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The potential role of heparin in assisted conception

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BACKGROUND: Heparan sulphates play key roles in conception and early pregnancy events. The role of heparin, a structural analogue, and its application to assisted conception, is largely unknown. METHODS: Relevant studies were identified by searching PubMed 1966–November 2007 and Google Scholar without limitations. Sensitive search strategies were combined with relevant medical subject headings and text words. RESULTS: The similarities of heparin and heparan, the haemostatic changes induced by ovarian stimulation and the risk of thrombosis, the contribution of thrombophilia to pregnancy and infertility outcomes, early embryo-maternal dialogue and how these various aspects of assisted conception may be modified by heparin are reviewed. CONCLUSIONS: Heparin can alter the haemostatic response to controlled ovarian stimulation and modify the risk of thrombosis. It can also modulate many of the fundamental physiological processes required for blastocyst apposition, adherence and implantation and as well as trophoblast differentiation and invasion due to its similarities with heparan sulphates and has the potential to improve pregnancy rates and outcomes.

Keywords: heparin; thrombosis; infertility; implantation; assisted conception

Introduction

Artificial reproductive techniques have continued to evolve to become part of routine care with up to 4.2% of babies in Europe born as a consequence of these techniques. However, despite the initial dramatic improvements in success rates and significant increments in uptake of ART, the live birth rate resulting from these techniques has recently plateaued (Andersen *et al*., 2007). Furthermore, it is increasingly recognized that pregnancies, including singletons, occurring through ART are at increased risk of adverse perinatal outcomes (Jackson *et al*., 2004). Consequently, greater emphasis is now being placed on experimental strategies to further improve oocyte and embryo number and quality, implantation rates and successful transition to live births. One such strategy is the use of heparin, which as a product of its significant impact on live birth rates in women with acquired thrombophilia (Fiedler and Wurfel, 2004; Empson *et al*., 2005), is now being considered as a potential therapy for all ART patients. In this paper, we discuss the rationale for the use of heparin in assisted reproduction, with particular emphasis on low molecular weight heparin (LMWH) and its role in women with thrombophilia in addition to potential roles irrespective of autoimmune or thrombophilic status. These benefits will include reductions in the thrombotic risks associated with exogenous gonadotrophins, and, given the accumulating evidence that the beneficial effects of heparin are not solely mediated via an anticoagulant effect (Girardi *et al*., 2004), the potential for heparin to optimize implantation and trophoblast development.

Materials and Methods

Relevant studies were identified by searching PubMed 1966–November 2007 and Google Scholar without limitations. Sensitive search strategies were combined with relevant medical subject headings and text words (details available from the authors). In addition, bibliographies of all located articles were scanned for previously unidentified references.

Proteoglycans: the common link

Heparin and heparan: the structural similarities

Heparin is a linear polydisperse polysaccharide consisting of 1→4-linked pyranosyluronic acid and 2-amino-deoxyglucopyranose (glucosamine) residues (Comper, 1981) (Fig. 1). The uronic acid residues typically consist of 90% L-iodopyranisyluronic acid (L-iduronic acid) and 10% D-glucopyranosyluronic acid (D-glucuronic acid). Structural variations of this disaccharide exist, leading to heparin microheterogeneity. The amino group of the glucosamine residue may be substituted with an acetyl or sulpho group or unsubstituted. The 3 and 6 positions of the glucosamine residues can be either substituted with an *O*-sulpho group or unsubstituted. The uronic acid, which can be either L-iduronic or D-glucuronic acid, may also contain a 2-*O*-sulpho group.

Heparan sulphate is also a linear copolymer of uronic acid 1→4 linked to glucosamine but has a more varied structure (Fig. 1). D-glucuronic acid predominates in heparan sulphate, although substantial amounts of L-iduronic acid can be present. Additionally, heparan sulphate is much less substituted in sulpho groups than heparin.

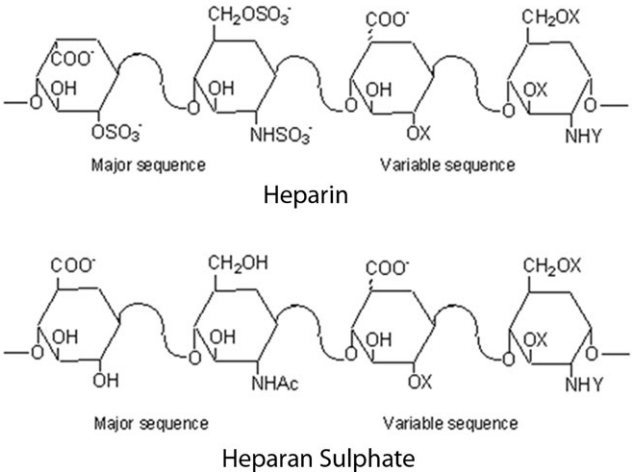

Figure 1: Structural similarities of heparin and heparan sulphate.Major and minor disaccharide-repeating units in heparin and heparan sulphate (X, hydrogen or sulphate; Y, acetyl, sulphate or hydrogen).

Table I. Heparin-binding proteins.

Heparin-binding protein	Physiological/pathological role
Protease/esterase inhibitors	
Antithrombin	Coagulation cascade serpin
Proteinase nexin-1	Inhibitor of trypsin-like serine proteases such as thrombin
Protein C inhibitor	Procoagulant
Plasminogen activator inhibitor 1	Regulates activities of plasminogen activators
Secretory leukocyte protease inhibitor	Inhibits elastase and cathepsin G
C1 inhibitor	Inhibits c1 esterase
Enzymes	
Factors Xa, IXa, IIa	Coagulation cascade serine protease
Neutrophil elastase	Inflammation and pulmonary diseases
Cathepsin G	Inflammation and pulmonary diseases
Superoxide dismutase	Antioxidant enzyme
Growth factors	
Fibroblast growth factor	Cell proliferation, differentiation, morphogenesis and angiogenesis
Hepatocyte growth factor	Cell proliferation
Chemokines	
Platelet growth factor 4	Coagulation, inflammation and wound healing
Interleukin 8	Proinflammatory cytokine
Stromal-derived factor 1a	Proinflammatory mediator
Lipid-binding proteins	
Annexin V	Anticoagulant activity, influenza and hepatitis B viral entry
Apolipoprotein E	Lipid transport, Alzheimer's disease risk factor
Pathogen proteins	
HIV envelope protein glycoporotein 120	Viral entry
Herpes simplex virus envelope proteins	Viral entry into cell and fusion
Dengue virus envelope protein	Viral localisation and tropism
Malaria circumsporozoite protein	Sporozoite attachment to hepatocytes

Muñoz and Linhardt (2004).

Heparin has an average of 2.7 negative charges per disaccharide when compared with <2 negative charges for heparan sulphate, provided by the sulpho and carboxyl groups and consequently the most prominent type of interaction between heparin or heparan sulphate and proteins is ionic. Heparin-binding sites in proteins are characterized by the presence of clusters of positively charged basic amino acids that form ion pairs with spatially defined negatively charged sulpho or carboxyl groups of the glycosaminoglycan (GAG) chain. Non-electrostatic interactions such as hydrogen bonding and hydrophobin interactions can also contribute to the stability of heparin–protein complexes. Owing to the highly anionic nature, heparin and heparan sulphate can bind to a plethora of proteins (Table I), and although initially thought of as predominantly non-specific binder of proteins, they both have defined sequences within their binding domain that facilitate high affinity binding to anti-thrombin, growth factors, growth factor receptors, viral envelope proteins and extracellular matrix (ECM) molecules, with almost every ECM molecule containing binding sites for heparan sulphate.

Heparan sulphate proteoglycan

There are three subfamilies of heparan sulphate proteoglycans (HSPGs): the membrane spanning proteoglycans (namely, syndecans, betaglycan and CD44v3), the glycophosphatidylinositol-linked proteoglycans (namely, glypicans) and the secreted ECM proteoglycans (namely agrin, collagen XVIII and perlecan) (Fig. 2). HSPGs are widely expressed throughout the reproductive tract, with evidence of them involved in the regulation of folliculogenesis (Rodgers *et al*., 2003), modulation of sperm viability and capacitation during sperm transport in the oviduct (Tienthai *et al*., 2000; Bergqvist and Rodriguez-Martinez, 2006), regulation of endometrial cycling (Potter and Morris, 1992; Kelly *et al*., 1995; San Martin *et al*., 2004; Germeyer *et al*., 2007; Lai *et al*., 2007; Xu *et al*., 2007), genital HIV transmission (Bobardt *et al*., 2007; Saidi *et al*., 2007), cervical neoplasia (Numa *et al*., 2002; Shinyo *et al*., 2003; Sobel *et al*., 2005) and vaginal growth (Cano-Gauci *et al*., 1999; Inki, 1997). These interactions primarily occur due to the properties of their heparan side chains; syndecan-3 on dendritic cells is capable of directly binding HIV-1 in a heparan suphate-dependent manner, thereby promoting transmission to T-cells (de Witte *et al*., 2007). Similarly, the effect on decondensing of sperm nuclei is largely determined by the degree of sulphation of the heparan side chains (Romanato *et al*., 2005).

One of the key pieces of evidence showing that HPSGs play a critical role in reproduction is the demonstration that mice deficient in *Hs3st1*, the enzyme which encodes the rate-limiting enzyme hs-3-*O*-sulfotransferase-1 (3-OST-1) essential for heparan sulphate synthesis, demonstrate markedly decreased fertility (HajMohammadi *et al*., 2003). *Hs3st1 null* (−/−) mice exhibit a reduction in the number of preimplantation embryos, shortened reproductive period and altered estrous cyclicity at 7 months of age consistent with a deficiency of proteoglycans impairing ovulation (de Agostini, 2006). For those embryos that did implant, there was significant mortality with 50% of embryos dying due to massive haemorrhage and inflammation of the genital tract, and those that survived exhibiting a phenotype consistent with intrauterine growth restriction (Shworak *et al*., 2002; HajMohammadi *et al*., 2003). Analysis of the placenta of *Hs3st1 null* mice has demonstrated significant inflammation on Day 9.5, suggesting that if this placental inflammation is able to be controlled, the placenta is retarded and growth restriction occurs; however, if it is extensive and uncontrolled, miscarriage and maternal death occurs (de Agostini, 2006). In addition to these

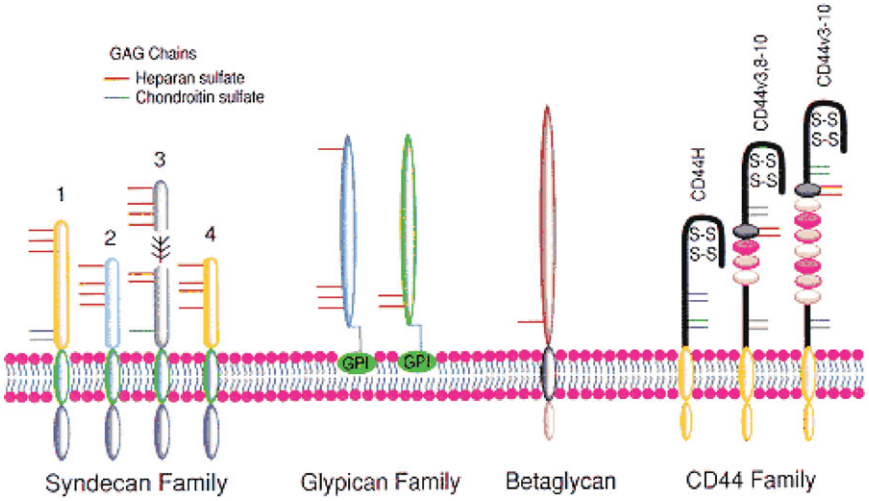

Figure 2: Membrane-bound proteoglycan families.
Family of proteoglycans like syndecan, glypican, betaglycan and CD44, which vary in their anchoring to the membrane, and the kind of GAGs organized on protein core.

pregnancy-related phenotypes, despite heparan sulphate anticoagulant dramatically enhancing the neutralization of coagulation proteases by antithrombin, *Hs3st1 null* mice do not show a procoagulant phenotype with no reduction in tissue fibrin accumulation, generation of vessel thrombosis or generation of thrombin–antithrombin complexes (Haj-Mohammadi *et al.*, 2003), and indeed express the ability to have massive haemorrhage. Collectively, these data suggest that heparan sulphate is not the sole endothelial anticoagulant and/or alternative mechanisms exist to maintain a set anticoagulant tone on the endothelial surface, but also that heparan sulphates may have a non-anticoagulant-dependent role in the physiological processes intrinsic to reproduction, and therefore amenable to manipulation by structurally similar molecules.

