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Nanoparticles in pregnancy: the next frontier in reproductive therapeutics

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TABLE OF CONTENTS

- Introduction
- Principles of nanoparticles in pregnancy
  - Nanoparticle fundamentals
  - Types of nanoparticles investigated in reproductive medicine
  - The placenta: a gatekeeper to the foetal compartment
  - Safety of nanoparticles in pregnancy
  - Practical challenges to clinical translation of nanoparticle treatment in pregnancy
- Nanoparticle treatment for infertility
  - Treatment of conditions impacting fertility
  - Treatment of the gamete or embryo
  - Male factor infertility
- Nanoparticle therapy in early pregnancy
  - Disorders of abnormal trophoblastic growth
- Nanoparticle treatment in late pregnancy
  - Foetal growth restriction
  - Preeclampsia
  - Preterm labour
  - Selective treatment of the mother or foetus
- The prospects for clinical translation
- Conclusions

BACKGROUND: Nanotechnology involves the engineering of structures on a molecular level. Nanomedicine and nano-delivery systems have been designed to deliver therapeutic agents to a target site or organ in a controlled manner, maximizing efficacy while minimizing off-target effects of the therapeutic agent administered. In both reproductive medicine and obstetrics, developing innovative therapeutics is often tempered by fears of damage to the gamete, embryo or developing foetus or of negatively impacting a woman's reproductive

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potential. Thus, nanomedicine delivery systems may provide alternative targeted intervention strategies, treating the source of the disease and minimizing long-term consequences for the mother and/or her foetus.

OBJECTIVE AND RATIONALE: This review summarizes the current state of nanomedicine technology in reproductive medicine and obstetrics, including safety, potential applications, future directions and the hurdles for translation.

SEARCH METHODS: A comprehensive electronic literature search of PubMed and Web of Science databases was performed to identify studies published in English up until February 2020. Relevant keywords were used to obtain information regarding use of nanoparticle technology in fertility and gene therapy, early pregnancy complications (ectopic pregnancy and gestational trophoblastic disease) and obstetric complications (preeclampsia, foetal growth restriction, preterm birth and gestational diabetes) and for selective treatment of the mother or foetus. Safety of specific nanoparticles to the gamete, embryo and foetus was also investigated.

OUTCOMES: Pre-clinical research in the development of nanoparticle therapeutic delivery is being undertaken in many fields of reproductive medicine. Non-hormonal-targeted nanoparticle therapy for fibroids and endometriosis may provide fertility-sparing medical management. Delivery of interventions via nanotechnology provides opportunities for gene manipulation and delivery in mammalian gametes. Targeting cytotoxic treatments to early pregnancy tissue provides an alternative approach to manage ectopic pregnancies and gestational trophoblastic disease. In pregnancy, nanotherapeutic delivery offers options to stably deliver silencing RNA and microRNA inhibitors to the placenta to regulate gene expression, opening doors to novel genetic treatments for preeclampsia and foetal growth restriction. Restricting delivery of teratogenic drugs to the maternal compartment (such as warfarin) may reduce risks to the foetus. Alternatively, targeted delivery of drugs to the foetus (such as those to treat foetal arrythmias) may minimize side effects for the mother.

WIDER IMPLICATIONS: We expect that further development of targeted therapies using nanoparticles in a reproductive setting has promise to eventually allow safe and directed treatments for conditions impacting the health and reproductive capacity of women and for the management of pregnancy and serious pregnancy complications.

Key words: nanoparticles / nanotechnology / reproduction / pregnancy / ectopic pregnancy / infertility / selective treatment / targeted therapeutic delivery

Introduction

The ultimate aim of all human reproduction is to achieve a live, healthy term birth. This requires a healthy mother, who can successfully conceive, sustain the pregnancy and subsequently evade or navigate many pregnancy-related complications. Many factors have led to increased perinatal complications, including increasing maternal age (Cohen, 2014), obesity (Marchi *et al.*, 2015) and increased use of ARTs (Pandey *et al.*, 2012; Pinborg *et al.*, 2013), all of which increase the risk of obstetric complications.

Nanotechnology involves the manipulation and engineering of structures at the molecular level, often <100 nm in size (Kim *et al.*, 2010a). As nanoparticles are of a similar size to proteins and intracellular structures, they are able to utilize existing cellular mechanisms to help facilitate their function (Bamrungsap *et al.*, 2012). The development of tissue-targeted nanotherapeutic delivery has largely been pioneered by the oncology field (Peer *et al.*, 2007), where nanoparticles have been successfully exploited to load, carry and deliver drugs. Importantly, nanoparticles can be engineered to selectively target a site, organ or tumour, maximizing efficacy while minimizing the dose, and thus the side effects, of the drugs administered. For the field of reproductive medicine where the consequences of off-target effects can be significant, targeted nano-delivery systems have the potential for great impact.

In infertility, nanoparticle use must be carefully considered, especially since the developing gamete and embryo are exceptionally sensitive, and any damage could have profound consequences. The influence of drugs on a single gamete may cause substantial developmental effects (Gandolfi and Brevini, 2010); however, it is in these early stages that interventions such as genetic manipulation also have the potential to offer great hope. The treatment of gynaecological conditions impacting fertility is also tempered by concerns of any adverse toxicological effects that may impact the female reproductive tract. It is in fertility treatments that one must tread carefully, but in which targeted drug delivery systems have the potential for considerable impact while reducing invasive surgical treatments. This approach would offer important advances to the field.

The first trimester is a potentially sensitive period since at this time the foetus's major organ systems are forming; therefore, the foetus is most sensitive to the teratogenic effects of drugs (Kinch, 1982; Byrd and Francis, 1998; Choi *et al.*, 2013; Vargesson, 2015). For most, this has created a natural hesitancy in manipulation of the human embryo or administering interventions in early pregnancy. Even beyond the first trimester, active development of novel therapeutics remains very limited (Fisk and Atun, 2008, 2009). The likely reason for this is that there is fear that any medication given may inadvertently cause harm to the developing foetus, thus tempering innovation and investment. This is despite the fact that new treatments are desperately needed to avert the lifelong legacies of preeclampsia (Williams, 2011), preterm birth (Chan *et al.*, 2010; Miller *et al.*, 2016), gestational diabetes (Damm *et al.*, 2016) and foetal growth restriction (Chan *et al.*, 2010), for both the infant and mother (Barker *et al.*, 1989; Ganguly *et al.*, 2020).

In addition to increases in pregnancy-specific complications, more women than ever of reproductive age are entering pregnancy with pre-existing medical conditions that require ongoing treatment (Spurgeon, 2008; Umesawa and Kobashi, 2017). Conversely, some foetal conditions, such as foetal arrhythmias, require treatments that have no direct benefit for the mother (Singh, 2004). Targeting treatments, to either prevent placental transfer or facilitate transport to treat the foetus, would have enormous advantages, providing optimal precision care for both the mother and her baby. It is time that the

medical field stops seeing pregnant women as a population where drug therapies should be avoided, but instead, as a population in which safe and efficacious treatments are desperately needed (Fisk and Atun, 2009; Keelan et al., 2015). Embracing nanotechnology may provide novel treatment options for obstetric medical conditions, which overcome the anxieties arising from off-target effects to either foetus or mother. Further development of nanotechnology offers hope for currently untreatable obstetric conditions (Harris, 2016).

Principles of nanoparticles in pregnancy

Nanoparticle fundamentals

Nanoparticles (or nanocarriers) used for targeted delivery of therapeutics need to be able to stably encapsulate or carry a drug, avoid destruction in the maternal circulation, identify and enter a target cell and dissemble and release the contents within the target cell. Furthermore, they need to do this without causing adverse effects of toxicity or immune system activation to either the mother or the foetus (Kim et al., 2010a; Wilczewska et al., 2012) (Table I).

Nanoparticles have relatively large surface area to volume ratio, allowing their physical and chemical properties to be engineered, and their surface to be coated with other molecules. Physical characteristics affecting the pharmacokinetics of nanoparticles include their chemical composition, size, shape and structure, surface charge and pH (Peer et al., 2007; Xia et al., 2009). Most nanoparticles are either inorganic or organic. In reproductive medicine, greater focus has been placed on the use of organic nanoparticles, due to their biocompatibility and biodegradability (Keelan et al., 2015). Organic nanoparticles can either encapsulate the drug inside or integrate the drug on the surface of the nanoparticle (Kim et al., 2010a).

When carrier nanoparticles break down, the payload (e.g. drug) is released. Controlling the rate of degradation of nanoparticles can be a useful strategy to prevent rapid release into the bloodstream, allowing controlled drug release at the target site. For most organic nanoparticles, the half-life depends largely on renal excretion and the ability to evade the reticuloendothelial system (Dobrovolskaia et al., 2008). PEGylation (inclusion of negatively charged hydrophilic polyethylene glycol (PEG) into the formulation) is a commonly used strategy to enable the nanoparticles to move through the body undetected by the immune system. This can extend the nanoparticles' half-life, lowering the therapeutic dose required, increasing the chance that it reaches the target organ and reducing off-target effects (Salata, 2004; Alexis et al., 2008).

Nanoscale particles exhibit unique properties, making it very challenging to predict their likely pharmacokinetic effects (Saunders, 2009). Nanoparticles that are <50 nm in diameter become subject to the laws of quantum physics (Saunders, 2009). This can make predicting their exact effects difficult, for example whether they will end up inducing oxidative stress or acting as antioxidants (Saunders, 2009). However, working at a nanoscale provides options to treat pathology on a molecular level and enables exploitation of existing cellular mechanisms to facilitate drug delivery (Bamrungsap et al., 2012; Patra et al., 2018).

Non-targeted nanoparticles passively accumulate in organs with high blood flow, such as the liver, kidney, spleen and in pregnancy, the placenta, as well those with high levels of vascular permeability, such as tumours (Jahan et al., 2017). A major advantage with nanotherapy is the ability to perform surface modification, conjugating the nanoparticle to targeting peptides or antibodies. These targeting ligands work through binding to a moiety ideally expressed only on the target tissue. This allows the nanoparticles to accumulate and degrade at the target organ, maximizing the delivery of drug to the desired location (Friedman et al., 2013).

Table I Characteristics of nanoparticles that would be beneficial in treating pregnancy complications.

Characteristic	Benefit
Targeting ability	Allows selective targeting of the mother, placenta or foetus, reducing risk to the foetus when trialling experimental drugs.
Increased efficacy and/or bioavailability of drugs	A lower concentration of drug will achieve the same biological effect, reducing the dose required, thus limiting the potential adverse side effects that the mother or foetus are exposed to.
Prevention of drug degradation and onset of action via maternal clearance mechanisms	Concealing drugs within nanoparticles prevents first pass metabolism as they pass through the liver and limits uptake by cells other than the intended target. Enables delivery of drugs to the placenta that would otherwise have been cleared by maternal circulation.
Encapsulation of otherwise unstable or insoluble therapeutic agents	This enables advanced treatments, such as the use of siRNA to treat placental conditions, to be utilized.
Nanoscale properties	Nanoparticles can be modified to exploit existing physiological pathways and internalization mechanisms, which could allow for design of a systemically delivered drug engineered to be taken up or act through a specific route in the placenta during pregnancy.
Large carrying capacity due to large surface area to volume ratio	Improved drug loading and production of more effective formulations. Allowing reduced dosing of drugs and limiting the adverse side effects that the mother or foetus is exposed to.

