



Nanomedicines: An approach to treat placental insufficiency and the current challenges

C.M. van Kammen^{a,*}, S.J. van Woudenberg^b, R. Schiffelers^a, F. Terstappen^b, A.T. Lely^b

^a University Medical Center Utrecht, Department CDL research, Nano medicine, Heidelberglaan 100, 3584 CX Utrecht, the Netherlands

^b University Medical Center Utrecht, Wilhelmina Children's Hospital, Department of Woman and Baby, Lundlaan 6, 3584 EA Utrecht, the Netherlands

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ABSTRACT

Introduction: Preeclampsia and fetal growth restriction are common pregnancy complications that significantly impact perinatal health and offspring development later in life. The origin of these complex syndromes overlap in placental insufficiency. Progress in developing treatments for maternal, placental or fetal health is mainly limited by the risk of maternal and fetal toxicity. Nanomedicines are a promising approach to safely treat pregnancy complications since they can regulate drug interaction with the placenta to enhance efficacy of the treatment while minimizing exposure of the fetus.

Methods: This narrative review discusses the current developments and challenges of nanomedicines during pregnancy with a focus on preclinical models of placenta insufficiency syndromes. Firstly, we outline the safety requirements and potential therapeutic maternal and placental targets. Secondly, we review the prenatal therapeutic effects of the nanomedicines that have been tested in experimental models of placental insufficiency syndromes.

Results: The majority of liposomes and polymeric drug delivery system show promising results regarding the prevention of trans-placental passage nanomedicines in uncomplicated and complicated pregnancies. The others two studied classes, quantum dots and silicon nanoparticles, have been investigated to a limited extent in placental insufficiency syndromes. Characteristics of the nanoparticles such as charge, size, and timing of administration have been shown to influence the trans-placental passage. The few available preclinical therapeutic studies on placental insufficiency syndromes predominantly show beneficial effects of nanomedicines on both maternal and fetal health, but demonstrate contradicting results on placental health. Interpretation of results in this field is complicated by the fact that results are influenced by the choice of animal species and model, gestational age, placental maturity and integrity, and nanoparticle administration route.

Conclusion: Nanomedicines form a promising therapeutic approach during (complicated) pregnancies mainly by reducing fetal toxicity and regulating drug interaction with the placenta. Different nanomedicines have been proven to effectively prevent trans-placental passage of encapsulated agents. This can be expected to dramatically reduce risks for fetal adverse effects. Furthermore, a number of these nanomedicines positively impacted maternal and fetal health in animal models for placental insufficiency. Demonstrating that effective drug concentrations can be reached in the target tissue. While these first animal studies are encouraging, more research is needed to better understand the influence of the pathophysiology of this multi-factorial disease before implementation in clinical practice can be considered. Therefore, extensive evaluation of safety and efficacy of these targeted nanoparticles is needed within multiple animal, in vitro, and/or ex vivo models. This may be complemented by diagnostic tools to assess the disease status to identify the best time to initiate treatment. Together these investigations should contribute to building confidence in the safety of nanomedicines for treating mother and child, as safety has, understandably, the highest priority in this sensitive patient groups.

* Corresponding author.

E-mail address: c.m.vankammen@umcutrecht.nl (C.M. van Kammen).

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1. Introduction

Preeclampsia (PE) and fetal growth restriction (FGR) majorly impact perinatal morbidity and mortality. Annually, PE causes approximately 50,000 maternal deaths worldwide [1] and half of the stillbirths are linked to FGR [2,3]. The etiology of these syndromes overlap in placental insufficiency. There is no therapy for PE and FGR available apart from termination of pregnancy. Therefore, these pregnancy complications often result in prematurity and (mainly neurological) neonatal sequela. Therefore, novel therapeutics to improve maternal and placental health issues remain an important research area in obstetrics.

While preclinical studies demonstrated the effectiveness of several therapeutic agents to improve uteroplacental blood flow or placental health, they have not (yet) reached clinical application due to several challenges [4]. One of the largest challenges concerns the safety risks of the new therapeutic intervention for both mother and unborn child [5–7]. A solution to increase the safety of a treatment, could be to prevent trans-placental passage and support placental-specific drug delivery.

Nanomedicines can control drug interaction with the placenta and enhance efficacy of the treatment while minimizing exposure of the fetus [8–13]. Nanoparticles are colloidal systems with a size below 100 nm; although, generally in drug delivery, the definition extends to include all particles below 1 μm . The tissue distribution profile of nanoparticles can be steered by regulating physicochemical characteristics of the nanoparticle and by decorating the surface of the nanoparticle with targeting ligands. For each therapeutic strategy nanomedicines need to be optimized for material composition, size, and surface charge, to take full advantage of potential to enhance efficacy and safety of the encapsulated drug. Nanomedicines could be a promising approach to safely treat pregnancy complications illustrated by the rise in studies on different characteristics in pregnancy. Here, we provide an overview of nanomedicines used in pregnancies complicated by PE and FGR to identify the most promising characteristics to prevent trans-placental passage. Firstly, we outline the safety opportunities and

potential therapeutic maternal and placental targets in pregnancy with a focus on pregnancies complicated by PE and FGR. Secondly, we discuss the prenatal therapeutic effects of the nanomedicines in experimental models of placental insufficiency.

2. Multifactorial pathophysiology of placental insufficiency: complexity limits development of effective therapies

The etiology of PE and FGR originates in poor placentation. The pathophysiology of placental insufficiency remains complex and multifactorial in which the development of maternal and fetal symptoms can be described as a process in three-phases [14–17] (Fig. 1). During phase 0, the maternal interaction with fetal or paternal antigens plays an important role in healthy placentation [14,15,17]. Incompatible tolerance is believed to elicit impaired remodeling of the placental spiral arteries [17]. During phase one, the impaired vascularization exposes the placenta to oxidative stress and hypoxia which in turn results in placental release of anti-angiogenic factors and pro-inflammatory cytokines into the maternal blood circulation [14–17]. The imbalance of these circulating factors, such as soluble FMS-like tyrosine kinase-1 (sFlt1), Placental like Growth Factor (PlGF), and Tumor Necrosis Factor- α (TNF- α), induces maternal systemic endothelial dysfunction. While the origin of placental insufficiency syndromes can be traced back to early gestation or even preconceptually, the onset of symptoms presents late during gestation which is marked as phase 2. Preeclampsia is defined as hypertension after the 20th week of gestation in combination with maternal organ failure and/or hemolysis (including proteinuria) or fetal growth restriction [14–17], FGR results from reduced nutrient and oxygen supply due to the reduced utero-placental blood flow [15–17]. Understanding the pathophysiology better, offers multiple targeting options for prophylactic agents during phase zero and one, or therapeutic agents during phase two. The therapeutic window for intervention will lie between the onset of symptoms around 20 weeks till the end of pregnancy. The potential therapeutic targets which can counter the aberrant pathways of the pathophysiology of PE are the oxidative stress, inflammation and endothelial dysfunction pathways

