vaginal progesterone gel only (Kwon et al., 2013).

**Recommendation(s):** Follicular priming with oestrogen or the COCP cannot be recommended as an adjuvant in IVF cycles. In addition, the available evidence does not recommend routine oestradiol supplementation for endometrial development in the luteal phase support of fresh IVF cycles.

**Metformin**

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women during their reproductive years, and accounts for approximately 80–90% of women with anovulatory infertility (Thessaloniki ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2008). Both obese and non-obese women with PCOS are more insulin-resistant and hyperinsulinemic than age- and weight-matched women with normal ovaries. Thus there appear to be factors in women with PCOS which promote insulin resistance that are independent of obesity. Women with PCOS who are oligomenorrheic are more likely to be insulin resistant than those with regular cycles, irrespective of their body mass index.

Algorithms for induction of ovulation are well established, and IVF remains the last resort unless there are other factors of infertility for which this treatment is indicated. Women with polycystic ovaries are at increased risk of OHSS and care is required to minimise this potentially fatal complication of ovarian stimulation. Some of the strategies to reduce the risk include low-dose stimulation protocols, freezing all embryos and the use of GnRH antagonist cycles with a GnRH agonist to trigger final oocyte maturation.

Insulin-sensitizing agents such as metformin were thought to have potential in the management of PCOS. Indeed early studies suggested an improvement in reproductive function and long-term health, but the results of large RCTs have failed to demonstrate such benefits (Tang et al., 2012). Interestingly, metformin appears to act as a brake on the response of polycystic ovaries to exogenous gonadotrophin stimulation, which parenthetically may explain its lack of efficacy in ovulation induction.

The first RCT (Tang et al., 2006) to compare metformin with placebo in IVF cycles in women with PCOS comprised a randomised group of 101 consecutive cycles using a conventional long GnRH agonist protocol and metformin 850mg twice daily for 4 weeks before oocyte retrieval. There were no differences in total FSH dose, number of oocytes retrieved or overall fertilisation rate; however, a significant increase in clinical pregnancy rate beyond 12 weeks (38.5% vs. 16.3%, P = 0.023) and a clinically significant reduction in severe OHSS were reported (3.8% vs. 20.4%, P = 0.023). Metformin was also shown to attenuate the ovarian secretion of vascular endothelial growth factor (VEGF), which is thought to be crucial in the pathophysiology of OHSS. A subsequent study suggested a benefit only for women with PCOS but not...
for those with polycystic ovary morphology alone (Swanton et al., 2011). A Cochrane review (Tso et al., 2009) concluded that the main benefit of metformin in the context of IVF for women with PCOS was for the prevention of OHSS (OR: 0.27, 95%CI: 0.16–0.47). Moreover, a small study of metformin administration in GnRH antagonist cycles, although underpowered, showed a trend towards a reduced incidence of OHSS (Doldi et al., 2006).

The potential benefit of metformin supplementation may also extend into subsequent frozen embryo transfer cycles. A retrospective study (Brewer et al., 2010) found that for women who used metformin in the fresh attempt, the subsequent frozen cycle had a significantly increased live birth rate (28.6% vs. 12.3%). This was most significant in those who had all embryos frozen due to OHSS risk, in whom a 9-fold increase in live birth was seen.

Further, some early studies suggested that metformin may achieve a reduction in miscarriage, but this has since been shown not to be the case (Palomba et al., 2009). Women with PCOS are prone to increased risks for complications in pregnancy including gestational diabetes, pregnancy-induced hypertension, pre-eclampsia and neonatal morbidity (Boomsma et al., 2006). A large Norwegian multi-centre RCT (Vanky et al., 2010) found no improvement in these complications with continued use of metformin throughout pregnancy, although there appeared to be a reduction in late miscarriage and pre-term delivery rates, which is now the subject of a large ongoing RCT. It is reassuring that metformin has a good safety profile in healthy women and early pregnancy with no evidence of teratogenicity, although the gastrointestinal side effects that occur in approximately 10% of patients are well recognised.

**Recommendation(s):** The available evidence suggests that metformin may have some beneficial effects in women with PCOS undergoing IVF by reducing the risk of developing OHSS and increasing clinical pregnancy rates.

**Summary of recommendations for good clinical practice**

Fertility physicians should always be aware of the best available evidence regarding efficacy and safety profile of adjuvants used in IVF cycles, and should provide the information to their patients before commencing treatment. The difference between evidence of the absence of benefits and the absence of evidence itself is recognised and for those recommending an adjuvant empirically, caution is advised. It is of paramount importance that physicians who decide to prescribe unproven therapeutic agents should discuss the available evidence for clinical benefit and the potential adverse effects with the patient.

This document is based on the current best available evidence in the literature for each of the adjuvants considered. For the majority of adjuvants under consideration, there is a need for basic research into potential mechanisms of action to provide weight to the rationale for their use. Adequately powered and well-designed RCTs are also needed.

In summary, consideration should be given to the following recommendations:

1. There is no convincing evidence for the use and safety of IVIg as adjuvants in women with recurrent implantation failure embarking on IVF.
2. There is a lack of evidence to indicate the effectiveness and safety of using anti-TNF-α agents as adjuvants in IVF cycles.
3. There is a lack of evidence to recommend intralipid infusion therapy as an adjuvant in IVF cycles.
4. There is a lack of robust evidence to support the routine use of corticosteroids as adjuvants in IVF cycles. There is limited evidence that corticosteroids may improve pregnancy rates in women undergoing conventional IVF and in the subgroup of women with auto-immunity or unexplained implantation failure.
5. There is a lack of evidence that NTG and sildenafil have significant beneficial effects on IVF outcome and their routine use as adjuvants in IVF cycles is not recommended.
6. There is a lack of robust evidence to support the use of uterine relaxants (NTG, selective β2-adrenergic blockers and progesterone) as adjuvants in IVF cycles.
7. There is a lack of proven efficacy for routine use of aspirin as an adjuvant in IVF cycles.
8. There is a lack of robust evidence to warrant routine use of LMWH in the wide population of women undergoing IVF treatment, but its administration should be carefully considered in women with thrombophilia.
9. The available evidence does not recommend routine use of GH as an adjuvant in IVF cycles.
10. There is a lack of evidence to support the use of DHEA as an adjuvant in IVF cycles.
11. There is a lack of evidence to recommend oestrogen as an adjuvant for follicular priming and/or for endometrial development in the luteal phase of fresh IVF cycles.
12. The available evidence suggests that metformin may have some beneficial effects in women with PCOS undergoing IVF by reducing the risk of developing OHSS and increasing clinical pregnancy rates.

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

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