Low molecular weight heparin: a pharmacological heparan sulphate

LMWHs are derived from heparin by enzymatic (e.g. tinzaparin) or chemical (e.g. dalteparin, nadroparin and enoxaparin) depolymerization of unfractionated heparin (UFH), which in itself cannot be synthesized *in vitro*. Consequently, the principle sources of heparin are porcine intestine and bovine lung (Warda *et al.*, 2003). LMWHs have a mean molecular weight of 4300–5000 kDa (range 1000–10 000 kDa), compared with 15 000 kDa for UFH. Owing to the reduced interaction between LMWH and the acute phase proteins in blood, there are significant alterations in the pharmacokinetic, anticoagulant and biological effect of LMWH compared with UFH (Table II). Like UFH, LMWHs facilitate antithrombin's anticoagulant effect (Bick *et al.*, 2005), but compared with UFH, LMWH has reduced anti-factor IIa activity because the smaller fragments cannot bind simultaneously with antithrombin and thrombin to form ternary complexes, a necessary step in the efficient inhibition of thrombin by antithrombin. However, bridging between antithrombin and factor Xa is not critical for anti-factor Xa activity, and, therefore, the smaller LMWH fragments inactivate factor Xa with equal efficacy. LMWH clearance is principally by the renal route, with a prolonged half-life in patients with renal failure (Mosenkis and Berns, 2004). LMWH has several advantages over UFH; a longer half-life and a more predictable antithrombotic response, allowing administration to patients in fixed-weight adjusted doses without the need for laboratory monitoring, and a substantially lower risk of HIT (Warkentin *et al.*, 1995; Warkentin and Greinacher, 2004) and osteoporosis (Murray *et al.*, 1995).

Monitoring of LMWH therapy for anticoagulation is usually unnecessary given the efficacy and safety of fixed, weight-based doses, with comparable pharmacokinetics in obese and non-obese patients. Chromogenic factor Xa assays are the most widely used assays for LMWH monitoring, although the inter-laboratory accuracy of the assays is also poor (Greer and Hunt, 2005), with debates over peak or trough level monitoring or validity of tests ongoing (Greer and Hunt, 2005). Although the anti-factor Xa level has been shown to be inversely related to thrombus propagation (Levine *et al.*, 1989), the minimal therapeutic level has not been accurately defined and anti-factor Xa levels have not been demonstrated to be good predictors of bleeding risk or anti-thrombotic efficacy (Nieuwenhuis *et al.*, 1991; Leizorovicz *et al.*, 1993; Alhenc-Gelas *et al.*, 1994).

Table II. Pharmacological properties of unfractionated and low-molecular weight heparin.

Properties	Unfractionated heparin	Low-molecular weight heparin
Anticoagulant target	Thrombin and factor Xa	Factor Xa and thrombin
Route of administration	Intravenous/ subcutaneous	Subcutaneous
Half-life (h)	0.5	3–6
Monitoring requirements	aPTT (partial thromboplastin time)	None unless creatine clearance <30 ml/min
Bioavailability	Low and unpredictable	Very high
Plasma protein binding	High and unpredictable	Low
Clearance	Renal	Renal
Dose-dependent clearance	Yes	No
Neutralized by protamine sulphate	Yes	Partial
Risk of heparin-induced thrombocytopaenia	High	Low/negligible
Safe in pregnancy	Yes	Yes

Haemostasis and thrombosis

Haemostatic changes during ovarian stimulation

In an attempt to maximize the number of embryos available for transfer and freezing, women are being exposed by many units to high doses of exogenous gonadotrophins (Tarlatzis *et al*., 2003; Rombauts, 2007; Siristatidis and Hamilton, 2007) with a concurrent increase in thrombotic risk. The changes in coagulation and fibrinolysis observed during ovarian stimulation are similar to those observed during pregnancy (Clark *et al*., 1998), with the drive for these haemostatic changes potentially being the rapid increase of estradiol levels which occur with ovarian stimulation—estradiol levels potentially increase more than 100-fold within a 2 week period. Correlation with estradiol levels has been found for fibrinogen, D-dimers and activated protein C (APC) resistance (Curvers *et al*., 2001a,b; Rogolino *et al*., 2003). However, in general, ovarian stimulation is associated with increases in several circulating coagulation factors; factor V, fibrinogen, von Willebrand factor, increased coagulation activation markers; prothrombin fragment 1+2 and D-dimers, and impairment of endogenous anticoagulants; decreased antithrombin and protein S levels (Phillips *et al*., 1975; Kim *et al*., 1981; Aune *et al*., 1991; Biron *et al*., 1997). There is no reported alteration in platelet function *in vitro* (Richard-Davis *et al*., 1997). In a temporal analysis of APC resistance during an IVF cycle (Curvers *et al*., 2001a); APC resistance increased with down-regulation, then increased further with ovarian stimulation and remained elevated during luteal support. Consistent with these pro-coagulant changes, analysis of whole blood clotting by thromboelastograph, demonstrated a significant reduction in clotting time, although this remained within normal limits and fibrinolysis was not altered (Harnett *et al*., 2002).

In addition to these haemostatic changes that most women experience, if an excessive ovarian response occurs as in ovarian hyperstimulation syndrome (OHSS), the coagulation changes are usually more pronounced; higher levels of fibrinogen, von Willebrand factor, D-dimers, thrombin–antithrombin complexes and prothrombin fragment 1+2, low levels of pre-kallikrein and tissue factor (TF) inhibitor (Phillips *et al*., 1975; Kodama *et al*., 1996; Rogolino *et al*., 2003). In particular in women who become pregnant with OHSS, the delay for normalization of pre-kallikrein is 3 weeks; in contrast, a rapid return to normal occurs within 1 week after OHSS in women who are not pregnant (Kodama *et al*., 1996). D-dimers and thrombin–antithrombin complexes are also higher in women with OHSS who have unsuccessful pregnancy outcomes, despite D-dimer levels positively associated with estradiol levels and oocyte yield (Rogolino *et al*., 2003). This association between excessive coagulation activation and poorer IVF outcomes, despite higher oocyte yields (conventionally a good prognostic factor; HFEA, 2007), suggests that haemostatic mechanisms have an important role in implantation. In women who experienced OHSS and do achieve pregnancy, increased coagulation activation is still present 3 weeks after OHSS onset (Kodama *et al*., 1996) and protein S levels (Curvers *et al*., 2001a) are still lower.

Thrombosis and ovarian stimulation

The data discussed above show that activation of the coagulation cascade along with impairment of fibrinolysis occurs in conjunction with ovarian stimulation, and that these changes are exaggerated in OHSS. Further, there is a delay in returning to baseline of several weeks. Despite these significant alterations in haemostatic parameters, thromboembolic disease associated with ovarian stimulation is an uncommon complication, with an incidence of 0.08–0.11% of treatment cycles (Mara *et al*., 2004; Chan and Ginsberg, 2006). This reflects the 'multihit' nature of venous thromboembolism (VTE) disease where a number of risk factors usually require to coexist before a clinical event occcurs. Thus, a risk assessment is required in all patients undergoing procedures associated with an increase incidence of VTE. The magnitude of this risk for VTE in patients undergoing ovarian stimulation is low in absolute terms and is similar to that reported during pregnancy (Toglia and Weg, 1996; Lindqvist *et al*., 1999a). However, this represents at least a 10-fold increase in relative risk on top of a baseline risk of 2–3 VTE events per 10 000 person years for women of reproductive age (Fowkes *et al*., 2003). In the presence of OHSS, this risk is substantially increased with ~1 in 128 women with severe OHSS developing a thromboembolic complication (Delvigne *et al*., 1993), thereby representing a 20–40-fold increase (Rao *et al*., 2005). It is important to note that these are the risks associated with the procedure and a specific complication (OHSS) and must be put in the context of other risk factors for VTE which the woman may have such as a previous VTE, family history of VTE, concurrent medical condition or obesity. As risk factors interact, combinations of risk factors will lead to substantial elevations in risk meriting specific thromboprophylaxis.

A review of all cases of thrombosis occurring in conjunction with assisted reproduction identified only 97 reported cases by 2005 (Rao *et al*., 2005). We have subsequently identified an additional nine cases using a similar search strategy (Bedarida *et al*., 2006; Cupisti *et al*., 2006; Ergas *et al*., 2006; Giner *et al*., 2007; Kitao *et al*., 2006; Sinha *et al*., 2006). Notably, all cases apart from one were after hCG administration (Ludwig *et al*., 2000). Of the 106 cases to date 69% were venous, with a predominance of upper extremity deep venous thrombosis (UEDVT) including the subclavian and internal jugular veins, which is in stark contrast to the classical left ilio-femoral deep vein thrombosis (DVT) of pregnancy (Nelson and Greer, 2006b). The preponderance of UEDVT in association with assisted reproduction has been recently highlighted (Chan and Ginsberg, 2006), with the pathophysiology being attributed to the unique drainage of the lymphatic system, with high levels of abdominal fluid containing estrogen drained via the thoracic duct (Bauersachs *et al*., 2007). This anatomical predisposition will interact with the observed haemoconcentration, increased activation of coagulation particularly in association with pregnancy and potentially underlying thrombophilia (Nelson and Greer, 2006a). A more recent review using a different search strategy identified 71 thromboembolic cases (Chan and Dixon, 2008), with a similar predominance of venous and UEDVT events. Furthermore, it is identified that 31% of thromboembolic events occurred even in women who did not get pregnant, and that the timing of presentation for venous events was well beyond the perceived period of risk, with a meant time to presentation of 40 days after embryo transfer and 27 days after ovulation induction. Notably, this clinical presentation of VTE was also several days to weeks after OHSS had resolved (Chan and Dixon, 2008). Although less frequent, arterial thrombotic complications did occur, and presented earlier—approximately 10.5 days post-embryo transfer and 8.2 days post-hCG administration in ovulation induction cycles (Chan and Dixon, 2008). Notably, 41% of women experiencing a venous thrombosis had known thrombophilias which is similar to background rates for women with proven VTE (Nelson and Greer, 2006b). Thrombophilias were less prevalent in conjunction with arterial thromboses, with 19% of women affected, consistent with them having a lesser role in arterial complications (Boekholdt and Kramer, 2007).