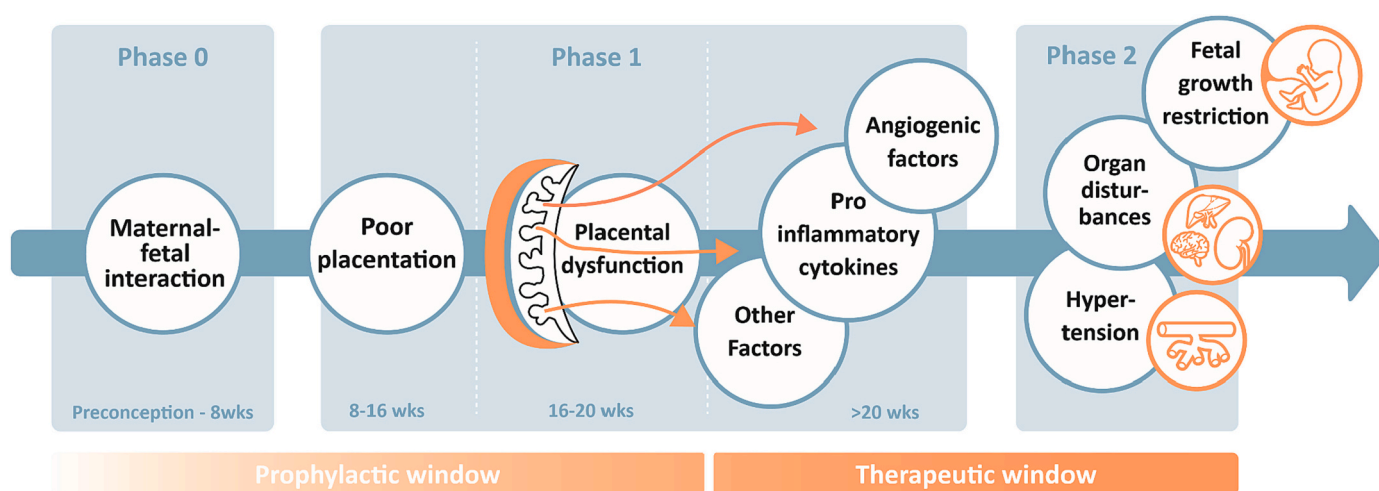


Fig. 1. Three phase model of pathophysiology of placenta insufficiency syndromes.

Three phase model of pathophysiology of placenta insufficiency syndromes. Poor placentation is believed to be driven by an inadequate maternal tolerance to fetal or paternal antigens during implantation [14,15,17]. The partial intolerance creates an adverse determinative milieu, which impairs remodeling of the placental spiral arteries into vascular sinuses and hamper extravillous cytotrophoblast (EVT) invasion [17]. The subsequent uteroplacental blood flow impairs nutrient and oxygen supply towards the fetus which results in FGR or stillbirth. The sustained oxidative stress and hypoxia in the placenta, as a consequence of poor placentation, also triggers placental release of anti-angiogenic factors and pro-inflammatory cytokines, such as sFlt1, PlGF, TNF- α , into the maternal circulation [14–17]. The angiogenic and inflammatory imbalance induces maternal systemic endothelial dysfunction, which eventually gives a rise to the cardinal features of preeclampsia, namely hypertension after the 20th week of gestation accompanied by maternal organ failure and/or hemolysis [15–17]. In the first 16 weeks during development of the disease there is a prophylactic window option. The therapeutic window option will lie between the onset of symptoms around 20 weeks till the end of pregnancy. Related to pathophysiology of PE the potential therapeutic targets are the oxidative stress, inflammation and the endothelial dysfunction. Adaption of figure from paper Terstappen et al. [70].

that dominate the disease. Currently the complexity and multifactorial origin of the disease hampers the development of novel therapies for placental insufficiency. Most likely a multi-targeted drug and potentially individualized therapy is needed for this multi-factorial disease in which nanomedicines might form the solution.

3. Strategies to treat placental insufficiency: currently used treatments and future opportunities

Currently, there is no treatment available for placental insufficiency syndromes. Therefore, obstetricians are subdued to induce labor when stabilization management with anti-hypertensive drug and magnesium to prevent eclampsia becomes insufficient. However, preventive therapies are available, started before 16 weeks of pregnancy, such as low dose aspirin and calcium supplementation for woman with higher risk to develop PE [18].

Considering the etiology of placental insufficiency, novel therapeutic strategies aimed at either placental or maternal (vascular) health or even better in combination, would improve maternal and fetal outcome by prolonging the duration of pregnancy. Examples of some promising agents that have been studied in preclinical and clinical setting include anti-oxidants, statins [19] and molecules with pro-angiogenic properties [20–22].

Unfortunately, therapies that appear promising in preclinical setting have so far been unsuccessful in reaching clinical practice. This is mostly because of unavoidable limitations of the animal models that do not fully recapitulate the human disease, in combination with suboptimal efficacy in humans. Safety concerns in clinical studies make it is difficult to arrive at similar therapeutic conditions as those used in animal studies. These limitations can be related to a variety of reasons including: inadequate dosage that can be ascribed to possible differences in metabolism per species, suboptimal timing, and individual responses to therapy [4]. Nanomedicines could help to enhance efficacy and safety of the therapeutic strategy by preventing trans-placental passage and supporting the placental-specific drug delivery [11].

4. Nanomedicines as a promising approach during pregnancies: towards pathophysiology of PE and FGR

Nanotechnology enables specific applications in medicine, ranging from diagnostics with improved sensitivity to targeted therapeutics. Nanomedicines provide an opportunity to improve prenatal therapy with direct and long-lasting benefit for both mother and child. By encapsulating or associating a drug molecule to a nanoparticle, the fate of the drug molecule is no longer dictated by its own physicochemical characteristics, but determined by the characteristics of the nanoparticle. This offers control over the destination of the drug and the timing of release. For example, many water-soluble small molecular weight drugs are cleared fast from the circulation by the kidneys, in turn limiting exposure to the desired tissue. Encapsulation of such a drug in a nanoparticle avoids renal clearance and generally extends circulation time providing a chance to engage with targeted cells [23].

The ability to modify the pharmacokinetics and bio-distribution of drugs enables specific delivery of drugs at placental or maternal endothelium. Improving target accumulation provides the benefit of enhanced local therapeutic effects and improved drug safety profiles by averting trans-placental passage and thereby fetal exposure. Besides the therapeutic role, nanomedicines might be deployed for diagnostic or theragnostic purposes as well. These systems combine chemical versatility in labeling reaction to support imaging with a capacity to transport medicines. In contrast to other research fields, this topic remains to be explored on optimal design of diagnostic or theragnostic nanoparticles in the research field of PE and FGR.

Optimizing nanoparticle design for placental delivery is a multi-parametric challenge since each characteristic plays a key role in bio-distribution, biocompatibility, and safety. The following important

characteristics will be discussed: nanoparticle class, nanoparticle size and shape, surface charge, and modifications.

4.1. Nanoparticle class

There are four main classes of nanoparticles that has been evaluated over the last years for prenatal treatment with maternal and placental target in pregnancies: liposomes, polymers, quantum dots, and silicon nanoparticles. In this review we are going to discuss these four groups (Table 1).

4.1.1. Liposomes

Liposomes were among the first nanoparticles to be investigated as a pharmaceutical carrier with the first formulation being approved for clinical application in 1992. The amphiphilic (phospho)lipid molecules contain a hydrophilic head and a hydrophobic tail which self-assemble into a bilayered spherical vesicle. Drugs can be encapsulated inside the hydrophilic core or embedded in the lipophilic membrane. The first generation of liposomes were rapidly recognized and taken up by macrophages in liver and spleen providing only a very short time window to reach target tissues. Therefore, the second generation of liposomes known as poly(ethylene glycol) (PEG)-coated or “stealth” liposomes were designed to diminish this limitation. They expose PEG-chains on their surface which decelerates macrophage uptake and prolongs their blood circulation time improving chances of target tissue engagement.

