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DOI:

[10.1016/j.jri.2017.09.009](https://doi.org/10.1016/j.jri.2017.09.009)

Document Version

Peer reviewed version

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Citation for published version (APA):

Girardi, G. (2017). Pravastatin to treat and prevent preeclampsia. Preclinical and clinical studies. *Journal of Reproductive Immunology*, 124, 15-20. <https://doi.org/10.1016/j.jri.2017.09.009>

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Accepted Manuscript

Title: Pravastatin to treat and prevent preeclampsia. Preclinical and clinical studies.

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PII: S0165-0378(17)30069-4
DOI: <https://doi.org/10.1016/j.jri.2017.09.009>
Reference: JRI 2503



To appear in: *Journal of Reproductive Immunology*

Received date: 16-7-2017
Revised date: 26-9-2017
Accepted date: 26-9-2017

Please cite this article as: Girardi, Guillermina, Pravastatin to treat and prevent preeclampsia. Preclinical and clinical studies. *Journal of Reproductive Immunology* <https://doi.org/10.1016/j.jri.2017.09.009>

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Pravastatin to treat and prevent preeclampsia. Preclinical and clinical studies.

Running title. Pravastatin to treat preeclampsia

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HIGHLIGHTS

- **Mouse models have been instrumental in defining pathogenic mechanisms in preeclampsia and in the identification of statins as a potential treatment.**
- **Numerous epidemiological studies provided robust evidence demonstrating that pravastatin exposure during pregnancy does not affect fetal development.**
- **Several pilot studies suggest that pravastatin may be a good option to prevent and treat preeclampsia in women.**
- **Rnandomised clinical trial are necessary to confirm the effectiveness of pravastatin to treat preeclampsia.**

SUMMARY

Pre-eclampsia is a disease of pregnancy affecting 5%–8% of all pregnancies and leading cause of maternal and fetal mortality. Despite improvements in the diagnosis, there is no effective method of prevention and treatment. While studies in women are of critical importance, investigation of pathological mechanisms in pregnant women is necessarily limited, and the ability to establish cause and effect relationships, difficult. Mouse models have been instrumental in defining pathogenic mechanisms in preeclampsia and in the identification of statins as a potential treatment to prevent pregnancy complications associated with placental dysfunction.

Numerous epidemiological studies provided robust evidence demonstrating that pravastatin exposure during pregnancy does not affect fetal development. In addition, pravastatin is hydrophilic and has a limited passage through the placenta, diminishing any safety concerns. Several pilot studies suggest that pravastatin may be a good option to prevent and treat preeclampsia in women. While these studies are promising, the effectiveness of pravastatin to treat preeclampsia needs to be confirmed by randomized clinical trials.

Key words: pravastatin, preeclampsia, obstetric antiphospholipid syndrome, animal models, translational studies

INTRODUCTION

Preeclampsia (PE) is a pregnancy complication characterized by high blood pressure and damage to organ systems such as the liver and kidneys. PE usually begins after 20 weeks of pregnancy in women whose blood pressure had been normal. Left untreated, PE can lead to serious - even fatal - complications for both mother and fetus. The only available treatment for PE is the management of the high blood pressure in the mother with antihypertensive medication and lastly, the delivery of the placenta and fetus with the serious risks associated with prematurity of the neonate. Identifying a beneficial treatment to improve maternal and fetal outcomes in PE remains paramount. In the last 10 years, numerous preclinical studies demonstrated that statins might be a beneficial treatment in PE.

Emerging data from several pilot experiments validated these results in women. RCT are currently ongoing to confirm these results.

Lessons from the mouse

While studies in women are of critical importance, investigation of pathological mechanisms in pregnant women is necessarily limited, and the ability to establish cause and effect relationships, difficult. Mouse models of PE that closely resemble the clinical cases have been essential in defining pathogenic mediators in PE and identifying targets for prevention and therapy. Several studies in mice identified pravastatin as a potential treatment for pregnancy complications associated with placental malperfusion. In this review article we will discuss both the preclinical studies and the translational studies that support the use of statins to prevent and treat preeclampsia.