This clear association with thromboembolic events in the context of ovarian stimulation, even in the absence of pregnancy or known thrombophilias, is consistent with a major hormonally driven effect

on the haemostatic system. This is also consistent with epidemiological studies noting an increase in VTE in women exposed to exogenous estradiol via the contraceptive pill (Vandenbroucke *et al*., 2001) or hormone replacement therapy (Wu, 2005), and the association between increased lifetime exposure to estrogen and increased incidence of thrombosis (Simon *et al*., 2006). Similarly, this relationship with estradiol would be consistent with enhanced risk in association with OHSS, where elevated estradiol levels are commonly observed (Aboulghar, 2003). Consequently, milder stimulation and tailoring of ovarian stimulation regimens (Fauser *et al*., 2007) will potentially lower thrombotic risk, with studies urgently needed to assess the impact of these newer regimens on the haemostatic system.

Prevention of ovarian stimulation-related thrombosis

Heparin should ameliorate the risk of thrombotic complications associated with OHSS, and thromboprophylaxis using pregnancy-related LMWH doses (e.g. 40 mg enoxaparin) is now part of many recommended treatment protocols (RCOG) (Al-Shawaf and Grudzinskas, 2003). However, despite prophylactic (Hignett *et al*., 1995; Arya *et al*., 2001) and even therapeutic anticoagulation (McGowan *et al*., 2003), thrombosis has been described in association with OHSS. This resistance to heparin may reflect localized excessive activation of coagulation and elevated concentrations of estradiol impairing the endothelium's antithrombotic properties (Bauersachs *et al*., 2007). This raises the question as to whether consideration should be given to antecubital intravenous administration of UFH to target those venous areas at greatest risk. There are no controlled studies in this area. Clinically, progression of thromboembolism is seen in ~10% of cases and along with the presentation in unusual sites suggests that adequate anticoagulation must be implemented promptly. As in pregnancy (Greer and Hunt, 2005), the appropriate duration of therapy for VTE in this situation is not yet established, and although idiopathic VTE, especially with ongoing risk factors for recurrence, in this case pregnancy, is generally treated for 6 months, for those with additional risks (e.g. immobility or multiple risk factors), anticoagulation may be for life and specialist advice taking into account risk factors and site is warranted (Guidelines on Oral Anticoagulation, 1998; Hirsh *et al*., 2004; Ost *et al*., 2005). Further, prophylactic treatment should be used for the remainder of any pregnancy and for 6 weeks post-partum. The future risk of recurrence in patients who develop these complications is unknown; however, extrapolation from pregnancy-related studies suggest a 2–8% risk in the absence of anticoagulation which would be useful in counselling such patients (Brill-Edwards *et al*., 2000; Pabinger *et al*., 2005; Stefano *et al*., 2006).

At present, given the absence of clinical trials, a pragmatic approach to prevention of life-threatening thrombotic complications during controlled ovarian stimulation and in patients who develop OHSS is warranted. Notably, given the multifactorial aetiology of VTE with thrombosis resulting from the synergistic effects between separate predisposing conditions (Table III), all women undergoing controlled ovarian stimulation should undergo risk assessment. Given the lack of large randomized studies on the optimal strategy for thromboprophylaxis and acute management of VTE in conjunction with ovarian stimulation and subsequent pregnancy, the authors suggest the following approach (Table IV); however, specialist advice for individualized management of patients is advisable in many of these situations, with the use of weight-adjusted dosing strategies recommended (Table VIII).

Heparin and the pathophysiology of OHSS

Although the aetiology of OHSS is complex and many aspects remain unclear, the proangiogenic factor vascular endothelial growth factor (VEGF) plays a major role in its pathogenesis (Levin *et al*., 1998).

Table III. Risk factors for VTE in association with ART.

Risk factors identifiable prior to and during ovarian stimulation	
Previous venous or arterial thrombembolism	Age over 35 years
Obesity (body mass index $\geq 30\ kg/m^2$)	Prolonged travel
Gross varicose veins	Dehydration
Previous intravenous drug abuse	Ovarian hyperstimulation
Prolonged bed rest	Immobility
Medical conditions	Inflammatory conditions, systemic lupus erythematosus, hyperlipidaemia, sickle cell anaemia, ulcerative colitis, diabetes mellitus, Cushing's syndrome, nephrotic syndrome, malignancy, myeloproliferative disorders and liver disease
Inherited thrombophilia[§]	Odds ratio (95% confidence intervals)
Factor V Leiden heterozygous	9.32 (5.44–12.70)
Factor V Leiden homozygous	34.40 (9.86–120.05)
Antithrombin deficiency	4.69* (1.30–16.96)
Protein C deficiency	4.76 (2.15–10.57)
Prothrombin G20210A heterozygous	6.80 (2.46–19.77)
Prothrombin G20210A homozygous	26.36 (1.24–559.29)
Family history of VTE in one or more first degree relatives[γ]	2.7 (95% CI, 1.8–3.8)
Acquired thrombophilia	
Lupus anticoagulant[†]	Five associations with odds ratio of 5.7–9.4 and all significant at 95% CI
Anticardiolipin antibodies[†]	Eight associations with deep vein thrombosis were analysed in four studies: one had a significant 95% CI but only for IgG anticardiolipin antibody titers exceeding the 95th percentile (i.e. 33 GPL units)

[§]Data derived from pregnancy related risk of VTE (Robertson *et al*., 2006).
[γ]Data derived from non-pregnant related risk of VTE (Noboa *et al*., 2008).
[†]Data derived from non-pregnant related risk of VTE (Galli *et al*., 2003b).
*Antithrombin deficiency odds ratio is a serious underestimate of risk for VTE, given 73% of affected individuals have a VTE (see text for full details).

Table IV. Suggested management strategies for various clinical situations prior to and after controlled ovarian stimulation.

Clinical situation	Suggested management[†]
Single previous VTE (not pregnancy- or 'pill' related) associated with a transient risk factor and no additional current risk factors, such as obesity	Surveillance or prophylactic doses of LMWH (e.g. 40 mg enoxaparin or 5000 IU dalteparin daily) ± graduated elastic compression stockings. Discuss decision regarding LMWH with the woman
Single previous *idiopathic* VTE or single previous VTE with underlying thrombophilia and not on long-term anticoagulant therapy, or single previous VTE and additional current risk factor(s) (e.g. BMI ≥ 35)	Prophylactic doses of LMWH (e.g. 40 mg enoxaparin or 5000 IU dalteparin daily) commenced in conjunction with controlled ovarian stimulation and continued throughout pregnancy ± graduated elastic compression stockings. With antithrombin deficiency, there is a strong case for more intense LMWH therapy (e.g. enoxaparin 0.5–1 mg/kg 12-hourly or dalteparin 50–100 IU/kg 12-hourly)
More than one previous episode of VTE, with no thrombophilia and not on long-term anticoagulant therapy	Prophylactic doses of LMWH commenced (e.g. 40 mg enoxaparin or 5000 IU dalteparin daily) and fitted with graduated elastic compression stockings at time of starting controlled ovarian stimulation and continued throughout pregnancy
Previous episode(s) of VTE in women receiving long-term anticoagulants (e.g. with underlying thrombophilia)	Switch from oral anticoagulants to LMWH therapy (e.g. enoxaparin 0.5–1 mg/kg 12-hourly or dalteparin 50–100 IU/kg 12 hourly) prior to controlled ovarian stimulation and continue throughout pregnancy and fit graduated elastic compression stockings
Thrombophilia (confirmed laboratory abnormality) but no prior VTE	Surveillance or prophylactic LMWH ± graduated elastic compression stockings. The indication for pharmacological prophylaxis in the antenatal period is stronger in AT-deficient women than the other thrombophilias, in symptomatic family members compared with asymptomatic relatives and also where additional risk factors are present
Risk factors for VTE present prior to controlled ovarian stimulation but no previous VTE or thrombophilia	Carry out risk assessment for VTE. If multiple risk factors present, such as high BMI, immobility and pre-eclampsia, or if single major risk factor present, such as morbid obesity, consider LMWH thromboprophylaxis (e.g. 40 mg enoxaparin or 5000 IU dalteparin but dose may need to be increased with extreme levels of BMI) ± graduated elastic compression stockings
OHSS	Prophylactic doses of LMWH (e.g. 40 mg enoxaparin or 5000 IU dalteparin daily) ± graduated elastic compression stockings. In women who do not conceive, thromboprophylaxis may be discontinued with resolution of OHSS. For women who do conceive continuation until the end of the first trimester, or even longer, depending on the presence of additional risk factors and course of the OHSS
Develops VTE	Therapeutic doses of LMWH (e.g. 1 mg/kg 12-hourly or dalteparin 90 IU/kg 12-hourly) with specialist advice sought for duration of therapy, but usually for at least 6 months with therapeutic or prophylactic treatment continued until at least 6 weeks post-partum

[†]Day of oocyte retrieval—refrain from administering LMWH for 12 h prior to oocyte retrieval if prophylactic dose and 24 h if on therapeutic dose. Both regimens can be restarted 3 h after oocyte retrieval.

VEGF increases capillary membrane permeability, resulting in the large fluid shifts characteristic of severe OHSS. A rise in plasma VEGF concentration is a marker for subsequent development of OHSS, and plasma VEGF levels correlate with the clinical severity of OHSS. Changes in the VEGF levels in the ascitic fluid have also been correlated with the clinical course in OHSS (Abramov *et al*., 1997; Agrawal *et al*., 1999; Chen *et al*., 1999). VEGF-A is known to be heparin binding and VEGF-A-mediated angiogenesis can be inhibited by tinzaparin 5 kDa fragments (Norrby, 2000). Furthermore, heparin fragments with molecular weights of 4.8–5.5 kDa inhibit the binding of ^{125}I-VEGF-A to the VEGF-A receptors on cultured bovine aortic arch endothelial cells, whereas fragments with molecular weights of >6.9 kDa enhance the binding (Soker *et al*., 1994).