Types of nanoparticles investigated in reproductive medicine

There are many types of nanomaterials and nanocarriers used for drug transfer. A select few are discussed below, with a focus on those that have been investigated for the treatment of reproductive issues.

Liposomes

Liposomes are organic engineered vesicles ranging between 50 and 1000 nm in size with a phospholipid bilayer wall, similar to cell membranes (Fig. 1A). They can be used to increase the solubility of lipophilic drugs and improve the pharmacokinetic properties by reducing systemic toxicity or slowing drug elimination. These properties make them ideal packaging vectors for drugs that are otherwise unstable in circulation or with unacceptable systemic toxicity (Lian and Ho, 2001; Torchilin, 2005; Bamrungsap *et al.*, 2012). An advantage to liposomes is their ability to encapsulate not only macromolecules but also DNA, siRNA (to therapeutically regulate gene expression) and small proteins (Majzoub *et al.*, 2016). This has made them one of the most established and tested nanoparticle drug delivery systems (Zylberberg and Matosevic, 2016).

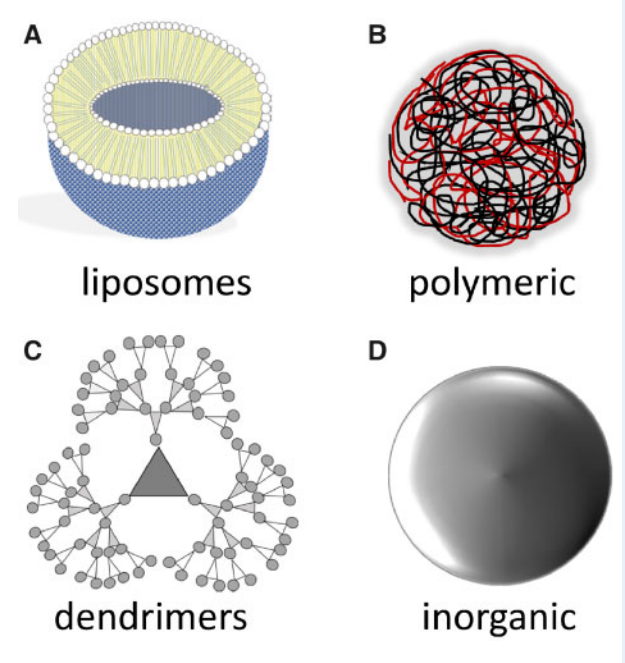

Figure 1. Different types of nanoparticles used in reproduction. (**A**) Liposomes are organic engineered vesicles that range between 50 and 1000 nm in size and are characterized by their phospholipid bilayer. They can be used to encapsulate macromolecules, DNA, siRNA and small proteins. (**B**) Polymeric nanoparticles are organic nanoparticles with varying structures and can be either biodegradable synthetic polymers, or naturally occurring. The physical and chemical structure of polymers can be engineered for specific purposes, or they can be modified to incorporate drugs for delivery. (**C**) Dendrimers are large synthetic nanoparticles that branch to form a tree-like structure. Their chemical composition can be easily customized for predictable pharmacokinetics and drugs can be loaded onto the external branching surfaces for delivery. (**D**) Inorganic nanoparticles cover a broad range of substances such as metal oxides, metal salts and elemental metals. They do not degrade and thus their safety in pregnancy is still under scrutiny.

However, they still have limitations such as a relatively low encapsulation efficiency, their instability in storage and their quick drug release if orally administered (Bamrungsap *et al.*, 2012). Strategies to control the pharmacokinetics through enhanced circulation time in the blood have included PEGylation and other stealth mechanisms (Sihorkar and Vyas, 2001; Stadler *et al.*, 2009) or the design of environmental triggers for drug release, such as degradation of the nanoparticle and release of the drug in response to a certain pH or temperature (Guo and Szoka, 2003). Conjugation with targeting ligands such as antibodies or peptides can facilitate enriched targeted nanoparticle therapeutic delivery, resulting in a significantly lower amount of the therapeutic required compared to systemic administration, reducing patient side effects and increasing the tissue-specific delivery, since the targeting mechanism concentrates the liposomes administered at the target organ.

Current clinical uses of liposomes include drug delivery of chemotherapeutics, such as doxorubicin for ovarian cancer and breast cancer (Gabizon *et al.*, 1989; Rivera, 2003), packaging cytarabine enhancing sustained release for treatment of lymphomatous meningitis (Glantz *et al.*, 1999) and daunorubicin for solid tumours (Lowis *et al.*, 2006).

Polymeric nanoparticles

Polymeric nanoparticles describe a group of organic nanoparticles with varying structures, such as spherical or branched (Fig. 1B). They can be composed of either biodegradable synthetic polymers or naturally occurring polymers such as albumin or chitosan (Bamrungsap *et al.*, 2012). They are particularly efficient for conjugating macromolecular drugs, such as protein or peptide drugs, which can substantially increase in their bioavailability by extending their half-life (Greenwald *et al.*, 2003). An advantage to polymers is that their versatility allows engineering of their physical or chemical properties, so they can respond to environmental signals (e.g. changing temperature, different pH and biological signals with enzymes) (Bamrungsap *et al.*, 2012).

Polymers can be categorized based on their method of incorporating drugs, including direct conjugation, hydrogen bonds formed from hydrophobic interactions (such as polymeric micelles) and hydrogels, which encapsulate hydrophilic drugs within a water-filled depot (Bamrungsap *et al.*, 2012). Not all are easily biodegradable that limits their widespread clinical use (Bamrungsap *et al.*, 2012); however, there are polymers currently used in clinical practice. These include polymer-mediated delivery of chemotherapy drugs (Meerum Terwogt *et al.*, 2001; Rosen *et al.*, 2003; Sabbatini *et al.*, 2004; Duncan, 2006; Duncan *et al.*, 2006; Etrych *et al.*, 2011) and PEGylated interferon (IFN)-α-2a to treat hepatitis C (Glue *et al.*, 2000).

Dendrimers

Dendrimers are large synthetic nanoparticles that branch to form a tree-like structure (Fig. 1C) . An advantage of dendrimers is that their chemical composition can be easily customized, allowing the developer to make pharmacokinetics more predictable (Lee *et al.*, 2005). Drugs are loaded on to the external branching surface of the polymeric nanoparticle (Fig. 1C), which provides a very large surface area, meaning the loading capacity is higher than expected for such a small molecule (Svenson and Tomalia, 2005). One of the more commonly used

dendrimers are the polyamidoamine (PAMAM) dendrimers. PAMAM can be conjugated with antibodies to allow targeted therapy (Bamrungsap et al., 2012). Although dendrimers are not yet in current clinical use, they have potential for translation. Specifically their utilization would have advantages for delivery of targeted chemotherapeutic medicine (Wolinsky and Grinstaff, 2008; Myc et al., 2010), improving oral administration of drugs that are otherwise easily broken down in the gastrointestinal tract (Najlah et al., 2007), enhancing intracellular delivery (Najlah and D'Emanuele, 2006) and delivery of antihypertensive agents via antibody nanocarriers (Menjoge et al., 2010a).

Inorganic nanoparticles

The safety of inorganic nanoparticles (Fig. 1D), including metallic nanoparticles, in pregnancy has been the subject of significant interest. However, this research has focussed on safety of the nanoparticle rather than their development as a drug delivery system in pregnancy. Since they do not biodegrade and may cause damage to the placenta, translation to treat pregnancy complications is unlikely (Muoth et al., 2016a). Further work is required to determine which inorganic nanoparticles exhibit low to no toxicity and might be suitable for imaging applications and thermal treatments. Thus, inorganic nanoparticles will not be a major focus of this review, but some examples of potential uses however will be given.

The placenta: a gatekeeper to the foetal compartment

Pharmacologically, the placenta has many parallels to the blood–brain barrier in that it acts as a gatekeeper to the foetal compartment and prevents indiscriminate exchange of materials between the mother and foetus (Rosenfeld, 2017). Teleologically, this protects against infection and an immune response by the mother against the developing conceptus (because it has many antigens that will be foreign) but facilitates the transport of nutrients and oxygen to the foetus and the elimination of waste materials away from the foetus (Jashvant et al., 2004).

The maternal–foetal barrier is the structure separating the foetal circulation from the intervillous space. The surface of the placenta, the syncytiotrophoblast, forms a very large surface area, due to the extensive microvillous nature, allowing for optimal diffusion and exchange and significant endocytic uptake (Frank, 2017). The maternal blood first interacts with the syncytiotrophoblast on the placenta, a multinucleated layer covering the villi and lining the intervillous space. Directly beneath the syncytiotrophoblast layer is the cytotrophoblast layer, which is thick in the first trimester and thins out by the second and third trimesters (Sibley et al., 2018). Beneath this is the trophoblastic basal lamina, connective tissue from the extraembryonic mesoderm and the foetal vascular endothelium (Hannan et al., 2006; Frank, 2017) (see Fig. 2).

Throughout pregnancy, the ability of drugs to cross the placenta is not constant. In the early first trimester, most drugs or chemicals in the maternal bloodstream cross to the embryo through passive diffusion as the placenta is not yet perfused with maternal blood. By the end of the first trimester, the placental barrier is up to 20-µm thick, thinning to 2–5 µm at term (Syme et al., 2004). This, as well as decreasing expression of efflux transporters such as P-glycoprotein that pump drugs out of cells (Iqbal et al., 2012) and increased expression of active transporters (Hayward et al., 2017), means that closer to term the foetus has a greater potential to be exposed to maternal drugs. Moreover, placental blood flow and surface area increases dramatically with gestation and is highest close to term. This also increases the possibility of placental transfer (Jashvant et al., 2004). On the other hand, the foetus undergoes developmental changes as gestation advances, making it more robust and at less risk of being adversely affected by different drugs. These factors should be taken into consideration when considering the potential impact of candidate therapeutics (Marin et al., 2004).

There are two main routes of transfer across the maternal–foetal barrier. The first is a transcellular route, where drugs cross the surface of the plasma membrane and pass through to the cytoplasm of the syncytiotrophoblast. Lipophilic drugs diffuse passively across the lipid membrane, while hydrophilic drugs usually require active transport mechanisms (Fig. 3). The second is the paracellular route, which is an extracellular pathway (Fig. 3). The paracellular route is the mode by which many small molecule drugs pass through via passive diffusion, occurring often due to the incomplete integrity of the maternal–foetal barrier (Hayward et al., 2017; Sibley et al., 2018).

Simple diffusional transfer is the transfer between the maternal and foetal compartments passively along a concentration gradient and varies based on the composition of the molecule in question, in particular if they are hydrophilic or lipophilic. Hydrophilic molecules cannot cross the trophoblast membrane in the absence of specific transport mechanisms, although they can cross very slowly through extracellular pores. They are limited by the size of the molecule, and the permeability and pore size of the membrane, and so are considered to be 'membrane limited' (where small hydrophilic molecules diffuse more easily). Lipophilic substances, however, diffuse more readily through the trophoblast bilayer. The syncytiotrophoblast cell membrane is covered in microvilli, resulting in a vast surface area, which facilitates high rates of transfer of lipophilic substances. In particular, this is important for facilitating transfer of lipophilic gases, such as oxygen (Hayward et al., 2017).