Pravastatin prevents adverse pregnancy outcomes in a mouse model of antiphospholipid syndrome (APS)

Placental insufficiency is one of the most severe APS-related complications for pregnant women. Several *in vitro* studies demonstrated the binding of antiphospholipid (aPL) antibodies to trophoblasts, either cell lines or primary cells from placentas (Di Simone et al 2015, D'Ippolito et al 2007). However, until recently there was no direct evidence that aPL antibodies bind to the placenta *in vivo*. aPL antibodies, isolated from patients by affinity purification were labelled with radioactive indium-111 and single photon emission computed tomography (SPECT/CT) was used to visualize the tissue distribution and binding of aPL antibodies in a pregnant mouse (Bertolaccini et al 2016). After an intravenous injection, radiolabelled antibodies were rapidly cleared from circulation and accumulated in the fetal sacs (Bertolaccini et al 2016). Within the fetal sacs, a high count was found in the placenta (Bertolaccini et al 2016). Using a non invasive *in vivo* real time imaging method the placenta as a main target organ in APS was demonstrated.

Given that thrombosis is one of the most common manifestations in APS, pregnancy complications in APS have been erroneously attributed to placental thrombosis and infarcts. Animal models demonstrated that inflammation, in particular complement activation,

rather than thrombosis plays a crucial role in trophoblast injury (Girardi et al 2004). Furthermore, histological studies in human placentas also suggest that inflammation contributes to placental injury (Viall et al 2015, Stone et al 2006). Regardless of the evidence suggesting that APS is a proinflammatory disorder, the primary treatment for pregnant women with APS is aimed to preventing thrombosis. Despite drugs such as heparin and low dose aspirin, pregnant women with APS are at increase risk for severe preeclampsia and intrauterine growth restriction (IUGR) associated with impaired placentation (Laskin et al. 2009, Branch et al 1992). Animal models of APS allowed the identification of the pathogenic mechanisms of aPL and therapeutic approaches to prevent adverse pregnancy outcomes. Binding of aPL antibodies to the placenta was associated with complement deposition, measured using USPIO-labelled anti C3 antibodies and magnetic resonance imaging (Girardi et al, 2015). aPL-induced complement activation in the placenta was associated with signs of placental insufficiency characterized by increased neutrophil infiltration, decreased blood flow, increased levels of isoprostane STAT-8 –indicative of oxidative stress- and angiogenic imbalance characterized by diminished levels of vascular endothelial growth factor (VEGF) (Girardi et al 2015, Redecha et al 2008 **Figure 1**). A cross talk between coagulation and inflammation has been described in the pathogenesis of abnormal pregnancy outcomes in APS (Redecha et al 2007, Redecha et al 2008). Tissue factor (TF) was identified as a downstream mediator of complement activation (Redecha et al, 2007). TF, the major cellular activator of the coagulation cascade, is a key mediator in inflammation and trophoblasts injury in the mouse model of obstetric APS (OAPS-mice) (Redecha et al, 2008). Using genetically modified mice with selective expression of TF in different tissues, an important role of complement-mediated TF expression in maternal neutrophils was demonstrated in OAPS-mice (7, **Figure 2**). aPL-induced TF expression modulates neutrophil activity towards a proinflammatory phenotype. In OAPS-mice, TF increases the respiratory burst and phagocytosis leading to subsequent trophoblast oxidative injury and adverse pregnancy outcomes. TF-mediated proinflammatory phenotype in OAPS involved the activation of protease activated receptor 2 (PAR-2). Mice deficient in PAR-2 and treated with aPL antibodies exhibited reduced neutrophil activation and normal pregnancies, indicating that PAR-2 plays an important role in the pathogenesis of aPL antibody-induced fetal injury (Redecha et al, 2008). Statins, simvastatin and pravastatin, downregulate TF and PAR-2 expression in neutrophils, prevent neutrophil activation, trophoblast injury and fetal death

(Redecha et al, 2008). By downregulating TF and PAR-2, statins target the proinflammatory phenotype of neutrophils in OAPS leading to normal pregnancies. Pleiotropic effects of statins include improvement of endothelial dysfunction, increased nitric oxide bioavailability, antioxidant properties, inhibition of inflammatory and prothrombotic responses, and proangiogenic responses. All these cholesterol-independent properties (**Figure 3**) may also contribute to the preventive effects of statins in pregnancy complications associated with placental insufficiency. In this line, pravastatin prevented oxidative stress, placental angiogenic imbalance and neutrophil infiltration in the 15 dpc placentas from the surviving fetuses in the OAPS-mouse model (**Figure 2**)