In addition to VEGF, TF is also important in angiogenesis. VEGF-A will upregulate TF expression on endothelial cells, which do not normally express TF. Moreover TF further enhances VEGF-A expression (Bobek and Kovarik, 2004). TF is the primary cellular trigger of the coagulation cascade, and is closely regulated by TF pathway inhibitor (TFPI). In severe OHSS, TF plasma levels are significantly increased with a concomitant reduction in plasma TFPI (Rogolino *et al*., 2003). Support for a key role of TF in OHSS has been derived from interventional studies with angiotensin-converting enzyme (ACE) inhibitors, which decrease the risk of OHSS (Morris *et al*., 1995) and are known to modulate TF synthesis in monocytes and reduce TF in *vivo* and *vitro* (Soejima *et al*., 1999; Napoleone *et al*., 2000). However, ACE inhibitors are contraindicated in pregnancy and preferentially avoided during early embryonic development. Thus, heparin, which is also capable of inhibiting TF release (Bobek and Kovarik, 2004) and inducing the secretion of TFPI (Ma *et al*., 2007), may be an alternative strategy to reduce VEGF expression and its widespread effects in OHSS. To explore this concept, a temporal analysis of VEGF, TF and TFPI in OHSS relative to LMWH therapy would provide biological plausibility for the use of LMWH in OHSS to prvent not only thrombosis but also the other manifestations of the condition.

Haemostasis and adverse perinatal outcome

The association between placental-mediated pregnancy complications and inherited and acquired thrombophilia and the improvement with antithrombotic therapy in antiphospholipid syndrome with recurrent

Table V. Inclusion criteria for definitive antiphopholipid syndrome (Miyakis *et al*., 2006).

Antiphospholipid antibody syndrome is present if at least one of the clinical criteria and one of the laboratory criteria that follow are met
Clinical criteria
1. Vascular thrombosis
One or more clinical episodes of arterial, venous or small vessel thrombosis[†], in any tissue or organ. Thrombosis must be confirmed by objective validated criteria (i.e. unequivocal findings of appropriate imaging studies or histopathology). For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall
2. Pregnancy morbidity
(a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
(b) One or more premature births of a morphologically normal neonate before the 34th week of gestation because of: (i) eclampsia or severe preeclampsia defined according to standard definitions[§], or (ii) recognized features of placental insufficiency, or
(c) Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Laboratory criteria
1. Lupus anticoagulant (LA) present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Haemostasis (Scientific Subcommittee on LAs/phospholipid-dependent antibodies)
2. Anticardiolipin (aCL) antibody of IgG and/or IgM isotype in serum or plasma, present in medium or high titer (i.e. >40 GPL or MPL, or >the 99th percentile), on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA
3. Anti-b2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or plasma (in titer >the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardized ELISA, according to recommended procedures

[†]Superficial venous thrombosis is not included in the clinical criteria.
[§]Generally accepted features of placental insufficiency include: (i) abnormal or non-reassuring fetal surveillance test(s), e.g. a non-reactive non-stress test, suggestive of fetal hypoxemia, (ii) abnormal Doppler flow velocimetry waveform analysis suggestive of fetal hypoxaemia, e.g. absent end-diastolic flow in the umbilical artery, (iii) oligohydramnios, e.g. an amniotic fluid index of 5 cm or less, or (iv) a post-natal birth weight less than the 10th percentile for the gestational age.

miscarriage has led to renewed interest in the use of heparin and in particular LMWH for such pregnancy complications—a concept initially proposed by John Bonnar in 1969.

Inherited thrombophilia and pregnancy outcome

To date, meta-analyses of relatively small case–control studies have demonstrated a small but significant increase (OR 1.5–4.0) in embryonic and fetal loss, abruption, intrauterine growth restriction and preeclampsia in association with inherited thrombophilias (Rey *et al*., 2003; Robertson *et al*., 2004; Howley *et al*., 2005; Lin and August, 2005; Wu *et al*., 2006). Prospective cohort studies have similarly supported a minor contribution of inherited thrombophilias on perinatal outcomes (Lindqvist *et al*., 1999b; Murphy *et al*., 2000; Dizon-Townson *et al*., 2005).

Acquired thrombophilia amd pregnancy outcome

Antiphospholipid syndrome (Miyakis *et al*., 2006), the most common acquired thrombophilia, is associated with an unusually high proportion of pregnancy losses (Lockshin *et al*., 1985; Oshiro *et al*., 1996) and an increased risk of recurrent miscarriage (Silver and Branch, 1994; Levine *et al*., 2002). Despite an updated consensus definition (Wilson *et al*., 1999; Miyakis *et al*., 2006), the diagnosis is difficult as it is dependent on only one clinical event and only one laboratory marker (Table V) with the latter poorly standardized, particularly if titres are weak (Brandt *et al*., 1995; Reber *et al*., 1995). Lupus anticoagulant (LAC) is a much better correlate of thrombo-embolic events and pregnancy morbidity than anticardiolipin or anti-β_2-glycoprotein-I (anti-β_2-GPI) assays (Galli *et al*., 2003b; Opatrny *et al*., 2006). This may reflect that although there is overlap of antibody subsets responsible for the LAC effect, these are not the same as those determined by the anticardiolipin assay (McNeil *et al*., 1989). Discrimination of LAC subsets by characterization of β_2-GPI phospholipid binding (Simmelink *et al*., 2003; Pengo *et al*., 2004) has confirmed that anti-β_2-GPI-dependent LACs correlate better with thromboembolic events (de Laat *et al*., 2004), consistent with β_2-GPI being the predominant target in the syndrome. Although the more recent consensus definition no longer requires β_2-GPI dependency (Wilson *et al*., 1999; Miyakis *et al*., 2006), thereby reducing specificity by incorporation of non-autoimmune-related anticardiolipin antibodies, anti-β_2-GPI antibodies were added to the diagnostic criteria (Miyakis *et al*., 2006). Theoretically and indeed practically, the direct anti-β_2-GPI performs better than the standard anticardiolipin assay (Reber *et al*., 2005; Pengo *et al*., 2007). However, they are not without weaknesses due to artificial cut-off limits, use of bound bovine β_2-GPI, differing types of enzyme-linked immunoabsorbent assay (ELISA) plate altering β_2-GPI orientation (Iverson *et al*., 2002) and relatively widespread prevalence of low affinity anti-β_2-GPI antibodies in the population (De Laat *et al*., 2006).

Despite these limitations of commercially available assays, it is clear that detection of antiphospholipid antibodies identifies patient subgroups at risk of thrombosis and pregnancy complications. The relative merits of any individual anti-phospholipid antibody are, however, unclear, with a meta-analysis and subsequent studies not demonstrating an association between anticardiolipin antibodies and VTE (Runchey *et al*., 2002; Galli *et al*., 2003b, 2007; Naess *et al*., 2006), despite a positive association with LAC (Galli *et al*., 2003b) and potentially anti-β_2-GPI (Galli *et al*., 2003a; De Groot *et al*., 2005). In contrast, a pregnancy-related meta-analysis found a significant association between anticardiolipin antibodies and both early and late fetal recurrent fetal loss (Opatrny *et al*., 2006), but this was stronger with respect to recurrent fetal loss for LAC. Presence of anti-β_2-GPI antibodies is also more strongly associated with fetal loss (Ulcova-Gallova *et al*., 2001; Gris *et al*., 2003; Obermoser *et al*., 2004; Danowski *et al*., 2006; Sailer *et al*., 2006; Zammiti *et al*., 2006). Additional antiphospholipid antibodies including those against annexin A5 have been described and associated with thrombosis (Vora *et al*., 2007) and fetal loss (Nojima *et al*., 2001; Zammiti *et al*., 2006; Galli *et al*., 2007). It is clear however that that patients testing positive for more than one antibody have a substantive increase

Table VI. Trials of heparin for fetal loss in antiphospholipid syndrome.

Study	Intervention	Inclusion criteria	Outcomes	Number of subjects	Results
Cowchock *et al*. (1992)	Multicentre, non-blinded, non-placebo controlled RCT of Heparin 10 000 units twice daily s.c. plus aspirin 80 mg/day versus prednisone 20 mg twice daily plus aspirin 80 mg/day	(1) ≥2 unexplained fetal losses. (2) Exclusion of other causes of recurrent miscarriage or fetal death. (3) ≥2+ve APL tests over at least a 6 week period determined by IgG ACL > 30 GPL units, IgM ACL>11 MPL units, or presence of LA (APTT or dRVVT at least 2 SDs greater than the mean and lack of correction with 1:1 fresh frozen plasma)	Medical and obstetric complications, e.g. fetal distress, preterm delivery (<37 weeks), low birthweight (<10th percentile gestational age), and maternal morbidity	45	Live birth rates were the same (75%) with either treatment, but 'serious' maternal morbidity and the frequency of preterm delivery were significantly higher among women randomly assigned to prednisone (P=0.02 versus P=0.006)
Kutteh and Ermel (1996)	Single centre, quasi-randomised (alternatively assigned to treatment) non-blinded, non-placebo controlled of heparin 5000 units twice daily sc plus aspirin 81 mg/day versus aspirin 81 mg/day. Heparin dose increased by 1000 units/dose weekly until APTT 1.2–1.5 times baseline.	(1) Desire to become pregnant. (2) Agreement to be completely evaluated. (3)≥3 consecutive pregnancy losses. (4) Consent to alternative treatment assignment. (5) +ve APL antibody on at least 2 occasions determined by IgG ACL or antiphosphotidylserine >27 GPL units or IgM ACL or antiphosphotidylserine >23 MPL units	Multiple obstetric and neonatal outcomes.	50	Viable infants were delivered from 20/25 (80%) women treated with higher dose heparin versus 19/25 (76%) of women treated with lower dose heparin. There were no significant differences between groups with respect to gestational age at the time of delivery (37.2 ± 3.4 versus 37.7 ± 1.6 weeks), maternal complications, or fetal complications
Kutteh (1996)	Single centre, quasi-randomised (sequentially assigned to treatment) non-blinded, non-placebo controlled of Heparin 5000 units twice daily sc adjusted to maintain the PTT at 1.2 to 1.5 times the baseline (high-dose) plus aspirin 81 mg/day versus heparin 5000 units twice daily adjusted to maintain the PTT at the upper limit of normal (low-dose) plus aspirin 81 mg/day	(1) Desire to become pregnant. (2) Agreement to be completely evaluated. (3) ≥3 consecutive pregnancy losses. (4) Consent to treatment protocol. (5) +ve APL antibody on at least 2 occasions determined by IgG>27 GPL units (>2.5 multiples of the median)	Multiple obstetric and neonatal outcomes	50	Viable infants were delivered of 11 of 25 (44%) women treated with aspirin and 20 of 25 (80%) women treated with heparin and aspirin ($P < 0.05$). There were no significant differences between the low-dose aspirin and the heparin plus low-dose aspirin groups with respect to gestational age at delivery (37.8 ± 2.1 versus 37.2 ± 3.4 weeks), number of Caesarean sections (18% versus 20%), or complications
Rai *et al*. (1997)	Single centre, non-blinded, non-placebo controlled RCT of calcium heparin 5000 units twice daily sc plus aspirin 75 mg/day versus aspirin 75 mg/day alone. Although aspirin commenced in all when positive pregnancy test.	(1) ≥3 consecutive miscarriages. (2) +ve APL antibody on at least 2 occasions >8 weeks apart determined by ACL IgG>5 GPL units or ACL IGM>3 MPL units or a positive LA (APTT, dRVVT ratio ≥1.1 confirmed by platelet neutralization—decrease of ≥10% of ratio)	Live birth, gestational age and weight, congenital abnormality, admission to neonatal ICU, bone mineral densitometry and maternal morbidity	90	The rate of live births with low-dose aspirin and heparin was 71% (32/45 pregnancies) and 42% (19/45 pregnancies) with low-dose aspirin alone [odds ratio 3.37 (95% confidence interval 1.40–8.10)]. There was no difference in outcome between the two treatments in pregnancies that advanced beyond 13 weeks' gestation