4.1.2. Polymers

Polymeric nanoparticles are perhaps the most versatile among the various drug delivery systems. The choice of synthetic or natural monomers is infinite and different monomers can be combined within a single polymer. Even more so, these monomer combinations can be linked in, for example, random, alternating, diblock, or triblock arrangements. These arrangements can be biodegradable or stable, based on their lengths and connectivity in a linear or branched fashion. Several types of polymer nanoparticles have been characterized in the context of placental drug delivery. These include poly(amidoamine)(PAMAM) dendrimers and nanoparticles composed of Poly(Lactic-co-Glycolic Acid) (PLGA) and polystyrene.

4.1.3. Quantum dots

The smallest nanoparticles applied in drug delivery concerns the quantum dots (QDs). These nanoparticles of only a few nanometers contain unique optical and electronic properties. Quantum dots convert a spectrum of light into different colors, which endows them with inherent fluorescent properties. These properties can be highly relevant for theragnostic applications where diagnosis and therapy are combined [24,25]. The fluorescence provides information on localization. When target accumulation is reached a trigger for drug release can be provided.

4.1.4. Silicon nanoparticles

At small sizes, silicon (Si) nanoparticles are a type of quantum dots that possess the same attractive features for theragnostic applications as described above. The larger porous Si nanoparticles have a high internal surface area and a rich surface chemistry that allows for high loading capacity and modification of surface properties. Electrochemical synthesis allows construction of tailored pore sizes and volumes that are controllable from the scale of microns to nanometers. In addition, porous Si surfaces can be chemically engineered to control the drug type that can be encapsulated, the amount and the release rate of drug payloads.

4.2. Size

Nanoparticle size is, just like nanoparticle class, an important

Table 1

Overview of nanomedicines aiming to prevent trans placental passage during pregnancy.

Type	Size (nm)	Surface charge	Target/Surface modifier (ligands)	Encapsulated agent (Drug, gene, label)	Experimental model(s)	Transplacental passage	Localisation and uptake (compared to control)	Ref.
Liposomes	124	Cationic	OTR	Indomethacin	<i>In vitro, in vivo, ex vivo</i>	No	Uterus, minimally in liver and placenta	35
Liposomes	CGKRRK 156, iRGD 146, ARA 142	Neutral	Integrines GKRRK, iRGD or ARA	IGF-2 (therapeutic effect FGR only with iRGD)	<i>In vivo, ex vivo</i> ^h	No	Placenta, liver, spleen	34
Liposomes	197	Neutral	OTR	Nifedipine, salbutamol, rolipram, dofetilide, indomethacin	<i>In vivo, ex vivo</i> ^h	No	Uterus, mammary glands, liver	36
Liposomes	N/S	Cationic	Epitope hemagglutinin (HA)	Mitochondrial antioxidant manganese superoxide dismutase-plasmid	<i>In vivo</i>	No	Liver	37
Liposomes	N/S	Neutral	Endothelium of the uterine spiral arteries and placental labyrinth CNKGLRKN-peptide	SE175 NO donor	<i>In vivo, ex vivo</i> ^h	No	Placenta, not in vascular bed of any other major organ, but small amounts seen in liver, kidneys and spleen	38
Liposomes	50-100	Cationic	1) Neutral,	Insulin 2 (gene transfer)	<i>In vivo</i>	No	Lung, liver, spleen	39
Liposomes	33-98	2) Anionic, 3) Cationic	–	Inulin, penicillin	<i>In vivo</i>	No/yes	Uterus, placenta, liver (less than placenta), spleen, kidney	33
Liposomes	114	Cationic	Trophoblast, megalin; Gentamicin	Cy5.5 siRNA, Nr2 siRNA, and sFlt-1 siRNA	<i>In vitro, in vivo</i>	No	Placentas, maternal kidneys,	41
Liposomes	N/S	Cationic	Trophoblast, pICSA-BP	–	<i>in vitro, in vivo</i>	No	–	42
Liposomes and Polymers-PEI	N/S	1) Neutral 2 + 3) Cationic	–	Nuclear (gene transfer); LacZ	<i>In vivo</i>	Yes	Liver, spleen, lungs, 1) placenta 2 + 3) fetal membrane	40
Liposomes, PEGylated	252	Neutral	–	Hemoglobine	<i>In vivo</i>	No	Spleen, liver, kidney, placenta	43
Liposomes, PEGylated	201	Neutral	OTR, anti-OTR monoclonal antibodies (OTR-Lipo) or OTR antagonist (ATO-Lipo)	Atosiban (prevention contraction delivery)	<i>In vitro</i>	N/A	–	58
Liposomes, PEGylated	1) 121 2) 86 3) 317	Cationic	–	siRNA (gene transfer)	<i>In vitro</i>	N/A	–	61
Polymers	N/S	N/S	PLAC1-hIGF1 plasmid	–	<i>In vitro, ex vivo</i>	No	–	49
Polymers	180 nm	Cationic	–	Mitochondrial antioxidant manganese superoxide dismutase-plasmid	<i>in vivo</i>	No	–	54
Polymers	N/S	N/S	PLAC1-hIGF1 plasmid	–	<i>in vitro, in vivo</i>	N/S	–	69
Polymers, ELP	N/S	N/S	–	human VEGF-B167 (ELP-VEGF-B)	<i>In vitro, in vivo</i>	No	1. Kidney, liver, brain, spleen, lung, placenta 2. kidney, liver, placenta,	55
Polymers, ELP	N/S	N/S	–	VEGF Chimera	<i>In vivo</i>	No	Kidneys, aorta, liver, placenta	44
Polymers, ELP	N/S	Cationic	–	CPP SynB1	<i>In vivo, ex vivo</i> (imaging)	No	Kidneys, liver, hart, milt, placenta	46
Polymers, ELP	N/S	Cationic	–	CPP SynB1- NF-κB inhibitory peptide (p50i)	<i>In vitro, in vivo, ex vivo</i> (imaging)	No	Kidneys, lever, placenta	47
Polymers, ELP	4.1-6.8	N/S	–	–	<i>In vivo</i>	No	Placenta, kidneys, liver, brain, lungs, heart	45
Polymers, PAMAM	6	Neutral	–	–	<i>Ex vivo</i> ^h	Yes	–	52
Polymers, PAMAM	N/S	N/S	–	N-acetyl-L-cysteine	<i>In vivo, ex vivo</i> (imaging)	Yes	Restricted to yolk sac and ↑ with more exposure of LPS	53
Polymers, PAMAM	70-80	Cationic, neutral, anionic	–	siRNA -sFlt-1	<i>In vitro, In vivo</i>	N/S	–	62
Polymers, pHPMA-b-pDMAEMA	N/S	Cationic	–	Trophoblast (gene transfer) Cyp19a or PLAC1 human IGF1 plasmid DNA	<i>In vitro, in vivo</i>	No	–	48
Polymers, pHPMA-b-pDMAEMA	N/S	Cationic	–	Trophoblast (gene transfer) PLAC1 human IGF1 plasmid DNA	<i>In vitro, Ex vivo</i> ^h	No	–	49

(continued on next page)

Table 1 (continued)