For substances that do not cross the placenta via simple diffusion, active transport mechanisms exist. These include endocytosis, where the cell surface invaginates to form an intracellular, membrane-bound vesicle, containing molecules tethered to the surface and the surrounding extracellular fluid (Besterman and Low, 1983), which is transported across the placenta to the foetal side where it is released. This is the mechanism of transfer for molecules such as IgG. Transporter protein-mediated transport mediates transfer of glucose and other hydrophilic solutes such as amino acids across the placenta; this is an active process that requires ATP (Hayward et al., 2017; Sibley et al., 2018). These larger molecules are much less likely to enter foetal circulation as their ability to cross is dependent upon more than a concentration gradient.

Thus, the physiological placental transport systems described above could be exploited to carefully design placental-targeted nanomedicine delivery, to control uptake of molecules by the placenta and retention within the placenta. This would prevent cross over to the foetus and, if desirable, reduce systemic levels in the maternal circulation and organs.

Nanoparticles crossing the placenta

The placenta shares many common features with solid tumours. It is able to rapidly proliferate, generate a large variety of hormones,

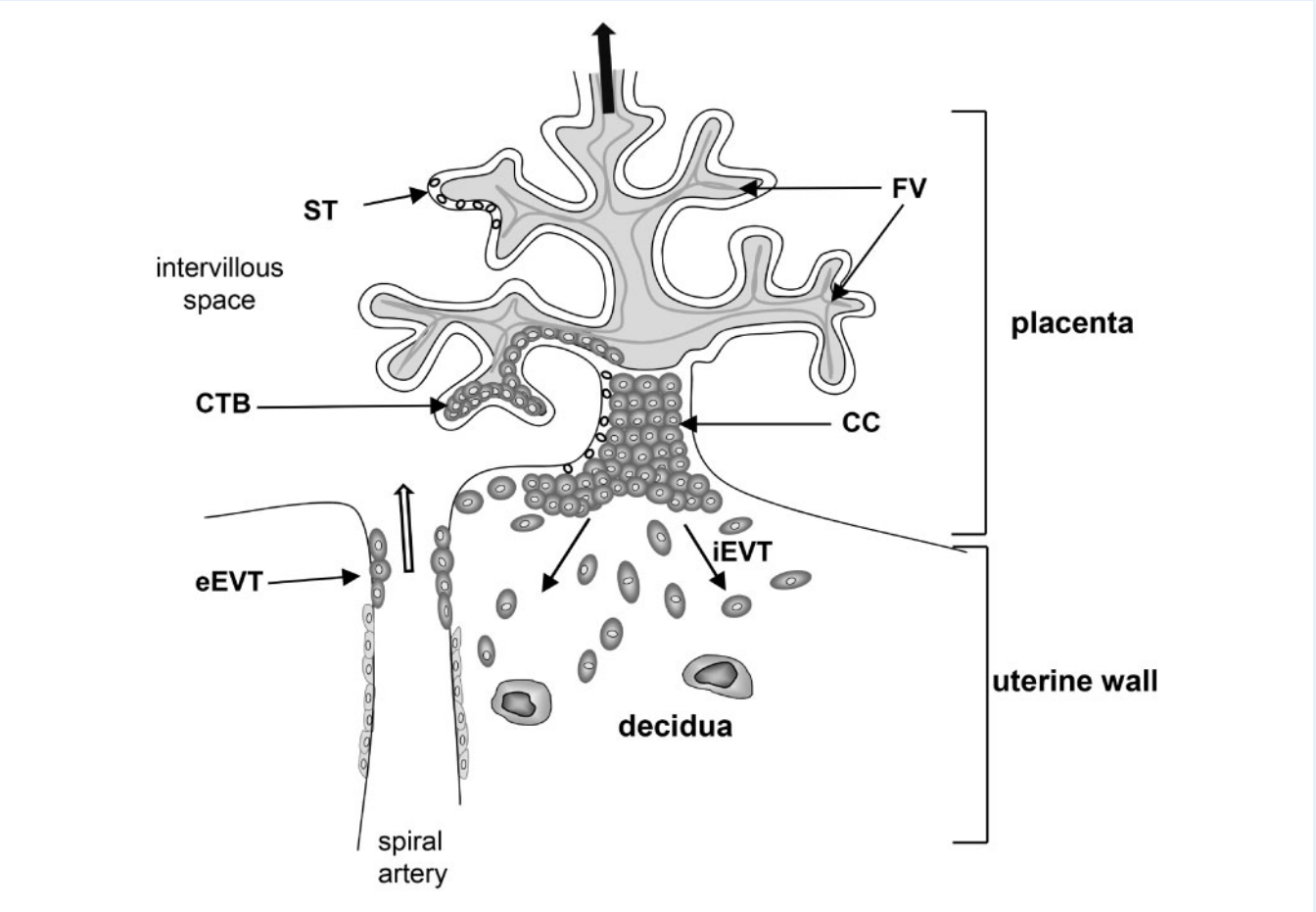

Figure 2. Diagrammatic representation of the human placental implantation site. The placenta is depicted in the intervillous space, above the uterine wall, adjacent to a maternal spiral artery. The maternal blood first interacts with the ST, a multinucleated layer covering the villi and lining the intervillous space. Directly beneath the ST layer, is the CTB layer. The villous stroma forms the layer below (*shaded in grey*), surrounding the FV. Blood flows through to the foetus (*see black arrow*) via the umbilical cord. Invasive extravillous trophobast (EVT)s (interstitial: iEVTs and endovascular: eEVTs) are depicted breaking through the CS and invading through the decidua. eEVTs plug the spiral artery. ST, syncytiotrophoblast; CTB, cytotrophoblast; DC, decidual cell; CC, cell column; FV, foetal vasculature; CS, cytotrophoblast shell. Adapted from Hannan *et al.* (2006).

growth factors and cytokines, evade immune detection and destruction and demonstrate behaviour similar to metastatic cells when extravillous trophoblasts invade the decidua and remodel the uterine spiral arteries (see Fig. 2) (Hannan *et al.*, 2006). Thus, a lot can be learned from the development of targeted therapeutic delivery via nanoparticles in oncology and applied to treating major complications of pregnancy where placental dysfunction is central, such as selective targeting of highly proliferative tissue (Peer *et al.*, 2007). The ways in which nanoparticles interact with the placenta depend on their intended action. They may be designed to cross easily in order to access the foetal circulation or be designed to prevent crossing and remain in the maternal compartment, in order to maximize dosage to the mother while minimizing harmful effects on the foetal compartment. Alternatively, for disorders originating from the placenta such as pre-eclampsia, they can be designed to directly target the surface layer of the placenta, the syncytiotrophoblast, the source of pathogenic anti-angiogenic factors that play a significant role in driving the pathogenesis of the disease (such as soluble fms-like tyrosine kinase-1; sFlt1) (Keelan *et al.*, 2015). Factors influencing nanoparticle transfer include size, surface charge and shape, any of which can be used to achieve the desired clinical effect (Muoth *et al.*, 2016a).

Many traditional transfer studies in the placenta have demonstrated that the capacity for molecules to cross is size-dependent. Small molecules $<$600 Da passively diffuse across the human placenta to reach the foetus; however, substances $>$1000 Da, such as IgG, are restricted and depend on specific syncytiotrophoblast receptors to actively facilitate transport within and across (Jashvant *et al.*, 2004; Schneider and Miller, 2010; Iqbal *et al.*, 2012). There is now substantial evidence from both animal and human models that the ability of nanoparticles to easily cross the placenta into the foetal compartment is size-dependent.

Many nanoparticle pharmacokinetic studies in pregnancy have been performed with the intention of investigating whether nanoparticle encapsulation of a drug is able to limit placental transfer of drugs to the foetal compartment and so minimize the foetal exposure. Organic silicon nanovectors (SNVs) of varying sizes (519, 834 and 1000 nm) have

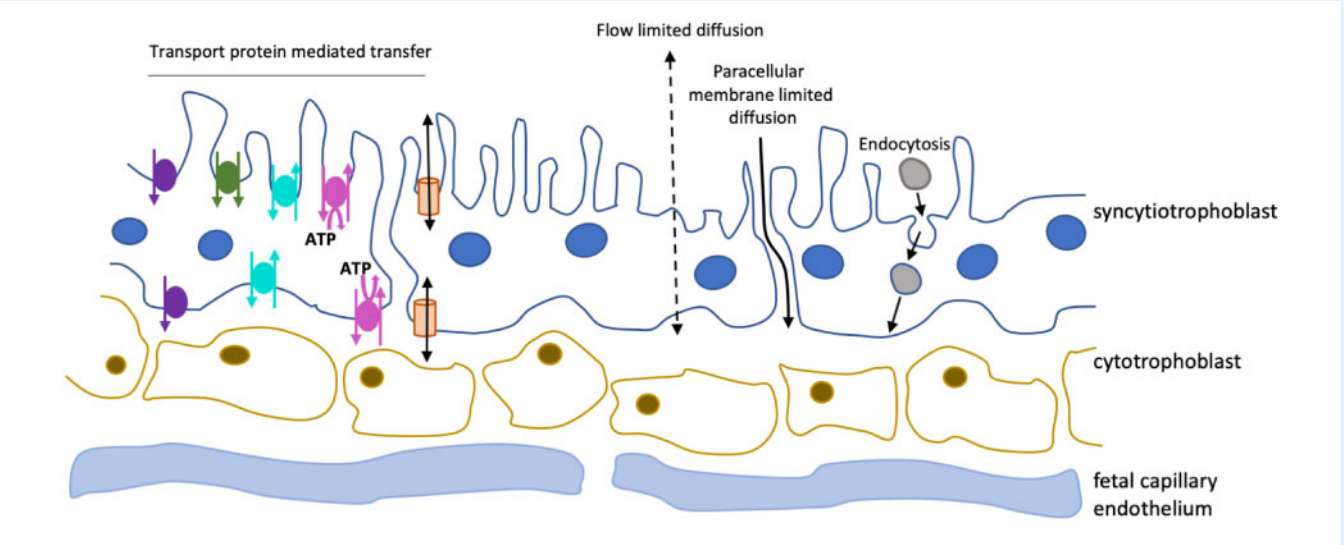

Figure 3. Diagram representing main modes of transport across the maternal–foetal interface. There are four main ways that substances can cross the maternal–foetal interface. Transport protein-mediated transfer involves the use of active transporters that may require ATP. They are found on both the apical and basal surfaces of the syncytiotrophoblast and mediate transfer of molecules such as glucose and amino acids. Flow-limited diffusion represents a transcellular mode of transport, where drugs can cross the surface of the plasma membrane and pass through the syncytiotrophoblast. It usually occurs along a concentration gradient and varies based on the composition of the molecule in question. Paracellular or extracellular transport may also be possible when there is incomplete integrity of the maternal–foetal barrier and represents the most common mode by which many small molecule drugs enter the placenta. Finally, endocytosis represents the other active transport mechanism. It involves the invagination of the cell surface to form an intracellular membrane-bound vesicle, which can then move through the cytoplasm where it may degrade, or be transferred to the foetal side. Adapted from Sibley et al. (2018).

been tested in pregnant rats. It was found that those given SNVs of 519 nm had foetal silicon levels significantly higher than those given the larger nanovectors (Refuerzo *et al.*, 2011). In pregnant mice, another group compared silica nanoparticles of diameters 35, 70, 300 and 900 nm and found only the 35 and 70 nm nanoparticles reached the foetus or accumulated in the placenta. In mice treated with the smaller nanoparticles, toxicity was demonstrated through smaller uteri and foetuses than the untreated controls; however, this was not seen with larger sized SNV (Yamashita *et al.*, 2011).