Branch *et al.* (2000)	Multicentre, double-blind, placebo controlled RCT of intravenous immunoglobulin (10%) 1 g/kg versus placebo (albumin 5%), on 2 days every 4 weeks. All participants also received aspirin 81 mg/day and heparin 7500 units twice daily s.c.	(1) A single live fetus of ≤12 weeks' gestation. (2) Either IgG ACL ≥20 GPL units and a history of fetal death and/or venous/arterial thromboembolism or IgG ACL ≥40 GPL units or LA, but no history of fetal death or thromboembolism	Multiple obstetric and neonatal outcomes	16	All women delivered live born infants after 32 weeks' gestation. The birthweight, gestational age at delivery and rates of pre-eclampsia, fetal growth restriction, and placental insufficiency not significantly different between groups
Farquharson *et al.* (2002)	Single centre, non-blinded, non-placebo controlled RCT of aspirin 75 mg/day versus aspirin 75 mg/day and LMW heparin 5000 units s.c./day	(1) 18–41 years. (2) >2 consecutive pregnancy losses or 2 consecutive losses with proven fetal death after 10 weeks. (3) 2+ve APL antibodies >6 weeks apart determined by LA (dRVVT>1.09 with >20% correction with platelets) or IgG ACL>9 GPL units or IgM ACL>5 MPL units	Embryo loss (no visible crown rump length or fetal heart activity) and fetal loss (loss of fetal heart activity)	98	The live-birth rate was 72% with aspirin alone and 78% in for LMWH plus aspirin (odds ratio 1.39, 95% confidence interval 0.55, 3.47)
Triolo *et al.* (2003)	Single centre, non-blinded, non-placebo controlled RCT of IVIG 400 mg/kg/day for 2 consecutive days then single monthly dose versus LMWH (Seleparina) 5700 IU/day and aspirin 75 mg/day	(1) 18–39 years. (2) ≥3 consecutive fetal losses <10 weeks' gestation. (3) ≥+ve results for ACL (intervals ≥3 months) determined by IgG ACL>40 GPL units	Pregnancy loss, maternal side effects, preterm delivery (<37 weeks), neonatal ICU admission, low birthweight and neonatal bleeding or bruising	42	The women treated with LMWH plus low-dose aspirin had a higher rate of live births (84%) than those treated with IVIG (57%)

in risk of thrombosis (De Groot *et al*., 2005; Zoghlami-Rintelen *et al*., 2005) or pregnancy morbidity (Ruffatti *et al*., 2006; Sailer *et al*., 2006).

Hameostasis and infertility

Heritable thrombophilia: contribution to infertility and outcomes

The association between thrombophilia and adverse perinatal events, including embryonic loss, has led to speculation that thrombophilia may also contribute to infertility and in particular recurrent implantation failure. With respect to inherited thrombophilia, the studies are largely underpowered, as due to the low prevalence of inherited thrombophilias more than 1000 patients would be required for a 1:1 case–control study. Despite this, two studies have demonstrated an association between inherited thrombophilia and unexplained infertility. The prevalence of Factor V Leiden but not methylenetetrahydrofolate reductase (MTHFR) C677TT (associated with increase homocysteine levels) or prothrombin G20210A (associated with increased prothrombin levels) was increased 6-fold in a cohort of Iranian women with unexplained infertility present in 30.6% (11 out of 36 women affected), when compared with a background rate of 3–5.5% (Behjati *et al*., 2006; Rahimi *et al*., 2007). A small but significant risk of infertility (OR 1.5–2.5) was also observed for Factor V Leiden heterozygotes in a case–control study incorporating 128 cases and 461 controls (Bare *et al*., 2000). Conversely, two studies with 31 patients (Bellver *et al*., 2007) and 37 patients (Martinelli *et al*., 2003), respectively, with unexplained infertility did not show any alteration in prevalence of heritable thrombophilia. Factor V Leiden status was also not associated with fecundity in a retrospective cohort of 80 Factor V Leiden heterozygotes and 140 controls (van Dunne *et al*., 2005). Clearly, much larger studies are required to definitively answer the question of association of whether inherited thrombophilas contribute to infertility *per se*; however, an effect, if any, is likely to be small.

Heritable thrombophilias may also contribute to implantation failure after ART, with an increased prevalence of Factor V Leiden and prothrombin G20210A heterozygotes reported in women failing to conceive after three or more embryo transfers (Grandone *et al*., 2001). An increase in MTHFR C677TT and combined thrombophilias, but not isolated Factor V Leiden, was observed in the largest study to date of 90 women who failed to achieve a pregnancy after three embryo transfers when compared with two separate control groups, namely women who achieved a pregnancy after their first attempt ($n = 90$) and a group of women who conceived spontaneously ($n = 100$)(Qublan *et al*., 2006). The Factor V Leiden mutation was, however, more prevalent (14.4%) in women with repeated IVF failure than controls (1%), although this was not statistically significant, it is similar to the 11.1% previously reported (Grandone *et al*., 2001). These findings for Factor V Leiden were not replicated by other studies (Martinelli *et al*., 2003; Azem *et al*., 2004); however, Azem *et al*. again showed a significantly higher prevalance of heritable thrombophilias in women with four or more failed IVF cycles when compared with spontaneous conceptions (OR 3.6, 95% 1.25–10.6), or women who conceived after their first cycle (OR 2.9, 95% CI 1.02–8.4) (Azem *et al*., 2004), findings replicated by Coulam (Coulam *et al*., 2006). In conclusion, heritable thrombophilia does appear to contribute to repeated ART failure and although the contribution of any single thrombophilia to implantation failure is likely to be small, it is likely that for any given individual, this association will reflect the total number of mutations rather than the involvement of specific genes.

Acquired thrombophilia: contribution to infertility and outcomes

The role of antiphospholipid antibodies in contributing to infertility is contentious. In experimental models, immunized females demonstrate reduced fecundity (Bakimer *et al*., 1992), and there is an increased antiphospholipid prevalence in general IVF populations, with rates of 10–48% (Gleicher *et al*., 1994; Sher *et al*., 1994; Fisch *et al*., 1995; Nip *et al*., 1995; Birdsall *et al*., 1996; Denis *et al*., 1997; Kowalik *et al*., 1997; Kutteh *et al*., 1997) when compared with background rates in general obstetric populations of 1–4% (Lockwood *et al*., 1989). The latter are comparable with those found in healthy young control subjects where the prevalence is 1–5% (Levine *et al*., 2002). Despite this higher prevalence and suggestions that this may translate to an adverse effect on IVF outcomes in particular implantation failure (Birkenfeld *et al*., 1994; Geva *et al*., 1995; Kaider *et al*., 1996), this finding was not supported by a meta-analysis (Hornstein *et al*., 2000). This included 7 out of 16 studies (el-Roeiy *et al*., 1987; Sher *et al*., 1994; Birdsall *et al*., 1996; Denis *et al*., 1997; Kowalik *et al*., 1997; Kutteh *et al*., 1997), with 703 cases and 1350 controls and did not demonstrate an adverse effect of antiphospholipid antibodies on the IVF outcomes of clinical pregnancy (OR 0.99; 95% CI 0.64–1.53) or live birth (OR 1.07; 95% CI 0.66–1.75) (Hornstein *et al*., 2000). Subsequent studies with 121 cases and 418 controls have similarly not shown a detrimental effect of antiphospholipid antibodies in first IVF cycles and pregnancy rates (Chilcott *et al*., 2000; Buckingham *et al*., 2006; Caccavo *et al*., 2007; Lee *et al*., 2007). Significant heterogeneity in the definition of the antiphospholipid antibody positivity was noted across the seven studies within the meta-analysis and the subsequent studies. In addition to these problems with assays, power remains an issue as a single study of 1240 women would be required to definitively address the role of antiphospholipid antibodies with an agreed *a priori* definition of which antibodies will be tested for and what level corresponds to a positive result (Buckingham and Chamley, 2007; Matsubayashi *et al*., 2007). At present, routine antiphospholipid antibody evaluation in the general assisted conception population, outwith a large prospective trial, still does not appear to be warranted (Hornstein, 2000), a view shared by the Practice Committee of the American Society for Reproductive Medicine (2006).

Antiphospholipid antibodies have also been demonstrated in follicular fluid, although they are at lower levels than sera (el-Roeiy *et al*., 1987; Nip *et al*., 1995; Buckingham *et al*., 2006; Matsubayashi *et al*., 2006). There are no data to link this observation to an adverse effect on fertilization (el-Roeiy *et al*., 1987; Nip *et al*., 1995; Buckingham *et al*., 2006), apart from women with repeated failures (Buckingham and Chamley, 2007; Matsubayashi *et al*., 2007). The clinical relevance of these findings is difficult, as, at present, no treatments have been shown to ameliorate follicular fluid antibody levels, and knowledge of antiphospholipid positivity prior to controlled ovarian stimulation will be determined from serology.

Although these studies have questioned the role of antiphospholipid antibodies in infertility, they do not address the outcome of assisted conception for women who experience repeated failure or consecutive fetal losses after IVF. Indeed, similar to inherited thrombophilia, it would appear that women who do not achieve pregnancy after three embryo transfers do exhibit an increased prevalence of antiphospholipid antibodies (Balasch *et al*., 1996; Qublan *et al*., 2006; Vaquero *et al*., 2006). We would therefore suggest thrombophilia screening prior to embarking on a fourth embryo transfer, as intervention with heparin may be warranted as discussed below. With respect to pregnancy loss, thrombophilia screening after one IVF-related early miscarriage is not supported (Balasch *et al*., 1998). However, antiphospholipid seropositivity was similar in women with two IVF-related early miscarriages compared with women with spontaneous recurrent miscarriage (Egbase *et al*., 1999), a group with a high risk of recurrence (~90%) (Rai *et al*., 1995). This may be amenable to therapy (Empson *et al*., 2005). It would therefore seem appropriate that screening is undertaken after two IVF-related early miscarriages, rather than waiting until the usual definition of recurrent miscarriage is achieved.

Anticoagulants as a treatment strategy for pregnancy

A thrombotic model has dominated the potential mechanisms underlying adverse pregnancy outcome. This reflects observations of extensive placental infarction and thrombosis in women with acquired and inherited thrombophilia (Dizon-Townson *et al*., 1997; Heller *et al*., 2003; Sebire *et al*., 2003; Van Horn *et al*., 2004), along with their known associations with VTE (Greer, 1999; Rosendaal, 1999; Seligsohn and Lubetsky, 2001). Several alternative or additional mechanisms have been proposed, particularly with respect to antiphospholipid antibodies (Di Simone *et al*., 2007b). These include: displacement of annexin V from the trophoblast surface (Rand *et al*., 1997, 1998), although others dispute this (Lakasing *et al*., 1999; Bevers *et al*., 2000; Donohoe *et al*., 2000); antiphospholipid antibody inhibition of trophoblast proliferation (Chamley *et al*., 1998) and invasion (Di Simone *et al*., 2000); and antiphospholipid antibody activation of complement (Girardi *et al*., 2004).