Type	Size (nm)	Surface charge	Target/Surface modifier (ligands)	Encapsulated agent (Drug, gene, label)	Experimental model(s)	Transplacental passage	Localisation and uptake (compared to control)	Ref.
Polymers, PLGA	109	Anionic	Trophoblast, pICSA-BP	Methotrexate (immunosuppression)	<i>In vivo, Ex vivo</i> ^h	No	Placenta, binding specifically to trophoblast cells, SCR also in liver	50
Polymers, PLGA	109	Cationic	Trophoblast; pICSA-BP	Doxorubicin	<i>In vitro</i>	N/A		57
Polymers, γ -PGA-Phe	N/S	Anionic	Mitochondrial; Anti oxidant mitoQ	–	<i>In vitro, in vivo</i>	No		51
Polymers, γ -PGA-Phe	130	Cationic	Mitochondrial; Anti oxidant mitoQ	–	<i>In vivo, ex vivo</i>	N/S		68
QD	15–20	N/S	OTR; integrins iRGD	IGF-1	<i>ex vivo</i> ^h	N/A		26
QD, PEG/PEI coated	171	Cationic	–	–	<i>In vitro, in vivo</i>	No/yes	Lungs, liver, kidneys, reproductive organs (GD7 none, GD11 only uterus, GD19 uterus placenta and fetus)	27
Silicon particles	519, 834, 1000	Anionic	–	–	<i>In vivo</i>	No/yes	Liver, uterus, placenta	28

Legend: Current research studies with nanomedicines preventing trans placental passage during pregnancy. Abbreviations CD = cyclodextrin; COOH = carboxylate; CPP = cell penetrating peptide; CGKRK = tumor homing peptide, composed of amino acids Cys-Gly-Lys-Arg-Lys; DMAEMA = mmol), 2-(dimethylamino) ethyl methacrylate; DMAPAP = dimyristoylaminopropylaminopropyl; ELP = elastin-like polypeptide; GHRH = growth hormone release hormone; HPMA = 2-Hydroxypropyl methacrylamide; IGF = insulin-like growth factor; iRGD = tumor homing peptide, composed of amino acids Cys-Arg-Gly-Asp-Lys-Gly-Pro-Asp-Cys; N/A = not applicable; N/S = not specified; OTR = oxytocin receptor; PAMAM = poly-amidoamine; PC = phosphatidylcholine; PEG = polyethylene glycol; PEI = poly-ethylenimine; PLAC1 = trophoblast specific promoters; Phe = phenylalanine; pICSA-BP = placental chondroitin sulfate A binding peptide; PLGA = polylactic-co-glycolic acid; PS = polystyrene; Psi = Silicon nanovector, SCR = scrambled peptide; SE175 = NO donor 2-[[4-[(nitrooxy)methyl]benzoyl]thio]-benzoic acid methyl ester; sFlt-1 = soluble fms-like tyrosine kinase-1; NF = nuclear factor; VEGF = vascular endothelial growth factor; Qd = Quantum dots; NOTE: h = in human tissue.

parameter that affects circulation time, bio-distribution profile, placental transport, and fetal exposure. The size influences the clearance route in which small nanoparticles (< 5 nm) will likely be rapidly cleared by the kidneys, whereas larger nanoparticles tend to be cleared by the reticuloendothelial system, predominantly the macrophages in liver and spleen. From a clinically perspective, the size forms a relevant characteristic in designing drug delivery systems to prevent transplacental passage. Physiological evidence suggests that healthy hemochorial placental pore sizes measure around 10 nm, which would prevent accumulation for the majority of drug delivery systems. For selected tissues, e.g. the liver, spleen and bone marrow, endothelial fenestrae from blood to tissue have diameters in the range of 40–100 nm that allow access [29]. The capillary permeability increases under inflammatory conditions, in which the formed endothelial gaps allow entry of particles up to several hundreds of nm and also endothelial transport capacity is increased [30]. The accumulation of nanoparticles at these sites of inflammation is augmented by reduced lymphatic drainage of nanoparticles. This phenomenon is known in cancer research as the enhanced permeability and retention (EPR) effect. It is plausible that this EPR effect also occurs in preeclamptic women, since increased placental factors induced endothelial permeability forms the key mediator in the pathogenesis of PE [31,32].

4.3. Charge

The charge of nanoparticle components influences the ability to self-assemble through electrostatic interactions. For nucleic acids, the nanoparticles charge will be cationic to bind the negatively charged phosphate groups present in the polynucleotides. Surface charge has also been identified to majorly affect nanoparticle translocation into a cell or through a physiological barrier. Most likely this indirectly results from opsonization. Nanoparticles also rapidly accumulate a corona of proteins in a biological environment due to their high surface area. The identity of proteins that adhere to the nanoparticle surface are dependent on particle surface characteristics, such as charge. As a result, the 'new' surface, acquired by the nanoparticles surface, mediates barrier and cellular interactions. Major protein classes involved in opsonization include apolipoproteins, complement factors, and immunoglobulins.

Diseased states may alter blood protein composition, because of the opsonization pattern and hence the interaction profile.

4.4. Surface modifications

Modifications to the nanoparticle surface can increase or decrease the likelihood of particle-placental interaction. Surface modifications can be used to acquire an altered protein corona on the surface that in turn affect the tissue distribution or cellular interactions. For example, second generation liposomes possess a PEG-coating that reduces corona formation and reduces recognition and uptake by macrophages. Surface modifiers can also be added to prevent placental transfer during maternal treatment, protecting the fetus from direct pharmacological interaction [8].

Decoration of the nanoparticle surface with targeting modalities for site-specific delivery to the placenta is still emerging.

The important characteristics nanoparticle class, nanoparticle size and shape, surface charge, and modifications are the key factors in biodistribution and safety of the nanomedicines. These characteristics affect circulation time and nanoparticle-cell interactions which in turn can contribute too safety and efficacy of the approach. Surface modifiers and targeting ligands can enhance tissue-specific interactions and improve binding and internalization into placental cells, which will be discussed in chapter six. Effective encapsulation of drugs into the delivery systems increases the cost-effectiveness of nanomedicines and when combined with appropriate drug retention helps to increase the therapeutic efficacy of nanoparticles.

In the next section we will discuss nanomedicines specifically developed for use during pregnancies in which we will focus on pregnancies complicated by PE and FGR. In this section we will relate the characteristics (as mentioned in this current section) of these nanodrug delivery strategies to tissue distribution profiles or biological outcomes.

5. Nanomedicines preventing trans-placental passage in uncomplicated and complicated pregnancies

The total number of studies on nanoparticles in pregnancy, with maternal or placental target, either uncomplicated and complicated, is

limited (Table 1). The most frequently studied nanoparticles in this line of research are liposomes and polymers. In this chapter we will describe general observations from *in vitro*, *in vivo* and/or *ex vivo* studies, concerning uptake/clearance and trans-placental passage in pregnancies as part of the proof of concept in our pursuit towards treatment options in complicated pregnancies.

5.1. Clearance and accumulation of nanodrug during pregnancy

Many animal studies in pregnancies, observe accumulation of the nanoparticles in the canonical organs for uptake, e.g. in the liver and spleen similar to non-pregnant animal models [30]. These tissues house active phagocyte populations that continuously sample the circulation for particulate matter. In line with results reported for nanoparticles in other fields, particles that are large and charged are recognized faster and to a higher degree increase hepatosplenic uptake. Conversely, kidney clearance dominates for very small structures, such as the elastin-like polypeptide (ELP) conjugates with hydrodynamic radii ~5 nm.

In relation to clearance, target tissue interaction opportunities are limited mostly due to fast removal from the circulation, irrespective of which route of clearance. Two animal studies analyzed placental accumulation in relation to clearance in two different approaches: a study on liposomes showed that liposomal accumulation in the placenta could be increased >15-fold by switching from a bolus intravenous injection protocol to an infusion-based approach in which a continuous administration has shown to increase placental exposure [33]. Placental exposure can also be increased by shielding of the particle surface to reduce phagocytic recognition. The most popular strategies to achieve this are PEGylation or increasing molecular weight of the ELP-conjugate and thereby limiting kidney clearance enhancing placental accumulation.