Pravastatin prevents the onset of preeclampsia in mouse models

The last decade has seen the emergence of abundant experimental evidence supporting a protective role for statins in preventing preeclampsia in mice. Pravastatin, hydrosoluble statin with limited transport across the placenta and thus favorable pharmacokinetic profile in pregnancy (Balan et al 2017, Nanovskaya et al 2013, Zarek et al 2013), prevented the onset of PE in several animal models listed below (a-c). The numerous studies in mice paved the way for using pravastatin in pregnant women.

a- C1q KO mouse model

Complement component C1q has an important role in trophoblast migration, spiral arteries remodeling, and normal placentation (Agostinis et al 2010, Singh et al 2011). Pregnant C1q-deficient (C1qKO) mice recapitulate the key features of human PE: hypertension, albuminuria, endotheliosis, decreased placental vascular endothelial growth factor (VEGF) and elevated levels of soluble VEGF receptor 1 (sVEGFR1 or sFlt-1) that correlate with increased fetal death (Singh et al 2011). In addition, decreased blood flow and increased oxidative stress are observed in placentas from C1qKO mice. Treatment of C1qKO mice with pravastatin restored trophoblast invasiveness, improved placental blood flow, and angiogenic balance and, thus, prevented the onset of PE (Singh et al 2011). sVEGFR1 levels were reduced and placental VEGF levels were significantly increased in C1qKO mice treated with pravastatin compared with untreated C1qKO mice (16). Pravastatin treatment reduced

hypertension and albuminuria, signs of preeclampsia associated with adverse pregnancy outcomes. Renal damage and endothelial dysfunction were significantly attenuated with pravastatin (Singh et al 2011). Studies using the C1qKO mouse model of PE highlighted the causative role of impaired trophoblast invasion in the pathogenesis of PE and identified pravastatin as a good therapeutic option to prevent PE.

b- CBA/J x DBA/2 mouse model

While C1qKO pregnant mice exhibit the wide spectrum of clinical features observed in human PE, the CBA/J x DBA/2 mouse model of recurrent miscarriage show most of the clinical signs except hypertension (Ahmed et al 2010). CBA/J females impregnated by DBA/2 males develop endothelial dysfunction and angiogenic factors dysregulation leading to adverse pregnancy outcomes (Ahmed et al 2010). Provocatively, as observed in humans with PE, the concept of primigravidity - epidemiological landmark of this disease - is also observed in CBA/J x DBA/2 mice, in which only the first pregnancy is affected (Ahmed et al 2010). CBA/J x DBA/2 mice exhibit albuminuria, endotheliosis, increased sensitivity to angiotensin II and increased plasma leptin levels during the first pregnancy that correlates with bad pregnancy outcomes. Despite not having hypertension, CBA/J x DBA/2 females show severe endothelial dysfunction (Ahmed et al 2010). Antagonism of VEGF signaling by sFLT1 seems to be involved in placental and fetal injury in CBA/J x DBA/2 mice. Pravastatin restored angiogenic balance, ameliorated glomerular injury, diminished hypersensitivity to angiotensin II and prevented intrauterine growth restriction leading to favorable maternal and fetal outcomes (Ahmed et al 2010). The CBA/J x DBA/2 model was another helpful tool, in the identification of pravastatin as a candidate therapy to prevent preeclampsia and its related maternal and fetal complications.

c- sFLT1-induced preeclampsia mouse models

The human placenta undergoes high levels of angiogenesis during pregnancy. Failure of placental angiogenesis has been linked to the pathogenesis of preeclampsia. It is currently believed that soluble factors released by the diseased placenta lead to an antiangiogenic state and the onset of preeclampsia. Placenta-derived anti-angiogenic factors, such as sFLT1 and soluble endoglin (sENG), have been related to the cause and progression of

preeclampsia (Maynard et al 2008, Maynard et al 2011). Indeed, systemic administration of adenoviral vectors expressing sFLT-1 to mice resulted in PE (Costantine et al 2010). Interestingly, pravastatin improved the vascular reactivity in this murine model of PE and decreased sFLT-1 levels (Costantine et al 2010). Using this model of PE induced by general overexpression of sFLT-1, the authors suggested that pravastatin's ability to prevent the preeclamptic phenotype may be mediated through pleiotropic mechanisms involving a prosurvival/ antiapoptotic mitogen-activated protein kinase (MAPK) pathway in the trophoblasts (Saad et al, 2016).