Anticoagulants and acquired thrombophilia

As a consequence of the early predominance of theories involving haemostasis, treatment of affected patients with antithrombotics agents, in particular low-dose aspirin and heparin, was initiated. With respect to antiphospholipid syndrome and recurrent pregnancy loss, a systematic review (Empson *et al*., 2002) and subsequent Cochrane analysis (Empson *et al*., 2005), with no new trials since publication, have summarized the data. They reported six studies with 391 patients incorporating LMWH or UFH in their design (Table VI). Owing to the significant heterogeneity in terms of trial design, the interventions studied, outcomes measured and small study numbers, definitive conclusions are difficult. Of the interventions examined, only UFH combined with aspirin was shown to reduce the incidence of pregnancy loss [relative risk (RR) 0.46, 95% CI 0.29–0.71], the composite adverse pregnancy outcomes of 'pregnancy loss or IUGR' (RR 0.57, 95% CI 0.39–0.83) and 'pregnancy loss or premature delivery' (RR 0.65, 95% CI 0.47–0.91). However, the numbers of patients used to draw these conclusions were small (Empson *et al*., 2005). Pooling of trials using either LMWH or UFH was associated with a 35% reduction in pregnancy loss or premature delivery (RR 0.65, 95% CI 0.49–0.86). This conclusion was despite one study showing no additional benefit of aspirin and LMWH combined compared with aspirin alone (Farquharson *et al*., 2002); however, this study had limited power to detect a difference because of a high crossover rate with 25% of women not receiving allocated treatment. Also definitions of seropositivity have been questioned in two studies (Rai *et al*., 1997; Farquharson *et al*., 2002), with criticisms of inclusion of women with relatively low levels of IgG antiphospholipid antibodies (Branch and Khamashta, 2003). Despite the quite different biological effects of UFH and LMWH, no study compared these two agents; however, one recent study reported that the combination of LMWH and aspirin is as effective as UFH and aspirin in women with recurrent pregnancy loss, with 21 of 25 patients on LMWH delivering a

viable fetus compared with 20 out of 25 in the UFH group (Noble *et al*., 2005).

For women with antiphospholipid antibodies undergoing assisted conception, there are only a few studies examining the role of heparin (Sher *et al*., 1994; Schenk *et al*., 1996; Kutteh *et al*., 1997; Stern *et al*., 2003; Qublan, personal communication) (Table VII). These six studies comprise 1792 patients, with 563 pregnancies out of 1344 (41.8%) in the heparin and aspirin arms and 122 out of 448 (27.2%) in control arms, suggesting that heparin does have a positive impact on live births rate ($P < 0.001$). However, again these studies are heterogenous in design and entry criteria. The largest of these studies (Stern *et al*., 2003) included a crossover design, thereby not excluding potential biological hangover effects of aspirin, and the study population comprised women with recurrent failed embryo transfers (>10 embryos). This study has been criticized for its inclusive nature with an alternative definition of recurrent implantation failure proposed; age <35 years, normal ovarian response (≥8 oocytes), fertilization rate >60% and transfer of 2–3 embryos of adequate morphology quality (Fiedler and Wurfel, 2004). In this context, the authors noted an unspecified improvement in outcome with heparin and aspirin from the day of embryo transfer, with a non-quantified improvement also seen in older women undergoing standard care in conjunction with heparin and aspirin (Fiedler and Wurfel, 2004). In support of this potential improvement, a recent prospective randomized placebo-controlled trial examining women with cases with repeated IVF failure has just been completed. This trial included 42 women who received enoxaparin 40 mg/day from the day of embryo transfer and continued until delivery, and 41 women who received placebo. LMWH treatment was associated with a significant increase in implantation (20.9% versus 6.1%; $P < 0.001$) and pregnancy (31% versus 9.6%; $P < 0.05$) rates compared with the placebo group, with translation to a significant increase in live birth rates (23.8% versus 2.8%; $P < 0.05$), but not at the expense of treatment complications (Qublan *et al*., personal communication).

Collectively, these data suggest that for women with either definitive antiphospholipid syndrome (Miyakis *et al*., 2006) or repeated implantation failure and antiphospholipid seropositivity, LMWH and aspirin should be commenced in conjunction with ovarian stimulation and continued throughout pregnancy. For women who are seropositive for one antiphospholipid antibody undergoing their first cycle of ART, treatment with LMWH or aspirin would not be appropriate, given the current lack of evidence and wide prevalence of antiphospholipid antibodies in infertility and general populations. In contrast, if multiple antiphospholipid antibodies have been detected, given the strong association with VTE (De Groot *et al*., 2005; Zoghlami-Rintelen *et al*., 2005) and pregnancy morbidity (Ruffatti *et al*., 2006; Sailer *et al*., 2006), pragmatic treatment with LMWH and aspirin would be appropriate, accepting the current lack of trials in this area.

Anticoagulants and heritable thrombophilia

For women with heritable thrombophilia, only two randomized trials have been published. The first study, despite methodological limitations (Rodger, 2004), suggested benefit with LMWH compared with aspirin alone in women a heritable thrombophilia with single unexplained fetal loss after 10 weeks (OR 15.5; 95% CI 7–34, $P < 0.0001$) (Gris *et al*., 2004). This study can also be criticized for the lower than expected survival rate in the aspirin arm (23 live births out of 80 treated patients), suggesting that aspirin may have had a detrimental effect—unfortunately with the absence of an appropriate control group we cannot be sure. The second study compared two different doses of enoxaparin, 40 mg once a day versus twice daily in women with recurrent fetal loss and heritable thrombophilia. Both groups had live birth rates exceeding 78%, with the authors suggesting a beneficial effect of enoxaparin compared with their historical cohorts (Brenner *et al*., 2005). These trials are very limited and further trials in the setting of thrombophilia in women with at least two spontaneous miscarriages or fetal death are required (Di Nisio *et al*., 2005) along with appropriate controls. Although a higher prevalance of heritable thrombophilia was noted in women with repeated IVF failure (Azem *et al*., 2004; Coulam *et al*., 2006; Qublan *et al*., 2006), and included in the study by Qublan, the relative contribution from women with acquired or inherited thrombophilia is not known.

Despite the recognized limitations of the data, and in particular with respect to LMWH, many women with recurrent miscarriage and implantation failure are regularly screened for antiphospholipid syndrome and if affected treated with low-dose aspirin and LMWH (Branch and Khamashta, 2003; Heilmann *et al*., 2003). This is based on biological plausibility rather than any proven mechanism or intervention. Further study of heparin, both in terms of mode of action and its potential therapeutic role in women with thrombophilia and implantation failure are warranted, with multicentre trials required to achieve meaningful numbers (Empson *et al*., 2005). Notably, the optimal agent has also to be defined, however, at present enoxaparin, with its established pregnancy safety record (Greer and Nelson-Piercy, 2005) and use in many of the studies would potentially be the first agent of choice.

Role of heparin outside of haemostasis

Modulation of implantation and trophoblast development

As discussed in this review, the role of heparin in assisted conception and also now in improving outcomes in women with inherited and acquired thrombophilia has been thought classically to be prevention of thrombosis in relation to implantation and placental development. There is, potentially, a much wider role for heparin in assisted conception due to its ability to interact with a wide variety of proteins (Table I), which can alter the physiological processes of implantation and trophoblast development, a process that may be adversely influenced by assisted conception *per se*. Certainly, there is increasing evidence from matched cohort studies that pregnancies achieved by assisted reproduction are at significantly higher risk of adverse perinatal outcomes. Although risks have been attributed largely to multiple pregnancies, two meta-analyses have confirmed that singleton IVF/ICSI pregnancies are at significantly higher risk of preterm delivery before 32 weeks and 32–36 weeks, small for gestational age, and perinatal mortality (Helmerhorst *et al*., 2004; Jackson *et al*., 2004). Maternal risk was also increased with increments in the rates of pre-eclampsia, gestational diabetes, placenta praevia and consequently need for Caesarean section (Helmerhorst *et al*., 2004; Jackson *et al*., 2004). All of these conditions are being increasingly recognized as having their origins in the first trimester (Smith *et al*., 1998, 2002), with abnormal implantation and trophoblast development being the key pathophysiological processes. Although the process of implantation is enigmatic, anticoagulant therapy is now being examined as a preventative measure for women with a history of placental-mediated pregnancy complications. Given the increased risk of adverse outcome associated with ART pregnancies, clinics are embarking on the use of LMWH, again based on biological plausibility rather than evidence of efficacy.

Heparin has, however, been examined in other model systems, which show some similarity to the basic physiological processes of

Table VII. Studies of heparin in assisted conception.

Study	Intervention	Outcome of patients receiving treatment	Outcome of patients in control arm	Conclusion
Kutteh *et al.* (1997)	Two centre comparator study. In centre 2 women with positive APA initiated aspirin 81 mg/day at the time of gonadotrophin start and s.c. heparin 5000 units twice daily beginning on the night of aspiration	10/19 (52.6%) pregnant 8/19 (42.1%) clinical pregnancies	8/17 (42.1%) pregnant 6/17 (35.3%) clinical pregnancies	No significant differences in implantation, pregnancy or clinical pregnancy rates
Qublan, personal communication	Double-blind placebo controlled RCT of 81 women with inherited or acquired thrombophilia and $\geq$3 failed embryo transfers. Women started s.c. enoxaparin 40 mg once daily on day of embryo transfer.	31% pregnant 23.8% live births	9.6% pregnant 2.8% live births	LMWH improves pregnancy and live birth rate in women with thrombophilia and $\geq$3 failed embryo transfers ($P < 0.05$)
Schenk *et al*. (1996)	Single centre non-randomised study. APA positive women given heparin and LDA compared with seronegative women	18/35 (51.4%) pregnant	12/40 (30%) seronegative women pregnant	Heparin and aspirin improves implantation rate in APA positive women ($P < 0.05$)
Sher *et al.* (1994)	Single centre non-randomized study. APA positive women initiated aspirin 81 mg/day and s.c. heparin 5000 units twice daily beginning on Day 2 of stimulation	82/169 (49%) pregnant	4/25 (16%) seropositive women pregnant 47/171 (27%) seronegative women pregnant	Heparin and aspirin improves implantation rate in APA positive women ($P < 0.05$) and when compared with seronegative women ($P < 0.001$)
Sher *et al.* (1998)	Multiphase study. APA positive women initiated aspirin 81 mg/day and s.c. heparin 5000 units twice daily beginning on Day 2 of stimulation. Those that declined treatment were control group	417/923 (46%) live births	22/127 (17%) live births	Heparin and aspirin improves live birth rate in APA positive women ($P < 0.001$)
Stern (2003)	Double-blind placebo controlled RCT crossover trial of women either APA or ANA and $\geq$10 embryos transferred and not achieved pregnancy. Women initiated aspirin 100 mg/day and s.c. heparin 5000 units twice daily beginning on day of embryo transfer	23/158 (15%) pregnant 18/296 (6%) live births per embryo	25/142 (18%) pregnant 17/259 (7%) live births per embryo	Heparin and aspirin from day of embryo transfer does not improve pregnancy or live birth rates in women seropositive for APA or ANA and $\geq$10 embryos transferred

LDA=low dose aspirin.
APA=antiphospholipid antibody.
ANA=anti nuclear antibody.