A relation between plasma residence time and target tissue accumulation is often observed in the nanomedicine field suggesting an greater statistical likelihood of target tissue interaction. The fact that placental tissue is a highly active tissue with strong endothelial cell involvement makes placental accumulation substantial.

5.2. Preventing trans-placental passage

Twenty-six of the thirty-three studies with nanosized drug delivery during pregnancy reported on trans-placental passage. The majority studied liposomes [33–43] and polymers [44–55], twenty of these twenty-six studies show promising results regarding preventing trans-placental passage for both liposomes and polymers without superiority. Since there is no superiority in relation to trans-placental passage between the two classes, the choice of nanoparticle is based on other characteristics.

Several characteristics influence trans-placental passage in pregnancy, most notably size and charge, as described in a review about liposomes [10]. The influence of composition and charge of liposomes is studied in pregnant rats and show high uptake for negatively charged composed SUV liposomes of egg PC:Chol:DCP in the placenta and almost no uptake in the fetus. Furthermore, their results show that the type of encapsulated drug also affects the uptake in the placenta and small differences on the trans-placental transfer to fetus [33].

Evaluation of size differences is performed in a few studies demonstrating that increased size correlates to increased uptake [28,35,45]. For example, a study with polymers [45] of various sizes ranging from 4.1 to 6.8 nm hydrodynamic radius were administered as *intravenously* (i.v.) bolus to pregnant Sprague Dawley rats resulting in increased placental uptake for larger sizes. Little to no ELP was detected in the pups. Moreover, assessment of silicon nanovectors is performed in pregnant rats with relatively large particles (519,834 and 1000 nm) and demonstrated that larger silicon nanoparticles (834 and 1000 nm) will not transfer over the placenta in contrast to the smaller particles (519 nm) [28]. It is worth noting that the selective permeability of the

placenta is different to nanoparticles made of different materials.

Timing of injection during pregnancy is also an important characteristic in preventing trans-placental passage, as blood volume, renal blood flow, intestinal transit, protein binding and a developing placenta differ during pregnancy. Moreover the variation on biodistribution and release rates of the different nanomedicines also need to be considered in combination with the developmental stages of pregnancies and pathophysiology of PE/FGR. For instance, the importance of timing of injection is illustrated by a study in mice showing that injection of QD's *via* the tail vein in mice, resulted in accumulation in the reproductive organs (uterus, placenta, and fetus) only when administered on gestational day (GD) 19, but not when administered on GD7 [27]. Controversially, on GD9.5 a higher accumulation of gold nanoparticles was observed in the placenta and small amounts in the fetus when compared to i.v. injection on GD14 in mice [56]. Placental development most likely plays a key role in timing dependent accumulation. The class of nanoparticles and differences in placentation between rodents and humans should be taken into consideration.

The inherent variability in uncomplicated and complicated pregnancy study set-ups make interpretation of results challenging. Apart from the variability regarding nanoparticle class and characteristics, the type of animal species and model, gestational age, placental maturity and integrity, nanoparticle administration format, and choice for label or drug can all affect the trans-placental passage. Consequently, it is virtually impossible to draw firm conclusions on most promising characteristics of nanosized drug delivery system from the tables presented (Table 1). Nevertheless, many nanomedicines are indeed able to favorably steer tissue distribution of encapsulated material towards the placenta while limiting or preventing distribution to the fetus.

6. Evidence on cellular uptake in placenta

Several *in vitro* and *in/ex vivo* studies investigated the uptake in the placental cells (Table S4 and S5) with focus on binding and internalization, functionalization, and different pathways of uptake of nanomedicines in pregnancy.

6.1. Binding, accumulation and internalization

Functionalized nanoparticles with surface modifiers able to recognize a membrane receptor of targeted cells can be an interesting concept. Which can induce tissue-specific interactions and enhance binding and internalization into placental cells. Using ligands to target the syncytiotrophoblast is one of the approaches, from the mother's blood circulation, because this is the first layer of the placenta that is available. As far as known in literature no other specific ligand has been validated for placental drug delivery purposes, overview of targets shown in Table 2. Improving binding and internalization raises the efficacy and diminishes (off-target) side effects.

Zhang et al. described *in vitro* and *in vivo* experiments with the pLCSA-BP-conjugated lipid-polymer nanoparticles targeting the placental chondroitin sulphate A (pLCSA) which are expressed in syncytiotrophoblast. pLCSA-BP could rapidly bind to JEG3 cells and increase uptake [50,57]. Moreover, they demonstrated a biotinylated pLCSA-BP that binds specifically to trophoblasts in the *ex vivo* placental tissue of mouse and human.

In four other studies they developed functionalized liposomes targeting the oxytocin receptor (OTR) in different *in vitro* and *in vivo* settings to deliver drugs to the myometrial cells or tissue as a goal to allow therapeutic modification of myometrial contractions in obstetric settings [35,36,58]. All three studies showed binding and a higher uptake. Hua et al. demonstrated significantly increased cellular internalization [58]. A study with liposomes targeted to megalin receptors showed a dose dependent 1,5 and 2 fold enhanced internalization [41].

King et al. evaluated a library of tumor-homing peptides and selected the pentapeptide CGKRK and the cyclic peptide iRGD to develop

Table 2

An overview of targeting approaches of nanomedicines in complicated pregnancies for placenta insufficiency syndromes.

Type of nanoparticle	Target/Surface modifier (ligands)	Type of surface modifier	Ref.
Liposomes	Endothelium of the uterine spiral arteries and placental labyrinth CNKGLRNK-peptide	Targeting peptide	37
Liposomes	Epitope; hemagglutinin	Targeting peptide	36
Liposomes	Integrines; GKRK, iRGD or ARA	Membrane protein	33
liposomes	Trophoblast, megalin; Gentamicin	Ligand/substrate for receptor	40
liposomes	Trophoblast; pICSA-BP	Ligand for receptor	41
Polymer	Trophoblast; PLAC1-hIGF1 plasmid	Ligand for receptor	48
Polymer	Trophoblast; PLAC1-hIGF1 plasmid	Ligand for receptor	65
Polymers, PLGA	Trophoblast; pICSA-BP	Ligand for receptor	49
Polymers, PLGA	Trophoblast; pICSA-BP	Ligand for receptor	56
Polymers, γ -PGA-Phe	Mitochondrial; Anti oxidant mitoQ	Targeting antioxidant	50
Polymers, γ -PGA-Phe	Mitochondrial; Anti oxidant mitoQ	Targeting antioxidant	64
QD	Oxytocin receptor; integrines iRGD	Lipophilic cation	
		Membrane protein	26

Legend: The different types of ligands e.g. targeting peptide, membrane protein, ligands for receptors and targeting antioxidant on nanomedicines in complicated pregnancies models placenta insufficiency.

peptide-coated nanosized drug delivery system that binds specifically to the maternofetal interface [34]. The selected peptides were tested on human and mice placental explants, they accumulated in the syncytiotrophoblast of the tissue explant. Then, the peptide coated nanoparticles with encapsulated marker was injected into mice. The nanoparticles were mostly localized at the maternofetal interface, the liver and the spleen. Another study with the tumor homing peptide iRGD targeting coupled to QD's, showed in human placental explants internalization of the encapsulated IGF (insulin-like growth factor) mainly in the cytotrophoblasts after two hours, at 24 h IGF was localized at basal membrane of the syncytium [26].