The beneficial effects of statins in PE were also observed in a mouse model of preeclampsia that highlights the importance of the placental origin of antiangiogenic factors in the pathogenesis of PE. Kumasawa et al developed a model of PE that faithfully reproduces many of the human findings of late-onset PE by using a lentiviral vector-mediated placenta-specific expression system (Kumasawa et al 2011). Transduction of the trophectoderm - that provides most of the main and functional components of the future placenta - of blastocyst-stage embryos with HIV-I-based self-inactivating lentiviral vectors expressing sFLT-1 resulted in the development of PE in mice. In this model, hypertension, proteinuria and intrauterine growth restriction were observed (Kumasawa et al 2011). Pravastatin treatment of mice with sFLT-1-induced PE, ameliorated symptoms by increasing VEGF-like angiogenic factor placental growth factor and diminishing sFLT1 (Kumasawa et al 2011).

The protective effects of statins in pregnancy were also observed in rats with reduced utero-placental perfusion pressure (RUPP)-induced hypertension (23). In the rat, pravastatin attenuated hypertension, oxidative stress and angiogenic imbalance after placental-ischaemia was induced by reduction of utero-placental perfusion pressure (RUPP) (Bauer et al, 2013).

In vitro studies

In vitro studies using isolated trophoblasts or endothelial cells have been used to complement in vivo studies in the identification of pravastatin mechanism/s. In this line, several studies investigating the effects of pravastatin on the release of angiogenic/antiangiogenic factors by trophoblasts and endothelial cells were performed. The results obtained from these studies

need to be interpreted with caution as concentrations of pravastatin, that do not mimic the in vivo plasma concentrations measured in pregnant women, were used in many of these in vitro studies. The pharmacokinetic data regarding the use of pravastatin for preventing preeclampsia in high-risk pregnant women were reported in a recent study by Costantine and colleagues (Costantine et al 2016). This study determined the biodistribution of pravastatin in pregnant women for the first time. The maximum (or peak) serum concentration (C_{max}) that was achieved after an oral dose of 10mg of pravastatin was 14.9±11.3 ng/mL at 18-24 weeks and 11.1 ±6.2 ng/mL at 30-34 weeks (Costantine et al 2016). The half life of pravastatin was 2.1±0.9 h and 3.0 ±1.6 h respectively (Costantine et al 2016). In a previous study performed in males, the C_{max} after oral administration of 20 mg of [14C]-pravastatin was 27.4 ± 10.7 ng/mL (Singhvi et al 1990). The in vitro studies by Brownfoot and collaborators incubated primary placental cells and endothelial cells with doses of pravastatin ranging from 2 to 2000 μM (pravastatin MW: 424,52 g/mol) (Brownfoot et al 2014, 2015 , 2016). A study by Odiari et al, reported that pravastatin augments aPL-induced release of inflammatory and antiangiogenic factors and also affects migration in a trophoblast cell line (Odiari et al 2012). This study used 500 to 2500 ng/ml of pravastatin, significantly higher than the C_{max} (≈ 10-15 ng/ml) measured in pregnant women. Thus, these observations might not resemble the clinical physiological conditions.

From mice to women – Pilot clinical studies and RCT

Based on the pathophysiologic similarities between cardiovascular disease and preeclampsia and the promising results observed in different animal models in the last 10 years, treating pregnant women with statins to prevent preeclampsia seems like a good therapeutic option. Despite the abundant information provided by animal studies regarding the beneficial effects of statins in preventing pregnancy complications and its safety during pregnancy in mice and women, translational studies and the organization of RCT was significantly delayed. This delay was caused in part because of the misinterpretation and misuse of the Food and Drug administration (FDA) categories. Statins were classified as category X in 1979 not because they were teratogenic but because there was no indication for a woman to take statins while pregnant and no data relating to the effects on a pregnant

women and/or the fetus were available at that time (as opposed to the existence of evidence of harm) (Manson et al 1996, Girardi 2014).