A study examining fluorescently labelled polystyrene beads showed that particles with diameters of 50, 80 and 240 nm crossed the placental barrier, but beads with a 500-nm diameter did not. Glucose consumption, lactate production and hCG release from the perfused tissue were not significantly affected by any of the particles tested. Polystyrene beads with a 240-nm diameter had no impact on the viability of the placental explant (Wick *et al.*, 2010). Placental transport of large PAMAM dendrimers (16 kDa) was compared to that of antipyrine (188 Da), a molecule known to freely diffuse across the placenta. Only a small amount of PAMAM was found within the foetal compartment, comparable to large macromolecules like IgG, demonstrating that this approach had the potential to selectively deliver drugs to the mother, while minimizing foetal exposure (Menjoge *et al.*, 2011). PEGylated gold nanoparticles 10–30 nm in diameter were also tested in perfused human placentas and found to reach the trophoblast cell layer, but not the foetal compartment (Myllynen *et al.*, 2008). Although the exact size of nanoparticle that crosses the placenta does appear to vary with the composition, in general the ability of larger molecules to cross the placenta appears greatly limited.

Studies of liposomes in a human placental transfer model again confirmed that transplacental passage can be modulated by altering the size of the liposomes (Bajoria and Sooranna, 1998), as well as the surface charge. Cationic (positive) charged liposomes were minimally transferred compared to uncharged liposomes (Bajoria and Contractor, 1997; Bajoria *et al.*, 1997b). The effect of surface charge was also demonstrated in a pregnant rat model using both anionic (negatively charged) and cationic multimodal polymeric nanoparticles in mid and late gestation. Cationic nanoparticles preferentially accumulated in the chorionic plate of the placenta, compared to anionic nanoparticles in late gestation. However, passage of both occurred more readily in early gestation, which highlights the importance of both charge and gestation in nanoparticle uptake (Ho *et al.*, 2017).

Furthermore, whether targeted delivery to the placenta can be enhanced by exploiting cellular internalization pathways to increase nanoparticle and therapeutic uptake is of keen interest to many in the field. One study developed a method for targeted delivery of lipid-polymer nanoparticles, utilizing the abundant chondroitin sulphate A (CSA) peptide on the syncytiotrophoblast (Zhang *et al.*, 2018b) to specifically internalize the nanoparticle via endocytosis. A synthetic CSA-binding peptide was conjugated to the surface of nanoparticles loaded with infrared fluorescence and the drug methotrexate. Distribution and localization following intravenous injection of nanoparticles was monitored in live animals. CSA-nanoparticles were found to specifically deliver their payload to trophoblast cells, but not to other cell types in the

mouse placenta. This demonstrates that CSA can be exploited for the targeted delivery and uptake by endocytic internalization mechanisms to enhance intracellular drug delivery in the trophoblast (Zhang *et al.*, 2018b).

Tumour-homing peptides, which have a C-terminal arginine (or less often lysine) that specifically recognize the endothelium of tumour vessels, offer delivery advantage via tissue-specific internalization mechanisms for both tumours and the placenta. These peptide sequences, CGKRK and iRGD, have been shown to penetrate deep into the extravascular tumour and placental tissue through binding to neuropilin-1 (NRP-1) on the target tissue (Teesalu *et al.*, 2009; King *et al.*, 2016). Our work has also shown that the CGKRK peptide binds to calreticulin on the placental surface that is normally intracellular but is expressed on the surface of the syncytium (King *et al.*, 2016) and tumour cells. We identified that membrane-associated calreticulin could be utilized as a specific placental receptor for the CGKRK peptide and offers mechanisms for internalization for drug delivery to the placenta, both mouse and human (King *et al.*, 2016).

The challenges of modelling nanoparticle transfer

The ability to accurately determine the pharmacokinetics of nanoparticle transfer across the human placenta is desirable. However, this is challenging given that the ability to cross the placenta is altered with gestation. During pregnancy, there are significant physiological changes, such as greater total blood volume, increased renal blood flow, slower intestinal transit and changes in the degree of protein binding in the circulation. These factors may limit the validity of translating pharmacokinetic findings from a non-pregnant to pregnant population, making pregnancy-specific studies all the more necessary (Syme *et al.*, 2004). Because of the challenges, both *in vivo* and *in vitro*/*ex vivo* studies should be utilized in order to better gauge the levels and breakdown of nanoparticles in the placenta.

Like mice, rats, rabbits and guinea pigs, humans have a haemochorial placenta where maternal blood makes direct contact with the trophoblastic tissue. Thus, rodents provide a good model for studying nanoparticle placental transfer (Bhosle *et al.*, 2017). It is important to note however that while mouse models do have similarities to human placenta (in that trophoblasts and endothelium separate maternal and foetal blood supplies), there are still significant differences in the structure, the number of cell layers and the thickness of the diffusion barrier (which is greater in mice than in humans). Thus, care must be taken in assuming applicability and interpretation of results to humans (Keelan, 2011).

It is important to be able to test the transfer of different nanoparticles across the human placenta as extrapolation from animal models, though important, may not always mimic human tissue (Muoth *et al.*, 2016a). Several models have utilized the BeWo (human choriocarcinoma) cell line to examine transport of nanoparticles *in vitro* (Cartwright *et al.*, 2012; Aengenheister *et al.*, 2018b), while others have used the hanging drop method to create a core of placental fibroblasts surrounded by a trophoblast cell layer, which more closely resembles the complexity of the multi-layered and multi-cell type nature of the placenta (Muoth *et al.*, 2016b; Karolczak-Bayatti *et al.*, 2019). Dual perfusion of a human placental lobule *ex vivo* possibly best replicates the transfer across the trophoblast cells, however is limited in its ability to measure pharmacokinetic factors in the mother (Hutson *et al.*, 2011; Aengenheister *et al.*, 2018a).

As all potential methods to study nanoparticles have certain limitations, it is important that a combination of these strategies (*in vitro*, *ex vivo* and *in vivo*) is used when evaluating the likely degree of transplacental delivery, pre-clinical efficacy and safety of nanoparticle therapeutics proposed for use in pregnancy.

Safety of nanoparticles in pregnancy

Attempting to directly treat the placenta is similar in many ways to treating solid tumours. However, in pregnancy, the tolerance for adverse off-target effects is much lower than in oncology. Any genetic or epigenetic changes to the foetus caused by nanoparticles delivered in utero have the potential to cause long-term detrimental effects across generations. This makes safety of paramount importance when considering nanoparticle use in reproductive medicine. As such, several reviews have focussed on the safety of nanoparticles in reproduction (Keelan *et al.*, 2015; Das *et al.*, 2016; Ema *et al.*, 2016; Muoth *et al.*, 2016a; Hou and Zhu, 2017; Zhang *et al.*, 2017). As direct nanoparticle use in reproduction is in its infancy, it needs further in-depth studies. In particular, it is essential to examine the toxicity of nanoparticles at the different stages of pregnancy given this toxicity may be dependent on developmental stage, dose (physiological) and frequency of delivery (single versus repeated exposure), as well as particle type and properties.

While placental translocation and direct toxicity within the foetus has been a major focus, indirect toxicity of the nanoparticles themselves on the placenta and placental function as well as maternal or foetal mediators that impact foetal development and long-term health requires further exploration (Ema *et al.*, 2016; Buerki-Thurnherr *et al.*, 2018; Dugershaw *et al.*, 2020). Findings from a multi-layered BeWo cell line model demonstrated toxicity within the adjoining layers when BeWo cells were exposed to cobalt and chromium nanoparticles, despite the lack of direct physical contact with the nanoparticles (Sood *et al.*, 2011). Subsequent work in the same cell line model has demonstrated that this is through impairment of the autophagic flux and release of interleukin-6 (Hawkins *et al.*, 2018). In animal models, no pathological changes were observed in the placenta in animals exposed to nanoparticles; however, there was neuronal toxicity (Hawkins *et al.*, 2018) and increased DNA damage in the neonatal liver and blood (Sood *et al.*, 2011).

Furthermore, there is some trepidation within the field regarding the possible endocrine disrupting effects of certain nanomaterials used in the generation of the nanoparticles. Rat studies have demonstrated that inhalation of titanium dioxide showed reduced levels of oestrogen in exposed dams compared to sham controls, with impaired endothelium-dependent uterine arteriolar dilation (Bowdridge *et al.*, 2019) in the titanium dioxide exposed rats. Placental weights were increased but placental efficiency was decreased, and pup weight was reduced in exposed animals compared to sham controls (Bowdridge *et al.*, 2019). Given that the placenta has key roles in steroidogenesis during pregnancy, endocrine disruption via engineered nanomaterials deserves careful consideration (Larson *et al.*, 2014). This work indeed highlights that further work and careful assessment is needed in human primary trophoblast and placental explant tissue to determine which are the safest nanomaterials to ensure both direct and indirect toxicity does not affect the placenta and placental function.

The adverse effects of nanoparticles on the developing embryo or foetus would depend on its developmental stage (Das et al., 2016; Hou and Zhu, 2017) and the size and chemical composition of the nanoparticles as these factors influence how readily the nanoparticles can cross the placenta. Any damage to the vulnerable developing gametes or embryo in early pregnancy can have significant developmental impacts (Gandolfi and Brevini, 2010; Juch et al., 2013). Maternal nanotoxicity also needs to be considered, particularly as it will indirectly impact the health of the foetus. A relative state of immunosuppression, such as the suppression of cell-mediated immunity that occurs during pregnancy, may exacerbate nanoparticle-induced toxicity (Barkalina et al., 2014a). This was demonstrated in experiments where mice were given titanium dioxide nanoparticles: there was no inflammation in non-pregnant females, but persistent acute inflammation in pregnant mice (Lamoureux et al., 2010). Another study in a pregnant mouse model demonstrated that the presence of intrauterine inflammation elicited a toxic response from nanoparticles, which did not occur in healthy pregnant mice (Tian et al., 2013).

Nanoparticles can cause reproductive toxicity through various mechanisms (Table II). Smaller molecules have a relatively larger surface area, which helps with their drug carrying capacity but makes them more reactive than larger particles (Das et al., 2016). Different chemical compositions affect cellular uptake, subcellular localization and reactivity of the nanoparticle, which in turn impacts its potential toxicity (Das et al., 2016). Positively charged nanoparticles are associated with greater toxicity (Das et al., 2016). The impact on the foetus in later pregnancy to some degree depends not only on whether the nanoparticles reach the foetal compartment but also on whether the nanomaterials used have toxic effects on the placenta. An understanding of the mechanisms by which toxicity can occur, and the factors influencing them, enables drug carriers with the safest pharmacological profiles to be chosen.

Overall, the safe use of nanoparticle therapy in pregnancy requires several factors. It requires an understanding of human embryonic and foetal development throughout pregnancy and an understanding of the transfer of drugs and nanoparticles across the placenta and of what happens to both the cargo and nanoparticle carrier with transfer across the placenta. The likely safety profile of a particular nanoparticle requires important consideration, and thorough testing should be performed to assess toxicity on maternal and placental tissue, using placental transfer modelling with combinations of cells and cell lines and perfused tissue. Assessment of adverse and toxic effects on the foetus requires specific examination *in vivo* in carefully designed animal studies, as translocation to the foetus is not necessarily associated with foetotoxicity.