Gene transfer therapy to trophoblast cells could also be a promising way to (over)express or silence specific proteins. However, their instability in biological fluids as well as their hydrophilicity, hinder delivery to the target tissue and cellular uptake. Nanomedicines could offer the opportunity (i) to protect fragile nucleic acids from nuclease degradation; (ii) to target gene delivery to the syncytiotrophoblast; and (iii) to restrict off-target side effects especially towards the fetus. Wilson et al. used polymers and demonstrated uptake and delivery of the human insulin-like growth factor 1 (hIGF1) transgene decorated with the trophoblast-specific (PLAC1) promoter to syncytiotrophoblast cells [49].

Another strategy in gene delivery is presented using oligonucleotides like siRNA and antisense that can silence mRNAs [59]. A recent publication has shown that silencing three sFlt1-mRNA isoforms in the placenta by siRNA reduces circulating sFlt1 up to 50% and improves the PE phenotype in baboons [60]. Valero et al. performed research on liposomes as a gene delivery vector for human placental cells in 3 formulation carrying rhodamine-labeled siRNA [61]. All tested formulations led to fluorescent siRNA uptake in the primary cytotrophoblasts. Yu et al. siRNA-sFlt1-PAMAM complexes showed high cellular uptake and excellent siRNA encapsulation ability [62].

6.2. Functionality of encapsulated strategy

Several studies described functionality of their encapsulated

strategy. Eddy et al. was able to block NF- κ B activation and induce TNF- α production of endothelin with Elastin like polypeptide with NF- κ B inhibitor (SynB1-ELP-p50i) [47]. The group of Jones developed two non-viral, trophoblast-specific gene delivery systems with hIGF-1 and showed higher IGF-1 expression with both formulations *in vitro* in BeWo cells and *in vivo* mouse placentas [48,49]. Consecutive in human placental villous fragments *ex vivo*, the PLAC1 increased hIGF1 expression in villous fragments resulting in the translocation of glucose transporter 1 to the syncytiotrophoblast cell membrane. Li et al. demonstrated, using pICSA-bp targeted liposomes, an efficient simultaneous downregulation of placental target genes Nrf2 and sFlt1 in primary mouse trophoblast cells [42]. In another study, siRNA-sFlt1-PAMAM polymers were tested *in vitro* for functionality and showed significantly decreased sFlt1 secretion from HTR-8/SVneo cells [62].

6.3. Placental exchange, pathway of uptake

So far only very few studies have investigated whether nanoparticles pass the human placenta, and yet there is almost no information on the trans placental uptake mechanisms involved. Several mechanisms are already known for the placental exchange of endogenous substances including passive diffusion, facilitated diffusion, active transport, endocytotic pathways and trans trophoblastic channels [11,63]. Passive diffusion is the prevailing transfer mechanism for most small substances (<500 Da) and pharmacologically active compounds. Passive transfer is profoundly influenced by the concentration gradient, the properties of the placenta and the physicochemical characteristics of the substance.

Nanoparticles designed with the purpose of not passing the placental barrier will be too large for passive or facilitated diffusion. Nanoparticles uptake by active transporters is questionable as similarity to other endogenous substances is unlikely. Most likely, different endocytotic pathways may be involved in the potential trans-placental transfer of certain nanoparticles. Endocytosis is an active process that is facilitated by cells for the uptake of large, polar molecules that cannot pass through the hydrophobic membrane of a cell. This includes clathrin-mediated endocytosis, caveolae, macropinocytosis and phagocytosis. In all cases, molecules either transport over cell membranes or bind to specific receptors (receptor-mediated endocytosis), followed by the invagination of cell membranes and formation of intracellular vesicles. Expected size restriction for the uptake is >120 nm for clathrin-mediated endocytosis, around 60 nm for caveolae and up to 1 μ m for macropinocytosis respectively.

Energy-dependent clathrin-mediated endocytosis is probably the primary characterized mechanism for the cellular uptake of nanoparticles. At the placental barrier endocytic vesicles. Nanoparticles often prevail in membrane-bound vesicles in the cytoplasm, indicating an active uptake process. Interestingly, nanoparticles may even be actively directed towards receptor-mediated endocytic uptake by functionalizing their surface with selected ligands [64].

Hua et al. evaluated the mechanistic pathway of cellular uptake of liposomes conjugated with anti-OTR monoclonal antibodies(OTR-Lipo) or atosiban (ATO-Lipo, OTR antagonist) *in vitro* (size approx. 200 nm) [58]. They concluded that both different types undergo internalization through clathrin-mediated and caveolin-mediated mechanisms.

7. Research on perinatal therapeutics in complicated pregnancies for PE and FGR

The focus of this review is to highlight the potential that nanomedicines as perinatal therapeutics for PE and FGR. Perinatal therapy aims to restore maternal and fetal health, and additionally intervening during a critical period of fetal development provides a window of opportunity to prevent developmental diseases later in life [65–67].

The distribution of nanomedicines can vary between healthy and complicated pregnancies due to a change *in utero*-placental blood flow or high maternal blood pressure, for example. Altered utero-placental

blood flow is a consequence of failed spiral artery adaption and high blood pressure results from maternal systemic endothelial dysfunction induced by angiogenic and inflammatory imbalance. The multifactorial pathophysiology of PE and FGR requires research with several preclinical models providing insightful information on therapeutic effect of nanomedicines. However, given the anatomical differences in placentas between the used animal models compared to humans and the way of inducing the phenotype in animal model, extrapolations must be made with caution.

There are a number of recent publications focusing on functionality and therapeutic outcome of nanomedicines performed in PE and FGR phenotype animal models (Table 3). Seven different phenotype models were used including surgically induced models [44,47,48,55], hypoxic rat models [51,54,68], irradiation mouse model [37], genetically modified mouse model [34,38,42,69], and inflammatory models [35,36,53,62]. In addition, three studies used preterm birth models on top of FGR/PE models [35,36,53]. Most studies reported beneficial effect on the fetal phenotype (FGR) [34,38,42,48,51,54,62,68], three studies showed attenuated effects on the maternal phenotype (PE) [42,44,62], and only two study showed data on both maternal and fetal phenotype (PE/FGR) [42,62] (Fig. 2).

7.1. Effect of nanomedicines on preeclamptic phenotype

The administration of different nanoparticles has shown to improve maternal phenotype by lowering mean arterial pressure. Studies carried out with polymers encapsulated genes (VEGF or siRNA-sFlt-1) ameliorated hypertension in placental insufficiency-induced rat models [44,62]. Similar results were shown with liposomes encapsulating Nrf2 and sFlt-1 siRNA [42]. Two studies additionally reported decreased proteinuria [42,62]. Moreover, the angiogenic factor sFlt-1 and pro-inflammatory factor TNF- α were decreased in two different placental insufficiency rat models, namely RUPP [44] and an inflammatory rat model [62].

7.2. Effect of different nanomedicines on prolonging pregnancy

PE and FGR often results in preterm birth in the human condition. Improving the preeclamptic symptoms (as described in the previous paragraph) could prolong the pregnancy which also offers more time for fetal development. The effect of nanosized drug delivery strategies on prolongation of gestation has not been specifically investigated in animal models of PE and FGR since the majority of these animal models do not provoke preterm birth. However, several nanomedicines have shown to prolong pregnancy in animal models of induced preterm birth and provide helpful information in preventing trans placental passage, effect of administration route and on effectiveness of therapy. The administration of targeted liposomes encapsulating indomethacine has shown to prolong the pregnancy. For instance, two studies in animal models for preterm birth showed that administration of targeted liposomes encapsulating indomethacine prolonged pregnancy [35,36]. Paul et al. evaluated the ability of oxytocin receptor targeted liposomes to localize to uterine tissue and the capability of preventing lipopolysaccharide-induced preterm birth in mice [36]. By injecting the targeted liposomes intravenously preterm birth rate was reduced from 67% to 18% compared to LPS animals. Notably, they observed no significant difference in free indomethacine compared to encapsulated indomethacine in OTR-targeted liposomes. Refuerzo et al. used the same strategy with oxytocin receptor targeted liposomes, through an intraperitoneal injection, length of pregnancy in hours was prolonged by 31% in mice treated with LIP-IND-ORA and preterm birth rate was reduced from 87,5% to 46,2%, compared to control [35]. There was a trend towards reduced rate of preterm birth by 15% with LIP-IND-ORA compared to free indomethacine. Interestingly, comparing the results of the described studies with the same targeting strategy, the administration route of liposomes (intravenously vs intraperitoneal) can

possibly have influence the therapeutic effect.