The use of drugs in pregnancy for off-label indications such as pravastatin for preeclampsia prevention is still challenging (Cleary et al 2014). Importantly, while atorvastatin and simvastatin were included in category X, pravastatin was never included in this classification. Epidemiological data collected to date demonstrate that statins are not major teratogens (Zarek et al 2014, Karalis et al 2016). Several reports described normal fetal outcomes in women that were inadvertently exposed to statins during pregnancy (Bateman et al 2015, Lecarpentier et al 2012, Winterfeld et al 2013). In 2015, based on the fact that the five-letter system resulted in erroneous assumptions about the actual meaning of the letters, the FDA replaced the former pregnancy risk letter categories on prescription drug labelling with new and clearer information for both patients and healthcare providers (www.drugs.com/pregnancy-categories.html) strong evidence of fetal safety is now available. Furthermore, recent studies showing a limited transfer of pravastatin across the dually perfused placental supports pravastatin's favourable pharmacokinetic profile in pregnancy (Balan et al 2017). That several clinical studies are currently being performed in the USA and Europe clearly shows a better understanding of clinicians and patients regarding the safe use of statins during pregnancy.

In humans, the first report to suggest the beneficial effects of pravastatin in preventing preeclampsia described a patient with antiphospholipid syndrome (APS). The APS-patient, with a history of early preeclampsia leading to a still birth at week 26, developed preeclampsia in her second pregnancy, despite anticoagulant treatment (Lefkou et al 2014). Uterine artery Dopplers showed increased resistance and bilateral notching. To prevent intrauterine fetal death as in the previous pregnancy, the patient was supplemented with pravastatin. Addition of pravastatin (20mg/day) to standard of care therapy low molecular weight heparin plus low dose aspirin normalised maternal disease, blood pressure and proteinuria and reversed abnormal uterine blood flow (Lefkou et al 2014). A live and healthy baby girl weighing 2830 g was delivered vaginally at 38 weeks with no complications. The patient stopped taking pravastatin prior to delivery, in preparation for breastfeeding. Interestingly, preeclampsia relapsed shortly after delivery. Pravastatin

therapy (20 mg/day) was started again and the preeclamptic features disappeared (**Figure 4**, Lefkou et al 2014).

A pilot clinical study to examine the effects of pravastatin in women diagnosed with PE between 24 and 29 weeks was conducted in Australia (Brownfoot et al 2015). In this study, 4 patients –significantly hypertensive (90-105/155-200 mm Hg) and with proteinuria ranging from 840 to 2990 mg/24h- were treated with daily pravastatin (40 mg) from the day of admission. All women presented growth restricted fetuses. The patients also received betamethasone and magnesium sulfate for lung maturation and neuroprotection. After pravastatin treatment symptoms of PE resolved. Pravastatin stabilized blood pressure in 3 of the patients and only one patient required antihypertensive medication nifedipine. Delivery in these women were triggered by fetal indications rather than worsening of maternal disease. Furthermore, serum sFLT-1, sENG and endothelin-1 levels remained stable after pravastatin treatment (Brownfoot et al 2015). The authors concluded that pravastatin might be a candidate therapeutic strategy for preeclampsia.

Another study in women with OAPS that did not respond to anticoagulation and develop PE was recently published (Lefkou et al 2016). As mentioned before, many women with OAPS do not respond to low dose aspirin (LDA) and heparinoids (LMWH) (Laskin et al 2009) and develop PE. In an attempt to improve obstetrical outcome, the effects of pravastatin in this group of women with OAPS was assessed. 21 pregnant women with OAPS and an adverse obstetric history - that developed PE and/or severe intrauterine growth restriction (IUGR) despite being treated with LDA+LMWH since the beginning of pregnancy - participated in this study. A group of 10 women with APS and severe PE and/or IUGR received only conventional LDA+LMWH treatment and served as control. Pravastatin (20mg/day) was added to conventional treatment in 11 women when signs of preeclampsia and or IUGR were observed (Lefkou et al 2016). In the group supplemented with pravastatin, placental blood flow increased and hypertension and proteinuria stabilized as early as 10 days after treatment (median 14, IQR[10-15]) leading to live birth in all patients (Lefkou et al 2016, **Table 1**).