Practical challenges to clinical translation of nanoparticle treatment in pregnancy

Despite the theoretical benefits to nanoparticle treatments in fertility treatments and pregnancy, there remain realistic challenges to translation into clinical practice. Within the oncology field, clinical translation of nanoparticle delivery to solid tumours has been less than expected, based on laboratory and animal findings (Wilhelm et al., 2016). There are of course many reasons impacting the delay in translation of any therapeutic from the laboratory to the clinic, with one of the major reasons being the requirement of substantial financial investment to do so. Despite an improvement in side effect profiles of nanoparticle delivered drugs, the few oncological drugs translated have not appeared to show significant therapeutic improvements, and thus the cost associated may be unappealing in comparison to other known drugs (Barenholz, 2012; Park, 2013).

Initial nanoparticle targeting to solid tumours was often based on the enhanced permeability and retention effect. This passive targeting mechanism occurs given leaky capillary systems feeding the tumours allow access for the nanoparticles, and poor lymphatic drainage impairs its removal, thus leading to an accumulation within the tissues (Golombek et al., 2018). More recent targeting mechanisms studied, as discussed in further detail below, may provide greater targeting efficiency, and be what is required to attract future financial investment. Safety in pregnancy is of paramount importance. Any natural reticence about drug safety outside of pregnancy is multiplied and intensified in reproductive medicine.

Nanoparticle treatment for infertility

Studies on the use of nanoparticles for drug delivery in infertility treatment are limited, perhaps since there are concerns that the gamete and early developing embryo are highly sensitive and subtle changes could have a profound impact on reproductive outcomes. Concern about toxicological effects within the female reproductive tract impacting future fertility generally tempers the introduction of any innovative gynaecological treatments. We suggest targeted drug delivery systems should be actively explored as they offer the potential for considerable therapeutic benefits. However, they must be thoroughly tested to demonstrate that they are safe.

Treatment of conditions impacting fertility

Fibroids

A great dilemma in the treatment of gynaecological conditions that can cause subfertility is that the treatment itself can impair fertility. Many hormonal therapies are themselves contraceptive, and surgical treatments at best carry inherent surgical risks, and at worst may add risk to subsequent pregnancies. This is demonstrated in the management of fibroids or leiomyomas, which are hormone-dependant tumours arising from the myometrium. Fibroids have a lifetime prevalence of 70–80% (Baird et al., 2003) and can have significant impacts on a woman's quality of life, affecting menstruation (Buttram and Reiter, 1981), causing pelvic pain and leading to sequalae including infertility, recurrent pregnancy loss (Bajekal and Li, 2000) and later obstetric complications (Shavell et al., 2012). Effective surgical treatment can be challenging and can cause obstetric complications such as an increased risk of uterine rupture, particularly if the uterine cavity is breached (Gambacorti-Passerini et al., 2016). The effects of hormonal therapy are temporary and largely render the woman unable to conceive while they are being used (Moroni et al., 2014). They are therefore wholly unsuitable for women desiring pregnancy. Therefore effective, fertility sparing treatments are highly desirable.

Extracellular matrix remodelling and accumulation is increasingly recognized to be part of the pathology of uterine leiomyoma

Table II Potential risk of nanoparticles in reproduction (Campagnolo et al., 2012; Das et al., 2016; Zhang et al., 2017).

Mechanism of damage	Explanation	Examples
Direct induction of maternal inflammation or oxidative stress	Increases in ROS and inflammation can activate cytokines or other secondary messengers within the mother that can then cause alterations in placental function (Yamashita *et al.*, 2011).	Higher maternal oxidative stress levels associated with subsequent developmental neurotoxicity in animal studies (Powers *et al.*, 2013). Nanoparticle-induced germ cell toxicity is mediated primarily through oxidative stress (Gao *et al.*, 2012; Pawar and Kaul, 2014; Meena *et al.*, 2015; Yoisungnern *et al.*, 2015).
Internalization and accumulation inside cells	Some nanoparticles can cross biological barriers, and if not biodegradable can accumulate within cells remaining for a long duration, causing inflammation or toxicity. For gametes, this could have a direct damaging effect or cause damage to their surrounding cellular environment.	Nanoparticles can enter the testis, increasing proinflammatory responses that weaken the blood testes barrier (Lan and Yang, 2012). Nanoparticles can pass into ovaries and accumulate within ovarian cells (Austin *et al.*, 2012; Gao *et al.*, 2012; Zhao *et al.*, 2013; Tassinari *et al.*, 2014).
Direct placental or foetal oxidative damage	Direct passage of nanoparticles can induce ROS and inflammation in either placental or foetal tissues.	Foetal exposure to titanium dioxide led to dysregulation of apoptotic genes, neurotransmitters and brain development (Shimizu *et al.*, 2009). Amine-functionalized MWCNTs were injected into pregnant mice, inducing DNA damage in foetal liver, while addition of an antioxidant reduced these effects (Huang *et al.*, 2014). Oxidized MWCNTs were shown to cross the placental barrier and reach the foetus. Placental blood vessels were narrowed and reduced in number (Qi *et al.*, 2014).
Genotoxicity and DNA damage	Severe DNA damage, such as DNA deletions, DNA strand breaks, mutations and oxidative DNA adducts can occur.	Sperm DNA damage after nanoparticle exposure can cause male infertility (Pawar and Kaul, 2014; Xu *et al.*, 2014; Meena *et al.*, 2015; Preaubert *et al.*, 2016). Transplacental transfer of gold nanoparticles has caused size-dependant epigenetic effects (Balansky *et al.*, 2013). Maternal exposure to toxic diesel nanoparticles led to DNA deletions in the offspring (Reliene *et al.*, 2005). Maternal exposure of cobalt and chromium nanoparticles increased DNA damage in the neonatal liver (Sood *et al.*, 2011).
Direct foetal exposure after placental transfer	After entering the foetus, nanoparticles may accumulate in foetal organs such as liver, kidneys, heart and brain.	Small silica and titanium dioxide nanoparticles were found in foetal liver and foetal brain and also had smaller uteri and foetuses than controls (Yamashita *et al.*, 2011).
Teratogenicity	Exposure to either the drug or the nanoparticle can cause teratogenicity.	Carbon nanotubes have been shown to cause foetal malformations and placental injury in pregnancy studies (Mallatt *et al.*, 1986; Pietroiusti *et al.*, 2011; Fujitani *et al.*, 2012; Keelan *et al.*, 2015; Ema *et al.*, 2016).

ROS, reactive oxygen species; MWCNT, multi-walled carbon nanotube.

(Islam *et al.*, 2018). Ali *et al.* (2013b) engineered polymeric nanoparticles to deliver 2-methoxyestradiol, an anti-tumour drug for fibroid treatment (Ali *et al.*, 2013b), that can inhibit the synthesis of key extracellular matrix molecules (Salama *et al.*, 2006). They found that delivery with polylactic acid and polylactic-*co*-glycolic acid nanoparticles increased the anti-tumour activity of the drug when compared to the free drug, in a human leiomyoma cell line model (Ali *et al.*, 2013b). Another group assessed magnetic nanoparticles complexed to adenovirus in an *in vitro* human fibroid cell model, which acted as a suicide gene system. They found that magnetic nanoparticles, when targeted in the presence of an external magnetic field, reduced the total viral load required while maintaining the inhibitory effects of the system (Shalaby *et al.*, 2016). Jiang and Bischof (2010) used a female mouse model of uterine leiomyoma to demonstrate that the use of PEGylated gold core nanoparticles to deliver tumour necrosis factor-alpha delayed tumour growth after cryosurgery (Jiang and Bischof, 2010). In humans, this could improve the efficacy of surgery, lowering the complication rate and allowing less invasive surgery to be performed.

Endometriosis

Endometriosis provides another therapeutic challenge for women. Endometriosis is a condition where fragments of the endometrial functionalis layer adheres to and persists in the peritoneal cavity. It is often associated with pain and infertility. It is driven by oestrogen and is complicated by a spectrum of pathology including adhesions and anatomical distortion, angiogenesis and neuronal infiltration (Giudice, 2010). Endometriosis affects up to 10% of all reproductive aged women, but as many as half of those with infertility (Giudice, 2010). It has a considerable economic (Simoens *et al.*, 2007) and functional burden and is associated with a higher risk of miscarriage, preterm birth, placenta praevia, small for gestational age infants and caesarean delivery if a pregnancy is achieved (Zullo *et al.*, 2017).

Current medical therapies for endometriosis are suppressive rather than curative, centring on ovarian suppression to inhibit menstruation and the formation or persistence of oestrogen-dependent endometriotic lesions. However, such medical treatments are not compatible with attempted conception (Kennedy *et al.*, 2005). Given that surgical treatments are equivocally successful, the final treatment option is often ARTs (Practice Committee of the American Society for Reproductive Medicine, 2012). Nanoparticle therapy may provide an alternative option. Cerium oxide nanoparticles, a potent free radical scavenger, have been proposed as a treatment for endometriosis through reducing oxidative stress and inhibiting angiogenesis. In a murine model, successful treatment of endometriosis was demonstrated using this approach, importantly without oocyte toxicity (Chaudhury *et al.*, 2013). Iron oxide nanoparticles have previously been used as a thermal ablation treatment (Zhang *et al.*, 2014) and it has been suggested that they could form the future basis for hyperthermia-related endometriosis treatment. Further studies are needed to determine if this could be effective in women.

Non-invasive diagnosis of endometriotic lesions with nanoparticles could avoid many unnecessary laparoscopies in infertility investigations (Brosens *et al.*, 2004). To date, although circulating biomarkers have been linked to endometriosis, none have the sensitivity or specificity to be incorporated into routine clinical care (May *et al.*, 2010; Ahn *et al.*, 2017). Endometriosis is highly angiogenic and circulating endothelial progenitor cells are recruited into endometriotic lesions. These could be used as novel diagnostic biomarkers, avoiding the need for a diagnostic surgery, or targeted with anti-angiogenic or vascular disrupting compounds (Laschke and Menger, 2018). Ultrasmall supermagnetic iron oxide has been intravenously injected into mice with experimentally induced endometriosis and has improved the magnetic resonance imaging detection rate of endometrial implants (Lee *et al.*, 2012). Hyaluronic acid-modified magnetic iron oxide nanoparticles were also tested in another experimental group, and they too were found to increase the detection of deep infiltrating endometriosis (Zhang *et al.*, 2014).