7.3. Effect of different nanomedicines on fetal growth

Fetal growth in most studies is determined by fetal or pup weight. In thirteen studies fetal or pup weight was reported on different time points (from GD18 up to at time of birth) (Table 3). Eight studies showed beneficial therapeutic effects on increased fetal or pup weight. The group of Jones performed, besides their *in vitro* functionality experiments, preclinical experiments by injecting their trophoblast-specific gene delivery systems with hIGF-1 in an invasive FGR mouse model (uterine branch ligation). They found increased fetal birth weights on GD20 [48]. Yu et al. evaluated, in addition to the attenuated maternal outcome by siRNA-sFlt1-PAMAM, that the weights of fetuses were also increased compared to the control rat group [62]. Cureton et al. reported improved fetal outcome on GD18 by multiple administrations of nitric oxide donor (SE175) encapsulated in targeted liposomes within an endothelial nitric oxide synthase knockout (eNOS-/-) mice model [38]. King et al. encapsulated insulin growth factor II (IGFII) in the decorated liposomes and injected it i.v. multiple times into gene knock out (deletion U2 Exon IGF2-gene) mouse model to investigate therapeutic effect on GD18.5 [34]. Fetal weight gain was observed with data points represented as individual conceptuses. They showed significant results of altered fetal weight distribution, with fewer of the smallest (lowest weight) pups being observed. Furthermore, no adverse effect of the treatment on the pups were observed. In another study, they reported no significant improvement of fetal weight in a endothelial nitric oxide synthase knockout (eNOS-/-) mouse model on GD18 [69]. Similarly, the group of Bidwell showed no significant increase in fetal weight in three studies with invasive RUPP model using ELP polymers and encapsulated genes (VEGF [44,55] or NF- κ B inhibitor [47]). Maternal treatment of antioxidant MitoQ loaded polymers (nMitoQ) showed a positive effect on fetal outcome in a gestational hypoxia model [51,54,68]. Besides improving fetal and birth weight, nMitoQ also improved long-term outcomes of neurocognitive, vascular, or mitochondrial function of the offspring. While one study revealed sex-specific differences in birth weight in females only [68], contradictory results were found in another study by the same group in which they suggest that the robustness of the phenotype in that animal model might have played a role [54].

7.4. Effect of different nanomedicines on placental health

Placental health is mainly expressed in studies by placental weight and/or ratio of fetal placental weight, with a lower ratio marking placental insufficiency. Placental weights in healthy rodents increase with pregnancy progression with a plateau on GD 19. Fetal-placental weight ratios can decrease by both lower fetal weight and increased placental weight. Placental weight or fetal-placental weight ratio was described in ten studies. The effects on placental weight and the ratio fetal-placenta weight were divergent. A study [38] reported decreased placental weight and increased fetal-placental weight ratio after multiple administrations of nitric oxide donor encapsulated in targeted liposomes on GD18. Their peptide-coated nanosized drug delivery system was mostly localized at the maternofetal interface. The polymers with siRNA-sFlt1-PAMAM increased placental weight, but no significant improvement in fetal-placental weight ratio [62]. Liposomes with tumor homing peptides [34] or PICSA-BP [42] resulted in no improvement in placental weight, however they both showed an increased fetal placental weight ratio. Three other studies found no effect on placental weight [48,55,69]. nMitoQ treatment only improved placental weights of the female fetuses [68]. No placental health improvement was reported in other study from same group [51,54]. However, nMitoQ treatment prevented increase of placental oxidative stress by the hypoxic condition in the placenta [51,68].

Table 3
Perinatal therapeutics in complicated pregnancies for PE and FGR.

Type	Size (nm)	Target/Surface modifier (ligands)	Encapsulated agent (Gene, Drug)	Animal model/phenotype	Administration time nanocarrier	Route of administration	Therapeutic effect Maternal /placental Fetal		Other outcomes	Ref.
Liposomes	N/S	Epitope hemagglutinin (HA)	Mitochondrial antioxidant manganese superoxide dismutase-plasmid	Irradiation mouse model Total body	GD13-14	IV	–	No significant improvement of birth weight or number ↑ pup survival at birth *	–	37
Liposomes	N/S	Endothelium of the uterine spiral arteries and placental labyrinth, CNKGLRKN-peptide	SE175 NO donor	Genetically modified mouse model, gene knockout, endothelial nitric oxide synthase knockout IUGR	GD11, GD13, GD15 and GD17	IV	↓ placenta weight ↑ F:P weight ratio ↑ mean spiral artery diameter	↑ fetal weight	–	38
Liposomes	iRGD 146	Placental iRGD (therapeutic effect FGR only with iRGD)	IGF-2	Genetically modified mouse model, gene knockout, deletion U2 Exon IGF2-gene, IUGR	GD11; GD13; GD15 and GD17	IV	NS in placental weight ↑ F:P weight ratio	↑ fetal weight	↓ the rate of cytotrophoblast apoptosis	34
Liposomes	N/S	conjugation of pLCSA-bp	siRNA, Nrf2 siRNA, and sFlt-1 siRNA	Genetically modified model, Pregnancy associated hypertension mouse model, not specified how model is induced.	GD13; GD15; GD17	IV	↓ maternal mortality ↓ hypertension ↓ urine protein level ↑ F:P weight ratio	↓ fetal mortality ↑ fetal weight	↑ placenta expression of angiogenic factors (CCL2, CCL5, CXCL9, and CXCL10).	42
Polymers	180 nm	–	Mitochondrial antioxidant manganese superoxide dismutase-plasmid	Hypoxic rat model 11% Oxygen	GD15	IV	No significant improvement F:P weight ratio	↑ Male:fetal weight No significant improvement crown-rump length, abdominal girth No significant improvement female weight	–	54
Polymers, pHMA-b-pDMAEMA	N/S	–	Trophoblast (gene transfer) PLAC1 human IGF1 plasmid DNA	Genetically modified mouse model, gene knockout, endothelial nitric oxide synthase knockout IUGR	GD15	Intra-placental, LZ region	No significant improvement placenta weight	No significant improvement fetal weight	–	69
Polymers	130	Mitochondrial Anti oxidant mitoQ	–	Hypoxic rat model 11% Oxygen	GD15	IV	Female: ↑ placenta weight No significant improvement in F:P weight ratio	Female: ↑ fetal weight Male: No significant improvement fetal weight ($p = 0.47$)	↓ oxidative stress (ROS), both male and female fetuses.	68
Polymers	N/S	Mitochondrial Anti oxidant mitoQ	–	Hypoxic rat model 11% Oxygen	GD15	IV	No significant improvement in placental weight	Fetal weight not described ↑ birth weight	↓ oxidative stress	51
Polymers, ELP	N/S	–	human VEGF-B167 (ELP-VEGF-B)	Invasive ischaemic placenta RUPP rat model	GD13,15,17	SC	No significant improvement hypertension or placental weight	No significant improvement of fetal weight or fetal reabsorption rate	–	55
Polymers, ELP	N/S	–	VEGF Chimera	Invasive ischaemic placenta RUPP rat model	GD14-19, daily	IP infusion	↓ hypertension (dose-dependent)	No significant improvement fetal weight	↑ sFlt-1 plasma levels, ↓ free sFlt-1, ↑ NO production	44
Polymers, ELP	N/S	–	CPP SynB1- NF-κB inhibitory peptide (p50i)	Invasive ischaemic placenta RUPP rat model	GD 14-19, Continuous	IP infusion	trend ↓ hypertension	trend ↑ fetal weight	–	47
Polymers, PAMAM	70-80	–	siRNA -sFlt-1	Inflammatory rat model Pre-eclampsia TNF-α GD10-GD14	GD10-17	IV	↓ hypertension ↓ Urine protein level ↑ placental weight	↑ fetal weight	↓ free sFlt-1 *	62
Polymers, pHMA-b-pDMAEMA	N/S	–	Trophoblast (gene transfer) Cyp19a or PLAC1 human IGF1 plasmid DNA	Invasive IUGR mouse model, uterine branch ligation	GD16	Intra-placental	No significant improvement placenta weight	↑ birth weight	↑ IGF-1 expression	48