In the control group that received only antithrombotic therapy maternal disease did not improve and deliveries occurred preterm and only 6 of the 11 neonates survived. Neonates spent several months at the neonatology intensive care unit and 3 show abnormal development (40, **table 1**). In the group treated with pravastatin pregnancies were

significantly prolonged after diagnosis (13 weeks IQR[8-14]) compared to the group that only received LDA+aspirin (4.5 weeks, IQR[2-6] (Lefkou et al 2016, **Table 1**). Delivery dates in the group supplemented with pravastatin were close to term allowing appropriate fetal development and preventing admission to the NICU. No late sequelae were reported for the infants in the pravastatin group (Lefkou et al 2016). This study suggests that women with refractory OAPS may have improved pregnancy outcomes with pravastatin taken at the time of onset of PE or severe IUGR until the end of pregnancy.

The studies mentioned above were aimed to ameliorate preeclampsia after the onset of the disease. Interestingly, a study by Costantine et al (24) investigated the effectiveness of pravastatin in preventing preeclampsia in high risk patients. An initial pilot study in a multicenter, double-blind, placebo-controlled, randomized trial (clinicaltrials.gov NCT01717586) (Costantine et al 2016), evaluated the utility of pravastatin in preventing preeclampsia in women with a history of severe preeclampsia in a prior pregnancy that required delivery before 34 weeks. Twenty subjects between 12 and 16 weeks of pregnancy, were randomized to pravastatin (10 mg) (n=10) or placebo (n=10). Four women in the placebo group developed preeclampsia; three of them showed severe disease compared to the pravastatin group, suggesting a beneficial effect of pravastatin in preventing the onset of preeclampsia (Costantine et al 2016). In line with the proangiogenic effects of pravastatin, a slight but not significant diminution in antiangiogenic factors sFlt-1 and sEng and increase in PlGF was observed in the pravastatin group compared to placebo (Costantine et al 2016). Birth weight was similar in both groups and no congenital abnormalities or developmental abnormalities were observed in the two groups. No maternal, fetal or neonatal death was observed (Costantine et al 2016). As previously stated, this study provided important information regarding preliminary safety and pharmacokinetic data regarding the use of pravastatin in early pregnancy to prevent preeclampsia in high risk women. This pilot study was part of a larger RCT that is currently taking place at Eunice Kennedy Shriver National Institute of Child Health and Human Development Obstetric-Fetal Pharmacology Research Units Network. A proof of principle, double-blind, randomised placebo-controlled, multicentre trial of pravastatin to ameliorate early onset pre-eclampsia (sTAMP) was recently completed in the United Kingdom (<https://www.clinicaltrialsregister.eu/ctr-search/trial/2009-012968-13/GB>). The aim of this

trial was to establish whether a significant reduction of angiogenic markers by pravastatin will alleviate the severity of early-onset pre-eclampsia in women.

CONCLUSION

The preclinical studies showed promising results on the beneficial effects of pravastatin in treating placental insufficiency and laid foundation for the human studies. The mouse studies and the pilot human studies emphasize the need of a Randomised Clinical Trials (RCT) to confirm these observations. Pravastatin was never included in category X by the FDA and due to its hydrophilic characteristics has a minimal transplacental transfer, diminishing fetal safety concerns. The new FDA classification that replaced the five-letter system together with the abundant evidence demonstrates that pravastatin is safe during pregnancy. Other therapeutic strategies to prevent and/or treat preeclampsia have had limited success and the only current available therapy is the delivery of the baby, associated with high risks due to prematurity. Treatment and prevention of PE with pravastatin is a promising option to prevent and treat preeclampsia but RCT should confirm its effectiveness.

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FIGURE AND TABLE LEGENDS**Figure 1. Placental dysfunction in OAPS-mice – protective effects of pravastatin.**

Increased levels of 8- isoprostane (STAT-8), marker of oxidative stress and decreased levels of proangiogenic molecule VEGF are observed in 15 dpc placentas from the surviving fetuses in aPL-treated mice (OAPS-mice). Increased infiltration of neutrophils is also observed in these placentas. Control placentas belong to mice that received normal human IgG (NHlgG). Pravastatin prevented all these signs associated with placental dysfunction.