MicroRNAs (miRNAs) are short non-coding RNAs that can induce mRNA cleavage or suppress protein translation. Expression of miRNAs in women with endometriosis are altered, both in circulating levels (Cho *et al.*, 2015; Rekker *et al.*, 2015; Wang *et al.*, 2015) and within the eutopic (Ramon *et al.*, 2011) and ectopic endometrial tissue (Ohlsson Teague *et al.*, 2009; Filigheddu *et al.*, 2010). This has raised the possibility that miRNA may be useful as diagnostic biomarkers (Ghazal *et al.*, 2015; Wang *et al.*, 2016) or therapeutic targets (Panir *et al.*, 2018). However, administration of miRNA remains a barrier because miRNAs are susceptible to degradation by circulating RNases, reducing their half-life and distribution (Panir *et al.*, 2018). This is why there are few RNA-based treatments in clinical practice. One approved example in clinical practice utilizes an RNA aptamer that antagonizes the pro-angiogenic factor vascular endothelial growth factor (VEGF) and is administered via intravitreal injection for treatment of macular degeneration (Sankar *et al.*, 2018). Stable miRNA delivery could provide a useful diagnostic tool or potential treatment for endometriosis.

siRNAs are double-stranded RNA oligonucleotides that induce silencing of a target gene through RNA interference. Studies of siRNA in endometriosis have also identified several potential targets; if the right delivery system could be utilized there would be exciting potential. Jiang *et al.* (2012) used *EZRIN*-silencing siRNA, in ectopic endometrial cells from women with and without endometriosis (Jiang *et al.*, 2012). In a chick embryo model of endometriosis, Liu *et al.* (2009) demonstrated siRNA knock-down of nuclear factor kappa-light-chain-enhancer of activated B cells in human eutopic endometrial fragments, which increased apoptosis and reduced vascularization (Liu *et al.*, 2009). Reduction of VEGF-C through siRNA transfection resulted in inhibition of angiogenesis and lesion development (Xu *et al.*, 2013), demonstrating further potential providing the siRNA therapeutics could be stably and selectively delivered using nanoparticle technology. However, the obvious major hurdle in such approaches is that there are no obvious avenues to target nanoparticles so that such drugs can preferentially accumulate within the endometriotic lesions (which are much smaller relative to the other tissues in the body). Preferably, there is a surface antigen unique to endometriotic lesions (or at least very highly expressed relative to other tissues) against which a targeting mechanism could be developed, but one has yet to be discovered.

The above examples demonstrate how nanoparticle therapy may provide novel diagnostic and therapeutic options in gynaecological conditions impacting fertility.

Treatment of the gamete or embryo

ART has provided revolutionary treatment for couples suffering from infertility (Faddy *et al.*, 2018). It has rapidly progressed over four decades from an experimental therapy to a routine treatment, as well as a tool for preimplantation genetic testing and fertility preservation (Barkalina *et al.*, 2015). However, limitations still exist. In ART, *in vitro* handling of gametes may cause oxidative stress in oocytes (Martin-Romero *et al.*, 2008; Otsuki *et al.*, 2009) or damage to sperm through DNA fragmentation and oxidation (Matsuura *et al.*, 2010; Rougier *et al.*, 2013).

Nanoparticle therapy may prove useful in this setting in several ways. The mature oocyte in an *in vitro* setting is resistant to the uptake of exogenous materials, as intracellular delivery currently requires membrane disruption (Barkalina *et al.*, 2015). A less destructive approach, utilizing targeted nanoparticles to exploit the existing cellular uptake mechanisms of the oocyte, may provide a more effective and less invasive approach to deliver exogenous molecules to the oocyte. Targeted delivery could also lead to treatment with compounds that may improve sperm or oocyte quality, or protect the gametes from deterioration *in vitro*, particularly if there is a planned delay before the use of either the sperm (Yun *et al.*, 2013; Tardif *et al.*, 2014), oocyte (Telfer and McLaughlin, 2012) or embryo (Kawamura *et al.*, 2012).

Sperm delivers genetic material or proteins into the oocyte, and it has been proposed that if they could be loaded with exogenous DNA or proteins, then they could efficiently transfer this material into the oocyte (Kim *et al.*, 2010b; Campos *et al.*, 2011a,b). Sperm may in fact be the ideal transfer medium as they are able to transfer genetic material into the oocyte, then degrade. This would overcome uncertain long-term impacts of using non-biodegradable nanocarriers to carry DNA or proteins into the oocyte or embryo. Further refinement of the process to ensure there was no carry over of nanomaterial during sperm-mediated transfer, could offer promising strategies to deliver to the oocyte intracellular compartment (Kim *et al.*, 2010b; Feugang *et al.*, 2012; Courbiere *et al.*, 2013; Barkalina *et al.*, 2014b; Barchanski *et al.*, 2015).

Extracellular vesicles (EVs) are naturally occurring phospholipid bilayer-enclosed particles released by many cell types. Epididymosomes (EVs of epididymal origin) can fuse with the sperm membrane (Schwarz *et al.*, 2013), and prostasomes (EVs of prostate origin) have been shown to 'supplement' sperm with complement-inhibitory molecules, shielding the sperm from immune recognition in the female genital tract (Rooney *et al.*, 1993), or molecules that enhance sperm motility (Wang *et al.*, 2001) or impact capacitation (Piehl *et al.*, 2013). These physiological roles demonstrate the potential of artificial EVs to be used to transfer proteins or genetic material into the sperm, and subsequently to the embryo (Barkalina *et al.*, 2015). In the female, EVs are known to regulate ovarian function through delivery of granulosa and cumulus cell-derived miRNAs and cell-specific proteins (da Silveira *et al.*, 2012, 2014; Santonocito *et al.*, 2014), and exosomes from oviducts (Al-Dossary *et al.*, 2013) and the uterine cavity fluid (Ng *et al.*, 2013; Ruiz-Gonzalez *et al.*, 2015) have been shown to transport molecular cargo (miRNAs and proteins) with functions impacting fertilization, implantation and early pregnancy.

Although artificial EVs have not yet been developed for translation into infertility treatments, they have been a focus of research in cancer and inflammatory and neurodegenerative diseases (Andaloussi *et al.*, 2013). Several research groups have successfully created miRNA and intracellular protein containing artificial EVs (JaNg *et al.*, 2013; Jo *et al.*, 2014). Early success in Alzheimer's and Parkinson's diseases (Alvarez-Erviti *et al.*, 2011; El-Andaloussi *et al.*, 2012; Cooper *et al.*, 2014), oncology (Mizrak *et al.*, 2013; Ohno *et al.*, 2013; Tian *et al.*, 2014) and autoimmune disease (Zhuang *et al.*, 2011) shows promise that this could be a realistic treatment approach in fertility in future.

While still remote from human clinical translation, the applications of nanoparticles to deliver genes into germ cells to produce transgenic animals may also one day be utilized for the treatment of various genetic conditions (Das *et al.*, 2016). It is an area of avid pre-clinical interest.

Male factor infertility

The proportion of healthy spermatozoa in semen could be improved by the use of nanoparticles. Cryopreservation of semen increases oxidative stress and reduces the fertilization capacity of sperm (Khalil *et al.*, 2018). Cerium oxide (CeO_2) nanoparticles are able to store oxygen and act as reactive oxygen species (ROS) scavengers, which has increased cell viability during cryopreservation in rams (Falchi *et al.*, 2018a). Nano-selenium also act as a ROS scavenger and have improved post-thawing parameters in rooster semen (Safa *et al.*, 2016) and have protected against oxidative damage by cisplatin, a known anticancer agent (Rezvanfar *et al.*, 2013). Nano-zinc has been shown to improve semen quality (Isaac *et al.*, 2017) and mitochondrial activity and increase seminal plasma anti-oxidase activities (Falchi *et al.*, 2018b) and activity of antioxidant enzymes in testicular tissue (Afifi *et al.*, 2015). Biocompatible magnetic nanoparticles have been used to target apoptotic and acrosome-reacted boar spermatozoa, allowing sperm selection to occur, and increasing the proportion of motile, viable, fertile sperm in semen (Durfey *et al.*, 2019). These animal studies demonstrate significant potential for nanoparticles in the improvement of semen quality prior to transfer.

Nanoparticle therapy in early pregnancy

Disorders of abnormal trophoblastic growth

Disorders of trophoblastic growth can occur in several forms, including ectopic pregnancy, when a fertilized ovum implants outside the uterine cavity (van Mello *et al.*, 2012), and gestational trophoblastic disease (GTD), which is a term encompassing abnormal trophoblastic growth ranging from premalignant hydatidiform mole to invasive choriocarcinoma (Seckl *et al.*, 2010). Untreated ectopic pregnancies can rupture, causing fatal intra-abdominal bleeding. Definitive surgical management, through removal of the Fallopian tube, risks leaving only one tube for natural conception, or none in the case of contralateral tubal damage (van Mello *et al.*, 2012). Medical treatment with methotrexate is an alternative, but as a folic acid antagonist, methotrexate can have significant side effects and is not always successful, particularly if the beta-hCG (β-hCG) levels are high or the ectopic pregnancy large (Practice Committee of American Society for Reproductive Medicine, 2013). GTD treatment can also be challenging, sometimes requiring high doses of systemic chemotherapy, which can cause significant toxicity to the mother. Development of effective, non-toxic, non-invasive treatment options is urgently needed.

In many ways, early trophoblastic cells have remarkable similarities to malignant tissue in their proliferative and invasive properties (Ferretti *et al.*, 2007). To test the potential of targeted delivery of a chemotherapeutic agent, to effectively destroy trophoblastic tissue/cells, our team examined the potential for targeted delivery of doxorubicin, a chemotherapeutic drug via EnGeneIC delivery vehicles (EDVs). EDVs are bacterially derived organic nanospheres, coated with bispecific antibodies targeting cell surface epidermal growth factor receptor (EGFR) (MacDiarmid *et al.*, 2007), which is expressed 3000-fold higher in placenta compared to other human tissues. EGFR coated EDVs, bind to the cell surface receptor on the cell membrane and are endocytosed by receptor-mediated endocytosis. The phospholipid bilayer wall is broken down releasing the pay load. We found that EGFR-targeted EDVs loaded with doxorubicin were more readily taken into human placental explants *ex vivo* and they induced greater apoptosis than non-targeted EDVs (Kaitu'u-Lino *et al.*, 2013). In a murine model utilizing JEG-3 (choriocarcinoma cells) xenografts, EGFR-targeted EDVs were more effective at reducing JEG-3 xenograft size, compared to non-targeted EDVs or naked doxorubicin not encapsulated by an EDV. *In vitro* studies confirmed reductions in cell viability, increased

apoptosis and reduced proliferation (Kaitu'u-Lino et al., 2013). Naked doxorubicin was minimally taken up by the placental explant tissue; uptake was significantly higher when delivered in the targeted EDVs (Kaitu'u-Lino et al., 2013). Previous biodistribution data suggested that targeted EDVs do not have adverse effects in mice and evoke little inflammatory response *in vivo* (MacDiarmid et al., 2007). Thus, these data were the first to suggest that nanoparticle delivered therapies may be a novel avenue to pursue for the treatment of ectopic pregnancy.

These EDVs have been progressed to clinical trials in oncology. Early phase trials have shown that they are safe (Solomon et al., 2015; van Zandwijk et al., 2017) and multiple trials are in progress or are planned (ACTRN 1261900038514S; further trials planned are listed at https://engeneic.com/pipeline/). If these subsequent larger oncology trials confirm that they are safe in humans, then it would pave the way to evaluate EDVs in trials for ectopic pregnancy and GTD.

Zhang et al. (2018a,) used CSA-binding nanoparticles to also target the delivery of doxorubicin to JEG-3 choriocarcinoma cell lines (Zhang et al., 2018a) via endocytotic uptake detailed earlier. They found that CSA doxorubicin nanoparticles significantly increased the percentage of cells undergoing early and late apoptosis, compared to untargeted or free doxorubicin, and promoted a greater reduction in the primary tumour burden, as well as metastatic tissue, in *in vivo* mouse models (Zhang et al., 2018b).