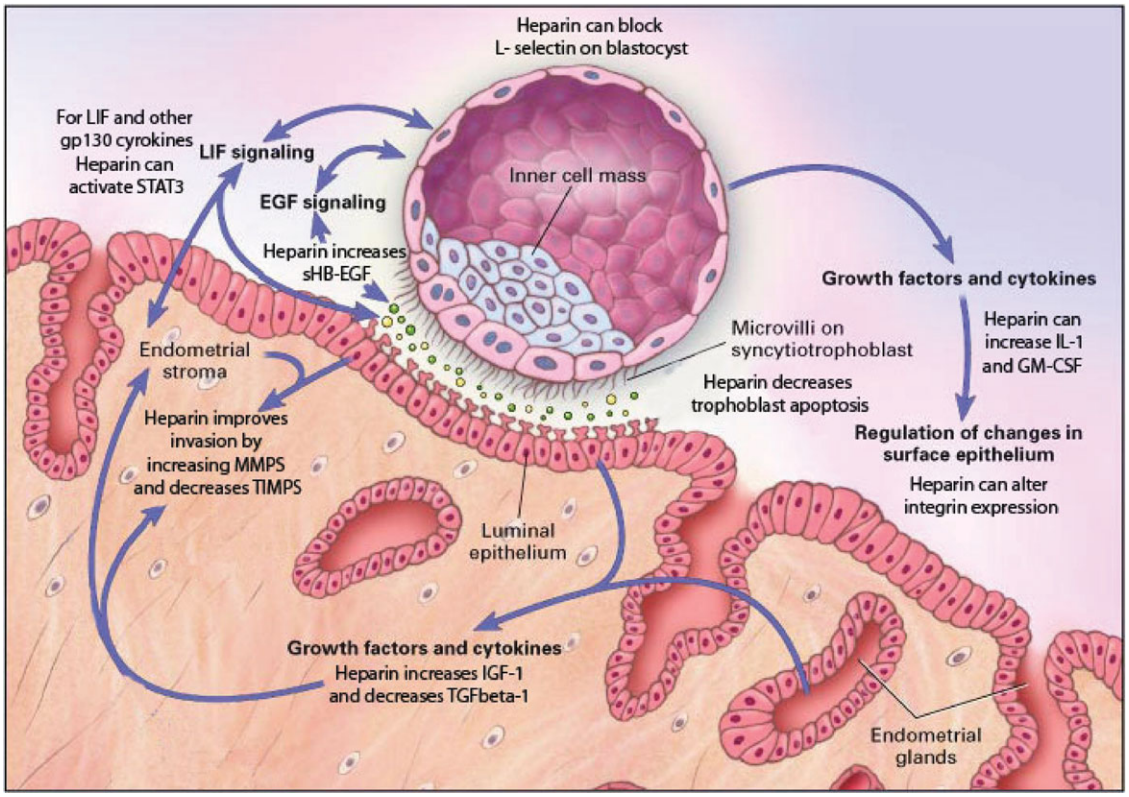

Figure 3: Potential actions of heparin on implantation.
The diagram shows a preimplantation blastocyst and the processes by which heparin may alter the processes necessary for uterine receptivity and blastocyst apposition and adhesion. Figure adapted with permission from (Norwitz *et al*., 2001). Copyright © *2001* Massachusetts Medical Society. All rights reserved. EGF, epidermal growth factor; MMPS, metallo-matrix proteinases; TIMPS, tissue inhibitors of MMPs; IGF, insulin-like growth factor; TGF,transforming growth factor; STAT3, signal transducer and activator of transcription 3; GM-CSF, granulocyte-macrophage colony stimulating factor.

implantation and trophoblast invasion. The remainder of this review will address these potential similarities, with a brief outline of the role of key systems and their potential interaction with heparin (Fig. 3). Although there is now widespread use of heparin and LMWH during pregnancy, there is little solid evidence for it use and thereby we anticipate this narrative review will generate hypotheses, and may guide future clinical trials of heparin in patients undergoing assisted conception.

Selectins and heparin

The initial interaction of the blastocyst with the endometrial epithelial surface is analogous to that of leukocyte trafficking. Leukocyte rolling is an important step for the successful recruitment of leukocytes into tissue and is mediated by a group of C-type lectins, termed selectins. Three different selectins have been identified: P-, E- and L-selectin—which recognize and bind to crucial carbohydrate determinants on selectin ligands. On the blastocyst side, strong L-selectin staining has been observed over the entire embryo surface (Genbacev *et al*., 2003). Conversely on the maternal side, the expression of the selectin oligosaccharide-based ligands, e.g. MECA-79 and HECA-452 are up-regulated during the window of implantation, with expression predominantly on luminal epithelium (Genbacev *et al*., 2003). The selectin adhesion system may therefore constitute an initial step in the implantation process.

Heparin may modulate selectins detrimentally as heparin can block selectin-mediated cell adhesion (Wang *et al*., 2002). UFH at *in vivo* concentrations has the greatest degree of inhibition in model systems including U937 cell adhesion to activated human lung microvascular endothelial cells (Wang *et al*., 2002). However, like many of the effects of heparin, this ability to modulate selectin binding is molecular weight dependent. Tinzaparin, which is known to have 22–36% of fragments greater than 8 kDA, also significantly impaired L-selectin binding. In contrast, enoxaparin with 0–18% fragments >8 kDa did not impact on L-selectin expression (Stevenson *et al*., 2005). Therefore, care with the choice of LMWH would be required, as the use of UFH or a LMWH with a high contribution of large molecular weight fragments such as tinzaparin could impair selectin expression and implantation. Confirmation of the potential effects of heparin on blastocyst selectin expression may be achieved by heparin supplementation of *in vitro* embryo culture medium in model systems, and secondly in primary cultures of endometrium and related cell lines, with assessment of selectin expression and adhesive function.

Cadherins and heparin

Cadherins are a group of glycoproteins for the calcium-dependent cell-to-cell adhesion mechanism. E-cadherin is expressed by a variety of tissues including endometrium and suppression of E-cadherin expression is associated with disruption of cell-to-cell adhesion, cell dissociation and dispersion and acquisition of invasive growth (Cavallaro and Christofori, 2004). E-cadherin is up-regulated by estradiol via the estrogen receptor beta (Wada-Hiraike *et al*.,

2006) and down-regulated by progesterone (Jha *et al*., 2006). This dynamic nature with respect to sex steroids and consequently menstrual cycle is consistent with E-cadherin down-regulation facilitating trophoblast invasion. E-cadherin expression has been noted to be decreased in gestational trophoblastic disease when compared with control first trimester placenta (Xue *et al*., 2003), and E-cadherin expression undergoes a temporospatial shift in extravillous trophoblasts possessing a migrating and invasive potential (Floridon *et al*., 2000).

UFH and enoxaparin have recently been shown to down-regulate decidual E-cadherin expression (Erden *et al*., 2006), thereby potentially explaining the observations that UFH and LMWH can promote extravillous trophoblast differentiation (Quenby *et al*., 2004). LMWH also induces *in vitro* trophoblast invasion in some (Di Simone *et al*.), but not all studies (Ganapathy *et al*., 2007), with work ongoing.

Heparin-binding epidermal growth factor-like growth factor and heparin

Heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF), a member of the EGF family, functions as a mitogen and potent survival factor during stress (Pillai *et al*., 1998; Iwamoto and Mekada, 2000), with apoptosis induced by either transforming growth factor (TGF)-β or tumour necrosis factor (TNF)-α in human endometrial stromal cells reduced by HB-EGF (Chobotova *et al*., 2005). HB-EGF expression is induced by sex steroids during the secretory phase of the endometrial cycle, and persists during early pregnancy (Leach *et al*., 1999). Its expression on the surface of pinopodes (Stavreus-Evers *et al*., 2002) suggests an early role in blastocyst implantation and placentation. In humans, it has been shown that cells expressing the transmembrane form of HB-EGF adhere to human blastocysts displaying cell surface ErbB4 (Chobotova *et al*., 2002), and that HB-EGF is a potent growth factor for enhancing the development of IVF-derived embryos to blastocysts and subsequent zona hatching (Martin *et al*., 1998). Studies of mice expressing non-cleavable HB-EGF indicate that the major functions of HB-EGF are mediated by the soluble form (Yamazaki *et al*., 2003), with generation of sHB-EGF achieved by several methods including cleavage by matrix metalloproteinases (MMPs) (Prenzel *et al*., 1999).

sHB-EGF, however, requires heparin or cell surface HSPGs for binding to and activation of the EGF receptor (Aviezer and Yayon, 1994). Therefore, LMWH may potentiate sHB-EGF binding, but may also up-regulate sHB-EGF via increased MMP activity. Furthermore, cleavage of HB-EGF by MMPs could be critical in prevention of trophoblast apoptosis in the low oxygen environment that predates full trophoblast invasion (Armant *et al*., 2006). Consistent with this, the addition of HB-EGF to explant cultures of first trimester chorionic villi enhances extravillous trophoblast differentiation and invasive activity (Leach *et al*., 2004) as does LMWH (Di Simone *et al*., 2007a; Quenby *et al*., 2004).

Insulin-like growth factors and heparin

Insulin-like growth factors I (IGF-I) and II (IGF-II) are potent mitogenic and differentiation-promoting growth factors and are also implicated in implantation and fetal development (Constancia *et al*., 2002; Fowden, 2003). Importantly, they have their activity modulated by IGF binding proteins (IGFBPs), which possess binding sites for GAGs (Clemmons, 2001; Bach *et al*., 2005), including heparin and LMWH. Consequently, heparin and LMWH increase free IGF-I in a dose-dependent manner without altering total IGF-I or IGFBP levels (Arai *et al*., 1994; Moller *et al*., 2006). As IGF-I promotes the migration of trophoblast cells *in vitro* (Lacey *et al*., 2002), it is possible that localized elevations in free IGF-1 with concomitant reductions in TGF-β due to LMWH administration may promote trophoblast invasion. Similarly, increased expression of IGF-II may facilitate human extravillous cytotrophoblast cells invading the decidua and its vasculature (Han *et al*., 1996; Hamilton *et al*., 1998) as observed in mice (Pringle and Roberts, 2007).