Figure 2. *Statins prevent neutrophil activation, trophoblast injury and adverse pregnancy outcomes in a mouse model of obstetric APS - proposed mechanism.*

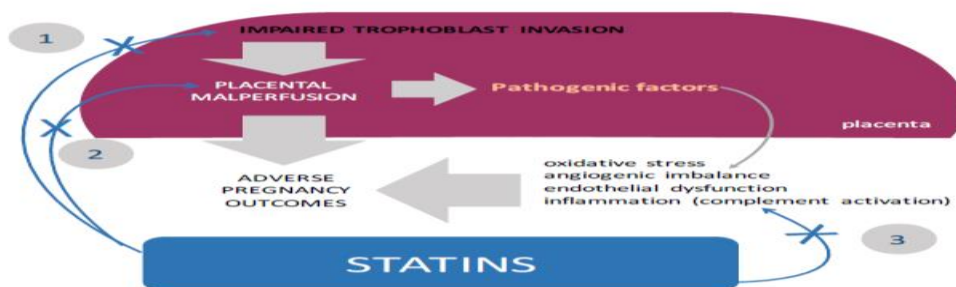
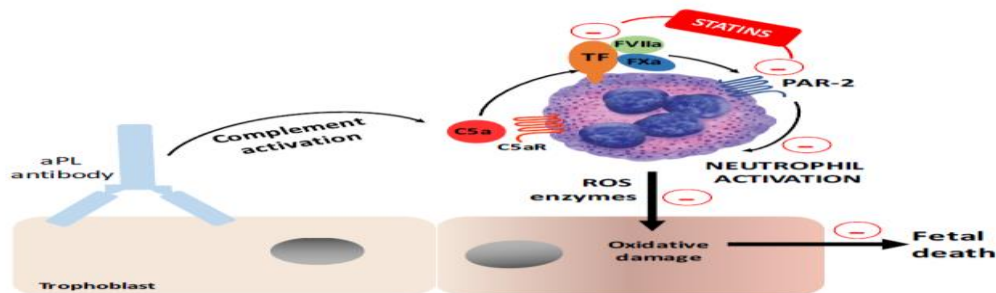
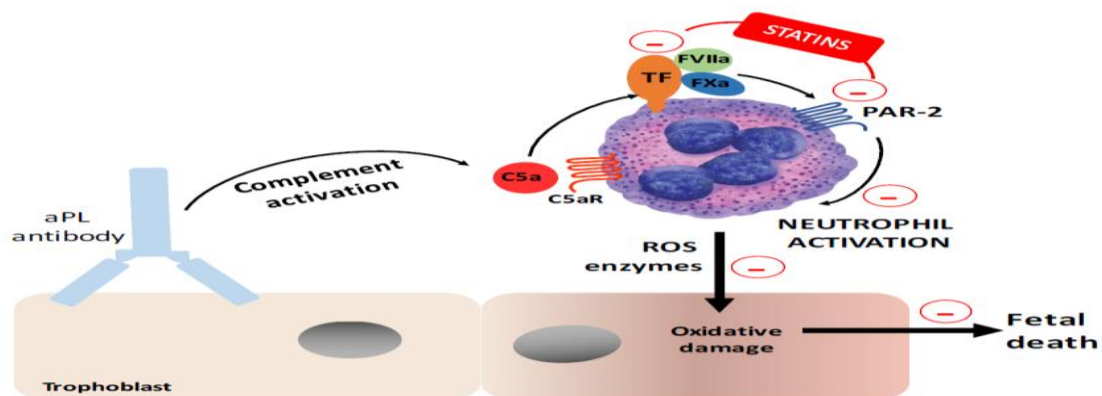
Maternal aPL antibodies bind to the placenta and activate the complement system. Interaction of complement split product C5a with its receptor C5aR triggers expression by neutrophils of tissue factor (TF), resulting in increased phagocytic capacity and generation of reactive oxygen species. Increased neutrophil activity leads to trophoblast injury and ultimately fetal death. TF-mediated neutrophil activation proceeds through engagement of protease activated receptor 2 (PAR2). Pravastatin prevent C5a-induced upregulation of TF and PAR2 expression, thereby inhibiting the release of reactive oxygen species and trophoblast damage, protecting pregnancies.