Jingting et al. (2011) prepared magnetic iron oxide (Fe_3O_4)-dextran nanoparticles decorated with -anti-β-human chorionic gonadotrophin monoclonal antibodies, which could absorb significant amounts of DNA with limited toxicity and could be targeted to specific sites using external magnets. The β-hCG antibodies coating the Fe_3O_4 nanoparticles were successfully able to deliver the nanoparticles to JEG-3 cell lines, as demonstrated with flow cytometry. Use of magnetic targeting led to greater transfection efficiency than that of liposomes (Jingting et al., 2011). This suggests another potential targeted treatment option for disorders of trophoblastic cells.

Unlike the EDV concept, both iron oxide (Fe_3O_4)-dextran and CSA-binding nanoparticles will require pre-clinical further toxicology studies before they could be considered candidates for first in persons clinical trials.

Nanoparticle treatment in late pregnancy

Over the past two decades, significant progress in the understanding of molecular pathways underlying the pathophysiology of pregnancy-related disorders has occurred. This greater understanding has opened up the potential for targeted therapies (Ilekis et al., 2016; Refuerzo et al., 2017; Groom and David, 2018), which could be stably delivered using nanoparticles. As placental dysfunction is the cause of many pregnancy complications, the ability to treat the placenta could lead to better outcomes for the foetus, with resulting improvements in long-term health (Ganguly et al., 2020).

Foetal growth restriction

Foetal growth restriction is a serious complication in pregnancy where placental dysfunction is associated with impaired foetal growth, caused by a combination of impaired placental perfusion, reduced placental surface area, altered cell turnover and reduced nutrient transfer capacity (Sibley et al., 2005). The consequences of foetal growth restriction include iatrogenic preterm birth (the need to deliver the baby preterm because placental function is insufficient) and stillbirth (Kady and Gardosi, 2004). Treatment options to enhance placental function including targeting the placental growth factor (PlGF) pathway to increase the number of terminal villi and available surface area for nutrient and oxygen transfer (Ilekis et al., 2016) or stimulating the mammalian target of rapamycin pathway to increase nutrient transporters (Ilekis et al., 2016).

King et al. (2016) identified two tumour-homing peptide sequence, CGKRK and iRGD, that bound selectively to placental tissue of mice, in particular the decidual spiral arteries and the placental labyrinth, without interfering with placental function or causing off-target effects (King et al., 2016). iRGD-conjugated liposomal nanoparticles were used to package insulin-like growth factor-2 (IGF-2) and were injected into pregnant mice. This resulted in a significant increase in placental weight in wild-type mice when compared to empty liposomes, non-targeted liposomes or free IGF-2, and improved foetal weight distribution in a mouse model of foetal growth restriction (King et al., 2016).

The same group identified a further targeting peptide, CNKGLRNK, which selectively bound to the placental tissue as described above. Nitric oxide was packaged in order to act as a vasodilator and enhance uteroplacental perfusion. *In vitro* studies demonstrated relaxation of both mouse and term human placental vessels using myography. *In vivo* mouse studies in a mouse model of foetal growth restriction (nitic oxide synthase knockout mice) showed greater foetal weights and spiral artery diameters, as well as a reduction in placental expression of COX-1, COX-2 and 4-hydroxynonenenal, as markers of placental oxidative stress (Cureton et al., 2017).

IGF-1 as a potential treatment for foetal growth restriction has been a source of interest for another group, as it is known to be critical for healthy foetal and placental growth. Adenoviral-mediated overexpression of IGF-1 was demonstrated in cultured trophoblast cell lines to improve placental glucose transport (Jones et al., 2013) and enhance amino acid transporter expression (Jones et al., 2014), in both *in vitro* human studies and *in vivo* mouse studies of foetal growth restriction (Jones et al., 2014). Subsequently IGF-1 was complexed to a deblock copolymer (pHPMA-*b*-pDMAEMA), under the control of Cyp19a or PLAC1, trophoblast-specific promoters. These complexes were tested in trophoblast cell lines and in a mouse model of foetal growth restriction (FGR), where they rescued the FGR phenotype in the mouse model and significantly increased placental IGF-1 expression, compared to those treated with the same complexes but with empty plasmids (where IGF-1 was not encoded) (Abd Ellah et al., 2015).

Chronic placental insufficiency leads to a state of oxidative stress, and it has been proposed that antioxidant therapy may reduce this. Phillips et al. (2017) investigated the use of mitochondrial antioxidant (MitoQ) bound to non-targeted γ-PGA-Phe polymeric nanoparticles. Using a hypoxic rat model where exposure to a low oxygen environment in pregnancy results in reduced birthweights, administration of MitoQ bound to nanoparticles (that accumulated in the placenta) rescued 60% of the deficit, without affecting foetal brain or placental weight (Phillips et al., 2017). Importantly, the liposomes were found in high concentrations in the cytotrophoblast and were also detected in the maternal liver and brain. Notably, they were not detected in the

foetal brain, thoracic or abdominal tissues. This demonstrates that the dysfunctional placenta can be specifically treated to rescue the FGR phenotype, without direct entry into the foetal circulation (Phillips *et al.*, 2017). Liposome-encapsulated haemoglobin was also investigated as a potential treatment for foetal hypoxia, as an indirect route of supplying oxygen to the placenta. A proof of principle study in pregnant rats showed no adverse effects on the pregnant mother, nor on the haemoglobin in the placenta, but there was an accumulation of liposomes in the foetus (Kaga *et al.*, 2012). Further studies are required to demonstrate possible clinical effects.

Preeclampsia

Preeclampsia is a serious complication of pregnancy with significant consequences for both the mother and child. In early pregnancy, there is inadequate remodelling of the maternal spiral arterioles that perfuse the intervillous space. This leads to chronic placental ischaemia and hypoxia (Roberts and Cooper, 2001; Powe *et al.*, 2011; Tomimatsu *et al.*, 2017). In later pregnancy, there is excess release of anti-angiogenic factors from the placenta into the maternal circulation, such as sFlt1 (Maynard *et al.*, 2003) and soluble endoglin (Ilekis *et al.*, 2016; Zeisler *et al.*, 2016). There is also a reduction in the placental production of pro-angiogenic factors, especially PlGF. This anti-angiogenic profile causes maternal endothelial dysfunction and the clinical preeclampsia, which includes hypertension, proteinuria, foetal growth restriction and multi-system maternal organ injury.

The pathophysiology of preeclampsia, in particular the excessive placental production and secretion of anti-angiogenic factors (which is one of the main drivers of clinical disease), lends itself to potential modification via gene therapies. However, the clinical utility of siRNA to date has been limited because naked unmodified siRNAs are unstable in the bloodstream, limiting their ability to reach the target tissue, and have negatively charged hydrophilic phosphate backbones, limiting their ability to enter the target cells (Yu *et al.*, 2017). Nanoparticles offer the potential to safely encapsulate the siRNA, which improves its stability, and allows internalization of the siRNA by the cytotrophoblast, which minimizes potential adverse effects to the foetus by reducing the transfer to the foetus (Valero *et al.*, 2018a,b). Valero *et al.* (2018a,b) used three liposomal formulations with varied charged states to stably deliver negatively charged siRNA; they demonstrated minimal toxicity and good internalization of siRNA into human primary villous cytotrophoblasts (Valero *et al.*, 2018a) as well as improved biocompatibility compared to the transfection reagent lipofectamine. This group previously demonstrated that their liposomes showed good potential for placental drug delivery, as they were able to selectively deliver to the placenta without passing into foetal circulation, as modelled with a dual-perfused placenta (Valero *et al.*, 2017). This, in conjunction with the ability of liposomes to internalize siRNA into cytotrophoblasts, demonstrates their ability as a delivery system.

The application of sFlt1 siRNA was examined in both *in vitro* and *in vivo* pregnant rat studies. siRNA-sFlt1 was conjugated to PAMAM dendrimers, and these nanoparticles were able to significantly reduce sFlt1 secretion from cultured trophoblastic cell lines, as well as reduce circulating sFlt1 levels, mean arterial pressure and urine protein level in their *in vivo* preeclamptic rat model (Yu *et al.*, 2017). These early data suggest that PAMAM dendrimers could act as a novel carrier for sFlt1 siRNA, offering a novel therapeutic strategy for the potential of treating preeclampsia. Turanov *et al.* in 2018 used a pregnant mouse model to demonstrate that sFlt1 siRNAs could effectively reduce circulating and placental sFlt1 levels and were able to reduce clinical manifestations of preeclampsia and sFlt1 overexpression in a baboon model of preeclampsia (Turanov *et al.*, 2018). These studies offer exciting insights into the potential of stably delivering siRNAs to establish a new treatment model for women with preterm preeclampsia.

Preterm labour

Preterm birth remains a challenge to treat, as multiple aetiologies lead to the same terminal pathway of excessive uterine contractility, cervical dilatation, decidual activation and membrane rupture (Romero *et al.*, 1994; Goldenberg *et al.*, 2008). However, despite the challenges, the importance of treatment is not diminished, as it occurs in over 1 in 10 pregnancies (Goldenberg *et al.*, 2008), and is a major cause of short term neonatal morbidity and mortality, as well as long-term neurodevelopmental and metabolic consequences (Saigal and Doyle, 2008). Targeted delivery of drugs to the uterus, especially to the myometrium or placenta, may provide a new avenue for treatment, given the limited efficacy of currently available treatments to reduce uterine contractility and halt preterm labour (i.e. tocolytic agents).

Uterine inflammation and infection, often associated with decidual haemorrhage, can activate the preterm pathway (Simhan and Caritis, 2007). Preterm birth can have long-term adverse neurological sequalae, particularly when associated with inflammation (Saigal and Doyle, 2008). *N*-Acetyl-L-cysteine (NAC) therapy has been evaluated as a potential treatment for foetal neuroinflammation, but if given systemically, the doses administered to the mother to deliver a sufficient concentration of drugs to the foetus can cause significant systemic maternal side effects. By packaging NAC using targeted dendrimer nanoparticles in a murine model of intrauterine inflammation, Lei *et al.* (2017) were able to reduce preterm birth rates and reduce CD8+ T-cell infiltration. Most importantly, they showed reduced inflammation in the foetal brain and improved neuro-behavioural outcomes (Lei *et al.*, 2017). The dendrimer was conjugated with the Cy5 fluorophore and used to successfully administer NAC doses of only one-tenth of what was required with the free drug (Lei *et al.*, 2017).