Cytokines and heparin

Transforming growth factor. Various cytokines have been implicated in controlling trophoblast invasion (Staun-Ram and Shalev, 2005; Achache and Revel, 2006). TGF-β1 to -3 are expressed both in endometrial and trophoblast cells, and been shown to inhibit trophoblast proliferation and invasion (Graham *et al*., 1994; Caniggia *et al*., 1999; Lash *et al*., 2005). LMWH inhibits TGF-β1 expression by mesangial cells in response to insult, by prevention of enhanced binding of nuclear proteins to the regulatory AP-1 site of the TGF-β1 promoter (Weigert *et al*., 2001). Furthermore, in animal models of renal disease, LMWH attenuates collagen and fibronectin deposition, and decreases TGF-β (Pecly *et al*., 2006).

Interleukin-1. Interleukin-1, a pro-inflammatory cytokine, has also been suggested to be involved in implantation (Salamonsen *et al*., 2000; Dimitriadis *et al*., 2005b), with intraperitoneal injections of the natural inhibitor IL-1 receptor antagonist (IL-1ra) preventing blastocyst implantation in mice due to down-regulation of integrins at the luminal epithelial surface (Simon *et al*., 1994). Supplementation of IL-1 to blastocysts in culture increases endometrial epithelial cell β3 integrin expression with an improvement blastocyst adhesion (Simon *et al*., 1997). Although the effect of LMWH on trophoblast or blastocyst IL-1 expression has not been examined, heparin and LMWH are reported to increase IL-1 expression in activated leukocytes (McBride *et al*., 1996; Call and Remick, 1998) again raising the possibility that modulation of integrin expression may be possible.

Gp130 cytokines. A number of other cytokines that share an accessory signal transducing subunit (gp130) are also concerned with implantation, namely leukaemia inhibitory factor (LIF), interleukin-6 (IL-6) and interleukin-11 (IL-11). All of these activate the janus kinase/signal transducer and activator of transcription (JAK/STAT) signal transduction pathway (Heinrich *et al*., 1998), with attenuation of their signal transduction via suppressors of cytokine signalling facilitating a negative feedback loop (Alexander, 2002; Alexander and Hilton, 2004). LIF is known to regulate differentiation, proliferation and survival of various cells in the embryo as well as in the adult (Gearing, 1993). LIF has been shown to be involved in a number of reproductive steps including sperm survival (Attar *et al*., 2003), ovulation (Arici *et al*., 1997), enhancement of blastocyst formation and hatching (Lavranos *et al*., 1995; Dunglison *et al*., 1996) and ovine and murine implantation (Fry *et al*., 1992; Stewart *et al*., 1992). LIF is also likely to have a role in human implantation (Dimitriadis *et al*., 2005b), and although predominantly regulated by progesterone(Cameron *et al*., 1997; Danielsson *et al*., 1997), TGF-β1 and HB-EGF (Arici *et al*., 1995; Lessey *et al*., 2002) are also important, with the latter absent in LIF null mice (Song *et al*., 2000). To date, no studies have examined the interaction of LIF and heparin, although given the interactions of HB-EGF and TGF-β1 with heparin, potential up-regulation of LIF expression is feasible with experimental analysis urgently required.

Interleukin-11 (IL-11) is another pleiotropic gp130 cytokine (Trepicchio and Dorner, 1998) which functions as a hematopoietic growth factor and immunoregulator (Opal and DePalo, 2000),

Table VIII. Antenatal prophylactic and therapeutic doses of LMWH.

Prophylaxis			
Normal body weight (50–90 kg)	Enoxaparin 40 mg/daily	Dalteparin 5000 IU daily	Tinzaparin 4500 IU daily
Body weight<50 kg	Enoxaparin 20 mg/daily	Dalteparin 2500 IU daily	Tinzaparin 3500 IU daily
Body weight>90 kg	Enoxaparin 40 mg/12 hourly	Dalteparin 5000 IU 12 hourly	Tinzaparin 4500 12 hourly
Therapy			
Therapeutic dose	Enoxaparin 1 mg/kg 12 hourly	Dalteparin 90 IU/kg 12 hourly	Tinzaparin 90 IU/kg 12 hourly

exhibiting anti-inflammatory effects by regulating immune effector cell function (Trepicchio *et al*., 1997). IL-11 has additional positive roles in decidualization (Dimitriadis *et al*., 2005a), decidual specific maturation of natural killer cells (Ain *et al*., 2004) and potentially implantation (Dimitriadis *et al*., 2006) and regulation of trophoblast invasion (von Rango *et al*., 2004).

Heparin acts synergistically with IL-11 to induce STAT3 activation (Walton *et al*., 2002) via the mitogen-activated protein kinase pathway (Rajgopal *et al*., 2006). The importance of the JAK/STAT pathway has been demonstrated by the impaired implantation associated with STAT3 inhibition (Catalano *et al*., 2005) and the embryonic lethality of STAT3 deficient mice, with STAT3-deficient embryos implanting but subsequently dying due to placental defects (Takeda *et al*., 1997; Ernst *et al*., 2001). A role for STAT3 activity in trophoblast invasion has also been reported (Corvinus *et al*., 2003; Poehlmann *et al*., 2005). Given the observations that IL-11 is reduced in glands in ectopic non-viable tubal pregnancies compared with viable ectopic and normal intrauterine pregnancies (von Rango *et al*., 2004) and that relative to fertile women IL-11 is reduced in endometrium from women with infertility (Dimitriadis *et al*., 2006), the augmentation of IL-11 signalling and induction of STAT3 by heparin may prove to beneficial both in terms of implantation and placental development.

IL-6 in addition to its roles as a gp130 adipokine, regulation of the acute phase response and haematopoiesis is also implicated in reproduction with IL-6 deficient mice exhibiting reduced fertility and a decrease in viable implantation sites (Dimitriadis *et al*., 2005b). In humans, production of Th2 cytokines including IL-6 by peripheral blood mononuclear cells is significantly lower and Th1 cytokines are significantly elevated with miscarriage (Makhseed *et al*., 1999, 2001; Raghupathy *et al*., 2000). Furthermore, serum IL-6 levels have been reported to be significantly lower in cases with recurrent miscarriages (Hattori *et al*., 2007) and mRNA expression for IL-6 is significantly lower during the mid-secretory phase in the endometrium of patients with recurrent miscarriage (Lim *et al*., 2000; Bates *et al*., 2002).

The effect of heparin on endometrial IL-6 production is not known; however, LMWH stimulates IL-6 production by peripheral blood mononuclear cells in a dose-dependent manner (Köller *et al*., 2001) and more importantly the LMWH enoxaparin had identical effects to recombinant IL-6 in reducing embryonic absorption in a pro-abortive murine model (Gutierrez *et al*., 2004). IL-6 signalling occurs via gp130 and the JAK/STAT pathway (Heinrich *et al*., 2003), and therefore the beneficial effects of heparin may be via its involvement in this pathway (Walton *et al*., 2002; Rajgopal *et al*., 2006).

Granulocyte-macrophage colony-stimulating factor. Granulocyte-macrophage colony-stimulating factor stimulates proliferation and differentiation of myeloid precursors into several cell types, such as monocytes/macrophages, granulocytes (Metcalf, 1991). GM-CSF is also an important determinant of pregnancy outcome, through its actions as an immune-regulatory agent contributing to maternal immune tolerance of the fetal–placental tissues, and as a trophic growth and viability factor in preimplantation embryo development and regulation of placental morphogenesis (Robertson, 2007). A high affinity protein receptor for GM-CSF has been described (Hayashida *et al*., 1990) and localized to human endometrium (Chegini *et al*., 1999; Zhao and Chegini, 1999) with its low affinity variant localized to trophoblast (Loke *et al*., 1992; Hampson *et al*., 1993; Jokhi *et al*., 1994). Several lines of evidence indicate that these are not the only receptors involved in the cellular responses to GM-CSF, with sulphated GAGs also implicated in the co-activation of growth factor receptors upon ligand binding (Harmer *et al*., 2003). Moreover, cell-surface proteoglycans, mainly through their GAG moieties, participate in GM-CSF signalling and mitogenic activity (Modrowski *et al*., 1998, 2000), with heparan sulphate and heparin in particular, regulating the biological functions of GM-CSF (Sebollela *et al*., 2005). Notably, LMWH is also capable of binding to GM-CSF (Liang *et al*., 2006). Again although direct evidence for heparin is lacking, a positive effect on this pathway is possible.

Matrix metalloproteinases and heparin. MMPs are a family of 22 endopeptidases capable of degrading all components of the ECM and are important mediators of cell behaviour including cell–matrix and cell–cell interactions. *In vitro* studies suggest that successful implantation and placentation result from the balance between secretion of MMPS from the trophoblast and their inhibition by their natural antagonist tissue inhibitors of metalloproteinases (TIMPs) (Niu *et al*., 2000). With respect to the MMPs, gene knockout studies in mice suggest that MMP-9 is critical for implantation as MMP-9 deficient mice have a decreased litter size and an increase in percentage of infertile mice (Dubois *et al*., 2000). MMP-9 is known to degrade collagen IV, the main component of the basement membrane, and in conjunction with MMP-2 may enable the invasion of trophoblast cells through the decidua and into the maternal vasculature. Consistent with this concept several *in vitro* studies have shown that MMP-2 and MMP-9 are required for trophoblast invasion (Bischof *et al*., 1995; Isaka *et al*., 2003; Staun-Ram *et al*., 2004; Xu *et al*., 2000), with TGFβ1 down-regulating MMP expression (Zhao *et al*., 2006) and increasing TIMP-1 (Chakraborty *et al*., 2002).

The role played by MMPs in modulating invasion may not solely be tissue degradation as they have also been shown to break down IGFBPs thereby increasing IGF-1 bioavailabilty and promotion of invasion (Martin *et al*., 1999). They also regulate expression of the angiogenesis inhibitor angiostatin (Chung *et al*., 2006), thereby allowing a tight and balanced control on angiogenesis during the initial stages of implantation. Although divergent effects of heparin on MMPs have been observed (Kenagy *et al*., 1994; Putnins *et al*., 1996; Tyagi *et al*., 1997; Gogly *et al*., 1999; Hornebeck *et al*., 1999), LMWH at therapeutic doses has been shown to induce

trophoblast MMP-2 and MMP-9 transcription and protein expression *in vitro*, with a concomitant reduction in TIMP-1 and TIMP-2 expression (Di Simone *et al*.). Therefore, LMWH appears capable of improving the invasive capacity of trophoblast cells by regulating their degradative capacity.

Conclusions

Although it is clear that heparin can modify the thrombotic response associated with assisted reproduction, implantation is a complex process involving spatiotemporally regulated endocrine, paracrine, autocrine and juxtacrine modulators that span cell–cell and cell–matrix interactions. The dynamic coordination of these events and the interaction between the developing embryo and the endometrium continues to be elucidated. It is clear, however, that heparin can potentially modulate many of the known mechanisms that underlie successful apposition, adhesion and penetration of the developing embryo. Furthermore, there is now evidence that in women with repeated IVF failure and thrombophilia, heparin can improve pregnancy rates. Confirmation of the outlined potential of heparin and its associated compounds to alter the molecular processes underpinning successful implantation is urgently required given the potential for clinical translation to increased pregnancy and live birth rate and a reduction in adverse perinatal outcomes for all women undergoing assisted reproduction.

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