Legend: Therapeutic effect of nanosized drug delivery system in different complicated pregnancies models, with described route and time of administration *studies observed also longterm effect offspring, not described in this review. Abbreviations: GD gestational day; IV intra venous; IP intraperitoneal; N/S = not specified.

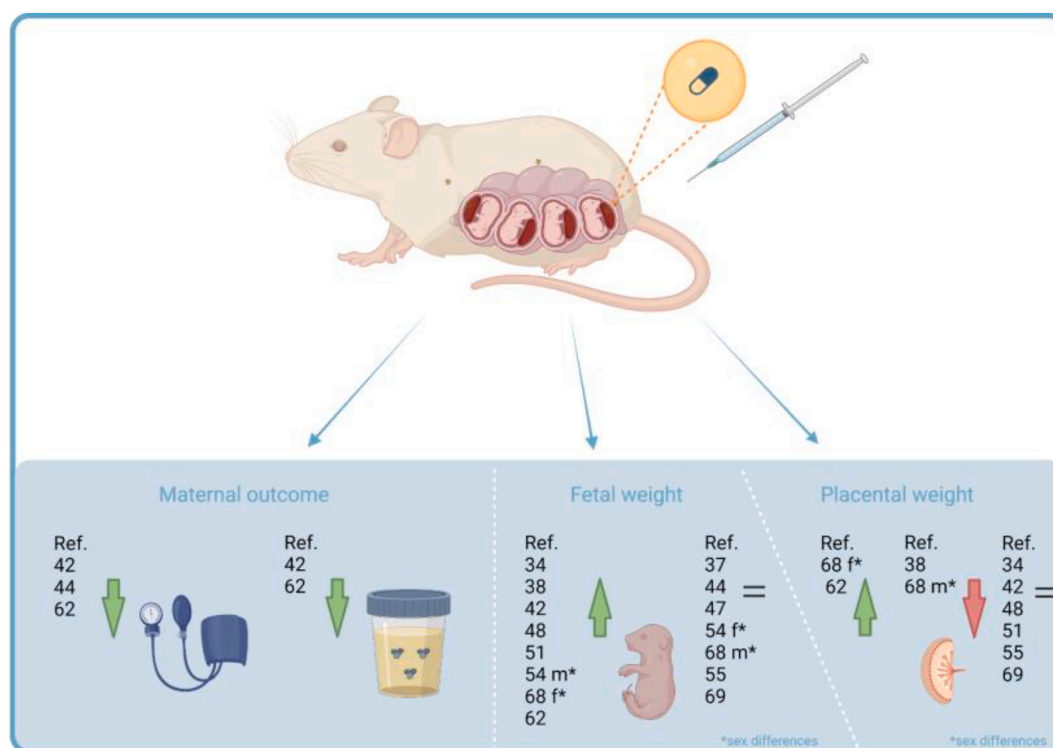


Fig. 2. Therapeutic outcome of nanomedicines performed in PE and FGR phenotype animal models

Overview of maternal, placental, and fetal outcome in PE/FGR models. Green arrow stands for improvement of phenotype. Sex- specific differences in fetal and placental weight are displayed with male(m) or female(f). Figure created in Biorender.

8. Discussion and future perspectives

At present, no therapy is available for PE and FGR. Progression in the field is hampered due to safety challenges. The use of nanomedicines targeting the placenta and delivering the drug at its site of action provides a chance to improve therapy efficacy and safety. On top of improving maternal health with placental-targeted strategies, these early interventions also provide an opportunity to impact fetal development and improve offspring health later in life.

8.1. A promising approach

The studies summarized in this review gives an outline of the recent developments in innovative approaches with the focus to safely treat PE and FGR. By encapsulating or associating a drug molecule to a nanoparticle, the fate of the drug molecule is no longer dictated by its own physicochemical characteristics but determined by the characteristics of the nanoparticle. The particle size and surface charge of these nanomedicines can be altered, to allow for controlled drug release targeted to specific locations over an extended period of time in order to achieve an increased therapeutic efficacy. This permits the use of lower dosages of therapeutic drugs; reduced repetition of intervention and an effective therapeutic dose will properly be achieved earlier. Importantly, placental retention rate and trans-placental passage in the studied animal models seem to depend on the nanosized drug delivery system characteristics but also on the encapsulated drug characteristics, gestational age, fetal sex differences, placental maturity, and integrity. The nanosized drug delivery system choice should be based, primarily on biodegradability and biocompatibility. Secondly, on limited trans placental passage and specific placental tissue targeting. In this context, both liposomes and polymers seem to ensure the safety of mother and fetus. Yet, firm conclusions cannot be drawn due to the limited number of studies available for complicated pregnancies, the variability in design of the nanomedicines together with the lack of information on

size and charge of the systems in some publications. Therefore, it is complex to compare and determine the best approach to prevent trans-placental passage and a high therapy efficacy.

8.2. Current challenges

Pinpointing the exact window to therapeutically intervene in humans is challenged by the difficulty to translate animal data to human pregnancy. All these animal studies started the treatment with these nanomedicines at time of induction of the model, whereas in the clinical situation, administration of therapy would most likely be subsequent to diagnosis. Moreover, animal models often mimic placental insufficiency syndromes by manipulation of a specific pathway whereas the human disease is multifactorial. In addition, placental development and function differ between species e.g. trophoblast layers, pore sizes and differences in electro potential. Accordingly, in humans choosing the most relevant time point, dosages and frequency of the therapeutic intervention will be a demanding process and has yet to be determined and possibly needs individual based therapy. Imaging of labeled nanoparticles could be a useful tool to identify the best timing for personalized targeted therapy.

In summary, potential placental-specific ligands to functionalize nanomedicines must be investigated to promote the targeting specificity. The nanomedicines feature control over placental uptake and their trans-placental passage are keys to progress. Safe, biocompatible, and biodegradable nanomedicines should be rationally designed. Moreover, they need to be extensively evaluated using robust analytical tools in different relevant human and animal models. This may be complemented by diagnostic tools to assess the disease status to identify the best time to initiate treatment. Along these lines we could overarch the various pathophysiological pathways of preeclampsia, to specifically treat the placental insufficiency causing preeclampsia and fetal growth restriction.

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Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jconrel.2023.06.003>.

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