Indomethacin is a commonly used tocolytic agent, which works by reducing prostaglandin production, thus lessening uterine contractions and prolonging pregnancy. However, it freely crosses the placenta and can cause substantial adverse foetal side effects, including premature closure of the ductus arteriosus (which may lead to progressive heart failure and foetal death), necrotizing enterocolitis (infection and inflammation leading to bowel ischaemia and death) and intraventricular haemorrhage (bleeding into the ventricular system of the brain) (Abou-Ghannam *et al.*, 2012). A drug packaging system that can target delivery of indomethacin to the uterine myometrium (the site of prostaglandin production that incites preterm contractions) while minimizing the amount that crosses the placenta could reduce foetal risk without affecting efficacy. Refuerzo *et al.* (2015) examined the selectivity of targeting and efficacy of delivery of indomethacin packaged in small liposomes (Refuerzo *et al.*, 2015). Enhanced liposome fluorescence was observed in the uterus compared to the placenta and foetus, and liposomal packaging of indomethacin resulted in a 7.6-fold reduction in the amount that entered the foetus, compared to systemically administered indomethacin. However, the encapsulated indomethacin had the

same efficacy to reduce prostaglandin E2 levels compared to naked drug (Refuerzo et al., 2015). Addition of an oxytocin receptor (OTR) antagonist to the surface of the liposomes enabled selective targeting to the uterus, which expresses high levels of OTRs; this approach resulted in a 15% reduction in preterm birth in a mouse model (Refuerzo et al., 2016).

Paul et al. (2017) showed similar results using liposomes decorated with an anti-OTR antibody. These targeted liposomes were able to effectively deliver nifedipine, salbutamol, indomethacin and rolipram (all drugs known to reduce uterine contractions), as well as a contraction enhancing agent (dofetilide) to spontaneously contracting human and mouse myometrial tissue *in vitro* (Paul et al., 2017). Non-targeted liposomes loaded with the same agents did not demonstrate any effect on myometrial contractions *in vitro*, and empty OTR-targeted liposomes did not have any effect on uterine activity (Paul et al., 2017). When administered to pregnant mice, there was no evidence of transplacental passage of the liposomes to the foetus; however, localization to the maternal breast was noted. These studies demonstrate the potential for liposomes to selectively deliver therapeutic agents that can suppress preterm uterine contractions and treat threatened preterm birth.

Intrauterine infection is another cause of preterm birth and can be difficult to treat as antibiotics that are administered systemically have varying penetrance to the amniotic cavity and can also have long-term side effects for the foetus. Burd et al. (2014) created a murine model of intrauterine inflammation and preterm birth and tested whether intrauterine administration of hydroxyl-terminated PAMAM dendrimers could act as a potential treatment for associated foetal neuroinflammation. They were able to demonstrate accumulation of the dendrimer in the foetal gut and brain, suggesting dendrimers could be an appropriate carrier for foetal neuroinflammation treatments (Burd et al., 2014). Wang et al. (2010) also demonstrated that PAMAM dendrimers have natural antimicrobial properties, and when topically applied to the cervix were able to eliminate *Escherichia coli* in the amniotic fluid of infected guinea pigs and reduce cytokine levels (Wang et al., 2010). These properties are ideal in a treatment for uterine infection.

Selective treatment of the mother or foetus

Despite the foetus being completely dependent on the mother, there are circumstances or conditions in which only one part of the foetoplacental unit requires treatment. In these unique circumstances, either being able to target the mother, by blocking placental transfer of drugs to the foetus, or by targeting the foetus, through enhancing transfer of drugs that may otherwise not cross, offers a way forward in developing targeted therapies.

Selective treatment of the mother

Over the last three decades, there has been a dramatic increase in the number of pregnant women with chronic pre-existing medical diseases as a result of increasing maternal age (Cohen, 2014) or higher rates of obesity (Marchi et al., 2015). Moreover, advances in assisted reproductive techniques enable women who previously may have not been able to conceive, to become pregnant (Jølving et al., 2016). Conditions that affect the mother in which the foetus would ideally have no exposure to treatments include malignancy, cardiovascular diseases, renal disease, epilepsy, psychiatric disorders, thyroid disease and infections (Keelan et al., 2015). This poses a dilemma, because treatment of the mother leads to optimal control of her underlying comorbidities, but pharmacotherapy in pregnancy opens the foetus up to potential teratogenicity.

A study examining the transfer of a PAMAM-dendrimer and conjugates across a perfused human placental (*ex vivo*) model found concentrations that were 18 times higher in the maternal side placental perfusate than in the foetal side placental perfusate (Menjoge et al., 2011). The same PAMAM dendrimers were evaluated for intravaginal application to treat ascending genital infections using an *ex vivo* model and showed <3% transplacental passage, compared to almost 50% using intravaginal fluorescein, a known marker of transplacental passage (400 Da) (Menjoge et al., 2010b). Overall, this suggests that drugs conjugated to dendrimers are restricted from crossing the human foetal membranes when administered intravaginally, thus this may offer new methods to selectively deliver therapeutics to the mother without risking exposure to the foetus.

Women with mechanical heart valves are best protected against thromboembolic events with warfarin, which unfortunately can cause foetal warfarin syndrome (including low birth weight, small head size, developmental delay, deafness and congenital heart defects) when given systemically in pregnancy (Chan et al., 2000). Small liposomes were tested as a drug carrier for warfarin (Bajoria and Sooranna, 1998; Bajoria et al., 2013); because liposomes are metabolized in the liver, the target organ of warfarin, and do not easily cross the placenta, they are an ideal carrier of warfarin. Encapsulating warfarin using lecithin (F-SUV) or steryl-amine (S-SUV) with cholesterol and stearylamine reduced transplacental passage of warfarin compared with naked drug in an *ex vivo* placental perfusion model (Scherphof and Kamps, 2001).

Valproic acid is a known highly teratogenic medication that is sometimes critical for epileptic control in pregnancy. Liposomal encapsulation has also been demonstrated to significantly reduce the amount of valproic acid in foetal circulation and to increase the ratio of maternal to foetal valproic acid in *in vitro* placental transfer studies (Barzago et al., 1996). These examples of encapsulating teratogenic drugs and reducing the levels reaching the foetus would have significant clinical benefits.

Selective treatment of the foetus

Foetal conditions requiring pharmacotherapy create a unique therapeutic and ethical dilemma. Treatment either needs to be administered to the mother (with potential maternal side effects and no direct maternal benefit) or to be administered directly to the foetus (via invasive and potentially hazardous procedures such as ultrasound guided injections directly into the umbilical cord through the maternal abdomen). Examples of situations in which the foetus requires treatment with no clear benefit to the mother include administration of corticosteroids for foetal lung maturity (Roberts et al., 2017) and treatment of foetal supraventricular tachycardia by administering anti-arrhythmic medications via the mother (Zoeller, 2017), foetal hypothyroidism by administering thyroxine (Bajoria and Sooranna, 1998) and foetal congenital adrenal hyperplasia (Ali et al., 2013a).

Treatment of foetal hypothyroidism is difficult, as the placenta converts active free thyroxine (T4) to its less active component reverse triiodothyronine (rT3). Early studies examining transplacental passage

of liposomes have demonstrated that anionic stearylamine from lecithin enhanced T4 uptake across the placenta and reduced rT3 (Bajoria *et al.*, 1997a). This gives an example of nanoparticle encapsulation enhancing placental transfer.

Congenital adrenal hyperplasia, most commonly due to an inherited genetic defect, can be life threatening for the foetus or cause disorders of sexual differentiation. Delivery of encapsulated dexamethasone via polylactic-*co*-glycolic acid nanoparticles led to a 10-fold increase in transfer to the foetal compartment, as modelled using the BeWo cell line (Ali *et al.*, 2013a), which again demonstrates the ability for nanoparticle encapsulation of drugs to alter their inherent potential to cross the placental barrier.

The prospects for clinical translation

It is evident that there has been a substantial body of pre-clinical studies describing the safety and potential clinical applications of nanoparticle technologies to treat major gynaecological conditions and pregnancy complications. Most have been done with a translational goal in mind. Distinctly lacking, however, are clinical trials of nanoparticle technologies in our field. It would be disappointing if our field never manages to translate the expanding pre-clinical knowledge into the clinic. This raises the question of whether clinical translation for nanoparticles is realistic for obstetrical and gynaecological indications, and whether it will ever happen. As discussed throughout this review, testing any new therapies (including nanoparticles) for pregnancy conditions is challenging. A major barrier is concerns regarding foetal safety. The further challenge is that for many of the nanoparticle concepts, trials will need to be 'first in human studies', which are not often done in pregnant cohorts (most trials evaluate drugs where some human safety data already exist). Second, it has been long recognized that pharmacological industries have avoided therapeutic opportunities in obstetrics as it is perceived that the risks may be high (particularly the litigation risk arising from foetal harm) and that the market may be small (use of any drug is time limited during pregnancy) (Chappell and David, 2016).

However, there are grounds for optimism that clinical trials of nanoparticles to treat pregnancy conditions could happen within the next decade.

First, as reviewed, the safety of nanoparticle technologies has been thoroughly interrogated in many pre-clinical studies. For instance, we now understand that manipulating the size (Myllynen *et al.*, 2008; Wick *et al.*, 2010; Refuerzo *et al.*, 2011; Yamashita *et al.*, 2011) and charge (Bajoria and Contractor, 1997; Bajoria *et al.*, 1997b) of the nanoparticles can limit transplacental passage. There are now many *in vitro*, *ex vivo* and *in vivo* models available that can be used pre-clinically to interrogate tissue (and foetal) distribution of nanoparticles. Thus, it is realistic to generate a nanoparticle that targets a disease-causing pathway and to generate sufficient pre-clinical data that ethically justifies translation to phase I trials in pregnant humans.

Second, there is now increasing commentary that rightly laments the lack of therapeutic development for pregnancy conditions (Fisk and Atun, 2009; Keelan *et al.*, 2015; Chappell and David, 2016). Notably, the Royal College of Obstetricians and Gynaecologists (UK) released a position statement arguing that therapeutic development to treat pregnancy conditions continues to be neglected (Royal College of Obstetricians and Gynaecologists, 2015). There is a clinical and commercial inertia that needs to be overcome. Thus, over the last decade, there has been a positive cultural in attitude towards running trials during pregnancy, which offers hope for the translation of nanoparticles for pregnancy trials.

Conclusions

As discussed, the development of therapies for the treatment of serious reproductive conditions and obstetric complications poses formidable and unique challenges. However, it is clear that there is an urgent need for new treatment options and new ways to deliver these treatments for many reproductive disorders and major complications of pregnancy. This review highlights how targeted nanomedicine delivery systems may help to overcome some of the limitations with current treatment options.

Nanoparticles targeted to specific sites may offer non-invasive options for treating gynaecological complications that impact fertility, such as endometriosis and fibroids, and the possibility of early genetic modifications of the embryo or gamete. Specifically, delivery of therapeutics to the dysfunctional placenta or uterus, especially agents that are unstable in the maternal circulation such as siRNA, may provide opportunities to treat serious obstetric complications at the molecular level. Furthermore, delivery to the individual maternal or foetal compartments in pregnancy may substantially improve the safety of the treatment, compared to when they are administered systemically.

Despite the recent advances in targeted delivery of nanomedicines and novel methodologies for diagnostics, this technology is still in an early phase of development. Incorporation into other fields of medicine particularly oncology give rise to hope that in time, with advances in nanomedicine regulatory mechanisms as well as enhanced safety/toxicity assessments, nanoparticle therapy in reproduction may become part of exciting new strategies to alleviate much of the suffering arising from many reproductive disorders and major complications of pregnancy.

Data availability

No new data were generated or analysed in support of this research.

Authors' roles

The review was initially planned and finalized by N.P., S.T. and N.H. N.P. and N.H. performed extensive literature searches. Written sections, figures and critical discussions were contributed by N.P., T.K.-L., L.H., S.T. and N.H.

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Conflict of interest

The authors have no conflicts to declare.

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