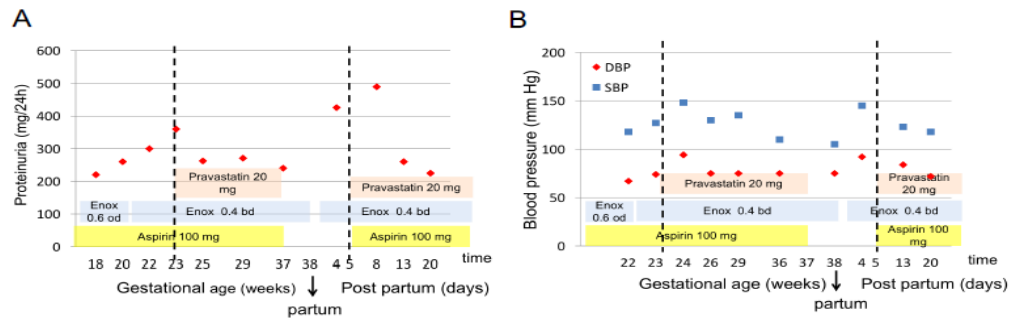
Figure 3. *Pleiotropic effects of statins.* The beneficial effects of pravastatin in preventing placental malperfusion/insufficiency might not be entirely due to cholesterol reduction.

There is now compelling evidence that statins: 1- stimulate trophoblast invasion, 2- increase placenta blood flow preventing the release of pathogenic factors. 3- Other beneficial effects include: antioxidant properties, release of nitric oxide, protection of the endothelium, anti-inflammatory and anticoagulant.

Figure 4. *Pravastatin improved blood pressure and proteinuria during pregnancy and postpartum in a patient with APS refractory to anticoagulation (enoxaparin and aspirin).*

- A- Urinary protein levels (mg/24h) in the patient during pregnancy and postpartum. Proteinuria dropped significantly after pravastatin treatment was administered at 23+1 weeks and at day 5 in the postpartum (Lefkou et al 2014).
- B- Systolic (SBP) and diastolic (DBP) blood pressure variations during pregnancy and postpartum. A significant diminution in SBP and DBP was observed after pravastatin was added at 23+1weeks and 5 days postpartum. Ninety days after delivery, proteinuria and blood pressure remained within normal values (Lefkou et al 2014).





APS	PE / IUGR	Doppler studies	LMWH + LDA	Pravastatin - time of administration (weeks)	Threshold achievement (BP <130/90 mmHg; Prot < 300mg/dl, normal Dopplers) (days)	Pregnancy survival after diagnosis (weeks)	Time of delivery (weeks)	Neonatal outcome
YES	Yes severe	↑uterine arteries PI 4 bilateral Notching 2rEDV	yes	NO	-	4.5 IQR[2-6] 3 stillbirths (25-26 w)	26.5 IQR[26-32]	BW: 900gr IQR[580-1100] All admitted at NICU: 3 deaths 3 abnormal development
YES	Yes severe	↑uterine arteries PI 3 bilateral notching 2rEDV	yes	YES 24 IQR [23-26]	14 IQR[10-15]	13 IQR[8-14]	36 IQR[35-38]	BW: 2390gr IQR[2065-2770] 2 admitted at NICU (twins) All normal now

Table 1. Pravastatin improved maternal and fetal outcomes in OAPS refractory to

APS	PE/IUGR	Dopplers studies	LMWH + LDA	Pravastatin time of administration (weeks) (median, IQR)	Threshold Achievement (BP<130/90 mmHg; prot <300 mg/dl, normal Dopplers (days) (median, IQR)	Pregnancy survival after diagnosis (weeks)(median, IQR)	Time of Delivery (weeks) (median , IQR)	Neonatal outcomes
YES (n=10)	Yes, severe	↑ uterine arteries PI 4 bilateral notching 2REDV	yes	NO	-	4.5 IQR [2-6] 3 stillbirths (25-26w)	26.5 IQR [26-32]	BW:900g IQR[580-1100] All admitted at NICU 3 deaths 3 abnormal development
YES (n=11)	Yes, severe	↑ uterine arteries PI 3 bilateral notching 2REDV	yes	YES 24 IQR [23-26]	14 IQR [10-15]	13 IQR [8-14]	36 IQR[35-38]	BW: 2390g IQR[2065-2770] 2 admitted at NICU (twins) normal development in all

antithrombotic therapy.

Maternal and fetal improvement was observed at 14 days (IQR[10-15])(Lefkou et al, 2016).

Pregnancy survival improved significantly with pravastatin treatment and deliveries occurred close to term diminishing the risks associated with prematurity. LMWH=low molecular weight heparin; LDA=low dose aspirin; PE preeclampsia; IUGR=intrauterine growth restriction; PI= pulsatility index, REDV= reverse end diastolic volume (Umbilical arteries); BP=blood pressure; Prot=proteinuria; BW=birth weight; NICU=neonatal intensive care unit